VOL. 27 1986

MALACOLOGIA

International Journal of Malacology

Revista Internacional de Malacologia

Journal International de Malacologie

Международный Журнал Малакологии

Internationale Malakologische Zeitschrift

Z. A. Filatova

K. Hatai

A. Myra Keen
1905–1986
see pp. 375–404

C. M. Yonge

Publication dates
Vol. 26, No. 1–2—9 July, 1985
Vol. 27, No. 1–7 March, 1986

MALACOLOGIA, VOL. 27

CONTENTS

VOL. 27, NO. 1

1986

MALACOLOGIA

International Journal of Malacology

Revista Internacional de Malacologia

Journal International de Malacologie

Международный Журнал Малакологии

Internationale Malakologische Zeitschrift

MALACOLOGIA

Editors-in-Chief:

GEORGE M. DAVIS

ROBERT ROBERTSON

Editorial and Subscription Offices:

Department of Malacology
The Academy of Natural Sciences of Philadelphia
Nineteenth Street and the Parkway
Philadelphia, Pennsylvania 19103, U.S.A.

Associate Editors:

JOHN B. BURCH
University of Michigan, Ann Arbor

ANNE GISMANN
Maadi, A. R. Egypt

Editorial Assistant:

MARY DUNN

Assistant Managing Editor:

CARYL HESTERMAN

MALACOLOGIA is published by the INSTITUTE OF MALACOLOGY, the Sponsor Members of which (also serving as editors) are:

KENNETH J. BOSS, *President-Elect*
Museum of Comparative Zoology
Cambridge, Massachusetts

JOHN B. BURCH

MELBOURNE R. CARRIKER, *President*
University of Delaware, Lewes

GEORGE M. DAVIS
Secretary and Treasurer

PETER JUNG, *Participating Member*
Naturhistorisches Museum, Basel, Switzerland

JAMES NYBAKKEN, *Vice-President*
Moss Landing Marine Laboratories
California

OLIVER E. PAGET, *Participating Member*
Naturhistorisches Museum, Wien, Austria

ROBERT ROBERTSON

CLYDE F. E. ROPER
Smithsonian Institution
Washington, D.C.

W. D. RUSSELL-HUNTER
Syracuse University, New York

NORMAN F. SOHL
United States Geological Survey
Washington, D.C.

SHI-KUEI WU
University of Colorado Museum, Boulder

J FRANCIS ALLEN, *Emerita*
Environmental Protection Agency
Washington, D.C.

ELMER G. BERRY, *Emeritus*
Germantown, Maryland

EDITORIAL BOARD

AMERICAN MALACOLOGICAL UNION SYMPOSIUM PROCEEDINGS
MOLLUSCAN EXTINCTIONS IN THE GEOLOGIC PAST
AND AT THE PRESENT TIME

Organized by Geerat J. Vermeij
9 August 1983 Seattle, Washington, U.S.A.

MALACOLOGIA, 1986, 27(1): 1–2

MOLLUSCAN EXTINCTION: INTRODUCTION TO A SYMPOSIUM

Geerat J. Vermeij

Department of Zoology, University of Maryland, College Park, MD 20742, U.S.A.

Most of the species that have ever lived are extinct. This simple fact may seem a tired cliché yet it has profound implications for the study of evolution and, perhaps even more importantly, for study of the effects that humans are having on the world's biota. Few topics are as theoretically interesting and as important to human welfare as extinction.

Molluscs are an exceptionally attractive group for the study of extinction. Their fossil record is better than that of most other groups, and shelled molluscs are ecologically and biogeographically perhaps the best understood animals among invertebrates.

A host of important questions awaits the willing investigator. Which factors bring about the extinction of species? How do extinct species differ morphologically, ecologically, and biogeographically from survivors? Which features enable species to persist through crises, and how do these features affect post-crisis evolution? What is the tempo of extinction; does extinction occur continuously, or is it concentrated during certain brief intervals of time? Are there qualitative differences between the famous mass extinctions, such as those at the end of the Permian and Cretaceous Periods, and other less devastating events such as those in the Pliocene? What effects has extinction had on surviving species? Can the extinctions of the geological past be held up as useful models for the extinctions taking place in the present day as humans are eliminating species and habitats on an ever increasing scale?

During a symposium that was held on the morning of August 7, 1983, at the annual meeting of the American Malacological Union in Seattle, Washington, five papers dealing with various aspects of extinction were presented. Four of these papers are published in this issue.

P. Ward examines the pattern of selectivity and timing of extinction in ammonoids during the Cretaceous Period. His analysis goes a long way toward understanding the eventual global failure of these externally shelled animals.

G. J. Vermeij and E. J. Petuch also examine the pattern of selectivity of extinction. Their analysis highlights the extinction of gastropods and pelecypods during the Pliocene in tropical America. Perhaps the most important conclusion is that the striking architectural differences currently existing between the gastropod faunas of the tropical Western Atlantic and Eastern Pacific are, at least in part, attributable to differential extinction after the uplift of the Panama Isthmus in the Pliocene.

D. Jablonski and K. Flessa present an analysis of the distribution of molluscan and echinoderm families on oceanic islands and continental shelves. They show that a reduction in shallow-shelf area owing to a catastrophic lowering of sea level, such as the one that is believed by some to have contributed to the mass extinctions near the close of the Permian and Cretaceous Periods (or the Paleozoic and Mesozoic eras), would not cause a major extinction at the family level among living molluscs. They suggest that changes in sea level have in general been of secondary importance as direct agencies of extinction. The close association between sea level and extinction in the fossil record may be fortuitous, or an indirect consequence of biogeographic or climatic perturbations.

The final paper, by M. Hadfield, is an elegant analysis of the way in which populational characteristics have rendered several Hawaiian endemic land snails exceptionally susceptible to decimation and extinction as a result of chronic harvesting by overzealous human collectors. His work is a beautiful example of how theoretical ecology can be applied to the practical problems arising when humans tamper with the species and environments around them. Hadfield's work is also important in that it highlights the value of the scholarly gathering together of anecdotal accounts from unpublished notebooks and other obscure sources more familiar to the historian of human affairs than to a biologist.

These contributions illustrate the important role that the study of molluscs can play in enhancing our understanding of extinction. They also forcefully remind us that results and implications of great practical significance often flow from studies of seemingly abstruse and esoteric topics. The man in the street might be disinclined to fund research on ammonoids or theoretical ecology, yet such research provides the essential groundwork for the resolution of problems of great practical significance, such as the human impact on habitats and species.

MALACOLOGIA, 1986, 27(1): 3–28

CRETACEOUS AMMONITE SHELL SHAPES

Peter Ward[1]

University of California, Davis

ABSTRACT

Cretaceous ammonite genera, and Cretaceous ammonite species of the Great Valley Sequence, California, are analyzed for shell morphology on a stage by stage basis. Each taxon was measured for shell ornamental rugosity (shell rib width divided by whorl height), and overall shell shape (either planispiral or heteromorphic, with planispiral taxa being measured for the S statistic of Raup, 1967). For both generic and species level data, shell ornamental rugosity for the Cretaceous stages was compared by placing all taxa into one of four ornamental categories, based on the rib width/whorl height measurement. It was found that at both the species and generic level, shell ornamental rugosity increased progressively during the lower Cretaceous, and reached a peak in the Cenomanian-Turonian. During the remainder of the Cretaceous, rugosity of the generic and species level populations diminished. By placing all members of the two data sets into one shell shape category (either *ornamented*, with rib width/whorl height ratios of .15 or greater), *streamlined planispiral* (S values of .5 or less), *heteromorphic* (all non-planispiral shells) or *non-streamlined, non-coarsely ornamented planispiral*, stage by stage examination of the relative proportions of these shell shape categories was carried out. In addition to the mid-Cretaceous peak of coarsely ornamented forms, these analyses revealed two peaks of heteromorphic diversity, during the Lower and Upper Cretaceous. Streamlined forms also showed their highest relative abundances during the latest Cretaceous.

INTRODUCTION

Perhaps the greatest difference between marine ecosystems of the Mesozoic and Cenozoic Eras was the presence of thousands of species of chambered cephalopods, mainly ammonites, in the former. During the Mesozoic Era the ammonoids were numerically abundant in all of the world's seas, and judging from their commonality in a variety of sedimentary facies, must have been important constituents in a wide variety of marine ecosystems. They cannot be excluded from any discussion of evolution within the pelagic realm, for they played a vital part in the marine Mesozoic history of the earth.

The mode of life of Mesozoic ammonoids has been the topic of scientific inquiry for over a century, but remains controversial because of the complete lack of living ammonoid species. Our nearest living analogue is the Indo-Pacific cephalopod *Nautilus,* the last vestige of the Nautiloidea, and last surviving externally shelled cephalopod. As in ammonoids, the external shell in *Nautilus* provides not only protection of the soft parts, but perhaps more importantly serves as a buoyancy producing organ, equivalent in function to the swimbladder of a fish. *Nautilus* and ammonoid shells are separated into two parts: a large cavity that contains the living soft parts, and a closed portion that is gas and liquid filled, and is partitioned by numerous calcareous septa that serve as strengthening structures. The chambered shell region is of sufficiently low density to buoy up the heavy, calcareous shell and soft parts so as to provide neutral buoyancy for the active shell and animal.

With this buoyancy system, and presumed squid-like water jet propulsion system, Mesozoic ammonoids must have been functional equivalents of fish. The recent, exhaustive survey of paleobiological research on the functional morphology and paleoecology of ammonites (Jurassic and Cretaceous ammonoids) recently published by Kennedy & Cobban (1976) emphasized many aspects of the convergence between ammonites and fish, and also listed several aspects of ammonoid paleobiology that are relevant to understanding Mesozoic pelagic ecosystems. These can be summarized as follows:

[1]Present address: Department of Geological Sciences, University of Washington, Seattle, WA 91895, U.S.A.

1. All ammonites passed through a planktonic post-hatching stage, spending some unknown period in the surface regions of the seas, thus being affected by changing evolutionary conditions within the plankton. While in the plankton, hatchling ammonites may have fed on other zooplankton species, and in turn been prey of still others. In contrast to the hatchlings, juvenile and adult ammonites inhabited a variety of environments (based on interpretations of shell morphologies). Planktonic, nektonic, and nektobenthonic species have all been identified.

2. All living cephalopods are capable of mantle-powered swimming. Ammonites were probably no exception, and may have swum in a fashion very similar to that of Recent *Nautilus*. Studies on musculature and hydrodynamic properties of the shell, however, suggest that the majority of species were relatively weak or slow swimmers. Some ammonite species (heteromorphic ammonites) had shell shapes that seem adapted to anything but swimming. The current opinion among ammonite specialists is that many species were very slow swimmers, and may have been better adapted for slow vertical excursions, rather than lifestyles necessitating rapid horizontal swimming.

3. Most ammonites appear to have exploited low levels in marine food chains, based on functional morphology of jaw parts, and exceptionally preserved fossils with crop contents still preserved. While all ammooids are thought to have been carnivores (like all extant cephalopods), many species have been interpreted as zooplankton feeders.

The ammonites disappeared at the end of the Cretaceous Period. Their final extinction coincided with the extinction of other prominent groups of Mesozoic organisms, such as dinosaurs, other molluscs (most notably the majority of belemnites and all inoceramid and rudistid bivalves) and many groups of marine plankton. Extinction of the last remaining ammonite species came at the end of a long-term (10 to 15 million years) diversity decline. During this last phase in the history of the ammonites, shell morphologies as well as characteristic evolutionary tempos of the constituent ammonite species were also changing (Ward, 1983; Ward & Signor, 1983). The purpose of this paper is to better document the nature of the Cretaceous ammonite record in terms of shell morphologie.

MATERIALS AND METHODS

Ammonite shell morphology has been examined and described in the following way. To describe shell shape, Cretaceous planispiral ammonite genera illustrated in the *Treatise on Invertebrate Paleontology* (Pt. 4) have been measured, and the W, D, and S parameters of Raup computed (Appendix 1). From these same specimens and from Cretaceous heteromorphic genera from the same source, ornamental rugosity has been computed by dividing rib width by whorl height (Appendix 1). Based on these measurements, ammonite taxa have been placed in shell shape categories, grouped according to one descriptor (ornament rugosity or streamlining), or together (by assigning taxa to one shell shape category for each time period examined and then graphing the percentages of these distributions).

These graphs and table only provide a model for ammonoid shell shape distributions, and the limitations of the data cannot be underestimated. The generic data base is old, and the methodology of assigning a genus to an entire stage if it appears in any part of that stage greatly inflates the ranges of the taxa. Most ammonite genera ranged far less than a single stage. Secondly, the use of genera to construct shell shape distributions tends to give equal weight to all taxa. Obviously, some genera were far more speciose than others, so that diverse taxa are penalized. Thirdly, the data measured for a given genus are based on measurement of a single species of that genus; values for other species of the same genus could be different. In defense of the data base, however, it can be demonstrated that similar trends in shell shape distributions appear to be present at the species level of ammonite genera occurring in a single depositional system (the Great Valley Sequence of California). These species have been tabulated and categorized as above.

RESULTS

1. Generic level analyses

A. Cretaceous ammonite ornamental rugosity—generic level Shell ornament rugosity has previously been discussed by Ward (1981), who quantitatively demon-

strated that shell ornamental rugosity increased among ammonoid genera throughout the history of the group. That study was made at a system or series level of resolution. In this study I have examined the Cretaceous ammonite genera at the stage level. The methodology used here is also somewhat different than in my previous study, in that here the rugosity measure is computed by dividing average rib width (for non-major ribs, taken at mid flank) by whorl height, rather than by whorl diameter at the point of measure. Using this new methodology, heteromorphic as well as planispiral ammonites can be directly compared.

The rugosity of combined heteromorphic and planispirally coiled genera for Cretaceous stages is listed in Table 1, and figured in Fig. 1. The four categories of rugosities are 0–.049, .05–.099, .10–.149, and greater or equal to .15. These are approximately equivalent to the four ornamental categories of Ward (1981). The combined groups as a percentage of the entire fauna for each stage illustrated in Fig. 1 clearly show that there was a maximum of shell ornamental rugosity during the middle part of the Cretaceous, with minima at the start and end of the Cretaceous, a trend described earlier by Ward (1983). The major expansion of more coarsely ornamented ammonoid genera began during the Hauterivian, and increased in numbers progressively through the Turonian. The Albian, Cenomanian, and Turonian all had ammonite faunas in which the majority of taxa belonged to the two highest ornamental categories. Following the Turonian, coarsely ornamented forms gradually lessened in number (as a percentage of the entire fauna). By Campanian and Maastrichtian time, the two highest ornamental categories comprised less than 20% of the entire fauna.

Ward (1983) suggested that Lower and Upper Cretaceous heteromorphic ammonite faunas would be distinguished from one another on the basis of shell ornament. Shell ornamental values for heteromorphic ammonite genera are listed in Table 2. From these data, it is clear that shell ornament rugosity in the Lower and Upper Cretaceous heteromorphic faunas is differentiable.

B. Streamlining

Streamlining in ammonites is related to both whorl profile and ornamentation. Raup (1967) showed there to be an inverse correlation between highly streamlined forms and degree of shell ornamental rugosity. Such a correlation is also observable in my data. The shell shapes best designed for high streamlining efficiency are those with involute shells (low D) and especially low whorl breadth to height ratios (S), and nonexistent or only weak ribbing and tuberculation. To search for trends in streamlining during the Cretaceous, S values for the planispiral ammonite sample from Appendix 1 have been tabulated, and placed into categories of either 0–.49, .5–.99, 1.0–1.49, and greater than, or equal to 1.5. These data are listed in Table 3. The most highly streamlined forms, with very compressed cross sections having S values less than .5, show maxima during the Hauterivian, Campanian, and Maastrichtian. The Maastrictian shows the highest individual level of highly streamlined forms, with 29%. The Late Cretaceous trend of increasing proportions of highly streamlined forms appears to be real at the species level as well. I have recently been able to study types of many Maastrichtian ammonite species in European and North American museums; within these collections, the great abundances of highly streamlined species belonging to *Sphenodiscus, Libycoceras, Hauericeras,* and *Pachydiscus* are quickly evident, and in my mind indicate a significant trend in ammonite shell shapes. Other than the Hauterivian maximum, highly compressed ammonites appear to remain at approximately constant levels throughout the Lower Cretaceous, and then increase in numbers during the Upper Cretaceous.

Highly depressed ammonite shells are those with S values of greater than 1.5. These types of shells show maxima during the middle part of the Cretaceous, and perhaps not surprisingly, many of the highly depressed species are also those with the coarsest shell ornament. The Maastrichtian also shows a high percentage of depressed forms, but this appears to be an artifact of the very small Maastrichtian sample, rather than a real trend.

To compare Cretaceous streamlining efficiency with the set of Jurassic ammonites (Ward, 1980), percentages of shells having low D (O–.33), and either S values of less than .5, or between .5 and 1.0 have been computed for Lower, Middle and Upper Jurassic and Lower and Upper Cretaceous (Table 3). From this table, it is apparent that the

TABLE 1. Rugosity of shell ornament, Cretaceous planispiral and heteromorphic genera. Values in absolute numbers and percentages of total for each stage (parentheses). Values derived from division of rib width by whorl height.

Stage	n	0 – .049	.05 – .099	.10 – .149	≥ .15
Maastrichtian	23	13(56)	6(26)	2(09)	2(09)
Campanian	48	17(35)	21(44)	4(08)	6(12.5)
Santonian	44	14(32)	18(41)	4(09)	8(18)
Coniacian	45	13(29)	11(24)	8(18)	13(29)
Turonian	43	12(30)	8(19)	8(19)	15(35)
Cenomanian	55	18(33)	6(11)	15(27)	16(29)
Albian	88	23(26)	13(15)	25(28)	27(31)
Aptian	43	13(30)	12(28)	9(21)	9(21)
Barremian	42	14(33)	10(24)	10(24)	8(19)
Hauterivian	35	12(34)	12(34)	6(17)	5(14)
Valanginian	37	14(38)	14(38)	5(14)	4(11)
Berriasian	24	7(29)	12(50)	2(08)	3(12.5)

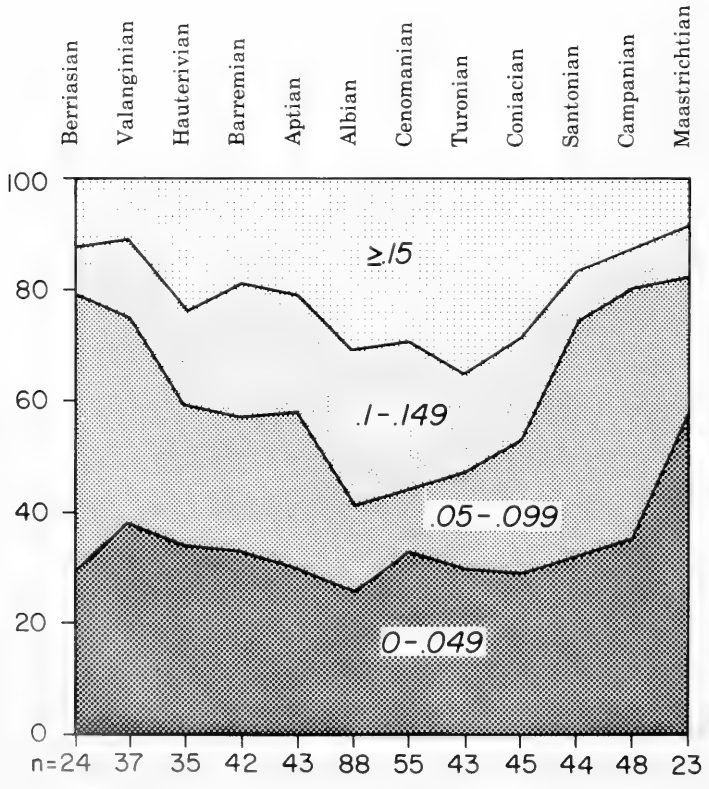


FIG. 1. Shell ornamental rugosity of Cretaceous ammonite genera listed in Appendix 1. For each stage, taxa have been placed in one of four categories. Shell rugosity was determined by dividing rib width by whorl height at the point of measure. These categories are then given a percent frequency value. N values at bottom of graph refer to number of taxa used in each stage.

TABLE 2. Rugosity of shell ornament, Cretaceous heteromorphic ammonite genera. Because of the low number of taxa, values are in absolute numbers, rather than percentages.

Stage	n	0 – .049	.05 – .099	.10 – .149	≥ .15
Maastrichtian	9	4	4	1	0
Campanian	14	4	8	2	0
Santonian	10	2	8	0	0
Coniacian	7	1	5	1	0
Turonian	8	2	4	0	2
Cenomanian	9	1	2	2	4
Albian	12	1	2	6	2
Aptian	13	1	3	4	5
Barremian	18	2	5	7	5
Hauterivian	12	1	7	2	2
Valanginian	3	0	0	2	1
Berriasian	2	0	0	0	2

TABLE 3. The temporal distribution of streamlined ammonites.

Time	D = 0 – .33, S = ≤ 0.5	D = 0 – .33, S = .5 – 1.0	1 and 2 combined
Lower Jurassic	4.5%	22.5%	27.0%
Middle Jurassic	10.4%	33.1%	43.5%
Upper Jurassic	8.3%	29.0%	37.0%
Lower Cretaceous	12.3%	38.3%	54.2%
Upper Cretaceous	18.4%	40.8%	59.2%

percentage of highly streamlined taxa was higher in the Cretaceous than any time in the Jurassic.

