PROCEEDINGS OF THE 5TH INTERNATIONAL SPONGE SYMPOSIUM

ORIGIN & OUTLOOK

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5TH INTERNATIONAL SPONGE SYMPOSIUM, 'ORIGIN & OUTLOOK'

In 1998 the Queensland Museum, Brisbane, hosted the *5th International Sponge Symposium, 'Origin & Outlook' (5th ISS)* (27 June–4 July), including two concurrent pre-Symposium workshops on '*Systema Porifera*' and '*Early Evolution of Sponges and Biomarkers*', and a subsequent post-Symposium field excursion to Heron Island, Capricorn-Bunker Group, Great Barrier Reef (5-9 July).

The Brisbane Symposium was the largest of the five international symposia to date, with 165 delegates and accompanying partners present, from 25 countries. This was also the first occasion this series of international symposia has been held in the southern hemisphere, with previous meetings hosted (and Proceedings published) by: the Natural History Museum, London (1968) (Fry, 1970); Muséum National d'Histoire Naturelle, Paris (1978) (Lévi & Boury-Esnault, 1979); National Museum of Natural History, Smithsonian Institution, and Peabody Museum of Natural History, Yale University (held at Woods Hole Oceanographic Institute, Massachusetts, 1985) (Rützler, 1990); and the Zoölogisch Museum, University of Amsterdam (1993) (Van Soest et al., 1994). Over the past decade two smaller international meetings were also held in Berlin (1988), hosted by the Institut für Paläontologie, Freie Universität (Reitner & Keupp, 1991), and Lake Biwa, Japan (1996), hosted by a consortium of Japanese researchers lead by Yoko Watanabe (Ochanomizu University, Tokyo) (Watanabe & Fusetani, 1998). Additionally, over the past few decades, there have been several workshops conducted by North American and European workers, producing several substantial volumes of collective papers: Society for Developmental Biology, Albany, New York (1975) (Harrison & Cowden, 1976); Sherkin Island Marine Station, Ireland (1983) (Jones, 1987); Station Marine d'Endoume, Marseille, France (1986) (Vacelet & Boury-Esnault, 1987); Centre for Advanced Studies, Blanes, Spain (1992) (Uriz & Rützler, 1993); and Royal Belgian Institute of Natural Sciences, Brussels, Belgium (1995) (Willenz, 1996). Including the present volume, these conference and workshop proceedings contain over 550 refereed papers, signalling an abnormally high productivity and communication amongst sponge workers over a relatively short period, certainly considering the small size of our group.

The theme of the Brisbane Symposium, '*Origin & Outlook*' — reflected in the motif depicted on the cover ('Morphology to Molecules') — refers to the adage that scientific progress rests on knowledge of the past, and reflects the productive interaction between palaeontology, biology, chemistry, ecology, cytology, molecular biology and other disciplines as multidisciplinary approaches to the strange but innovative world of sponges. We are, perhaps, fortunate to work on a phylum that is important to the pharmaceutical industry, with its associated political and economic agendas, but the strength of our collective research lies in its diversity of approaches, opportunities and collaborative outcomes. This multidisciplinary synergy has escalated over the past decade: a trend reflected in many of the papers published here.

Despite the tyranny of distance, nearly 30% of delegates at the *5th ISS* in Brisbane were recent graduates or post-graduate students, from many countries, providing optimism for the future of sponge-related research in an otherwise aging population of established workers. The quality and diversity of their presentations, published here in 71 refereed papers and 69 additional abstracts, is testimony to this optimism.

Over the five-day Symposium, 95 scientific papers and approximately 60 posters were presented in 10 sessions: one session of invited papers and nine of general contributions. Claude Lévi was invited to deliver the keynote address on the central theme '*Origin & Outlook*'. Three invited plenary speakers were then presented with the challenge to explore this theme through the dimension of time: the *Past* (Françoise Debrenne), *Present* (Patricia Bergquist) and *Future* (Jean Vacelet) of sponges and those who work on them. Each of these keynote and plenary speakers were assigned their general themes, but given 'carte blanche' on how to approach them. As shown here, their perspectives were very different, but their conclusions were similar. The *Origin* is sound. We have an extensive and firm scientific knowledge-base to 'deal' with sponges in our various ways, even if many data are still missing, we are at least becoming increasingly aware of the 'right' sorts of questions we should be asking of these data. And, the *Outlook* for sponges and spongers is a bright one.

Papers presented during the nine general sessions encompassed a broad range of topics, including: the production of chemicals and the chemical ecology of sponge metabolites with pharmaceutical potential; commercial sponge fisheries and their human impacts; sponge cell behaviour and their immunological implications; the role of sponges as pollution indicators and their ecological interactions with other communities; the role of 'living fossil' sponges in coral reef geomorphology; advances in the origins and relationships of Porifera as evidenced by molecular biology; recent discoveries in biodiversity, evolution, biogeography and palaeontology of the phylum; and the physiology, ultrastructure and interactions of sponges with symbiotic microbes.

It was originally intended to publish these contributions under several, broad themes (palaeontology, systematics, ecology, etc.), but this became nearly impossible to organise given that the boundaries between many disciplines have become blurred through increasing multidisciplinary studies, and this strict arrangement is becoming less relevant.

Compared to the institutions that have hosted previous symposia, the Queensland Museum has no tradition of sponge research prior to 1991. However, thanks to pioneering efforts of previous generations of scientists working on Australian sponges (1813-1950) — Lamarck, Bowerbank, Haeckel, Selenka, Saville-Kent, Marshall, Carter, Dendy, Ridley, Poléjaeff, Lendenfeld, Kieschnick, Kirkpatrick, Whitelegge, Hentschel, Hallmann, Row, Shaw, Burton, Guiler — we know that Australian waters contain a megadiverse fauna of more than 1,500 published ('valid') species of sponges (Hooper & Wiedenmayer, 1994; with subsequent updates since 1994), mostly unique. We also now know that more than double this number of species live in these waters (Queensland Museum database), representing about 30% of the world's known sponge diversity, and of these more than 50% were collected from Queensland waters. It was therefore appropriate for the Queensland Museum to host the *5th ISS*, as a relatively new, active and expanding proponent for marine sponge biodiversity research and conservation. The *5th ISS* provided an important forum to disseminate this new knowledge to the international scientific community; in promoting the phylum as an important, productive target for future research; promoting the quality and diversity of our international collaborations, and to facilitate these in the future; and demonstrating the substantial amount of work that still remains to be done to achieve even an adequate 'basic knowledge' of this simple, but complex, phylum of animals.

It is greatly anticipated that escalated progress in many fields of sponge science will be revealed at the next symposium, to be hosted by the Istituto di Zoologie dell'Universita, University of Genova, Italy, early in the new millenium.

The achievements of the *5th ISS* were largely made possible through the generous support of our sponsors: the Board of the Queensland Museum; Astra Pharmaceutical (Australia) Pty Ltd and the Queensland Pharmaceutical Research Institute, Griffith University; the Commonwealth Bank of Australia; the Ian Potter Foundation, and the Queensland Government Travel Centre. The United States National Science Foundation is also gratefully acknowledged for supporting the attendance of many students to participate in the *5th ISS* and in sponsoring a workshop on 'Regional research opportunities and career development in the sponge sciences' for these students. I would also personally like to thank the following colleagues for providing assistance in organising and running the Symposium, workshops, field trips, providing publicity, documentation, logistic support, and in assisting with the production of this publication: Stephen Cook, Cheryl Cook, John Kennedy, Sue List-Armitage, Gert Wörheide, Joachim Reitner, Sally Leys, Nelson Lauzon, Christi Adams, Andrew McGown, Derrick Griffin, Jenny Utz, Debra Luk, Adrian Gibb, Paul Avern, Tim Avern, and the Queensland Museum Association.

John N.A. Hooper, Convenor and Editor, 5th ISS, Queensland Museum, Brisbane, Australia, 30 June 1999.

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Left to right. 1st row: Alex Cook, Clare Valentine, Michelle Kelly, Jean Vacelet, Clive Wilkinson, Françoise Debrenne, Catherine Chombard, Welton Lee; **2nd row:** Matthias Bergbauer, Stephen Cook, Cheryl Cook, Stewart Fallon, Jerry Bakus, Pierre Kruse; **3rd row:** Gert Wörheide, John Kennedy, Sue List-Armitage, Adam Burja, Mary Wakeford, Libby Evans-Illidge, Shirley Sorokin, Carsten Wolff, Jane Fromont, Guilherme Muricy; **4th row:** Anthony Wright, Christine Schönberg, Adrienne Grant, Donelle Trautman, Rosalind Hinde, Michael Borowitzka, Yoshihiro Fujimoto, Yoshihisa Kamishima, Jason Ritter, Malcolm Hill; **5th row:** Kathleen Smith, Lisa Goudie, Ann Knowlton, Yuriko Sato, Yoshiki Masuda, Andrew Flowers, Nicole de Voogd, Jose Lopez, Nathan Knott; **6th row:** Michaël Manuel, Murray Munro, Renata Manconi, Carlo Cerrano, Maurizio Pansini, Roberto Pronzato. **Absent from photo:** Carla da Silva, Clea Lerner, Ron Quinn, Andy Davis, Peter Steinberg, Tony Carroll, Bernie Degnan, Peter Murphy, Nicole Webster, David Miller, Roland Pitcher.

Left to right. 1st row: Maria Uriz, John Hooper, Nicole Boury-Esnault, Bill Austin, Janie Wulff, Patricia Bergquist, Sally Leys, Gisèle Van de Vyver, Evelyn Richelle-Maurer, Ruth Martí Lluch; **2nd row:** Ernie Kovacs, Michele Sarà, Claude Lévi, Steve de C. Cook, Belinda Alvarez de Glasby, Ruth Desqueyroux-Faúndez, Benjamin Wheeler, Bettina Kübler, John Fuerst; **3rd row:** Eduardo Hajdu, Gisele Lôbo-Hajdu, Phillipe Willenz, Henry Reiswig, Monica Puyana, Rochelle Newbold, Matt Dunlap, Mary Garson, Alison Colby, Scott Roush, Scott Nichols, Cécile Debitus, Rick Webb; **4th row:** Manuel Maldonado, Brian Chanas, Micha Ilan, Joseph Pawlik, Chung-Ja Sim, Yoko Watanabe, George Schmahl, Christi Adams, Adele Pile, Sheila McKenna, Russell Hill; **5th row:** Alan Duckworth, Ronald Osinga, Peter Schupp, Michael Assman, Mary Kay Harper, John Faulkner, Calhoun Bond, Jennifer Carroll, Miranda Sanders, Cristina Diaz, Junichi Tanaka, Joachim Reitner, Klaus Ruetzler, Sven Zea, Shirley Pomponi; **6th row:** Gregory Nishiyama, Herbert Peveling, Dorte Mehl, Grace McCormack, James McInerney, Toufiek Samaai, Andrzej Pisera, Manfred Krautter, Helmut Lehnert, Theo Eugeser, Cecilia Volkmer Ribeiro, Xavier Fernàndez-Busquets, Jochen Gugel.

SPONGE SCIENCE, FROM ORIGIN TO OUTLOOK

CLAUDE LÉVI

Ian Potter Foundation Keynote Address

Lévi, C. 1999 06 30: Sponge science, from Origin to Outlook. *Memoirs of the Queensland Museum* **44**: 1-7. Brisbane. ISSN 0079-8835.

Much of the important data, and most of the progress made on sponge biology has come from careful *in vivo* and *in vitro* studies on living populations. These techniques were used in studies conducted over a century ago, but much of this early work has been overlooked, or distorted during its transmission to the present time. This review revisits the scientific philosophy and techniques of our predecessors. It evaluates the quality of their observations, experimental prowess and originality in thought, and highlights the pivotal discoveries that have produced our present concept of 'what is a sponge', underlining those fields of study, such as developmental biology, where information is incomplete or lacking altogether. ❐ *Porifera, historical review, sponge biology, animality.*

Claude Lévi (email: levi@mnhn.fr), Laboratoire de Biologie des Invertébrés Marins et Malacologie, Muséum National d'Histoire Naturelle 57, Rue Cuvier, 75013 Paris, Cédex 05, France; 19 October 1998.

In 1948, as I began my career in science, I asked some of my mentors in sponge biology, Emile Topsent, Odette Tuzet, Henriette Meewis and Paul Brien, whether or not working on sponges, particularly their developmental biology, was still a worthwhile pursuit for a young scientist. Now, 50 years later, despite the tremendous progress we have made in our understanding of the general and molecular biology of sponges, it is clear to me that we need much more work to better understand development, growth and morphogenesis of sponges.

Scientific knowledge is a human collective and diachronic phenomenon, with each of us adding our own observations, thoughts and experiences (sometimes orally transmitted, but mostly in written form and as artwork), to the accumulated body of information from the past. Reading ancient documents is often amusing for their apparent naive contents, but often the answer is not obvious without many years of experimentation and observation. Moreover, is the answer definitive?

For this plenary address on the 'Origin & Outlook' of sponge biology, it is necessary to revisit the scientific philosophy and techniques available to our predecessors, in order to evaluate the quality of their observations, their experimental prowess and originality of thought. Indeed, personal reading of the older literature is invaluable; one can discover observations which have been forgotten or distorted by their transmission and subsequent interpretation, and one can also find hypotheses which more contemporary experiments subsequently invalidated or confirmed. It is essential to read original documents in order to better understand the great debates on 'animality', individuality, diblasty and inversion of layers, origin of the phylum, origin of bathyal and abyssal fauna, cellular differentiation and re-differentiation, internal transmission of information within this multicellular organism, self-not-self recognition, and so forth.

Studying living populations of marine animals is difficult at best, but unequivocally *in vivo* and *in vitro* observations on sponges over the centuries have contributed most to what we know about the phylum today. It was no accident that sponge science began somewhere in the eastern Mediterranean during an earlier millenium, in the province of sponge fishers, subsequently reaching the western Mediterranean, then the French coasts, and then the British Isles at the end of the 18th century. Discoveries made from field observations have had a substantial influence on our collective thinking about the Porifera, and in some cases these discoveries have changed our perception of the phylum completely.

Two such extraordinary events have occurred since the previous conference (4th International Porifera Congress, University of Amsterdam, 1993), both widely reported by the international media: 1) the existence of carnivorous sponges with neither aquiferous system nor choanocytes (Vacelet & Boury-Esnault, 1995); and 2) the

presence of soft sponge elements and embryos in southern Chinese Guizhou deposits some 580 million years old (Chia-Wei Li, Ju-Yuan Chen, Tzu-En Hua, 1998). Both these discoveries have made us re-evaluate two key aspects of sponge structure and biology: the interaction between, and the respective functions of, flagellated cells and amoebocytes.

First, Jean Vacelet and Nicole Boury-Esnault (1995) found that *Asbestopluma* lacked choanocytes yet it could breath, eat, and reproduce successfully. They found that *Asbestopluma* uses microscleres embedded along long filaments that are supported by long, aligned megascleres to actively capture the prey; the prey is enveloped and ingested by epithelial cells on the filaments. This system of macrophagy replaces the microphagous suspension-feeding by choanocytes, unique to the Porifera. Typically, the development of the aquiferous system and differentiation of choanocytes are the final ontogenetic events in sponge development. From the pioneering work of H.V. Wilson (1932) we know that choanocytes and canals may disappear from a severely distressed sponge (termed involution bodies, resting bodies or reconstitution diamorphy). Similarly, we also know from the pioneering work of J.S. Huxley (1911), that involution bodies in Calcarea are clearly made of archeo-amoebocytes that lack basal bodies. Perhaps early sponges did not require an aquiferous system?

The characteristic synapomorphy of the Porifera is the possession of an aquiferous system and choanocytes, yet this appears to be the most labile part of a sponge. By trying to understand how the aquiferous system is formed and reformed during morphogenesis, can we hope to approach the phylogenetic origin of sponges ? J.S. Huxley (1911) thought not, yet carnivorous sponges live and survive perfectly well without this characteristic synapomorphy of the phylum. It would be of great interest to know whether there is a transitional aquiferous system present during growth and development of *Asbestopluma*. Is *Asbestopluma* some kind of permanent diamorphy?

The second discovery, of ancient Guizhou sponges by Chia-Wei Li, Ju-Yuan Chen and Tzu-En Hua (1998), suggests that larval flagellated cells and amoebocytes were already present in sponges as old as 580MY. Does this mean that modern species do not differ substantially from those early in the evolution of the group ? How much further does the group extend into the past?

To understand these contemporary viewpoints in the correct context, it is informative to review early historical interpretations of the structure of the aquiferous system and the anatomy of the soft parts of a sponge before modern histological techniques and descriptive embryology provided us with our current understanding of sponge morphogenesis.

At the end of the 18th century Peter Pallas (1766) and John Ellis (1755, 1786) suggested that sponges were of animal nature, contrary to popular opinion in those times. Debates on the 'animality' of sponges continued for at least a century.

Animality, as understood in those times, was assigned to organisms which were capable of voluntary movement, muscular response, and were sentient. Observers sought evidence of responsiveness and of motion, which would be similar to muscular contraction, for sponges (which by nature are a fixed animal). Some investigators, like Donati (1750), observed that the contact between any object and the sponge caused the sponge to contract, whereas others found no such response. In the absence of any sensory reaction or of motion by the sponge, some philosophers suggested they could detect animality based on the nature of the smell arising during sponge decomposition: this smell depends, after all, on the 'animal' chemical structure or pattern of the organism. This approach is complicated by the fact that most naturalists at that time were only familiar with very few marine sponges (at that time assigned to the greatly misused genera *Spongia*, '*Alcyonium*' and *Tethya*), in some cases only one species, or they worked exclusively on freshwater sponges. Freshwater spongillids are typically green when alive, which led observers to think they were plants.

Convergence of viewpoints on the nature of sponges gradually emerged, especially following the works of Grant (1825-26), Dutrochet (1828), Dujardin (1838) and Laurent (1844) on freshwater and marine sponges. Prior to these authors' works it was clear to the casual observer that the sponge surface was perforated. This feature was the most consistent amongst the known species of sponges, which eventually led to the phylum being named Porifera (pore-bearing) by Robert Grant (1836). But few authors had any idea on the nature of these pores. Were they normal apertures produced by the sponges or were they caused by foreign organisms, such as worms or polyps,

P.S. PALLAS 1766

Animal ambiguum, crescens, torpidissimum. Stirps polymorpha, e fibris contexta, gelatina viva obvestitis. Oscula oscillantia, seu cavernae cellulae ve superficiei

C.v. LINNE 1767

Flores. Foraminibus respirant aquam. Stirps radicata, pilis contexta, flexilis bibula

J.ELLIS 1786

Animal fixum, flexile, polymorphum, torpidissimum, contextum vel e fibris reticulatis, vel e spinulis, gelatina viva vestitis, osculis seu foraminibus superficiei aquam respirans.

FIG. 1. Definitions of a sponge from the earliest literature: Pallas (1766), Linneaus (1767), Ellis & Solander (1786).

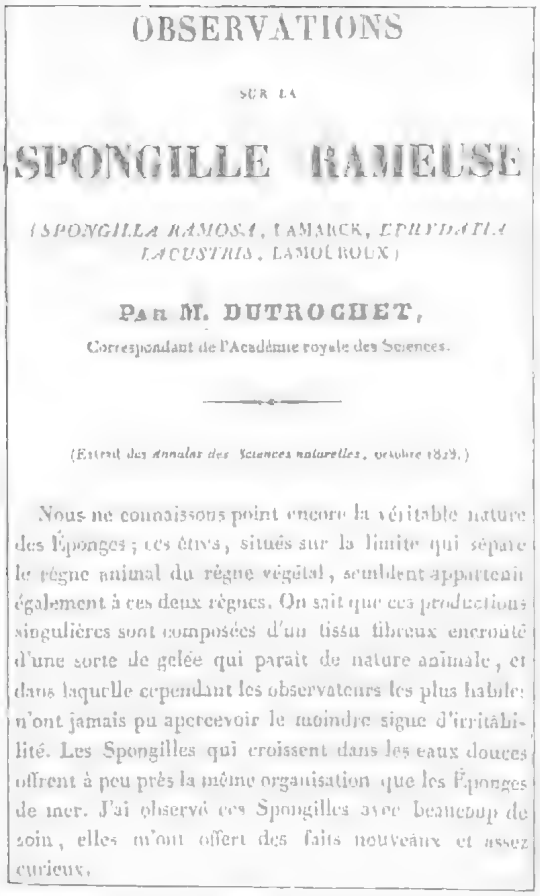
burrowing into the sponge? Many, including Jean Baptiste Monet de Lamarck (1814-16), favoured the latter hypothesis. In fact Lamarck's (1814) classification of the Zoophytes was centred on the presence or absence of polyps: with sponges included in the latter group, and defined as Polypiers empâtés — made of a common substance and ... without polyps.

Those who observe a living sponge, however, immediately realise that the pores are an integral part of the sponge anatomy, conducting water flow into and out of the body. Some authors, such as Marsigli (1711) and Ellis (1755), imagined (probably moreso than they actually observed), a double flow into and out of the same pore — a systolic-diastolic phenomenon. Others, like Grant (1825) and Dutrochet (1828), observed a unidirectional, continuous water flow exiting the sponge at constant speed. But, they wondered, if water keeps flowing out of the sponge, where and how does it go in?

Dutrochet (1828) was convinced that the freshwater spongillid was a plant. He perceived that it was green, it formed a membranous extension that grew through expansion at its edges, like the algae *Ulva*, it didn't appear to have food cavities and therefore it probably fed by absorbing solutions of water enriched with nutrients, much like a plant. To him it was a plant whose chemical structure was identical to some extent to that of animal tissues. However, Dutrochet observed cavities in sponges with a transparent membrane covering them. He noted that this membrane also covered the entire external surface; it was not sensitive when touched by a foreign object; and it was capable of creating a conical protuberance (oscules) capable of continuously expelling water through the apical part. Dutrochet dismissed the hypothesis that water was expelled from the sponge by commensal Crustacea, and instead he proposed that water was expelled from *Spongilla* via 'some kind of a force' produced by the living animal itself. He did not, however, discover the inhalant pores, but he hypothesised instead that water was drawn slowly into the sponge by adsorption over the whole surface. According to his theory, the expulsion of water from the sponge depended on endosmosis, with the continuous introduction of surrounding water into the cavities of *Spongilla*, which he said were filled with a denser organic fluid.

Grant (1825-26) was also sure that sponges were animals. To him it was obvious that sponges had two types of orifices: 1) larger faecal orifices, through which water was forcefully expelled; and 2) numerous smaller pores through which water entered. Grant, who studied living populations of several sponge species in coastal Scotland, observed a continuous water circulation flowing through many internal canals. Although he was unable to suggest what the 'motor' was that drove this circulation, he was none-the-less certain that something like this existed. In fact he commented that minuscule bodies, or granules, organised along the canals might be directly involved, and that water flow is similar to that which might possibly be generated via a flagellar system. Demonstrating remarkably modern vision Grant was sure that water current was one of the vital functions of the pore-bearing animal, and that it helped the animal to feed, breath and even reproduce.

In addition to the aquiferous system Grant also noted the sponge soft parts were differentiated into a general cellular substance and a body of material unifying the spicules. This cellular substance, he wrote, twenty years before the cell-theory was proposed by Virchow and others,



OBSERVATIONS

SUR LA

SPONGILLE RAMEUSE

(*SPONGILLA RAMOSA*, LAMARCK, *EPHYDATIA LACUSTRIS*, LAMOUROUX)

PAR M. DUTROCHET,

Correspondant de l'Académie royale des Sciences.

(Extrait des *Annales des Sciences naturelles*, octobre 1828.)

Nous ne connaissons point encore la véritable nature des Éponges ; ces êtres, situés sur la limite qui sépare le règne animal du règne végétal, semblent appartenir également à ces deux règnes. On sait que ces productions singulières sont composées d'un tissu fibreux encroûté d'une sorte de gelée qui paraît de nature animale, et dans laquelle cependant les observateurs les plus habiles n'ont jamais pu apercevoir le moindre signe d'irritabilité. Les Spongilles qui croissent dans les eaux douces offrent à peu près la même organisation que les Éponges de mer. J'ai observé ces Spongilles avec beaucoup de soin, elles m'ont offert des faits nouveaux et assez curieux.

FIG. 2. Definition of a sponge, from Dutrochet (1828).

was mostly located in the spaces between the walls of internal canals, and appeared to be most obvious during the reproductive period of the sponge.

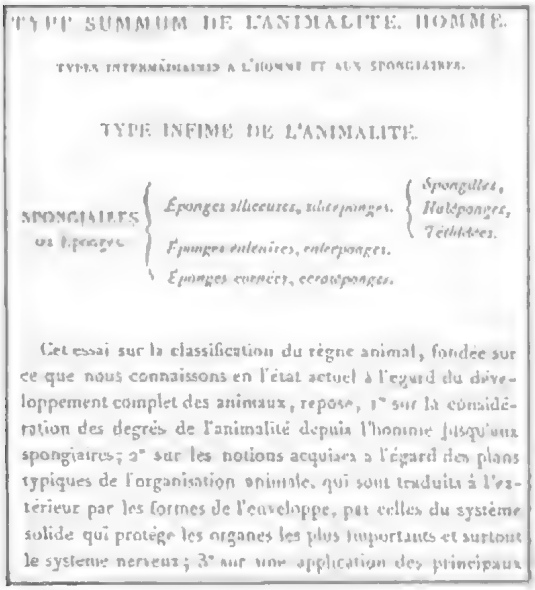
Dutrochet (1828) was the first to explain shape changes of sponges through cellular movements. He focused on the transparent membrane and membranous ducts which provided a pathway for the continuous flow of water out of the sponge. Through regular observations he noticed that these ducts changed shape and length, stretching and shrinking periodically. He suggested these movements were not the result of sensitivity but a mechanism for transporting substances from one part of the tube to another. To him the membranes were made of vesicular globules, and changes to their shape and length were caused by the transport of elementary globules. These globules were not static but moved over each other without detaching, in a predefined direction, using a kind of sliding motion. Changes in shape and movement were too slow to be visually observed, much like the hands of a clock, but shape changes could be seen over time. 'This fact is physiologically of the highest importance' stated Dutrochet, 'It is a new vital action which plays one of the main roles in stretching the plant's size, the immediate agent responsible for the vital motion uncovered in its very nature and action mode'.

Felix Dujardin (1838) was well known for his studies on Rhizopods and Amoebae, in which he had observed very slow, micrometrical movements. He began studies on sponges during 1835-1837, working on *Cliona celata*, *Halichondria*, *Halisarca* and *Spongilla* species, to try and understand sponge organization. From these studies he observed irregular globules made of a contractile substance which, when drawn 20 times at 5 minute intervals, gave 20 different profiles. These globules periodically generated round expansions and thin appendages, much like shape changes to amoebae. He subsequently demonstrated the animal nature of sponges before the French Academy of Sciences, based on the presence of these contractile expansions and by the crawling motion of these 'packets'. Moreover, in a *Spongilla* from the Seine River in Paris, Dujardin also saw packets with flagellated filaments which he said 'determined the water flow and streams in the oscules'.

Dujardin and Dutrochet were clearly at odds, and before the French Academy, Dujardin stated 'Mr Dutrochet, who refuses to admit sponge contractility, explains all their shape changes by the motion of molecules, probably vesicular according to him, which make up the tissues of external membrane'. This controversy was further complicated by the ambiguity of the words contractility, motion and movement, mandatory to the concept of animality.

Whereas Dutrochet was the first to describe shape changes of the sponge caused by directed cell motion, and also showed the importance of the external pinacoderm, Dujardin described for the first time the presence of amoebocytes with pseudopodial extensions. In 1844 Jean Laurent suggested that the presence of this external membrane was the sign of the beginning of life for a perfect state of *Spongilla* species. In that same year Laurent published this classification, which has been completely forgotten today.

These early, careful, time-lapse ocular observations of Dutrochet and Dujardin demonstrated that the sponge and its constituents were able to



TYPE SUMMUM DE L'ANIMALITE. HOMME.

TYPES INTERMÉDIAIRES A L'HOMME ET AUX SPONGIAIRES.

TYPE INFIME DE L'ANIMALITE.

SPONGIAIRES ou Eponges
- Éponges siliceuses, silicispongiæ.
  - Spongillées, Halléponges, Téthidées.
- Éponges calcaires, calcispongiæ.
- Éponges cornées, cornispongiæ.

Cet essai sur la classification du règne animal, fondée sur ce que nous connaissons en l'état actuel à l'egard du développement complet des animaux, repose, 1° sur la considération des degrés de l'animalité depuis l'homme jusqu'aux spongiaires; 2° sur les notions acquises à l'égard des plans typiques de l'organisation animale, qui sont traduits à l'extérieur par les formes de l'enveloppe, par celles du système solide qui protége les organes les plus importants et surtout le système nerveux; 3° sur une application des principaux

FIG. 3. Succinct classification of the animal kingdom, from Laurent (1844).

move and displace, although the speed of these phenomena was visually undetectable. A century later, using time-lapse cinematography, Ankel (1965) studied cellular motion of spongillid fragments sandwiched between two glass-slides. Subsequently, Efremova (1967), Pavans de Ceccatty (1979), Rasmont (1975) and their teams produced a wealth of fascinating observations on cell motility. For Pavans de Ceccatty, who essentially focused on information transfer systems and integration, cell mobility and displacement were basic features of sponge organisation. For Rasmont, who was more interested in experimental morphogenesis, the most striking characteristic to him was the extreme mobility of all sponge constituents. For example, using a frame-by-frame analysis he concluded that the movement of spongillid amoeboid cells during gemmulation could be statistically tested for random versus directed movement.

It is strange though, that although there has been so much research on embryology, postlarval, postgemmular and postdiamorphic development, we still have so little data on growth and true morphogenesis (i.e. shape achievement), a character so important to the taxonomist but still largely speculative.

We have some good models, such as *Leucosolenia*, whose growth has been thoroughly studied by W. Clifford Jones (1964, 1965), and particularly from work on the spongillids — a group intensively studied since the beginning of sponge science (e.g. Grant, Dutrochet, Dujardin, Laurent, Ankel, Rasmont, van de Vyver and many others). Nevertheless, there are many questions still unanswered. In particular, we know little about intercellular events and chronology during the simultaneous organisation of the skeleton and the aquiferous system (so different in the three classes of Porifera); the role of primary external physical factors, such as light and hydrodynamics, in triggering, orienting and maintenance of cell movements; the regulatory processes influencing the relative proportions of cell populations and the local conditions prevailing during their differentiation; the movements of sclerocytes and factors that determine which types of megascleres are produced, where they are localised and distributed within the skeleton (such as in prismatic or cubic meshes, isotropic prismatic system, or in ascending dendritic fibres), and the consequences of their localisation within the sponge.

More than a century after Grant, I also spent a great deal of time observing the littoral sponge fauna around Roscoff, including the same species studied by Grant. My curiosity led me to follow the shape changes that sponges undergo over many years. I noted that fragmentation of individual specimens was frequent, as was the susequent fusion of these same sponge fragments. It was clear that fragmentation was not only produced by catastrophic events, such as storms or predation, but also occurred as a much more gradual process, presumably linked to adaptation of the sponge to local environmental conditions. Sometimes these processes can be observed in the aquarium, and sometimes it is possible to generate them experimentally. Slow fragmentation is the result of massive cellular movements, which are not very different from those described by Dutrochet in *Spongilla*. Both fragmentation and fusion are opposite and complementary and are 'the two fundamental tendencies of the sponge to concentrate and to isolate from the external environment', as stated by Borojevic (1971) and Wilson (1932) before him. Through the manipulation of light and water circulation it is possible to generate the partial or complete motion of the sponge, whose cells are able to move and leave the existing skeleton to build another in a more physiologically favourable environment. Ankel (1965), Ankel & R. Eigenbrodt (1950) and Rasmont (1975, 1979) provided pivotal data on these processes.

What other organisms are easily able to get rid of their skeleton? This phenomenon is unthinkable in more mobile organisms, and certainly does not occur within the plants, and is perhaps unique amongst the Porifera. Of course, morphological freedom associated with cellular migration has constraints and genetic limitations; but why is shape more stable in some species despite their constant local morphological re-adjustments? Even *Tethya*, a sponge universally characterised by it 'golf ball' shape, can distort and move. In a compact spherical sponge that lacks cavities and has a severely localised aquiferous system, an internal equilibrium is set up between cell populations which are using internal energy stocks. Growth under cell proliferation requires an increase in exchange between external and internal surfaces. Growth of the exchange zone occurs through folding or multiplication of choanocyte chambers, and by regulating inhalant cavities. Growth of the external surface can be horizontal, polyaxial and peripheral, or, vertical, monaxial and apical, and all intermediate situations exist between the prolate and oblate states.

Recently, Jaap Kandoorp (1995) described a new fractal approach to *Haliclona* morphology, which in the future should be coupled to 3-dimensional analyses of the aquiferous system, following the method of corrosion casts developed by Bavestrello et al. (1988). But we also have to investigate the signals which determine the orientation of migrating cells, and we have to know the control mechanisms that determine the motile behaviour of cells of this 'torpidissimum' animal.

We enter a new millenium with new and fantastic technology. Widespread use by researchers and increasing speed of nucleic acids sequencing technology, together with computerised analysis of sequences, provides more and more information on the genetic make up of sponges, and at this rate it might be reasonable to hope that by the next century we will know the complete sponge genome, a current witness of a primitive multicellular organism. It will be the result of teamwork which has to be carefully organised and planned. The choice of the reference model species could well be *Ephydatia fluviatilis*. But not everything will be explained by knowing all about the genes, although certainly it is a mandatory step. Progress in developmental biology, one of the most important scientific fields of the next century, shows that the chronology of gene expression (also an essential subject), does not yet explain the topological evolution of the blastula, a primarily spherical organism, towards increasingly complex stages possessing multiple compartments and under the constant influence of environmental factors. It be equally necessary to direct team efforts to the central theme of growth and form, following the trail pioneered such a long time ago by d'Arcy Thomson (1917).

As Grant wrote, 'This animal affords many curious and interesting subjects of inquiry to those who [like John Hooper] have leisure and opportunities of examining the more perfect species of tropical seas. Though probably the simplest of animal organisation, the investigation of its living habits, its structure and vital phenomena, and the distinguishing characters of its innumerable polymorphous species is peculiarly calculated to illuminate the most obscure part of Zoology, to exercise and investigate our intellectual and physical powers, and to gratify the mind with the discovery of new scenes of infinite wisdom in the economy of Nature'.

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THE PAST OF SPONGES — SPONGES OF THE PAST

FRANÇOISE DEBRENNE
Astra Pharmaceuticals (Australia) Plenary Address

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Fossil sponges lack many of the features seen in living sponges, with the consequence that their traditional taxonomy was nearly completely reliant on preserved skeletal architectural characteristics, producing a fossil sponge classification that had diverged considerably from that of living sponges. Subsequent discoveries of 'living fossil' sponges with hypercalcified basal skeletons, representing some of the groups thought to be long extinct, provided a revolutionary basis to solve some of the palaeontological enigmas and to comprehensively revise the groups themselves. Ancient groups sphinctozoans, stromatoporoids and chaetitids, with species in Recent seas, are now recognised as grades of construction rather than clades of taxa. The existence of these 'living fossil' sponges provided an unique opportunity to compare tissues, spicules and microstructures of the basal skeleton with well preserved fossil material; to understand the influences of biomineralisation and diagenetic alterations affecting mineral composition and microstructures in fossil sponges and to infer the systematic position of Paleozoic to Recent sponges with a calcified skeleton. Similar conclusions were reached for the archaeocyaths, with no living representative yet recorded, but with structural features consistent with the Phylum Porifera. More recent discoveries of ancient sponge tissues and larvae from Precambrian phosphorites provide even more valuable data on the early history and development of Demospongiae and Calcarea, extending the age of the latter group considerably. ❐ *Porifera, palaeontology, hypercalcified basal skeleton, sphinctozoans, stromatoporoids, chaetitids, archaeocyaths, taxonomic overview.*

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We know from the old literature that living sponges have been known since Ancient Times, being familiar household items in ancient Greece and Rome. During the Middle Ages burned sponges were reputed to have therapeutic value in the treatment of various diseases, perhaps anticipating their present pharmaceutical use! Conversely, discoveries of fossil sponge-like 'objects' occurred much latter. These were first figured and described as 'mushrooms' at the end of the 16th century in the Moscardo collection, according to Zittel (1883). Other scattered examples of sponge-like objects were published later, but these authors did not know whether these forms were plants or zoophytes (Fig.1). The first valuable observations were made in the second half of the 18th century by Guettard (1768-1783) and several other authors at the beginning of the 19th century. These authors compared their fossils to Alcyonaria or horny corals, but not to recent sponges. Goldfuss (1826) first suggested these fossil forms may be related to living horny sponges, which subsequently mineralised into silica or calcium carbonate, and they attributed known fossil forms to Recent sponge genera.

With the ensuing discovery of Hexactinellida (or Hyalosponges) from deepwater dredgings, the exact position of some fossils was established (auguring the impact of the future discovery of 'living fossil' hypercalcified sponges or sclerosponges).

D'Orbigny (1849-1850) proposed an initial classification of fossil sponges based on external characters. He considered that these fossil sponges, the *Petrospongia*, a nearly extinct group, had a mainly calcareous 'stony' skeleton, contrary to previous interpretations whereby the horny skeleton became secondarily mineralised. De Fromentel's (1889) classification took into account the interlocking pattern of fibers, the shape of spicules and characteristics of the canal system, but it still kept separate the fossil group *Spongitaria*, amorphozoans with 'testacean' skeleton, and the extant group *Spongia*, amorphozoans with horny skeleton.

The existence of siliceous sponges in the fossil record was confirmed by the discovery of spicules in Jurassic and Cretaceous rocks. The

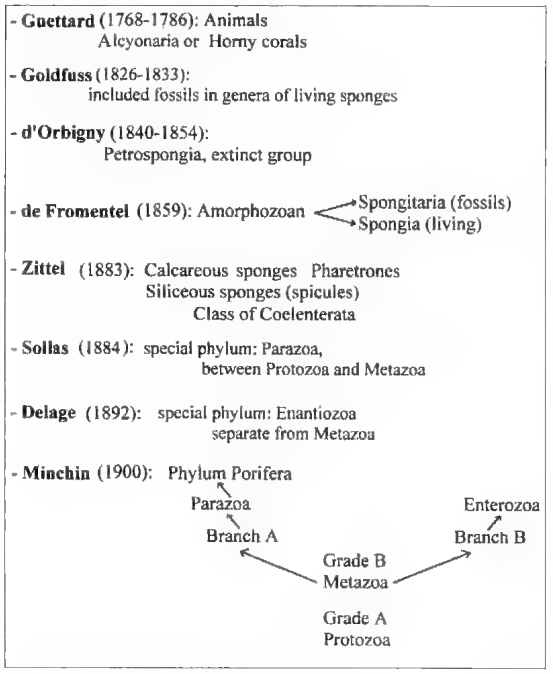

FIG. 1. Plants or zoophytes?

existence of calcareous and siliceous fossil sponges was recognised in the 1870s, but at that time specialists were unable to distinguish the two groups because of secondary replacement of calcium by silica, and vice versa. Zittel (1885), pioneering microscopic studies on sponge structures, described the anastomosing fibers in the skeleton of calcareous sponges (pharetrones), clearly differentiating them from siliceous spicules of other sponges. He concluded from studies on microstructures of fossil and recent forms that they both belonged to the same 'Class' among Coelenterata. By comparison, Sollas (1884) included them in the Phylum Porifera, in a group Parazoa intermediate between the Protozoa and Metazoa, whereas Delage (1892) created a special group, the Enantiozoa, separated from Metazoa.

By the end of 19th century the first act of the 'Fossil Sponge Story' had closed. Minchin (1900) established the essential features: sponges were animals and the most primitive phylum of the Metazoa. The main lines of classification were recognised: those with calcareous spicules or skeletons were included in the class Calcarea; those with siliceous spicules bearing 3 axes arranged to form hexactines were included in the class Hexactinellida; and those with a spongin skeleton, or a spongin skeleton and siliceous spicules, or only with siliceous spicules lacking 3 axes were included in the class Demospongiae.

The description of new genera in time and space raised the problem of their systematic position within families and orders. During Zittel's (1883) time there were few taxa or only the non-identifiable remains of sponges available on which to base a classification. The predominance of Cainozoic and Mesozoic forms reflected the bias of stratigraphical investigations moreso than an evolutionary trend. Rapidly, however, the number of genera increased as monographs were published throughout the world. De Laubenfels (1955) noted that more than 1,000 genera have been established for fossil sponges.

Since that time techniques in preparation and methods of investigations had improved progressively such that the number of new taxa, and the number of 'significant characters' upon which to differentiate taxa, had both significantly increased. Similarly, and inevitably, there has been disagreement amongst authors concerning the relative importance of certain characters over others, and different interpretations of the development of new structures and new forms from the existing ancestral forms. As a consequence, the systematics of living and fossil sponges have diverged substantially, developed independently, and are now based on largely different criteria.

Living sponges have a relatively large pool of morphological and other biological characters that are potentially useful for classification. Their skeletons are made of various materials ranging from organic spongin to mineralised spicules or aspicular elements. In addition to skeletal characteristics, they are also classified on the basis of their biological activity, biochemistry, methods of reproduction, and several other useful characters related to their soft parts and cellular constituency. The fossilisation potential of sponges is also very variable. With some rare exceptions, sponges with isolated spicules are fossilised only as scattered skeletal elements, accounting for the numerous gaps in the fossil record. After death spicules are usually dispersed amongst the sediments and sometimes dissolved in the seawater, but in some cases rapid sedimentation has buried or winnowed sponges in favourable environments (such as in back reef lagoons and volcanic products), with a few fossils much better preserved. Moreover, the diagnostic value of isolated spicules may be poor given that many of the major spicule types are

present in several orders, even in different classes. The best fossils concern species with a skeleton built by fused spicules (such as 'lithistid' construction), and most sponges with solid skeletons (such as compressed skeletons or hypercalcified sponges) also provide reasonable fossil material. Bodily preserved sponges are often diagenetised, the spicules in place also often dissolved or recrystallised.

By comparison, fossil sponges lack many of the features useful for taxonomy of living sponges, relying largely on features of preserved skeletal architecture. Fortunately, some fossil forms are known through a miracle of preservation (lagerstäten), and comparisons between these fossil species and so-called 'living fossil' sponges from Recent seas provide opportunities to reinterpret the palaeo-environment. The importance and meaning of calcification in sponges became evident following the discovery of the Jamaican 'coralline sponges'. These hypercalcified sclerosponges have a compound basal skeleton of aragonite together with organic fibers and free siliceous spicules (Hartman & Goreau, 1970), demonstrating that there were several permutations to the concept of calcitic skeletons, not limited to possession of only calcified spicules or to possession of a solid calcareous skeletons devoid of spicules. The class Sclerospongiae was erected for these sponges, with an indication they may be the living representatives of some Mesozoic and Paleozoic cnidarian-like fossils.

It was a conceptual revolution: the systematic position of some enigmatic groups long thought to be extinct, such as the reef-building archaeocyaths, stromatoporoids, sphinctozoans and chaetetids, each previously attributed to independent phyla or to Cnidaria in the case of the latter, were considered in a completely new light. As an ancient sponge fauna has living remnants in Recent seas, it is possible to compare the tissue and the spicules of these 'living fossils' with those of other modern forms, and to infer the systematic position of Paleozoic to Recent forms with a hypercalcified skeletons. I will discuss each of these groups separately.

ARCHAEOCYATHS. One of the main problems in assigning archaeocyaths to the Porifera is the absence of spicules in the hypercalcified skeleton, but Jean Vacelet's (1964) work on *Petrobiona massiliana* provided a basis for direct comparison between Recent and fossil sponges with hypercalcified skeletons but lacking free spicules. Nevertheless, at that time we were still uncertain of their affinities, so we left the archaeocyaths in their own, extinct phylum, close to, but different from Porifera. At the London Symposium in 1967 Ziegler & Rietschel (1970) stated that none of the features shown by archaeocyaths really conflict with the possibility they may be sponges. In contrast, in the same volume Zhuravleva (1970) created a new subphylum, the Archaeozoa, of equal rank with Parazoa and Enterozoa, more similar to Protozoa than to Porifera, and included in it the Sphinctozoa and other enigmatic extinct multicellular animals. This latter group, called the 'Archaeata', included archaeocyaths, sphinctozoans, aphrosalpingidids and receptaculitids, and resided somewhere between animals and plants. Finally, this kingdom was subdivided into Aphrosalpingata and Inferibionta (Fig. 2), which combined archaeocyaths and sphinctozoans. This view was not so far from the general opinion of the time, except the suggestion that Inferibionta might have originated from the Eukaryotes, independently from all other kingdoms.

At the Washington 'Fossil Cnidarian Symposium' in 1980, in light of recent discoveries, Jean Vacelet and I re-examined the question of archaeocyath affinities (Debrenne & Vacelet, 1984). Much progress had been made on archaeocyath studies between 1967 and 1980. Studies on their functional morphology (indicating that they were filter feeders), ontogenetic stages, microstructural analysis of primary and secondary skeletons (supporting the concept of their monophyly, despite the great diversity of morphologies), allowed more precise comparisons to be made between archaeocyaths and sponges. Moreover, discoveries of Antarctican archaeocyaths and of Australian sphinctozoans in the Upper Cambrian narrowed the stratigraphic gap between the two groups. Detailed comparisons with the Recent species *Vacelettia crypta* (Vacelet, 1977) led us to conclude that secretion of both the primary and secondary skeleton proceeds by rapid mineralisation, and that none of the structural features of an archaeocyath were inconsistent with a sponge model. Further studies by A.Yu. Zhuravlev (1989) and P. Kruse (1990) reinforced the hypothesis that archaeocyaths are poriferans. The pattern of immune reactions, the type of asexual reproduction and the presence of crypt cells suggest that they are closer to demospunges than to other classes of sponges (Debrenne & Zhuravlev, 1994).

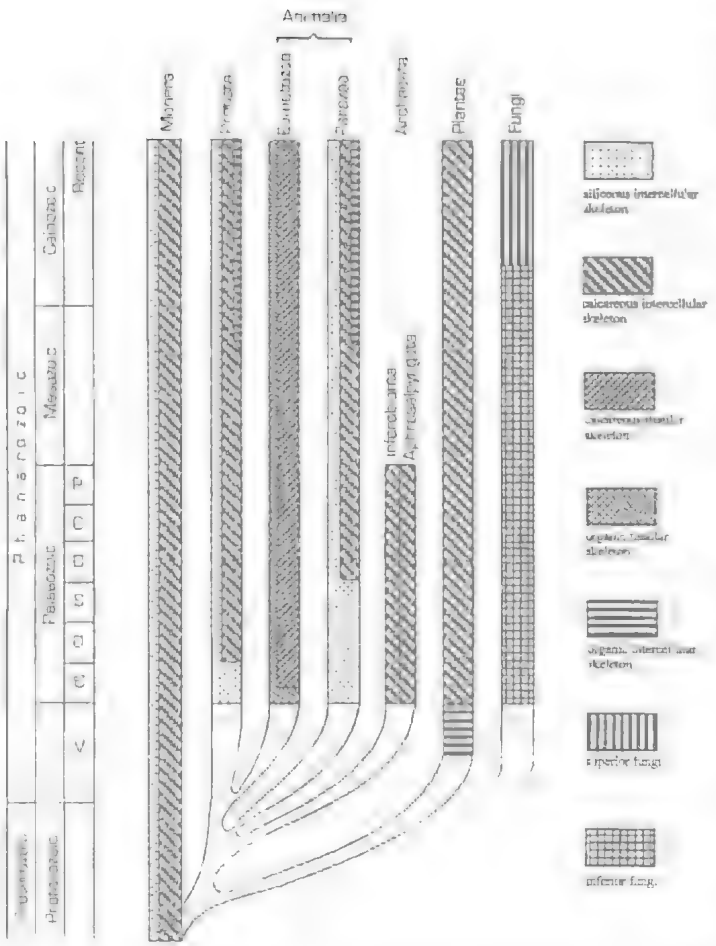

FIG. 2. Archaeatha in the organic world, after I.T. Zhuravleva & E.I. Miagkova, 1979, modified.

SPHINCTOZOANS. *Vaceletia crypta*, and its possibly intraspecific colonial form (Vacelet et al., 1992), were discovered in cryptic habitats. Both presented a series of successive hemispherical chambers, reminiscent of the Sphinctozoa, and at that time they were included as one of the two orders of Pharetronida. For palaeontologists, Pharetronida (simple Inozoa and segmented Sphinctozoa) belong to the Calcarea. However, the histology, cytology and sexual reproduction of *Vaceletia* are similar to those of the Ceractinomorpha in the class Desmospongiae. Consequently, the systematic position of sphinctozoan sponges is questionable and must be re-evaluated.

The lack of spicules in *Vaceletia* could explain the absence of spicules in some fossil sphinctozoan forms. Vacelet (1979, 1985), Pickett (1982) and Picket & Jell (1983) placed most of the Sphinctozoa (including those lacking spicules) into Demospongiae, whereas segmented sponges with calcite spicules were retained in Calcispongea (Calcarea). For H. & G. Termier (Termier & Termier, 1975, 1977) all Pharetronida (Sphinctozoa and Inozoa) belonged to a primitive group Ischyrospongia, originating from stromatoporoid-chaetetid stock, and with archaeocyaths as a close group of ancestors stemming from the Cambrian. This proposal has been heavily criticised by many workers due to the highly polyphyletic nature of this collection of fossils.

It is now admitted that the chambered calcareous skeleton seen in sphinctozoans is a convergent feature, having arisen many times within the classes Demospongiae and Calcarea. Evidence indicated that sponges were able to produce these sorts of skeleton with relatively ease (Vacelet, 1985; Wood, 1987; 1990), and that the concept of Sphinctozoa was artificial, a grade of construction, and not a systematic clade. This grade of organisation can also be found in archaeocyaths (Debrenne & Wood, 1990).

Sphinctozoa has been included in Calcarea since Steinman (1882); the problem was only to move them within the classes of Porifera; but it was not easy to admit for some time that most sphinctozoans were Demospongiae, as indicated by more reliable taxonomic criteria concerning the soft tissue and spicule form.

STROMATOPOROIDS AND CHAETETIDS. It was even more difficult to assess the affinities of these groups, whose systematic positions have long been disputed. Palaeontologists had generally accepted that Stromatoporoidea and Chaetetida had affinities to Hydrozoa. This position required reassessment, however, with the discovery of *Acanthochaetetes* by Hartman & Goreau (1975), with this new genus assigned to a Mesozoic chaetetid. As a consequence,

Paleozoic and Mesozoic chaetetids were considered to have Poriferan affinities due to their similarity with these 'living fossils'. Like sphinctozoans, the stromatoporoids and chaetetids were polyphyletic and represented grades of organisation rather than systematic clades. These grades are also known in the archaeocyaths (Table 1).

TABLE 1. List of the various proposal of affinity for Stromatoporoids, after R.A. Wood, 1987, modified.

Anthozoa (not including tabulate corals)	Porifera	Bryozoa
Goldfuss 1826 De Blainville 1833 Lonsdale 1840 Römer 1843 Von Keyserling 1843 Hall 1847 McCoy 1851 Billings 1862 Lindstrom 1880 Mori 1976,1984	Steininger 1834 D'Orbigny 1850 Eichwald 1860 Von Rosen 1869 Salter 1873 Nicholson 1873 Sollas 1877 Nicholson & Murie 1878 Solomko 1886 Kirkpatrick 1912 (Aug) Heinrich 1912 Twitchell 1929 Hartman & Goreau 1970,1972 Steam 1972,1975 Wendt 1975,1979,1984 Hartman 1979 Stock 1984 Wood 1986	Röemer 1851 Sandberger & Sandberger 1850
Hydrozoa	Cyanobacteria	Tabulate corals
Linström 1873 Carter 1877,1880 Zittel 1877 Steinmann 1878 Champernowne 1879 Bargatsky 1880 Nicholson 1886 Yabe & Sugiyama 1920, 1935 Dehome 1920 Steiner 1935 Lecompte 1952,1956 Hudson 1955,1960 Flugel 1958 Turnšek 1960,1974 Kazmierczak 1971 Turnšek & Masse 1974	Kazmierczak 1976, 1983	Röemer 1856 Nestor 1981
Foraminifera	'Vegetable'	Cephalopoda
Dawson 1875, 1879 Lindström 1870 Kirkpatrick 1912 (Sept) Hickson 1934 Parks 1935	Billings 1857	Hyatt 1865

'LIVING FOSSILS'. New discoveries in the Mesozoic and the Paleozoic fossil record since the 1970s, by researchers such as Cuif, Dieci and their teams, Wendt, Kazmierczak, H. & G. Termier and others, dramatically increased the number of forms assigned to 'sclerosponges'. These discoveries provided a larger diversity of taxa to further compare with the few known Recent species, but they also led to many different hypotheses on their affinities and systematics, sometimes leading to further confusion.

The discovery of 'living fossils' certainly settled some enigmas, but it also led to the recognition that the existing taxonomy and phylogenetic grouping within Porifera required substantial revision. Vacelet (1985) showed that living sclerosponges were a collection of assorted demosponges, which can be distributed easily within pre-existing orders and families, and that the class Sclerospongiae was polyphyletic and unnecessary. He also found that many hypercalcified forms had closely related non-calcified equivalents. As a result, he invited palaeontologists to apply and test his phylogenetic proposals to the fossil record.

Because they lack many of the characteristics seen in living species, fossil forms are difficult to compare directly to living taxa, and thus it is difficult to test all of Vacelet's (1985) criteria. 1) The presence of siliceous spicules in hypercalcified skeletons is still a matter of debate, as the structures observed in fossil forms are moulds which could be interpreted equally as well as either cavities or calcareous modified spicules (argument used by Rigby & Webby, 1985 to maintain the Sphinctozoa in the Calcarea). 2) Minute details of macroscleres, such as small ornamentations important for differentiating living taxa, are rarely observed in fossils. 3) Similarly, the large diversity of spicules (including microscleres) so common in living species is generally unknown in the fossil record. 4) The possession of a hypercalcified skeleton remains the principal source of information for palaeontologists to assess relatedness, whereas gross morphological characters cannot be used, given the high probability of architectural convergences. 5) As a consequence of these problems, palaeontologists have devised other ways to investigate affinities, such as growth pattern, type of skeletal microstructures, mineralogy, biochemistry of intraskeletal organic material (Gautret, 1989). 6) The systematic importance of the microstructure of hypercalcified skeletons has also been disputed. Wendt (1979)

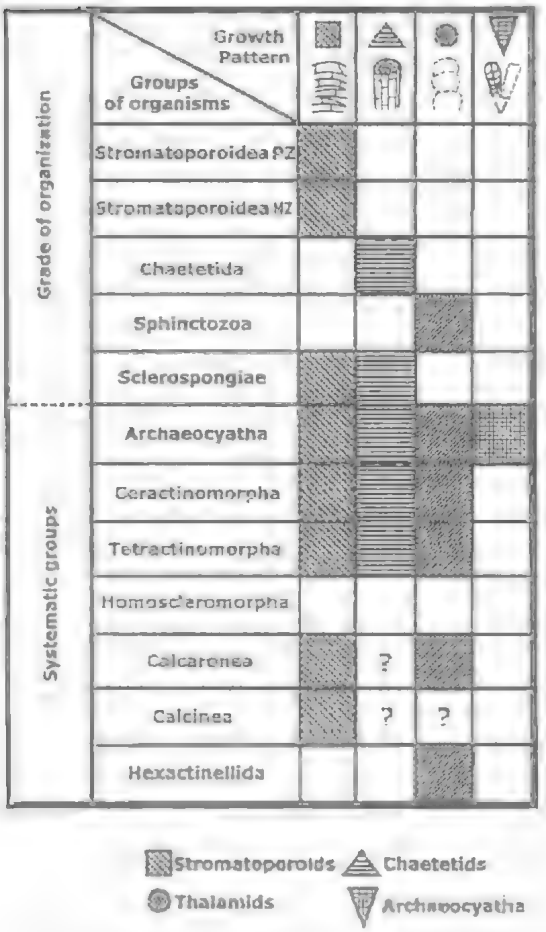

FIG. 3. Grades of organisation in the different systematics groups, after F. Debrenne & A. Zhuravlev, 1992, modified.

proposed that diagenetic modifications to primary skeletal structures might be useful. He suggested through carefully study of the size, shape and arrangement of microstructural units, and the composition of intraskeletal organic compounds, that these characters appear to be biologically controlled. 7) Another problem concerns inconsistencies in the terminology used by different authors to describe hypercalcified sponge skeletons, whereby the same term can be used to describe different skeletal types. For example, spherolitic structures in *Petrobiona* and *Astrosclera* are clearly distinct and may define these taxa (Gautret, 1986), yet global statements such as 'non-taxonomic value of calcareous microstructures' have been proposed since the 1970s.

Thus, the challenge to palaeontologists proposed by Jean Vacelet (1985) seemed impossible to address: we were unable to use structural morphology and microstructural features were not really recognised.

MICROSTRUCTURAL FEATURES. Two questions were asked by Jean-Pierre Cuif and his team in Paris-Sud-Orsay University: 1) Is it possible to obtain significant data on microstructure of the various calcified tissues, at the same time avoiding confusion between them, even in fossils suffering some diagenetic alterations? 2) What is the probability that identical modes of secretion of skeletal structures exist in distantly related, or unrelated, taxa?

The microstructural elements on fossils are 'biologically finished' and more-or-less diagenetically transformed structures. Pascale Gautret had already been studying skeletal structures of Recent hypercalcified sponges since 1986, examining in particular the living tissues responsible for their secretion, and not restricting research to the typology of fossils microstructures as most of those before her. She re-examined the different microstructures known to occur during ontogenetic development of skeletal formation, as well as the growth pattern of microstructural elements. She used the same methodology for living and fossil taxa, and was able to redefine the concept of 'microstructure' and to resolve differences in microstructures at a higher resolution. Validation of microstructural criteria was confirmed through biochemical analysis and ultrastructural analysis of organo- mineral components, through selective separation of mineral and the organic intraskeletal material using different reagents and appropriated observation techniques (Gautret & Marin, 1990; Marin & Gautret, 1994).

At about the same time as Gautret's team was working on this problem, Cuif's group completed an ultrastructural analysis of microcrystals using chromatography (evolution curves, molecular weights, comparison of the soluble matrix) and X-ray mapping (used for in situ characterisation of fossil skeletal material based on the premise that there is a reduction of the mean molecular weight during their diagenetic evolution). Cuif's group also examined amino acid and monosaccharide composition of the soluble organic matrix of both fossils and Recent sponges. They found that each type of biomineralisation process involved specific organic material, confirming that particular combinations of organic components may be characteristic of particular skeletal types.

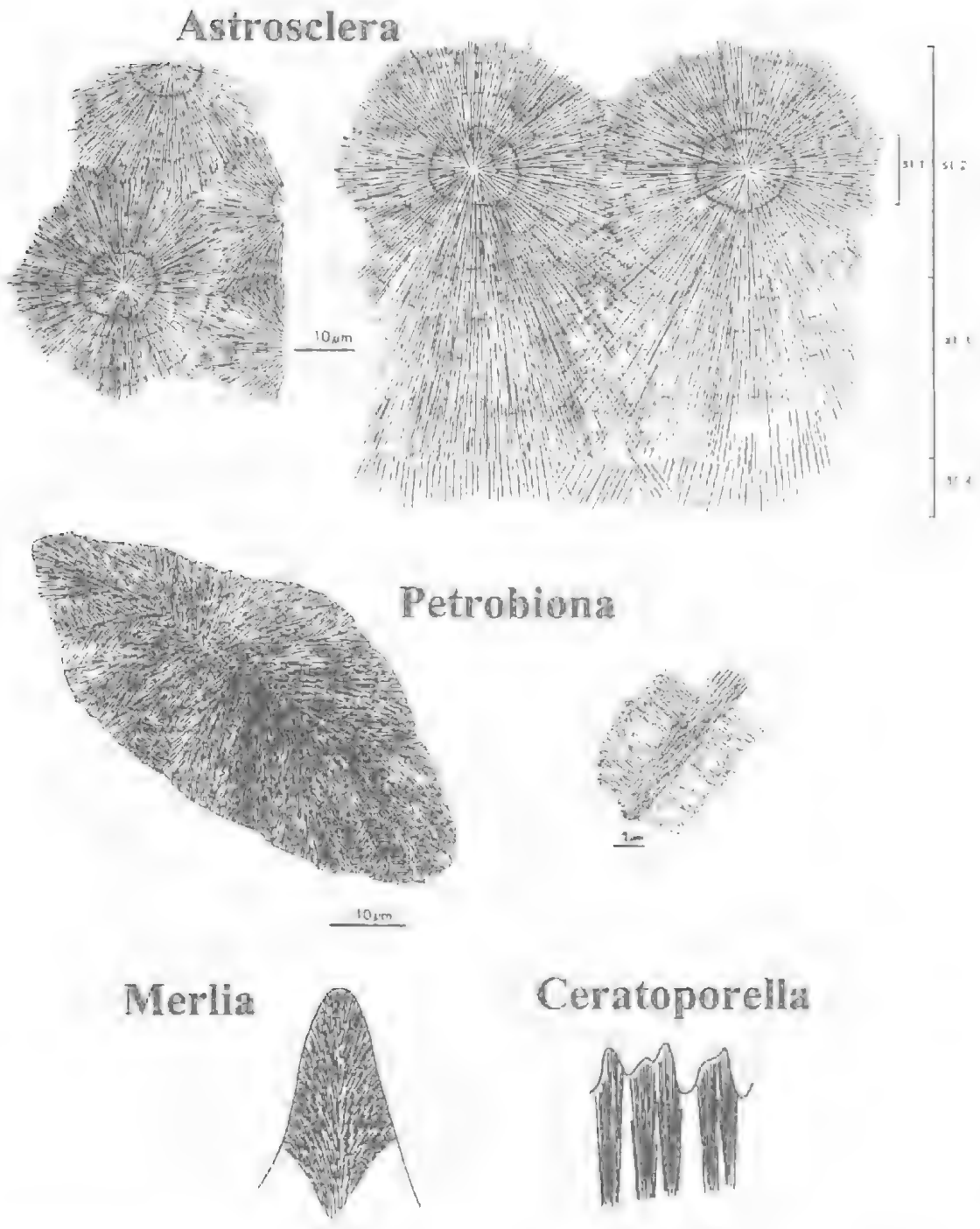

FIG. 4. Microstructural features of fibrous tissues in the skeleton of sponges of different systematic position. 'Spherolitic' microstructural type: *Astrosclera* (real spherolitic) and *Petrobiona* (fibro-radial microstructure) after P. Gautret, 1986; 'clinogonal': *Merlia* (water-jet longitudinal arrangement of the fibers) and *Ceratoporella* (penicillate arrangement of the fibers) after J.P. Cuif & P. Gautret, 1993.

Thus, it is now possible to answer Cuif's first question positively. As for the second question, it appears that the specificity of intraskeletal structures confirms the phylogenetic value of the biomineralisation processes. Using these methods Cuif's group was able to provide precise definitions of microstructural elements for unresolved cases: 1) *Astrosclera* and the Triassic fossils *Follicatea*, developing from an unique center of mineralisation, with periodic growth by addition of prismatic units in the prolongation of similar units produced during the anterior growth stages, have typical spherulitic microstructure; 2) the Calcarea *Petrobiona* and *Murrayonia* are characterised by composite microstructural elements with a continuous growth pattern of parallel fibril-like particles. No fossil forms are known at the moment with this type of microstructure.

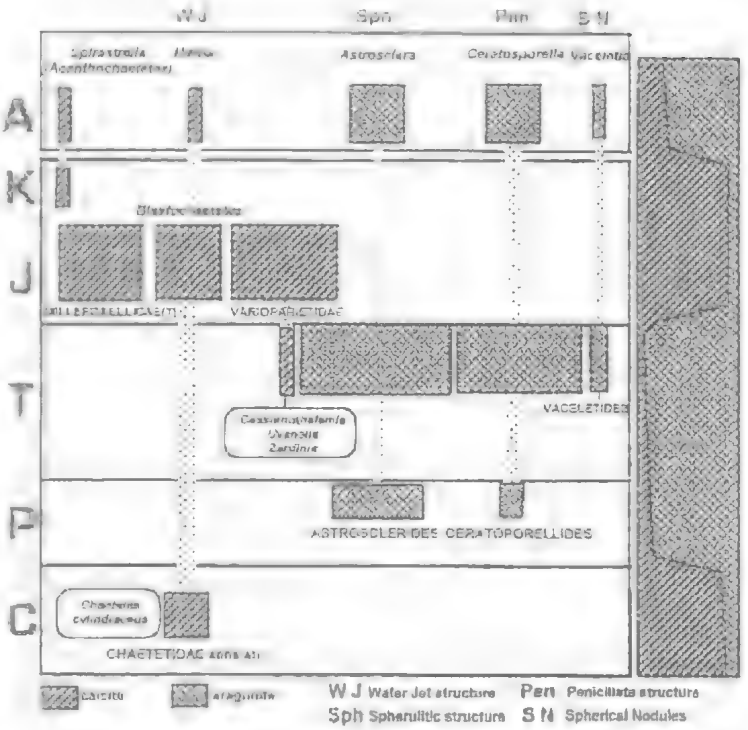

FIG. 5. Correspondence between skeletal mineralogy of sponges and deposits of carbonate sediments, after J.P. Cuif & P. Gautret, 1991, modified.

For a long time the term 'clinogonal' has included the concepts of 'trabecular', 'water-jet' and 'penicillate' microstructures. Through accurate microstructural analysis Cuif & Gautret (1993) were able to show that these three types are distinct, and that the term 'clinogonal' is misleading and redundant. A 'water-jet structure' can be seen in *Merlia*, *Blastochaetetes s. str.* and *Chaetetes*; a 'penicillate' structure is seen in the Ceratoporellids (both Recent and fossil taxa); whereas true 'simple trabecular' microstructure has never been discovered in hypercalcified sponges (Fig. 3). Furthermore, chronologically there appears to be a synchronic alternating occurrence of microstructural types (spherulitic-astroclerid-like; water-jet merliid-like; penicillate ceratoporellid-like), correlated with the alternation in skeletal aragonitic-calcitic mineralogy. These biological alternations correspond to the Sandberg thresholds (i.e. the repartition of the mineralogy of carbonate cements during the same geological time) (Fig. 4). The external constraints of oceanic parameters can influence the reactions by which calcium carbonate crystals are formed, although not the whole biological sequence of skeletal construction. During times when the water chemistry was unfavourable for mineral precipitation, sponges may have had only an entirely organic skeleton.

Diagenetic alterations affect mineral composition and microstructures, and this was one of the arguments previously used to dismiss the value of microstructural features for sponge systematics. This problem was carefully considered by the Orsay team (Marin & Gautret, 1994). The diagenesis of biogenic carbonates could not be solely estimated based on changes to the mineral phase. The amino acid content of the soluble organic matrices of different groups of sponges and other groups of fossils with hypercalcified skeletons, now required investigated.

Thus, the answers to Jean Vacelet's (1985) challenge could be obtained by palaeontologists, studying first the corresponding structures of living sponges, then applying these results to fossil sponges using the same methods, but applying necessary adjustments to compensate for diagenetic processes. Progress in these methods have been of mutual benefit to both palaeontologists and neontologist.

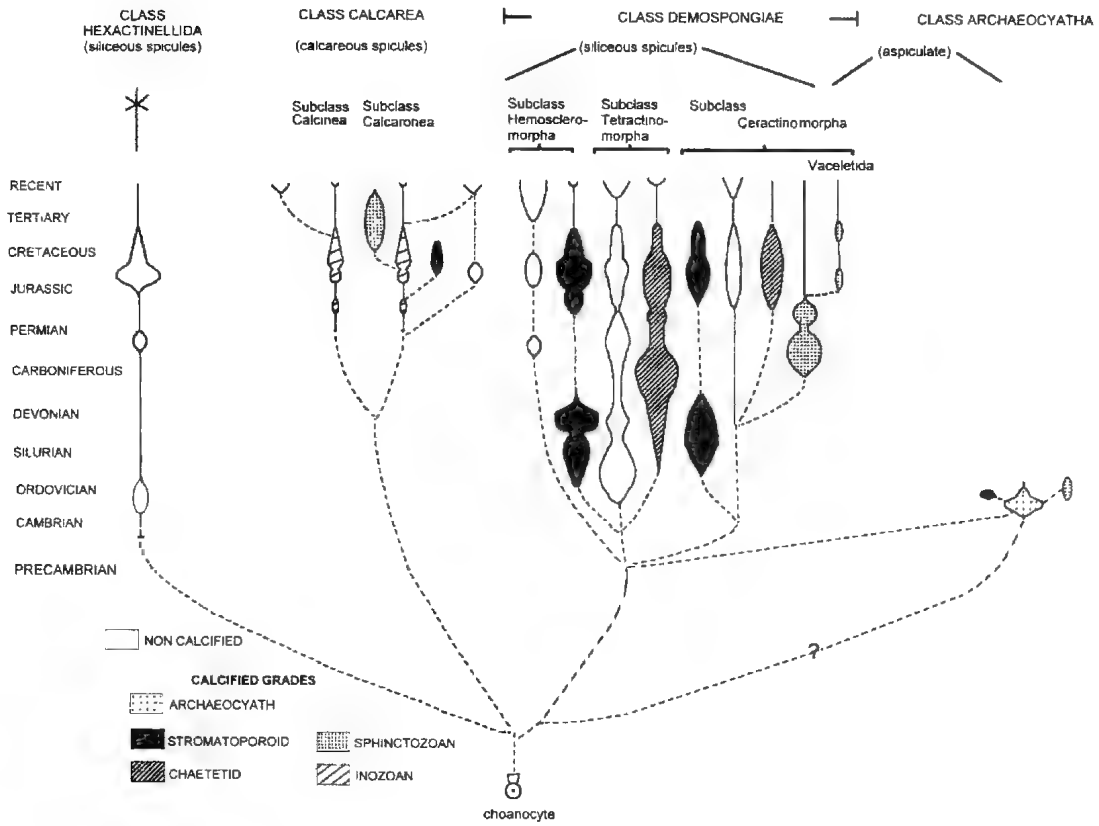

FIG. 6. Possible relationships of fossils and Recent sponges, after R.A. Wood, 1989, modified.

'THE PAST'. Fossil sponges might contribute to a better understanding of the history of the phylum, using palaeontological data to trace Recent families far back in time (Fig. 5). With the progress made in investigations into the terminal Precambrian and Lower Cambrian rocks (thanks to the successive international programs of IUGS since 1972), we can now trace the oldest preserved fossils (Fig. 6).

Only rare occurrences of hexactins have been found in pre-trilobitic sequences, in the Tommotian of Siberia and Meishucunian of South China. Genuine demosponge spicules are present in the upper Atdabanian as tetractines, with various additional elements in a much higher diversity than previously recognised, and some calcareous spicules are known from Australia (Bengtson et al., 1990). Calcified skeletons of archaeocyaths are present since the Tommotian. A cryptic pharetronid, *Gravestockia pharetronensis* Reitner, 1992, anchored on the inner wall of an archaeocyath cup and partially overgrown by its secondary skeleton, occurs in Atdabanian of Australia.

The discovery of Lower Cambrian soft fauna at Chengjian in Yunnan (Zhang & Hou, 1985) and at Shansha in Hunan (Steiner et al., 1993), containing completely preserved sponges, provide important indications on the origin and ecology of the first sponges. After arthropods, sponges represent the most diverse metazoan group in the Chengjiang fauna, with at least 11 genera and 20 species of hexactinellids (Chen & Erdtmann, 1991; Rigby & Hou, 1995). Those described previously as demosponges are also now considered to be hexactinellids (Reitner & Mehl, 1995). The soft bodied Chengjiang sponges, embedded in mudstone layers of a low-energy environment, displayed different architectures and they represent a sessile, suspension-feeding epifauna.

Precambrian remains were under discussion for a long time. Of the many reported spicules from proterozoic sediments most have proven to be volcanic shards, or other inorganic crystals, apart from some indubitable spicules from the Upper Precambrian of China. Until recently the oldest sponges known were late Ediacarian

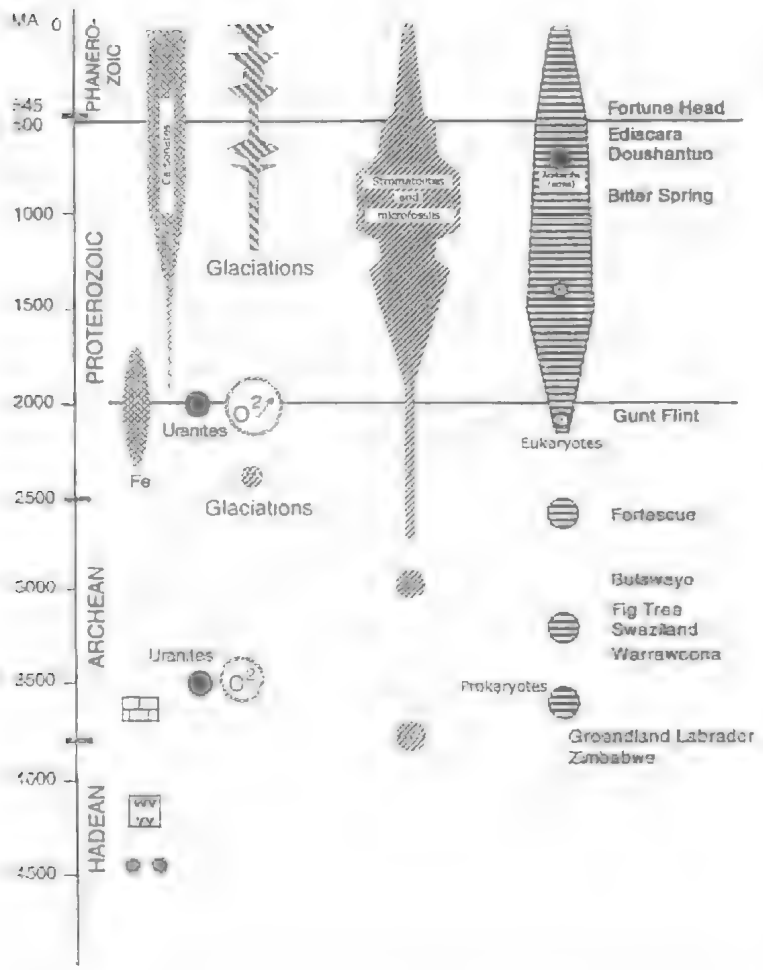

FIG. 7. Biotic and abiotic events since the Earth formation; position of the first fossil assemblages (Doushantuo & Ediacara) containing sponge remains.

hexactinellids, *Paleophragmodictya* (Gehling & Rigby, 1996), characterised by disc shape impressions preserving characteristic spicular network. This sponge is slightly older (565my) than the 'Cambrian explosion' (545my), when practically all the principal animal phyla appeared over a period of a few tens of million of years in the form of skeletised bodies. More recent discoveries in Weng'an, China, of spectacularly preserved embryos and tissues in rocks that are about 570my old, provide new data for the early animal evolution and particularly for sponges.

Since Haeckel (1877) it was thought that sponge ancestors might have been microscopic, soft bodied, and therefore not preserved in the fossil record. Now such fossils have been found in Doushantuo phosphorites (Xiao et al., 1998): the constant size of fossils, irrespective the number of compartments they have (two-cell stage; four-cell stage; polyhedral blastomeres) fits a pattern of developing early embryos with a constant cytoplasmic volume. Li, Chen & Hua (1998) figured and described a tubular and globular phosphatised sponge, some plasmolised epidermal cells, a young morula with spherical blastomeres, some embryos at the blastula stage, a parenchymella larva with peripheral flagella, a less convincing fragment of an amphiblastula larva, and a bud connected to its parent. They interpreted these as sponges: the needle shaped spicules in Doushantuo sediments are regularly arranged in distinct bodies built up of cell-like objects, some of which adhere to the spicule, much the same way as sclerocytes do in living sponges. Preserved soft tissues found in the Doushantuo material include sclerocytes, porocytes, amoebocytes; the most abundant fossilised embryos were at the blastula stage of development; three specimens were identified as parenchymella larvae with preserved flagella (demosponges); and the putative presence of one amphiblastula suggests that the calcareous sponges may extend into the Precambrian.

THE FUTURE OF THE PAST. This is a small precis of what can be said about fossil sponges, their connections to Recent ones, and of the interactions between the two domains. Other topics are now promising: the history of reef-building, the evolution of their communities, the influence of nutrients and predators (Wood, 1993; 1995), and the importance of the cryptos since the Cambrian (Wood & Zhuravlev, 1993).

Advances in molecular biology, sequencing and gene cloning applied to well-chosen Recent sponges is a promising new path for research. The ability to apply these techniques to some fossil material has already been demonstrated, although the highly degraded nature of 'fossil DNA' makes the choice of the material critical, and careful attention must be paid when interpreting group relationships. As in the past, in the future there is hope of discovering new and exciting fossil material. We are only at the beginning of investigations into the Precambrian phosphorites, in which were found the exceptional record of early multicellular life. Precambrian phosphorites containing soft cellular tissue and embryos preserved in calcium phosphate, equivalent of Doushantuo Formation, are known throughout the world. It is hoped that their continued investigation will offer endless resources for a new comprehension of primitive evolution of animal life. Are palaeoembryology and palaeohistology the future of Palaeontology?

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THE PRESENT STATE OF SPONGE SCIENCE

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The status of sponge sciences is assessed over recent decades, examining their strengths, weaknesses, opportunities and threats. The strength of sponge research lies in the organisms themselves, including the very complex array of features this supposedly 'simple metazoan' presents to researchers. We still know very little even about the basic 'bauplan', let alone the myriad of processes associated, and the phylum presents many undiscovered challenges. One of the greatest challenges, and potentially a weakness, is the difficulty in using sponges as experimental subjects outside their home environments, with the likelihood that many in vitro investigations have been flawed. But the future is optimistic, with technology approaching that will allow manipulation of sponge environments sufficient to study various processes in sponges from a range of environments. Multidisciplinary approaches to sponge sciences provides workers with significant opportunity to investigate fundamental biological and chemical problems. This provides us with an opportunity to respond to the political and academic climate by identifying current and future themes, and guiding project directions to meet the demands of the marketplace. Threats to current and future progress in sponge sciences may include the persistence of a narrow focus during disciplinary investigations, and failing to meet the challenge of being dynamic and innovative (with the caution against becoming superficial or 'trendy'). Irrespective of current diminishing funding, agency restructuring and shifts in research priorities, sponge sciences are flourishing and provide reason for current and future optimism. ❐ *Porifera, status of research, strengths, weaknesses, opportunities, threats.*

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The first question is, what are the boundaries of the 'Present' in relation to a group of organisms, which, if you consider just Demospongiae, have been in existence for at least 580 million years in close to their present form and which, judged by their success in recent environments, look set to well outlast those who study them?

After some thought and consultation I have decided to construe the 'Present' as the period since regular sponge conferences began (1968), and extending forward five years from the 5th International Sponge Symposium in Brisbane. In the final keynote paper (Vacelet, this volume), Jean Vacelet will then at least know where the future starts. I will use this forum simply to provide a very brief snapshot of the attention the sponges are receiving and have received through active research programs over that period, and the major achievements.

It was hard to decide how to focus and organise this presentation. I have decided to use a device beloved of our omnipresent bureaucrats and to present the talk as a SWOT analysis which addresses the Strengths, Weaknesses, Opportunities and Threats we, as an international community of sponge scientists, experience. This, of course, will be a personal view, someone else could come up with a very different scenario.

We can think under each of these headings in terms of the 'discipline', things which arise directly from the biology of the organisms we study, and then, 'in context', referring to the dynamics of our group, the scientific trends impacting upon us and the political realities of the day (Table 1).

STRENGTHS. To set a note of optimism, nothing is more obvious than the fact that the major strength of sponge biology lies in the organisms themselves. Sponges, as the simplest true Metazoans, are just incredibly intriguing. They also provide insights into the development of systems which characterise more complex organisms. If we look back over the international conferences since 1968 we can record many milestones passed in our understanding of sponge function and relationships. Many simple elegant

experiments and investigations have been presented and have served to demonstrate sophisticated cellular systems, complex developmental processes, endogenous rhythmic behaviour, cellular communicating networks, incredible versatility in feeding behaviour, amazing chemistry and biosynthetic alternatives, incredible survival of ancient forms, a striving for tissue organisation. Sponges may be the simplest Metazoa but we, as a group of researchers, have demonstrated beyond any doubt that they are also complex organisms.

In light of recent discoveries it occurs to me to wonder whether we have found all the variants upon the basic defining sponge structure or 'bauplan'. May something yet again cause us to reappraise the boundaries of the term Porifera — I can imagine one or two possibilities — but that is for Dr Vacelet, speaking of the 'Future', to tell us about. I think it is justifiable at this stage to contend that the spectrum of disciplines which constitute a holistic approach to sponge biology is being addressed, some disciplines more comprehensively than others — I will return to that.

Strengths contextually arise from the fact that, because of their cellular and chemical basis of operation, it is an absolute necessity to approach questions of sponge function and relationships from a multidisciplinary perspective. Some may have been slow to embrace the molecular methodologies which are simply the tools which can help elucidate cell and organismal function across a broad spectrum. Some may have felt 'rolled over' by the molecular bandwagon, but, now that molecular biology has rediscovered the whole organism, as developmental biology takes centre stage, it is an opportune time to promote the benefits of basic research on sponges.

Lastly, there is the commitment and cohesion of our body of researchers. This is not true of all fields, malacologists are always at war within their community, and entomologists, well, the least said soonest mended! This has been, and hopefully will remain, a communicative, co-operative, congenial and exciting community within which to work.

WEAKNESSES. To temper this, what do I perceive as weaknesses? As you will observe (Table 1), I could not think of many. Sponges are not the most tractable experimental animals, requiring as they do large volumes of sea water to maintain feeding and body form. In the absence of adequate understanding of how they function in nature, many investigations on feeding, response to environmental stresses, cell differentiation and cellular function have been flawed. There is a serious weakness here and the root cause has been the persistent consigning of some biological parameters to the 'too hard' to study basket. This has significantly hampered investigation of ecological physiology and reproductive behaviour, to give just two examples. It is possible that ingenuity in experimental design and/or ability to utilise expensive land based systems can overcome this problem. One possibility I can suggest here is to establish some multidisciplinary collaboration with JAMSTEC, the Japanese Association for Marine Science & Technology, which I was fortunate enough to visit prior to the Otsu Conference. Technology exists there to maintain invertebrates in the laboratory, collected from the deep oceanic vents, and to take them through reproductive cycles. The controls that can be applied in this experimental system surely would permit manipulation of sponge environments sufficient to study physiological and reproductive processes in sponges from a range of environments. The worst thing, however, would be to continue to ignore these areas. A few workers who have 'done the hard yards' in the field have greatly enhanced our knowledge; much more effort is required.

Many would perceive the ageing population of established workers as a weakness in the present context — on the other hand it could be seen as an opportunity. If established positions are retained and deployed in the broad field of sponge biology I perceive no problem. That then becomes the challenge; a test of your political skills in defining and promoting sponge biology in the modern context. Older workers may also, in line with environmental trends, be recycled at greatly reduced cost, surely this is a benefit!

OPPORTUNITIES. I have already noted that the organisms we work on dictate a multidisciplinary approach to almost any serious study. This provides sponge workers with a significant opportunity when presenting applications to granting agencies, which increasingly are requiring such approaches. Sponge models can provide an insight into many fundamental biological and chemical problems. The training that this broadly-based research gives, opens doors for graduates specialising in sponge topics into medicine, particularly in the fields of cell adhesion, cancer biology, immunology, cell differentiation, in the broader field of developmental biology particularly its molecular

aspects, in environmental and conservation management and aquaculture, to name just a few areas where my graduate students have gone.

The fact that sponges inhabit all aquatic environments from the deep ocean to fresh water makes a knowledge of their ecology and reproductive biology an integral part of many multi-agency environmental programs. This provides opportunity to pursue basic sponge research as part of a team.

At present there is opportunity to respond to the new climate in political and academic circles by identifying current and future 'themes' and merging your own interest with these, not being submerged, but guiding project directions to meet the demands of the marketplace and to provide the answers you want as well.

An example from my own experience has been to combine my interest in taxonomy and phylogenetic relationships of sponges with the requirements of those funding marine pharmacological research, always ensuring that I could obtain the data I required through this involvement. Others have taken up similar collaborations. I suggest, however, that biological interests beyond taxonomy, phylogenetics and biogeography can be supported and pursued through selective participation in pharmacologically directed programs.

THREATS. Coming then to actual and potential threats, in many ways the following points apply very generally and are not confined to sponges. However, my thinking is generated from sponge examples. Central to all research is a striving to better and more completely understand how the organisms function and relate to each other and to their environment. Asking the questions — what can a sponge do; what must it have to survive; what can a sponge experience and still survive? — can be enlightening in most, if not all, areas of research.

Such thinking requires, no matter what one's particular specialisation, that you are conversant with developments across the discipline. It is no longer adequate to maintain a narrow focus. These suggestions apply with most force to those of us who are practitioners of the older biological subdisciplines. There has been a tendency for workers to wrap themselves in the mantle of their disciplinary antiquity, new workers being proclaimed not 'true' systematists or 'real' marine biologists if they deploy new techniques or new conceptual approaches to their study. This applies less to sponges than some other disciplines. It is essential that old learning be maintained, but this most often has to take place in new contexts. The eminent philosopher, Alfred North Whitehead, once remarked, 'Knowledge does not keep any better than fish'. There is a challenge then as evolutionary, ecological or systematic biologists, to reilluminate old facts with new insights as well as to make new discoveries. This approach brings a convincing dynamism to our science and is a protection against being declared obsolete. The very real threat lies in failing to meet this challenge.

Having argued the need to keep up with the pace, a caution must be sounded against becoming superficial or 'trendy'. The tools to be applied must be understood and directed to properly formulated questions. To take one example, some of us have engaged in molecular systematic studies attempting to obtain objective data to expand the base upon which classification can be built and relationships can be postulated. Most have worked with the ribosomal gene. However, how many sponge biologists understand the complex, underlying assumptions upon which the tools to deal with sequence analysis rest? That is a discipline in itself, and a highly genetical and mathematical one.

Molecular phylogenies based on ribosomal sequences have implicitly been accorded a higher authority than those phylogenies derived from morphological data sets. Yet, we now know, that particularly for ancient branches, they can be significantly misleading, if not downright wrong. This is particularly so when the number of taxa sampled is low, as has often been the case. There is a significant cost in this work. It is now acknowledged that for deep evolutionary branches it is difficult to have confidence in 18s rRNA trees in the absence of corroborating morphological phylogenies. Sponges are an ancient group already diversified in Pre-Cambrian time. Because of the length of this history, many of our most vexatious higher order taxonomic problems, which rRNA phylogenies hoped to address, probably are subject to a number of artefacts, long branch attraction effects to name just one.

Looking to the future, as molecular systematics comes of age, it seems likely that protein coding genes, which make up a much larger proportion of the genome than RNA coding genes, will provide more reliable phylogenies. Thus it becomes a matter of, choose your question, choose your molecules, choose your

collaborators, and then generate a broadly based morphological and molecular study. It takes time and money; however, superficial exercises waste everyone's time.

Contextual threats can be dealt with quickly. One point arises in part from what I have just said. Failure to develop appropriate collaborations and to determine when to cooperate and when to compete can be a threat to the credibility of the discipline.

Further, and most importantly, it is incumbent on us all to encourage and assist new recruits to the study of sponges. Help at the right time can mean a great deal. Certainly it did for me when as a PhD student, the only person in the Southern Hemisphere working on sponges, I received a letter from Willard Hartman confirming and/or correcting my identifications of a small collection of sponges I had sent him. It made the difference between my continuing with sponges or working in fisheries ecology. Any discipline where helping new workers is ignored is under threat.

Following from this, in view of a perceived lack of present employment, is it ethical to encourage students to study sponges? I think so, provided the training their projects deliver is sufficiently broad to allow adjustment of direction, and we encourage students to think in such terms.

I think the greatest threat to our discipline lies in adopting the common down-beat attitude that years of parsimonious funding and ill-informed managerial changes in direction and philosophy have engendered in universities, museums, and government science agencies. As a group of scientists, devoting research time to organisms the new right would certainly regard as insignificant, we have survived and indeed are flourishing. There is reason for optimism, sponges can almost speak for themselves.

TABLE 1. SWOT analysis of the current status of sponge sciences.

STRENGTHS – DISCIPLINARY
1. the organisms themselves
2. something exciting is always just around the corner
3. the disciplinary spectrum is being covered
4. simple elegant experiments have been done and remain to be done
5. milestones are being passed

STRENGTHS - CONTEXTUAL
1. necessity to take a multidisciplinary approach
2. molecular biology is now rediscovering whole organisms
3. commitment and cohesion of our membership

WEAKNESSES – DISCIPLINARY
1. consigning some biological questions to the 'too hard' basket (e.g. ecological physiology, reproductive behaviour)
2. sponges are difficult material for in vivo laboratory work – expensive systems may be needed

WEAKNESSES – CONTEXTUAL
1. ageing population of established workers

OPPORTUNITIES – DISCIPLINARY
1. multidisciplinary approaches are being demanded by granting agencies
2. sponge models provide an approach to fundamental questions
3. to present basic biological questions in terms and context that can be funded
4. linkages/synergies with many groups possible

OPPORTUNITIES - CONTEXTUAL
1. identifying and manipulating current and future 'fashionable' themes (e.g. sustainability, biodiversity)
2. being able to respond to new academic/political climates and merge your interest with these

THREATS – DISCIPLINARY
1. taking and maintaining a narrow focus
2. failure to understand the organisms
3. becoming superficial and 'trendy'

THREATS – CONTEXTUAL
1. failure to encourage and mentor new workers
2. failure to discriminate when to compete and when to collaborate
3. lack of employment opportunities – is this real?
4. adopting the pervasive down-beat attitude

OUTLOOK TO THE FUTURE OF SPONGES

JEAN VACELET
Astra Pharmaceuticals (Australia) Plenary Address

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Evidence from the past and recent discoveries provide material for a philosophical review and possible scenarios, for the future of sponges, their essential characters, their evolutionary potential and direction, and their survival. Their short and long term futures appear secure. Species are capable of coping with the outcomes of human impacts on the oceans (survival in highly polluted, warmer waters, in dark and oligotrophic conditions), whereas increased sedimentation is a potential problem to the deep-sea species. Recent species have an ancient, simple 'bauplan' more-or-less unchanged since Precambrian times and are capable of simplifying (independently losing the essential poriferan characters of the aquiferous system and choanocytes), much like the newly discovered Precambrian fossils, to adopt a carnivorous life style. To date, 'complexification' in sponges has been restricted to their considerably complex biochemical constituency and numerous biosynthetic pathways and their ability to develop a canal system, filter-feeding habit and single layer of choanocytes which permit them to attain larger sizes and to have considerable ecological success. But the oldest fossils show that Precambrian sponges did not have such filtering devices and new findings show that carnivorous sponges can certainly live without them. These characters, therefore, are probably not fundamental characteristics of Porifera, which may be better defined by their characteristic cell motility, plasticity of body organisation, absence of tissues and organs and presence of spicules (although the latter optional). ❐ *Porifera, body plan, carnivory, Precambrian fossils, evolutionary trends, defining characters.*

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The future of sponges, in the context of this review, could be seen from different viewpoints depending on the time scale that is adopted, and whether we look at the question from the human or the sponges' perspective. I could focus on the immediate future of sponge studies: such as emphasising the possible development of molecular tools in reconstructing phylogenies; the expected increase in knowledge of sponge biology; the implications of knowing a complete sponge genome (as predicted by Claude Lévi, this volume); or the impact that machines of the future, successors to our current primitive computers, will have on taxonomy. These concepts have already been considered by Patricia Bergquist (this volume), predicting the next five years. Any prediction over a longer term is not reasonable, because over the next decade techniques are likely to change so spectacularly that the mere extrapolation of existing trends will not provide the insight necessary to make forecasts. We all feel that considerable changes are occurring, and I could predict that molecular taxonomy will demonstrate that Halichondrida are to be merged with Hadromerida, or that Homoscleromorpha is another phylum, but such predictions cannot be taken seriously or would be based on preliminary results, and that concerns the Present.

This paper, instead, considers the potential evolution of sponges, both in the relatively short term (i.e. during the dramatic changes that humanity has imposed on our planet) and in the far longer term (the future of life on Earth). These predictions are even more uncertain than those concerning the immediate future of sponge research and are matters of philosophy rather than science. I feel more comfortable in this role: there is no risk that I will be disproved in my lifetime. Claude Lévi (this volume) noted that in the early 18th century Dutrochet was wrong when he thought that sponges functioned as an osmotic pump; but who will be there to remember that I was wrong when predicting that sponges would develop a nervous system, or a locomotory apparatus, during the next 200my?

SHORT TERM. The relatively short term future of sponges will certainly be modified by humanity. They will have to face disruptions to the

ecological equilibrium caused by the proliferation of our species and activities. We can infer from the rate of extinction over the last two centuries that we are facing a major extinction period of the same order of magnitude as the seven or eight recorded in the fossil record. Sponges have survived these major extinction periods, with up to 80% of marine species known from the fossil record disappearing at the end of Permian. It is therefore likely that they will have a chance of being able to cope with this new threat. We fear a rise in sea-level due to global warming. This is not a problem for sponges. They have seemingly failed in their role, discovered by a French humorist, Alphonse Allais, around 1900, who maintained that sponges were placed in the oceans by Providence to prevent overflowing from all the rivers, but this failure may be turned to their advantage, as more seabed surface will become available for colonisation. A little more seriously, the colonisation or urbanisation of the ocean by mankind, as predicted by some visionary architects, could also turn to the advantage of sponges, which generally prefer solid substrates to soft sediments. These futuristic developments could include submarine cities, with house walls covered by brilliantly coloured sponges, cleaning the water by their filtering activity, with equipment for farming genetically modified species producing molecules of exceptional biological interest.

Many sponges are able to live in polluted areas, so that a scenario of general pollution of the oceans may cause change in species composition but is unlikely to compromise the survival of the phylum. One cause for concern, however, is a global increase in sedimentation rates, which most sponges do not appreciate. This could be a problem for deep-sea sponges impacted by the run-off from intensive land deforestation and erosion, or in the case of careless exploitation of abyssal mineral nodules, a project which is at present discarded but which reappears periodically. Increase in sedimentation is likely to affect first the Hexactinellida, which have a highly efficient but more delicate aquiferous system. A nuclear winter of two or three years would probably decrease sponge diversity, but again without compromising the survival of species that are presently able to live in dark, oligotrophic conditions.

LONG TERM. The phylum Porifera thus has a good chance of surviving this new extinction crisis, certainly better than the species causing it — man. After an extinction crisis biodiversity recovery apparently requires 5-10my (Jablonski, 1994), which is long in our history but very short in the history of life on Earth (which may last up to 5 billion years). What will happen during this time for Porifera? The main question is: will this evolution modify the fundamental body plan, or 'bauplan', of Porifera? This presupposes that we agree on the definition of this fundamental body plan.

The current view of the evolution of metazoans is that the fundamental bauplans which define the various phyla appeared suddenly, that is 5-10my, during a highly imaginative period which has been called the Cambrian explosion. The subsequent evolution of taxa, over approximately 550my, entailed the extinction of many of these types of organisation, whereas a few survivors impressively diversified, but without any fundamental change or creation of any new fundamental types. This pattern of evolution was believed to have occurred mostly by 'complexification' and 'progress', partly because one of nature's most recent products, the 'summum', is *Homo sapiens*. These views of 'progress' are now strongly challenged, as popularised by Gould (1997). Although it is difficult to be entirely free of this intellectual bias, evolution no longer appears to be directed toward complexification and progress, but alternating between complexification and 'simplification', which thus makes the outcome very difficult to predict.

The phylum Porifera conforms to a very simple body plan, succinctly defined by Bergquist (1978): '... a sedentary, filter-feeding metazoan which utilises a single layer of flagellated cells (choanocytes) to pump a unidirectional water current through its body'. A long accepted view is that this type of organisation, which is the simplest to be found among the successful metazoan survivors, was the first to appear. As a consequence, Porifera is considered as an old phylum, whose evolution has been completed, and whose simple body plan could not complexify like those of other metazoans which developed tissue, organs, guts, eyes, nervous system, etc.

What is true in these assumptions? Their discussion in the knowledge of recent findings on sponge biology could modify the view of the astrologer. Several lines of evidence confirm that sponges are very old metazoans. Biochemical data indicate that although they are true metazoans, several molecules appear as phylogenetically the oldest within the metazoans (review in Müller, 1998). For instance, the time

of divergence of galectin or of the cell-surface RTK (Receptor Tyrosine Kinase) from those of other metazoans has been estimated by Müller from 800–650my The fossil record, as shown by Françoise Debrenne (this volume), gives increasing evidence that sponges very similar to the modern Demospongiae, Hexactinellida and possibly Calcarea, were already present in the early Cambrian, with spicules and presumably body structures not very different from the Recent fauna (Bengtson et al., 1990; Zhang & Pratt, 1994; Gehling & Rigby, 1996). Moreover, the extraordinarily well preserved fossils from Guizhou in South China (Li et al., 1998) indicate that Demospongiae, and probably other sponges, were already present 580mya. This estimate places the modern sponge bauplan significantly earlier than the Cambrian explosion, which is usually understood to have lasted 5-10my and to have occurred 540-550mya. (Bengston, 1998; Kerr, 1998). In fact the Cambrian explosion concerned a diversification of the Bilateria phyla moreso than the diploblasts such as sponges and cnidarians. Therefore, one conclusion could be that if sponges did not evolve spectacularly during the last 500my, they are also unlikely to evolve so much during the next 5000my — in which case my argument is finished.

Nevertheless, let us consider the poriferan 'bauplan'. As Claude Lévi has already noted (this volume), we have probably over-stressed the canal system and choanocyte in the definition of the Porifera.

The Precambrian Chinese fossils are evidently sponges because their miraculously preserved cells closely resemble those of modern sponges, and because they have spicules. Although spicules are not an indisputable character of sponges, being absent in some Recent Demospongiae, the similarity of the oxeas in the Chinese fossils with those of the Recent Haplosclerida is striking. In passing, it is worth noting that preliminary molecular taxonomic data indicates the order Haplosclerida to be one of the earlier branchings (Lafay et al., 1992). In contrast with these evident poriferan characters, choanocytes and a complex aquiferous system have not been recognised in these Chinese fossils. This is not due to the processes of fossilisation, as the other cell categories (pinacocytes, porocytes, archaeocytes, sclerocytes), are perfectly recognisable in the fossil material. The individuals are also subspherical, with an unusually small size compared with more recent sponges. Does this mean that these first 'sponges' were devoid of an aquiferous system and had another mode of life that did not allow them to grow larger than about 750mm? This would throw some doubt on the plesiomorphic character of the aquiferous system (which characterises sponges so clearly among metazoans), and of choanocytes (which is so similar to choanoflagellates). But the present observations deal with a few square centimeters of thin sections, and there are still 57km^2 of phosphatite to explore in the Guizhou deposit in South China.

A second case is the carnivorous mode of life in some Recent sponges. These 'sponges' are devoid of an aquiferous system and choanocytes and develop appendages or filaments covered by hook-like microscleres which trap small crustaceans (Vacelet & Boury-Esnault, 1995). Fortunately they have spicules, so we can recognise that they are sponges, and more specifically sponges closely allied to well known families of Poecilosclerida. Their cytology would be typical of Demospongiae, were it not for the absence of choanocytes. In the absence of a digestive cavity, the digestion of the prey occurs by means of a cellular system which is unique among metazoans, with cells individually migrating toward the prey and digesting it. Intense cell migration and dramatic reshaping of the body occur during the processes of prey capture, engulfment and digestion. These animals thus have a cytology of sponge, with the extreme mobility of all the sponge constituents emphasised by Claude Lévi, but without the conventional diagnostic characters of the phylum Porifera.

This adaptation to carnivory is present in several evolutionary lines of the Poecilosclerida, with chelae microscleres indicative of close affinities with Mycalidae or Esperiopsidae. This adaptation also seems to occur in the family Guitarridae, genus *Eucheliplumа*, in which the placocheles are disposed along long appendages with the teeth oriented towards the surface (Vacelet, unpublished observations). A special case is the genus *Chondrocladia*, classified in the Cladorhizidae because of its morphology, but belonging to a different line than *Asbestopluma* and *Cladorhiza* as indicated by its isancorae microscleres, and characterised by inflated spheres which collapse when the sponge is collected (Tendal et al., 1993). From preliminary results, although carnivorous, this genus appears to have retained its choanocyte chambers and an aquiferous system, which is probably used in both filter-feeding and inflating the turgescent spheres which trap the prey (Kübler & Barthel, 1999, this volume).

As in the Precambrian Chinese fossils, these animals are clearly 'sponges' that lack choanocytes and an aquiferous system. Contrary to the Chinese fossils, however, this seems to be a relatively recent adaptation which has appeared independently in several evolutionary lines of Poecilosclerida, probably one of the most recent orders in Demospongiae. Based on their spiculation carnivorous taxa are closely allied to normal littoral sponges such as Mycalidae or Guitarridae. The development of carnivory has been described in other deep-sea invertebrates, such as tunicates or gastropods, and appears to be related to the present conditions of the deep sea, which are relatively recent and in any case not older than the Cretaceous. Carnivory in sponges could be older than the Cretaceous, as suggested by *Esperiopsis desmophora*, a deep-sea sponge whose morphology suggests carnivory and for which a possible affinity with the Ordovician *Saccospongia* has been suggested (Hooper & Lévi, 1989). However, in any case, carnivory does not appear to be a plesiomorphy of Porifera.

There are therefore two indications that sponges could be permanently devoid of choanocytes and an aquiferous system. In the first case, which is still to be confirmed, it appears as a plesiomorphy. In the second case, it appears as a relatively recent loss in closely related evolutionary lines, under environmental constraints.

This second case is of interest for another reason. Carnivorous sponges have been able to discard the filter feeding system otherwise characteristic of poriferans and to develop a unique organisation. Is this a new bauplan? If yes, then this would be a unique case of an appearance of a new body plan after the Cambrian explosion, and of the development of such a novelty arising from an existing phylum. This scenario would be promising for the future: if sponges succeeded once in such a dramatic change, they may be capable of other changes.

My preference is for another interpretation, already suggested by Claude Lévi (this volume). Our definition of the poriferan body plan is not appropriate. Possession of a canal system, filter-feeding habit and presence of a single layer of choanocytes in fact may not be the fundamental characteristics of sponges. Sponges have the ability to develop these structures that allow them to attain larger sizes and to have considerable ecological success, but the Chinese fossils suggest that the oldest known sponges in the Precambrian did not have such filtering devices, and the carnivorous sponges also show that they can live without them.

Now the question is: what is the true definition of sponges? Cell motility, plasticity of the organisation, absence of tissues and organs, and presence of spicules (although optional), are good candidates, although it is very difficult to write something simple and not rely too much on characters that are absent. Even so, we must not forget that the development of a unique aquiferous system occurs in more than 99.9% of the species. I will leave that to the future of spongology and future advances on these topics. In this context, an animal such as *Asbestopluma hypogea*, which compensates for the absence of filter system through an increased plasticity (Vacelet, 1998), which is able to live and reproduce in 1/2 litre of sea water with a monthly water change and a monthly feeding with a deep frozen piece of shrimp, without the expensive and sophisticated JAMSTEC (referred to by Patricia Bergquist, this volume), appears to me *the* experimental animal for the future.

Let us now suppose that in the next century we will achieve a definition of the bauplan of Porifera taking these new data into account. If the loss of an aquiferous system during development of carnivory is only a return to square one, then the fundamental organisation of the sponge has not changed so much since Precambrian times. Sponges successfully diversified, but they did not attain a high level of 'complexification' as compared with other metazoan phyla. They were unable to develop a nervous system, motility, etc. in 580my. They still have nearly 10-fold this amount of time before the sun boils the oceans, in approximately 5 billion years. What will happen during this vast expanse of available time ? Is greater complexification likely? Two prerequisites are required: they must be capable of complexifying, and they must need to do it.

With evolution now seen as a contingent alternating process between complexification and simplification, sponges will certainly complexify again in the future. What are the possibilities? There are some indications that sponges could already be more complex than previously thought. For example, sponges have only primitive cell junctions, but this seems to be rather for functional reasons than for a lack of genetic potential (Müller, 1982). Indeed, during spiculogenesis in Calcarea, which needs tight occlusion of a space between several cells, these sponges could develop septate junctions, which

are absent in normal circumstances (Ledger, 1975). There are several examples in biological evolution where the development of a structure precedes its function; for instance several dinosaurs had feathers before they were able to fly. Carnivorous sponges also provide a good example of this phenomenon. The anisochelate microscleres, isancorae or placocheles of *Mycale*, *Esperiopsis*, *Guitarra*, that have no evident function in littoral sponges, were most probably developed before carnivory, for which they appeared perfectly suited to the capture of prey with only a small change in orientation. An exercise for the future could be: what is the potential for evolution of the structures, genes, molecules, that we are discovering in sponges without having any precise knowledge of their present function?

Recent developments in biochemistry suggest that the phylum already has many requisites for 'complexification'. We know that sponges have receptors and their ligands homologous to those of other metazoans, suggesting the possibility of developing true tissue (Mehl et al., 1998). Collagen type IV specific to the basal membrane has recently been identified in the Homoscleromorpha (Boute et al., 1996), indicating that a true basal lamina, which is required for the establishment of true tissue and organs, is present in sponges. Neurotransmitters are found in sponges, but they are apparently not engaged presently in cell-communication (Mackie, 1990). Another recent discovery is g-crystallins, a protein of vertebrate eye lens, in *Geodia* (Krasko et al., 1997), which presently has no eye, as far as known. There are many other examples, mostly found in the famous *Geodia cydonium*, and our colleague Werner Müller is adding day after day molecules and genes involved in signal transduction, immunorecognition, neurotransmission, etc. These molecules may suggest potentiality for complexification, although it is more likely that in most cases they are plesiomorphies shared with the other metazoans, which, contrary to sponges, were able to develop and diversify functionality for such precursors. Another interpretation is that the molecule is not a precursor, but a vestige of a more complex stage which evolved towards simplicity with loss of function. This is certainly less general, but is worth keeping in mind for some cases.

Thus, sponges may have some potential for complexification, although it is probably limited. It is not certain, however, that a higher degree of complexity will be necessary for their future success. Two points need to be made here.

Firstly, compare the Cambrian archaeocyathids and the Recent calcarean genus *Clathrina*. The first have a sophisticated solid calcareous skeleton, with an extraordinary complex system of openings in the outer wall, and probably a complex soft tissue system for filter-feeding (Debrenne et al., 1990; Debrenne & Zhuravlev, 1992). They became extinct in the Middle and Upper Cambrian. In contrast, it is difficult to imagine a filter-feeding metazoan simpler than a live *Clathrina*, with its asconoid tube, simple spicules and reduced number of cell types. However, Recent species of *Clathrina* are certainly not archaic survivors of primitive sponges. Their number and diversification, their distribution in highly competitive littoral environments, all indicate that they originate from a relatively recent burst in evolution. So, the complex archaeocyathids were highly successful in the Cambrian, but evolution at present retains the ultrasimple *Clathrina*. During the alternating processes of complexification and simplification, such asconoid sponges have reached the simplest possible stage. They are hitting against the wall of simplicity, as Gould (1997) would say, and it may be predicted that simpler sponges will never occur.

Secondly, conditions in the deep-sea apparently favour carnivory versus filter-feeding. Carnivory usually develops through highly sophisticated devices and behaviour patterns, which need a high degree of complexity. Sponges succeeded in developing this mode of life without a spectacular increase in complexity. Why bother to develop a nervous system, digestive cavity, nematocysts or other weapons when there is the ability to efficiently catch the prey and digest it by other means, as is already done by carnivorous plants or some foraminiferans? So it is not certain that Porifera will really need to complexify while maintaining their success and possibly again diversifying in the ocean of the future.

A last wild thought as a conclusion. Metazoans, including Porifera, are monophyletic. They share the same ancestor. This means that some metazoans may have derived directly from a sponge, which is so difficult to define. Could this happen again? It is easier to imagine such a derivation from a sponge that lacks the specialised anatomy of a filter-feeder, such as the extinct Precambrian Chinese sponges, or the carnivorous sponges, some of which have been captive for three years in my laboratory and may be preparing a new burst in evolution ...

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INDICATIONS OF RELATIONSHIPS BETWEEN PORIFERAN CLASSES USING FULL-LENGTH 18S rRNA GENE SEQUENCES

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Porifera is traditionally viewed as monophyletic, yet recent molecular data indicate it may be paraphyletic or even polyphyletic. In this study full-length 18S rRNA sequences were derived from two hexactinellid sponges (Class Hexactinellida), four demosponges (Class Demospongiae) and one calcareous sponge (Class Calcarea), in order to test the evolutionary hypotheses of relationship between them, and ultimately, to test the monophyly of Porifera. Phylogenetic analyses yielded congruent polyphyletic topologies with Demospongiae and Hexactinellida, forming a well-supported clade, which excluded the Calcarea. The Calcarea was hypothesised to be more closely related to other diploblasts, forming a clade with the comb-jellies (Phylum Ctenophora). The Kishino-Hasegawa test was applied to explore alternative evolutionary relationships between the sponge classes. Constraining the Calcarea as sister taxon to either Demospongiae or Hexactinellida was rejected in this test, although a monophyletic sponge phylum could not be rejected using this dataset. ❐ *Porifera, Demospongiae, Hexactinellida, Calcarea, molecular phylogeny, evolution, 18S rRNA.*

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Sponges are regarded as the most primitive multicellular animals, primarily due to their 'simple' body plans and evidence from the fossil record, which together suggest they were the earliest lineage to diverge within the Metazoa. Sponges have attained a 'multicellular' grade of construction, with no development of tissues or organs, and their fossil record extends for at least 600 million years. Due to their shared possession of unique choanocyte cells, the Porifera is generally considered to be a monophyletic phylum. Recent ultrastructural and molecular evidence, however, suggests they may be a paraphyletic or even polyphyletic group of animals (Mackie & Singula, 1983; Reiswig & Mackie, 1983; Cavalier-Smith et al., 1996; Borchiellini et al., 1998; Kruse et al., 1998).

Phylum Porifera, as it is presently recognised, consists of three classes; Demospongiae, Hexactinellida and Calcarea, differentiated primarily by differences in composition and geometry of skeletal components and cellular organisation of the soft parts. Hexactinellida and Demospongiae are characterised, in part, by the common possession of inorganic skeletons composed of siliceous spicules, although some demosponges (Dictyoceratida, Dendroceratida and Verongida), have skeletons composed of only proteinaceous (spongin) fibres and collagen fibrils. Demospongiae and Hexactinellida differ in that the former have spicules with one to four rays (monactine to tetractine), whereas the latter always have triactine or triaxial-derived (pentactinal and hexactinal) spicules. In contrast, Calcarea include sponges with calcium carbonate spicules in the form of calcite. Calcarea and Demospongiae both have representative species of 'coralline sponges' possessing a calcareous aragonitic base, in addition to calcareous or siliceous spicules, respectively. These sponges, now referred to as 'hypercalcified', formerly comprised the Class Sclerospongiae, whereas it is now believed that this grade of construction has evolved independently in different lineages within the two classes (Vacelet, 1985; Reitner, 1992).

Demospongiae and Calcarea have three major cellular layers. The first layer, the pinacoderm, lines all external surfaces of the sponge and is composed of a single layer of pinacocyte cells. The second layer is the choanoderm, composed of choanocytes which are the collared cells that draw water, and hence nutrition, into the sponge via the aquiferous canal system. Lastly, is the mesohyl, a proteinaceous matrix lying between

the pinacoderm and choanoderm, where the skeletal material is found with all other cell types.

Morphologically, Hexactinellida are considerably different from Demospongiae and Calcarea, with syncytial cellular organisation. Instead of pinacocytes, these sponges have a syncytial surface dermal membrane which is contiguous with an inner trabecular membrane that drapes through the sponge interior. The mesolamella in hexactinellids is equivalent to the mesohyl in other sponges (Mackie & Singula, 1983). The mesolamella is composed of collagenous sheets which form a suspensory network for attachment and support of trabecular tissues (Reiswig & Mackie, 1983). Hexactinellids do not possess a choanoderm as in the other two classes, but have a choanosyncytium composed of numerous collared bodies sharing a common nucleus and joined by stoloniferous cytoplasmic bridges. Hexactinellids also possess a unique secondary suspensory network that supports the collars of the collar bodies, which is not present in Demospongiae or Calcarea (Reiswig & Mackie, 1983). Because of these major differences Bergquist (1978) and Reiswig & Mackie (1983) proposed separate phylum and subphylum status, respectively, for the Hexactinellida.

Two major hypotheses have been developed explaining the evolutionary relationships between the sponge classes (Fig. 1). The first suggests that Demospongiae and Hexactinellida are more closely related to each other than to Calcarea, based on their respective similarities in the chemical composition of spicules (Möhn, 1984; Böger, 1988; Ax, 1996). Calcareous spicules are formed extracellularly by several sclerocytes (Ledger & Jones, 1977), whereas siliceous spicules of Demospongiae originate intracellularly within a single sclerocyte, and those of Hexactinellida are formed intrasyncytially by a 'scleroblast mass' containing many nuclei. The central axial filaments of spicules from Demospongiae and Hexactinellida differ in their cross sectional geometry (hexagonal and triangular vs. square, respectively), while calcareous spicules are devoid of a central filament. These differences in the axial filament could be indicative of differences in chemical composition (Reitner & Mehl, 1996). Most authors believe that spicules were derived independently in each of the three classes, and consequently are not useful as phylogenetic character at the class level except to show that spiculogenesis is not homologous in each class.

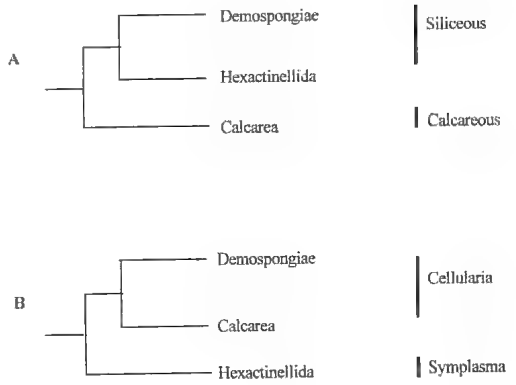

FIG. 1. Diagrammatic representation of the two major hypotheses of relationship between the three poriferan classes. A, Class Demospongiae and Class Hexactinellida are more closely related to each other than to the Calcarea based on chemical composition of the spicules (after Möhn, 1984; Böger, 1988; Ax, 1996). B, Class Calcarea and Class Demospongiae (Subphylum Cellularia) are more closely related to each other than to the Hexactinellida (Subphylum Symplasma) due to their differing cellular condition (after Reiswig & Mackie, 1983).

The second hypothesis proposes that Calcarea and Demospongiae are more closely related to each other than to Hexactinellida, emphasising major differences in cellular condition between these groups. It was proposed that the Subphyla Symplasma (representing hexactinellids) and Cellularia (demosponges and calcareans) be erected to distinguish between these groups (Reiswig & Mackie, 1983). Reitner & Mehl (1996) proposed the term Pinacophora, stating that it was more appropriate than the term Cellularia when comparing sponges with other metazoans. They identified three apomorphies for the group which separated it from Hexactinellida: 1) the presence of a pinacoderm, 2) ball-shaped choanocyte chambers in the adult, and 3) the ability to produce a calcareous ('hypercalcified') basal skeleton, as in the coralline sponges (Reitner & Mehl, 1996).

Hexactinellida first appeared in the fossil record during the Late Proterozoic, approximately 540my ago, whereas Calcarea and Demospongiae did not appear until some 50my later, in the Early Cambrian (Finks, 1970). It has since been proposed, however, that precursors to extant sponges were devoid of spicules and therefore would not have fossilised easily (Vacelet, 1985), which lends apparent support to the second hypothesis. Moreover, the known fossil history for

sponges has recently been challenged by Li et al. (1998), who claim to have found fossil demosponges 580my old. They suggest that demosponges were the first class to evolve, rather than hexactinellids (Li et al. 1998), supporting the first hypothesis.

As demonstrated by Van Soest (1987), sponge classification at the higher levels is problematic due to a lack of synapomorphic characters and numerous assumed homoplasies. Characters such as spicule composition and cellular construction may be valid and truly indicative of phylogeny, but it is difficult to determine which character should be given more weight, if at all.

Recent molecular studies have been very helpful in providing additional characters to assist with phylogenetic reconstructions. In an attempt to elucidate the phylogenetic history for Porifera, various gene sequences have been explored including the small subunit of the ribosomal 18S gene (18S rRNA), heat shock protein 70 (Hsp70) and Protein Kinase C.

Cavalier-Smith et al. (1996) analysed full-length 18S rRNA genes which yielded a paraphyletic Porifera. A Demospongiae- Hexactinellida clade formed a sister group to a Calcarea-Ctenophora clade. This suggests that Demospongiae and Hexactinellida are more closely related to each other than they are to Calcarea. This hypothesis contradicts the proposal for subphylum status for Hexactinellida, as suggested by Reiswig & Mackie (1983) and Reitner & Mehl (1996).

Analysis of the two protein coding genes, Hsp70 (Borchiellini et al., 1998) and Protein Kinase C (Kruse et al., 1998) produced a polyphyletic and paraphyletic Porifera, respectively. Koziol et al. (1997) also examined the Hsp70 gene, but considered it to be too conservative for resolution within the Porifera. Borchiellini et al. (1998), however, examined the Hsp70 gene using the first and second codon positions and found, with low bootstrap support, that Calcarea and Demospongiae formed a clade to the exclusion of Hexactinellida. Their results are controversial in that sponges were shown to be a group derived from other Metazoa. Cnidarians were hypothesised as being the first metazoans to diverge, followed by Ctenophora which formed a sister group to sponges. In some analyses, hexactinellids formed a clade with ctenophores, rather than with other sponge groups, while the 18S rRNA gene showed ctenophores to be more closely related to Calcarea (Cavalier-Smith et al., 1996). Analyses of the Hsp70 gene always resulted in a Demospongiae-Calcarea clade, which supports the concept of the Subphylum Cellularia.

Results from analysis of the Protein Kinase C gene were more similar to the 18S rRNA data, with Calcarea forming a clade with the lower metazoans. These data also showed that hexactinellids were the first to diverge from the metazoans, while demosponges formed a sister group to a calcarean-metazoan clade (Kruse et al, 1998). Hexactinellida did not form a clade with demosponges, but instead formed a sister group to all other metazoans. Unfortunately, analyses for each gene yielded differing topologies, each with low bootstrap support, leaving the alleged phylogenetic relationships of sponges unresolved. Even though these studies yielded conflicting phylogenetic patterns, it is still possible that Porifera may not be monophyletic, as traditionally believed.

To date, these are the only molecular studies which have included representatives from each of the three classes of sponges (although the only hexactinellid 18S rRNA sequence has not yet been made available in GenBank; West & Powers, 1993). The 18S rRNA gene is the most extensively studied gene, with the largest database available for comparison. Universal primers have also been developed, making this gene relatively easy to obtain sequences in a short period of time. Although it has been suggested that the 18S rRNA gene does not have enough signal to address the phylogenetic history of the lower metazoans, due to over saturation (Rodrigo et al., 1994), it has been demonstrated that increased taxon sampling can assist in resolving phylogenetic relationships, principally by spreading homoplasic signal among a greater diversity of internal branches (Hillis, 1996).

The aims of this study were to test the monophyly of Porifera by examining relationships between Demospongiae, Calcarea and Hexactinellida. In addition to those six sequences already available in GenBank, we generated six additional full-length 18S rRNA sequences, strengthening inference from all available data. Phylogenetic trees inferred from Distance Matrix (DM), Maximum Likelihood (ML) and Maximum Parsimony (MP) methods were compared with hypotheses generated from other genes, utilising Constraint Analysis via the Kishino-Hasegawa test (Kishino & Hasegawa, 1989).

TABLE 1. List of sponges used in this study with corresponding museum voucher number and collection locality (identified by MK).

Classification	Voucher number	Collection locality
Class Demospongiae		
Subclass Tetractinomorpha		
Vetulina stalactites Schmidt, 1879	BMNH 1998.3.19.1	Caribbean Sea
Acanthochaetetes wellsi Hartman & Goreau, 1975	BMNH 1995.11.2.2	Palau, Micronesia
Subclass Ceractinomorpha		
Clathria (Thalysias) reinwardti Vosmaer, 1880	MKB 142	Pohnpei, Micronesia
Negombata corticata (Carter, 1879)	BMNH 1998.3.19.2	Red Sea
Class Hexactinellida		
Subclass Hexasterophora		
Sympagella nux Schmidt, 1870	HBOM 003:00925	Turks & Caicos
Margaritella coeloptychioides Schmidt, 1880	HBOM 003:00929	Turks & Caicos
Class Calcarea		
Subclass Calcinea		
Leucetta sp.	HBOM 27:X:96:3:305	Bahamas

MATERIALS AND METHODS

SAMPLE COLLECTION AND SELECTION. Specimens were collected by SCUBA and manned-submersible from various localities between 1989-1996 (Table 1). Immediately upon collection a small piece of the sponge, approximately $3cm^3$, was removed from the interior with a clean scalpel blade to minimise surface epibiont contamination. This voucher was either frozen at -20°C or diced as finely with a sterile razor blade and immediately placed in Guanidium Chloride (GnCl) Buffer [6M GnCl, 5% Tween 20, 0.5% Triton X-100 in 1L Tris-EDTA buffer, pH 8.0 (100mM Tris, 30mM EDTA)] for stable storage of the lysed cells and DNA. A representative piece of the sponge was placed in 70% ethanol for subsequent taxonomic identification. Voucher specimens of all taxa were deposited at The Natural History Museum, London (BMNH), the Harbor Branch Oceanographic Institution Museum, Fort Pierce, Florida (HBOM) and in the personal collection (MKB) of MK.

EXPERIMENTAL PROCEDURES. Sponge cell buffered lysate was diluted four-fold with autoclaved analar H_2O, and frozen specimens were thawed and ground with a micropestle to form a slurry prior to DNA extraction, using standard phenol:chloroform extraction procedures (Sambrook et al., 1989). Full-length (approximately 1300bps) 18S rDNA was amplified with the forward and reverse primers 18Sf20 and 18Sr21, respectively (McInerney et al., in press). PCR conditions for 1.6ng/µl DNA in 50µl reactions were: initial denaturation at 94°C for 5mins, followed by 35 cycles of denaturation at 94°C for 1min, annealing at 55°C for 1min, and extension at 72°C for 1min. The product was electrophoresed on an 0.8% agarose gel stained with 1µg/µl ethidium bromide to check band size, and then purified from the gel with the Qiaex II PCR purification kit (Qiagen Ltd, UK), following manufacturers instructions. In addition to the two PCR primers, eight internal primers were used to sequence both chains automatically, utilising the dideoxy chain termination method (Sanger et al., 1977) (forward primers 377F CCGGAGARGGAGCCTGA, 577F GCCAGC MGCCGCGGT, 1262F GGTGGTCGATG GCCG and 1510F CAGGT CTGTGATGCC and their complementary reverse primers called 377R, 577R, 1262R and 1510R). Each contiguous sequence fragment was replicated with at least one overlapping fragment (Amersham Cycle Sequencing Kit). Ambiguous nucleotide positions were coded according to the International Union of Pure and Applied Chemistry (IUPAC) nomenclature. Sequences were managed utilising Sequencher 3.0 software (Gene Codes Corporation, 1995). New sequences were deposited in the GenBank sequence repository (http://www2.ncbi.nlm.nih.gov) under accession numbers *Vetulina stalactites* (AF084236), *Acanthochaetetes wellsi* (AF084237), *Clathria (Thalysias) reinwardti* (AF084238), *Negombata corticata* (AF084239), *Sympagella nux* (AJ224123), *Margaritella coeloptychioides* (AJ224124) and *Leucetta* sp. (AF084240).

PHYLOGENETIC RECONSTRUCTION. To test the monophyly of Porifera, sequences from the lower metazoans were included in the analysis: Phylum Porifera - *Tetilla japonica* (D15067), *Microciona prolifera* (L10825) (which Hooper (1996) referred to the subgenus *Clathria (Clathria)* based on taxonomic re-evaluation of type material, and we assume that GenBank L10825 belongs to this species), *Axinella polypoides* (U43190), *Clathrina cerebrum* (U42452), *Scypha ciliata* (L10827) (which belongs to *Sycon*, follwing Dendy & Row, 1913; Gert Woerheide, pers.comm.), *Sycon calcaravis* (D15066); Phylum Placozoa -

Trichoplax adhaerens (L10828); Phylum Ctenophora - *Beroe cucumis* (D15068), *Mnemiopsis leidyi* (L10826); Phylum Cnidaria - *Anemonia sulcata* (X53498), *Tripedalia cystophora* (L10829). Representatives of each of the major fungal groups were chosen as the outgroup taxon, due to their inferred position as a sister group to Metazoa, according to rRNA data (Wainright et al., 1993) and protein data (Baldauf & Palmer, 1993): Fungi - *Aureobasidium pullulans* (M55639), *Saccharomyces cerevisiae* (Z75578), *Athelia bombacina* (M55638), *Blastocladiella emersonii* (X54264). Taxa selected for this study were retrieved from a secondary structure alignment maintained on the Ribosomal Database Project (RDP) database (http://rdpwww.life.uiuc.edu/ index2.html; Maidak et al., 1996). The profile alignment option of ClustalW was then used to combine the two alignments (Higgins & Sharp, 1988). Sequences were aligned using ClustalW 1.7 and then modified by eye in the Genetic Data Environment, GDE, (Smith et al., 1994) on a SUN workstation. A conservative approach was used for alignment. Only those positions whose alignment was ambiguous were chosen for analysis, thus ruling out a significant number of potential positions. Approximately 1300bps were sequenced. The new alignment (including other sponges, metazoans and fungi), was 2590bps in length, and after removal of ambigous positions the resulting alignment was 987bps long.

Phylogenetic hypotheses were constructed, and sequence statistics were evaluated, using PAUP* 4.0.0d64 test version (Swofford, in press). The likelihood ratio test statistic was used to evaluate which evolution model fit the data best (Goldman, 1993). This was achieved by first constructing a neighbour joining tree calculated by the Jukes & Cantor (1969) method. Using this guide tree and maximum likelihood criteria, the transition-transversion ratio, base composition and proportion of invariable sites were estimated using the Newton-Raphson method implemented in Paup*4.0. Each of these variables were calculated separately, and then entered into the model to calculate the next variable. This process was repeated until the best maximum likelihood value was reached. The chosen evolutionary model was that which yielded the optimal likelihood value without compromising model complexity. The reliability of internal branches was evaluated by the bootstrap resampling method (Felsenstein, 1985). In each analysis, 100 iterations were carried out for each optimality criterion. A 50% majority rule consensus tree was inferred from the resulting bootstrap partition table.

The inferred phylogenetic relationships derived from the new 18S rRNA sequences were compared with the major phylogenetic hypotheses derived from morphological characters and other genes. Using MacClade (Maddison & Maddison, 1992), we constructed trees representing competing hypotheses. The constrained trees were examined using the Kishino-Hasegawa test (Kishino & Hasegawa, 1989), for both ML and MP methods.

ABBREVIATIONS. DM, Distance Matrix; ML, Maximum Likelihood; MP, Maximum Parsimony methods.

RESULTS

Prior to bootstrapping, MP, DM and ML methods yielded trees with virtually identical topologies, except for the branch arrangement within Demospongiae which was largely unresolved. For the DM method, the F84 model was used (Felsenstein, 1984), with the minimum evolution objective function. The proportion of sites assumed to be invariable = 0.686332. For the MP method, (parsimony informative sites = 155), the characters were treated as unordered and equally weighted. The ML analysis was conducted with a Two-type substitution model with an estimated transition/transversion ratio of 1.553481, estimated base frequencies of A = 0.270333, C = 0.206219, G = 0.258425 and T = 0.265023, and an estimated proportion of invariable sites = 0.686760 (with equal rates of variation for all sites). Using the MP method as representative, two equally parsimonious trees with minimal differences in tree topology were recovered (CI = 0.669, RI = 0.723, tree length = 465, total number of characters = 987, parsimony informative characters = 155) (Fig. 2). The only difference in branch arrangement between the two MP trees was the position of *Acanthochaetetes* within the Demospongiae. In Tree A (Fig. 2), *Acanthochaetetes* is the earliest separation within Tetractinomorpha, whereas in Tree B (Fig. 2), *Acanthochaetetes* is the earliest separation of the Ceractinomorpha clade. The three sponge classes are monophyletic in both trees, with Demospongiae as sister taxon to Hexactinellida, while Calcarea formed a sister taxon to Ctenophora.

Within Demospongiae, Subclass Tetractinomorpha is represented by *Vetulina* and *Tetilla*,

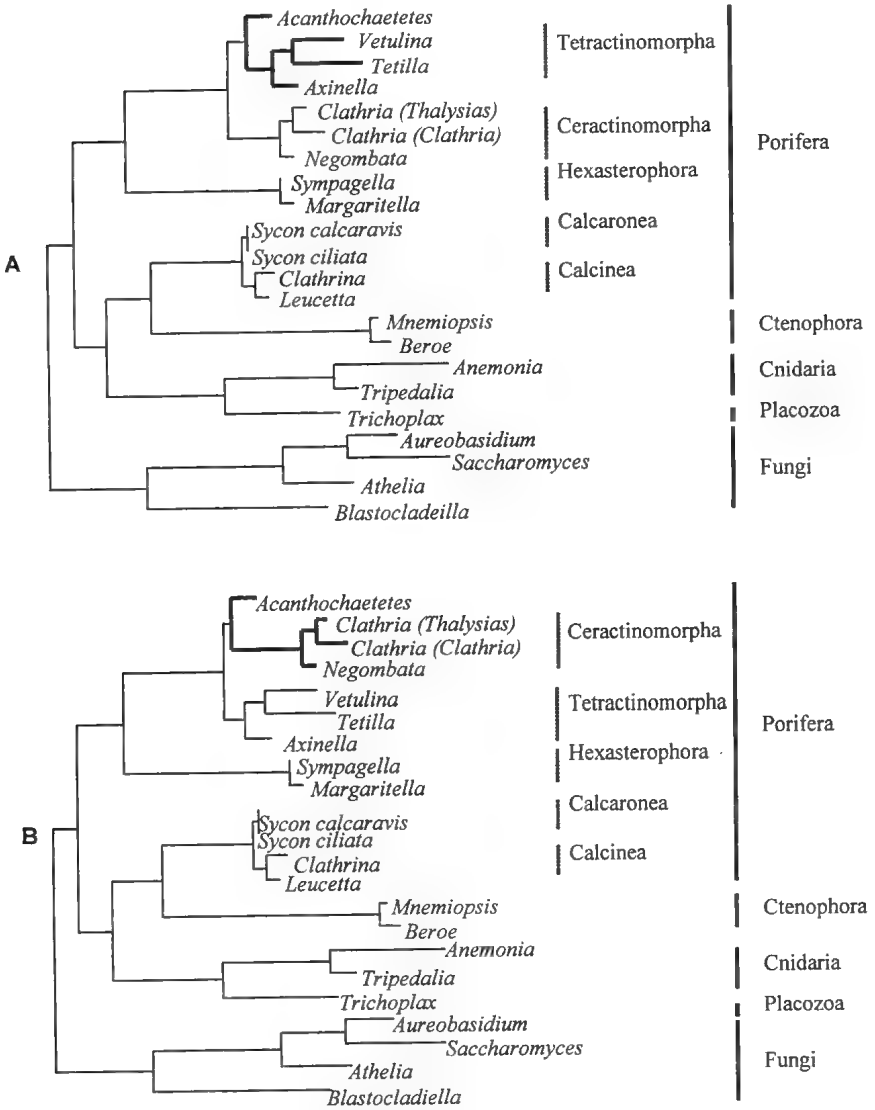

FIG. 2. Two most parsimonious trees derived from molecular data analysed using the assumptions of MP (CI = 0.669 , RI = 0.723, tree length = 465, total number of characters = 987, parsimony informative characters = 155). A, *Acanthochaetetes* is the earliest taxon to diverge from the Tetractinomorpha. B, *Acanthochaetetes* is the earliest taxon to diverge from the Ceractinomorpha.

which form a common clade. *Axinella* was also located within the tetractinomorphan clade. The family Axinellidae was formerly considered to be a tetractinomorph on the basis of their reproductive strategies, but Van Soest et al. (1990) suggested that, based on morphology, there was more support for their placement among Ceractinomorpha. Our dataset, however, places Axinellidae (represented by *Axinella*), in Tetractinomorpha. *Acanthochaetetes* is also traditionally recognised as a tetractinomorph sponge, but this position is unstable, grouping with tetractinomorphan Tree A, and with Ceractinomorpha in Tree B. Ceractinomorpha were represented by two species of *Clathria,* and *Negombata*, where the latter taxon has had a questionable affinity with switches between Tetractinomorpha (Bergquist, 1978) and Ceractinomorpha (Topsent, 1922). Kelly-Borges & Vacelet (1995) suggested that based on morphology, chemistry and reproduction, *Negombata* has a closer affinity to Ceractinomorpha, Order

TABLE 2. Results of the K-H test for alternatives to the MP/ML tree. Tree number 1 is the best tree in both analyses. Tree 2 is a constrained tree that retains a clade containing all of the sponge taxa. Tree 3 is a constrained tree that places Calcarea with Demosponges to the exclusion of all other taxa. Tree 4 places Calcarea with Hexactinellida. The second column gives the ML score (negative log-likelihood) for each tree. The third column gives the difference in Log-likelihood between alternatives. The fourth column indicated whether or not an alternative is significantly worse than the optimal tree ($P<0.05$). The fifth column gives the tree length of the various trees. Column number six gives the difference in tree length between alternative trees. The last column indicates the significance of the result (an asterisk indicates a P-value < 0.05).

Tree	Likelihood			Parsimony		
	-Ln L	Diff -ln L	P*	Length	Diff.	P*
1	3960.28210	(best)		466	(best)	
2	3964.75361	4.47151	0.4971	469	3	0.3176
3	4128.96432	168.68222	<0.0001*	514	48	<0.0001*
4	4122.38473	162.10263	<0.0001*	510	44	<0.0001*

Poecilosclerida. Our data lend support to this hypothesis.

There were only two representative taxa for Hexactinellida, each belonging to the same subclass, Hexasterophora, forming a monophyletic clade in all three analyses. Hexactinellida consistently form a sister group with Demospongiae.

The topology of trees obtained from the three methods of analysis varied only slightly from that of the DM tree, recovering a topology which suggests that the calcareous Subclass Calcaronea arose from within the Subclass Calcinea (not shown). Both MP and ML methods yielded a topology with the two calcareous subclasses positioned as sister taxa. Our data show the Subclasses Calcaronea and Calcinea to be valid groupings, but at least three taxa per group are needed to infer relationships. Calcarea form a sister group relationship with Ctenophora. This clade is hypothesised to be derived from the diploblastic animals (Cnidaria and Placozoa).

A total of 100 bootstrap replicates were constructed for each of the three methods of analysis; DM, MP and ML (Fig. 3). Each of the three classes is consistently recovered, with high bootstrap support as a monophyletic class. Hexactinellida formed a clade with 100% bootstrap support for each method, and Demospongiae formed a monophyletic group with bootstrap values of 100, 99 and 88 for DM, MP and ML methods, respectively. There were no representatives of the Subclass Homoscleromorpha in these analyses due to difficulties we encountered during PCR. The topology of trees relating most Demospongiae taxa received less than 50% bootstrap support. The *Negombata* - *Clathria* clade was the only relationship that remained intact and formed a monophyletic group, with bootstrap values of 98 for DM, 100 for MP and 99 for ML, yielding further support to the hypothesis that *Negombata* is more closely related to Poecilosclerida than to the tetractinomorph Hadromerida.

Support for a Demospongiae-Hexactinellida clade was relatively high (DM 89%, MP 81% and ML 70%), while Calcarea formed a monophyletic group with bootstrap support of 100 for the DM and MP trees and 98 for the ML tree. Calcarea consistently formed a sister taxon with Ctenophora, although there was only low bootstrap support of 64, 55 and 56, for DM, MP and ML methods, respectively.

In order to test alternative hypotheses on possible relationships between the sponge classes, the Kishino-Hasegawa (K-H) test was employed for both the MP and ML methods. Constraint trees were designed and compared against the optimal tree inferred from our data, which showed Calcarea was more closely related to Ctenophora, and Demospongiae and Hexactinellida formed a sister-group. The optimal tree for each constraint analysis was found using the MP and ML methods, and then the resulting tree was compared with the optimal, unconstrained tree to test for significant differences (Table 2).

For the K-H test using ML and MP methods, three constrained trees were designed and tested against the optimal tree derived from the molecular data (Tree 1): a monophyletic clade containing all three classes (Tree 2); a clade containing the Hexactinellida as a sister taxon to a Demospongiae-Calcarea clade (Tree 3); and a clade containing the Demospongiae as a sister taxon to a Hexactinellida-Calcarea clade (Tree 4). All alternative trees were rejected when compared to the optimal tree, with the exception of a monophyletic Porifera (Tree 2).

The optimal tree from ML analysis has a log-likelihood value of -3960.28210, while the best tree which retained a monophyletic sponge clade had a log-likelihood score of -3964.75361. A two-tailed T-test did not reject this hypothesis as

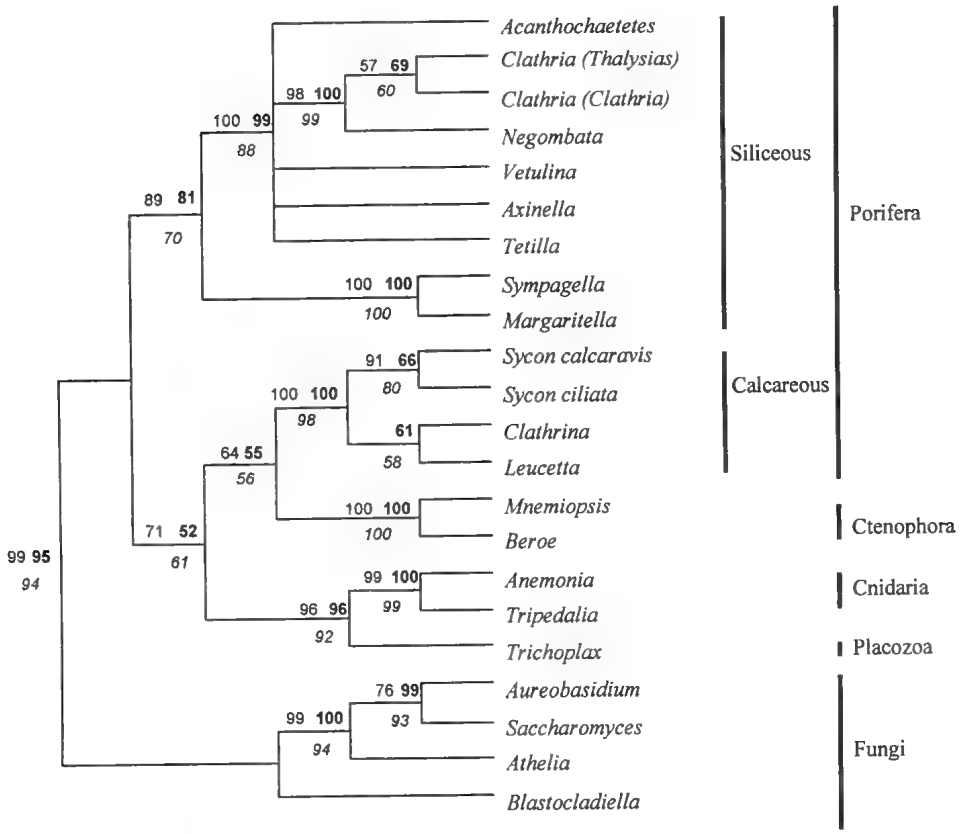

FIG. 3. Phylogenetic consensus tree for DM, MP and ML with bootstrap values corresponding to each method. 50% majority rule consensus trees are displayed. DM values are presented in normal text, MP values are in bold and ML bootstrap values are in italics.

being significantly worse than the ML tree. Similarly, using the MP analysis method, the most parsimonious tree was only three steps shorter than a tree which contained a monophyletic sponge clade, and the Log-likelihood of a monophyletic tree was only 0.1% less likely than the optimal tree. In both instances, the alternative hypothesis was not significantly worse than the optimal tree ($P<0.05$). Given that we cannot reject the alternative hypothesis, it would be unwise to suggest that the phylum is not monophyletic.

DISCUSSION

Bootstrap results indicate a polyphyletic arrangement for Phylum Porifera, and support the theory that siliceous sponges evolved separately from calcareous sponges. This arrangement has previously been suggested in the literature, supported by data from 18S rRNA (Kobayashi et al., 1993; Cavalier-Smith et al., 1996), 28S rRNA (Lafay et al., 1992) and Protein Kinase C genes (Kruse et al., 1996). In these earlier studies, the sponge classes and subclasses were not extensively represented in analyses, whereas our data doubles the number of sponge 18S rRNA sequences analysed, and yet yields the same topology. Our results also show that calcareous sponges are derived from other metazoans, which are generally considered to have evolved later than the siliceous sponges, based on morphological data and the fossil record.

The poriferan classes have several apparent apomorphies, but upon closer examination these may be convergent characters. Differences in spicule geometry and spiculogenesis are obvious between calcareous and siliceous sponges, but possibly also between the Hexactinellida and Demospongiae. There are notable differences in choanocyte size, shape and arrangement within choanocyte chambers at the class and order

levels. While species of Demospongiae and Calcarea share similar cellular construction and mesohyl characteristics, when compared to Hexactinellida, Calcarea lack the collagen and proteinaceous fibre development found in Demospongiae. Larval morphology is also very different within and between the three classes. It is possible that convergent evolution has taken place between these three classes due to a common benthic, filter-feeding lifestyle.

It is also quite difficult to explain why Calcarea are consistently grouped with the Ctenophora according to molecular data, even with low bootstrap support. Morphologically, Ctenophora are much more complex than Porifera, with true tissue structure such as mesodermal muscle, gonoducts, and an anal pore; however increased complexity does not necessarily equate with a more derived evolutionary state. It is conceivable that Calcarea have secondarily lost characters that are present in ctenophores. Cavalier-Smith et al. (1996) suggest that calcareous spicules found in Calcarea may be homologous to those found in Cnidaria. A loss of spicules could have occurred in Ctenophora. They also suggest that larvae found in the subclass Calcinea (Calcarea) are morphologically much more similar to those of other animals than they are to other sponges. Additionally, the position of ctenophores with respect to other diploblasts, based on molecular data, rests on analysis of only two representatives of the phylum.

Although we failed to confirm whether the Porifera was a monophyletic taxon using our expanded molecular dataset, the hypothesis of poriferan monophyly is not rejected based on molecular data. The exact placement of calcareous sponges is problematic and requires further empirical support from sequences derived from a greater diversity of sponges and ctenophorans. Surprisingly, when ctenophores were removed from analyses (data not shown), Calcarea remained a sister taxon to other diploblasts, and not with siliceous sponges (Demospongiae, Hexactinellida). Although these results are congruent with previous data, they are difficult to explain on the basis of 'classical' morphological characters and the paleontological record.

Using the bootstrap resampling method, we have shown that a partition separating the Classes Hexactinellida and Demospongiae from all other taxa is reasonably well supported. Although bootstraps are not statistically high, any alternative arrangement receives very little support. This arrangement does, however, refute the hypothesis that Hexactinellida merit phylum or subphylum status. The conclusions of this study do not rule out the monophyletic nature of the phylum. Until data are gathered that can yield high confidence levels for a polyphyletic phylum, the possibility of sponges being monophyletic cannot be dismissed.

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APPROACH TO THE PHYLOGENY OF AXINELLIDAE (PORIFERA: DEMOSPONGIAE) USING MORPHOLOGICAL AND MOLECULAR DATA. *Memoirs of the Queensland Museum 44: 43. 1999:-* A set of 27 species of marine sponges of the Axinellidae and related families was selected with the aim of testing the monophyly of Axinellidae and investigating their phylogenetic relationships using cladistic methods. Partial 28S rDNA sequences, including the D3 domain, and traditional morphological characters were used independently to construct phylogenetic trees. Alignment of the sequences using the appropriate model of secondary structure of the RNA was compared to that produced by the ClustalW. The alignment using secondary structure constraints produced a better estimate of the phylogeny and was demonstrated to be an effective and objective method.

The results from the analyses of the molecular and morphological data sets are not fully congruent; the morphological data suggest that Axinellidae is monophyletic, however the molecular data suggest that it is not monophyletic. In both cases the sampled members of the family are closely related to those of Halichondriidae and Dictyonellidae. Tests of heterogeneity (reciprocal T-PTP and partition homogeneity test) shown that the data partitions are heterogeneous, which could be due to sampling errors (in either data set) or differences in the underlying phylogenies, and therefore data were not combined in a single analysis. ❐ *Porifera, Axinellidae, secondary structure, D3 domain 28S ribosomal DNA, phylogeny.*

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PECULIARITIES OF FERTILIZATION PROCESS IN THE SPONGE *LEUCOSOLENIA COMPLICATA* MONTAGU (CALCISPONGIAE: CALCARONEA) FROM THE BARENTS SEA. *Memoirs of the Queensland Museum 44: 44. 1999:-* The fertilization process in the Barents Sea sponge *Leucosolenia complicata* Mont. (Calcispongiae: Calcaronea) was studied on the ultrastructural level and light microscopy level with histochemical methods being applied (tests for the total content of proteins, lipids, mucopolysaccharides, nucleic acids were carried out). As with all other Calcispongiae (Hexactinellida), fertilisation is conducted with the special carrier cell. At present, carrier-cell fertilisation is found in a series of species, mainly in sycon- and leucon-structured Calcaronea. *L. complicata* has an anatomical organisation of the ascon type and specific fertilisation processes in *L. complicata* might be due to its sperm's unique organisation.

The mature spermatozoon has neither acrosome nor flagellum, but is just a spherical cell 4.8μm in diameter, occupied mainly with the nucleus (d=4.6nm). In *L. complicata* spermiogenesis takes place in choanocytes where the protein capsule around the sperm nucleus is synthesised. During the massive sperm release, any cell from the nurse cells complex, can seize a sperm and transform into a sperm-carrying cell *in situ*. The transformation of a seized sperm into the spermiocyst is accompanied with rapid isolation of the sperm's nucleus from its protein capsule. These processes are correlated with protein-dying tests and might be a result of either protein accumulation or structural transformation of the proteins that comprise the capsule and nuclear chromatin. The spermiocyst formation goes along with the hypertrophic changes of the carrier cell, i.e. its diameter increases from 8.8-19.4μm and the nurse-cells increase in their size (from 8.8-11μm diameter), evident from transmission electron micrographs.

The fertilisation process begins with the protein capsule penetrating the oocyte and gradually resolving in its ooplasm. The extra swelling of the sperm nucleus within the carrier cell coincides with this process. The sperm nucleus penetrates into the oocyte's cytoplasm and the maturation divisions in the egg proceed. They take place in the egg's animal part, turned towards the choanoderm. Both meiotic divisions from metaphase1 to telophase2 follow. The chromosomes' arrangement within the metaphasal plates during both maturation divisions appear to be most characteristic of the *L. complicata* oocytes' meiosis: the chromosomes arrange themselves annulus-like (ring-like). During either the first or the second maturation divisions the annulus-like metaphasal plates turn 90° around and only then the chromosomes begin to move towards the maturation spindle poles. The polar bodies are separated under the choanoderm. The completion of the oocyte's maturation divisions coincide with the beginning of the sperm-nucleus's transformation into the male pronucleus. Therefore the processes of male and female pronuclei formation is concurrent.

The sperm nucleus begins transforming into the male pronucleus with its own nuclear membrane destruction, male chromatin swells and loosens, and the building of the pronucleus membrane follows. After the maturation divisions are completed, the chromosomes are condensed into a tight spherical chromatin mass, which then gradually loosens and is transformed into a chromatin net. The female-pronucleus membrane is then formed. Definitive pronuclei are similar in their size and show up as large (d=22nm) bubble-like nuclei filled with finely-granulated chromatin net. In *L. complicata,* pronuclei do not fuse together. As a rule, two groups of chromosomes are formed in the zygote, they unite into one and arrange into a metaphasal plate of the first cleavage division. ❐ *Porifera, Calcispongiae, fertilisation, spermatozoon, carrier cell, spermiocyst, oocyte, meiosis, zygote.*

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THE RELATIONSHIP OF SILICATE LEVELS TO THE SHALLOW WATER DISTRIBUTION OF HEXACTINELLIDS IN BRITISH COLUMBIA. *Memoirs of the Queensland Museum 44: 44. 1999:-* The boot sponge, *Rhabdocalyptus dawsoni*, occurs at depths as shallow as 10m in the Strait of Georgia, British Columbia. The cloud sponge, *Aphrocallistes vastus*, typically occurs in slightly deeper water but has been found as shallow as 5m in Johnstone Strait, British Columbia. These species also form bioherms several meters thick in some localities. Initial surveys of the literature indicate that shallow water silicate levels in regions of British Columbia are high compared to levels in other shallow marine waters. Areas of the Antarctic are an exception and hexactinellids also occur here in shallow water. Hexactinellids are absent in habitats which might be expected to support populations, such as Norwegian fjords, but where silicate levels are low. The recently described shallow water occurrence of hexactinellids in the Mediterranean may be an exception. Additional input from anecdotal or unpublished data from the community of sponge researchers may help support or refute a relationship between shallow water occurrence of hexactinellid populations and high levels of silicates. ❐ *Porifera, hexactinellid, silicates, British Columbia, shallow water.*

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SPONGE DISTRIBUTION AND CORAL REEF COMMUNITY STRUCTURE OFF MACTAN ISLAND, CEBU, PHILIPPINES

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Coral reef community structure was studied during 1994-95 at Mactan Island, off Cebu City, Cebu, Philippines. Three transect lines perpendicular to the shore were surveyed from depths of 7-32m. Transect slack line distances were 55-68m long. Live hard coral represented 29-42% (mean=36%) of categories intercepted and sponges 1-5% (mean=3%), representing the two most abundant groups of benthic organisms. All remaining benthic taxa together comprised only an average of 1% of the intercept distance. The number of sponges intercepted along each line ranged from 4-19 (mean=12). Approximate sponge densities from line intercept data ranged from 1/20m^2 to 1/250m^2 (mean=1/40m^2) and were typically large specimens. Sponge densities off Mactan Island were considerably lower and species richness much higher than that of the Caribbean. A transition frequency matrix was calculated for all line intercepts and a test for a Markov chain was conducted. The most frequently encountered sequence was coral rubble followed by live hard coral (11% sequence frequency). Live hard coral followed by sponges was 5% and sponges followed by live hard coral was 5%. In a similar study of the benthos with five line intercepts parallel to the shore at depths of 7-12 m, the most frequently encountered sequence was live hard coral to sponge and sponge to live hard coral (36%). Sponge to sponge transitions represented 4%. None of the sequences were significant at P=0.05; i.e. the succession of substratum types were independent of each other, supporting the null hypothesis. ❐ *Porifera, Philippines, coral reef, community ecology, benthic, densities, Markov Chains, transects.*

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Field studies on sponges in the Philippines by our group have been ongoing since 1994. Several species of sponges were found to be toxic to fishes and hard corals and research on the effects of three species of allomone-secreting subtidal sponges on adjacent corals are continuuing (Nishiyama, 1999, this volume). Sponges and other marine taxa of the Philippines are poorly known. Gomez (1980) lists 16 publications on marine sponges for the Philippines and few have appeared since that time (e.g., Raymundo & Harper, 1995). No quantitative studies on sponges have been carried out in the Philippines, so far as is known. Therefore, the major objectives of this study were: 1) to determine sponge distribution and abundance and compare it with similar data from other tropical regions, and 2) to characterise coral reef community structure as a basic framework for our continuing research on toxic sponges.

MATERIALS AND METHODS

Coral reef community structure and sponge distributions were studied between 1994-1996 off the Tambuli Resort, at Mactan Island (an 18km × 6km, 7750ha, flat, low limestone reef), off Cebu City, Cebu, Philippines (Fig. 1) (10°17'N, 124°0'E). This site is located approximately 400m N of the University of San Carlos Maribago Marine Station. The Hilutangan Channel between Mactan and Olango Islands is a 300m deep trench (von Bodungen et al., 1985). A gently sloping reef extends to a depth of 10m on each side of the channel then typically, abruptly plunges steeply downward. The slope off the Tambuli resort is considerably less steep. Oceanographic characteristics of channel waters are found in von Bodungen et al. (1985), Anon. (1991a,b) and Ilano & Dacles (1994).

Three line intercepts perpendicular to the shoreline were surveyed 10m apart from 7-32m depth with slack line distances of 55-68m. Five additional line intercepts were surveyed parallel to the shore at depths of 7, 8, 10, 11 and 12m, with slack line distances of 100m, excepting the 12m line, which was 70m in slack length (slack length partially follows the reef contour and the tape is

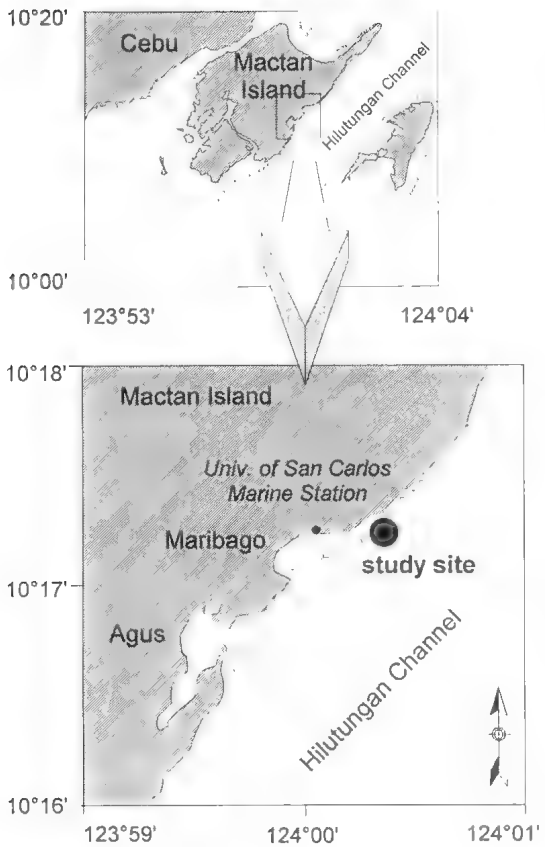

FIG. 1. Mactan Island, Cebu, Philippines, showing study site.

easy to deploy for measurements; see Reichelt et al., 1986, for methodology on reef transects).

Organisms and substrata encountered along transects were recorded as line intercept distances. Most organisms were individuals or single colonies rather than conspecific patches. Toxic sponges reported by Nishyamand Bakus (1999, this volume) were most abundant at depths of 7-12m. The number of transitions between sequences of organisms and substrata was tallied. The null hypothesis was that the sequence of occurrences of benthic organisms and substratum types along transect lines (depth gradients) are random. The results were tested for a Markov chain (i.e. tendency of one thing to be followed by another, as in repeated textile patterns) by the non-parametric chi-square statistic. Markov chain analyses of sequences are commonly used in stratigraphy (Davis, 1986) and in the prediction of plant succession after fires (Isagi & Nakagoshi, 1990). The present study is the second use of Markov chain analysis in coral reef community structure (after Licuanan & Bakus, 1992), so far as is known. There do not appear to be null models developed for Markov-type chain analyses dealing with sequences of substratum types where the species are unidentified (Gotelli & Graves, 1996).

Approximate sponge densities were calculated using a modified Strong's equation (Cox, 1996), as follows:

$$D = (\Sigma\ 1/M)(A/T)$$

where D=density of sponges (no./m^2); M= maximum animal or plant orthogonal width (m) of an organism intercepting the transect line; A= unit area (1m^2); T= total transect length (m).

RESULTS

Line intercept or line transect surveys are recognised as one of the best methods of studying coral reef organisms (e.g. Loya, 1978; Marsh et al., 1984; Reichelt et al., 1986). We compared different sampling methods in the field in Kenya and found that line intercepts gave us density estimates closest to those of actual counts. For example, the densities (no./m^2) of *Padina* (P) on a reef near Mombasa in 1997 and *Ipomoea* (I) leaves on a flat berm at Malindi in 1998 were as follows: actual count (P-10, I-14), point-center quarter (P-2, I-32), stratified random sampling (P-20, I-19) and line intercept (P-16, I-14). The major advantage of using intercept width measurements for density estimates is that the technique is about an order of magnitude more rapid than are direct counts or quadrat studies of organisms underwater. One advantage of using transects both perpendicular and parallel to the shore is that allelochemical effects can be directional (i.e. directional currents) and using only one transect orientation might overlook transitions resulting from such chemicals.

TRANSECTS PERPENDICULAR TO SHORELINE. Live hard coral represented 29-42% (mean=36%) of categories intercepted and sponges 1-5% (mean=3%), representing the two most abundant groups of benthic organisms (Table 1). The standard errors (SE) of the percentage intercept of common categories were relatively small whereas those of the least abundant categories were considerable, due mostly to small numbers. Obviously, increased sample size would improve the results. All remaining large benthic taxa together comprised only an average of 1% of the intercept distance. The number of sponges along each intercept ranged from 4-19 (mean=12). Sponge

TABLE 1. Mactan Island transects, perpendicular to shoreline (7-32m depth regime), showing percentage of total intercept distance ([1]=seagrass was avoided after the first transect).

Category	Line 1	Line 2	Line 3	Mean % ± SE
Live Coral	35	42	29	36 ± 4
Sand	19	24	32	25 ± 4
Coral Rubble	25	22	22	23 ± 1
Dead Coral	7	6	15	9 ± 3
Seagrass[1]	9	-	-	3 ± 3
Sponges	3	5	1	3 ± 1
Other Organisms	2	1	1	1 ± 0.03
Total %	100	100	100	100
Slack Line Distance (m)	55	62	68	62
No. Sponges Measured	14	19	4	12
Total Sponge Width (cm)	111	323	33	156
Sponge Density (No./m^2)	0.02	0.05	0.004	0.025
Sponge Density Converted (No./m^2)	1/50	1/20	1/250	1/40

approximate density from line intercept data ranged from 1/20m^2 to 1/250m^2 (mean=1/40m^2) and were typically large specimens (Table 1). The most frequently encountered sequence was coral rubble followed by live hard coral (11% sequence frequency) (Table 2A). Live hard coral followed by sponges was 5% and sponges followed by live hard coral was 5%. None of the sequences were significant at P=0.05 (calculated χ^2=0.19; DF=24); i.e. the succession of organisms and substrata were independent of each other. This supports the null hypothesis.

TRANSECTS PARALLEL TO SHORELINE. The sponge percentage of the total transitions increases rapidly from 7-10m depth, then changes rapidly again between 11-12m depth (Table 2B). The most frequently encountered sequence was live hard coral to sponge and sponge to live hard coral (total combined=36%) (Table 2C). Sponge to sponge transitions represented 4%. Sponges were most frequently associated with live coral, dead coral and coral rubble. Again, the sequence of organisms and substrata were independent of each other at P=0.05 (calculated χ^2=12.9; DF=25). This supports the null hypothesis.

DISCUSSION

Beyond the shallow water seagrass community (0-8m depth; principally *Thalassia hemprichii*), sponges represented the second most dominant invertebrate taxon (after hard corals), comprising 1-5% (mean=3%) of intercept distances (depth 7-32m) and 5-28% of transitions. The transition percentage between sponges and live hard corals was 10% for depths of 7-32m and 36% for depths of 7-12m. The dominance of sponges (after hard corals) is typical of the Caribbean but sponge abundance can be considerably greater in the Caribbean than in the Philippines (Table 3). For example, Zea (1994) found that sponges of Santa Marta, Colombia, were an average of about four times as abundant as the sponges of Mactan Island. The species richness and irregular distribution of individual sponges at Mactan Island, however, were considerably greater than at Santa Marta. Species richness can be high in the Caribbean. Alcolado (1979) reported 47 species of sponges at depths of 11-16m in Havana, Cuba. This rough inverse relationship between species richness and abundance was expected (Krebs, 1994).

As in the Caribbean, the sponges of Mactan Island represent the second most important taxon of benthic invertebrates after hard corals. Although there is no universally recognised system of classifying coral reefs based on percentage cover, one frequently used rule of thumb would consider that a 36% coverage (the Mactan Island study site) represents a moderately rich coral reef.

The reefs at Mactan Island are probably not as rich as they were 20 years ago because of dense development of coastal resorts and concomitant anthropogenic effects (principally sewage effluents). The 1997-98 El Niño may have caused the death of many hard corals and the disappearance of numerous toxic sponges in our study area (Nishiyama, unpublished data).

TABLE 2. Mactan Island transects. A, transects perpendicular to shoreline, running from onshore to offshore, showing transition frequencies ([1]=unbroken dead coral substratum). B, transects parallel to shoreline, showing sponge transitions by depth ([2] =100m slack length except for 12m depth transect which was 70m long). C, transects parallel to shoreline, showing sponge transition frequencies.

A. Category	% Total transition frequencies
Coral Rubble to Live Coral	11
Live Coral to Coral Rubble	9
Live Coral to Sand	8
Sand to Live Coral	8
Live Coral to Live Coral	8
Dead Coral[1] to Live Coral	6
Live Coral to Sponges	5
Sponges to Live Coral	5
Live Coral to Dead Coral	5
Others (numerous transition categories)	35
Total	100

B. Depth (m)	Total no. of transitions[2]	Sponge % of total transitions	No. sponges	Mean (± SE)
7	256	5	6	
8	371	11	16	
10	426	15	32	27±7
11	396	16	31	
12	365	28	48	

C. Category	Percentage of total transitions
Live Coral to Sponge	18
Sponge to Live Coral	18
Sponge to Dead Coral	12
Coral Rubble to Sponge	10
Sand to Sponge	8
Sponge to Sand	8
Dead Coral to Sponge	6
Sponge to Coral Rubble	6
Sponge to Algae	5
Sponge to Sponge	4
Algae to Sponge	3
Others	2
Total	100

The somewhat abrupt change in sponge percentage of total transitions (Table 2B) between depths of 7-10m was the result of the disappearance of seagrass (i.e. it is the edge of the shallow water seagrass community) with a concomitant increase in sponges. The marked increase in sponge transitions at a depth of 10-12m was similar to that recorded by Alcolado (1979) for sponges at depths of 11-16m off Havana, Cuba (Table 3). Presumably this depth is optimal because it provides sufficient light for photosynthesising symbionts and is below the depth of major destruction by hurricanes and typhoons.

None of the transitional sequence patterns of organisms or substrata at Mactan Island were statistically significant, thus the null hypothesis is supported. This was because species richness was very high and individual distributions highly irregular. Tropical island forests show the same patterns (e.g. Fiji Islands, Bakus, pers. obs.). Significant sequential patterns of the benthos would be expected to occur only at larger scales (e.g. community scale changes such as transitions from coral to seagrass) (Isagi & Nakagoshi, 1990).

Bradbury & Young (1983) concluded that hard coral distributions on the Great Barrier Reef were random. This was based on the fact that only 5 species pairs out of 545 species pairs were not random. They concluded that coral interactions on a small scale do not produce random neighbours. They also concluded that random neighbours express the combined workings of many effects but did not specify what those factors were. Licuanan & Bakus (1992) found random distributions of Philippine reef organisms at a depth of 12m at Puerto Galera but a significant non-random unidirectional pattern at 6m depth, possibly the result of strong longshore currents. The possible reasons for the random distributions of benthic organisms are complex and include high species richness (Bakus & Ormsby, 1994), fish predation and grazing on benthic organisms (Bakus, 1964, 1967, 1969), predation on planktonic stages of benthic species (Gili & Coma, 1998), perhaps allelochemical defenses (Bakus et al., 1986, 1989/90) and other factors such as currents (Licuanan & Bakus, 1992). A discussion of these topics is beyond the scope of the present paper.

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TABLE 3. Comparisons of sponge densities (as percentage of intercept distance, ID) between Indo-Pacific and Caribbean localities (?=densities could not be determined from the published data).

Location	Depth (m)	Method	Density (range)	Density (mean)	Source of data
Indo-Pacific					
Cebu, Philippines	7-32	Line intercept	1-5% ID 1/50-1/250 m^2	3% ID 1/40 m^2	this study
Flinders Reef flats, Coral Sea, Australia	1-4	Quadrats	7.3% ID	?	Daniel et al. in Wilkinson (1987)
Caribbean					
Santa Marta, Colomia	17-22	Chain transects	5-24% ID	13% ID	Zea (1994)
Roques National Park, Venezuela	1-35	Quadrats	?	1/2 m^2	Alvarez et al. (1990)
Biscayne National Park, Florida Keys, USA	1-18	Quadrats	3-18/m^2	8/m^2	Schmahl (1990)
Havana, Cuba	1-17 11-16	Quadrats Quadrats	0-15/m^2 15/m^2	6/m^2 ?	Alcolado (1979)

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SPONGES, INDICATORS OF MARINE ENVIRONMENTAL HEALTH. *Memoirs of the Queensland Museum 44: 50. 1999:-* There is an urgent need for marine ecosystem indicators to facilitate management aimed at either ameliorating impacts or guiding sustainable utilisation of marine resources. We propose that qualitative and quantitative examination of marine benthic communities will provide robust indication of responses to short and long term environmental conditions, and further suggest that information exists which permits the creation of a hierarchy of indicators for establishing ecosystem health in a regional context. These are in the form of identifiable marine community assemblages, together with biomass and growth indices determined from morphological parameters associated with the characterising species for each assemblage. Examples are provided to demonstrate the sensitivity of such indicators by focusing on sponge characterised communities. The composition of assemblages and population statistics of key species reflect ecosystem disturbances following catastrophic sediment deposition following cyclones, and in response to more recent and relatively short-term impacts. The latter include responses to sediment disruption from trawling and sand mining, and responses to water quality change during algal bloom events.

Marine environmental indicators are likely to take the form of well-defined ecotypes described by characterising species presence. These species have known ranges of tolerance to environmental variables such as light, current, food supply, turbidity, BOD, and sediment regime. They are by their very nature, relevant at a regional level and will be set in the context of a biogeographic classification for any coast or shelf. They can be further refined by interrogation of models relating population structure of key species to biological and physical attributes of the environment. ❐ *Porifera, growth, morphology, indicators, environmental health, marine resources, benthic communities.*

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FEEDING BIOLOGY OF *POLYMASTIA CROCEUS*

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Polymastia croceus is a yellow, encrusting, marine sponge endemic to New Zealand's coastal waters. It is currently of great interest due to its production of a proteinaceous secondary metabolite which has potential for use in anti-cancer and anti-HIV pharmaceuticals. An examination of feeding in *P. croceus* was undertaken, to determine its importance in coastal ecology and implications for aquaculture, using *in situ* flow cytometry. High proportions of ultraplankton (cells <5µm) were consumed and *P. croceus* appeared to be selective in its feeding at one of the sites sampled. The ultraplankton species best retained were *Synechococcus*-type cyanobacteria (up to 94%) and picoeukaryotes (up to 88%), in contrast to previous studies where sponges were found to retain *Prochlorococcus* spp. most efficiently. Using a microthermistor-based flow meter, attempts were made to quantify the rate at which *P. croceus* processes water. From initial results *P. croceus* was shown to process large quantities of water at rates (up to 8.82cm^3 s^{-1}), well in excess of those previously recorded for other sponge species. These preliminary data indicate that *P. croceus* has potential to process large quantities of water in short periods of time. The highly efficient retention of ultraplankton species, together with the large volumes of water processed, indicate that sponges like *P. croceus* are likely to be a major component in the benthic-pelagic carbon cycle. *Polymastia croceus* is an abundant species and therefore likely to play a significant role in coastal foodwebs. Furthermore, we suggest that the contribution of sponges to coastal production deserves more attention. ❐ *Porifera, feeding biology, ultraplankton diet, water flow rates, Polymastia croceus.*

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Research on *in situ* pumping rates and diets of sponges has been minimal worldwide, with most effort attributed so far to Reiswig (1971-1974) and Pile et al. (1996, 1997). Nevertheless, these few studies show that sponges can process large amounts of water, extracting high percentages of the available plankton, in particular the fraction less than 10µm cell size. Pile et al. (1996) further defined the fraction of diet less than 10µm to show that there was high retention (>70%) of hetertrophic bacteria, *Prochlorococcus* spp., *Synechococcus*-type cyanobacteria, picoeukaryotes and nanoeukaryotes. These observations have implications for the distribution and abundance of sponges, and the marine ecosystems in which they exist. The high rates of feeding activity described in this literature indicate that sponges are important components of benthic-pelagic coupling.

Polymastia croceus is an abundant, yellow, encrusting, marine sponge, endemic to New Zealand's coastal waters. It has recently attracted interest due to its production of a proteinaceous secondary metabolite, which has potential for use in anti-cancer and anti-HIV pharmaceuticals. As the metabolite is present in sponges in only trace levels, alternative modes of production are being examined. Wild harvest and aquaculture are options for producing the large quantities of sponge biomass required to supply sufficient metabolite for continuing research. However, it is generally considered that harvesting the required biomass directly from wild stocks is unsustainable and thus artificial supply options have to be considered. Of these options, aquaculture appears to be the one most likely to be rapidly developed (Battershill & Page, 1996). Knowledge of the feeding biology is of fundamental importance to the design and implementation of any aquaculture regime, and the assessment of the impact of removal or addition of *P. croceus* to the ecosystem.

Previous research on *P. croceus* has been restricted to its reproductive ecology (Battershill & Bergquist, 1999a, in press) and taxonomy (Kelly-Borges & Bergquist, 1997). The work

FIG. 1. Map showing the location of study sites in NE New Zealand, approximately 36°16'S, 174°48'E.

reported here is based on the approaches used by Pile et al. (1996, 1997), and investigates the following hypotheses: 1) That, like the temperate sponge *Mycale lingua* (Pile et al., 1996), *P. croceus* would efficiently consume large quantities of ultraplankton, in particular *Prochlorococcus* spp.; and 2) The rates at which water is processed by *P. croceus* would be high and relatively constant over diel periods. *Polymastia croceus* is a sponge capable considerable contraction, closing pores and withdrawing oscula, and for unknown reasons alternates between inflated and deflated forms. When deflated no oscula are visible and there is no apparent pumping activity taking place. In contrast, when inflated, large volumes of water appear to be turned over (confirmed visually using dye trace; Bell, 1998).

MATERIALS AND METHODS

Studies on diet and processing ability were undertaken at two sites on the NE coast of New Zealand where extensive *Polymastia croceus* biomass is found: Sponge Garden, within the Cape Rodney to Okakari Point (Leigh) Marine Reserve, and Takatu Point further to the south (Fig. 1). *Polymastia croceus* occurred between 16-18m below MLWS at both sites on sand covered base rock (Battershill & Bergquist, 1999b, in press).

DIET DETERMINATION. Flow cytometry was used to determine the diet of *P. croceus*. Following methods used by Pile (1997), five samples of ambient water (within 5cm of the sponge), and five samples of water being exhaled from oscula were taken *in situ* (using 5cc syringes) from each of five sponges at each site. Samples were fixed in 10% paraformaldehyde and frozen at -80°C, following the protocol described by Campbell et al. (1994). The samples were transported to Macquarie University (Sydney, Australia) for analysis of the ultraplankton composition of each sample using a FACScan Flow Cytometer unit (Becton Dickinson). The analysis technique was similar to that used by Marie et al. (1997). Two light scatter parameters were analysed: 1) forward light scatter, which relates to particle size; and 2) side light scatter, which relates to cell complexity. Three fluorescence parameters were also analysed: 1) green fluorescence from the SYBR Green I DNA stain (Molecular Probes Inc.); 2) orange fluorescence from the photopigment Phycoerythrin; and 3) red fluorescence from the photopigment Chlorophyll A. Each sample was run twice for all of these parameters. The first run was 100µl with autofluorescence being recorded, and the second was a 1 minute run of sample that had been stained with SYBR Green I (5µl SYBR Green I to 450µl sample).

The resultant data from the flow cytometry were then analysed using the custom designed Cytowin software (Vaulot, 1989), used to identify and enumerate the cells. Retention efficiency (RE) was determined by applying the following formula to each ultraplankton species:

$$RE=100\times(CCA-CCE)/CCA$$

where CCA is mean cell count ambient water, and CCE is mean cell count exhalant water.

TABLE 1. Summary of exhalant and ambient cell concentrations (mean number of cells ml^{-1} ± 1 SD) and resultant retention of ultraplankton species by *Polymastia croceus* at Sponge Garden. P-values for Students t-test, α=0.05.

Ultraplankton Species	Ambient ($x10^3$)	Exhalant ($x10^3$)	P-value	Percent Retained
Heterotrophic Bacteria	103.7 ± 37.7	55.8 ± 35.1	0.0000	46
Prochlorococcus spp.	20.5 ± 24.4	6.8 ± 4.1	0.0063	74
Synechococcus-type cyanobacteria	184.6 ± 26.6	11.7 ± 8.1	0.0000	94
Picoeukaryotes	8.1 ± 2.3	1.0 ± 0.4	0.0000	88

TABLE 2. Summary of exhalant and ambient cell concentrations (mean number of cells ml^{-1} ± 1 SD) and resultant retention of ultraplankton species by *Polymastia croceus* at Takatu Point. P-values for Students t-test, α=0.05.

Ultraplankton Species	Ambient ($x10^3$)	Exhalant ($x10^3$)	P-value	Percent Retained
Heterotrophic Bacteria	88.5 ± 28.7	70.8 ± 45.1	0.4759	20
Prochlorococcus spp.	13.1 ± 13.7	15.1 ± 24.2	0.3658	-15
Synechococcus-type cyanobacteria	117.0 ± 22.5	33.1 ± 30.0	0.0000	72
Picoeukaryotes	5.1 ± 2.3	1.8 ± 1.7	0.0000	65

PROCESSING RATE. To measure sponge pumping rates, a microthermistor-equipped datalogger was built for submarine use based on the design by Pile & Young (in prep.; modified from LaBarbera & Vogel, 1976). Colloquially known as a 'Medusa', the unit consists of a 12V battery, data logger and six microthermistor probes. The microthermistors were each placed over an osculum (five probes over oscula and one probe 20cm above the sponge in the ambient flow), and Fluorescein dye was used to visualise the outflow from the oscula to ensure that the probes were correctly in place perpendicular to the flow. The 'Medusa' was left in place for 24hr periods to log any changes in pumping rate over time. A Hobotemp temperature logger was also deployed, attached to the 'Medusa' housing, to enable the data to be calibrated for temperature variation. The logged data were down-loaded and calibrated with the temperature log data and calibration coefficients to allow conversion of voltage draw into flow rates of cm s^{-1}. All the sampled oscula were photographed and the images digitised to allow area measurements, which in turn permitted volume per unit time to be calculated.

RESULTS

DIET DETERMINATION. The most abundant ultraplankton (cells ml^{-1}) available to *Polymastia croceus* at both sites were *Synechococcus*-type cyanobacteria, followed by heterotrophic bacteria, *Prochlorococcus* spp. and autotrophic picoeukaryotes (Tables 1-2). Retention efficiencies, however, were highest for *Synechococcus*-type cyanobacteria followed by picoeukaryotes. At Sponge Garden (Table 1), retention efficiencies of *Prochlorococcus* spp. were next highest, followed by heterotrophic bacteria, while the opposite occurred at Takatu Point (Table 2). The absolute amounts of ultraplankton in ambient water differed between the two sites, although relative proportions were constant. Significant differences (one-way ANOVA with Bonferroni's pairwise comparisons, $P<0.05$) occurred between sites for the ambient concentrations of *Synechococcus*-type cyanobacteria, which averaged $184.6x10^3$ cells ml^{-1} at Sponge Garden but only $117.0x10^3$ cells ml^{-1} at Takatu Point. The ambient concentrations of the other species at Takatu Point were not significantly different from those at Sponge Garden ($P>0.05$). The retention efficiencies of sponges differed between the two sites with the mean retention of *Synechococcus*-type cyanobacteria, for example, being 94% at Sponge Garden and 72% at Takatu Point. The largest difference in retention occurred with *Prochlorococcus* spp.; 67% at Sponge Garden and -15% at Takatu Point. Differences between ambient and exhalant concentrations were tested (Students t-test, α=0.05) to confirm that the retention efficiencies were significant. Only heterotrophic bacteria and *Prochlorococcus* spp. at Takatu Point had insignificant differences.

PROCESSING RATE. Due to technical difficulties only two oscula had (at the time of writing), produced reliable results over a reasonable period (Fig. 2), but it is clear that *P. croceus* can pump at high velocities (Table 3). One oscule, for example, processed on average 26L an hour, or 304L an hour for every cm^2 of oscule area. The oscula showed a fairly constant pumping rate, with a period of heightened

TABLE 3. Summary of pumping rates (cm s^{-1}) of two oscula measured with the 'Medusa' at Sponge Garden. The estimates of volume pumped (cm^3 s^{-1}) were derived from the area of the oscule (8.53mm^2 for 1 and 6.96mm^2 for 2).

Oscule	Measure	Average ± 1 SD	Min	Max
1	Velocity (cm s^{-1})	84.48 ± 4.78	80.15	103.44
2	Velocity (cm s^{-1})	64.14 ± 2.55	60.68	77.65
1	Volume (cm^3 s^{-1})	7.21 ± 0.41	6.84	8.82
2	Volume (cm^3 s^{-1})	4.45 ± 0.18	4.22	5.4

activity around midday hinting at periodicity in pumping rate. The sponges from which these results were derived were not fully inflated when studied, with only a few oscula per sponge open, and many of the surrounding sponges deflated. Thus, we believe these rates are likely to be conservative as an inflated sponge is likely to have greater pumping potential.

DISCUSSION

The hypothesis that *Polymastia croceus* would consume high percentages of ultraplankton, especially *Prochlorococcus* spp., proved to be partially correct. High percentages of ultraplankton were indeed consumed, but these consisted of *Synechococcus*-type cyanobacteria and picoeukaryotes as preferred dietary species, rather than *Prochlorococcus* spp., as reported in previous studies (Table 4). Heterotrophic bacteria and *Prochlorococcus* spp. were considerably less favoured, particularly at the Takatu Point site where their retention was statistically insignificant. The ambient samples showed that the most abundant ultraplankton species at both sites was *Synechococcus*-type cyanobacteria, correlating with it being the most retained species. However, picoeukaryotes, the least available ultraplankton species, had the second highest retention efficiency. This trend is most obvious at Takatu Point and suggests that a certain level of feeding selectivity by *P. croceus* may be present. Unselective feeding would be expected to show that those ultraplankton species occurring in higher numbers (cells ml^{-1}) in the water column would also be retained in proportionally higher numbers simply due to the higher probability of encounter. Although *Prochlorococcus* spp. appeared to be in higher abundance in exhalant water at Takatu Point, this was not significant and possibly an artefact of sampling, or due to the sponges concentrating patchily distributed *Prochlorococcus* spp. into

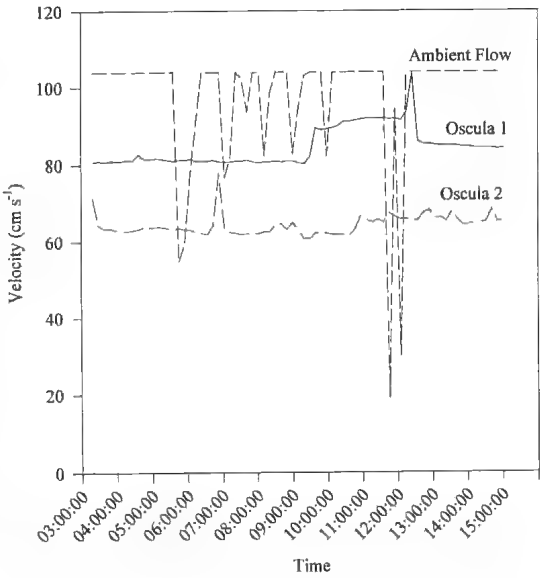

FIG. 2. Oscula velocity (cm s^{-1}) from *Polymastia croceus* at Sponge Garden. Velocity readings were taken at 10 minute intervals. Flow is a record of the ambient water velocity. The large spikes in the flow record are most likely the result of fish bites.

their exhalant currents, with few or no *Prochlorococcus* spp. cells being taken up. *Synecococcus*-type cyanobacteria and picoeukaryotes are also smaller than heterotrophic bacteria or *Prochlorococcus* spp. and to extract them from the internal current especially at high velocities would be difficult. The general perception (e.g. Kilian, 1952; Bergquist, 1978; Pile, 1996) is that sponges are unselective filter feeders, whereas our findings that there is selectivity in dietary retention have implications for the distribution and abundance of *P. croceus* in the wild.

The Takatu Point sponges had lower retention of ultraplankton species (both in cells ml^{-1} and percent retained), than the sponges at Sponge Garden. The reasons for this can only be postulated at this stage, but there are three primary, interrelated possibilities. 1) Sponges may have a cycle of pumping, and the time of day at which they were sampled (mid-morning for Takatu Point and mid-afternoon for Sponge Garden), may be associated with different periods of pumping activity. 2) Sponges were inflated to different degrees at the different sampling sites. Inflation and deflation occurs in *P. croceus* in relation to unknown environmental conditions, which in itself may suggest that different microclimates exist at the two sites. The

TABLE 4. Comparison of ultraplankton retention efficiencies (%) and mean exhalant velocities between *Polymastia croceus* at Sponge Garden and previously studied species. Key: Hbac, heterotrophic bacteria; Pro, *Prochlorococcus* spp.; Syn, *Synechococcus*-type cyanobacteria; Peuks, autotrophic picoeukaryotes; 1, Pile et al. (1996); 2, Pile (1997); 3, Pile et al. (1997) with velocity for *B. bacilifera* from Savarese et al. (1997); 4, Reiswig (1971).

Sponge Species	Ultraplankton Species				Mean Exhalant Velocity (cm s^{-1})
	Hbac	Pro	Syn	Peuks	
Polymastia croceus	46	74	94	87	84.48 (SD = 4.78)
Ircinia felix[2]	30	26	48	-91	
Ircinia strobilina[2]	56	52	53	32	
Baikalospongia bacilifera[3]	71	NA	58	NA	4.3 (SD = 12.3)
Baikalospongia intermedia[3]	84	NA	66	99	
Mycale lingua[1]	74	93	89	86	14 (SD = 9.7)
Mycale sp.[4]	NA	NA	NA	NA	7.8
Tethya crypta[4]	NA	NA	NA	NA	15

Takatu Point sponges were observed to be less 'open' than those at Sponge Garden, so they may have been going into, or coming out of, a period of deflation and thus processing at a lower, less efficient rate. The lower cells ml^{-1} available at Takatu Point may be linked to the suppressed pumping/retention activity, as it is possible that food availability may be a cue for inflation/deflation (pers. observations). 3) The Takatu Point sponges, which are positioned adjacent to a constant long shore current, have some other nutrient source which can be, for example, absorbed through the pinacoderm making filtering for food less necessary. Other studies (e.g. Kilian, 1952) have shown that particles can be ingested by the pinacoderm and thus direct uptake of nutrients from the ambient water is possible, although unlikely to be significant to the sponge's nutritional requirements.

In situ sampling provided a realistic insight into the feeding ecology of sponges, although this insight is still limited given the lack of temporal and spatial variation in sampling. Certainly, differences between the two sites sampled suggest that there may be considerable spatial variation. The inference of selectivity shown at Takatu Point is something that has yet to be demonstrated in sponges and is an exciting find, but its verification requires considerably more work.

We hypothesised that *P. croceus* would have relatively high pumping rates that were fairly constant over time. This cannot be verified due to the lack of data, but we can confirm the average flow velocities of 84.48cm s^{-1} and 64.14cm s^{-1} are high compared to those found by Reiswig (1971), Pile et al. (1996) and Savarese et al. (1997) (Table 4). It was not possible to determine mean pumping rates for *P. croceus* per unit biomass, a relationship which would have allowed more relevant comparisons to be made with data from the literature. As the sponges were not fully inflated at the time, pumping rates — particularly those in relation to sponge biomass — were likely to have been depressed. Variations in pumping velocities over time suggest that there may be periods of increased velocity which are independent of the ambient flow. Previous studies confirm that pumping rates can vary over time, although this appears to be species dependant. *Mycale* sp., for example, maintained a fairly constant level of water transport, whereas *Tethya crypta* had a highly changeable pumping rate, apparently determined by light intensity (Reiswig, 1971). Savarese et al. (1997) also noted high variability in pumping rates over time and with location for the freshwater sponge *Baikalospongia*. While the results for *P. croceus* are far from conclusive, it is possible that there was some variability in pumping over short periods of time. The inflation/deflation phenomenon certainly shows that over longer periods of time there is large variation in the water processing potential of any given biomass. Further work with the 'Medusa' would allow both short- and long-term variability to be better defined, and thus enhance the potential for making predictions on the amount of water that can be turned over in any given period of time.

Determination of the variability in pumping performance with site would allow some gauge of the influence of environmental factors on pumping performance. Variation in ambient flows between sites may show the extent to which flow can assist pumping, and whether this assistance is related to differences between sites, such as the distribution and biomass of sponges: in other words, whether or not assisted flow enhances the growth and distribution of *P. croceus*. Ecological studies (Bell, 1998) have provided estimations of pumping rates per unit biomass during an inflation (and thus maximum

pumping) event. The calculated mean oscula area per m^2 at Sponge Garden, when combined with the mean velocity from oscule 2 (64.14cm s^{-1}), produced an estimate of 54ml s^{-1} m^{-2}. This estimated rate is probably conservative, but its extrapolation suggests that the water column directly above the sponges is turned over approximately every 9.3hrs at Sponge Garden.

In summary, *P. croceus* appears to be able to process large volumes of water over short periods. This could lead to extremely high rates of carbon consumption by this species, which has potentially significant implications for the benthic marine ecosystem. For example, removing or adding *P. croceus* to habitats such as during harvesting or aquaculture ventures, would impact significantly on these habitats, particularly in terms of the availability of primary production.

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PUSHING THE BOUNDARIES: A NEW GENUS AND SPECIES OF DICTYOCERATIDA

PATRICIA R. BERGQUIST, SHIRLEY SOROKIN AND PETER KARUSO

Bergquist, P.R., Sorokin, S. & Karuso, P. 1999 06 30: Pushing the boundaries: a new genus and species of Dictyoceratida. *Memoirs of the Queensland Museum* 44: 57-62. Brisbane. ISSN 0079-8835.

Descriptive parameters used to segregate sponges into genera, families and orders must always be subject to re-evaluation. A rare foliose sponge usually found between 20-24m depth on reefs in the central Great Barrier Reef, Australia, has occasioned such reappraisal. The distinct thick, organised sand cortex and surface characters of the oscular and poral faces, the regular, simple primary and secondary fibres and the absence of a tertiary skeleton provide the basis for the diagnosis of a new genus. The lamellate form, brilliant white colouration and regular skeletal arrangement are diagnostic of a new species. A new subfamily, Phyllospongiinae, is established within the Thorectidae, to encompass the new genus and the other foliose dictyoceratid genera *Phyllospongia*, *Carteriospongia*, *Strepsichordaia* and *Lendenfeldia*. In addition to fibre structure, chemotaxonomic characters and choanocyte chamber morphology support the establishment of the new subfamily. Members of the Phyllospongiinae contain homoscalaranes and a unique series of bishomoscalaranes. While the structure of the new sponge has precipitated a subfamilial rearrangement within the Thorectidae, the task of assigning species and genera to other thorectid subfamilies is not complete at this time. ❒ *Porifera, Dictyoceratida, new genus, new species, Phyllospongiinae new subfamily, chemotaxonomy, sesterterpenoids, bishomoscalaranes.*

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Descriptive parameters used to segregate sponges into genera, families and orders are always subject to re-evaluation, particularly so given the fact that in large areas of the world's oceans numerous species remain undiscovered or, if collected, undescribed. This means that a taxonomist must always be prepared to confront unexpected combinations of characters in new species and to revise systematic arrangements accordingly.

The sponge described in this short communication has, with its combination of structural characteristics, required that the diagnoses of two dictyoceratid families, Spongiidae and Thorectidae, be refined and that a subfamily structure be established within the latter. The revised diagnoses of all families and subfamilies will be included in a separate publication. Abbreviations: QM, Queensland Museum, Townsville branch, the Museum of Tropical Queensland; AM, Australian Museum, Sydney.

SYSTEMATICS

Class **Demospongiae** Sollas
Order **Dictyoceratida** Minchin
Family **Thorectidae** Bergquist
Subfamily **Phyllospongiinae** s. fam. nov.

DIAGNOSIS. Foliose, or folio-digitate Dictyoceratida in which the spongin fibres making up the anastomosing skeleton are finely laminated and contain a differentiated pith. Zones of disjunction between successive fibrous layers remain tightly adherent, producing an overall homogeneous structure with visible contiguous laminae. Pith is not sharply disjunct from the investing spongin fibre but rather merges into it. Secondary fibres show a surface striation. Skeleton is made up of cored primary and uncored secondary fibres to which uncored vermiform or reticulate tertiary elements are added in most genera. Choanocyte chambers are large, spherical and diplodal. Matrix is only very lightly infiltrated with collagen and appears fibrous macroscopically rather than fleshy. Cyanobacteria are always

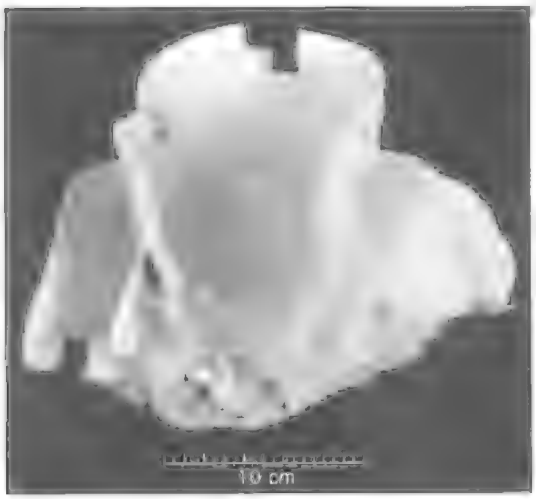

FIG. 1. *Candidaspongia flabellata*. Holotype, in situ view is of the poral face and shows the verrucoses caused by barnacles.

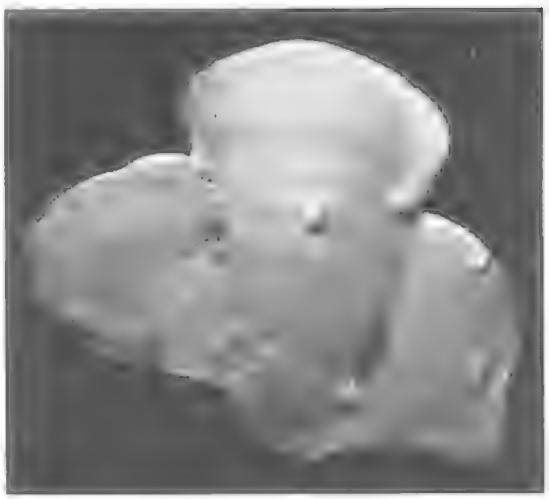

FIG. 2. *Candidaspongia flabellata*. Holotype, view of the oscular face.

incorporated. Secondary metabolites include a series of unique bishomoscalaranes.

REMARKS. In addition to the new genus described below, the subfamily contains four other genera presently classified in the family Spongiidae: *Phyllospongia* Ehlers, *Carteriaspongia* Hyatt, *Strepsichordaia* Berquist, Ayling & Wilkinson and *Lendenfeldia* Bergquist, with the first being the type genus of the new subfamily. *Lendenfeldia* can now be included following the discovery of fresh specimens from the Central Pacific and Australia, which have yielded further information on their morphology. However, it is possible that some species presently assigned to *Lendenfeldia*, particularly those still relatively poorly known, belong in other genera, while others including the type species *L. frondosa*, properly belong in the Phyllospongiinae.

As a result of the transfer of these genera from Spongiidae to Thorectidae (Phyllospongiinae), only seven genera now remain in Spongiidae (*Coscinoderma* Carter, *Dactylospongia* Bergquist, *Hippospongia* Schulze, *Hyattella* Lendenfeld, *Leiosella* Lendenfeld, *Rhopaloeides* Thompson, Murphy, Bergquist & Evans and *Spongia* Linneaus)

The nominotypical thorectid subfamily, Thorectinae, contains 13 genera (*Aplysinopsis* Lendenfeld, *Cacospongia* Schmidt, *Collospongia* Bergquist, Cambie & Kernan, *Fascaplysinopsis* Bergquist, *Fasciospongia* Burton, *Fenestraspongia* Bergquist, *Hyrtios* Duchassaing & Michelotti, *Luffariella* Thiele, *Petrosaspongia* Bergquist, *Smenospongia* Wiedenmayer, *Taonura* Carter, *Thorecta* Lendenfeld and *Thorectandra* Lendenfeld); Phyllospongiinae contains five genera.

Candidaspongia gen. nov.

ETYMOLOGY. For the brilliant white colouration.

DIAGNOSIS. Lamellate Phyllospongiinae with slightly roughened oscular face and finely conulose poral face. Both surfaces invested by a thick, organised sand cortex, deeper on the oscular face. The compact arrangement and uniform composition of the sand which makes up the cortex is remarkable and produces a brilliant white colour over all surface areas.

Skeleton is a network of moderately cored, evenly spaced, simple primary fibres which are disposed regularly, and oriented from attachment base to the surface. Conules on the poral face are aligned along the primary fibres. Primary fibres are connected by a polygonal network of uncored secondary fibres, with a striated braided surface texture. There are no vermiform tertiary fibre elements. Texture is compressible, firm but flexible. Choanocyte chambers are large, wide mouthed and spherical.

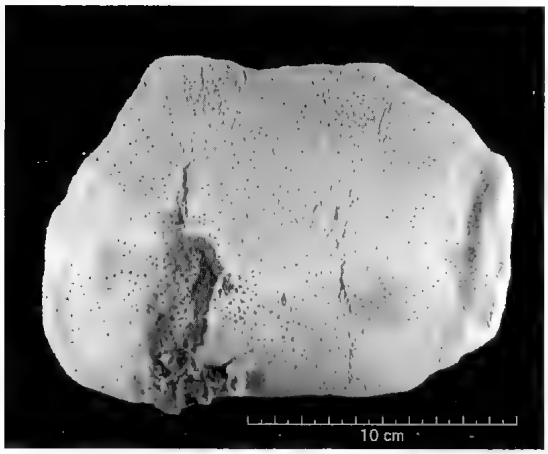

FIG. 3. *Candidaspongia flabellata*. Detail of the regular, sand-encrusted oscular face of a flat, spreading specimen.

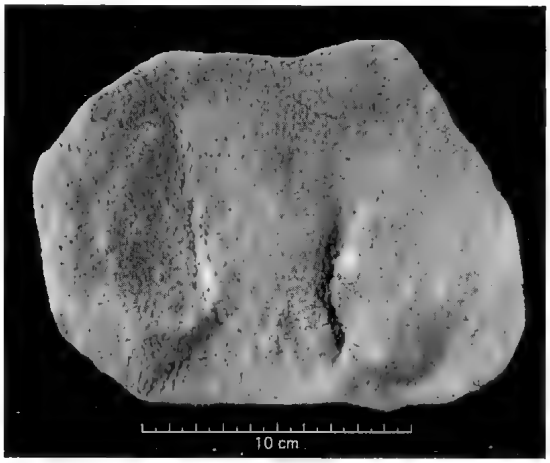

FIG. 4. *Candidaspongia flabellata*. Detail of the sand-encrusted but conulose poral face of the same specimen as in Fig. 3.

Candidaspongia flabellata sp. nov.
(Figs 1-5)

MATERIAL. HOLOTYPE: QMG25081: Bowden Reef, 19°02'S, 147°56'E, 24m depth, 7.xi.1994. Coll. S. Sorokin. PARATYPES: QMG25082: Bowden Reef, 19°2'S, 147°56'E, 23m depth, 21.xi.1993. Coll. J Wolstenholme. AMZ5286: Davies Reef, 19°01'S, 148°50'E, 20m depth, 11.x.1980. Coll. C. Wilkinson.

ETYMOLOGY. For the fan-like shape.

DIAGNOSIS. *Candidaspongia* with brilliant white colouration, lamellate form, extremely regular primary skeleton and strongly developed sand cortex on both faces.

DESCRIPTION. *Shape*. The sponge is a slightly concave, erect fan or a concave plate with a broad base of attachment (Figs 1-4). The lamella is most frequently simple and undissected but small fan-like accessory lobes are sometimes located near the base. Specimens attached under overhangs are thin flattened plates, while those attached above are erect, spreading and thicker. The body is 3-4.5mm thick, up to 25cm high and 35cm wide. Ocular and poral faces are differentiated, oscules are flush with the surface, 0.1-1.5mm diameter and scattered evenly over the entire face. The pores make up an even delicate network over the entire poral face and are 0.1-0.3mm diameter. The colour in life is brilliant white out of water and in ethanol, golden brown internally. A slight purplish tinge can develop, resulting from leaching cyanobacterial pigments. Dry specimens are cream externally, pale golden brown internally. The texture is firm, pliant and compressible, dry to the touch with no mucus exuded in life or on collection.

Surface. The oscular face is thrown into very low rounded mounds, oscules are situated on or between these. The poral surface is very finely conulose with rows of conules following the line of the underlying primary fibre tracts. Near the edge of the fan this produces a very regular pattern which is enhanced in dry specimens (Fig. 4). A well developed sandy cortex 0.3-0.6mm deep occurs on the oscular face; the poral face has a similar but thinner cortex, 0.2-0.4mm deep.

Skeleton. The skeleton is a network of slender, uncored secondary fibres with irregular mesh extending between thin, cored primary fibres which are evenly spaced 1-2mm apart and which run from the attachment base to the margin of the sponge. Primary fibres 65-120µm in diameter, secondaries 30-69µm. As seen in SEM, the surface of the secondary fibres has a fine braided texture (Fig. 5).

Soft tissue organisation. An ectosomal region is developed on both faces, and is collagen-reinforced in addition to supporting the sand cortex. The endosome is open, lightly infiltrated with collagen and with significant volume devoted to canal and choanocyte chamber space and little to matrix. Choanocyte chambers are spherical to oval, 50-90µm in longest dimension, with a wide mouth.

Reproduction. Sperm follicles and eggs are present in specimens collected in spring.

Associations. Light microscope thin sections show that the sponge has an associated cyanobacterium.

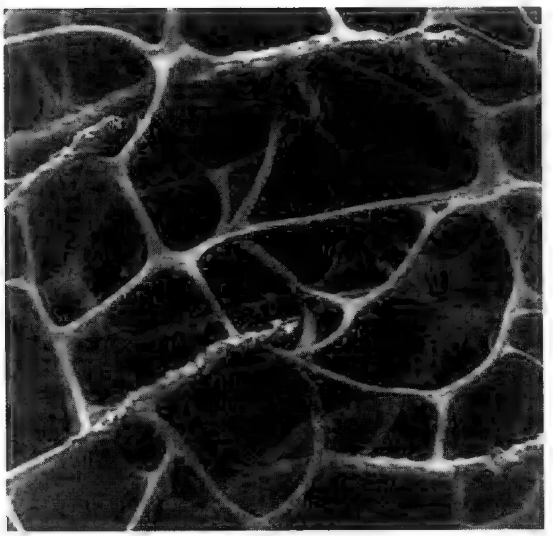

FIG. 5. *Candidaspongia flabellata.* Scanning electron micrograph of the primary and secondary fibre skeleton (×80).

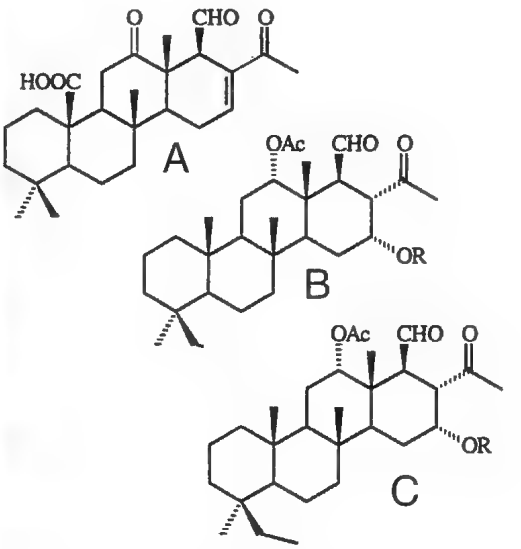

FIG. 6. A, Homoscalarane structure. B, Bishomoscalarane structure. C, Scalarane structure.

This is very similar to *Oscillatoria spongeliae*, but it is one third of the size of *O. spongeliae* as found in *Dysidea herbacea* and does not appear to have green pigment. The surface of the sponge is generally clean, ridges in some specimens are indicative of a polychaete burrow, and occasional surface nodules indicate the presence of a barnacle. A small (2-3mm) pale mauve nudibranch, *Chromodoris* sp., is often found on the sponge.

Chemistry. The sponge contains a novel homosesterterpene, a bishomoscalarane, the structure of which will be published elsewhere, and also a cytotoxic macrolide that may have pharmacological potential (McKee et al., 1993). The sponge can also cause severe dermatological reaction if handled.

REMARKS. The brilliant colour and strongly developed, evenly dispersed sand cortex on both lamellar faces make this species easy to recognise in nature. In addition to the type material referred to above, histology has been examined for a further thirty specimens.

DISTRIBUTION. Specimens of this sponge are uncommon, occurring on rock surfaces on reef slopes between 6-25m depth; most commonly between 12-25m depth. It is found on middle and outer reefs in the central region of the Great Barrier Reef. It has also been found on one reef in the Coral Sea (Wreck Reef) and on most reefs in the Capricorn-Bunker Group, S Great Barrier Reef (Hooper, pers.comm.). It has also allegedly been sighted in the Philippines although this record has not yet been verified by the authors.

DISCUSSION

Taxonomy. Several points of interest are raised by the structure of this sponge. As a consequence it has taken time to decide on the assignment made here and, in doing so, to address references made by other workers to the integrity or otherwise of the families Spongiidae and Thorectidae. We do not regard the task of reassigning species and genera to be complete at this time. This will be done in a contribution to the forthcoming 'Systema Porifera' (Hooper & Van Soest, in prep.).

Bergquist et al. (1988) indicated clearly that the affinities of *Phyllospongia*, *Carteriospongia, Strepsichordaia* and *Lendenfeldia* lay with the Thorectidae, rather than the Spongiidae. The major simple point of distinction is the presence of homogeneous fibres as seen in cross section in the latter group, compared to the concentrically laminate and lightly pithed fibres of the former. Until the 1988 study was done it was not possible to properly distinguish these foliose genera and thus to determine the integrity of the group with respect to fibre structure. A subfamilial status within the Thorectidae was indicated but not formally proposed in that paper, the authors

preferring to wait until further material of *Lendenfeldia* could be examined.

A subsequent paper dealing with a sponge termed, among other things, *'Spongia' mycofijiensis* (Sanders & Van Soest, 1996) again drew attention to the difficulties in defining the boundaries between the Spongiidae and Thorectidae using the existing criterion of laminated versus homogeneous fibre construction. These authors did not refer to the position stated by Bergquist et al. (1988). The sponge Sanders and Van Soest were dealing with provides a perfect example of the problem we encountered in attempting to assign *Candidaspongia* to an appropriate taxon. There were too few established genera to accommodate the range of morphologies being discovered, and existing terminology was not sufficiently refined, or perhaps understood, to differentiate between taxa. *'Spongia' mycofijiensis* belongs to none of the genera considered by Sanders and Van Soest or other authors reported to have expressed prior opinion on that sponge. It is a species of *Petrosaspongia* Bergquist (Bergquist, 1995), and is here formally assigned to that genus.

In both instances, that of *Candidaspongia flabellata* and of *Petrosaspongia mycofijiensis*, new genera were required to accommodate the species. In both instances, pressure was applied to 'give the beast a name'. Acquiescing to such pressure before certain about a generic assignment, merely creates confusion and obscures valid morphological or chemical patterns which may exist.

One further contribution which should be referred to is that of Jaspars et al. (1997). They deal with the group of sponges which have been reported to contain scalarane sesterterpenes; this includes the Phyllospongiinae. Many of the problems in the literature that they cite stem from identifications being published without the necessary study of histology and fibre structure to verify generic identifications. In addition, the authors acknowledge that in their collections, voucher specimens are inadequate to resolve those essential morphological details. That has been confirmed by study of those vouchers. The same authors cite confusion over the reported occurrence of homosesterterpenes in *Dysidea* species which usually contain sesquiterpenes. In all cases this stems from the misidentification of *Lendenfeldia* species as *Dysidea herbacea*. The gross morphologies are similar, the histology, fibre structure and chemistry are not. Study of relevant vouchers confirms the above assignment.

With regard to "*Phyllospongia vermicularis*" (Jaspars et al., 1997: ref. no. 94028) the sponge, as originally described by Lendenfeld (1889), is unrecognisable. Bergquist et al. (1988) referred to a sponge from the Great Barrier Reef which is very similar in morphology but with thicker branches. It has a different histology and skeletal arrangement and proves to be an as yet undescribed species of *Dysidea*. The status of Lendenfeld's species cannot be further determined without new material. The sponge from the Great Barrier Reef is a tangle of very fine branches with a grooved surface when dry, and is quite distinct from the sponge 94028 referred to by Jaspars et al. (1997). The other sponge cited by Jaspars et al. (1997; ref. no. 94515) is *Carteriospongia contorta* Bergquist et al. (1988). Nothing, therefore, in the contribution of Jaspers ed al. (1997) disrupts the integrity of the group of homosesterterpene containing Dictyoceratida.

Sesterterpene chemistry. The terpene chemistry of the Phyllospongiinae is characterised by the presence of structures known as homoscalaranes (Fig. 6A) and bishomoscalaranes (Fig. 6B). These are scalaranes (Fig. 6C) which are alkylated at C19, or C19 and 24 respectively. Homoscalaranes are typical of species of *Lendenfeldia* (Kazlauskas et al., 1982; Alvi & Crews, 1992). *Phyllospongia dendyi*, from which these metabolites have also been recorded, is a species of *Lendenfeldia*. The compounds have also been reported to occur in *Carteriospongia flabellifera* (Schmitz & Chang, 1988). There is one record of a Red Sea sponge containing both homoscalaranes and bishomoscalaranes (Kashman & Zviely, 1979). It was identified as *Dysidea herbacea* but examination of vouchers shows that it is *Lendenfeldia dendyi*.

Bishomoscalaranes are also reliably known to occur in *Carteriospongia foliascens* and *Strepsichordaia lendenfeldi*, and a second, undescribed species of this genus. All the bishomoscalaranes so far described have the ethyl group at C4 b-disposed to the ring system. The only exception is in *Candidaspongia* which has a-stereochemistry at C4. This is the only example of alkylation at C25 (cf. C24 for all others), and it serves to emphasise the separation of *Candidaspongia* from its nearest relatives, the other bishomoscalarane or homoscalarane containing sponges.

ACKNOWLEDGEMENTS

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MICROBIAL SYMBIONTS OF GREAT BARRIER REEF SPONGES

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Microbial symbionts of two sponges, *Rhopaloeides odorabile* (Dictyoceratida: Spongiidae) and a new species 'Very White Fan' (VWF) (Dictyoceratida: Phyllospongiidae), are being studied in detail. Bacteria isolated from *R. odorabile*, VWF, and the surrounding ambient seawater were characterised using morphological, biochemical, and molecular techniques. In the case of *R. odorabile*, a single bacterium, designated NW001, was found to dominate the culturable bacterial community associated with the sponge but was absent from ambient seawater samples. Strain NW001 was predominant in all individual sponges sampled (N=40) from different regions of the Great Barrier Reef, generally at more than an order of magnitude greater than the second most common bacterium (NW002). The bacterial community associated with *R. odorabile* appears to be highly stable. In the case of VWF, the culturable bacterial community was more diverse and showed greater variation between individuals. This community generally comprised eight predominant bacteria, rarely isolated from water samples and constituting ca. 70% of the total culturable bacteria. Extensive biochemical testing was performed on all isolates to give data for cluster analyses to identify the major groups of bacteria present. One isolate from each sponge was characterised at the molecular level by PCR amplification and sequencing of 16S ribosomal RNA gene fragments. Analysis of sequence from NW002 indicates it is a *Pseudoalteromonas* sp. Strain E30004315 from VWF is a microalga, with 16S rRNA sequence from its plastid closely related to that of other plastids of marine eukaryotic algae. This study produced an array of well-characterised microbes for natural products screening, in particular for important compounds known to be produced by these sponges. ❐ *Porifera, sponge, symbiont, Dictyoceratida, Demospongiae, 16S rRNA, Rhopaloeides odorabile, microalgae, Vibrio.*

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Symbiosis is considered a permanent association between organisms of different species. This term is not restricted to mutualistic associations but encompasses all associations, regardless of the type of interaction between the individuals. Mutualistic bacterial-invertebrate symbioses have been reported from many invertebrate taxa. Examples of these include cellulolytic nitrogen fixing bacteria from wood boring bivalves (Shieh & Lin, 1994), methanotrophic bacteria of bivalves (Dubilier et al., 1995) and bacterial symbionts of echinoderms (Burnett & McKenzie, 1997; Kelly & McKenzie, 1995). Although chemoautotrophic symbiosis has received the most attention, there are also many symbioses where the type of interaction between the host and their symbiont remains unknown. Most symbiotic bacteria from sponges have been located within the intercellular matrix and can occupy up to 60% of the sponge volume (Wilkinson, 1978a).

The biology of bacterium-sponge associations has elicited considerable interest from researchers investigating novel chemicals derived from sponges. The term symbiont has been broadly applied and few investigators have explored metabolic relationships and capabilities of the symbiont-host complex. One approach that will contribute to understanding these relationships is to isolate symbiotic bacteria and investigate their metabolic and taxonomic characteristics.

Cosmopolitan microbial symbionts associated with marine sponges include heterotrophic bacteria, cyanobacteria and unicellular algae. Numerous studies have described three general classes of heterotrophic bacterial-sponge associations (Wilkinson, 1978a). 1) Small

populations of cosmopolitan bacteria with a species composition similar to that found in the ambient seawater. These are most likely utilised as a food source by the sponge. 2) Species- specific population inhabiting the mesohyl region, not found in seawater, and most likely comprising true symbionts. 3) Fairly ill-defined, consisting of bacteria located within the sponge cells, also likely to be true symbionts. Phenotypically related bacterial symbionts have been described from taxonomically disparate sponges collected from geographically remote locations. Described sponge symbionts have included members of the genera *Pseudomonas*, *Alteromonas*, *Vibrio*, *Aeromonas*, *Acinetobacter*, *Micrococcus* and *Moraxella* (Santavy et al., 1990). It has been determined that facultative anaerobic symbionts metabolise a wide range of compounds and may be important in removing waste products whilst the sponges are not circulating water (Wilkinson, 1978a). It has also been postulated that sticky mucoid colonies may be important contributors to sponge structural rigidity (Wilkinson, 1978c). Other functions that have been suggested for sponge bacterial symbionts include digestion of material not available to the host sponge, direct incorporation of dissolved organic matter from the seawater, and digestion and recycling of insoluble sponge collagen.

Sponge symbionts are of biotechnological interest since bioactive compounds of potential medical importance isolated from sponges may be microbial in origin (Bewley & Faulkner, 1998; Bewley et al., 1996; Stierle et al., 1988). There are several practical advantages in isolation of symbionts which produce bioactive compounds, including consistent yield and large-scale production in fermenters, obviating the need for collection of sponges from natural ecosystems (Zilinskas et al., 1995).

In this study, microbial symbionts were investigated in two Great Barrier Reef sponges, *Rhopaloeides odorabile* Thompson et al. (Dictyoceratida: Spongiidae) and a new species, termed here 'Very White Fan' (VWF) (see Bergquist et al., 1999, this volume). This is a first step towards ascertaining whether these symbionts are implicated in the production of important bioactive compounds. *Rhopaloeides odorabile*, common throughout the GBR, produces novel norsesterterpenes (thopaloic acids) which exhibit potent cytotoxic activities (Ohta et al., 1996), and VWF contains the compound fanolide (P. Murphy, unpublished data), which retards the growth of several tumour cell lines.

MATERIALS AND METHODS

SPONGE COLLECTION AND BACTERIAL ISOLATION. Material examined in this study was collected using SCUBA (0-30m depth). Seasonal sampling for bacterial community studies was conducted over 12 months at Davies Reef (50 nautical miles off Townsville, Queensland, Australia, 18°49.6'S, 147°34.49'E). Immediately after collection, specimens were processed for bacterial isolation. Using aseptic technique, a $1cm^3$ section of sponge tissue was excised and surface-sterilised. Sponge tissue was homogenised in sterile artificial seawater (ASW) using a mortar and pestle. Serial dilutions (10^0, 10^{-1} and 10^{-2}) were prepared in ASW and plated onto several media for isolation of microbes.

Media for isolation of heterotrophic bacteria were used in this study. Difco Marine Agar 2216 as a non-selective marine medium, TCBS for enteropathogenic vibrios (Oxoid) and SBA, a selective medium for bacterial pathogens (Oxoid Columbia Blood Agar Base). BG-11 (Stanier et al., 1971) and MN + B12 (Waterbury & Stanier, 1978) were used for isolation of oxygenic phototrophs. Plates were incubated at 27°C for a period ranging between 2-4 weeks. Total culturable colony counts were obtained from Difco Marine Agar 2216 spread plates. Representatives of each morphotype were cultured from initial isolation and cryopreserved for further studies. Total bacterial counts were determined by fluorescent microscopic enumeration of cells stained with 4'6-diamidino-2-phenylindole (DAPI) as described by Porter & Feig (1980).

SCANNING AND TRANSMISSION ELECTRON MICROSCOPY. Sponge sections were prepared using a scalpel blade to cut 1-1.5mm thick sections of sponge tissue, ensuring that both the ectosome and choanosome were represented. Sections were fixed in 2.5% glutaraldehyde made in 0.1M sodium cacodylate buffer for 20hrs. Fixed samples were transferred into 0.1M sodium cacodylate and stored at 4°C until further processing. Sections of sponge tissue were placed in a 1% osmium tetroxide solution (prepared in 0.2M potassium phosphate buffer, pH 7.4) for 3.5hrs. Sections were removed and washed thoroughly in sterile distilled water, dehydrated in a graded ethanol series (15%, 35%, 55%, 75%, 85% and 95%), placed in embedding capsules and covered with Spurr's resin. Thin sections were cut and stained with 2% uranyl acetate followed by 0.2% lead citrate. Sections were mounted on 200 mesh copper TEM grids

TABLE 1. Type cultures used as control strains in biochemical analyses. Key: ACMM, Australian Collection of Marine Microorganisms; ATCC, American Type Culture Collection.

Collection number	Organism
ACMM 667	*Vibrio parahaemolyticus*
ACMM 668	*V. parahaemolyticus*
ACMM 89	*Vibrio alginolyticus*
ACMM 90	*V. parahaemolyticus*
ATCC 33809	*Vibrio fluvialis*
ATCC 33807	*V. fluvialis*
ATCC 7966	*Aeromonas hydrophila*
ATCC 35624	*Aeromonas* group 77
ATCC 33509	*Vibrio ordalii*

coated with carbon and Formvar. Samples were visualised following standard scanning and transmission electron microscopy techniques at James Cook University and University of Queensland.

BIOCHEMICAL TESTING OF BACTERIAL ISOLATES. The isolates were characterised by determining biochemical characteristics in 96-well microtitre-plates based on methods described by Hansen & Sorheim (1991). Several dye indicator tests were performed: Moller's arginine, lysine, ornithine, base; nitrate reduction, ONPG, indole, acetoin, tellurite, aesculin, alginate, acid-arabinose, arbutin, glucose, inositol, mannose, salicin, sorbitol, sucrose, and urea. The following tests were performed to determine the ability to utilise different carbon sources in assimilation broth: arabinose, cellobiose, galactose, glucose, mannose, melibiose, lactose, melizitose, sucrose, trehalose, xylose, ethanol, glycerol, propan-1-ol, sorbitol, gluconate, glucuronate, amygdalin, arbutin, citrulline, hydroxyproline, leucine, glucosamine, hydroxybutyrate, α-ketoglutarate, succinate, base (control), adenine, aminovalerate, N-acetyl-D-glucoseamine, ethanolamine, m-erythritol, D-fructose, D-galacturonate, glutarate, inositol, malonate, maltose and valerate. The assimilation broth contained (per litre) 0.015g of yeast extract, 1.0g of ammonium chloride, 0.075g of di-potassium hydrogen orthophosphate, 6.1g of Tris (hydroxymethyl) aminomethane and 15g of ASW salts (pH 7.5). After autoclaving, the carbon sources were filter sterilised and added aseptically to a final concentration of 8% (wt/vol.). Inoculations were performed by suspending colony material in ASW and inoculating 100µl of this suspension into each test. In addition, the following morphological characteristics were determined: colony morphology, gram stain and cell morphology, plate swarming, oxidation/fermentation (Leifson, 1963; Lemos et al., 1985), oxidase, catalase and growth at various salt concentrations (0%, 1%, 6%, 8%). In addition several antibiotic susceptibility tests (O/129 10 & 150µg, ampicillin 10µg & polymixin B 50iu) were performed along with growth on different media (Lecithinase, DNase, TCBS, SBA). Several American Type Culture Collection (ATCC) and Australian Collection of Marine Microorganism (ACMM) strains were included as controls (Table 1). Isolates were tentatively identified to either the genus or species level by comparing their phenotypic characteristics with those of type cultures and by comparing biochemical test results, carbohydrate utilisation patterns, and cell morphologies to those of species described in *Bergey's Manual of Systematic Bacteriology* (Holt, 1986) and *Bergey's Manual of Determinative Bacteriology* (Buchanan & Gibbons, 1974).

DATA ANALYSIS. The levels of relatedness of the bacteria were determined from the phenotypic data using Jaccard's similarity index (Zar, 1984).

$$S_j = a/(a + b + c)$$

where S_j =Jaccard's similarity coefficient, a = no. species in sample A and sample B (joint occurrences), b = no. species in sample B but not in sample A, c = no. species in sample A but not in sample B.

Phenograms were constructed by using unweighted pair group mean average (UPGMA) linkage (Sokal & Michener, 1958), Euclidean distances and the computer software package STATISTICA (StatSoft Inc., Tulsa, Oklahoma).

BACTERIAL IDENTIFICATION BY 16S RIBOSOMAL RNA (rRNA) ANALYSIS. Two microbial isolates, NW002 from *R. odorabile* and the microalga E30004315 were identified using a molecular approach, partial sequencing of the 16S rRNA gene fragments amplified from these isolates using the polymerase chain reaction (PCR). Total DNA was prepared from strains NW002 and E30004315 using a method modified from Ausubel et al. (1987). Oligonucleotide primers with specificity for eubacterial 16S rRNA genes [Forward primer 8-27:5'AGAGTTT GATCCTGGCTCAG -3' (modified from FD1) (Weisburg et al., 1991) and Reverse primer 1492:5'-GGTTACCTTGTTACGACTT-3' (Reysenbach et

al., 1992)] were used to amplify a 16S rRNA gene fragment from NW002. The cyanobacterial and plastid-specific 16S rRNA primers described by Nübel et al. (1997) were used for E30004315, since this isolate, although unialgal, may have been contaminated with low numbers of heterotrophic bacteria. PCR fragments were purified using the Microcon 30 system (Amicon, Beverly, MA), and sequenced using the PRISM Ready Reaction Kit (PE Applied BioSystems, Foster City, CA) and an ABI 310 sequencer (PE Applied BioSystems). Sequencing data were analysed by comparison to 16S rRNA genes in the Ribosomal Database Project (Maidak et al., 1999; Maidak et al., 1997) and the Genbank database, and aligned manually using the Phydit software (Chun, 1995).

Evolutionary trees were inferred using the neighbour-joining (Saitou & Nei, 1987), Fitch-Margoliash (Fitch & Margoliash, 1967) and maximum-parsimony (Kluge & Farris, 1969) algorithms in the PHYLIP package (Felsenstein, 1993). Evolutionary distance matrices for the neighbour- joining and Fitch-Margoliash methods were generated as described by Jukes & Cantor (1969). Tree topologies were evaluated by performing bootstrap analyses of the neighbour-joining data, based on 1000 re-samplings (Felsenstein, 1985).

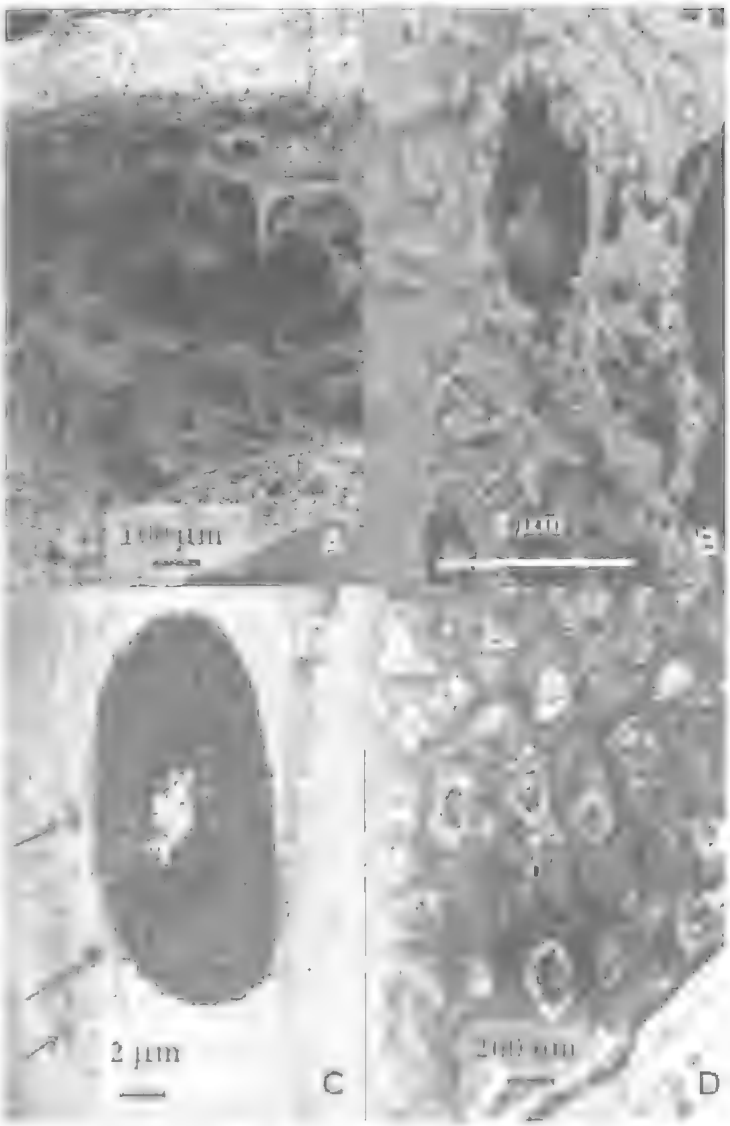

FIG 1. Electron micrographs of VWF sponge sections. A, Low magnification scanning electron micrograph of sponge section. B, High magnification of sponge section showing cells identified as putative cyanobacteria on morphological criteria. C-D, Low and high magnification, respectively transmission electron micrograph of sponge mesohyl section showing location of bacteria within 'bacteriocytes' and putative cyanobacteria, indicated by arrows.

Abbreviations: AIMS, Australian Institute of Marine Science; ASW, Artificial seawater; DAPI, Diamidino-phenylindole; 16S rRNA, 16S ribosomal ribonucleic acid; SBA, Sheep Blood Agar; TCBS, Thiosulphate Citrate Bile Salts Medium; VWF, Very White Fan.

RESULTS

ELECTRON MICROSCOPY. A large and complex bacterial community was shown by electron microscopy to be present within the sponge VWF (Fig. 1A-D). Bacteria closely associated with the sponge tissue, possibly embedded in a polysaccharide matrix, were presumed to be cyanobacteria based on morphological criteria (Fig. 1B), since these cells resemble filamentous cyanobacteria (e.g. genus

Oscillatoria). Cells presumed to be other eubacteria, based on the standard morphological criteria of size, shape and membrane structure, were also in close contact with the sponge tissue and contained in cellular organelles resembling the 'bacteriocytes' described by Vacelet & Donadey (1977) (Fig. 1C-D). Sand grains were observed which appeared to be incorporated into the sponge external structure (also reported by Bergquist, et al., 1999), possibly performing the function of increasing structural integrity (Shaw, 1927).

The bacterial community within *R. odorabile* was also large and appeared to be comprised of many different bacteria (Fig. 2). The bacteria appear to be dispersed throughout the sponge mesohyl and no bacteriocytes were evident. In contrast to VWF, cells resembling cyanobacteria were not seen.

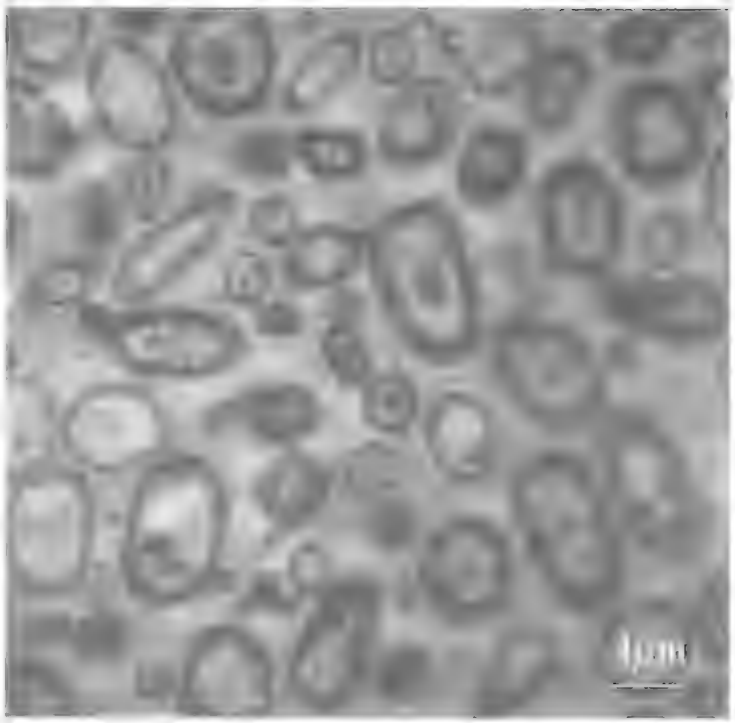

FIG. 2. Transmission electron micrograph showing the diversity of bacterial morphologies and the density of bacterial cells within the tissue of the sponge *R. odorabile*.

BACTERIAL ENUMERATION.
The average number of culturable bacteria from direct plate counts obtained from VWF was 3.6×10^4/ml and the average total count observed from DAPI staining was 6.3×10^7/ml. Only 0.06% of total bacteria were able to be recovered using traditional culture techniques. The range of total and culturable bacterial counts found in samples from eight individual VWF sponges are shown in Figure 3.

The average percentage of culturable bacteria from *R. odorabile* was only 0.1% with a range of 0.001-0.8%. The average percentage of bacteria able to be cultured from the water column was 0.23% with a range from 0.003-0.9%. Total and culturable bacterial counts found in samples from four individual *R. odorabile* sponges and the ambient seawater surrounding each sponge are shown in Figure 4.

BIOCHEMICAL CHARACTERISATION OF BACTERIAL ISOLATES. Morphological and biochemical data indicated that, at least as judged from the culturable fraction, the bacterial community within VWF differed from that present in the water column. Culture results from 15 VWF individuals from different locations revealed several similarities. Eight eubacteria, designated AB001 to AB008, were frequently observed as being part of the culturable bacterial community of VWF and were found to be present only in small numbers in samples from the water column.

A total of 220 isolates were isolated from VWF samples collected between June 1997-May 1998 from locations between Trunk Reef and Davies Reef, Great Barrier Reef. These isolates conformed to one of eight clusters (Fig. 5). Two clusters were Gram-positive with the remainder being Gram-negative. The Gram-positive bacteria were further sub-divided by means of cellular morphology. Approximately 40% of all bacteria (including strains AB004, AB007, and AB008) isolated from VWF clustered in phenons 6 and 7 (Fig. 5); these phenons contained the vibrio and aeromonad representatives, respectively, of the *Vibrionaceae* type strains used in this study. In addition, approximately 30% of the total bacterial community fell in a single phenon (phenon 8), which contained the strain AB005. Of the remaining three Gram-negative

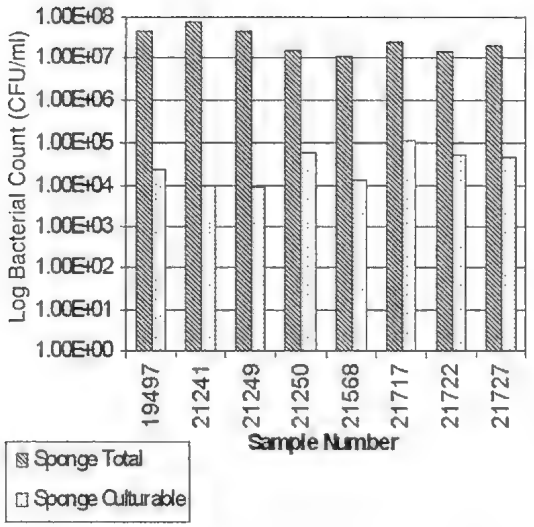

FIG. 3. Total and culturable bacterial counts from tissue of eight VWF individuals collected at Davies Reef, Great Barrier Reef.

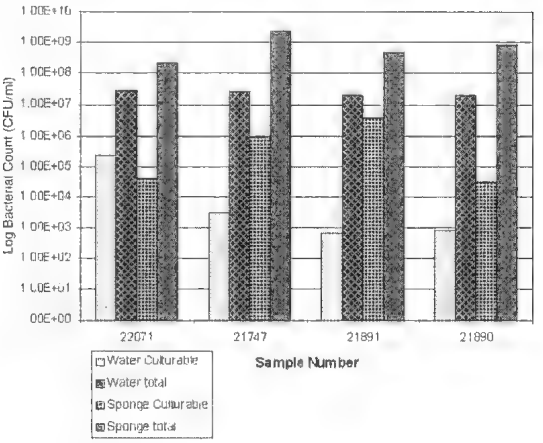

FIG. 4. Total and culturable bacterial counts from tissue of four *R. odorabile* individuals and seawater surrounding each individual collected at Davies Reef, Great Barrier Reef.

clusters, phenon 3 contained pigmented bacteria, phenon 4 included strains AB003 and AB004, and phenon 5 included strains AB001 and AB002. Strain AB001 appears closely related to NW001 from *R. odorabile*, with both strains being representatives of the alpha-Proteobacteria (data not shown).

From biochemical and morphological observations, it was apparent that the bacterial community within *R. odorabile* was quite distinct from the bacterial assemblages associated with the ambient water column. In general, both total and culturable counts from sponges exceeded counts from the corresponding water samples. The sponge microbiota was dominated by an organism designated NW001, whereas this isolate was completely absent in the surrounding water column. A small component of the microbial community was observed in both the sponge tissue and the ambient seawater.

A total of 223 isolates were collected from 40 *R. odorabile* samples collected between August 1997-May 1998. These isolates conformed to one of ten major clusters (Fig. 6). Two clusters (phenons 9 & 10) were Gram-positive and these were distinguished from each other on the basis of the oxidase reaction. Two of the Gram-negative clusters (phenons 1 & 2) were oxidase-negative and showed profiles linking them to the Enterobacteriaceae. One of the Gram-negative, oxidase-positive clusters (phenon 3) was catalase-negative and the remaining five clusters were catalase-positive and separated on the basis of carbon source utilisation. One of the five clusters contained an *Aeromonas* sp. type culture (phenon 6) and a second cluster contained the *Vibrio anguillarum* type culture (phenon 4). NW001 was a Gram-negative rod; oxidase, catalase, urease, VP and indole positive; utilised adenine dihydrogenase and had the ability to utilise glucose and gluconate as carbon sources. NW002 was a Gram-negative rod, oxidase, catalase, VP, indole and acid arabinose positive. It was urease--negative and did not utilise any of the tested carbon sources. Both NW001 and NW002 clustered within phenon 5.

MICROALGAL ISOLATES. In addition to the heterotrophic bacterial isolates, eight strains of oxygenic phototrophs were isolated from VWF and one of these strains, designated E30004315 was characterised by 16S rRNA sequencing (below). A single phototroph strain was isolated from *R. odorabile*. Phototroph strains were not characterised by biochemical testing because of the difficulty in identification of microalgae by this means; instead 16S rRNA sequencing was used as a method for identification of strain E30004315.

PHYLOGENETIC POSITIONS BASED ON 16S rRNA SEQUENCING. Phylogenetic relationships for the plastid of microalga E30004315 from VWF and heterotrophic bacterial strain NW002 from *R. odorabile* are shown in Figures 7 and 8,

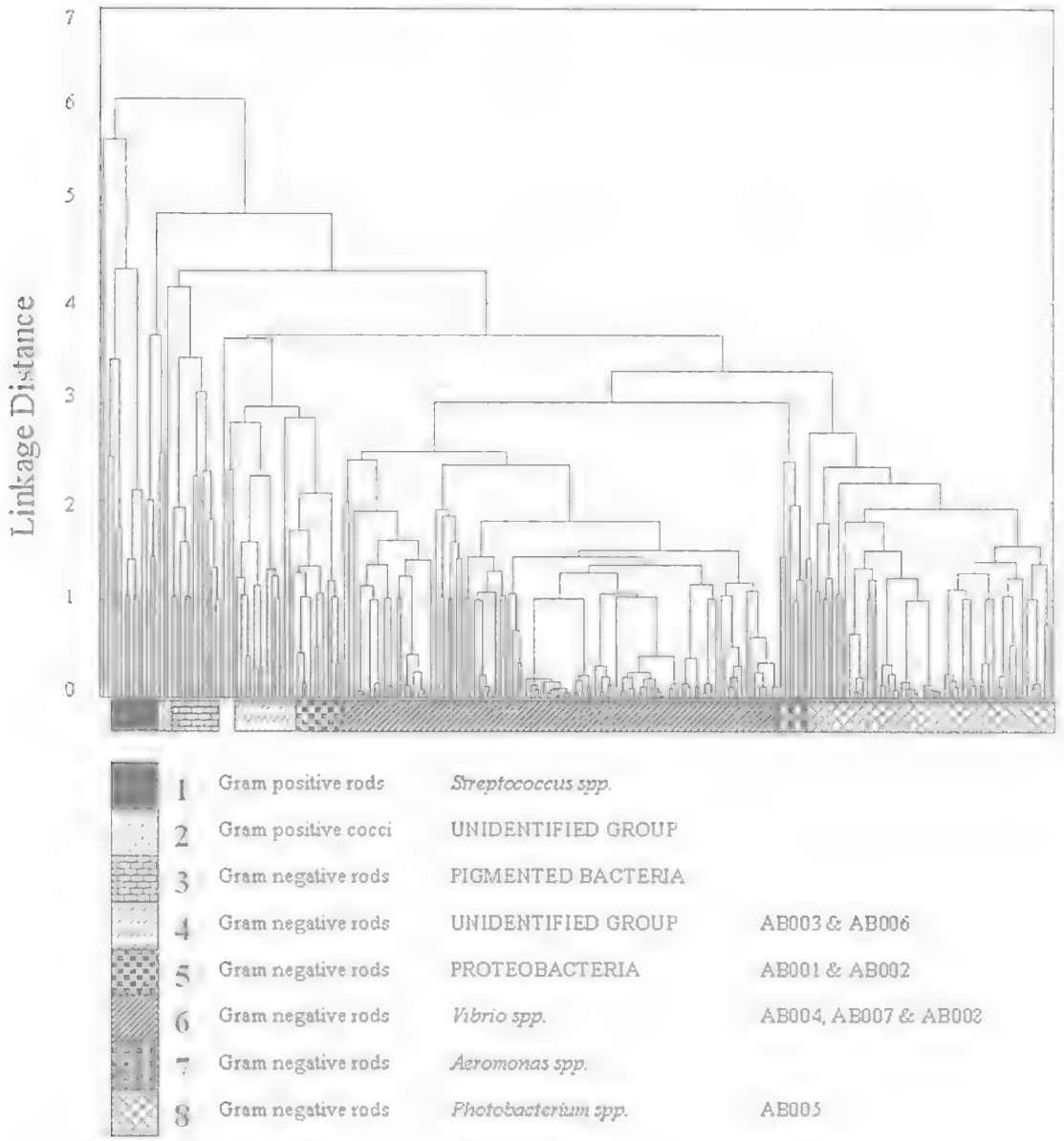

FIG. 5. Similarity dendrogram for isolates obtained from VWF.

respectively. The plastid from isolate E30004315 from VWF is closely related to plastids of other marine eukaryotic algae. NW002 is a *Pseudoalteromonas* sp.

DISCUSSION

This comprehensive biochemical and morphological analysis of bacteria isolated from two Great Barrier Reef sponges further emphasises the variability in microbiota associated with marine invertebrates. Several studies have documented diverse microbial communities associated with sponges (Vacelet, 1970, 1975; Vacelet & Donadey, 1977; Wilkinson, 1978a,b,c; Santavy, 1985; Willenz & Hartman, 1989; Santavy & Colwell, 1990; Santavy et al., 1990; Lopez et al., 1999). These communities are generally comprised of large numbers of heterotrophic bacteria that often occupy up to 60% of the sponge volume (Santavy, 1985; Wilkinson, 1978a,b).

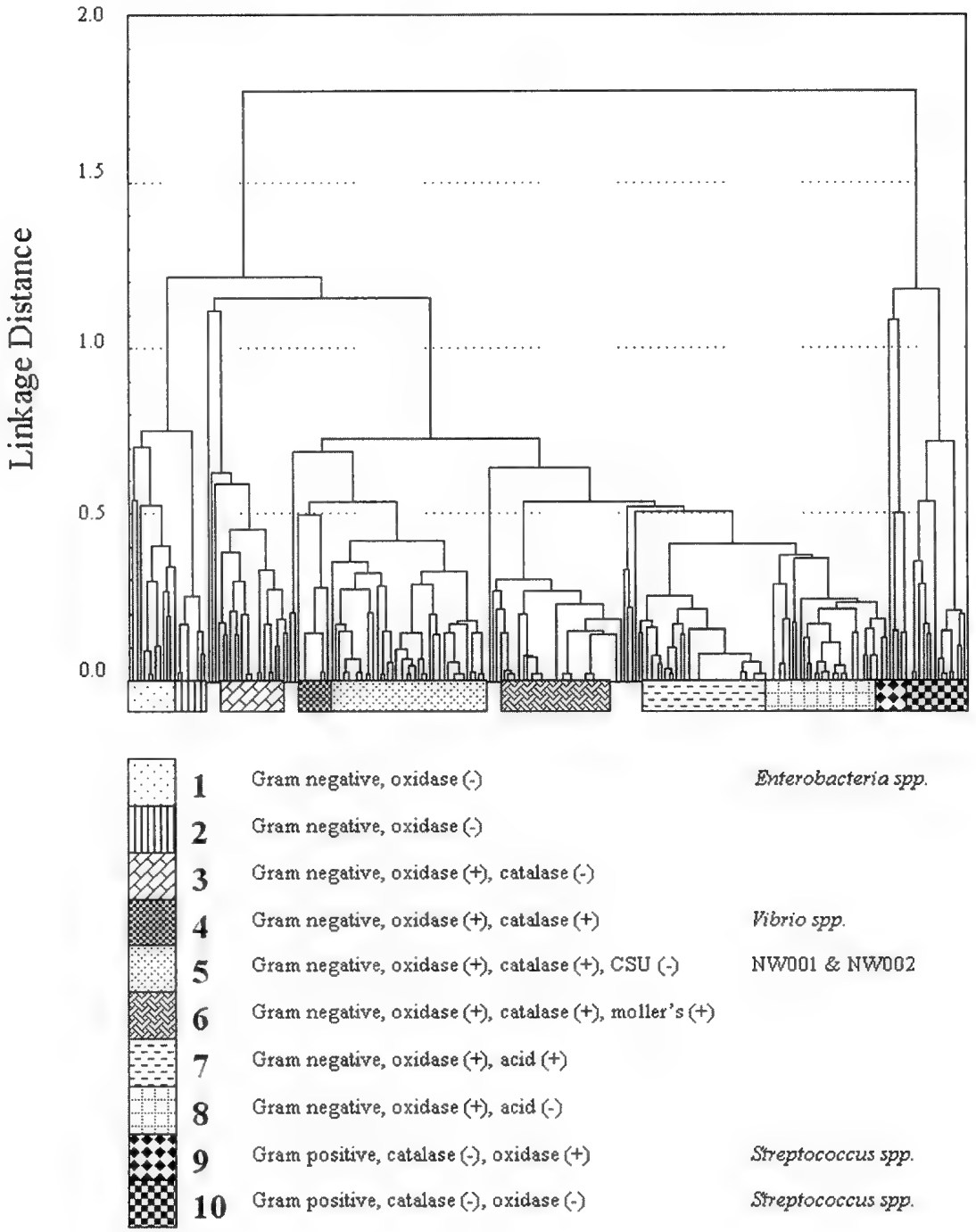

FIG. 6. Similarity dendrogram for isolates obtained from *R. odorabile.*

Phenotypic analysis of bacteria from the Caribbean sclerosponge, *Ceratoporella nicholsoni* revealed significant differences in sponge and seawater phenotypes (Santavy & Colwell, 1990). It is evident from the present study, that the sponges *Rhopaloeides odorabile* and VWF support taxonomically diverse microbial assemblages. High microbial diversity is not surprising when

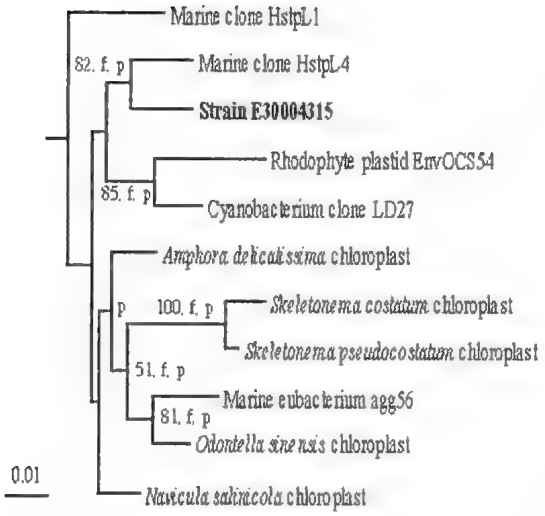

FIG. 7. Neighbour-joining tree for 631 bp of sequence obtained using cyanobacterial and plastid-specific primers from strain E30004315 isolated from VWF. Key: f and p indicate branches that were also found using the Fitch-Margoliash or maximum-parsimony methods, respectively. The numbers at the nodes are percentages (only values over 50% shown) indicating the level of bootstrap support, based on a neighbour-joining analysis of 1,000 re-sampled data sets. Scale bar represents 0.01 substitutions per nucleotide position.

considering that sponges derive their nutrition from filtering the ambient seawater. It has been demonstrated that marine sponges are capable of discriminating between food bacteria and bacterial symbionts. The mechanisms for this recognition are not clear but it has been postulated that sponge phagocytic cells do not recognise the capsule coating of symbionts (Wilkinson et al., 1984).

Eight predominant heterotrophic bacteria were evident in the culturable community isolated from VWF and these isolates were generally absent from the surrounding seawater samples. These culturable bacteria clustered in several phenons on biochemical analysis. The culturable community of VWF was more diverse than that observed in *R. odorabile* and showed greater fluctuations between individual sponges. One notable feature was the prevalence of cells resembling cyanobacteria within the VWF matrix, observed by microscopy. Also, eight strains of phototrophs were isolated from this sponge. It is postulated that VWF is a phototrophic sponge, deriving a component of its carbon budget from photosynthetic symbionts. Wilkinson (1992) has reported that many sponge phototrophs are morphologically flattened to increase surface area for interception of light. This is consistent with the structural morphology of VWF. In contrast, only a single phototroph was isolated from *R. odorabile* and cells with characteristic cyanobacterial morphology were not observed on microscopic examination of *R. odorabile* tissue.

The culturable bacterial community of *R. odorabile* was dominated by strain NW001, which comprised 74% of the total culturable bacterial community in this sponge but was consistently absent from the seawater samples. This is the first report of a single bacterium comprising such a high proportion of the culturable bacteria from a sponge. Previously, a specific bacterial symbiont was found in nine of ten sponges of two classes and seven orders, and a second symbiont was specific to the sponge *Verongia*, but only as one component of a large mixed bacterial community (Wilkinson et al., 1981). The relationship between NW001 and *R. odorabile* provides an ideal model system for investigating the relationship between this strain and its host sponge because this isolate is predominant and has a characteristic colony morphology which facilitates enumeration of strain NW001 based solely on colony morphology. Initial indications are that this relationship persists over spatial and temporal scales and is highly stable (work in progress). Although the relationship between NW002 and *R. odorabile* appeared less stable, NW002 was frequently the second most predominant culturable bacterium (after NW001) present in *R. odorabile*, and was present in the sponge tissue at much higher concentrations than detectable in the ambient water surrounding the sponges. Similarly, strains AB001-AB008 were consistently present in the sponge VWF at higher concentrations than detected in the surrounding seawater. The mechanism whereby the sponges acquire these symbionts is a topic of current research.

It appears as though the two sponges adopt different strategies for harboring their microbial communities. *Rhopaloeides odorabile* maintains the bacterial cells in the loose matrix of the mesohyl, whereas VWF appears to incorporate the cells into structures referred to as bacteriocytes. In VWF, the bacteria are also closely associated with the sand grains just below the cuticle. The reasons for these different approaches are uncertain but may relate to the structural composition of the sponge or the function of the bacteria within the sponge tissue. *Rhopaloeides odorabile* maintains

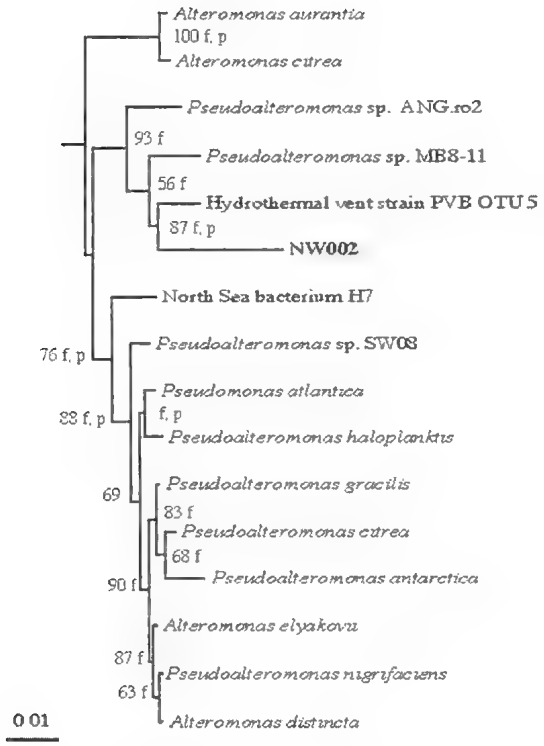

FIG. 8. Neighbour-joining tree for over 1,000 bp of sequence obtained using eubacterial-specific primers from strain NW002 isolated from *R. odorabile*. Key: f and p indicate branches that were also found using the Fitch-Margoliash or maximum-parsimony methods, respectively. The numbers at the nodes are percentages (only values over 50% shown) indicating the level of bootstrap support, based on a neighbour-joining analysis of 1,000 re-sampled data sets. Scale bar represents 0.01 substitutions per nucleotide position.

a bacterial community two orders of magnitude greater than that present in tissue of VWF.

Biochemical characterisation of all culturable isolates from VWF and *R. odorabile* was useful in clustering each of these assemblages into distinct phenons. In some cases, the presumptive identity of isolates could be deduced by comparison with type cultures which clustered in the same phenon. However, this approach must be used with caution because of the difficulty in identifying marine isolates based on criteria generally established for readily-culturable isolates of medical significance. In addition, many isolates scored negative against almost the entire biochemical profile, as is frequently the case with marine environmental isolates. Interestingly, two phenons from each sponge comprised Gram-positive bacteria, which made up approximately 10% of the total isolates in each case. Early studies of marine microbiology found that about 95% of marine isolates were Gram--negative (ZoBell, 1946) but recently 30% of the bacteria associated with a marine alga were found to be Gram-positive (Jensen & Fenical, 1995) and it is likely that the proportion of Gram-positive bacteria in most marine habitats has been underestimated (Jensen & Fenical, 1994). Gram-positive bacteria include actinomycetes, a group of particular importance in natural products discovery.

Because of the difficulties in accurately identifying marine bacteria by conventional biochemical characterisation, molecular techniques are the most appropriate for unequivocal identification of marine bacteria. A single isolate (NW002) from phenon 5 of the assemblage from *R. odorabile* was selected to demonstrate the utility of this approach and as a first step in the molecular identification of one isolate from each phenon. In addition, because of the difficulty in identification of marine microalgae by conventional techniques, phototroph isolate E30004315 was characterised by 16S rRNA sequence analysis of its plastid.

Sequence analysis of the plastid of microalgal strain E30004315 revealed that this plastid was most closely related to sequences of clones HstpL1 and HstpL4, cloned from a library of the uncultured microbes associated with the seagrass *Halophila stipulacea*, a ubiquitous seagrass from the subtidal zone of the Gulf of Elat (Weidner et al., 1996). Another close relationship was to clone OCS54, a plastid rRNA sequence from a natural phytoplankton population collected in the Pacific Ocean, off the mouth of Yaquina Bay, Oregon (Rappe et al., 1998). The identified microalgae with plastids clustering close to E30004315 fall in the genera *Odontella* and *Skeletonema* (Fig. 7). Microscopic examination of E30004315 revealed morphology consistent with identification as a microalga in the eukaryotic phytoplanton, with cigar-shaped cells about 10µm long.

Strain NW002 clearly belongs to the genus *Pseudoalteromonas*, a genus with many marine representatives, on the basis of the close phylogenetic relationship between this isolate and sequences of strains classified in the genus *Pseudoalteromonas*. The 16S rRNA sequence most closely related to that of NW002 was derived from a clone derived from a microbial mat at a hydrothermal vent site, the Loihi Seamount,

Hawaii and reported as an alteromonad (Moyer et al., 1995).

The percentage of culturable bacteria associated with these sponges was only 0.06% in VWF and 0.1% in *R. odorabile*. These percentages are considerably lower than the 3-11% culturable bacteria associated with the sclerosponge *Ceratoporella nicholsoni* (Santavy, et al., 1990), and may indicate that a high proportion of the bacteria associated with *R. odorabile* and VWF are obligate symbionts, requiring a close association with the sponge tissue to grow. These results illustrate the importance of molecular genetic techniques for total community analysis. Once more is known about the total microbial community associated with sponges, it may be possible to use this knowledge for rational selection of culture conditions appropriate for growth of additional, presently unculturable, strains. This study has resulted in an array of well-characterised microbes for natural products screening, in particular for important compounds known to be produced by these sponges. Compounds of potential pharmaceutical importance from *R. odorabile* include diterpenes (Kazlauskas et al., 1979) and rhopaloic acid A (Ohta, et al., 1996), although it is likely that these particular compounds are not of microbial origin (Thompson et al., 1987). VWF produces a potent antitumor compound lanolide. It is clear that marine sponges have the potential to be a major source of microbes for natural products screening programs.

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DISCOVERY AND SUSTAINABLE SUPPLY OF MARINE NATURAL PRODUCTS AS DRUGS, INDUSTRIAL COMPOUNDS AND AGRO-CHEMICALS: CHEMICAL ECOLOGY, GENETICS, AQUACULTURE AND CELL CULTURE. *Memoirs of the Queensland Museum 44: 76. 1999*:- Using chemical ecological clues, it is now possible to target habitats and eco-taxonomic groups of marine organisms to increase the likelihood of discovery of species which elicit natural compounds with chemotherapeutic or industrial application. Using the same clues, combined with Geographic Information System interrogation of the benthic geomorphology and oceanography associated with target species, it is possible to identify locations allowing recollection of species of interest. The information gained from both primary collections and focused recollections, provides the basis for hypothesis-driven experiments examining sustainable supply options for extracted target metabolites where synthesis is not practicable.

We describe recent results from an integrated multi-disciplinary programme designed to develop sustainable production options for a variety of marine natural products that have interesting biological activities. Three species of sponge from the genera *Lissodendoryx*, *Mycale* and *Latrunculia*, produce novel metabolites with anti-tumour activity. The natural abundance of each would not support a production industry based on wild harvest should their metabolites be required for drug production. Each has been successfully cultured in-sea demonstrating very good to excellent growth parameters. Each can be cultured with maintenance of target metabolite biosynthesis. In addressing the question of how to optimally produce target compounds, it has been necessary to examine a number of key biological issues pertaining to each species. These include genetic identity of populations supplying seed material, correlates with variable target metabolite biosynthesis in natural populations, origin of target metabolite biosynthesis (symbiont or sponge), and the efficacy of artificial production techniques (sea or land aquaculture or cell culture).

We conclude that the guess-work can now be taken out of artificial culture of sponges with a view to produce desirable natural products. It is possible to select for a high yielding culture stock and provide techniques to enhance biosynthesis or target metabolites. ❐ *Porifera, marine natural products, aquaculture, genetics, cell culture.*

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CHARACTERIZATION OF CALCIUM-BINDING MATRIX PROTEINS FROM DISTINCT CORALLINE DEMOSPONGES. *Memoirs of the Queensland Museum 44: 76. 1999*:- Calcified sponges played an important role as reef building organisms during different geological time periods. Living relatives of this group investigated here, *Spirastrella (Acanthochaetetes) wellsi*, *Astrosclera willeyana* and *Vaceletia n. sp.*, can be found in cryptic niches of indopacific coral reefs. The first known relatives of some of these sponges are known since the upper permian.The mode of biomineralization of the examined species seems to be extremely conservative, since they are phylogenetically very old and exhibit merely minor alterations in their calcareous skeletons. Each of the three species exhibits a unique type of basal skeleton with its own specific modifications of carbonate crystals. Each species was shown to have a specific array of calcium-binding macromolecules enclosed within its intraskeletal matrix. The proteins are separated by SDS polyacrylamide gel electrophoresis. A single protein was detected in *S. wellsi*, two proteins in *A. willeyana*, and four proteins in *Vaceletia n. sp.*. All proteins were characterized by their molecular weight and isoelectric point. The soluble matrix constituents of each species were tested for their potential to decrease precipitation of calcium and strontium carbonate, respectively, in a saturated solution. The findings strongly suggest that these soluble proteins function as the template for skeletal formation and are responsible for determining the particular type of calcium carbonate polymorphs. ❐ *Porifera, biomineralization, organic matrix, calcium-binding proteins, calcite, aragonite.*

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BIOLOGY OF THE MASSIVE SYMBIOTIC SPONGE *CLIONA NIGRICANS* (PORIFERA: DEMOSPONGIAE) IN THE LIGURIAN SEA

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Calcinai, B., Cerrano, C., Bavestrello, G. & Sarà, M. 1999 06 30: Biology of the massive symbiotic sponge *Cliona nigricans* (Porifera: Demospongiae) in the Ligurian Sea. *Memoirs of the Queensland Museum* **44**: 77-83. Brisbane. ISSN 0079-8835.

Cliona nigricans is a boring Atlanto-Mediterranean sponge, which on the Gallinara Island cliffs (Ligurian Sea, Italy), exhibits different growth forms: endolithic specimens bore the coralligenous cliff whereas massive specimens grow on the detritic bottom. In the latter habitat, large massive specimens of *C. nigricans* live partially burrowed in sediment. The sponges incorporate large amounts of sediment, selecting the greater size classes (>5mm). Several incorporated carbonatic fragments, particularly mollusc shells, are bored and crossed by canals of the aquiferous system. The distribution of the massive specimens of *C. nigricans* is affected by the distribution of coarser fractions in the sea bottom sediments. On the detritic bottom *C. nigricans* produces a large extension of secondary solid substrata, hosting a rich biocoenosis of sessile and vagile organisms. Differences in the structure of the aquiferous system between boring and massive stages are shown by corrosion casts, particularly in regard to the shape of exhalant canals. Boring forms possess cylindrical canals while those in massive specimens are moniliform. The density of the symbiotic zooxanthellae, evaluated by chlorophyll analysis of sponge papillae, is related to the seasonal solar radiation and depth. ❐ *Porifera, Cliona nigricans, growth forms, incorporation, selectivity, boring sponges, zooxanthellae, Ligurian Sea, Italy.*

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There are some species of boring sponges that develop different growth strategies, during their life cycles. After larval fixation, young boring sponges live endolithically with inhalant and exhalant papillae arising from the bored substratum (α form); in the following stage (β form), the papillae progressively form a thin sheet of sponge tissue; when the calcareous substratum is entirely etched away, the sponge grows into a massive form (χ) (Sarà & Vacelet, 1973).

The Gallinara Island (Ligurian Sea, W Mediterranean) hosts a dense population of *Cliona nigricans* which grows from the surface level to the detritic bottom (40-50m depth). The coralligenous cliffs are strongly eroded by *C. nigricans* (α and β forms) producing large tunnels which weaken and fracture the bioherme. At the base of the island cliffs, on the detritic bottom, the χ form of this species grows. The α and β forms are morphologically very different from the χ form, particularly in their exhalant papillae which, in massive forms, have oscular chimneys higher than 10cm. In spite of these morphological differences, electrophoretical analysis has clearly proven that the two forms belong to the same species (Bavestrello et al., 1996a).

All morphotypes of *C. nigricans* harbour zooxanthellae. This symbiosis is known to be present only in a small number of sponge species (Sarà & Liaci, 1964; Sarà, 1966; Rützler, 1985) whose boring ability has been correlated to the presence of the symbionts (Hill, 1996; Vacelet, 1981).

In this work we consider the relationships of the massive χ forms with the bottom sediment and their influence on the bottom communities in providing secondary solid substrata. Moreover, we compare the symbiont density in these sponges and the anatomy of their aquiferous system to those of boring specimens.

MATERIALS AND METHODS

Cliona nigricans was studied at Gallinara I. (Ligurian Sea), situated about 1.5km from the coast, with underwater cliffs reaching a maximum depth of 37m on the southern side, and a *Posidonia oceanica* bed located between the northern side of the Island and the coast (Fig. 1).

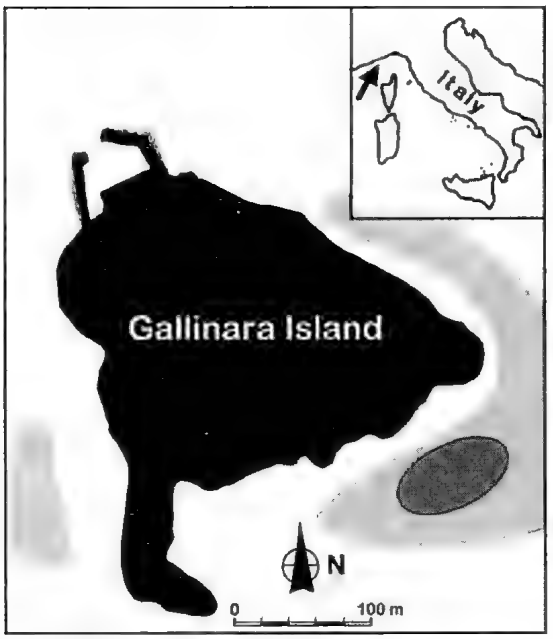

FIG. 1. Density of massive specimens of *Cliona nigricans* (gray areas) around Gallinara I. Key: dark grey >20 specimens/10m^2; light grey 5-10 specimens/10m^2.

The Island consists of greyish quartzitic rocks, together with pelitic layers and cretacic pudding stones (Balduzzi et al., 1994).

The density of massive *C. nigricans* specimens was determined along the eastern, southern and western sides of the islands (Fig. 1) on the detritic bottom at 40-45m depth. Densities were evaluated directly under water by counting the specimens present in a rectangular frame of 10m^2. The size of some specimens was estimated under water measuring the two main axis of their surface cleaned by sediments. Moreover, the thickness of sediments covering the sponges was measured.

The granulometric features of the bottom detritus (obtained by sieving) were studied on samples collected in areas where sponge density was higher and, for comparison, in areas where massive sponges were absent. In addition, the granulometric characteristics of the bottom sediments were compared to those of the sediments incorporated by the sponges by dissolution of sponge tissues in H_2O_2 (120 vol.). The environmental sedimentation rate was estimated by placing four conical sediment traps in the area with the highest sponge density.

To verify the role of C. *nigricans* in potentially harbouring macrobenthic organisms, specimens were photographed and collected for direct observation in the laboratory. Samples were enclosed in plastic bags and fixed directly underwater in 4% formalin.

Variation in the density of symbiotic zooxanthellae population in *C. nigricans* were determined from fresh tissue samples, taken from peripheral portions of sponges (especially papillae). Sampling took place at different seasons (with five collections made between October 1997 to August 1998), for all morphs and along a bathymetric transect (with five replicas per specimen at 5, 10, 20, 30, 37 and 42m depth). Spectrophotometric analyses of acetonic extracts of sponge tissue were conducted according to Gilbert & Allen (1973) to quantify chlorophyll-a concentrations.

Anatomical differences of the aquiferous system in different growth forms were evaluated using corrosion casts (Bavestrello et al., 1995a; Burlando et al., 1990) which were studied under stereomicroscope and, ultrastructurally, by SEM.

RESULTS

Large, massive specimens of *C. nigricans*, growing on the soft detritic bottom, are cushion-shaped or lobate, with a characteristic mamillate surface (Fig. 2C). Their maximal surface ranges from about 200-1000cm^2 and they are buried in the sediments up to 3-5cm deep. Their inhalant papillae are similar in size and shape to those of endolithic forms (Fig. 2A,B), whereas very high oscular chimneys (up to 10cm high) constitute the exhalant structures. In many specimens the inhalant papillae develop on the wall of the oscular chimney (Fig. 2D).

Tissues of these massive forms are very rich in incorporated bottom sediments which constitute 95% of the sponge dry weight. The mechanism of incorporation appears to be non-selective regarding the origin of foreign bodies: i.e. quartzitic or pelitic particles, rhodoliths, and organogenous detritus are collectively ingested (Fig. 3A-C). Nevertheless, the comparison between the granulometries of bottom sediment and those of sediments incorporated by the sponges, clearly indicates that *C. nigricans* actively selects the coarse fractions larger than 5mm diameter (Fig. 3D).

Corrosion casts demonstrate that calcareous fragments incorporated in the bodies of massive sponges are bored and often crossed by the canals

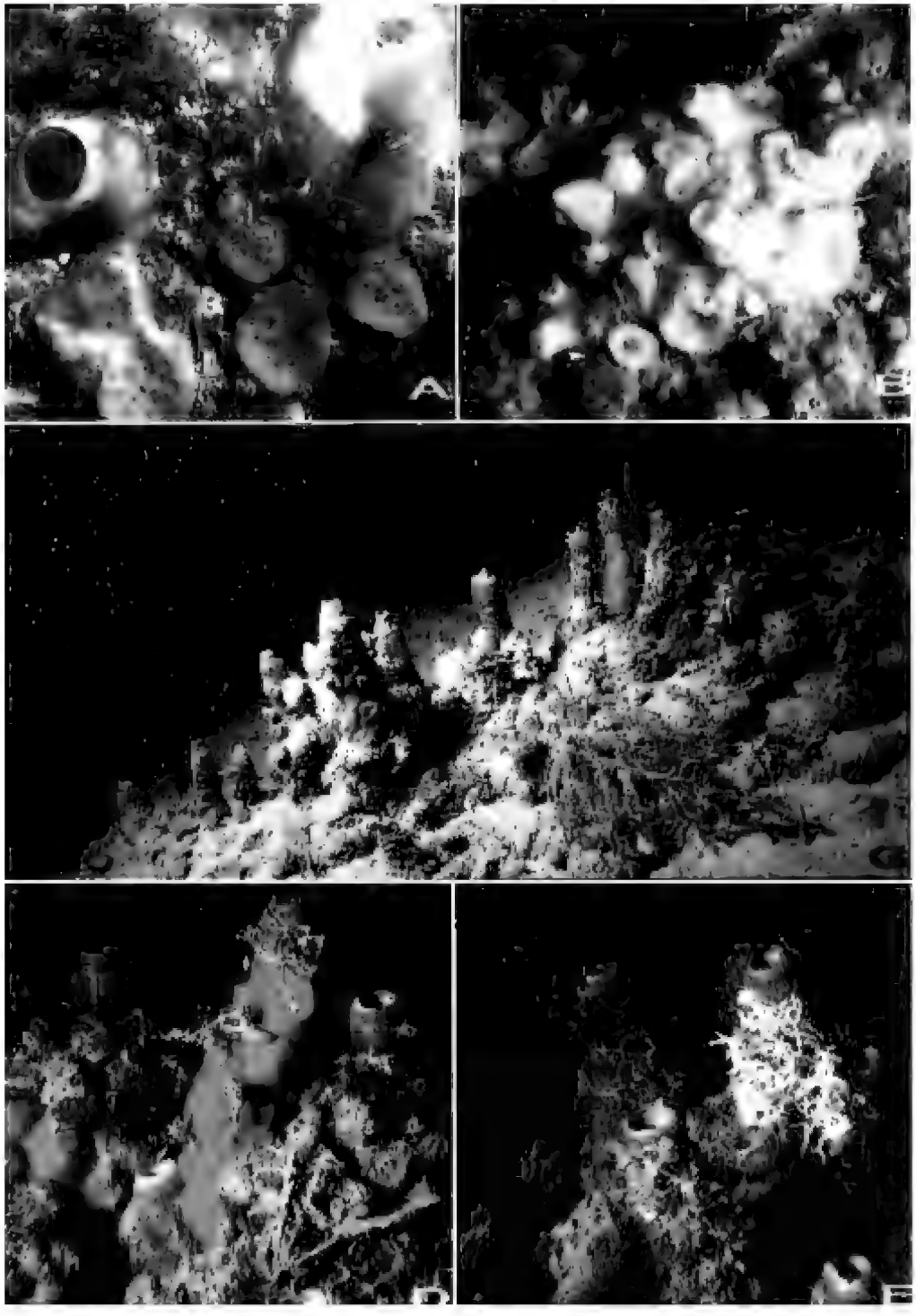

FIG. 2. *Cliona nigricans* specimens. A, Boring α stage. B, Boring β stage. C, Massive χ stage specimens on the detritic bottom characterised by high ocular chimneys and mamillate surface. D, Epibiotic bryozoan *Schizobrachiella* sp.). E, Epibiotic serpulid *Filograna* sp. (E) growing on massive specimens.

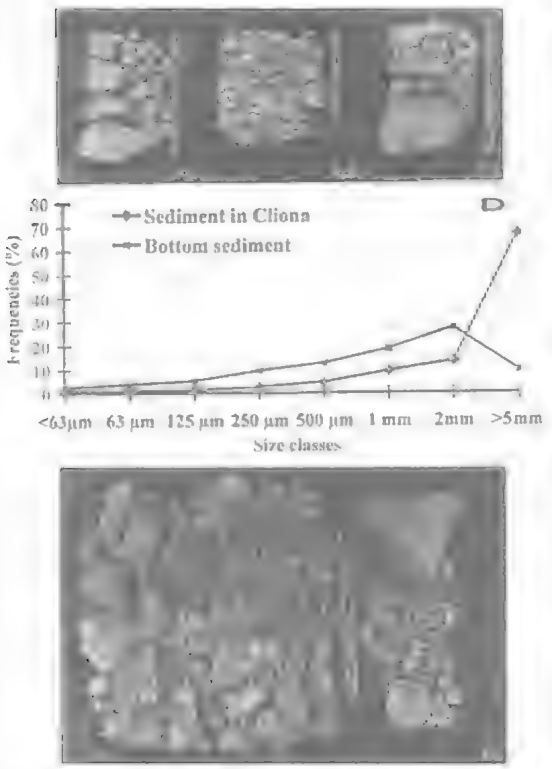

FIG. 3. Incorporation of foreign material by massive specimens of *Cliona nigricans*. A-C, Foreign material incorporated by different specimens. D, Size frequency distribution of material incorporated by sponges compared to that occurring in the surrounding sea bed. E, Fraction of the bottom sediment with a size >5mm occurring where sponge density is highest (left), compared to the same fraction in an area without sponges (right). Scale bars in cm.

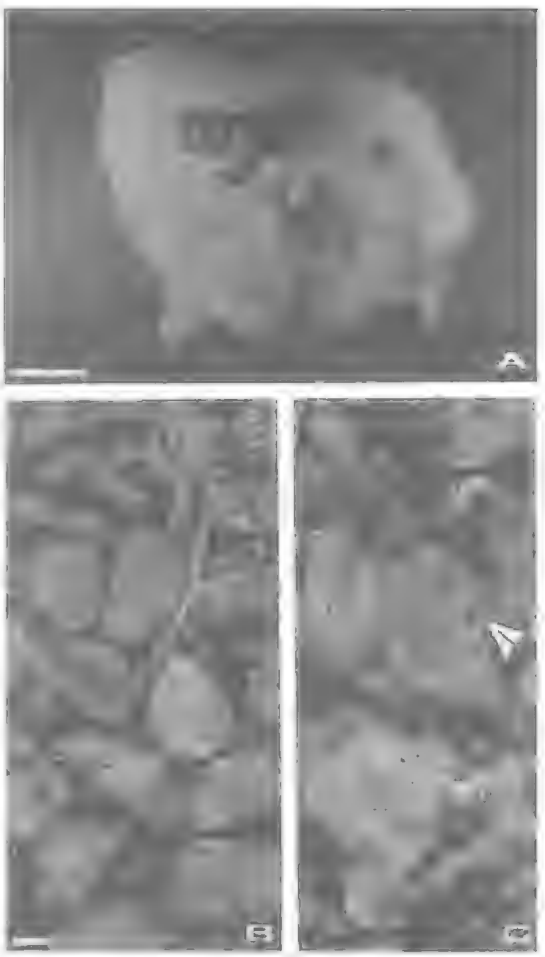

FIG. 4. Boring activity of massive specimens of *C. nigricans*. A, Portion of a corrosion cast of a massive specimen showing a calcareous fragment incorporated by the boring sponge and crossed by an exhalant canal. Scale bar 3mm. B-C, Comparison of the same granulometric fraction of the bottom sediments (B), with those incorporated by the sponges (C). The latter shows evidence of the perforations produced by the sponge (arrow). Scale bar = 8mm.

of the aquiferous system (Fig. 4A). A comparison between free bottom sediments and those entrapped within the sponge reveals erosion traces in the latter (Fig. 4B-C).

Coarse sediments incorporated by massive specimens derive from fragmentation of the overhanging cliffs, while the thin fraction of terrigenous origin, collected by the traps, reveals an average sedimentation rate of about 10kg/m^2/year.

The sponge population density is related to the amount of coarse sediment fraction present in the bottom sediments (Fig. 3E). In areas where sponges show a density greater than 20 specimens/10m^2 the coarse matter represents 10-15% of the bottom sediments. By comparison, sponges are scarcer (5-10 specimens/10m^2) in areas where the coarse fraction is 3-7% of the total, and they are absent where the coarse fraction is less than 3%.

On the soft bottoms of the Gallinara I. massive specimens of *C. nigricans* occupy a large surface on the soft bottom on which they constitute a secondary solid substratum, where a coralligenous-like assemblage lives. This assemblage (Fig. 2C-E) is mainly composed of sessile organisms such as other sponges, hydroids, anthozoans, bryozoans and serpulids that, in turn,

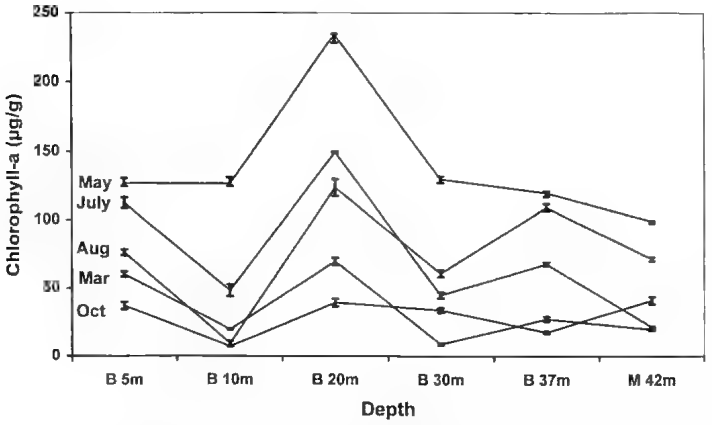

FIG. 5. Average chlorophyll-a concentrations during different periods of the year in specimens living at different depths (N = 5). Key: B = boring specimens; M = massive specimens.

support a vagile fraction represented mainly by nudibranchs, polychaetes, harpacticoids, amphipods and decapods (Table 1).

The concentration of symbiotic zooxanthellae does not vary significantly among massive and boring specimens, but rather exhibits a trend influenced both by season and depth distribution. In October the values are homogeneously low among the different growth forms and at different depths. In March and May these values progressively increase, then subsequently decrease in the following summer months. In all sampling periods a peak in values always occurs at 20m depth (Fig. 5).

Corrosion casts of the aquiferous system reveal differences between the massive and boring specimens in the shape of their exhalant canals. These canals are cylindrical in endolithic specimens (Fig. 6A) and moniliform in massive ones (Fig. 6B). Moreover, endolithic sponges differ from massive ones in the arrangement of choanocyte chambers, which are clustered inside the boring chambers. In massive forms the choanocyte chambers are homogeneously distributed in the sponge body.

DISCUSSION

It is probable that the initial stages of larval development in *Cliona nigricans*, as in all clionids (Sarà & Vacelet, 1973), are linked to the boring activity on a suitable substratum. In coastal detritic bottoms, however, the carbonate fragments are small, and sponge size exceeds the bored fragment very precociously. From this stage, sponge growth is linked to the incorporation of sediment into its tissue. It is also possible that massive sponges living on the soft detritic bottom originate from the boring specimens higher up on the cliffs which, through fragmentation of the substratum, fall down with a portion of the sponge tissue. In this case, the activity of the boring specimens of *C. nigricans* is an agent for asexual reproduction and spatial dispersal.

Our data indicate that massive specimens of *C. nigricans* select larger fractions (>5mm) of sediment and that high concentrations of these coarse sediments are necessary for successful sponge development. Cellular mechanisms which control this selection are still poorly known (Teragawa, 1986; Bavestrello et al., 1998). The selective ability of sponges to incorporate foreign matter is currently a subject of debate in the literature, with empirical support only recently available (Bavestrello et al.,1995b, 1996b).

Studies in chlorophyll-a concentrations in *C. nigricans* give some indication of the quantitative changes in the symbiotic community of zooxanthellae in relation to depth and seasonal variation. The zooxanthellae population correlates more to the seasonal cycle rather than to depth. Only in

TABLE 1. List of the main phyla living on massive *Cliona nigricans* as sessile epibionts. Key: + occasional; ++ common; +++ present on almost each specimen.

Phylum	Species	Abundance
Porifera	*Oscarella lobularis*	+
	Dysidea sp.	+
Cnidaria	*Clythia hemisphaerica*	+++
	C. lincaris	+++
	Paralcyonium sp.	+
	Caryophyllia smithi	++
Bryozoa	*Smittina cervicornis*	+
	S. mammillata	+
	Schizobrachiella sp.	+++
	Hippellozoon mediterraneus	+
	Schizomavella auricolata	+++
	Turbicellepora avicularis	+++
Polychaeta	*Filograna* sp.	++
	Serpula vermicularis	++
Tunicata	*Halocynthia papillosa*	+

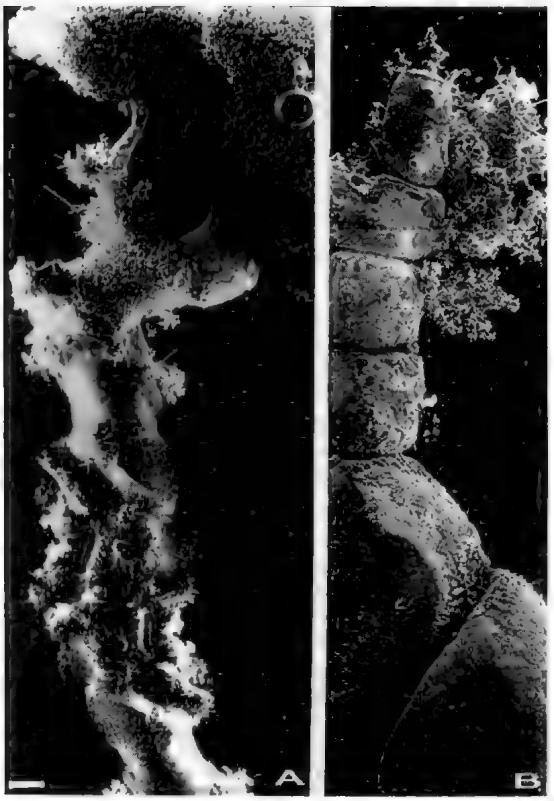

FIG. 6. Corrosion casts made of the aquiferous system of *C. nigricans*. A, Cylindrical shape of boring specimens. Scale bar 250µm. B, Characteristic moniliform structure of the exhalant canals of massive specimens. Scale bar = 100µm.

autumn, the density of zooxanthellae population in massive forms is similar to the density in all boring samples independent from depth. During spring zooxanthellae density increases in all of the sponge morphotypes reaching its maximum in May, in samples collected from 20m depth, and decreasing, subsequently, during the summer. These data indicate significant differences in the behaviour between the zooxanthellae of *C. nigricans* and the cyanobacteria of *Petrosia ficiformis* in the same area (Bavestrello et al., 1992). Cyanobacteria density in *P. ficiformis* is very sensitive to light variations related to depth and, from 10 to 40m, the chlorophyll concentration decreases by about four times. In contrast, the zooxanthellae population in *C. nigricans* remains relatively constant, suggesting a control of the host cells on their reproduction, as in other algae-invertebrate symbioses (e.g. Cook, 1983). Rosell (1993) showed how reproductive process (sexual and asexual) can modify the density of zooxanthellae in *C. viridis* populations through digestion or expulsion, and how at the end of the sexual process few zooxanthellae were present. Further data are necessary to clarify how reproduction periods affect the population of symbiotic zooxanthellae in *C. nigricans*.

Hill recently (1996) showed how symbiotic zooxanthellae are related to boring activities and growth of a tropical boring sponge (*Anthosigmella varians*). Vacelet (1981) also demonstrated that the most active boring sponges harbour zooxanthellae. Some authors (Hartman, 1958; Sarà & Vacelet, 1973) suggested a decrease in the boring power of endolithic versus massive growth forms, whereas our data indicate that even if fragments, incorporated by massive forms, are widely bored,both endolithic and massive morphotypes have comparable amounts of zooxanthellae.

Differences between the structure of the aquiferous system of boring and massive morphotypes were found through the study of their corrosion casts. The particular beaded shape of the exhalant canals in massive specimens may be determined by a system of contractile elements regularly disposed along the endopinacoderm of the canals.

The two alternative forms of *C. nigricans* (endolithic and massive) are linked to different habitats (coralligenous cliffs and detritic bottoms, respectively), and may be considered in the context of developmental modulation (Smith-Gill, 1983). Morphological variability is common among many sponge species (e.g. Barthel, 1991; Bavestrello et al., 1992), and is generally thought to be linked to variations in the intensity of water movement influencing food supply, and the probability of re-inhalation of filtered waste-water (Fry, 1979). The differences we observed in the behaviour of sponges in relation to their choice of substrata, and of their pumping physiology, between endolithic and massive form of *C. nigricans* stress this variability.

From an ecological perspective *C. nigricans* from Gallinara I. impacts on its environment in two alternative ways: 1) on the cliffs, where boring activity destroys the calcareous substrata, causing fragments to roll down onto the sea floor; and 2) on the detritic bottom, where this same material is gathered up by the massive specimens, which in turn provide a secondary hard

substratum that hosts an unusual biocoenosis, otherwise not present on the soft bottom.

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CARBON ISOTOPE TIME SERIES OF CORALLINE SPONGES FROM THE CORAL SEA, PHILIPPINES AND CARIBBEAN. *Memoirs of the Queensland Museum 44: 84. 1999:-* Live coralline sponges (*Ceratoporella nicholsoni, Astrosclera willeyana, Spirastrella* (*Acanthochaetetes) wellsi*) were collected from reef caves and deeper reef slopes of the Caribbean, the Visaya Sea (Philippines) and the Coral Sea (Great Barrier Reef). The specimens were dated by either radiocarbon or uranium-thorium methods. Age ranges were from 200-600 years. We tested the reproducibility of $\delta^{13}C$ values measured on the aragonite of *Ceratoporella nicholsoni* by investigating variations along single layers of a well-laminated specimen. We also compared values measured on the outermost layers of several specimens. The reproducibility for $\delta^{13}C$ is excellent in most cases. Only few samples show depletion by up to 0.2 permil. Two parallel transects through a specimen of *Astrosclera willeyana* also display excellent reproducibility of $\delta^{13}C$ values. All specimens show the well-known industrial decline in $\delta^{13}C$ values starting ca. in 1850 A.D (e.g. Druffel & Benavides, 1986; Böhm et al., 1996). In comparing the magnitude of this decline measured in our samples and in $\delta^{13}C$ of atmospheric CO_2 we can estimate the local degree of isotopic equilibration between atmosphere and sea-water. We find values range from 40% of the atmospheric change at the Great Barrier Reef and in the Philippines to 65% in Jamaica. For each site we compared the preindustrial $\delta^{13}C$ from total CO_2 (DIC) of the surface water, calculated from our sponge records, with published phosphate concentrations. The values agree with a high input of nutrient-rich subsurface water at the Philippine site and at the Great Barrier Reef. At the Great Barrier Reef local upwelling at the reef front has been reported. However, the measured $\delta^{13}C$ values are much lower than expected for average phosphate concentrations. Either the upwelling is much more intense than assumed, or the Astrosclera record is affected by secondary processes and/or a vital/kinetic effect. ❐ *Porifera, coralline sponges, Philippines, Great Barrier Reef, Caribbean.*

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INCORPORATION OF INORGANIC MATTER IN *CHONDROSIA RENIFORMIS* (PORIFERA: DEMOSPONGIAE): THE ROLE OF WATER TURBULENCE

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Cerrano, C., Bavestrello, G., Benatti, U., Cattaneo-Vietti, R., Giovine, M. & Sarà, M. 1999 06 30: Incorporation of inorganic matter in *Chondrosia reniformis* (Porifera: Demospongiae): the role of water turbulence. *Memoirs of the Queensland Museum* **44**: 85-90. Brisbane. ISSN 0079-8835.

The role of sedimentation and sea conditions in relation to the amount of the foreign matter (sand grains and opaline sponge spicules) present in the body of the demosponge *Chondrosia reniformis* was evaluated monthly at two sites, each characterised by different sedimentary conditions along the rocky cliff of the Portofino Promontory (Ligurian Sea). Contrary to the process in keratose ('horny') sponges, the mineral particles incorporated by *Chondrosia* are subjected to an evident turnover probably linked to its unusual ability to dissolve quartz. The quantity and size of the particles taken up by the sponge are linked to environmental sedimentation and sea conditions. These data indicate that settlement of particles on the sponge is affected by the stickiness of the sponge's mucous surface. The large amount of quartz grains continuously incorporated and dissolved by *Chondrosia*, suggests a possible role played by the sponge in the local silica flux in shallow coastal waters. ❐ *Porifera, foreign matter, mineral selectivity, uptake, water turbulence, silica.*

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Sedimentation on rocky bottoms influences the distribution of organisms, impacting significantly on larval settlement and its further development, and compromising the filtering structures of filter feeders, even with the extreme result of total exclusion from their habitat (Loosanoff & Tommers, 1948; Wilber, 1971; Rogers, 1990). Porifera, living under high sedimentation regimes, might also be subjected to both abrasion by coarse sediment particles and occlusion of inhalant pores by fine ones (Sarà & Vacelet, 1973; Verdenal & Vacelet, 1985). The filtered water volume decreases proportionally to the amount of particulate matter present in the water column; e.g. in *Aplysina* (=*Verongia*) *lacunosa* (Gerodette & Flechsig, 1979). Sponges can live in oligotrophic waters owing to their high filtering efficiency, but cannot survive for long periods of reduced pumping (Reiswig, 1974). Some species have developed defense mechanisms against high sedimentation, as in the fresh-water species *Ephydatia fluviatilis,* where amoeboid cells of the exopinacoderm have endocytosis capabilities (Willenz & Van de Vyver, 1982) and can remove foreign particles (Harrison et al., 1985). Several species, such as the keratose sponge *Dysidea etheria,* can select sedimentary particles from their habitat, incorporating proper-sized ones in their primary fibers and removing others through the selective action of their external amoeboid cells (Teragawa, 1986a, 1986b).

Chondrosia reniformis does not produce its own spicules but engulfs foreign siliceous materials (i.e. siliceous sponge spicules present in the water column and sand grains), into its collagenous ectosome. Moreover, it recognises the mineralogical features of particulate material, dissolving quartz particles and reducing their original size (Bavestrello et al., 1995a, 1996, 1999).

The aim of this study is to explore the relationships between the amount of allochtonous matter engulfed by *C. reniformis* during an annual cycle, comparing this to the different sedimentation conditions, which are closely related to the local sea conditions in two different sites of the Portofino Promontory (Ligurian Sea, Tigullio Gulf, Italy): Punta del Faro and Paraggi Bay (Fig. 1).

These stations are well known from a biocoenotical (Tortonese 1961; Morri et al., 1986)

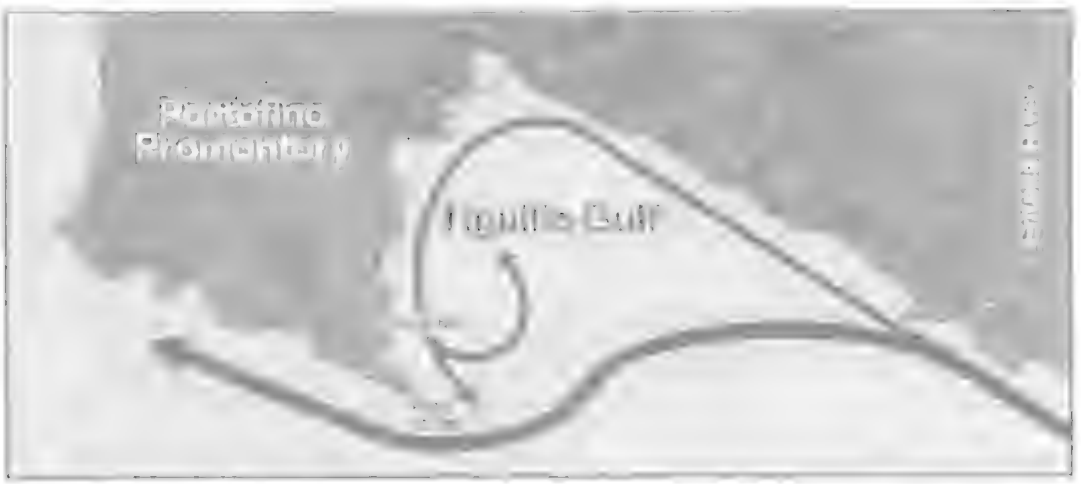

FIG. 1. Schematic figure showing the main current patterns in the studied area. At Punta del Faro, the current from the Golfo Tigullio meets the main cyclonic stream of the Ligurian Sea. Paraggi Bay represents a decantation area consequent to an eddy.

and a sedimentological point of view (Bavestrello et al., 1991; 1995b). The sedimentation rate is about seven times higher at Paraggi Bay than at Punta del Faro, owing to differenees between their local hydrodynamic features (Esposito & Manzella 1982; Marullo et al., 1985). In fact, Paraggi represents a decantation area, while Punta del Faro is the meeting point of two currents, one from the Ligurian Sea and the other one flowing outwards from the Tigullio Gulf (Fig. 1).

MATERIALS AND METHODS

At Punta del Faro, where the cliff ends at 55m depth, specimens of *C. reniformis* were sampled monthly by SCUBA diving during March 1994-June 1995, at depths of 3m, 12m and 25m. At these last two depths two sediment traps, as described by Bavestrello et al. (1991), were installed to collect the fraction of sediments available for sponges. At Paraggi Bay, where the cliff ends at 25m depth, sponges were collected from 3m and 15m depths, and a sediment trap was installed at this last depth only. At both localities, a superficial (3m depth) sediment trap was also installed, but strong wave action prevented a sufficient continuity in data collection at this station.

At each station, $1cm^2$ fragments of the sponge ectosome were collected monthly from six specimens. To analyze quantity and granulometry each fragment was dissolved in boiling hydrogen peroxide (39% weight/volume; about 130 vol.). The dissociated foreign material was centrifuged at 5000G for 5mins, washed twice in 95% ethanol, resuspended in 0.5ml of 100% ethanol, and finally two subsamples of 0.1ml were mounted on two slides. All particles (sand grains and spicules) were counted on each slide. The main axis of 100 sand grains per slide from Punta del Faro specimens was measured using a GRAPHTEX KD 4300 digitiser connected to an IBM PC. The area of incorporated particles was expressed per square centimeter of sponge surface.

Sediments collected from traps were evaluated monthly as dry-weight after combustion at 550°C for 4hrs in order to reach the inorganic fraction. Three slides were prepared from each sediment sample to collect the granulometric data.

Wave height data (cm above the free sea surface) were kindly provided by the Meteorological Observatory of Chiavari. Measurements of wave height commenced 10 days prior to each sampling date in order to compare trends. This period was chosen after initial trials of 7, 10 and 15 days prior to sampling, as it provided the best comparison between environmental conditions and collected sediments.

Additionally, investigation of the sponge ectosome was conducted by SEM analyses to evaluate morphological relationships between sponges and settled sediments. Samples were collected and fixed underwater in 2.5% glutaraldehyde. After rinsing in artificial seawater, samples were dehydrated in an ethanol gradient, followed by critical-point drying in a CO_2 Pabish CPD apparatus. They were mounted on stubs with

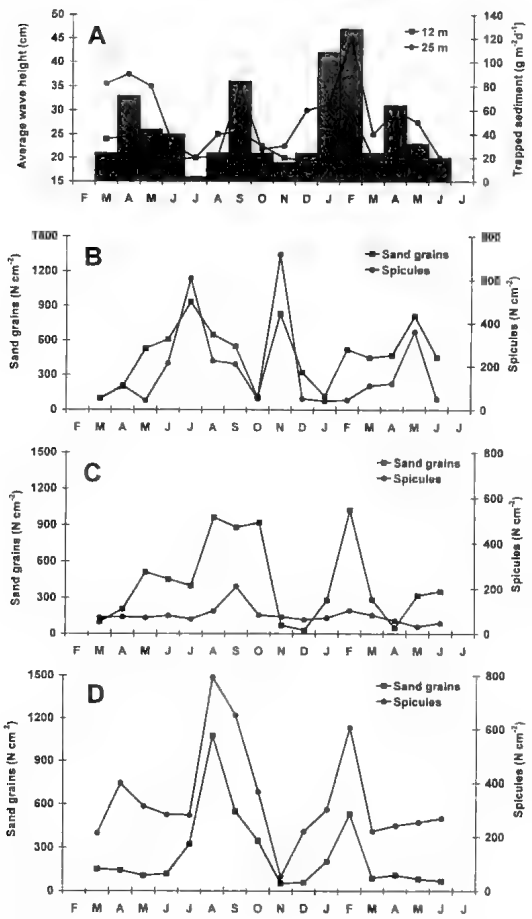

FIG. 2. Punta del Faro. A, Relationship between annual trend in sea conditions (histogram) and sediments collected by traps at 12 and 25m depths. B-D, Foreign matter (spicules and sand grains) incorporated by *Chondrosia reniformis* at 3, 12 and 25m depths, respectively.

silver conducting paint, sputter-coated with gold-palladium in a Balzer Union Evaporator, and observed using a Philips EM 515 electroprobe microscope.

To estimate the silica production by *C. reniformis* in the studied area, the sponge abundance and its surface were evaluated by visual census along 10 vertical 1m belt transects from the base of the cliff of the Promontory (50m depth) to the sea surface, following Hiscock's (1987) method.

RESULTS

At Punta del Faro, a high energy site, the amount of sediments collected by traps was directly related to the sea conditions at both depths (Fig. 2A). Sand grains and spicules (number cm^{-2}) incorporated by *Chondrosia reniformis* at 3m depth, peaked during periods of calm sea and declined during rough seas (Fig. 2B). Conversely, at intermediate (12m) and deep (25m depth) stations, higher quantities of sand and spicules were incorporated by the sponge during periods of rough seas, when sediment availability was higher (Fig. 2C-D).

Similarly, at Paraggi Bay, a decantation site, the amount of sediments collected by traps was strongly related to the sea conditions (Fig. 3A). Both sponge stations (at 3m and 15m depths) showed the same phenomenon as did the most shallow station at Punta del Faro: high values of incorporated particles were recorded during calm periods (Fig. 3B-C), even if the available sedimentary material was greater during periods of rough seas, as shown by our data on the trapped matter (Fig. 3A).

The granulometries of the incorporated sand grains by *C. reniformis* at Punta del Faro showed a similar trend for all depths sampled (Fig. 4). Average values ranged between 18-51µm diameter, with maxima occurring in July and November-December and minima occurring mainly from August to October and during winter. Comparison between these granulometries and sea conditions reveals an inverse relationship: large particles were present exclusively following periods of calm water.

SEM observations on the intact sponge surface showed that numerous crystals, organised in spherical-like balls (of about 5-15µm), and enveloped by a thin mucus web, emerge from the sponge ectosome (Fig. 5). Electroprobe analysis of crystals (indicating silica as the major constituent) and their shape, allowed us to conclude that these are quartz crystals.

DISCUSSION

Many demosponges are able to incorporate allocthonous inorganic material into their skeletons, a mechanism that is generally considered to provide additional strength to their organic fibrous skeleton. This phenomenon occurs most widely in the 'horny' keratose sponges (Lendenfeld, 1889; Teragawa, 1986a; Pronzato et al., 1998), comprising the orders Dictyoceratida, Dendroceratida and Verongida. In keratose sponges the uptake seems to be irreversible, since foreign matter is cemented into primary fibers. Conversely, *Chondrosia reniformis* shows an evident turnover

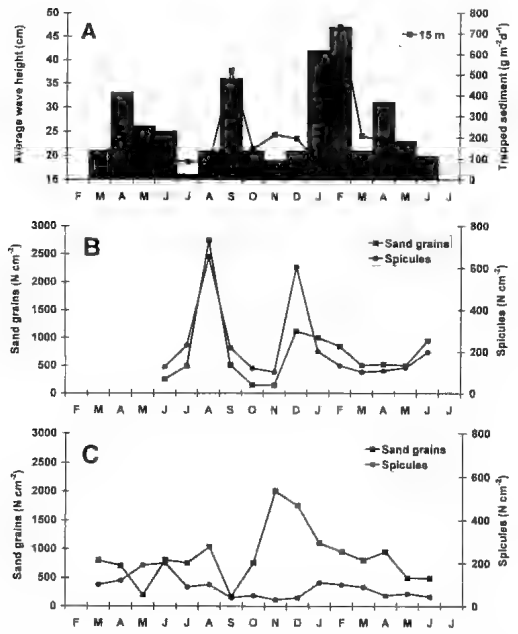

FIG. 3. Paraggi Bay. A, Relationship between annual trend in sea conditions (histogram) and sediments collected by the trap at 15m depth. B-C, Foreign matter (spicules and sand grains) incorporated by *Chondrosia reniformis* at 3 and 12m depths.

of incorporated foreign material and a capability to discriminate amongst the incorporated particles. This finding opens new perspectives on sponge behaviour. Although influenced by environmental parameters, these phenomena suggest a continuous utilisation of the incorporated matter as evidenced by the quartz dissolution ability (Bavestrello et al., 1995a), and the production of quartz 'pellets' on the ectosome.

Annual trends in the amount and size of sedimentary matter incorporated by *C. reniformis* appear to depend mainly on the local sea conditions and on the sponge etching. During calm periods, mainly in shallow waters, the sponge also uptakes large particles, as suggested by the inverse relationship between particle size and sea conditions. These phenomena are most evident in the shallow stations, where the highest amounts and largest sizes of incorporated foreign materials are present, corresponding to periods of calm waters. Conversely, during rough periods, the sponge surface is not sticky enough to retain large particles and consequently the quantity and size of engulfed matter decrease. In deeper water, where wave disturbance is reduced and resuspension processes are higher, populations of *C. reniformis* respond to these environmental conditions, incorporating higher amounts of siliceous matter. This is evident at Paraggi Bay, a more protected site than Punta del Faro, where swell conditions are frequent. In this way, it is possible to assume that sea conditions influence this phenomenon in two ways: on one hand, rough sea conditions can limit the uptake of particles, where the effect of waves action is strong, but on the other hand, the same conditions increase the availability of sediment material, owing to resuspension processes. This causes a higher amount of incorporated sediments in sponges living in deeper waters, where wave action is not strong enough to detach particles from the sponge surface.

In *C. reniformis* the mechanism of incorporation of inorganic matter involves different physical, mineralogical, and biological aspects: the settled particles are transferred, at variable speeds, to special areas of the sponge ectosome where they are quickly engulfed and, after incorporation, the collected material remains scattered in the fibrous ectosome, where particle sizes are re-elaborated (Bavestrello et al., 1995a, 1996, 1999).

Selectivity in the incorporation of foreign bodies in sponges has long been debated (Haeckel, 1872; Schulze, 1879; Lendenfeld, 1889; Sollas, 1908; Shaw, 1927; Teragawa 1986a). The uptake of particles in *C. reniformis* seems to be determined by an active selection of the minerals (Bavestrello et al., 1998b), and a passive one regarding their size. In agreement with Schulze's hypothesis (1879), it is possible that the uptake

FIG. 4. Relationship between annual trends in sea conditions (histogram) and granulometries of sand grains incorporated by *Chondrosia reniformis* at 3m (circles), 12m (squares) and 25m (triangles) depths at Punta del Faro (Portofino Promontory).