C. Cretaceous shell shapes

The nature of the Cretaceous ammonite shell categories can be better understood by combining the ornamental, streamlining, and heteromorphic categories into a single stage by stage compilation. To do this, four shell shape categories have been tabulated as a percentage of the total sample of each stage. Shells were placed in the category of *ornamental planispiral* if shell rugosity was .15 or more. Shells were placed in the category of *streamlined planispiral* if they had S values of less than .5. The remainder were *planispirals*, or *heteromorphic*. These shape categories were then plotted as a percent-fraction of the entire sample for each stage. If a taxon appeared in any part of a stage, it was considered to range throughout that stage.

Shell shape categories for the Cretaceous stages are shown in Fig. 2. Frequencies of the different shapes are shown in Fig. 3. Perhaps the most significant aspect of this diagram is found within the category of ornamented ammonites. As described above, coarsely ornamented ammonites comprised a minority of the ammonite faunas at the beginning and end of the Cretaceous. The Berriasian Stage, and to a lesser extent the Valanginian Stage as well, are composed of taxa that are continuations of Jurassic families. Almost all showed the Jurassic pattern of moderately ornamented, non-streamlined shell shapes. This overall shell shape pattern suggests that neither speed, nor shell defensive measures were necessary or advantageous in the pre-Cretaceous ammonite record. By the middle of the Cretaceous, however, and into the lower part of the upper Cretaceous, these coarsely ornamented forms became among the most diverse ammonite taxa. Beginning in the Coniacian Stage, and increasing in tempo in the Santonian and Campanian Stages, the coarsely ornamented forms showed marked reductions in diversity through extinction. By late Campanian and Maastrichtian times, this shell shape category was virtually non-exis-


2
3
4
5
6
7a
7b
8
10

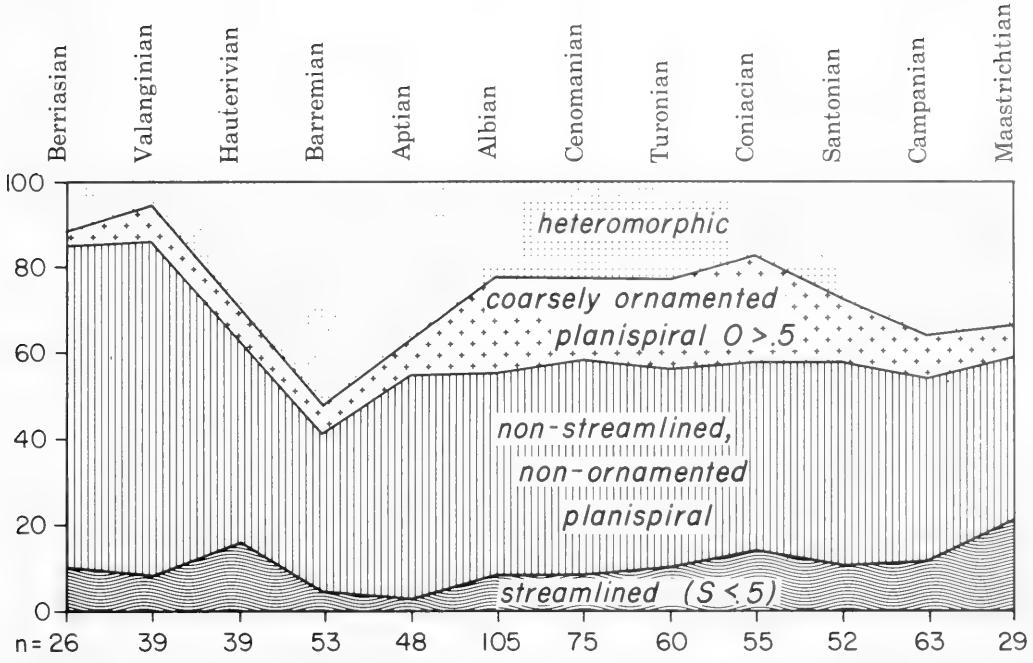


FIG. 3. Shell shape categories for generic level data. All taxa were placed in one of four shell shape categories: *streamlined,* for planispiral shells with S values (whorl breadth divided by whorl height) less than 0.5; *non-streamlined, non-ornamented planispirals*, for planispiral shells with S values greater than 0.5, and shell ornament rugosities of less than 0.15; *coarsely ornamented planispirals*, with shell rugosity of greater than or equal to 0.15; and *heteromorphic*, for non-planispirally coiled shells. For each stage, the number of taxa (in value at bottom of graph) are placed in one of the shell shape categories, and the percent frequency for the entire stage sample calculated and graphed.

tent. The reduction in the ornamented shell category can also be observed in other ways. Ward & Signor (1983) have recently examined the nature of evolutionary tempo in Jurassic and Cretaceous ammonites. In a companion piece (Ward & Signor, in press), we looked at the nature of the clade shapes. At the same time as overall ammonite diversity declined during the latest part of the Cretaceous, the average shapes of the majority of family clades as replicated by spindle diagrams also began to change. Characteristic clade shapes for the highly ornamented ammonites were short but wide, indicating high origination and high extinction rates. The extinctions of the ornamented ammonites near the end of the Cretaceous left mainly long-ranging, low diversity ammonite families in the final stages of the Cretaceous. These groups included the Upper Cretaceous he-

FIG 2. Upper Cretaceous ammonites from the North Pacific Province, illustrating shell shape categories used in this paper. 1. *Pseudoxybeloceras nanaimoense*, a large Campanian heteromorphic ammonite. Shell ornamental rugosity between .06 and .08. 2. Three ammonites from the same concretion. Two specimens of the heteromorphic ammonite *Hyphantoceras venustum*, and a single planispiral *Tetragonites popetensis.* Note the short spines on the heteromorphs. Santonian of Mill Creek, California. 3. *Bostrychoceras elongatum*, a Santonian heteromorphic ammonite. This species has fairly coarse ornament for a heteromorph. O = .09 to .10. 4. *Gaudryceras denseplicatum*, a Santonian planispiral. This ammonite is neither streamlined, nor ornamented. Ornamentation is very fine ribs. 5. *Glyptoxoceras subcompressum*, a Santonian through Maastrichtian heteromorph. 6. *Desmophyllites diphyloides*, a Campanian desmoceratid. This ammonite is neither highly streamlined, nor ornamented. 7a, b. *Hauericeras gardeni*, a highly streamlined (S = .49) desmoceratid planispiral of the Santonian. 8. *Epigoniceras epigonum*, a Santonian and Campanian tetragonitid ammonite. 9. *Submortoniceras chicoense*, a coarsely ornamented planispiral from the Campanian. 10. *Eupachydiscus haradai*, a moderately ornamented planispiral from the Santonian.

teromorph families, Nostoceratidae and Diplomoceratidae, and non-ornamented planispirals such as the Phylloceratidae, Desmoceratidae and Tetragonitidae.

Heteromorphic ammonites are the other shell shape category that distinguishes the Cretaceous from all previous periods. Although hetermorphics are known for the Triassic and Jurassic as well, they occur at these times at very low diversity. Their diversifications during the Cretaceous set this system apart from the rest of ammonoid history.

Although heteromorphic ammonites are here categorized as one shell grouping, the enormous range of morphology evolved by the disparate heteromorphic families suggests a wide range of adaptation. In this regard this category is probably much more artificial than either the streamlined or ornamented planispiral categories. Within the Lower Cretaceous the more massive, heavily sculpted forms such as members of the Ancyloceratidae and Crioceratidae are ammonites that appear closely allied to the coarsely ornamented planispirals of the time, and in some cases are phylogenetically related (Wiedmann, 1969). Wiedmann's finding that planispiral and heteromorphic shell shapes freely transformed from one category to the other is further evidence that these two categories, the coarsely ornamented heteromorphs and planispirals of the Aptian, Albian, and Cenomanian stages were ecologically allied. In contrast, the more finely sculpted, delicate heteromorphic forms of the Upper Cretaceous, initiating with the Hamitidae and continuing with the Nostoceratidae and Diplomoceratidae, suggest different adaptations. These will be discussed further below.

2. The Cretaceous ammonite record of the Great Valley Sequence, California

During the Late Jurassic and throughout the Cretaceous Period, subduction of ocean crust along the present day California coastline produced the Sierran Arc. Thick wedges of clastic sediments were deposited along the continental shelf and slope west of this north-south trending arc. The stratal record of this event, the Great Valley Sequence, contains a rich ammonite record. The fossils are often preserved unaltered, and in many areas, field locales are locally abundant. All of the stages of the Cretaceous are represented, and ammonite zonation for the entire Cretaceous has been proposed (Ward & Signor, 1983).

A. Stage level ornamentation rugosities

Using the same method as for the generic level data, the Sacramento Valley ammonite species of the Valanginian to Maastrichtian Stages have been measured for S (whorl width divided by whorl height), and for ornamental rugosity (rib width divided by whorl height at the point of measure). For the latter measure, the heteromorphic ammonite species assignable to *Baculites* have been omitted. Species of this genus are either non-ornamented, or can have crescent-shaped swellings on the flanks that are not really true ribs, even though this shell ornament, as in other ammonites, probably served to strengthen the shell.

The results of the ornamental analyses, tabulated as for the generic data, are shown in Fig. 4 (from data in Appendix 3). Heteromorphic as well as planispiral species have been included. As for the generic level data, the lower and upper Cretaceous stages show a lower degree of average shell ornamentation than do the middle stages. The ammonites of the Aptian through Turonian of the Great Valley Sequence can be characterized as having a higher number of more coarsely ornamented species than did the ammonite faunas of earlier and later stages.

The four shell shape categories used for the generic level data are also used to analyze the shell shape distributions of the species level data (Fig. 5). The only major difference between this figure for species of the Great Valley Sequence, and the figure for the generic level data (Fig. 3) is in the category of streamlined planispiral ($S \leq .5$). In the Great Valley Sequence, there appeared to be a relatively lower percentage of highly streamlined species than for the Cretaceous ammonite record as a whole. This may be due to the differing taxonomic levels of analysis (generic vs. species level), or may be real. It is my feeling that the Great Valley Sequence did have fewer, highly streamlined species relative to other ammonite shell shapes at any given time, due to the deepwater facies and environments that characterized most Great Valley Sequence paleoenvironments. Most highly streamlined genera of the Cretaceous, such as *Placenticeras* and the numerous neoceratites, appear to have been restricted to

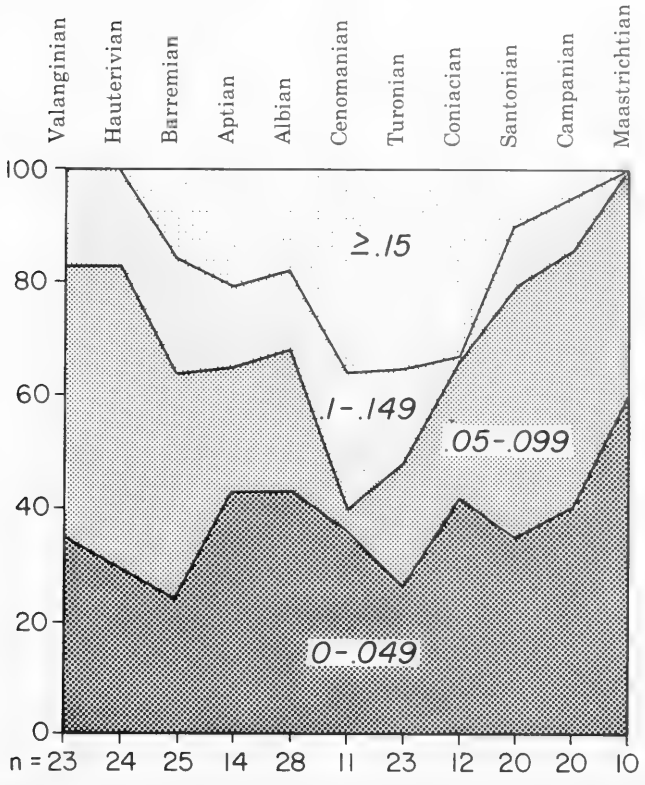


FIG. 4. Shell ornamental rugosity for Great Valley Sequence species data. Ornamental categories as in Fig. 1.

extremely shallow water (Kennedy & Cobban, 1976). These genera are completely absent from the Great Valley Sequence.

B. Shell shape categories

The stage-level percentages of major shell categories from the great Valley Sequence of California are shown in Fig. 5 (from data in Appendix 3). The three earliest Cretaceous stages in California have ammonite faunas that are more closely allied to Jurassic faunas than to those of the rest of the Cretaceous. The majority of taxa are from the ammonitidid families Olcostephanidae, Craspeditidae, and Berriasellidae. The dominant shell forms are non-streamlined, with prominent (but not coarse) ornament of finely incised ribs and small tubercles. In California the Berriasian, Valanginian, and Hauterivian Stages are best represented in the Paskenta region of the northwestern Great Valley, where strata are dominated by species of *Neocosmoceras* and *Kilianiceras* in the Berriasian, and large numbers of *Thurmanniceras* in the Valanginian. This trend of large numbers of moderately sculpted, non-streamlined planispiral species continued into the early Hauterivian age. In the later Hauterivian, however, the seeds of the coming revolution in shape were already sown; early heteromorphic genera such as *Crioceratites, Acrioceras,* and the first *Shasticrioceras* signalled the end of the dominance of ammonitinid planispiral ammonites, which had made up nearly the entirety of ammonite faunas throughout the Jurassic and into the lowermost Cretaceous of California.

During the Barremian and Aptian ages, heteromorphic ammonite shapes were clearly increasing numerically in terms of the total spectrum of shell shapes present. Barremian heteromorphic ammonites included common gyroconic species of very large size, with planispiral shells of whorl expansion rates so

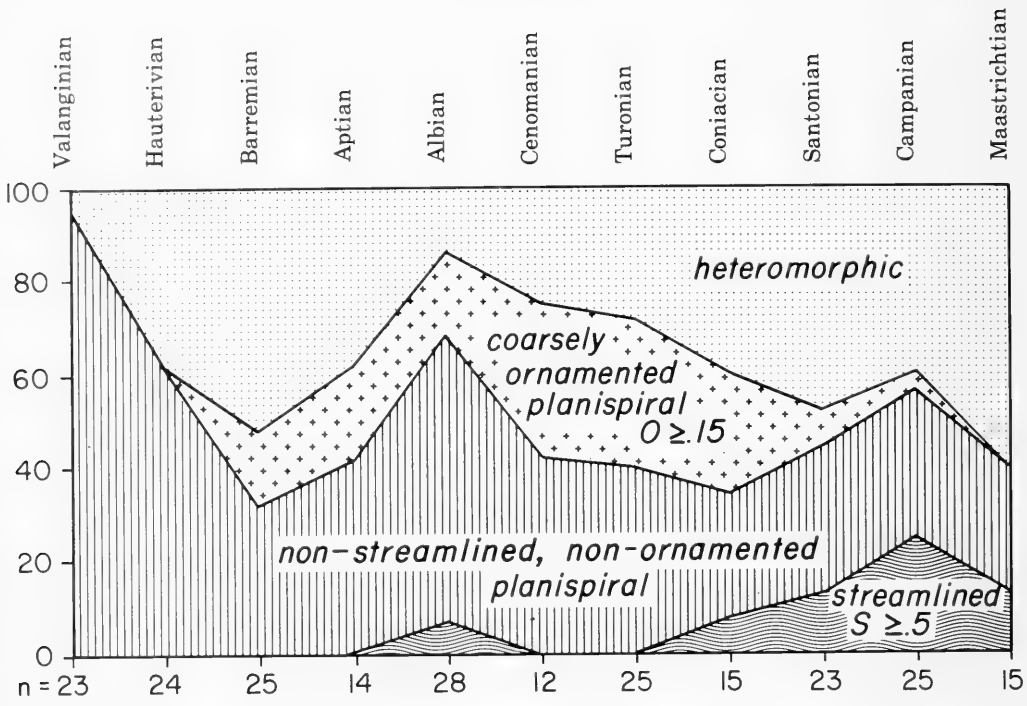


FIG. 5. Shell shape categories for Great Valley Sequence species. Shell shape categories as in Fig. 3.

large that the outer shell wall was no longer in contact with previously secreted shell. Most important of these were specimens of *Shasticrioceras*, which were endemic to, but extremely diverse in the North Pacific Province, and important in biostratigraphic zonation because of their ubiquity and short stratigraphic ranges. Many of these species attained more than a meter in diameter. The second most abundant group of heteromorphs was the ancyloceridS, some of which also attained very large size. Aptian heteromorphic shell faunas were largely similar to those of the Barremian, but with the addition of large, hook-like shell species assigned to *Hamulina* and *Anahamulina*. Another important shape trend initiated in the Barremian-Aptian was the emergence of coarsely ornamental planispiral species, such as *Pulchellia* and *Cheloniceras*. These massively armored species continued to increase in diversity in the Great Valley Sequence until the end of the Turonian.

The Albian and Cenomanian ages, in contrast to the Aptian, are widespread throughout the North Pacific Province; Albian and Cenomanian strata with ammonite faunas are known from Alaska, several areas in British Columbia, and in many areas of California, although the Ono region of northern California is by far the most complete and fossiliferous. No single ammonite shell group dominates Californian Albian-Cenomanian shell shape distributions. New additions to these faunas were by the successive radiations of desmoceratid and tetragonitid ammonites, to be diverse components from the Albian until the very end of the Cretaceous. These species had smooth, extremely well-streamlined shells. Ornamented species also continued to diversify in the North Pacific Province. Among heteromorphic ammonites the crioceratid and ancylocerid species of the Barremian-Aptian were replaced by torticonic forms, such as *Turrilites*, and, for the first time, large numbers of delicately ornamented hamitids, assignable to *Hamites* and *Stomohamites*.

The remainder of the Upper Cretaceous Stages in the Great Valley Sequence, the Coniacian, Turonian, Santonian, Campanian, and Maastrichtian, show three main trends. First, the large numbers of coarsely ornamented species diminished in numbers fol-

lowing the Turonian. Secondly, large numbers of small to medium sized heteromorphic ammonites, especially the baculitids, became increasingly important. Finally, streamlined planispirals increased in abundance until the end of the Maastrichtian.

The Maastrichtian of the North Pacific realm is nowhere near as widely exposed as is the Campanian. Only Lower Maastrichtian strata have been definitely identified on the basis of molluscs, and much confusion still exists about the placement of the Campanian-Maastrichtian boundary in the North Pacific Province (see Jones, 1963, for a discussion), let alone the Lower-Upper Maastrichtian boundary. By Maastrichtian time ammonite diversity in the North Pacific Realm had dropped markedly. Only 14 Maastrichtian species are known from California as compared to 37 during the Campanian. The Maastrichtian species are almost all holdovers from the Campanian. They make up an interesting assemblage of shape categories, for the morphologic make-up of the Maastrichtian ammonites sheds light on selective conditions apparently operating on ammonites immediately prior to their complete, world-wide, extinction at the end of the Maastrichtian. Of the 14 California Maastrichtian species, 10 are heteromorphic (5 baculitids and 5 nosto- and diplomoceratids).

5. Numerical abundance of North Pacific Cretaceous shell shapes

The diversity of specific shell shapes discussed above for each stage of the California Cretaceous does give a picture of evolutionary trends occurring within ammonite communities of the North Pacific Province. Of equal interest is information about the relative abundance of each taxon.

Biofacies distributions of Cretaceous ammonites from various times and places around the globe have been studied by Scott (1940), Kauffman (1967) and Kennedy & Cobban (1976), and most recently by Tanabe (1979). Tanabe's paper is significant in its thoroughness and detail, as well as being the only study of Cretaceous faunas similar to those discussed in this paper. Tanabe followed the works of Ziegler (1967) and Wendt (1971) in sampling individual outcrops, and tabulating the percentage of each species or morphotype within the sample.

I have made similar studies on selected outcrops of North Pacific Cretaceous strata in California, Washington, and British Columbia (Fig. 6). Although these studies have been very few, they do illustrate trends in abundances of various Cretaceous shell shapes through time.

Abundance trends from absolute numbers of individual species show the same trend illustrated by diversity trends: heteromorphic ammonites became increasingly abundant (as a percentage of the entire ammonite fauna at any locality) as well as increasingly diverse through time. In the late Cretaceous the most abundant of all ammonites were species of *Baculites*.

The dominance of baculitid species in virtually all marine, Late Cretaceous facies of California, Washington, and southeastern British Columbia is in marked contrast to the facies patterns of similarly aged strata in Japan. Matsumoto (1960) first noted that abundance patterns in California and Japan showed significant differences: "The apparent difference may be due to ecological or sedimentary environment. Members of the Tetragonitidae, such as *Tetragonites, Anagaudryceras, Gaudryceras*, and some of the Desmoceratidae, such as *Desmoceras (Pseudouhligella), Damesites*, and *Mesopuzosia* are persistent and occur abundantly in various stages of the Japanese Upper Cretaceous. They do occur in California but the occurrence is sporadic and restricted to particular beds. The Baculitidae are fairly common and occur at various levels of the Upper Cretaceous of California, being often predominant over other ammonites. In the Japanese Cretaceous they are not rare but never so abundant as the desmoceratids and tetragonitids."