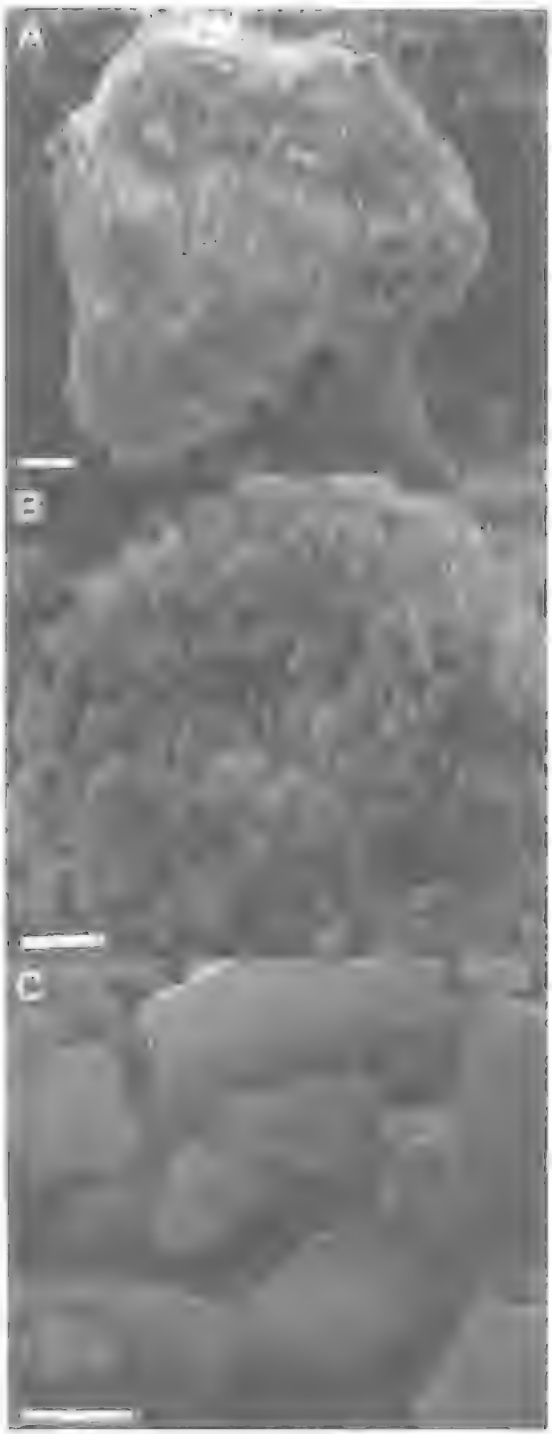

FIG. 5. SEM photographs, at three different magnifications (A-C), of spherical-like structures showing quartz particles expelled by the ectosome and enveloped by a thin mucus web. Scale bars: A = 11mm; B = 9mm; C = 2 mm.

mechanism is determined by the interaction between the stickiness of the sponge surface and the intensity of water movement, and that the biological activity of the sponge towards the quartz particles affects the granulometric trend and the amount of incorporated sediments.

An important consequence of this unusual behaviour is the output of dissolved silica, thus biologically available to other organisms. Under experimental conditions (Bavestrello et al., 1995a; 1996), with excess quartz grains available on its ectosome, *C. reniformis* engulfs about 0.2mg cm^{-2} day^{-1} of quartz and produces 0.1mg cm^{-2} day^{-1} of dissolved silica. On the Portofino Promontory cliff, the average daily quartz availability, evaluated with sediment traps, is 0.4mg cm^{-2}. This suggests that quartz availability is not a limiting factor, allowing us to hypothesise that the sponge maintains the same ratio of incorporation and dissolution shown in laboratory experiments.

Considering that the population density of *C. reniformis* along the Portofino Promontory is about 5,000cm^{-2} per meter of coast, and that the Promontory coast is about 13km long, it is possible to estimate a production of dissolved biologically available silica of about 2106g yr^{-1}.

Even if the most important contribution of silica to the Mediterranean Sea comes from the Gibraltar Strait (De Master, 1981), input from rivers into the Mediterranean, although generally modest, may also be locally important. In the Tigullio Gulf (Ligurian Sea), the Entella River, with an average annual flow rate of 14.8m^3 sec^{-1}, carries about 214x106g yr^{-1} of dissolved silica. However, the production by populations of *Chondrosia* at the Portofino Promontory, of about 2106g yr^{-1}, suggests that this species has a significant role in silicate turn-over, in rocky littoral areas, far removed from river input.

ACKNOWLEDGEMENTS

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CARBON ISOTOPE HISTORY OF CARIBBEAN SURFACE WATERS REVEALED BY CORALLINE SPONGES. *Memoirs of the Queensland Museum 44: 91. 1999*:- Live coralline sponges of the species *Ceratoporella nicholsoni* were collected from caves of north Jamaican reefs (20m depth) and from the deeper slope of Pedro Bank (125m depth). These sponges build a very dense aragonitic basal skeleton in apparent isotopic equilibrium with ambient water. Uranium-thorium dating of four specimens resulted in ages of 450-600 years. Within that timeframe, the sponge skeletons provide a continuous carbon isotope record, which starts at the end of the medieval warm period (1400AD) and covers the 'Little Ice Age' (about 1550-1850AD), as well as the industrial period (since ca. 1850AD). With a sample resolution of 0.7mm and growth rates of 0.2-0.4mm/year the temporal resolution is about 2-4 years. The carbon isotope records show an excellent linear correlation with the atmospheric pCO_2 history, as recently reconstructed from Antarctic ice cores (Etheridge et al., 1996). We find no significant difference between the preindustrial and the industrial regression slopes (-0.013 permil/ppm) which agrees with a common mechanism for the observed surface water carbon isotope variations, i.e. addition/removal of isotopically 'light' organic carbon to/from the atmosphere-surface ocean-biosphere system. The 'Little Ice Age' is characterized by a slight increase of $\delta^{13}C$ values (+0.1 permil), peaking around 1700AD During the same period, pCO_2 was about 6ppm lower than during the medieval warm period. Both can be explained by an increase in the terrestrial organic carbon reservoirs or in oceanic productivity. The Pedro Bank specimen, collected from the uppermost thermocline, shows only a dampened $\delta^{13}C$ increase during the Little Ice Age and a slightly subdued industrial $\delta^{13}C$ decline. This is expected because of the greater influence of deep-water at this depth. A comparison of the observed variation of marine $\delta^{13}C$ values and $\delta^{13}C$ of atmospheric CO_2 included in Antarctic ice allows one to constrain the maximum global average cooling of the ocean surface layer during the Little Ice Age to ca. -0.7K (possible range 0 to -2K). Further comparison to the simultaneous pCO_2 decrease of 6ppm suggests an even smaller cooling. Alternatively, an enhanced oceanic export productivity could partly explain the observations. ❐ *Porifera, carbon isotope history, coralline sponges.*

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TIME-LAPSE STUDIES OF SPONGE MOTILITY AND ANATOMICAL REARRANGEMENTS. *Memoirs of the Queensland Museum 44: 91. 1999*:- Sponges have a general reputation as sessile and static animals, but this view has been contradicted by time-lapse microscope studies of live intact sponges belonging to several taxa (2 freshwater and 5 marine genera). These studies have demonstrated that adult sponges form leading margins made of crawling cells (pinacocytes and mesohyl cells), and these crawling margins appear capable of generating shape changes and locomotion of the entire sponge. These together with tracing studies have shown that sponges can move up to 160µm/hr (4mm per day). Observed sponges also display continuous cell movements and anatomical rearrangements in their marginal regions. These rearrangements produce slow continuous changes in the spicule skeleton and in the canal systems. Both whole-sponge motility and the internal rearrangements appear to be strongly affected by factors such as substratum adhesiveness, grooves, internal tensile forces, and water flow patterns. These ongoing changes may be an important source for plasticity in a sponge's life history. ❐ *Porifera, anatomy, cells, crawling, locomotion, motility, anatomical rearrangement, time-lapse.*

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DO CARIBBEAN SPONGES HAVE PHYSICAL DEFENSES ? *Memoirs of the Queensland Museum 44: 92. 1999*:- Sponges are conspicuous members of the Caribbean marine ecosystem, but are preyed upon by a very select group of consumers called spongivores. Like other sessile reef invertebrates such as ascidians and octocorals, sponges possess a variety of novel secondary metabolites and as well as mineral and organic skeletal components. Several studies have shown that sponges possess chemical defenses that inhibit feeding by browsing generalist fish, but no study to date has demonstrated that sponge skeletal components deter predation. Sponges are soft-bodied and seem to lack an obvious physical defense, such as a mineralized shell. However, the tissues of most sponges often contain a collagen-like substance called spongin and sharp siliceous spicules in high concentrations. Spicules serve as important structural components by increasing tissue rigidity and could potentially act as a defense by irritating the mouth parts and the digestive system of predators. Calcified structures, similar in size to spicules, from octocorals and algae have been shown to reduce feeding by fish and invertebrates. Surprisingly, field and laboratory aquarium assays of sponge spicules employing predatory reef fish did not support a defensive function. Consumption by reef fish was reduced only when spicules were assayed using foods of low nutritional quality. In assessing the chemical defenses of Caribbean sponges, 31% of the species we studied possessed organic extracts palatable to reef fish. Interestingly, many of these undefended sponge species are abundant and consumed only by spongivores. Sponges lacking a chemical defense may be protected from generalist predators by having tissues of low nutritional value. Protein, carbohydrate, lipid, ash, and caloric content of 71 Caribbean sponge species were measured to investigate the relationship between chemical defense and nutritional value. Except for lipid content, no significant differences in nutritional quality were found between chemically defended and undefended species. Sponges lacking a chemical defense may rely on tactics other than a physical or 'nutritional' defense, such as faster growth rates, to avoid predation by generalist consumers. ❐ *Porifera, chemical defenses, physical defenses, spicules, silica, nutritional quality, predatory-prey interactions, Caribbean reef ecosystems.*

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SPONGE DISTRIBUTION AND LAKE CHEMISTRY IN NORTHERN WISCONSIN LAKES: MINNA JEWELL'S SURVEY REVISITED

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Colby, A.C.C., Frost, T.M. & Fischer, J.M. 1999 06 30: Sponge distribution and lake chemistry in northern Wisconsin lakes: Minna Jewell's survey revisited. *Memoirs of the Queensland Museum* **44**: 93-99. Brisbane. ISSN 0079-8835.

Minna Jewell conducted an extensive survey of the regional distribution of freshwater sponges in Northern Wisconsin, USA, during the 1930's, and examined factors that controlled the occurrence of sponges. We returned to 18 of her original 102 study lakes in 1996-97 to evaluate the long-term stability of the sponge distribution patterns that she reported. Comparisons of Jewell's data and our recent survey reveal a decline in the distribution of *Spongilla lacustris* in N. Wisconsin lakes during the past 60 years. Jewell had originally reported *S. lacustris* present in 10 of the 18 lakes that we re-visited. As of 1996, we were unable to find *S. lacustris* in 5 of these 10 lakes. In addition, we observed only 1 invasion by *S. lacustris* in a lake that previously had not contained this species. To test how effectively four chemical variables reported by Jewell (pH, colour, conductivity and SiO_2) could predict the distribution of *S. lacustris*, we applied a discriminant model to the historical data set. Based on these four variables, we found that discriminant models poorly predicted sponge distribution patterns in Jewell's original survey lakes and in 17 additional lakes surveyed in 1996. Our analyses indicate that *S. lacustris* can grow under a wide range of chemical conditions and suggest that other environmental variables are probably influencing sponge distribution in N Wisconsin lakes. ❐ *Porifera, ecology, freshwater sponges, fauna survey, Spongilla lacustris, Wisconsin.*

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Freshwater sponges are present in many aquatic ecosystems and may comprise a major component of a lake's benthic community (Frost, 1991). Currently, 27 species of freshwater sponges have been identified in North America (Jewell, 1959; Penney & Racek, 1968; Harrison, 1974; Frost, 1991; Ricciardi & Reiswig, 1993). Most of these species have been reported from the N. United States and S. Canada, and regional distribution patterns indicate that biogeographic conditions may restrict the distribution of some sponge species (Penney, 1960; Penney & Racek, 1968; Jones & Rützler, 1975; Frost, 1991). At a more local scale, freshwater sponge distribution is influenced by environmental conditions within a particular lake or stream, however the relationship between the distribution of different sponge species and these environmental variables is not well understood.

In a classic and unusually detailed study for the times, Minna Jewell (1935, 1939) investigated the distribution of freshwater sponges in 102 lakes in the Northern Highland Lake District of Wisconsin, as a contribution to the comparative limnological efforts of Birge, Juday, and their co-workers (Frey, 1963). Jewell identified 10 different sponge species and related their distribution to chemical variables in lakes. For each lake Jewell (1935, 1939) recorded dissolved oxygen, free- and bound- CO_2, pH, residue, SiO_2, conductivity, colour, and secchi depth. Results of her study indicated considerable variation in habitat requirements among sponge species and reported some level of correlation between abiotic environmental variables and species' distributions.

We revisited Jewell's efforts to examine the long-term stability of sponge distributions, and applied more modern analytical techniques to her original dataset. Of the ten sponge species reported by Jewell, *Spongilla lacustris* was by far the most prevalent. It occurred in 76 of the 102 lakes sampled, and was distributed throughout the entire range of abiotic conditions surveyed. Because of the widespread distribution of this species in Northern Wisconsin lakes (Jewell, 1935), we re-surveyed a subset of Jewell's original study lakes to determine if *S. lacustris* occurred in the

TABLE 1. Distribution of *Spongilla lacustris* in 18 Northern Wisconsin lakes during Jewell's (1935) and 1996 surveys. Water chemistry data is presented for 1996 survey. Key: 0, sponges absent; +, sponges present; *, 1996 findings were different from the 1935 dataset; [2], Jewell referred to this lake as Muskelunge by Pickerel).

Lake	Survey 1935	Survey 1996	*	pH	colour (Pt, mg L^{-1})	Conductivity (µmho cm^{-1})	DRSi (µg L^{-1})
Anne	+	0	*	6.14	28.15	12	78
Bug	0	0		6.37	95.4	18	595
Crystal	0	0		6.57	6.04	12	14
Helmet	0	0		5.6	483.11	37	131
Joyce	+	0	*	6.03	32.39	16	32
Little John Jr.	0	0		5.78	36.2	13	13
Little Pickerel	+	+		6.85	157.74	63	4409
Little Rock	+	+		6.4	19.89	11	65
Mann	0	+	*	9.44	45.6	123	2219
Mary	+	+		6.26	314.05	26	961
Muskelunge[2]	0	0		7.55	114.11	80	7641
Nebish	+	+		7.04	23.03	18	147
Nixon	+	0	*	7.24	251.63	60	5973
Oswego	+	0	*	6.29	43.63	15	47
Street	0	0		6.00	23.91	15	47
Tamarack	+	+		7.34	129.04	73	889
U. Gresham	0	0		8.32	38.3	254	5191
Wishow	+	0	*	5.82	45.77	10	52

same habitats after 60 years, or if the distribution had shifted substantially. We focused our study on the relationship between *S. lacustris* and the lake chemical features, pH, colour, conductivity, and SiO_2, that Jewell (1935, 1939) suggested had the strongest apparent correlations with sponge distribution. Because Jewell's data were potentially limited by the analytical techniques available at that time, we applied modern statistical techniques to the original dataset to further test the degree to which a lake's chemistry could be related to sponge distribution. We applied discriminant analysis to her dataset, and used the resulting model to predict sponge distribution in a new set of 17 lakes surveyed during the summer of 1996. This provided a further test to determine how well chemical lake features are related to the occurrence of *S. lacustris*.

MATERIALS AND METHODS

Thirty five lakes in the Northern Highland Lake District of Wisconsin were surveyed for the presence of *S. lacustris* during the summer of 1996. Eighteen of these lakes were opportunistically selected from those in Jewell's (1935) survey. In addition, 17 new lakes were surveyed to expand the original dataset. Our survey techniques included shoreline and littoral zone sampling by snorkeling and boating. A small jonboat, rake, and net were used to complement specimens collected by snorkeling. Water samples were collected in open-water regions of the lakes for chemical analysis. Small portions of sponges were brought back to the laboratory where they were air-dried and stored until spicule processing and identification following procedures described in Frost (1991). For each sample, dried sponge tissue was placed in centrifuge tubes and boiled in concentrated nitric acid for one hour. The remaining spicules were rinsed in ethanol, centrifuged, and slides were prepared for examination on a compound light microscope.

Water samples were collected in polyethylene bottles and processed in the laboratory. An Oakton WD-35607-10 conductivity meter and an Accumet 900 pH meter were used for analyses. A spectrophotometer was used to determine water colour following procedures described in Cuthbert (1992). Dissolved reactive silica (DRSi) concentrations were determined colourimetrically by a Technicon Segmented Flow Auto Analyzer.

Discriminant analysis was used to examine the relationship between the distribution of *S. lacustris* and pH, SiO2, conductivity, and colour values reported by Jewell for the lakes she sampled in 1935. Our approach attempted to predict the presence or absence of *S. lacustris* in a study lake using an equation of the form:

$$F = di_1Z_1 + di_2Z_2 + ... + di_nZ_n ,$$

where di is the weighted discriminant coefficient, Z is the discriminating chemical variable, and F is a categorical variable reflecting the presence or absence of a sponge (Digby & Kempton, 1994). The magnitude of the discriminant coefficient indicates the influence that the associated variable has on the distribution of *S. lacustris*. We applied discriminant analysis to the entire data set on the presence or absence of *S. lacustris* reported by Jewell for 102 lakes. We tested the efficacy of the discriminant analysis by a cross-

TABLE 2. Performance of discriminant model fit to 99 lakes from Jewell's (1935) survey. * = Jewell reported water chemistry data for 99 of the 102 lakes that she surveyed.

Spongilla lacustris	Jewell's (1935) Results	Discriminant Analysis Predicted Results
No. of lakes with sponges	73	50
No. of lakes without sponges	26	49
Total no. of lakes surveyed*	99	99

validation of the predicted results compared to the actual observed results reported by Jewell in all the lakes that she surveyed. In addition, the resulting model was applied to the 17 new lakes that we surveyed during the summer of 1996 to test whether this model predicted current distribution patterns accurately.

RESULTS

The distribution of *S. lacustris* was found to be the same as reported by Jewell in 12 of the 18 lakes re-surveyed. We detected *S. lacustris* present in one lake in which it had not been previously recorded (Table 1). Conversely, we did not find *S. lacustris* in 5 of the 10 lakes in which Jewell had reported its presence. However, we found no dramatic changes in lake chemistry to account for the disappearance of *S. lacustris* from these lakes.

Graphical analyses of the lake chemistry and sponge distribution reported by Jewell (1935), and the 35 lakes we surveyed in 1996, showed no obvious patterns between the pH, colour, conductivity, and DRSi values in relation to the presence or absence of *S. lacustris* (Fig. 1A-H). A comparison between our survey and that of Jewell (1935) revealed a general decline in the distribution of *S. lacustris* during the last 60 years (Fig. 1).

Our discriminant analysis of Jewell's dataset did not reveal any significant relationships between the pH, colour, conductivity, or SiO_2, and the presence or absence of *S. lacustris*, as reported by Jewell (Table 2). The discriminant analysis of Jewell's original data assigned discriminant coefficients to each chemical variable of 1.16 for pH, 0.46 for conductivity, 0.35 for colour, and 0.23 for DRSi. We cross-validated with Jewell's actual dataset to test the ability of these 4 coefficients to correctly predict the presence or absence of *S. lacustris*. We found no significant relationship between actual sponge distribution and the predicted distribution. Jewell had reported *S. lacustris* to be present in 73 of her study lakes and absent in 26. Using the original chemical values that Jewell reported as predictors, the cross-validation of her dataset predicted sponges to be present in 50 of the surveyed lakes and absent in 49, with an error rate of 49% (Table 2).

Our more recent survey also indicated that chemical variables are ineffective predictors of the occurrence of *S. lacustris*. We found *S. lacustris* in just over half (9 of 17) of the lakes that we included in our new survey (Table 3). Using the discriminant model derived from Jewell's data, and the chemical data from the new survey lakes, we had predicted that 13 of the 17 new survey lakes would contain *S. lacustris*, with an error rate of 49% (Table 4). Furthermore, the absence of any significant relationship between the occurrence of *S. lacustris* and the chemical gradients that we evaluated, as illustrated by the lack of any indication of correlation in Jewell's dataset or our recent survey, strongly indicates that some factors besides the chemical variables may dictate the presence or absence of *S. lacustris*.

DISCUSSION

The notion that tolerance to a wide range of abiotic factors is a major feature of the niche of some species, is a well-recognized phenomenon (Dunson & Travis, 1991). Our research emphasises the ability of *S. lacustris* to tolerate a wide range of chemical conditions, setting this species apart from several other groups of aquatic organisms. Abiotic factors have been shown to limit the distribution of several fish and zooplankton species, and to directly influence aquatic macrophyte community structure (Brown & Jewell, 1926; Rahel & Magnuson, 1983; Tilman, 1988; Webster et al., 1992; Arnott & Vanni, 1993). Our results do not indicate any significant relationship between the distribution of *S. lacustris* and lake chemistry. Recent surveys conducted in Norway and Connecticut also note the ability of *S. lacustris* to tolerate a wide range of abiotic conditions (Økland & Økland, 1996; De Santo & Fell, 1996). This tolerance may be a very important adaptation for the survival of this species in freshwater habitats and may account for its reported cosmopolitan distribution.

We most frequently found *S. lacustris* in small, sheltered regions of lakes, growing directly up from bottom sediments. In lakes with less suitable bottom substrate, smaller specimens were found

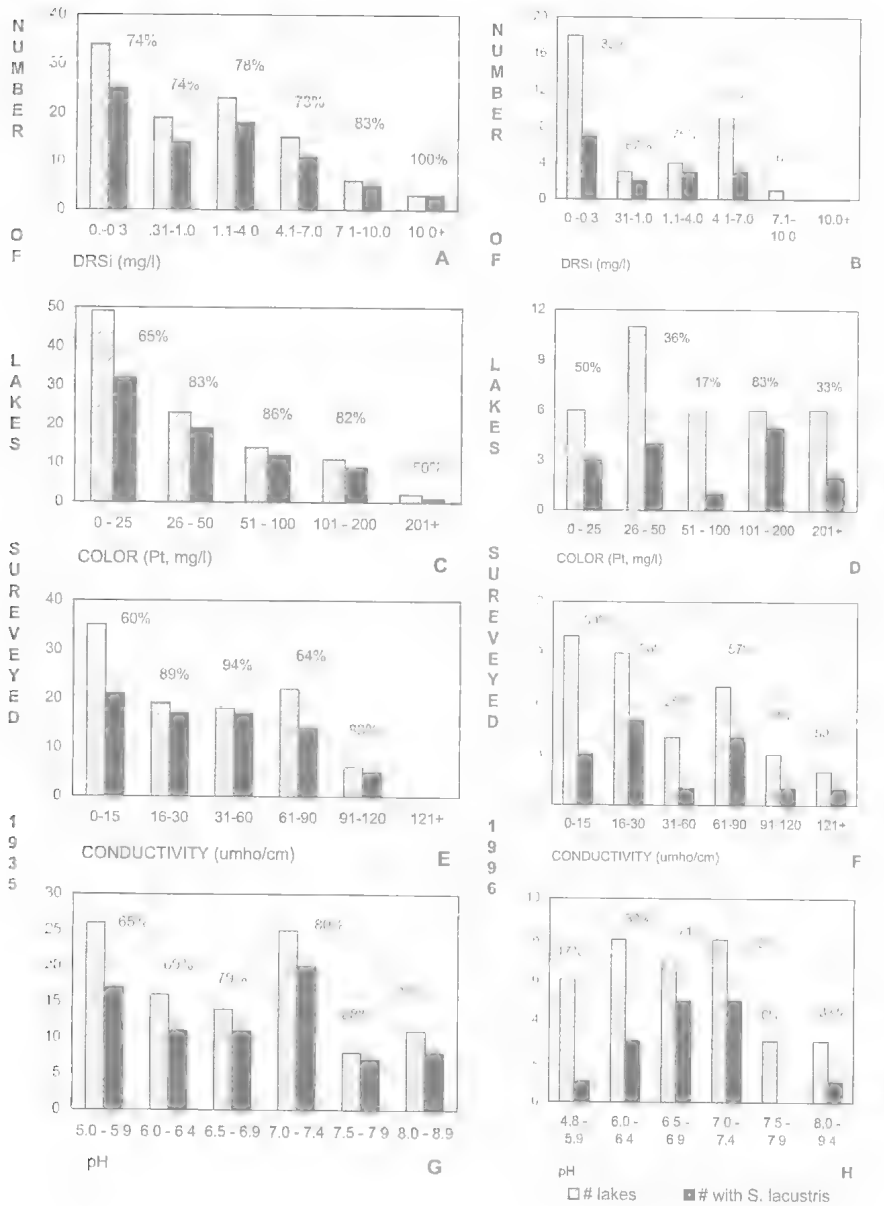

FIG. 1. Distribution of *S. lacustris* across chemical gradients for Jewell's (1935) survey (A,C,E,G) and 1996 survey (B,D,F,H). Lighter bars indicate the number of lakes surveyed; darker bars represent the number of lakes containing *S. lacustris*. Note the overall decline in percentage of lakes with *S. lacustris* present. A-B, Dissolved reactive silica concentrations; C-D, colour; E-F, Conductivity; G-H, pH.

TABLE 3. Distribution of *Spongilla lacustris* and water chemistry in 17 Northern Wisconsin lakes surveyed in 1996. These lakes were not included in Jewell's (1935) survey. Key: 0, sponges absent; +, sponges present.

Lake	Survey 1996	pH	colour (Pt. mg L^{-1})	Conductivity (μmho cm^{-1})	DRSi (μg L^{-1})
Aurora	+	6.86	190.67	93	6911
Benedict	+	5.99	48.37	8	136
Bittersweet	+	6.78	59.61	20	119
Crystal bog	0	5.1	98.71	8	158
Firefly	0	6.48	17.24	17	59
Fishtrap	0	7.7	40.27	93	4617
Frank	+	7.05	34.11	21	100
Goodyear spg	+	7.22	18.63	73	6390
Mystery	+	5.92	111.51	18	1348
Oberlin	+	6.68	27.48	15	153
Nixon creek	0	7.15	238.28	61	5712
Partridge	0	7.94	63.22	66	7015
Rainbow flwg	+	7.1	128.79	80	67
Round	0	8.61	85.96	106	4200
Sandy beach	+	6.87	239.17	34	3627
Tower	0	7.35	77.57	36	2845
Trout bog	0	4.76	202.67	17	60

encrusted on the underside of logs, and on the woody roots of cranberry bushes (*Vaccinium* spp.). Tiny specimens were found growing in very low silica and conductivity habitats, most often on the tips of aquatic macrophytes, usually *Myriophyllum* and *Isoetes* species. *Spongilla lacustris* appeared well-adapted to a wide range of light conditions, and depending upon the colour of the water, was found anywhere from just below the surface in Little Pickerel Lake to depths of 3m in Little Rock Lake (Frost & Elias, 1990).

The trace amounts of DRSi found in some northern Wisconsin lakes do not appear to limit the occurrence of *S. lacustris* (Table 1). Observations of freshwater sponge morphology suggest, however, that DRSi plays an important role in the growth and skeletal strength of a sponge (Jewell, 1935; Kratz et al., 1991). Limited silica availability may result in decreased strength of the spicule skeleton, causing indirect negative effects on the distribution of sponges, perhaps by providing less protection against predation (Frost, Kratz & Elias, personal communication). Jewell (1935) recognised that DRSi was an important factor in determining the degree of skeletal development in *S. lacustris*, and consequently differentiated two different growth forms, typical and atypical, correlated to the morphology of spicules. Jewell defined atypical specimens as those that had aberrant forms of spined microscleres from lakes with low silica concentrations. We also found several *S. lacustris* specimens from lakes with low silica concentrations to have finer, less robust spicules and smaller microscleres than those specimens from lakes with higher silica concentrations. For our analyses of Jewell's data we combined both the typical and atypical forms into one species classification.

Additional observations made during our field survey provided some insight into other environmental factors that may be influencing the distribution of *S. lacustris* in Northern Wisconsin lakes. We observed a slight decline in the presence of *S. lacustris* compared to its distribution in 1935. Many of the 12 lakes that we found no change in sponge distribution patterns are located in the Wisconsin State forest and have been protected from development for the past 60 years. Four of the five lakes that are now unoccupied by *S. lacustris* however (Anne, Joyce, Oswego and Wishow Lakes), have portions of their shorelines developed with privately owned cabins. Alteration of littoral habitats by development (e.g. removal of coarse woody debris; Christensen et al., 1996) may be negatively impacting the distribution of *S. lacustris* in these lakes.

Both our contemporary survey and that of Jewell (1935) focused on the occurrence of *S. lacustris,* but not on it's biomass and prevalence, which varies substantially among habitats. It can be quite abundant in some situations (Frost et al., 1982; Frost & Elias, 1990), and nearly absent in others (Colby & Frost, personal observations). Also, while the overall distribution of *S. lacustris* may appear stable in some lakes, undocumented observations of significant yearly fluctuations

TABLE 4. Predictions of *Spongilla lacustris* distribution in 17 Northern Wisconsin lakes surveyed for the first time in 1996. The discriminant model used to make these predictions was parameterised using Jewell's (1935) dataset.

Spongilla lacustris	1996 Survey Results	Discriminant Analysis: Predicted Results
No. of lakes with sponges	9	13
No. of lakes without sponges	8	4
Total no. of lakes surveyed	17	17

have been observed previously (Frost, personal observation), but could not be quantified in either survey. The fact that we have not clearly linked species occurrence patterns with lake chemistry strongly suggests that other physical or biological factors are influencing sponge distribution. These factors could include associated vegetation, available substrate, predation, dispersion, climatic conditions and disease.

Apart from some interesting results reported by Jewell (1935) there is generally little information available on interactions between *S. lacustris* and its surrounding communities, including interactions with other freshwater sponge species. Competition and mutualism between different species of marine sponges has been relatively well documented (e.g. Rützler, 1970; Sarà, 1970; Wulff, 1997), and it is possible that these interactions occur in freshwater as well. Jewell reported nine other species that are not as common as *S. lacustris* in these lakes, and that we did not include in our survey. These other freshwater species may be more strongly influenced by lake chemical factors, and could also be influencing the distribution of *S. lacustris*. Recognition of freshwater sponges as active members of aquatic communities could lead to a better understanding of the relationships between freshwater sponges, environmental factors important to their survival, and their associated surrounding communities.

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AN OVERVIEW OF STROMATOPOROID DOMINATED MIDDLE DEVONIAN REEF COMPLEXES IN NORTH QUEENSLAND.

Memoirs of the Queensland Museum 44: 99. 1999:- Middle Devonian stromatoporoid buildups are known from the Burdekin Subprovince and the Broken River Province in the Townsville hinterland, north Queensland.

Recent studies have placed these buildups within a reliable stratigraphic and sedimentologic framework. Buildups within the Burdekin Subprovince developed in a restricted near to proximal shore setting in a partially enclosed basinal setting. Those buildups within the Broken River province developed upon a more open marine shelf.

Major Burdekin stromatoporoid-coral buildups were of two types: low relief extensive biostromes and associated stromatoporoid pavements, and a biohermal system of one to two metres relief from the sea floor. Additional buildups of note are small patch reefs developed within nearshore siliciclastic muddy lagoons adjacent to granitic headlands. In a number of such metre scale buildups within dominantly siliciclastic settings, assemblages of stromatoporoids and corals show repetitive growth interruption surfaces suggesting episodic stress and killing events. Storm disturbance during development the biostromal pavements was high and an important sedimentologic factor for the 'reef' growth. Minor sponge s.s. buildups are known from the uppermost Burdekin Formation, but have not been studied.

In the Broken River Province, Givetian buildups are more extensive and can be traced on the hundreds of metre scale, these have received little detailed sedimentologic study, but are of similar style to biostromal pavements from the neighbouring Burdekin Basin. Minor biohermal occurrences are found within the Papilio Mudstone, and formed on a muddy shelf, and include both stromatoporoid and sponge s.s. buildups.

Stromatoporoid taxonomy has revealed the presence of eight stromatoporoid communities in the Burdekin Basin, comprising 35 taxa. Dominant stromatoporoids were dendroids *Amphipora*, *Stachyodes* and *Trupetostroma*, frame building. *Trupetostoma*, *Pseudotrupetostroma*, *Hermatostroma*, *Actinostroma* and *Ferestromatopora*. *Coenostroma*, *Clathrocoilona*, and *Stromatopora* were accessory to reef growth. In the Broken River detailed taxonomic work has only been partially completed. Significant overlap exists at generic level with the two adjacent provinces, but species level differences are strong suggesting distinct partitioning of open marine versus embayment faunas. This phenomenon is reflected in other faunal elements (gastropods, rugose corals). ❐ *Porifera, stromatoporoid, biostromes.*

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GOOD CONGRUENCE BETWEEN MORPHOLOGY AND MOLECULAR PHYLOGENY OF HADROMERIDA, OR HOW TO BOTHER SPONGE TAXONOMISTS. *Memoirs of the Queensland Museum 44: 100. 1999*:- Within Demospongiae, the order Hadromerida is well defined and there is a strong consensus among systematicians about its composition and validity. This order is characterised by the presence of tylostyles radially arranged at least in the periphery, and by microscleres, when present, of the aster type. All Hadromerida are oviparous and the choanocytes have a periflagellar sleeve. Ten families are without any doubt attributed to Hadromerida, six of which with microscleres of the aster type and four of which without microscleres.

The first work on molecular phylogeny of Porifera was made on the Hadromerida (Kelly-Borges, Bergquist & Bergquist, 1991). The molecule used was the 18S rRNA, which appeared to be not sufficiently informative to resolve the phylogeny at that taxonomic level.

In this work we have used the 5' end of the 28S rRNA (about 1000bp) to explore the internal phylogeny of this order. 15 species belonging to 12 genera and 8 families were sequenced. Five outgroup species were sequenced belonging to Axinellida, Tetractinellida, and Halichondrida. Parsimony and Neighbor-Joining analyses have been done. Trees were rooted by using Tetractinellida (*Cinachyrella* and *Discodermia*) as a monophyletic outgroup. Both analyses (Parsimony and Neighbor-Joining) show that the Hadromerida are composed of four monophyletic taxa. Taxon 1 is composed of 6 species belonging to the Spirastrellidae, Acanthochaetetidae, Clionidae, and Placospongiidae. All these families have microscleres of the spiraster-type. Taxon 2 is composed by 5 species of Timeidae and Tethyidae. These two families have microscleres of the euaster-type. Taxon 3 is composed of only one species *Polymastia mamillaris* belonging to the family Polymastiidae, which has no microsclere of aster type. The validity of this taxon has to be checked with other genera belonging to the Polymastiidae family. Taxon 4 is composed of three Suberitidae and an external species *Halichondria panicea*, which belongs to the family Halichondriidae (order Halichondrida). Neither the Suberitidae nor the Halichondriidae have microscleres of the aster type. The monophyly of each of these four taxa is well supported with high bootstrap proportions. The monophyly of the four taxa together is also well supported but the relationships between them cannot be ascertained.

The monophylies of taxa 1 and 2 are congruent with morphology, both taxa corresponding to the hadromerid families with spirasters and with euasters, respectively. An important and unexpected problem of classification appeared with taxon 4. The result obtained with our sequence of *Halichondria panicea* was confirmed with a shorter sequence of *Hymeniacidon heliophila* available in GenBank. When the sequence of *Hymeniacidon* is included, taxon 4 remains monophyletic and strongly supported by BP. From the morphological and cytological point of view there is no synapomorphy between the two groups. The Halichondrida are defined mostly by negative characters. However, we observed a fine morpho-molecular synapomorphy for taxon 4. This is the loss of a small loop of 15 bp in the secondary structure of the D2 domain, which is probably the result of only one deletion event. From the chemical point of view, there is another synapomorphy: a large amount of stanols have been described both in the Suberitidae and the Halichondrida.

The best hypothesis seems to reallocate Halichondriidae to the Hadromerida. The order Hadromerida remains monophyletic. With the exception of this reallocation the classification obtained with 28S rRNA is perfectly congruent with the existing classification. All the families are monophyletic. We propose a subordinal classification : Spirastrellina, Timeina, Polymastiina and Suberitina. ❐ *Porifera, Demospongiae, molecular phylogeny, 28S rRNA, Hadromerida, Halichondrida, monophyly.*

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REMARKS ON THE STATUS OF *MYXILLA* (PORIFERA: POECILOSCLERIDA) ON THE GALICIAN COAST (NW IBERIAN PENINSULA)

F.J.CRISTOBO, P. RÍOS AND V. URGORRI

Cristobo, F.J., Ríos, P. & Urgorri, V. 1999 06 30: Remarks on the status of *Myxilla* (Porifera: Poecilosclerida) on the Galician coast (NW Iberian Peninsula). *Memoirs of the Queensland Museum* **44**: 101-123. Brisbane. ISSN 0079-8835.

Myxilla Schmidt is represented on the Iberian Peninsula by six species, five of which, studied in this paper, were collected from the coast of Galicia (NW of Spain): *M. incrustans, M. iotrochotina, M. macrosigma, M. rosacea* and *M. fimbriata*, and the sixth (*M. tarifensis*), recently described from the Strait of Gibraltar. 188 specimens were collected from 72 stations along the coast of Galicia between 1979-1991. Illustrated descriptions of these species, their habitus, skeletal arrangement and spicules are provided, together with information on their autecology, distribution, and biometric studies of spicules. Morphological comparisons are made between these species and other *Myxilla* from the Atlantic region, and a taxonomic key to species of *Myxilla* in the NE Atlantic is provided. ❐ *Porifera, Poecilosclerida, Myxilla, Iberian Peninsula, NE Atlantic, taxonomy, ecology, key.*

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Only few studies have been made on Galician sponges (Solórzano & Rodriguez, 1979; Solórzano & Durán, 1982; Solórzano, 1991; Solórzano & Urgorri, 1991, 1993; Solórzano et al., 1991). Other records of sponges from the sublittoral benthos are also available in more general publications (Benito, 1976; Gili et al., 1979; Polo et al., 1979; Durán & Solórzano, 1982; Acuña et al., 1984), as well as from nudibranch - sponge dietary studies (Urgorri & Besteiro, 1984). Studies on *Myxilla* in the Ría de Ferrol (Cristobo, 1997) and Galician coast (this study) recorded five species: *M. incrustans, M. iotrochotina, M. macrosigma, M. rosacea*, and *M. fimbriata*. These are comprehensively described and discussed in this present study.

MATERIALS AND METHODS

Collections were made between 1979-1991 using direct sampling in the intertidal, and SCUBA and naturalist benthic dredge (Holme & McIntyre, 1984) in the sublittoral zones.

A total of 188 specimens of *Myxilla* were collected from 72 stations on the Galician coast (Fig. 1). Preparation and histological methods follow Rubió (1973), Rützler (1978), Uriz (1978, 1986) and Cristobo et al. (1993). Spicules were examined under a Hitachi S570 scanning electron microscope (SEM). Underwater photographs were taken with a Nikonos V camera and SB-102 flash. A biometric study of sponge spicules was made for specimens from the Ría de Ferrol and microscopic preparations of two paratypes of *M. macrosigma* (Museum National d'Histoire Naturelle, París (MNHN), Laboratoire de Biologie des Invertébrés Marins et Malacologie: DNBE282 from the Grotte des Calanques, and DNBE287 from Île Grosse). All specimens were deposited in the Departamento de Bioloxía Animal in the Facultade de Bioloxía at the Universidade de Santiago de Compostela, Spain.

SYSTEMATICS

Order **Poecilosclerida** Topsent
Family **Myxillidae** Topsent
Myxilla Schmidt, 1862

Myxilla incrustans (Johnston, 1842) (Figs 2-4, 17C)

MATERIAL. Stations 19, 20, 23, 43 (see Fig. 1).

AUTECOLOGY. In Galicia, this sublittoral species lives in a small bathymetric zone from 8-14m depth in the outer Ría area, settling on granite rock on exposed bottoms; also found on gravel bottoms (Topsent, 1913) and as epibiont on *Inachus* and *Cellaria* (Crawshay, 1912); elsewhere it may also be found intertidally (Stephens, 1921; Könnecker, 1973; Hoshino, 1981), in the sublittoral zone (Descatoire, 1969)

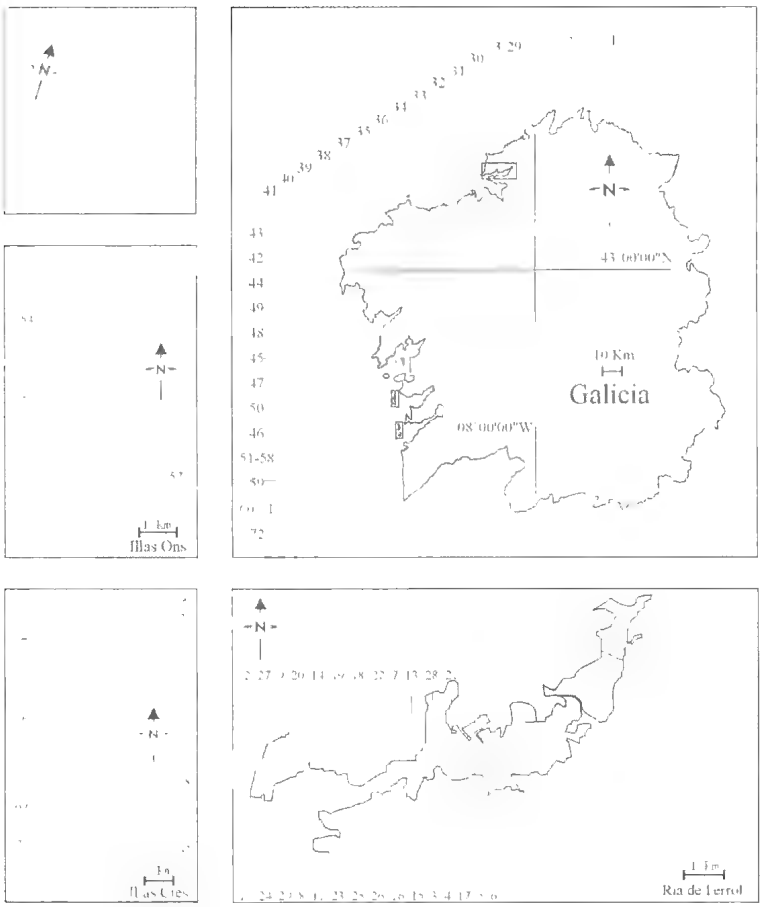

FIG. 1. Map of the study area showing location of collecting stations.

and on rocky circalittoral bottoms (Vidal, 1967; Borojevic et al., 1968; Topsent, 1913) up to 170m deep (Boury-Esnault et al., 1994).

DISTRIBUTION. Arctic, European Atlantic coasts, Gibraltar and Mediterranean Sea (Ackers et al., 1992); also allegedly reported from Senegal (Lévi, 1952), Japan (Hoshino, 1981), Korea (Sim, 1994) and Antarctica (Arndt, 1935), although the conspecificity of these records must be checked. In Galicia this species is known from the Ría de Ferrol, only the second record for the Iberian Peninsula, previously known from Punta Uhía, Ría de Muros (Solorzano, 1991).

DESCRIPTION. An encrusting sponge, sometimes massive, with a rough surface consisting of fine reticulation of spicules. Orange or yellow in colour. Skeletal arrangement: Choanosomal skeleton myxilloid with triangular and quadrangular meshes of acanthostyles forming ascending tracts of up to 20 spicules interconnected by transverse fascicles. The ectosome is made up of tornotes in paratangential brushes which extend out in a bouquet-like fashion. Microscleres are scattered throughout the sponge but anchorate chelae are more abundant in the ectosome, where they form a sub-superficial layer. Sigmas are dispersed within the choanosome. Megascleres: straight or slightly curved robust acanthostyles with conical spines. Dimensions: 150.3-209.0× 2.9-12.8µm. Smooth, straight or slightly curved tornotes, with asymmetrical terminations: one having a marked ellipsoidal tyle and the other with diverse irregular terminations, the most common of which is spear-shaped, in some cases bearing fine spines. Dimensions: 128.5-207.6×2.6-7.3µm. Microscleres: Sigmas with the typical c- and s- shapes. Dimensions: 22.2-39.4×0.7-3.2µm. Arched spatuliferous anchorate isochelae, of two different size categories: 11.3-19.2×3.5-6.3µm and 24.1--35.5 ×10.9-16.2µm.

Myxilla iotrochotina (Topsent, 1892)
(Figs 5-7, 17D)

MATERIAL. Stations 1, 2, 7, 9, 31, 34, 36, 37, 40, 43, 45, 52, 56, 58, 66 (see Fig. 1).

AUTECOLOGY. Cryptic species, occupying highly localised and well-concealed enclaves, perhaps explaining why it has been overlooked since it was first described by Topsent; in Galicia it is found in secluded places such as on the roofs of small caves and intertidal crevices in the mid-outer zone of the rías; the few references to this species describe it living in similar environments to a depth of up to 30m, such as detritic bottoms (Sará & Siribelli, 1960), and artificial breakwaters (Sará, 1961); also epibiont on other sponges such as *Geodia* (Ferrer-

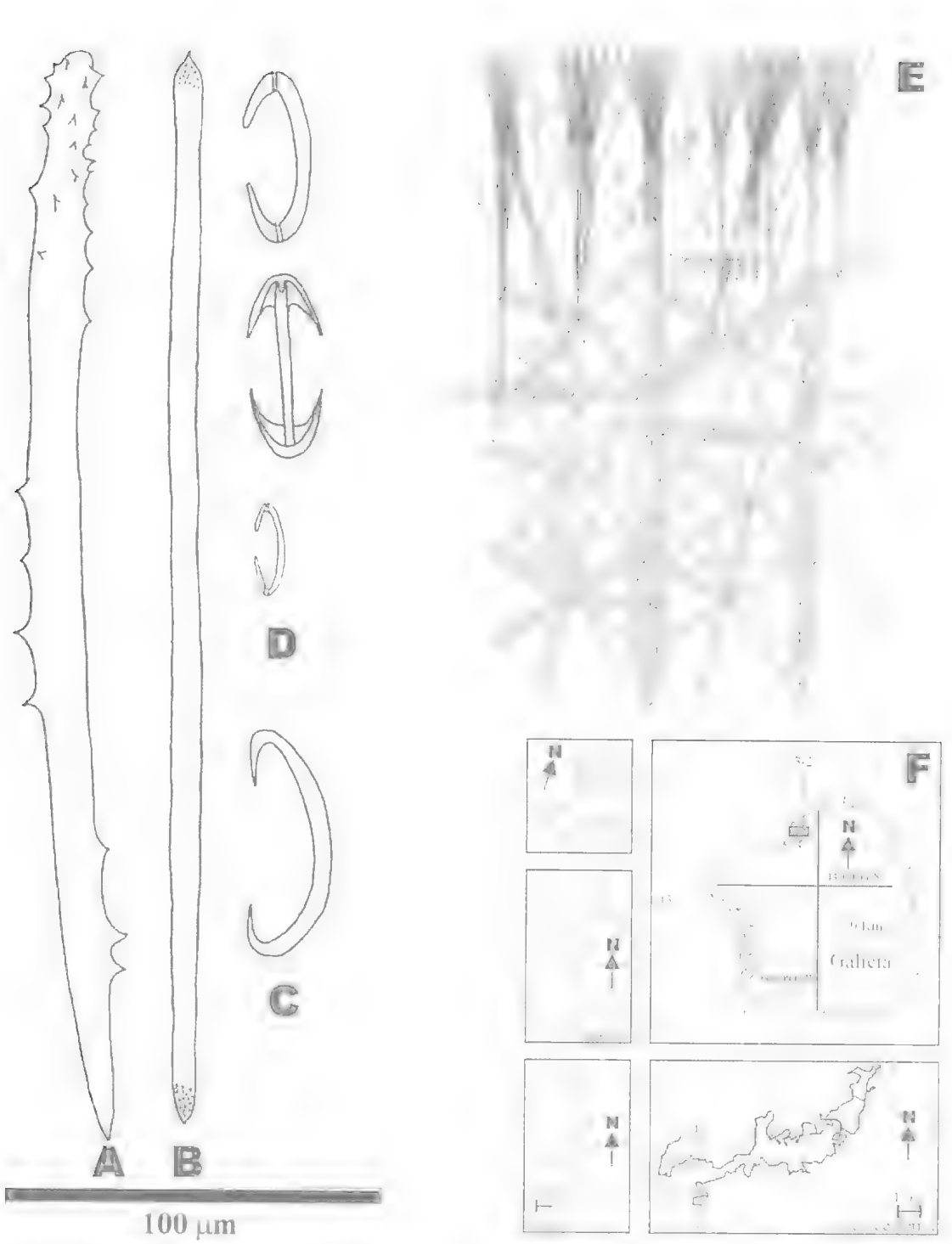

FIG. 2. *Myxilla incrustans*. Spicules: A, Acanthostyle; B, Tornote; C, Sigmas; D, Isochelae; E, Skeletal arrangement; F, Distribution in Galicia.

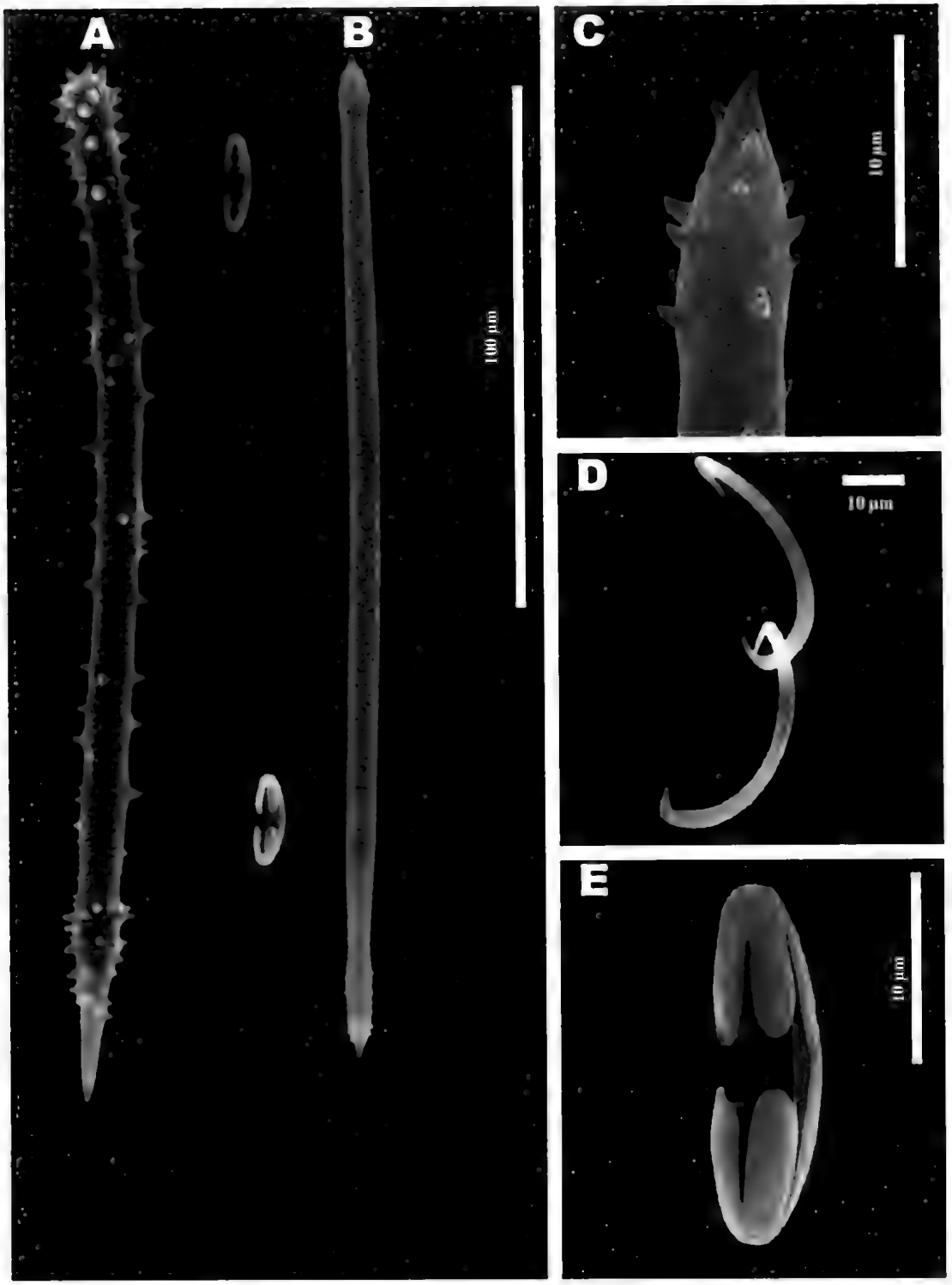

FIG. 3. *Myxilla incrustans*. Spicules: A, Acanthostyle; B, Tornote; C, Detail of the end of a tornote; D, Sigmas; E, Isochela.

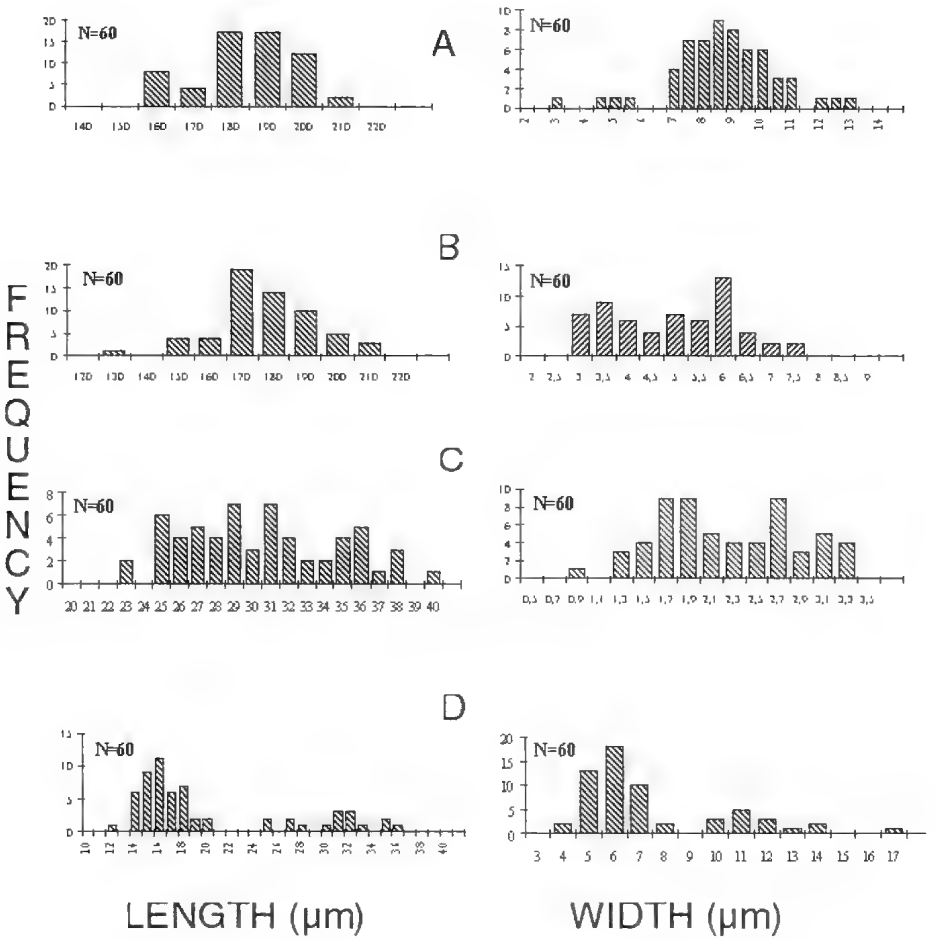

FIG. 4. Frequency histograms for spicules of *Myxilla incrustans*. All measurements are given in µm. (numbers in parentheses indicate means). A, Acanthostyles: 150.3-(179.5)-209.0×2.9-(8.4)-12.8µm. B, Tornotes: 128.5-(172.8)-207.6×2.6-(4.7)-7.3µm. C, Sigmas: 22.2-(30.0)- 39.4×0.7-(2.1)-3.2µm. D, Isochelae: 11.3-(19.6)- 35.5×3.5-(6.9)-16.2µm.

Hernández, 1918; Solórzano, 1991), *Erylus discophorus* (Solórzano, 1991), on *Pinna* (Topsent, 1892), and on Laminarian rhizoids (Descatoire, 1969). It has recently been reported by Carballo (1994) in the stomach contents of *Platydoris argo* (Mollusca: Opistobranchia) in the Bay of Algeciras.

DISTRIBUTION. Atlantic and Mediterranean, 0-30m depth (Carballo & García-Gómez, 1996). In Galicia it is known from the Ría de Ferrol (Cristobo, 1997), Punta Uhía, Centoleira, (Durán & Solórzano, 1982), Islas Cíes (Acuña et al., 1984), Morás, Espasante, Orzán, Caión, Malpica, Santa Mariña, Camariñas, Esteiro, Punta Pasante, Enseada Canibeliñas, Punta Cociñadoiro and Enseada do Lago (Solórzano, 1991).

DESCRIPTION. Forming small coverings on rocks. Rough surface; light cream in colour. Skeletal structure is typical for the genus with a choanosome made up of quadrangular or triangular polyspicular meshes and ectosomal tornotes in palisade; microscleres are widespread throughout the sponge. Megascleres: straight, robust acanthostyles with conical spines in a tangential arrangement over the entire spicule. Dimensions: 106.5-144.5×5.5-11.4µm. Smooth, straight fusiform tornotes with symmetrycal ends formed by several spines (from 3-6) which may be slightly divergent. Dimensions: 113.1-139.2× 3.9-8.7µm. Microscleres: sigmas typically c- and s- shape, differentiated into two sizes categories: 11.7-19.9×0.5-1.2µm and 20.7-43.1×1.3-3.6µm. Tridentate chela with a straight spicular stem;

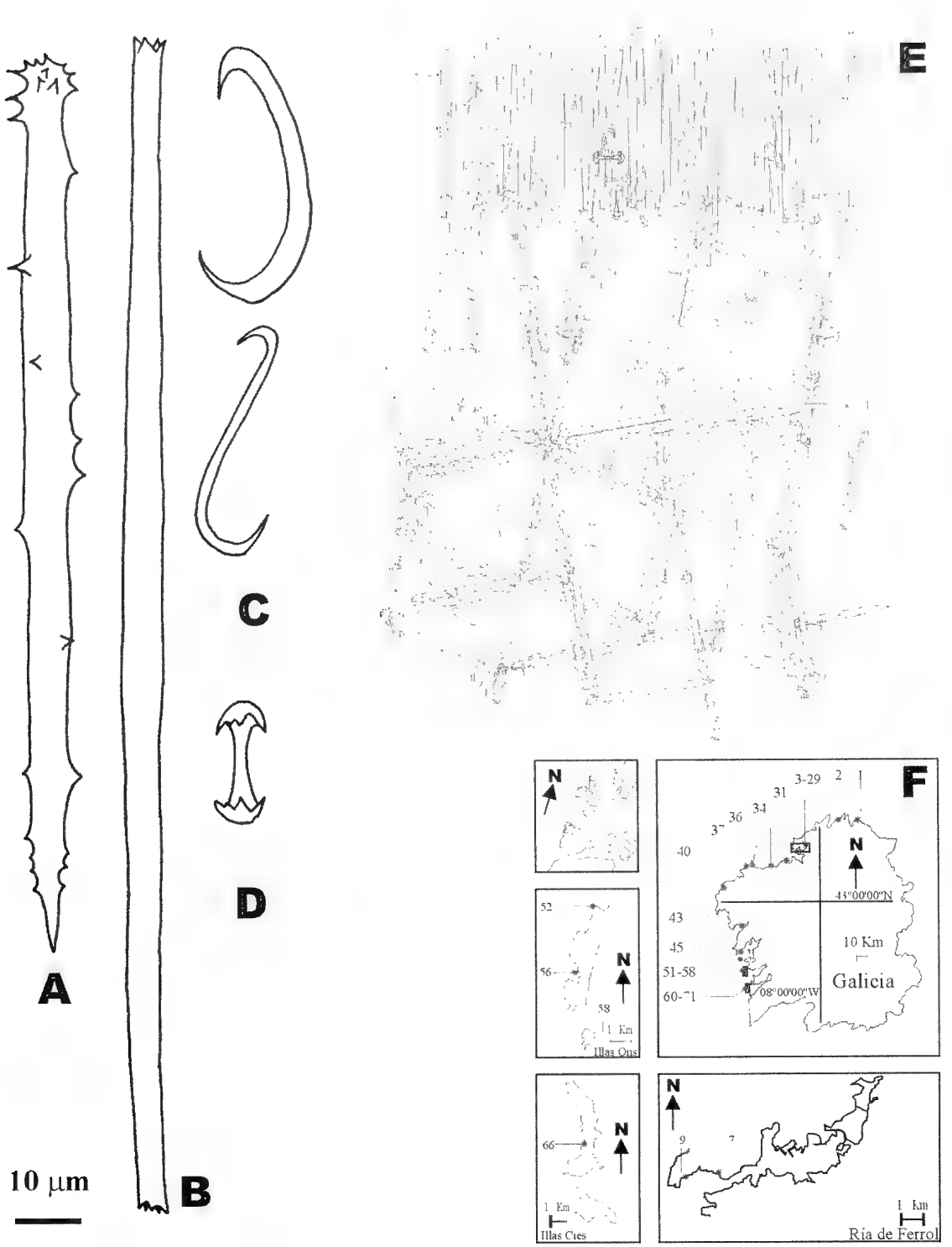

FIG. 5. *Myxilla iotrochotina*. Spicules: A, Acanthostyle; B, Tornote; C, Sigmas; D, Tridentate chela; E, Skeletal arrangement; F, Distribution in Galicia.

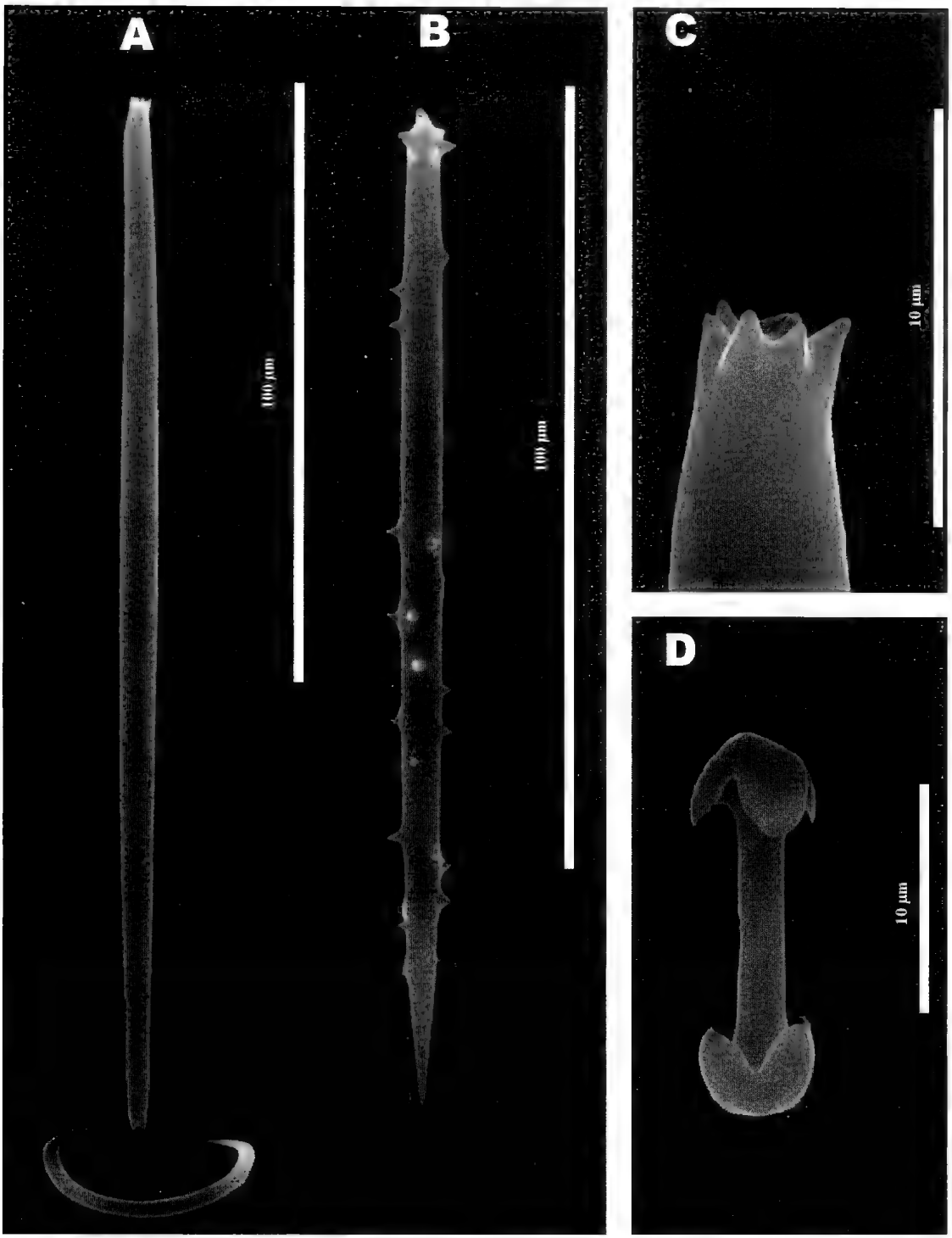

FIG. 6. *Myxilla iotrochotina*. Spicules: A, Tornote and sigma; B, Acanthostyle; C, Detail of the end of a tornote; D, Tridentate chela.

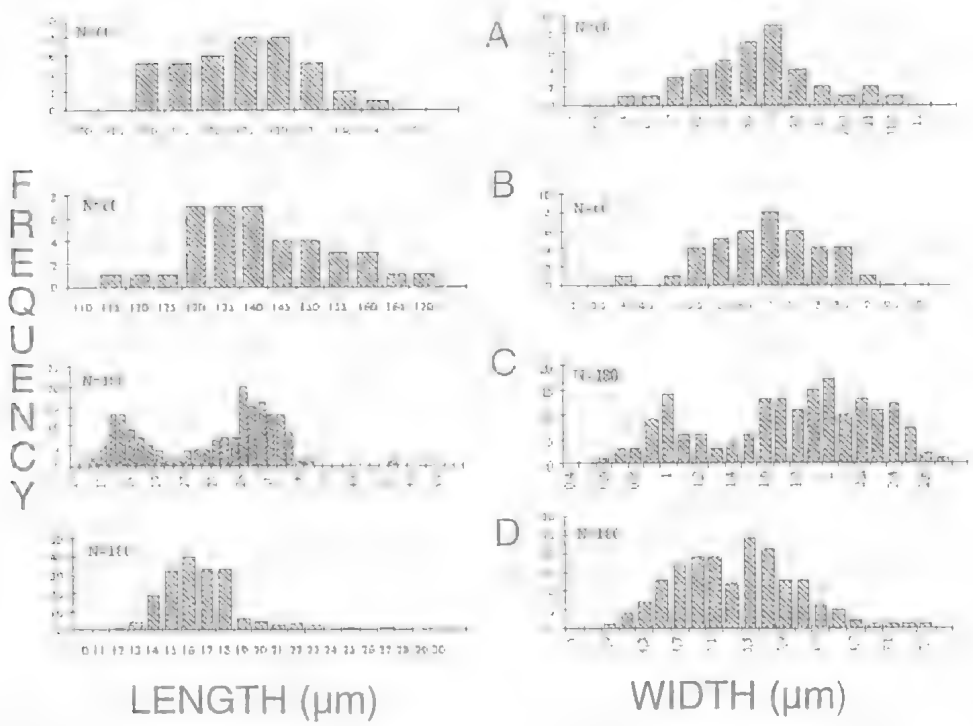

FIG. 7. Frequency histograms for spicules of *Myxilla iotrochotina*. All measurements are given in µm (numbers in parentheses indicate the mean). A, Acanthostyles: 106.5-(122.4)-144.5×5.5-(8.3)-11.4µm. B, Tornotes: 113.0-(167.6)-139.2×3.9-(6.6)-8.7µm. C, Sigmas: 11.7-(23.9)-43.1×0.5-(1.6)-3.6µm. D, Tridentate chelae: 11.2-(16.1)-26.0x3.8-(5.2)-7.3µm.

extremities bearing three short, wide teeth; abundant. Dimensions: 11.2-26.0× 3.8-7.3µm.

Myxilla macrosigma Boury-Esnault, 1971
(Figs 8-10, 17A)

MATERIAL. Stations 13, 25, 26, 66 (see Fig. 1).

AUTECOLOGY. In the Ría de Ferrol this species is found between intertidal to 11m depth, preferring to settle on vertical walls and in crevices in the outer Ría stations, with either a southern or northern orientation. Boury-Esnault (1971) first collected it on the upper and middle levels (2-13m) of dark biotopes in the area of Banyuls-sur-Mer (Mediterranean). Pouliquen (1972) collected it from the caves of Endoume (Marseille), and later Boury-Esnault & Lopes (1985) found it in the Azores on vertical walls between 8-20m depth. This species prefers to settle in areas with very little light, such as crevices and the roofs of caves. It was not found on soft substrates such as mud, sand, pebbles or gravel in the 78 stations sampled in the a Ría de Ferrol, unlike the closely related *Myxilla rosacea* which is found on maerl in the Ría de Arousa (Solórzano et al.,1991), and on gravel in the Ría de Ferrol (Cristobo et al., 1992), indicating that the two species have different ecological preferences.

DISTRIBUTION. Mediterranean and Atlantic. In Galicia it has only been found in the Ría de Ferrol and on the Cíes Islands, the first record for the Iberian Peninsula.

DESCRIPTION. Massive or encrusting sponge. The largest specimen (Station 25) measures 4cm long, 1cm thick. Surface is irregular, lobate, velvet to touch and highly perforated. The exhalant canals form a network of surface veins converging toward the circular osculum measuring up to 5mm diameter. Consistency is flexible and mucusy. Live specimens are yellowish-orange in colour, becoming light beige or brown in alcohol, staining the alcohol slightly yellowish. Specimens collected in August were reproductive. Choanosomal skeleton composed of a reticulation of acanthostyles. The networks are isodictyal with triangular or quadrangular

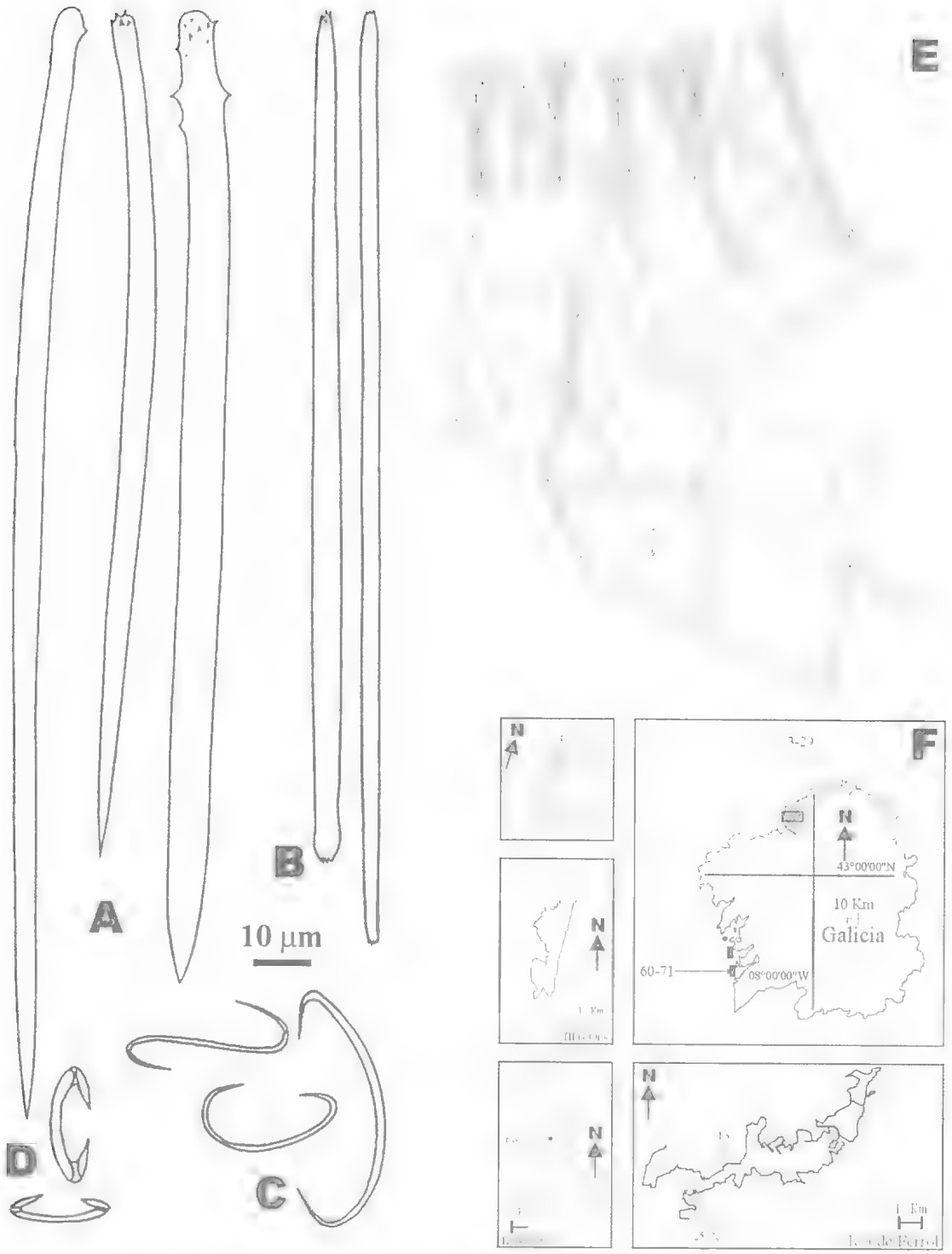

FIG. 8. *Myxilla macrosigma.* Spicules: A, Acanthostyles; B, Tornotes; C, Sigmas; D, Isochelae; E, Skeletal arrangement; F, Distribution in Galicia.

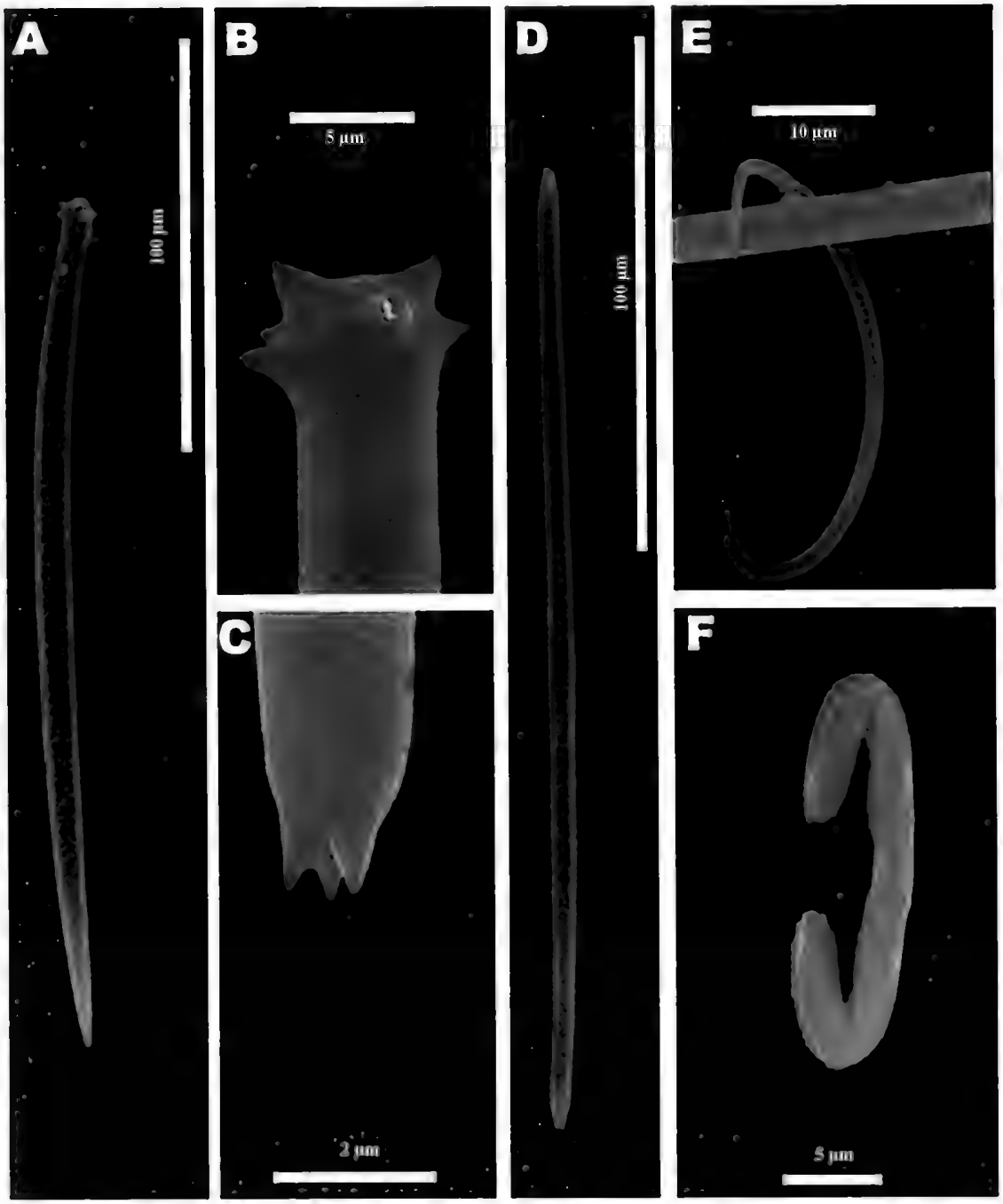

FIG. 9. *Myxilla macrosigma.* Spicules: A, Acanthostyle; B, Detail of the head of acanthostyle; C, Detail of the end of a tornote; D, Tornote; E, Sigma; F, Isochela.

meshes composed of 1-5 acanthostyles. The ectosomal skeleton is made up of tornotes which are tangentially arranged to the sponge surface, sometimes forming bouquets. Microscleres, sigmas and isochelae, are found throughout the sponge. Spicules. Megascleres: slightly curved acanthostyles, with curvature occasionally more pronounced near the head of the spicule. Spines

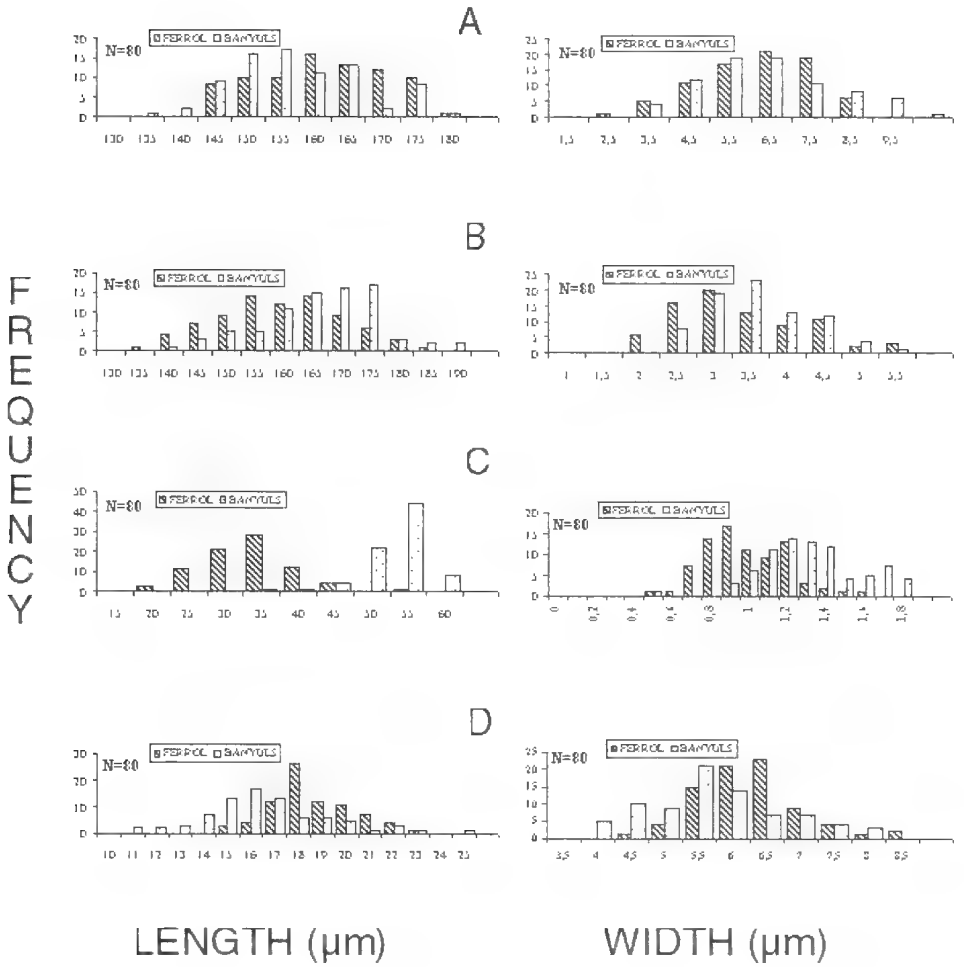

FIG. 10. Frequency histograms for spicules of *Myxilla macrosigma* from two different localities: Ferrol, Spain (Atlantic) and Banyuls-Sur-Mer, France (Mediterranean). All measurements are given in µm (numbers in parentheses indicate the mean). A, Acanthostyles: 141.1-(158.4)-177.3×1.9-(5.6)-8.3µm. B, Tornotes: 132.5-(157.0)-182.8×1.5-(3.1)-5.2µm. C, Sigmas: 18.4-(30.7)- 51.9×0.4-(0.9)-1.5µm. D, Isochelae: 14.5-(18.0)-22.2× 4.4-(5.9)-8.1µm.

are located on or near the head and few in number (1-20). Smooth stem. Dimensions: 141.1-177.3× 1.9-8.3µm. Smooth, straight tornotes fusiform, with spiny endings and occasionally swollen at the ends with short terminations. Dimensions: 132.5-182.8×1.5-5.2µm. Microscleres: sigmas typically c- and s- shape with a wide opening. Dimensions: 18.4-51.9×0.4-1.5µm. Isochelae with alae closed along less than a third of the total length of the spicule. Dimensions: 14.5-22.2 ×4.4-8.1µm.

Myxilla rosacea (Lieberkühn, 1859)
(Figs 11-13, 17B)

MATERIAL. Stations 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 64, 65, 66, 67, 68, 69, 70, 72 (see Fig. 1).

AUTECOLOGY. This species has been found on gravel in the mid-zone, on soft bottoms of the Ría de Ferrol, where tidal currents are predominant (Cristobo et al., 1992). In other locations in Galicia it is also abundant in both the intertidal zone in semi-exposed areas, and in protected areas such as on rocky bottoms of the sublittoral

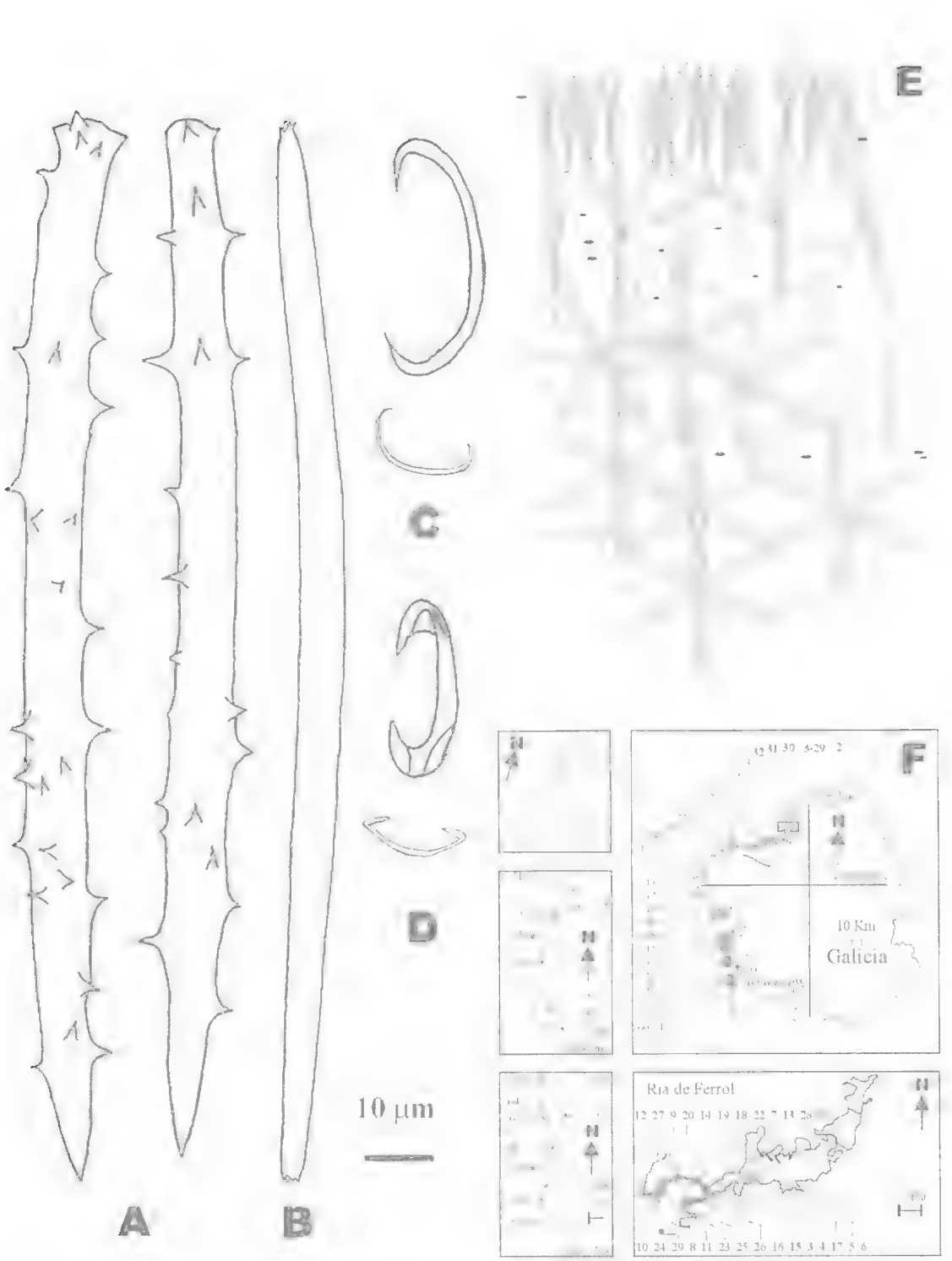

FIG. 11. *Myxilla rosacea.* Spicules: A, Acanthostyles; B, Tornote; C, Sigmas; D, Isochelae; E, Skeletal arrangement; F, Distribution in Galicia.

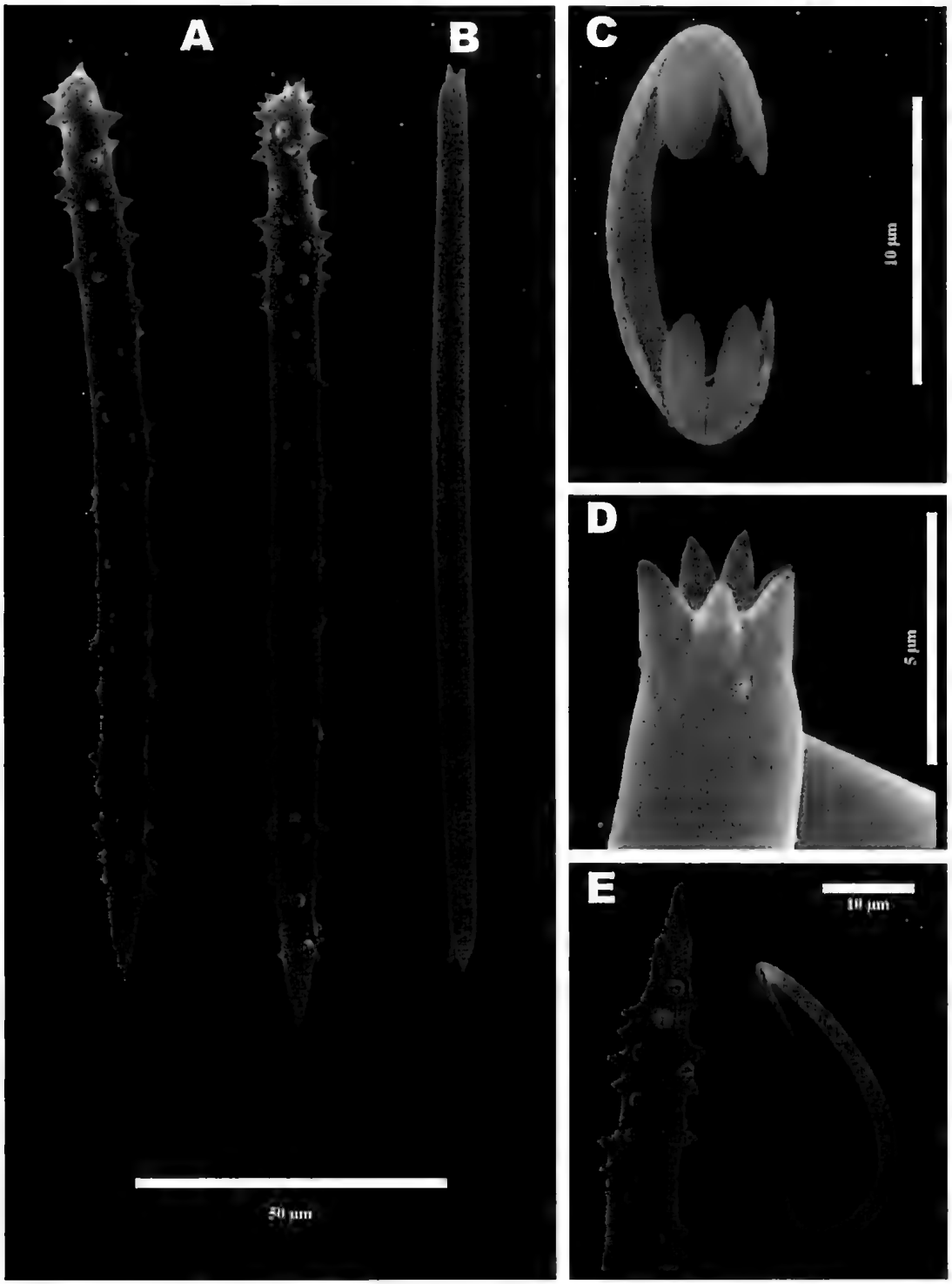

FIG. 12. *Myxilla rosacea.* Spicules: A, Acanthostyles; B, Tornote; C, Isochela; D, Detail of the end of a tornote; E, Detail of the end of an acanthostyle and sigma.

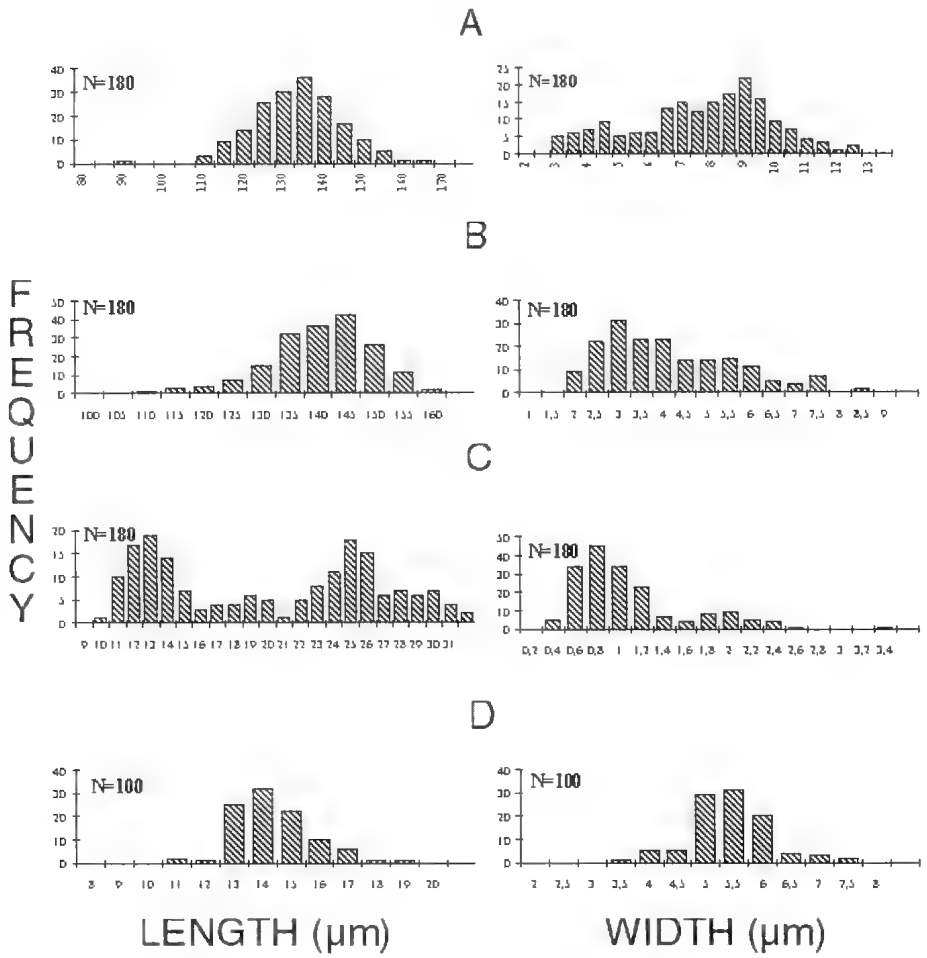

FIG. 13. Frequency histograms for spicules of *Myxilla rosacea.* All measurements are given in µm (numbers in parentheses indicate the mean). A, Acanthostyles: 89.6-(130.9)-162.0×2.5-(7.3)-12.2µm. B, Tornotes: 106.2-(137.9)-158.6×1.5-(3.9)-8.3µm. C, Sigmas: 9.8-(19.6)- 31.7×0.3- (0.9)-3.2µm. D, Isochelae: 10.5-(13.8)-18.7×3.0-(5.1)-7.3µm.

zone, where it is frequently associated with rhizoids of *Laminaria* and other seaweeds, and on soft bottoms such as the biocenosis of maerl (Solórzano et al., 1991). At greater depths on the circalittoral bottoms this species has been found associated with the biocenosis of *Dendrophyllia cornigera.* Abundant in both intertidal and sublittoral zones, with wide bathymetric range to 414m depth (Uriz, 1988). In the intertidal zone it is found in the middle and outer rías, particularly in semi-exposed enclaves and protected areas, with preference to settle on rocky granitic substrates, vertical and horizontal walls, and on seaweeds, especially laminarian rhizoids. The wide ecological range of this species is conducive to its colonisation of a wide variety of habitats: the intertidal zone (Stephens, 1921; Sará, 1961, 1964a, 1964b), seaweed rhizoids (Stephens, 1921; Benito, 1976; Rodriguez & Lorenzo, 1978). In the sublittoral zone it is found in caves and on extremely plumb surfaces (Labate, 1964; Descatoire, 1969; Boury-Esnault, 1971; Pouliquen, 1972; Bibiloni, 1981a), it incrusts on *Microcosmus sulcatus* (Sará & Melone, 1963), on ascidians, balanids, and *Sabellaria* tubes (Borojevic et al., 1968), on *Spondylus* (Benito, 1981); it also encrusts *Arca barbata* and *Cerithium vulgatum* (Pulitzer-Finali, 1978), on meadows of *Posidonia oceanica* (Benito, 1981; Pansini & Pronzato, 1985), on gravel, sand, calcareous seaweeds *Inachus* sp., and the sponge *Ircinia variabilis* (Babic, 1922), on the

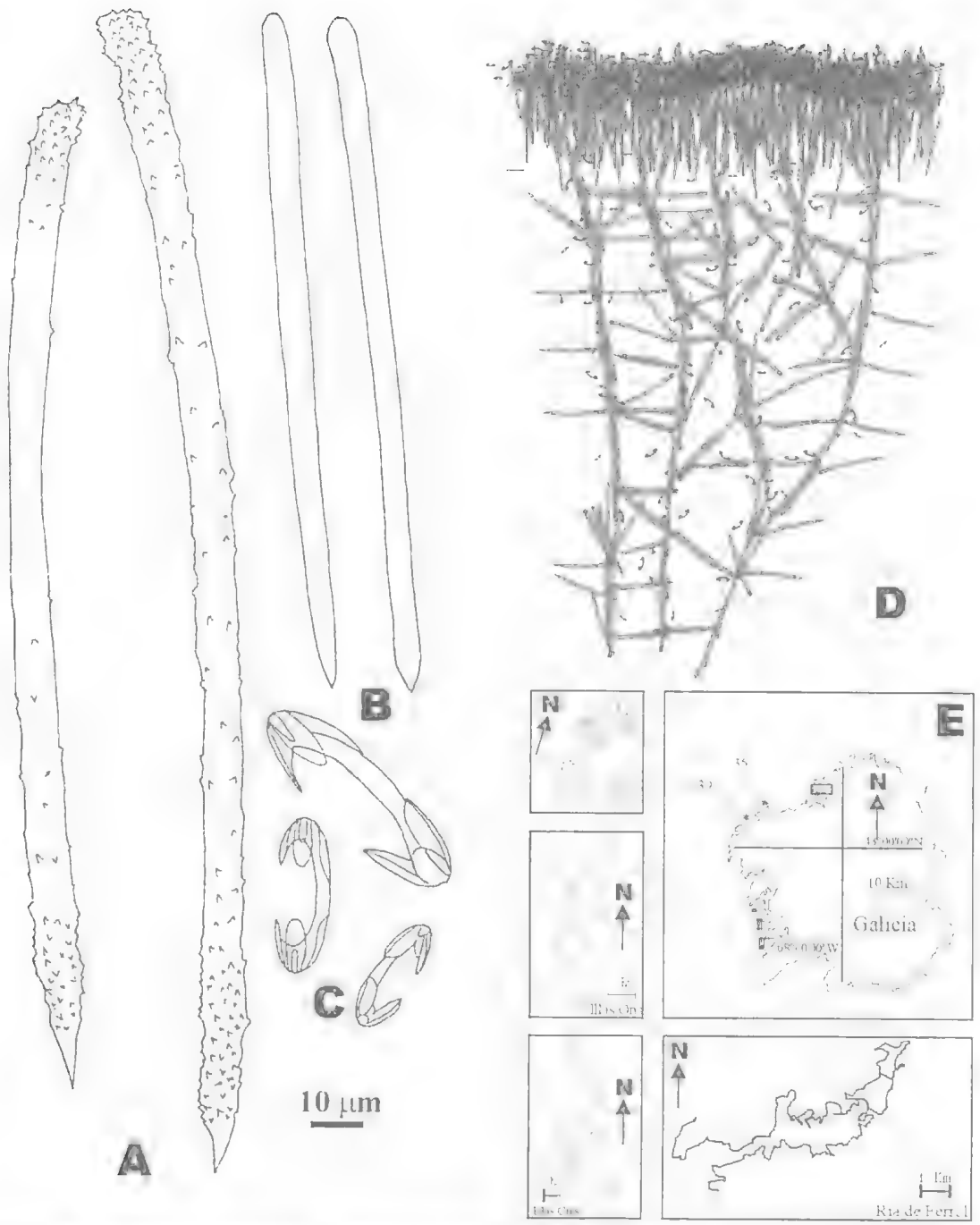

FIG. 14. *Myxilla fimbriata.* Spicules: A, Acanthostyles; B, Tornotes; C, Isochelae; D, Skeletal arrangement; E, Distribution in Galicia.

Mediterranean coralligen biocenosis (Sará, 1972; Bibiloni, 1981b), detritic and detritic-mud bottoms, (Poggiano, 1965) and forming part of the port fouling epifauna (Pronzato, 1972; Sará, 1974). This species is also found on circalittoral bottoms (Vidal, 1967), consisting of sand, pebbles, and gravel, between 130-160m depth (Topsent, 1928), on *Antipatharia* at 60-100m depth

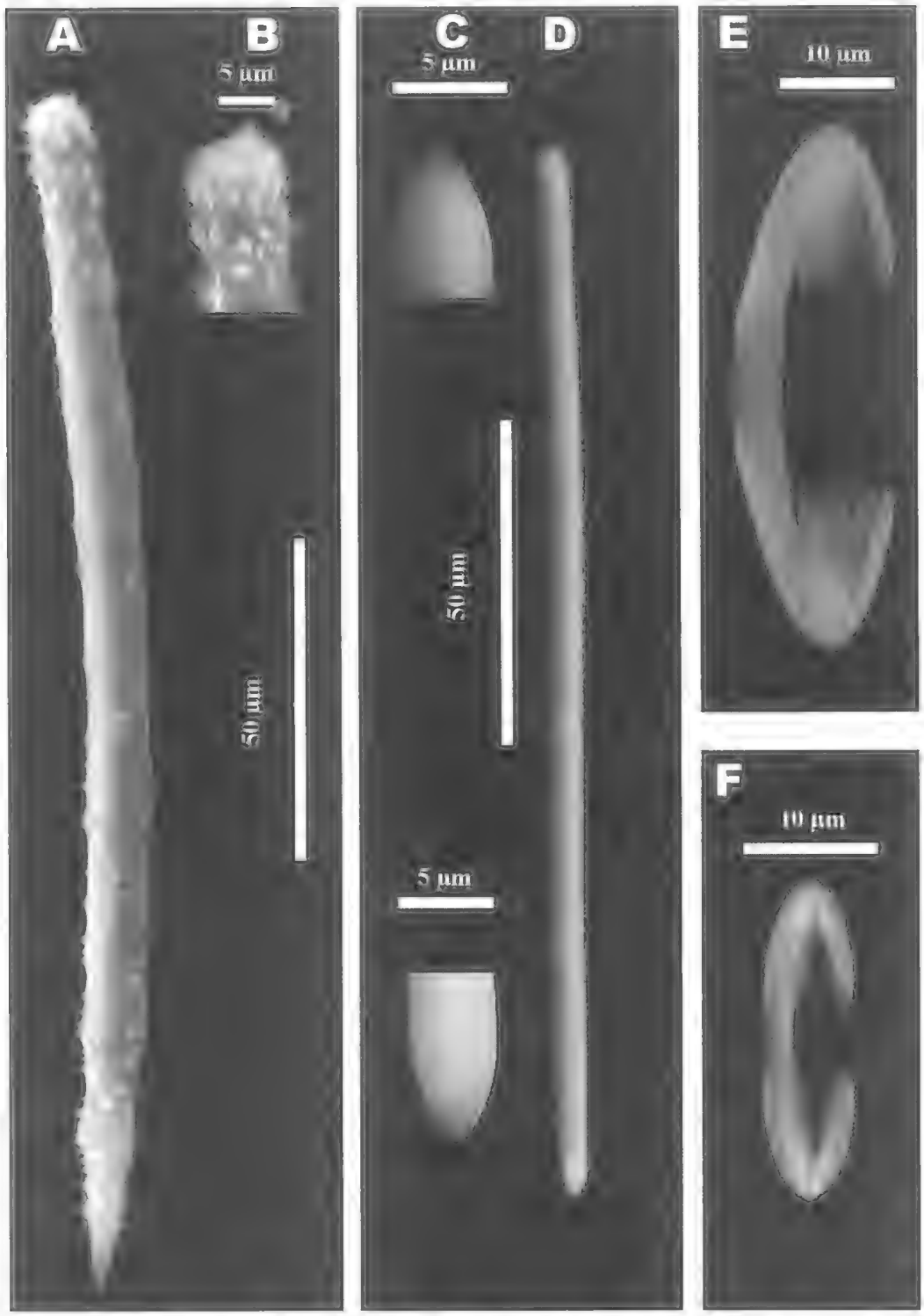

FIG. 15. *Myxilla fimbriata.* Spicules: A, Acanthostyle; B, Detail of the head of a acanthostyle; C, Detail of the ends of a tornote; D, Tornote; E-F, Isochelae.

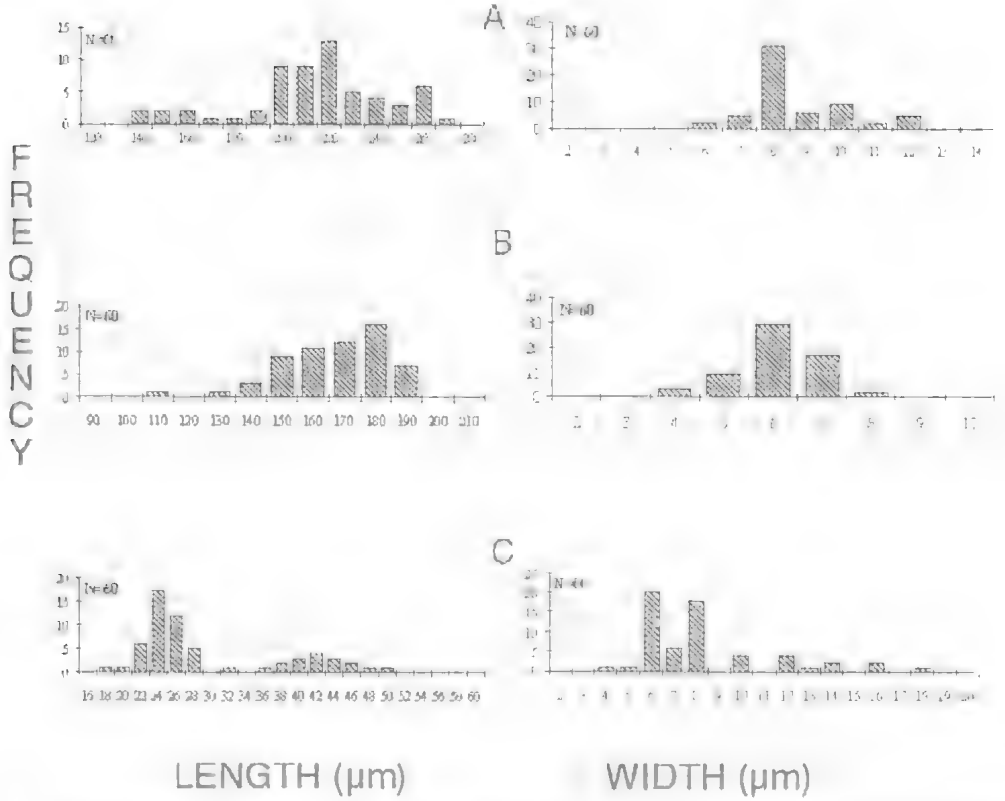

FIG. 16. Frequency histograms for spicules of *Myxilla fimbriata*. All measurements are given in µm (numbers in parentheses indicate the mean). A, Acanthostyles: 133.2-(210.2)-264.0×6.1-(8.6)-12.1µm. B, Tornotes: 106.5-(162.9)- 190.8×4.1-(5.9)-7.5µm. C, Isochelae 18.0-(29.3)- 58.3×4.0- (8.2)-18.1µm.

(Topsent, 1928), on the biocenosis of *Corallium rubrum* between 100-200m depth (Templado et al., 1986), on bottoms of dead Madreporaria with brachiopods, tubicolous polychaetes and anthozoans at between 260-269m depth (Uriz, 1985), on *Antipathes fragilis* between 130-180m depth, as well as on epibathyal mud (Vacelet, 1960).

DISTRIBUTION. Eastern Atlantic from the Arctic to South Africa; Pacific (Lambe, 1892); Mediterranean, (Carballo & García Gómez, 1996). In Galicia it is found in a number of locations: 43°44'50"N, 08°12'W - 43°40'N, 08°55'W (Topsent, 1892), Os Feitales (Benito, 1976), Aguiño, O Grove (Rodríguez & Lorenzo, 1978), San Ciprián de Burela (Gili et al., 1979; Polo et al., 1979), Suevos, Caión, Patos (Solórzano & Rodríguez, 1979), Punta Uhía, Queixal, Insuela, Corvasa, Corasa, Centolleira, Isla de Rúa, Airós, Sálvora, Isla de Ons (Durán & Solórzano, 1982), Laxe (Solórzano & Durán, 1982), Islas Cíes (Acuña et al., 1984) and Ría de Arousa (Solórzano et al., 1991).

DESCRIPTION. Morphologically variable, appearing as a massive, prominent covering on rocky substrates with osculiferous digitiform chimneys, and heart-shaped covering small-sized seaweeds. Dimensions: 2-20cm maximum diameter, 0.4-10cm thick. Rough surface, having several characteristic crests, in some places very occasionally smooth. Soft and slightly flexible consistency; delicate ectosome and choanosome with a spongy appearance, highly perforated. The oscula may not be apparent in smaller specimens, but they are generally abundant, located in conical elevations protruding from the sponge mass from 1-8cm, producing chimneys, commonly having ascending, superficial aquiferous ducts; the osculum is circular in shape, sometimes clover-shaped. Abundant ostia appear between the numerous ridges on the surface. colouration varies from various shades of orange, beige or light pink. The species frequently secretes

TABLE 1. Comparison between spicule dimensions of *Myxilla fimbriata* (Bowerbank, 1866). All measurements in µm.

Reference	Acanthostyles	Tornotes	Isochelae I	Isochelae II
Cristobo et al.	190-260	129-200	18-25	35-60
Lundbeck, 1910	260-430	230-320	22-35	64-90
Descatoire, 1966	210-310	160-250	25-30	60-75

mucus in formaldehyde during fixation. Skeletal arrangement: Choanosomal skeleton consists of quadrangular or triangular polyspicular meshes composed of 2-15 acanthostyles. Ectosomal tornotes form bouquets protruding externally less than one third of the length of the spicule. Microscleres, sigmas and isochelae, are distributed throughout the sponge. Megascleres are straight or slightly curved acanthostyles, with strong conical spines highly variable in number arranged perpendicularly to the axis of the spicules, ranging from smooth (i.e. styles) to completely bristled with spines covering the entire surface, and all intermediate gradations between. Dimensions: 89.6-162.0×2.5-12.3µm. Smooth, straight tornotes, slightly fusiform, symmetrical similar extremities ending in small straight spines. Dimensions: 106.2-158.6×1.5-8.3µm. Microscleres: sigmas in typical c- and s- shapes in two size categories: 9.8-20.7× 0.3-1.5µm and 21.3-31.7×1.6- 3.2µm. Arched isochelae. Dimensions: 10.5-18.7×3.0-7.3µm.

Myxilla fimbriata (Bowerbank, 1866)
(Figs 14-16, 17E)

MATERIAL. Stations 35, 39 (see Fig. 1).

AUTECOLOGY. In Galicia the species has been recorded from the biocenosis of *Dendrophyllia cornigera* between 50-58m depth, where it covers both the anthozoan and the brachiopod *Terebratulina caputserpentis*. This species is typical of the circalittoral and bathyal bottoms with bathymetric range between 50-3500m (Descatoire, 1966). It is abundant on bottoms characterised by the presence of *Dendrophyllia cornigera* at 60m depth, and less common in shallower waters less than 40m depth. In the sublittoral and circalittoral zones, the species is found in crevices (Descatoire, 1969). Also reported on *Caryophylia clavus, Lophoelia prolifera* and on rocky bottoms between 80-700m depth (Stephens, 1921).

DISTRIBUTION. North Atlantic and Arctic (Arndt, 1934). In Galicia: Laxe (Solórzano & Duran, 1982).

DESCRIPTION. Encrusting sponge with a smooth or slightly crateriform surface without aquiferous orifices visibles macroscopically. Consistency is elastic; live colouration is pale yellow, brownish-ochre in alcohol. Dimensions: 23×5×2mm. Skeletal arrangement: Choanosomal skeleton is arranged in ascending tracts of acanthostyles interconnected by other transverse fascicles, with isochelae arranged around the tracts. The ectosomal skeleton consists of tornotes forming a relatively regular palisade, together with abundant isochelae. Megascleres: slightly curved acanthostyles with the distal end terminating in a sharp tip and irregular ornamentation with profuse spines on the head and a third of the distal region, except for the tip which is smooth. Dimensions: 133.2-264.0×6.1-12.1µm. Straight tornotes with slightly swollen and tapered distal extremities. Dimensions: 106.5-190.8×4.1-7.5µm. Microscleres: spatuliferous isochelae, with two clearly differentiated size classes. Dimensions: 18.0-28.0 and 35.0-58.3µm.

KEY TO *MYXILLA* FROM THE NE ATLANTIC

1. With styles as choanosomal megascleres 2
   With acanthostyles as choanosomal megascleres . . . 4
2. With pluridentated subtylotes . . . . . *M. pluridentata* Lundbeck, 1905 . . . . . .
   With sharp tornotes 3
3. Only one class of spatuliferous anchorate chela . . . . . *M. pedunculata* Lundbeck, 1905
   Two classes of spatuliferous anchorate chela . . . . . *M. diversiancorata* Lundbeck, 1905
4. Without isochelae . . . . . *M. prouhoi* (Topsent, 1892)
   With isochelae 5
5. Without sigmas. . . . *M. fimbriata* (Bowerbank, 1864)
   With sigmas 6
6. With strongyles . . . . *M. tarifensis* Carballo & García Gómez, 1996
   Without strongyles. 7
7. Tornotes without ends having small divergent points. 8
   Tornotes with ends having small divergent points . . 9
8. Microspined tornote ends . . . . . *M. incrustans* (Johnston, 1842)
   Smooth tornote ends . . . . *M. fibrosa* Levinsen, 1893
9. With tridentate chela . *M. iotrochotina* (Topsent, 1892)
   Without tridentate chela 10
10. Sigmas of two size classes . . . . . *M. rosacea* (Lieberkühn, 1859)
    Sigmas of one size class . . . . . *M. macrosigma* Boury-Esnault, 1971

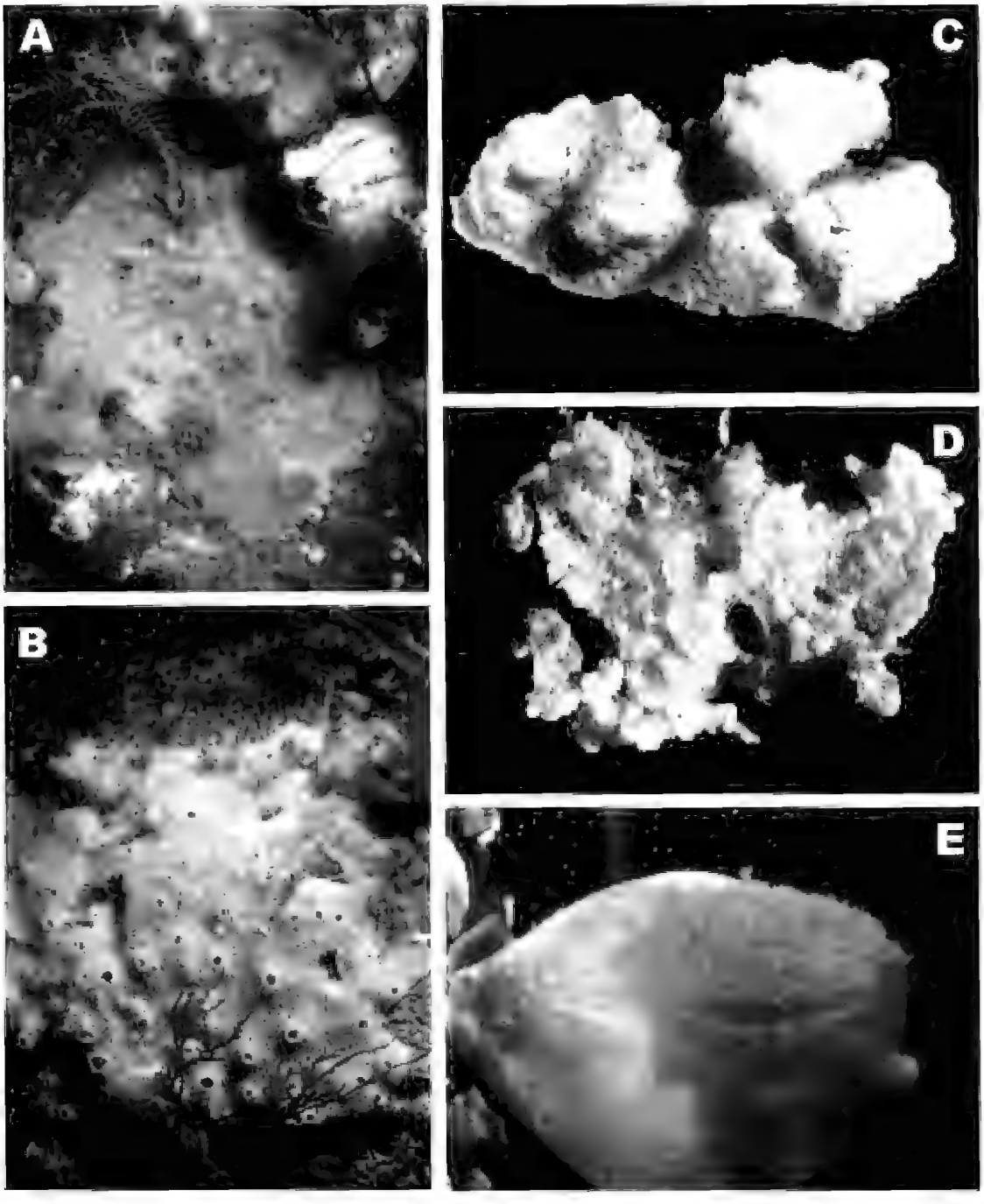

FIG. 17. Habitus of *Myxilla* species from Galicia. A, *M. macrosigma*. B, *M. rosacea*. C, *M. incrustans*. D, *M. iotrochotina*. E, *M. fimbriata*

DISCUSSION

Currently thirty-eight genera are assigned to Myxillidae (Hooper & Wiedenmayer, 1994), although recent revisions, such as Hajdu et al. (1994), Bergquist & Fromont (1988) and others, recognise fewer than these as correctly residing here. In Galicia species of *Myxilla* are amongst the more common sponges, both in terms of biomass and diversity. A key to species in the NE Atlantic is presented in Table 1. As compared to species from other latitudes, *Myxilla* from Galicia have certain unique characteristics in their morphology, habitat and distribution, as discussed below.

Specimens of *M. incrustans* from the Ría de Ferrol show some differences in morphology of their tornotes as compared to specimens from other locations in Galicia. In the samples from Ferrol the tornotes have asymmetrical ends, one forming a tyle with a small elliptic head, perfectly defined by a tiny pre-capitular narrowing, and the other having a certain degree of polymorphism ranging from a spear-shaped tip to irregular shaped tips as illustrated in Figures 3-4. Other authors (e.g. Boury-Esnault et al., 1994) have described smooth tornotes with almost no spines.

Myxilla iotrochotina is very similar to *M. rosacea* but differs externally in its much smaller size and the fact that it forms small scales. *Myxilla rosacea,* on the other hand, is usually massive and frequently has considerable osculiferous chimneys, whereas the skeletal arrangement in both species is similar. The spicular composition is also similar; with acanthostyles, tornotes, and sigmas, but whereas *M. rosacea* has arched spatuliferous isochelae, *M. iotrochotina* has characteristic tridentate chelae with a straight spicular stem and three short teeth on the ends. On this basis we question the identification of *Dendoryx iotrochotina* from the Baleric Islands (Bibiloni, 1990), as it lacks tornotes with spiny ends and the isochelae are also different.

The descriptions of *M. macrosigma* by Boury-Esnault (1971) and Boury-Esnault & Lopes (1985) agree with specimens described here from Galicia, highlighting one of the traits used to identify this species (viz. its mucus appearance). Acanthostyles have few spines, and the stem is practically bare; on the head spines are very scarce and may even be absent totally which gives the spicule the appearance of a true style. Comparisons between our samples from the Ría de Ferrol and paratypes collected from Banyuls-sur-Mer revealed greatly similar lengths, widths, and maximum and minimum values of acanthostyles and tornotes between these populations. In morphological appearance, however, specimens from Ferrol have a slight thickening on the ends of many tornotes. The morphology of sigmas is comparable to the original description. Sigma sizes are, curiously enough, those that show the greatest discrepancy of the four spicular types, even though the maximum and minimum values of the two populations are found to lie within the range of dimensions originally described for the species (20-70μm). The isochelae are similar in terms of size and shape.

Myxilla rosacea has considerable polymorphism in its external morphology, which may be attributed to microhabitat differences between localities (Bidder, 1923), among other factors. The most common form found in Galicia is massive, encrusting rocks and forming an irregular cushion 1-4cm thick, sometimes producing up to 20 digitiform oscular chimneys. Spicules also undergo great variations in morphology from one specimen to another, especially the acanthostyles as previously noted by Descatoire (1969), where the hispidation may be sparse, moderate or dense, with all intermediate stages. Other spicular elements (tornotes, isochelae) have a greater morphological homogeneity, whereas sigmas may be separated into two size classes. The skeletal arrangement also presents variations in terms of the geometry and arrangement of the meshes of choanosomal acanthostyles, which may be relatively slack and confused, related to the number of spicules forming skeletal meshes. The ectosomal skeleton is bouquet-shaped, a structure which has not been cited frequently in previous records of this species.

Myxilla fimbriata on the coast of Galicia is typical of this species from other localities in its habitus, habitat and size although showing slight differences in spicule sizes as compared to those provided by Lundbeck (1910) and Descatoire (1966). In specimens from Galicia all types of spicules are generally smaller than those from Ingolf and Glenan, as demonstrated in Table 1.

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NITROGEN FLUX IN A SPONGE-MACROALGAL SYMBIOSIS. *Memoirs of the Queensland Museum 44: 124. 1999*:- Nutrient cycling between corals and their zooxanthellal symbionts is important to the conservation of limiting nutrients, such as nitrogen, in oligotrophic reef waters. The tropical reef sponge *Haliclona cymiformis* forms an intercellular symbiosis with the red macroalga *Ceratodictyon spongiosum*, but it is unknown whether this association also promotes the cycling and conservation of essential nutrients. We therefore determined the potential importance of ammonium excreted by the sponge to the nitrogen-status and growth of the macroalga, using specimens collected from One Tree Island Lagoon on the Great Barrier Reef.

The association possessed the capacity to take up ammonium, nitrate and perhaps nitrite from the ambient seawater in the light. However these nutrients were commonly present at concentrations of less than 3 µM in the water at One Tree Island and so this seawater was probably only a minor source of nitrogen for the macroalga. In contrast, when the association was pre-incubated in darkness for 24hrs and its dark ammonium excretion rate measured, ammonium levels in the surrounding seawater (1 litre) increased from 0.25± 0.1µmol/g dry weight to 2.2±0.6µmol/g dry weight over a 6hr period; shorter dark pre-incubations resulted in lesser rates of ammonium excretion. It therefore appears that sponge waste is a major source of inorganic nitrogen for *C. spongiosum*, and this will be illustrated by means of a preliminary nitrogen budget. However, the enhancement of dark carbon fixation by ammonium (20µM NH_4Cl), which increases as algae become more nitrogen limited, suggested that *C. spongiosum* was still nitrogen-limited in One Tree Island Lagoon. The ammonium enhancement ratio of freshly-collected *C. spongiosum* was 1.4±0.06, which compared to a ratio of 1.5±0.07 for cultured *C. spongiosum* when deprived of inorganic nitrogen for one week; the ratio ranged from 1.1± 0.1 for cultured *C. spongiosum* supplemented with a regular source of nitrogen (100µM NH_4Cl) to 2.1±0.6 for cultured *C. spongiosum* deprived of nitrogen for 6 weeks.

We therefore propose that the situation in the *Haliclona-Ceratodictyon* symbiosis is analogous to that in corals and other zooxanthellate invertebrates, with the animal partner being an important source of nitrogen for the alga. Furthermore, when combined with evidence for the translocation of nitrogenous compounds from the macroalga to the sponge (Grant et al., in prep), it is evident that the cycling and conservation of nitrogen within the symbiosis may be an important factor in the success of this association in nutrient-poor habitats. ❐ *Porifera, sponge-macroalgal, nitrogen flux, nitrogen conservation, nitrogen budget.*

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DOES THE LARGE BARNACLE *AUSTROBALANUS IMPERATOR* (DARWIN, 1854) STRUCTURE BENTHIC INVERTEBRATE COMMUNITIES IN SE AUSTRALIA ?

ANDREW R. DAVIS AND DAVID W. WARD

Davis, A.R. & Ward, D.W. 1999 06 30: Does the large barnacle *Austrobalanus imperator* (Darwin, 1854) structure benthic invertebrate communities in SE Australia ? *Memoirs of the Queensland Museum* **44**: 125-130. Brisbane. ISSN 0079-8835.

The shallow subtidal zone of SE Australia is dominated by urchin-grazed barrens, created and maintained by a large urchin, *Centrostephanus rodgersii* (A. Agassiz). We sought to determine how benthic invertebrates, such as sponges and colonial ascidians, maintain space in the face of this intense grazing pressure. Our data indicate that the cover of invertebrates on vertical substrata was positively correlated with the density of a large barnacle *Austrobalanus imperator* and are consistent with this barnacle providing a refuge from urchin grazing. The exception was the common sponge *Clathria pyramida* which showed a strong negative relationship with barnacle density. We speculate that as aggregations of barnacles may represent foci for competitive interactions among sessile invertebrates, *C. pyramida* seeks to avoid these sites. It appears that recruitment of *A. imperator* is sporadic and hence the conditions which allow the establishment of high densities of this barnacle remain unclear. As our data are correlative they must be interpreted cautiously. Experimental manipulation of barnacle density will provide a much clearer indication of the role of *A.imperator* in structuring these communities and is the focus of current work. ❐ *Porifera, Crustacea, Echinodermata, grazing refugia, structural habitat complexity, urchin grazing, Clathria pyramida.*

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On subtidal rock walls, sessile invertebrates, such as sponges and ascidians, may cover a substantial area of the substratum. Typically in these communities habitable space is at a premium and competition for this resource is frequently intense. The loser in these spatial interactions is usually overgrown and killed. In addition to the intense spatial interactions characteristic of these communities, it is also clear that the action of predators (we use the term in its broadest sense and include grazing sea urchins) can have dramatic effects on community structure and dynamics. Urchins are capable of completely removing encrusting invertebrates and fleshy algae, thereby producing a community dominated by crustose algae (Lawrence, 1975; Vance, 1979; Ayling, 1981; Sebens, 1985; Witman, 1985).

Some organisms can resist grazing by urchins and may provide refuges for less resistant species. Several studies have stressed the importance of refugia from urchin grazing in determining the structure of algal (e.g. Dayton, 1985) and invertebrate communites (e.g. Witman, 1985). For example, encrusting communities in the rocky subtidal of New England, USA, reach their greatest profusion either in the absence of urchins, or within beds of the mussel *Modiolus modiolus* when urchins are abundant (Sebens, 1985, 1986; Witman, 1985). This large bivalve adds significantly to the structural complexity of these rock wall habitats and serves as an important refuge for organisms against grazing urchins and predators (Witman, 1985; Ojeda & Dearborn, 1989). Structural habitat complexity may not only modify the foraging activities of predators it may also influence the settlement and recruitment of marine invertebrates (Keough & Downes, 1982).

An examination of the structure of rock wall assemblages, must therefore consider the potential for grazer-resistant species (such as *Modiolus*) to form refuges, both from grazing and the activities of other predators. On the other hand, refuge forming species will take up space - a limiting resource - and may thus be in direct competition with those species that do not require a refuge. The expectation is then, that some species will be positively associated with refugia, while other species will be negatively associated.

On the New South Wales (NSW) coast the sea urchin, *Centrostephanus rodgersii*, is the most

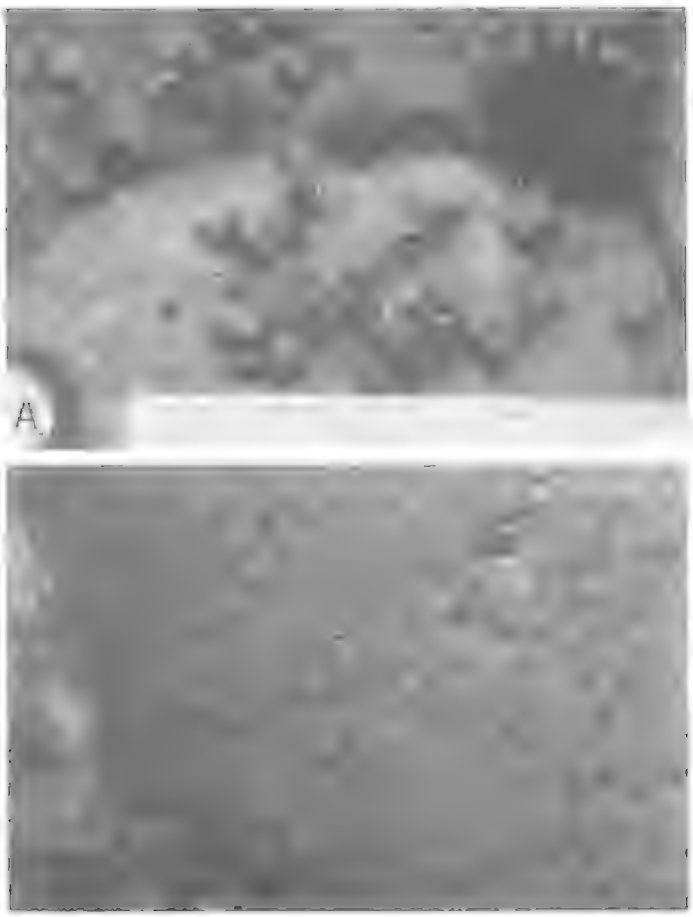

FIG. 1. A, The sponge *Darwinella australiensis* with a clump of *Austrobalanus imperator* as the urchin *Centrostephanus rodgersii* lurks nearby (Photo A. Davis). B, A high density *of A. imperator* overgrown by the sponge *Tedania* sp. Only the opercular plates of the barnacles are visible (Photo D. Ward).

conspicuous generalist grazer in the shallow rocky subtidal zone. This urchin creates and maintains extensive areas of substratum dominated by grazer-resistant encrusting coralline algae (Fletcher, 1987; Andrew, 1991). In these urchin grazed areas, sponges and colonial ascidians, reach their greatest profusion on rock walls or the vertical faces of large boulders. The large barnacle *Austrobalanus imperator*, which can measure over 4cm in height, also forms dense aggregations on shallow subtidal rock walls (Davis & Ward, unpublished data), thereby adding significantly to the structural complexity of these habitats (Fig. 1A). Another barnacle, *Balanus trigonus*, also often occurs in high densities in the subtidal zone, but is relatively small.

As part of a study to examine determinants of the structure and dynamics of natural rock surfaces in SE Australia we sought to determine how sessile invertebrates might maintain space in the face of intense grazing pressure by sea urchins. The sheer size of *A. imperator* combined with the high densities that occur on some rock walls should, we reasoned, represent a significant hinderance to grazing urchins and for that reason these barnacles are likely to play an instrumental role in determining the structure of subtidal rockwall communities. We initiated this study by collecting quantitative data on the relationship between invertebrate cover and the density of *A. imperator*.

MATERIALS AND METHODS

The relationship between barnacle density and invertebrate cover was investigated at three sites on the S coast of NSW. Sites were selected to encompass a length of the coast and for their convenient access. From north to south the sites were Henry Head (34°0.0'S, 151°14.2'E) at the N entrance to Botany Bay, the N end of Flinders Islet (34°27.3'S, 150°55.7'E) near Wollongong, and Longnose Point (35°4.5'S, 150°47.0'E) within Jervis Bay (Fig. 2). These sites spanned almost 150km of coastline and we considered them representative of a much larger number of potential sites. Vertical surfaces, ranging from rock walls to the faces of large boulders were haphazardly photographed at all sites, usually between a depth of 5-15m. A camera (Nikonos V), mounted on a frame with twin strobes (Nikonos SB102 and Ikelite Ai strobes) to provide even illumination, produced photographs of an area of 0.08m². Between 24-36 haphazard photographs were taken at each site after applying the criterion that the surface was vertical or near vertical.

The total cover of invertebrates was estimated from the photographs using a transparency overlay with 100 systematically arranged dots. Only sessile invertebrates were considered and so the anemone *Anthothoe albocincta*, although

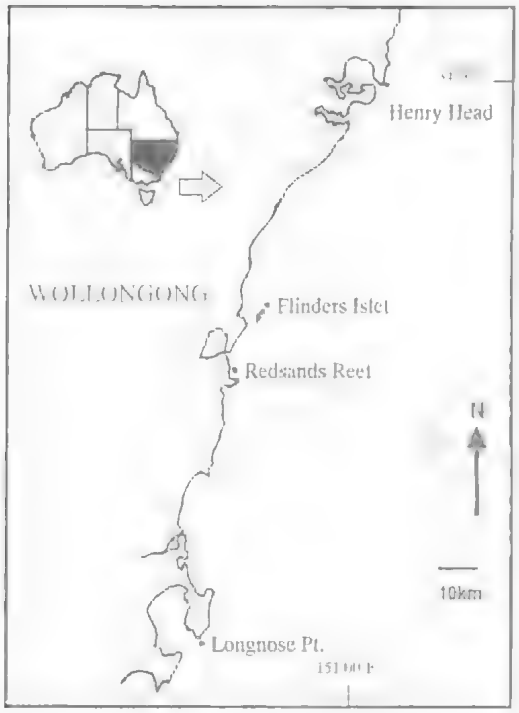

FIG. 2. Study sites on the S coast of NSW, Australia. The relationship between invertebrate cover and barnacle density were assessed at Henry Head, Flinders Islet and Longnose Point. The size frequency of *Austrobalanus imperator* was determined at Flinders Islet and Redsands Reef.

quite common in some areas was excluded owing to its mobility. The number of barnacles was also counted on each photograph. We were initially concerned that barnacles may be obscured by overgrowth, but found no evidence of this; the opercular plates of *Austrobalanus imperator* were always visible (Fig. 1B). Barnacles down to a size (basal diameter) of 5mm could be reliably recorded from the photographs. Correlation coefficients (Pearson) were calculated for each site.

Close examination of the photographs indicated that the pattern of distribution of one sponge, *Clathria pyramida*, contrasted with that of the other sessile invertebrates. To better assess the relationship between this sponge and the barnacles a series of haphazard photographs were taken of *C. pyramida* at Flinders Islet in September, 1995. Sponge cover was again estimated using the overlay transparency on the photographs and barnacles were also counted and recorded.

In order to get a clearer picture of the potential of *A. imperator* to form a refuge from urchin grazing we determined the size frequency of this barnacle at two sites; Flinders Islet and Redsands Reef (34°35.7'S, 150°54.3'E, Fig. 2). The maximum diameter of the base of each barnacle was measured in the field with calipers. To ensure that basal width was a good estimator of barnacle tissue biomass we regressed barnacle tissue dry weight against basal width. Data were pooled from collections at Flinders Islet and Redsands Reef.

RESULTS

Clathria (Dendrocia) pyramida Lendenfeld (Porifera, Demospongiae, Poecilosclerida, Microcionidae), showed a significant negative relationship with the density of the barnacle *A. imperator* ($r=-0.54$, $n=24$, $P<0.05$, one tailed test, Fig. 3). In contrast, the total cover of invertebrates, excluding *C. pyramida*, was strongly positively correlated with barnacle density at all three sites. Barnacle density explained more than 30% of the variation in invertebrate cover at two of these sites, Henry Head ($r=0.548$, $P<0.001$) and Flinders Islet ($r=0.586$, $P<0.001$). The positive relationship between barnacle density and cover of invertebrates was not statistically significant by a one tailed students t-test at Longnose Point

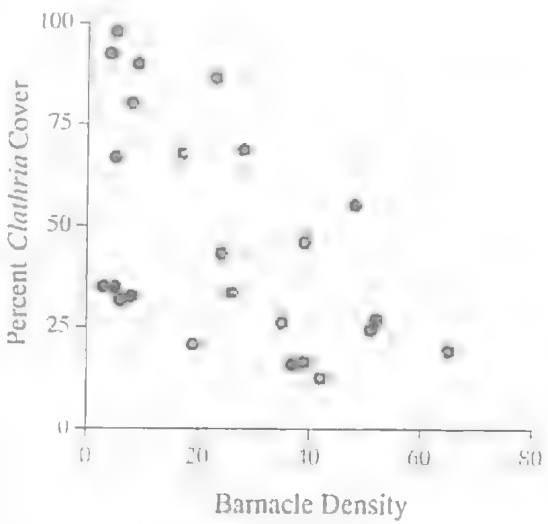

FIG. 3. The cover of *Clathia pyramida* was negatively correlated with the density of the barnacle *Austrobalanus imperator* within photographic plots. Each data point represents a plot photographed on 13 September, 1995 at Flinders Islet. ($r=-0.54$, $n=24$, $P<0.05$, one tailed test).

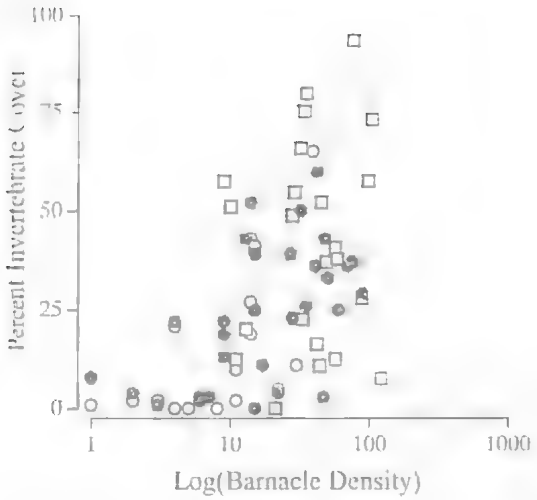

FIG. 4. Positive correlation between total invertebrate cover (excluding *Clathria pyramida*) and the density of the barnacle *Austrobalanus imperator* within photographic plots. Each data point represents a plot photographed at Henry Head (solid circle), Flinders Islet (open circle) and Longnose Point (square). All tests of significance were one tailed tests, Henry Head ($r=0.548$, $P<0.001$), Flinders Islet ($r=0.586$, $P<0.001$) and Longnose Point ($r=0.107$, $P>0.05$).

($r=0.107$, $P>0.05$). We present the data with the barnacle density log transformed to provide a more even spread of data on the *x* axis (Fig. 4).

The modal size of adult barnacles at both sites was between 35-40mm, with the largest individuals having a basal width of around 55mm. Recruits of *A. imperator* were not recorded at either site from which we collected size frequency data. A cohort of 'sub-adults' were observed at the Redsands Reef site, but these animals were still quite large with a modal basal width of around 15mm (Fig. 5A). No such cohort was observed at the Flinders Islet site (Fig. 5B). Maximum basal width of the barnacles was an excellent estimator of animal biomass (Fig. 6). The resultant power function produced a very strong correlation ($y=0.0000069^{x3.043}$, $r=0.95$).

DISCUSSION

With increasing densities of barnacles the cover of sessile invertebrates was also seen to increase. These findings are consistent with our initial suspicion that aggregations of *Austrobalanus imperator* form a refuge from sea urchin grazing. The rocky intertidal provides several examples of how habitat structure influences the activities of grazers (Hawkins & Hartnoll, 1982; Creese, 1982; Dungan, 1986). For example, Creese (1982) reported that surface heterogeneity provided by barnacle shells can markedly influence the ability of grazers to feed. Limpets caged with high densities of a common intertidal barnacle starved to death (Creese, 1982).

In addition to modifying patterns of invertebrate mortality, habitat structure may influence patterns of invertebrate colonisation. Bros (1987) reported a positive relationship between invertebrate recruitment and the addition of barnacle shells to glass slides in Tampa Bay Florida, although he noted that the treatments did not greatly affect the colonisation of sessile species. The responses of colonists to the modification of habitat structure on natural substrata remains unclear.

Although it is tempting to ascribe our findings to an increase in structural heterogeneity produced by the presence of these large barnacles an equally plausible alternate explanation is that the barnacles simply enhance recruitment rather

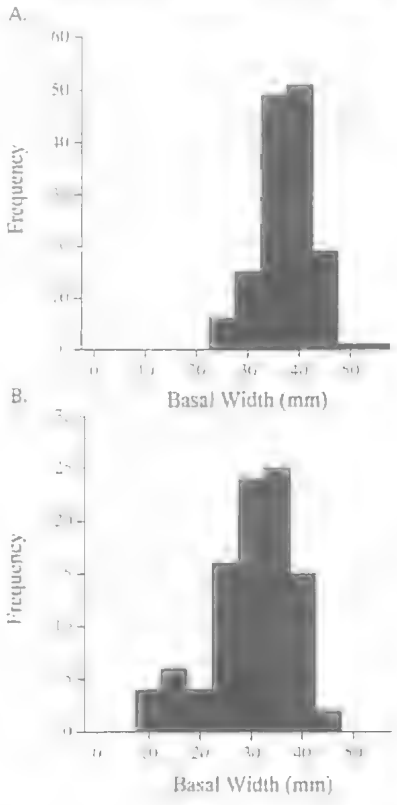

FIG. 5. Size frequency of *Austrobalanus imperator* at: A, Flinders Islet; and B, Redsands Reef. Data were collected on a single day in October, 1994 at each site.

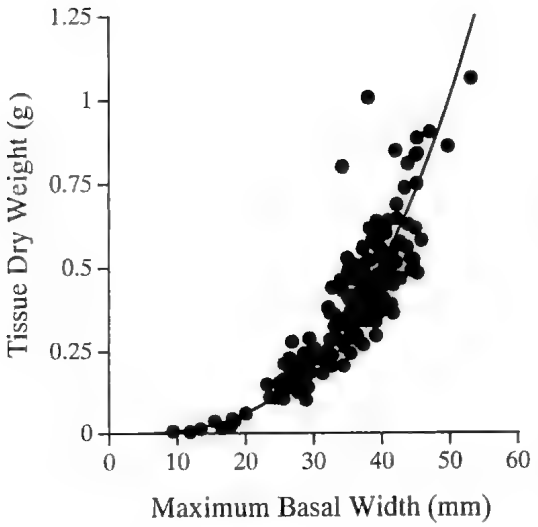

FIG. 6. Relationship between the maximum width of *Austrobalanus imperator* at their base with dry tissue weight (r=0.949).

than reducing mortality. Changes in hydrodynamics near the rock surface or the additional surface area provided by barnacles when compared to a smooth rock surface are two potential mechanisms by which invertebrate recruitment may be enhanced. Nevertheless our data are correlative and in the absence of experimental evidence we can only speculate as to the processes which have produced the patterns we have observed. Notably, though, we have produced a conservative test of our initial hypothesis as a high density of barnacles will leave less space for other sessile invertebrates, yet we see higher invertebrate cover in the presence of high densities of barnacles.

The striking negative relationship that we observed between barnacle density and the cover of *C. pyramida* suggests that this sponge is not reliant on the presence of barnacles to establish itself successfully and maintain space. *Clathria pyramida* clearly contains novel metabolites (Capon & Macleod, 1987) and field bioassays with crude solvent extracts of this sponge have revealed the presence of antifeedant natural products that dissuade urchins (*Centrostephanus rodgersii*) from grazing (Wright et al., 1997). The nature of the deterrent metabolites is currently unknown, but is under investigation (Davis & van Altena, work in progress). It is likely that the presence of biologically active metabolite(s) explains why this sponge does not rely on the presence of barnacles. However, the strong negative relationship we observed is consistent with the avoidance of barnacles by *C. pyramida*. This may be an appropriate strategy if this sponge is likely to encounter competitively superior species among aggregations of barnacles. Several studies reveal that some invertebrate larvae can detect competitive dominants and subsequently avoid sites where their survivorship is likely to be compromised (Grosberg, 1981; Davis, 1987). There is no need to invoke avoidance of barnacles or competitive dominants by the larvae of *C. pyramida* to explain the observed pattern; directional growth by adults could produce the same pattern. The reasons why *C. pyramida* avoids barnacles and the mechanisms used to do this remain speculative as nothing is known of the competitive ability of *C. pyramida* relative to other sessile species it is likely to encounter in SE Australia.

It appears that the presence of *A. imperator* is an important contributor to the structure and dynamics of encrusting communities on vertical surfaces in SE Australia. Unfortunately, little is known of the biology of this cirripede or the determinants of its distribution and abundance; of particular interest for example is how recruits of the barnacle withstand grazing by urchins. Our data reveal that recruitment of *A. imperator* is sporadic as we did not detect reliable recruitment to the barnacle population in the course of this study. There are also no data on the growth rates of this barnacle and therefore the time taken to reach a size that may interfere with the grazing activities of urchins, thereby forming a refuge. Experimental manipulation of the density of this barnacle is an important step in better understanding its role in these benthic communities and is the focus of current work.

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CONVENIENT GENERA OR PHYLOGENETIC GENERA ? EVIDENCE FROM CALLYSPONGIIDAE AND NIPHATIDAE (HAPLOSCLERIDA)

RUTH DESQUEYROUX-FAÚNDEZ

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A taxonomic revision of all nominal genera in the haplosclerid Callyspongiidae and Niphatidae (Porifera; Demospongiae), is based exclusively on type species and discusses the taxonomic value of traditional characters. Of 27 available nominal genera in both families only 19 available genera are recognised. For some of them I tentatively propose subgenera (of *Callyspongia*), or synonymise them with other genera (e.g. –*Cladochalina*). Type species of 6 'chalinid' genera in the early literature of Lendenfeld 1886-1888 are comprehensively revised and their taxonomic status confirmed, as previously suggested by Burton in 1934, or changed, and some are illustrated for the first time. Morphometric characters important in defining a species group (genus or subgenus) include specific modifications to a specialised ectosomal skeleton, structure and distribution of choanosomal fibres, presence and width of the spongin sheath in the fibres, presence of foreign material and free spicules in the skeleton, and presence and amount of free spongin in the skeleton. General characters, only useful as a reference for identification of a species group, but not essential to establish their taxonomical status are also indicated. ❐ *Porifera, Demospongiae, Haplosclerida, Callyspongiidae, Niphatidae, generic revision, taxonomy, morphology.*

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The taxonomy of Haplosclerida is still controvertial as far as recognition, interpretation and definition of genera, subgenera and species groups are concerned. One reason for this is the small number of unequivocal taxonomic characters available (including secondary metabolite structures). The recognition of 'reliable' characters, which are discrete and consistent for a genus group, is a first condition for the construction of a classification based on natural affinities.

Recent revisions of Haplosclerida provided some new insights to the taxonomy of this group. A study of West Indian Haplosclerida, notably re-examination of the Duchassaing & Michelotti collection, lead Van Soest (1980) to propose a new classification of families and genera, and to erect three new families: Niphatidae, Petrosiidae and Oceanapiidae (pro Phloeodictyidae). A phylogeny of Haplosclerida was proposed, based principally on features of the ectosomal skeleton, and nearly completely disregarding microscleres.

A rival classification based on five descriptive criteria (colour, growth form, consistency, spicules and skeleton) was published by Bergquist (1980) and Bergquist & Warne (1980), who distinguished two orders (Haplosclerida and Nepheliospongida), and five families (Haliclonidae, Callyspongiidae, Adociidae, Nepheliospongiidae and Oceanapiidae (pro Phloeodictyidae).

The monophyly of Haplosclerida was supported by de Weerdt (1985, 1986, 1989), in a study of three families of Eastern Atlantic Haplosclerida: Oceanapiidae (pro Phloeodictyidae), Chalinidae and Petrosiidae. De Weerdt (1985, 1986, 1989) based her phylogeny principally on synapomorphies of skeletal architecture. Van Soest (1990) claimed that Bergquist (1980) emphasised some of the apomorphic characters shared by Nepheliospongida and Haplosclerida, while at the same time recognising Nepheliospongida (pro Petrosida) as a distinct order. Van Soest (1990) proposed to keep Nepheliospongida (pro Petrosida) within the Haplosclerida, as suborder or superfamily. Presently, the taxonomic position of Petrosida is still unresolved.

Sponge secondary metabolites also appear to be useful in characterising different groups of sponges (e.g. reviews by Bergquist & Wells, 1983; Van Soest & Braekman, 1999, this volume), and based on an analysis of sterols in Haplosclerida and Petrosida there is no chemotaxonomic support for a division of Haplosclerida into two orders (Fromont et al., 1994). A phylogeny of

TABLE 1. Callyspongiidae genera and their taxonomic assignments.

Genus	Type species	Original assignment	Actual assignment	Proposed subgenus assignment	Synonymy
Callyspongia Duchassaing & Michelotti, 1864	*C. fallax*	*C. fallax* Duchassaing & Michelotti, 1864	*C. (Callyspongia) fallax*	*Toxochalina, Spinosella, Chalinopora, Patulascula Euplacella*	*Cavochalina* Carter, 1885, *Placochalina* Lendenfeld, 1887, ?*Platychalina* Esper, 1797, *Ceraochalina* Lendenfeld, 1887, *Chalmella* Lendenfeld, 1887
Toxochalina Ridley, 1884	*T. foliodes*	*Desmacidon foliodes* Bowerbank, 1875	*C. (Toxochalina) foliodes*		
Spinosella Vosmaer, 1885	*S. sororia*	*Tuba sororia* Duchassaing & Michelotti, 1864	*C. (Spinosella) sororia*		*Claudochalina* Schmidt, 1870
Chalinopora Lendenfeld, 1887	*C. typica*	*C. typica* Lendenfeld, 1887	*C. (Chalinopora) typica*		*Euchalina* Lendenfeld, 1887
Siphonochalina Schmidt, 1868	*S. coriacea*	*S. Coriacea* Schmidt, 1868	*Siphonochalina coriacea*		*Sclerochalina* Schmidt, 1868, *Siphonella* Lendenfeld, 1887, *Tubulodigitus* Carter, 1881
Patulascula Carter, 1882	*P. procumbens*	*P. procumbens* Carter, 1882	*C. (Patulascula) procumbens*		
Euplacella Lendenfeld, 1887	*E. australis*	*E. australis* Lendenfeld, 1887	*C. (Euplacella) australis*		
Arenosclera Pulitzer-Finali, 1982	*A. heroni*	*A. heroni* Pulitzer-Finali, 1982	*A. heroni*		
Dactylia Carter, 1885	*D. chaliniformis*	*D. chaliniformis* Carter, 1885	*D. chaliniformis*		*Chalinopsis* Lendenfeld, 1886 *Chalinopsilla* Lendenfeld, 1888

Haplosclerida based on molecules of the 3-alkylpiperidine alkaloid types appears incongruent with the phylogenetic tree of haplosclerid families, probably because of the uncertainty in identification of species analyzed and also ignorance of metabolite occurrence throughout a range of taxa (Andersen et al., 1996). Straight-chain acetylene compounds of Haplosclerida are efficient markers for the whole order, and in some cases can be characteristic of certain genera and families (Van Soest et al., 1997), but these compounds are found among species of other orders, and their presence or absence is not an absolute indicator of phylogenetic relationships.

Moreover, difficulties encountered in the application of biochemical methods can also be attributed to the diversity of other organisms living within sponges (e.g. symbiotic cyanobacteria), making it arduous to identify the origin of chemical compounds in some instances (Bergquist & Wells, 1983). Consequently, chemotaxonomic data must be treated with caution, and these data are not considered in this present paper.

In this paper, therefore, I am constrained to using to relatively few 'reliable' morphometric characters traditionally described for Haplosclerida, but this raises three problems. 1) Superficially at least, some of these 'reliable' morphological characters appear to be lacking in some taxa included in the five recognised families of Haplosclerida: Chalinidae, Niphatidae, Callyspongiidae, Phloeodictyidae and Petrosidae. 2) Conversely there are a number of nominal taxa, doubtfully available or presently considered as synonyms, which are clearly distinct from each other in their respective 'reliable' characters. 3) Frequently characters have been difficult to differentiate because their variability occurred within very narrow bounds, especially related to the structure of fibres.

In order to clarify the status of these taxa I undertook a thorough analysis of the structural characters, especially at the skeletal level, in two families of Haplosclerida: Callyspongiidae and Niphatidae. I focused on the use of shared morphological characters as determinant tools to establish the taxonomic position of genera.

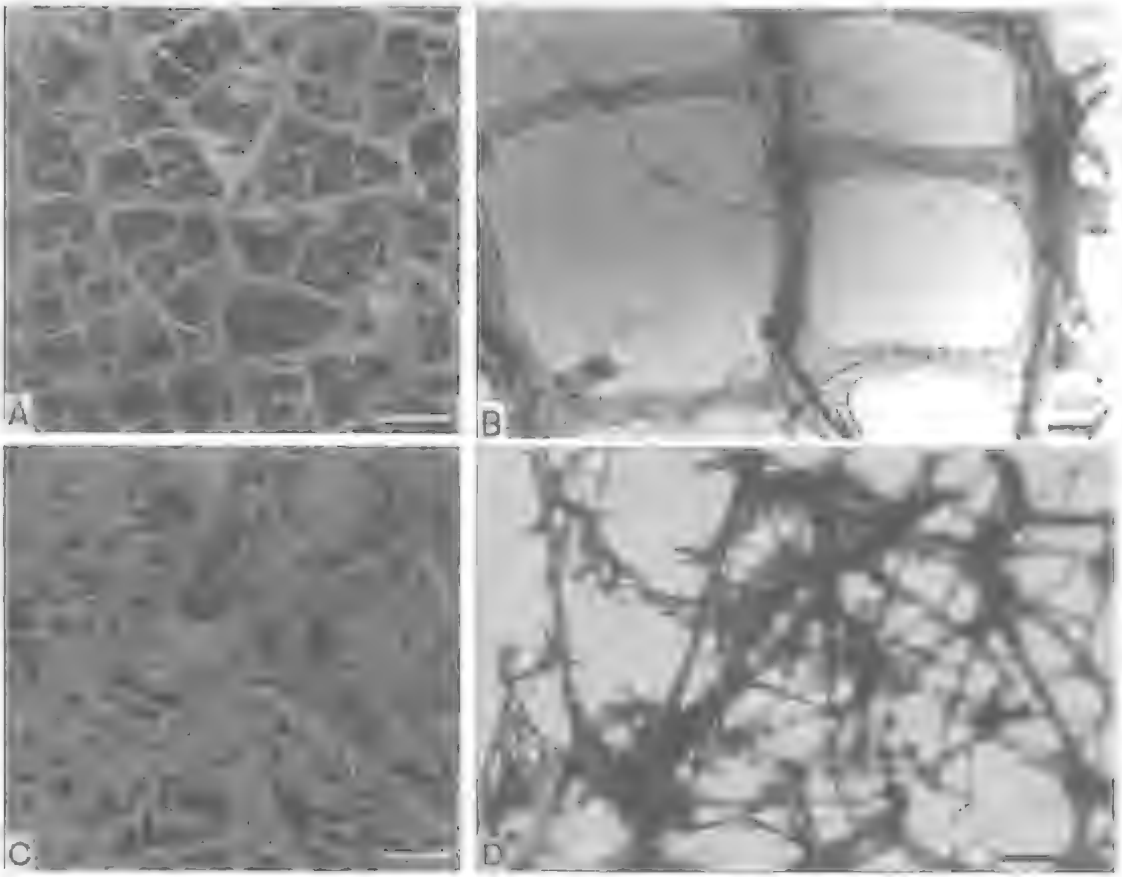

FIG. 1. *Callyspongia* and *Toxochalina*. A, B, *Callyspongia* Duchassaing & Michelotti, 1864. Type species *Callyspongia fallax* Duchassaing & Michelotti, 1864. St. Thomas. Schizolectotype BMNH1928:11:12:5. A, Ectosomal network. Large, triangular to polygonal meshes, subdivided in smaller secondary and tertiary meshes. B, Choanosomal regular meshes, longitudinal principal and transversal connecting fibres. (Scales bars A = 200µm; B = 8.2µm). C, D, *Toxochalina* Ridley, 1884. Type species *Desmacidon foliolides* Bowerbank, 1875, Straits of Malacca, 'Bowerbank Collection'. Lectotype BMNH1887:5.21.2034. C, Ectosomal network, three types of meshes. D, Choanosomal network. (Scale bars C = 100µm; D = 20µm).

subgenera and/or groups of species in Haplosclerida.

I present here morphological evidence obtained exclusively from the study of type species of each nominal genus.

MATERIALS AND METHODS

Type material from the following Institutions was studied: BMNH, The Natural History Museum, London; MNHN, Muséum National d'Histoire Naturelle, Paris; IRSNB, Institut Royal de Sciences naturelles de Belgique, Brussels; ZMA, Zoological Museum, Amsterdam; MSNG, Museo de Storia Naturalle, Genova; AM, Australian Museum, Sydney; QM, Queensland Museum, Brisbane; MHNG, Muséum d'histoire naturelle, Geneva .

Described characters, and taxonomic relationships based on them, reflect my own critical observations. Consequently, character analyses were completed with the inclusion of remarks from the original author's descriptions. Specimens were studied by light and SEM microscopy. Lists of structural characters and character states of genera were established by comparing differences between genera. The following characters were used to compare genera: 1) variations in external morphology; 2) surface features; 3) type of ectosomal and choanosomal skeletons; 4) fibre structure and width variations of the spongin sheath; 5) presence of free spicules

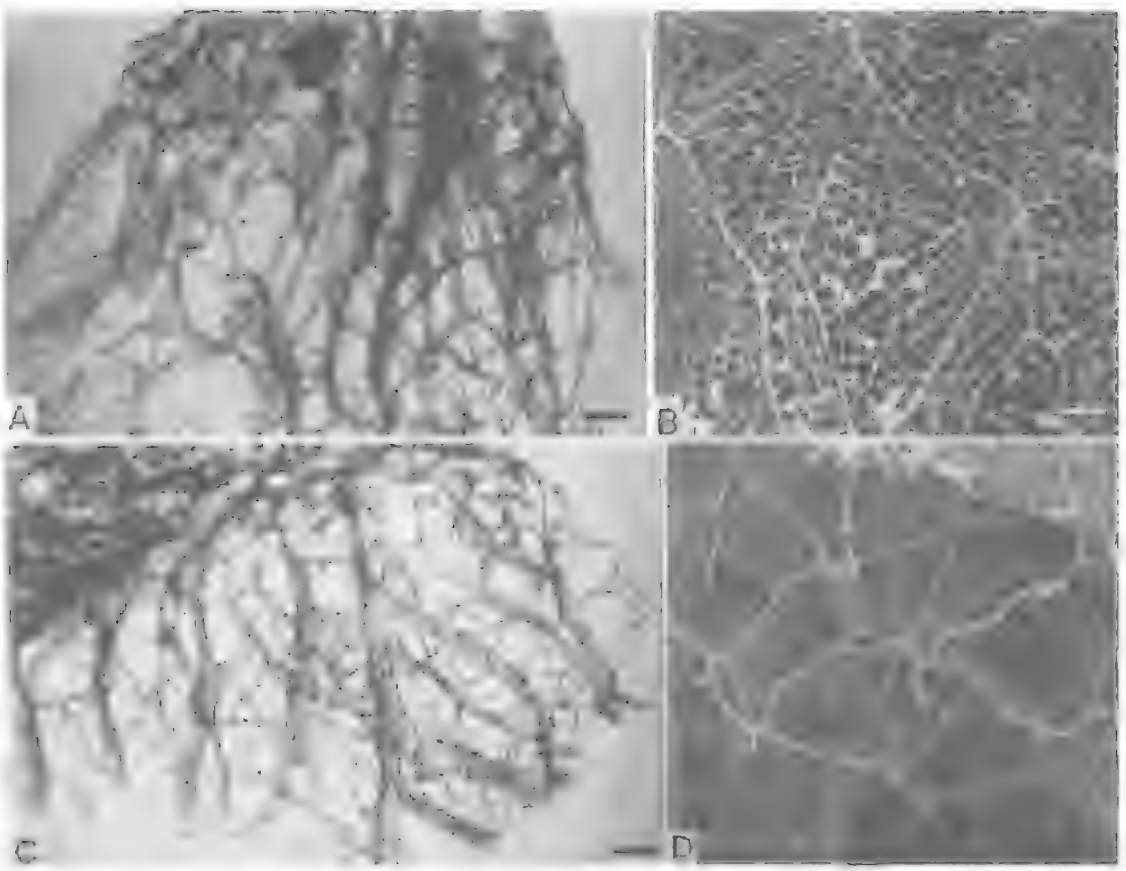

FIG. 2. *Spinosella (=Cladochalina)* and *Chalinopora (=Euchalina)*. A, C, *Spinosella* Vosmaer, 1885. Type species *Tuba soreria* Duchassaing & Michelotti, 1864. St. Thomas. Paralectotype. MUS. TORINO POR118. A, Longitudinal section through conular fascicle, surface at both sides of the figure. C, Three different sizes of triangular to polygonal ectosomal meshes and fasciculated subectosmal longitudinal fibres. (Scale bars A, C 8.2µm). B, D, *Chalinopora* (=*Euchalina*) Lendenfeld, 1887. Type species *Chalinopora typica* Lendenfeld, 1887. Port Jackson, NSW. Syntype BMNH1886:8:27:411 (AMG3408, slide). B, Simple ectosomal network, one size of fine triangular to rectangular meshes. Conules not visible. D, Choanosomal network, fasciculated underlying longitudinal primary fibres. Fragment of surface on top. (Scale bars B = 200µm; D = 500µm).

in fibre meshes; and 6) amount of free spongin and type of spicules. Each character was checked for its presence or absence; and when present, each character was scored objectively as to the expresion of the character, with at least three different states recognised per character. In the present work only the three most significant ('reliable') characters are presented. Other allegedly 'inconsistent' or 'unreliable' characters, deemed by previous authors to have little or no taxonomic value at the supraspecific level (e.g. external morphology), are omitted from this work. A more complete, phylogenetic analysis of these taxa will is in progress (Desqueyroux-Faúndez, in prep.).

Whenever a character used in an original description was not sufficiently informative for the present work, a detailed description from a larger haplosclerid study was used (Desqueyroux-Faúndez, in prep.). In some cases, re-examination of type specimens did not closely follow the published description, or there was mistaken identification of type material by the original author, with the consequence that the actual concept of some genera had to be changed (e.g. *Hemigellius*).

Taxonomy and classification of families and genera are based on the most recently accepted classification of Porifera: Haplosclerida of Wiedenmayer, in Hooper & Wiedenmayer (1994).

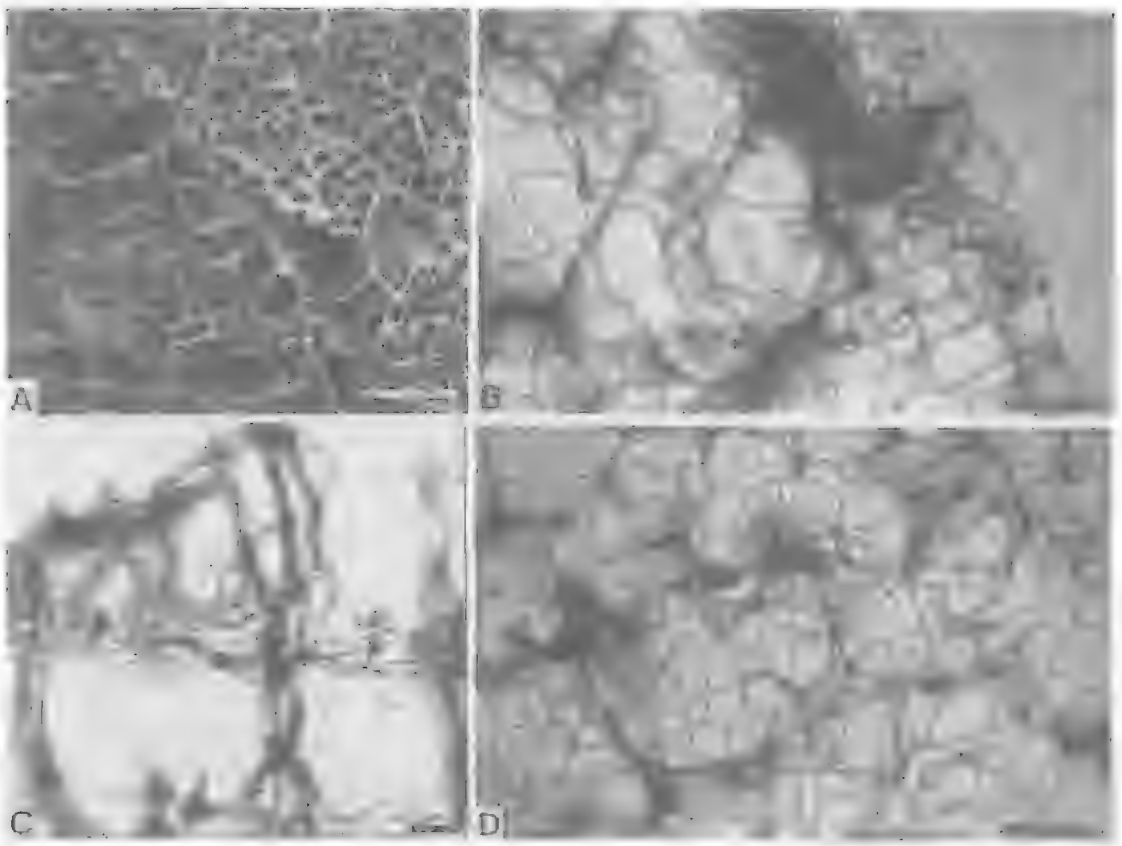

FIG. 3. *Ceraochalina (=Chalinella)* and *Tubulodigitus*. A, C, *Ceraochalina* Lendenfeld, 1887. Type species *Ceraochalina typica* Lendenfeld, 1887. Port Phillip, Victoria. Holotype BMNH1886:8:27:439. A, Transverse section of ectosomal network with one size of triangular meshes, subectosomal longitudinal fibres, intercalate short longitudinal fibres, small meshes and transverse fibres (arrow). C, Longitudinal section of surface, intercalate fibres and transversal fibres with echinating oxea. (Scale bars A = 500μm; C = 8.2μm). B, D, *Tubulodigitus* Carter, 1881. Type species *Tubuludigitus communis* Carter, 1881. Bass Strait. Neotype BMNH-1889:1:21:1. B, Longitudinal section through ecto and choanosomal skeleton. D, Transverse section of ectosomal skeleton, with one size of meshes. (Scale bars B, D = 20μm).

Modification of this classification in this work, and actual taxonomic assignments are indicated in each case (Table 1). Terminology for descriptive morphological characters is taken from Boury-Esnault & Rützler (1997). New morphometric terms were introduced and defined, when necessary.

SYSTEMATICS

A list of the major 'reliable' characters and their character states is presented as follows.

CHARACTER 1. *Ectosomal skeleton.* 1(1), Tangential, regular network, three sizes of large triangular to polygonal meshes subdivided in smaller secondary and tertiary meshes (triple ectosomal network). Inconspicuous conules formed by free end of only one longitudinal primary fibre (*Callyspongia*, Fig. 1A; *Toxochalina*, Fig. 1C). 1(2), Tangential regular network around a conspicuous central conule, with three sizes of large triangular to polygonal meshes subdivided in smaller secondary and tertiary meshes (triple ectosomal network). Central conspicuous conule produced by ends of the fascicle branches of longitudinal primary fibres (*Spinosella (= Cladochalina)*, Figs 2A, C). 1(3), One size of fine triangular to rectangular meshes (simple ectosomal network) over confusely fasciculated underlying longitudinal primary fibres. Conules not visible (*Chalinopora* (=*Euchalina*), Fig. 2B, D). 1(4), One size triangular

TABLE 2. Niphatidae genera and their taxonomic assignments.

Genus	Type species	Original assignment	Actual assignment	Proposed subgenus arrangement	Synonymy
Gelliodes Ridley, 1884	*G. fibulata*	*Axos fibulatus* Carter, 1881	*Gelliodes fibulata*		
Microxina Topsent, 1916	*M. charcoti*	*M. charcoti* Topsent, 1916	*Microxina charcoti*		
Niphates Duchassaing & Michelotti, 1864	*N. erecta*	*N. erecta* Duchassaing & Michelotti, 1864	*Niphates erecta*		
Dasychalina Ridley & Dendy, 1887	*D. fragilis*	*D. fragilis* Ridley & Dendy, 1886	*Pachychalina (Dasychalina) fragilis*	*Dasychalina*	
Pachychalina Schmidt, 1868	*P. rustica*	*P. rustica* Schmidt, 1868	*Pachychalina rustica*		
Amphimedon Duchassaing & Michelotti, 1864	*A. compressa*	*A. compressa* Duchassaing & Michelotti, 1864	*Amphimedon compressa*		*Hemihaliclona* Burton, 1937
Hemigellius Burton, 1932	*G. rudis*	*Gellius rudis* Topsent, 1901	*Hemigellius rudis*		
Cribrochalina Schmidt, 1870	*C. infundibulum*	*C. infundibulum* Schmidt, 1870	*Cribrochalina infundibulum*		
Haliclonissa Burton, 1932	*H. verrucosa*	*H. verrucosa* Burton, 1932	*Haliclonissa verrucosa*		

meshes (simple ectosomal network); isolated underlying longitudinal fibres connected by 2-3 transverse fibres with echinating brushes of oxeas. Intercalate longitudinal fibres present to form small subectosomal meshes (*Ceraochalina*, Fig. 3A, C (= *Chalinella*); *Siphonochalina*, Fig. 4A (= *Sclerochalina*, Fig. 4B, D; *Siphonella; Tubulodigitus*, Fig. 3B, D). 1(5), One size rounded meshes (simple ectosomal network); isolated underlying subectosomal ends of longitudinal primary fibres profusely divided to form uniform tangential network of poorly delimited unispicular fibres (*Patuloscula*, Fig. 5A, B). 1(6), One size rounded meshes (simple ectosomal network); ends of longitudinal primary fibres connected by three tangential successive layers of parallel fibres, echinated by numerous surface spicular brushes of oxeas. In longitudinal section subectosomal meshes appear smaller than choanosomal (peripheral condensation) (*Euplacella*, Fig. 5D). 1(7), Tangential irregular network of fragmentary unispicular fine fibres, one size rounded meshes (simple ectosomal network); string of foreign material (*Arenosclera*, Figs 6A). 1(8), Tangential irregular meshes; fine aspicular fibres finely cored by foreign material. No distinction between primary and secondary fibres (*Dactylia*, Figs 6C, D; *Chalinopsilla*, Fig. 7A). 1(9), Tangential irregular fibre network of secondary multispicular fibres, interrupted by ends of longitudinal primary fibres (ramified spines or conules); free oxeas and sigmas abundant (*Gelliodes*, Fig. 7B). 1(10), Strong free unordered oxea network, interrupted by ends of longitudinal primary fibres (brushes of spicules or strong spines); microscleres (sigma or microxea) abundant or scarce (*Microxina*, Fig. 8A; *Niphates*, Fig. 8C). 1(11), Tangential irregular network of abundant free oxeas and fibres interrupted by strong ends of longitudinal primary

TABLE 3. Presence of ectosomal and choanosomal characters and their distribution amongst genera of Callyspongiidae.

Genus	Ectosomal meshes	Subectosomal fibres	Longitudinal intercalate ectosomal fibres	Parallel connecting ectosomal fibres
Callyspongia	three sizes	isolated	absent	absent
Toxochallina	three sizes	isolated	absent	absent
Spinosella	three sizes	fasciculated	absent	absent
Ceraochalina (=*Chalinella*)	one size	isolated	present	absent
Chalinopora (=*Euchalina*)	one size	fasciculated	absent	absent
Siphonochalina (=*Sclerochalina, Siphonella, Tubulodigitus*)	one size	isolated	absent	absent
Patuloscula	one size	isolated	absent	present
Euplacella	one size	isolated	absent	present (3 layers)
Arenosclera	one size	absent	absent	absent
Dactylia (=*Chalinopsilla*)	one size	absent	absent	absent

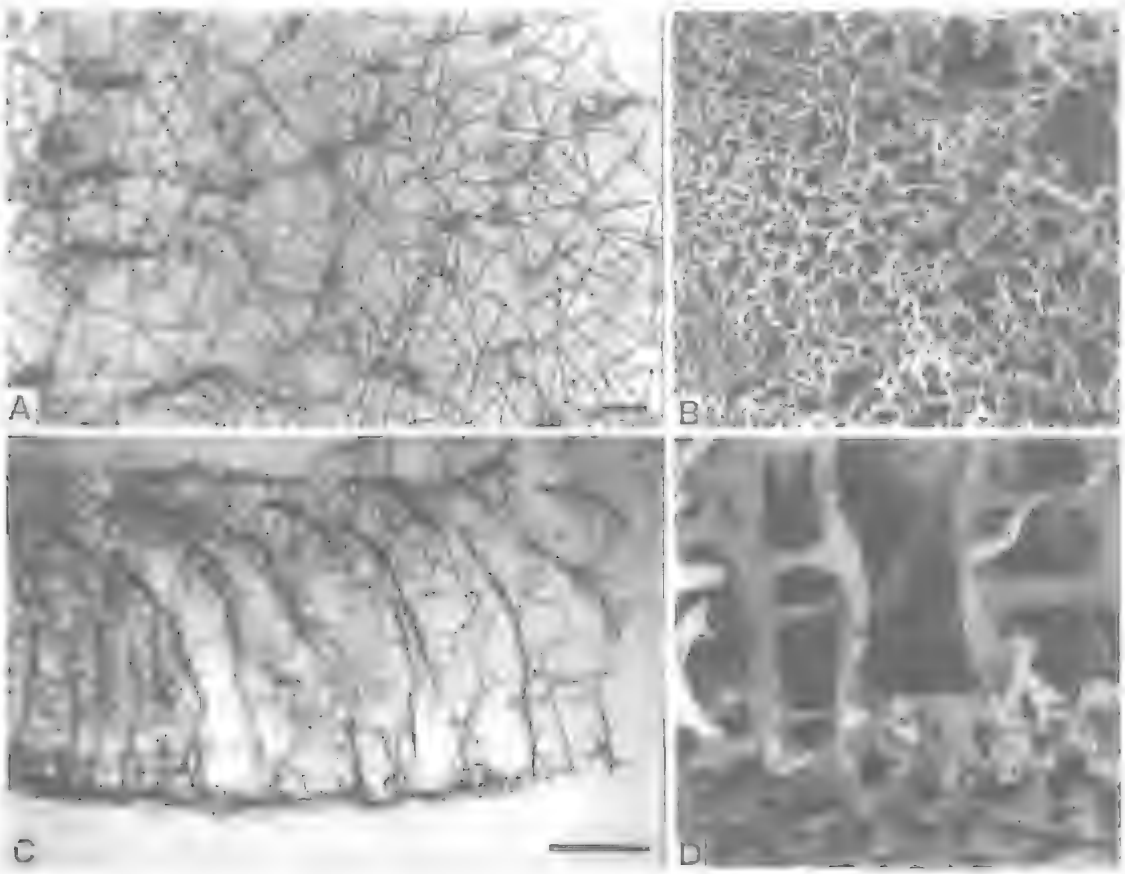

FIG.4. *Siphonochalina and (=Sclerochalina).* A, C, *Siphonochalina* Schmidt, 1868. Type species *Siphonochalina coriacea* Schmidt, 1868. La Calle, 'Lacaze-Duthier' collection. Syntype MNHN LBIM DT77. A, Dense tangential network of fine unispicular fibres with uniform meshes of only one type. C, Longitudinal section through choanosomal and ectosomal networks. Parallel, strong primary fibres with large spongin sheath. Ectosomal skeleton at the base of the figure (Scale bars A = 8.2µm; C = 20.0µm). B, D, *Sclerochalina* Schmidt, 1868. Type species *Sclerochalina asterigena* Schmidt, 1868. La Calle, 'Lacaze-Duthier' collection. Holotype MNHN LBIM DT 89-11. B, Simple ectosomal network with one size of triangular to rectangular small meshes. D, Longitudinal section through subectosomal and surface regions. Isolated parallel, strong longitudinal fibres, abundant spongin, surface below. (Scale bars B = 500µm; D = 100µm).

fibres (aculeations, prickles or stings) (*Dasychalina*, Fig. 9A). 1(12), Tangential regular fibre network with uniform rounded meshes. Ends of longitudinal primary fibres barely protruding (*Amphimedon*, Fig. 9C). 1(13), Perpendicular ill-defined extremely irregular network of spicule brushes in between the ends of primary fibres, riddled by aquiferous orifices (*Pachychalina*). 1(14), Tangential dense irregular network of free oxeas and sigmas forming continuous layer over ends of primary fibres (*Hemigellius*, Fig. 10B). 1(15), Perpendicular ends of longitudinal primary fibres expanded as strong continuous palisade of free oxeas (strongly hispid crust) (*Cribrochalina*, Fig. 10D). 1(16), Perpendicular ends of longitudinal primary fibres expanded to form wart-like elevations (verrucose surface), abundant free oxeas in between (*Haliclonissa*).

CHARACTER 2. *Choanosomal skeleton.* 2(1). Regular network of longitudinal parallel strong primary fibres, regularly connected by short secondary fibres to form empty rectangular very regular meshes. Large spongin sheath present (*Callyspongia* Fig. 1B; *Ceraochalina* (= *Chalinella)*, Fig. 3C; *Siphonochalina*, Fig. 4C). 2(2). Strong irregular network with large triangular to irregular meshes, poorly oriented; compact multispicular primary fibres irregularly

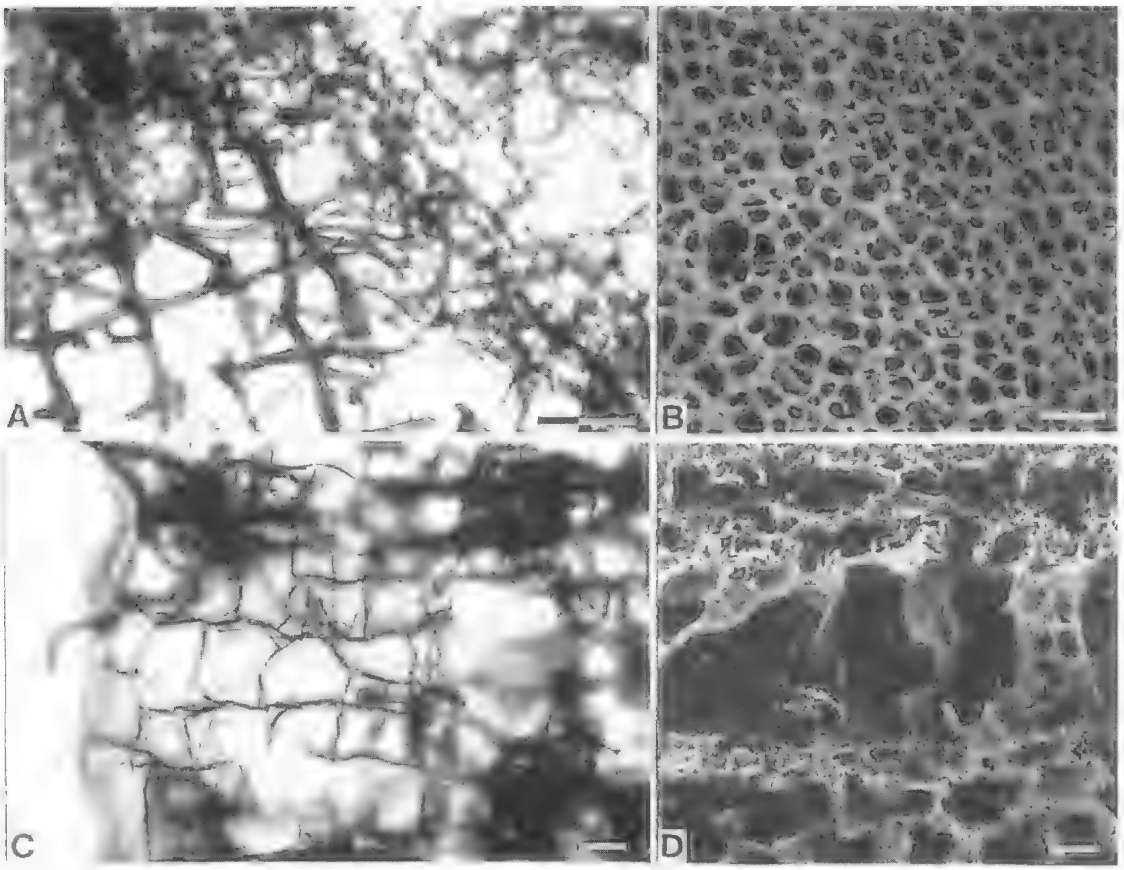

FIG. 5. *Patuloscula* and *Euplacella*. A, B, *Patuloscula* Carter, 1882. Type species *Patuloscula procumbens* Carter, 1882, Grenada, West Indies. Syntypes BMNH:1845:5:12:13, 15, 16. A, Longitudinal section, choanosomal region, isolated parallel subectosomal ends of primary longitudinal fibres at the base of the figure. B, Transverse section, continuous ectosomal layer, one size rounded meshes of poorly delimited unispicular fibres (simple network). (Scale bars A = 20μm; B = 500μm). C, D, *Euplacella* Lendenfeld, 1887. Type species *Euplacella australis* Lendenfeld, 1887. Torres Straits, Qld. Lectotype BMNH1886:8:27:591. C, Choanosomal network, paucispicular primary fibres with large spongin sheath, surface at left. D, Longitudinal section through triple ectosomal layer or 'peripheral condensation', with surface brushes of oxea, three layers indicated by arrows. (Scale bars C = 8.2μm; D = 200μm).

split up to form connective fibres. Spongin sheath absent from all types of fibres, present only at fibre nodes. (*Toxochalina*, Fig. 1D). 2(3), Irregular confused network of multispicular fasciculated longitudinal primary fibres, irregularly split up to form short connective fibres. Empty meshes of only one type but of different sizes. All fibres with narrow spongin sheath (one 25 % of fibre diameter) (*Chalinopora* (= *Euchalina*), Fig. 2D). 2(4), Strong network of stout longitudinal paucispicular, primary fibres and irregular short connecting fibres, irregular empty meshes. Fibres with large spongin sheath (*Euplacella*, Fig. 5C; Patuloscula, 5A). 2(5), Strong dense network of longitudinal primary fibres gathered to form fibrofascicles and split up to form free secondary fibres. Irregularly elongate to roundish meshes, always subdivided by tertiary finer fibres (*Spinosella* (= *Cladochalina*), Fig. 2C). 2(6), Aspicular network of longitudinal divergent primary fibres and perpendicular connecting fibres, abundantly cored by foreign material. Irregular to rectangular empty meshes (*Dactylia* (= *Chalinopsilla*), Fig. 7C). 2(7), Intricate network of undifferentiated meshes and fibres with no preferential direction, not clearly distinguishable,abundantly cored by foreign material (*Arenosclera*, Fig. 6B). 2(8), Regular network of longitudinal primary fibres and short connecting fibres, isotropic to elongate

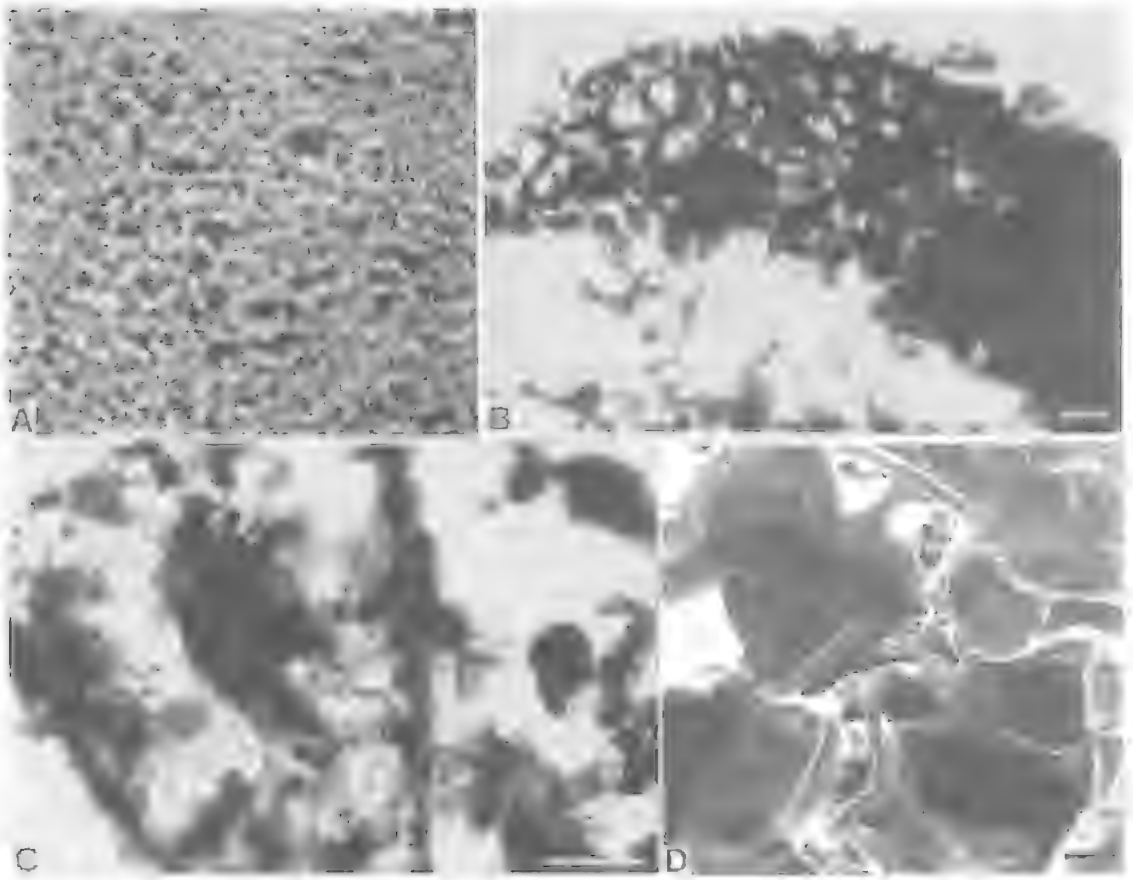

FIG. 6. *Arenosclera* and *Dactylia*. A, B, *Arenosclera* Pultizer-Finali, 1983. Type species *Arenosclera heroni* Pultizer-Finali, 1982. Heron Island, GBR, Qld. Holotype MSNG 46949. A, Tangential, irregular network of string of foreign material, one size rounded meshes (simple ectosomal network). B, Longitudinal section through ectosomal and choanosomal networks. (Scale bars A = 200μm; B = 8.2μm). C, D, *Dactylia* Carter, 1885. Type species *Dactylia chaliniformis* Carter, 1885. Port Phillip Heads, Victoria. Holotype BMNH 1886:12:15:196. C, Ectosomal network, abundant foreign material. D, Fine aspicular ectosomal fibres, finely cored by foreign material. (Scale bars: C = 20μm; D = 50μm).

meshes. Abundant free spicules (*Cribrochalina*, Fig. 10C). 2(9), Diffuse longitudinal primary fibres, tight and ill-defined meshes, abundant free spicules. Spongin abundant: free and coring the fibres (*Amphimedon*, Fig. 9D. 2(10), Poorly defined meshes with numerous free spicules in between the fibres; longitudinal primary fibres divergent, intermingled. Spongin inconspicuous (*Haliclonissa*). 2(11), Strong compact, multispicular primary fibres split up to form non connecting secondary fibres lacking orientation, irregular meshes, scattered spicules (*Dasychalina*, Fig. 9B). 2(12), Open and loose, formed by compact longitudinal primary fibres and abundant free secondary fibres, oxeas and sigmas, irregular meshes, free spicules (*Gelliodes* Fig. 7D). 2(13), Confused compact formed by abundant unordered strong spicules, no clear fibres or meshes. No visible spongin. Abundant sigmas (*Hemigellius*). 2(14), Confused, irregular lacuna with thick longitudinal primary fibres repeatedly divided to form isolated finer free ramifications, abundant free spicules, no clear meshes (*Pachychalina*, Fig. 10A). 2(15), Abundantly ramified with long thick non oriented fibres, large meshes and free spicules, no visible spongin, abundant microxea (*Microxina*, Fig. 8B). 2(16), Radiating longitudinal fasciculate primary fibres, connecting secondary fibres regularly distributed to form rounded to irregular meshes. Free spicules abundant (*Niphates*, Fig. 8D)

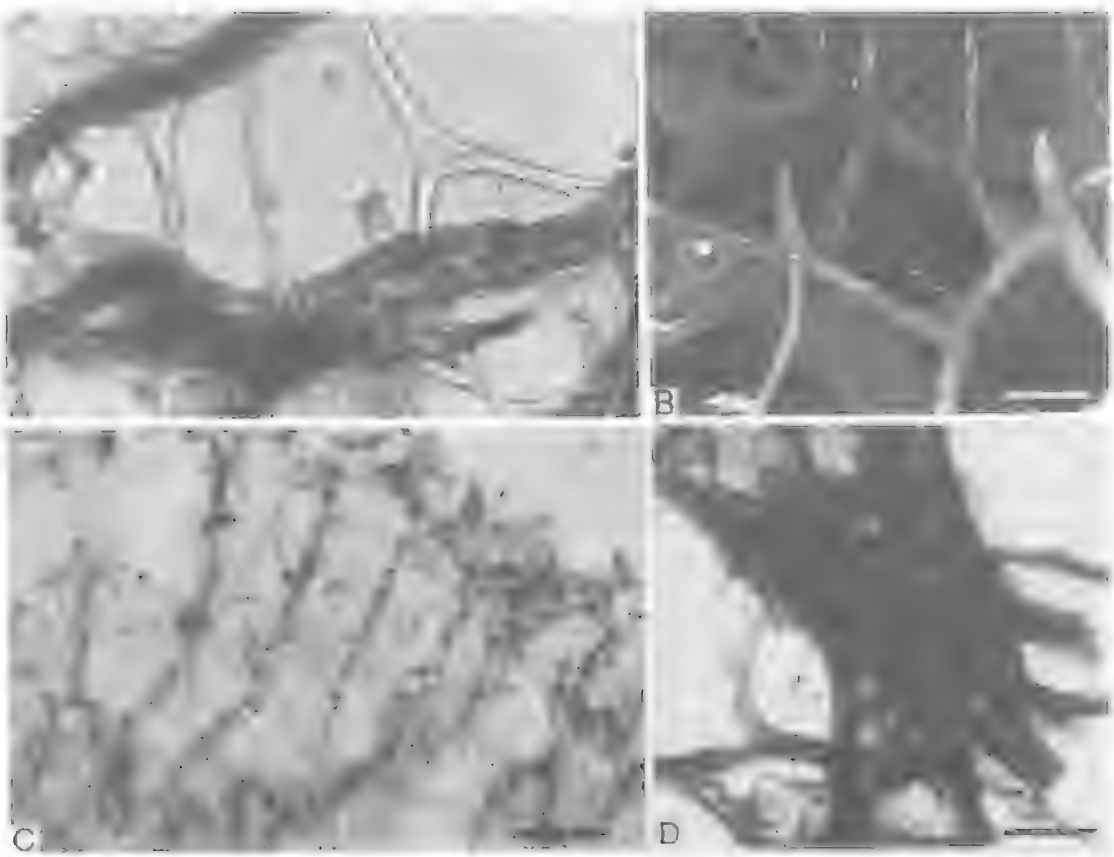

FIG. 7. *Chalinopsilla* and *Gelliodes*. A, C, *Chalinopsilla* Lendenfeld, 1888. Type species *Chalinopsilla dichotoma* Lendenfeld, 1886. West Coast of Australia. Lectotype BMNH1886:8:27:62. Schizolectotype: AM-G8969(MNHNLBIMDCL2661). A, Fine aspicular ectosomal fibres, abundantly cored by foreign material. C, Aspicular network abundantly cored by foreign material, divergent primary longitudinal fibres, and perpendicular connecting fibres. (Scale bars A = 8.2μm; C = 20μm). B, D, *Gelliodes* Ridley, 1884. Type species *Axos fibulatus* Carter, 1881. Bass Strait, Victoria. Syntype BMNH: 1882:2:23:202. B, Tangential view of surface, protruding ectosomal spines, from ends of longitudinal primary fibres (conules). D, Choanosomal skeleton of compact primary fibres and free secondary fibres. Free spicules abundant (Scale bars B = 500μm; D = 20μm).

CHARACTER 3. *Primary fibre structure*. 3(1), Strong longitudinal, pauci to multispicular primary fibres (5-15 or more spicules), parallel to divergent, regular in width, isolated, not ramified or moderately ramified, not fasciculate. Spicules sparsely distributed at center of fibre. Large spongin sheath, at least 66 % of fibre diameter (*Callyspongia*; *Patuloscula*; *Ceraochalina* (=*Chalinella*). 3(2), Strong large irregular spongin sheath cored by 3-5 spicules, some of them fused as observed by the presence of 2 or 3 central spicule rows; short, slender secondary fibres regularly split up from primaries (*Euplacella*). 3(3), Multispicular primary fibres densely cored; spongin sheath absent, only with nodal spongin; secondary fibres of the same type. Unispicular tertiary fibres with very scanty spongin (*Toxochalina*). 3(4), Ascending parallel radially distributed primary fibres extending from internal to external sponge wall. Fibres paucispicular (3-5 spicules), not ramified. Subectosomal longitudinal intercalate fibres present (*Siphonochalina* (=*Sclerochalina*; *Siphonella*; *Tubulodigitus*). 3(5), Aspicular to paucispicular primary fibres radiating from the sponge base to form fibrofascicles, surface conules and finer secondary and tertiary fibres. Large spongin sheath (*Spinosella* (=*Cladochalina*)). 3(6), Multispicular primary fibres with very narrow spongin sheath, less than 33 % of fibre diameter, or absent;

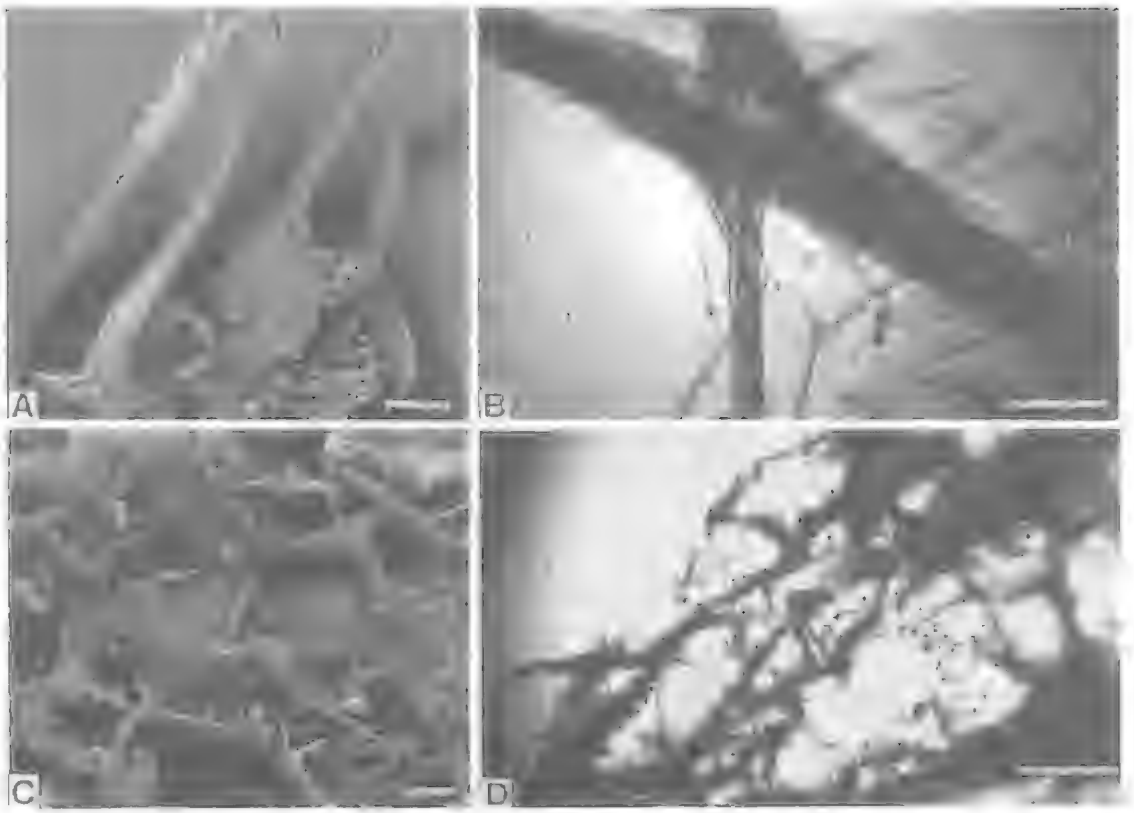

FIG. 8. *Microxina* and *Niphates*. A, B, *Microxina* Topsent, 1916. Type species *Microxina charcoti* Topsent, 1916. Antarctica. Holotype MNHN LBIM DT692, schizoholotype BMNH1926:10:26:339a. A, Ectosomal network of strong end brushes of primary fibres (strong spines), microxeas (arrows) in between. B, Strong ramified choanosomal tracts, abundant microxea. (Scale bars A = 500µm; B = 20µm). C, D, *Niphates* Duchassaing & Michelotti, 1864. Type species *Niphates erecta* Duchassaing & Michelotti, 1864 St.Thomas. Paralectotype MUS.TORINO POR51; ZMA POR1633. C, Ectosomal network, tangential section. Strong free unordered oxea network, interrumpted by ends of primary fibres, brushes of spicules. D, Choanosomal network, fasciculated primary fibres, secondary fibres and rounded meshes. (Scale bars: C = 200µm; D = 20µm).

secondary fibres of the same type (*Chalinopora* (=*Euchalina*)). 3(7), Primary and secondary fibres not clearly differentiated. All fibres lacking preferential direction. Spicules and foreign material variably present. Thicker fibres cored only by foreign debris, or both foreign debris and proper spicules. Thinner tracts with sparse spicules or uncored. Proper spicules absent if foreign material abundant (*Arenosclera*). 3(8), Aspicular, primary fibres isolated longitudinal, not ramified, slightly branched to form short, fine, aspicular, amber-like, connecting fibres. All fibres with abundant foreign material (*Chalinopsilla*). 3(9), Multispicular, inconspicuous, plumose, anastomosing, radially ascending primary fibres producing diffuse irregular secondary fibres *(Amphimedon)*. 3(10), Strong, regular, well-defined multispicular and radially ascending primary fibres not ramified. Spongin sheath narrow or missing. Short interconnecting fibres of the same structure (*Cribrochalina*). 3(11), Very stout compact, multispicular, primary fibres split up to form paucispicular secondary fibres. No visible spongin sheath (*Dasychalina*). 3(12), Very stout, compact, ascending, radiating, branching and anastomosing, splitting up to produce irregularly oriented, secondary fibres. No distinct spongin sheath except at bifurcation points (*Gelliodes*). 3(13), Pauci to multispicular longitudinal primary fibres, divergent, diffuse, isolated and rarely ramified. Connecting fibres not defined, only abundant free spicules (*Haliclonissa*). 3(14), Confused, compact primary tracts, with no clear fibres, only strong abundant

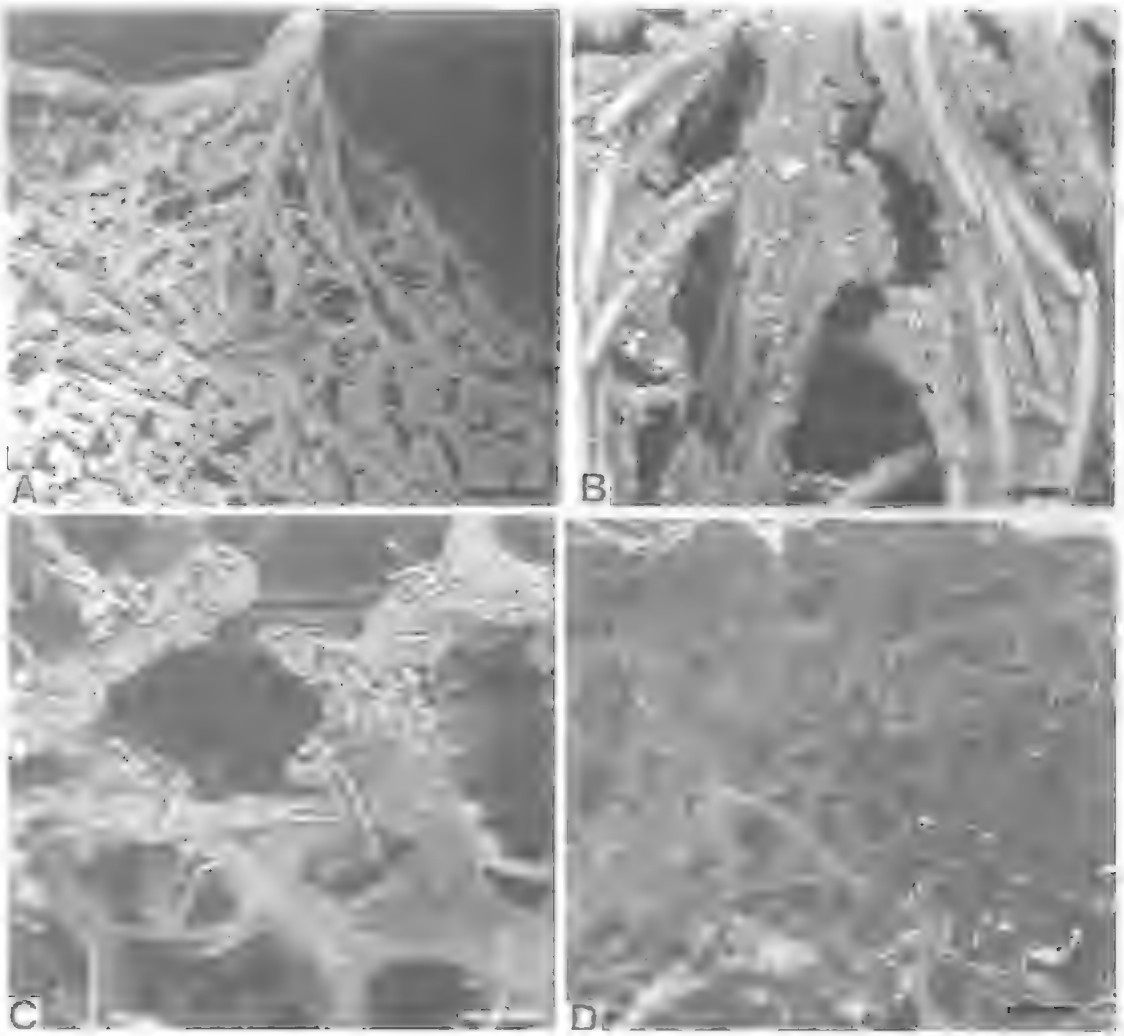

FIG. 9. *Dasychalina* and *Amphimedon*. *A, B, Dasychalina* Ridley & Dendy, 1886. Type species *Dasychalina fragilis* Ridley & Dendy, 1886. 'Challenger' Collection, Philippine Islands. Schizotype BMNH1887:2:170. A, Surface aculeations, spines or prickles, from ends of primary fibres, abundant free oxeas in between. B, Strong spicules, choanosomal fibres without distinct sheath of spongin (Scale bars A = 500μm; B = 50μm). C, D, *Amphimedon* Duchassaing & Michelotti, 1864. Type species *Amphimedon compressa* Duchassaing & Michelotti, 1864. St. Thomas. Lectotype MCS.TORINO POR.35; Schizoparalectotype BMNH1928:11:12:42. C, Tangential, regular ectosomal network with uniform rounded meshes, barely protruding ends of primary fibres. D, Choanosomal skeleton, plumose, anastomosing primary fibres, irregular secondary fibres, abundant spongin (Scale bars C = 50μm; D = 200μm).

spicules, unordered and cemented by no visible scarce spongin (*Hemigellius*). 3(15), Very stout, multispicular primary longitudinal, irregularly ascending, compact, large, occasionally fasciculated, without spongin sheath (*Microxina*). 3(16), Pauci to multispicular primary longitudinal fibres, diffuse, abundantly branched to form fibrofascicles, and pauci-to multispicular secondary connecting fibres. Spongin dominant between loose spicules, inside the fibres or free in meshes (*Niphates*). 3(17), Thick, irregular and compact multispicular primary fibres, with no preferential orientation; spongin sheath absent. Free spicules abundant. Thinner ill defined multispicular secondary fibres (*Pachychalina*).

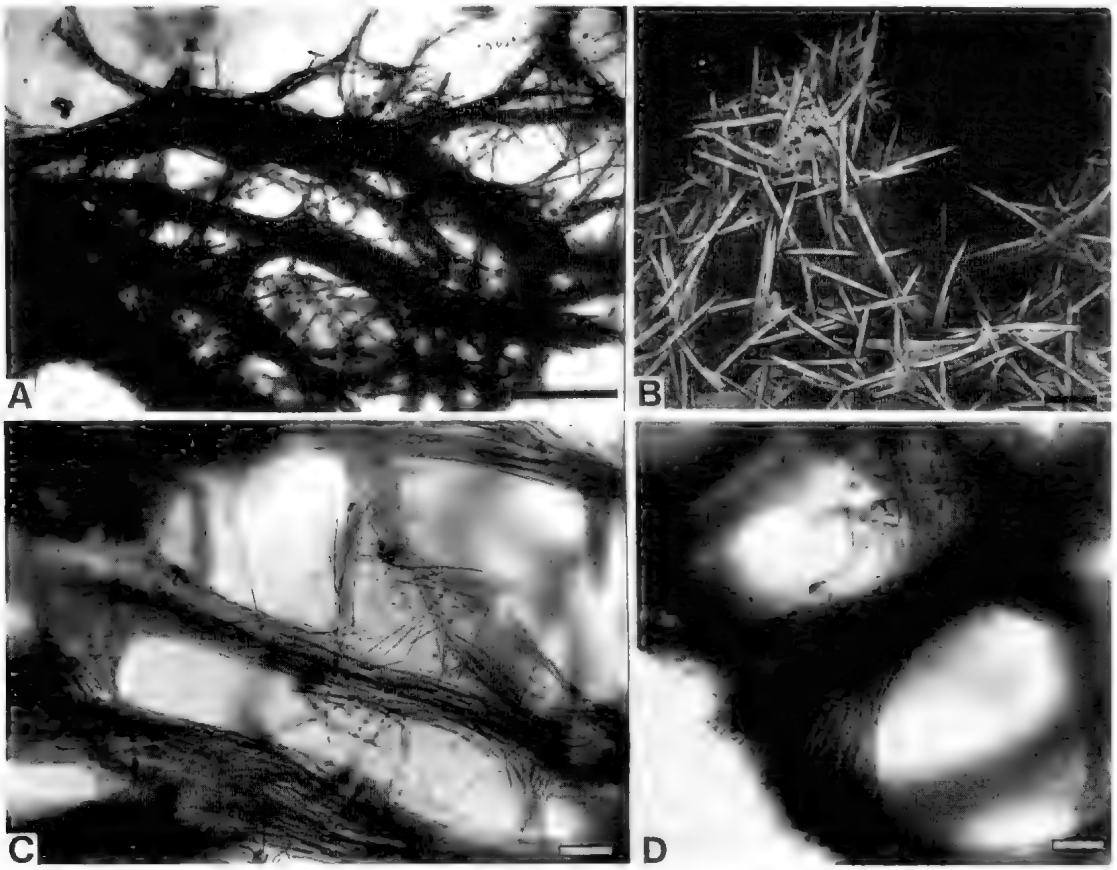

FIG. 10. *Pachychalina, Hemigellius, Cribrochalina.* A, *Pachychalina* Schmidt, 1868. Type species *Pachychalina rustica* Schmidt, 1868. La Calle, 'Lacaze-Duthier' collection. Syntype MNHN LIBM DT47. Confused, irregular, lacunar choanosomal network, thick longitudinal primary fibres, repeatedly divided to form isolated finer free ramifications, abundant free spicules. (Scale bar = 20μm). B, *Hemigellius* Topsent, 1901. Type species *Hemigellius rudis* Topsent, 1901. Antarctica, 'Belgica' Expedition. Holotype RBINSC POR033. Ectosomal skeleton, tangential dense, unordered network of free oxeas and sigmas, no clear fibres or meshes, no visible spongin. (Scale bar = 200 μm). C, D, *Cribrochalina* Schmidt, 1870. Type species *Cribrochalina infundibulum* Schmidt, 1870. West Indies. Lectotype BMNH1870:5:3:165. C, Longitudinal section through choanosomal elongate meshes, abundant free spicules. (Scale bar = 8.2μm). D, Longitudinal section through ectosomal and subectosomal skeleton, ectosomal palisade (crust) at left. (Scale bar = 8.2μm).

DISCUSSION

In both Callyspongiidae and Niphatidae studied here, 'reliable' generic characters matched structural differences within the group: ectosomal skeleton, choanosomal skeleton structure, and fibre structure. It is therefore important to determine the presence of these characters and their stability amongst genera, in order to use them as diagnostic characters.

In Haplosclerida the essential concept of 'genus' taxon is often misinterpreted or confused. Citations of genera as: "*Petrosia* Vosmaer 'sensu' Ridley & Dendy, 1887", or "*Callyspongia* Duchassaing & Michelotti, 1864, 'sensu' Burton, 1932" (Wiedenmayer, 1977), have no special validity or status in formal taxonomy. These terms have often a very different meaning than the one given by the original author. It is clear that this kind of citation should be avoided, particularly if it is not based on personal re-examination of type material. Such citations result in a new concept of the genus, an unnecessary widening of the generic concept without corroboratory evidence from the type specimen, and where 'sensu' the new author gives a new subjective diagnosis of the genus - all of which tend to become 'fixed' in the literature with their tacit acceptance by

contemporary authors. It is true that these modifications certainly make genera more 'convenient' for taxonomic identification of species, particularly in cases where the original concept of the genus is clouded or questionable. However, they omit the phylogeny and frequently lead to very heterogeneous taxa.

The question is then: do we accept genera or subgenera for their 'convenience', or do we have to find evidence for phylogenetically valid taxa ? What are the characters to consider when we split or fuse a nominal genus into an actual genus?

Some of the genera studied here are difficult to delimit, because of the large variability in their structural characters. For example *Callyspongia*, the type genus of Callyspongiidae, shares its habit with other genera of the same family: the lectotype of *Callyspongia: C. fallax*, has been described as massive, repent and lobate (Van Soest, 1980), but many species (including the type species) vary in habit from massive, ramose, lobate, repent to tubular. Today the genus is contains species with a great diversity and variability in growth forms, such that the concept of 'shape' has little taxonomic importance at the supraspecific level in this case. Nevertheless, *Callyspongia* exhibits a typical skeleton with a very stable fibre structure. Hooper & Wiedenmayer (1994) include 16 nominal genera as synonyms of *Callyspongia*, and Wiedenmayer (1989) included 21 nominal generic synonyms of *Callyspongia*. This is symtomatic of the biggest problem in Haplosclerida whereby the formulation of exact definitions and delimitation of generic boundaries is nearly impossible. Large revisions of problematic genera, following a strict interpretation of the genus' original concept, lead in some cases to the creation of genera with similar characters, or conversely to merge genera displaying some mutual characters ('splitting' versus 'lumping'). In the present work comparisons between type species of accepted generic synonyms of *Callyspongia* provided a clear mandate to differentiate them, based on their ectosomal features, whereas in some cases differences in their choanosomal skeletons were so minor as to be inconsequential.

So far, differences between type species of genera considered as synonyms of *Callyspongia* by Hooper & Wiedenmayer (1994) are too subtle to retain them as available genera (e.g. *Ceraochalina*; *Chalinella*; *Chalinopora*; *Euchalina*) whereas, conversely, the existence of several species showing consistent similarities in some of their characters confirm the potential validity of some of their nominal species groups, for which 'convenient' subgenera may be appropiate and 'useful' for classification (although perhaps not always phylogenetically sound taxa). A similar solution was adopted for a revision of the large family Microcionidae, with 73 nominal genera included (Hooper, 1996).

Tentative conclusions summarised in Tables 1 and 2 provide a convenient, practical classification, but they require confirmation from other sources using more objective methods (e.g. molecular studies).

Skeletal characters analyzed here were very often difficult to objectively differentiate, because variability occurred between very narrow limits, especially concerning the choanosomal skeleton and the structure of fibres and their variations. In these cases it is necessary to determine character priority in generic diagnoses as a first intent to delimit problematic genera.

The next stage in this analysis, an objective interpretation of characters and the distribution of character states amongst taxa, in a phylogenetic framework, should incorporate some of the more variable morphometric characters of Haplosclerida (e.g. habit, texture, surface ornamentation and aquiferous system). Authors have discarded these features as being 'not useful' at the generic level (e.g. Van Soest, 1980), but certainly some higher taxa such as *Cribrochalina* (Niphatidae) can be defined by a 'sticky texture', reflecting consistency in both skeletal and chemical characters, whereas in others this feature is completely inconsistent and discarded. Work is continuing in this regard (Desqueyroux-Faúndez, in prep.).

CONCLUSIONS

Grouping genera of Callyspongiidae and Niphatidae appears to be feasible using the structural characters defined above. Differences between genera are stable, consistent and deemed to be diagnostically important at the generic level.

In Callyspongiidae (Table 3), two groups of genera are distinguished, based on comparison of the form and size of ectosomal meshes with the structure of underlying longitudinal fibres: 1) Genera with three different sizes of triangular ectosomal meshes: *Callyspongia*, *Toxochalina* and *Spinosella* (=*Cladochalina*). 2) Genera with one size of ectosomal meshes: *Chalinopora* (=*Euchalina*), *Ceraochalina* (=*Chalinella*), *Siphonochalina* (=*Sclerochalina*, *Siphonella*,

Tubulodigitus), *Patuloscula*, *Euplacella*, *Arenosclera* and *Dactylia (=Chalinopsilla)*.

Furthermore, in this first group of genera this ectosomal character is associated with (linked to) the choanosomal character comprising 'fasciculated or isolated subectosomal longitudinal fibres'. In contrast, the character comprising the 'presence of transversal subectosomal connecting fibres' appears only in some genera displaying one size of meshes: *Siphonochalina* (=*Sclerochalina*, *Siphonella*, *Tubulodigitus*); *Patuloscula*. Similarly, *Ceraochalina* (=*Chalinella*), with one size of meshes, presents a different modification of subectosomal isolated longitudinal fibres in the form of 'presence of intercalate fibres and small subectosomal meshes', whereas *Euplacella* has one size of meshes and isolated longitudinal fibres, but it represents a modification of this character with three successive and isolated layers of ectosomal skeleton. This is considered here to represent different degrees in the development of the ectosomal layer, similar to those observed in genera of Phloeodictyidae (*Oceanapia* (=*Rhizochalina*). *Arenosclera* appears to be atypical of Callyspongiidae, having an irregular and disorganised skeleton without proper fibres and fibres strongly cored by foreign material. Wiedenmayer (1989) considered that the presence/absence of foreign material in fibres is a poorly correlated feature and only occurs as a gradual transition within some species of Callyspongiidae, with no clear boundaries between present/ absent. Nevertheless, there is a precedent for recognising a subgeneric taxon with incorporated detritus in the skeleton in Microcionidae (*Clathria* (*Wilsonella*)) (Hooper, 1996), as this feature was consistent within the species group and corroborated by the consistent morphological features. In this regard *Arenosclera* might form a 'convenient' and phylogenetically valid subgenus in group 2 Callyspongiidae.

In Niphatidae, the ectosomal skeleton appears to be a stable ans supraspecific character at the generic level, and in this regard two groups can be distinguished. 1) Genera with tangential ectosomal skeleton that may be formed by: a) a fibre network interrupted by ramified ends of longitudinal fibres (*Gelliodes*) or by barely protruding ends of longitudinal fibres (*Amphimedon*); b) a network of free oxea, interrupted by ends of longitudinal fibres, or spicule brushes with additional sigma or microxea (*Microxina*, *Niphates*); or c) both a fibre network and free oxeas interrupted by ends of longitudinal fibres or aculeations (*Dasychalina*). 2) Genera with perpendicular ectosomal skeletons that may be formed by: a) spicule brushes and ill-defined ends of longitudinal fibres (*Pachychalina*); b) a spicule palisade and expanded ends of longitudinal fibres *(Cribrochalin*a); or c) free spicules issued from the expanded ends of longitudinal fibres or wart-like conules (*Haliclonissa*).

Although, the characters used here appear to be useful to genus groups amongst Callyspongiidae and Niphatidae, their treatment is equivocal in the absence of data about their potential interrelations. Further studies on these two families is still necessary prior to accepting these criteria for a definitive classification. Nevertheless the present analysis provide a positive beginning towards a resolution of a very difficult and slightly chaotic group of sponge taxa.

ACKNOWLEDGEMENTS

For many years, Professor Claude Lévi (MNHN) helped me to understand the taxonomy of Haplosclerida. John Hooper (QM) and Rob van Soest (ZMA) assisted me with their valuable time encouraging this work in taxonomic discussions. I thank many colleagues from different institutions, for providing constructive comments on this manuscript and assisting me to obtain type material studied in this work: Philippe Willenz (IRSNB), Clare Valentine (BMNH), Valter Raineri and Enrico Borgo (MSNG). Technical work was undertaken at MHNG and I am grateful to J. Wüest (SEM), C. Ratton (photography) and I. Juriens (histology). Thanks to both reviewers who greately improved my manuscript.

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CROSS-SHELF DISTRIBUTION OF SOUTHWEST SULAWESI REEF SPONGES

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The quantitative distribution of open reef sponges (number of individuals per square meter) was studied across various reefs in the Spermonde Archipelago, southwest Sulawesi, Indonesia from April-July, 1997. The reefs are situated in 4 shelf zones that vary in distance offshore. Those closest to shore are subjected to freshwater inflow, nutrient input and sedimentation, whereas outer reefs are subjected to wave-action and upwelling from the Makassar Strait. Sponge individuals visible to the eye were identified and counted in 23 100×1m^2 belt transects distributed over the four shelf zones at depths of 3m, 6m, 9m and 15m. Distribution patterns of reef sponges were investigated in three spatial scales: 1) distance from land; 2) depth; 3) orientation to wind direction. The highest number of sponge species (richness) and individuals (abundance) was found in a middle-shelf reef, whereas the lowest richness and abundance was found in an inner-shelf reef. Lowest species richness and abundance occur in shallow transects (3-6m), and the highest were found in deeper transects (9-15m). More sheltered sites of reefs are lower in species richness than more exposed sites. Most species appear to have a wide distribution across the Spermonde shelf, and few are restricted to specific reef zones or depths. In contrast, the number of phototrophic sponge species and individuals increases with increasing distance from shore, with the highest numbers in the reefs farthest from shore. The occurrence of these sponges seems to be related to more clear, oligotrophic waters, such as found in the open Makassar Strait. ❐ *Porifera, Spermonde Shelf, Indonesia, sponge distribution, species diversity, phototrophy.*

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Many marine invertebrate species appear to reach their highest diversity in the Indo-Malayan region (Briggs, 1987), and sponges are no exception (Van Soest, 1994). However, sponges in Indonesia have been largely neglected by science for a long time. The few publications on sponges from this area were descriptions of small collections picked up almost casually, published during the first half of the century. Most of our knowledge of the Indonesian sponge fauna is based on collections made by large expeditions, such as the 'Siboga' expedition (1899-1900), and more recently from the Indonesian-Dutch 'Snellius II' expedition (1984-1985). Thousands of specimens were collected and identified, but remained unpublished (Van Soest, 1989). A database of these species, including manuscript names assigned to specimens by the late Maurice Burton (BMNH collections), appear in Hooper et al. (1999). Nevertheless, published information available suggests that a high degree of dissimilarity exists between sponge faunas in various reef locations within Indonesia (Van Soest, 1989; Amir, 1992). Differences in species composition has been mostly attributed to the degree of exposure to waves and currents (Amir, 1992), but these conclusions are based on very few data. Several studies have been made on differences in sponge population relation to various physical factors on the Great Barrier Reef. Wilkinson & Cheshire (1989) studied sponge distribution patterns across a broad continental shelf, related to distance offshore and depth. They concluded that highest biomass occurred on inner-shelf reefs and decreased further away from the mainland; abundance was highest on middle-shelf reefs; and this appeared to be correlated with different environmental factors across the continental shelf. Inner-shelf reefs are influenced by terrigenous run-off, high nutrient concentrations and fresh water inflow, whereas outer-shelf reefs are susceptible to oceanic features such as wave energy, oligotrophic conditions and upwelling. Wilkinson & Trott (1985) showed that light was also a factor determining sponge distribution across a continental shelf. Light transmittance varied considerably across a longitudinal continental shelf, and thus influenced the distribution of

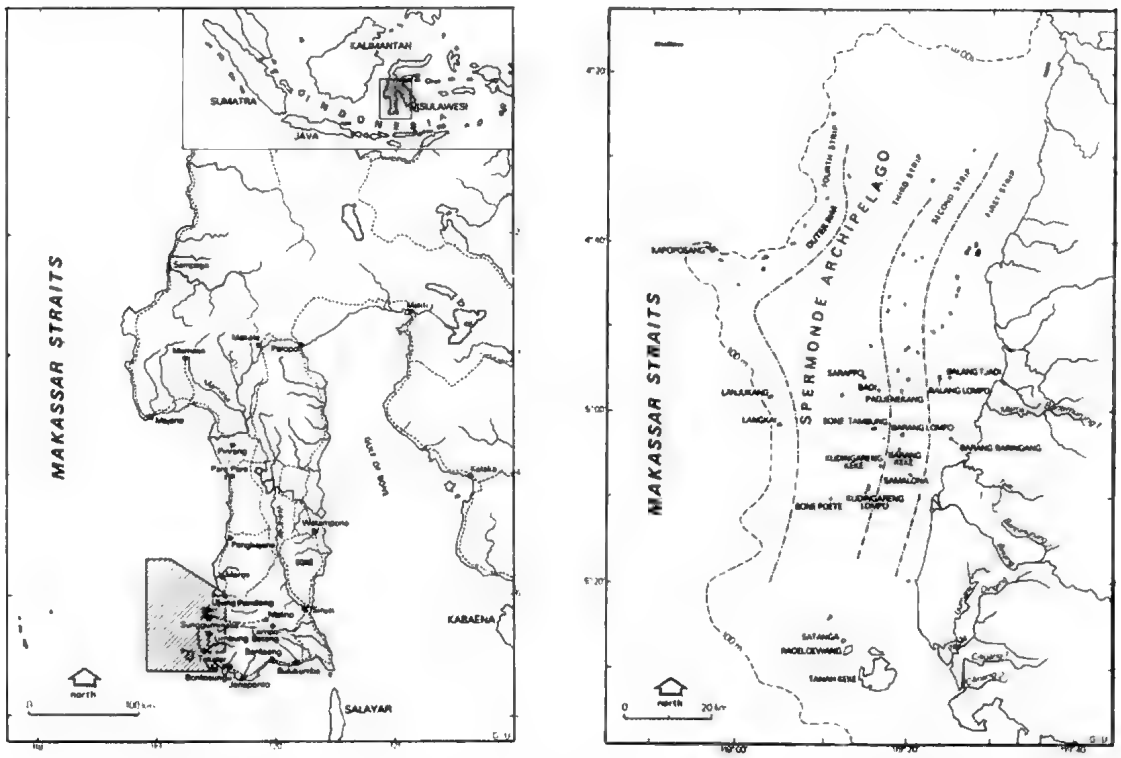

FIG. 1. Location of the Spermonde Archipelago (after de Klerk, 1983). The west side of the archipelago borders the deeper part of the Makassar Strait at the end of the shelf, whereas the east side borders the mainland of Sulawesi.

predominantly phototrophic sponges (i.e. those which rely on translocation of nutrients from cyanobacterial symbionts). Inner-shelf reefs harbour sponges which are r narily heterotrophic, whereas in outer-reefs a rger proportion of sponges are phototrophics (Wilkinson, 1987a). Variation in physical factors such as light, depth and turbulence were suggested as being major factors influencing the local composition of sponge populations across a middle-shelf reef of the central Great Barrier Reef (Wilkinson & Evans, 1989).

The Spermonde Archipelago, SW Sulawesi, is an exception to our otherwise limited knowledge of reefs in the Indo-Malayan region. Extensive marine biological (De Klerk, 1983; Moll, 1983; Hoeksema, 1990; Verheij, 1993) and physical geographic studies (Best & Zonneveld, 1989) have been made in this region, and this presently constitutes one of the better-explored marine regions of Indonesia. It provides a suitable area to conduct a study on distribution patterns of reef sponges similar to those carried out on the Great Barrier Reef, and to test the generality of trends observed by Wilkinson et al. (l.c.). The present paper reports on the distribution of open reef sponges, in relation to potential influences of depth, distance offshore and wind direction.

MATERIALS AND METHODS

The present study was conducted in the Spermonde Shelf off the SW coast of Sulawesi, Indonesia (Fig. 1). The Spermonde Shelf is 40-60km wide and up to 60m deep, and harbours some 150 coral reefs, most cay-crowned. The shelf is divided in four parallel zones (Fig. 2), which vary in distance offshore (Hutchinson, 1945). The study was conducted over a period of 3 months from April to July 1997. Surveys were conducted at four reefs, each located in different shelf zones: an inner-shelf reef, located in the first shelf zone (Lae-lae); middle-shelf reefs, the inner one located in the second shelf zone (Samalona) and the outer one in the third shelf zone (Kudingareng Keke); and an outer-shelf reef located in the fourth shelf zone (Langkai).

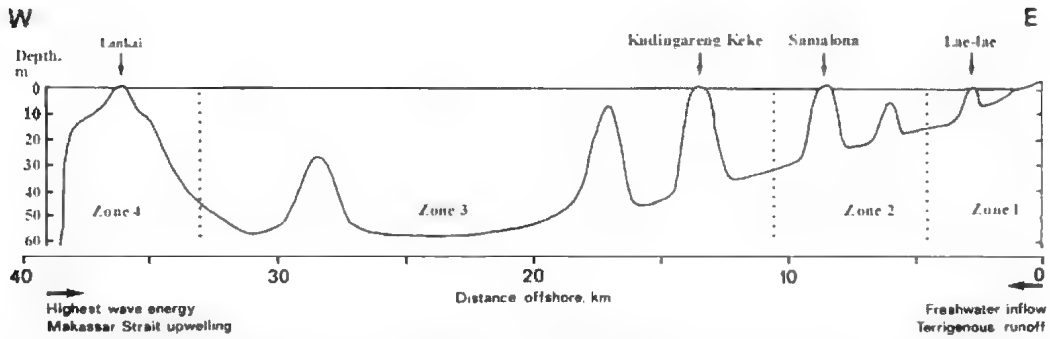

FIG. 2. Schematic cross-section of the Spermonde Shelf (approximately W-E) from Langkai to Ujung Pandang, with the proximate distances from the mainland (after Hoeksema, 1990).

Reefs were surveyed on the exposed side; these are most prone to waves generated by the NW monsoon (November to March). Two reefs (Samalona and Kudingareng Keke) were also surveyed on the sheltered lee side, which is less prone to wave-action. Surveys were conducted at 3, 6, 9 and 15m depth along a 100m transect line. With the aid of a $1m^2$ quadrat laid at each consecutive 1m section, sponge species visible to the eye were noted and their numbers counted. Smaller (cryptic, boring and thinly encrusting) specimens were excluded from this study. Representative samples of all sponge species were collected, and after preliminary identification (spicule preparations and hand section) each individual species was given a unique field-code. These samples are currently deposited in the collections of the Zoological Museum, University of Amsterdam (ZMA), where the specimens were identified definitively by the second author (RvS). Six presumed phototrophic sponges were recognised based on their similarities to published descriptions (Bergquist et al., 1988; Wilkinson, 1983, 1987, 1988): *Carteriospongia foliascens, Dysidea aff. herbacea, Halichondria cartilaginea, Phyllospongia papyracea, Phyllospongia* sp. and *Strepsichordaia aliena*. Minimum sampling area was determined by means of the quadrat-area method; whereby the number of species was counted in one m^2. This was repeated after doubling the area, adding species not encountered previously. The number of species was plotted against the area size. Further repetition was undertaken until doubling of the previous area resulted in less than 10% increase in species number (Kaandorp, 1984). In our study, the minimal area was achieved at $64m^2$. Since tropical marine ecosystems are far from homogenous, it was considered appropriate to maintain a larger minimal sampling area, $100m^2$.

For each transect, sponge species diversity, Shannon-Weaver index H' (Shannon & Weaver, 1949) and evenness index J' (Pielou, 1975) were calculated.

Species composition and relative abundance of species at various reef sites and depths were compared using an agglomerate hierarchical classification based on a (dis-)similarity matrix (CLUSTAN; Wishart, 1978). CLUSTAN was carried out with logarithmically transformed data and the average-linkage method (Sokal & Michener, 1958) in combination with the Bray-Curtis coefficient.

RESULTS

One hundred and fifty-four sponge species were recorded, belonging to 75 genera and 34 families. H' values were found to be in the range of 2.18 (Lae-Lae, 3m depth) to 3.83 (Kudingareng Keke, 15m depth). J' values varied between 0.79-0.92. Twenty-seven sponge species occurred at each of the four reefs ('common species') in the Spermonde Archipelago.

CROSS SHELF DISTRIBUTION PATTERNS. Both the number of species (species richness) and number of individuals (abundance) increased from inshore reefs to the middle-reef region, but further off-shore, at the outer shelf rim, these values decreased. The exposed side of the outer middle-shelf reef at Kudingareng Keke showed the highest species richness and highest abundance averaged over all four depths (Figs 3a, 4a). For each individual depth (transect), the same site also showed the highest species richness. However, the highest abundance of individuals in a transect was observed at the outer-shelf reef of Langkai at 15m (Fig. 4b).

DEPTH DISTRIBUTION. Generally, both species richness and abundance increased with

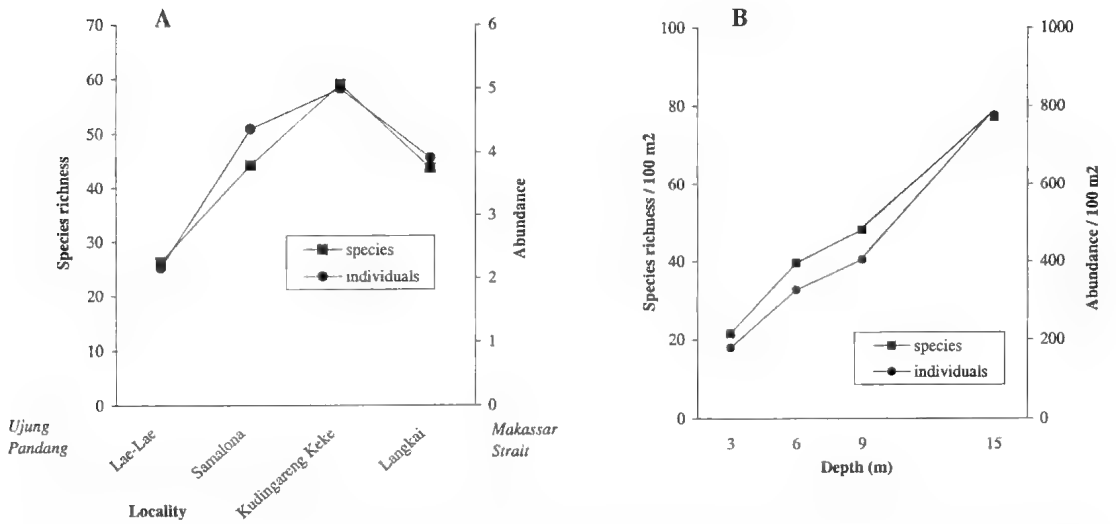

FIG. 3. Species richness (left axis) and abundance (right axis) per reef. A, localities arranged according to distance from the mainland at the capital Ujung Pandang; B, species richness and abundance per depth in metres. Indices are mean values.

increasing depth (Fig. 3b). The situation at the nearshore reef at Lae-Lae differed from that on other reefs (Fig. 4b) in showing a higher richness and abundance at 6m than 9m depths, where the bottom predominantly consisted of sand and silt as compared to hard substratum on other reefs. The 15m depth habitat was absent at Lae-Lae.

EXPOSED VS SHELTERED REEF SITES. Reefs at which both the exposed (west) and sheltered (east) sides were monitored, Samalona (shelf zone 2) and Kudingareng Keke (shelf zone 3), showed the lowest richness and abundance occurring on the sheltered side (Fig. 5). This difference is especially obvious at Kudingareng Keke, where species richness and abundance on the E side are about half that of the W side. Differences in both richness and abundance are most distinct between 9 and 15m depth contours.

PHOTOTROPHIC SPONGE DISTRIBUTION. In contrast to the general trends observed for all sponges, abundance of phototrophic sponges continued to increase with distance offshore (Fig. 6). This trend was also reflected in species abundance, increasing from 2 at the inshore-reef, 4 at both middle-shelf reefs, to 6 at the outer-shelf reef. Conversely, abundance of individual phototrophic species deviated from this trend: *Dysidea* aff. *herbacea* was slightly less abundant at Samalona (middle-shelf reef) than at Lae-Lae (inshore reef), whereas *Halichondria cartilaginea* was slightly more abundant at Kudingkareng Keke (outer middle-shelf reef) than at Langkai (outer-shelf reef). Averaged for all reef locations, the highest abundance of phototrophic species was found at 15m, and of individual transects sampled the highest abundance was found at the 15m transect at the outer-shelf reef of Langkai.

SPONGE COMMUNITY DISTRIBUTION. Similarities in species composition and abundance (number of individuals) of all reef locations and depths were determined by means of cluster analysis (Fig. 7). Three distinct clusters were recognised. Cluster 1 contains all Lae-lae sites. This reef was generally characterised by low species diversity and abundance. The high abundance of *Paratetilla bacca* and *Dysidea* aff. *herbacea* was notable, but these species also occurred elsewhere. Three species were confined to Lae-Lae, but they were rare and represented by only few individuals. Cluster 2 contains all shallow sites of 3m depth, with the exception of Lae-Lae (cluster 1), and is also characterised by low species richness and abundance. Relatively high abundance of *Gelliodes callista, Halichondria cartilaginea* and *Phyllospongia papyracea* were notable, but these also occur in cluster 3. One species is confined to the 3m zone, but only a single individual was found. Cluster 3 comprises the remaining transects. Five species (*Xestospongia ashmorica, Haliclona fascigera, Niphates olemda, Clathria vulpina* and *Ulosa* sp.) meet the criteria to be considered as 'characteristic and dominant species' of this large cluster, occurring in at least 66% of the transects and an average

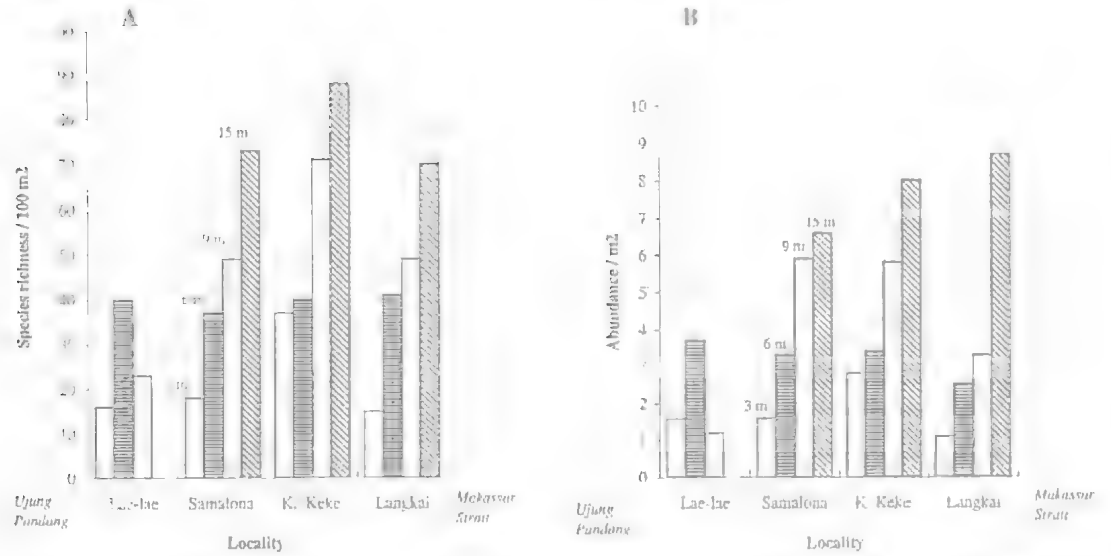

FIG. 4. Species richness (A) and abundance (B) per reef at each depth profile. Each shelf zone is represented by a reef and arranged according to distance from the shoreline at the capital Ujung Pandang.

abundance exceeding 5 % (Kaandorp, 1986). Some species only occur in subsets of this cluster, but their occurrence invariably overlaps only partially with that of other clusters, causing a dissimilarity index too low for confident recognition of distinct clusters. Some patterns are nevertheless worth noting: e.g. all transects on the east side of Kudingareng Keke are grouped together, having in common low species richness and abundance. The dendrogram clearly indicates that the first shelf zone and all the shallow (3m) sites are different from the remaining sites.

DISCUSSION

CROSS-SHELF DISTRIBUTION PATTERNS. Sponge species richness and abundance increase with distance offshore to the third shelf zone, decreasing further offshore. The Spermonde Archipelago is affected by the NW monsoon, both geomorphologically and ecologically (de Klerk, 1983). These differences seem to be related to distance from land, and clearly suggest an environmental gradient across the shelf (Hoeksema, 1990). The lowest number of coral species was found in the outer reef zone, but at the same time the outer-rim high energy environment provides a higher coral cover than on the inner-rim reefs (Moll, 1983). These patterns include all scleractinian corals. A study of cross-shelf distribution patterns in fungiid corals revealed that the number of species increased with increasing distance from shore until the third shelf zone. The fourth reef zone exhibited a lower species richness and abundance. Hoeksema (1990) concluded that the third reef zone was the most optimal reef zone for fungiid species richness. Similar to these trends our results found that the third reef zone was also the most optimal for sponge populations. Moll (1983) found no clear differences in

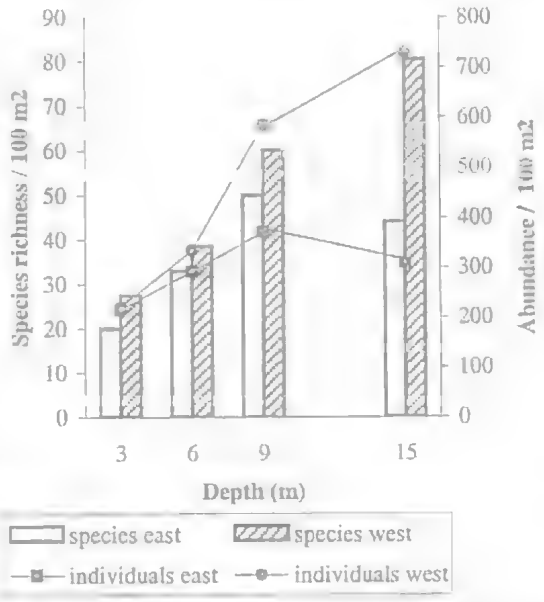

FIG. 5. Species richness (bars: left axis) and abundance (line: right axis). Values are mean values for transects on east and west sides of Samalona and Kudingareng Keke.

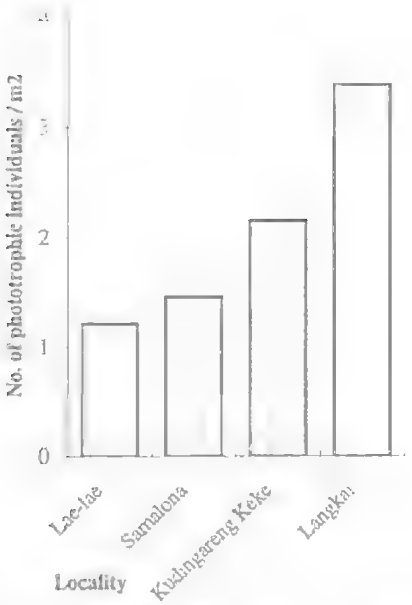

FIG. 6. Numbers of individuals of presumed phototrophic sponges in the four reef areas arranged according to distance offshore.

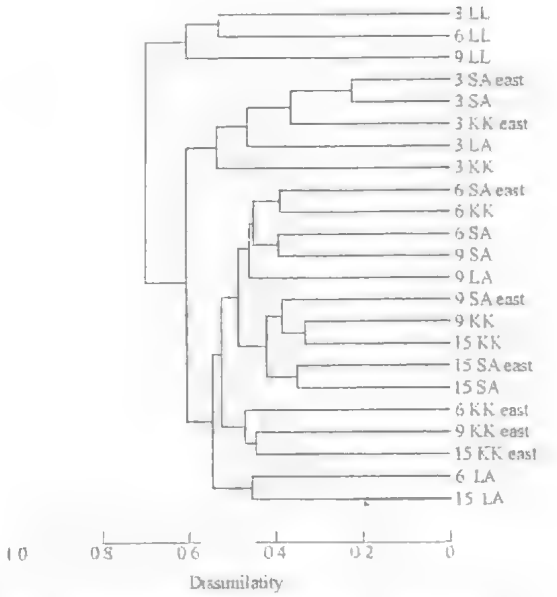

FIG. 7. Dendrogram of a hierarchical classification on species composition and sponge abundance for all transects. Transects are listed with depth in metres. Abbreviations: LL, Lae-lae; SA, Samalona; KK, Kudingareng Keke; LA, Langkai.

scleractinian species richness between reef zones 2 and 3, although the third zone had slightly higher number of species. The largest differences were found between reef zones 2-3 and zone 4. In our study, we found the highest proportion of sponge species with the most restricted distributions occurred at Langkai in the fourth reef zone.

From earlier studies on cross-shelf distribution patterns of sponges on the Great Barrier Reef Wilkinson & Cheshire (1988, 1989) found that sponge biomass showed an inverse relationship with distance from land. They concluded that this was due to high amounts of nutrients in near-shore waters, decreasing with distance from land. Their data correlated with the hypothesis that the clear-water sponge fauna depends predominantly on nutrition from their cyanobacterial symbionts. Abundance of sponges on the Great Barrier Reef was highest in middle-shelf reefs, whereas the pattern of richness appeared more complicated. Sponge abundance on the Spermonde shelf increased similarly with distance from land until the midshelf reefs. Species richness also followed this pattern on Spermonde reefs, whereas this was not the case on the Great Barrier Reef. However, the Great Barrier Reef constitutes a much broader area, with the outer-shelf reefs in the Townsville region located some 200km from land, whereas the inner-shelf reef is almost 20km from shore. Species compositions of Great Barrier Reef zones were not compared between zones, and it is conceivable that distinct faunal assemblages exist over the broad shelf, making ecological comparisons more complicated. The inner-shelf reefs of the Great Barrier Reef are perhaps more comparable with the second and third reef zone in the Spermonde Archipelago, where the inner-shelf reef is located only 1km from urban Ujung Pandang. Presumably, this area is far more prone to terrestrial influences than an inner-shelf reef on the Great Barrier Reef.

Like on the Great Barrier Reef (Wilkinson & Cheshire, 1988; Wilkinson & Evans, 1989), the abundance of presumed phototrophic sponges in the Spermonde area appears to be related to the presence of clear, oligotrophic waters.

DEPTH DISTRIBUTION. On the Great Barrier Reef, high irradiance, high UV light and high wave energy were thought to exclude most species from shallow waters. These factors decrease with increasing depth, hence species richness and abundance increase with depth (Wilkinson & Cheshire, 1988; Wilkinson & Evans, 1989). The depth distribution of sponges at the Spermonde shelf follows this general pattern closely, and it is probable that these

mechanisms are also responsible for differential distribution patterns in this region.

WITHIN REEF ZONE VARIATION. Moll (1983) observed that species richness and abundance in scleractinian corals was significantly lower on the E side than at the W side of Spermonde shelf islands. Hoeksema (1990) found that circumreef patterns in fungiid corals were mainly determined by water movement. The W (exposed) side is, in general, the most exposed to wave- energy caused by the strong NW monsoon, whereas the E (sheltered) side shows more sediment accumulation. This was evident from the low species richness and abundance on the E sides of reefs (Hoeksema, 1990). In our study we found that species richness and abundance of sponges are lower on E sides than on W sides of reefs. The slopes of E sides of the islands of Samalona and Kudingareng Keke are very steep and contain high amounts of sediment in comparison with the W side.

In conclusion, most species appear to have a wide range of distribution across the Spermonde Archipelago, and few are restricted to specific zones and depths. However, both species richness and abundance increase with depth, and also with increasing distance offshore until the third reef zone. Specific measurements to correlate with these variations were not made, thus it is not possible at this moment to characterise these habitats.

ACKNOWLEDGEMENTS

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PERSPECTIVES ON SPONGE-CYANOBACTERIAL SYMBIOSES. *Memoirs of the Queensland Museum 44: 154. 1999*:- Insights on the evolution of sponge-cyanobacterial symbioses are drawn from biogeographic and molecular data. The taxonomic and geographic distribution of sponge-cyanobacteria associations is analysed after surveying their occurrence at eight localities in the Eastern and Western Tropical Pacific, and the Caribbean. Three methods - fluorescent microscopy, thin layer chromatography and transmission electron microscopy - were used to infer the existence of endosymbiotic cyanobacteria.

Thirty-eight species, representing 17 families and 11 orders of Demospongiae, and one family and order of Calcarea, are added to the list of sponges involved in these associations. This number represents an increase of more than 50% over previously known occurrences of this type of metazoan-microbial association. However this increase of species numbers represents only an addition of twelve genera and two families to the taxonomic distribution of these associations. Species from 26 of the 72 recognised Demospongiae families, and 3 of the 17 recognised Calcarea families are found to harbour cyanobacterial endosymbionts. These data suggest a rather restricted taxonomic range for sponge-cyanobacterial assemblages, and invites a search for evolutionary trends among the families involved. The genera with highest number of species harbouring cyanobacteria are: Aplysina (10 spp.), Xestospongia (7 spp.), Dysidea (5 spp.), and Theonella (5 spp.). Although the updated list of sponge-cyanobacterial assemblages shows a few biogeographic trends, the understanding of the evolution of these associations requires the study of more extensive geographic areas.

The use of 16S rDNA analysis to understand the phylogenetic relationships of endosymbiotic cyanobacteria is discussed. Genetic analyses promise to shed light on the understanding of the evolution and specificity of these associations. 16S rDNA gene analyses carried out so far suggest that sponge-cyanobacterial assemblages comprise diverse and complex evolutionary histories, some of which might share evolutionary pathways with other important marine symbiotic assemblages involving cyanobacteria. ❐ *Porifera, cyanobacteria endosymbioses, biogeography, evolutionary trends, 16S ribosomal genes.*

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FARMING SPONGES FOR THE PRODUCTION OF BIOACTIVE METABOLITES

A.R. DUCKWORTH, C.N. BATTERSHILL, D.R. SCHIEL AND P.R. BERGQUIST

Duckworth, A.R., Battershill, C.N., Schiel, D.R. & Bergquist, P.R. 1999 0o 30: Farming sponges for the production of bioactive metabolites. *Memoirs of the Queensland Museum* **44**: 155-159. Brisbane. ISSN 0079-8835.

For successful aquaculture of sponges, with the aim of producing metabolites, a farming method is required that promotes sponge growth and survival, and produces high yields of target metabolites. To help develop a suitable farming method growth and survival were compared for two New Zealand sponges, *Latrunculia brevis* (Ridley & Dendy) and *Polymastia croceus* (Kelly-Borges & Bergquist), experimentally grown in a variety of ways. Explants were farmed in mesh, on rope, and with rope threaded through them. For both species of sponge, survival was greatest for explants farmed in mesh, probably because this produces little tissue damage and prevents explants from dislodging and 'escaping'. This method also promoted highest growth of *L. brevis*, with some explants doubling their weight in two months. The growth of *P. croceus*, however, was highest in explants with rope threaded through them. Explants of both sponges farmed on rope did not attach and had poor growth and survival. These findings are a major step forward in developing a method for farming sponges in temperate waters of New Zealand. ❐ *Porifera, aquaculture, farming method.*

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A major obstacle facing sponge aquaculture in the production of metabolites is the lack of a suitable farming method or on-growing structures (Shimizu, 1995; Osinga, 1998). To be suitable for large scale commercial use a structure must be inexpensive, have a low surface area to reduce drag and bio-fouling, and allow cost-effective and efficient harvesting. It must also promote high sponge growth and survival while also maintaining high metabolite production.

Farming structures used to grow bath sponges have historically involved attaching explants to concrete discs, or threading wire through explants so that they hang in mid-water (Cotte, 1908; Moore, 1908; Crawshay, 1939). This last method was modified slightly by Verdenal & Vacelet (1990), who successfully grew commercial bath sponges by first threading plastic-coated metal wires through explants and then attaching them to vertical ropes. Development of new farming structures to grow bath sponges was constrained by market forces determining acceptable shape and size of products (Storr, 1964; Bergquist & Tizard, 1969). In contrast, explant shape has no bearing on efficient metabolite production, and consequently there is considerable flexibility in the development of new farming structures for metabolite aquaculture.

We identified three general farming methods: 1) explants placed in mesh; 2) explants attached to and farmed on rope; 3) explants farmed with thin rope threaded through them (Fig. 1). The first method has already been tested with some success (Duckworth et al., 1997). For each method of farming it was necessary to test variation in structures and materials used. For example, rope thickness and composition were important considerations using methods 2 or 3 - as rope thickness increases, drag pressure as well as capital cost increases accordingly, whereas a decrease in rope thickness produces a decrease in available surface area for explant attachment. Rope composition is also important, because explant growth, survival and metabolite concentration may differ between ropes made of different materials.

In this study, we tested the potential of each farming method using two New Zealand sponges: *Latrunculia brevis* (Ridley & Dendy, 1886), a green massive sponge found throughout New

Zealand waters usually in exposed areas (Battershill & Bergquist, 1999a), and *Polymastia croceus* (Kelly-Borges & Bergquist, 1997), a common orange massive sponge. Both sponges contain metabolites with potential pharmaceutical properties (Lill et al., 1995; National Cancer Institute, personal communication).

The results described here are preliminary and part of a larger, ongoing experiment (October 1998). We focus here on the overall patterns of explant growth and survival between the three farming methods tested. Full results will be published after all relevant experiments are completed.

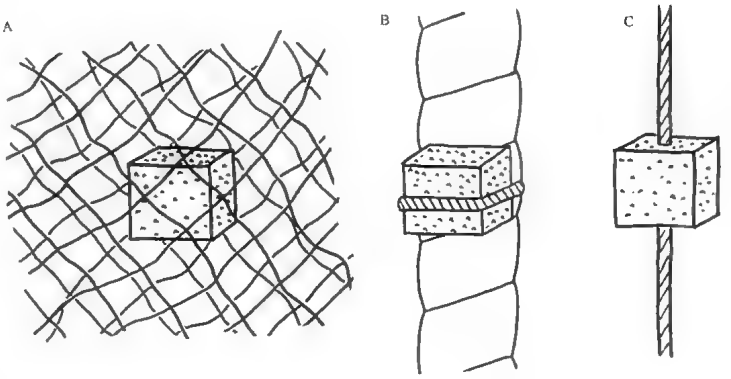

FIG.1. Schematic drawing of the 3 farming methods tested. A, explants placed in mesh; B, explants attached to rope; C, explants with thin rope threaded through them.

MATERIALS AND METHODS

For both *L. brevis* and *P. croceus*, we collected approximately forty sponges of similar size at 10-20m depth off the coast of Wellington (41°21S, 174°50E), situated at the southern end of the North Island of New Zealand. These sponges were cut, leaving approximately 30% of the original sponge intact to regenerate. Cut sponges left *in situ* had high survival and quickly healed. All collected sponges were cut under running seawater in a laboratory into cube-shaped explants, approximately 27cm^3 in size and 16g in weight. All explants had at least one side uncut, with the pinacoderm intact.

Three farming methods were tested for each species. Explants were: 1) placed in mesh; 2) attached directly to thick rope; 3) or had thin rope threaded through them (each method has several sub-methods, but full analysis at this stage is not yet possible given that the experiment is still in progress) (Fig. 1). Under method 2, each explant was firmly secured with cotton thread to an individual length of rope measuring 15x2.5cm. All explants in this method had their uncut side (with intact pinacoderm and oscules) facing outwards, away from the rope. Under method 3, to thread thin rope through explants, we carefully pushed a large needle, with rope attached, through each explant. Rope used in this treatment was 2-3mm thick. We used 40 explants of each species for each method. Explants were randomly selected and tied at intervals of 15cm to a rope back-line, and farmed at a depth of 12m.

We farmed *L. brevis* and *P. croceus* in Wellington Harbour from October 1997 to January 1998, and compared explant growth and survival. Growth was determined by wet-weighing the explants (to 0.1g) at the start and at the end of each experiment. We discovered that explants disturbed 30mins before weighing would expel all excess water, allowing us to weigh their true tissue weight.

Comparisons between the different methods of farming on growth and survival in *L. brevis* and *P. croceus* were made using one-way ANOVA.

RESULTS

In both species growth rates were not significantly different between the three farming methods tested (F_{df2}=0.24 and 0.04, N=68 and 110, P>0.05, for *L. brevis* and *P. croceus*, respectively). Conversely, survival of explants was significantly different between the methods used (F_{df2}=31.28 and 23.79, N=120, P<0.001, respectively) (Figs 2B,D). Survival of both *L. brevis* and *P. croceus* farmed in mesh, under method 1, was excellent. Only one of the forty explants of *L. brevis* died and all *P. croceus* survived. The growth of *L. brevis* explants farmed in mesh was relatively good with an average weight gain of 1.2g over the 95 days of experimentation (Fig. 2A). Some of these replicates doubled their weight from 16g to over 32g during this period, a promising result given the brief time of experimentation. Many of these explants grew through the mesh, incorporating it into their tissue. In comparison, average growth of *P. croceus* farmed in mesh was poor, increasing only 0.1g in weight over 95 days (Fig. 2C).

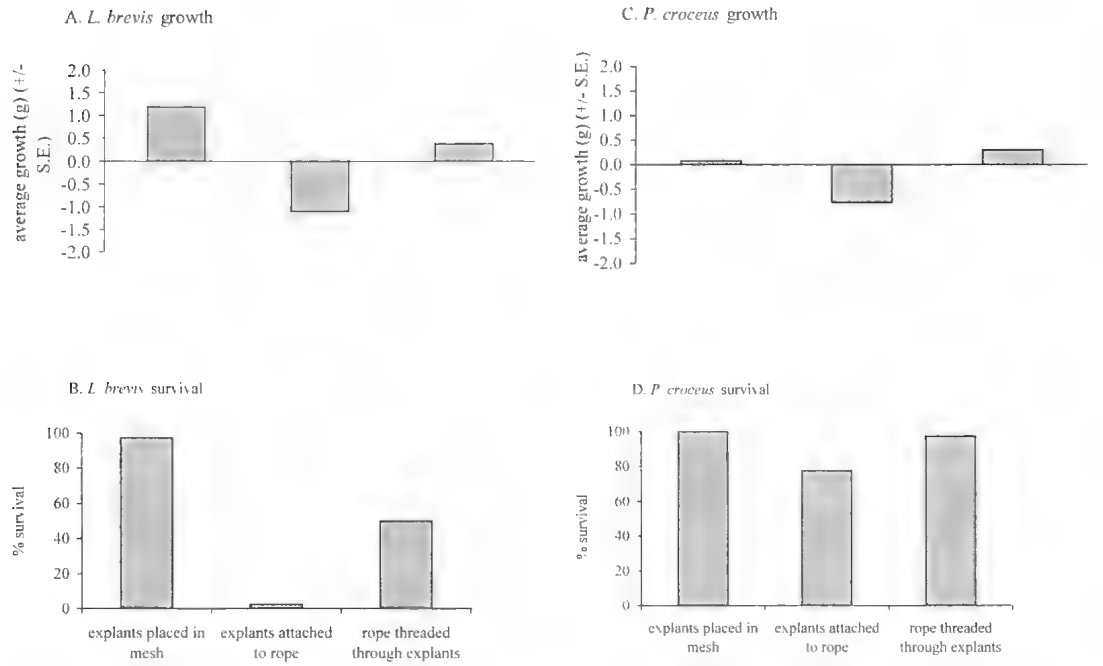

FIG. 2. Comparison in growth and survival of *L. brevis* and *P. croceus* between the three farming methods tested. Growth represents average explant weight gain or loss (+/- S.E.) over 95 days. Survival represents percent survival of the forty explants transplanted in each farming method.

Neither species grew well on rope (method 2). On average, *P. croceus* lost 0.8g while *L. brevis* lost 1.1g over 95 days (Figs 2A,C). Under method 2, survival on rope was also poor. *Polymastia croceus* had 78% survival but only 1 of 40 *L. brevis* explants survived (Figs 2B,D). Under this farming method no explants of either species attached to the rope. The explant side, in contact with the rope, was similar in appearance (morphology and colour) to the other healed sides. We also observed many explants moving or growing away from the rope, ultimately becoming dislodged.

Under method 3, when rope was threaded through explants, all but one *P. croceus* survived the 95 days experiment, whereas only 50% of *L. brevis* survived (Figs 2B,D). Average weight gain for both sponges was similar, approximately 0.3g (Figs 2A,C). Few explants of either species attached to the threaded rope. After 95 days, most explants had changed shape and were moving away from the rope.

DISCUSSION

The importance of choosing a suitable method of farming for sponge aquaculture is well demonstrated in this study. Survival of two species of sponges was greatly affected by the method used. Average growth of both species was generally low for all methods, most probably due to the short (95 day) period of experimentation, and factors inherent to each method mentioned below.

The high survival of *P. croceus* and *L. brevis* farmed in mesh (method 1) may be explained by two factors: 1) Explants experienced the least initial damage, as they are simply placed in mesh. By comparison, explants grown under the other methods had greater disturbance, with rope either pushed through or squeezed around them, causing tissue damage and increased mortality; 2) Even in cases where mesh method is not ideal, explants were effectively trapped in mesh. We noticed many explants in the rope methods moving or growing away from the rope, ultimately becoming dislodged. For farming this is effectively the same as mortality (i.e. the sponge is lost).

One disadvantage of the mesh farming method is a higher rate of fouling of mesh by sediment and sessile organisms, particularly bryozoans, reducing water flow and possibly influencing

poor explant growth or even weight loss (Bakus, 1968; Duckworth et al., 1997). Restricted water movement due to fouling probably caused poor growth of *P. croceus* farmed in mesh. Unlike *P. croceus*, many explants of *L. brevis* quickly grew through and over the mesh, reflecting inherent species differences. This reduced the effect of fouling and, combined with low explant stress and damage, probably explains the better growth of *L. brevis* farmed in mesh. Harvesting sponges growing in mesh would involve cutting away tissue growth, leaving the explant behind to grow back through the mesh.

Sponges farmed with rope threaded through them (method 3) were less effected by fouling because they were directly exposed to water. Whereas this may have promoted growth, mortality may have increased because of increased tissue damage. It is likely that increased tissue damage and rejection of the threaded rope caused poor survival of *L. brevis*. In contrast, *P. croceus* farmed with threaded rope survived well. Differences in growth and survival between the two sponges suggest that *P. croceus* is a hardier species and more amenable to different farming methods. However, given a suitable method, *L. brevis* achieved the best combination of growth and survival.

Neither species attached well to the threaded rope, which probably caused reduced growth. Other studies have shown that only explants attached to their fastening wire or identification tag grew well (Verdenal & Vacelet, 1990). The ability of sponges to change shape (Bond & Harris, 1988; Bond, 1992) allows them to move away from unpleasant conditions and can result in loss of explants and low overall survival. This farming method will not succeed unless a rope material is found to which explants will attach. We are currently investigating this, testing explant growth, survival and attachment on threaded rope made of different natural and artificial materials. It is unlikely that this farming method will be suitable in exposed areas where high water movement can easily tear sponges away from the rope.

Many studies have shown that sponges will attach well to a wide variety of natural and artificial substrata (Cotte, 1908; Moore, 1908; Crawshay, 1939; Wulff, 1984, 1985, Barthel & Theede, 1986; Bond & Harris, 1988; Rosell & Uriz, 1992). Unfortunately, both species of sponge in our study failed to attach to any of the ropes tested, perhaps a result of high substrate selectivity shown by some sponges (Battershill & Bergquist, 1999b).

Differences in growth and survival observed in the two species, *L. brevis* and *P. croceus*, probably point to inherent differences in sponge species ability to be successfully farmed. Thus, the findings of this study do not preclude the possibility of farming other New Zealand sponge species on rope. It may be possible to modify this method of farming to improve sponge attachment. For example, Battershill & Bergquist (1999b) discovered that *P. croceus* settles preferentially on rock chips, and it may be possible to incorporated these into the warp of a rope to promote explant attachment. Various types of rope substrate should also be tested.

Many factors have to be considered in the development of a method or on-growing structure suitable for farming sponges for metabolite production. These include cost, bio-fouling, harvesting procedures, explant growth and survival, and metabolite yield. The findings of this study, which concentrated on explant growth and survival using three farming methods, will help develop a suitable on-growing structure for farming massive sponges, such as *P. croceus* and *L. brevis*.

ACKNOWLEDGEMENTS

We would like to thank the many divers that helped in this study, in particular Chris Woods, Pete Notman and Phil James.

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POLLY WANT A SPONGE? : FIELD EXAMINATION OF SPONGIVORY BY CARIBBEAN PARROTFISHES IN REEF AND MANGROVE HABITATS. *Memoirs of the Queensland Museum 44: 160. 1999*:- Caribbean sponge species such as *Xestospongia muta* frequently display linear grazing scars that appear to have been made by parrotfishes, yet there are few scientific reports of parrotfish spongivory. We used a video camera to monitor 40 specimens of *X. muta* for a minimum of 0.5 hr/sponge to determine the frequency of parrotfish bites on this species. Ten hours of taping captured 45 bites on normally coloured sponges, and 527 bites on four bleached sponges. Also, the guts from parrotfishes collected in mangrove and reef habitats were digested in nitric acid and analysed for spicule content. Parrotfishes collected in the mangroves (*Sparisoma aurofrenatum*, *Scarus croicensis*, and *Sc. taeniopterus*) had a significantly greater mass of spicules in their guts than did parrotfishes collected on the reef (*Sp. aurofrenatum*, *Sp. viride*, *Sp. chrysopterum*, *Sc. vetula*, *Sc. coelestinus*, and *Sc. taeniopterus*). Up to 148mg of spicules were present in the guts of mangrove parrotfishes. The spicules of *Geodia gibberosa*, a sponge that is common in the mangroves but rare in exposed locations on the reef, were abundant in the gut samples. Our results suggest that some sponge species are palatable not only to specialist predators such as sea turtles and angelfishes, but also to species that are not usually recognised as sponge predators. ❐ *Porifera, spongivory, parrotfishes, Xestospongia muta, Geodia gibberosa, Sparisoma spp., Scarus spp., spicules, ecology, predation.*

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DEVELOPMENT OF *HALISARCA DUJARDINI* JOHNSTON 1842 (PORIFERA, CERACTINOMORPHA: HALISARCIDA) FROM EGG TO FREE LARVA. *Memoirs of the Queensland Museum 44: 160. 1999*:- Embryonic development in the sexual viviparous sponge *Halisarca dujardini* from the White Sea (Arctic) shallow water was studied. Complete, equal, asynchronal cleavage is characterised with variability of analogous developmental stages and the lack of the strictly determined clevage spindles position. The cytoplasm is filled with numerous yolky granules with heterogenic contents. At the 16-24 cell-stage a small cavity is formed. Blastomeres and the embryo polarity are not expressed. Large nuclei containing pronucleolar bodies are situated at the central parts of the cells. From the 16-24 cell- stage, true nucleolus formation starts. The polarisation of blastomeres is expressed by the distal movement of nuclei and changes in cell form. Cleavage furrow planes obtain the similar radial pattern forming roundish coeloblastula 130-170μm in diameter with the small cavity restricted with long wedge-shaped cells.

The internal layer of the larva is formed at the 100-130 cell-stage owing to the individual cells' apolar migration out of the blastula walls. At the same time flagella are formed on the cells' apical surfaces, yolk granules being concentrated basally. Internal cells proliferate actively, differentiating into nucleolated amoebocytes, granular cells and collencytes.

The larvae are is disphaerula it consists of two flagellated sphaerae external and internal. The disphaerula is completely covered with flagella.

Flagellated cells are less numerous at the posterior pole. Flagellated epithelial cells are wedge-shaped. At their apical parts they contain a drop-like nucleus with nucleolus and a flagellum embedded into a pocket-like cytoplasmic invagination. The basal 2/3 of the cell volume is filled with numerous yolk granules. Flagellated cells are connected at their apical end by outgrowths of the plasma membrane embedded into similar invaginations of the neighbouring membrane. Posterior flagellated cells are trapeziform or rectangular, and contain numerous yolk granules. The nuclei are roundish, with large nucleoli. The internal sphaera is formed by invagination of lateral cells. These sphaera are formed by a layer of cylindrical cells that have flagella inside the cavity. Their piriform nuclei contain nucleoli, and there are yolk granules in the cytoplasm. There are no specialized cell contacts between blastomeres and larval cells. The spiral symbiotic bacteria are present in the central part of the larva and in intercellular spaces. Some peculiarities of *H. dujardini* embryogenesis are unique among Ceractinomorpha and are a matter of principle for comparative embryological studies of Porifera. They are: 1) total equal asynchronic cleavage; 2) equal, apolar coeloblastula with a small cavity; 3) unexpressed polarity of blastomeres; 4) subsequent of the same type radial cleavage leading to the cell polarisation and coeloblastula formation; 5) formation of an internal cell mass in the embryos by multipolar cell ingression at the 100-130 cell-stage; 6) development of special larva disphaerula; 7) formation of internal sphaera by invagination. All the features mentioned can serve as additional arguments for separation of the Halisarcida as an order (Bergquist, 1996). ❐ *Porifera, Halisarca dujardini, embryology, cleavage, larva, cells, ultrastructure.*

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A PRELIMINARY ASSESSMENT OF 'SPACE WARS' AS A DETERMINING FACTOR IN THE PRODUCTION OF NOVEL BIOACTIVE INDOLES BY *IOTROCHOTA* SP.

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Evans-Illidge, E.A., Bourne, D.J., Wolff, C.W.W. & Vasilescu, I.M. 1999 06 30: A preliminary assessment of 'space wars' as a determining factor in the production of novel bioactive indoles by *Iotrochota* sp. *Memoirs of the Queensland Museum* 44: 161-166. Brisbane. ISSN 0079-8835.

Iotrochota sp. from Salamander Reef, North Queensland, has yielded a plethora of at least ten bioactive indoles including mono-, di- and non-brominated variants. Metabolite composition varies within and between individual sponges, and competition for hard substrate was suspected as a determining factor in this variability. To provide a preliminary assessment of this, profiles of six identified indoles were compared between tissue samples categorised according to neighbour contact and growth thickness. Five of these compounds contained either two indole moieties (indolyl) or one indole and one benzene ring (benzoyl). The sixth indole, by virtue of its structure, was identified as the putative precursor of the other compounds. There were no significant differences between tissue category and abundance of either indolyl or benzoyl product, or their putative precursor. However, two predominant populations of metabolites were identified. Diminished precursor and increased indolyl product occurred in tissue from sponge edges with direct neighbour contact and thick fleshy projections. This relationship was not absolute, and some samples from these tissue categories contained increased precursor and diminished indolyl product. Tissue from thin central sponge areas and edge samples without direct neighbour contact exclusively contained chemistry in the latter group. The quantity of benzoyl product remained constant between tissue categories. These results neither clearly support nor discount the potential role of space competition in determining production of these compounds. The issues involved are more complex than those examined here, and courses for further investigation are suggested. ❐ *Porifera, chemical ecology, indole, bioactivity, competition.*

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Porifera continue to be the most prolific source of marine derived bioactive compounds in published literature (Marinlit, 1998). Numerous authors have sought to attribute sponge secondary metabolites to a role in the source organism, and many have supported correlations between metabolite variability and environmental parameters such as depth, UV light, and chemical defence (Thompson et al., 1987; Kreuter et al., 1992; Pawlik, 1993; Pawlik et al., 1995; Swearingen & Pawlick, 1995). This report presents some preliminary findings on the possible role of competition for space in variability of some novel indoles produced by a north Queensland sponge from the genus *Iotrochota*.

A sample of this species of *Iotrochota*, a black thin encrusting sponge with occasional thick vertical fleshy projections, was initially collected from Salamander Reef, North Queensland in 1988. The sample was part of the Australian Institute of Marine Science biodiversity collection for natural products screening, and was initially erroneously assigned to the genus *Ircinia*. In 1995, a crude extract of this sponge was found to be highly active in a neuronal nitrous oxide synthase inhibition bioassay (authors' unpublished data). Initial fractionation yielded a plethora of novel indoles including mono-, di- and non-brominated variants. Seven of these compounds have been isolated and identified to date (Bowden et al., 1998; this report; and authors' unpublished data), but several still await further attention. The sponge was recollected in 1996 in order to provide sufficient material for follow-up bioassay and structure elucidation. While both samples contained a similar range of novel indoles, the relative abundance of each compound varied substantially between samples.

An understanding of the cause of this variability will become important if one of these

TABLE 1. Characteristics (HPLC, structure and mass) of compounds examined in this study. Key: A= Brominated species showing two isotopes (^{79}Br and ^{81}Br); B= Nonbrominated species.

Peak no.	Figure no.	HPLC chromatophore		Structural class	Ion species [M-1]
		280nm	360nm		
7	4A	Yes	No	Precursor	306.9/308.9 [A]
9	4B	Yes	Yes	Indolyl	402.1 [B]
14	4C	Yes	Yes	Indolyl	356.1 [B]
16	4E	Yes	Yes	Benzoyl	411.0/413.0 [A]
19	4D	Yes	Yes	Indolyl	434.0/436.0 [A]
21	4F	Yes	Yes	Benzoyl	395.0/397.0 [A]

compounds progresses to become a new drug candidate and there is a need to optimise its yield through manipulative culture or selective recollection. Field observations indicated that competition for hard substrate at Salamander Reef was fierce, yet *Iotrochota sp.* remained abundant. Hence 'Space Wars' was suspected to be a potential controlling factor in metabolite variability in this sponge.

This study aims to provide a preliminary assessment of variability in the production by *Iotrochota* sp. of six bioactive novel indoles, with respect to direct neighbour contact and tissue thickness. It also aims to create a basis for further work to develop an understanding of factors that determine indole variability in this sponge.

MATERIALS AND METHODS

Sponge tissue samples were collected from Salamander Reef, 19°10.91'S, 147°03.76'E, a small rocky inshore reef off Cape Cleveland near Townsville, North Queensland, in March 1998. 29 samples from 8 individual sponges where collected from 10-15m depth.

Small biopsies of sponge tissue (approx $1cm^2$) were taken and assigned one of four categories according to the degree of direct neighbour contact and tissue thickness, as follows: 1) Edge Interaction (edge of sponge in direct contact with a neighbouring organism); 2) Edge No Interaction (edge of sponge without direct neighbour contact); 3) Centre Thin (centre of sponge, no fleshy projection); and 4) Centre Thick (thick fleshy projection in the centre of sponge).

Tissue samples were freeze dried, and 80mg (dry weight) of tissue was extracted in 5ml of a solvent solution made up of equal parts dichloromethane and methanol, with sonication for 80mins. Extract was carefully decanted into clean vials, dried, then redissolved in 1ml methanol for High Performance Liquid Chromatography (HPLC) analysis. Where less than 80mg tissue was available for extraction, solvent quantities were adjusted to achieve the same extraction concentration.

Extracts were analysed for brominated indoles of interest using HPLC with an Alltima C18 column (250x4.6mm, Alltech Australia). A linear gradient from 60-100% of methanol in water was used. UV spectra were recorded with a Shimadzu MXA diode array and absorbance monitored at 280 and 360nm. Major components of HPLC peaks were then characterised by negative-ion electrospray mass-spectrometry to confirm that common compounds could be identified between different sponge extracts.

Areas under HPLC peaks were then used as a measure of relative amount of each fraction, for comparison between samples. These estimates were not suitable to compare quantities of different fractions within individuals, as a full analysis of extinction-coefficients of each compound was not undertaken.

One-way analyses of variance (ANOVA) with $\alpha=0.05$ was used to compare HPLC peak areas of each fraction group of interest, between sample categories. Four samples from each of the four tissue categories were selected from the available sample pool. Samples were independently selected in this way for analysis of each fraction group of interest. Where a result was non-significant, the detectable effect size (standard deviation between means) with 80% power was calculated according to Cohen (1977) and expressed as a percentage of the overall mean.

TABLE 2. Power analysis results for non-significant ANOVAs on HPLC peak areas between tissue categories.

Compound Type	Grand Mean (arbitrary units)	Mean Square Within Groups (from ANOVA)	Standard Deviation between detectably different means	
			(Arbitrary units)	(% of grand mean)
Precursor	42621802	2.37143E+14	14937464.6	35
Indolyl Product	3707935	3.94114E+13	6089514.452	164
Benzoyl Product	14993786	1.08893E+13	3200897.12	21

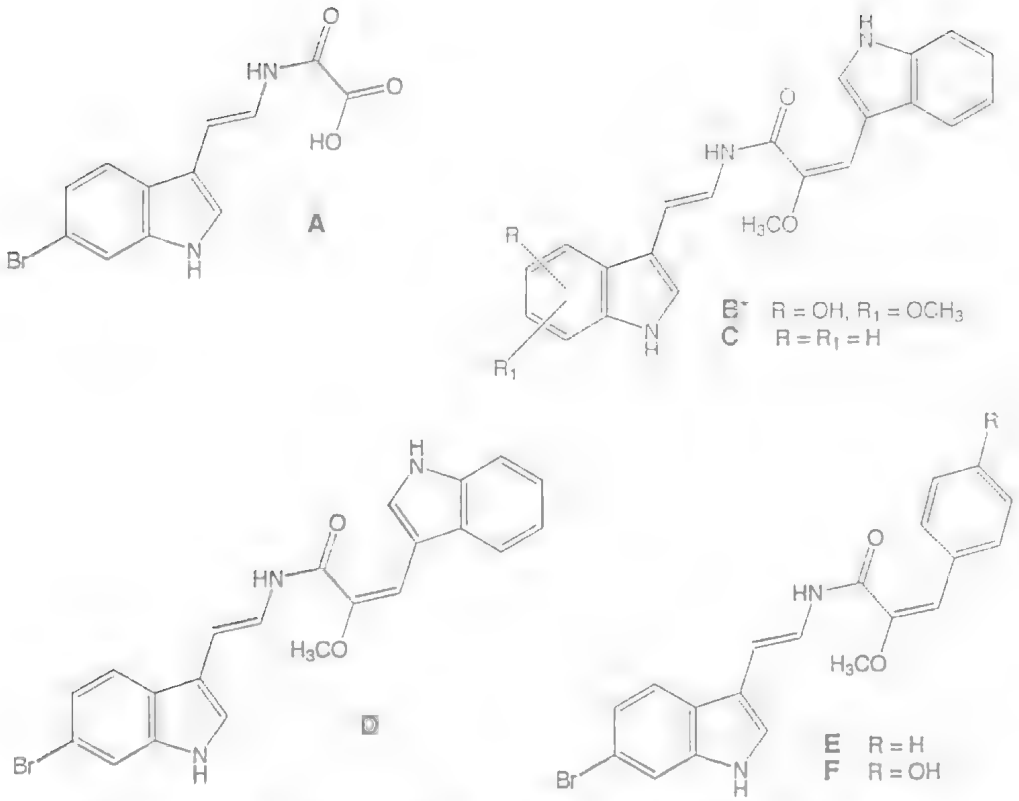

FIG. 1. Structure of the six indoles considered in this study: A, Putative precursor (HPLC Peak 7); B – D, Indolyl product (HPLC Peaks 9,14,19); E – F, Benzoyl product (HPLC peaks 16,21). * Assignment of substituent position not established

RESULTS

Six compounds of interest were separated using the HPLC system described above. Fourier Transform Mass Spectrometry and NMR confirmed that these indoles were the major components of the peaks listed and characterised in Table 1 and depicted by the structures shown in Figure 1. These data suggest that the low molecular weight indole in peak 7 (Fig. 1A) is the precursor of the more complex compounds in the other five peaks. These 'product' indoles fall into two structural classes based on whether the addition to the peak 7 core contains another indole (Fig. 1B-D) (= indolyl product) or a benzene group (Fig. 1E-F) (= benzoyl product).

ANOVAs on HPLC peak areas for precursor (peak 7), indolyl product (sum of peaks 9, 14 and 19) and benzoyl product (sum of peaks 16 and 21) found no significant difference in the amount of precursor or product present in tissue samples from the different tissue categories, with α=0.05. However, with 80% power, these non-significant tests were only capable of detecting differences between groups with a standard deviation between their means of 35% of the overall mean (precursor), 164% of the overall mean (indolyl product) and 21% of the overall mean (benzoyl product) (Table 2).

On the basis of HPLC, the 29 tissue sample extracts fell into two distinct groups. Figure 2 depicts a typical chromatograph of each group. When compared to Group 1, Group 2 contained more of peak 7 (Precursor) and less of peaks 9, 14 and 19 (indolyl product), while the quantity of peaks 16 and 21 (benzoyl product) were fairly consistent between the two groups. These relationships are summarised and quantified further in Figure 3.

Table 3 presents group membership with respect to tissue category. Only four tissue samples from three individuals had Group 1 chemistry (more indolyl product, less precursor). Two of these came from edges of direct interaction, and two

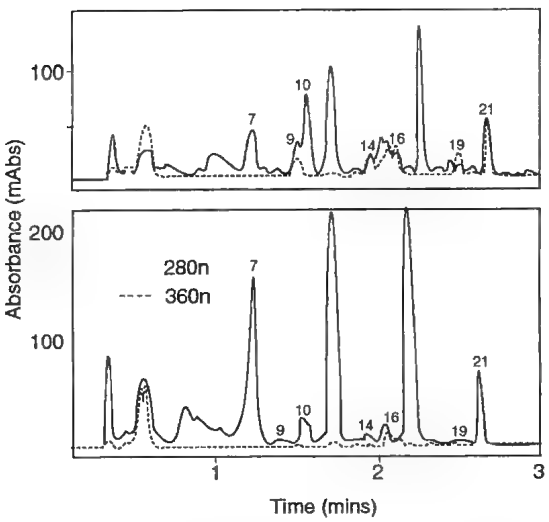

FIG. 2. Typical chromatograms of group 1 and group 2 samples. Peaks numbered for compounds considered in this study.

came from thick fleshy projections. While there were other samples from these categories with Group 2 chemistry (less indolyl product and more precursor), samples from either central sponge tissue or edge sites without direct interaction exclusively belonged to Group 2. Most samples analysed (25 out of 29) belonged to Group 2, and only two of the ten individuals examined contributed samples to both groups.

TABLE 3: Tissue categories sampled with respect to chemistry group membership.

Growth Type	Group 1	Group 2
Edge Interaction	2	10
Centre Thick	2	2
Edge No Interaction	0	6
Centre Thin	0	7

DISCUSSION

This work does not clearly support a direct relationship between neighbour interaction and indole chemistry in *Iotrochota* sp.. However, significance tests had moderate to low resolution at 80% power, and aspects of the distribution of samples containing Group 1 and Group 2 indole chemistry are consistent with an hypothesis of space competition influence. These are discussed below with respect to appropriate future directions for work in this area, and are not represented as definitive conclusions.

Morphological strategies are important to sessile benthic invertebrates in their struggle for substrate (Jackson, 1979; Hoppe, 1988; Vicente, 1990; Becerro et al., 1994). Becerro et al. (1994) suggested that another thin encrusting sponge, *Crambe crambe*, employed directional growth to either avoid stronger or confront weaker space competitors. Jackson (1979) suggested that vertical growth is another non-confrontational strategy in space competition. Whereas *Iotrochota* sp. is generally a thin encrusting sponge,

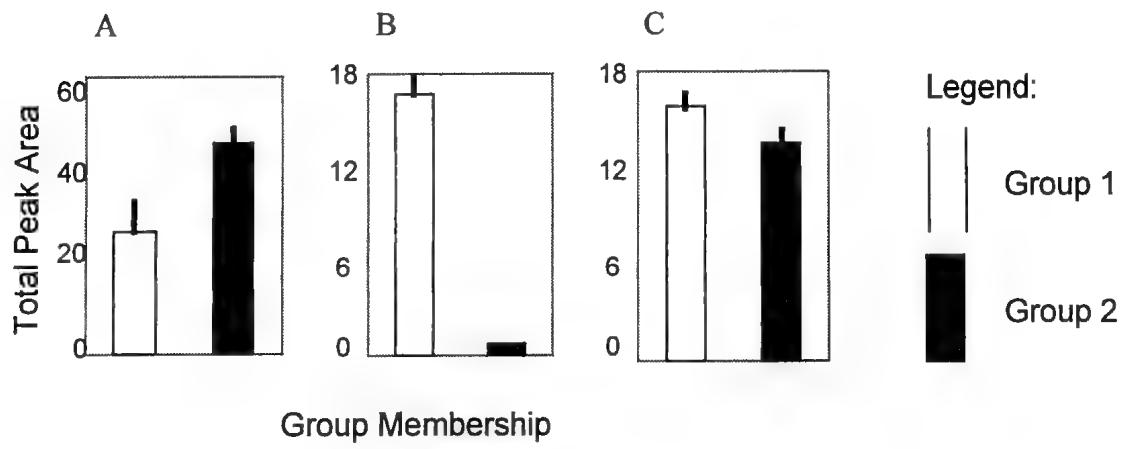

FIG. 3. Relative amounts (area under HPLC peak) of A. Precursor, B. Indolyl product and C. Benzoyl product in the two sample groups.

individuals typically sport several, thick, fleshy, vertical projections. A possible interpretation of this growth form is that the sponge utilises both confrontational and non-confrontational strategies to compete for hard substrate, whereas vertical growth in this otherwise thinly encrusting species may be a product of, or avoidance from encounters with superior space competitors at their outer growth margins.

It is therefore possible that samples containing Group 1 chemistry (i.e. more indolyl product, less precursor) had assumed a space competition strategy, either through direct confrontation at their margins, or non-confrontational vertical growth. However, this trend was not consistent, where both samples at the margins of neighbour contact (i.e. confrontational samples), and thick fleshy projections (i.e. non-confrontational samples), were included in chemistry Group 2. Further investigations into patterns of indole chemistry, which address growth form with respect to different neighbour interactions and the nature of these interactions, are essential to develop appropriate hypotheses.

Allelochemical interactions do not necessarily require direct contact between two individuals (Porter & Targett, 1988, Turon et al., 1996), and any non-contact interaction would be dependant on water flow. Thus, future work should also account for contact, distance and direction data (the latter with respect to currents). This species is amenable to transplantation (authors' unpublished data) and thus a candidate for controlled manipulative experimentation.

Patterns of variability in the other indole compounds known to occur in this *Iotrochota* sp. (authors' unpublished data), may also be important in understanding total metabolite variability in this species. More than 40 additional compounds which can be identified tentatively as indoles on the basis of mass spectrometry evidence, await characterisation, structural elucidation, and quantification. Also, the putative precursor-product relationship proposed in this work needs to be confirmed before any strong assertions about the invocation of a secondary metabolite from its precursor can be attributed to ecological factors.

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LOCALISATION OF BIOACTIVE METABOLITES IN MARINE SPONGES

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Marine natural product chemists have often proposed that bioactive sponge metabolites are produced by symbiotic micro-organisms. This paper discusses the rationale for these proposals, reviews the strengths and weaknesses of methods that are available to test such hypotheses and reports some experimental studies. The conclusion reached from the research to date is that it is too early to make generalisations concerning either the role of symbionts in the biosynthesis of sponge metabolites or even the best techniques for studying the cellular localisation of bioactive metabolites. ❐ *Porifera, bioactive metabolites, cyanobacteria, filamentous eubacteria, symbiosis, Aplysina fistularis, Dysidea herbacea, Theonella swinhoei, Oceanapia sagittaria, Jaspis splendens.*

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Sponges are exceptionally good synthetic chemists. They can make chemicals of extreme toxicity and/or deterrent value that have undoubtedly contributed to their survival over the ages. But they may not always have acted alone. We now know that some sponges harbor populations of symbiotic micro-organisms that produce the chemicals thought to defend the sponge from competition or predation. However, it is clear that this situation is less common than the marine natural products literature would have us believe. This paper reviews the methods used to determine the cellular location of natural products in sponges and presents some recent results from our laboratory that either confirm or deny the production of 'sponge metabolites' by symbiotic microbes.

SYMBIOSIS AS SEEN FROM THE VIEWPOINT OF CHEMISTRY. The history of natural products chemistry has been driven by the use of natural products to treat diseases. First came an interest in plant products such as digitalis and morphine, but this was superseded in the second half of this century by the discovery of a plethora of immensely important antibiotics and other drugs obtained by the fermentation of microbes. Chemists became indoctrinated with the concept that micro-organisms could provide the needs of the pharmaceutical industry, which for a long period of time was not far from the truth. Then came the discovery that marine organisms could provide many new classes of natural products that incorporated new and unexpected structural motifs. Within this group, sponges have provided not only the best source of novel compounds but also the greatest occurrence of potential pharmaceuticals (Faulkner, 1998, and references therein). However, when chemists compared the structures of sponge metabolites with those of compounds in the literature, they found many structures that were very similar to those of microbial metabolites. When chemists saw scanning electron micrographs of sponges that contained large numbers of micro-organisms, they felt justified in proposing that compounds resembling microbial metabolites were in fact of microbial origin. Furthermore, when closely related or identical compounds were found in different phyla and there was no evidence of transmission of the chemicals through the food chain, they proposed that these compounds might be produced by the same or similar micro-organisms endemic to hosts of different phyla. These hypotheses set the stage for a careful investigation of the role of symbiotic microbes in the production of 'sponge metabolites'.

LOCALIZATION OF SPONGE METABOLITES. There are two basic strategies for determining the location of specific metabolites in sponges: detection of compounds using microscopy, or cell separation followed by chemical analysis of each cell fraction. The strategy selected generally depends on the molecular properties of the compound to be investigated. Compounds that contain heavy elements such as bromine or iodine

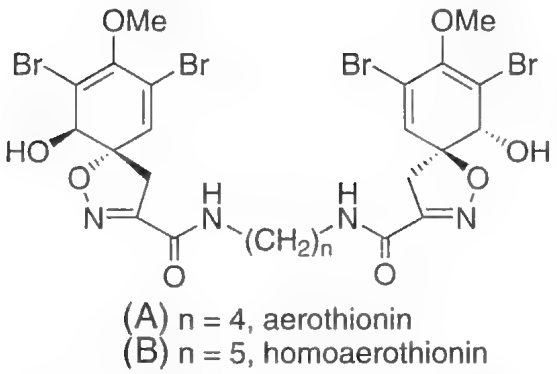


FIG. 1. Tetrabrominated metabolites. A, aerothionin. B, homoaerothionin.

can be detected by using an energy dispersive spectroscopy (EDS) detector on a scanning electron microscope (SEM) or a scanning transmission electron microscope (STEM). In theory, one could use the same technique to determine the location of compounds containing chlorine or sulfur but, in practice, the levels of chloride and sulfate ions in seawater preclude its use with marine specimens. Fluorescent compounds can be conveniently detected by fluorescence microscopy and by laser scanning confocal microscopy, but this technique is susceptable to problems caused by background fluorescence due to photosynthetic pigments and general autofluorescence of cells. The method of immunostaining using polyclonal antibodies to bind to a specific compound is common in cellular biology but prior to a report at this symposium (Ilan, 1998) and one other recent paper (Perry et al., 1998) had not been applied to study sponge metabolites. Finally, there is the possibility that specific compounds may be detected in cell preparations using secondary ion mass spectrometry in conjunction with tandem mass spectrometry. The latter two methods, both of which can be fine-tuned to detect individual compounds, could offer considerable advantages over methods that rely on detecting a class of compounds.

Cell separation methods take advantage of our ability to analyse the chemical content of fixed cells but suffer from the disadvantage that fractions containing only a single cell type may be difficult to prepare. Sponge tissues can be dissociated by enzymatic digestion or mechanical disruption in calcium-magnesium free seawater using squeezing, sieving, simple mincing, vigorous stirring, or even a juicer. The dissociated cells can then be fixed, which stabilises the cells during the period between collection and analysis. It is a relatively simple matter to separate cyanobacteria using a cell sorter to distinguish fluoresecent from non-fluorescent cells but this method does not distinguish between sponge and eubacterial cells. Cell types can also be separated by density using either differential centrifugation or Ficoll or Percoll density gradients. It has been our experience that repeated centrifugation is required to produce fractions of reasonable purity and that the different sponge cell types are difficult to separate on the basis of density. Nonetheless, filamentous bacteria, mixed sponge cells and mixed unicellular bacterial cells can all be enriched to ca. 90% purity using centrifugation. To detect the compounds of interest, each cell fraction is then extracted individually and analyzed using two or more of the following techniques: mass spectrometry (MS), which can be combined with high performance liquid chromatography (HPLC) or gas chromatography (GC), HPLC using a diode array detector to measure the UV spectrum, and ^{1}H NMR spectrometry.

RESULTS AND DISCUSSION

The tetrabrominated metabolites aerothionin (Fig. 1A) and homoaerothionin (Fig. 1B), which occur as a 10:1 mixture in a shallow-water specimen of *Aplysina fistularis* from La Jolla, were ideal candidates for study using energy dispersive spectroscopy because the molecules contain such a high concentration of bromine. Analysis of the STEM images using energy dispersive X-ray analysis revealed a 20-fold larger concentration of bromine in spherulous cells than in bacterial or other sponge cells, which were both at background levels. We therefore argued that the brominated metabolites (Fig. 1A-B) were produced and stored in spherulous cells (Thompson et al., 1983).

There are two major chemotypes of *Dysidea herbacea*; one contains both sesquiterpenes and metabolites biosynthesised from polychlorinated amino acids, the other produces only polybrominated biphenyl ethers and lacks terpenes. Very significant populations of cyanobacteria are found in both chemotypes and in both cases, the cyanobacterium is considered to be *Oscillatoria spongelliae*. The fluorescent cyanobacterial cells were separated from all other non-fluorescent cells using a cell sorter and

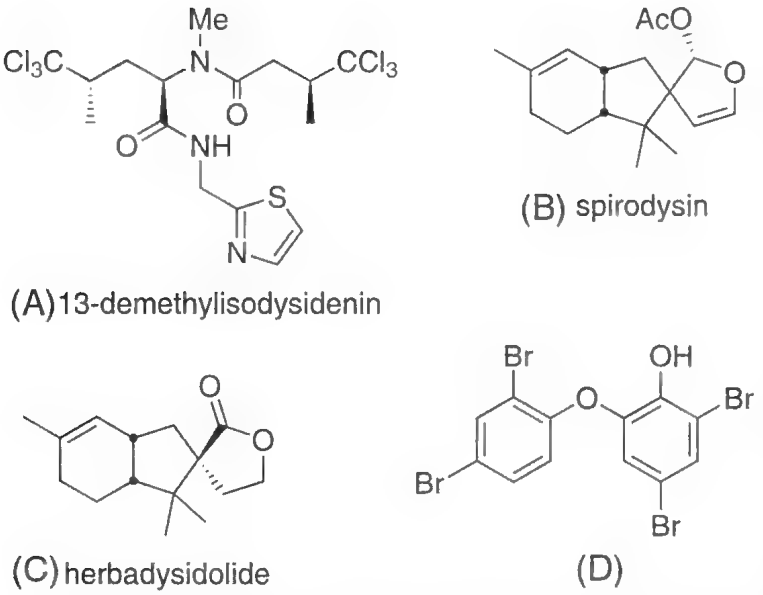


FIG. 2. Metabolites from two major chemotypes of *Dysidea herbacea*. A, 13-demethylisodysidenin. B, spirodysin. C, herbadysidolide. D, polybrominated biphenyl ether.

the chemical content of each cell type was analysed by ^{1}H NMR spectroscopy and GC-MS. In a specimen of *D. herbacea* from Heron Island, 13-demethylisodysidenin (Fig. 2A), a polychlorinated amino acid derivative, was extracted from the cyanobacterial cell fraction while the sesquiterpenes spirodysin (Fig. 2B) and herbadysidolide (Fig. 2C) were detected in the fraction that contained sponge and bacterial cells (Unson & Faulkner, 1993). Garson and coworkers recently separated the sponge cells using a Percoll density gradient and showed that the sesquiterpenes were located in a fraction containing archaeocytes and choanocytes (Flowers et al., 1998). A similar analysis of a specimen of *D. herbacea* from Palau revealed that the polybrominated biphenyl ether (Fig. 2D) was located not only in the cyanobacterial fraction but also as crystals situated just below the surface of the sponge (Unson et al., 1994).

The sponge cells were considered to be devoid of brominated metabolites, although it was possible to detect a very low level of the polybrominated biphenyl ether (Fig. 2D), which was consistent with the presence of a small number of cyanobacterial cells that remained as contaminants in the sponge cell fraction. Having shown that cyanobacteria are responsible for the halogenated chemicals in the two chemotypes of *D. herbacea*, there is now a need to analyse the 16S rRNA sequences of representative samples to determine whether the cyanobacteria represent two different strains of *O. spongelliae* or different cyanobacterial species, which are two of several possible explanations of the chemical diversity. The diversity of chemistry assigned to *Dysidea* spp. may also provide a good rationale for a sponge taxonomist to re-examine the genus and particularly the chemists' voucher specimens.

The lithistid sponge *Theonella swinhoei* has provided chemists with an almost unequalled source of highly bioactive chemicals (Bewley & Faulkner, 1998). Our interest in this sponge was piqued by the structural similarity between swinholide A (Fig. 3A), which had previously been isolated from *T. swinhoei*, and the cyanobacterial product scytophycin C (Fig. 3B) and by the fact that the cyclic peptides of *T. swinhoei*, such as theopalauamide (Fig. 3C)(Schmidt et al., 1998), contain aromatic ß-amino acids similar to those found in some cyanobacterial cyclic peptides (Ishibashi et al., 1986; Kitagawa et al., 1990; Bewley & Faulkner, 1998).

This led to a suggestion that the prominent filamentous micro-organisms in *T. swinhoei* were cyanobacteria that produced both groups of compounds (e.g. Kobayashi & Ishibashi, 1993; Fusetani & Matsunaga, 1993). We had reason to suspect that this assumption was incorrect because the sponges were often found in caves, the filaments were found in the interior of the sponge, away from the light, and extracts of the endosomal tissue of the sponge did not appear to contain sufficient chlorophyll pigments. In a specimen of *T. swinhoei* from Palau, there were unicellular cyanobacteria (*Aphanocapsa feldmanni*) in the ectosome, which was peeled away and examined separately. The ectosomal tissues were dissociated and the cyanobacteria were separated using differential centrifugation, but they did not contain the metabolites of interest. The endosomal tissues were dissociated, fixed in a mixture of formalin and glutaraldehyde in artificial seawater, and separated using

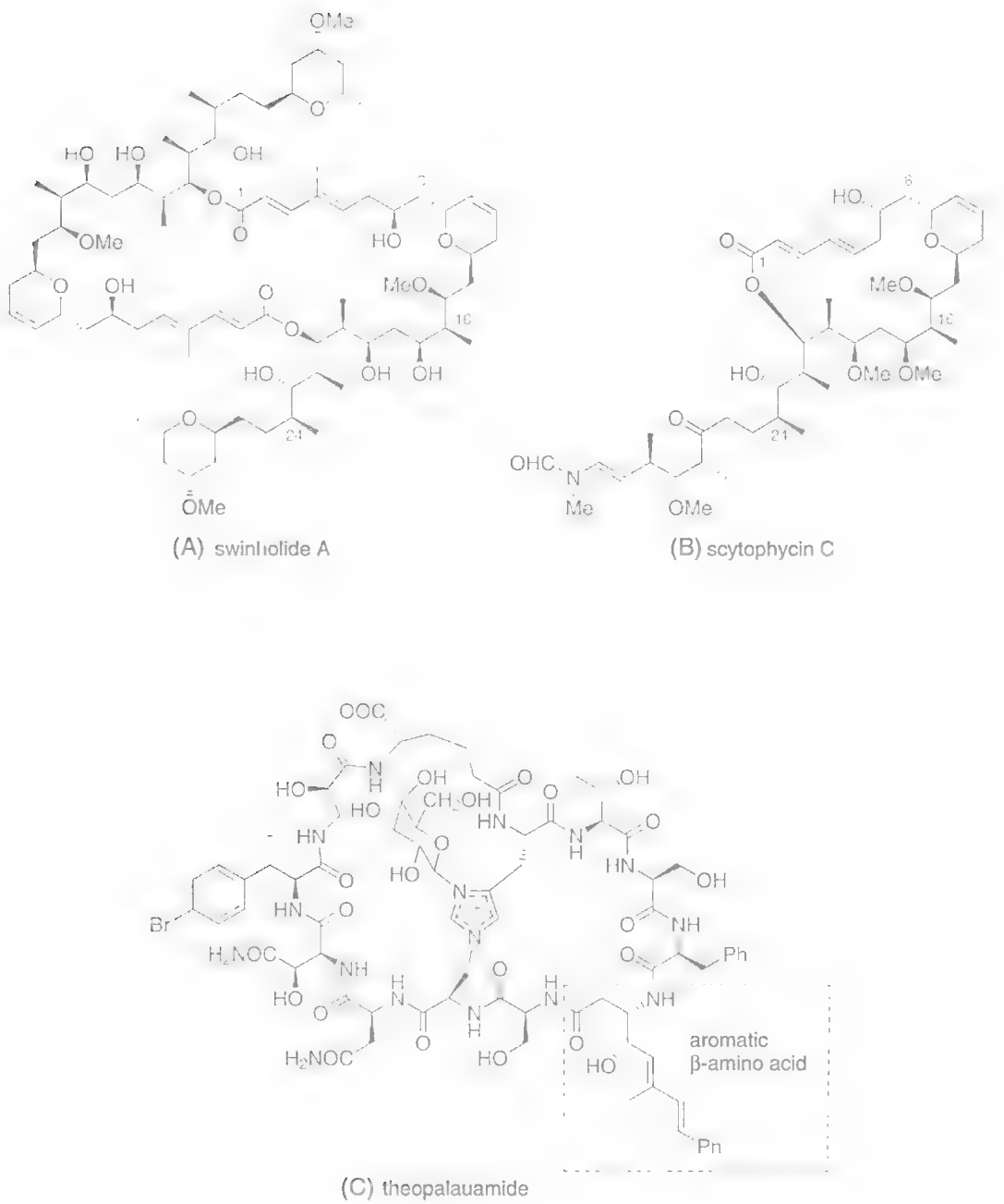


FIG. 3. Metabolites from the lithistid sponge *Theonella swinhoei*. A, swinholide A, which partially resembles scytophycin C. B, cyanobacterial product scytophycin C. C, theopalauamide.

differential centrifugation into three fractions containing mixed sponge cells, a filamentous bacterium, and mixed unicellular bacteria. The cell fractions were thoroughly washed, then extracted to obtain crude extracts that were analysed by ^{1}H NMR and HPLC. Theopalauamide (Fig. 3C) was found to be present in about 4% dry weight in the filaments, which were examined by TEM and found not to be cyanobacteria, since they lacked the thylakoid

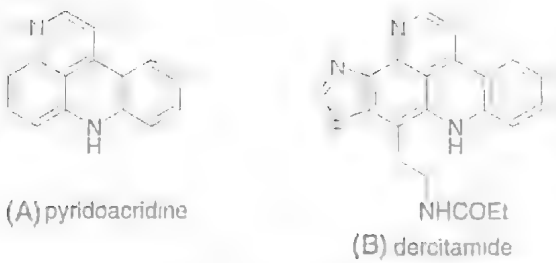
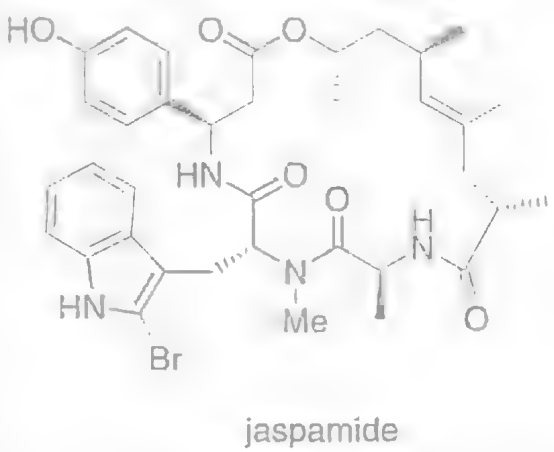


FIG. 4. A, pyridoacridine skeleton. B, dercitamide.

structures that house the photosynthetic apparatus of cyanobacteria (Bewley et al., 1996). We are currently characterising the eubacterial filaments using 16S rRNA analysis. Swinholide A (Fig. 3A) was extracted from the unicellular bacterial fraction, which contained many morphologically distinct bacteria. A recent re-examination of the ^{1}H NMR spectrum of the unicellular bacterial fraction revealed the presence of the 4-methylene sterols that are typical of *Theonella* spp., but we need to reconfirm that result because sterols are not usually produced by cultured bacteria. Both the sponge cells and the cyanobacterium *Aphanocapsa feldmanni* appeared to be devoid of bioactive metabolites.