Matsumoto attributed these distribution differences to facies differences between California and Japan. *Baculites* is the dominant ammonite in virtually all ammonitiferous, post-Turonian strata in California and British Columbia, irrespective of facies. Only strata of earliest and latest Santonian age (Venustum and Elongatum Zones, and Schmidti Zone) have facies where baculitids are not the numerically dominant species. If the Upper Cretaceous of Japan can be characterized as being composed of high diversity, high equitability ammonite faunas, with tetragonitids and desmoceratids as the most abundant species, then this is a marked contrast with the Californian Upper Cretaceous. The Cre-

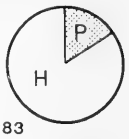
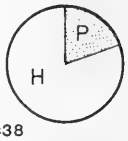
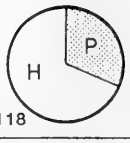
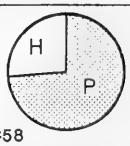
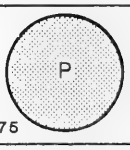
AGE	LOCALITY	FACIES	DOMINANT GENERA
Middle Campanian	Sucia Island, Washington	concretionary siltstone	*Baculites* *Canadoceras* *Hoplitoplacenticeras* 
Early Campanian	Trent River, Vancouver Island	mudstone	*Baculites* *Canadoceras* *Gaudryceras* 
Late Coniacian or Early Santonian	Cache Creek, Colusa County California	mudstone	*Baculites* *Protexanites* 
Late Albian	McCarty Creek, Tehama County, California	turbidites	*Puzosia* *Desmoceras* *Hamites* *Lytoceras* 
Valanginian	McCarty Creek, Tehama County, California	mudstone	*Thurmanniceras* 

FIG. 6. Shell shapes and dominant genera from selected Cretaceous outcrops, Great Valley Sequence and Nanaimo Group, Vancouver Island, Canada. P = planispiral, H = heteromorphic.

taceous record in the Great Valley Sequence shows a steady increase in the numbers of heteromorphic as compared to planispiral ammonites. During the Late Cretaceous the heteromorphic ammonites are by far the most abundant species in most Cretaceous outcrops of California, Washington, and British Columbia.

DISCUSSION

The shell shapes themselves of the North Pacific ammonites can be used as a clue to mode of life. Raup (1967) and Chamberlain (1976, 1980) examined the spectrum of evolved ammonoid shell shapes, and identified streamlining and stability as major adaptive influences on shell shape. Perhaps one of the most surprising findings of these studies was the presence of so many ammonite species which were so poorly streamlined. For many species, evidently, rapid swimming speeds or great maneuverability, which would be possible with weakly ornamented, compressed shells of high stability, were not of overriding selective value in their particular habitats. This generalization is especially evident in reviewing the evolutionary history of heteromorphic, or non-planispirally coiled ammonites. These forms were anything but streamlined (although most had very high shell stability). Many workers have considered heteromorphic species as secondary benthonic adaptations, ammonites which became benthonic in ways analogous to living octopus. Certainly this argument holds for the massive heteromorphs of the Lower Cretaceous. The greatest single argument against this hypothesis, however, lies in the morphology of the heteromorphic shells themselves, which have lost none of the components of the buoyancy maintenance system of all other

ammonites, and indeed have undergone adaptations of the shell which appear to have increased efficiency of the shell as a buoyancy organ (Ward, 1979; 1983; Klinger, 1980). Packard (1972) has argued that many heteromorphic ammonites were passive, nearly planktonic organisms that lived in the mesopelagic regions in ways analogous to the present day cranchiid squids. This view was reiterated by Ward (1976), Ward & Westermann (1977), Ward (1979), and Klinger (1980). If this generalization is true for a majority of heteromorphic species, it would suggest that a major evolutionary trend in ammonites of the North Pacific Cretaceous was towards increasing diversities and abundances of mesopelagic species.

An important breakthrough in interpreting the mode of life and living environment of ammonites was the discovery by Westermann (1971) that relative siphuncle strength in ammonites could be used as a measure of overall shell strength, and hence be useful as a key to bathmetry. This view has been challenged (Saunders & Wehman, 1977; Chamberlain & Moore, 1983); however, it is my belief that this measure may indeed yield information on maximum, potential depth limits (Westermann & Ward, 1980), if not in terms of strength, than perhaps in terms of siphuncular water movement (Ward, 1983).

Westermann showed that ammonitinid ammonites, which made up the bulk of Jurassic ammonite faunas, had large diameter, thin-walled siphuncles, and they may be restricted to shelf environment of one to two hundred meters maximum depth because of the potential weakness against ambient pressure explosion of these structures. These siphuncles contrasted markedly to supposed siphuncular strengths of phylloceratids and lytoceratids, and to that of *Nautilus*, all of which show a siphuncular strength potential of at least 700 m. Since Westermann's studies, other siphuncular diameters have been measured, including those for a number of species of the North Pacific Province Cretaceous ammonites (Ward & Westermann, 1977; Tanabe, 1979; Ward & Signor, 1983).

Based on generalizations from published ammonitonid siphuncle strength figures, all ammonitid species of the California Berriasian, Valanginian, and Hauterivian should be assigned to shallow water habitats, with maximum submersion limits of perhaps 100 to 200 m (Westermann, 1971). In these stages, only the lytoceratids and phylloceratids may have been deepwater forms. Interestingly, Tanabe (1979) showed that all coarsely ornamented ammonites he examined also showed similarly weak siphuncles, and thus, perhaps, restriction to shallower water. Certainly the facies distributions of these ammonites support such a generalization (Tanabe, 1979). Other shallow water species (based on siphuncular strength) include the Upper Cretaceous Placenticeratidae, which in the North Pacific are represented by the Campanian genera *Metaplacenticeras* and *Hoplitoplacenticeras*. Lytoceratids, phylloceratids, desmoceratids, and tetragonitids have had much stronger siphuncles (Ward & Signor, 1983) and thus the capability to inhabit much deeper habitats, perhaps to depths as deep as 1000 m. Many of these species may have been shallow water species by choice; nevertheless, the capability of deeper habitats was present.

Heteromorphic shells provide additional problems of interpretation, and should be examined on a case by case basis, rather than be categorized as one large group. Clearly, large, ornamented heteromorphic species of the lower Cretaceous, such as *Ancyloceras, Heteroceras*, and *Toxoceras* seem much more analogous to the large, coarsely ornamented planispiral ammonites of the lower and middle Cretaceous than they do to their less robust, finely sculpted descendents of the Upper Cretaceous. Siphuncle strength measurements of these Lower Cretaceous heteromorphic ammonites (Westermann, 1982; Ward & Signor, 1983) also show them to be similar to the ornamented ammonites in having large but thin-walled, and thus weak siphuncles. The Upper Cretaceous heteromorphs, such as *Baculites, Hyphantoceras, Bostrychoceras*, and *Glyptoxoceras* had stronger siphuncles (Ward & Signor, 1983) and are here interpreted as mesopelagic forms, while the more massive Lower Cretaceous forms may have been nektobenthonic, or perhaps entirely benthonic.

The major conclusion to be drawn from the published strength figures is that shallow water species, such as the early Cretaceous ammonitinids, and mid to upper Cretaceous coarsely ornamented species became increasingly restricted in number during the Cretaceous, and were completely replaced by deeper water species (such as nostoceratids, phylloceratids, desmoceratids, tetragonitids) by the latest Cretaceous. It may be that the

absolute number of ammonites living in shallow water habitats never changed during the Cretaceous, or perhaps even increased. It is apparent, however, that even if ammonites inhabited the same shallow water habitats throughout the Cretaceous, by Late Cretaceous time many were *capable* of inhabiting deeper habitats as well. It is my feeling that shallow water, nektonic ammonites gradually decreased in diversity and abundance throughout the Cretaceous. The ammonites may have moved off the shallow shelves in the late Cretaceous, either to deeper water benthic habitats or into the water column. Those that remained in shallow waters, such as the Placenticeratidae and Sphenodiscidae, were all highly streamlined.

CONCLUSION

Evolution of Cretaceous ammonites appears to show several trends. Early Cretaceous faunas are dominated by shallow water species, which were gradually replaced by deeper water species during the Cretaceous. Those species which continued to live in shallow water either became coarsely ornamented, with protective ribbing and spines, or evolved shells of high streamlining efficiency. By latest Cretaceous time shallow water species made up only a small fraction of the total ammonite fauna.

The increase in diversity and abundance of deep water planispiral forms (Phylloceratidae, Desmoceratidae, Tetragonitidae) and heteromorphic forms (Nostoceratidae, Baculitidae, Diplomoceratidae) suggests that ammonites migrated from their Jurassic–Lower Cretaceous shelf habitats into deeper benthic or mesopelagic habitats. This trend could have been due to increased competition in shallow shelf areas by newly evolving teleost fishes and/or dibranchiate cephalopods, or increasing predation in shallow water by teleosts, sharks, shell-crushing crustaceans, or marine reptiles.

The Cretaceous evolution of heteromorphic ammonites distinguishes the Cretaceous ammonite faunas from those of all preceding systems. I believe that the high diversity and abundance of heteromorphic ammonites is a signal that fundamental reorganization was occurring within the Cretaceous pelagic realm. I would interpret many of the Late Cretaceous heteromorphic species as mesopelagic zooplankton feeders (Ward & Westermann, 1977; Ward, 1979).

Within this context, the final extinction of the ammonites at the end of the Cretaceous may be due in part to environmental changes within the plankton. By Maastrichtian time all North Pacific shallow water ammonites were already extinct. All remaining species may have been deep-water forms, and most were heteromorphic. If those heteromorphic forms were indeed vertically migrating, mesopelagic zooplankton feeders, then they would have been very susceptible to the catastrophic changes within the zooplankton communities that occurred at the end of the Cretaceous either as adults, or in their planktonic hatchling stages. It is perhaps no coincidence that the only externally shelled cephalopods that survived the terminal Cretaceous extinction were nautilids, which are high in the food chain (large crustacean carnivores) without planktonic larval stages.

REFERENCES CITED

CHAMBERLAIN, J., 1976, Flow patterns and drag coefficients of cephalopod shells. *Palaeontology*, 19: 539–563.

CHAMBERLAIN, J., 1980, Hydromechanical design of fossil cephalopods. In: HOUSE, M. & SENIOR, J., eds., *The Ammonoidea*, Academic Press, p. 289–336.

CHAMBERLAIN, J. A. Jr., & MOORE, W. A. Jr., 1982, Rupture strength and flow rates of *Nautilus* siphuncular tubes. *Paleobiology*, 8: 408–425.

JONES, D., 1963, Upper Cretaceous ammonites from southern Alaska. [*United States*] *Geological Survey Professional Paper*, 432: 53 p.

KAUFFMAN, E. G., 1967, Coloradoan macroinvertebrate assemblages, central Western Interior United States. In KAUFFMAN, E. G. & KENT, H. G., eds., *Paleoenvironments of the Cretaceous Seaway—a symposium*. Colorado School of Mines, p. 67–143.

KENNEDY, W. J. & COBBAN, W. A., 1976, Aspects of ammonite biology, biogeography, and biostratigraphy. *Special Papers in Paleontology*, 17: 94 p.

KLINGER, H., 1980, Speculations on buoyancy control and ecology in some heteromorph ammonites. In HOUSE, M. & SENIOR, S., eds., *The Ammonoidea*, Academic Press, p. 337–355.

MATSUMOTO, T., 1960, Upper Cretaceous ammonites of California, Pt. III. *Memoirs of the Faculty of Science Kyushu University*, ser. D: 91–171.

PACKARD, A., 1972, Cephalopods and fish, the

limits of convergence. *Biological Reviews*, 47: 241–307.
RAUP, D. M., 1967, Geometric analysis of shell coiling: coiling in ammonoids. *Journal of Paleontology*, 40: 1178–1190.
SAUNDERS, B. & WEHMAN, D., 1977, Shell strength of *Nautilus* as a depth limiting factor. *Paleobiology*, 3: 83–89.
SCOTT, G., 1940, Paleoecological factors controlling the distribution and mode of life of Cretaceous ammonoids in the Texas area. *Journal of Paleontology*, 14: 299–323.
TANABE, K., 1979, Paleoecological analysis of ammonoid assemblages in the Turonian *Scaphites* facies of Hokkaido, Japan. *Palaeontology*, 22: 609–630.
WARD, P., 1976, Upper Cretaceous ammonites (Santonian-Campanian) from Orcas Island, Washington. *Journal of Paleontology*, 50: 454–461.
WARD, P., 1979, Functional morphology of Cretaceous helically-coiled ammonite shells. *Paleobiology,* 5: 415–422.
WARD, P., 1981, Shell sculpture as a defensive adaptation in ammonoids. *Paleobiology,* 7: 96–100.
WARD, P., 1983, The extinction of the ammonites. *Scientific American*, 249: 136–147.
WARD, P. & SIGNOR, P., 1983, Evolutionary tempo in Jurassic and Cretaceous ammonites. *Paleobiology*, 9: 183–198.
WARD, P. & WESTERMANN, G., 1977, First occurrence, systematics, and functional morphology of *Nipponites* from the Americas. *Journal of Paleontology*, 51: 367–372.
WENDT, J., 1971, Genese und Fauna submariner sedimentärer Spallenfüllungen im mediterranen Jura. *Palaeontographica*, A136: 121–192.
WESTERMANN, G. E. G., 1971, Form, structure and function of shell and siphuncle in coiled Mesozoic ammonoids. *Life Sciences Contributions, Royal Ontario Museum*, no. 78: 39 p.
WESTERMANN, G. E. G., 1982, The connecting rings of *Nautilus* and Mesozoic ammonoids: implications for ammonoid bathymetry. *Lethaia*, 15: 373–384.
WESTERMANN, G. E. G. & WARD, P., 1980, Septum morphology and bathymetry in Cephalopoda. *Paleobiology*, 6: 48–50.
WIEDMANN, J., 1969, The heteromorphs and ammonite extinction. *Biological Reviews*, 44: 563–602.
ZIEGLER, B., 1967, Ammoniten-Ökologie am Beispiel des Ober-Jura. *Geologische Rundschau*, 56: 439–464.

APPENDIX 1

Geometry and ornament of Cretaceous ammonite genera.

Taxon	Age	W	D	S	Or
LYTOCERATIDAE					
Lytoceras Suess	Sin-UpCr	1.67	.42	.9	
Pterolytoceras Spath	Tith-Val	2.25	.44	1.2	.0
Eulytoceras Spath	Haut-Bar	1.93	.4	.67	.04
Metalytoceras Spath	Val				
Ammonoceratites Rafinesque	Apt-Cen	2.25	.42	1.11	.03
Argonauticeras Anderson	Apt-Alb	2.42	.29	1.18	.03
Pictetia Uhlig	Alb	10.94	.35	1.14	.02
Megalytoceras Buckman	Baj	7.84	.36	.64	.02
Metrolytoceras Buckman		2.35	.39		.02
PROTETRAGONITIDAE					
Protetragonites Hyatt	Tith-Val	1.87	.54	1.08	.02
Leptotetragonites Spath	Ber-Val	2.07	.5	.68	.02
Hemitetragonites Spath	Haut-Alb	2.33	.41	1.06	.02
TETRAGONITIDAE					
Eogaudryceras Spath	Bar-Alb	2.14	.32	1.15	.02
Eotetragonites Breistroffer	Bar-Alb	2.01	.41	1.30	.02
Anagaudryceras Shimizu	Alb-Maas	3.51	.41	1.10	.03
Mesogaudryceras Spath	Ceno	3.72	.37	1.59	.03
Zelandites Marshall	Alb-Maas	2.71	.21	.59	.02
Parajaubertella Matsumoto	Ceno	1.49	.30	2.04	
Vertebrites Marshall	Sant-Maas	1.89	.36	.79	
Gaudryceras De Grossouvre	Tur-Maas	1.53	.57	2.78	.03
Kossmatella Jacob	Alb-Ceno	1.89	.36	.79	
Gabbioceras Hyatt	Apt-Ceno	2.51	.5	2.27	0
Tetragonites Kossmat	Alb-Ceno	2.37	.35	1.38	0

APPENDIX 1 continued

Taxon	Age	W	D	S	Or
Epigoniceras Spath	Tur-Maas	1.74	.24	1.12	0
Pseudophyllites Kossmat	Camp-Maas	2.78	.2	.93	.02
PHYLLOCERATIDAE					
Phylloceras Suess	Sin-Val	2.85	.06	.62	.02
Partschiceras Fucini	Sin-Bar	4.34	.02	.65	.04
Phyllopachyceras Spath	Bar-Maas	1.21	.09	.73	.03
Hypophylloceras Salfield	Haut-Maas	2.32	.06		0
Calliphylloceras Spath	Hett-Alb	1.78	.08	.68	.02
Holcophylloceras Spath	Baj-Apt	2.33	.10	.70	.02
CRASPEDITIDAE					
Subcraspedites Spath	Infravalang	1.69	.23	.7	
Paracraspedites Swinnerton	Infravalang	1.93	.36	1	
Platylenticeras Hyatt	Valang	1.96	.18	.48	
Tolypeceras Hyatt	Valang	2.08	.31	.78	
Proleopoldia Spath	Neocom	1.78	.41	.5	
Paquiericeras Sayn	Valang	2.56	.44	.56	
Temnoptychites Pavlow	L. Neocom	2.08	.27	1.2	
Tollia Pavlow	Infravalang	1.46	.48	1	.07
Praetollia Spath	Infravalang	1.10	.38		.12
Hectoroceras Spath	Infravalang	1.63	.13	.45	.12
OLCOSTEPHANIDAE					
Spiticeras Uhlig	U. Jur (U. Tithon)	1.69	.38	1	.09
S. (Negreliceras) Djanlidze	L. Cret (Berrias)	1.82	.44	.93	
Groebericeras Leanza	L. Cret (Berrias)	1.68	.34	.70	.09
Aspidostephanus Spath	L. Cret (Berrias)	2.44	.48	1.69	.29
Olcostephanus Neumayr	L. Valang	1.63	.27	1.33	.04
O. (Rogersites) Spath	U. Valang	1.31	.29	1.47	
Subastieria Spath	L. Haut	1.78	.33	1.88	.06
Parastieria Spath	L. Haut	3.36	.27	1	.12
Saynoceras Munier-Chalmas	U. Valang	2.04	.3	1.51	. 25
Polyptychites Pavlow	L. Valang	1.50	.34	1.32	.09
P. (Euryptychites) Pavlow	U. Valang	1.81	.37	2.05	
Valanginites Kilian	U. Valang	1.42	.44	2.4	
Dichotomites Koenen	U. Valang-L. Haut	1.91	.21		.04
Neocraspedites Spath	U. Valang-Haut	1.94	.15	.79	
Speetoniceras Spath	L. Haut	1.94	.44	1.18	.09
Simberskites Pavlow	L. Haut	1.94	.47	1.59	.18
Craspedodiscus Spath	U. Haut	1.98	.18	.55	.06
Subthurmannia Spath	L. Neocom	1.91	.38	.94	.08
Subalpinites Mazenet	Berrias	2.07	.30	.69	.07
BERRIASELLIDAE					
Berriasella Uhlig	Tithon-Ber	1.99	.42	.64	
Pseudargentiniceras Spath	U. Tithon-Ber	2.17	.43	.81	.07
Argentiniceras Spath	Berrias	.80	.32	1.22	.12
Thurmanniceras Cossmann	Berrias-Val	2.52	.26	.55	.08
Neocomites Uhlig	Berrias-Val	2.39	.24	.62	.08
Odontodiscoceras Spath	Valang	1.93	.28	.56	.07
Calliptychoceras Spath	Valang	2.25	.33	.81	.08
Lissonia Gerth	Berrias				.08
Cuyaniceras Leanza	Berrias	1.70	.43	.82	.09
Limaites Lisson	Berrias-Val	2.56	.31	.73	
Parandiceras Spath	Valang	2.07	.48	.83	.08
Frenguelliceras Leanza	Valang	1.83	.48	.83	.08
Lyticoceras Hyatt	Valang-Haut	2.07	.48	.83	.08
Favrella Douvillé	L. Haut	2.64	.5	.92	.09
Neocosmoceras Blanchet	Berrias	2.35	.39	.43	
Kilianella Uhlig	Berrias-Val	2.25	.42	.71	.07
Sarasinella Uhlig	L. Valang	2.17	.32	.84	

APPENDIX 1 continued

Taxon	Age	W	D	S	Or
Neohoploceras Spath	Valang	1.56	.25	1.29	.08
Wichmanniceras Leanza	Valang	1.67	.59	1.33	.1
Distoloceras Hyatt	U. Valang-Haut	1.89	.36	.89	.14
Acanthodiscus Uhlig	L. Haut	2.25	.29	1.2	.12
Leopoldia Mayer-Eymer	U. Valang-Haut	3.11	.23	.51	.1
Saynella Kilian	L. Haut	2.64	.15	.41	0
Hatchericeras Stanton	L. Haut	2.60	.17	.58	
Suboosterella Spath	L. Haut	2.25	.38	.47	.13
Delphinites Sayn	Valang	2.42	.21	.73	0
OOSTERELLIDAE					
Oosterella Kilian	U. Valang	2.91	.28	.48	
Pseudoosterella Spath	U. Valang	2.15	.41	.85	.08
DESMOCERATIDAE					
Eodesmoceras Spath	Valang-Bar	2.12	.38	1	
E. (Miodesmoceras) Wright	Bar	1.62	.25	.71	
Barremites Kilian	L. Haut-Bar	2.19	.15	.38	
B. (Raspailiceras) Wright	Haut-Bar	2.10	.17	.71	
B. (Barremites)	Bar	2.19	.15	.38	
Subsaynella Spath	U. Haut-Bar	3.06	.14	.5	.04
Valdedorsella Breistroffer	L. Haut-Apt	1.96	.29	1.27	.07
Pseudohaploceras Hyatt	Bar-Apt	2.16	.36		.05
Callizoniceras Spath	U. Bar	1.78	.19	.54	.12
Melchiorites Spath	L. Apt-Alb	2.37	.25	.8	
Puzosia Bayle	L. Alb-U. Tur	2.25	.33	.8	.1
P. (Anapuzosia) Matsumoto	L. Alb-Cen	1.44	.47		
Bhimaites Matsumoto	U. Alb-Cen	2.17	.32	.84	
Lytodiscoides Spath	U. Alb	2.25	.31	1.12	.07
Pachydesmoceras Spath	U. Alb-Tur	2.40	.29		.04
Silesitoides Spath	L. Alb-M. Alb	1.47	.53		.04
Jimboiceras Matsumoto	Turon-L. Sant	1.78	.38	1	.06
Parapuzosia Nowak	U. Cenom-Camp	1.99	.35		.06
Mesopuzosia Matsumoto	Tur-Coni	1	.26	.86	.08
Kitchinites Spath	Santon-Camp	2.40	.32	.65	
K. (Neopuzosia) Matsumoto	Santon-Camp		.32		.07
Zurcherella Casey	U. Bar-U. Apt	3.33	.26	.70	.07
Uhligella Jacob	U. Apt-M. Alb	1.25	.26	1	.09
Pseudosaynella Spath	L. Apt-L. Alb	2.39	.12	.57	
Beudanticeras Hitzel	L. Alb-U. Alb	2.19	.18	.5	
Desmoceras Zittel	U. Apt-Cenom	2.42	.21	1.18	
D. (Pseudouhligella) Matsumoto	U. Alb-Tur	2.83	.19	.7	
Tragodesmoceroides Matsumoto	Tur	2.04	.3	1.07	
Damesites Matsumoto	Cenom-Camp	2.25	.12	.69	
Desmophyllites Spath	Santon-Camp	1.92	.13	.49	
Hauericeras De Grossouvre	Coni-Maas	1.59	.29	.46	
HOLCODISCIDAE					
Spitidiscus Kilian	Haut	2.89	.29	1.25	
Plesiospitidiscus Breistroffer	U. Haut	2.10	.21	.65	
Holcodiscus Uhlig	Bar	1.91	.34	.89	.05
Metahoplites Spath	Bar	3.16	.19	.77	
Astieridiscus Kilian	Bar	2.85	.30	1	.07
SILESITIDAE					
Silesites Uhlig	Bar	1.92	.44	.75	.08
Neosilesites Breistroffer	U. Apt-L. Alb	1.89	.55		
KOSSMATICERATIDAE					
Hulenites Matsumoto	U. Alb	2.25	.29		.06
Holcodiscoides Spath	Tur	2.32	.38	1.05	.06
Yokoyamaoceras Wright/Matsumoto	Tur-Coni	2.37	.3	.86	

APPENDIX 1 continued

Taxon	Age	W	D	S	Or
Kossmaticeras De Grossouvre	U. Tur-Camp	1.71	.38	.95	.08
K. (Natalites) Collignon	U. Camp	1.88	.32		.08
Grossouvreites Kilian & Reboul	Camp	2.09	.18	.75	.03
Gunnarites Kilian & Reboul	Camp	2.20	.28	1.06	.05
Maorites Marshall	Camp		.31	1	.01
Pseudokossmaticeras Spath	U. Camp-Maas	2.25	.5		.07
Neograhamites Spath	Camp	1.88	.38		.09
Jacobites Kilian & Reboul	Camp			1.67	.06
Brahamites Kossmat	Maas	1.36	.49		.11
PACHYDISCIDAE					
Eopachydiscus Wright	U. Alb	2.25	.2		.07
Lewesiceras Spath	U. Cenom	2.33	.28	1.28	.11
Pseudojacobites Spath	U. Turon				
Pachydiscoides Spath	Coni-Santon	3.00	.31	1.33	.16
Nowakites Spath	Coni-Santon	1.89	.30	1	.12
Canadoceras Spath	U. Santon-Camp	2.05	.28	.87	.075
Patagiosites Spath	U. Camp-Maas	2.35	.22	.8	.1
Anapachydiscus Yabe & Shimuzu	Coni-Maas	2.04	.28		.04
Pachydiscus Zittel	Camp-Maas	2.25	.22	.57	.08
Menuites Spath	U. Santon-Camp	2.78	.17	.56	.08
Pseudomenuites Matsumoto	Camp				
Eupachydiscus Spath	Coni-Camp	1.7	.3	.76	.12
Bayleites Collignon	Santon-L. Camp	2.35	.39	1.64	.07
Tragodesmoceras Spath	L. Turon-Coni	2.35	.20	.49	.05
MUNIERICERATIDAE					
Muniericeras De Grossouvre	Coni	1.56	.54	.88	.12
PULCHELLIIDAE					
Nicklesia Hyatt	Bar	2.12	.06	.6	.12
Pulchellia Uhlig	U. Haut-U. Bar	2.19	.09	.58	.17
Coronites Hyatt	Bar	1.47	.5	.96	
Subpulchellia Hyatt	Bar-Apt	2.86	.09	.55	
Psilotissotia Hyatt	U. Haut-Bar	3.36	.09	.4	
Lopholobites Hyatt	Bar	2.25	.17		
TROCHLEICERATIDAE					
Trochleiceras Fallot & Termier	U. Apt-L. Alb	2.254	.42	1.14	
DOUVILLEICERATIDAE					
Paraspiticeras Kilian	Bar	2.42	.29	.13	.12
Procheloniceras Spath	L. Apt-U. Apt	2.04	.3	1.48	.17
Roloboceras Casey	L. Apt	1.75	.32	1.18	.2
Cheloniceras Hyatt	U. Apt	1.9	.31	1.6	.11
C. (Epicheloniceras) Casey	U. Apt	2.70	.26	1.41	.23
Diadochoceras Hyatt	U. Apt	1.84	.32	1.23	.12
Parahoplites Anthula	U. Apt	2.42	.25	.90	.15
Acanthohoplites Sinzow	U. Apt-L. Alb	1.87	.31	1.28	.11
Paracanthoplites Stoyanow	L. Alb	2.04	.2	.88	.13
Hypacanthoplites Spath	L. Alb	2.12	.34	.57	.12
Gargasiceras Casey	U. Apt	2.25	.33	1.67	
Colombiceras Spath	U. Apt	1.71	.35	1	.08
Douvilleiceras De Grossouvre	L. Alb-M. Alb	2.11	.31	1.33	.18
Astiericeras Parona & Bonarelli	Low M. Alb	1.69	.46	1.86	
DESHAYESITIDAE					
Deshayesites Kazansky	L. Apt-U. Apt	3.13	.22	1	.12
Dufrenoyia Burckhardt	U. Apt	2.64	.23	.5	.18
ENGONOCERATIDAE					
Knemiceras Böhm	M. Alb-U. Alb	2.17	.21	1.09	.27
Parengonoceras Spath	L. Alb-M. Alb	1.71	.18	.57	
Engonoceras Neumayr & Uhlig	M. Alb-Cenom	1.98	.18	.45	.1
Protengonoceras Hyatt	M. Alb	1.64	.09	.43	

APPENDIX 1 continued

Taxon	Age	W	D	S	Or
Metengonoceras Hyatt	U. Alb	2.07	.07		
Epengonoceras Spath	Cenom-L. Turon	2.31	.13	.58	
Neolobites Fischer	Cenom	2.25	.12	.43	.05
PLACENTICERATIDAE					
Proplacenticeras Spath	Cenom-Con	2.32	.09	.53	
Metaplacenticeras Spath	Santon-Camp				.06
Placenticeras Meek	U. Santon-L. Camp	1.64	.16	.59	
Stantonoceras Johnston	U. Santon-L. Camp	1.75	.22	1.03	.05
Diplacmoceras Hyatt	L. Camp	2.32	.17	.45	
Haresiceras Reeside	U. Santon	2.94	.08	.64	
Hoplitoplacenticeras Spath	L. Camp-Maas	2.10	.21	1.04	.17
LYMERIELLIDAE					
Proleymeriella Breistroffer	L. Alb	2.16	.28	.78	.13
Leymeriella Jacob	L. Alb-M. Alb	2.16	.36	.94	.25
Epileymeriella Breistroffer	L. Alb	3.06	.29	.8	.11
Aioloceras Whitehouse	U. Apt-L. Alb	2.51	.18	.65	
HOPLITIDAE					
Cleoniceras Parona & Bonarelli	L. Alb-M. Alb	2.54	.12	.42	
C. (Neosaynella) Casey	L. Alb	2.42	.11	.52	
Puzosigella Casey	Up. L. Alb	2.10	.21	.73	.075
Farnhamia Casey	L. Alb	1.89	.27		
Tetrahoplites Casey	L. Alb	1.86	.37	1.32	.25
Pseudosonneratia Spath	L. Alb-M. Alb	1.64	.38	.95	.17
Hoplites Neumayr	M. Alb	1.91	.2	.69	.14
Anahoplites Hyatt	M. Alb-U. Alb	2.64	.15	.55	.13
Epihoplites Spath	M. Alb-U. Alb	2.66	.29	1.05	.11
Discohoplites Spath	U. Alb	2.07	.30	.69	
Hyphoplites Spath	U. Alb-L. Cenom	2.35	.22	.56	.21
Sonneratia Bayle	Up. L. Alb	2.32	.31	.45	.11
Tetrahoplitoides Casey	L. Alb	3.31	.25		
Protohoplites Spath	Up. L. Alb-Low M. Alb	2.09	.35	1.65	
P. (Hemisonneratia) Casey	M. Alb	2.51	.26	1.14	.2
Otohoplites Steinmann	Up. L. Alb-L. M. Alb	2.25	.27	.96	.19
Dimorphoplites Spath	M. Alb-U. Alb	2.12	.31	.86	.16
Lepthoplites Spath	U. Alb	1.96	.25	.69	.08
Pleurohoplites Spath	U. Alb	2.01	.32	.9	.09
P. (Arrhaphoceras) Whitehouse	U. Alb	2.35	.30	1.31	.11
Cyamhoplites Spath	L. Alb	2.32	.31	.41	.14
Lemurcoceras Spath	L. Alb	1.99	.38	.73	
Arcthoplites Spath	L. Alb	1.99	.26	.91	.2
Gastropolites McLearn	M. Alb	1.96	.19	.76	.14
SCHLOENBACHIIDAE					
Schloenbachia Neumayr	Up. U. Alb-U. Cenom	1.96	.26	.96	.12
Euhystrichoceras Spath	L. Cenom	1.65	.33	1.17	
Prionocycloides Spath	Cenom	2.25	.2	.67	
Tropitoides Spath	Cenom	2.32	.06	.53	.07
Prohauericeras Nowak	Turon	2.19	.18	.46	
FORBESICERATIDAE					
Forbesiceras Kossmat	Cenom	2.91		.48	.03
BRANOCERATIDAE					
Eubrancoceras Breistroffer	Up. L. Alb-L. M. Alb	2.33	.38	.83	.25
E. (Parabrancoceras) Breistroffer	L. Alb	1.62	.43	1	
Brancoceras Steinmann	Up. L. Alb-M. Alb	2.15	.36	.86	.16
Hysteroceras Hyatt	Up. M. Alb-L. U. Alb	1.89	.41	.85	.25
Mojsisoviczia Steinmann	M. Alb	1.65	.25	.46	.2
Venezoliceras Spath	Up. M. Alb	1.6?	.25	.65	
Oxytropidoceras Sticler	Up. L. Alb-M. Alb	3.06	.21	.64	.06
Dipoloceras Hyatt	Up. M. Alb-L. U. Alb	2.06	.33	1.55	.08

APPENDIX 1 continued

Taxon	Age	W	D	S	Or
Mortoniceras Meek	Up. M. Alb-Up. Alb	1.72	.45	1.52	.22
M. (Dieradoceras) Van Hoepen	Low. U. Alb	3.10		1.19	
M. (Durnovarites) Spath	Up. U. Alb	2.68	.44	1.10	
M. (Cantabrigites) Spath	Up. U. Alb	2.51	.5	.82	
Neokentroceras Spath	Low. U. Alb	3.24	.39	1.18	
Aresoceras Van Hoepen	Low. U. Alb		.27	.65	
Cainoceras Van Hoepen	Low. U. Alb	1.94	.34	.76	.25
Prohysteroceras Spath	Low. U. Alb	2.10	.43	1.04	.09
Neoharpoceras Spath	Up. U. Alb	3.10	.14	.47	.07
Spathiceras Whitehouse	Top. U. Alb-L. Cenom	1.60	.33	.63	.33
FLICKIIDAE					
Flickia Pervinquiere	U. Alb-L. Cenom	2.42	.25	.57	
Ficheuria Pervinquiere	U. Alb-L. Cenom	2.01	.29		
Adkinsia Böse	L. Cenom	2.01	.29		
Prolyelliceras Spath	L. Alb	2.06	.36	1	.16
LYELLICERATIDAE					
Lyelliceras Spath	L. Alb-M. Alb	1.78	.47	1.29	.33
Tegoceras Hyatt	L. Alb-M. Alb	2.14	.26	.86	.15
Neophlycticeras Spath	M. Alb-C. Alb	2.91	.24	.86	.17
Stoliczkaia Neumayr	U. Alb-L. Cenom	2.32	.28	.18	
Budaiceras Böse	L. Cenom	2.31	.24	.66	.22
Salaziceras Breistroffer	U. Alb	1.69	.31		.22
Mantelliceras Hyatt	L. Cenom	2.25	.26	1.25	.12
M. (Cottreauties) Collignon	L. Cenom	2.47	.18		
Sharpeiceras Hyatt	Cenom	3.14	.26	.89	.11
Acompsoceras Hyatt	L. Cenom	2.25	.31	.74	.21
Calycoceras Hyatt	Cenom	3.09	.25	1.16	.18
Paracalycoceras Spath	Cenom	2.25	.27	1.14	.14
Eucalycoceras Spath	U. Cenom-basal Tur	2.40	.23	.83	.11
Acanthoceras Neumayr	Up. L. Cenom-U. Cenom	2.82	.36	1.3	.18
Neosaynoceras Breistroffer	L. Cenom	1	.33	1.75	
Euomphaloceras Spath	U. Cenom	3.06	.36	1.33	.13
Kanabiceras Reeside & Weymouth	U. Cenom-L. Tur	1.87	.23	1.25	.11
Romaniceras Spath	U. Cenom	2.78	.34	1.13	.19
Protacanthoceras Spath	U. Cenom	1.92	.33	.75	.27
Dunveganoceras Warren & Stelck	U. Cenom		.42	.96	.21
Utaturiceras Wright	U. Cenom	2.78	.18	.45	.1
Metoicoceras Hyatt	L. Turon	2.78	.17	.48	.24
Watinoceras Waren	L. Turon	2.39	.41	.9	.11
Benueites Reyment	L. Turon	1.99	.25	.67	.08
Mammites Laube & Bruder	Turon	2.19	.22	.79	.17
Kamerunoceras Reyment	L. Turon	2.33	.28		.15
Pseudaspidoceras Hyatt	L. Turon	2.73	.34	1.08	.13
Metasigaloceras Hyatt	L. Turon	1.29	.39	1.8	.26
Borrissjakoceras Arkangelsky	U. Cenom-Tur	2.64	.31	.56	
BINNEYITIDAE					
Binneyites Reeside	Coni	2.33	.07	.41	.04
VASCOCERATIDAE					
Spathites Kummel & Decker	L. Turon	2.36	.12		
Gombeoceras Reyment	L. Turon	1.72	.29	1.30	
Paravascoceras Furon	L. Turon	2.32	.17		.17
Vascoceras Choffat	L. Turon	2.04	.35	.88	
Paramammites Furon	L. Turon	2.78	.38	1.25	.14
Fagesia Pervinquiere	L. Turon	1.78	.32	2.32	.2
Thomasites Pervinquiere	L. Turon	2.25	.21	1.15	
Neoptychites Kossmat	L. Turon	1.47		.74	

APPENDIX 1 continued

Taxon	Age	W	D	S	Or
TISSOTIIDAE					
Pseudotissotia Peron.	L. Turon	1.96	.23	.78	.2
P. (Bauchioceras) Reyment	L. Turon	2.14	.16	.81	.15
P. (Wrightoceras) Reyment	L. Turon	2.17	.18	1.13	
Choffaticeras Hyatt	Turon	1.28	.24	.46	
C. (Leoniceras) H. Douvillé	Turon	1.27	.31	.84	
Plesiotissotia Peron.	Coni	2.20	.13	.53	.11
Heterotissotia Peron.	U. Turon-Coni	1.66	.15	.79	
Tissotia H. Douvillé	Coni-L. Santon	1.47	.09	.67	.11
T. (Metatissotia) Hyatt	Coni-L. Santon	2.42	.07	.92	
Hemitissotia Peron.	Coni				.14
Buchiceras Hyatt	Coni	1.70	.2	1.25	.17
Hoplitoides von Koenen	L. Turon-Coni	1.87	.05		
COILOPOCERATIDAE					
Glebosoceras Reyment	L. Turon	1.81	.03	.47	
Coilopoceras Hyatt	L. Turon-Coni	2.10	.07	.54	.33
COLLIGNONICERATIDAE					
Collignoniceras Breistroffer	Turon	1.84	.37		
C. (Selwynoceras) Warren & Stelck	L. Turon	2.34	.35	.76	
Prionocyclus Meek	Turon	2.47	.36	.79	.2
Subprionocyclus Shimizu	U. Turon	2.37	.3		.2
Germaniceras Breistroffer	U. Turon	2.25	.42	1.57	
Niceforoceras Basse	Coni	3.06	.21	.45	
Subprionotropis Basse	Coni	3.06	.21	.45	
Gauthiericeras De Grossouvre	U. Turon-Coni	2.25	.33	1.06	.12
Peroniceras De Grossouvre	Coni	2.20	.53	.96	.18
Yabieceras Tokunaga & Shimizu	Coni				
Protexanites Matsumoto	L. Coni-L. Santon	2.04	.4	1.06	.21
Texanites Spath	U. Coni-L. Camp	1.84	.39	.70	.20
Paratexanites Collignon	L. Coni-L. Santon				.22
P. (Parabevahites) Collignon	U. Coni-L. Santon	1.99	.38	1	.16
Bevahites Collignon	U. Santon-M. Camp	2.36	.40	1.15	.19
Submortoniceras Spath	Camp				.2
Menabites Collignon	U. Santon-M. Camp	1.73	.4	1.23	.2
Barroisiceras De Grossouvre	Coni	2.09	.17	.3	.16
Solgerites Reeside	Coni	1.60	.17	.55	.21
Forresteria Reeside	Coni	2.64	.15	1.41	.26
F. (Reesideoceras) Basse	Coni	2.42	.21	.77	
F. (Harleites) Reeside	Coni	1.83	.09	.43	
Lenticeras Gerhardt	Coni-L. Santon	1.14		1.13	
Paralenticeras Hyatt	U. Coni-L. Santon			.38	
Eulophoceras Hyatt	U. Coni-L. Camp	3.06		.31	
Pseudoschloenbachia Spath	U. Santon	2.64	.12	.52	.2
Diaziceras Spath	U. Santon	1.70	.10	1.11	
Manambolites Hourca	U. Camp-Maas	1.50		.39	
SPHENODISCIDAE					
Daradiceras Sornay & Tessiuer	Maas	2.33	.45	1.12	.25
Sphenodiscus Meek	Maas	2.25	.06	.41	
Libycoceras Hyatt	Maas	2.04	.07	.43	
Indoceras Noetling	Maas	1.99	.02	.45	

APPENDIX 2

Ornamental rugosities of heteromorphic ammonites.