The pyridoacridine alkaloids, which all possess the same underlying tetracyclic aromatic ring system (Fig. 4A), are examples of a class of metabolites that have been found in four different marine phyla, but predominantly in sponges and ascidians (Molinski, 1993). They have frequently been proposed to be metabolites of undesignated microbial populations that might occur as symbionts in the different phyla. We felt that there might be an alternative explanation based on the evolution of similar biosynthetic schemes in different phyla, in part because polyaromatic compounds are the most stable products that can arise from their presumed mode of biosynthesis (Steffan et al., 1993). Dercitamide (Fig. 4B) has been reported from both sponges and ascidians (Gunawardana et al., 1992; Carroll & Scheuer, 1990) — the latter authors referring to dercitamide as kuanoniamine C - and we have isolated it as the major metabolite of the sponge *Oceanapia sagittaria* (Salomon & Faulkner, 1996). Dercitamide is an interesting pigment that changes color from yellow in neutral or basic solution (pH > 7) to red in acidic solution (pH < 6) and has a fluorescence spectrum that is also pH dependent. Using a light microscope, one can observe a change in the color of the sponge tissue when a section is acidified using trifluoroacetic acid vapor. A similar pH dependency was noted when sections were observed using fluorescence microscopy, but there was so much fluorescence from cells that were out-of-plane that it was impossible to clearly image the cells containing dercitamide. Examination of both thick sections and enriched cell fractions using a confocal microscope under both neutral and acidic pH conditions led to the conclusion that dercitamide was localised in sponge cells containing between ten and twenty spherical inclusions. Transmission electron microscopy was employed to show that there were no intracellular bacteria that could be responsible for the chemistry (Fig.6). The dercitamide-containing cells were characterised by TEM analysis, although they have not been classified as a particular type of sponge cell. This appears to be the first time that confocal microscopy has been employed to locate marine natural products on the basis of their autofluorescence. Research is in progress to determine the cellular location of pyridoacridine alkaloids in ascidians.

Not every study has resulted in an unambiguous localisation of metabolites. The cyclic depsipeptide jaspamide (Fig. 5) is a cytotoxic metabolite of *Jaspis splendens* (De Laubenfels, 1954), referred to as *Jaspis* sp. in our earlier chemistry paper (Zabriskie et al., 1986), that has been proposed both as a chemotaxonomic marker and to be of microbial origin. It is interesting to note that jaspamide (Fig. 5) was also isolated from a completely different sponge, *Auletta* cf. *constricta* (Crews et al., 1994). The sponge was dissociated in a juicer, a technique previously used successfully on *T. swinhoei*, followed by



jaspamide

FIG. 5. Cyclic depsipeptide jaspamide from *Jaspis splendens*.

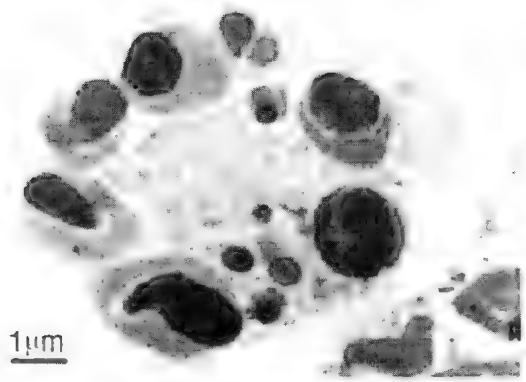


FIG. 6. Micrograph of a dercitamide-containing inclusional sponge cell from *Oceanapia sagittaria* that shows the absence of intracellular symbionts.

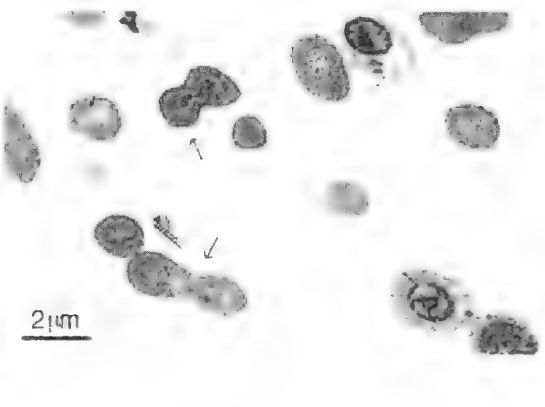


FIG. 7. Micrograph of dissociated cells of *Jaspis splendens*. Arrows indicate the unidentified symbionts that do not contain jaspamide.

fixation and cell separation using differential centrifugation. Jaspamide was not detected in extracts of an unidentified extracellular 'symbiont' (Fig. 7) or associated micro-organisms, which represented a large proportion of the whole sponge biomass, but was isolated in nearly 4% yield from a fraction containing small (ca. 500nm) orange bodies and cellular debris. The identity of the orange bodies is uncertain, but we have evidence that the dissociation process may have ruptured the sponge cells with concomitant release of the orange bodies. Examination of newly acquired sponge material by light microscopy revealed the presence of numerous small orange inclusions within the sponge cells. We now believe that jaspamide (Fig. 5) is located within sponge cells and further research is in progress to test this hypothesis.

CONCLUSIONS

The major conclusion that we have reached during our studies of the role of symbionts in the production of sponge metabolites is that it is extremely dangerous to make any general statements about the sources of bioactive metabolites. In essence, each compound of interest requires an individual study to determine its source. The results that we and others have generated indicate that it is possible to detect specific compounds or classes of compounds in either symbiont or sponge cell fractions and that the concentrations of secondary metabolites in isolated cell fractions can be spectacularly high. However, it is often difficult to determine which sponge cell type produces the metabolite and it is nearly impossible to define a particular unicellular bacterium as the source of a bioactive compound. The latter will undoubtedly require the culture of symbiotic micro-organisms, which is the goal of several research groups. In order to accomplish this goal, we have proposed a strategy that involves identification of the symbionts from their 16S rRNA sequence (Brantley et al., 1995) before attempting to culture them using media that are suitable for culturing their nearest relatives. While the ultimate goal is to culture symbionts that produce pharmacologically-active sponge metabolites in order to speed their pharmaceutical development, information gained from cellular localisation studies can also be useful in chemotaxonomy and the understanding of bio-synthetic pathways that may have influenced the evolution of symbioses within sponges.

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TRACE ELEMENT AND STABLE ISOTOPE PROFILES FROM THE CORALLINE SPONGE (*ASTROSCLERA WILLEYANA*). *Memoirs of the Queensland Museum 44: 174. 1999-* Techniques developed for laser-ablation-ICP-MS analysis of corals have now been utilised for the analysis of trace elements in the coralline sponge *Astrosclera willeyana*. In scleractinian corals the elements B, Mg, Sr, Ba and U show seasonal variations consistent with environmental parameters, predominantly sea surface temperature and variations in upwelling. We report here a preliminary investigation to determine whether elemental distributions in sclerosponges will provide meaningful proxy information about past oceanographic conditions.

Samples from Taveuni, Fiji, Ruby Reef, GBR and Truk, Caroline Islands have been analysed at a sampling resolution of ~40µm. With current techniques and data reduction methods, sampling at this resolution produces too much variation to show any elemental correlations. When samples are filtered to ~100µm resolution, longer-term (annual to several year) patterns appear, which are consistent between the B/Ca, Mg/Ca, Sr/Ca and Ba/Ca cycles. This suggests a common incorporation mechanism between these four elements. If this variation is temperature related, the method of incorporation is markedly different than corals. The boron, magnesium and barium concentrations in sclerosponges are 2-5 times lower than in corals, with concentrations of ~20ppm, ~200ppm and ~4ppm, respectively. The strontium and uranium concentrations are 1-2.5 times higher than in corals with concentrations of ~9000ppm and ~7ppm respectively. We will also present preliminary stable isotope data ($\delta^{18}O$ and $\delta^{13}C$) to compare with the trace element profiles. ❐ *Porifera, Astrosclera, Sr/Ca, Mg/Ca, Ba/Ca, laser ablation, ICP-MS, $\delta^{18}O$, $\delta^{13}C$, environmental parameters.*

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DEMOSPONGES OF THE HOUTMAN ABROLHOS

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The Houtman Abrolhos lie off the west coast of Australia within a biogeographic zone that has overlapping temperate and tropical components, and a significant proportion of endemic species. The islands are situated in the path of the warm, southward flowing Leeuwin current. Studies on marine biota of these islands found a dominant tropical component to the fauna. Marine sponges of the Houtman Abrolhos are poorly studied. A field program was established to collect sponges, document the biodiversity, and determine if this biota was principally tropical or temperate in origin. 77 demosponge species are reported from the two localities examined in this study, 28 of which are known to science, 14 are identified to known species but require confirmation by comparison with type material, and 35 species are probably new. Three genera are reported for the first time from Australia. This study brings the total number of demosponge species documented from the Houtman Abrolhos to 109. Preliminary assessment of tropical versus temperate affinities indicated more species of temperate than tropical origin were present. This is contrary to comparable studies on other components of the marine biota of these islands. ❐ *Porifera, Demospongiae, Houtman Abrolhos, Western Australia, biogeography.*

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The Houtman Abrolhos (herein referred to as the Abrolhos) are 65-90km off the W coast of Australia at 28°-29°S, 113°35'-114°03'E, near the edge of the continental shelf (Wells, 1997). There are 122 islands in 4 island groups (Fig.1).

The marine habitats of these islands are unique. 1) They are the southernmost area of major coral reef development in the eastern Indian Ocean (Wells, 1997). 2) There is a co-dominance of reef building corals and macroalgae in the upper photic zone. Macroalgae (often *Ecklonia*) dominates on windward (W facing) reefs and hard coral (*Acropora*) on leeward slopes. In some lagoons there may be a mixture of the two community types (Wells, 1997). 3) The western rock lobster, *Panulirus cygnus*, a species endemic to Western Australia, is commercially fished in the Abrolhos system. This is a seasonal fishery at the Abrolhos open for three months each year when the fishers and their families occupy huts on the islands (Wells, 1997). For the rest of the year the islands are largely unoccupied. 4) The islands have high conservation value. In 1994 the Marine Parks and Reserves Selection Working Group acknowledged the islands as the most significant area on the WA coast, and the most worthy of reservation (Anon., 1994).

These islands are considered to be within, and towards, the northern limit of the Western Overlap Zone (Wells, 1997), a biogeographic region on the WA coastline which has temperate and tropical components, and a significant proportion of endemic species. Studies on the marine biota of the islands found a higher proportion of northern tropical species than southern temperate species, compared to the adjacent mainland coastline at Geraldton. This high proportion of tropical species in the Abrolhos is due to the southward flowing Leeuwin Current, which carries tropical water from NW Australia (Cresswell & Golding, 1980). This is a relatively warm seasonal current that flows southward most strongly in autumn and winter, hence retaining higher sea temperatures at the islands than in adjacent mainland coastal waters (Pearce, 1997). However, geographically these islands are temperate, hence the co-occurrence of both tropical and temperate species.

The aims of this study were to document the poorly known demosponge fauna of these islands, to assess their biogeographic affinities, and to compare their biogeography to other marine phyla reported from there.

Seven previous publications have reported on the sponge fauna of these islands, with a total of 57 demosponges described from the Abrolhos prior to this study (Table 1).

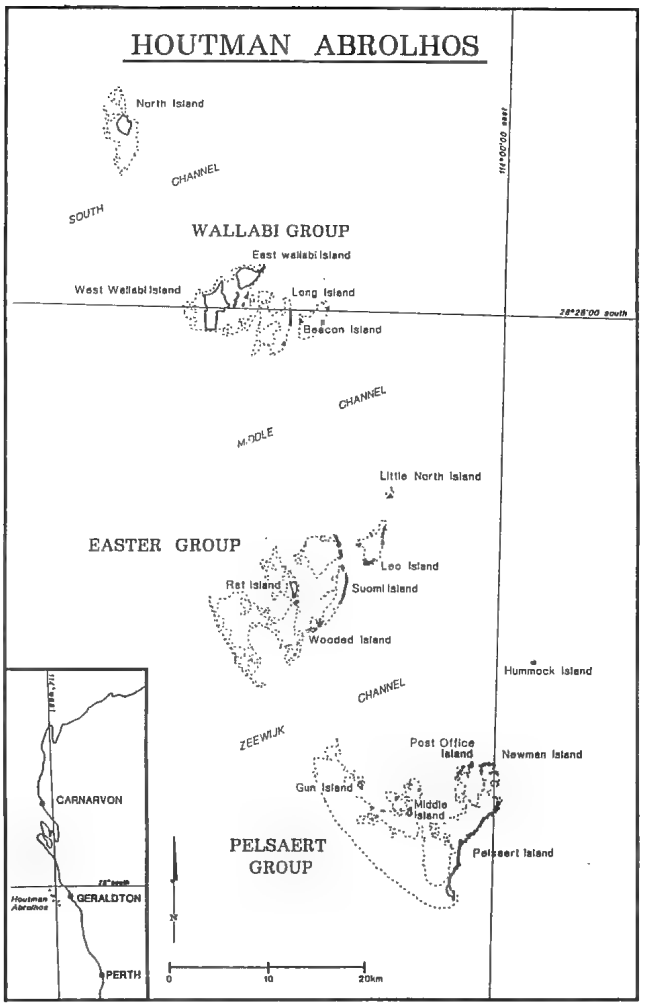

FIG. 1. Map of the Houtman Abrolhos. (Reproduced with permission of Fisheries Western Australia)

TABLE 1. Publications on Demosponges reported from the Abrolhos.

Author	Number of species	Field collection
Dendy & Frederick (1924)	38	Dakin
Hooper (1989)	1	Hooper, USSR Research Vessel Akademik Oparin, NCI
Hooper (1991)	2	USSR Research Vessel Akademik Oparin, NCI
Hooper & Bergquist (1992)	1	USSR Research Vessel Akademik Oparin, NCI
Hooper & Lévi (1993)	1	USSR Research Vessel Akademik Oparin, NCI
Hooper (1996)	12	Hooper, USSR Research Vessel Akademik Oparin, WAM, NCI
Fromont (1998)	2	WAM, this study
TOTAL	57	

MATERIALS AND METHODS

Field trips were made to the islands in 1996 and 1997 to examine demosponges of the Abrolhos, and to determine timing and mode of reproductive activity of species collected. The latter results will be reported elsewhere. Two islands in two island groups were visited, Rat I. in the Easter Group (December 1996), and Beacon I., Wallabi Group (March 1997) (Fig.1). Sponge species were photographed in situ or soon after collection, a representative specimen of each species from each site was preserved in 70% ethanol, and deposited in the collections of the WAM. Relative abundance of species was estimated for each dive and summarised into 3 broad categories, + = 0-5 specimens, ++ = 5-10 specimens, and +++ = 10+ specimens seen per dive. The dominant habitat type studied on these field trips was coral reef, and intertidal reef flats at Rat I. (Table 2).

Abbreviations: CSIRO, Commonwealth Scientific and Industrial Research Organisation, Perth; NCI, Marine Bioproducts Group, Australian Institute of Marine Science, Townsville; QM, Queensland Museum, Brisbane; UWA, University of Western Australia, Perth; WAM, Western Australian Museum, Perth.

RESULTS

Seventy-seven species were recorded from the two localities examined in this study. Fourteen (18%) of these were common to both localities, 40 (52%) were reported only from Rat I. and 23 (30%) only from Beacon I. Of these, 28 (36%) have already been described in the literature (Table 3). Of the remaining 49 (64%) species, 14 were tentatively assigned to a known species but require

TABLE 2. Summary of fieldwork program undertaken at the Abrolhos for this study.

Island/ Island group	Subtidal SCUBA dives	Maximum depth (m)	Intertidal reef walks	Habitat (subtidal dives)
Rat I./ Easter Group	5	18	4	Coral reef slope (4), deep hole (1)
Beacon I./ Wallabi Group	5	25	0	Coral reef slope (2), deep outcrop (1), patch reefs (2)

TABLE 3. Sponge species previously reported in the literature and collected during this study at the Abrolhos. * original species name that has since been synonymised with species name given in the table (Hooper & Wiedenmayer, 1994); # probable species complexes. Localities: GBR = Great Barrier Reef, NA = N Australia, NSW = New South Wales, SA = S Australia, NT = Northern Territory, IPM = Indo Pacific/Malay, Vic = Victoria, IO = Indian Ocean, Tas = Tasmania, NWA, = NW Australia, Abrolh = Houtman Abrolhos, WA = W Australia, New Cale = New Caledonia. Abundance estimates: + = 0-5 specimens seen in 1 dive; ++ = 5-10 specimens; +++ = >10 specimens.

Identification	WAM no.	Rat I. Easter Group	Beacon I. Wallabi Group	NA	SA	IPM	IO	Other areas
Plakinastrella mammillaris Lendenfeld, 1907	Z1280	+	-	NWA	WA			
Plakinastrella minor (Dendy, 1916)	Z1279	+	-				X	Abrolh
**Ancorina acervus* (Bowerbank, 1862)	Z1198, 1199	++	-	GBR, NT		X	X	Red Sea
Erylus lendenfeldi Sollas, 1888	Z1278, 1281, 2241	+	+		SA		X	
Caulospongia amplexa Fromont (1998)	Z53	-	+	NWA				
Chondrilla australiensis Carter, 1873	Z1167, 1168, 1169, 1170, 1171, 1172, 1173	+++	+++		NSW, Vic	X	X	Red Sea
#*Spirastrella vagabunda* Ridley, 1884	Z1204	+	-	GBR, NT		X	X	
Sigmosceptrella fibrosa (Dendy, 1897)	Z1160, 1161, 1162, 1163, 1164	++	++		Vic, Tas			
Trachycladus laevispirulifer Carter, 1879	Z1185, 1186, 1187	-	+		NSW, Vic, Tas			
Axinella aruensis (Hentschel, 1912)	Z1275, 1276, 1284, 1285, 1376	++	+	NWA		X		
Cymbastela marshae Hooper & Bergquist, 1992	Z1176, 1177, 1178	++	+					Abrolh
Halichondria phakelliodes Dendy & Frederick, 1924	Z1180, 1181, 1182, 1183, 1184	++	-					Abrolh
Holopsamma favus (Carter, 1885)	Z1191	+	-	NWA	Vic, Tas			
Crella incrustans thielei Hentschel, 1911	Z1196, 1197	+	-		WA			
Crella spinulata (Hentschel, 1911)	Z1179	-	+	NWA, GBR, NT				New Cale
Phorbas fictitioides (Dendy & Frederick, 1924)	Z1158	-	+					Abrolh
Forcepia biceps (Carter, 1885)	Z1159	+	-		WA, Vic			
Iotrochota acerata Dendy, 1896	Z16	-	+		WA, Vic			
Iotrochota baculifera Ridley, 1884	Z1286, 1287, 1288, 1289, 1290	+++	+++	NWA, NT		X	X	
Zyzzya massalis (Dendy, 1922)	Z1302	+	-			X	X	Abrolh
Mycale cockburnia Hentschel, 1911	Z1255, 1256, 1257, 1258	+	+		WA			
Mycale sulcata Hentschel, 1911	Z1297	+	-		WA			
#*Clathria (Thalysias) cactiformis* (Lamarck, 1814)	Z1259	-	+		WA, SA, NSW, Vic, Tas		X	
Clathria (Isociella) selachia Hooper, 1996	Z1282, 1283	+	+	NWA				
Echinodictyum clathrioides Hentschel, 1911	Z1195	-	+	NWA	WA			
Haliclona amboinensis (Lévi, 1961)	Z1406	+	-	GBR		X		
Haliclona cymaeformis (Esper, 1791)	Z1400, 1401, 1402	-	+++	NT, GBR		X	X	
**Lendenfeldia plicata* (Esper, 1806)	Z1201, 1202, 1203	++	-	NWA, Qld	WA, NSW	X	X	

TABLE 4. Undescribed species, and unconfirmed species identifications, collected during this study from the Abrolhos. Distributions: TR = tropical, ST = subtropical, TE = temperate, CO = cosmopolitan, Carrib = Carribean, Aust = Australia, NZ = New Zealand, Sth Afr = South Africa.

Identification	WAM no.	Rat I. Easter Group	Beacon I. Wallabi Group	TR	TR ST	TR ST TE	CO	Other regions
Plakortis sp. 1	Z1272, 1273, 1274	+	+		X			
Plakortis sp. 2	Z14, 1269, 1270, 1271, 2240	++	++		X			
Corticium cf. *simplex* Lendenfeld, 1907	Z1392	++	-		X			
Corticium cf. *candelabrum* Schmidt, 1862	Z1380	++	-		X			
Penares? cf. *intermedia* (Dendy, 1905)	Z1397	+	-			X		
Stelletta cf. *brevis* Hentschel, 1909	Z10, 1190	-	++			X		
Tethya cf. *multistella* Lendenfeld, 1888	Z1277	+	-			X		
Anthosigmella sp.	Z1303	+	-	X				Carrib
Aaptos sp.	Z1165, 1166	+++	-			X		
Theonella sp.	Z1242	+	-	X				
Agelas cf. *mauritiana* (Carter, 1883)	Z1200	+	-		X			
Agelas sp. 1	Z1193, 1194	++	-		X			
Agelas sp. 2	Z17	-	+		X			
Phycopsis sp.	Z1254	-	+	X		X		
Pararhapoxya sp.	Z1261	+	-			X		NZ
Dragmatella? sp.	Z1298	-	+					?
Cymbastela cf. *vespertina* Hooper & Bergquist, 1992	Z1174, 1175	+	-	X				
Neofibularia sp.	Z1391	-	+	X				
Iotrochota sp.	Z1291, 1292	+++	-	X				
Tedania cf. *anhelans* (Lieberkühn, 1859)	Z1296	+	-			X		
Ectyodoryx sp.	Z1301	+++	-			X		Bi-polar
Phoriospongia sp.	Z8, 1294	+++	-			X		Aust/ NZ
Guitarra sp.	Z1265, 1266, 1267	-	+			X		NZ/ Sth Afr
Liosina sp.	Z1205	+	-	X				
Clathria (Thalysias) cf. *abietina* (Lamarck, 1814)	Z1260	-	+				X	
Haliclona cf. *toxotes* (Hentschel, 1912)	Z1393, 1394, 1395, 1396	+	+				X	
Haliclona sp. 1	Z9, 11, 12, 1405, 2239	+	+				X	
Haliclona sp. 2	Z13	-	+				X	
Haliclona sp. 3	Z1399, 1403	-	++				X	
Reniera sp. 1	Z1375	+	-				X	
Reniera sp. 2	Z1384	+	-				X	
Reniera sp. 3	Z1383	++	-				X	
Reniera sp. 4	Z1381	+	-				X	
Gellius sp. 1	Z1379	-	+			X		
Niphates cf. *nitida* Fromont, 1993	Z1377, 1378	-	+			X		
Gelliodes cf. *obtusa* Hentschel, 1912	Z1250, 1251, 1252, 1253	+++	+			X		
Aka sp. 1	Z1263, 1264	+	+	X				

Table 4 *cont.*

Callyspongia sp. 1	Z1385	+	-				X		
Callyspongia sp. 2	Z1386	+	-				X		
Callyspongia sp. 3	Z1387	-	+				X		
Callyspongia sp. 4	Z1382	+	-				X		
Petrosia cf. *cancellata* Thiele, 1903	Z1388	-	+				X		
Strongylophora cf. *strongylata* (Thiele, 1903)	Z1389	-	+	X					
Spongia sp.	Z15	-	+		X				
Psammocinia sp.	Z1262	+	-				X		
Dysidea sp.	Z1299	+	-				X		
Spongionella sp.	Z1390	+	-					X	
Dendrilla sp.	Z1300	+	-				X		
Pseudoceratina sp.	Z1192	+	-	X					

confirmation by comparison with type material, and 35 are probably new (Table 4).

Of the 28 known species reported from the Abrolhos in this study 15 (13%) had previously been reported from this locality. Two of these, *Ancorina brevidens* Dendy & Frederick (1924) and *Megalopastas arenifibrosa* Dendy & Frederick (1924), have since been synonymised with more widespread species, *A. acervus* (Bowerbank, 1862) and *Lendenfeldia plicata* (Esper, 1806) respectively, by Hooper & Wiedenmayer (1994). Three species are apparent endemics for the Abrolhos: *Cymbastela marshae* Hooper & Bergquist (1992), *Halichondria phakelloides* Dendy & Frederick (1924) and *Phorbas fictitioides* Dendy & Frederick (1924). For 2 species, *Plakinastrella minor* (Dendy, 1916) and *Zyzza massalis* (Dendy, 1922), the Abrolhos is so far their only Australian locality (Hooper & Krasochin, 1989 & this study). Two species are new records for WA; *Haliclona amboinensis* (Lévi, 1961) and *H. cymaeformis* (Esper, 1791).

A further 14 species are known in the literature, but either some of their taxonomic characters were significantly different from published descriptions, or conspecificity would have produced highly disjunct distributions. In both these cases examination of type material is required to confirm identities, this has not yet been possible. These species are presently prefixed with 'cf' (Table 4). Thirty five species could only be identified to genus and are probably new (Table 4). Generic distributions are presented as per Van Soest (1994) and in the case of the 14 unconfirmed identifications, the known distribution of these species as reported in the literature.

Three of the genera reported here represent new records for Australia. *Anthosigmella* has been previously reported only from the Carribbean (see Wiedenmayer, 1977), *Guitarra* from South Africa (Lévi, 1963), New Zealand (Brondsted, 1924; Dendy, 1924; Bergquist & Fromont, 1988), and E Pacific coast (Desqueyroux Faundez & Van Soest, 1997) and *Liosina* from Papua New Guinea (Kelly Borges & Bergquist, 1988) and Bawi Island, Zanzibar, Tanzania (Kelly-Borges, 1998).

DISCUSSION

This study increases the total number of demosponge species reported from the Abrolhos from 57 to 109. This number of species is likely to represent only a small proportion of the total sponge fauna of these islands considering only two islands in two of the four island groups were surveyed; none of the algal-dominated areas have yet been visited; and depths were restricted to less than 18 and 25m respectively. A similar style of sponge collection, but with a much larger number of sampling sites, was undertaken on the NW. Australian oceanic reefs of Ashmore, Cartier and Hibernia, from which 138 species were reported (Hooper, 1994). The 109 species so far reported from the Abrolhos therefore indicates there is a very rich sponge fauna around these islands.

Fourteen of the 77 species collected during this study were common to both sites, but 40 (52%) of the remainder of species occurred only at Rat I. and 23 species (30%) at Beacon I. These differences in species compositions may indicate fundamental differences between the islands in a proportion of their sponge biota. For example, the 4 island groups are separated by channels of approximately 40m depth which may restrict movement between island groups of gametes of some species. It is also possible that there are

TABLE 5. Species previously described from the Abrolhos but not recollected during this study. * original species name that has since been synonymised with the species name given in the table (Hooper & Weidenmayer, 1994); # may be a species introduced via shipping. Localities: NA = N Australia, SA = S Australia, IPM = Indo Pacific/Malay, IO = Indian Ocean, NWA = NW Australia, WA = W Australia, GBR = Great Barrier Reef, Qld = Queensland, NSW = New South Wales, NT = Northern Territory, Vic = Victoria, Tas = Tasmania, Abrolh = Abrolhos, NZ = New Zealand, Sth Afr = South Africa, Subant = Subantarctic.

Species	NA	SA	IPM	IO	Other areas
Stelletta debilis Thiele, 1900			X		Abrol
Stelletta sigmatriaena Lendenfeld, 1907	NWA				
Ancorina australienesis (Carter, 1883)		WA			
Rhabdastrella rowi (Dendy, 1916)				X	Abrol
Asteropus simplex (Carter, 1879)	Qld, NWA	WA, Vic	X		NZ, Easter I
Erylus proximus Dendy, 1916				X	Abrol
Tethya robusta (Bowerbank, 1859)	Qld	WA	X	X	Red Sea
**Xestospongia similis* (Ridley & Dendy, 1886)	NT	WA, NSW	X	X	Subant
**Callyspongia mollis* (Lendenfeld, 1887)		NSW			Abrol
**Callyspongia ramosa* (Gray, 1843)	Qld	Tas, NSW, Vic	X		NZ, Subant
Oceanapia abrolhosensis (Dendy & Frederick, 1924)					Abrol.
**Mycale parasitica* (Carter, 1885)		TAS, NSW, Vic		X	
Mycale trichophora (Dendy & Frederick, 1924)					Abrol
#**Mycale parishi* (Bowerbank, 1875)	NT, NWA	WA, NSW	X	X	Sth. Afr
Biemna tubulata (Dendy, 1905)				X	Abrol
Waldoschmittia schmidti (Ridley, 1884)		WA, Tas, NSW, Vic	X	X	
Holopsamma crassa Carter, 1885		Tas, NSW, Vic			
Dysidea dakini (Dendy & Frederick, 1924)					Abrol
Hyatella intestinalis (Lamarck, 1814)	GBR, NWA	WA,	X	X	
**Coscinoderma pesleonis* (Lamarck, 1814)		Vic, Tas, WA			
Echinodictyum nidulus Hentschel, 1911	NWA, NT				
Clathria (Wilsonella) abrolhosensis Hooper, 1996					Abrol
Clathria (Wilsonella) australiensis (Carter, 1885)		NSW			
Clathria (Microciona) grisea (Hentschel, 1911)	NWA				
Clathria (Dendrocia) pyramida Lendenfeld, 1888		NSW			
Clathria (Axociella) patula Hooper, 1996	NWA				
Clathria (Thalysias) aphylla Hooper, 1996					Abrol
Clathria (Thalysias) cancellaria (Lamarck, 1814)	NWA				
Clathria (Thalysias) styloprothesis Hooper, 1996		WA			
Antho (Antho) tuberosa (Hentschel, 1911)	NWA			X	
Holopsamma arborea (Lendenfeld, 1888)	NWA	WA, NSW			
Caulospongia plicata Saville Kent, 1871	NWA				

significant microhabitat differences between these islands, thus influencing species composition of each island (cf. Hooper, 1994), but this has not been investigated.

One of the aims of this study was to determine if the sponges of the Abrolhos were principally tropical or temperate in origin. For this reason a list of species previously recorded from the Abrolhos, but not recollected during this study, is included (Table 5). Inclusion of this dataset (Table 5) brings the number of species known to be endemic to the Abrolhos to a total of 8, 3 recollected during this study (noted above) and 5 others: *Mycale trichophora* Dendy & Frederick (1924), *Dysidea dakini* Dendy & Frederick (1924), *Oceanapia abrolhosensis* (Dendy & Frederick, 1924), *Clathria (Wilsonella) abrolhosensis* Hooper (1996), and *C. (Thalysias) aphylla* Hooper (1996). Whether these species are true endemics to the islands, or more widely distributed but not yet reported, will not be

TABLE 6. Proportion of tropical, temperate and endemic species of sponges occurring at the Abrolhos.

No. of species	Trop.	Trop. & Temp.	Temp.	Endemic Abrol	Endemic WA
In this study	9	5	11	3	6
Reported in previous studies	8	7	12	5	7
Total	17 (28%)	12 (20%)	23 (38%)	8 (13%)	13

known until further work is undertaken in adjacent temperate and tropical localities in WA.

In addressing tropical and temperate origins of species, those identified only to genus, or with unconfirmed identifications, were excluded (Table 4). The majority of known species (Tables 3, 5) are of temperate origin. Twenty-three species (38%) are known from temperate waters, 17 species (28%) are tropical and 12 (20%) have a more widespread tropical and temperate distribution. Thirteen species are only known so far from WA, and appear to be endemic to the State. Their distribution as either temperate or tropical, or both, is incorporated into these categories in Table 6.

This biogeographic analysis of sponges of the Abrolhos is considered preliminary given that a large component of the fauna is presently excluded from the assessment. However, these data on proportions of temperate versus tropical species are in marked contrast to other marine biota reported from these islands. For most phyla there is a greater component of tropical than temperate species in the fauna (Table 7); echinoderms, molluscs and fishes have similar proportions of tropical versus temperate species. In contrast, sponges have a greater temperate component; amongst other phyla only seagrasses show a temperate species dominance. Should this apparent dominance of temperate sponge species be eventually confirmed, it may be the result of: 1) The reproductive biology of the sponges, whereby some species are known to have benthic larvae which may not have the temporal capability to survive a migration on the Leeuwin current. This would reduce the proportion of tropical species able to recruit to the islands. 2) The Leeuwin current is known to have been in existence since the Eocene (40 m.y.a.), and has continued to occur in pulses since this time. Periods when the current has not flowed may have allowed for recruitment of temperate species from the adjacent coastline.

In summary, this study doubles the number of demosponges reported from the Abrolhos. First indications are that the sponge fauna is relatively species rich, with a larger number of temperate than tropical species. Much work remains to fully document the fauna, including in the North and Pelsaert Island groups, algal dominated reefs, and greater depths than sampled here.

Until the sponge fauna of localities both N and S of the Abrolhos, and on the adjacent W coast of Australia are better documented, this work remains a study in isolation. Consequently, conclusions on species endemicity remain tentative, and the affinities of the undescribed component of the fauna are not presently known.

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TABLE 7. Proportions of tropical and temperate components of marine phyla studied at the Abrolhos. * total number of different species, including 49 as yet unnamed and 60 named.

Phylum	Tropical (%)	Subtropical (%)	Temperate (%)	Temp. & Trop. (%)	Endemic Abrol (%)	Endemic WA (%)	Total species
Porifera (Demospongiae)	28		39	20	13		60 (109*)
Echinoderms (Marsh, 1994)	64		15			21	172
Molluscs (Wells & Bryce, 1997)	69		20			11	492
Fish (Hutchins, 1997)	67	13	20				389
Seagrasses (Brearley, 1997)	30		70				10

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SPONGE CELL ADHESION: AN EVOLUTIONARY ANCESTOR OF HISTOCOMPATIBILITY SYSTEMS ? *Memoirs of the Queensland Museum 44: 184. 1999*:- Sponges have been traditionally used as models to study cell adhesion because their rather loose and porous extracellular matrix allows a mild cell dissociation and the recovery of intercellular components in virtually native state. Species-specific cell recognition and adhesion in sponges is mediated by extracellular proteoglycan-like complexes termed aggregation factors (AFs), still not identified in higher animals. Polyvalent glycosaminoglycan interactions are involved in the species-specificity, representing one of the few known examples of a regulatory role for carbohydrates.

A surprising characteristic of sponges, considering their low phylogenetic position, is that they possess an exquisitely sophisticated histocompatibility system. Any grafting between two different sponge individuals is almost invariably incompatible in the many species investigated, exhibiting a variety of transitive qualitatively and quantitatively different responses, which can only be explained by the existence of a highly polymorphic gene system regulating sponge allogeneic reactions. The development of variable-region molecules is thought to have been a crucial event in the evolution of primordial vertebrate immune systems, followed by gene rearrangement to provide more diversity. Early in the evolution of the immune system, then, a gene must have duplicated to allow such diversity to arise. Unfortunately, there is an absolute lack of protein sequence information concerning the molecules involved in invertebrate histoincompatibility reactions. Recently, we deduced from cDNA the sequence of the aggregation factor core protein from the red beard sponge, *Microciona prolifera*, and Southern blot analysis suggested the existence of several related genes.

We have screened individual sponge cDNA libraries, identifying multiple related forms for the AF core protein (MAFp3). Northern blots show the presence in several human tissues of transcripts strongly binding a MAFp3-specific probe. We have studied tissue histocompatibility within a sponge population, finding 100% correlation between rejection behaviour and the individual-specific restriction fragment length polymorphism pattern using AF-related probes. PCR amplifications with specific primers showed that at least some of the MAFp3 forms are allelic and distribute in the population used. A pronounced polymorphism is also observed when analysing purified AF in polyacrylamide gels. Protease digestion of the polymorphic glycosaminoglycan-containing bands indicates that glycans are also responsible for the variability. The data presented reveal a high polymorphism of aggregation factor components which matches the elevated sponge alloincompatibility, suggesting an involvement of the cell adhesion system in sponge allogeneic reactions. Our present work will be discussed in the context of the evolution of histocompatibility systems and their possible divergence from primitive cell-cell interaction molecules. ❐ *Porifera, graft rejection, proteoglycans, invertebrate immunity, aggregation factors, cell adhesion, porifera genes, cDNA, histocompatibility.*

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REPRODUCTION OF SOME DEMOSPONGES IN A TEMPERATE AUSTRALIAN SHALLOW WATER HABITAT

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Species of *Tethya, Chondrilla, Mycale* and *Echinodictyum* were monitored for two years at South Mole, Fremantle, Western Australia (32°04'S, 115°45'E) to determine onset of reproductive activity, sex phenotype, and reproductive mode. Most reproductive activity occurred from late spring through summer and autumn (November-April). Most species appear to be gonochoric with both ovipary and vivipary recorded. Details of their reproductive development is reported and discussed in relation to sea temperature data. ❐ *Porifera, Demosponges, Fremantle, Western Australia, reproduction.*

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The only previous study on the reproductive biology of temperate Australian marine demosponges (Hoskins, 1992), found low numbers of oocytes present between March and September in populations of *Phyllospongia* sp. from Rottnest Island, Western Australia (WA). This lack of basic biological information for this region is surprising given that sponges are a dominant component of the sessile fauna in these temperate marine habitats. Consequently, the present study aimed to collect baseline reproductive data for some of the more common species of demosponges in this region, examining species of *Echinodictyum, Mycale, Haliclona, Coelosphaera, Tethya* and *Chondrilla.* Both *Tethya* and *Chondrilla* have two distinctive colour morphs at the study site, possibly indicating sympatric species, so these colour morphs were monitored as separate populations.

Several studies undertaken in Europe and USA have described reproductive characteristics for temperate species belonging to some of the genera examined here. For instance, Elvin (1976) reported on the reproductive biology of *Haliclona permollis* in Oregon; Fell (1976) on *H. loosanoffi* in Connecticut; and Wapstra & Van Soest (1987) on *H. oculata* and *H. xena* in Holland. *Mycale micracanthoxea* from Holland (Wapstra & Van Soest, 1987) is the only temperate species of *Mycale* previously examined. Temperate populations of *Chondrilla nucula, Tethya aurantium* and *T. citrina* were studied by Liaci (1971a, b), whereas there are no published studies on the reproductive biology of *Coelosphaera* or *Echinodictyum*.

Increasing temperature is generally accepted as a major environmental factor regulating the onset of reproductive activity in sponges occurring in regions of large seasonal change (Fell, 1983; Simpson, 1984). Only four species are presently known where gametogenesis is associated with a decrease in temperature: *Halisarca dujardini* (Lévi, 1956; Chen, 1976) *Desmacidon fructicosum* (Lévi, 1956), *Tethya crypta* and *Aplysina gigantea* (Reiswig, 1973). In this study seasonal reproductive activity is discussed in relation to sea temperature data, and results are presented on the mode of reproduction and sexual phenotype of the species examined, estimates of development time of gametes, and timing of product release.

MATERIALS AND METHODS

The six species of sponges investigated here live subtidally on the ocean side of South Mole, an artificial groyne that forms the southern flank of the entrance to Fremantle Harbour (32°04'S, 115°45'E). All six species at this site ranged in abundance from common to abundant (i.e. ≥10 specimens of each species seen during a dive of 1 hour duration). Sponges were prolific from 4-7.5m depth, occurring between the shallow seaweed (*Ecklonia*) fringe found at 0-4m depth and the sand flat with seagrass at the base of the groyne.

Sampling was conducted over two years from October 1996 to April 1998. Sampling ceased in

TABLE 1. Reproductive activity of the species studied at South Mole. Key: -, not sampled; NR, not reproductive; X, not found in the field; O, oocytes; E, embryos; L, larvae; S, sperm; (sample size).

Species (year of survey)	Month								
	A	S	O	N	D	J	F	M	A
Echinodictyum clathrioides (96-97)	- (0)	- (0)	NR (3)	- (0)	NR (2)	NR (3)	NR (4)	NR (2)	X (0)
Echinodictyum clathrioides (97-98)	X (0)	- (0)	NR (4)	NR (5)	NR (3)	NR (4)	O (4)	NR (3)	NR (4)
Coelosphaera sp. (96-97)	- (0)	- (0)	NR (2)	- (0)	NR (2)	NR (2)	NR (6)	NR (2)	OE (2)
Coelosphaera sp. (97-98)	NR (2)	- (0)	NR (3)	NR (3)	NR (3)	NR (3)	NR (3)	NR (3)	S (3)
Haliclona sp. (96-97)	- (0)	- (0)	X (0)	- (0)	NR (4)	NR (6)	X (0)	X (0)	NR (2)
Haliclona sp. (97-98)	EL (2)	- (0)	NR (2)	NR (4)	NR (4)	NR (4)	NR (3)	O (3)	S (4)
Mycale sp. (96-97)	- (0)	- (0)	NR (2)	- (0)	OELS (5)	ES (7)	NR (3)	NR (2)	NR (2)
Mycale sp. (97-98)	NR (2)	- (0)	NR (4)	S (6)	ELS (3)	ELS (5)	NR (4)	NR (2)	NR (4)
Chondrilla australiensis (ochre morph) (96-97)	- (0)	- (0)	NR (2)	- (0)	NR (2)	NR (5)	O (4)	NR (5)	NR (3)
Chondrilla australiensis (ochre morph) (97-98)	NR (2)	- (0)	NR (4)	NR (9)	NR (7)	NR (7)	O (6)	NR (2)	NR (2)
Chondrilla australiensis (maroon morph) (96-97)	- (0)	- (0)	NR (2)	- (0)	NR (4)	NR (2)	O (3)	X (0)	NR (4)
Chondrilla australiensis (maroon morph) (97-98)	NR (2)	- (0)	NR (2)	NR (2)	NR (2)	NR (4)	O (4)	NR (3)	NR (7)
Tethya sp. (pink morph) (96-97)	- (0)	- (0)	NR (2)	- (0)	X (0)	O (2)	O (4)	X (0)	NR (2)
Tethya sp. (pink morph) (97-98)	X (0)	- (0)	X (0)	NR (4)	O (10)	NR (9)	O (7)	O (9)	NR (8)
Tethya sp. (orange morph) (96-97)	- (0)	- (0)	X (0)	- (0)	NR (4)	O (4)	O (4)	O (5)	NR (2)
Tethya sp. (orange morph) (97-98)	NR (4)	- (0)	NR (7)	NR (5)	O (8)	O (6)	O (8)	O (10)	NR (9)

the winter months of May, June and July 1997 and no sampling was possible in November 1996 and September 1997 due to bad weather and sea conditions. From October 1996 to April 1997 monthly samples of random individuals of each species were collected. With the resumption of sampling in August 1997 two regimes were adopted: sampling of random individuals as for the previous season, and sampling of known individuals of each species to monitor for sequential hermaphroditism. Two different techniques were used. Specimens of *Tethya* were sampled with a 0.5mm diameter cork borer, and ramose branching, encrusting, massive and fan shaped species had a small piece incised from them with a scalpel. Numbers of specimens of each species that were collected and examined by light microscopy are indicated in Table 1.

After collection, individual samples were placed in labelled glass vials and on return to the laboratory were fixed in a gonad fixative, FAACC (100ml = 10ml 37-40% formaldehyde solution: 5ml glacial acetic acid: 1.3gm calcium chloride dihydrate: 85ml tap water) for ≤48 hours, and then transferred to 75% ethanol. Sections cut at 8µm were stained with haematoxylin-eosin, mounted, and surveyed by light microscope for presence and development of eggs and sperm.

Average sizes of gametes were calculated, using an ocular micrometer, by measuring gametes from each gravid individual for each sampling period. To decrease sampling variation only oocytes sectioned through the nucleus, and sperm cysts sectioned through the midline, were measured.

Temperatures at 7m depth adjacent to the Fisheries Western Australia Marine Research Laboratories at Waterman were recorded twice daily (M. Rossbach, Fisheries Western Australia, pers.comm.). Monthly averages of this data, supplied by Fisheries WA, were calculated for the sampling months outlined above.

RESULTS

1. *Echinodictyum clathrioides* Hentschel, 1911 (Poecilosclerida: Raspailiidae). Adults of *E. clathrioides* are erect, fan shaped individuals that are at least 30cm in diameter. Female gametes were only found in specimens collected in February 1998, when oocytes were 45μm diameter (Fig. 1A). There were no oocytes in either January or March suggesting rapid oocyte development and release of products (Table 1). Small sponges ≤8cm diameter were found in April 1998.

2. *Coelosphaera* sp. (Poecilosclerida: Coelosphaeridae). Individuals of this species are rounded mounds with apical oscules and are bright orange alive. Reproductive products were found in April of both sampling seasons (Table 1). Oocytes and embryos (Fig. 1B) were present in April 1997 and sperm in April 1998 implying that reproductive development of embryos and larvae occurs in autumn and possibly winter.

3. *Haliclona* sp. (Haplosclerida: Chalinidae) is a maroon, ramose branching sponge with apical oscules. Specimens of this species rarely had reproductive products during this sampling program. Large embryos and larvae (Fig. 1C) were present in August 1997, and developing oocytes were found in March and sperm in April 1998 (Table 1). Presence of gametes at this time suggest that this species is reproductively active throughout winter. This species is viviparous and individuals are either gonochoric or successive hermaphrodites.

4. *Mycale* sp. (Poecilosclerida: Mycalidae). This species exudes large amounts of mucous upon collection. In the field the sponge has short, thick erect lobes with prominent conules, and is irridescent mauve or vivid blue. Sampling of the mesohyl of this species for reproductive products was difficult as most of the mesohyl oozed away prior to the sponge being placed in the collection vial, and the mesohyl that remained was detached from the skeleton prior to fixation. However, this species has particularly obvious and abundant female gametes visible in the field as orange spheres of about 2mm diameter. Because of the mucous mesohyl, few of these products were successfully sectioned. Female gametes were found in the field during December and January in 1997 and 1998, and were absent in February of both years (Table 1). Sperm cysts were found interspersed amongst embryos and larvae (Fig. 1F). Reproductive activity occurred for a minimum period of 62 days from first development noted in November to the last date when gametes were present in January. This species is viviparous and contemporaneously hermaphroditic.

Two colour morphs were observed in the remaining two genera examined, *Tethya* and *Chondrilla*. Consequently, replicate specimens of each morph were monitored separately to determine if reproductive timing or sex determination differed between them.

5. *Chondrilla australiensis* Carter, 1873 (Hadromerida: Chondrillidae). Specimens were either ochre to brown or maroon. The ochre colour morph was the dominant morph at the study site with extensive mats, up to 1m across, living in full light. Although both morphs tended to occur either in full light or shade under *Ecklonia*, the maroon morph occurred more frequently in shaded areas. Few reproductive products were found in either morphotype. Individuals were found with oocytes in late February 1997 (Table 1). No products were seen in the next sampling in late March. Oocytes measuring 30-40μm were abundant in February 1998 and had cellular extensions between the mesohyl and the oocytes (Fig. 1E). Oocyte development in this species is rapid with 34 days elapsing between the January sampling (when no female products were visible) and the February sampling (when oocytes were 30-40μm). No oocytes were present in the March sampling 21 days later. No sperm were seen in either sampling year. It is assumed therefore that spawning occurs in late February or early March, approximately 2-4 weeks earlier than in *Tethya*. These sponges are oviparous and probably gonochoric. Asexual reproduction by fragmentation appeared to be occurring in *C. australiensis* in April 1998, whereby elongated tear shaped droplets of sponge tissue were found extending from the edges of some of the adults. The tissue was thinnest at the point of attachment to the adult sponge and thickest at the furthest edge. It is likely that these droplets would detach from the adult and settle on the substrate beneath.

6. *Tethya* sp. (Hadromerida: Tethyidae). Specimens were either pink or orange and individuals of both colour morphs had numerous oocytes in February and March of 1997 and 1998. Oocyte development was first detected in early December when oocytes were 10-12μm in diameter. Ninety eight days later, in early March, oocytes were 50-70μm in diameter (Fig. 1D).

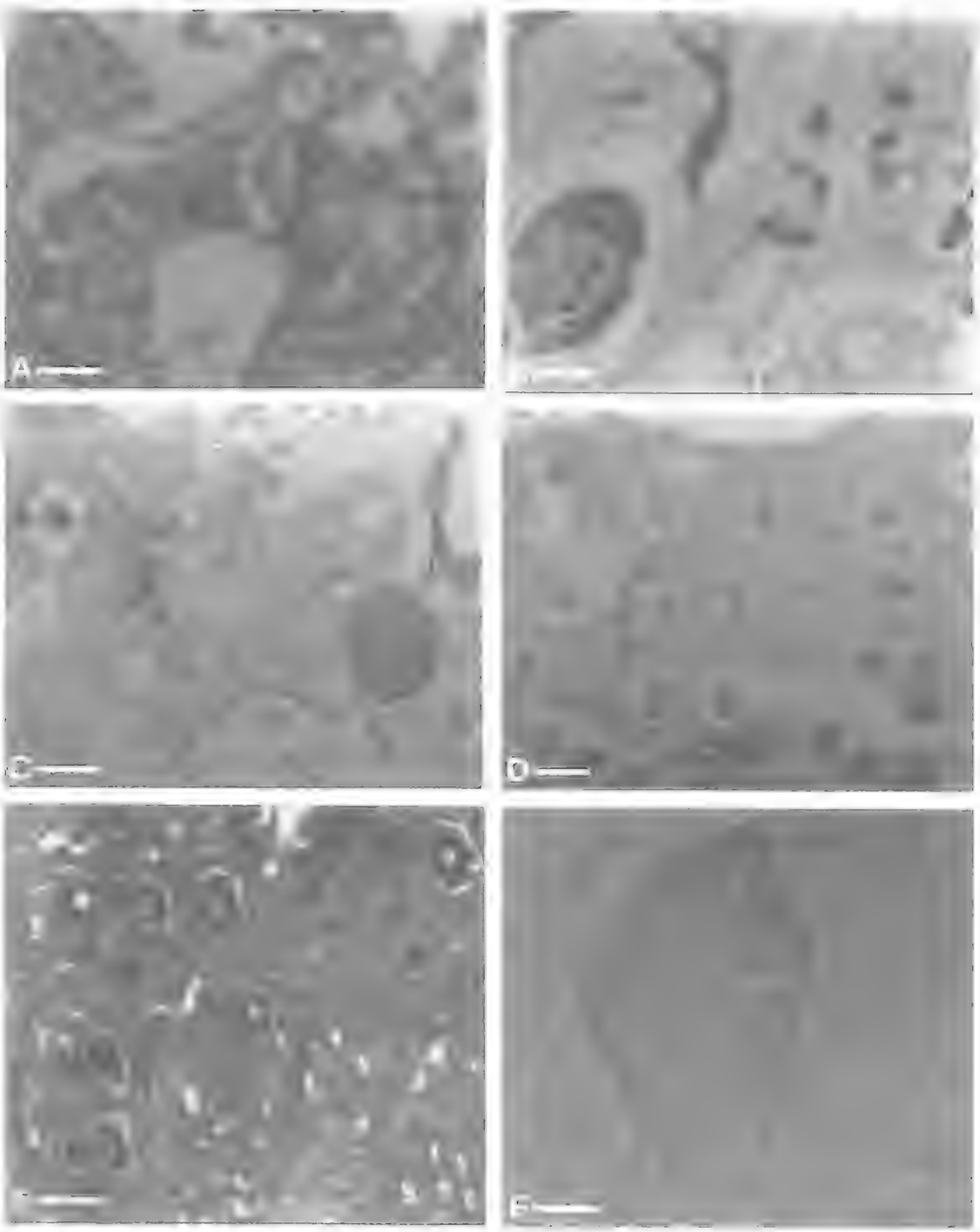

FIG. 1. Reproductive products of species examined in this study. A, Oocytes in *Echinodictyum clathrioides* 17/2/98. B, Embryo and oocytes in *Coelosphaera* sp. 29/4/97. C, Larvae and embryo in *Haliclona* sp. 26/8/97. D, Oocytes in *Tethya* sp. 25/3/97. E, Oocytes in *Chondrilla australiensis* 17/2/98. F, Larvae and sperm cysts (←) in *Mycale* sp. 20/12/96 (scale bar: E = 50μm, A-D, F = 100μm).

TABLE 2. Developmental mode of some sponge species in Western Australia. Abbreviations: X = developmental mode, ? = suspected developmental mode but awaits confirmation

Species	Oviparity	Vivipary	Gonochorism	Contemporaneous hermaphroditism	Successive hermaphroditism
Tethya sp.	X		?		
Chondrilla australiensis	X		?		
Echinodictyum clathrioides	?		?		
Mycale sp.		X		X	

There were no oocytes in samples taken at the next sampling period in April, either in 1997 or 1998 (Table 1). It is therefore likely that spawning occurs in mid to late March. No sperm were found in either sampling years. These sponges are oviparous and probably gonochoric. Thin filaments were seen extending from one individual of *Tethya sp.* in April 1998. This individual had been sexually reproductive with oocytes visible in March.

In summary, two types of reproductive mode were observed amongst these six species: ovipary in *Tethya* sp. and *Chondrilla australiensis*, and vivipary in *Mycale* sp., *Coelosphaera* sp. and *Haliclona* sp. (Table 2). Only one sex phenotype was determined, contemporaneous hermaphroditism was found in *Mycale* sp.

Two opposing trends are apparent when sea temperatures are compared with timing of reproductive activity (Fig. 2). In *Tethya* sp., *Chondrilla australiensis, Mycale* sp. and probably *Echinodictyum clathrioides* reproductive activity occurs in late spring or summer when sea temperatures are increasing or reaching a maximum. Conversely, in *Coelosphaera* sp. and *Haliclona* sp. reproductive activity occurs in autumn when sea temperatures are falling.

DISCUSSION

REPRODUCTIVE BIOLOGY. Sponges can be either gonochoric or hermaphroditic, oviparous or viviparous. In Haplosclerida both gonochorism and hermaphroditism have been reported, although all species examined to date have been viviparous. Tropical species of *Haliclona* from the Great Barrier Reef (*H. amboinensis* and *H. cymaeformis*; Fromont, 1994), and temperate intertidal species from the Oregon coast (*H. permollis*; Elvin, 1976), and from a Connecticut estuary (*H. loosanoffi*; Fell, 1976) are all viviparous and gonochoric. However, two species from a temperate European locality were reported as viviparous with contemporaneous hermaphroditism (Wapstra & Van Soest, 1987). In this study, *Haliclona* sp. is viviparous but is not contemporaneously hermaphroditic. This species is either gonochoric or a successive hermaphrodite.

Ilan & Loya (1990) reported finding aggregations of female reproductive products in species of the haplosclerid families Chalinidae and Niphatidae, and suggest these brooding chambers may be common amongst haplosclerids. In this study *Haliclona* sp. did not have brood chambers but had reproductive products aligned along the midline of the branches.

Mycale sp. conformed to previous reports of sex determination and reproductive mode for this genus, being viviparous and hermaphroditic. The temperate European species, *Mycale micracanthoxea*, the tropical Red Sea species, *M. fistulifera*, and *Mycale* sp. reported here are all viviparous and contemporaneous hermaphrodites (Wapstra & Van Soest, 1987, Meroz & Ilan, 1995, Reiswig, 1973).

There are no previous reports on mode of reproduction or sex determination in species of the genus *Coelosphaera* or *Echinodictyum*.

Chondrilla australiensis from South Mole was oviparous and probably gonochoric conforming to published reports of the reproductive biology of this genus. *Chondrilla nucula* from Italy has been reported to be oviparous and gonochoric (Liaci et al., 1971a).

Sponges possess high regenerative capacities and have been reported to reproduce asexually by budding, fragmentation and gemmulation (Fell, 1993). Fragmentation is possible because of the structural homogeneity and morphological flexibility of sponges so that even small fragments are likely to possess all essential functional elements and can readily reorganise to function as independent entities (Wulff, 1991). The droplets of tissue I found extending from the edges of some adults of *C. australiensis* in April 1998 appear to be a form of asexual reproduction through fragmentation.

In *Chondrilla nucula* cellular extensions between the mesohyl and oocytes are described as long thin filipodia connecting the nurse cells surrounding the developing eggs to the egg

A.

Species	Month								
	A	S	O	N	D	J	F	M	A
Tethya sp.					X	X	X	X	
Chondrilla australiensis							X		
Echinodictyum clathrioides							X		
Mycale sp.				X	X	X			
Coelosphaera sp.									X
Haliclona sp.	X							X	X

B.

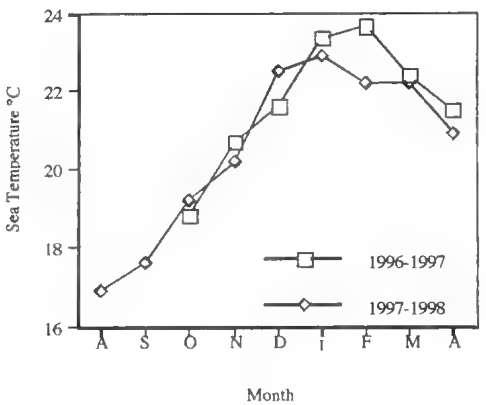

FIG. 2. Reproductive activity and sea temperature. A, Timing of reproductive activity. X = months when the species had reproductive products. B, Average monthly sea temperatures.

surfaces, and Liaci et al. (1971a) suggested that these connections allow a direct, nonphagocytic transfer of nutrients. Similarly, the cellular extensions I observed surrounding eggs in *Chondrilla australiensis* are likely to have the same function.

Tethya sp. individuals were oviparous and probably gonochoric, conforming to previous reports in the literature for both tropical species *T. crypta* (Reiswig, 1973), and temperate species *T. aurantium* and *T. citrina* (Liaci et al., 1971b).

Asexual budding has been reported for species of *Tethya.* Thin filaments containing spicules extend outwards from the adult and a spherical bud forms distally. This bud detaches from the adult and can attach to the substratum (Simpson, 1984). Thin filaments were seen extending from one individual of *Tethya* sp. in April 1998 but distal buds were not apparent at this time.

COLOUR VARIATION WITHIN SPECIES. *Chondrilla australiensis* and *Tethya* sp. each had two distinctive colour morphotypes at the study site. In *C. australiensis* the usual occurrence of the maroon morph in shaded or cave habitats suggests that its colour difference to the ochre morph, growing in full light, may be a response to reduced light conditions. The Northern hemisphere species *C. nucula* usually colonises illuminated bottoms and is generally brownish (Gaino et al., 1976). Arillo et al. (1993) found that its colouration is a consequence of the presence of the cyanobacteria *Aphanocapsa* sp. Similarly, it is speculated that colour differences between morphs at South Mole could be the result of either different cyanobacterial symbionts within the two morphs, or differing abundances of the symbionts in the two morphs. Individuals of both morphs occurred side by side with marked non-overlap zones between them. These zones were also common between specimens of the same colour morph, suggesting the occurrence of different genotypes within the same colour morphs, and that sexual reproduction is occurring to some extent in the population.

In *Tethya* sp. individuals of each colour morph occurred side by side in full light and under *Ecklonia.* Therefore, colour differences between the morphs cannot be attributed to differences in light regimes. The coexistence of more than one species in a restricted area has been found previously in the genus *Tethya* (Sarà et al., 1993), and similar analyses of genetic data and niche differentiation of the *Tethya* species at South Mole may find these colour morphs to also be distinctive at the species level.

TIMING OF REPRODUCTIVE DEVELOPMENT. The timing of reproductive activity in sponges has previously been related to sea temperature, with many species found to

initiate activity as sea temperatures increase (Simpson, 1984 and references therein). Fewer studies have found sponges to be reproductively active as temperatures fall or are at a minimum. In the present study most sponge species were reproductive as sea temperatures were increasing or reaching their summer maximum (i.e. *Tethya* sp., *Chondrilla australiensis*, *Mycale* sp. and possibly *Echinodictyum clathrioides*). At this stage there is not enough information about the reproductive activity of *E. clathrioides* to say with certainty when release of reproductive products occurs. In contrast, two species commenced reproductive activity as sea temperatures were decreasing (i.e. *Coelosphaera* sp. and *Haliclona* sp.). *Haliclona* sp. appears to develop embryos and larvae throughout the winter.

Light regimes are another environmental factor that could influence onset of reproductive activity in sponges. Elvin (1976) found that initiation of oogenesis in the temperate intertidal species *Haliclona permollis* was most closely related to an increase in incident light. Ilan & Loya (1990) speculated that the reproductive activity of *Niphates* sp. may be related to the seasonal disappearance of algae, thereby increasing incident light to the sponges. At South Mole, biomass of the *Ecklonia* fringe appears to increase during the summer months when the photoperiod has increased, and may therefore increase shading of sponges.

A third exogenous factor implicated in the timing of reproductive activity is food availability (Sarà, 1992). The occurrence of two different periods of reproductive activity of sponges at South Mole may coincide with two peaks in the abundance of ultraplankton (A. Pile, Flinders University of South Australia, pers.comm.), one peak occurring in late summer/autumn when most of the species release their products, and a second peak in spring when reproductive products are released by *Haliclona* sp.

Two explanations are possible to explain these opposing trends in timing of reproductive activity: 1) species are responding to different environmental cues which trigger initiation of reproductive activity, or 2) species are responding differently to the same environmental cues.

SUMMARY

This study shows that modes of reproduction in sponges from South Mole, southern WA, conform to modes already documented in the literature for these respective genera. More work is required to unequivocally determine sex phenotype of these species, but preliminary data indicate that this aspect of their reproductive biology also appears to conform to the majority of reports in the literature. Reproductive activity in two of the species in autumn and winter is unusual, and possible reasons for this require further investigation. For the present, a baseline has been established in the timing of activity which will support future investigations on many other aspects of the reproductive biology of these species. Larval biology, diurnal timing of spawning for species that broadcast their products, and analysis of the partitioning of resources in species known to have both sexual and asexual reproduction, would all be useful studies. Species with colour morphs should be examined using genetic methods, or monitored for reproductive isolating mechanisms, to establish whether they are distinct species occurring sympatrically at the study site or if some other factors are responsible for their observed differences.

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MEMBRANE-BOUNDED NUCLEAR BODIES IN A DIVERSE RANGE OF MICROBIAL SYMBIONTS OF GREAT BARRIER REEF SPONGES

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Fuerst, J.A., Webb, R I., Garson, M.J., Hardy, L. & Reiswig, H.M. 1999 06 30: Membrane-bounded nuclear bodies in a diverse range of microbial symbionts of Great Barrier Reef sponges. *Memoirs of the Queensland Museum* **44**: 193-203. Brisbane. ISSN 0079-8835.

Thin sections of chemically fixed tissue of several sponge species collected from Heron Island, Great Barrier Reef, including *Jaspis stellifera, Pseudoceratina crassa* and *Axinyssa* sp., were examined to investigate the cell organisation of bacteria-like microbial symbionts present. Such symbionts have been observed in these sponges to occur as a diverse range of morphotypes based on cell shape and cell wall type. A variety of different symbiont morphotypes were found to possess a membrane-bounded nucleoid, a feature not expected in prokaryotes. These had been previously observed by us in one symbiont morphotype in the Micronesian coralline sponges *Stromatospongia micronesica* and *Astrosclera willeyana.* Several distinct microbial morphotypes containing membrane-bounded nuclear bodies were observed in Great Barrier Reef sponges, only one of which resembled the type which we have previously observed in the two Micronesian sponges. In all these forms, the fibrillar nucleoid was surrounded by a single bilayer membrane, in most morphotypes defining a compartment also containing electron-dense particles resembling ribosomes or other nucleoplasmic pre-ribosomal material; such material was sometimes less dense and sometimes more dense than the cytoplasmic particulate material. Cell wall structure of the morphotypes broadly included both Gram-negative, outer membrane-bounded types, as well as a clear subunit S-layer type structure resembling that of known Archaea including crenarcheotes. Cytoplasmic membranes can be clearly seen in some cases as distinct from nuclear body membranes, excluding plasmolysis as an explanation for membrane-boundedness of nuclear bodies. The phylogenetic relationships of these microbes may be diverse if reflecting wall type, but at least some appear to be most likely to represent members of the Domain Archaea, perhaps resembling the crenarcheote *Cenarchaeum symbiosum* described from North American *Axinella* sp. ❐ *Porifera, Bacteria, Archaea, nucleoids, membrane-bounded, sponge symbionts, electron microscopy, ultrastructure.*

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There are 2 known major types of cell organisation, prokaryotic where the DNA of the genome is free in the cytoplasm and not confined to a special compartment, and the eukaryotic, where the genomic DNA is confined to a double membrane-bounded organelle, the nucleus, and in addition any other of several double-membrane-bounded organelles such as mitochondria and chloroplasts may be present (but not in all eukaryotes, e.g., archezoan protozoa such as *Giardia*). In the prokaryote the naked genomic DNA in chemically fixed cells often appears to be folded or condensed into a fibrillar structure and this ultrastructural entity is termed the '*nucleoid*'. The prokaryotic form is characteristic of most known species within two of the three great Domains of life defined by contemporary molecular systematics, the Bacteria and the Archaea, while the eukaryotic form is known so far only within the Domain Eucarya and not in the other two Domains (Woese et al., 1990). Several questions about such a classification of cell organisation can be posed, however. Are these the *only* forms of cell organisation which have evolved, or might there not be intermediate forms or even more complex ones, hitherto undiscovered due to our limited knowledge of biodiversity?

Related to this is a second question- are membrane-bounded nuclei or their analogues

exclusive to the Domain Eucarya, or might they or some analogous form of organelle occur in those two Domains of life thought to harbour only prokaryotic cells?

The first indication that there might be alternative forms of cell organisation to those classical known ones was discovered in a distinct division or phylum of the Bacteria, the planctomycetes (Order *Planctomycetales*), where one species, *Gemmata obscuriglobus*, possesses a genome bounded by two membranes (Fuerst & Webb, 1991) while in another two, *Pirellula marina* and *Pirellula staleyi*, a single membrane separates the compartment containing the genomic DNA from the rest of the cell (Lindsay et al., 1997). We present here evidence that several distinct morphotypes of sponge symbionts (only one of which has been described by us previously; see Fuerst et al., 1998), reveal further examples of structurally novel types of cell organisation in which the genomic DNA appears compartmentalised by a single membrane from the rest of the cell cytoplasm, and that these may occur in microorganisms resembling members of the Domain Archaea, and present new data to support these findings.

MATERIALS AND METHODS

Stromatospongia micronesica and *Astrosclera willeyana* were collected from Guam (Micronesia), and *Pseudoceratina crassa*, *Jaspis stellifera* and *Axinyssa* sp were collected from Heron Island (Great Barrier Reef), at sites previously described in detail (Fuerst et al., 1998). Sponge tissue samples were chemically fixed with glutaraldehyde followed by osmium tetroxide before resin embedding and thin sectioning, via protocols including hydrofluoric acid treatment of either tissue blocks or Epon resin-embedded block faces, and uranyl acetate-lead citrate stained thin sections were examined via transmission electron microscopy, as described previously (Fuerst et al., 1998). For immunolabelling experiments, samples of *Jaspis stellifera* were fixed in 2% glutaraldehyde-4% paraformaldehyde fixative in 0.1M cacodylate buffer, and further processed as described in Fuerst et al. (1998). Immunolabelling of thin sections was via use of an anti-ds+ss DNA antibody (Boehringer-Mannheim) and goat anti-mouse IgM conjugated to either 10nm or 5nm colloidal gold, as described previously (Lindsay et al., 1997), and sections were then stained with uranyl acetate and lead citrate. Labelling of sections to localise RNA using an RNase-colloidal gold (10nm) conjugate was performed essentially as described in Lindsay et al. (1997), followed by staining as above. In cases where double labelling was performed, RNase gold labelling was performed first, followed by anti-DNA gold immunolabelling. Goat anti-mouse IgM conjugated to 5 nm colloidal gold was used for labelling of DNA when double-labelling experiments involving both DNA and RNA labelling were performed.

Voucher samples of *Axinyssa* sp. nov. and *Pseudoceratina crassa* are held at the Queensland Museum as QMG312575 and QMG304915 respectively (identified by Dr John Hooper).

RESULTS AND DISCUSSION

Bacteria-like symbionts of varying morphotype were common in mesohyl of the tissue of the sponge species collected from Heron Island, including *Jaspis*, *Pseudoceratina* and *Axinyssa* spp. (see Fig. 1A). These associates were found to include a diversity of morphotypes displaying a novel form of compartmentation, in which either the fibrillar nucleoid representing the prokaryote chromosomal DNA was surrounded by a single membrane, or , in one type, where an inverse of this compartmentation topology was displayed. In the latter type, a single membrane separates one organelle-like region of the cytoplasm from a second region of cytoplasm containing the nucleoid. The compartmented morphotype in which the nucleoid is bounded by a single membrane has been previously described by us in sponges from Pacific Micronesia, *Stromatospongia micronesica* and *Astrosclera willeyana* (Fuerst et al., 1998). The dramatic distinction of this morphotype from bacteria with a classical prokaryotic 'naked' fibrillar nucleoid, free in the cytoplasm, is seen in Figure 1B of the mesohyl of *S. micronesica*. This first kind of compartmented morphotype, here seen in an actively dividing cell, displays a characteristically 'butterfly' shaped nuclear body surrounded by a single membrane. The cell wall structure is of a regular subunit type consistent with membership of the Domain Archaea (see Fuerst et al., 1998, and the discussion of this wall type in other morphotypes below). This morphotype is typical of all the nucleated symbiont morphotypes, in the consistency of features such as the cell size, the absence of membrane-bounded organelles other than the nuclear body, and cell wall structure,

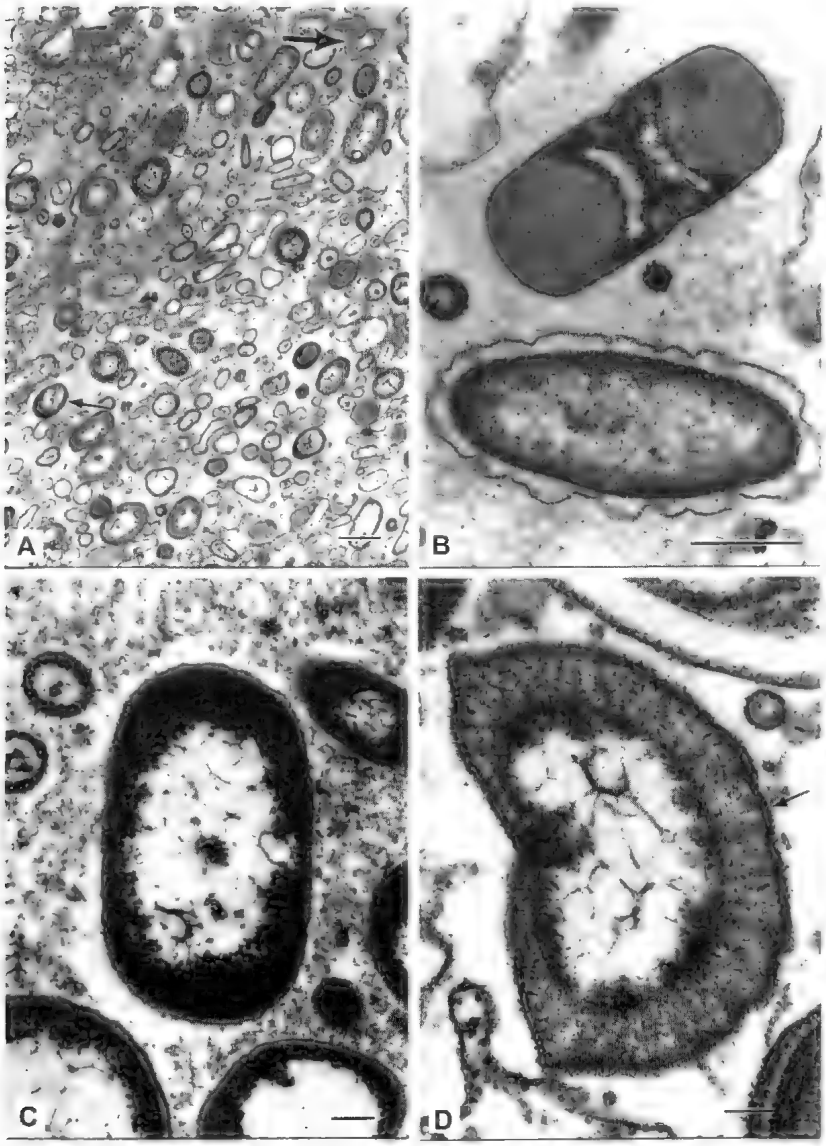

FIG. 1. A, Electron micrograph of thin section of mesohyl from sponge tissue of *Jaspis stellifera* from the Great Barrier Reef, with a diverse range of symbiont morphotypes apparent, based on a combined consideration of cell size, shape and internal structure. For example, the large arrow indicates a Morphotype 1 cell and the small arrow indicates a Morphotype 2 cell. (Scale bar 1 μm). B, Electron micrograph of thin-section of sponge tissue from *Stromatospongia micronesica* showing two symbiont morphotypes of contrasting internal structure. One morphotype (Morphotype 1 in the typing scheme used in this paper), the uppermost cell in this figure, possesses a membrane-bounded nuclear body region in a dividing cell (evidence for active viable cell growth of this type in the tissue) and the second morphotype is a cell with normal bacterial (prokaryotic) ultrastructure with fibrillar nucleoid DNA free in the cytoplasm. Note in the Morphotype 1 cell that the nuclear body in each cell half displays an outer electron dense region as well as a central fibrillar nucleoid region. (Scale bar 1 μm). C, Electron micrograph of thin-sectioned Morphotype 2 (short fat rod) symbiont from *Pseudoceratina crassa* from the Great Barrier Reef; note the membrane-bounded nuclear body and that this type has a relatively electron-dense cytoplasm external to the nuclear body compared with other morphotypes (e.g., Morphotype 1 seen in Fig. 1B). (Scale bar 200nm). D, Electron micrograph of thin-sectioned Morphotype 3 (D-shaped cell) symbiont from *Pseudoceratina crassa*. This D-shaped cell has a clear membrane-bounded nuclear region as well as radiating fibres in the cytoplasm outside the nuclear region. The cell wall displays a regular subunit periodic structure (arrow). (Scale bar 200nm).

with most probable phylogenetic relationships of these symbionts to non-eukaryote organisms such as Bacteria or the Archaea. This morphotype with 'butterfly' nuclear body has now been found also in sponges from the Great Barrier Reef, including *Jaspis stellifera*, *Pseudoceratina crassa* and *Axinyssa* sp. This morphotype has thus now been found to be distributed among at least 5 different sponge genera (*Stromatospongia, Astrosclera, Jaspis, Pseudoceratina* and *Axinyssa*) and in at least two different geographical locations in the Western Pacific. However, not only was this morphotype found in both Great Barrier Reef and Micronesian sponges, but it turns out to constitute only one of several different morphotypes which display membrane-bounded nuclear regions, or at least membrane-bounded compartments, separating the cell interior into a nucleoid- and non-nucleoid-containing region. These compartmented cell symbiont morphotypes could be distinguished from each other on the basis of the following criteria or combinations of such criteria; cell wall structure, cell shape, texture (fine structure) of the cytoplasm outside the nuclear body, and type of cell compartment (e.g. nucleoid-containing versus nucleoid-devoid, or butterfly-lobed versus round in outline). In most of these morphotypes, the nucleoid is surrounded by a single membrane separating the nuclear region from the rest of the cell, as exemplified most dramatically in the type with butterfly-shaped nuclear body found first in *S. micronesica* and *A. willeyana*. In one morphotype only, the nucleoid appears external to a central single-membrane-bounded compartment effectively separating the cell into two compartments, one with nucleoid and the other without.

In the morphotype classification used in this paper, Morphotype 1 is considered to be the type with a butterfly-shaped membrane-bounded nuclear region. The second type, referred to as Morphotype 2, is a short, fat, rod morphotype (Fig. 1C). In Morphotype 2, the nuclear region is bounded by a single membrane (Fig. 3A), but has a relatively electron-dense cytoplasm compared with Morphotype 1.

Morphotype 3 is a D-shaped cell; this type not only shows a characteristic cell shape and a very clear membrane-bounded nuclear region but also displays radiating fibres in the cytoplasm outside this region (Fig. 1D). Most interesting of all, the cell wall displays a structure of regular subunits, compatible with possible membership of the Domain Archaea (Fig. 1D). Members of the Archaea have been classically considered to include organisms inhabiting very hot hydrothermal and volcanically heated waters as well as methane-generating anaerobes and organisms growing in saturated salt (Woese et al., 1990). However, recently they have been reported from less extreme marine habitats (e.g., Atlantic, Pacific and Antarctic seawater; see DeLong, 1992, DeLong et al., 1994 and Fuhrman et al., 1992), including, very significantly for this context, a report of the cold water archaeon sponge symbiont *Cenarchaeum symbiosum* from a Californian coastal *Axinella* species, confirmed as an archaeon by gene probing (Preston et al., 1996). These Archaea all belong to the so-called marine group I cluster which is part of the Kingdom Crenarcheota within the Domain Archaea (DeLong, 1992). In addition to their occurrence in a sponge, such bacteria have also been recently found to occur in holothurian gut and marine fish gut (McInerney et al., 1995; Van Der Maarel et al., 1998).

Another morphotype (Morphotype 4) displays a cell wall with a structure consistent with Gram-negative bacteria - those known to give a negative Gram stain reaction. The wavy nature of the outer cell wall membrane is very clear, consistent with Gram-negative type cell wall; in this type the DNA fibrils occupying most of the nuclear body are very obvious and enclosed within a very distinct membrane (Fig. 2B).

Morphotype 5 is another morphotype with subunit cell wall consistent with membership of the Archaea, this time a rod without a D-shape bias (Fig. 2B-C). Note also the regular subunit 2-D lattice structure visible in the grazing section portion of wall (arrow in Fig. 2B). Morphotype 6 exhibits a characteristically blebbed cell wall membrane, containing a membrane-bounded internal body but in this case without a nucleoid - the nucleoid is in the other 'cytoplasmic' cell compartment (Fig. 2D). Thus the cell is still divided into a non-nucleoid-containing and a nucleoid-containing compartment, albeit in a reverse topological sense to that found in the other morphotypes with a membrane-bounded internal or centrally located nucleoid-containing compartment.

To summarise the symbiont morphotypes described above, there are six morphotypes occurring in Great Barrier Reef sponges *J. stellifera, P. crassa* and *Axinyssa* sp. The first five are Morphotype 1 (rods with "butterfly" nuclear

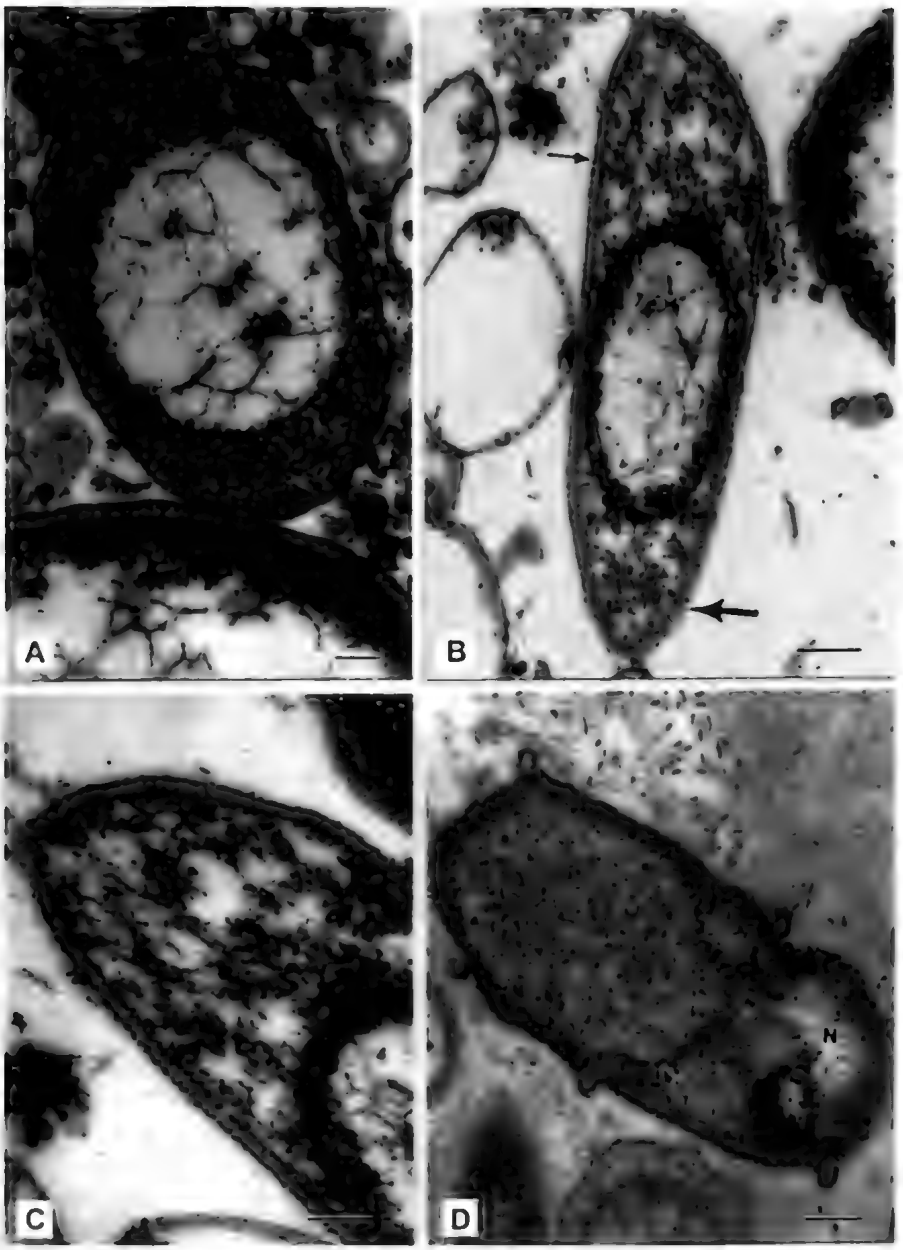

FIG. 2. A, Electron micrograph of thin-sectioned Morphotype 4 (Gram-negative walled cell) symbiont from *Pseudoceratina crassa* displaying a wavy outer cell wall membrane similar to that in walls of Gram-negative bacteria, as well as a single-membrane-bounded nuclear region with DNA fibrils occupying most of the nuclear body. (Scale bar 100nm). B, Electron micrograph of thin sectioned Morphotype 5 (regular subunit walled normal rod) symbiont from *Pseudoceratina crassa* displaying a cell wall consisting of regular subunits (small arrow), especially visible as a periodic lattice in a portion in which a grazing section has occurred (large arrow). (Scale bar 200nm). C, Enlargement of thin sectioned Morphotype 5 (regular subunit walled normal rod) symbiont from *Pseudoceratina crassa* shown in Fig. 2B displaying a cell wall consisting of regular subunits. Note also the portion of the nuclear body and clearly displayed single membrane envelope of this body towards lower right hand side of figure. (Scale bar 100nm). D, Electron micrograph of thin sectioned Morphotype 6 symbiont from *Pseudoceratina crassa* displaying a characteristic blebbed cell wall outer membrane. A membrane-bounded internal body (arrow) is present but the nucleoid (N) is situated in the cell compartment external to this inner body, in contrast to all other morphotypes described in these figures. (Scale bar 200nm).

bodies); Morphotype 2 (short fat rods with electron-dense cytoplasm); Morphotype 3 (D-shaped cells with subunit ("archaeal") walls); Morphotype 4 (rods with Gram-negative outer membrane walls); and Morphotype 5 (cells with large subunit ('archaeal') walls). All of these 5 types contain a membrane-bounded nucleoid-containing nuclear body, more or less centrally located within the cell. Note again that 'nucleoid' is here being used in the bacteriological sense of a fibrillar genomic DNA bundle, which is not normally membrane-bounded. Morphotype 6 differs from the first five. It consists of rods with blebbed cell wall membrane but with a membrane-bounded internal compartment without nucleoid (Fig. 3A-B). The cell is effectively divided into two compartments, however, in an analogous manner to the compartmentalisation in the first five morphotypes, but with a reversed topology.

It should be noted that every morphotype has been seen in all these Great Barrier Reef sponges examined (*P. crassa, J. stellifera* and *Axinyssa sp.*). Thus the diversity of morphotypes possessing membrane-bounded nuclear bodies we have seen may be a widely distributed phenomenon unrelated to host specificity.

The morphotypes described here appear to be sub-types of the types E and 4, previously described in published studies (Vacelet, 1975; Wilkinson, 1978). In those studies, the appearance of types E and 4 was explained by the occurrence in these bacteria of an unusually large periplasm, that is, the region between cell wall and cytoplasmic membrane (Vacelet, 1975; Wilkinson, 1978). In this interpretation, the membrane-bounded nuclear body we have seen would merely represent the cell protoplast (cell cytoplasm contents) surrounded by a retracted cytoplasmic membrane, and the space between nuclear body membrane and cell wall would represent a very wide 'periplasm' rather than true cytoplasm. We favour an alternative interpretation, in which the membrane bounding the nuclear region is a true internal membrane rather than representing cytoplasmic membrane, which is closely appressed to the cell wall in the symbionts we have observed and therefore often difficult to detect. Supporting this is the clear indication of cytoplasmic membrane for at least three Morphotypes as shown in Morphotypes 2 and 3 (Fig. 3A-B) and Morphotype 1 (Fig. 2c in Fuerst et al., 1998). Also consistent with this interpretation in Morphotype 1, and several other of the Morphotypes, is the uniform distribution of cytoplasm within the space between the cell wall and internal nuclear body-bounding membrane, a distribution unlikely if plasmolysis and retraction of cytoplasmic membrane were responsible for this space.

To investigate this problem further, we employed immunogold labelling methods to localise DNA and RNA within the cell and thus determine the location of true cytoplasm. Figure 3C shows a nucleated symbiont Morphotype 1 cell from *J. stellifera* in which the DNA has been localised via immunogold labelling using mouse monoclonal antibody against single-stranded and double-stranded DNA detected via goat anti-mouse antibody conjugated to 10nm colloidal gold particles. All the cell's DNA is localised exclusively within the membrane-bounded nuclear body, suggesting that this must be the location of the chromosomal, genomic DNA. Intracellular RNA was localised in this morphotype using the slightly different enzyme cytochemistry approach employing RNase conjugated directly to colloidal gold. By this method, RNA in these cells is located throughout the cytoplasmic region external to the membrane-bounded nuclear body (Fig. 3D), as well as being present to minor extent in the nuclear body, as would be expected if transcription is to occur using a genomic DNA template. This occurrence of RNA in the cytoplasm outside the membrane-bounded nuclear region supports an interpretation of symbiont ultrastructure in which the space between the nuclear body membrane and the cell periphery is occupied by true cytoplasm and is not merely an unusually large periplasm between a retracted cytoplasmic membrane and the cell wall, and in which the nuclear body is thus a true intracytoplasmic membrane-bounded compartment of the cell. Some RNA also appears in the electron-dense-particle-rich outer zone within the nuclear body itself, as would be expected if cell RNA is transcribed from DNA in the nuclear body. Double-labelling using both DNA and RNA labelling methods with differently sized gold particles confirms the distribution of DNA and RNA relative to the nuclear body found via separate use of DNA and RNA labels (Fig. 4A)

Gold labelling was also used to examine the problem posed by Morphotype 6 symbionts, where there appears to be a non-nucleoid-containing internal membrane-bounded body. A combination of RNase-gold and anti-ss and dsDNA antibody immunogold labelling demonstrated that most of the cell RNA appeared

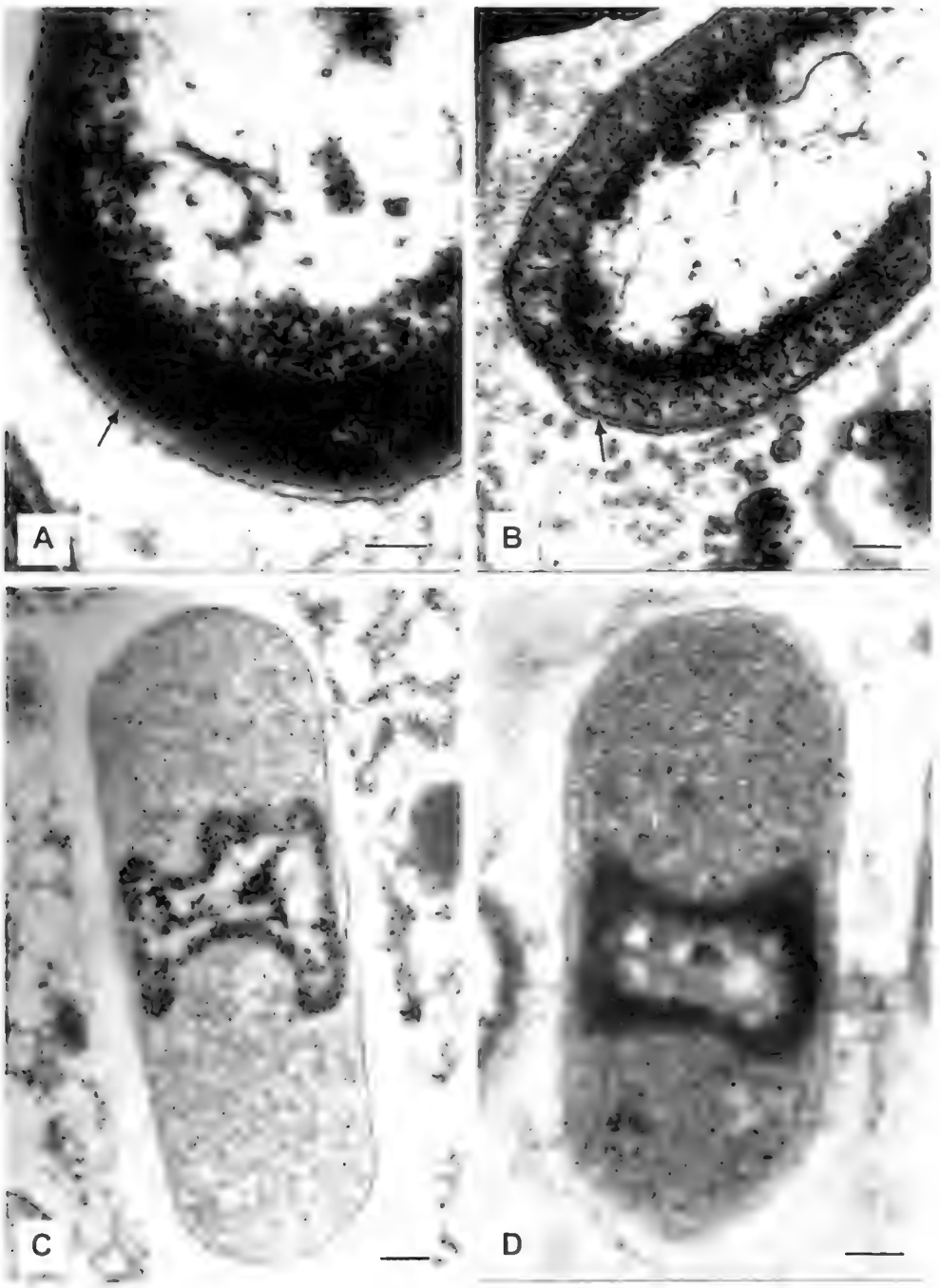

FIG. 3. A-B, Electron micrographs of thin-sectioned Morphotypes 2 and 3. A, Morphotype 2 cell (same cell as shown in Fig. 1C) showing a clear cytoplasmic membrane (arrow) adjacent to the cell wall and external to and widely separated by electron-dense cytoplasm from the nuclear body membrane. (Scale bar 100nm). B, Morphotype 3 showing a cytoplasmic membrane (arrow) closely appressed to the cell wall. (Scale bar 100nm). C, Electron micrograph of thin-sectioned nucleated symbiont Morphotype 1 from *Jaspis stellifera* showing labelling of DNA only within nuclear body, via immunogold detection of mouse monoclonal antibody against single stranded and double stranded DNA (10nm colloidal gold particles). (Scale bar 200nm). D, Electron micrograph of thin-sectioned nucleated symbiont Morphotype 1 from *Jaspis stellifera* showing location of intracellular RNA via labelling with RNase-gold. Note the absence of labelling over the central nucleoid. (Scale bar 200nm).

to be confined to the membrane-bounded internal body, and that all the cell DNA was found outside that body (Fig. 13). Although there is a reverse topology to the compartmentation of DNA found in the other symbiont morphotypes, it would appear that the cell's DNA is still restricted to a separate compartment within the cell as also occurs in the other Morphotypes, via a different compartment. The inner compartment of Morphotype 6 appears superficially to be similar structurally to the nuclear body in the other morphotypes, in the sense of being a single-membrane-bounded inner compartment, but in this case is devoid of DNA.

In their possession of a membrane-bounded nucleoid, the internal organisation of the sponge symbiont Morphotypes 1-5 described here contrasts with that known for most members of Domains Bacteria and Archaea. However, it is most similar to that found previously in planctomycete species of the genus *Pirellula*, where there is enclosure of a major nucleoid-containing cell compartment, the pirellulosome, by a single membrane dividing the cell interior into two regions and where a zone of electron-dense particles around the nucleoid occurs within the pirellulosome (Lindsay et al., 1997). Also relevant may be the double membrane-bounded nucleoid compartment found in another planctomycete, *Gemmata obscuriglobus* (Fuerst & Webb, 1991). In contrast to the cell structure in *Pirellula* species, the sponge symbionts described here do not display any polar differentiation or 'polar cap'. In natural habitat samples, the only bacterial cells appearing to show any similar ultrastructure to those described here are 0.3-0.5μm diameter cells from soil, described with an internal membrane surrounding the nuclear material (Bae & Casida, 1973).

There are also significant similarities, concerning cell shape and nuclear body shape during division, between the symbionts observed here and cells of *Cenarchaeum symbiosum*, a symbiont of the sponge *Axinella* sp. from the Californian coast of the Pacific Ocean determined by *in situ* hybridisation with oligonucleotide probes to be a member of the kingdom Crenarcheota of Domain Archaea (Preston et al., 1996). If the sponge symbionts exhibiting membrane-bounded nucleoids that we have observed prove to be related closely to *C. symbiosum*, this would be highly significant from an evolutionary perspective. This is because cell organisation in Domain Archaea is thought to be exclusively prokaryotic, even though there are many molecular and phylogenetic similarities with eukaryotes and Domain Eucarya (Keeling et al., 1995), and because a membrane-bounded nucleus would have been demonstrated in all three Domains of Life, suggesting its possible status as an ancestral character of the last common ancestor of the three Domains retained only in some lineages of contemporary organisms. *Cenarchaeum symbiosum* has been determined by both 16S rRNA sequencing and DNA polymerase sequencing (Preston et al, 1996; Schleper et al., 1997) to be a member of the Kingdom Crenarcheota within the Domain Archaea, and it may be relevant to the possible occurrence of nucleated cell organisation in sponge symbionts that a crenarcheote origin for the eukaryotes, or at least a crenarcheote/eukaryote clade, has been suggested from phylogenetic analyses based on amino acid sequences from the highly conserved duplicated genes for protein synthesis elongation factors, EF-Tu and EF-G (Baldauf et al., 1996).

Possible identities for the sponge symbionts with membrane-bounded nucleoids include those of a crenarcheotal Archaea member, a planctomycete member of the Bacteria, or a member of the Eucarya. If the latter, however, it must be a mitochondrion-less representative and one which has lost one membrane of the nuclear envelope. It seems most probable that at least some of the symbionts with a membrane-bounded nucleoid are members of the Archaea, since the cell wall in Morphotypes 1, 3 and 5 exhibit subunit structure consistent with an S-layer wall, the most common wall type in the Archaea (König, 1994). The fluorescent probe-labelled symbionts in *Axinella mexicana* identified by Preston et al. (1996) are non-thermophilic members of the *Crenarcheota* within the Domain Archaea, and these show DNA-containing regions with similar morphology via fluorescence microscopy to those seen in the relevant symbionts studied here by electron microscopy. Archaeal nucleoids in the hyperthermophilic crenarcheotes *Sulfolobus acidocaldarius* and *Pyrodictium abyssi* appear to be naked rather than membrane-bounded (Bohrmann et al., 1994; Rieger et al., 1995), but the mesophilic or even psychrophilic crenarcheotes, which have not been cultured or examined via electron microscopy, may well possess different internal structure from those hyperthermophilic representatives of the same Kingdom. Intracellular lamellar membranes

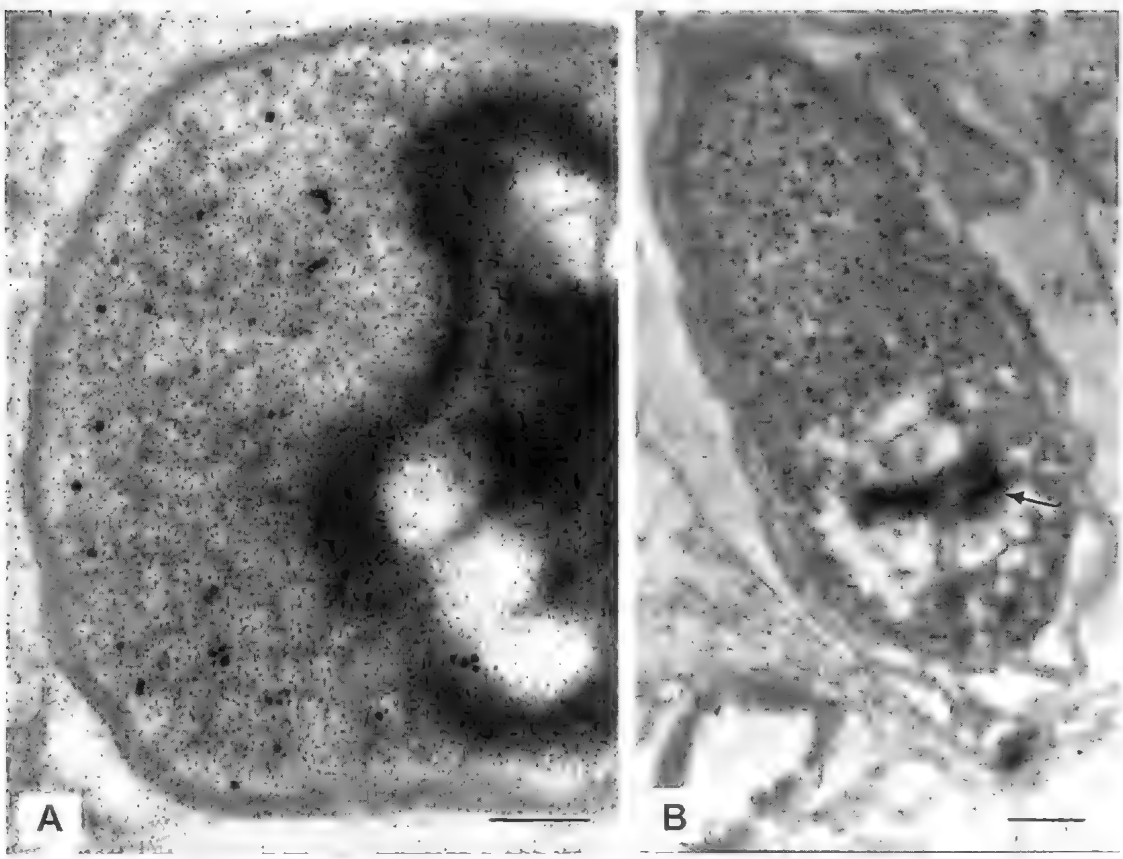

FIG. 4. A, Electron micrograph of portion of a thin-sectioned nucleated symbiont Morphotype 1 from *Jaspis stellifera* which has been double-labelled for both DNA and RNA via use of different sizes of gold particle (10nm for RNase gold and 5nm for anti-DNA antibody labelling via goat anti-mouse IgM Ab conjugated to colloidal gold). (Scale bar 100nm). B, Electron micrograph of thin-sectioned Morphotype 6 symbiont from *Jaspis stellifera* labelled using RNase-gold (large 10nm gold particles) and anti-ss- and ds-DNA antibody immunogold (small dot-like 5nm gold particles) showing the occurrence of RNA but not DNA within the internal membrane-bounded body and the exclusive occurrence of DNA associated with fibrillar nucleoid (arrow) outside the inner membrane-bounded body. (Scale bar 200nm).

found in Type I methanotroph-like Bacterial symbionts of deep-sea carnivorous sponges in methane-rich waters (Vacelet et al., 1996) do not appear to be similar to the membranes enclosing the nucleoids described above, which do not display membrane over-folding or multiple layering. Possible Domain membership of the symbionts can be resolved by direct probing of cells in sections using probes specific for 16S rRNA of specific Domains, or via cloning of PCR-amplified 16S rRNA genes from the symbiont community combined with hybridisation of sectioned or whole cells with probes designed from clone sequences. It can be predicted that the symbiont Morphotypes 1, 3 and 5 with regular subunit walls should be found by such methods to be members of the Kingdom Crenarcheota within the Domain Archaea.

CONCLUSIONS

At least 6 morphotypes of bacteria-like symbionts in the mesohyl of the sponge genera *Jaspis*, *Pseudoceratina*, *Axinyssa*, found in the waters of Heron Island, Great Barrier Reef, possess membrane-bounded nuclear regions. In at least 2 of these morphotypes, cell walls composed of subunits are present, consistent with membership of the Archaeal Domain. In this

context it is of great relevance and interest that members of the Domain Archaea belonging to the kingdom Crenarcheota have been found by direct gene probing using *in situ* fluorescent oligonucleotide hybridisation of whole cells to be present in sponges within genus *Axinella* (Preston et al., 1996). These associates or symbionts of this sponge have been referred to as *Cenarchaeum symbiosum*, and phylogenetic analysis using sequences from at least two genes from this species have confirmed membership of the Crenarcheota within the Domain Archaea (Preston et al., 1996; Schleper et al., 1997). In all but Morphotype 6, the blebbed cell wall morphotype, all the cell DNA is in a nuclear body bounded by a single membrane. The extranuclear cytoplasm possesses most of the cell RNA (i.e., it does not appear to be periplasm but true cytoplasm).

The diversity of morphotypes which differ in cell wall type, cell shape, cytoplasm texture and cell compartment type, yet all share compartmentalisation of the cell into two compartments, a nucleoid-containing and a non-nucleoid-containing one, suggests either that somewhat phylogenetically diverse organisms are perhaps phylogenetically related to a common ancestor with a similar form of compartmentalisation which was retained in otherwise structurally diverse descendants, or alternatively that some environmental factor in the sponge tissue selects for or induces nuclear compartmentalisation. It is also possible that nucleated organisms, or organisms with correlated cell wall structure, are selected for by a sponge tissue factor such as a compound with antibiotic activity, for example, a compound inhibiting enzymes involved in DNA synthesis or supercoiling as performed in cells with prokaryote structure and naked chromosomal DNA or one inhibiting synthesis of the peptidoglycan cell wall polymer found in most members of the Domain Bacteria. Classical prokaryotic Bacteria with peptidoglycan walls and naked cytoplasmic DNA may not compete efficiently with nucleated peptidoglycan-less organisms of whatever Domain.

Aspects of these results are significant to our understanding of cell organisation and are fundamental to biology in general. Our results from sponge symbionts may constitute a challenge to the major structural classification of cell types based on cell organisation - that of the prokaryote and eukaryote- since at least some otherwise bacteria-like cell types appear to contain membrane-bounded DNA in a manner analogous to eukaryote cell nuclei. This extends the challenge to that classification first revealed by the discovery of double- and single-membrane bounded nuclear bodies in the planctomycete members of the Division Bacteria. If the Archaeal nature of some of the sponge symbiont morphotypes with nuclear regions can be demonstrated by molecular sequence-based methods, then membrane-bounded nuclei may well be shown to occur at least rarely within members of all three Domains of life, Bacteria, Archaea and Eucarya, a finding which would be of fundamental significance to our understanding of how eukaryote cells may have evolved their initial defining structure. Insights from study of sponge biology may thus yet again contribute to our fundamental understanding of cell biology and evolution.

ACKNOWLEDGMENTS

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EVIDENCE OF TRANSFER OF PHOTOSYNTHATE FROM A RED ALGAL MACROPHYTE TO ITS SYMBIOTIC SPONGE.
Memoirs of the Queensland Museum 44: 204. 1999:-
Symbiotic cyanobacteria are quite common in coral reef sponges providing much of the sponge's supply of carbon. There are also several sponge species with macroalgal symbionts. In these sponges, the role of the algae is unknown. One of these symbioses is that of the sponge, *Haliclona cymiformis* (Haplosclerida) and the red alga, *Ceratodictyon spongiosum* (Rhodymeniales) which is common in the shallow tropical waters of fringing reefs of the Indo-Pacific region. The sponge tissue comprises about one third of the dry weight of the association and grows over the external surface of the alga and between the algal branchlets. In the field, the alga is dark green to purple with thick branches of tightly anastomosed (fused) branchlets. However, in culture, the branchlets are red and thin and do not fuse. Neither symbiont has been found growing separately in nature suggesting that the symbiosis is obligate. The physiological basis of this well integrated association is not yet known.

The sponge obtains nutrients from the water column in the form of dissolved and particulate organic matter at rates that are similar to those of free-living sponges (Trautman, 1997). We have found that some photosynthate is transferred from the alga to the sponge, in a time-dependent manner. After 1h incubation in the light with $Na_2{}^{14}C0_3$ the amount of photosynthetically fixed carbon transferred to the sponge (range 22.77- 48.3nmol carbon/mg dry wt. of sponge) represents 0.6-1.28% of the total carbon fixed by the alga during this period. When the fixed carbon in the sponge tissue is extracted using methanol/chloroform/water (24/10/4 v/v/v), to give an aqueous-soluble fraction (low molecular weight metabolites) and a chloroform-soluble fraction (lipids, sterols, chlorophyll etc.) followed by extraction in 2 M KOH (high molecular weight metabolites such as proteins, polynucleotides, polysaccharides) 75-88% of the ^{14}C-labelled carbon is found in the aqueous fraction, about 11-20% in the KOH-soluble fraction, 2-3% in the chloroform-soluble fraction and <3% in KOH-insoluble material.

When the aqueous-soluble fraction is further fractionated by ion exchange chromatography into neutral (sugars), basic (amino acids), acidic (organic acids) and phosphate ester fractions, most of the fixed carbon is found in the basic (47%) and neutral (38%) fractions. Some fixed carbon is found in organic acids (14%) with very little in phosphate esters (<2%). Our data suggest that while the alga may supply the sponge with some essential nutrients, the major source of organic carbon is the particulate and dissolved organic matter in the ambient seawater. It may be that the primary role of the algal symbiont is structural rather than nutritional. ❐ *Porifera, symbiosis, red alga, carbon metabolism, photosynthate, translocation.*

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ECOLOGICAL ROLE OF CYTOTOXIC ALKALOIDS: *HALICLONA* N.SP., AN UNUSUAL SPONGE/ DINOFLAGELLATE ASSOCIATION

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Garson, M.J., Clark, R.J., Webb, R.I., Field, K.L., Charan, R.D. & McCaffrey, E.J. 1999 XX XX: Ecological role of cytotoxic alkaloids: *Haliclona* sp. nov., an unusual sponge/ dinoflagellate association. *Memoirs of the Queensland Museum* **44**: 205-213. Brisbane. ISSN 0079-8835.

Light microscopy and electron microscopy studies of the tropical marine sponge *Haliclona* sp. nov. (Haplosclerida; Chalinidae) from Heron Island, Great Barrier Reef, have previously revealed the characteristic presence of a dinoflagellate symbiont and nematocysts. The dinoflagellates are morphologically similar to *Symbiodinium microadriaticum*, the common intracellular zooxanthellar symbiont of corals. The sponge grows on coral substrates, from which it may acquire the dinoflagellates and nematocysts. Chemical investigations found the sponge contained a suite of cytotoxic alkaloids, the haliclonacyclamines. Our investigations showed that these alkaloid metabolites cause significant coral tissue necrosis at concentrations of 5ppm after 160mins exposure in laboratory-based assays. At higher concentrations (10ppm and above) toxic effects were noted within 10mins exposure to the alkaloid fraction. Coral tissue necrosis was also observed after 40mins exposure to the major alkaloid component haliclonacyclamine A. In field experiments, the alkaloids were absorbed onto synthetic pads which were tied onto coral fingers. In both short term (10hrs) and long term (30hrs) experiments, coral tissue necrosis was observed at concentrations of 0.025% and above. We determined that a dose of 0.24% was equivalent to the natural exposure of coral pieces to sponge tissue, with our data indicating that haliclonacyclamines are effective toxins against coral tissue at lower than natural concentrations. When tested against natural populations of reef fish, the haliclonacyclamines were found to be potent feeding deterrents at ecologically-relevant concentrations (0.1% of sponge wet weight). ❐ *Porifera, Haliclona, Acropora, alkaloids, dinoflagellates, feeding deterrent, haliclonacyclamines, percoll density gradient fractionation, secondary metabolites, toxins, Symbiodinium microadriaticum.*

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Sponges are a major component of benthic fauna, representing the second largest biomass on tropical reefs after corals. The production of bioactive chemicals by marine sponges is a factor which likely enhances their competitiveness in coral reef environments. The secondary metabolites may act as chemical defenses against predation by fish, molluscs or other carnivores (reviewed in Paul, 1992), or act to prevent other marine species growing adjacent to or on top of the sponge tissue (Davis et al., 1989; Clare, 1996; Fusetani, 1997). Although sponge-derived chemicals have been implicated in allelochemical interactions with neighbouring corals (Sullivan et al., 1983; Porter and Targett, 1988), few rigorous ecological studies have yet been undertaken.

Field studies by McCaffrey (1988) discovered a haplosclerid sponge, *Haliclona* sp. nov. (Haplosclerida: Chalinidae), which grows on coral substrates in the channel zones of Heron island at 10-14m depth. Although the sponge tissue was soft and easily torn, there were no feeding scars to indicate predation by fish or other scavengers, nor was its surface fouled by epiphytes; these facts suggested the presence of inhibitory chemicals. The sponge also exuded mucus upon collection. McCaffrey (1988) showed that the sponge contained antimicrobial components toxic to hydroids, corals, crustaceans and fishes, although she did not identify the

chemicals involved. Our subsequent research found crude organic extracts of this species exhibited potent antifungal and antimicrobial activity and an IC50 of 5µg/mL in a P388 mouse leukaemia assay. The aqueous methanol phase of a toluene:methanol (3:1) sponge extract was therefore extracted with chloroform, and the combined organic extracts processed to give a suite of novel alkaloids, the haliclonacyclamines A-D (Fig. 1) (Charan et al., 1996; Clark et al., 1998). Using Percoll gradient centrifugation of fixed cells, we demonstrated that the alkaloids are stored in, and are therefore likely biosynthetic products of, sponge cells (Garson et al., 1998).

Haliclona sp. nov. has been reported to grow on coral substrate, usually *Acropora nobilis*, but also other corals such as *Poecillopora* sp. and *Seriatopora hytrix* and also on sand-covered coral rock (McCaffrey, 1988). When the sponge tissue was examined by light microscopy, nematocysts of mean length 12-15µm were detected, as was a dinoflagellate which morphologically resembled *Symbiodinium microadriaticum*, the dinoflagellate symbiont of reef corals (McCaffrey 1988, Garson et al., 1998).

Haliclona sp. nov. is a versatile sponge in that it appears to have evolved multiple defense strategies. In addition to the potential physical defense provided by mucus exudation, and the presence of nematocysts, the associated alkaloids may provide an additional chemical defence *in situ*. In this paper, we present some preliminary evidence on the ecological roles of the haliclonacyclamine alkaloids.

MATERIALS AND METHODS

CHEMICALS AND BIOCHEMICALS. Agar was purchased from Sigma Chemical Company (MO, USA) while brine shrimp eggs and dried krill were purchased from an aquarium supply shop. Solvents used in ecological experiments and in the extraction of compounds from whole tissue or cell separation experiments were glass distilled.

BIOLOGICAL MATERIALS. Samples of *Haliclona* sp. nov. were collected by hand using SCUBA at the Coral Gardens (10-15m depth), Heron Island (23°27'S, 151°55'E), S Great Barrier Reef, Australia, under permit numbers G96/050, G97/097, G98/037 issued jointly by the Great Barrier Reef Marine Park Authority (GBRMPA) and the Queensland National Parks and Wildlife Service; and at North Point, Lizard Island (14°39'S, 145°27'E), N Great Barrier Reef under GBRMPA permit G98/227. Sponge samples used in biological experiments were maintained in running sea water at ambient temperature and light conditions prior to use. Coral samples used for ecological studies were collected under GBRMPA permits G97/097 and G98/037. For a brief description of the sponge and the dinoflagellate symbiont, see Charan et al. (1996) and Garson et al. (1998). A voucher specimen of the sponge is accessioned in the Queensland Museum, Brisbane, collections (QM G304086).

ISOLATION OF METABOLITES. A crude alkaloid extract (600mg) was prepared from frozen sponge (250g wet wt.) as described by Clark et al. (1998) and further purified by normal phase HPLC using EtOAc/hexanes/Et_3N (30:65:5 or 80:15:5) to give haliclonacyclamines A (Fig. 1A; 162mg, 0.065%), B (Fig. 1B; 144mg, 0.057%), C (Fig. 1C; 26mg, 0.0012%) and D (Fig. 1D; 5mg, 0.002%).

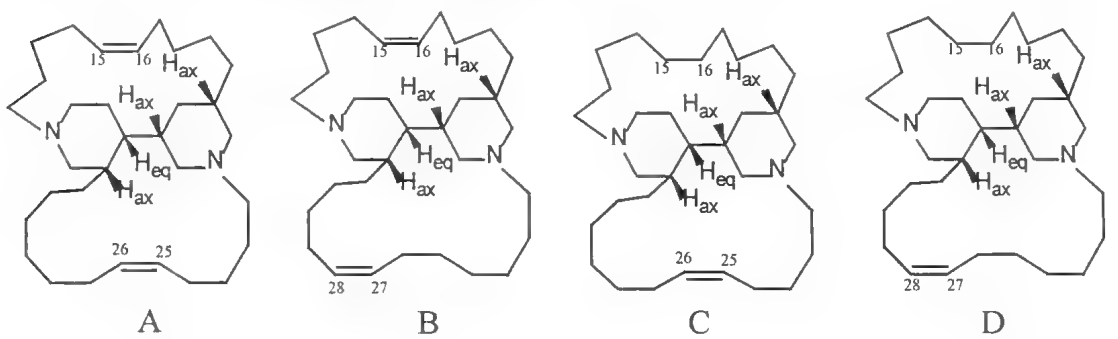

FIG. 1. Structures and stereochemistry of the haliclonacyclamines. A, Haliclonacyclamine A. B, Haliclonacyclamine B. C, Haliclonacyclamine C. D, Haliclonacyclamine D.

ESTIMATIONS OF NATURAL CONCENTRATIONS. To determine the average natural concentration of metabolite in the sponge, three different samples of *Haliclona* sp. nov. collected from the Coral Gardens dive site were extracted, and the alkaloid content estimated. The ratio of the different haliclonacyclamines in each extract was assessed by normal phase HPLC using EtOAc/hexanes/Et_3N (30:65:5). For comparison, a specimen collected at Coral Spawning dive site, 500m further along the reef, was extracted. For toxicity trials, a thin slice of frozen sponge (10mm x 20mm x 1mm; approx 1g wet wt.) was extracted; this piece of sponge was equivalent in volume to a pad used for the *in situ* coral toxicity trials. The exudation rate of alkaloids from the sponge was assessed by aerating a 33g piece of sponge in seawater for 3hrs 45mins in ambient temperature and light under flow conditions. The sponge was carefully removed, and the residual sea water filtered through a 0.22µm filter, then passed through a C_{18} Seppak cartridge, which was flushed with 100ml DCM to flush out organic components. Removal of the DCM solvent left a residue (3.9mg) which was analysed by TLC and NMR.

MICROSCOPY STUDIES. Tissue samples were processed as described previously (Garson et al., 1998). Sections were viewed using Hitachi H-800 and Jeol 1010 transmission microscopes. Light microscopic observations of tissue or cell preparations were made on an Olympus BH-2 microscopic using Nomarski interference optics.

FISH FEEDING DETERRENCY STUDIES. Agar cubes were prepared by combining 30g of agar, 2.7g of brine shrimp eggs and 2.7g of krill in one litre of Milli-Q water. This mixture was heated to 85°C and allowed to cool to approximately 50°C when the alkaloids were added to the agar mixture at 0.1% wt/vol (half the estimated natural concentration). The mixture was then cooled to approximately 40°C before being poured into ice cube trays. Each cube contained a $1cm^2$ piece of wire gauze to which a length of dental floss was attached. Seven cubes of either treatment or control were then attached to a polypropylene rope by the dental floss with a 25cm gap between each cube. Eleven sets of paired ropes (one control and one treatment rope) were placed at the Coral Gardens field site at a depth of approximately 14m and with no more than 0.5m between the ropes. Divers stayed in the water to monitor feeding. When approximately half the cubes were eaten (approx 1hr), the ropes were collected and the number of cubes eaten counted. Data was analysed with Wilcoxon's signed rank test (Zar, 1984); two-tailed *p*-values are reported. The haliclonacyclamine alkaloids remained present in the agar cubes throughout the assay (TLC confirmation at the end of the experiment).

CORAL TOXICITY STUDIES. *Laboratory experiments.* Pieces of *Acropora* sp. were collected and placed in an aquarium with continuous water flow for a period of 12hrs under ambient conditions of temperature and light. Pieces (approximately 2cm long) were broken off carefully and left in the aquarium for a further 10hrs to acclimatise. Treatments were prepared by dissolving the crude alkaloid extract in ethanol (at a concentration of 6mg/mL), then aliquots were dispensed into voucher jars containing 100mL of filtered sea water to give final concentrations of 40ppm, 10ppm, and 5ppm. A fourth treatment was prepared consisting of haliclonacyclamine A at 10ppm. A control experiment contained 666µL EtOH. There were ten replicates of each treatment and of the control. The solutions were aerated throughout the duration of the experiment. A single piece of coral was placed in each voucher jar and observed after 10mins, 20mins, 40mins, 80mins, 160mins and finally after an interval of 9.5hrs. The condition of the coral pieces was graded according to the following five point scale (Aceret et al, 1995): 1=75-100% of colony exhibiting normal polypal activity, with extended tentacles, no change in pigmentation, no mortality; 2=50-75% as above; 3=less than 50% as above; 4=tissue still evident, obvious loss of pigmentation, decreased water clarity, and no visible signs of life; 5=little or no remaining tissue evident, complete loss of pigmentation, mortality.

Field experiments. Absorbent pads (1x2cm; thickness 0.1cm) were impregnated with alkaloid at concentrations of 0.005, 0.01, 0.025, 0.05, 0.1 and 0.4% (10 replicates at each concentration, dissolved in 200µL DCM per pad). The control consisted of a pad impregnated with 200µL of DCM. The pads were taken underwater in plastic bags and attached to coral fingers with cable ties. The pads were left for 24hrs after which the coral was carefully detached using small pliers, taken back to the lab and left in the aquarium for 6hrs to acclimatise. The pads were removed and the condition of the coral graded (using the same scale as for the lab experiments). In a second

shorter term experiment using 0.01, 0.025, 0.035, 0.05 and 0.075% alkaloid, the pads were left underwater for 8hrs, then acclimatised in aquaria for 2hrs prior to grading. At the end of each experiment, the pads were extracted with dichloromethane to confirm the presence of residual alkaloids. In the 10hr experiment there were residual alkaloids present at all concentrations tested while in the 30hr experiment only the 0.4% treatment still contained residual alkaloid.

RESULTS

CHEMISTRY. The structures and stereochemistry of the haliclonacyclamine metabolites A-D are shown in Figure 1A-D. The metabolites were characterised by 2D-NMR spectroscopy and by single crystal x-ray analysis (Charan et al., 1996, Clark et al., 1998). Haliclonacyclamines A and B (Fig.1A-B) have $\delta^{25,26}$ or $\delta^{27,28}$ double bonds respectively in addition to a $\delta^{15,16}$ double bond, while haliclonacyclamines C and D (Fig. 1C-D) were found to be analogous to haliclonacyclamines A and B respectively, but lacking the $\delta^{15,16}$ double bond. The stereochemistry of the $\delta^{15,16}$, $\delta^{25,26}$ or $\delta^{27,28}$ double bonds was found to be *Z* in all metabolites (Charan et al., 1996, Clark et al., 1998).

ESTIMATION OF NATURAL CONCENTRATIONS. The average yield of alkaloid crude extract from the three Coral Gardens samples of *Haliclona* sp. nov. was 0.25% ± 0.01% of sponge wet weight; the ratio of the haliclonacyaclamines estimated by HPLC was found to be consistent between all three extracts. The yields of individual alkaloids were: 1A, 0.065%; 1B, 0.057%; 1C, 0.012%; and 1D, 0.002%, giving a combined isolation yield of 0.136%. Some losses of compound are expected to occur during purification. The alkaloid yield from the specimen collected at Coral Spawning dive site was 0.24% ± 0.01% of sponge wet weight; the composition of haliclonacyclamines was similar to that at Coral Gardens by HPLC. The 1g piece of sponge of equivalent volume to a single pad used in the *in situ* trials contained 2.4mg alkaloid. The rate of leaching of chemicals from *Haliclona* sp. nov. was assessed in laboratory experiments under flow conditions. An organic extract was obtained from sea water in which the sponge was immersed; TLC and ^{1}H NMRanalysis detected the haliclonacyclamines in this extract. The amount of alkaloid detected using these analytical techniques is estimated to be 1.58mg. Therefore if the haliclonacyclamines were present in the surrounding water, as suggested by McCaffrey (1988), then the rate of exudation was estimated to be 0.013mg/hr/g of sponge.

BIOLOGY. *Haliclona* sp. nov. is one of the dominant sponges of the channel zone at Heron Island, where it commonly occurs at depths of 10-15m at the base of the reef slope. The sponge grows on coral substrate, and when damaged or collected, exudes abundant mucus. Its preferred substrate is *Acropora nobilis*, however specimens have also been observed to grow on *Stylopora pistillata*, *Pocillopora* sp., *Seriatopora hytrix* and on sand-covered coral rock (McCaffrey, 1988). Usually the sponge is a uniform olive-brown colour, but infrequently the tips are bleached. By light and transmission electron microscopy, dinoflagellates, usually intracellular, and nematocysts were present throughout preparations from sponge samples collected growing on acroporid substrates (Garson et al., 1998). Infrequently samples contained low populations of nematocysts, for example when collected from sand-covered coral rock.

In June 1998, after an El Niño period, a bleached specimen of *Haliclona* sp. nov. was found growing on bleached acroporid coral at Heron Island. By microscopy, the sample was free of nematocysts, but contained some dinoflagellates; these were not healthy in appearance and were free-living rather than intracellular. Samples of the sponge were also found on dead coral substrate at North Point, Lizard Island; these specimens were small in size and always had bleached tips relative to the brown body colour. By microscopy, the bleached tips were free of both dinoflagellates and nematocysts; in contrast, the body of the sponge, which was a brown colour, contained healthy dinoflagellates but had no nematocysts.

ECOLOGY. *Fish deterrency.* The crude alkaloid fraction of *Haliclona* sp. nov. deterred feeding (mean deterrency = 4.6±0.6 of 7 cubes eaten; N=11; p=0.002) by natural populations of reef fish in field assays conducted at 14m depth in the channel at Heron Island (Fig. 2). The alkaloid fraction was tested at half average natural concentration (0.1% of wet weight; 16mg per agar cube). Rabbitfish (*Siganeus argenteus*) were a major consumer of cubes in this experiment.

Toxicity towards scleractinian corals. Figure 3 shows the results of laboratory experiments in which an alkaloid fraction from *Haliclona* sp.

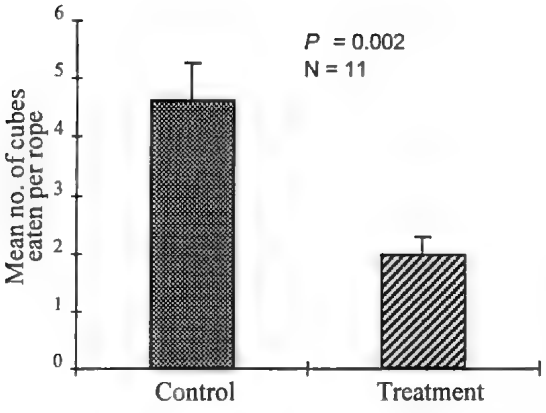

FIG. 2. Field assays of a crude alkaloid extract (tested at half natural concentration, 0.1% of wet wt.) from *Haliclona* sp. nov. at Heron Island. *P*-values calculated using a Wilcoxon two-tailed sample test.

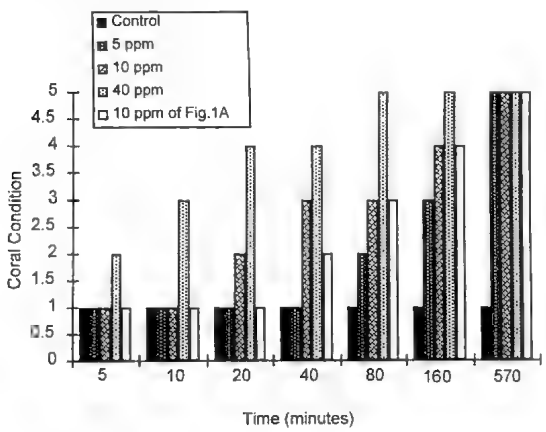

FIG. 3. Toxicity of an alkaloid fraction from *Haliclona* sp. nov. towards tips of *Acropora* sp. in laboratory experiments. Alkaloid concentrations ranged from 5-40ppm. Number of corals per concentration=10.

nov. was added at varying concentrations to small aquaria of filtered seawater containing tips of *Acropora* sp. At 5ppm concentration, less than 50% of the corals were fully viable, that is exhibiting normal polypal activity, with tentacles extended and no loss of pigmentation after 160mins. At higher concentrations (10ppm and above), toxic effects were noted within 10mins of exposure to the alkaloid fraction. At the highest concentration tested (40ppm), all the corals were killed within 80mins. The major alkaloid component haliclonacyclamine A (Fig.1A) was tested at a single concentration of 10ppm. The toxic effect of haliclonacyclamine A (Fig. 1A) was not as rapid as the alkaloid mixture was at 10ppm, but was equally effective in inhibiting coral and polypal activity after 80mins. Significant coral tissue necrosis was detected after 40mins exposure to this metabolite. In control experiments, dichloromethane solvent alone was added to the aquarium water without adverse effect to the coral pieces.

Field assays were carried out using a method based on the work of Porter & Targett (1988). Alkaloid extracts were coated onto synthetic sponge pads and tied to healthy coral pieces growing in the vicinity of *Haliclona* sp. nov. in the channel at Heron Island. Control pads (coated with dichloromethane solvent only) produced no effects, whereas pads containing 0.025% alkaloid resulted in less than 50% of the corals tested remaining viable after 30hrs (Fig. 4). In a shorter term experiment (10hrs duration; Fig. 5), the corals became unviable at concentrations of 0.025% alkaloid.

DISCUSSION

In her PhD work, McCaffrey (1988) demonstrated feeding deterrency of crude extracts from *Haliclona* sp. nov. by the bream, *Acanthopagrus australis*. Our field experiments have now demonstrated the deterrency of the haliclonacyclamine alkaloids to reef fish at ecologically-relevant concentrations. There is an increasing body of experimental evidence which demonstrates the deterrency of sponge secondary metabolites to fish predators (Rogers & Paul, 1991; Paul, 1992; Pennings et al., 1994; Pawlik et al., 1995; Chanas et al., 1996; Uriz et al., 1996). There is no obvious correlation between metabolite type and feeding deterrency since the range of structures which have been identified as feeding deterrents includes alkaloids (Chanas et al., 1996), sesterterpenes (Rogers & Paul, 1991; Duffy & Paul, 1992; Pennings et al. 1994), sesquiterpenes (Pennings et al., 1994; Uriz et al., 1996), and brominated metabolites (Paul, 1992; Pennings et al. 1994; Chanas et al., 1996). A number of these studies have considered other factors which may impact on palatability such as nutritional quality (Duffy & Paul, 1992; Chanas & Pawlik, 1995), the presence of spicules or the texture of the sponge tissue (Chanas & Pawlik, 1995; Chanas & Pawlik, 1996; Uriz et al., 1996). Some chemically-defended sponges contain spicules (Uriz et al., 1992; Chanas & Pawlik, 1995; Uriz et al., 1996). We have not yet investigated whether the spicules present in

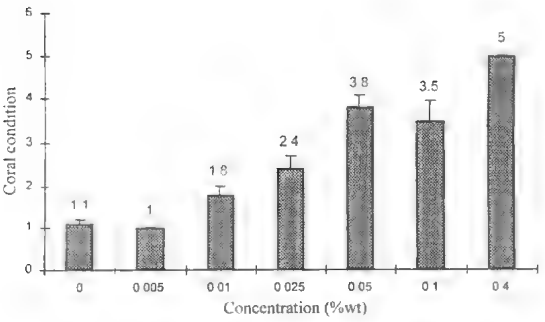

FIG. 4. Toxicity of an alkaloid fraction from *Haliclona* sp. nov. towards the periphary of *Acropora* sp. in field experiments. Alkaloid concentrations ranged from 0.005-0.4%. Number of corals per concentration=10. Length of experiment 30hrs. See text for coral toxicity gradation scale.

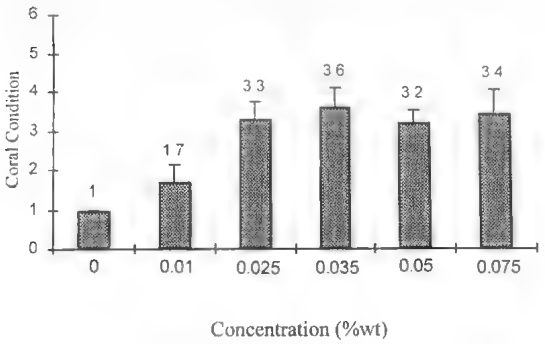

FIG. 5. Toxicity of an alkaloid fraction from *Haliclona* sp. nov. towards the periphery of *Acropora* sp. in field experiments. Alkaloid concentrations ranged from 0.01-0.075%. Number of corals per concentration=10. Length of experiment 10hrs. See text for coral toxicity gradation scale.

Haliclona sp. nov. are an additional deterrent to fish, or whether they simply play a structural role in this fragile sponge - although it is speculated that the former is unlikely given that spicules are small, smooth, homogeneous oxeas contained completely within the choanosome (J. Hooper, pers. comm.). Further experiments are also required to assess the effect of nutritional quality on the anti-feedant properties of the haliclonacyclamines.

Marine sponges are known to release metabolites directly into the water column (Walker et al., 1985) or indirectly through a mucus exudate (Sullivan et al., 1983). McCaffrey (1988) investigated the exudation of biologically-active compounds from *Haliclona* sp. nov., but did not identify the chemicals or measure the exudation rate. In preliminary laboratory experiments we have estimated the exudation of *Haliclona* metabolites is 0.013mg/hr/g wet weight of sponge under flow conditions. Our estimates took no account of the effect of water throughput or current or the concentration of alkaloids in the mucus exudate. Measurement of the natural leaching rate of organic extracts from marine sponges has not yet been addressed in the literature, although Henrikson et al. (1995) have measured the release of sponge chemicals from a range of artificial substrates. A detailed quantitative study of alkaloid leaching from *Haliclona* sp. nov. is in progress in our laboratory.

In laboratory experiments, a mixture of the haliclonacyclamine alkaloids exhibited toxic effects towards pieces of acroporid coral and caused the corals to release mucus and to shed symbiotic zooxanthellae. When a sample of haliclonacyclamine A was tested at 10ppm, coral tissue necrosis was observed, however the purified metabolite was less toxic than the alkaloid mixture tested at the same concentration during short term exposure. These data suggest that the alkaloid mixture may be a more effective toxin than the individual chemicals. Further experiments will be required to confirm this synergistic effect.

Our field results also confirmed the effective toxicity of the alkaloids. These experiments showed that the metabolites in *Haliclona* sp. nov. actively inhibit the metabolism and tissue survival of adjacent acroporid corals. Our experiments used a range of alkaloid concentrations up to 0.075% (10hr experiment) or 0.4% (30hr experiment). If it is assumed that the alkaloids leach out of the artificial pads at the same rate as from sponge tissue, our experiment suggests that the metabolites are effective toxins at lower than natural concentrations. The field results cannot easily be related to the laboratory toxicity trials. The metabolite concentrations used in the coral pad experiment were equivalent to 250-20,000ppm, however the effective metabolite concentration that the coral may experience is much lower.

Since both direct contact (using synthetic sponge pads to mimic the effects of sponge tissue), and indirect contact (addition of metabolites to aquarium water), resulted in toxic effects on corals, we conclude that the *Haliclona* alkaloids are effective allelochemicals which enable the sponge to compete successfully for space with coral substrates (Jackson & Buss,

1975; Wulff & Buss, 1979; Porter & Targett, 1988). *Haliclona* sp. nov. is an aggressive sponge which may preferentially select coral substrates as habitat. Although some studies have demonstrated the effectiveness of inhibitory substances in improving the competitiveness of sponges for space among benthic organisms (Becerro et al., 1997; McCaffrey & Endean, 1985; Thompson, 1985; Thompson et al., 1985; Walker et al., 1985; Clare, 1996; Wright et al., 1997), other studies have shown a contrasting ecological effect, for example that the presence of sponge chemicals may induce marine invertebrate larvae to settle (Bingham & Young, 1991). The ecological effectiveness of *Haliclona* metabolites on benthic invertebrates other than corals, or on their larvae, is yet to be tested in our laboratory. This study will enable us to determine if the haliclonacyclamines are selectively toxic or not.

The *Haliclona* metabolites possess a liphophilic carbon backbone together with a polar amine functionality, and are therefore amphiphilic in character. The compounds, thus, have partial water solubility; for example, NMR spectra can be obtained in deuteriated water. The sponge pads placed underwater for 10hrs still retained alkaloids at the end of the experiment. In the long term exposure study, we observed loss of metabolites from the artificial pads at the lower concentrations. The ongoing release of a polar, diffusible substance by a sponge is of no value as a mechanism to inhibit settlement (Becerro et al., 1997), and is also metabolically uneconomic; the most suitable chemical candidates for defense or for use as an anti-fouling agent are likely to be water-insoluble. Some recent studies have attempted to better simulate natural conditions in field trials by embedding sponge extracts onto artificial matrices which can be placed in the field for long periods. Chemicals may leach out of these gel matrix at rates which may mimic their natural release (Morse et al., 1994; Hendrikson & Pawlik, 1995). Experiments of this type are currently in progress in our laboratory.

In corals, photosynthesis is performed uniquely by dinoflagellate (zooxanthellae) symbionts which supply the host with nutrients by translocation (Muscatine & Cernichiari, 1969). Cyanobacteria are the most common sponge photosynthetic symbionts, however some groups of sponges, notably the boring sponges of the order Hadromerida (e.g. *Cliona* spp.), have been shown to contain zooxanthellae (Vacelet, 1982; Rützler 1990), although it is not yet known whether the dinoflagellate partners supply the hadromerid sponges with photosynthetic products. We propose that *Haliclona* sp. nov. may poison or kill the coral tissue on which it grows in order to acquire dinoflagellate symbionts, which provide additional metabolic benefits to the sponge, thereby enhancing its competitiveness. Sullivan et al. (1983) showed that the boring sponge *Siphonodictyon* sp. (=*Aka*, family Phloeodictyidae), uses toxin-containing mucus to kill surrounding tissue. *Haliclona* sp. nov. exudes abundant mucus on collection and so may perhaps use a similar process. We are currently investigating the chemistry of *Haliclona* sp. nov. mucus to determine if it contains the haliclonacyclamines.

To our knowledge, no other marine sponge has been reported to contain nematocysts. Perhaps the nematocyst capture represents an additional serendipitous defense mechanism in *Haliclona* sp. nov. The high numbers of nematocysts found intracellularly in healthy sponge tissue samples are inconsistent with their casual acquisition from the surrounding water. Both nematocysts and dinoflagellates were absent in a bleached sample of *Haliclona* sp. nov. found growing on bleached coral at Heron Island. This sample was clearly stressed since the dinoflagellates appeared unhealthy and were free-living rather than intracellular. Partially-bleached samples of *Haliclona* sp. nov. collected from dead coral rock at Lizard Island were also free of nematocysts even if the body of the tissue was healthy and contained dinoflagellates. The distribution and specific source of the nematocysts present within the sponge tissue is currently under further investigation, as is their impact on predation.

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SPONGIVORY BY THE BRAZILIAN STARFISH *ECHINASTER BRASILIENSIS*. *Memoirs of the Queensland Museum 44: 214. 1999:-* The feeding ecology of *Echinaster brasiliensis* has been studied on a temporal gradient (January 1995 - September 1996, 11 observation periods), along a shallow-water transect parallel to the coastline (1.5-6m depth, 2000m^2) at Ponta do Baleeiro (23°49.727'S - 45°25.364'W), São Sebastião Channel (São Sebastião, SP, Brazil). In total, 3025 starfish were observed, 44% of which were feeding (1337/3025). Of these, 42% (557/1337) were feeding on sponges, a significantly higher proportion than the real availability of sponges in terms of area coverage by organisms. Of the 33 sponge species recognised, the most wanted prey was *Mycale aff. americana*, representing 40% (221/557) of the total number of observed spongivory events. Other common sponge prey items were *Amphimedon* sp., *Haliclona* sp.n., *Mycale angulosa*, *Mycale microsigmatosa* and *Tedania ignis*, with ca. 5% of the spongivory events each. Semiquantitative arbitrary estimations point toward these species' high abundance in the study area. Therefore, we cannot discard the possibility of a direct link between the sponge's abundance and apparent starfish preferences. Of the 33 sponge species eaten, at least 61% (20/33) belong to genera from which species were found (literature) to possess toxins, thus raising the question: 'What are these toxins good for?' The conspicuous habit of *Echinaster brasiliensis* suggests that it may be unpalatable to many potential predators, perhaps through the use of sequestered toxins of dietary origin. The temporal gradient studied did not reveal clear patterns, thus suggesting that inter-annual climatic oscillations may play an important role in shaping the starfish's feeding ecology. Acknowledgement of financial support: CNPq, FAPERJ, FAPESP, FUJB-UFRJ. ❐ *Porifera, spongivory, SW Atlantic, Echinaster, Mycale, feeding ecology, chemical ecology.*

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ECOLOGICAL ADAPTIONS OF A FRESHWATER SPONGE ASSOCIATION IN THE RIVER RHINE, GERMANY (PORIFERA: SPONGILLIDAE)

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The species composition and autecology of freshwater sponges (Porifera, Spongillidae) were investigated in the Rhine between Karlsruhe and Bonn (Germany) between 1993 and 1995. *Ephydatia fluviatilis*, *E. muelleri*, *Trochospongilla horrida*, *Spongilla lacustris*, *Eunapius fragilis* and *E. carteri* were found. *Ephydatia fluviatilis* was classified as an r-strategist due to its high ability to colonise new habitats, whereas other species placed emphasis on successful establishment in more stable habitats and should therefore be classified as K-strategists (among freshwater sponges). Similarly, the production of larvae was an integral part of the life cycle only in *E. fluviatilis*, whereas other species put their main efforts in producing gemmules as distribution-units. Asexual vs. sexual reproductive strategies in freshwater sponges in running-water habitats is discussed in terms of their prevalence, periodicity and influence of limnological factors. ❐ *Porifera, Spongillidae, life cycle, adaptions, central Europe, running water, river Rhine.*

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A considerable number of publications on life cycles of freshwater sponges are now available (e.g. Gilbert et al., 1975, Frost et al., 1982, Courrèges & Fell, 1989, Bisbee, 1992). Mostly these focus on single life cycle events, such as formation of larvae or gemmulation, and often include only a single species. Only a few papers deal with associations of several species, their colonisation strategies and spatial competition (e.g. Williamson & Williamson, 1979; Mukai, 1989; Pronzato & Manconi, 1991). The present investigation reports on five sympatric species occuring in the Rhine between Karlsruhe and Bonn (Germany), their reproductive strategies and colonisation.

At the beginning of the 20th century Lauterborn distinguished 83 macrobenthic animals in the Rhine (Tittizer et al., 1990). In the 1970's these numbers decreased to 12 species (Conrad et al., 1977), due to an extremely high level of pollution. Since that time great efforts have been undertaken to purify the water, and the number of macrobenthic animals has again risen steadily (Schöll et al., 1995). This species diversity now exceeds that of Lauterborn, whereas the species composition is not the same as in the beginning of the century (Tittizer et al., 1990), due to the huge changes in the Rhine. In recent years ongoing invasions of foreign species (Neozoa: Kinzelbach, 1995) have been taking place, and have influenced the biocoenosis considerably.

Results of Franz (1992) indicate that the Rhine is a highly suitable habitat for sessile filter feeders. Not only is the nutritional situation excellent for these animals due to its eutrophic waters, but the banks are entirely covered by rocks which provide a suitable substrate. Filter feeding is not restricted to sessile animals - in particular many insects also gain their nutrition from filter feeding - and Mann et al. (1972) stated that the productivity of filter feeders is extremely high within waters disturbed by anthropogenic influences.

Nevertheless, our knowledge about freshwater sponges in the Rhine is still fragmentary, despite regular, general studies on the macrobenthic fauna. Such studies usually include sponges, although they are often given only cursory considerations.

This seems surprising since repeatedly high abundances of single species have attracted the attention of researchers in the past (Schön, 1957; Bartl, 1984). Large rivers are characterised by unpredictable changing water levels. This offers and destroys new habitats - a situation which seems difficult to deal with for sessile organisms.

TABLE 1. Collecting sites and dates of collections Key: 1, Near the facilities of the BASF AG. 2, Outflow of the cooling water circuit of the power plant, the temperature is here up to 10°C higher than the surrounding river. 3, Slightly polluted stagnant water. *, Collection undertaken with a grap dredger on board of the research ship 'Argus'.

Locality	22-23.i.93	08-11.v.93	24-26.viii.93*	7-8.ix.93*	25-26.ix.93	6-7.xi.93	22.ii.94	11.v.94*	8.vi.94*	30.vi.94*	15-26.vii.94*	10-11.viii.94	14-15.x.94	15.iii.95	3.viii.95	8.x.95	12-14.x.95
Iffezheim																	x
Neuburg	x	x										x					x
Leimersheim	x	X										x					x
Sondernheim	x	x										x					x
Altrip	x	x										x					x
Ludwigshafen[1]	x	x										x					x
Lampertheim-Rosen-Garten					x	x	x				x		x	x	x	x	
Worms				x													x
Biblis, nuclear power plant[2]					x	x					x		x	x	x		
Biblis, downstream n.p.pl.						x					x						
Worms-Rheinduerk-Heim	x	x										x					X
Gross-Rohrheim											x		x	x			
Gross-Rohrheim, 2km downstream					x	x					x		x	x	x	x	
Gernsheim			x	x	x	x	x				x		x	x		x	
Kornsand			x	x		x	x		x	x	x	x	x			x	
Nierstein	x	x	x														x
Oxbow of Ginsheim[3]					x	x	x				x	x	x				
Mainz-Laubenheim	x	x	x	x					x	x							x
Mainz			x	x				x	x	x							
Heidenfahrt									x			x					
Bingen	x	x						x	x			x					x
Bacharach	x	x										x					x
Boppard	x	x										x					x
Urmitz/Kaltenenger	x	x										x					x
Bad Breisig	x	x										x					x
Remagen																	x
Bonn-Bad Godesberg																	x

METHODS

COLLECTION. Samples were collected from many sites in the Rhine (Germany) (Fig. 1), with dates of collection for each site listed in Table 1. Collections were mostly made from the banks of the river, by wading in the water and removing substrate by hand. Some collections were made with a grab dredger aboard the research ship 'Argus' of the federal state Hesse (indicated with an asterisk in Table 1).

Sponges were removed from the substrate with a knife and preserved immediately in 70% ethanol. From each individual sponge, one microscope slide was prepared.

Individual sponges growing on approximately 1.3m^2 available substrate at each collecting site were counted.

The average number of individual sponges per m^2 of available substrate were calculated (Figs 4-9). Only active colonies were counted, no dead colonies or gemmules without their living mother-sponge.

Methods of preparation for microscopy followed Arndt (1928), with slight modifications. Sponge identification was based

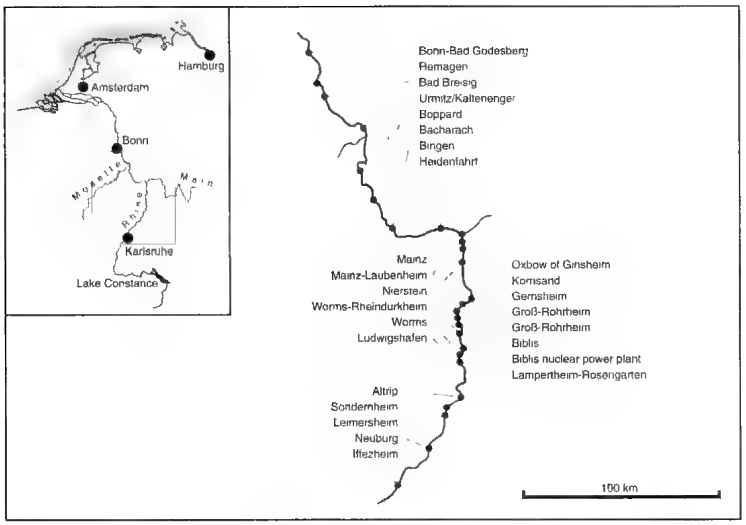

FIG. 1. Rhine collecting sites.

on Arndt (1926, 1928) and Penney & Racek (1968).

Slides prepared during this study are deposited in the Senckenberg Museum of Frankfurt (SMF).

EVALUATION OF THE PERIOD OF FLOODING BEFORE COLLECTION. At each collection site the actual water depth was recorded for all sponge samples. Daily information on water levels at the stations of Worms and Mainz were recorded (Fig. 2), thus for each site the period of flooding before collection could be calculated.

RESULTS

FAUNISTICS. Six species were found in the present study, listed according to their prevalence from abundant to rare: *Trochospongilla horrida* Weltner, 1893; *Ephydatia fluviatilis* (L., 1758); *Spongilla lacustris* (L., 1758); *Ephydatia muelleri* (Lieberkühn, 1855); *Eunapius fragilis* (Leidy, 1851); *Eunapius carteri* (Bowerbank, 1863)

SUBSTRATE. Sponges were found settling on all kind of solid substrates. Within the Rhine this mainly consists of rocks placed to support the river banks; wood is rarely found. Aquatic macrophytes are almost non existent in the investigated area, only once was a small *E. fluviatilis* found epizootic on *Fontinalis* sp. (Bryophyta, Fontinalaceae).

As a rule, the larger rocks (immovable by average currents) were more likely to be colonised with sponges. Smaller rocks (often moved by average currents) were rarely settled by sponges or other sessile organisms. These preliminary data agree with those of Rützler (1965), who studied colonisation by marine sponges in the Mediterranean Sea.

DISTRIBUTION IN RELATION TO DIFFERENT FLOODING REGIMES. The species-assemblages varied considerably between sites dependent on flooding events, comparing sites flooded more than six months before collecting, and those flooded only nine weeks prior to collection (Fig. 3). *Ephydatia fluviatilis* was the only species occurring regularly at the more recently flooded sites, whereas at sites flooded more than six months before collecting this species had the same absolute abundance in colony-counts, but colonies grew much larger. The other species, *T. horrida*, *S. lacustris*, *E. fragilis* and *E. muelleri*, also appeared at both these categories of sites, but only in very small numbers and small size at recently flooded sites (with most having a diameter of less than 1cm).

It was also apparent that differences in flooding events between the sites is a major factor responsible for different depth preferences of sponge species. Places recently flooded were very shallow, often dry, whereas places flooded over 6 months ago were deeper, below the levels affected during river-level fluctuations.

Other factors show no depth dependent variations in the river environment. Nutrient levels should be evenly distributed within the waterbody, due to turbulent currents, and light can only penetrate about 0.75m through the water column due to high turbitidy.

In Figure 3 and subsequent figures only colony-counts are given, where no distinction has been made according to the size or local abundance of colonies.

SEASONAL DEVELOPMENT. The general outline of the development of sponge species associations is given in Figure 4. A generalised model of a life-cycle of a freshwater sponge in the

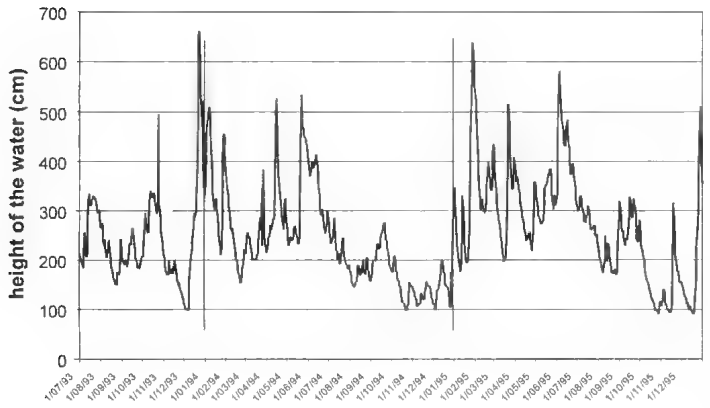

FIG. 2. The niveau of daily water levels at Worms from July 1993-December 1995; the vertical lines indicate the turn of the years (the scale is relative, without a defined zero point).

Rhine is as follows (Fig. 4). In March-April young sponges hatched from their overwintering gemmules. Sponges grew until midsummer (July-August), sexually produced larvae may occur from May to July. Asexual gemmules were produced year-round, but more regularly towards the autumn. In September-October the colonies declined and desintegrated, thus producing overwintering units (gemmules).

SPECIES AUTECOLOGY. Statements about the presence or absence of sexual reproduction usually require histological analysis of specimens, which was not conducted in this study. Under low magnification only fully developed larvae were clearly visible, not the preceding states of larval development, but it was not the goal of this study to describe the life history of these sponges, only to report on the presence or absence of mature larvae, as important indicators on the ecology of species.

Spongilla lacustris. This species formed thick crusts (1-3cm thick); the outline was irregular with rounded edges. Colonies could reach a considerable size (up to $1m^2$). Only very few colonies showed tendencies towards branching growth forms. Colonies of *S. lacustris* always disintegrated in late autumn (October-November). In winter (December-February) only gemmules survived. The first young sponges hatched from gemmules in spring (beginning of April), the number of colonies then rose steadily until October (Fig. 5). The high numbers of colonies reported during the period from October-December were mainly due to these colonies being present at the beginning of October, whereas by the end of October their numbers had declined rapidly. Furthermore, the larger colonies observed in October fragmented into several smaller colonies before dying, so that counts of number of colonies rose before they dropped, and eventually disappeared completely in December-March.

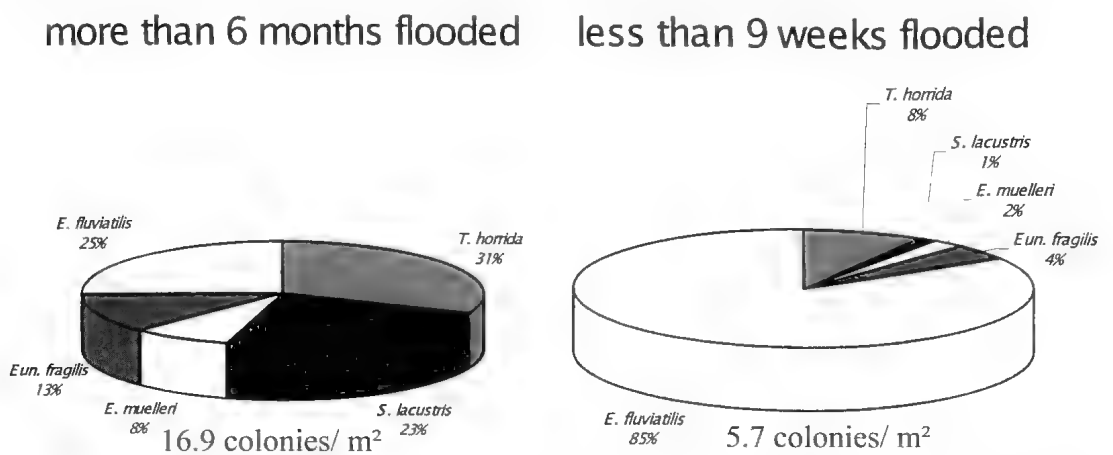

FIG. 3. Species-assemblages at places with different times of flooding before collection (numbers are calculated for $1m^2$): 26 collections were made at sites more than 6 months prior to flooding of collection sites; 28 collections took place at sites less than 9 weeks prior to flooding.

TABLE 2. Life cycle data and ecological strategies of the five sympatric sponge species.

Event	*E. fluviatilis*	*S. lacustris*	*E. muelleri*	*E. fragilis*	*T. horrida*
Time of greatest abundance	October (autumn)	October (autumn)	June (early summer)	May-June (early summer)	August-September (late summer)
Overwintering units	Whole colony	Weakly fixed free gemmules	Fixed free gemmules	Attached gemmule crusts	Attached gemmule crusts
Distribution units	Larvae	Drifting gemmules	Drifting gemmules	Larvae	?
Colonisation of newly established habitats	Through active swimming larvae	Not observed	Not observed	Not observed	Not observed
Ecological strategy	r-strategy	K-strategy	K-strategy	K-strategy	K-strategy

Gemmules generally appeared from August, but their appearance seemed to be less dependent on seasonality and more dependent on colony size. This was also true for other species (see Rasmont, 1962, 1963; Simpson, 1980). Colonies of *S. lacustris* larger than 3cm in diameter always contained at least some gemmules; smaller colonies were mostly free of gemmules, at whatever time of the year they were encountered. Gemmules were built singly within the tissue of the mother-sponge, always within the basal parts of the colonies. The gemmulation process was more regular towards the end of the life span of intact colonies. There were often dense, single-layered carpets of gemmules, resting where they formed. The whole sponges disintegrated after death, but sheltered parts of the skeleton still remained intact so that gemmules resting in these patches of skeletal refugia were bound together and weakly fixed to the substrate. Green gemmules, due to an infestation with unicellular symbiotic algae and a gemmule-polymorphism, as described by Gilbert & Simpson (1976) and Brondsted & Brondsted (1953), were not observed in this study. Larvae were not observed in this species from the Rhine. This was very intriguing given that in other habitats colonies of *S. lacustris* containing larvae were regularly found (e.g. within the outflow of the 'Steinbrücker Teich', a eutrophic pond near Darmstadt, Germany, nearly 50% of the colonies in July 1994 contained larvae).

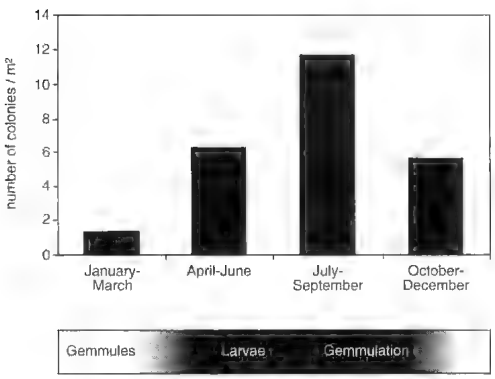

FIG. 4. Seasonal appearance of Spongillidae in general in the Rhine study sites. The white bar indicates when only gemmules are present; the black bar represents times of the year when active colonies are present; the time-scale of the chart corresponds with that of the bar.

In early autumn about 30% of colonies were bright green due to the presence of symbiotic algae (Fig. 5). Only during this part of the year were water levels low enough to provide the preferred habitats for *S. lacustris* (i.e. in slightly deeper, permanently flooded water, Fig. 3), with sufficient light for the successful photosynthesis of symbionts.

Eunapius fragilis. This species formed low crusts (1-2cm thick), with an irregular outline and rounded edges. Rarely it exceeded a diameter of 5cm. Colonies of *E. fragilis* usually disintegrated in summer (July-September). In winter (December-February) intact colonies were rarely found (Fig. 6). The first sponges hatched from gemmules in spring (April). Immediately after hatching the highest numbers of colonies appeared (Fig. 6). The species completed its gemmulation process up until summer (July), after which colonies began to disintegrate. Gemmules were formed *in situ* producing a pavement-like gemmule crust, tightly fixed to the substrate. These gemmules are virtually immovable and it is difficult to perceice how they could contribute to the dispersal within the habitat, whereas in May-June 1994 free movable larvae were found in about 10% of colonies. Colonies containing symbiotic algae were not found within the Rhine, probably because their preferred distribution was in permanently flooded,

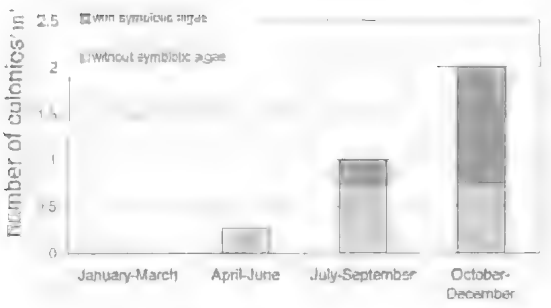

FIG. 5. Seasonal appearance and number of symbiotic colonies in *Spongilla lacustris*.

deeper habitats, where light regimes may be insufficient for photosynthesis (Fig. 3).

Eunapius carteri. This species record from the Rhine is the first time it has been encountered in central Europe (see Gugel, 1995). It was found November 1993 within the cooling-water outflow of the nuclear power plant in Biblis. A detailed description and discussion about its dispersal are given in Gugel (1995).

Ephydatia fluviatilis. This species forms more-or-less thin encrustations (1-2cm thick). Smaller colonies (less than 5cm diameter), had a circular outline, whereas larger ones (more than 7cm diameter), were more irregularly shaped. Colonies of this species grew up to 20cm diameter. *Ephydatia fluviatilis* was regularly seen alive in winter (December-February), in contrast to the other species. Overwintering colonies were small crusts, only 1.5cm diameter, in which no canal systems were visible. These probably survive in a reduced state, as suggested by Arndt (1928) and Weissenfels (1989). In early spring (April) their abundance was only slightly increased in comparison with winter (Fig. 7), probably due to hatching of gemmules (see below), whereas the number of colonies dramatically increased during June-July (Fig. 7), at which time a large-scale production of larvae

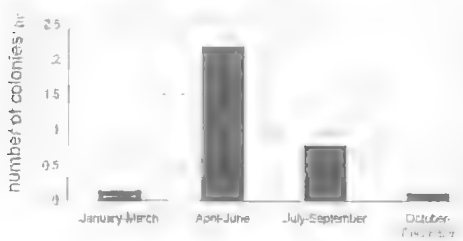

FIG. 6. Seasonal appearance of *Eunapius fragilis*.

took place (June). In July these larvae had settled and built new colonies. The gemmulation process was irregular throughout the whole year and a considerable number of colonies was always devoid of gemmules. When gemmules were present their numbers were reduced: in colonies of 5cm diameter not more than 10 gemmules were found. During fragmentation of sponges few gemmules were freed from the mother-sponge and these 'hatched' in spring. The highest number of colonies was encountered during October-December. As in the case of *S. lacustris*, the large colonies present in autumn fragmented into several smaller colonies, many of which died towards winter (December-February); the overwintering colonies were also small.

In May-June about 25% of colonies produced larvae. This seemed to be the most important event in the life cycle of *E. fluviatilis*, as in July many very small colonies were seen in close proximity to each other, a phenomen quoted as 'Sprühinfektion' (spray infection) by Steusloff (1938). As already indicated, *E. fluviatilis* was the only species which occurred in higher numbers at sites flooded only a few weeks prior to collection (Fig. 3). This was probably due to the more active dispersal of larvae. Symbiotic colonies were never found.

Ephydatia muelleri. Colonies of this species were mostly thickly encrusting (2-4cm thick), with irregular outline and rounded edges. The diameter was rarely more than 10cm. The first colonies of *E. muelleri* appeared in spring (at the beginning of April), and soon after hatching colonies were found in large numbers, peaking during summer (July/August). After completing gemmulation colonies died, usually from the beginning of August to October (Fig. 8). Active colonies were not observed during winter (November-March), only dead colonies with gemmules. *Ephydatia muelleri* often used its entire tissue for gemmule-production, whereas its skeleton remained intact for considerable period of time after the death of the maternal sponge. Large numbers of gemmules were fixed by the skeleton to the place of production. In this way a successful recolonisation at the same site was ensured in the following year. In addition, when single gemmules became free and were no longer fixed to the substrate, they could be distributed by the current within the habitat, providing an effective mechanism for dispersal and recolonisation of adjacent habitats. Sexually produced larvae were not observed in this

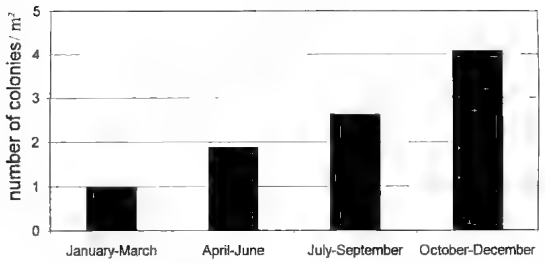

FIG. 7. Seasonal appearance of *Ephydatia fluviatilis*.

species, and only a single symbiotic colony was found (from Sondernheim, in August 1994), close to symbiotic colonies of *S. lacustris*. *Ephydatia muelleri* is mainly distributed in deeper waters, below the levels affected during river-level fluctuations (Fig. 3).

Trochospongilla horrida. Colonies of this species formed thin encrustations (less than 1cm thick), with a very irregular outline. Large colonies may cover an area of $0.5m^2$. *Trochospongilla horrida* began hatching from gemmules in spring (early April). The highest abundance, in both numbers and size of colonies, was reached in summer (August; Fig. 9). In autumn (September-October) colonies always disintegrated and left the gemmule crusts tightly adhered to the substrate. As in *E. fragilis*, gemmules remain fixed to their place of production and it is difficult to imagine that they might be dispersed within the river. Gemmulation commenced in early summer (June-July), and at this time especially *T. horrida* was a successful space-competitor against the otherwise dominating neozoan crustacean *Corophium curvispinum* (Amphipoda). When growing, small colonies tended to fuse with other colonies of the same species, thus forming larger 'super-colonies'. Neither larvae nor colonies containing symbiotic algae were observed. The species was mainly distributed in permanently flooded habitats (Fig. 3).

Ecological strategies for each species are summarised in Table 2.

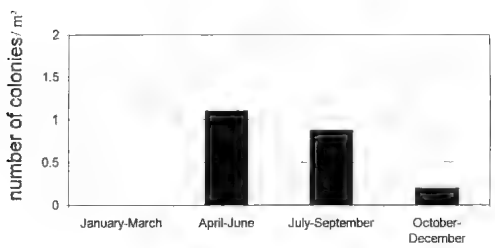

FIG. 8. Seasonal appearance of *Ephydatia muelleri*.

DISCUSSION

Freshwater sponges often display a considerable plasticity in their ecological strategies (Pronzato & Manconi, 1994a), and their life cycles are often adapted to the special requirements of their habitats (e.g. *E. fluviatilis* in temperate regions is usually active during summer and inactive during winter). In hot, arid regions the pattern of activity/inactivity is reversed (Harsha et al., 1983; Corriero et al., 1994). This shows that the life cycle is very adaptable to specific climatic conditions (Pronzato & Manconi, 1994b). According to Pronzato et al. (1993) the life cycle of *E. fluviatilis* seems to be controlled by exogenous factors in regions with strongly oscillating environmental conditions. In more stable habitats endogenous control seems to dominate.

For several species the data presented in the literature differ from those presented here. For example, Bisbee (1992) reported the presence of active colonies year-round in *S. lacustris* from North Carolina. He observed gemmulation in late spring-early summer, sexual reproduction in April, and some sponges disappeared during summer. According to Cheathum & Harris (1953) both *E. fragilis* and *T. horrida* were active year-round in Texas. Pronzato & Manconi (1995) counted up to 324 gemmules cm^{-2} of tissue in *E. fluviatilis* from Sardinia.

Ecological strategies of these species are given in Table 2. *Ephydatia fluviatilis* is considered to be an r-strategist, mainly for its ability to colonise new habitats. In contrast, the remaining species are characterised as k-strategists because they lack this ability.

This general tendency is congruent with results of Pronzato & Manconi (1991), who compared *E. fluviatilis* and *S. lacustris* showing the former to be more successful in colonising new habitats, whereas the latter was more successful as a competitor.

Details in the life cycle of *E. fluviatilis* seem to contradict this classification as an r-strategist: the dominance of sexual vs. asexual reproduction and its year-round presence; these are usually quoted as typical for k-strategists (Pianka, 1970).

The successful colonisation of new habitats is here considered to be due mainly to active

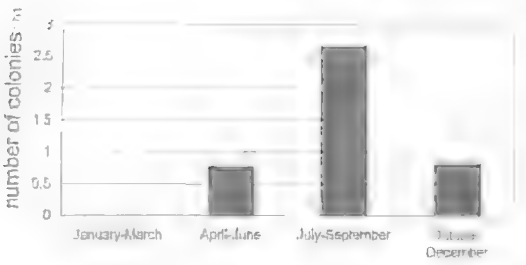

FIG. 9. Seasonal appearance of *Trochospongilla horrida*.

distribution of free larvae. The production of larvae in Spongillidae is mostly controlled by endogenous factors (Gilbert et al., 1975). The seasonal appearance of larvae is confined to a few weeks in the year, and this timing is synchronous between various localities. Leveaux (1941, 1942) reported that production of larvae in *E. fluviatilis* in central Europe is confined to May-June, which corresponds to the results presented here. Since timing in the production of larvae is severely constricted to certain weeks in the year and cannot be altered in the short term, producing larvae does not appear to be an effective strategy to react to unpredictable environmental changes.

The so-called k-strategists (*T. horrida*, *S. lacustris*, *E. muelleri* and *E. fragilis*) have the potential to produce a large number of offspring via their gemmules, traditionally an r-strategy feature. These species do produce a lot of gemmules, but these usually stay at the place of their production (see Table 2).

According to Manconi & Pronzato (1991) *S. lacustris* follows the r-strategy in the short-term, but is essentially a k-strategist in the long term. In many Spongillidae both strategies occur simultaneously (Pronzato & Manconi 1995), even within the same structures: the gemmules serve as distribution-units under an r-strategy, and as resting bodies under a k-strategy.

It was surprising to see that asexual reproduction was clearly dominant over sexual reproduction. Larvae were only observed in *E. fluviatilis* and to a lesser degree in *E. fragilis*.

In the special limnological environment of running water it must be questioned whether or not gemmules are more suitable distribution-units than larvae, aside from their role as resting bodies. They are more robust than larvae, and dispersal is passive via currents. There is no need or advantage in having a capacity for active dispersal.

Van de Vyver & Willenz (1975) reported that in *E. fluviatilis* from Belgium sexual reproduction is confined to overwintering colonies. In that species the development of oocytes commenced in autumn of the year prior to larval production, which occurred the following June. In Belgium, parts of colonies of *E. fluviatilis* survived winter as living, but reduced colonies, similar to the Rhine populations. These results are also confirmed by Weissenfels (1989). Williamson & Williamson (1979) discussed whether or not sexual reproduction was triggered by a pheromone in Spongillidae. According to these authors sexual reproduction occurs rarely in running water because the postulated pheromone would be diluted and ineffective (in contrast to situations in stagnant water). This hypothesis would explain the occurence of many larvae in colonies of *S. lacustris* within the outflow of the 'Steinbrücker Teich' (see above), where the water flows quickly but is only 5-10cm deep. Here, the population of *S. lacustris* is so dense that a pheromone would not be diluted too much. In all colonies of this species at least some gemmules occurred in addition to larvae. The few reports of larvae in *E. muelleri* and *T. horrida* also originate from populations in stagnant water.

The report of larvae in *E. fragilis* occurring in only one year (1994) suggests that sexual reproduction occurs in some years, even in species without regular sexual reproductive strategies.

Many of the different strategies and life-cycles mentioned above help species avoid competition or enhance their competitive abilities. Competitive interactions among sponges, or between sponges and other organisms, are regularly observed in the field. As shown above, species have their highest abundance at different times of the year (Figs 5-9, Table 2). Many details of life histories can be interpreted as mechanisms to enhance species' competitive abilities, including the fact that during periods of highest abundance of *S. lacustris* the proportion of colonies with symbiotic algae is also considerable (Fig. 5). These symbionts strongly enhance the growth of their host (Frost & Williamson, 1980). In *T. horrida* smaller colonies regularly fuse to form larger ones. Neubert & Eppler (1991) discussed wether the competitive ability was reduced, and therefore *T. horrida* was relatively rare compared to other freshwater species. However, my data show that it was the

most abundant sponge in the Rhine, and is also very competitive among sponges and in competition with other organisms. It is concluded that competition for space is the species' main challenge.

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TOWARD A PHYLOGENETIC CLASSIFICATION OF THE MYCALIDS WITH ANISOCHELAE (DEMOSPONGIAE: POECILOSCLERIDA), AND COMMENTS ON THE STATUS OF *NAVICULINA* GRAY, 1867

EDUARDO HAJDU

Hajdu, E. 1999 06 30: Toward a phylogenetic classification of the Mycalids with anisochelae (Demospongiae: Poecilosclerida), and comments on the status of *Naviculina* Gray, 1867. *Memoirs of the Queensland Museum* **44**: 225-238. Brisbane. ISSN 0079-8835.

Phylogenetic relationships for mycalids with anisochelae are revised. Several likely monophyletic species groups are included, currently assigned subgeneric rank or lower, totalling 12 groups, with special reference to *Naviculina*. The type species of *Naviculina*, *N. cliftoni* from SW Australia, is redescribed and its alleged relationship to *Arenochalina mirabilis* is contested, with more suitable affinities to *Aegogropila*. Its main anisochelae are termed here naviculichelae. A preliminary revision of over 230 published species names for *Mycale* and allied taxa with anisochelae was undertaken looking for *N. cliftoni*'s kinship, yielding four likely candidates: *M. cleistochela* (from the W Indian Ocean and Indonesia), *M. diastrophochela* (from the Vema Seamount, SE Atlantic), *M. obscura* (from Indonesia and pan Australia), and *M. peculiaris* (from Papua New Guinea). A phylogeny is proposed for mycalids with anisochelae, although not fully resolved, and alternative phylogenetic classification schemes are hypothesised with discussion on the relative merits of each one.❐ *Porifera, phylogenetic classification, Mycale, Naviculina, phylogeny, Linnean hierarchy.*

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Naviculina was erected by Gray (1867) for Bowerbank's (1864: 252, pl. XXXVII, fig. 152) 'naviculoid spiculum', thought by him to belong to *Hymedesmia*. Gray (1867) was mistaken in calling the spicule 'equibianchorate', since its anisochelate condition was apparent in Bowerbank's illustration. Gray's (1867) concept of Esperiadae, to which he associated *Naviculina*, was essentially based on the possession of sigmas and/or chelae than on any other feature. Despite the excellent state of preservation of the type slide of *N. cliftoni* (Fig. 1), revealing a neat ectosomal reticulation of bundles of mycalostyles, no closer relationship was suggested by Gray (1867) between *Naviculina* or any other mycalid assemblage: *Mycale*, *Aegogropila*, *Grapelia* and *Carmia*. Hooper & Wiedenmayer (1994), on the contrary, considered *Arenochalina* Lendenfeld, 1887, a junior synonym of *Naviculina*, thus postulating a closer relationship between both taxa.

It is the purpose of this article to explore the probable relationships of *Naviculina* and *Aegogropila* Gray, 1867 (as inferred from their sharing of a neat ectosomal reticulation), none of which is a synonym of the other in phylogenetic terms. It is postulated that *Arenochalina* deserves status as a valid subgeneric assemblage of *Mycale*, in view of its possession of a choanosomal stout quadrangular reticulation of spiculofibres (cf. Hajdu & Rützler, 1998). Worldwide records of *Mycale* and allied genera are revised and a list of species which are best allocated to *Mycale (Naviculina)* is proposed. Hajdu & Desqueyroux-Faúndez's (1994) cladistic analysis of the Mycalidae is reconsidered, with the inclusion of *Mycale (Naviculina)*, and the likely monophyletic species-groups of *Mycale*.

PHYLOGENETIC TAXONOMY. The need for a phylogenetic taxonomy has been recently stressed by de Queiroz & Gauthier (1992, and references therein), who claim that taxon names will never be explicit, universal and stable, as envisaged by the implementation of the diverse biological codes of nomenclature, if definitions continue to be assembled from lists of characters (but see Wiley, 1979). By accepting that characters may be reduced (lost), it is easily seen that groupings defined on these terms will frequently be artificial (Sundberg & Pleijel, 1994).

The matching of evolution and systematics implies in the equation of species with population lineages, and of higher taxa with clades

(Christoffersen, 1995, Cantino et al., 1997). De Queiroz & Gauthier (1992) proposed three ways of defining higher taxon names phylogenetically [as ammended by Schander & Thollesson (1995), for the definition of taxon AB as implied by the phylogeny (C, (A,B))]: 1) stem-based definitions, where taxon names are defined as the most inclusive clade that contains taxon A but not taxon C; 2) node-based definitions, where taxon names are defined as the least inclusive clade that contains taxa A and B; and 3) apomorphy-based definitions where taxon names are defined as the most inclusive clade containing some synapomorphy of AB.

Biological classifications are built over categories created over 200 years ago by Linnaeus (1758). Evolutionary ranking adds retrievable information content to the classification, and is thus necessary, but Linnean categories are based on the essentialistic logic of Aristotelis, where ranks are absolutely arbitrary, with no implied meaning across distinct taxonomic groupings (de Queiroz & Gauthier, 1992; Christoffersen, 1995). Is there any sense in comparing an Order of Demospongiae with one such taxon of the Polychaeta? Taxa placed at the same categorical level do not represent equivalent entities (Sundberg & Pleijel, 1994). The Linnean hierarchy has proved a constraint where diversity is considerable, hence the need for a super-subtribe, for instance. Linnean categories add no stability to the names of taxa as changes in rank imply changes in suffixes (at least). It promotes redundancy via mandatory categories and the principle of exhaustive subsidiary taxa (e.g. Cantino et al., 1997). Biological classification might benefit from a change in paradigm.

Given the above rationale, phylogenetic classificatory schemes are proposed for the mycalid phylogeny where evolutionary hierarchy is preserved (retrievable) following some guidelines revised in Amorim (1997). In two of these the Linnean ranking is preserved. In the last proposed classification Linnean ranking is abandoned altogether.

MATERIALS AND METHODS

Specimens were studied under light and scanning electron microscopy. Preparations of thick sections and dissociated spicules were made using procedures described elsewhere (Hooper, 1991, 1997; Hajdu, 1994). Spicule measurements are given as minimum - *mean* - maximum dimensions in micrometres. SEM study was partly performed in a Jeol JSM 35-L machine, under an accelerating voltage of 25KV, working distance of 15mm, and magnifications of up to 3600x; partly in a ZEISS DSM-940 machine, under accelerating voltages between 17 and 19KV, working distance around 8mm, and magnifications up to 10000x.

Phylogenetic analyses were performed using PAUP 3.1.1 (Swofford, 1993) with a choice for the ACCTRAN algorithm. Characters were treated as unordered and equally weighted on a first run. Subsequent weighting was applied on the basis of character's rescaled consistency indices. The phylogenetic classificatory schemes proposed are based on the guidelines revised by Amorim (1997). The four basic rules are: 1) every taxon must be monophyletic, or alternatively, if doubt persist, it must be stated clearly; 2) all the known levels of generality must be recognisable from the classification; 3) sister-group relationships must be recognisable; and 4) it must be possible to know to which larger clade a smaller clade pertains.

Nelson (1972) identified two ways of assigning less general clades to more general ones: subordination and 'sequenciation'. In subordinated classifications sister taxa share the same taxonomic category, and less inclusive clades are always associated to lower ranks than the more universal clades. In sequenced classifications, sister taxa need not share the same rank, but rather, successive lateral branches are associated to the same rank. Subordination has thus the advantage of naming every clade, what may turn into a disadvantage if there are more known levels of generality than taxonomic categories are available (but see Farris, 1976). Still, the finding of additional levels of generality (i.e. the inclusion of new taxa in the phylogeny) implies in considerable rearrangement of categories in a subordinated system. A more basic idea concerns the use of indentation to reflect distinct levels of universality (Wiley, 1979), a strategy adopted below.

In the alternative phylogenetic classifications proposed, no clade is given a new name due to the preliminarity of the exercise undertaken. In the classifications furnished below no choice was made for either subordination or 'sequenciation', as no new name is advanced and no Linnean category changed. Some procedures suggested by Wiley (1979), Amorim (1982, 1994), Christoffersen (1988) and Papavero et al. (1992),

TABLE 1. Rules adopted for the build-up of phylogenetic classifications from phylogenies, and their reference sources.

References	Rules
Wiley (1979)	'sedis mutabilis' for taxa pertaining to politomies
Amorim (1982)	'group+' for the more inclusive unnamed clade, which includes a less inclusive named group and its sister-group. 'group++' for the more inclusive unnamed clade, which includes a less inclusive 'group+' and its sister-group.
Christoffersen (1988)	'[taxon X]' for the lower rank taxon of a monotypic redundant higher rank one, where intermediary categories are simply ommited
Papavero et al. (1992)	'group-1, -2, -3, ...' - clades receive the names of their oldest included genus or species (every other rank is abandoned), to which a negative index is added to indicate the number of speciation events occurred between it and the actual taxon
Amorim (1994)	'group*' for the more inclusive unnamed clade, which includes a less inclusive named group pertainig to a polytomy, and its sister-group

were used in the construction of the classifications advanced below (see Table 1).

Abbreviations: BMNH, The Natural History Museum, London; INV-POR, Instituto de Investigaciones Marinas de Punta de Betin, Santa Marta, Colombia - Porifera collection; MNHN-LBIM, Muséum National d'Histoire Naturelle, Paris, Laboratoire de Biologie des Invertébrés Marins et Malacologie; MNRJ, Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro; SMF, Senckenberg Museum, Frankfurt; UERJ, Universidade do Estado do Rio de Janeiro, Rio de Janeiro; USNM, National Museum of Natural History, Smithsonian Institution, Washington D.C.; USP, Universidade de São Paulo, São Paulo; ZMA, Zoologisch Museum Amsterdam, Amsterdam; ZMH-S, Zoölogisch Museum Hamburg, Schwam/Sponge collection.

SYSTEMATICS

Class **Demospongiae** Sollas
Order **Poecilosclerida** Topsent
Suborder **Mycalina** Hajdu, Van Soest & Hooper
Family **Mycalidae** Lundbeck

Naviculina Gray, 1867

DIAGNOSIS. *Mycale* with an ectosomal skeleton composed of a reticulation of megasclere budles. Anisochelae include naviculichelae (complete or near fusion of both frontal alae, falx markedly expanded along the shaft, lateral alae of the head project backward and upward). Type species: *N. cliftoni* Gray, 1867 (by monotypy).

Naviculina cliftoni Gray, 1867
(Figs 1-2)

Hymedesmia sp.; Bowerbank, 1864: pl. 37, fig. 152.
Naviculina cliftoni Gray, 1867: 538; Hooper & Wiedenmayer, 1994: 293.

MATERIAL. HOLOTYPE: BMNH 1877.5.21.270: Freemantle, Western Australia (type slide). COMPARATIVE MATERIAL. HOLOTYPE of *Mycale diastrophochela* Lévi, 1969: MNHN LBIM DCL1447: Vema Seamount, SE Atlantic. HOLOTYPE of *Mycale cleistochela* Vacelet & Vasseur, 1971: MNHN LBIM DJV36: Tulear, Madagascar. SPECIMENS: *M. cleistochela* ssp. *flagellifer*: MNHN LBIM DJV35: det. J. Vacelet & P. Vasseur, Tulear, Madagascar. ZMA 8512: det. R.W.M. van Soest, Sumbawa, Indonesia. ZMA 8896, 8897, 8912, 8917: det. R.W.M. van Soest, Tarupa Kecil, Indonesia. ZMA 12660: det. R.W.M. van Soest, Mahé, Seychelles. HOLOTYPE of *Mycale obscura* (Carter, 1882): BMNH 1881.10.21.318: Torres Strait, Queensland. SPECIMENS: BMNH 1925.11.1.732: det. M.E.Shaw, Tasmania, Australia. SMF 1041: det. E. Hentschel, Aru, Indonesia. ZMA 1602: det. M. Burton, Indonesian 'Siboga' material. ZMH-S 1670: det. E. Hentschel, Sharks Bay, Western Australia. SPECIMENS of *Mycale* spp.: INV-POR 2198: det. S. Zea, Colombian Caribbean. USNM 34348: det. Mote Marine Lab., off Florida, Gulf of Mexico. USNM 41555: det. E. Hajdu, Florida, Gulf of Mexico. MNRJ 263, 362, 425, 773: det. E. Hajdu, São Sebastião, Brazil.

REDESCRIPTION OF *NAVICULINA* GRAY, 1867. One single thick-section slide preparation remains. It contains a perfectly preserved fragment of the specimen's surface peel, from which it is possible to gather the whole series of spicules in *Mycale*. This peel contains an ectosomal skeleton characterised by a neat reticulation of megasclere bundles (2-6 spicules across) or single megascleres, forming meshes which are mostly triangular (40x70-240x350µm across), and inside which pores are clearly visible (60µm across). Naviculichelae abound inside the

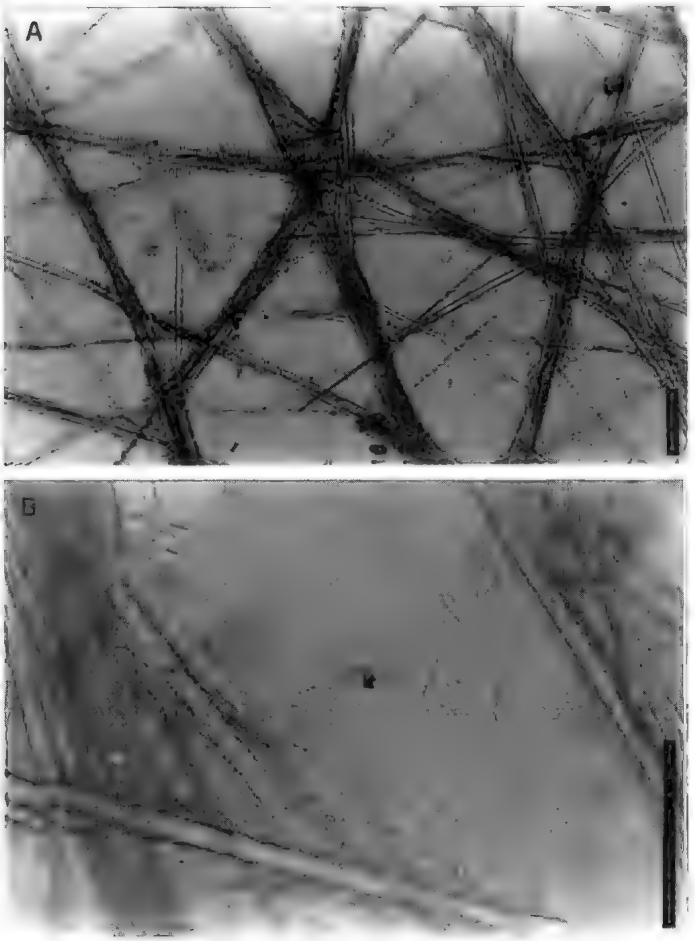

FIG. 1. *Mycale (Naviculina) cliftoni* (Gray, 1867). BMNH 1877.5.21.270. A, Neat ectosomal reticulation of megasclere bundles or single megascleres, most meshes triangular, pores clearly visible. Naviculichelae abound inside the meshes, specially around the bundles of megascleres. B, Mycalostyles, naviculichelae and a single sigma (arrow). Scale bars 100μm.

meshes, especially around the bundles of megascleres (Fig. 1A).

Spicules (light microscopy only, Fig. 1B). Megascleres: Mycalostyles, smooth, mostly straight, slightly fusiform, with elliptic or oval heads, and points which taper more-or-less gradually. Dimensions: 330-*357.4*-388μm long (N=20), 4.8-8.4μm thick (head, N=10), 6-9.6μm thick (shaft, N=10). Microscleres: Naviculichelae, head 60-70% the total spicule length, with narrowing and complete regression of the frontal alae of the head, which may touch the one of the foot, lateral alae of the head projecting backward and slightly upward, downward expansion of the upper falx along the shaft. Dimensions: 12-*17.3*-21.6μm long (N=100). Sigma, slender, smooth, sharp endings. Dimensions: 14.4μm long (N=1).

REMARKS. The term naviculichela is proposed here for Bowerbank's (1864) "naviculiform spiculum". It is a type of anisocleistochela where there is complete or near fusion of both frontal alae (cf. Boury-Esnault & Rützler, 1997), the falx is markedly expanded along the shaft (Fig. 2A), and the lateral alae of the head project backward and upward (Fig. 2B) encircling the shaft. Another common feature is the extreme narrowing of the frontal ala, in such a way that it becomes thinner than the shaft itself (Fig. 2C). The term cleistochelae was first used by Topsent (1925) for the isochelae of a *Clathria*, a much simpler morphotype than that observed in *Naviculina cliftoni*, and related forms (e.g. *Mycale cleistochela* Vacelet & Vasseur, see below).

In *N. cliftoni* over 80% of the naviculichelae are 16.8-19.2μm long, and it is possible there are two categories [possibly 12-16.8 (N=15) and 18-21.6μm long (N=85)], but this is unclear from the distribution of spicule size categories. The origin of the single sigma observed is also dubious, possibly a contaminant.

DISCUSSION

A survey of nearly 230 published descriptions of *Mycale* revealed there were four species bearing naviculichelae-like anisochelae. These are: *M. cleistochela* Vacelet & Vasseur, 1971, *M. diastrophochela* Lévi, 1969, *M. obscura* (Carter, 1882), and *M. peculiaris* Pulitzer-Finali, 1997. However, the status of *M. cleistochela* ssp. *flagellifer* Vacelet & Vasseur, 1971 remains uncertain. It is possibly a separate species based on its distinctive microsclere complement, but a decision is not possible until detailed morphological comparisons are made of both taxa, which is beyond the scope of this present contribution.

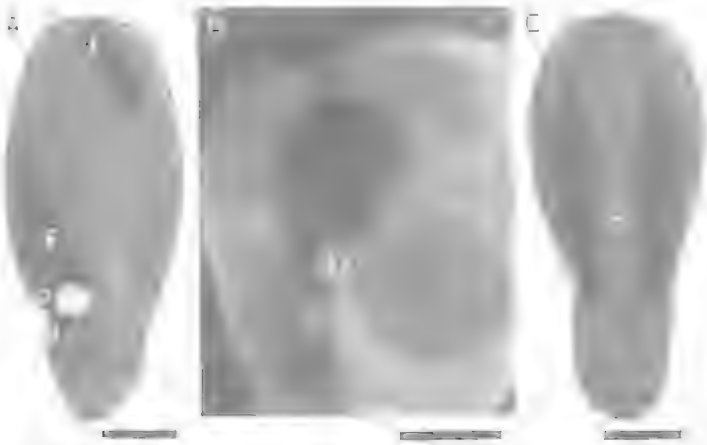

FIG. 2. A, Naviculichelae - anisochelae characterised by the complete or near fusion of both frontal alae (a), falx (f) markedly expanded along the shaft. B, Lateral alae (la) of the head projecting backward and upward, encircling the shaft (s). C, Extreme narrowing of the frontal ala (a), in such a way that it becomes thinner that the shaft itself. (A, C, MNRJ 773, *Mycale (Naviculina)* sp.. B, USNM 41555, *Mycale (Naviculina)* sp. All scales bars 5µm.

All five taxa have neat ectosomal reticulations, potentially referable to *Mycale (Aegogropila)* (i.e. differentiated from other mycalids in having a neat ectosomal reticulation, but no serrated sigmas, and no isochelae). However, this act would be inconsistent with the use of *M. (Paresperella)*, for example, used for species with serrated-sigmas, to my knowledge, all bearing an ectosomal reticulation (Hajdu & Desqueyroux-Faúndez, 1994). A parallel can be made with the use of *M. (Grapelia)* for species with unguiferate anisochelae, all of which possess a confused tangential ectosomal architecture, typical of *M. (Mycale)* (Hajdu, 1995). Despite the fact that these names have been variably used as genera or subgenera in the past, it appears obvious that they comprise assemblages at distinct levels of universality. This is a common problem in the Linnean hierarchy, which has traditionally been ignored by the mere proposal of new scientific names for every assemblage recognisable on the basis of some more-or-less conspicuous trait; coupled to the acceptance of dustbin/plesiomorphic assemblages (i.e. *incertae sedis*).

PROPOSAL OF A FRAMEWORK FOR THE CLASSIFICATION OF THE MYCALIDS WITH ANISOCHELAE. The phylogenetic analysis of the Mycalidae undertaken by Hajdu & Desqueyroux-Faúndez (1994) has been reconsidered in light of the information derived from re-examination of *Mycale (Naviculina)*. Characters and taxa included in the analysis were reevaluated in view of decisions taken in Hajdu (1995). *Mycale (Anomomycale)*, *M. (M.) immitis*-group, *M. (Naviculina)*, *M. (Oxymycale)*, *M. (Rhaphidoteca)*, and *M. (Zygomycale)* have been added to the list of taxa considered here. *Esperiopsis*-I and -II were used as the outgroups, referring to those species conforming to *E. villosa* Carter, 1882 and *E. fucorum* (Esper, 1794), respectively. A list of 22 characters and their 50 states used in the cladistic analysis is given in Table 2. Taxa and their character states are tabulated in the datamatrix shown in Figure 3.

Figure 4 shows the preferred tree, selected with the purpose of advancing a discussion on phylogenetic classifications in mind (see below). It is a majority-rule consensus of 81 trees (50 steps, CI=0.94, RI=0.87, RC=0.82), filtered for more-resolved topologies from 1981 most parsimonious trees generated by PAUP's Branch and Bound exact algorithm for the datamatrix in Figure 3. Characters were treated as unordered, and multistate taxa were considered to be polymorphic. Following the suggestion by Nixon & Davis (1991), both *Mycale (Aegogropila)* and *M. (Carmia)* were split into terminal taxa -I and -II, to account for presence vs. absence of micracanthoxeas, respectively. In this way, the discussion advanced by Carballo & Hajdu (1998) on the status of micracanthoxeas within the mycalids can hopefully be refined. Ideally, this procedure would have been extended to every taxon polymorphic for one or more characters, but this would further reduce the resolution attained in Figure 4, through the addition of many more terminal taxa.

From this analysis neither *Mycale (Aegogropila)* nor *M. (Carmia)* are indicated as likely to be monophyletic. Carballo & Hajdu's (1998) hypotheses 2 and 4 appear more probable explanations for the observed distribution of micracanthoxeas. These hypotheses state, respectively, that either species that possess micracanthoxeas form a monophyletic clade, and one or both subgenera are polyphyletic; or poor taxonomic resolution (and/or interpretation)

TABLE 2. Morphological characters and their character-states used to build the datamatrix in Figure 3.

Characters	Character states
1. Categories of megascleres	0: one, 1: two or more rare, 2: two or more common
2. Main megascleres	0: (mycalo)styles only, 1: exotyles too; 2: oxeas only
3. Three categories of chelae	0: absent, 1: present
4. Basic shape of chelae	0: isochelae, 1: anisochelae
5. Rosettes	0: absent, 1: one category (maybe rare), 2: two categories maybe rare
6. Anisochelae-I with shaft markedly curved on profile view	0: absent, 1: present
7. Anisochelae-I ratio height of the head/total height of the spicule in %	0: > 35, 1: 25-35, 2: < 25
8. Anisochelae-I unguiferate	0: absent, 1: present
9. Anisochelae-I shape of the foot	0: normal (falx basal), 1: with pore (falx hidden within the alae), 2: contorted and denticulated
10. Anisochelae-II acanthose	0: absent, 1: present
11. Anisochelae-II	0: larger than III, 1: can be smaller than III
12. Anisochelae-II and/or III (naviculichelae) with falx extending downward along the shaft considerably	0: absent, 1: present
13. Anisochelae-II and/or III (naviculichelae) with frontal ala of the head extremely narrow (as thick as the falx itself)	0: absent, 1: present
14. Anisochelae-II and/or III (naviculichelae) with lateral alae of the head bent backward encircling the shaft	0: absent, 1: present
15. Anisochelae-III with a basal spur-like projection	0: absent, 1: present
16. Micracanthoxeas	0: absent, 1: present
17. Serrated sigmas	0: absent, 1: present
18. Toxas	0: absent, 1: present
19. Raphides	0: absent or one category, 1: maybe two categories
20. Ectosomal skeleton	0: absent, 1: reticulated; 2: confused
21. Choanosomal skeleton	0: absent, 1: stout quadrangular reticulation
22. Pore-grooves	0: absent, 1: present

prevents us from accessing the occurrence of micracanthoxeas in *M. (Aegogropila*-II) and *M. (Carmia*-II), which would be monophyletic instead.

The strict consensus for the 81 trees selected holds the monophyly of (*Aegogropila*-I, *Carmia*-I) and of (*Anomomycale*, *Mycale* (*Grapelia*, *immitis*-group, *Rhaphidoteca*)). If we exclude the micracanthoxeas as potentially good synapomorphies, due to their largely underestimated occurrence, we are left with: 1) a confused tangential ectosomal skeleton, and 2) anisochelae-I markedly curved in profile view, being the only real synapomorphies within the mycalids with anisochelae.

A posteriori weighting of characters in Figure 3 by their rescaled consistency indices does reduce the number of most parsimonious trees to 416 (36 after filtration), but this occurs at the expense of resolution. The majority-rule consensus is similar to Figure 4, but (*Oxymycale*), (*Naviculina, Paresperella, Zygomycale*), and ((*Aegogropila*-I, *Carmia*-I), *Carmia*-II) compose a polytomy next to the mycalids with a confused tangential ectosomal architecture.

It is interesting to note from the present analysis that the absence of an ectosomal skeleton in *Mycale (Carmia)* appears as a possible subsequent loss, as opposed to the findings reported by Hajdu & Desqueyroux-Faúndez (1994). As argued elsewhere (e.g. Hajdu & Van Soest, 1996), some losses are likely to be easily achieved, and conversely it could be expected that parallel developments might also have occurred. Hajdu & Rützler (1998) reported on a *M. (Aegogropila?)* which can have an ectosomal reticulation, or may lack any ectosomal skeleton whatsoever, thus supporting the hypothesis that such ectosomal architectures have a low adaptive value. In other words, a careful study of species currently assigned to *M. (Carmia)* may indicate a more appropriate allocation in several distinct monophyletic assemblages, related to assemblages bearing ectosomal specialisations. In these cases assemblages sharing the absence/loss of ectosomal specialisation would not form a monophyletic clade, as already foreseen by the inferred relationships between *Arenochalina* and *Carmia*.

PHYLOGENETIC CLASSIFICATION EXERCISES. Several proposals have been made in the specialist systematics literature, as to how a phylogenetic classification (i.e. one that reflects the relationships among taxa, should be

Taxa\Char.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Aegogropila	0	0	01	1	1	0	0	0	0	0	0	0	0	0	0	1	0	1	0	01	0	0
Anomomycale	2	0	0	1	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	2	0	0
Arenochalina	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Carmia	0	0	01	1	1	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0
Grapelia	0	0	1	1	2	1	2	1	1	1	01	0	0	0	1	0	0	0	0	2	0	0
immitis-group	2	0	1	1	1	12	0	1	0	0	0	0	0	0	01	0	0	0	01	2	0	0
Mycale	2	0	01	1	1	0	0	0	0	0	0	0	0	0	01	0	0	0	01	2	0	01
Naviculina	0	0	01	1	1	0	0	0	0	0	0	1	1	1	0	0	0	1	0	1	0	0
Oxymycale	0	2	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Paresperella	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0
Rhaphidoteca	2	1	0	1	1	01	1	0	1	0	0	0	0	0	0	0	0	0	01	2	0	0
Zygomycale	0	0	1	01	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0
Esperiopsis-I	0	0	01	0	01	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Esperiopsis-II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

FIG. 3. Datamatrix showing the 14 mycalid taxa and their character states, for 22 characters used in the phylogenetic analysis undertaken here. (Refer to Table 2 for a list of characters and character states).

constructed (e.g. Nelson, 1972; Griffiths, 1974; Wiley, 1979; de Queiroz & Gauthier, 1990, 1992; Papavero et al., 1992; Papavero & Llorente-Bousquets, 1993; Amorim, 1997). These classifications were proposed under the protocols of subordination and sequenciation, with a minor or major relation to Linnean categories. Both paleontologic, as well as biogeographic data have been variously included in several proposals, thus enhancing enormously the information content of classifications.

There are at least six distinct levels of universality (for clades inferred here to be monophyletic), for mycalids with anisochelae (Fig. 4). If the terminal taxa are considered species-groups, the next five hierarchic levels (each one successively more inclusive than the preceding), could be, for instance subgenera, genera, tribes, subfamilies and families. This is the outcome of extreme commitment to Linnean hierarchy in a subordinated system, where sister taxa are always assigned similar taxonomic rank (Nelson, 1972; Amorim, 1997). In such an arrangement, *Mycale* (mycalids with anisochelae), would be named a family instead. Every time a new clade is found through refining phylogenetic analyses, considerable changes would have to be implemented in the Linnean hierarchies. In an extreme situation, especially applicable for speciose groups, there might be more recognised hierarchic levels than Linnean categories. Farris (1976) proposed a series of prefixes (Super-, Hiper-, Mega-, Giga-, and Sub-, Infra-, Micro-, Pico-) to allow the establishment of a nearly infinite number of categories, but is this what we seek in a pragmatic systematics?

There are alternatives. Amorim (1982, 1994) suggested a coding strategy through which inclusive taxa (e.g. a 'potential family') would receive the name of their most basal taxon (e.g. a genus), coupled with a '+' to state that the 'potential family' includes the mentioned genus plus its sister-group. This occurs when relationships are resolved within the inclusive taxon, whereas if they are not, the chosen name would be that of the oldest taxon coupled to an '*'. Figure 5 shows the translation of the cladogram in Figure 4 into one such classification, using the suggestion by Wiley (1979) regarding the labelling of taxa pertaining to politomies.

The advantages of this system are that phylogeny is retrievable, and changes in hierarchic levels need not reflect changes in Linnean categories, thus confering stability to names used day-by-day by non-specialists. Moreover, taxa may be kept at the hierarchic level to which they are currently assigned, and new names need not be established for every new clade. Instead, use is made of available names coupled to a symbol.

The disadvantage is not exclusive. The same Linnean category may have several distinct ontologic meanings (Fig. 5). What does a subgenus mean? Subgenera represent five distinct levels of generality in this classification. They are employed for the sake of stability only. However, suggestions were made in the past to confer ontologic meaning to Linnean categories.

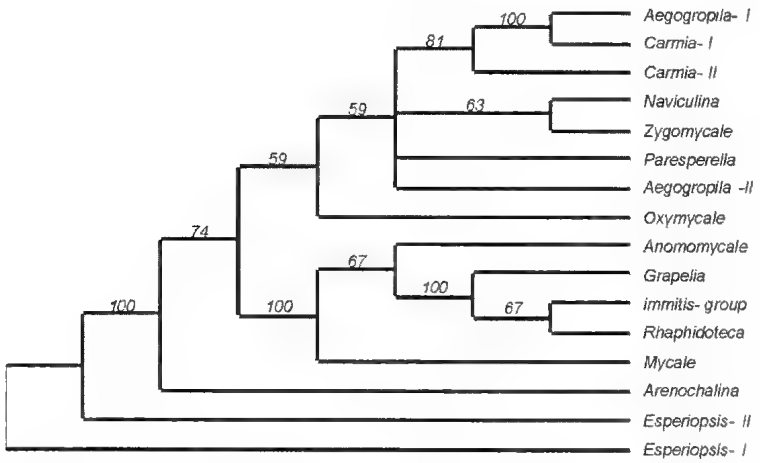

FIG. 4. Majority-rule consensus tree obtained from 81 trees filtered for more resolved topologies, and from 1981 most-parsimonious trees, showing the phylogenetic relationships of mycalids with anisochelae, obtained by analysing the datamatrix in Figure 3 using PAUP. Refer to Table 2 for a list of characters and states.

These include an association between Linnean categories and a palaeontological age (e.g. Hennig, 1966), and the association of categories to their supposed biogeographic origin (Amorim, 1992). Unfortunately, neither source of evidence is readily available for the mycalids, or for that matter for most of the Poecilosclerida.

The oldest likely anisochelae-bearing mycalid (evidenced from a single palmate anisochela), is, to my knowledge, from the Early Cretaceous (Albian) (Wiedenmayer, 1994). This anisochela is only tentatively assigned to *Mycale*, and no finer allocation is possible. The next anisochela in the geologic scale is a curved, palmate form described by Gruber & Reitner (1991) (Lower Campanian, Cretaceous). This anisochela was assigned to a group within *Mycale*, the curved-assemblage of Hajdu (1995; as 'sp.2'). Its position in the cladogram (Hajdu, 1995, fig. 7.4) is more basal than both *M. (Grapelia)* and *M. (M.) immitis* species-group, but it is more derived in comparison to the remaining *M. (Mycale)*. In other words, there are subgenera of *Mycale* that are probably younger than the Campanian (ca. 80 Myr), and others that are probably older. Hajdu's (1995) 'sp. 3' is from the Eocene-Oligocene transition, thus younger than both records cited above, but sits in a more basal position in the cladogram. It is, therefore, pointless to assign geologic ages to categories within *Mycale*, on the basis of such a meager and patchy database.

An alternative methodology is to look for biogeographic origin. According to Van Soest (1994), most demosponge higher taxa (suprageneric taxa) have notably wide distributions, an indication of their probable early ancestry (e.g. Van Soest & Hajdu, 1997). Knowledge of global tectonic events prior to the Triassic is fragmentary, so that no precise link would be possible between clades supposed to have originated before the break-up of Pangea and some likely original crustal plate. Additionally, Amorim (1997) stressed that any implementation of a classification in which clades are directly linked to biogeographic categories is clearly dependent on the elaboration of a well established general area cladogram.

Marine areas have been dealt with recently by many sponge specialists (Hooper & Lévi, 1994; Hajdu, 1995; Van Soest & Hajdu, 1997), and the general area cladograms generated would certainly form a framework over which to advance a classification along the lines suggested by Amorim (1992). Further speculation in this direction, however, is not possible until we draw a much clearer picture on the distribution of mycalids, as well as obtain well-supported cladograms for marine areas: all necessary prerequisites for the implementation of Amorim's (1992) suggestions.

For the time being, I propose a working hypothesis (Fig. 6), as a way of overcoming the problem of multiple significance of Linnean categories arrived at in Figure 5. This scheme takes into consideration the suggestion of Chiristoffersen (1988) on redundant taxa (Table 1), used here as an artifact to respect the hierarchic level of Linnean categories. The fundamental taxon is the genus *Mycale*. The terminal taxa are either subgenera or monophyletic species-groups, such as the *M. (M.) immitis*-group. Intermediate hierarchic levels are simply named 'groups', with their terminal taxa included within brackets, if monotypic.

Finally, a phylogenetic classification (Fig. 7), based on the cladogram in Figure 4, was built

Genus *Mycale* Gray, 1867
.....Subgenus *Arenochalina* Lendenfeld, 1887
.....group *Mycale*++
.....group *Mycale*+
.....Subgenus *Mycale*
.....group *Anomomycale*+ Topsent, 1924
.....Subgenus *Anomomycale*
.....group *Grapelia*+
.....Subgenus *Grapelia*
.....group *Rhaphidoteca*+ Kent, 1870
.....Subgenus *Rhaphidoteca*
.....*Mycale immitis* (Schmidt, 1870) species-group
.....group *Oxymycale*+ Hentschel, 1929
.....Subgenus *Oxymycale*
.....group *Aegogropila*-II* Gray, 1867
.....Subgenus *Aegogropila*-II *s. m.*
.....Subgenus *Paresperella* Dendy, 1905 *s. m.*
.....group *Carmia*-II+ *s. m.*
.....Subgenus *Carmia*-II
.....group *Aegogropila*-I+
.....Subgenus *Aegogropila*-I
.....Subgenus *Carmia*-I
.....group *Naviculina*+ Gray, 1867 *s. m.*
.....Subgenus *Naviculina*
.....Subgenus *Zygomycale* Topsent, 1930

FIG. 5. Phylogenetic classification of the mycalids with anisochelae built from the cladogram in Figure 4, by subordination, incorporating the coding strategy of Wiley (1979), and Amorim (1982). Key: 'group+', more inclusive taxon containing the taxon formally described with that name (the basalmost taxon, preserving its current Linnean hierarchic status) and its sister-group; 'group*', more inclusive taxon (the oldest available name included), the relationships of its included taxa being unresolved. The priority of *Rhaphidoteca* Kent, 1870 over the *Mycale immitis* (Schmidt, 1870) species-group was decided not on the basis of knowledge of the actual dates of publication of both works, but on the fact that the species-group is bound to be named in the future, becoming then a much younger taxon; *s.m., sedis mutabilis*.

under the provocative protocol of Papavero et al. (1992) and Papavero & Llorente-Bousquets (1993), in which Linnean categories are simply abolished. Names considered are only those of genus- and/or species-level taxa, eventually coupled to an index which indicates their hierarchic level on the phylogeny.

For example, *Mycale immitis* (Schmidt, 1870) was used instead of *M. (M.) immitis*-group of Hajdu (1995). Its coupling to a '-5' index means that the supposed ancestor of the *M. (M.) immitis*-group (the terminal taxon used in the present analysis), is 5 hypothetic ancestral species away from the true *M. immitis* species (Hajdu, 1995; fig. 7.4, 'species 10'). Thus, ancestor '-1' is the ancestor of *M. immitis* + its sister group ('species 6-9' of Hajdu, 1995); ancestor '-2' refers to 'species 1-10'; ancestor '-3' to 'species 1-12'; ancestor '-4' to 'species 1-16'; and, ancestor '-5' to the entire *M. (M.) immitis*-group (viz. 'species 1-17' of Hajdu, 1995).

Accordingly, *Mycale*$_{-5}$ is the appropriate nomenclature in this case because *Mycale* is the oldest available name within the studied clade (page and line priority considered), coupled to the observation that the root of the clade containing all the mycalids with anisochelae is three ancestors away from the terminal *M. (Mycale)*. Ancestor '-1' is the ancestor of (*Myc*, (*Ano*, (*Gra*,(*Rha*, *imm*-group)))). Ancestor '-2' refers to the latter clade and its sister taxon. (*Aeg*-II, *Par*, (*Nav*, *Zyg*), ((*Aeg*-I, *Car*-I), *Car*-II)). And, ancestor '-3' to the whole ingroup.

PHYLOGENETIC DIAGNOSES FOR THE TERMINAL TAXA CONSIDERED. The cladogram in Figure 4 is a weakly supported working hypothesis. Accordingly, there are unnamed, more-inclusive clades remaining because relationships are bound to shuffle with the inclusion of additional terminal taxa. Nevertheless, this does not preclude the establishment of phylogenetic diagnoses for the taxa considered, as any phylogenetic hypothesis is better than no hypothesis at all. The cladogram (Fig. 4) is viewed as an improvement over the hypothesis put forward by Hajdu & Desqueyroux-Faúndez (1994) because it is a more comprehensive sample of probably monophyletic

Genus *Mycale* Gray, 1867
.....group *Arenochalina* [Subgenus *Arenochalina* Lendenfeld, 1887]
.....group *Mycale*+ +
.....group *Mycale*+
. ...group *Mycale* [Subgenus *Mycale*]
.....group *Anomomycale*+ Topsent, 1924
.....group *Anomomycale* [Subgenus *Anomomycale*]
.....group *Grapelia*+ Gray, 1867
.....group *Grapelia* [Subgenus *Grapelia*]
.....group *Rhaphidoteca*+ Kent, 1870
.....Subgenus *Rhaphidoteca*
....*Mycale immitis* (Schmidt, 1870) species-group
.group *Oxymycale*+ Hentschel, 1929
.....group *Oxymycale* [Subgenus *Oxymycale*]
.....group *Aegogropila*-II* Gray, 1867
.....group *Aegogropila*-II [Subgenus *Aegogropila*-II] *s. m.*
.....group *Paresperella* Dendy, 1905 [Subgenus *Paresperella*] *s. m*
.....group *Carmia*-II+ Gray, 1867 *s. m.*
.....group *Carmia* [Subgenus *Carmia*-II]
.....group *Aegogropila*-I+
.....Subgenus *Aegogropila*-I
.....Subgenus *Carmia*-I
.....group *Naviculina*+ Gray, 1867 *s. m.*
.....Subgenus *Naviculina*
.....Subgenus *Zygomycale* Topsent, 1930

FIG. 6. Phylogenetic classification of the mycalids with anisochelae built from the cladogram in Figure 4, by subordination, incorporating the coding strategy of Wiley (1979), Amorim (1982) and a parallel of Christoffersen's (1988). Key: 'group+', more inclusive taxon containing the taxon formally described with that name (the basalmost taxon, preserving its current Linnean hierarchic status) and its sister-group; 'group*', more inclusive taxon (the oldest available name included), the relationships of its included taxa being unresolved; *s.m.*, *sedis mutabilis*.

species-groups within *Mycale* than the earlier attempt.

The proposed scheme is as follows:

Subgenus *Aegogropila*-I - *Mycale* with a reticulated tangential ectosomal skeleton and micracanthoxeas (many with toxas, and three categories of anisochelae).

Subgenus *Aegogropila*-II - *Mycale* with a reticulated tangential ectosomal skeleton (many with toxas, and three categories of anisochelae).

Subgenus *Anomomycale* - *Mycale* with a confused tangential ectosomal skeleton and anomochelae.

Subgenus *Arenochalina* - *Mycale* without any ectosomal skeletal specialisation, and with a stout choanosomal architecture composed of spiculofibres arranged in quadrangular meshes.

Subgenus *Carmia*-I - *Mycale* without any ectosomal skeletal specialisation and micracanthoxeas (many with toxas, and three categories of anisochelae).

Subgenus *Carmia*-II - *Mycale* without any ectosomal skeletal specialisation (many with toxas, and three categories of anisochelae).

Subgenus *Grapelia* - *Mycale* with a confused tangential ectosomal skeleton, three categories of anisochelae, anisochelae-I with a curved shaft in profile view, ratio height of the head/total height of the spicule < 25%, alae of the foot projecting downward forming a pore, and rosettes built both by anisochelae-I and -II (many with unguiferate anisochelae-I, acanthose anisochelae-II, and basally-spurred anisochelae-III).

Subgenus *Mycale* - *Mycale* with a confused tangential ectosomal skeleton (many with pore-grooves, three categories of anisochelae, basally-spurred anisochelae-III, and rhaphides in two categories).

Mycale (Mycale) immitis-group - *Mycale* with a confused tangential ectosomal skeleton, anisochelae-I with a curved shaft in profile view, ratio height of the head/total height of the spicule > 25% and < 35%, alae of the foot projecting downward forming a pore (many with pore-grooves, three categories of anisochelae, basally-spurred anisochelae-III, and rhaphides in two categories).

Subgenus *Naviculina* - *Mycale* with a reticulated tangential ectosomal skeleton, and naviculichelae (many with three categories of anisochelae, and toxas).

Subgenus *Oxymycale* - *Mycale* with a reticulated tangential ectosomal skeleton and megascleres which are oxeas exclusively.

Subgenus *Paresperella* - *Mycale* with a reticulated tangential ectosomal skeleton and serrated sigmas (many with toxas).

1. *Mycale*$_{-3}$ Gray, 1867

2. *Mycale*$_{-2}$ Gray, 1867; *Arenochalina* Lendenfeld, 1887

3. *Mycale*$_{-1}$ Gray, 1867; *Aegogropila*-II$_{-2}$ Gray, 1867

4. *Mycale* Gray, 1867; *Grapelia*$_{-2}$ Gray, 1867

5. *Grapelia* $_{-1}$ Gray, 1867; *Anomomycale* Topsent, 1924

6. *Grapelia* Gray, 1867; *Rhaphidoteca*$_{-1}$ Kent, 1870

7. *Rhaphidoteca* Kent, 1870; *Mycale immitis*$_{-5}$ (Schmidt, 1870)

8. *Aegogropila*-II$_{-1}$ Gray, 1867; *Oxymycale* Hentschel, 1929

9. *Aegogropila*-II Gray, 1867: *Paresperella* Dendy, 1905; *Aegogropila*-I$_{-2}$ Gray, 1867; *Naviculina*$_{-1}$ Gray, 1867

10. *Aegogropila*-I$_{-1}$ Gray, 1867: *Carmia*-II Gray, 1867

11. *Aegogropila*-I Gray, 1867; *Carmia*-I Gray, 1867

12. *Naviculina* Gray, 1867; *Zygomycale* Topsent, 1930

FIG. 7. Phylogenetic classification of the mycalids with anisochelae built from the cladogram in Figure 4 under the protocol of Papavero, Llorente-Bousquets & Abe (1992) and Papavero & Llorente-Bousquets (1993). Linnean categories are abolished, only genus- and species-level taxa are considered, hierarchy is retrievable from a numbered sequence attributed to the oldest taxon included (priority is applied to pages and lines also). The priority of *Rhaphidoteca* Kent, 1870 over *Mycale immitis*$_{-5}$ (Schmidt, 1870) was decided not on the basis of knowledge of the actual dates of publication of both works, but on the fact that the species-group represented by *M. immitis*$_{-5}$ is bound to be named in the future, becoming then a much younger taxon. (Refer to the text for further explanations).

Subgenus *Rhaphidoteca* - *Mycale* with a confused tangential ectosomal skeleton, exotyles, and anisochelae-I with alae of the foot projecting downward forming a pore (ratio height of the head/total height of the spicule may be > 25% and < 35%, rhaphides may be in two categories).

Subgenus *Zygomycale* - *Mycale* with a reticulated tangential ectosomal skeleton and isochelae next to anisochelae (many with three categories of anisochelae, and toxas).

Phylogenetic definitions for the above taxa based on apomorphies can be obtained by referring each clade to all the species sharing that clade's synapomorphies, and those of all its descendants. Apomorphy-based definitions have been severely criticised, however, because subsequent discovery of homoplasies can lead to substantial reshuffling of clades (e.g. Schander & Thollesson, 1995). The alternative option - using node-based definitions for the terminal taxa considered above - would be premature at this stage. The definition of more-inclusive taxa is dependent upon an unambiguous understanding of the less-inclusive taxa it contains. Cantino et al. (1997) chose to build their node-based definitions using only species level taxa, which were selected in such a way so that the more basal genera included in the clade would be represented. These kind of data are absent, or nearly so, for most of the terminal taxa considered here. Where this information is available, node-based definitions can be powerful taxonomic tools (explicit, universal and stable). Hajdu (1995) published a phylogeny for the curved-assemblage of *Mycale*, which permits the derivation of node-based phylogenetic definitions for the *immitis*-group, *Rhaphidoteca* and *Grapelia*.

This scheme is as follows:

Subgenus *Grapelia* - the least inclusive clade that contains *Mycale myriaselera* Lévi & Lévi, 1983 and *Mycale burtoni* Hajdu, 1995.

Subgenus *Rhaphidoteca* - the least inclusive clade that contains *Mycale marshallhalli* (Kent, 1870) and *Mycale loricata* (Topsent, 1896).

Mycale (Mycale) immitis-group - the least inclusive clade that contains *Mycale trichela* Lévi, 1963 and *Mycale paschalis* Desqueyroux-Faúndez, 1990.

CONCLUSIONS

This discussion illustrates that current poriferan classifications may be very distant from truly phylogenetic schemes. While debate

persists on the merits and pitfalls of retaining the Linnean hierarchy, this does not excuse any proposal based on non-phylogenetic definitions for poriferan taxa. It is imperative that taxa are always diagnosed on the basis of their synapomorphies. This makes them more likely to be natural, and more relevant to future phylogenetic classification schemes. This is especially important when dealing with more-inclusive taxa, from which less-inclusive groups are extracted on the basis of their clearer monophyletic status. If, as is the current trend, effort is made toward defining such inclusive, plesiomorphic taxa (but excluding the extracted, less-inclusive taxa), it is likely that a paraphyletic assemblage will be recognised instead. In the phylogenetic system, groups such as these are going through a metaphorical 'mass-extinction episode' right now.

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PHOTOSYNTHESIS AND RESPIRATION OF THE CYANOBACTERIUM-CONTAINING SPONGE, *DYSIDEA HERBACEA*. *Memoirs of the Queensland Museum 44: 238. 1999*:- Marine sponges containing cyanobacterial endosymbionts are common in tropical waters, and the dictyoceratid sponge, *Dysidea herbacea*, is one of the most abundant sponges in the shallow lagoon at One Tree Reef, Great Barrier Reef. This sponge contains large numbers of the filamentous cyanobacterium, *Oscillatoria spongeliae*. The *O. spongeliae* trichomes are located free in the sponge mesohyl, although they are often in contact with archaeocytes. The high biomass of the cyanobacteria is illustrated by the chlorophyll *a* content of the association, which is about 335µg.mL^{-1} sponge volume, or 180.3µg.g^{-1} sponge wet weight. These values are much higher than for any other sponges so far studied.

Photosynthetic and dark respiration rates were measured using an oxygen electrode in summer and winter at ambient lagoon temperatures and at saturating irradiances. The compensation point for photosynthetic O_2 production is reached at about 30-50µmol photons.m^{-2}.sec^{-1} and photosynthesis saturates at about 300µmol photons.m^{-2}.sec^{-1}. No seasonal differences in the photosynthetic and respiration rates could be detected indicating that the sponge adapts to changing environmental conditions. The *D. herbacea/ O. spongeliae* association, does however respond to changes in temperature, with a Q_{10} for photosynthesis of about 5. Photosynthesis and respiration rates are also sensitive to the O_2 concentration in the seawater. The implications of these results for the ecology of this symbiotic association will be discussed. ❐ *Porifera, Dictyoceratida, cyanobacterium, symbiosis, photosynthesis, respiration, temperature.*

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MORPHOLOGICAL AND GENETIC EXAMINATION OF PHENOTYPIC VARIABILITY IN THE TROPICAL SPONGE *ANTHOSIGMELLA VARIANS*

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Hill, M.S. 1999 06 30: Morphological and genetic examination of phenotypic variability in the tropical sponge *Anthosigmella varians*. *Memoirs of the Queensland Museum* **44**: 239-247. Brisbane. ISSN 0079-8835.

Sponges commonly exhibit phenotypic variation in response to a heterogeneous environment. Determining the ecological causes and understanding the evolutionary consequences of this variation is a primary goal of biologists. Three ecotypes of the common Caribbean sponge *Anthosigmella varians* (Demospongiae: Hadromerida: Spirastrellidae) have been identified, and they provide a model system for exploring morphological variation. *Anthosigmella varians* forma *incrustans* is an encrusting bioeroder located on fore- and back-reef environments at depths ranging from 3->30m; *A. varians* forma *varians* is an irregularly lobate branching ecotype found in shallow (<1m) water near-shore; and *A. varians* forma *rigida* is a branching form found in sympatry with *incrustans* but restricted to shallower depths. In this paper, a detailed examination of ecologically important characters (e.g. tissue strength, skeletal properties, distribution) for all 3 morphs is presented. Using allozyme electrophoresis, fixed differences at two loci were discovered indicating potential reproductive isolation between branching (*rigida* and *varians*) and encrusting (*incrustans*) morphotypes. Results from a transplantation experiment indicate that sediment load may be an important factor in branch production. Sedimentation may also explain the competitive aggressiveness of *incrustans* which is often found growing over coral species (i.e. *A. varians* grows over corals to gain access to $CaCO_3$ skeletons which are typically low sediment zones). It is proposed that *rigida* populations can exist on the reef due to the production of a spicule- and collagen-rich cortex that provides a structural defense against predators. It is suggested that wave energy may have less important effects on branch production in *A. varians* than either predation or sedimentation. ❐ *Porifera, phenotypic plasticity, spongivory, population structure, cryptic species, Anthosigmella varians.*

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Sponges present a unique challenge to our understanding of evolutionary processes in the sea. This ancient group of metazoans has few traits that are taxonomically useful for distinguishing closely related species, and those that are employed in assigning taxonomic positions can often be modified by environmental parameters. For example, wave energy has been demonstrated to affect gross morphological characteristics of intertidal sponges (Palumbi, 1984, 1986) and changes in spicule size and shape can often reflect the influence of extrinsic factors (e.g. Jones, 1970, 1971). Thus, there is a danger of misinterpreting the evolutionary history of this metazoan group. Determining when morphological variation in sponges represents phenotypic plasticity or true speciation is a significant challenge.

Several investigators have shown that morphological variants previously included within a single sponge taxon represent distinct species (Sole-Cava & Thorpe, 1986, 1990; Sole-Cava et al., 1992; Bavestrello & Sara, 1992; Barbieri et al., 1995). These studies indicate that we know little about sponge diversity, and ultimately, evolutionary processes in the ocean. These studies also highlight how poorly we understand the importance of phenotypic variation in the ecology and evolution of sponges. When faced with morphological varieties of a species, two questions must be addressed. First, are the phenotypic variants reproductively isolated? This can be inferred by examining the genetic constitution of populations. Second, if the variants are part of an interbreeding population, are environmental factors responsible for the observed variation, and if so, which are most important? These questions are of particular concern on tropical coral reefs since ongoing debate concerns the relative importance of equilibrial and nonequilibrial processes in the

maintenance and origin of diversity (Sale, 1994; Knowlton & Jackson, 1994a, b). If environmental parameters are highly predictable, then niche partitioning via habitat specialisation may be a common product of evolution on coral reefs (Knowlton & Jackson, 1994a; Palumbi, 1994). If, however, larvae settle randomly into diverse habitats, or disturbance frequencies differ among habitats, then phenotypic plasticity may evolve (e.g. Lively, 1986).

Sponges present a highly tractable system for examining ecological conditions necessary for the evolution of phenotypic plasticity. They also provide a model for identifying factors important in the evolution of reproductive isolation (i.e. speciation) between morphologically divergent populations. Here, I present an analysis of morphological and genetic differences among morphotypes of the common Caribbean sponge *Anthosigmella varians* (Demospongiae: Hadromerida: Spirastrellidae). In addition, I discuss the roles that phenotypic plasticity and habitat specialisation may have played in the evolution of this species.

NATURAL HISTORY. *Anthosigmella varians* (Duchassaing & Michelotti) is a common sponge of Caribbean coral reefs (Wiedenmayer, 1977; Vicente, 1978). This sponge harbors intracellular dinoflagellate zooxanthellae, bores into calcium carbonate structures (Hill, 1996a) and exhibits two distinct morphologies: branching and encrusting (Wiedenmayer, 1977). It is included in the diets of angelfish (Randall & Hartman, 1968; Hourigan et al., 1989) and at least some parrotfish species (pers. obs.; Dunlap & Pawlik, 1996; Wulff, 1997). Taxonomists recognise two morphotypes: an encrusting growth form (forma *incrustans*) and an amorphous, irregularly lobate, branching growth form (forma *varians*), and consider these to be ecophenotypes based on their occurrence in different habitats (Wiedenmayer, 1977). *Anthosigmella varians* forma *incrustans* (hereafter referred to as *incrustans*) is conspicuous on fore- and back-reefs while *A. varians* forma *varians* (hereafter referred to as *varians*) is typically found in shallow, lagoonal areas.

In the Florida Keys, USA, *varians* is found close to shore on both bay and ocean sides of many islands; *incrustans* is only found on the reefs which run parallel to the islands approximately 8km from shore (Fig. 1). Wave energy has been proposed as the factor responsible for this distribution: *varians* is presumed to be unable to handle the strong currents and periodic heavy wave action that open reefs receive (Vicente, 1978). During this study, a number of branching morphs sympatric with encrusting morphs were encountered on both Tennessee and Alligator fore-reefs in the Florida Keys (as well as Molasses Reef; J. Pawlik, pers. comm.) (Fig. 1, Table 1). This morph has lobate branches like *varians* but is easily distinguishable due to its much stiffer skeletal construction. It was often found less than a meter from *incrustans* individuals. I refer to this morph as *A. varians* forma *rigida* (hereafter as *rigida*).

MATERIALS AND METHODS

There were three components to this study: 1) comparison of morphological and ecological characteristics of the three morphs of *A. varians*; 2) transplant experiments to assess the effect of sedimentation on morphological variation in *varians* and *rigida*; and 3) allozyme analysis, used to estimate genetic relatedness among *A. varians* morphs.

MORPHOLOGICAL CHARACTERISTICS. *External*. The following external features were measured in situ; surface area of attachment, number of branches, branch length, maximum height, mound height and tissue strength. Sample sizes for all parameters are listed in Table 1. Surface area of attachment was estimated using a $1m^2$ quadrat marked off in $25cm^2$ increments. Branch length was measured as the distance from tip to node. Maximum height was measured from the highest point of the sponge perpendicular to the substratum. Mound height was measured from the substratum to the highest point on the mounding portion of branching individuals.

The method for determining tissue strength was adapted from Palumbi (1984). A barbless hook, attached to a spring scale, was embedded to a depth of 0.5cm into the surface tissue of an individual. Care was taken to ensure that there was no foreign material in the sponge tissue. The spring scale was pulled perpendicularly until the hook tore free, and the maximum force required to extract the hook was recorded. Three pulls were averaged for each sponge, sample sizes for each morph are shown in Table 1. One-way ANOVA was used to compare morphotypes (Zar, 1984).

Internal. Several internal characters were also compared among morphs. Cortex thickness was measured for seven individuals from each morph, 10 measurements were averaged for each individual. The cortex of a sponge is defined as

TABLE 1. Distribution and morphological characteristics of three morphotypes of *Anthosigmella varians* in the Florida Keys. Values represent means (±SE); sample sizes are shown. Values sharing a superscripted letter are not significantly different at P >0.05.

Parameter	*incrustans*		*varians*		*rigida*	
Distribution	Reef	n	Bay/near island	n	Reef	n
Depth (m)	8 - 27		1 - 3		8 - 13	
Number of branches	0a	48	2.95 (0.3)^{b}	55	1.92 (0.62)^{c}	13
Branch length (cm)	n/a	-	10.37 (0.51)^{a}	161	4.36 (0.79)^{b}	25
Mound Height (cm)	n/a	-	3.47 (0.28)^{a}	38	3.38 (0.64)^{a}	13
Maximum Height (cm)	n/a	-	12.95 (1.22)^{a}	55	6.67 (1.8)^{b}	13
Area of attachment (cm^2)	1430.6 (300.5)^{a}	48	68.1 (14.4)^{b}	32	36.3 (4.79)^{c}	13
Tissue strength (N)	4.9 (0.36)^{a}	28	2.3 (0.14)^{b}	61	>10^{c}	8
Cortex thickness (mm)	0.99 (0.14)^{a}	7	n/a	-	5.7 (1.33)^{b}	7
Open Space in choanoderm	10.1% (1.8)^{a}	7	32.7% (2.1)^{b}	7	21.1% (1.9)^{c}	7
Spicule conc. (mg cm^{-3})	52 (2)^{a}	7	112 (5)^{b}	8	168 (11)^{c}	5
Zooxanthella cm^{-2} (H 10^{6})	0.86 (0.053)^{a}	8	1.37 (0.092)^{b}	13	1.03 (0.82)$^{a/b}$	3
Wet weight (g cm^{-1})	1018 (104)^{a}	6	833 (39)^{a}	10	759 (105)^{a}	4
Sponge biomass (g cm^{-3})	80.8 (8.2)^{a}	6	100.8 (3.8)^{a}	10	97.2(7.6)^{a}	4
Spicule (mg cm^{-3})	70.5 (13.9)^{a}	6	132.6 (15.0)^{b}	10	199.7 (17.4)^{c}	4
Calcareous debris (g cm^{-3})	553.9 (66.8)^{a}	6	-2 (7.3)^{b}	10	-3 (1.1)^{b}	4

the layer of ectosome consolidated by a distinctive skeleton (Kelly-Borges & Pomponi, 1992). Measurements were made from the surface along the growth axis to the point where the cortex met the choanosome. Zooxanthellae densities were also compared among morphs. Densities were quantified using methods described in Hill (1996a). Spicule concentrations were estimated as follows: samples of known volume were dissolved in nitric acid until all tissue was removed; the resulting solution was washed several times with ddH^2O and then dried at 60°C for 48hrs. Results were reported as mg of spicule cm^{-3} of sponge tissue.

To determine if there were potential differences in pumping capabilities among morphs, I estimated the percentage of open space in the choanosome of each morph. Five estimates were taken at different locations within the choanosome of a single individual, and these were averaged to give one value per sponge. Seven individuals were measured from each morph. Percentages were arcsin transformed before they were compared using one-way ANOVA (Zar, 1984).

Differences were compared among morphs in the relative content of spicules, biomass and calcareous debris. Wet weights and volumes were recorded for individuals from each morph. Sample sizes are shown in Table 1. Samples were placed in crucibles and weighed after drying for 48hrs at 60°C. Crucibles were then placed in a furnace for 6hrs at 450°C. This procedure removed sponge tissue but left behind spicules, calcareous debris and ash. Ash was washed from crucibles with ddH_2O, and crucibles were then dried for 48hrs at 60°C. After weighing, crucibles were treated with 5% HCl (which removed all $CaCO_3$), washed with ddH_2O, dried for 48hrs at 60°C and weighed. At this point, all that remained in the crucibles was spicular material.

The distribution and concentration of collagen was compared among morphotypes using the Mallory-Heidenhain's connective tissue stain (Humason, 1979). Immediately after collection, samples were fixed in Bouin's solution. Fixed samples were run through a series of dehydration steps prior to embedding. Samples were placed in paraplast embedding media, allowed to harden, and then sectioned. After staining, sections were placed on slides for viewing.

Spicule measurements were conducted following methods described in Palumbi (1986) with the following modifications. Spicules from 10 *varians* individuals, 6 *incrustans* individuals and 4 *rigida* individuals were cleaned of tissue using concentrated nitric acid (Kelly-Borges & Pomponi, 1992). Samples were then washed with ddH_2O and dried. Spicules were placed on glass slides and a cover slip was mounted over the preparation. Measurements of spicules were

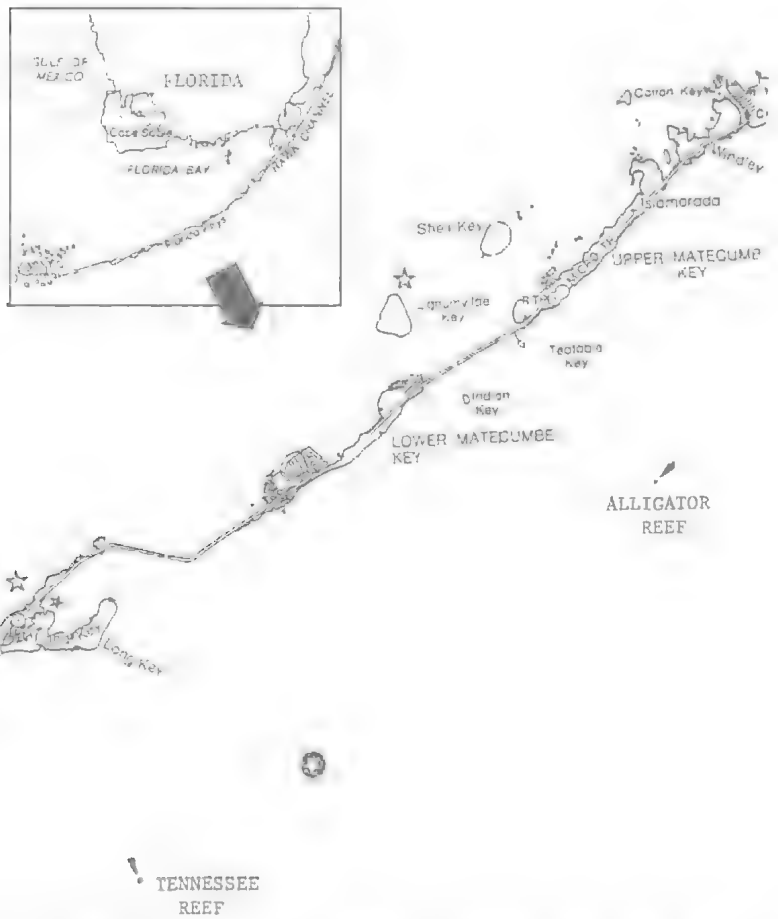

FIG. 1. Map of Florida's Middle Keys; all collections for allozyme analysis were made in areas with stars as well as on Tennessee and Alligator reefs. Reef habitats (i.e. reefs between, and including, Tennessee and Alligator reefs) harbored *incrustans* and *rigida* populations, while near-shore areas harbored *varians* populations.

made from digitised images. Total length and head and shaft widths of subtylostyles were measured for 50 spicules from each individual from each morph. A qualitative assessment of anthosigma shape was also performed at this time. Comparisons of spicule size and shape were made using one-way ANOVA (Zar, 1984).

TRANSPLANT EXPERIMENTS. To examine the effect of sedimentation on morphological variation, several *varians* and *rigida* individuals were transplanted onto platforms that were raised above heavily sedimented substrata. Transplanted *varians* individuals (n=8) were placed on the upper surface of cinder blocks at a depth of <1m. These sponges were monitored for 6 months. Morphological changes were monitored in 10 *rigida* individuals that were affixed to ceramic tiles (15cm×15cm) by fishing line. The ceramic tiles were placed above the substrata and were attached to wooden planks that had been cemeted to the reef. Both of these transplantations provided low sediment zones that represented uncolonised (i.e. competitor-free) space.

ALLOZYME ANALYSIS. Samples of each morphotype were collected in July, 1996. Sympatric individuals of *incrustans* and *rigida* were collected on both Alligator and Tennessee reefs at a depth of 8m (Fig. 1). *Anthosigmella varians* forma *varians* individuals were collected from the bayside of Long Key and Lignumvitae islands at a depth of 1m (Fig. 1). Small sections (<5cm in length) were sampled from each individual and transported to the Key Largo Marine Research Laboratory. Within 4hrs of collection, portions of the endosome were removed from each specimen and frozen in liquid

nitrogen. The possibility of banding artefacts due to zooxanthellae was reduced by avoiding the pinacosome (general location of the symbiont). Samples were stored at -80°C after shipping.

Samples were processed by grinding a small ($<15mm^3$) piece of sponge with a stainless steel rod in a 0.1% aqueous solution of ß-mercapto-ethanol with NADP (2mg ml^{-1}) added. Grinding wells were kept on ice at all times. Approximately 0.25µl of the supernatant was applied to cellulose acetate gels. All samples were run at 200V for 15mins in a Tris-glycine buffer (pH 8.5). Staining procedures followed those described in Richardson et al. (1986) and Hebert & Beaton (1993)

Preliminary screening of 30 enzyme systems revealed seven that showed good resolution and could be reliably scored. The enzyme systems employed in this study are shown in Table 2. Genotypes at eight loci were scored directly by examining stained gels. At each locus, alleles were distinguished based on the relative mobilities of their products.

Abbreviation: EC, Enzyme Commission number.

RESULTS

GROSS MORPHOLOGY. There were significant differences in the majority of morphological characteristics measured for the three morphs (Table 1). The most conspicuous trait that differed among morphs was the presence of branches: *incrustans* lacked branches entirely, but covered far greater surface area on average than either *rigida* or *varians*. Hook extraction force (i.e. tissue strength), cortex thickness and spicule concentration were greatest in *rigida*. Zooxanthellae density, number and height of branches, as well as mound height were greatest in *varians*. The percentage of open space in the choanosome was greatest in *varians* and lowest in *incrustans*. The non-transformed percentages are shown in Table 1, but significance values were based on arcsin transformed data.

Standardising ratios of biomass, spicule weights and calcareous debris to dry weights revealed interesting trends. Greater than 75% of *incrustans* tissue was composed of calcareous debris whereas there was no evidence of $CaCO_3$ within the tissues of either *rigida* or *varians* (Table 1). Residual material that could not be washed out with ddH_2O after the acid treatment resulted in slight increases in crucible weights for both *rigida* and *varians* (hence the negative values in Table 1). Sponge biomass represented approximately 43% of dry weight for *varians* compared to only 11% for *incrustans*. However, wet-weights were statistically indistinguishable among *varians*, *rigida* and *incrustans*. Spicule concentrations standardised to dry weight showed the following trend: *rigida* > *varians* > *incrustans*. These values matched spicule concentrations measured previously (Table 1).

Collagenous fibers stained bright blue using the Mallory-Heidenhain's connective tissue stain. Collagen was densely concentrated within the cortex of both *incrustans* and *rigida*, but was more extensive in *rigida* since the cortex was thicker in this morph (Table 1). Collagen content in *varians* was negligible and was widely scattered throughout the choanosome.

There were no differences in the lengths of subtylostyles in any morph (Fig. 2), but *varians* had significantly wider megascleres (i.e. head and shaft; Fig. 2). There were no significant differences in widths between *incrustans* and *rigida*. The subtylostyles of *incrustans* were more curvaceous than *rigida* or *varians*. Sigmata, termed anthosigmas in this group, typically had a single bend (i.e. bow shaped) in *incrustans* and *rigida* but often had two or more bends (i.e. sigma shaped) in *varians*. Samples taken from the cortex and choanosome of *rigida* appeared to have no differences in lengths or widths.

TRANSPLANT EXPERIMENTS. Both *varians* and *rigida* began to encrust the surfaces on to which they were transplanted. The manner of encrustation was the same. A thin sheet proceeded to take over the unoccupied area spreading from the base of the branch that had been attached to the substratum. This growth was relatively rapid and may have represented tissue reorganisation rather than true increases in biomass (for example, see Jackson & Palumbi, 1979).

ALLOZYME ANALYSIS. Heterozygotes at loci Glucose-6-Phosphate Isomerase (EC 5.3.1.9; *Gpi*), Malate Dehydrogenase (EC 1.1.1.37; *Mdh-1*) and 6-Phosphogluconate Dehydrogenase (EC 1.1.1.44; *6Pgdh*) all showed 3-banded zymograms typical of a dimeric enzyme. Heterozygous individuals exhibited a multi-banded zymogram for Fumarate Hydratase (EC 4.2.1.2; *Fum*) as would be expected for a tetrameric enzyme; individuals assumed to be heterozygous at Malic Enzyme (EC 1.1.1.40; *Me*) (a suspected tetrameric enzyme) exhibited a smear. No heterozygote was detected at Arginine Kinase (EC 2.7.3.3; *Ark*) or Creatine Kinase (EC

TABLE 2. Allele frequencies for the 8 enzyme loci scored in this study categorised by morphotype of sponge. *Incrustans* = encrusting morph, *varians* = bay branching morph and *rigida* = ocean branching morph. N = number of individuals used for each enzyme system.

Locus	EC #	Allele	*incrustans*	*varians*	*rigida*
Ark	2.7.3.3	1	0.11	0	0
		2	0	1.0	1.0
		3	0.22	0	0
		4	0.67	0	0
		N	9	8	9
Ck	2.7.3.2	1	0.22	1.0	1.0
		2	0.78	0	0
		N	9	8	9
Fum	4.2.1.2	1	0.78	1.0	1.0
		2	0.22	0	0
		N	9	8	9
Gpi	5.2.1.9	1	0	0.03	0
		2	0.14	0.40	0.09
		3	0.86	0.57	0.91
		N	14	15	11
Me	1.1.1.40	1	0.67	0	0
		2	0.33	0	0
		3	0	1.0	1.0
		N	9	7	6
Mdh1	1.1.1.37	1	0.28	1.0	1.0
		2	0.72	0	0
		N	9	8	9
Mdh2	1.1.1.37	1	0	0.75	0.94
		2	0.11	0	0
		3	0.22	0.25	0.06
		4	0.67	0	0
		N	9	8	9
6Pgdh	1.1.1.44	1	0.33	0.5	0.25
		2	0	0.08	0
		3	0.17	0	0.67
		4	0.28	0.25	0.08
		5	0.22	0.17	0
		N	9	8	9

2.7.3.2; *Ck*) loci. Neither *varians* nor *incrustans* populations had heterozygotes at the *Mdh*-2 locus. Encrusting populations appear to be genetically distinct from both branching morphs. There were fixed differences at *Ark* and *Me* loci (Table 2). In addition, there were large differences between encrusting and branching morphs in frequencies for the following loci: *Ck*, *Fum*, *Mdh1* and *Mdh2*. However, allelic frequencies for *Gpi* were more similar in oceanside populations.

DISCUSSION

Anthosigmella varians represents a morphologically diverse species with substantial phenotypic differences among the three morphotypes (Table 1). The distribution of the three morphs (Table 1) indicates that *varians* and *rigida* individuals prefer shallower and more sediment-laden habitats than *incrustans*. Despite significant differences among morphs, the presence of branches is the most useful diagnostic trait available in the field: *rigida* and *varians* have branches while *incrustans* assumes only an encrusting form.

Preliminary grafting experiments demonstrated that all three morphs were capable of attaching to one another, but connections between *rigida* and *varians* were strongest (unpublished results). Although sample sizes are small, genetic analysis supports this distinction since *rigida* and *varians* populations were fixed for alleles at two loci that were not present in the *incrustans* population. Any conclusions about reproductive isolation must be tentative, however, given the absence of heterozygotes at the loci *Ark*, *Ck* and *Mdh*-2. For this reason, the potential reproductive isolation in *A. varians* is being further explored using larger sample sizes and DNA-based molecular techniques which provide access to a larger number of polymorphic loci.

Transplant experiments indicated that sediment may be responsible for the branching phenotype since *varians* and *rigida* individuals that were placed on sediment-free substrate (in both the Florida Bay and reef) began to encrust. Sedimentation rates are highest in the shallow lagoonal areas where *varians* is found while on the reef sedimentation rates appear to decrease with depth (J. Schmerfeld, pers. com.). Although correlative at this stage, this information supports the claim that branching is a response to sediment load.

There is a strong positive correlation between tissue strength and cortex thickness (Table 1); *varians* lacks any sort of cortex, *incrustans* has a well defined but relatively thin cortex, and *rigida* has a thick cortex. The cortex was shown to be collagen rich (with Mallory-Heidenhain stain), and it is probable that the collagen accounted for the dramatic increases in tissue strength in *rigida*. The observed differences among the three morphs may be due to a number of environmental parameters. Wave energy has been hypothesised to prevent *incrustans* from producing branches (Vicente, 1978). Another possibility that has not been considered to date is that predation causes

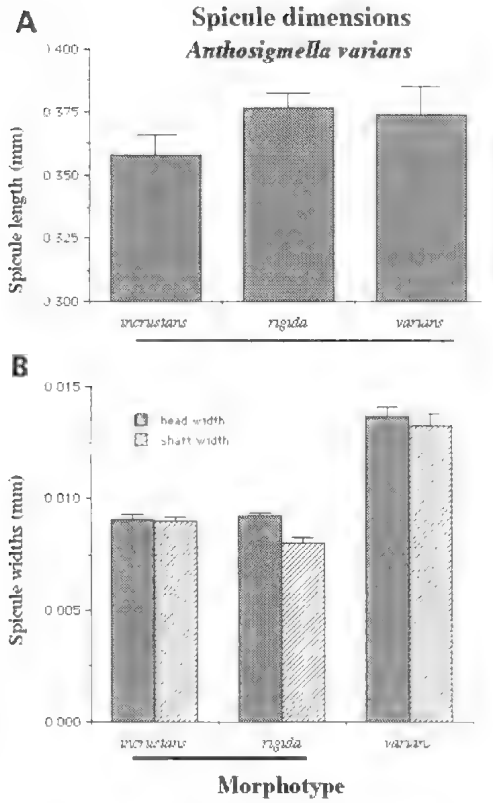

FIG. 2. Measurements of total length and widths of head and shaft in subtylostyles of *A. varians* morphs. Lengths and widths of 50 randomly chosen spicules were averaged for each individual examined. The number of individuals used were: *incrustans* = 6, *rigida* = 4 and *varians* = 10. Histobars represent means (±SE); bars connected by an underline are not significantly different at P >0.05 using one-way ANOVA and Tukeys' multiple comparisons test.

increases in collagen synthesis in the cortex. Several lines of evidence suggest that predators, and not wave energy, influence tissue strength and branch production in *A. varians*. For instance, numerous *rigida* individuals were found on the high wave energy reef crest. Qualitative support for this hypothesis came when *rigida* and *incrustans* individuals were cut open to expose the choanosome. Angelfish immediately consumed large quantities of the interior portions of these individuals while completely avoiding the cortex. Caging and transplant experiments indicate that predation is probably more important than wave energy (Hill, 1996b), but the influence of wave energy on collagen production cannot be ruled out.

Encrusting and branching morphs play very different roles in coral reef communities. Over 40% of a surveyed *incrustans* population (n=48) was involved in competitive encounters while none of the branching morphs were ever observed growing over corals (Hill, 1996b). Furthermore, by occupying large areas of reef, *incrustans* indirectly affects invertebrate recruitment by usurping space that could be used for successful settlement (Table 1; Vicente, 1978). Given that *incrustans* appears to devote less tissue volume to pumping activities (i.e. fewer choanosomal open spaces; Table 1), it seems that *rigida* and *varians* individuals should have a larger impact on bacterioplankton communities than *incrustans*. Finally, *incrustans* penetrates deeper into carbonate structures than either *varians* or *rigida*. Neither *varians* nor *rigida* devoted as high a percentage of their biomass to boring activities (Table 1), and sponge-produced sediment is often seen on the surfaces of *incrustans* individuals indicating active boring. These observations indicate that *incrustans* plays a larger role in bioerosional processes than either *rigida* or *varians*.

If the branching and encrusting morphs of *A. varians* are truly reproductively isolated populations, then the speciation process must be explained (see discussion of Sarà, 1990). Two distinct, but not exclusive, schools of thought have been adopted by biologists to explain how the great diversity of tropical coral reefs has originated and is maintained. Many attribute observed patterns of diversity to non-equilibrial processes such as disturbance and chance (Sale, 1977, 1988; Connell, 1978, 1979; Hughes, 1989; Doherty & Fowler, 1994; Aronson & Precht, 1995). Hypotheses involving equilibrial processes, such as niche diversification, have recently received increased attention (e.g. Jackson, 1991; Knowlton & Jackson, 1994a). However, there is no clear non-equilibrial mechanism proposed to explain the origin of diversity on coral reefs (Sale, 1988). Although they are assumed to play a major role (Knowlton, 1993; Knowlton & Jackson, 1994a), it is unknown how important habitat specialisation and niche diversification have been in the origin of diversity. The results presented here suggest that niche diversification may have played a role in the separation of *incrustans* from *rigida* and *varians*. That is, *varians* and *rigida* populations are capable of utilising sediment laden environments that *incrustans* is unable to utilise.

Recent research has demonstrated that Poriferan diversity may be greater than indicated by current classifications. For example, Boury-Esnault et al. (1992) measured genetic divergence among sympatric morphotypes of the Mediterranean sponge *Oscarella lobularis* using protein electrophoresis. They found fixed differences at seven loci among sympatric morphotypes indicating a significant reduction (or cessation) in gene flow among populations. The results presented here, in addition to several other studies asking similar questions (e.g. Boury-Esnault et al., 1992; Stobart & Benzie, 1994; Barbieri et al., 1995), suggest that niche partitioning may be a very important diversifying process for tropical sponges.

It is clear that examination of Poriferan diversity, especially morphologically diverse species, is essential if we are to understand evolutionary trends in the simplest metazoans. The small number of informative taxonomic traits in this Phylum has hindered interpretation of patterns and processes. Greater attention must be focused on elucidating microevolutionary processes operating in marine environments, and identifying barriers to gene flow should be a priority. Sponges such as *A. varians* provide a model system to address these questions.

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REGULATORY MECHANISMS OF IMMUNE CELLS IN SPONGES. *Memoirs of the Queensland Museum 44: 248. 1999*:- Gray cells, large granular wandering cells present throughout the tissues of many species of sponges, have been identified as immunocytes in two species of sponges, *Microciona prolifera* and *Callyspongia diffusa*. When the tissues of two different sponge individuals are apposed, the gray cells accumulate at the boundary of contact at the time of tissue rejection. I have suggested that these cells may be viewed as the most primordial examples of evolutionary predecessors of the well-known vertebrate lymphocytes. This comparison implies that gray cells share features of vertebrate lymphocytes and I have examined this idea with studies on two prominent aspects of activation of T and B cells. The primary signalling event upon activation of a lymphocyte by recognition of an appropriate immune target is the synthesis and release of cytokines that alert and coordinate the activity of other lymphocytes in the surrounding tissue and throughout the body. In addition the activation of lymphocytes involves internal second messenger pathways converging on the transcription factor, NFkB, that are inhibited by Cyclosporin A, a drug often used medically to prevent rejection in human transplants. Using Boyden Chamber assays, the assays originally used to identify vertebrate immune system cytokines, I have succeeded in establishing in *M. prolifera* that contact with foreign tissue stimulates the release of cytokines activating the migration of gray cells toward the contacting tissue. Similarly, doses of Cyclosporin A commonly used to inhibit the activation of vertebrate T cells, suppresses histoincompatibility in *M. prolifera* and allows the healing together of tissue from two individual sponges that would normally undergo tissue rejection. These results provide further evidence that the foundations of the cellular immune system of animals were already established in the sponges and that study of gray cells will provide insight into the course of evolution of animal immunity. ❐ *Porifera, immunology, immunocytes, gray cells.*

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***NEGOMBATA MAGNIFICA* – A MAGNIFICENT (CHEMICAL) PET.** *Memoirs of the Queensland Museum 44: 248. 1999*:- *Negombata magnifica* (*Latrunculia*) is a Red Sea sponge known to produce the toxin latrunculin (Lat). Since synthesis of this compound is economically non-viable, we evaluated various ways of producing it, while determining its natural mechanism of production and ecological relevance.We examined the possibility of: 1) identifying the cells which produce and harbour latrunculin; 2) establishing cell cultures; 3) forming an underwater sponge 'garden'; and 4) takingadvantage of the sponge's own reproduction and larval settlement. Early in the study it became evident that *N. magnifica* might actually comprise two closely related species of *Negombata*, one of them an undescribed, new species. The work reported here refers to the original *N. magnifica*. 1) The location of Lat B, was studied using specific rabbit anti-Lat B antibodies. Rabbits were immunised with a conjugate of Lat B with Keyhole Limpet Hemocyanin (KLH), and the antibodies were affinity purified over a Lat B-Sepharose column.Thick and thin sections of the sponge were analysed by immuno-histochemical and immuno-gold techniques using light and transmission electron microscopy, respectively. Latrunculin B was prominently labelled in the sponge ectosome -endosome border, especially in the dense cell layer beneath the cortex. Immuno-gold localisation within the sponge revealed that Lat B resides in the sponge cells and not in its prokaryotic symbionts. The labelling density of gold particles in the archeocytes and choanocytes was significantly higher than that of the other sponge cell types (special cells and skeleton associated cells). The antibodies labelled primarily archeocytes and choanocytes, membrane-limited inclusions which are perhaps Lat B secretory and/or storage vesicles. The concentration of Lat B in the sponge periphery correlates with the defensive role of the toxin, since encounters with epibionts, predators and competitive neighbours take place through the surface layer. It may, therefore, be useful to isolate these cells for culture. 2) Primary cell cultures were established from adults and embryos. Mechanical dissociation of inner parts (without the external layer) proved to be superior (less contamination and more cell types) to other techniques. Primary cultures from embryos lasted significantly longer (up to 280 days) and cells survived a freezing phase. Cell lines, however, have not yet been established. 3) Initial steps were taken toward establishing an *in situ* 'garden' of *N. magnifica* from sponge fragments. Although growth rate of sponge fragments was superior to that of natural sponges in their vicinity, fragment survival over a year proved to depend on sponge handling, water depth and environmental conditions (currents, sedimentation etc.). 4) *Negombata magnifica* had a peak in sexual reproduction during the summer. Sexually produced, naturally released, larvae were settled on plates and their growth and development were followed for up to 4 months. ❐ *Porifera, latrunculin, natural product, localization, antibodies, reproduction, immuno-histochemical and immuno-gold techniques, cell culture,Negombata magnifica.*

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SPONGES OF THE LOW ISLES, GREAT BARRIER REEF: AN IMPORTANT SCIENTIFIC SITE, OR A CASE OF MISTAKEN IDENTITY ?

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Much of our early, reliable scientific knowledge on marine taxonomy, biological and other processes of coral reefs in general, and the Great Barrier Reef (GBR) in particular, comes from the 1928-29 GBR Expedition based on the Low Isles. 106 species of sponges were collected from northern reefs of the GBR Expedition and described by Burton in 1934, 36 from the Low Isles. Burton concluded that the sponge fauna contained: 'species characteristic of the Indo-Pacific' (38% of his species); many 'common also to the coasts of Australia' (17%) 'with a mixing of the Australian and Malayan sponge-faunas'; substantial cosmopolitanism (12%) with species 'also found in the West Indies, Azores and Mediterranean'; and only few indigenous species (14% unique to the Low Isles, 19% exclusive to N Australia). Re-examination of BMNH voucher and type material found 42% of these species were misidentified, mainly concerning the so-called 'widely distributed' taxa. Recent collections from the Low Isles by the Queensland Museum (QM) discovered 109 species, and together with the revised Burton collection indicate a sponge fauna of 134 species (in 63 genera and 35 families). Surprisingly only 12 species (9% of the Low Isles fauna) were common to both the Burton and QM collections. Taxonomic comparisons with other provinces show several major trends for Low Isles sponges: 1) The fauna contains a generalist element comprising 'typical GBR species', found on virtually all reefs surveyed so far (23% of Low Isles species). 2) The fauna also contains an indigenous component of species unique to the northern GBR (48% of Low Isles species), with 32% of these not yet recorded from anywhere else, and another 16% known only from both the Low Isles and Lizard Island (200km to the north). 3) Affinities with coastal faunas are low, contrary to Burton's hypothesis, with only 13% of Low Isles species also found on adjacent coastal regions. 4) Affinities with oceanic coral reef species are also low, with only 10% of Low Isles species found on the Coral Sea seamounts. 5) The concept of an 'east Australian coast' sponge fauna is not supported, contrary to both earlier collections described by Lendenfeld in 1888 and 1889, and Burton, with only 10% of Low Ises species extending southwards into more temperate Queensland waters, and only 2% extending further into southern New South Wales. 6) The concept of 'cosmopolitan' species is unsubstantiated. ❐ *Porifera, Low Isles, Great Barrier Reef, faunal survey, biodiversity, biogeography, taxonomy.*

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The Low Isles, Cairns Section, Great Barrier Reef (GBR), is an historically important site for coral reef research in Australasia, being the base for the 1928-29 Great Barrier Reef Expedition. These islands (16°23'S, 145°34'E) lie about 15km off the coast of far northern Queensland, 70km N of Cairns, approximately midway between the mainland and outer barrier reef (Fig. 1A), and easily accessible from both Port Douglas and Cairns. They consist of two small coral islets (Fig. 1B), one with a sand cay and the other a coral 'shingle' islet with mangroves, both with extensive fringing reef and connected by an expansive coralline reef flat. The geomorphology and many other aspects of these reefs have been described in detail in the *Scientific Reports of the Great Barrier Reef Expedition 1928-29*.

Since at least the 1880s these small islands have been frequented by recreational and commercial fishermen, tourists and government authorities (e.g. meteorological bureau, coast-watch, and scientists). The islands owe their

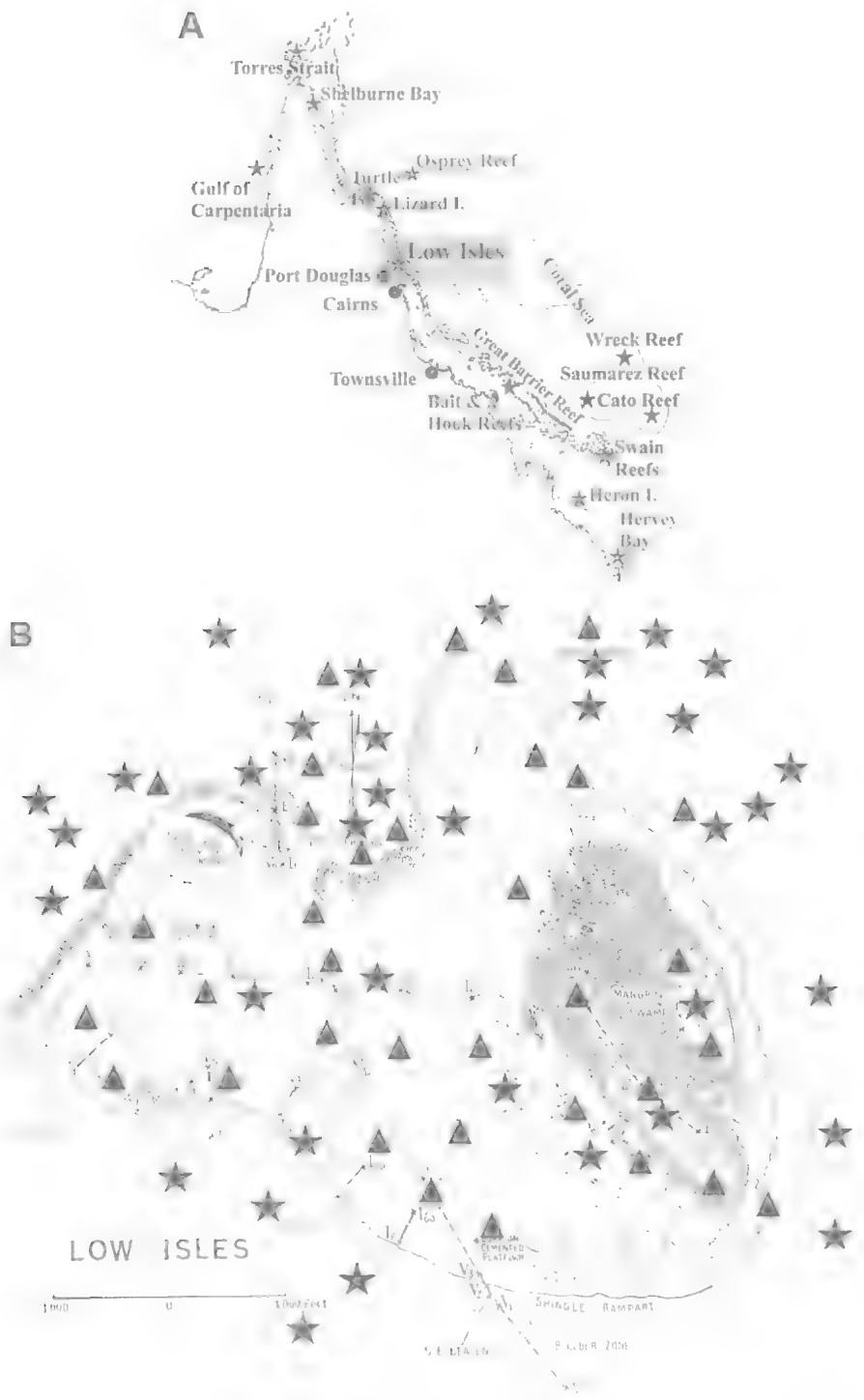

FIG. 1. A, Location of the Low Isles on the GBR and other localities mentioned in the text (dots indicate adjacent coastal settlements; stars indicate sites of major sponge collections undertaken by the QM). B, Low Isles (from Stephenson et al., 1931), showing collection localities of the 1928-29 expedition (triangles; taken from Burton's text) and 1997 QM expedition (stars).

popularity largely to their close proximity to human settlement, their wide variety of habitat types (typical of the chain of about 50 low woody islets on the far northern sector of the GBR, of which the Low Isles are the most southern), including sandy beaches, a vegetated sand cay, extensive coral reef flat and lagoon, fringing reefs, and large stands of 'uninhabitable mangrove swamp' (Yonge, 1928), as well as a permanent settlement on the sand cay since 1878 associated with the operation and maintainance of the lighthouse — now a heritage listed building (Anon., 1993).

Between the early 1880s and the early 1900s William Saville-Kent and Robert von Lendenfeld actively collected and described sponges from far northern Queensland. Unfortunately, neither author provided specific or reliable locality or habitat data, with the exception of collections made during the pearl oyster surveys off Cape York in the late 1800s (in which case the locality 'Torres Strait' was usually quoted). Where locality data did exist on specimen labels it was often contradicted in the corresponding museum register and again in the published records, and therefore all of these data must be treated as suspect (Hooper & Wiedenmayer, 1994). Nevertheless, it is likely that some of their material was collected from reefs in the vicinity of Cairns and Port Douglas given the close proximity of the GBR to the coast in this region, and the popularity of these reefs. Their collections were deposited in both the Natural History Museum, London (BMNH), and Australian Museum, Sydney (AM), but much of this early material is dry and virtually useless for modern taxonomic determination.

In 1925 the Great Barrier Reef Committee proposed a concerted program to explore the 'origin, growth and natural resources of the Great Barrier Reef' (Yonge, 1928), with the Low Isles subsequently chosen as the site for a major expedition to undertake in situ studies of coral reefs and their processes, led by C.M. Yonge. The expedition remained on the Low Isles for just over twelve months during 1928-29. During this time they surveyed most of the available habitats on and surrounding the two islands of the Low Isles (Stephenson et al., 1931). From Stephenson's description of sampling localities and methods, this effort was rigorous and comprehensive, even by today's standards. Collection of biological samples included reef-walking, dredging and diving via surface supply air (SSA) ('tin-hat' diving).

The *Scientific Reports of the Great Barrier Reef Expedition 1928-29* (British Museum (Natural History): London), were published in six volumes between 1928 and 1950, representing the most comprehensive study on coral reef biology, physics, chemistry and geology of the GBR system at that time, and perhaps of coral reefs in general. The sponge fauna from this expedition was published by Burton (1934), who described 36 species from the Low Isles and another 70 species from coral reefs and inter-reef habitats further north (mostly in the vicinity of Lizard I.). Discounting the publications from the 'Alert' (Ridley, 1884) and 'Challenger' expeditions (e.g. Ridley & Dendy, 1887), which mostly concerned the coast and islands of the Torres Straits and not the GBR proper, Burton was the first author to provide accurate locality and habitat data for GBR species, unlike his predecessors Saville-Kent and Lendenfeld. It was not until 35 years later that Bergquist (1969) published the next paper on GBR sponges, and another 10 years after that with the subsequent work of Wilkinson (1978). These latter publications described only a few intertidal and shallow subtidal species, from the southern end of the GBR (Heron I.), and consequently Burton's (1934) species have stood for over 50 years as being 'typical' or 'representative' of the entire GBR. Until this current decade his work has represented virtually the sum-total of our knowledge of the GBR sponge fauna.

Burton's (1934) species were divided into two groups: 1) 'Common Indo-Malay', with 'Indo-Malayan' species (38% of his collections), allegedly 'cosmopolitan' species (12%), and 'typical east Australian coast' species (17%); and 2) 'Indigenous', with apparent 'endemic' species (14%), and exclusively northern Australian species (19%), described from one or only few localities. Of the former group he rarely provided descriptions or referred to any museum voucher specimen to validate his identifications; of the latter group only relatively few have been subsequently recollected or redescribed in the literature (e.g. de Laubenfels, 1954), some of which we suspect, or now know, are misidentifications.

It was the intention of this study, therefore, to revisit the Low Isles to: 1) 'Rediscover' Burton's GBR species, locate and re-examine his voucher specimens (if they existed), of the allegedly 'cosmopolitan' species in particular, and ultimately to assign Burton's species names to

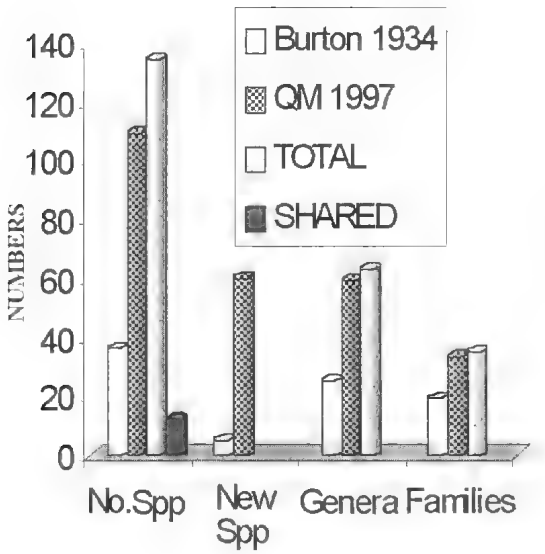

FIG. 2. Comparison of species diversity and taxonomic composition between Low Isles sponges collected by the GBR Expedition 1928-29 (Burton, 1934) and collections of the QM in 1997, indicating the total number of species collected (and species common to both expeditions), the number of new (or unnamed) species, numbers of genera and families.

living populations — a theoretically simple but practically elusive task for many Australian sponge faunas. 2) Compare sponge biodiversity and species composition between the Low Isles and other reefs of the GBR from our contemporary collections (see Fig. 1A), to ascertain whether this fauna is indeed representative of the GBR fauna in general as has been interpreted by many contemporary authors. To achieve these aims, without having to revise the entire northern GBR fauna, we restricted this study to include only the Low Isles, ignoring for the time being those species Burton described from the more northern reefs of Eagle, Direction, Lizard, Turtle and Howick Is.

MATERIALS AND METHODS

All sponges were collected using SCUBA, by hand for the intertidal fauna, or a small dredge for deeper subtidal soft-bottom species. All specimens are housed in the permanent collections of the QM (prefix QMG). Methods of preservation, histological preparation and taxonomic identification are published elsewhere (e.g. Hooper, 1996). Abbreviations: BMNH, The Natural History Museum, London; GBRMPA, Great Barrier Reef Marine Park Authority; QM, Queensland Museum, Brisbane; SSA, surface supplied air.

RESULTS AND DISCUSSION

BIODIVERSITY. The published sponge fauna of the entire Queensland region, including coastline, Great Barrier Reef, Queensland Plateau, and the Coral Sea, so far consists of only 428 named species and subspecies (Hooper & Wiedenmayer, 1994, including literature updated since 1994). Fewer than this, perhaps 250 named species, actually belong to the GBR fauna, with the remainder restricted to coastal waters, soft sediments in the Gulf of Carpentaria, the inter-reef region in the Torres Straits, and deeper-waters off the continental shelf. Recent collections by the QM from the GBR have subsequently recorded 507 species, many of which are probably new to science (Hooper et al., 1999, this volume).

Since Burton's (1934) work there were no subsequent publications of GBR sponges until Bergquist's (1969) description of a small intertidal collection from Heron I. Since Bergquist (1969), only relatively few other publications containing descriptions or redescriptions of GBR sponges have appeared, although these seem to be slowly escalating, perhaps reflecting the renewed interest in the phylum and in biodiversity in general (Wilkinson, 1978; Ayling, 1982; Pultizer-Finali, 1982; Thompson et al., 1987; Hooper, 1987, 1990, 1991, 1996; Bergquist et al., 1988; Stoddart, 1989; Wilkinson & Cheshire, 1989; Fromont, 1989, 1991, 1993, 1995; Van Soest et al., 1991; Hooper & Bergquist, 1992; Reitner, 1992; Hooper et al., 1993; Hooper & Lévi, 1993a, b, 1994; Van Soest & Hooper, 1993; Fromont et al., 1994; Bergquist, 1995; Bergquist & Kelly-Borges, 1995; Kelly-Borges & Vacelet, 1995; Reitner & Woerheide, 1995; Van Soest et al., 1996; Reitner et al., 1997).

Burton (1934) recorded 36 species from the Low Isles, collected over a 12 month period by the GBR Expedition, consisting of 5 new species, 25 genera and 19 families. By comparison, collections made by the Queensland Museum in 1997 over 7 days, from similar habitats encircling the islands as described by Stephenson et al. (1931), yielded 109 species (in 59 genera and 33 families; Fig. 2), of which only 46 (42%) can be accurately assigned to a known species — i.e. the remainder are possibly new to science or perhaps belong to species described by Lendenfeld (1888, 1889) but whose identity is still a 'mystery'

(Hooper & Wiedenmayer, 1994). Surprisingly, only 12 species were common to both the Burton and QM collections from the Low Isles (although we also collected another 12 species from the Low Isles that were reported by Burton (1934) from the GBR Expedition collections made at Lizard, Turtle and Direction Islands, but not previously found on the Low Isles).

In order to verify conspecificity between these two collections we undertook a search for Burton's (1934) Low Isles voucher specimens in the BMNH, of which all but three species were found (Table 1). Re-examination of this material found 15 species (42%) were misidentified, 12 belonging to completely different species than supposed by Burton (1934), and 3 split into different species (i.e. allopatric sibling species, as opposed to so called 'widespread' species); 1 is uncertain (i.e. the voucher specimen is missing and no description was provided by Burton); and 14 are transferred to other genera (based on more recent systematic revisions). Most of these 15 misidentified species were assigned by Burton (1934) to species that had 'wide Australian distributions' (i.e. temperate Australian, Northern Territory, and/or tropical Western Australian), 'widespread Indo-Pacific' (e.g. Indo-Malay archipelago, Sri Lanka and W Indian Ocean), or 'cosmopolitan species' (e.g. Mediterranean, Caribbean, Atlantic). These misidentifications were detected and confirmed by comparing Burton's samples with the type material (and/or contemporary specimens) of his named species from these other localities (QM and BMNH collections).

Quantitative differences in species diversity between the GBR Expedition (36 spp.) and QM collections (109 spp.) are not surprising given the greater technological advances made in contemporary collecting techniques (SCUBA, underwater photography), and the probable ineffectual use of generalist (non-specialist) biological collectors to undertake sponge faunal surveys, irrespective of the substantial differences between time scales of two collections (12 months versus 7 days duration, respectively). For example, Burton (1934) described *Raphidotethya enigmatica* and recorded *Ianthella flabelliformis* from more northerly reefs in the Lizard Island region (but not from the Low Isles), whereas we found both these species were relatively common on the Low Isles subtidal reefs. It is possible (but not explicit in their reports), that the GBR Expedition did not commonly use SSA and dredging around the Low Isles themselves (whereas we do know they used these techniques on the more northerly reefs), and it is likely that many or most of the Low Isles sponges were collected from the intertidal reef flat (Fig. 1B).

Thus, based on the recent QM collections and the revised Burton (1934) collections, the total species diversity for the Low Isles now consists of 134 species (in 63 genera and 35 families) (Fig. 2).

SPECIES COMPOSITION. The low similarity in species composition between the GBR Expedition and QM sponge collections is more surprising. Only 12 species or 33% of Burton's (1934) published fauna were common to both collections, consisting mainly of widespread GBR species (e.g. *Druinella purpurea*, *Carteriospongia foliascens*, *Haliclona cymaeformis*, *Cinachyra australiensis*). Several explanations are apparent. 1) Perhaps the more recent QM collection did not find the other 66% of Burton's (1934) species because of the shorter time-scale for collection (7 days versus 12 months), whereby these other species might represent the rare or cryptic species? This explanation is highly unlikely, however, given that we have found some of Burton's Low Isles species elsewhere on the GBR, from collections of similar duration, and in some cases (e.g. *Spirastrella inconstans*, *Callyspongia diffusa*) these species are common. 2) It is also possible that the GBR Expedition mainly, or perhaps exclusively, targetted the easily accessible intertidal fauna, whereas QM collections were predominantly (although not exclusively) subtidal. 3) Nevertheless, there are several species (particularly some of the *Haliclona* and *Callyspongia* described by Burton) which are common on the intertidal reef flats of other reefs on the GBR, but apparently not present on the Low Isles today. It is possible that some of these species may be 'locally extinct' due to anthropogenic or natural causes.

Burton's (1934) misidentifications are less easily explained. Burton had ready access to the vast BMNH collections, containing types, fragments, or representative samples of most species known at that time from the Australian, Oriental, Afrotropical, Neotropical and Palaearctic provinces, yet 42% of his species are not conspecific with these allegedly 'widely distributed' or 'cosmopolitan' species. For example, Burton recorded *Jaspis stellifera* from the Low Isles, noting that it did not contain asters, whereas

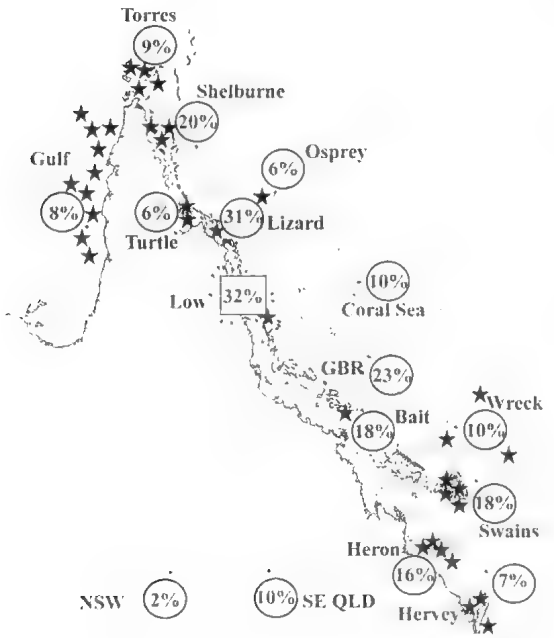

FIG. 3. Biogeographic comparisons in sponge diversity and species composition between the Low Isles and adjacent provinces (data from Hooper et al., 1999, this volume). Square = percentage of Low Isles species that are 'apparent endemics'; circles = percentage of Low Isles species also found in other provinces.

our re-examination of his material found that it did contain asters, and moreover was not conspecific with *J. stellifera*, differing significantly in growth form, surface features, skeletal structure, megasclere and microsclere dimensions from southern Australian populations. Burton's specimen appears to be a new species. Other authors have also recorded similar discrepancies. Bergquist and Warne (1980) found a 25% difference between Burton's spicule measurements from the holotype of *Callyspongia diffusa* and their own re-examination of this specimen. Burton also appears to have overemphasised the importance of external characters in identifying some of his material, overlooking other important skeletal characters. For example, his record of *Haliclona camerata* appears to be solely based on external features (growth form, surface features), whereas Ridley's (1884) holotype has a multispicular skeleton with spicules 25% larger than Burton's voucher specimen, which has a unispicular skeleton — again Burton's specimen appears to be a new species.

BIOGEOGRAPHY. A comparison of species diversity and composition between the Low Isles (including Burton's (1934) revised species list and our more recent QM collections), with sponge faunas of other reefs in the northern part of the GBR, indicate several patterns (Fig. 3).

1) 31 species (or 23% of the Low Isles fauna) are distributed throughout the GBR (annotated '3' on Table 1). These species were recorded on virtually every reef we have surveyed so far on the GBR, and they can be defined as a 'typical GBR sponge fauna'. Thus, the concept of a 'GBR sponge fauna' is partially substantiated.

Conversely, QM collections recorded several other species common throughout the GBR but notably absent from the Low Isles: *Acanthella costata*, *Amphimedon terpenensis* and another (new) species of *Amphimedon*, *Callyspongia carens* and several other *Callyspongia* spp., *Crella calypta*, *Echinochalina intermedia*, *Hippospongia elastica*, *Hyrtios erecta*, *Phakellia flabellata*, *P. klethra*, *Phyllospongia papyracea*, and several apparently undescribed species of *Dysidea*, *Haliclona*, *Niphates*, *Pericharax*, *Psammoclemma*, *Pseudoceratina* and *Siphonochalina*. In addition, the cryptic, cave-dwelling coral species *Levinella prolifera*, *Astrosclera willeyana*, *Acanthochaetetes wellsii*, *Sycetta* sp. and *Hypograntia* sp. are also absent from the Low Isles, probably because these specialised habitats are not present (e.g. Woerheide & Reitner, 1998).

2) Recent collections from Lizard Island, about 200km N of the Low Isles and closer to the outer barrier reef, found 176 species (Hooper et al., 1999, this volume). Of the Low Isles fauna 41 species (31%) are also found on Lizard I., with these two islands showing the highest affinities in their sponge faunas.

3) Recent collections from the adjacent northern coastal province (including fringing coral reefs, intertidal rock reefs, embayments and muddy reefs near the shore, extending along the Queensland coast from the Cooktown region into the Gulf of Carpentaria), found 142 species (Hooper et al., 1999, this volume). A comparison between the Low Isles sponges and this coastal fauna shows that only 17 species (13% of the Low Isles fauna) were common to both provinces (annotated '4' on Table 1). Furthermore, when considered separately each of these provinces usually had an even lower similarity in species composition: Gulf of Carpentaria (8% of Low Isles species), Torres Strait (9%), Shelburne Bay

including the Cockburn and Fast I. groups (20%), Turtle I. (6%) (Fig. 3). These data suggest that the Low Isles contain a greater proportion of 'coral reef species' than 'inshore coastal species', despite their closer proximity to the coast.

4) Recent collections of sponges from the coral reefs on seamounts in the Coral Sea (Osprey, Wreck, Cato and Saumerez Reefs), found 95 species (Hooper et al., 1999, this volume). A comparison between the Low Isles fauna and Coral Sea sponges shows that only 13 species (10% of the Low Isles fauna) were common to both provinces (annotated '5' on Table 1).

5) Only 4 species were found in all 3 regions (*Coscinoderma matthewsi*, *Halichondria* n.sp. #1227, *Myrmekioderma granulata*, and *Xestospongia testudinaria*).

6) Recent collections from the SE Queensland fauna (extending from Hervey Bay to Moreton Bay), found 233 species (Hooper et al., 1999, this volume). Comparisons with these SE Queensland faunas found only 14 species of Low Isles sponges (10% of the fauna) extended southward into this region: *Chondrilla australiensis*, *Echinodictyum mesenterinum*, *Ianthella basta*, *I. flabelliformis*, *Iotrochota foveolaria*, *Leucetta microraphis*, *Myrmekioderma granulata*, *Pericharax heterorhaphis*, *Phakellia cavernosa*, *Pseudaxinella australis*, *Xestospongia testudinaria* and 4 undescribed species of *Dysidea*, *Spirastrella*, *Timea* and *Clathria* (*Microciona*). Similarly, recent collections from N NSW (Byron Bay to Gold Coast) and S NSW (Sydney, Illawarra and Port Stephens regions) found 69 and 131 species from these regions, respectively (Hooper et al., 1999, this volume). Only 4 species living on the Low Isles also extend into S New South Wales.

7) A large number of species on the Low Isles are either 'apparent endemics' or have very restricted distributions here and on adjacent reefs. 43 species (32% of the Low Isles fauna) have not yet been found anywhere else, and another 22 (16%) are known only from the Low Isles and one other reef in the northern part of the GBR (mostly from Lizard Island). Thus, nearly 50% of the sponge fauna on the Low Isles is unique to this N GBR region.

It is possible that this high 'apparent species endemism' might be related to true regional endemism (such as the concept of a 'northern GBR fauna'). There is some empirical support for this through comparisons with S GBR reefs: 18% of Low Isles species were recorded on Bait and Hook Reefs (central GBR); 18% from the Swain Reefs (S GBR, outer reefs); and 16% from reefs in the vicinity of Heron I. (S GBR, inner reefs) (Fig. 3). It is also probable that some of this 'apparent endemism' is due to the heterogeneous distributions of many coral reef sponges (Hooper, 1994), perhaps related to particular habitat requirements and local geomorphological differences between individual reef systems (such as the availability of specialised habitats on particular reef systems).

COMMERCIAL 'BATH' SPONGES. Scientific investigation and commercial 'exploitation' of the Low Isles may have commenced as early as the 1890s, with the alleged introduction of commercial 'bath' sponge cuttings, apparently imported from the Mediterranean, seeded on the reef flat between the two islets ('Thalamita Flat' and 'Mangrove Park'). Surviving remnants (or decendents) of these populations are still common in this area, with some more-or-less 'organised' into vague rows. Burton (1934) identified this species as the Mediterranean *Spongia officinalis*. Its status as a possible remnant of a commercial 'sponge farm' is supported to some extent by our 1997 observations of its 'organised' distribution into 'vague rows' on the reef flat.

It is possible that Saville-Kent may have been responsible, directly or indirectly, for introducing these 'bath' sponges onto the Low Isles, given the popularity of 'translocating' exotic species during his era; he was also the Queensland Commissioner of Fisheries around this time (Harrison, 1997); and there is an anecdotal record of commercial sponge beds occuring on the Isles dating back to about the 1890s (Port Douglas Historical Society; pers. comm.). However, this evidence is inconclusive. It is more probable that these 'bath' sponge beds are remnants of the 'seeding experiments' conducted on the Low Isles and Murray Islands (Torres Strait) by Moorehouse during the GBR Expedition, and described in his report on the investigation of the potential viability of commercial sponge farming on the Great Barrier Reef (Moorhouse, 1933). Moorhouse noted that he made cuttings of wild populations of a 'black, dome-shaped *Hippospongia*', fitting the description of Burton's (1934) *S. officinalis*, which he seeded on the reef flat using various commercial methods of his day. This suggests that these commercial 'bath' sponges may be native to the GBR and not introduced, and therefore probably not conspecific

with the Mediterranean *S. officinalis*. Re-examination of Burton's (1934) voucher specimen of *S. officinalis* from the Low Isles (Table 1) showed that it belonged to *Hippospongia* (our sp. #1983), and not to *Spongia*. This surviving population on the Low Isles possibly represents the first attempt at sponge culture on the GBR.

CONCLUSIONS

Patterns in species diversity and composition of Low Isles sponges (Fig. 3) indicate a greater proportion of both 'typical GBR species' and 'indigenous species' (most similar to Lizard I. than other reefs); only a small proportion of species shared with adjacent coastal and oceanic provinces; and very few species shared with more southern Australian provinces. In fact Burton (1934: 513) acknowledges that '[although] the sponges collected by the [GBR] Expedition belong ... to species characteristic of the Indo-Pacific ... many common to the coasts of Australia ... [with] mixing of the Australian and Malayan sponge-faunas ... this broad generalization [is] in itself inconclusive and unsatisfactory, [but] is the most that can be said'. He states further that comparison between the collections of the GBR Expeditions and those of Saville-Kent (the latter comprising an overwhelming number of indigenous species, but unfortunately with no locality data), suggests that generalizations about a 'GBR sponge fauna' based on the Low Isles species list are probably invalid. In this conclusion he is undoubtedly correct, given the peculiar nature of the Low Isles (inshore coastal reef), as compared with outer barrier reefs of the GBR in particular. However, to some extent there does appear to be a 'typical GBR sponge fauna' of about 20% of regional species' compositions, and some of these (perhaps up to 10%) are truly widely distributed throughout the Indo-west Pacific (although this latter estimate still lacks good empirical support). There is also indication that closer similarities between northern GBR reefs than with southern GBR reefs suggests the concept of a 'typical GBR fauna' may be too simplistic, and that the GBR itself comprises more than one province.

Burton's (1934) assumption that a significant number (12%) of GBR species may be 'cosmopolitan', also found in the West Indies, Azores and Mediterranean, is rejected. His voucher specimens of all these allegedly 'cosmopolitan' species are misidentifications. The concept of a generalised 'east coast Australian sponge fauna' (Lendenfeld, 1888, 1889; Burton, 1934) is also not supported (with the exception of 4 species). Nevertheless, despite the fact that 42% of Burton's species were misidentified, and only relatively few species were reported from the Low Isles themselves, Burton's (1934) report still stands as a valuable taxonomic contribution and a reasonable precis of faunal relationships of GBR sponges in general.

ACKNOWLEDGEMENTS

This study would not have been possible without the special permission of Great Barrier Reef Marine Park Authority (GBRMPA permit no. G96/005), to undertake extractive research from reefs of the Low Isles. Since the late 1800s the popularity of the Low Isles has led to inevitable environmental degradation. Consequently, GBRMPA and the Department of Environment proposed a strict plan of management for the Isles (Anon., 1993). Now implemented, the plan includes designated zones for specific use, a restriction of daily visitor numbers, and (more importantly) restrictions on the types of research activities now permitted. These latter restrictions include the curtailment of manipulative and extractive research (i.e. no collecting). The School of Marine Science, University of Queensland, now operates a small research station housed in some of the refurbished lighthouse buildings (Low Isles Research Station), with the intention to repeat the marine chemistry and physics experiments pioneered by the GBR Expedition. This present study was undertaken by the Queensland Museum in a similar spirit to revisit the pioneering work of Yonge, Burton and coworkers, and for this opportunity we are grateful to the Department of Environment and the Low Isles Preservation Society, Port Douglas. We are also grateful to the Department of Primary Industries, Fisheries, Cairns, for access to their facilities and charter of the FV 'Gwendolyn May'.

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TABLE 1. List of species collected from the Low Isles during the GBR Expedition 1928-29, described by Burton (1934), with revised nomenclature from re-examination of relevant BMNH voucher specimens, and list of species collected in 1997 by the QM. Key to codes: 1 = species collected by the QM from other reefs in the GBR but not found in our collections from the Low Isles. 2 = species reported by Burton from other more northerly reefs but not present in his Low Isles collection. 3 = species now known to be widespread throughout the Great Barrier Reef and some other Indo-west Pacific reefs. 4 = species found on both the Low Isles and the adjacent coast. 5 = species found on both the Low Isles and Coral Sea reefs. # = species identification presently unknown, possibly new, with unique QM species number indicated.

GBR Expedition 1928-29 collection from Low Isles (Burton, 1934)	BMNH voucher numbers	Revised name	QM 1997 collection from Low Isles
CALCAREA			
-	-	-	*Pericharax heteroraphis* Poléjaeff, 1884 (2,3,5)
Sycon gelatinosum (Blainville, 1834) (1)	1930.8.13.29a	*Sycon gelatinosum* (Blainville, 1834)	-
-	-	-	*Leucetta microraphis* Haeckel, 1872 (3,5)
ASTROPHORIDA			
Jaspis stellifera (Carter, 1879) (1)	1930.8.13.23a	*Jaspis* n.sp. (not *Jaspis stellifera* (Carter, 1879))	-
-	-	-	*Jaspis* n.sp. #2242
-	-	-	*Jaspis* n.sp. #1005
-	-	-	*Jaspis splendens* (de Laubenfels, 1954)
SPIROPHORIDA			-
Cinachyra australiensis (Carter, 1886)	1930.8.13.14a	*Cinachyra australiensis* (Carter, 1886)	*Cinachyra australiensis* (Carter, 1886) (3,5)
-	-	-	*Cinachyra* sp. #1870
-	-		*Cinachyrella* sp. #2270
-	-	-	*Raphidotethya enigmatica* Burton, 1934 (2,3,5)
-	-	-	*Raphidotethya* sp. #2045
HADROMERIDA			
Pseudosuberites andrewsi Kirkpatrick, 1900 (1)	1930.8.13.20a	*Pseudosuberites andrewsi* Kirkpatrick, 1900	-
-	-	-	*Suberites peleia* (de Laubenfels, 1954)
Laxosuberites proteus Hentschel, 1909	1930.8.13.111a	*Laxosuberites proteus* Hentschel, 1909	-
Polymastia megasclera Burton, 1934	1930.8.13.155a	*Polymastia megasclera* Burton, 1934	-
-	-	-	*Polymastia* sp. #2258
Tethya robusta Bowerbank, 1859 (1)	1930.8.13.199a	*Tethya robusta* Bowerbank, 1859	-
-	-	-	*Tethya coccinea* Bergquist & Kelly-Borges, 1991
-	-	-	*Tethya* sp. #2249
-	-	-	*Timea* sp. #1389
Spirastrella inconstans (Dendy, 1887) (1,3)	missing	*Spirastrella inconstans* (Dendy, 1887) (some description provided)	-
Spirastrella aurivillii Lindgren, 1897 (1)	missing	? (no description provided)	-
-	-	-	*Spirastrella* sp. #1385
-	-	-	*Chondrilla australiensis* Carter, 1873 (2,3)
Chondrilla nucula Schmidt, 1862	1930.8.13.23a	*Chondrilla* cf. *nucula* Schmidt, 1862	-
-	-	-	*Chondrilla* sp. #492
HAPLOSCLERIDA			
Haliclona camerata (Ridley, 1884) (1,3)	1930.8.13.60a	*Haliclona* sp. (not *Haliclona camerata* (Ridley, 1884))	-
Haliclona clathrata (Dendy, 1895)	1930.8.13.57	*Reniera* sp. (not *Haliclona clathrata* (Dendy, 1895))	-
Haliclona exigua (Kirkpatrick, 1900)	1930.8.13.53a	*Xestospongia exigua* (Kirkpatrick, 1900)	*Xestospongia exigua* (Kirkpatrick, 1900) (3)
Haliclona pigmentifera (Dendy, 1905) (1)	1930.8.13.55a	*Haliclona* sp. (not *Haliclona pigmentifera* (Dendy, 1905))	-

Haliclona tenuispiculata Burton, 1934	1930.8.13.59a	*Haliclona tenuispiculata* Burton, 1934	-
-	-	-	*Haliclona* sp. #1954 (5)
-	-	-	*Haliclona* sp. #2246
-	-	-	*Haliclona* sp. #2247
-	-	-	*Haliclona* sp. #2248
Adocia fibulatus var. *microsigma* Dendy, 1916	missing	*Haliclona cymaeformis* (Esper, 1791) (no description but ID probable from Burton's remarks)	*Haliclona cymaeformis* (Esper, 1791) (3)
-	-	-	*Haliclona* (*Toxadocia*) sp. #2253
Adocia toxius (Topsent, 1897)	1930.8.13.38a	*Haliclona* sp. (not *Haliclona toxius* (Topsent, 1897)	-
Adocia minor (Dendy, 1916) (1)	1930.8.13.62a	*Adocia* sp. (not *Haliclona minor* (Dendy, 1916))	-
Adocia pumila (Lendenfeld, 1887) (1)	1930.8.13.32a	*Gelliodes pumilus* (Lendenfeld, 1887)	-
Adocia sagittaria (Sollas, 1902)	1930.8.13.40a	*Oceanapia sagittaria* (Sollas, 1902)	*Oceanapia sagittaria* (Sollas, 1902)
-	-	-	*Aka* sp. #1373
-	-	-	*Aka* sp. #2254
-	-	-	*Aka* sp. #2255
-	-	-	*Aka* sp. #2259
-	-	-	*Gelliodes* sp. #1215
-	-	-	*Gelliodes* sp. #2244
-	-	-	*Gellius* sp. #2269
-	-	-	*Niphates* sp. #2245
Callyspongia diffusa (Ridley, 1884) (1)	1930.8.13.47a	*Callyspongia* (*Euplacella*) *diffusa* (Ridley, 1884)	-
Callyspongia ridleyi Burton, 1934 (1)	1930.8.13.165a	*Callyspongia ridleyi* Burton, 1934	-
-	-	-	*Callyspongia* sp. #981
Oceanapia fistulosa (Bowerbank, 1873) (1)	1930.8.13.50a	*Oceanapia* sp. (not *O. fistulosa* (Bowerbank, 1873)	-
Oceanapia reneiroides Burton, 1934	1930.8.13.49a	*Oceanapia reneiroides* Burton, 1934	*Oceanapia renieroides* Burton, 1934
-	-	-	*Petrosia* sp. #2252
-	-	-	*Strongylophora* sp. #1580
-	-	-	*Xestospongia testudinaria* (Lamarck, 1815) (3,4,5)
-	-	-	*Xestospongia nigricans* (Lindgren, 1897)
-	-	-	*Xestospongia pacifica* Kelly-Borges & Bergquist, 1988 (3,5)
POECILOSCLERIDA *Desmapsamma anchorata* (Carter, 1882) (1,3)	1930.8.13.151a	*Ceratopsion* n.sp. (Raspailiidae)	-
-	-	-	*Desmapsamma* sp. #1528
Iotrochota purpurea (Bowerbank, 1875)	1930.8.13.90a	*Iotrochota foveolaria* (Lamarck, 1814)	*Iotrochota foveolaria* (Lamarck, 1814) (4)
-	-	-	*Iotrochota* sp. #377
-	-	-	*Iotrochota* sp. #2256
-	-	-	*Iotrochota* sp. #2263
Clathria aculeata Ridley, 1884	1930.8.13.93a	*Clathria* (*Thalysias*) *abietina* (Lamarck, 1814)	*Clathria* (*Thalysias*) *abietina* (Lamarck, 1814)
Tenacia coralliophila (Theile, 1903)	1930.8.13.107	*Clathria* (*Thalysias*) n.sp. (not *Clathria* (*Thalysias*) *coralliophila* (Thiele, 1903))	-
-	-	-	*Clathria*(*Thalysias*) *cervicornis* (Thiele, 1903)
-	-	-	*Clathria* (*Thalysias*) *lendenfeldi* Ridley & Dendy, 1886 (4)
-	-	-	*Clathria* (*Thalysias*) *tingens* Hooper, 1996
-	-	-	*Clathria* (*Thalysias*) *vulpina* (Lamarck, 1814) (2,3,4)

Ophlitaspongia rimosa (Ridley, 1884) (1)	1930.8.13.17a	*Clathria (Isociella) eccentrica* (Burton, 1934)	-
Ophlitaspongia eccentrica Burton, 1934	1930.8.13.109a	*Clathria* (*Isociella*) *eccentrica* (Burton, 1934)	*Clathria* (*Isociella*) *eccentrica* (Burton, 1934)
-	-	-	*Clathria* (*Microciona*) n.sp. #1882
-	-	-	*Clathria* (*Microciona*) n.sp. #2265
-	-	-	*Echinochalina* (*Echinochalina*) *tubulosa* (Hallmann, 1912) (4)
-	-	-	*Raspailia* (*Raspaxilla*) *reticulata* Hooper, 1991
-	-	-	*Echinodictyum mesenterinum* (Lamarck, 1814)
-	-	-	*Endectyon elyakovi* Hooper, 1991
-	-	-	*Raspailia* (*Raspaxilla*) n.sp. #2264
-	-	-	*Thrinacophora* n.sp. #1993 (5)
-	-	-	*Biemna* sp. #2260
-	-	-	*Coelocarteria singaporensis* (Carter, 1883) (2,3,4)
-	-	-	*Crella* sp. #2243
-	-	-	*Strongylacidon* sp. #1533
-	-	-	*Zyzzya* sp. #1653
HALICHONDRIDA			
-	-	-	*Acanthella* n.sp. #1562
-	-	-	*Auletta constricta* Pulitzer-Finali, 1982 (3)
-	-	-	*Axinella* n.sp. #2267
-	-	-	*Axinella carteri* (Dendy, 1889) (3,4)
-	-	-	*Axinyssa* n.sp. #2257
-	-	-	*Cymbastela concentrica* (Lendenfeld, 1887) (3)
-	-	-	*Cymbastela coralliophila* Hooper & Bergquist, 1992 (3)
-	-	-	*Phakellia cavernosa* (Dendy, 1921) (2,3,4)
-	-	-	*Pseudaxinella australis* Bergquist, 1970 (3)
-	-	-	*Reniochalina* cf. *stalagmitis* sp. #417 (4)
-	-	-	*Reniochalina stalagmitis* Lendenfeld, 1888 (4)
Leucophloeus fenestratus Ridley, 1884 (1)	1930.8.13.153a	*Ciocalypta fenestratus* (Ridley, 1884)	-
-	-	-	*Ciocalypta* n.sp. #2251
-	-	-	*Halichondria* sp. #1227 (4,5)
-	-	-	*Halichondria stalagmites* (Hentschel, 1912)
-	-	-	*Hymeniacidon* n.sp. #2261
-	-	-	*Myrmekioderma granulata* (Esper, 1830) (3,4,5)
-	-	-	*Liosina paradoxa* (Thiele, 1899) (3)
DICTYOCERATIDA			
Phyllospongia dendyi Lendenfeld, 1889	1930.8.13.199a	*Lendenfeldia plicata* (Esper, 1806)	-
Carteriospongia foliascens (Pallas, 1766)	1930.8.13.203a	*Carteriospongia foliascens* (Pallas, 1766)	*Carteriospongia foliascens* (Pallas, 1766) (3)
-	-	-	*Coscinoderma mathewsi* (Lendenfeld, 1886) (3,4,5)
Spongia officinalis Linnaeus, 1759	1930.8.13.188a	*Hippospongia* sp. (not *S. officinalis* Linn.)	*Hippospongia* sp. #1983 (5)
-	-	-	*Spongia* cf. *officinalis* Linnaeus sp. #262 (3)
-	-	-	*Dactylospongia elegans* (Thiele, 1899) (3,5)
-	-	-	*Ircinia* sp. #1534
-	-	-	*Ircinia* sp. #1876 (5)
-	-	-	*Ircinia* sp. #2268
-	-	-	*Ircinia* cf. *ramosa* #1377

-	-	-	*Psammocinia* sp. #487
	-		*Fascaplysinopsis* sp. #2170
-	-	-	*Fascaplysinopsis reticulata* (Hentschel, 1912) (3,5)
Dysidea herbacea (Keller, 1889)	1930.8.13.175a	*Dysidea herbacea* (Keller, 1889)	*Dysidea herbacea* (Keller, 1889) (3)
-	-	-	*Dysidea* sp. #229 (4)
-	-	-	*Dysidea* sp. #1214
-	-	-	*Dysidea* sp. #2250
-	-	-	*Dysidea* sp. #2262
-	-	-	*Dysidea* sp. #2266
VERONGIDA -	-	-	*Aplysinella rhax* (de Laubenfels, 1954) (3)
Druinella purpurea (Carter, 1880)	1930.8.13.198a	*Druinella purpurea* (Carter, 1880)	*Druinella purpurea* (Carter, 1880) (3)
-	-	-	*Pseudoceratina* sp. #1565
-	-	-	*Pseudoceratina* sp. #2196
-	-	-	*Pseudoceratina* sp. #2399
-	-	-	*Ianthella basta* (Pallas, 1766) (4)
-	-	-	*Ianthella* cf. *flabelliformis* sp. #196 (4)
-	-	-	*Ianthella flabelliformis* Pallas, 1766) (2,3,4)
TOTAL BURTON SPECIES = 36 spp. (5 new)		15 misidentified spp., 1 sp. uncertain, 14 revised generic assignments	TOTAL QM SPECIES = 109 spp (61 spp. unnamed possibly new)
Comparison between Burton & QM collections = 12 spp. in common. Total Low Isles fauna: 134 species, 63 genera, 35 families.			

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NEW DATA ABOUT MORPHOLOGY AND FEEDING PATTERNS OF BARENTZ SEA *HALICHONDRIA PANICEA* (PALLAS). *Memoirs of the Queensland Museum 44: 262. 1999*:- Visual observations in the marine aquaria and transmission electron microscopy studies on the larvae of the intertidal sponge *Halichondria panicea* demonstrated individual variations in external and internal morphology, behaviour and type of metamorphosis. Parenchymulae of this species were found to possess the ability to actively feed by endocytosis (phago- and pinocytosys). The larvae crawled over the substrate and cast numerous unicellular organisms (bacteria and flagellates from 2 - 4µm in size) onto the body surface by a flagellum. During this, the apical parts of the flagellated cells formed large lobopodia that served for catching and ingesting food particles. I monitored the consequent patterns of contact of the flagellates with the surface of lobopodia, their entrapment, submersion, the formation and transport of the digestive phagosomes into the basal parts of the surface cells. Each surface locomotory cell was capable of catching and ingesting food. No morphological and/or functional differences between the surface cells were found. Nevertheless, singular flagellated cells packed with the phagosomes submerged inside the larva. Here these cells could be easily distinguished by the presence of a flagellum and the typical shape of the nucleus. Later on, the submerged flagellated cells withdrew the flagellum and acquired an amoeboid shape. Final digestion of the caught organisms occurred only inside the larva. It was suggested that endosymbionts found in the surface and inner cells of the larvae served as an additional food source for the larvae. Presence of the numerous pinocytosis vacuoles in the apical parts of the flagellated cells suggested that the sponge larvae are also able to absorb dissolved low-molecular matter.