Taxon	Age	Or
BOCHIANITIDAE		
Protancyloceras	Ti-Be	.18
Bochianites	Ti-Haut	.25
Juddiceras	Val	.125
ANCYLOCERATIDAE		
Aegocrioceras	Haut	.09
Crioceratites	Ha-Ba	.045
Balearites	Ha	.05
Paracrioceras	Ha-Ba	.09
Menuthiocrioceras	Ha	.08
Hoplocrioceras	Ha-Ba	.14
Shasticrioceras	Ba	.13
Pedioceras	Ba-Apt	.05
Parancyloceras	Ba	.1
Acrioceras	Ha-Ba	.07
Aspinoceras	Ba	.13
Uhligia	Ba-Apt	.07
Ancyloceras	Ba	.11
Toxoceras	Apt	.16
Australiceras	Apt	.11
Tropaeum	Ba-Apt	.2
Hamiticeras		.21
HETEROCERATIDAE		
Heteroceras	Ba-Apt	.15
Colchidites	Ba-Apt	.17
Dissimilites	Ba	.1
HEMIHOPLITIDAE		
Pseudothurmannia	Ha-Ba	.11
Hemihoplites	Ha-Ba	.17
PTYCHOCERATIDAE		
Anahamulina	Ha-Ba	.05
Hamulina	Ba	.11
Ptychoceras	Apt-Alb	.11
HAMITIDAE		
Hamites	Apt-Alb	.05
Hemiptychoceras	Alb	.1
Stomohamites	Alb-Tur	.09
ANISOCERATIDAE		
Protanisoceras	Alb	.11
Anisoceras	Alb-Tur	.2
Idiohamites	Alb-Ceno	.11
Allocrioceras	Tur	.22
Phlycticrioceras	Coni	.12
TURRILITIDAE		
Proturrilitoides	Alb	.11
Turrilitoides	Alb	.13
Ostlingoceras	Alb-Ceno	.125
Pseudohelicoceras	Alb	.25
Hypoturrilites	Alb	.3
Turrilites		.13
NOSTOCERATIDAE		
Bostrychoceras	Ceno	.07
Nipponites	Tur-Sant	.08
Nostoceras	Camp	.08
Exiteloceras	Camp	.12
Solenoceras	Ca-Ma	.1
Neocrioceras	Camp	.07
Didymoceras	Ca-Ma	.08
DIPLOMOCERATIDAE		
Glyptoxoceras	S-Ma	.09
Diplomoceras	Ca-Ma	.04
Polyptychoceras	Coni-Camp	.12
Pseudoxybeloceras	Coni-Ca	.09
SCAPHITIDAE		
Clioscaphites	S-C	.12
Hoploscaphites	Ca-Ma	.05
Discoscaphites	Ca-Ma	.07
Acanthoscaphites	Camp	.07

APPENDIX 3

S and Or values for Great Valley Sequence ammonites, California.

Taxon	Age	S	Or
PACHYDISCIDAE			
Anapachydiscus californicus	Camp	1.0	
Eupachydiscus teshioensis	Sant	1.3	.15
Eupachydiscus hardai	Sant	1.0	.14
Pachydiscus egertoni	Camp	.8	.08
Pachydiscus subcompressus	Maas	.7	.05
Pachydiscus buckhami	Sant-Camp	1.0	.06
Canadoceras yokoyami	Sant-Camp	.9	.09
Canadoceras newberryanum	Camp	.8	.10
Patagiosites arbucklensis	Camp	.8	.07
Menuites sp.	Camp	1.1	
ACANTHOCERATIDAE			
Graysonites wooldridgei	Ceno	.65	.17
Calycoceras spinosum	Ceno	1.02	.17
Calycoceras orientale	Ceno	.9	.15
Calycoceras boulei	Ceno	1.2	.15
Calycoceras stoliczkai	Ceno	1.48	.11
Acanthoceras whitei	Ceno	1.2	.18
Acanthoceras sp.	Ceno		.11
Romaniceras deveroide	Tur	1.25	.21
Romaniceras pseudodeverianum	Tur		.23
Eucalycoceras shastense	Tur	1.0	.17
Kanabiceras septemseriatum	Tur	1.4	.19
VASCOCERATIDAE			
Plesiovascoceras californicum	Tur	1.8	.3
COLLIGNONICERATIDAE			
Collignoniceras woollgari	Tur	1.0	.15
Subprionocyclus branneri	Coni	.8	.20
Subprionocyclus neptuni	Coni	.7	.25
Subprionocyclus normalis	Coni	.57	.15
Peroniceras tehamense	Coni?	1.0	.23
Texanites kawasakii	Coni?		.15
Protexanites thompsoni	Coni		.16
Submortoniceras chicoense	Camp	.7	.23
Pseudoschloenbachia boulei	Sant-Camp	.5	.02
Pseudoschloenbachia sp.	Sant		0
Metaplacenticeras pacificum	Camp	.45	.08
Hoplitoplacaticeras vancouverense	Camp	.48	.12
PULCHELLIIDAE			
Pulchellia popenoi		.6	.14
BRANCOCERATIDAE			
Mortoniceras sp.	Alb	1.14	.30
Oxytropidiceras packardi	Alb	.55	.12
DOUVILLEICERATIDAE			
Douvilleiceras spiniferum	Alb	1.33	.19
Acanthoplites gardneri	Apt	.66	.16
Paraphoplites dallasi	Apt	.94	.09
Cheloniceras sp.	Bar		.15
DIPLOMOCERATIDAE			
Scalarites mihoensis	Tur-Coni		.075
Ryugusella ryugasensis	Sant-Camp		.09
Glyptoxoceras subcompressum	Sant-Maas		.08
Glyptoxoceras indicum	Camp		.06
Polyptychoceras vancouverense	Sant		.25
NOSTOCERATIDAE			
Bostrychoceras elongatum	Sant		.13
Bostrychoceras otsukai	Sant		.07

APPENDIX 3 continued

Taxon	Age	S	Or
Pseudoxybeloceras lineatum	Sant-Camp		.09
Didymoceras vancouverensis	Maas		.09
Hyphantoceras venustum	Coni-Sant		.05
Neocrioceras sp.	Coni-Sant		.07
Nipponites occidentalis	Tur		
HAMULITIDAE			
Euptychoceras jordanense	Bar		0
Anahamulina aldersona	Apt		.04
Anahamulina wilcoxensis	Apt		.07
CRIOCERATIDAE			
Acrioceras hamlini	Haut		.10
Acrioceras voyanum	Haut		.05
Acrioceras vespertinum	Haut		.11
Shasticrioceras pontiente	Bar		.09
Shasticrioceras whiteneyi	Bar		.09
Crioceratites sp.	Bar		.06
Crioceratites latus	Haut-Bar		.08
Hemibaculites sp.	Bar		.13
Shasticrioceras patricki	Bar		.08
Hoplocrioceras wintunium	Haut		.09
Hoplocrioceras redmondi	Haut		.036
Hoplocrioceras duncanense	Haut		.06
HAMITIDAE			
Hamites sp.	Alb		<.05
Stomohamites sp.	Alb		<.05
TURRILITIDAE			
Pseudohelicoceras	Ceno		<.15
ANCRYLOCERATIDAE			
Ancyloceras elephans	Bar		.09
Ancyloceras thomeli	Bar		.25
Dissimilites sp.	Bar		.12
Toxoceras blandi	Bar		.12
Toxoceratoides royerianus	Bar		.3
Toxoceratoides saulae	Bar		.10
Toxoceratoides starkingi	Bar		.10
Toxoceratoides corae	Bar		.14
Toxoceratoides greeni	Bar		.10
Heteroceras jeletzkyi	Bar		.09
Hemihoplites popenoi	Bar		.08
Arquethites sp.	Bar		.15
TETRAGONITIDAE			
Eogaudryceras hertleini	Apt	1.0	0
Eogaudryceras aurarium	Alb	1.0	0
Anagaudryceras whitneyi	Alb	1.0	.14
Eotetragonites wintunius	Apt	1.03	.02
Eotetragonites shoupi	Apt	1.2	.02
Eotetragonites gainesi	Alb	.97	.02
Gabbioceras angulatum	Apt	1.4	.01
Protegragnoites crebrisulcatus	Alb	1.0	0
Anagaudryceras mikobokense	Maas	.9	
Gaudryceras denseplicatum	Tur-Camp		.03
Gaudryceras denmanense	Sant-Camp	.8	.03
Vertebrites kayei	Maas	1.1	
Zelandites inflatus	Coni-Sant?	.78	0
Tetragonites glabrus	Sant	1.03	0
Tetragonites popetensis	Sant-Camp	1.03	0
Epigoniceras epigonium	Camp	1.02	0
Pseudophyllites indra	Camp-Maas		.02

APPENDIX 3 continued

Taxon	Age	S	Or
PHYLLOCERATIDAE			
Phylloceras aff. *P. thetys*	Bar	.6	.02
Partschiceras occidentale	Bar	.9	.07
Phyllopachyceras trinitense	Val-Haut		.07
Phyllopachyceras umpquanum	Val-Haut	.94	.03
Hypophylloceras onoense	Haut	.54	.02
Neophylloceras ramosum	Coni-Maas	.48	.02
Neophylloceras hetonaiense	Maas	.56	.02
Calliphylloceras sp.	Alb	.6	0
Phylloceras aldersona	Apt		.02
Hypophylloceras californica	Alb		.03
Neophylloceras serititense	Alb		.02
LYTOCERATIDAE			
Lytoceras batesi	Bar-Alb		.04
Lytoceras saturnale	Val-Bar		.02
Eulytoceras phestum	Bar	1.0	
KOSSMATICERATIDAE			
Marshallites sp.	Camp	.60	.06
BOCHIANITIDAE			
Bochianites sp.	Val-Haut		.125
DESMOCERATIDAE			
Desmoceras kossmati	Ceno	1.0	0
Desmoceras japonicum	Ceno		0
Desmoceras merriami	Alb	.92	0
Barremites sp.	Bar		.05
Desmoceras dawsoni	Alb	.6	0
Desmophyllites diphylloides	Sant-Camp	.75	0
Damesites damesi	Sant	.7	0
Damesites hetonaiensis	Sant	.65	0
Hauericeras angustatum	Sant-Camp	.5	0
Hauericeras rembda	Maas	.5	0
Puzosia intermedia	Alb	.73	.05
Puzosia subquadrata	Alb	1.0	.05
Puzosia hofmanni	Alb	1.06	.05
Melchiorites indigenes	Bar	.93	.09
Mesopuzosia pacifica	Alb	.75	.07
Mesopuzosia indopacifica	Sant	.75	.08
Pachydesmoceras pachydiscoide	Alb	.85	.05
Leconteites lecontei	Alb	.74	.07
Beaudanticeras breweri	Alb	.56	.07
Brewericeras hulenensis	Alb	.48	.06
Cleoniceras susukii	Alb	.57	.06
BERRIASELLIDAE			
Thurmanniceras stippi	Val	.55	.07
Thurmanniceras californicum	Val	.64	.07
Thurmanniceras wilcoxi	Val	.74	.04
Hannaites riddlensis	Haut	.55	.04
Hannaites truncatus	Haut	.64	.10
Neocomites indicus	Val		.07
Kilianella crassiplicata	Val	.82	.08
Kilianella besairiei	Val		.11
Sarasinella angulata	Val		.07
Sarasinella hyatti	Val		.08
Sarasinella densicostata	Val		.06
HOLCODISCIDAE			
Spitidiscus oregonensis	Val	.64	.04
OLCOSTEPHANITIDAE			
Homolsomites stantoni	Val	.72	.05

APPENDIX 3 continued

Taxon	Age	S	Or
Homolsomites mutabilis	Val	.68	.09
Olcostephanus pecki	Val	1.0	.04
Speetoniceras agnessense	Haut		.15
Spitidiscus oregonensis	Haut	.64	.04
Polyptychites trichotonus	Val		.07
Wellsia packardi	Haut		.08
Wellsia oregonensis	Haut	.63	.04
Wellsia vigorosa	Haut		.06
Neocraspedites giganteus	Haut	.67	.04
Simbirskites lecontei	Haut	1.0	.07
Simbirskites broadi	Haut	1.27	.12
Hertleinites aguila	Haut	.65	.06
H. rectoris	Haut	1.04	.06
Hollisites lucasi	Haut	1.1	.07
Hollisites inflatus	Haut	1.07	.04

MALACOLOGIA, 1986, 27(1): 29–41

DIFFERENTIAL EXTINCTION IN TROPICAL AMERICAN MOLLUSCS: ENDEMISM, ARCHITECTURE, AND THE PANAMA LAND BRIDGE

Geerat J. Vermeij & Edward J. Petuch

Department of Zoology, University of Maryland, College Park, MD 20742, U.S.A.

ABSTRACT

The uplift of the Central American isthmus during the Pliocene triggered a substantial impoverishment in the marine biota of tropical America. We tabulated all Pliocene subgeneric taxa and their living descendants in 18 families of gastropods and three families of pelecypods for each of three regions: (1) the Caloosahatchian Province, centered in Florida; (2) the Atlantic Gatunian region, comprising the rest of the tropical Western Atlantic, and (3) the Pacific Gatunian, corresponding to the modern tropical Eastern Pacific. Extinction affected molluscs more in the Caloosahatchian (32%) and Atlantic Gatunian regions (32%) than in the Pacific Gatunian (15%). In all regions, endemic taxa suffered more than 50% extinction. Because the Atlantic faunas were richer in endemics than was the Pacific Gatunian, part of the interoceanic difference in the impact of extinction is attributable to the high susceptibility to extinction of narrowly distributed taxa.

The tendency for hard-bottom gastropods to be somewhat more resistant to extinction than were soft-bottom taxa is shown to be partly the result of an artifact of geographical range, there being relatively few endemic taxa among hard-bottom gastropods. Hard-bottom taxa with a narrow or thick-lipped aperture were more susceptible to extinction in the Atlantic than were their wide-apertured counterparts. This pattern, which is not an artifact of geographical range, resulted in a post-Pliocene decline in the incidence of apertural protective devices among Atlantic hard-bottom gastropods, while the incidence of these features in the Eastern Pacific remained at the high Pliocene level. Among soft-bottom gastropods, the incidence of narrow and thick-lipped apertures has increased from the Pliocene to the Recent in each of the three regions of tropical America.

An examination of refuges to which previously more widely distributed taxa have become restricted shows that high productivity could have played a role in the persistence of many populations. The Eastern Pacific is the most important of these refuges, but the north and east coasts of South America have also been important.

Key words: extinction; Panama land bridge; productivity; predation; molluscs; endemism.

INTRODUCTION

The uplift of the Central American isthmus, which created a continuous land bridge between North and South America between 3.5 and 3.1 million years ago (Saito, 1976, Keigwin, 1978), was one of the most important events in recent earth history. It permitted a large-scale two-way migration of land mammals (Marshall *et al.,* 1982), intensified north-south circulation in the oceans (Emiliani *et al.,* 1972; Holcombe & Moore, 1977; Kaneps, 1979), altered the chemistry of the Atlantic Ocean (Keigwin, 1982), and perhaps created conditions favorable for northern-hemisphere glaciation (Weyl, 1968). Together with periodic glaciations which raised and lowered sea levels and sea surface temperatures, the uplift triggered a substantial biotic impoverishment by the extinction of numerous shallow-water lineages of molluscs (Woodring, 1966; Vermeij, 1978; Petuch, 1982), barnacles (Spivey, 1981), corals (Porter, 1972; Dana, 1975; Frost, 1977; Heck & McCoy, 1978), and other groups.

It is evident from the distribution of Recent molluscs that the extinctions in marine tropical America did not affect all regions equally. Many molluscan taxa which in the Pliocene occurred in both the Atlantic and Pacific areas of the Americas are today confined to the Eastern Pacific. The number of taxa that have become confined to the Atlantic is only 1/8 as large as that of taxa confined to the Pacific (Woodring, 1966; Vermeij, 1978). Extinction therefore seems to have affected the Atlantic biota far more profoundly than it affected the

Pacific. For scleractinian corals, however, the trend may have been the reverse (Porter, 1972; Heck & McCoy, 1978).

The study of extinction in tropical America might shed light on several interesting problems. The Recent faunas on the Atlantic and Pacific coasts of tropical America differ in many important respects. A study of the incidence of cognate species (closely similar Atlantic and Pacific congeners) showed that hard-bottom gastropods are taxonomically more divergent than are gastropods from unconsolidated (soft) bottoms (Vermeij, 1978). Does this pattern mean that extinction affected hard-bottom gastropods more than it affected species on soft bottoms, or is the pattern the result of differential diversification on the two coasts? Moreover, there is a considerable difference between Western Atlantic and Eastern Pacific hard-bottom gastropods in the incidence of certain antipredatory features. Caribbean faunas show a lower incidence of narrow apertures (aperture length/aperture width greater than 2.5), strong sculpture, low spires (apical half-angle greater than 45°), and thick outer lips than do assemblages in the Eastern Pacific (Vermeij, 1978). Ecological studies have shown that the architectural contrast reflects a higher intensity of predation by shell-breakers in the Eastern Pacific (Bertness, 1982). When did these differences arise, and did differential extinction contribute to them? What was the architectural history of soft-bottom gastropods, among which regional differences in predation-related architecture are not evident today in tropical America (Vermeij, 1978; Vermeij *et al.*, 1980)?

New reconstructions of pre-isthmian biogeography (Petuch, 1982) and the discovery of a relict fauna in northern South America (Petuch, 1976, 1981a) now permit a refinement of our understanding of marine extinctions in tropical America. During the Miocene and Pliocene, tropical America was divided into two biogeographical provinces: (1) the Caloosahatchian Province, centered in Florida and extending to the Carolinas, Yucatan, and northern Cuba; and (2) the Gatunian Province, extending in the Atlantic from Nicaragua and the West Indies to central Brazil and in the Pacific from the Gulf of California to northern Peru (Petuch, 1982). After the formation of the Central American isthmus, the Pacific and Atlantic parts of the Gatunian Province became mutually isolated and acquired distinctive though closely allied biotas.

We ask three questions in this paper. First, what proportion of the Pliocene fauna is still extant in Florida, the Atlantic Gatunian Region, and the Eastern Pacific (Pacific Gatunian Region)? Second, what factors have influenced differential extinction on the two coasts of tropical America? Does a narrow range during the Pliocene imply a higher than average susceptibility to extinction and, if so, does the small number of endemic Pacific taxa during the Pliocene explain the relatively small impact of extinction in that region? Finally, were the extinctions selective with respect to habitat type and predation-related architecture?

METHODS

Petuch (1982) compiled a list of all subgenera and species groups of larger mesogastropods and neogastropods that have been described from Pliocene strata of tropical America. Because many of these families have not been studied comprehensively in recent years, we have chosen to restrict our analysis to 18 of the families treated by Petuch. Our revised and emended compilation (see the Appendix) is based on a reevaluation of several general systematic treatments (Olsson, 1964; Keen, 1971) and on recent papers on Strombidae (Abbott, 1960), Tonnacea (Abbott, 1968; Beu, 1980), Muricidae (Vokes & D'Attilio, 1982), Columbellidae (Radwin, 1977a, b), Buccinidae (Vokes, 1970; Cernohorsky, 1981), Mitridae (Cernohorsky, 1976), and Cancellariidae (Petit, 1967, 1970, 1976). In addition, we have compiled a list for the pelecypod families Arcidae (Reinhart, 1935; Olsson, 1961, 1964; Woodring, 1973), Cardiidae (Keen, 1980), and Lucinidae (Bretsky, 1976). We have followed Woodring's (1973) interpretation of the arcid groups established by Olsson (1961). We accept Olsson's (1964) lucinid taxa together with Woodring's (1982) range extensions despite Bretsky's (1976) uncertainty about the status of some of the fossil forms. Many of the subgenera that we have tabulated may be subject to reinterpretation and revision in the future.

Some readers will object that we have taken only a subset (indeed, a minority of

families) of gastropods and pelecypods. Unfortunately, many groups have not received serious attention from investigators on one or both coasts of tropical America, so that the stratigraphical and geographical distribution of many genera and subgenera remains in doubt. Some families, like the Naticidae, are well monographed in the Eastern Pacific (Marincovich, 1977), but remain little understood in the Western Atlantic. Small-shelled families and most opisthobranchs are poorly known. In order to have more control over the quality of our data, we elected not to include these families. We must assume that the evolutionary behavior of the families which we have studied is similar to that of families that we omitted.

Six biogeographical categories of tropical American Pliocene molluscs may be recognized: (1) Caloosahatchian endemics; (2) Atlantic Gatunian endemics; (3) Pacific Gatunian endemics; (4) Gatunian taxa, found in both the Atlantic and Pacific parts of the Gatunian Province; (5) Atlantic taxa, those found in both the Caloosahatchian and Atlantic Gatunian regions; and (6) pan-American taxa, those found in all three regions. Many Pliocene taxa that during the Pliocene were found in both the Atlantic and the Pacific have in the Recent fauna become restricted to the Pacific; they have been referred to as Paciphilic (Woodring, 1966). Caribphilic taxa are those that have become restricted in the Recent fauna to the Western Atlantic.

Although a more quantitative treatment of geographical range would be desirable, we believe that artifacts of preservation mitigate against greater precision. Although the Pliocene fauna is known from many localities throughout tropical America, some environments may be represented by only a few sites. Taxa confined to these environments would then seem to be endemic when in fact their limited distribution represents the rarity or poor preservability of shells in some environments.

We calculated the impact of extinction as follows. For each of the three regions of tropical America, the number of taxa that became locally extinct was divided by the total number of taxa that were present in that region at or before the time of the isthmian uplift.

Throughout this paper, we treat the Recent faunas of America as impoverished versions of the Pliocene fauna. At the supraspecific level this is a valid procedure, because very few subgenera appear in the Pleistocene that were not already present during the Pliocene. The few new Pleistocene genera, such as *Charonia* (Cymatiidae) and *Mammilla* (Naticidae), appear to be immigrants from the Indo-West-Pacific or Eastern Atlantic (Marincovich, 1977; Emerson, 1978; Vermeij, 1978; Petuch, 1982). Some groups became extinct in part or all of tropical America but were later re-introduced from the Western Pacific. We believe that the number of such groups is small, and that we have only slightly underestimated the levels of extinction.