To conclude, parenchymula of *H. panicea* could be recognised as a living embodiment (a living model) of the hypothetical phagocytella of Mechnikov in which the differentiation of the body layers into kinoblast and phagocytoblast is only primordial, purely functional and still reversible. ❐ *Porifera, intertidal, larva, feeding, digestion, endocytosis, digestive phagosomes, Halichondria panicea.*

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BIODIVERSITY, SPECIES COMPOSITION AND DISTRIBUTION OF MARINE SPONGES IN NORTHEAST AUSTRALIA

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Biodiversity, species composition and biogeographic relationships were compared between 18 regional populations of marine sponges along the NE Australian coastline (extending northwards from Byron Bay to the E Gulf of Carpentaria, including the southern fauna from Sydney-Illawarra as an outgroup comparison), based exclusively on samples of living populations. Much of the older literature concerning Australian sponge taxonomy is too unreliable to be used effectively as a tool to determine conspecificity and explore faunistic relationships, and consequently this literature was ignored completely in our analyses using instead recent collections made throughout the study area, all documented in situ.

Levels of biodiversity varied considerably between many regions, related in part to the size and diversity of habitats present in particular regions, but also to differences in collection effort. Several regions with apparently low sponge diversity (e.g. 3 seamounts in the Coral Sea) were clearly biased by correspondingly low collection efforts, whereas in other regions these biodiversity data appear to be more realistic indicators of species richness. Faunas of the Gulf of Carpentaria and Turtle Islands were more intensively sampled but had relatively low sponge diversity, whereas those of the Swain Reefs, Capricorn-Bunker Group, Lizard Island and Moreton Bay regions had much higher species diversity with equivalent (and sometimes lower) collection effort. Five of the seven relatively highly diverse regions lay in the south (Swain Reefs, Capricorn-Bunker Group, Moreton Bay, Sydney-Illawarra, Sunshine Coast), with only two northern regions showing comparable diversity (Lizard Island, Low Isles), contrary to latitudinal trends in diversity found in some other marine phyla. Statistically these trends do not appear to be artifacts of sampling effort but reflect true differences in provincial diversity.

The number of unique (apparent endemic) species within each of the 18 regions had a median value of about 33%, although this value varied considerably between particular regional faunas. Species endemism was seen to be largely a function of their biogeographic isolation or proximity to other regional faunas and to ecological factors such as the possession of unique habitat types. Regions with highest levels of relative endemism were Sydney-Illawarra (the most southern region; 81% of species), Wreck Reef (the most isolated oceanic region; 46%), and the Gulf of Carpentaria (differing substantially from all other regions in its habitat composition; 45%). Consistent discovery of about 33% of new (i.e. not previously encountered) species from each reef system surveyed suggests that the possible sponge biodiversity in NE Australia greatly exceeds previous estimates of about 1,500 species.

Within NE Australia (ignoring the S NSW outgroup), five provincial faunas were recognised, grouped hierarchically based on parsimony analysis, each showing greater similarities in species composition within-regions, and fewer similarities between-regions: 1) Tweed River (Byron Bay to the Gold Coast) (with 30% provincial species endemism); 2) SE Qld (Moreton Bay to Hervey Bay) (49%); 3) GBR (Capricorn-Bunker Group to the Cockburn Is) (70%); 4) far northern region (coastal reef and islands in the vicinity of northern Cape York, extending into the Gulf of Carpentaria) (52%); 5) Coral Sea (49%) (although not yet substantially surveyed). Lendenfeld's concept of a homogeneous E Australian coastal fauna is rejected, and the possibility that both the GBR and Coral Sea regions each comprise more than single provinces requires further investigation. ❐ *Porifera, biodiversity, biogeography, fauna survey, species distribution patterns, endemism, NE Australia, Great Barrier Reef, Coral Sea.*

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This study examines the biodiversity, species composition and biogeographic relationships between regional populations of marine sponges along the NE Australian coastline, extending northwards from the Byron Bay region (N New South Wales (NSW)) to the E Gulf of Carpentaria (N Queensland (Qld)), with the southern fauna of Sydney-Illawarra region used as an outgroup comparison. Our study is based exclusively on our samples of living populations, documented using contemporary methods. For reasons explained below we ignored the published literature completely in these analyses.

Biogeographic relationships of Great Barrier Reef (GBR) sponges in particular, and of the Qld fauna in general, have been speculative ever since the pioneering studies in this region by Ridley (1884), Poléjaeff (1884a,b), Ridley & Dendy (1887), Sollas (1888), Lendenfeld (1883, 1885a,b, 1887, 1888, 1889), Thiele (1898, 1903), Schulz (1900), Kieschnick (1900) and Burton (1934). Together these earlier authors indicated that a large proportion of this fauna consisted of 'widely distributed Indo-Malay', 'Indo-Pacific', 'cosmopolitan' or 'general east Australian coastal' species, with a much smaller proportion of indigenous species.

There is some evidence from contemporary collections to support this contention in the older literature that a certain proportion of tropical and subtropical Indo-Pacific sponges extensively range in distribution from the Red Sea to the central west Pacific islands. These species are thought to comprise between 5% (Hooper & Lévi, 1994) and 15% of regional faunas (Hooper, 1994), and they mostly concern species associated with coral reefs, belonging to many different families and orders (i.e. demonstrating a diversity of reproductive strategies and mechanisms for dispersal). They occupy a diversity of coral reef habitats, including the reef flats and lagoons (e.g. *Hyrtios erecta* (Keller), *Carteriospongia foliascens* (Pallas)); coral rubble (e.g. *Iotrochota baculifera* Ridley, *Spirastrella* (*Spheciospongia*) *vagubunda* (Ridley), *Tethya robusta* Bowerbank); deeper fringing reefs (e.g. *Axinella carteri* (Dendy), *Ianthella basta* (Pallas)); and specialised habitats such as coral caves (e.g. *Astrosclera willeyana* Lister). The occurrence of these species in a particular region may be linked to the presence or absence of these habitats on each reef (e.g. Hooper, 1994), and in some cases dispersal has been assisted through anthropogenic activities (such as ship bilge water (e.g. *Mycale* (*Zygomycale*) *parishii* (Bowerbank)), and oyster farming (e.g. *Cliona vastifica* Hancock) (e.g. Wesche et al., 1997)).

Morphometrically these widely dispersed populations appear to be conspecific and in some cases they do not appear to vary morphologically across this vast geographic range. But it is still unknown to what extent these discontiguous regional populations differ genetically, their potential capabilities for interbreeding or re-hybridising, or any realistic estimates of what proportion of these species are truly widely dispersed and what proportion consist of complexes of closely related, but genetically distinct, species (sibling species). Increasingly, however, many of these allegedly widely distributed morphospecies are being found to consist of heterogeneous allopatric populations, with biochemical and genetic diversity not necessarily manifested at the morphological level (e.g. Solé-Cava and Thorpe, 1986, 1994; Hooper et al., 1990, 1992; Solé-Cava et al., 1991, 1992; Bavastrello & Sarà, 1992; Boury-Esnault et al., 1992; Kerr and Kelly-Borges 1994; Kelly-Borges et al. 1994; Klautau et al., 1994; Solé-Cava et al., this volume). To date only one allegedly widely distributed species, *A. willeyana*, has been sampled across the entire Indo-Pacific system, including populations from the GBR (Woerheide, 1997). Chemical and genetic analyses suggest that regional populations of this morphospecies may consist of several discrete sibling species, corresponding to subtle but consistent morphometric differences between them. No other data are yet available for other species from the GBR.

The possibility that regional endemism amongst the GBR and Qld species may be higher than previously recognised (Hooper & Lévi, 1994) is supported from three sources.

1) In the more recent literature on GBR sponges local populations of so-called widely distributed species are recognised as belonging to distinct species (Wilkinson, 1978; Pulitzer-Finali, 1982; Thompson et al., 1987; Hooper, 1987, 1990, 1991, 1996; Bergquist et al., 1988, 1990; Sarà, 1990; Fromont, 1991, 1993; Van Soest et al., 1991, 1996; Hooper & Bergquist, 1992; Van Soest & Hooper, 1994; Bergquist & Kelly-Borges, 1991, 1995; Kelly-Borges & Vacelet, 1995). These contemporary studies differ from the older literature largely through their recognition that consistent (and sometimes subtle) morphometric differences between regional populations may constitute valid interspecific differences, as opposed to merely recognition of (sometimes substantial) intraspecific variability. A common

feature of these contemporary studies is that they were largely based on living populations and not solely reliant on often antiquated, preserved or dry, museum voucher specimens (which lose most of their useful field characteristics). Some of these studies also include chemical and genetic data to support their morphological hypotheses. By comparison, very few authors of the older literature had access to living populations, with few (if any) data on living species' characteristics. For many taxa (particularly Chalinidae, Callyspongiidae, Halichondriidae), such data are mandatory, and consequently, as stated long ago by Hallmann (1912), many of the identifications in the older literature have long been doubtful.

Unfortunately, however, these species described in the contemporary literature comprise only a relatively small proportion of the published fauna of the entire GBR and Qld, with most species names established in the older literature (see Hooper & Wiedenmayer, 1994).

2) Our re-examination of some museum voucher specimens described in the older literature has found many instances where species were misidentified, with regional populations being unjustifiably 'lumped' into a single widely distributed or so-called cosmopolitan taxon (e.g. Hooper, 1991, 1996; Hooper & Weidenmayer, 1994; Hooper et al.,1999, this volume). Unfortunately, again, relatively few of these older species have yet been revised — a long and arduous process — and the status of many nominal species throughout the GBR and Qld faunas is still in doubt. Until identifications can be confirmed, estimates of endemism are equivocal, and endemism is referred to as 'apparent'.

3) Throughout the Indo-Pacific there are published regional faunas which have much higher levels of species endemism and relatively fewer widely distributed species than has been suggested for the GBR and Qld in the older literature. This extra-limital literature includes both earlier authors (e.g. Topsent (1897) and Thiele (1900, 1903) in describing the Ambon and Ternate faunas; de Laubenfels (1954) on the central west Pacific island and atoll faunas) and more contemporary publications (e.g. Bergquist (1968 et seq.) on the New Zealand fauna; Lévi (1967 et seq.) on the New Caledonia fauna). Theoretically, levels of species endemism amongst Qld and GBR faunas might also be expected to approach these other regions, but the existing taxonomic literature is largely unreliable to serve as a basis to analyse faunistic relationships of sponges in this region.

For these reasons, it has not been possible to develop any reliable hypothesis on the biogeographic affinities of the GBR and Qld sponge faunas, even though 428 'valid' species of sponges have already been published from this region (Hooper & Wiedenmayer, 1994; including literature published since 1994). Many of these species are still poorly known, with relatively few subsequently recorded since they were first described (particularly those of Lendenfeld). Moreover, recent collections from this region now consist of >1,500 species, most documented from living populations (Queensland Museum (QM) collections), but most cannot yet be assigned reliably to a known taxon given the largely inadequate descriptions in the older literature, their lack of published data on living characteristics, the significant proportion of misidentifications amongst the so-called widely distributed species, and the inaccessibility, scattered and time-consuming task of locating and re-examining type collections.

Consequently, we chose to make use of these comprehensive, but still largely unnamed QM sponge collections to explore the biogeographic affinities within the Qld regional faunas by ignoring the published literature completely. This literature, concerning the 428 described species from Qld waters, is summarised in Hooper & Wiedenmayer (1994). The QM collections were primarlily obtained from shallow coastal waters of the Qld coast, GBR and the Coral Sea (0-70m depth), with accurate GPS locality data, habitat descriptions and underwater photography. They were obtained using SCUBA and trawling, and have been identified and documented to species level (with many already known to be new to science). Our standardised method of collection and documentation provides us with the ability to unequivocally differentiate between closely related sibling species and not to rely solely on the literature to determine conspecificity and faunistics relationships. It is well beyond the scope of this paper to provide a comprehensive list of raw species data used to compare regional and provincial faunas. These raw data have been included (in tabular format) on the senior author's personal web page at the QM web site (http://www.qmuseum.qld.gov.au).

Of these QM collections we selected 17 discrete regions within the Qld fauna (i.e. ignoring some of the dispersed inter-reef regions sampled such as the collections described by Cannon et al., 1987). Together these collections consisted of approximately 800 species. As an

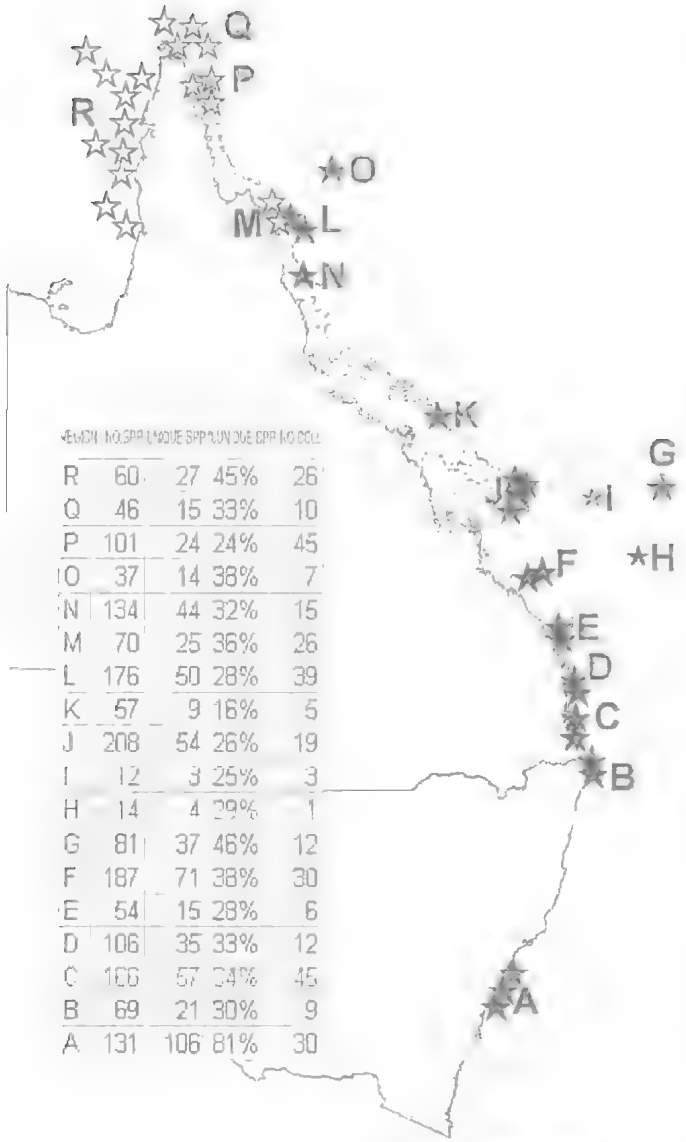
TABLE 1. Regional species diversity (bold numbers in the diagonal row) and similarities in species composition between sponge faunas of central and NE Australia (upper half of matrix showing the numbers of species shared between each region; lower half of matrix showing the percentage similarity between regional faunas (Greig-Smith Similarity Index; Krebs, 1978)). Key to regions: A, Sydney-Illawarra region; B, Tweed River region, from Byron Bay to the Gold Coast; C, Moreton Bay region, within the bay and outside the bay from South Stradbroke I. to Flinders Reef, N of Moreton I.; D, Sunshine Coast region, from Mooloolaba to Noosa Heads; E, Hervey Bay region, including W side of Fraser I.; F, N islands of the Capricorn-Bunker Group, S Great Barrier Reef; G, Wreck Reef, S Coral Sea; H, Cato Reef, S Coral Sea; I, Saumarez Reef, S Coral Sea; J, Swain Reefs, S Great Barrier Reef; K, Bait and Hook Reefs, Whitsunday Is region, central Great Barrier Reef; L, Lizard I. region, including the Direction Is and MacGilvray Reef, N Great Barrier Reef; M, Turtle Is region, N Great Barrier Reef (trawled fauna); N, Low Isles, N Great Barrier Reef; O, Osprey Reef, Far N Coral Sea; P, Shelburne Bay region, Far N Great Barrier Reef, including the Cockburn and Fast Is (trawled fauna); Q, Torres Strait region (trawled fauna); R, E Gulf of Carpentaria region (trawled fauna).

Number of shared species																		
Region	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
A	**131**	10	10	9	2	1	2	0	0	3	2	2	1	1	0	5	1	1
B	10	**69**	33	14	5	7	1	0	0	8	2	5	4	1	0	6	3	3
C	6.7	28	**166**	31	18	19	6	1	3	28	8	13	3	7	2	16	4	9
D	7.6	16	23	**106**	11	19	7	1	1	25	10	23	3	12	6	12	4	5
E	2.2	8.1	16	14	**54**	11	1	0	0	11	3	7	3	6	3	8	2	4
F	0.6	5.5	11	13	9.1	**187**	21	5	7	61	22	44	5	25	6	23	5	4
G	1.9	1.3	4.9	7.5	1.5	16	**81**	7	4	25	7	20	1	12	5	5	6	1
H	0	0	1.1	1.7	0	5	15	**14**	2	4	1	2	0	2	0	1	0	0
I	0	0	3.4	1.7	0	7	8.6	15	**12**	3	3	3	0	3	0	2	0	0
J	1.8	5.8	15	16	8.4	31	17	3.6	2.7	**208**	28	62	6	29	13	27	11	6
K	2.1	3.2	7.2	12	5.4	18	10	2.8	8.7	21	**57**	31	5	16	4	19	4	3
L	1.3	4.1	7.6	16	6.1	24	16	2.1	3.2	32	27	**176**	16	39	16	32	12	9
M	1	5.8	2.5	3.4	4.8	3.9	1.3	0	0	4.3	7.9	13	**70**	6	0	13	6	8
N	0.8	1.1	4.9	11	6.9	16	12	3	4.5	18	18	26	6.3	**134**	6	22	9	8
O	0	0	2	8.4	6.6	5.4	8.5	0	0	11	8.5	15	0	7.6	**37**	5	0	1
P	4.3	7.1	12	12	10	16	5.5	1.7	3.5	17	24	23	15	20	7.2	**101**	16	16
Q	1.1	5.2	3.8	5.3	4	4.3	9.4	0	0	8.7	7.8	11	10	11	0	22	**46**	9
R	1	4.7	8	6	7	3.2	1.4	0	0	4.5	5.1	7.6	12	8.8	2.1	20	17	**60**
Similarity Index (%)																		

outgroup comparison to check on species relationships throughout the Qld faunas we used recent collections of 131 species from the Sydney-Illawarra region, NSW, all of which we have documented, identified and described in the same manner as the Qld voucher material (i.e. again ignoring the published NSW fauna of Lendenfeld (1884 et seq.), Whitelegge (1889 et seq.), Hallmann (1912 et seq.) and others).

MATERIALS AND METHODS

Species diversity, composition and distributions were compared between 17 discrete regional faunas within NE Australia, extending from Byron Bay (N coast of NSW) northwards to the E Gulf of Carpentaria (Qld), including several seamounts in the Coral Sea, and comparing these with the Sydney-Illawarra region (S NSW; see Fig. 1). From the QM sessile marine invertebrate databases we retrieved 913 species collected from these regions. Some of these species have been described and recorded previously from Qld waters in the literature whereas most cannot be identified with a known taxon (i.e. probably new to science). Only species (known and unknown) for which we have collected a voucher specimen during our contemporary collections were considered in this study. Other species records from the literature from the Qld fauna, for which we do not yet have a voucher specimen in QM collections were ignored and are not included in this study. Hence, the potential diversity of regional sponge faunas is much larger than we consider here, whereas the uncertainty still surrounding some of these taxa preclude us from using them reliably in our species' inventories.



REGION	NO.SPP	UNIQUE SPP	%UNIQUE SPP	NO.COLL.
R	60	27	45%	26
Q	46	15	33%	10
P	101	24	24%	45
O	37	14	38%	7
N	134	44	32%	15
M	70	25	36%	26
L	176	50	28%	39
K	57	9	16%	5
J	208	54	26%	19
I	12	3	25%	3
H	14	4	29%	1
G	81	37	46%	12
F	187	71	38%	30
E	54	15	28%	6
D	106	35	33%	12
C	166	57	34%	45
B	69	21	30%	9
A	131	106	81%	30

FIG.1. Distribution of NE Australian regional sponge faunas, showing collecting localities grouped into regional faunas, table of the total number of species collected, the number and percentage of unique species for each region (apparent endemics), and number of collection stations in each region for which sponges were present (see Table 1 for key to regions).

Species lists were generated for each of the regional faunas from the QM databases. Species were then tabulated as present/absent for each of the 18 regions, producing a pairwise matrix of the number of shared species between individual regions, and a simple index of similarity calculated for each pairwise comparison (Greig-Smith 1964, in Krebs, 1978; as (2*X/Y+Z) where Y and Z are the total number of species in each region and X is the number of shared species between regions Y and Z, expressed as a percentage). A cluster analysis was performed on all pairwise comparisons and plotted using UPGMA (Group- Average) sorting, and data were checked for consistency using non-parametric Spearman's rank correlation analysis on all pairwise correlations. A hierarchical classification based on a heuristic distance matrix was calculated from all pairwise comparisons using PAUP 3.1.1 (Swofford, 1993) with dendrograms plotted using MacClade (Maddison & Maddison, 1992). Based on these comparisons, regions of highest similarity in species composition were then combined into six provincial faunas and reanalysed using these same methods.

Together these analyses provided information on biodiversity (total number of species within each of the 18 regions); regional endemism (number of unique species within each region); similarities and differences between regional faunas (through a pairwise comparision of similar species for each region, expressed as a total number of shared species and a similarity index); and a biogeographic model (using cluster analysis and distance matrices producing a hierarchical classification of regional faunistics similarities).

RESULTS AND DISCUSSION

SPECIES DIVERSITY. Levels of biodiversity in each of the 18 selected regions (Fig. 1) varied considerably (Table 1), undoubtedly related in part to the size and diversity of habitats present in each reef system. In some cases these differences were biased by differences in the collection effort between regions, whereas in other cases they reflect more true indications of diversity (table in Fig. 1).

TABLE 2. Levels of species endemism for each province (combined regions), showing total number of species, number and percentage of unique species (apparent endemics) in each province (see Table 1 for key to regions).

Region	No. spp	Unique spp	% Unique
A	131	106	81
B	69	21	30
CDE	233	114	49
GHI	95	47	49
FJKLNOP	507	356	70
MQR	142	74	52

Few collections were made at Cato and Saumarez Reefs in the Coral Sea, and these reefs were also relatively homogeneous compared to other regions sampled, both factors reflecting their low sponge diversities (14 and 12 spp. respectively).

In contrast, higher diversities in Moreton Bay (166 spp.), the Capricorn-Bunker Group (187 spp.) and Shelburne Bay (including the Cockburn and Fast Islands) (101 spp.) are undoubtedly related to the presence of larger and more diverse habitats in these regions, although there were also more collections undertaken from each region.

By comparison, Lizard Island and the Low Isles are both relatively small reef systems but contain relatively high sponge diversities (176 and 134 spp., respectively), although the former region had over twice the collection effort of the latter.

The Sunshine Coast (106 spp.) and Swain Reefs (208 spp.) had relatively fewer collections than these other regions but relatively high diversity, the latter the most diverse region yet sampled.

Two inshore faunas, the Turtle Islands (70 spp.) and eastern Gulf of Carpentaria (60 spp.) were characterised by shelly and soft sediments and murky waters, yielding only few species despite relatively higher collection efforts.

These trends are summarised in Figure 4. In the case of Cato Reef, Saumarez Reef, Osprey Reef, Torres Strait and Bait and Hook Reefs, apparent low biodiversity is obviously related directly to collection effort, whereas for other reef systems our collections are more valid indicators of existing sponge diversity. This is particularly evident for Lizard Island, the Capricorn-Bunker Group and the Swain Reefs in which species diversity increased despite a consecutive decrease in collection effort (Fig. 4). These differences are confirmed through one-way ANOVA, comparing the numbers of species collected, the number of unique species, and the number of collections made (Table 2), showing significant differences between their means ($P<0.001$).

From our data there is no evidence that species diversity increases at lower latitudes, contrary to some other phyla of marine invertebrates in which biodiversity generally increases towards the equator, especially within the GBR system (e.g. Rohde, 1979). In fact the reverse appears to be true for sponges, in which five of the seven most diverse regions lay in the south (Swain Reefs, Capricorn-Bunker Group, Moreton Bay, Sydney-Illawarra and Sunshine Coast regions), and only two northern regions had comparable sponge diversity (Lizard Island, Low Isles).

SPECIES COMPOSITION. Affinities between regional faunas generally appear to be related to their proximity to each other, such that adjacent regions usually had higher proportions of similar species than did those further apart (Fig. 2). Regions containing the highest proportions of unique species (i.e. apparent endemics) were not necessarily those containing the highest biodiversity; nor were they always artifacts of higher collection efforts ($P<0.001$; Fig. 4), but were those that were either more isolated or contained substantially different habitats than other regions (table in Fig. 1). The southernmost region, Sydney-Illawarra, had 81% endemic species; the most isolated oceanic coral reef, Wreck Reef, had 46%; and the Gulf of Carpentaria, with mainly soft substrata, had 45% unique species (Fig. 1). By comparison, levels of species endemism for most other regions were consistent (between 24-38%), with the exceptions of Bait and Hook Reefs in the central GBR (16%) which probably contains a more even mixture of species from both northern and southern GBR faunas.

These data support previous contentions that sponge species distributions are notoriously heterogeneous, particularly in coral reef faunas, with differences in faunal composition partly attributed to differences in geomorphology between reefs (Hooper, 1994), but also with biogeographic factors influencing composition (as indicated by the correlation between proximity and similarity in species composition).

For each new reef system visited about 30% of species had not previously been encountered, with many of these possibly also new to science. Thus, our prediction of sponge biodiversity for Qld. (about 1500 species; Hooper & Lévi, 1994; Hooper & Wiedenmayer, 1994), may be a gross

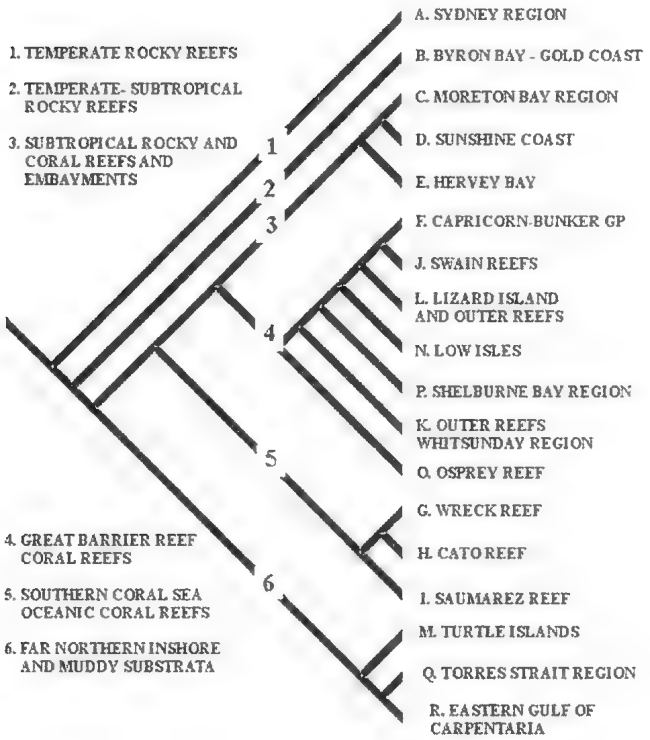


FIG. 2. Cladogram illustrating the hierarchical classification of affinities between regional sponge faunas, based on heuristic distance matrices computed using PAUP 3.1.1, indicating 6 mjaor faunistic provinces. Major provinces are: 1, Temperate rocky reefs; 2, Temperate-subtropical rocky reefs; 3, Subtropical rocky and fringing coral reefs and embayments; 4, Coral islands and reefs of the Great Barrier Reef; 5, S Coral Sea oceanic coral seamounts; 6, Far N coastal islands around Torres Strait and Gulf of Carpentaria, including fringing coral reefs and inter-reef soft substrata.

underestimate, and it is conceivable that twice this number may live in this region.

Parsimony analysis, producing a hierarchical classification of similarities between regions based on regional species compositions (Fig. 2), grouped the 18 regional faunas into 6 logical provinces. These were generally (but not exclusively) correlated with their proximity to each other, their distance from the coast (and terrestrial influences), and possession of similar habitat types in each, such that these provincial groups appear to be valid indicators of biogeographic affinities: 1) Temperate rocky reefs; 2) Temperate-subtropical rocky reefs; 3) Subtropical rocky and fringing coral reefs and embayments; 4) GBR and island coral reefs; 5) Southern Coral Sea oceanic coral reefs; 6) Far northern inshore, fringing coral reefs and inter-reef soft substrata.

REGIONAL BIOGEOGRAPHY. On the basis of these trends we combined the data for the 18 regional faunas into 6 provincial faunas, and repeated this analysis (Fig. 3, Tables 2-3). Ignoring for the time being the most southern region (Sydney-Illawarra, used as an outgroup comparison), and the most isolated region (oceanic southern Coral Sea), similarities in species composition between the other four provinces along the NE coast ranged from only 18-25%.

Highest species diversity and apparent endemism was found in the GBR provincial fauna (507 spp. and 70%, respectively). It has been suggested, based on more subjective criteria (Hooper et al., 1999, this volume) that recognition of a single GBR fauna may be artificial, with the possible existence of separate northern and southern GBR provinces. To test this we compared the two southern GBR regions (Capricorn-Bunker Group and Swain Reefs), showing a 31% similarity in their species compositions, with the three major northern GBR regions (Low Is, Lizard I. and Cockburn and Fast Is), showing similarities between 20-26%. We then compared the combined data sets for the two southern reefs with those of the three northern reefs, discovering that there were 88 species in common, with a similarity index of 22%. Thus, between-group comparisons clearly overlap the within-group comparisons, providing no statistical support for a proposal to subdivide the GBR province. Breaking the data down even further and re-examining all the pair-wise comparisons between species similarities for each of the individual GBR regions was also uninformative (Table 1).

Similarly, any biogeographic trends in sponge distributions that may be useful as a basis for subdivision may also be partially masked by the well-known heterogeneity amongst coral reef sponges (Hooper, 1994), with the two factors difficult to separate.

Nevertheless, from present data we can clearly differentiate at least five provincial sponge faunas within NE Australia, each having high levels of within-group regional endemism and

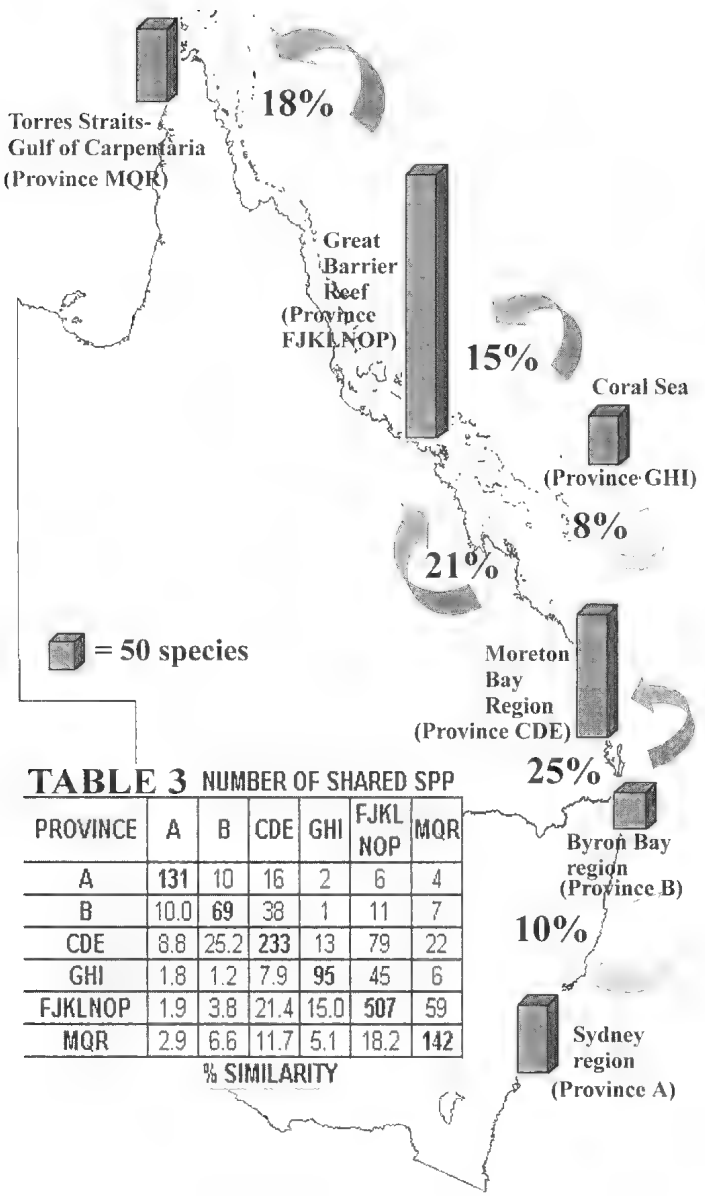


TABLE 3 NUMBER OF SHARED SPP

PROVINCE	A	B	CDE	GHI	FJKL NOP	MQR
A	**131**	10	16	2	6	4
B	10.0	**69**	38	1	11	7
CDE	8.8	25.2	**233**	13	79	22
GHI	1.8	1.2	7.9	**95**	45	6
FJKLNOP	1.9	3.8	21.4	15.0	**507**	59
MQR	2.9	6.6	11.7	5.1	18.2	**142**

% SIMILARITY

FIG. 3. Distribution of NE Australian provincial sponge faunas (18 regional sponge faunas amalgamated into 6 major provinces based on PAUP analysis), showing species diversity (bar graphs), percentage similarity in species composition between adjacent provincial faunas (arrows) (see Table 1 for key to regions).

TABLE 3. Similarities in species composition between the 6 major provinces (upper half of matrix showing the numbers of species shared between each province; lower half of matrix showing the percentage similarity between provincial faunas using Greig-Smith Similarity Index; Krebs, 1978); and total number of species in each province (bold numbers in the diagonal row)) (see Table 1 for key to regions).

relatively low levels of between-group similarities in species composition: 1) Tweed River region (Byron Bay-Gold Coast), with 30% of species not yet found outside this province (apparent endemic species) (Table 2). 2) SE Qld (Moreton Bay – Hervey Bay), with 49% provincial endemism. This fauna is relatively homogeneous in comparison with the other provincial faunas. 3) GBR (Capricorn-Bunker Group – Cockburn Is), with 70% provincial endemism. 4) Far northern coastal and islands region (Torres Strait – E Gulf of Carpentaria), with 52% provincial endemism. It is also likely that this province could be further subdivided, given that the combined Torres Strait – Shelburne Bay regions have only a 17% similarity in their species composition with the Gulf of Carpentaria region (Table 1). 5) Coral Sea, with 49% provincial endemism. Further collections from these seamounts are necessary to determine whether they contain a single homogeneous fauna or several distinct provincial faunas.

The concept of an E Australian coastal sponge fauna, mentioned frequently by Lendenfeld (1888, 1889) is rejected, and the concept of homogeneous GBR and Coral Sea coral reef faunas (cf. Burton, 1934) is also questionable, although more extensive sampling of regional faunas within each of these provinces is required to further investigate any proposed biogeographic subdivisions.

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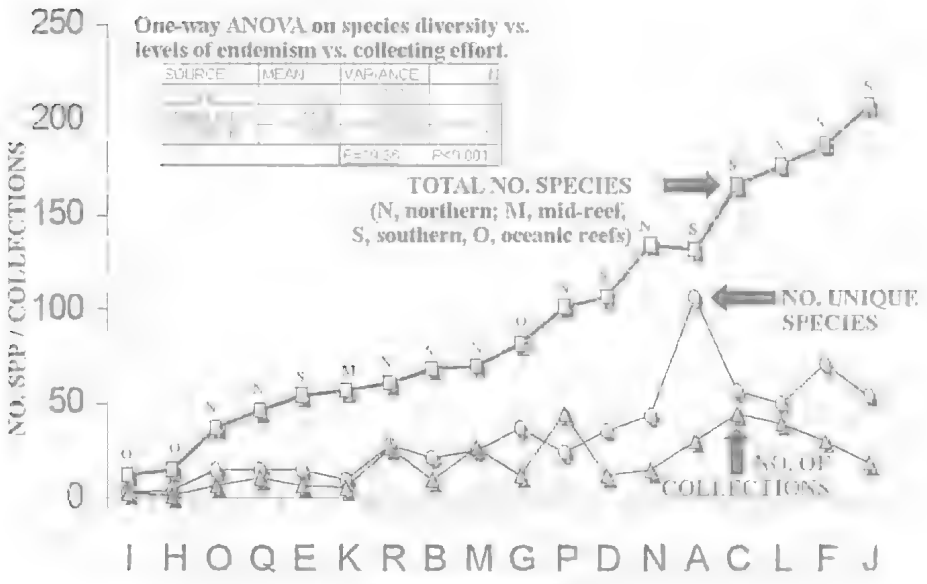

FIG. 4. Comparison between regional species diversity (total number of species collected in each region), endemism (number of unique species in each regional fauna), and collection effort (number of collecting stations in each region that yielded sponges), with regions arbitrarily sorted on increasing species diversity. Results of one-way ANOVA between species diversity, levels of endemism and collecting effort are tabulated.

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MORPHOLOGY AND MOLECULES IN LITHISTID TAXONOMY: NEW SOLUTIONS FOR OLD PROBLEMS. *Memoirs of the Queensland Museum 44: 274. 1999*:- Most lithistid sponges lack an adequate range of taxonomic characters for differentiation, and in most genera these characters are extremely plastic. Consequently, the generation of morphological hypotheses in comparison with molecular phylogenies is nearly impossible due to the absence of reliable synapomorphies. Historically, lithistids have been grouped together in a single order on the basis of common possession of an interlocking siliceous skeleton. Recent morphological and palaeontological data indicate, however, that lithistid sponges are polyphyletic; several genera possess skeletal characters that suggest affinity with non-lithistid demosponges. We have found that in many cases these characters are probably non-homologous and misleading.

Ongoing research on the phylogeny of lithistid sponges has revealed some interesting 'anomalies' of identification. Although our data collection is still incomplete, we have already found unexpected phylogenetic affinities between three lithistid species in Theonellidae and Corallistidae, comparing morphological and 28S rDNA analyses. Surprisingly, the nearest relatives of de Laubenfel's (1954) '*Plakinalopha*' *mirabilis* are *Theonella* spp.; *Theonella atlantica* is more closely related to *Corallistes* spp. than to *Theonella* spp.; and *Theonella tubulata* Van Soest is more closely related to *Macandrewia azorica* (in the Corallistidae) than to other *Theonella*.

What is to be done in this situation ? To what extent can molecular hypotheses be accepted over morphological hypotheses or vice versa ? We have found that rather than having to 'accept' one over the other, which often goes against 'instinctual phylogeny', molecular data makes us re-examine these problems by reciprocal illumination, through the generation of higher quality morphological research and the examination of characters that are often, not at first, obvious. With this group of lithistid sponges, triaene rhabd and clade morphology, microsclere ornamentation, and the patterns of desma zygoses, and shaft ornamentation become crucially important in differentiating taxa.

Thus, for this particular group of organisms, we have found that morphological hypotheses between closely related taxa are often strongly informative and can lend crucial evidence for the acceptance of certain molecular phylogenies over others. Molecular data can clearly indicate relationships between organisms where morphological data had previously failed, and molecular data often require us to re-examine morphological characters from new perspectives, leading the discovery of new taxonomic discriminators. ❐ *Porifera, phylogeny, 28S rDNA, morphology, congruence, lithistid, Theonellidae, Corallistidae.*

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PHYLOGENETIC RELATIONSHIPS BETWEEN LUBOMIRSKIIDAE, SPONGILLIDAE AND SOME MARINE SPONGES ACCORDING PARTIAL SEQUENCES OF 18S rDNA

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Itskovich, V.B., Belikov, S.I., Efremova, S.M. & Masuda, Y. 1999 06 30: Phylogenetic relationships between Lubomirskiidae, Spongillidae and some marine sponges according partial sequences of 18S rDNA. *Memoirs of the Queensland Museum* **44**: 275-280. Brisbane. ISSN 0079-8835.

Two families of Porifera are represented in Lake Baikal, Russia: cosmopolitan Spongillidae and endemic Lubomirskiidae. Systematics and phylogeny of Lubomirskiidae are still poorly known. Indeed, there is little agreement on the origin of freshwater sponges in general, and this group is considered to be polyphyletic. Latest morphological and embryological data indicate that Lubomirskiidae and Spongillidae are closely related. Using molecular data we explored the possible origins of Lubomirskiidae and determined the closest relatives of Spongillidae and Lubomirskiidae among marine sponges. Partial sequences of 18S rDNA for *Halichondria japonica*, *Lubomirskia abietina*, *Swartschewskia papyracea*, *Spongilla lacustris* and *Ephydatia muelleri* were compared with available sequences of 18S rDNA of other Porifera from the GenBank. Parsimony and neighbour-joining analyses gave trees of similar topology. Molecular data were in accordance with the notion of close relationships of endemic and cosmopolitan families. Some marine sponge families are assumed to be related to freshwater sponges.❐ *Porifera, Lake Baikal, Spongillidae, Lubomirskiidae, 18S rRNA, phylogeny, freshwater sponges.*

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Three families of Porifera inhabit freshwater: Spongillidae, Lubomirskiidae and Potamolepidae. The problem of the origin of endemic and cosmopolitan freshwater sponges, their relationships with each other and with marine sponges, repeatedly attract the attention of scientists. Marshall (1885) suggested freshwater sponges were polyphyletic, with Renieridae (Haplosclerida) possibly being their closest marine relative. The idea of a polyphyletic origin for freshwater sponges was subsequently discussed and emphasised by many authors. In describing the genus *Sterastrolepis*, believed to be a Neotropical representative of Potamolepidae, Volkmer-Ribeiro & De Rosa-Barbosa (1978) noted that the characteristics of its gemmoscleres were too different to assign this family to Haplosclerida. On the basis of gemmule structure, gemmosclere and skeleton peculiarities, they confirm Briens' (1970) assumption about the close relationship of Potamolepidae with Hadromerida. They also favour the hypothesis of a passive mechanism of invasion into freshwater habitats by marine sponges, noting that endemic freshwater genera (e.g. *Ochridaspongia*, *Pachydictium*, *Lubomirskia*) have been recorded from ancient lakes, remnants from past sea levels, but not from estuaries. Evidence for a hadromerid origin of some freshwater sponges (Volkmer-Ribeiro & Watanabe, 1983) is also provided by the Japanese sponge *Sanidastra yokotonensis*. Volkmer-Ribeiro (1990) also hypothesised that the Neotropical genus *Metania* may be related to the marine poecilosclerid genus *Acarnus*. Conversely, Racek & Harrison (1975), using palaeontologic data, suggest that *Spongillidae* was monophyletic having evolved from *Radiospongilla* stock.

The endemic family Lubomirskiidae, inhabiting Lake Baikal, has approximately 10 species belonging to 3 genera: *Lubomirskia*, *Baikalospongia* and *Swartschewskia* (Rezvoi, 1936). At present the systematics and phylogeny of this family is still poorly known. The history of study on the origin of Lubomirskiidae shows a number of contrary opinions. Dybowsky (1882), Swartschewsky (1902), Annandale (1913) and Rezvoi (1936) believed Lubomirskiidae was closely related to marine sponges and not to Spongillidae, owing to their considerable morphological differences. Later palaeontological studies (Martinson, 1940) hypothesised that

Lubomirskiidae were representatives of the mezolimnological fauna, originating much later than the usual palaeolimnological fauna to which Spongillidae belongs. In contrast to these beliefs, the latest comparative morphological data indicate a close relationship between Spongillidae and Lubomirskiidae (Efremova, 1981), supported by data on their loss of sexual reproduction by gemmules as an adaptive feature (Efremova, 1994). To solve contradictions in the systematic and phylogenetic interpretation of morphological data rDNA analysis is now widely used (e.g. Christen et al., 1991; Halanych, 1991). Although this method has been succesfully used for some marine sponge families (Lafay et al., 1992; West & Powers, 1993; Kelly-Borges & Pomponi, 1994) there are no previous studies on molecular phylogeny of freshwater sponges. In this study we apply partial 18S rDNA sequence analysis, firstly to explore the origin of Lubomirskiidae, and secondly to obtain new data on the origin of freshwater sponges in general.

TABLE 1. Classification of the species used in this study.

Classification	GenBank accession number	References
CNIDARIA: ANTHOZOA		
Parazoanthus axinella (Zoantharia: Zoantidae: Parazoanthida)	U42453	Cavalier-Smith, 1996
PORIFERA: DEMOSPONGIAE		
Axinella polypoides (Axinellida: Axinellidae)	U43190	Cavalier-Smith, 1996
Tetilla japonica (Spirophorida: Tetillidae)	D15067	Kobayashi et al., 1993
Microciona prolifera (Poecilosclerida: Microcionidae)	L10825	Wainright, 1993
Halichondria japonica (Halichondrida: Halichondridae)	AF058946	this study
Lubomirskia abietina (Haplosclerida: Lubomirskiidae)	AF058947	this study
Swartschewskia papyracea (Haplosclerida: Lubomirskiidae)	AF058948	this study
Ephydatia muelleri (Haplosclerida: Spongillidae)	AF058949	this study
Spongilla lacustris (Haplosclerida: Spongillidae)	AF058945	this study
PORIFERA: CALCAREA		
Clathrina cerebrum (Calcinia: Clathrinida: Clathrinidae)	U42452	Cavalier-Smith, 1996
Scypha ciliata (Calcaronia: Sycettida: Sycettidae)	L10827	Wainright, 1993
Scypha calcaravis (Calcaronia: Sycettida: Sycettidae)	D15066	Kobayashi et al., 1993

MATERIALS AND METHODS

Specimens of *Lubomirskia abietina*, *Swartschewskia papyracea*, *Spongilla lacustris* and *Ephydatia muelleri* were collected from Lake Baikal (Russia) and specimens of *Halichondria japonica* were collected from Desaki seashore (Japan) by SCUBA diving in depths between 0.5-13.5m. All specimens were photographed alive. Data on ecology, habitat and texture were recorded. Part of each sample was fixed in 70% ethanol for taxonomic identification, another part was frozen in liquid nitrogen for molecular analysis. Total genomic DNA extraction was performed with standard phenol method (Sambrook et al., 1989) and with CTAB method (Gustincich et al., 1991). PCR primer design was performed by alignment of Porifera 18S rRNA sequences available from GenBank (see Table 1). As sponges harbour a large number of symbionts, in addition to universal primers, sponge-specific primers were also designed. The primers correspond to the V4 and V5 regions of 18S rRNA: R1 (5'-TAAAAAGCTCGTAGTTGGAT-3'; forward, universal, correspond to positions 629-647 in *Axinella polypoides* 18S rRNA (GenBank accession number U43190)); L1 (5'-GGACTACGACGGTATCTGAT-3'; reverse, universal (1008-1026)); R2 (5'-GTAGTGGC CTACCATGGTTGC-3'; forward, sponge-specific (342-361)); L2 (5'-CTAATTTTTTCAAAG TAAACGTCCCGA-3'; reverse, sponge-specific (749- 777)).

The primers were synthesised by H-phosphonate method. Two overlapping fragments of the 18S rRNA gene (400bp each) were amplified. A 25µl PCR reaction mix contained 2.5µl of 10×PCR Buffer (Promega), 3µl of $MgCl_2$ (25mM), 0.5µl of each primer (10pmol/µl), 1µl of dNTP mix (100mM each), 1µl of DNA (~0,1µg), 0.2µl of Taq DNA polymerase, 25µl of ddH_2O. Cycle parameters were: initial denaturation at 94°C for 120secs, followed by 40 cycles of denaturation at 94°C for 60secs, anneling at 45°C for 60secs, and extension at 72°C for 60secs, followed by a final extension of 8mins at 72°C. About 6 tubes of each PCR reaction were purified by electrophoresis in low melting agarose. PCR fragment purification was carried out twice with equal volume of phenol, followed by precipitation by 2 volume of ethanol and 0.1 volume of 10M ammonium acetate and washing in 70% ethanol (Sambrook et al., 1989). PCR

FIG. 1. Alignment of partial 18S rDNA sequences (630 bp) obtained. Only nucleotides that differ from those of *Lubomirskia abietina* are indicated (identities are denoted by points and deletions by hyphens). GenBank accession numbers are: *Halichondria japonica* AF058946, *Lubomirskia abietina* AF058947, *Swartschewskia papyracea* AF058948, *Spongilla lacustris* AF058945 and *Ephydatia muelleri* AF058949.

fragments were sequenced on both strands using fmol DNA sequencing System (Promega) according to the published protocol. Cycle parameters were: initial denaturation at 95°C for 120secs, followed by 30 cycles of denaturation at 95°C for 30secs, anneling at 42°C for 30secs, and extension at 70°C for 60 secs. The structures obtained were aligned manually with the help of the GeneTools package (Resenchuk, 1991). Neighbour-joining analysis was derived using Treecon for Windows (Van de Peer, 1994). The distance estimation was carried out using the formula of Kimura (1980). Bootstrap values were calculated from 100 replicates. *Parazoanthus axinellae* was used as the outgroup. Programs SEQBOOT, DNAPARS and CONSENSE of PHYLIP 3.5c package (Felsenstein, 1995) were used to construct maximum parsimony trees. Bootstrap analyses with 100 replications were carried out.

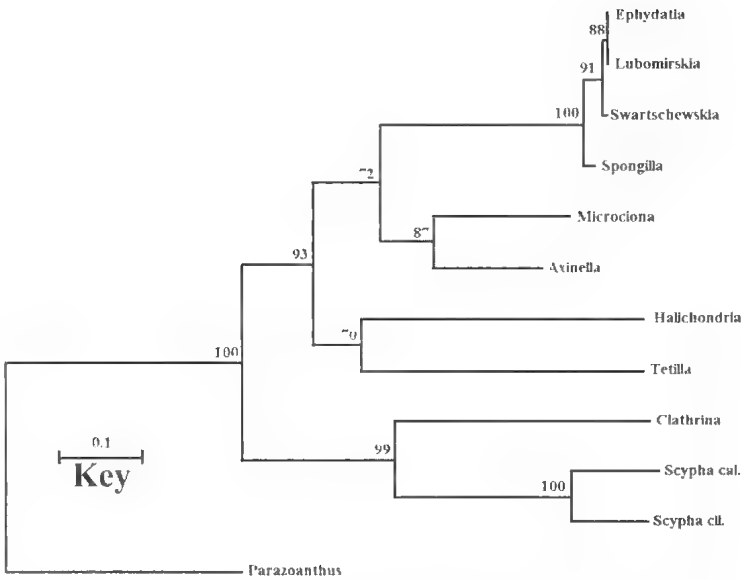

FIG. 2. Phylogenetic relationships of the Lubomirskiidae, Spongillidae and other Porifera based on neighbour-joining analysis of 18S rDNA (630bp). Bootstrap percentages are shown at the nodes for 100 resamplings. *Parazoanthus axinellae* used as the outgroup.

RESULTS AND DISCUSSION

We obtained partial 18S rRNA gene sequences (~630bp) for five species of Porifera. GenBank accession numbers are as follows: *Halichondria japonica* (AF058946), *Lubomirskia abietina* (AF058947), *Swartschewskia papyracea* (AF058948), *Spongilla lacustris* (AF058945) and *Ephydatia muelleri* (AF058949). Two specimens of each species were used to obtain sequences. All structures were aligned successfully, and common length of alignment was 630bp (Fig. 1).

There are a few nucleotide differences between 18S rDNA structures obtained for freshwater sponges compared to those from marine sponges. Sequences from the marine sponge *H. japonica* have many more transitions/ transversions events, and insertion/deletion events were observed only this species. *Lubomirskia* and *Ephydatia* show no nucleotide differences in their 18S rDNA sequences, indicating a very high level of genetic relationships between them.

To study the molecular relationships between freshwater and marine sponges, sequences from other Porifera available from GenBank (see Table 1) were included in the alignment.

Figure 2 shows a tree obtained by neighbour-joining analysis with *Parazoanthus axinellae* as the outgroup. High bootstrap values show that all clusters are statistically significant. *Spongilla, Lubomirskia, Swartschewskia* and *Ephydatia* form a common clade. A sister branch formed by *Axinella* and *Microciona* is the most closely situated to this clade. Parsimony analysis, performed on the basis of these sequences, provides a similar topology (not shown here). These data confirm that freshwater sponge genera form a closely related group and, except for *Axinella* and *Microciona*, *Halichondria* and *Tetilla*, also refer to the neighbouring cluster.

These molecular data are in accordance with the notion of a close relationship between endemic and cosmopolitan families. They do not support the idea that Lubomirskiidae has an independent origin from Spongillidae. These data also suggest that the assumption of Racek & Harrison (1975), that endemic genera in the ancient lakes appeared independently of the cosmopolitan fauna, is invalid as far as Baikalian Lubomirskiidae is concerned.

Branch length shows that divergence of Lubomirskiidae and Spongillidae took place much later than divergence of their common ancestor. It

provides support for Efremova (1981) that Lubomirskiidae is not a relic fauna, but a flourishing group of Lake Baikal organisms. This also confirms Talievs' (1955) opinion about the relatively fast evolution of the Lake Baikal fauna. It will be interesting to check this assumption using palaeontological studies of sponge spicules in the bottom sediments of Lake Baikal.

It is possible that the scenario of Baikalian sponge fauna formation is similar to that of the Baikalian Turbellaria, which is closely related to cosmopolitan species (Timoshkin, 1995). Thus, although a part of Lake Baikal fauna really has marine origin, Baikalian sponges have a typical freshwater origin.

However, as the evolution of animal 18S rDNA is non-clock-like, it is advisable to conduct investigations into the cytochrome oxidase genes whose sequences are not yet available for Porifera. This study would allow estimates to be made of divergence times between Lubomirskiidae and Spongillidae. Our tree also demonstrated an earlier divergence of *Spongilla* from the common branch of freshwater sponges. However, the few freshwater genera yet analysed, and insufficient variability of 18S rDNA, does not yet provide any unequivocal support to hypothesise relationships between certain freshwater genera. To study relationships between closely related freshwater genera, we need data from more variable regions of the gene. Work on internal transcribed spacers (ITS1 and ITS2) is currently in progress. *Trochospongilla* is likely to be a possible direct ancestor of Lubomirskiidae. This genus has no microscleres, and spicules have maximal mutability. According to preliminary data, *Axinella, Microciona, Halichondria* and *Tetilla* are the most closely related to the present freshwater sponges. It is probable, however, that obtaining new data on the other marine sponge sequences, for example other Haplosclerida, will substantially change the scheme presented here.

ACKNOWLEDGEMENTS

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CYMBASTELA HOOPERI AND *AMPHIMEDON TERPENENSIS*: WHERE DO THEY REALLY BELONG?

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König, G.M. & Wright, A.D. 1999 06 30: *Cymbastela hooperi* and *Amphimedon terpenensis*: where do they really belong? *Memoirs of the Queensland Museum* **44**: 281-288. Brisbane. ISSN 0079-8835.

A sponge sample identified as *Cymbastela hooperi* collected from Kelso Reef, the Great Barrier Reef, Australia, yielded a series of natural products, mainly diterpene isonitriles, which demonstrated significant in vitro antimalarial activity. As a result of these compounds being consumed in a number of bioassays it was considered desirable to have more of them so as to enable further and more detailed biological testing to be undertaken. Subsequently three *Cymbastela* samples (two of *C. concentrica* and one of *C. coralliophila*) and two of *Amphimedon* (*Cymbastela*) *terpenensis* were tested for antimalarial activity and investigated for their natural product content. The results of these investigations provided further evidence that either, *Amphimedon terpenensis* is more appropriately *Cymbastela terpenensis*, or that both *C. hooperi* and *A. terpenensis* belong to an as yet undefined genus and may perhaps be the same species. ❐ *Porifera, Cymbastela, Amphimedon, Acanthella, biological testing, malaria, cytotoxicity, taxonomy, Great Barrier Reef, diterpene isonitriles, marine natural products.*

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Since the discovery by Angerhofer et al. (1992a) of the antimalarial activity of axisonitrile-3 (Fig. 1A) isolated from the sponge *Acanthella klethra*, much of our research activity has focused on finding further marine natural products with this biological activity. These efforts resulted in the identification of other marine derived natural products having selective antimalarial activity (König et al., 1998, Wright et al., 1996), particularly the compounds isolated from *Cymbastela hooperi* (König & Wright, 1995, 1997a; König et al., 1996; Linden et al., 1996; Wright et al., 1996). In order to obtain further amounts of these natural products it was decided to investigate some sponge samples likely to contain this class of compound. In the present paper we provide a discussion of these biologically-guided isolations, the results of chemical analyses, as well as the possible taxonomic implications of these findings.

MATERIALS AND METHODS

General methodology follows Wright et al. (1996). Abbreviations: DCM, dichloromethane; MeOH, methanol; EtOAc, ethyl acetate; VLC, vacuum liquid chromatography; HPLC, high performance liquid chromatography; TLC, thin layer chromatography; GC, gas chromatography; GC-MS, gas chromatography coupled mass spectrometry; ^{1}H NMR, proton detected nuclear magnetic resonance spectroscopy.

MATERIAL. All sponges were collected using SCUBA from the Great Barrier Reef, in the vicinity of Lizard Island between 11-30m depth. All specimens were frozen, then freeze dried. Five samples of 3 sponge species were: *Cymbastela coralliophila* Hooper & Bergquist, 1992 (Demospongiae, Halichondrida, Axinellidae) (specimen CTA); *C. concentrica*, Lendenfeld, 1887 (Demospongiae, Halichondrida, Axinellidae) (specimens CTD and CTE); *Amphimedon terpenensis*, Fromont, 1993 (Demospongiae, Haplosclerida, Niphatidae) (specimens CTB and CTC).

EXTRACTION AND ISOLATION. Initially, a small piece (~5g of freeze dried tissue) from each sample was exhaustively extracted with DCM followed by MeOH. A portion of the resultant extracts was then sent for antimalarial and cytotoxicity testing (~2mg). ^{1}H NMR and TLC investigations of these extracts were also made. On the basis of the results obtained from the biological testing and the ^{1}H NMR and TLC investigations, specimens CTA, CTC and CTD were subsequently selected for bulk extraction and fractionation.

TABLE 1. Antimalarial activity, towards clones D6 and W2 of *Plasmodium falciparum*, of the dichloromethane (D) and methanol (M) extracts from 5 sponge samples. SI = the ratio of the KB cell cytotoxicity to the *Plasmodium flaciparum* toxicity. * Extract was non-toxic only towards KB cells, in other cell lines it was at least 100 times more toxic.

Sample	Species	IC_{50} (ng/ml)	Clone D6		Clone W2	
		KB cells	IC_{50} (ng/ml)	SI	IC_{50} (ng/ml)	SI
CTA (D)	*C. coralliophila*	>20,000	>10,000	-	>10,000	-
CTA (M)	*C. coralliophila*	>20,000	5150	>3.9	6380	>3.1
CTB (D)	*A. terpenensis*	>20,000	4820	>4.1	>10,000	-
CTB (M)	*A. terpenensis*	>20,000	3240	>6.2	9680	2.1
CTC (D)*	*A. terpenensis*	>20,000	<41	>490	<41	>490
CTC (M)	*A. terpenensis*	>20,000	540	>37	5250	>3.8
CTD (D)	*C. concentrica*	>20,000	1360	>14.7	>10,000	-
CTD (M)	*C. concentrica*	>20,000	2730	>7.3	1360	>14.7
CTE (D)	*C. concentrica*	>20,000	>10,000	-	>10,000	-
CTE (M)	*C. concentrica*	>20,000	8470	>2.4	5700	>3.5

1) Specimen CTA: Freeze-dried tissue (108.4g) was exhaustively extracted with DCM (2L) and MeOH (2L) to yield 15.9g (14.7%) of DCM soluble material, and 11.3g (10.4%) of MeOH/H_2O solubles. VLC separation of the DCM solubles over silica, employing gradient elution from hexane to acetone to MeOH, yielded 15 fractions each of approximately 90ml. Fractions 3-14 were predominantly compounds depicted in Figure 1C-D. The remaining fractions and the MeOH/H_2O solubles were ubiquitous lipids and a number of other sterols.

2) Specimen CTC: Freeze-dried tissue (38.8g) was exhaustively extracted with DCM (1.5L) and MeOH (2L) to yield 3.0g (7.7%) of DCM soluble material. VLC separation of the DCM solubles over silica, employing gradient elution from hexane to EtOAc to MeOH, yielded 12 fractions each of approximately 100ml. Fractions 1-5 were found by GC-MS analysis to contain compounds depicted in Figure 1E-Q. Fractions 6 and 7 were essentially the pure compound shown in Figure 1B. Fractions 10 and 11 were found to contain the compounds depicted in Figures 1R-X and 2A. The remaining fractions and the MeOH/H_2O solubles were ubiquitous lipids and a number of sterols.

3) Specimen CTD: Freeze-dried tissue (57.5g) was exhaustively extracted with DCM (2L) and MeOH (2L) to yield 600mg (1.1%) of DCM soluble material. VLC separation of the DCM solubles over silica, employing gradient elution from hexane to ethyl acetate (EtOAc) to MeOH, yielded 13 fractions, each of approximately 80ml. All fractions and the MeOH/H_2O solubles were ubiquitous lipids and a number of sterols. One of the major components of fractions 8-12 was a sterol of the type represented by the compound shown in Figure 2A.

GC SEPARATIONS. GC analyses were done according to methods previously described (Witte et al., 1993). From each of VLC fractions 1 and 2, obtained from the DCM extract of sponge specimen CTC approximately 1mg of material was taken and analysed my GC-MS. The results of these analyses indicated VLC fraction 1 to contain the compounds depicted in Figures 1E-H, and VLC fraction 2 to contain the compounds depicted in Figures 1I-Q.

BIOLOGICAL TESTING. The antimalarial (antimalarial activity is defined as the ability of some substance, pure or mixture, to inhibit the growth of, or be lethal to, one or other strains of *Plasmodium falciparum*) and cytotoxicity testing was undertaken as previously described (Angerhofer et al., 1992b, Likhitwitayawuid et al., 1993).

RESULTS

A small piece (~5g of dry tissue) from each of 5 sponge samples thought likely to contain diterpene isonitriles of the type represented by diisocyanoadociane (Fig. 1B), was exhaustively extracted with DCM, followed by MeOH, and the resultant extracts sent for antimalarial activity assessment. Out of the 10 extracts only 2 were found to have significant activity, the DCM and MeOH extracts of sample CTC (*A. terpenensis*) (Table 1). The only other extracts to show some promise in terms of their activity and selectivity were the DCM and MeOH extracts of sample CTD (*C. concentrica*), and to a lesser extent the

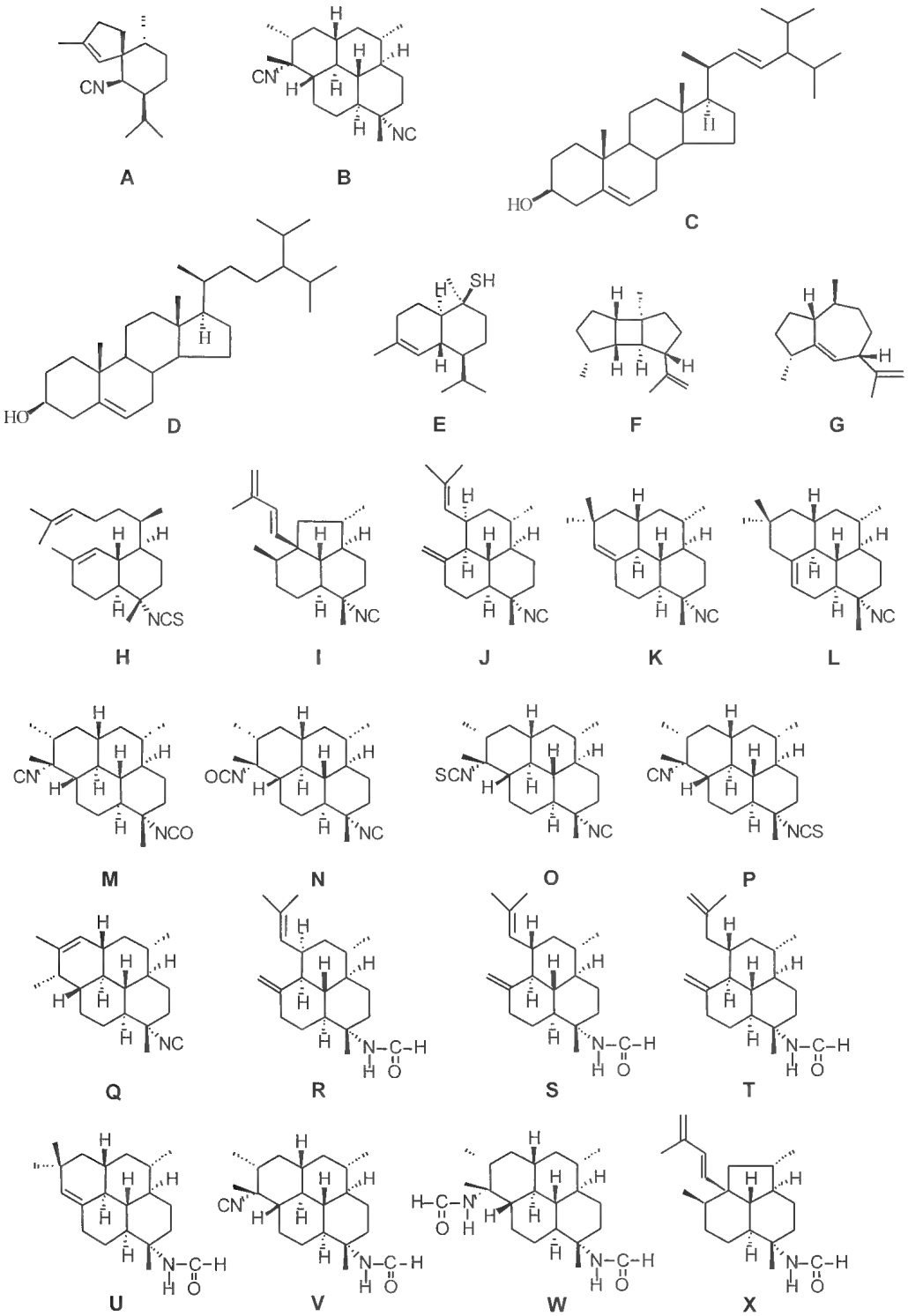

FIG. 1. A-X, chemical structures of secondary metabolites derived mainly from sponges of the genera *Cymbastela* and *Amphimedon* (refer to text for further information).

DCM and MeOH extracts of sample CTB (*A. terpenensis*) (Table 1). On the basis of results presented in Table 1, TLC and ^{1}H NMR examinations of the extracts, detailed investigations were made of samples CTA (*C. coralliophila*), CTC (*A. terpenensis*) and CTD (*C. concentrica*). For this purpose DCM and MeOH extracts were prepared from bulk material of each of the 3 samples to identify the major components present.

SAMPLE CTA (*C. CORALLIOPHILA*). The DCM extract of this sample (CTA, *C. coralliophila*) was found to contain predominantly lipids and 2 sterols (Fig. 1C-D), previously isolated from *Pseudaxinyssa* sp. The latter sample was collected from several mid-shelf reefs on the Great Barrier Reef, by Hofheinz & Oesterhelt (1979). These 2 sterols have also been isolated from another *Pseudaxinyssa* sp. collected from a reef fringing Pelorus Island on the Great Barrier Reef (König, G. M. & Wright, A. D., unpublished data). An interesting observation concerning these compounds (Fig. 1C-D) is, that they always seem to occur as a 1:1 mixture which is essentially inseparable, even by GC (Bergquist et al., 1980). The MeOH extract was composed of ubiquitous lipids and a number of other sterols (Bergquist et al., 1980).

SAMPLE CTC (*A. TERPENENSIS*). Chromatographic and ^{1}H NMR analyses indicated the MeOH extract of CTC (*A. terpenensis*) to contain many of the components to be found in the DCM extract. The MeOH extract was therefore partitioned between water and DCM and the resulting DCM solubles combined with the DCM extract. These DCM solubles were fractionated as outlined in the experimental section. ^{1}H NMR analysis of the resultant fractions indicated a similarity in composition to those produced by the fractionation of the DCM solubles obtained from the previously investigated *C. hooperi* (König et al., 1996; König & Wright, 1997b). Based on this observation GC-MS investigations of selected VLC fractions were undertaken. These analyses indicated the sample to contain compounds shown in Figures 1E-Q, and thus, to be almost identical in secondary metabolite content to *C. hooperi* (König et al., 1996; König & Wright, 1997b). This finding also explained the observed antimalarial activity of its DCM extract. As a result of these studies it was also observed that VLC fraction 11 contained a number of resonances in the 8.0-8.3ppm region of the proton NMR spectrum. Comparison of this ^{1}H NMR spectrum with an equivalent VLC fraction from *C. hooperi* (König et al., 1996; König & Wright, 1997b; Wright et al., 1996) showed the two VLC fractions to be almost identical. Purification of the main components from both VLC fraction 11s has resulted in the identification of 7 diterpene formamide derivatives (Fig. 1R-X), a number of which are new natural products, and a mixture of peroxide containing sterols of the type represented by Figure 2A; the detailed results of this investigation will be presented elsewhere (König et al., in preparation).

SAMPLE CTD (*C. CONCENTRICA*). Both the DCM and MeOH extracts of this sample (CTD, *C. concentrica*) were found to be complex mixtures of ubiquitous lipids and sterols. In VLC fractions 8-12, made from the DCM solubles of CTD, sterols of the type represented by Figure 2A were abundant.

TLC and ^{1}H NMR of the extracts of the two remaining sponge samples, CTB (*A. terpenensis*), and CTE (*C. concentrica*), clearly showed that specimen CTB is very similar in all respects to CTC (*A. terpenensis*) and that sample CTE shows the greatest similarity to samples CTA and CTD, particularly with respect to their ^{1}H NMR spectra. The reduced activity of the DCM and MeOH extracts of CTB when compared to the activity of the equivalent extracts of CTC, appears to be due to the relatively large amounts of lipids present in the extracts of CTB.

DISCUSSION

Of the 3 species of *Cymbastela* investigated none were shown to contain terpenoids substituted with isonitrile based functionalities. This is in direct contrast to results obtained for *C. hooperi* (König & Wright, 1995, 1997a; König et al., 1996; Linden et al., 1996; Wright et al., 1996). In this respect it is of interest to note that *C. hooperi* is also morphologically distinguished from other *Cymbastela* species (Van Soest et al., 1996). The investigation of the two samples of *A. terpenensis* (CTB and CTC), however, led to the identification of secondary metabolites identical, or closely related, to those obtained from *C. hooperi.*

Literature relating to the secondary metabolite chemistry of sponges from *Amphimedon* and *Cymbastela* shows sponges from the former to have received the most attention. In the 40 or so publications on *Amphimedon* the compounds which are typically reported are: various classes of alkaloids (e.g. Fig. 2B-F; Chehade et al., 1997; Kobayashi et al., 1994a; Kobayashi et al., 1994b; Schmitz et al., 1983; Tsuda et al., 1994), long

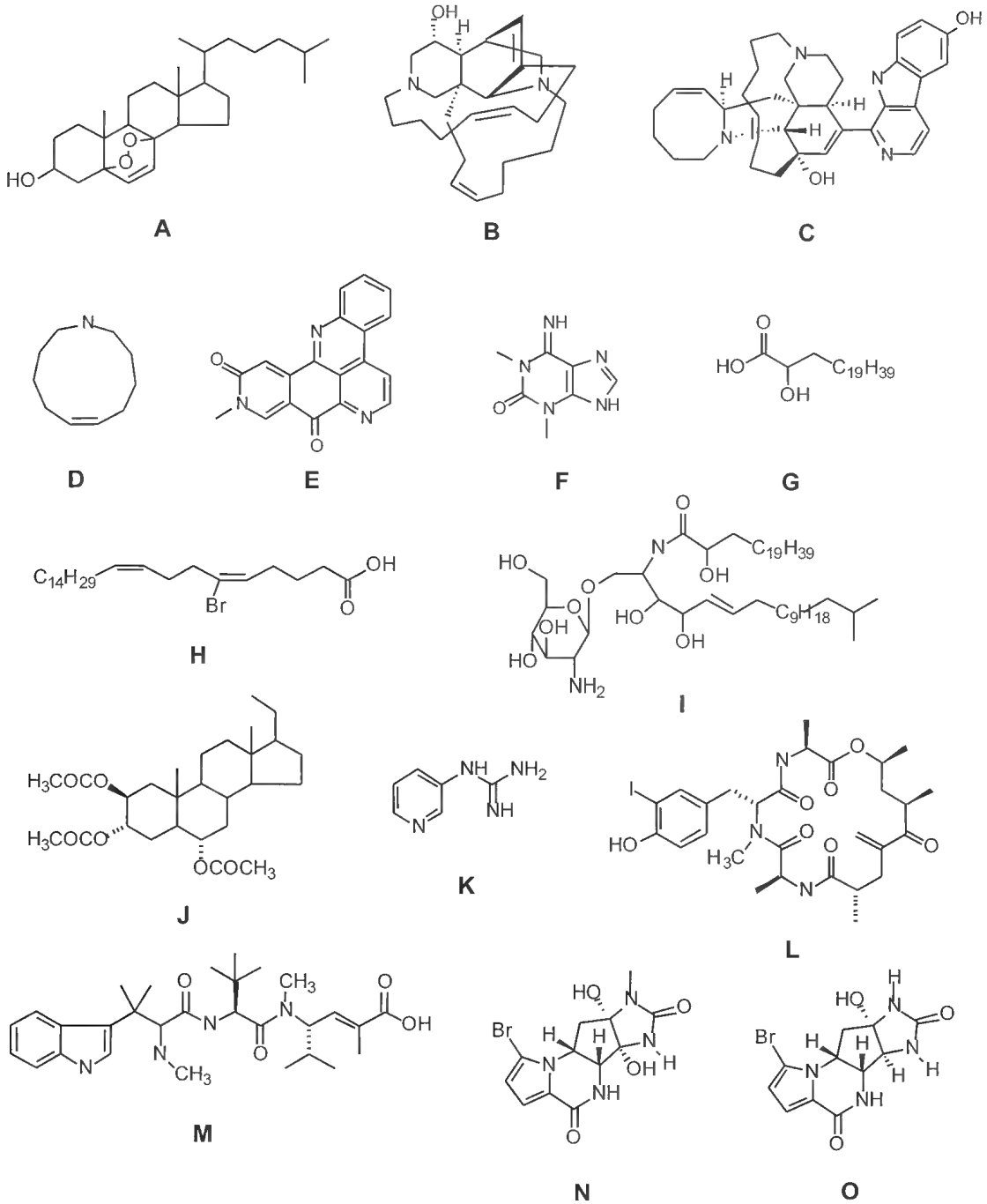

FIG. 2. A-O, chemical structures of secondary metabolites derived mainly from sponges of the genera *Cymbastela* and *Amphimedon* (refer to text for further information).

chain fatty acid derivatives (e.g. Fig. 2G-H; Carballeira & Lopez, 1989; Garson et al., 1994), glycosphingolipids (e.g. Fig. 2I; Hirsh & Kashman, 1989) and some isonitrile containing diterpenes (e.g. Fig. 1B, J, K; Fookes et al., 1988; Kazlauskas et al., 1980; König & Wright, 1995). As there have only been about 11 reports on the secondary metabolite chemistry of sponges from

Cymbastela it is possible to show most of the isolates in this contribution. From *C. coralliophila* steroids of the type represented by Figure 2J were isolated (Makarieva et al., 1995). Pyraxinine (Fig. 2K), a novel alkaloid was isolated from *C. cantharella* (Mourabit et al., 1997), while from two unidentified species of *Cymbastela* two peptides (Fig. 2L-M; Coleman et al., 1995) and two pentacyclic bromopyrroles (agelastatins C and D; Fig. 2N-O, respectively; Hong et al., 1998) were obtained. The present authors have also published a number of works about secondary metabolites from *C. hooperi* (König & Wright, 1995, 1997a; König et al., 1996; Linden et al., 1996; Wright et al., 1996) and the typical metabolites described are mainly isonitrile containing diterpenes (e.g. Fig. 1B, I-Q).

When the literature is considered for *Cymbastela* and *Amphimedon* it is evident that there is currently no class of secondary metabolite one might designate as being 'characteristic' for one or the other of these genera. What is evident, however, is that in both genera only one species produces isonitrile containing diterpenes; *C. hooperi* and *A. terpenensis*.

Two observations can be made: *A. terpenensis* is positioned in an order (Haplosclerida) and family (Niphatidae) where no other sponges are known to produce secondary metabolites that have isonitrile or similar functionalities, and *C. hooperi* is located in an order (Axinellida or Halichondrida; see Van Soest, 1996) and family (Axinellidae) that are known to contain sponges that produce isonitrile containing secondary metabolites. It is clear that '*A. terpenensis*' does not fit in *Amphimedon* as currently defined (Bergquist, Fromont, Hooper, Van Soest, pers. comm.), nor is it clearly a haplosclerid, it is possibly an axinellid close to *Cymbastela*, as suggested by Van Soest et al. (1996), but it's life characteristics do not conform well with the other species of *Cymbastela* (e.g. growth form, texture, mucus production, surface features and amount of spongin to spicule ratio). *Cymbastela* as defined by Hooper & Bergquist is a fairly homogeneous genus, and '*A. terpenensis*' clearly disrupts that homogeneity.

CONCLUSIONS

These observations and the fact that *C. hooperi* and a specimen of *A. terpenensis* have been shown to have almost identical secondary metabolite chemistry, lead to three possible conclusions concerning their current taxonomic classification. 1) *A. terpenensis* belongs to *Cymbastela*, as proposed by Van Soest et al. (1996); 2) both *A. terpenensis* and *C. hooperi* belong to another, possibly new genus located in the family Axinellidae; 3) they are the same species with *hooperi* representing an unusual morphotype of *terpenensis*. The results of the current work indicate that the taxonomic classification of *C. hooperi* and *A. terpenensis* needs to be clarified, particularly since sponges belonging to both of these species produce so many interesting and biologically active compounds. This study also serves to further highlight the significance of secondary metabolite chemistry as an important taxonomic tool. It is also hoped that continued investigations into the biologically active secondary metabolites produced by both of these sponge species will eventually lead to the development of an agent suitable for the treatment of malaria and/or some other disease.

ACKNOWLEDGEMENTS

We thank J.N.A Hooper, Queensland Museum, Brisbane, for identification of all sponges. C.K. Angerhofer, Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, and N. Lang-Unnasch, Division of Geographic Medicine, University of Alabama at Birmingham, provided the antimalarial and cytotoxicity data associated with this study. L. Witte, Institute for Pharmaceutical Biology, Technical University Braunschweig, Germany, performed the GC-MS analysis associated with sample CTC. All NMR measurements made were undertaken by V. Wray and his team, Gesellschaft für Biotechnologische Forschung mbH (GBF), Braunschweig. We are indebted to J. Fromont, Western Australia Museum, Perth, P. Bergquist, University of Auckland and R. Desqueyroux-Faúndez, Museum d'Histoire Naturelle, Geneva, for their constructive comments concerning the possible taxonomic significance of our findings.

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THE REPLACEMENT OF NATURAL HARD SUBSTRATA BY ARTIFICIAL SUBSTRATA: ITS EFFECTS ON SPONGES AND ASCIDIANS. *Memoirs of the Queensland Museum 44: 288. 1999:-* Subtidal reefs around coastal cities such as Sydney are composed of a variety of natural and artificial substrata. Commonly these are natural rocky reefs, breakwalls, seawalls and pier pilings. These types of hard substrata differ in their structure. Most natural hard substrata consist of horizontal surfaces; most surfaces on artificial hard substrata are vertical. Therefore, replacing natural hard substrata with artificial hard substrata is likely to change the surface of substrata from predominantly horizontal to mostly vertical. To understand and predict the potential effects of these changes on the assemblage of sponges and ascidians it is important to determine their distribution on horizontal and vertical surfaces.

The few ecological studies on the distribution of algae and invertebrates on horizontal and vertical surfaces have reported that there are more sponges and ascidians on vertical than on horizontal surfaces. It has not been tested whether these patterns exist in the temperate waters around Sydney. Furthermore, of the studies that have examined the effects of horizontal and vertical surfaces on the distribution of sponges and ascidians, none has experimentally tested the factors that cause these distributions.

Here, I present results of my tests of the hypothesis that sponges and ascidians are more abundant on vertical than horizontal surfaces in the shallow subtidal zone around Sydney. I will also discuss future manipulative experiments to determine which factors are important in creating these distributions. □ *Porifera, Ascidiacea, distribution, hard substrata, shallow subtidal, habitat.*

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CONVERGENCE IN THE TIME-SPACE CONTINUUM: A PREDATOR-PREY INTERACTION. *Memoirs of the Queensland Museum 44: 288. 1999:-* Community structure is influenced by many biotic and abiotic factors. Predation is a key structuring mechanism for some marine communities. Prey abundances may fluctuate with strength of predator recruitment and persistence, except in cases where some of the prey population has a refuge in space or time from predation. Consistent, moderate predation levels on a predictably available prey resource should lead to stable community structure with relatively small fluctuations in predator and prey population densities. Conversely, prey species lacking a refuge from predation are subject to major population fluctuations commensurate with strength of predator recruitment and abundance.

The sponge *Halichondria panicea* is patchily distributed in the rocky intertidal on the south shore of Kachemak Bay, southcentral Alaska, and in certain locations is the spatial dominant. At one site approximately 55m in horizontal length, *H. panicea* has dominated the mid-intertidal for at least 10 years, with low densities of potential molluscan predators such as *Archidoris montereyensis*, *Katherina tunicata*, and *Diadora aspera* present. Percent cover estimates of primary space occupiers at the site were collected from 10 0.25m^2 permanent quadrats established in August 1994. *H. panicea* averaged 53.4% +/-9.9% cover through August 1996. Other major cover categories were algae, 14.6% +/-6.4%, and open rock, 26.1% +/-10.2%. Visits to the site in early spring of 1997 revealed that the sponge colonies overwintered with few indications of major mortality events. No percent cover data were collected at that time.

Total numbers of the nudibranch *Archidoris montereyensis*, which is a specialist predator on *H. panicea*, present at the site were recorded and ranged from 12-42 from 1994-1996. In the spring of 1997, strong recruitment resulted in an average population of 151 *A. montereyensis* on site from May to July. Percent cover of *H. panicea* declined from visual estimates of 40% in May to 15% in July. By August 1997, when the 10 permanent quadrats and 10 haphazardly placed quadrats were measured, essentially no sponge could be found at the study site. After July, the abundance of nudibranchs declined to 32 individuals commensurate with sponge reduction. By September, only one small sponge colony and 7 predatory nudibranchs were present at the site. Even though *H. panicea* is abundant in the region and potential recruits should be numerous, as of April 1998, the site once dominated by *H. panicea* is predominantly open rock with some recruitment of annual macroalgae occurring. The predator-prey relationship of *A. montereyensis* and *H. panicea* is an example of a chase through space and time with convergence resulting in extreme population fluctuations and an unstable community. □ *Porifera, predation, nudibranch, intertidal, predator/prey interaction, community structure, Alaska, recruitment.*

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A CARNIVOROUS SPONGE, *CHONDROCLADIA GIGANTEA* (PORIFERA: DEMOSPONGIAE: CLADORHIZIDAE), THE GIANT DEEP-SEA CLUBSPONGE FROM THE NORWEGIAN TRENCH

BETTINA KÜBLER AND DAGMAR BARTHEL

Kübler, B. & Barthel, D. 1999 06 30: A carnivorous sponge, *Chondrocladia gigantea* (Porifera: Demospongiae), the giant deep-sea clubsponge from the Norwegian Trench. *Memoirs of the Queensland Museum* **44**: 289-298. Brisbane. ISSN 0079-8835.

The ultrastructure of the deep-sea sponge *Chondrocladia gigantea* from the Norwegian Sea, North Atlantic, was studied for the first time. Club-shaped, erect *C. gigantea* has a unique form of aquiferous system, not previously observed in Porifera, consisting of rows of large choanocyte chambers running through the main axis of the sponge, which explains the numerous, normally extended water-filled spheres sitting on little stalks in the upper external part of the main body. These previously enigmatic translucent spheres serve as surface extensions of the sponge to trap prey in the food-poor, deep-sea environment. In addition, they release male reproductive cells into the water. Sexual reproduction seems to play an important role in *C. gigantea*, since spermatocysts were found at different stages of maturity in two out of six samples examined. No mature oocytes were encountered, leading to the assumption that this species may be hermaphroditic (probably with a seasonal reproductive cycle). The phylogenetic relationship of *Chondrocladia* to the other genera of the Cladorhizidae is discussed, based on the presence of an aquiferous system with choanocyte chambers as the basic 'bauplan' of sponges, which is lost in the other genera. ❐ *Porifera, Cladorhizidae, deep-sea, food-poor environment, adaption, aquiferous system, choanocyte chambers, macrophagy, carnivory.*

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Carnivory is an extremely rare feeding strategy among Recent sponges, with confirmed records so far only from the Cladorhizidae, which is a typical deep-sea family restricted to the bathyal, abyssal or even hadal zones. However, Vacelet (1999, this volume) suggests that several other poecilosclerid families are also likely to contain carnivorous species, as judged by their published descriptions. Carnivory was first discovered in *Asbestopluma hypogea* (Vacelet & Boury-Esnault, 1995, 1996), from a Mediterranean shallow-water cave where the habitat resembles that of the deep-sea (Vacelet et al., 1994). In adaption to its food-poor environment *A. hypogea* developed a carnivorous feeding strategy. The organism is no more than 15mm high, carrying long, thin filaments on its slim main axis, on which swimming prey is captured and overgrown by sponge cells within hours. Vacelet et al. (1995) also described carnivory in a *Cladorhiza* sp., a sponge from a mud volcano in the Barbados Trench that has developed a symbiosis with methanotrophic bacteria: 'The sponge morphology, erect with branching processes bearing a cover of hook-like spicules, suggests that they may also feed on swimming prey ... This was supported by the presence of debris from small crustaceans on the sponges. So far, nothing is known about the feeding strategy of the third genus of the Cladorhizidae, *Chondrocladia*.

The deep-sea sponge *C. gigantea* (Lundbeck, 1905) has a remarkable morphology that has always fascinated scientists. The giant clubsponge, whose skeleton is built by styli and collagen, carries spheres filled with water on little stalks. These are situated mostly on the upper part of its main body, which is slim and erect, rooted in the muddy substratum. The tallest known specimen is 600mm long and has a maximum width of 50mm. From in situ photographs thin-walled spheres are seen to be translucent, whereas when brought to the water surface 'the spheres at the tip of the branches are shrunk into the somewhat oblong, clavate, relatively massive structures characteristic of the branches of a number of *Chondrocladia* species ...'(Tendal et al., 1993) (Fig. 1). Like the main body and stalk these spheres have a certain firmness attributed to the presence of collagen and styli, but are additionally covered by hook-like isochelae.

FIG. 1. In situ photograph of *Chondrocladia* sp., by H. Sahling at 4,900m depth, 54°18'N, 157°11'W. Estimated extended sponge diameter is approx. 50mm (reproduced with permission from Tendal & Sahling).

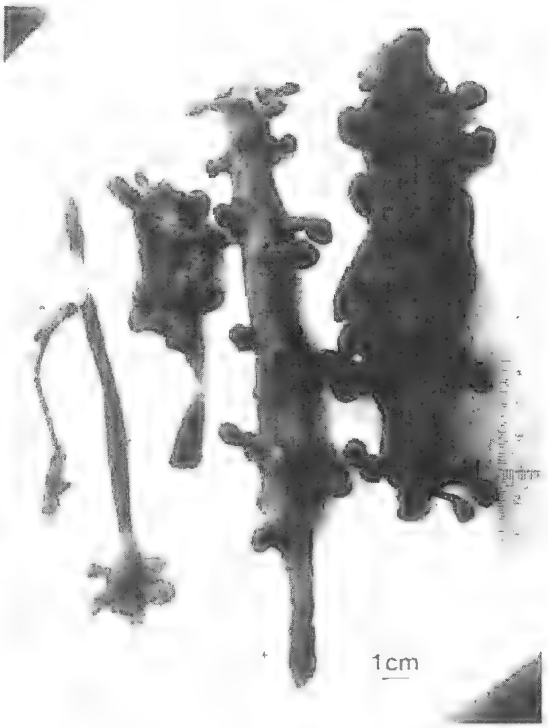

FIG. 2. Five different individuals of *C. gigantea* examined in the present study, prior to TEM-fixation. The compact 'bulbs' on the main axis are deflated spheres. The largest specimen measures 20cm in length.

MATERIALS AND METHODS

Five specimens of *Chondrocladia gigantea* were dredged from 480m depth at BIOICE station 2792 in the North Atlantic (67°15.17'N; 18°52.01'W) on 15 August 1995 (Fig. 2). Their lengths varied from 78-98mm and widths of their main axes between 3-22mm. On board samples were preserved for electron microscopy with a double fixation in 1% glutaraldehyde and 1% osmium tetroxyde according to the procedure developed by Langenbruch (1983). Afterwards, samples were desilicified with 5% hydrofluoric acid in sea water. The samples were then stored in 100% ethanol at 5°C.

After two years the samples were embedded in acrylic resin (Unicryl, British Biocell International, Cardiff, UK), and cut into semi-thin (1μm) and ultrathin (60-150nm) sections. The semi-thin sections were examined with a Leitz DM RB light microscope (phase contrast) after staining with toluidine blue, eosin and haematoxylin (all stains provided by British Biocell).

For transmission electron microscopy, the ultra-thin sections, cut with freshly made glass knifes on a Reichert OM U3 ultramicrotome, were placed on slot-grids and the contrast was enhanced by uranyl acetate and lead citrate (adapted from the method of Reynolds, 1963). The photographs were taken on a Zeiss EM9 S2 electron microscope using photo plates. The samples were also used for scanning electron microscopy on a Zeiss DSM 940 microscope with a Nikon camera system.

A formalin-fixed sample of *C. gigantea* collected by Ole S. Tendal at BIOICE station 2085, 754m depth, 4 July 1992 was embedded in paraffin (AgarScientific Ltd, Stansted, UK), sectioned on a Leitz microtome (3-7μm) and stained with toluidine blue.

RESULTS

The main axis of all specimens had an extended aquiferous system with wide canals (200-300μm diameter) (Fig. 3) and oval-shaped choanocyte chambers measuring up to 100μm diameter length (Fig. 4). The canals of the aquiferous system appeared to run through the whole length of the main axis of the stalk, while the choanocyte chambers were found in rows along them. The surface of the main axis seemed to carry pores

FIG. 3. A cross section through the main axis of *C. gigantea*. The widths of canals measure between 200-300µm. Spermatocysts (sc) gather in the centre of the axis, whereas choanocyte chambers (chc) are situated mostly between canals (c) and surface of the sponge. Phase contrast microscopy.

that were about 160µm wide, narrowing to 20µm diameter. Through these pores diatoms could pass, some of which were found on the walls of the canals. These inlets covered the outside in a regular order, approximately 500µm apart. No choanocytes or ostia were found in the spheres, but canals ran through them and at the distal end of one sphere we noted a few openings (approximately 13) likely to be oscules. However, these structures could as well be caused by deflation.

The main axis and stalks contained only styli, whereas spheres carried styli as well as isochelae and, occasionally, sigmata. The outsides of the spheres were covered entirely with hook-shaped spicules, the microsclerid isochelae, standing closely together like a palisade (Fig. 8). The palisade was then underlain by styli which formed lateral layers or upward protrusions. The thickness of the palisade measured about 70µm. Because of its inflexibility the palisade was partially folded up into the deflated sphere.

Many spermatocysts at different stages of maturity were found in the main axis (Figs 3, 5, 6) and spheres (Fig. 7), all enclosed in cysts. In the middle of the main axis the gametocytes were globular (Figs 5-6) but appeared to elongate when migrating towards the spheres losing their protoplasm (Fig. 7). In one case, a distinct area was visible where cells appeared to form cysts (up to 80µm diameter) and develop into spermatogonia (Fig. 5). More mature stages of

FIG. 4. Oval-shaped choanocyte chamber with apopyle (a) from the main axis. Phase contrast microscopy.

FIG. 5. Spermatocysts from a spermatogonia region in the main axis. Within the cysts (c) the cells (sc) develop simultaneously, but the individual cysts are at different stages of maturity. They are enveloped in epithelial cells (e). Around the cysts are many cells with inclusions (ic) whose function is still unknown. Phase contrast microscopy.

spermatocytes were found on the periphery of this area, the cysts there measuring only 60µm diameter. Even in the spheres the mature spermatozoa remained in cysts. Using semi-thin sections of mature cysts, we estimated that a single cyst contained at least 500 spermatozoa.

Crustaceans in various stages of digestion were abundant only in the spheres, with up to 19 individuals per sphere (Fig. 9). No other prey organisms were noted. Due to their small size or advanced state of digestion, only two species, *Calanus finmarchicus* and *Calanus hyperboreus*, could be determined exactly and were found in greatest abundance (Fig. 10). In one sphere 16 specimens were found probably belonging to *C. finmarchicus*, up to 5.8mm long, and most in the copepodit stage V (Fig. 9).The largest item of prey measured 6.5mm long.

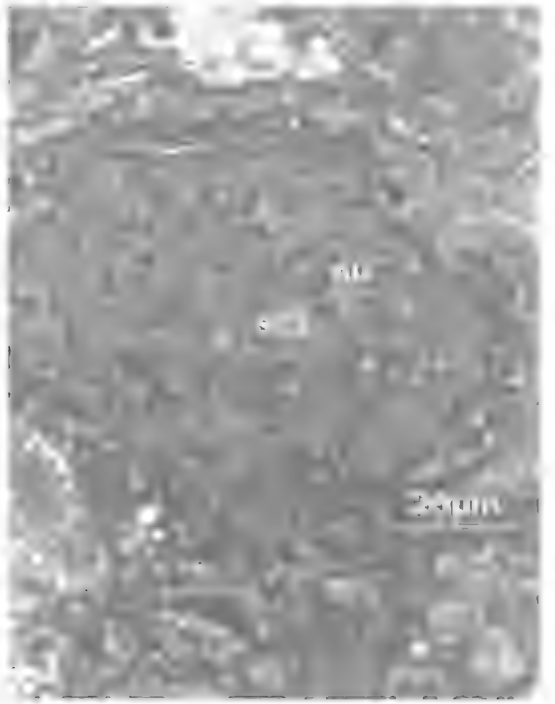

FIG. 6. Spermatocyst in the main axis with spermatocytes that could be in their reduction phase (sc2). The nucleoli (nu) are visible. Condensed heterochromatin can be seen as dark rings on the nuclei. Phase contrast microscopy.

Muscle tissue was found both inside the chitin cuticule of crustaceans surrounded by archaeocytes (Fig. 11) that had migrated towards the prey, and as inclusions in cells (Fig. 12a). Whereas in the first case the tissue still appeared to be intact, showing its striation, pieces of muscle tissue within inclusion cells showed different stages of digestion. In the ones most digested striation was no longer visible, and the inclusions consisted of a more-or-less homogenous mass.

In the outer layer of the main body globular structures of up to 5mm diameter were found being distinctly different from the surrounding sponge tissue. Consisting only of inclusions, they were enveloped in a layer of collagen with an average thickness of 30µm.

Bacteria were found extracellularly in the sponge tissue as well as in massive gatherings of different sizes, especially inside the chitin cuticule of half-way digested crustaceans where they were no longer intact.

FIG. 7. Cyst with elongated, more mature spermatocytes inside a sphere. Phase contrast microscopy.

DISCUSSION

Our investigations showed that *C. gigantea* has developed carnivory like other members of the family, but with a major difference: whereas the other genera have apparently lost their aquiferous system, *Chondrocladia* still possesses it.

AQUIFEROUS SYSTEM. Water is drawn into the main body through pores and then 'pumped' through canals into the spheres. The water current probably flows unidirectionally, from the base of the organism to the top, since the spheres are predominantly in the upper part of the sponge. It is probably not only collagen and spicules that keep this slim organism upright, but also the water pressure within the sponge. The spheres must be filled with water via the stalks. This is the reason why they appear translucent in in situ photographs and possess little biomass in relation to surface area. Water is assumed to be expelled through oscules on the spheres.

FUNCTION OF THE AQUIFEROUS SYSTEM AND THE SPHERES. The presence of wide canals running through the main axis and rows of

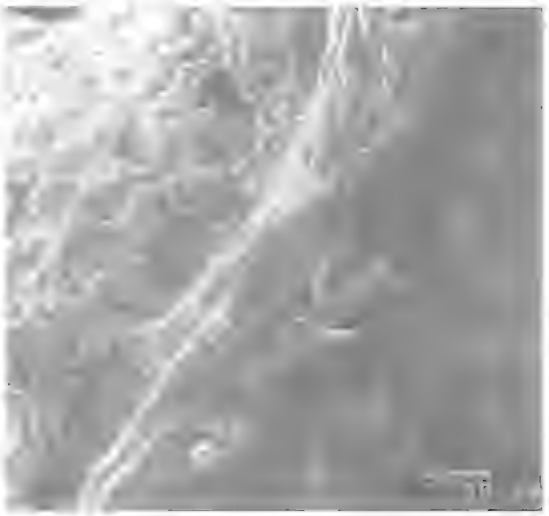

FIG. 8. A palisade of isochelae (isp) on the surface of a sphere. Underneath a criss-cross layer of megascleres (ms), which are simple styli. Scanning electron microscopy (SEM).

choanocyte chambers allow this tall, but slim, sponge to perform an exceptional feeding mode. The spheres can be explained as structures used to catch prey. When observed in situ (Fig. 1) they are usually filled with water and are seen as extended, thin-walled, translucent spheres. It is likely that passing crustaceans are caught on the protruding palisade of microscleres with their many thin appendages. As suggested by Tendal & Sahling (1997), a sphere can probably collapse within tens of seconds. Through the mechanical stimulus of the prey the sphere ejects its water contents through openings at its distal end. Collapsing very quickly, the crustacean is hooked and completely surrounded by sponge tissue so it cannot escape. The crustacean is then digested. Apparently archaeocytes migrate towards the prey and ingest pieces of muscle tissue (estimated size 200µm³). These archaeocytes are then inclusion cells and migrate through the sponge tissue of the spheres into the main axis. The muscle tissue was often found in rectangular shapes leaving the impression that it was dissected into distinct pieces. Aggregations of pieces of muscle tissue that were observed close to the areas where spermatogonia were formed suggest that their protein content may be utilised for the build-up of reproductive cells.

REPRODUCTIVE CELLS. Only male gametogonia could be clearly recognised, although the presence of very young oocytes in the same

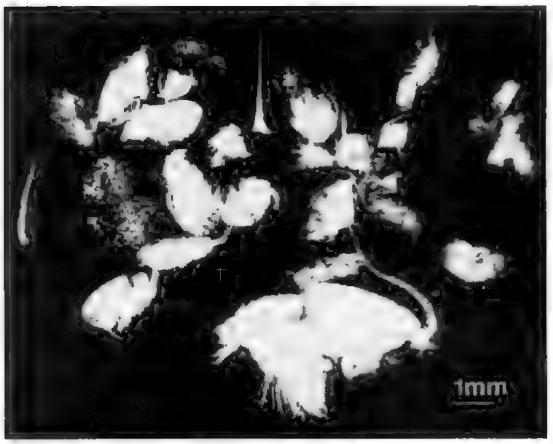

FIG. 9. Contents of a sphere from a formalin-fixed sample. The largest crustacean is a *Calanus* species, copepodit stage V, measuring 5.8mm.

specimen where spermatocytes were noted cannot be precluded (Kübler, 1998). The presence of spermatocytes in at least two out of six individuals and the lack of mature oocytes may indicate successive hermaphroditism. Spermatocytes were obviously produced very quickly, while oocytes seemed to take more time. Our investigations support the hypothesis that sexual reproduction plays an important role even in deep-sea organisms, as stated by Witte (1996) for other deep-sea demosponges. Finding mature stages of male reproductive cells and very young oocytes confirms the idea that seasonal reproduction also takes place in greater depths due to dependence on food availability, i.e. productivity of the surface waters, in this case in the form of crustaceans. The hypothesis that asexual reproduction in the form of budding could take place in this species (Tendal & Barthel, 1993) nevertheless cannot be precluded.

Since the most mature stages of spermatocytes were found in cysts within the spheres, complete cysts may be released into the surrounding water, possibly ejected through the distal openings on the spheres concurrent with the expulsion of water. This would be a mode of reproduction similar to that described by Vacelet & Boury-Esnault (1996) for the related species *Asbestopluma hypogea*.

According to Tuzet (1930), Fincher (1940) and others, archaeocytes constitute the source of spermatogonia in Porifera. Subsequently it was proposed that choanocyte chambers could be their predecessors (e.g. Tuzet et al., 1970; Paulus, 1989; Barthel & Detmer, 1990). Although in our

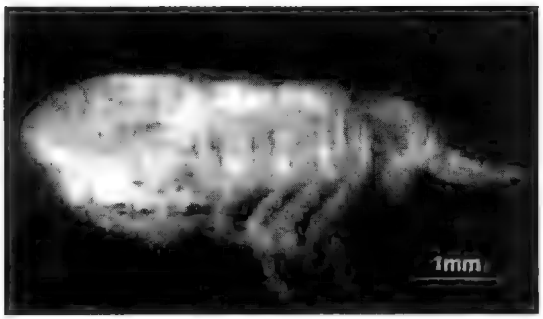

FIG. 10. *Calanus hyperboreus* Kröyer, 1838, copepodit stage V, length: 6.5mm.

samples there were choanocyte chambers that apparently disintegrated to almost the same size as spermatocysts, spermatogonia could also be formed from archeocytes that contain inclusions. Theoretical considerations to calculate cell numbers in choanocyte chambers and spermatocysts (spermatocytes 2 and spermatids) led to the result that choanocyte chambers (with about 1,500 cells) possess roughly two to three times more cells than mature spermatocysts, with the consequence that they probably would not originate directly from choanocyte chambers. In addition, young spermatocysts gathered in certain areas towards the centre of the main axis, whereas choanocyte chambers were regularly distributed and orientated towards the surface of the main axis (Fig. 3). Thus, choanocytes would have to migrate towards the centre to form cysts. In addition to spermatogenesis another reason for disintegration of choanocyte chambers may have been poor preservation of parts of the sponge tissue.

CRUSTACEANS. Most of the crustaceans found in the spheres belonged to two species that are very common in this area of the Norwegian Sea. Almost all of these were in the last copepodite stage (V), which usually sink to the bottom of the sea in late summer to hibernate (e.g. Orr, 1934; Raymont, 1963). It can be assumed that the sponge neither selects its food nor has any food preferences.

GATHERINGS OF INCLUSIONS. The function of gatherings of inclusions to build globular structures up to 5mm diameter in the main body could not be clarified. Originally, they were interpreted as embryos by Lundbeck (1905), but this could not be subsequently proven since no embryonic structures were found. Possible alternative interpretations are that these are places where by-products are deposited, or they may be

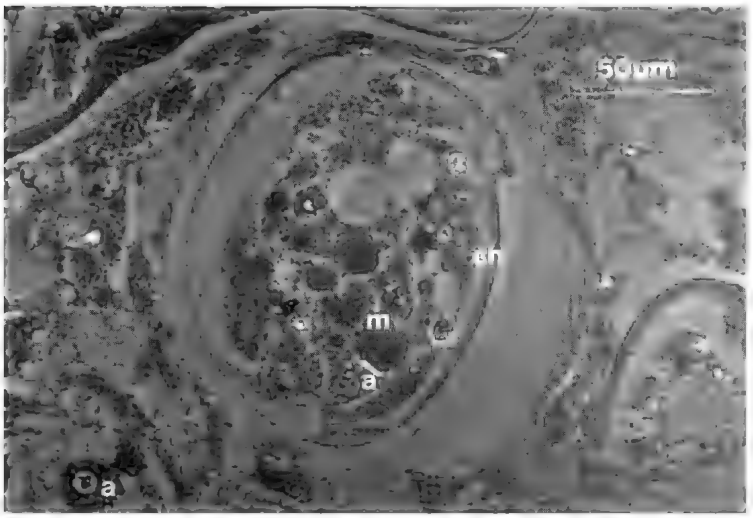

FIG. 11. Pieces of intact muscles (striated) within an extremity of a crustacean found in a sphere (m, muscle; a, archaeocytes; ch, chitin cuticle). Phase contrast microscopy.

depots of nutrients. The latter interpretation makes most sense given the food-poor environment in which *C. gigantea* lives, and the presumably vicarious seasonal food availability, but there is so far no empirical support for either hypothesis.

BACTERIA. The role of bacteria in this species was not clarified from our investigations. The fact that masses of bacteria, which did not look intact, were found close to or within prey in the sponge tissue, provides two possible assumptions: 1) either bacteria are digested by the sponge, which would mean, that this species is optionally bacterivor, or 2) bacteria facilitate digestion of prey organisms.

We assume that in *C. gigantea* the presence of an extended aquiferous system, which in other sponges is used to filter food particles (e.g. Simpson, 1984), is modified to an elaborate mechanism to catch prey. The spheres, which are part of this mechanism, also distribute the sponges' sexual products into the surrounding water.

Whereas closely related species like *A. hypogea* catch their prey (also consisting of crustaceans) by overgrowing it, *C. gigantea* catches its prey by inflating its spheres (probably within tens of seconds; Tendal & Sahling, 1997). Both species react to the mechanical stimulus induced by crustaceans landing on the external surface.

Living as a predator (macrophagy) instead of a filter feeder (microphagy) is a typical adaption to the deep-sea environment (Gage & Tyler, 1991). However, as noted above, feeding on bacteria and/or food particles in the water column, subject to their seasonal availability, cannot be excluded. Thus, the sponge would have maintained its original feeding strategy as a filter feeder and added carnivory as a new, supplementary method.

The peculiar morphology of this sponge may reflect its adaptation to the extreme, food-poor, deep-sea habitat in which it lives by reducing its main axis to a thin stalk that reaches into currents above the bottom and forming surface extensions through thin-walled spheres, thus providing a maximum chance to catch prey utilising minimal sponge material, i.e. body mass, as possible.

Our interpretation of the presence of choanocyte chambers and canals in *C. gigantea*, which are not present in other carnivorous sponges such as *A. hypogea* or *Cladorhiza* sp., is that *Chondrocladia* possesses the basic sponge 'bauplan' and thus stands at the base of the Cladorhizidae. The affiliation of Cladorhizidae within Porifera has been questioned due to its lack of the 'typical' sponge feature (viz. filter feeding using an aquiferous system with choanocyte chambers creating a water current; Vacelet & Boury-Esnault, 1995). Our data show that this feature is certainly present in *C. gigantea*, and consequently the family certainly belongs to Porifera. *Chondrocladia* is a binding link between the original microvorous and the specialised carnivorous species which have lost important anatomical characteristics. In contrast to related cladorhizid species the conversion from particle-feeding to carnivory in *C. gigantea* is not fundamentally linked to the loss of the aquiferous system, but is a functional modification (and maybe optional use) of existing structures.

ACKNOWLEDGEMENTS

We are indebted to the Danish Government for financial support of the BIOICE and BIOFAR Expeditions, to Ole S. Tendal (Zoological Museum, University of Copenhagen) for providing sponge material and for always being open

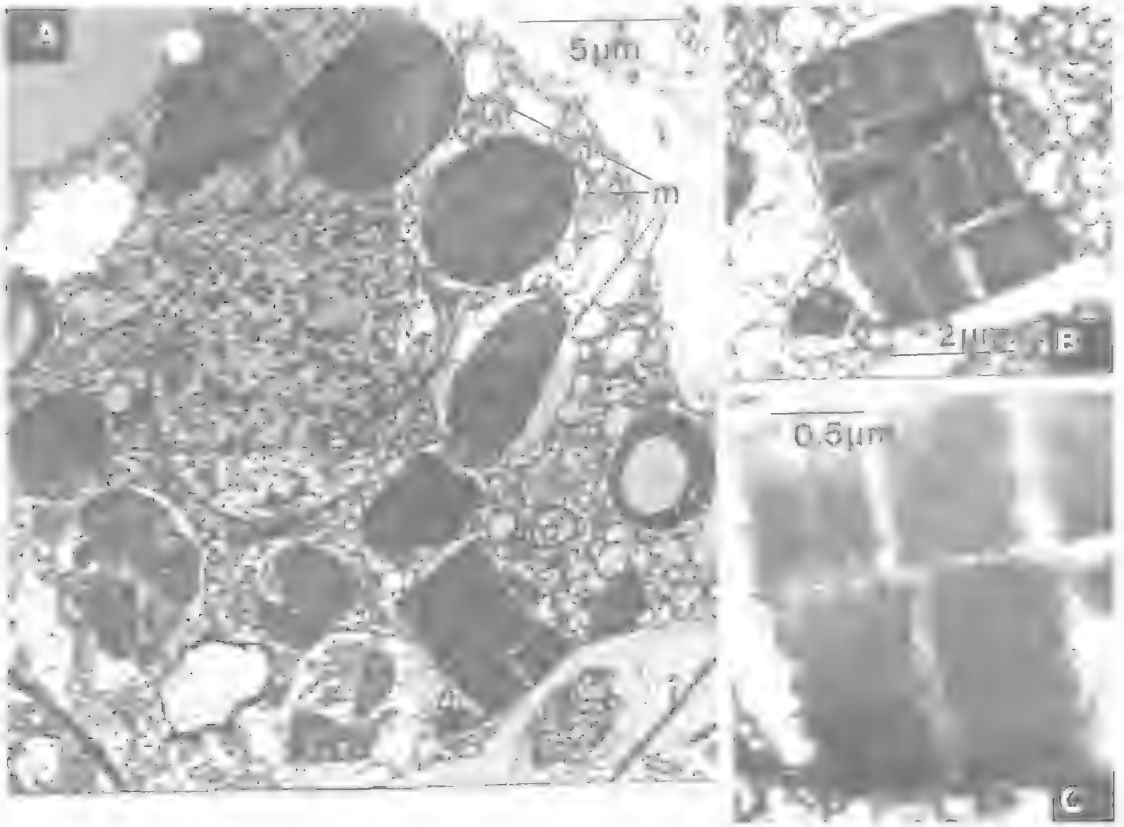

FIG. 12. A, inclusion cell from the main axis containing at least twelve pieces of muscle tissue (m). From the bottom right to the top left a succession of increasingly digested pieces can be observed. While the least digested ones still possess their striation, the more digested ones are homogenous inclusions. In the middle of the cell there is a nucleus. Transmission electron microscopy (TEM). B, enlarged view of a piece of muscle least digested in this cell. TEM. C, enlarged view of the striation of the muscle fibre. TEM.

to discussions, and to Heiko Sahling (GEOMAR, Kiel) for providing the in situ photograph of *Chondrocladia* sp. We are also grateful to H. Flügel for technical advice on the TEM and R. Schmaljohann (both Institut für Meereskunde Kiel) on the SEM. Thanks also to J. Hoeg for introducing us to his methods for preparing material for electron microscopy and P.V. Jensen (both Department of Cell Biology, University of Copenhagen) for assistance in histological questions. The manuscript benefitted from the comments of two anonymous reviewers.

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REMARKS ON THE PALEOECOLOGY AND REEF BUILDING POTENTIAL OF LATE JURASSIC SILICEOUS SPONGES. *Memoirs of the Queensland Museum 44: 297. 1999:-* In the early Late Jurassic (Oxfordian) siliceous sponges developed extensively. They formed a discontinous siliceous sponge reef belt extending over more than 7000km from New Foundland, Iberia, France, Switzerland, Germany, Poland, Romania to the Caucasian Mountains.

Siliceous sponges are no systematic unit but belong to the different taxonomic groups Hexactinellida and the polyphyletic lithistid demosponges. Due to their different organisation and biology, the ecological demands of the different siliceous sponges groups differ remarkably. The two major groups must be carefully distinguished for paleoenvironmental interpretations.

In general, lithistid demosponges are active filter feeding organisms. They feed on nannoplancton mainly bacteria. The bathymetric distribution of demosponges corresponds to a great extent with the bathymetric distribution of bacteria. The fairly high preservation potential of rigid demosponges is explained by a high amount of mesohyl-dwelling bacteria, causing rapid calcification after death.

Osmotrophy is an important feeding strategy of Hexactinellida. Dissolved organic matter is enriched in deeper water low-energy settings, causing the majority of Hexactinellida to dwell in such habitats. As the mesohyl of Hexactinellida consists of very thin collagenous material, there is hardly any room to harbour bacteria. This easily explains why microbially induced post-mortem calcification of the sponge by microbial automicrites occurs at a much lower rate so that fossilisation potential is much lower in comparison with rigid demosponges.

The taxonomic composition of fossil siliceous sponge populations is mainly controlled by sedimentation rate, nutrition and hydrodynamics. The dominance of major taxa is strongly influenced by bathymetry, due to changes of hydrodynamics and nutrition along a bathymetrical gradient. However, the quality of substrates, water energy or extreme oligotrophy may strongly modulate bathymetric distribution. ❐ *Porifera, Late Jurassic, Hexactinellida, lithistid Demospongiae, paleoecology, calcification, fossilisation.*

Manfred Krautter (email: manfred.krautter@geologie.uni-stuttgart.de), Institut für Geologie und Paläontologie der Universität,, Herdweg 51, D-70174 Stuttgart, Germany; 1 June 1998.

DISTINCTIVE MIDDLE CAMBRIAN SPONGE-CALCIMICROBE REEFS IN IRAN. *Memoirs of the Queensland Museum 44: 298. 1999*:- Following the virtual demise of archaeocyaths in the Toyonian stage and consequent collapse of the Early Cambrian archaeocyath-calcimicrobe reef consortium, Middle and Late Cambrian reefs remained generally devoid of metazoan input, being almost entirely microbial. The few exceptions in this interval generally include some minor contribution by spiculate sponges. One such spiculate sponge-calcimicrobe reef system in the Middle Cambrian of northern Iran is distinctive in that the spiculate sponges constitute a major component of the reef framework.

The reefs are in units 2 and 3 of the Mila Formation in the eastern Elburz (Alborz) Mountains. The Mila Formation consists of five units, together ranging in age from Middle Cambrian to Early Ordovician. Trilobites permit correlation of unit 2 and the reef-bearing lower unit 3 with the late Middle Cambrian, and upper unit 3 with the Chinese Kushanian (terminal Middle to earliest Late Cambrian) and Changshanian (Late Cambrian) stages. The reefs are well exposed in a road section 3 km north of Shahmirzad.The reefs are constructed by a consortium of the anthaspidellid sponge *Rankenella* and a presumed variety of microbes including the calcimicrobe *Girvanella*. *Rankenella* is otherwise known only from the Ordian-early Templetonian stage of the Northern Territory, Australia.That stage is equivalent respectively to the late Toyonian-early Amgan and Longwangmiaoan-Maozhuangian stages of Siberia and China.Unit 2 comprises fossiliferous interbeds of grey limestone/dolostone and yellow-brown marly shale, with desiccation cracks, bidirectional ripples and probable tempestites and hardgrounds.

The stratigraphically lowest known appearance of *Rankenella* is in upper unit 2, in a single decimetre-thick limestone bed of abundant eocrinoid ossicles. Scattered, widely conical *Rankenella* are preserved upright in life position, suggesting attachment to a hardground. Sponges, ossicles, trilobites and hyoliths are encrusted by *Girvanella*, which also forms rafts and onkoids. Texture within this biostromal bed ranges from floatstone-rudstone to *Girvanella* boundstone, with evidence of microbial and oxea-bearing sponge-body automicrites.The lower, reef-bearing portion of overlying unit 3 is massive, comprising pale grey stacked bioherms of similar texture and composition to the unit 2 *Rankenella* bed. In this interval, *Rankenella* adopts the entire range of co-occurring cup shapes from narrowly conical through to explanate. Clotted-peloidal biohermal mud is interpreted as automicrite. Substrate, peribiohermal and overlying sediment is commonly a bioclast rudstone rich in orthide brachiopod valves. Sponges are contributors to bioconstruction in a reef tract toward the top of lower unit 3. Component bioherms of this reef tract are constructed by ramose *Rankenella* encrusted by thick coatings of *Girvanella* to form a *Rankenella-Girvanella* framestone with only minor lime mud pockets. Interstices are rimmed by one to two generations of columnar cement and occluded by coarse equant cement. By comparison with Early Cambrian reefs, *Rankenella* and *Girvanella* played the roles of archaeocyaths and calcimicrobes: framework/substrate and encrusting/binding respectively. In many Early Cambrian reefs, however, lime mud represents a much greater component, while calcimicrobes were capable of building massive framework unaided by metazoans. ❐ *Porifera, Middle Cambrian, Iran, calcimicrobe, Rankenella, Girvanella, reef, automicrite.*

Peter D. Kruse (email: Pierre.Kruse@dme.nt.gov.au), Northern Territory Geological Survey, PO Box 2901, Darwin NT 0801, Australia; 1 June 1998.

GENETIC CONFIRMATION OF THE SPECIFIC STATUS OF TWO SPONGES OF THE GENUS *CINACHYRELLA* (PORIFERA: DEMOSPONGIAE: SPIROPHORIDA) IN THE SOUTHWEST ATLANTIC

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Lazoski, C., Peixinho, S., Russo, C.A.M. & Solé-Cava, A.M. 1999 06 30: Genetic confirmation of the specific status of two sponges of the genus *Cinachyrella* (Porifera: Demospongiae: Spirophorida) in the Southwest Atlantic. *Memoirs of the Queensland Museum* **44**: 299-305. Brisbane. ISSN 0079-8835.

In the Caribbean *Cinachyrella alloclada* and *C. apion* are readily distinguished by their different spicule types and sizes, and by the presence of buds in the former. In contrast, in the SW Atlantic both species can reproduce by budding, and also have identical chemical profiles in lectins, fatty acids and steroids. Verification of whether *C. alloclada* and *C. apion* were different biological species or were morphotypes of a single polymorphic species on the Brazilian coast was undertaken using allozyme electrophoresis. Samples collected in the intertidal zone of Pituba beach, Salvador, Brazil, and studied independently by morphological and allozyme analyses, showed a high congruence between morphology and allozymes, and 11 (of 19) loci were diagnostic of each species. *Cinachyrella apion* has smooth oxeas of only one size class, protriaenes of two sizes, anatriaenes of one size, small sigmaspires and raphides. *Cinachyrella alloclada* has smooth oxeas of two or three size classes, protriaenes and anatriaenes with one size, and sigmaspires like those of *C. apion*. The unbiased genetic identity between the two species was very low (I=0.28), as often found for congeneric sponge species. The consistent morphological and genetic differences between the two putative species confirm that, in spite of their high chemical similarity, they are distinct biological species. This indicates that, at least in these species, evolutionary rates for allozymes and secondary metabolites are clearly unrelated. ❐ *Porifera, Demospongiae, Spirophorida, Cinachyrella, morphology, allozymes, molecular systematics, Brazil.*

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Cinachyrella apion (Uliczka, 1929) and *C. alloclada* (Uliczka, 1929) are common tetillid marine sponges found in the Caribbean and Brazilian coast (Mothes de Moraes, 1980; Rützler, 1987; Rützler & Smith, 1992). Because of their ubiquity and abundance, they have been the subject of many chemical and pharmacological studies (Atta et al., 1989; Barnathan et al., 1992a; Bergquist & Bedford, 1978; Kaul et al., 1977; Portugal, 1992; Rodriguez et al., 1997). Although the two species can be separated on the basis of reproductive and spicular characters in Caribbean populations (Rützler & Smith, 1992), their differences are less obvious on the Brazilian coast (Peixinho, unpublished results). For example, reproductive buds, reported by Rützler & Smith (1992) as occurring only in *C. apion*, are very common in both species in Brazil. Furthermore, samples of *Cinachyrella* from Brazil, identified on the basis of spiculation as *C. apion* or *C. alloclada*, had identical sterol (Rodriguez et al., 1997), lectin (Portugal, 1992) and fatty acid patterns (Jiménez, unpublished results), as well as proteases with the same chromatographic and electrophoretic profiles (Portugal, 1992). Therefore, it became important to verify whether *C. apion* and *C. alloclada* on the Brazilian coast comprised two species with a high chemical and reproductive similarity, or whether they represented the product of phenotypic polymorphism or plasticity of one single species.

The method of choice for the determination of specific status of sympatric populations is the genetic interpretation of allozyme patterns (Thorpe & Solé-Cava, 1994), a complementary approach to morphology that has been used with great success to identify cryptic species in sponges (Solé-Cava & Thorpe, 1986; Solé-Cava et al., 1991a, 1991b; Bavestrello & Sarà, 1992;

Boury-Esnault et al., 1992; Klautau et al., 1994; Muricy et al., 1996). In this paper we compare electrophoretically sympatric populations of *C. apion* and *C. alloclada* to verify their specific status.

MATERIALS AND METHODS

COLLECTION. Fifteen samples each of *C. alloclada* and *C. apion* were collected in June 1995 from the intertidal zone at Pituba beach, Bahia, Brazil (13°27'S, 38°26'W). After collection, the presence of buds on each individual was verified, and the specimens were transported to the laboratory, where each one was immediately divided into two parts: one part was fixed in ethanol for morphological analysis, at the Federal University of Bahia; and the other was stored at –20°C for electrophoresis, in the Federal University of Rio de Janeiro. Both parts of each sponge were given the same code, prior to their putative identification, and the genetic and morphological analyses of the samples were performed in different laboratories. The electrophoresis laboratory, therefore, did not have any information as to the species identity of the samples. This blind-analysis helped to minimise any possible bias in the interpretation of genetic patterns, which might be critical given the supposed high similarity between the two species.

To verify the consistency of the diagnostic loci for discriminating each species, a second collection from the same locality was made in July 1996, consisting of 33 samples of *C. alloclada* and 66 samples of *C. apion*. These samples were then analysed for 4 of the 11 diagnostic loci found in the first study. The results of the first and second experiments were merged for the final analysis.

ALLOZYME ANALYSES. Horizontal 12.5% starch gel electrophoresis was carried out as previously described for sponges (Solé-Cava & Thorpe, 1986). The buffer systems used were the 0.25M Tris 0.06M citrate, pH 8.0 (Ward & Beardmore, 1977) and the discontinuous 0.03M Tris 0.005M citrate, pH 8.5 (gel), 0.30M borate, pH 8.1 (buffer tank; Poulik, 1957).

Twenty enzyme systems were investigated, of which ten: acid phosphatase (*Acp*; E.C.3.1.3.2); catalase (*Cat*; E.C.1.11.1.6); esterases (*Est*; E.C.3.1.1.1); glutamate dehydrogenase (*Gdh*; E.C.1.4.1.4); hexokinase (*Hk*; E.C.2.7.1.1); leucine aminopeptidase (*Lap*; E.C.3.4.11.1); malate dehydrogenase (*Mdh*; E.C.1.1.1.37); 6-phosphogluconate dehydrogenase (*Pgd*; E.C.1.1.1.44); phosphoglucose isomerase (*Pgi*; E.C.5.3.1.9); and superoxide dismutase (*Sod*; E.C.1.15.1.1), gave reproducible results for 19 loci. The staining of the gels followed standard procedures (Manchenko, 1994).

Genotype frequency data from both species were used to estimate gene frequencies, fits to Hardy-Weinberg equilibrium, and the unbiased gene identity between them (Nei, 1978) using the BIOSYS-1 programme (Swofford & Selander, 1981).

MORPHOLOGY. The overall morphology of the sponge was analysed under a binocular microscope, and the presence of porocalices and sub-ectosomal cavities (vestibules sensu Boury-Esnault & Rützler, 1997) were visualised in histological sections of paraffin-embedded samples.

Small pieces of each sponge were boiled in nitric acid to obtain clean preparations for spicule analysis. A qualitative analysis of mounted spicule preparations was made of every individual collected. Furthermore, 30 measurements of length and width of the eight types of spicule were made, using a light microscope in one individual of each putative species. Spicular and morphological nomenclature follow Boury-Esnault & Rützler (1997).

RESULTS

ELECTROPHORESIS. Of the 19 gene loci observed, 11 (*Acp*-2, *Acp*-4, *Cat*, *Est*-2, *Est*-3, *Est*-5, *Gdh*, *Lap*, *Mdh*-2, *Pgd* and *Sod*-1; Table 1) unambiguously separated the analysed sponges into two groups, which corresponded perfectly well with the species separated by the morphological analyses. These loci were, therefore, diagnostic of each species (sensu Ayala, 1983).

Levels of heterozygosity (h) within each population were high: h=0.13 in *C. apion* and h=0.15 in *C. alloclada*, as often seen in marine sponges (Solé-Cava & Thorpe, 1989, 1991). No significant deviations of genotype frequencies from Hardy-Weinberg expectations were observed at any of the loci studied (P>0.05; Fisher's exact test, using a Bonferroni transformation for multiple tests; Lessios, 1992). The unbiased genetic identity (Nei, 1978) observed between the two species was 0.28.

Locus	Allele	*C. alloclada*	N	*C. apion*	N
Acp-1	1	1.00	12	1.00	15
Acp-2	1	1.00	12	0.00	15
	2	0.00		1.00	
Acp-3	1	1.00	12	1.00	15
Acp-4	1	1.00	12	0.00	15
	2	0.00		1.00	
Cat	1	0.00	13	0.29	14
	2	0.00		0.46	
	3	0.00		0.25	
	4	1.00		0.00	
Est-1	1	1.00	13	1.00	14
Est-2	1	1.00	46	0.00	80
	2	0.00		1.00	
Est-3	1	1.00	46	0.00	80
	2	0.00		1.00	
Est-4	1	0.65	13	0.00	14
	2	0.00		0.86	
	3	0.35		0.14	
Est-5	1	0.77	31	0.00	46
	2	0.23		0.00	
	3	0.00		1.00	
Gdh	1	1.00	46	0.00	80
	2	0.00		1.00	
Hk	1	0.00	12	0.42	13
	2	0.00		0.42	
	3	0.25		0.12	
	4	0.75		0.04	
Lap	1	1.00	12	0.00	15
	2	0.00		1.00	
Mdh-1	1	0.25	10	0.03	14
	2	0.50		0.04	
	3	0.15		0.89	
	4	0.10		0.00	
	5	0.00		0.04	
Mdh-2	1	0.50	11	0.00	14
	2	0.50		0.00	
	3	0.00		1.00	
Pgd	1	1.00	12	0.00	15
	2	0.00		1.00	
Pgi	1	0.00	13	0.31	13
	2	0.00		0.04	
	3	0.46		0.61	
	4	0.00		0.04	
	5	0.54		0.00	
Sod-1	1	0.00	13	0.96	14
	2	1.00		0.00	
	3	0.00		0.04	
Sod-2	1	1.00	6	1.00	6

TABLE 1. *Cinachyrella alloclada* and *C. apion*. Gene frequencies at the 19 loci studied. **N** = number of individuals analysed.

MORPHOLOGY. The two species had similar overall (round) shape, yellow colour, hispid surface and firm consistency, being virtually indistinguishable on the field. Both species had porocalices, as is typical of the genus, but vestibules were only observed in *C. apion*. Calcareous precipitates were observed in both species. The spicular composition of *C. apion* consists of one size class of anatriaenes and oxeas, and two size classes of protriaenes, small sigmaspires and raphides (Table 2). Conversely, the spicular composition of *C. alloclada* consists of two or three size classes of smooth oxeas, one size class of protriaenes and anatriaenes, which varied in abundance from rare to abundant, and sigmaspires (Table 3). The sizes of the sigmaspire microscleres of the two species in Brazil were clearly not different (both had about the same average: 10µm), and more like those of *C. apion* from the Caribbean (Table 2). Reproductive buds were observed on the surface of most specimens of both species.

DISCUSSION

The presence of 11 diagnostic loci and the consequent very low genetic identity, associated with the sympatry of the samples, clearly demonstrate that the Brazilian *C. apion* and *C. alloclada* are reproductively isolated, regardless of their chemical similarity. Therefore, they must be evolving independently and belong, thus, to different biological and phylogenetic species (Mayr, 1981; Cracraft, 1987). The genetic identity (I=0.28) found between these two species is, in fact, as small as that often found between species belonging to different genera in other invertebrate groups (Thorpe, 1982; Knowlton, 1993; Thorpe & Solé-Cava, 1994). Similarly, high levels of gene divergence have been found for some aster-bearing hadromerid genera and *Oscarella* (Boury-Esnault et al., 1992; Sarà et al., 1993; Barbieri et al., 1995; Boury-Esnault et al., 1999; Solé-Cava & Boury-Esnault, 1999, this volume). However, further data are necessary before overall generalisations can be made about gene divergence in sponges, and surely before decisions as to the taxonomic rank (above species level) can be directly inferred from genetic

TABLE 2. Spicule micrometry of *Cinachyrella apion*. Measurements are given in micrometers, as minimum-*mean (standard deviation)*-maximum. Triaene measurements refer only to the rhabdomes. 30 spicules were measured for each type, except in the case of anatriaenes, which were rare so only 9 spicules were measured. (A = absent).

Spicule type	Present data	Rützler & Smith, 1992
Oxeas 1 length	2217 - *3797(660)* - 5478	3500 - *4100* - 4600
diameter	21.7 - *65.9(20.9)* - 108.7	35 - *41* - 45
Oxeas 2 length	A	A
diameter	A	A
Oxeas 3 length	A	A
diameter	A	A
Protriaenes 1 length	1587 - *3907(1045)* - 5761	1800 - *3500* - 8000
diameter	8.6 - *16.9(5.3)* - 25.9	4 - *8.3* - 10
Protriaenes 2 length	588 - *1079(186)* - 1400	400 - *1350* - 1800
diameter	3.6 - *4.3(1.4)* - 7.2	1 - *2.3* - 4
Anatriaenes length	2196 - *2560(222)* - 2880	1800 - *2900* - 3500
diameter	10.8 - *12.8(1.9)* - 14.4	3 - *4.6* - 5
Sigmaspires length	3.4 - *10.0(2.6)* - 15.5	12 - *13.4* - 16
Raphides	212 - *238(14)* - 259	200 - *244* - 270

identities (Solé-Cava & Boury-Esnault, 1999, this volume).

Spicule sizes of the Brazilian specimens of *C. apion* and *C. alloclada* were similar to those described by Rützler & Smith (1992) for Caribbean populations. Conversely, samples of *C. alloclada* were very different from those described by Mothes de Moraes (1980) (see Table 3). Sponges from the SE coast of Brazil identified as *C. alloclada* by Mothes de Moraes, had much smaller protriaenes and larger anatriaenes than those found by us and by other authors (Uliczka, 1929; Wiedenmayer, 1974; Rützler & Smith, 1992). This difference might be judged large enough to warrant the creation of a new species. However, it could also be the result of phenotypic polymorphism, since spicule size can be very variable and dependent of environmental conditions (Simpson, 1978; Bavestrello et al., 1993; Schrönberg & Barthel, 1998). Further studies, possibly using genetic markers, should be done on *Cinachyrella* samples from the same region studied by Mothes-de-Moraes in SE Brazil, to verify their specific status.

The very large genetic divergence (*I*=0.28) observed between *C. alloclada* and *C. apion* contrasts markedly with their overall chemical similarity (Atta et al., 1989; Portugal, 1992; Rodriguez et al., 1997). However, this is to be expected, since the evolutionary rates of the enzymes involved in the housekeeping metabolism (like those used on allozyme analyses) are not necessarily related to those of secondary metabolites. Allozyme polymorphisms are genetically based and usually neutral, since they involve small changes in areas of the protein that do not significantly affect its function (Kimura, 1991). For this reason, these polymorphisms are expected to evolve at constant rates and unlinked to environment conditions, which is very important when dealing

TABLE 3. Spicule micrometry of *Cinachyrella alloclada*. Measurements are given in micrometers, as minimum-*mean (standard deviation)*-maximum. Triaene measurements refer only to the rhabdomes. 30 spicules were measured for each type. (A = absent).

Spicule type	Present data	Rützler & Smith, 1992	Mothes de Moraes, 1980
Oxeas 1 length	1900 - *1932(26)* - 2016	1500 - *3500* - 5900	2644 - *4923* - 6256
diameter	14.4 - *18.2(1.9)* - 21.6	20 - *50* - 65	17 - *38* - 56
Oxeas 2 length	1144 - *1217(78)* - 1440	900 - *1800* - 2800	A
diameter	10.8 - *12.8(1.8)* - 14.4	1 - *13* - 20	A
Oxeas 3 length	756 - *837(81)* - 1008	100 - 355 - 950	74 - *159* - 223
diameter	7.2 - *7.2(0)* - 7.2	2.5 - *5.4* - 8	4 - *7* - 9
Protriaenes 1 length	1296 - *2164(423)* - 3197	2400 - *4200* - 6500	407 - *437* - 462
diameter	3.6 - *4.6(1.6)* - 7.2	4 - *10.7* - 20	3 - *11* - 23
Protriaenes 2 length	A	A	A
diameter	A	A	A
Anatriaenes length	1051 - *1225(102)* - 1440	2200 - *3200* - 4000	7259 - *8289* - 9061
diameter	4.0 - *7(2.0)* - 14.0	3 - *8.3* - 14	5 - *9* - 14
Sigmaspires length	7.0 - *10.1(1.3)* - 11.2	10 - *14.3* - 23	10 - *14* - 22
Raphides	A	A	A

with problems on taxonomic status of organisms. On the other hand, studies with secondary metabolites necessarily deal with the products of enzyme function, which are much more constrained by natural selection and therefore are not expected to evolve in a clock-wise manner (Nei, 1987). Lectins and secondary metabolites are often involved in strong interspecies interactions, playing a very important role in the survival of the species (Kennedy et al., 1995; Hirabayashi & Kasai, 1998). Therefore, most of the time they are likely to be under strong normalising or directional selection, which are highly dependent on environmental conditions. This may explain, for example, why steroids in three species of *Cinachyrella* from W Africa are not overtly different except for small variations in their proportions (Barnathan et al., 1992b), and the presence of cholest-4-en-3-one steroids, both in the Brazilian *Cinachyrella* (Rodriguez, et al., 1997), and in *C. tarentina* (Aiello et al., 1991). A high intrageneric similarity has also been observed in sterols of the genus *Aplysina* (Kelecom & Kannengiesser, 1979) and in lectin composition of *Axinella* spp. (Bretting et al., 1980). Structures or molecules of adaptive importance and, hence, under strong normalising selection can also present large evolutionary shifts due to directional selection. These shifts, albeit rare, can generate homoplasy by evolutionary convergence. It is not uncommon, for example, to find species from different phyla that share a secondary metabolite which is not found in other members of the same family or order (Tursch et al., 1978). At the species level, therefore, secondary metabolites are not indicated for phylogenetic studies, since they can be evolutionarily too conserved at that level (i.e. plesiomorphic) or prone to homoplasy due to convergence. At this taxonomic level, thus, the use of allozymes or neutral DNA genes is more indicated (Hillis et al., 1996).

The two Brazilian species of *Cinachyrella* can be positively identified as the Caribbean *C. apion* and *C. alloclada*, based on morphological data. However, the use of molecular systematics for the study of sponge species has unveiled a large amount of hidden biodiversity in the phylum (reviewed in Solé-Cava & Thorpe, 1994), especially when applied to so-called cosmopolitan species and many of those with very large and/or disjunct geographical distributions. In fact, all allegedly cosmopolitan sponge species studied to date by allozyme electrophoresis have been shown to consist of clusters of morphologically similar, but evolutionarily distinct species. This means that levels of endemism in marine sponges may be much larger than assumed by taxonomists using only morphometric data (Solé-Cava et al., 1991a, 1992; Klautau et al., 1994; Klautau et al., in press). It would be very interesting, therefore, to verify whether *C. alloclada* and *C. apion* from the Bermudas, Senegalese coast (Barnathan et al. 1992a), Brazil (including the samples from Bahia studied here), and those from SE Brazil (Mothes de Moraes, 1980) are indeed conspecific.

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HOMEOBOX GENES EXPRESSED IN THE ADULT AND REAGGREGATING SPONGE. *Memoirs of the Queensland Museum 44: 306. 1999:-* Homeobox genes encode a large family of conserved transcription factors that control a range of important developmental decisions, and can be interspersed or organised in clusters (e.g. HOX genes) within metazoan genomes. A striking feature of many homeobox orthologues is how they are expressed in a similar fashion during the development of phylogenetically-disparate animals. In recent years, interest has developed in homeobox genes in the lower metazoans, such as platyhelminths, cnidarians, and sponges. Instead of studying embryological development, focus has often been on the role of homeobox genes during regeneration. Interestingly, these genes exhibit comparable expression patterns during the regeneration of lower metazoans as they do during normal embryological development of higher metazoans. In this study, homeobox genes expressed in the adult sponge (newly dissociated cells) *Iotrochota baculifera* (Demospongiae: Poecilosclerida: Myxillidae) and during reaggregation were identified by RT-PCR with degenerate primers and sequencing. As with regeneration in other taxa, investigating the molecular genetics of reaggregation in the sponge, apart from providing many practical advantages, gives us insight into the level of conservation between embryological and other developmental processes. To confirm the poriferan origin of the homeobox genes isolated, the identical procedure was applied to another sponge and some homologous genes were obtained. As sponges appear to be monophyletic with the rest of the Metazoa and the first lineage to diverge during metazoan evolution, the study of their homeobox genes provides insight into the initial role of metazoan-specific homeobox genes in governing the multicellular state and cellular differentiation. ❐ *Porifera, reaggregation, development, homeobox genes, regeneration.*

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ANTIMICROBIAL ACTIVITY OF SPONGES FROM SOUTHERN BRAZIL, ATLANTIC COAST. *Memoirs of the Queensland Museum 44: 306. 1999:-* Within the research program of biologically active natural products, we have investigated different species of marine sponges collected by scuba diving in the South-western Atlantic region, near the coast of South-Brazil, aiming at the evaluation of their potential as a source for new drugs. In order to achieve this goal, the antimicrobial activity of five species, *Tedania ignis* Duchassaing & Michelotti, *Pseudaxinella reticulata* (Ridley & Dendy), *Polymastia janeirensis* (Boury-Esnault, 1973), *Batzella* sp. and *Petromica* sp. were analysed. The sponge species found in this particular region constitute a group of shallow-water Demospongiae in which a pharmacological usage has not previously been evaluated. Aqueous extracts obtained by grinding and maceration for 30mins following freeze-drying, as well as organic solvent extracts (toluene: methanol 3:1 v/v) were prepared from organisms frozen since the harvesting. Antimicrobial activity was evaluated by the agar diffusion method on paper disks (6mm diameter). Each sponge extract was tested for growth inhibition of five bacteria species: *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 6538P), *Staphylococcus epidermidis* (ATCC 12228), *Baccillus subtilis* (ATCC 6633), *Micrococcus luteus* (ATCC 9341) and two yeast species: *Candida albicans* (ATCC 110231) and *Saccharomyces cerevisiae* (ATCC 1600). None of the tested extracts analysed by this method could inhibit the growth of these microorganisms, an exception was the *Petromica* sp organic extract which showed an inhibitory activity for *Bacillus subtilis*. The same extracts were analysed for antitumour activity by *in vitro* inhibition of proliferation of different tumour cell lines. Some of the extracts showed promising results. The preliminary antimicrobial assay can be useful for pointing out sponge species to be further analysed. ❐ *Porifera, antimicrobial, Atlantic coast, southern Brazil, biologically active natural products, antitumor.*

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DISTRIBUTION PATTERNS OF SPONGES AND CORALS DOWN TO 107 M OFF NORTH JAMAICA

HELMUT LEHNERT AND HAGEN FISCHER

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Sixty species of sponges (23 new) were collected from the deep fore-reef (60-107m depth) off the North Jamaican Discovery Bay area using trimix diving. Comparison with the shallow water sponge fauna shows only 15% of shallow water sponges extend down to the deep fore-reef and 60% of deep fore-reef sponges are not found in shallow water. Mapping sponge and coral distributions around Discovery Bay to 40m depth revealed a database of 102 sites with a surveyed area of 1659m^2. Multivariate analysis of this database recognizes three large scale habitats: Reef-surfaces, lagoon, and undersides of platy corals. Separate analyses of subsets indicate internal differences within habitats. Benthic colonization on reef-surfaces are continuous along depth and inclination gradients, except around river mouths. Within lagoon habitats there are subhabitats: blue hole, *Thalassia* seagrass-beds, ridges with freshwater outflow and protected (eastern) backreef. Zonation of Jamaican reefs appears to have changed over 34 years in comparision to data of Goreau (1959). ❐ *Porifera, distribution patterns, depth zonation, habitat specialisation, Jamaica, coral reefs.*

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Zonation patterns of corals have been studied by several workers (Goreau, 1959; Geister, 1977). Several authors (Liddell & Ohlhorst, 1987; Rützler, 1971, 1974, Wilkinson & Cheshire, 1989; Wilkinson & Evans, 1989; Zea, 1993) mentioned the importance of sponges in coral reefs, only few (Alcolado, 1990; Alvarez et. al., 1990; Diaz et. al., 1990; Schmahl, 1990) have attempted to describe the zonation of sponges, probably due to taxonomic difficulties within this group (Rützler, 1987; Böger, 1988, Van Soest, 1991). The first extensive study of Jamaican sponges was attempted by Hechtel (1965). He investigated the area of Port Royal on the Jamaican south coast, recording 57 species and listing the common species in each of his ten collecting areas. Few details were given on the nature of these localities but he did mention that a considerable number were restricted to certain habitats. This is surprising considering that his survey extended down to only 6.1m (20ft) depth. This implies sponges may have strong zonation patterns.

Previous studies on zonation of coral reefs recognized more intuitive morphological differences in reefs and based their zonation only in part on the sessile organisms occurring there, mainly on scleractinian corals. Geister (1977) wrote that in Caribbean reefs there was a "distinct coral zonation controlled by exposure to wave activity. Based on this zonation, six basic reef types can be distinguished, ..." But he admitted that "Influence of factors other than wave exposure, however, may considerably disturb the regular zonation pattern". Geister (1983) gave an excellent overview about reef definitions, classifications and geological aspects of recent reefs, but worked on relatively large temporal and spatial scales.

The present paper is based on mapping of species from selected sites and subsequent multivariate analyses. The mapping of sponges and corals provides an estimate of the importance of sponges compared to corals. The aim of this study is to determine if similar species communities occur on different sites investigated, and if these similarities can be explained by environmental factors.

MATERIALS AND METHODS

Thirteen trimix dives to depths between 60-107m were undertaken in Discovery Bay, Jamaica in May-June 1993 and June-July 1996, to collect and photograph sponges of the deep fore-reef.

TABLE 1. Outline of classification undertaken on data, using methods proposed by Wildi (1989).

Program	Action	Comment
TRAFOA (PPS)	Histogram equation (Fischer, 1994)	Compensation of the extreme right-skewed distribution of population data.
	Species selection by variance.	Only species with highest variance, with total variance of 95% of the whole data set, were selected to reduce the data set and to reduce "noise".
INIT (MULVA)	Transform attribute vectors to unit length.	Avoid undesired effects caused by unequal species variance
RESE (MULVA)	Calculate similarity matrix using similarity ratio.	Recommended for sites
CLTR (MULVA)	Classify sites with minimum variance classification.	Minimizes in-group variance and maximizes between-group variance
INIT (MULVA)	Transform site vectors to unit length.	Avoid undesired effects caused by unequal species numbers in sites
RESE (MULVA)	Calculate dissimilarity matrix using chord distance.	Recommended for species
CLTR (MULVA)	Classify species with minimum variance classification.	Minimizes in-group variance and maximizes between-group variance
DIAN (MULVA)	Analysis of variance (Jancey's F-rank see Wildi, 1990) with F>2.6 (a=1%)	Select only significantly different species
INIT (MULVA)	Transform data for correspondance analysis	Required for correspondence analysis
RESE (MULVA)	Calculate similarity matrix using non centered cross product	Is required for correspondence analysis
PCAB (MULVA)	Compute correspondence analysis	Normal version choosen
AOCL (MULVA)	Analysis of concentration	Ordination of species and site- groups to get meaningful sequences
EDGR (MULVA)	Rearrange groups internally according to correspondence analysis	Obtain meaningful within group sequence of species and sites
TABS (MULVA)	Display the ordered table	-

Between January-July 1993 coral reefs in the Discovery Bay area, from the mouth of the Rio Bueno in the west to the mouth of the Pear Tree River in the east, were mapped using SCUBA, using the method described by Braun-Blanquet (1964). This method, originally developed for botanical surveys, was used to estimate the abundance and percentage cover of sponge and coral species in different habitats of reefs. Site surface area was measured with a plastic tape measure, and percentage cover of sessile species was estimated using the following procedure:
r=one individual specimen in the site surveyed; +=cover below 1%; 1=cover below 5%; m=cover below 5%, but species abundant; a=cover 5-15%; b=cover 15-25%; 3=cover 25-50%; 4=cover 50-75%; 5=cover 75-100%.

Large differences in size in both habitat and species occurrence (especially within sponges) necessitated adjustment of the size of sites according to prevailing conditions. Some habitats (e.g. undersides of platy corals), were very limited in their extent, whereas habitats like *Thalassia* sea-grass beds inside the lagoon, in shallow water, were far more extensive. Another factor limiting size of sites was decreasing bottom-time with increased depth. Evaluation of these data was performed using MULVA (Wildi & Orlóci, 1990), CANOCO (ter Braak, 1988, 1990) and PPS (Fischer, 1994).

Classification of data was made using the standard strategy for the analysis of phytosociological data, suggested by Wildi (1989) with some modifications. Table 1 summarizes the analysis path.

Ordination was performed with CANOCO (ter Braak 1988, 1990) based on redundancy analysis (RDA), analyzing the influence of environmental factors on the fauna and providing graphical representation of the data. CANOCO offers two methods for canonical analysis: RDA and canonical correspondence analysis (CCA). CCA is preferable if the data set demonstrates large ß-diversity, (i.e. if it contains several very different habitat types with very few or no species occurring in all of these types). RDA, in contrast, is applicable for small ß-diversity. Our sites were recorded from a geographically small area from similar habitats. Several species were found in most of these habitats. Consequently, RDA is the preferable method for our data set. Graphical representation of canonical ordination (RDA) depicts similarity in distance between sites based on their faunistic and ecological affinities. Metric

TABLE 2: Jamaican corals and sponges. Columns represent site groups. The numbers are percentage frequency of the species. Site groups and species are arranged according to classification and correspondence analysis. Species groups (Sp. Gps) are indicated for each species. Site-groups are sites with similar species; co-occurring species are species-groups. 85 originally mapped species underwent an analysis of variance (see Table 1). The displayed 36 species have significantly different occurrence. Species not displayed are either very rare or run through all or most sites.

	Site-Group		4	3	8	5	6	7	10	9	1	2
	Number Of Sites		3	3	12	9	22	13	22	9	3	6
	Mean Number Of Species		3	5	12	12	16	12	10	9	2	4
ID	SPECIES NAME	Sp. Gps										
40	*Plakortis simplex* (olive)	17		100	17		5					
12	*Clathrina primordialis*	17	33	100								
9	Unidentified demosponge	17		100								
85	*Stylaster roseus*	16	100			22		23				
55	olive incrusting	16	100	67								
84	*Helioseris cucullata*	10			33				5			
28	*Agelas sceptrum*	10		67	75		9					
11	*Ectyoplasia ferox*	9			50		32		9	11		
61	*Agaricia agaricites var.unifaciata*	11			92	56	59	31	55	11		
60	*Montastrea cavernosa*	11			67	11	50	38	32	22		
58	*Acropora cervicornis*	4			8	22	55	15	14			
25	*Agelas dispar*	3			25	89	82	38	50	11		
71	*Siderastrea radians*	3			8	33	73	31	45	11		
41	*Erylus formosus*	2			17	11	55	8		11		
81	*Millepora complanata*	19			8	11	14	85	9	11		
57	*Acropora palmata*	19						23				
35	*Iotrochota birotulata*	6			8		45	15	55	33		
10	*Niphates erecta*	6				11	27	8	45	44		
13	*Ircinia strobilina*	6			17	67	77	31	36	33		
69	*Diploria clivosa*	20						46	9	44		
43	*Anthosigmella varians var.incrustans*	20			25		18	54	27	56		
44	*Chondrilla nucula*	20				33	5	54	5	44		
1	*Neofibularia nolitangere*	5					9	8	45	22		
79	*Porites furcata*	1			17	89	82	69	23	44		17
78	*Porites astreoides*	1			58	100	77	92	32	56	33	
62	*Agaricia agaricites var.massiva*	1			33	44	64	54	14	22		
59	*Montastrea annularis*	1			83	78	100	92	59	22		
82	*Millepora alcicornis*	1			17	56	59	38	18	11		
15	*Aiolocroia crassa*	1			42	44	82	31	32	11		
46	*Aka coralliphaga*	1			8	78	9	38	9			
68	*Diploria labyrinthiformis*	1					32	54				
72	*Siderastrea siderea*	12			17		9	8	9	78		
49	*Xestospongia carbonaria*	18									100	67
54	*Haliclona coerula*	18										50
48	*Myrmekioderma rea*	18			8					11	33	83
30	*Amphimedon erina*	18						8	5	22		100

environmental variables are displayed as arrows, indicating the direction of average increase of each variable, whereas categorical variables are displayed as points (indicating the center of the occurrence of each category). The scores on the axis represent relative distance units in the

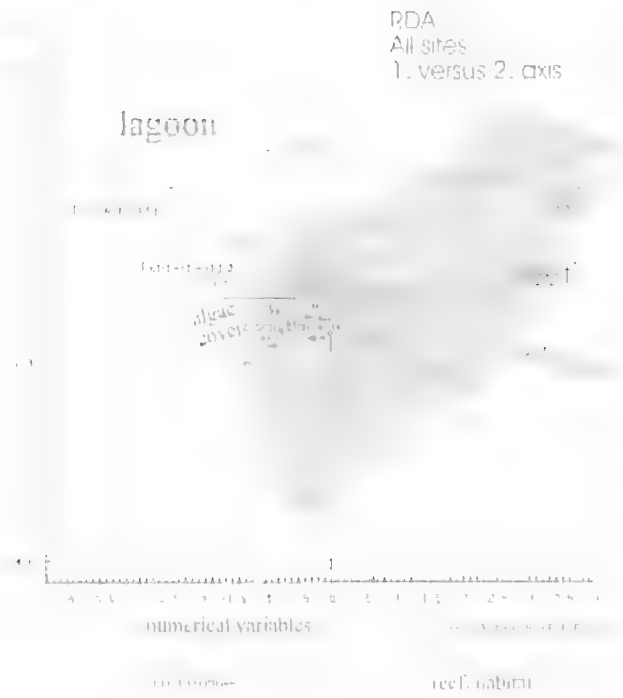

FIG. 1. Redundancy Analysis (RDA) of all sites. Similarity of sites is displayed as distance. Lagoonal and reef sites are differentiated on the first two axes with lagoonal sites on the upper left and reef-sites with grey background. Note that there is a mixing zone between lagoonal and reef-sites, indicating sites influenced by both environments. Numerical variables are shown as arrows. Differences in sizes of arrows reflect different influence of environmental variables. Ordinal variables are printed as centroids without direction but influence sites nearby. Numbers refer to site-numbers.

similarity matrix from the center of the data set. The following environmental variables were used in these analysis: Depth, size of site, total cover, sponge cover, coral cover, algae cover, inclination of substrate, ridges with freshwater outflow, *Thalassia* seagrass, backreef, bluehole, ship channel, fore-reef, deep fore-reef, pinnacle, undersides of platy corals, reef-flat, Discovery Bay, Rio Bueno, Pear Tree River, sediment cover, coral rubble.

An *a priori* selection of environmental variables was carried out. Only variables with $p<0.05$ were retained for further analysis, to ensure that only statistically significant variables influenced the analysis.

RESULTS

COMPARISON BETWEEN SHALLOW WATER (0-40M) AND DEEP FORE-REEF (60-107M) SPONGES. The relatively well known Jamaican shallow-water sponge fauna consists of now 157 sponge species (Lehnert & Van Soest, 1998). 133 species (85%) are restricted to shallow water, 5 of them were new to science, and 24 species (15%) also occur in the deep fore-reef. From the deep fore-reef 60 sponge species were collected from 13 trimix dives. 23 of these are new (Lehnert & Van Soest, 1996, in press), and with 13 known species a total of 36 species (60%) are restricted to the deep fore-reef, 24 species (40%) are shared with shallow water habitats. The inventory of deep fore-reef sponges is far from being complete, with additional undescribed and described species expected. However, there are striking differences in species composition between deep water and shallow water habitats, and it is improbable that any additional deep fore-reef species will be found in the well known shallow water fauna.

INTERNAL ANALYSIS OF THE SHALLOW WATER DATA SUBSETS. *Classification.* Table 2 shows the results of the classification obtained by the analysis outlined in Table 1, indicating ten groups of sites attributed to three large-scale habitats. Site-groups 3 & 4 are from undersides of platy corals with the characteristic species-groups 10 & 16. Lagoonal environments are represented by site-groups 1, 2, 9 & 10. Characteristic lagoonal species include species-groups 14 & 18, restricted to lagoonal environments. Many species frequently occur within the lagoon, but have their focal points within reef-environments, like species-groups 1, 2, 3, 6, 11 and 12. The remaining site-groups 5, 6, 7 and 8 are from different reef-environments. Species-group 6 is most abundant in general reef environments. Consequently these species can be used for large scale characterization of habitats only, but not for subhabitats.

Ordination analysis using RDA was performed on a square-root transformation of the percentage substrate cover values, with centered and normalized species. The following environmental variables were statistically significant ($p<0.05$): Depth, algae cover, inclination of substrate, ridges with freshwater outflow, *Thalassia* seagrass,

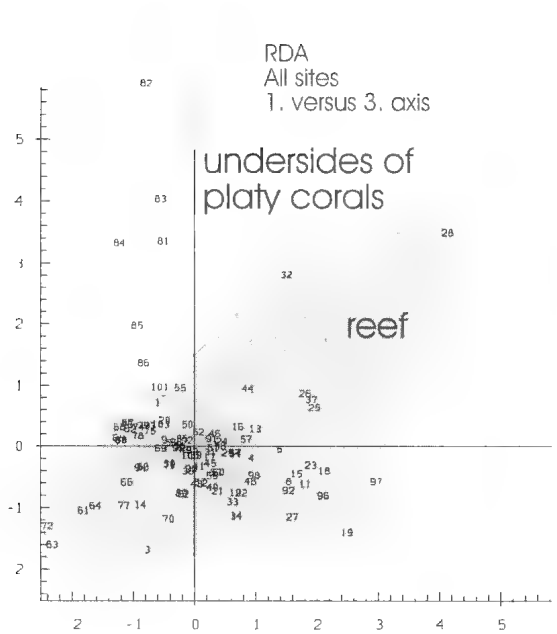

FIG. 2. Redundancy Analysis (RDA) of all sites. Representation of first and third axes show that undersides of platy corals is a valid large scale habitat distinguished in the third dimension. Numbers refer to site-numbers.

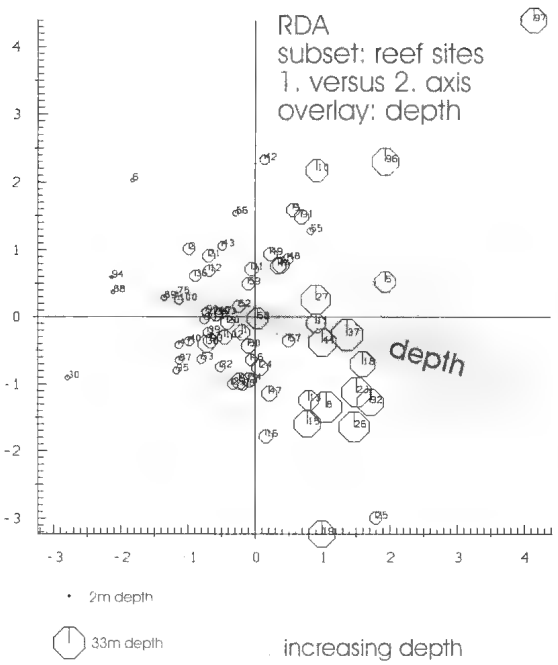

FIG. 3. Redundancy Analysis (RDA) displaying only reef sites. No distinct site-groups are recognizable, but depth as overlay (larger symbols indicate greater depth) shows arrangement of the sites along a depth gradient. Grey arrow indicates general direction of depth gradient (increasing depth from upper left to lower right). Numbers refer to site-numbers.

back-reef, Bluehole, Ship-channel, fore-reef, deep fore-reef, pinnacle, undersides of platy corals, Discovery Bay, coral rubble.

A plot of sites of the first two axes (Fig. 1) shows two distinct faunistic groups. Group 1 contains all sites from lagoonal environments, whereas group 2 contains sites from reef habitats. Sites and species are printed as numbers. For species names consult Table 2.

Looking at the third dimension of the sites plot (Fig. 2) a third group of sites is clearly separated from other habitats. All sites included in this group derive from undersides of platy corals where a completely different assemblage of sponge species occurs (Table 2, species groups 10 & 16). The lagoonal and reef-surface habitats were analyzed separately, whereas too few members of the 'undersides of platy corals group' excluded it from further investigation.

Reef surface. Excluding sites from the lagoon and the undersides of platy corals, the remaining sites from reef surfaces show a more-or-less continuous distribution along a gradient increasing depth, from the upper left to the lower right (Fig. 3). The sample areas 28, 32, 33 and 34 did not fit into this gradient, and have in common that they derive from reefs near river mouths. The location 'river mouth' is obviously different from other reef localities and therefore, these sites were omitted from further analysis. This area may be greater influenced by freshwater, sediments and turbid water although this is speculative and based on few sites only. Figure 4 shows the reef surface sites with substrate inclination overlayed, with an increasing trend towards inclination to the right indicated. The corresponding plot of the species (Fig. 5) shows preferences for species with regard to water depth and inclination. (e.g. species on the upper left are from more horizontal, shallow environments, whereas species on the lower right are from more vertical, deep environments). The two striking gradients, depth and inclination, have more-or-less the same direction, they are not

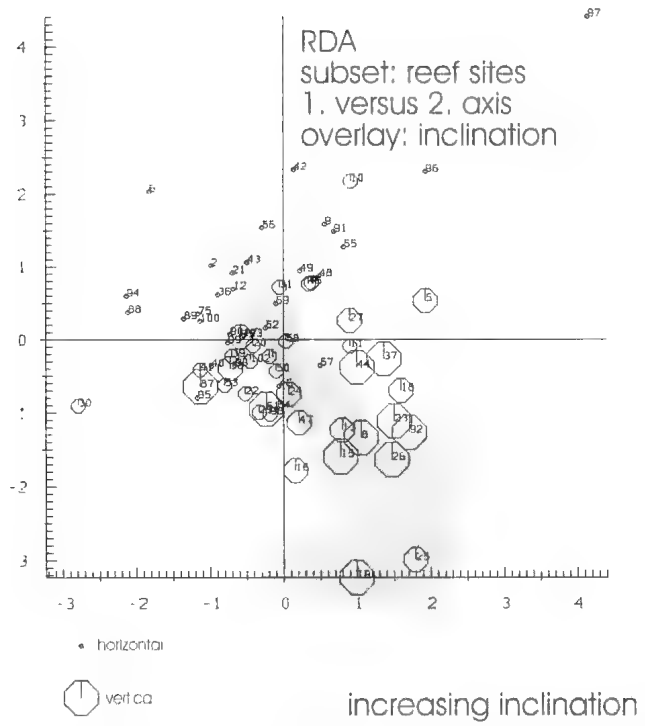

FIG. 4. Redundancy Analysis (RDA) of reef sites, with inclination of substrate as overlay. Larger symbols indicate greater inclination. No site-groups can be recognized, but a continuous arrangement of sites along a gradient of inclination from horizontal sites (upper left) to vertical (lower right). Grey arrow indicates general direction of increasing inclination. Numbers refer to site-numbers.

independent variables. There is undoubtedly an increase in steeper habitats with increasing depth, especially at the deep fore-reef. In these deeper waters there is a steep wall extending down to several hundred meters. However, depth alone is not sufficient to explain species' distributions because less inclined deep water habitats are settled by different species than more inclined deep water habitats. These two gradients do not produce clearly separated groups but the sites appear to be arranged along continuous gradients.

Lagoon. The separation of lagoonal sample areas from reef sites is shown in Figure 1. Inside Discovery Bay several subhabitats are evident. In shallow parts of the lagoon, close to the coast, many ridges occur where freshwater flows out. Two 'blue holes' are the deepest parts of the lagoon (13m and 50m), with very turbid water, fine sediments on the sea bed and probably also some freshwater outflow. Extensive *Thalassia* seagrass-beds occur here and the eastern part of the bay is protected by land and reef from NE trade winds. Site-groups 1, 2, 9 and 10 (Table 2) are mainly from lagoonal environments. The site-groups 9 and 10 of the classification (Table 2) deviate somewhat from the results of the ordination (Fig. 6) whereby there is mixing between some sites from shallow fore-reef habitats with sites from the lagoon. This is probably due to recent hurricane destructions in which the topography of seaward lagoonal-, reef flat- and shallow fore-reef -habitats were more or less equalized, and therefore subsequently settled by similar species. For site-groups 1 (*Thalassia* seagrass) and 2 (freshwater ridges) classification and ordination analyses are in complete agreement. According to the ordination analysis of the lagoon subset (RDA, square root transformation, species normalized and centered), five groups of sites (a-d) are distinguishable and shown in Figure 6.

1) Blue hole: the sites 77, 78, 79, 80, 50 and 3 (site group 10) are from the large blue hole (the smaller blue hole mostly consists of bare sediments, and only a small part at the SE end is overgrown by sessile organisms). Sites 77-80 are from NE to W parts of the blue hole where seawater streams into the bay from the ship-channel. Clearly separated from these are sites 3 and 50 which are from the SE slope of the blue hole, locally known as the 'Columbus Park' locality. Here, influences of freshwater and pollution with bauxite are probable, the latter because of the proximity of the docking area of bauxite freightships. The remaining sample areas from group 10 are from shallow fore-reef habitats, as mentioned above.

2) *Thalassia* seagrass-beds: Very close to the 'blue hole' group is a group of sites from *Thalassia* seagrass-beds. The long leaves of the *Thalassia* seagrass slow down water velocity and lead to higher sedimentation comparable to the situation within the blue hole, and this is the most probable explanation for its statistical similarity to the 'blue hole' group. *Thalassia* seagrass often grows within the back-reef, and the seaward margin of the blue hole is also proximate to back-reef environments, so that these two habitats are not clearly differentiated in their faunistic composition.

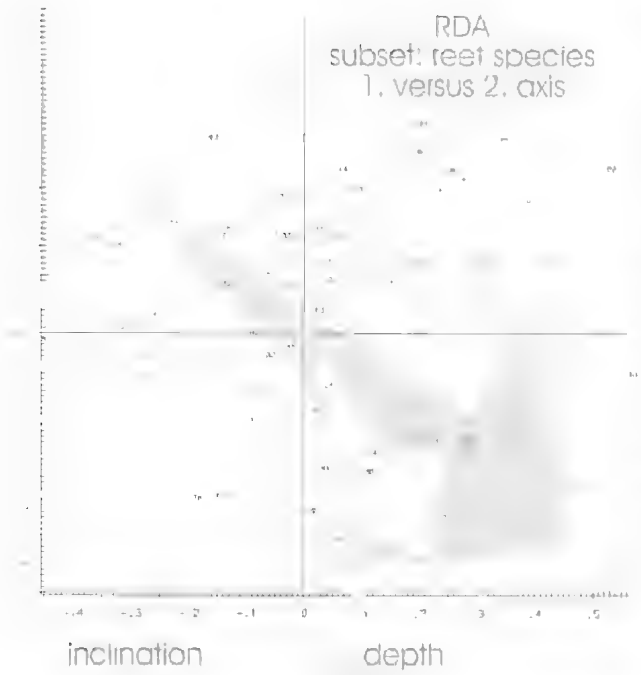

FIG. 5. Redundancy Analysis (RDA) of reef sites, showing a complementary plot of species data from Figures 3-4. Within reef habitats there are no distinct groups of species but species are continuously arranged along gradients. Upper left: shallow reef-species; center: species from intermediate depths or with indistinct depth distribution; lower right: deep reef-species. Numbers refer to species numbers in Table 2.

3) Ridges with freshwater outflow: (group 1, Table 2). These ridges are often surrounded by *Thalassia* seagrass-beds but the influences of the freshwater are strong enough to promote settlement of a different fauna here.

4) Protected back-reef: Clearly separated from other lagoonal environments are two sites from the eastern back-reef. This environment is very close to the blue hole. Consequently, the water here is more turbid than in western parts of the reef. Furthermore, the eastern back-reef is in lee of prevailing waves from the NE trade winds.

DISCUSSION

Hechtel (1965) investigated only 10 (sometimes very small) localities on the south coast and one cannot be sure that individual random differences are responsible for the observed distributions. Because there are considerable differences in sponge species along the north coast, a direct comparison between Hechtel's and our results is not appropiate.

Alcolado (1990) differentiated reef-sponge communities, which he subdivides into less than 10m depth and 10-30m depth, mangrove-sponge communities, macrolagoons and bathyal-sponge communities (150-608m depth). He described the common species in each community and compared diversities, but obviously chose depth-classes before sampling. Alvarez et al. (1990) also focused on the importance of depth gradients in influencing species distributions, and found that there were "a few abundant species and many uncommon ones... The most frequent species are also the most widely distributed along the depth gradient." They also conclude that "For all species, the values of density and area coverage varies along the transects but seems independent from depth". These data seem to contradict our results But their investigation obviously focuses on the few abundant and dominant species, whereas our results are based on the analysis of the whole species composition with a data transformation avoiding dominance types.

Díaz et al. (1990) also studied community structure of sponges along depth gradients. They compared species number, area coverage, density, diversity and evenness. Again, in contradiction to our results, they found that "The results of the cluster analysis confirm that the sponges in the study area lack a well defined pattern of zonation", and the "predominance of encrusting species at almost all depths". However, in looking for differences between transects they restricted their question to zonation based on depth alone. Schmahl (1990) investigated the distribution and abundance of sponges in southern Florida reefs at three depth zones and found that "distributional patterns of sponges may be used to identify the ecological factors that influence the communities in this area".

Whereas all these investigations focused on depth zonation only and comparing pre-defined regions of the reef, the present paper makes no *a priori* assumptions about reef zonation, irrespective of bathymetric or habitat bias, instead using subsets of sites with similar species composition, provided by multivariate analysis.

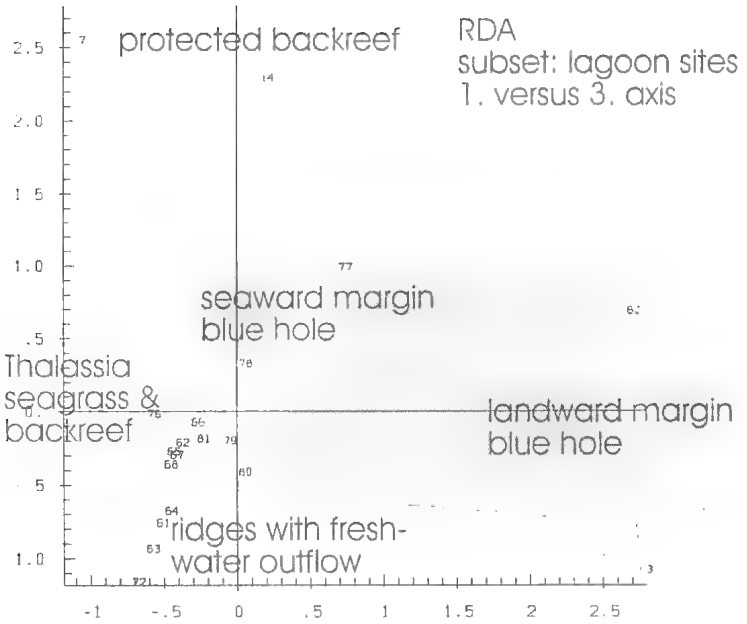

FIG. 6. Redundancy Analysis (RDA) of lagoon sites. Several groups are distinguished, characterising different subhabitats, however, small sample sizes of group-members weakens the interpretation. Numbers refer to site numbers.

Only then we began interpretation of these groups of sites with field data.

The analyses made are objective and repeatable, but we have to admit, that for our conclusions there is no objective test. We think the conclusions are well justified because in most cases there are enough members of the group, making our conclusions probable. Exceptions are the subhabitats within the lagoon. The groups have only few members and we are aware of the risks of interpreting natural variation. But the groups are easily explainable and fit very well in environmental differences, observed during dives, that we think it worth, to include them here.

COMPARISON WITH GOREAU'S ZONATION OF JAMAICAN CORAL REEFS. Goreau (1959) described a reef near Ocho Rios, N Jamaica, 34 year ago, in relatively close proximity to Discovery Bay. He claimed the Ocho Rios reef to be "typical of the large fringing barrier reef communities found along the north coast of Jamaica." and was also familiar with the Discovery Bay reefs. Furthermore, Goreau & Wells (1967) wrote "... that extensive surveys carried out in other parts of Jamaica over more than 10 years have shown that the Discovery Bay reefs are representative for the island as a whole...". Consequently we believe that comparison between their results and ours is well justified. Goreau made a more intuitive approach, naming zones either after dominant species or after striking morphological structures, while in the present paper we tried to find characteristic species from different habitats (which need not to be dominant) and their correlations to some abiotic factors. We consider it is worthwhile comparing these results to see what is still recognizable and what has changed. Goreau distinguished three regions, back-reef, reef-crest and fore-reef, which were also divided into 7-9 different zones. These are considered separately below with remarks as to the present status of these reefs and differences between these two data sets.

1) Goreau divided the back-reef region into a shore zone, with a variety of hermatypic corals, and a lagoon zone, with less corals. The shore zone has almost disappeared. Now, only one small protected area within Discovery Bay has living corals close to shore. *Acropora palmata* has disappeared, *Millepora complanata* dominates this small spot. The upper parts of this 'inshore reef' is now dominated by the green zoanthid *Zoanthus sociatus*, a species which is described by Goreau for the reef flat (which he called also the *Zoanthus* zone), but where it is barely present today. Additional to Goreau's zones the present paper distinguishes four lagoonal subhabitats (blue hole, *Thalassia* seagrass-beds, ridges with freshwater outflow and protected back-reef) separated on the basis of faunistic data. Obviously Goreau worked on a larger scale. Goreau's 'inshore reefs' were probably destroyed by the hurricanes, and he did not divide the lagoon-zone into subhabitats.

2) Goreau's reef-crest region is divided into rear-zone, reef-flat (zoanthus-zone), palmata-

zone (with breaker and lower palmata zone), and buttress-zone. Probably, again due to hurricane destruction, it is now not possible to recognize all of these zones. Rudiments of his rear-zone can be recognized in some parts, with still large *Montastrea annularis*, *Diploria strigosa* and *Sideratrea siderea*. The reef-flat or zoanthus-zone has changed very much. *Zoanthus sociatus* can only barely be found. There are still some *Millepora,* but *Gorgonia* and *Lithothamnium* are rare. Large dead coral rocks, often above sea-level, occur instead. On the sides of coral rocks some small *Solenastrea* sp. occur. His palmata-zone also does not exist any more, replaced by hargrounds, settled by the sponges *Anthosigmella varians* and *Chondrilla nucula*. The previously dominant *Acropora palmata* exists only with some scattered (sometimes large) colonies. The buttress-zone can still be easily identified but, the sediment canals, described by Goreau as "...very narrow, somewhat winding, canyons the walls of which are perpendicular or even overhanging" are now wide sediment streams, the walls less inclined. Exceptions are found in front of the mouths of the Rio Bueno and the Pear Tree River, where some buttresses come close to Goreau´s description. However, these localities seem to have suffered less destruction than any other reefs investigated. Another striking difference are the depths given by Goreau. He wrote: "At the buttress crests, the depth averages only about 2 meters whereas the canyons are between 8 and 10 meters deep." Now the buttress crests range between 5-20m depth and the sediment areas between 7-23m. The uppermost region of the buttresses has probably been destroyed or buried and considerable amounts of the buttresses have been removed. This seems very probable because Goreau described *Acropora palmata* on top of the buttresses where they no longer exist, even as dead colonies. While Goreau stated the cover of living coral was 90% of the available surface, it is now about 15%, except for a few small areas at the walls of the buttresses where 90% coral cover occurs.

3) The seaward slope or fore-reef was divided by Goreau into the cervicornis-zone and the annularis-zone. Both zones have completely disappeared. He located the cervicornis-zone at 'the uppermost region of the seaward slope', seaward of the buttress-zone. Now there are large sandy areas between the buttress zone and the shelf break. *Acropora cervicornis* is now found only sporadically in the buttresses. At the shelf break there are some pinnacles, the edge, and wall of the break itself below 30m depth, covered with numerous large *Montastrea annularis*. This area is much deeper than Goreau described as the annularis-zone, where he gave an average depth of 15m. The 'undersides of platy corals-habitat' described here was not mentioned by Goreau, probably because he did not investigate these depths and he was also mainly interested in hermatypic corals. This habitat is a very small component of the 'area scale' used by Goreau, although relatively large from our faunistic approach. Some differences between our results and those of Goreau are related to our different approaches and methodologies (e.g. our lagoon subhabitats, or the undersides of platy coral habitat). Other differences, like the missing palmata zone, cervicornis-zone or the different depths of the buttress zone seem to be due to destruction by hurricanes.

To summarize the changes since Goreau´s investigations published in 1959, it is obvious that the fringing reefs with three distinguishable main structures (back-reef, reef crest and fore-reef) have changed into a situation where the back-reef has lost its inshore reefs and has gradually merged into a long seaward slope with nearly no living reef crest in between. Goreau´s internal zonation of reef crest and seaward slope can now only be recognized in parts while several striking structures described by him have completely disappeared. Extensive growth of algae seems to inhibit recovery of the reefs.

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RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) ANALYSIS CAN REVEAL INTRASPECIFIC EVOLUTIONARY PATTERNS IN PORIFERA

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Random amplified polymorphic DNA (RAPD) polymerase chain reaction (PCR) techniques were used to generate polymorphic markers to investigate evolutionary patterns in Porifera. Three primers were selected based on their amplification profiles using genomic DNA from 18 sponge species (17 Demospongiae and 1 Calcarea), collected from various localities along the Brazilian coast. The total number of amplified scorable bands per primer varied from 9 (primers OPS-17 and UBC-322) to 32 (primer OPG-19). The level of genetic polymorphism was measured in a population (N=9) of *Hymeniacidon heliophila* from Itaipu Beach (Rio de Janeiro State). The percentage of polymorphic fragments for primer UBC-322 was 31% (mean intrapopulation genetic distance=0.32). The interpopulational genetic diversity was calculated using two individuals from each of five different populations of *H. heliophila*. The percentage of polymorphic fragments varied from 50-68% (mean genetic distance between populations=0.53). A monomorphic band isolated from an individual of the Itaipu population of *H. heliophila* was labelled and used to probe dot blots containing genomic DNA from 18 species of sponges, fruit fly, rat and 15 individuals from the 5 populations of *H. heliophila*. Only *H. heliophila* DNA was hybridised with this marker. We also generated RAPD band patterns using genomic DNA from 6 different species of the genus *Mycale* and primers OPG-19 and OPS-17, with an average percentage of polymorphic bands of 91 and 98%, respectively. The genetic distance between species of *Mycale* varied from 0.45-0.93 (mean genetic distance=0.76) for primer OPG-19 and 0.64-0.93 (mean genetic distance=0.80) for OPS-17. The RAPD technique was shown to be a useful tool to generate molecular markers and to measure polymorphism and genetic distances in Porifera. ❐ *Porifera, molecular markers, RAPD, genetic diversity, SW Atlantic, Hymeniacidon heliophila, Mycale.*

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Sponges (phylum Porifera) show considerable morphological variability. At the biochemical level this variability is supported by heterogeneous allozyme banding patterns reflecting high genetic heterozygosity in sponges (Solé-Cava & Thorpe, 1991, 1994). These characteristics together with the limited data on aspects of reproduction, life history, ecology and cell biology make the study of sponge genetics and systematics complicated tasks. In fact, these problems are general to many marine organisms where complexes of sibling species are known to be common (Knowlton, 1993).

Very few molecular genetics studies have been initiated on sponges, the majority of them using allozyme electrophoresis or ribosomal RNA sequence comparisons (Kelly-Borges et al., 1991; Kelly-Borges & Pomponi, 1994; Solé-Cava & Thorpe, 1994; Collins, 1998). The application of molecular techniques to study sponge genetics and the evolution of the phylum is a difficult proposition due to the lack of knowledge on the complexity of the sponge genome.

On the other hand, fingerprinting techniques have been widely used in studying ecology and taxonomy of several marine phyla, and constitute a good integration bridge between environmental and molecular sciences (Burton, 1996). These techniques provide possibilities to reach more stable classifications by quickly assessing vast series of polymorphic molecular characters of potential phylogenetic significance. Characterisation of sponge genomes by fingerprinting techniques allow better estimation of their intra- and interspecific diversity. This estimation is essential for effective management of biological resources and sound species delimitations, particulary in sibling species complexes.

The study of polymorphisms in the eukaryotic genome is a way to discover the evolutionary relationships between populations or species. Among the many molecular methods currently available for genetic diversity studies, the random amplified polymorphic DNA (RAPD) polymerase chain reaction (PCR), also known as arbitrarily primed (AP) PCR (Williams et al., 1990; Welsh & McClelland, 1990), appears particularly suitable for population genetics. In RAPD analysis there is no need for a priori knowledge of DNA sequences of samples, cost and efforts are reasonable, and many individuals and loci can be efficiently assayed.

In RAPD analysis a single nonspecific primer is used to randomly amplify DNA segments in the genome. The DNA sequence between inverted hybridisation sites is amplified, and, because the distance between primer sites can vary among individuals, different length fragments (or fingerprints) are generated. As the primers are small (normally 10-mers) and not specific to any given gene, many different fragments are produced in an amplification. Fragments present in one individual (or species), but not in others, are defined as polymorphic markers. The fingerprint generated is consistent for the same primer, DNA, and PCR conditions used. This fingerprint is useful in the assessment of affinities along the lower levels of biological and classificatory hierarchies, from individuals and populations to species within a genus (Hillis et al., 1996).

The primary goal of this study was to generate molecular markers by RAPD analysis to demonstrate their usefulness in the investigation of genetic variation within, and among populations of sponges, and in the assessment of species relationships. This technique has already been used successfully for population genetics in, for example, leguminous trees (Chalmers et al., 1992), prosobranch snails (Jacobsen et al., 1996) and weevil insects (Taberner et al., 1997). It has also been effective in phylogenetic studies of plants of the genus *Brassica* (Demeke et al., 1992), papaya cultivars (Stiles et al., 1993) and mangrove species (Lakshmi et al., 1997). These studies have shown that RAPD is a rapid, accurate and sensitive method to detect genetic variation, and to assist in taxonomic investigation at levels from populations to species.

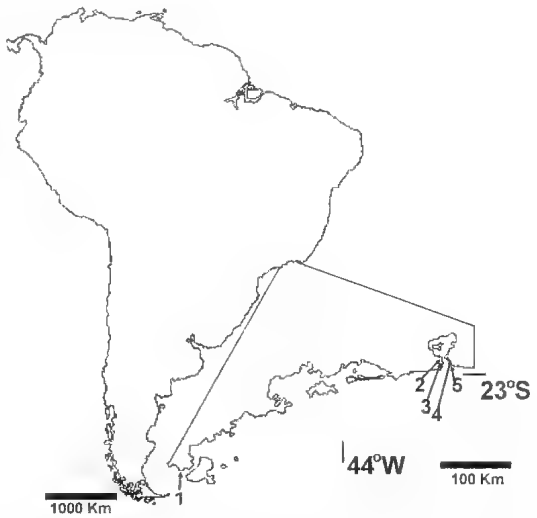

FIG. 1. Map of the Brazilian coast, showing collection sites for populations of *Hymeniacidon heliophila* (inset). 1) Cabelo Gordo Beach, São Sebastião, São Paulo State (23°48'S, 45°26'W); 2) Praia Vermelha Beach; 3) Urca Beach; Rio de Janeiro City, Rio de Janeiro State (23°0'S, 43°12'W); 4) Boa Viagem Beach; 5) Itaipu Beach, Niterói, Rio de Janeiro State (22°52'S, 43°0'W). The distances between beaches are: Itaipu-Boa Viagem=20km; Boa Viagem-Urca–10km; Urca-Praia Vermelha=3km; Praia Vermelha-Cabelo Gordo=300km.

In this report we used RAPD analysis to: 1) assess the genetic diversity within and between populations of the sponge *Hymeniacidon heliophila* Parker, 1910; 2) generate a potential species-specific molecular marker for *H. heliophila*; and 3) generate molecular markers which could be used to investigate interspecific relationships within the genus *Mycale* Gray, 1867.

MATERIALS AND METHODS

SPONGE COLLECTIONS. Samples of *Hymeniacidon heliophila* were collected using SCUBA at Praia Vermelha Beach, Urca Beach

TABLE 1. Sponge species, classification, collection sites, and voucher numbers. Abbreviations: Ba = Bahia State, PE = Pernambuco State, RJ = Rio de Janeiro State, SP = São Paulo State. MNRJ and UFRJPOR = Porifera Collections of the Museu Nacional, Universidade Federal do Rio de Janeiro.

Species	Classification	Collection site	Voucher number
Aiolochroia crassa (Hyatt, 1875)	Verongida, Demospongiae	Fernando de Noronha, PE	UFRJPOR 4766
Amphimedon viridis Duchassaing & Michelotti, 1864	Haplosclerida, Demospongiae	Praia do Sono, RJ	UFRJPOR 4747
Aplysina fulva (Pallas, 1766)	Verongida, Demospongiae	Abrolhos, BA	UFRJPOR 4699
Arenosclera sp.	Haplosclerida, Demospongiae	Búzios, RJ	UFRJPOR 4628
Callyspongia sp.	Haplosclerida, Demospongiae	Fernando de Noronha, PE	UFRJPOR 4783
Cinachyrella alloclada (Uliczka, 1929)	Spirophorida, Demospongiae	Parati, RJ	UFRJPOR 4755
Ectyoplasia ferox (Duchassaing & Michelotti, 1864)	Poecilosclerida, Demospongiae	Fernando de Noronha, PE	UFRJPOR 4772
Gastrophanella sp.	Lithistida, Demospongiae	Fernando de Noronha, PE	UFRJPOR 4773
Hymeniacidon heliophila Parker, 1910	Halichondrida, Demospongiae	Itaipu, RJ	UFRJPOR 4759
Hymeniacidon heliophila Parker, 1910	Halichondrida, Demospongiae	Boa Viagem, RJ	MNRJ 809
Hymeniacidon heliophila Parker, 1910	Halichondrida, Demospongiae	Urca, RJ	UFRJPOR 4605
Hymeniacidon heliophila Parker, 1910	Halichondrida, Demospongiae	Praia Vermelha, RJ	UFRJPOR 4246
Hymeniacidon heliophila Parker, 1910	Halichondrida, Demospongiae	São Sebastião, SP	MNRJ 1307
Leucilla sp.	Leucosoleniida, Calcarea	Praia Vermelha, RJ	UFRJPOR 4838
Mycale (Aegogropila) aff. americana Van Soest, 1984	Poecilosclerida, Demospongiae	São Sebastião, SP	MNRJ 1584
Mycale (Aegogropila) escarlatei Hajdu, Zea, Kielman & Peixinho, 1995	Poecilosclerida, Demospongiae	São Sebastião, SP	UFRJPOR 4451
Mycale (Arenochalina) laxissima (Duchassaing & Michelotti, 1864)	Poecilosclerida, Demospongiae	São Sebastião, SP	MNRJ 1027
Mycale (Carmia) microsigmatosa Arndt, 1927	Poecilosclerida, Demospongiae	São Sebastião, SP	MNRJ 1331
Mycale (Mycale) arenaria Hajdu & Desqueyroux-Faundez, 1994	Poecilosclerida, Demospongiae	Búzios, RJ	UFRJPOR 2438
Mycale (Zygomycale) angulosa (Duchassaing & Michelotti, 1864)	Poecilosclerida, Demospongiae	Parati, RJ	UFRJPOR 4743
Mycale (Zygomycale) angulosa (Duchassaing & Michelotti, 1864)	Poecilosclerida, Demospongiae	São Sebastião, SP	MNRJ 429
Plakortis sp.	Homosclerophorida, Demospongiae	Fernando de Noronha, PE	UFRJPOR 4774
Pseudaxinella reticulata (Ridley & Dendy, 1886)	Halichondrida, Demospongiae	São Sebastião, SP	MNRJ 289
Topsentia ophiraphidites (de Laubenfels, 1934)	Halichondrida, Demospongiae	Fernando de Noronha, PE	UFRJPOR 4779

(Rio de Janeiro City, Rio de Janeiro State), Boa Viagem Beach, Itaipu Beach (Niterói, Rio de Janeiro State) and Cabelo Gordo Beach (São Sebastião, São Paulo State) (Fig. 1). Samples of *Mycale* (*Mycale*) *arenaria* were collected at João Fernandinho Beach (Búzios, Rio de Janeiro State), while *M.* (*Aegogropila*) *aff. americana*, *M.* (*A.*) *escarlatei*, *M.* (*Arenochalina*) *laxissima*, *M.* (*Carmia*) *microsigmatosa* and *M.* (*Zygomycale*) *angulosa* were collected at Cabelo Gordo Beach and surroundings (São Sebastião, São Paulo State). The other twelve species used were collected in several sites along the Brazilian coast (Table 1).

Voucher specimens were deposited in the Porifera Collections of the Museu Nacional of the Universidade Federal do Rio de Janeiro (MNRJ and UFRJPOR) (Table 1). Specimens were frozen in dry ice immediately after removal from sea water and kept frozen at -20°C or -70°C prior to DNA extraction.

EXTRACTION OF DNA. After careful dissection to remove macroscopic symbionts on an ice-cooled Petri dish, specimens were ground

with a glass rod in a solution of 4M guanidine hidrochloride, 0.1M Tris-HCl pH 8.0, 0.5% sarcosil and 1% ß-mercaptoethanol. The suspension was incubated at 50°C for 1hr and centrifuged at 3000g/20mins. The supernatant was extracted with an equal volume of phenol: chloroform:isoamyl alcohol (25:24:1) and nucleic acids were precipitated with 1 volume of isopropanol. The pellet was washed in 70% ethanol and air dried. The dried pellet was dissolved in 0.01M Tris-HCl pH 8.0, 0.005M EDTA, 0.5% SDS, 50µg/ml proteinase K and 20µg/ml RNAse A and incubated at 50°C for 2hrs. Another extraction with phenol:chloroform: isoamyl alcohol (25:24:1) was followed by a single extraction with chloroform/0.3M sodium acetate pH 5.2 and the DNA was precipitated with two volumes of ethanol. After centrifugation the DNA pellet was washed in 70% ethanol, air dried and dissolved in sterile water. The DNA concentration was estimated in 1% agarose gels and, followed by dilution in water to a final concentration of 1ng/µl.

RAPD- PCR PROCEDURE. RAPD analysis of genomic DNA was undertaken using a set of three 10-mer random oligonucleotide primers selected on the basis of the high reproducibility of the patterns and the signal intensity of the bands from a total of seventeen decamer primers screened (Table 2). Each PCR amplification reaction mixture of 25µl contained 2ng of genomic DNA, 2.5µl of 10 x buffer (0.5M KCl, 0.1M Tris-HCl pH 8.4, 1mg/ml gelatin), 1.5µl of 50mM $MgCl_2$, 0.5µl of 5mM dNTPs (Pharmacia), 20ng of primer and 1 unit of Taq DNA polymerase (USB). The reaction mixture was overlaid with one drop of mineral oil, and amplification was carried out in a DNA thermal cycler (Perkin Elmer 480). An initial denaturation step of 3mins at 94°C was followed by 35 cycles of 30secs at 94°C, 30secs at 37°C and 90secs at 72°C, with an additional final step of 10mins at 72°C. The amplified bands were separated by electrophoresis on 6% vertical non-denaturing polyacrylamide gels (PAGE) and visualised after silver staining following the procedure of Sanguinetti et al. (1994). The size of amplified fragments was estimated by comparison with a standard DNA marker: λ (lambda) DNA, double digested with *Eco*RI-*Hind*III.

RAPD DATA INTERPRETATION AND ANALYSIS. Relationships between RAPD profiles obtained from DNA amplifications of sponge individuals with each primer were explored by comparing the presence or absence of shared bands. We scored bands using sharpness and differentiation from the background, and although this is an empirical technique, it produced a consistent outcome which was reproduced by at least two people in blind tests. This process of fragment selection was used throughout all experiments reported in this study. From these assays we recorded the selected markers as either present (1) or absent (0) in each DNA sample. Data recording and calculations were performed using the RAPDistance package, version 1.03 (Armstrong et al., 1994), which allowed us to construct a matrix of pairwise distances. Data from these comparisons were used to calculate similarities between pairs of samples using the Jaccard coefficient (Jaccard, 1901, 1908). This algorithm provides similarity values in the range 0-1, and the software converts these to genetic distances as (1-s).

TABLE 2. Name/number, sequence and G+C content of the primers used.

Name/ No. of primer	Sequences 5`-3`	G+C content
OPS-17/ no. 6	TGG GGA CCA C	70%
OPG-19/ no. 13	GTC AGG GCA A	60&
UBC-322/ no.15	GCC GCT ACT A	60%

The first requirement, and a potential problem of the RAPD method, is its reproducibility. Small changes in reaction conditions are known to cause variation in the amplification pattern (Khandka et al., 1997, and references therein). These difficulties may be overcome if care is taken to ensure consistent PCR reaction conditions during amplification. To optimise these conditions and demonstrate RAPD reproducibility we conducted multiple (four or more) PCR amplifications with the same primer and individual samples (Lobo-Hajdu, unpublished data). In the conditions standardised in our laboratory the PCR-RAPD reactions were reproducible.

Many studies have shown Mendelian inheritance of RAPD markers (Lewis & Snow, 1992; Levitan & Grosberg, 1993), with a few exceptions (e.g. Riedy et al., 1992). Although RAPD analysis of DNA does provide a random sample of the genome, the banding patterns cannot be interpreted allelically because bands are dominant markers and preclude the discrimination of heterozygotes and homozygotes (Welsh & McClelland, 1990; Williams et al., 1990). Consequently, following Williams et al. (1990), we assumed that DNA profiles were the

result of single alleles when computing RAPD data in this study.

DOT BLOTS. Aproximately 500ng of genomic DNA from fruit fly, rat, 18 species of sponges and 15 individuals of *H. heliophila* were denatured with NaOH and bound to a Gene Screen plus nylon membrane (Dupont) using a Bio Dot microfiltration apparatus (Bio Rad), following the manufacturer's recommendations. The membrane was dried in an oven at 80ºC for 1hr and pre-hybridised for 1hr at 65ºC in 10ml of buffer containing 50mM Tris-HCl pH 7.4, 1M NaCl, 10% PEG-8000, 1% SDS and 100µg/ml of heat denatured salmon sperm DNA. A monomorphic band obtained with primer UBC-322 in the interpopulation assay was excised after separation on a 1.6% agarose gel. The fragment contained in the agarose plug was isolated using a Geneclean Kit (BIO 101) following the manufacturer's recommendations, and then reamplified under standard conditions with the same primer originally employed to generate the band (UBC-322). The reamplified product was labelled with α–^{32}P dCTP using a T7 Quick Prime Kit (Pharmacia Biotechnologies) and added to the pre-hybridisation solution. Hybridisation was carried out for another 12hrs. The membrane was then washed with 0.2 × SSC at 65ºC and exposed overnight to an X-Ray film (Kodak) in a cassete at -70ºC.

RESULTS

Fourteen of 17 primers used to amplify DNA of 12 specimens of *Hymeniacidon heliophila* from Praia Vermelha Beach, produced clear and distinct banding patterns in a standard RAPD reaction. Three primers were selected to compare banding patterns within and among different sponge species and to test if these primers would work in general for sponges. Each primer generated a different number of fragments for the same species of sponge. The total number of bands per primer ranged from 9-32 (average =22). The majority of fragments scored were found in the molecular weight range between 300-2500 base pairs (bp) (Table 3). Commonly, bands with molecular weight above 2000bp are very compressed and difficult to score.

DNA amplification profiles in 9 individuals of *H. heliophila* from Itaipu Beach, produced using primer UBC-322 (Fig. 2), yielded both monomorphic and polymorphic bands (arrows in Fig. 2). The diversity of RAPD markers generated for this sample of a natural sponge population was estimated by the percentage of polymorphic fragments which ranged between 15-42% (average =31%; Table 4). Estimated genetic distance between individuals ranged from 0.17-0.45 (average =0.32; Table 5).

TABLE 3. Sponge species and average number of bands, polymorphic plus monomorphic, obtained with each primer used (range of molecular weight in base pairs).

Sponge sample	OPS-17	OPG-19	UBC-322
Amphimedon viridis	20 (400-2100)	23 (400-2200)	22 (100-2100)
Aplysina fulva	22 (200-2100)	32 (200-2500)	13 (100-2000)
Arenosclera sp.	23 (400-2500)	30 (200-2500)	27 (650-2500)
Callyspongia sp.	22 (450-2000)	21 (150-1900)	09 (700-1900)
Ectyoplasia ferox	20 (500-3000)	13 (500-1600)	23 (400-1900)
Gastrophanella sp.	28 (400-2500)	25 (600-3000)	28 (600-2500)
Leucilla sp.	17 (700-2300)	26 (600-3000)	19 (600-2500)
Mycale (Aegogropila) aff. *americana*	11 (600-3000)	27 (400-2500)	20 (300-2100)
Mycale (Aegogropila) escarlatei	23 (300-2500)	29 (400-2500)	21 (600-2500)
Mycale (Arenochalina) laxissima	24 (300-2500)	32 (300-2500)	24 (400-2500)
Mycale (Carmia) microsigmatosa	23 (400-2500)	30 (450-2500)	10 (700-2500)
Mycale (Mycale) arenaria	18 (300-2100)	31 (200-2500)	24 (400-2000)
Mycale (Zygomycale) angulosa	21 (400-2500)	28 (300-2500)	25 (300-2500)
Plakortis sp.	09 (400-1700)	22 (400-3000)	17 (600-1600)

The percentage of polymorphic fragments obtained from RAPD amplification profiles of 2 individuals from each of the 5 different populations of *H. heliophila* (Fig. 3) varied from 50-68% (mean of 5 populations=61%; Table 6). Genetic distances between individuals ranged from 0.22-0.73 (average=0.53; Table 7). Genetic distances between individuals of the same population are within the range of intrapopulation variation (0.17-0.45; Table 5): PV1-PV2=0.22; U1-U2=0.32; BV1-BV2=0.32; IT1-IT2=0.27 and SS1-SS2=0.42 (Table 7).

A monomorphic band of approximately 700bp (Fig. 3, middle left arrow) isolated from one individual of *H. heliophila* from Itaipu Beach was purified, reamplified, labelled and used as a probe in dot blots. These blots contained genomic DNA from 18 sponge species, fruit fly, rat and from 15 individuals of the 5 populations of *H. heliophila* (Fig. 4). The labelled monomorphic band hybridised only with *H. heliophila* DNA.

TABLE 4. Number and percentage of polymorphic fragments amplified with primer UBC-322 for each individual of *Hymeniacidon heliophila* shown in Fig. 2. Fragments scored in the range between 200-2000bp.

Specimens of *H. heliophila*	No. polymorphic fragments	Total no. fragments	% polymorphic fragments
Specimen H1	6	17	35
Specimen H2	3	14	21
Specimen H3	8	19	42
Specimen H4	5	16	31
Specimen H5	5	16	31
Specimen H6	8	19	42
Specimen H7	6	17	35
Specimen H8	2	13	15
Specimen H9	4	15	27
Mean	5	16	31

Genomic DNA from 6 species of *Mycale* was amplified with primers OPG-19 and OPS-17 (Fig. 5A, B). Genetic distances between *Mycale* species ranged from 0.45-0.93 (average=0.76; Table 8A) when using primer OPG-19 and from 0.64-0.93 (average=0.80; Table 8B) when using primer OPS-17. Genetic distances between the two *Mycale* (*Zygomycale*) *angulosa* from two different populations (Parati and São Sebastião) was equal to 0.65 with both primers used. Interestingly, this genetic distance was within the range obtained for interpopulation variation in *Hymeniacidon* (0.22-0.73; Table 7), suggesting that the range for interpopulation variation on *Mycale* could be similar.

DISCUSSION

In this study we tested the utility of the RAPD method to estimate genetic variation in sponges and to generate molecular markers that could be used in population genetics, species delimitation and intrageneric phylogenetic studies. These markers were used to quantify the genetic diversity at the intrapopulation, interpopulation and interspecific levels in experiments undertaken on populations of one species, *Hymeniacidon heliophila*, and in six species within *Mycale*.

Major advantages in using RAPD techniques include their simplicity, reduced running time, and low cost. Moreover, the technique does not make use of radioactive probes and does not require large amounts of DNA or foreknowledge of the regions to be amplified. Another simple and popular way to analyse genetic variation, is to make use of allozyme electrophoresis (Shaw & Prasad, 1970; Avise, 1994; Hillis et al., 1996). This technique revealed different degrees of polymorphism than did the RAPD analysis, which can be explained by the nature of genetic markers generated by both methods. Allozymes are products of structural genes, subjected to relatively strict selective processes, and variation could also be due to post-translational modifications. In contrast, the RAPD amplification technique scans anonymous DNA sequences, whether or not they are coding regions, unique or repetitive, which are randomly distributed in the genome. RAPD markers generate a wide picture of the genome and are not strictly subjected to natural selection.

One of the advantages of RAPD analysis is that RAPD patterns can be easily generated from DNA from any living organism. However, this can also be a major disadvantage, especially when the target organism harbours symbionts. Sponges are notorious for the presence of a number of different organisms living in them, such as bacteria, cyanobacteria, protozoa, fungi, algae and several invertebrates (Berquist, 1978; Wilkinson, 1978; Cheshire & Wilkinson, 1991; Preston et al., 1996; Vacelet & Donadey, 1997).

A potential problem with the data and interpretations of RAPD analysis was the amplification of symbiotic DNA together with sponge DNA which could result in the presence of non-homologous, yet co-migrating bands. We

TABLE 5. Genetic distances (1-s, where s = Jaccard's similarity values in the range 0-1) between 9 individuals of *Hymeniacidon heliophila* from Itaipu Beach (see Fig. 2), calculated from the presence and absence of the amplification products of primer UBC-322. Complete identity is 0 and complete difference is 1. Mean genetic distance among the 9 individuals H1-H9 was 0.32.

	H1	H2	H3	H4	H5	H6	H7	H8
H2	0.28							
H3	0.36	0.35						
H4	0.35	0.24	0.41					
H5	0.35	0.24	0.25	0.22				
H6	0.29	0.26	0.35	0.33	0.41			
H7	0.38	0.28	0.29	0.17	0.17	0.36		
H8	0.33	0.20	0.40	0.29	0.29	0.40	0.24	
H9	0.40	0.29	0.30	0.45	0.28	0.38	0.40	0.35

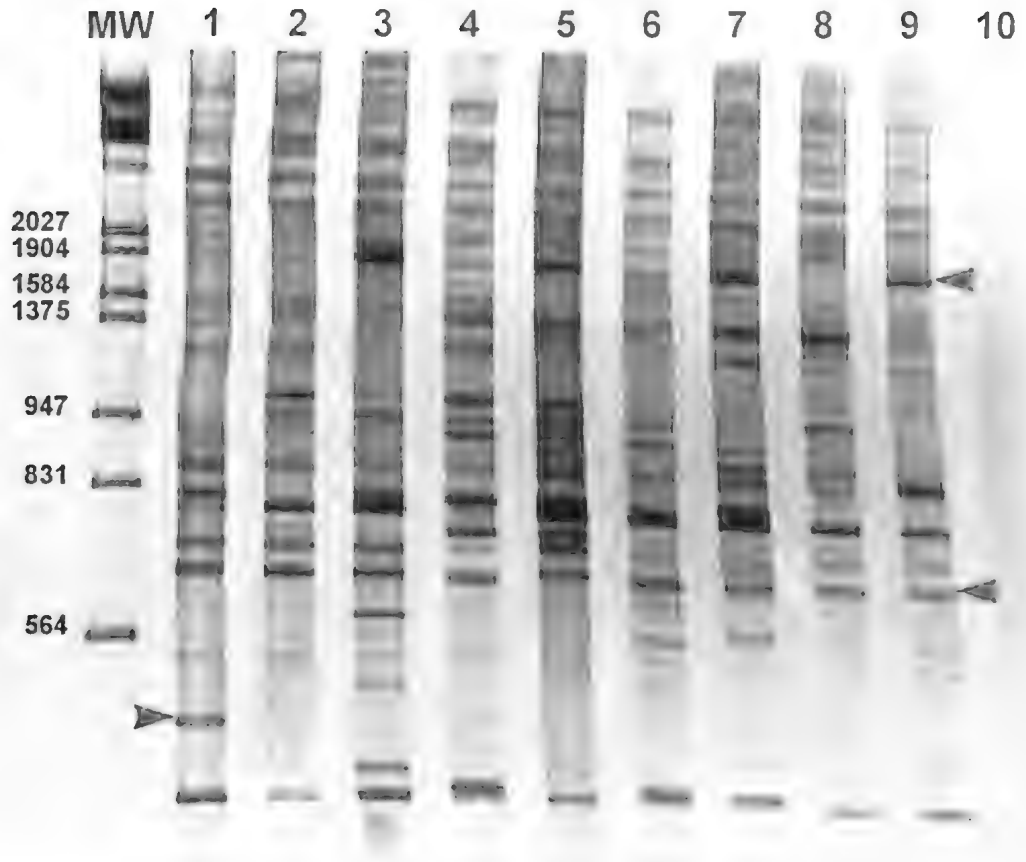

FIG. 2. DNA amplification profiles obtained by RAPD analysis of *Hymeniacidon heliophila* specimens using primer UBC-322. Key: MW, molecular-weight size markers (λ DNA digested with *Eco*RI and *Hind*III) in base pairs. Lanes 1-9, nine individuals from the Itaipu Beach population, lane 10, PCR control reaction without DNA. Arrows show polymorphic (upper right and lower left), and monomorphic (middle right) bands.

TABLE 6. Number and percentage of polymorphic fragments amplified with primer UBC-322 for each individual of *Hymeniacidon heliophila* shown in Fig. 3. Fragments scored in the range 200-1200bp. Abbreviations: PV, Praia Vermelha Beach; U, Urca Beach; BV, Boa Viagem Beach; IT, Itaipu Beach; SS, Cabelo Gordo Beach, São Sebastião.

Specimens of *H. heliophila* / beach site	No. polymorphic fragments	Total no. fragments	% polymorphic fragments
specimen 1/PV	11	17	65%
specimen 2/PV	9	15	60%
specimen 3/U	12	18	67%
specimen 4/U	13	19	68%
specimen 5/BV	12	18	67%
specimen 6/BV	8	14	57%
specimen 7/IT	6	12	50%
specimen 8/IT	8	14	57%
specimen 9/SS	8	14	57%
specimen 10/SS	10	16	63%
Mean	10	16	61%

account for this by taking into consideration that one of the first original works describing the technique (Williams et al., 1993), showed that in a competitive amplification assay using up to 460-fold molar excess of a cyanobacterial genomic DNA mixed with DNA from a soybean genome, all of the detectable amplified products were from soybean. This result suggests that the outcome of an amplification reaction is determined in part by competition for priming sites in the genome, and that a genome of high complexity (soybean) should have more target sites with a better complement to a RAPD primer, as compared to a genome of low complexity

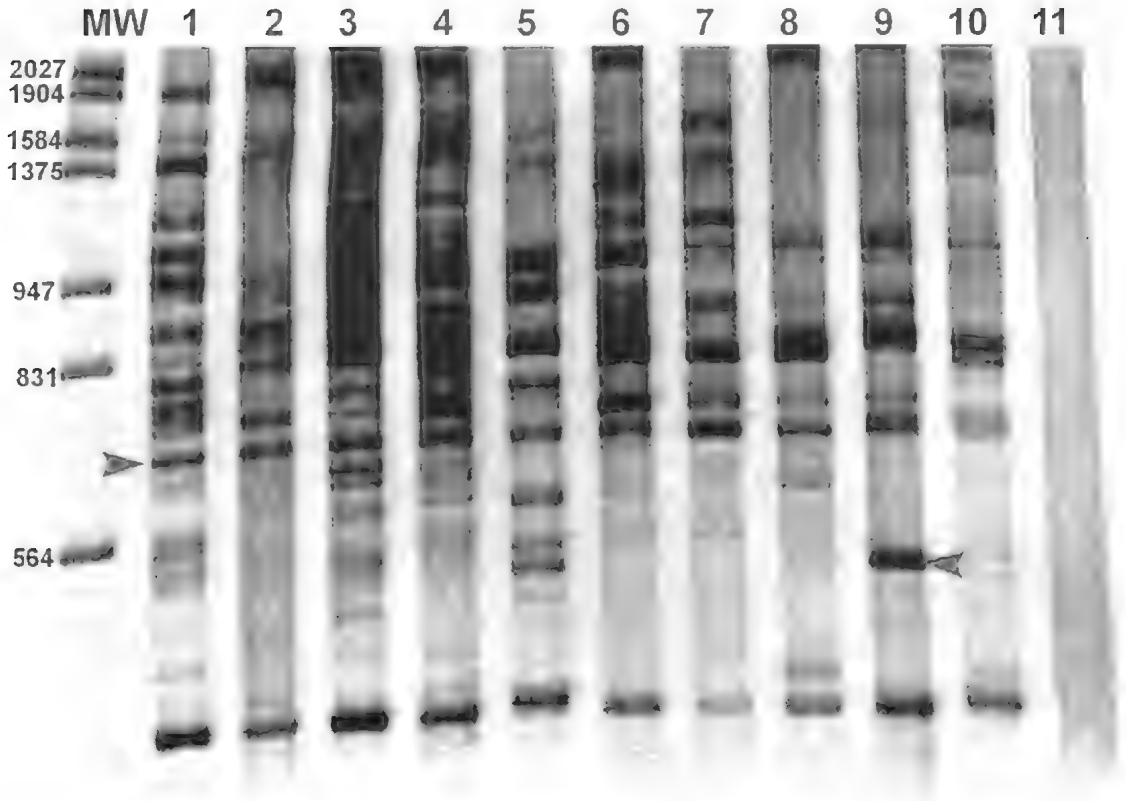

FIG. 3. DNA amplification profiles of individuals from different populations of *H. heliophila* obtained by RAPD analysis with primer UBC-322. Key: MW, molecular-weight size markers in base pairs. Lanes 1-2, Praia Vermelha Beach; lanes 3-4, Urca Beach; lanes 5-6, Boa Viagem Beach; lanes 7-8, Itaipu Beach; lanes 9-10, Cabelo Gordo Beach; lane 11, PCR control reaction without DNA. The arrows show polymorphic (lower right) and monomorphic (middle left) (this band was used on dot-blot) bands.

(cyanobacteria). Symbionts are a commom problem for the majority of the molecular techniques including allozyme analysis and DNA sequencing. Although, RAPD gels can be blotted and markers can be checked with sponge derived probes, we are currently approaching the 'symbiont problem' through the purification of sponge cells, the use of dissociated sponge cells in RAPD amplification reactions, and the comparison of the resulting RAPD pattern with the one obtained for the whole sponge.

Hymeniacidon heliophila showed high levels of genetic variation within and between populations. Allozyme electrophoresis studies

TABLE 7. Genetic distances, based on Jaccard's similarity index, among 10 individuals of *Hymeniacidon heliophila* from 5 populations calculated from the presence or absence of the amplification products of primer UBC-322. Complete identity is 0 and complete difference is 1. Mean genetic distance among the 10 individuals from the 5 beaches was 0.53. Abbreviations: PV, Praia Vermelha Beach; U, Urca Beach; BV, Boa Viagem Beach; IT, Itaipu Beach; SS, Cabelo Gordo Beach, São Sebastião.

	PV1	PV2	U1	U2	BV1	BV2	IT1	IT2	SS1
PV2	0.22								
U1	0.60	0.57							
U2	0.56	0.52	0.32						
BV1	0.65	0.73	0.62	0.58					
BV2	0.65	0.62	0.55	0.50	0.32				
IT1	0.62	0.65	0.50	0.52	0.57	0.38			
IT2	0.65	0.62	0.48	0.50	0.55	0.44	0.27		
SS1	0.71	0.68	0.48	0.50	0.48	0.44	0.38	0.35	
SS2	0.57	0.65	0.58	0.48	0.45	0.64	0.60	0.57	0.42

TABLE 8. Genetic distances, based on Jaccard's similarity index, among 6 species of *Mycale* calculated from the presence and absence of the amplification products of primers OPG-19 (A) and OPS-17 (B). Mean genetic distance among the 6 species (considering just *M. angulosa* SS) was 0.76 (A) and 0.80 (B). Abbreviations: PT, Parati, Rio de Janeiro State; SS, Cabelo Gordo Beach, São Sebastião.

(A) OPG-19	*M. arenaria*	*M.* aff. *americana*	*M. escarlatei*	*M. laxissima*	*M. microsigmatosa*	*M. angulosa* PT
M. aff. *americana*	0.85					
M. escarlatei	0.74	0.62				
M. laxissima	0.81	0.63	0.77			
M. microsigmatosa	0.80	0.87	0.93	0.92		
M. angulosa PT	0.75	0.67	0.71	0.78	0.76	
M. angulosa SS	0.79	0.45	0.70	0.67	0.86	0.65
(B) OPS-17						
M. aff. americana	0.90					
M. escarlatei	0.78	0.83				
M. laxissima	0.81	0.86	0.88			
M. microsigmatosa	0.78	0.78	0.64	0.69		
M. angulosa PT	0.90	0.90	0.93	0.68	0.93	
M. angulosa SS	0.89	0.85	0.84	0.71	0.71	0.65

have already shown that sponges are among the most genetically variable organisms (Solé-Cava & Thorpe, 1994, and references therein). For example, Solé-Cava & Thorpe (1994) showed that the average proportion of polymorphic loci for 21 sponge species was 0.50 (range 0.11-0.86). Our results, with an average genetic distance of 0.32 for the intrapopulation variation (Itaipu samples) and of 0.53 for the interpopulation samples, show that RAPD analysis also points to the existence of high levels of genetic variation, at least for this sponge species.

The dot-blot experiment was used to determine whether the isolated RAPD marker was specific to *Hymeniacidon*, or also present in other species. Our results indicate that this marker is specific to populations of *Hymeniacidon* within the regions studied, or perhaps to the genus *Hymeniacidon*. To determine more accurately the taxonomic value of this marker, we are presently expanding our sponge samples to other species of *Hymeniacidon* and other genera of the Halichondriidae. Cloning and sequencing of this marker is also in progress.

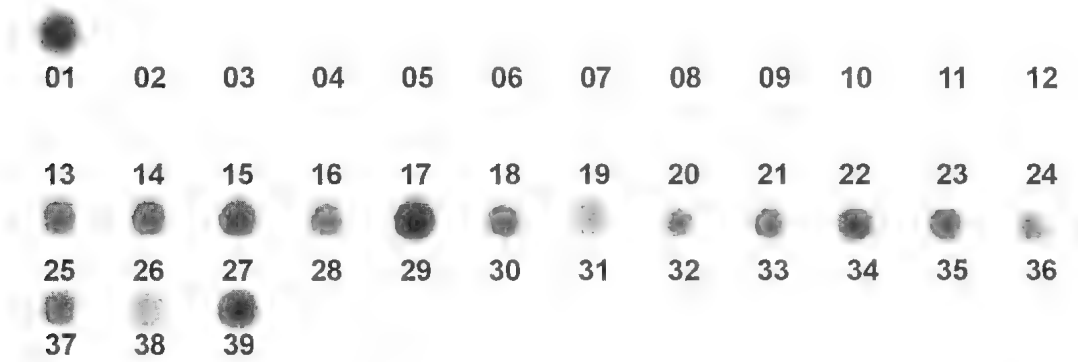

FIG. 4. Dot-Blot hybridised to a RAPD monomorphic marker (shown in Fig. 3) from *Hymeniacidon heliophila*. Key: Wells: 01, *H. heliophila*; 02, *Callyspongia sp.*; 03, *Cinachyrella alloclada*; 04, *Ectyoplasia ferox*; 05, *Gastrophanella* sp.; 06, *Aiolochroia crassa*; 07, *Mycale* (*Mycale*) *arenaria*; 08, *Mycale* (*Aegogropila*) *aff. americana*; 09, *Mycale* (*Aegogropila*) *escarlatei*; 10, *Mycale* (*Arenochalina*) *laxissima*; 11, *Mycale* (*Carmia*) *microsigmatosa*; 12, *Mycale* (*Zygomycale*) *angulosa*; 13, *Plakortis* sp.; 14, *Pseudaxinella reticulata*; 15, *Topsentia ophiraphidites*; 16, *Aplysina fulva*; 17, *Amphimedon viridis*; 18, *Leucilla* sp.; 19, fruit fly; 20, rat; 25-39, *H. heliophila* from five populations (25-27, Praia Vermelha; 28-30, Urca; 31-33, Boa Viagem; 34-36, Itaipu; 37-39, Cabelo Gordo).

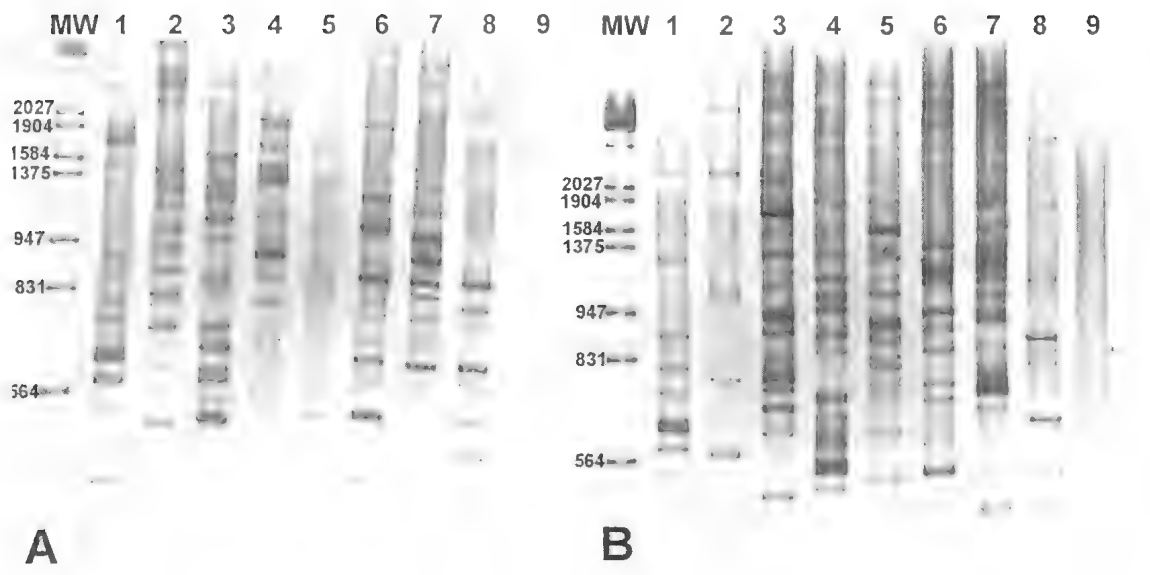

FIG. 5. DNA RAPD amplifications of *Mycale* species. A, Amplification with primer OPG-19. B, Amplification with primer OPS-17. Key: MW, molecular-weight size markers in base pairs. Lanes: 1, *Mycale* (*Mycale*) *arenaria*; 2, *M.* (*Aegogropila*) aff. *americana*; 3, *M.* (*A.*) *escarlatei*; 4, *M.* (*Arenochalina*) *laxissima*; 5, *M.* (*Carmia*) *microsigmatosa*; 6, *M.* (*Zygomycale*) *angulosa* (Parati, Rio de Janeiro State); 7, *M.* (*Z.*) *angulosa* (Cabelo Gordo Beach, São Sebastião, São Paulo State); 8, *Arenosclera* sp. (João Fernandinho Beach, Búzios, Rio de Janeiro State).

Markers generated by RAPD analysis have been used extensively to distinguish species (Bardakci & Skibinski, 1994; Coffroth & Mulawka, 1995; Appa Rao et al., 1996; Partis & Wells; 1996, De Bustos et al., 1998). We applied this method to a set of species of *Mycale* to check its utility in exploring phylogenetic affinities. *Mycale* is well represented at the SE Brazilian coast and phylogenetic affinities within this genus were recently generated (Hajdu & Desqueyroux-Faúndez, 1994; Hajdu & Rützler, 1998; Hajdu, 1999, this volume). Due to the high levels of intra- and interpopulational genetic diversity obtained for *Hymeniacidon*, it will be necessary to survey several (5-10 at least) specimens of each *Mycale* species before deriving conclusions at the interspecific level. This work is in progress, even though the levels of interspecific variation for the six *Mycale* studied here were consistently high for the two primers used. We recognise that our results are still preliminary and stress that our experiments have been conducted to explore the potential utility of RAPD-PCR techniques to assess phylogenetic relationships of congeneric species. We are currently directing our efforts towards increasing the number of primers, specimens and species considered.

RAPD-PCR divergence rates cannot be used as a universal clock with an invariant rate in all animals (Borowsky, 1998). However, a strong relationship seems to exist between degrees of RAPD pattern divergence and time since separation of isolated taxa (Borowsky, 1998). Data from this study show that the mean genetic distance increases from the intrapopulation (0.32) through the interpopulation (0.53) to the interspecific level (0.76 and 0.80). In fact these results support our assumption that there was no amplification of symbiotic DNA, as the presence of symbionts would not correlate well with the degree of relatedness of individuals; in other words, would not be homogeneous at all levels. Our main conclusion is that RAPD patterns show a good correlation between genetic distances and taxonomic level.

Our results demonstrate that molecular markers generated by the PCR-RAPD technique provide an effective tool to assess the existing genetic polymorphism within and between populations and species of sponges. As a future outcome of the present study, specific markers for each sponge population or species can be isolated and sequenced, and population/species-specific primers can be generated. This would allow rapid screening of a larger number

of individuals and thus ease the genetic identification of field samples, more detailed study of the genetic structure of sponge populations, and construction of phylogenies for congeneric species.

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MOLECULAR TECHNIQUES REVEAL WIDE PHYLETIC DIVERSITY OF HETEROTROPHIC MICROBES ASSOCIATED WITH *DISCODERMIA* SPP. (PORIFERA: DEMOSPONGIAE)

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Sponges are well known to harbor large numbers of heterotrophic microbes within their mesohyl. Studies to determine the diversity of these associated microbes have been attempted for only a few shallow water species. We cultured various microorganisms from several species of *Discodermia* collected from deep water using the 'Johnson-Sea-Link' manned submersibles, and characterised them by standard microbiological identification methods. Characterisation of a small proportion (ca. 10%) of the total and potential eubacterial isolate collection with molecular systematics techniques revealed a wide diversity of microbes. Phylogenetic analyses of 32 small subunit (SSU) 16S-like rRNA gene sequences from different microbes indicated high levels of taxonomic diversity associated with this genus of sponge. For example, bacteria from at least five eubacterial subdivisions — gamma, alpha, beta, *Cytophaga* and Gram positive — were isolated from the mesohyl of *Discodermia*. Several strains were unidentifiable from current sequence databases. No overlap was found between sequences of 24 isolates and 8 sequences obtained by PCR and cloning directly from sponge samples. The abundance and diversity of microbes associated with sponges such as *Discodermia* suggest that they may play important roles in marine microbial ecology, dispersal and evolution. ❐ *Porifera, Discodermia, microbial diversity, bacterial symbionts, in vitro culture, gene sequencing, 16S rRNA.*

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Many marine sponges harbor numerous microbial symbionts or 'associates'. Researchers enumerating and characterising sponge-microbial interactions have shown that bacterial biomass can reach over 50% in some marine sponges with corresponding phenomenally huge diversity (Vacelet & Donaday, 1977; Simpson, 1984; Wilkinson, 1987; Santavy et al., 1985; Fuerst et al., 1999, this volume). Although studies of sponge-bacterial associations are hampered by upper microorganism culturability limits, estimated to be below 1-10% (Austin, 1988; Eguchi & Ishida, 1990; Button et al., 1993), focus on marine microbial ecology and taxonomy is increasing. For example, novel aspects of diverse microbes and their habitats are being revealed by molecular genetics approaches (Amann et al., 1995; McInerney et al., 1995; Distel et al., 1988; Delong, 1998; R. Hill pers.comm.).

An understanding of the biology of marine sponges in the polyphyletic order 'Lithistida' would be enhanced by characterisation of the constituent organisms coexisting with these sponges, e.g. species richness, ecological function, metabolic load, etc. Lithistids in general, and *Discodermia* in particular, are the source of several compounds with pharmaceutical potential (Longley et al., 1991; Kelly-Borges et al., 1994; Gunasekera et al., 1994). Identification of microbial associates in *Discodermia* could lead to a better understanding of the ecological or physiological role of these compounds. In this study, we provide a preliminary description of eubacterial species diversity associated with species of *Discodermia*, using both microbiological and molecular tools. A primary goal of this exercise was the identification and matching of *Discodermia*–associated microbe sequences to the closest rRNA relative in current sequence databases. Potentially uncultivable microorganisms were characterised using the polymerase chain reaction (PCR), cloning and DNA sequencing of cloned 16S-like small subunit (SSU) rRNA gene segments (Pace et al., 1986; Lane et al., 1991; Delong, 1998).

Molecular phylogenetic analyses of these sequences highlight the diversity of lineages found within a single sponge genus. Final systematic resolution of each isolate based on rRNA sequence data alone is not attempted in this study.

MATERIALS AND METHODS

COLLECTION AND ISOLATION OF MICROORGANISMS FROM MARINE MACROORGANISMS. Specimens belonging to *Discodermia* spp. used as sources of microbial isolates characterised in this study are listed in Table 1. Isolates from several non-*Discodermia* sponges (*Dercitus*, *Halichondria* and *Corticium* spp.) were included for a cursory comparison with microbe profiles of *Discodermia* spp. (Table 2). All specimens were obtained in accordance with the permits and rules granted by the cooperating sovereign governments. Marine macroorganisms were collected either by SCUBA or through the use of the 'Johnson- Sea-Link' manned submersibles. On return to the surface, sponge samples used for microbial isolation were immediately sub-sampled and prepared for plating using the following method. The sponge was surface-sterilised by rinsing with 70% (v/v) ethanol. A cube of mesohyl (approx. $1cm^3$) was removed using aseptic technique and placed in 20ml sterile artificial sea water (ASW). The sample was then ground in a Waring blender pre-sterilised with 70% (v/v) ethanol, and the resulting suspension was serially diluted with sterile ASW. The dilution series was used as the inoculum for a series of isolation plates. Typically, 100µl of the 10^0, 10^{-3} and 10^{-5} dilutions were used since these were found to bracket the range at which discrete colonies were found.

Sponge cell dissociation and selective cell enrichment were performed by dissociating fresh sponge sections in calcium- and magnesium-free artificial seawater (CMF) (Pomponi & Willoughby, 1994) in a Virtis grinder for 10secs. The resulting slurry was filtered through a 70µm strainer and cells were checked for viability. Cell suspensions were then placed into 15ml tubes and centrifuged to obtain enriched fractions of sponge cells, unicellular bacteria and filamentous bacteria.

Several microbial isolates (e.g. K200, K202, K261) were obtained by fractionating dissociated sponge mesohyl through Percoll/CMF Gradients. A 10ml working solution of 15% Percoll/CMF was diluted with 5M Tris [pH 8.0] from a 90% Percoll/CMF stock solution, and placed in 15ml centrifuge tubes at -75°C for 30mins. About 1ml of sponge mesohyl (5–10g ground in 20ml of filtered sea water) was layered on top of the thawed Percoll/CMF working solutions and centrifuged at 2,000 RPM for 20mins. Tubes were punctured approximately 2.5cm from the bottom, and five 2ml fractions were collected into 24 well plates. About 100µl of each fraction was then plated onto the appropriate isolation media (see below).

Isolation media used in this study were: 1) Chitin Sea Water: Colloidal chitin (in approx. 25ml deionised H_2O), 0.25g dry weight/liter medium; agar, 20g; ASW, 975ml. 2) LN: Bacto Peptone (Difco), 0.5g; Yeast Extract (Difco), 0.5g; agar, 16g; 75% (v/v) ASW, 1L. 3) M3: K_2HPO_4, 0.466g; NaH_2PO_4, 0.732g; KNO_3, 0.1g; $MgSO_4.7H_20$, 0.1g; Na propionate, 0.2g; NaCl, 0.29g; $CaCO_3$, 20mg; $FeSO_4$, 200mg; $ZnSO_4$, 180mg; $MnSO_4$, 20mg; agar, 18g in 1L deionised H_2O. Cycloheximide (50mg) and thiamine (4mg) were added after autoclaving. 4) ISP2: Difco ISP2 prepared with 75% (v/v) ASW. 5) HSV (humic acid, sodium salt), 1g; glycerol phosphate, 110mg; 75% (v/v) ASW, 1L; 10ml BME Vitamin Mix (Sigma) added after autoclaving.

Inoculated plates were incubated at ambient temperature (approx. 25°C) for 2-4 weeks. After this period of incubation, discrete colonies were transferred to fresh plates of the isolation medium, incubated and then re-streaked until the isolate was axenic. Isolates were then transferred to Marine Agar 2216 (Difco). All strains used in this study were maintained as Marine Agar 2216 slant cultures.

DNA ANALYSIS. Genomic DNA from bacterial isolates and sponge mesohyl fractions was extracted using standard purification methods (Sambrook et al., 1989), although some of this DNA required alternative purification methods or modifications which have been previously described (Pitcher et al., 1989). Segments of the 16S-like small subunit (SSU) nuclear rRNA gene amplified from bacterial cultures were sequenced directly after purification of the PCR product. Products derived from sponge mesohyl fractions were cloned before sequencing based on the following procedure.

The universal eubacterial primers, Ecoli9 [5' GAG TTT GAT CAT GGC TCAG 3'] and Loop27rc [5' GAC TAC CAG GGT ATC TAA TC 3'], amplify about 800bp of the 5' end of SSU rRNA gene under standard PCR conditions (Lane, 1991). The segments encompass variable

TABLE 1. Profile of *Discodermia* samples used for microbial characterisation. Key: 1, sample was obtained at a similar location and depth as for the samples of species not belonging to *Discodermia* (as indicated in Table 2). 2, *Discodermia* sample 20-XI-97-1-001 is listed twice because it was used to both a) isolate 51 microorganisms of which two have been analysed, and b) to derive the eight PCR-amplified products comprising the 28 and 29 clone series, which were not cultured.

Discodermia sponge sample ID	Location collected	Depth (ft.)	Total no. microbial isolates	No. sequences analysed
21-III-87-3-014	Bahamas	100	5	2
18-III-87-3-001	Bahamas	515	2	2
8-XI-90-1-001	Bahamas	592	9	2
1-XII-92-2-001	Bahamas	110	13	1
7-XII-92-2-001 [1]	Bahamas	540	8	1
17-XII-92-1-005	Bahamas	535	10	1
15-I-96-2-012	Bahamas	520	1	1
27-X-96-1-003	Bahamas	543	59	7
29-X-96-4-006	Bahamas	520	8	1
9-XI-97-3-008	Honduras	383	65	2
16-XI-97-1-004	Honduras	440	100	2
20-XI-97-1-001 [2]	Honduras	415	51	2
20-XI-97-1-001 [2]	Honduras	415		8
Totals			331	32

regions V1–V4 of bacterial SSU rRNA (*E. coli* positions 9–804) and were expected to provide sufficient genetic variation for phylogenetic analyses (Lane, 1991; Liesack et al., 1991). This size also facilitated complete sequencing of both strands with a minimal number of sequencing reactions, using the above amplification primers and internal primers int-250f [5' GAC TCC TAC GGG AGG CAG 3'] and int-275rc [5' CAC GCG GCG TCG CTG CAT 3']. The typical PCR amplification profile was 94°C denaturation for 45secs, 53-55°C annealing for 60secs, and 72°C extension for 60secs, repeated for 30 cycles. PCR products conforming to expected molecular weights were purified by gel isolation or Qiagen columns (Qiagen), and sequenced by the dye-terminator cycle sequencing method (Applied Biosystems Inc - ABI) run on ABI 373 automated DNA sequencers (University of Florida, ICBR, DNA Sequencing Core Lab, Gainesville FL). To identify potentially uncultivable microbes, PCR products derived from either total mesohyl preparations or enriched mesohyl fractions were 'shotgun' cloned into TA vectors according to manufacturers' instructions (Invitrogen). Clones derived in this manner were designated a number beginning with either 28 (enriched fraction) or 29 (total mesohyl preparation).

All rRNA gene sequences were analysed with the GCG DNA analysis package (GCG, 1994) and SEQED data editor (ABI). The most conserved rRNA sequences relative to *Discodermia*- derived microbes were identified using queries generated by SIMILARITY-RANK in the Ribosomal Database Project (Maidak et al., 1994), or by BLAST using GenBank (Altschul et al., 1990). Preliminary alignments of sequences were made using PILEUP (GCG, 1994), followed by a manual verification for the presence of canonical rRNA secondary structures and compensatory base changes (Neefs et al., 1993; Gutell et al., 1994) (also see Fig. 1). A gap extension penalty of 1, rather than the default of 4, maximised similarity by allowing longer gaps. Maximum parsimony analysis was performed with PAUP, version 3.1.1, while neighbour-joining (NJ) and maximum likelihood (ML or DNAML) analyses were performed with PHYLIP 3.572 software, (Felsenstein, 1993; Swofford, 1993; Hillis et al., 1996). Bootstrap replications of datasets were performed a minimum of 100 times and individual taxa (or operational taxonomic units, or OTUs) which appeared to be problematic (exhibiting long branch lengths or many uninformative nucleotide substitutions) were jackknifed (Efron, 1982). Typical heuristic searches in PAUP utilised evaluations of at least 50 replications of random sequence additions, tree bisection-reconnection (TBR), and decay index assessments (Hillis et al., 1996). Subsets of the total dataset of over 40 OTU's (including

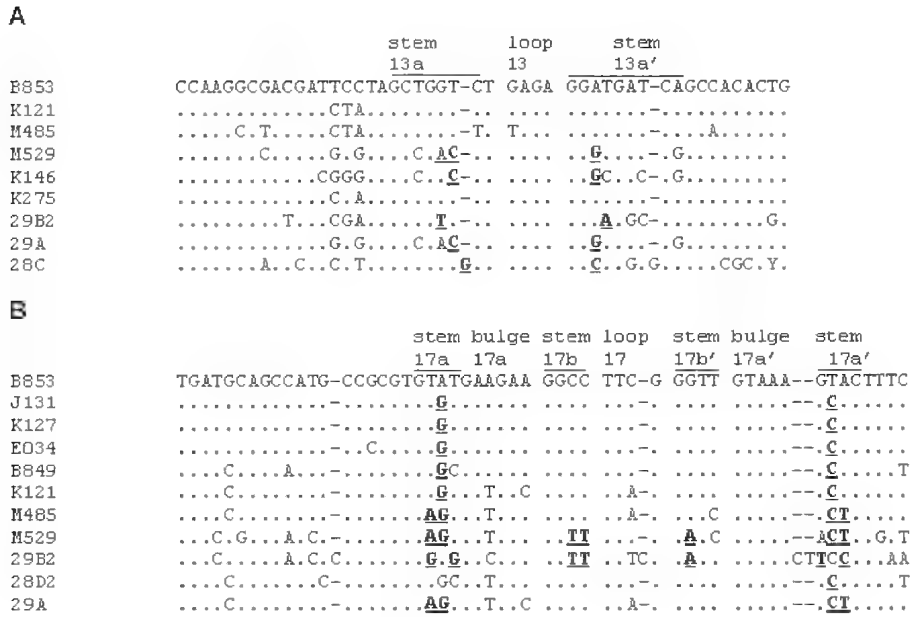

FIG. 1. Representative partial alignments of SSU rRNA regions with conserved secondary structures (Neefs et al., 1993; Gutell et al., 1994). Structures corresponding to: A, Stem/Loop 13; B, Stem /Loop and Bulge 17 are shown over the nucleotide sequences. Both strands of a stem are underlined with the downstream structure marked by a (') above the sequences. Stem 13 in (A) and stem 17 (B) begin at *E. coli* position 288 and 405, respectively. Stem 13a' contains an asymmetric bulge, lengthening it relative to upstream 13a. Nucleotides identical to the first reference sequence are shown below as dots (.); gaps are indicated by (-); compensatory mutations in all stems are underlined.

outgroups listed below) were analysed to verify support for each clade. For NJ, genetic distances were calculated with DNADIST, also in PHYLIP, using Kimura's 2N parameter correction for multiple substitutions and empirically derived transition/transversion ratio of 2.0. Although each maximum likelihood analysis was limited to a subset of 20 OTUs, different taxa within major clades were interchanged and substituted in separate runs of the program to monitor consistency of the consensus topology. NJ and DNAML analyses were performed on a Digital AlphaServer 8400 mainframe computer maintained at the Frederick Biomedical Supercomputer Center in Frederick, Maryland.

Chimeric sequences were detected by comparing the identity of 5' and 3' ends separately before making contiguous constructs, or by using the CHECK-CHIMERA option in RDP (Liesack et al., 1991; Maidak et al., 1994; Rheims et al., 1996). When chimeric products were found, each respective terminal sequence was analysed as a single entity up to the artificial crossover junction and included in phylogenetic analyses despite the shorter length of rRNA sequence.

Sequences (with GenBank accession numbers) of the following representative eubacterial strains and genera were used as outgroups or as reference sequences for the major clades observed in phylogenetic reconstructions: *Bacillus firmus* (X60616), *Pseudomonas straminea* (D84023), *Capnocytophaga* sp. (X97245), *Alteromonas macleodii* (X82145), *Vibrio alginolyticus* (X74690), *Ridgeia piscesae* (U77480), *Thermotoga maritima* (M21774), *Actinomycetes* spp. (X92705), *Rhodobium marinum* (M27534), *Burkholderia solanacearum* (U28232), *Clostridium* sp. (L09175, X77837), *Chloroflexus* (D38365), *Lyngbya* (AJ000714) and an unidentified marinobacter (U61848).

RESULTS

MICROBIAL ISOLATIONS. Phenotypic identifications of microbial isolates were made by analyses of colony morphology, microscopic observation and Gram staining. A general

TABLE 2. Comparison of isolates from different sponge species collected at similar depths, locations and times as for samples of *Discodermia* (listed in Table 1). The number given for *Discodermia* is the average for the three samples analysed.

Sponge taxon	No. of sponge samples used for analysis	No. of Isolates		
		Gram-positive bacteria	Gram-negative bacteria	Fungi
Discodermia	3	5	3	1.5
Dercitus	1	4	4	
Corticium	1	8	1	
Halichondria	1	3	1	3

taxonomic grouping of microbial isolates was obtained from different sponge taxa proximal to, or at similar location, depth and time as *Discodermia* samples (Tables 2, 3). Although major microbial groups such as eubacteria, actinomycetes and fungi were identified, archaebacteria and protists were not cultured. Fungal isolates and their sequences will not be discussed in this paper. To investigate any possible trends or biases in microbial isolations, the number of isolates in different microbial categories obtained from different sponge taxa and different subsamples are summarised in Table 3. These values indicate that there are different profiles of microbial populations among different species of sponges, and that there is also potential variation in microbial yields among different samples of a particular sponge species. For example, some variation in isolate yields appeared to be related to geographical differences in sample collection, whereas specimens of particular species from different depths did not exhibit any strong trends. Some variation may also be attributed to changes in the criteria used to select colonies for isolation and in the media used for certain experiments.

EUBACTERIAL STRAIN IDENTIFICATION. The names of eubacterial strains resulting from highest identity scores are listed in Table 4, together with their corresponding percentage sequence identities. Relatively good agreement was observed between the two major sequence datasources, RDP and GenBank databases, with novel bacterial rRNA sequences from cultured isolates used to infer possible taxonomic placements. However, several caveats to this procedure should be emphasised. 1) Database searches revealed only the most similar sequence in the respective database, and these were not considered as an absolute identification of a particular 'species' or strain of bacteria, even when sequence identity exceeded 99% (Fox et al., 1992). Indeed, it is probably more appropriate to make reference to a specific 'rRNA type' or strain than to infer that these identifications are homologous to species-level taxonomy. 2) Since it was not possible to obtain multiple operon sequences or samples of any given isolate, it was therefore not possible to assess possible intra-strain or intraspecific variation amongst the microbial taxa (Clayton et al., 1995).

The wide taxonomic diversity observed in this survey was striking. For example, at least three major eubacterial divisions, or five classes (Woese, 1987; Balows et al., 1992), are represented in the microbial isolates obtained from samples of *Discodermia*: the gamma-, beta-, alpha- proteobacteria, cytophaga, and Gram-positive eubacteria. Of the 24 isolate sequences obtained from *Discodermia* the most commonly observed bacterial subdivisions were gamma-proteobacteria (9) and alpha-proteobacteria (8), followed by Gram -positive (4), beta-proteobacteria (2) and possibly a single *Cytophaga*-like isolate (K279) (Table 4). This Gram-negative isolate matched most closely a psychrophilic marine *Cytophaga*, with some regions reaching 93% correspondence in identity, although these sequences were still not fully confirmed at the time of writing. Nonetheless, detection of *Cytophaga* primarily from the surfaces of marine aggregate particles in marine systems has been previously described (Delong, 1998).

There were only a few cases where sequence database matches appeared unequivocal: e.g. K169 showed high similarity to *Pseudomonas stutzeri*, a common Gram-negative microbe in RNA group 1 (Balows et al., 1992), and E034 appeared to be related to a bacterium first characterised from Pele's hydrothermal vents in the Pacific ocean. In other instances *Discodermia* isolates matched sequence database entries at highly significant identity levels (97-100% similarity), but these matches were made to 'anonymous' strains identified only to the genus or even subdivision level. For example, the closest relatives to isolates K171 and J131 were an 'alpha-proteobacterium MBIC3368' and *Alteromonas* sp., respectively. Moreover, comparison of these two isolates maintained high sequence conservation across the whole rRNA segment, in contrast to 28D which had a range of conservation values (77-93%) largely dependent on the region of the gene under comparison (Table 4). Since several species of *Vibrio* exhibited equally

TABLE 3. Profile of isolates from different sponge species collected at depths and habitats similar to *Discodermia*. Bold face type indicates samples that were obtained at similar locations or on the same expedition, and thus reliable for more direct comparisons between samples.

Sponge taxon	Number of samples used for isolation	Depth (ft.)	Collection location	Bacteria		Actinomycetes	Fungi
				Gram Pos.	Gram Neg.		
Halichondria	21	390	W. Barbados				1
		526	Canary Islands				1
		477	Bahamas	2	4		
		393	Bahamas	4	1	1	2
		1386	Bahamas	5		1	
		750	Bahamas	1	1	1	1
		1803	Bahamas	5	1		1
		803	Bahamas	2	1		1
		440	Bahamas	1	1		1
		576	Bahamas				4
		543	**Bahamas**	**3**	**1**		**3**
		473	Bahamas		1		
		479	Jamaica	4	9		1
		520	Jamaica		3		2
		1497	Jamaica				1
		410	Jamaica				1
		480	Bahamas	6			3
		230	Bahamas	1			
		149	Florida, east coast	12	33	3	5
		472	Puerto Rico	3	73		2
		450	Puerto Rico		1		
Dercitus	10	20	Venezuela			2	3
		455	Bahamas			1	4
		504	Bahamas				
		540	Bahamas			1	1
		432	Bahamas	6	1	1	
		700	Bahamas	6	3		1
		525	**Bahamas**	**4**	**4**		
		806	Jamaica	3		1	
		400	Jamaica	15	1		3
		384	US Virgin Islands		3		
Corticium	9	581	Bahamas			1	
		525	**Bahamas**	**8**	**1**		
		462	Bahamas	1	2		1
		443	Bahamas	4	8		1
		305	Bahamas		2		
		2819	Bahamas	1	3	1	
		377	Bahamas	8			1
		1585	Turks & Caicos		1		
		90	Puerto Rico	5	11	2	3
Discodermia	10	592	Bahamas				1
		110	Bahamas	7	5		1
		540	**Bahamas**	**4**	**3**		**1**
		535	Bahamas	3	2		5
		520	Bahamas	8	5		1
		543	Bahamas	21	31	5	2
		520	Bahamas	3	2		3
		383	Honduras	9	51	1	4
		440	Honduras	36	61	1	2
		415	Honduras	14	25		12

high scores in comparison to the isolate K261 (ca. 98%), only the genus is listed in Table 4.

Although we did not deliberately attempt to detect or amplify cyanobacteria, this group is well known amongst sponges living in the photic zone (Wilkinson, 1987; Ruetzler, 1990; Diaz, 1997), and some of our samples of *Discodermia* were collected in or near this zone. Clone 28C exhibited strong sequence similarity to a *Leucothrix*, which has been described as a large-diameter, morphologically distinct, marine gliding bacteria related to cyanobacteria (Balows et al., 1992).

To facilitate direct comparisons between our sample isolates and known sequences, and to monitor sampling variation, we undertook parallel rRNA sequence analysis of potentially different microbial isolates from other sponge taxa living in geographical proximity to *Discodermia* (Table 2). These isolates (indicated in boldface in Table 3) were derived from sponge samples collected from the same habitats and depths as *Discodermia* (indicated in bold in Table 1). These few data, although preliminary, suggest a higher frequency of a *Bacillus* in non- *Discodermia* sponges, which is possible circumstantial evidence for sponge-specific microbial associations (Althoff et al., 1997) (see Table 3). More extensive comparisons could be designed to determine the optimal and natural conditions of *Discodermia*-associated microbes, perhaps by wider sampling of proximal sponge and non-sponge habitats (e.g. sediments, seawater, etc.).

PCR was also used to directly amplify rRNA sequences from a) a total sponge cell preparation, and b) a dissociated mesohyl fraction enriched for specific populations of microorganisms. Although only a small number of rRNA clones were obtained by shotgun cloning from these two sources (labelled 28A–Z for the enriched fraction and 29A–Z for the total 'crude' mesohyl preparation), their identities and overall compositions appeared to be different from those derived from cultured isolates (Table 4). In spite of the fact that these sequences also exhibited the highest frequency of chimeric PCR artifacts (Liesack et al., 1991), precluding analysis of the total rRNA fragment, some of these clones may represent 'uncultivable' microbes. Up to 99% of naturally occurring microbes may be overlooked by standard culturing techniques (Button et al., 1993; Amann et al., 1995; Hulgenhotz & Pace, 1996). For example the 5' ends of two clones in the 29 series appeared to significantly match *Flectobacillus*, along with some conservation (82-91%) to *Ridgea* and *Riftia* hydrothermal vent bacteria (Feldman et al., 1997). Sequences of other *Discodermia* isolates exhibited significant similarity to bacteria associated with hydrothermal vent habitats and organisms. Also, clone 29B2 appeared to be distantly related to *Clostridia*. Furthermore, many microbes have not yet been analysed or may not have been isolated from the original plates. Interestingly, the sequences of most of the PCR-derived rRNA clones exhibited identity levels below 90%, and thus it may be recommended that unidentified strains with this level of conservation to any entry in either database be considered strong candidates for 'novel' species designation.

PHYLOGENY OF NOVEL ISOLATES. Phylogenies constructed from the rRNA sequences were used to characterise the diversity and relatedness of *Discodermia* bacterial strains. Figures 2 and 3 display dendrograms constructed with two different phylogenetic algorithms: maximum parsimony and neighbour-joining (NJ), respectively. Maximum likelihood (ML) analyses were also performed with smaller subsets of taxa. Relationships constructed under the principal of parsimony use a criterion of minimum evolution (shortest tree), while NJ uses a clustering algorithm based on overall similarity or distance in a comprehensive OTU×OTU matrix of corrected pairwise distances (Hillis et al., 1996). Although NJ and ML reconstructions better compensate for rate variation among different lineages (Hasegawa & Fujiwara, 1993), ML analyses involved fewer taxa due to computational limitations for large datasets, and thus are not discussed further in this work.

Despite relatively large differences in rRNA sequences among some of the taxa, sequence alignments appeared to be robust. Multiple invariant positions and highly conserved regions corresponding to previously described secondary structures (e.g. loop 20, loop 14-15, and stem 5) were observed by eye along the nearly 800bp of rRNA sequences. Observation of compensatory mutations in stem 13 and bulge 17, among others, in the novel bacterial rRNAs corroborate the conservation of those structures (Fig. 1). Only one highly variable region corresponding to loop 11 (Neefs et al., 1993; Gutell et al., 1994) required removal due to ambiguous alignment and its effect on nucleotide site homology.

For the 30 microbial taxa analysed by maximum parsimony (Fig. 2), an estimation of

TABLE 4. *Discodermia*-associated microbes identified by 16S-like rRNA sequences. Key: 1, the list of organisms was derived directly from the output of GenBank or RDP queries (Altshchul et al., 1990; Maidak et al., 1994). The names of the closest relative may refer to species, genus or common names. 2, percent identity was derived from BLAST scores only, and may reflect identities of different segments of a single query rRNA sequence. Thus, variable conservation of different regions of the rRNA sequence is indicated in the ranges of identity values shown. 3, since these clones were shown to be chimeric, whole contigs were not analysed and query results reflect identities for 5' termini only. 4, five microbial sequences derived from non-*Discodermia* sponge microbes are underlined - M234 and M196 (*Corticium*), M162 and M099 (*Halichondria*) and M119 (*Dercitus*). 5, clonal sequences indicated with an asterisk (*) represent the 28/29 clonal series which was derived from PCR amplification of sponge mesohyl fractions. Therefore, these sequences were not derived from cultured isolates.

Most closely related genus or group[1]	Microbial ID no.	% Sequence identity[2]
ALPHA		
Alpha proteobacteria	K200	90
Alpha proteobacteria MBIC3368	K202	97
Alpha proteobacteria	K121	88
Alpha proteobacteria	K126	91
Alpha proteobacteria	K275	88-96
Alpha proteobacteria	M485	94
Alpha proteobacteria	M162[4]	97
Erythrobacter	E035	98
Alpha proteobacteria MBIC3368	K171	99
Phodospirillum	29A*	90
GAMMA		
Hydrothermal vent bacterium	M119[4]	97
Vibrio	B853	96
Alteromonas	J131	100
Hydrothermal vent bacterium	E034	99
Vibrio	K261	98
Pseudoaltermonas	K127	97
Vibrio alginolyticus	K141	97
Microbulbifer	C724	89
Unidentified gamma	C723	91
Pseudomonas stutzeri	K169	99
Leucothrix mucor	28C[3] *	80-94

Most closely related genus or group[1]	Microbial ID no.	% Sequence identity[2]
BETA		
Unidentified marine proteobacterium	K255	92 -96
Alcaligenes	28D2*	85–94
Beta proteobacteria	B849	92–99
GRAM-positive		
Bacillus (low GC)	M680	96
Bacillus (low GC)	M529	97
Bacillus firmus	M099[4]	91
Bacillus fusiformes	28X*	81
Bacilllus firmus	M196[4]	97
Bacillus firmus	M234[4]	91
Unknown actinomycete	28D*	91
Nocardia, actinomycete	K146	97
Nocardia, actinomycete	K145	80
CYTOPHAGA		
Marine psychrophile	K279	94
AMBIGUOUS GROUPING		
Flectobacillus	29W*	85
Flectobacillus	29B[3]	82–91
Clostridia	29B2*	76–81

skewness of tree length distributions (i.e. for 10,000 random trees using the Random Trees option in PAUP), yielded a g_1 statistic of -0.64. This value is above the 99% significance level for the corresponding critical value of g_1 for more than 25 taxa, indicating a strong leftward skewness and high phylogenetic signal in a four-state character dataset (Hillis & Huelsenbeck, 1992). Weighting transversions over transitions by a factor of 2 shortened the overall length of MP trees. There were only two and four more trees that were one or two steps longer, respectively, than the shortest tree shown in Figure 2 using the same dataset. The clade containing beta and gamma bacteria shows the weakest support (55%), and is thus depicted as a polytomy. Low support is likely due to the uncertain placement of clones 29B, 29W and 28C. Proximal *Ridgeia* and *Marinobacter* groups are typically grouped with gamma proteobacteria.

In parallel, neighbour-joining analyses of rRNA sequence data yielded very similar conclusions to parsimony (Fig. 3). Pairwise genetic distances of all OTU's based on Kimura's 2N parameter correction (Hillis et al., 1996), ranged from 0.04->0.70. The major differences between the NJ and MP trees were: 1) higher bootstrap support for individual clades with NJ relative to MP; 2) fewer collapsed nodes and polytomies with NJ, providing clearer groupings of major proteobacteria subdivisions; 3) inclusion of *Rhodobium* and clone 29A with the cluster of Alpha eubacteria;

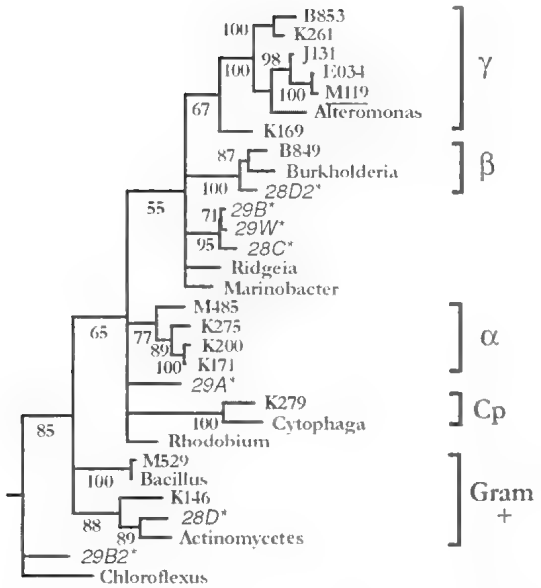

FIG. 2. Representative maximum parsimony phylogeny of *Discodermia*-associated microbes. Cultured isolates are shown in standard bold font. PCR-derived/uncultivated clones are shown with asterisks (*) and in italics, while non-*Discodermia* microbe M119 is underlined. Numbers below each node refer to the bootstrap value after 500 iterations. Representatives of genera or families are listed in the Methods and have names written out. Preliminary heuristic searches using the same dataset, 10 random stepwise additions of taxa with tree bisection reconnection (TBR), found only 2 most parsimonious trees. Length of the two most parsimonious heuristic trees was 1864 steps, with a consistency index (CI) of 0.495. Sub-optimal trees that were longer by 1 or 2 steps numbered only 2 and 4, respectively, and retained the same basic topology of the bootstrap consensus. Morever, the heuristic trees did not collapse the beta and gamma proteobacteria clades into a single polytomy, but rather showed the beta proteobacteria as a distinct group relative to the gamma bacteria (McDonald et al., 1997). Preliminary groupings in the proteobacteria subdivisions and *Cytophaga* (CP) were based on the identities obtained from BLAST sequence database searches.

and 4) monophyly of all representative Gram-positive bacteria. Phylogenetic assignment of clone 29A, which appeared most closely related to the alpha subgroup, was problematic with all three algorithms (ML tree not shown). The decay index for any group that included 29A was always low (<3). Since it is not within the scope of this study to evaluate the strengths and weaknesses of various phylogenetic methods, or to make definitive conclusions on the taxonomic status of each novel isolate, some of these taxonomic placements are likely to be revised in the future.

Nevertheless, the topologies of parsimony and distance trees were generally consistent in showing at least 5 major clades. Isolates K261 and K171 were at the tips of the MP tree and thus their omission to accelerate computation times did not have a significant effect on NJ tree topology. Several features in the present reconstructions of *Discodermia* microbes, such as the monophyly of all proteobacteria, strong bootstrap support for the gamma and beta subdivisions, and the outgroup status of the Gram-positive clade, are all in agreement with current bacterial taxonomy. More specifically, a long branch characterised the lineage of K279 and its strong association with *Cytophaga* bacteria. This branch is considered by us to be a fifth major bacterial clade of the sponge. Relatively long branch lengths were prominent for several other lineages (e.g. K146 and some clones in the 28/29 series).

Although uncultivated clones 28C, 29B and 29W were grouped significantly with other marine bacteria discovered from previous environmental surveys (Moyer et al., 1995; Fuhrman & Davis, 1997), the database matching of 29W and 29B to *Flectobacillus* (a Cytophagales) indicates that accurate placement of these bacteria require more refined determination. However, the tight clustering of taxa observed also suggests possible endemism or ecological specificity with respect to *Discodermia*. Consistent with the database matches to *Clostridia*, clone 29B2 was placed repeatedly near outgroup taxa at the base of all trees. The weak and unresolved positions of some taxa, such as 29B2, 29A and K146, connote another level of diversity. The inclusion of non-*Discodermia* isolates (M119, M169, M234, M162 or M099) in some reconstructions did not significantly alter tree topologies, nor did it suggest any evidence of taxon-specific symbioses occurring in the current dataset. Overall, these results parallel earlier descriptions (Santavy et al., 1990) describing major bacterial groups such as *Vibrio*, *Aeromonas*, and coryneform/actinomycete (Gram-positive) strains derived from marine sponges.

DISCUSSION

Phenotypic, comparative DNA sequence and molecular phylogenetic analyses confirmed the presence of at least five distinct eubacterial clades of 16S-like SSU rRNA sequences from microbial

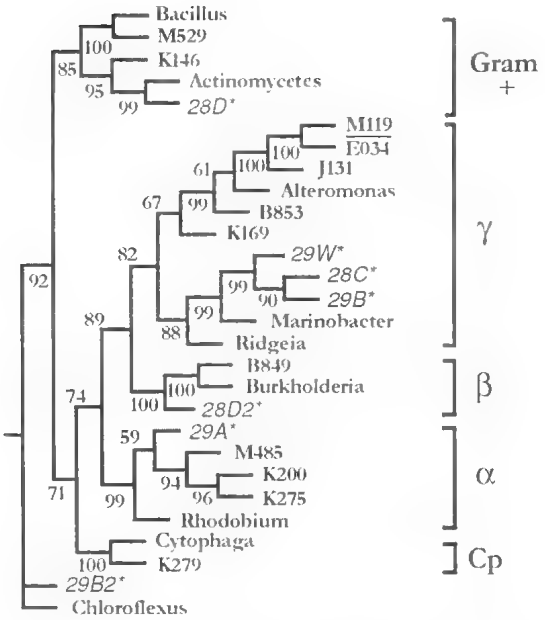

FIG. 3. Distance-based phylogeny reconstructed with the Neighbour-joining method. The same taxa (except K171 and K261) and annotations as that shown in Fig. 2 were analysed.

isolates of the lithistid sponge genus *Discodermia*. Several of the identified *Discodermia* bacterial groups, such as the gamma proteobacteria, are consistent with previous characterisations of deep-sea microbes (Moyer et al., 1995; Feldman et al., 1997; Fuhrman & Davis, 1997), while other lineages (e.g. K279, 28C, 29W, 29A) appear unallied or novel. This situation may have arisen via accelerated substitution rates, while group- or strain-specific synapomorphies were maintained (Hillis et al., 1996; Peek et al., 1998). More likely, however, no close relatives exist in current prokaryotic rRNA sequence databases, which underscores possible missing links in current bacterial rRNA taxonomies. Similar to many earlier surveys of bacterial diversity from the environment (Pace et al., 1986; Giovannoni et al., 1990; Amann et al., 1995; Rheims et al., 1996), PCR and molecular methods have probably revealed unique microorganisms which are otherwise uncultivable under traditional methods. Although some variation may be attributed to differences among species of *Discodermia* and between individual samples of particular species, this characterisation also likely underestimates total diversity in the genus, since only about 10% of the *Discodermia* isolate collection has been sequenced at the time of writing. Nevertheless, our finding that many different bacterial strains and rRNA 'types' stem from only one sponge genus is novel and distinguishes the present study from earlier results.

Bacterial symbionts occur both intracellularly and extracellularly with respect to their sponge host mesohyl (Vacelet & Donaday, 1977; Simpson, 1984; Wilkinson, 1987). However, without positive identification of the types of interacting organisms, elucidating symbiotic parameters such as nutrient transfer, detoxification or gene exchange will not be as meaningful as those made for well-established cnidarian-dinoflagellate associations (Trench, 1993). It is possible that some of the microbes identified here stem fortuitously from the microbial pool derived from sponge filter-feeding activities (Reiswig, 1971; Pile et al., 1996). A long-standing question in sponge-microbial symbioses has been how do so many different symbionts coexist and seemingly thrive in the relatively inhospitable (phagoctye-filled) environment of the marine sponge mesohyl (Simpson, 1984; Wilkinson, 1987)? One answer may stem from the advantages of 'ecto-symbioses' and bacterial communities (Bull & Slater, 1982).

Conversely, it is not unreasonable to suppose that a fraction of the bacterial species isolated and characterised here represent bona fide obligate symbionts of *Discodermia*, an expectation which has been confirmed in other sponges (Burlando et al., 1988; Althoff et al., 1998). Although not trivial, the question of determining specific microbial symbionts could be approached by probing for consistent rRNA (or other genetic) signatures among a matrix of geographically separated samples of *Discodermia* present in our collection. Definitive conclusions on the relative abundance of a particular bacterial strain in *Discodermia* are precluded, since the quantitative recovery of several microbial types during sequencing may suggest any of the following: 1) a dominant presence in the host sponge and concomitant functional role in *Discodermia* physiology; 2) habitat-specific differences; or 3) experimental bias of PCR primer binding sites, genomic DNA quality, etc. (Rheims et al., 1996). It would be interesting in the future to determine whether the mode of molecular evolution in these microbes matches other observations of faster nucleotide substitution rates in symbiotic versus free-living marine bacteria, which may be a function of small population sizes of some symbiotic communities (Peek et al., 1998).

The relatively large breadth and depth of phylogenetic diversity found among *Discodermia*-associated microbes, as revealed by SSU rRNA sequences, has significance in several areas. Firstly, the data reiterate previous studies showing that some marine sponges either maintain or tolerate high levels of microbial species richness. Consequently, this study supports claims that current numbers of catalogued bacterial species are underestimated (UNEP, 1995; Hawksworth & Colwell, 1992; Colwell, 1997). Moreover, the low frequency of duplicate rRNA sequences observed in this survey supports the large diversity of microbes in some sponge taxa.

Lastly, these results may have ramifications for microbial and deep water ecology. Since viable deep sea habitats are generally 'patchy' (Grassle, 1991; Cavanaugh, 1994; Snelgrove & Grassle, 1995), sponges such as *Discodermia* may represent essential stepping stones for bacterial dispersal across large expanses of the seafloor bottom. Such functions are often attributed to less common and more random sinking detritus, animal carcasses (e.g. whale falls), or hydrothermal vent habitats (Showstack, 1998; Tunnicliffe & Fowler, 1998). At the level of the organism sponges may embody oases of species richness, rather than oases of biomass, which is the perception often associated with hydrothermal vents (Snelgrove & Grassle, 1995). The detection of hydrothermal vent-like microorganisms in *Discodermia*, regardless of whether or not they are actual sponge symbionts, suggests a possible source of colonisers for deep water habitats. Thus, *Discodermia* and similar marine sponges should be re-evaluated in the context of a potentially pivotal role in marine microbial ecology, dispersal, and evolution.

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PROPAGATED ELECTRICAL IMPULSES IN A SPONGE. *Memoirs of the Queensland Museum 44: 342. 1999*:- Previous work has shown that *Rhabdocalyptus dawsoni*, a hexactinellid sponge, can arrest its feeding current following mechanical or electrical stimuli. Although a propagated impulse was suspected as the signal triggering arrests, numerous attempts to record such an event failed due to the porous character of the tissue and extreme fragility of the surface membranes. Using a new approach, which involves dissociating sponge tissue, letting it reaggregate, and grafting it back on to the sponge as an autograft, we have found it possible to record propagated electrical impulses. The grafts fuse with the trabecular reticulum, a syncytial tissue that penetrates all parts of the body, including the flagellated chambers, and are eventually absorbed into the sponge. But for a while they form solid lumps that can be used for attachment of suction recording electrodes. Impulses are all-or-none events evoked by single electrical shocks that propagate diffusely through the entire preparation at 0.27 ± 0.1cm·s^{-1} at 10°C, presumably in the trabecular reticulum. The preparation shows an absolute refractory period of 29 s, and is relatively refractory for a further 95-100 s. Intracellular recordings have not been carried out but the wave form recorded extracellularly is suggestive of a conventional, overshooting spike. Pharmacological evidence suggests that it is calcium-based. Thus, despite its long refractory period and low conduction velocity the system is functionally equivalent to the through-conducting nerve nets and excitable epithelial conduction systems of other animals. ❐ *Porifera, hexactinellid, Rhabdocalyptus, conduction, electrophysiology, behaviour, pumping.*

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THEONELLAPEPTOLIDES FROM THE DEEP-WATER NEW ZEALAND SPONGE *LAMELLOMORPHA STRONGYLATA*. *Memoirs of the Queensland Museum 44: 342. 1999*:- The deep-water marine sponge, *Lamellomorpha strongylata*, was collected by benthic dredging at 80m on the Chatham Rise (200km off the E Coast of the South Island of New Zealand). Besides the previously reported calyculins, calyculinamides and swinholide H, five new tridecapeptides, theonellapeptolides IIIa, b, c, d and e, were obtained (Fig. 1).

The following strategy was used for determining the structures of the theonellapeptolides: 1) the amino acids were established by GC/MS following acid hydrolysis and derivatization; 2) methanolysis gave a linear peptide, which was sequenced by tandem mass spectrometry; 3) isobaric residues were distinguished by 2D NMR experiments; 4) detailed analysis led to the complete NMR assignment; 5) the absolute stereochemistry of IIIe was determined by X-ray crystallography coupled with chiral HPLC; 6) the stereochemistry of the other peptides were established by an LC/MS method.

Theonellapeptolides IIIb, c, d and e showed mild cytotoxicity against P388 cell line, but IIIa was very much less cytotoxic. This implied that the second residue from N-terminus (X) plays a key role in maintaining bioactivity.

A comparison with the known theonellapeptolide Id suggested that the crystal structure of IIIe is similar to that of Id although four residues are changed and the ring size is 36 in IIIe, not 37 as in Id.

The theonellapeptolides from the I and II series have all been isolated previously from Lithistid sponges, while those from the III group are nominally from a different sponge order. A key question still to be adressed is whether or not all three groups of peptolides have a similar, or comparable, symbiont origin ? ❐ *Porifera, peptolides, amino acids, nmr spectroscopy, lc/ms, gc/ms, chiral hplc, X-ray crystallography, symbionts, Lithistida, Theonella sp., Lamellomorpha strongylata, New Zealand.*

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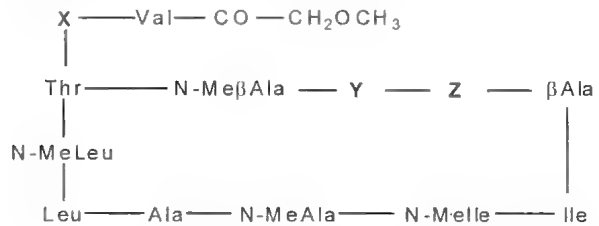

Theonellapeptolides IIIa to e:

(a)	X=N-MeHyMet,	Y=Val,	Z=N-MeIle
(b)	X=N-MeAla,	Y=Val,	Z=N-MeIle
(c)	X=Leu,	Y=N-MeAla,	Z=N-MeIle
(d)	X=N-MeLeu,	Y=Val,	Z=N-MeVal
(e)	X=N-MeLeu,	Y=Val,	Z=N-MeIle

FIG. 1. Structures of theonellapeptolides from *Lamellomorpha strongylata*.

PHYLOGENETIC RESOLUTION POTENTIAL OF 18S AND 28S rRNA GENES WITHIN THE LITHISTID ASTROPHORIDA

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Kelly-Borges et al. (1991) and Kelly-Borges & Pomponi (1994) utilised partial 18S rRNA gene sequences to resolve relationships within hadromerid and lithistid sponges (Porifera: Demospongiae). While their results clarified several specific systematic problems, their conclusions were hampered by low levels of sequence variation. This study sought primarily to evaluate the resolution potential between regions of the 18S rRNA gene used in previous studies on sponges, and 28S rRNA genes used in more recent work. Six lithistid sponge taxa were chosen to represent a gradient of taxonomic relationships, ranging through genus, family, order and class. Approximately 1,300bp of the 18S rRNA gene and a 700bp region at the 5' end of the 28S rRNA gene were compared with the data of Kelly-Borges & Pomponi (1994). We found that the 700bp region of the 28S rRNA gene presented the greatest potential for resolution of this group of Porifera at the genus and family level, and that the resultant molecular phylogeny was congruent with morphological hypotheses for the group.
❐ *Porifera, molecular phylogeny, evolution, Lithistida, Theonella, Discodermia, Corallistes.*

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For the past twenty years, organismal phylogenies have been inferred from the primary sequence of a portion of their genomes. The small subunit ribosomal RNA (SSU rRNA) or 18SrRNA gene has dominated the field as molecule of choice. The analysis of this molecule has been singularly instrumental in elucidating the phylogenetic relationships and natural history of almost all known prokaryotic species where attempts using other methods have failed (see Woese, 1987). Following the success of microbiologists adopting this approach, the systematics of eukaryotic taxa has been addressed by sequencing the 18S rRNA gene (Sogin et al., 1986). This gene is probably still the most frequently used for this purpose.

It is often desirable to use more than a single gene region for the reconstruction of a phylogeny, to supply additional, potentially corroborative phylogenetic hypotheses. For example, the 5' region of the 28S rRNA gene has also been used with great effectiveness (Baroin et al,. 1988; Chombard et al., 1998), as have Elongation Factor genes (Iwabe et al., 1989; Rivera & Lake, 1992), ATPases (Iwabe et al., 1989) and DNA-dependent RNA polymerases (Puhler et al. 1989), among others. There are a number of additional prerequisites for choosing a gene for the purposes of reconstructing a phylogeny. It is desirable that a constancy of function (functional orthology) is maintained throughout the evolution of the taxa of interest. Problems associated with long branches may be observed on trees where some genes have experienced a relaxation in selective pressures. The possibility of mistakenly isolating a paralogous homologue must be minimal. A suitable gene must show signs of having enough variability to discriminate between taxa at the desired taxonomic level. It must also be conserved enough to permit robust alignments and comparisons across the deepest divisions.

Sponge phylogenies, in particular, have given rise to a number of contentious arguments, most of which result from a lack of suitably variable morphological characters which distinguish sponges at the species level and higher (Van Soest, 1987; Hadju et al., 1993; Kelly-Borges & Bergquist, 1997; Sandford & Kelly-Borges, 1997). The primary diagnostic morhological characters that differentiate sponge genera and species are spicule morphology and their arrangement within the sponge body. Characters often influenced by environmental factors, such

as texture, surface features and colouration are less reliable as they are frequently plastic.

Although the construction of poriferan phylogenetic hypotheses using molecular sequence data is still at a preliminary stage, with very few studies completed, it is likely that many future sponge systematic projects will incorporate a molecular moiety. For this reason, it is necessary to establish the taxonomic levels at which certain gene regions will be appropriate. For example, some stretches of DNA might be informative about phylogenetic relationships at the genus and species level, but they may be unsuitable for studies of ordinal relationships and so on. All studies so far have used different genes and gene regions for phylogenetic purposes (Kelly-Borges et al., 1991, 1994; West & Powers, 1993), preventing any useful links between these data towards the construction of larger phylogenies. The advantage that may be gained in future by using the same gene region in all studies is therefore obvious.

The primary goals of our study were to evaluate the resolution potential between various regions of sponge 18S and 28S rRNA genes, in order to determine which genes would efficiently resolve phylogenies at several taxonomic levels. Because of past difficulties in resolution using 18S rRNA (Kelly-Borges et al, 1991; Kelly-Borges & Pomponi, 1994), we took a positive approach in our more recent research to determine which gene would successfully provide resolution within a group of lithistid sponges, and thus, potentially within other taxonomic groups.

In this study we evaluate the relative utility of four alignments with different gene origin, sequence length, and method of analysis. To do this, we extended the 18S rRNA gene data of Kelly-Borges & Pomponi (1994) for six species up to approximately 1,300bp, and in addition, using the same taxa, we have sequenced approximately 700bp of the 5' end of the 28S rRNA gene. Taxa were selected to encompass a range of taxonomic levels including genus, family, order, and class. The criteria by which the sequence data sets were evaluated for their potential utility included counting the number of variable sites and the number of parsimony-informative sites, using maximum likelihood in order to estimate the proportion of constant sites that might be invariable, and also conducting an objective analysis of the resulting tree topologies. The resulting topologies were compared with a hypothetical reconstruction based upon morphological characters.

For the purpose of this exercise four lithistid sponges (Class Demospongiae) were selected from a much larger study on sponge phylogeny, and two hexactinellid sponges (Class Hexactinellida) were chosen as an outgroup. Lithistid sponges represent relict forms of an ancestral fauna from which, it is thought, most demosponges have evolved. These sponges are characterised by the possession of a rigid siliceous skeleton made up of irregular branching desma spicules, the ends of which interlock (zygose) with neighbouring spicules (see Kelly-Borges & Pomponi, 1994, Figs 1,2). In some cases there are additional spicules present, providing clues as to their affinities with non desma-bearing sponges, and the polyphyly of at least some of these genera has been recently confirmed by Kelly-Borges & Pomponi (1994). Although very difficult to differentiate morphologically, the most reliable diagnostic characters that can be used are the morphology, ornamentation, and pattern of zygosis of the desma spicules, and the morphology of the additional spicules if they are present.

MATERIALS AND METHODS

TAXA SELECTION. Sponge species were selected from a broader study of sponge phylogeny, specifically for the purpose of examining capability of taxonomic resolution of poriferan sequence data (Table 1). All sponges were collected using Harbor Branch Oceanographic Institution's 'Johnson-Sea-Link II' manned submersible, except *Theonella* spp. which were collected using SCUBA. Samples were identified through histological examination of skeletal structures, the procedures for which are detailed in Kelly-Borges et al. (1994). Voucher specimens have been deposited in the collections of either The Natural History Museum, London (BMNH), or the Harbor Branch Oceanographic Museum, Florida (HBOM). Registration numbers are given in Table 1. Hexactinellid sponges *Margaritella coeloptychioides* and *Sympagella nux* (Table 1) were selected to provide outgroup sequences for the molecular phylogeny reconstruction.

MORPHOLOGICAL PHYLOGENY RECONSTRUCTION. A phylogenetic analysis of morphological characters (Table 2) was carried out to examine relationships within and between the theonellid and corallistid taxa for the purpose of comparison with trees gained from reconstruction of molecular data. The analysis used the

TABLE 1. Collection data and taxonomic position for sponge taxa sequenced in this study.

Taxon	Museum Registration	Locality
Class Hexactinellida		
Subclass Hexasterophora		
Order Hexactinosida		
Family Euretidae		
Margaritella cocloptychioides Schmidt, 1870	HBOM 003:00925	Turks and Caicos
Order Lyssacinosida		
Family Caulophacidae		
Sympagella nux Schmidt, 1870	HBOM 003:00929	Turks and Caicos
Class Demospongiae		
Subclass Tetractinomorpha		
Order Astrophorida		
Family Theonellidae		
Theonella sp. 1	BMNH1998.3.4.1	Belau, Micronesia
Theonella sp. 2	BMNH1998.3.4.2	Belau, Micronesia
Discodermia sp.	BMNH1998.3.4.3	Bahamas, Caribbean
Family Corallistidae		
Corallistes typus	Schmidt, 1870	BMNH1998.3.4.4

TABLE 2. Morphological characters (A) and their character-states (B) for taxa in this study (see Kelly-Borges & Pomponi, 1994, for an explanation of characters). Characters indicated as * are absent from the outgroup in that they do not possess desmas.

A. Character number	Character	Character state
1	Desmas.	a, present; b, absent
2	Desma development.	a, tetraclonal; b, dicranoclonal; c, absent*
3	Zygosis architecture.	a, articulated at ends of zygomes (Fig. 2A,C); b, articulated along zygomes (Fig. 2E); c, absent*
4	Monactinal megascleres.	a, oxea; b, tylostrongyles (Fig. 2C); c, whispy oxeotes (Fig. 1A)
5	Triaene megascleres.	a, short-shafted discotriaenes (Figs 1C, 2B); b, short-shafted phyllotriaenes (Fig. 2D); c, long-shafted ornamented dicho- and trichotriaenes (Fig. 1A,B); d, long-shafted ortho- and dichotriaenes
6	Euasters.	a, present; b, absent
7	Streptasters (Fig. 2F).	a, present; b, absent
8	Small acanthose microrhabds (Fig. 2B).	a, present; b, absent
9	Large acanthose microrhabds (Fig. 2B).	a, present; b, absent

Branch and Bound search option of PAUP 3.1.1, and data were unordered and unweighted. In order to obtain a directed analysis, members of two non-lithistid astrophorid families, *Geodia* (Family Geodiidae) and *Stelletta* (Family Ancorinidae) were chosen as outgroups. The major skeletal characters that separate these lithistids from their outgroups are the possession of desmas, unique ornamented dichotriaenes, and certain types of microscleres (see Kelly-Borges & Pomponi, 1994).

MOLECULAR EXPERIMENTAL PROCEDURES. Sample collection, preservation and DNA extraction have been previously described (Kelly-Borges & Pomponi, 1994). PCR primers and sequencing oligonucleotides are listed in Table 3 for both genes. PCR reactions were carried out in a 50µl reaction volume which contained a one-tenth volume of 10x PCR buffer (500mM KCl, 100mM Tris-HCl, 1% Triton X-100), dNTPs to a final concentration of 200µM, primers at a concentration of 200µM and 2.5mM MgCl. The PCR protocol began with an initial denaturation at 94°C for 5mins, followed by 35 cycles of denaturation at 94°C for 1min, annealing at 55°C for 1min and extension at 72°C for 1min. This PCR regime was used for both genes. Following cycling, the success of the amplification was determined by electrophoresing on an ethidium bromide-stained agarose gel and visualised by short-wave UV illumination. For each taxon, a total of 10 PCR reactions were carried out and the products were pooled. This was an effort to reduce the potential for an amplification-induced error in the sequences. The pooled amplification products were electophoresed on a single agarose gel and the band was excised using a clean scalpel blade. The DNA

B. Taxon	1	2	3	4	5	6	7	8	9
Discodermia	a	a	a	a	a	b	b	a	a
Theonella sp. 1	a	a	a	b	b	b	b	a	b
Theonella sp. 2	a	a	a	b	b	b	b	a	b
Corallistes sp.	a	b	b	c	c	b	a	b	b
Geodia (outgroup)	b	c	c	a	d	a	b	b	b
Stelletta (outgroup)	b	c	c	a	d	a	b	b	b

fragment was extracted from the agarose using the Qutaex II (Quaigen Ltd, U.K.) PCR purification kit. DNA sequencing reactions were carried out using the Amplitaq FS sequencing kit (Applied BioSystems, Inc.). The sequencing protocol for both genes was carried out as per the manufacturer's instructions. Sequencing was carried out on an Applied Biosystems 373 automated sequencing apparatus and the data was analysed using the Sequence Navigator software (Applied Biosystems Inc.). We estimate that 98% of gene regions were covered by more than one sequencing read, with approximately 25% being covered by three or more sequencing overlaps. The sequences have been deposited in the EMBL sequence repository under the accession numbers AJ224646-AJ224651 (demosponge 18S rRNA), AJ224123-AJ224124 (hexactinellid 18S rRNA), AJ005911-AJ005918 (28S rRNA).

MOLECULAR PHYLOGENY RECONSTRUCTION. Nucleotide positions whose identities were not possible to establish unambiguously, were coded according to the International Union of Pure and Applied Chemistry (IUPAC) nomenclature. The sequences were aligned using the Genetic Data Environment (Smith, 1993). In the majority of cases, the positional orthology of the nucleotides was relatively easy to establish. A conservative approach to the alignment was taken, with those positions whose homology was not possible to establish with absolute certainty, being excluded from subsequent analyses. Attempts in the most difficult cases, to relate the sequences to each other, on the basis of RNA secondary structure proved recalcitrant. In the absence of conclusive grounds for establishing homology, the sites in question were excluded. Phylogenetic hypothesis construction and sequence statistics were evaluated using PAUP*4.0d54 (Swofford, 1993). Transition-transversion ratios were calculated from each dataset by first constructing a neighbor-joining tree from LogDet distances (Lockhart et al., 1994). This tree was considered a working hypothesis of relationships. The estimate of transition-transversion ratio might be influenced to some extent by tree topology, but this influence was not thought to be significant. Using maximum likelihood criteria, the transition-transversion ratio was chosen that yielded the highest likelihood value. The gamma shape parameter for each dataset was calculated using the optimised transition-transversion ratio, again using maximum likelihood as the optimisation criterion. The gamma shape parameter that yielded the highest likelihood was chosen.

RESULTS

MORPHOLOGICAL PHYLOGENY RECONSTRUCTION. A single minimum length tree was obtained of length 14 and a very high consistency index (CI) of 1.0. The phylogenetic tree hypothesises that species of *Theonella* are more recently derived than *Discodermia*, and that these two genera form a clade more recently derived than *Corallistes*. This topology is identical to one of the reconstructions derived from sequence data (Fig. 3A), and supports the current classification that recognises the differentiation of *Discodermia* and *Theonella* from *Corallistes* in the Family Theonellidae and Family Corallistidae, respectively (see Kelly-Borges & Pomponi, 1994).

Morphological characters 2,3,7, 8 and 9 (Table 2) differentiate *Discodermia* and *Theonella* from *Corallistes*, and the states of characters 4 and 5 differentiate *Theonella* from *Discodermia*, and both from *Corallistes*. In *Discodermia* and *Theonella* the desmas are tetracrepid (character 2a) with four clones (zygomes) which clasp (zygose) at their very ends (character 3a; Fig. 2A,C). *Corallistes*, on the other hand, has dicranoclonal desmas (character 2b) in which zygosis spreads along the zygomes (character 3b; Figs 1A, 2B).

The desma skeleton of these genera is supplemented with monaxonal megascleres - oxea in *Discodermia* (character 4a), long and curved with blunt hammer-like ends (tylostrongyles) in *Theonella* (character 4b; Fig. 2C), and whispy roughened oxea-like spicules in *Corallistes* (character 4c, Fig. 1A). Triaenose megascleres are found at the surface of the sponge with their head rays parallel with the surface and the rhabd perpendicular to the surface (Fig. 1A,C). These are discotriaenes in *Discodermia* (character 5a; Fig. 1C, 2B), phyllotriaenes in *Theonella* (character 5b; Fig. 2D), and ornamented (character 5c; Fig. 1A,B) or plain (Fig. 2F) dichotriaenes in *Corallistes*. Microscleres are scattered throughout the sponge body and often form a thick surface crust. In *Discodermia* there are two size categories of roughened microrhabds (characters 8a, 9a; Fig. 2B), whereas in *Theonella* there is only one (character 8a; Fig. 2C). *Corallistes* lacks microrhabds but possess streptasters (Fig. 2F).

TABLE 3. Oligonucleotide names and their corresponding sequences. The first and second oligonucleotides are designed to amplify a large portion of the 18S rRNA gene, whilst the third and fourth oligonucleotide sequences are designed to amplify an approximately 700bp stretch of the 28S rRNA gene.

Oligonucleotide name	Sequence
18Sf20	TGG TAC GGT AGT GGC CTA CCA TGG
18Sr21	ACG GGC GGT GTG TAC AAA GGG CAG
RD3A	GAC CCG TCT TGA AAC ACG A
RD5B2	ACA CAC TCC TTA GCG GA

MOLECULAR PHYLOGENY RECONSTRUCTION. In total, six taxa had a portion of their 18S genes sequenced. The final alignment was in excess of 1,300bp in length. The positions whose alignment could not be determined unambiguously were removed. When all taxa were considered, the final alignment was 1,135 positions in length, whereas with the exclusion of the hexactinellid sequences, the number of alignable positions increased to 1,269. For the 28S rRNA gene dataset, almost 700bp were sequenced for each taxon. The final alignment lengths were 505 positions for the eight taxon dataset and 583 positions for the ingroup taxa alone.

A total of eight alignments were analysed for their information content. Statistics that were evaluated included the number and percentage of variable sites, the number and percentage of parsimony-informative sites, the estimated gamma shape parameter for rate variation across sites and the transition-transversion ratio. The results of these analyses are given in Table 4. The top half of the table refers to the alignments that were used when the two hexactinellid outgroup sequences were included, whereas the bottom half of the table refers to the ingroup only (in this instance lithistid demosponges only).

In most cases, the exclusion of the hexactinellid outgroup sequences facilitated the use of a longer gene region. This was due to the difficulty of aligning some hexactinellid regions with their equivalent location in the demosponge genes. On the other hand, removal of the hexactinellid sequences had the effect of reducing the numbers of variable and informative sites, with a consequent increase in the number of constant sites. The combined dataset always contained the highest number of variable and informative sites as would be expected, but in neither instance did it contain the greatest percentage. One of the striking features of both kinds of analysis is the performance of the 28S rRNA gene dataset. This region had the highest

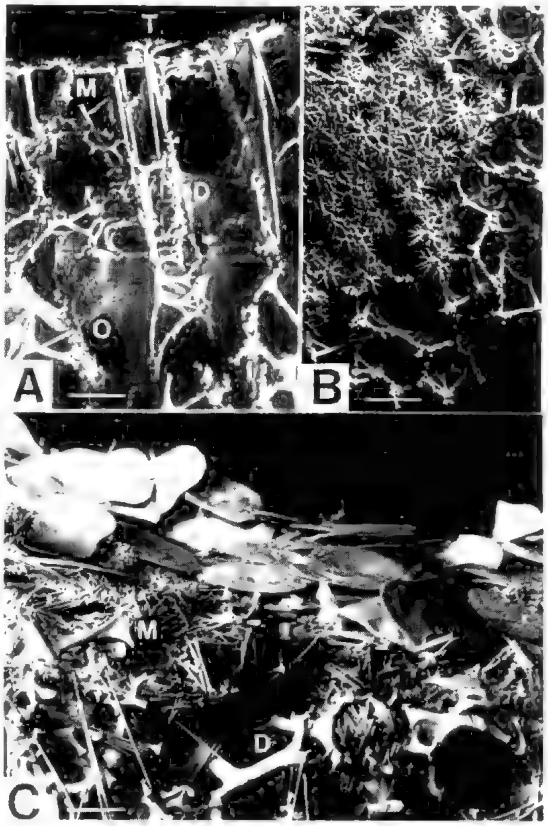

FIG. 1. Skeletal architecture of lithistid demosponges. Transverse sections have been taken through the sponge surface, cellular material removed using hydrogen peroxide, and viewed by SEM. A, *Corallistes nolitangere*. Transverse section through surface of sponge showing dicranoclonal desma reticulation (D). Long-shafted ornamented dichotriaenes (T) emerge from the desma reticulation with the shaft perpendicular to the surface and the cladomes (head) parallel with the surface. Oxeote spicules (O) resembling fine hairs can be seen in residue cellular material. Streptaster microscleres pack the surface (M). Scale=238μm. B, *Corallistes nolitangere*. View of the sponge surface showing the ornamented heads of the dichotriaenes, with the desma reticulation visible beneath. Scale=400μm. C, *Discodermia* sp. Transverse section through surface of sponge showing tetracrepid desma reticulation (D) and above this a dense crust of two sizes of acanthose microrhabd microscleres (M). Short-shafted discotriaenes line the surface with discs overlapping. Scale=111μm.

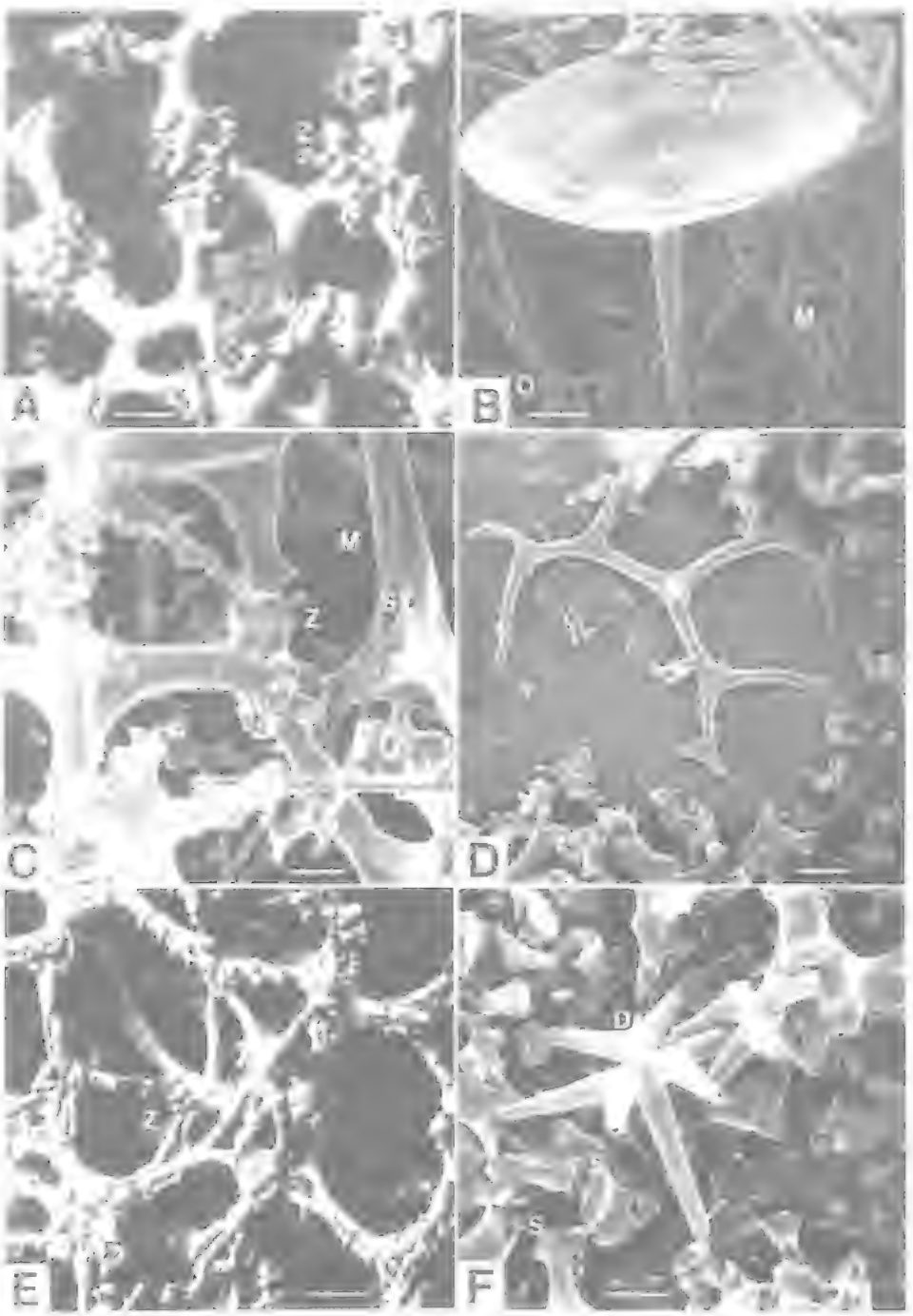

FIG. 2. Desma, triaene and microsclere morphology in *Discodermia*, *Theonella* and *Corallistes*. A-B, *Discodermia* sp. A, Tetracrepid desmas with tuberculate zygoses (Z) at the ends of the zygomes. Scale=125μm; B, Short-shafted discotriaene (D), large and small acanthose microrhabd microscleres (M), and large regular oxea (O). Scale=20μm. C-D, *Thenonella* sp. C, Tetracrepid desmas with zygoses (Z) at the ends of the zygomes. Acanthose microrhabd microscleres are of one size (M) and strongyles have tylote ends (S). Scale=30μm. D, Short-shafted phyllotriaene. Scale=50μm. E-F, *Corallistes nolitangere*. E, Dicranoclonal desmas with zygoses (Z) along the zygomes. Scale=100μm; F, Long-shafted dichotriaene (D), streptaster microscleres (S). Scale=50μm.

TABLE 4. Results from analyses of eight alignments. The top half of the table refers to the alignments that were used when the two hexactinellid outgroup sequences were included, whereas the bottom half of the table refers to the ingroup only (i.e. lithistid demosponges only), where *Sympagella nux* and *Margaritella coeloptychioides* were excluded. The first column contains the gene region that was used for each particular analysis. The words 'all taxa' in parentheses indicates that all six taxa were used in that particular analysis. 18S (short) refers to the data of Kelly-Borges & Pomponi (1994).

Gene region	Length	Variable	Informative	Gamma	Ti/Tv
18S (long) (all taxa)	1138	446 (39%)	163 (14%)	0.31	0.98
18S (short) (all taxa)	473	137 (29%)	75 (16%)	0.24	1.20
28S (all taxa)	505	142 (28%)	84 (17%)	0.24	1.79
Total (all taxa)	1643	588 (36%)	247 (15%)	0.29	1.18
18S (long) (ingroup)	1269	214 (17%)	37 (3%)	0.54	1.16
18S (short) (ingroup)	473	72 (15%)	8 (2%)	0.79	1.46
28S (ingroup)	583	169 (29%)	40 (7%)	0.40	1.26
Total (ingroup)	1852	383 (21%)	77 (4%)	0.34	1.19

percentage of parsimony-informative sites in both datasets and when outgroup taxa were removed, it also had the highest percentage of variable sites.

The gamma shape parameter, which is an estimate of the rate variation across the sites in the alignment, ranged from 0.24-0.31 for alignments that included all taxa, and from 0.34-0.79 for ingroup alignments. The increased estimate of rate variation across sites (lower gamma value) in the eight taxon datasets indicates that the addition of more sequences caused more variation at sites that were already variable, whilst conserved sites remained so even with the addition of more distant taxa.

Of the twelve possible substitution types, there are twice as many possible transversion mutations as transition mutations. For these datasets, the maximum likelihood transition-transversion ratio is slightly above 1.0 in most cases (except for the 18S rRNA dataset with the eight taxon alignment). This is indicative of a bias towards transition substitutions. At very extreme genetic distances, the transition-transversion ratio will converge to 0.5. This is not apparent in any of the datasets in these analyses.

The short 18S alignments generally produced trees with low amounts of resolution. Frequently none of the hypothesised phylogenies in Figure 3 were seen in the resulting bootstrap partition tables. The longer 18S alignments provided a greater amount of resolution, with bootstrap proportions sometimes becoming quite high. The 28S alignments were also quite well resolved and these also yielded high bootstrap values, particularly for the phylogeny that is

Topology		18S (long)		28S		18S (short)		Total	
		All taxa	Ingroup	All taxa	Ingroup	All taxa	Ingroup	All taxa	Ingroup
a Theonella sp1, Theonella sp2, Discodermia, Corallistes	LD	8	42	77	96	47	49	54	90
	ML	8	4	87	86	9	0	59	57
	P	7	0	84	62	0	0	45	13
b Theonella sp1, Theonella sp2, Discodermia, Corallistes	LD	51	43	18	2	0	0.33	42	10
	ML	50	65	13	14	23	49	32	38
	P	83	97	16	38	0	0	55	87
c Theonella sp1, Theonella sp2, Corallistes, Discodermia	LD	31	14	0	0	34	11	1	0
	ML	42	31	0	0	0	0	9	5
	P	10	3	0	0	0	0	0	0

FIG. 3. Phylogenetic reconstructions of the relationships between *Discodermia*, *Theonella* and *Corallistes*. The first column (topology) indicates the topology that is under consideration and the open circle indicates the internal branch whose support is being assessed. The second column indicates the type of analysis that was undertaken: LD - LogDet; ML - Maximum Likelihood; P - Parsimony. The results are in four consecutive blocks according to the gene region that was used in the analysis. Within each block, the left side indicates the bootstrap proportions for the alignment that included all taxa and the right side indicates the results when only the ingroup sequences were used.

favoured by the morphological data (Fig. 3A). The combined sequence dataset alignments also displayed a reasonable amount of signal.

DISCUSSION

The analysis of sequence statistics showed that the 28S rRNA gene region provides the greatest amount of information per unit sequence length (Table 4). Despite the fact that the 28S alignment was hampered by the necessity of removing a large hypervariable portion, it still contained a high number and percentage of variable and parsimony-informative sites. The 18S gene behaved in a less efficient way. The combined dataset always contained the largest number of variable and parsimony-informative sites, but it did not contain the highest percentage of these sites in any of the analyses.

It is curious to note the behaviour of the estimated shape of the gamma parameter, a. The gamma shape parameter is an estimation of rate variation across sites. Lower gamma parameters indicate a more severe amount of rate variation whilst higher numbers indicate that the evolutionary rate is more equivalent at all sites. There was an obvious difference in values between the taxon-inclusion sets. When all taxa were considered, the gamma shape parameter was always lower than when only ingroup taxa were analysed. The addition of more taxa is simply increasing the amount of variability at sites that are already free to vary. Conserved sites remain unchanged with the addition of more taxa. Although the addition of more taxa has the effect of increasing the percentage of variable and parsimony-informative sites, the gamma shape of rate variation across sites is more marked.

Not all of the three phylogenetic trees were congruent with the morphological phylogeny. The shorter of the two 18S rRNA alignments was unable to resolve the relationships of the four taxa of interest with any degree of confidence. Indeed during some bootstrap replicates, some other topologies, not considered in Figure 3 were found. The main reason for this was a complete lack of variability in the dataset.

The longer 18S gene region was slightly more decisive about branching order. The topology that received strong support using this region was a pairing of *Discodermia* and *Corallistes* to the exclusion of the other taxa. However, this high level of support was only achieved using parsimony tree reconstruction. Given the general lack of confidence using the other methods, and the inability of parsimony to compensate for superimposed substitutions, it is possible that these findings are a result of long branch artifacts. This clade is rooted by a particularly long branch leading to the other demosponge taxa. The topology that was observed the least number of times during bootstrapping was the pairing of *Discodermia* with *Theonella*, and this was irrespective of type of analysis or taxon-inclusion set.

The results for the 28S gene sequences were considerably different to those seen in the 18S analyses. For this gene, the placement of *Corallistes* as the sister taxon to *Theonella* was never seen in any analysis (Fig. 3C) Of the two remaining alternative topologies, the placement of *Discodermia* as sister taxon to *Theonella* (Fig. 3A) received considerably more support than the placement of *Discodermia* with *Corallistes* (Fig 3B). Reasonably high bootstrap support was seen for *Discodermia* and *Theonella* as sister-groups using all methods of analyses irrespective of whether the outgroup taxa were used or not.

The alignment combining 18S and 28S rDNA data also yielded ambiguous results. The topology that suggests a sister taxon relationship between *Corallistes* and *Theonella* is very poorly supported (Fig. 3C). The other two topologies are more strongly supported, but neither was supported with any degree of confidence and the differences in the levels of support do not justify acceptance of one over the other. It is likely that the ambiguous nature of the results from the 18S rRNA gene have a detrimental effect on the combined alignment. During bootstrapping, a character can be selected from either gene region. Given that the 18S gene region is approximately 150% larger than the 28S rRNA gene region, it will probably contribute more to each replicate on the whole. The result of this seems to be the carry-over of the ambiguous results from the separate analysis that used only the 18S rRNA gene.

The topology that was most strongly supported using the 28S gene is consistent with the hypotheses of relationships deduced from morphological characters; *Discodermia* and *Theonella* are more closely related to each other than they are to *Corallistes*, and they are the more derived taxa. The 28S rRNA gene generally has a higher proportion of variable and parsimony-informative sites and can provide the best possibility of resolving poriferan phylogenetic relationships, at least at the sub-ordinal level.

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THE PHYLOGENETIC POSITION OF THE SPONGE *SPONGOSORITES SUBERITOIDES* DETERMINED BY ANALYSIS OF 28S RRNA GENE SEQUENCE. *Memoirs of the Queensland Museum 44: 352. 1999:-* A number of problems exist in the phylogenetic consideration of the sponge *Spongosorites suberitoides* that cannot be resolved on morphological grounds alone. Placing the sponge in the genus *Spongosorites* divides this genus into two groups; a single shallow water species and many deep-water species. Described differences between these groups include; oxea size, aerophobic colour-change and surface texture. Further, *S. suberitoides* shows an affinity with hadromerid sponges such as colour in life, texture, arrangement of anastomosing choanosomal tracts and the lack of an aerophobic reaction. In its association with hermit crabs and gastropods it resembles the genus *Suberites*. It also shows similarities to species of *Aaptos* and some Polymastiidae. The DNA sequence of the five prime region of the 28S ribosomal gene of *S. suberitoides* is compared with DNA sequences from hadromerid and halichondrid species in a phylogenetic analysis to resolve the position of this species. ❐ *Porifera, phylogeny, morphology, DNA, 28S ribosomal gene.*

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PHYLOGENY OF LITHISTID SPONGES. *Memoirs of the Queensland Museum 44: 352. 1999:-* Kelly-Borges and Pomponi (1994) utilised partial 18S rRNA gene sequences to resolve relationships within lithistid sponges (Porifera: Demospongiae). While their results lent weight to the growing realisation that the Order Lithistida is polyphyletic, their conclusions were hampered by low levels of sequence variation. Our initial study sought to evaluate the resolution potential between regions of the 18S and 28S rRNA genes within a group of selected Porifera. Approximately 1,300bp of the 18S rRNA gene and a 5' region of the 28S rRNA gene were compared with the data of Kelly-Borges and Pomponi (1994). Six taxa were selected which represented a gradient of relationships, ranging through the taxonomic levels of genus, family and class. We found that the 700bp of the 28S rRNA gene presented the greatest potential for resolution of this group of porifera at the genus and family level, and that this molecular phylogeny is congruent with morphological hypotheses for the group. The study has progressed to include a number of other lithistid and non-lithistid taxa. ❐ *Porifera, Lithistida, 18S rRNA, 28S rRNA, phylogeny.*

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A NEW DENDROCERATID SPONGE WITH RETICULATE SKELETON

MANUEL MALDONADO AND MARIA J. URIZ

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A new encrusting dendroceratid sponge characterised by a skeletal network of ascending primary spongin fibers transversally interconnected by secondary fibers is described from the Alboran Sea (W Mediterranean). Primary fibers, provided with a subcircular basal plate for attachment to the substratum, are unbranched or poorly branched, fasciculate in most cases, with a pith containing foreign material, and a laminated bark. Secondary fibers are also laminated, usually lack any coring material, and may form fenestrated plates around the point where they anastomose to a primary fiber. The reticulation of the skeleton is loose and irregular, so that some primary fibers are interconnected through their basal portions only, some are interconnected along their apical portion, and some are even isolated, lacking any transversal interconnection. Although skeletal features of both the darwinellid genus *Aplysilla* and the dictyodendrillid genus *Igernella* are recognisable in this new sponge, it cannot be taxonomically assigned to either genus, unless the current diagnosis of one of them is modified. A reanalysis of the chemical, histological and skeletal evidence available to date gives little support to the hypothesis that reticulate skeletons appeared in Dendroceratida as a single evolutionary event. Consequently, we propose expanding the diagnosis of the genus *Pleraplysilla* to include species with an irregular network of secondary fibers, such as *Pleraplysilla reticulata* sp. nov. ❐ *Porifera, Keratose sponges, Dendroceratida, Pleraplysilla, Darwinellidae, Dictyodendrillidae, new species.*

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The siliceous skeleton that characterises most sponges in the class Demospongiae is replaced by a skeleton of spongin fibers in a group of sponges that comprises the orders Verongida, Dictyoceratida and Dendroceratida. To provide skeletal support to the bulk of soft sponge tissue, spongin fibers may be either anastomosed to form a network or organised as sets of diversely dendritic, unconnected structures. Dendritic skeletons occur in some Verongida and some Dendroceratida, whereas reticulate skeletons occur in all three orders.

Reticulate skeletons display a wide variety of models of organisation not only within orders, but also families. Differences involve morphology, orientation and dimensions of the basic mesh, as well as several degrees of diversification, both in size and structure, of the anastomosing fibers (e.g. Van Soest, 1978; Bergquist, 1980, 1995, 1996; Bergquist et al., 1998). Given such a structural diversity, the acquisition of a reticulate skeleton is likely to have evolved independently in Dictyoceratida, Verongida and Dendroceratida. This idea is implicitly assumed in the historical classification of these so-called 'fibrous' or 'keratose' sponges, which are distributed in three different orders. Nevertheless, it is also assumed that the acquisition of a reticulate skeleton within the order Dendroceratida was a single, synapomorphic evolutionary step. Consequently, dendroceratid genera with reticulate and non-reticulate skeletons are placed in two different families (e.g. Bergquist, 1980, 1995, 1996; Bergquist et al., 1998): Dictyodendrillidae Bergquist (with a reticulate skeleton made of primary fibers interconnected by secondary fibers) and Darwinellidae Merejkowsky (with exclusively dendritic skeletons made of a single type of fiber). However, some chemical and histological studies have reported unexpected affinities between members of these two families. For example, studies on the diterpenoid chemistry (Bergquist et al.,1990) suggested a relationship between the dictyodendrillid genus *Igernella* Topsent (with a reticulate skeleton and spongin spicules) and darwinellid genera with a typically dendritic skeleton, such as *Darwinella* Müller (with spongin spicules) and *Pleraplysilla* Topsent (without spongin spicules). Similarly, studies on choanocyte chamber structure (Dendy, 1905; Vacelet et al., 1989; Boury-Esnault et al.,

1990) revealed that the genus *Dysidea* Johnston, traditionally included in Dictyoceratida, and members of the family Darwinellidae share a remarkable feature: the presence of eurypylous chambers. At first sight, these non-skeletal affinities do not appear to be consistent with the subdivision of Dendroceratida into Darwinellidae and Dictyopleraplysillidae on the basis of the skeletal pattern. Rather, chemical and histological affinities suggest that reticulate skeletons may have arisen independently in Dendroceratida more than once, so that families diagnosed on the basis of this skeletal trait would not represent monophyletic groups. Nevertheless, these non-skeletal features should not be considered as conclusive evidence for a new classification within Dendroceratida. Bergquist (1996) claimed that the reliability of chamber structure as a character to support a high level classification of keratose sponges is not great, since eurypylous chambers are not exclusively found in *Dysidea* and Darwinellidae, but they also occur in the verongid genus *Ianthella* Gray. Furthermore, although the available body of information from the diterpenoid chemistry and histology is clearly in conflict with a taxonomic scheme based on a separation between reticulate and dendritic dendroceratid genera, it fails to reveal any robust alternative pattern of taxonomic relationships. Thus, despite the fact that skeletal pattern remains the only exclusive familial characteristic supporting a division of Dendroceratida into Dictyodendrillidae and Darwinellidae (Bergquist,1996), such a taxonomic scheme persists as the best option to date.

Here we describe a new dendroceratid sponge in which the skeletal traits of the Darwinellidae and the Dictyodendrillidae are combined, suggesting that even the skeletal criterion may not be as robust as first thought to maintain the current familial scheme in Dendroceratida. Unfortunately, the contribution of the new material described here to an understanding of the relationships in Dendroceratida has been undermined by two unfortunate mishaps. First, although several individuals of this new species occurred at the collection site, we only collected one specimen because we mistook them for material belonging to the common species *Darwinella muelleri* Schulze, which closely resembles the new species when under water. This would not be an insurmountable problem, if the collection site (the Alboran Island) had not been a remote Mediterranean location. Unfortunately, the Alboran Island was only accessible via a costly scientific cruise. Second, the tissue of the holotype became somewhat macerated and therefore useless for providing information on choanocyte chamber structure, since, when we realised the importance of this specimen, it had already been exposed to air during dissection to obtain its skeletal fibers. In 1996, that is eight years after this collection, a second scientific cruise ('Fauna Ibérica - IV') visited the same collection site, but the team of divers failed to find any individuals from this new species. Ten years after its collection and despite the problems mentioned above, we have finally decided to record this material in the scientific literature for two reasons. First, the well-preserved skeletal traits of the specimen clearly indicate that it belongs to an undescribed species. Second, this material provides skeletal information that may be crucial in retracing the path of skeletal evolution in Dendroceratida as further information is gained.

MATERIALS AND METHODS

The material was collected by SCUBA from the sublittoral bottom of the Alboran Island (W Mediterranean: 35°56'45"N, 3°01'38"W; 24m deep) during the 'Ecopharm-I' Cruise in 1988. The specimen was fixed in a 4% formalin solution and stored in 70% alcohol. The skeletal arrangement was studied under dissecting and compound microscopes after partial dissection of the specimen. Its fibers were also studied under a Hitachi S-2300 SEM, after a process of dehydration in a graded series of ethanol, critical point and coating with gold palladium in an E-5000 sputtering.

The holotype is deposited in the collection of the Museo Nacional de Ciencias Naturales (MNCN), Madrid, Spain. For comparative purposes, we also examined several individuals of *Pleraplysilla spinifera* (Schulze) from the Alboran Island and the NE coast of Spain (authors' collection), the holotype of *Igernella mirabilis* Lévi from Indonesia (Zoölogisch Museum Amsterdam, ZMA: POR9316), Caribbean specimens of *Igernella notabilis* (Duchassaing & Michelotti) (authors' collection and ZMA POR6938 specimen), and a specimen of *Dendrilla cirsioides* Topsent from Banyuls (ZMA POR74).

SYSTEMATICS

Class **Demospongiae** Sollas
Order **Dendroceratida** Minchin
Familia **Darwinellidae** Merejkowsky

Pleraplysilla Topsent, 1905

Encrusting Darwinellidae with a fiber skeleton of ascending primary fibers that may be either isolated or diversely fused to each other with or without the development of secondary fibers. Primary fibers are short (few mm), simply or partially fasciculate, poorly branched or unbranched, with a laminated bark and a pith filled with debris, and stand on the substratum on which the sponge grows, attached by means of a small basal plate. Secondary fibers, when present, are concentrically laminated, without coring material or with scarce scattered inclusions. Spongin spicules are absent (emended).

Pleraplysilla reticulata sp. nov.

MATERIAL. HOLOTYPE: MNCN 1.01/182. W Mediterranean, Alboran Island, 35°56'45"N, 3°01'38"W; 24m depth.

ETYMOLOGY. Named for the reticulate condition of the fiber skeleton.

DESCRIPTION. Encrusting, 2x3cm, 0.5cm thick; dull yellow alive, with some lemon yellow zones; soft, slippery to touch, with some mucus on the surface; surface sparsely conulose; conules 1.5-2mm high and 2-4mm apart; oscules and ostia punctiform, grouped in depressed areas located among conules.

Choanosome fleshy, with tiny aquiferous channels; skeleton as a loose, irregular network made of ascending primary fibers transversally interconnected by secondary fibers (Fig. 1A-B); primary fibers non-branched or poorly branched, erect, attached to the substratum by means of a small basal plate (Fig. 2A, C). Fibers have a concentrically laminated bark and a pith containing foreign material (Fig. 1C). Two or more adjacent primary fibers usually fused, yielding fibers with fasciculate appearance (Fig. 1A-C); primary fibers <1cm long, 30-85μm wide, although they can reach 150μm in the fasciculate portions; basal plates 300-700μm in diameter; secondary fibers 20-40μm wide, concentrically laminated, usually lacking any coring material (Fig. 1D); fenestrated plates up to 300μm wide are formed around the point where a primary and a secondary fiber contact (Figs 1B-C, 2B, D); spongin is almost colorless and highly transparent, especially in the secondary fibers and fenestrated plates.

The reticulation of the skeleton is quite irregular; some primary fibers are interconnected through their basal portions only, whereas others are interconnected along their apical portions, just under the ectosome; some primary fibers, especially those newly formed at the growing margins of the sponge, are even isolated, lacking any secondary interconnection; isolated fibers strongly resemble those of *Pleraplysilla spinifera.*

HABITAT. The specimen was found on the vertical side of a rocky block at 24m depth. Although only one specimen was collected for this study, at least six others were observed at the sampling site along with various specimens of the species *Clathrina clathrus* (Schmidt), *Aplysilla sulfurea* Schulze and *Pleraplysilla spinifera.*

REMARKS. To our knowledge, the species described in this study is the first dendroceratid sponge with reticulate skeleton recorded in the Mediterranean.

DISCUSSION

The skeletal features of this species suggest that it is closer to the genera *Pleraplysilla* and *Igernella* than to any other dendroceratid. Its primary fibers strongly resemble those of the genus *Pleraplysilla*, which also contain foreign material in the pith. Furthermore, anastomoses of adjacent primary fibers forming fasciculate primaries are not exclusive to this new species. Van Soest (1978) reported anastomoses of primary fibers to yield "vague meshes here and there" in the Caribbean species *Pleraplysilla stocki* Van Soest, 1978. However, the occurrence of a reticulate skeleton made of ascending primaries and interconnecting secondary fibers has not been reported in this genus to date. Such a trait does not coincide with the current diagnosis of either the genus *Pleraplysilla* or the family Darwinellidae (e.g. Bergquist, 1980). Rather, the network model suggests a relationship between the specimen collected and some dictyodendrillids, such as *Igernella* species that are characterised by an irregular reticulation made of ascending primaries transversally interconnected by secondary fibers (Van Soest, 1978; Uriz & Maldonado 1996). However, this new species does not match the current diagnosis of *Igernella*,

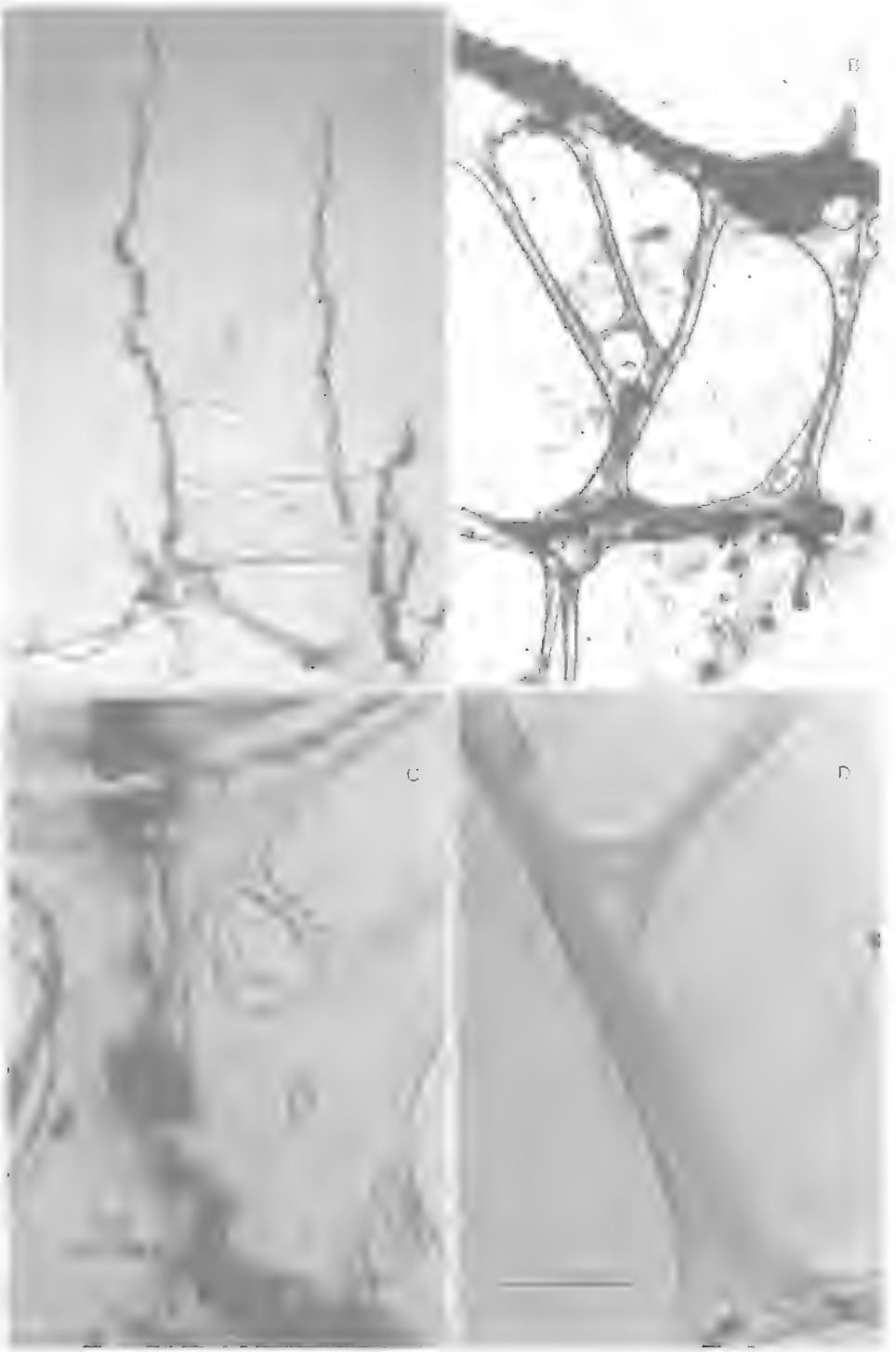

FIG. 1. *Aplysilla reticulata* sp. nov. A, General organisation of the skeleton. B, Detail of secondary reticulation interconnecting two cored primary fibers. C, Detail of a primary fiber showing a pith filled with debris. Fenestration can also be seen. D, Detail of the laminated structure of a secondary fiber. Scale bars A, 950µm; B, 420µm; C-D, 50µm.

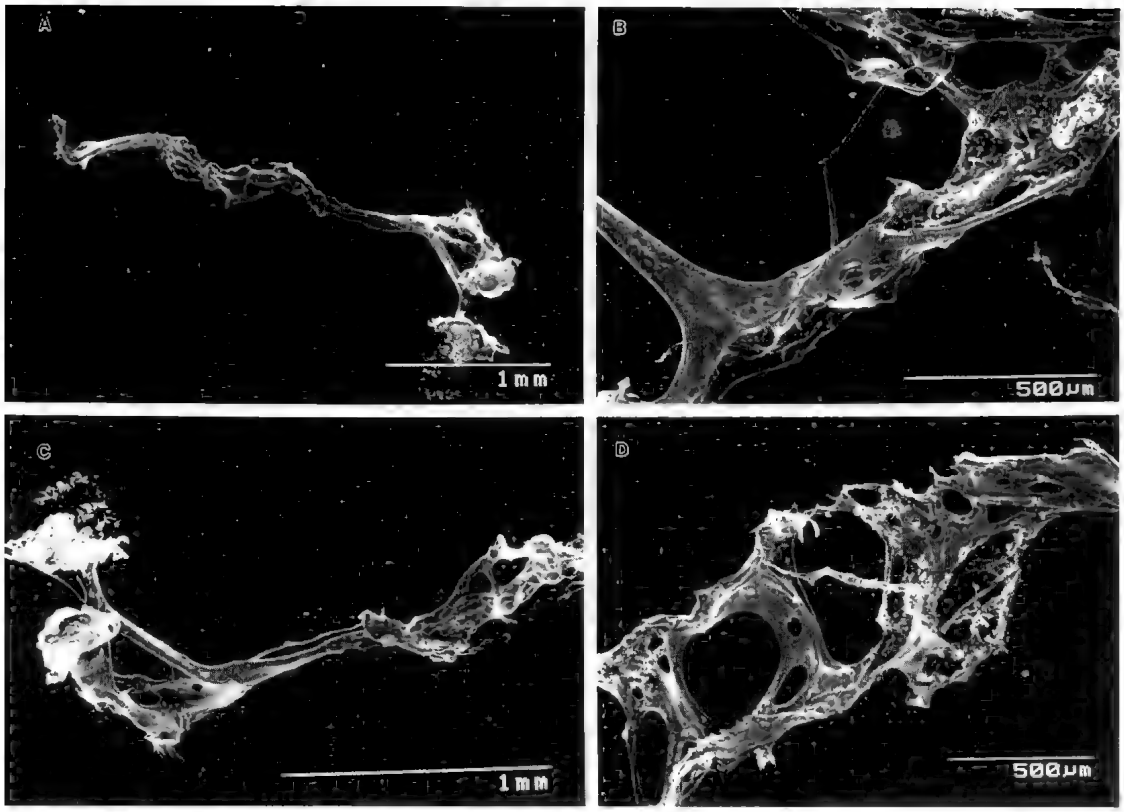

FIG. 2. *Aplysilla reticulata* sp. nov. A, Fasciculate fiber resulting from the fusion of two primary fibers, as shown by the presence of two basal plates. B, Detail of a primary fiber. C, Detail of the basal plates of a fasciculate primary fiber. D, Detail of secondary reticulation between two adjacent primary fibers. Fenestrated plates can also be seen.

because it lacks both the spongin spicules and the basal continuous plate of spongin that are characteristic of this genus (Bergquist, 1980). Moreover, *Igernella* species usually have massive habits and relatively large oscules, a much more regular and denser network of secondary fibers, and longer and less fasciculate primary fibers (e.g. Van Soest, 1978; Bergquist, 1980; Uriz & Maldonado, 1996).

Therefore, this new sponge combines the skeletal characteristics of the darwinellid genus *Pleraplysilla* and the dictyodendrillid genus *Igernella*, yet it cannot be assigned to either genera, unless the diagnosis of one of them is modified. On the one hand, if the acquisition of interconnecting secondary fibers is assumed to be a polyphyletic condition, the new sponge could be included in *Pleraplysilla* on the basis of its similarity in the structure of the primary fibers and the absence of spongin spicules. However, should this assumption be accepted, the dictyodendrillid genus *Dictyodendrilla* Bergquist (with highly dendritic fibers anastomosed to form a network) and the darwinellid genus *Dendrilla* Lendenfeld (with highly dendritic fibers that do not anastomose) would have to be fused into a single taxonomic unit, since taxonomic separation is based solely on the skeletal pattern. The genera *Darwinella* and *Igernella* would remain distinct genera, not on the basis of different skeletons (dendritic versus reticulate), but on the presence of distinctive spongin spicules, which appear not to be homologous (Garrone, 1978; Bergquist, 1996). Spongin spicules have a concentric laminated structure in *Darwinella*, whereas they have helicoidal structure and incorporate deposits of lepidocrocite in *Igernella* (Garrone, 1978; Bergquist, 1996). Conversely, if the presence/absence of both spongin spicules and a basal plate of spongin is assumed not to be taxonomically relevant at the generic level, the new species

could also be assigned to *Igernella* on the basis of its reticulate skeleton. Again, to be consistent in the application of the taxonomic criterion, the genera *Darwinella* and *Aplysilla*, which are distinguished on the presence-absence of spongin spicules, would have to be fused into a single taxonomic unit.

This dilemma raises the same question that has troubled taxonomists for the past century: which set of skeletal features should be emphasised in Dendroceratida? Obviously, we do not have conclusive evidence to offer a definitive solution. Nevertheless, an analysis of available evidence suggests that certain assumptions on which the current classification of Dendroceratida is based need to be reassessed. It is a fact, for example, that reticulate skeletons display a wide variety of models of organisation not only within orders, but also families (e.g. Van Soest, 1978; Bergquist, 1980, 1995, 1996; Bergquist et al., 1998). Therefore, the acquisition of a reticulate skeleton is likely to have evolved independently in Dictyoceratida, Verongida and Dendroceratida. In this context, the hypothesis that interconnecting secondary fibers also evolved independently within Dendroceratida cannot be discarded. The taxonomic interpretation given for the pattern of chemical affinities (Bergquist et al., 1990) conflicts with that given for the histological findings (Vacelet et al., 1989; Boury-Esnault et al., 1990). However, it is noteworthy that both patterns of affinities agree in suggesting a relationship between the dictyodendrillid genus *Igernella* and some darwinellid genera, such as *Aplysilla, Darwinella, Pleraplysilla* and *Chelonaplysilla* de Laubenfels. In contrast, they do not support any relationship between *Igernella* and *Dictyodendrilla* (Bergquist et al., 1990), although both genera are currently allocated in the same family. Furthermore, evidence from both non-skeletal differences and skeletal differences are consistent in suggesting that *Igernella* and *Dictyodendrilla* may have acquired their reticulate skeleton through independent evolutionary pathways. In fact, as previously suggested by Topsent (1905), and further stated by Bergquist et al. (1990), "there is no real similarity between the fine, regular skeletal reticulum of *Dictyodendrilla* and the coarse, sparse and irregular pattern of *Igernella*".

This reanalysis of the available evidence, although still only offering a partial view of the evolutionary relationships, appears to support the hypothesis that the acquisition of a secondary reticulate skeleton was probably a convergent process in Dendroceratida. This is of little surprise, since the anastomosis of skeletal elements (either spicules or ascending fibers), in the building of skeletal networks, is a condition that may have evolved independently many times in Porifera. Among other possibilities, the development of a skeletal network appears to be the result of selective pressure on thinly encrusting growth habits to increase body size. For example, several lines of encrusting axinellids and poecilosclerids share a similar, non-reticulate (typically hymedesmoid) skeletal architecture that has obviously evolved independently and that converges towards reticulate and plumo-reticulate patterns in massive and erect genera (e.g. Hooper, 1991, 1996). Similarly, Bergquist (1996) noted that most darwinellid sponges are encrusting, while the reticulate structure of the skeleton allows the Dictyodendrillidae to attain a large size. In this context, and by linking chemical, histological and skeletal data, it would be interesting to consider the possibility that some reticulate patterns, such as those of *Igernella*, may have evolved from skeletal models similar to those of *Pleraplysilla, Darwinella* and *Chelonaplysilla*. The genus *Dictyodendrilla*, however, appears to be chemically, cytologically, and skeletally unrelated to this set. Therefore, the possibility that the reticulate pattern of this genus, as well as that of the genus *Acanthodendrilla* Bergquist, may have evolved from a skeletal state similar to that of the genus *Dendrilla*, as fibers and morphology suggest, should be kept in mind. Indeed, some degree of fiber anastomosis has already been reported in species of both *Aplysilla* and *Dendrilla* by Van Soest (1987: *A. stocki*) and by Vacelet (1960: *D. cirsioides* Topsent), respectively.

In order to allocate our new species taxonomically, we have opted to expand the diagnosis of the genus *Pleraplysilla* to include species with an irregular network of secondary fibers, such as *Pleraplysilla reticulata* sp. nov. Nevertheless, this taxonomic allocation must be considered tentative, since the material studied was unsuitable for providing information on intraspecific skeletal variability, the diterpenoid chemistry, and choanocyte chamber structure. Such a taxonomic allocation comes into conflict with the current subdivision of Dendroceratida based on reticulate and dendritic skeletons. Although some preliminary ideas for a new classification have been put forward here, we

explicitly refuse to make any familial re-arrangement for several reasons: 1) the information available at present, though in conflict with the current taxonomic scheme, is still insufficient to support a robust alternative pattern of relationships at the family level; 2) re-arrangements at the suprageneric level must be based on re-examination of abundant type material, and fall beyond the scope of this study; and 3) the ideas proposed here can be discussed and reassessed in the context of the wide-ranging revision of the Dendroceratida that is being prepared, as announced by Bergquist et al (1998).

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CLIONA LAMPA AND DISTURBANCE ON THE CORAL REEFS OF CASTLE HARBOUR, BERMUDA. *Memoirs of the Queensland Museum 44: 360. 1999:-* On reef regions of Bermuda, one of the most abundant populations of the boring sponge *Cliona lampa* occurs within Castle Harbour. Historically, dredging and landfill for the construction of an airfield (1941-1944) in the harbour caused changes in the coral reef community structure. The resulting increase in sedimentation and turbidity led to mass mortality, changes in species composition and age distribution of the coral (especially for *Diploria* sp.; Dryer & Logan, 1978, Cook, et al., 1994). We hypothesised another outcome of this disturbance was an increase in the distribution and abundance of *Cliona lampa* within Castle Harbour. If the sponge was able to invade the space made available by death of the coral colonies, it should be reflected in the extent and type of substrate infested. An *a posteri* field survey of Castle Harbour was conducted to determine the extent and substrate type (coral vs. non-coral in origin) of *C. lampa* infestation. A field experiment was conducted to test the ability of *C. lampa* to colonise *Diploria* sp. with and without live tissue coverage. Results from the field survey and experiment support our hypothesis. Following the disturbance, it appears the increase in substrate availability combined with the decrease in competitors occurred at a time and place that favoured colonisation by the sponge. In addition to algae and corals, sponges may be important to consider when examining alternative states following disturbances in coral reef communities. ❐ *Porifera, Cliona, coral reef communities, disturbance, Bermuda, colonisation.*

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SPATIAL AND TEMPORAL VARIATION OF THE NATURAL TOXICITY IN SPONGES OF A MEDITERRANEAN CAVE: IS THERE A TREND ? *Memoirs of the Queensland Museum 44: 360. 1999:-* Strong intraspecific variation of chemical defenses has been documented for marine seaweeds and, less often, for some benthic invertebrates. The causes and the extent of this variation still remain poorly studied. We present here an extensive study on natural toxicity of sponges inhabiting a sublittoral cave at the Balearic Islands, (Mediterranean). We looked over spatial and temporal patterns at both community and species level

First, we performed an exhaustive semi-quantitative census of the benthic species present along the cave walls and analysed the species/ abundance matrix by cluster techniques. Three clearly different zones with distinct species assemblages came out from the analysis. We characterised the main abiotic factors of these zones by measuring irradiance, water movement, and particulate organic matter of the water. For the toxicity analysis, we collected at the three zones a minimum of three specimens per species (33 species, 291 specimens). We looked at seasonal variation in toxicity by sampling in June and November 1998. Toxicity was measured by the Microtox assay, which has shown a high performance for assessing natural toxicity in previous studies. We also ran sea-urchin bioassays over randomly selected samples to determine whether toxicity against marine bacteria correlates with toxicity against invertebrate cells. Correlation between both bioassays was always high. Whenever a given concentration of crude extract resulted in Gamma values (=toxicity units in the Microtox assay) above 0.5, it also featured some toxicity against sea-urchin embryos. Thus, we chose this value as a threshold to separate toxic from non-toxic sponges.

We found contrasting trends in the variation of toxicity along the cave with season. At the community level, toxicity values had a tendency to increase as irradiance and substrate occupation decreased (increasing distances from the cave entrance) in June. This trend reversed in November, when lower toxicity was found in the innermost zone. High variances prevented ANOVAs from detecting significant differences in mean toxicity between zones or seasons. In contrast, we detected significant changes (Chi-square statistic) in the number of toxic species among zones between seasons. At the species level, we found significant differences in toxicity among zones and the pattern of variation along the cave also changed seasonally.

Our results proved that spatial and temporal variability in toxicity is remarkably high in Mediterranean sponges. This variability, either genetically determined or environmentally induced, may have important ecological and evolutionary implications in benthic communities. ❐ *Porifera, natural toxicity, spatial and temporal variation, Microtox assay, caves, Mediterranean Sea, benthic communities.*

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AFRICAN FRESHWATER SPONGES: *MAKEDIA TANENSIS* GEN. ET SP. NOV. FROM LAKE TANA, ETHIOPIA

RENATA MANCONI, TIZIANA CUBEDDU & ROBERTO PRONZATO

Manconi, R., Cubeddu, T. & Pronzato, R. 1999 06 30: African freshwater sponges: *Makedia tanensis* gen. et sp. nov. from Lake Tana, Ethiopia. *Memoirs of the Queensland Museum* **44**: 361-367. Brisbane. ISSN 0079-8835.

The new genus *Makedia* is described and illustrated from shallow waters of Lake Tana, Ethiopia. Its morphological distinguish traits are characterised and discussed in comparison with those genera belonging to genera *incertae sedis* from ancient lakes of the world. ❐ *Porifera, Demospongiae, Makedia tanensis, new genus, new species, taxonomy, scanning electron microscopy, Ethiopian region, biodiversity, endemism, ancient lakes, geographic distribution.*

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Africa has a rich freshwater sponge fauna, so far containing about 60 species of Spongillidae (8 genera), Potamolepidae (2 genera), Metanidae (1 genus), and 4 genera with still undefined taxonomic status (Hilgendorf, 1883ab; Marshall, 1883a,b; Weltner, 1895, 1898, 1913; Evans, 1899; Kirkpatrick, 1906, 1907; Annandale, 1909, 1914; Jaffè, 1916; Schouteden, 1917; Stephens, 1919; Burton, 1929ab, 1934, 1938; Seurat, 1930; Topsent, 1932ab; Arndt, 1933,1936; Schroeder, 1934; Tuzet, 1953; Brien & Govaert-Mallebrancke, 1958; Lévi, 1965; Brien 1967, 1968abc, 1969abc, 1970ab, 1972, 1973, 1974, 1975; Boury-Esnault, 1980; Vacelet et al., 1991; Gugel, 1993).

The most extensive reviews of this fauna are the synopsis of Arndt (1936) and the worldwide revision of Penney & Racek (1968). The cosmopolitan family Spongillidae Gray, 1867, is widespread in African freshwater habitats ranging from: wadi in Sahara; large perennial rivers such as Nilo, Zambesi and Congo; to ancient lakes and man-made basins. Nevertheless, there are some genera and/or species of Potamolepidae Brien, 1967, and Metanidae Volkmer-Ribeiro, 1986, that are endemic to few hydrographic basins in Western and Central Africa. Finally, Brien (1972, 1973) suggested the erection of the new sub-family Globulospongillinae to define the status of the endemic genus *Malawispongia* Brien, 1972, known from the Mid-Pleistocenic Lake Malawi/Nyasa.

In this paper we describe a new genus *Makedia* n.g., with type species *Makedia tanensis* sp. nov., from the Pleistocenic Lake Tana in the NW marginal area of the African Rift Valley. Prior to the present study only Arndt (1936) had reported on sponges from Abyssinia, recording an unidentified freshwater species.

MATERIALS AND METHODS

In a preliminary survey of the freshwater sponge fauna of N Ethiopia and Eritrea, in 1988-1989 ten water courses and lakes were sampled. Of these sites sponges where found only in Lake Tana, although sampling was performed under severe constraints represented by the civil war and particularly by the endemic schistosomiasis in the lake.

Sponges were collected in shallow waters along the S coast of Lake Tana at Bahir Dar (11°36'N, 37°23'E), NW Ethiopia in May 1988 (Fig. 1). Specimens were preserved dry. All specimens, microscope slides and SEM stubs are presently registered in the senior author's collection at the Istituto di Zoologia dell'Università, Genova (IZUG), to be deposited in the Museo Civico di Storia Naturale G. Doria, Genova (MCSNG), Italy.

An entire specimen (FW250) and fragments of specimen FW280 were sputtered coated with gold and observed under scanning electron microscopy (SEM) to define skeletal characters. Spicules from FW250, 251, 279, 280 were dissociated by boiling sponge fragments in nitric acid, washing in water, and dehydrating in

TABLE 1. Morphological diagnostic traits of some freshwater sponges from ancient lakes of the world that do not produce gemmules. Data based on original descriptions and a preliminary study of holotypes by the authors.

Species	Lakes	Characteristics
Makedia tanensis gen. et sp. nov.	Lake Tana Ethiopia	1) undifferentiated ectosome; 2) alveolate isotropic choanosomal skeleton with paucispicular fibres; 3) sparse spongin; 4) oxeas ranging from slender to stout, straight to slightly curved, from smooth to variably ornamented; acerate tips; 151-285/5-22µm;
Balliviaspongia wirrmanni Boury-Esnault &Volkmer-Ribeiro, 1992	Lake Titicaca Perù	1) ectosome an irregular network of uni- or bi-spicular meshes tangential to the surface; 2) reticulate irregular choanosomal skeleton with multispicular primary tracts and paucispicular irregular secondary tracts; 3) sparse spongin; 4) oxeas ranging from slender to stout, from straight to slightly curved, from smooth to spined; acerate tips; 153-450/2.6-13µm
Cortispongilla barroisi Topsent, 1892	Lake Kinneret Israel	1) undifferentiated ectosome; 2) reticulate choanosomal skeleton with multispicular primary tracts and paucispicular secondary tracts more dense toward the surface; 3) sparse spongin; 4) oxeas ranging from slender to stout, from straight to slightly curved, from smooth to granulated; acerate tips; 180-370/30-33µm
Ochridaspongia rotunda Arndt, 1937	Lake Ohrid Macedonia	1) undifferentiated ectosome; 2) reticulate choanosomal skeleton with multispicular primary tracts, diverging in tufts toward the apical surfaces and paucispicular irregular secondary tracts; 3) sparse spongin; 4) oxeas ranging from slender to stout, from straight to slightly curved, from smooth to spined; acerate tips; 180-367/5-23µm
Malawispongia echinoides Brien, 1972	Lake Malawi Malawi	1) undifferentiated ectosome; 2) reticulate multispicular choanosomal skeleton with primary tracts diverging in tufts toward the apical surfaces and irregular secondary tracts; 3) abundant spongin; 4) oxeas ranging from slender to stout, from straight to slightly curved, from smooth to spined; acerate to blunt tips; 190-240/4-10µm
Ohridospongilla stankovici Gilbert & Hadzisce, 1984	Lake Ohrid Macedonia	1) undifferentiated ectosome; 2) alveolate isotropic skeleton with pauci-(multi-?)spicular fibres; 3) sparse spongin; 4) oxeas ranging from slender to stout, from straight to slightly curved, from smooth to spined; acerate tips; ?
Pachydictyum globosum Weltner, 1901	Lake Poso Sulawesi	1) undifferentiated ectosome; 2) reticulate multispicular skeleton with primary tracts, diverging in tufts toward the apical surfaces, and multispicular irregular secondary tracts; 3) sparse spongin with abundant foreign material; 4) stout oxeas, straight to slightly curved, varying from smooth to spined; tips range from acerate to blunt; 220-410/30-64µm
Spinospongilla polli Brien, 1974	Lake Tanganika Zaire	1) ectosome an irregular network of uni- or bi-spicular meshes tangential to the surface; 2) alveolate isotropic multispicular choanosonal skeleton with rare scattered smaller oxeas (128/2,3µm); 3) sparse spongin; 4) oxeas ranging from slender to stout, from straight to slightly curved, from smooth to spined and granulated; tips range from acerate to blunt; 160-208/11-12µm

alcohol. Suspended spicules from each specimen were dropped on slides for light microscopy; SEM analysis was performed on spicules sputter-coated in gold sputtering. Seventy spicules, photographed by SEM, were measured from specimens FW251 and FW280; mean and standard deviation of measurements were calculated (in µm).

SYSTEMATICS

Class **Demospongiae** Sollas
Order **Haplosclerida** Topsent
Family **incertae sedis**

Makedia new genus

TYPE SPECIES. *Makedia tanensis* sp. nov., monotypic.

ETYMOLOGY. Named for the Abyssinian Queen of Saba, Makeda.

DIAGNOSIS. Spongillid-like sponge with skeleton shaped as an alveolate isotropic paucispicular network with sparse spongin. No ectosomal differentiation present. Megascleres are oxeas ranging from completely smooth to granulated, tuberculated and/or strongly spined; spines acutely slanting with a globular base, named drop-like spines, with an evident axial canal. Microscleres and gemmules absent.

Makedia tanensis new species
(Figs 2-3)

MATERIAL. HOLOTYPE. IZUG-FW251: Bahir Dar, Lake Tana, NW Ethiopia, coll. R. Manconi, -.v.1988. PARATYPES. IZUG-FW250, IZUG-FW279, IZUG-FW280: same locality, coll. R. Pronzato, -.v.1988.

ETYMOLOGY. Named for the type locality, Lake Tana.

DESCRIPTION. FW250. Whitish encrusting sponge (0.5x0.2x0.1cm) on a pebble at a depth of 15cm. FW251. Whitish encrusting sponge (0.5x0.8x0.5cm) on the lateral side of a boulder at a depth of 5cm. FW 279.Two whitish thin contiguous crusts (0.5x0.7cm and 0.3x0.3cm), within the same concavity of a cobble, on the dried shoreline. FW280. Brown crust (1x2x 0.2cm) covered by unidentified epi- and

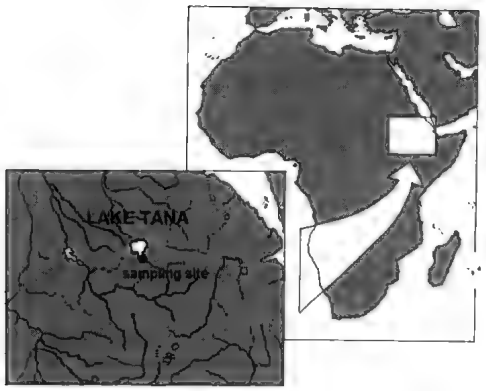

FIG. 1. Geographical position of the type locality of *Makedia tanensis* along the coast of Tana Lake in NW Ethiopia.

endobionts on the lateral surface of a cobble at 20cm depth. All specimens share the following traits. Encrusting body shape small in size, up to 0.5cm thick, 2cm diameter (Fig. 2A). Consistency was soft and fragile. Surface was hispid (Fig. 2B,C), oscules were not conspicuous (Fig. 2A). Ectosome was undifferentiated from choanosome macroscopically. Isotropic alveolate choanosomal skeleton with paucispicular fibres and scanty spongin (Fig. 2B,C). Megascleres range from slender to stout, straight or slightly curved oxeas, smooth to variably spined with apices from smooth to spiny acerate (Fig. 3A). Spicules have a wide morphological variety of irregularly scattered sculpturing, ranging from granules (Fig. 3C-H), granulated tubercules (Fig. 3C-E), drop-like granulated spines (Fig. 3C), to large acute spines (Fig. 3E). Two or more of these sculptures are associated on the same or in different oxeas. Atypical apices were also frequently observed on spicules (Fig. 3B). Lengths/widths of megascleres are as follows: FW251, 151-285/6-16μm (mean 210/11; standard deviation 26/2.5); FW280, 184-289/5-22μm (233/13; 27/3). Microscleres and gemmules are absent.

HABITAT. Dry and living sponges were found on the dried shoreline and in shallow standing waters up to 20cm depth, associated with gastropods, bivalves and triclads on the lower or lateral surfaces of littoral volcanic pebbles, cobbles and boulders. The Pleistocenic volcanic Lake Tana, the largest in Ethiopia with a surface of about 3150km^2 and a maximum depth of 14m (mean 8m), is tributary of the Nilo basin with its single outlet the Blue Nile (River Abbay); located in the N highlands of Ethiopia at an altitude of about 1800m the lake is characterised by a strong seasonality in rainfall and water level; the rainy and wet seasons occur in summer and winter, respectively, with recurrent long lasting drought periods; water level range is about 0.4-2.30m and reaches its maximum about 2 months after the peak of the rainy season (Bini, 1940a; Nagelkerke & Sibbing, 1996).Waters are characterised as oligotrophic; water temperature range is 15.6-20°C; silica is 9-16 mg/l (Bini, 1940a; Rzoska, 1976). The lake is isolated downstream by the Tis Issat Falls, and hosts a scarcely diversified fauna with the exception of fishes and nematods (Bini, 1940b; Brunelli, 1940; Brunelli & Cannicci, 1940; Nagelkerke et al., 1995; Abebe, 1996).

DISCUSSION

Cyclic disturbances produced by seasonal water level fluctuations in the littoral zone largely influences sponge populations, notably inducing stressed conditions and small body size of specimens. In spite of the suboptimal habitat, where unfavourable conditions are linked to the alternation of the wet and dry seasons, gemmules were absent in all specimens of *M. tanensis* on the dried shoreline, or in very shallow waters of Lake Tana. The absence of gemmules in May, at the end of the dry season, strongly supports the hypothesis that this species is not able to produce resistant bodies. Several data, however, suggest that the production of gemmules is not necessary obligatory in the life history of all freshwater sponges, as shown in Lubomirskiidae from Lake Baikal (Rezvoi, 1936), which have lost their ability to reproduce asexually by means of gemmules (Efremova, 1994), and in other genera from ancient lakes of the world.

The recognition of a new species, *Makedia tanensis*, in a new monotypic genus, is supported by the possession of peculiar ornamentations on oxeas. This isolated monotypic taxon fits the trend shown by several authors, such as Topsent (1892), Weltner (1901), Arndt (1937), Brien (1974), Gilbert & Hadzisce (1984), Boury-Esnault & Volkmer-Ribeiro (1992), indicating that most freshwater sponges in isolated, ancient lakes belong to monotypic genera - with the sole exception of *Ochridaspongia* Arndt *(O. rotunda* Arndt, 1937, and *O. interlithonis* Gilbert &

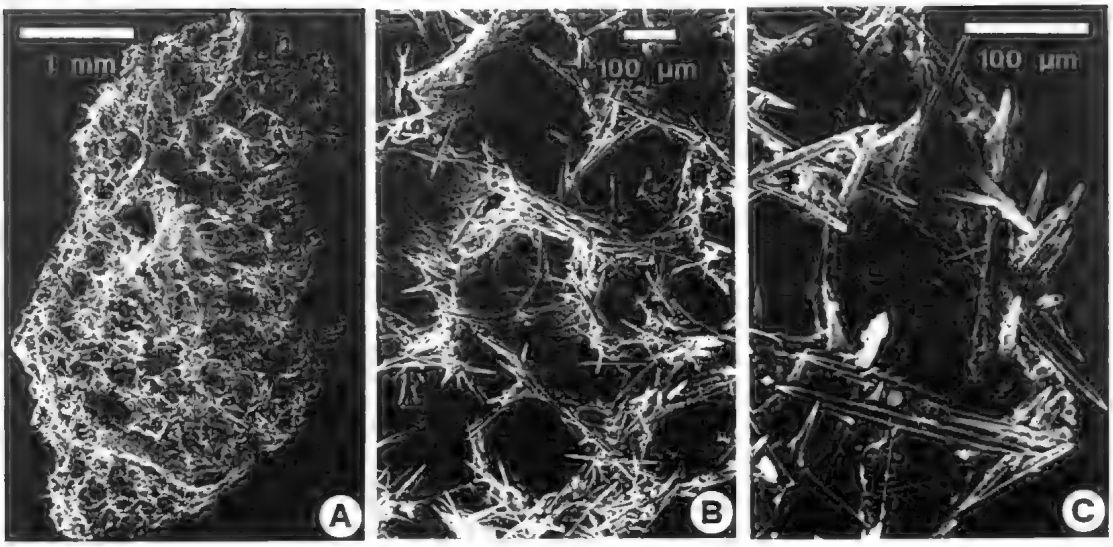

FIG. 2. *Makedia tanensis* sp. nov. (paratype IZUG-FW250). A, Entire specimen. B, Choanosomal alveolate skeleton. C, Surface hispidation.

Hadzisce, 1984), presently assigned to Lubomirskiidae. These genera have some morphological affinities, but their life histories are poorly known, they are highly disjunct in their distributions. The question, therefore, remains as to their higher taxonomic affinities.

The new genus *Makedia* is characterised by a spongillid-like skeleton, with a peculiar range of ornamentations on spicules, and the absence of microscleres and gemmules. A comparative analysis, in the framework of a general revision of freshwater sponge taxa, of the type material and original descriptions of genera from ancient lakes, show that these taxa share the following traits (Table 1) with the genus *Makedia*: 1) they are monotypic; 2) known only from the type locality; 3) inhabit tectonic or volcanic lakes with high levels of endemism; 4) the skeleton is a network of ornamented oxeas with multi- or pauci-spicular choanosomal tracts or fibres; 5) ectosomal skeleton, if present, uni- or bi-spicular; 6) they do not produce microscleres; 7) they do not produce gemmules. However diagnostic skeletal traits highlight a morphological divergence within this group of sponges from ancient lakes. *Makedia*, *Spinospongilla* and *Ohridospongilla* share the spongillid-like alveolate skeletal trait; on the other hand *Cortispongilla, Ochridaspongia, Pachydictyum, Malawispongia* and *Balliviaspongia* (Table 1) share the reticulate choanosomal skeleton. All these genera are amalgamated provisionally into one *incertae sedis* group because of their disjunct distribution and the high possibility of convergence as occur in other taxa of ancient lakes.

Some other genera and species *incertae sedis*, such as *Metschnikowia* and *Nudospongilla* (in part), could also be included in this group in the future but this requires more detailed examination of their type material as to their true morphological characters (Manconi & Pronzato, in preparation).

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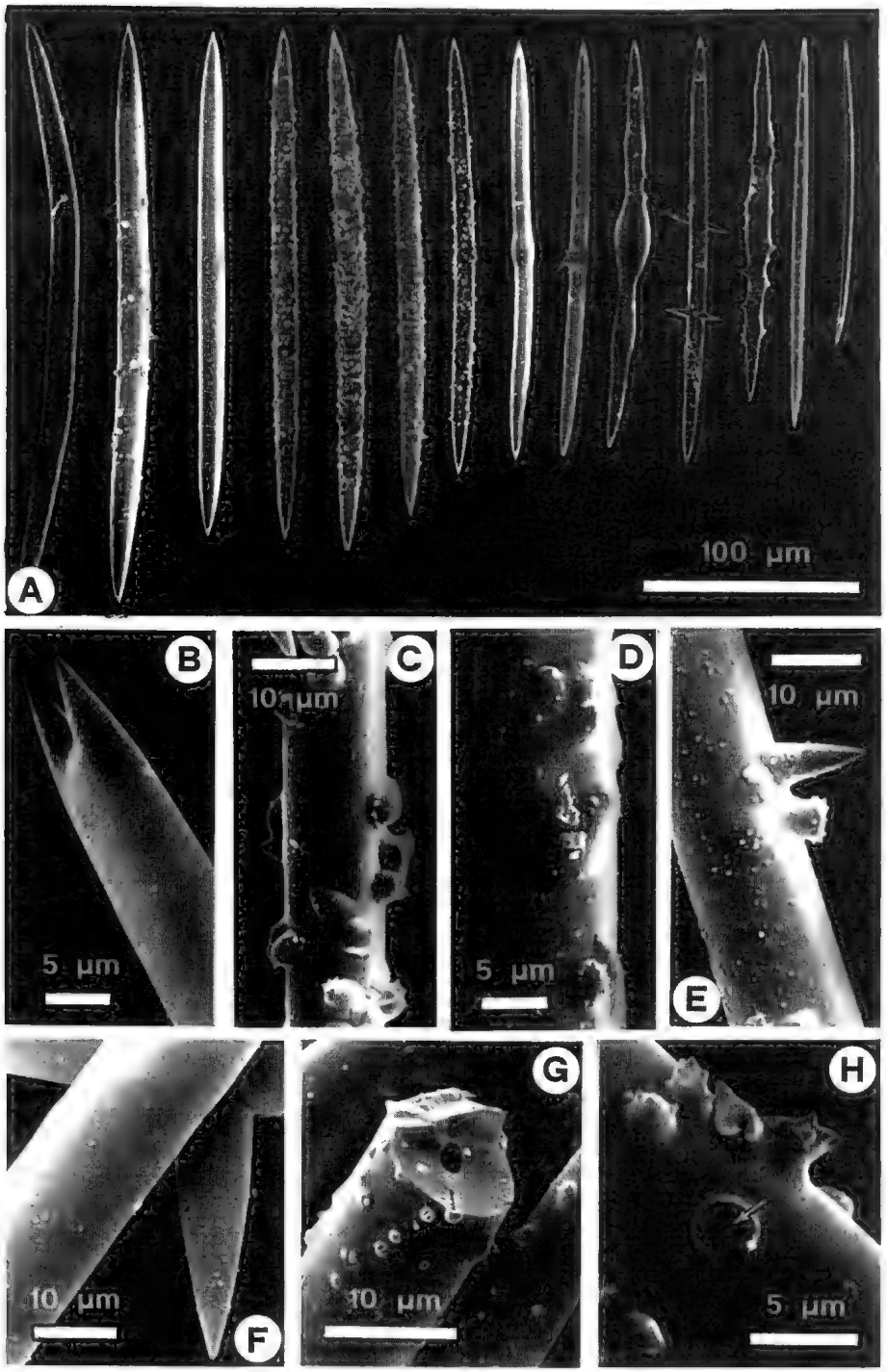

FIG. 3. *Makedia tanensis* sp. nov. (holotype IZUG-FW251). A, Dimensional and morphological variety of megascleres ranging from smooth to granulated, tuberculated and spined oxeas. B, Atypical apex of an oxea. C-D, Surface of oxeas with granules, tubercules and drop-like spines. E, Isolated large spine and tubercule on a finely granulated oxea. F, Surface of a finely granulated oxea. G-H, section of a granulated oxea with an axial canal extending toward a granule (arrow).

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STUDY ON THE DISTRIBUTION OF BAIKALIAN SPONGES. *Memoirs of the Queensland Museum 44: 368. 1999*:- Freshwater sponges are classified into three families. Spongillidae, Potamolepidae and Lubomirskiidae. Spongillidae are cosmopolitan sponges and widely distributed throughout the world, Potamolepidae are found in lakes of Africa and South America, and Lubomirskiidae inhabit only Lake Baikal. One of the characteristics of Spongillidae is gemmule formation. Gemmules are asexual bodies with a structure in highly resistant resting stages. Most Japanese sponge species form gemmules, but a certain species which forms in the shallow zone of lakes, does not form gemmules in deeper zones. Therefore we have a great interest in the Lubomirskiidae of Lake Baikal which does not produce any gemmules. At present, the taxonomy of some Lubomirskiidae is in a chaotic state. Furthermore, the recent distribution of Baikalian sponges has not been recorded. We decided to collect as many Baikalian sponges as possible and to review their taxonomy and their distribution in Lake Baikal. About 700 specimens were collected, mainly from the entire littoral zone of Lake Baikal, but some specimens were collected from the Academishan ridge by a dredge survey, and others collected on diving surveys. Most of the specimens belonged to the family Lubomirskiidae, with a few belonging to the family Spongillidae. Lubomirskiidae were classified into three genera and eight species according to Rezvoy. Based on the results of our study, our Lubomirskiidae specimens were tentatively classified into 4 genera and 11 species. At the present, Lubomirskiidae are classified mainly by their spicules and skeletons, not by the form of the sponges (changeable due to substrate and water current), oscula or colour (thought to not contain pigment cells). The colour of green sponges is owing to symbionts, zoochlorellae.

The Lubomirskiidae species were distributed throughout the entire littoral zone of Lake Baikal, except where the substratum was sand, mud or pebbles. On the other hand, Spongillidae species (*Spongilla lacustris*, *Ephydatia muelleri* and *Eunapius* sp.) were collected from only four stations. Lake Baikal may be an in appropriate habitat for Spongillidae species. Spongillidae lack of presence throughout the entire littoral zone may be due to; the amount of nutrients, wave action and water temperature. Regarding nutrients, certainly Lake Baikal is characterised by oligotrophy when compared with other lakes where many Spongillidae species live, but the limits within which the Spongillidae species can not live is unknown. Lubomirskiidae may be accustomed to poor food. Due to the weak and fragile bodies of Spongillidae, they cannot live in an area of strong wave action. But the wave action in deeper zones is weaker than that in shallow zone. In Lake Biwa, in Japan, Spongillidae species can live at a depth of 30m, where the wave action is weak. In Lake Baikal, wave action is also weak at such a deeper zone. But we could not find Spongillidae even at a depth of 30m. We compared the maximum temperature in Lake Baikal and Lake Biwa during the year at a depth of 30m. In Baikal, the maximum temperature is about 6°C. On the other hand, in Lake Biwa, it is about 10°C. The maximum temperature may be an important factor for the survival of Spongillidae species. More detailed information on differences in the two family habitats is necessary to resolve this problem, which is important in the analysis of spicules in old sediment.

The spicules of freshwater sponges are very stable in old sediment because they consist of silica components, such as diatoms, and we are now studying the spicules of old sediment obtained from drilling cores. Some spicules from sediments, believed to have been deposited there 4,500,000 years ago, were found about 180m under Lake Baikal. If Spongillidae spicules are found, we might hypothesise the lake's conditions as being similar to the Little Sea near Olkhon Island at present. If we examine spicules along the drilling core from surface to bottom, we might find successive changes in the circumstances. Furthermore, if we should find new spicules not seen in recent sponges, the new finding would help us in drawing up the phylogenetic tree of freshwater sponges. ❐

Porifera, freshwater sponges, Lake Biwa, Lubomirskiidae, Spongillidae, taxonomy, distribution, environmental conditions, sediment studies.

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REVISION OF BRAZILIAN *ERYLUS* (PORIFERA: ASTROPHORIDA: DEMOSPONGIAE) WITH DESCRIPTION OF A NEW SPECIES

BEATRIZ MOTHES, CLÉA B. LERNER AND CARLA M. M. DA SILVA

Mothes, B., Lerner, C.B. & Silva, C.M.M. da 1999 06 30: Revision of Brazilian *Erylus* (Porifera: Astrophorida: Demospongiae) with description of a new species. *Memoirs of the Queensland Museum* **44**: 369-380. Brisbane. ISSN 0079-8835.

Prior to the present study only four species of *Erylus* were described for the Brazilian coast: *E. formosus* Sollas, 1886, *E. corneus* Boury-Esnault, 1973, *E. topsenti* Lendenfeld, 1903 and *E. oxyaster* Lendenfeld, 1910. Re-examination of these species, and additional material using scanning electron microscopy, detected new characters necessitating a revision of the genus in Brazilian waters. Collections were made by SCUBA or narghile (0-30m) or dredging (13-918m depth). Re-examination of material detected the presence of *E. alleni*, a Caribbean species with southern limit at the coast of Rio Grande do Sul state (31°20'S, 48° 40'W) and three new species, one described here, E. *diminutus* sp. nov., a sister-species of *E. oxyaster* (Galapagos), and two others still undescribed, one of which was previously misidentified as *E. topsenti* by Mothes-de-Moraes (1978) from the Brazilian coast. ❐ *Porifera, Demospongiae, Astrophorida, Geodiidae, Erylus, revision, new species, taxonomy, Brazilian coast.*

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Erylus Gray is a genus with Tethyan distribution restricted to tropical and subtropical areas (Van Soest, 1994). Gray (1867) originally described this genus: as "Sponge expanded, mammillated, ending in an oscule. Spicules of three kinds: 1.stellate; 2.ternate, rays forked; 3.subcylindrical, waved. With oblong ovisacs, formed of claviform spines". Subsequent authors enlarged the definition. Ridley (1884): "Comprising Choristid Tetractinellid with the surface covered by a layer of detached discoid trichite globate, and having besides a zone – zone spicule and small stellates with slender few rays. Form lobate. Vents single or multiple"; Sollas (1888): "The sterraster is seldom spherical; the somal microsclere is a centrotylote microrhabd. The incurrent chones are uniporal; and the oscule is the patent opening of a cloaca"; Topsent (1894): 'Sterrasters rarely spherical. Somal microsclere is a microxea usually centrotylote. Poral cone typical uniporal; larger oscule'; Lendenfeld (1903, 1907): "Geodiidae with tetractines megascleres (triaene and derivates) radially arranged; disc-shaped sterrasters at the surface covered by microrhabds"; Lendenfeld (1910): "With uniporal afferents and uniporal efferents or larger oscules. Without ana- or protriaenes"; Dendy (1916) defined genus as family Erylidae, with diagnosis: "Astrotetraxonida with a cortex containing aspidasters. The typical megascleres are triaenes and oxea (or strongyla). The microscleres include microrhabs and choanosomal euasters"; Wilson (1925): "The afferent orifices are uniporal apertures into chone canals efferent orifices also the uniporal openings of chone canals, or in other cases larger oscula. The megasclere-complex includes orthotriaenes and rhabds; anatriaenes and protriaenes absent. The sterraster is more or less flattened, often so flattened as to be a thin plate. Microrhabds (here spicules of good size, reaching a length of 70μ), typically centrotylote, form a dermal layer. Euasters also occur, but not at the surface"; de Laubenfels (1936): "*Erylus* Gray is a very different sort of sponge entirely, with the sterrasters derived in a different way from peculiar disc-shaped beginnings. Even when fully developed they are much more disc-shaped than are those in *Geodia*"; Van Soest & Stentoft (1988): "Geodiidae with flattened or disc-shaped sterrasters and ectosomal microrhabds"; Desqueyroux-Faundez & Van Soest, (1997): "Geodiidae with uniporal afferent and efferent surfaces or larger oscules. Triaenes short-shafted ortho- or plagiotriaenes; no ana- or protriaenes. Sterrasters usually flattened into aspidasters".

The foregoing shows the gradual evolution of a definition for *Erylus*, and the different interpretations made by various authors on importance of certain characters over others.

The present study revises the species of *Erylus* from the Brazilian coast (Fig.1), based on re-examination of existing and new material, using scanning electron microscopy (SEM). Prior to this study only four species were recorded for the region: *E. formosus* Sollas, 1886, *E. corneus* Boury-Esnault, 1973, *E. topsenti* Lendenfeld, 1903 and *E. oxyaster* Lendenfeld, 1910.

MATERIALS AND METHODS

Two specimens were collected by SCUBA or Narghilé (0-30m). Most material examined was dredged from 13-918m depth, carried out under the auspices of Diretoria de Hidrografia e Navegação da Marinha (DHNM); Departamento de Recursos Pesqueiros da Superintendência de Desenvolvimento do Nordeste (SUDENE); Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS); Projeto Recursos Vivos da Zona Econômica Exclusiva (REVIZEE) score Norte II supported by Universidade Federal do Maranhão (UFMA), and score Nordeste supported by Universidade Federal de Pernambuco (UFPE); Superintendência do Desenvolvimento da Pesca (SUDEPE), Ministério da Agricultura, Brasília; "Programa Rio Grande do Sul-I" (PRGS-I), supported by Universidade de São Paulo and Governo do Estado do Rio Grande do Sul; Projeto Talude, Fundação Universidade do Rio Grande (FURG), Brazil; Britannic Expedition H.M.S. 'Challenger'; and French Expedition 'Calypso'.

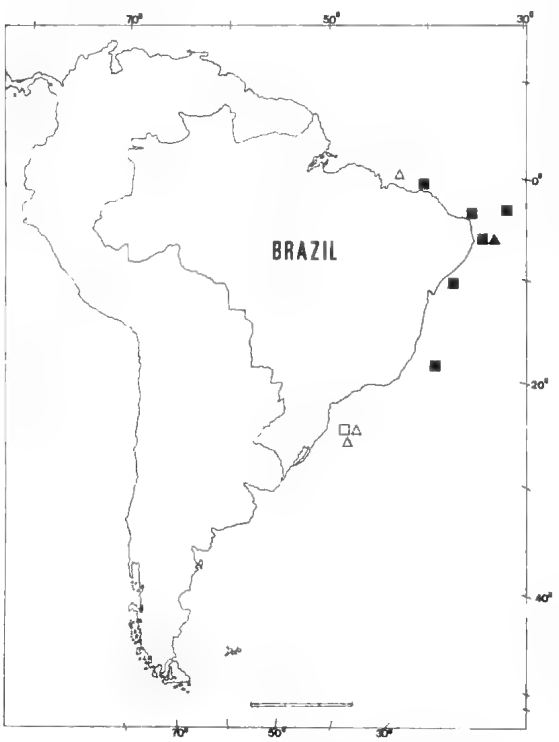

FIG.1. Map showing known distribution of *Erylus* species along the Brazilian coastline: ❐ *E. diminutus sp.n*, Δ *E. alleni*, ■ *E. formosus*, ▲ *E. corneus*. Scale bar 1200km.

Dissociated spicule mounts, thick sections and preparations for SEM study were made according methods described by Mothes (1996). Spicule measurements are given as minimum-mean-maximum, N=20 (except for the new species with N=50), and mean measurements are not supplied when N was smaller than 20. Spicule measurements are given in μm.

Abbreviations cited in the text: BMNH, The Natural History Museum, London; FZB, Fundação Zoobotânica do Rio Grande do Sul, Brazil; MCN, Museu de Ciências Naturais of FZB, Brazil; MCNPOR, MCN Porifera collection; MHNG, Muséum d'Histoire Naturelle, Genéve; MNHN, Muséum National d'Histoire Naturelle, Laboratoire de Biologie des Invertébrés Marins et Malacologie, Paris (DNBE, Boury-Esnault collections); USNM, National Museum of Natural History, Smithsonian Institution, Washington DC; ZMA, Zoölogisch Museum, Universiteit van Amsterdam, Amsterdam; ZMB, Museum für Naturkunde an der Humboldt-Universität zu Berlin, Berlin.

SYSTEMATICS

Class **Demospongiae** Sollas, 1885
Order **Astrophorida** Lévi, 1973

DEFINITION. Sponges with astrose microscleres sometimes accompanied by microxeas or rod-shaped spicules. The megascleres are tetractines, frequently triaenes, often occurring together with oxeas. The skeletal framework is radially arranged at least peripherally, but spicules may occur in confusion in the interior. Either tetractinal megascleres or microscleres or both may be lost to give genera having oxeas and astrose microscleres or only oxeas for spicules. A radial skeletal architecture and generally coarse texture permit recognition of these forms as astrophorids (Hartman, 1982).

Family **Geodiidae** Gray, 1867

DEFINITION. Sponges with either long (or short-shafted) triaenes and oxeas or strongyles as megascleres. Microscleres always include sterrasters (these are modified aspidasters in *Erylus*)] which form closely packed cortical armour at the surface. Other microscleres that may be present are euasters, microrhabs, and spherules. The shape varies from thickly encrusting to massive to shallow-bowl-shaped (Hartman, 1982).

Genus **Erylus** Gray, 1867

Erylus Gray, 1867; Wiedenmayer, 1977; Van Soest & Stentoft, 1988; Desqueyroux-Faundez & Van Soest, 1997. Type species: *Stelletta mammillaris* O. Schmidt, 1862 by monotypy. Fragments of type material examined: BMNH 1867.3.11.32, Adriatic; BMNH 1868.3.2.42, Algiers.

DIAGNOSIS. Geodiidae with ectosomal microrhabds and aspidasters or sterrasters with the following forms: elliptical to disc-shaped, flattened to globose, irregular (with lobes) or regular outline and microspined to smooth surface. Incurrent channels are uniporal; oscules are large.

KEY TO BRAZILIAN *ERYLUS*

1. Orthotriaenes present 2
   Dichotriaenes present with short rhabd (cladome 285.0-418.0/ 38.0-57.0μm; rhabd 256.5-304.0/ 38.0-57.0μm) 4
2. Digitiform aspidasters present (95.0-305/ 11.5-52.2μm) and smooth centrotylote microstrongyles . *E. formosus*
   Elliptical aspidasters and smooth centrotylote microxeas present 3
3. One category of oxyaster present (9.2-23μm) . *E. corneus*
   Two categories of oxyasters present (oxyaster I 23.0-57.5/ oxyaster II 8.1-27.6μm) . . . . . *E. alleni*
4. Strongyles present varying to strongyloxeas (460-920/ 9.5-23.8μm); aspidasters with slightly irregular outline (159-228.8/ 105.8-151.8μm) . . . *E. diminutus* sp.nov.

Erylus diminutus sp. nov.
(Figs 2A-B, 3A-H)

MATERIAL. HOLOTYPE: MCNPOR 347: Rio Grande do Sul, Brazil, 30°50'S, 49°13'W, 183m depth, x.1968, coll. N/Oc. Prof. W. Besnard. SCHIZOHOLOTYPE: ZMA (microscope slides).

ETYMOLOGY. Named for the presence of dichotriaenes and microrhabds smaller than those described in *E. oxyaster*.

DESCRIPTION. *Shape*. Irregular to sublobate fragment, massive sponge with 3.4cm length, 2.3 cm width and 1.9 cm height.

Colour. Gray-white in alcohol.

Oscules. Small, not conspicuous.

Texture and surface characteristics. Fragile consistency with a slight hardening only in the cortex. Smooth surface. Small openings uniformly distributed.

Ectosome. Centrotylote microstrongyles are slightly tangential to the surface and become obliquely oriented internally in the interstices between the aspidasters. Aspidasters have a compact and irregular regional distribution in the inner cortex.

Choanosome. Dichotriaenes with cladome oriented tangentially to cortex. Strongyles, in bundles of 2-12, bundles 76-190μm wide, scattered among the dichotriaenes. Oxyasters, centrotylote microstrongyles and sterrasters in several stages of development are randomly distributed throughout the choanosome.

Megascleres. Strongyles, sometimes varying to strongyloxeas, thick, straight to slightly curved, sometimes mucronate at one side or with unilateral expansion near their extremity, axial canal visible (460.0-732.6-920.0/ 9.5-18.0-23.8μm). Dichotriaenes are strong with short, straight and gradually pointed rhabd; deuteroclad with variable extremities: from acerate to blunt, curved or sometimes bifurcate; Cladome 684.0-855.0μm, rhabd 256.5-304.0/38.0-57.0μm, clads 285.0-418.0μm, deuteroclad 213.8-289.8μm, protoclad 118.8-171.0μm.

Microscleres. Centrotylote microstrongyles smooth, straight or slightly curved, extremity blunt or rarely mucronate, rare microxeas. Central swelling very distinct (39.1-48.0-59.8/ 3.5-5.3-6.9μm). Elliptical aspidasters, rarely disc-shaped, generally with distinct hilum. In the young stage spicules are radially striated discs. Their outline presents discrete lobose marginal protuberances. Adult spicules present serrated margins because microspine density increases towards the edges. Sometimes spicules have only few spines. The outline of aspidasters is irregular with slight digitiform or lobulate expansions. Surface with stellate microspination, divided by 2 striae producing 4 lateral bifurcate projections, totalling 8 conical microspines (159.0-203.9-228.8/ 105.8-128.7-151.8/ 14.0μm). Oxyasters with gradually pointed rays and conical microspines in the middle; centre with 6-8 rays 11.5-15.6 –23.0μm, diameter of centre 2.3-2.9-4.6μm.

Ecology. Associated with polychaete tubes, bryozoan colonies and colonial foraminiferans.

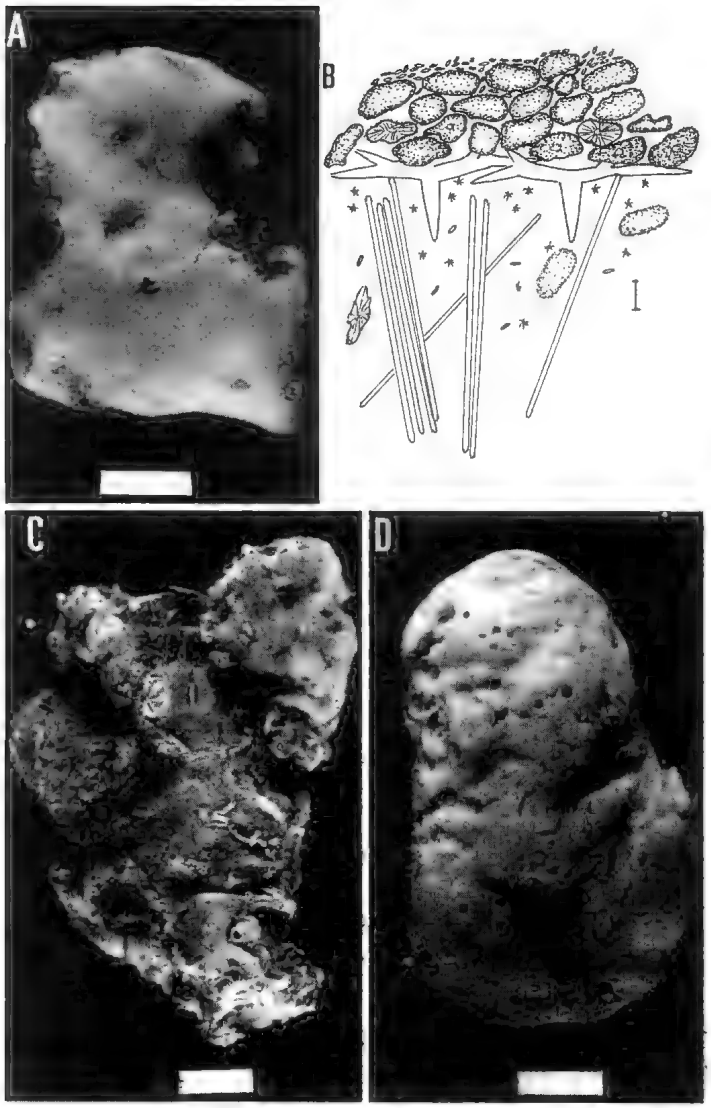

FIG. 2. Photographs of preserved material. A-B, *Erylus diminutus* sp. nov.: A, Holotype MCNPOR 347. Scale bar 5mm. B, Schematic representation of the skeleton arquitecture. Scale bar 0.1mm. C, *Erylus alleni*: MCNPOR 193. Scale bar 5mm. D, *Erylus formosus:* MCNPOR 2439. Scale bar 10mm.

REMARKS. The new species was identified by Mothes-de-Moraes (1978) as *Erylus oxyaster* Lendenfeld, 1910. This material was re-examined using SEM, and a comparative material was also studied: *Erylus oxyaster* Lendenfeld described by Weltner, 1927 (ZMB 6636), and *Erylus* cf. *oxyaster* sensu Desqueyroux-Faúndez & Van Soest, 1997 (MHNG Ga III 8 from Coast James Is of the Galapagos, 00°37'S-90°51'W, 78m depth). These studies revealed that our material was closely allied to, but clearly different from the Galapagos species, and new to science. *Erylus oxyaster* differs from the present species in the possession of much larger dichotriaenes and larger categories of oxyasters and microrhabs. It is, nevertheless, a sister species of *E. oxyaster*.

Erylus alleni de Laubenfels, 1934
(Figs 2C, 4A-G, Table 1)

Erylus alleni de Laubenfels, 1934: 7.

MATERIAL. HOLOTYPE: USNM 22268: Porto Rico, West Indies, 18°29'40"N, 66°08'30"W - 18°31'N, 66°10'15"W, 69.5-173.7m depth, coll. First Johnson-Smithsonian Deep-Sea Expedition. SCHIZOHOLOTYPE: MCNPOR 3449: (slides). OTHER MATERIAL. MCNPOR 1824: Maranhão, Brazil, 00°22'00"S, 44°12'00"W, 43m depth, iii.1973, coll. Barco Pesqueiro IV (SUDENE). MCNPOR 193: Rio Grande do Sul, 30°25'S, 48°48'W, 165m depth, 25.xi.1971, coll. N.P. Mestre Jerônimo (SUDEPE). MCNPOR 2202: Rio Grande do Sul, 31°20'S, 48°40'W, 150m depth, coll. N. Oc. Atlântico Sul (FURG).

DESCRIPTION. Adequate description is provided by de Laubenfels (1934), and expanded here.

Megascleres (refer to Table 1 for dimensions). Oxeas with hastate to acerate ends, few blunt, usually slightly curved, sometimes straight. Orthotriaenes: rhabd and clads with blunt ends.

Microscleres (refer to Table 1 for dimensions). Centrotylote microxeas, smooth, usually slightly curved with pointed ends, seldom with blunt ends. Aspidasters disc-shaped or elliptical, nearly regular outline; surface microspines stellate-shaped with conical points; developmental forms are visible. Oxyasters I with 6-7 slightly microspined rays, bigger spines are located close to the distal ends. Oxyasters II with 12-16 microspined rays, spines more concentrated at the distal extremities.

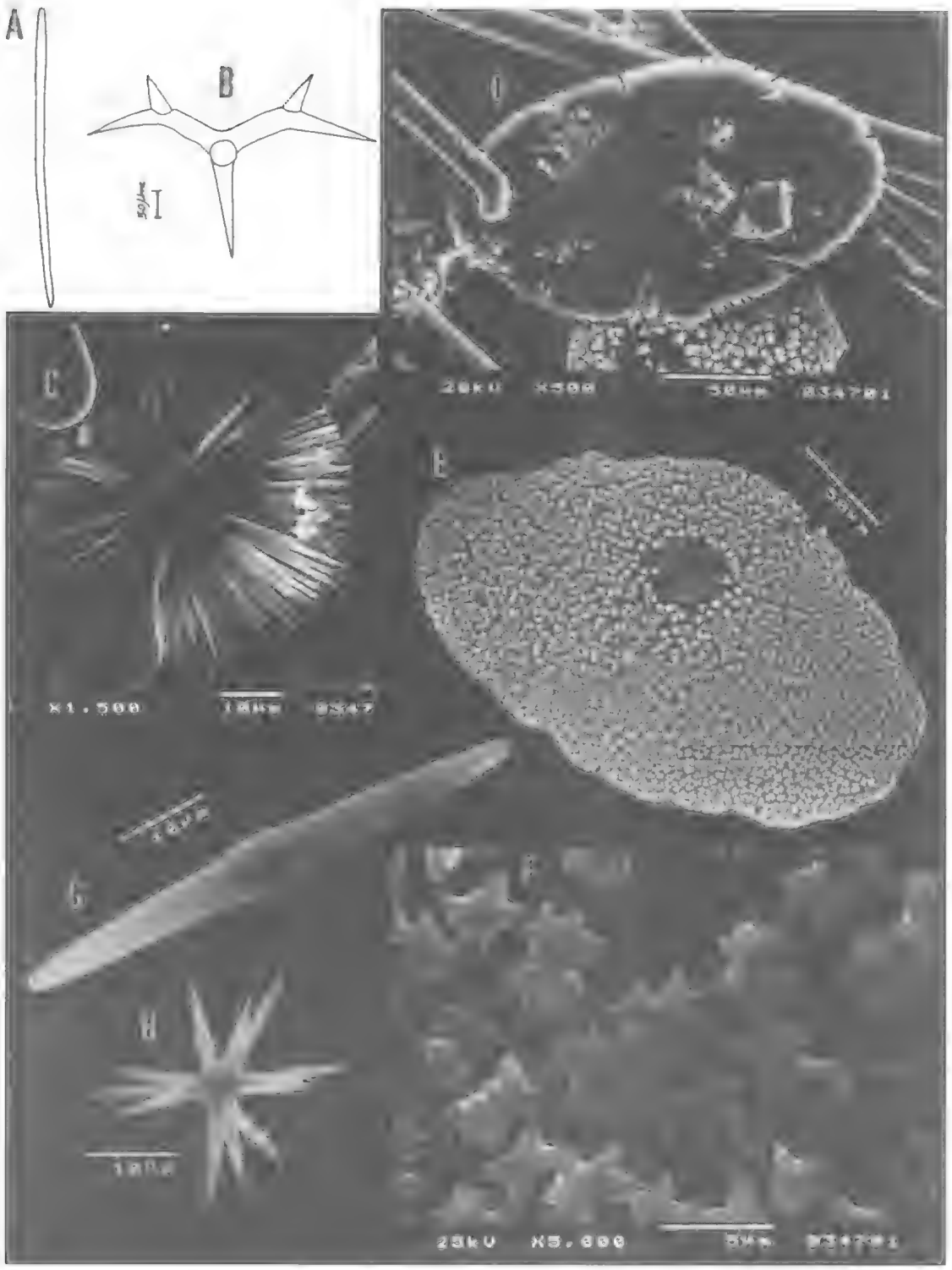

FIG. 3. *Erylus diminutus* sp. nov. (Holotype MCNPOR 347). A, strongyle. B, dichotriaene. C-D, aspidaster developmental stages. E, adult aspidaster. F, aspidaster surface. G, microstrongyle. H, oxyaster.

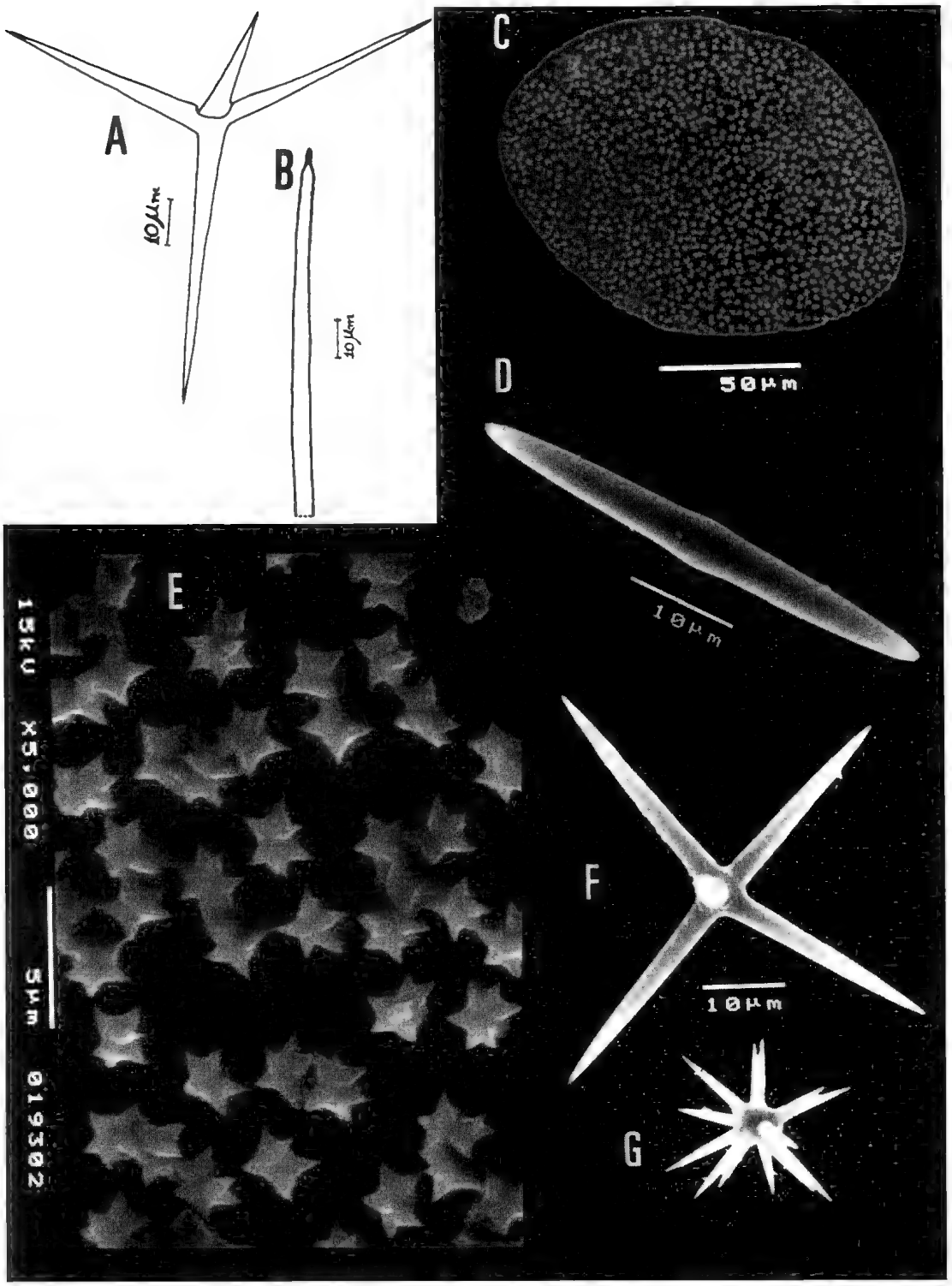

FIG. 4. *Erylus alleni* de Laubenfels (MCNPOR 1824). A, orthotriaene. B, oxea extremity. C, aspidaster. D, microxea. E, aspidaster surface. F, oxyaster I. G, oxyaster II.

TABLE 1. Comparative data on the spicule measurements of *Erylus alleni* de Laubenfels, 1934, holotype and additional material. Orthotriaene measurements refer to shaft length/width, cladome length/ width. Measurements given in µm. Key to material of *E. alleni*: 1, Holotype USNM 22268 (data from the author); 2, Schizoholotype MCNPOR 3449 [slides]; 3, MCNPOR 1824; 4, MCNPOR 193; 5, MCNPOR 2202.

Material	Orthotriaenes	Oxeas	Aspidasters	Microxeas	Oxyasters I	Oxyasters II
1	250-300/ 13 200-300/ 13	660/12	35/70/5	37/1	30	7
2	171-465.5/ 6.9-20.7 256.5-484.5/ 9.2-18.4	465.5-608.4-684/ 4.6-9.1-13.8	69.0-108.2-126.5/ 50.6-70.8-80.5	29.9-42.2-48.3/ 2.3-2.6-3.5	25.3-38.0-48.3	8.1-11.3-16.1
3	211.6-240.0/4.6 119.6-195.0/4.6	617.5-670.9-788.5 / 6.9-11.9-16.1	92.0-105.8-133.4/ 52.9- 87.4-96.6	41.4-50.6-71.3/ 1.1-4.1-4.6	23.0-32.2-39.1	9.2-11.5-16.1
4	323.0-464.3-589.0/ 18.4-33.7-50.6 266.0-418.9-570.0/ 16.1-29.9-48.3	437.0-564.6-807.5 / 4.6- 9.9-20.7	112.7-134.5-144.9/ 66.7- 92.2 -105.8	39.1-45.6-59.8/ 4.6	46.9-34.5-59.8	11.5-18.3-27.6
5	437.0-577.6-665.0/ 27.6-37.8-46.0 408.5-505.4-617.5/ 27.6-33.1-41.4	598.5-775.2-950.0 / 9.2-15.9-20.7	85.5-117.3-142.5/ 57.0-90.3-114.0	34.5-43.2-52.9/ 4.6-5.8-6.9	25.3-39.6-57.5	9.2-13.2-16.1

REMARKS. The specimens examined above from Brazil appear to be conspecific with *E.alleni* de Laubenfels, 1934. Van Soest & Stentoft (1988) suggested this species was a synonym of *E.transiens* (Weltner, 1882), whereas we suggest that *E. alleni* differs from *E. transiens* in having two distinct size categories of oxyasters, the usual small ones and a larger one with fewer rays. *Erylus alleni* is closely related to *E. transiens.*

DISTRIBUTION. Caribbean: Porto Rico (de Laubenfels, 1934); Brazil: Maranhão and Rio Grande do Sul (present study).

Erylus formosus Sollas, 1886
(Figs 2D, 5A-I, Table 2)

Erylus formosus Sollas, 1886: 195; 1888: 209, pl.28; Wiedenmayer, 1977: 181 (full synonymy); Boury-Esnault, 1973: 267, fig. 3, pls I-II; Solé-Cava, Kelecom & Kannengiesser, 1981: 125, fig. 1; Mothes & Bastian, 1993: 18, figs 7-12, 38.

MATERIAL. HOLOTYPE: BMNH 1889.1.1.77: Bahia, Brazil, 12.8-36.6m depth, ix.1973, coll. H.M.S. 'Challenger' Expedition. SCHIZOHOLOTYPE: MCNPOR 3769: Curaçao, 5-15m depth, 1.ii.1981 (slides ZMA POR 4587, MCNPOR 2586). OTHER MATERIAL. MCNPOR 2439: Fernando de Noronha, Baía do Sueste, Brazil, 03°50'S, 32°25'W, <30m depth (Mothes & Bastian, 1993). MCNPOR 3807: Off Maranhão State, 02°07'35"S, 41°55'46"W, 72m depth. MCNPOR 3379: Rio Grande do Norte, 03°54'S, 37°38'W, 43.6m depth. MNHN: Paraíba, 07°29'S, 34°30'W, 45m depth (Boury-Esnault, 1973). MCN: Espírito Santo, Três Ilhas (near Guarapari), 20°36'S, 40°23'W, 3-12m depth (Solé-Cava et al., 1981).

DESCRIPTION. Adequate descriptions are provided by Sollas (1888), Boury-Esnault (1973), Solé-Cava et al. (1981) and Mothes & Bastian (1993), and expanded here.

Megascleres (refer to Table 2 for dimensions). Oxeas with acerate to hastate ends, usually slightly curved. Orthotriaenes: rhabd conical, clads and rhabd with slightly blunt ends.

Microscleres (refer to Table 2 for dimensions). Centrotylote microstrongyles, smooth, usually slightly curved. Aspidasters usually digitiform, regular to very irregular outline; surface microspines rosette-shaped with conical points; developmental forms are visible. Oxyasters with 4-7 microspined rays, bigger spines are located close to the distal ends. Strongylaster / tylaster with 4-16 usually microspined rays.

REMARKS. This species differs from other Brazilian *Erylus* in having aspidasters usually digitiform and proportionally 1:7. Two specimens were first collected at 02°07'35"S, 41°55'46"W and 03°54'S, 37°38'W, expanding the distribution of this species along the Brazilian coast.

Erylus corneus Boury-Esnault, 1973
(Fig. 6A-F, Table 3)

Erylus corneus Boury-Esnault, 1973: 268, fig. 4.

MATERIAL. HOLOTYPE: MNHN-NBE 973: Paraíba, Brazil, 07°29'S, 34°30'W, 45m depth, 1961-1962, coll. 'Calypso' Expedition. SCHIZOHOLOTYPE: MCNPOR 2505: (slide).

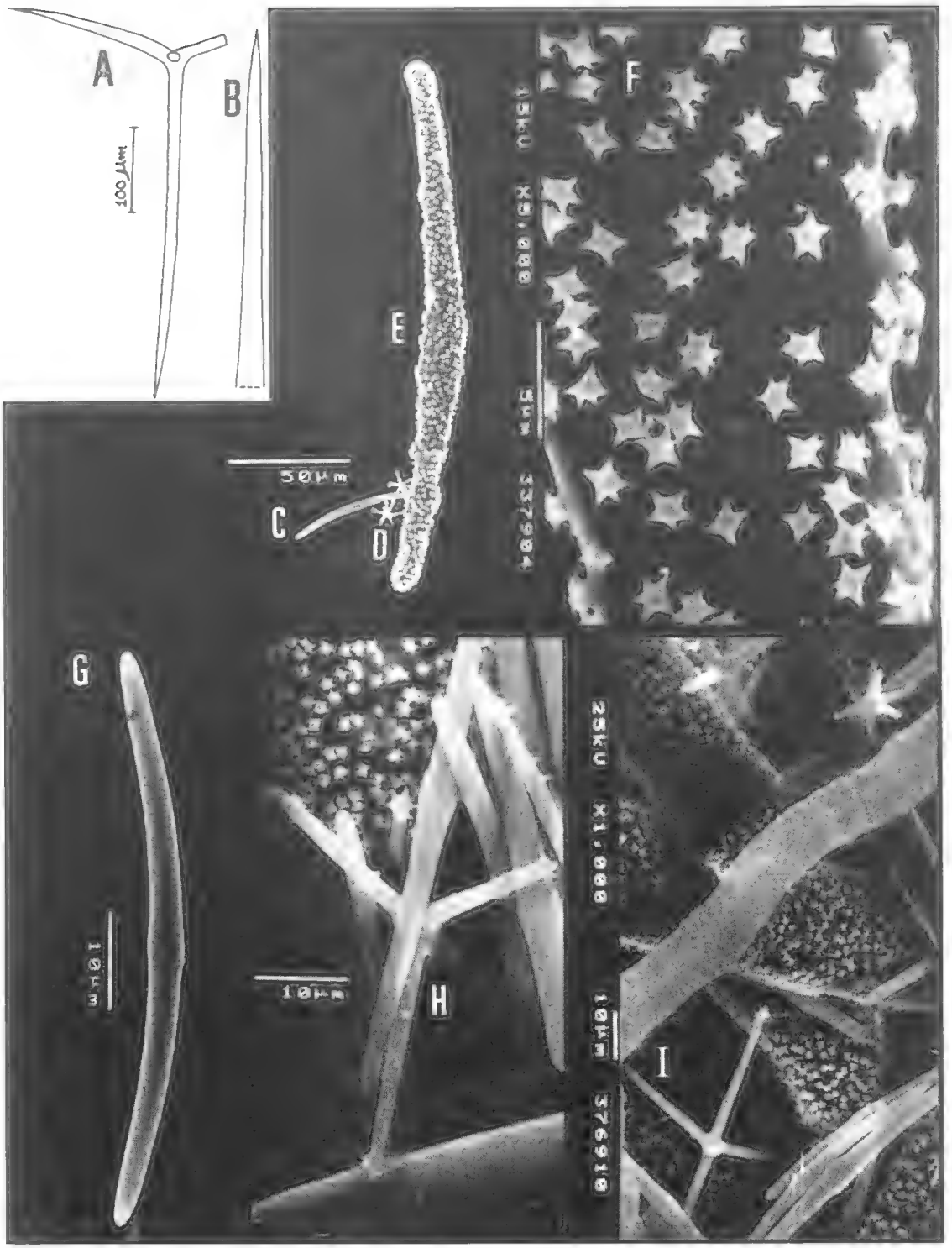

FIG. 5. *Erylus formosus* Sollas (MCNPOR 3379). A, orthotriaene. B, oxea extremity. C, microstrongyle. D, tylaster. E, aspidaster. F, aspidaster surface. G, microstrongyle. H, oxyaster. I, tylaster.

TABLE 2. Comparative data on spicule measurements of *Erylus formosus* Sollas, 1886, holotype and additional material. Orthotriaene measurements refer to shaft length/width, cladome length/ width. Measurements given in µm. Key to material of *E. formosus*: 1, Holotype – BMNH 1889.1.1.77 [data from the author]; 2, Schizoholotype MCNPOR 3769 [slides]; 3, MCNPOR 3807; 4, MCNPOR 2439 (Mothes & Bastian, 1993); 5, MCNPOR 3379; 6, Boury-Esnault (1973) [data from the author]; 7, Solé-Cava et al. (1981) [data from the author]; 8, ZMA POR 4587 [MCN POR 2586 slides]. ?=dimensions unknown, not cited by original author).

Material	Orthotriaenes	Oxeas	Aspidasters	Microstrongyles	Oxyasters	Strongylasters/ Tylaster
1	393/23.7 (21cladi)	892/23.7	14/32–175/26, 197/23.6, 122/47.4 (8-1 thickness)	70/6	63	12-16
2	180.5-304.0/ 9.2-16.1, 266.0-446.5/ 13.8-19.6	644-824.6-989.0/ 9.5-15.0-19.0	128.3-177.2-204.3 / 12.7-21.0-31.1	40.3-53.4-66.7/ 2.3-3.7-4.6	34.5-46.0-62.1	9.2-13.9-18.4
3	Not observed (Rare or Absent)	475.0-681.7-950.0 / 15.0-21.1-27.6	95.0-227.1-285.0/ 25.3-45.1-55.2	55.2-63.3-71.3/ 2.3-3.6-4.6	25.3-39.4-50.6	9.2-14.4-23.0
4	285.0-351.5/ 6.9-9.2, 171.0-247.0/ 6.9	522.5-581.8-665.0 / 6.9-11.2-13.8	133.0-153.4-171.0 / 28.5-38.9-47.5	41.4-53.8-69.0/ 2.3	23.0-28.4-39.1	6.9-13.0-18.4
5	332.5-475.0 / 9.2-11.5, 237.5-332.5/9.2	598.5-711.6-817.0 / 9.2-12.4-16.1	114.0-172.4-218.5 / 11.5-14.8-20.7	46.0-53.2-69.0/ <2.3	16.1-23.5-34.5	9.2-12.7-18.4
6	450.0-550.0/ ?, 250.0-350.0/ ?	600.0-900.0/ 9.4-12.5	188.0-256.0/ 12.5-19.0	45.0-80.0/ ?	37.0-41.0	9.4-12.5
7	313.0-504.0-625.0/ ?, 250.0-363.0-625.0/ ?	597.0-761.0-955.0 / 7.5-16.4-21.3	171.0-210.0-305.0 / ?	45.0-61.0-83.0/ ?	27.0-47.0-64.0	8.5-12.5-16.0
8	361.0-522.5/ 9.2-13.8, 256.5-418.0/ 9.2-16.1	674.5-781.3-931.0 / 9.2-12.6-18.4	103.5-170.5-253.0 / 27.6-41.7-52.9	39.1-48.6-66.7/ 2.3-3.5-4.6	29.9-44.3-59.8	6.9-10.8-13.8

DESCRIPTION. A complete description is provided by Boury-Esnault (1973), and expanded here.

Megascleres (refer to Table 3 for dimensions). Orthotriaenes with short rhabd-like calthrops; rhabd hastate and mucronate on one side; cladome with clads slightly curved. Oxeas hastate or mucronate, slightly curved, sometimes straight or strongly curved; axial canal visible.

Microscleres (refer to Table 3 for dimensions). Centrotylote microxeas smooth and slightly curved with acerate ends. Aspidasters elliptical-shaped, nearly regular outline, surface microspines stellate-shaped with 6-10 slightly conical points; developmental forms are visible with serrated margins because of stria that radiate from its central point; small hilum. Oxyasters with 10-14 microspined rays, spines more concentrated at the distal extremities.

REMARKS ON CARIBBEAN *ERYLUS*

The Brazilian coast is a continuity of the Caribbean biogeographic Province. Warm and shallow-water species have their southernmost limits along the coast of Santa Catarina State (27°S) (Fig.1), and some species extend up to the subtropical region of the coast of Rio Grande do Sul State (30°S) (Fig.1) and neighbouring areas (Mothes, 1996), such as *E. alleni*. Nine species of *Erylus* were listed in the Caribbean fauna by Pulitzer-Finali (1986). 1) *E. goffrileri* Wiedenmayer, 1977. 2) *E. amphiastera* Wintermann-Kilian & Kilian, 1984. 3) *E. ministrongylus* Hechtel, 1965. 4) *E.alleni* de Laubenfels, 1934, considered by Van Soest & Stentoft (1988) to be synonymous with *E. transiens* (Weltner, 1882), but reinstated here, for reasons described above, as a distinct species and sister species of *E. transiens*. 5) *E. clavatus* Pulitzer-Finali, 1986, also considered by Van Soest & Stentoft (1988) as a probable synonym of *E. transiens*, apparently differing only in the narrower width of the aspidasters; *E. clavatus* could also be considered as a synonym of *E. formosus*, however it has aspidasters (with proportion 1:3), which are not comparable with those of the latter species. 6) *E. formosus* Sollas, 1886. 7) *E. trisphaera* (de Laubenfels, 1953) (originally described in *Unimia*), and 8) *E. bahamensis* Pulitzer-Finali, 1986, both have much narrower aspidasters (with proportion 1:9) than other Caribbean species, however, *E. formosus* and *E. trisphaera* differ by the presence of oxyasters, and *E. trisphaera* has trilobate aspidasters. 9) *E. discophorus* (Schmidt, 1862) and *E. euastrum* (Schmidt, 1868), both originally described in *Stelletta* from the Adriatic, are certainly not conspecific with Caribbean species given their disjunct distributions. *Stellettinopsis*

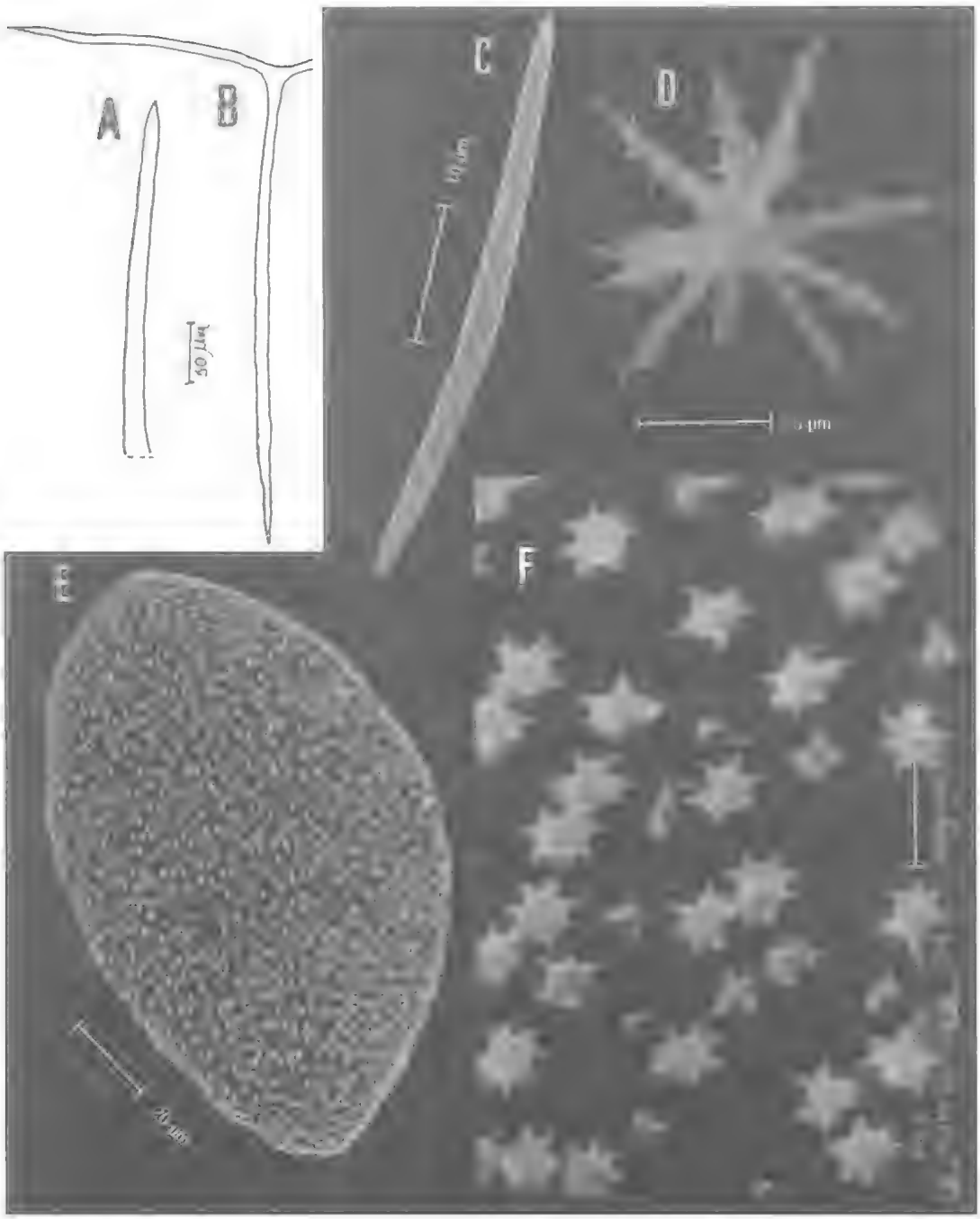

FIG. 6. *Erylus corneus* Boury-Esnault (Schizoholotype MCNPOR 2505). A, oxea extremity. B, orthotriaene. C, microxea. D, oxyaster. E, aspidaster. F, aspidaster surface.

euastrum Schmidt, 1880 was cited from Grenada by Van Soest & Stentoft (1988), but this specimen may belong to *E. transiens*. Of all these species *E. diminutus* sp. nov. is closest to *E. ministrongylus* in having strongyles, dichotriaenes and elliptical aspidasters (with proportion 1:2), although differing by the presence of microstrongyles and a single

TABLE 3. Data on spicule micrometries of *Erylus corneus* Boury-Esnault, 1973. Holotype and Schizoholotype. Measurements given in µm. Key to material of *E. corneus*: 1, Holotype - MNHN-NBE 973 [data from the author]; 2, Schizoholotype – MCNPOR 2505 [slides].

Material	Orthotriaenes	Oxeas	Aspidasters	Microxeas	Oxyasters
1	56.0-125.0 actines	546.0-673.0 / 9.0 –19.0	125.0-153.0 / 69.0-84.0	37.0-56.0 / 1.0-3.0	12.5-22.0
2	126.5-380.0/ 11.5 , Clads 119.6-213.7 / 5.7-9.2	494.0–680.0/ 8.0–19.5	119.6-147.2/ 72.4-87.4	27.6-57.5/ <2.3-3.5	9.2-23.0

category of oxyasters. A taxonomic revision of these Caribbean species is currently in progress.

DISCUSSION

Lendenfeld (1910) introduced the term 'aspidaster' for the special spicules of *Erylus*, being smooth in the young stages, shield-like shape (flattened) and oval, rarely round or irregular discs. However, some species have globiform, either circular or ellipsoidal aspidaster spicules, identical to sterrasters of *Geodia*. Consequently, we propose to enlarge the definition of the genus here, given that both sterrasters and aspidasters may be found in some species of *Erylus* (i.e. *E. polyaster*, *E. geodioides* and *E. topsenti*). The proposition to enlarge the scope of the genus to include additional forms of spicules is based only on adult spicules described here, and from species previously recorded in the literature.

In the present revision, a 'provisionally endemic' new species is described; the distribution of *E. formosus* is enlarged; the presence of *E. alleni* is recorded for the first time for the Brazilian coast; and zoogeographical data on marine demosponges from Brazil, recorded by Hechtel (1976) and Mothes (1996), are expanded.

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ORIGIN OF THE METAZOA: A REVIEW OF MOLECULAR BIOLOGICAL STUDIES WITH SPONGES

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The phylogenetic position of Porifera is near the base of the kingdom Metazoa. During the last few years rRNA sequences, and more importantly cDNAs/genes coding for proteins, have been isolated and characterised from sponges, especially from the marine demosponge *Geodia cydonium*. Analyses of their deduced amino acid sequences allowed a molecular biological approach to solve the problem of monophyly of the Metazoa. Molecules of the extracellular matrix/basal lamina, with the integrin receptor, fibronectin and galectin as prominent examples, cell-surface receptors (tyrosine kinase receptor), elements of nerve systems (crystallin, metabotropic glutamate receptor) as well as homologs/modules of an immune system (immunoglobulin-like molecules, SRCR- and SCR-repeats, Rhesus system) unequivocally classify Porifera as true Metazoa. As living fossils sponges also show pecularities not known in other metazoan phyla provided with simple, primordial molecules allowing cell-cell and cell-matrix adhesion as well as processes of signal transduction known in a more complex manner from higher Metazoa. Tissues of sponges are rich in telomerase activity, suggesting a high plasticity in the determination of cell lineages. Based on this experimental background a first successful approach to establishing a cell culture from a sponge was possible. It is concluded that molecular biological studies using sponges as models will not only help us to understand the evolution of the Metazoa from the Protista, but also the complex, hierarchial regulatory network of cells in higher Metazoa. ❐ *Porifera, evolution, monophyly, receptors, phylogeny, molecular biology, (Eu)Metazoa.*

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The transition from unicellular to multicellular organisms has taken place in all five kingdoms of life; this process took place separately in Fungi (Ascomycota), Plantae (Chlorophyta) and in Metazoa. The origin of plants appears to be well established within the phylum Chlorophyta (Margulis & Schwartz, 1995), whereas the origin of Fungi, and especially of Metazoa, is perhaps the most enigmatic of all phylogenetic problems (Willmer, 1994).

The origin of the Metazoa remained uncertain until a few years ago. At that time two questions were paramount: 1) what were the relationships between the different metazoan phyla in general, and between the lowest metazoan phylum, the Porifera (sponges), and those of higher invertebrates in particular; and 2) what are the ancestor(s) of the Metazoa among the Protista ? Some authors favoured the idea that sponges had unicellular ancestors different from those of other Metazoa [polyphyly] (Margulis & Schwartz, 1995), while other scientists (e.g. Morris, 1993), believed that multicellular animals evolved only once [monophyly].

One powerful approach particularly helpful in answering questions on the presence or absence of corresponding structures in sister groups is to gather molecular data from the respective taxa. Here, a clear distinction must be made. Nucleotide [nt] sequence data have been gathered from genes of species in different phyla, encoding small and large ribosomal RNA. These data have been used to build phylogenetic trees to resolve deep branches. The outcome in most reports was that bootstrap statistics supporting mono- or polyphyly is low (e.g. Cavalier-Smith et al., 1996), or even not at all significant (Rodrigo et al., 1994).

In a second, separate concept, amino acid [aa] sequences deduced from nt sequences from genes and proteins that are known from metazoan systems (e.g. immune, adhesion, sensory systems), have been used to obtain reliable

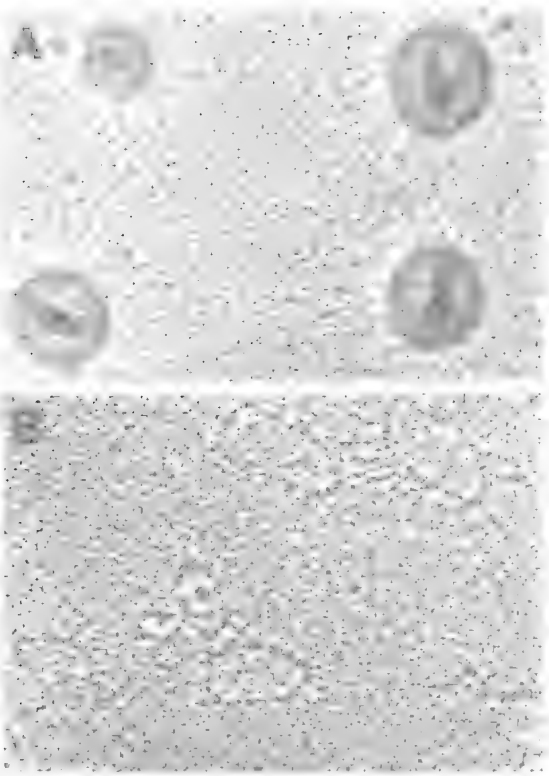

FIG. 1. Adhesion molecule of the sponge *G. cydonium*; electron micrograph. A, Native form of the aggregation factor. B, Aggregation factor core structure with the circular center and the 25 radiating arms. Preparation shadowed with platinum (magnification x140,000).

insight into the branching of metazoan phyla from a potential common ancestor (Pfeifer et al., 1993). Our research group has introduced this approach to establish the phylogenetic position of Porifera, within the Metazoa. Our data are compatible with the view that all Metazoa are monophyletic in origin (Müller et al., 1994; Müller, 1995).

We also discuss evidence for evolution of cell lineages in early Metazoa; we demonstrate the use of a new type of sponge 'organotypic' cell culture in cell proliferation and cell death; and finally we discuss evidence for the separation of the Porifera into two subphyla.

DISCUSSION

GENOME SIZE OF PORIFERA. Using the method of cytometry and DAPI staining, the DNA content of haploid cells (C-value) from *Geodia cydonium* and *Suberites domuncula* was found to be 1.7pg, corresponding to $1.7x10^6$kb (Imsiecke et al., 1995). This unexpectedly high value is further exceeded by the result which came from a separate technique, the determination of DNA reassociation kinetics. In a recent calculation, based on the determination of genetic complexity, a value of 3.3pg DNA/haploid genomic set was calculated (Bartmann-Lindholm et al., 1997). In comparison, the C value for human cells is $3.4x10^6$kb (see Li & Graur, 1991). The number of chromosomes in the diploid state in *S. domuncula* is 32 (Imsiecke et al., 1995).

The recent finding that five major subcomponents of DNA could be distinguished in *G. cydonium* by density gradient centrifugation (Bartmann-Lindholm et al., 1997) was surprising as it indicates a heterogeneity which has not been reported in any other metazoan. The genetic complexity within these subcomponents was determined by reassociation kinetics to vary from $2.1x10^8$bp to $1.4x10^9$bp, corresponding to a content of single copy DNA of >93% (Bartmann-Lindholm et al., 1997). The extreme heterogeneity in DNA composition of the genome of *G. cydonium* suggests that an unusually high exchange of well-defined DNA regions occurred in this animal.

METAZOAN GENES/PROTEINS IN PORIFERA. *Molecules/modules of the extracellular matrix/basal lamina in G. cydonium.* It is now well acknowledged that repeated sequences or modules (Patthy, 1995) are found in proteins of the complement system, extracellular matrix and also in various cell-surface receptors. Mobile modules, according to the definition of Patthy (1995), are protein-coding domains that are flanked by introns of identical phase, facilitating a dispersal within the genome. It is shown here that most polypeptides deduced from cDNA sequences from sponges are assembled by an unusually large variety of such modules.

Two types of adhesion systems have been described in sponges, cell-cell and cell-matrix systems. With respect to the first system, the aggregation factor (AF) is considered to be the major extracellular molecular complex (Fig. 1). AFs were enriched from two sponge species, *Microciona prolifera* and *G. cydonium* (reviewed in Müller, 1982). The AF is a multiprotein complex which interacts with a membrane component, the aggregation receptor (AR), that has been identified but not yet purified.

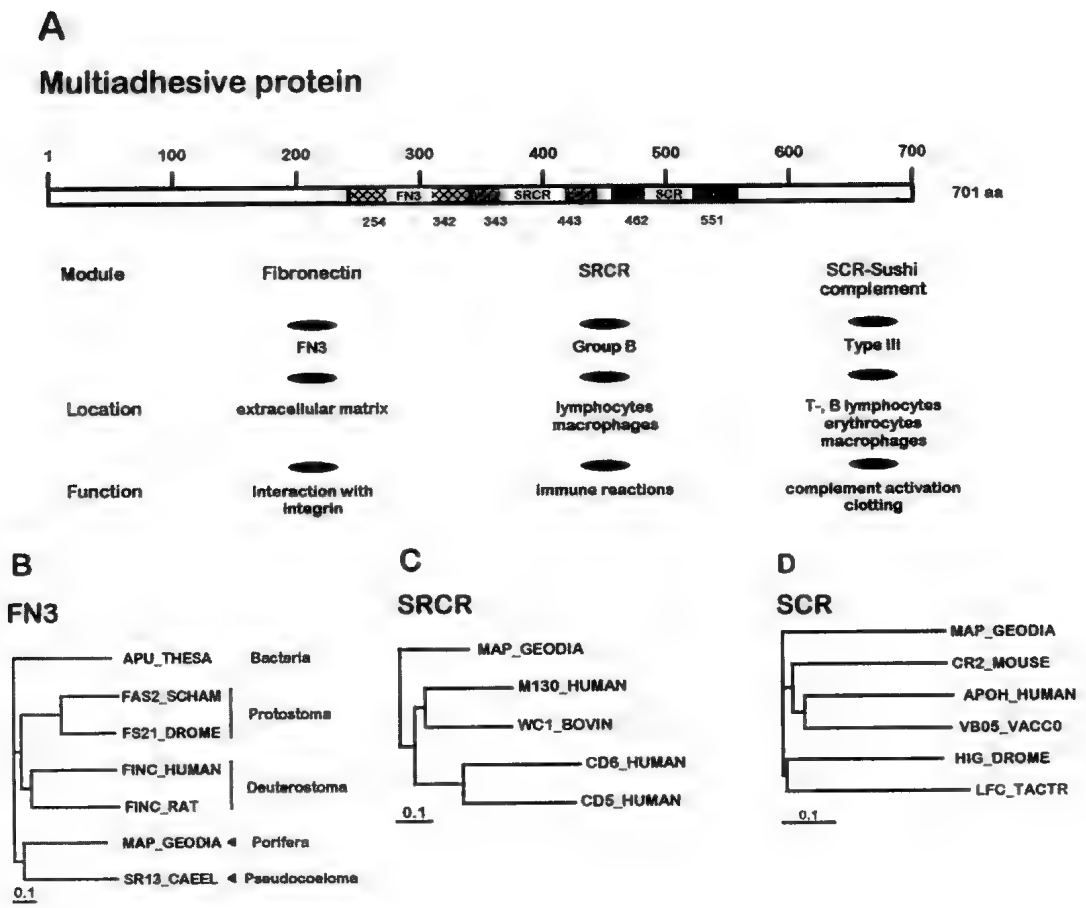

FIG. 3. A, Scheme of the putative 'multiadhesive protein' (MAP_GEODIA), cloned from *G. cydonium*. Three modules can be identified in this protein; the fibronectin- (FN3), the a scavenger receptor cysteine-rich (SRCR)- and the a short consensus repeat (SCR; Sushi) module. B-D, Phylogenetic analyses of the modules. B, Unrooted phylogenetic tree composed from the deduced aa sequences of FN3 modules found in 1) Metazoa, from deuterostomes human (FINC_HUMAN), and rat (FINC_RAT; module 6), from protostomes FN3 from *D. melanogaster* (FS21_DROME) and the arthropod *S. americana* (FAS2_SCHAM; module one), the pseudocoelomate *C. elegans* (SR13_CAEEL) and the sponge *G. cydonium* (MAP_GEODIA), as well as in 2) Bacteria *T. saccharolyticum* (APU_THESA; module one). C, Phylogenetic tree computed from five SRCR scavenger molecules of group B; the modules from the sponge MAP_GEODIA, the human CD6 antigen (CD6_HUMAN), human CD5 surface glycoprotein (CD5_HUMAN), human M130 antigen (M130_HUMAN) and bovine WC1 surface antigen (WC1_HUMAN) were analyzed. D, Phylogenetic tree constructed from SCR modules of the following six polypetides; the module from MAP_GEODIA together with the corresponding type III SCRs from human beta-2-glycoprotein I precursor (APOH_HUMAN), mouse CR2 complement receptor type 2 precursor (CR2_MOUSE), *D. melanogaster* locomotion-related protein (HIG_DROME), *Limulus* clotting factor C precursor from *Tachypleus tridentatus* (LFC_TACTR) and the host range protein precursor from the vaccinia virus strain LC16MO (VB05_VACCO). The evolutionary distance of 0.1 aa substitutions per position in the sequence is given.

matrix (ECM): 1) fibronectins, 2) collagens, and 3) galectin.

Fibronectin. Fibronectins are high molecular weight glycoproteins, present in most ECM and also in blood plasma. A typical fibronectin

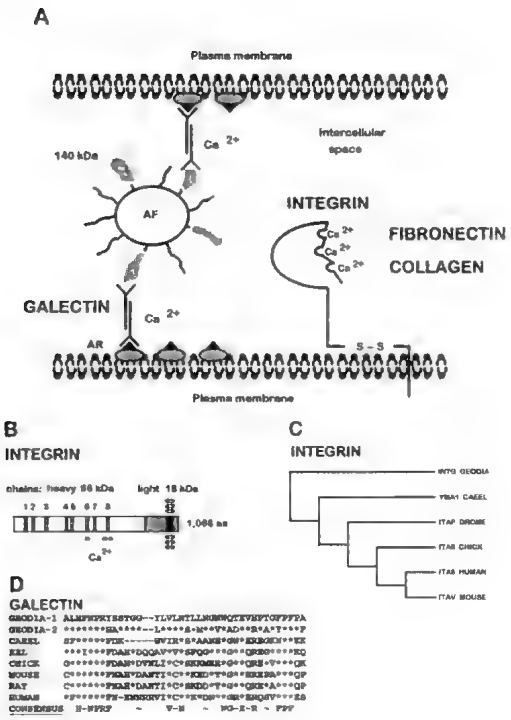

FIG. 2. A, Scheme of aggregation factor (AF)-mediated cell recognition in *G. cydonium*. A 29-kDa aggregation receptor (AR) is inserted into the plasma membrane to which one galectin molecule binds. In the presence of Ca^{2+} a second galectin molecule binds to the first one. Then these two molecules form a bridge between the AR and the 140 kDa polypeptide, associated with the AF. Following this scheme, the interactions between the AF and the AR involves the galectin which might bind to carbohydrate both at the 140 kDa polypeptide and at the AR. In addition, the sponge contains an integrin receptor which is assumed to interact with fibronectin and collagen. B, Sponge integrin receptor. Schematic presentation of the structural features in *G. cydonium* integrin α subunit. The heavy and light chains are indicated (the light chain is shaded). Positions of the 8 characteristic repeats of integrins are marked 1 to 8. Three putative Ca^{2+}-binding sites as well as the C-terminal transmembrane region are indicated. C, Dendrogram computed from the deduced aa sequences of integrin α subunits found to be most homologous to sponge integrin α (INTG_GEODIA) with the corresponding sequences from the invertebrate species, *Caenorhabditis elegans* (YMA1_CAEEL) and *Drosophila melanogaster* (ITAP_DROME), and the vertebrate species, mouse integrin αV (ITAV_MOUSE), chicken integrin αVIII (ITA8_CHICK) and human fibronectin receptor α subunit (ITA5_HUMAN). D, Multiple alignment of the conserved region within the galectin carbohydrate binding domains. The asterisks (*) mark sequence identities between *G. cydonium* galectin-1 and -2 (GEODIA-1 and -2) and other galectins from *C. elegans* (CAEEL), eel (EEL), chicken (CHICK), mouse (MOUSE), rat (RAT) and human (HUMAN). The consensus sequence for galectins is given.

Monoclonal antibodies have been used as tools to identify the binding domains of the AF (Wagner-Hülsmann et al., 1996). A 140kDa polypeptide was found to participate in the AF-mediated reaggregation process. This polypeptide interacts with a galectin that links individual AF molecules to the AR at the plasma membrane, and consequently bridges two cells together (Müller et al., 1997) (Fig. 2A). Confocal laser scanning microscopical analysis demonstrated that both the galectin and the AF are present at the rim of the cells (Wagner-Hülsmann et al., 1997).

Within the last few years, major elements characteristic of a basal lamina have also been discovered in sponges. Besides cDNAs coding for two proteins with similar complexity as those found in higher Metazoa, collagen type IV, integrin, and one fibronectin module (FN3) were identified.

Integrin. One major class of extracellular matrix (ECM) receptors are the integrin receptors. Integrins are membrane-anchored heterodimer receptors composed of α- and β subunits. At least 16 different α- and 8 different β subunits have been identified in vertebrates which yield more than 20 heterodimeric integrin receptors.

We have isolated and characterised cDNA clones encoding the α subunit of an integrin from the marine sponge *G. cydonium* (Pancer et al., 1997a). The open reading frame encodes a 118,628Da polypeptide (Fig. 2B). Most α subunits of integrins, including the one from the sponge, contain 7-8 repeating domains (Fig. 2B). Like other α subunits of integrins the sponge molecule also contains putative divalent cationic-binding sites. A dendrogram was computed from the deduced aa sequences of integrin α subunits (Fig. 2C).

The integrin receptor binds primarily molecules of the following three families, present in the extracellular

consists of more than 10 type I-, approximately 2 type II-, and more than 15 type III (FN3) modules. Protein(s) have been isolated from *G. cydonium* that immunologically cross-react with human anti-fibronectin antiserum (Pahler et al., 1998). The main bands have sizes of 230 and 210kDa. In addition, a cDNA was cloned, encoding a putative 'multiadhesive protein' which comprises three interesting modules: 1) a fibronectin, 2) a scavenger receptor cysteine-rich (SRCR), and 3) a short consensus repeat (SCR) module (Fig. 3A).

The fibronectin module of the deduced sponge protein (Pahler et al., 1998) comprises the characteristic topology and aa found in fibronectin type-III (FN3) elements. Even though it remains to be proven in Porifera that this FN3 module functions as an adhesion molecule, the finding supports the immunochemical data on the presence of fibronectin-like molecules in sponges. FN3 modules have been primarily described in Metazoa; in addition they are found in a related sequence in extracellular glycohydrolases from soil bacteria (Bork & Doolittle, 1992). The unrooted phylogenetic tree (Fig. 3B) reveals that the FN3-related sequence of the bacterium branches off first from a common ancestor, while the deuterostomes (human and rat) and the protostomes (*Drosophila melanogaster* and *Schistocerca americana*) are grouped within one branch, and the acoelomate (*Caenorhabditis elegans*) and the sponge (*G. cydonium*) in another (Fig. 3B). From these data we conclude that the sponge FN3 module from *G. cydonium* is phylogenetically the oldest one within Metazoa.

Collagen. Collagens constitute a superfamily of extracellular matrix proteins. Until recently it was accepted that collagens are present only in Metazoa. However, a new class of collagens recently identified in fungi is assumed to have arisen by convergence (Celerin et al., 1996). In sponges, primitive fibrillar collagens have been seen in several species; *Chondrosia reniformis* (Garrone et al., 1975), and *G. cydonium* (Diehl-Seifert et al., 1985a). The finding that sponges contain basement membrane collagen type IV was spectacular, as this is known to be the scaffold of the basal lamina (Boute et al., 1996).

Galectin. As mentioned, the sponge AF interacts with the AR via galectin and the 140kDa polypeptide (Fig. 2A). The galectin, which occurs in isoforms, was studied in detail. The purified molecules reveal forms of Mr 13 to 18kDa (Bretting et al., 1981) which bind specifically to the sugars dGalNAc, dGalβ1→4dGlcNAc, dGalβ1→3dGlcNAc and dGalNAc. In the presence of Ca^{2+} or glycoconjugates the sponge galectins undergo conformational changes and 'polymerise' to large three-dimensional clumps (Diehl-Seifert et al., 1985b). The cDNAs of two isoforms of the galectins from *G. cydonium* were cloned (Pfeifer et al., 1993; Wagner-Hülsmann et al., 1996). The predicted proteins deduced from the complete sequences display high similarity with the corresponding molecules from vertebrates and *C. elegans* (Hirabayashi & Kasai, 1993; Müller et al., 1997). The deduced aa sequences of the two isoforms feature the characteristic carbohydrate-recognition domain **LHFNPR-G-V-N-W-E-R[H]**-PF (the aas given in bold are those directly involved in binding to the carbohydrate); this domain is conserved from sponge to human (Pfeifer et al., 1993) (Fig. 2D). Based on the extent of aa substitutions the two sponge galectins were calculated to have

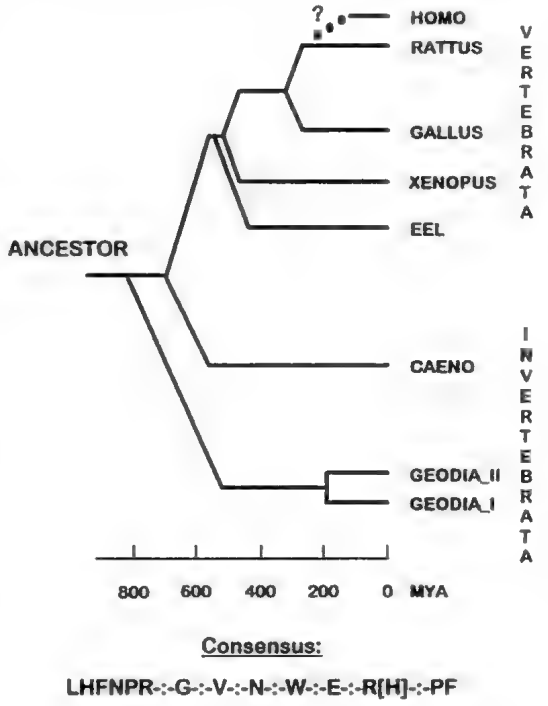

FIG. 4. Phylogenetic tree computed from the deduced aa sequences of galectins from: 1) vertebrates - human (HOMO), rat (RATTUS), chicken (GALLUS), frog (XENOPUS), conger eel (EEL); and 2) invertebrates - nematode (CAENO) and *G. cydonium* (GEODIA; isoform I and II). The consensus sequence for galectins are given. Time scale indicates the time of divergence, based on aa substitution analysis.

diverged from the galectin isolated from the nematode *C. elegans*, 800 MYA (Hirabayashi & Kasai, 1993; Pfeifer et al., 1993) (Fig. 4).

CELL-SURFACE RECEPTORS. Besides adhesion receptors, receptors involved in signal transduction, or elements of signal transduction pathways coupled to them have also been cloned from *G. cydonium*. The results have been recently reviewed (Müller & Müller, 1997; Müller, 1997a); so only a brief summary is given here.

One-transmembrane-segment receptor-receptor tyrosine kinase. Protein tyrosine kinases (PTKs) play important roles in the response of cells to different extracellular stimuli. PTKs are divided into two major groups, the receptor tyrosine kinases (RTKs), which are membrane spanning molecules with similar overall structural topologies, and the non-receptor TKs, also composed of structurally similar molecules. The first RTK from lower Metazoa was identified and cloned from *G. cydonium* (Müller & Schäcke, 1996). The putative aa sequence comprises 1) the extracellular part with a Pro/Ser/Thr-rich region, and two complete immunoglobulin (Ig)-like domains, 2) the transmembrane domain, 3) the juxtamembrane region, and 4) the catalytic tyrosine (TK)-domain. A similarity search with the *G. cydonium* TK-domain aa sequence showed that all RTKs fall in one branch of the tree while the non-receptor TKs are grouped in a second one; sponge RTK is placed in a separate branch, which splits-off first from the common tree of metazoan PTKs. An estimation of the time of divergence of the sponge RTK from RTKs of other metazoans was 650-665 MYA (Gamulin et al., 1997).

Seven-transmembrane-segment receptors. The first seven-transmembrane-segment receptor in sponges was isolated from *G. cydonium*. It is the metabotropic glutamate receptor (see description below).

Most seven-spanning receptors transmit extracellular signals through G-proteins. G-proteins, coupled to the putative receptors, have been identified in *G. cydonium*. G-proteins are heterotrimers composed of α-, β- and χ subunits (Seack et al., 1998).

Several secondary effector enzymes of the seven-transmembrane-segment receptor/G protein-linked receptor have been cloned from *G. cydonium*: the Ser/Thr kinases (STKs). These kinases are ubiquitously present in animal tissues; they express their activities in response to second messengers (e.g. Ca^{2+} or diacylglycerol). The STKs have been sequenced from *G. cydonium* (Kruse et al., 1996, 1997, 1998). A comparison of the complete structures of the sponge STKs, which are identical to those of nSTKs and cSTKs from higher Metazoa, with the structures of protozoan, plant and bacterial Ser/Thr kinases, indicates that the metazoan STKs are different from the non-metazoan enzymes. These data imply that metazoan STKs have a universal common ancestry with the non-metazoan STKs with respect to the kinase domain, but they differ from them in the overall structural composition.

NEURONAL-LIKE ELEMENTS IN PORIFERA. Until now no molecular evidence has been presented in demosponges for the existence of a nervous-like cell system. Recently our group identified two elements (crystallin and a metabotropic glutamate receptor) in sponges, which are characteristic for sensory systems in higher Metazoa.

Crystallins. Crystallins are categorised into two classes, the ubiquitous crystallins and the taxon-specific crystallins. No structural or functional characteristics are common to all crystallins. The α-, β- and χ-crystallins are classed as ubiquitous crystallins and are found in almost all vertebrate species. The second class, the taxon-specific crystallins, includes a series of 'enzyme crystallins', which display catalytic functions.

Until recently, no molecular sequence data was available for βχ-crystallins in invertebrates. The cDNA coding for the βχ-crystallin molecule was isolated from *G. cydonium* (Krasko et al., 1997). The sponge sequence comprises the four repeated motifs which compose the two domains of the βχ-crystallin. The peptide shows striking homologies to vertebrate βχ-crystallins. Each motif is composed of the four β-strands and one 'Greek key' signature (Fig. 5A). Like in other crystallins a signal peptide is missing in the sponge sequence, suggesting that it is an intracellular structural protein (Krasko et al., 1997). Thus, molecules from light-sensory organs, in this case crystallins, are also present in sponges.

Nerve cell receptors - presence of sensory cells: Metabotropic glutamate receptor. Sponges are (according to the literature) not provided with nerve cells. However, recently we showed that isolated cells from the marine sponge *G. cydonium* react to the excitatory neurotransmitter glutamate with an increase in the concentration of intracellular Ca^{2+}, $(Ca^{2+})_i$. This effect was also measured if the compounds L-quisqualic acid

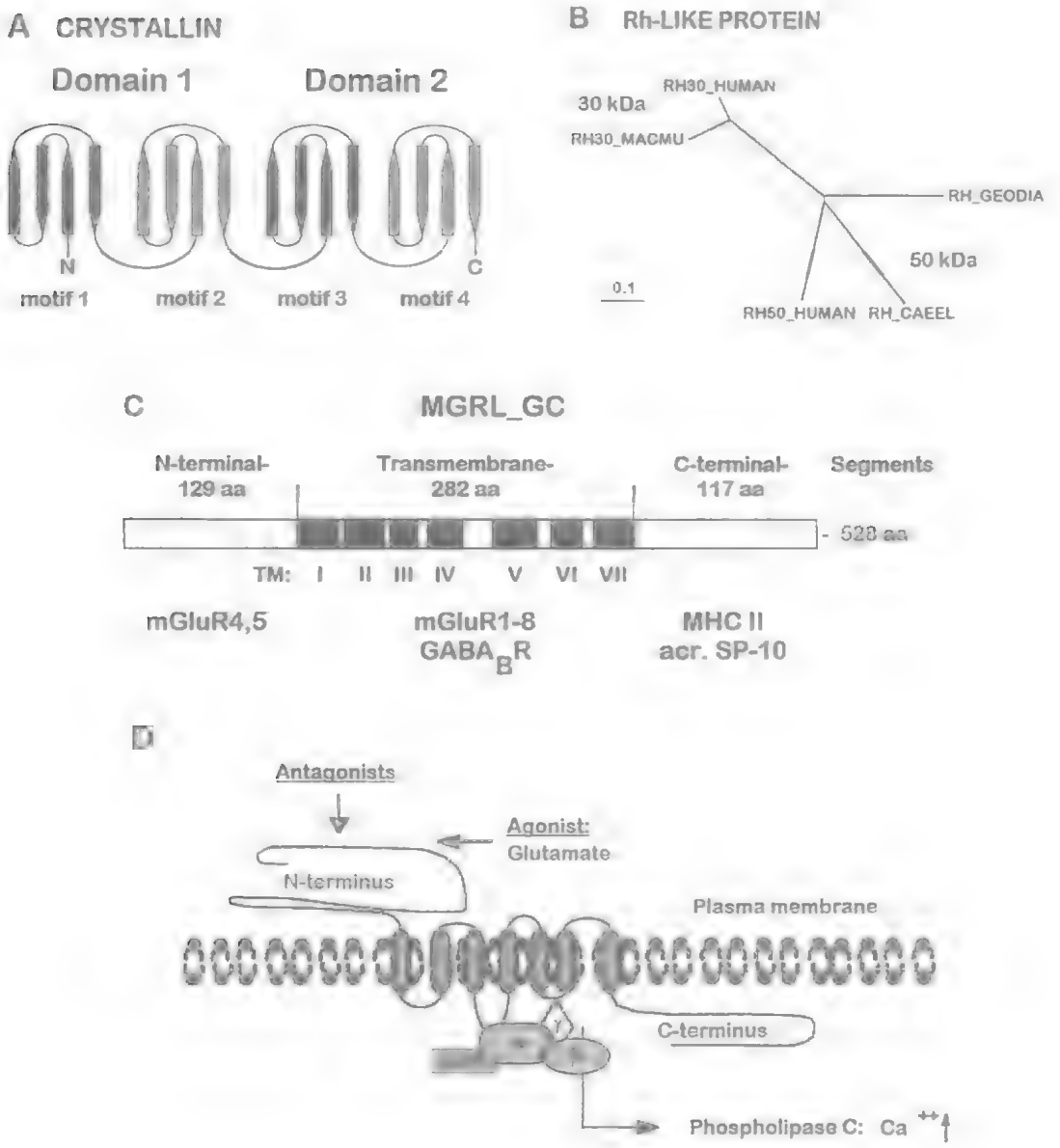

FIG. 5. A, Crystallin: Sponge βχ-crystallin from *G. cydonium*. Schematic representation of the βχ-crystallin folding pattern. Two Greek key motifs form one domain. Domains 1 and 2 form the monomeric βχ-crystallin. B, Rhesus-like antigen: Relationship of the sponge Rhesus(Rh)-like protein to animal Rh- and Rh-like antigens. Unrooted phylogenetic tree computed from the sponge Rh-like protein (RH_GEODIA) and Rh-related proteins: the Rh30 Ag from human (RH30_HUMAN) and rhesus monkey (RH30_MACMU), the RhD-like polypeptide from *C. elegans* (RH_CAEEL) and the human Rh50 Ag (RH50_HUMAN). Two clusters, comprising the ~30 000 Mr and the ~50 000 Mr Rh polypeptides, are grouped. C, Metabotropic glutamate receptors (mGluRs): Schematic presentation of the sponge receptor (*G. cydonium*), showing its three segments. The sequences with the highest similarity to the sponge segments are listed. D, mGluRs: Scheme of the sponge mGluR, inserted by seven transmembrane segments into the cell membrane; it is coupled to G-proteins.

(L-QA) or L-(+)-2-amino-4- phosphono- butyric acid (L-AP-4) were used. The effects of L-QA and L-AP-4, both agonists for metabotropic glutamate receptors (mGluRs), could be abolished by the antagonist of group 1 mGluRs, (RS)-α-methyl-4-carboxyphenyl- glycine. These data suggest that sponge cells contain a mGluR-like protein - hence it is justified to state that sponges are provided with sensory-like cells. The demonstration of a neuronal-like receptor in sponges also allows to challenge the question for the underlying molecules, involved in the coordination of the cells for contraction. It is interesting to note that some sponge species are known to migrate in a directed manner, e.g. the sponge *Tethya* spp (Fishelson, 1981) or *Ephydatia fluviatilis* (Bond & Harris, 1988).

Using a cDNA encoding the rat mGluR subtype 1, a complete nucleotide sequence of *G. cydonium* cDNA coding for a 528 aa long protein (59kDa) was identified which displays high overall similarity to mGluRs as well as to GABABRs. The deduced sponge polypeptide, termed a putative mGlu/GABA-like receptor, displayed the highest similarity to the two families of metabotropic receptors within the transmembrane segment. The N-terminal part of the sponge sequence shows similarity to the mGluR4 and -5. These findings suggest again that the evolutionarily earliest metazoan phylum, Porifera, possesses a complex intercellular communication and signaling system as known from the neuronal network of higher Metazoa (Perovic et al., 1999) (Fig. 5C and D).

HOMOLOGUES/MODULES OF THE IMMUNE SYSTEMS. Little is known about natural challenges to self integrity in sponges (reviewed in Pancer et al., 1996). In their extensive review Smith & Hildemann (1986) have grouped sponge alloimmune responses seen in experimental transplantations into two major rejection processes. Some species form barriers to separate themselves from non-self tissue, while others react by cytotoxic factors which destroy the transplant. However, until recently, no molecule has been identified which can be considered to be involved in self/non-self responses in sponges.

Three modules are now known in deduced aa sequences of cDNAs isolated from *G. cydonium*, which are present also in immune molecules of higher Metazoa; 1) immunoglobulin-like domains, 2) proteins featuring scavenger receptor cysteine-rich domains, and 3) molecules comprising short consensus repeats.

Immunoglobulin-like (Ig-like) domains. To determine if the two immunoglobulin-like (Ig-like) domains of the RTK from *G. cydonium* display sequence polymorphism (Pancer et al., 1996), allo- and autografting experiments were performed using two grafting methods: 1) parabiotic attachment, and 2) insertion technique. Thirty-six pairs of auto- and allografts were assayed. All of the autografts fused, while only two allografts fused and 34 pairs were incompatibile. At the molecular level the two Ig-like domains of RTK were analyzed from two pairs of fusing and one pair of rejecting sponges (Pancer et al., 1996). High nt and aa polymorphism was recorded.

Proteins featuring scavenger receptor cysteine-rich domains. Proteins featuring scavenger receptor cysteine-rich (SRCR) domains comprise a superfamily, which includes one invertebrate and several vertebrate proteins. The SRCR domain consists of a 110 aa-residue motif with conserved spacing of six to eight cysteines, which apparently participate in intradomain disulfide bonds. Proteins of this superfamily feature 1-11 SRCR domain repeats.

We identified the putative SRCR protein - belonging to group A of this family (Wijngaard et al., 1992) - from the marine sponge *G. cydonium* (Pancer et al., 1997b; Müller, 1997b). Three forms of SRCR molecules were characterised, which apparently represent alternative splicing of the same transcript. The long putative SRCR protein features twelve SRCR repeats, a C-terminal transmembrane domain and a cytoplasmic tail. The sequence of the short form is identical with the long form except that it lacks a coding region near the C-terminus, without the transmembrane domain. Homology searches revealed that the sponge putative SRCR protein shares with bovine T-cell antigen WC1 29.2% identity in 1054 aa overlap, 33.9% identity in 475 aa overlap with sea urchin speract, and 56% identity in 110 aa overlap with macrophage scavenger receptor type I.

Recently, the SRCR module of the group B (Wijngaard et al., 1992) was also identified in the 'multiadhesive protein' (Pahler et al., 1998) (Fig. 3). The percentage of identity (homology) of this module of MAP_GEODIA is most similar to the mammalian sequences M130 with 63% (44%) and WC1 with 50% (40%) and lower for CD5 32% (25%) and CD6 36% (25%).

Phylogenetic analysis shows that the sponge MAP_GEODIA scavenger module branches off first from a common ancestor, whereas two other modules, the mammalian macrophage antigen M130 and the antigen WC1 (which is expressed on gamma/delta T lymphocytes) as well as those of the CD6 and the CD5 antigen of lymphocytes, branch off later (Fig. 3C).

Molecules comprising short consensus repeats. The short consensus repeats (SCR) also termed 'Sushi domain', with 11-14 conserved aa residues and four conserved cystein residues, are classified according to the consensus aa pattern into four types. In the course of seeking further splice forms of the sponge putative SRCR, a protein sequence was identified which contains the 12 SRCR repeats mentioned above plus two others that are linked to six SCR, the SRCR-SCR molecule (Pancer et al., 1997b; Müller, 1997b). The SCR modules present in this putative polypetide belong to group II of the SCR family.

The presence of an SCR module of type III in a sponge molecule, 'multiadhesive protein', was surprising (Fig. 3A). This module belongs to the SCR repeats, which are dominant building blocks in the complement receptor of type 1, type 2, and factor H, but also in a few non-complement proteins (e.g. β2-glycoprotein I; reviewed by Reid & Day, 1989). The phylogenetic tree built from the selected SCRs of group III revealed that the sponge SCR module forms the basis for the two related SCRs from mammals, mouse complement receptor and human beta-2-glycoprotein I precursor, and the two invertebrate sequences from the locomotion-related protein of *Drosophila melanogaster* and the *Limulus* clotting factor (Fig. 3D). The SCR from the vaccinia virus displays closest relationship to the mammalian sequences, suggesting horizontal gene transfer from host to the virus (Bishop, 1981).

Rhesus-like protein. Vertebrate red blood cells display a variety of cell-surface molecules. Some, like the ABO system and the Rhesus (Rh) system of higher mammals, exhibit extensive polymorphism. Although the function of these antigens is poorly known, their role has been implicated in severe human disorders due to abnormal functioning or immunological destruction of the red blood cells (Nash & Shojania, 1987). A breakthrough in the analysis of the Rh system was marked by cloning of the Rh cDNA encoding the D antigen (Cherif-Zahar et al., 1990; Avent et al., 1990), and later also of the associated and closely related Rh50 protein (Ridgwell et al., 1992; Le van Kim et al., 1992). Surpringly, a Rhesus-like protein (cDNA) of 57,000Mr was isolated from *G. cydonium* (Seack et al., 1997). Both the hydropathy profile of the sponge Rh-like protein and its high similarity to the aa sequence clearly show that the sponge molecule shares a common ancestor with the human and rhesus monkey Rh30 antigen, and with the ~50,000Mr Rh-like polypeptides from humans and *Caenorhabditis elegans* (Fig. 5B).

CELL LINEAGES. In contrast to higher metazoan phyla, sponges are characterised by a pronounced plasticity in the determination of cell lineages. In a first approach to elucidate the molecular mechanisms controlling the switch from the cell lineage with a putative indefinite growth capacity to senescent, somatic cells, the activity of the telomerase as an indicator for immortality has been determined. The studies were performed on two demosponges, *Suberites domuncula* and *Geodia cydonium* (Koziol et al., 1998).

High activity for the telomerase in tissue of both sponges was found, reaching about 30% of that seen in telomerase-positive mammalian reference cells. In contrast, dissociated spherulous cells from *G. cydonium*, after an incubation period of 24hrs, contain no detectable telomerase activity. From earlier studies it is known that isolated sponge cells do not proliferate (Gramzow et al., 1989). From this it is assumed that the separation of the senescent sponge cell lineage from the immortal germ-/somatic cell lineage is triggered by the loss of contact to cell adhesion factors. Preliminary evidence exists which suggests that the final progress of the senescent, telomerase-negative cells to cell death is caused by apoptosis (Fig. 6).

ESTABLISHMENT OF A PRIMARY CELL CULTURE FROM A SPONGE. Despite the fact that cells from sponges contain high levels of telomerase activity, no successful approach to cultivate sponge cells has yet been described; in phyla which are evolutionary higher than sponges the somatic cells are telomerase-negative. One reason may be seen in the observation that after dissociation the cells lose their telomerase activity. In addition, no nutrients and metabolites have been identified that would stimulate sponge cells to divide.

In close collaboration with the group of R. Borojevic and M.R. Custodio (Departamento de Histologia e Embriologia, Instituto de Ciêcias Biomédicas, Universidade Federal, Rio de

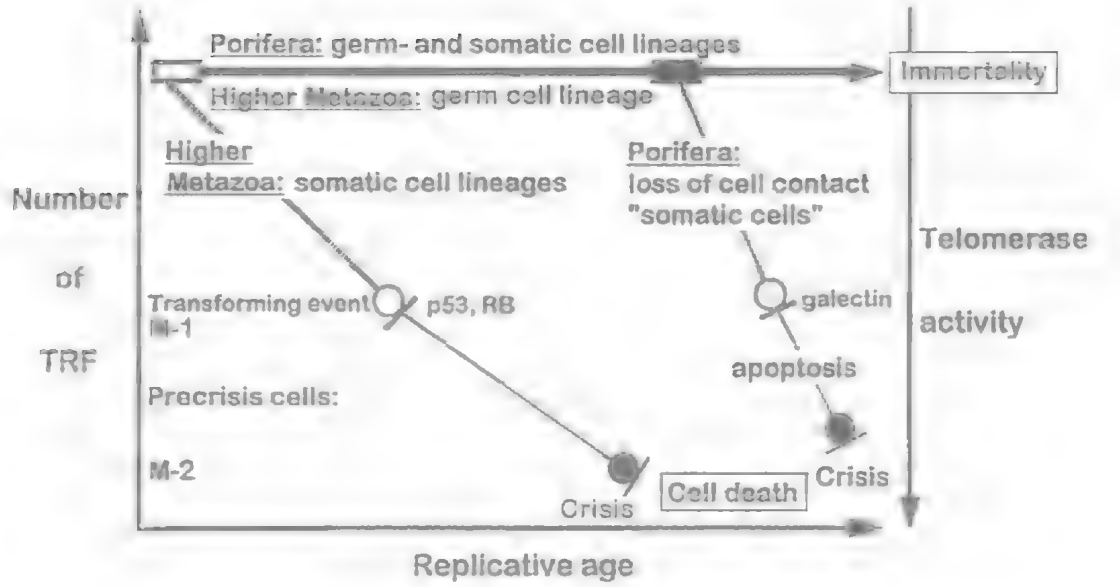

FIG. 6. Hypothetical consequence of telomere loss on senescence of metazoan cells. The reduction of telomeres is given in number of loss of terminal restriction fragments (TRFs). It has been shown experimentally that the cells of the germ line in higher Metazoa and the cells of both the germ- and the somatic lineage in Porifera contain high levels of telomerase thus allowing the maintenance of stable telomeres. In higher Metazoa an early loss of telomerase activity determines the fate of the somatic cells to senescence (open box) via the two phases. 1) 'Mortality Phase 1' (M-1) - cell cycle arrest - during which factors controlling life span via recognition of 'damaged' DNA, e.g. the RB protein or p53 protein, are activated. 2) After transformation (downregulation of RB and/or p53) the second process, 'Mortality Phase 2' (M-2), is initiated during which the telomeres reach in the precrisis cells a critical length from which a signal for cell death arises. In sponges it is proposed that the switch from immortal somatic cells to mortal 'somatic' cells occurs after a loss of adhesion factors for a given set of cells (closed box). The M-1 point is assumed to be reached after only a few rounds of cell replication. The growth arrest might be bypassed by addition of adhesion factor(s), e.g. galectin. The second phase towards cell senescence is supposed to be induced by central death signal(s), e.g. activation of the expression of the *MA-3* gene, which are under the control of either extrinsic- or an intrinsic factors. (Adapted from Harley, 1991; Harley et al., 1994).

Janeiro; Brazil), we succeeded in defining the culture conditions required for the formation of multicellular aggregates of *S. domuncula* from dissociated single cells; these are termed 'primmorphs', resembling organotypic cell cultures (Custodio et al., 1998; Müller et al., 1999). These aggregates, formed in seawater supplemented with antibiotics, have a tissue-like appearance, and have been cultured for more than five months. Cross sections through the primmorphs revealed an organised zonation into a distinct unicellular epithelial-like layer of pinacocytes and a central zone composed primarily of spherulous cells. After their association into primmorphs, the cells turn from the telomerase-negative state to the telomerase-positive state. Important is the finding that a major fraction of the cells in the primmorphs undergoes DNA synthesis and hence has the capacity to divide.

We propose that the primmorph system developed by us is a powerful novel model system to study basic mechanisms of cell proliferation and cell death; it can also be used in

aquaculture for the production of bioactive compounds and as bioindicator system.

SYSTEMATIC CONSIDERATIONS ON THE TWO SPONGE SUBPHYLA: HEXACTINELLIDA AND CELLULARIA. It has been proposed that Porifera should be divided into two subphyla, Cellularia (comprising Demospongiae and Calcarea), and Symplasma (containing only Hexactinellida) (Reiswig & Mackie, 1983). This classification reflects the fact that species belonging to the Cellularia are composed of uninuclear cells, while those in the Hexactinellida have syncytial tissues (Mackie & Singla, 1983). This fundamental structural difference raises the question whether the ancestors of the Metazoa in general, and the Porifera in particular, were colonial flagellates or syncytial ciliates.

The cDNAs coding for proteins which have been used to establish the classification of subphyla within the Porifera, in particular, and the monophyly of Metazoa in general, came from two Demospongiae: *Geodia cydonium* (Jameson) (Demospongiae, Tetractinomorpha, Astrophorida, Geodiidae), *Suberites domuncula* (Olivi) (Demospongiae, Tetractinomorpha, Hadromerida, Suberitidae); one Calcarea: *Sycon raphanus* (Schmidt) (Calcarea, Calcaronea, Leucosoleniida, Sycettidae); and two Hexactinellida *Rhabdocalyptus dawsoni* (Lambe) (Hexactinellida; Hexasterophora; Lyssacinosida; Rossellidae), *Aphrocallistes vastus* (Schulze) (Hexactinellida, Hexasterophora, Hexactinosida, Aphrocallistidae).

THE PHYLOGENETIC POSITION OF THE TWO SUBPHYLA OF PORIFERA. Two alternative hypotheses have been proposed to explain relationships between the major sponge classes. One groups the Porifera into the adelphotaxa Hexactinellida and Demospongiae/Calcarea based on the gross difference in tissue structure and on differences in the structure of the flagellae, whose beating generates the feeding current through sponges (Mehl & Reiswig, 1991). The other hypothesis assumes that the Demospongiae are more closely related to Hexactinellida based on presumed larval similarities (Böger, 1988).

MOLECULAR APPROACH: PROTEIN KINASE C. In order to approach this question the cDNA encoding a protein kinase C, belonging to the C subfamily from the hexactinellid sponge *R. dawsoni*, has been isolated and characterised (Kruse et al., 1998). The two conserved regions, the regulatory part with the pseudosubstrate site, the two zinc fingers and the C2 domain, as well as the catalytic domain were used for phylogenetic analyses. Sequence alignment and construction of a phylogenetic tree from the catalytic domains revealed that the hexactinellid *R. dawsoni* branches off first among the metazoan sequences; the other two classes, Calcarea (using the sequence from *S. raphanus*) and Demospongiae (using sequences from *G. cydonium* and *S. domuncula*) branch off later. The statistically robust tree also shows that the two cPKC sequences from the higher invertebrates *D. melanogaster* and *Lytechinus pictus* are most closely related to the calcareous sponge (Kruse et al., 1998) (Fig. 7A).

This finding was also confirmed by comparing the regulatory part of the kinase gene.

MOLECULAR APPROACH: 70KDA HEAT SHOCK PROTEIN. Previous analyses of the 70kDa heat shock protein isolated from the same sponge species (Koziol et al., 1997) justify the conclusion that: 1) within Porifera, the subphylum Hexactinellida diverged first from a common ancestor to the Calcarea and the Demospongiae, which both appeared later; and 2) the higher invertebrates are more closely related to the calcareous sponges (Müller et al., 1998).

ADDITIONAL SUPPORT FOR TWO SUBPHYLA: INSULIN-LIKE RECEPTORS. Further support came from the analysis of the autapomorphic character restricted to all the Metazoa including Porifera, the transmembrane receptor tyrosine kinases (RTKs). Recently, we screened for the presence of molecules grouped into one specific subfamily within the superfamily of the RTKs (which includes the insulin receptors (InsR), the insulin-like growth factor I receptors and the InsR-related receptors), all found in vertebrates, as well as the InsR-homologue from *D. melanogaster*. The cDNAs, encoding the putative InsR-homologues, were isolated from the hexactinellid sponge *A. vastus*, the demosponge *S. domuncula* and the calcareous sponge *S. raphanus* (Skorokhod et al., submitted).

Phylogenetic analyses of the catalytic domains of the putative RTKs showed that sponge polypeptides have to be grouped to the putative InsR-homologues. Relationships revealed that all sponge sequences fall into one branch, while the related sequences from higher Metazoa, including the invertebrate sequences from insects and molluscs, or polypeptide(s) from one

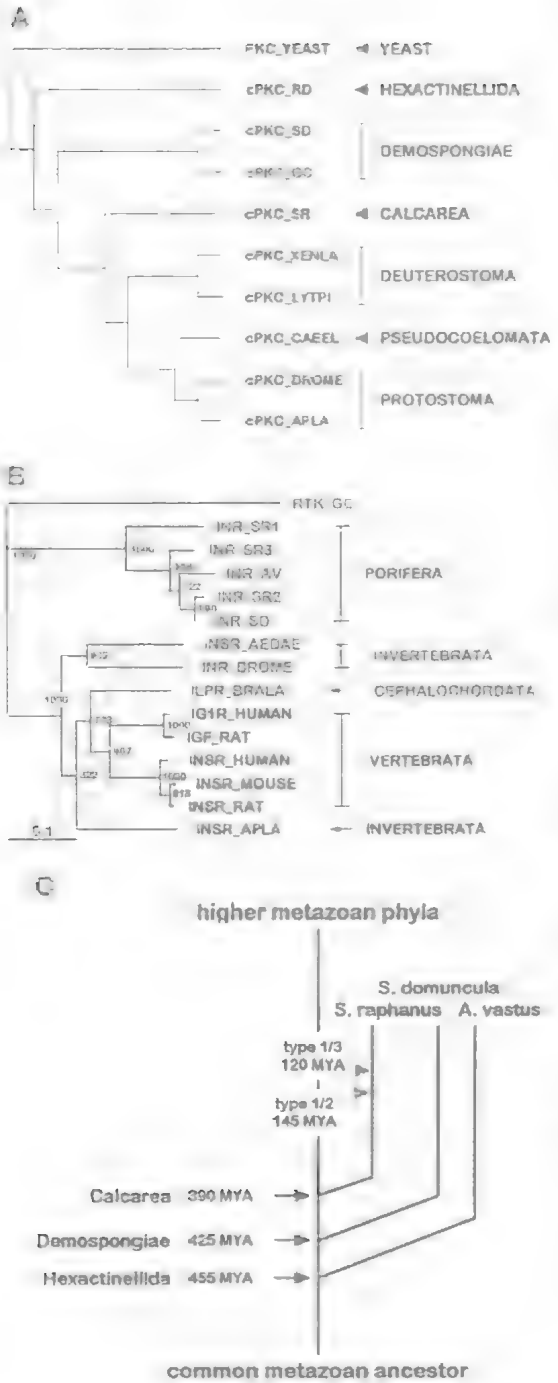

FIG. 7. Evolutionary position of Hexactinellida, Demospongiae and Calcarea. A, Phylogenetic tree based on the alignment of the catalytic domains of the deduced PKCs from: 1) Metazoa - cPKCs from the deuterostomes *Xenopus laevis* (frog - cPKC_XENLA) and *Lytechinus pictus* (sea urchin - PKC_LYTPI), from the protostomes cPKC *Drosophila melanogaster* (fruit fly - PKC_DROME) and *Aplysia californica* (mollusc, PKC_APLA); 2) Sponges of the classes Demospongiae, *Geodia cydonium* (CPKC_GC) and *Suberites domuncula* (CPKC_SD), Calcarea, *Sycon raphanus* (CPKC_SR), and Hexactinellida, *Rhabdocalyptus dawsoni* (CPKC_RD); 3) Yeast *Saccharomyces cerevisiae* (PKC_YEAST). B, Analysis of the insulin receptors (InsR), the insulin-like growth factor I receptors and the InsR-related receptors from vertebrates and invertebrates together with those from sponges. The deduced aa sequences of InsR homologues from the polypeptides of the three classes of Porifera: 1) Demospongiae: *S. domuncula* (INR_SD); 2) Calcarea *S. raphanus* type 1 (INR_SR1), *S. raphanus* type 2 (INR_SR2), *S. raphanus* type 3 (INR_SR3), and 3) Hexactinellida: *Aphrocallistes vastus* (INR_AV). All have been aligned with the related sequences for invertebrates: the insulin-like receptor precusor from the mosquito *Aedes aegypti* (INSR_AEDAE) and the InsR homologue from the fruit fly *Drosophila melanogaster* (INR_DROME) as well as the mollusc *Aplysia californica* InsR (INSR_APLA), one cephalochordate: the insulin-like peptide receptor precursor from amphioxus *Branchiostoma lanceolatum* (ILPR_BRALA) and from selected vertebrates: the human insulin-like growth factor 1 receptor precursor (IG1R_HUMAN), the human InsR precusor (INSR_HUMAN), the InsR precursor from the house mouse *Mus musculus* (INSR_MOUSE), the IGF-I-R I receptor precursor from the rat *Rattus norvegicus* (IGF_RAT) and the InsR precusor from *R. norvegicus* (INSR_RAT). The RTK domain from the sponge *G. cydonium* (accession number X77528) was used for comparison. The rooted phylogenetic tree of the catalytic domains of these sequences is shown. C, Proposed branching order of the three classes of Porifera (Hexactinellida, Demospongiae and Calcarea), from a common metazoan ancestor. In addition, the separtion of the three types of the *S. raphanus* InsR-homologues, type 1 from type 3, and type 1 from type 2, are also indicated. The dates of the approximate divergence time are indicated (MYA).

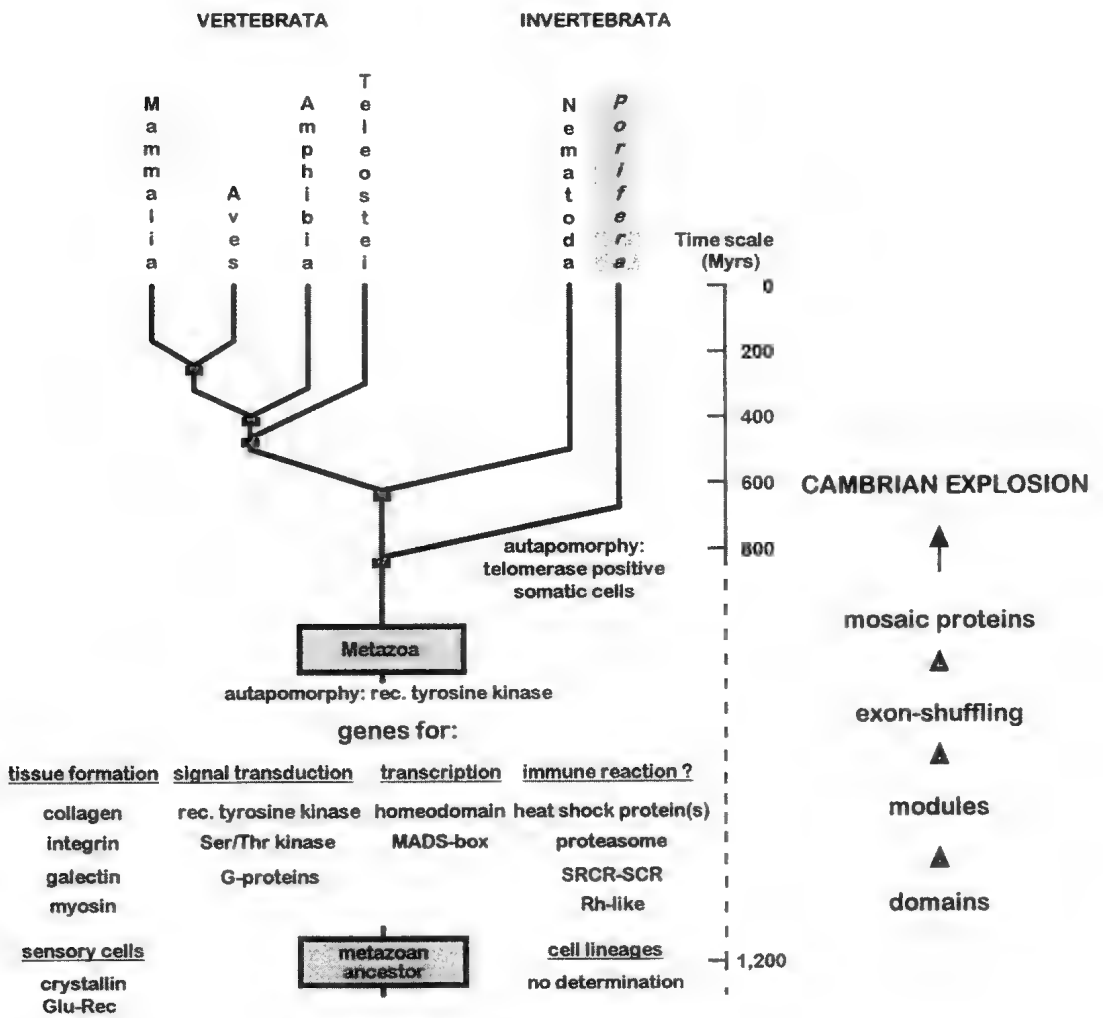

FIG. 8. Phylogenetic relationship of Porifera within the animal groups based on molecular biological data, obtained from sequences of 'metazoan' proteins required for tissue formation, signal transduction, transcription, immune reaction (potential) and sensory cells. The cell lineages in sponges are less determined than in higher Metazoa. It is proposed that the Cambrian explosion of metazoan radiation became possible after the creation of the evolutionary mechanism of modularisation of distinct protein domains, thus allowing the formation of mosaic proteins by exon-shuffling; this process happened approximately 1,000MYA. It is indicated that the presence of the telomerase activity is one autapomorphic character of Porifera.

cephalochordate and from selected vertebrates (human, mouse and rat) fall together into a second one.

Full length clones have been isolated from *S. raphanus*, that in addition to having the characteristic signatures for InsR-homologues, have one complete and one incomplete calcium binding epidermal growth factor receptor (EGF)-like domain in the extracellular regions.

Estimation of the rate of evolution of InsR-homologues revealed that the InsR-homologue of the hexactinellid sponge *A. vastus* is the phylogenetically oldest one (455MYA), while the molecules from the demosponge *S. domuncula* (425MYA) and the calcareous sponge *S. raphanus* (390MYA) are phylogenetically younger (Fig. 7B-C).

SPONGES AS LIVING FOSSILS. Based on the sequence data presented here it is reasonable to

state that Porifera should be placed into the kingdom Animalia together with the (Eu)Metazoa (Müller et al., 1994; Müller, 1995, 1997a). In addition, from the analysis of these first sponge genes, especially the one coding for RTK, it is now established that modular proteins, composed by exon-shuffling, are common to **all** metazoan phyla; a detailed description is given elsewhere (Müller & Müller, 1997). This mechanism of exon-shuffling is apparently absent in plants and protists (Patthy, 1995). If this view can be accepted then the burst of 'evolutionary creativity' (Patthy, 1995) during the period of Cambrian explosion, which resulted in the big bang of metazoan radiation (Lipps et al., 1992), was driven by the process of modularisation. During this process the existing domains were transformed into mobile modules, allowing the composition of mosaic proteins (Fig. 8).

As an example, the Ig-like domain is not an invention of Metazoa. Molecules featuring Ig-like domains appeared early in eukaryotic evolution (e.g. they are present in yeast a-agglutinin cell wall-associated protein; Chen et al., 1995). However, their use as modules, as building blocks, for the creation of mosaic proteins only became possible after a new step in evolution was acquired which allowed exon-shuffling. The mechanism of modularisation is more universal and more versatile - it can be applied to all preexisting domains - than the process of forming new domains. Therefore, it can be assumed that during the transition from Protozoa to Metazoa, a process which lasted approximately 1,000 million years, the formation of domains with distinct folds was at the center of evolution. After having reached a critical number of domains the mechanism of modularisation allowed a rapid formation of a series of mosaic proteins by exon-shuffling.

During the transition from unicellular Protista to multicellular Metazoa, the primary pattern of differentiation implies the presence of at least two different cell types, and as such the simplest multicellular organism could have consisted of one cell type specialised for feeding and the other for reproduction (Wolpert, 1990). This is in agreement with Roux-Weismann's original concept of primary separation of somatic and germinal cell lineages (Weismann, 1892), in which the immortal germen produces a mortal soma that will sustain the growth and reproduction of the organism but will necessarily perish. In view of the proposed monophyletic evolution of metazoans, and the position of sponges at the base of the evolution of multicellularity, we have addressed the question for those molecular mechanisms which underlie the evolution of the germ cell- and somatic cell-lineages, and the potential control of their immortality or their programmed senescence and death.

Sponges reproduce both asexually, by bud- and gemmule-formation, and sexually by production of gametes (reviewed in Simpson, 1984). But they lack special reproductive organs. The identification of putative stem cells for primordial germ cells in sponges has not been clearly provided, and the compelling morphological evidence for the origin of gametes from the somatic fully differentiated cells, such as choanocytes, argues against the clear separation of the germinal and somatic cell lineages. Preliminary experimental evidence has now been presented which reveals that sponge tissue is rich in telomerase activity, suggesting that the separation of cell lineages of somatic and germ stem cells has not been established, the determination of the fate of given sponge cells is still dynamic and might, under different physiological conditions, be reversible. It is proposed that the presence of telomerase activity is one autapomorphic character of Porifera (Fig. 8).

CONCLUSION

Our data show that sponges contain, as taken from deduced aa sequences, most structural elements known from higher Metazoa. It was intriguing to realise during the last three years that, while belonging to Metazoa, sponges 1) do have in some respect primitive, primordial metazoan characteristics, (e.g. simple elements of an immune system, with the 'multiadhesive protein' as an example), and 2) are already provided with complex and highly structurally evolved molecules not yet described from higher Metazoa such as the SRCR molecule. It is fortunate that sponges are not extinct. Assuming that Porifera were not the first metazoan phylum to evolve, they were witnesses to an evolutionary step that occurred during the maturation of the Metazoa near the Proterozoic-Phanerozoic boundary, close to 1 billion years ago. In this respect they can be considered to be living fossils.

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AN ULTRASTRUCTURAL STUDY ON THE CONTRACTILE PINACOCYTE OF A FRESHWATER SPONGE. *Memoirs of the Queensland Museum 44: 398. 1999:-* Contractile cells in sponges were first observed in the oscular diaphragm of marine species, *Microciona* and *Tedania* (Bagby, 1961). They consisted of well-differentiated myocytes having myosin and actin filaments, whereas in the freshwater sponges, there are no reports of contractile apparatus or myocytes.The oscular diaphragms and body walls of freshwater sponges are contractible when stimulated. Stimulation is effective whether it is osmotic, thermal, electric, etc. According to these observations on sponges we hypothesise that pinacocytes in the oscular diaphragm and body wall must bear the contractile apparatus, and we suggested in a previous report that these cells have a network of filamentous bundles. We subsequently attempted to structurally identify the contractile filaments of the cell using the following methods: 1) Observations under light microscopy; 2) Observations under electron microscopy; 3) SDS-PAGE; 4) NBD-phallacidin staining; 5) Anti-actin gold conjugation.Pinacocytes in these sponges showed a flat and multiangular shape, measuring about 5µm in diameter and 0.1µm thick. Pinacocytes in the outer layer of the oscular diaphragms and body wall had many bundles extending radially from the central nuclear zone to the peripheral region of the cell, whereas these bundles were not observed in the inner pinacocytes. Bundles were easily stained using NBD-phallacidin. Observations of thin sections showed these bundles are composed of many thin filaments of about 4-6nm diameter. These bundles ran straight in the contracted state, and were distributed in the basal region of the cell. Thin filaments in the bundle were clearly decorated with anti-actin gold conjugation. SDS-PAGE analysis of the diaphragm revealed a protein band of 45kD. These results support the idea that thin filaments of about 4-6nm in diameter in pinacocytes are composed of actin molecules. A freshwater sponge, *Ephydatia fluviatilis*, has no myocytes but has contractile pinacocytes with actin bundles. ❐ *Porifera, freshwater sponges, contractile filaments, ultrastructure, actin, pinacocytes.*

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AN EVALUATION OF MORPHOLOGICAL AND CYTOLOGICAL DATA SETS FOR THE PHYLOGENY OF HOMOSCLEROPHORIDA (PORIFERA: DEMOSPONGIAE)

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Muricy, G. 1999 06 30: An evaluation of morphological and cytological data sets for the phylogeny of Homosclerophorida (Porifera: Demospongiae). *Memoirs of the Queensland Museum* **44**: 399-409. Brisbane. ISSN 0079-8835.

Congruence and resolution of two independent data sets (morphology and cytology) were compared in a phylogenetic analysis of ten Mediterranean species representing four genera of Homosclerophorida (Demospongiae). Two aspiculate genera (*Oscarella*, *Pseudocorticium*) and two with a siliceous skeleton (*Plakina*, *Corticium*) were studied to assess the validity of the traditional classification recognizing two families Plakinidae and Oscarellidae, respectively with and without a skeleton, with *Discodermia polydiscus* (Tetractinomorpha) used as an outgroup. Cytological data showed highest consistency but poor resolution, support, and average performance, probably due to low number of informative characters. It was the only data set that supported the monophyly of Oscarellidae. Morphological data had better congruence and support than cytological data, and indicated the non-monophyly of Oscarellidae. Combined analyses yielded a 'total evidence' tree topologically similar to that of morphological data, although with greater bootstrap support (average 64.7% per branch). The combined data set should be chosen for classification of Homosclerophorida because it includes all evidence available and showed the best general performance with the indices measured. Therefore, a classification including *Oscarella* and *Pseudocorticium* within Plakinidae is supported over the alternative, less parsimonious option which recognizes Oscarellidae as monophyletic. ❐ *Porifera, Homosclerophorida, phylogeny, morphology, cytology, W Mediterranean.*

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Over the last few decades several new, non-morphological characters have been proposed for sponge taxonomy (e.g., embryological, cytological, biochemical and molecular data), in an effort to overcome long standing problems in traditional classification based solely on morphology. However, the relative merits of different types of characters have rarely been evaluated in a phylogenetic framework (but see Hajdu & Van Soest, 1996). The present study compares the phylogenetic information content of morphological and cytological data sets in the resolution of a particular phylogenetic problem in Porifera, viz. the monophyly of Oscarellidae Lendenfeld, 1887.

Homoscleromorpha Lévi, 1973, with its single order Homosclerophorida Dendy, 1905, is widely accepted as a monophyletic group (e.g., Bergquist, 1978; Hartman, 1982; Van Soest, 1987; Diaz & Van Soest, 1994). Its synapomorphic traits include viviparous cinctoblastula larvae, a basement membrane lining the pinacoderm and choanoderm, and both exo- and endopinacocytes being flagellated (Boury-Esnault et al., 1984, 1995; Diaz & Van Soest, 1994). Its outgroup relationships are still debated; despite the fact that a relationship to Calcarea, Calcaronea, has been postulated based on shared tetractinal symmetry of spicules and possession of amphiblastula larvae (Van Soest, 1984; Grothe & Reitner, 1988), it is more generally accepted that these are homoplastic traits and Homosclerophorida is more closely related to Demospongiae, Tetractinomorpha, due to the shared presence of siliceous tetractinal calthrops spicules (Diaz & Van Soest, 1994). Homosclerophorida is usually divided into two families, Plakinidae Schulze, 1880 with five genera bearing tetractinal spicules and derivatives (*Corticium* Schimdt, 1862, *Plakina* Schulze, 1880, *Plakinastrella* Schulze, 1880, *Plakortis* Schulze, 1880, and *Placinolopha* Topsent, 1897; see Diaz & Van Soest, 1994), and the monotypical Oscarellidae with genus *Oscarella* Vosmaer, 1884 without a skeleton (Lévi, 1973; Bergquist, 1978; Hartman, 1982). The recently described genus *Pseudocorticium*

TABLE 1. Species of Homosclerophorida studied from Marseille, France. *Discodermia polydiscus* is an outgroup (Tetractinomorpha: Lithistida).

Species	Author	Site of Collection	Depth (m)	Specimens examined
Pseudocorticium jarrei	Boury-Esnault et al., 1995	Jarre Cave	15-20	25
Corticium candelabrum	Schmidt, 1862	La Vesse, Jarre Is.	5-20	30
Oscarella lobularis	(Schmidt, 1862)	La Vesse	15-35	27
Oscarella tuberculata	(Schmidt, 1868)	La Vesse, Jarre Is., Gameau Cave	5-35	32
Oscarella microlobata	Muricy et al., 1996	Jarre and Gameau Caves	15-20	8
Oscarella viridis	Muricy et al., 1996	Jarre Cave	15-20	6
Plakina monolopha	Schulze, 1880	Grand Congloue	15	3
Plakina trilopha	Schulze, 1880	Jarre Cave	15-20	12
Plakina endoumensis	Muricy et al., 1998	Endoume Cave	2-5	8
Plakina jani	Muricy et al., 1998	Gameau Cave	15-20	15
Discodermia polydiscus	Du Bocage, 1869	Gameau Cave	15-20	10

Boury-Esnault et al., 1995, although aspiculate, is more similar to *Corticium* than to *Oscarella* in its biochemical characters (allozyme patterns; Solé-Cava et al., 1992, as *Corticium* sp.) and in several morphological characters (presence of a cortex, leuconoid aquiferous system with diplodal chambers, cartilaginous consistency, perforated surface; Boury-Esnault et al., 1995). This led to a proposal to abandon the taxon Oscarellidae and to merge all homoscleromorph genera into a single family Plakinidae (Solé-Cava et al., 1992; Diaz & Van Soest, 1994; Boury-Esnault et al., 1995). The problem at the level of character evolution is whether the absence of skeleton in *Oscarella* and *Pseudocorticium* is a convergence, a plesiomorphy, or a synapomorphy. Phylogenetic relationships among genera in Homosclerophorida remain unstudied, and therefore the validity of Oscarellidae, containing *Oscarella* and *Pseudocorticium* (both aspiculate), is questionable. This is currently a major phylogenetic problem in the classification of Homosclerophorida.

In this study, the consistency and resolution of two data sets (morphological and cytological) were compared in a phylogenetic analysis of ten Mediterranean species in four genera of Homosclerophorida (viz., *Oscarella*, *Plakina*, *Corticium* and *Pseudocorticium*). *Discodermia polydiscus* Du Bocage, 1869 (Tetractinomorpha: Lithistida: Theonellidae), was used as outgroup in all analyses. Although *Discodermia* is part of a problematic group, the lithisthid sponges (family Theonellidae Lendenfeld), its close relationships with the Astrophorid families Ancorinidae Schmidt and Geodiidae Gray have been demonstrated by analyses of both morphological data and DNA sequences (Kelly-Borges & Pomponi, 1994). The hypothesis that ectosomal discotrienes of *Discodermia* are homologous (although distantly related) to plakinid calthrops, both having derived from a common tetractinal ancestor spicule, is implicit in the choice of this particular outgroup. Cytological characters have been proposed as useful taxonomic tools at the species level, both in Homosclerophorida (Boury-Esnault et al., 1984, 1992, 1995; Muricy et al., 1996, 1999) and in other sponge groups (e.g. Simpson, 1968; Pomponi, 1976; Vacelet & Donadey, 1987; Boury-Esnault et al., 1994). The present study aimed to compare the congruence and resolution of phylogenetic estimates derived from morphological and cytological data sets at the genus and family levels in Homosclerophorida. The two individual and combined data sets were also compared as to their support for the monophyly of Oscarellidae. Alternative hypotheses on phylogenetic relationships between the four genera of Homosclerophorida studied are discussed.

MATERIALS AND METHODS

Samples of ten homosclerophorid species and one outgroup species (Table 1) were collected using SCUBA between 1984-1995 from caves and vertical walls at 10-30m depth along the coast of Provence, Western Mediterranean, France (5°20'W, 43°10'N). For the morphological study, samples were fixed in formalin or glutaraldehyde/ sodium cacodylate, dehydrated in an alcohol series and included in Araldite. Thick and semi-thin sections were stained with toluidine blue and studied under light microscopy (LM). Spicules were observed under LM or scanning electron microscopy

(SEM) after sputter-coating with gold-palladium. Complete descriptions of each species studied and their important morphological and cytological characters are given elsewhere (Schulze, 1880; Ridley & Dendy, 1887; Topsent, 1895; Boury-Esnault et al., 1984, 1992, 1995; Muricy et al., 1996, 1998, 1999). Vouchers were deposited at the sponge collections of the Muséum National d'Histoire Naturelle, Paris (MNHN) and the Museu Nacional of the Universidade Federal do Rio de Janeiro, Brazil (UFRJPOR). For the cytological study, specimens were fixed as described by Boury-Esnault et al. (1984): glutaraldehyde 2.5% in a mixture of 0.4M cacodylate buffer and sea water (4 vol.: 5 vol.; 1120mOsm) and postfixed in 2% osmium tetroxide in sea water. Thin sections, contrasted with uranyl acetate and lead citrate, were observed under TEM in a Hitachi Hu 600 microscope. Cytological data on *Discodermia polydiscus* are based on the author's unpublished observations and those of N. Boury-Esnault (pers. comm., 1993).

All parsimony analyses were carried out using PAUP 3.0 (Swofford, 1991). In all searches, the following options were used: algorithm = branch and bound; keep minimal trees only; addition sequence furthest; collapse zero-length branches; multistate taxa = polymorphisms; optimization = ACCTRAN; rooting by outgroup (=*Discodermia polydiscus*); all characters unordered, with equal weights. Dependence between characters was checked by searching the data matrix for characters with identical distribution of states among taxa (Appendices 1,2). Although some characters were excluded by this method (e.g. presence/absence of spicules and of an ectosomal skeleton), total similarity in distribution of several characters was considered fortuitous (i.e. not artifacts of character coding), and these traits were thus kept in the phylogenetic analyses (characters 7, 18; 13, 15, 32, 33, 34; 16, 17; 19, 20; 21, 22; 23, 24; 38, 40; 41, 42). Presence/ absence of spicules was excluded from the analyses because it was a key-character in the hypotheses tested, and to avoid redundancy with characters 19-26 (spicule types). Its evolution was traced *a posteriori* using MacClade 3.0 (Maddison & Maddison, 1992), as well as that of all other unambiguous character-state changes. One thousand minimal random trees (MRTs) were generated and their length distribution was compared to the most parsimonious trees obtained for the real data. The observed length of the most parsimonious trees never exceeded that of the shortest randomized trees, and therefore it was accepted that the original data differed significantly ($P<0.001$) from randomness in all data sets. The following information was recorded for each individual and combined data sets: number of characters, number of informative characters, number of most parsimonious trees (MPTs), length of MPTs, number of extra steps (e), consistency index (CI), homoplasy index (HI), rescaled consistency index (RC), difference in steps between MRTs and MPTs, majority-rule consensus tree, number of branches involved in polytomies, bootstrap support for each branch, and unambiguous character-state changes supporting each branch (Swofford, 1991). These parameters measure either the internal consistency (congruence) of each data matrix, the resolution of phylogenetic estimates, or the support for the phylogenies derived from the different data sets. Bootstrap support for branches in consensus trees (Felsenstein, 1985; Li & Zharkikh, 1994) was calculated by 100 replicated branch and bound searches, with the same options held constant as described above. The number of extra steps in each analysis was used to measure incongruence due to disparity within and between data sets, analogous to analysis of variance (Mikevich & Farris, 1981; Kluge, 1989). Individual and combined data sets were ranked according to their relative performances on average for all indices measured, with the exception of extra steps and rescaled CI, which were redundant with HI and CI, respectively.

RESULTS

Of 30 morphological characters, 24 (80%) were phylogenetically informative for parsimony analysis, which resulted in three MPTs 67 steps long, with a fully-resolved majority-rule consensus tree (Fig. 1). MPTs of morphological data set required 14 extra steps, with intermediate consistency (CI=0.79, RC=0.61), and relatively high homoplasy (HI=0.25; Table 2). The majority-rule consensus tree (Fig. 1) supported monophyly of both *Plakina* and *Oscarella* (bootstrap=82-89%), but suggested non-monophyly of Oscarellidae. The alternative clade [*Plakina* + *Oscarella*] is supported by relatively low bootstrap (49%), but also by seven synapomorphies: small lobes, surface wrinkled, soft consistency, sylleibid canal system, eurypylous choanocyte chambers, ectosome between 5-50mm thick, and proportion of mesohyl to chambers less than 1:1 (Fig. 1). *Corticium*

TABLE 2. Summary of information on Homosclerophorida congruence. Abbreviations: MPTs, most parsimonious trees; MRTs, minimal random trees; e, number of extra steps; CI, consistency index; HI, homoplasy index; RC, rescaled consistency index.

Information	Morphology	Cytology	Combined
Character numbers	1-30	31-47	1-47
No. of characters	30	17	47
No. of informative characters	24	9	33
No. of MPTs (length)	3 (67)	7 (25)	1 (95)
CI	0.79	0.84	0.78
RC	0.61	0.65	0.57
HI	0.25	0.16	0.25
e	14	4	21
No. of MRTs (length)	1 (80)	1 (28)	4 (115)
MRTs minus MPTs	13	3	20
No. of branches in polytomies	0	2	0
No. of unambiguous changes	25	4	32
Mean bootstrap per branch (%)	62.2	40.7	64.7

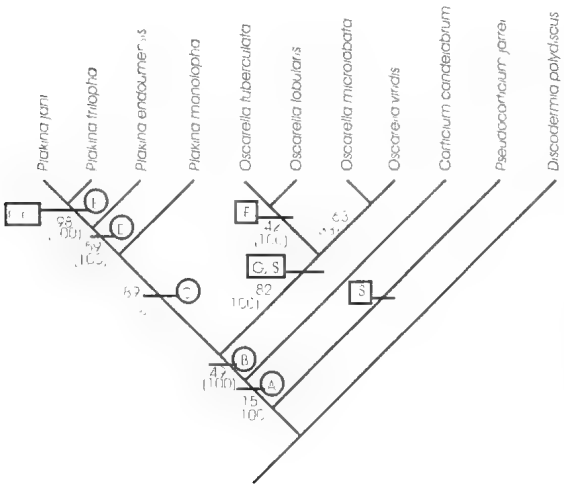

FIG. 1. Majority-rule consensus of 3 most parsimonious trees of the morphological data set. Numbers beside branches are bootstrap support for the branch, and the percentage of MPTs displaying the branch (within parenthesis). Synapomorphies (within circles) are indicated as 'character.state': A = 4.1; B = 5.1, 6.1, 10.4, 11.1, 12.1, 16.2, 17.2; C = 19.1, 20.1, 27.2; D = 23.1, 24.1, 25.1; E = 1.1, 6.0, 7.1, 10.3, 14.1, 16.1, 17.1, 18.1. Homoplasies (within rectangles) are: F = 5.2; G = 2.1, 4.2; S = absence of spicules. For the names of characters and states see Appendices 1-2.

candelabrum is placed as sister-group of the clade [*Plakina* + *Oscarella*], with *Pseudocorticium jarrei* as the most basal ingroup branch. Complete loss of spicules appeared convergently in *Oscarella* and *Pseudocorticium*. Bootstrap support per branch was intermediate among all analyses (average 62.2%). Morphological data were more informative for skeleton-bearing than for aspiculate species, as expected, since skeletal characters comprised 13 out of 30 morphological characters included in the analysis.

Parsimony analysis of cytological data yielded seven MPTs, of length 25 (4 extra steps). The cytological data set showed the smallest amount of homoplasy among all analyses (CI=0.84, RC=0.65, HI=0.16), but also the smallest number of informative characters (9 of 17; Table 2). All MPTs and their consensus (Fig. 2) supported the monophyly of Oscarellidae (with 54% of bootstrap support), with both genera *Oscarella* and *Plakina* indicated as paraphyletic, and *Corticium* placed in the most basal ingroup branch. Absence of spicules appeared as a synapomorphy for *Oscarella* and *Pseudocorticium*. Support to all resolved branches was generally low (average bootstrap 40.7%, 0-2 synapomorphies per branch). This was the only data set that suggested monophyly of Oscarellidae, including *Pseudocorticium jarrei* within the genus *Oscarella* as sister-species of *O. microlobata*. The clade [*Oscarella* + *Pseudocorticium*] is supported by synapomorphies 31.1 (triangular apopylar cells) and 44.0 (absence of sclerocytes). Cytological characters allowed better resolution and stronger support to the aspiculate homosclerophorid species than to the skeleton-bearing *Plakina* and *Corticium*.

The combined data set provided 33 informative characters (70.2%), which resulted in one MPT, 95 steps long (21 extra steps). As the combined data included all the homoplasy (incongruence) hypothesized within each data set and between data sets, consistency was slightly lower than that of any individual analyses (CI=0.78; RC=0.57; HI=0.25; Table 2). The single MPT (Fig. 3) supported the monophyly of both *Plakina* and *Oscarella*, with significant bootstrap (72-87%). It also supported the non-monophyly of the Oscarellidae, showing *Corticium* as the sister group of a clade [*Plakina* + *Oscarella*] which was supported by

TABLE 3. Rankings of data sets according to their performance in each index measured (data from Table 2) and on average. Abbreviations as for Table 2.

Index	Morphology	Cytology	Combined
No. of characters	2	3	1
No. of informative characters	2	3	1
No. of MPTs	2	3	1
CI	2	1	3
HI	2	1	2
MRTs minus MPTs	2	3	1
No. of branches in polytomies	1	3	1
No. of unambiguous changes	2	3	1
Mean bootstrap per branch (%)	2	3	1
Sum of rankings	17	23	12
Average ranking	2	3	1

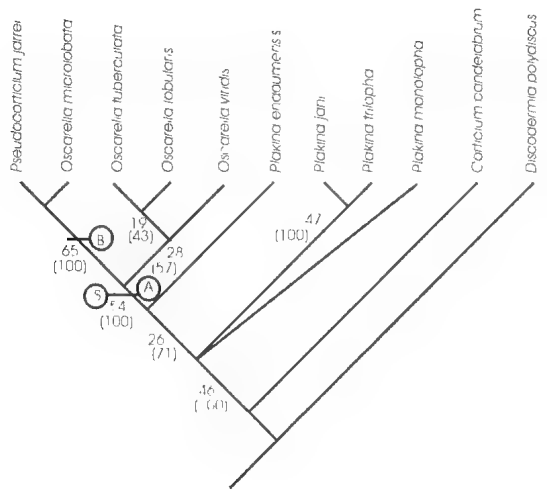

FIG. 2. Majority-rule consensus of 7 most parsimonious trees of the cytological data set. Key as for Figure 1. Synapomorphies (within circles) are indicated as 'character.state': A = 31.1, 44.0; B = 38.1, 40.1; S = absence of spicules. For the names of characters and states see Appendices 1-2.

66% of bootstrap and by 8 synapomorphies (small lobes, wrinkled surface, soft consistency, sylleibid canal system, eurypylous choanocyte chambers, ectosome between 5-50mm thick, proportion of mesohyl to chambers less than 1:1, and collencytes absent). Absence of spicules appeared homoplastic in *Oscarella* and *Pseudocorticium*, which was placed as the most basal ingroup branch.

Although the topologies suggested by the two data sets analyzed in this study were heterogeneous, their combination slightly increased the repeatability (as measured by the mean bootstrap support per branch) of the phylogenetic estimate when compared to the individual analyses. In general, the results of the combined data set were very similar in topology, resolution and support to those of the morphological data set, differing only in the relationships within *Oscarella*. The number of extra steps in each individual and combined analyses provided a direct measure of the incongruence within and between data sets. Of a total of 21 extra steps required by the most parsimonious trees for the combined data set, 86% (14+4) were due to incompatibility between characters within each data set, leaving 3 extra steps (14%) which reflect the incongruence between the two data sets (i_{mf}, Mikevich & Farris, 1981). Incongruence between sets was lower than within sets (16-20.1% in each of the individual analyses). A ranking of the average performance of individual and combined data sets (Table 3) was calculated based on their performance in each index measured (Table 2). The combined data set ranked best, followed closely by the morphological data set, and more distantly by the cytological data set.

DISCUSSION

Each of the two independent and combined data sets analyzed suggested a different phylogenetic reconstruction for Homosclerophorida, with varying degrees of resolution and support. The morphological data set showed better general performance when compared to the cytological data set (Tables 2, 3). Morphological data (particularly skeletal characters) are traditionally the major source of phylogenetic information for sponge taxonomists. Skeletal morphology provides valuable information in groups with high spicule diversity, such as Astrophorida and Poecilosclerida (e.g. Maldonado, 1993; Hajdu & Van Soest, 1996), but is of little use in skeleton-lacking species or in groups with low skeletal diversity such as Haplosclerida (e.g. Boury-Esnault et al., 1995; Muricy et al., 1996; Vacelet & Donadey, 1987; De Weerdt, 1989). Non-skeletal morphological characters (e.g., shape, surface, aquiferous system) comprised 17 out of 30 morphological characters studied, and 14 were informative for phylogenetic analysis.

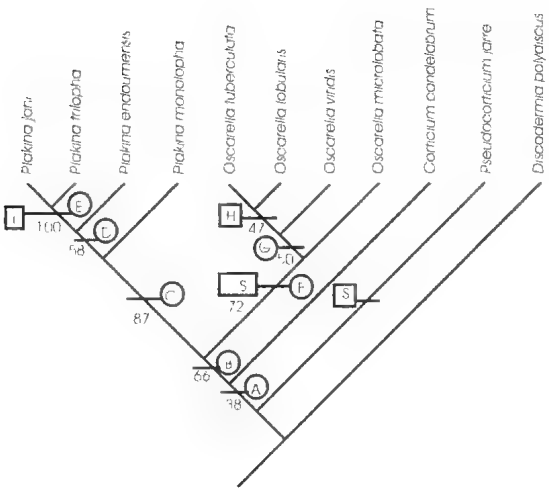

FIG. 3. Most parsimonious tree of the combined data set. Numbers beside branches are bootstrap support for each branch. Synapomorphies (within circles) are indicated as 'character.state': A = 4.1; B = 5.1, 6.1, 10.4, 11.1, 12.1, 16.2, 17.2, 45.0; C = 19.1, 20.1, 27.2; D = 23.1, 24.1, 25.1; E = 1.1, 5.2, 6.0, 7.1, 10.3, 14.1, 16.1, 17.1, 18.1, 47.1; F = 44.0; G = 46.0; H = 5.2. Homoplasies (within rectangles) are: I = 2.1, 4.2; S = absence of spicules. For the names of characters and states see Appendix 1.

Such non-skeletal characters, particularly the anatomy of the aquiferous system, can also be useful for the taxonomy of Homosclerophorida and other sponge taxa (e.g. Langenbruch, 1991; Bavestrello et al., 1995), and should be taken into account more often in sponge taxonomy.

The cytological data set showed poorest resolution, support, and general performance, although it had the highest consistency indices of all data sets. This was probably due to the low number of informative cytological characters. Cytology was more informative in *Oscarella* and *Pseudocorticium* than in *Plakina* and *Corticium*. This was expected since, in Homosclerophorida, the aspiculate species show greater diversity of cells with inclusions than the skeleton-bearing species (Boury-Esnault et al., 1992, 1995; Muricy et al., 1996, 1999). Cytological data also have proved useful for the systematics of other sponge groups such as Poecilosclerida, Haplosclerida and Hadromerida (e.g., Simpson, 1968; Pomponi, 1976; Boury-Esnault et al., 1994), and should be more frequently used in phylogenetic studies of all Porifera. However, phylogenetic interpretation of sponge cytology is presently difficult due to the scarcity of information available on cell function and chemistry, particularly of the so-called 'cells with inclusions' (Simpson, 1984). An increase in number of functional and cytochemical studies of sponge cells would greatly add phylogenetic information to cytological data.

How to choose the 'best' data set upon which classification should be based? One approach is to follow the principle of maximum evidence (e.g. Kluge, 1989), according to which the results of the combined analysis should be taken as a basis for classification of Homosclerophorida, since it was built with the maximum amount of independent evidence available. This approach has only been criticized when high between-set incongruence is found, because it may increase phylogenetic 'noise' and therefore reduce the accuracy of the combined analysis (Shaffer et al., 1991; Chippindale & Wiens, 1994; Wiens & Chippindale, 1994). Incongruence between data sets in Homosclerophorida (i_{mf}=0.14) is relatively low when compared to that found in other organisms (Shaffer et al., 1991; Olmstead & Sweere, 1994; Omland, 1994, and references therein). Therefore, it seems advisable in this case to base the classification on a combined data set. Another logical approach is to rank the data sets by their relative performances in each index of congruence, resolution and support recorded, and the data set with the best average performance would be chosen for classification. This approach is shown in Table 3, in which the sets were ranked according to data in Table 2. It shows that, on average, the combined data set performed better than any individual data sets. This approach also supports using the combined data set as a basis for classifying the Homosclerophorida.

The topology derived from the combined data set supported monophyly of *Oscarella* and *Plakina* and non-monophyly of Oscarellidae, with absence of spicules appearing as a homoplastic character in *Oscarella* and *Pseudocorticium*. Therefore, with the currently available data, a classification of Homosclerophorida with a single family Plakinidae Schulze (including Oscarellidae Lendenfeld and Corticiidae Vosmaer), as recently adopted by Solé-Cava et al. (1992), Diaz & Van Soest (1994), and Boury-Esnault et al. (1995), is preferred over the traditional classification with two families Plakinidae and Oscarellidae (Lévi, 1973; Bergquist, 1978; Hartman, 1982).

The analysis of independent data sets

obviously help to increase the amount of independent evidence upon which the phylogenetic hypotheses are based, and to reveal conflicts between data that can have strong influence in the choice of a classification. Furthermore, comparison of different data sets allows the determination of a 'phylogenetic confidence interval' for the phylogenetic hypothesis, which includes the topologies suggested by the individual and combined data sets (Bull et al., 1993; Huelsenbeck et al., 1994). Incongruence between data sets suggests that probably none of them has given the exact answer, and this should caution against changes in classification in face of new, conflicting data, before the congruence of the new evidence is more carefully evaluated. Therefore, the monophyly of Oscarellidae suggested by old classifications and by the cytological data set, although with relatively low support, cannot be completely ruled out at the current state of knowledge on biology of Homosclerophorida. It is kept as an alternative, less parsimonious hypothesis of phylogeny, together with the possibility of paraphyly of both *Oscarella* and *Plakina* suggested by cytological data, which should also be taken into account in studies on phylogeny of Homosclerophorida.

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APPENDIX 1. Morphological and cytological characters and character-states used in phylogenetic analysis. Uninformative characters are marked with an asterisk (*).

MORPHOLOGICAL CHARACTERS.

1. Shape: 0-spherical; 1-thin spreading; 2-lobate/spreading; 3-small circular; 4-cushion; 5-lobate/pending.

2. Size (surface cover in cm^2): 0-small (<10); 1-large (>10).

3. Thickness (mm): 0-thin (1-5); 1-medium (5-20); 2-thick (20-50).

4. Fixation: 0-firm; 1- by thin filaments; 2-loose but without filaments.

5. Size of lobes (mm): 0-absent; 1-small (1-5); 2-medium (5-20); 3-large (20-50).

6. Surface: 0-smooth; 1-wrinkled; 2-perforated.

7. Arrangement of ostia: 0-dispersed; 1-alveolar pattern.

8*. Oscula: 0-low; 1-with elevated rim.

9. Colour: 0-white/cream; 1-light brown; 2-yellow; 3-green; 4-variable.

10. Consistency: 0-rigid; 1-cartilaginous; 2-semi-cartilaginous; 3-firm but compressible; 4-soft; 5-fragile.

11. Canal system: 0-leuconoid; 1-sylleibid.

APPENDIX 1 (cont.). Morphological and cytological characters and character-states used in phylogenetic analysis. Uninformative characters are marked with an asterisk (*).

12. Choanocyte chambers type: 0-aphodal or diplodal; 1-eurypilous.

13*. Chamber diameter (μm): 0-small (10-30); 1-large (30-70).

14. Subdermal cavities: 0-absent; 1-present.

15*. Basal cavity: 0-absent, 1-present.

16. Ectosome thickness (μm): 0-greater than 100; 1-between 50 and 100; 2-between 5 and 50.

17. Proportion of mesohyl to chambers: 0-greater than 2; 1-between 1 and 2; 2- less than 1.

18. Spicule malformations: 0-absent; 1-present.

19. Diods: 0-absent; 1-present.

20. Triods: 0-absent; 1-present.

21. Smooth calthrops: 0-absent; 1-present.

22. Monolophose calthrops: 0-absent; 1-present.

23. Dilophose calthrops: 0-absent; 1-present.

24. Trilophose calthrops: 0-absent; 1-present.

25. Tetralophose calthrops: 0-absent; 1-present.

26*. Candelabra (heterolophose calthrops): 0-absent; 1-present.

27. Position of first ramification in lophose actines: 0-absent; 1-proximal; 2-medial.

28*. Second ramification in lophose actines: 0-absent; 1-distal.

29. Lophose actine ends: 0-absent; 1-simple; 2-with terminal spines.

30*. Desmata, oxea, and discotriaenes: 0-absent; 1-present.

CYTOLOGICAL CHARACTERS.

31. Apopylar cells: 0-absent; 1-triangular; 2-ovoid, with large osmiophilic inclusions.

32*. Flagellum in pinacocytes: 0-absent; 1-present.

33*. Pinacocyte shape: 0-'T-shaped'; 1-flat/ovoid.

34*. Basement membrane: 0-absent; 1-present.

35. Vacuolar cells type A: 0-absent; 1-present.

36*. Vacuolar cells type B: 0-absent; 1-normal, sparse; 2-turgid, in groups.

37. Vacuolar cells type C: 0-absent; 1-present.

38. Paracrystallin inclusions cells: 0-absent; 1-present.

39*. Homogeneous inclusions cells: 0-absent; 1-present.

40. Single inclusion cells: 0-absent; 1-present.

41*. Crescent cells: 0-absent; 1-present.

42*. Microgranular inclusions cells: 0-absent; 1-present.

43*. Spherulous/microgranular inclusions cells: 0-absent; 1-present.

44. Sclerocytes: 0-absent; 1-present.

45. Collencytes: 0-absent; 1-present.

46. Endosymbiont bacteria: 0-few, sparse; 1-diverse, abundant.

47. Distribution of endosymbiont bacteria in the mesohyl: 0-random uniform; 1-patchy; 2-close to choanocytes; 3-far from choanocytes.

APPENDIX 2. Data matrix of the combined data set (characters 1-24). Names of characters and character-states are listed in Appendix 1.

Taxon	Character																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Pseudocorticium jarrei	5	1	1& 2	0	3	2	0	1	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0
Plakina jani	1	1	1	2	2	0	1	1	2	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Plakina trilopha	1	1	1	2	2	0	1	1	0	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Plakina endoumensis	3	0	0	1	1	1	0	1	0	4	1	1	1	0	1	2	2	0	1	1	1	1	1	1
Plakina monolopha	3	0	0	1	1	1	0	1	0	4	1	1	1	0	1	2	2	0	1	1	1	1	0	0
Oscarella tuberculata	2	1	1& 2	2	2	1	0	1	4	2	1	1	1	0	1	2	2	0	0	0	0	0	0	0
Oscarella lobularis	2	1	1& 2	2	2	0	0	1	4	4	1	1	1	0	1	2	2	0	0	0	0	0	0	0
Oscarella microlobata	2	1	1	2	1	1	0	1	1	4	1	1	1	0	1	2	2	0	0	0	0	0	0	0
Oscarella viridis	2	1	1	2	1	1	0	1	3	5	1	1	1	0	1	2	2	0	0	0	0	0	0	0
Corticium candelabrum	4	0	1	1	0	2	0	1	1	1	0	0	1	0	1	0	0	0	0	0	1	1	0	0
Discodermia polydiscus	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

APPENDIX 2 (continued). Data matrix of the combined data set (characters 25-47). Names of characters and character-states are listed in Appendix 1.

Taxon	Character																						
	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47
Pseudocorticium jarrei	0	0	0	0	0	0	1	1	1	1	0	0	1	1	1	1	0	0	0	0	1	1	3
Plakina jani	1	0	2	1	2	0	2	1	1	1	0	0	1	0	0	0	0	0	0	1	0	1	1
Plakina trilopha	1	0	2	0	1	0	2	1	1	1	0	0	0	0	0	0	0	0	1	0	1	1	1
Plakina endoumensis	1	0	1	0	2	0	2	1	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0
Plakina monolopha	0	0	2	0	1	0	2	1	1	1	0	0	0	0	0	0	0	0	0	1	0	1	0
Oscarella tuberculata	0	0	0	0	0	0	1	1	1	1	0	2	0	0	0	0	0	0	0	0	0	0	0
Oscarella lobularis	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0
Oscarella microlobata	0	0	0	0	0	0	1	1	1	1	1	0	0	1	0	1	0	0	1	0	0	1	0
Oscarella viridis	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	1	1	0	0	0	0	0
Corticium candelabrum	1	1	1	0	1	0	2	1	1	1	0	0	0	0	0	0	0	0	0	1	1	1	2
Discodermia polydiscus	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0

THE PHYLOGENETIC HISTORY OF SPONGES IN PALAEOZOIC TIMES. *Memoirs of the Queensland Museum 44: 410. 1999*:- According to molecular biological analysis, the origin of the phylum Porifera dates back about 800MY. The first sponges were probably aspicular, like the so far oldest definite sponge *Palaeophragmodictyon* from the Late Proterozoic of Ediacara (the even older Duoshantuo fossils may reverse the picture again if the sponge interpretation holds true). Early Cambrian sponge assemblages were dominated by the Hexactinellida, but by the time of the Atdabanian the Pinacophora (Demospongiae/Calcarea-taxon) were also well represented. The Archaeocyatha can be considered as a stem lineage representative of this group. Early Cambrian Calcarea comprise modern-appearing forms as well as the exclusively Palaeozoic Heteractinellida and Polyactinellida, the latter group exhibits triradiate calcitic spicules, which are probably a constituent character of the taxon Calcarea. Within the Demospongiae, the tetraxon is considered the basic spicular symmetry, from which the other spicula-types have derived. Oxyasters from the Early Cambrian, which are in the size range of megascleres and show well-developed central canals, may have evolved from tetraxone mesotriaenes, whereas the large Middle Cambrian sigmata are probably derived oxeas. This means that the differentiation in mega-and microsclerocytes known from recent demosponges may have taken place at a later stage of poriferan evolution. The first desmata-bearing demosponges ('Lithistida') of the group Anthaspidellidae, Orchocladina-known since the Middle Cambrian-probably originated from reticulated monaxonid precursors close to the Hazeliidae. During the Late Ordovician, the chiastoclones developed from anthaspidellid dendroclones, and the Palaeozoic groups Tricranocladina and Sphaerocladina may have derived from chiastoclonellid ancestors. Contrary to widely accepted hypotheses, there seems to be no direct phylogenetic line from the Orchocladina to the modern Tetracladina, since the origin of tetraxial desmata from anaxial chiastoclones is very unlikely. The earliest true tetraclones with definite axial canals are documented from the Permian *Jereina robusta*, whereas the first phyllotriaenes of the modern spirasterophoran type are known since the Late Triassic. Because of their skeletal architecture, the Palaeozoic Saccospongiidae and Orchocladina, as well as theTricranocladina and Sphaerocladina which most probably evolved from the Orchocladina, are now attributed to the Sigmatophora. The 'megamorine' Saccospongiidae probably originated from a monaxonid group close to the Halichondritidae, but the Palaeozoic heloclones and megaclones as well as the elongate rhizoclones of the Haplistiidae are probably not phylogenetically linked to the modern Megamorina or Rhizomorina. Mesozoic and Recent Rhizomorina are characterised by skeletons of exclusively small rhizoclone desmata with sigmaspires as microscleres. But the sigmaspire is unknown from the fossil record and almost certainly has no connection with the sigmatophoran sigmata, which are known since the Middle Cambrian. At the end of the Permian, the Palaeozoic 'Lithistida', maybe with the exception of the Sphaerocladina, had all become extinct. Against widely accepted ideas, there is probably no phylogenetic link from the Tricranocladina to the modern Corallistidae (Dicranocladina). The Sphaerocladina, which have recently been documented also from the Early Palaeozoic, may have lead to the Mesozoic Neosphaerocladina, but no connection can be documented between these groups and Recent genera sometimes attributed to the Sphaerocladina, such as *Crambe* or *Vetulina*. Lophocalthropses of the Plakinidae first occurred in the Early Carboniferous and are connected by transitional forms to the candelabra of the modern Homoscleromorpha, known since the Early Cretaceous. The characteristic Plakinidae spicules probably originated from the same type of tetraxones, which lead to the first dichotriaenes in the Early Carboniferous. Thus the Plakinidae/ Homoscleromorpha are probably the sister group of the Spirasterophora, to which most modern 'Lithistida' belong. ❐ *Porifera, phylogeny, Palaeozoic sponges, skeletal architecture, Calcarea, Demospongiae, Hexactinellida.*

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RELEASE OF ALLELOCHEMICALS BY THREE TROPICAL SPONGES (DEMOSPONGIAE) AND THEIR TOXIC EFFECTS ON CORAL SUBSTRATE COMPETITORS

GREGORY K. NISHIYAMA AND GERALD J. BAKUS

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Three sponge species (*Xestospongia*, *Acervochalina*, *Plakortis* spp.) from Mactan I., Philippines, were shown to release allelochemicals directly into the water. These allelochemicals were demonstrated to be toxic to one or more scleractinian coral species (*Acropora*, *Pocillopora*, *Porites* spp.) and, for one of the sponges tested, to one hydrozoan coral (*Millepora* sp.). The five coral species tested (including *Montipora*) were both numerically and spatially dominant organisms at the study site. Toxicity tests involved exposing corals brought into the laboratory to water that had been conditioned by the sponges. Responses of each coral species to each sponge allelochemical varied. The allelochemical from *Acervochalina* was found to be highly toxic (51-75% tissue death) to both *Pocillopora* and *Acropora*, and had only a moderate effect (26-50% tissue death) on *Porites*. Allelochemicals of *Xestospongia* and *Plakortis* were moderately and weakly toxic (11-25% tissue death) to *Millepora*, respectively. Neither sponge was toxic towards the other coral species. *Montipora* was not affected by allelochemicals from any of the sponges. Dead coral was noted in many positions around the sponges in the field, but mainly in the direction of the current. This might support, although not confirm, an allelopathic effect. The influence of allelochemicals on the small scale and large scale spatial structuring of coral reefs is discussed. ❐ *Porifera, corals, allelochemical, coral reef, toxicity, chemical ecology, Philippines, Xestospongia, Acervochalina, Plakortis.*

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Release of allelochemicals into the surrounding water, as a defense against benthic spatial competitors, fouling organisms, or microorganisms, has been demonstrated in several groups of marine organisms (see Bakus et al., 1986). These organisms include sponges (Thompson, 1985; Walker et al., 1985; Porter & Targett, 1988; Targett, 1988; Bingham & Young, 1991), soft corals (Coll & Sammarco, 1983; Sammarco et al., 1983, 1985; La Barre et al., 1986; Maida et al., 1995), an anemone (Bak & Borsboom, 1984), possibly an alga (Littler & Littler, 1997) and hard corals (Fearon, 1997; Koh, 1997). Although only few studies have yet been undertaken, allelochemicals are believed to play a role in structuring particular marine habitats (Jackson & Buss, 1975; Davis et al., 1989; Turon et al., 1996; Thacker et al., 1998), whereas the magnitude of this role is still uncertain.

La Barre et al. (1986) demonstrated that when three species of soft corals were relocated in pairs under contact and non-contact conditions, tissue necrosis was observed in all contact pairs. Only one species of soft coral produced tissue necrosis when placed near, but not in contact with, the other two species of soft coral. They suggested that avoidance behavior, such as those caused by allelopathy, may contribute to the dispersion patterns of plants and animals in space. In another study on soft corals, Sammarco et al. (1985) found that scleractinian corals varied in their susceptibility when exposed to soft corals in both contact and non-contact conditions.

Porter & Targett (1988) demonstrated that the sponge *Plakortis halichondroides* was capable of damaging or destroying tissue in all coral species examined, with almost half the corals growing naturally in contact with, or near to, these sponges experiencing bleaching or tissue necrosis. It was suggested that by creating dead zones on the corals *P. halichondroides* was subsequently able to overgrow them. In a more recent study, Turon et al. (1996) suggested that *Crambe crambe* could have an impact on adjacent organisms by possibly releasing allelochemicals

into the surrounding water. The allelochemical effect of this sponge was at the small scale level (centimeters) (Turon et al., 1996), where patterns such as an increase in the amount of dead coral or tolerant species found adjacent to the producer of allelochemicals became evident. Turon et al. (1996) suggested that at different scales different patterns might be recognised. On previous trips to the Philippines we observed similar bleached areas or dead zones up to 1cm in width in areas where some of the species of sponges came in contact, or close contact, with coral species.

The purpose of the present study was to: 1) Determine if three tropical sponges were releasing allelochemicals potentially toxic to five hard corals on a coral reef; 2) Identify patterns due to allelochemical effects, such as the presence or absence of dead space adjacent to sponges (given that sponges releasing toxic allelochemicals would have a preponderance for dead coral, or tolerant species adjacent to them, and these might be predominantly located in the direction where the allelochemicals were most concentrated), and 3) Determine if the allelochemicals were toxic to common substrate competitors.

MATERIALS AND METHODS

Our study site was located on a limestone reef approximately 0.25km off the Tambuli Resort on Mactan I., Philippines. The study site was chosen on the basis of its high marine diversity and close proximity to the Maribago Marine Station, operated by the University of San Carlos. Depths in the vicinity of the study site ranged from 5m, where a seagrass bed began, to 15m, which bordered the start of a steep slope. However, most of the experiments conducted in this study were located between 8-11m depth. The study was conducted in May and June, 1996. (For a detailed description and map of the site see Bakus & Nishiyama, 1999, this volume). Species of sponges included: *Xestospongia* sp. (Haplosclerida: Petrosiidae), *Acervochalina* sp. (Haplosclerida: Chalinidae), and *Plakortis* sp. (Homosclerophorida: Plakinidae), and the corals: *Acropora* sp. (Scleractinia: Acroporidae), *Millepora* sp. (Milleporina: Milleporidae), *Montipora* sp. (Scleractinia: Acroporidae), *Pocillopora* sp. (Scleractinia: Pocilloporidae) and *Porites* sp. (Scleractinia: Poritidae).

ALLELOCHEMICAL DETECTION AND ISOLATION. To determine if sponges were releasing chemicals into the water, an allelochemical collecting apparatus was constructed from a battery operated bilge pump and SEP paks (Fig. 1). This apparatus was a modified version of one used by Coll et al. (1982), and later by Schulte et al. (1991). The battery operated bilge pump was fitted at its outflow hose with a step-down tubing connector (2cm to 0.7cm), which allowed two plastic screw valves to be connected (diameter 0.7cm). C18 SEP paks were initially conditioned by passing 10ml of Etoh followed by 10ml of deionised water through each SEP pak using a plastic pipette. Conditioning SEP paks before use was critical, otherwise flow would be

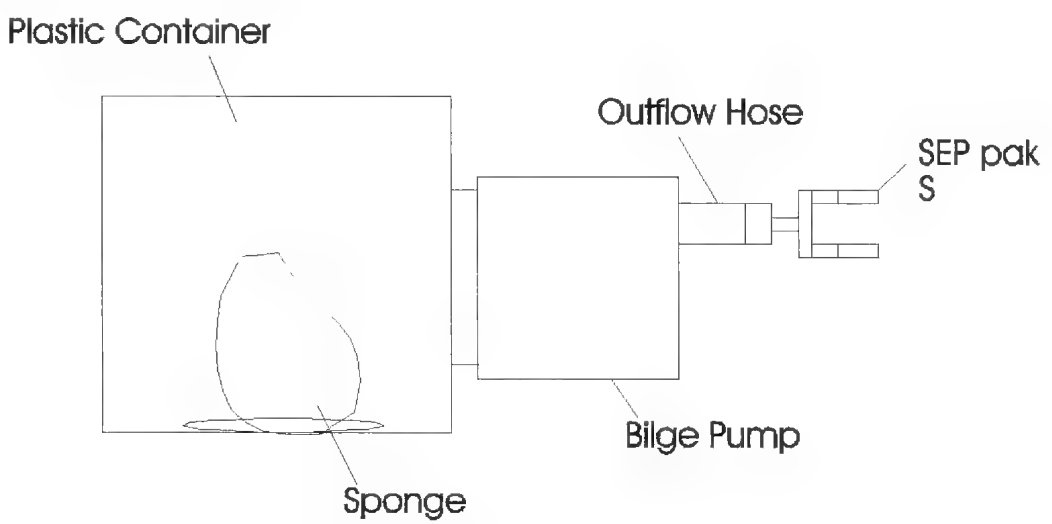

FIG. 1. Allelochemical isolating apparatus used to isolate allelochemicals from sponges.

interrupted. *In situ*, one conditioned SEP pak was inserted into each end of the plastic screw valve. At the inflow end of the pump, a plastic collapsible container (20x30x50cm) was attached which had a large hole cut at the bottom (diameters 20x30cm; Fig. 1). The purpose of the container was to assist in concentrating allelochemicals to increase probability of detection. Once the SEP paks were attached, the apparatus was placed over the sponge, with the sponge protruding into, but not touching, the plastic container. Although flow rate was determined both at sea level and 10m depth by allowing the output water to flow into an empty container for 30mins, no flow meter continuously measured flow rates in this initial model. Water surrounding eight unidentified sponges was sampled for 1.5hrs, coinciding with the maximum continuous run-time for the pumps. A control apparatus was set-up approximately 0.5m upstream from each apparatus covering a sponge. A newer model is currently being constructed with a flow meter and an added battery included for longer run times and continuous monitoring. Eight different sponge species were sampled at the study site using this apparatus to detect the release of any allelochemicals. After each run, the SEP paks and the whole sponge specimens being sampled were immediately sealed separately in small plastic bags, and upon arrival to the laboratory (not more than 20mins later), were placed in a freezer (-5°C). Upon departure from the Philippines the SEP paks and sponge specimens were placed in dry ice, and upon arrival in the USA the items were placed into a deep freezer (-20°C). Chemicals were initially eluted out by passing 20ml of dichloromethane, followed by 20ml of Etoh through SEP paks. Extractions were air evaporated for approximately 24hrs. It was subsequently discovered that extraction with 50ml acetone produced higher extraction yields. Thereafter, only acetone extractions were used. Thin layer chromatographies (TLC) were conducted using acetone as the mobile phase throughout the extraction process to insure that chemicals were not lost during the evaporation . TLCs were visualised using both long and short UV light. There was no noticeable changes in chemical composition of the extract within this period. TLCs were run on SEP pak extracts, control SEP paks, and whole sponge extracts (5gm samples of each sponge species extracted in acetone).

SMALL SCALE PATTERNS AROUND SPONGES. Patterns of organisms found adjacent to, or within approximately 5cm from, sponges were investigated by first taking photographs of between 29-39 individuals of each species and their adjacent organisms *in situ* with a Nikonos V camera. A scale bar with a waterproof compass attached to it was laid parallel to the direction of the current and placed next to each sponge before photographs were taken. Sponges in each photograph were divided into four quadrants and the presence or absence of dead coral in each quadrant was recorded. The widths of the dead zones on corals ranged from 1-10mm. Seven categories were devised to quantify the positions of dead coral: 1) Horizontal: dead coral only on two quadrants that faced currents (roughly in the E and W positions); 2)Vertical: dead coral in two quadrants perpendicular to the current (roughly in the N and S directions); 3) Dead coral found more in horizontal than vertical positions; 4) Half-half: dead coral found equally in horizontal and vertical positions; 5) More dead coral in vertical than horizontal positions; 6) Dead coral found completely around the sponge; and 7) Only live organisms completely surrounding the sponge.

TOXICITY OF ALLELOCHEMICALS ON HARD CORALS. Fifty pieces (approximately 3-4cm long) from each of two individuals of the five coral species were obtained at the study site using a geological pick, and placed in separate plastic bags for each coral species. These plastic bags were then placed into buckets to ensure minimal mechanical stress during transport. Sponges were removed from the substrate by chiselling around each sponge and carefully removing them. Sponges were then positioned and left on dead coral for one week to allow recuperation following their removal. When ready for use, two whole sponges of each of the three sponge species were placed in separate plastic bags. Care was taken to minimise damage when removing and transporting corals and sponges. Both were returned to the laboratory and sponges were immediately placed in a 1L beaker filled with filtered seawater obtained from the study site (ambient water, gravity filtered with a #1 Whatman filter). Corals were placed in separate plastic buckets with unfiltered seawater from the site until ready for use. Sponges were allowed to condition the water for 1hr before the water was passed through a Whatman #1 filter and gravity filtered. This filtration process required less than 30mins. Two sponge individuals from each species were allowed to condition water separately to test for individual variability

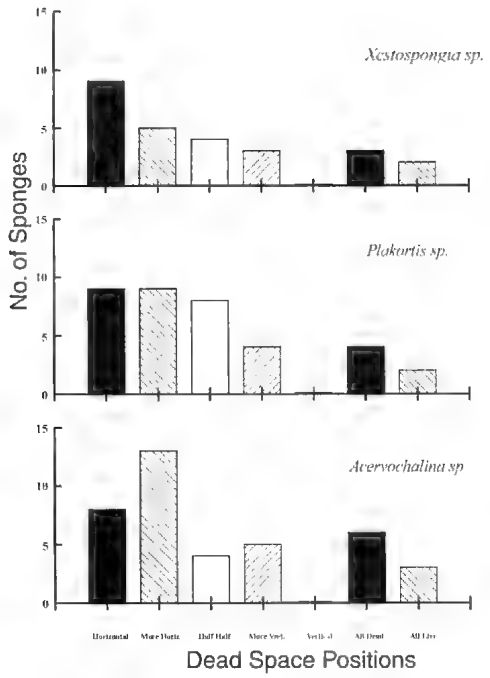

FIG. 2. Positions of dead space found around sponges from Mactan Island, Philippines. (See text for detailed descriptions of categories).

towards allelochemical toxicity. While this filtration process was carried out, corals were placed in glass finger bowls along with 100ml of ambient water. When sponge-conditioned water was ready, water in finger bowls was decanted off and replaced with an equal volume of sponge-conditioned water. A control treatment of 1L of non-conditioned, filtered seawater was also initially set aside for 1hr. The condition of corals and water temperature were noted. Corals were exposed to conditioned and control water experiments for 24hrs. A change in temperature from 25°C to 22°C was recorded for the water in the bowls. The ambient water temperature at the study site during the experiment was 27°C. After this 24hr period, corals were returned to their original site of collection and placed under a metal cage (5x30x100cm; mesh size approx. 1cm). The condition of coral fragments was monitored for 4 days thereafter. Following this period, close-up photographs were taken, both *in situ* and after the corals were returned to the laboratory. These photographs were enlarged (approximately 200%), and the area of the exposed surface of each piece of coral, as well as the area of dead tissue, traced onto acetate. The percentage of dead tissue was determined after estimating the total area of each coral fragment and the area of dead tissue, using an image analysis program (SigmaScan [SPSS Inc], 1998). Detailed notes and sketches of each piece of coral *in situ* were made earlier and were compared to the calculated dead tissue areas. In all instances both estimates were comparable. Coral tissue was considered to be dead or dying if there were signs of cell death or, as in most cases, actual detachment of tissue from the skeletal base. Loss of coloration was also observed in all dead tissue. One-way ANOVA and post hoc Tukey tests were conducted on the percentage tissue death of each coral species, with the allelochemical and control treatments being the main factor.

RESULTS

Of the eight unidentified sponges sampled for allelochemical release at our study site, three were shown to be releasing allelochemicals. These species were subsequently identified as *Acervochalina*, *Plakortis*, and *Xestospongia* spp. TLC comparisons between water immediately surrounding these three sponges, control water samples, and whole sponge extracts confirmed that allelochemicals were found within the respective sponges but not in the water column upstream from the sponges.

The presence and position of dead space adjacent to the the species of sponges occurred predominantly in the direction facing (either partially or completely) the current flow (Fig. 2). Since the direction of water flow was reversed periodically, depending on whether the tide was rising or falling, dead space occurred on both sides of the sponge facing these currents. In *Xestospongia* dead space occurred in horizontal positions (i.e. facing currents) and positions mostly facing current flows in 71% of specimens (Fig. 2). Dead space was located in these positions in 73% of *Plakortis*, and 66% of *Acervochalina*. In no instance for any of the three species of sponges was dead space found only in the vertical position (i.e. not facing current flow).

Water conditioned individually by each of the three species of sponges was toxic to one or more of the five coral species tested (Fig. 3). Responses of each coral species towards each sponge

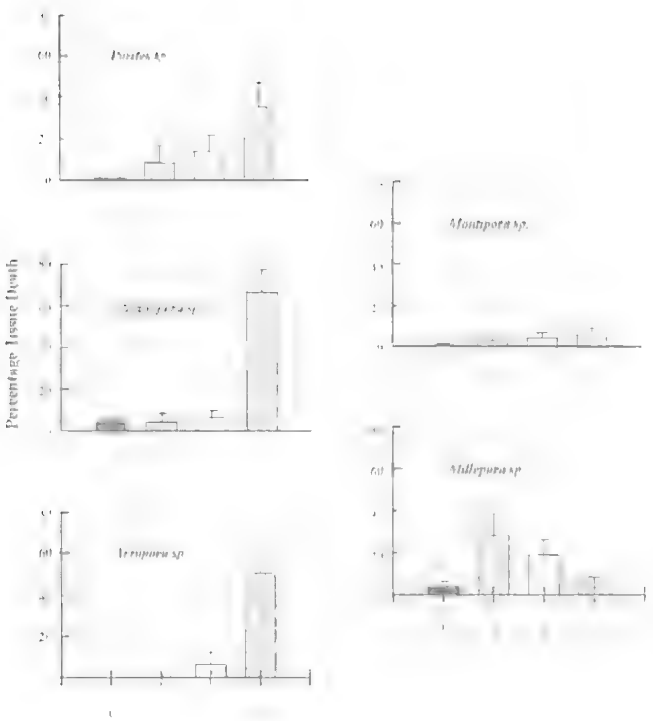

FIG 3. Average percentage tissue death of five coral species exposed to water conditioned by three sponge species (Key: 1=*Xestospongia*; 2=*Plakortis*; 3=*Acervochalina*; C= control treatments, representing corals exposed to filtered seawater). (Bar represents 1 S.E.).

allelochemical varied. Control corals experienced minimal tissue death, but not always less death than all the other treatments (Fig. 3). For all coral species, except *Montipora*, significant differences existed among the treatments (one-way ANOVA). Results of the Tukey tests and average percentage coral tissue death show that when compared to controls, the allelochemical of *Acervochalina* was highly toxic (51-75% tissue death) to *Pocillopora* ($P<0.05$) and *Acropora* ($P<0.05$), and had a moderate effect (26-50% tissue death) towards *Porites* ($P<0.05$) The sponge was not toxic towards *Millepora*. *Xestospongia* and *Plakortis* were moderately (26-50% tissue death; $P<0.05$) and weakly toxic (11-25% tissue death) ($P=0.05$) to *Millepora*, respectively. Neither sponge was toxic towards the other coral species. *Montipora* was not affected by allelochemicals from any of the sponges ($P>0.05$).

DISCUSSION

Results show that filtered seawater conditioned by all three species of sponges, *Xestospongia*, *Plakortis* and *Acervochalina*, were toxic to at least one species of hard coral (*Porites*, *Pocillopora*, *Acropora* and *Millepora*). Responses of corals towards sponge-conditioned water varied, as expected. Susceptibility of corals in contact with, or in proximity to sponges suggests the possibility that sponge allelochemicals may influence patterns of distributions of organisms adjacent to sponges. In other words, certain tolerant species of corals may grow adjacent to sponges, whereas others may never or rarely be found in close proximity.

In a parallel study we conducted at our study site, whole sponges were transplanted and placed into direct contact with corals. We determined that several species of corals were highly susceptible to (i.e. damaged by) several species of sponges. Under natural conditions, these corals were rarely found growing in direct contact with these sponges (Nishiyama & Bakus, unpublished information). In rare cases where they did grow naturally adjacent to these sponges, a zone of dead or bleached tissue was often noted at the site of contact. Furthermore, if a species of sponge was not found to be deleterious to a species of coral, incidences of growth with direct contact between the two species were more often noted. This corresponds to field observations made by Porter & Targett (1988) where almost half the corals growing next to *Plakortis halichondroides* experienced bleaching or tissue necrosis. Both these studies support our hypothesis that water borne allelochemicals may deter particular substrate competitors from growing in direct contact with sponges. It should be cautioned, however, that laboratory bioassays using sponge-conditioned water were conducted on corals in still water, whereas in nature currents probably have a major influence in dissipating allelochemicals, and thus, their physiological impact may not be as extensive as those observed in the laboratory.

Although particular sponges may have an impact on adult corals, these toxic effects may also have a greater impact in preventing coral larvae settling adjacent to sponges. The occurrence of dead coral space around sponges, mainly in the direction facing currents, may reflect the directions that highest allelochemical concentrations occur given that toxins are carried away from sponges. Although only suggestive, this supports the notion that toxic allelochemicals were being released by sponges. Maida et al. (1995) provided evidence to suggest that a soft coral could influence the direction from which recruitment occurred. An alternative hypothesis is that some hydrodynamic effect prevents settlement of larvae in areas facing the current. It may also be advantageous for a sponge to encourage adjacent settlement and growth of coral species which are susceptible to the sponges' allelochemicals. This would ensure that the sponge would be able to grow and expand into the adjacent area.

Through their release of allelochemicals sponges may stop the growth of adjacent, adult substrate competitors, such as corals. However, this effect may operate only on a local or small scale, at least for the three species of sponges investigated here, because zones of dead or bleached tissue on corals growing adjacent to the three species of sponges extended for only 1cm at most. *Acervochalina*, however, may also possibly overgrow corals, with an observed high density in comparison to the other two species, generally having the highest toxicity towards corals, and being thinly encrusting with possibly greater lateral growth. *Acervochalina* was also observed growing on branches of corals that were dead at the bases (where the sponges occupied), yet alive at the tips (where the sponge had not yet extended). Allelochemicals of *Acervochalina* may operate to stop corals from overgrowing it and as a mechanism to kill coral tissue, to open up space for its own growth.

In a study conducted by Bakus & Nishiyama (1999, this volume), data from transects at the study site show that no apparent sequence existed where a particular substratum type (i.e. live hard coral, sponges, coral rubble, etc.) was found next to sponges; that is, the succession of organisms and substrata were independent of each other. In that study, however, individual species of both corals and sponges were not differentiated, and therefore, specific species pairs may exist. Using transect line data techniques, Turon et al. (1996) investigated the possibility of allelochemicals being released by sponges. They determined that *Crambe crambe* had toxic effects up to 1cm from particular coral substrate competitors, corresponding to the effective distance of allelochemicals suggested in the present study. Turon et al. (1996) also suggested that these small scale effects might only be detected by sampling at a small scale (centimeters), whereas sampling at 3cm intervals produced a different outcome. Where allelochemicals have effective distances at a scale of less than 1cm, observations made at 1cm intervals may not suffice in detecting these chemical interactions. In the present study, extreme care was taken to accurately determine dead space and organisms adjacent to sponges, and in most cases, measurements were determined to the nearest millimeter.

The chemical nature of allelochemicals released by sponges, as measured by SEP paks, are currently being ascertained, as are the toxicities of these isolated chemicals towards the five species of corals. Although SEP paks retained chemicals released by sponges, this does not necessarily confirm any toxicity by the sponge. Only isolation of the active chemicals from sponges and verification of their toxicity towards corals would confirm that these are allelochemicals deleterious to corals. Data from another study conducted by the authors are currently being analyzed (Nishiyama & Bakus, unpublished data), involving the deterrence of settlement of substrate competitors by sponges placed next to plates kept in the water at the site for approximately one month.

After hard corals, sponges were the dominant organisms at Mactan I. (Bakus & Nishiyama, 1999, this volume), also showing relatively high diversity (Bakus & Nishiyama, unpublished data). Of eight sponges investigated, three released allelochemicals into the water column, suggesting that many more sponges, not investigated here, may release allelochemicals. Thus, sponge allelochemicals may play an important role in structuring the coral reef community at a small scale, local level. More work is needed, however, to determine the extent of this role.

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TOWARDS A PHYLOGENETIC SYSTEMATICS OF THE FOSSIL HEXACTINELLIDA. *Memoirs of the Queensland Museum 44: 418. 1999*:- The vast majority of all fossil hexactinellid taxa has been described from the Mesozoic. This is due to the rich occurrence of Mesozoic hexactinellids, especially in the well-exposed Jurassic and Cretaceous strata of Europe, and to the generally larger preservation potential of the rigid Mesozoic hexactinellids compared to the predominantly non-rigid Palaeozoic ones. Nevertheless, most of the main hexactinellid taxa can be traced back to the Early Palaeozoic. Isolated hexasters of the Hexasterophora occur in the Early Ordovician, and the first hexactinosans are known from the Late Devonian, whereas the earliest definite amphidiscophorans are documented from the Late Silurian. However, the bulk of the Palaeozoic hexactinellid sponges, although well established as monophyletic groups, cannot definitely be attributed to any recent taxon and require an exclusively fossil-based systematics. The Early Palaeozoic Protospongiidae and Hintzespongiidae are derived from a reticulate hexactine-bearing ancestors, probably close to the *Mattaspongia-Microstaura*-group, which can be regarded as adelphotaxon of the Hexactinosans. The Dictyospongiidae (s.str.), which are hexasterophorans, probably also originated from the *Mattaspongia*-stem lineage, as did the modern Sceptrulophora (Clavularia-Scopularia-taxon), which recently have been traced back to the Early Palaeozoic through the documentation of Ordovician scopules. The Brachiospongiidae, including the Stiodermatidae, may be attributed to the amphidiscophorans, because of the great similarity in skeletal architecture between *Strobilospongia* and the modern Hyalonematidae. However, the systematic affinity of many Palaeozoic lyssacine hexactinellids which appear (or were in fact) primitive, including most Early Cambrian genera such as *Quadrolaminiella*, *Solactiniella* and *Hyalosinica*, is still uncertain, and these taxa have to be classified within the probably non-monophyletic grouping 'Rossellimorpha'. At the end of the Permian, all major Palaeozoic hexactinellid groups had become extinct, and from the Mesozoic onwards, the Hexactinellida are represented by modern forms, mainly Hexactinosans and Lychniscosans. '*Lyssacinosa*', which comprise the majority of Recent hexactinellid taxa, are not commonly found in Mesozoic strata, but nevertheless there are some important occurrences, from which recent genera can be identified. *Regadrella* of the Euplectellidae is known with several species from the Cretaceous, and the first species of the Hyalonematidae, *Hyalonema cretacea*, has been described from the Campanian. But more, new, Late Cretaceous representatives of these groups and also of the Rosselliidae from the section of Arnager (Bornholm, Denmark) are still to be described. The earliest definite lychniscosans are known since the Middle Jurassic, and the group reached its maximal diversity during Late Cretaceous time. Probably, this group did not arise from the hexactinosans, but it is the adelphotaxon of some lyssacine group, maybe the Euplectellidae. Today the Lychniscosans have become almost extinct, so the exact systematic attribution of the Mesozoic families and genera to recent ones is problematic and in many cases impossible. The same thing is true to many Mesozoic hexactinosans, although many Recent genera have now been identified from the Late Cretaceous, and this allows an approach of the zoological systematics at least for Late Mesozoic and Tertiary sponge fossils. However, still many Cretaceous and most Jurassic hexactinosans classified by Schrammen in the grouping 'Inermia', such as the *Casearia-Porospongia*-group, cannot be definitely attributed to any taxa within the Recent systematics, but have to be subject to a phylogenetic-systematic approach based on fossil representatives only. ❐ *Porifera, phylogeny, systematics, Hexactinellida, fossils, Mesozoic.*

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MEASUREMENT OF SPONGE GROWTH BY PROJECTED BODY AREA AND UNDERWATER WEIGHT

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In vitro growth rates of the Indo-Pacific demosponge *Pseudosuberites andrewsi* (Kirkpatrick) were measured using two alternative techniques to estimate biomass: determination of projected body area, and determination of underwater weight. Four small explants of *P. andrewsi* were fed regularly with the microalgae *Rhodomonas* sp. and *Chlorella sorokiniana*, and growth was monitored over a period of 24 days. Three explants showed considerable increase in both projected body area and underwater weight, but the growth pattern was irregular. Although the observed trends in growth were similar for both methods, the absolute values were not in general agreement, which may be due to the fact that photographic data were two-dimensional. It was concluded that determination of underwater weight is a promising method for measuring growth of sponges if the size of the explants used is sufficiently large. Measuring projected body area has a higher precision when explants are smaller than 10mg and is a preferred method when small explants are used. ❐ *Porifera, Pseudosuberites andrewsi, growth monitoring, projected body area, underwater weight.*

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Due to the rich potential of marine sponges as producers of interesting natural compounds, there is a growing need for methods to produce large amounts of sponge biomass (Munro et al., 1994; Osinga et al., 1998a). *In vitro* cultivation of sponges in bioreactors may be an interesting option for mass production of sponge metabolites, because such systems can easily be manipulated and optimised. An essential prerequisite for studying and optimising *in vitro* growth of sponges, is to have a good method to monitor this growth.

Sponge growth can be monitored by measuring increases in biomass. Thus, to detect slight changes in growth rates, a method is required to precisely measure sponge biomass. This method should not negatively affect the survival or growth of sponges, and in this respect it is important to keep sponges continuously underwater. Although some sponge species can tolerate short exposure to air, it is generally assumed that exposure to air can be harmful to living sponge tissue. Air entering the aquiferous system can irreversibly damage choanocyte chambers (Fosså & Nilsen, 1996). Therefore, determination of sponge volume (by water replacement), wet weight, or drip dry wet weight, although often applied (e.g. Barthel, 1986; Thomassen & Riisgård, 1995), are not preferred methods to measure living sponge biomass and to simultaneously maintain viable experimental populations.

In some studies, growth rates are determined by measuring the area of two-dimensionally projected images of the sponge body. Ayling (1983) used this technique to measure *in situ* growth and regeneration rates of several encrusting sponge species in the coastal water of New Zealand. Series of photographs were taken underwater over a period of time and the images were projected on graph paper. Poirrier et al. (1981) used similar methods to measure *in vitro* growth of the freshwater sponges *Ephydatia fluviatilis* and *Spongilla alba*. Although these methods may be suitable to measure growth in almost two-dimensionally growing encrusting species, or for regularly shaped species such as the sphere-shaped *Cinachyrella* spp. and *Tethya* spp., problems may occur when the two-dimensional surface area data are converted into three-dimensional volumes. Especially with more irregularly shaped species, increases in surface area can easily under- or overestimate increases in body volume, especially in more irregularly shaped species.

FIG. 1. A 200dm^3 air-lift bioreactor for maintaining the culture of *P. andrewsi*.

In this study, we introduce a new, three-dimensional measure of sponge biomass: underwater weight. Determination of underwater weight is used to measure *in vitro* growth rates of the Indo-Pacific demosponge *Pseudosuberites andrewsi* (Kirkpatrick). These results are compared with two-dimensional growth rates obtained from projected body areas. The value of underwater weight as a measure of sponge biomass is further evaluated by correlating these data to other biomass parameters (volume, wet weight, dry weight and ash-free dry weight).

MATERIALS AND METHODS

SPONGES. On the basis of previous results (Osinga et al., 1998b), *P. andrewsi* was selected as a model species for further experiments to improve the methodology for *in vitro* sponge culture. Living material of *P. andrewsi* was obtained from Blijdorp Zoo (Rotterdam, The Netherlands), where it was growing in a large, shallow basin, in which a strong water current was generated to simulate an intertidal environment. We are uncertain about the location were these sponges originally came from. They had been introduced in the zoo coincidentally on so called 'living stones', which were presumably collected from Indonesian coastal waters. In our laboratory, we have been able to maintain small colonies of this species for more than a year under the conditions described below.

Sponges were held in a 200dm^3 airlift bioreactor (Fig. 1) containing artificial seawater (using Instant Ocean Reef Crystals artificial sea salt) with a salinity of ~32‰. This water was replaced continuously ($D=0.033d^{-1}$). The temperature in the bioreactor varied between 25-29°C. In order to provide the sponges with a source of silica, 0.25mM $Na_2O_3Si\ 9H_2O$ was added to the artificial seawater. Measurements of the silica concentration in outflow water showed that this addition was sufficient to cope with sponge demands. Non-axenic batch-cultures of two species of microalgae were regularly added as a food source for the sponges. Twice a week, 1dm^3 of a culture of the freshwater alga *Chlorella sorokiniana* (Chlorophyceae, average size ~3µm) was added, containing ~$1x10^7$ cells cm^{-3}. In addition, 1dm^3 of a culture of marine *Rhodomonas* sp. (Cryptophyceae, average size ~6µm), containing ~$1x10^6$ cells cm^{-3}, was added weekly. The algae were cultured at a temperature varying between 17-20°C. A light-dark cycle of 14hrs light and 10hrs darkness was applied. The growth media for the algae are given in Table 1. When the cultures were added to the sponge reactor, the algae were usually near the end of their logarithmic growth phase.

The choice to use these two algae in current experiments was based on the literature. Additions of *Chlorella sorokiniana* were used successfully to enhance the growth of the temperate sponge *Halichondria panicea* in semi-controlled cultures (Barthel & Theede, 1986). *Rhodomonas* sp. was used by Thomassen & Riisgård (1995) to feed *in vitro* cultures of *H. panicea*.

Growth experiment. Comparative growth rate measurements were performed on four explants colonies of *P. andrewsi*. Explants were prepared using razor-sharp knifes. Pieces of sponge tissue were tied onto perspex slides with nylon fishing-line. Explants were placed in temperature controlled, 1.58dm^3 bioreactors, equipped with a

TABLE 1. Growth media for algae (a freshwater medium for *Chlorella sorokiniana* and a seawater medium for *Rhodomonas* sp.). The freshwater medium was based on the A9 medium described by Lee & Pirt (1981). Concentrations are given in mM, unless indicated otherwise.

Component	Freshwater medium concentration	Seawater medium concentration
$NaHCO_3$	10.0	5.00
KNO_3	1.00	0.50
NaH_2PO_4	0.10	0.05
Instant Ocean Reef Crystals artificial seasalt		~ 33 g dm^{-3}
$MgSO_4 \cdot 7H_2O$	4.99	
$CaCl_2 \cdot 2H_2O$	0.272	
$EDTANa_2 \cdot 2H_2O$	0.391	
$FeCl_3$	0.148	
$Na_2B_4O_7 \cdot 10H_2O$	$4.72 \cdot 10^{-2}$	
$ZnSO_4 7H_2O$	$3.13 \cdot 10^{-2}$	
$CuSO_4 \cdot 5H_2O$	$3.20 \cdot 10^{-2}$	
$MnSO_4 \cdot H_2O$	$3.59 \cdot 10^{-2}$	
$Na_2MoO_4 \cdot 2H_2O$	$2.07 \cdot 10^{-2}$	
$NiSO_4 \cdot 6H_2O$	$2.85 \cdot 10^{-3}$	
$NaVO_3$	$2.85 \cdot 10^{-3}$	
thyamin-HCl		$5.93 \cdot 10^{-5}$
cyanocobalamin		$5.90 \cdot 10^{-6}$
biotin		$1.64 \cdot 10^{-6}$

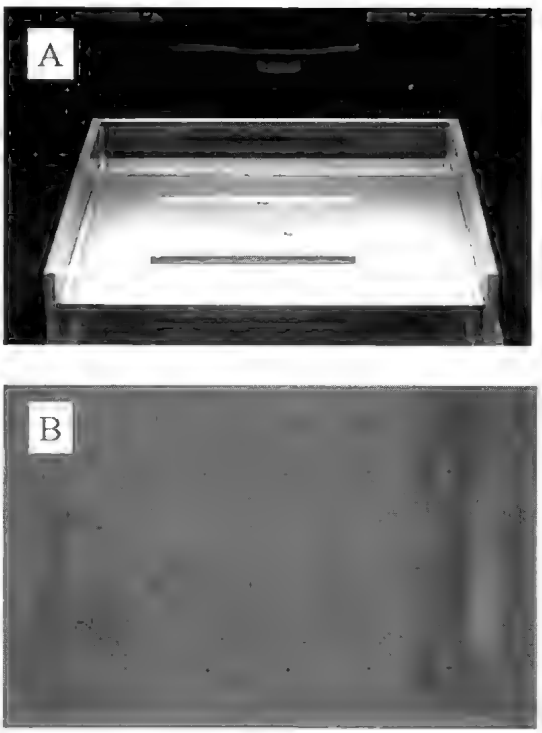

FIG. 2. Photographic method. A, Overview of the system. B, Detail of the explants laying on the perspex rack. The black dots on the rack indicate a known distance.

sparger for air-supply and a magnetic stirrer to keep food particles in suspension.

Sponges were fed with *C. sorokiniana* (twice a week, 50cm^3) and *Rhodomonas* sp. (once a week, 50cm^3) using material from batch-cultures described in the previous section. Temperature and salinity in the bioreactors were kept constant at 25°C and 33‰, respectively. The experiment was run for a period of 24 days. Monitoring of the growth of the explants was performed according to the procedures described below.

Determination of projected body area. During the experiment, several photographs of the explants were made to determine changes in the projected body area. To take photographs, explants were removed from the bioreactor (kept underwater, in a beaker glass) and placed onto a rack (also underwater) on which black dots were painted to indicate a known distance (Fig. 2). Photographs were taken under a straight angle with a digital camera (Hewlett Packard Photo-Smart Model C5340A). Digital images were printed, the areas of sponges were cut out with scissors and these cuttings were weighed. Weights of these cuttings were converted to areas by comparing them to the weight of a cutting of a known area. These values were converted to real body areas using the marked distances on the rack as a reference. Photographs were taken at Days 1, 7, 10, 20 and 22.

Determination of underwater weight. Underwater weight was measured using a A&D HR300 analytical balance (weighing range: 0.0001-300g) equipped with an underweighing-possibility. A hanger was connected to the balance, in which the slides with the explants could be placed. This balance was placed over a small basin filled with artificial seawater in such a manner that the part of the hanger containing the explant would remain underwater (Fig. 3). It is important to keep the level and salinity of the seawater in the basin constant. Changes in salinity will change the density of the seawater. Since sponge tissue is not much denser than seawater, a slight change in salinity will affect the underwater weight of sponges. The salinity of the seawater in the basin was always maintained at 33‰.

FIG. 3. Underwater weight measurement. Detail of an explant placed in the hanger under the balance.

The underwater weight was calculated by subtracting the weight of the carrier slide from the combined weight of the explant + carrier slide. It was therefore important that the weight of the carrier slide remained constant throughout the experiment, especially since the slides in this study were much heavier than the explants. Therefore, an inert material was required to be used as carrier, and consequently we chose to use perspex slides instead of the commonly used glass slides (e.g. Simpson, 1963; Poirrier et al., 1981; Vethaak et al., 1982), because glass was found to dissolute slowly in seawater, causing a slow, but steady decrease of the underwater weight of the carrier slide.

Explants were transported from the bioreactors to the weighing basin underwater in a beaker glass. Measurements were performed on Days 1, 2, 5, 9, 12, 22 and 24.

Determination of volume, wet weight, dry weight and organic carbon and nitrogen content. In order to evaluate the utility of underwater weight as a measure of biomass, underwater weight data were correlated to other non-destructive biomass parameters, volume and wet weight (WW).

To determine volume and WW, sponge-explants were removed from the water and firmly shaken until they no longer dripped. Volume was then determined by putting an explant into a graded cylinder filled to a certain reference level with artificial seawater. After addition of the explant, all water in excess of this reference level was removed with a syringe and transferred into a $1cm^3$ glass pipette, in which the volume of excess water could be determined. In this way, sponge volumes of about $0.1cm^3$ could be determined with reasonable precision (the methodological error was less than 10%). The corresponding WW of explants was measured on an analytical balance. For these measurements, it was imperative that explants were not attached to carrier materials, and hence, volume and WW determinations of experimental explants were undertaken immediately prior to growth experiments. Some additional explants were measured to obtain more reliable correlations/ conversion factors.

Dry weight (DW) organic carbon content and organic nitrogen content of sponge tissue were also determined, but only for a single sample, since these measurements are destructive and only limited amounts of sponge material were available. For the determination of DW, pieces of sponge were dried for 24hrs in an oven at 80°C and weighed. The dried material was ground and analysed for organic carbon and nitrogen on a Fisons EA 1108 Elemental Analyser.

RESULTS AND DISCUSSION

GROWTH RATES AND KINETICS. Results of growth experiments are presented in Figure 4, showing changes in surface area and underwater weight. Three of the four explants showed growth during the experimental period, both when measured with two-dimensional photography and with the underwater weighing technique. The fourth explant did not show obvious changes in projected body area or underwater weight. This explant failed to attach to the perspex slide, while the other three explants firmly attached within a few days. Explants used for the experiment were made shortly before the experiment started. In future work, only healthy (attached) explants should be used, demonstrating viability after a period of acclimatisation

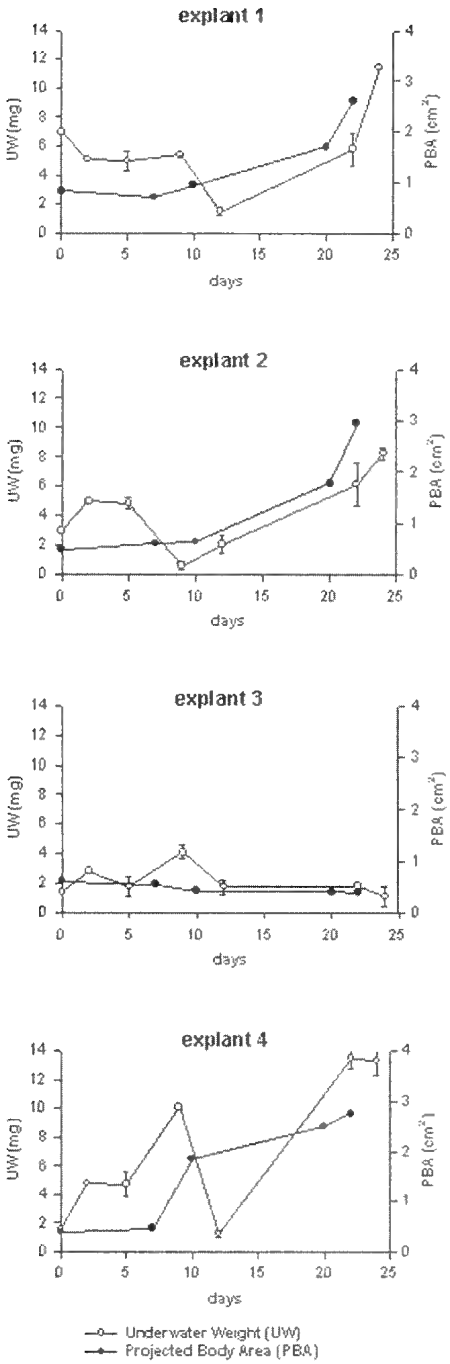

FIG. 4. Results of the growth experiment. Changes in underwater weight (mg, open symbols) and projected body area (dm^2, black symbols) of the four explants. Error bars for underwater weight data indicate the standard deviation of two replicate measurements.

in aquaria. However, some explants (e.g. explants in Fig. 3) attach to the glass only at one point, and start to form lateral processes that are not attached. These explants do grow, but this growth is difficult to quantify using photographic techniques. As these processes can easily break off from the explant, such explants are also not very suitable for growth experiments using determination of underwater weight or other weight parameters.

It is not easy to deduce general statements about the kinetics of growth in *P. andrewsi* from the data presented in Figure 4. Explants 1 and 2 seem to exhibit a kind of lag-phase, followed by a period of exponential growth after Day 12. The lag-phase may be some kind of response to cutting sponge tissue: the tissue must rearrange and attach to the substratum before growth can start. This process may have also caused the observed decrease in underwater weight of explants 1, 2 and 4 that occurred around Day 10-12. Rearrangement of the body into a functional, pumping sponge will cost energy that is probably obtained from respiring sponge tissue. More data are needed to give a reliable description of sponge growth in this species.

It is difficult to estimate a specific growth rate for *P. andrewsi* under the given experimental conditions, due to the strong variability in our data. We calculated specific growth rates only for explants 1 and 2, based on data measured after the lag-phase. These data tend to show exponential growth, which justifies calculation of a specific growth rate μ according to the formula:

$$\mu = t^{-1} \cdot \ln C_0/C_t$$

where C_0 is sponge biomass at the start of the exponential growth, C_t is sponge biomass at the end of the experiment, and t is the number of days between the start of the exponential growth and the end of the experiment. The calculated specific growth rates (Table 2) were between 0.08-0.10d^{-1} for data of projected body area and 0.16d^{-1} for underwater weight. These values are considerably higher than previously reported growth

TABLE 2. Specific growth rates (d^{-1}) for explants 1 and 2 during the period of exponential growth. Calculations are performed with data for projected body area (PBA) and underwater weight (UW).

Explant	Period used for calculation	μ(PBA)	μ(UW)
1	Days 7-22	0.08	
1	Days 12-24		0.16
2	Days 7-22	0.10	
2	Days 9-24		0.16

rates, which range from 0.01-0.058 (see Table 2, in Thomassen & Riisgård, 1995). Hence, *P. andrewsi* is able to grow faster under the applied conditions.

PROJECTED BODY AREA VS. UNDERWATER WEIGHT. Although general trends in results are similar between the two methods, some differences are apparent. The calculated specific growth rates for explants 1 and 2 (Table 2) are almost twice as high when underwater weight is compared to projected body area. Furthermore, data of underwater weight show a more irregular pattern than data of projected body area. The steep decrease in underwater weight for explants 1, 2 and 4 around Day 12 was not reflected in surface area. Shrinking of more massive body parts may not be reflected in changes in projected body area. Finally, the absolute growth after 22 days of the explants in projected body area is different from the growth in underwater weight (Table 3). These differences could be caused by the projection of three-dimensional growth onto two dimensional body areas. Explants 1 and 2 may have spread out horizontally without a corresponding increase in body mass, leading to an overestimation of actual growth. In contrast, explant 4 may have formed vertical outgrows that are difficult to quantify as increase in body area on a two-dimensional image, thus leading to an underestimation of growth.

A possible improvement for the photography method would be to use so-called 'sandwich-cultures', flat sponge tissue cultures growing in a narrow space between a glass slide and a cover slip. This method, introduced by Ankel & Eigenbrodt (1950) to study development of freshwater sponges, was successfully applied to seawater sponges by Langenbruch (1983) and Sanchez-Moreno (1984). Sandwich-cultures can be viewed as forced two-dimensional explants, and may thus be very suitable for growth rate measurements based on changes in projected body area. However, growth of sandwich-cultures may not mimic that of normal explants, which could be a major drawback when using this type of culture.

Despite the precision of 0.1mg provided by the analytical balance, the methodological error in the weighing technique (expressed in Fig. 4 as the standard deviation of two replicate measurements) is usually around 1mg. Hence, the precision of the weighing method for determining growth rates decreases when small explants are used. A better stabilised weighing device could probably improve this precision, but it is probably more practical to work with bigger explants with an underwater weight of at least 10mg. The methodological error in photographic measurements is not shown in Figure 4. A previous study in our lab (D. Redeker, unpublished data), set up to develop the photographic method, showed that this error is generally less than 10%, even when small explants are used. Images can be easily enlarged without losing too much contrast, which makes the photographic method more favourable over the weighing method when small explants are used.

VOLUME AND WEIGHT PARAMETERS. Volume (V), wet weight (WW) and underwater weight were compared in order to determine conversion factors for these parameters and to evaluate the utility of underwater weight as a measure of sponge biomass. Correlations are shown in Figure 5, and the corresponding conversion factors can be found in Table 4. Both V and WW of *P. andrewsi* showed a moderate positive correlation (Fig. 5A; $r=0.78$), that is highly significant ($n=11$, $\alpha=0.001$). In a study on *Halichondria panicea*, Barthel (1986) also found that the correlation between V and WW was not very strong, probably due to variability in the water- and spicule-content of sponge tissue. In contrast, we found considerably stronger

TABLE 3. Growth of the sponge explants after 22 days (projected body area and underwater weight) and 24 days (underwater weight), Growth is defined as the newly formed sponge biomass, expressed as a percentage of the initial projected body area (PBA) or underwater weight (UW).

Explant	Increase in PBA after 22 days	Increase in UW after 22 days	Increase in UW after 24 days
1	215 %	- 30 %	63 %
2	500 %	105 %	175 %
3	- 37 %	7 %	- 21 %
4	605 %	745 %	735 %

TABLE 4. Wet Weight (WW), Underwater Weight (UW) and Dry Weight (DW) of 1cm^3 tissue of *P. andrewsi*, and the percentages of Organic Carbon Content (OCC) and Organic Nitrogen Content (ONC) in the dried sponge material. Key: 1, Not significant; 2, Reliability unknown (one sample only).

WW(mg)	UW(mg)	DW(mg)	OCC(% of DW)	ONC(% of DW)
0.68	0.044[1]	0.01[2]	13.9	3.15

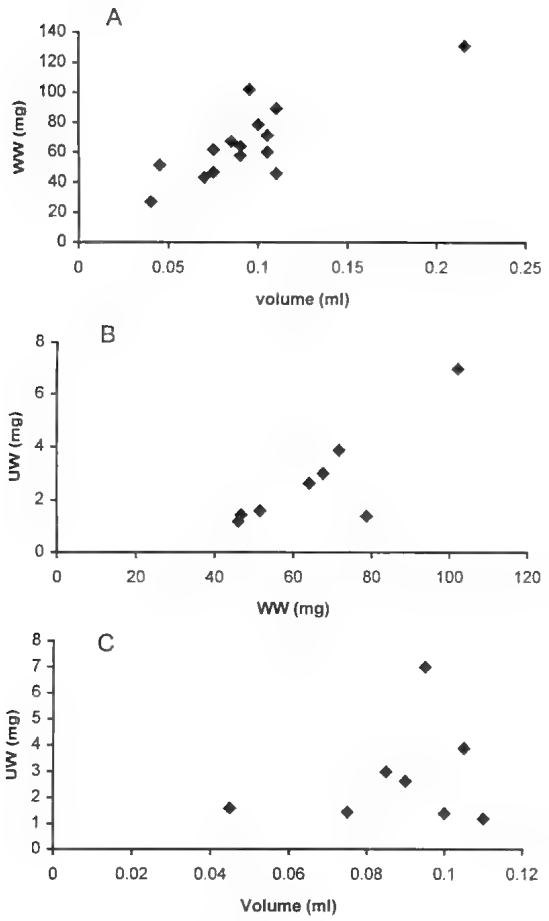

FIG. 5. Comparison between the three techniques for measuring biomass. A, correlation between WW and V. B, correlation between WW and underwater weight (UW). C, correlation between V and UW.

correlations for *Axinella polycapella* (0.98) and *Cinachyrella apion* (0.99) (R. Osinga & E. Planas Muela, unpublished results), and for these species conversion factors are much more reliable.

To evaluate the use of underwater weight as a measure of sponge biomass, the underwater weight data were compared to corresponding measurements of WW and V (Fig. 5B-C). Here, WW showed a significant ($r=0.73$; $n=8$; $\alpha=0.025$) correlation with underwater weight. Hence, our underwater weight data may be converted to WW, using the conversion factor given in Table 4, and underwater weight therefore seems to be an acceptable measure of sponge biomass. However, no significant relation between underwater weight and V could be detected, despite the weak, but significant correlation found for volume and WW. This indicates that tissue of *P. andrewsi* might be subject to a large intraspecific variation in density, which implies that the other data in Table 4 (DW, organic carbon content, and organic nitrogen) must be seen as a first indication only.

CONCLUSIONS

We found that under the applied food regimen (batches of the microalgae *Rhodomonas* sp. and *Chlorella sorokiniana*), the sponge *Pseudosuberites andrewsi* is able to grow rapidly. However, we have not yet succeeded in creating artificial circumstances that enable a constant growth rate; fluctuations in time and intraspecific differences between explants were large. In further studies, this may be improved by using only those explants that have already shown the ability to grow and by adding food particles continuously using continuous cultures of algae.

The two methods used in this study to determine growth have both proven their value in studying sponges. Photography of the body area is the most suitable technique when the availability of sponge material is limited (i.e. when small explants are used). Determination of underwater weight is a promising alternative for photography. Underwater weight has the advantage of being a direct measure of biomass, and therefore, the accuracy of this method to measure growth may be better. The method has a detection limit of ~1mg, which makes it less suitable for small explants.

More data are needed to provide a reliable picture of the relation between volume and weight parameters for tissue of *P. andrewsi*, as this species seems to exhibit a high variability in tissue density.

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PREDATION ON CARIBBEAN SPONGES: THE IMPORTANCE OF CHEMICAL DEFENSES. *Memoirs of the Queensland Museum 44: 426. 1999*:- The conventional view has been that the impact of predation on Caribbean reef sponges is minimal: generalist predatory fishes are deterred by sponge spicules and chemistry, while the few spongivorous fishes are 'smorgasbord' feeders that circumvent chemistry by eating small amounts of many different sponge species. New data suggest that this traditional view needs to be re-examined. Generalist predatory fishes are deterred by chemistry, but not by structural elements, toughness, or nutritional quality of sponge tissue. Spongivorous fishes are not smorgasbord feeders, but instead choose to eat chemically undefended sponge species. Transplantation experiments reveal that the grazing activity of spongivorous fishes restricts certain sponge species to refugia, including cryptic habitats on the reef and mangrove and grassbed environments, where these fish are absent. Chemical defense plays an important role in the ecology of sponges on Caribbean reefs. ❐ *Porifera, chemical defense, predation, Caribbean, ecology.*

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RELATIONSHIP BETWEEN SPONGES AND A TAXON OF OBLIGATORY INQUILINES: THE SILIQUARIID MOLLUSCS

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Some coenogastropod molluscs are adapted to living embedded in a matrix of sediment, coral or sponge tissue. In the latter case the siliquariid molluscs are obligatory inhabitants of the sponge hosts. Siliquariidae is a small family with three extant genera with circum-tropical and temperate distribution. Information on their association with Porifera is so far limited to 15 records. The present study analyses 35 sponge species hosting siliquariids from the Mediterranean Sea, E. Atlantic, New Zealand, Philippines and New Caledonia, living in a water depth between 10-440 m. A close species-specific association was not found, although only a restricted number of sponge families host siliquariid molluscs. From these data it is apparent that siliquariids prefer hosts with a compact and rigid sponge skeletal structure, produced by a radial organisation and/or high spicule density. Commensal siliquariids show different growth rates. When their larvae settle on the sponge surface larval shells (protoconchs) are partially overgrown by the host sponge. As soon as the mollusc begins development it opens a slit along its entire length, hence commencing close interactions with the sponge. The mollusc is able to modify the shape of the longitudinal slit, adapting it to the sponge aquiferous system by transforming the slit into a series of contiguous holes that communicate with the sponge's excurrent canals. Based on the trend that there is a successively decreasing diameter of these canals, it seems evident that the siliquariid conveys self-drained water into the sponge incurrent canal system. This behaviour was studied using x-ray photography and casts obtained from resin injections into the aquiferous system. It is clear that the mollusc obtains most benefit from this association, achieving: protection against predators, defence from sediment clogging, and increased feeding efficiency. Minor benefits are obtained by the sponge host: increased water inflow with an energy saving, and a secondary source of food from the mollusc's expelled water. The sponge does not seem to be negatively affected by the siliquariid presence and is able to maintain, through its plasticity, its original skeletal structure. This form of strict and integrated association between filter-feeders may well be interpreted as commensalism and probably as facultative mutualism. ❐ *Porifera, siliquariid molluscs, association, commensalism, symbiosis, adaptations, behaviour.*

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Symbiotic associations between sponges and other organisms involve a diversity of taxa, from bacteria to large crustaceans (see review by Sarà et al., 1998), whereas other associations, less integrated or intimate, involve other sponge dwellers which, according to cases, may be regarded as commensals or inquilines. Most studies on these latter associations (e.g. Pearse, 1932; Pansini, 1970; Bacescu, 1971; Rützler, 1975; Koukouras, 1996) have examined the descriptive aspects of the association whereas only a few have focused on the interaction between host and commensal (Forbes, 1966; Connes et al., 1971; Uriz et al., 1992).

Concerning molluscan commensals, natural suspension feeders such as bivalves are most frequently associated with sponges (e.g. *Chlamys varia* in *Halichondria panicea* (Forester, 1979), *Hyatella arctica* in *Geodia cydonium* (Santucci, 1922), *Ostrea permollis* in *Stelletta grubii* (Forbes, 1966)), whereas associations between sponges and gastropods are rare and restricted only to sessile gastropods with a filter feeding strategy that requires important shell adaptations (Seilacher & Gunji, 1993; Savazzi, 1996). The shells of gastropods living embedded in sponges do not exhibit a fixed geometrical constraint but a 'heteromorph' growth pattern (Morton, 1951, 1955; Gould, 1966; Savazzi, 1996; Schiaparelli

et al., 1998), as seen in several Vermiculariinae and in all slit-bearing Siliquariidae (genera *Tenagodus* and *Pyxipoma*).

The obligatory association of these molluscs with sponges is supported by the absence of any scar on the shells from attachment to a substrate (Deshayes, 1864; Savazzi, 1996). Slit-bearing Siliquariidae represent a separate unit from other uncoiled gastropods (as Vermetidae, Vermiculariinae and *Stephopoma*, the third siliquariid genus), due to the presence of the shell's longitudinal slit that drains the incoming water-flow from the shell aperture. In the adult mollusc the slit is only partially open and functional, with partial closure due to secondary carbonate deposition. During its growth the living mollusc shifts its position along the shell aligning the mantle cavity opening with the functional apertures of the slit. As for most ciliary feeders the water incurrent flow is produced by means of cilia on numerous filamentous gills; these gills also retain food particles (Morton, 1951).

According to Bieler (1992, 1996) three genera are presently included in the family Siliquariidae: *Pyxipoma* Mörch, 1861, with a short longitudinal slit and a smooth shell; *Tenagodus* Guettard, 1770, with a longer slit and either a smooth or a spiny shell; and *Stephopoma* Mörch, 1861, which is devoid of slit and shows a vermetid-like ecology. Siliquariid molluscs have a wide range of shell coiling patterns with whorls developing either on a single or on several planes. In addition, some *Tenagodus* species are able to produce a series of transversal cracks in their smooth shells, thus allowing adjustment to the curvature of shell coils (Savazzi, 1996). This capacity to modify adult shell shape is unique amongst molluscs (Savazzi, 1996).

Little is known about the biology, ecology and geographical distribution of these obligate sponge dwellers because most reports in the literature concern descriptions of empty shells or shell fragments (Bieler & Hadfield, 1990). Virtually nothing is known about their reproduction, larval longevity and dispersal capacity. In addition, siliquariids are rather rare and live mainly at considerable depths. For these reasons, their associations with host sponges have never been extensively studied: only Morton (1955) remarked on the association between the mollusc slit and the sponge aquiferous system, and Savazzi (1996) hypothesised the existence of a unidirectional water outflow from the mollusc into the sponge aquiferous system.

This study aims to clarify the different aspects of the sponge-siliquariid association, notwithstanding the lack of access we have to living material available for study. More specific topics involving the functional morphology of siliquariid molluscs are treated in a separate paper (Schiaparelli et al., in preparation).

MATERIALS AND METHODS

To date only 35 sponge specimens associated with siliquariid molluscs were studied, collected from the W Mediterranean, E Atlantic and the Pacific area including Philippines, Japan, New Caledonia and N New Zealand. Most of this material was collected by the Muséum National d'Histoire Naturelle, Paris from several deep-water expeditions, and kindly trusted us for study. Several small lots of specimens were also obtained from other sources (Museo di Zoologia of Bologna and private collections). Most of the studied material comes from relatively deep waters (10-550m depth).

All the massive sponge specimens with siliquariids embedded in their bodies were carefully studied in toto. X-ray photographs of some *Penares* and *Spongosorites* specimens were made using health diagnostic X-ray facilities, to ascertain the distribution and alignment of molluscs within the sponge body. Casts of the water flow routes were made by injecting a setting resin into both the shell's main aperture and the sponge's oscule in alcohol preserved specimens and, after the compound had set, by dissolving the sponge body by soaking it in HCl (Bavestrello et al., 1988). Much better results would have been obtained through *in situ* application of resin into living specimens, but this has not yet been possible due to the restricted material available to us.

Sub-samples of the two associated organisms were then separated for species identification. Spicules were prepared by dissolving pieces of sponge in nitric acid in a vial, then dehydrated and mounted either on slides with Eukitt resin or directly on stubs. The skeletal arrangement was studied by hand cut (tangential and transversal) sponge sections.

The abundant siliquariid material available, allowed us to leave specimens intact to study in toto and to dissect and prepare the main diagnostic parts for ultramicroscopy (protoconchs,

TABLE 1. Literature of sponges associated with siliquarid molluscs. References refer to papers reporting whole animals, not just empty shells.

Sponge Species	Mollusc Species	Locality	Depth	Reference
Erylus amorphus Burton, 1926	unidentified Siliquariid	South Africa	-	Burton, 1926
Erylus burtoni Lévi & Lévi, 1983	unidentified Siliquariid	New Caledonia	425-430m	Lévi & Lévi, 1983b
Erylus carteri Sollas, 1888	unidentified Siliquariid	Gulf of Manaar	-	Lévi & Lévi, 1983b
Erylus geodioides Burton & Rao, 1932	unidentified Siliquariid	Mergui Archipelago	119m	Burton & Rao, 1932
Erylus nigra Bergquist, 1968	unidentified Siliquariid	New Zealand	129m	Bergquist, 1978
Erylus proximus Dendy, 1916	unidentified Siliquariid	Cargados	55m	Dendy, 1916
Penares schulzei (Dendy, 1905)	unidentified Siliquariid	New Caledonia, Ceylon	182-430m	Dendy, 1905
Penares sp.	*Pyxipoma weldii* (Tennison Woods, 1876)	New Zealand	-	Morton & Miller, 1968
Racodiscula sceptrellifera (Carter, 1881)	*Tenagodus cumingii* (Mörch, 1861)	Indian Ocean	27-55m	Annandale, 1911
Racodiscula sceptrellifera (Carter, 1881)	*Tenagodus trochlearis* Mörch, 1861	Indian Ocean	-	Annandale, 1911
Siliquariaspongia japonica Hoshino, 1981	*Tenagodus cumingii* Mörch, 1861	Japan	Intertidal	Hoshino, 1981
Spongosorites topsenti Dendy, 1905	*Tenagodus muricatus* (Born 1778)	Indian Ocean	55-69m	Annandale, 1911
Spongosorites ruetzleri (Van Soest & Stentoft, 1988)	unidentified Siliquariid	Barbados	108-153m	Van Soest & Stentoft, 1988
Spongosorites siliquaria Van Soest & Stentoft, 1988	unidentified Siliquariid	Barbados, Jamaica	108-170m	Van Soest & Stentoft, 1988
Unidentified (?) sponge	*Tenagodus modestus* Dall, 1881	Bermuda	-	Dall, 1881
Unidentified (?) sponge	*Tenagodus obtusus* (Schumacher, 1817)	South Africa	-	Barnard, 1963
Unidentified sponge	*Pyxipoma lacteus* Lamarck, 1818	Indian Ocean	-	Morch, 1860
Unidentified sponge	*Pyxipoma weldii* (Tennison Woods, 1876)	New Zealand	-	Morton, 1951
Unidentified sponge	*Tenagodus anguinus* (L., 1758)	Philippines	2-3m	Savazzi, 1996
Unidentified sponge	*Tenagodus armatus* Kuroda et al., 1971	Japan	50-100m	Kuroda et al., 1971
Unidentified sponge	*Tenagodus bernardi* Morch 1860	? Senegal	-	Gould, 1966
Unidentified sponge	*Tenagodus chuni* Thiele, 1925	South Africa	40-155m	Barnard, 1963
Unidentified sponge	*Tenagodus cumingii* Morch, 1860	Philippines	-	Morch, 1860
Unidentified sponge	*Tenagodus cumingii* Morch, 1860	Western Pacific	10-100m	Kuroda et al., 1971
Unidentified sponge	*Tenagodus obtusus* (Schumacher, 1817)	Mediterranean	-	Philippi, 1836
Unidentified sponge	*Tenagodus squamatus* Blainville, 1827	Bermuda	549-732m	Gould, 1966
Unidentified sponge	*Tenagodus squamatus* Blainville, 1827	Bermuda	732m	Abbott, 1974
Unidentified sponge	*Tenagodus wilmanae* Tomlin, 1918	South Africa	150m	Kenseley, 1973
Unidentified sponge	*Tenagodus wilmanae* Tomlin, 1918	South Africa	-	Barnard, 1963

opercula and radulae). A Philips 515 microscope was used for SEM observations.

RESULTS

Twenty nine records of sponges associated with siliquariids have been recorded in the literature (Table 1), but the identification of both partners was complete only in five cases. Nevertheless, from these data, 13 sponge species in total, belonging to 6 genera and 5 families (Ancorinidae, Coppatiidae, Geodiidae, Halichondriidae, Theonellidae) have been identified (Table 1). By comparison, in the present study, preliminary identifications of 35 sponge specimens associated with siliquariids differentiated 19 sponge species belonging to the same 5 families cited above (Table 2), in addition to a fragment of an unidentified horny 'keratose' sponge. Siliquariids studied belonged to 6 species of the genus *Tenagodus* and to a single species of *Pyxipoma* (Table 2). The taxonomic part of the study, including the description of several new species of both sponges and siliquariids, will be the object of future papers.

Geographic and bathymetric distributions of material showed that 5 specimens were from temperate and 30 from tropical regions, and 34 specimens out of 35 were collected at more than 50m depth (Table 2). A similar trend is shown in literature, with 11 temperate and 18 tropical records and 12 specimens out of 17, with known depths of collection, coming from waters deeper than 50m (Table 1).

In all cases but one the sponge specimens hosted a variable number of molluscs belonging to a single species. The exception is a sponge specimen from New Caledonia, collected around 230m depth, and tentatively attributed to the genus *Epipolasis*, that hosted two species of spiny *Tenagodus* that were also recorded in association with other sponge species, indicating that their association is facultative. Some siliquariids may be associated with as many as six different sponge species, as the case of the two spiny *Tenagodus* (*Tenagodus* sp.5 and *T.* cf. *anguinus*) (Table 2).

Different specimens of *Holoxea furtiva* Topsent from distant localities hosted slightly different siliquariid species. Two Mediterranean specimens from Sardinia and Tunisia were associated with *Tenagodus obtusus*, whereas a specimen from Cabo Verde hosted *T. senegalensis*. Different specimens of the same sponge species collected in the same area (e.g. *Spongosorites* cf. *solomonensis*, *Spongosorites* sp.3 and *Topsentia* sp.1) may host different siliquariid species (Table 2).

Considering the five families of sponges that host these siliquariids, three types of skeletal patterns were distinguished: a radial structure in Ancorinidae, Coppatiidae and Geodiidae; a disordered structure in Halichondriidae, and the usual articulated, solid 'lithistid' structure in Theonellidae. Analyzing the distribution of siliquariids belonging to the genus *Tenagodus*, which has species with either smooth or spiny shells, we found a remarkable correlation with the sponge skeletal architecture. 1) Smooth *Tenagodus* species were always associated with sponges that had radial structure (Table 2). These molluscs were completely embedded in the sponge body with only the shell apertures protruding from the sponge surface (Fig. 1A). X-ray photographs showed that the direction of shell growth is straight, determined largely by the radial pattern of the sponge skeleton (Fig. 1B). According to the position of the shell apertures, which are almost flush with the sponge surface, it may be inferred that the growth rate of the associated organisms is nearly the same. This behaviour was observed only in small and medium sized siliquariid species with smooth shells. 2) Conversely, spiny *Tenagodus* species were always associated with sponges having disorderly arranged skeletons (Table 2). In these cases the molluscs were not completely embedded in the sponge body because part of the shell laid on the sponge surface (Fig. 1C). X-ray photographs showed that spiny siliquariids shorten as much as possible the ray of curvature of their first coils, and that a precise direction of shell growth cannot be defined (Fig. 1D). Shell uncoiling is more accentuated towards the sponge surface. The mollusc growth rate certainly exceeded that of the sponge when the shell develops on the host surface. The same behaviour was observed in large size smooth *Tenagodus* specimens (Fig. 2B), which, instead of laying on the sponge surface, raise the terminal part of their shells (Schiaparelli et al., in prep.).

Siliquariids associated with Theonellidae were completely entrapped among desmas. Here they are so constrained by the rigid skeletal structure that they are unable to transversally crack their shells, varying their shape as described by Savazzi (1996), and consequently obliged to grow very irregularly.

Siliquariid protoconchs (larval shells which

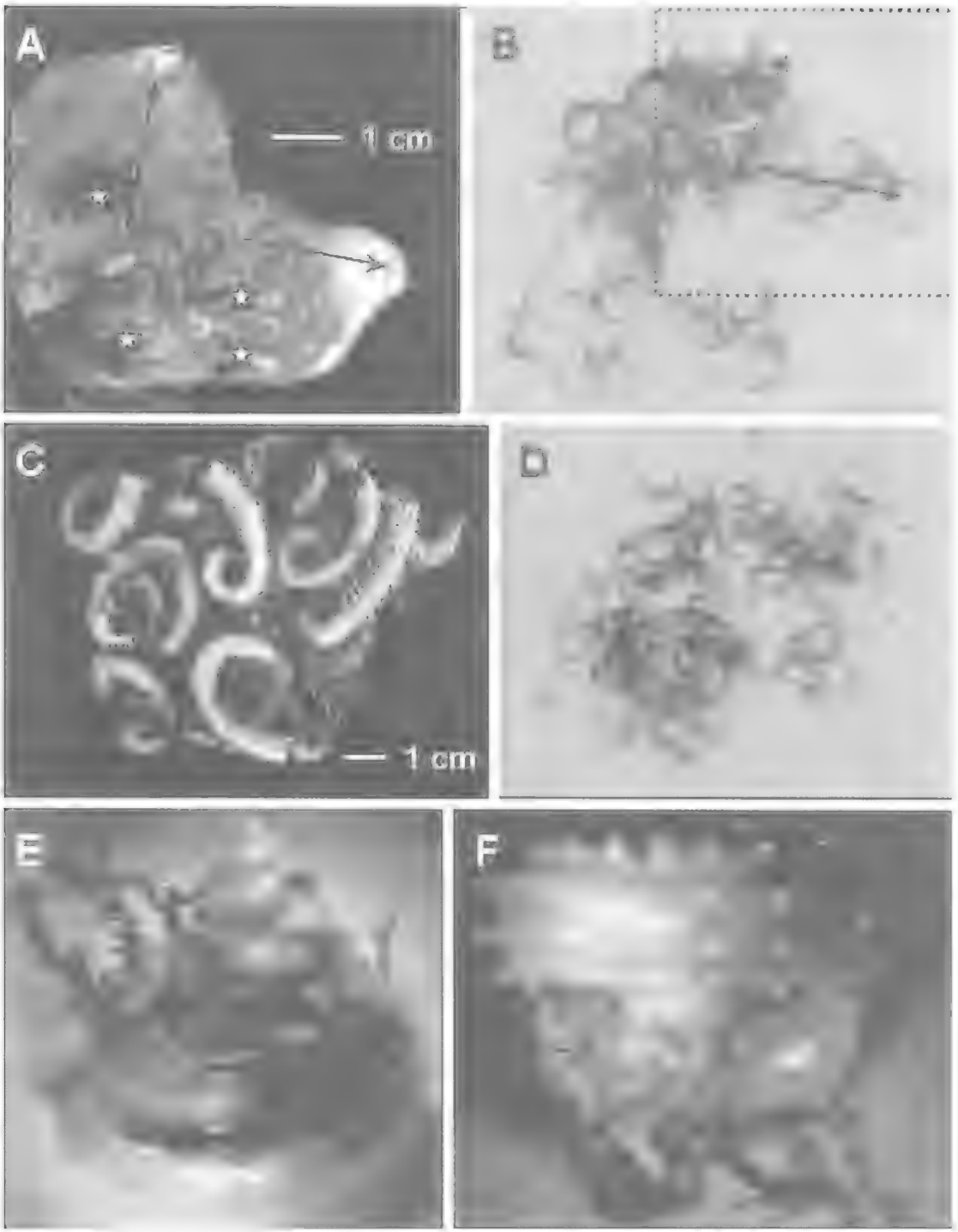

FIG. 1. Associations between siliquariids and sponges. A, Specimen of *Penares intermedia* (Dendy, 1905): the shell apertures are indicated by arrows, whereas the oscules are marked by stars. B, X-ray photograph of the same specimen of *P. intermedia* showing the associated siliquariids (*Tenagodus* sp. 4): the arrows mark the axes of two coiled shells. C, Specimen of *Spongosorites* sp.1 associated with *Tenagodus* cf. *anguinus*. D, X-ray photograph of *Spongosorites* sp.1. E-F, Two aspects of an aquiferous system cast of a *Topsentia* sp.1 specimen associated with *Tenagodus* cf. *anguinus*.

TABLE 2. List of sponge species associated with siliquarid molluscs. Different shades of grey refer to the sponge skeletal structure and to the external morphology of the shells.

Specimen	Sponge Family	Sponge Species	Mollusc Species	Locality	Depth
SI 23/30	Theonellidae	*Discodermia cf. laevidiscus* Carter, 1880	*Tenagodus* sp. 4	Philippines	92-97m
SI 13/31	Geodiidae	*Erylus* sp. nov.	*Tenagodus* sp. 4	New Caledonia	234-242m
SI 25	Geodiidae	*Erylus* sp. nov.	*Tenagodus* sp. 4	Philippines	92-97m
SI 33	Geodiidae	*Erylus nigra* Bergquist, 1968	*Tenagodus* sp. 4	New Caledonia	415m
SI 5	Geodiidae	*Geodia cf. parasitica* Bowerbank, 1873	*Tenagodus senegalensis*	Senegal	-
SI 4	Coppatiidae	*Holoxea furtiva* Topsent, 1892	*Tenagodus obtusus*	Italy	-
SI 1/2	Coppatiidae	*Holoxea furtiva* Topsent, 1892	*Tenagodus obtusus*	Tunisia	9; 32m
SI 3	Coppatiidae	*Holoxea furtiva* Topsent, 1892	*Tenagodus senegalensis*	Cabo Verde	55-60m
SI 97	Coppatiidae	*Jaspis* sp.	*Tenagodus ponderosus*	(?) Australia	-
SI 12/34	Ancorinidae	*Penares intermedia* (Dendy, 1905)	*Tenagodus* sp. 4	New Caledonia	430m
SI 19/26	Ancorinidae	*Penares* sp. nov.	*Tenagodus* sp. 4	New Caledonia	270-300m
SI 20	Ancorinidae	*Penares* sp. 1	*Tenagodus* sp. 4	Philippines	92-97m
SI 35	Ancorinidae	*Penares* sp. 2	*Pyxipoma weldii*	New Zealand	-
SI 8	Halichondriidae	(?) *Epipolasis* sp.	*Tenagodus cf. anguinus* + *Tenagodus* sp. 3	New Caledonia	234-242m
SI 15	Halichondriidae	*Spongosorites cf. salomonensis* Dendy, 1921	*Tenagodus cf. anguinus*	New Caledonia	243m
SI 32	Halichondriidae	*Spongosorites cf. salomonensis* Dendy, 1921	*Tenagodus* sp. 6	New Caledonia	440m
SI 7	Halichondriidae	*Spongosorites* sp. nov.	*Tenagodus* sp. 3	New Caledonia	242m
SI 16	Halichondriidae	*Spongosorites* sp. nov.	*Tenagodus* sp. 3	New Caledonia	300m
SI 9	Halichondriidae	*Spongosorites* sp. 1	*Tenagodus cf. anguinus*	New Caledonia	270-300m
SI 17	Halichondriidae	*Spongosorites* sp. 1	*Tenagodus cf. anguinus*	New Caledonia	260m
SI 27	Halichondriidae	*Spongosorites* sp. 1	*Tenagodus cf. anguinus*	New Caledonia	237-550m
SI 18	Halichondriidae	*Spongosorites* sp. 2	*Tenagodus* sp. nov. 2	New Caledonia	397-439m
SI 24	Halichondriidae	*Spongosorites* sp. 3	*Tenagodus cf. anguinus*	Philippines	92-97m
SI 29	Halichondriidae	*Spongosorites* sp. 3	*Tenagodus* sp. 5	Philippines	183-187m
SI 11	Halichondriidae	*Topsentia* sp. nov.	*Tenagodus* sp. 3	New Caledonia	233m
SI 14	Halichondriidae	*Topsentia* sp. 1	*Tenagodus* sp. nov. 1	Philippines	186-187m
SI 21	Halichondriidae	*Topsentia* sp. 1	*Tenagodus cf. anguinus*	Philippines	92-97m
SI 22	Halichondriidae	*Topsentia* sp. 1	*Tenagodus cf. anguinus*	Philippines	92-97m
SI 28	Halichondriidae	*Topsentia* sp. 2	*Tenagodus cf. anguinus*	New Caledonia	410-440m
SI 6	?	fragment of a horny sponge, dark violet	*Tenagodus maoria*	New Zealand	-

	Sponges with radial skeletal growth		Sponges with disordered skeletons

	Siliquariids with smooth shells		Siliquariids with spiny shells

are separated by a boundary (concave septum) from the adult shells, called teloconchs), have been observed on the surface of several sponge specimens. According to characteristics of their coils (number and size), they belong both to planctotrophic and lecitotrophic species (Schetelma, 1978). Planctotrophic larvae have been observed in *Tenagodus senegalensis* to settle preferentially near the mollusc slit, where they probably find the most suitable water-movement conditions. Recruits are covered by the host sponge and develop in the remaining space between the adult siliquariids.

The functional associations between sponges and associated molluscs were also ascertained by the study of casts. Resin injected into the oscule of a specimen of *Topsentia* sp.1 containing *Tenagodus* cf. *anguinus* came out from the main aperture of the shell and vice versa (Fig. 1F). Casts show that the water pushed by the mollusc ciliary movement seeps through the slit (Fig. 1E) and enters the sponge aquiferous system. There is a reciprocal morphological adaptation of the two associated organisms because the sponge moulds its aquiferous system on the continuous slit aperture and then the mollusc divides this simple slit into a series of holes (Fig. 2AD). Spicule tracts correspond to the carbonate pillars separating the holes (Fig. 2CD). The wide aquiferous system canals (0.6mm diameter) conveying water from the mollusc into the sponge, fit perfectly with the slit holes (Fig. 1E). Thereafter these canals divide either dichotomously or by emitting transverse branches (Fig. 1F). Their size decreases continuously up to a minimum detectable diameter of 0.1mm.

Casts, however, are interpreted as single moments of a continuous growth process which involves both the partners in the association. Particularly important is the shifting of the living mollusc, as far as it grows, towards the shell opening, which causes the moulding of a new part of a functional slit. The growth process also determines a rapid closure by carbonate deposition of the non-functional slit apertures behind the mollusc body: holes in *Tenagodus* (Fig. 2E) and a continuous slit in *Pyxipoma* (Fig. 2F). It was also observed that whenever an open part of the slit accidentally lost its sponge covering it was immediately closed by the mollusc.

DISCUSSION

A siliquariid mollusc living within a sponge has three primary needs: 1) to be at least partially covered by the sponge in order to get support and protection; 2) to have the water-outflow drained through the sponge body; and 3) to maintain the shell opening free for the water-inflow, laying on the sponge surface or variously raised. Such requirements may be fulfilled only by sponges with peculiar characteristics, as demonstrated by the restricted number of sponge taxa currently known to host siliquariids. Some of these characteristics may be tentatively identified as: a massive growth form, assuring an adequate volume to host the molluscs; and a solid structure, generally bound to a high spicule content, contributing to maintain a constant space ratio between the associated organisms, in order to guarantee a plain water outflow. A soft, elastic sponge which continually moves is probably less adapted to maintain a steady association – involving the aquiferous system - with a host dwelling in a rigid shell. The host molluscs, however, display a remarkable adaptive capacity to different situations as demonstrated by the fact that two species of siliquariids were found associated with six different sponge species. As a rule each siliquariid species colonises a single species of sponge (with the exception of the New Caledonian sponge mentioned above), with the number of successful mollusc recruits related to sponge size. Siliquariid recruitment is either through lecithotrophic larvae, which develop *in situ*, or planktotrophic larvae that are released into deep, relatively still waters, and probably do not disperse over large areas. Several factors may favour the recruitment of young siliquariids in this association. One of these is the combined pumping activity of the sponge and associated molluscs, that produces a water current from the surroundings towards the sponge surface which may attract the swimming larvae.

According to our observations it seems probable that the association between sponges and siliquariids is not species specific. This hypothesis is supported by the behaviour of *Holoxea furtiva*, a sponge with a wide geographic distribution that hosts two siliquariid species in geographically distant localities. Similarly, different specimens of the same sponge species in the same geographic locality host different siliquariids (e.g. *Spongosorites* cf. *salomonensis*, *Spongosorites* sp. 3 and *Topsentia* sp. 1), also support this contention.

From present knowledge the association between sponges and siliquariids seems to be relatively frequent in the tropics and in deeper waters – where the latter taxon is more abundant –

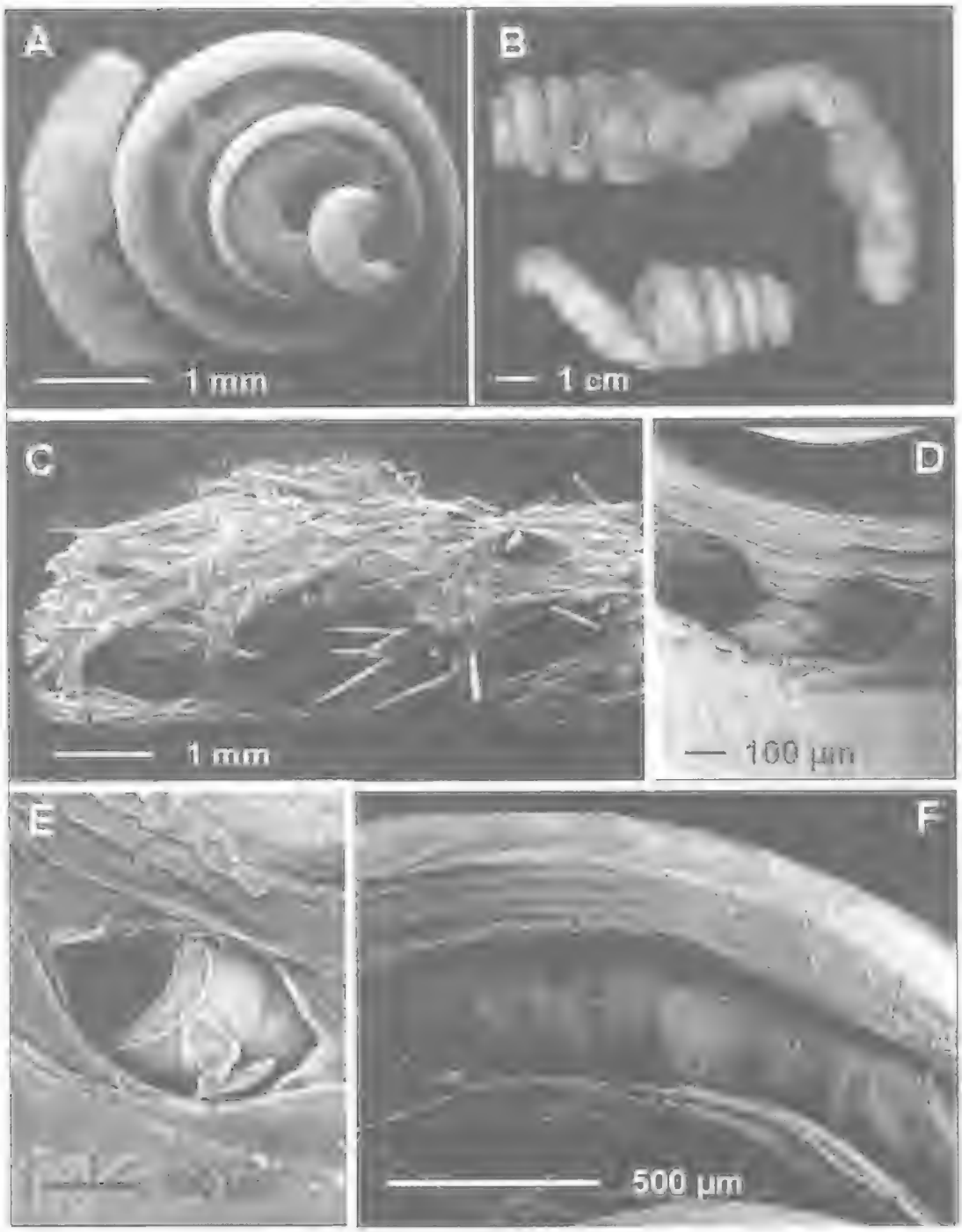

FIG. 2. Associations between siliquariids and sponges. A, Specimen of *Tenagodus* with a slit divided into holes. B, Large, smooth specimens of *Tenagodus senegalensis* with a continuous slit and the terminal part of the shell uncoiled. C, Portion of a *Jaspis* sp. specimen overgrowing a siliquariid slit. D, Formation of holes in the slit of a *Tenagodus* specimen by carbonate denticulation. E, Closure of a slit hole in a *Tenagodus* specimen by a carbonate lunula during the molluscan growth. F, Continuous slit closed in a specimen of *Pyxipoma weldii*.

but these conclusions are based on restricted samples, and future surveys of the shallow-water areas might alter this presumed distribution pattern.

The relationship between the shell morphology (smooth or spiny) and the sponge skeletal architecture (radial or disordered) is particularly strong. Of possible hypotheses to explain why smooth siliquariids are associated with radially structured sponges, and spiny ones with disorderly arranged skeletons, the most consistent seems to be that the choanosomal space is so reduced due to high spicule density, and the physical constraints so strict due to the presence of radial spicule tracts, that only smooth shells can adapt to the radial structure. Smooth siliquariids, in fact, which have transversally cracked shells, may adapt their shells to extremely confined spaces by changing the curvature of their coils. However, when smooth *Tenagodus* specimens are tightly entrapped into an articulated 'lithistid' desma reticulation (e.g. *Discodermia*), they are unable to modify their shell shape and must change their normal growth habit becoming uncoiled (Schiaparelli et al., in prep.). Spiny siliquariids, on the contrary, which very rarely show shell cracks, cannot modify their shape in order to adapt to very hard sponges and are associated with disorderly arranged skeletal structures (such as those found in Halichondriidae), where the available space inside the skeleton is certainly wider. Since the main factor that forces all siliquariids to live permanently within sponges is the demand for protection against predators (Vermej, 1987), the production of spines by these molluscs may be interpreted as a reaction against an inadequate protection from the host sponge. The fact that the last coils of spiny species lay uncovered on the sponge surface is due to the mechanical protection offered by the spines against muricid molluscs which, according to the shape of perforations (Carriker & Jockelson, 1968), seem to be the most common siliquariid predators. Smooth species, on the contrary, are much more vulnerable, as demonstrated by the high number of unsuccessful muricid holes (in the uncovered shell portions) and by the attitude to close, by means of a calcareous lamina, every portion of their slit accidentally left uncovered by the host sponge.

The prompt responses shown by siliquariids to new situations, together with the ability to shift their position along the shell during growth, determine a continuous and complex variation of the slit morphology (Schiaparelli et al., in prep.). Casts show that close relationships are established with the sponge aquiferous system to obtain an effective drain of water pumped by the mollusc. Water entering the shell aperture is pushed by ciliary beating through the slit towards the sponge canals, thus obtaining an obligate flow direction. The sponge does not try to clog the slit but, on the contrary, seems to mould its skeletal structure on it. The mollusc, on the other hand, is able to modify the slit shape by forming holes that correspond exactly to the sponge canals. The dichotomous branching and ever-decreasing diameter of canals, even if negligible as an absolute figure given its variation between specimens (Bavestrello et al., 1988), are typical of the sponge incurrent system (Bavestrello et al., 1990, 1995). Therefore the sponge receives from each associated siliquariid a continuous water flow.

CONCLUSIONS

Associations between sponges and siliquariids are examined in terms of benefits and disadvantages for either partner. A sponge associated with siliquariids may obtain two major benefits: 1) a considerable energy-saving for the pumping activity, due to the water flow pushed by the mollusc; 2) an additional food supply coming from the fine edible particles that a gastropod ctenidium is unable to hold. The presence of shells, on the contrary, could be an obstacle to the formation of the normal skeletal frame, but the sponge plasticity seems to easily overcome this constraint.

Siliquariids may obtain a greater amount of benefits from their association with sponges: 1) an effective defence from predators, which is certainly mechanical and possibly chemical (in the latter case, however, the mollusc should have developed a form of resistance to the sponge bioactive products, with the assumption that the association between both partners has a significant evolutionary history); 2) in terms of space the sponge primarily offers the mollusc a steady platform of support, even on unstable, detritic bottoms, and secondly a raised position, less disturbed by the sediment, which may confer trophic advantages to the filter-feeder; and 3) the sponge pumping activity, determining a continuous water flow towards the sponge surface, certainly brings food particles that the siliquariid can consume and, possibly, even attracts molluscan larvae.

Theoretically, the main potential disadvantage for a siliquariid associated with a sponge is the risk of being killed by the host growth overwhelming its shell apertures. However, even if some sponges, under special conditions, are able to increase their growth rates several-fold (Ayling, 1983), such cases of overwhelming by the host would probably occur very rarely, because the growth of the terminal part of the shell, bearing the aperture, is probably very rapid, especially in spiny species.

Finally, siliquariid molluscs have developed such basic structural adaptations to live in association with sponges (see also Schiaparelli et al., in prep.), that the obligatory nature of their relationship is a logical and predictable consequence. On the contrary, sponges may or may not react negatively to the 'invasion' or colonisation of their body by siliquariids, but they are surely getting remarkable benefits in terms of food, and are obviously free to live without associated siliquariids. The association, therefore, may be viewed as a form of commensalism and probably even of facultative (for the sponges at least) mutualism.

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ANTIMICROBIAL ACTIVITY OF CARIBBEAN SPONGE EXTRACTS. *Memoirs of the Queensland Museum 44: 438. 1999*:- Marine sponges produce a diversity of unusual chemical compounds, but the ecological functions of these metabolites remain largely unknown. Organic extracts from 33 Caribbean sponges were assayed against a panel of 8 marine bacterial strains to determine if sponge secondary metabolites have ecologically significant antimicrobial effects. The test panel was comprised of an opportunistic pathogen (*Vibrio parahaemolyticus*), a common fouling bacterium (*Deleya marina*), and strains isolated from seawater and healthy and necrotic Caribbean sponges. Extracts were tested for antibiotic activity at concentrations that were volumetrically equivalent to those found in sponge tissues (i.e., whole-tissue concentrations). Bioassay results revealed that 16 species extracts (48% of those tested) exhibited antibiotic activity against at least one bacterial isolate and that the necrotic sponge isolates were the most sensitive test strains (inhibited by 40% of the extracts). Extracts from *Amphimedon compressa*, *Amphimedon erina*, *Aplysina lacunosa*, *Ptilocaulis spiculifera* and *Axinella corrugata* inhibited the largest numbers of test strains and exhibited the most potent antibiotic activities with values frequently exceeding that of the control antibiotic (Gentamicin). The pattern of antimicrobial activity was different for 15 of the 16 active species indicating that diverse taxa do not produce similar antibacterial metabolites. In total only 23% of the extracts/bacterial interactions tested generated antimicrobial activity indicating that conspicuous members of the Caribbean sponge community do not generally produce broad-spectrum antibacterial metabolites. All the extracts from species that exhibited antibacterial activity also deterred feeding by reef fish in a previous study, suggesting that some secondary metabolites may have evolved with multiple functions. Stevensine, a compound from *Axinella corrugata* known to deter feeding by predatory reef fishes, exhibited weak antibacterial activity, suggesting that this potent feeding deterrent is not solely responsible for the antimicrobial activity detected in the crude sponge extract. ❐ *Porifera, antimicrobial, antibiotic, secondary metabolites, chemical defense, ecology.*

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ANNOTATED CHECKLIST OF MARINE SPONGES OF THE INDIAN REGION

J.G. PATTANAYAK

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An annotated checklist of marine sponges from the Indian region was compiled primarily from: identified sponges in the holdings of Zoological Survey of India; published descriptions of species from this region; and personal correspondence with experts in the field both from European and Oriental regions. 451 species are recorded from the 3 classes, 17 orders, 64 families and 168 genera. Few taxonomic studies on sponges have been undertaken in the Indian region over the past two decades, but there is a large early literature commencing in 1873. Consequently most of the literature and species descriptions are relatively old, sometimes too brief, and sometimes entirely inadequate to differentiate between related species. It is also well known that sponges are notoriously difficult to identify and misidentifications are common. Unfortunately re-examination of many type or voucher specimens was not possible given that they are scattered throughout many different museums and institutions. The preparation of this checklist therefore relies heavily on the published literature, with only relatively few species yet checked from original material. ❐ *Porifera, taxonomic checklist, Indian sponges, sponge identification.*

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Published literature on the Indian region sponge fauna has a rich and productive history, commencing with the work of Bowerbank (1873), Carter (1880-1887), Dendy (1887-1916) and Schulze (1894-1904) followed by the present century workers Annandale (1911-1915), Kumar (1924-1925), Dendy & Burton (1926), Burton (1928-1937), Burton & Rao (1932), Rao (1941), Ali (1956), Thomas (1968-1993) and Pattanayak (1995-1998), with some revisions of this fauna also made by Hooper (1996). With the exception of several papers by Thomas few taxonomic studies have been undertaken in the Indian region over the past two decades. Consequently most of the literature and species descriptions are relatively old, sometimes too brief, and sometimes entirely inadequate to differentiate between related species. It is also well known that sponges are notoriously difficult to identify and misidentifications are common. Unfortunately re-examination of many type or voucher specimens was not possible given that they are scattered throughout many museums and institutions.

Consequently, this annotated checklist of marine sponges from the Indian region is based primarily on: a) identification of sponges in the holdings of Zoological Survey of India by several workers, including the author; b) cross-referencing of all available literature pertaining to this region; and c) personal correspondence with experts in the field both from European and Oriental regions. This heavy reliance on the published literature is presently unavoidable, with only relatively few species yet checked from original material, although it is anticipated that this checklist will be both expanded (with current work being undertaken by the Zoological Survey of India) and revised (as type material becomes available to check species' identities, and as contemporary authors increase their published revisions).

For convenience the Indian region is divided into 9 zones (see Fig. 1). In the species checklist each published record is accompanied by one or more literature citation, the locality and region of collection, as defined above in brackets following the citation. For brevity, only the Indian literature is cited in this paper. Literature pertaining to the higher taxa can be found in Hooper & Wiedenmayer (1994).

SYSTEMATIC CHECKLIST OF SPONGES

Phylum **Porifera**
Class **Demospongiae**
Subclass **Homoscleromorpha**
Order **Homosclerophorida**
Family **Plakinidae** Schulze

Genus *Corticium* Schmidt. *C. acanthastrum* Thomas, 1968e: 260, fig. 1a-b; Thomas, 1985: 353, pl. VIII, fig. 21 (Palk Bay) (5). *C. candelabrum* Schmidt; Thomas, 1968e: 261, fig. 2a-b;

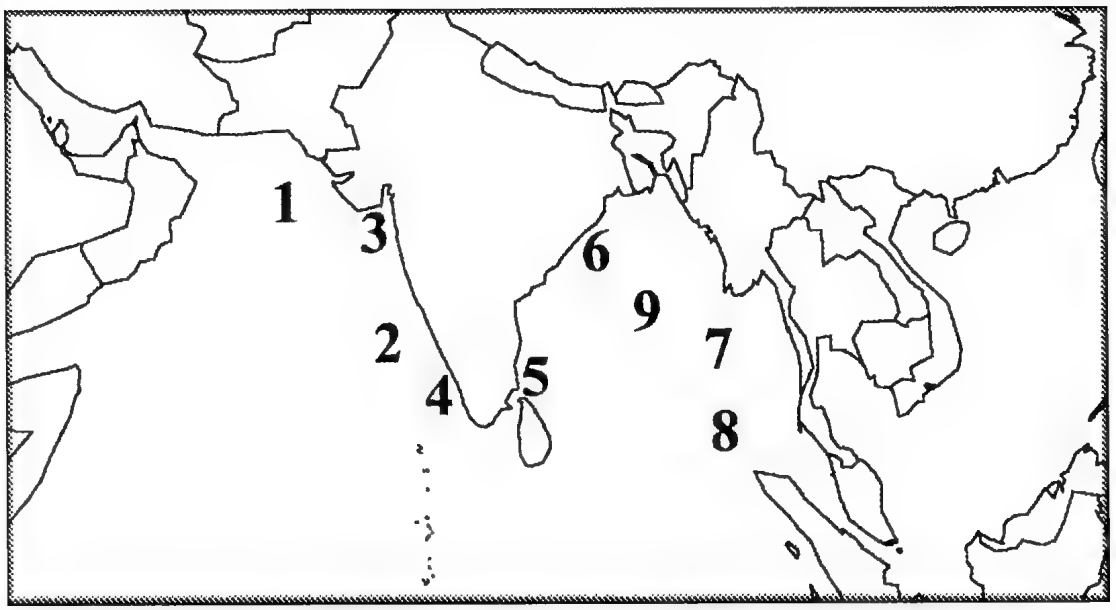

FIG. 1. Study zones of the Indian region. 1, Arabian sea - off-shore waters; 2, Laccadive Islands; 3, northern W coast - Gujarat and Maharashtra; 4, southern W coast - Karnataka and Kerala; 5, SE coast - Tamil Nadu and adjacent islands in the Gulf of Mannar; 6, NE coast - Andhra Pradesh, Orissa and West Bengal; 7, Andaman Islands; 8, Nicobar Islands; 9, Bay of Bengal - off-shore waters.

Thomas, 1985: 352, pl. VIII, fig. 20 (Gulf of Mannar and Palk Bay) (5).

Genus *Plakina* Schulze. *P. acantholopha* Thomas, 1970c: 53, fig. 3; Thomas, 1985: 354, pl. IX, fig. 3 (Palk Bay) (5). *P. monolopha* Schulze; Thomas, 1970c: 208, fig. 10; Thomas, 1970c: 52, fig. 1; Thomas, 1985: 353, pl. VIII, fig. 22 (Palk Bay) (5). *P. trilopha* Schulze; Thomas, 1970c: 53, fig. 2; Thomas, 1985: 353, pl. IX, fig. 1 (Gulf of Mannar) (5).