The habitats of gastropods were established by analogy with the known habits of living forms. Taxa considered to have narrow apertures or a thick lip include all Cypraeacea (Cypraeidae, Eratoidae, Ovulidae), Tonnacea (except Ficidae and *Tonna*), Columbellidae (except *Mitrella*), Olividae (except *Ancilla*, *Eburna*, and *Agaronia*), Mitridae, Marginellidae, Conidae, Strombidae, and some Volutidae (*Plicoliva*), Muricidae (*Eupleura, Vitularia*), and Buccinidae (*Bailya, Engina, Northia*).

We have chosen not to employ statistical tests to our data. Our intent is to discover trends and effects whose significance depends less on statistical cut-off points than on biological impact.

RESULTS

Before the establishment of the Central American isthmus, the Atlantic part of the Gatunian Province was slightly richer in taxa than was the Pacific portion. Of the groups tabulated in the Appendix, only the Tonnacea and Cancellariidae had a slightly higher Pacific than Atlantic diversity in the Pliocene Gatunian Province (Table 1). The Caloosahatchian Province was generally less rich than the Atlantic Gatunian region, although the Muricidae, Fasciolariidae, and Volutidae had a larger number of taxa in the Caloosahatchian. Diversities in the Pacific Gatunian and Caloosahatchian regions were roughly comparable (Table 1).

The patterns of diversity are somewhat different today. The Atlantic and Pacific portions of the former Gatunian Province now support roughly the same number of taxa (Table 1). The region corresponding to the Neogene Caloosahatchian Province still has a gener-

TABLE 1. Diversity and extinction of tropical American molluscs. N_P = number of subgenera present in the Pliocene. N_R = number of subgenera surviving from Pliocene to Recent. E = percentage of extinction of Pliocene taxa.

	Caloos.			Atl. Gatun.			Pac. Gatun.		
Taxon	N_P	N_R	E	N_P	N_R	E	N_P	N_R	E
Gastropoda									
Turritellidae	7	4	43%	8	4	50%	7	4	43%
Strombidae	3	3	0	4	2	50%	2	2	0
Cypraeacea	8	3	63%	17	11	35%	13	11	15%
Tonnacea	7	6	14%	13	11	15%	14	9	36%
Muricidae	30	19	27%	24	19	21%	18	16	11%
Columbellidae	13	8	38%	24	19	21%	16	14	13%
Buccinidae	14	7	50%	18	8	56%	17	14	18%
Fasciolariidae	8	6	25%	7	6	14%	4	4	0
Mitridae	4	3	25%	10	5	50%	8	8	0
Olividae	9	5	44%	17	11	35%	9	9	0
Volutidae	6	5	17%	4	4	0	3	2	33%
Marginellidae	12	11	8%	12	12	0	5	5	0
Cancellariidae	10	4	60%	18	6	67%	19	16	16%
Conidae	6	4	33%	7	6	14%	7	6	14%
Total	137	88	36%	183	124	32%	142	121	15%
Pelecypoda									
Arcidae	15	10	33%	20	12	40%	20	18	10%
Lucinidae	16	14	13%	18	16	11%	16	12	25%
Cardiidae	8	7	13%	13	6	54%	13	11	15%
Total	39	31	21%	51	34	33%	49	41	17%
Total	176	119	32%	234	158	32%	191	162	15%

ally lower diversity than does either portion of the former Gatunian Province, but the Strombidae and Volutidae reach their highest subgeneric number in Florida and surrounding waters.

Differential extinction was responsible for the equalization of diversity in the two portions of the former Gatunian Province. Of the 234 taxa tabulated in the Appendix from the Pliocene Atlantic Gatunian region, 32% have become regionally extinct. For the 191 Pliocene Pacific Gatunian gastropods and pelecypods, the corresponding percentage is 15%. These estimates suggest that the impact of regional extinction was roughly two times greater in the Atlantic part of the Gatunian Province than in the Pacific part. The only families that suffered greater extinction in the Atlantic portion were the Volutidae, Lucinidae, and those in the Tonnacea (Table 1).

Even more taxa would have become regionally extinct in the Atlantic Gatunian region were it not for two refuges where taxa whose distribution was much broader during the Pliocene survive today as relicts. Atlantic representatives of six taxa (*Broderiptella, Muracypraea, Panamurex, Sincola, Aphera,* and *Subcancilla*) persist in the waters off eastern Colombia and Venezuela (Petuch, 1976, 1981a). Five taxa survive in the Western Atlantic only in Brazil (*Pusula, Northia, Plicoliva, Bullata,* and *Miltha*). *Ancilla* and *Eburna* are found today only in northern and eastern South America, but like the other taxa mentioned above they had much wider distributions in tropical America during the Pliocene. Vermeij (1978) and Petuch (1979, 1981a) have listed additional taxa that have become restricted in the Recent fauna to the Brazilian and Colombo-Venezuelan refuges.

The Caloosahatchian Province suffered substantial impoverishment of subgenera, especially among gastropods (Table 1). Of the 176 Pliocene taxa tabulated in the Appendix, 32% have become regionally extinct. Impoverishment was therefore similar in magnitude to that in the Atlantic Gatunian region and greater than that in the Eastern Pacific. The only families suffering less extinction in the Caloosahatchian Province than in the Eastern Pacific are the Volutidae, Lucinidae, and groups in the Tonnacea, the same groups which also did relatively well in the Atlantic Gatunian (Table 1).

Vermeij's (1978) suggestion that the Florida region serves as a refuge is not substantiated by our data. Of the taxa we studied, only three (*Metulella, Stewartia,* and *Dinocardium*) have survived in Florida after disappearing from the Gatunian region. Petuch (1981b) regarded the area around Roatan Island, Honduras, as a refuge for several Caloosahatchian relicts, but only one taxon (*Pleioptygma*) among the 176 Pliocene taxa we studied seems to have become restricted to this area.

As Woodring (1966) and Vermeij (1978) recognized, the Eastern Pacific serves as a refuge for many tropical American taxa which during the Pliocene lived in both the Atlantic and the Pacific. Of the 57 taxa that became regionally extinct in the Caloosahatchian Province, 16 (28%) are Paciphiles, whereas 11 (*Broderiptella, Pusula, Panamurex, Sincola, Strombina, Ancilla, Eburna, Subcancilla, Bullata, Aphera,* and *Miltha*) (19%) have become restricted to refuges in the former Atlantic Gatunian region. Paciphilic taxa comprise 39 of 76 regionally extinct subgenera (51%) in the Atlantic Gatunian region; these taxa constitute 15% of the Pliocene fauna in that region. Caribphilic taxa comprise 9 (17%) of 30 taxa which became regionally extinct in the Eastern Pacific. These data support the earlier finding that the Atlantic refuges are less important as sanctuaries than is the Eastern Pacific for Pliocene relicts.

Several taxa listed in the Appendix as being extinct in the Americas still persist in the Indo-West-Pacific, Australia, or southern Africa. They include *Dolomena, Labiostrombus, Cypraeovula, Pustularia, Subpterynotus, Harpeola, Dibaphus, Omogymna, Strephona, Neocylindrus,* and *Hawaiarca.* Of the 62 taxa that became globally extinct in the Americas, 11 (18%) belong to this relict category. That the Indo-West-Pacific has acted as a refuge for corals and other animals throughout the Cenozoic is well known (Vermeij, 1978; Heck & McCoy, 1978).

Our data show clearly that endemism (defined as occurrence during the Pliocene in only one of the three regions in tropical America) is associated with a high probability of post-Pliocene extinction. Regional extinction affected 25 of 50 Caloosahatchian taxa (50%), 19 of 34 Atlantic Gatunian taxa (56%), and 6 of 10 Pacific Gatunian taxa (60%). By contrast, the 102 pan-American taxa suffered only 24% local extinction in the Caloosahatchian, 20% extinction in the Atlantic Gatunian, and 14% regional extinction in the Pacific Gatunian region.

Habitat and apertural form clearly influenced the likelihood of extinction in tropical American gastropods. Soft-bottom gastropods were somewhat more affected by extinction than were hard-bottom forms in each of the three regions. This results chiefly from the large number of extinction-vulnerable wide-apertured taxa among soft-bottom gastropods.

As Vermeij (1978) already suspected, Atlantic Gatunian and Caloosahatchian hard-bottom gastropods with a narrow or thick-lipped aperture were more prone to extinction than were co-occurring hard-bottom forms with a wide or thin-lipped aperture (Table 2). In the Pacific Gatunian region, the situation for hard-bottom gastropods was the reverse; wide-apertured taxa were more prone to extinction than were narrow-apertured taxa (Table 2). Among gastropods from soft (unconsolidated) bottoms, taxa with a narrow or thick-lipped aperture were less susceptible to extinction than were broad-apertured taxa. This difference was evident in all three regions (Table 2).

The architectural difference between hard-bottom gastropod assemblages of the modern-day Western Atlantic and Eastern Pacific is at least in part attributable to the selective

TABLE 2. Effect of habitat and apertural shape on susceptibility of gastropods to extinction.

	Caloos.		Atl. Gatun.		Pac. Gatun.	
Category	N_P	E	N_P	E	N_P	E
Soft-bottom, broad aperture	47	53%	53	49%	46	22%
Soft-bottom, narrow aperture	43	23%	67	28%	44	16%
Soft-bottom, total	90	39%	120	38%	90	19%
Hard-bottom, broad aperture	27	26%	24	8%	20	15%
Hard-bottom, narrow aperture	20	35%	37	32%	31	3%
Hard-bottom, total	47	30%	61	23%	51	8%

extinction of narrow-apertured and thick-lipped gastropod taxa in the Atlantic. During the Pliocene, the Atlantic and Pacific parts of the Gatunian Province had about the same incidence of modified apertures (61% and 60% respectively). Among the Pliocene survivors in the modern fauna, the incidence of narrow-apertured and thick-lipped forms has remained at the Pliocene level in the Eastern Pacific (64%), but has fallen in the Caribbean to 53% (Table 3). In the Caloosahatchian Province, the incidence of modified apertures has remained rather low (40% and 37% respectively for the Pliocene and Recent) (Table 3).

TABLE 3. Incidence (I) of narrow-apertured taxa among Pliocene and Recent gastropods in tropical America.

Category	N_P	I	N_R	I
Caloosahatchian				
Hard-bottom	48	40%	32	37%
Soft-bottom	90	48%	55	60%
Atlantic Gatunian				
Hard-bottom	61	61%	47	53%
Soft-bottom	122	55%	75	64%
Pacific Gatunian				
Hard-bottom	51	60%	47	64%
Soft-bottom	90	49%	73	51%

For the soft-bottom component of the gastropod fauna, there was a modest increase in the incidence of narrow apertures and thick lips in all three regions, though in the Eastern Pacific this increase was slight (Table 3). The higher incidence of modified apertures that characterized the Atlantic Gatunian soft-bottom gastropods relative to those of the Pacific Gatunian has been maintained in the Recent fauna. Although Vermeij (1978) was unable to detect this present-day difference, he had very few Recent assemblages, particularly from the Eastern Pacific.

DISCUSSION

In general, the two Atlantic provinces of tropical America were more affected by extinction than was the Eastern Pacific. This conclusion applies to gastropods and pelecypods (Table 1), to soft-bottom and to hard-bottom gastropods (Table 2), and to narrow-apertured and wide-apertured gastropods (Table 2). Our findings are in excellent agreement with earlier inferences drawn from distributional patterns of extant species (Woodring, 1966; Vermeij, 1978).

Conclusions about the impact of extinction inevitably depend on the quality of information about the stratigraphical and geographical distribution of the taxa in question. One possible source of error in our data is the distribution of sites from which Pliocene fossils have been collected in tropical America. As Woodring (1966) has pointed out, a majority of localities is located in the Caribbean part of the Gatunian region. Although Eastern Pacific localities in Nicaragua, Costa Rica, Panama, Colombia, Ecuador, and Peru are rich in species, they are outnumbered by Caribbean localities in Central America, northern South America, and the West Indies. If sampling of the Pliocene Pacific Gatunian region was less complete than that in the Caribbean, some extinction-prone endemic taxa might have been missed, and some taxa now known only from the Atlantic Gatunian might be found in the Pacific portion of that province as well. Estimates of regional extinction in the Eastern Pacific would therefore probably be somewhat higher if more fossil-bearing localities were available. Given the large differences between present estimates of Pacific and Atlantic Gatunian extinctions, however, we do not believe that new fossil discoveries will alter our findings significantly.

Another source of error, or more precisely of variation, is the choice of taxonomic group. Families differed widely in their susceptibility to extinction (Table 1). Nevertheless, the geographical, habitat, and architectural patterns of selectivity in extinction are evident in many families. We therefore take these patterns to reflect selectivity that transcends taxonomic considerations.

The difference in the impact of extinction between the Atlantic and the Pacific may be due in part to the high incidence of extinction-vulnerable endemic taxa in the Pliocene Atlantic. Our data show clearly that Pliocene endemic taxa (those confined to a single region during the Pliocene) had a much higher probability of extinction than did pan-American taxa (those occurring in all three regions during the Pliocene). Because the Pacific Gatunian faunas contained fewer endemics (5%) than did either the Atlantic Gatunian (14%) or the Caloosahatchian fauna (28%), the greater collective resistance of

eastern Pacific taxa to extinction can be interpreted partly as an artifact of geographical range. The correspondingly large contribution of pan-American taxa to the Pliocene Eastern Pacific fauna (53%) adds further support to this interpretation.

Geographical artifacts also explain some other features of post-Pliocene extinction in tropical America. Hard-bottom gastropods were less affected by extinction than were gastropods in unconsolidated sediments (Table 2). The percentage of endemic taxa among soft-bottom gastropods is higher both in the Caloosahatchian Province (31%) and Atlantic Gatunian region (21%) than it is among hard-bottom gastropods from these two regions (27% and 15% respectively). More importantly, the percentage of pan-American taxa is substantially lower in soft-bottom gastropods both in the Caloosahatchian Province (44%) and Atlantic Gatunian region (33%) than among hard-bottom forms (66% and 51% respectively). The collectively greater resistance of hard-bottom gastropods to extinction therefore seems to be associated with relatively broad geographical ranges.

We do not know why hard-bottom snails should tend to have broader geographical distributions than soft-bottom gastropods in tropical America. It is interesting that many of the hard-bottom gastropods belong to families that have planktonically dispersing larvae. Any statistical association between distribution, habitat, and dispersibility may be quite fortuitous, but our present understanding of these relationships is still rudimentary.

Several aspects of selective extinction cannot be explained as artifacts of geographical range. In each of the three regions of Pliocene tropical America, soft-bottom gastropods with a narrow or thick-lipped aperture were more resistant to extinction than were broad-apertured forms from the same habitats (Table 2). If this pattern were the consequence of a geographical artifact, narrow-apertured forms should show a lower incidence of endemism than should broad-apertured taxa. This is indeed so in the Caloosahatchian Province (26% and 36% endemism, for narrow-apertured and broad-apertured subgenera respectively), but not in the Atlantic Gatunian region (19% and 11% respectively). Moreover, the incidence of pan-American taxa, which should be higher in the more extinction-resistant narrow-apertured forms, is either the same for the two groups (44% in the Caloosahatchian Province), or lower in the narrow-apertured forms (28% versus 42% in the Atlantic Gatunian region).

Among hard-bottom gastropods, extinction affected narrow-apertured taxa more profoundly than wide-apertured forms in both Atlantic regions of tropical America, whereas in the Eastern Pacific the narrow-apertured forms were less affected by extinction than were gastropods with a broad aperture. Again, this complex pattern would not have been predicted from the incidences of endemic and pan-American taxa. In the Caloosahatchian Province, for example, endemics comprise 56% of broad-apertured taxa and only 10% of the more extinction-prone narrow-apertured forms.

The relative reduction of narrow-apertured and thick-lipped gastropods from the Pliocene to the Recent in hard-bottom Atlantic environments suggests that selection in favor of shell armor decreased. This hypothesis is consistent with the observation that the ecological impact of shell-breaking predators is less on the Atlantic coast of Panama than on the Pacific side (Bertness, 1982). The rise in incidence of narrow-apertured taxa among gastropods from unconsolidated bottoms in all three regions suggests an increase in selection for armor in this habitat. No ecological or other data have yet come to light which support this interpretation. Vermeij *et al.* (1980) found no temporal change in the frequency of repaired injuries in terebrid gastropods in tropical America, as would have been expected if the hypothesis were correct.

Predators probably did not play a direct role in bringing about the extinction of broad-apertured taxa. Plausible instances of extinction due to biotic agents are rare, and invariably involve the bringing together of two biotas with very different evolutionary histories (Vermeij, 1978). Post-Pliocene immigration into the Atlantic seems to have been of negligible magnitude, and all the available evidence suggests that the chief predators of modern Atlantic gastropods were already present in tropical America during the Pliocene.

We believe instead that narrow-apertured forms may, on average, have lower individual and population growth rates than do broad-apertured taxa, at least on hard bottoms, and that their populations rebound less rapidly after being decimated by a catastrophe. Direct estimates of individual growth rates, egg

production, and other life-history characteristics are required for the evaluation of this hypothesis. No data of this kind currently exist. The hypothesis is, however, consistent with the properties of the tropical American refuges that we have documented in this study. Both the Pacific and the Atlantic refuges are characterized by high productivity (Vermeij, 1978; Petuch, 1981a, 1982; Antonius, 1980). Birkeland (1982) has marshalled an impressive body of evidence to support his view that massive starvation is typical for many marine invertebrate larvae under conditions of low productivity, whereas mass survival of larvae is possible when nutrient levels are increased either through upwelling or by rain-induced terrestrial runoff. High-productivity environments might therefore protect many species from repeated decimation to dangerously low population levels by providing conditions for rapid population expansion. Species with high individual growth rates might be less affected by decimation and routine starvation than those with slower growth, smaller internal volume, and other features associated with the production of armor.

There is some evidence for the hypothesis that the Caribbean Sea suffered a reduction in productivity after the uplift of the Panama land bridge. Keigwin (1982) has documented a Late Neogene decline in nutrient levels in deep Caribbean waters, whereas deep waters in the Eastern Pacific have remained consistently rich in nutrients. This pattern is also consistent with the history of scleractinian corals, which, unlike most molluscan groups, suffered more extinction in the Eastern Pacific than in the Atlantic (Heck & McCoy, 1978). Birkeland (1977) has shown that corals are outcompeted by suspension-feeding animals lacking algal symbionts when they co-occur as newly settled juveniles on panels under conditions of upwelling. A catastrophe such as a sudden drop in temperature could therefore have had much more profound effects on corals in the Eastern Pacific than in the Caribbean, where recruits would stand a better chance of success in the generally less productive waters.

The reasons for selective extinction with respect to gastropod aperture shape remain shrouded in mystery, but the consequences of selectivity are clear. Selective extinction helped to bring about a change from an architecturally homogeneous Pliocene fauna of hard-bottom gastropods in Gatunian America to architecturally divergent faunas on the Pacific and Atlantic coasts. Selective diversification has perhaps exaggerated this divergence between the faunas, as suggested by Vermeij (1978), but we have no new evidence on this point.

The patterns of extinction that we have uncovered in the Late Neogene molluscan faunas of tropical America may have properties that apply to other extinction events. The greater susceptibility of endemics to extinction may be generally true and is well known for human-caused extinctions (Vermeij, 1985). Valentine (1973), Boucot (1975), and many others have pointed out that residual faunas after major episodes of extinction show low provinciality and cosmopolitan distributions of taxa.

Our study suggests that extinction and its consequences depend on geography. Not only was extinction less profound in the Eastern Pacific than in the Atlantic, but the patterns of selectivity were different. These results provide good reasons for caution. It is unsafe to generalize from single studies of extinction. Not only do we need to understand why certain morphological traits are associated with stratigraphical persistence, but we need to know how these possibly fortuitous associations are influenced by geographical and historical peculiarities.

ACKNOWLEDGMENTS

We are grateful to the (U.S.) National Science Foundation for supporting our research. The senior author is indebted to Egbert G. Leigh for instilling an early interest in the history of tropical America. We have greatly profited from critical appraisals of our manuscript by M. L. Reaka, D. Jablonski, J. W. Valentine, and two anonymous reviewers.

REFERENCES CITED

ABBOTT, R. T., 1960, The genus *Strombus* in the Indo-Pacific. *Indo-Pacific Mollusca*, 1: 33–146.

ABBOTT, R. T., 1968, The helmet shells of the world (Cassidae). *Indo-Pacific Mollusca*, 2: 2–202.

ANTONIUS, A., 1980, Occurrence and distribution of stony corals in the Gulf of Cariaco, Venezuela.