Genus *Plakinastrella* Schulze. *P. ceylonica* (Dendy, 1905); Thomas, 1985: 351, pl. VIII, fig. 17 (Gulf of Mannar, as *Dercitopsis ceylonica*) (5). *P. minor* (Dendy, 1905); Thomas 1985: 351, pl. VIII, fig. 18 (Gulf of Mannar and Palk Bay, as *Dercitopsis minor*) (5).

Subclass **Tetractinomorpha**
Order **Spirophorida**
Family **Tetillidae** Sollas

Genus *Cinachyra* Sollas. *C. arabica* (Carter); Burton & Rao, 1932: 326; Pattanayak, 1998 (Camorta I., Nicobars, Krusadai I., Andamans) (5,7). *C. australiensis* (Carter); Burton & Rao, 1932; Pattanayak, 1998 (Andamans) (7). *C. cavernosa* (Lamarck); Burton, 1937: 12; Rao, 1941: 425, (Gulf of Mannar and Palk Bay, as *Chrotella australiensis*); Thomas, 1985: 341, pl. VII, fig. 21 (Gulf of Mannar and Palk Bay); Thomas, 1984: 101, fig. 1h (SE coast of India) (5,9). *C. hirsuta* (Dendy); Burton, 1930: 666 (Gulf of Mannar) (5).

Genus *Paratetilla* Dendy. *P. bacca* (Selenka); Kumar, 1925: 218; Burton & Rao, 1932: 325 (Kilakarai, Ramnad Dt.); Burton & Rao, 1932: 325, Pattanayak, 1998 (Andamans); Thomas, 1980b: 16 (Minicoy I.); Thomas, 1985: 342, pl. VII, fig. 22 (Gulf of Mannar and Palk Bay)) (2,5,7).

Genus *Samus* Gray. *S. anonyma*, Gray; Carter 1880: 59; Thomas, 1985: 354, pl. IX, fig. 3 (Gulf of Mannar) (5).

Genus *Tetilla* Schmidt. *T. barodensis* Dendy, 1916b: 105, pl. 1, fig. 3a-d (Off Dwarka, Okhamandal) (3). *T. cranium* (Müller); Burton & Rao, 1932: 326 (Invisible bank, Andamans; 2-3 miles W of Cape Comorin) (5,7). *T. dactyloidea* (Carter); Annandale, 1915c: 53, pl. V, fig. 4 (Chilka Lake, Orissa, as *T. dactyloidea* var. *lingua*); Dendy, 1916b: 102, pl. II. fig. 10a-c (Balapur, Okhamandal); Burton & Rao, 1932: 326; Pattanayak, 1998 (Port Blair, Andamans) (3,6,7). *T. hirstua* (Dendy): Dendy, 1916b: 104 (Vamiani Point, off Okhamandal) (3). *T. pilula* Dendy, 1916b: 104, pl. I, fig. 2a-c (Okhamandal) (3).

Family **Scleritodermidae** Sollas

Genus *Aciculites* Schmidt. *A. orientalis* Dendy, 1905; Thomas, 1985: 323, pl. VI, fig. 26 (Gulf of Mannar) (5).

Genus *Amphibleptula* Schmidt. *A. herdmani* (Dendy, 1905); Thomas, 1985: 255, pl. II, fig. 28 (Gulf of Mannar, as *Taprobane herdmani*).

Order **Astrophorida**
Family **Ancorinidae** Schmidt

Genus *Ancorina* Schmidt. *A. simplex* (Lendenfeld); Kumar, 1925: 213 (Ramnad Dist., S India) (5).

Genus *Asteropus* Sollas. *A. simplex* (Carter); Dendy, 1916b: 98 (Okhamandal); Kumar, 1925: 212 (Orissa Coast); Thomas, 1985: 326, pl. VI, fig. 30 (Gulf of Mannar and Palk Bay, as *Stellettinopsis simplex*) (3,5,6).

Genus *Ecionemia* Bowerbank. *E. acervus* Bowerbank, 1863; Burton & Rao, 1932: 318 (Andaman and Nicobar Is, as *E. carteri*; Kilakarai); Burton, 1937: 5 (Krusadai I., as *E. bacilifera*); Thomas, 1980b: 15 (Minicoy I.) ; Thomas, 1985: 334, pl. VII, fig. 6 (Gulf of Mannar) (2,5,7,8). *E. laviniensis* Dendy, 1905; Thomas, 1985: 335, pl. VII, fig. 7 (Gulf of Mannar) (5). *E. thielei* Thomas, 1980b: 15, fig. 2c (Minicoy I.) (2).

Genus *Myriastra* Sollas. *M. clavosa* (Ridley); Dendy & Burton, 1926: 246; Burton & Rao, 1932: 331 (off Cinque I., Andamans, as *Stelletta clavosa*); Thomas, 1985: 336, pl. VII, fig. 9 (Gulf of Mannar) (5,7). *M. purpurea* (Ridley); Burton & Rao, 1932: 310 (Tuticorin, Nicobar I., as *Stelletta purpurea*); Burton, 1937: 4 (Krusadai I., as *Stelletta purpurea*); Rao, 1941: 418; Thomas, 1985: 336, pl. VII, fig. 8 (Gulf of Mannar); Pattanayak, 1998 (Andaman and Nicobar Is) (5,7,8).

Genus *Penares* Gray. *P. intermedia* (Dendy, 1905); Thomas, 1984: 100, fig. 1j,k (SE coast of India); Thomas, 1985: 334, pl. VII, fig. 5 (Gulf of Mannar (5,9).

Genus *Rhabdastrella* Thiele. *R. globostellata* (Carter); Burton & Rao, 1932: 317 (Aberdeen Reef, Andaman, as *Aurora globostellatta*); Thomas, 1984: 100 (SE coast of India, as *Aurora globostelletta*); Thomas, 1985: 336, pl. VII, fig. 10 (Palk Bay, as *Aurora globostelletta*) (5,7,9). *R. providentiae* (Dendy); Thomas, 1985: 337, pl. VII, fig. 11 (Gulf of Mannar and Palk Bay, as *Aurora providentiae*) (5). *R. rowi* Dendy, 1916b; Burton & Rao, 1932: 317 (Ganjam Coast, as *Aurora rowi*) (6).

Genus *Stelletta* Schmidt. *S. aruensis* Hentschel, 1912; Burton & Rao, 1932: 312 (Ganjam coast) (6). *S. cavernosa* (Dendy, 1916b); Burton & Rao, 1932: 311 (Nicobar I.) (8). *S. haeckeli* (Sollas); Dendy, 1916b: 97 (Okhamandal, as *Myriastra (Pilochrota) haeckeli*); Dendy & Burton, 1926: 246 (Interview I., Andamans (3,7). *S. herdmani* Dendy, 1905; Thomas, 1985: 338, pl. VII, fig. 13 (Gulf of Mannar) (5). *S. orientalis* Thiele; Burton and Rao, 1932: 311 (Andamans) (7). *S. tethyopsis* Carter, 1880: 137, pl. 6, figs 39,40; Thomas, 1985: 337, pl. VII, fig. 12 (Gulf of Mannar) (5). *S. validissima* Thiele; Burton and Rao, 1932: 310; Dendy & Burton, 1926: 241; Pattanayak, 1998 (Andamans) (7). *S. vestigium* Dendy, 1905; Thomas, 1985: 338, pl. VII, fig. 14 (Gulf of Mannar) (5).

Family **Coppatiidae** Topsent

Genus *Cryptotethya* Dendy. *C. agglutinans* Dendy, 1905; Thomas, 1985: 328, pl. VI, fig. 36 (Gulf of Mannar) (5).

Genus *Jaspis* Gray. *J. bouilloni* Thomas, 1973: 65, pl. 3, fig. 15; Thomas, 1985: 327, pl. VI, fig. 37 (Gulf of Mannar) (5). *J. investigatrix* (Annandale, 1915b: 460, pl. 34, figs 1,2) (Gulf of Mannar, as *Coppatius investigatrix*); Thomas, 1985: 327, pl. VI, fig. 33 (Gulf of Mannar) (5). *J. penetrans* (Carter, 1880: 141, pl. 7, fig. 44) (as *Tisiphonia penetrans*); Thomas, 1972: 352, pl. I, fig. 6A,B; Thomas, 1985: 326, pl. VI, fig. 32 (Gulf of Mannar) (5). *J. reptans* (Dendy, 1905); Thomas, 1985: 327, pl. VI, fig. 31 (Gulf of Mannar); Dendy, 1916b: 97 (Okhamandal) (3,5).

Genus *Zaplethea* de Laubenfels. *Z. diagnoxea* var. *diastra* Vacelet and Vasseur; Thomas, 1985: 328, pl. VI, fig. 35 (Gulf of Mannar and Palk Bay) (5).

Family **Geodiidae** Gray

Genus *Erylus* Gray. *E. carteri* Sollas; Carter, 1880: 15, pl. 7, fig. 41 (as *Stelletta euastrum*); Thomas, 1985: 341, pl. VII, fig. 20 (Gulf of Mannar) (5). *E. lendenfeldi* Sollas; Burton & Rao, 1932: 320; Pattanayak, 1998 (Andamans) (7).

Genus *Geodia* Lamarck. *G. areolata* Carter, 1880: 133, pl. 6, figs 36-37; Thomas, 1985: 339, pl. IX, fig. 8 (Gulf of Mannar); Burton, 1937: 8, pl. 1, fig. 3 (Krusadai I.) (5). *G. globostellifera* Carter, 1880: 134, pl. 6, fig. 38; Thomas, 1985: 340, pl. VII, fig. 18 (Gulf of Mannar) (50). *G.*

inconspicua (Bowerbank); Burton & Rao, 1932: 322 (Kilakarai, Romnad Dist.; Ganjam coast; Gulf of Mannar); Thomas, 1985: 339, pl. VII, fig. 16 (Gulf of Mannar) (5,6). *G. lindgreni* (Lendenfeld); Thomas, 1980b: 16 (Minocoy I.); Thomas, 1985: 340, pl. VII, fig. 1a (Gulf of Mannar and Palk Bay) (2,5). *G. perarmata* Bowerbank; Carter, 1880: 131, pl. 6, figs 32-35; Thomas, 1985: 339, pl. VII, fig. 15 (Gulf of Mannar) (5). *G. picteti* (Topsent); Burton, 1937: 9 (Krusadai I.) (5). *G. ramodigitata* Carter, 1880: 133, pl. 34, fig. 31; Thomas, 1985: 340, pl. VII, fig. 17 (Gulf of Mannar) (5). *G. variospiculosa* Thiele; Dendy, 1916b: 99 (Okhamandal) (3).

Family **Pachastrellidae** Carter

Genus *Dercitus* Gray. *D. simplex* (Carter); Burton & Rao, 1932: 309 (Invisible Bank, Andamans, as *D. plicatus* var. *simplex*).

Genus *Halina* Bowerbank. *H. extensa* (Dendy, 1905); Thomas, 1985: 349, pl. VIII, fig. 13 (Gulf of Mannar) (5). *H. plicata* (Schmidt); Carter, 1880: 60 (as *Samus simplex*); Thomas, 1970a: 207, fig. 9; Thomas, 1985: 349, pl. VIII, fig. 12 (Gulf of Mannar) (5).

Genus *Pachamphilla* Lendenfeld. *P. dendyi* Hentschel; Thomas, 1977: 119, fig. 1D,E; Thomas, 1985: 351, pl. VIII, fig. 19 (Gulf of Mannar) (5).

Genus *Pachastrella* Schmidt. *P. parasitica* Carter, 1880: 60 (as *Samus (Pachastrella) parasitica*); Thomas, 1985: 350, pl. VIII, fig. 15 (Gulf of Mannar) (5). *P. nana* (Carter, 1880: 138, pl. 7, fig. 4) (as *Tisphonia nana*); Thomas, 1985: 350, pl. VIII, fig. 16 (as *Nethea nana*) (Gulf of Mannar) (5).

Genus *Poecillastra* Sollas. *P. eccentrica* Dendy & Burton, 1926: 238; Pattanayak, 1998 (Andamans) (7). *P. schulzei* Sollas; Thomas, 1970: 208, fig. 8, 8a, 8b; Thomas, 1985: 355, pl. IX, fig. 4 (Gulf of Mannar) (5). *P. tenuilaminaris* Sollas; Dendy and Burton, 1926: 238; Burton & Rao, 1932: 309; Pattanayak, 1998 (Andamans) (7).

Genus *Sphinctrella* Schmidt. *S. annulata* (Carter, 1880: 140, pl. 5, fig. 28) (as *Tisiphonia annulata*); Thomas 1985: 349, pl. VIII, fig. 14 (Gulf of Mannar)(5).

Family **Theneidae** Sollas

Genus *Thenea* Gray. *T. andamanensis* Dendy & Burton, 1926: 235; Pattanayak, 1998 (Andamans) (7).

Order **Hadromerida**
Family **Chondrillidae** Gray

Genus *Chondrilla* Schmidt. *C. agglutinans* Dendy, 1916a: 102, pl. I, fig. 1a,b (Okhamandal) (3). *C. australiensis* Carter; Dendy, 1916b: 101 (Okhamandal); Burton and Rao, 1932: 325 (Ganjam coast, Krusadai I.); Thomas, 1985: 355, pl. IX, fig. 5 (Gulf of Mannar) (3,5,6). *C. kilakaria* Kumar, 1925: 214, fig. 1 (Kilakarai, Ramnad Dt.); Thomas, 1985: 356, pl. IX, fig. 7 (Gulf of Mannar) (5). *C. sacciformis* Carter; Thomas, 1985: 356, pl. IX, fig. 6 (Palk Bay) (5).

Genus *Chondrosia* Nardo. *C. reniformis* Nardo; Burton & Rao, 1932: 324 (Pearl Oyster Bank, Tuticorin); Burton, 1937: 10 (Krusadai I.); Rao, 1941: 424; Thomas, 1985: 358 (Gulf of Mannar) (5).

Family **Clionidae** Gray

Genus *Annandalea* Thomas. *A. laeviaster* (Annandale, 1915: 462, fig. 2); Thomas, 1979: 179, fig. 5F (Bay of Bengal) (6).

Genus *Cliona* Grant. *C. anulifera* Annandale, 1915; Thomas, 1979a: 178, fig. 1N, 4G, 5I,J,K, pl. 2, fig. 9 (Gulf of Mannar) (5). *C. carpentari* Hancock; Thomas, 1975: 122 (Zuari and Mandovi Estuary, Goa); Thomas, 1979a: 175 (Port Blair, Andamans); Thomas, 1985: 319, pl. VI, fig. 17 (Gulf of Mannar and Palk Bay); Thomas, Ramadoss & Vincent, 1993: 145 (Coast of Kerala and Tamil Nadu) (3,4,5,7). *C. celata* Grant; Thomas, 1975: 120 (Zuari and Mandovi Estuary, Goa); Thomas: 11 (Minicoy I.); Thomas, 1985: 317, pl. VI, fig. 12 (Gulf of Mannar and Palk Bay); Thomas, Ramadoss & Vincent, 1993: 145 (Coast of Kerala and Tamil Nadu) (2,3,4,5). *C. coronaria* (Carter); Dendy, 1916b: 132 (Okhamandal) (3). *C. ensifera* Sollas; Thomas, 1979a: 176; Thomas, 1985: 320, pl. VI, fig. 18 (Gulf of Mannar); Pattanayak, 1998 (Andamans) (5,7). *C. kempi* Annandale, 1915b: 462, fig. 2; Thomas, 1979a: 179, fig. 5E (Andamans) (7). *C. lobata* Hancock; Burton, 1937: 16; Thomas, 1979a: 172, fig. 2N; Thomas, 1985: 318, pl. VI, fig. 14 (Gulf of Mannar); Thomas, Ramadoss & Vincent, 1993: 145 (Coast of Kerala and Tamil Nadu); Pattanayak, 1998 (Andamans) (4,5,7). *C. margaritifera* Dendy, 1905; Annandale, 1915b: 9; Thomas, 1972: 348, pl. III, fig. 1; Thomas, 1985: 321, pl. VI, fig. 21 (Palk Bay); Thomas, 1975: 122 (Zuari and Mandovi estuary, Goa); Thomas, Ramadoss & Vincent, 1993: 145 (Coast of Kerala and Tamil Nadu) (3,4,5). *C. mucronata* Sollas; Thomas, 1972: 347, pl. 1, fig. 8A,B,C,D;

Thomas, 1979a: 175, fig. 32, fig. 4I,J; Thomas, 1985: 320, pl. VI,fig. 19 (Gulf of Mannar); Pattanayak, 1998 (Andamans) (5,7). *C. orientalis* Thiele; Thomas, 1972: 347, pl. II, fig. 2A,B; Thomas, 1979a: 177, fig. 1M, fig. 4F, pl. IV, fig. 1; Thomas 1985: 321, pl. VI, fig. 20 (Gulf of Mannar) (5). *C. quadrata* Hancock; Carter, 1880: 370, pl. 18, fig. 6; Thomas, 1972: 349, pl. III, fig. 1; Thomas, 1979a: 174, fig. 5,D; Thomas, 1985: 319, pl. VI, fig. 15 (Gulf of Mannar); Pattanayak 1998 (Andamans) (5,7). *C. vastifica* Hancock; Annandale, 1915b: 37, pl. IV, fig. 7 (Chilka Lake, Orissa); Thomas, 1972: 345, pl. I, fig. 3,3A,3B; Thomas, 1985: 318, pl. VI, fig. 13 (Gulf of Mannar and Palk Bay); Thomas, 1975: 121; Thomas and Thanapati, 1980: 54 (Zuari and Mandovi Estuary, Goa); Thomas, 1980b: 12 (Minicoy I.); Thomas, Ramadoss & Vincent, 1993: 145 (Coast of Kerala and Tamil Nadu); Pattanayak, 1998 (Andamans) (2,3,4,5,6,7). *C. viridis* Schmidt; Thomas, 1972: 349, pl. II, fig. 1; Thomas, 1979a: 174, fig. 1F; Thomas, 1985: 319, pl. VI, fig. 16 (Gulf of Mannar and Palk Bay); Kumar, 1925: 228 (Kilakarai, Ramnad Dt.) (5).

Genus *Delectona* de Laubenfels. *D. higgini* (Carter, 1880: 58, pl. 5, fig. 25); Thomas, 1972: 351, pl. III, fig. 4; Thomas, 1985: 322, pl. VI, fig. 24 (Gulf of Mannar) (5).

Genus *Donotella* Dendy. *D. acustella* (Annandale, 1915b: 14, fig. 2) (Ganjam coast) (6).

Genus *Dotona* Carter. *D. pulchella* Carter, 1880: 57, pl. 5, fig. 24; Thomas, 1979a: 180, fig. 5f; Thomas, 1985: 323, pl. VI, fig. 25 (Gulf of Mannar) (5).

Genus *Thooce* de Laubenfels. *T. socialis* (Carter, 1880: 56, pl. 5, fig. 23) (as *Thoosa socialis*); Thomas, 1972: 350, pl. 3, fig. 5; Thomas, 1985: 322, pl. VI, fig. 23 (Gulf of Mannar) (5).

Genus *Thoosa* Hancock. *T. (Cliothosa) armata* (Topsent); Thomas, 1979a: 181, figs 2A, 3B (Bay of Bengal) (9). *T. (Cliothosa) fischeri* (Topsent); Thomas, 1985: 321 (Gulf of Mannar and Palk Bay) (5). *T. (Clithosa) hancocki* (Topsent); Thomas, 1979a: 180, fig. 1p (Bay of Bengal); Pattanayak, 1998 (Andamans) (7,9). *T. (Cliothosa) investigatoris* (Annandale, 1915b); Thomas, 1985: 322, pl. VI, fig. 22 (Gulf of Mannar and Palk Bay) (5).

Family **Latrunculiidae** Topsent

Genus *Latrunculia* Bocage. *L. tenuistellata* (Dendy, 1905); Thomas, 1985: 299, pl. V, fig. 12 (Gulf of Mannar) (5).

Family **Placospongiidae** Gray

Genus *Placospongia* Gray. *P. corinata* (Bowerbank); Dendy, 1916b: 132 (Okhmandal); Burton, 1937: 16; Thomas, 1985: 314, pl. VI, fig. 8 (Gulf of Mannar); Thomas, 1980: 11 (Minicoy I.) (2,3,5). *P. melobesioides* Gray; Thomas, 1985: 313, pl. VI, fig. 7 (Gulf of Mannar) (5).

Family **Polymastiidae** Gray

Genus *Polymastia* Bowerbank. *P. gemmipara* Dendy, 1916b: 135, pl. I, fig. 9a,b (Okhamandal) (3).

Family **Spirastrellidae** Ridley & Dendy

Genus *Spirastrella* Schmidt. *S. andamanensis* Pattanayak, 1998 (Andamans) (7). *S. aurivilli* Lindgren; Thomas, 1972: 340, pl. 1, fig. 4A-C; Thomas, 1979a: 185, figs 10,4L; Thomas, 1985: 306, pl. V, fig. 25 (Gulf of Mannar) (5). *S. coccinea* (Duch & Mich.); Thomas, 1985: 304, pl. V, fig. 21 (Gulf of Mannar) (5). *S. cuspidifera* (Lamarck); Thomas, 1972: 338; Thomas, 1979a: 183; Thomas, 1985: 305, pl. V, fig. 22 (Gulf of Mannar and Palk Bay); Thomas, 1980b: 9 (Minicoy I.) (2,5). *S. florida* Lendenfeld; Kumar, 1925: 227 (Kilakarai and Gulf of Mannar) (5). *S. inconstans* (Dendy); Burton, 1937: 14; Thomas, 1972: 339; Thomas, 1979a: 183, fig. 1F, 4M; Thomas, 1985: 305, pl. V, fig. 23 (Gulf of Mannar); Thomas, 1980b: 10 (Minicoy I.); Pattanayak, 1998 (Andaman and Nicobar I.) (2,5,7). *S. pachyspira* Levi; Thomas, 1968f: 265; Thomas, 1985: 306, pl. V, fig. 24 (Gulf of Mannar) (5). *S. punctulata* Ridley; Kumar, 1925: 228 (Kilakarai and Gulf of Mannar) (5). *S. vagabunda* var. *tubulodigitata* Dendy, 1916b: 132 (Okhamandal) (3).

Family **Suberitidae** Schmidt

Genus *Aaptos* Gray. *A. aaptos* (Schmidt, 1864); Dendy, 1916b: 101 (Okhamandal, as *Tuberella aaptos*); Burton, 1937: 13; Thomas, 1985, 313, pl. VI, fig. 5 (Gulf of Mannar); Thomas, 1980b: 10 (Minicoy I.) (2,3,5). *A. unispiculus* (Carter, 1880: 45, pl. 4, fig. 8); Thomas, 1985: 313, pl. VI, fig. 6 (Gulf of Mannar) (5).

Genus *Laxosuberites* Topsent. *L. aquaedulcioris* (Annandale); Annandale, 1915c: 42, pl. iv, figs 5,6 (Chilka Lake, Orissa) (6). *L. conulosus* Burton, 1930: 669; Thomas, 1985: 311 (Gulf of Mannar) (5). *L. cruciatus* (Dendy, 1905); Dendy, 1916: 135 (Okhamandal as *Suberites cruciatus*); Kumar, 1925: 229 (Waltair, as *Suberites cruciatus* var. *depressa*); Burton, 1937: 14;

Thomas, 1985: 310, pl. VI, fig. 1 (Gulf of Mannar); Thomas, 1980b: 10 (Minicoy I.) (2,3,5,6). *L. lacustris* Annandale, 1915: 45, pl. V, figs 2,3 (Chilka Lake, Orissa); Burton, 1937: 14; Thomas, 1985: 311, pl. VI, fig. 2 (Gulf of Mannar) (5,6). *L. proteus* (Hentschel); Burton, 1930: 669 (Gulf of Mannar) (5).

Genus *Pseudosuberites* Topsent. *P. andrewsi* Kirkpatrick; Burton, 1937: 14; Rao, 1941: 425; Thomas, 1985: 312, pl. VI, fig. 3 (Gulf of Mannar) (5).

Genus *Suberites* Nardo. *S. carnosus* (Johnston); Dendy, 1916b: 134 (Okhamandal); Rao, 1941: 426; Thomas, 1985: 310, pl. V, fig. 35 (Gulf of Mannar); Thomas, 1980b: 10 (Minicoy I.) (2,3,5). *S. flabellatus* Carter; Dendy, 1916b: 135 (Okhamandal) (3). *S. sericeus* Thiele; Annandale, 1915c: 36, pl. IV, fig. 4 (Chilka lake, Orissa) (6). *S. tylobtusa* Lévi; Thomas, 1985: 310, pl. V, fig. 36 (Gulf of Mannar) (5).

Genus *Terpios* Duchassaing & Michelotti. *T. fugax* Duch. & Mich.; Burton, 1937: 15; Thomas, 1985: 312, pl. VI, fig. 4 (Gulf of Mannar) (5).

Family **Tethyidae** Gray

Genus *Tethya* Lamarck. *T. andamanensis* (Dendy & Burton, 1926: 248) (as *Donatia andamanensis*); Pattanayak, 1998 (Andamans) (7). *T. diploderma* Schmidt; Burton, 1937: 12; Thomas, 1985: 331, pl. VII, fig. 2 (Gulf of Mannar); Pattanayak, 1998 (Andaman and Nicobar Is) (5,7,8). *T. japonica* Sollas; Burton, 1937: 13; Thomas, 1985: 331, pl. VII, fig. 3 (Gulf of Mannar); Thomas, 1980b: 14 (Minicoy I.) (2,5). *T. repens* (Schmidt); Dendy & Burton, 1926: 247 (as *Donatia repens*); Pattanayak, 1998 (Andamans); Burton, 1937: 12; Thomas, 1985: 332, pl. VII, fig. 4 (Gulf of Mannar, as *Tethytimea repens*); Thomas, 1980b: 14 (Minicoy I.) (2,5,7). *T. robusta* Bowerbank; Burton, 1937: 13; Thomas, 1985: 330, pl. VII, fig. 1 (Gulf of Mannar); Thomas, 1980b: 12 (Minicoy I.), Pattanayak, 1998 (Andamans) (2,5,7). *T. seychellensis* (Wright); Dendy, 1916b: 100 (Okhamandal, as *Donatia seychellensis*) (3).

Genus *Xenospongia* Gray. *X. patelliformis* Gray; Thomas, 1985: 309, pl. V, fig. 34 (Gulf of Mannar) (5).

Family **Timeidae** Topsent

Genus *Kotimea* de Laubenfels. *K. moorei* (Carter, 1880: 50, pl. 4, fig. 11); Thomas, 1985: 309, pl. V, fig. 33 (Gulf of Mannar) (5).

Genus *Timea* Gray. *T. capitatostellifera* (Carter, 1880: 51, pl. 4, fig. 12); Thomas, 1985: 307, pl. V, fig. 30 (Gulf of Mannar) (5). *T. curvistellifera* (Dendy, 1905); Thomas, 1985: 308, pl. V, fig. 31 (Gulf of Mannar) (5). *T. spinatostellifera* (Carter, 1880: 51, pl. 4, fig. 13); Thomas, 1985: 307, pl. V, fig. 29 (Gulf of Mannar) (5). *T. stellata* (Bowerbank); Burton, 1937: 15; Thomas, 1985: 307, pl. V, fig. 26 (Gulf of Mannar) (5). *T. stelligera* (Carter); Thomas, 1985: 307, pl. V, fig. 27 (Gulf of Mannar and Palk Bay) (5). *T. stellivarians* (Carter, 1880: 50, pl. 4, fig. 10a-c); Thomas, 1985, pl. v, fig. 32 (Gulf of Mannar) (5).

Order **Lithistida**
Suborder **Triaenosina**
Family **Corallistidae** Sollas

Genus *Corallistes* Schmidt. *C. aculeata* Carter, 1880: 143, pl. 7, fig. 45; Thomas, 1985: 343, pl. VIII, fig. 1 (Gulf of Mannar) (5). *C. elegantissima* Carter, 1880: 144, pl. 7, fig. 47; Thomas, 1985: 343, pl. VIII, fig. 2 (Gulf of Mannar) (5). *C. verucosa* Carter, 1880: 144, pl. 4, fig. 46; Thomas, 1985: 343, pl. VIII, fig. 3 (Gulf of Mannar) (5).

Family **Theonellidae** Lendenfeld

Genus *Discodermia* du Bocage. *D. enigmatica* Dendy, 1905; Thomas, 1985: 345, pl. VIII, fig. 9 (Gulf of Mannar) (5). *D. gorgonoides* Burton, 1928: 109; Pattanayak, 1998 (Andamans) (7). *D. interspersa* Kumar, 1925: 215 (Ganjam coast) (6). *D. laevidiscus* Carter, 1880: 149, pl. 8, fig. 51; Thomas, 1985: 344, pl. VIII, fig. 6 (Gulf of Mannar) (5). *D. papillata* Carter, 1880: 146, pl. 8, fig. 48; Thomas, 1985: 344, pl. VIII, fig. 4 (Gulf of Mannar); Burton & Rao, 1932: 305; Pattanayak, 1998 (Andamans) (5,7). *D. spinispirulifera* Carter, 1880: 148, pl. 8, fig. 50; Thomas, 1985: 344, pl. VIII, fig. 5 (Gulf of Mannar) (5). *D. sceptrellifera* Carter, 1881: 372, pl. 18, fig. 2; Thomas, 1985: 345, pl. VIII, fig. 8 (Gulf of Mannar); Burton & Rao, 1932: 307 (Ganjam coast) (5,6). *D. sinuosa* Carter, 1887: 372, pl. 18, fig. 1; Thomas, 1985: 345, pl. VIII, fig. 7 (Gulf of Mannar) (5).

Genus *Theonella* Gray. *T. coupla* Burton, 1928: 110 (Laccadive I.) (2). *T. swinhoei* Gray; Burton & Rao, 1932: 308; Thomas, 1985: 346, pl. VIII, fig. 10 (Gulf of Mannar); Burton & Rao, 1932: 308; Pattanayak (Andamans) (5,7,8).

Suborder **Anoplina**
Family **Azoricidae** Sollas

Genus *Leiodermatium* Schmidt. *L. pfefferae*; Burton, 1928 (Andamans, as *Azorica pfefferae*) (7).

Family **Desmanthidae** Topsent

Genus *Lophocanthus* Hentschel. *L. rhabdophorus* Hentschel; Burton, 1937: 11; Thomas, 1985: 348, pl. VIII, fig. 11 (Gulf of Mannar); Thomas, 1980b: 16, fig. 2f (Minicoy I.) (2,5).

Subclass **Ceractinomorpha**
Order **Agelasida**
Family **Agelasidae** Verril

Genus Agelas Duchassing & Michelotti. *A. ceylonica* Dendy, 1905; Thomas, 1980: 3 (Minicoy I.); Thomas, 1985: 256, pl. II, fig. 30 (Gulf of Mannar) (2,5). *A. mauritiana* (Carter); Thomas, 1980a: 2 (Minicoy I.); Thomas, 1985: 256, pl. II, fig. 29 (Gulf of Mannar) (2,5).

Incertae Sedis Genus *Acanthostylotella* Burton & Rao. *A. cornuta* (Topsent); Thomas, 1985: 257, pl. II, fig. 31 (Gulf of Mannar) (5).

Order **Poecilosclerida**
Suborder **Microcionina**
Family **Iophonidae** Burton

Genus *Acarnus* Gray. *A. souriei* (Levi); Thomas, 1970b: 46, figs 1,2a-h; Thomas, 1985: 263, pl. III, fig. 4 (Gulf of Mannar, as *Acanthacarnus souriei*) (5). *A. ternatus* Ridley; Thomas, 1985: 262, pl. III, fig. (Gulf of Mannar) (5). *A. thielei* Lévi; Thomas, 1970b: 44, figs 3a-g, 4; Thomas, 1985: 263, pl. III, fig. 3 (Gulf of Mannar) (5). *A. tortilis* Topsent; Dendy, 1916: 130 (Okhamandal) (3).

Genus *Cornulum* Carter. *C. vesiculatum* (Dendy, 1905); Thomas, 1968e: 260, fig. 1a-b; Thomas, 1985: 238, pl. I, fig. 33 (Gulf of Mannar) (5).

Genus *Damiria* Keller. *D. simplex* Keller; Thomas, 1985: 243, pl. II, fig. 8 (Gulf of Mannar) (5). *D. fistulatus* (Carter, 1880: 53, pl. 15, fig. 22); Thomas, 1985: 236, pl. I, fig. 31 (Gulf of Mannar, as *Xytopsene fistulatus*) (5).

Genus *Zyzzya* de Laubenfels. *Z. papillata* (Thomas, 1968e: 252, fig. 6-8); Thomas, 1985: 243, pl. II, fig. 12 (Gulf of Mannar, as *Damirina papillata*) (50).

Family **Microcionidae** Carter

Genus *Antho* Gray. Subgenus *Antho (Antho)* Gray. *A. (A.) mannarensis* (Carter, 1880: 37) (as *Dictyocylindrus mannarensis*); Thomas, 1985: 257, pl. II, fig. 32 (as *Plocamilla mannarensis*) (Gulf of Mannar); Burton & Rao, 1932: 255 (Laccadive sea, as *Plocamia mannarensis*); Burton & Rao, 1932: 355 (off Mangalore, off Karwar) (2,4,5). *A. (A.) tuberosa* (Hentschel); Burton & Rao, 1932: 341 (Ganjam coast, E coast of India) (6).

Genus *Artemisina* Vosmaer. *A. indica* (Thomas, 1974: 312; Thomas, 1985: 287, pl. IV, fig. 19 (Gulf of Mannar, as *Qasimella indica*) (5).

Genus *Clathria* Schmidt. Subgenus *Clathria (Clathria)* Schmidt. *C. (C.) decumbens* (Ridley); Burton, 1937: 29; Thomas, 1985: 278, pl. IV, fig. 3 (Gulf of Mannar) (5). *C. (C.) indica* Dendy, 1905; Burton & Rao, 1932: 336; Thomas, 1985: 278, pl. IV, fig. 4 (Gulf of Mannar). *C. (C.) maeandrina* Dendy, 1905; Burton, 1930: 668 (Gulf of Mannar) (5). *C. (C.) prolifera* (Verrill); Burton & Rao, 1932: 344 (Mangalore, off Karwar, Arabian sea) (1,4).

Subgenus *Clathria (Dendrocia)* Hallman. *C. (D.) antyaja* Burton & Rao, 1932: 348 (Gulf of Mannar, as *Dendrocia antyaja*) (5).

Subgenus *Clathria (Microciona)* Bowerbank. *C. (M.) aceratoobtusa* Carter; Thomas, 1985: 273, pl. III, fig. 20 (Gulf of Mannar) (5). *C. (M.) affinis* Carter, 1880: 41, pl. 4, fig. 15; Thomas, 1985: 273, pl. III, fig. 19 (Gulf of Mannar) (5). *C. (M.) atrasanguinea* Bowerbank; Burton, 1937: 30 (Krusadai I.); Burton & Rao, 1932: 344 (off Karwar, Arabian sea; Travancore, Kilakarai, Puri coast, Andamans); Thomas, 1985: 273, pl. III, fig. 18 (Gulf of Mannar); Pattanayak, 1998 (Andamans) (1,5,7). *C. (M.) fascispiculifera* (Carter, 1880: 44, pl. 4, fig. 7a-g); Thomas, 1985: 275, pl. III, fig. 24 (Gulf of Mannar) (5). *C. (M.) rhopalophora* (Hentschel); Thomas, 1970a: 206, fig. 7; Thomas, 1985: 274, pl. III, fig. 23 (Gulf of Mannar) (5).

Subgenus *Clathria (Thalysias)* Duch & Mich. *C. (T.) amiranteiensis* Burton, 1937: 29, pl. 3, fig. 20; Thomas, 1985: 276, pl. III, fig. 26 (Gulf of Mannar, as *Colloclathria ramosa)* (5). *C. (T.) encrusta* Kumar, 1925: 221 (Orissa coast) (6). *C. (T.) lendenfeldi* Ridley & Dendy, 1886; Burton & Rao, 1932: 334 (Tuticorin, Ganjam Coast) (5,6). *C. (T.) longitoxa* (Hentschel); Burton, 1937: 30; Thomas, 1985: 274, pl. III, fig. 22 (Gulf of Mannar) (5). *C. (T.) micropunctata* Burton & Rao, 1932: 340 (off Tuticorin) (5). *C. (T.) procera* (Ridely); Burton & Rao, 1932: 340 (Tuticorin, Cape Comorin, as *Tenacia procera*); Burton, 1937: 28; Thomas, 1985: 277, pl. IV, fig. 2 (Gulf

of Mannar) (5). *C. (T.) procera* var. *tessellata* (Dendy, 1905); Burton, 1937: 29 (Gulf of Mannar) (5). *C. (T.) reinwardti* (Vosmaer); Dendy, 1916b: 128 (Okhamandal) (3). *C. (T.) spiculosus* Dendy, 1889; Dendy, 1916b: 128 (Okhamandal) (3). *C. (T.) toxifera* (Heantschel); Burton, 1937: 31; Thomas, 1985: 274, pl. III, fig. 21 (Gulf of Mannar). *C. (T.) vulpina* (Lamark); Dendy, 1916b: 128 (Okhamandal, as *Clathria coralliticta*); Burton & Rao, 1932: 337 (Tuticorin, Kilakarai, Andamans, as *Tenacia frondifera*); Burton, 1937: 27; Thomas, 1985: 277, pl. IV, fig. 1 (Gulf of Mannar); Pattanayak, 1998 (Andamans) (3,5,7).

Genus *Echinochalina* Thiele. Subgenus *Echinochalina (Echinochalina)* Thiele. *E. (E.) barba* (Lamarck); Thomas, 1977: 115; Hooper, 1996: 521 (Andaman sea) (7).

Genus *Echinoclathria* Carter. *E. rimosa* (Ridley); Thomas, 1985: 276, pl. III, fig. 27 (Gulf of Mannar) (5).

Genus *Holopsamma* Carter, 1885. *H. crassa* Carter; Thomas, 1985: 268 (Gulf of Mannar and Palk Bay) (5)

Family **Raspailiidae** Hentschel

Genus *Aulospongus* Norman. *A. sessilis* (Carter, 1880: 38) (as *Dictyocylindrus sessilis*); Thomas, 1985: 270, pl. III, fig. 11 (Gulf of Mannar) (5). *A. tubulatus* (Bowerbank); Burton & Rao, 1932: 347 (Tuticorin); Burton, 1937: 32; Thomas, 1985: 269, pl. III, fig. 10 (Gulf of Mannar) (5).

Genus *Cyamon* Gray. *C. quadriradiata* Carter, 1880: 42, pl. 4, fig. 4; Thomas, 1985, pl. II, fig. 33 (Gulf of Mannar) (5). *C. quinqueradiata* (Carter, 1880: 43, pl. 4, fig. 5); Thomas, 1985: 258, pl. II, fig. 34 (Gulf of Mannar) (5). *C. vickersii* (Bowerbank) Burton & Rao, 1932: 355 (Mangalore) (4).

Genus *Echinodictyum* Ridley. *E. asperum* Ridley & Dendy; Burton & Rao, 1932: 348; Pattanayak, 1998 (Andamans) (7). *E. clathrum* Dendy, 1905; Burton, 1937: 31; Rao, 1941: 451; Thomas, 1985, pl. II, fig. 18 (Gulf of Mannar) (5). *E. gorgonoides* Dendy, 1916b; 129 (Okhamandal); Burton & Rao, 1932: 348 (Tuticorin); Thomas, 1985: 251, pl. II, fig. 19 (Gulf of Mannar) (3,5). *E. longistylum* Thomas, 1968b: 246, pl. 2, fig. A,B; Thomas, 1985: 251, pl. II, fig. 20 (Gulf of Mannar); Thomas, 1980a: 1 (Minicoy I.) (2,5). *E. nervosum* Ridley, 1881; Burton & Rao, 1932: 348 (Ganjam coast) (6).

Genus *Endectyon* Topsent. *E. fruticosa* (Dendy, 1905); Thomas, 1985: 271, pl. III, fig. 15 (Gulf of Mannar) (5). *E. lamellosa* Thomas, 1976a: 170, pl. I, figs1A,B,C, figs 1a-d; Thomas, 1985: 272, pl. III, fig. 16 (Gulf of Mannar) (5). *E. thurstoni* (Dendy); Burton & Rao, 1932: 347 (Tuticorin, as *Hemectyon thurstoni*); Burton, 1937: 34; Thomas, 1985: 272, pl. III, fig. 17 (Gulf of Mannar) (5).

Genus *Eurypon* Gray. *E. clavatum* (Bowerbank); Thomas, 1985: 275, pl. III, fig. 25 (Gulf of Mannar) (5).

Genus *Raspailia* Nardo. Subgenus *Raspailia (Raspailia)* Nardo. *R. (R.) anastomosa* Kumar, 1925: 223 (Ganjam coast) (6). *R. (R.) fruticosa* Dendy; Kumar, 1925: 224 (Waltair) (6). *R. (R.) fruticosa* var. *tenuiramosa* Dendy, 1905; Dendy, 1916b: 130 (Okhamandal) (3). *R. (R.) hornelli* Dendy, 1905; Burton, 1937: 33; Thomas, 1985: 269, pl. III, fig. 9 (Gulf of Mannar) (5). *R. (R.) viminalis* (Schmidt); Burton & Rao, 1932: 342 (Puri coast, Andamans); Pattanayak, 1998 (Andamans) (6,7).

Genus *Thrinacophora* Ridley. *T. cervicornis* Ridley & Dendy; Dendy, 1916: 117 (off Dwarka) (3).

Family **Rhabdermiidae** Topsent

Genus *Rhabderemia* Topsent. *R. acanthostyla* Thomas, 1968b: 247, pl. 2, figs 4,5; Thomas, 1985: 271, pl. III, fig. 14 (Gulf of Mannar) (5). *R. indica* Dendy, 1905; Burton, 1937: 33; Thomas, 1985: 270, pl. III, fig. 12 (Gulf of Mannar) (5). *R. prolifera* Annadale, 1915b: 464, pl. 34, fig. 3 (Gulf of Mannar, Andamans, E coast of India); Thomas, 1985: 271, pl. III, fig. 13 (Gulf of Mannar) Pattanayak, 1998 (Andamans) (5,7,9).

Suborder **Myxillina**
Family **Anchinoidae** Topsent

Genus *Ectyobatzella* Burton & Rao. *E. enigmatica* Burton & Rao, 1932: 332, pl. XVIII, fig. 6; Pattanayak (1998) (Nicobar I.) (8).

Genus *Kirkpatrickia* Topsent. *K. spiculaphila* Burton & Rao, 1932: 332, pl. XVII, figs 5,5a; Pattanayak, 1998 (Andamans) (7).

Genus *Phorbas* Duchaissaing & Michelotti. *P. dubia* Burton; Thomas, 1985: 252, pl. II, fig. 21 (Gulf of Mannar, as *Anchinoe dubia*) (5).

Family **Coelosphaeridae** Hentschel

Genus *Coelosphaera* Thomson. *C. encrusta* (Kumar, 1925: 220, fig. 3) (Kilakarai, as *Histoderma encrusta*); Thomas, 1985: 254, pl. II, fig. 24 (Gulf of Mannar) (5). *C. navicelligera*

(Ridley & Dendy); Thomas, 1985: 253 (Gulf of Mannar, as *Siderodermella navicelligera*) (5).

Genus *Ectyodoryx* Lundbeck. *E. lissostyla* Thomas, 1970a, p. 203, figs 1-3; Thomas, 1985: 260, pl. II, fig. 36 (Gulf of Mannar) (5).

Genus *Lissodendoryx* Topsent. *L. balanophilus* Annandale; Thomas, 1985: 266, pl. II, fig. 5 (Gulf of Mannar) (5). *L. similis* Thiele; Ali, 1956: 293 (Madras) (5). *L. sinensis* Brondsted; Burton, 1937: 26; Thomas, 1985: 266, pl. III, fig. 6 (Gulf of Mannar) (5). *L. ternatensis* (Thiele); Burton & Rao, 1932: 332 (Madras coast) (5).

Genus *Waldoschmittia* de Laubenfels. *W. schmidti* (Ridley); Thomas, 1980a: 2 (Minicoy I.); Thomas, 1985: 252, pl. II, fig. 22 (Gulf of Mannar) (2,5).

Family **Crambeidae** Lévi

Genus *Psammochela* Dendy. *P. elegans* Dendy, 1916: 126, pl. I, fig. 6, pl. III, fig. 22 (Okhamandal); Pattanayak, 1998 (Andamans) (3,7). *P. fibrosa* (Ridley); Thomas, 1985: 268, pl. III, fig. 8 (Gulf of Mannar) (5).

Family **Hymedesmiidae** Topsent

Genus *Hymedesmia* Bowerbank. *H. dendyi* Burton; Burton & Rao, 1932: 350 (Andamans) (7). *H. mannarensis* Thomas, 1970a: 204; Thomas, 1985: 261, pl. II, fig. 38 (Gulf of Mannar) (5). *H. mertoni* Hentschel; Thomas, 1968f: 264; Thomas, 1985: 260, pl. II, fig. 37 (Gulf of Mannar) (5). *H. stylophora* Thomas, 1970a: 204; Thomas, 1985: 261, pl. II, fig. 39 (Gulf of Mannar) (5). *H. tenuissima* (Dendy); Thomas, 1985: 261, pl. II, fig. 40 (Gulf of Mannar) (5).

Family **Myxillidae** Topsent

Genus *Damiriopsis* Burton. *D. brondstedi* Burton, 1928: 124 (Andamans) (7).

Genus *Desmapsamma* Burton. *D. anchorata* (Carter); Thomas, 1985: 267, pl. III, fig. 7 (Gulf of Mannar) (5).

Genus *Iotrochota* Ridley. *I. baculifera* Ridley; Dendy, 1916b: 123 (Okhamandal); Burton & Rao, 1932: 353 (Nicobars); Thomas, 1985: 235, pl. I, fig. 29 (Gulf of Mannar) (3,5,8).

Genus *Myxilla* Schmidt. Subgenus *Myxilla (Myxilla)* Schmidt. *M. (M.) arenaria* Dendy, 1905; Dendy, 1916b: 127 (Okhamandal); Thomas, 1985: 259, pl. II, fig. 35 (Gulf of Mannar) (3,5).

Family **Phoriospongiidae** Lendenfeld

Genus *Chondropsis* Carter. *C. kirkii* (Carter); Dendy, 1916b: 127 (Okhamandal) (3).

Genus *Strongylacidon* Lendenfeld. *S. stelliderma* (Carter); Rao, 1941: 449; Thomas, 1985: 237(Gulf of Mannar) (5).

Family **Tedaniidae** Ridley & Dendy

Genus *Tedania* Gray. *T. (T.) anhelans* (Liberkühn); Burton & Rao, 1932: 353 (Andamans, Gulf of Mannar); Burton, 1937: 27; Ali, 1956: 293 (Madras); Thomas, 1980a: 4 (Minicoy I.); Thomas, 1985: 261, pl. III, fig. 1 (Gulf of Mannar); Pattanayak, 1998 (Andamans) (2,5,7).

Suborder **Mycalina**
Family **Guitarridae** Burton

Genus *Guitarra* Carter. *G. indica* Dendy, 1916b: 124, pl. I, fig. a-b, pl. III, fig. 21 (Okhamandal) (3).

Family **Desmacellidae**
Ridley & Dendy

Genus *Biemna* Gray. *B. annexa* (Schmidt); Burton, 1928: 120 (Laccadive sea) (2). *B. fistulosa* Topsent, Burton, 1937: 25; Thomas, 1985: 288, pl. IV, fig. 22 (Gulf of Mannar) (5). *B. fortis* (Topsent); Burton & Rao, 1930: 327 (Puri coast); Thomas, 1980a: 6 (Minicoy I.); Thomas, 1985: 288, pl. IV, fig. 21 (Gulf of Mannar) (2,5,6). *B. lipsosigma* Burton, 1928: 120; Pattanayak, 1998 (Andamans) (7). *B. microstyla* Thomas, 1984: 95, fig. 1b (Bay of Bengal, SE coast of India) (9). *B. tubulata* (Dendy, 1905); Burton & Rao, 1932, p.327 (Andamans, Nicobars, Tuticorin); Thomas, 1985: 287, pl. IV, fig. 20 (Gulf of Mannar, as *Toxemna tubulata*) (5,7).

Genus *Desmacella* Schmidt. *D. tubulata* Dendy, 1905; Dendy, 1916b: 116 (Okhamandal) (3).

Family **Mycalidae** Lundbeck

Genus *Mycale* Gray. Subgenus *Mycale (Carmia)* Gray. *M. (C.) madraspatna* Annandale; Burton, 1937: 24 (Krusadai I.); Ali, 1956: 295 (Madras); Thomas, 1985: 285, pl. IV, fig. 15 (Gulf of Mannar, as *Carmia madraspatna*) (5). *M. (C.) monanchorata* (Burton & Rao, 1932: 329 (Kilakarai)); Rao, 1941: 447; Thomas, 1985: 284, pl. IV, fig. 13 (Gulf of Mannar) (5). *M. (C.) sulevoidea* Sollas; Thomas, 1968d: 256; Thomas, 1985: 284; pl. IV, fig. 14 (Gulf of Mannar and Palk Bay) (5).

Subgenus *Mycale (Mycale)* Gray. *M. (M.) aegagropila* (Johnston); Rao, 1941: 445 (Gulf of Mannar) (5). *M. (M.) aegagropila* var. *militaris* (Annandale); Ali, 1956: 295 (Madras) (5). *M. (M.) coronata* Dendy; Burton, 1928: 121 (Andamans) (7). *M. (M.) crassissima* (Dendy); Thomas, 1985: 282, pl. IV, fig. 10 (Gulf of Mannar) (5). *M. (M.) grandis* (Gray); Burton, 1937: 23; Thomas, 1985: 279, pl. IV, fig. 5 (Gulf of Mannar); Thomas, 1980a: 6 (Minicoy I.) (2,5). *M. (M.) gravelyi* Burton, 1937: 24; Thomas, 1985: 283, pl. IV, fig. 9 (Gulf of Mannar) (5). *M. (M.) indica* (Carter, 1887); Burton & Rao, 1932: 328; Pattanayak, 1998 (Andamans); Rao, 1941: 445 (Pamban, Gulf of Mannar) (5,7). *M. (M.) mannarensis* Thomas, 1968a: 255, fig. 1-2; Thomas, 1985: 283, pl. IV, fig. 12 (Gulf of Mannar) (5). *M. (M.) mytilorum* Annandale; Burton, 1937: 24; Thomas, 1985: 282 (Gulf of Mannar); Ali, 1956: 294 (Madras) (5). *M. (M.) plumosa* (Carter); Dendy, 1916b: 121 (Okhamandal) (3). *M. (M.) spongiosa* (Dendy); Burton, 1928: 119; Thomas, 1985: 279, pl. IV, fig. 6 (Gulf of Mannar) (5). *M. (M.) tenuispiculata* (Dendy, 1905); Burton, 1937: 23; Thomas, 1985: 280, pl. IV, fig. 7 (Gulf of Mannar) (5). *M. (M.) tricomaliensis* Rao, 1941: 447, pl. 12, fig. 19; Thomas, 1985: 283, pl. IV, fig. 11 (Gulf of Mannar) (5).

Genus *Paresperella* Dendy, 1905. *P. bidentata* Dendy, 1905; Burton, 1937: 26; Thomas, 1985: 286, pl. IV, fig. 18 (Gulf of Mannar) (5). *P. serratohamata* (Carter, 1880: 49, pl. 5, fig. 20a-d); Thomas, 1985: 286, pl. IV, fig. 17 (Gulf of Mannar) (5).

Genus *Zygomycale* Topsent. *Z. parishii* (Bowerbank); Burton & Rao, 1932: 328; Thomas, 1985: 285, pl. IV, fig. 16 (Gulf of Mannar and Palk Bay); Thomas, 1980: 6 (Minicoy I.) (2,5).

Order **Halichondrida**
Family **Axinellidae** Carter

Genus *Acanthella* Schmidt. *A. cavernosa* Dendy; Burton, 1937: 36 (Gulf of Mannar); Thomas, 1980b: 9 (Minicoy I.); Thomas, 1984: 97 (Bay of Bengal, SE coast of India) (2,5,9). *A. megaspicula* Thomas, 1984: 99 (Bay of Bengal, SE coast of India) (9). *A. ramosa* Kumar, 1925: 224 (Ganjam coast) (6).

Genus *Auletta* Schmidt. *A. andamanensis* Pattanayak, 1998 (Andamans) (7). *A. elongata* Dendy, 1905; Burton, 1937: 37; Thomas, 1985: 302, pl. V, fig. 19 (Gulf of Mannar) (5). *A. elongata* var. *fruticosa* Dendy, 1916: 119 (Okhamandal) (3). *A. lyrata* (Esper); Dendy, 1889: 92 (as *A. aurantiacea*); Burton, 1937: 34 (as *Axinella lyrata*); Thomas, 1985: 303 (as *Acanthella lyrata*) (Gulf of Mannar) (5). *A. lyrata* var. *glomerata* Dendy, 1905; Dendy, 1916b: 119 (Okhamandal) (3).

Genus *Axinella* Schmidt. *A. acanthelloides* Pattanayak, 1998 (Andamans) (7). *A. agaricioformis* (Dendy, 1905); Thomas, 1985: 291, pl. IV, fig. 27 (Gulf of Mannar) (5). *A. bubarinoides* Dendy; Burton, 1937: 36; Thomas, 1985: 293, pl. V, fig. 3 (Gulf of Mannar) (5). *A. carteri* (Dendy, 1889); Burton, 1937: 35; Thomas, 1985: 290, pl. IV, fig. 24 (Gulf of Mannar) (5). *A. ceylonensis* (Dendy, 1905); Burton, 1937: 35; Thomas, 1985: 293, (Gulf of Mannar) (5). *A. conulosa* Dendy; Burton, 1937: 36 (Kruasadai I., Gulf of Mannar) (5). *A. crassistylifera* (Dendy, 1905); Thomas, 1985: 293, pl. V, fig. 4 (Gulf of Mannar) (5). *A. donnani* (Bowerbank); Dendy, 1916b: 118 (Okhamandal, as *Phakellia donnani*); Burton, 1937: 35, Thomas, 1970a: 207; Thomas, 1985: 289 (Gulf of Mannar) (3,5). *A. durissima* (Dendy, 1905); Thomas, 1985: 292 (Gulf of Mannar) (5). *A. halichondroides* Dendy, 1905; Thomas, 1985; 293, pl. V, fig. 1 (Gulf of Mannar) (5). *A. labyrinthica* Dendy, 1889: 88; Thomas, 1985: 290 (Gulf of Mannar) (5). *A. lamellata* (Dendy, 1905); Thomas, 1985: 291 (Gulf of Mannar) (5). *A. manus* Dendy, 1905; Thomas, 1985: 292, pl. IV, fig. 29 (Gulf of Mannar) (5). *A. symmetrica* Dendy, 1905; Thomas, 1985: 291, pl. IV, fig. 26 (Gulf of Mannar) (5). *A. tenuidigitata* Dendy, 1905; Thomas, 1985: 290, pl. IV, fig. 25 (Gulf of Mannar) (5). *A. virgultosa* Carter, 1887; Dendy, 1916b: 118 (off Dwarka) (3).

Genus *Bubaris* Gray. *B. columnata* Burton, 1928: 130 (Andaman sea) (7). *B. gorgonoides* Thomas, 1984: 96, fig. 1 f,g, pl. 1A (Bay of Bengal, SE coast of India) (9). *B. radiata* Dendy, 1916b: 131, pl. I, fig. 8a,b, pl. IV, figs 24a,b (Okhamandal). *B. vermiculata* (Bowerbank); Carter, 1880: 46 (as *Hymerhaphia vermiculata* var. *erecta*); Thomas, 1985: 296, pl. V, fig. 6 (Gulf of Mannar) (5).

Genus *Monocrepidium* Topsent. *M. eruca* (Carter, 1880: 46, pl. 4, fig. 9a-c); Thomas, 1985: 297, pl. V, fig. 8 (Gulf of Mannar) (5).

Genus *Phakettia* Bowerbank. *P. ridleyi* (Dendy); Thomas, 1985: 294, pl. V, fig. 5 (Gulf of Mannar) (5).

Genus *Rhabdoploca* Topsent. *R. curvispiculifera* (Carter, 1880: 43, pl. 4, fig. 6) (as *Microciona curvispiculifera*); Thomas, 1985: 296, pl. V, fig. 7 (Gulf of Mannar) (5).

Family **Desmoxyidae** Hallmann

Genus *Higginsia* Higgin. *H. higgini* Dendy; Thomas, 1985: 298, pl. V, fig. 10 (Gulf of Mannar) (5). *H. mixta* (Hentschel); Thomas, 1977: 116 (Bay of Bengal); Thomas, 1985: 297, pl. V, fig. 9 (Gulf of Mannar) (5).

Genus *Myrmekioderma* Ehlers. *M. granulata* (Esper); Burton, 1930: 668 (as *Acanthoxifer ceylonensis*); Burton, 1937: 39; Thomas, 1985: 298, pl. V, fig. 11 (Gulf of Mannar); Thomas, 1980b: 8 (Minicoy I.) (2,5).

Family **Dictyonellidae**
Van Soest, Diaz & Pomponi

Genus *Liosina* Thiele. *L. paradoxa* Thiele; Burton, 1937; 39; Thomas, 1985: 236, pl. I, fig. 30 (Gulf of Mannar) (5).

Family **Halichondriidae** Vosmaer

Genus *Amorphinopsis* Carter. *A. arcotti* (Ali, 1956: 296) (Rayapuram coast, Madras, as *Prostylissa arcotti*) (5). *A. excavans* Carter; Thomas, 1972: 341, pl. 2, fig. 3; Thomas, 1979a: 168; Thomas, 1985: 316, pl. VI, fig. 9 (Gulf of Mannar) (5). *A. excavans* var. *digitifera* Annandale, 1915; Kumar, 1925: 225 (Waltair coast) (6). *A. foetida* (Dendy); Burton, 1937: 37; Thomas, 1985: 324, pl. VI, fig. 27 (Gulf of Mannar); Thomas, 1980b: 12 (Minicoy I.); Thomas, 1984: 99, fig. 1a (Bay of Bengal, SE coast of India); Pattanayak, 1998 (Andamans) (2,5,7,9). *A. kempi* Kumar, 1925: 226 (Waltair) (6). *A. oculata* (Kieschnick); Burton, 1937: 38; Thomas, 1985: 325, pl. VI, fig. 28 (Gulf of Mannar, as *Prostylyssa oculata*) (5).

Genus *Axinyssa* Lendenfeld. *A. flabelliformis* (Keller); Burton, 1937: 35; Thomas, 1985; 309, pl. VI, fig. 39 (Gulf of Mannar) (5).

Genus *Ciocalypta* Bowerbank. *C. dichotoma*, Dendy, 1916: 119, pl. III, fig. 18 (Okhamandal) (3). *C. penicillus* Bowerbank; Burton, 1937: 38 (as *Prostylissa heterostyla*); Thomas, 1985: 301, pl. V, fig. 17 (Gulf of Mannar) (5). *Ciocalypta polymastia* (Lendenfeld); Thomas, 1973b: 441; Thomas, 1980b: 8 (Minicoy I.) (5).

Genus *Collocalypta* Dendy. *C. digitata* Dendy, 1905; Thomas, 1985: 303, pl. V, fig. 20 (Gulf of Mannar) (5).

Genus *Epipolasis* de Laubenfels. *E. lapidiformis* (Dendy, 1905); Thomas, 1985: 329, pl. VI, fig. 38 (Gulf of Mannar) (5). *E. topsenti* (Dendy, 1905); Thomas, 1985: 329, pl. VI, fig. 37 (Gulf of Mannar) (5).

Genus *Halichondria* Fleming. *H. glabrata* Keller, 1891; Burton, 1937: 37; Thomas, 1985: 300, pl. V, fig. 14 (Gulf of Mannar and Palk Bay) (5). *H. panicea* Johnston; Dendy, 1916b: 112 (Okhamandal); Thomas, 1985: 300, pl. V, fig. 13 (Gulf of Mannar) (3,5). *H. reticulata* Baer; Dendy, 1916b: 113, pl. II, fig. 14a,b (Okhmandal) (3).

Genus *Hymeniacidon* Bowerbank. *H. petrosioides* Dendy, 1905; Thomas, 1985: 302, pl. V, fig. 18 (Gulf of Mannar) (5).

Genus *Petromica* Topsent. *P. massalis* Dendy, 1905; Thomas, 1985: 304 (Gulf of Mannar); Burton, 1928: 110; Pattanayak, 1998 (Andamans) (5,7).

Genus *Spongosorites* Topsent. *S. aplysinoides* (Dendy); Burton, 1937: 38; Thomas, 1985: 301, pl. V, fig. 16 (Gulf of Mannar as *Trachyopsis aplysinoides*) (5). *S. andamanensis* Pattanayak, 1998 (Andamans) (7). *S. cavernosa* (Topsent, 1896); Burton, 1937: 38 (Gulf of Mannar) (5). *S. halichondrioides* Dendy, 1905; Burton, 1928: 118; Pattanayak, 1998 (Andamans); Thomas, 1985: 300, pl. V, fig. 15 (Gulf of Mannar, as *Trachyopsis halichondroides*) (5,7). *S. solida* Topsent; Burton, 1937: 38 (Gulf of Mannar) (5).

Genus *Topsentia* Berg. *T. nigrocutis* (Carter); Thomas, 1985: 325, pl. VI, fig. 29 (Gulf of Mannar) (5).

Order **Haplosclerida**
Family **Callyspongiidae**
de Laubenfels

Genus *Callyspongia* Duchassaing & Michelotti. *C. barodensis* Burton; Thomas, 1985: 250 (Gulf of Mannar) (5). *C. ceylonica* (Dendy, 1905); Thomas, 1985: 249, pl. II, fig. 17 (Gulf of Mannar) (5). *C. clathrata* (Dendy, 1905); Thomas, 1985: 249 (Gulf of Mannar) (5). *C. diffusa* (Ridley); Burton, 1930: 20; Rao, 1941: 432; Thomas, 1985: 247, pl. II, fig. 13 (Gulf of Mannar); Ali, 1956: 292 (Madras); Thomas, 1979b: 15 (Minicoy I.) (2,5). *C. fibrosa* (Ridley & Dendy); Burton, 1930: 669; Burton, 1937: 21; Thomas, 1985: 248, pl. II, fig. 14 (Gulf of Mannar) (5). *C. fistularis* (Topsent); Burton, 1937: 21; Thomas, 1985; 248 (Gulf of Mannar) (5). *C. pambanensis* Rao, 1941: 441; Thomas, 1985: 249, pl. II, fig. 16 (Gulf of Mannar) (5). *C. spinosissima* (Dendy, 1887); Burton, 1937: 21; Thomas, 1985: 248, pl. II, fig. 15 (Gulf of Mannar) (5).

Genus *Siphonochalina* Schmidt. *S. crassifibra* Dendy, 1889; Dendy, 1916b: 114 (Okhamandal) (3). *S. minor* Dendy, 1916b: 115, pl. II, fig. 15 (Okhamandal) (3).

Family **Chalinidae** Gray

Genus *Adocia* Gray. *A. carnosa* (Dendy, 1889); Burton, 1937: 19 (Gulf of Mannar) (5). *A. semifibrosa* (Dendy, 1916b: 110) Okhamandal, as *Reniera semifibrosa*); Burton, 1937: 19 (Gulf of Mannar) (3,5).

Genus *Gellius* Gray. *G. flagellifer* Ridley & Dendy; Burton, 1928: 114; Pattanayak, 1998 (Andamans) (7). *G. fibulatus* (Schmidt); Kumar, 1925: 218 (Waltair) (6). *G. fibulatus* var. *microsigma* Dendy, 1916b: 107 (Okhamandal) (3). *G. megastoma* Burton, 1928: 115; Pattanayak, 1998 (Andamans) (7). *G. ridleyi* Hentschel; Dendy, 1916b: 107 (Okhamandal) (3). *G. toxius* Kumar, 1925: 219 (Waltair) (6).

Genus *Haliclona* Grant. *H. camerata* (Ridley); Burton, 1937: 17; Thomas, 1985: 233, pl. 1, fig. 23 (Gulf of Mannar) (5). *H. implexa* (Schmidt); Thomas, 1985: 233, pl. I, fig. 22 (Gulf of Mannar) (5). *H. madrepora* (Dendy, 1889); Burton, 1937: 17; Thomas, 1985: 233, pl. I, fig. 24 (Gulf of Mannar) (5). *H. obtusispiculifera* (Dendy, 1905); Burton, 1937: 17; Thomas, 1985: 234, pl. I, fig. 27 (Gulf of Mannar) (5). *H. occulata* (Linnaeus); Rao, 1941: 429; Thomas, 1985: 232, pl. I, fig. 20 (Gulf of Mannar) (5). *H. pigmentifera* (Dendy, 1905); Burton, 1937: 19 (as *Adocia pigmentifera*); Thomas, 1985: 234, pl. I, fig. 26 (Gulf of Mannar) (5). *H. tenuiramosa* (Burton, 1930: 666) (as *Chalina tenuiramosa*); Burton, 1937: 17; Thomas, 1985: 235, pl. I, fig. 28 (Gulf of Mannar) (5). *H. viridis* (Duch. & Mich.); Burton, 1937: 18; Thomas, 1985: 232, pl. I, fig. 21 (Gulf of Mannar) (5).

Genus *Reniera* Nardo. *R. delicatula* Ali, 1956: 290, figs 1,4 (Madras) (5). *R. fibroreticulata* Dendy, 1916b: 110 (Okhamandal) (3). *R. hornelli* Dendy, 1916b: 110 (Okhamandal) (3). *R. permollis* (Bowerbank); Dendy, 1916b: 109 (Okhamandal) (3). *R. topsenti* Thiele; Dendy, 1916b: 109 (Okhamandal) (3). *R. tuberosa* (Dendy); Kumar, 1925: 220 (Ganjam coast) (6).

Genus *Sigmadocia* de Laubenfels. *S. carnosa* (Dendy, 1905); Thomas, 1985: 240, pl. II, fig. 4 (Gulf of Mannar) (5). *S. fibulata* (Schmidt); Carter, 1880: 48; Thomas, 1985: 239, pl. II, fig. 1 (Gulf of Mannar); Thomas, 1979b: 15 (Minicoy I.) (2,5). *S. petrosioides* (Dendy, 1905); Thomas, 1985: 240, pl. II, fig. 3 (Gulf of Mannar) (5). *S. pumila* (Lendenfeld); Burton, 1937: 20, Rao, 1941: 431; Thomas, 1985: 240, pl. II, fig. 2 (Gulf of Mannar); Thomas, 1979: 15 (Minicoy I.) (2,5).

Genus *Toxadocia* de Laubenfels. *T. dendyi* (Burton); Thomas, 1985: 241 (Gulf of Mannar) (5). *T. ridleyi* (Dendy); Thomas, 1985: 241 (Gulf of Mannar) (5). *T. toxius* (Topsent, 1897); Thomas, 1985: 241, pl. II, fig. 5 (Gulf of Mannar) (5).

Family **Niphatidae** Van Soest

Genus *Aka* de Laubenfels. *A. diagnoxea* Thomas, 1968c: 250; Thomas, 1979a: 168; Thomas, 1985: 317, pl. VI, fig. 11 (Gulf of Mannar) (5). *A. minuta* Thomas, 1972: 343, pl. 2, figs 4,4a; Thomas, 1979a: 170; Thomas, 1985: 317, pl. VI, fig. 10 (Gulf of Mannar) (5).

Genus *Amphimedon* Duch. & Mich. *A. multiformis* (Dendy); Ali, 1956: 290 (Madras, as *Pachychalina multiformis* var. *mannarensis*) (5).

Genus *Gelliodes* Ridley. *G. cellaria* (Rao, 1941: 439) (as *Callyspongia cellaria* var. *fusca*); Thomas, 1985: 238, pl. I, fig. 32 (Gulf of Mannar) (5). *G. fibrosa* Dendy, 1905; Dendy, 1916b: 108 (Okhamandal); Thomas, 1985: 237 (Gulf of Mannar) (3,5). *G. fibulatus* (Carter); Burton, 1928: 115, Pattanayak, 1998 (Andamans) (7). *G. incrustans* Dendy, 1905; Thomas, 1985: 238 (Gulf of Mannar) (5).

Family **Phloeodictyidae** Carter

Genus *Calyx* Vosmaer. *C. clavata* Burton, 1928: 117; Pattanayak, 1998 (Andamans) (7).

Genus *Oceanapia* Norman. *O. arenosa* Rao, 1941: 443; Thomas, 1985: 255 (Gulf of Mannar); Ali, 1956: 292 (Madras) (5). *O. fistulosa* (Bowerbank); Carter, 1880: 37 (as *Desmacidon jeffreysii*); Rao, 1941: 443; Thomas, 1985: 254, pl. II, fig. 25 (Gulf of Mannar) (5). *O. media* (Thiele); Burton, 1937: 22; Thomas, 1985: 254, pl. II, fig. 26 (Gulf of Mannar) (5). *O. putridosa* Burton, 1928: 118 (Orissa coast, as *Phloeodictyon putridosa*) (6). *O. sagittaria* (Sollas); Thomas, 1985: 242, pl. II, fig. 6 (Gulf of Mannar) (5). *O. zoologica* (Dendy); Thomas, 1985: 254, pl. II, fig. 27 (Gulf of Mannar) (5).

Family **Petrosiidae** Van Soest

Genus *Petrosia* Vosmaer. *P. nigricans* Lindgren; Thomas, 1985: 246, pl. II, fig. 11 (Gulf of Mannar) (5). *P. similis* Ridley & Dendy; Burton, 1930: 666 (as *Chalina similis*); Thomas, 1985: 246, pl. II, fig. 10 (Gulf of Mannar) (5).

Genus *Strongylophora* Dendy. *S. durissima* Dendy, 1905; Thomas, 1985: 242, pl. II, fig. 7 (Gulf of Mannar) (5).

Genus *Xestospongia* de Laubenfels. *X. exigua* (Kirkpatrick); Burton, 1937: 17; Thomas, 1985: 234, pl. I, fig. 25 (Gulf of Mannar) (5). *X. testudinaria* (Lamarck); Burton, 1937: 22; Thomas,

1985: 246, pl. II, fig. 9 (Gulf of Mannar, as *Petrosia testudinaria*); Pattanayak, 1998 (Andamans) (5,7).

Order **Dictyoceratida**
Family **Ircinidae** Gray

Genus *Ircinia* Nardo. *I. aruensis* (Hentschel); Burton, 1937: 40 (Gulf of Mannar, as *Hircinia aruensis*) (5). *I. cactiformis* Rao, 1941: 459 (Gulf of Mannar) (5). *I. fusca* (Carter, 1880: 36); Burton, 1937: 40; Rao, 1941: 458 (Gulf of Mannar, as *Hircinia fusca*); Thomas, 1985: 224, pl. I, fig. 6 (Gulf of Mannar) (5). *I. ramodigitata* Burton; Rao, 1941: 458 (Gulf of Mannar) (5). *I. ramosa* (Keller); Burton, 1937: 40 (as *Hircinia ramosa*); Thomas, 1985: 224, pl. I, fig. 7 (Gulf of Mannar) (5). *I. tuberculata* (Poléjaeff); Thomas, 1985: 225, pl. I, fig. 8 (Gulf of Mannar) (5).

Family **Thorectidae** Bergquist

Genus *Cacospongia* Schmidt. *C. mollior* Schmidt; Thomas, 1985: 226, pl. I, fig. 11 (Gulf of Mannar) (5). *C. scalaris* Schmidt, 1862; Thomas, 1985: 227, pl. I, fig. 12 (Gulf of Mannar) (5).

Genus *Fasciospongia* Burton. *F. anomala* (Dendy, 1905); Thomas, 1985: 228, pl. I, fig. 14 (Gulf of Mannar) (5). *F. cavernosa* (Schmidt); Burton, 1937: 42 (as *Aplysinopsis reticulata)* Rao, 1941; 465 (as *Aplysinopsis reticulata*); Thomas, 1985: 227, pl. I, fig. 13 (Gulf of Mannar) (5).

Genus *Hyrtios* Duch. & Mich. *H. erecta* Keller; Burton, 1937: 43 (as *Duriella nigra*); Thomas, 1985: 220, pl. I, fig. 2 (Gulf of Mannar) (5).

Family **Spongiidae** Gray

Genus *Hyattella* Lendenfeld. *H. cribriformis* (Hyatt); Dendy, 1916b: 141 (Okhamandal, as *Hippospongia clathrata*); Burton, 1937: 41 (as *Luffariospongia clathrata*); Rao, 1941: 464 (as *Luffariospongia clathrata*); Thomas, 1985: 221, pl. I, fig. 4 (Gulf of Mannar); Thomas, 1979b: 13 (Minicoy I.) (2,3,5). *H. intestinalis* (Lamarck); Thomas, 1985: 221, pl. I, fig. 3 (Gulf of Mannar) (5).

Genus *Phyllospongia* Ehlers. *P. dendyi* Lendenfeld; Thomas, 1973b: 440; Thomas, 1979: 14 (Minicoy I.). *P. foliascens* (Pallas); Thomas, 1979b: 14 (Minicoy I.); Pattanayak, 1998 (Andamans) (2,7). *P. papyracea* (Esper); Rao, 1941: 454 (Gulf of Mannar) (5). *P. papyracea* var. *polyphylla* de Laubenfels; Thomas, 1985: 222, pl. I, fig. 5 (Gulf of Mannar) (5).

Genus *Spongia* Linneaus. *S. hispida* Lamark; Thomas, 1985: 220 (Gulf of Mannar) (5). *S. officinalis* var. *ceylonensis* Dendy, 1905; Burton, 1937: 39; Thomas, 1985: 219, pl. I, fig. 1 (Gulf of Mannar); Thomas, 1979: 12 (Minicoy I.) (2,5). *S. officinalis* var. *fenestrata* Rao, 1941: 455 (Gulf of Mannar) (5).

Order **Dendroceratida**
Family **Dysideidae** Gray

Genus *Dysidea* Johnston. *D. cineria* (Keller); Dendy, 1916b: 140 (Okhamandal as *Spongelia cinerea*) (3). *D. elegans* Nardo; Dendy, 1916b: 140 (Okhamandal as *Spongellia elegans*) (3). *D. fragilis* (Montagu); Burton, 1937: 41; Rao, 1941: 463; Thomas, 1985: 228, pl. I, fig. 15 (Gulf of Mannar); Thomas, 1979b: 14 (Minicoy I.) (2,5). *D. fragilis* var. *ramosa* (Schulze, 1879); Dendy, 1916: 139 (Okhamandal) (3). *D. herbacea* (Keller); Thomas, 1985: 329, pl. I, fig. 16 (Gulf of Mannar); Thomas, 1979b: 14 (Minicoy I.) (2,5).

Genus *Spongionella* Bowerbank. *S. nigra* Dendy, 1889; Burton, 1937: 42 (Gulf of Mannar) (5). *S. tuberosa* Burton, 1937; 42, pl. 9, fig. 58; Thomas, 1985: 301, pl. V, fig. 16 (Gulf of Mannar) (5).

Family **Darwinellidae**
Merekowsky

Genus *Darwinella* Müller. *D. australiensis* Carter; Dendy, 1916b: 139 (Okhamandal) (3). *D. mulleri* Schulze; Thomas, 1985: 230, pl. I, fig. 19 (Gulf of Mannar) (5).

Genus *Dendrilla* Lendenfeld. *D. cactos* (Selenka); Burton, 1937: 42 (as *Spongionella pulvilla*); Thomas, 1985: 229, pl. I, fig. 17 (Gulf of Mannar) (5). *D. membranosa* (Pallas); Burton, 1937: 43 (Gulf of Mannar) (5). *D. nigra* (Dendy, 1889); Burton, 1937: 43; Thomas, 1985: 230, pl. I, fig. 18 (Gulf of Mannar) (5).

Genus *Hexadella* Topsent. *H. purpurea* Burton, 1937: 43; Thomas, 1985: 231 (Gulf of Mannar) (5).

Family **Dictyodendrillidae**
Bergquist

Genus *Dictyodendrilla* Bergquist. *D. retiaria* (Dendy, 1915: 137, pl. IV, fig. 27) (Okhamandal) (3).

Order **Verongida**
Family **Aplysinidae** Carter

Genus *Aplysina* Nardo. *A. lacunosa* (Lamarck); Thomas, 1985: 225, pl. I, fig. 9 (Gulf of Mannar) (5).

Family **Druinellidae** Lendenfeld

Genus *Druinella* Lendefeld. *D. purpurea* (Carter, 1880: 36) (as *Aplysilla purpurea*); Thomas, 1985: 231 (Gulf of Mannar as *Prammaplysilla purpurea*) (5).

Class **Calcarea**
Subclass **Calcinea**
Order **Clathrinida**
Family **Clathrinidae** Minchin

Genus *Clathrina* Gray. *C. coriacea* (Montagu); Pattanayak, 1998 (Andamans) (7).

Family **Leucettidae** de Laubenfels

Genus *Pericharax* Poléjaeff. *P. heteroraphis* Poléjaeff; Burton & Rao, 1932: 304; Pattanayak, 1998 (Andamans) (7).

Subclass **Calcaronea**
Order **Leucosolenida**
Family **Grantiidae** Dendy

Genus *Leucandra* Haeckel. *L. donani* var. *temiradiata* Dendy, 1916a: 86, pl. I, fig. 4a,b (Okhamandal) (3). *L. dwarkaensis* Dendy, 1916a: 86, pl. I, fig. 6, pl. II, fig. 10 a-e (Okhamandal) (3). *L. wasinensis* (Jenkin); Dendy, 1916a: 87, pl. I, fig. 5 (off Dwarka) (3).

Genus *Ute* Schmidt. *U. syconoides* (Carter); Burton & Rao, 1932: 305 (Tuticorin) (5).

Family **Heteropiidae** Dendy

Genus *Grantessa* Lendenfeld. *G. hastifera* (Row); Dendy, 1916a: 81, pl. I, fig. 2,2a, pl. II, fig. 7a-f (off Dwarka) (3).

Genus *Heteropia* Carter. *H. glomerosa* (Bowerbank); Dendy, 1916a: 83, pl. I, fig. 3a-b, pl. II, fig. 8a-g (Okhamandal) (3).

Family **Sycettidae** Dendy

Genus *Sycon* Risso. *S. grantioides* Dendy, 1916a: 79, pl. I, fig. 1 (off Dwarka) (3).

Class **Hexactinellida**
Suborder **Amphidiscophora**
Order **Amphidiscosida**
Family **Hyalonematidae** Gray

Genus *Hyalonema* Gray. *H. aculeatum* Schulze; Schulze, 1902: 31, pl. II, figs 1-14; Pattanayak, 1998 (Andamans) (7). *H. affine* Marshall; Schulze, 1902: 27, pl. VII; Pattanayak, 1998 (Andamans) (7). *H. alcocki* Schulze, 1895; Schulze, 1902: 23, pl. VI, figs 1-8 (Laccadive Sea) (2). *H. indicum* Schulze, 1895; Schulze, 1902: 10, pl. III, figs 1-13, pl. IV, figs 1-14 (Andamans, Laccadives); Pattanayak; 1998 (Andamans) (2,7). *H. lamella* Schulze, 1900; Schulze, 1902: 15, pl. XIX (Cape Comorin); Pattanayak, 1998 (Andamans) (5,7). *H. martabanense* Schulze, 1900; Pattanayak, 1902 (Andamans) (7). *H. masoni* Schulze, 1895; Schulze, 1902: 13, pl. V; Pattanayak, 1998 (Andamans) (7). *H. nicobaricum* Schulze, 1904 (Nicobars) (8). *H. rapa* Schulze, 1900; Schulze, 1902: 18, pl. XVII (Bay of Bengal); Pattanayak, 1998 (Andamans) (7,9). *H. weltneri* Schulze, 1900; Schulze, 1902: 36, pl. IV, figs 15-24 (Laccadive Sea) (2).

Genus *Lophophysema* Schulze. *L. inflatum* Schulze, 1902: 38, pl. XX, XXI; Pattanayak, 1998 (Andamans) (7).

Family **Pheronematidae** Gray

Genus *Pheronema* Leidy. *P. raphanus* Schulze, 1895; Schulze, 1902: 5, pl. I; Pattanayak, 1998 (Andamans) (7).

Genus *Semperella* Gray. *S. cucumis* Schulze, 1895; Schulze, 1902: 41, p.VIII; Pattanayak, 1998 (Andamans) (7).

Subclass **Hexasterophora**
Order **Hexactinosida**
Family **Aphrocallistidae** Gray

Genus *Aphrocallistes* Gray. *A. beatrix* Gray; Schulze, 1902: 87, pl. XV, figs 1-13; Burton & Rao, 1932: 302; Dendy & Burton, 1926: 227, Pattanayak, 1998 (Andamans) (7). *A. bocagei* Wright; Schulze, 1902: 93, pl. XVI (Andamans, Cape Comorin); Pattanayak, 1998 (Andamans) (5,7). *A. ramosus* Schulze; Schulze, 1902: 97, pl. XV, fig. 14; Pattanayak, 1998 (Andamans) (5).

Family **Farreidae** Gray

Genus *Farrea* Bowerbank. *F. occa* Bowerbank; Schulze, 1902: 86 (Andamans); Dendy & Burton, 1926: 226 (Cape Comorin, Andamans) (5,7).

Family **Tretodictyidae** Schulze

Genus *Hexactinella* Carter. *H. minor* Dendy & Burton, 1926: 227; Pattanayak, 1998 (Andamans) (7).

Order **Lyssacinosida**
Family **Euplectellidae** Gray
Sub family **Corbitellinae** Ijima

Genus *Dictyaulus* Schulze. *D. elegans* Schulze, 1895; Schulze, 1902: 70, pl. XII (Laccadive, Cape Comorin) (2,5).

Genus *Regadrella* Schmidt. *R. decora* Schulze, 1900; Schulze, 1902: 67, pl. XXII, figs 10-18 (Cape Comorin) (5).

Subfamily **Euplectellinae** Ijima

Genus *Euplectella* Owen. *E. aspera* Schulze, 1895; Schulze, 1902: 59, pl. XI (Laccadive Sea) (2). *E. aspergillum* Owen; Burton & Rao, 1932: 302 (Andamans) (7). *E. regalis* Schulze, 1900; Schulze, 1902; 61, pl. XXII, figs 1-9; Pattanayak, 1998 (Andamans). *E. simplex* Schulze, 1895; Schulze, 1902: 51, pl. X; Pattanayak, 1998 (Andamans) (7).

Family **Rossellidae** Gray
Sub family **Lanuginellinae** Schulze

Genus *Lophocalyx* Schulze. *L. spinosa* Schulze, 1900; Schulze, 1902: 82, pl. XXIII; Pattanayak, 1998 (Andamans).

INCERTAE SEDIS

Cryptospongia enigmatica Burton, 1928: 133, pl. 2, fig. 5; Pattanayak, 1998 (Andamans) (7).

Protoschmidtia cerebrum Burton, 1928: 116, pl. 1, fig. 2; Pattanayak, 1998 (Andamans) (7).

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THOMAS, P.A., RAMADOSS, K. & VINCENT, S.G. 1993. Invasion of *Cliona margaritifera* Dendy and *C. lobata* Hancock on the molluscan beds along the Indian coast. Journal of the Marine Biological Association of India 35(1&2): 145-156.

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THE IMPACT OF TRAWLING ON SOME TROPICAL SPONGES AND OTHER SESSILE FAUNA. *Memoirs of the Queensland Museum 44: 455. 1999*:- Following a replicated repeated-trawl depletion experiment, which removed 70-90% of the initial biomass of sessile fauna, non-destructive methods have been used to measure recovery of the structurally dominant species. To date, three post-impact surveys have been completed (1 month, 1 year, 2 years) on 6 trawled swathes and 6 controls. These involved quantitative video observations from a tow-sled and from an ROV with positioning precision of 2m. More than 80 taxa of sessile fauna were recorded and about 15 taxa, including sponges, gorgonians, and alcyonarians and hard corals, were abundant enough for analysis. The attributes measured for each organism were species, position, size (height, width, and area), and condition (proportion intact, dead, or encrusted). These data enabled analyses of density, size, condition and species composition, with high precision. We have demonstrated statistically that the methods are very powerful for detecting recovery on the trawled tracks, but the time series is, as yet, too short to identify any recovery. Nevertheless, in treatment vs control or before vs after comparisons, all taxa analysed for which sample size was adequate, showed significant impact due to trawling, for at least one or more of the measured attributes. For all benthos taxa combined, the rate of decrease in benthos density with trawl intensity corresponded to ~10% per trawl, so that in areas trawled 13 times, the benthos density was only ~25% of that in un-trawled areas. This conclusion was very similar to the overall estimate of removal obtained from depletion-regression analysis of the catch obtained during the repeat-trawl experiment. The results also indicated the nature in which trawl impact was manifested for different organisms with different structure and morphology. The sea whips were most resilient to removal, though they could be damaged. Sponges and soft corals were relatively easily removed and hard corals were easily broken. The gorgonians were intermediate and variable in resilience. The interaction between these differences and trawl impact caused a marked change in, and degradation of, the community composition of the seabed habitat. ❐

Porifera, sessile fauna, impact, condition, recovery, trawling, ROV, video, resilience.

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SPONGE SHAPE AS A TAXONOMIC CHARACTER: THE CASE OF *SPONGIA OFFICINALIS* AND *SPONGIA AGARICINA*.

Memoirs of the Queensland Museum 44: 456. 1999:-

The extreme phenotypical plasticity is one of the main characterizing traits of Porifera at all organizational levels (West Heberard, 1986, 1989; Gaino et al., 1995). Body shape is highly variable both in time and space particularly among demosponges living in shallow waters with high selective pressures exerted by fluctuations of water movement and light, by substrate stability and shape and by spatial competition (Bidder, 1923; Hartman, 1950; Reiswig, 1973; Wilkinson & Vacelet, 1979; Palumbi, 1986; Barthel, 1986, 1991; Pansini & Pronzato, 1990; Gaino et al., 1991; Pronzato & Pansini, 1994). Moreover external morph can be influenced, in some cases, by the age of the sponge and therefore by its life cycle (Barthel, 1986; Manconi & Pronzato, 1991).

Spongia officinalis is reported as being very variable both in colour and body shape not only by spongologists (de Laubenfels, 1948; Storr, 1976; Pronzato et al., in press), but also by fishermen that encounter difficulties distinguishing it from other species as *S. zimocca* or *Ircinia spinosula* and *Cacospongia scalaris*. On the other hand *S. agaricina* displays such a peculiar shape that it is easily identified also by students of zoology. Such a different degree of body shape variation in these two close species is an intriguing case.

The present paper aims to investigate the temporal and spatial evolution of body shape of *Spongia officinalis* and *Spongia agaricina* in order to ascertain if and what external morphological traits can have a diagnostic value to clarify their taxonomic status. The trait of body shape could considered as the result of several associated sub-traits as growth in height; growth in width; number and shape of oscules; distribution of oscules; presence and distribution of lobes; differentiation of inhalant and exalant area; presence and distribution of conules. A comparative analysis was performed between Eastern and Western Mediterranean populations of *S. officinalis*. The rarity of *S. agaricina* in the Ligurian Sea meant it was not possible to carry out a comparison with the studied Aegean population.

The following material was considered: *S. officinalis*: 56 specimens collected at 15m depth on hard bottoms by diving at Portofino; 63 specimens at 5-10m depth on hard bottom around the island of Crete; 50 living specimens from Portofino: 25+25 sponges settled, respectively, at 8-10 and 20-25m depth. *S. agaricina:* 37 specimens collected, at 50-100m depth, on sand bottoms by gangava around the island of Kalymnos.

Living sponges were monitored by under-water photography from 1994-1995 in September, July and November to follow the temporal evolution of body shape. To avoid morphological variations linked to pumping activity and to rhythmic contraction/expansion processes, cleaned skeletons were studied at the laboratory. The identification of specimens was performed by SEM at the level of the skeletal net.

Results highlight that *S. agaricina* displays a constant body plan in spite of a wide size variation within the considered population. In the case of *S. officinalis* a constant body plan is displayed by living *sponges* within the same population at different depth. *Spongia agaricina*: body shape is relatively constant; cup-shaped with distal margin ondulati, lateral profile trapezio-like; height and max diameter (at the cup aperture) show an isometric growth; diameter at the base seems to be allometric with respect to the other growth axis and constrained by the substrate morphology; the fan-like shape is absent, with the exception of two specimens, in this population; the distal margin is constantly elliptic with a low difference among the two axis; the irregular growth of the distal margin seems not linked to sponge size. It is possible to hypothesize a shape shifting during ageing from an opened cup toward a tronco-conic shape (*un po' tirata*). ❐ *Porifera, bath sponges, body shape, variability, Spongia officinalis, Spongia agaricina.*

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RESOURCE PARTITIONING BY CARIBBEAN CORAL REEF SPONGES: IS THERE ENOUGH FOOD FOR EVERYONE ?

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Pile, A.J. 1999 06 30: Resource partitioning by Caribbean coral reef sponges: is there enough food for everyone? *Memoirs of the Queensland Museum* **44**: 457-461. Brisbane. ISSN 0079-8835.

Sponges are known to graze primarily on the ultraplankton fraction (plankton < 5μm) of the water column community and have been implicated as primary coral reef consumers of ultraplankton, but it is unknown if there is inter- or intraspecies competition for food resources. I characterised diet and retention efficiency of three co-occuring species of sponge at Chub Cay Reef, Bahamas (25°22`82"N, 77°51`93"W). The erect tube sponge *Callyspongia vaginalis*, the mounding sponge *Spongia tubulifera*, and small *Aplysina fistularis* were conspicuous and common members of the benthic community, and had mean heights above the substrate of 22.5, 7.0, and 1.2cm, respectively. Ambient and exhalent current water samples were collected by snorkelers and analyzed for ultraplankton using flow cytometry. *Callyspongia vaginalis* retained only *Synechococcus*-type cyanobacteria with an efficiency of 90%. In contrast, the diets of *S. tubulifera* and *A. fistularis* were more reflective of the overall water column community consisting of heterotrophic bacteria, *Prochlorococcus*, *Synechococcus*-type cyanobacteria and autotrophic picoeucaryotes. *Spongia tubulifera* had retention efficiencies of 41, 29, and 86% for heterotrophic bacteria, *Prochlorococcus*, and *Synechococcus*-type cyanobacteria, respectively. Retention efficiencies were highest for *A. fistularis*, the smallest sponge, with 96% for heterotrophic bacteria, 95% for *Prochlorococcus*, 99% for *Synechococcus*-type cyanobacteria and 100% for autotrophic picoeucaryotes. Food availability increased closer to the benthos such that an order of magnitude more ultraplankton cells were available to *S. tubulifera* and *A. fistularis*. Overall low abundance of food particles ($<10^5$ cells ml^{-1}) 22cm above the benthos may prevent effective capture by choanocytes. Competition for food resources between phylla is most likely the cause of the resource partitioning found at this location rather than competition between sponges. ❐ *Porifera, feeding, ultraplankton, Caribbean, coral reef, competition.*

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Given that oligotrophic conditions inherently characterise coral reefs, it is not surprising that they are net sinks for all types of planktonic foods, such as zooplankton (Glynn, 1973), nanoplankton (Glynn, 1973), and picoplankton (Buss & Jackson, 1981; Ayukai, 1995; Charpy & Blanchot, 1998), consumed by sessile benthic organisms. However, difficulties in measuring food availability at a scale relevant to these organisms themselves has restricted our understanding of the role of competition for food by benthic suspension feeders. This primarily has been limited by large sample sizes required to quantify naturally occurring, low densities of many food types. Ultraplankton (plankton < 5μm; Murphy & Haugen, 1985) is the most abundant food source on coral reefs both numerically and in terms of total carbon (Ayukai, 1992, 1995; Pile, 1997; Charpy & Blanchot, 1998), and has recently been found to be the major component of the diet of sponges (Reiswig, 1971; Pile, 1997), ascidians (Pile & Young, in review), and soft corals (Fabricius et al., 1995a, b; Ribes et al., 1998) common to coral reefs. Considering that the potential guild of active and passive suspension feeders that will graze on ultraplankton is quite large it is reasonable to suspect that competition for food resources could limit the distribution of some organisms.

Sponges are known to graze primarily on the ultraplankton fraction of the water column community (Pile et al., 1996, 1997; Pile, 1997), and have been implicated as the primary coral reef consumers of ultraplankton (Reiswig, 1971; Pile, 1997; Charpy & Blanchot, 1998) . On Pacific reefs, 90% of the ultraplankton is removed from water that passes over a reef and it

TABLE 1. Mean ultraplankton availability (10^3 cells ml^{-1} ± sd, n=10) at Chub Cay, Bahamas.

Type of ultraplankton	Mean height above bottom (cm)		
	22	7	1.4
Procaryotes			
Heterotrophic bacteria	4.99 (1.37)	77.1 (48.9)	116.0 (49.2)
Prochlorococcus	2.00 (0.44)	45.8 (26.6)	37.2 (17.9)
Synechococcus-type cyanobacteria	8.59 (12.2)	93.8 (81.9)	177.0 (15.3)
Eucaryotes			
Autotrophic eucaryotes (<3µm)	0.10 (0.11)	16.2 (9.37)	63.9 (48.3)

has been suggested that this is the result of grazing by the benthos (Ayukai, 1995). In the Caribbean, sponges are the dominant benthic invertebrate, contributing up to 2.5kgm^2 of the benthic biomass (Wilkinson, 1987). Concurrent with this high biomass the sponge community is very diverse with morphologies ranging from encrusting to massive (Wilkinson, 1987). High abundance and species diversity of sponges coupled with oligotrophic conditions common to coral reefs could require partitioning of food resources between sponges or with other members of the guild of primary consumers of ultraplankton which is not found in more eutrophic ecosystems (Stuart & Klumpp, 1984; Lesser et al., 1992).

Abelson et al. (1993) hypothesised that the morphology of coral reef organisms modifies the flow patterns around them such that it predisposes their diets. In their model, organisms with a high slenderness ratio (the ratio between body height and downmost width > 1) will graze on fine particulate matter whereas organisms with a low slenderness ratio (< 1) will feed primarily on bed load particles. Upright tubular sponges, gorgonians and other soft corals all have high slenderness ratios and it is highly likely that they will utilise the same food resources. Small mounding, massive, and encrusting sponges all have low slenderness ratios and would be able to exploit an unoccupied niche by grazing on ultraplankton if all other low slenderness ratio organisms (i.e. flattened types of corals, solitary fungiid coral species, and bryozans) grazed primarily on bed load particles. Therefore, in this study I quantified the food availability and diet of three co-occuring species of demosponges on a coral reef with varying slenderness ratios to determine if there was greater competition for food resources for species with high slenderness ratios.

MATERIALS AND METHODS

Diets and retention efficiencies were measured for three co-occuring species of sponge at Chub Cay Reef, Bahamas (25°22'82"N, 77°51'93"W). Chub Cay Reef is a patch reef that has a maximum depth of 5m. The erect tube sponge *Callyspongia vaginalis*, the mounding sponge *Spongia tubulifera*, and very small *Aplysina fistularis* were conspicuous and common members of the benthic community and had mean heights (n=10) above the substrate of 22.5 (± 3.8 sd), 7.0 (± 1.3 sd), and 1.2 (± 0.4 sd)cm respectively.

Retention of ultraplankton was quantified from 1ml water samples collected using 5cc syringes from 10 individuals of each species while snorkeling to a depth of no greater than 3m. Samples were taken from water adjacent to the sponge and from the exhalent current of each individual and preserved for flow cytometry using standard protocols (Campbell et al., 1994). Ultraplankton populations were quantified using an Epic Elite flow cytometer (Coulter Electronics Corporation, Hialeah, Florida) at Harbor Branch Oceanographic Institution, following the techniques of Marie et al. (1996). Orange fluorescence (from phycoerythrin), red fluorescence (from chlorophyll), and green fluorescence (from DNA stained with SYBR Green) were collected through band pass interference filters at 575, 680, and 450nm, respectively. The five measured parameters (forward- and right-angle light scatter (FALS and RALS), orange, red, and green fluorescence) were recorded on 3-decade logarithmic scales, sorted in list mode, and analyzed with a custom-designed software (CYTOWIN; Vaulot, 1989). Ultraplankton populations were identified to general cell types of heterotrophic bacteria (HBac), *Prochlorococcus* (Pro), *Synechococcus*-type cyanobacteria (Syn), and autotrophic eucaryotes <3µm (Peuc), visually confirmed (except for *Prochlorococcus*), and mean cell diameter measured (n=50) using epifluorescence microscopy.

Differences between cell counts from ambient and exhalent current water of each type of ultraplankton were analyzed using two tailed t-tests for each species of sponge with a Bonferroni-transformed experimentwise ; of 0.00625 to determine the effects of sponges on

TABLE 2. Mean 10^3 cells ml^{-1} (± sd, n=10) in the exhalent current demonstrating the effect of each sponge on the four types of ultraplankton. Individual t-tests comparing mean cell concentrations to ambient cell concentrations (Table 1). * $p < 0.00625$.

Species of Sponge	Height (cm)	Heterotrophic bacteria	*Prochlorococcus*	*Synechococcus*-type cyanobacteria	Autotrophic picoeucaryotes
Callyspongia vaginalis	22.5	5.08 (0.96)	2.12 (0.58)	0.90* (0.23)	0.04 (0.05)
Spongia tubulifera	7.0	45.8 (0.49)	32.3 (0.49)	13.5* (0.29)	9.09 (0.21)
Aplysina fistularis	1.2	4.2* (0.42)	1.70* (0.24)	2.03* (1.43)	0.17* (0.06)

ultraplankton (Zar, 1984). The mean retention efficiency for each sponge was calculated as ((mean cell count ambient - mean cell count exhalent)/mean cell count ambient)x100 for each type of ultraplankton. Student t tests, one tailed, were used to determine if the retention efficiency for each type of ultraplankton was significantly >0 employing a Bonferronni transformed experimentwise error of α=0.0001, p=0.00625.

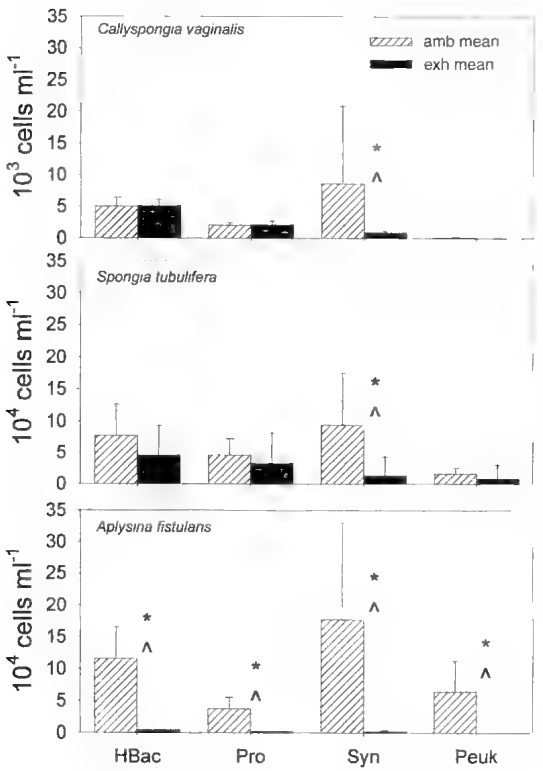

FIG. 1. Effect of each sponge on ultraplankton populations. Concentration of each type of ultraplankton in ambient water and water from the exhalent currents of each sponge. Stippled bars are for ambient water and black bars for water from the exhalent current. Abbreviations: Hbac= heterotrophic bacteria, Syn= Synechococcus-type cyanobacteria, and Peuk= autotrophic eukaryotes <3µm. Note that the y axis is an order of magnitude less for *C. vaginalis*. * Cell concentrations between ambient water and exhalent current water which are significantly different (paired t-test with a Bonferroni transformed experimentalwise α<0.00625).

RESULTS

Ultraplankton abundance decreased with height above the benthos (Table 1). Abundance at all three heights followed the pattern of *Synechococcus*-type cyanobacteria as the most abundant cell type followed by heterotrophic bacteria, *Prochlorococcus*, and autotrophic eucaryotes < 3µm were the least abundant. Ultraplankton abundance increased from $1.57x10^4$ cells ml^{-1} at 22cm to $29.1x10^4$ cells ml^{-1} at 1.4cm from the benthos.

Callyspongia vaginalis retained only *Synechococcus*-type cyanobacteria (Table 2, Fig. 1) with an efficiency of 90% (Fig. 2). In contrast, the diets of *S. tubulifera* and *A. fistularis* were more reflective of the overall water column community consisting of heterotrophic bacteria, *Prochlorococcus*, *Synechococcus*-type cyanobacteria and autotrophic picoeucaryotes (Table 2, Fig. 1). *Spongia tubulifera* had retention efficiencies of 41, 29, and 86% for heterotrophic bacteria, *Prochlorococcus*, and *Synechococcus*-type cyanobacteria respectively (Fig. 2). Retention efficiencies were highest for *A. fistularis*, the smallest sponge, with 96% for heterotrophic bacteria, 95% for *Prochlorococcus*, 99% for *Synechococcus*-type cyanobacteria and 100% for autotrophic picoeucaryotes (Fig. 2).

DISCUSSION

Typical of other demosponges all three species grazed primarily on the ultraplankton fraction of the water column community (Reiswig, 1971; Pile et al., 1996, 1997; Pile, 1997). Retention efficiencies by *C. vaginalis* and *S. tubulifera* were substantially lower than those previously reported for demosponges and this may be related

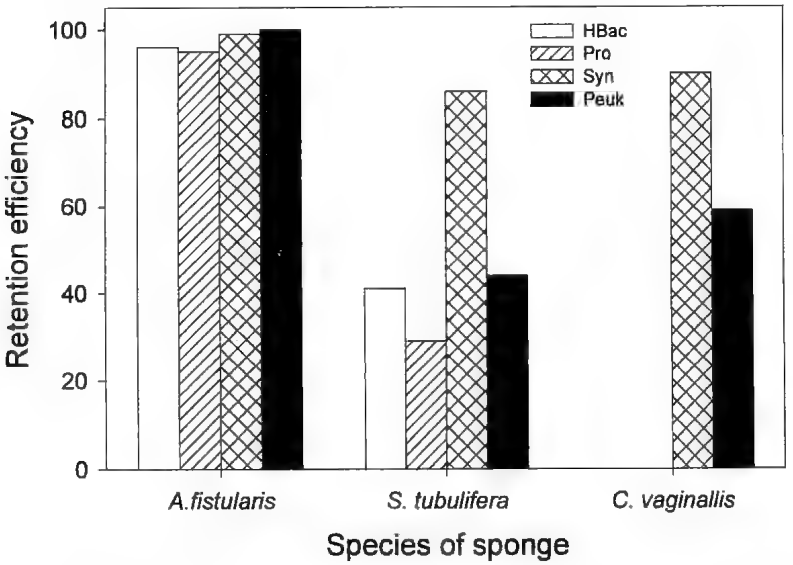

FIG. 2. Retention efficiency (x ± sd, n = 10) for each species of sponge for each type of ultraplankton. Abbreviations: Hbac = heterotrophic bacteria, Syn = *Synechococcus*-type cyanobacteria, and Peuk = autotrophic eukaryotes <3µm

to the low abundance of cells available to the sponges (Reiswig, 1971; Pile et al., 1996, 1997; Pile, 1997). When the abundance of ultraplankton approached those normally found on coral reefs (Ayukai, 1995; Pile, 1997), such as those in water surrounding *A. fistularis*, retention efficiencies are similar to those previously observed (Reiswig, 1971; Pile, 1997). It should be noted that at Chub Cay Reef *Synechococcus*-type cyanobacteria was the most abundant food source, which is unusual in that bacteria are normally the most abundant food source on coral reefs (Ayukai, 1995; Pile, 1997).

Increasing ultraplankton availability nearer to the benthos opposes the pattern of ultraplankton community structure in shallow waters found in the Red Sea (Yahel et al., 1998) and Lake Baikal (Pile et al., 1997) where abundance decreases closer to the benthos. As predicted by the model of Abelson et al. (1993) ultraplankton availability increased closer to the benthos and this trend is most likely due to decreasing competition for it as a food source. Ultraplankton abundance was extremely low (< 10^5 cells ml^{-1}) 22cm above the bottom and availability increased closer to the benthos such that an order of magnitude more ultraplankton cells were available to *S. tubulifera* and *A. fistularis*. Overall low abundance of food particles 22cm above the benthos may be preventing effective capture by the choanocytes and merits further investigation.

Competition between phylla for food resources is most likely the cause of the resource partitioning found at this reef rather than competition between sponges. The other major benthic organisms at Chub Cay Reef are gorgonian corals *Gorgonia flabellum*, *G. ventalina*, *Plexaura flexuosa*, and *P. porosa*. Recently, soft corals have been found to significantly impact ultraplankton communities. In the Caribbean *Plexaura flexuosa* and *P. porosa* graze on the ultraplankton fraction >3µm (Ribes et al., 1998) while in the Red Sea the soft corals *Dendronephthya hemprichi*, *D. sinaiensis*, and *Scleronephthya corymbosa* and the gorgonian *Acabaria sp.* have been found to graze on plankton down to *Synechococcus*-type cyanobacteria (typically 1.2-1.8µm) (Fabricius et al., 1995b). Soft coral biomass is considerable in some communities were sponges are also prolific (Kinzie, 1973) and may be a significant competitor for ultraplankton. Since soft corals and gorgonians typically have a higher s/r ratio they will most likely impact a zone of water that is higher from the benthos than sponges with a low s/r ratio. Most other organisms with low s/r ratios, such as hard corals, bryozans, and bivalves are typically bed load feeders (e.g. Abelson et al., 1993; Jørgensen, 1996; Riisgård & Manriquez, 1997). Sponges with a low s/r ratio may be the only group of organisms to graze on ultraplankton. If this is true, then they have cornered a niche which has allowed for their success in benthic communities.

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'MUD MOUND' STRUCTURES AND CORALLINE SPONGES FROM OSPREY REEF (QUEENSLAND PLATEAU, CORAL SEA, AUSTRALIA). *Memoirs of the Queensland Museum 44: 462. 1999:-* Osprey Reef is located at the NW tip of the Queensland Plateau. This reef represents an open oceanic reef platform on a drowned carbonate platform (Queensland Plateau). The metamorphic basement was drilled to a depth of about 450m below the sea floor (Betzler et al., 1995).

The reef caves of Osprey Reef were studied during two one-week expeditions in 1995 and 1996 using the DPI RV 'Gwendoline May'. At Osprey Reef a very patchy distribution of coralline sponges was observed. At some dive sites caves and reef internal surfaces (RIS) were free of this fauna, whereas a few hundreds of meters away, the walls of caves and the RIS were covered with coralline sponges. The reasons for this very patchy distribution are not presently clear. The structure of the cave community is the same as found on the GBR. The caves with abundant coralline sponges are mainly located between 15-20m depth.

At all sites, *Astrosclera* was the most dominant sponge, and similar to populations on the outer barrier reefs of the GBR, it sometimes lives in semi-dark conditions and is colored red or green. Its size never exceeds 5cm. *Spirastrella (Acanthochaetetes)* was rare at Osprey Reef compared to the GBR.

We found two new species of colonial variations of the 'sphinctozoan' sponge *Vaceletia* at Osprey Reef which still remain unnamed and largely undescribed, although preliminary notices of their occurrence and brief descriptions are provided by Reitner & Wörheide (1995) and Wörheide & Reitner (1996). These new 'sphinctozoans' are common in most of the caves and are mostly associated with *Vaceletia crypta*. They occur in only the darkest parts of the caves, and from their large biomass and 'insinuating habit' (i.e. growing between dead coral), they appear to be important components of reef structure. This type of coralline sponge shows similarities to Permo-Triassic reef building sphinctozoans.

At 250-300m depth aggregates of medium sized mound structures were observed. These structures are located at the NW steep escarpment of Osprey Reef and are ca. 10-20m long, 1-2m wide, and approximately 0.5-2m high. The surface is rigid and sometimes overgrown with sponges and gorgonians. Between the elongated mounds groove systems are developed where reef sediments are transported. Little sediment 'snow' is fixed by the mound surfaces. These mound structures are comparable with micritic sponge reefs known from Mesozoic reef sites. ❐ *Porifera, coralline sponges, mud-mounds, Vaceletia, reef-building sphinctozoans, Osprey Reef, Coral Sea.*

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POSTPALEOZOIC HISTORY OF THE SILICEOUS SPONGES WITH RIGID SKELETON

ANDRZEJ PISERA

Pisera, A. 1999 06 30: PostPaleozoic history of the siliceous sponges with rigid skeleton. *Memoirs of the Queensland Museum* **44**: 463-472. Brisbane. ISSN 0079-8835.

Most of the Mesozoic groups of siliceous sponges with rigid skeletons (Hexactinosa, Lychniscosa and 'Lithistida') have Paleozoic roots, except the Lychniscosa known from the Middle Jurassic to Recent, and the 'lithistid' Didymorina known only from the Jurassic. The Triassic record of these groups is poor, and all become common only in the Middle - Late Jurassic, but probably reach maximum diversity and frequency during the Late Cretaceous. The Tertiary record of all these groups is much poorer than for the Mesozoic. Hexactinosa and 'Lithistida' are common elements of Recent deeper water faunas, while Lychniscosa, which were very common during the Mesozoic, are very rare and of low diversity in modern seas. Known and newly discovered Tertiary faunas show many affinities with Cretaceous ones indicating lesser susceptibility of these sponges to K/T boundary disturbances, than seen in other organisms. Large faunas of siliceous sponges with rigid skeletons occur in the fossil record in a punctuated manner, and are correlated with high sea level stands. ❐ *Porifera, Hexactinosa, Lychniscosa, Lithistida, PostPaleozoic history, K/T boundary.*

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Siliceous sponges with rigid skeletons are virtually the only ones which have sufficient fossil records to allow some generalisations to be deduced about their history and patterns of distribution during the Mesozoic. Here belong the Hexactinosa, Lychniscosa and the polyphyletic assemblage we currently know as 'Lithistida'. Even these records, however, are very punctuated. Sponges with skeletons consisting of loose spicules that fell apart after death, such as lyssacinosan hexactinellids and soft demosponges, usually have much poorer records. What is worse, these records are nearly exclusively represented by mixtures of spicules belonging to various taxa (see for example Hinde, 1880; Reif, 1967; Pisera, 1997, and literature therein). This does not mean, of course, that such sponges are not preserved intact at all. There are numerous examples of both demosponges (*Propachastrella* from the Upper Cretaceous; Schrammen, 1910-1912), or lyssacinosan hexactinellids having loose spicules (*Stauractinella* from the Upper Jurassic; Mehl, 1992; Pisera,1997) whose preservation as body fossils was facilitated either by very early cementation and/or collagen cement. Another example is a rosselid sponge from the Upper Cretaceous of Denmark (Mehl, 1992). In the Eocene of Catalonia intact demosponges and hexactinellids with loose spiculation (Busquets et al., 1997; Pisera unpublished) are preserved, most probably due to catastrophic burial. All these, however, are relatively rare cases showing only how much information is missing in the fossil record.

Some lyssacinosan hexactinellids may have entirely, or partly fused skeletons and such sponges are quite common among fossils, examples can include Triassic *Cypellospongia* from the USA (Rigby & Gosney, 1983), *Hexactinoderma* and *Silesiaspongia* (Fig. 1C) from Poland (Pisera & Bodzioch, 1991), Cretaceous *Proeuplectella* from France (Moret, 1926) and *Regadrella* from Germany (Salomon, 1990) and Tertiary (Brimaud & Vachard, 1986b). The fusion in these sponges, however, is of irregular type (at points of contact and without formation of dictyonal strands; Fig.1C), and happens rather late in ontogeny, in opposition to Hexactinosa and Lychniscosa where the fusion is an early ontogenetical phenomenon. I will not refer to these unusual sponges any further.

STRATIGRAPHICAL DISTRIBUTION OF HEXACTINOSA, LYCHNISCOSA AND LITHISTIDA

HEXACTINOSA. Hexactinosa are hexasterophoran hexactinellids having skeletons fused in a regular way, i.e., particular hexactines form longitudinal dictyonal strands (Reid, 1963) (Fig. 1B, D) (fusion of hexactines happens by

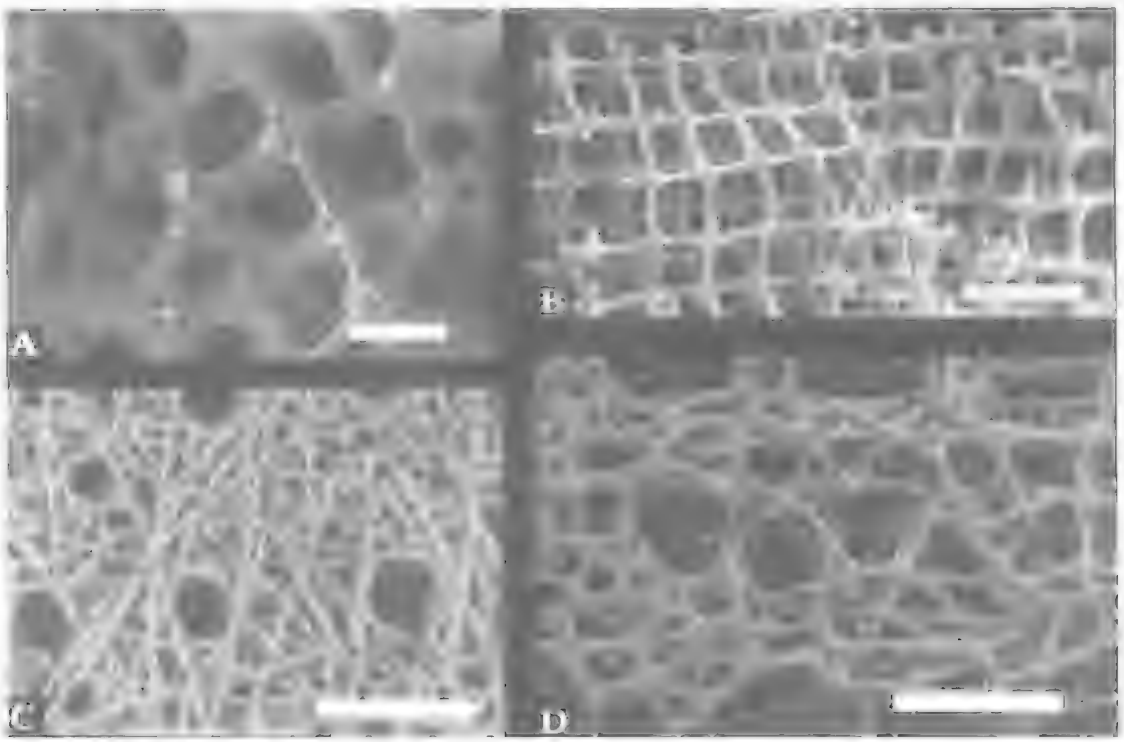

FIG. 1. Skeletons of hexactinellid sponges. A, Lychniscosan choanosomal skeleton, *?Rhogostomium* sp., Late Jurassic, Germany, (scale bar 200µm). B, Hexactinosan choanosomal skeleton (note that hexactines are fused together with the help of silica cement), *Sphenaulax* sp., Late Jurassic, Germany, (scale bar 500µm). C, Surface of lyssacinosan choanosomal skeleton (note the irregular fusion of spicules which are mostly diactines), *Silesiaspongia rimosa* Pisera & Bodzioch, Middle Triassic, Poland, (scale bar 1mm). D, Hexactinosan choanosomal skeleton, *Dactylocalyx* sp., Recent, (scale bar 1mm).

joining two rays into a common silica envelope). Fusion between particular strands may be less regular. For a long time Hexactinosa were regarded as a typical Mesozoic group (see Rigby, 1983); during the last several years, however, numerous reports appeared about the presence of this group in Devonian rocks (Rigby et al., 1981). Much earlier reports of their presence in the Paleozoic (Fraipont, 1911; Mayr, 1930) have been mostly overlooked.

The best and the richest fauna of these sponges I know so far is from the Late Devonian (Frasnian) of the Holy Cross Mountains in Poland (Rigby et al., 1981, and in prep.). These sponges are already very close to Jurassic and Cretaceous representatives of Hexactinosa, which suggests that they have an even longer, but unfortunately unknown Paleozoic history. (There is a chance that some sponges described in the literature from the Ordovician as belonging to sphaerocladine lithistids are in fact Hexactinosan sponges. It is their extremely regular structure, typical of Hexactinosa, but unknown in lithistids, which suggest such a possibility. We know of both, fossil and Recent hexactinosan sponges, that have enlarged sphaerical nodes. This problem needs further study). Smaller faunas of Devonian hexactinosans were reported from Germany, Belgium and Australia. There is a large gap between this late Devonian fauna and the next undoubted hexactinosans that occur only in the Mesozoic.

Undoubted Triassic hexactinosan sponges occur in Sichuan, China, Caucasus and in the Alps (Keupp et al., 1989; Wend et al., 1989; Boiko, 1990; Rigby et al., 1998). It is interesting that some of them belong to genera established from the Upper Jurassic. Among them are characteristic genera *Casearia* and *Sphenaulax*.

Hexactinosa become very diversified and common starting from the Late Jurassic (Schrammen, 1936-37; Trammer, 1982, 1989; Mehl, 1992; Pisera, 1997, and literature therein).

For example, a Late Jurassic fauna of Hexactinosa from the Swabian Alb is composed of at least of 23 genera and 48 species (Pisera, 1997). Rich hexactinosan faunas are known from the Early Cretaceous (Lagneau-Hérenger, 1962) and Late Cretaceous of Europe (Schrammen, 1910-1912; Moret, 1926). Tertiary hexactinosans are infrequent and reported mostly in recent times (Rigby, 1981; Rigby & Jenkins, 1983; Brimaud & Vachard, 1986b; Busquets et al., 1997). Today hexactinosans form important and diversified elements of deep-water sponge faunas mainly in tropical areas, and include about 40 genera and 135 species (Reid, 1968, and literature therein).

LYCHNISCOSA. Lychniscosan sponges also display development of dictyonal strands and fusion of spicules, but they have lychnisc (octahedral) nodes (Fig. 1A), in contrast to the solid nodes of hexactines in Hexactinosa. Their history is quite different. There is no trace of this group before the Middle Jurassic. Earlier reports of Triassic lychniscosan sponges (Vinassa de Regny, 1911; Keupp et al., 1989; Wendt et al., 1989; Wu Xi Chun, 1990) were proven to be erroneous (Mostler, 1990; Pisera & Bodzioch, 1991; Mehl, 1992). Also the report of Early Jurassic lychniscosans (Broglio Loriga et al., 1991) seems very doubtful.

The oldest known bodily preserved lychniscosan sponge (Pisera, 1993, and in prep.) belongs most probably to the Late Jurassic genus *Pachyteichisma* and occurs in the uppermost Bajocian of the Mečsek Mountains in southern Hungary. This genus is common in the Callovian of Kutch, India (Mehl & Fürsich, 1997, referred to as *Sporadopyle*) and it occurred also during the Late Jurassic (Pisera, 1997).

Lychniscosan sponges are an important part of the large Late Jurassic fauna from the Swabian Alb (34 species and 15 genera; Pisera, 1997) and Upper Cretaceous faunas of Northern Germany (81 species and 34 genera; Schrammen, 1912). Even if there is an 'oversplitting' of taxa this diversity is impressive, especially when compared with Recent diversity of this group.

For a long time very little was known about Tertiary lychniscosan sponges, and only one genus, *Manzonia* from the Miocene of Italy and Spain, was known. To this Pomel's genus *Pachychlaenium* (=*Tremabolites*) was added by Mehl (1992). More recent discoveries in the Eocene of Catalonia (Pisera in prep.) show that their diversity was lower than during the Late Cretaceous, but was still higher than today, for they are represented there by at least 5-6 species and 5 genera, which are quite prevalent. Today, lychniscosan sponges are a relict group represented only by 3 species and 2 genera (Mehl, 1992) and are rare. The reason why Lychniscosa developed during the Cenozoic differently than the Hexactinosa remains unknown.

'LITHISTIDA'. Lithistids are demosponges characterised in having choanosomal skeletons composed of desmas joined by articulation without cementation by silica (Fig. 2A). There is no doubt that they are a polyphyletic group, and when we speak about 'lithistids' we should split them into smaller units that have the same geometry of desmas, with a greater probability of being monophyletic (i.e., Tetracladina Zittel, 1878, Rhizomorina Zittel, 1878, Dicranocladina Schrammen, 1924 (=Corallistidae Sollas, 1888), Sphaerocladina Schrammen, 1910, Megamorina Zittel, 1878 and Didymorina Zittel, 1878). Lithistids as a group are known from the Lower Palaeozoic (Rigby, 1983), and some Mesozoic groups, i.e., Rhizomorina (if the Paleozoic rhizomorines are the same lineage as Mesozoic one), Sphaerocladina and Megamorina have their Palaeozoic representatives. The Dicranocladina are most probably closely related to Paleozoic hindiids (Finks, 1971). Diversity of fossil and Recent lithistids, as a whole group, is probably comparable because in the Upper Jurassic of the Swabian Alb about 42 species have been found by me, whereas a Recent fauna of lithistids from the New Caledonia region is composed of 23 species according to Lévi & Lévi (1983). Taking into account that the Recent fauna represents only one time plane, the diversity may be regarded as similar. Diversity of particular groups, however, differs considerably. Tertiary lithistids, as a whole, are rather poorly known (Moret, 1924; Brimaud & Vachard, 1986a). For excellent and more detailed review of lithsitids distribution than presented below see Wiedenmayer (1994).

The oldest bodily preserved Rhizomorina Zittel, 1878, characterised by irregular, usually thorny desmas called rhizoclones (Fig. 2B-E), based on monaxons, are known from the Early Jurassic from Georgia (Nutsubidze, 1965), although rhizoclone spicules are known from the Triassic (Wiedenmayer, 1994). Rhizomorine sponges are common only from the Late Jurassic on (Schrammen, 1910-1912, 1937; Moret, 1924; Brimaud & Vachard, 1986a; Pisera, 1997). They are probably the most common group of

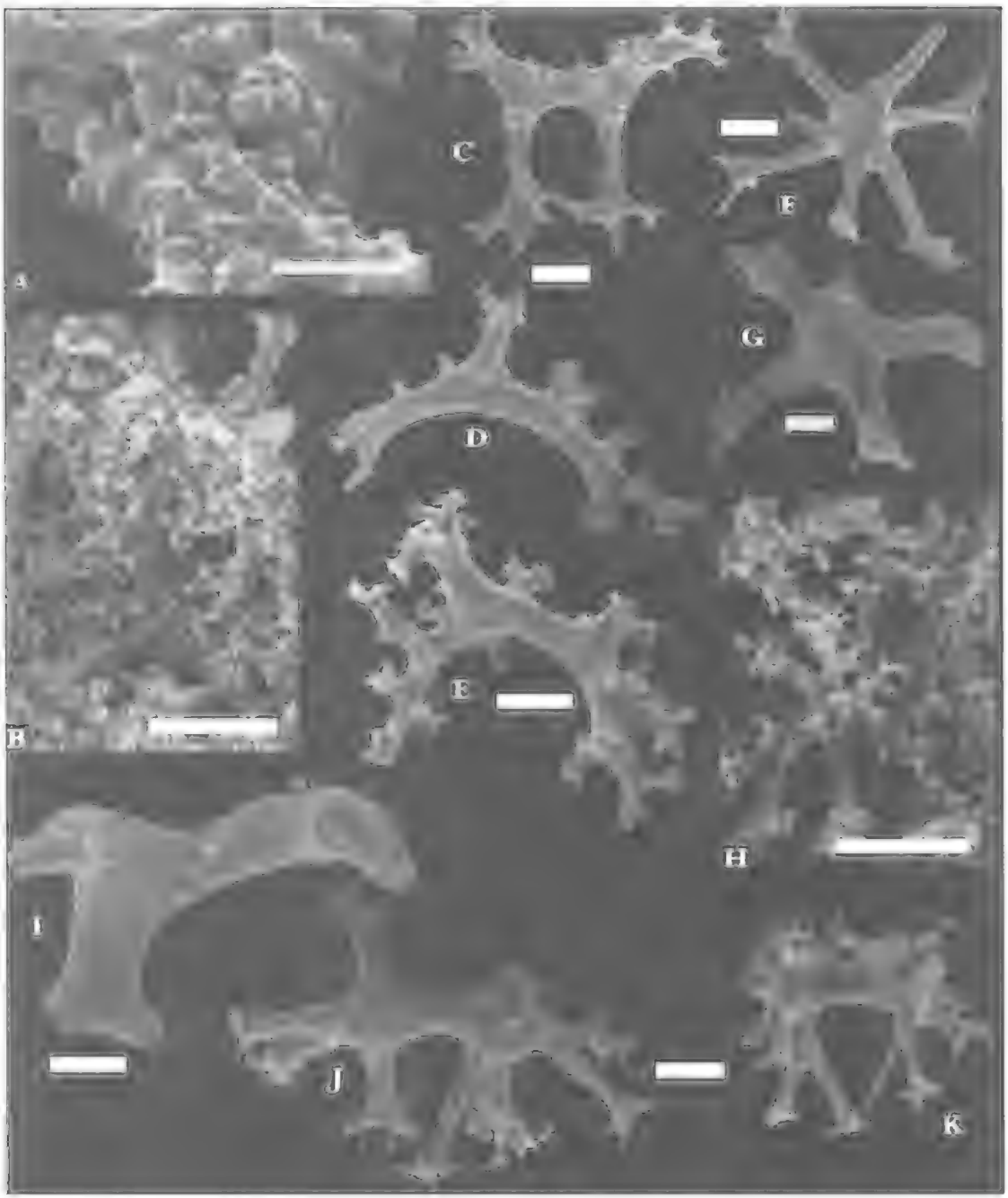

FIG. 2. Lithistid sponge spiculation: A, Articulation of desmas (note that desmas are joined only by articulation without cementation), Recent tetractinellid lithistid, Caribbean, (scale bar 100µm). B, Choanosomal skeleton of Recent rhizomorine sponge *Setidium* sp., (scale bar 200µm). C-E, Desmas (rhizoclones) of the Late Jurassic rhizomorine sponges, Germany, (scale bars 100µm). F-G, Desmas of the fossil sphaerocladine sponges, Late Jurassic, Germany, (scale bars 100µm). H, Choanosomal skeleton of the Recent sphaerocladine lithistid *Vetulina* sp., Caribbean, (scale bar 100µm). I, Desma (megaclone) of fossil megamorine sponge, Late Jurassic, Germany, (scale bar 100µm). J-K, Desmas (didymoclones) of fossil didymorine lithistid *Cylindrophyma* sp., Late Jurassic, Germany, (scale bar 100µm).

Mesozoic lithistids known in Europe. Rhizomorines are quite common in the Tertiary (Moret, 1924), and today they are represented by 8-10 genera.

Bodily preserved Tetracladina Zittel, 1878, characterised by usually regular desmas based on a tetraxon called tetraclone (Fig. 3F-G), already exist in the Triassic (known from the thin sections only; Keupp et al., 1989), but they are rare. They become common only in the Late Jurassic (5 genera and 6 species), with maximum fossil diversity in the Late Cretaceous where Moret (1926) cited 26 genera and 62 species from France alone. In Recent seas they are also common and represented by about 130 described species worldwide (Wiedenmayer, 1994 and the literature therein). In more resticted areas like New Caledonia 23 species and 16 genera were reported (Lévi, 1991).

The earliest Dicranocladina Schrammen, 1924, characterised by regular strongly tuberculated, usually tripodal or tetrapodial, desmas (Fig. 3A-E), based on monaxons, are known from 5 species and 3 genera of the Late Jurassic. They are distinguished with difficulty from Recent ones. Dicranocladina diversity is high in the Late Cretaceous (Moret, 1926, lists 8 genera and 15 species). Tertiary Dicranocladina are rarely reported (Rigby, 1981; Brimaud & Vachard, 1986a), but they are quite common in Recent seas (Lendenfeld, 1903; Levi & Levi, 1983; Levi, 1991).

The next group of lithistids, i.e., Megamorina Zittel, 1878 (which corresponds to Recent Pleromatidae Sollas, 1888), are characterised by desmas called megaclones (Fig. 2I). They appear as early as the Ordovician but their Mesozoic record starts in the Middle Triassic (Wiedenmayer, 1994, and literature therein). They become common and diversified in the Jurassic and Cretaceous (Schrammen, 1910-1912, 1937; Moret, 1926; Lagneau-Hérenger, 1962; Pisera, 1997). Their Tertiary record is poor and only one bodily preserved, recently discovered, but still undescribed species, has been found in the Eocene. Today Megamorina is also a small group with only 2 genera (Wiedenmayer, 1994).

Similar is the history of the Sphaerocladina Schrammen, 1910, which are characterised by desmas called sphaeroclones or astroclones (Fig. 2F-H). They first appear in the Paleozoic (Wiedenmayer, 1994, and references therein) (if astylospongids are included here, and that may be questioned), but their Mesozoic record starts in the Late Jurassic (3 genera and 5 species). Sphaerocladina have the same diversity in the Cretaceous (Moret, 1926, lists 3 genera, 5 species). They have a poor Tertiary record, and in Recent seas are represented most probably only by *Vetulina stalactites* Schmidt (Fig. 2H). This species has been suggested to be the rhizomorine (Gruber, 1993), but more recent studies of the holotype suggests that it is not.

The last group to be considered is Didymorina Zittel, 1878, with desmas called didymoclones (Fig. 2J-K). They are extinct and undoubted representatives occur only in the Middle and Late Jurassic - 2 genera and 3 species. Similar loose spicules of uncertain affinity were reported by Mostler (1976) from the Triassic.

PATTERNS IN THE HISTORY OF SILICEOUS SPONGES WITH RIGID SKELETON.

Longevity of sponge genera. It has been known long that numerous Recent hexactinellid genera are long ranging and occur even in Upper Cretaceous rocks. For example Mehl (1992) found that of 31 genera from the Late Cretaceous Hexactinosa, 11 survive in Recent seas. The hexactinosan genus *Laocoetis* (=*Craticularia*) ranges most probably from the Early Jurassic (Nutsubidze, 1965) until today (Lévi, 1986) - a duration of nearly 200 million years. The Recent genus *Dactylocalyx* has been reported by Trammer (1989) from the Late Jurassic. Recently discovered sponge faunas strengthen this pattern by showing the presence of the genera known from the Upper Jurassic also in the Triassic (e.g., the hexactinosan *Casearia* and *Sphenaulax;* Rigby et al., 1998), and other Cretaceous genera in the Tertiary (e.g., the lychniscosan *Becksia*, *Sporadoscinia*, *Brachiolites* in newly discovered Eocene faunas from Spain; Pisera, unpublished), thus pointing to the extremely conservative nature of these sponges at the generic level.

Lithistids also include several long ranging genera. *Cnemidiastrum*, for example, is typical of the Upper Jurassic but has been recently recorded in the Miocene, while the Cretaceous genus *Aulaxinia* has been discovered by Lévi & Lévi (1988) in Recent waters around New Caledonia. The Recent rhizomorine genus *Amphibleptula* from the Atlantic has been recognised in the Late Jurassic (Pisera, 1997). Numerous lithistids of the Late Cretaceous (especially rhizomorine sponges) show no recognisable differences when compared with Late Jurassic forms. It seems that different names given to these different faunas

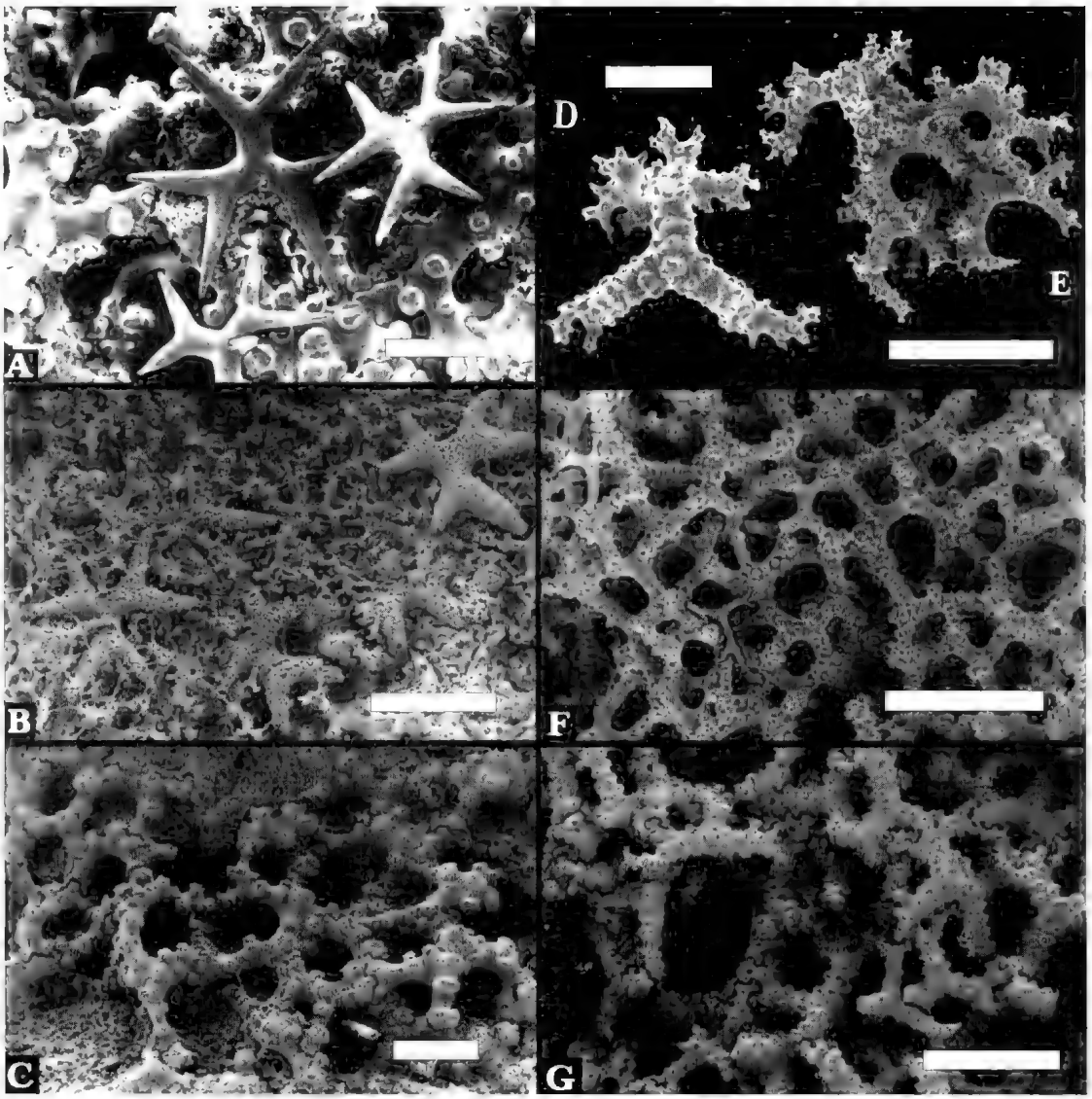

FIG. 3. Lithistid sponge spiculation; A, Recent *Corallistes* sp., choanosomal skeleton composed of strongly tuberculated dicranoclones, and dermal dichotriaenes, Gulf of Mexico, (scale bar 100μm); B-E, Fossil *Dicranoclonella* sp., Late Jurassic, Germany. B, Upper surface showing dermal dichotriaenes and rhizoclone-like modified dicranoclones between them, (scale bar 200μm); C, Choanosomal skeleton composed of strongly tuberculated dicranoclones, (scale bar 200μm); D, Isolated typical dicranoclone, (scale bar 200μm); E, Fragment of choanosomal skeleton, (scale bar 500μm). F, Choanosomal skeleton of Recent tetracladine sponge, Carribean, (scale bar 500μm); G, Choanosomal skeleton of fossil tetracladine sponge, Oligocene, the Ukraine, (scale bar 500μm).

stem from the philosophy that large age differences are enough to justify establishing a new genus. This approach is questionable. It appears that lithistids are also rather conservative and slowly evolving.

Sponges and K/T boundary. The Cretaceous-Tertiary (K/T) boundary was a time when most fossil groups of marine organisms were severely decimated. The pattern of distribution of siliceous sponges with rigid skeleton across this boundary is interesting. There are no sponge

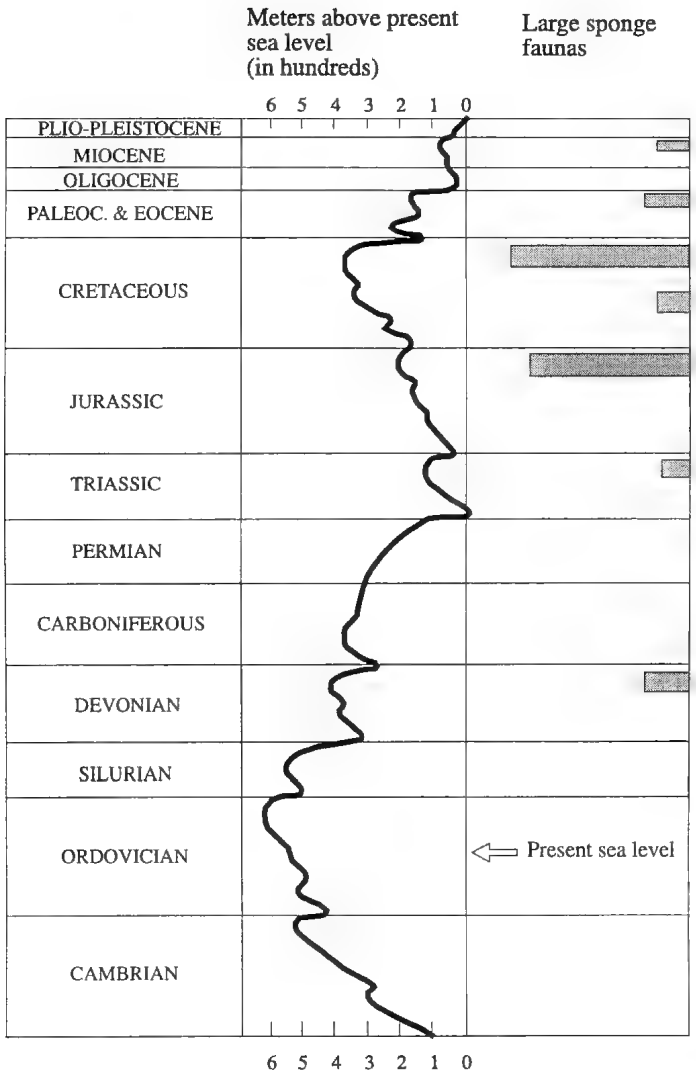

FIG. 4. Sea level curve for the Phanerozoic and the distribution of large faunas of siliceous sponges (sea level curve from Hallam, 1992).

faunas known directly above the boundary, but when one considers the newly discovered Eocene faunas from the Pyrenees (Busquets et al., 1997) composed of both lithistids (mostly tetractinellids, one megamorine and some rhizomorine sponges have been also found; Pisera, unpublished data) and hexactinellids with rigid skeletons, including both hexactinosans and lychniscosans. Such characteristic forms as *Guettardiscyphia*, a typical Cretaceous hexactinosan, are important elements (rock forming in places) of these Eocene faunas. Among lychniscosan sponges the so called meandrispongids (*Brachiolites*, *Plocoscyphia*), and *Sporadoscinia*, which are characteristic of the Late Cretaceous, dominate the Eocene faunas (Pisera, unpublished data). A similar fauna has been reported also from the Eocene of the USA (Rigby, 1981; Finks, 1983, 1986). Miocene faunas from Algeria, described by Moret (1924), and from Spain described by Brimaud & Vachard (1986a, b) have many Mesozoic elements. Recent faunas of sponges with rigid skeleton are also considered by Reid (1967) as of the Tethyan (Mesozoic) origin. All this indicates that sponges were less strongly affected by K/T boundary disturbances than other organisms.

How to explain such behaviour of the siliceous sponges discussed here? It follows because they are (and were) rather deep-water creatures. They were at least probably protected by a water column from disturbances occurring at the surface. The rather simple character of sponges, which fed on colloidal matter and bacteria, may have also played a role, for they were less influenced by supposed disturbances of the food chain during the K/T event (whatever its cause). On the other hand, it is difficult even to speculate at the moment, upon what differences, other than chance, caused different behaviour of particular groups of sponges (like Hexactinosa and Lychniscosa) in relation to K/T boundary event.

Large sponge faunas. So far I have been concentrated on the stratigraphic ranges of particular groups or lineages of sponges, but there are some interesting patterns in distribution of large sponge faunas as such. In this context large sponge faunas refer to faunas that are diverse, have wide geographical distribution, and in which sponges occur in profusion, where

sponges are usually a rock forming element. During the Late Jurassic, for example, they formed biostromal or reefal structures, and the sponge facies extends across the whole Europe from Portugal to Romania (Trammer, 1982, 1991; Leinfelder et al., 1994; Krautter, 1997; Pisera, 1997, and literature therein).

Distribution of large faunas of bodily preserved (intact) fossil sponges during the Meso-Cenozoic is rather punctuated and limited to certain periods of time. The largest such faunas known for a long time are associated with the Upper Jurassic, Upper Cretaceous, and the Miocene rocks (Fig. 4). Because of large gaps separating these faunas, they appear at the genus level to be composed of very different taxa, which makes interpretation of evolution of these sponges difficult. Such a pattern of distribution has been interpreted, at least for lithistids, by Rigby (1983) as the result of "selective preservation and discovery, not one of original limited diversity and density". This interpretation has partly found support in more recent important discoveries from the Late Triassic (China - Wendt et al., 1989; Wu Xi Chun, 1990), Middle Jurassic (Spain - Scheer, 1988; Hungary - Pisera, 1993; India - Mehl & Fürsich, 1997), Eocene (Spain - Busquets et al., 1997) and Miocene (Spain - Brimaud & Vachard, 1986a, b) and a smaller one from the Oligocene (Antigua - Wiedenmayer, 1994; Ukraine - Pisera, unpublished). Generally, however, if we look at the distribution of large sponge faunas, the pattern of punctuated record is preserved. The largest are of Late Jurassic and Late Cretaceous age (across the whole of Europe); smaller ones occur in the Upper Triassic (The Alps, Sichuan), Middle Jurassic (Spain, France, Hungary, Kutch in India), Eocene (Spain, Italy, Turkey) and the Miocene (Algeria, Spain, Italy). When one compares all these occurrences with the sea level history (Fig. 4), a clear correlation appears: the occurrence of the large siliceous sponge faunas is correlated with the high sea level times during the Mesozoic and Tertiary (and it seems that this pattern is valid also for the Paleozoic in the case of Hexactinosa in the Frasnian/Famenian). It points to the importance of sea level in controlling distribution of large sponge faunas. This relationship, however, is mostly of environmental character, although some evolution, took place especially at the species level. The sponges considered here are deep-water creatures and their widespread development may be interpreted as a possibility to colonise new, vast, relatively deep-water areas. These areas were not available during lower sea level periods, and when sponges of these groups existed only in relatively narrow refugia along continental and island slopes, as it is in many cases today. Such new areas of relatively deep-water were at a distance from shore, had low sedimentation rates and low hydrodynamic energy, and thus were suitable for sponge colonisation.

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Note added in proof.

Eocene sponge faunas should be supplemented with a lithistid fauna from the southern Western Australia (Pickett, 1983, and references therein), of which I was earlier unaware. After preliminary examination of a new collection of sponges from this region (thanks to Dr. P. Gammon, Canada) it seems to be the largest, the most diversified, and the best preserved lithistid fauna of the Tertiary.

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LITHISTID SPONGE *SETIDIUM OBTECTUM* SCHMIDT, 1879, REDISCOVERED

ANDRZEJ PISERA

Pisera, A. 1999 06 30: Lithistid sponge *Setidium obtectum* Schmidt, 1879, rediscovered. *Memoirs of the Queensland Museum* **44**: 473-477. Brisbane. ISSN 0079-8835.

The poorly known genus and species *Setidium obtectum* Schmidt, 1879 was revised based on the holotype and newly collected material from the Caribbean, and referred to the family Scleritodermidae Sollas. ❐ *Porifera, Rhizomorine lithistids, Setidium, systematic position.*

Andrzej Pisera (email: apis@twarda.pan.pl), Instytut Paleobiologii PAN, ul. Twarda 51/55, 00-818 Warszawa, Poland; 6 January 1999.

The rhizomorine lithistid genus and species *Setidium obtectum* was established by Schmidt (1879) based on a unique specimen dredged off Havana from 234-439m depth. The occurrence of remarkable, numerous bundles of very long oxeas protruding from the inner (upper) surface of the sponge confers upon it a very characteristic form. As a result of its incomplete description and single previous record its systematic position remained obscure. Recently, new rich material of this sponge was discovered in the collections of the Marine Invertebrates Museum, University of Miami, Florida (MIM-RSMAS), dredged from several localities in the Caribbean (Fig. 1). Investigation of this new material and re-examination of the holotype in Schmidt's collection at the Museum of Comparative Zoology, Harvard University (MCZ), permitted determination of its characteristics and establishment of its taxonomic position.

SYSTEMATICS

SYSTEMATIC POSITION. Lendenfeld (1903) regarded *Setidium* a synonym of *Leiodermatium* Schmidt, and placed it within Leiodermatidae Lendenfeld, which encompasses rhizomorine lithistids without microscleres. Van Soest & Stentoft (1988) placed *Setidium obtectum* among rhizomorine lithistids of the family Siphonidiidae Sollas, which is characterised by an ectosomal skeleton composed of desmas without zygosis, and absence of microscleres as well.

The present investigation found that choanosomal desmas of *Setidium obtectum* are typical thorny rhizoclones, and ectosomal spicules are mostly amphioxeas that form a dense tangential layer, showing all transitional forms to rhizoclones. Numerous thorny sigmaspire microscleres were found both in the holotype and the new material. As a result, *Setidium obtectum* Schmidt, 1879, should be placed in Scleritodermidae Sollas, 1888. The presence of sigmaspires, rhizoclone type, as well as the ectosomal skeleton of amphioxeas, make this genus very close to the genus *Scleritoderma* Schmidt, 1879.

Family **Scleritodermidae** Sollas, 1888
Setidium Schmidt, 1879

DIAGNOSIS. Rhizomorine sponges bearing on the surface (mostly the upper one) bundles of long oxeas protruding from the choanosome; ectosomal spicules are amphioxeas that show a complete transition to rhizoclone desmas. Sigmaspire microscleres concentrated around oscules. Choanosomal skeleton very dense, confused and composed of strongly "thorned" rhizoclones.

REMARKS. This is so far a monotypic genus. It is close to *Scleritoderma*, but differs in having smooth ectosomal spicules that are amphioxeas and their derivatives while *Scleritoderma* has acanthose microstrongyles (Van Soest & Stentoft, 1988).

Setidium obtectum Schmidt, 1879
(Figs 2-4)

Setidium obtectum Schmidt, 1879: 30-31, Pl. 1, fig. 9, Pl. 2, fig. 14; Lendenfeld, 1903: 145-148; Van Soest & Stentoft, 1988: 74.

MATERIAL. HOLOTYPE MCZ6462: off Havana, 234-441m depth, collected Blake Expedition. MIM-RSMAS G688: off Miami, 26°53'N, 78°16'W, 492m depth (two specimens); G1312: off Miami, 26°38'N, 79°02'W, 516m depth (one deciduous specimen); P1141: off Great Inagua, 20°51'N, 73°16'W, 429m depth (4 specimens).

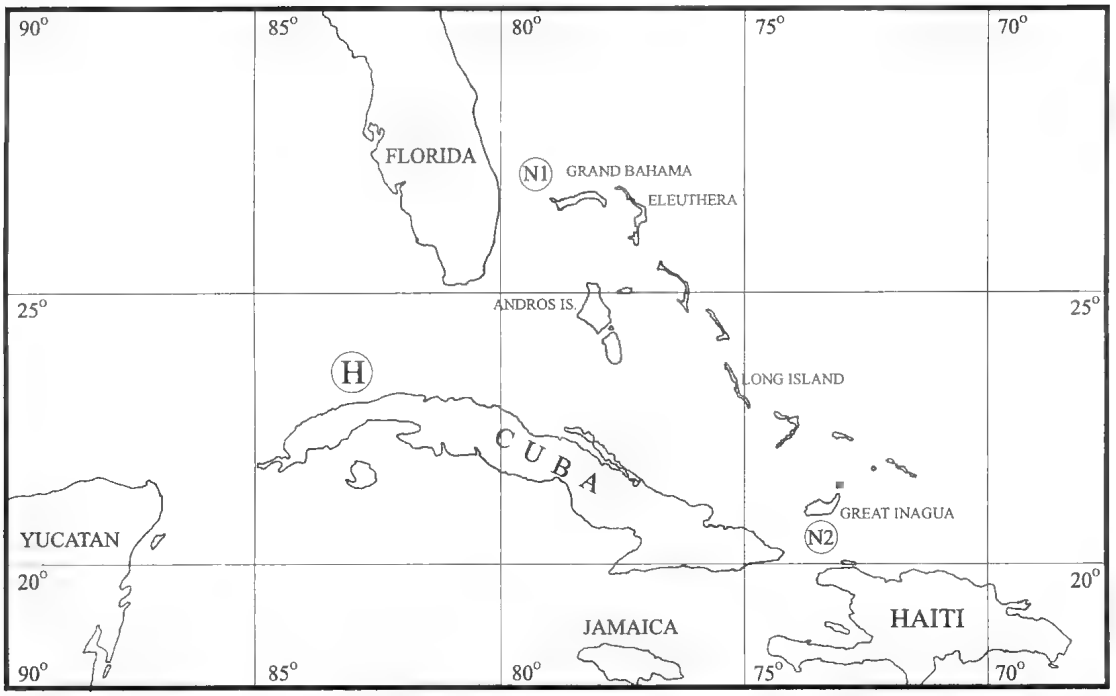

FIG. 1. Distribution of the known specimens of *Setidium obtectum* Schmidt. H=holotype, N1, N2=new material.

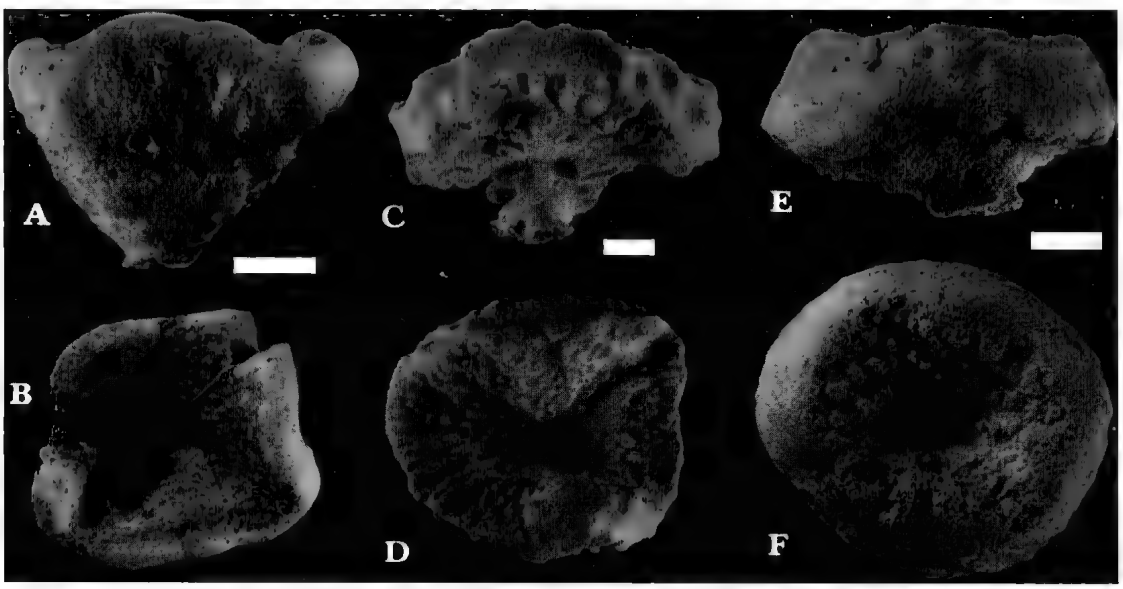

FIG. 2. Morphology of *Setidium obtectum* Schmidt (scale bars 1cm). A-B, Holotype, lateral and upper side views, MCZ 6462, off Havana. C-D, Large deciduous specimen, lateral and upper side views, G1312, off Miami. E-F, Living specimen with ectosomal spicules, lateral and upper side views, P1141/2, off Great Inagua.

DESCRIPTION. *Shape and structure of the skeleton*. The holotype is irregular vase-shaped (or turbinate - see Boury-Esnault & Rützler, 1997) about 5cm wide, 3cm high, with a wall about 1cm thick and rounded margin. The base shows a very short peduncle. The new specimens

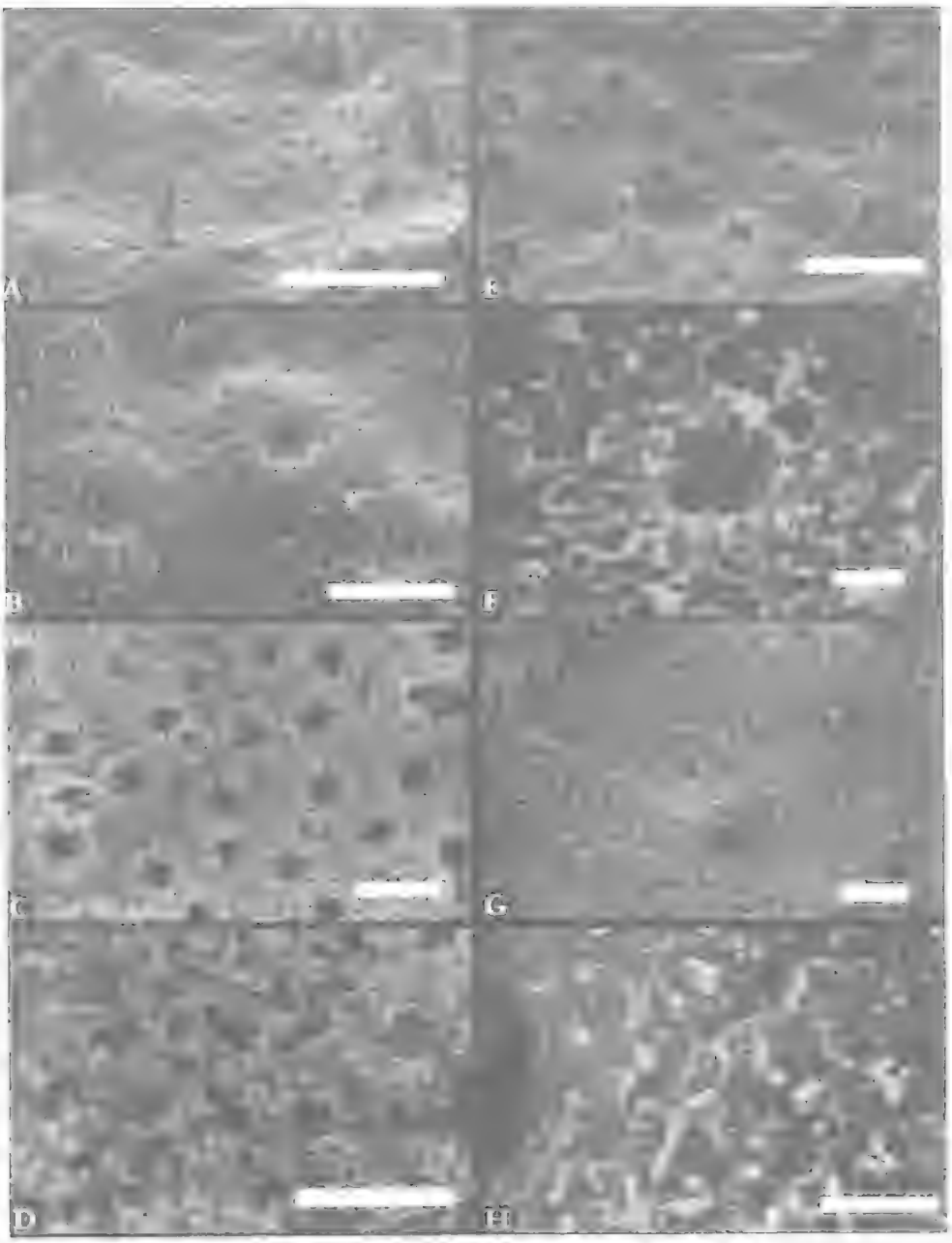

FIG. 3. *Setidium obtectum* Schmidt. A, Upper surface, bundles of oxeas protruding from the choanosome, ?oscula and ectosomal spiculation, P1141/1, off Great Inagua (scale bar 2mm). B, Details of upper surface showing ?oscules and tangentially arranged ectosomal spicules, P1141/1, off Great Inagua (scale bar 500µm). C, Upper surface of the choanosomal skeleton (HNO_3 preparation) showing ?oscula and bundles of oxeas (broken) protruding from choanosomal skeleton, P1141 1, off Great Inagua (scale bar 1mm). D. Rhizoclones on the upper surface of choanosomal skeleton (HNO_3 preparation), note partly incorporated young desma, P1141/1, off Great Inagua (scale bar 200µm). E, Lower (outer) surface showing ostia protected by a tepee-like organised oxeas, P1141/1, off Great Inagua (scale bar 1mm). F, Close-up of ?osculum with numerous sigmaspires, P1141 1, off Great Inagua (scale bar 20µm). G, Upper surface showing ?osculum and ectosomal spicules, holotype, MCZ 6462, off Havana (scale bar 200µm). H, Close-up of ?osculum with sigmaspires, the holotype, MCZ 6462, off Havana (scale bar 20µm).

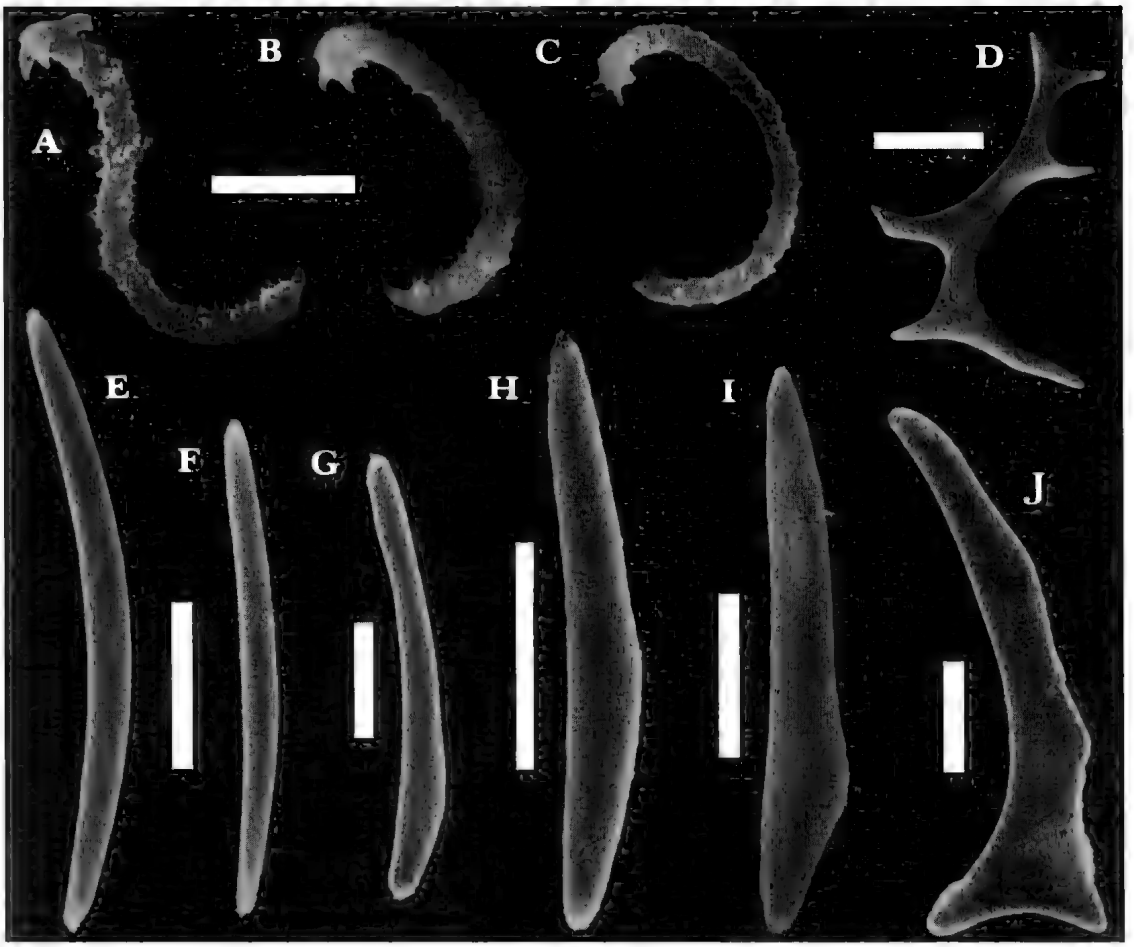

FIG. 4. *Setidium obtectum* Schmidt, P1141/1, off Great Inagua. A-C, Sigmaspires (scale bar 5μm). D, Young desma (scale bar 100μm). E-J, ectosomal spicules showing transition to desma (scale bars 50μm).

range in shape from deep vase or turbinate (especially when young), to shallow widely open vase-shaped with very thick walls, about 1-1.5cm thick, and with a short massive peduncle. The margin of the vase is more-or-less square (rounded in the holotype) and its surface is nearly smooth or bearing low, wide elevations from which protrude bundles of long oxeas (now all broken). Both surfaces are rugose as a result of numerous irregularly to slightly radially distributed conules, 2-5mm high, from which protrude bundles of long oxeas, up to 20 spicules each, that are at least 20mm long (now all broken). Because the conules are slightly oblique to the surface, especially in old individuals, they may form kind of short, radially aligned ridges.

Except for the tips of conules both surfaces are covered with small openings - presumably ostia and oscules. These are less densely distributed on the upper surface and are located in shallow depressions, sometimes surrounded by a slightly rised rim of ectosomal spicules. On the lower surface ostia are very densely distributed, practically touching each other, and placed on tops of small elevations; these elevations are not visible on the surface of choanosomal skeleton and thus they are probably formed only by ectosomal spiculation.

Ostia are surrounded and covered by a tepee-like structure formed by delicate oxeas up to 0.5mm long (length of visible part only) protruding from the surface. These openings on the upper surface measure 160-220μm (when ectosomal spicules are present) and corresponding canal openings in the choanosomal skeleton are 230-300μm. Those on

the lower surface are slightly larger and measure 200-260µm (when ectosomal spiculation preserved) and corresponding canal openings in the choanosomal skeleton are 230-280µm. Ectosomal spicules are tangentially arranged amphioxeas. No other structures are present on the surface of the choanosomal skeleton beyond these canal openings that lead to ostia and oscula. On the surface of the margin slightly sinuous and partly open canals, 0.20-0.25mm wide, run close to the surface of the skeleton from the lower to the upper surface of the sponge. Similar canals pierce the wall in cross section. Close to the outer (lower) surface of the choanosomal skeleton there are numerous large connected lacunes 0.4-0.5mm diameter. In cross section are also visible bundles of oxeas that protrude from the conules on the surface and enter deeply into the choanosomal skeleton.

Colour. White or yellow when dry or in alcohol, white when devoid of ectosomal spicules.

Desmas. These are strongly branched and thorny rhizoclones measuring 200-400µm long; rhizoclones around canals are only tuberculated on the canal side.

Other megascleres. Large oxeas protrude from conules on both surfaces (but are more developed on the upper one) and deep in the choanosome. They are invariably broken so total length is unknown but they are at least 1cm long and 15-23µm thick. Ectosomal spicules are tangentially arranged, smooth, regular amphioxeas that are 120-210µm long and 10-14µm thick. They show all transitions to desmas. Derivatives with shapes modified toward rhizoclone desmas may be 270µm long and 40µm thick or more. Ectosomal spicules may be concentrically arranged around oscula.

Small oxeas protect ostia on the lower surface forming tepee-like structures around the ostia. These spicules are usually broken but they appear to be 300-500µm long and 3.5-5µm diameter.

Microscleres. Thorny sigmaspires measure 8-13µm long and up to 1.7µm thick.

ACKNOWLEDGEMENTS

The material was kindly loaned for study by Prof. Nancy A. Voss (Marine Invertebrates Museum, RSMAS, University of Miami, Miami, Florida). All SEM photos were made using a Philips XL-20 scanning microscope at the Institute of Paleobiology, Polish Academy of Sciences. Special thanks are given to Ardis Johnston (Museum of Comparative Zoology, Harvard University) for loan of Schmidt's material.

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AN UNUSUAL SUBERITID DEMOSPONGE FROM A MARINE ALKALINE CRATER LAKE (SATONDA ISLAND, INDONESIA). *Memoirs of the Queensland Museum 44: 477-478. 1999*:- To date, the Satonda crater lake, Indonesia, is the only 'marine' lake known to have an increased alkalinity compared to seawater (Kempe & Kazmierczak, 1990, 1993; Kempe et al., 1997). The lake was originally filled with freshwater, as evidenced by the presence of fossil peat deposits (3,150 ^{14}C-yrs BP). Later, the lake was rapidly filled with seawater, as indicated by the settlement of a marine fauna. Today the lake is divided into three water bodies with differing salinities, separated by two pycnoclines (Kempe & Kazmierczak, 1990, 1993; Kempe et al., 1997). Both bottom water layers are anaerobic due to intensive oxygen consuming bacterial decomposition processes, linked to the large input of organic matter from the surrounding vegetation. As a result, an intense sulfate reduction occurs in both bottom water bodies, producing high amounts of bicarbonate ions. As a result of seasonal mixings events, waters from the upper layers of these high-alkalinity bottom waters are transferred to the well oxygenated mixolimnion, causing a slight rise in alkalinity to 4-5 meq/l in the brackish (32 ‰ salinity) surface waters ('alkalinity pump'; Kempe, 1990; Kempe & Kazmierczak, 1993; Kempe et al., 1997). The pH values of mixolimnion waters range between 8.3-8.6. As a consequence of raised carbonate alkalinities, the lake generally contains a decreased amount of Ca^{2+}.

(Cont. over)

The red-algal-microbialite reefs exhibit a vertical development which started with a serpulid framework, followed by microstromatolites encrusting former green algal filaments, and loose crusts largely composed of the aragonitic squamariacean red alga *Peyssonnelia* (Kempe & Kazmierczak, 1990, 1993; Kempe et al., 1997). The uppermost calcareous crust is formed by a framework of *Lithoporella*, *Peyssonnelia* and intercalated micrite layers of presumed microbial origin. The living reef community is located on top of this layer. The special hydrochemical situation might be responsible for the very specific and endemic development of the biota. Cyanobacteria and heterotrophic microbes exhibit large diversities in contrast to just one sponge taxon (*Laxosuberites* n. sp.). Common are cyanobacteria of the morphological taxa *Phormidium*, *Calothrix*, *Pleurocapsa*, *Hyella*, and *Spirulina*, in addition to unidentified taxa (Arp et al., 1996)

The steep slopes of the red algae reefs are entirely covered by a dense curtain of *Cladophora* tufts extending to 15-16m depth. Sponges grow underneath this curtain, where light regimes are between 200-300lux. In 1993, the depth limit of sponges was recorded at 20m, i.e. slightly above the upper pycnocline. The sponge fauna is represented by different morphotypes of the hadromerid taxon *Suberites*, which is characterized by tylostyle megascleres only. The dermal layer of the sponge is constructed of plumose bundles of short tylostyles (150-200µm long), the choanosomal spicules are randomly orientated and much larger than the dermal ones (300-500µm). Most of the sponges observed exhibit a lateral, encrusting growth habit and therefore show a well developed exhalant canal system. The exhalant system is differentiated into star-shaped units ('astrorhizae'-pattern). In each unit the main exhalant canals conjugate in one large osculum. A second sponge morphotype exhibits a more-or-less erect growth habit and does not show any star-shaped outer exhalant system. This sponge forms tubes with a central osculum, reminiscent of *Polymastia* and which is phylogenetically closely related to *Suberites*. The new species is referred to *Laxosuberites*, based on its spicule inventory, geometry and arrangement. This species shows many color variations: from dark green, brown, yellow/brown to yellow. Different colors are related to microorganisms within the soft tissue. The dark green color is restricted to specimens in extremely shallow water (20-50cm depth), produced by living unicellular green algae enclosed within the mesohyle of the sponge. These algae are part of the plankton and filtered by the sponge. The brownish color variation is restricted to few specimens from deeper water (18-20m depth), with coloration related to the presence of large populations of a still-unidentified mesohyle bacterium. The presumably symbiotic, native bacterial flora of the sponge is rare and very small (less then 1µm – 'nanobacteria'). The size and abundance of these bacteria are comparable to those observed in the marine hadromerid coralline sponge *Acanthochaetetes*. In many cases the encrusting sponges form very thin films (ca. 50µm thick), growing within interspaces of dead red algal knobbs. The sponges occupy large spaces between the dead portions of the algal reef surface. Apparently, they prefer light protected areas, except for the algaebearing specimens.

Theoretically these sponges are particle feeders. Within vacuoles of archaeocytes, for example, the remains of diatoms were observed. However, the sponges also have abundant ostia in their basopinacoderm, that is, in all observed cases, growing on active heterotrophic biofilms. This may suggest a close relationship between the biofilms and the sponge. We assume that the biofilms release metabolic products consumed by the sponge. This behavior may also explain the enormous lateral growth of thin sponge sheets. Of further significance is the ability of these sponges to build resting bodies, which are located in small protected cryptic niches between coralline algae or in small caverns 200-500µm diameter. The resting bodies are hemispherical or sack-shaped, and filled with archaeocytes/ thesocytes.The sponge fauna seems perfectly adapted to this extreme environment. Pending additional ultrastructural studies, we assume that these sponges are new and restricted to this special environment. ❐ *Porifera, suberitids, alkaline crater lake, Indonesia.*

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INNOVATIVE NEW METHODS FOR MEASURING THE NATURAL DYNAMICS OF SOME STRUCTURALLY DOMINANT TROPICAL SPONGES AND OTHER SESSILE FAUNA

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Pitcher, C.R., Wassenberg, T.J., Smith, G.P., Cappo, M., Hooper, J.N.A. & Doherty, P.J. 1999 06 30: Innovative new methods for measuring the natural dynamics of some structurally dominant tropical sponges and other sessile fauna. *Memoirs of the Queensland Museum* **44**: 479-484. Brisbane. ISSN 0079-8835.

Population dynamics (growth, mortality, recruitment, and reproduction) of large sessile fauna (including sponges, gorgonians, and corals), that dominate and provide structure on patches of seabed habitat in open shelf waters, 20-50m depth, is currently under study at several sites in the Great Barrier Reef region. Sites, chosen to contain representative benthos habitat fauna, are being surveyed using an ROV to document dynamics, including: mapping the large sessile fauna at each site; tagging a size range of individuals of each of several dominant species; measuring growth and mortality rates through time; observing the occurrence of new small individuals for measurements of recruitment; taking samples to confirm taxonomy and for histological examination in the laboratory to determine reproductive strategies. By tagging a full size-range of individuals of each study species, we aim to estimate lifetime growth curves in three years. From these data we will construct models of the population dynamics of sessile fauna, which can be used to estimate how fast the seabed habitat might recover in new reserve areas. This study will also document the usage of living seabed habitat by key fish species. ❐ *Porifera, sessile fauna, dynamics, growth, mortality, recruitment, ROV, video, tagging.*

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Communities of large sessile epibenthic fauna (such as sponges, gorgonians, alcyonarians and corals), provide structural complexity to the seabed - an important component of habitat for a myriad of other species - and also contribute to the biodiversity of the marine environment (Van Dolah et al., 1987; Hutchings, 1990; Pitcher, 1997). Further, they form the basis of 'bioprospecting' to discover natural products of pharmaceutical promise (Hooper et al., 1998). However, megabenthos communities are vulnerable to damage by sedimentation, dredging and extensive trawling on the seabed (Pitcher et al., 1997; Sainsbury et al., 1997). A recently completed project (Poiner et al., 1998) has demonstrated the significance of impacts of prawn trawling on tropical seabed habitats. One method of managing these impacts for ecological sustainability will be spatial closures (Sainsbury et al., 1997), e.g. establishing refuge areas to preserve representative seabed habitats. Predicting the response of megabenthos to the establishment of refuge areas, and acquiring an understanding of the ecological interactions between trawled and refuge areas, are both essential steps in the design of effective refuges for fisheries habitat and the stocks and biodiversity they support. To achieve these goals, it is first necessary to obtain information on the recovery rates of habitat and the processes that link trawled areas and refuges.

Estimation of recovery rates requires information on population dynamics, but these are virtually unknown for large sessile epibenthic fauna (Hutchings, 1990). We are investigating the fundamental population dynamics (recruitment, growth, mortality, reproduction) of structurally dominant megabenthos habitat fauna and documenting the relationship between benthic habitat and ecological usage by some commercially important finfish species.

MATERIALS AND METHODS

STUDY AREA. Seabed areas located in the Great Barrier Reef off Townsville, Australia, were surveyed for suitable study sites in August 1997, during a voyage of the AIMS vessel RV 'Lady Basten'. Towed video cameras were used in grided surveys of four areas, each about 4,000km^2, in the main lagoon and among the mid-shelf reef matrix.

Four sites were chosen in an inshore area around the Palm Islands (18.7°S, 146.5°E), in depths ranging from 20-30 m. Another four sites were chosen in a mid-shelf area in the vicinity of the Slashers Reefs (18.5°S, 147.1°E), in depths ranging from 30-50 m. There is evidence that inshore areas have higher productivity of food organisms for filter feeders, than offshore areas (Adele Pile, pers. comm.) – consequently dynamics can be expected to differ between these sites. Each site was chosen to contain benthos habitat with species representative of those found on the types of seabed that are trawled for prawns or fin-fishes.

DEMOGRAPHIC MEASUREMENTS OF MEGABENTHOS. The population dynamics of sessile fauna that provide structural habitat is being documented by mapping the dominant fauna at each site; tagging several dominant species of sponges, gorgonians, and alcyonarians to identify individuals; making video measurements of individual growth and mortality rates through time; observing the occurrence of new small individuals in quadrats for measurements of recruitment; taking samples to confirm taxonomy; and histological examination in the laboratory to determine reproductive strategies.

At each site, a 4×3m quadrat was established to measure recruitment. Initially, all individuals of all species of sessile fauna within each quadrat were tagged so that the appearance of new individuals can be detected, mapped and tagged. Typically, 10-20 individuals were present and tagged in the quadrats. The settlement of any new individuals on 0.25m^2 concrete marker blocks placed at one corner of each quadrat will also be recorded.

To estimate lifetime growth curves in three years, we tagged across the full size-range of individuals of several species common in the area, and for the next three years we will measure tagged individuals every six months. The dominant species included sponges (*Yestospongia, Ianthella, Cymbastela, Ircinia*), gorgonians (*Ctenocella, Subergorgia, Semperina, Menella, Junceella, Muricella, Echinogorgia*) and the hard coral *Turbinaria*. We attempted to cover the size spectrum available at each site by tagging 3-5 individuals, varying in size from small to large, of each species. The absolute size range depended on the species. After tagging individuals in the recruitment quadrat, the size spectrum was completed by choosing animals within a 20-30m radius of the quadrat. Typically 35-50 individuals were tagged at each site. Large and/or old sessile fauna may grow very slowly and, in a three-year study, their growth may not be measured as precisely as small or young fauna. To counter this, benthos are being measured as accurately as possible, using laser scaling equipment and video image capture and analysis techniques (see below).

The latitude/longitude position on the seabed of each tagged individual was recorded with an underwater tracking system and differential GPS. The positions of tagged individuals were mapped so they could be found easily on subsequent

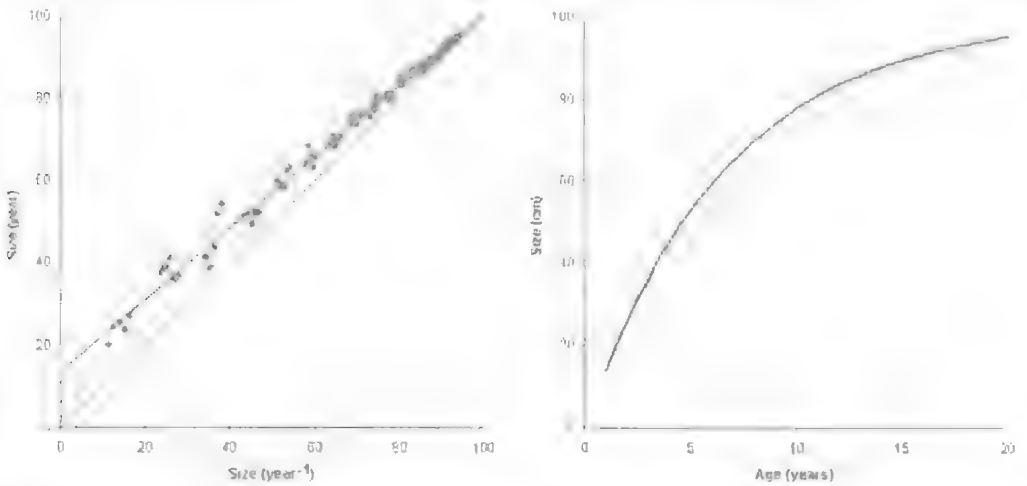

FIG. 1. A, Example of a Ford-Walford regression plot of size (yr^{-1}) vs. size (yr) to estimate the parameters growth rate K (from slope=e^{-K}) and asymptotic size L_∞ (from intersection of regression with Y=X) for a hypothetical species of sessile benthic fauna. B, The corresponding estimated von Bertalanffy growth curve for the species that may reach a size of 100cm after more than 20 years.

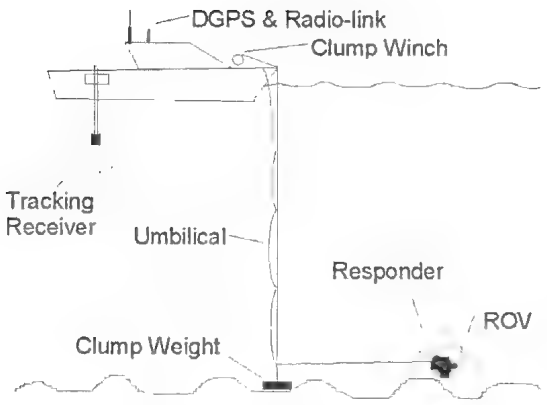

FIG. 2. Schematic diagram showing the method of anchoring a 20 m vessel over the study sites and deployment of the ROV on nylon rope.

occasions, for measurement. Growth of tagged individuals will be estimated by measuring increments in linear and/or areal dimensions seasonally. Growth curves of the von Bertalanffy form will be parameterised by statistical analysis of Ford-Walford Plots (e.g. Fig. 1; Gulland, 1983). Mortality will be estimated by the disappearance of tagged individuals. Mortality, when not directly observed from skeletal or decayed remains, can be separated from tag loss by cross-checking any apparent losses with accurate position information and the photographic record.

Every six months, separate specimens of the same suite of species are being collected for histological studies of reproductive condition. The taxonomy, identification and reproductive studies of the sessile fauna are being undertaken at the Queensland Museum. We have concentrated on relatively few species of structurally dominant fauna and, even if we cannot assign scientific names to each, we will be able to separate different species and determine which different forms belong to the same species.

LOGISTICS OF RESEARCH. Tagging in the marine environment is typically troubled by fouling and grazing by fish, which lead to difficulties with tag reading and tag loss and associated ambiguities. To minimise these problems and facilitate identification, the tags used in the study were radio-frequency identification tags in 23×4mm glass capsule form (Texas Instruments RI-TRP-RRHP), that were read automatically by an induction transceiver (Texas Instruments TIRIS Series 2000 module) mounted in an underwater housing. The tags were attached to sessile epifauna by cable-ties, or inserted into sponges with a large needle, or moulded into epoxy pucks placed at the base of the target animal.

A small remotely operated vehicle (ROV — Hydrovision 'Offshore HYBALL') is being used to conduct most of the underwater tasks. SCUBA divers assist to a maximum depth of 30 m, by setting up quadrats and tagging benthos in shallow sites. The ROV conducted these tasks on deeper sites, where it can operate for virtually unlimited periods. An acoustic underwater tracking system and differential GPS navigation enables accurate (±1m) latitude/longitude position fixing and location of tagged fauna for measurement at each sampling time. The ROV telemetry link also allows data such as tag numbers to be automatically acquired in real time, displayed, logged to database along with corresponding position, video frame numbers and captured image filenames. A pair of parallel lasers fitted in the ROV provide a 100mm scale on the video images of megabenthos for measurements. A manipulator on the ROV is used to apply tags and take samples. Deployment of the ROV involves anchoring the vessel precisely over the study sites with a 800kg weight as an anchor on a 25mm plaited nylon rope that can absorb up to 30% rise and fall of the vessel on the waves (Fig. 2). The ROV umbilical is clipped onto the rope to minimize the drag due to currents. This method is simple and effectively enables repeated, accurate positioning of the vessel over the study sites.

Integrated data acquisition, storage and retrieval are central to the logistics of the field operations and analysis. Custom software controls this integration of data for vessel position and orientation, ROV tracking, video frame, and tag numbers (Fig. 3). It also provides a navigation system that gives accurate coordinate positions of the vehicle, which are overlaid on the video tape record and displayed as an ROV track on a plotter window. The positions of tagged fauna are shown as waypoints to facilitate their location (Fig. 4). When a tagged animal is detected, previous images of that subject are displayed for confirmation and to enable the same image orientation and perspective to be captured.

The laser scaled images of fauna recorded from the ROV's video camera are captured live or from tape. The lasers are calibrated by projecting onto scaled grids to check accuracy and precision of measurements of size through time. Image analyses are achieved efficiently by using a custom software application to control, link and synchronise the field

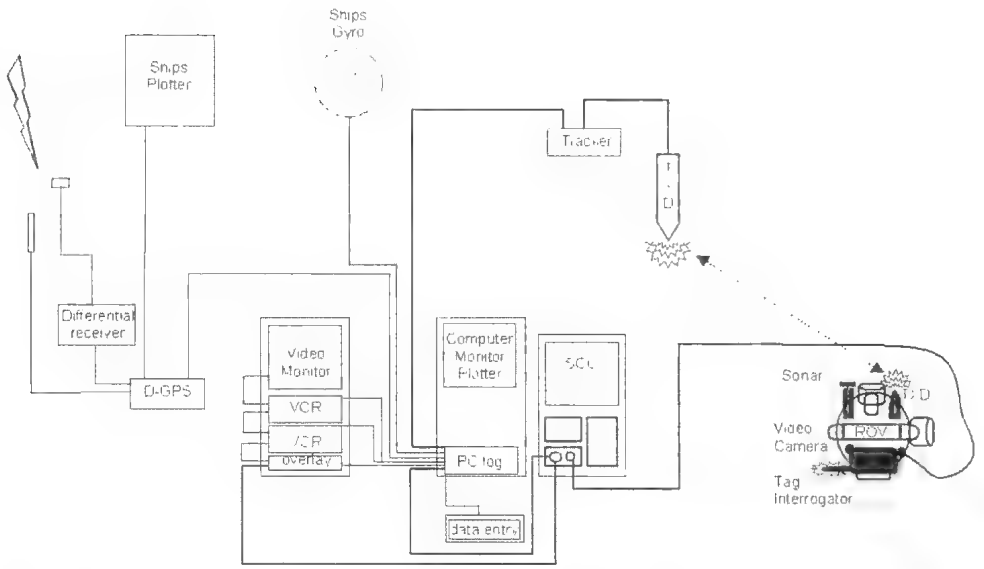

FIG. 3. System diagram showing integration of components necessary for automated tracking of the ROV and synchronous logging of position, tracking, tag numbers and video data, to facilitate post-analysis and measurement of sessile benthic fauna. DGPS: differential global positioning system, VCR: video recorder, PC: logging computer, SCU: surface control unit for ROV, TXD: tracking system transducers.

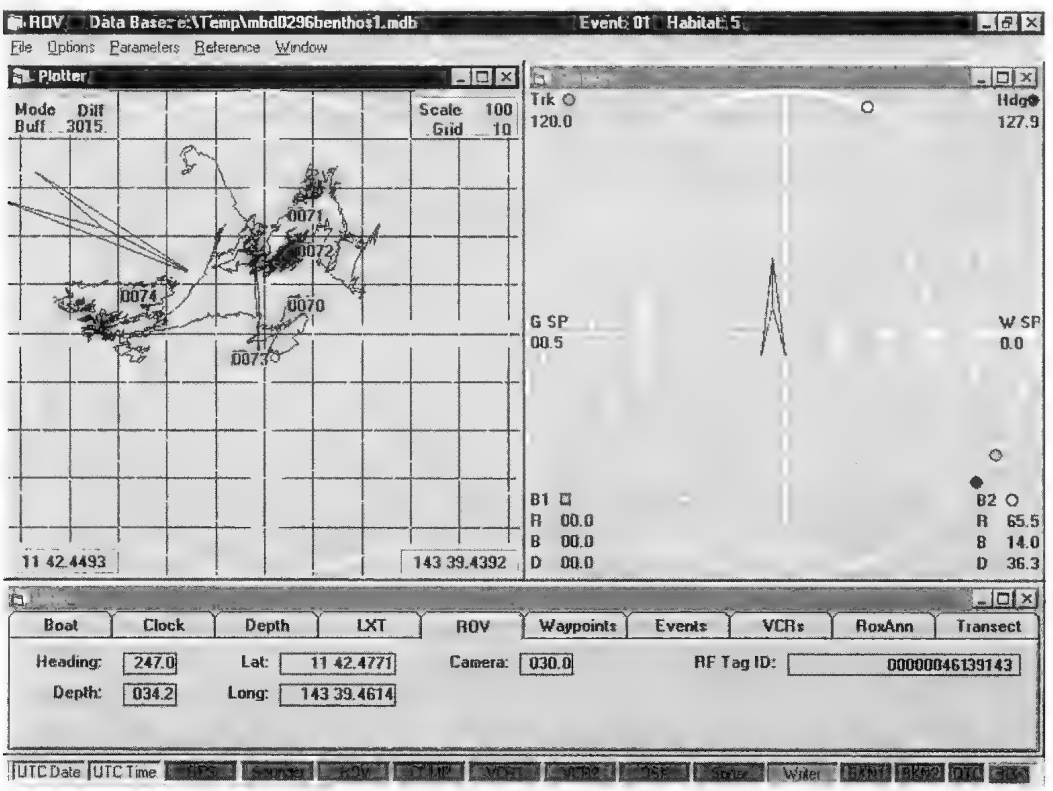

FIG. 4. User interface of the custom acquisition, tracking and logging software, showing vessel-relative ROV tracker (right window) and ROV navigation plotter (left window) with waypoints (e.g. 0070 to 0074), ROV track (irregular black line), vessel position (arrowhead), ROV position (pale circle off starboard bow near 0073). The lower window shows data acquired and status.

databases (of tracking, positioning, and tag numbers) with the video images and execute macros on the Optimas® image analysis software. An operator digitises the lines for the laser points (100 mm scale), height, width, and area of profile as appropriate for the growth form (Fig. 5) and chooses species and condition information from a select list. The software transfers the measurement data to a database along with the image filename and corresponding field data. This provides a semi-automated method for extracting the required quantitative data in the form of date/time, site, tag-number, species, position, morphometrics and condition.

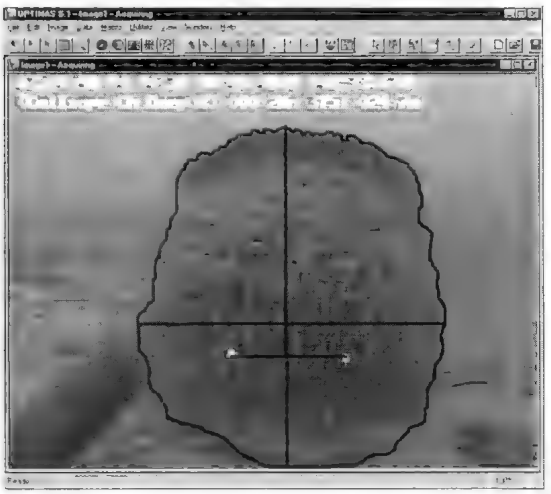

FIG. 5. Captured image of a computer screen showing results of the macro run on Optimas by the custom control software, to measure benthic fauna – *Xestospongia testudinaria* in this example. The tag interrogator antenna is visible in the lower left of the image.

RESULTS

The first tagging fieldwork was conducted in March 1998. Eleven of the most abundant species were targeted for tagging and a total of 174 sessile fauna were successfully tagged, including 26 putative species. The available size-spectrum of each species was successfully covered in most cases (Table 1). Specimens of these species were collected for taxonomic confirmation.

The second tagging field trip is currently underway (December 1998). In the four study sites around the Palm Islands, 95% of tagged individuals have been re-measured, 9 individuals were not re-located, 5 incidences of mortality were confirmed, and 13 new recruits were observed in the quadrats.

To date, results have demonstrated that the logistics of the project are working as planned. Tagged individuals can be re-located successfully, tags can be re-interrogated and cross-referenced in the database, captured images can be measured with mm accuracy, recruitment can be observed and mortalities confirmed. The methodological protocols that have been established for ROV deployment and for benthos measurement will be used for future repeated visits to individual tagged fauna to provide a consistent series of measurements.

TABLE 1. List of species targeted for tagging during fieldwork in March 1998, with statistics of each species for height (cm) of tagged specimens, count of numbers tagged by size-categories in classes of one standard deviation (sd) unit relative to the mean, and total count.

Species	Min Height	Mean Height	Max Height	Small <-1 sd	Med-small -1<sd>0	Med-large 0<sd>1	Large >1 sd	Total Count
Ctenocella pectinata	10.6	34.1	69.9	4	6	8	4	22
Xestospongia testudinaria	9.8	23.2	42.1	3	5	6	4	18
Menella	5.0	18.0	28.9	2	3	10	1	16
Cymbastela concentrica	9.4	21.6	43.5	3	4	4	2	13
Subergorgia reticulata	14.6	43.9	108.1	2	4	4	3	13
Turbinaria	2.0	24.4	62.0	1	5	6	1	13
Semperina brunei	10.0	34.6	53.3	1	4	7	1	13
Ianthella basta	16.8	31.0	46.7	4	1	4	2	11
Junceella divergens	9.0	27.5	41.2	2	2	7	0	11
Muricella	13.1	25.9	40.7	1	4	4	2	11
Echinogorgia	20.4	33.6	45.8	1	2	2	1	6
Others								27

DISCUSSION

Our study has developed techniques for *in situ* investigations of the types of large sessile fauna that provide structural habitat in deeper areas, where access by divers is limited or impossible using conventional breathing equipment. These methods open the opportunity for understanding the poorly known

ecology of these faunas, and will lead to greater appreciation of their role and importance. The study will also document usage of living habitat by key fin-fish species, in terms of species micro-distribution, shelter requirements, and food chain links.

The growth, mortality and recruitment rates estimated by this study will be used for developing population dynamics models of the large sessile fauna. The structure of these models will be of a size-based matrix form (e.g. Hughes, 1984). Basically, for each species, the models will have several size categories, the number of which will depend on life history characteristics; recruitment to the smallest size-category will be the probability of settlement of larvae; each larger category will receive recruitment due to growth from lower categories; and individuals within each size category will have a probability of dying or growing to the next size category (the possibility of negative growth will also be included if required. Other factors to be included in the models include the possible effects of density of the same and other benthos taxa, and the reproductive potential and proximity of source populations on supply of larvae.

Results of this study can be used to examine a number of issues, including: the establishment of refuge areas on the seabed, trawling strategies, habitat restoration, stakeholder conflicts, and conservation. These issues revolve around the impact of trawling on seabed habitat and associated stocks, and the rate of recovery of habitat if areas were reserved. In particular, models will contribute to the development of management strategies to improve the environmental sustainability of trawling, by simulating the interaction of the dynamics of habitat fauna with the dynamics of trawl impacts and estimating levels of trawl effort that do not cause continuing degradation.

Information of this kind will become increasingly important as the requirement for ecologically sustainable fisheries management is implemented in trawl fisheries from the temperate zone to the tropics. The lessons learned from this study in the form of knowledge of habitat dynamics, and methods for monitoring habitats and the commercial stocks they support, will contribute to a rational balance between ecologically sustainable fishing and biodiversity conservation when ESD related management objectives are implemented in those Australian fisheries dependent on seabed habitat.

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SPONGE FARMING IN THE MEDITERRANEAN SEA: NEW PERSPECTIVES

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Some Mediterranean species of *Spongia* and *Hippospongia* are characterised by a soft and absorbent skeleton and usually harvested for commercial purposes. Recently, the synergetic effect of a widespread epidemic, together with overfishing, has strongly reduced their density, leading local populations of these species to the brink of extinction. Recovery of populations takes a long time and even now, after several years, sponge density is still very low. A simple solution to this problem is sponge-farming. Sponges are sessile filter feeding organisms and through their pumping activity they are able to retain bacteria and suspended organic matter from the entire water-column in littoral marine environments. This ability provides the basis for an integrated aquaculture of sponges and fish in coastal areas: floating-cage fish farms release a lot of organic wastes that can be recycled as a rich source of food for surrounding intensive commercial sponge cultures. Moreover, the interest shown by chemists and pharmacologists in regard to natural products extracted from sponges creates new possibilities for sponge farming. ❐ *Porifera, aquaculture, organic pollution, overfishing, bath sponges, natural products, cicatrisation.*

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Commercial 'horny' or Keratose sponges have been harvested and utilised as bath sponges since ancient times. Phoenicians and Egyptians collected sponges stranded along the seashore, while the millenary history of sponge fishery takes root in the ancient Greek civilisation. Traditionally fishermen used a heavy stone as ballast to easily reach the sea bottom and gather sponges into a net basket. At the end of the last century this harvesting system was replaced by 'hard hat' diving-suits. The introduction of this device rapidly increased fishing effort, although many divers died from decompression sickness, and as a consequence many Governments banned this technique. Modern developments in hyperbaric medicine and diving equipment have since solved both legal and medical problems associated with commercial diving activities, but have created a new suite of problems for sponge fisheries.

In recent times sponge population density has continually decreased, both through overfishing and from the so-called sponge disease. Older professional divers relate the existence of an incredible abundance of commercial species during the 1930s, along the coasts of Cyprus, Crete and Sardinia, consisting of more than 200-300 specimens/100m^2. Prior to the sponge disease epidemic, unexploited commercial sponge banks contained sponge densities of about 100 specimens/100m^2, whereas at present, the mean density is often less than 50 specimens/100m^2 (Pronzato et al., 1996, 1999; Pronzato, 1999).

Sponge diseases do not occur frequently, but have been recorded in populations from both the Mediterranean and Caribbean Seas. Between 1985-1988 commercial sponges practically disappeared in many of these areas, especially in the eastern Mediterranean Basin, with consequent heavy economic losses. Sick sponges are easily recognisable through exposure of their internal skeleton. Sponge disease is caused by invasive pathogenic micro-organisms: first they destroy the sponge's external fibrous layer, then proceed rapidly into the sponge body, destroying living tissues. Fibres become fragile and flake off, losing their characteristic durability and

TABLE 1. Percentage of survival of monitored species farmed in Kalymnos and Paraggi. *Hippospongia communis*, *Petrosia ficiformis* and *Cacospongia mollior* are perfectly suited, while *Axinella damicornis* and *Ircinia variabilis* are unsuitable. Abbreviation: N=no. of transplanted fragments.

Species	N	Survival after 48 hours (%)	Survival after 2 months (%)
Kalymnos (Dodecanese, Greece)			
Spongia officinalis	75	69.4	69.4
Hippospongia communis	252	100	100
Paraggi (Ligurian Sea, Italy)			
Agelas oroides	46	45.8	44
Axinella damicornis	50	0	0
Cacospongia mollior	60	83.3	83.3
Ircinia variabilis	50	2	1
Petrosia ficiformis	40	100	98
Spongia officinalis	50	66	66
Spongia agaricina	49	42.8	40

softness (Gaino & Pronzato, 1989; Pronzato & Gaino, 1991; Gaino et al., 1992). There is undoubtedly a synergetic effect between overfishing and sponge disease in reducing populations, given that overexploitation may lower the sponge's self-defence mechanisms, increasing the risk of environmental aggravation (Pronzato, 1999). Moreover, it is also known that types of pollution are responsible for decreasing biodiversity amongst Porifera (Pansini & Pronzato, 1975; Carballo et al., 1996).

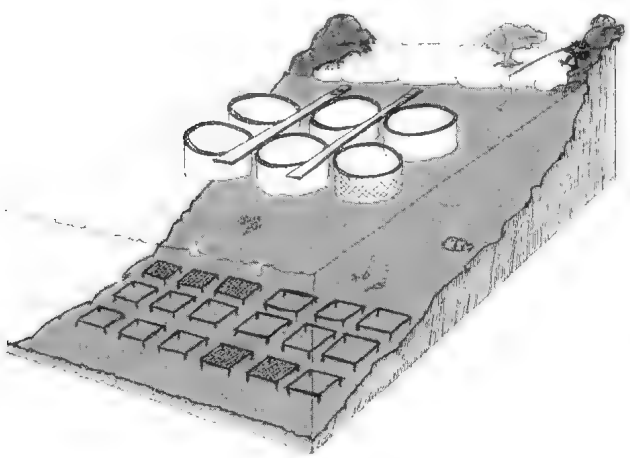

FIG. 1. The sponge experimental plant in Kalymnos: arrows indicate the structures for sponge farming, moored on the bottom.

Due to depletion of sponge populations from 1960-1990 many commercial producers have gone out of business, with exports from many Mediterranean countries diminishing substantially. The decrease in catch has produced a sharp increase in price for Mediterranean sponges, with the consequence that lower quality, cheaper Caribbean and Pacific stocks have invaded the market (Verdenal & Vacelet, 1990). Recovery of the sponge banks is a long term process (Rizzitello et al., 1997). Ten years after the onset of Mediterranean sponge disease, commercial sponges are still rare in many sites we examined during our experiments (this paper).

In the last few years chemical researchers have also shown an interest in sponge culture, owing to the presence of natural products useful in pharmacology. Metabolites extracted from Porifera are providing promising results in the prevention and treatment of tumours (De Flora et al., 1995), antiphlogistic compounds (De Rosa et al., 1995) and other properties (Uriz et al., 1991). Moreover, sponge extracts appear in catalogues of laboratory products at very high prices. Our goal is to satisfy the market request for these products without reducing natural populations.

This paper reports on the preliminary results from two different experiments in sponge farming. The first aimed to reconvert sponge fishery toward a more profitable and environmentally sustainable activity, located in Kalymnos Island (Dodecanese, Greece), during March 1998. The two target species were *Spongia officinalis* and *Hippospongia communis*, the most common commercial species in this area. The second experimental sponge culture was directed towards pharmacology, located in the W Mediterranean (Paraggi, Ligurian Sea), testing the survival of different non-commercial sponge species under farming conditions.

MATERIALS AND METHODS

The study site in Kalymnos (Dodecanese, Greece: 36°58'N, 27°02'E), was situated in the sheltered Bay of Vathi where there was a fish farming plant hosting 30 floating cages. During March 1998 four metallic

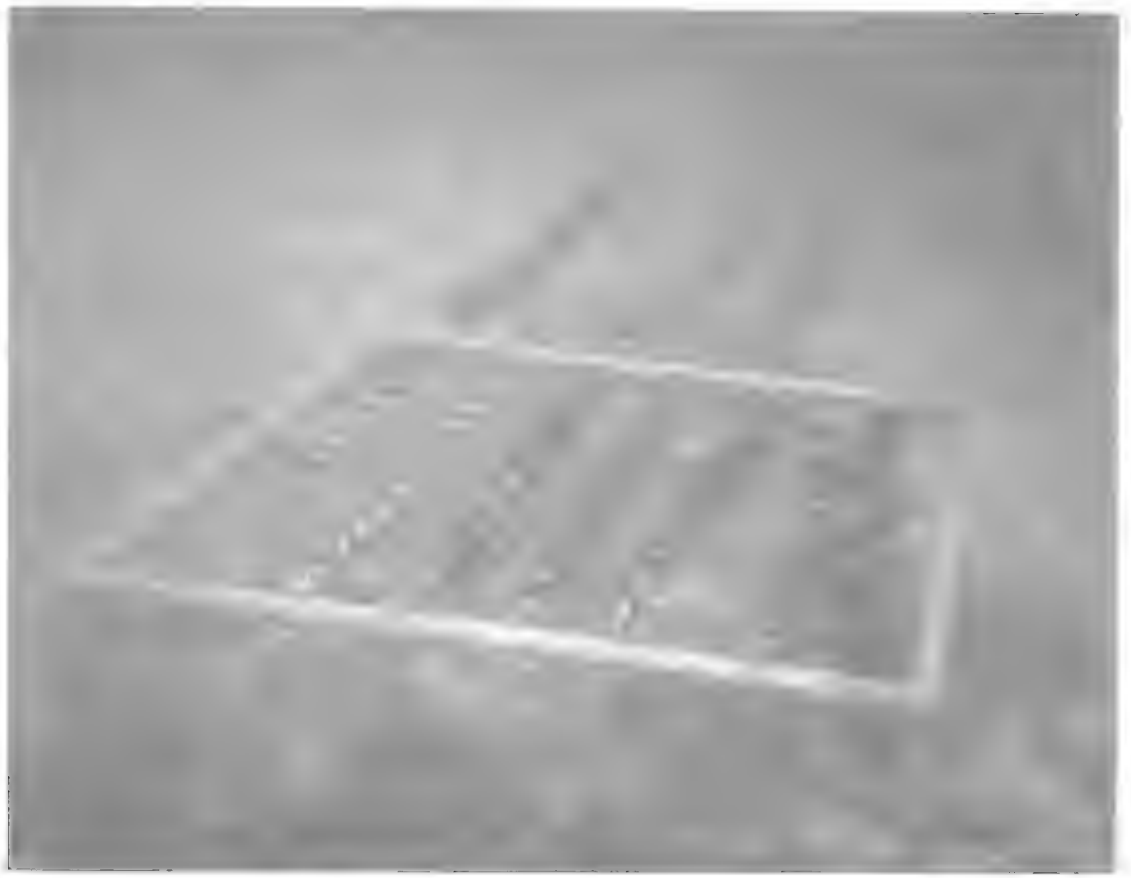

FIG. 2. The horizontal structures moored on the bottom with sponge fragments fixed onto lines.

horizontal structures were moored on a flat bottom 200-500m away from the floating fish cages at 15m depth (Figs 1-2). Specimens of *S. officinalis* var. *adriatica* and *H. communis* were cut into 4x4cm fragments and threaded onto a nylon line, separated by plastic tube spacers (7x0.6cm) (Figs 2, 3A,B). In total, 350 fragments were attached to lines. A team of operators monitored the plant of Kalymnos daily for the first week, and subsequently every ten days for the following two months.

Using the same method, the plant at Paraggi (Ligurian Sea, Italy: 44°18'N, 9°09'E), was situated on a flat bottom, at a depth of 25m. During May 1998 fifty fragments were obtained from each of the following species: *Agelas oroides*, *Axinella damicornis*, *Cacospongia mollior*, *Chondrosia reniformis*, *Ircinia variabilis*, *Petrosia ficiformis*, *S. agaricina* and *S. officinalis* var. *adriatica*. These were fixed onto horizontal structures using the methods described above. These species are the most common sponges living on the rocky cliffs of the Ligurian Sea.

During the first week of experiments samples from the cut surfaces were collected daily from both plants (Paraggi and Kalymnos), fixed in Glutaraldehyde 2.5% in ASW, dehydrated in a graded series of ethanol, critical-point dried using a Pabish CPD 750 drier coated with gold using a Balzers SCD 004 coater, and observed under a Philips EM 515 scanning electron microscope.

RESULTS AND DISCUSSION

Many experimental approaches have been applied to investigate the problem of producing a profitable sponge culture since the beginning of the century, and at present, some data are well-grounded (see Pronzato, 1999, for a review). From these data, on average, over two years sponges increase their volume by 100-200%.

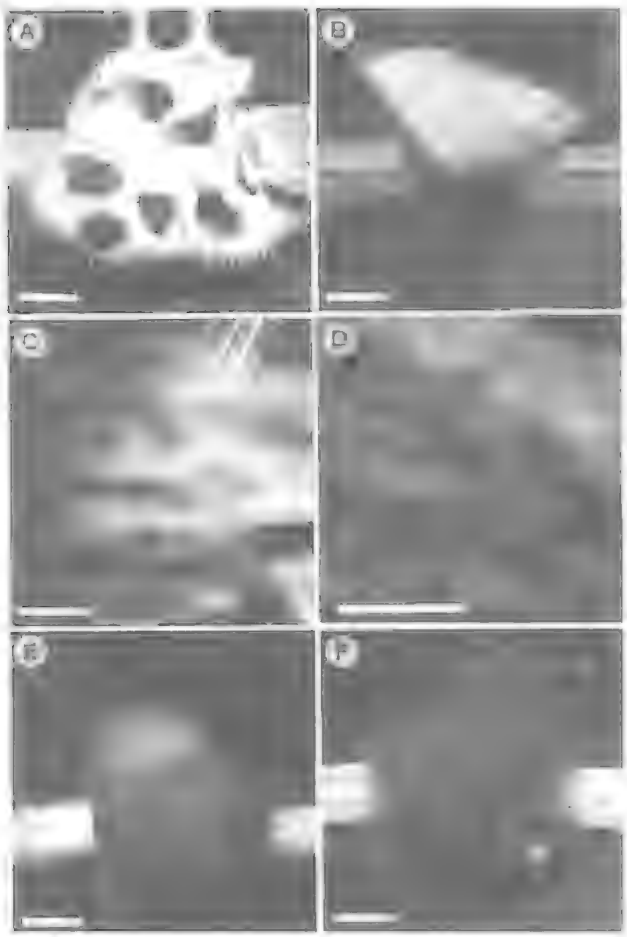

FIG. 3. A, Fragments of *Hippospongia communis*. B, *Spongia officinalis* just after transplantation: portions of the dark original external fibrous layer are maintained. C, Details of the thin cell layer in *H. communis* after 24hrs recovery. D, Thin cell layer recovering in *S. officinalis*. E, *Spongia officinalis* three weeks after transplantation: the characteristic dark pigmentation and the rounded shape have been restored. F, A dead specimen of *S. officinalis*. Death occurs mainly after the first 48hrs of transplantation. (Scale bars=1cm).

Generally, smallest fragments show the highest growth rates (Verdenal & Vacelet, 1985).

KALYMNOS PLANT. Mean mortality, less than 20%, was limited to the first 48hrs after transplantation, and *H. communis* seemed to be more resistant than *S. officinalis*. In fact, *S. officinalis* showed a survival rate of 69.4% whereas *H. communis* gave excellent results with a survival of 100% (Table 1). Mortality may be due to high sedimentation rates which favours bacterial proliferation: only naked skeletons of dead fragments remained on the ropes (Fig. 3F).

The regenerative process starts immediately after transplantation. Within 2-3 days sponges rebuild their external protective layer; after 24hrs a thin transparent cell layer covers the cut surfaces (Fig. 3C,D). After one week the characteristic dark external pigmentation of the sponge was restored. After one month sponge fragments assumed a rounded shape, with the external fibrous layer and the aquiferous system of the cut surfaces completely reorganised (Fig. 3E).

Among all the phyla of filter feeders, sponges play a remarkable role in the auto-epurative processes of the sea (Sarà, 1973). In accordance with modern integrated aquaculture systems, the association of sponge culture with floating cage fish farms have the potential to reduce environmental impact on coastal areas due to pollution produced by intensive fish farming (Manconi et al., 1998; Pronzato et al., 1998). The major impact occurs on the sea bed, under floating cages, where a rain of particles falls on benthic organisms causing rapid eutrophication (i.e. decrease in dissolved oxygen and increase in nutrient levels) (Wu, 1995). Food wastes and faecal pellets released by captive fish are rapidly colonised and degraded by bacteria (Ilonjo & Roman, 1977). Filtering activity of sponges has the potential to contribute to reduce this pollution within the precinct of fish farms. Sponges can retain about 80% of organic particulate material suspended in water, and about 70% of bacteria (Reiswig, 1971, 1975), with sponges filtering the entire water column in a single day (Reiswig, 1974). This integrated aquaculture provides a double bonus: purified water and commercial bath sponges.

Following our first attempt the Municipality of Kalymnos is presently planning to farm many thousands of sponges within the boundaries of floating fish cages along the island's coast.

PARAGGI PLANT. *Petrosia ficiformis* was the most productive species at this site. The cut surface produced a new pinacoderm within 4-5 days, and survival of fragments was close to 100% (Table 1).

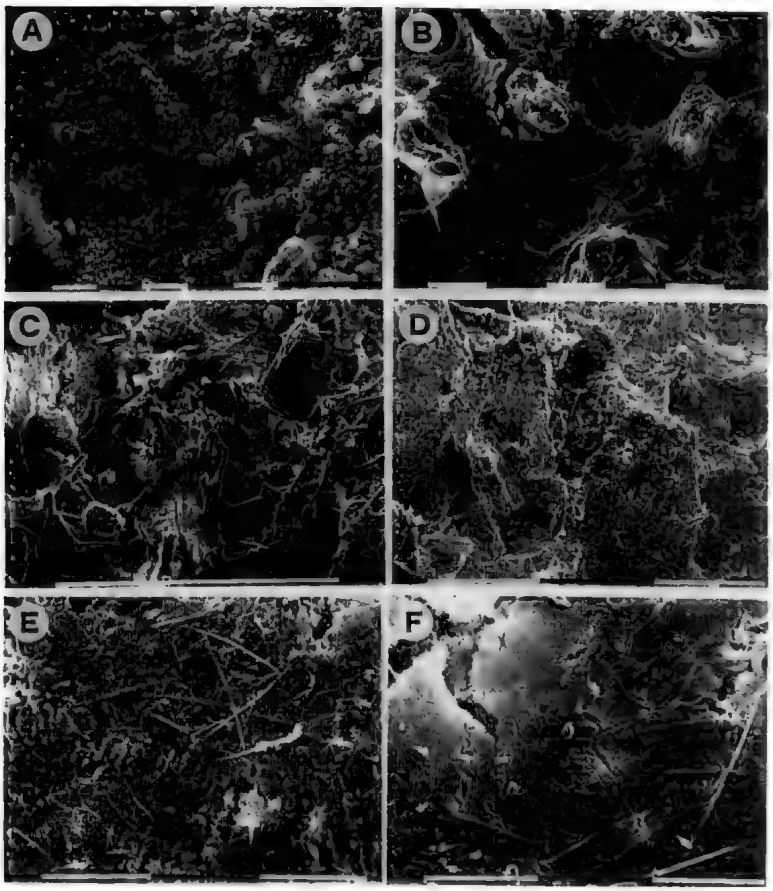

FIG. 4. The cicatrisating process of some investigated species. A, The cut surface on the day of transplantation in *Agelas oroides*. B, Cut surface of *Agelas oroides* after three days when the exopinacoderm is completely restored. C, Cut surface of *Axinella damicornis* after the first day. D, Cut surface of *Axinella damicornis* after the third day, where the external layer has not yet rebuilt. E, Cut surface of *Petrosia ficiformis* immediately after cutting. F, Cut surface of *Petrosia ficiformis* after three days, where the external cell layer is perfectly reconstructed. (Scale bars: A, D, E, F=100μm; B=10μm; C=1mm).

Percentage survival rates of *S. officinalis* and *C. mollior* were satisfactory (about 60-80% over two months), and data on *S. officinalis* were concordant between the Kalymnos and Paraggi plants (Table 1). It is important to underline that environmental conditions, the state of health of the mother sponge, and techniques used in transplantation all influence survival and growth rate of farmed specimens, as also noted by Verdenal & Vacelet (1990). For instance, in their Marseilles farm, Verdenal & Vacelet (1990) found that *S. agaricina* showed a survival of 100% whereas we observed a mortality rate of about 60%.

Axinella damicornis and *I. variabilis* showed a high mortality rate, probably due to damage incurred during the cutting process. In fact, *A. damicornis* is very fragile and must be handled with care, whereas *Ircinia* is so compact that it is difficult to cut without squeezing and potentially damaging tissues (Table 1).

Of all species tested, *C. reniformis* was completely unsuitable for our experimental conditions. The collagen matrix cut itself on the thread and in 1-2 days the sponge 'dripped' down from the thread. This behaviour, reminescent of the variable structure of Echinoderms (Candia Carnevali et al., 1990), is very interesting and the subject of a recent study (Bavestrello et al., 1998).

Recovery of the exposed choanosome starts from the borders of the cut and increases concentrically. The reconstruction process differs between species, depending on whether the external layer is a real pinacoderm, or, as for bath sponges (*Spongia* and *Hippospongia*), it is a fibrous layer without cells. Our experience shows that in *A. oroides* (Fig. 4A,B) and *P. ficiformis* (Fig. 4E,F) the restoration of the exopinacoderm is complete in 2-3 days, whereas in *A. damicornis* (Fig. 4C,D) this process does not occur at all.

Commercial bath sponges show the lowest mortality rate, probably due to the possession of a fibrous layer in which the recovery process is different from other species. Spherical cells, with long pseudopodia, travel along the cut edges producing collagen fibrils and completing the cicatrisation process after only a few days (Fig. 5).

The rapidity of reconstitution of the new external layer on cut portions of sponges varies

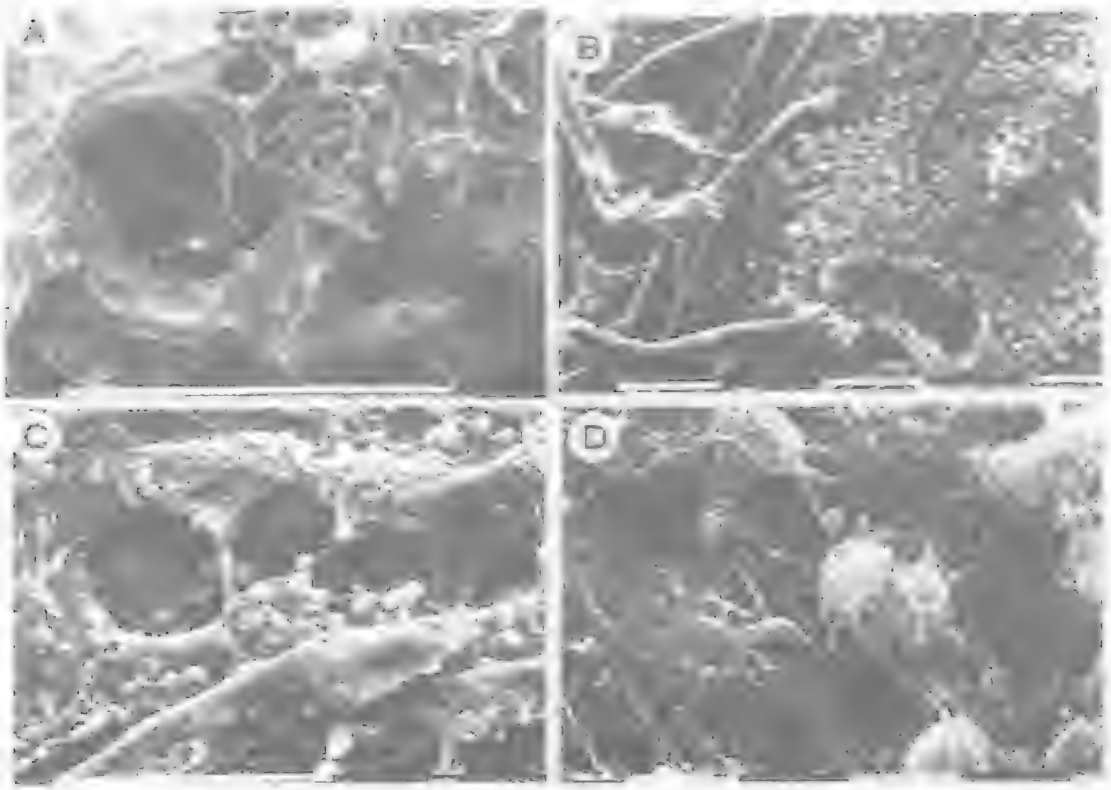

FIG. 5. A, The regenerative process of *Spongia agaricina* shows recovery of a new fibrous layer on exposed spongin fibers. B, Many globous mobile cells occur on the sponge surface. C, Elongated pseudopodia actively produce the collagen deposited on sponge fibers. D, After a few days the superficial fibrous layer is more-or-less completely restored. (Scale bars: A =1mm; B, C=100μm; D=10μm).

between species (e.g. 2-3 days in *A. oroides* and *P. ficiformis;* to about 10 days in *C. mollior*).

CONCLUSIONS

Commercial bath sponges have practically disappeared from the E Mediterranean Sea, owing to both overfishing and sponge disease. Sponge aquaculture has the potential to decrease fishing pressure, thus facilitating the natural repopulation of these affected areas.

Pharmacological research on marine natural products extracted from sponges are providing promising results, opening new perspectives in the exploitation of new species of Porifera. However, there are virtually no data on the densities of wild sponge banks for most species, and the risk of overfishing of potentially commercially valuable species could become real. An evaluation of the adaptability of the most common Mediterranean species to farming conditions could provide a valuable resource to any future exploitation of these species for pharmacological or other products.

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NEW APPROACHES TO THE BIOMINERALIZATION PROCESSES OF CALCIFIED SKELETONS IN CORALLINE DEMOSPONGES. *Memoirs of the Queensland Museum 44: 492. 1999*:- Biomineralization of calcareous basal skeletons in coralline sponges is a strongly phylogenetically convergent character (Reitner, 1992). However, the basic mineralization process is ancestral and exhibits similarities with mineralization processes known in bacterial biofilms and organo-mineralization via controlled taphonomy (Reitner et al, 1997). The main biocalcification events in the phylogenetically distinct taxa *Vaceletia* sp., *Astrosclera willeyana*, *Ceratoporella nicholsoni*, and *Spirastrella (Acanthochaetetes) wellsi* are discussed. *Vaceletia*, a demosponge with a thalamid basal skeleton, exhibits the most ancestral mode to build a calcareous skeleton via controlled taphonomy. The stromatoporoid *Astrosclera willeyana* intracellularly forms egg-shaped aragonitic asters in a first step which grow together via an epitactical process. The chaetetid *S. (Acanthochaetetes) wellsi*, phylogenetically the most evolved coralline sponge taxon, forms its unique high-Mg calcitic skeleton in extracellular acidic organic mucilages in the presence of collagen. In all cases the mineralization is controlled by acidic matrix proteins. All aragonitic basal skeletons are characterized by high amounts of Sr and U. In *Ceratoporella nicholsoni* an increase of Mg, Sr and U in the old skeletal parts is observed using ICP-MS based geochemical analyses. The decrease of P concentrations is probably linked to the collapse of the intracrystalline matrix proteins. In *Ceratoporella* two distinct Ca^{2+} - binding matrix proteins are observed which are enriched in the amino acids asp (20 mol%) and glu (15 mol%) (18kd, >100kd). The uppermost growing zones exhibit relatively light $\delta^{13}C$ 3.8 and $\delta^{18}O$ –0.4 values on average.

Astrosclera differs in many aspects from the phylogenetically closely related *Ceratoporella*. The newly formed aragonite asters are depleted in ^{13}C ($\delta^{13}C$ 3.5) in comparison with the mature basal skeleton. The spherulites are enriched in Sr, P, Li and Mo. In the youngest cementing area of the spherulites the content of ^{13}C increases to $\delta^{13}C$ 4.03. *Astrosclera* exhibits five acidic matrix proteins, the smaller ones (17kd, 30kd) controlling the spherulite growth, and a large one (120kd) which probably controlls the cementation process.

The young portion of the basal skeleton of *Vaceletia* n.sp. differs from the aragonitic basal skeletons of the other coralline sponges. Mg and P is extremly enriched and the carbon isotope composition is relatively light ($\delta^{13}C$ 3.8), *Vaceletia* exhibits 10 acidic matrix proteins.

Acanthochaetetes has the most evolved basal skeleton which exhibit five acidic matrix protein. The small ones (ca. 20kd) control the initial calcification. The basal skeleton is a Mg rich calcite (19-20mol% $MgCO_3$). The uppermost growing zones exhibit light $\delta^{13}C$ values (2.65). The simultaneously growing inner cements have $\delta^{13}C$ 3.03. Based on the measured geochemical and isotopic data a vital effect during the early formation of the basal skeletons is very probable. The old or mature basal skeleton portions exhibit in all cases signals of a mineralization in equilibrium with the ambient sea water. ❑ *Porifera, coralline sponges, Vaceletia, reef-building sphinctozoans, Acanthochaetetes.*

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EVIDENCE FOR SYMBIOTIC ALGAE IN SPONGES FROM TEMPERATE COASTAL REEFS IN NEW SOUTH WALES, AUSTRALIA

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The symbiotic relationships between tropical reef sponges and cyanobacteria (microalgae) has been well documented. Preliminary evidence suggests that these relationships may be just as common in temperate reef species. Screening of sponges from temperate reefs in New South Wales, Australia, found 5 out of 8 species tested were 'chlorophyll positive'. Of those tested, *Cymbastela concentrica* (Lendenfeld) had the greatest concentration of chlorophyll-*a* pigment within its tissue (139.4±9.4µg/g). An estimate of the percentage of temperate reef sponges that potentially contained symbiotic algae was made based on their in situ colour pigmentation. It was predicted that over 65% of temperate reef sponges potentially contain symbiotic algae, although it is unknown how many may be phototrophic. ❐ *Porifera, symbiotic algae, cyanobacteria, temperate sponges, ecology.*

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Many sponges from tropical reefs contain and benefit from symbiotic relationships with cyanobacteria or microalgae (Sarà, 1971; Vacelet & Donadey, 1977; Wilkinson, 1978, 1981, 1983; Larkum et al., 1988). Wilkinson (1983) suggested that in some species these algae can provide a substantial source of nutrition for the host sponge (see also Wilkinson, 1981; Wilkinson et al., 1988). In tropical regions, light levels may limit the maximum depth to which phototrophic sponges are distributed. Photosynthetic symbionts are unlikely to provide nutrition beyond the net photosynthetic compensation point (Cheshire & Wilkinson, 1991). In striking contrast, symbiotic algae may even provide photo-protection to some species of sponges from excessive light in shallow water (Sarà, 1971; Wilkinson, 1983).

Until recently, phototrophic sponges were thought to be restricted to sub-tropical and shallow tropical reef habitats (Wilkinson, 1983), however a temperate reef phototrophic sponge (*Cymbastela* sp.) is now known to be an exception (Cheshire et al., 1995). At least one tropical sponge with symbiotic algae, *Cymbastela concentrica* (Wilkinson, 1983; Larkum et al., 1988; Hooper & Bergquist, 1992; Hooper & Lévi, 1994), also has a temperate range where it is found between 10-60m depth or more (Roberts & Davis, 1996).

We predicted that symbiotic relationships between temperate reef sponges and algae may be widespread because of the number of species exhibiting a colour, indicative of microalgae within their tissue (Hooper & Bergquist, 1992). Here we report the results of a screening study where eight common temperate reef sponges were tested for the presence of symbiotic algae. Furthermore, we extrapolated from our records on pigmentation for over 100 temperate species to gain some estimate of the potential for temperate reef sponges to contain symbiotic algae.

MATERIALS AND METHODS

To test for general evidence of symbiosis in temperate reef sponges we sampled a range of common species, some of which were replicated to estimate variation and consistency in both colour and chlorophyll concentration. Eight species of Demospongiae (Table 1) were haphazardly collected from the subtidal reefs at Henry Head and Inscription Point at the entrance to Botany Bay, New South Wales (NSW) Australia (Fig. 1), and the concentration of chlorophyll-*a* within their tissue was determined.

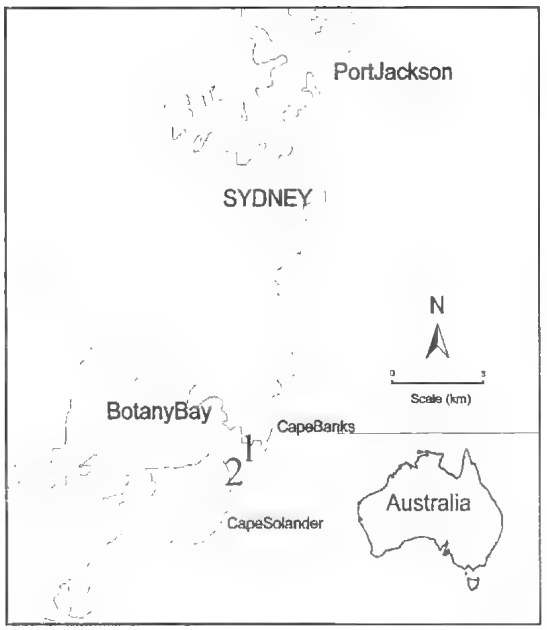

FIG. 1. Location of reefs at Inscription Point and Henry Head, NSW, where sponges were collected to determine the presence of symbiotic algae (1=Henry Head; 2= Inscription Point).

The collections were made on sponge-dominated reefs between 10-20m depth. Other common sessile macrobenthic organisms found on these reefs included ascidians, bryozoans, cnidarians, foliose macroalgae and a nondescript matrix of microorganisms and silt (Davis et al., 1997). Spatial and temporal patterns of abundance for these assemblages have been described elsewhere (Davis et al., 1997).

The sponges were photographed, their in situ colour recorded, brought to the surface, field weighed (to the nearest gram), and immediately transported back to the laboratory under dark conditions in fresh seawater. Variation in the concentration of chlorophyll-*a* within a species was also examined for two of the eight sponges, by collecting five replicate specimens of each of *C. concentrica* and *Clathria striata*. All sponges were identified and were included in a voucher collection lodged with the Queensland Museum.

To determine whether symbiotic algae were present in each of the sponges, 1g of tissue was removed and processed with 90% aqueous acetone solution. A sub-sample of 1g was found to give a suitable chlorophyll-*a* absorbance reading for a variety of sponge samples. To ensure the complete extraction of the chlorophyll-*a* pigment, the sponge tissue was mechanically broken down using a mortar and pestle. The resultant extracted slurry was transferred to a screw-cap bottle and the total volume was adjusted to a constant volume (10ml) and stored at -20°C until analysis.

Immediately before spectrophotometric determination samples were removed from the freezer and returned to room temperature in the dark. The sample extracts were filtered through a solvent-resistant disposable filter (Whatman GF/C 47mm diameter), and 3ml of the solution was transferred to a 1cm cuvette. Absorbance was measured on a Cary 1E UV-visible spectrophotometer at 664nm for chlorophyll-*a*, and 750nm, 647nm and 630nm for turbidity, chlorophyll-*b* and chlorophyll-*c* corrections, respectively. Before sample analysis, the spectrophotometer absorbance was adjusted to zero by inserting a 90% acetone blank. The concentration of chlorophyll-*a* was calculated using the methods described in Clesceri (1985). It should be noted that the samples were not subsequently acidified and analysed for pheophytin-*a* (a common degradation product of chlorophyll-*a*). All work with chlorophyll extracts was undertaken in cool, dark conditions to minimise potential degradation of chlorophyll.

The in situ colour of a sponge may be a good preliminary test or indicator for the presence of symbiotic algae (Hooper & Bergquist, 1992). To estimate the percentage of temperate reef sponges that potentially contained symbiotic algae we examined our earlier collections of over 100 temperate reef species from Sydney to Port Stephens, NSW (Roberts & Davis, 1997; Roberts et al., 1998). Subjective estimates were made on the presence or absence of symbiotic algae based on the in situ colour of each species from field records and photographs.

RESULTS

Five of the eight species of temperate reef sponges we tested (in four orders and seven families) were found to be 'chlorophyll positive'. These were *Clathria striata*, *Phoriospongia cf. kirki*, *Cymbastela concentrica*, *Callyspongia* sp. and *Spirastrella areolata* (Table 1). Of the five species, *C. concentrica* had the greatest concentration of chlorophyll-*a* within its tissue, followed by *Callyspongia* sp. (Table 1). Little within-species variation was found in the mean concentration of chlorophyll-*a* (±S.E.) in the tissue of *C. concentrica* (139.4±9.4) and *C. striata* (19.3±1.4) (Table 1).

The other three common species tested were also chlorophyll positive (Table 1), although

TABLE 1. Summary of sponges from temperate reefs (10-20m depth) screened for the presence of symbiotic algae (* n = 5 sponges).

Order	Family	Species	Chlorophyll-a (µg/g)
Hadromerida	Spirastrellidae	*Spirastrella areolata* Dendy	20.7
Halichondrida	Axinellidae	*Cymbastela concentrica* (Lendenfeld)	139.4 ± 9.4 *
Haplosclerida	Callyspongiidae	*Callyspongia* sp.	85.3
Poecilosclerida	Tedaniidae	*Tedania digitata* (Schmidt)	Nil
	Microcionidae	*Clathria striata* Whitelegge	19.3 ± 1.4 *
		Holopsamma arborea (Lendenfeld)	Nil
	Phoriospongiidae	*Phoriospongia* cf. *kirki* (Bowerbank)	10.2
	Raspailiidae	*Ceratopsion aurantiaca* (Lendenfeld)	Nil

chlorophyll-*a* concentrations were considerably lower than in *C. concentrica* and *Callyspongia* sp. *Spirastrella areolata* is a massive ridge shaped sponge, which has an olive-yellow colour, whilst *C. striata* is a fan-shaped, orange-tan coloured sponge. *Phoriospongia cf. kirki* grows as a massive- ridged shaped form and is characteristically a cream-tan colour throughout. It had the lowest measurable concentration of chlorophyll-*a* and contained small dark-brown nodules along the side of each ridge within the ectosomal outer layer. We are not certain whether the chlorophyll positive result we obtained for this species was due to these dark-brown nodes and further work would be required to identify the distribution of symbiotic algae within this species.

Other species *T. digitata*, *C. aurantiaca* and *H. arborea* all returned a 'chlorophyll negative' response for the presence of symbiotic algae (Table 1). Both *T. digitata* and *C. aurantiaca* were bright orange in colour, which probably reflects metabolically produced carotenoid pigments within the sponge (Hooper, 1996). These pigments may be photo-protective and have been predominately observed in the Poecilosclerida and Axinellida (Hooper, 1996). *Holopsamma arborea*, a common white, honeycombed reticulated sponge (Hooper, 1996) on shallow and deep reefs in NSW (Roberts & Davis, 1996) was also chlorophyll negative.

Four orders of sponges examined in this preliminary study had chlorophyll positive species (Table 1). Wilkinson (1993) also found chlorophyll positive species in each of 5 orders he examined from tropical reefs (see Table 2). Over 100 species of temperate reef sponges were examined for their potential to contain symbiotic algae based on their in situ colour. It was estimated that of these species, 65% potentially contained symbiotic algae.

DISCUSSION

Our findings show that over 60% of sampled temperate reef sponges have chlorophyll levels consistent with the presence of microalgae. Of the 5 species which we identified as being 'chlorophyll positive', we expected *C. concentrica* and *Callyspongia* sp. to contain chlorophyll-*a*, based entirely on the colour of their external tissue. Since *Cymbastela* spp. were reported as phototrophic in temperate and tropical waters (Cheshire et al., 1995; Wilkinson, 1983; Larkum et al., 1988), it was highly likely that this would be the case for related species in NSW.

Wilkinson (1983) found that nine out of ten of the most common sponges on a tropical reef (representatives from five orders and six families) contained symbiotic algae. It has been estimated that up to 50% of tropical sponges may rely on this symbiosis because of the relatively low levels of available nutrients in these waters (Cheshire & Wilkinson, 1991). It is our opinion that a large proportion of shallow water temperate reef sponges may also have these associations, given that five of the eight species we screened were 'chlorophyll positive'. Furthermore, in our subjective examination of over 100 species from these temperate waters, at least 65% had similar colour to those that proved to be chlorophyll positive in this preliminary analysis.

On shallow water temperate reefs, *C. concentrica* was found to have on average 139.4±9.4µg/g of chlorophyll-*a* within its tissue. Wilkinson (1983) reported a concentration of 93.4µg/g of chlorophyll-*a* in *C. concentrica* (described as *Pseudaxinyssa* sp. in his work) from a tropical reef. Seddon et al. (1993) investigated the ability of *C. concentrica* to photoacclimate in shallow water, and suggested that factors other than visible light were important in restricting its distribution. A preliminary study by Cheshire et al. (1995) on a *Cymbastela* sp. from southern Australian waters (possibly *C. notiana* Hooper & Bergquist), demonstrated that these sponges were capable of maintaining themselves

TABLE 2. Sponge orders found to be chlorophyll positive on temperate versus tropical reefs (nt = not tested).

Sponge Order	Temperate (this study)	Tropical (Wilkinson, 1983)
Astrophorida	nt	1/1
Dictyoceratida	nt	5/6
Hadromerida	1/1	nt
Halichondrida	1/1	1/1
Haplosclerida	1/1	nt
Poecilosclerida	2/5	1/1
Clathrinida	nt	1/1

photosynthetically. Given the results we have obtained in this study, we anticipate that *C. concentrica* and many other temperate reef sponges may have this ability. Whether or not temperate reef sponges rely on symbionts to enhance nutrition is unknown, however the work by Cheshire et al. (1995) in South Australia would suggest that this is the case.

Cymbastela concentrica is typically an olive-brown colour (Hooper & Bergquist, 1992) at its surface, but variations in the shade of colour have been observed between shallow and deep waters. This may indicate changes in the concentration of symbiotic algae within the sponge associated with light gradients. Initial experiments with *C. concentrica* suggest that its colour can 'lighten' within days of manipulating its position with respect to incident light. *Cymbastela concentrica* and a number of other temperate reef sponges have been shown to be adversely affected by the discharge of sewage effluent into shallow and deep-water habitats (Roberts, 1996; Roberts et al., 1998). We speculate that any symbiotic relationship between sponges and algae may be altered through reductions in available light and/or increased nutrients as a result of sewage effluent (Roberts et al., 1998).

We believe that many temperate reef sponges contain symbiotic algae however the significance of any symbiosis has to be quantified. We need to identify the types of symbionts within various species and examine these relationships with light gradients. Although temperate reef sponges may contain symbiotic algae, their role in the nutrition of the sponge needs to be quantified.

ACKNOWLEDGEMENTS

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NEW COLONIAL *VACELETIA*-TYPE SPHINCTOZOAN FROM THE PACIFIC. *Memoirs of the Queensland Museum 44: 498. 1999*:- Three new morphotypes of a Recent colonial sphinctozoan coralline sponge are presented. All types show close relationships to the taxon *Vaceletia crypta*, a non-colonial form from Indo-Pacific reef caves. The first two types were discovered in shallow water reef caves of Osprey Reef, N Queensland Plateau in the Coral Sea. These sponges are common in these caves. The third type of colonial sphinctozoan was found only at two localities at North Astrolabe Reef and Great Astrolabe Reef in Fiji. This variety shows similarities with a previously described deep water variation of *Vaceletia* from New Caledonia.

The first two morphotypes of colonial *Vaceletia* from Osprey Reef show more similarities to the cryptic, non-colonial form *V. crypta* from reef caves of the Great Barrier Reef and reefs of the Indo-Pacific, than to the deep-water colonial species described by Vacelet (1988) and Vacelet et al. (1992) from New Caledonia. The third variation from Astrolabe Reef is more similar to this deep water variation from New Caledonia. All three variations will be described elsewhere in detail as multidisciplinary taxonomic and geochemical investigations of these taxa are still in progress (Reitner & Wörheide, 1995; Wörheide & Reitner, 1996).

The discovery of these three new colonial variations from shallow water reef caves of the SW Pacific clearly demonstrates that colonial forms of Recent *Vaceletia* are not restricted to deep waters, as previously thought.

Sphinctozoan sponges were primary reef building organisms during the Permo-Triassic. They are chambered calcified sponges with morphological similarities to Cambrian Archaeocyaths. The *Vaceletia*-type of coralline sponges occured first in the middle/late Triassic (Reitner. 1992). Sphinctozoans were considered to be rare since the end of the Triassic, and were thought to be extinct since the end of the Cretaceous; that is until the 'living fossil' *Vaceletia* was discovered by Vacelet (1979) in the Indian Ocean.

The solitary, non-colonial form *Vaceletia crypta* has no reef building potential and is found only sparsely dispersed in the darker areas of Indo-Pacific reef caves.

These recently discovered colonial variations of *Vaceletia* from shallow water reef caves retain a colonial growth mode and a reef building capability. They provide, therefore, clues to understand the modalities of skeletal construction and biocalcification, as well as the ecology of Permo-Triassic sphinctozoan sponges. ❐ *Porifera, coralline sponges, mud-mounds, Vaceletia, colonial reef-building sphinctozoans, Osprey Reef, Coral Sea.*

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NEW HEXACTINELLID SPONGES FROM THE MENDOCINO RIDGE, NORTHERN CALIFORNIA, USA

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Reiswig, H.M. 1999 06 30: New hexactinellid sponges from the Mendocino Ridge, Northern California, USA. *Memoirs of the Queensland Museum* **44**: 499-508. Brisbane. ISSN 0079-8835.

Two hexactinellid sponges collected by ROV from the Mendocino Ridge by Dr. Andrew G. Carey Jr, are representatives of new taxa of Hexactinellida. The first, *Poliopogon mendocino* sp. nov. (Amphidiscophora, Pheronematidae) is an orange, sheet-like fragment from a 60cm wide, flaring, funnel-shaped sponge sampled at 2332m depth. The lower part of the specimen, and thus the basal spicules, were not collected and are unavailable. A new generic diagnosis for *Poliopogon* is provided. The second sponge, *Nubicaulus careyi* gen. nov., sp. nov. (Hexasterophora, Euplectellidae, Corbitellinae) is a nearly complete specimen in the form of a soft white cup on long hollow stalk encountered at 2102m depth. It bears distinctive drepanocomes, spirodiscohexasters and aspidoplumicomes, a combination previously unknown among hexactinellids. A reformed diagnosis of *Trachycaulus* is provided from re-inspection of the type specimen. ❐ *Porifera, Hexactinellida, new species, new genus, Mendocino Ridge, Poliopogon, Nubicaulus, Trachycaulus, California.*

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Hexactinellid sponges from northern California (San Francisco to the Oregon border) are known from only three publications. Schulze (1899) recorded *Rhabdocalyptus dawsoni* from Albatross stn 3349 (1890) W of Point Arena, 437m depth. Talmadge (1973) reported three species, *Aphrocallistes vastus*, *Chonelasma tenerum* (now *Heterochone tenera*) and *Bathyxiphus subtilis*, along with numerous other partially identified specimens obtained by the dragboat fishing fleet out of Humboldt Bay, N. California; specific collection sites were not included. The specimens were reportedly housed at Stanford University, but repeated attempts by the author to locate them have failed. Since the specimens' identities cannot be authenticated, and the original identifier is unknown, Talmadge's report cannot be considered adequate for acceptance as documented species records. Carey et al. (1990) listed hexactinellids collected on Gorda Ridge, at the California/Oregon border. Among the 10 forms reported, only 3 of them — *Staurocalyptus fasciculata*, *Farrea aculeata* and *Aphrocallistes vastus* — were identified to species by a recognised authority. The seven other incompletely resolved forms are still under review; their number is indicative of considerable diversity remaining to be documented in the poorly known hexactinellid fauna of this region. All of the above deal with new occurrences of taxa originally known from other locations. The present report is the first description of previously unknown taxa of hexactinellid sponges from the N. California region.

MATERIALS AND METHODS

Large sponges were encountered during operations of the Remotely Operated Vehicle (ROV) Advanced Tethered Vehicle (ATV) of the US Navy Deep Submergence Group on a NOAA Undersea Research Program on the Mendocino Fault Ridge off N. California (Fig. 1). This fauna was recorded on video tape and specimens collected by A.G. Carey Jr. in the course of a local faunal survey. After manipulator collection and recovery to shipboard, the specimens were air dried. The two specimens that form the basis of this present report were deposited to the invertebrate zoology collections of the California Academy of Sciences, San Francisco (CASIZ). Comparative material from The Natural History Museum, London (BMNH) was also reviewed.

Sections of the sponge body wall as well as fragments of dermal and gastral surfaces were either whole-mounted in balsam for light microscopy or digested in hot nitric acid. Large spicules in the resulting spicule suspensions were rinsed, spread on microscope slides and mounted

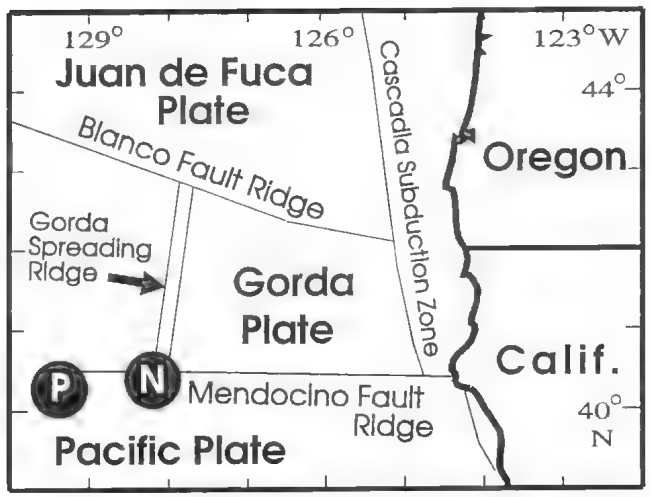

FIG. 1. Collection sites of *Poliopogon mendocino* sp. nov. (P) and *Nubicaulus caryi* sp. nov. (N) and major physiographic features of the northern California / Oregon region.

in balsam. Smaller spicules were dispersed on 25mm diam., 0.2mm pore-size, nitrocellulose filters by filtration; the filters were rinsed, dried and mounted in balsam. Spicules were measured by computer via a microscope-coupled digitiser. Data are reported as: mean ± st. dev. (range, number of measurements). Spicule drawings were prepared from video-captured microscope images imported into a computer drawing program and traced on-screen. Samples for scanning electron microscopy (SEM) were nitric-acid-cleaned and either filtered onto 13mm diam., 0.2mm pore-size, membrane filters or deposited directly onto cover-glasses after rinsing in distilled water. Following gold-palladium coating, specimens were viewed and photographed with a JEOL JSM-840 SEM.

SYSTEMATICS

Subphylum **Symplasma**
Reiswig & Mackie, 1983
Class **Hexactinellida** Schmidt, 1870
Subclass **Amphidiscophora** Schulze, 1886
Order **Amphidiscosa** Schrammen, 1924
Family **Pheronematidae** Gray, 1872
Poliopogon Thomson, 1873

TYPE SPECIES. *Poliopogon amadou* Thomson, 1873:29, Fig. 1.

DIAGNOSIS (summarised from Schulze, 1893:166 and Ijima, 1927:9; emended here). Body lamelliform, either ear-shaped involute plate, disc attached on edge, or widely open funnel attached centrally. Attached to hard substrate by short, broad, brush-like pad of thin bidentate basalia with shafts, extending into the body, entirely smooth; columella lacking. Conspicuous marginal fringe composed of sceptres and uncinates as marginal prostalia. Lateral surfaces smooth; lateral prostalia absent.

REMARKS. *Poliopogon*, established by Thomson (1873) for the type species, *P. amadou*, was augmented by Schulze's (1886) addition of *P. gigas*. It was briefly synonymised with *Pheronema* from 1894 through 1902 due to Schulze's (1894) discovery of two *Pheronema* lacking lateral prostalia, thus removing the only sustaining difference between the genera. After discovery of the missing lateral prostalia in other specimens of those species, Schulze (1902) re-established *Poliopogon* with its earlier complement of two species. Ijima (1927) suggested removal of *P. gigas* from the genus because of its different body shape (a barrel), an action taken up by Tabachnick (1990). The genus presently includes only the type species, *P. amadou*, *P. maitai* (Tabachnick 1988), and the new species, *P. mendocino*, described below. The form erected as *P. amadou pacifica* by Tabachnick (1988) cannot be accepted as a member of the genus due to poor condition of the specimen and/or incomplete description, e.g., lack of sceptres. On the basis of available information, even its family placement cannot be confirmed. It is relegated to Amphidiscosa *incertae sedis* until more details are revealed. Proper rhabdodiactin megascleres (excluding uncinates) are completely absent in this genus as in all Pheronematidae, a point needing reinforcement.

Poliopogon mendocino sp. nov.
(Figs 2A, 3A-B, 4A-J, 5)

MATERIAL. HOLOTYPE: CASIZ 113631: Mendocino Ridge, 300km W. of Cape Mendocino, N. California, 40°21.6'N, 129°23.7'W, 18.ix.1995, 2,332m depth, coll. A.G. Carey Jr., US Navy Deep Submergence Advanced Tethered Vehicle from R/V 'Laney Chouest', stn. MRF-1, dive no. 95-52-153 (Fig. 1).

ETYMOLOGY. Named after the type locality, Mendocino Ridge.

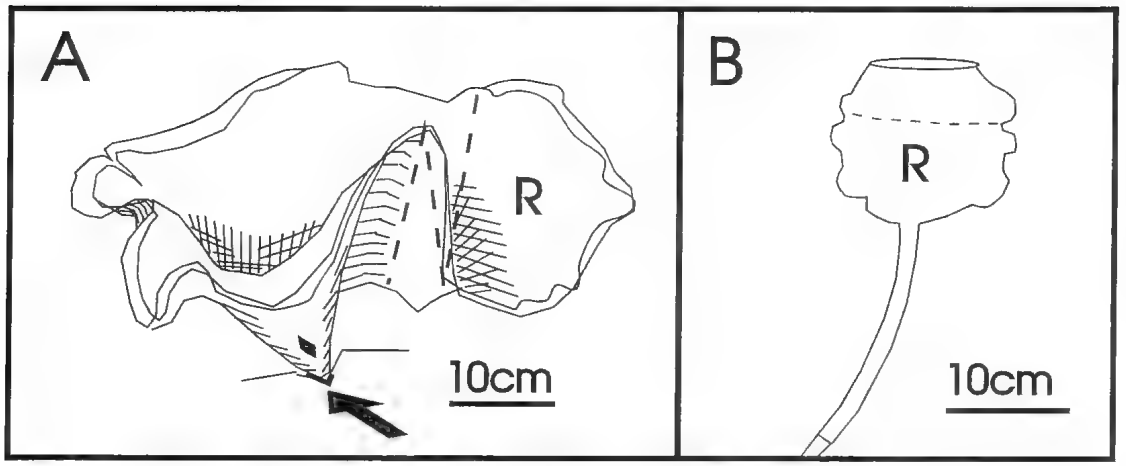

FIG. 2. Gross morphology of the holotypes of A, *Poliopogon mendocino* sp. nov. and B, *Nubicaulus caryi* sp. nov. *in situ* before collection, traced from video clips; the recovered samples are indicated by "R" and the dashed lines; arrow indicates point of attachment.

DESCRIPTION. *Shape.* Single specimen recorded live *in situ* on video tape before sampling (Fig. 2A) with broad flaring funnel, ca. 60cm diam., 20cm high. Robust marginal fringe to 2cm wide extends continuously around strongly undulated distal edge. Large openings of exhalant channels, about 4mm wide, spaced 2cm apart, clearly evident through transparent gastral cover layer within funnel (upper surface). Attachment point at base of a central cone which carried an inferior lateral opening. Approximately one third of the specimen was torn from one side and recovered for study.

Recovered dried sample (Fig. 3A-B) a sheet, 23x29x1.8cm maximum dimensions, with marginal fringe intact on one-half of edge. Gastral surface smooth with exhalant channels clearly evident through gastral cover layer. Irregular, vein-like network of white lines formed by strands of overlapping tangential rays of hypodermal pentactins. Dermal surface more irregular and opaque, with openings of inhalant canals visible through thin transparent dermal cover only in marginal areas. Hypodermal strand system not evident to eye. Both dermal and gastral surfaces lack lateral prostalia.

Colour. Gold-colored *in situ* (video recording). Dry sample pumpkin orange, intensified when wetted.

Skeleton. Skeleton composed of completely separate spicules; synapticular fusion does not occur. Both surfaces lined by pinnular pentactins forming a delicate and fragile quadrate lattice by overlapping of basal rays; square meshes have

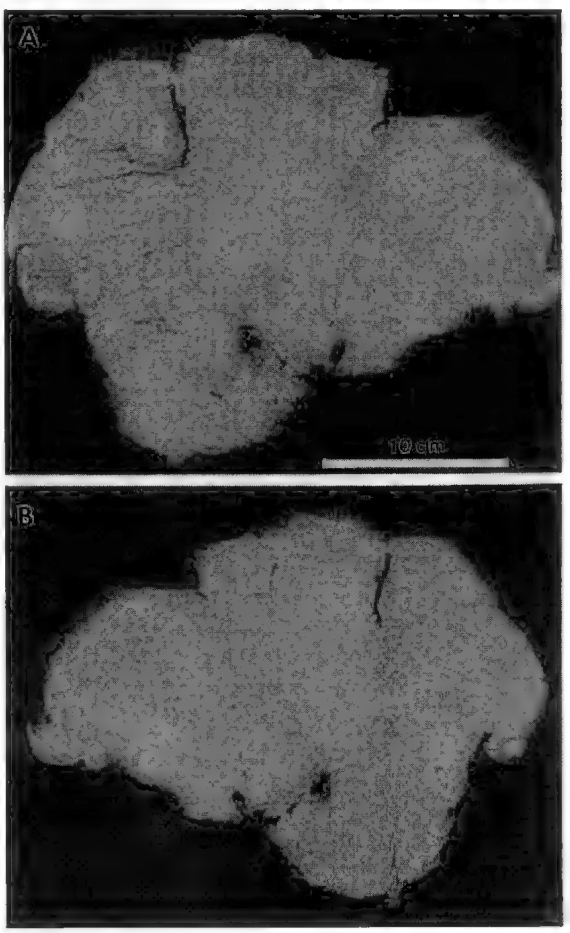

FIG. 3. *Poliopogon mendocino* sp. nov. Holotype. A, Dermal surface. B, Gastral surface.

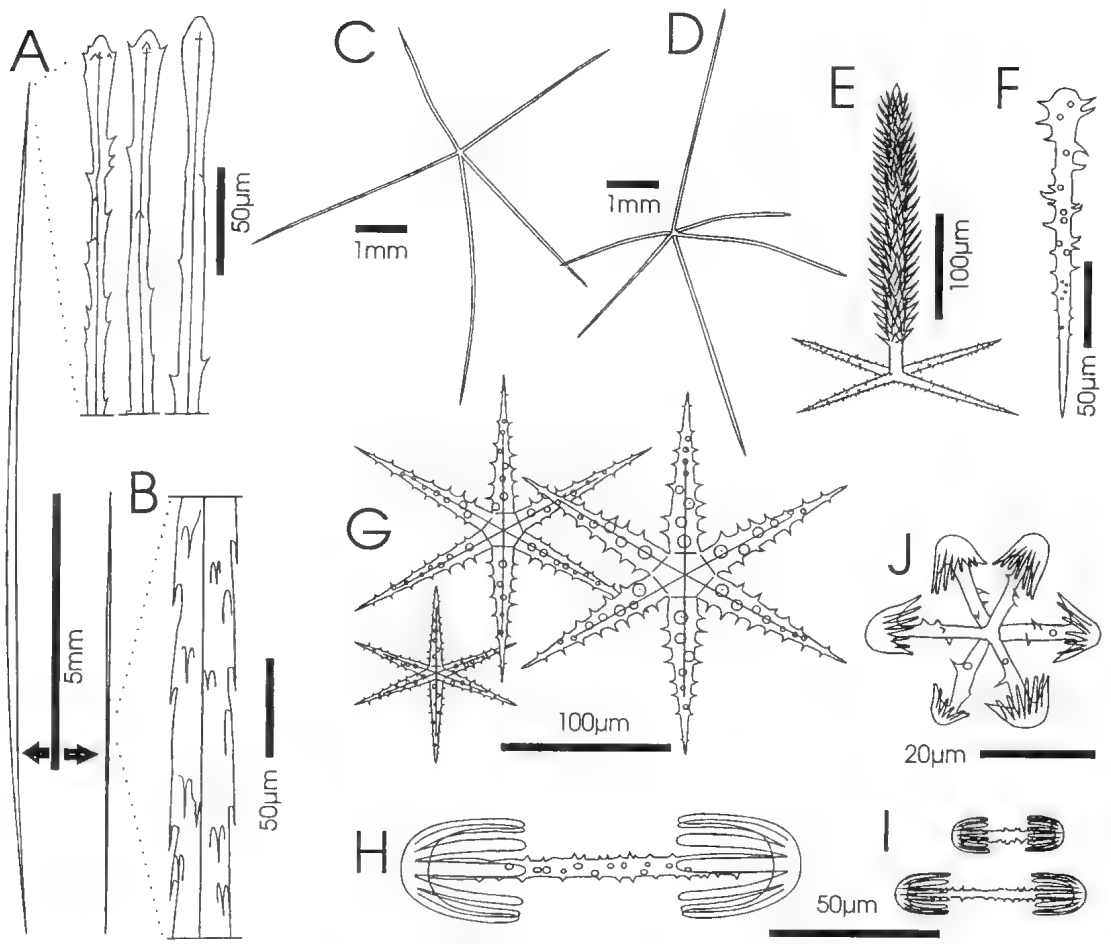

FIG. 4. *Poliopogon mendocino* sp. nov. Holotype spicules. A, Sceptre with three distal tips magnified. B, Uncinate with magnified central segment. C, Macropentactin. D, Three macrohexactins. E, Pinnular pentactin. F, Spiny mesomonactin. G, Mesohexactins. H, Mesamphidisc. I, Two micramphidiscs. J, Microhexadisc.

sides of ca. 100mm. These are supported on tangential rays of large, irregular hypodermal pentactins, with rays overlaping to form subdermal strands on both surfaces, but only macroscopically evident on gastral side. Choanosome supported by principalia which are pentactins and hexactins, both irregular in form and shape of rays. Marginal fringe composed mainly (ca. 95%) of large sceptres, with distal tips mostly broken off, and small component (ca. 5%) of uncinates. Proper rhabdodiactin megascleres (excluding uncinates) absent. Attachment point and associated basal spiculation not included in sample and unavailable for characterisation.

Megascleres. Principalia large pentactins and hexactins with thin irregular, curved rays; the same pentactins as hypodermalia and hypogastralia; pentactin (Fig. 4C): tangential ray length 3.1±0.1.3mm (range 1.5-7.3mm; n=50), ray width 37.7±8.4µm (range 19.7-55.5µm; n=50), proximal ray length 2.5±1.2mm (range 0.5-5.9mm; n=50); hexactin (Fig. 4D): ray length 3.4±1.4mm (range 1.2-7.2mm; n=50), ray width 39.3±7.9µm (range 22.8-63.2µm; n=50). Sceptres (Fig. 4A), restricted to marginal fringe, have mainly smooth shafts when mature, with varying degrees of proclined spination on distal extremity just proximal to the tip which bears the axial cross (centrum); the proximal tenth usually roughened; smaller (younger) sceptres entirely spined; length 12.2±2.4mm (range 7.6-19.4mm; n=50), width 41.3±9.9µm (range 19.2-58.6µm; n=50). Uncinates (Fig. 4B) with very low barbs,

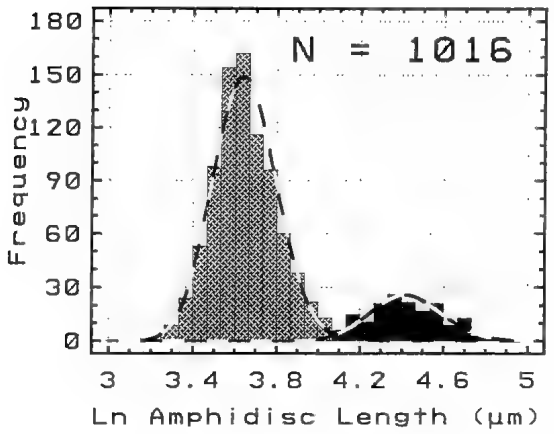

FIG. 5. Frequency distribution of amphidisc length (natural logarithm) from *Poliopogon mendocino* sp. nov. Holotype.

not projecting from spicule profile, also restricted to marginal fringe; length 6.1±1.3mm (range 3.6-9.0mm; n=50), width 20.4±3.8µm (range 8.3-29.1µm; n=50).

Mesoscleres. Dermal and gastral pinnular pentactins (Fig. 4E) similar but differ significantly in all dimensions ($P<0.05$); pinnulus has cylindrical profile and basals are long, moderately spined throughout, straight, taper uniformly to a sharp tip, and cross at right angles; no evidence of ray curvature or 'figure-8' form. Dermalia: pinnulus length 226±25µm (range 180-292µm; n=50), pinnulus total width 47±5.1µm (range 36.3-56.3µm; n=50), basal ray length 148±11µm (range 124-171µm; n=50), basal ray width 9.6±1.3µm (range 6.8-12.0µm; n=50). Gastralia: pinnulus length 204±24µm (range 145-238µm; n=50), pinnulus total width 42.9±5.7µm (range 26.2-53.8µm; n=50), basal ray length 128±13µm (range 104-155µm; n=50), basal ray width 8.7±1.1µm (range 6.6-12.2µm; n=50). Mesohexactins (Fig. 4G) exceedingly abundant throughout entire wall thickness; rays perfectly straight, regularly arrayed, strongly spined and highly variable in size and robustness; ray length 84±20µm (range 49-144µm; n=50), ray width (excluding spines) 6.3±2.5µm (range 3.1-12.2µm; n=50). Spiny mesomonactins (Fig. 4F) occur throughout body wall in low numbers, probably representing extreme reduction of mesohexactins; length 152±29µm (range 105-243µm; n=50), width at head (excluding spines) 12.9±4.2µm (range 7.3-25.9µm; n=50).

Microscleres. Amphidiscs of a single shape occur in large numbers in surface layers and throughout wall; frequency distribution (Fig. 5) shows two distinct size classes. Mesamphidiscs (Fig. 4H) have uniformly spined shafts and umbels with 8 round-tipped tines, slightly in-turned at tips, length 85±15µm (range 61-131µm; n=166), width 22±3µm (range 14-35µm; n=100). Micramphidiscs (Fig. 4I) similar but umbels carry 11-14 tines, length 39±6µm (range 26-60µm; n=850), width 12±1.5µm (range 9-18µm; n=100). Hexadiscs (Fig. 4J) rare; umbels differ from those of micramphidiscs and invariably differ in size on the two ends of each axis; diameter 33±5µm (range 26-44µm; n=35).

REMARKS. This species differs qualitatively from the other two members of the genus, *P. amadou* and *P. maitai*, in body form, lack of microdiactins and perpendicular junction of pinnule basal rays. It also differs from them in size of largest amphidiscs, and pinnulus ray length. The closer relative of *P. mendocino* appears to be *P. maitai*, the form also occurring in the northern Pacific basin, but detailed similarity cannot be assessed on the basis of the sparse data so far available on *P. maitai*.

Subclass **Hexasterophora** Schulze, 1886
Order **Lyssacinosa** Zittel, 1877
(sensu Ijima 1927)
Family **Euplectellidae** Gray, 1867
Subfamily **Corbitellinae** Ijima, 1902
Nubicaulus gen. nov.

TYPE SPECIES. *Nubicaulus careyi* sp. nov.

ETYMOLOGY. Descriptive combination from Greek: *nubis* = cloud and *caulus* = stalk; the cloud-stalk or stalked cloud sponge.

DIAGNOSIS. Body form a cup on a long, thin, hollow stalk. Principalia are diactins and hexactins. Dermalia and gastralia are pinnulate hexactins. Microscleres are drepanocomes, spirodiscohexasters, and aspidoplumicomes.

DISTRIBUTION. Known only from the type locality of the type species: Mendocino Ridge off Cape Mendocino, N. California, U.S.A. (Fig. 1).

Nubicaulus careyi sp. nov.
(Figs 2B, 6A-G, 7A-F)

MATERIAL. HOLOTYPE: CASIZ 113632: Mendocino Ridge, 300 km w of Cape Mendocino, northern California, 40°22.5'N, 128°08.4'W, 23.ix.1995, 2,074m depth, coll. A.G. Carey, Jr., US Navy Deep Submergence Advanced

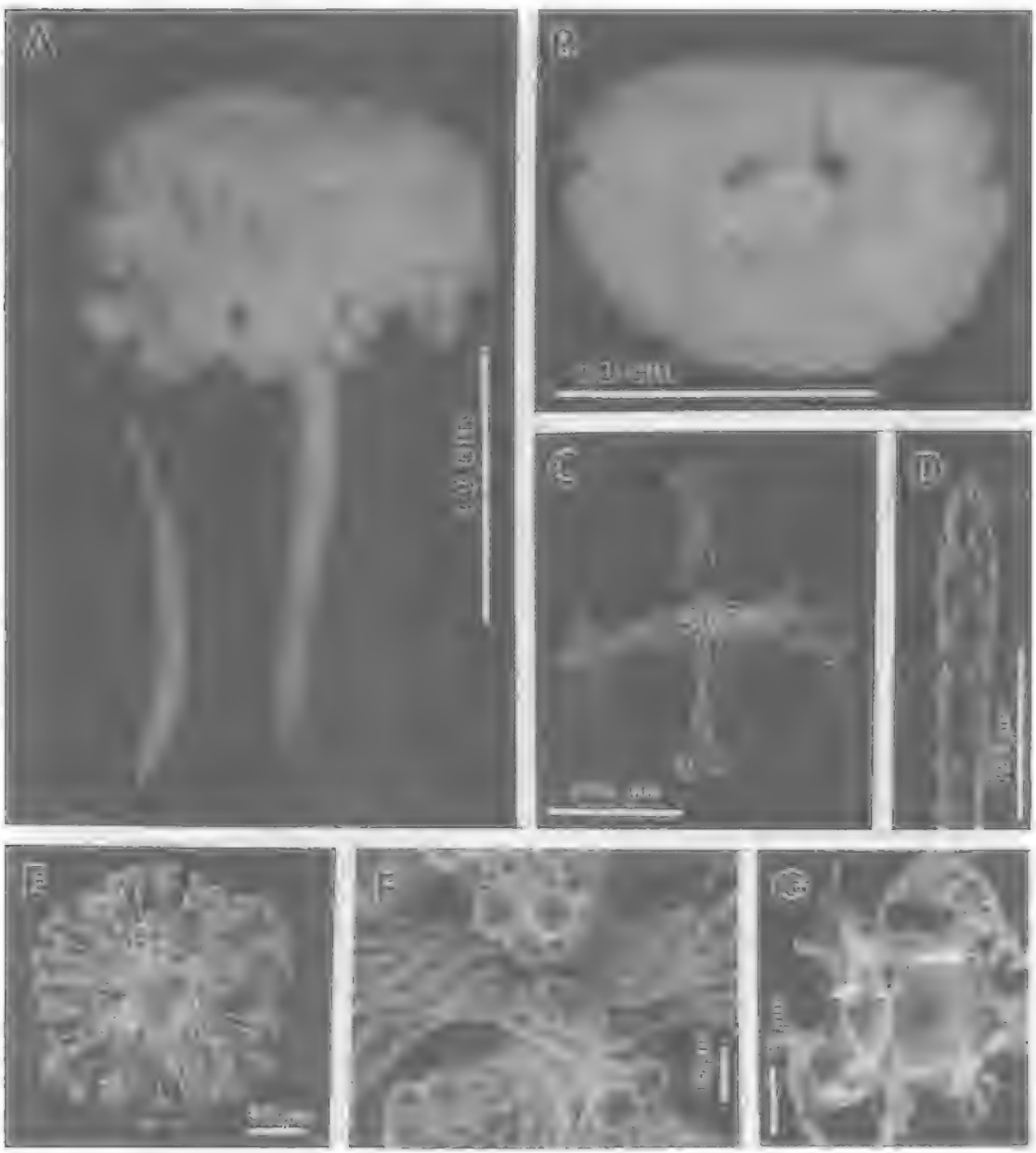

FIG. 6. *Nubicaulus caryi* sp. nov. Holotype, specimen and spicules (SEM). A, Recovered sample in lateral view. B, View of the distal surface. C, Drepanocome. D, Distal pinnulus tip of a dermal pinnule. E, Spirodiscohexaster. F, Magnified view of central secondary ray bundles showing counterclockwise spiralling. G, Aspidoplumicome showing secondary rays originating in a single marginal whorl.

Tethered Vehicle from R/V *Laney Chouest*, stn. MRF 5-10, dive no. 95-54-155 (Fig. 1).

ETYMOLOGY. Named in acknowledgment of the extensive effort and accomplishment made by the collector, Andrew G. Carey, Jr., in his numerous surveys of the NE Pacific deep-water benthos.

DESCRIPTION. *Shape.* White *in situ*, nearly spherical goblet, 15cm diam., on a very long, thin stalk, estimated from video records as 60cm long, attached to hard bottom. During collection most of stalk left in place and terminal 2-3cm of main body lost during manipulation (Fig. 2B). Dried

recovered specimen has basic form of a calyx (body) with convoluted surface on a long, thin, hollow stalk (Fig. 6A-B). Squat, slightly laterally flattened calyx is 14.7x10.5cm diam., 9.4cm tall. Large superior upper opening, 4.2cm diam., is the upper margin of a cylindrical atrial cavity, the osculum proper and its marginal structures lost during collection. Atrial cavity extends axially 5cm down into body dividing into 4 large, radial, exhalant canals, 1.7cm diam., separated by broad tissue septa, and into stalk lumen aperture located on a central conical prominence on floor of atrium (Fig. 6B). Smaller exhalant canals, 0.4-1.0cm diam. extend radially from lateral atrial walls and four main exhalant canals deep into diverticula of lateral and inferior body wall. Diverticula are manifest on external body surface as softly rounded protrusions, 0.8-2.5cm diam., often joined as ridges which circumscribe deep embayments of outer surface; protrusions and ridges exhibit no regular arrangement. On lower third of body, protrusions lengthen to 4.2cm, and terminate in parietal oscula 0.5-0.8cm diam. Proper body wall only 3.3-4.1mm thick at any point, but due to protrusions and their anastomosis, the convoluted and cavernous wall is effectively 3.5-4.3cm thick. A thin, delicate hydrozoan colony permeates entire wall of body, with unprotected terminal polyps located on all sponge body surfaces: external, atrial and larger exhalant canals.

Two recovered stalk pieces total 23.3cm long, ca. 40% of entire stalk in place; tapers from base of body, 1.5cm diam., to lower broken end, 1.1cm diam. Stalk wall thickness is uniform, 1.0mm. All recovered stalk living (sponge tissues present throughout).

Colour. White alive (video tape) and dried.

Skeleton. Dermal surface of body smooth and covered by a tight quadratic lattice of pinnular hexactins of 200µm mesh. Immediately below are openings of inhalant canals, mean diameter 0.6mm (range 0.5-0.9mm). Spirodiscohexasters abundant in, and just below, dermal layer. Gastral surface not covered by a spicule lattice, but consists of open ends of small calibre exhalant canals, mean diam. 1.2mm (range 0.6-2.0mm). Ridges between adjacent canal apertures carry 2-4 ranks of overlapping pinnular hexactins, pinnular rays spaced 150µm apart; ridges densely tufted. Ridges supported by conspicuous bundles of diactins coursing sinuously between exhalant canals in plane parallel to, but just below, aperture margins. Parenchyme supported by loose network of macrohexactins and diactins, the latter as single spicules or in small bundles of 2-6 spicules. No discernable layering or orientation of parenchymal megascleres.

Stalk skeleton mainly composed of tightly synapticula-joined diactins, oriented randomly with respect to stalk axis; diacts oriented longitudinally only in outermost layer. A few loose pinnules remain on outer surface, but most lost by abrasion during collection and subsequent handling. Some hexactine pinnules fused into outer layer by synapticulae. Internal surface without free gastralia; their absence not attributable to abrasion.

Megascleres. All megascleres sparsely and inconspicuously spined throughout, not apparent below 100x magnifications. Principalia are parenchymal diactins and hexactins. Diactins (Fig 7A) smooth (at low magnification), thin, with 4 conspicuous central tubercles; tips rounded or parabolic, not densely microspined; length 2.78±0.66mm (range 1.18-5.06mm; n=100), width 11.5±2.1µm (range 7.1-18.2µm; n=100). Parenchymal hexactins (Fig. 7C) have thin, often curved rays, ray length 738±239µm (range 258-1,341µm; n=100), ray width 11.8±2.0µm (range 5.3-16.7µm; n=100). Dermalia and gastralia are pinnulate hexactins; most have long, sharp-tipped, spindle-form pinnulus (Figs 6D, 7B, left) but 10% of dermalia have shorter, blunt-tipped, club-shaped pinnulus (Fig. 7B right); dermal and gastral spicules significantly different in dimensions (t-test; $P<0.05$). Dermalia pinnulus length 496±32µm (range 392-595µm; n=100), tangential ray length 203±33µm (range 116-297µm; n=100), proximal ray length 373±106µm (range 106-604µm; n=100). Gastralia pinnulus length 478±67µm (range 338-730µm; n=100), tangential ray length 319±75µm (range 193-655µm; n=100), proximal ray length 502±158µm (range 122-841µm; n=100).

Microscleres. Spirodiscohexasters (Figs 6E, 7E) usually spherical in aspect but secondary bundles often restricted in angular splay and central-most secondaries are longer than peripherals, producing noticeable cruciate profile. Secondaries number 15-24 and spiral is sinistral (counterclockwise) (Fig 6F). Terminal discs hemispherical with 15-20 marginal teeth, occurring most commonly in and near dermal and gastral surfaces, but found throughout wall and in stalk; diameter: 147±13µm (range 118-176µm; n=100). Drepanocomes (Figs 6C, 7D) are large oxyhexasters with recurved secondary rays (hooks); secondaries 4-8, occasionally branched

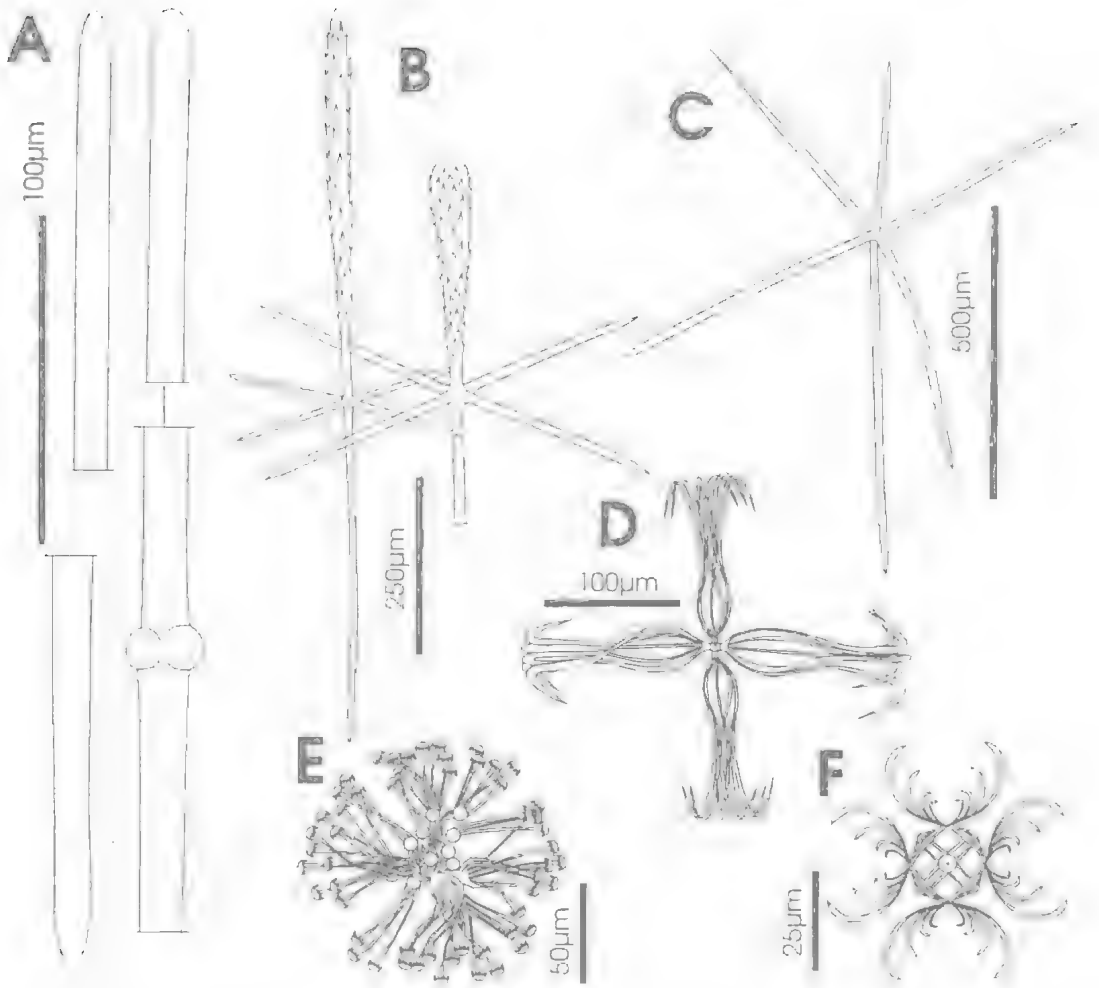

FIG. 7. *Nubicaulus caryi* sp. nov., spicules of the Holotype. A, Diactins. B, Dermal and gastral pinnular hexactins. C, Macrohexactin. D, Drepanocome, two rays perpendicular to the page omitted. E, Spirodiscohexaster. F, Aspidoplumicome, two rays perpendicular to the page omitted.

at terminal bend; uncommon, occuring in stalk and throughout body wall, but not closely associated with surface layers. Body drepanocomes slightly smaller than those of stalk (t-test; $P<0.01$). Body drepanocome: diameter 265±40µm (range 142-329µm; n=68); stalk drepanocome: diameter 297±19µm (range 234-326µm; n=38). Aspidoplumicomes (Figs 6G, 7C) delicate hexasters with shield-like primary terminations nearly contacting adjacent shields. About 60 secondaries emanate from each primary shield in a single marginal whorl but differ in length and angle of curvature, thereby forming a series of 4-5 apparent 'layers' when seen in profile (Fig. 7F); distributed throughout body wall and stalk. Diameter 65±6µm (range 50-76µm; n=72), primary ray length (to distal surface of shield) 11.2±0.9µm (range 8.9-14.2µm; n=87).

REMARKS. Drepanocomes have been reported from three monospecific genera of Corbitellinae, *Dictyaulus* Schulze, *Hertwigia* Schmidt, and *Trachycaulus* Schulze, and from a single Euplectellinae, *Holascus belyaevi* Koltun, 1970. The new species differs from *Dictyaulus* in at least 10 significant characters, including (in *Dictyaulus*): gastral pentactins, floricomes, codonhexasters, non-spiral discohexasters. The

genus *Hertwigia* differs in at least four characters including its possession of gastral pentactins, floricomes, oxyhexasters, and codonhexasters, all of which are absent in *Nubicaulus careyi*. Koltun's *H. belyaevi* differs from *N. careyi* in at least seven characters, including its tubular (*Euplectella*-like) body form, possession of tetractins as principalia, oxyhexasters, oxyhexactins, and graphiocomes as well as absence of spirodiscohexasters and aspidoplumicomes. Thus the new species, *N. careyi*, shows little affinity with any of these taxa. The poorly known genus and species, *Trachycaulus gurlitti*, consists entirely of only two short sections of hollow stem totalling 9cm length, collected by the 'Challenger' in the mid-southern Pacific Ocean (Schulze, 1887). Schulze reported its spiculation as including pinnular hexactins, parenchymal hexactins, and drepanocomes, all compatible with the new species. He also reported simple oxyhexasters which were never figured and are here considered to have been a mistaken earlier statement from Schulze's original description (Schulze, 1886). The holotype of *T. gurlitti* (BMNH 1187.10.20.42) was re-examined using filtration methods to recover small pieces of spicules lodged in the stalk framework. It was found to contain, in addition to the spicules reported by Schulze, two classes of codonhexasters. There was no indication of the presence of any other microsclere such as plumicomes, graphiocomes, floricomes, etc. If any of these were present in the remaining stem tissues, their secondary rays or parts of them, would certainly have been detected by this method. The presence of codonhexasters and absence of spirodiscohexasters and aspidoplumicomes in *Trachycaulus gurlitti* are considered sufficient differences to prevent inclusion of the new species in that genus. This new information on *Trachycaulus*, which is added to its revised diagnosis (below), pertains to a suggestion by Lévi (1964) that *T. gurlitti* be synonymised with *Hertwigia falcifera*. The combination of dermal pinnular hexactins, drepanocomes and codonhexasters is shared by both of these species, but the same are also shared by *Dictyaulus elegans*. The absence of any evidence of floricomes in *T. gurlitti*, as well as its geographic location, argue against Lévi's suggestion. For the present, it is recommended that both the genus and species represented by *T. gurlitti* be maintained as distinct, but still poorly known, entities.

Trachycaulus Schulze, 1886

TYPE SPECIES. *Trachycaulus gurlittii* Schulze, 1886:46.

DIAGNOSIS (based on re-inspection of holotype BMNH 1887.10.20.42, and modification of the description summary by Schulze, 1887:373). Corbitellinae with principalia as long diactins and thin oxyhexactins. Known only as a hollow stalk; upper body remains unknown. Diactins arranged in parallel longitudinal series in stalk and united by profuse synapticula. Dermalia are thin pinnular hexactins; gastralia unknown. Microscleres include large drepanocomes with 4 secondary rays per primary, and two classes of small codonhexasters.

DISTRIBUTION. Mid south Pacific, 4665m depth.

ACKNOWLEDGEMENTS

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THE SPONGES OF PARAISO NEARSHORE FRINGE REEF, COZUMEL, MEXICO. *Memoirs of the Queensland Museum 44: 508. 1999:-* Although sponges as a group are an easily recognisable life form, in ecological studies the identification of individual species of this phylum can be problematic. The objective of this study was to identify and describe the sponge species of the Paraiso nearshore fringing reef off the island of Cozumel, Mexico. A survey of sponges living within an 80m x 40m permanent study site was conducted using underwater video. Sponge tissue samples were also collected. A field guide based on morphological characteristics was compiled describing 42 different sponges, representing 9 orders, 18 families and 21 genera of the class Demospongiae. Comparing the results of this study with earlier descriptions of the diversity of this sponge community indicate the importance of correct sponge identifications for accurate evaluation of changes in reef community structure. The results of this study suggest that regional identification guides are necessary for life forms such as sponges that have a plastic morphology that can be dramatically affected by environmentally induced variables. ☐ *Porifera, taxonomy, species list, reefs, biodiversity, Cozumel, Mexico, field guide.*

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EXPRESSION OF HOMEOBOX-CONTAINING GENES IN FRESHWATER SPONGES

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Homeoboxes have been particularly valuable to identify genes involved in development. This prompted us to look for homeobox-containing genes in sponges, the most primitive metazoans, and to explore the potential role of these genes in sponge development. Using RT-PCR, we have shown that two homeobox-containing genes, *EmH-3* and *prox1* are present in five freshwater sponge species: *Ephydatia muelleri, E. fluviatilis, Spongilla lacustris, Eunapius fragilis* and *Trochospongilla horrida. EmH-3* is expressed differentially during gemmule germination and hatching in *E. muelleri* as well as in *E. fluviatilis*. The expression pattern of *EmH-3* suggests a role during cell differentiation. Hydroxyurea, which specifically blocks the differentiation of choanocytes and the aquiferous system, seems not to affect the expression pattern of *EmH-3*. Contrary to *EmH-3*, prox1 is expressed almost at the same level throughout development. ❐ *Porifera, homeobox-containing genes, development, expression.*

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Homeobox-containing genes are important developmental genes that play a central role in the early development of a variety of organisms. It was thought for a time that they were only involved in spatial and temporal organisation in segmented animals, whereas it is now known that they are also active in non-segmental organisms and systems, and are implicated in axial patterning and cell-fate decisions during differentiation (Davidson, 1995; Dolecki et al., 1986; Garcia et al., 1993; Lawrence & Morata, 1994; Salser & Kenyon, 1996).

Homeobox-containing genes have been identified throughout the animal kingdom, from primitive phyla such as cnidarians, nematodes, flatworms and more recently sponges, to chordates (Ruddle et al., 1994). They have been isolated both from freshwater sponges (Coutinho et al., 1994; Richelle et al., 1998; Seimiya et al., 1994; Seimiya et al., 1997), and from marine sponges (Degnan et al., 1995; Kruse et al., 1994). The presence of homeobox-containing genes in Porifera is of particular interest, and of evolutionary significance, as sponges are considered to be the most primitive metazoans: they do not display any type of symmetry nor polarity, nor do they contain distinct organs or a nervous system. Therefore, elucidating the structure, function and role of homeobox-containing genes in sponges is essential to comprehend the evolution of these genes in metazoans.

As previously reported, we have isolated and sequenced three homeobox-containing genes: *EfH-1* and *EfH-2* from *Ephydatia fluviatilis* using the PCR reaction and degenerated *Antennapedia* primers (Coutinho et al., 1994), and *EmH-3* from *Ephydatia muelleri* by screening an *E. muelleri* genomic library with *EfH-1* (Richelle et al., 1998).

The nucleotide and predicted amino acid sequences of *EfH-1* and *EfH-2* are very different whereas *EfH-1* is very similar to *EmH-3* (85%-86%).

The comparison of *EfH-1* and *EmH-3* homeodomains with all known sponge homeodomains *prox1*, *prox2*, *prox3* from *E. fluviatilis* (Seimiya et al., 1994), *SHOX* from *Geodia cydonium* (Kruse et al., 1994), *SpoxTA1* from *Tethya aurantia*, and *SpoxH1* and *SpoxH2* from *Haliclona* sp. (Degnan et al., 1995) has revealed the highest similarity with *prox2* and *SpoxTA1* (Table1). *EfH-1* and *EmH-3* share a lesser degree of similarity with *prox3* and *prox1*, and are not more closely related to them than to *Cnox3*, *Cnox2* and *Cnox1* from *Hydra* (Schummer et al., 1992; Shenk et al., 1993). They exhibit only a low level of similarity (19%) with *SHOX* homeodomain which seems at present not to belong to homeobox genes as it does not contain the critical sequence of standard homeodomains (Seimiya et al., 1998).

TABLE 1. Levels of similarity between sponge and hydra homeodomains in percent of identical amino acids including conservative substitutions.

Gene	*Efh-1* (37 aa)	*EmH-3*
prox2	92	98
SpoxTA1 (23aa)	96	96
prox3	73	70
SpoxH2 (23aa)	74	70
prox1	67	70
SpoxH1 (23aa)	52	52
SHOX	19	17
Cnox3	70	68
Cnox2	70	66
Cnox1	65	60

Phylogenetic studies have shown that *EmH-3* is closer to metazoan homeodomains than to those of yeast/ fungi and plants (Richelle et al., 1998). *EfH-1*, *EmH3*, *prox2* and probably *SpoxTA1* are representatives of the *Hox11/ Om* (1D) class; *prox1*, *prox3* and *SpoxH2* representatives of the *NK-3*, *msh* and *Chox7* class respectively and *SpoxH1* may be a representative of the *Antp*-class (Degnan et al., 1995). Nevertheless, no clustered homeobox genes have yet been reported in sponges.

A realignment of msh related genes by Master et al. (1996) has indicated that all four *NK*-class homeoboxes from *D. melanogaster* clustered with the sponge homeoboxes *prox1*, *prox2* and *prox3* to the exclusion of all other homeodomain family. The *NK* family is a large widespread family of non-clustered genes that appears to have been conserved throughout the evolution of animals and may be involved in specifying cell fate rather than specifying regional patterns (Shenk & Steele, 1993).

In the present study, we investigate the occurrence of *EmH-3* and *prox1* genes in three freshwater sponge species, common in Belgium: *Spongilla lacustris*, *Eunapius fragilis* and *Trochospongilla horrida* in addition to *E. fluviatilis* and *E muelleri* from which they where initially isolated. The expression of these genes was followed during gemmule germination and hatching. The effect of hydroxyurea on the expression of *EmH-3* was analyzed.

MATERIALS AND METHODS

SPONGE CULTURE. sponges were raised in the laboratory from gemmules in Petri dishes filled with sterile mineral medium (Rasmont, 1961) and incubated at 20°C. For some experiments, they were grown in mineral medium containing hydroxyurea at a final concentration of 100µg/ml (HU-medium). Finally some sponges were cultivated in the field. For this purpose, six-day-old sponges, hatched from gemmules on glass plates, were transferred to the outflow of a pond and were allowed to grow for several weeks.

RT-PCR EXPERIMENTS. This sensitive method for the detection and estimation of the levels of RNA transcripts was applied to analyse *EmH-3* gene expression during development. The expression of two other genes was followed in the same conditions: *prox1* homeobox-containing gene isolated from *E. fluviatilis*, known to be expressed at all stages of development for comparison (Seimiya et al., 1994); *EmA 1* actin gene isolated from *E. muelleri* as a control (Ducy, 1993). Total RNA was extracted at different stages of development, from gemmules to functional sponges, using TRIzol reagent as described in the instructions for use (Life Technologies). Before hatching, gemmules were collected and ground in a Potter homogeniser, on ice, in the presence of TRIzol reagent. After hatching, sponges were scraped and mechanically dissociated by pipetting. The gemmules were discarded, the dissociated cells were pelleted by low speed centrifugation (500g, 10mins, 4°C) and resuspended in TRIzol reagent. For sponges grown in the field, a small piece of the sponge was squeezed in cold mineral medium and the cells dissociated as described for laboratory sponge cultures. The quantity and purity of RNA was estimated by optical absorbance at 260nm and 280nm according to standard procedures (Sambrook et al., 1989). Its quality was checked on an agarose gel.

RT-PCR reactions were carried out using the Promega single tube, two-enzyme Access RT-PCR System which provides quick and reproducible analysis of even rare RNAs. All components necessary for RT-PCR were mixed in one tube with 10ng of total RNA and reverse transcription was automaticaly followed by PCR cycling without additional steps according to the manufacturer's protocol. The conditions were: 1 cycle of 45mins at 48°C; 1 cycle of 2mins at 94°C; 40 amplification cycles: 30sec at 94°C, 1min at 55°C (EmH-3, EmA 1) or 57°C (prox1) and 2mins at 68°C; followed by a final extension cycle of 7mins at 68°C. The amplification products were analysed by agarose gel (1%) electrophoresis of 10% of the total reaction.

The primers were supplied by Eurogenetec (Belgium). They were all gene specific and designed to flanked introns in order to discriminate between products that had been amplified from RNA and those that had been amplified from DNA. 1) For the study of *EmH-3*, the upstream primer, 5'-ATGGACAACTGCAGGG GTGA-3', was complementary to nt 1-20 of the first exon of the genomic sequence and the downstream primer, 5'-CATTCTCCTATTTTG GAACC-3', was complementary to nt 716-736 of the third exon containing the homeobox. 2) For the study of *prox1*, primers were those chosen by the authors Seimiya et al. (1994): the upstream primer, 5'-GGACAGATACGCTTCCGATCT-3', was complementary to nt 19-39 of the genomic sequence of the first exon and the downstream primer, 5'-ATATCGTCTGTTCTGAAA CCA -3', was complementary to nt 347-367 of the second exon. 3) For the study of *EmA 1*: the upstream primer, 5'-AACTGGGACGACATGG AGAA-3', was complementary to nt 15-35 of the published *EmA 1* Actin cDNA sequence (Ducy, 1993) and the downstream primer, 5'-GATCCA GACACTGTACTTGC-3', was complementary to nt 787-807. According to the author, there must be at least one intron between the sequences chosen for the two primers.

The nature of the amplified products was checked by digestion with specific restriction enzymes. The length of transcripts were: about 440bp for *EmH-3*, 240bp for *prox1*, 390bp for *prox2* and 792bp for *EmA 1*.

RESULTS

Gemmules hatched after 3-4 days incubation according to the species. Subsequently, choanocytes and aquiferous system became differentiated and the osculum appeared around 4-5 days of incubation. Seven-day-old sponges were considered to be fully functional.

In HU-medium, hatching was postponed by about 2 days and sponges had a typical hollow-dome structure (Rozenfeld & Rasmont, 1976). Neither choanocytes nor an aquiferous system were differentiated.

The investigation of *EmH-3* and *prox1* in *S. lacustris*, *E. fragilis*, *T. horrida* indicated that these genes were expressed in fully functional sponges in the three species as in *E. muelleri* and *E. fluviatilis* (Fig. 1). However, as far as *EmH-3* expression was concerned, there was a noticeable difference between the length of *E. fluviatilis* transcripts and those of *S. lacustris*, *E. fragilis* and *T. horrida* (Fig. 1A). The latter were

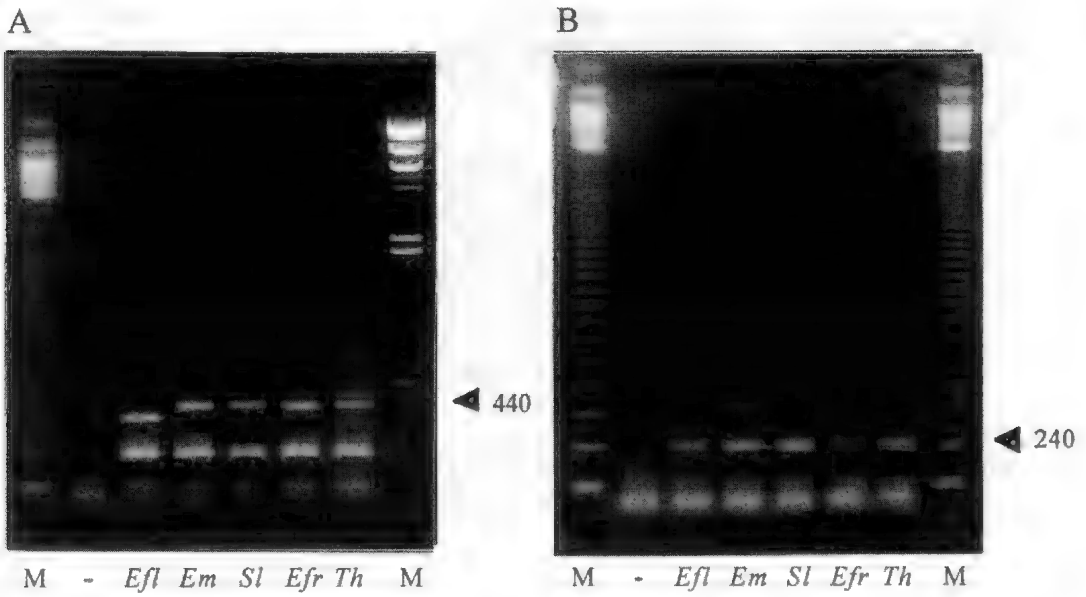

FIG.1. Expression of homeobox-containing genes in five freshwater species. Amplified products of RT-PCR of total RNA isolated from 7-day-old sponges. A, expression of *EmH-3* gene. B, expression of *prox1* gene. Abbreviations: *Efl=Ephydatia fluviatilis*; *Em=Ephydatia muelleri*; *Sl=Spongilla lacustris*; *Efr=Eunapius fragilis*; *Th=Trochospongilla horrida*; - = negative control without RNA template; M=molecular-size marker. Arrows indicate the size in bp of the amplified products for each gene.

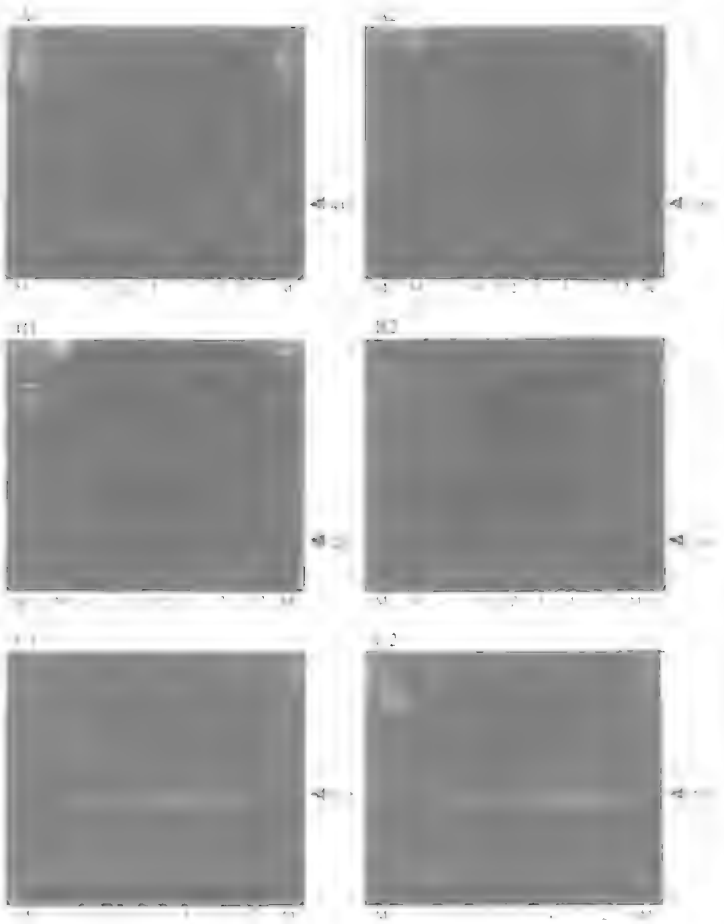

FIG.2. Expression of homeobox-containing genes in the course of development. Amplified products of RT-PCR of total RNA isolated from gemmules to the formation of fully functional sponges. A,Expression of *EmH-3*. B, Expression of *prox1*. C, Control, expression of *EmA 1* (Actin gene from *E. muelleri*). 1=*E. muelleri*; 2=*E. fluviatilis*. Developmental stages are expressed as days after incubation at 20°C in mineral medium; Abbreviations: - =negative control without RNA template; M=molecular-size marker. Arrows indicate the size in bp of the amplified products for each gene.

approximately 440bp long, the same size as *E. muelleri* transcripts and about 50bp longer than *E. fluviatilis* transcripts. *E. fluviatilis* transcripts were 390bp long, the expected *prox2* transcript size according to Seimiya et al. (1994).

We noticed also that *prox1* was expressed at a slightly lower level in *E. fragilis* (Fig. 1B).

The study of the temporal expression of *EmH-3*, *prox1*, and *EmA 1*, summarised in Figure 2, reveals a clear-cut difference in the level and pattern of expression of these genes, though the absolute amounts of expression cannot been directly compared from one gene to another because of possible differences in amplification efficiency between the different sets of primers.

EmH-3 gene was expressed differentially in the course of development in both species (Fig. 2, A1 and A2). In gemmules, transcripts were present in very small amounts as they were almost undetectable by RT-PCR. The level of expression increased very slightly until hatching, 3 days and 4 days of incubation, respectively. At that time, a high level of expression was observed. This level was maintained during several days, even in sponges transferred to the field for three weeks (27-day-old sponges).

On the other hand, *prox1* gene appeared to be expressed at nearly the same level throughout development: transcripts were already discernible in the gemmules and their level varied little although a slight enhancement could be detected at the moment of hatching (Fig. 2, B1 and B2).

In the control set of experiments, *EmA 1* Actin gene from *E. muelleri* (Ducy, 1993), was strongly expressed at all stages of development (Fig. 2, C1 and C2).

In HU-treated sponges, the evolution of the expression of *EmH-3* was roughly the same as in non-treated sponges (Fig. 3). The level of transcripts, very low during the first days of incubation reached already high values one day before hatching (6th day of incubation). Actin expression was high at all stages.

DISCUSSION

The results of the RT-PCR survey of *EmH-3* and *prox1* in 5 freshwater species corroborate previous Southern hybridisations realised with *EfH-1* as a probe (Richelle et al., 1995). They indicate that *S. lacustris*, *E. fragilis* and *T.*

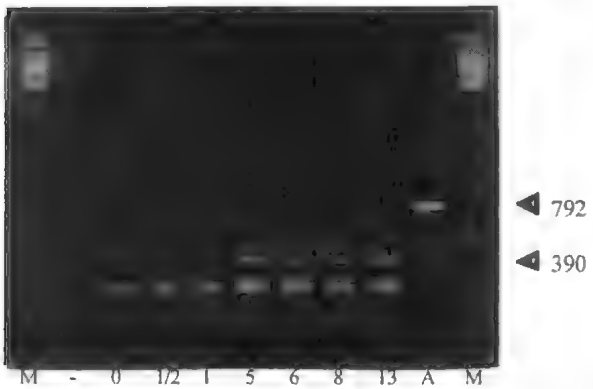

FIG. 3. Expression of *EmH-3* in hydroxyurea-treated sponges in the course of development. Amplified products of RT-PCR of total RNA isolated from gemmules to the formation of fully functional sponges in *E. fluviatilis*. Developmental stages are expressed as days after incubation at 20°C in mineral medium. Abbreviations: A=Expression of *EmA 1* (Actin gene from *E. muelleri*); -=negative control without RNA template; M=molecular-size marker. Arrows indicate the size in bp of the amplified products for each gene.

horrida possess an *EmH-3-like* gene but that this gene differs in structure from the *E. fluviatilis EfH-1/ prox2* gene. This is clearly evidenced by the difference in length of their transcripts. This difference could be explained by a differential splicing as is the case for *E. muelleri* the first exon of which is 54bp longer than that of *E. fluviatilis* (Richelle et al., 1998).

The presence of an *EmH-3*-like gene in 5 species of freshwater sponges together with the high identity of sequence with *SpoxTA1* from *Tethya aurantia*, a marine sponge, may indicate that this type of gene could be widespread among Porifera and could represent one of their ancestral homeobox-containing gene. This hypothesis is supported by the data of Larroux & Degnan (1999), showing that a *prox2*-like gene is present in two other marine sponge species, *Iotrocota baculifera* and *Tedania digitata*.

On the contrary, prox1 although present in the 5 species of freshwater sponges, does not show a high degree of similarity with other sponge homeobox-containing genes isolated to date.

The temporal pattern of expression of *EmH-3* clearly demonstrates a differential expression of *EmH3* gene during gemmule germination and hatching. The enhancement of the expression at the moment of hatching suggests that this gene is particularly involved at that stage of development and provides evidence for a role in cell-fate decisions during differentiation. Actually, at hatching, all cells began to differentiate from the undifferentiated gemmular archaeocytes in a definite sequence: first the pinacocytes and the sclerocytes, then the choanocytes which arise by repeated divisions undergone by the archaeocytes (Rasmont & Rozenfeld, 1981). The persistence of the expression of *EmH-3* in the adult sponge is probably related to the continuous replacement and/ or differentiation of cells occurring in the organism, in particular the turnover of the choanocytes (Rozenfeld & Rasmont, 1976).

In their work, Seimiya et al. (1994) concluded that *prox2* transcripts were identified at all stages of differentiation in *E. fluviatilis*. This discrepancy with our results arises from the fact that the authors studied only one undefined stage before hatching and that obviously, as demonstrated by our results, the main events occur during gemmule hatching.

On the other hand, the kinetics of expression of *prox1* show that this gene is expressed almost at the same level at all stages of development in *E. muelleri* as in *E. fluviatilis*.