Internationale Revue der Gesamten Hydrobiologie, 65: 321–338.
BERTNESS, M. D., 1982, Shell utilization, predation pressure, and thermal stress in Panamanian hermit crabs: an interoceanic comparison. *Journal of Experimental Marine Biology and Ecology*, 64: 159–187.
BEU, A. G., 1980, Australian gastropods of the family Bursidae. 1. The families of Tonnacea, the genera of Bursidae, and revision of species previously assigned to *Tutufa* Jousseaume, 1881. *Records of the Australian Museum*, 33: 248–324.
BIRKELAND, C., 1977, The importance of rate of biomass accumulation in early successional stages of benthic communities to the survival of coral recruits. *Proceedings of the Third International Coral Reef Symposium*, 1: 15–21.
BIRKELAND, C., 1982, Terrestrial runoff as a cause of outbreaks of *Acanthaster planci* (Echinodermata: Asteroidea). *Marine Biology*, 69: 175–185.
BOUCOT, A. J., 1975, *Evolution and extinction rate controls*. Elsevier, Amsterdam, 427 p.
BRETSKY, S. S., 1976, Evolution and classification of the Lucinidae (Mollusca; Bivalvia). *Palaeontographica Americana*, 8: 219–337.
CERNOHORSKY, W. O., 1976, The Mitridae of the world. I. The subfamily Mitrinae. *Indo-Pacific Mollusca*, 3: 273–528.
CERNOHORSKY, W. O., 1981, The family Buccinidae. Part I: the genera *Nassaria, Trajana* and *Neoteron*. *Monographs of Marine Mollusca*, 2: 201–284.
DANA, T. F., 1975, Development of contemporary Eastern Pacific coral reefs. *Marine Biology*, 33: 355–374.
EMERSON, W. K., 1978, Mollusks with Indo-Pacific faunal affinities in the eastern Pacific Ocean. *Nautilus*, 92: 91–96.
EMILIANI, C., GARTNER, S. & LIDZ, B., 1972, Neogene sedimentation on the Blake Plateau and the emergence of the Central American isthmus. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 11: 1–10.
FROST, S. H., 1977, Miocene to Holocene evolution of Caribbean Province reef-building corals. *Proceedings of the Third International Coral Reef Symposium*, 2: 353–359.
HECK, K. L., Jr. & McCOY, E. D., 1978, Long-distance dispersal and the reef-building corals of the Eastern Pacific. *Marine Biology*, 48: 349–356.
HOLCOMBE, T. L. & MOORE, W. S., 1977, Paleocurrents in the eastern Caribbean: geological evidence and implications. *Marine Geology*, 23: 35–56.
KANEPS, A. G., 1979, Gulf Stream: velocity fluctuation during the Late Cenozoic. *Science*, 204: 297–301.
KEEN, A. M., 1971, *Seashells of tropical West America*. Stanford University, Palo Alto, Calif., 1064 p.
KEEN, A. M., 1980, The pelecypod family Cardiidae: a taxonomic summary. *Tulane Studies in Geology and Paleontology*, 16: 1–40.
KEIGWIN, L. D., Jr., 1978, Pliocene closing of the Isthmus of Panama, based on biostratigraphic evidence from nearby Pacific Ocean and Caribbean Sea cores. *Geology*, 6: 630–634.
KEIGWIN, L. D., Jr., 1982, Isotopic paleoceanography of the Caribbean and East Pacific: role of Panama uplift in Late Neogene time. *Science*, 217: 350–353.
MARINCOVICH, L., Jr., 1977, Cenozoic Naticidae (Mollusca: Gastropoda) of the northeastern Pacific. *Bulletins of American Paleontology*, 70: 160–494.
MARSHALL, L. G., WEBB, S. D., SEPKOSKI, J. J., Jr. & RAUP, D. M., 1982, Mammalian evolution and the great American interchange. *Science*, 215: 1351–1357.
OLSSON, A. A., 1961, *Mollusks of the tropical eastern Pacific, particularly from the southern half of the Panamic-Pacific faunal province (Panama to Peru). Panamic-Pacific Pelecypoda*. Paleontological Research Institute, Ithaca, New York, 574.
OLSSON, A. A., 1964, *Neogene mollusks from northwestern Ecuador*. Paleontological Research Institute, Ithaca, New York, 256 p.
PETIT, R. E., 1967, Notes on Cancellariidae (Mollusca: Gastropoda). *Tulane Studies in Geology*, 5: 217–219.
PETIT, R. E., 1970, Notes on Cancellariidae (Mollusca: Gastropoda)—II. *Tulane Studies in Geology and Paleontology*, 8: 83–88.
PETIT, R. E., 1976, Notes on Cancellariidae (Mollusca: Gastropoda)—III. *Tulane Studies in Geology and Paleontology*, 12: 33–43.
PETUCH, E. J., 1976, An unusual molluscan assemblage from Venezuela. *Veliger*, 18: 322–325.
PETUCH, E. J., 1979, New gastropods from the Abrolhos archipelago and reef complex, Brazil. *Proceedings of the Biological Society of Washington*, 92: 510–526.
PETUCH, E. J., 1981a, A relict Neogene caenogastropod fauna from northern South America. *Malacologia*, 20: 307–347.
PETUCH, E. J., 1981b, A volutid species radiation from northern Honduras, with notes on the Honduran Caloosahatchian secondary relict pocket. *Proceedings of the Biological Society of Washington*, 94: 1110–1130.
PETUCH, E. J., 1982, Geographical heterochrony: contemporaneous coexistence of Neogene and Recent molluscan faunas in the Americas. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 37: 277–312.
PORTER, J. W., 1972, Ecology and species diversity of coral reefs on opposite sides of the isthmus of Panama. *Bulletin of the Biological Society of Washington*, 2: 89–116.
RADWIN, G. E., 1977a, The family Columbellidae in the Western Atlantic. *Veliger*, 19: 403–417.
RADWIN, G. E., 1977b, The family Columbellidae

in the Western Atlantic. Part II A. Pyreninae. *Veliger*, 20: 119–133.

REINHART, P. W., 1935, Classification of the pelecypod family Arcidae. *Bulletin du Musée royal d' Histoire naturelle de Belgigue*, 11(3): 1–68.

SAITO, T., 1976, Geologic significance of coiling direction in the planktonic Foraminifera *Pullenatina*. *Geology*, 4: 305–309.

SPIVEY, H. R., 1981, Origins, distribution, and zoogeographic affinities of the Cirripedia (Crustacea) of the Gulf of Mexico. *Journal of Biogeography*, 8: 153–176.

VALENTINE, J. W., 1973, *Evolutionary paleoecology of the marine biosphere*. Prentice Hall, Englewood Cliffs, New Jersey, 511 p.

VERMEIJ, G. J., 1978, *Biogeography and adaptation: patterns of marine life*. Harvard University, Cambridge, Mass., 332 p.

VERMEIJ, G. J., 1985, The biology of human-caused extinction of species. In NORTON, B. G. & SHUE, H., ed., *The preservation of species*. In press.

VERMEIJ, G. J., ZIPSER, E. & DUDLEY, E. C., 1980, Predation in time and space: peeling and drilling in terebrid gastropods. *Paleobiology*, 6: 352–364.

VOKES, E. H., 1970, The genus *Trajana* (Mollusca: Gastropoda) in the New World. *Tulane Studies in Geology and Paleontology*, 7: 75–83.

VOKES, E. H. & D'ATTILIO, A., 1982, Review of the muricid genus *Attiliosa* (Mollusca: Gastropoda). *Veliger*, 25: 67–71.

WEYL, P. K., 1968, The role of the ocean in climatic change: a theory of the ice ages. *Meteorology Monographs*, 8: 37–62.

WOODRING, W. P., 1966, The Panama land bridge as a sea barrier. *Proceedings of the American Philosophical Society*, 110: 425–433.

WOODRING, W. P., 1973, Geology and paleontology of Canal Zone and adjoining parts of Panama: description of Tertiary mollusks (additions to gastropods, scaphopods, pelecypods: Nuculidae to Malleidae). [*United States*] *Geological Survey Professional Paper*, 306-E: 453–539.

WOODRING, W. P., 1982, Geology and paleontology of Canal Zone and adjoining parts of Panama: description of Tertiary mollusks (Pelecypods: Propeamussiidae to Cuspidariidae; additions to families covered in P 306-E; additions to gastropods; cephalopods). [*United States*] *Geological Survey Professional Paper*, 306-F: 541–759.

APPENDIX

Pliocene molluscs and their living descendants in tropical America. 1 = Caloosahatchian endemic. 2 = Atlantic Gatunian endemic. 3 = Pacific Gatunian endemic. 4 = Pacific and Atlantic Gatunian. 5 = Caloosahatchian and Atlantic Gatunian. 6 = Pan-American. A = Western Atlantic. P = Eastern Pacific. ex = extinct. s = soft-bottom. h = hard-bottom.

Taxon	Pli	Rec	Habitat
Turritellidae			
Bactrospira	6	ex	s
Broderiptella	6	A P	s
Eichwaldiella	6	ex	s
Lemintina	4	ex	s
Springvaleia	2	ex	s
Torcula	6	A P	s
Torculoidella	1	ex	s
Turritella	6	A P	s
Vermicularia	6	A P	h
Strombidae			
Dolomena	2	ex	s
Labiostrombus	2	ex	s
Lentigo	3	P	s
Strombus	6	A P	s
Tricornis gigas group	1	A P	s
T. gallus group	5	A P	s
Cypraeacea			
Cymbula	4	A P	h
Cyphoma	4	A P	h
Cypraeovula	1	ex	h
Eocypraea	2	ex	h
Erato	6	A P	h
Erosaria	4	P	h
Jenneria	6	P	h
Luria	4	A P	h
Macrocypraea	4	A P	h
Marginocypraea	2	ex	h
Muracypraea	4	A	s
Propustularia	2	A	h
Pseudocyphoma	2	A	h
Pseudozonaria	4	P	h
Pustularia	6	ex	r
Pusula	6	A P	h
Simnia	6	A P	h
Siphocypraea	1	ex	s
Trivia	6	A P	h
Tonnacea			
Casmaria	4	A P	s
Cassis	6	A	s
Dalium	4	A	s
Cypraecassis	4	A P	s
Echinophoria	4	A P	s
Ficus communis group	1	A	s
F. lanza group	3	ex	s
F. ventricosa group	4	A P	s
Malea	6	P	s
Miogalea	2	ex	s
Morum	4	A P	h
Neosconsia	3	ex	s
Oniscoidea	6	A P	h
Sconsia	6	A	s
Semicassis	6	A P	s
Tonna	6	A P	s

APPENDIX CONTINUED

Pliocene molluscs and their living descendants in tropical America. 1 = Caloosahatchian endemic. 2 = Atlantic Gatunian endemic. 3 = Pacific Gatunian endemic. 4 = Pacific and Atlantic Gatunian. 5 = Caloosahatchian and Atlantic Gatunian. 6 = Pan-American. A = Western Atlantic. P = Eastern Pacific. ex = extinct. s = soft-bottom. h = hard-bottom.

Taxon	Pli	Rec	Habitat
Muricidae			
Acantholabia	1	ex	h
Acanthotrophon	1	A P	h
Aspella	6	A P	h
Attiliosa	4	A P	h
Calotrophon	6	A P	h
Chicoreus florifer group	1	A	h
C. brevifrons group	2	A	h
C. shirleyae group	1	ex	h
Dermomurex	1	A P	h
Eupleura caudata group	1	A	h
E. thompsoni group	4	P	h
Favartia	6	A P	h
Laevityphis	5	A	h
Microrhytis	1	ex	h
Miocenebra	1	ex	h
Muricanthus	6	A P	h
Muricopsis	1	A P	h
Murex	6	A P	s
Murexiella	6	A P	h
Murexsul	1	A	h
Neurarhytis	1	ex	h
Panamurex	6	A	h
Phyllonotus	6	A P	s
Pilsbrytyphis	2	ex	s
Pteropurpura	4	A P	h
Pterorhytis	1	ex	h
Pterotyphis	4	A P	s
Pterynotus	4	A P	h
Purpurellus	4	P	h
Rugotyphis	1	ex	s
Siphonochelus	2	A	h
Siratus	2	A	h
Subpterynotus	6	ex	h
Talityphis	6	A P	s
Trachypollia	6	A P	h
Tripterotyphis	6	A P	s
Typhinellus	5	A	s
Urosalpinx	c	A	h
Vitularia	6	P	h
Genus unnamed	1	ex	h
Columbellidae			
Alcira	2	ex	s
Anachis	6	A P	h
Astyris	6	A P	h
Cigclirina	4	P	s
Columbella	6	A P	h
Columbellopsis	2	A	h
Conella	2	A	h
Costoanachis	6	A P	h
Cotonopsis	4	A P	s
Eurypyrene	6	P	h
Litotrema	2	ex	s
Macgintopsis	1	ex	s
Mazatlania	4	A P	s
Metulella	5	A	s
Mitrella	6	A P	h
Nassarina	1	A P	s
Nitidella	2	A	h
Parametaria	6	P	h
Parvanachis	6	A P	h
Sincola	6	A P	s
Streptorygma	2	ex	s
Strombina	6	A P	s
Strombinella	4	ex	s
Zafrona	4	A P	h
Zanassarina	3	P	s
Buccinidae			
Agassitula	2	A	s
Antillophos	4	A P	s
Bailya	6	A P	h
Calophos	6	ex	s
Celatoconus	1	ex	s
Cymatophos	6	P	s
Engina	6	A P	h
Floritula	1	ex	s
Fusinosteira	4	P	h
Gemophos	6	A P	h
Gordanops	3	ex	h
Metaphos	4	A P	s
Metula	6	A P	s
Minitula	1	A	s
Monostiolum	1	A P	h
Nerva	2	ex	s
Nicema	4	P	s
Northia	4	A P	s
Pisania	2	A	h
Ptychosalpinx	1	A	h
Rhipophos	4	ex	s
Solenosteira	1	ex	s
Strombinophos	4	P	s
Thiarinella	2	ex	s
Trajana	6	P	s
Fasciolariidae			
Cinctura	1	A	s
Dolicholatirus	2	A	h
Fasciolaria tulipa group	5	A	s
F. gorgasiana group	2	ex	s
Fusinus	6	A P	h
Heilprinia	1	A	s
Luecozonia	4	A P	h
Liochlamys	1	ex	s
Pleuroploca	4	A P	s
Polygona	6	A P	h
Terebraspira	1	ex	s
Triplofusus	1	A P	s
Olividae			
Agaronia	4	A P	s
Ancilla	5	A	s
Callianax	4	A P	s
Dactylidella	4	A P	s
Dactylidia	5	A	s

APPENDIX CONTINUED

Pliocene molluscs and their living descendants in tropical America. 1 = Caloosahatchian endemic. 2 = Atlantic Gatunian endemic. 3 = Pacific Gatunian endemic. 4 = Pacific and Atlantic Gatunian. 5 = Caloosahatchian and Atlantic Gatunian. 6 = Pan-American. A = Western Atlantic. P = Eastern Pacific. ex = extinct. s = soft-bottom. h = hard-bottom.

Taxon	Pli	Rec	Habitat
Eburna	5	A	s
Jaspidella	5	A	s
Macgintiella	1	A	s
Mansfieldella	1	ex	s
Minioliva	4	A P	s
Neocylindrus	1	ex	s
Niteoliva	4	A P	s
Oliva	6	A P	s
Olivella	6	A P	s
Omogymna	2	ex	s
Pachyoliva	4	P	s
Strephona	2	ex	s
Strephonella	4	P	s
Toroliva	5	ex	s
Mitridae			
Atrimitra	4	P	h
Dibaphimitra	1	A	s
Dibaphus	2	ex	s
Fusimitra	6	A P	s
Isara	4	A P	s
Nebularia	4	A P	h
Pleioptygma	1	A	s
Prochelaea	2	ex	s
Scabricola	4	A P	h
Strigatella	4	P	h
Subcancilla	6	A P	s
Tiara	4	P	s
Volutidae			
Aurinia	1	A	s
Calliotectum	3	P	s
Clenchina	1	A	s
Enaeta	6	A P	s
Harpeola	1	ex	s
Lyria	5	A	s
Mysterostropha	3	ex	s
*Plicoliva**	2	A	s
Scaphella	1	A	s
Voluta	2	A	s
Marginellidae			
Bullata	5	A	s
Cypraeolina	1	A	s
Eburnospira	5	A	s
Egouena	5	A	s
Eratoidea	1	A	s
Gibberula	5	A	s
Leptegouana	5	A	s
Marginella	2	A	s
Microspira	6	A P	s
Persicula	6	A P	s
Prunum	6	A P	s
Radicea	4	A P	s
Serrata	5	A	s
Volvarina	6	A P	s
Cancellariidae			
Admetula	4	A P	s
Agatrix	4	A P	s
Aphera	6	A P	s
Bivetiella	4	P	s
Bivetopsia	4	P	s
Bonnelitia	4	ex	s
Calcarata	6	P	s
Cancellaria	6	A P	s
Charcolleria	4	ex	s
Euclia	4	P	s
Extractrix	6	P	s
Hertleinia	4	P	s
Marksella	3	ex	s
Massyla	1	P	s
Narona	6	P	s
Olssonella	6	A P	s
Perplicaria	1	P	s
Pyruclea	4	P	s
Sveltia	4	P	s
Trigonostoma	6	P	s
Ventrilia	6	A P	s
Conidae			
Asprella	4	A	s
Conasprella	6	A P	s
Contraconus	1	ex	s
Leptoconus	6	A P	s
Lithoconus	6	A P	s
Pyruconus	6	P	s
Stephanoconus	4	A P	h
Ximeniconus	6	A P	s
Arcidae			
Acar	4	A P	
Arca	6	A P	
Arcopsis	4	A P	
Arcoptera	1	ex	
Barbatia	6	A P	
Caloosarca	6	A P	
Cucullaearca	6	A P	
Cunearca	6	A P	
Eontia	6	A P	
Fugleria	6	A P	
Grandiarca	4	P	
Granoarca	1	ex	
Hawaiarca	6	ex	
Larkinia	6	P	
Lunarca	6	A P	
Noetia	4	P	
Obliquarca	2	ex	
Potiarca	6	A P	
Rasia	6	A P	
Sheldonella	4	P	
Taeniarca	6	ex	
Tosarca	4	P	
Lucinidae			
Anodontia	6	A P	
Armimiltha	1	ex	
Bellucina	6	A P	
Callucina	6	A	

APPENDIX CONTINUED

Pliocene molluscs and their living descendants in tropical America. 1 = Caloosahatchian endemic. 2 = Atlantic Gatunian endemic. 3 = Pacific Gatunian endemic. 4 = Pacific and Atlantic Gatunian. 5 = Caloosahatchian and Atlantic Gatunian. 6 = Pan-American. A = Western Atlantic. P = Eastern Pacific. ex = extinct. s = soft-bottom. h = hard-bottom.

Taxon	Pli	Rec	Habitat
Cavilinga	6	A P	
Codakia	6	A P	
Ctena	6	A P	
Divalinga	6	A P	
Eulopia	2	A	
Here	3	P	
Lepilucina	4	ex	
Levimyrtea	3	ex	
Lucina	5	A	
Lucinisca	6	A P	
Lucinoma	6	A P	
Miltha	6	A P	
Myrtea	2	A	
Parvilucina	6	A P	
Phacoides	6	A	
Pleurolucina	6	A P	
Stewartia	5	A	
Cardiidae			
Acrosterigma	1	P	
Americardia	6	A P	
Apiocardia	4	P	
Dallocardia	6	A P	
Dinocardium	6	A	
Laevicardium	6	A P	
Lophocardium	4	P	
Mexicardia	4	P	
Microcardium	4	A P	
Nemocardium	4	ex	
Papyridea	6	A P	
Phlogocardia	4	P	
Trachycardium	6	A P	
Trigoniocardia	6	A P	

*Although originally placed in the Olividae by Petuch (1979), the genus *Plicoliva* Petuch 1979 now appears to belong to the Volutidae, subfamily Lyriinae. This change in familial placement was made in order to accommodate a number of volutid characteristics that are found in *Plicoliva*, such as an unchanneled suture, few but evenly sized columellar plications, and a lyriine papillate protoconch. Furthermore, this volutid genus has been found in the fossil record of the Caribbean. An examination of the type of *Prochelaea gabbi* Pilsbry & Johnson 1917 showed that this species is not referable to that mitrid genus, but belongs to *Plicoliva*. *P. zelindae* Petuch 1979 is a Brazilian relict.

MALACOLOGIA, 1986, 27(1): 43–66

THE TAXONOMIC STRUCTURE OF SHALLOW-WATER MARINE FAUNAS: IMPLICATIONS FOR PHANEROZOIC EXTINCTIONS

David Jablonski[1] & Karl W. Flessa[2]

ABSTRACT

The taxonomic and biogeographic structure of Recent shallow marine faunas provides a means of evaluating the causes and magnitudes of extinctions in the fossil record. We assembled data on the distribution of families of marine gastropods, bivalves, echinoderms and scleractinians and on the number of species within families in gastropod, bivalve and echinoid faunas. The 22 oceanic islands for which we collected data harbor a very large proportion (87%) of the global, shallow water marine fauna, and 78% of the families are at two or more of the 22 islands. This suggests that even if eustatic lowering of sea level ravaged the continental shelf faunas, oceanic islands would provide a safe haven for representatives of the great majority of the shallow marine benthic families.

Continental shelf bivalve and echinoid faunas have significantly more species per family than island bivalve and echinoid faunas (a proportion of 1.5:1 and 1.3:1, respectively), though gastropod faunas show no such difference. Gastropod faunas display persistently higher species-family ratios than bivalve faunas, and echinoid faunas have the lowest ratios of all three classes. Species-family ratios are diversity-dependent, so that island-continental shelf and class-to-class differences in species-family ratios appear to be a direct consequence of differing species richness among the faunas and classes.