In HU-treated sponges, the overall pattern of expression of *EmH-3* is similar to that in untreated sponges. The time-dependent increase in expression of *EmH-3* is not delayed in the presence of hydroxyurea, even though hatching is delayed to day 6 rather than day 4. Thus, in contrast to control sponges, the increased expression of *EmH-3* in HU-treated sponges appears to precede hatching, since it occurs before the migration of the cells through the micropyle. Consequently, it would be interesting to determine if differentiation processes observed in control sponges at hatching have already started in unhatched HU-treated gemmules.

These experiments are of special interest because hydroxyurea inhibit the differentiation of only one type of cells, i.e. the choanocytes, the other cell types being insensitive to its action. In addition, HU-blocked sponges provide a suitable source for the isolation of pure populations of embryonic archaeocytes that can be brought to differentiate and achieve normal development by removal of hydroxyurea from the medium (Rozenfeld & Rasmont, 1976).

Indeed, to gain more understanding of the role played by *EmH-3* and *prox1* in sponge development, it would be essential to determine what

happens when archaeocytes differentiate into other cells, in particular into choanocytes but also to determine in which cells these genes are expressed.

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ORIGIN AND EARLY FOSSIL RECORD OF SPONGES - A GEOBIOLOGICAL APPROACH. *Memoirs of the Queensland Museum 44: 515. 1999*:- The Porifera are Precambrian active filter feeding metazoans which exhibit a reproductive strategy as known from the Eumetazoa. However, most morphological characters of the sponges differ from those of the Eumetazoa. The defining unique character of Porifera is the possession of aggregates of choanocytes, which demonstrate a phylogenetic relationship with the protozoan taxon Choanoflagellata (Reitner & Mehl, 1996). Sponges have various amounts of symbiotic bacteria (e.g. Reitner, 1993; Schumann-Kindel et al., 1996, 1997)which control metabolic processes. As an hypothesis, sponges originated from biofilms which were associated with choanoflagellates. The first remains of sponges are known from Middle Proterozoic (1.8bya) blackshales (biomarker C30-sterane, 24-isopropylcholestane) (Moldowan et al., 1994; Thiel et al 1999). First spicules and entirely preserved sponge bodies are known from the Late Proterozoic (Ediacaran, various microbialite reefs) (Steiner et al., 1993; Gehling & Rigby, 1996). In the early Cambrian all main groups of sponges were known to exist, including the Calcarea (Reitner & Mehl, 1995). During the Phanerozoic six major sponge events are noticed. The first one is represented by the development of the Archaeocyaths in the Lower Cambrian. The occurrence of typical stromatoporoids started in the Ordovician. Rigid hexactinellids are known since the Late Devonian. First modern demosponge taxa occurred after the Late Devonian extinction event. Modern types of coralline sponges, e.g. Ceratoporellida, occured in the Permian, and have an optimum diversity in the Late Triassic. The last significant development is seen in the Jurassic - starting point of the fresh water sponges - when some marine taxa (Haploscerlida: Poecilosclerida: ?Hadromerida) moved into fresh water environments. Sponges were important in reef buildung, and many are still specialised reef dwelling organisms. Their importance as main reef buildung organisms decreased in the Late Jurassic - Lower Cretaceous, when fast growing modern zooxanthellate corals became more important. ❐ *Porifera, symbionts, reef-building sponges, fossil sponges.*

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TAPHONOMY AND PRESERVATION POTENTIAL OF SPONGE TISSUE. *Memoirs of the Queensland Museum 44: 516. 1999*:- The preservation potential of sponge tissues is mainly controlled by sponge related bacteria (Reitner, 1993; Reitner & Neuweiler, 1995). *In situ* hybridization of the associated microbial populations in few modern demosponges (*Petrosia, Chondrosia*) showed that the majority of bacteria are members of the gamma-subclass of Proteobacteria (Schumann-Kindel et al., 1996, 1997). Using highly specific oligonucleotide probes for detecting sulfate-reducing bacteria, distinct signals were found scattered in native sponge tissue of both investigated sponges. Also, other fermentative bacteria are involved in the degradation of sponge tissue. Sulfate reducing bacteria may control the calcification of the sponge tissue during degradation, increasing the carbonate alkalinity (Schumann-Kindel et al., 1997; Reitner & Schumann-Kindel, 1997). Therefore, isolated pyrite crystals are common in mineralized (automicritic) sponges tissues. In the surrounding sediment, pyrite is absent or rare. The sponge tissue automicrites are often dark-coloured due to statistically distributed very fine pyrite crystals (ca. 1µm diameter). Besides the small pyrite, larger crystals often exhibit patchy concentrations or they are arranged in rows. Pyrite formation is probably linked with sulfate reducing symbiotic bacteria in the sponge mesohyle. During early decaying processes of the sponge tissue the internal sponge space becomes entirely anaerobic which favours the growth of the sulfate reducing bacteria.

This process may explain the rapid calcification of sponge tissue in modern marine microbialites and ancient sponge mud mounds. In mud mounds siliceous sponges contribute to buildup development with considerable amounts of sponge body-related micrite produced in place. These sponge container automicrites form during the biodegradation of soft tissues, resulting in various 'classical' microbial fabrics. The initial formation of carbonate crystals is controlled by reactive organic compounds (macromolecules) during conditions of elevated carbonate alkalinity (ammonification) (Reitner, 1993; Reitner et al., 1995). The resulting carbonate microfabrics correlate with different soft tissue precursors (mesohyle). The mesohyle structure varies from: bacteria-containing (minipeloidal); bacteria-bearing, rich in choanocyte chambers (peloidal); to bacteria-poor or syncytial structures (aphanites). Intermediate reactive states of organic matter also lead to the *in situ* preservation of non-rigid demosponges, which are recognized by spicular architecture, spatially restricted occurrences of unsorted spicules, or even by spicule bearing minipeloids, peloids or aphanites. Principally non-spicule bearing sponges should be recognized by the outer (e.g. nodular) shape of microbial fabrics. Organically induced automicrites (organomicrites) are high-Mg calcites with an inorganic signature of $\delta^{13}C$ (+3 to +4). The enhanced identification of an autochthonous sponge fauna within mud mounds provides new insight into the nature and origin of these structures. Semi-quantitive data of Cretaceous mounds reveal that 50-80% of mound micrites were produced in place from which up to 60% of automicrites can be related to metazoans. Therefore, the origin of reactive organic matter is the crucial point to evaluate the pure microbial vs metazoan character of Paleozoic and Mesozoic mud mounds, as well as Precambrian micrites within biostromal and biohermal deposits (Reitner & Arp, 1999). ❐ *Porifera, mud-mounds, micrites.*

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MORPHOLOGICAL PHYLOGENETIC CONSIDERATIONS ON THE RELATIONSHIPS OF *ISODICTYA* BOWERBANK, 1864

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Isodictya Bowerbank was recently transferred from the Order Poecilosclerida to the Order Haplosclerida, based upon the hypothesis that skeletal architecture and spiculation are homologous between *Isodictya* and some genera in the haplosclerid family Niphatidae. We examined this hypothesis by determining the diagnostic morphological characters of *Isodictya*, comparing these with a selection of poecilosclerid and haplosclerid genera, which also have reticulate spongin skeletons. A phylogenetic analysis of diagnostic morphological characters such as spicule morphology, choanosomal skeletal architecture, and surface fibre ornamentation was carried out to determine the ordinal affinities of *Isodictya*. The analysis of this data set produced 21 equally parsimonious trees of 29 steps with a consistency index (C.I) of 1.000 and retention index (RI) of 1.000. Major characters that separate *Isodictya* from the haplosclerid genera include the nature of the surface skeletal outgrowths, the amount of spongin associated with these outgrowths, the absence of a paratangential skeleton, the presence of chelae, and the presence of small cigar-shaped oxeas. Results strongly suggest the retention of *Isodictya* and allied taxa *Cercidochela* and *Esperiopsis* within the Order Poecilosclerida. ❐ *Porifera, Isodictya, Poecilosclerida, Haplosclerida, phylogeny.*

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The order Poecilosclerida is the largest and most taxonomically difficult and unstable of all Demospongiae (Van Soest, 1984; Bergquist & Fromont, 1988; Hajdu et al., 1994a); at present there is little consensus on family composition and classification. One major debate in poecilosclerid classification has questioned the integrity of Desmacididae Schmidt, 1870 (also incorrectly known as Desmacidonidae Gray, 1872). It is now considered to be polyphyletic (Van Soest, 1984; Hajdu et al., 1994a,b) because the family contains species with monactinal and/or diactinal megascleres, with myxillid, mycalid, and microcionid skeletal architecture, and in some genera sand replaces the megascleres. *Isodictya* Bowerbank, 1864 has been traditionally placed in this family because it contains diactinal megascleres in a reticulate skeleton and possesses chelae. Recent rearrangement of the Desmacididae by Van Soest (1984), Bergquist & Fromont (1988) and Hajdu et al. (1994a), resulted in the transfer of all desmacidid genera to other poecilosclerid families, leaving *Isodictya* unassigned.

Recently Van Soest (1987) and de Weerdt (1989) postulated that Desmacididae was a sister group of Haplosclerida, with the primary synapomorphy being the presence of small oxeas (100-250µm) (de Weerdt, 1989). Hajdu et al. (1994a ,b) subsequently transferred *Isodictya* to the order Haplosclerida arguing that the skeletal architecture is homologous to that of genera in the haplosclerid family Niphatidae. The presence of chelae in *Isodictya*, which are absent in all haplosclerids as presently defined, was considered by Hajdu et al. (1994b) to have been secondarily lost in other haplosclerids, *Isodictya* alone retaining this plesiomorphic character. A comprehensive historical overview of this problem is given in Hadju et al. (1994a) and Bergquist and Fromont (1988).

The validity of this transfer rests on the question of whether oxeas and the pattern of their reticulation in *Isodictya* and the Haplosclerida are truly homologous. Our objectives were firstly to determine the diagnostic morphological characters for *Isodictya*, and then to consider this genus in the light of other poecilosclerid genera

TABLE 1. Material examined and locality data for *Isodictya* spp., *Esperiopsis informis* Kirkpatrick, *Cercidochela lankesteri* Kirkpatrick, *Niphates* spp., *Amphimedon* spp., *Cribrochalina* spp.

Species	Registration Number	Locality
Isodictya palmata	BMNH 1830.7.3.381	NW. Atlantic
Isodictya multiformis	BMNH 1997.5.12.18	Ouderkraal, South Africa
Isodictya sp.	BMNH 1895.6.8.140	Indo-Pacific
Esperiopsis informis	BMNH 1997.5.12.30	Ouderkraal, South Africa
Cercidochela lankesteri	BMNH 1826.10.26.179	Winter Quarters, Antarctica
Niphates digitalis	BMNH 1928.5.12.202	Bahamas
Niphates sp.	0CDN 4130-X	Micronesia
Amphimedon compressa	BMNH 1928.5.12.921	St Thomas
Amphimedon sp.	0CDN 4249-C	Micronesia
Cribrochalina vasculum	BMNH 1997.3.20.1	Bahamas
Cribrochalina sp.	0CDN 4159-G	Micronesia

such as *Esperiopsis* and *Cercidochela*. Finally, a selection of reticulate spongin skeletons in haplosclerid genera were examined and compared to those in species of *Isodictya*.

MATERIALS AND METHODS

Collections were made by the authors using SCUBA unless otherwise stated. Methods of collection, preservation, histological preparation for light microscopy examination, and scanning electron microscopy, were carried out according to Bergquist & Kelly-Borges (1995). Material examined and considered in this study is listed in Table 1. Taxa for comparison with *Isodictya* and allied genera were selected on the basis of their superficial similarity to haplosclerid genera. Genera in the haplosclerid families Chalinidae, Callyspongiidae, and Oceanapiidae were not considered here as their reticulate skeletons are quite separate from the Niphatidae whose genera have been strongly compared to *Isodictya*. Abbreviations:BMNH, Natural History Museum, London; 0CDN, Specimen sample numbers for the United States National Cancer Institute shallow-water collection programme contracted to the Coral Reef Research Foundation, Micronesia. Twelve morphological characters (Table 2) were identified from direct examination of the specimens listed in Table 1. Parallelism may be quite a common feature in sponges (de Weerdt, 1989), at least when simple characters such as consistency, colour and habitats are concerned. We have avoided this by excluding them from the phylogenetic analysis. Each character was scored for each species in a taxon/character data matrix (Table 3). The data were analysed using PAUP Version 3.1.1 (Swofford, 1993). Characters were coded as unordered and multistate and were unweighted. Wagner parsimony (Kluge & Farris, 1969) was used as it minimises evolutionary steps by making no assumptions about the direction of character changes. Analyses were performed using an exhaustive search to find the minimum length trees. *Halichondria moorei* (Halichondrida, Halichondriidae) was defined as the outgroup, data obtained from Bergquist (1970). One hundred bootstrap replicates (Felsenstein, 1985) were carried out to provide confidence estimates on groups contained in the most parsimonious trees.

RESULTS

Phylogenetic analysis produced 21 equally parsimonious trees of 29 steps with a consistency index (CI) of 1.000, a retention index (RI) of 1.000, and homoplasy index (HI) of 0.000 (Fig. 1A,B). Even under the hypothesis of polymorphisms for multiple character states, the CI was 1.000. The advantage of using multiple states with the hypothesis of polymorphism is that this procedure permits the detection of hidden homoplasies and reversions in a study at the generic level, which otherwise would be omitted by exclusively affecting isolated species in each genus (Maldonado, 1993).

Isodictya spp. form a monophyletic clade in all 21 equally parsimonious trees, with essentially the same arrangement of species in each tree (Fig. 1). *Cercidochela lankesteri* is grouped with *Esperiopsis* and *Isodictya* in 15 of the 21 reconstructions, but in the remaining 6 trees the position of *Isodictya* is unresolved with respect to *Cercidochela* and *Esperiopsis*. The overall position of *Isodictya* is stable within the poecilosclerid clade, and this is clearly separated from haplosclerid genera which also form a distinct, yet internally unresolved clade. Removing *Cercidochela* from the analysis resolves *Isodictya* spp. as a single clade that it is more closely related to *Esperiopsis informis* than to haplosclerid genera. A strict consensus tree yielded a polytomy between the different orders but *Isodictya* remains clearly separated from Niphatidae (Fig. 2).

TABLE 2. Characters and character states of *Isodictya* spp., *Esperiopsis informis* Kirkpatrick, *Cercidochela lankesteri* Kirkpatrick, *Niphates* spp., *Amphimedon* spp., *Cribrochalina* spp.

CHARACTERS 1-2. GENERAL SKELETAL STRUCTURE: **1**. Body compression: a. three-dimensional b. planar; **2**. General skeletal organisation: a. plumoreticulate with interstitial isodictyal reticulation, b. square-meshed reticulation; c. confused halichondroid.

CHARACTERS 3-7. FIBRE DEVELOPMENT: **3**. Primary fibres: a. small square-meshed reticulation, b. large polygonal reticulation, c. fine plumose fibres, d. robust plumose fibres, e. Absent; **4.** Secondary fibres: a. regular spongin-bound ladder-like fibres, b. irregular fascicular spongin-bound fibres, c. primary fibres bridged by single spicules and tracts, d. primary fibres bridged by semi-isodictyal reticulation of spicules, e. absent; **5.** Mesh shape: a. small square, b. large square, c. large elongate, d. small irregular, e. absent; **6**. Ornamentation associated with termination of primary fibre: a. low blunt conule, b. large spiky conule, c. plumose tuft, d. absent; **7**. Spongin development in primary fibres: a. spongin joining spicules; b. spongin entirely enclosing fibres; c. absent.

CHARACTER 8. ECTOSOME: **8**. Ectosome (between primary fibres): a. tangential fibres, b. ectosomal brushes, c. tangential detachable ectosome.

CHARACTER 9. MEGASCLERES: **9**. Morphology: a. small hastate oxeas, uniformly thick; b. small centrally angulate and thickened fusiform oxeas; c. styles, d. large fusiform oxeas.

CHARACTER 10. MICROSCLERES: **10**. Chelae: a. palmate isochelae, normal form; b. palmate isochelae, modified; c. canonochelae; d. absent.

CHARACTER 11. BIOCHEMISTRY: **11**. Manzamine alkaloids: a. present; b. absent. (Magnier & Langlois, 1998)
CHARACTER 12. REPRODUCTION: **12**. Viviparity: a. present; b. Absent.

Isodictya palmata (Fig. 3A-D) and *Isodictya* sp. are characterised primarily by a plumoreticulate skeleton (character 2a) (Fig. 3B) with tufted surface outgrowths (character 6a), the possession of small fusiform oxeas (character 12c) which are often angulate and thickened centrally (Fig. 3C), and palmate isochelae (character 13a) (Fig. 3D). *Isodictya multiformis* is separated from other species of *Isodictya* by the nature of the secondary fibres (character 4b->c), the differences in mesh shape and size (character 5a->b) and morphology of the palmate isochelae (character 13a->b).

Esperiopsis informis (Fig. 3L-N) and *Cercidochela lankesteri* (Fig. 3H-K) are joined in a common clade linked by the possession of an irregular anastomosing interstitial network (character 3d) (Fig. 3I,M), absent in all other taxa except *Isodictya*. These two genera form a common clade with *Isodictya* spp. sharing plumose surface outgrowths (character 6a), absence of special dermal skeleton found only in Niphatidae, and isochelae. Canonochelae (Fig. 3K) are unique to *Cercidochela lankesteri* (character 13a->c) while *Esperiopsis* has palmate isochelae (character 13a) (Fig. 3H). *Esperiopsis informis* is unique in this analysis as it has styles (character 12a->d).

TABLE 3. Character state matrix. Characters and states are described in Table 2. For certain characters some taxa may not logically possess a given state, or the authors are unsure of the character state assignment, in which case these character states are coded as 'unknown' which is indicated by '?'; * = the outgroup.

Species	Character											
	1	2	3	4	5	6	7	8	9	10	11	12
Isodictya palmata	b	a	c	c	c	c	a	b	b	b	b	a
Isodictya multiformis	b	a	c	c	d	c	c	b	b	a	b	a
Isodictya sp.	b	a	c	c	c	c	a	b	b	a	b	a
Esperiopsis informis	b	a	c	d	d	c	c	b	c	a	b	a
Cercidochela lankestri	b	a	d	d	d	c	c	b	d	c	b	a
Niphates digitalis	a	b	b	b	b	b	b	a	a	d	a	a
Niphates sp	a	b	b	b	b	b	b	a	a	d	a	a
Cribrochalina sp	a	b	a	a	a	a	b	a	a	d	a	a
Amphimedon sp	a	b	a	a	a	a	b	a	a	d	a	a
Amphimedon compressa	a	b	a	a	a	a	b	a	a	d	a	a
*Halichondria moorei**	?	c	e	e	e	d	c	c	d	d	b	a

Niphatidae are united by several synapomorphies. They all have hastate oxeas (Fig. 3G) (character 12b->a), a paratangential ectosome and surface conules surrounded by spongin (character 7a->b). The interstitial skeleton of Niphatidae is limited to individual spicules rather than an anastomosing network as in the *Isodictya* group. Major characters that separate *Isodictya* from Niphatidae include the nature of the surface skeletal outgrowths (plumose tufts in *Isodictya* and conules in *Niphates*), the amount of spongin associated with these outgrowths in Niphatidae, spongin being absent in

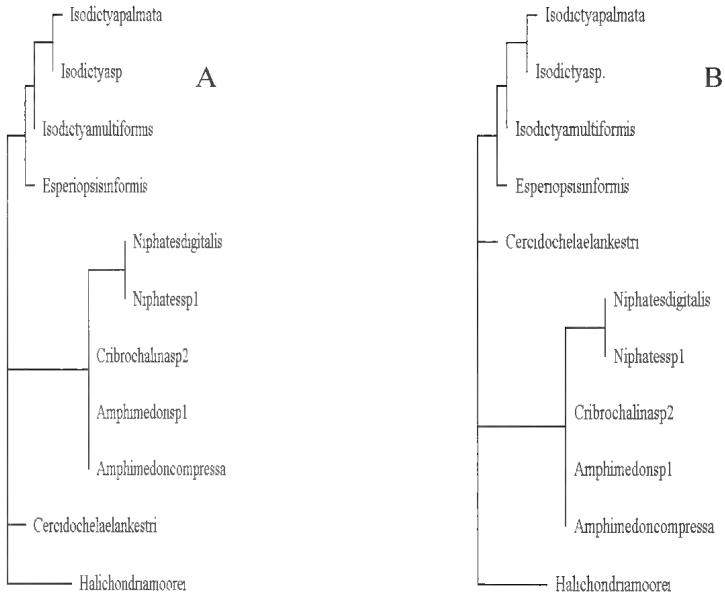

FIG. 1. A-B, Alternative, equally likely hypothetical phylogenies for *Isodictya* spp. with respect to allied genera *Cercidochela* and *Esperiopsis*, and selected haplosclerid genera.

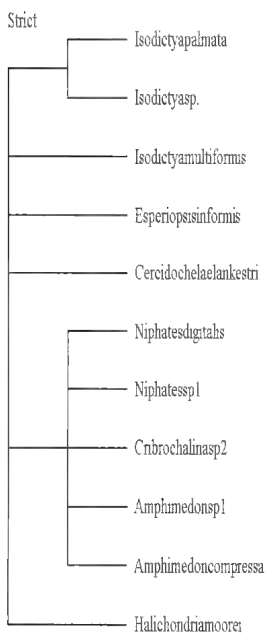

FIG. 2. Strict consenses tree of the 21 MP trees generated from the morphological data for the 12 sponge species.

Isodictya outgrowths, the presence of a paratangential ectosomal skeleton in Niphatidae, the presence of chelae in *Isodictya*, and the presence of small curved hastate oxeas of uniform thickness, in Niphatidae. A bootstrap 50% majority rule consensus cladogram (Fig. 4) provides good support for separation of the *Isodictya* group from niphatid genera, strongly suggesting that they belong to separate orders.

DISCUSSION

The diagnostic character reversal suggested by Hajdu et al. (1994b) as being synapomorphic (viz. loss of chelae) is inconsistent with the present analysis. It is more parsimonious to regard the appearance of chelae in the Poecilosclerida, rather than their loss, as a subsequent achievement in the evolution of this order. Thus, the presence of chelae is a synapomorphic character for the poecilosclerid genera under study. Moreover, although suggested as symplesiomorphic at the species level, palmate

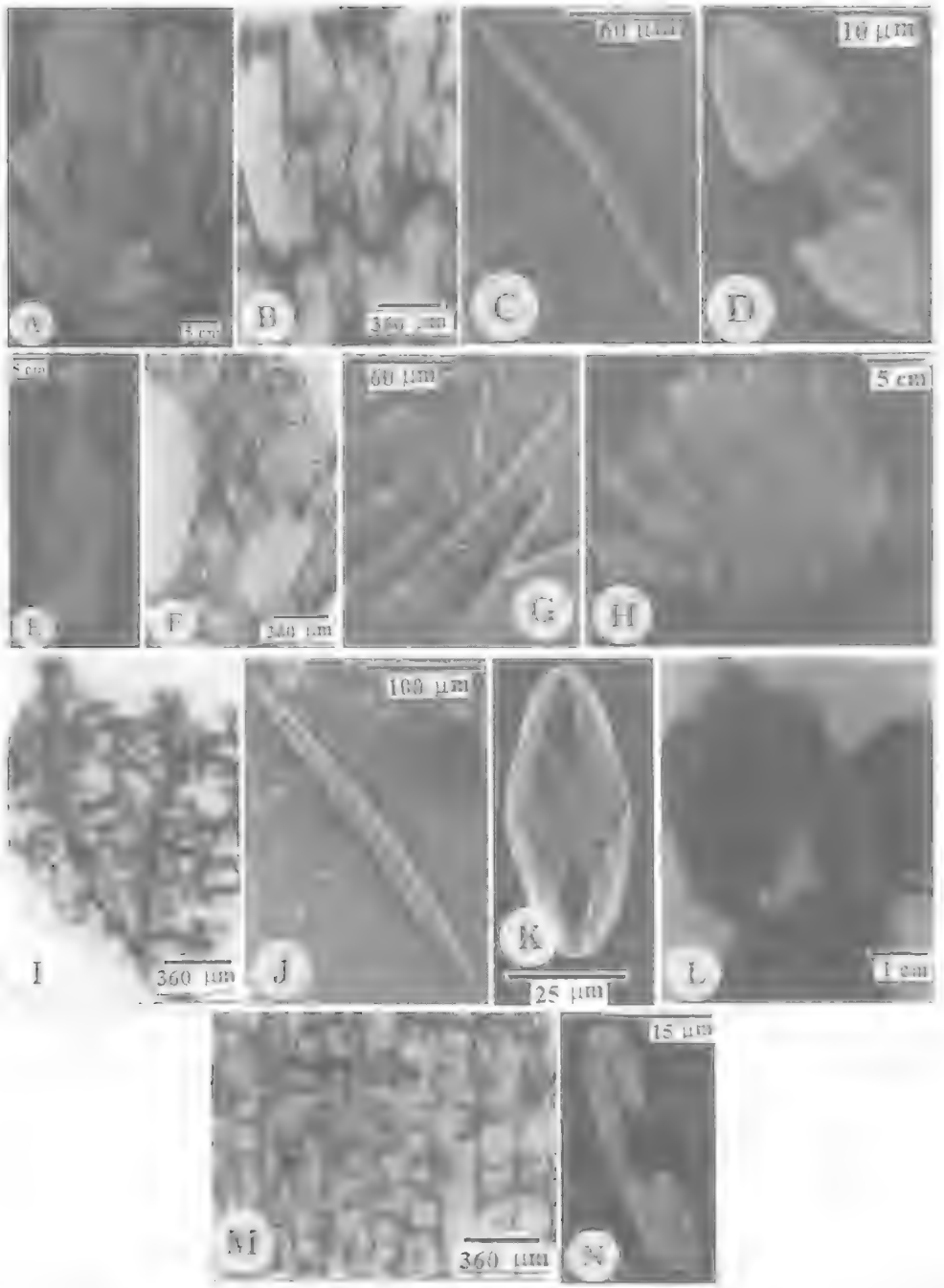

FIG. 3. Morphological characters of *Isodictya*, *Amphimedon*, *Cercidochela* and *Esperiopsis*. A-D, *Isodictya palmata* Bowerbank, 1864 (BMNH 1895.6.8.140). A, Holotype. B, Skeletal architecture (×31). C, Oxea morphology (×500). D, Profile view of palmate isochela (×3,000). E-G, *Amphimedon compressa* Duch. & Mich., 1864 (BMNH 1928.5.12.921). E, Specimen. F, Skeletal architecture (×31). G, Oxea morphology (×700). H-K, *Cercidochela lankesteri* Kirkpatrick, 1906 (BMNH 1826.10.26.179). H, Holotype. I, Skeletal architecture (×31). J, Oxea morphology of (×300). K, Profile view of canonochela (×3,000). L-N, *Esperiopsis informis* Stephens, 1915 (BMNH 1997.5.12.30). L, Specimen. M, Skeletal architecture (×31). N, Profile view of palmate isochela (×3,000)

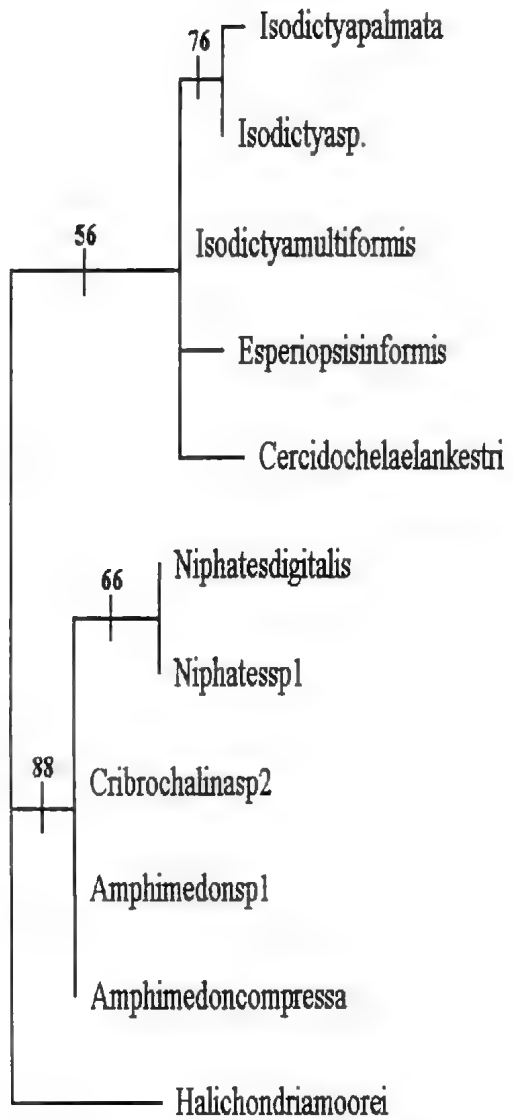

FIG. 4. Bootstrap 50% majority rule tree of the 21 MP trees generated from the morphological data for the 12 sponge species. The percentage of these trees that contain each component is shown along each branch.

isochelae can be treated as a synapomorphy when discussing generic or familial affinities (Hajdu et al., 1994b).

The similarity between the principal morphological characters of *Isodictya*, *Esperiopsis* and *Cercidochela* are striking; the independent development of the skeletal architecture, ocolourea morphology, and chelae morphology is considered to be unlikely. Hajdu et al. (1994b) based their transfer of *Isodictya* to Niphatidae primarily on the presence of what they regarded to be an 'isodictyal' reticulation, and secondarily on the presence of palmate isochelae in *Isodictya*. These microscleres are absent in all other Haplosclerida, and are considered to be simply an underlying synapomorphy to both orders by Hadju et al. (1994a). Close examination of the reticulate skeletons of pertinent genera within these two groups shows here that niphatid skeletons are not isodictyal in the strict sense, a term that should be reserved for genera within the haplosclerid family Chalinidae, and that they are very different from the reticulate skeletons of *Isodictya, Esperiopsis* and *Cercidochela*. Moreover, additional characters examined here, such as the nature of surface ornamentation, megasclere morphology, interstitial spiculation, nature of the actual fibres and the exclusive presence of manzamine alkaloids (Magnier & Langlois, 1998) in the haplosclerid family Niphatidae and Chalinidae add confidence to the separation of these to groups.

Even though palmate isochelae are present in *Esperiopsis*, it is presently placed in the poecilosclerid family Mycalidae whose genera all contain anisochelae. If other skeletal characters are considered, such as the nature of the primary fibres and surface ornamentation, *Isodictya* may also fit within this family (see Hooper, 1997).

Many of these decisions on affinities may be further corroborated and illuminated with additional taxa and additional tools such as molecular systematics. Until further studies are carried out on the detailed nature of the skeletal morphology, spiculation, and chemistry of *Isodictya*, this genus should be retained within Poecilosclerida.

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EFFECTS OF PHOTOSYNTHETIC ACTIVITY IN ENDOSYMBIOTIC ZOOCHLORELLAE ON GEMMULE GERMINATION OF A FRESHWATER SPONGE, *RADIOSPONGILLA CEREBELLATA*. *Memoirs of the Queensland Museum 44: 524. 1999*:- Many of freshwater sponges thrive in oligotrophic clear waters in cooperation with photosynthetic endosymbionts, such as zoochlorellae in their mesenchymal cells. They also withstand unfavourable winter season in dormant forms as gemmules. Annandale sponge *Radiospongilla cerebellata* is a green freshwater demospongiae with zoochlorellae in their archaeocytes and flourishes only in warmer season in southern district of Japan. Gemmules of the Annandale sponge also contain zoochlorellae in thesocytes and germinate only under illumination even if all other conditions are properly provided. Although the light sufficient to induce the germination was very low in intensity and extremely short in illuminating period, photosynthesis seems to be essential for the germination, because a photosynthetic inhibitor, atrazine, strongly inhibited the germination under optimal condition.

Since gemmules of the Annandale sponge contain a rich storage of nutrients in the thesocytes, photosynthetic nutrients, produced during the incubating period under very low intensity and short length of illumination, seem to have little effect on the induction of the gemmule germination. We undertook to observe the effects of other factors on gemmule germination, that is, gaseous components such as oxygen evolved and carbon dioxide consumed by photosynthesis. To accomplish gas experiments, we devised a glass slide with a hollow chamber of $3cm^3$ sealed with a glass plate. In gas experiments, gemmules were placed in the chamber with M-medium (previously boiled to eliminate dissolved gaseous components). The chamber was tightly sealed with a glass plate and the desired amount of gas was introduced to the medium as a bubble under the glass plate. Gemmules were illuminated at 3000 lx through the glass plate by ordinal fluorescent tubes for 10hrs daily and kept at 24°C for 8 days. When gemmules were incubated in degassed medium that was tightly sealed and isolated from the atmospheric gases, no gemmules were germinated. When an air bubble was introduced to the incubating chamber by one tenth or more the volume of the incubation medium 100% germination was achieved.

Of the major elements of air, only oxygen induced germination efficiently, and brought full germination at much less quantity than the air (about one hundredth of the incubating medium in volume). On the other hand, nitrogen and carbon dioxide, showed no effects on the gemmule germination under the optimal condition. On the contrary, carbon dioxide showed strong inhibition of gemmule germination in the oxygenated or aerated media. These results show that gemmules of the Annandale sponge are induced to germinate by cytoplasmic oxygen concentration under the favourable condition, but can be substantially suppressed by carbon dioxide. Thus, it is regarded that light applied to the gemmules would initially promote photosynthesis in the symbiotic chlorellae in the thesocytes, which would absorb cytoplasmic carbon dioxide and block the initiation and/or progression of gemmule germination, and evolve oxygen that promotes gemmules to germinate. To confirm this assumption, we have tried to induce germination in darkness with fully oxygenated and carbon dioxide free media. However, no gemmules have germinated in total darkness, in spite of other optimal conditions.

The failure of germination in darkness can be understood as follows: when optimal temperature and oxygen were provided to the gemmule cells, (which had been dormant under the cold temperature), they seemed to rouse and begin to respire, and generate carbon dioxide. The carbon dioxide could not be eliminated from the cytoplasm readily, due to the lack of photosynthesis under darkness, so its accumulation in the thesocytes appears to inhibit germination of the gemmules. ❐ *Porifera, freshwater sponges, gemmule, germination, symbionts, photosynthesis, O_2, CO_2.*

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TAXONOMIC EVALUATION OF JASPLAKINOLIDE-CONTAINING SPONGES OF THE FAMILY COPPATIIDAE

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Much interest has been generated by the isolation of jasplakinolide (or jaspamide), a novel cyclodepsipeptide originating from a variety of marine sponges, but there have been taxonomic problems in assigning specimens from the South Pacific and Indo-Pacific, possessing a similar spicule composition of oxeas and euasters and sharing parallel chemical profiles. From a comparative study of museum types and recent collections of jasplakinolide-containing sponges in the family Coppatiidae, skeletal and external morphology indicate that only one genus (*Jaspis* Gray) and five species are valid (*J. splendens* (de Laubenfels), *J. digonoxea* (de Laubenfels), *J. johnstoni* (Schmidt), *J. serpentina* Wilson, and a *Jaspis* sp. probably new to science), with the genera *Dorypleres* Sollas and *Zaplethea* de Laubenfels relegated into synonymy. ❐ *Porifera, jasplakinolide, cyclodepsipeptide, Coppatiidae, Jaspis, Dorypleres, Zaplethea.*

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Jasplakinolide (jaspamide) represents a structurally novel compound which has been the focus of several natural products and synthetic studies (Zabriskie et al., 1986; Crews et al., 1986; Braekman et al., 1987; Grieco et al., 1988; Rao et al., 1993; Imaeda et al., 1994). The anticancer activity identified in the NCI 60 cell assay promoted this marine secondary metabolite as a potential therapeutic lead. Of particular value is jasplakinolide's biological profile against PC-3 (human prostate cancer) cells. Although several synthetic derivatives have been examined, jasplakinolide proves to be the most potent agent against cancer models. Mechanism based studies have shown this compound to be a specific actin inhibitor which stabilises microfilaments (Senderowicz et al., 1995). Its potential for drug development may therefore be hindered by its inherent toxicity. However, as a tool for study of the cytoskeleton and the numerous processes mediated by actin in eukaryotes, jasplakinolide provides unique qualities.

Three genera have been used to refer to jasplakinolide-containing sponges, all very closely related morphologically and chemically: *Jaspis* Gray, 1867, *Dorypleres* Sollas, 1888 and *Zaplethea* de Laubenfels, 1950. *Jaspis*, with type species *Vioa johnstoni* Schmidt, 1862, was originally described as having two spicule types: fusiform and stellate (Dendy, 1916), but now contains several disparate species justifying its division into at least two separate genera (Boury Esnault, 1973; Hajdu & Van Soest, 1992). Currently species with oxeas, a single category of euasters, and a confused choanosomal arrangement are included in *Jaspis* (Bergquist, 1968; Wiedenmeyer, 1989). *Dorypleres* contains species like *Jaspis* that have more than one category of asters, but the genus has been used by few authors. Topsent (1904) synonomised *Dorypleres* with *Jaspis*, an action subsequently reversed by de Laubenfels (1954), and reversed again by Bergquist (1968) on the basis that the type species, *D. dendyi* Sollas, lacked two categories of asters. *Zaplethea* includes species with oxeas and euasters, in which the oxeas were characteristically bent (twice-bent), but only one species, *Z. digonoxea* de Laubenfels, 1950, was assigned to it. A report of a jasplakinolide producing sponge from Laing Island (Madang, PNG) refers to *Z. digonoxea* as a synonym of *Jaspis johnstoni* Schmidt, 1862 (Braekman, 1987), implicitly merging the two genera.

At least four taxonomic names have been used for thick encrusting jasplakinolide-containing sponges in the South Pacific and Indo-Pacific with a skeleton of oxeas and euasters: *Jaspis* sp. (Zabriske, 1986; Crews, 1986), *Jaspis johnstoni*, *Zaplethea digonoxea* (Braekman, 1987), and *Dorypleres splendens* (Schmitz and Kelly-Borges, pers. comm.). The present study aims to determine the appropriate generic assignment of

these species and their relative conspecificity. Two other species (*J. serpentina* Wilson, 1925, and an unidentified species referred to here as *Jaspis* sp. 2), have similar spiculation and external morphology to the jasplakinolide-containing species, and are included here as a comparison to these species.

MATERIALS AND METHODS

Specimens were collected from various localities in the South Pacific and Indo-Pacific. All samples were collected using SCUBA at approximate depths of 10-25m. Sponges were preserved in 3.7% formalin for one week, and then transferred and stored in 70% ethanol. Morphological characterisation was made from thick sections (Permount embedded) and spicule preparations of each specimen. Spicule sizes represent mean and range values (minimum and maximum) of the spicule length and width for the oxeas, ray length and width for oxyasters I and II and total diameter for oxyasters III. 15 spicules were measured per spicule type. Special attention was given to surface details in order to assess whether this was a valid taxonomic character for *Dorypleres splendens* in particular.

TABLE 1. List of voucher and type specimens examined (*=specimen known to contain jasplakinolide).

Reference/institution	Material	Locality
USNM		
USNM 23037	*Dorypleres splendens* de Laubenfels 1954	Caroline Islands, Ponape
USNM 21270	*Jaspis serpentina* Wilson 1925	Philippines
USNM 22746	*Zaplethea digonoxea* de Laubenfels 1950	Oahu, Hawaii
MHNG		
LMJG 15256/0	*Vioa johnstoni* Schmidt 1862	Sebenico, Adriatic
LMJG 15257/0	*Vioa johnstoni* Schmidt 1862	Sebenico, Adriatic
LMJG 15258/0	*Vioa johnstoni* Schmidt 1862	Sebenico, Adriatic
SME		
SME E104	*Zaplethea digonoxea diastra* Vacelet & Vasseur 1976	Tuléar, Madagascar
SME Tu 120	*Jaspis cf. johnstoni*	Tuléar, Madagascar
	**Jaspis johnstoni* (*Z. digonoxea*) Braekman 1987	Laing Island, Papua New Guinea
UO		
35-T-93	**Dorypleres splendens*	Palau
UCSC		
91601	**Jaspis* sp.1	Walindi, Papua New Guinea
91629	*Jaspis* sp. 2	Walindi, Papua New Guinea
92102	**Jaspis* sp. 1	Pacific Harbour, Fiji
92204	**Jaspis* sp. 1	Tioman Island, Malaysia
97238	**Jaspis* sp. 1	Madang, Papua New Guinea
92402	**Jaspis* sp. 1	Bali, Indonesia
92405	*Jaspis* sp. 2	Bali, Indonesia
94541	**Jaspis* sp. 1	N Sulawesi, Indonesia
95077	**Jaspis* sp. 1	Milne Bay, Papua New Guinea
96555	**Jaspis* sp. 1	N Sulawesi, Indonesia
96591	*Jaspis* sp. 2	N Sulawesi, Indonesia

Specimens examined are listed in Table 1. These were acquired from the United States National Museum (USNM), the Landes-Museum Joanneum of Graz (on loan to the Museum d'Histoire Naturelle Genève (MHNG)), the Station Marine d'Endoume, Centre d'Oceanologie de Marseille (SME), the University of Oklahoma (UO) and the University of California, Santa Cruz (UCSC).

RESULTS AND DISCUSSION

Of the material examined (Table 1) five species were differentiated on the basis of their external morphology and skeletal structure (Table 2). In all this material there is great similarity in skeletal composition and arrangement (Figs 1-6). All species possess oxeas and euasters, a confused choanosomal skeletal arrangement and paratangential arrangement of small spicules at the surface. The external morphology of *Jaspis* sp. 1, (UCSC collections) *J. johnstoni*/*Z. digonoxea* (Braekman, 1987) and *D. splendens* (both USNM 23037 and UO 35-T-93) is very similar, consisting of an encrusting growth form (2-4cm thick) with oscules on lobate protrusions (1-2cm high). The texture is dry and crumbly and the sponges easily torn. The dry voucher of *J. serpentina* (USNM 21270) is similar except that it appears to have grown away from the substrate, anchored by a stalk (Fig. 3A). External morphology of voucher specimens *Z. digonoxea* (USNM 22746) and *Vioa johnstoni* (LMJG 15258/0, 15256/0) are quite different from the other species described above: they are much more delicate and thinly encrusting (1-3mm thick).

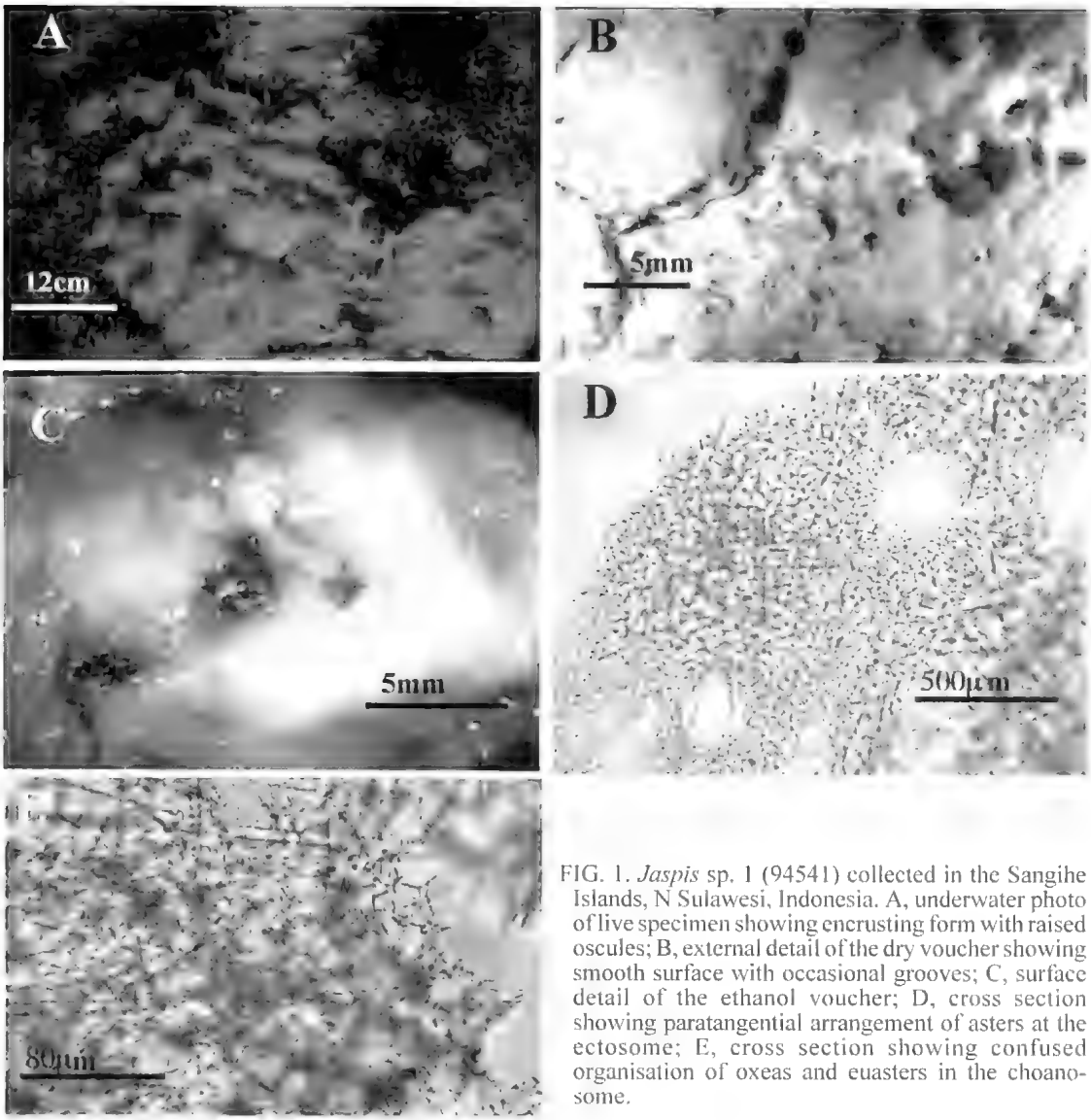

FIG. 1. *Jaspis* sp. 1 (94541) collected in the Sangihe Islands, N Sulawesi, Indonesia. A, underwater photo of live specimen showing encrusting form with raised oscules; B, external detail of the dry voucher showing smooth surface with occasional grooves; C, surface detail of the ethanol voucher; D, cross section showing paratangential arrangement of asters at the ectosome; E, cross section showing confused organisation of oxeas and euasters in the choanosome.

We propose to synonymise all these species under the senior generic name *Jaspis* Gray. The twice-bent oxeas of *Z. digonoxea* de Laubenfels, 1950, are interpreted here as a diagnostic character at the species level only, similar to the serpentine rhabds of *J. serpentina* (Fig. 3B). The proposed assignment of the studied species are: *J. splendens* (de Laubenfels, 1954; Figs 1, 4); *J. digonoxea* (de Laubenfels, 1950; Fig. 5); *J. johnstoni* (Schmidt, 1862; Fig. 6); *J. serpentina* Wilson, 1925, (Fig. 3) and *Jaspis* sp. 2 (Fig. 2), which is probably new to science.

All of the jasplakinolide-containing sponges studied here (Table 1) were found to be conspecific. *Jaspis splendens* (de Laubenfels, 1954) is the senior-most available name for these specimens. The species description requires emendation to de-emphasise the significance of certain surface characteristics given importance by de Laubenfels (1954): conules or protruberances occur as a rather uncommon feature both in the type (Fig. 4B) as well as in more recent collections (Fig. 1B,C).

Figure 2 shows an additional *Jaspis* sp., which although very similar to the others, does not

TABLE 2. Skeletal analysis of specimens of *Jaspis* mentioned in this paper. Measurements given in micrometres as mean (range) of lengths and widths or diameter. 1 = in some specimens this category represents chiasters; 2 = this sample also has serpentine rhabds, 1625 (1550-1700) × 30 (20-40)μm.

Specimen	Reference #	Large oxeas	Small oxeas	Oxyasters I	Oxyasters II	Oxyasters III[1]
Jaspis sp. 1	Crews 92102	741 (530-900) H 8 (5-10)	170 (100-275) H 4 (3-5)	19 (15-30) H 3 (1-4)	16 (13-20)	11 (8-15)
Jaspis sp. 1	Crews 94541	686 (550-780) H 9 (8-10)	130 (88-198) H 3 (2-4)	22 (18-28) H 3 (2-5)	21 (15-28)	8 (5-10)
Jaspis sp. 1	Crews 95077	675 (590-750) H 10 (10)	1222 (80-188) H 3 (2-4)	22 (15-25) H 3 (3)	18 (13-23)	8 (5-10)
Jaspis sp. 1	Crews 96555	637 (520-700) H 10 (10)	120 (78-168) H 3 (2-4)	20 (15-25) H 3 (2-3)	20 (15-30)	10 (8-13)
Jaspis sp. 1	Crews 97238	844 (710-970) H 14 (8-20)	113 (78-160) H 3 (2-5)	23 (18-30) H 3 (3-4)	17 (13-23)	10 (8-13)
Jaspis sp. 2	Crews 96591	777 (650-900) H 11 (10-15)	115 (88-130) H 3 (2-3)	21 (18-28) H 2 (2-3)	19 (15-28)	11 (8-13)
Jaspis sp. 2	Crews 91629	859 (820-920) H 20 (15-25)	156 (105-218) H 5 (3-8)	23 (15-28) H 4 (3-5)	17 (13-20)	10 (8-13)
Dorypleres splendens	Schmitz 35-T-93	665 (600-730) H 9 (7-10)	121 (83-175) H 3 (3-4)	17 (10-23) H 3 (2-3)	13 (8-20)	11 (8-15)
Dorypleres splendens	USNM23037	631 (580-720) H 9 (5-13)	124 (108-175) H 3 (3-5)	19 (13-23) H 3 (2-4)	18 (13-25)	10 (8-13)
Jaspis johnstoni (*Z. digonoxea*)	Braekman, 1987	651 (580-740) H 9 (8-10)	119 (88-163) H 3 (3-4)	18 (13-25) H 2 (2-3)	20 (15-25)	10 (8-13)
Jaspis cf. johnstoni	Vacelet Tu 120	548 (370-920) H 7 (3-15)	98 (73-128) H 3 (2-3)	none	22 (18-30)	11 (8-15)
Zaplethea digonoxea diastra	Vacelet E104	643 (250-1070) H 10 (5-15)	112 (80-148) H 3 (2-4)	none	24 (18-30)	11 (8-15)
Zaplethea digonoxea	USNM22746	751 (560-980) H 10 (5-13)	354 (220-540) H 4 (3-5)	none	21 (15-28)	10 (8-15)
Vioa johnstoni	LMJG15257/0	444 (300-700) H 8 (6-12)	96 (80-110) H 4 (3-5)	none	11 (8-20)	none
Jaspis serpentina	USNM21270[2]	225 (150-350) H 6 (5-8)	81 (63-113) H 3 (2-4)	none	13 (8-20) H 3 (2-5)	8 (6-10)

contain jasplakinolide and differs in external morphology and growth form: it is generally found growing away from the substrate in an encrusting to fan-like form. The topside is very similar to *J. splendens* shown in Fig. 1, but the reverse side is dominated by regularly spaced holes (Fig. 2B). Based on these differences, we suggest that this species is also probably new to science.

CONCLUSIONS

Comparison of type and recently collected material previously assigned to three genera (*Dorypleres splendens*, *Jaspis johnstoni*, *Zaplethea digonoxea*, *Jaspis serpentina*, *Jaspis* sp.) are all referred to *Jaspis*. All *Jaspis*-like species reported in the literature to contain jasplakinolide were examined and referred to *J. splendens* (de Laubenfels), and it is probable that jasplakinolide-containing sponges belong to this species.

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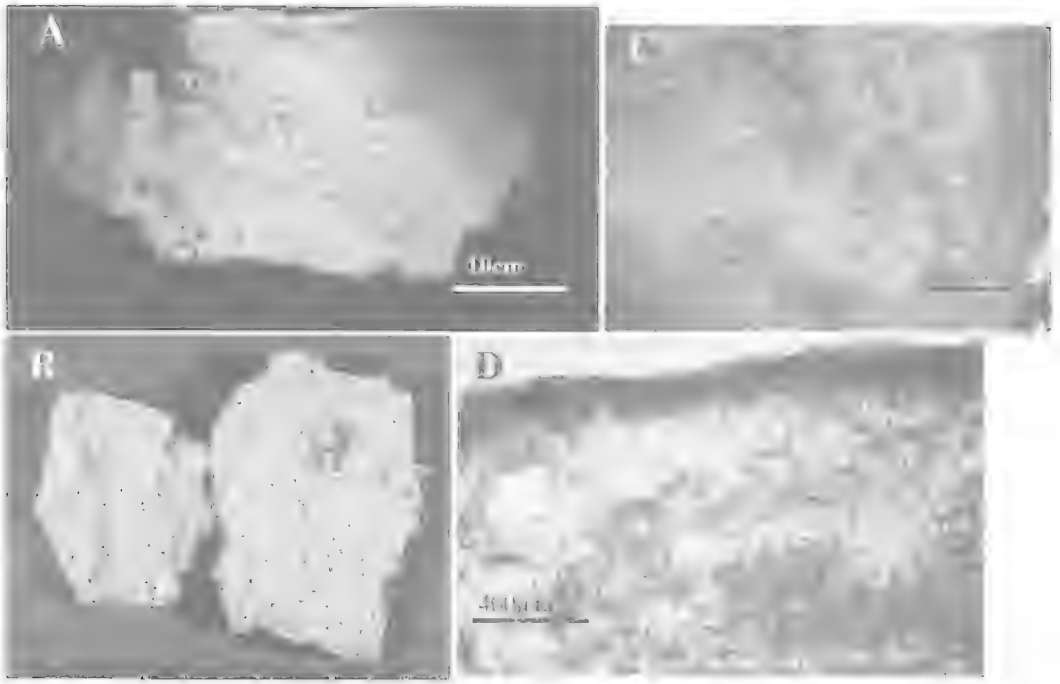

FIG. 2. *Jaspis* sp. 2 (96591) collected at Tifore Island, N Sulawesi, Indonesia. A, underwater photo of live specimen showing encrusting to fan-like morphology; B, external morphology of the dry voucher, topside (left) and underside (right) (scale in cm); C, surface detail of the ethanol voucher; D, cross section showing the concentration of asters at the ectosome and confused organisation of the choanosome.

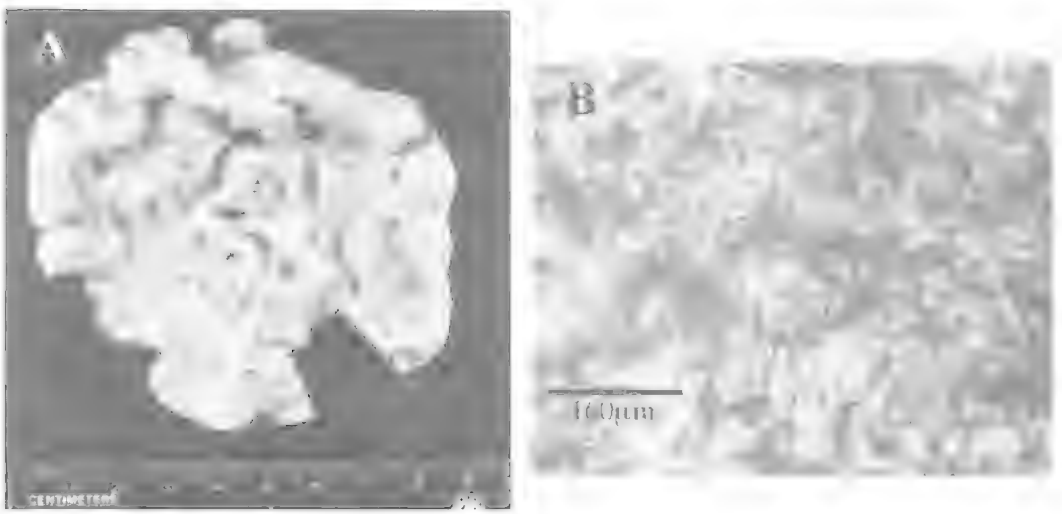

FIG. 3. *Jaspis serpentina* Wilson 1925 (USNM21270). A, dry voucher showing raised oscules and smooth surface; B, cross section of choanosome, dominated by serpentine rhabds.

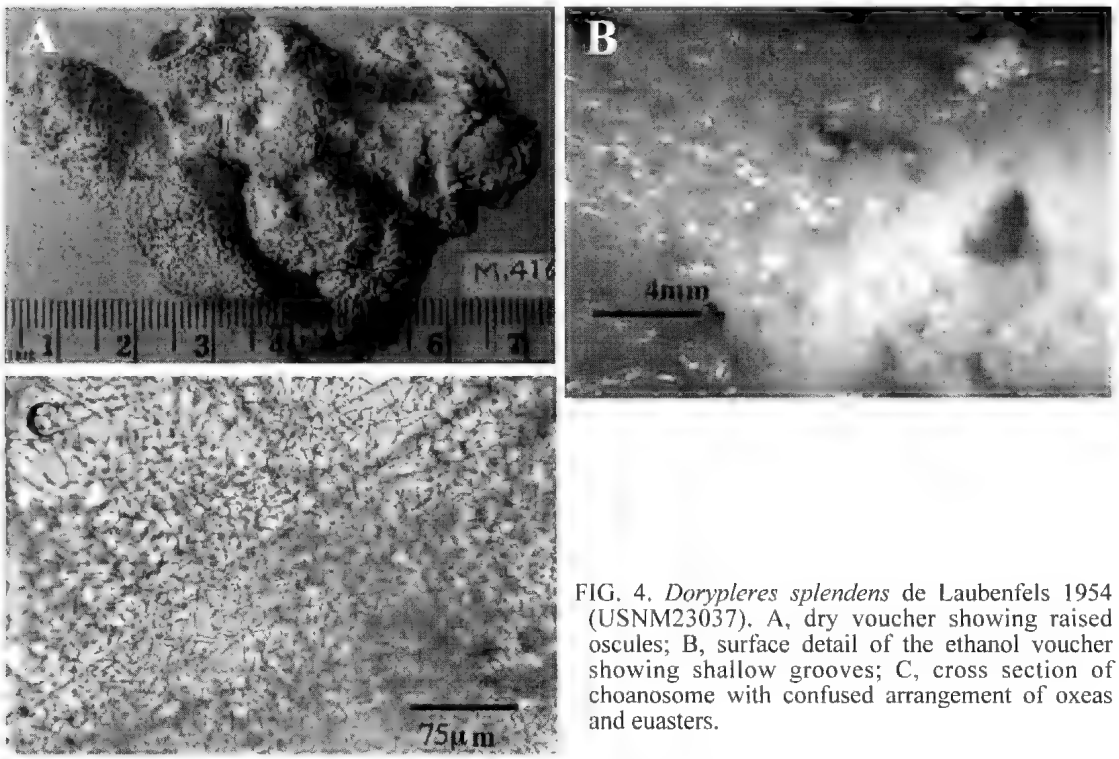

FIG. 4. *Dorypleres splendens* de Laubenfels 1954 (USNM23037). A, dry voucher showing raised oscules; B, surface detail of the ethanol voucher showing shallow grooves; C, cross section of choanosome with confused arrangement of oxeas and euasters.

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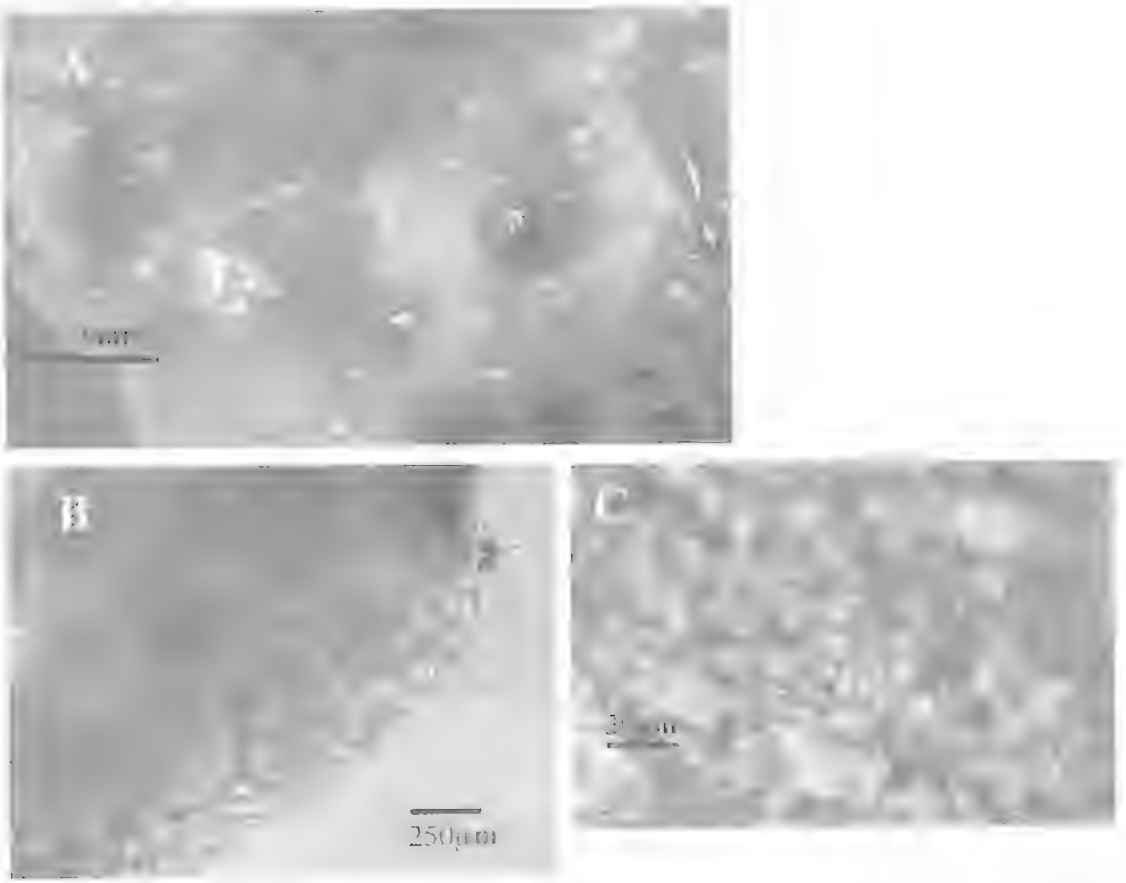

FIG. 5. *Zaplethea digonoxea* de Laubenfels 1950 (USNM22746). A, smooth surface of the ethanol voucher; B, cross section of the ectosome; C, cross section of the choanosome.

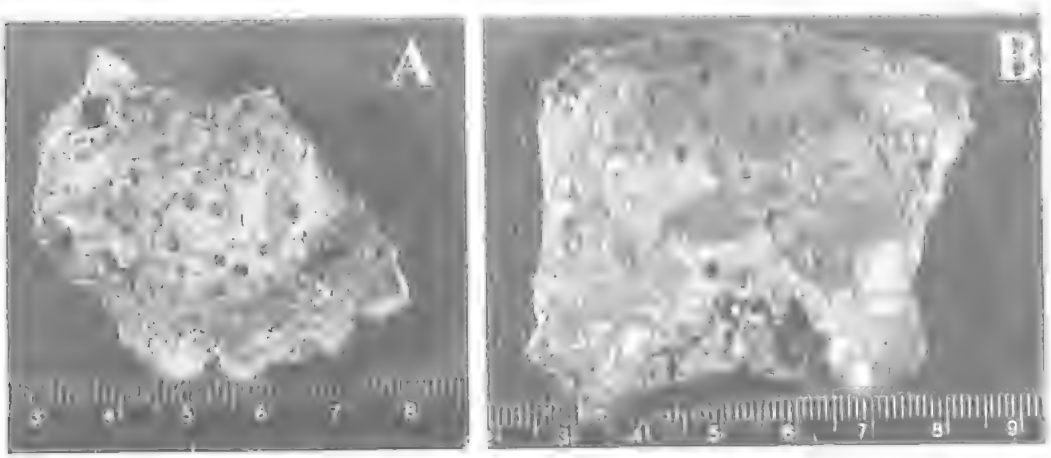

FIG. 6. *Vioa johnstoni* Schmidt 1862. A, dry voucher encrusting on coralline rock (LMJG15258/0); B, dry voucher encrusting on coralline rock (LMJG15256/0). Scales in cm.

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RECOVERY AND GROWTH OF THE GIANT BARREL SPONGE (*XESTOSPONGIA MUTA*) FOLLOWING PHYSICAL INJURY FROM A VESSEL GROUNDING IN THE FLORIDA KEYS. *Memoirs of the Queensland Museum 44: 532. 1999:-* On February 2, 1997, the 187m (614 feet) container ship 'Contship Houston' ran aground on the Florida reef tract near Maryland Shoal within the Florida Keys National Marine Sanctuary. This incident resulted in significant injury to coral reef resources over an area 650m (2,132 feet) in length. Hundreds of the Giant Barrel Sponge (*Xestospongia muta*) were damaged or destroyed as the ship approached the final grounding site, along with thousands of scleractinian corals and other reef organisms. A major coral reef restoration project is currently underway to address the physical and biological injury caused by the grounding. Over 3,000 broken and dislodged corals were reattached to the substrate within the inbound tract of the vessel, and large areas of rubble created by the ship's hull have been stabilised through a variety of techniques.

The purpose of this study was to assess the response of injured *Xestospongia* to the physical injury caused by the vessel grounding. As the vessel approached the grounding site, sponges which were in the path of the ship were subjected to various degrees of injury. This injury ranged from the minor breaking off of the tops of the sponges to the complete destruction of the sponge except for the basal tissue attached to the substrate. I located and marked 37 injured specimens with individual tags attached to plastic cable ties positioned tightly on the upper injured surface at two locations of each sponge. I monitored the sponges at two to three month intervals and measured upward linear growth from the cable ties. I also observed the condition and vitality of each sponge and the method by which the sponges responded to their injuries. All sponges were photographed at regular intervals.

During the course of the study, seven of the tagged sponges disappeared from the study site. Four of these were observed to have died from a wasting disease that was reported from numerous locations in the Florida Keys and the Caribbean. The causes of the disappearance of the other three were not directly observed. The 30 remaining sponges have survived and recovered from the direct physical injury at a minimum by healthy tissue regeneration of the damaged areas. The rate of upward linear growth ranged from zero to 4.48cm over 13 months, with an average upward linear growth of 1.42cm for all sponges. Eight specimens (27%) showed no upward growth over the observation period. The average growth rate for the sponges that did exhibit upward growth was 1.94cm. The most significant period of growth was in the late summer and throughout the fall, which corresponds to the period of warmest seawater temperatures. Upward linear growth was correlated with the degree of injury, with the moderate or slightly injured specimens growing at a faster rate than the badly injured ones. Of the four sponges that died from the wasting disease, three had been categorised as badly injured, which may suggest that injured sponges may be more susceptible to disease than non-injured sponges. ❐ *Porifera, Giant Barrel Sponge, Xestospongia muta, sponge growth rates, recovery from physical injury, coral reef injury and restoration, Florida Keys, Florida Keys National Marine Sanctuary.*

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AN IMPROVED METHOD OF TISSUE DIGESTION FOR SPICULE MOUNTS IN SPONGE TAXONOMY

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Schönberg, C.H.L. 1999 06 30: An improved method of tissue digestion for spicule mounts in sponge taxonomy. *Memoirs of the Queensland Museum* **44**: 533-539. Brisbane. ISSN 0079-8835.

Digestion of bioeroding sponges is difficult as the tissue of many samples cannot be dissolved easily using traditional techniques involving 70% nitric acid. A number of factors reduce the effectiveness of the acid, such as dilution by water from fresh and alcohol preserved specimens, and buffering effects, particularly contamination with calcium carbonate. In these preparations spicules are often obscured by a white precipitate and tissue/spongin residues. This traditional method was emended to produce clean preparations, with the added benefit that digestion time was greatly reduced. In contrast to repeated treatments with 70% nitric acid in an 80°C sandbath under the traditional method, I propose the use of alternate applications of 70% aqua regia and 70% nitric acid in a 140°C sandbath with whirl shaking after each application. Drying the tissue samples prior to digestion was important to speed up the process. Nevertheless, under the modified method some impurities remained resistant to acid digestion, including diatoms, clay and quartz particles. ❐ *Porifera, Demospongiae, bioeroding sponges, tissue digestion, taxonomy, spicule preparations.*

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Spicule morphology remains a fundamental criterion in sponge identification, yet their preparation for examination under light microscopy has changed very little since last century. Traditionally, demosponge spicule preparations have been obtained by digesting sponge tissue and calcareous particles in either sodium hypochlorite ('bleach') or heating in nitric acid, leaving the siliceous spicules remaining. Nitric acid digestions have been applied in several ways: 1) a small piece of tissue placed directly onto a microscope slide and digested by dropping small amounts of acid and boiling off the supernatant, with the spicules fixed in place with a mounting medium; 2) tissue digested in a test tube, then spread over slides by burning a drop of the resuspended mixture, and subsequentially fixed onto the slide with a standard mountant.

The first method, henceforth referred to as the traditional method, has been widely used over time and is adequate for sponges containing few or no foreign particles. It also has the advantage over the second method in minimising the potential loss of rare spicules or microscleres from slides, given that preparation occurs directly on the slide medium, whereas using separate platforms for digestion and viewing introduces the possibility that some spicules may be lost during their transfer to glass slides (usually via pipette). However, this traditional method is clearly inadequate for arenaceous species and bioeroding species (i.e. those that bore into calcitic substrata). Under the traditional method calcitic debris is retained on slides, obscures spicules, and often make spicule preparations too thick to be useful. The second method, henceforth referred to as the modified method, was described most recently by Schönberg & Barthel (1997, 1998), and enables clean spicules to be pipetted from the test tube onto the slide, leaving behind the contaminating material.

However, recent tests on bioeroding sponges boiled in 70% nitric acid (Schönberg, unpublished data), found both methods were unsatisfactory for this group of sponges. The present study aimed to identify the reasons for the reduced effectiveness of acid during digestion, and to develop improvements in the modified method.

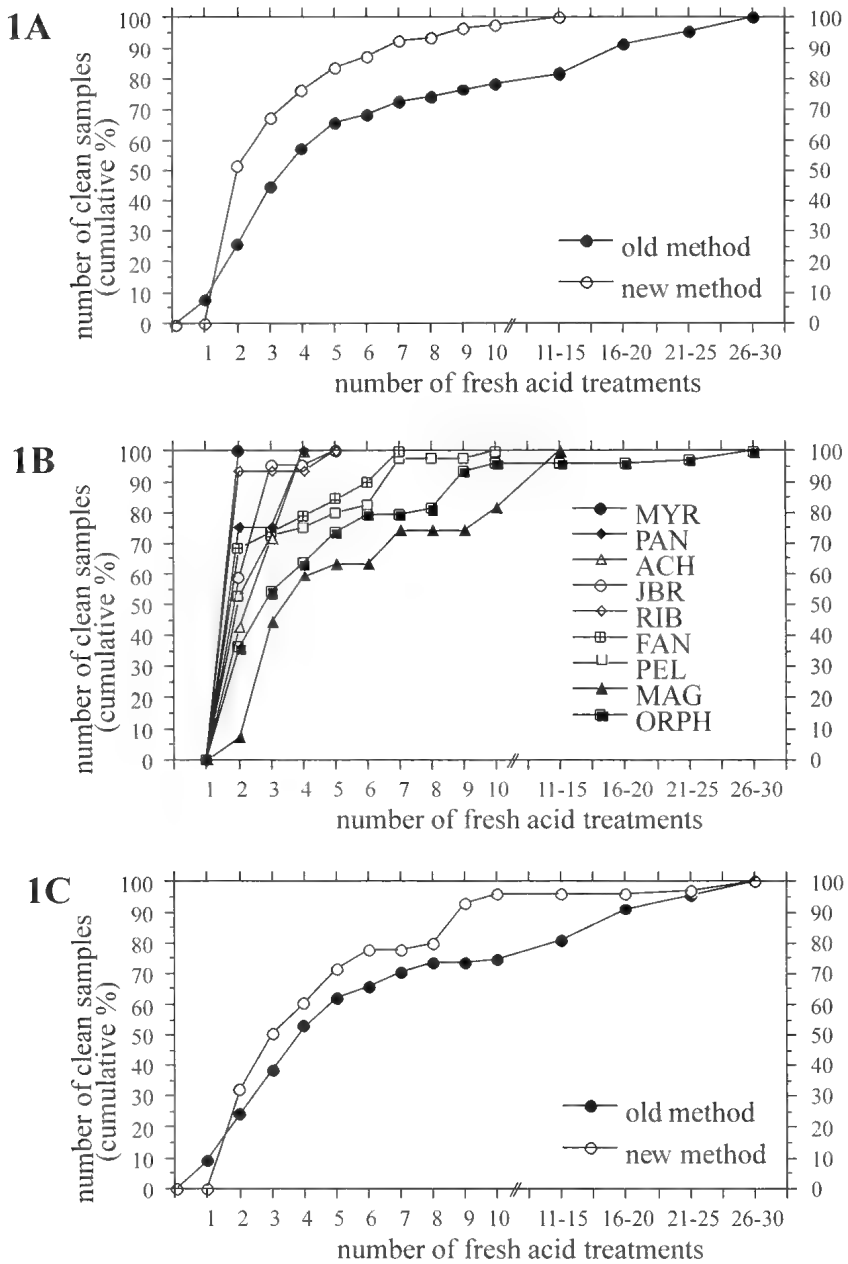

FIG. 1 A-C. Bioeroding sponge tissue digestion speed expressed in number of acid washes necessary to obtain clean spicule samples. A, Digestion speed for samples from all sample sites combined. Black circles - traditional method 2a (N=255); white circles - new method 2b (N=217); B, Digestion speed ordered by sample site (digestion under method 2b only). Black circles - Myrmidon Reef (MYR; N=14), white circles - John Brewer Reef (JBR, N=22), white rhombus - Rib Reef (RIB, N=15), white squares - Pelorus Island (PEL; N=40), black squares - Orpheus Island (ORPH; N=107), subdivided white squares - Fantome Island (FAN; N=19), white triangles - Acheron Island (ACH; N=7), black triangles - Magnetic Island (MAG; N=27), black rhombus - Pandora Reef (PAN; N=8). C, Digestion speed for samples from a single sample site, Little Pioneer Bay, Orpheus Island. Black circles - method 2a (N=200), white circles - method 2b (N=99). Values represent counts, hence no error bars are included.

TABLE 1. Relationship of bioeroding sponge tissue digestion speed and distance to the shore through possible uptake of fine terrestrial sediments.

Sample site	Distance to shore (km)	Visibility during dive (m)	% of samples clean after a given number of acid washes		
			50%	80%	100%
Myrmidon Reef	112	>25	2	2	2
John Brewer Reef	72	~20	2	3	5
Rib Reef	55	~20	2	2	5
Fantome Island	20	5-7	2	5	7
Acheron Island	18	~10	3	4	4
Orpheus Island	16	<5	3	8	>30
Pandora Reef	15	~5	2	2	4
Pelorus Island	14	5-7	2	5	10
Magnetic Island	5	2-5	4	10	10-15

MATERIALS AND METHODS

Bioeroding sponges were collected from the central region of the Great Barrier Reef, Queensland. Material was acid digested using three methods.

1) Traditional method of placing a small fragment of sponge in 70% nitric acid directly on a microscope slide, and boiling under low heat (e.g. alcohol flame or a sand bath). This simple traditional method is described by Hooper (1996).

2a) Modified method of Schönberg & Barthel (1997) whereby pieces of sponge tissue 1-3mm^3 were digested in test tubes using repeated washes with 70% nitric acid in an 80°C sand bath. Each application of fresh acid was allowed to react over night. This method is similar to that described by Hooper (1996) for digesting sponge tissue for examination under scanning electron microscopy.

2b) Pieces of sponge tissue 1-3mm^3 were dried at room temperature for 2 days or overnight at 80°C, then pre-digested in test tubes in a 140°C sand bath using aqua regia (1 volumetric unit of 70% nitric acid and 3 units of 70% hydrochloric acid). The high temperature was chosen to maximise acid reactivity by keeping it at boiling level (hydrochloric acid: 110°C; nitric acid: 122°C; Falbe & Regitz, 1992), allowing for lower temperatures inside the test tubes than at the bottom of the sandbath. Spent acid was removed after 24hrs and replaced by 70% nitric acid. The samples were then again left overnight in the 140°C sandbath. This combined acid treatment was repeated as required. Samples were mixed on a whirl shaker with each fresh application of acid (Fig. 1A).

Using both methods, digestion was considered to be finished when the solution looked clear (i.e. without any yellow colouration or white debris remaining in the spicule sediment). In a few cases, digestion was stopped after more than 20 acid applications even though the sample still appeared whitish, and there was no evidence of nitrous gases indicating that the acid was still reacting with organic material. The supernatant acid was removed, and residual acid in the test tubes was allowed to react off with 70% ethanol added dropwise. The re-settled spicules were washed twice with 100% ethanol to remove remaining acid and to dehydrate the sample. Spicules were stored in 100% ethanol until mounting. The proportion of sample concentrations of spicules to alcohol was adjusted to a ratio of about 1:10 in volume, or less, to ensure optimal spicule concentrations on the microscope slides when applying the same amount of spicule suspension (i.e. 300µl). The suspension was haphazardly spread on a level slide and then burnt off (Schönberg & Barthel, 1998). Before fixing the cover slip with DPX mountant, a flame was held underneath the slide to remove vapour still adhering to the spicules and the microscope slide.

The efficiency of both methods was compared by noting the number of acid washes necessary to obtain clear suspensions. In addition, the quality of the preparations was checked by noting whether microscleres or spines on megascleres were still obscured, and if so by what. In 69 sponges, ectosome and choanosome regions were sampled separately to assess whether it was possible that different proportions of spongin and cell material were responsible for producing different digestion results.

RESULTS

The traditional method of tissue digestion, as well as the modified method of Schönberg & Barthel (1997, 1998), were both found to be inadequate in producing clear, useful slide preparations for bioeroding sponge spicules, irrespective of whether digestion was attempted

directly on a microscope slide (method 1) or in a test tube (method 2a).

The traditional method (1) usually produced a thick, white precipitate on the microscope slide, which obscured the spicules, especially microscleres. It was almost impossible to obtain clean preparations, even when carefully washing with alcohol. Moreover, washing increased the risk of losing spicules.

The modified digestion method (2a) was extremely slow, requiring many fresh acid washes, and not always dissolving all the tissue. As a consequence, microscleres and spines on megascleres were often obscured, minimising their usefulness of these slides for sponge identification. With up to 20-30 acid washes there was also the risk of losing small and rare spicules during repeated pipetting off the supernatant spent acid. Using method 2b proposed here, the efficiency of sponge tissue digestion was markedly improved.

A comparison of methods 2a and 2b is as follows.

1) After two acid applications, one each of aqua regia and 70% nitric acid, spicules were clean in 50% of samples using method 2b. By comparison, using 70% nitric acid in an 80°C sand bath, an average of four acid applications were required to produce 50% of clean samples under method 2a.

2) After four acid applications 75% of samples were clean under method 2b; whereas nine applications were required to produce the same result under method 2a. After nine acid applications 95% of samples were clean under method 2b.

3) After 10 acid applications using method 2b all samples were generally clean (Fig. 2B), whereas using method 2a samples still

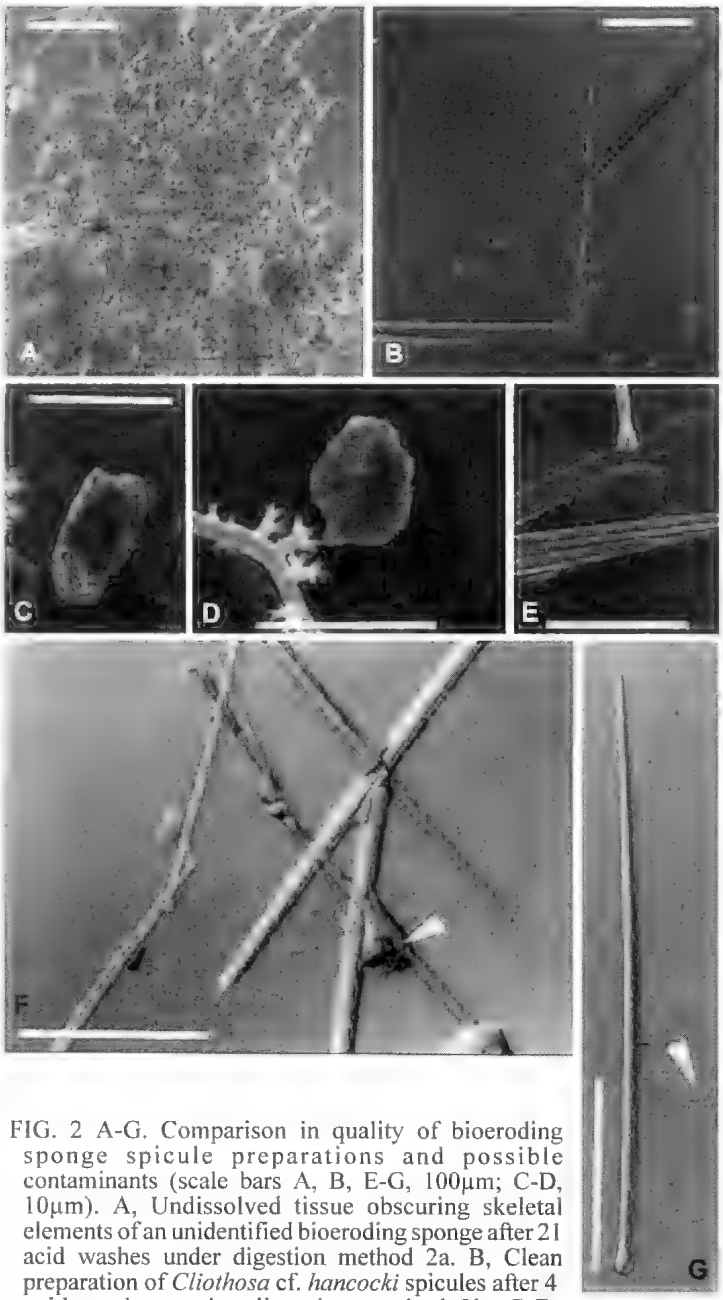

FIG. 2 A-G. Comparison in quality of bioeroding sponge spicule preparations and possible contaminants (scale bars A, B, E-G, 100μm; C-D, 10μm). A, Undissolved tissue obscuring skeletal elements of an unidentified bioeroding sponge after 21 acid washes under digestion method 2a. B, Clean preparation of *Cliothosa* cf. *hancocki* spicules after 4 acid washes under digestion method 2b. C-E, Contaminating particles in SEM spicule preparations of *Cliona viridis* sensu Bergman (1983). C, Diatom. D, Clay particle. E, Quartz particle. F, Permanent preparation of *C. viridis* skeletal elements with residual water adhering to the spicules, obscuring finer details and microscleres (arrow). G, Clear spicule preparation of *C. viridis* which has been dried by heating with a flame prior to mounting (arrow = clearly visible spirasters).

contained debris, obscuring microscleres in particular, which adhered to debris or remained embedded in tissue (Fig. 2A).

4) Under method 2a 50/255 (20%) of samples contained incompletely digested material after more than 10 acid washes, whereas only 5/217 (2%) of samples contained 'impurities' under method 2b.

Clearly, using the modified method 2b there are far fewer 'impurities' in samples than under method 2a. Moreover, any 'impurities' that did remain in spicule suspensions were inorganic debris rather than organic tissue remains. This remaining debris was checked under the electron microscope and identified as diatoms, clay and quartz particles (Ross Freeman pers. comm.; Rothwell, 1989; Fig. 2C-E). Therefore, it is possible that part of the cloudiness remaining in samples may be a product of the habitats from which samples were collected, such as differing levels of turbidity and sedimentation rates in different localities. Empirical support for this hypothesis comes from a comparison between samples collected from offshore and mid-shelf sites, which were digested much faster than samples collected from inshore sites. These latter samples were the only ones which could not be cleaned entirely of debris (Fig. 1B). This finding largely correlates with visibility recordings made during field collections, although samples from Pandora Reef and Acheron Island digested surprisingly better than expected (Table 1).

The distribution of data comparing the different methods was not random in terms of sample sites. To test for bias in site-effect, data were re-evaluated for one site (Little Pioneer Bay, Orpheus Island), which had both the lowest visibility and highest number of samples. These data provided weaker support for method 2b over 2a, than did analysis of the entire data set, although both data sets followed the same trend (Fig. 1C). For the site-effect data, methods 2a and 2b showed 50% of the samples were clear of debris after 4 and 3 acid washes, respectively, 75% after 10-15 and 6 washes, 90% after 15-20 and 9 washes, and 100% of samples were clear after more than 30 washes of acid. Whereas under method 2a 51/200 samples (26%) were still cloudy after 10 washes, in method 2a only 4/99 samples (4%) were cloudy.

Too few data were available to statistically evaluate the effect of collection localities (zones) on coral reefs, but visually the data suggest that acid digestion was slightly better from samples collected from the fore-reef zones than from lagoons or back reefs, possibly related to differential sedimentation rates between the various zones. Potential bias due to seasonal effects were not tested given the patchy collection schedule.

Differences in the type of sponge tissue did not appear to effect the speed of digestion except in *Aka* cf. *mucosa*, in which tissue samples from the soft choanosome digested on average twice as fast as those taken from of the long, brittle papillae. In general, it was found that tissue samples with minimum calcium carbonate digested better than more calcitic samples, although this is often difficult to achieve for most bioeroding sponges which often incorporate calcitic debris into the choanosome. Nevertheless, some samples that contained more than 50% of their volume with incorporated coral skeleton were sometimes clean within the first two acid washes.

No differences were observed in acid digestion rates between the several different (but still unidentified) species of *Cliona* and *Aka* sampled, apart from the example of *A.* cf. *mucosa* mentioned above. In general, however, samples which contained more soft tissue (such as those eroding large chambers), generally digested slightly better than those in which tissue penetrated carbonate substrates in honeycomb patterns.

Finally, in the modified method 2b, repeated alcohol washes were necessary after digestion to avoid residual acid that masked spicule features (such as spines). These washes also reduced the amount of water in samples, as did heating microscope slides before adding the mountant, which can obscure finer details of spicules (Fig. 2F-G).

DISCUSSION

A general problem in sponge tissue digestion is the high water content of fresh tissue samples, which dilutes the acid. Similarly, the readily available 'concentrated nitric acid' is actually 70% concentrated, whereas the more effective 'fuming nitric acid' is both extremely expensive and unavailable in certain countries. However, dehydration is easily achieved by fixing samples in ethanol, or drying fresh samples, prior to acid digestion, with the latter method being the most effective.

Another explanation for the reduced effect of acid in tissue digestion is the buffering effect of

calcium carbonate debris, which is a particular problem in bioeroding sponges. Most samples examined in this study contained a relatively high proportion of calcium carbonates, particularly those that produce small excavations in which the tissue cannot be separated entirely from the host coral skeleton. In species which excavate large chambers, where tissue can be more easily be separated from carbonate, eroded coral fragments (termed "sponge chips" by Cobb (1969) are always present in the tissue.

Inorganic contaminants also occur in samples, mostly skeletal remains of diatoms, clay and quartz particles (Fig. 2C-E). Clay minerals may also produce a decreased effectiveness in acid digestion, because they are phyllosilicates, which are negatively charged and thus attract cations resulting in a specific ion-exchange capacity depending on the nature of the mineral (Brownlow, 1979). In acidic solution, these cations can be substituted against hydrogen ions until an equilibrium is reached, which could produce an additional buffering effect (Schröder, 1978).

Silicate contaminants produce a cloudy supernatant in acid digestions, superficially appearing to be a problem involving undissolved organic matter and suggesting that digestions should be repeated. This is incorrect, however, and repeated digestions with nitric acid or aqua regia do nothing further to siliceous particles given that these are chemically similar to the demosponge spicules. One solution to this problem is foresight in the design of the sampling program, armed with the knowledge that specimens living close to the shore are likely to contain higher amounts of such debris than those sampled further away (Fig. 1B, Table 1).

Tissue of bioeroding sponges was surprisingly resistant to acid digestion. There is evidence that bioeroding sponges are able to shift the calcium carbonate solubility equilibrium with the aid of carbonic anhydrase in favour of substrate dissolution ($CO_2 + H_2O \Leftrightarrow H_2CO_3 \Leftrightarrow 2H^+ + CO_3^{2-}$; Pomponi, 1980). The production of hydronium ions in close vicinity to the etching cells of the sponge would require a special resistance to their corrosiveness. However, the etching process is not yet entirely explained.

The improved method for sponge tissue digestion described here is primarily based on increasing the efficiency of acid by reducing its dilution and buffering effects as far as possible.

1) The greatest improvements were gained by drying tissue samples and increasing the sand bath temperature from 80°C to 140°C.

2) Alternating rinses using aqua regia and nitric acid provided some improvement, although the reason for this is not clear. Due to the development of activated chlorine and nitrose chloride ($HNO_3 + 3\,HCl \Leftrightarrow NOCl + Cl_2 + 2\,H_2O$), aqua regia is more corrosive than both nitric acid or hydrochloric acid alone (Falbe & Regitz, 1990). Curiously, aqua regia alone seemed to be less efficient than when used in alternation with nitric acid. The reason for this is also not clear.

3) Repeated stirring during acid digestion, using a whirl shaker, makes some small difference to the efficiency of the process by providing a better saturation of organic shreds with acid, and by physically breaking down larger particles.

ACKNOWLEDGEMENTS

All field and laboratory work was conducted at the Australian Institute of Marine Science, and I thank the staff for their frequent assistance, particularly scientists in Module 4 for their patience and provision of equipment and space. D. Barthel and O. Tendal first showed me how to digest sponge tissue in test tubes. J. Hooper, S. Cook and J. Kennedy at the Queensland Museum, Brisbane, provided demonstrations of digestion directly on a microscope slide and discussed possible improvements. H. Windsor at the James Cook University, Townsville, assisted with electron microscopy. C. Wilkinson, H. K. Schminke and two anonymous reviewers provided useful comments on the manuscript. Studies of bioeroding sponges were supported by a scholarship from the German Academic Exchange Service. This is AIMS publication no. 931.

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THE INFECTIVENESS OF A BIOERODING CLIONID SPONGE. *Memoirs of the Queensland Museum 44: 540. 1999*:- Sponges play a major role in reef bioerosion. Early impressions suggested that only dead coral skeleton was infected. Cores of a very abundant Great Barrier Reef clionid sponge,*Cliona* sp., probably new, were removed with an underwater drill, allowed to heal and fixed onto living surfaces of nine coral spp. at Orpheus Island. Sponge survival varied greatly. It was best on control surfaces of dead massive *Porites*, on live massive *Porites* and on *Astreopora myriophtalma*. It was least on *Lobophyllia hemprichii* and two branching *Porites* spp. Several individuals in seven of the nine coral species were infected within eight weeks. The areas of infection varied widely. However, after removal of grafts, the sponges died, regardless of their size. The risk of epidemics by fragmentation of this sponge species is considered to be low. ❐ *Porifera, Cliona, bioerosion, Great Barrier Reef, Coral Sea.*

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WANTED: THE NAMES OF COMMON BIOERODING SPONGES OF THE CENTRAL GREAT BARRIER REEF. *Memoirs of the Queensland Museum 44: 540. 1999*:- Bioeroding sponges have been well studied in the Mediterranean and the Caribbean Seas. Only few bioeroding sponge species are properly described from the Australian Great Barrier Reef. All other descriptions of Australian species are based on von Lendenfeld (1884-85) and de Laubenfels (1954). Detailed surveys in the central section of the Great Barrier Reef resulted in a large amount of new reference material suitable for revisions and new descriptions. Field descriptions and preliminary studies of spicule mounts made it possible to clearly distinguish 15 species from the rest of the samples, which are harder to categorise. Some of the sampled species appear to be sponges described previously and occurring in other oceans, some new species are likely to be endemic to the Coral Sea. Descriptions of the most common species are presented with preliminary names, distinguishing morphological features, and spicule assemblages with a request that participants assist by comparing with species they are familiar with or have observed elsewhere. ❐ *Porifera, Cliona, Cliothosa, Aka, bioerosion, Great Barrier Reef, Coral Sea, taxonomy.*

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FRESHWATER SPONGES (PORIFERA: SPONGILLIDAE) OF THE GUANACASTE CONSERVATION AREA, COSTA RICA: A PRELIMINARY SURVEY. *Memoirs of the Queensland Museum 44: 540. 1999*:- A survey of freshwater sponges in the Guanacaste Conservation Area (GCA), NW Costa Rica, was conducted in August and December of 1996 and March of 1997. The GCA occupies 110,000 hectares and includes Pacific dry forest, cloud forest and Atlantic rain forest. Objectives were to find out what sponges occur in the GCA, their distribution and preferred habitats. Sites were chosen to represent aquatic habitats from each biome in the GCA. At each site water temperature, pH, specific conductance and current velocity were measured. A unique aspect of this project entailed the measurement of particulate organic carbon (POC). POC can be useful for estimating food availability and has not been used to describe habitat preferences of freshwater sponges. To estimate POC, methods outlined by Wetzel & Likens (1979) were used.

Listed in order of decreasing frequency, the following taxa were observed: *Radiospongilla* sp., *Dosilia* sp., *Corvomeyenia* sp., *Spongilla cenota*, *Trochospongilla* sp., and unidentifiable colonies without gemmules. The *Radiospongilla* species is currently being described in a separate paper (Poirrier, in prep.), and prior to this survey, *Spongilla cenota* was known only as far south as Florida and Mexico and therefore represents a significant range extension and a new record for Central America. The other genera are of uncertain taxonomic position and will be considered in a future paper. Sponges in the GCA were restricted to temporary, slow moving streams and ponds in dry tropical forest. These habitats have a drought season of up to six months and have POC content greater than 560g/l. No sponges were found in the clear, fast, permanent streams of the cloud and rain forests. Due to the high volume of fast flowing water, POC values are extremely low in these streams. These data suggest that natural aquatic habitats within evergreen tropical forests do not provide adequate food for freshwater sponges and that more favourable habitats are found in the dry tropical forest biome. This may be an important point for the conservation of Central American freshwater sponges, because dry tropical forest is considered the most endangered of all tropical ecosystems. ❐ *Porifera, Costa Rica, ecology, habitat, POC, Spongillidae, taxonomy, conservation.*

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CHEMISTRY, ECOLOGY AND BIOLOGICAL ACTIVITY OF THE HAPLOSCLERID SPONGE *OCEANAPIA* SP.; CAN ECOLOGICAL OBSERVATIONS AND EXPERIMENTS GIVE A FIRST CLUE ABOUT PHARMACOLOGICAL ACTIVITY?

P. SCHUPP, C. EDER, V. PAUL AND P. PROKSCH

Schupp, P., Eder, C., Paul, V. & Proksch, P. 1999 06 30: Chemistry, ecology and biological activity of the haplosclerid sponge *Oceanapia* sp.; Can ecological observations and experiments give a first clue about pharmacological activity? *Memoirs of the Queensland Museum* 44: 541-549. Brisbane. ISSN 0079-8835.

A new species of *Oceanapia* was discovered around Moen Island, Chuuk Lagoon, Micronesia, during collections made in 1994-1995. The appearance and growth form closely resembled that of *Oceanapia sagittaria*, which is widely distributed throughout the Western Pacific. Unlike the Chuuk species, however, *O. sagittaria* has microscleres (sigmas and toxas) and thicker oxeas, suggesting to us it may be new. We examined whether this conspicuous, red sponge was chemically defended against generalist and more specialized fish predators. Methanol extracts of the sponge were highly deterrent in field feeding assays against generalist reef fish at physiological concentrations. This extract also deterred feeding by the spongivorous angelfish, *Pomacanthus imperator*, in laboratory feeding experiments at the same concentration. Based on our field observations and fish feeding experiments, different pharmacological screens were undertaken to demonstrate possible targets of these compounds. Kuanoniamine C and D showed insecticidal activity against the polyphagous larvae of *Spodoptera littoralis*, and toxic effects against the brine shrimp *Artemia salina*; N-deacyl derivative did not show a pronounced activity; whereas cytotoxicity screens against HELA and MONO-MAC-6 tumor cells found that all three compounds revealed considerable cytotoxicity. □ *Porifera, Oceanapia, secondary metabolites, predation, feeding deterrency, Micronesia, kuanoniamines.*

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Despite considerable progress achieved in modern synthetic chemistry, natural products are still an indispensable source for the development of new pharmaceuticals and plant protectants. The frequent occurrence of bioactive compounds in nature is due to selective forces (e.g. predation or attack by pathogens), which have shaped and optimized a highly efficient chemical defense armory in many plants, animals and micro-organisms (Harborne, 1993). For decades scientists have relied on ethno-pharmacological knowledge to obtain initial indication on the biological and pharmacological properties of terrestrial plants. In contrast, there is almost no equivalent information available for the marine environment, requiring the implementation of other strategies to achieve fast and environmentally sensible bioprospecting.

Marine organisms are a rich source of novel, often unusual, secondary metabolites (Faulkner, 1996). Researchers often focus only on the ecology of secondary metabolites from marine organisms (Pawlik, 1993; Hay, 1996; Wink, 1998), or on the chemistry and pharmacological activity of the new compounds (Eder et al., 1998; Faulkner, 1996; Eder et al., 1999). Ecological studies focus mostly on assumed defensive functions of secondary metabolites, such as predator deterrence (Paul, 1992; Pawlik et al., 1995), prevention of fouling (De Nys et al., 1991), or inhibition of overgrowth (Thacker et al., 1998). Chemical studies report mainly on the structure elucidation of new compounds and their pharmacological activities, such as cytotoxicity (Steube et al., 1998) and anti-microbial activity (Edrada et al., 1996).

Paul (1988) suggested that ecological experiments could be useful as a first indication for the presence of pharmacological active compounds, although at that time there was no clear empirical support for this idea. In this study, we show that ecological observations and

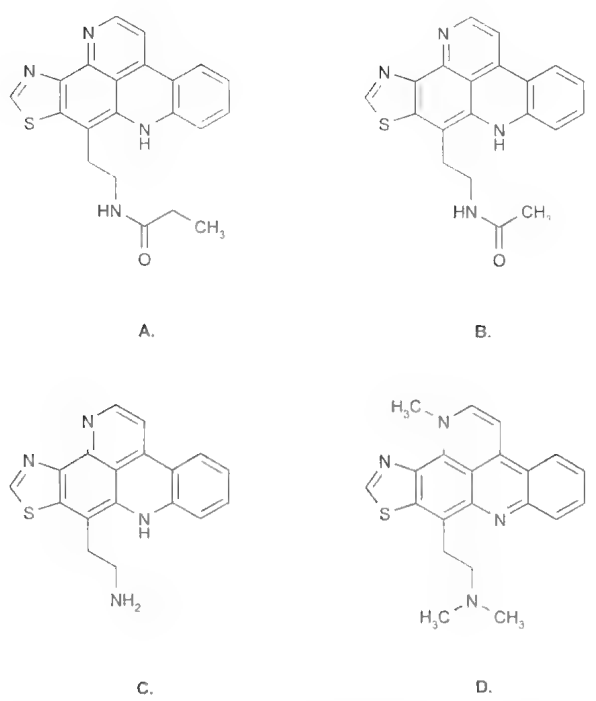

FIG. 1. Major metabolites isolated from *Oceanapia* sp. A, kuanoniamine C; B, kuanoniamine D; C, N-acetyl-kuanoniamine D; D, dercitin.

experiments can indeed be used as initial indicators of possible, potential pharmacological properties of the compounds involved (Schupp et al., 1999a, 1999b).

MATERIALS AND METHODS

COLLECTION AND ISOLATION. *Oceanapia* sp. was collected at 1-3m depth on reef flats near the southern tip of Moen Island, Chuuk Lagoon, Federated States of Micronesia. Immediately after digging the basal 'root' of the sponge from the substrate, samples were separated *in situ* into the translucent capitum, fistule and base. After separation the different sample components were frozen and stored at - 20°C, until subsequent freeze-drying and extraction in 100% methanol.

CHEMISTRY. The crude methanol extract was evaporated under reduced pressure and chromatographed on a silica gel column (elution with CH_2Cl_2/MeOH/NH_4OH, 70:30:3). The pyridoacridine alkaloids kuanoniamine C (Fig. 1A), kuanoniamine D (Fig. 1B), and N-deacetyl-kuanoniamine D (Fig. 1C) were obtained after a final purification on a RP-18 silica gel column (MeOH/H_2O/TFA, 70:30:0.5)(Eder et al., 1998).

The N-deacyl derivative could only be isolated from the sponge in trace amounts. Therefore, we synthesized the compound through acid hydrolysis of kuanoniamine C, which provided a sufficient quantity of the compound for subsequent bioassays. Kuanoniamine C (65mg) was dissolved in 120mL MeOH and the same volume of 2N HCl was added. The reaction mixture was stirred for 48hrs at 85°C under reflux. The sample was dried and the resulting red solid was purified by RP-18 column chromatography, using the solvent system described above, to yield 49mg of the new compound. Structure elucidation was accomplished by NMR and mass spectrometry (Eder et al., 1998).

BIOLOGICAL ACTIVITY. *Experiments with insects.* Neonate larvae of the vigorous pest insect, *Spodoptera littoralis*, were used to test if the isolated compounds had any insecticidal properties. This assay allowed determination whether compounds were toxic towards insect larvae, or if they reduced or inhibited larval growth. It was not possible to determine whether reduced growth was due to inhibition of physiological processes and metabolism, or reduced food consumption caused by feeding deterrence. Larvae of *S. littoralis* were obtained from a laboratory colony, reared on an artificial diet under controlled conditions, as described by Srivastava & Proksch (1991). Feeding studies were conducted with neonate larvae (n=20) maintained on an artificial diet which had been treated with various concentrations (13-373ppm) of the compounds under study. *Spodoptera littoralis* was offered the spiked diet over a range of 5-6 concentrations in a chronic feeding experiment. After 6 days, surviving larvae were counted, weighed, and compared to controls. LC_{50}s were calculated from the dose-response curves by probit analysis (Eder et al., 1998).

Brine Shrimp Assay. This assay was used to determine if the isolated compounds were toxic towards small marine organisms, although no information was forthcoming on the mode of action, or on how specific the toxicity was directed towards the test organisms (*Artemia salina*). Eggs of this species were kept for 48hrs in artificial

seawater, as described previously (Meyer et al., 1982), and the nauplii (n=20) were introduced into vessels with brine, containing various concentrations (5-100µg/mL) of the compounds under test (each concentration in triplicate). DMSO (10µL/mL brine) was added to improve solubility. After 24hrs the surviving larvae were counted and compared to controls. LC_{50}s were calculated from the dose-response curves by probit analysis (Eder et al., 1998).

Cytotoxicity Studies. Information on the selectivity and possible intracellular targets were tested by analysing the effects of kuanoniamine C and D, and the new compound N-deacetyl-kuanoniamine D, on cell growth and differentiation of two different human cell lines. These cell lines, MONO-MAC 6 and HELA, were deposited in the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). Cultures were mycoplasma-free, and were cultivated under standardized conditions (Drexler et al., 1995). For all experiments, exponentially-growing cells were used, with a viability exceeding 90%, as determined by trypan blue staining. The final concentration used in experiments was 10^5cells/mL. Experiments were conducted using 100IU/mL penicillin G and 100mg/mL streptomycin. Concentrated stock solutions of the test compounds were prepared in ethylene glycol monomethyl ether, and stored at -20°C. For the cytotoxicity analysis, cells were harvested, washed, and resuspended in a final concentration of 10^5cells/mL. These solutions were seeded in triplicate, in 90µL volumes, in 96-well flat bottom culture plates (Nunc). Test compounds in 10µL, obtained by diluting the stock solution with a suitable quantity of growth medium, were added to each well (Eder et al., 1998). Cultures were incubated for 48hrs at 37°C in a humidified incubator with 5% CO_2. Cytotoxicity was determined by incorporation of [^{3}H]-thymidine (Steube et al., 1992). Radioactive-incorporation was carried out for the last 3hrs of the 48hr incubation period. One µCi of [methyl-^{3}H]-thymidine (Amersham-Buchler, Braunschweig, Germany; specific activity 0.25mCi/µmol), was added in 20µL volumes to each well. Cells were harvested on glass fiber filters with a multiple automatic sample harvester, and radioactivity was determined in a liquid scintillation counter (1209 Rackbeta, LKB, Freiburg, Germany). Media with 0.2% ethylene glycol monomethyl ether were included in the experiments as controls (Eder et al., 1998).

ECOLOGY. *Distribution of secondary metabolites.* Extract and secondary metabolite concentrations were determined for the basal, fistular and capitum subsamples of *Oceanapia* sp., to see whether there were any differences in compound concentrations between these subsamples of the sponge, correlated with differences in exposure of these sponge structures (e.g. burrowing versus exposed). This strategy was essential to test the extract and sponge compounds at ecological relevant concentrations. These freeze-dried sponge subsamples were weighed before extraction, and the extract weights were also determined after removing all solvents with a rotary evaporator. Concentrations of kuanoniamine C and D in the different subsamples of the sponge were determined by HPLC quantification, following methods described by Schupp et al. (1999b).

Field feeding assays. Field feeding experiments using natural fish assemblages were conducted as an initial test for biological activity of the crude extract. We used reef fishes to determine whether the crude extract had antifeedant properties. The methanol fraction was incorporated in an artificial diet at a concentration of 7.4 % of dry mass, which represents the methanol extract concentration in the basal part of the sponge. We also tested the three major metabolites (Fig. 1A-C) at their respective fistule concentrations, against several fish species in the field at Western Shoals, Guam. Kuanoniamine C and D and the N-deacyl derivative were tested at fistule concentrations since this was considered to be the most easily accessible structure of the sponge susceptible to fish feeding (Schupp et al., 1999b).

The food was prepared according to Schupp et al. (1999b). Four food cubes, with or without one of the metabolites, were attached to a polypropylene rope by a safety pin. Ropes were placed on the reef in pairs of one treated (with one metabolite), and one control (without the metabolite) rope, and attached to coral heads at 3-5m depth. Several pairs of ropes (replicates) were set at the same time. Ropes were removed when approximately half the cubes were completely eaten. These assays were scored as the number of cubes completely eaten, and the results analyzed with a Wilcoxon signed-rank test for paired comparison (two-tailed; Schupp et al., 1999b).

Laboratory feeding assays. The angelfish, *Pomacanthus imperator*, was used as an experimental subject to determine whether or not there were differences in susceptibility between generalist and specialist predators (Thacker et al.,

TABLE 1. *In vitro* cytotoxicity of the kuanoniamine derivatives (IC_{50} values, µg/mL) to HELA and MONO-MAC-6 tumor cells in the [^{3}H]-thymidine incorporation assay (MTT), (mean ± SD, n=3; Eder et al., 1998).

Cell line	Compound		
	1	2	3
HELA	5.1 ± 1.3	1.4 ± 0.7	1.2 ± 0.2
MONO-MAC-6	1.2 ± 0.4	0.8 ± 0.1	2.0 ± 0.5

1998). Experimental food was prepared following methods described by Schupp et al. (1999b),resulting in strips of window screens that had one rectangle of treated food and one rectangle of control food (2.5x2.0cm) embedded in the screens. To determine the amount of control and treated food eaten by angelfish, the squares in the window screen served as a grid, and the number of squares where the food had been completely removed were counted (Hay et al., 1998). These results were analyzed with a paired t-test (Schupp et al., 1999b).

RESULTS AND DISCUSSION

TAXONOMY. While diving on reef flats, in sandy areas and coral rubble, a bright-red, short stocked capitum was observed protruding from the sand. The capitum was found sitting on top of a long fistule, buried in the sand, and attached to the turnip-shaped base of the sponge burrowed in the substrate (Fig. 2). Sometimes this basal portion can be buried up to 20cm deep into the substrate. The capitum is easily broken off by stronger water movements, found 'rolling' on the substrate. The capita are between 0.5-1.5cm diameter. The hollow fistules vary in length from 6-12cm and up to 1.3cm diameter. Fistules are attached to an irregular turnip-shaped main body (base), up to 10cm high, 6cm diameter, and sometimes several fistules are attached to the base of the sponge. In some cases one fistule branches into several smaller ones, protruding from the sand. The burrowing basal portion often grows around large pieces of coral rubble, or completely incorporates smaller pieces of rubble, shells, or sand into the base. The color of the sponge ranges from bright- to dull-red. The surface of the sponge is smooth, with a distinct skin-like ectosomal layer, and a very crumbly, fragile choanosome, with an abundance of foreign material. Spicules are exclusively curved oxeas, with pointed or blunt tips, ranging in size from 260-320x3-5µm. This material probably belongs to an undescribed species of *Oceanapia* (M. Kelly, pers. comm.), subsequently verified by Dr. Rob W.M. van Soest (Eder et al., 1998), and a voucher of the sponge is deposited in the Zoölogisch Museum, Amsterdam (registration number ZMA POR 11007).

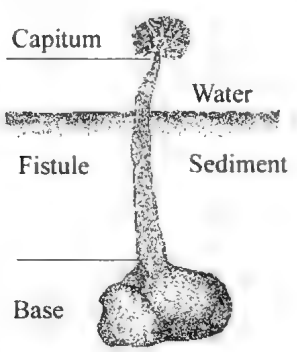

FIG. 2. Schematic drawing of *Oceanapia* sp. (Redrawn from Hooper et al., 1993).

CHEMISTRY. Three pyridoacridine alkaloids were isolated from *Oceanapia* sp. (Eder et al., 1998): kuanoniamine C (Fig. 1A), kuanoniamine D (Fig. 1B), and N-deacetylkuanoniamine D (Fig. 1C), of which kuanoniamine C and D have been previously described from an unidentified tunicate and its prosobranch mollusc predator, *Chelynotus semperi* (Carroll & Scheuer, 1990). Additionally, kuanoniamine C has also been isolated from a deep water sponge of the genus *Stelletta* (Gunawardana et al., 1989, 1992), and from *Oceanapia sagittaria* (Salomon & Faulkner, 1996). The fact that these alkaloids occur in different species, comprising more than one major phylum, gives support to the speculation that marine microorganisms could possibly be the true source of these compounds (Molinski, 1993). Conversely, there is some evidence that sponge cells are the actual producers of the alkaloids (Faulkner et al., 1999, this volume).

BIOLOGICAL ACTIVITY. The insecticidal activity of the three metabolites (Fig. 1A-C) was determined by assessing survival and growth rates after six days of exposure. From the dose-response curves obtained, LC_{50} values of 156ppm (±0.46 S.E.) for kuanoniamine C, and

TABLE 2. Results of the fish feeding experiments testing the deterrent effect of the extract and the kuanoniamine derivatives against an array of reef fish at Western Shoals, Guam.

Sponge part (compound/ extract concentration, % of dry mass)	Mean number of cubes eaten STD		N	P
	Control food	Treated food		
Methanolic extract from base , 7.4 %	3.1 ± 0.2	0.7 ± 0.7	17	<0.001
Kuanoniamine C from fistule, 1.2 %	3.5 ± 0.5	0.5 ± 0.6	19	<0.001
Kuanoniamine D from fistule, 0.4 %	3.6 ± 0.5	1.1 ± 1.2	19	<0.001
N-deacetylkuanoniamine D from fistule, 0.04 %	2.8 ± 0.7	2.5 ± 1.1	20	=0.27

59ppm (±0.30) for kuanoniamine D, were calculated by probit analysis. The new compound N-deacetylkuanoniamine D (Fig. 1C), was tested up to a concentration of 934ppm, although, in contrast to the other two compounds, no significant insecticidal activity was observed. Only the growth of larvae was reduced. This can be expressed by an ED_{50} value of 141ppm (±0.13) for the new compound (Fig. 1C).

General cytotoxicity was assessed by testing the three alkaloids (Fig. 1A-C), in the brine shrimp assay. After 24hrs of exposure, the number of surviving nauplii was counted. LC_{50} values calculated from the dose-response curves by probit analysis were 37µg/mL (±17) for one compound (Fig. 1A), and 19µg/mL (±4) for the second compound (Fig. 1B). The third compound (Fig. 1C), did not show any toxic effect up to 100µg/mL. No LC_{50} of this alkaloid was obtained due to its limited solubility at concentrations greater than 100µg/mL.

Several pyridoacridine alkaloids have been reported to exhibit significant cytotoxicity towards murine and human tumor cell lines (Molinski, 1993). In this study, we analyzed the effects of the three compounds (Figs 1A-C), on cell growth towards two different human cell lines using [^{3}H]-thymidine incorporation. This cytotoxicity assay is used to determine the capability of cells to synthesize DNA during the cell cycle. The validity of the method applied in this study has been documented previously (Arnould et al., 1990). Table 1 summarizes the results obtained with the two cell lines. The small amount of ethylene glycol monomethyl ether (0.1%), used to solubilize the compounds, did not affect growth of the tumor cells. Each alkaloid was tested for its cytotoxic activity at a range of concentrations (0.1-20µg/mL).

Suppression of [^{3}H]-thymidine incorporation into cells treated with the three compounds (Fig. 1A-C), was observed for each of the two cell lines.

Intercalation with DNA has been demonstrated previously for dercitin (Fig. 1D) (Gunawardana et al., 1988; Burres et al., 1989), a marine natural product closely related to the kuanoniamines. Based on obvious structural similarities of these three alkaloids (Fig. 1A-C) with dercitin, it may be hypothesized that the kuanoniamines also interact with DNA by intercalation.

In addition to our own testing, kuanoniamine C was tested with the *in vitro* disease-oriented primary antitumor screen on a panel of 60 cell lines at the National Cancer Institute (NCI), in Bethesda, Maryland, U.S.A.

The *in vitro* assays showed that most cell lines were fairly sensitive against kuanoniamine C at concentrations, ranging from 4.93×10^{-05} molar to 3.11×10^{-08} molar for the GI_{50}, and a concentration of 1.86×10^{-06} molar as the mean graph midpoint (mean concentration required over all cell lines; Monks et al., 1991). The GI_{50} is an interpolated value representing the concentration at which the percentage growth of exposed cells used in the assay is 50% compared to that of non-exposed cells (Monks et al., 1991). One of the breast cancer cell lines (MCF7), was extremely sensitive against kuanoniamine C, with a concentration of 3.11×10^{-08} molar for the GI_{50}.

Based on the successful performance of the *in vitro* screen, NCI subsequently requested further material for *in vivo* studies.

The cytotoxicity data are not corroborated by insecticidal activity towards neonate larvae of *S. littoralis*, or the toxic activity against *A. salina*. In the latter tests, the new derivative was nearly inactive, but it showed similar activity in the

cytotoxicity assay to kuanoniamine C and D. Thus, it is possible that the different activities observed are not caused by a general cytotoxicity, but may be due to a different mode of action.

ECOLOGY. *Distribution of secondary metabolites in the different sponge parts.* Extraction of the base, fistule and capitum of the sponge yielded significantly different crude extract concentrations. Concentrations increased from the base, with an extract yield of 26.4% of dry mass, 34.2% in the fistule, up to 52.7% crude extract concentration in the capitum (Schupp et al., 1999b).

Concentrations of kuanoniamine C and D increased sharply from the base to the capitum of the sponge. The lowest concentrations of these alkaloids were found in the base, with 0.4% of dry mass for kuanoniamine C and 0.1% of dry mass for kuanoniamine D. The fistule showed a four fold increase of these metabolites, with 1.2% of kuanoniamine C and 0.4% of kuanoniamine D. Highest concentrations were found in the translucent capitum, with 3.5% of dry mass for kuanoniamine C and 1.2% of dry mass for kuanoniamine D, representing a 9 to 12-fold increase in secondary metabolite concentration compared to the base (Schupp et al., 1999b). This huge increase of the two major metabolites kuanoniamine C and D in *Oceanapia* sp. stresses the importance not only to determine an overall yield of secondary metabolites, but also to look at compound concentration at an intraspecimen level. This is important for both ecological investigations and pharmacological screening for new bioactive compounds.

Secondary metabolites are often concentrated in parts where they are first encountered by predators, such as the skin of mollusks or in biologically valuable parts like reproductive regions (Pawlik et al., 1988; Avila & Paul, 1997). When whole organisms are extracted, compounds used by animals to deter possible predators might show lower yields than they actually have in the organs or regions where they are deposited. Therefore, it is possible that bioactive compounds are overlooked in pharmacological screens, because their concentrations are too low.

Fish feeding assays. The methanol fraction was highly deterrent towards reef fishes at base concentration (Table 2). It also deterred feeding by the angelfish *Pomacanthus imperator* in laboratory feeding experiments at the same concentration, although the deterrent effect was not as pronounced as with other reef fishes ($p = 0.047$; $N = 7$; Schupp et al., 1999b).

The field and laboratory fish feeding experiments showed that even the base is chemically well protected. This is surprising, since the base is normally not accessible to fish because it is buried in the sand. A possible explanation could be that the high extract concentrations might be a defense against crabs, flatworms and other sediment-dwelling invertebrates (Schupp et al., 1999b). The laboratory feeding experiments with *P. imperator* demonstrated the potency and broad effectiveness of the compounds, since they deterred the more specialized angelfish in addition to being a deterrent towards generalist reef fishes. Angelfishes are known to feed primarily on ascidians and sponges, and are thought not to be as susceptible to sponge secondary metabolites as are other fishes (Randall & Hartman, 1968; Wulff, 1994).

The field feeding assays with the pure compounds demonstrated that the pyridoacridine alkaloids were at least partly responsible for the deterrent effect of the crude extract. Kuanoniamine C and D clearly deterred fish feeding at fistule concentration (Table 2). Each compound was by itself effective in deterring reef fishes. Schupp et al. (1999b) suggested that it might be important for a sponge to have more then one deterrent chemical, or to increase the overall concentration of defensive compounds, to prevent feeding by different predators. The N-deacyl derivative did not reduce feeding (Table 2). One possible explanation is the low concentration of the compound in the sponge. It was tested at natural concentrations, which is approximately 1/10 of the concentration used for kuanoniamine D.

There are numerous studies showing that benthic invertebrates can reduce predation through the production of secondary metabolites (Bakus et al., 1986; Wylie & Paul, 1989; Paul, 1992; Pawlik, 1993; Ebel et al., 1997; Thacker et al., 1998), but there is little information on the pharmacological activity of these compounds - this study being one of them. One problem might be that researchers looking at the ecological importance of new compounds, seldom perform pharmaceutical screenings, and conversely, those searching for new pharmacologically active substances seldom conduct ecological experiments with the substances or organisms. Fish feeding assays could be used as a first indication for the presence or absence of pharmacologically

active substances, and also providing information on the ecological relevance of these compounds as feeding deterrents.

Schupp et al. (1999b) demonstrated that the intraspecimen distribution of secondary metabolites in *Oceanapia* sp. was in accordance with the optimal defense theory (McKey, 1974, 1979; Baldwin & Ohnmeiss 1994). This theory suggests that organisms optimize the production of secondary metabolites and deter possible predators with the least amount of energy, assuming that the production of secondary metabolites is costly. Therefore, biologically important parts (with a high contribution to fitness), like reproductive regions (e.g. the capitum in *Oceanapia* sp.; Hooper et al., 1993), and parts that are first encountered by predators (e.g. the fistule and capitum in *Oceanapia* sp.), should show higher secondary metabolite concentrations. This is certainly the case in *Oceanapia* sp. (Schupp et al., 1999b).

It might be advantageous to keep the optimal defense theory in mind, while collecting and screening for pharmacologically active compounds. By collecting mostly exposed and vulnerable parts of marine invertebrates, it is highly probable that biologically active compounds are present and that they are tested at ecologically relevant concentrations. This could be also interesting from a conservation standpoint, since it should be possible to collect the exposed parts of certain organisms (e.g. sponges), and leave the remainder to regrow. *Oceanapia* sp. can be used again as an example, since it is possible to collect the capitum without severely damaging the sponge. The capitum grows back and can be harvested repeatedly. The harvested amounts are small, but show on the other hand the highest secondary metabolite concentrations.

CONCLUSION

This study demonstrates, that ecological observations and experiments can be useful as initial indicators for possible pharmacological properties of secondary metabolites (Schupp et al., 1999a, 1999b). Field observations and fish feeding experiments demonstrated the biological activity of the pyridoacridine alkaloids found in *Oceanapia* sp. Through different pharmacological screens we were able to show possible physiological targets of the compounds, although these results do not explain the mode of action as a fish deterrent agent.

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MICROBIAL INFLUENCED PYRITISATION OF MARINE SPONGES. *Memoirs of the Queensland Museum 44: 550. 1999:-* Although biomineralisation plays an important role in the history of earth, our knowledge about the involved processes is rather limited. In this context

microorganisms take a significant part within these global processes. Pyrite crystals are often found in taphonomically mineralised sponge tissues but are absent or rare in the surrounding sediment. In terms of microbiology, the role of sponge-associated, anaerobic species such as sulfate reducing and fermentative bacteria is fairly unknown. During early decaying processes of the sponge tissue the internal sponge becomes entirely anaerobic which is a necessary prerequisite for the growth and metabolical activity of the sulfate reducers. Therefore, pyrite formation is probably linked with sulfide producing bacteria. In the marine environment, sulfate reducing bacteria are most likely to play this role besides heterotrophic sulfidogenic bacteria. In this study enrichment cultures of native sponge tissue of the mediterranean sponges *Chondrosia reniformis* and *Petrosia ficiformis* under sulfate reducing conditions were investigated using a combination of rRNA-targeted *in situ* probing and classical cultivation techniques. Recently developed specific oligonucleotide probes for sulfate reducing bacteria elucidated the occurrence and abundance of various sulfate reducing-species affiliated to different phylogenetic taxa within the Desulfovibrionaceae and Desulfobacteriaceae. Furthermore, 16S-rRNA based phylogeny revealed to hitherto unknown anaerobic bacteria inhabiting the sponge mesohyl. Additionally, the sulfate reducing activity was confirmed by established physiological methods. In living sponges, sulfate reducing bacteria were evenly distributed within the tissue. This indicates the presence of anoxic microniches, which allow not only the survival, but even the subsequent growth of these anaerobic bacteria. Further investigations of culturable sponge-associated sulfate reducing bacteria are currently carried out to investigate their ecology and ability to produce pyrit in culture. ❐ *Porifera, biomineralisation, pyritisation, anaerobic microbes, sulphate reducing bacteria, phylogeny, molecular biology, microniches.*

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RELATIONSHIP OF SAND AND FIBRE IN THE HORNY SPONGE, *PSAMMOCINIA*

CHUNG JA SIM AND KYUNG JIN LEE

Sim, C.J. & Lee, K.J. 1999 06 30: Relationship of sand and fibre in the horny sponge, *Psammocinia*. *Memoirs of the Queensland Museum* **44**: 551-557. Brisbane. ISSN 0079-8835.

Five species of the genus *Psammocinia* (Irciniidae) are described from Chejudo Island, Namhaedo Island and Wando Island, Korea (*Psammocinia jejuensis, P. mosulpia, P. mammiformis, P. samyangensis* and *P. wandoensis*). *Psammocinia* is characterised by large quantities of sand in spongin fibres, mesohyl matrix and as a thick superficial cortex. In addition to the primary and secondary branching fibres, fine filaments emerge from individual pores in the fibres. Occasionally short secondary fibres are connected to large sand grains, forming bridges between adjacent sand grains. The skeleton formed from sand grains associated with fibres provides additional support for the body of the sponge. ❐ *Porifera, horny sponge, Dictyoceratida, Psammocinia, Korea.*

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Psammocinia (Irciniidae) is characterised in having many sand grains within spongin fibres and the mesohyl matrix, and a surface crust of sand (Bergquist, 1980; Cook & Bergquist, 1996). Lendenfeld (1888, 1889) reported eight species in *Psammocinia*, at that time included as a subgenus of *Hircinia* (*Ircinia*). Of these, only four are currently included in *Psammocinia*: *H. rugosa* Lendenfeld, 1889, *H. arenosa* Lendenfeld, 1888, *H. tenella* Lendenfeld, 1889 and *H. halmiformis* Lendenfeld, 1888, and of these two (*H. rugosa* and *H. tenella*) are synonyms of *P. vesiculifera* (Poléjaeff, 1884) (Hooper and Wiedenmayer, 1994). More recently, Bergquist (1995) reported one new species from New Caledonia, and Cook & Bergquist (1998) described five new species from New Zealand. In Korea, three species of *Psammocinia* were reported from Chejudo Island and Namhaedo Island (Sim, 1998), and two new species, *P. samyangensis* Sim & Lee, 1998 and *P. wandoensis* Sim & Lee, 1998, were described from the South Sea of Korea (Sim & Lee, 1998).

In the present study we examine five species from Korean waters with the aim to determine the extent of skeletal support provided by these foreign skeletal elements, showing that sand is closely associated with the fibres of the sponge, sometimes providing support to the sponge as bridges of primary and secondary fibres connect sand grains in the body.

The principal diagnostic characteristic of Irciniidae is the possession of a third element of the skeleton consisting of fine collagenous filaments beyond the fasciculate primary fibres and uncored secondary fibres (Bergquist, 1980). Filaments of *Psammocinia* emerge from pores in the fibres.

MATERIALS AND METHODS

Specimens of *Psammocinia* were collected from Chejudo Island, Namhaedo Island and Wando Island in Korea; *P. mammiformis* (Manjaedo, Namhaedo Island), *P. mosulpia* (Mosulp'o, Chejudo Island), *P. jejuensis* (Kimnyung, Chejudo Island), *P. samyangensis* (Samyang 1 dong, Chejudo Island) and *P. wandoensis* (Wando Island). Specimens were collected by SCUBA, 10-25m depth, and by fishing-net. For identification of horny sponge, light microscopy and SEM (AKASHI ISI-SS40) were used to determine fibre arrangement.

RESULTS

DISTRIBUTION OF SAND. In all species examined the primary fibres are completely filled with sand grains that form a loosely packed sand core. Secondary fibres may be either partially cored with sand, or lack any sand grains within their cores (Fig. 1D, E). Larger size sand grains are attached to the outside of the fibres (Fig. 2G). Beneath the surface, a sand crust is mixed with foreign spicules (Fig. 1A, B). In one species (*P. jejuensis*) the external surface of the sponge is armoured with pieces of shell debris.

SAND ON THE ECTOSOMAL CRUST. Surfaces of *P. wandoensis, P. mosulpia* and *P. mammiformis* have a sand crust mixed with foreign spicules, whereas *P. samyangensis* has a

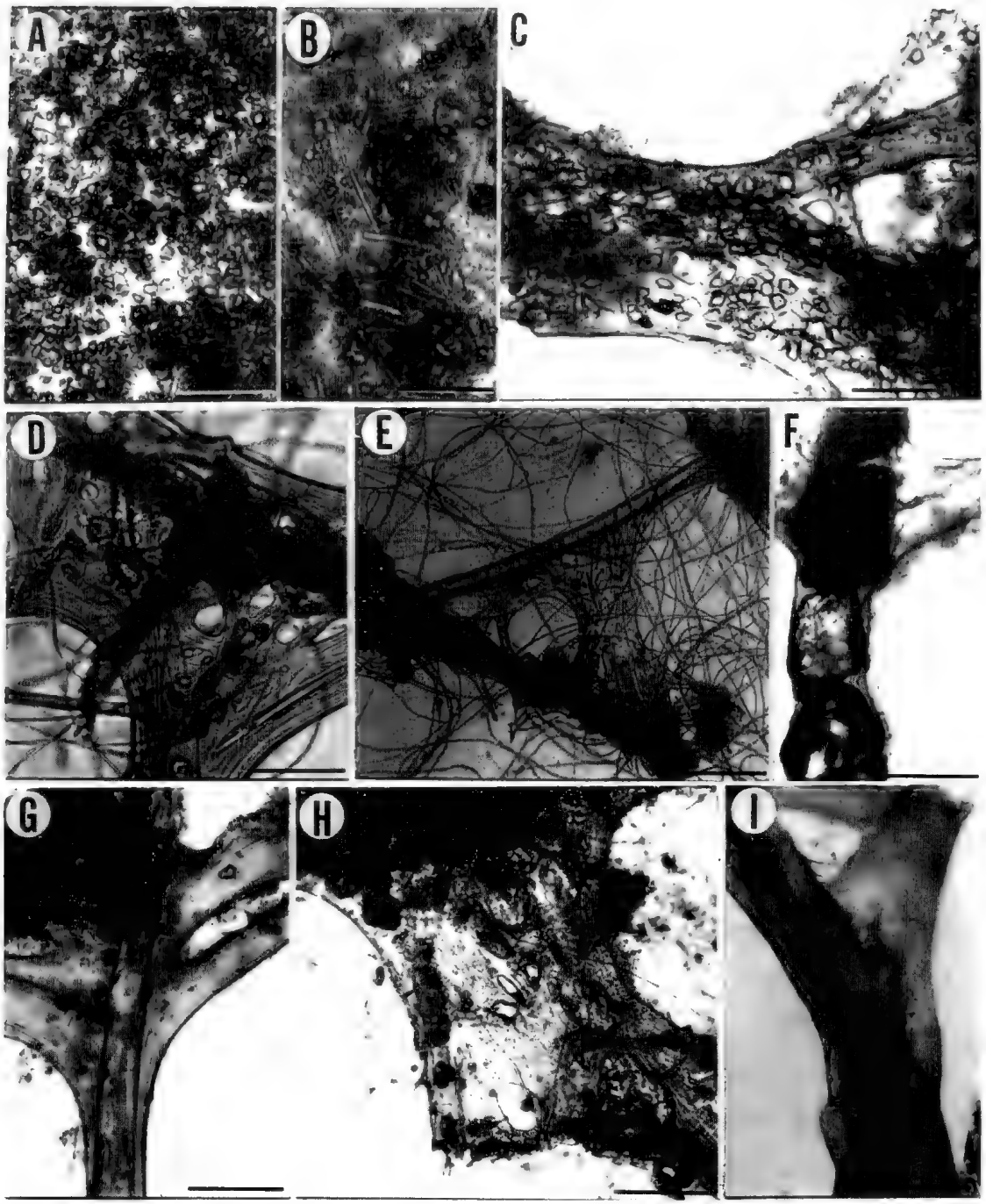

FIG. 1. A-B, *Psammocinia mosulpia*; A, sand crust; B, under-crust mixed with sponge spicules. C, *P. wandoensis*, sand attached inside of fibres. D-E, *P. Samyangensis*; D, primary fibre with sand; E, large oxea supporting fibre. F, *P. mosulpia*, primary fibre with sand. G-H, *P. jejuensis*; G, large oxea in fibre; H, sand in fibre. I, *P. mammiformis*, primary fibre with sand. (Scale bars; A-B, 300μm; C-D, 150μm; E, 300μm; F- I, 150μm)

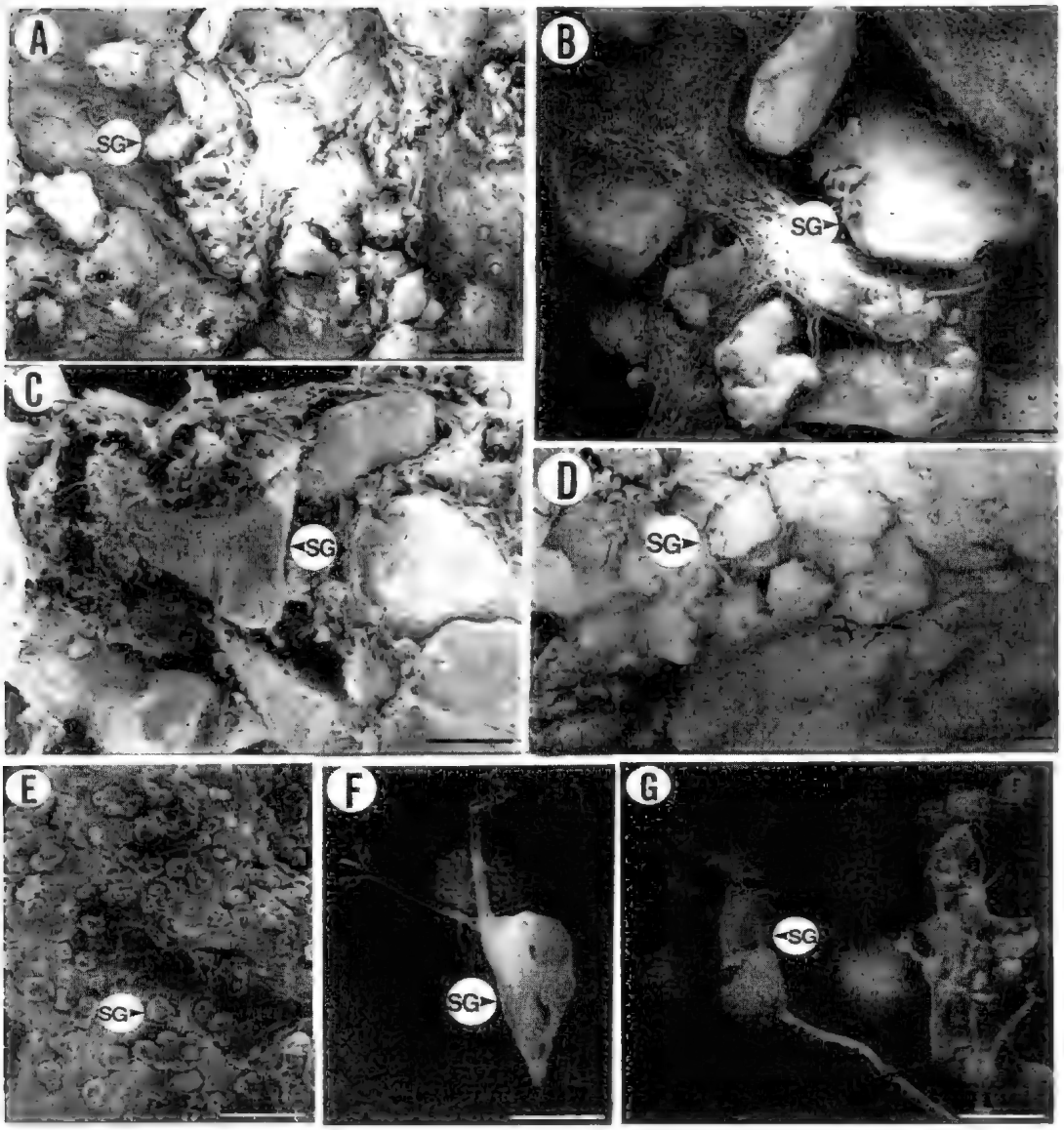

FIG. 2. A, *Psammocinia mammiformis,* choanosome with sand grains (SG). B, *P. samyangensis*, choanosome. C, *P. jejuensis*, choanosome. D, *P. mosulpia*, choanosome. E, *P. wandoensis*, choanosome. F, *P. samyangensis*, choanosome secondary fibre with sand. G, *P. mosulpia*, choanosome fibre with sand (Scale bars: A-D, 400μm; E, 100μm; F, 80μm; G, 300μm).

thin filamentous membrane mixed with large sand grains and pieces of shell, each 1-2.5mm diameter, not strictly a sand crust. The texture of this species is soft and easily torn because the fibre and filament arrangement is very loose. *Psammocinia jejuensis* also has filamentous membrane instead of true sand crust, with large grains of sand and pieces of shell distributed within the surface armour. In *P. mosulpia* there is a black sand crust making this species appear darkly pigmented (Fig. 1A, B).

SAND IN THE MATRIX. The choanosomal matrix also contains many sand grains, with sizes of sand grains varying between each species. In *P. jejuensis* and *P. samyangensis* large sand grains, 0.7-4.0mm diameter, are combined with the filament network (Fig. 2B). In *P. wandoensis* the

mesohyl matrix contains smaller size sand grains, 10-70μmdiameter (Fig. 2E), whereas *P. mosulpia* and *P. mammiformis* have sand grains of intermediate size, 350-600μm diameter.

SAND IN SPONGIN FIBRES. Sand grains in *P. wandoensis* are attached to the inside of fibres, with grains approximately 50μm diameter and uniformly distributed in a unidirectional plane. In this species the fibres are difficult to differentiate from the closely packed, amalgamated sand grains.

In *P. samyangensis* the accumulation of large sand grains, 10-180μm diameter, completely obscure the axis of primary fibres, and often a single foreign oxea connects adjacent primary fibres like a bridge, further supporting fibres. Rarely, smaller sand is distributed among the primary fibres (Fig. 1D, E).

Psammocinia mosulpia has large sand grains, 100-400μm diameter, contained within transparent fibres which are simple, not fasciculated (Fig. 1F). Sand grains are attached in and outside of fibres.

Psammocinia jejuensis has sand grains within its primary fibres that make up stout fasciculate columns. Sand grains measure 20-220μm diameter. Fibres are easily torn. Secondary fibres may have a large single foreign oxea included, up to approximately 1,440μm long and 80μm wide, appears to support the sponge (Fig. 1G, H). These oxeas are unbroken within fibres.

In *P. mammiformis* there are thick, strong fibres with chain-like, small sand grains included in the centre of fibres (Fig. 1I). Secondary fibres connected to large sand grains, form bridges between adjacent sand grains (Fig. 2F). Sands and fibres are tightly bound together (Fig. 2G).

FILAMENTS AND FIBRES. In all five species of *Psammocinia* we observed that filaments emerge from pores on fibres (Figs 3A-F, 4A-H). These filaments are visible under light microscopy, but are more clearly observed using SEM. In *P. samyangensis* fibre pore sizes vary greatly, apparently correlated to the thickness of filaments emerging. At their base several adjacent filaments fuse to form a central filament (Fig. 3B). At their terminal ends filaments are usually composed of a single terminal knob (Figs 3C, 4H). The fibres seem to be in the form of a branch but we are able to confirm that there is an opening on the end of the branch from which the filament emerges (Figs 3E, F, 4D).

DISCUSSION

In *Psammocina* spongin fibres are thin and either simple or weakly fasciculated. As such, fibres probably do not provide sufficient support for the sponge body. Through the incorporation of sand grains into fibres and at the fibre core, sufficient structural integrity is achieved by these species. In addition to the rigidity received from association with sand grains, fibres of *Psammocinia* are also supported by a large, single oxea in many places within the mesohyl (Fig. 1E). Filaments also serve an important role in the sponge. If the sponge surface lacks a true sand crust, filaments produce a filamentous, cotton-like membrane at the surface. Together these structures provide some measure of skeletal support for the sponge, perhaps compensating for some inadequacy in their own organic skeletal elements.

Bergquist (1995) stated that organic filaments were separated from spongin fibres, whereas we have shown that filaments emerge from the numerous pores throughout the fibre, and thus integral to the sponge fibre system. We also noted that several filaments emerge from a pore on the fibres (Fig. 3A), merge, and continue as a single filament ending in a terminal knob (Fig. 3D, E,

TABLE 1. Main characteristics of the five *Psammocinia* species.

Species	Consistency	Surface	Mesohyl	Fibre and Sand	Filament
P. wandoensis	Very resilient	Thick sand crust	Small sand grains, 10-120μm diameter	Much small sand inside fibres	Filament pores difficult to detect
P. mosulpia	Resilient	Thin sand crust	Medium sand grains, 400-600μm diameter	Sand in and outside of fibre	Filament pores difficult to detect
P. mammiformis	Resilient	Sand crust	Medium sand grains, 350-600μm diameter	Small sand in fibre, chain-like	Filament pores slightly visible
P. jejuensis	Hard but not resilient, easily torn	No sand crust, filamentous membrane	Large sand grains, 700-4,000μm diameter	Much sand in fibre	Filament pores easily visible
P. samyangensis	Very soft, easily torn	No sand crust, filamentous membrane	Large sand grains, 700-4,000μm diameter	Much sand in fibre	Filament pores easily visible

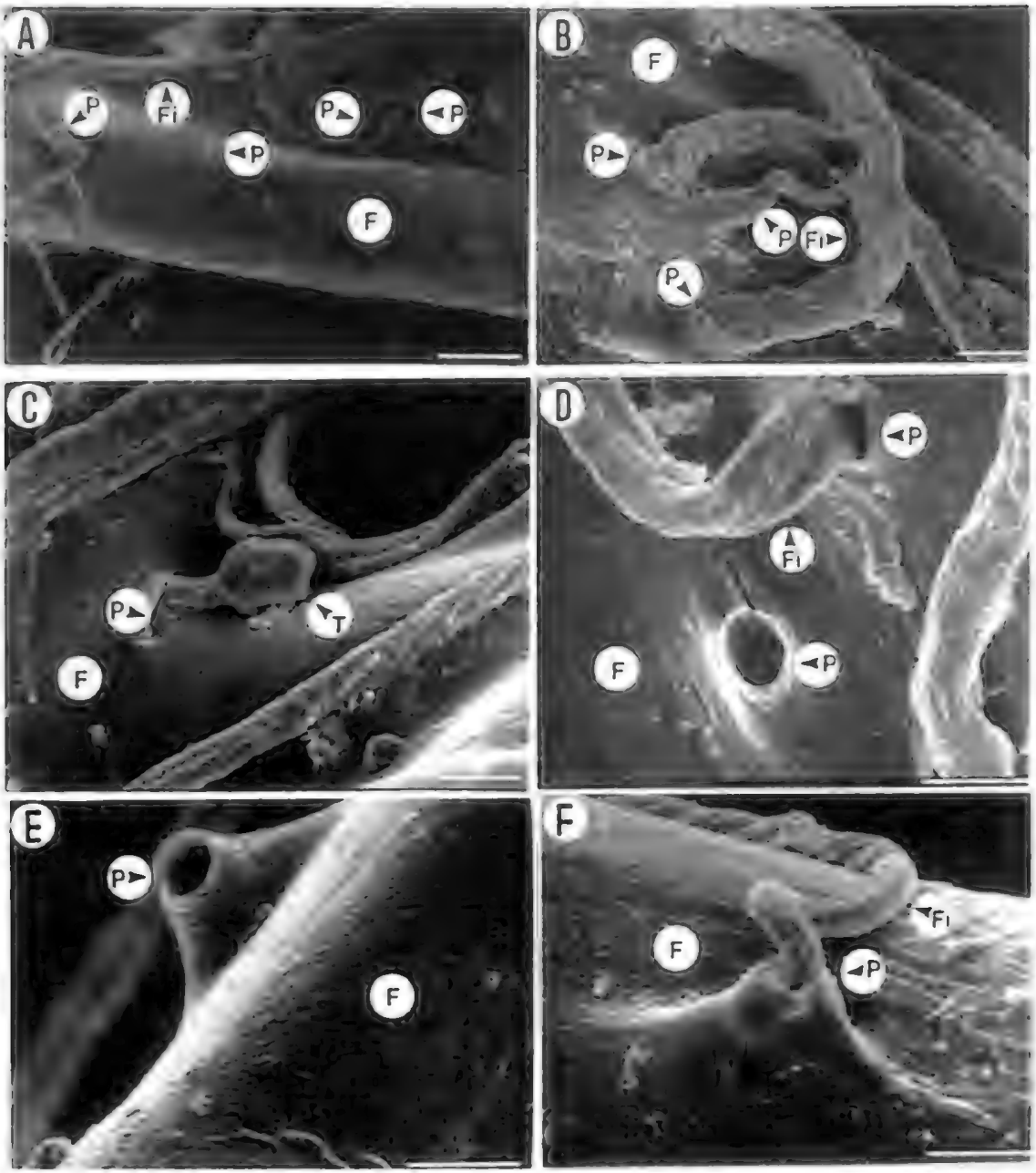

FIG. 3. A-F, *Psammocinia samyangensis*; A, many pores on a fibre (F, fibre; Fi, filament; P, pore); B, base of filaments and pores; C, filament emerging from pore on the fibre (T, terminal knob); D-E, filament and pore on a fibre; F, base of filament and pore (Scale bars: A, 100μm; B-C, 20μm; D-F, 10μm).

H). Very rarely, we noted filaments emerging from both fibre pores and longitudinal slits along the fibres (Fig. 3C), but most commonly filaments appear to emerge only from pores.

Cook & Bergquist (1998) stated that the fibre skeleton in *Psammocinia* is supplemented at fine collagenous filaments, each enlarged terminally at both ends, whereas in the five species examined here these terminal knobs appear at one end only.

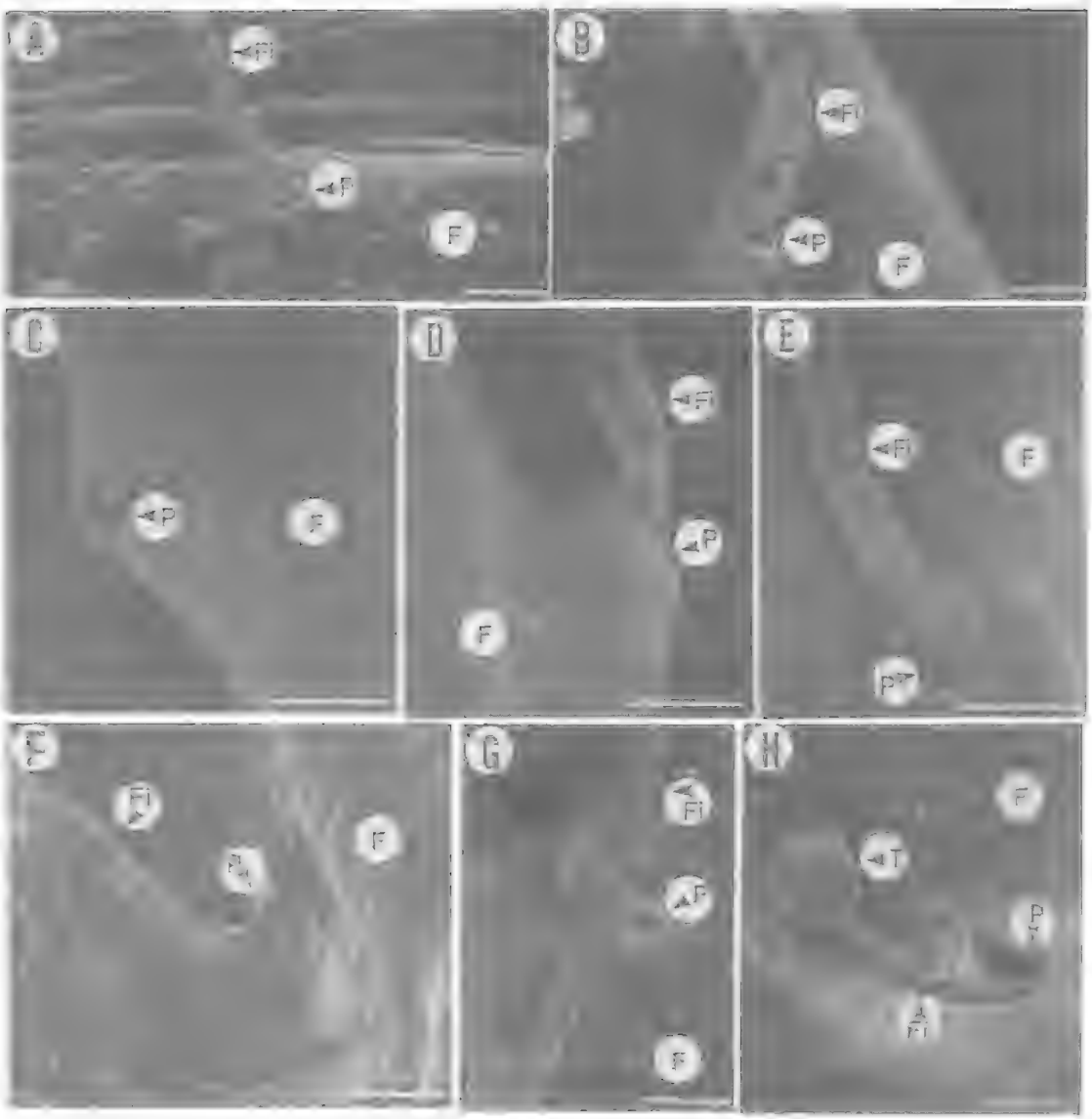

FIG 4. A-B, *Psammocinia jejuensis*, base of filament (F, fibre; Fi, filament; P, pore). C-E, *Psammocinia mammiformis*; filament and pore on a fibre. F, *Psammocinia wandoensis*, base of filament and pore. G-H, *Psammocinia mosulpia*; G, base of filament and pore; H, filament emerging from pore on a fibre (T, terminal knob) (Scale bars: A-B, 20μm; C-E, 10μm; F-H, 20μm).

Several questions still remain regarding the nature of the filaments of *Psammocinia*. One such question concerns the origin and development of the filaments along the fibres, which is a topic for further study. Another question concerns the quantity of filaments in relation to the quantity of fibre pores. In all sponges we examined we observed a large number of filaments, whereas there were far fewer pores from which the filaments emerged, and we assume, with empirical support from SEM studies, that a single pore can produce several filaments over time.

Due to the complex morphology of the fibres and filament arrangement in *Psammocinia*, we were fortunate to observe the multi-based filaments (Fig. 3A) not previously described for this genus. Further studies are required, however, to

determine whether this type filament is exclusively a characteristic of *Psammocinia*, or is also found within other sponges of Irciniidae.

As noted in Table 1, all species with a true sand crust are tough and it is difficult to observed filament pores given that so many sand grains are attached to fibre. The two species without a true sand crust are not tough, easily torn, have many filaments and many filament pores were observed.

ACKNOWLEDGEMENTS

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***CHONDROSIA RENIFORMIS*: HITHERTO UNKNOWN BACTERIA.** *Memoirs of the Queensland Museum 44: 558. 1999:-* Marine sponges as evolutionarily ancient Metazoa are nowadays in the focus of great interest. However, the implication of closely associated bacterial populations within the sponge tissue is completely unknown. One of the sponges investigated to clarify this relationship is the Mediterranean species *Chondrosia reniformis*. Bacteria associated with this sponge were examined by aerobic and anaerobic cultivation, culture-independent methods as whole cell *in situ* hybridization, PCR assisted rDNA sequence retrieval and comparative sequence analysis. *In situ* hybridization of bacteria within the sponge tissue with fluorescently labeled rRNA-targeted oligonucleotides for the major subclasses of Proteobacteria revealed a great part of the population to be affiliated to the alpha-, gamma- and delta-subclasses. Interestingly, no organism was found to be a member of the beta-Proteobacteria. On the other hand, there are also many microorganisms that only gave signals with the universal probe for all bacteria, whereas group- or species-specific probes did not hybridize with these bacteria. Some of them are culturable and could successfully be characterised using the polyphasic approach. 16S rDNA-sequencing and subsequent analysis of the cultivated bacteria obtained from sponge-tissue showed that these metazoa are closely associated with a great diversity of hitherto unknown bacteria. Further studies of culturable sponge-associated bacteria are currently carried out to investigate the ecology of sponge-associated bacteria. This survey will elucidate the physiological properties and, by molecular analysis, the phylogenetical affiliation of these organisms. Occurrence and spatial distribution of even unculturable bacteria in sponge tissue will be analyzed by in situ hybridization with specifically designed rRNA-targeted oligonucleotides. ❐ *Porifera, Bacteria, in situ sequencing, phylogeny, PCR, microecology.*

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CYANIDE AND THIOCYANATE-BASED BIOSYNTHESIS IN TROPICAL MARINE SPONGES

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The sponge *Axinyssa* n.sp. incorporates both sodium [^{14}C] cyanide and sodium [^{14}C] thiocyanate into 2-thiocyanatoneopupukeanane as well as into 9-isothiocyanatopupukeanane, however these 2 precursors were not incorporated into 9-isocyanopupukeanane. The specificity of incorporation into the thiocyanate carbon was confirmed by chemical degradation. *Stylotella aurantium* incorporates sodium [^{14}C] cyanide and sodium [^{14}C] thiocyanate into the dichloroimine functionality of the stylotellanes A and B, as well as into farnesyl isothiocyanate. The specificity of incorporation into the dichloroimine carbon atom was confirmed by chemical degradation. These experiments represent the first detailed study of the biosynthetic origin of organic thiocyanates and dichloroimines, and extend the range of functionality known to be biosynthesised from cyanide and thiocyanate. Our results raise the interesting question of the interconversion of inorganic cyanide and thiocyanate and/or the interconversion of the resulting organic metabolites in marine sponges. An isothiocyanate-isocyanide conversion was demonstrated in *Amphimedon terpenensis* by incorporation of a ^{14}C-labelled sample of diisothiocyanatoadociane into diisocyanoadociane. ❐ *Porifera, Amphimedon terpenensis, Axinyssa, Stylotella aurantium, biosynthesis, cyanide, dichloroimines, isocyanides, isothiocyanates, secondary metabolites, terpenes, thiocyanates.*

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Marine sponges of the order Axinellida, Halichondrida and Haplosclerida often contain bioactive terpenes with isocyanide, isothiocyanate and formamide functionality; the rarer isocyanate and thiocyanate substituents are also known (Scheuer, 1992; Chang & Scheuer, 1993; Garson et al., 1998). These unique metabolites have been novel targets for study with ^{14}C- and ^{13}C-labelled precursors to determine the biosynthetic origin of the non-terpenoid carbon atom (Garson, 1989; Chang & Scheuer, 1990; Garson, 1993; Garson et al., 1999). Work by our research group on the sponge *Amphimedon terpenensis* has shown that marine isocyanides such as diisocyanoadociane (Fig. 1A) are derived by functionalisation of a terpene precursor with inorganic cyanide (Garson, 1986; Fookes et al., 1988). Karuso & Scheuer (1989) subsequently showed that both diterpene (eg. Fig. 1B) and sesquiterpene (eg. Fig. 1C) isocyanides are cyanide-derived, and further demonstrated the intact incorporation of the N_1-C_1 unit. Our recent work with *Acanthella cavernosa* has shown the utilisation of cyanide for the biosynthesis of both a sesquiterpene isocyanide (Fig. 1D) and an isothiocyanate (Fig. 1E) in this axinellid sponge. Furthermore inorganic thiocyanate was shown also to be a precursor to both the isocyanide and the isothiocyanate metabolites in this sponge. From these experimental results a biosynthetic link was inferred between the two inorganic precursors or between the two metabolite types (Dumdei et al., 1997).

The origin of the thiocyanato group has been the subject of much biosynthetic speculation (Garson, 1993). Pham et al. (1991) suggested the cyanation of a thiol, which appears to be a reasonable pathway to the amino acid-derived psammaplin thiocyanate (Jimenez & Crews, 1991). In contrast, in those sponges in which thiocyanates co-occur with isocyanides or with isothiocyanates, the involvement of the ambident thiocyanate ion has been invoked (He et al., 1989; 1992; Walker & Butler, 1996). The dichloroimine (= carbonimidic dichloride) moiety represents a rare example of a functional group containing both nitrogen and carbon which has previously been found in terpene metabolites of the Indo-Pacific sponge *Pseudaxinyssa pitys* (Wratten & Faulkner, 1977; 1978a; 1978b). The coocurrence of an isothiocyanate together with dichloroimines in *P. pitys* suggested to us the involvement of

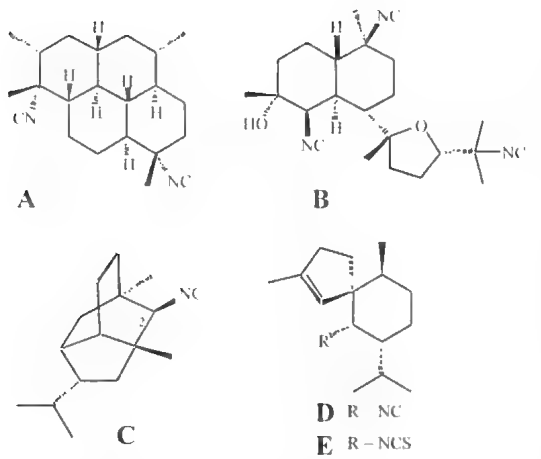

FIG. 1. Structures of isocyanide and isothiocyanate metabolites investigated in biosynthetic experiments. A, diisocyanoadociane. B, kalihinol F. C, 2-isocyanopupukeanane. D, axisonitrile-3. E, axisothiocyanate-3.

cyanide/thiocyanate in the biosynthesis of the dichloroimine group.

In this paper we present the results of biosynthetic experiments with the sponge *Aximyssa* n. sp. which provide evidence for a cyanide/thiocyanate origin of the thiocyanato functionality. We test the possibility using *Stylotella aurantium* that dichloroimine metabolites are biosynthesised from farnesyl pyrophosphate using cyanide or thiocyanate to supply the N_1-C_1 moiety. The role of inorganic thiocyanate and of an organic isothiocyanate in diisocyanoadociane biosynthesis are also explored.

MATERIALS AND METHODS

Abbreviations. GC-MS, gas chromatography- mass spectrometry; TLC, thin layer chromatography; NMR, nuclear magnetic resonance; HPLC, high performance liquid chromatography.

Chemicals and biochemicals. Solvents used in the extraction of compounds from sponge samples were glass distilled. All radioactive precursors were purchased from Sigma Chemical Co. (St Louis, MO).

Biological materials. Samples of *Axinyssa* n. sp. (Halichondrida: Halichondriidae), *Stylotella aurantium* (Halichondrida: Halichondriidae), Kelly-Borges & Bergquist, 1988, and *Amphimedon terpenensis* (Haplosclerida: Niphatidae) Fromont, 1993, were collected using SCUBA at Coral Gardens, Experimental Gardens or Coral Spawning dive sites (12-16m depth), Heron Island (23°27'S, 151°55'E) or at North Point (12-16m depth), Lizard Island (14°39'S, 145°27'E) on the Great Barrier Reef, Australia under permit numbers G96/050, G97/097, G98/037 and G98/227 issued jointly by the Great Barrier Reef Marine Park Authority and the Queensland National Parks and Wildlife Service. Sponge samples used in biosynthetic experiments were maintained in running seawater at ambient temperature and light conditions prior to use. Voucher specimens of the sponges *Axinyssa* n. sp., (accession number QMG312575), *Stylotella aurantium* (QMG307133) and *Amphimedon terpenensis*, (AMZ4978; QMG314228), are held at the Queensland Museum (QM), Brisbane or the Australian Museum (AM), Sydney.

Isolation of metabolites. 1) Axinyssa n. sp. An organic extract was prepared from frozen sponge (49.6g wet wt) and further purified by normal phase flash chromatography (gradient elution with hexanes/EtOAc) and normal phase HPLC using 0.25% EtOAc in hexanes to give (-)-9-isocyanopupukeanane (Fig. 2A; 107.6mg), (-)-9-isothiocyanatopupukeanane (Fig. 2B; 3.5mg), and (-)-2-thiocyanatoneopupukeanane (Fig. 2C; 31.4mg) together with smaller amounts of other isocyanides and isothiocyanates as described by Simpson et al. (1997b). 2) *Stylotella aurantium*. An organic extract was prepared from frozen sponge (204g wet wt) and further purified by normal phase flash chromatography (gradient elution with hexanes/EtOAc) and by normal phase HPLC using 0.2% EtOAc in hexanes to give stylotellane A (Fig. 2E; 9mg), stylotellane B (Fig. 2F; 75.6mg), and farnesyl isothiocyanate (Fig. 2G; 2mg) as described by Simpson et al. (1997a). 3) *Amphimedon terpenensis*. Diisocyanoadociane (Fig. 1A; 16mg) was isolated from frozen sponge (25g wet wt) as described by Fookes et al. (1988).

Biosynthetic experiments. 1) Pieces of *Axinyssa* n. sp. (approx. 80g wet wt) were placed in an aquarium containing 200ml aerated seawater at ambient temperature (20-23°C). Sodium [^{14}C] cyanide (100μCi) or sodium [^{14}C] thiocyanate (25μCi) was added and the sponge allowed to assimilate radioactivity for 12hr. The sponge was kept in running seawater in a 10 litre aquarium at ambient temperature for 16 days, then frozen for subsequent radiochemical analysis. Metabolites were purified according to the above protocol. The radioactivity content was monitored at each stage of the purification sequence, and terpenes were subjected to repeated HPLC until the specific activity was constant. 2) *Stylotella aurantium* (24g

wet wt) was placed in an aquarium containing 200ml aerated seawater at ambient temperature (20-23°C). Sodium [^{14}C] cyanide (50μCi) was added and the sponge allowed to assimilate radioactivity for 12hr overnight. The sponge was kept in running seawater in a 10L aquarium at ambient temperature for 9 days, then frozen for subsequent radiochemical analysis. Metabolites were purified according to the above protocol. The radioactivity content was monitored at each stage of the purification sequence. A sodium [^{14}C] thiocyanate (13μCi; 9 days incorporation) experiment, used a 12g piece of sponge. 3) *Amphimedon terpenensis* (26g wet wt) was placed in an aquarium containing 400mL aerated seawater at ambient temperature (20-23°C) [^{14}C]-Diisothiocyanatoadociane (11μCi) was added and the sponge allowed to assimilate radioactivity for 12hrs overnight. The sponge was kept in running seawater in a 20L aquarium at ambient temperature for 19 days, then frozen for subsequent radiochemical analysis. Metabolites were purified according to the above protocol. The radioactivity content was monitored at each stage of the purification sequence. A sodium [^{14}C] thiocyanate (50μCi; 19 days incorporation) experiment used a 45g piece of sponge.

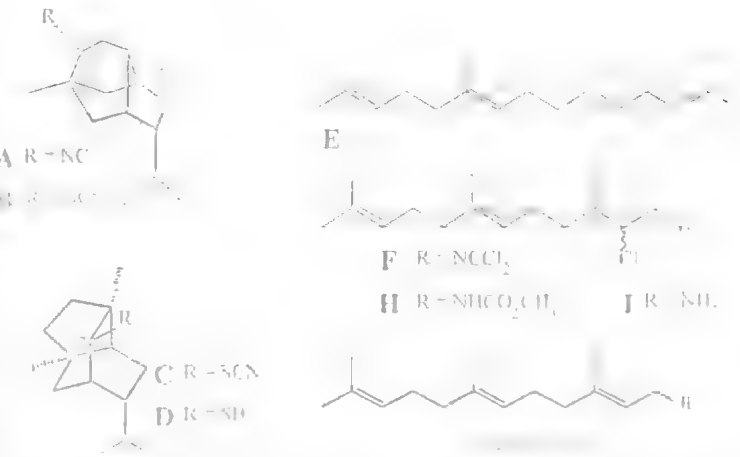

FIG. 2. Structures of terpenes isolated from *Axinyssa* sp., *Stylotella aurantium* and degradation products. A, 9-isocyanopupukeanane. B, 9-isothiocyanatopupukeanane. C, 2-thiocyanatoneopupukeanane. D, thiol from 2-thiocyanatoneopupukeanane. E, stylotellane A. F, stylotellane B. G, farnesyl isothiocyanate. G, methyl carbamate from stylotellane B. I, amine from stylotellane B

Procedures used in the synthesis of [^{14}C]-diisothiocyanatoadociane will be described elsewhere (Simpson & Garson, in preparation).

RESULTS

1) *Axinyssa* n.sp. collected at Heron I. contained sesquiterpene metabolites by GC-MS, TLC and NMR; the hexane-solubles were processed as described in Simpson et al. (1997b) to give the 9-pupukeanane isocyanide/isothiocyanate pair (Fig. 2A,B) and 2-thiocyanatoneopupukeanane (Fig. 2C). The GC-MS profile of the sesquiterpene fraction showed a number of other peaks including isocyanides and isothiocyanates. Light and electron microscopic inspection of *Axinyssa* n.sp. revealed the presence of microbial symbionts. The outer layers of sponge tissue were rich in cyanobacteria of a type morphologically similar to *Aphanocapsa feldmanni* while the inner tissue contained high populations of diverse bacterial cell types in addition to sponge cells. An Archaea-like symbiont with a membrane-bound nucleoid was found (see Fuerst et al., this volume; Fuerst et al., 1998).

25μCi Sodium [^{14}C] thiocyanate was supplied to a specimen of *Axinyssa* n.sp. maintained in a small aquarium (Dumdei et al., 1997; Simpson & Garson, 1998). After 16 days aquarium incubation, the sponge sample was frozen and 2-thiocyanatoneopupukeanane (Fig. 2C) was isolated and rigorously purified by HPLC to constant specific radioactivity. The thiocyanate (Fig. 2C) was significantly radioactive, as shown in Table 1, consistent with the use of thiocyanate for the biosynthesis of the thiocyanato group as shown in Fig. 3. To test the specificity of incorporation, 2-thiocyanatoneopupukeanane (Fig. 2C) was degraded to the thiol (Fig. 2D) using $LiAlH_4$. The thiol product was not radioactive (Table 2) therefore the [^{14}C] label was exclusively associated with the thiocyanato carbon. Incorporation of sodium [^{14}C] cyanide into a second piece of sponge also gave radioactive 2-thiocyanatoneopupukeanane (Table 1). Degradation resulted in unlabelled thiol product (Table 2) indicating the label was again exclusively associated with the thiocyanato moiety.

Our experiments also allowed us to monitor isocyanide/isothiocyanate biosynthesis in this sponge. When the isocyanide/isothiocyanate pair

TABLE 1. Molar specific activities of *Axinyssa* n. sp. metabolites. a, published incorporation values were not percentage values (Simpson & Garson, 1998); b, incorporation of 25μCi; c, $<10^{-2}$ %; d, incorporation of 100μCi.

Compound (Fig. no.)	Precursor	Molar specific activity (mCi/mMole)	Incorporation[a] (%)
2A	Na[^{14}C]SCN[b]	0.004	[c]
2B	Na[^{14}C]SCN[b]	2.630	0.08
2C	Na[^{14}C]SCN[b]	0.150	0.02
2A	Na[^{14}C]CN[d]	0.014	[c]
2B	Na[^{14}C]CN[d]	13.900	0.3
2C	Na[^{14}C]CN[d]	1.230	0.2

(Fig. 2A,B) were isolated, the isothiocyanate samples were radioactive (>150,000dpm/mg), whereas the isocyanide samples from both thiocyanate and cyanide feedings were not significantly labelled (<100dpm/mg). The specificity of labelling of 9-isothiocyanato-pupukeanane is currently under investigation.

2) Extracts of the sponge *Stylotella aurantium* weakly inhibited the growth of a P388 mouse leukaemia cell line and contained terpenes by TLC and NMR. The DCM-soluble components of the extract were processed as described in Simpson et al. (1997a) to give the stylotellanes A and B (Fig. 2E,F), together with farnesyl isothiocyanate (Fig. 2G). Light microscopic inspection of sponge tissue revealed the absence of microbial symbionts other than bacteria.

50μCi Sodium [^{14}C] cyanide was supplied to a specimen of *S. aurantium* according to our established protocols (Dumdei et al., 1997; Simpson et al., 1997a). After 9 days aquarium incubation, the sponge sample was frozen and stylotellanes A and B were isolated and rigorously purified by HPLC to constant specific radioactivity. The samples of stylotellanes A and B (Fig. 2E,F) were significantly radioactive, as shown in Table 3, consistent with the use of cyanide for the biosynthesis of the dichloroimine group (Fig. 4, route notation 'a'). The percentage incorporation levels measured were low as a result of loss of volatile metabolites during the purification process combined with the chemical instability of the dichloroimine group. To test the specificity of incorporation, stylotellane B was degraded to the methyl carbamate (Fig. 2H) and the amine (Fig. 2I) using 0.1N phosphoric acid in 95% methanol. The carbamate product was radioactive, whereas the amine was devoid of radioactivity (Table 4), therefore the [^{14}C] label was exclusively associated with the imine carbon. Incorporation of sodium [^{14}C] thiocyanate into a second piece of sponge also gave radioactive metabolites (Table 3), however there was insufficient material for chemical degradation. In each experiment, the isolated farnesyl isothiocyanate (Fig. 2G) was also radioactive.

TABLE 2. Molar specific activities of *Axinyssa* n. sp. degradation products.

Compound (Fig. no.)	Precursor	Molar specific activity (mCi/mMole)	Radioactivity (%)
2C	Na[^{14}C]SCN	0.150	100.0
2D	Na[^{14}C]SCN	<0.001	0.3
2C	Na[^{14}C]CN	1.230	100.0
2D	Na[^{14}C]CN	0.001	0.1

3) 50μCi Sodium [^{14}C] thiocyanate was then supplied to a specimen of *A. terpenensis* according to our established protocols (Fookes et al., 1988; Dumdei et al., 1997). After 19 days aquarium incubation, the sponge sample was frozen and diisocyanoadociane isolated and rigorously purified by HPLC, then recrystallised to constant specific radioactivity. The sample was significantly radioactive consistent with the use of thiocyanate for the biosynthesis of the isocyanide group (Fig. 5). Degradative experiments are in progress to confirm the specific labelling. When a sample of diisothiocyanatoadociane, ^{14}C-labelled in both isothiocyanate groups, was provided to *A. terpenensis*, the diisocyanoadociane isolated was found to be radioactive. The specificity of labelling is under investigation.

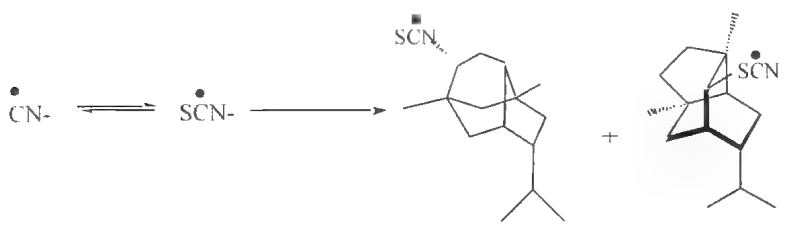

FIG. 3. Incorporation into isothiocyanate and thiocyanate metabolites of *Axinyssa* n. sp.

TABLE 3. Molar specific activities of *S. aurantium* metabolites. a, incorporation of 50μCi; b, incorporation of 13μCi.

Compound (Fig. no.)	Precursor	Molar specific activity (mCi/mMole)	Incorporation (%)
2E	Na[^{14}C]CN[a]	1.136	0.004
2F	Na[^{14}C]CN[a]	1.472	0.033
2E	Na[^{14}C]SCN[b]	0.354	0.00034
2F	Na[^{14}C]SCN[b]	0.224	0.00056

TABLE 4. Molar specific activities of *S. aurantium* degradation products. a, after dilution with unlabelled metabolite.

Compound (Fig. no.)	Precursor	Molar specific activity (mCi/mMole)	Radioactivity (%)
2F	Na[^{14}C]CN	0.332[a]	100.0
2H	Na[^{14}C]CN	0.326	98.2
2I	Na[^{14}C]CN	0.004	1.2

DISCUSSION

Our biosynthetic experiments with *Axinyssa* n. sp. and with *S. aurantium*, together with the earlier work on *A. terpenensis* and *A. cavernosa* (Garson, 1986; Fookes et al., 1988; Dumdei et al., 1997), reveal that cyanide and thiocyanate are precursors involved in the biosynthesis of four N_1-C_1 functional groups found in marine terpenes, namely isocyanides, isothiocyanates, thiocyanates and dichloroimines.

A number of different biosynthetic pathways can be invoked to explain the origin of the thiocyanate group. Pham et al. (1991) suggested the cyanation of a terpene thiol, however this proposal does not adequately explain the co-occurrence of thiocyanates and isothiocyanates in the same sponge. The insertion of sulphur into an organic cyanide or isocyanide to give a thiocyanate is mechanistically unprecedented. Sulphur insertion into an isocyanide to give an isothiocyanate (Hagadone et al., 1984), perhaps using an enzyme functionally equivalent to rhodanese (Westley, 1973), followed by isomerisation of the isothiocyanate to the thiocyanate is a plausible biosynthetic pathway. In the laboratory however, the isothiocyanate-thiocyanate equilibrium usually favours an isothiocyanate over a thiocyanate (Hughes, 1975). A final biosynthetic possibility is the use of an ambident thiocyanate anion to attack a terpene carbenium ion or its functional equivalent (He et al., 1989; 1992; Walker & Butler, 1996). Thiocyanate either reacts through the nitrogen centre generating an isothiocyanate derivative or through the sulphur generating a thiocyanate.

Results on the biosynthesis of the thiocyanate moiety are particularly informative. The incorporation of inorganic thiocyanate into the thiocyanate and isothiocyanate metabolites (Fig. 2B,C) of *Axinyssa* n. sp. is consistent with direct utilisation of this ambident precursor. Likewise cyanide is utilised for both thiocyanate and isothiocyanate biosynthesis. Our proposal is that cyanide is converted in *Axinyssa* n. sp. to thiocyanate by the action of an enzyme similar to rhodanese (Scheivelbein et al., 1969; Westley, 1973) and then incorporated into either 9-isothiocyanatopupukeanane or 2-thiocyanatoneopupukeanane. The alternative possibility that cyanide is converted first to the isocyanide then by sulphur insertion to an isothiocyanate is less likely since cyanide was not utilised for the biosynthesis of 9-isocyanopupukeanane in the same specimen of *Axinyssa* n. sp.

Stylotella aurantium uses both cyanide and thiocyanate as precursors for the biosynthesis of the dichloroimine and isothiocyanate groups. Fig. 4 shows two plausible biosynthetic routes to the stylotellanes A and B, one route (a) using an isonitrile intermediate and the other (b) invoking an isothiocyanate intermediate. The isolation of farnesyl isothiocyanate, but not of farnesyl isocyanide (Fig. 4A), from this sponge is consistent with the operation of path (b). The dichloroimine metabolites are among the most unusual of the cyanide and thiocyanate-derived terpenes. In the laboratory, isocyanide dihalides can be synthesised by addition of chlorine to isocyanides or by chlorination of isothiocyanates (Kühle et al., 1967). The biosynthetic mechanisms proposed invoke the use of a chloroperoxidase enzyme to chlorinate intermediates (Butler & Walker, 1993; Walker & Butler, 1996).

In *A. terpenensis*, our results are consistent with the use of both thiocyanate and of cyanide for isocyanide biosynthesis. Thiocyanate may perhaps be converted to cyanide by use of a peroxidase enzyme, as has been demonstrated in some bacteria (Ohkawa et al., 1971; Pollock & Goff, 1992; Westley, 1981), which is then utilised for isocyanide biosynthesis (Fig. 5).

We have previously suggested that *A. cavernosa* is able to interconvert inorganic cyanide and thiocyanate (Dumdei et al., 1997). Our current

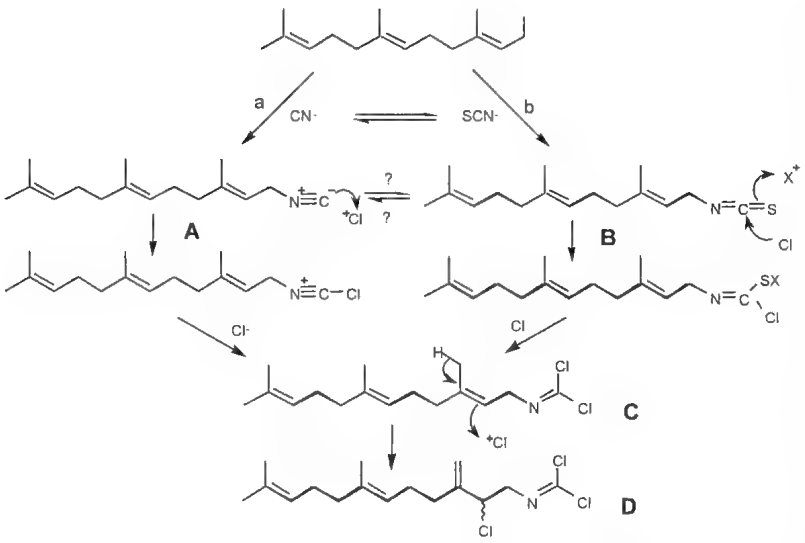

FIG. 4. Biosynthesis of dichloroimines in *Stylotella aurantium*. A, farnesyl isocyanide. B, farnesyl isothiocyanate. C, stylotellane A. D, stylotellane B.

results suggest *Axinyssa* n. sp., *S. aurantium* and *A. terpenensis* are able to interconvert these 2 inorganic precursors. We have also speculated that enzymic transformations which parallel the cyanide-thiocyanate interconversion may transform organic isocyanides into isothiocyanates, or the reverse, in marine sponges (Dumdei et al., 1997). Figure 6 illustrates these suggested biosynthetic relationships for *Axinyssa* n.sp. Thiocyanate is used to make 9-isothiocyanatopupukeanane which then undergoes desulphurisation to give 9-isocyanopupukeanane; alternatively, cyanide is used for isocyanide biosynthesis, then the isocyanide is converted into the isothiocyanate by an enzyme functionally equivalent to rhodanese.

In pioneering biosynthetic experiments, Hagadone et al. (1984) inferred the precursor status of an isocyanide terpene metabolite in isothiocyanate formation in *Ciocalypta* sp. They explored the *in vivo* conversion of 2-isocyanpupukeanane into the corresponding formamide and isothiocyanate metabolites. The natural product status of formamide metabolites has however been questioned by Tada et al. (1988). A second concern with the work of Hagadone et al. (1984) is their use of the relatively insensitive ^{13}C label in conjunction with mass spectrometric detection.

Our preliminary results with *A. terpenensis* suggest that an isothiocyanate to isocyanide transformation may occur in this sponge. The sponge contains isothiocyanates as minor metabolites (unpublished results). When radiolabelled diisothiocyanatoadociane was supplied to samples of *A. terpenensis*, the diisocyanoadociane isolated was shown to be radioactive. Chemical degradation is currently in progress to confirm the specificity of labelling in this advanced precursor experiment.

In view of the previous successful experiments with both diterpene isocyanides (Garson, 1986; Fookes et al., 1988; Karuso & Scheuer, 1989) and sesquiterpene isonitriles (Karuso & Scheuer, 1989; Dumdei et al., 1997), it is quite extraordinary that we have not demonstrated the incorporation of cyanide into the major isocyanide component (Fig. 6) of *Axinyssa* n. sp. Likewise thiocyanate appears not to be used for isocyanide biosynthesis in this sponge, in contrast to *A. cavernosa* in which thiocyanate is used for isocyanide biosynthesis (Dumdei et al., 1997) and also in contrast to *A. terpenensis* (this paper). The lack of incorporation of thiocyanate into 9-isocyanopupukeanane (Fig. 6) suggests that either the thiocyanate to cyanide conversion is inefficient in this sponge or that the conversion of isothiocyanate into isocyanide does not occur. We are currently isolating some of the other minor isocyanide metabolites from *Axinyssa* samples labelled by thiocyanate or cyanide in order to investigate the role of cyanide and thiocyanate in isocyanide biosynthesis in this sponge. A clearer picture of the complex metabolic interrelationships in *Axinyssa* n. sp. will emerge when we test the utilisation of

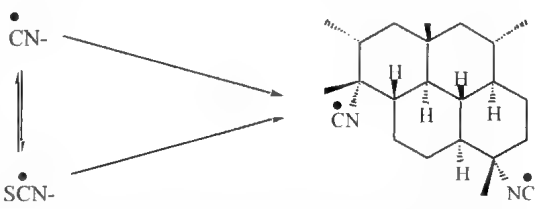

FIG. 5. Biosynthesis of diisocyanoadociane in *A. terpenensis*.

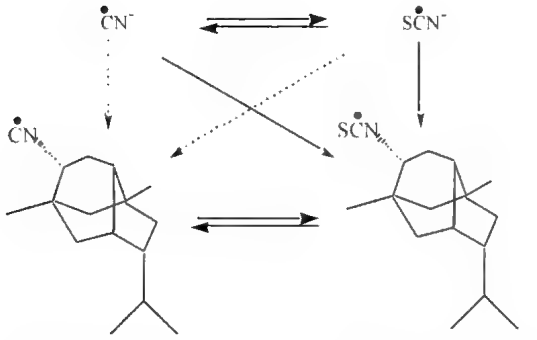

FIG. 6. Possible biosynthetic interconversions in *Axinyssa* n. sp. Solid lines indicate incorporation results or possible conversions, dotted lines indicate non-incorporation.

^{14}C-labelled isocyanide and isothiocyanate precursors by this sponge.

The origin of the cyanide or the thiocyanate used by marine sponges remains a tantalising mystery. Plants generate hydrogen cyanide by hydrolysis of cyanogenic glycosides (Seigler, 1975). Some bacteria are known to produce hydrogen cyanide (Knowles, 1976) or to convert the amino acid cysteine to thiocyanate (Voet & Voet, 1995), while methionine has been implicated in the formation of cyanide as a byproduct of ethylene biosynthesis (Pirrung, 1985). To date experiments to determine an amino acid origin for the isocyano group in diisocyanoadociane have been unsuccesful (Fookes et al., 1988).

Two sponges used in our biosynthetic experiments have interesting symbiotic profiles. *Amphimedon terpenensis* has previously been shown to contain high bacterial populations of eubacteria together with a cyanobacterial symbiont which morphologically resembles *Aphanocapsa feldmanni* (Garson et al., 1992). *Axinyssa* n.sp. contains a cyanobacterial symbiont together with numerous bacteria, in particular an archaeal-like bacteria which contains a highly unusual membrane-bound nucleoid (Fuerst, this volume; Fuerst et al., 1998). We have previously demonstrated that *A. terpenensis* isocyanides are localised in sponge cells, primarily archaeocytes and choanocytes, and infer that this is the site of synthesis of the metabolites (Garson et al., 1992). Terpene metabolites in 2 other sponges have been shown to be localised in sponge cells rather than symbiont cells (Uriz et al., 1996; Flowers et al., 1998). The range of sponges with which we are now exploring N_1-C_1 biosynthesis provide us with additional candidates to study the cellular localisation of terpene metabolites and to explore the role of symbionts in biosynthesis.

TAXONOMIC NOTE. The sponge which we have identified as '*Amphimedon*' *terpenensis* in this paper has a chequered taxonomic history. It was first named in the literature as an *Adocia* sp. by the Roche group (Baker et al., 1976). Fromont (1993) placed the sponge within *Amphimedon* in her taxonomic studies on haplosclerid sponges of the Great Barrier Reef and proposed the species name for the large proportion of terpene metabolites. Van Soest et al. (1996) considered the skeletal characteristics were too irregular to be compatible with *Amphimedon*. Based on structural characteristics and spicule analysis, they proposed the combination *Cymbastela terpenensis*, but acknowledged however that the skeletal morphology, growth form and texture for the sponge were not typical of *Cymbastela*, as described by Hooper & Bergquist (1992). The documented secondary metabolite chemistry of *Cymbastela* spp. consists of pyrrole metabolites from a New Caledonian species (Ahond et al., 1988). Samples of *Cymbastela* sp. collected from Heron I. and Lizard I. do not have a secondary metabolite profile by NMR and GC-MS, but have been shown to contain 24-isopropyl-δ^5 sterols (Stoilov et al., 1986), whereas *A. terpenensis* contains $\delta^{5,7}$-sterols (Garson et al., 1988). A more thorough taxonomic assessment of '*A.*' *terpenensis* (and the related *C. hooperi*), including consideration of live specimen characteristics, growth form, texture and spongin content and skeletal structure is required. It is possible that a new genus is required for these species but this requires substantially more corroborative evidence than is presently available (e.g. genetic analyses). For the present we retain the taxon '*A.*' *terpenensis*, but acknowledge it does not belong with typical members of *Amphimedon* (Haplosclerida; Niphatidae).

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REGENERATION ABILITIES OF SPONGILLID LARVAE. *Memoirs of the Queensland Museum 44: 568. 1999:-* Free-swimming larvae of *Spongilla lacustris* were cut into two halves with a razor blade under a binocular microscope. Two samples of the larvae were used. In the first sample, larvae were cut in the tangential plane (two equal halves with a similar set of cells and structures). In the second sample, larvae were cut in the transverse plane (two unequal parts). The 'anterior' fragment contained a large cavity lined with pinacocytes, the halved amount of the surface flagellated cells, and underlying collencytes. The 'posterior' part of the larva had a halved amount of the surface flagellated cells and all of the internal structures typical for the fully developed spongillid larvae. Each half of the larva was maintained in a separate Petri dish with the celloidine-covered bottom in well-aerated river water.

Visual observations and transmission electron microscopy yielded the following preliminary results. The halves of the larvae closed the edges of the wound immediately after dissection while continuing to move. However, trajectory, velocity and direction of the movements differed in different types of experimentally cut larvae. This was directly related to the presence or absence and the development of the larval cavity. Thirty minutes following the dissection, the tangentially split halves of the larvae looked normal (movement, attachment and metamorphosis generally similar), but half the size of the control larvae. In 2-3 days after the settlement these half-larvae metamorphosed into normally functioning small sponges. Developmental capabilities of the transverse halved larvae were different. The anterior half-larvae soon closed the cut edges, acquired a shape of a hollow sphere and swam easily and rapidly in the water. They maintained activity for two or more days, attached, formed pinacoderm and few flagellated chambers, and the sponges died. The posterior halves recovered the integrity of the flagellar cover in an hour following the dissection. They acquired the shape of spheres tightly packed with cells, covered with slightly elongated flagellated cells, and swam heavily and slowly near the bottom, with a maximum free life of 18 hours. After the settlement and attachment they formed pupae covered with pinacoderm and within 2 days developed into normal sponges.

Transmission electron microscopy showed the surface flagellated cells played an important role. These cells provided restoration of the surface cell cover, however, their role greatly differed in development of the 'anterior' and 'posterior' half-larvae. In the 'anterior' halves, the flagellated cells migrated inside; some were ingested by underlying collencytes (phagocytosis); some transformed into choanocytes giving rise to few flagellated chambers. During development of the 'posterior' half- larvae, some surface flagellated cells transformed into the pinacocyte-like cells *in situ* and still retained flagella for a long time. The leading role in the transformation of the flagellated cells belonged to the centrioles (both flagellated and flagellum-less) and to the root structures of flagellum connected with the centrioles.

Collencytes played the important role in the attachment and development of the settled half-larvae. These cells actively migrated to the surface of the settled larva, phagocyted the cells damaged during dissection, secreted a large amount of collagen and contributed to the flattening of the half-larvae and their attachment to the substrate. The post- settlement fate of flagellated surface cells of the half-larvae was partially dependent on the amount and the activity of collencytes. The next major morphogenetic role belonged to archaeocytes, the main source for the formation of choanoblasts, spiculocytes, collencytes, pinacocytes and other cells. The archaeocytes mitotically divided several times, losing their storage inclusions, and thus gave rise to several differentiated cell lineages. Probably, the lack of the necessary amount of the cells is responsible for the developmental retardation of the settled 'anterior' half-larvae. ❐ *Porifera, Spongillidae, larva, metamorphosis, development, ontogeny, transmission electron microscopy.*

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CHEMOSYSTEMATICS OF PORIFERA: A REVIEW

R.W.M. VAN SOEST AND J.C. BRAEKMAN

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All compounds isolated from Porifera were reviewed in an attempt to discover what level of reliability may be attached to chemistry data when applied to sponge systematics. To date (May 1998) more than 3500 different compounds have been described from 475 species of marine sponges, belonging to two of the three classes (Calcarea and Demospongiae), all major orders of Demospongiae, 55 families and 165 genera. Previous studies suggested that several ordinal, family and genus patterns may exist, with unique types of compounds apparently restricted to discrete sponge taxa. Based on this premise, the impressive chemical dataset is potentially valuable in solving persistent problems and disagreements over the systematics of various taxa. However, compounds may be produced by sponge cells (and thus regarded as sponge characters), or by microsymbionts (which may not be necessarily species- or group-specific). Large numbers of proven or suspected microsymbiont compounds appear to be present from the lack of correspondence between sponge identity and compound structure, e.g. macrolides and cyclic peptides dispersed amongst most demosponge groups are suspected products from various microbes. Reported chemistry is distributed heterogeneously over the various sponge taxa, with highest diversity of compounds reported from Dictyoceratida and Dendroceratida (1,250 compounds from 145 species), Haplosclerida s.l. (665 from 85 species) and Halichondrida s.l. (approximately 650 from 100 species); other groups have an intermediate (Astrophorida-Lithistida, Hadromerida and Poecilosclerida) or very low (Calcarea) diversity of compounds. Despite previous claims that particular compounds occur exclusively in particular sponge taxa, we found that in most, if not all, cases compound distribution does not exactly match sponge classification. Some classes of compounds are predominant in particular taxa (e.g. bromotyrosines in Verongida, furanoterpenes in Dictyo- and Dendroceratida, straight-chain acetylenes and 3-alkylpiperidine derivatives in Haplosclerida s.l.), but almost invariably there are also reports of these classes of compounds from unrelated sponges. Furthermore, in rare cases where a compound type is restricted to a certain sponge group (e.g. pyrrole-2-carboxylic derivatives in Halichondrida s.l.), their distribution amongst the families within the group appears to be inconsistent. Possible reasons for this fuzzy distribution include: 1) parallel biosynthetic pathways leading to the same structure; 2) involvement of microsymbionts; 3) careless specimen handling (contamination by epibionts, confused labels, etc.); 4) incorrect identification/classification. Currently, the degree of inconsistency is such that direct use of chemical data to solve classification problems, or to erect new higher taxa, is inadvisable. Inconsistent occurrence of compounds cannot be dismissed without further study. Large scale re-examination of voucher specimens, or recollection and chemical analysis, as well as cooperative studies between systematists, microbiologists and bio-organic chemists, are necessary to demonstrate whether or not chemical characters are true indicators of sponge systematics. ❐ *Porifera, chemistry, chemotaxonomy, bioactive compounds, review.*

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Natural products chemistry described from sponges is reminiscent of terrestrial plant chemistry in its diversity and distribution throughout the phylum. Secondary metabolites, such as terpenoids, alkaloids and peptides, as well as bioactive fatty acid-, polyketide- and sterol derivatives, are common amongst most sponge groups. Biological activity of sponge compounds is very diverse (MarinLit lists more than 20 activity categories for various sponge compounds; Blunt & Munro, 1998), but cytotoxic (see also Schmitz, 1994), antibiotic, antifungal, antitumour, antiviral, antifouling and enzyme- inhibitory activities are the most common.

Among marine organisms, sponges are the most productive sources of bioactive compounds: they

have so far yielded more than twice the number of structures reported from Cnidaria and from Algae, five times the number from Mollusca and Echinodermata, and seven times the number from Ascidiacea (Garson, 1994; Baker, 1996; Blunt & Munro, 1998).

Geographic areas and habitats with the highest reported numbers of bioactive compounds from sponges are the Indo-West Pacific (ca. 800 structures), Australian - South Pacific (600) and Caribbean coral reefs (600). The Mediterranean (550) and Japanese waters (750) are also prolific source areas. East Pacific (250), East Atlantic (150), Indian Ocean (150), Red Sea (150) and New Zealand waters (100) are intermediate in diversity. This pattern is similar to the pattern of sponge species diversity over the seas and oceans of the world (Van Soest, 1994), and thus cannot be directly linked to ecological phenomena such as increased predation and competition (e.g. Green, 1977).

Natural products continue to be described from sponges at an increasing rate (Table 1), such that the extent of sponge bioactivity is not yet apparent. So far, chemical structures have been elucidated from about 475 species of sponges, but many more have been shown to be bioactive in various bioassays.

Previous reviews (e.g. Bergquist, 1979; Bergquist & Wells, 1983; Sarma et al., 1993), demonstrated that many types of compound are restricted to discrete groups of sponges, the prime example being bromotyrosine derivatives which appeared to be restricted to Verongida. A large body of literature has appeared since these last reviews of sponge chemotaxonomy. This literature is now easily accessible through the MarinLit database (Blunt & Munro, 1998), which provides bibliographic references, structures and key words to virtually all marine natural products publications since the early sixties.

The origin of bioactive compounds isolated from sponges is still a controversial issue. Many bio-organic chemists believe that microsymbionts are likely to be the source of most compounds rather than sponge cells themselves. In some recent studies (e.g. Faulkner et al., 1994), it has been demonstrated that sponge microsymbionts may indeed be the source of bioactive compounds, but that sponge cells themselves also appear to produce them. It is possible, although yet to be demonstrated, that endosymbionts living inside the sponge cells are the true source of such compounds. In that case, such symbionts may be obligatory symbionts which have co-evolved with the sponge hosts and sponge-symbiont chemical interactions may be indicative of chemo-taxonomic affinities. An example of such a scenario is explored in a recent study by Van Soest et al. (1998).

TABLE 1. Numbers of published articles in which sponge chemistry is described and numbers of chemical structures reported in the past decades since 1960 (source MarinLit data base; Blunt & Munro, 1998).

Year of publication	No. articles	No. structures
1960-1969	4	2
1970-1979	314	361
1980-1989	1016	1275
1990-1997	1691	2484
total (1998)	3025	4122

It is the purpose of the present paper to review conclusions of previous chemotaxonomic studies and to determine whether new chemotaxonomic evidence has come forward to support these conclusions. For this purpose we reviewed all sponge compounds and examined their distribution over the classes, orders, families and genera of sponges and, if relevant, over other marine phyla.

METHODS AND DATA SOURCES

The MarinLit database (Blunt & Munro, 1998) was consulted using taxonomic keywords for the various taxonomic groups yielding lists of references, species, and trivial names of compounds, as well as drawings of structures of compounds extracted from the species. The card system built up by one of us (JCB) was used as a supplementary source. These data provided a compilation of sponge chemistry arranged taxonomically by order, family and genus. Subsequent searches were made using trivial names of compounds or compound types as key words, to establish the distribution of classes of compound over the various sponge groups and other marine phyla. Since there is still no firmly established classification for sponges nor for secondary metabolite chemistry, chemosystematic significance of the various compounds and classes of compound was also determined by ad hoc discussions between the two authors.

Compounds considered to be related and occurring in two or more clearly different taxa (species, genera, families, orders) are listed

below arranged according to the sponge group in the order given in Table 2. The usually recognised sponge orders and families (Bergquist, 1978) are employed, with the exception of orders Halichondrida s.l. (following Van Soest et al., 1990), and Haplosclerida (following Van Soest, 1980). Orders Dictyoceratida and Dendroceratida are treated together for reasons explained below.

TABLE 2. Numbers of structures reported from various sponge taxa, arranged by ordinal group and the numbers of species, genera and families from which the compounds were isolated. * including Chondrosiidae; **including Halichondriidae, Axinellidae, Bubaridae, Agelasidae, Ceratoporellidae; *** including 'Nepheliospongida'/ Petrosida.

Taxon	No. Structures	No. Species	No. Genera	No. Families
Homosclerophorida	120	14	5	1
Astrophorida	200	38	14	4
Spirophorida	14	5	1	1
Lithistida	200	20	11	5
Hadromerida*	185	40	13	7
Halichondrida s.l**	650	100	23	7
Poecilosclerida	350	63	33	14
Haplosclerida s.l***	665	85	17	5
Lubomirskiidae	3	3	3	1
Dictyo/Dendroceratida	1,250	145	36	6
Verongida	240	22	8	3
Calcarea	40	9	3	2

Relatedness of compounds, in a phylogenetic sense, is not unequivocal as most compounds consist of building blocks and side-chains with often diverse biosynthetic origin. Unless biosynthetic experiments have been performed, homology of the seemingly related compounds remains tentative in most cases. In accordance with the chemical literature and to acknowledge discrepancies between chemical and morphological characters, we use the term 'markers' for shared compounds rather than 'synapomorphies'. Examples of structures of 'markers' for the various taxa are included (Figs 1-43). Unique compounds reported from single species, though possibly significant as fingerprints, are ignored here, because they cannot be used for classification.

RESULTS

NUMBERS OF COMPOUNDS ISOLATED FROM SPONGES. To date (May 1998) more than 3,500 different chemical compounds have been extracted from 475 species of marine sponges belonging to two of the three classes (Demospongiae and Calcarea), all major orders of Demospongiae, one major order of Calcarea, ca. 55 families and 165 genera (Blunt & Munro, 1998). The various orders demonstrate large differences in numbers of compounds (Table 2).

The total diversity of species compounds listed in Table 2 (3,917) maybe misleading because it has not been possible to check whether the same compounds were sometimes isolated from different sponge taxa. However, since that is of relatively rare occurrence, a conservative estimate is approximately 3,500 different structures. Similarly, the total number of sponge species from which these compounds have been isolated (544) is inaccurate because of the large numbers of indeterminate identifications; quite a few of these may concern the same species. A conservative estimate, based on arguments of geographic nearness of localities of indeterminate identifications, is approximately 475 different species of sponges.

GENERALLY DISTRIBUTED COMPOUNDS. The first category delineated includes compounds which are apparently found over several or many different sponge groups, without any clearly restricted distribution amongst any particular sponge group. Some of these are suspected to be products of microsymbionts because sponges are known to have a rich bacterial flora which they use as food. The chemosystematic significance of these types of compounds is usually limited to be, at most, a fingerprint for individual species. Frequently, however, even that cannot be confirmed because symbionts may not be species specific.

The following classes of compounds do not generally have much value for sponge classification because of their distribution amongst unrelated groups of sponges:

Fatty acids and derived lipids. (Fig. 1A). These are ubiquitous and are often primary metabolites, although some specialised branched or unsaturated fatty acids appear to be restricted in their distribution (see below). The chemosystematic significance of the presence and concentration of fatty acids with particular carbon-chain lengths has been explored by Bergquist et al. (1984), but from their patterns of distribution there is no hard

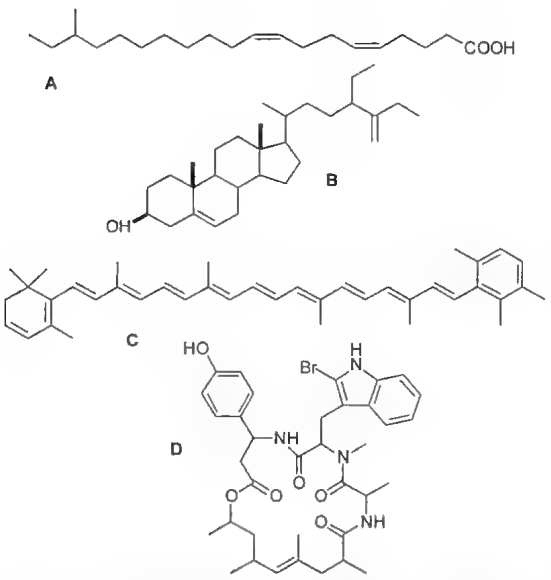

FIG. 1. Sponge chemistry. A, branched unsaturated fatty acid. B, sterol. C, carotenoid. D, cyclic peptide.

evidence for their applicability to sponge systematics.

Sterols. (Fig. 1B).These are ubiquitous and are often primary metabolites. Some sterols with specific side chains or functionalities (e.g. cyclopropene-, polyhydroxylated- or sulfated sterols) have a more restricted distribution and may have chemotaxonomic significance. Cyclopropene sterols have been used as a chemotaxonomic character to support the erection of a new order (Nepheliospongida or Petrosida; Bergquist, 1980), although subsequent research (Fromont et al., 1994) failed to demonstrate the consistent presence of these sterols amongst members of this 'order', whereas several similar cyclopropene sterols were isolated from the disparate taxa *Spheciospongia* (Hadromerida) (Catalan et al., 1982), *Halichondria* sp. (Halichondrida) (Ravi et al., 1978), and *Lissodendoryx topsenti* (Poecilosclerida) (Silva & Djerassi, 1991). The chemosystematic significance of the presence and concentration of sterols with particular side-chains and functionalities has been explored by Bergquist et al. (1986) and Fromont et al. (1994), but again there is no hard evidence for their consistency and applicability for sponge classification. They may be useful for fingerprinting at the species level, but even then care must be exercised (see e.g. Kerr & Kelly-Borges, 1994).

Carotenoids. (Fig. 1C). These are basically derived from ingested autotrophic organisms and modified in various ways by the sponges. A review is found in Liaaen-Jensen et al. (1982). The distribution of sponge carotenoids coincides with orange colour. They are reported from Astrophorida, Hadromerida, Halichondrida, Agelasida, Poecilosclerida, Haplosclerida, Dictyoceratida and Verongida.

Cyclic and linear peptides. (Fig. 1D). These elaborate molecules have been reported from Astrophorida, Spirophorida, Lithistida, Halichondrida, Poecilosclerida, Haplosclerida, Dictyoceratida and Dendroceratida. In a review published by Fusetani & Matsunaga (1993) it is concluded that microsymbiont involvement is the most likely explanation for the widespread occurrence of these compounds. Compounds similar to those isolated from sponges are also reported from Cyanobacteria and several other marine invertebrates, notably ascidians.

Macrolides. (Fig. 2). Similarly, these elaborate molecules have a wide distribution among Porifera: Calcarea, Astrophorida, Spirophorida, Lithistida, Hadromerida, Halichondrida, Poecilosclerida, and Dictyoceratida. Their apparent absence from Haplosclerida, Dendroceratida, Dysideidae and Verongida is noteworthy. Some of the molecules reported from sponges are almost identical to those of terrestrial Cyanobacteria or marine bacteria (Kobayashi & Kitagawa, 1998).

Acridine derivatives. (Fig. 3A). These compounds are not common, but recorded from Homosclerophorida, Astrophorida, Haplosclerida and ascidians. Some of these sponges and ascidians are brightly coloured due to the possession of acridine derivatives.

Nucleosides. (Fig. 3B). These have been recorded from Astrophorida, Hadromerida and Poecilosclerida. They are very likely to be microbial.

Sesquiterpene quinones. (Fig. 3C). Related structures have been recorded from *Chondrosia* (Hadromerida or Chondrosida), *Halichondria* (Halichondrida), *Strongylophora* (Haplosclerida) and many Dictyoceratida and Dendroceratida, and even from Verongida. No taxonomic significance or pattern can be attributed to this distribution.

Tetracyclic triterpenes. (Fig. 3D). Similar structures have been reported from *Siphonochalina siphonella* (Haplosclerida), *Axinella weltneri* (Halichondrida) and *Raspaciona aculeata* (Poecilosclerida). No taxonomic significance can be attributed to this distribution.

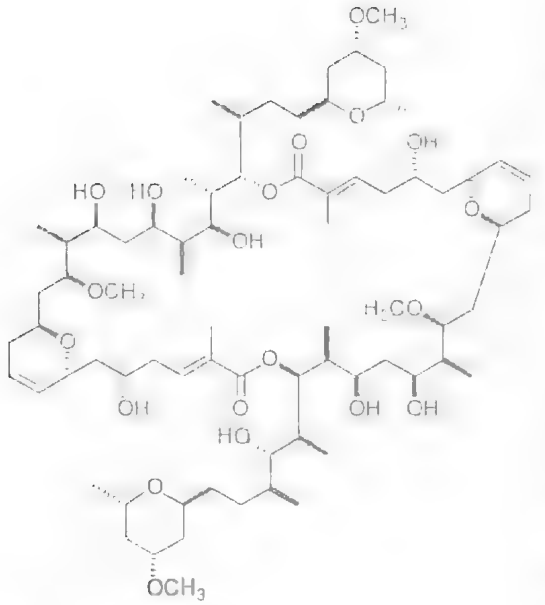

FIG. 2. Sponge macrolide.

TAXONOMICALLY DISTRIBUTED COMPOUNDS

*Homoscleroph*ori*da compounds*. About 120 different secondary metabolites have been reported from about 14 species belonging to 5 genera:

Peroxy-polyketides (recorded from at least 9 species belonging to at least 2 genera) and acridine derivatives (recorded from 4 species belonging to 2 genera), are common constituents of Homosclerophorida. However, both these types of compounds are also found in other sponge groups. The *Plakortis* peroxy-polyketides (e.g. Higgs & Faulkner, 1978) (Fig. 3E) are particularly similar to those isolated from *Callyspongia* sp. (Toth & Schmitz, 1994), *Cladocroce incurvata* (D'Auria et al., 1993) (both Haplosclerida) and from *Chondrosia* and *Chondrilla* (order Hadromerida or Chondrosida) (Wells, 1976; Stierle & Faulkner, 1979). So, despite the apparent concentrated occurrence of these structures in Homosclerophorida, it is not entirely justified to consider peroxy-polyketides as valid markers for the group. Further investigations are required as to why similar structures can be found in the unrelated Haplosclerida and Hadromerida/Chondrosida.

Aminosteroids isolated from *Plakina* sp. (Rosser & Faulkner, 1984: Fig. 3F) and *Corticium* sp. (Jurek et al., 1994), have an unusual nitrogen-bearing side chain, and these may be considered to be a valid marker for these two taxa despite their being sterols.

Astrophorida compounds. More than 200 secondary metabolites have been reported from at least 38 species belonging to 14 genera and all major families. Chemosystematic markers are listed below.

Saponines (steroid-saccharides, e.g. Carmely et al., 1989: Fig. 4A) are reported across families: from 6 species of *Erylus* (Family Geodiidae), 1 species of *Melophlus* (as *Asteropus*) (Family Ancorinidae) and 1 species of *Pachastrella* (Family Pachastrellidae). Related compounds are very common in Echinodermata, notably Asteroidea and Holothuroidea. Possibly the number of saccharides attached to the sterol part is species specific. The apparent absence of saponines in the other genera of the Astrophorida make them of dubious value for classification.

Triterpenes (malabaricane and derivatives, e.g. McCabe et al., 1982) (Fig. 4B) were reported from 2 species of *Stelletta*, 1 species of *Rhabdastrella* and from *Jaspis stellifera*. The latter is probably a *Stelletta* lacking triaenes and not a true *Jaspis*. Thus, these triterpenes are a good marker for *Stelletta* s.l. (including closely related

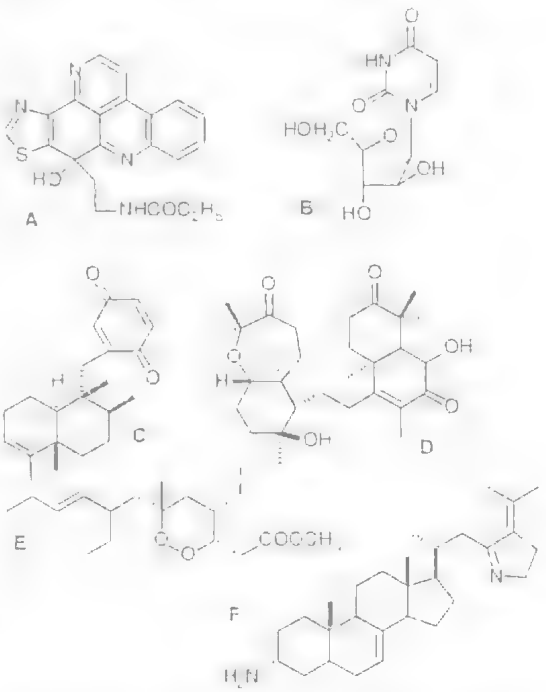

FIG. 3. Sponge chemistry. A, acridine alkaloid. B, nucleoside. C, sesquiterpene quinone. D, triterpene. E, peroxy-polyketide from *Plakortis halichondrioides*. F, aminosteroid from *Plakina* sp.

Rhabdastrella and '*Jaspis*' *stellifera*) (family Ancorinidae).

Penaresidins, peculiar straight-chained azetidine alkaloids (e.g. Kobayashi et al., 1991) (Fig. 4C) were independently isolated from two species of *Penares* (Ancorinidae) and thus may be a good marker for that genus.

There are also compounds suspected or proven to be of microsymbiont origin. Sulfated sterols were reported from *Pachastrella* and *Poecillastra*, and thus could be a potential marker for the family Pachastrellidae. However, sulfated sterols are also found in *Polymastia* (Hadromerida), *Hymedesmia* (Poecilosclerida) and several Halichondrida, so their value as marker is dubious. Also, like saponines, these compounds are very common in Echinodermata.

Cyclic peptides, macrolides and polyketides are commonly reported from species of *Geodia* (Geodiidae) and *Jaspis* (Coppatiidae), but these are often similar to Lithistid compounds and very probably of microsymbiont origin (discussed elsewhere in this review).

Geodia barretti and *Pachymatisma johnstonia* (Geodiidae) share a bromoindole compound of very similar structure, and thus these may be considered to be a valid marker for these two genera.

Dercitus (Pachastrellidae) and *Stelletta* (Ancorinidae) share similar acridine derivatives; however, related compounds are also reported from Homosclerophorida and Haplosclerida, as well as from ascidians as noted above.

Common-place sterols and (un)saturated fatty acids were reported from many species, but their chemosystematic value is low and they will not be discussed further here.

Spirophorida compounds. Fourteen secondary metabolites have been reported from about 5 species belonging to a single genus, *Cinachyrella* (Tetillidae). The fatty acids, sterols and macrolides shared between species do not seem to have chemosystematic value. The macrolides are similar to those of 'Lithistida' (e.g. *Theonella*), and to those isolated from the marine bacterium *Vibrio* sp. (Kobayashi & Kitagawa, 1998). No compounds are shared with the 'lithistid' family Scleritodermidae which is — on morphological grounds — assumed to be closely related to Spirophorida.

'Lithistida' compounds. Approximately 200 structures have been reported from at least 20 species belonging to 11 genera and 5 families. 'Lithistida' are certainly polyphyletic, with some

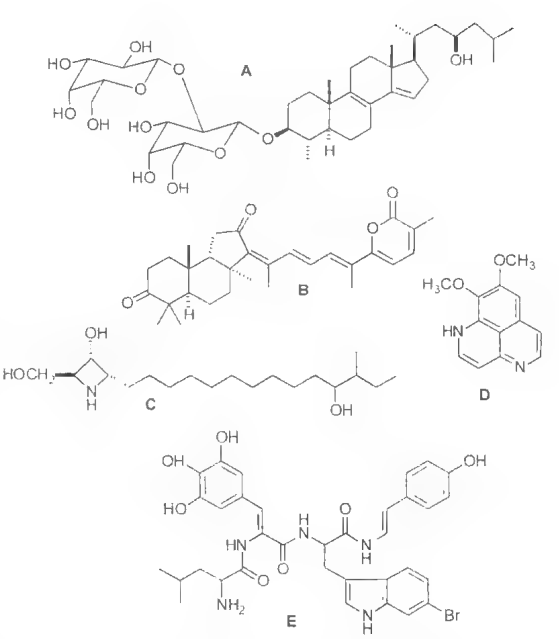

FIG. 4. A, Saponine from *Erylus lendenfeldi*. B, malabaricane-type triterpene from *Stelletta* sp. C, penaresidine from *Penares* sp. D, aaptamine from *Aaptos aaptos*. E, clionamide from *Cliona celata*.

families showing distinct synapomorphies with Spirophorida (e.g. Scleritodermidae) and Astrophorida (e.g. Corallistidae). Commonplace sterols were reported from several species, but their chemosystematic value is low and they will not be discussed further here.

Dominant compounds are cyclic peptides and macrolides, shared between families and genera. Related cyclic peptides are shared between several species of *Theonella*, 3 species of *Discodermia* and 1 species of *Neosiphonia* (Theonellidae), 1 species of *Callipelta* (Corallistidae), 1 species of *Aciculites* and 1 species of *Microscleroderma* (=*Amphibleptula*) (Scleritodermidae). Thus, it would seem that these cyclic peptides are straightforward markers for the 'Lithistida'. However, similar compounds are found in unrelated Astrophorida (*Geodia, Jaspis*), Hadromerida (*Hemiasterella*) and several Halichondrida (*Halichondria, Stylissa*). Discodermin E, the cyclic peptide isolated from *Discodermia kiiensis* (Ryu et al., 1994) is almost identical to the halicylindramides of *Halichondria cylindrata* (Li et al., 1995). These facts, coupled to the recorded presence of a rich microsymbiont flora in 'lithistids', support Fusetani & Matsunaga's (1993) conclusion of probable microsymbiont origin of these peptides.

Related macrolides are shared between several species of *Theonella*, 2 species of *Discodermia*, 1 species of *Neosiphonia*, 1 species of *Reidispongia* (Theonellidae) and 1 species of *Callipelta* (Corallistidae). Again, however, similar macrolides have been isolated from many different sponges belonging to widely divergent orders. As with cyclic peptides the chemosystematic value of 'lithistid' macrolides is thus compromised.

Hadromerida compounds. Approximately 185 secondary metabolites have been reported from at least 40 species belonging to 13 genera and 7 families. Fatty acids (except those mentioned below), sterols and carotenoids occur across several families and genera, but will not be discussed further. Cyclic peptides, macrolides and polyketides have been reported from a few Hadromerida and will also be left out of consideration.

No distinct hadromerid compounds can be identified. However, several compounds appear to be useful markers for families (or at least genus groups) and genera.

Aaptamine-type alkaloids (e.g. Nakamura et al., 198) (Fig. 4D) have been isolated from several species of *Aaptos* and *Suberites* and thus may be considered tentative markers for Suberitidae. They have been reported previously as markers for the order Hadromerida (Bergquist et al., 1991), but this is unwarranted in view of their limited occurrence.

Carballeira et al. (1989) maintained that 4,8,12-trimethyltridecanoic acid was a useful marker for the families Spirastrellidae and Clionidae, as they isolated this fatty acid from both *Anthosigmella varians* and *Cliona aprica*. The occurrence of this admittedly unusual fatty acid needs further investigation before this can be accepted.

Two presumably different species of *Cliona* apparently share the possession of clionamides (e.g. Stonard & Andersen, 1980) (Fig. 4E), which could serve as a useful marker for the genus. However, the amide shows some structural relationships with cyclic peptides and is thus a suspect microsymbiont-produced compound.

Peroxy-sesterterpenoids and derivatives (e.g. Capon et al., 1987) (Fig. 5A) have been isolated from 1 species of *Sigmosceptrella* and 3 species of *Latrunculia*. These genera were previously considered synonymous. But the value of these compounds as markers for Latrunculiidae is diminished by the isolation of closely related sesterterpenoids from 3 species of *Mycale* (Mycalidae) and from *Prianos* spec. (a name of uncertain affinity) (Manes et al., 1984).

Pyrroloquinoline alkaloids (e.g. Perry et al., 1986) (Fig. 18) have been isolated from 5 species of *Latrunculia*, but again the value of these compounds as markers for this genus is compromised by the reports of very similar and undoubtedly related compounds from another group of Poecilosclerida, viz. *Zyzzya* (Iophonidae) (see Van Soest et al., 1996; Dumdei et al., 1998).

If both compound types were genuine sponge compounds then their shared occurrence in Latrunculiidae - Mycalidae and Latrunculiidae - Iophonidae could indicate that 1) *Latrunculia* s.l. has poecilosclerid affinities; and 2) *Latrunculia* is polyphyletic supporting the data of Kelly-Borges. However, Perry et al. (1988) suggested that microsymbionts may be involved in the production of the pyrroloquinoline alkaloids.

Chondrosida compounds. This group is considered a family of the order Hadromerida in most previous classifications, but the apparent absence of morphological synapomorphies may justify their separation as an order of their own. Approximately 18 secondary metabolites have been reported from at least 4 species belonging to 2 genera.

Apart from straight-chained and branched unsaturated fatty acids and sterols, two other compound types have been reported from this group:

Peroxy-polyketides similar to, or some identical to, those of Homosclerophorida (e.g. Fig. 3E) were isolated from Australian *Chondrilla* (Wells, 1976) and Caribbean *Chondrosia* (Stierle & Faulkner, 1979). Mistaken identification is unlikely, though not impossible, and corroboration of the occurrence of peroxy-polyketides in Hadromerida/ Chondrosida would be welcome.

Halichondrida s.l. compounds. For morphological and chemosystematic reasons Halichondrida are here treated in a very wide sense, including the families Halichondriidae, Desmoxyidae, Dictyonellidae, Axinellidae, Bubaridae, Agelasidae and Ceratoporellidae. The nominal orders Axinellida (Hemiasterellidae and Raspailiidae excluded), Agelasida and Halichondrida s.s. are here included but not separately treated because the groups are in a taxonomic flux, with several recent revisions and proposed rearrangements. Morphologically, the recognised families are perceived by us to intergrade from Agelasidae at one end to Halichondriidae at the other end.

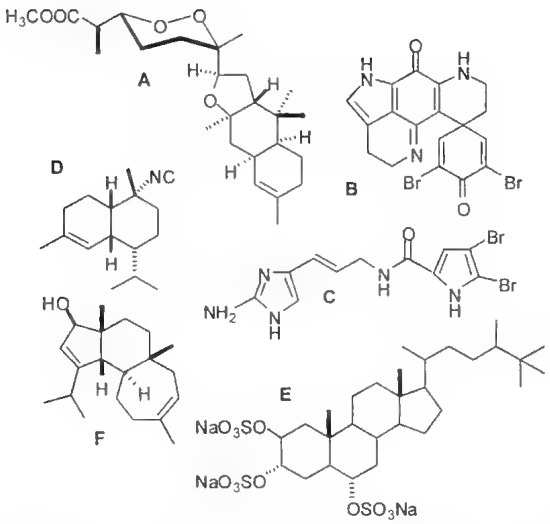

FIG. 5. A, trunculin-type sesterterpene from *Latrunculia brevis*. B, discorhabdin from *Latrunculia* sp. C, oroidin from *Agelas oroides*. D, isocyanosesquiterpene from *Halichondria* sp. E, sulfated steroid from *Halichondria* cf.*moorei*. F, cyclic terpene from *Myrmekioderma styx*.

Chemically there appear to be shared classes of compounds amongst family clusters with an overlap between Halichondriidae and Axinellidae (cf. Braekman et al. 1992, and see below). Many identifications of sponges with secondary metabolites are suspected or proven to be slightly or widely off the mark, which makes detailed re-examination of vouchers an essential prerequisite.

From Halichondrida, as employed here, approximately 650 structures have been reported from at least 100 species belonging to 23 genera and 7 families. Compounds with chemosystematic significance include the following.

Pyrrole-2-carboxylic derivatives (e.g. Braekman et al, 1992) (Fig. 5C) have been isolated from 12 species of *Agelas* (Agelasidae), 1 species of *Astrosclera*, 1 species of *Goreauiella* (Ceratoporellidae), 3 species of *Axinella*, 3 species of *Stylissa*, 1 species of *Phakellia*, 1 species of *Cymbastela*, 1 species of *Ptilocaulis* (as *Teichaxinella*) (Axinellidae) and 3 species of *Hymeniacidon* (Halichondriidae). At least one of the *Hymeniacidon* species (*H. aldis*) is a suspect *Hymeniacidon* as *H. aldis* is a junior synonym of *Stylissa massa*. It is possible that true *Hymeniacidon* (i.e., those with a detachable tangential skeleton) do not synthesise this class of compounds, and all reported *Hymeniacidon* with that compound type are in reality *Stylissa*. Thus, it appears that pyrrole-2-carboxylic derivatives are at least a marker for Agelasidae-Ceratoporellidae-Axinellidae, illustrated for example by the shared possession in *Agelas oroides* (Agelasidae), *Goreauiella* sp. (Ceratoporellidae) and *Stylissa carteri* (Axinellidae) of the same compound oroidin (Fig. 5C) (e.g. Braekman et al., 1992; Rinehart, 1989; Supriyono et al., 1995). A possibly related pyrrole compound is recorded from *Pseudoceratina purpurea* (Verongida) (Tsukamoto et al., 1996), but it may also be a case of convergent synthetic pathways.

Isocyanoterpenes (Burreson et al., 1975) (Fig. 5D) have been isolated from 2 species of *Axinella*, 1 species of '*Stylotella*', 2 species of *Cymbastela*, 5 species of *Acanthella* (all Axinellidae), 1 species of *Bubaris* (Bubaridae), 5 species of *Halichondria*, 3 species of *Hymeniacidon*, 2 species of *Ciocalypta*, 1 species of *Topsentia*, 1 species of '*Leucophloeus*', 3 species of *Axinyssa* (partly as *Trachyopsis*), and 1 of *Epipolasis* (all Halichondriidae). Even though it is suspected that identifications may not be entirely accurate, this is overwhelming evidence, that isocyanoterpenes are shared between families Axinellidae and Halichondriidae.

Sulfated sterols (Fusetani et al., 1981) (Fig. 5E) were isolated from 2 species of *Halichondria*, 4 species of *Topsentia*, 1 species of *Axinyssa*, 1 species of *Epipolasis* and 1 Halichondriidae not further identfied. Thus it would seem that they are a marker for the family Halichondriidae. However, sulfated sterols are also common in Pachastrellidae (Astrophorida) and have been isolated from a species of *Polymastia* (Kong & Andersen, 1996) and a species of *Hymedesmia* (as *Stylopus*) (Prinsep et al., 1989); they are also common in Echinodermata.

Cyclic diterpenes (Sennett et al., 1992) (Fig. 5F) occur in *Myrmekioderma* and *Higginsia* and thus may be a potential marker for the family Desmoxyidae.

Linear diterpenes (Albrizio et al., 1992: Fig. 6A) described from *Myrmekioderma* and *Didiscus* appear to be unrelated or only distantly related to the cyclic diterpenes. Moreover, the record from *Didiscus* is a suspect identification because it concerns an E. Pacific species, and so far the genus *Didiscus* is not known from that area. It could be a case of a mistaken *Myrmekioderma*, because *Myrmekioderma* and *Didiscus* share similar habit characters. Consequently, the linear diterpenes may be a marker for *Myrmekioderma* only.

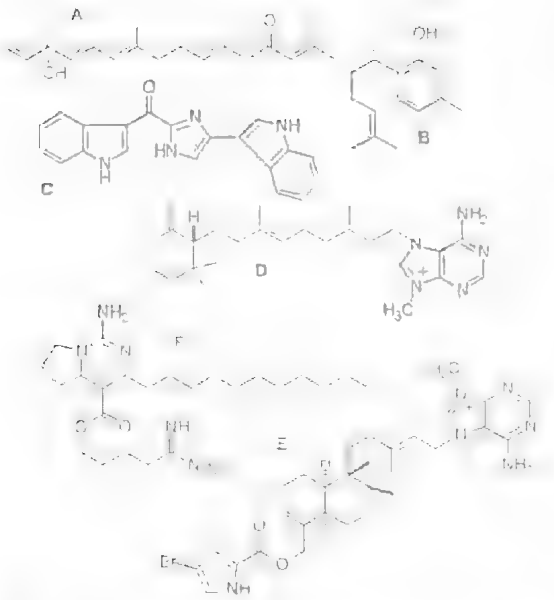

FIG. 6. A, linear diterpene from *Myrmekioderma styx*. B, curcuphenol from *Didiscus oxeata*. C, topsentin from *Spongosorites genitrix*. D, agelasine from *Agelas nakamurai*. E, agelasine G from *Agelas* sp. F, crambescine A from *Crambe crambe*.

Curcuphenol (Wright et al., 1987) (Fig. 6B) and related sesquiterpenes have been recorded from *Didiscus flavus* and *Epipolasis* sp. Some *Didiscus* specimens may have few of the characteristic didiscorhabs and then are easily mistaken for related genera such as *Topsentia* or *Epipolasis*. If that has been the case, it would mean curcuphenol and related sesquiterpenes are markers for *Didiscus*.

Topsentins (Bartik et al., 1987) (Fig. 6C) were considered a marker for *Spongosorites* since 4 species of that genus have yielded these compounds. However, this neat marker is threatened by the record of topsentins from 2 species of the Axinellidae genus *Dragmacidon*, which shows no close relationship with *Spongosorites*. The reported occurrence of both bromotyrosine derivatives (a Verongida marker compound) and topsentins in *Hexadella* (Dendroceratida) (Morris & Andersen, 1989; Morris & Andersen, 1990) is one of the more intriguing inconsistencies. It is also possible that bisindole compounds isolated from *Hamacantha* (Poecilosclerida) (Komoto & McConnell, 1988) are related to topsentins.

Terpene compounds (diterpenes (Wu et al., 1984) (Fig. 6D) and sesquiterpenes) are found in several *Agelas* species and thus may be markers of species groups within that genus (Braekman et al., 1992). A remarkable and significant compound was isolated from *Agelas* sp. (Ishida et al., 1992) (Fig. 6E): an apparent combination of a terpene and a pyrrole-2-carboxylic substructure. This indicates that in Agelasidae, in contrast to Axinellidae, some species have the ability to synthesise both terpenes and pyrrole-2-carboxylic acid moieties and even to combine these.

Compounds with low chemosystematic significance are the following: Macrolides and polyethers are commonly reported from *Halichondria*, cyclic peptides from *Halichondria*, *Axinella*, *Phakellia*, *Cymbastela* and *Stylissa*. Carotenoids are found in *Acanthella* and *Agelas*. Sterols and fatty acids are ubiquitous in this group. A host of unrelated smaller and larger compounds have so far been isolated from single species. Further exploration is needed to assess their potential as taxonomic markers.

Poecilosclerida compounds. Approximately 350 secondary metabolites have been reported from at least 63 species belonging to 33 genera and 14 families. Despite this large number of compounds, very few appear to have chemosystematic significance. Apart from sterols, fatty acids, macrolides and cyclic peptides, many indoles, pyrroles and carotenoids have been reported from Poecilosclerida, but in most cases there is no consistent taxonomic pattern.

Polycyclic guanidine alkaloids (Berlinck et al., 1990) (Fig. 6F) have been isolated from 2 species of *Monanchora* and 1 species of *Crambe* and are thus a potential marker for the family Crambeidae (Van Soest et al., 1996). However, these are also reported from *Arenochalina mirabilis* (Barrow et al., 1996), which is supposedly a Mycalidae. The voucher must be verified because *Arenochalina* has subtylostyles rather similar to those of *Monanchora* or *Crambe*, and reduced spiculation is very common in those genera.

Peroxy-sesterterpenoids and related derivatives (Capon & Macleod, 1987) (Fig. 7A) have been isolated from about 5 species of *Mycale*, but very similar compounds are known from *Latrunculia* (Hadromerida, see above and Fig. 5A). The chemosystematic value of these compounds is thus dubious.

Trikentrin (Capon et al., 1986) (Fig. 7B) and related compounds were isolated from two species of *Trikentrion* (Raspailiidae) and these might be a marker for that genus. But similar indoles are also reported for a species identified as *Axinella*

(Halichondrida) (Herb et al., 1990). Re-examination of the voucher might reveal that the characteristic triactines have been overlooked (they are often rare in various *Trikentrion* species and the further skeletal characters are similar to those of *Axinella*).

Haplosclerida compounds. Haplosclerida are here considered in a wide sense, including the order Nepheliospongida or Petrosida. The issue of one or two orders has been debated at several occasions using morphological, life cycle and chemistry arguments. Since both groups appear to share unique chemistry it is practical to unite the two groups for our purpose. From this group of 5 (marine) families, approximately 665 secondary metabolites have been reported from at least 85 species belonging to 17 genera and 5 families.

Chemosystematic markers appear as follows: straight-chain acetylenic compounds occur across 4 of the 5 families, they appear to be lacking in Niphatidae (see an extended review in Van Soest et al., 1998). The compounds are a clear marker for Haplosclerida s.l. However, related compounds have been described from *Phakellia carduus* (Halichondrida) and *Raspailia ramosa* (Poecilosclerida), which make it likely that the compounds are produced by microsymbionts. There are distinct types of acetylenic compounds based on the number of carbon atoms, the number and position of acetylenic bonds and the nature and position of the side chains. For example, *Petrosia* characteristically has hydroxyl-groups as side chains (e.g. Fusetani et al., 1983) (Fig. 7C), whereas the massive *Xestospongia* species (*X. muta, X. testudinaria*) characteristically have terminal bromine atoms (e.g. Patil et al., 1992) (Fig. 7D).

3-Alkylpiperidine derivatives have been reported from all 5 families of Haplosclerida and thus are a good marker for the order (see review in Andersen et al., 1996). Their occurrence in Phloeodictyidae is based on *Pellina* and *Pachypellina*, the family assignment of which is considered dubious. The type of *Pellina* is considered to be a *Halichondria*, but most species assigned to *Pellina* are either *Haliclona* (Chalinidae) or *Oceanapia* (Phloeodictyidae). The type of *Pachypellina* is considered to be a *Xestospongia* (Petrosiidae). It appears as if straight alkylpiperidines such as niphatesines (e.g. Kobayashi et al., 1992) (Fig. 7E) and halitoxins occur in families Niphatidae and Callyspongiidae, whereas cyclic alkylpiperidines (e.g. Sakai et al., 1986) (Fig. 7F) occur in Chalinidae, Petrosiidae

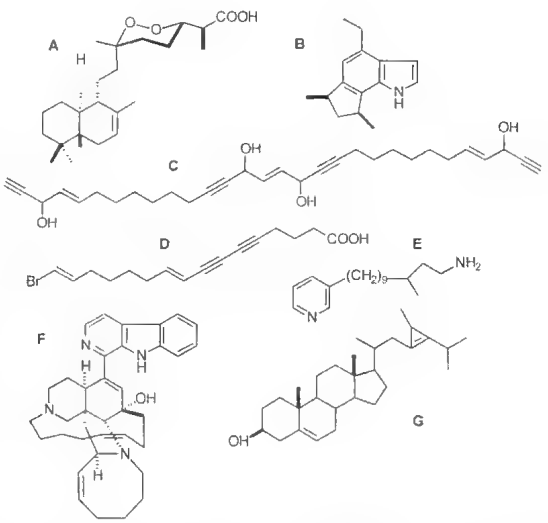

FIG. 7. A, sigmosceptrelline from *Mycale ancorina*. B, trikentrine from *Trikentrion flabelliforme*. C, straight-chain acetylene from *Petrosia* sp. D, straight-chain acetylene from *Xestospongia muta*. E, niphatesine D from *Niphates* sp. F, manzanine A from *Haliclona* sp. G, calysterol from *Calyx nicaensis*.

and (perhaps) in Phloeodictyidae. Much remains uncertain, because identification and assignment of species in this order are a specialist job. Voucher re- examination and repetition of collection and extraction is necessary to properly assess the chemotaxonomic significance of this group of compounds at the genus and family level. 3-alkylpiperidine derivatives have been reported from *Stelletta* (Astrophorida), *Theonella* ('Lithistida') and *Ircinia* (Dictyoceratida), but these are very likely cases of overgrowth by epibiont Haplosclerida, because identical compounds were also isolated from Haplosclerida. Unpublished information (M.K. Harper, in litteris) indicates that a particular 3-alkylpiperidine derivative, manzamine A, occurs also in a few Poecilosclerida (*Clathria* and *Mycale*). Thus, some doubts exist as to the true origin of these compounds, but no microsymbiont sources have so far been identified (see Kobayashi & Kitagawa, 1998).

A rather striking observation is that straight-chain acetylenes are recorded from several massive volcanoe-shaped *Xestospongia*, whereas 3-alkylpiperidines are recorded from compact, fine-grained and less elaborate *Xestospongia*. Previous authors have employed different names (*Xestospongia* s.l. and *Neopetrosia*) for these

sponges and chemistry appears to support this subdivision.

Cyclopropene sterols (e.g. Itoh et al., 1983) (Fig. 7G) are a marker for the 'order' Nepheliospongida/Petrosida (Bergquist, 1980), as they have been recorded from 2 species of *Xestospongia*, 2 species of *Petrosia*, 1 species of *Cribrochalina* (all Petrosiidae), 1 species of *Oceanapia* and from 2 species of *Calyx* (Phloeodictyidae). However, a recent study (Fromont et al., 1994) has shown that they are absent in most investigated members of these families. Moreover, similar sterols have been reported from species in the Hadromerida, Halichondrida and Poecilosclerida. Their chemosystematic significance is probably low and certainly debatable.

Tetrahydropyrans (e.g. Ciminiello et al., 1992) (Fig. 8A) are independently recorded from two species of *Haliclona*. They may be a marker for that genus, although such functionalities are widely distributed in natural compounds.

Low value markers for Haplosclerida are as follows: acridine alkaloids of closely related structure were isolated from *Amphimedon* sp. (Niphatidae) (Schmitz et al., 1983), *Petrosia* sp. (Petrosiidae) (Molinski et al., 1988), *Oceanapia sagittaria* (Salomon & Faulkner, 1996) and *Oceanapia* sp. (Eder et al., 1998) (Phloeodictyidae), and would seem to be a potential marker for those three genera/families. However, they contain an acridine moiety and are closely related to similar acridine compounds reported from Homosclerophorida and Astrophorida (as reported above).

Sesquiterpene and diterpene quinones are rather commonly found in Chalinidae (3 species), Petrosiidae (6 species) and Phloeodictyidae (2 species). However, similar compounds are also common in Dictyoceratida and Dendroceratida and are reported occasionally from Halichondrida and Chondrosida.

Isoquinolinoquinones are recorded from blue sponges assigned to *Reniera*, *Haliclona* (Chalinidae), *Petrosia*, *Xestospongia* and *Cribrochalina* (Petrosiidae). In view of the rather unusual blue colour, it is possible that these records all concern only a single species. In any case, identical or closely similar compounds are produced by a terrestrial *Streptomyces* (bacteria), and thus the chemistry is probably symbiont-derived.

The usual complement of fatty acids, sterols, cyclic peptides, polyketides and carotenoids have been reported across the families of Haplosclerida, but no taxonomic significance can be attributed to them. Many unrelated compounds were isolated from single species.

Freshwater sponge compounds. Only fatty acids and sterols have been reported from several species of the family Lubomirskiidae. These seem to have no taxonomic value.

Dictyoceratida and Dendroceratida compounds. We choose here to treat the orders Dictyoceratida and Dendroceratida in tandem because there is shared chemistry between the two and there is also a 'border conflict' over the assignment of the family Dysideidae. Bergquist (e.g. Bergquist, 1996) retains Dysideidae in Dictyoceratida, whereas Boury-Esnault et al. (1990) assign it to Dendroceratida. From this assemblage approximately 1,250 structures have been recorded from at least 145 species belonging to about 36 genera and 6 families.

Chemosystematic markers are as follows: Furan- or lactone terpenes (sesterterpenes: e.g. De Giulio et al., 1989 (Fig. 8B); sesquiterpenes, e.g. Guella et al., 1985 (Fig. 8C); and diterpenes: e.g. Bobzin & Faulkner, 1989 (Fig. 8D)) are shared by many species and genera of the group. Although there is a predominance of sesterterpenes in families Spongiidae, Irciniidae and Thorectidae (undisputed Dictyoceratida), a predominance of sesquiterpenes in Dysideidae, and a predominance of diterpenes in Darwinellidae and Dictyodendrillidae (both undisputed Dendroceratida), the occurrence is never absolute and many inconsistent records exist. Bergquist (e.g. Bergquist, 1996) chose to dismiss these inconsistencies announcing that they can be resolved by reassigning species to different families and genera. However, in view of the number of inconsistencies, this seems rather too optimistic. The evidence for this opinion is as follows. Sesterterpenic furans or lactones are found in: 11 species of *Spongia*, 3 species of *Hippospongia*, 3 species of *Carteriospongia*, 3 species of *Phyllospongia*, 1 species of *Strepsichordaia*, 1 species of *Collospongia*, 1 species of *Leiosella*, 1 species of *Dactylospongia*, 1 species of *Rhopaloeides*, 1 species of *Hyattella* (all Spongiidae), about 9 species of *Ircinia*, 3 species of *Sarcotragus*, 1 species of *Psammocinia* (all Irciniidae), 3 species of *Cacospongia*, 3 species of *Luffariella*, 3 species of *Fasciospongia*, 2 species of *Hyrtios*, 2 species of *Lendenfeldia*, 2 species of *Thorecta*, 1 species of *Petrosaspongia*, 1 species of *Fascaplysinopsis* (all Thorectidae), 2 species of *Dysidea*, 1 species of *Spongionella*

TABLE 3. Distribution of furan- and lactone terpenes over Dictyoceratida and Dendroceratida.

Compound	Spongiidae	Thorectidae	Irciniidae	Dysideidae	Darwinellidae	Dictyodendrillidae
Sesterterpene	26	17	13	3	0	2
Sesquiterpene	1	0	0	14	1	2
Diterpene	8	1	0	3	11	2

(Dysideidae), and 2 species of *Igernella* (Dictyodendrillidae).

Sesquiterpenic furans or lactones are found in: 1 species of *Spongia* (Spongiidae), 12 species of *Dysidea*, 2 species of *Euryspongia* (Dysideidae), 1 species of *Pleraplysilla* (Darwinellidae) and 2 species of *Dictyodendrilla* (Dictyodendrillidae).

Diterpenic furans or lactones are found in: 5 species of *Spongia*, 1 species of *Hippospongia*, 1 species of *Dactylospongia*, 1 species of *Hyattella* (all Spongiidae), 1 species of *Luffariella* (Thorectidae), 2 species of *Dysidea*, 1 species of *Spongionella* (Dysideidae), 3 species of *Aplysilla*, 3 species of *Chelonaplysilla*, 3 species of *Darwinella*, 2 species of *Dendrilla* (Darwiellidae), 1 species of *Igernella* and 1 species of *Dictyodendrilla* (Dictyodendrillidae).

From the overview presented in Table 3 it is evident that the number of cases that do not match the simple scheme presented by Bergquist (1996), viz. Dictyoceratida: sesterterpenes, Dendroceratida: diterpenes, Dysideidae: sesquiterpenes, is substantial, involving about 20% of all investigated species. It will take more than just reassigning a few possible mistakes. Moreover, the sesterterpenes, diterpenes and sesquiterpenes are biogenetically related. Several species (e.g. *Spongia agaricina*) apparently are able to synthesise both furanosesterterpenes and furanosesquiterpenes, or (e.g. *Spongia officinalis*) both furanosesterterpenes and lactone diterpenes. Because all three terpene types basically originate from a common biosynthetic pathway, which only at the end part of the synthesis of the terpenes will have divergent pathways, it is conceivable that the inconsistent occurrence of the terpenes is the product of independent (convergent) development. It seems best at present to emphasise the shared presence of furan- and lactone terpenes as a marker for both Dictyoceratida and Dendroceratida. A possible use of this compound type as marker for family or genus levels will have to await further studies combining voucher re-examination, morphological and molecular taxonomy, microsymbiont research and biosynthetic experiments. Pending this, it would be unwise to rearrange Dictyo- and Dendroceratida species and genera based only on terpene chemistry. Of course, emphasis of shared compounds which appear to confirm morphological synapomorphies remains a justified course of action.

A single inconsistent occurrence of sesterterpenic lactones is reported from a Japanese *Amphimedon* spec. (Ishibashi et al., 1993). This is possibly a case of mistaken labelling as the same group of chemists reported the occurrence of a typical Haplosclerid compound, manzamines, from an *Ircinia* spec. (Kondo et al., 1992).

Low value markers are as follows: Sesquiterpene quinones are reported from *Spongia* (4 species), *Hippospongia*, *Coscinoderma*, *Dactylospongia*, *Hyattella*, *Ircinia*, *Sarcotragus*, *Fasciospongia*, *Smenospongia*, *Hyrtios*, *Thorectandra*, *Fenestraspongia*, *Dysidea* (6 species) and *Euryspongia*. Thus, they seem to be good markers for Dictyoceratida including Dysideidae. However, these compounds are closely similar to the sesquiterpene quinones reported from Haplosclerida, Halichondrida and Chondrosida. Their apparent absence from Darwinellidae and Dictyodendrillidae is noteworthy.

Diketopiperazine derivatives, resulting from the condensation of two aminoacids, and diphenylether derivatives have been isolated from several species of *Dysidea*. However, sophisticated research by Faulkner et al. (1994) proved beyond doubt that bacteria are responsible for the production of these compounds. Diketopiperazines isolated from *Tedania* (although with different amino acid building blocks than those of *Dysidea*) also appeared to be produced by a bacterium (Stierle et al., 1991).

Polyhydroxylated sterols have been isolated from *Spongia* (2 species), *Hippospongia*, *Ircinia* (2 species), *Dysidea* (4 species), *Euryspongia* and *Spongionella*. Thus, they seem to be a marker for the Dictyoceratida including the family Dysideidae. However, these compounds are reported from isolated species belonging to almost all orders of the Demospongiae. Moreover they are commonly reported from Echinodermata and soft corals.

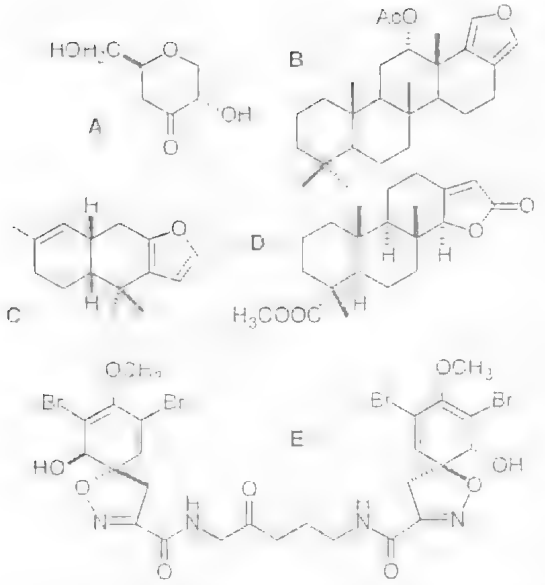

FIG. 8. A, halichondrol from *Haliclona hogarthi*. B, scalarine from *Spongia officinalis*. C, furanosesquiterpene derivative from *Dysidea avara*. D, polyrhaphin from *Aplysilla polyrhaphis*. E, bromotyrosine derivative from *Aplysina aerophoba*.

Indole derivatives occur scattered over all families. These compounds occur in many different sponge groups (and indeed other animal phyla), and they are assumed to be of microsymbiont origin. In any case, they appear quite diverse in the various species and genera.

Sterols and fatty acids have been isolated from many Dictyoceratida and Dendroceratida. Macrolides occur scattered over a few species in the families Spongiidae and Thorectidae; their absence in Dendroceratida is perhaps noteworthy. Cyclic peptides occur sparsely (here and there) over all families.

The family Halisarcidae is usually attributed to Dendroceratida, but was recently raised to ordinal level: Order Halisarcida Bergquist, 1996. No compounds have been isolated from members of this group so far.

Verongida compounds. Approximately 240 secondary metabolites are reported from at least 22 species belonging to 8 genera and 3 families.

Bromotyrosine derivatives (e.g. Ciminiello et al., 1997) (Fig. 8E) are uniformly present in all families and genera of Verongida (11 species of *Aplysina*, 2 species of *Verongula*, 2 species of *Ianthella*, 1 species of *Anomoianthella*, 2 species of *Pseudoceratina*, 2 species of *Suberea*, 1 species of *Aiolochroia* and 1 species of *Aplysinella*). Within this large group of derivatives, macrocyclic bromotyrosines (e.g. Pordesimo & Schmitz, 1990) (Fig. 9A) are shared between two species of *Ianthella*, so they could be a marker for that genus; however, a macrocyclic bromotyrosine is also recorded from *Pseudoceratina purpurea* (Carney et al., 1993).

The value of bromotyrosine derivatives as a marker for the Verongida is diminished by the isolated occurrence of similar bromotyrosine compounds in *Iotrochota birotulata* (Poecilosclerida) (Constantino et al., 1994) and *Agelas* (Agelasidae) (König & Wright, 1993). Related bromotyrosines have been found also in an ascidian, *Botryllus* (McDonald et al., 1995) and a green alga, *Avrainvillea* (Colon et al., 1987). The reported occurrence in *Hexadella* (Dendroceratida) of both bromotyrosine derivatives (Verongida) (Morris & Andersen, 1989) and topsentins (*Spongosorites* compounds) (Morris & Andersen, 1990) is one of the more intriguing inconsistencies.

Sterols, fatty acids, carotenoids, nucleosides and sesquiterpene quinones have been reported across families and genera. Their value for taxonomy is low. The apparent absence of cyclic peptides and macrolides in this order is noteworthy.

Calcarea compounds. Approximately 40 secondary metabolites were reported from at least 9 species. So far the subclass Calcaronea did not yield any compounds (the record of phospholipid fatty acids and sterols from Caribbean

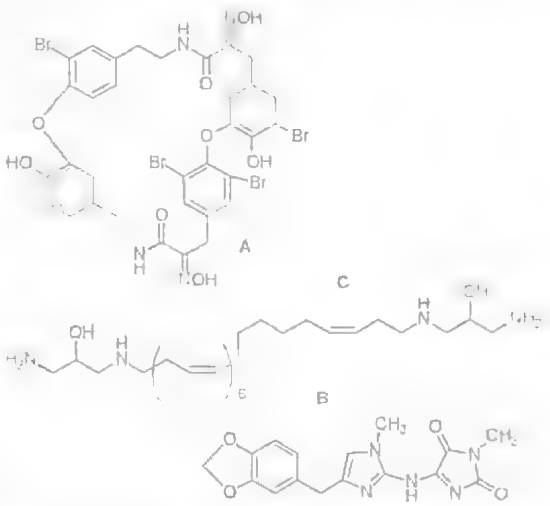

FIG. 9. A, bastadine from *Ianthella basta*. B, clathridine from *Clathrina clathrus*. C, rhapsamine from *Leucetta leptorhaphis*.

TABLE 4. Chemical markers (bold print) and non-exclusive markers (plain print) for sponge taxa, with numbers of species from which the compound was isolated. Figure numbers refer to figures presented in this review. For further comments and explanations see text.

Sponge group (No. spp. studied)	Compound type (example of structure)
Homosclerophorida (9)	**peroxy-polyketides** (Fig. 3E)
Plakina-Corticium (2)	**steroid-amines** (Fig. 3F)
Astrophorida (8)	saponines (Fig. 4A)
Stelletta s.l. (4)	**triterpenes** (Fig. 4B)
Penares (2)	**penaresidins** (Fig. 4C)
Pachastrellidae (2)	sulfated sterols (Fig. 5E)
Suberitidae (3)	**aaptamines** (Fig. 4D)
Spirastrellidae/Clionidae (2)	4,8,12-trimethyltridecanoic acid
Cliona (2)	**clionamides** (Fig. 4E)
Latrunculiidae (4)	peroxy-sesterterpenoids (Fig. 5A)
Latrunculiidae (5)	pyrroloquinoline alkaloids (Fig. 5B)
Axinellidae- Agelasidae-Ceratoporellidae (26)	**pyrrole-2-carboxylic derivatives** (Fig. 5C)
Axinellidae-Bubaridae-Halichondriidae (32)	**isocyanoterpenes** (Fig. 5D)
Halichondriidae (9)	sulfated sterols (Fig. 5E)
Desmoxyidae (3)	**cyclic diterpenes** (Fig. 5F)
Myrmekioderma (2)	linear diterpenes (Fig. 6A)
Didiscus (2)	sesquiterpene phenols (Fig. 6B)
Spongosorites (4)	topsentins (Fig. 6C)
Agelas (6)	**di- and sesquiterpenes** (Fig. 6D)
Crambeidae (3)	**polycyclic guanidine alkaloids** (Fig. 6F)
Mycale (5)	peroxy-sesterterpenoids (Fig. 7A)
Trikentrion (2)	**trikentrin indoles** (Fig. 7B)
Haplosclerida s.l. (ca. 17)	**straight-chain acetylenes** (Figs 7C-D)
Haplosclerida s.l. (ca. 22)	**3-alkylpiperidine derivatives** (Figs 7E-F)
Petrosia (ca. 7)	**polyhydroxylated acetylenes** (Fig. 7C)
Xestospongia s.s. (ca. 3)	**brominated acetylenes** (Fig. 7D)
Niphatidae + Callyspongiidae (ca. 6)	**linear 3-alkylpiperidines** (Fig. 7E)
Chalinidae + Petrosiidae (ca. 8)	**cyclic 3-alkylpiperidines** (Fig. 7F)
Petrosiidae + Phloeodictyidae (8)	cyclopropene sterols (Fig. 7G)
Haliclona (2)	tetrahydropyrans (Fig. 8A)
Dictoceratida + Dendroceratida (102)	**furano-or lactone terpenes** (Figs 8B-D)
Spongiidae + Thorectidae + Irciniidae (56)	furano-or lactone sesterterpenes (Fig. 8B)
Dysideidae (14)	furano-or lactone sesquiterpenes (Fig. 8C)
Darwinellidae + Dictyodendrillidae (13)	furano - or lactone diterpenes (Fig. 8D)
Verongida (22)	**bromotyrosine derivatives** (Fig. 8E)
Ianthella (2)	macrocyclic bromotyrosines (Fig. 9A)
Clathrinida (4)	**guanidine-imidazoles** (Fig. 9B)
Clathrinida (3)	**long-chained aminoalcohols** (Fig. 9C)

Leucosolenia canariensis (Carballeira & Shalabi, 1995) almost certainly concerns *Clathrina*, which is a member of the Calcinea). Within the subclass Calcinea compounds were isolated only from members of the order Clathrinida.

Guanidine-imidazoles (e.g. Ciminiello et al., 1989) (Fig. 9B) are recorded across families: from 1 species of *Clathrina* (Clathrinidae) and 3 species of *Leucetta* (Leucettidae), and are thus markers for the order Clathrinida.

Long-chained aminoalcohols (Jayatilake et al., 1997) (Fig. 9C) are recorded across families: from 1 species of *Clathrina* (Clathrinidae) and 2 species of *Leucetta* (Leucettidae), and are thus also markers for the order Clathrinida.

Sterols, fatty acids, a macrolide (similar to those of various Demospongiae) and a pteridine (similar to compounds from terrestrial organisms) make up the remaining compounds reported from

Calcarea. These do not appear to have chemotaxonomic significance.

SUMMARY

Table 4 summarises the conclusions, showing that a total of 38 chemical markers have been identified for 35 sponge groups of different taxonomic levels (7 orders, 8 family groups, 7 families and 13 genera). However, only 22 of these markers show some consistency, the remaining 16 presenting substantial inconstencies preventing their use as reliable markers ('non-exclusive markers'). The overview presented above clearly demonstrates that, despite huge numbers of compounds isolated from sponges, only a fraction shows potential as chemical markers for larger or smaller groups. When the distribution of the thousands of compounds over the various sponge groups is viewed as a whole, most demonstrate an erratic, scattered distribution, occurring either in single species only, or shared by unrelated species.

Thus, the 22 markers and 16 non-exclusive markers comprise only a small part of the chemical database. Moreover, markers often are not well-founded because only a handful of species have so far been recorded to contain them. Further exploration may or may not establish their consistent occurrence in the group.

Nevertheless, about 260 sponge species (out of a total of about 475) appear to contain secondary metabolites belonging to the 22 markers with utility for sponge chemosystematics.

DISCUSSION

CHEMISTRY AS A CHARACTER FOR SYSTEMATICS. The major problem preventing the use of chemotaxonomic markers as characters for sponge classification is their lack of consistency. Not one marker is problem-free, either because it is reported to occur outside the group for which it is supposed to be a marker, or because the marked groups overlap partially. To be useful for classification, markers should either include each others groups or exclude them completely.

Seemingly solid markers known from dozens of closely related species in a particular taxonomic group have also been reported from a few sponges phylogenetically unrelated from that particular sponge group. Examples include: bromotyrosine compounds (Verongida markers) in the poecilosclerid *Iotrochota*; furanoterpenes (Dictyo- and Dendroceratida markers) in the haplosclerid *Amphimedon*; straight-chain acetylenes (Haplosclerida markers) in halichondrid *Phakellia* and poecilosclerid *Raspailia*; isocyanoterpenes (Halichondrida marker) in the haplosclerid *Amphimedon*.

The same lack of consistency is apparent when the distribution of marker derivatives is viewed within the group of which they are assumed to be characteristic. Examples include: furanosesterterpenes concentrated in Dictyoceratida overlap in distribution with furanoditerpenes concentrated in Dendroceratida (as discussed in detail above); linear 3-alkylpiperidine derivatives concentrated in Callyspongiidae and Niphatidae overlap in distribution with macrocycle 3-alkylpiperidines concentrated in Chalinidae and Petrosiidae; isocyano-diterpenes, concentrated in most Halichondrida s.l. (including Axinellidae) are lacking in Agelasidae.

These inconsistencies may be the result of one or more of the following four explanations.

Parallel biosynthetic pathways. These may lead to compounds with structural similarity. Secondary metabolites are built from very generally distributed precursors of the primary metabolism. Different enzymes may have the property for allowing the biosynthesis of structurally related compounds. Such an explanation may be valid for the occurrence of bromotyrosine derivatives in *Iotrochota*, a genus which cannot conceivably be mistaken for a verongid. Tyrosine and bromine are very general molecules in marine organisms and their combination may be achieved by different enzymes. This explanation, however, requires empirical support through biosynthetic experiments.

Microsymbiont involvement. Although evidence for the involvement of microsymbionts in production of sponge secondary metabolites is largely circumstantial, several studies have established that compounds suspected to be of microsymbiont origin were indeed produced by bacteria and fungi isolated from sponges. Examples of sponges known to harbour microsymbionts which are the source of bioactive compounds are: *Theonella swinhoei*, *Halichondria okadai*, *Mycale* sp., *Tedania ignis*, *Calyx podatypa*, *Dysidea herbacea* and *Darwinella rosacea*. Schmitz (1994) provides a review of such proven or suspected cases.

On the basis that compounds are universally inconsistently distributed, it is conceivable that all natural products isolated from sponges are of microsymbiont origin. Studies which have allegedly identified sponge cells as the source of

a given compound (Faulkner et al., 1994; Garson, 1994; Uriz et al., 1996) did not address the real possibility that isolated sponge cell fractions, free from bacterial cells, contained endosymbionts ultimately involved in the production of compounds.

Microsymbionts involved in natural products biosynthesis may be species- or group-specific, in which case a close correspondence between sponge phylogeny and compound type is most likely. Such cases would not be easily distinguished from true sponge cell origin of compounds. It is possible that symbionts may be occasionally transferred to other organisms, including other sponges, which would explain 'pockets' of concentrated occurrence of compound types in unrelated organisms.

Careless specimen handling. During earlier days of natural products exploration, in particular, collected specimens were not always treated in a way required to get unequivocal results. Specimens that were not 'cleaned' from overgrowing algae and epizootic invertebrates were quoted as sources for compounds not actually produced by them. There are quite a few of these suspected cases, which can only be solved if a voucher including the epibionts has been retained. Labels have also been confused, resulting in reciprocal mismatch of compounds and sponge identities. Such cases are unlikely to be easily solved and will continue to 'pollute' the database.

Incorrect identification/classification. Some sponge groups are extremely difficult to identify to family or genus level and considerable taxonomic experience is needed. Moreover, classification of such groups is often a source of disagreement among reigning classification systems. Specimens have often been identified by dozens of taxonomists with very diverse experiences and views on the classification, resulting in an almost Babylonic confusion of sponge sources of interesting chemistry, especially in certain genera (e.g. *Batzella*, *Hymeniacidon*, *Halichondria*, *Amphimedon*, *Reniera*, *Xestospongia* and *Cribrochalina*). It is obvious that re-examination of vouchers — if at all retained — is needed to correct the more obvious mistakes. It is essential that voucher specimens of important sources of compounds be lodged in museums or collections maintained in perpetuity, and not thrown away after a certain period.

IMPACT OF CHEMISTRY ON CURRENT CLASSIFICATION. Several taxonomists have used chemical data to underbuild existing classifications or to support proposals for changes in the classification. Bergquist (1978) erected the Verongida on the basis of the universal occurrence of bromotyrosines in the group, which was earlier recognised only at the family level. This proposal remains unchallenged. Bergquist (1980) erected Nepheliospongida (later to be renamed Petrosida for nomenclatorial reasons), based on the occurrence of cyclopropene sterols. This proposal has been challenged on morphological (e.g. Van Soest, 1990) as well as chemical grounds (Fromont et al., 1994). Recent chemosystematic analyses (Andersen et al., 1996; Van Soest et al., 1998) yielded strong chemical arguments for the integrity of the Haplosclerida s.l. Van Soest (1991) also used chemical data to unite Axinellidae and Halichondriidae into a Halichondrida s.l. Braekman et al. (1992) on the basis of chemical arguments, suggested including Agelasidae into that group, although without making a formal proposal to do so. Chemical data are additional proof that soft bodied *Agelas* and the sclerosponges *Goreauiella* and *Astrosclera* are closely related, supporting morphological indications (Rinehart, 1989; Braekman et al., 1992; Williams & Faulkner, 1996).

It appears as if the chemosystematic evidence for order-level relationships are more-or-less exhausted. A final proposal could be made to formally unite Dictyoceratida and Dendroceratida into a taxon of the ordinal level, because both groups contain furanoterpenes. Such a proposal has the added advantage of avoiding border disputes at the ordinal level over the position of *Dysidea* (Boury-Esnault et al., 1990; Bergquist, 1996). Further rearrangement of suborders, families and genera may well be necessary, but will remain within unchallenged ordinal boundaries.

Promising chemosystematic conclusions may be expected in the near future especially at the genus level. Examples are *Crambe* - *Monanchora* (Poecilosclerida: Crambeidae) sharing the same compounds; some morphological subgroups of *Xestospongia* (Haplosclerida: Petrosiidae) appear to share similar chemistry. Intriguing problems to be solved are *Latrunculia* (Hadromerida or Poecilosclerida) - *Mycale* (Poecilosclerida) relationships, which share similar chemistry but lack morphological correspondence.

CHEMOSYSTEMATICS AS A FUTURE DISCIPLINE. It seems imperative that microsymbiont involvement in the production of

compounds used to underpin classifications is investigated exhaustively. It goes without saying that classifications of sponges should be based on hypotheses of evolutionary developments in the group and not — unwittingly — on those of microsymbionts. Techniques to investigate whether or not microsymbionts are involved in this process are available, but these are sophisticated, and lie beyond the reach of the average sponge taxonomist. Thus, certainty of this outcome will be slow in arriving and may depend heavily on non-relevant factors, such as pharmacological interest in the compounds. In view of the observed widespread inconsistencies it is judged to be unwise at the present time to propose new classification schemes that depend heavily on chemical information. Conversely, however, in the face of overwhelming morphological evidence, support from chemistry should also be underlined.

In cases where microsymbionts are demonstrated to be the source of the compounds, chemosystematic conclusions may still be possible on the basis that many microsymbionts may be obligatory and co-evolved with their sponge hosts. However, different analytical techniques are necessary to arrive at such conclusions, which involve 'mapping' phylogenetic data of microsymbionts on those of the sponge hosts (see for example Van Soest et al., 1998).

Chemosystematic studies are hampered by certain aspects of current practice of natural products chemical research. Examples are: bias caused by limited biosassays and extraction procedures, and a widespread reluctance to report on the re-discovery of already known structures. Future studies would benefit greatly from broad-spectrum bioassays including organisms or cell-lines from all five kingdoms, to maximise efforts of discovery of useful compounds. Natural products chemists should report their results, irrespective of the news value for chemists, through networks and databases. Confirmation of repeated occurrence of particular compounds in particular sponges has much greater strength of conviction.

Chemosystematic conclusions should take into account inconsistent results of previous studies. Rather than dismissing inconvenient data, it should be attempted to find out why inconsistencies are there on a case-by-case basis (parallel biosynthetic pathways, microsymbiont involvement, careless specimen handling, or mistaken identification). Such attempts can be made only fruitful in cooperative efforts of taxonomists, chemists, cell biologists and microbiologists.

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BIOLOGY OF SPONGE NATURAL PRODUCTS. *Memoirs of the Queensland Museum 44: 590. 1999*:- This is to announce the EC-MAS 3 project, which started April 1, 1998. The biological and chemical aspects of selected sponge natural products (secondary metabolites) of interest to human use will be studied to obtain understanding of: 1) the cellular origin and possible microsymbiont involvement, and 2) the ecological significance of sponge secondary metabolites, and 3) the patterns in these processes enabling rationalisation of exploration for and exploitation of sponge secondary metabolites. The results will have a direct bearing on policy decisions concerning industrial production of sponge secondary metabolites, which are too difficult or too costly to synthesise. A major deliverable product of the proposed research will be the formulation of a standard protocol of research steps needed as a basis for such policy decisions.

The research will be structured in three phases: 1) exploration and pattern recognition, 2) testing of hypotheses using experiments with selected sponges, 3) protocol construction. Initially, investigations will be directed towards two sponge groups (Haplosclerida and Halichondrida) and towards a limited number of molecule types. Known secondary metabolite occurrence will direct exploration for related sponges and related secondary metabolites. For the experimental phase a choice for 3-4 target sponges will be made based on suspected production of secondary metabolites by own sponge cells (1-2 target sponges) or microsymbionts (1-2 target sponges). The biological aspects include: determination of the identities and phylogenetic relationships of bioactive sponges; within-sponge spatial distribution of sponge cells and microsymbiont cells; experimental observation of variability of biological activity of selected sponges in various environmental (biotic and abiotic) situations; identification of target microsymbiont cells; fractionation, isolation and culture of target sponge cells. The chemical aspects include: extraction, isolation and structure determination of selected bioactive compounds; development of qualitative and quantitative analytical methods for their spatial distribution in the sponge and for their distribution between and within different species.

Summary of methodologies: Sponges will be collected using SCUBA (shallow water) and/or dredges (deep water) and photographed upon collection. Various types of fixations of material will be made immediately after removal from the water. Voucher specimens will be studied for identity and phylogeny using routine morphological as well as molecular (18S / 28S rDNA) characters. Collected sponges preserved in methanol or as freeze-dried material will be extracted with methanol and dichloromethane. The primary extracts will then be tested for their biotoxicity using an invertebrate bioassay organism, the Artemia toxicity test, and several prokaryote and eukaryote bioassay organisms (bacteria, fungi, yeast). Cytological analyses will consist of two different approaches, one using glutaraldehyde-fixed material, the other using live sponges: 1) sponges will be fixed in glutaraldehyde, (a) for microsymbiont detection; thick sections will be stained with suitable fluorochromes and viewed by fluorescence microscopy and confocal scanning light microscopy. If microsymbionts are present, populations will be characterised by different parameters. Microsymbionts will be further identified by fluorescence in situ hybridisation using rRNA-targeted oligonucleotides as probes; (b) for sponge cell spatial distribution, samples will be postfixed in 1% osmium tetroxide and thin sections will be studied by Transmission Electron Microscopy (TEM). 2) Live sponges will be dissociated into single-cell suspensions. Recognition of secondary metabolite production will be realised using two advanced techniques: (a) cell fractionation into pure cell populations using continuous or discontinuous Percoll gradients; (b) symbiont-free sponge cultures, initiated either from pure cell populations or from dissociated sponge cell suspensions. Experimental observations will be made in situ using various types of manipulations (caging, artificial standard lesions, confrontation with substrate competitors, crude extract assays with substrate competitors and potential predators). ❐ *Porifera, secondary metabolites, microsymbionts, chemical ecology, exploration and exploitation.*

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PATTERNS OF INTRA AND INTERSPECIFIC GENETIC DIVERGENCE IN MARINE SPONGES

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Since the first molecular systematic studies on marine sponges in the 1980's, many papers have been published about levels of allozyme divergence between conspecific and congeneric sponge populations. Those genetic studies have indicated that sponges are more divergent than other marine invertebrates, a fact that was attributed to the high levels of genetic variation and morphological conservativeness found in Porifera. However, an analysis of 55 interspecific and 87 intraspecific pairwise genetic identity (I) values indicates a more complex picture. This study found that the average of I over all interspecific comparisons (I=0.42) was not much smaller than that found among other marine invertebrates (I=0.54), and the frequency distribution of I, for intraspecific comparisons, appears to be bimodal. Some genera were consistently highly divergent (I<0.30; *Cinachyrella*, *Oscarella*, *Cliona*, *Spirastrella* and *Tethya*), whereas others were within the normal range of gene divergence (0.40 < I < 0.80; *Chondrosia*, *Suberites*, *Petrosia*, *Plakina* and *Phyllospongia*). Furthermore, in the genera *Axinella*, *Chondrilla* and *Clathrina*, both low and high levels of intrageneric genetic differentiation were found (0.13 < I < 0.82). This pattern may reflect a large variance in the evolutionary age of genera in sponges, with very large levels of intrageneric gene divergence for some. We conclude with two non-mutually exclusive scenarios: a) genetic identity levels are too variable among sponge species to be of any use to evaluate taxonomic rank above species, or b) the range of evolutionary divergence in some genera of sponges is so broad that they may need revision. ❐ *Porifera, gene divergence, allozymes, heterozygosity, molecular systematics, larval dispersal.*

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For marine organisms genetic markers have been extremely useful both for estimating levels of gene flow in structured populations (Burton, 1996), and for the detection of sibling species (Knowlton, 1993; Thorpe & Solé-Cava, 1994). Allozyme electrophoresis has become the method of choice for alpha (i.e. at the species level) molecular systematics of marine organisms (Thorpe & Solé-Cava, 1994; Knowlton & Weigt, 1997). The main advantage of allozyme electrophoresis for taxonomic studies is that it represents an independent set of characters for the detection of sibling species (Solé-Cava & Thorpe, 1987). Genetic markers such as allozymes are particularly powerful for alpha-taxonomy (Hillis et al., 1996) because they can be used to detect reproductive isolation in sympatry (i.e. the biological species concept of Mayr, 1981), and describe unambiguous diagnostic characters (i.e. the phylogenetic species concept of Cracraft, 1987). In addition, as they are ubiquitous, allozymes offer a yardstick to compare levels of evolutionary divergence in relation to taxonomic rank in widely different taxonomic groups. Through molecular methods, it has become easier to verify whether ichthyologists, entomologists and spongologists infer the same thing when they talk about generic taxa in their respective groups. Since 1978, over 3000 intraspecific and interspecific allozyme comparisons have been performed between marine populations (literature data based on search on the Aquatic Sciences and Fisheries Abstracts database, between 1978 and 1998). The most commonly used measure of genetic similarity is the index of gene identity (I; Nei, 1972), which varies from 1.0 (=complete identity) to zero. An analysis of the large database of genetic studies,

mostly for terrestrial vertebrates and *Drosophila*, demonstrated that mean levels of gene identity were, as expected, very different when conspecific populations, congeneric species or confamilial genera were compared (Thorpe, 1982; Thorpe, 1983). It was shown that less than 5% of all conspecific comparisons fell below an identity level of 0.8 (Thorpe, 1982). Consequently, the *I* value of 0.8 has been used as a threshold for deciding about specific differentiation using allozyme data to define species, especially for comparing allopatric populations, where the more straightforward use of diagnostic loci (*sensu* Ayala, 1983) is not possible, and the biological species concept (Mayr, 1981) is not practical (Aron & Solé-Cava, 1991; Claridge et al., 1997). However, that value may be still too high for making decisions about the taxonomic rank of some marine invertebrates from geographically distant populations. This is because the number of allozyme loci detectable in marine invertebrates is usually smaller than in other organisms, with a consequent increase in the variance of estimates of gene identity (Nei, 1978), and also because gene flow is expected to be limited by geographical distance, with a consequent lowering of gene identities (Palumbi, 1992). Considering that decisions about species' borders in complex groups, using genetic attributes, are best taken using what has become known as 'fuzzy logic' (Van Regenmortel, 1997), the use of a threshold value becomes very important for the comparison of allopatric sponge populations.

Allozyme electrophoresis was first employed for molecular systematics of sponge populations by Solé-Cava & Thorpe (1986) and recently for sponge population genetics (Benzie et al., 1994).

Molecular data are also very useful for inferring patterns of genetic flow linked to larval dispersal (Burton, 1996). Sponge larvae are usually short lived (e.g. Borojevic, 1970; Fry, 1971; Sarà & Vacelet, 1973), which suggests that geographical distance could determine levels of gene differentiation in sponge populations. On the other hand, the pattern of gene flow observed in many marine invertebrates is often chaotic, depending mostly on rare but long-ranging broadcasting events (Johnson & Black, 1984). It would be interesting, therefore, to verify whether gene flow among sponge populations is also chaotic or supports the 'isolation by distance' model of genetic differentiation (Wright, 1978).

It has been suggested that Porifera might display much higher levels of interspecific gene divergence than other invertebrates, possibly due to the presence, in the former, of high levels of gene variation (Solé-Cava et al., 1991a; Klautau et al., 1994; Boury-Esnault et al.,1999). If this is true, then a re-calibration of the threshold value of conspecific gene identity should be performed, in order to reduce possible type I errors (i.e. deciding that putative species are different when they are not), due to a shift in gene identities between sponge populations in relation to other organisms. This calibration would be fundamental both for the analysis of evolutionary rates in the Porifera and for the continuing study on putative cosmopolitanism in the group.

The aims of this paper are to: 1) correlate levels of intraspecific gene identity with geographical distance, in order to estimate the importance of larval dispersal to the composition of sponge populations; 2) verify whether patterns of interspecific gene similarity in sponges are indeed different from those of other marine invertebrates; and 3) re-evaluate the threshold gene identity value for making taxonomic decisions for sponges.

MATERIALS AND METHODS

Data were gathered from the literature and from unpublished studies made by our laboratory (see references listed in the table legends). Whenever necessary, values of mean heterozygosity and genetic identity (Nei, 1978) were calculated from tables of gene frequency. Geographical distances were measured as the shortest distances by sea, using a large scale map (1 cm=60km; Christie et al., 1995). The possible relationship between pairwise geographical and genetic distances for intraspecific populations was tested using a Mantel test, with 1,000 replicates (Sokal & Rohlf, 1995). Pooled data of pairwise gene identity measures of intraspecific, interspecific and intergeneric comparisons were used to construct frequency histograms, in a similar way as those built by Thorpe (1982, 1983). The significance of differences between mean identity levels in interspecific (intrageneric) and intergeneric comparisons was tested using a Mann-Whitney U test (Sokal & Rohlf, 1995).

RESULTS

From all available data, 87 intraspecific, 55 interspecific and 8 intergeneric comparisons were compiled (Tables 1-3 respectively). No significant correlation (Mantel test; $P>0.40$) was found

TABLE 1. Levels of gene identity between conspecific populations. Key: Km, distance in kilometers; NL, number of loci; *I*, unbiased mean genetic identity (Nei, 1978); H, mean Hardy-Weinberg expected heterozygosity (Nei, 1972). References: 1, Benzie et al. (1994); 2, Klautau et al. (in press); 3, Cristiano Lazoski (unpublished results); 4, Solé-Cava et al. (1992); 5, Boury-Esnault et al. (1992); 6, Bavestrello & Sarà (1992); 7, Boury-Esnault et al. (1999); 8, Sarà et al. (1992).

Species	Locality 1	Locality 2	Km	NL	*I*	h	Ref
1. *Carteriospongia flabellifera*	Willis Island (Aust)	Middle Island	8.7	6	1.00	0.19	1
	Willis Island	Magdelaine	44	6	0.90	0.26	1
	Middle Island (Aust)	Magdelaine	52	6	0.89	0.27	1
	Lihou NE (Aust)	Lihou SW	80	6	0.93	0.22	1
	Magdelaine (Aust)	Lihou SW	175	6	0.69	0.28	1
	Magdelaine	Lihou NE	200	6	0.77	0.22	1
	Willis Island	Lihou SW	210	6	0.59	0.25	1
	Middle Island	Lihou SW	210	6	0.62	0.21	1
	Willis Island	Lihou NE	245	6	0.64	0.20	1
	Middle Island	Lihou NE	245	6	0.67	0.16	1
2. *Chondrilla nucula*	Marseille (Fr)	Ligurian (It)	350	9	0.91	0.11	2
3. *Chondrilla* sp.3	Anjos (Braz)	Praia do Forno	2	9	0.99	0.27	2
	Búzios (Braz)	Anjos	30	9	0.87	0.56	2
	Búzios	Praia do Forno	30	9	0.90	0.30	2
	Itacuruca (Braz)	Picinguaba	60	9	0.95	0.24	2
	Búzios	Itacuruca	240	9	0.95	0.30	2
	Anjos	Itacuruca	240	9	0.92	0.27	2
	Praia do Forno (Braz)	Itacuruca	240	9	0.94	0.30	2
	Picinguaba (Braz)	Ilha do Mel (Braz)	280	9	0.91	0.22	2
	Anjos	Picinguaba	300	9	0.95	0.22	2
	Praia do Forno	Picinguaba	300	9	0.89	0.25	2
	Búzios	Picinguaba	310	9	0.89	0.25	2
	Itacuruca	Ilha do Mel	340	9	0.91	0.27	2
	Anjos	Ilha do Mel	560	9	0.89	0.25	2
	Praia do Forno	Ilha do Mel	560	9	0.98	0.27	2
	Búzios	Ilha do Mel	700	9	0.98	0.28	2
	Noronha (Braz)	Búzios	2400	9	0.84	0.34	2
	Noronha	Anjos	2400	9	0.88	0.31	2
	Noronha	Praia do Forno	2400	9	0.91	0.34	2
	Noronha	Itacuruca	2600	9	0.88	0.33	2
	Noronha	Picinguaba	2700	9	0.90	0.28	2
	Noronha	Ilha do Mel	3200	9	0.84	0.59	2
4. *Chondrosia reniformis*	La Ciota (Fr)	Callelongue	17	13	0.96	0.14	3
	La Ciota	Endoume	25	13	1.00	0.16	3
	Callelongue (Fr)	Endoume	8	13	0.99	0.12	3
	La vesse (Fr)	Endoume	10	13	0.99	0.11	3
	La vesse	La Ciota	15	13	0.97	0.12	3
	La vesse	Callelongue	2	13	0.99	0.08	3
5. *Chondrosia* sp.	Bermudas	Recife	6640	13	0.89	0.27	3
	Bermudas	Búzios	8300	13	0.95	0.33	3
	Bermudas	Forno	8330	13	0.95	0.30	3
	Bermudas	Angra	8600	13	0.92	0.28	3
	Recife (Braz)	Búzios	1860	13	0.94	0.27	3

TABLE 1. Continued.

5. *Chondrosia* sp. (cont.)	Recife	Forno	1890	13	0.94	0.25	3
	Recife	Angra	2160	13	0.94	0.22	3
	Búzios	Forno	30	13	0.93	0.30	3
	Búzios	Angra	300	13	0.93	0.28	3
	Forno (Braz)	Angra	270	13	0.93	0.25	3
6. *Collospongia auris*	Willis Island	Middle Island	8.7	6	1.00	0.30	1
	Willis Island	Lihou SW	210	6	0.95	0.31	1
	Middle Island	Lihou SW	210	6	0.91	0.28	1
7. *Corticium candelabrum*	La Vesse	Riou (Fr)	25	16	0.97	0.24	4
8. *Oscarella lobularis*	La Vesse	Riou	25	16	1.00	0.11	4
	La Vesse	Riou	25	12	0.98	0.12	5
9. *Petrosia clavata*	Paraggi (It)	Zoagli (It)	2	9	0.96	0.12	6
10. *Petrosia ficiformis*	Paraggi	Zoagli	2	9	0.90	0.09	6
11. *Phyllospongia alcicornis*	Willis Island	Middle Island	8.7	6	0.96	0.38	1
	Willis Island	Holmes	210	6	0.89	0.35	1
	Willis Island	Lihou SW	210	6	0.85	0.26	1
	Middle Island	Holmes	210	6	0.86	0.34	1
	Middle Island	Lihou SW	210	6	0.87	0.32	1
	Holmes (Aust)	Osprey	300	6	0.74	0.34	1
	Holmes	Lihou SW	370	6	0.77	0.27	1
	Willis Island	Osprey	430	6	0.74	0.31	1
	Middle Island	Osprey	430	6	0.76	0.38	1
	Osprey (Aust)	Lihou SW	630	6	0.49	0.24	1
12. *Phyllospongia lamellosa*	Willis Island	Middle Island	8.7	6	0.91	0.30	1
	Diamond (Aust)	Lihou SW	50	6	0.89	0.25	1
	Lihou NE (Aust)	Lihou SW	80	6	0.93	0.26	1
	Diamond	Lihou NE	120	6	0.86	0.31	1
	Willis Island	Diamond	175	6	0.96	0.35	1
	Lihou NE	Marion	175	6	0.91	0.26	1
	Lihou SW (Aust)	Marion	175	6	0.99	0.19	1
	Willis Island	Holmes	210	6	0.96	0.34	1
	Willis Island	Lihou SW	210	6	0.85	0.27	1
	Middle Island	Holmes	210	6	0.90	0.24	1
	Middle Island	Lihou SW	210	6	0.76	0.19	1
	Diamond	Marion	220	6	0.93	0.24	1
	Willis Island	Lihou NE	245	6	0.85	0.36	1
	Middle Island	Lihou NE	245	6	0.80	0.27	1
	Holmes	Diamond	300	6	0.93	0.29	1
	Holmes	Lihou SW	370	6	0.85	0.27	1
	Willis Island	Marion	380	6	0.87	0.30	1
	Middle Island	Diamond	380	6	0.91	0.24	1
	Middle Island	Marion	380	6	0.80	0.19	1
	Holmes	Lihou NE	420	6	0.85	0.31	1
	Holmes	Marion	500	6	0.85	0.24	1
13. *Spirastrella hartmani*	San Blas 1 (Pan)	San Blas 2	1	8	0.95	0.30	7
	San Blas 1	Galeta (Pan)	100	8	0.87	0.28	7
	San Blas 2	Galeta	100	8	0.95	0.29	7
14. *T. citrina*	Marsala (It)	Torbay (GB)	3600	11	0.74	0.15	8
		Average	-	-	0.89	0.26	-

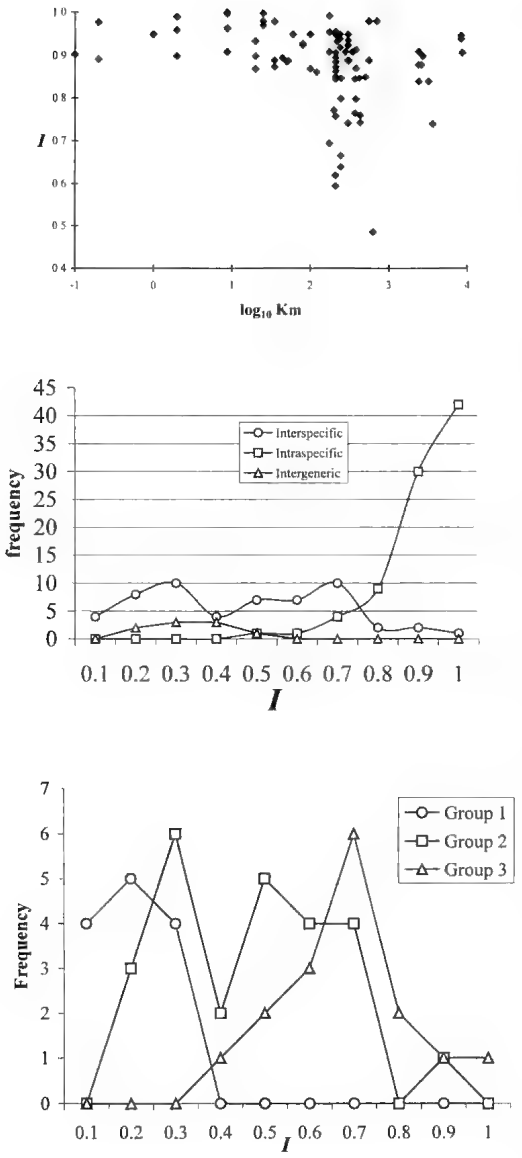

FIG. 1. A, Relationship between geographical distance (in $\log_{10}$ km) and pairwise gene identities (Mantel test; 1,000 replicates; P>0.40). B, Frequency histogram of gene identity (*I*) and taxonomic rank for species of sponges. C, Frequency histogram of gene identity (*I*) and taxonomic group. Group 1 - *Chondrosia, Suberites, Petrosia, Plakina* and *Phyllospongia*; Group 2 - *Axinella, Chondrilla* and *Clathrina*; Group 3 - *Oscarella, Cinachyrella, Tethya, Cliona* and *Spirastrella*.

between geographic distance and genetic identity (Fig. 1A).

The empirical frequency distribution of intraspecific (Table 1), interspecific (Table 2) and intergeneric (Table 3) gene identities studied on the different genera of Demospongiae and Calcarea (Fig. 1B) was similar to that found for other organisms (Thorpe & Solé-Cava, 1994). The average of *I* over all interspecific sponge comparisons was 0.42, which is similar to that found among other marine invertebrates (I=0.54). However, the distribution of interspecific pairwise gene identities in sponges was bimodal (Fig. 1C). Species of some genera were consistently highly divergent (I<0.30; 'Group 3': *Cinachyrella, Oscarella, Cliona, Spirastrella* and *Tethya*), whereas others were within the normal range of gene divergence (0.40<I<0.80; 'Group 2': *Chondrosia, Suberites, Petrosia, Plakina* and *Phyllospongia*). Furthermore, in the genera *Axinella, Chondrilla* and *Clathrina* ('Group 2'), species displayed both low and high levels of genetic differentiation in relation to their congeners (0.13<I<0.82). Some supposedly congeneric species had significantly lower (Mann-Whitney U test, z=2.94; P<0.004) levels of gene identity (mean I=0.16; Table 2), than species of different genera (mean I=0.30; Table 3). However, because genetic analyses have so far only focused on taxa with depauperate morphological characters or other groups presenting difficult systematic problems for Porifera, a complete pattern cannot be provided by the available data.

DISCUSSION

Two very interesting results are evident from the gene Identity analyses. 1) Generally, levels of gene identity were not correlated to geographic distance (i.e. it appears that potential for dispersal is not a key component in the structuring of sponge populations). 2) Levels of interspecific gene identities in the few sponge taxa so far examined are within the normal range found between species of other invertebrates, although some sponge genera have species that are extremely divergent from each other.

The low correlation observed between geographical distance and gene identity of intraspecific populations suggests that the length of larval life is not an essential factor in the structuring of sponge populations. Episodic recruitment events by rafting or some forms of asexual reproduction may play a more important

TABLE 2. Levels of gene identity between congeneric species. Group 1, *Chondrosia, Suberites, Petrosia, Plakina* and *Phyllospongia;* Group 2, *Axinella, Chondrilla* and *Clathrina*; Group 3, *Oscarella*, *Cinachyrella, Cliona, Spirastrella* and *Tethya*. See Table 1 for key to abbreviations. References: 1, Cristiano Lazoski (unpublished results); 2, Bavestrello & Sarà (1992); 3, Benzie et al. (1994); 4, Muricy et al. (1996); 5, Solé-Cava & Thorpe (1986); 6, Solé-Cava et al. (1991b); 7, Klautau et al. (in press); 8, Klautau et al. (1994); 9, Solé-Cava et al. (1991a); 10, Lazoski et al. (in press, this volume); 11, Barbieri et al. (1995); 12, Boury-Esnault et al. (1992); 13, Solé-Cava et al. (1992); 14, Boury-Esnault et al. (1999); 15, Sarà et al. (1992); 16, Sarà et al. (1993).

Group	Species 1	Species 2	NL	*I*	Ref
1	*Chondrosia reniformis*	*Chondrosia* sp.	12	0.48	1
1	*Petrosia ficiformis*	*Petrosia clavata*	9	0.62	2
1	*Phyllospongia lamellosa*	*Phyllospongia alcicornis*	6	0.50	3
1	*Plakina A*	*Plakina* sp.C	11	0.49	4
1	*Plakina A*	*Plakina* sp.D	11	0.73	4
1	*Plakina A*	*Plakina trilopha*	11	0.83	4
1	*Plakina C*	*Plakina* sp.D	11	0.79	4
1	*Plakina monolopha*	*Plakina* sp.C	11	0.35	4
1	*Plakina monolopha*	*Plakina* sp. D	11	0.58	4
1	*Plakina monolopha*	*Plakina trilopha*	11	0.61	4
1	*Plakina monolopha*	*Plakina* sp.A	11	0.66	4
1	*Plakina trilopha*	*Plakina* sp.C	11	0.54	4
1	*Plakina trilopha*	*Plakina* sp.D	11	0.61	4
1	*Suberites pagureorum*	*Suberites luridus*	19	0.66	5
1	*Suberites pagureorum*	*Suberites rubrus*	19	0.67	5
1	*Suberites rubrus*	*Suberites luridus*	19	0.98	5
2	*Axinella damicornis*	*Axinella verrucosa*	8	0.13	6
2	*Axinella damicornis*	*Axinella* sp.	8	0.70	6
2	*Axinella verrucosa*	*Axinella* sp.	8	0.13	6
2	*Chondrilla nucula*	*Chondrilla* sp.4 (Salvador)	9	0.23	7
2	*Chondrilla nucula*	*Chondrilla* sp.1 (Noronha)	9	0.28	7
2	*Chondrilla nucula*	*Chondrilla* sp.3 (Brazil)	9	0.33	7
2	*Chondrilla nucula*	*Chondrilla* sp.2 (Panama)	9	0.53	7
2	*Chondrilla* sp.1 (Noronha)	*Chondrilla* sp.2 (Panama)	9	0.32	7
2	*Chondrilla* sp.1 (Noronha)	*Chondrilla* sp.3 (Brazil)	9	0.48	7
2	*Chondrilla* sp.1 (Noronha)	*Chondrilla* sp.4 (Salvador)	9	0.58	7
2	*Chondrilla* sp.2 (Panama)	*Chondrilla* sp.3 (Brazil)	9	0.24	7
2	*Chondrilla* sp.2 (Panama)	*Chondrilla* sp.4 (Salvador)	9	0.25	7
2	*Chondrilla* sp.3 (Brazil)	*Chondrilla* sp.4 (Salvador)	9	0.30	7
2	*Clathrina aspina*	*Clathrina ascandroides*	9	0.57	8
2	*Clathrina aspina*	*Clathrina cylindractina*	9	0.65	8
2	*Clathrina aspina*	*Clathrina primordialis*	9	0.82	8
2	*Clathrina brasiliensis*	*Clathrina cylindractina*	9	0.43	8
2	*Clathrina brasiliensis*	*Clathrina ascandroides*	9	0.43	8
2	*Clathrina brasiliensis*	*Clathrina primordialis*	9	0.55	8
2	*Clathrina brasiliensis*	*Clathrina aspina*	9	0.69	8
2	*Clathrina cerebrum*	*Clathrina brasiliensis*	7	0.29	9
2	*Clathrina clathrus*	*Clathrina aurea*	11	0.13	9
2	*Clathrina cylindractina*	*Clathrina ascandroides*	9	0.43	8
2	*Clathrina primordialis*	*Clathrina ascandroides*	9	0.44	8
2	*Clathrina primordialis*	*Clathrina cylindractina*	9	0.65	8

TABLE 2. Continued.

3	*Cinachyrella apion*	*Cinachyrella alloclada*	19	0.27	10
3	*Cliona viridis*	*Cliona nigricans*	4	0.00	11
3	*Oscarella lobularis*	*Oscarella tuberculata*	16	0.27	12,13
3	*Spirastrella subogae*	*S. hartmani*	8	0.12	14
3	*Tethya citrina*	*Tethya aurantium*	11	0.18	15
3	*Tethya citrina*	*Tethya norvegica*	11	0.20	15
3	*Tethya norvegica*	*Tethya aurantium*	11	0.10	15
3	*Tethya orphei*	*Tethya robusta* "B"	8	0.01	16
3	*Tethya robusta* "A"	*Tethya robusta* "B"	8	0.18	16
3	*Tethya robusta* "A"	*Tethya orphei*	8	0.27	16
3	*Tethya seychellensis*	*Tethya robusta* "B"(Red Sea)	8	0.03	16
3	*Tethya seychellensis*	*Tethya orphei*	8	0.19	16
3	*Tethya seychellensis*	*Tethya robusta* "A" (Maldives)	8	0.28	16

role in sponges, as observed in other marine invertebrates (Johnson & Black, 1984; Johnson et al., 1993; Burnett et al., 1995). This indicates that sponges follow the islands model, rather than the isolation by distance model of genetic differentiation (Wright, 1978). Levels of population structuring, measured both by pairwise gene identities (Nei, 1972) and FST inbreeding indices (Wright, 1978) are very high in sponge species (F_{ST}=0.05–0.36; Benzie et al., 1994; Klautau, unpublished results). This indicates that sponge larvae either have low capacity for dispersal, or are philopatric, as also observed for some species of ascidians (Grosberg & Quinn, 1986). In any case, the high levels of population structuring observed indicate that sponge populations are continuously diverging genetically even over small geographic scales. Possible consequences of the high level of population differentiation are the adaptation of local populations to micro-environmental conditions, and the scope for a high speciation rate in sponges (Benzie et al., 1994).

TABLE 3. Levels of gene identity between confamilial genera. See Table 1 for key to abbreviations. References: 1, Benzie et al. (1994); 2, Solé-Cava et al. (1992); 3, Guilherme Muricy & Antonio Solé-Cava (unpublished results).

Genus 1	Genus 2	NL	I	Ref
Phyllospongia	*Carterospongia*	6	0.32	1
Phyllospongia	*Collospongia*	6	0.19	1
Carterospongia	*Collospongia*	6	0.20	1
Oscarella	*Corticium*	16	0.32	2
Oscarella	*Pseudocorticium*	16	0.28	2
Corticium	*Pseudocorticium*	16	0.47	2
Plakina	*Oscarella*	11	0.22	3
Plakina	*Corticium*	11	0.30	3
Plakina	*Pseudocorticium*	11	0.40	3

In general, the frequency distribution of the values of gene identity, in relation to taxonomic rank in sponges (Fig. 1B), shows a similar pattern to that observed for other species of animals (Thorpe & Solé-Cava, 1994). The main differences observed were a slight shift to the left in the distribution of intraspecific gene identities, and the bimodal distribution of interspecific gene identities (Fig. 1B). The higher levels of intraspecific differentiation may be related to high levels of gene variation (Skibinski & Ward, 1982) as those usually observed in sponges (Solé-Cava & Thorpe, 1989; Solé-Cava & Thorpe, 1991), although no significant association between heterozygosity and gene identity was observed for the sponge data (Table 1; Spearman's Rank Correlation, $P>0.10$). The bimodal distribution of interspecific gene identities is more puzzling, and seems to result from different patterns of gene divergence in different sponge genera. The genera analysed can be roughly broken into three groups in relation to levels of interspecific gene identities: 1) genera whose species have similar levels of gene identity as other invertebrates (*Chondrosia, Petrosia, Phyllospongia, Plakina* and *Suberites*); 2) genera where some pairwise species comparisons give very low identity values ($I<0.3$), whereas others have levels of gene identity comparable to those of other organisms ($0.4<I<0.8$ *sensu* Thorpe, 1983; Thorpe & Solé-Cava, 1994) (*Axinella, Chondrilla* and *Clathrina*); and 3) genera where interspecies comparisons consistently give extremely low (<0.3) identity values

(*Cinachyrella*, *Cliona*, *Oscarella*, *Spirastrella* and *Tethya*). The three groups are significantly different from each other (Mann Whitney's U test, $P<0.001$ for each pairwise comparison). In relation to levels of intra and intergeneric divergence, group 1 genera showed a profile similar to that observed in other animals, that is, species of group 1 were more similar to each other than to species of other genera (Mann-Whitney's U test, $P<0.0001$). On the other hand, levels of gene identity between group 3 species were lower than those observed between different genera of invertebrates (Fig. 1B-C; Thorpe, 1983). More interestingly, they were also significantly lower (Mann-Whitney's U test, $P<0.004$) than those found between different genera of marine sponges (Table 3).

Consequently, what is the threshold gene identity value used for deciding about specific differentiation in sponge populations ? Taxonomic decisions about the comparison of morphs living in sympatry should be based on the presence of diagnostic loci (sensu Ayala, 1983; i.e. loci for which the probability of wrongly identifying one individual as belonging to one species of a pair is smaller than 1%), rather than using gene identities. The use of diagnostic loci is preferred because it is more consistent from the theoretical point of view, and decisions based on them amalgamate the power of virtually all currently accepted species concepts (Claridge et al., 1997). Diagnostic loci can, of course, also be found between allopatric populations, but in that case making taxonomic decisions about their conspecificity is not as straightforward, since allopatric populations are expected to diverge if levels of gene flow are not very large (Wright, 1978). Under the phylogenetic species concept (Craeraft, 1987), diagnostic loci indicate independent evolution, and therefore speciation. However, given the very low levels of gene flow that seem to exist between sponge populations, if we make an orthodox use of that concept we will be forced to create new species of sponges for almost every allopatric population that we analyse. A more reasonable alternative is to use, for the comparison of allopatric populations, levels of gene identity, since they take into account the overall level of divergence, on which diagnostic loci do have a heavy weight (I for diagnostic loci = 0), but that is less biased by episodic events of selection or drift. Given the shift to the left in the intrageneric gene identity distribution, a value of about 0.7 of gene identity can be chosen as a threshold for making decisions about interspecific differentiation between allopatric sponge populations. We chose this value because it corresponds to the point where the distribution curves of intraspecific and interspecific gene identities meet, clearly separating the two groups (Fig. 1B). If we observe the distribution of interspecific identity levels (Table 2) and consider only the comparison of sympatric populations, thus avoiding the potential circularity of using allopatric comparisons, we can see that the average of I is 0.52, and only 16% of the interspecific gene identity values are above 0.70. Using this threshold value, *Phyllospongia alcicornis* from Lihou and Osprey Reefs (Coral Sea, Australia), and some of the populations of *Carteriospongia flabellifera* from Lihou Reef, considered by Benzie et al. (1994) to be conspecific with those of Middle Island and Willis Island, would have to be considered as separate species (I=0.49-0.67).

The belief that sponge species have a higher level of genetic differentiation than other organisms (Solé-Cava et al., 1991a; Klautau et al., 1994; Boury-Esnault et al., 1999) may simply be the result of an over-representation of species of group 3 in the literature. The picture that emerges from this study using a larger set of data, indicates that levels of gene divergence among presently recognised sponge genera vary broadly, which may be the result of two different, but not mutually exclusive, phenomena. 1) Those sponge genera with genetic identities below 0.3 are so old that there has been a saturation of gene divergence leading to the accumulation of homoplasic changes (as discussed by Thorpe, 1989). 2) Some sponge genera, notably those of groups 2 and 3 above, are polyphyletic. In the first case, allozymes would be considered to be of little use above the species level in sponges, but it would remain to be explained why some sponge genera can diverge at so different rates (Solé-Cava & Thorpe, 1994). In the second instance, some sponge genera require revision, and possibly splitting up into smaller, monophyletic units. In the first case the congeneric species of group 3 should be much more different from each other than those of different genera of practically all other groups of animals (including sponges).

The high levels of gene divergence observed between conspecific sponge populations and between species in some genera of sponges should be further investigated, as they have important consequences for the taxonomic framework for the whole group. If the gene identity found

between species of *Cliona* is zero (Barbieri et al., 1995), and between species of *Spirastrella* is 0.12, what would be the identity between *Cliona* and *Spirastrella* species? Likewise, what levels of gene identity would be observed between species of *Tethya* and *Tectitethya* or *Timea* ? At this very low level of gene identity, intergeneric species may have some alleles in common by simple homoplasic convergence, due to the saturation of possible alleles detectable by the technique (Thorpe & Sole-Cava, 1994). Those convergent alleles are often found in taxonomic comparisons above the genus level, but their presence is usually detected because they conflict with a much larger number of true synapomorphies within each genus (Hillis et al., 1996). However, given the very low gene identity found between species of group 3 genera, these few convergent alleles could be misinterpreted as synapomorphies, and lead to wrong taxonomic conclusions. For example, considering the lack of synapomorphies in the molecular data within *Cliona* or *Cinachyrella*, and the possible alleles in common between species from those genera, what should be our decision about their taxonomic status? Further genetic studies, possibly linked to independent DNA analyses, are needed to determine whether allozyme data are sufficently objective to distinguish sponges at the genus level.

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POPULATION DYNAMICS OF A SPONGE/MACROALGAL SYMBIOSIS: POSSIBLE CAUSES FOR A PATCHY DISTRIBUTION AT ONE TREE REEF, GREAT BARRIER REEF. *Memoirs of the Queensland Museum 44: 602. 1999*:- *Haliclona cymiformis* is a tropical marine sponge that is found only in association with a red macroalga, *Ceratodictyon spongiosum*. This association is commonly found on coral reef flats where it is frequently the most prominent macroscopic organism. At One Tree Reef, populations of *Haliclona/Ceratodictyon* are generally restricted to the rubble banks just inside the reef crest that surrounds One Tree Lagoon. Only one population of the association is found in the centre of the lagoon. It is likely that the lack of rocky substrata in the centre of the lagoon limits the recruitment of the association into new areas. Sexual reproduction by the sponge appears to be rare at OneTree Reef. At the rubble bank sites, populations of *Haliclona/Ceratodictyon* appear to be maintained by fragmentation and the size-frequency distribution is skewed toward smaller individuals. In the centre of the lagoon, clumps of the association grow to much larger sizes. Fusion experiments between individuals collected from different sites showed some histocompatibility, suggesting that existing populations of *Haliclona/Ceratodictyon* may have originated from the same parent population. ❐ *Porifera, Haliclona cymiformis, Ceratodictyon spongiosum, symbiosis, distribution, size-frequency, reproduction, fragmentation, histocompatibility.*

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ABOLISHMENT OF THE FAMILY CAULOPHACIDAE (PORIFERA: HEXACTINELLIDA)

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The hexactinellid family Caulophacidae possesses no unique features to distinguish it from Rossellidae, and hence it is proposed to revise the order Lyssacinosa by abandoning the family Caulophacidae and assigning its genera to Rossellidae, subfamily Rosellidae (*Caulophacus*, *Caulodiscus* and *Caulophacella*) and subfamily Lanuginellinae (*Sympagella*). ❐ *Porifera, Hexactinellida, Hexasterophora, Caulophacidae, revision.*

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The history of Caulophacidae is complex and intricately connected with that of Asconematidae Gray, 1872. Gray initially assigned a single genus, *Asconema*, to the family he but included no Recent representatives of Caulophacidae. Caulophacidae was erected by Ijima (1903) for two genera (both now considered valid): *Caulophacus* (including *Balanites* (Schulze, 1886; 1887) and *Balanella* (Schulze, 1887)) and *Sympagella* (including *Aulascus* (Schulze, 1886; 1897)). Ijima considered that Caulophacidae had important systematic characters which distinguished it from the allied Rossellidae, whereas earlier Schulze (1886) included them in Asconematidae, together with *Asconema*, *Hyalascus*, *Calycosoma* and *Calycosaccus*. Schulze (1886) further developed Gray's (1872) concept of Asconematidae in establishing Asconematinae and Rossellidae (1886), whereas he considered later (1899) that Asconematidae and Rossellidae should not continue as separate families. Taxa included in Asconematidae were determined to be lyssacinosan Hexasterophora, having a pinular ray in dermal and atrial spicules. They were divided into three subfamilies: Asconematinae, Sympagellinae and Caulophacinae. Thus, formally (according to the International Code of Zoological Nomenclature, Article 50-C-1; Anon., 1985), Caulophacidae must be attributed to Schulze (1886) because he had already erected the taxon Caulophacinae at the subfamily level, whereas Ijima (1903) had simply elevated it to family level. In doing so Ijima (1903) assigned four other genera of Asconematidae (*Asconema*, *Hyalascus*, *Calycosoma* and *Calycosaccus* (later synonymised with *Aulosaccus*)) to Rossellidae, and creating two subfamilies Rossellinae and Lanuginellinae.

In differentiating the new family Ijima (1903) provided a diagnosis, defined its scope and nominated a type genus. Apart from *Caulophacella* (Lendenfeld, 1915) which was described later and assigned to the same family, *Sympagella* was synonymised with *Calycosoma gracile* of Schulze (1903) and a new genus *Caulodiscus* was distinguished from *Caulophacus* (Ijima, 1927). Hence the scope of the family had increased and its diagnosis (sensu lato) had to be changed because of the inclusion of taxa with new combinations of microscleres. Caulophacidae is currently defined as: Lyssacinosa of goblet-like or mushroom-like body, always stalked and firmly attached at base; solitary or forming small branching stock. Ectosomal skeleton of small hexactines, seldom pentactines, pinular dermalia and of strong pentactine hypodermalia; the latter generally alone, seldom supplemented by rhabdodiactine hypodermalia. Choanosomal megascleres of hexactins and rhabdodiactins. Hexasters various, with or without sytrobiloplumicome (Ijima, 1927). The same diagnosis with minor amendments was used later by Koltun (1967) and Hartman (1982).

DISCUSSION

Comparing the diagnoses of all Lyssacinosan families the only unique feature for Caulophacidae appears to be possession of a sort of stolonial branching of stalks in some representatives of *Caulophacus* and *Sympagella*. I have investigated some of these specimens, none of which had a common cavity system in their tubular stalks. Hence, it is possible that these are separate

individuals rather than a whole organism. Some of these specimens had settled on the rigid, or perhaps dead, stalk of another specimen. Another possibility is that they may be products of asexual budding from the living tissues on the periphery of the stalk. The latter hypothesis is doubtful, however, as it is still uncorroborated that asexual reproductive processes occur in Hexactinellida. The abnormal dichotomous branching of tube-like bodies of some Rossellidae and Euplectellidae may be the result of compensation for marginal growth, known for many Hexactinosa with rigid skeletons (Reid, 1964). Thus, these sponges with two or more main oscules are single specimens rather than a colony. The classic example often cited is budding in *Lophocalyx philippinensis* and *Anoxycalyx ijimai*, but this too may be a result of larval settlement and growth on the prostalia spicules of a large specimen. Similarly, I do not consider the experimental aggregation of dissoluted fragments of *Rhabdocalyptus dawsoni* (Pavans de Ceccatty, 1982) to be connected with asexual reproduction as was suggested by Bartel & Tendal (1994).

As for other features of Caulophacidae given in its diagnosis, they overlap with those of many genera of Euplectellidae and Rossellidae. The funnel-like body of *Sympagella* is characteristic for many genera. The rare mushroom-like body form is also known for a new genus of a true Euplectellidae and some representatives of *Crateromorpha* (e.g. *C. meyeri* off New Caledonia). Moreover, the mushroom-like body may be easily developed in pedunculate sponges through marginal growth, consequently having a tendency to develop the inverted atrial cavity. The presence of a peduncle is certainly not a unique character since it is known in a number of Euplectellidae (Corbitellinae) and in *Crateromorpha* and *Aulochone* of the Rossellidae. Dermal pentactine and hexactine spicules are found in Rossellidae (i.e. *Aulascus*, *Hyalascus*). Possession of a pinular ray in dermal spicules is characteristic for *Asconema*, *Aulosaccus pinularis*, *Lophocalyx* and *Calycosoma* — all doubtful representatives of Rossellidae. Hypodermal pentactines are recorded for most Rossellidae. Choanosomal diactines and hexactines are known for Lanuginellinae (a subfamily of Rossellidae), in *Vitrollula*, *Schaudinnia*, *Crateromorpha*. 'Various hexasters' are known for all Hexasterophora. Strobiloplumicomes, spicules which in the Caulophacidae are known from *Sympagella* only, are also characteristic for Lanuginellinae.

One feature supporting the close affinity between Caulophacidae and Rossellidae is the discovery of 'discomultiasters', spicules with more than eight primary rays (Tabachnick, in prep.), among lophodis cohexasters of *Caulophacus latus*. Discomultiasters closely resemble discoctasters in shape, being derived in parallel from discohexasters. Discoctasters are characteristic of all members of the subfamily Acanthascinae (Rossellidae), although the former have more than eight primary rays (Tabachnick, in prep.).

Thus, no single feature seems to be unique for Caulophacidae, nor are there any complexes of characters which could be used to define, or sufficient to support the family as a valid taxon. I propose here to abandon Caulophacidae.

CONCLUSIONS

The well-defined genera presently included in Caulophacidae, often easily recognisable superficially, should be transferred to other taxa. *Sympagella* is most appropriately placed in Rossellidae, subfamily Lanuginellinae. This change in systematic position does not require any changes to the family or subfamily diagnosis. The three remaining genera should be included in Rossellinae, which also does not require any emendment to its diagnosis.

The abolishment of Caulophacidae makes the suborder Hypodermalia of Reid (1958) monotypic, with a single family Rossellidae. The presence of hypodermal pentactines distinctly separates Rossellidae from two other families of Lyssacinosa, Euplectellidae and Leucopsacidae, which belong to the suborder Autodermalia.

ACKNOWLEDGEMENTS

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PHOTOSYNTHESIS AND RESPIRATION BY THE SYMBIOTIC ASSOCIATION BETWEEN A CORAL REEF SPONGE AND ITS MACROALGAL SYMBIONT. *Memoirs of the Queensland Museum 44: 606. 1999:-* In the association between the haplosclerid sponge *Haliclona cymiformis* and the red macroalga *Ceratodictyon spongiosum*, the algal thallus comprises the bulk of the organism, while the sponge fills in the spaces between the algal branchlets and forms a thin layer around the outside of the association. The alga is exposed only at the very tips of the branches of the association. Measurements of photosynthesis and respiration of the symbiotic association have shown that this association makes a significant contribution to the primary productivity of the fringing reefs of One Tree Lagoon, southern Great Barrier Reef, in areas where few large primary producers (corals or algae) can live.

Light entering the branches of the association is rapidly attenuated and, as a result, the compensation and saturating irradiances are high; approximately 750 and 315µmol photons $m^{-2}s^{-1}$ respectively. Photoinhibition at higher irradiances does not occur. Maximum rates of photosynthesis reach 435µmol O_2mg chl $a^{-1}h^{-1}$ during summer, while respiration consumes up to 220µmol O_2 mg chl $a^{-1}h^{-1}$. These rates decrease by about half during the winter. Photosynthetic and respiratory rates were unaffected by changes in ambient oxygen concentration or by nutrient enrichment with nitrogen or phosphorus. Prolonged periods of heavy shading lead to an increase in pigment concentration in the alga, but no changes in maximum photosynthetic or respiratory rates were found when compared to control samples. There were no significant differences in the rates of respiration or photosynthesis between cultured *C. spongiosum* and the intact *Haliclona/Ceratodictyon* association, so it was not possible to formulate a model as to how respiration was partitioned between the partners in the association. ❐ *Porifera, Haliclona cymiformis, Ceratodictyon spongiosum, symbiosis, photosynthesis, respiration, oxygen concentration, nutrient enrichment, photoadaptation, partitioning of respiration.*

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AN APPROACH TO THE PHYLOGENETIC RECONSTRUCTION OF AMPHIDISCOPHORA (PORIFERA: HEXACTINELLIDA)

K.R. TABACHNICK AND L.L. MENSHENINA

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Two phylogenetic lines characterise Amphidiscophora (Porifera: Hexactinellida): the Pheronematidae-Monorhaphididae line and Hyalonematidae line. Both are derived from a lophophytous, cup-like sponge with an atrial cavity and osculum. Changing the body shape of the ancestral form gives rise to difficulties in water transport, hence the development of special types of compensation is required. The Pheronematidae-Monorhaphididae line leads to a columnar body with compensation developed in the form of expansion of dermal and atrial surfaces which penetrate into each other. The Hyalonematidae line leads to a conical body shape in which compensations developed in the form of cavities, atrial depressions and septa. Various body shapes which correspond to all Recent genera and subgenera of Amphidiscophora are discussed, emphasising their phylogenetic relations. ❐ *Porifera, Hexactinellida, Amphidiscophora, Pheronematidae, Monorhaphididae, Hyalonematidae, phylogenetic reconstruction, phenetics, systematics, external body shape.*

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Amongst the hexactinellids Amphidiscophora is a distinct and relatively stable taxon. Its stability and monophyletic status has not been challenged by either the discovery of hexadiscs in some taxa, or by more recent discovery of amphidiscs (spicules which were earlier considered to be specific for Amphidiscophora) as in some representatives of Hexasterophora (Tabachnick & Lévi, 1997). Recent Amphidiscophora consists of three families: Hyalonematidae, Pheronematidae and Monorhaphididae. All sponges belonging to Amphidiscophora can be easily assigned to a family, even if represented by only a small fragment, through the distinctness of their choanosomal megascleres (Ijima, 1927). By comparision, assigning species to genera is more complex, often depending on consideration of the external shape of the body; whereas spicule composition is less important. Species identifications require analysis of spicules, particularly microscleres, dermal and atrial spicules. Cases where spicules are unique or specific to a single taxon are very rare within Amphidiscophora, and moreover, some spicules previously considered to be monospecific have since been found in other taxa (e.g. four-toothed anchorate basalia have since been found in a *Semperella*-like sponge (Pheronematidae) (Reiswig, pers. comm.); paradiscs and tylodiscs are reported in *Monorhaphis chuni*, *Hyalonema (Oonema)* n. sp. and *Pheronema conicum* (series of publications by Tabachnick & Lévi, in press a, b & c).

The palaeontological history of Amphidiscophora, as well as of other Hexactinellida with loose skeletons, is poorly known. The occurrence of almost-complete specimens in the fossil record is restricted to several descriptions: *Uralonema* (Librovich, 1929), *Hyalonema* (Mehl & Hauschke, 1995) and problematic findings of Recent genera *Pheronema* and *Monorhaphis* (Mehl, 1992). The division of Amphidiscophora into Amphidiscosa and Hemidiscosa (Reid, 1958) (their erlier names Amphidiscaria and Hemidiscaria (Schramen, 1924), commonly used in the palaeontological literature), is now doubtful. This division was based on a single type of loose spicule, the latter taxon being characterised by presence of hemidiscs only. The discovery of hemidiscs among common amphidiscs in Recent *Pheronema conicum* and *Hyalonema (Oonema)* n. sp. (Tabachnick & Lévi, in press a & b) raises doubts about the validity of Hemidiscosa. Hence, palaeontological records provide no further clues for the reconstruction of phylogenetic process within Amphidiscophora.

The lack of any unique spicules and uniformity in spicule combinations among Recent amphidiscophorans do not allow these characters to be

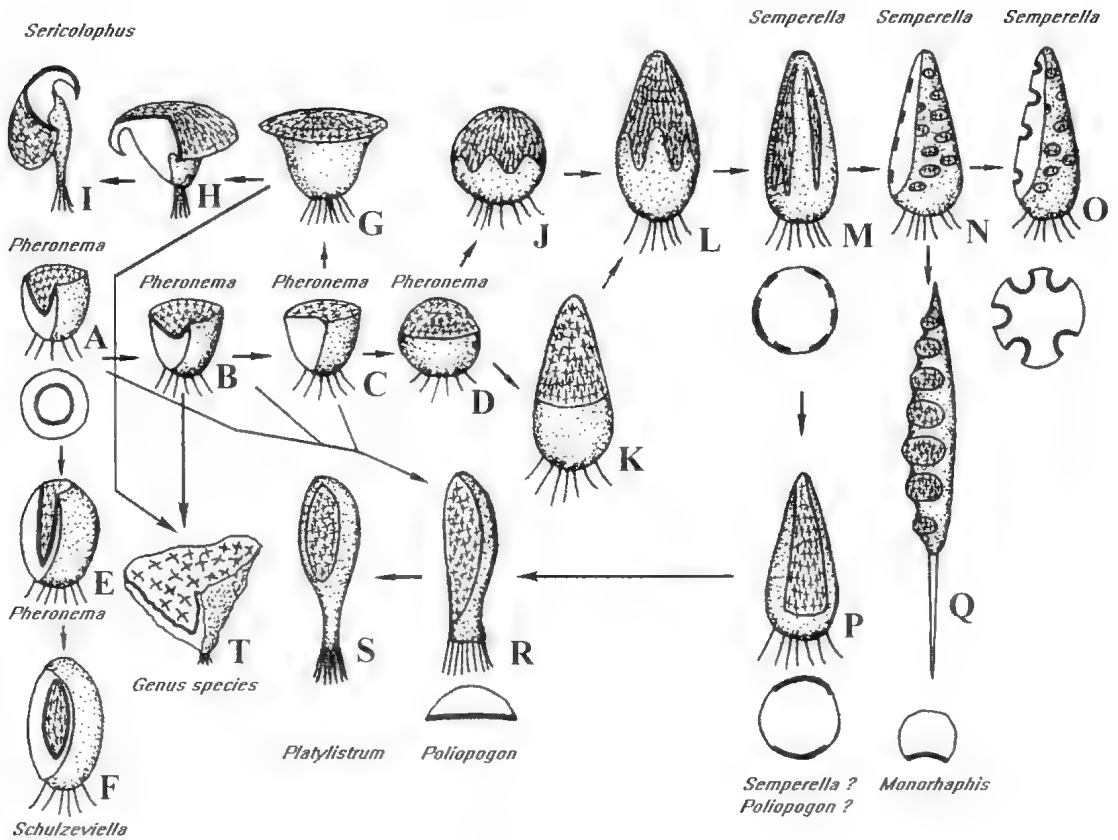

FIG. 1. Phylogenetic scheme for the Pheronematidae-Monorhaphididae line. A, cup-like, thin walled ancestral form and its transverse section; B-D, reduction of atrial cavity to a spherical form; E, barrel-like form with decreased osculum; F, oval form without osculum, atrial cavity inside the body; G, bell-like form with thickened marginalia; H, hypothetical form with thin marginalia twisted downwards and compact basal tuft; I, body form developed through bilateral deformation of the former form; J, hypothetical spherical form with deformed dermal-atrial border; K, hypothetical ovoid form; L, hypothetical ovoid form with deformed dermal-atrial border; M, ovoid form with atrialia divided into vertically distributed spaces; its transverse section; N, ovoid form with atrialia divided into numerous rounded spots; O, ovoid form with numerous atrial cavities and oscula; its transverse section; P, ovoid form with two vertical atrial spaces; its horizontal section; Q, columnar body with atrialia represented with oval spots organised in a linear vertical series; R, simple bilaterally, fan-like form; its transverse section; S, spoon-like form; T, thin-walled form (see Reiswig, 1999, this volume). Thin line in sections and dotted surface = dermalia; thick line in sections and surface covered with crosses = atrialia; arrows = suggested phylogenetic relationships.

used in any informative sense when considering their phylogeny. Conversely, variation in body shape among Recent species, which is very important in taxonomy, may be of greater value in reconstructing their evolution.

In this present work the systematics of Amphidiscophora is constructed using phenetic principles, developing the phylogenetic ideas of Reid (1964) who emphasised the significance of body form in the evolution of Hexactinellida. Cladistic analysis has only been applied to Hexactinellida in ranking higher taxa (Van Soest, 1985), and for constructing some cladograms of Hexasterophora (Mehl, 1992), but these methods seem to be useless for Amphidiscophora given their lack of any obvious or easily defined apomorphies within constituent taxa. Thus, the phylogenetic scheme proposed here is based on the traditional school of evolutionary systematics, using functional morphological analyses and morphogenetic reconstructions. Under this scheme, evolution from the ancestral form rests on the assumption that increases in absolute body

size lead to disproportionate size changes of related parts (Huxley, 1932), with consequent demands on the development of special mechanisms to compensate for water-flow and other problems. The method of rectangular coordination in deformations (Thomson, 1917) mostly shows variations in related body forms, whereas some hydrodynamic requirements may explain peculiarities in internal body constructions.

PHYLOGENETICS

ANCESTORS. In analysing the variation in external body shape in Amphidiscophora it is necessary to emphasise the poriferan function. To develop the ideas of Oken (1809), who suggested characterising taxa with short aphorisms such as 'animal-eyes', 'animal-gut' and 'animal- lungs', it is possible to offer the term 'animal-settled-kidney' for most sponges. From this aphoristic definition further evolution of the external shape of amphidiscophorans may be explored.

All representatives of Amphidiscophora are lophophytous sponges attached by anchorate spicules which often raise the body high above the substratum. This feature provides taxa with the ability to dwell on most types of oceanic substrata, from rocks to mud (Tabachnick, 1991). In body shape, the tube-like form is hypothesised to be the ancestral hexactinellidan form (Mehl, 1991; Tabachnick, 1991). The tube-like body allows filtered and unfiltered water to remain separate, also providing a means for passive filtration (Vogel, 1974). Thus, the amphidiscophoran ancestor would be a lophophitous sponge with numerous basalia, a cup-like body with atrial cavity and a single terminal osculum (Figs 1A; 4A). Two principal amphidiscophoran lines, Pheronematidae-Monorhaphididae and Hyalonematidae, are easily derived from this ancestral form. Tube-like or cup-like body forms are observed in most species of *Pheronema* (Pheronematidae) (Fig. 1A, E). Representatives of Hyalonematidae are more divergent except for a single monotypic genus, *Platella,* known from 3 specimens which preserve some of the ancestral features. One specimen is cup-like with compact untwisted basalia (Fig. 4B); 2 other specimens have probably the same form but the basalia are twisted (Fig. 4C) (Tabachnick, unpub. data).

PHERONEMATIDAE-MONORHAPHIDIDAE LINES OF EVOLUTION. The main tendency for most genera of Pheronematidae is to reduce the atrial cavity through several stages, probably through adaptation to high sedimentation conditions (Tabachnick, 1991). Some of these stages are present in several species of *Pheronema* (Fig. 1B-D): *P. circumpalatum*, *P. globosum* and *P. nascaniensis* have low atrial depression (Fig. 1B) or hemispherical body form (Fig. 1C). The spherical form is realised in some specimens of *P. nascaniensis* (Fig. 1D). This process, as well as wall thickening, is accomplished with intensive development of subdermal and subatrial cavities or canals, hence water must pass through larger distances inside the sponge between dermal and atrial surfaces. The extension of the evolutionary tendency for Pheronematidae to invert the atrial cavity and to elongate the spherical body form in the vertical direction should lead to an ovoid sponge where the dermal surface is represented on the lower part, and the atrial surface on the upper part of the ovoid body. Similar results were obtained using mathematical techniques applied to growth modelling, where the ovoid body form is a result of simulation of the radial accretive growth derived from an initially spherical object (Kaandorp, 1994). In visualising water flow through a sponge with an ovoid body, two distinct zones (the lower and upper) may not be sufficiently associated in pumping-filtering activity (Fig. 2). Even large cavity and canal development would not facilitate water transport because the total surface area is limited by sponge diameter. Conversely, the proportions of a spherical form seems to be theoretically more probable. Moreover, spherical body form is practically realised in some specimens of *Pheronema nascaniensis* whereas the closest theoretical descendant growth form — the ovoid one — has not been practically realised, and is probably 'unstable', hypertrophied or non-functional.

To avoid the problems connected with an ovoid body form (long distance to transport water), compensation takes place by development of deformation of the border between dermal and atrial surfaces (Fig. 1J-L). In such sponges each sector of the dermal surface in the middle part of the body is connected by two close atrial sectors, and vice versa. Thus, the distance required for water transport is more restricted (Fig. 3). This process enables an increase in body length in the vertical direction and leads to a number of theoretical body forms which in reality are seen united in *Semperella* (Fig. 1M-P). The most primitive of these forms seem to be hydrodynamically

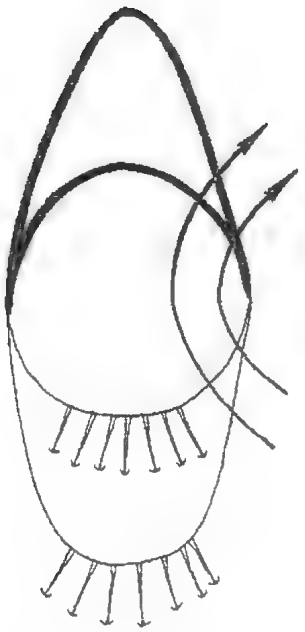

FIG. 2. Superposition of vertical sections of spherical and ovoid forms of Pheronematidae with the same maximal diameter. Thin line = dermalia; thick line = atrialia; arrows = water flow. The scheme shows the difficulties of water transport through distinct parts of the ovoid form compared to the spherical one.

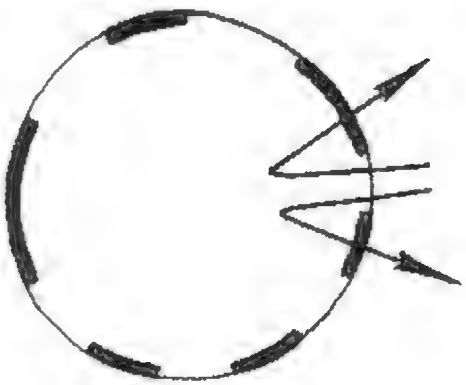

FIG. 3. Transverse section of a *Semperella*-like sponge (like Fig. 1M, N, P). Thin line = dermalia; thick line = atrialia; arrows = water flow. The scheme shows facilitation of water transport in forms with deformed dermal-atrial borders due to reduction of the distance of water transport which becomes possible in the horizontal direction.

plausible but it is not so far known among any Recent species (Fig. 1L). The more developed stages, with fragmentation of the atrial area, are quite common. The least specialised form with columnar body has several longitudinally directed atrial areas: *Semperella schulzei* and *S.* n. sp. (Fig. 1M) (Tabachnick & Lévi, in press a) and *S. alba* (Fig. 1P). A more advanced sponge body form has the same type of columnar body but where atrial surface consists of numerous rounded atrial spots separated by the dermal area: *Semperella cucumis* and *S.* n. sp. (Fig. 1N) (Tabachnick & Lévi, in press a), or forms with the rounded atrial spots bent inwards inside the body forming numerous atrial cavities with corresponding oscula: *Semperella stomata* and probably *S. spirifera* (Fig. 1O).

The growth form which apparently can be easily transformed from a columnar body with numerous rounded atrial spots is known for *Monorhaphis*, the atrial surface of which is represented by a linear vertical series of rounded spots (Fig. 1Q).

Another body form typical of Pheronematidae is bilaterally-symmetrical, fan-like, with dermal and atrial surfaces located on the opposite sides, as in the genus *Poliopogon* (Fig. 1R). Similar to it is the spoon-like body form which characterises *Platylistrum* (Fig. 1S), a slightly divergent variant of the fan-like form. The fan-like body may be interpreted as a result of irregular growth of a hemispherical body, or by asymmetrical growth of a wall sector in a tube-like body (both from a *Pheronema*-like ancestor), or from the columnar body with longitudinally directed atrial areas through to reduction of these atrial areas to a single one (from a *Semperella*-like ancestor). A body form with two atrial and two dermal surfaces on opposite sides is known in *Semperella alba* (Fig. 1P) (Tabachnick, 1988), which could be considered to be transitional between *Semperella* and *Poliopogon*.

A very peculiar bilateral body form known in *Sericolophus* (Fig. 1I) may also be derived from the hemispherical growth form, but with several transitional stages: a bell-like form with thin marginalia (Fig. 1G) — eversion of thin marginalia downwards (Fig. 1H) — irregular body growth. The transitional forms (Fig. 1G-H), which seem to be hydrodynamically plausible, are, however, so far unknown among Pheronematidae although common among the related Hyalonematidae.

The other theoretical possibility in the development of the Pheronematidae line is a body form with an entirely closed atrial cavity, as seen in the problematic genus *Schulzeviella* (Fig. 1F) (Tabachnick, 1990). This genus contains a single species with two subspecies: *S. gigas gigas* (pro

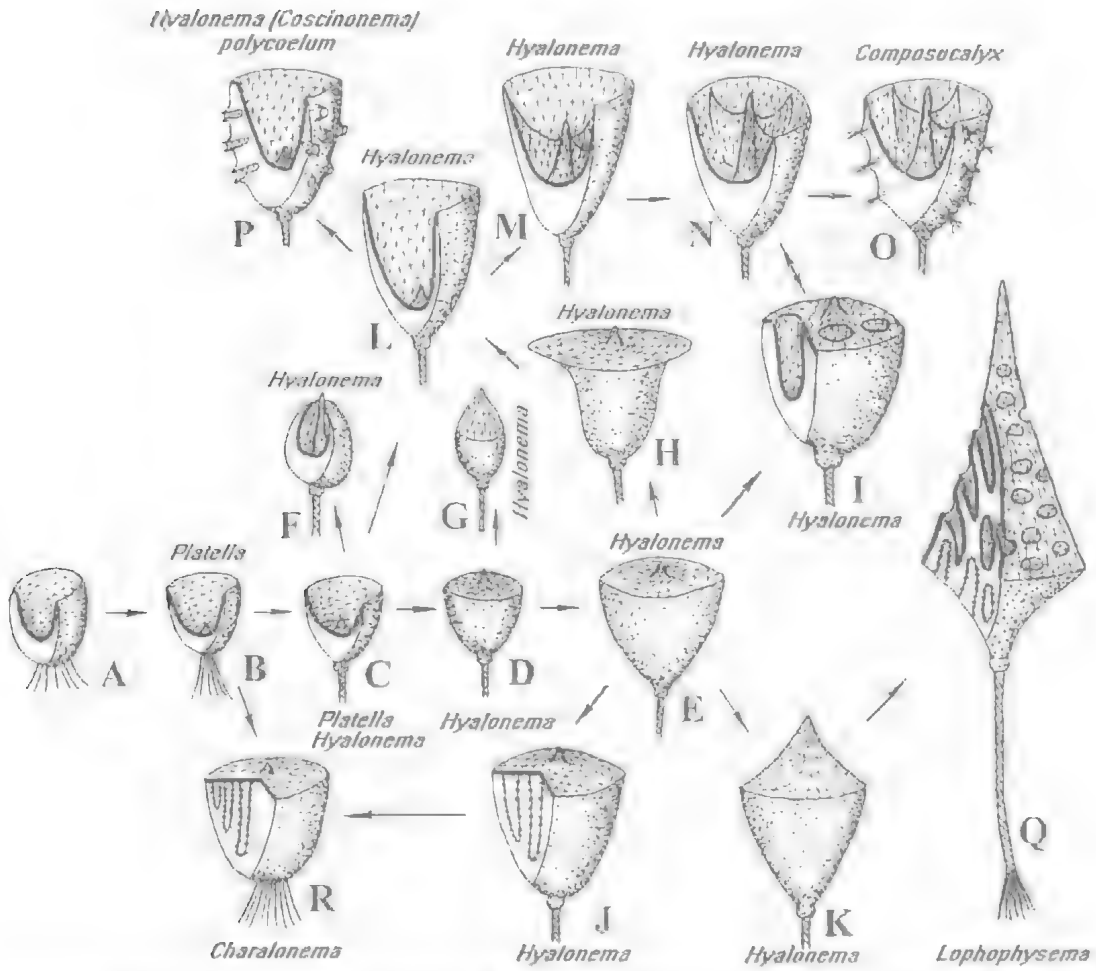

FIG. 4. Phylogentic scheme for the Hyalonematidae line. A, hypothetical cup-like, thin walled ancestral form; B, the same with compact tuft of basalia and apical cone; C, the same with twisted basalia; D, conical form with reduced atrial cavity; F, enlarged previous form; F, ovoid form with vast atrial cavity, narrow osculum and apical cone penetrating it; G, ovoid form in which atrial surface is present as the upper hemisphere and dermal surface as the lower one; H, bell-like form with thin marginalia; I, conical form with developed atrial depressions; J, conical form with developed subatrial cavities; K, body form of two cones fused to each other by their widest bases; L, body form which originated from the 'C' by its enlargement or from the 'H' by twisting marginalia upwards; M-N, septate cup-like forms; O, same with tufts of prostalia lateralia; P, cup-like form with dermal depressions; Q, body form of two cones fused to each other by their widest parts with subdermal cavities and atrial depressions; R, conical form with subatrial cavities and broad untwisted basalia. Thin line in sections and doted surface = dermalia; thick line in sections and surface covered with crosses = atrialia; dotted line in sections = subatrial cavities; arrows = suggested phylogenetic relationships.

Poliopogon gigas Schulze, 1887) and *S. gigas spinosum* (Tabachnick, 1990). The latter subspecies is represented by a slightly damaged specimen. The reconstruction of its body shape shows that it must be an oval sponge in which the atrial cavity is entirely enclosed inside the body (no osculum was observed). This body shape may be derived from the most primitive ancestral form by the overgrowth of the osculum with marginal walls. The intermediate body forms with large atrial cavity and small osculum (Fig. 1E) are known among species of *Pheronema*. If this interpretation of the body of *Schulzeviella* is correct it may be worthy of recognition as a separate genus, given that all genera of Pheronematidae are characterised by specific body forms. If not, however, these sponges should be returned

to *Pheronema* as suggested by Reiswig (1992) and Dawson (1993).

To understand the functionality of the body form seen in *Schulzeviella* it is necessary to study other sponges with analogous construction among the Hexasterophora (e.g. *Aphrocallistes* and *Euplectella*), given that this type of body form is otherwise absent among the other Amphidiscophora. *Euplectella* has thin walls with numerous lateral oscules and orifices in the sieve-plate which covers the main osculum. The sieve-plate of *Euplectella* is considered to appear from the lateral walls (Ijima, 1901) as in *Schulzeviella*. The out-flow of water must pass through the sieve-plate and lateral oscules. The walls in *Schulzeviella* are thin (in relation to its huge size) and they become thinner toward the upper end of the sponge. Inside these walls there are numerous canals. In *Schulzeviella* the out-flow of water may penetrate through the sieve-plate as it does in *Euplectella*-like sponges, although water movement from atrial to dermal surfaces is an uncommon mode among Hexactinellida. Similarly, comparison with *Hyalonema* body forms provide a clearer understanding of body functionality in *Schulzeviella*, whereby they develop large subatrial cavities beneath the atrial surface, which is represented by the sieve-plate (Fig. 4J).

A new type of pheronematid body-form is described by Reiswig (1999, this volume), consisting of a widely open funnel with thin walls and a short, thin tuft of basalia (Fig. 1T). This form might be interpreted as a descendant of cup-like *Pheronema*, or of a hypothetical ancestor of *Sericolophus* (Fig. 1G). The affinities of this sponge to *Poliopogon* may be ancestral, or vice versa, but based on evidence presented here I consider that this sponge is more probably allied to *Pheronema* then to *Poliopogon*.

HYALONEMATIDAE LINE OF EVOLUTION. Evolution of most Hyalonematidae is connected with the gathering of basalia into a compact tuft. This process is correlated with the appearance of an apical cone — a conical protrusion from the middle of the atrial cavity where the proximal parts of anchorate basalia are gathered beneath the atrial surface. In most hyalonematid representatives the basal tuft is compact and twisted, presumably a functionality to increase its strength and flexibility. A minute widening in the lower part of the body containing special spicules (acanthophores) also correlates with the appearance of a compact tuft of basalia. This body form is common for some species of most subgenera of *Hyalonema* (i.e. *H. (Pteronema)*, *H. (Thamnonema)*, *H. (Prionema)*, *H. (Paradisconema)*, *H. (Oonema)*, *H. (Leptonema)*, *H. (Cyliconema)*, *H. (Coscinonema)*, *H. (Corynonema)*; (Fig. 4C). A similar body form with numerous dermal depressions also appears to be present in *H. (Coscinonema) polycoelum* (Fig. 4P) (Lévi & Lévi, 1989). Two principal lines of evolution may be derived from this body form.

The first line leads to the appearance of an oval body with a small osculum, a deep atrial cavity and an apical cone which protrudes above the osculum penetrating it (e.g. *H. (Phialonema)*, some species of *H. (Oonema)*, *H. (Onconema)* and *H. (Leptonema)*; (Fig. 4F). The further development of several vertical septa between the lateral wall and the apical cone could also be anticipated (see below) (Fig. 4M-N).

The second line leads to a reduction of an atrial cavity and formation of a so-called sieve-plate from the atrial surface (Fig. 4D-E). These conical forms are known in some species of *H. (Thamnonema)*, *H. (Pteronema)*, *H. (Prionema)*, *H. (Paradisconema)*, *H.(Oonema)*, *H. (Leptonema)*, *H. (Cyliconema)* and *H. (Coscinonema)*. The extension of this process leads to oval forms, similar to the pheronematid tendency where the dermal surface corresponds to the lower hemisphere and the atrial surface to the upper one (Fig. 4G). This body form is rarely found among Hyalonematidae, represented only by small sponges in several species of *H. (Coscinonema)* and *H. (Corynonema)*. Forms with a reduced atrial cavity are common in most subgenera of *Hyalonema* and are typical for all its known juveniles. The proportional enlargement of these forms does not seem to be successful because of the concomitant water transport problems associated with increased body volume (Fig. 5). Hence such large forms require special types of compensation.

One form of compensation is the development a large body composed of two cones fused together at their widest part (Fig. 4K). Intensive development of subdermal cavities beneath thin, net-like dermalia and numerous atrial depressions with ocular openings on the upper cone are presented in the form seen in *Lophophysema* (Fig. 4Q). The development of large subdermal cavities is a rare event, hence the inhalant orifices near the dermal surface should be smaller then exhalant ones near the atrial surface to facilitate the water transport. That is why large subatrial cavities and atrial depressions are common while

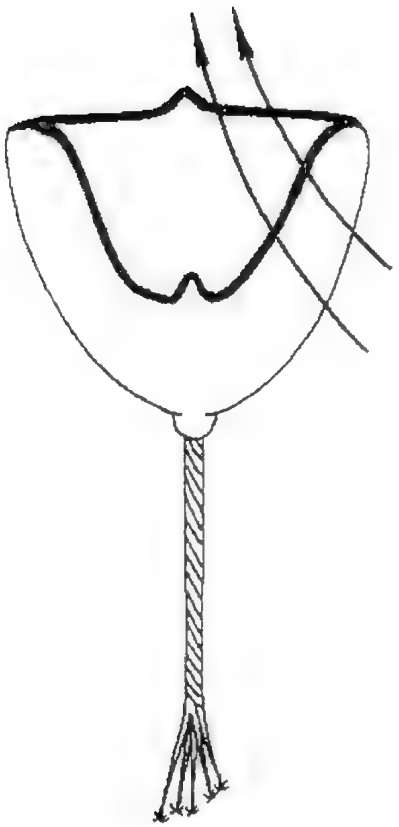

FIG. 5. Superposition of the sections of cup-like and conical forms of Hyalonematidae with coexisting external borders. Thin line = dermalia; thick line = atrialia; arrows = water flow. The scheme shows prolongation of water transport through central part of conical form in comparison to cup-like one.

such cavities and depressions in the vicinity of dermal area are rare.

A second possible form of compensation is the development of large subatrial cavities in large conical forms, seen in some *H. (Thamnonema)*, *H. (Pteronema)*, *H. (Hyalonema)* (pro *Hyalonema (Euhyalonema)*); (Hooper & Wiedenmayer, 1994) and *H. (Coscinonema)* (Fig. 4J). *Charalonema* may be considered to be a descendent of this form in which the tuft of basalia became less compact and untwisted (Fig. 4R). Conversely, the other possibility for the origin of *Charalonema* is from a *Platella*-like ancestor with a tendency, parallel to that of *Hyalonema*, to reduce the atrial cavity and to develop subatrial canals.

A third possible method of compensation for a large bell-like form without an atrial cavity is seem in the development of several (often four) atrial depressions directed downwards (Fig. 4I) (e.g. some species of *H. (Coscinonema) and H. (Corynonema)*). This body form may be considered to be a further step in the evolution towards septate forms.

The appearance of septate forms must take place mainly in thin-walled sponges with an elongate apical cone. Septa may have developed to increase the rigidity of the enlarged thin-walled body. Nevertheless other possibilities of septate-form appearance can be considered: from conical forms with atrial depressions (see above), or from bell-like forms with thin and well developed edges (Fig. 4H) (this form is known for *H. (Prionema)*). The septate sponges are known among *H. (Hyalonema)*, *H. (Onconema)*, *H. (Leptonema)*, *H.(Cyliconema)* and *H. (Coscinonema)* (Fig. 4M-N). *Composocalyx* also corresponds to the septate body type but it has specific tufts of prostalia lateralia composed of diactines situated on the conical prominences of dermal surface (Fig. 4O).

DISCUSSION

PROBLEMS IN TERMINOLOGY. There are several problems remaining in the terminology pertaining to sponge body form. 1) The sieve-plate described in some representatives of Hyalonematidae is not homologous to the sieve-plate of other Hexactinellida since it is constructed from the atrial surface and not from the wall, as suggested for *Euplectella* and *Regadrella* (Ijima, 1901), and even for *Schulzeviella*. 2) The origin of secondary oscula in Amphidiscophora also requires some clarification. In all known representatives of Pheronematidae, including *Semperella stomata* (Fig. 1O) there appear to be orifices and borders between dermally- and atrially-lined surfaces. The secondary oscules of Hyalonematidae, where known (Fig. 4I), are also orifices but they are surrounded with an atrially-lined surface while the border between dermalia and atrialia marks the ancestral position of the primary osculum.

PHYLOGENETICS. The common trend for both Hyalonematidae and Pheronematidae-Monorhaphididae lines of the Amphidiscophora is to reduce the atrial cavity. The former line mostly develops the conical body with corresponding compensation in large specimens through the appearance of depressions, septa and subatrial cavities. The latter line shows tendency to invert the atrial cavity, which leads to a columnar body and corresponding compensation through the changing position of dermal and

atrial areas which penetrate into each other; hence the ancestral form is represented by a large number of species of one genus (*Pheronema*). Nevertheless, *Sericolophus* (Pheronematidae) evolves into a form reminiscent of Hyalonematidae, while *Lophophysema* (Hyalonematidae) displays some pheronematid features of evolution. The presence of bilaterally symmetrical forms also emphasises the differences between these lines. In the Pheronematidae-Monorhaphididae line, forms with bilateral symmetry are known in many genera, while in the Hyalonematidae line they are entirely absent. This state of bilateral symmetry is achieved by curvation of the stalk in any direction, under the influence of water flow (Tabachnick, 1991).

PHYLOGENETICS AND SYSTEMATICS. Phylogenetic reconstruction of the Pheronematidae-Monorhaphididae line shows the distinct relationship between *Pheronema*, *Semperella* and *Sericolophus*. These genera can be connected only through hypothetical, functionally unstable forms (as in the two former genera), or through forms which have not so far been encountered in Recent representatives of this family (as in the latter genus). The numerous variants of these hypothetical forms (Fig. 1J-L) do not challenge the monophyly of *Semperella* since they all seem to originate from a common ancestor. The possibility of a polyphyletic origin of *Poliopogon* may be related to the difficulties connected with its identification (Tabachnick & Lévi, in press). Nevertheless, the phylogenetic reconstruction does not provide arguments to improve the situation with *Poliopogon*. The close relationship between *Monorhaphis* and *Semperella* was also recognised in the classical systematics literature, where *Monorhaphis* was placed in Semperellidae (Schulze, 1887) with a single genus *Semperella*, prior to the subsequent inclusion of *Monorhaphis* (Schulze, 1904). Later this family was rejected by Ijima (1927), and *Monorhaphis* was placed in a separate family. The distinct taxonomic position of *Monorhaphis* is a result of clear spicule divergence, due probably to its extremely elongate body. The choanosomal skeleton composed of tauactines and the single giant anchorate spicule are specific features which apparently justified its recognition as a separate family. The Monorhaphididae probably originated from Pheronematidae, as indicated by this present phylogenetic reconstruction. Another support for this suggestion is that only representatives of Pheronematidae (*Semperella* and *Poliopogon*) posses basalia of different kinds: a mixture of anchorate spicules and monaxons-diactins. The single basal spicule of *Monorhaphis* is likely to be the same monaxon-diactin, and the loss or reduction of all the other basalia in a *Semperella*-like ancestor is very probable. The solve the problem of the validity of *Schulzeviella* requires new data. Confirmation of its specific body shape is necessary to maintain the validity of the genus.

Most of the specific body-forms, or their close series, correspond to a single taxon of Amphidiscophora within the generic range. This emphasises the generic status of *Hyalonema* with its numerous subgenera, differing from each other by combinations of several features; the corresponding body forms are not specific for each subgenus. The subgenera of *Hyalonema* were raised to generic level by Lévi (1964), but this action was reversed in his subsequent papers. The generic status of *Composocalyx* and *Charalonema* is not obvious since these genera have close relations with the diverse complex of *Hyalonema*. The genus *Charalonema* was considered to be close to *H. (Pteronema) topsenti* (Ijima, 1927) and in the scheme suggested here it may be the ancestor of that subgenus. Nearly all extrapolated body forms of the hyalonematid line of evolution are present in nature. The absence of gaps explains the problems with their close relationships and difficulties in identification.

Amphidiscophoran representatives seem to realise all the possible body forms which can be derived from their suggested ancestors, excepting several forms which are unfavourable for pumping-filtering activity.

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In press b. Hyalonematidae (Porifera, Hexactinellida, Amphidiscophora) off the New Caledonia. The origin of uncinates and paradises in the Hexactinellida. In Resultats. des Campagnes Musorstom. Memoires du Muséum National d'Histoire Naturelle, Paris.

In press c. Revision of the genus *Monorhaphis* (Porifera, Hexactinellida, Amphidiscophora). Zoosystema.

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THE ROLE OF EARLY LIFE-HISTORY STAGES IN DETERMINING ADULT SPATIAL PATTERNS OF ENCRUSTING SPONGES. *Memoirs of the Queensland Museum 44: 616. 1999*:- We studied the abundance and spatial pattern of two Mediterranean encrusting sponges: *Crambe crambe* (highly toxic) and *Scopalina lophyropoda* (non-toxic) at three spatial scales (0.5, 1 and $2m^2$). We examined the reproductive output, larval behaviour and early recruitment in these species, and assessed the relative importance of these parameters in explaining the abundance and spatial patterns of adults. We also determined, in field experiments, whether the presence of adults induces or inhibits recruitment in these two species. We found that *C. crambe* was much more abundant than *S. lophyropoda* at the site studied in both number of individuals per square meter (67±2.7 vs. 10.2±2.1, mean±SE) and coverage (47±1.9% vs. 11.1±1.4%). At the smallest scale sampled ($0.5m^2$), both species showed an aggregated pattern. Aggregation was also detected for *S. lophyropoda*, but not for *C. crambe*, at the scales of 1 and $2m^2$. The number of embryos incubated per cm^2 by *C. crambe* and *S. lophyropoda* was 76.2±12.5 and 14±1.7 (mean±SE), respectively. We estimated that the potential number of larvae of *C. crambe* released into the water column was about 20 times higher than that of *S. lophyropoda*.

Larval behaviour was monitored in the laboratory and in the field. Larvae of *S. lophyropoda* did not swim away from the release point. They maintained a vertical posture that minimised horizontal dispersal, and soon began crawling. In contrast, the larvae of *C. crambe* swam actively and had a comparatively delayed crawling phase. Recruitment of the two species in scraped quadrats surrounded by individuals of *C. crambe* and *S. lophyropoda*, and in controls (rocky areas with no sponges) was monitored weekly for a month. Recruitment of both species was higher in scraped quadrats surrounded by conspecifics. This effect was notably more marked for *S. lophyropoda* than for *C. crambe* recruits. The toxicity of *C. crambe* did not inhibit settlement of *S. lophyropoda* with respect to controls. The mean number of recruits per surface unit after one month (all substrates pooled) was ca. 3.5 times higher for *C. crambe* than for *S. lophyropoda*. This difference was smaller than expected given that larval production of *C. crambe* was ca. 20 times higher. This indicates that a significant proportion of *C. crambe*'s offspring did not contribute to the maintenance of the local population. The aggregated pattern of *S. lophyropoda* at scales ranging from 0.5-$2m^2$ and its discontinuous geographic distribution may be partially explained by strong phylopatry of its larvae due to their poor swimming ability and limited dispersal. The dominance of *C. crambe* in littoral assemblages, its random distribution at scales larger than $0.5m^2$, and its ubiquity along the littoral are traits that are consistent with high reproductive output, the swimming behaviour of larvae which facilitates wide dispersal, and patterns of recruitment found in this study. Therefore, *S. lophyropoda* populations appear to be maintained by offspring supplied by autochthonous individuals while populations of *C. crambe* appear to be open, with a potentially significant flow of larvae between them. ❐ *Porifera, reproductive output, larval behaviour, settlement, recruitment, spatial patterns, encrusting, Mediterranean Sea.*

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CUTICULAR LININGS AND REMODELISATION PROCESSES IN *CRAMBE CRAMBE* (DEMOSPONGIAE: POECILOSCLERIDA)

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Turon, X., Uriz, M.J. & Willenz, Ph. 1999 06 30: Cuticular linings and remodelisation processes in *Crambe crambe* (Demospongiae: Poecilosclerida). *Memoirs of the Queensland Museum* **44**: 617-625. Brisbane. ISSN 0079-8835.

The common Mediterranean sublittoral sponge *Crambe crambe* goes through a resting, non-feeding period with cellular restructuring which may have biological and ecological significance. This red encrusting sponge reproduces in summer and larvae released during July-August. After reproduction, from the end of August until the end of October, some specimens appeared covered with a glassy cuticle, obliterating the ostia and oscula. No water pumping and, hence, no feeding occurs during this stage. At the end of October and during November some specimens displayed a strongly hispid surface, with spicules retaining entangled debris. This hispid form is interpreted as an intermediate stage between the resting phase and the active period. SEM examination of the surface during the non-feeding period confirmed the absence of inhalant orifices and the presence of an acellular cuticle markedly different from the glycocalyx layer associated with the pinacoderm of active specimens. In some individuals, micro-organisms were found adhering to the outer side of the cuticle which were absent from the surface of active specimens. In TEM, the cuticle appeared as a complex 2.5-3µm thick structure made up of three layers: a proximal dense layer (0.06-0.12µm), an intermediate amorphous layer (0.15-0.3µm), and an outer granular layer also of variable thickness (more than 2µm) which progressively disintegrated. Collagen debris appeared between the proximal and intermediate layers. The zone beneath this triple-layered cuticle was either completely devoid of cells or showed scarce degenerating cellular components (mainly from pinacocytes and spherulous cells), and sparse collagen fibrils. The choanosome appeared rather disorganised, with most choanocyte chambers disintegrated, with abundant phagocytosing archeocytes, sclerocytes, spherulous cells, degenerated cells and cell debris. Later in the season the cuticle appeared broken in many places. It was cast off and a new pinacoderm with ostia developed below; filtering activity of the sponges resumed. Spicules, previously protected by the cuticle, were uncovered, giving rise to a hispid phase. Subsequently the emergent spicules were cast off and smoothness of the sponge surface was restored. These changes in sponge cell structure and activity may be explained as reorganisation processes after reproduction, but other causes, such as adverse water temperature, may have similar effects. ❐ *Porifera, aquiferous system reorganisation, cuticle, glycocalyx, external surface, resting stage, SEM, TEM, fine structure, Crambe crambe, Demospongiae, reproduction, Mediterranean Sea.*

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External surfaces of sponges have an important role in the exchange of particles and gases between sponges and the environment. Pinacocytes are the main cells implicated in dermal structures of sponges (reviewed in Simpson, 1984), although spherulous cells can occasionally glide between exopinacocytes and temporarily remain on the surface of the sponge (Uriz et al., 1996; Willenz & Pomponi, 1996). However, non-cellular surface structures have also been described in several species of sponges. An external cuticle has been recorded in Dictyoceratida (Connes et al., 1971; Garrone, 1975; Donadey, 1982; Teragawa, 1986), Verongida (Vacelet, 1971), Chondrosiida (e.g. *Thymosia guernei* (Rosell, 1988; Boury-Esnault & Lopes, 1985; Carballo, 1994) and *Thymosiopsis cuticulatus* (Vacelet & Perez, 1998)), and Poecilosclerida (e.g. *Microciona prolifera* (Bagby, 1970)). When such a cuticle is present, the exopinacocytes either disappear or lose the ability to capture particles directly from the environment. When they

remain, their feeding depends on particles transferred by archeocytes.

It has been shown in several species of different taxonomic orders that both exopinacocytes and choanocytes produce an external layer of mucopolisaccharides, for example, *Oscarella lobularis* (Lévi & Porte, 1962), *Haliclona elegans*, *Chondrilla nucula* and *Hippospongia communis* (Garrone et al., 1971), *Hemimycale columella* (Willenz, 1981, 1982), *Ceratoporella nicholsoni* and *Stromatospongia norae* (Willenz & Hartman, 1989), and *Myceliospongia araneosa* (Vacelet & Perez, 1998). This glycocalyx is consistent along the surface of the pinacoderm, has a variable thickness and can have the appearance of a cuticle. It plays a role in the adhesion of external particles to cell surfaces, prior to their phagocytosis (Willenz, 1982).

In contrast to the well known function of glycocalyx coats, the nature and biological role of cuticles in sponges remains speculative. Cuticles have been seen as mechanisms that allow isolation of the sponge from the environment for cell repair or reorganisation, or for survival during adverse environmental conditions (Vacelet, 1971; Diaz, 1979). Another possible function is to get rid of harmful epibionts since this cuticle is periodically shed (Connes et al., 1971; Donadey, 1982).

The red, encrusting sponge *Crambe crambe* is a common species in the Mediterranean sublittoral. Its structure, biology, ecology and defense mechanisms are well known from several recent studies (reviewed in Becerro et al., 1997). This sponge reproduces in summer, with larval release occurring from the end of July until the end of August in the NE of Spain (Uriz et al., 1998). Here we describe the existence of a resting, non-feeding stage in *Crambe crambe* and compare its finestructure to that of active individuals.

MATERIALS AND METHODS

FIELD OBSERVATIONS. Field work was undertaken near Blanes (NE coast of Spain). Individuals in a resting stage were clearly discernible *in situ* from contracted normal individuals because their surface appeared glassy and in places showed a translucent, occasionally ridged, film. The extent of the phenomenon was determined by recording the number of individuals with a glassy surface in horizontal transects placed at random on vertical rocky walls (10m long x 1m wide, N=10). The number of specimens reproducing in 1998 was assessed by counting the number of individuals incubating larvae in the same study area. The timing and intensity of reproduction of this species in the area has been assessed regularly over recent years by monitoring both the number of individuals incubating larvae and the abundance of larvae in the water column (Uriz et al., 1998, and unpublished data).

FINE STRUCTURE. Specimens of *Crambe crambe* with different external appearance (normal, contracted, glassy and hispid) were collected from two Mediterranean localities: Calvi, Corsica (in winter 1982) and Blanes, NE coast of Spain (in winter 1993, and summer 1997 and 1998).

For transmission electron microscopy (TEM), specimens were fixed for 5hrs in 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer (osmolarity adjusted to 980mOsM with saccharose). Samples were washed several times in the buffer solution and postfixed for 90mins in 2% osmium tetroxide (OsO_4) in the same buffer, dehydrated in acetone series and embedded in ERL 4206 according to Spurr (1969). Ultrathin sections, double contrasted with uranyl acetate and lead citrate according to Reynolds (1963) were examined either with a Hitachi H-600 (University of Barcelona) or with an AEI transmission electron microscope (Université Libre de Bruxelles).

To obtain a contrast enhancement of the glycocalyx, ruthenium red was added for some samples (50mg/100ml) in each solution from glutaraldehyde to 70% alcohol (Garrone et al., 1971; Luft, 1971a, b, c; Willenz, 1982; Willenz & Hartman, 1989; Hartman & Willenz, 1990). The fixation time was threefold for those samples and postfixation was performed in the dark (Willenz, 1982).

For scanning electron microscopy (SEM), samples were fixed in a 6:1 mixture of 2% osmium tetroxide (OsO_4) in sea water and a saturated aqueous solution of mercuric chloride ($HgCl_2$) for 90mins (Johnston & Hildeman, 1982). They were then cryofractured in liquid nitrogen, thawed in amyl-acetate at ambient temperature, critical point dried from carbon dioxide, mounted and sputter-coated with gold following standard procedures, and finally examined with a Hitachi-2300 scanning electron microscope (University of Barcelona).

RESULTS

FIELD OBSERVATION. When observed in situ, *Crambe crambe* usually has a clean, smooth surface with conspicuous inhalant and exhalant

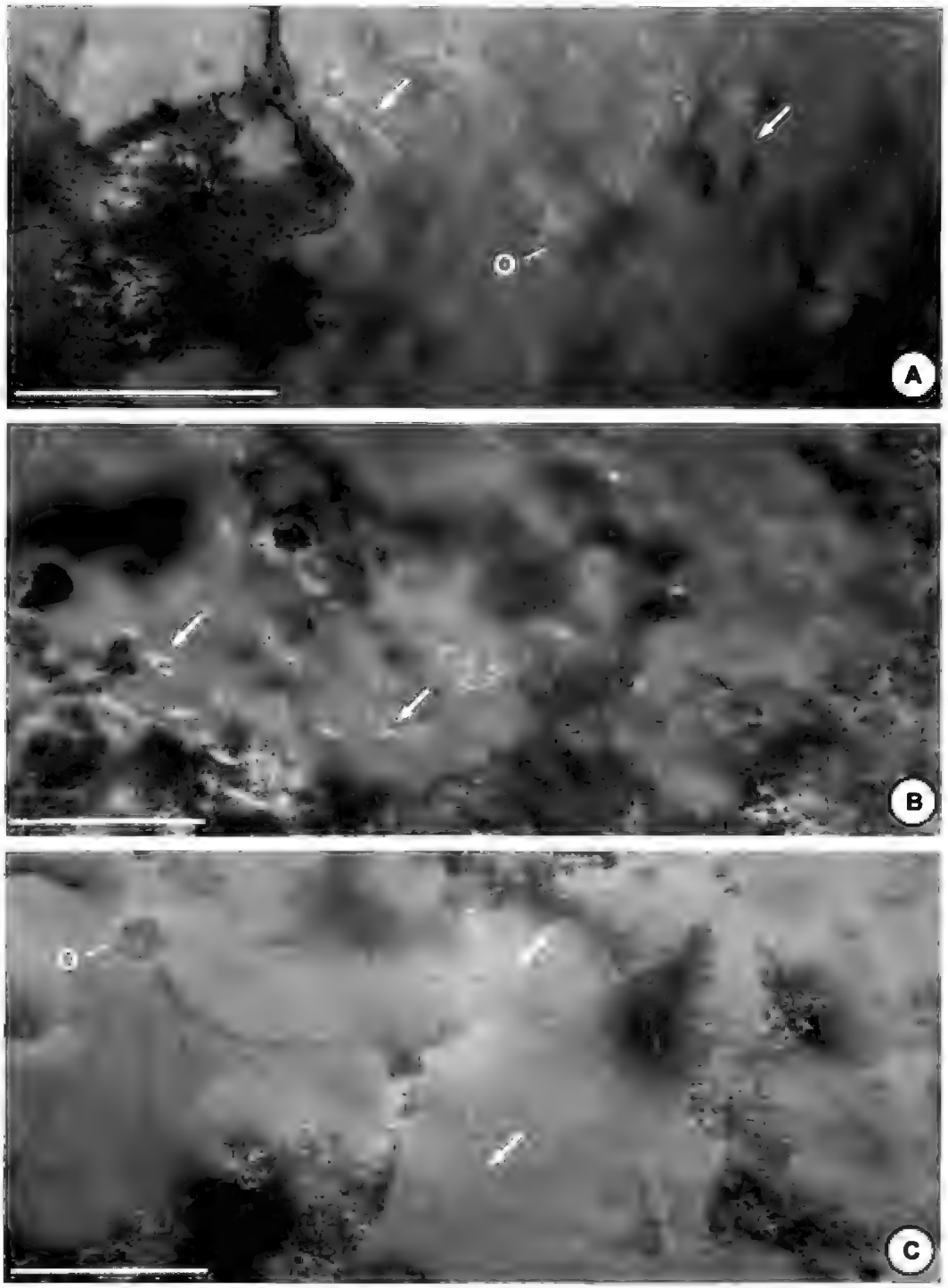

FIG. 1. *Crambe crambe*. A, Active specimen seen in situ. Arrows indicate prominent exhalant canals. O = Osculum. (Scale bar = 5cm). B, Inactive specimen seen in situ. Arrows indicate glassy surface. Oscula are sealed. (Scale bar = 2cm). C, Hispid specimen seen in situ. Arrows indicate debris entangled with spicules. O=Osculum. (Scale bar = 2cm)

orifices and prominent exhalant canals (Fig. 1A). However, in the Blanes littoral, 5% to 20% (in 1997 and 1998, respectively) of the *Crambe* population appeared strongly contracted and with a glassy surface from mid-August to the end of October (Fig. 1B). Specimens were covered by a translucent film. During that stage no inhalant orifices or oscula were perceptible and sponges seem unable to filter water or to feed. In October-November, the surface of some specimens was covered by noticeable amounts of particulate matter and debris (Fig. 1C), contrasting with the usually clean and smooth surface of this species. Upon closer observation, abundant spicules protruded from the surface of these specimens and retained debris. Unfortunately, no data on the percentage of specimens in the hispid phase could be gathered.

FINE STRUCTURE. *Active individuals.* SEM examination of active, water-pumping sponges showed a smooth surface, perforated by abundant ostia (Fig. 2A). No micro-epibionts were present on the sponge surface. The ectosome was rich in collagen fibrils, collencytes and spherulous cells. The exopinacoderm was covered by a dense layer of acid mucopolysaccharides (glycocalyx), 0.25-0.35µm thick, which exhibited a fibrillar structure (i.e. fibrils perpendicular to the cell surface traversed by two more dense bands parallel to the cell surface; Fig. 2B). This structure was enhanced by the ruthenium red staining.

The choanosome was formed by small choanocyte chambers with 7-13 choanocytes, as seen in histological sections, archeocytes, sclerocytes, spherulous cells and endopinacocytes. A central cell was often visible in the choanocyte chambers (Galera et al., in press). Choanocytes had a basal nucleus which often protruded from the cell, only covered by the cell membrane. They produced a conspicuous glycocalyx around the base of the flagellum (Fig. 2C). Transverse sections also revealed acid mucopolysaccharide secretions filling the space between collars, as well as extending from the flagellum (Fig. 2D). Conversely, the endopinacocytes did not appear to produce any glycocalyx.

Inactive individuals. SEM examination of the sponge surface during the non-pumping period confirmed the absence of ostia and revealed the presence of an acellular cuticle (Fig. 3A-B), as well as the almost total absence of an exopinacoderm. In TEM, the cuticle appeared as a complex 2.5-3µm thick structure, comprising three layers (Fig. 3C, E): a proximal dense layer (0.06-0.12µm), an intermediate amorphous layer (0.15-0.3µm), and an outer granular layer (2µm or more) in different stages of disintegration. Collagen debris appeared intermingled between the proximal and the intermediate layers. The region below this triple-layered cuticle was either devoid of cells or showed scarce degenerating cellular components (mainly pinacocytes and spherulous cells), and sparse collagen fibrils. The choanosome appeared rather disorganised; some choanocyte chambers were still recognisable, while others were severely disintegrated (Fig. 4A). Archeocytes, sclerocytes, spherulous cells, degenerated cells and cell debris were abundant, and phagocytosis by archeocytes was common (Fig. 4B).

At more advanced stages, the cuticle appeared broken in many places. Field observation and SEM images indicated that the cuticle was being cast off, with a new pinacoderm developing below. Spicules, previously covered by the cuticle, became exposed, giving rise to a hispid phase (Fig. 3D). New functional ostia developed on the dermal membrane and water pumping activity resumed. We assume that protruding spicules were expelled, as the sponge surface recovered its normal smooth appearance.

DISCUSSION

The resting stage of *Crambe crambe*, characterised by the presence of a well developed cuticle covering a disorganised choanosome, is followed by the formation of a new pinacoderm, the rejection of the cuticle and reorganisation of the choanosome. This sequence may be related to a reconstruction of the sponge canal system after larval release. Similar ultrastructural changes have been considered to be a reorganisation process after damage or reproduction in *Suberites massa* (Diaz, 1979). In *Crambe crambe*, however, these morphological changes do not occur in all specimens every year. This phenomenon was particularly intense during the last two years (1997 and 1998), although it had been occasionally observed before 1997. In 1997 it affected only an estimated 5% of the population at one time whereas 50% of individuals were engaged in sexual reproduction (authors, unpublished data). In 1998 the percentage of specimens going through reproduction was below one third of that in previous years and the number of released larvae was exceptionally low (3-5 larvae per m^3 of water vs. more than 200 larvae per m^3 of water seen in previous years).

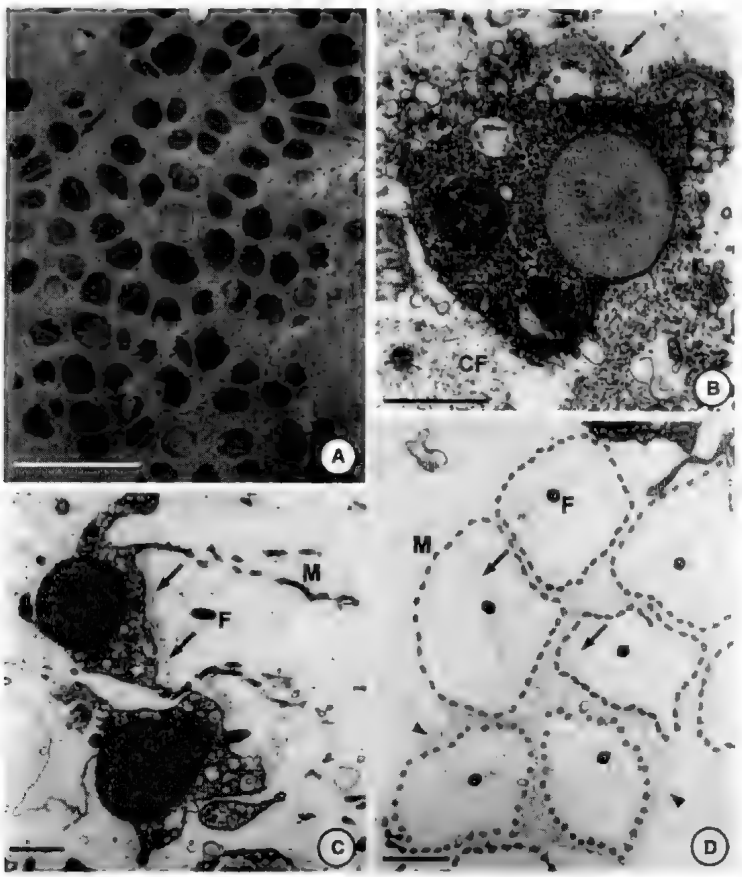

FIG. 2. *Crambe crambe*. A, SEM view of active specimen shows smooth surface and abundant wide open ostia (arrows). (Scale bar = 200μm). B, Active stage. Cross section of pinacocyte covered with dense glycocalyx (arrow). CF = collagen fibrils. (Scale ba r = 1μm). C, Active stage. Cross section of choanocytes with basal nucleus (N); arrows indicate well developed glycocalyx; F = flagellum; m = collar microvilli. (Scale bar = 1μm). D, Active stage. Transverse section through periflagellar collar. Muchopolysaccharide material (arrowheads) fills the space between collars and forms lateral expansions of the flagellum (F); m = collar microvilli. (Scale bar = 1μm).

Nevertheless, up to 20% of the population went into resting stages and related morphological changes. Environmental factors other than reproduction may be triggering this phenomenon. During summer 1997, the water temperature in the Mediterranean area was the highest of the past 25 years. In contrast, summer 1998 was characterised by a sharp drop of water temperature in the first half of August (from 25°C-19°C), just at the onset of larval release, caused by deep cold water mixing with shallow warm waters. It may be possible, therefore, that the resting stages develop not only in response of remodelisation following the process of sexual reproduction, but also as an effect of water temperature abnormalities.

There are no essential differences between the structure and thickness of permanent cuticles (Vacelet & Perez, 1998), and transient cuticles reported here and by Vacelet (1971) and Donadey (1982). Both permanent and transient cuticles are directly in contact with collagen fibrils and seem to replace the pinacoderm. When the transient cuticle is cast off, some collagen fibrils are also lost, along with cellular debris. Most sponges appear to be able to form a cuticle, for instance, *Aplysina* spp, *Microciona prolifera* and *Stelletta grubei* (Bagby, 1970; Vacelet, 1971; Boury-Esnault, 1975; Simpson et al., 1985). Other sponge species that exhibit the glassy appearance of resting stages mostly in autumn-winter, are *Spongia officinalis, S. agaricina, Ircinia fasciculata, Cacospongia scalaris, C. mollior* (authors, personal observation). Factors triggering resting stages are still poorly known, but seem to be related to adverse environmental conditions, such as insufficient water current (Vacelet, 1971), or strong water temperature variations (this paper), remodelisation after sexual reproduction (this paper), or injury (Diaz, 1979).

Most cuticles are described to last for relatively short periods of time. The biological functions of transient cuticles remains speculative. In keratose sponges, cuticles are periodically shed possibly to eliminate harmful epibionts (Vacelet, 1971; Donadey, 1982). Permanent cuticles were only rarely reported (Vacelet & Perez, 1998). Their role remains unknown, but they may help maintain the internal milieu of sponges containing a high density of symbiotic, extracellular bacteria, and separate it from the surrounding water.

Sponges isolate themselves from their

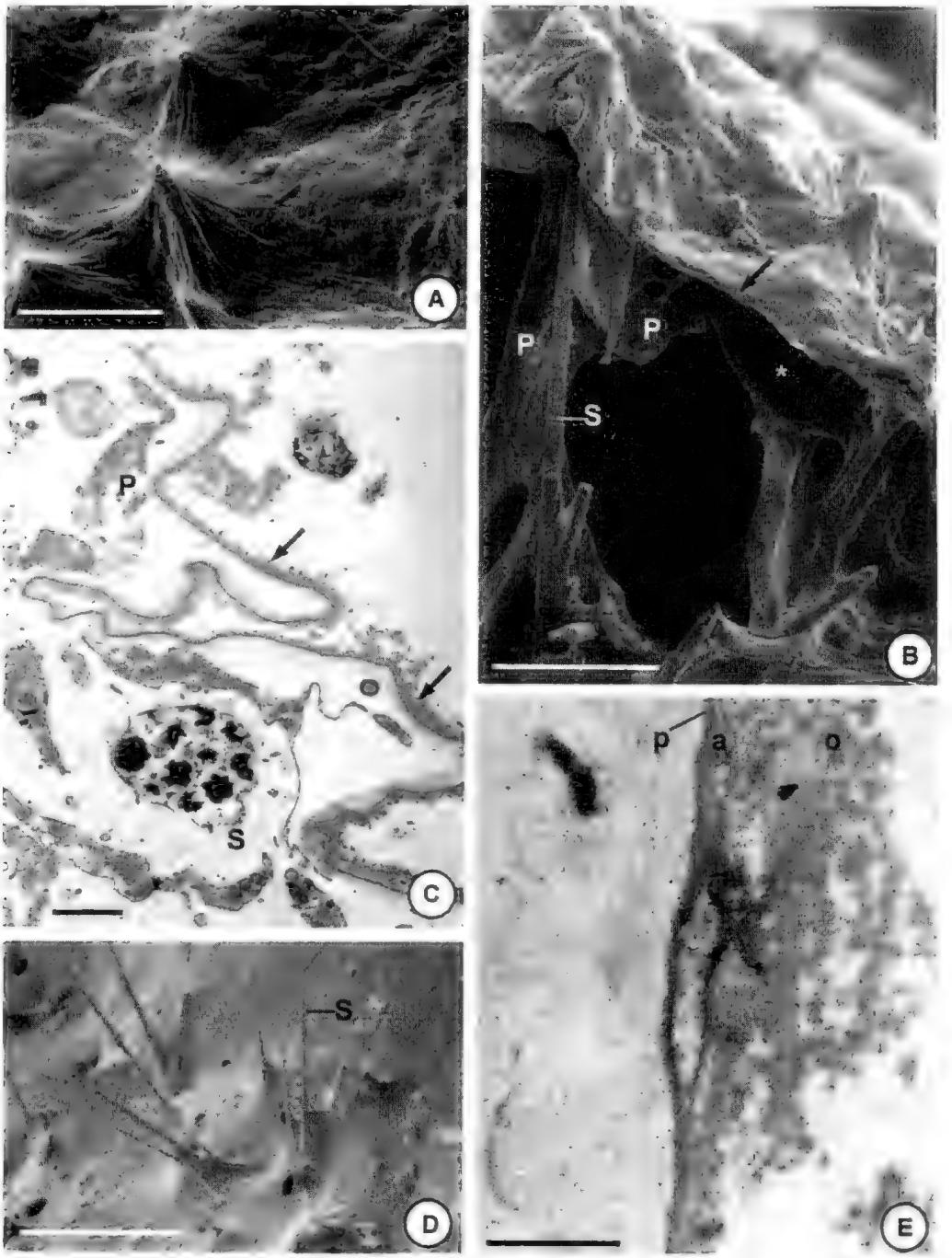

FIG. 3. *Crambe crambe*. A, Resting stage. Sponge surface devoid of ostia and covered by acellular cuticle. (Scale bar = 50μm). B, Resting stage. Cryofracture shows large empty space (asterisk) underneath cuticle (arrow). Fragments of pinacocytes (P) are attached to spicules (S). (Scale bar = 50μm). C, Resting stage. Cross section through cuticle (arrows) with both degenerating pinacocytes (P) and spherulous cells (S). (Scale bar = 10μm). D, Hispid stage. Detail of sponge surface with protruding spicules (S). (Scale bar = 100μm). E, Resting stage. Detail of a cross section through cuticle with triple layer. p = proximal dense layer; a = amorphous intermediate layer; o = outer layer. (Scale bar = 1μm).

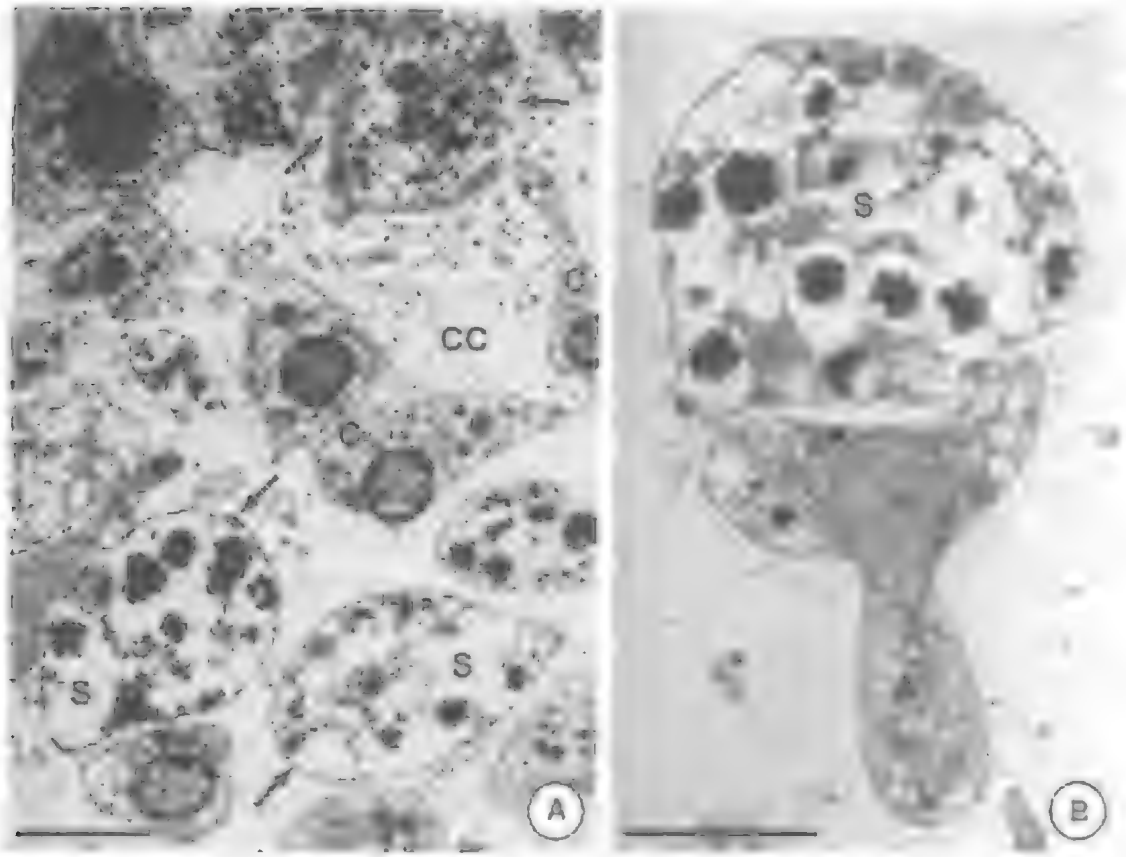

FIG. 4. *Crambe crambe*. A, Resting stage. Choanosome with disintegrated choanocyte chamber (cc) and degenerating cells (arrows); C = choanocytes; S = spherulous cell. (Scale bar = 10μm). B, Resting stage. Degenerating spherulous cell (S) in early stage of phagocytosis by archeocyte (A). (Scale bar = 10μm).

environment on many occasions. They deposit condensed collagen fibrils around harmful inhabitants such as Polychaeta, Cirripedia or Amphipoda (Connes, 1967; Sube, 1970; Connes et al., 1971; Uriz, 1983). The presence of micro-organisms adhering to the transient cuticle of resting specimens of *Crambe crambe* supports the protective role of this structure, isolating the sponge from the environment. In active specimens, the surface is usually free of bacteria (Becerro et al., 1994), owing to spherulous cells containing antimicrobial metabolites which cross the pinacoderm (Uriz et al., 1996). The chemical composition of these 'resistant' barriers is still unknown, but it is likely to be of a proteinaceous nature because collagen debris is visible in some places in the proximal layer. Furthermore, as shown here, it reacts similar to collagen and spongin if subjected to different stains (Vacelet, 1971).

The production of a dense cuticle allowing the sponge to become temporarily isolated from the environment may be a more widespread protective mechanism in sponges than previously thought (Bagby, 1970; Vacelet, 1971). The formation of temporary glassy pellicules is not restricted to sponges. It has also been described from colonial ascidian species belonging to different families (Turon, 1988, 1992), where it was interpreted as a product of a periodic rejuvenation process when the filtering thoraxes of the zooids are being replaced. Glassy cuticles are also reported in some cnidarian species (Garrabou, 1997) where they have been interpreted as protective structures to overcome adverse conditions (summer). Temporary isolation through production of an acellular cuticle may, therefore, be a common mechanism among soft, sessile invertebrates, allowing for survival during periods of adverse conditions or internal reorganisation.

ACKNOWLEDGEMENTS

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PHYLOGENETICS OF THE EURETIDS (EURETIDAE: HEXACTINELLIDA). *Memoirs of the Queensland Museum 44: 626. 1999*:- A long overdue phylogenetic analysis using 25 characters from 20 OTU's and two outgroups reveals several monophyletic groups within the Euretidae (Hexactinellidae) which may merit sub-family designation. The ordinal separation of Clavularia and Scopularia is disregarded on the basis of *Bathyxiphus subtilis* Schulze and *Claviscopulia furcillata* which contain both clavule and scopuline spicules. This analysis also suggests that an ancestral euretid closely resembling *Farrea occa* Schulze possessed a pre-scopuline spicule and that microsclere spicules and body form are prone to homoplasy and are therefore not stable phylogenetic indicators. This preliminary analysis will hopefully stimulate further research and interest into hexactinellid phylogeny and biology. ❐ *Porifera, Hexactinellida, Euretidae, classification, spicules, phylogenetics, homoplasy.*

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PLANKTONIC ARMOURED PROPAGULES OF THE EXCAVATING SPONGE *ALECTONA* (PORIFERA: DEMOSPONGIAE) ARE LARVAE: EVIDENCE FROM *ALECTONA WALLICHII* AND *A. MESATLANTICA* SP. NOV.

JEAN VACELET

Vacelet, J. 1999 06 30: Planktonic armoured propagules of the excavating sponge *Alectona* (Porifera: Demospongiae) are larvae: evidence from *Alectona wallichii* and *A. mesatlantica* sp. nov. *Memoirs of the Queensland Museum* **44**: 627-642. Brisbane. ISSN 0079-8835.

The armoured propagules of the excavating sponges *Alectona* and *Thoosa*, which are the only stage of reproduction of sponges consistently observed in full planktonic conditions, have been interpreted as asexual because of their unique morphology among sponge larvae. Evidence for their sexual origin is presented by new data for *Alectona wallichii* and a new species from the deep Atlantic, *A. mesatlantica* sp. nov. Stages in spermatogenesis and oogenesis are present at the same time as embryos in various stages of development, from early segmentation to advanced embryos possessing a discotriaene and style skeleton. Thick collagen strands surround the blastomeres from the beginning of segmentation. Collagen strands and spicules appear when the embryo cells are still undifferentiated. The larva is unique among Demospongiae in lacking surface flagella and having a larval skeleton, including spicules that are unknown in the adult. The name 'hoplitomella' is proposed for this special larva of alectonid sponges. The uniqueness of its sexual development and the tetraxonial nature of discotriaenes in larva of *Alectona* both indicate that these sponges do not belong to Hadromerida. A distinct family Thoosidae is supported for the genera *Thoosa*, *Alectona* and *Delectona*, although the family is considered presently as *incertae sedis* within Demospongiae. ❐ *Porifera, Demospongiae, Alectona wallichii, Alectona sp. nov., sexual reproduction, embryology, planktonic larva, excavating sponges, biocrosion.*

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The armoured propagules of the excavating clionid sponges *Alectona* Carter, 1879, and *Thoosa* Hancock, 1849, are intriguing from several points of view. These sponges produce unique reproductive bodies having a planktonic stage, and possessing a special skeleton of long protruding spicules and a cover of flattened spicules. Their sexual or asexual nature is unclear, although they have most often been considered as asexual products because of their unusual morphology, with the name 'armoured gemmules' generally applied to them.

These reproductive bodies have unique characteristics among Porifera. Firstly, the occurrence of a special spicule skeleton in propagules of sponges, otherwise not present in adults, is exceptional. Although spicules may be present in larvae, they are usually juvenile spicules resembling those of the adult (Bergquist & Sinclair, 1973; Simpson, 1984), or differing only in spination (Brien, 1967). The only other examples of a special skeleton in sponge reproductive bodies occur in Demospongiae, in the form of a microsclere skeleton in gemmules of the fresh-water Spongillidae (De Vos et al., 1991), and in Hexactinellida in the form of a stauractine skeleton in trichimella larva (Okada, 1928; Boury-Esnault & Vacelet, 1994). Moreover, these spicules in *Alectona* are discotriaenes with a clear tetraxonid origin. Tetraxon spicules are diagnostic for Spirophorida and Astrophorida, and are absent from Hadromerida in which *Alectona* is usually classified. *Alectona* and *Thoosa*, and by extension the family Clionidae in which they are usually included, have been considered intermediary between monaxinellids and tetractinellids (Topsent, 1891).

Secondly, the 'armoured gemmules' are the only example of organised sponge propagules consistently observed in the plankton. Dispersal stages of sponges are probably, but not certainly, present in a fully planktonic environment given that some species, especially Calcarea, are able to colonise experimental substrates several kilometres from the littoral in open sea conditions (Vacelet, 1981). Although several examples of 'pelagic plasmods' (Trégouboff, 1942), which

were interpreted as fragments of various adult demosponges (Topsent, 1948), could actually have a role in sponge dispersal (Wulff, 1985, 1991), typical sponge larvae are almost never observed in the plankton, possibly because they are lecithotrophic with a short free life span normally spent near the bottom. The armoured propagules of *Alectona* were first described as a planktonic radiolarian (Karawaiew, 1896, 1897), and then identified as the larvae or gemmules described by Topsent (1903) in the living tissue of *Alectona* and *Thoosa* (Trégouboff, 1939, 1942). The area of Villefranche/mer in the Mediterranean, where *Alectona* and possibly *Thoosa* propagules have been observed, is especially rich in planktonic organisms and famous for the upwelling of deep-sea planktonic species (Trégouboff & Rose, 1957).

Planktonic armoured gemmules were the subject of controversy with acrimonious debate between the planktonologist Trégouboff and the spongologist Topsent. Trégouboff (1939, 1942, 1957) claimed that the skeleton, which is well-adapted to a pelagic existence, with protruding styles constituting flotation devices and a cover of discotriaenes protecting the mass of cells, develops during their planktonic life. The gemmules would mature in the plankton, the tetraxonial plates changing to monaxonial plates while amphiaster microscleres develop. The plates would then dissolved, while the styles progressively disappear, and the gemmules, having lost their special skeleton, supposedly fall to the bottom and metamorphose. Topsent (1941, 1948) maintained that the alleged stages in evolution were gemmules belonging to two different genera, i.e. *Alectona*, in which the plates are tetraxonial, and *Thoosa*, in which the plates are monaxonial. Topsent also questioned the usefulness of a heavy armour and special skeleton for a planktonic existence. The question has not been resolved, due to the rarity of observations that furthermore concern only part of the complete cycle. The evolution of the tetraxonial plates into monaxonial ones, and their progressive dissolution described by Trégouboff, appear rather unlikely; but the stages presumably belonging to *Thoosa*, according to Topsent, do not have the characteristic nodulose amphiasters of this genus.

The sexual or asexual nature of the 'armoured gemmules' also remained uncertain. They were first interpreted as sexual embryos (Topsent, 1903, 1904). However, their unusual morphology later inclined sponge biologists to the opinion that they were asexual (Topsent, 1920), and the name 'armoured gemmules' has been generally used, although they would be better referred to as 'armoured buds' (Simpson, 1984). In a modern cytological study Garrone (1974) concluded that their structure was clearly distinct from any type of sponge larvae or gemmules, but showed similarities with external buds.

I report here on some new data provided by specimens of a rare species from the Indo-Pacific, *Alectona wallichii* (Carter, 1874), recently rediscovered (Bavestrello et al., 1998), and here redescribed, and by a new deep-sea species, *A. mesatlantica* sp. nov., in which stages in reproduction demonstrate the sexual origin of these propagules.

MATERIALS AND METHODS

Specimens of *Alectona wallichii* from Hawaii were collected by the author while SCUBA diving in caves at Oahu, Hawaii. Rocky pieces of the walls were detached with a hammer and preserved in formalin.

Five specimens of the same species were collected excavating the skeleton of *Acropora sp.* on the outer reef flat of the 'Grand Récif', Tuléar, Madagascar. These were also preserved in formalin.

Specimens of *Alectona mesatlantica* sp. nov. were collected by means of the submersible 'Nautile' near St Peter & St Paul Rocks on the south Mid-Atlantic Ridge, at 2030m depth, during the 'SAINT PAUL' cruise in 1998.

Fragments of specimens were embedded in Araldite, either prior to or following desilicification in 5% hydrofluoric acid. Semi-thin sections were stained with toluidine blue. Polished sections including spicules and living tissue were obtained by sawing the embedded specimens with a low speed saw using a diamond wafering blade and wet-grounded with abrasive paper. The dissociated spicules were sputter-coated with gold-palladium, and observed under a Hitachi S570 scanning electron microscope (SEM). Observations with transmission electron microscopy were not possible due to the poor cytological preservation of specimens. Spicule dimensions are given as length/width.

Abbreviations: BMNH, The Natural History Museum, London; MNHN, Muséum National d'Histoire Naturelle, Paris; SME, Station Marine d'Endoume, Marseille.

SYSTEMATICS

Order **Incertae sedis**
Family **Thoosidae** Rosell & Uriz, 1997
Alectona Carter, 1879

Alectona wallichii (Carter, 1874)
(Figs 1-2, Table 1)

Gummina wallichii Carter, 1874: 252.
Corticium wallichii; Carter, 1879a: 353.
Alectona wallichii; Carter, 1879b: 496.

MATERIAL. HOLOTYPE: BMNH (not seen): Cape of Good Hope, South Africa, 146-182m depth. SPECIMENS: Unregistered: Shark Cove, on the NW coast of Oahu Island, Hawaii, 27.vi.1978, in a cave at 10m depth, coll. J. Vacelet. SME 1111(2), 1119(1-2), 1371(1), Grand Récif, Tuléar, Madagascar, 1971, outer reef flat, intertidal, coll. Brunel.

DESCRIPTION. *Morphology.* Specimens from Hawaii, collected from limestone rocks, excavate large, subspherical cavities, irregular in shape, 5-6mm diameter. Cavities are usually single, without the cateniform arrangement of chambers present in most clionids. The flesh is yellowish, with rather cartilaginous consistency, and contains numerous white ovoid or round embryos, 200-300µm diameter. Cavities communicate with the outside by tunnels (papillary canals) 1.5mm diameter, ending in papillary perforations of the same size. In tunnels, the sponge tissue is tougher than in chambers, and is organised around a central longitudinal cavity, 0.5mm diameter, with the large diactine spicules longitudinally arranged and with a strong concentration of amphiasters, similar to that described for *A. millari* Carter, 1879. The papillae, which are probably contracted, are grossly circular, without raised rim. They are closed at their outer end and no ostia or oscules are visible. Specimens from Madagascar were excavating on *Acropora* corals. The flesh is softer, whitish or yellowish, and the cavities are approximately 4-8mm diameter. Papillae were not observed.

Surface. In specimens from both localities, cavities show the characteristic pitted surface of excavating sponges (Rützler & Rieger, 1973), with subcircular depressions 50-80µm diameter (Fig. 1A, B). A few small perforations, 1-8µm diameter, irregularly dispersed among the pits, are probably due to boring Cyanobacteria or to fungi. The pits bear concentric lines and a secondary system of radiating lines (Fig. 1B).

Spicules (Figs 1C-H; 2A-C, E-F; Table 1). Smooth diactines, irregularly flexuous or bent at the centre, frequently with a swelling on the convex side of the central angle. Abnormal, monstrous forms are frequent. Ends are most often acerate, rounded in the thicker spicules. The large axial canal frequently makes an angle or a loop in the middle of the spicule, corresponding to the swelling. The smallest spicules often make a loop in the centre. A few spicules with two lateral actines have also been observed. Size: 280-490/10-28µm in Hawaii, 170-500/5-20µm in Tuléar.

Tuberculate diactines, of general shape similar to the smooth diactines, but wholly covered with button-like spines, 10-12.5µm diameter, regularly arranged along 12 longitudinal rows in which the tubercles alternate in adjoining rows. Shaft of the button smooth, enlarged surface covered by irregular tubercles. Spicules usually slightly bent; some flexuous or strongly bent in the centre. The large axial canal (0.6-1.2µm) frequently displays an angle or a loop in the middle of the spicule. A prominent axial filament is clearly visible on semithin sections after desilicification (Fig. 2D). A few tripod-like triactines are present. Size 380-500/40-50µm in Hawaii, 290-550/33-53µm in Tuléar.

Bumped or spinose diactines, are intermediary between smooth and tuberculate diactines. Size 350-560/22-30µm in Tuléar, absent in Hawaii.

TABLE 1: Spicule sizes (in µm) of the various known specimens of *Alectona wallichii*.

Spicules	Material					
	Hawaii	Tuléar 1111(2)	Tuléar 1119(1-2)	Tuléar 1371/1	Bavestrello et al. 1998	Carter 1874-79
Tuberculate diactines	380-500/40-50	290-310/33-35	320-390/30-38	470-550/50-53	678-713/70-93	805/62-125
Smooth diactines	280-490/10-28	290-350/11-20	170-410/5-20	430-500/18-20	468-631/21-38	-
Spinose diactines	-	350-370 /22-25	380/30	540-560/30 (rare)	-	-
Amphiasters	15-35	15-30	15-40	10-30	20-47/7-10	25/8.3
Discotriaenes	110-120	90-120	-	-	-	-
Styles	280-610/2-3	370-650/2-3	-	-	-	-

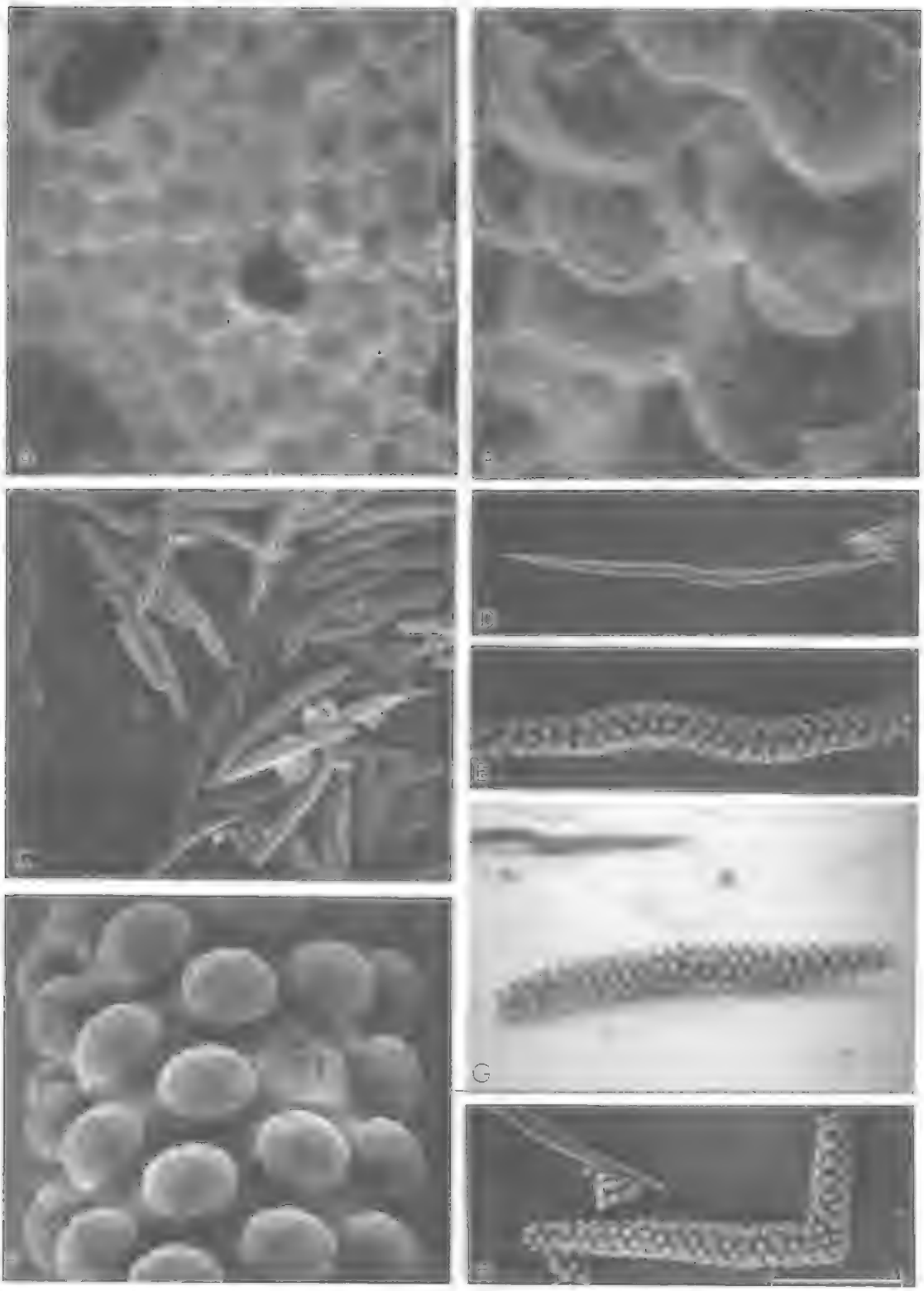


FIG. 1. *Alectona wallichii*. A-B, Pits on the surface of excavated scleractinian skeleton from Tuléar, showing a double system of concentric and radiating lines (Scale bar: A=152µm; B=51µm). C-H, Smooth and tuberculate diactines from Hawaiian specimens (Scale bar: C=374µm; D=126µm; E=126µm; F=19.6µm; G=136µm; H=142µm). Scale bar on bottom right.

Amphiasters, or more correctly sanidasteroid discorhabds, have short, blunt actines. Actines are generally equal and disposed in two whorls near the middle of the axis, but spicules with additional or unequal actines are frequent. Spicules are microspined, except in the central part of the axis between the two whorls of actines. A few small spicules, with an axis as short as 7.5μm, resemble the nodulose amphiasters found in the genus *Thoosa*. Size 15-35/2-2.5μm in Hawaii, 10-40μm in Tuléar.

Discotriaenes of the embryos have an irregularly circular outline, sometimes roughly triangular. Edge of the disc is slightly inwardly curved, with outer central part depressed. The inner surface bears a few small tubercles. The rhabdome is rarely acerate, most often with a blunt or inflated tip which may divide. An axial canal is clearly visible in the rhabdome, but cannot be traced in the disc. Size 110-120μm diameter with a rhabdome 20-30/8-10μm in Hawaii, 90-120μm in Tuléar (the latter observed in only a single specimen).

Styles of the embryos, are straight, slightly enlarged at some distance from the rounded end, with a large axial canal up to 1.2μm diameter. Two size categories are present in specimens containing advanced embryos: 280-360/1.5-3μm and 610-650/2-3μm, the former dominating in Hawaii and the latter in Tuléar.

The diactines are dispersed without order in the choanosome inside cavities. Walls of the papillary canals contain a greater concentration of longitudinally arranged tuberculate diactines (Fig. 2D). Amphiasters are dispersed throughout the whole tissue, although more numerous in papillary canals. The styles and discotriaenes are present only in embryos, the discotriaenes as an external layer, the styles as three fascicles made up of two spicules crossing in the centre of the embryo.

Living tissue. Living tissue of specimens from Hawaii is rather dense, with few canals (Fig. 3A-B). Choanocyte chambers are spherical, 14-20μm diameter. The mesohyl contains a large number of bacteria of the morphotypes usually found in bacteriosponges. The walls of the papillary canals are reinforced by dense, intertwined collagen fascicles containing elongated collencytes (Fig. 2D).

Specimens from Tuléar have the same general features as those from Hawaii, although their tissues are more poorly preserved. Choanocyte chambers are not visible. One specimen that has probably suffered from delayed fixation contains many rod-like bacteria, different in shape from the usual symbiotic bacteria.

Reproductive stages (Fig. 3A-F). Several stages of sexual reproduction are simultaneously present in the choanosome of most specimens. They are described here mostly from the Hawaiian specimens, which are better preserved and display more numerous stages.

Spermatogenesis occurs in spermatic follicles, which are spherical cavities, 25-40μm diameter, surrounded by a thin pinacocyte envelope. They contain densely stained spermatids or spermatocytes in various stages grouped as morulae at the centre of the follicle. In the most advanced stage observed (Fig. 3B), the spermatozoa are dispersed within the spermatic follicle. They have an elongated head measuring 2-3/0.5-0.6μm, and a long flagellum.

Oocytes are rare, with only two stages of their development observed. The youngest is a rounded cell lying in the mesohyl without a special collagen envelope, 20μm diameter, with the cytoplasm containing a few large inclusions, and a prominent, 6μm diameter nucleus containing a 2-3μm nucleolus. Another stage, which was observed only once in a thick section with poor definition, is 90μm diameter with an 18μm nucleus and a 4μm nucleolus. The cytoplasm contains large vitelline inclusions, and is surrounded by a follicular envelope of flattened cells and by a dense collagen envelope.

Embryos are spherical and uniform in size, approximately 200-320μm, regardless of their developmental stage (Fig. 3A). However, the most mature stages are slightly larger and elongated. Segmentation is total and equal. A four-cell stage has been observed, with apparently equal blastomeres 70-110μm diameter, filled up with heterogeneous vitelline inclusions 3-8μm diameter (Fig. 3C). Blastomeres divide without formation of a blastocoele. The early stages, up to the formation of the larval skeleton, are surrounded by a thin outer envelope made up of very thin, elongated cells, and by a dense inner layer, up to 10μm thick, made of collagen fascicles. Similar fascicles also individually wrap the blastomeres (Fig. 3C, D). This collagen development, which is highly unusual in sponge embryos, is maintained during the following stages. The most advanced embryos observed are made up of cells, 10-15μm size with inclusions 5μm diameter, which are widely dispersed in a loose collagen matrix also containing dispersed

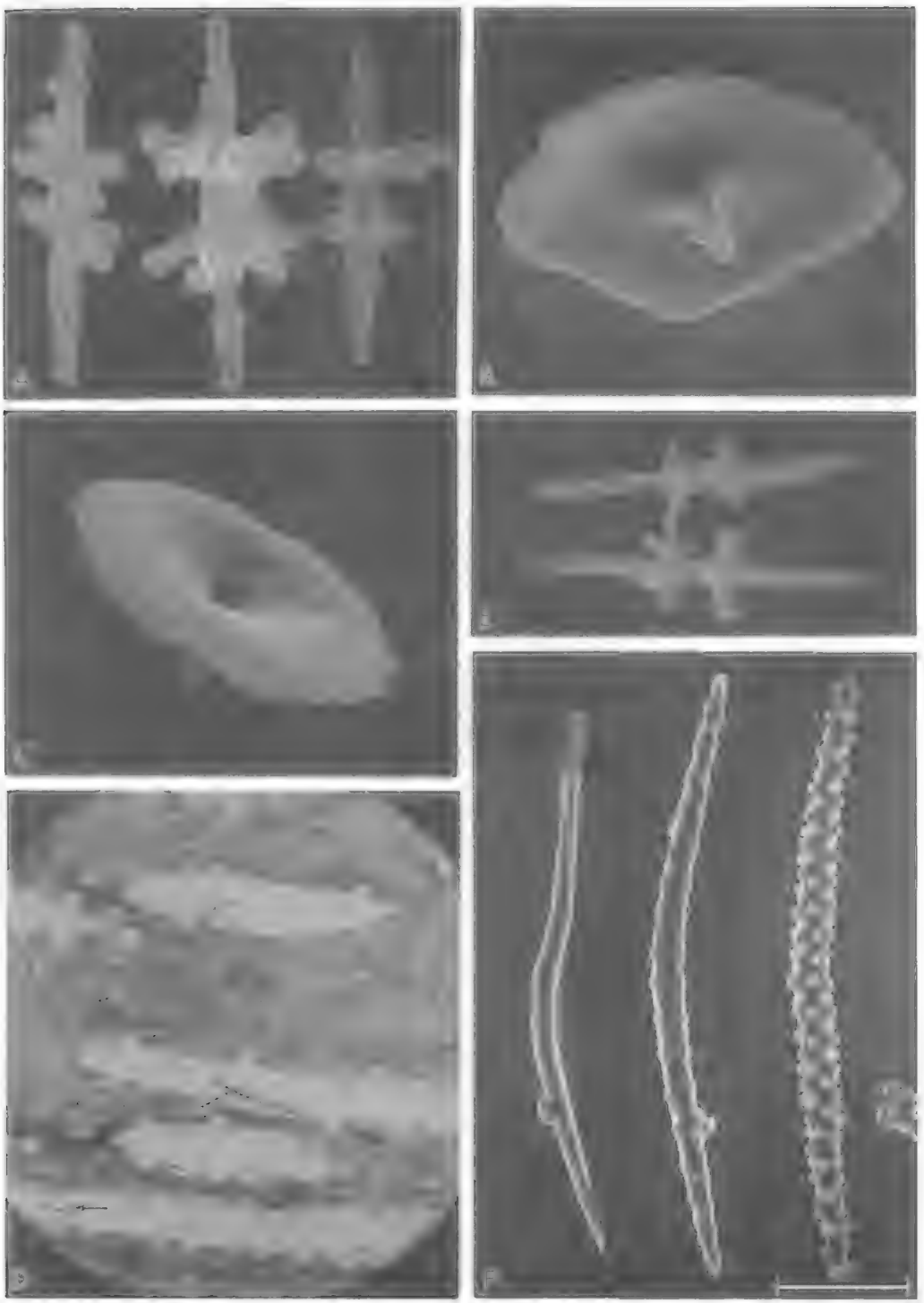


FIG. 2. *Alectona wallichii*. A-C, Spicules from Hawaiian specimens. A, Amphiasters. B, C, Discotriaenes (Scale bar: A=11μm, 6.2μm, 12.5μm from left to right; B=40μm; C=44μm). D, Semi-thin section through the papilla after desilicification, showing collagen strands, collencytes and axial filament (arrows) in the spicule ghosts (Scale bar=52μm). E-F, Spicules from Tuléar specimens. E, Amphiasters. F, Smooth, spinose and tuberculate diactines (Scale bar: E=15.8μm; F=86μm, 72μm, 98μm from left to right). Scale bar on bottom right.

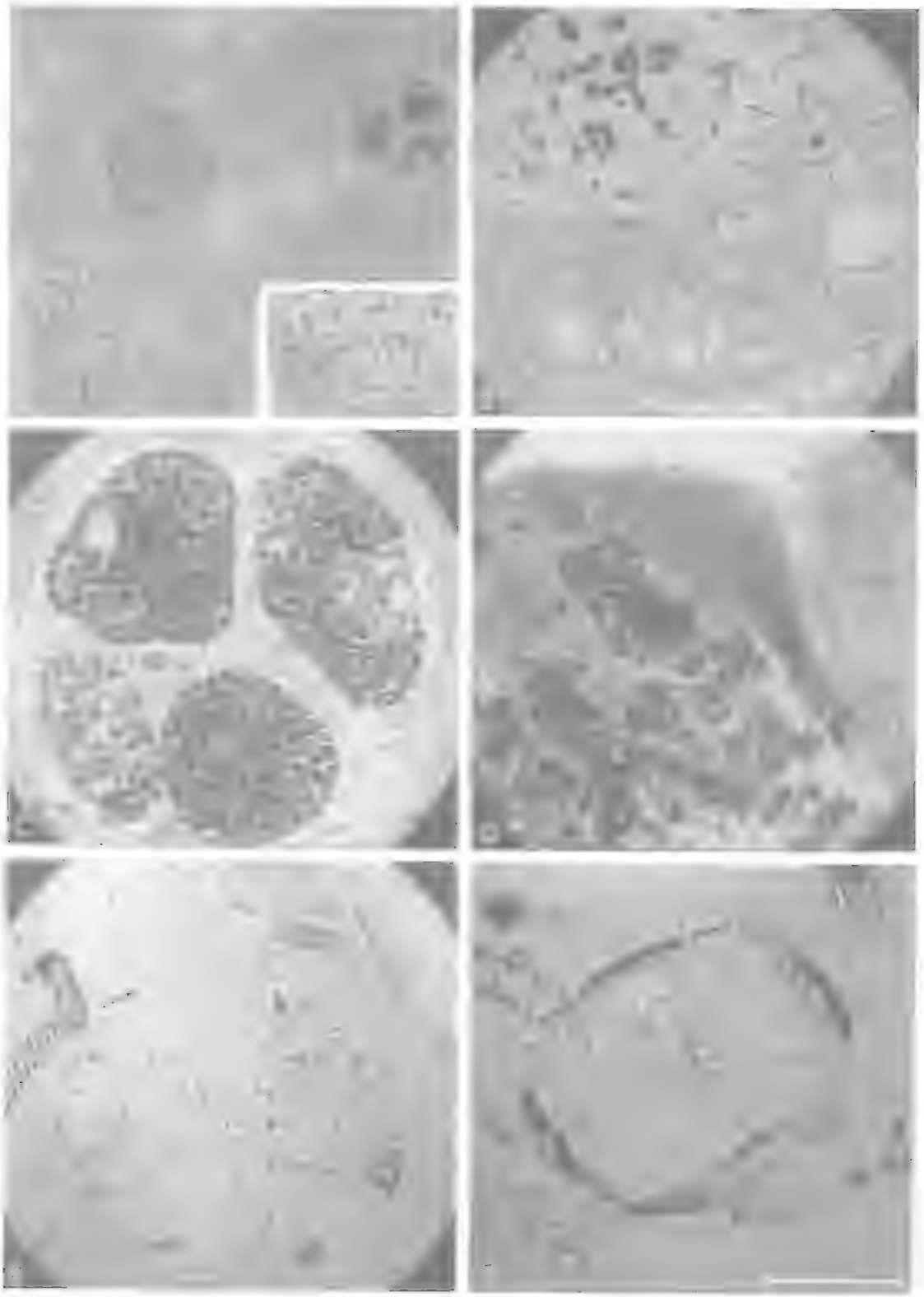
symbiotic bacteria. A group of elongated cells perpendicular to the embryo surface is observed near one pole of the mature embryo on a slightly prominent button (Fig. 3A). Most cells still appear to be undifferentiated. Contrary to the free-living larvae of *Alectona millari* (Garrone, 1974), these embryos possess neither choanocyte chambers nor spherulous cells.

The larval skeleton appears when the blastomeres have grown to approximately 20µm diameter and are still apparently undifferentiated. The three spicule categories appear simultaneously, first as very thin (1-1.5µm) styles and incomplete discotriaenes, in which the smaller disc observed attained only 20µm diameter (Fig. 3E). Amphiasters are recognisably the same as in later stages, but are less numerous than those found in the most advanced embryos. In advanced stages (Fig. 3F), the embryo is surrounded by a single layer of discotriaenes in which the shaft is inwardly directed. The cover is first discontinuous, then becomes continuous with a total number of approximately 12 discotriaenes. Styles, consistently six in number in each embryo, are disposed in three fascicles made up of two parallel spicules, which cross approximately at right angles to each other near the centre of the embryo. Amphiasters are mostly located near the discotriaene cover, although a few are dispersed within the internal tissue. The most advanced embryos observed had relatively short styles, which will probably eventually elongate and finally rearrange at one pole, as observed in *Thoosa* (Topsent, 1904), before being shed through the canals at later developmental stages.

Specimens from Tuléar also have several reproductive stages. Rare spermatic follicles were observed, with the same features as those seen in Hawaii. Embryos were mostly in early stages of development, with blastomeres 30-50µm diameter, and without spicules. They differ from those of Hawaiian samples by a greater development of the collagen envelope, which is up to 25µm thick and made up of intertwined collagen fascicles (Fig. 3D). The fascicles in between the blastomeres are also larger (approximately 10µm) than in Hawaiian material. Mature stages retaining a larval skeleton were not observed in sections, although they were present in one specimen, as indicated by the presence of larval spicules on dissociated spicule preparations (Table 1).

REMARKS. As summarised in Pang (1973, 1977), *Alectona wallichii* was originally described in the genus *Gummina* by Carter (1874), from isolated acanthodiactines collected from the Cape of Good Hope at 146-182m depth. These remarkable spicules were previously described by Bowerbank (1864), also as isolated spicules washed off corals. Carter (1879a) later succeeded in finding the whole sponge and its complete spiculation from the same skeletons of *Stylaster* studied by Bowerbank, and subsequently transferred the species to *Corticium*. He discussed its possible excavating habitus and gave three probable localities inferred from the known occurrences of the highly diagnostic spicules: the 'South Sea', Cape of Good Hope and the Seychelles. Carter (1879b) later transferred the species to *Alectona*, where it has been considered either as the type-species of the genus *Alectona* or as a synonym of *A. millari* (Laubenfels, 1936), although no additional material was known until the species was rediscovered in the NW Pacific by Bavestrello et al (1998). It is likely that *A. wallichii* was already present in the Early Miocene, as suggested by tuberculate spicules found in sediments from the W Atlantic figured by Wiedenmayer (1994).

Although some individual variations were observed between the various specimens from the Indian and Pacific Oceans (Table 1), the species appears to be well-characterised by its tuberculate diactines. These diactines are generally smaller in material from Tuléar (with the exception of specimen 1371/1, which has the largest megascleres). In all specimens, however, megascleres were smaller than in those recorded in the type-specimens by Carter (1874, 1879a) and in material from the NW Pacific (Bavestrello et al., 1998). The most important difference between material from Hawaii and Tuléar, however, is the presence of spinose or bumped diactines, which are intermediary stages between tuberculate and smooth diactines, in Tuléar specimens. The presence of these intermediary spicules in Tuléar specimens, which have few or no advanced embryos, could indicate that diactine production, first appearing as smooth spicules, is reduced in actively reproducing specimens. The discotriaenes and styles are present only in specimens containing advanced embryos.



Alectona mesatlantica sp. nov.
(Fig. 4)

MATERIAL. HOLOTYPE: MNHN D JV 63: Saint Peter & Saint Paul Rocks, mid-Atlantic Ridge, 00°94'N, 25°29'W, 2030m depth, 6.i.1998, coll. 'Nautile' submersible, R.V. 'Saint Paul' (sample SP13-16).

ETYMOLOGY. From *mes*, Greek, middle, and Atlantic, pertaining to the type locality on the Mid-Atlantic Ridge.

HABITAT. In deep water (2030m depth), near to Saint Peter & Saint Paul Rocks in the Equatorial Atlantic; excavating large cavities in calcareous rock of probable organic origin, encrusted by ferromanganese oxyhydroxides.

DESCRIPTION. *Morphology and living tissue* (Fig. 4A). The sponge is a fleshy mass growing in tunnels or in subspherical cavities, up to 5cm maximum dimension, single but irregular in shape. Communication with the outside is by a few papillary canals, 2-3mm diameter, ending in papillae of the same size with a single aperture, 1mm diameter. Colour is white in alcohol. The tissue is rubbery, compact with few aquiferous canals 2-3mm diameter. The mesohyl is typical of bacteriosponges, containing numerous, densely packed symbiotic bacteria. There are no visible cells with inclusions. Choanocyte chambers were poorly preserved, 22-30µm diameter. Aquiferous canals are lined by elongated cells aligned parallel to the canals, collagen fascicles and diactines.

The skeleton, composed of acanthodiactines and amphiasters, is developed only along the canals and near the border of the cavities. It is reduced and nearly absent in the choanosome. Styles and discotriaenes are found only in spiculate embryos.

Cavities display the characteristic pitted surface of excavating sponges, with subcircular pits 25-42µm diameter (Fig. 4B). Pits have an irregular surface, with concentric lines and secondary small holes. This structure is visible on all the components of the limestone construction, indicating that it is not related to the microstructure of the bored substrate.

Spicules (Fig. 4C-F). Acanthodiactines are of very irregular shape, usually with a swelling and bent in the middle, sometimes with a more-or-less developed third ray, or one of the two rays absent. Axial canal are large, frequently vesiculated or making a loop near the middle of the spicule or near the occasional branching of a third ray. Spines are acerate, short, irregularly distributed. Size: 410-530/21-28µm without spines.

Amphiasters, with a relatively thick axis, are microspined except near the centre of the axis, with rounded actines predominantly central, sometimes disposed in two irregular whorls especially in the shorter spicules. Size: 20-60/3-7µm. Amphiasters of the spiculate embryos have a thinner axis than those of the body.

Styles of embryos are very thin, flexuous, slightly enlarged at some distance from the rounded end, mostly broken on the slides. Size: up to 1125/4-5µm.

Discotriaenes of embryos have a short rhabd (20-40µm), frequently dichotomous or with lateral expansions. Disc is 130-150µm diameter, circular or slightly triangular, with the three branches of the axial canal clearly visible. Incomplete spicules, including a 'triaene' with free clads whose surface is irregular, were observed in embryos in the early stage of spiculogenesis.

A few large, irregular siliceous plates, probably of foreign origin, were observed in dissociated spicule preparations. Their position in the sponge tissue is unknown.

Acanthodiactines and asters are very rare or absent in the choanosome. They are more abundant near the walls of the excavation, and in the lining of the choanosomal and papillary

FIG. 3. *Alectona wallichii*. Stages in reproduction in specimens from Hawaii (except D, from Tuléar). A, Semi-thin section of desilicified tissue, general view of choanosome with young embryos at diverse stages in segmentation, and an embryo with ghost of discotriaene cover (arrow) enlarged in the inset (Scale bar=218µm, inset=142µm). B, Semi-thin section of desilicified tissue, nearly mature spermatic follicle and advanced embryo with ghost of discotriaene cover (arrows) (Scale bar=56µm). C, Semi-thin section of desilicified tissue, early stage in segmentation with collagen strands between the blastomeres (Scale bar=56µm). D, Semi-thin section of desilicified tissue, specimen from Tuléar, embryo with thick collagen strands (Scale bar=38µm). E, Non-desilicified thick section, early stage in segmentation (arrow) and three embryos with developing discotriaenes and styles; a fully developed discotriaene of a mature embryo on bottom left (Scale bar=200µm). F, Non-desilicified thick section, advanced embryo with discotriaene cover and three fascicles of two styles (Scale bar=132µm). Scale bar on bottom right.

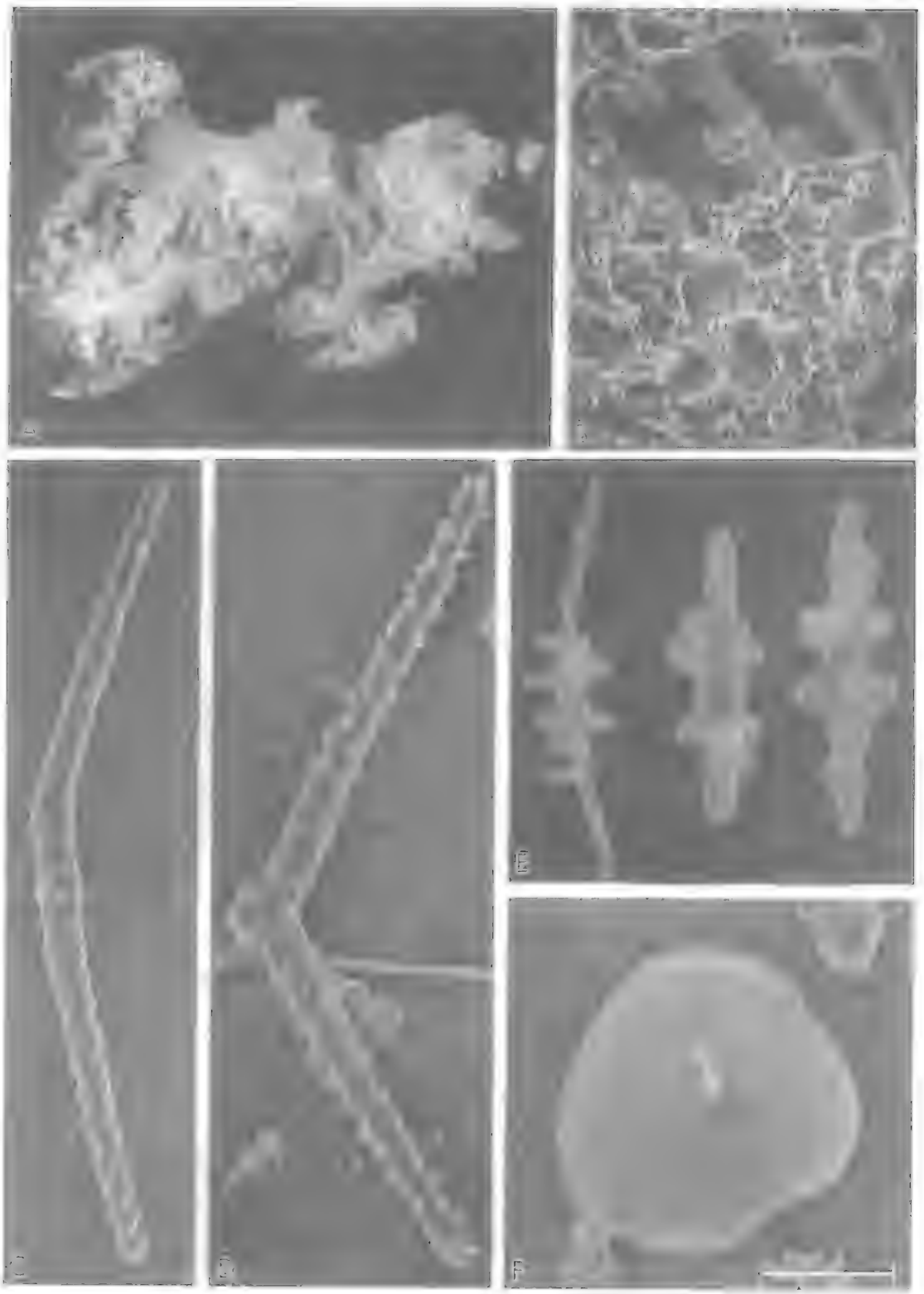


FIG. 4. *Alectona mesatlantica* sp. nov. A, Holotype (Scale bar=15.6mm). B, Pits on the excavated surface (Scale bar=64μm). C-D, Acanthodiactine (Scale bar: C=84μm; D=78μm). E., Amphiasters (Scale bar=20μm, 17.2μm, 17.2μm from left to right). F, Discotriaene (Scale bar=72μm). Scale bar on bottom right.

canals. Styles and discotriaenes are found only in advanced embryos, with the same arrangement as in *A. wallichii*.

Reproductive stages. The tissue contains numerous spermatic follicles, approximately 25μm diameter, at various stages of development ranging from a central mass of dense, 4μm diameter cells, to nearly mature spermatozoa with an elongated head 3/1μm.

The single stage of oogenesis observed was an oocyte with a poorly preserved cytoplasm, 40/20μm, with a nucleolate nucleus. Numerous ovoid embryos are present in the tissue. They display several developmental stages with a uniform size, 200-230/150μm. The earliest stage observed is a four-cell blastula with equal blastomeres, 100μm diameter, with numerous vitelline inclusions, 2-8μm diameter. These blastulae are surrounded by a thin envelope. Collagen strands, up to 35μm thick, are located between this envelope and the blastomeres. Thinner strands also wrap the blastomeres individually. Spicules first appear in embryos with an undetermined number of blastomeres, whose size is reduced to approximately 20μm, with inclusions smaller than 5μm. In these embryos the amphiasters are thinner than in the adult tissue, and the disc of the discotriaenes is incomplete, with the four axial filaments clearly visible. In the most advanced stages containing a complete larval skeleton, the blastomeres are smaller (approximately 10μm diameter), but apparently still undifferentiated and contain heterogeneous inclusions up to 4μm diameter. Intercellular bacteria are numerous in the embryo tissue. Discotriaenes are disposed as an outer cover over the embryo, and are surrounded by a thin tissue strand. Long styles are grouped either in a fascicle protruding from one pole into the maternal tissue, or in three perpendicular fascicles of two spicules.

REMARKS. The new species is characterised by the large size of its acanthodiactines, amphiasters with rounded spines, the unusually large cavities it excavates, the small size and peculiar aspect of the pits, and the distribution of megascleres which are virtually absent from the choanosome. The most closely related species appears to be the common North Atlantic-Mediterranean *A. millari* Carter, 1879, which bores smaller cavities with pits up to 100μm diameter, thus considerably larger than those of *A. mesatlantica*, which are smaller than 50μm, and whose diactines are smaller (Bavestrello et al., 1998) - although a Mediterranean specimen of *A. millari* has been recorded with tuberculate diactines 240-460/27-54μm (Pulitzer-Finali, 1983).

This is the deepest record for the genus. *Alectona millari*, previously considered to be a deep-sea species (Topsent, 1900), has now been found at 0.5m depth and is not recorded deeper than 1190m. The present species is also the deepest record for an excavating sponge after *Cliona levispira*, recorded at a depth of 2165m (Topsent, 1928b).

DISCUSSION

SEXUAL ORIGIN OF THE EMBRYOS. Observations made here have some similarities to those reported for *Thoosa armata* by Topsent (1904). Both these data demonstrate, in my opinion, that the 'armoured gemmules' are clearly larvae of sexual origin. However, this point is equivocal and requires further discussion because these are peculiar larvae, with characteristics generally found not in larvae, but rather in asexual gemmules or buds, and also because an asexual origin for 'larvae' of incubating demosponges has been alleged by several authors (Wilson, 1894; Sivaramakrishnan, 1951; Bergquist et al., 1970) – although their interpretation has been challenged and has never really gained general acceptance (Bergquist, 1978; Simpson, 1984). According to these authors, 'asexual larvae' may be found together with stages of sexual reproduction. The occurrence of sexual products (oocytes, spermatic follicles and indisputable embryos) simultaneously with the armoured bodies in *Alectona* is thus not full evidence that they are of sexual origin. However, considering the observed sequence it appears very unlikely that the armoured bodies would not be derived from the early segmentation stages. It may also be argued that oocytes are fewer in number than embryos, but this is a frequent phenomenon in incubating sponges. The presence of collagen fascicles between the blastomeres is highly unusual in sponge early embryos, as discussed below, but this is not a convincing argument for an asexual origin because collagen fascicles are also unknown between gemmular archaeocytes. Finally, the present data, particularly the observed sequence in development, leave no doubt as to the sexual origin of these reproductive bodies.

These embryos, however, display a number of unique peculiarities among poriferan larvae. 1)

They possess a larval spicule skeleton which is absent from the adult, a feature known only so far in the hexactinellid trichimella larva. 2) They are devoid of flagellae. 3) They have a strong development of collagen structures appearing at the end of the oocyte development and during the first segmentation stages. 4) They presumably have a long planktonic life with special flotation devices.

Such larvae clearly cannot be included in any other types of embryonic development in Porifera (Borojevic, 1970; Simpson, 1984; Fell, 1989), and the new term 'hoplitomella' (from *hoplites*, Greek, armoured, with heavy armour, and the suffix *mella*, used for some other types of sponge larvae), is proposed here. The hoplitomella is presently known only from the two genera *Alectona* and *Thoosa*.

MODE OF DEVELOPMENT. The early embryonic development of the hoplitomella larva is normal in that the first cleavages are equal and give rise to a solid stereoblastula, as in most incubating demosponges. However, a strong development of collagen occurs, both around the mature oocyte and in the embryo. The origin of collagen fascicles is not clear, and an ultrastructural study is needed to resolve this point. However, it is speculated that collagen fascicles surrounding the oocyte could be made either by the maternal sponge tissue, or by the oocyte itself. A synthesis of collagen fibrils by oocytes, which has been very rarely reported in animals (Wischnitzer, 1966), has been described in some oviparous demosponges (Gallissian & Vacelet, 1976; Watanabe & Masuda, 1990). The collagen envelope in early stages of the embryo may be derived from the oocyte envelope, as in the direct development of *Tetilla*, in which radiating fiber bundles of the egg surface are enclosed in the perivitelline space after fertilisation (Watanabe, 1978). This hypothesis, however, would neither explain the greater thickness of the embryo envelope, nor the presence of thick collagen fascicles between the blastomeres. It appears that in hoplitomella development the blastomeres are able to secrete thick collagen strands, even during the first cleavages. Although collagen fibrils are present in mature sponge larvae, they appear at a relatively late stage when most cell categories are already differentiated and they never form such thick, intertwined fascicles.

Another peculiarity is the early synthesis of the larval spicule skeleton, which also occurs at a time when most of the blastomeres are apparently still undifferentiated. The precocious appearance of siliceous spicules has been described in some demosponge embryos (Brien & Meewis, 1938; Fell, 1969; Simpson, 1984), but this occurs after differentiation of most cell types. It appears that the cells at the surface of the embryo, which in other sponges differentiate into a flagellated layer, differentiate here into discrete sclerocytes. As for precocious collagen synthesis, an ultrastructural study of the phenomenon would be of the greatest interest.

Differentiation of macromeres and micromeres, which is the subsequent step in embryonic development in other poriferans having a stereoblastula, resulting in differentiation of flagellated cells, appears to be skipped in this scheme. There is no early separation of a flagellated cell lineage. This separation probably occurs in a late developmental stage when the first choanocyte chambers differentiate in the free larvae, as described in *A. millari* (Garrone, 1974). Embryos observed here, as well as the free larval stages found in the plankton (Trégouboff, 1942; Garrone, 1974), are devoid of surface flagellae. This is again unique amongst the Porifera, where larvae are always more-or-less mobile due to the presence of flagellated cells, even in the creeping larvae of *Polymastia* (Borojevic, 1967). Flagellated cells are absent only in the development of *Tetilla*, whose zygotes develop directly without any larval stage (Watanabe, 1978). It remains to be confirmed, however, whether the mature larva is actually devoid of flagellae. The elongated cells grouped on a small protuberance in a pole of the embryo of *A. wallichii* (Fig. 3A) vaguely suggest a future small tuft of flagellae, although it has not yet been reported in free larva of *A. millari* (Trégouboff, 1942; Garrone, 1974).

After long debate, it has finally been convincingly demonstrated, both in Calcarea and in Demospongiae, that at metamorphosis the choanocytes of the young sponge may derive from larval flagellated cells (Amano & Hori, 1993, 1996, 1998). There is, however, evidence from other species for an absence of such a 'reversal of layers' (Misevic et al., 1990). The present observations, reporting the absence of flagellated cells in alectonid larvae, suggest that a direct lineage from larval flagellated cells to choanocytes cannot be retained in this development.

The absence of surface flagella, and consequently of swimming behaviour, is

surprising in these larvae which are the only sponge elements regularly found in full planktonic conditions. It might have been supposed that such larvae with a presumed relatively long pelagic life would have a well developed swimming apparatus. Although the swimming devices of sponge larvae do not allow significant movement, they could play an important role by changing the vertical position in the water column or for final microhabitat selection before settlement. Instead, the hoplitomella appears to rely on flotation devices produced by its long protruding styles and, at least at the stages which have been observed, is completely at the mercy of the currents. The possibility that a swimming apparatus will develop at the end of the pelagic life, assuring refinement in the choice of substratum as in other poriferan larvae, is unknown but cannot be excluded at present. The absence of surface flagella would exclude photonegative behaviour, which is nevertheless likely as most species of *Alectona* and *Thoosa* are sciaphilous. Whether or not this peculiar mode of larval life has some effect on their dispersal ability and geographic distribution remains to be investigated.

GALLERY SIZE AND PIT ORNAMENTATION. The shape, size, and organisation of the camerate borings of excavating sponges have been tentatively used in systematics of clionids and for identification of species of the ichnogenus *Entobia* Bronn, 1837, with an evident interest as indicators of palaeoenvironments (Bromley, 1970, 1978; Bromley & D'Alessandro, 1984; Pleydell & Jones, 1988; Bromley et al., 1990; Edinger & Risk, 1996). The chambers of the two species of *Alectona* appears, from present data, to be larger than in most clionids, especially those of *A. mesatlantica* which range up to 5cm diameter. Another difference is that these large chambers are simple, apparently without any growth stage in which they would be camerate or catenate as in clionids.

The possible use of the fine features of the pits for a correlation between extant species and ichnospecies of excavating sponges has yet to be explored. The genus *Alectona* appears to have a special ornamentation of the pits, with a double system of concentric and radiating lines whatever the nature of the substratum. This has been observed in the skeleton of the calcified calcareous sponge *Petrobiona massiliana* bored by *A. millari* (Omnes, 1991), and is confirmed here in *A. wallichii*. The small size and the peculiar ornamentation of pits in *A. mesatlantica*, whose spiculation is not very different from that of *A. millari*, appears as an interesting additional taxonomic feature. This demonstrates that characterisation of both Recent species and the ichnospecies of genus *Entobia* using microsculpture of the boring walls is worth considering.

SYSTEMATIC POSITION OF *ALECTONA*. The systematic position of *Alectona* and related genera (*Thoosa* and *Delectona* Laubenfels, 1936, possibly with *Thooce* Laubenfels, 1936 and *Anmandalia* Topsent 1928, which are probable synonyms of *Thoosa*) is puzzling. These genera are generally classified within Clionidae (Hadromerida) based on their excavating habit – a character of doubtful value given that it occurs within other orders of Demospongiae – and the presence of amphiasters that resemble those of *Cliothosa* Topsent, 1905, which undoubtedly belongs to the Clionidae (Topsent, 1928a; Rosell & Uriz, 1997). This classification has been questioned by several authors. Topsent (1891, 1900, 1928a) considered that the spinose diactines of *A. millari* were giant microscleres derived from oxyasters rather than from megascleres, and suggested that clionids, and in particular *Alectona*, were intermediary between Hadromerida and Tetractinellida, whereas Alander (1942) firmly classified *Alectona* and *Thoosa* in Tetractinellida. De Laubenfels (1936) suggested that Clionidae could be divided into two groups, possibly of the subfamily rank, i.e. *Cliona* and related genera, and *Thoosa* and related genera.

More recently, recognition of Thoosidae as a distinct family of Hadromerida has been proposed, first as Alectonidae (Rosell, 1996), then as Thoosidae (Rosell & Uriz, 1997). This distinction appears fully justified from my results. The presence of discotriaenes in larvae, to which may be added now the unique features of the sexual development, precludes their classification in Clionidae. These spicules are undoubtedly of tetraxonial origin, and thus also preclude the classification of *Alectona* in Hadromerida. Alternatively, they may be considered as an 'ancient adult character' (Jägersten, 1972), still present during embryonic development but disappearing in the adult during ontogeny. I will leave for the moment the family Thoosidae *incertae sedis*. Based on presently accepted criteria, its classification in Tetractinellida

would rest only on the presence of discotriaenes in larvae of *Alectona*, whereas the tetractinellid character is unclear in *Thoosa* and *Delectona*. Furthermore, the larval development is markedly different from both Tetractinellida and Hadromerida, which are oviparous. Possibly a new order will be needed for these sponges when additional data becomes available on their full life cycle, cytology and molecular sequences.

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FRESHWATER SPONGES FROM A NEOTROPICAL SAND DUNE AREA

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Volkmer-Ribeiro, C., Correia, M.M.F., Brenha, S.L.A. & Mendonça, M.A. 1999 06 30: Freshwater sponges from a Neotropical sand dune area. *Memoirs of the Queensland Museum* 44: 643-649. Brisbane. ISSN 0079-8835.

A survey of a freshwater sponge community from a sand dune belt area, NE coast of Brazil, is reported for the first time. *Corvoheteromeyenia heterosclera* was the only sponge living in crystal clear seasonal ponds nestled among the sand dunes. The sponge forms fan-shaped growths around the leaves of *Eleocharis* sp.(Cyperaceae) in the shallow border of ponds, or massive crusts on sporophytic plants of *Equisetum* sp.(Equisetaceae) in deeper parts of ponds. Towards the boundary, between sand dunes and savanna (cerrado), ponds are less subject to wind action, thus more stable and allowing the establishment of shrubby vegetation and palm trees. Accumulation of decaying vegetation produces brownish acid waters in which some of the five sponge species live, all characteristic of the savanna fauna: *Metania spinata, Corvomeyenia thumi, Dosilia pydanieli, Radiospongilla amazonensis* and *Trochospongilla variabilis*. An association between two of the five savanna species with *C. heterosclera* was observed in some ponds at the verge of the mobile sand dunes. This large ecotone seems to represent the 'patch concept' in the dynamics of its ponds and their sponge communities. ❐ *Porifera, freshwater ephemeral habitats, sand dunes, savanna, ecotone, survival, dispersal, adaptations.*

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The eastern part of Maranhão State, Brazil, is a coastal sand dune field containing an amazing array of seasonal freshwater ponds nestled among dunes, stretching along the municipalities of Tutóia, Barreirinhas and Humberto de Campos and penetrating 10-50km into the countryside where it borders with the 'cerrado' (Brazilian savanna). The National Park of 'Lençóis Maranhenses' was created in 1981 to protect the western part of this area. Under Koeppen's classification this region is classed as AW climate, with the rainy season between December-May, and the annual mean air temperature between 26.8-27.2°C.(Padua, 1983). Prior to the present work we knew nothing about the freshwater sponge fauna of this large seasonal lentic system, which appears to be unique in the world and certainly within the Neotropical Region. The study is part of ongoing investigations of the Neotropical freshwater sponge habitats and communities undertaken by the senior author and collaborators (Volkmer-Ribeiro, 1992; Volkmer-Ribeiro & Tavares,1993; Volkmer-Ribeiro et al., 1983, 1998).

MATERIALS AND METHODS

Our survey of freshwater sponges in the sand dune lentic system was undertaken at the E and W borders of the Lençóis Maranhenses, Maranhão State, Brazil (in proximity to the villages of Tutóia and Santo Amaro), with some sites inside the National Park, where sand dunes border with the savanna (Fig 1). In response to valuable information provided by local residents on current water levels (since dry and wet seasons do not occur exactly at the same period each year), our survey was conducted between 20-30 October 1995. At this time of year the largest ponds (approximately 1500m^2) contained only about 30% of their potential water capacity, which was considered an opportune time to sample because it was most likely that sponges would have gemmules.

Several variables were measured in some of the ponds in the western area (pH, temperature and salinity). The pH was taken in situ with Merck pH paper. Salinity was also recorded in situ with a salinometer. Samples of sediments were also

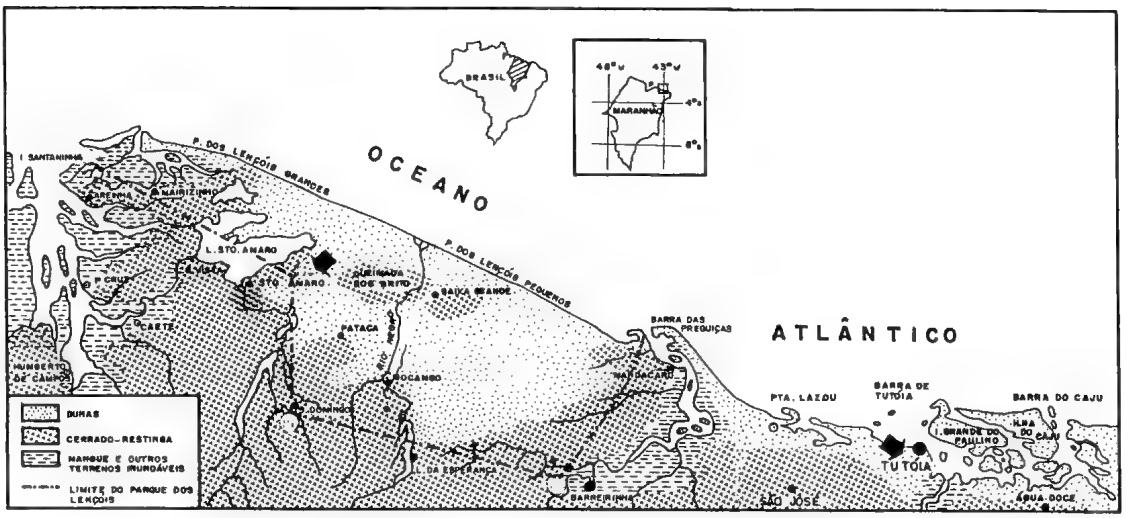

FIG. 1. The Lençóis Maranhenses. The villages of Tutóia and of Santo Amaro (arrows) are shown, at the E and W area of the Lençóis Maranhenses, respectively, as well as the National Park limits and the contact areas with the savanna.

collected from the top layer of pond beds, stored in small glass jars, for ponds in which sponges were not conspicuous. This was undertaken in order to determine, through the presence of spicule sediments, whether sponges had ever been present in these ponds (Volkmer-Ribeiro & Turcq, 1996). These sediment samples are also currently being used by the senior author to study recent and paleo sediments. Entire or cross-sectioned dry specimens of *C. heterosclera* were glued to a stub and gold-coated for scanning electron microscope (SEM) observations and photographed on a MCN JEOL-5200 microscope equipped with a Pentax SF7 35mm camera. Part of the dry specimens was deposited in the collection of Departamento de Biologia of Universidade Federal do Maranhão. The other part was deposited in the Porifera Collection of Museu de Ciências Naturais of Fundação Zoobotânica do Rio Grande do Sul (MCN/POR) with Catalog numbers listed in Tables 1 and 2.

RESULTS

A succession of ponds with different spatial areas (Fig. 2A-C) and water color was observed as one progressed from the mobile dunes towards the savanna boundary. Ponds situated among the

TABLE 1. Identification of the sponges sampled at the ponds from the E area of Lençóis Maranhenses, together with their environmental characteristics and substrates.

Ponds sampled around Tutóia Village	Type of sample	Environment	Substrate	Water color	Sponge species	Catalog number MCN/POR
Lagoa do Vidro	specimens	sand dunes	*Equisetum* sp	clear	*Corvoheteromeyenia heterosclera*	3829-3840
Lagoa da Ponta do Arpoador	specimens	sand dunes	*Eleocharis* sp	clear	*Corvoheteromeyenia heterosclera*	3830-3839
Lagoa da Pedra Hume	sediment	sand dunes		brownish clear	*Radiospongilla amazonensis*, *Trochospongilla variabilis*, *Corvoheteromeyenia heterosclera*	3854-3856
Lagoa da Ponta do Mangue	bottom sediment	oasis		brownish	*Radiospongilla amazonensis*, *Trochospongilla variabilis*	3859
Lagoa da Coceira	sediment	grassy field		black	*Corvomeyenia thumi*, *Metania spinata*	3858

FIG. 2. Study area. A, Lagoa do Vidro, at the E boundary of the Lençóis Maranhenses. B, Lagoa da Ponta do Arpoador, showing the stiff Cyperacean vegetation exposed to the sun as the pond becomes fully dry. C, Lagoa do Pico, at the western border of the Lençóis, with its surrounding dunes covered by bushy vegetation and palm trees (photos C. Volkmer Ribeiro).

mobile dunes had crystal clear waters whereas those approaching the savanna boundary had increasingly brownish color and acidic pH (Tables 1-2).

Lagoa do Vidro (Fig. 2A), Lagoa da Ponta do Arpoador (Fig. 2B) and Lagoa do Cajueiro are clear water ponds amongst the mobile dunes. The dominant plants in these ponds, as potential sponge substrates, were *Eleocharis* sp. and *Equisetum* sp. The former plant is a small but very abundant Cyperaceae which inhabits the shallow marginal area of ponds, and is the first to emerge from the water (Fig. 2B) as ponds begin to dry up. The second species is also relatively abundant in deeper parts of ponds, close to the leeward face of the barchan dunes (Fig. 2A).

Lagoa da Pedra Hume, Lagoa da Ponta do Mangue, Laguinho, Lagoa do Pico (Fig. 2C) and Lagoa da Coceira represent a succession of ponds having brownish clear to black water, and distributed from the mobile sand dunes into the almost flat, fixed dunes, covered with grassy or bushy vegetation. Lagoa da Ponta do Mangue is an oasis pond with surrounding patches or islands of palm trees and containing shrubby vegetation. Lagoa da Coceira was reduced to a fetid, tiny muddy pond with black water surrounded by grass. Laguinho was a relatively deep pond, and together with Lagoa da Ponta do Mangue, is a popular fishing site.

Taxonomic identification of the sponges sampled in the E and W dune areas are presented in Tables 1 and 2, respectively, together with their

TABLE 2. Identification of the sponges sampled at the ponds from the W area of Lençóis Maranhenses, together with their environmental characteristics and substrates.

Ponds sampled around Santo Amaro Village	Type of sample	Environment	Substrate	Water color	Sponge species	Catalog number MCN/POR
Lagoa do Cajueiro (pH 6; salinity 1%, water temperature 28.5°C at 10am)	Specimens & sediments	sand dunes	*Eleocharis* sp	clear	*Corvoheteromeyenia heterosclera*	3853
Laguinho	Specimens	sand dunes	stones	brownish clear	*Dosilia pydanieli*	3857
Lagoa do Pico (pH 5.5; salinity 0.5%, water temperature 32°C at 0.10pm)	Specimens & sediments	sand dunes with bushy vegetation	submersed branches of riparian bushes (*Crysobalanus icaco*)	brownish	*Metania spinata, Corvomeyenia thumi, Trochospongilla variabilis, Radiospongilla amazonensis*	3089

environmental characteristics and substrates. Ponds in which only sediments were reported indicate that living samples of sponges were not found. These tables indicate that *Corvoheteromeyenia heterosclera* and *M. spinata* were abundant, as were gemmules, particularly in the two extremes of the 'pond succession' (i.e. very clear ones and the brownish colored ones), confirming that the sampling period was well chosen and our prediction that this huge seasonal lentic system contained such a fauna.

The pond sponge communities also revealed interesting distribution patterns. The only species found in clear-water sand dune ponds was *C. heterosclera* (Ezcurra de Drago, 1974), with fan-shaped growths on the leaves of the Cyperaceae *Eleocharis* sp. (Fig. 3A) at the pond margins (Fig. 2B), or thick irregular growths on the sporophitic plants of *Equisetum* sp.(Fig. 3B) in deeper parts of the pond close to the leeward dune face (Fig. 2A). Sponges on both substrates were fully developed and full of gemmules. Many specimens were seen dried out, not far from the pond margin, their gemmules continually swept away by the wind. On the other hand specimens still submerged at the pond margins were already half buried by sand blown in from the dune, the same way as were specimens encrusting *Equisetum* located close to the dune inner wall. *Corvoheteromeyenia heterosclera* appears to be a species fully adapted to such an environment. SEM study of cross sections of *Eleocharis* leaves encrusted with this species (Fig. 3C) disclosed a skeletal structure of very slim fibers producing a very open and irregular network enclosing sand grains of variable sizes. The presence of oscular sieves (Fig. 3D) is another device used to prevent oscula being clogged with sand. Gemmoscleres were also seen to take part in skeletal fibers, together with megascleres and two categories of microscleres (Fig. 3E-F), which might be due to the accelerated production of gemmules during this time of the year. Living specimens of *C.heterosclera* had a light green color, probably due to its association with a microscopic green algae, and this green color could be seen around the pond margins where sponges had been buried and were decaying in the sand.

The sponge community towards the savanna boundary included one or more of the following species, but never all five of them: *Metania spinata* (Carter, 1881), *Corvomeyenia thumi* (Traxler, 1895), *Dosilia pydanielli* Volkmer-Ribeiro, 1992, *Trochospongilla variabilis* Bonetto & Ezcurra de Drago, 1973, and *Radiospongilla amazonensis* Volkmer-Ribeiro & Maciel, 1983. However, leaving this area and heading towards the clear-water ponds we observed associations of *C. heterosclera* with *R. amazonensis* and *T. variabilis*.

TAXONOMIC REMARKS. Ezcurra de Drago (1979) erected *Corvoheteromeyenia* for *Corvomeyenia australis* Bonetto & Ezcurra, 1966, and *C. heterosclera* Ezcurra de Drago,1974. The holotype of the first species comes from Laguna Setúbal, next to the town of Santa Fé, Argentina, whereas the holotype of the second species comes from NE Brazil, with no precise locality data. The distinction between the two species was based particularly on their respective gemmoscleres, which are composed of two categories differing in size and shape in the first species, and one category in the second species. Specimens from the Lençóis Maranhenses agree with the second species in spiculation and geographical origin. *Corvoheteromeyenia heterosclera* has also been collected from other sand dune or paleo sand dune areas in S Brazil (Volkmer-Ribeiro, unpublished data), and its spicular remains may be useful indicators of such environments.

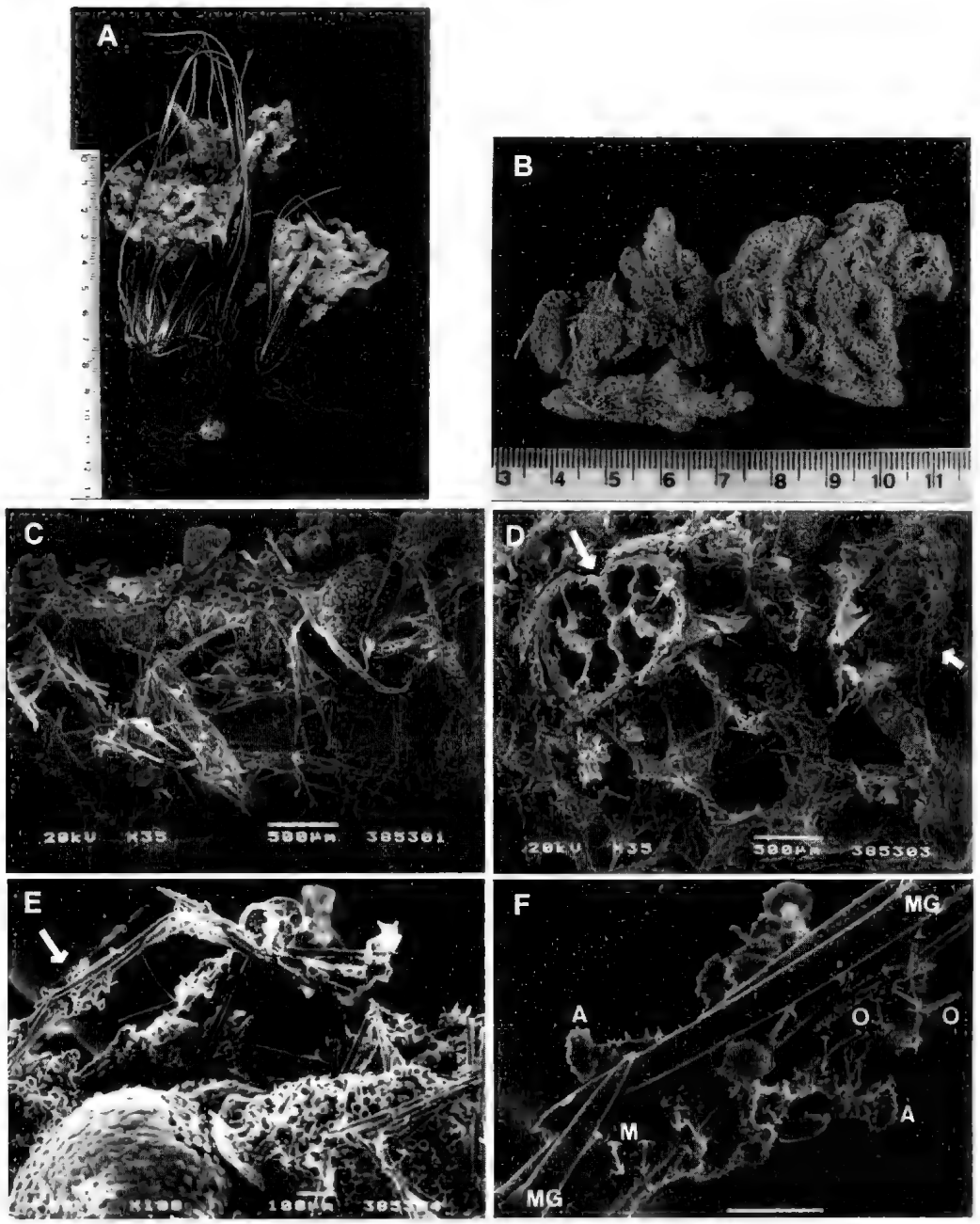

FIG. 3. Freshwater sponges. A, Fan-shaped specimens of *Corvoheteromeyenia heterosclera* full of gemmules growing on *Eleocharis* sp. B, Specimens of *Corvoheteromeyenia heterosclera* growing on *Equisetum* sp. C, Skeleton of *Corvoheteromeyenia heterosclera* with an open network where sand grains are enclosed side-by-side with the gemmules. D, Cross section of a leaf of *Eleocharis* sp. (arrow, upper left) encrusted by the skeleton of *Corvoheteromeyenia heterosclera*, showing an irregular distribution of the thin skeletal fibers and (arrow, upper right) the presence of an oscular sieve. E, Skeleton of *Corvoheteromeyenia heterosclera* with two gemmules in the process of completion at the bottom as well as abundant free gemmoscleres (arrow). F, Skeletal fiber of *Corvoheteromeyenia heterosclera* (see arrow in Fig. 3E) showing the reduced amount of spongin and the presence of the two categories of microscleres (O, microspined oxea; M, microanfidiscs) and several large anfidisc gemmoscleres (A) around the megascleres (MG).

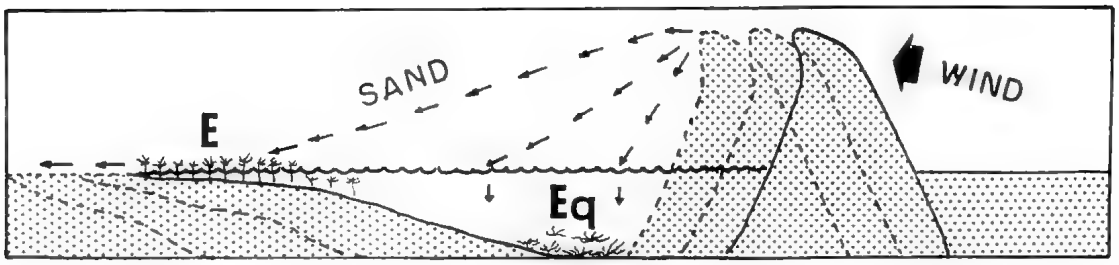

FIG. 4. Schematic drawing of the wind-driven dynamics that seasonal ponds around the moving dunes of Lençóis Maranhenses are subjected to, together with the vegetation and sponges they contain. E= *Eleocharis*, Eq= *Equisetum*.

DISCUSSION

Recent literature dealing with the study of Neotropical freshwater sponge communities has shown that the five species recorded in the present study, found in the ponds close to the savanna boundary, generally thrive in seasonal ponds N to S throughout the Brazilian savanna (Volkmer-Ribeiro, 1992; Volkmer-Ribeiro et al., 1998). Thus, two characteristic faunal assemblages are present: one in the savanna ponds with five species, and one which is monospecific and lives in the sand dune ponds.

Observations made here on the characteristics of *C. heterosclera* match well with our present understanding of the processes associated with constant disturbances imposed by dune movement on populations. The living barchan dunes, which move in the direction of the wind in coastal areas, is a well known geomorphological phenomenon (Termier & Termier, 1963). The Lençóis Maranhenses is, however, unique in its situation being in a tropical area with a marked rainy season, resulting in temporary accumulation of water in ponds on the leeward sides of moving dunes, with the ponds subsequently displaced next season. The wind, which was seen to blow the sand into the ponds, was also seen to blow ahead the gemmules from dry, exposed sponges growing on *Eleocharis* at the perimeter of ponds. Moreover, the erect position of plant leaves together with the fan-shaped morphology of the sponges appears to facilitate gemmule dispersal via wind action. These are the pioneers of new sponge populations, opportunistically waiting in place where the new border of the pond with *Eleocharis* will be situated next season. The same process applies to sponges encrusting on *Equisetum*, which will be passed over by the moving dune when again they will be set against the inner side of a barchan dune, protected against drought and ready to again form the *Equisetum/Corvoheteromeyenia* association (Fig. 4).

The region extending between the areas occupied by these two faunas thus biologically and physically defines a savanna/sand dune ecotone. This particular ecotone best fits the dynamic 'patch concept' (White & Picket, 1985), driven by constant disturbance through dune movement. The Lençóis Maranhenses is an outstanding example of the patch concept with its pond system, spatial and temporal relationships to dune movement, and dynamic sponge fauna. It also illustrates the remarkable ability of freshwater sponges to manage constant disturbance via asexual reproduction when gemmules produced by these sponges are especially selected mechanisms to withstand drought and to passively disperse via the wind, enabling all six species to opportunistically colonise new pond systems as they develop.

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BIOGEOGRAPHY AND TAXONOMY OF THE REEF CAVE DWELLING CORALLINE DEMOSPONGE *ASTROSCLERA WILLEYANA* THROUGHOUT THE INDO-PACIFIC. *Memoirs of the Queensland Museum 44: 650. 1999:-* *Astrosclera willeyana* Lister, 1900, is a pyriform-half spherical, predominantly bright orange coralline demosponge. The habitat of *Astrosclera* is generally restricted to cryptic and light reduced environments of the Indo-Pacific, found mainly in reef caves, but sometimes also in the dim-light areas of cave entrances and overhangs. Its spicule skeleton consists of megascleres only, whereas microscleres are absent. The basic spicule type is a sub-verticillate to verticillate acanthostyles, of the *Agelas* type, with a mean length of 80µm. The spicule morphology and size is highly variable, depending on the geographic origins of specimens. Variability in spicule morphology of *Astrosclera* from different geographic localities was previously reported by several authors (Vacelet, 1967, 1977, 1981; Ayling ,1982; Wörheide et al., 1997), and Vacelet (1981) discussed the idea that there might be more than one species of *Astrosclera*. Empirical testing of the question - whether variation in spicule morphology represents geographic variation or separate species - was undertaken in this study, examining the spicule morphology of specimens from 26 geographically distinct populations. Corroborative evidence from a restriction fragment length analysis of the ribosomal DNA was also undertaken for twenty specimens from five geographically distinct populations of *Astrosclera*.

Analysis of spicule morphology showed that variation was not random but specifically linked to geographical origin of the specimen. Six groups were recognized with similar spicule morphology (group with similar spicule morphology: GSSM's), based on spicule length, spination, proximal thickening, and abundance. These GSSM's comprise populations from adjacent localities. These spicule data, therefore, support the concept that there may be more than one species of *Astrosclera*, whereas analysis of ribosomal DNA did not lend support to this hypothesis. The RFLP-method of rDNA-analysis was sensitive enough to detect species-level differences in sponges, as shown by comparative studies on other demosponges, but until further divergent characters are found, the different GSSM's are still regarded as one species. The GSSM's seem to represent geographic subspecies, whose genetic differences, expressed by different (non-random) spicule morphology, were not detected by rDNA-analysis. It is supposed that *Astrosclera* is currently in the process of species separation. Each GSSM (or subspecies) is likely to have its own history with respect to radiation, isolation and evolution, and a model of the biogeographic and phylogenetic relationships of the GSSM's are presented. See Wörheide (1998) for details. ❐ *Porifera, biogeography, taxonomy, Astrosclera, ribosomal DNA, spicule morphology, phylogeny.*

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EXTANT AND FOSSIL SPONGIOFAUNA FROM THE UNDERWATER ACADEMICIAN RIDGE OF LAKE BAIKAL (SE SIBIRIA)

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Weinberg, E., Eckert, C., Mehl, D., Mueller, J., Masuda, Y. & Efremova, S. 1999 06 30: Extant and fossil spongiofauna from the underwater Akademician Ridge of Lake Baikal (SE Sibiria). *Memoirs of the Queensland Museum* 44: 651-657. Brisbane. ISSN 0079-8835.

Sediments of Lake Baikal contain unique and highly diverse siliceous assemblages. Sponge spicules and diatom frustules are both well preserved and abundant. A bottom sediment core STX3GC (4.8m long) was taken from the Academician Ridge using a gravity corer and studied at intervals of 10cm. At discrete intervals sediments consisted of 10-30% by weight of biogenic silica. Major contributors were diatoms, which are a good tool for stratigraphic assignments. Sponges were also widely distributed in space and time, and may also be valuable stratigraphic markers in Lake Baikal, in addition to their important ecological role. Analysis of core STX3GC revealed 4 genera of Lubomirskiidae consisting of 9 species, together with scattered mega- and microscleres of *Spongilla* sp. and *Ephydatia* sp. (Spongillidae). Spicule abundance and diversity were highest during periods of warm climate, whereas during cold intervals *Spongilla* sp. and *Swartschewskia papyracea* spicules were missing, and abundance and diversity of other spicules also significantly decreased. These observed morphometric changes may be applied in tracing palaeoecological and climatic changes in Lake Baikal during the Holocene and Pleistocene, or even earlier, and spicules of new species may hold information on the evolution of Lubomirskiidae and their probable spongillid roots. ☐ *Porifera, Lubomirskiidae, Spongillidae, freshwater sponges, spicules in sediments, palaeolimnology, Lake Baikal, Pleistocene, Holocene.*

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Compared to most existing fresh water lakes Lake Baikal has many unique features, one of which is its sedimentary fossil fauna. Spicules of siliceous sponges and diatom frustules are well preserved and abundant in sediments. In the Academician Ridge region the biogenic silica content of sediments reaches 10-30% by weight, the main part consisting of diatoms which are traditionally used in palaeostratigraphic analyses. According to Martinson (1936b) sponge spicules in deeper parts of Lake Baikal are of allochthonous origin because, in his opinion, freshwater sponges live on hard substrata in shallow waters. However, the sedimentary bed of the Academician Ridge represents a silt layer 1,000-1,500m thick, and the water column height above it is 300m. The present work was undertaken to find how sponge spicules have come into the Academician Ridge sediments — if they are autochtonous elements of sediments or have come in from other parts of the lake.

MATERIAL AND METHODS

SAMPLE COLLECTION. Living sponges were sampled between 1996-1997 from the Academician Ridge by dragging the lake bed using a deep water trawler, in a transect extending from the north part of Olchon Island to the south part of Great Ushkany Island. 38 specimens of sponges were collected using this method, with an additional 50 specimens collected by divers from the litoral zone of Great Ushkany I.

Sediment samples were collected by box corers and a gravity corer on board the RV Vereshagin, a scientific research vessel from the Limnological Institute, Irkutsk. Altogether 9 surface samples and a gravity core with a length of 480cm were sampled (see Fig. 5). Sampling was undertaken at

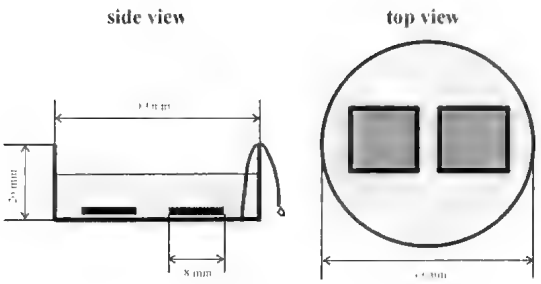

FIG. 1. Schematic illustrations of Petri dish with cover glases for sample sedimentation.

every 10cm interval of the gravity core, i.e. 48 samples for the entire core.

SAMPLE PREPARATION. 5g of each sediment sample was freeze dried for 12hrs, oxidised and disaggregated on a sample shaker using 100ml 3% hydrogen peroxide with a drop of conc. NH_4OH. The solution was then wet sieved through a 32mm mesh. The fraction <32mm was taken for further sedimentological analysis, whereas the fraction >32mm was carefully washed out from the sieve into PE bottles using 50ml distilled H_2O, and prepared as follows. Depending on numbers of samples several Petri dishes (4.9cm diameter) were put on a horizontal base (Fig. 1). Two cover glasses were placed into each dish and the dishes filled to approx. 2/3 with a gelatine solution (0.06g of 700ml distilled water). After shaking the PE bottles containing the >32mm fraction a 1ml aliquot was pipetted onto the gelatine solution, slowly stirring with a pipette in order that the suspension would ideally be distributed evenly within the Petri dish. Petri dishes were left undisturbed for at least 2hrs to ensure even settlement of the suspended material. Subsequently, prepared filter strips were inserted into the Petri dishes with one end touching the bottom and the other end touching the base outside. Thus, due to cohesive attraction, water from the basin flowed to the base and was picked up with a pipette or absorbing paper. For this purpose, a sedimentation 'stairway' was constructed at the Alfred Wegener Institute (Zielinsky, 1993), making this preparation step much easier. In the Petri dishes only the dry cover glasses remained, with an equally dispersed >32mm fraction. These were put onto glass slides using canada balsam as mounting medium.

$$N_{spicules/g} = (0.3925\,(n_1+n_2)\,V_{H2O}\,d^2)/(V_{al}\,l^2\,M_s)$$

(where N= amount of spicules per gram of freeze dried sediment; n_1+n_2=amount of spicules counted in the preparation; V_{H2O}=volume of distilled water added to the sample; V_{al}=volume of the sample aliquote; l=length of the cover glass; M_s=weight of the freeze dried sample; d=diameter of the Petri dish).

The coefficient of quantity, in this case 0.3925, has to be newly determined depending on the diameter of each Petri dish using the following formula:

$$N=(0.5(n_1+n_2)\,V_{H2O}\,(d/2)^2\,p\,d^2)/(V_{al}l^2M_s)$$

RESULTS

LIVING SAMPLES. Over the Academician Ridge transect we sampled 38 living sponges from two species, of which 31 specimens belonged to a new species *Baikalospongia* sp. nov.1 (identified by S. Efremova, in prep.). This species is pillow shaped with a strongly porous surface and very stable consistency. Lateral surfaces are covered by a cornea-like layer protecting the sponge body from silt penetration. Size varies from 1-5cm. Colour of the upper surface is green- blue or brownish. *Baikalospongia* sp. nov.1 has spicules of a characteristic shape: both ends of the spicule have unique crown of thorns. The sponges grow on large, compressed, iron-manganese concretions which are embedded abundantly in the silts of the Academician Ridge. The spicules of this species were found in all sediment samples here.

Two sponges externally similar to *Baikalospongia* sp. nov.1, but with different spicule forms — short strongyles 170-215µm long, 15-22µm wide, with tiny spines diverging on all sides — may be a subspecies of *Baikalospongia bacilifera*, based on similarities in their skeletal forms.

Also interesting is the find of 5 specimens of soft, friable, brown-blue sponges, 170-215µm long, 15-22µm wide, with round oscules and unattached to any substratum. In skeletal structure and spicule morphology this sponge is similar to a variety of *B. intermedia* (Dyb.) described by Rezvoy (1936) as *morpha profundalis*. Rezvoy noted that this variety differs from *B. intermedia* s.s. in its spicule size (330-470µm long, 20-25µm wide), spicule morphology (consisting of slightly curved strongyles covered with small spines, with a dense accumulation of small pointed spines at the rounded (basal) end), and possession of weak skeletal structure (with an irregular net-like reticulation of spicules bonded at their ends by collagen). This species is previously known only

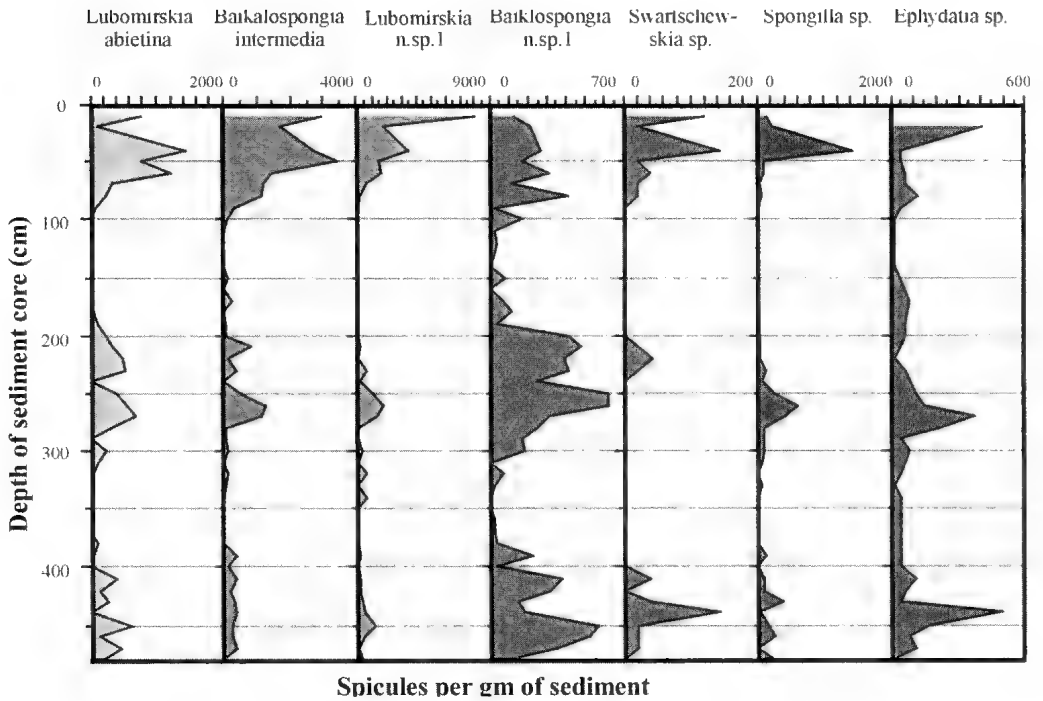

FIG. 2. Distribution of sponge spicules in sediments collected from the gravity core STX3GC.

from a single specimen collected in 1932 from 890m depth.

The taxonomy used here is based on the spicule classification system of E. Weinberg which is in some details controversal among the authors (e.g. the differentiation of isolated spicules between *Ephydatia* and *Spongilla*). However, we still consider the spicular signals sufficiently distinct to establish the specific ecological response among the fresh water sponges, Lubomirskiidae and Spongillidae.

SUBRECENT AND FOSSIL SAMPLE MATERIAL. Analysis of slides preparations of sediment sample revealed 7 different species of Lubomirskiidae. Analysis and description of the latter are in progress. The most prevalent and widely distributed spicules found in surface sediment samples came from *Baikalospongia intermedia*, followed by *Lubomirskia abietina*, *Baikalospongia* sp. nov.1, *Baikalospongia* sp. nov.2, *Baikalospongia* sp. nov.3 and Lubomirskia gen. et sp. nov., whereas spicules of *Swartschewskia* sp. were relatively rare.

The concentration of the spicules in surface sediment samples was inversely correlated with water depth, showing a general decrease in abundance of spicules in sediments as water depth increased (Fig. 5).

TABLE 1. Distribution of sponge species in the central part of Lake Baikal.

Sponges species	Ushkany Is	Akadem -ician Ridge	Surfacial ground	STX3GC
1. *Baikalispongia bacillifera*	+	-	-	-
2. *B intermedia*	+	-	+	+
3. *B.interm.* m. *profund.*	-	+	-	+
4. *B.bacillifera*, ssp. 1	-	+	-	+
5. *Baikalispongia* nsp. 1	+	+	+	+
6. *Baikalispongia* nsp. 2	+	-	+	+
7. *Baikalispongia* nsp 3	+	-	+	+
8. *Baikalispongia* nsp. 4	+	-	-	+
9. *Lubomirskia abietina*	+	-	+	+
10. *Lubomirskia* nsp. 1	+	-	-	+
11. *Swartschewskia* sp.	+	-	+	+
12. Lubomirskiidae n. G. n. sp.	-	-	+	+
13. *Spongilla* sp.	+	-	-	+
14. *Ephydatia*	+	-	-	+
15. New spicule 1	-	-	-	+
16. New spicule 2	-	-	-	+

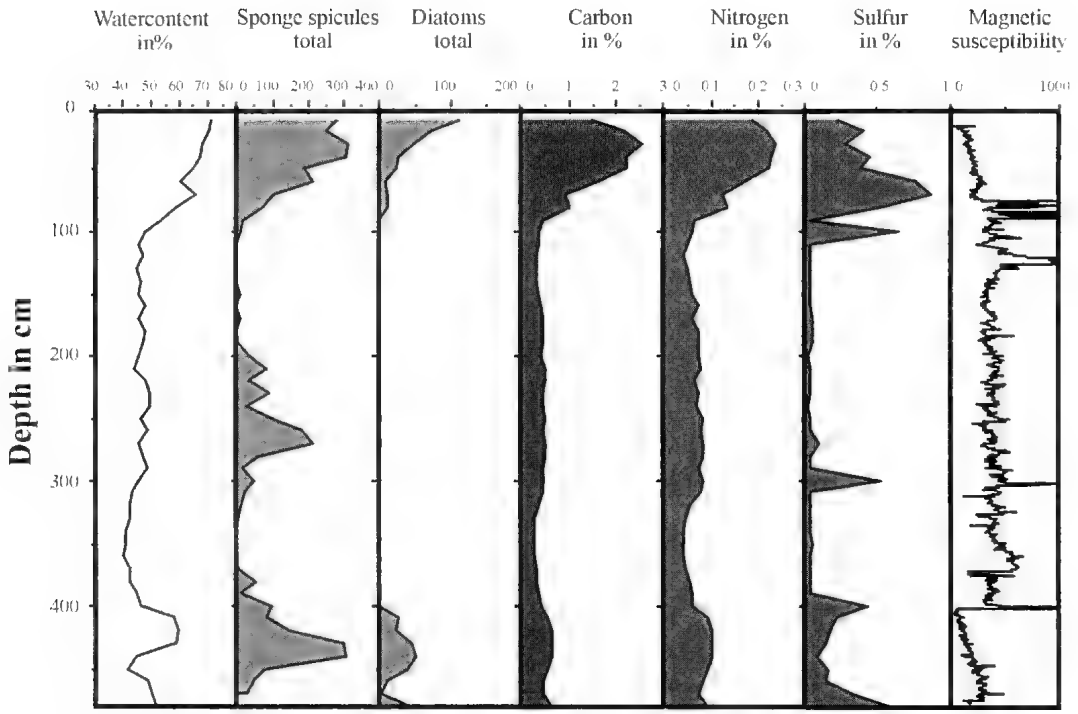

FIG. 3. Sediment parameters of the gravity core STX3GC.

Analysis of the gravity core STX3GC revealed four genera of Lubomirskiidae in nine species, in addition to mega- and microscleres of Spongillidae *Spongilla* sp. and only megascleres of *Ephydatia* sp. (Fig.3, Table 1). We also found spicules that could not be attributed to any known living species. Some of these were extraordinarily long (360-700μm), relatively thin (12-16μm) and with small spines spread over the whole rhabd. Others had a smooth surface and a relatively thick rhabd with a length of 225-280μm and width of 24-40μm, and small spines found only on the ends.

The abundance of sponge spicules throughout the core indicates that cyclic environmental changes had taken place between these sedimentary strata as indicated by maximum and minimum spicule concentrations. The first maximum concentration showed up in Holocene sediments, down to a depth of about 40cm. It is dominated by *Baikalospongia intermedia*, as for the subrecent surface sediment samples, and *Lubomirskia abietina*. The second maximum concentration commenced at a sediment depth of 200cm and ended at 300cm. This has to be classified as the Karginsk interstadial, and is indicated by the presence of spicules of *Baikalospongia* sp. nov. 1. The intervals, which according to diatom stratigraphy (Grachev et al., 1997) can be classified as glacials and stadials, are characterised by distinctly low spicule diversity and total spicule concentration, perhaps reflecting low species diversity and abundance during these periods. Spicules of *Swartschewskia* sp. and *Spongilla* sp. disappear completely during these intervals.

DISCUSSION

The presence of both living sponges and their spicules in subrecent sediments on the Academician Ridge suggest that sponge spicules are an autochtonous element of these sediments. This opinion is confirmed by the presence of large numbers of spicules from *Baikalospongia* sp. nov.1 in the surface samples of ST14, located near living populations.

Sponges have adapted to life on a loose silty substrata in different ways. Firstly, they may live without a fixed anchorage to the bottom, as seen in *Baikalospongia intermedia* morpha *profundalis* living on the Academician Ridge. Rezvoi (1936) described similar examples of sponges living on loose substrata without any fixed anchorage. Secondly, sponges may live on

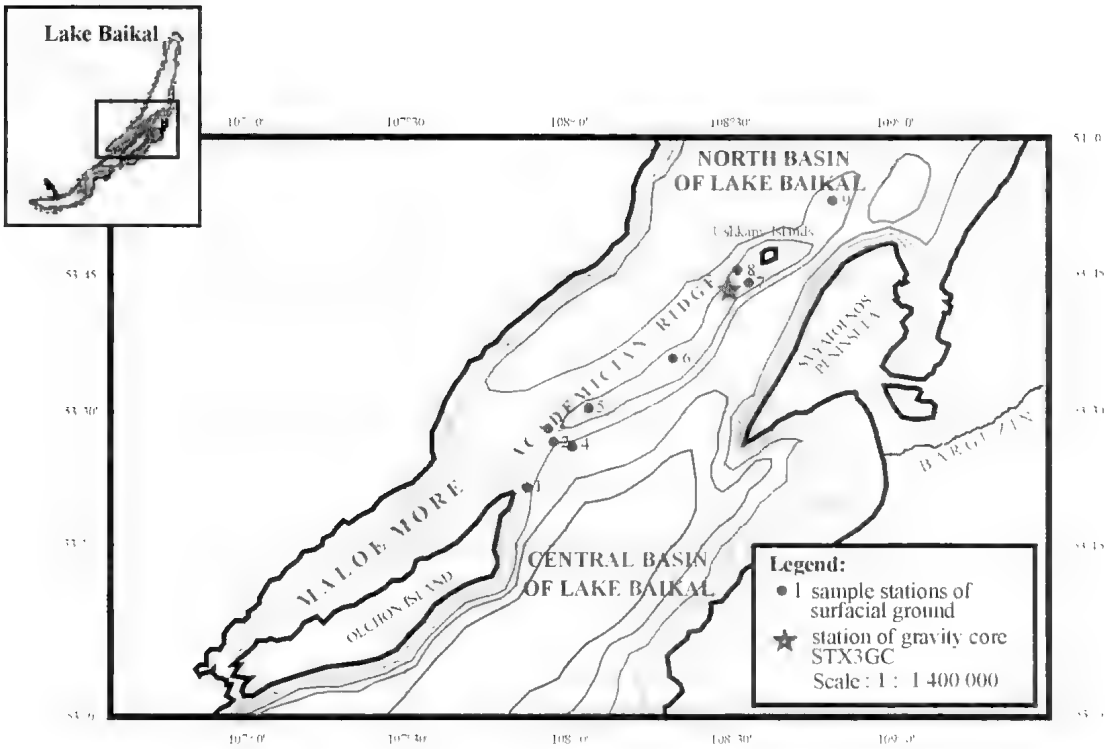

FIG. 4. Map of the study locality in Lake Baikal indicating station sites for surface sediments and gravity core samples.

ferro-manganese crusts in which they build up a horny layer for protection against sedimentation in the muddy environment. This strategy is seen in *Bakalospongia* n. sp 1 and *Baikalospongia bacillifera*. Thirdly, sponges may grow on other sponges, using their horny layer as a substrate. An example of this commensalism was seen in a specimen of *Baikalospongia* sp. nov. 1 covered by two smaller individuals of the same species living on its horny external layer, collected by our expedition to the Bolshye Koty area in 1998, at a water depth of 100m. The expedition of the Irkutsk State University found a similar specimen at Academician Ridge, kindly provided to us by Dr. V. Takhteev. In this case it is also possible that the two smaller individuals may be buds of the larger 'parent' sponge.

Spicules were found in both subrecent and fossil sediment samples, including those of species living today in near shore areas and shallow waters of Lake Baikal. These mainly concern Spongillidae but also some representatives of *Lubomirskia*. The Recent spectra of species in the area of the S part of Bolshye Ushkany I. is generally comparable with the spectra found in the gravity core STX3GC (see Table 1). Assuming that prevailing wind directions have changed from E to NE, these sponge spicules are probably allochthoneous material brought in from the Barguzin Bay and Ushkany Is. Even during periods of more prevalent SW winds the cyclonic centre remains above the Ushkany Is. Thus, sediment material is also transported from near shore areas into central parts of Lake Baikal. Based on these facts, we consider that a part of the spicules found by us from the Academician Ridge are allochtonous whereas the others are autochtonous in origin.

In general sponge spicules are not regularly distributed within sediments, showing distinctly different patterns during colder and warmer periods of the Lake. Maximum concentrations of spicules occur in the Holocene and late Pleistocene (Figs 3-4). During this peak there is also a maximum concentration of diatoms present in sediments, demonstrating that it was a period of longer lasting warm weather and high bioproductivity. In contrast, sediments laid down during the long lasting Sartan stadial (latest Pleistocene), a relatively cool period, show

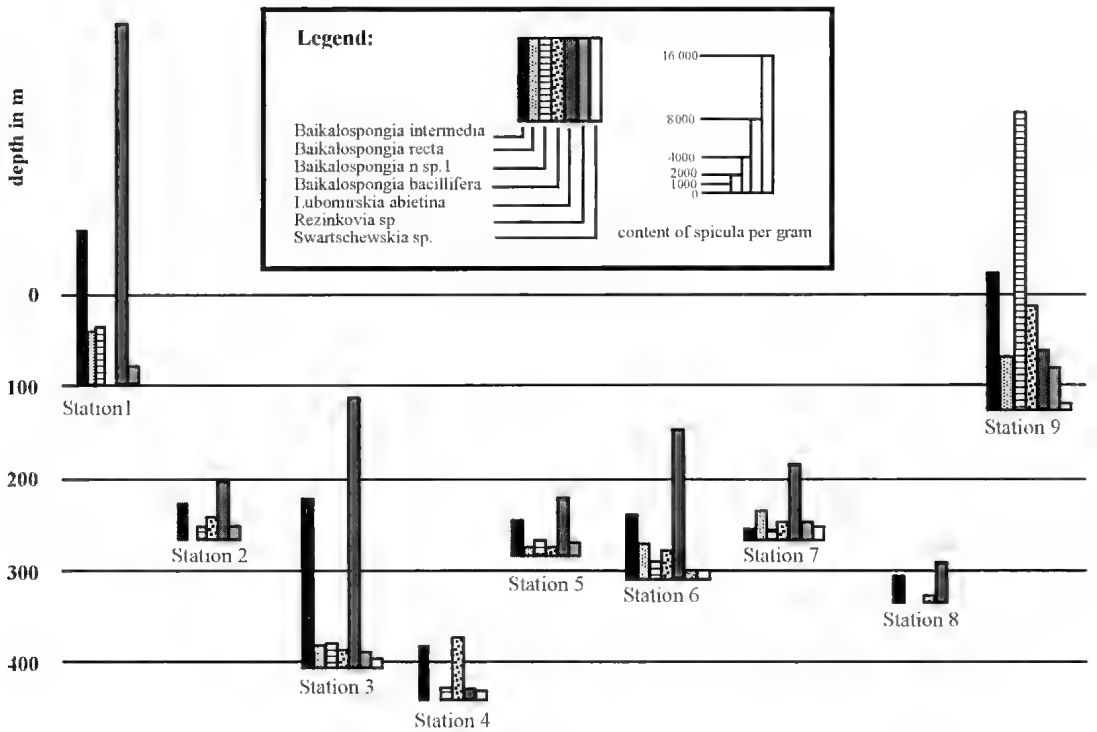

FIG. 5. Relative composition of sponge spicules in subrecent sediments in relation to water depth.

variable concentrations of diatoms ranging from very low to virtually absent (Fig. 4), whereas sponge spicules are present, and spicule abundance of *Baikalospongia* sp. nov. 1 is at a maximum, during this period. It is possible that this deep water species can better survive or adapt to changing environmental conditions due to particular nutrient regimes and possession of symbionts, because its growth is not strongly dependent on the availability of organic nutrient supplies. A change in water chemistry, resulting in dissolution of diatom frustules during colder climates, as hypothesised by some scientists (e.g. Grachev et al., 1997), seem unlikely given that the biogenic silica of sponge spicules and diatoms is identical. If this were so then it would be expected that during a change in pH conditions there would be dissolution of both sponge spicules and diatom frustules. A better explanation proposed by Back & Strecker (1998) is that during colder climates, and largely during glacial activity, high amounts of suspended material were transported into the Lake largely reducing light transmission in surface waters. This could have led to a distinct decrease in the diatom population, whereas its effect on the sponge fauna, if any, would at worst have led to a change in their symbiont relationships with little or no impact on their general living conditions.

Not all sponge species were present consistently over time. Spicules of *Swartschewskia* sp. 1 and *Spongilla* sp. occur only in the concentration maxima of the Holocene and the Karginsk interstadial. These species are typical representatives of the littoral environment, and are obviously more prone to environmental changes than deeper water species. These species are potentially useful palaeomarkers as indicators of relatively warm periods.

The occurrence of unidentified spicules in sediments, so far are unknown to any species, suggest that the Lake Baikal fauna may contain undescribed species of sponges, particularly in the endemic family Lubomirskiidae. Of special interest in this regard is our further investigations of deep drilling cores BDP-96 from the Academician Ridge, which contains nearly 5m.y. of sedimentary records. Thus, even single spicules of new species can provide information about the evolution of Lubomirskidae as well as their probable spongillid ancestry.

ACKNOWLEDGEMENTS

We are grateful to Mikhail A. Grachev, Limnological Institute of Irkutsk, whose devoted work on Lake Baikal made these international studies possible. We wish him all the best for a fast recovery. We also thank I.B. Mizandrontsev for help in the mathematical analysis of data. Thanks to John Hooper of Queensland Museum for organising the Sponge Symposium in Brisbane, for editing the proceedings and for his, and two other anonymous referees, critical review of the manuscript.

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CLIMATIC CHANGES OF THE LAST 450 YEARS RECORDED IN THE SKELETON OF THE CORALLINE DEMOSPONGE *ASTROSCLERA WILLEYANA*. *Memoirs of the Queensland Museum 44: 658. 1999*:- Stable isotope time series of $\delta^{18}O$ and $\delta^{13}C$ were measured in successive growth layers of the largest and oldest *Astrosclera* ever found (diameter of 25cm, max. age 550yrs) from Ribbon Reef #10 (GBR) (Wörheide et al., 1997; Wörheide, 1998). *Astrosclera* forms its skeletal aragonite in equilibrium with the ambient seawater, and represents, therefore, a high precision recorder of the isotopic history of the ambient seawater. $\delta^{13}C$ of surface water dissolved inorganic carbon in the northern Great Barrier Reef has apparently decreased continuously since the mid-16th century. The total decrease is 0.7‰. The major decline of 0.5‰ occurred during the industrial period of the 19th and 20th century, likely to be due to the increased release of CO_2 by deforestation and burning of fossil fuel during the period of industrialization after 1850 (increased input of lighter carbon isotopes). The oxygen isotope history shows a slightly colder (and/or dryer) phase before 1850, which correlates with the 'Little Ice Age'. A considerable shift to lighter values occurred during the 20th century (warming of SST). This may be due to an anthropogenic greenhouse effect. Most of the major climatic changes caused by ENSO/El Niño events, as reported by Quinn et al. (1987), as well as by large volcano eruptions (see LaMarche & Hirschbroek, 1984) in the last four and a half centuries seem to be recorded in the oxygen isotope record of *Astrosclera*. Further, more detailed isotope analyses on replicate samples are needed to corroborate present preliminary data. ❐ *Porifera, Astrosclera, growth layers, isotopes $\delta^{18}O$ and $\delta^{13}C$, seawater.*

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SEROTONIN IN PORIFERA? EVIDENCE FROM DEVELOPING *TEDANIA IGNIS*, THE CARIBBEAN FIRE SPONGE (DEMOSPONGIAE)

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Weyrer, S., Rützler, K. & Rieger, R. 1999 06 30: Serotonin in Porifera ? Evidence from developing *Tedania ignis*, the Caribbean fire sponge (Demospongiae). *Memoirs of the Queensland Museum* **44**: 659–665. Brisbane. ISSN 0079–8835.

Histochemical study of larvae and freshly settled juveniles of the Caribbean fire sponge *Tedania ignis* (Tedaniidae, Poecilosclerida) reveals evidence of serotonin-like immuno-reactivity, a possible indication for the presence of precursors of nerve cells in this species. Already in the earliest stages of its life, *T. ignis* is made up of two discernable cell types: monociliated cells arranged in quasi-epithelial fashion and covering the larva and the developing settled organism, and mesohylal cells (archeocytes). In the adult sponge, several mesohylal cell types can be distinguished which form a complex connective tissue. Serotonin-like immuno-reactivity demonstrated by us occurs only in two cell types: in some archeocytes of the parenchymella larvae, and in similar archeocytes and in a second, bipolar cell type of the settled, juvenile sponge. The discovery of a neuroactive substance in cells of developing sponges before and after metamorphosis provides new insights into the origin and evolution of nerve and muscle cells in the Eumetazoa. ❐ *Porifera, histochemistry, serotonin, Tedania ignis, larval development, evolution*

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Although sponges, one of the oldest metazoan groups, possess the greatest diversity of biologically active compounds of any marine phylum, the neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) has been reported only once, in myocyte-like cells of *Sycon ciliatum* (Sycettidae, Calcarea) (Lenz, 1966). Serotonin appeared early in the evolution of eucaryotes. For example, it is used in chemical signal chains in Protista where, in a species of the ciliate *Blepharisma*, a serotonin-like substance is known to function as a mating pheromone (Haldane, 1954; Miyake, 1984). It has also been shown that a number of lower organisms use serotonin as an internal messenger in their neurotransmitter-receptor systems (Carr et al., 1989) and that some of these characteristics of molecular structure that arose in unicellular organisms may have been inherited and modified by metazoans (Mackie, 1990; Van Houten, 1990).

It seems obvious that nerve cells developed gradually over a long period of time but the sequences of changes that must have occurred are difficult to establish. Being a primitive outgroup of the Eumetazoa, Porifera do not have neurons or myocytes that are present in organisms of higher levels of organisation. A common phylogenetic hypothesis such as the Planula or Phagocytella hypothesis (see Hyman, 1951; Ivanov, 1988; Rieger et al., 1991; Ax, 1995) encouraged the authors to search for precursors of nerve and muscle cells in sponge larvae in early developmental stages rather than in adults, a neglected area of research so far (Harrison & De Vos, 1991; Woollacott & Pinto, 1995). Such precursors of nerve cells and myocytes in sponges could represent the first stage in the evolution of integrative systems (e.g. Pavans de Ceccatty, 1974a, 1989; Mackie, 1990).

MATERIALS AND METHODS

Larvae of *Tedania ignis* (Durchassaing & Michelotti) (Tedaniidae, Poecilosclerida, Demospongiae) were sampled in the laboratory seawater system of the Smithsonian Coral Reef Field Station at Carrie Bow Cay, Belize, in March 1994 and November 1995. Larval release was induced in adult specimens collected in the nearby mangrove of Twin Cays (Rützler & Feller, 1996) and maintained in aerated seawater by exposing them to natural sunlight following a 12–24hr period of dark adaptation (Woollacott, 1993). The larvae were kept in seawater-rinsed glass dishes (10cm diameter) and fixed immediately after release and 80–100hrs after attachment to the substrate. To provide a

substrate suitable for fixation and removal for subsequent processing, the bottom of these dishes was coated with polymerised epoxy resin (Spurr).

Specimens were fixed in 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS; 0.1M, pH 7.4) for 6–8hrs, rinsed in PBS, and treated with Triton X-100 (0.2%, 1hr) to permeabilise membranes. After labelling with the primary antibody (rabbit anti-serotonin, IMMUNOTECH 0601; 2.5%) overnight at 4°C, fluorescence-labelling was done for 1hr with a tetrarhodamine-isothiocyanate-(TRITC)-conjugated secondary antibody (swine anti-rabbit; DAKO, 1%) for 1hr. Specimens were then rinsed in PBS, whole-mounted (Gelmount) on slides, and examined under a REICHERT Polyvar epifluorescence microscope. Incubation in bovine-serum albumin-Triton (BSA-T) without primary antibody was used as the control for nonspecific binding of the secondary antibody. Three larvae and three settled juvenile sponges were sectioned (epoxy-embedded, 1µm thick, stained with toluidin blue) and investigated in detail. The immuno-staining of both larvae and freshly settled sponges was carried out by the labelled streptavidin-biotin method (LSAB kit; DAKO). Histochemical staining of peroxidase with amino-ethyl-carbazole (AEC, substrate buffer) was used to enhance visibility of the labelled cells.

RESULTS

Tedania ignis has a parenchymella larva composed of two types of cells (Bergquist et al., 1979). Peripherally, flagellated epithelium-like cells cover the organism. This "epithelium" is monociliated and 10-25µm high. The free-swimming larva exhibits coordinated ciliary action. A distinct basal lamina and typical eumetazoan apical junctional complexes are apparently lacking (but see below). Interior, apparently motile mesenchymal cells (mesohyl cells) are arranged beneath the epithelium-like sheath (Woollacott, 1990, 1993; Amano & Hori, 1994) (Fig. 1A). The live larvae are ovoid and have a size of 700–900µm long, 500–600µm wide, but the necessary Triton X-100 treatment weakens the cell membranes and larvae usually shrink and collapse (Fig. 1A, B). In the juvenile, settled sponge too– as in the adult–the exopinacoderm which covers the ectosome acts as the protective layer. Inside the sponge, choanocyte chambers connected by canals lined with endopinacocytes lie embedded among mesohyl cells (Fig. 2A). Using a whole-mount fluorescence technique, we found serotonin-like immuno-reactivity in special mesohyl cells of both larval and juvenile *T. ignis*. Spherical serotonergic cells appear to be randomly distributed and occur alone or in clusters (Figs 1B, 2B). In one larva, for example, 6 clusters of such serotonin positive cells were found, each composed of 2–4 single spherical cells with a diameter of 4–6µm. In some clusters as well as in several single cells the nuclei are clearly visible and appear as non-fluorescent regions (Fig. 1B).

In the juvenile, settled sponge, a few bipolar cells were found in addition to the spherical cells that superficially resemble bipolar neurons or 'myocyte-like' cells (actinocytes) reported by Bagby (1966) (Fig. 2B, C). These bipolar cells have a maximum length of 20-50µm. Both types of serotonin-positive cells (spherical and bipolar) appear to be located in the mesohyl as spicules can be seen on top of the labelled cells (Fig. 2C).

No information is yet available on whether interactions between these morphological types of serotonin-positive cells occur, nor do we know whether the bipolar cells differentiate from the spherical type. If these serotonergic cells are part of an integrative system, both cellular communication at a distance (spherical cells) or cell–cell contacts (bipolar cells) could be expected.

DISCUSSION

Our study is the first to report serotonergic cells in Demospongiae, a spherical type in both larva and post-metamorphosed sponge, and a second bipolar cell type that is exclusive to the post-larval developing organism . Up to now, serotonin was only demonstrated histochemically in myocyte-like cells of Calcarea (see below). Among the most primitive Eumetazoa, serotonin is well known to act as a neurotransmitter (e.g. in Anthozoa, Umbriaco et al., 1990). Actually, 5-HT has a wide range of functions in invertebrates, such as control of regeneration processes in Planaria (Kimmel & Carlyon, 1990) and of beat of cilia in echinoderm embryo (Mogami et al., 1992), and as inhibitors and activators of muscle of molluscs (Welsh, 1953; Twarog, 1988). As in Porifera, members of the phylum Placozoa do not differentiate nerve or muscle cells and are therefore counted among the most primitive eumetazoans (Grell, 1974; Ax, 1989; Grell & Ruthmann, 1991). Schuchert (1993) demonstrated in *Trichoplax adherens*

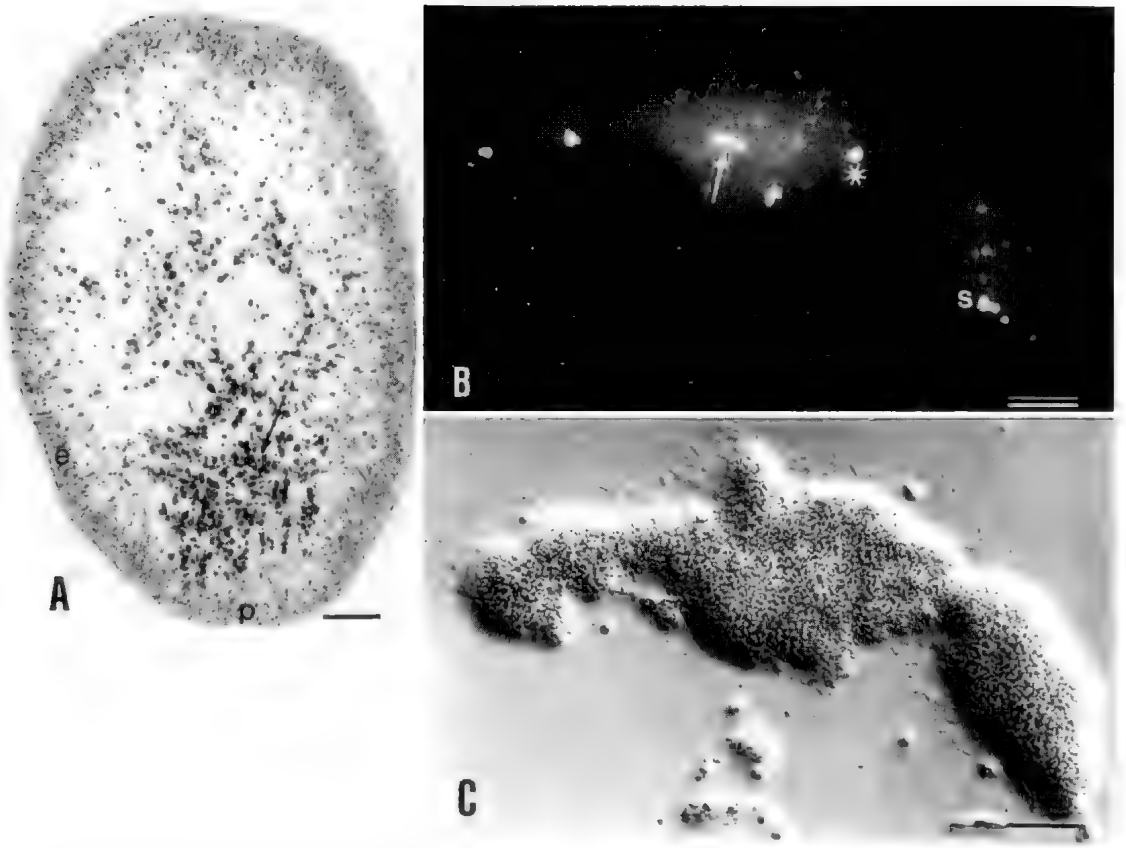

FIG. 1. *Tedania ignis,* histology of larva. A, Longitudinal section of an entire larva stained by toluidin blue showing epithelial-like cell layer (e) and dark-staining cells (archaeocytes, arrow) in clusters near the posterior pole (p) (scale bar=100μm). B, Serotonin-positive cells (s) are randomly distributed in the larval body; at least 6 clusters of 2-4 labelled cells are evident (one marked, asterisk). The nucleus in some of the cells is visible as a non-fluorescent region (arrow). As control for non specific binding of the secondary antibody specimen were incubated in BSA-T without primary antibodies. (Scale bar=100μm.) C, Nomarsky contrast view of the same specimen as in Fig. 1B. The collapsed and shrunk appearance is due to a necessary Triton X-100 treatment that weakens the cell membranes. (Scale bar=100mμ.)

(Placozoa) bottle-shaped cells (2.7–4μm) that stain specifically with the neuropeptide RF-amide. The author speculated about a possible sensory function of the bottle-shaped cells because the RF-amide positive cells were localised at the margin of the disc-like body of *T. adhaerens* and the neuropeptide RF-amide is regarded as functionally conservative in lower invertebrates.

Much effort has been made toward identifying attachment complexes between adjacent cells of the pinacoderm in adult sponges, as this layer controls the sponge's internal milieu which differs from the surrounding environment (Ledger, 1975; for review see Harrison & De Vos, 1991). This altered chemical composition in the tissue fluid of the sponge is regarded as a basic precondition for the evolution of nervous systems. Bandshaped, (epithelial-type) apical cell junctional complexes seem to be present in adult Porifera (e.g. apposed membrane junctions in Bagby, 1970; simple junctions in Ledger, 1975; parallel membrane junctions in Green & Bergquist, 1982; fig. 6 in Garonne & Lethias, 1990) in ultrastructural investigations of *Tedania ignis,* comparable structures seem to be evident (authors, unpublished). However, unequivocal clarification of the organisation of apical junctional complexes is still lacking in the Porifera. It has been stated repeatedly that permanent junctional complexes in epithelially organised cells, if present, are different in

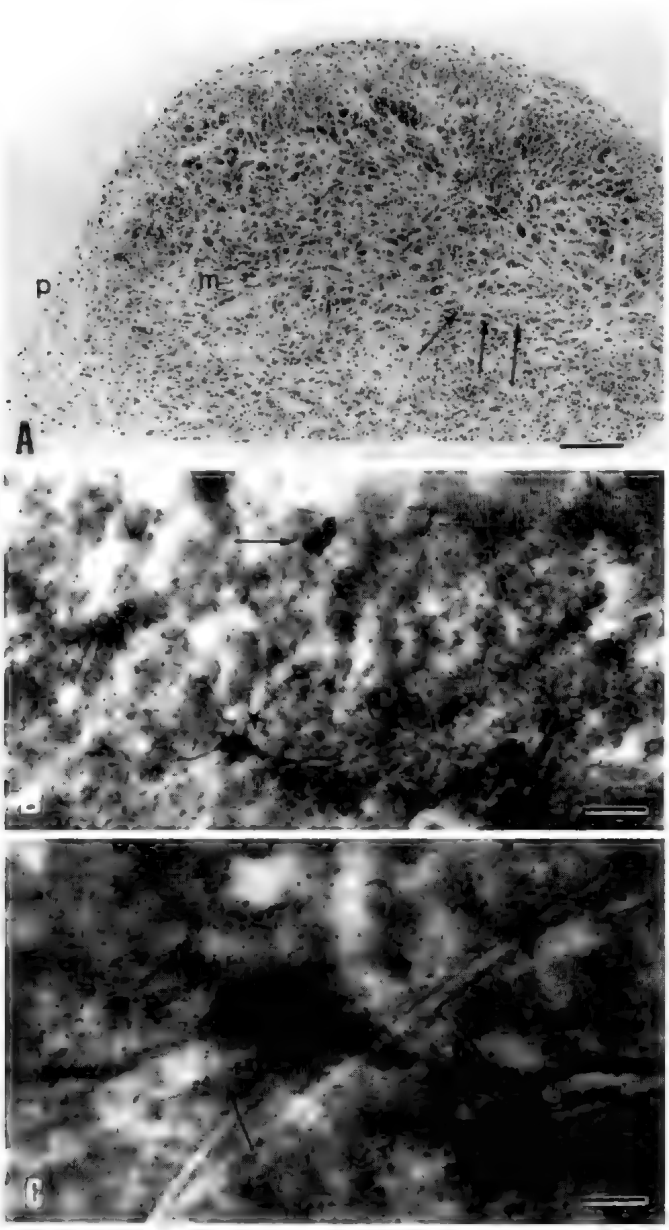

FIG. 2. *Tedania ignis,* histology of freshly metamorphosed juvenile sponge. A, Overview of a cross section stained by toluidin blue. The cells have begun to differentiate into pinacocytes (p) and multiple subtypes of mesohyl cells (m). A water canal in process of forming is indicated (arrow). (Scale bar=150µm.) B, Detail of the ectosome. Several spherical (arrow) and one bipolar (asterisk) serotonin-positive cells are visible. To enhance visibility of the labelled cells a histochemical peroxidase staining was used. (Scale bar=15µm.) C, Bipolar serotonergic cell (same specimen as in Fig 2A). Spicules (arrow) can be seen above the labelled cell. (Scale bar=4µm.)

construction from those of Metazoa (e.g. Green ,1978; Green & Bergquist, 1979). One reason for this difference is seen in the high motility and frequent rearrangement of sponge cells (e.g. Müller, 1982). However, it seems likely that development of permanent communicating structures goes hand in hand with less mobility and a highly differentiated state of cells. For example, Lethias et al. (1983) are of the opinion that additional freeze-fracture- and TEM-investigations of sponges might reveal membrane specialisations and connections to cytoskeletal components. On the other hand, several investigators of freshwater and marine sponges could not identify such junctional complexes (e.g. Lethias et al., 1983; Weissenfels, 1990; Woollacott, 1990).

While there is yet no evidence for chemical synapses in sponges, several reports discuss gap junction-like structures in the Porifera that may function as electrical synapses. For example, Green & Bergquist (1979) interpreted structures observed by SEM as temporary intercellular communication canals. Also, Revel (1988) and Garrone & Lethias (1990) describe various particle fields in freeze-fracture replicas, some of which superficially resemble gap junctions or rhomboid panicle fields of Eumetazoa but cannot unequivocally be claimed as typical gap junctions.

Contractile cells in the mesohyl of sponges–actinocytes (see Boury-Esnault & Rützler, 1997), but often called myocytes or myocyte-like cells –are arranged in networks (Bagby, 1966; Prosser, 1967; Bergquist, 1978; Burlando et al., 1984; Wachtmann et al., 1990; Harrison & DeVos, 1991) and have been seen as an early stage in the evolution of nerve and muscle cells (see literature in Lentz, 1966; Pavans de Ceccatty, 1974a; Mackie, 1990). Two types of microfilaments, that is, thin (5-7nm) and thick (15-25nm) filaments can be observed in the cytoplasm. In *Tedania ignis* and *Hippospongia communis*, only thick filaments have been reported (Harrison

& De Vos, 1991). The ability to contract or condense in response to endogenous events or external stimuli is a common feature in Calcarea and Demospongiae. It results in a decrease in body size and concurrent increase in number of cell contacts (review in Simpson, 1984; Weissenfels, 1989). One can speculate whether the increased number of cell contacts is only the result of a reduced body volume or possibly serves the intensification of 'signal transduction' in the network of actinocytes. Frequent cell–cell contacts between myocyte-like cells were also observed in *H. communis* (Pavans de Ceccatty et al., 1970). According to Pavans de Ceccatty (1974a), the microfilament-containing pinacocytes play an important role in this process, both for cell contraction and cell communication. Owing to the dynamic, 'loose' organisation of cellular features (Pavans de Ceccatty, 1974b; Bond, 1992) there are no nervous cells evident in sponges, but one can expect temporary, fixed pathways through connected mesohyl cells at the points of stable intercellular connections. Lentz (1966) reported acetylcholinesterase, monoamine oxidase, epinephrine, norepinephrin, and 5HT (serotonin) in 'myocyte-like' cells of *Sycon ciliatum*. These observations along with the association of cholinesterase and myofilaments in myocyte-like cells and the report of actin filaments in pinacocytes (Pavans de Ceccatty, 1989) may signify myoid and neuroid elements from a common integrative system that is coordinating 'tissue' contractions in sponges although electrophysiological evidence of a conducting mechanism is still lacking (Lawn, 1982).

In conclusion, we believe our findings of serotonergic cells in the Parazoa suggest an evolutionary specialisation of serotonin, separate from its function in Protists. We interpret our observations as supporting the recently emphasised sister-group relationship with the Eumetazoa (Morris, 1993; Müller, 1995; Ax, 1995) by exhibiting the very first steps in the evolutionary development of the integrative system of the Metazoa. Further research involving additional species and immuno-cytochemical, electrophysiological, and other approaches is clearly needed.

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BIOCALCIFICATION IN THE INDO-PACIFIC CORALLINE DEMOSPONGE *ASTROSCLERA WILLEYANA* LISTER - THE ROLE OF BASOPINACODERM. *Memoirs of the Queensland Museum 44: 666. 1999*:- The aragonitic calcareous basal skeleton of *Astrosclera* is composed of 20-60µm-sized aragonitic spherulites, produced by a combination of three processes. First, the spherulites are formed in large vesicle cells (LVC's) inside large vesicles in the ectosome. In a second process, after release from LVC's, basopinacocytes transport the spherulites to the tips of the skeletal pillars, where they fuse together by epitaxial growth; and in a third process, during upward growth, the soft tissue is slowly rejected from the lowermost-parts of the skeletal cavities and the remaining spaces are subsequently filled by epitaxially-growing aragonite fibers. In the second and third process, basopinacocytes produce either the insoluble intracrystalline organic matrix, which does not consist of collagen, as well as the soluble intracrystalline matrix, which consists of highly acidic Ca^{2+}-binding mucus substances. Basopinacocytes control speed and direction of epitaxial growth in both of the latter two biocalcification processes. It is hypothesized that *Astrosclera* is able to control the rate of calcification by the regulation of its bacterial population. The mean growth rate of *Astrosclera* was measured at 230µm per year. A detailed description of soft tissue ultrastructure and its cellular composition has recently been published by Wörheide (1998). ❐ *Porifera, Astrosclera, skeletal development, calcification regulation, ultrastructure.*

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NITROGEN FIXATION IN SYMBIOTIC MARINE SPONGES: ECOLOGICAL SIGNIFICANCE AND DIFFICULTIES IN DETECTION

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There has been considerable speculation, and some evidence, that coral reef sponges can fix atmospheric nitrogen through some of their microbial symbionts, particularly symbiotic cyanobacteria. Many Indo-Pacific coral reef sponges can satisfy much of their requirement for carbon energy compounds via translocation from photosynthetic symbionts, and a similar mechanism has been invoked to explain how some sponges could supplement the low amount of available nitrogen in clear tropical waters. Attempts to measure nitrogen fixation using the acetylene reduction test have proven technically difficult and given ambiguous results. However, fixation was demonstrated unambiguously with incorporation of the stable isotope $^{15}N_2$ into the amino acids glutamine, glutamate and aspartate of *Callyspongia muricina*, although at relatively low rates. The variability in measuring acetylene reduction in 23 sponge species is attributed to several factors: the number of cellular and matrix barriers that must be passed by acetylene and ethylene; the difficulty of maintaining sponges alive under experimental conditions; possible metabolism of ethylene by symbiotic bacteria; and possible toxicity of the reagents. ❐ *Porifera, nitrogen fixation, acetylene reduction activity, cyanobacteria, coral reef sponges, Callyspongia muricina.*

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Photosynthetic symbionts convey a distinct advantage to marine sponges living in low nutrient, tropical waters. These sponges have been shown to receive fixed nutrient carbon translocated from cyanobacterial symbionts (Wilkinson, 1979), such that some are virtually 'phototrophic', i.e. the symbionts can provide the bulk of carbon energy requirements (Wilkinson, 1983; Cheshire & Wilkinson, 1991). Such sponges are distinctly flattened to enhance light capturing, possibly at the expense of the ability to act as filter feeders (Wilkinson et al., 1988). These abilities are similar to other major photosynthetic symbioses on coral reefs: zooxanthellate corals (Muscatine et al., 1981); tridacnid clams (Trench, 1987); and didemnid ascidians (Griffiths & Thinh, 1983).

The potential to fix nitrogen was demonstrated in Red Sea sponges using the acetylene reduction test (Wilkinson & Fay, 1979). However, subsequent attempts to apply similar methods to a range of sponges from other coral reef areas proved to be highly variable. After many experiments showing equivocal results in 23 species, more direct and unambiguous nitrogen fixation methods were applied with the incubation of a sponge in the stable isotope $^{15}N_2$. We chose a sponge from the Great Barrier Reef that had previously shown some promise at fixing nitrogen and from which cyanobacterial cell preparations could be easily obtained.

Marine sponges also have many other photosynthetic and non-photosynthetic symbionts (Wilkinson, 1992). Many sponges have large populations of symbiotic bacteria, which may occupy up to 40% of the animal volume, comparable to the volume of the matrix and exceeding the volume of animal cells (Vacelet & Donadey, 1977; Wilkinson, 1978). These symbionts are particularly variable with the possibility of six or more 'species' occurring within the mesohyl matrix. However, few, if any, roles have been demonstrated for these symbionts, until recent experiments demonstrated a possible role in nitrogen metabolism within the sponge that also may be significant within coral reef ecosystems (Corredor et al., 1988; Diaz & Ward, 1997). Recently, many different bacterial forms have been observed, including Archaea-like cells (Preston et al., 1994; Fuerst et al., 1998) and methane-oxidising bacteria in sponges (Vacelet et al., 1996).

MATERIALS AND METHODS

All field experiments were performed at Davies Reef (18°15'S; 147°38'E) on the central part of the Great Barrier Reef aboard the RV Harry Messell (location in Wilkinson & Evans, 1989).

NITROGEN FIXATION BY ACETYLENE REDUCTION. The technique of Stewart et al. (1967) was used with incubations in 10-20% acetylene over acetylene saturated seawater (Flett et al., 1976). In some instances additional 5-20% oxygen gas was added to ensure that the sponges were not stressed through anaerobic conditions. Pieces (frequently transplanted several months prior to experimentation; Wilkinson & Thompson, 1997) or whole sponges of 23 species (116 replicates) were incubated in air tight containers, temperature buffered in running seawater and illuminated by filtered sunlight. Controls were either sponge tissue incubated in the dark, or boiled sponge tissue, or live coral rubble incubated in the light. Cyanobacterial cell preparations (see below) were incubated similarly.

Regular gas samples were collected over 4-6 hour incubation periods in evacuated tubes and assayed in a Tracor 222 gas chromatograph, with concentrations determined against an internal methane standard with corrections for gas solubility (Wilkinson & Sammarco, 1983).

NITROGEN FIXATION WITH $^{15}N_2$. The sponge *Callyspongia muricina* was chosen because it had frequently shown some acetylene reduction activity and because large quantities of cyanobacteria can be extracted from sponge tissues relatively easily. Seawater was degassed in a stream of argon gas and then saturated overnight under a headspace of 4 parts $^{15}N_2$ (Amersham) to 1 part O_2 with stirring.

Cyanobacterial preparations were obtained by gently crushing pieces of sponge, and blending the suspension in a glass blender with a teflon piston. After repeated centrifugation, washing and resuspension, a pellet was obtained that was predominantly cyanobacteria (examined microscopically). Replicate whole pieces of sponge and cell suspensions were incubated in closed chambers with no gas spaces in $^{15}N_2$ seawater (as above) for 20, 40 and 60mins.

Approximately 3cm^2 of sponge tissue was fixed in ethanol: water: acetic acid (50:45:5) and then pulverised using a 2cm probe diameter polytron probe blender in a 50ml polyethylene centrifuge tube in 25ml of distilled water. The mixture was filtered through 'Miracloth' until the spicule mass was colourless with all cyanobacterial cells removed. The suspension was extracted through Dowex 50 and 1 ion exchange columns to yield amino acid fractions. Centrifuged pellets of the cyanobacterial suspensions were similarly fixed and then blended.

Each fraction was reduced to 2ml and Kjeldahl digested at 150°C for 1.5hrs, followed by 330°C for 3hrs to remove all water (Bergensen, 1980). Digests were immediately converted to ammonium sulphate salts to prevent ambient contamination (Volk & Jackson, 1979) by digesting in 0.1M H_2SO_4 followed by 10M NaOH to convert to an alkaline solution. After 4 days incubation at 50°C, digests were evaporated to dryness under argon and analysed. The fixed nitrogen in samples was driven off by mixing with NaOBr under an argon atmosphere, and the gas injected directly into VG 602D (Cheshire, UK) computerised mass spectrometer through an ethanol-dry ice moisture trap. Calibration was with CIG ultra high purity nitrogen calibrated against N-1 and N-2 international standards (IAEA, Vienna) and results expressed as delta notation on air nitrogen scale with a reproductibility of 20 replicate samples being less than 0.1ppm. Control samples of unlabelled sponge, cell suspensions and coral rubble were similarly assayed to detect natural levels of $^{15}N_2$.

SPONGE PARAMETERS. Wet weight, dry weight, surface area and chlorophyll a content (Jeffrey & Humphrey, 1975) were as outlined in Wilkinson (1983).

RESULTS

NITROGEN FIXATION BY ACETYLENE REDUCTION. Assays of 23 sponge species revealed either negative, ambivalent or slightly positive results for ethylene production (Table 1). Three species, *Callyspongia muricina*, *Ircinia ramosa* and *Collospongia auris* frequently showed slight, or on occasions significant positive acetylene reduction. However, there was little consistency or reproducibility of positive results in these species, with or without symbiotic cyanobacteria. Similar ambiguous results were obtained with incubations of 6 sponge species from coral reefs off the coast of Western Australia (Wilkinson, unpublished data).

Variations in oxygen or acetylene concentrations on incubations of whole tissue or cell preparations had no apparent effect on acetylene reduction rates. Control incubations of coral

TABLE 1. Results of acetylene reduction assays on coral reef sponges, compared to pieces of rubble as positive controls. Negative controls of incubations in the dark or with dead sponge are not reported as all were negative. Results from experiments using different concentrations of oxygen and or acetylene are combined, as there were no discernible patterns. Symbiont = lists of the type of cyanobacterial symbiont. The Results are reported as the number of sponge incubations in each category: – = no evolution of ethylene, and less than contamination; +/– = inconclusive result with traces of ethylene; + = slight release of ethylene but less than 0.5 nM cm^{-2} hr^{-1}; ++ = significant increase in ethylene >2.0 nM cm^{-2} hr^{-1}. Nutrition = whether or not sponge can obtain the bulk of their carbon energy via symbiont photosynthesis (phototrophic), versus none (heterotrophic), or both (mixed).

Order	Species	Symbiont	Nutrition	–	+/–	+	++
Dictyoceratida	*Ircinia ramosa*	Unicellular	Phototrophic	1	9	9	0
	Phyllospongia lamellosa	Unicellular	Phototrophic	0	1	0	0
	Phyllospongia papyracea	Unicellular	Phototrophic	0	2	1	0
	Carteriospongia foliascens	Unicellular	Phototrophic	1	9	1	0
	Carteriospongia flabellifera	Unicellular	Phototrophic	2	2	0	0
	Strepsichordaia lendenfeldi	Unicellular	Mixed	0	3	0	0
	Collospongia auris	Unicellular	Phototrophic	1	1	2	0
	Ircinia sp. 1	Multicellular	Mixed	0	2	0	0
	Rhopaloeides odorabile	None	Heterotrophic	0	2	0	0
Dendroceratida	*Dysidea herbacea*	Multicellular	Phototrophic	0	3	0	0
	Dysidea sp. 1	Multicellular	Phototrophic	0	1	0	0
Haplosclerida	*Callyspongia muricina*	Unicellular	Phototrophic	7	23	4	0
	Callyspongia sp. 1	None	Heterotrophic	0	1	0	0
	Amphimedon sp. 1	Unicellular	Phototrophic	0	2	0	0
Petrosida	*Xestospongia exigua*	Unicellular	Mixed	0	2	0	0
Axinellida/ Halichondrida	*Cymbastela* sp. 1	Uni & Multi	Phototrophic	7	20	1	0
	Cymbastela sp. 2	Uni & Multi	Phototrophic	0	2	0	0
	Phakellia aruensis	None	Heterotrophic	0	1	0	0
	Acanthella sp.1	None	Heterotrophic	0	2	0	0
Poecilosclerida	*Neofibularia irata*	Unicellular	Phototrophic	0	3	0	0
Astrophorida	*Jaspis stellifera*	Unicellular	Mixed	0	2	0	0
Hadromerida	*Cliona* sp. BP	Zooxanthellae	Phototrophic	0	1	0	0
Class Calcarea	*Pericharax heteroraphis*	Unicellular	Mixed	0	1	0	0
None	Coral rubble controls	Turf algae		0	0	0	8

rubble with natural turf algal populations showed consistent, relatively high rates of acetylene reduction, comparable to those shown by Wilkinson et al. (1984).

NITROGEN FIXATION WITH $^{15}N_2$. Definite nitrogen fixation of $^{15}N_2$ was observed in whole sponge and cyanobacterial cell preparations of *C. muricina*. The highest rates of enrichment were observed in the amino acids: glutamine, glutamate and aspartate (Fig. 1). Similar rates of enrichment in the amino acid fraction were found in pieces of rubble incubated in $^{15}N_2$.

CELLULAR NATURE OF THE SYMBIONTS. The nature and location of cyanobacterial symbionts varies between sponges (Table 1). These were observed during transmission electron microscopic study of these sponges for other studies (Wilkinson, unpublished data). Three distinct categories can be observed: a) cyanobacteria free living in the mesohyl; b) cyanobacteria predominantly within large vacuoles within special mesohyl cells, cyanocytes; or b) and c) cyanobacteria both within vacuoles and in the mesohyl (Fig. 2; Wilkinson, 1978). In addition, some sponge species have other symbionts including multicullular cyanobacteria and zooxanthellae (Table 1; Wilkinson, 1992).

DISCUSSION

Two conclusions are evident from these studies: 1) at least one sponge with cyanobacterial

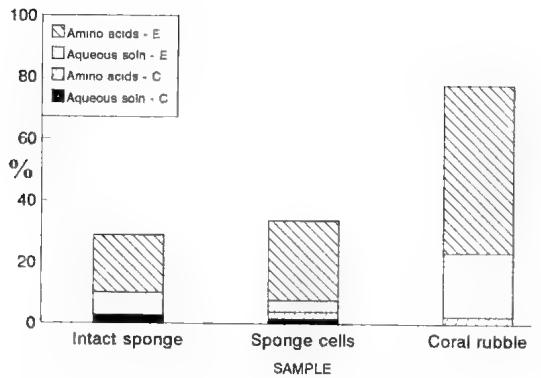

FIG. 1. Total enrichment of $^{15}N_2$ in whole sponge and cell suspensions of *Callyspongia muricina* compared to coral rubble controls. Data are delta enrichment values of $^{15}N_2$ compared to non-labelled N within control (C) aqueous and amino acid solutions and experimental (E) aqueous and amino acid fractions. Differences between E and C are significant at $P<0.001$.

symbionts fixes atmospheric nitrogen, but not at rapid rates, as indicated by direct fixation of $^{15}N_2$; and 2) the relatively easier technique of acetylene reduction is unreliable and inapplicable. A presumptive conclusion is that nitrogen fixation may occur in many other sponges with symbiotic cyanobacteria, but this can only be verified with individual testing of direct incorporation of $^{15}N_2$.

The first conclusion confirms the earlier observations of acetylene reduction in two Red Sea sponge species by Wilkinson & Fay (1979). We subsequently questioned those earlier results when repeated acetylene reduction tests on a larger number of species showed inconsistent results (Table 1). However, the direct incorporation of $^{15}N_2$ as gas has demonstrated that sponges with cyanobacterial (or possibly other prokaryotic) symbionts do contain active nitrogenase.

The progressive enrichment of glutamine, glutamate and aspartate demonstrate that this fixed nitrogen is of potential benefit to the host sponge as these compounds can be incorporated into sponge and symbiont protein, and metabolised for energy. Translocation of these amino acids, however, was not demonstrated, but may be assumed because the population size of microbial symbionts is usually stable with little need for protein for cell growth and there is a parallel release of fixed carbon as glycerol (Wilkinson, 1979).

Any nitrogen fixation would be valuable to those coral reef sponges that live in clear tropical waters, as it can supplement the particularly low levels of particulate nutrients and dissolved fixed nitrogen (Wiebe et al., 1975). Moreover, many of these sponges obtain the bulk of their energy from the photosynthetic symbionts as translocated glycerol, which is rich in carbon but devoid of nitrogen (Wilkinson, 1979, 1983). Without this added source of nitrogen, sponge growth rates could be reduced through a lack of nitrogen to produce proteins, particularly for the production of the fibrous protein skeleton. The majority of sponges in clean water on the Great Barrier Reef are distinctly flattened to enhance light capture (Wilkinson, 1988). These are sponges that exhibit particularly phototrophic nutrition and have the potential to obtain virtually all of their nutrition from the symbionts down to a depth of 30m (Cheshire & Wilkinson, 1991).

The following possible explanations are advanced to explain the inconsistency in acetylene reduction assays compared to coral rubble controls: 1) poor diffusion between multiple layers of cell and matrix; 2) disturbance to host sponges; 3) possible removal of ethylene by symbiotic bacteria; and 4) possible acetylene toxicity to sponges and cells.

1) The turf algae on the rubble are totally exposed to the seawater containing dissolved acetylene, with only the algal cell barriers remaining for ethylene to diffuse back into the water. Therefore, there is efficient and rapid transfer of both the acetylene into turf cyanobacteria and similar transfer of the ethylene back into seawater, evident as high and consistent rates of ethylene production (Table 1).

The situation in sponges is different as the symbionts in many sponges are contained within specialised vacuoles (cyanocytes; Wilkinson, 1978) embedded in the sponge mesohyl matrix (Fig. 2). For these symbionts, there are multiple cell and matrix layers that must be passed for both acetylene to diffuse into the cyanobacteria (or bacterial symbionts) and then for the ethylene to diffuse back out to the water, where it can be detected in water samples. This double diffusion process may be slow and inefficient, as it would rely on diffusion gradients across cell and matrix barriers.

Against this argument is the fact that low molecular weight gaseous molecules like acetylene (M.W. 26) and ethylene (M.W. 28) diffuse rapidly through membranes, comparable to other gases such as nitrogen (N_2 and $^{15}N_2$; M.W. 28 and 30) and oxygen (M.W. 32) (Cheung & Marshall, 1997).

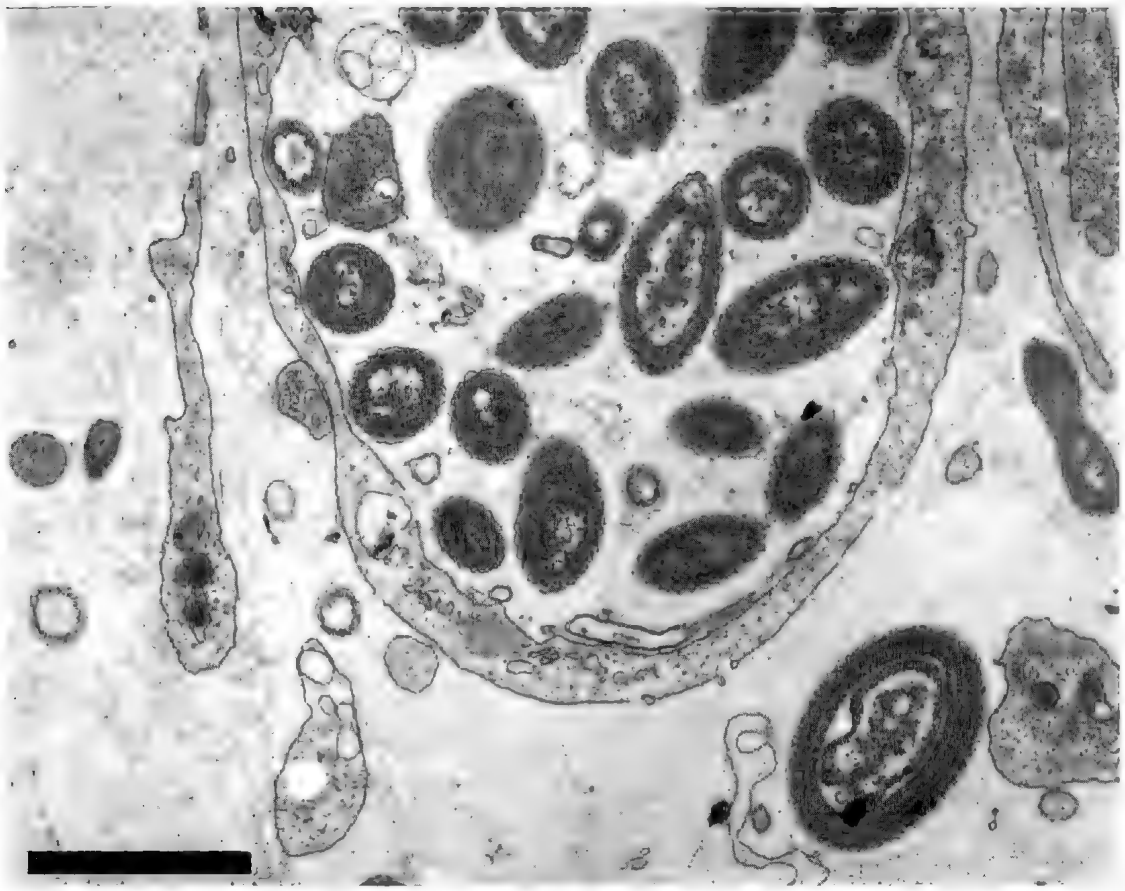

FIG. 2. Electron micrograph of the Great Barrier Reef sponge *Jaspis stellifera* showing unicellular cyanobacterial symbionts both free in the mesohyl matrix and contained within vacuoles of specialised cells, the cyanocytes. Scale bar 5μm.

2) Sponges have the ability to contract and cease pumping when disturbed, which would reduce water and gaseous exchanges. This was demonstrated by Reiswig (1971) and has been a consistent problem with physiological experiments with sponges on the Great Barrier Reef, because they frequently contract and close their oscules when placed in experimental chambers. This is most evident in massive species with large oscules, like *Rhopaloiedes odorabile* and *Jaspis stellifera*, but may also occur in small oscule species like the *Phyllospongia* and *Carteriospongia* spp., but observing any contraction in the field is particularly difficult. Cessation of pumping activity would restrict water movement through canals and prevent a free exchange of acetylene and ethylene, thereby reducing the potential for acetylene reduction.

3) There is the possibility that symbiotic bacteria exist which can oxidise either or both acetylene and ethylene and interfere with concentration measurements. Sponges contain a large variety of bacterial symbionts (Vacelet & Donadey, 1977; Wilkinson, 1978) and recently it has been shown that there are methane-oxidising bacteria in marine sponges (Vacelet et al., 1996), as well as a wide range of Archaea-like bacteria (Preston et al., 1994; Fuerst et al., 1998). The majority of bacterial symbionts cannot be isolated in culture and have only been observed using electron microscopy.

4) Acetylene toxicity has not been shown in these sponges, but has been demonstrated in other nitrogen fixing systems (David & Fay, 1977). Most acetylene reduction assays have been applied to plants, rather than animals. Thus acetylene toxicity may have a greater impact on

animal respiration and reduce or block the transfer of water through the canal system. This would result in the effects in 2) above.

Irrespective of the reason for inconsistencies with the acetylene reduction method compared to the use of the stable nitrogen isotope technique, it is concluded that acetylene reduction should not be used with these animals as a method to detect nitrogen fixation. One problem is that stable isotope analysis is more expensive and difficult to apply under field conditions. However, the ready availability of new continuous-flow isotope analyses methods for carbon, nitrogen and hydrogen isotopes means that enrichment experiments are very easily evaluated at the molecular level using compound-specific isotope analyses (Merrit & Hayes, 1994; Macko et al., 1997).

Verified nitrogen fixation in one sponge species has demonstrated the potential for fixation to be found in other sponges with microbial symbionts. Although it is more probable that the cyanobacterial symbionts are responsible for the activity, the possibility exists that bacterial symbionts may also fix atmospheric nitrogen in other sponges. More research is needed to confirm the origin of the nitrogen fixing enzyme, nitrogenase. Irrespective of the source, any fixed nitrogen would supplement nutrition in coral reef sponges that must make a living in low nutrient tropical waters.

ACKNOWLEDGEMENTS

Professor J.M. Hayes and Z. Roksandic are thanked for advice and assistance with nitrogen isotopic analyses.

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RAPID CHANGE AND STASIS IN A CORAL REEF SPONGE COMMUNITY. *Memoirs of the Queensland Museum 44: 674. 1999*:- Four censuses of a sponge community on a shallow coral reef in San Blas, Panama, have revealed a combination of both extreme change and also apparent stasis over the 14 years between 1984-1998. Total biomass of the sponge assemblage varied little over the first 11 years, with the exception of the first few years after a hurricane decreased sponge populations in 1988. However, relative contributions to total biomass by the different species have changed to the extent that over half of the original species are now altogether absent from the censused area. Species lost were not necessarily those that had been rare initially, and the hurricane does not appear to have been responsible for the loss of species. The most striking pattern of loss is that keratose species account for a disproportionately large number of the species and also of the volume of biomass lost. Growth form also seems to influence vulnerability to loss, as massive forms were lost disproportionately and no erect branching forms were lost. Pathogens appear to be the agents of at least some of the mortality, with high rates of infection by what seem to be species-specific pathogens in the most common species. ❐ *Porifera, coral reef sponges, population dynamics, disease, environmental ecology.*

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GROWTH AND REGENERATION RATES OF THE CALCAREOUS SKELETON OF THE CARIBBEAN CORALLINE SPONGE *CERATOPORELLA NICHOLSONI*: A LONG TERM SURVEY

PHILIPPE WILLENZ AND W.D. HARTMAN

Willenz, Ph. & Hartman, W.D. 1999 06 30: Growth and regeneration rates of the calcareous skeleton of the Caribbean coralline sponge *Ceratoporella nicholsoni*: a long term survey. *Memoirs of the Queensland Museum* **44**: 675-685. Brisbane. ISSN 0079-8835.

The growth rate of the aragonitic skeleton of the Caribbean 'sclerosponge' *Ceratoporella nicholsoni* has been studied by *in situ* staining of specimens with calcein in a reef tunnel, 28m depth, near Discovery Bay, Jamaica. Experiments were performed up to five times from 1984 to 1997 on a population of 10 specimens ranging from 10-20cm maximum diameter. In each experiment small skeletal samples were removed from the periphery of sponges, and specimens were left in place for further studies on growth and regeneration. Perpendicular sections, ground to a thickness of about 10µm, were photographed by fluorescence microscopy. Annual skeletal growth rates were calculated from measurements of the linear extension between calcein stained lines along growth axes. Data indicate that although average annual growth rates remained in the same range for different periods (214.6±54.5-233.3±33.0µm yr^{-1}), significant differences occurred from one individual to another within the same period. The annual growth rate of a given individual also varied significantly in time (191.1±30.0-269.9±37.0µm yr^{-1}). A second population of smaller individuals, measured after a single period of one year, revealed a strikingly lower average annual growth rate (124.4±35.0µm yr^{-1}). Regeneration of the skeleton of injured specimens was also characterised by an initial slower growth rate. Nevertheless, after the first year, it was comparable to normal growth, and exceeded it slightly thereafter. This first long term study of *Ceratoporella* provides important information on the variability in growth rates, with implications on the use of sclerosponges as paleoenvironmental proxies. ❐ *Porifera, sclerosponges, coralline sponges, growth rate, aragonite, skeleton, regeneration, calcein, Ceratoporella nicholsoni.*

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Although the first specimen of the coralline sponge *Ceratoporella nicholsoni* was dredged off Cuba in 1878, and described as a new alcyonarian coelenterate more than thirty years later (Hickson, 1911), it was not until the mid-1960s that this species was rediscovered (Hartman & Goreau, 1966), and subsequently shown to be a sponge (Hartman & Goreau, 1970). At the same time, thanks to the increased use of SCUBA diving, the extent of the diversity of 'sclerosponges' became evident (Hartman, 1969; Hartman & Goreau, 1970, 1975).

Among the nine known species of Caribbean coralline sponges, *Ceratoporella nicholsoni* secretes the most massive basal skeleton of calcium carbonate. Despite the fact that the ecology and ultrastructure of *Ceratoporella* have now been extensively investigated (Lang et al., 1975; Willenz & Hartman, 1989), the growth rate of its aragonitic skeleton is still unknown, more than a century after its discovery.

Both direct staining and indirect techniques have been used to evaluate the growth rate of *Ceratoporella*: the former using alizarin red (Dustan & Sacco, 1983) or calcein stains (Willenz & Hartman, 1985); the latter based on ^{14}C and ^{210}Pb chronologies (Benavides & Druffel, 1986; Druffel & Benavides, 1986) or focusing on carbon and oxygen isotope studies (Joachimski et al., 1995; Böhm et al., 1996). Considering the estimated slow calcification rate of this species, the latter techniques are the most convenient for elucidating long term information (time scales of tens of years to centuries). Direct methods, however, have the potential advantage of revealing data on growth rates for shorter time scales (years to decades).

Calcein was first used in invertebrates to mark

TABLE 1. *Ceratoporella nicholsoni.* Successive *in situ* labeling with calcein (*). Abbreviations: T1, 9.VII.1984; T3, 15.II.1985; T4, 29.IV.1986; T5, 1.V.1987; T6, 1.V.1997.

Specimen number	Experimental period				
	T1	T3	T4	T5	T6
4	*	*	*	*	*
7	*	*	*	*	*
8	*	*	*	*	
9		*	*	*	*
10		*	*	*	*
11		*	*	*	*
14		*	*	*	*
16	*	*	*	*	*
17			*	*	*
27				*	*
29				*	*
DURATION					
T1-T3	221 days				
T3-T4		438 days			
T4-T5			363 days		
T5-T6				10 years	

the newly deposited calcium carbonate of the basal skeleton of *Ceratoporella* (Willenz & Hartman, 1985). Subsequently, this chemical has been used to record calcification amongst a wide variety of taxa such as brachiopods, bryozoans, molluscs and echinoderms (reviewed in Rowley & Mackinnon, 1995). More recently it has also been employed in studies of the growth dynamics of calcareous sponge spicules (Ilan et al., 1996), or to estimate the growth rate of the Indo-Pacific coralline sponges *Acanthochaetetes wellsi* (Reitner & Gautret, 1996) and *Astrosclera willeyana* (Wörheide, 1998).

From these studies, calcein appears to be permanently bound to calcium carbonate that forms in the presence of the dye, although the chemistry of the process has yet to be studied. Calcein has the advantages of fluorescing brightly under UV light and having only weak toxicity.

Several specimens of *Ceratoporella nicholsoni*, including the four individuals used in the first experiment by Willenz & Hartman (1985), were repeatedly stained and sampled at different intervals over 13 years, in order to evaluate potential growth rate variations among the sponges during extended periods of time.

MATERIALS AND METHODS

EXPERIMENTAL DESIGN. Two size categories of *Ceratoporella nicholsoni* were studied in a reef tunnel at depths ranging from 25-29m at Pear Tree Bottom, 5km E of Discovery Bay, Jamaica. The largest individuals, 10-15cm diameter, were labelled with calcein (Fluka 21030) *in situ* without being removed from their substrate. Labelling was performed from 1984 to 1997 at intervals given in Table 1. After the initial labelling in July 1984 (T1) a second incubation was performed six days later (T2), to test the

FIG. 1. *Ceratoporella nicholsoni.* Natural growth pattern. Ground section from specimen no. 16 sampled in May 1997, viewed by epifluorescence microscopy. Successive labeling events with calcein are indicated at apex of walls separating pseudocalicles, along aragonitic skeleton (A) extension axis. Living tissue (T) is brightly fluorescent. N=natural growth axis, S=surface of living tissue. (Scale bar=500µm).

TABLE 2. *Ceratoporella nicholsoni*. Annual growth rates during the 10 year experimented period. A, Kruskal-Wallis ANOVA on ranks (H=379.5 with 9 degrees of freedom; P<0.0001). B, All pairwise multiple comparison procedures (Dunn's Method). NS indicates no significant difference and * indicates significant (P<0.05) difference. Both statistics indicate a significant variability between specimens (P<0.005).

A. Specimen	Median	25%	75%	Mean	N
4	236.0	228.0	242.0	234.6	42
7	224.0	221.5	228.0	224.3	29
9	280.0	275.5	284.0	279.4	33
10	219.0	210.0	224.0	216.9	36
11	248.0	242.0	254.5	249.5	37
14	290.0	281.5	296.0	287.3	61
16	232.0	228.0	242.0	234.9	85
17	215.0	212.0	222.0	215.8	18
27	217.1	214.7	219.6	216.5	28
29	173.7	170.4	178.2	173.6	59

B. Specimen	4	7	9	10	11	14	16	17	27	29
4	-									
7	NS	-								
9	*	*	-							
10	*	NS	*	-						
11	NS	*	NS	*	-					
14	*	*	NS	*	*	-				
16	NS	NS	*	*	NS	*	-			
17	*	NS	*	NS	*	*	*	-		
27	*	NS	*	NS	*	*	*	NS	-	
29	*	*	*	*	*	*	*	NS	NS	-

sensitivity of the method. Although distinct bands could be detected at a distance of about 4µm (Willenz & Hartman, 1985), interval T1-2 was omitted in this analysis because of the shortness of the time period involved. Additional smaller specimens (11-25mm diameter) were removed from the substrate and cemented *in situ* to Plexiglas plates (5 specimens /12x12cm plate) with epoxy underwater patching compound (Pettit Paint Co no. 7050 & 7055). Plates were stored in Plexiglas racks placed on a ledge of the tunnel at the depth of collection.

To label the sponges with dye, the large specimens were individually enclosed within a plastic bag (of 4L volume) that was secured around the base of the sponge with nylon cords or rubber bands. In the case of plates bearing small specimens, the Plexiglas racks securing the plates were enclosed in a plastic bag. Calcein, dissolved in sea water, was injected in each bag to reach a concentration of 100mg/l. Bags were removed from the sponges after 12 or 24hrs.

For large specimens, samples of the skeleton, with attached living tissue, about 1-3cm^3 in volume, were removed with hammer and cold chisel from the periphery of the sponge, each specimen was left in place for further growth and regeneration. Small specimens were sacrificed after one year. Following dehydration in a graded series of alcohols, samples were embedded in Spurr's medium (Spurr, 1969). Sections, cut with a low speed diamond saw (Bennet Labcut 1010) were mechanically ground on a series of diamond grinding disks (Buehler ultra-prepTM) using a semiautomatic grinder (Buehler Minimet 1000) to a thickness of 5-10µm and observed under epifluorescence microscopy (Nikon Optiphot-2 microscope, excitation filter 340-380nm, barrier filter 420nm).

Growth increments of the aragonitic skeleton were established by measuring the linear extensions (in micrometers) between stained lines along growth axes at the apical edges of the wall separating two pseudocalices, or, for the most recent period, between stained lines and the surface of the skeleton.

DATA ANALYSIS. Statistical analyses were performed using SIGMASTAT and SIGMAPLOT (Jandel Scientific) data analysis and graphics software. All linear extension measurements were normalised as annual growth rate prior to their analysis. Non parametric Kruskal-Wallis analysis of variance (ANOVA) on ranks was performed to test two null hypotheses. 1) H_0: there are no differences in the average growth rates among specimens of *Ceratoporella* within a given

TABLE 3. *Ceratoporella nicholsoni*. Comparison between linear annual growth rate and regeneration growth rate. Unavailable data due to bioerosion in a specimen are indicated (*).

Period	Linear growth rate	Regeneration growth rate	Specimens
T3-4	230.5 + 61.2	194.2 + 42.2	7 - 9 - 10 - 17*
T4-5	232.0 + 59.6	238.4 + 38.8	7 - 9 - 10 - 11 - 17
T5-6	244.4 + 25.10	272.2 + 36.9	9 - 10 - 11 - 16

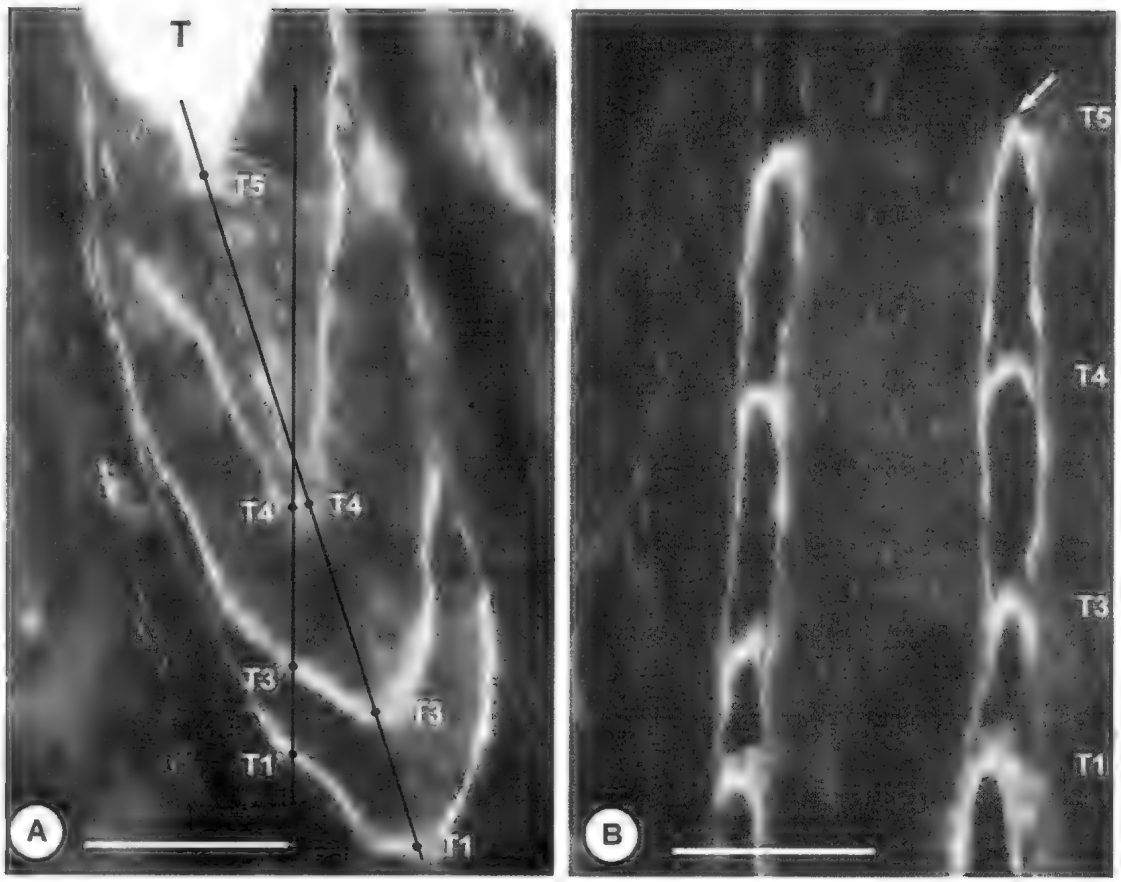

FIG. 2. *Ceratoporella nicholsoni*. A, ground section of fragment sampled at T5, view at base of pseudocalyx. Two possible orientations of section plane indicate that measurement can easily be biased when section is not parallel to growth axis (T1'-T3' < T1-T3; T3'-T4' < T3-T4). T= living tissue. (Scale bar=250μm); B, ground section of fragment sampled at T6, view at apex of wall separating two pseudocalices. Narrow structure of walls (arrow) prevents reading errors. Here, space between two walls has been filled as sponge grew upward. (Scale bar=250μm).

TABLE 4. *Ceratoporella nicholsoni*. Measurement reproducibility test. A Mann-Whitney test indicates that differences obtained from two different slides of the same sample are not significantly different (P < 0.005), except for specimen 16 in period T3-4.

Specimen slide no.	Period T3-4			Period T4-5		
	Mean	n	P	Mean	n	P
7 a	273.4	24	0.1120	287.6	24	0.0395
7 b	260.9	20		266.6	21	
9 a	268.1	35	0.0272	254.3	35	0.6680
9b	258.5	35		254.8	35	
10 a	160.7	26	0.7190	204.8	26	0.7490
10 b	159.1	24		202.4	24	
16 a	177.0	35	0.0054	211.6	35	0.7960
16 b	189.7	35		211.2	35	

period; 2) H_0: there are no differences in the average growth rates among various periods for a given specimen (Sokal & Rohlf, 1981; Fox et al., 1994). Where the ANOVA on ranks rejected the null hypothesis the Dunn's all pairwise multiple comparison procedure was used to determine the groups that differed from each other (P<0.005 level). A Mann-Whitney rank sum test was used where only two groups were to be compared. Significant differences were concluded at P<0.005 level. Data reproducibility was tested for four of the largest specimens by comparing measurements from a second ground section prepared from the same fragment.

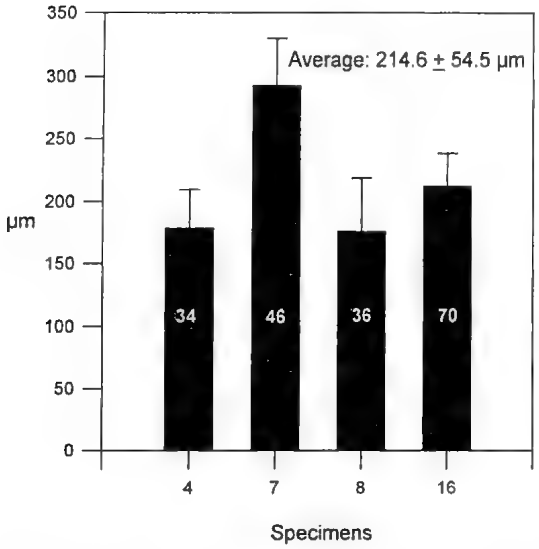

FIG. 3. *Ceratoporella nicholsoni*. Mean linear annual growth rates (µm yr $^{-1}$) of 4 specimens during period T1-3 (221 days). Average of all specimens is indicated. Numbers of measurements are indicated within bars. A Kruskal-Wallis ANOVA on ranks and all pairwise multiple comparison test (Dunn's method) indicate a significant variability between specimens ($P<0.005$), except between specimen numbers 4 vs 8.

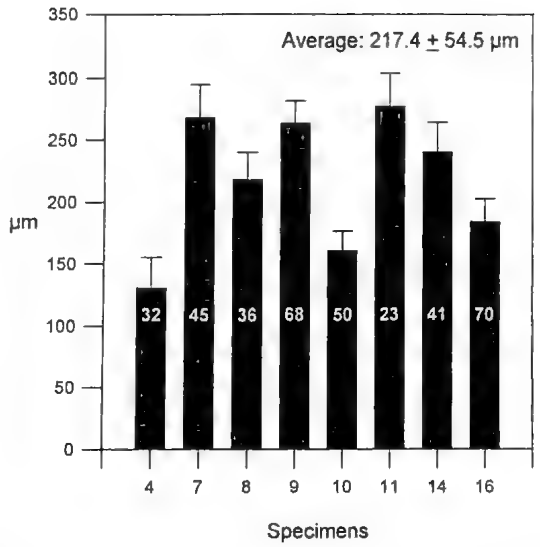

FIG. 4. *Ceratoporella nicholsoni*. Linear annual growth rates (µm yr $^{-1}$) of 8 specimens during period T3-4 (438 days). Conventions indicated as in Figure 3. A Kruskal-Wallis ANOVA on ranks and all pairwise multiple comparison test (Dunn's method) indicate a significant variability between specimens ($P<0.005$), except between specimens 11 vs 14, 14 vs 16, 8 vs 16, 16 vs 10, and 10 vs 4.

RESULTS

LINEAR ANNUAL GROWTH RATES. Observation of ground sections of fragments of *Ceratoporella nicholsoni* in epifluorescent microscopy revealed successive labelling with calcein (Fig. 1). Figure 2A-B shows details of the bright fluorescent bands at the base of a pseudocalicle and at the apex of walls separating two units, respectively. It is shown that variations in the orientation of sections can induce larger measurements errors at the base than at the apex. Consequently, only measurement at the apexes were considered.

Figure 3 presents the means of measurements of the four specimens of *Ceratoporella* successively marked during the first period T1-3 (221 days), as well as the average growth rate of the population. The results of Kruskal-Wallis ANOVA on ranks test indicate significant variability between the means ($P<0.0001$). However, a Dunn's all pairwise multiple comparison procedure determined that specimens no. 4 and 8 did not differ from each other.

Figures 4-6 present the same procedure for periods T3-4 (438 days), T4-5 (363 days) and T5-6 (10yrs), respectively. Identical statistical tests also indicate a significant variability between specimens, except for pairs indicated in the captions. In period T4-5, a batch of smaller samples gave rise to an average annual value (124.4±35.0µm yr^{-1}) reaching only half the average annual growth rate of the larger specimens (233.3±45.0µm yr^{-1}). However, individual measurements did not reveal a direct correlation between the size of sponges and their growth rate.

Table 2 presents the results of a Kruskal-Wallis ANOVA on ranks on data available from the longest interval (T5-6), showing a significant variability between specimens ($P<0.005$). An all pairwise multiple comparison procedure indicates in detail which pairs are significantly different.

Comparison of the mean annual growth rates of specimens from one period to the other (Fig. 7) also indicates a significant variability (191.1±30-269.9±37.0µm yr^{-1}), revealing that individual growth rate amongst *Ceratoporella* was not steady either.

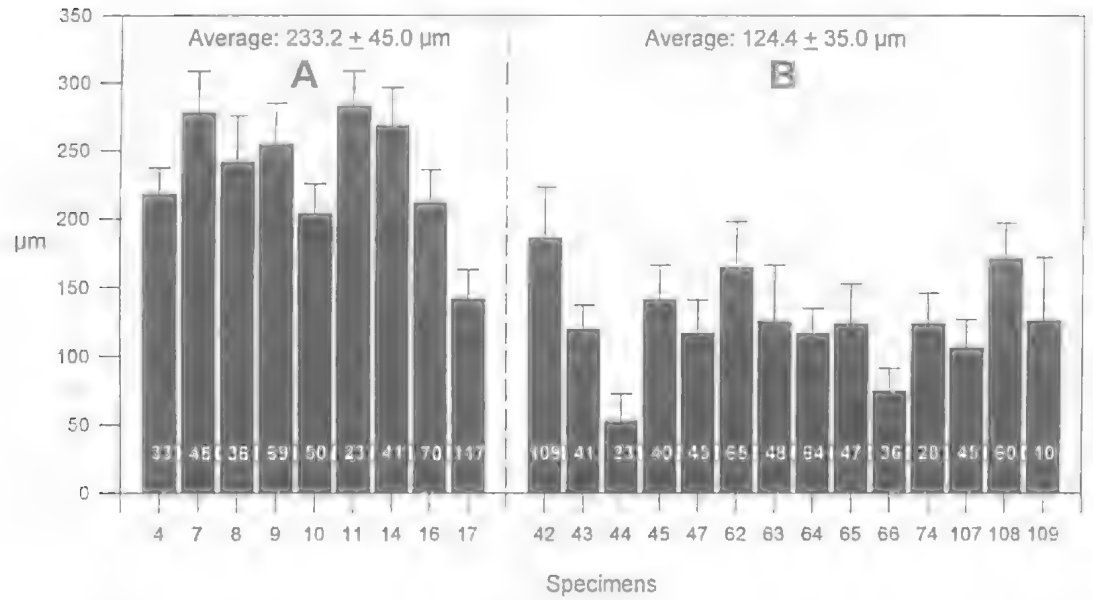

FIG. 5. *Ceratoporella nicholsoni*. Linear annual growth rates (µm yr^{-1}) of A, 9 large specimens and B, 14 smaller ones during period T4-5 (363 days). Conventions indicated as in Figure 3. A Kruskal-Wallis ANOVA on ranks and all pairwise multiple comparison test (Dunn's method) indicate a significant variability between specimens ($P<0.005$), except between specimens 4 vs 10, 8 vs 11, 8 vs 16, for the large specimens and specimens 42 vs 62, 45 vs 64, 108 vs 109, 109 vs 107, 107 vs 44 for the small ones. Both populations have distinct average annual growth rates.

REGENERATIVE ANNUAL GROWTH RATES. Each sampling caused an injury to the sponge, leaving a bare fracture of the skeleton (Fig. 8A). The living tissue rapidly extended over this fracture within a few weeks and covered it completely after a month (observations reported by a local diver). No detailed measurement was done, but at most, after 200 days the naked fracture was healed (shortest interval between personal observations).

Subsequent labelling followed by re-sampling in the healed zone (Fig. 8B) provided direct observation on the regeneration pattern of the skeleton. Ground sections show that new walls are progressively erected to form new pseudocalices perpendicularly oriented toward the fracture (Fig. 9).

Measurements of the extension of the skeleton show that in the first period following an injury, the average regeneration rate is lower than the average normal linear growth rate measured on the same specimens (Fig. 10, Table 3). In the subsequent periods, the regeneration rate increased, exceeding progressively the normal average growth rate.

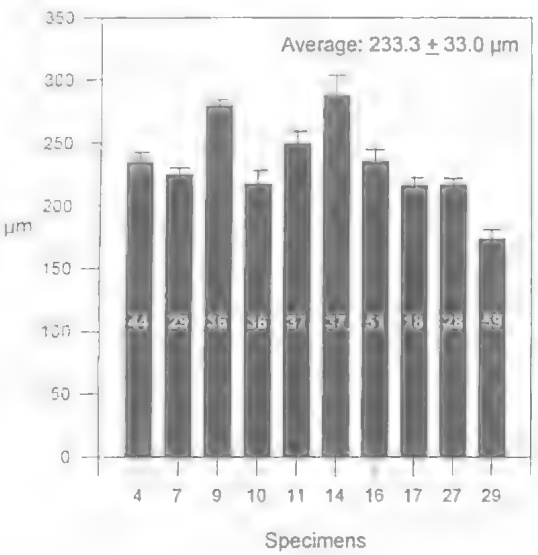

FIG. 6. *Ceratoporella nicholsoni*. Linear annual growth rates (µm yr^{-1}) of 10 specimens during period T5-6 (10 yr). Conventions indicated as in Figure 3. A Kruskal-Wallis ANOVA on ranks and all pairwise multiple comparison test (Dunn's method) indicate a significant variability between specimens ($P<0.005$), except as indicated in Table 2.

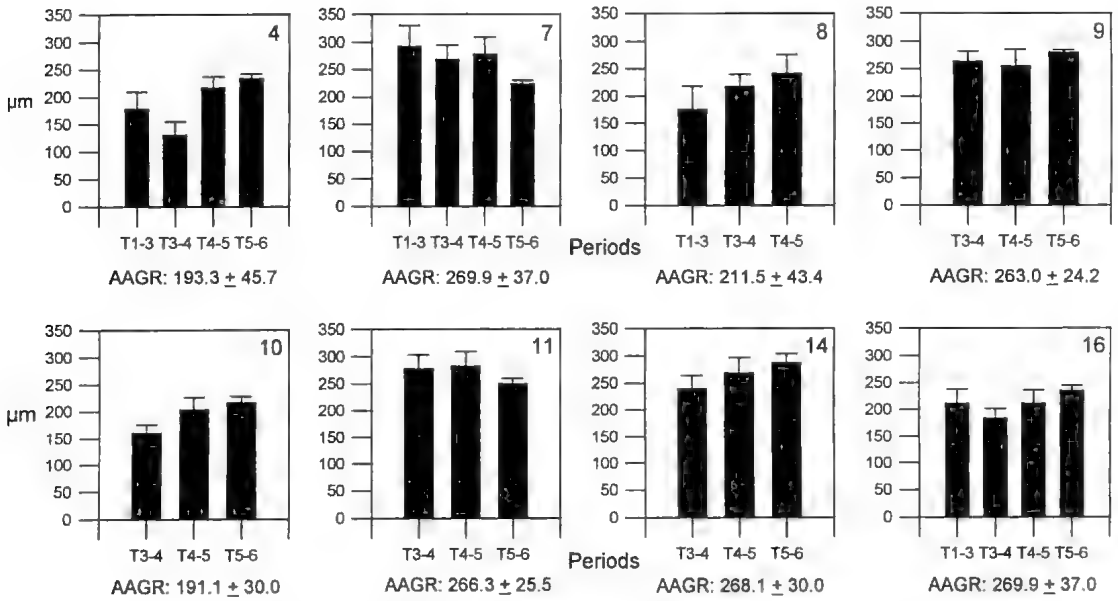

Fig. 7. *Ceratoporella nicholsoni*. Growth rate variability within samples, from one experimental period to another. A Kruskal-Wallis ANOVA on ranks and all pairwise multiple comparison test (Dunn's method) indicate a significant variability between means ($P<0.005$), except for the following specimens and periods: specimen 4 (T4-5 vs T5-6), specimen 7 (T1-3 vs T4-5; T 3-4 vs T4-5), specimen 10 (T4-5 vs T5-6), specimen 11 (T3-4 vs T 4-5), specimen 16 (T1-3 vs T4-5). Specimen numbers are indicated within each graph. AAGR= Average annual growth rate.

Obvious but unexpected marks from sampling were noticed on several specimens when revisiting them in May 1997 (Figs 8A-8B). Measurement of the extension of those particular regeneration areas indicates that someone had shown interest in our samples exactly one year before. Luckily, only one specimen had disappeared (specimen no. 8).

REPRODUCIBILITY OF THE MEASUREMENTS. In order to test the reliability of the method, measurements of pairs of sections prepared from the same fragment for four of the largest specimens were compared. A Mann-Whitney test indicates that differences obtained from two different slides were not significant, except for one specimen (Table 4).

DISCUSSION

Both tetracycline and calcein were used to initiate this study (Willenz & Hartman, 1985). First experiments found that tetracycline failed to label the skeleton of *Ceratoporella*. Although no harm was apparent to the organism, further experimentation in an attempt to adjust the concentration of the dye, to improve its recovery in the skeleton, was abandoned. This was decided in consideration of the potential effects the antibiotic might have on the abundant intercellular symbiotic bacteria present in sponge tissues (Willenz & Hartman, 1989; Hartman & Willenz, 1990). Calcein, first used then on invertebrates, was considered as a more appropriate benchmark to measure 'growth since marking'. At that time, there was no indication of the permanence of its strong fluorescence for long term measurements, whereas this study clearly showed that calcein is stable enough to mark aragonite for at least 13 years.

Based on calcein-labelling experiments, the average annual growth rates of *Ceratoporella nicholsoni* were shown to remain in the same range throughout the different experimental periods (214.6±54.5-233.3±33.0µm yr^{-1}). Measurements made over the largest time interval (10yrs) are obviously most indicative of average annual growth rates (233.3±33.0µm yr^{-1}). These values are comparable to other studies based either on indirect radiometric methods [270µm yr^{-1} using ^{14}C, and 220µm yr^{-1} using ^{210}Pb (Benavides & Druffel, 1986; Druffel & Benavides, 1986), 180-260µm yr^{-1} (Joachimski et al., 1995), and 220µm yr^{-1}

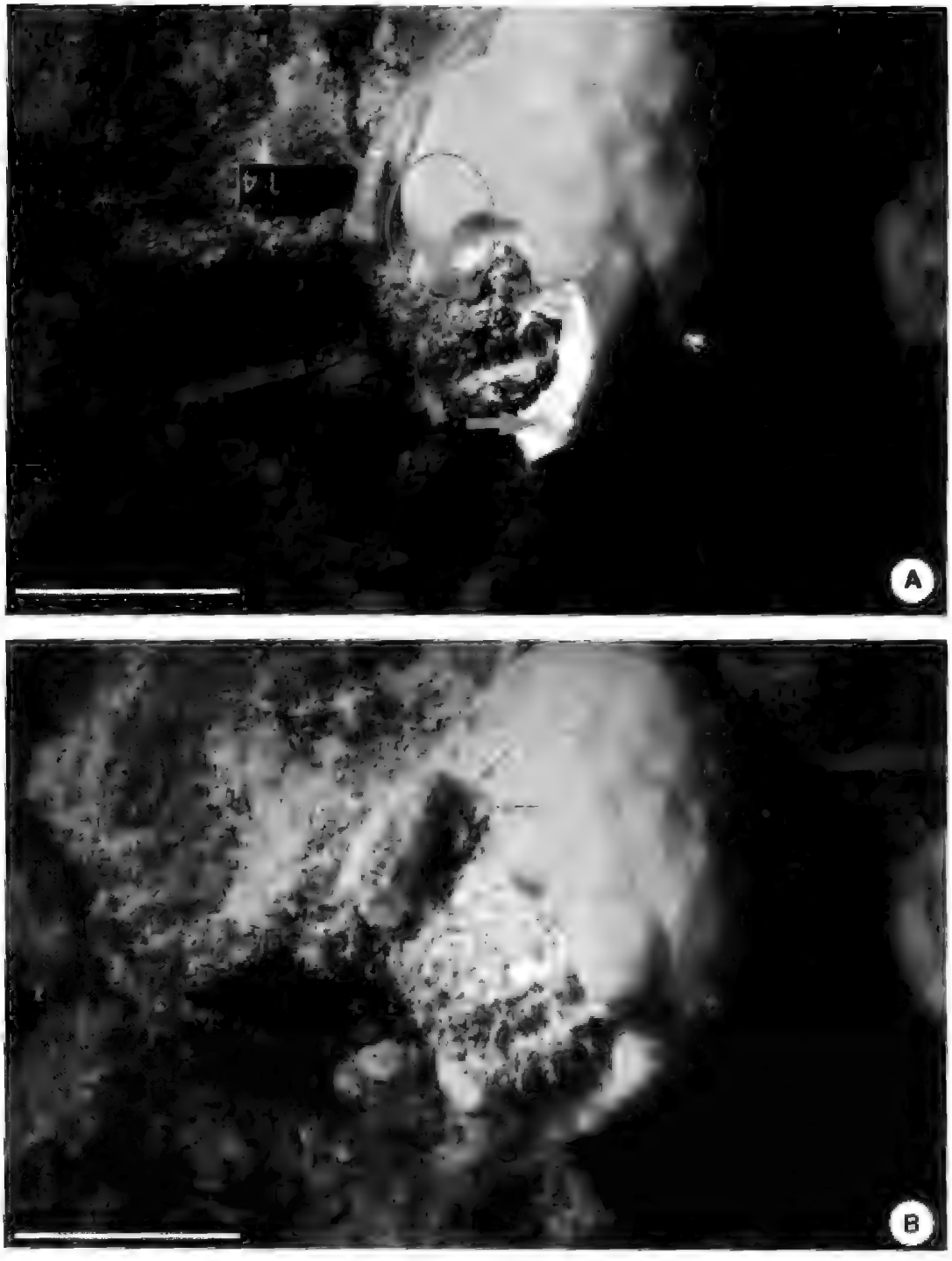

FIG. 8. *Ceratoporella nicholsoni*. A, Specimen no. 14 after a particularly severe sampling in May 1987. Arrow indicates fractured aragonitic skeleton. Encircled zone was found missing in 1997. (Scale bar=5cm); B, same specimen as in A, seen in May 1997. Zone fractured in 1987 has healed and shows round edges created by skeleton regeneration. Arrows indicate sharp edge of a more recent unexpected injury. (Scale bar=5cm)

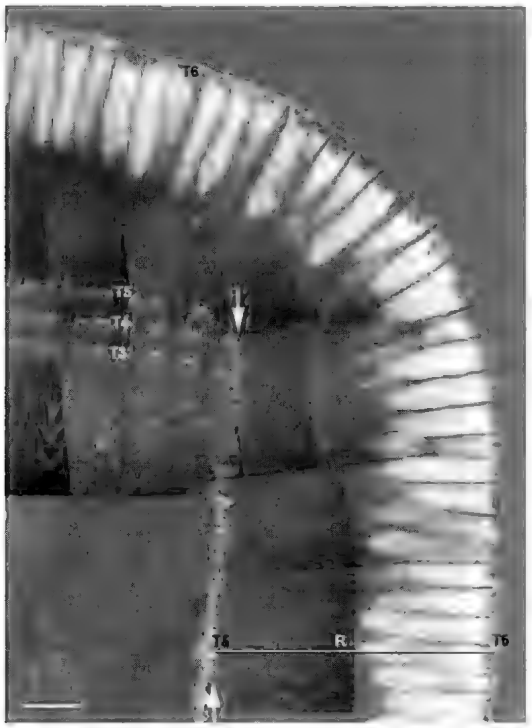

FIG. 9. *Ceratoporella nicholsoni*. Regeneration growth pattern. Ground section from specimen no. 10 sampled from its regeneration area in May 1997. Fracture, indicated by arrows occurred in May 1986, when a fragment was sampled. T6 indicates surface of skeleton marked with calcein 363 days later. R= regeneration growth axis. Epifluorescence microscopy. Captions as in Figure 1. (Scale bar= 500μm).

(Böhm et al., 1996)], or on direct methods (Dustan & Sacco, 1983; Willenz & Hartman, 1985). No details of analytical methods were reported by Dustan & Sacco (1983), who used alizarin red staining; their data remain approximate (0.1-0.2mm yr^{-1}). Initial values using calcein (Willenz & Hartman, 1985) were slightly lower (184.2±19.4μm yr^{-1}) than in the present study because measurements at the infilling zone at the base of pseudocalices were included in the latter study. Such artifacts were avoided here by rejecting measurements made in this zone, because they are too sensitive to minor deviations in the orientation of skeletal sections.

This study also provides clear evidence that statistically significant differences occur in the growth rate from one individual to another, within the same period of time. Moreover, the annual growth rate of a given individual also varied significantly with time.

Variations in growth rate were shown to occur in two other circumstances, suggesting that young tissues produce aragonite at a slower rate than tissues of older individuals. Firstly, measurements on a second population of smaller sized individuals, revealed a striking lower average annual growth rate (124.4±35.0μm yr^{-1}). Secondly, injuries made to large specimens induced horizontal regeneration zones which also appeared to reduce growth rate. In this latter case, however, after one year, growth was recorded but it was never higher than 18% of the normal growth figure. The impressive lateral regeneration growth rate (102-154 times faster) reported by Lehnert & Reitner (1997) corresponds to an ordinary extension of the living tissue produced to repair a damaged zone of the sponge prior to calcification. However, these authors did not record growth rates of the skeleton itself.

The present study examined populations of *Ceratoporella*, whereas most previous reports on growth rates were based on measurements of single specimens. These studies assumed a constant growth rate during the lifetime of the sponge. In this work we found that statistically significant variations are consequential, especially in researches relating growth rates and marine paleoenvironmental conditions such as water temperature or salinity, using skeletal chemistry of sclerosponges.

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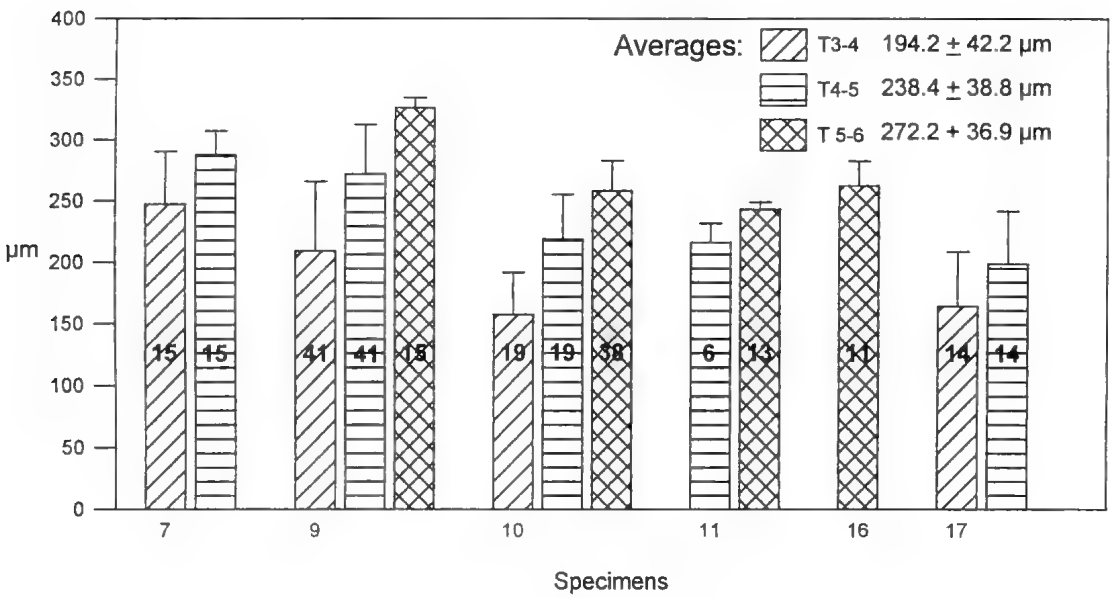

FIG. 10. *Ceratoporella nicholsoni.* Regenerative annual growth rate (µm yr^{-1}) of 6 specimens after injury caused by sampling. Conventions indicated as in Figure 3. A Kruskal-Wallis ANOVA on ranks and all pairwise multiple comparison test (Dunn's method) indicate a significant growth rate increment within a given specimen, from one period to the next one.

Marine Research of the American Museum of Natural History (1987), a subvention from Yale University (1987), and from the Léopold III Funds for Nature Exploration and Conservation (1997). Presentation of these data at the *5th International Sponge Symposium, Origin & Outlook,* was supported by the Queensland Museum and the Symposium sponsors, and a travel grant of the Fonds National de la Recherche Scientifique. Mr. Yves Barette patiently helped with slide preparations. We extend our gratitude to Dr Jackie Van Goethem for his encouragements and significant support in the completion of this work. This is Contribution No. 607 from the Discovery Bay Marine Laboratory, University of the West Indies, Discovery Bay, Jamaica, W.I.

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A SPONGE THAT CHEATS ON DIFFUSE MUTUALISM AMONG OTHER SPONGE SPECIES. *Memoirs of the Queensland Museum 44: 686. 1999*:- The demosponge *Desmapsamma anchorata* is frequently found growing on other organisms, especially gorgonians and other sponges. For paired *D. anchorata* individuals of the same genotype and initial size, growth rates were lower and mortality rates were higher on carbonate substrata than they were on sponges of other branching species. The three sponge species that served as hosts in the experiments, *Iotrochota birotulata*, *Amphimedon compressa*, and *Aplysina fulva*, grow and survive better when they are intimately intertwined with each other, and do not therefore discourage other sponges from adhering to them. However, *D. anchorata* does not improve the quality of life for these species when it participates in associations with them. *Desmapsamma anchorata* grows many times more rapidly than the other species, and appears to accomplish this by skimping on skeletal quality such that it requires skeletal support produced by other organisms in order to withstand physical disturbances. In the early stages of its growth on sponges of other species, *D. anchorata* does not decrease growth rates of its hosts, but as it continues to grow, it can entirely overwhelm the other sponges, smothering and killing enveloped tissue. The extreme fragility of *Desmapsamma anchorata* makes it vulnerable to being swept away by physical disturbance, and this prevents it from becoming a chronic hazard for the other sponges. Intimate association with *D. anchorata* may provide one benefit to other sponge species, which is to facilitate reattachment of loose fragments. Because *D. anchorata* is able to reattach to carbonate substrata within one day, fragments of other species to which it is attached are anchored for the few additional days that they require in order to establish their own stable attachments to solid substrata. ❐ *Porifera, mutualism, parasite, growth, mortality, asexual fragmentation.*

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PRODUCTION OF BIOACTIVE FURANOSESTERTERPENE TETRONIC ACIDS AS POSSIBLE INTERNAL CHEMICAL DEFENSE MECHANISM IN THE SPONGE *IRCINIA FELIX* (PORIFERA: DEMOSPONGIAE)

SVEN ZEA, FERNANDO J. PARRA, ALEJANDRO MARTINEZ AND CARMENZA DUQUE

Zea, S., Parra, F.J., Martinez, A. & Duque, C. 1999 06 30: Production of bioactive furanosesterterpene tetronic acids as possible internal chemical defense mechanism in the sponge *Ircinia felix* (Porifera: Demospongiae). *Memoirs of the Queensland Museum* **44**: 687-696. Brisbane. ISSN 0079-8835.

Marine sponges of the genus *Ircinia* (Porifera, Demospongiae, Ircinidae) are known to produce several linear furanosesterpene tetronic acids (FTAs) with antimicrobial, cytotoxic and antitumoral properties. *Ircinia felix* is a common and abundant sponge from Santa Marta, Colombian Caribbean Sea, containing FTAs in quantities up to 4.5% of its ash-free dry tissue weight. FTA concentration was quantified by HPLC after organic extraction in individuals of *I. felix*. The following results were obtained: 1) peripheral tissues had greater concentration than internal tissues; 2) total body FTA concentration was inversely and significantly related to the ambient illumination where individuals lived (in relation to depth, and comparing locations shaded vs. open to light, and localities differing in water turbidity); 3) there was no significant variation in FTA concentration throughout the time of study (June-December 1995); 4) over a 2 month period it was found that experimental shading induced a significant increase in total body FTA concentration; 5) there was a strong FTA increase (in a scale of 1 week-2 months) when sponges were manipulated in depth transference experiments and when they were purposely injured and; 6) intact or injured individuals did not exude measurable quantities of FTAs into the surrounding medium in laboratory conditions. Together, these results indicate that FTAs have some adaptive value, but probably not in mediating external ecological interactions, but instead acting as allomonal internal suppressors and/or antibiotics. The shade-dependent production of FTAs suggest that these substances may prevent parasitisation by photosynthetic *Aphanocapsa feldmani*-type endosymbionts, when the ambient illumination is below their compensation point. Additionally, as the sponge becomes more heterotrophic under lower light levels it may have an increased need for antibiotics in the choanosome to prevent bacterial food from becoming infectious. Finally, during wound healing, increased FTA levels may also act as internal antibiotic protection. ❐ *Porifera, furanosesterterpene tetronic acids, Ircinia, chemical internal defense, Caribbean.*

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The search for new drugs has led to the discovery of a variety of bioactive secondary metabolites in many terrestrial and aquatic organisms (Garson, 1994). In the marine realm, however, little is known about the use that source organisms make of these substances. It is commonly thought that sessile organisms use bioactive secondary metabolites as signals to communicate with conspecifics, to deter predators of adults (e.g. Bakus et al., 1986; Pawlik et al., 1995; Wulff, 1994; 1995), or propagules (Thompson et al., 1983), to actively or defensively compete for space (e.g. Sullivan et al., 1983; Aerts & Van Soest, 1997), or to prevent epibiosis or external damage by roaming browsers (e.g. Thompson, 1985; Walker et al., 1985; Thompson et al., 1987; Davis, 1991). Intraspecific variation in toxicity or in secondary metabolite composition has been documented in several groups of benthic sessile organisms (see review in Becerro et al., 1995). In sponges there are a few cases in which intraspecific variation of bioactive secondary metabolites, both in type and concentration, has been documented (Thompson et al., 1983, 1985, 1987; Kreuter et al., 1992; Becerro et al., 1995). This variation has been attributed to individual physiological defensive responses to differential environmental

pressures within its habitat range, especially the degree of spatial competitive interactions with neighboring macrobiota, and epibiosis prevention (Thompson et al., 1987; Becerro et al., 1995). Intra-individual (intercellular) variation in the location of bioactive secondary metabolites has been documented in a few sponges. In two cases, bioactive metabolites were found in inclusions within spherulous cells, located mostly near the surface of the sponge or around exhalant canals. These cells appear to disintegrate and release their inclusions, resulting in the exudation of metabolites through the pinacoderm, into the excurrent canals, and then into the boundary layer around the sponge. These metabolites are thought to be used for external defense-offense, but they may also be released into the mesohyl matrix for internal use (Thompson et al., 1983; Uriz et al., 1996b).

Species of *Ircinia* (Demospongiae, Dictyoceratida, Irciniidae) are known to produce linear furanosesterterpenes (Cimino et al., 1972a, b; Lumsdon et al., 1992; Urban & Capon, 1992; Capon et al., 1994; Davis & Capon, 1994; Murray et al., 1995). Recently we reported on three Caribbean species of *Ircinia* (*I. felix, I. strobilina* and *I. campana*), containing the novel (7E, 12E, 18R, 20Z)-variabilin as the major (58%-59.8%) furanosesterterpene tetronic acid, followed by a mixture of (8E, 13Z, 18R, 20Z)-strobilinin plus (7E, 13Z, 18R, 20Z)-felixinin (27.1%-28.6%) and a mixture of the new compounds (8Z,13Z,18R,20Z)-strobilinin and (7Z,13Z,18R, 20Z)-felixinin (13.1%-13.9%) (Martínez et al., 1995b, 1997). The greatest concentration of FTAs occur in *I. felix*, followed by *I. campana* and *I. strobilina* (Martínez, 1996). These FTAs were also found to occur as branched chain fatty acid esters, a unique combination never reported before in nature (Martínez et al., 1995a). FTAs have been demonstrated to have a variety of pharmacological properties: e.g. antibiotic (Faulkner, 1973; Martínez, 1996), cytotoxic (Martínez, 1996), antimicrobial and antitumoral (Gamboa & Pinzón, 1997), analgesic and anti-inflammatory (Del Valle & Vargas, 1997), calcium transport inhibition (Beveridge et al., 1995). Pawlik et al. (1995), argued that structurally complex secondary metabolites, which are usually present at high concentrations in sponges, can be physiologically expensive to produce, and thus must have an adaptive purpose. To test whether FTAs play an ecological role in *Ircinia* we studied the intraspecific and intra-individual variation in FTA concentration in *I. felix*, under various natural and experimental conditions. We found an inverse relationship between FTA concentration and ambient illumination, a greater concentration of FTAs in internal tissues, and an induced production of FTAs in wounded sponges. Here, we report on, and interpret these results in terms of internal defense mechanisms.

MATERIALS AND METHODS

STUDY AREA AND SOURCE ORGANISM. *Ircinia felix* (Duchassaing & Michelotti, 1864) was collected from rocky shores and mid-depth fringing reefs of Punta de Betín, and adjacent port dock-pilings in the bay of Santa Marta city (11°15'N, 74°13'W), and in rocky shores and fringing reefs of Isla Aguja, further to the NE (11°19'N, 74°12'W), Colombian Caribbean Sea. Compared to Isla Aguja, Punta de Betín generally has more turbid waters, and is subjected to greater sedimentation loads from an adjacent river, the city sewage outflows and commercial port activities. Reef corals in this locality have also suffered greater mortality, and reefs are amply colonised by sponges (Zea, 1994), especially by species of *Ircinia* (Parra, 1997). *Ircinia felix* has been described from this locality in detail by Zea (1987), and reference material is deposited in the collections of INVEMAR, Santa Marta, and the Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Bogotá. In the investigated localities this species lives in densities from 4-50 individuals/40m^2; it is usually thickly encrusting to cushion-shaped, occupying areas from about 30-100cm^2, and having maximal thickness from about 1-6cm; surface is conulose, usually clean and free of epibionts, with several interspersed oscules, 3-5mmdiameter, slightly raised by a membranous collar; external color in life varies from shades of maroon and amber in specimens in well illuminated locations, to dirty cream in shaded or deep locations; internal color is cream (Parra, 1997). Below a sand-filled ectosomal reticulation (cortex), there is a layer with dense aggregations of cyanobacteria of the *Aphanocapsa feldmani*-type, whose pigments are responsible for the color of the sponge (Rützler, 1990; Vicente, 1990). This species is typical for the genus in being very tough and difficult to cut or tear largely due to a dense reinforcement of spongin fibrils throughout the mesohyl. Species of *Ircinia* also yield a characteristic sulfur-garlic stench when handled (Bergquist, 1978), releasing several sulfur and cyanide volatiles (Bonilla, 1997). Two morphotypes are readily distinguishable in Santa Marta populations of *I. felix*: 1) encrusting,

dark amber surface, oscular skin collar dark brown, and,; 2) encrusting to cushion-shaped, maroon surface, oscular skin collar white (Zea, 1987). Only the latter morphotype, which is the most abundant in the study area (Parra, 1997) was used for the chemical ecology studies presented here.

EXTRACTION AND QUANTIFICATION OF FTAs. Whole sponges or fragments were immediately frozen upon return to the field laboratory base (INVEMAR, Santa Marta). Frozen material was air-shipped to the Natural Products Laboratory of the Universidad Nacional de Colombia at Bogotá, for chemical analyses. Extraction and quantification of bioactive FTAs was developed by Martínez (1996) and Martínez et al. (1997), and standardised for this study as follows: 5-10g of wet sponge were cut and macerated first in methanol (MeOH) and then in ethyl acetate (EtOAc), each for 15mins, removing the supernatant by filtration after each solvent addition. Each supernatant was separately vacuum-dried at 35°C in a rotatory evaporator, then diluted in EtOAc, mixed, and partitioned repeatedly with H_2O to eliminate sea-water salts. The EtOAc fraction was collected, dried with anhydrous sodium sulfate, filtered, vacuum-dried and weighed. Ash-free dry weight of the solid sponge residual was obtained by subtracting from the oven-dry weight (at 115°C) the ash weight obtained after combustion in a muffle furnace at 400°C. To prolong its stability during storage, the extract was then acetylated in a 20ml mixture of acetic anhydride-pyridine (1:1). The acetylated FTAs were then purified by silica-gel column chromatography, dried and stored under nitrogen atmosphere at 0°C until use. Acetylated FTAs were subjected to HPLC for final purification using MeOH-H_2O (85:15) as mobile phase at a flow rate of 1ml min^{-1} and a Capcell Pak C_{18} (250x46mm i.d.) column as stationary phase, monitoring at 270nm. Chromatograms typically gave three peaks between 9 and 12mins, whose subfractions were known to contain five different acetylated FTAs (Martínez et al., 1997). Since the largest and latest subfraction contained pure (7E, 12E, 18R, 20Z)-variabilin acetate (henceforth refered to as variabilin), a 10µg $µl^{-1}$ solution of this compound previously obtained was used to construct a calibration curve to calculate sample concentrations. Initial quantifications were done only on variabilin, but as the structure of all FTAs were being elucidated and found to be bioactive (Martínez, 1996), data for the three peaks were pooled for further analyses and calculated as mg FTA g^{-1} ash-free dry weight of sponge.

FTA CONCENTRATION IN *IRCINIA FELIX*. *Depth and ambient illumination factors.* To initially explore if there was variation in natural variabilin concentration in tissues between individuals across various environmental conditions, two specimens of *I. felix* were collected in June 1995 at each of four depths (5, 10, 15 and 20m), in conditions of open exposure to ambient illumination at the rocky shore and fringing reef of Punta de Betín, and two more at 4-9m depth in the adjacent well-shaded pilings of the Santa Marta port dock. Statistical differences in variabilin concentration between depths and in pilings were tested by one-way ANOVA; variabilin concentration in relation to depth was investigated by regression analysis. For all statistical tests, including those mentioned below, data was tested for homogeneity of variances between treatment combinations (Bartlett test), and for normality of residuals (Kolmogorov-Smirnov test); when suitable, transformations were applied and means and standard errors back-transformed for presentation (Sokal & Rohlf, 1981).

Variabilin concentrations in peripheral vs. choanosomal tissues. To compare variabilin and total FTA concentration in peripheral tissues (including pinacoderm and peripheral choanosomal tissues a few mm below the ectosome) vs. internal tissues (deeper within the choanosome), in open vs. shaded locations, two specimens were collected at 6-7m in the Punta de Betín rocky shore in June 1995 (variabilin only), and two more at 5-6m in the adjacent dock pilings in September 1995 (variabilin and total FTA). Peripheral tissues were dissected upon return to the laboratory and stored and processed separately from choanosomal tissues. Statistical differences in variabilin and FTA concentration between tissues were compared by one-way ANOVA for each habitat separately.

Exudation. Two assays were carried out to test whether FTAs are released by undisturbed and wounded *I. felix*. 1) Nine darkened and aerated aquaria filled with 0.5L of filtered sea-water were set up in the laboratory. Six specimens of *I. felix* were carefully collected with the substratum at 10m depth in Punta de Betín in October 1995 using hammer and chisel, and each placed in an aquarium. Three specimens were deeply wounded with a razor blade whereas the other three were left undisturbed; the three aquaria

without sponges were used as controls. Unwounded specimens were checked for vitality by observing the pumping of water through oscules. After 4 hours, sponges were removed and the water stored in cold (4°C), while it was being vacuum-filtered through RP-8 cartridges to retain organics. Cartridges were then kept frozen in the dark. 2) This assay was carried out in a similar manner to the first, in November 1995, with a single specimen for each treatment. The water was immediately partitioned in EtOAc, the organic fraction dried, put under nitrogen atmosphere and stored frozen in the dark. After shipment to Bogotá, cartridges were flushed with MeOH and EtOAc to release organics and the combined extracts dried. Extracts from both experiments were acetylated and quantified as mentioned above.

Ambient- and time-related differences, and experimentally induced production. To compare total FTA concentration in localities having different environmental conditions, three specimens of *I. felix* were collected at each of two depths (10 and 20m) in Punta de Betín and Isla Aguja, in September 1995. Three more specimens were collected at each of the same depths in Punta de Betín in December 1995.

Simultaneously, shading and depth transference experiments were carried out at Punta de Betín from September to December 1995 to test for additional production of FTAs. The above-mentioned specimens served as initial and final controls for natural FTA levels. At each of the two depths, three individuals were shaded by a canopy of wire nailed to the coralline bottom and covered by a black soft polyethylene plastic. As controls, two individuals were also covered by a canopy but with transparent plastic. Also, at each depth, a PVC tube frame holding four, 5cm-wide Plexiglas beds was nailed to the bottom. Four specimens at each depth were carefully collected together with the substratum using hammer and chisel, and fixed tightly on each bed with plastic cable ties; in each frame, two specimens came from the same depth as controls for manipulative experiments, and two specimens were transferred from the reciprocal depth. All sponges were collected simultaneously in December 1995, frozen and processed.

Natural, total FTA levels for two localities, two times (initial and final), and under shading and transference treatments and controls were statistically compared against depth in a two-way ANOVA (type III sums of squares), and a Tukey multiple comparisons procedure (separately for each depth), both appropriate for unbalanced designs (SAS Institute Inc., 1988). Time changes were tested, comparing in a separated one-way ANOVA, samples from Punta de Betín collected in June (see above), September and December.

Wound-induced production. To test for FTA production in tissues after wounding, six specimens were located and tagged at 10m depth from Punta de Betín in November 1995. Initially, a wound was produced by cutting free from the edge a 2-3cm wide fragment from each specimen, which was immediately frozen to measure initial, natural FTA levels. A similarly-sized fragment was again taken, cutting parallel to the initial wound, from each of three specimens seven days later, and from each of the other three specimens 14 days later; these were frozen to measure FTA concentration changes in the wound area in the intervening period as a result of the initial wound. Initial and final FTA concentrations were compared for each time interval set by a t-student test. FTA changes for individual sponges were compared (null hypothesis of no change) by a paired t-student test, separately for each time interval.

RESULTS

NATURAL FTA LEVELS. Total body content of FTAs in *I. felix* ranged from about 1-46mg g^{-1} of ash-free dry tissue weight (Table 1; 0.1%-4.6% by weight). Significant variation in total FTA and/or variabilin (58-60% of total) content in samples was found as follows.

Ambient illumination factor. There was a significant increase in variabilin concentration in tissues of *I. felix* with increased depth at the fringing reef of Punta de Betín in June 1995 (Fig. 1). This trend followed a potential regression model [variabilin] = $0.0453\ Z^{1.82}$, R^2=0.95, $0.01<P<0.025$ (see Martínez, 1996). Since light intensity decreases exponentially with depth in sea-water, ambient illumination (not measured) was assumed to at least relate partly to variabilin concentration. Deviations from regression, however, accounted for a significant part of the model (R^2=0.04, $0.025<P<0.05$), denoting that other factors apart from depth (and light) are important in determining variabilin concentration. In addition, variabilin concentrations similar to those found at 20m, were measured in sponges collected simultaneously at 4-9m in the shaded nearby pilings of Santa Marta port (Fig. 1). These values were significantly greater than those of the sponges collected at 5m in the open fringing reef.

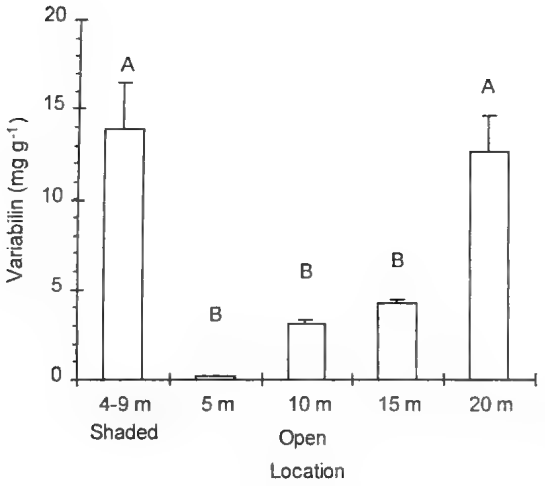

FIG. 1. Concentration of (7E,12E,18R,20Z)-variabilin (mg g^{-1} ash-free dry weight of sponge, mean±one standard error) in *Ircinia felix* under natural conditions, in shaded (commercial port dock pilings, 4-9m depth), and open (rocky-shore and fringing reef, 5-20m depth) locations at Punta de Betín in June 1995. Bars sharing the same letter are not significantly different from each other in a Tukey multiple comparison procedure (after one-way ANOVA, R^2=0.93, P=0.004, n=2 samples per location-depth).

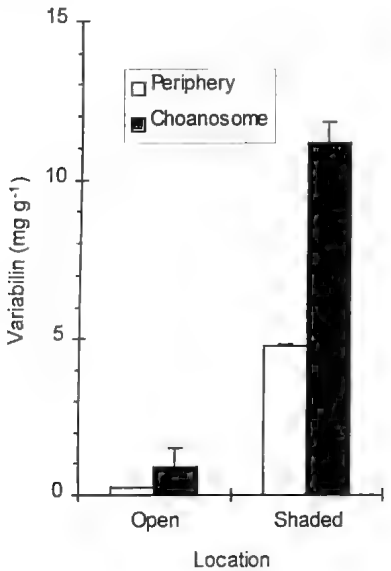

FIG. 2. Concentration of (7E,2E,18R,20Z)-variabilin (mg g^{-1} ash-free dry weight of sponge, mean±one standard error) in peripheral vs. choanosomal tissues of *Ircinia felix* in two different ambient illumination regimes (open rocky shore, 6-7m depth, June 1995; shaded dock pilings, 5m depth, Sept. 1995).

Further comparisons of total FTA concentration in September and December 1995 at Punta de Betín revealed a similar depth trend (Fig. 3A,D, representing 10 and 20m depths, respectively). The same was also true for Isla Aguja, although the absolute levels were much lower that at Punta de Betín at both depths, presumably due to greater light penetration in its generally more transparent waters. Together, these results show that ambient illumination is an important factor related to FTA natural concentration in tissues of *I. felix*.

Time factor. At 5m depth, both in open (Punta de Betín rocky shore) and shaded (dock pilings) locations, there was no significant difference in variabilin concentration between samples collected in June and September (two-way ANOVA, R^2 [time] =0.007, P= 0.48, for data, compare Fig. 1 and Fig. 2 choanosomal tissue). Similarly, for 10 and 20m depths, there were no significant differences in variabilin concentration between sponges collected in June, September and December 1995 at Punta de Betín (two-way ANOVA on time and depth, R^2 [time] =0.01, P=0.33, data from Fig. 1, and corresponding variabilin values of Fig. 3A, not shown), nor was there any difference in total FTA concentration between September and December (see Fig. 3A).

Variabilin in sponge tissues. Peripheral tissues of *I. felix* showed a significantly lower concentration of FTA than internal tissues, both in shaded (dock pilings, one-way ANOVA, R^2=0.96, P=0.0002, n=2) and open locations (Punta de Betín rocky shore, one-way ANOVA, R^2=0.99, P=0.003, n=2) at similar depths, although concentrations were significantly higher in shaded sponges in both tissues (approximately 23 times in peripheral and 12 times in choanosomal tissues to that of sponges open locations) (Fig. 2). This implies that lower FTA concentrations in peripheral tissues is not directly due to ambient illumination.

FTA PRODUCTION INDUCTION. *Exudation.* Waters surrounding individuals of *I. felix* kept in aquaria for a few hours, even when wounded, did not show traces of FTAs; both RP-8 cartridge filtration and direct EtOAc extraction gave negative results. Hence, it seems that FTAs are not released into the surrounding water of the sponge, at least in detectable quantities.

Shade-induced production. Shading experiments at 10 and 20m depths in Punta de Betín revealed a significantly greater FTA concentration when sponges were covered for 3 months with a dark

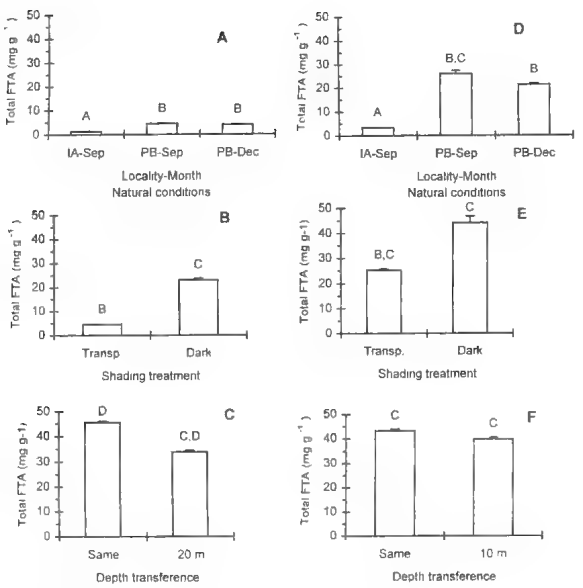

FIG. 3. Total FTA concentration (mean±one standard error, mg g^{-1} ash-free dry weight of sponge, mean±one standard error) at 10m (A-C) and 20m (D-F) depth in *Ircinia felix* under natural conditions (A and D) at Isla Aguja (IA-September 1995) and Punta de Betín (PB-September and December 1995) fringing reefs, and after shading (B and E) and depth transference experiments (C and F) at Punta de Betín. Bars on each depth having the same letter were not significantly different from each other in a Tukey multiple comparison procedure (after two-way ANOVA, Log_{10} transformed data, means and standard errors backtransformed, R^2 full model =0.99, P=0.0001, n=3 for each treatment-depth combination except for shading transparent (Transp.) controls and depth transference, in which n = 2).

shade, than when sponges were covered with transparent plastic, or were left alone under natural light. Initial and final natural levels, as well as transparent cover controls, were similar in FTA concentration at both depths, while the dark shade elicited a 5 times increase at 10m, and an about 2 times increase at 20m depth (Fig. 3B, E).

In contrast, further experiments failed to demonstrate an expected decrease in FTA concentration when sponges were transferred from 20m to 10m depth, due to a manipulation effect. Both control and reciprocal transference resulted in a significant increase after three months to a similar FTA concentration, when compared to untreated controls, regardless of depth (Fig. 3C, F).

Wound-induced production. A significant increase in FTA concentration from the initial, natural levels were found seven days (x 6 times, t-student test t=4.74, P=0.04, n=3) and 14 days (x 9 times, t=18.19, P=0.003, n=3) after wounding sponges at 10m depth in Punta de Betín fringing reef (Fig. 4). Initial levels were similar for the two sets of three sponges (t-student test, t=1.08, P=0.34). Throughout the experiment, the wound area was bleached, and a substantial release of mucus was noted while the wound was sealed.

Transference of depth experiments (see above, Fig. 3C, F) elicited FTA increases due to manipulation, probably also as a result of internal and external damaged done to sponges by squeezing them tight to the experimental frame. A few days after transference, partial bleaching and discoloration was noted; a few specimens were necrotic in areas contact to the cable ties. Slight differences between transference sets were probably due to the degree of manipulation, which elicited more-or-less damage.

DISCUSSION

This work is the first demonstration of intraspecific and between-tissue variation in FTA contents in the marine sponge *Ircinia felix*, in relation to several environmental factors and biological stress conditions. A slight degree of variation in deterrence of feeding by fish was found in pelletised extracts from Caribbean *Ircinia* species (including *I. felix*), indicating indirectly that toxic secondary metabolites may vary intraspecifically (Pawlik et al., 1995). Our results show that FTAs are not exuded free, in mucus, or after injuries, into the surrounding water, and that they also occur in a lower concentration in the peripheral tissues. Hence, an external defensive (epibiosis, predation) and offensive (competition for space) role cannot be directly ascribed to FTAs. This contrasts with the fact that *Ircinia* species usually have an epibiont-free surface (Parra, 1997), their pelletised crude extracts are not consumed by fish (Pawlik et al., 1995), and they have been found to be aggressive *in situ* against corals (Aerts & Van Soest, 1997), and these defensive--offensive mechanisms are thought to be chemically mediated. Perhaps FTAs are bad-tasting, or toxic if swallowed, hence the above-mentioned rejection by fish (J. Pawlik, pers. comm., 1998).

Nevertheless, other investigations carried out by us showed that *I. felix* passively and actively (upon injury) releases into the surrounding medium other noxious volatile compounds (Bonilla, 1997) which may be partly responsible for the above-mentioned ecological interactions.

Our results are also in contrast with those reported for other sponges that have so far shown environmentally induced variation of bioactive secondary metabolites, in which the higher concentrations of these metabolites have been found in the periphery of the sponge, or in cell types surrounding the internal canal system (e.g. *Aplysina fistularis, Rhopaloeides odorabile, Verongia aerophoba, Crambe crambe*; see Thompson et al., 1983, 1987; Kreuter et al., 1992; Uriz et al., 1996a; respectively). Some of these sponges contain metabolites which may be released into the milieu, either in areas of direct contact with other sessile organisms, or exuded into the water (inside the canals or into the surrounding medium), to act in various chemically-mediated interactions (Sullivan et al., 1983; Thompson et al., 1983; Thompson, 1985; Walker et al., 1985; Kreuter et al., 1992; Becerro et al., 1995).

However, similar to FTAs, the bioactive compound avarol and its derivatives, produced by the Mediterranean sponge *Dysidea avara,* are found only in the choanosome, specifically within choanocytes (Uriz et al., 1996b). In fact, the latter authors have cautioned against ascribing a natural function to avarol in lieu of the possibility of it being a by-product of extraction and manipulation procedures. In addition, there are no reports of environmentally induced variation in avarol. As for FTAs, the mild extraction and purification procedures used are not strong enough to either generate their functional groups, or, in case these molecules only occur in the sponge tissues as fatty acid esters (cf. Martínez et al., 1995a), to break their ester bond (Martínez, 1996). Hence, we argue for an adaptive, internal defense function of FTAs based on: 1) their presence in the tissues in free, bioactive form, and in relatively high concentrations, implying an energetic cost of production which should be balanced against other needs (cf. Pawlik et al., 1995); 2) natural (between individuals and between tissue areas of the body) and experimentally-induced variation in concentration, in relation to ambient illumination; and 3) injury-induced production.

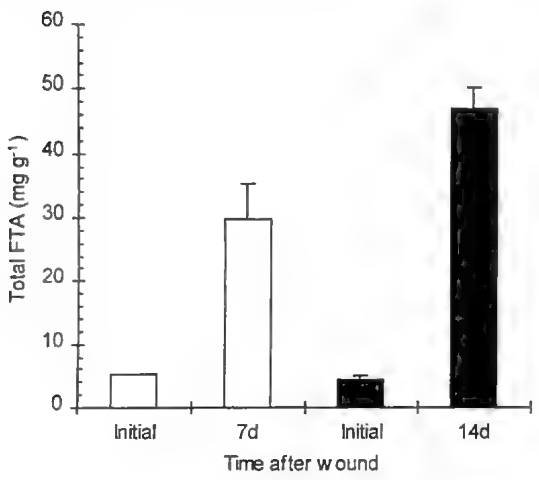

FIG. 4. Changes in total FTA concentration (mg g^{-1} ash-free dry weight of sponge, mean±one standard error) in *Ircinia felix*, 7 d (open bars) and 14 d (shaded bars) after eliciting a wound; n = 3 for each set.

One could also argue that the natural and shade-induced variations found in FTA concentration are an artifact of their lability to light, or to the result of a light-dependent biosynthetic mechanism (e.g. Kreuter et al., 1992). However, the relatively low FTA levels in choanosomal tissues in sponges located in open conditions cannot be ascribed to a light-dependent effect because the peripheral tissues have a strong pigmentation (Parra, 1997), that probably prevents light from reaching deeply into the choanosome (Wilkinson & Vacelet, 1979; Wilkinson, 1980). Similarly, finding a lower FTA concentration in peripheral vs. choanosomal tissues in discolored sponges from very dark habitats argue against light-dependent degradation.

The following are possible (and not mutually exclusive) explanations for our results in regard to internal defense.

FTA CONCENTRATION AND PRODUCTION INDUCTION IN RELATION TO AMBIENT ILLUMINATION. The causality of light (and tissue)-related FTA natural levels and shade-related production may be interpreted in the following ways.

1) FTAs are used for (partial) control of *Aphanocapsa feldmani*-type cyanobacterial symbionts. Apart from cellular mechanisms for repression (e.g. phagocytosis) of endosymbiont population growth (Simpson, 1984), antimicrobial secondary metabolites could help in their repression. Assuming an antibiotic or cytostatic affect of FTAs on *A. feldmani*, a lower

FTA concentration is retained in the ectosomal tissues of *I. felix*, where this bacteria is located, to allow it to carry out its symbiotic role. Also, an overall concentration of FTAs is maintained in inverse relation to light levels, to prevent *A. feldmani* from parasitising the sponge once its production/respiration ratio is below unity (i.e. below the compensation point). This role is difficult to test from our observations under natural conditions, since light itself may be directly responsible for *A. feldmani* control. In fact, in contrast to other, more phototrophic sponges (e.g. Seddon et al., 1992) and zooxanthellate corals (e.g. Titlyanov, 1981; Jaubert, 1981), in which symbiont density and total chlorophyll-a concentration tend to be inversely related to ambient illumination (and hence depth), in *I. felix* coloration intensity (and also chlorophyll-a concentration) decreases with decreasing illumination (unpublished results). Through cell dissociation procedures, we have failed to do precise direct counts of *A. feldmani* from *I. felix* collected at various depths and light regimes, because the dense network of spongin fibrils prevented a total separation of bacterial cells (histological procedures are needed). In short, to test this role, the sponge-cyanobacterial metabolism in relation to light, and the capacity of this cyanobacteria to live heterotrophically off the sponge, should be explored, as well as a direct, *in vitro* control of population growth by FTAs. We have succeeded in isolating the cyanobacteria, but have failed in its culture; trials are under way to test the effect of crude sponge extracts on recently isolated cyanobacteria.

2) FTAs are used to help in controlling bacterial food (e.g. Bergquist & Bedford, 1978). Assuming *I. felix* turns more heterotrophic with decreasing light (as translocation of photosynthates from cyanobacteria decreases), there may be an increased need for antibiotics in the choanosome to prevent bacterial food from parasitising the sponge. The sponge may also make use of its own stores of endosymbiotic heterotrophic bacteria as food when photosynthesis is off (Simpson, 1984; but see Wilkinson et al., 1984), and may use FTAs to regulate its bacterial flora (Thompson et al., 1983).

In the above context, the relative constancy of FTA concentration in *I. felix* throughout the study period may indicate that seasonal changes in ambient illumination and degree of phototrophy-heterotrophy are not enough to exert an important effect on the *Aphanocapsa*-FTA system. In contrast, other studied sponges have shown annual cycles of toxin production related to internal physiological cycles (e.g. reproduction), and external ecological pressures (e.g. competition for space) (Turon et al., 1996).

FTA PRODUCTION INDUCTION AS A RESULT OF INJURIES. FTAs may act as a mid-term (days to weeks) internal antibiotic protection during wound healing (e.g. after predator bites, sand scouring, etc.). However, this increase does not lead to exudation. Instead, rapid (minutes to hours) release of noxious volatile compounds (Bonilla, 1997) may act in the short term to protect wounded sponges of the genus *Ircinia*, as it has been found for other sponges (Thompson, 1985; Walker et al., 1985).

Regardless of its specific mechanism of use, it can be advanced that FTAs possess allomonal effects (cf. Whittaker & Fenny, 1977), acting as internal supressors and/or antibiotics against the sponge's own bacterial endosymbionts, or against external bacterial invaders from food or through injuries.

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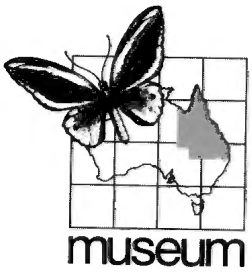

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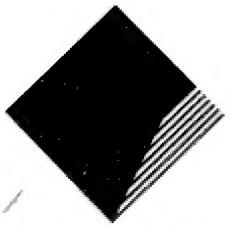

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