The fossil record suggests that species richness within clades may not be an adequate measure of resistance to mass extinction. Tropical clades appear to suffer disproportionately during times of mass extinction, and in general species-rich clades are no better represented among survivors than are species-poor clades. The linkage between speciation and extinction rates generates species-rich, but evolutionarily volatile clades. Species richness within clades may, however, contribute to a clade's resistance to background extinction. That different factors contribute to extinction-resistance during times of mass vs. background extinction suggests that macroevolutionary processes during those times are qualitatively as well as quantitatively different.

Key words: biogeography; extinction; species-family ratios; mollusks; echinoderms; corals.

INTRODUCTION

Extinction is the fate of all species. In addition to persistent levels of background extinctions, at least five mass extinction events of various magnitudes have plagued the marine biota since the beginning of the Phanerozoic (Newell, 1967; Raup & Sepkoski, 1982). Most recent analyses of extinctions have emphasized global tallies of higher taxa, but this approach yields few insights into the biogeographic or ecologic controls of such events. The biodistributional patterns of victims and survivors hold considerable promise as indicators of the processes underlying mass extinctions, but reliable data are difficult to accumulate on the appropriately large scales. Examination of present-day taxonomic, ecologic, and biogeographic patterns provides one complementary approach to the extinction problem. For example, by using the modern biota to estimate probable faunal response to a given perturbation, we can evaluate hypotheses available to explain the magnitudes and patterns of extinction observed in the fossil record. Here we analyze the taxonomic and biogeographic structure of recent marine organisms, with emphasis on bivalves, gastropods and echinoids, in order to test hypotheses

[1]Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona 85721, U.S.A. Present address: Department of Geophysical Sciences, University of Chicago, 5734 South Ellis Avenue, Chicago, IL 60637, U.S.A.
[2]Department of Geosciences, University of Arizona, Tucson, Arizona 85721, U.S.A.

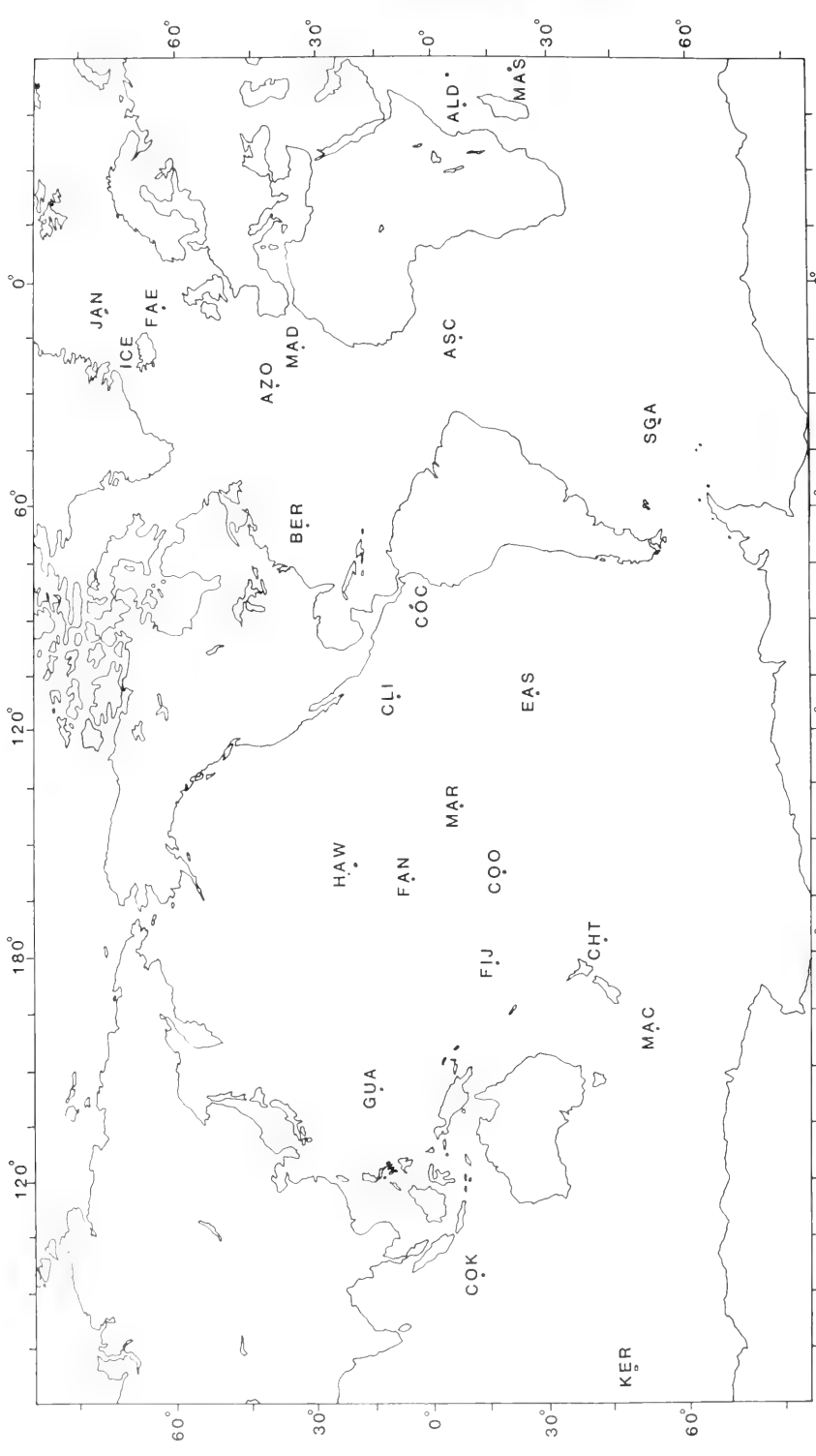


FIG. 1. Oceanic islands for which presence-absence data at the familial level were collected for bivalves, prosobranch gastropods, asteroids, ophiuroids, echinoids, and scleractinian corals. Abbreviations: ALD, Aldabra; ASC, Ascension; AZO, Azores; BER, Bermuda; CHT, Chatham; CLI, Clipperton; COC, Cocos; COK, Cocos-Keeling; COO, Cook; EAS, Easter; FAE, Faeroes; FAN, Fanning; FIJ, Fiji; GUA, Guam; HAW, Hawaii; ICE, Iceland; JAN, Jan Mayen; KER, Kerguelen; MAC, Macquarie; MAD, Madeira; MAR, Marquesas; MAS, Mascarenes; SGA, South Georgia.

and make inferences regarding mass extinctions in the geologic past.

Because the present-day fauna is by far the best-known biota of the Phanerozoic, it can provide a relatively complete—if still imperfect—assessment of patterns of species-level diversity and distribution, and how those patterns relate to the higher taxonomic levels that usually serve as bases for paleontological analysis (see Raup, 1979a, for a discussion of the biases hindering species-level studies of Phanerozoic diversity). In many respects the Recent biota represents a biogeographic extreme for Phanerozoic marine invertebrates: this is apparently a period of near-maximum latitudinal thermal gradients and maximum development of longitudinal land barriers, yielding very high provinciality and presumably minimum mean geographic ranges for species and higher taxa (see Valentine, 1969, 1973; Valentine *et al.*, 1978; Raup, 1982). Consequently, analysis of present-day geographic distributions can give us an end-member against which to test extinction hypotheses.

One extinction hypothesis for marine organisms is the proposed link between marine regression and mass extinction through species-area relationships. Some authors have emphasized climatic and other indirect effects of regression as a causal factor (e.g., Haq, 1973; Fischer & Arthur, 1977; Cavelier *et al.*, 1981), but Schopf (1974) and Simberloff (1974) have maintained that the Permo-Triassic extinction, the largest of the Phanerozoic, can be explained directly through loss of habitable shelf area (but see Schopf, 1979; Wise & Schopf, 1981, for a very different viewpoint). The species-area relationship is often best described by the power function $S = kA^z$, where S is the number of species, A is the area of the geographic isolate, and k and z are fitted constants, with z values usually clustering near 0.3 (Connor & McCoy, 1979; Flessa & Sepkoski, 1978). Though grounded in studies of island biogeography, its extrapolation to larger-scale questions of global extinction and evolution has met considerable acceptance among paleontologists (e.g. Flessa, 1975; Gould, 1976; McLaren, 1983). This is despite misgivings among some biologists regarding the general explanatory power of island biogeographic theory (e.g., Simberloff, 1976, 1981; Connor & McCoy, 1979; Gilbert, 1980) and the actual applicability of the species-area relationship to the fossil record (Flessa & Sepkoski, 1978).

In this paper we assess the probability that a marine regression could produce a mass extinction in the Recent biota through reduction in habitable shelf area. We test this hypothesis by comparing shallow marine faunas of continental shelves to those of oceanic islands. Islands would appear to be immune from the effects of lower sea level. While drops in relative sea level reduce habitable epicontinental and continental margin sea, conical oceanic islands will actually gain slightly in perimeter and therefore in shallow-water area (see Stanley, 1979; Jablonski, in press a). At the same time, previously-drowned seamounts emerge into the photic zone, tending to replace lagoonal habitats that may be exposed during regression, so that net destruction of habitat types also will be limited in oceanic settings.

In order to estimate the proportion of the marine biota inhabiting oceanic islands, and thus exempt from extinction by area effects during regression, we surveyed the literature of island faunas for representatives of the benthic families of six skeletonized invertebrate classes. There is, of course, no guarantee that those families would persist in their island refugia for geologically significant periods of time. For example, if all the families are monospecific on each island, normal attritional extinction could rapidly remove a significant number of families. To test for this possibility, we compare the frequency distribution of species within families for mainland and island faunas. These frequency distributions are simply the "hollow curves" of Willis (1922; see also Williams, 1964; Anderson, 1974). The shape of the species-family frequency distribution, or simple species-family ratios, may at least partly determine a clade's resistance to extinction; all other factors being equal, the more species in the family, the lower its probability of extinction. This probabilistic model of extinction has received few empirical tests, and we provide a preliminary comparison among bivalves, gastropods and echinoids to assess the role of taxonomic structure in shaping rates and patterns of extinction of major groups of marine organisms.

FIG. 2. Oceanic islands and mainland areas for which frequency distributions of species within families were collected for bivalves, shelled gastropods, and echinoids. Abbreviations: ALD, Aldabra; ARA, southeast Arabia; ARC, Arctic Canada; ASC, Ascension; BCO, British Columbia; BER, Bermuda; BON, Bonin; BRA, Brazil; BRI, Britain; CAY, Grand Cayman; CHA, Chatham; COK, Cocos-Keeling; EAS, Easter; FAE, Faeroes; FAL, False Bay; FAN, Fanning; FUN, Funafuti; GAQ, Gulf of Aqaba; GHA, Ghana; GOA, Arabian Gulf; GOM, Gulf of Mexico; GRE, Grenada; HAW, Hawaii; ICE, Iceland; ITA, Italy; JAM, Jamaica; JAN, Jan Mayen; JAP, Japan; KRG, Kerguelen; KRM, Kermadecs; KUT, Gulf of Kutch; MAL, Maldives; MAR, Marquesas; MAU, Mauritius; MED, Mediterranean; MEX, Pacific Mexico; MON, Monterey Bay; NIU, Niue; NZA, New Zealand, Aupourian Province; NZC, New Zealand, Cookian Province; NZF, New Zealand, Forsterian Province; OMA, Oman; PAG, Pagan; PAN, Atlantic Panama; PTB, Point Barrow, Alaska; RED, Red Sea; SAF, South Africa; SAG, Sagami Bay; SPM, Spanish Mediterranean; SUE, Gulf of Suez; SUR, Surinam; TEX, Texas; WFL, West Florida.

MATERIALS AND METHODS

Families. Distribution of families on islands was assessed using a revised and updated version of Jablonski's (in press a) data. The distributions are based on published records for 22 oceanic islands of three major phyla of benthic marine invertebrates (see Faunal References): Mollusca (bivalves, prosobranch gastropods), Echinodermata (asteroids, ophiuroids, echinoids), and Coelenterata (scleractinian corals). Only volcanic islands surrounded by oceanic crust were used. Classification follows the *Treatise on Invertebrate Paleontology* and, for gastropods, Taylor & Sohl (1962). To compensate for differences in sampling and preparation procedures among islands, families were included only if living representatives occur in depths of 100 m or less, and if maximum adult size exceeds 5 mm. A total of 276 families met these criteria. Sites ranged from one of the world's most northerly islands, Jan Mayen (71° N), to South Georgia and Macquarie Islands (both approximately 54° S), and include localities from most of the major marine biogeographic regions (Fig. 1).

Species. Frequency distributions of species within families were compiled from the literature for oceanic islands (15 molluscan faunas, 13 echinoid faunas) and continental shelves (22 molluscan faunas, 15 echinoid faunas) (see Faunal References). Owing to their proximity to continental shelves, Caribbean islands were recorded as continental rather than oceanic. In data compilations such as this, presence/absence of families is a far more reliable observation than the detailed apportionment of species within each family, and critical evaluation of the island faunas led to deletion of some that seemed complete at the family level but considerably less so for species; other islands were added that lacked the broad multi-phylum coverage needed for the familial analyses but included good accounts of one or more of the three classes of most interest. Consequently, the islands characterized at the species level do not constitute an exact subsample of those characterized at the familial level. Also, shelled opisthobranchs as well as prosobranchs were included in the gastropod species counts.

So that the data on the shelf biotas would be comparable to the island ones, we attempted to use continental shelf data from individual sites or small, well-defined areas rather than large-scale regional compilations. Continental shelf coverage ranges from the Canadian Arctic (up to about 80° N) to the Forsterian Province at the southern end of New Zealand (ca. 47° S). As with the oceanic islands, we were able to include most of the major marine biogeographic regions (Fig. 2).

Despite our critical approach, we recognize that the species-level data must still be considerably more heterogeneous and less reliable than the family data. Nevertheless, because families can go extinct only if their member species do so, we feel that our species-level data, however flawed, provide some insight into the processes of extinction.

RESULTS

Oceanic islands harbor a remarkably large proportion of today's shallow-water families (Fig. 3). Of the 276 families considered here, 239 (87%) have species recorded from one or more of the 22 oceanic islands in Fig. 1, and at least 200 (78%) are present on two or more

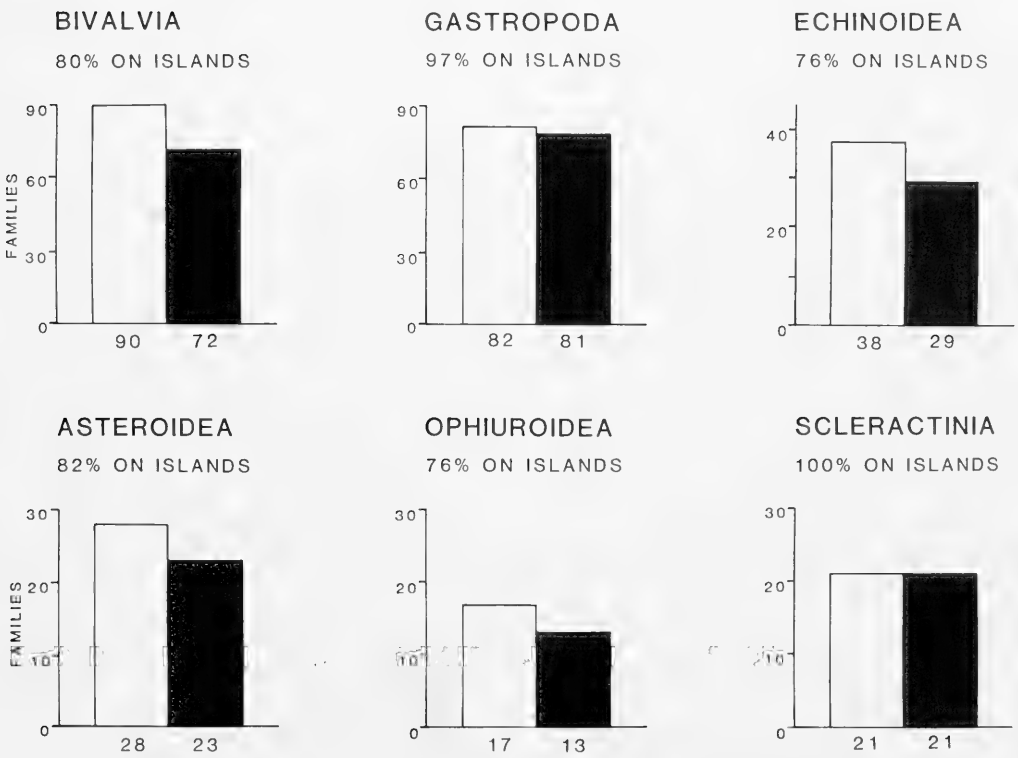


FIG. 3. Number of shallow-water families in the world biota (white bars) represented on 22 oceanic islands (black bars). At the family level, a high proportion of the living biota is represented on oceanic islands.

of the islands. These results are essentially identical to Jablonski's (in press a) earlier analysis of the same groups. Classes are not equally represented, but even the more poorly represented classes, the ophiuroids and echinoids, have about 76% of their families recorded on the 22 islands (70% and 66%, respectively, on two or more islands). Next come the bivalves and asteroids, each with about 80% of their families on the islands (70% and 64% on two or more islands). Especially well-represented are the prosobranch gastropods (99% on at least one island, 85% on two or more islands) and the scleractinian corals (100% on at least one island, 90% on two or more of the islands).

However, at the species level the mainland and oceanic island faunas exhibit different taxonomic structures for two of the three classes we examined in detail. Both the echinoids (Figs. 4–9) and the bivalves (Figs. 10–18) have fewer species per family in the oceanic island faunas than in the continental shelf faunas, and also have a higher proportion of monospecific families (in effect, a measure of the steepness of the hollow curve) on islands relative to mainlands. These differences are significant at $p = 0.05$ by the Wilcoxon two-sample test (Sokal & Rohlf, 1969: 391–395). Although means of such non-normally distributed ratios are difficult to interpret, they are a rough measure of the magnitude of the differences. Mainland bivalves have an average species-family ratio of 3.76 and an average proportion of monospecific families of 34%; for island bivalve faunas the average species-family ratio is 2.32 and the average proportion of monospecific families is 48%. Mainland families, then, exceed island bivalves in average number of species per family by a factor of approximately 1:1.5. Mainland echinoid faunas have an average species-family ratio of 2.29, and the average proportion of monospecific families is 42%; for island faunas the average species-family ratio is 1.74 and the average proportion on monospecific families is 58%. Mainland echinoid families, then, exceed island families in average number of species per family by a factor of about 1:1.3.

In contrast to the echinoids and bivalves, gastropods (Figs. 19–31) show no significant difference between island and mainland faunas in species-family ratios or percentage of monospecific families (Wilcoxon two-sample test).

Not only is there variation among areas for two of the three classes we analyzed, but there is significant variation among the three taxa, with species-family ratios significantly higher for gastropods than for bivalves, which in turn are significantly higher than echinoids. Accordingly, the percentage of monospecific families is significantly lower for gastropods than for bivalves, and significantly lower for bivalves than for echinoids (Wilcoxon signed ranks tests, $p = 0.01$).

DISCUSSION

The family data suggest that reduction in continental shelf area during marine regression is not sufficient in itself to produce a mass extinction at the familial level in marine invertebrates (see also Jablonski, in press a). Even if the entire continental shelf biota was eradicated, this would remove at most 13% of the Recent families; the rest are established on islands not subject to reduction in habitable shallow-water area during a drop in relative sea level. As Jablonski (*ibid.*) pointed out, this is a conservative estimate, because: (1) few scenarios include complete annihilation of the shelf fauna—some families should persist there as well; (2) only 22 islands out of the thousands actually present in the world ocean were surveyed, and even for these the faunas are doubtless incompletely monographed; and (3) provinciality is unusually high in today's oceans, even at the family level (e.g. Valentine, 1973; Campbell & Valentine, 1977), so that on average families in the geologic past can be expected to have been even more widespread and better-represented on oceanic islands than in the Recent. We do not claim, however, that oceanic islands have constituted the sole haven for shallow-water benthos during marine regressions. Because less radical environmental reductions than postulated here are surely the rule, other refugia will also be available. Therefore survivorship of taxa within provinces need not be closely related to the number of islands within the region [see, for example, Vermeij & Petuch's (1985) and Vermeij's (in press)] observation of greater survivorship of Neogene molluscan taxa in the island-poor tropical Eastern Pacific relative to the island-rich Caribbean.

Habitats will not have equal probabilities of

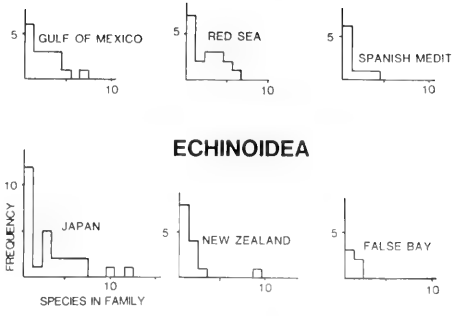


FIG. 4. Species-family frequency distributions in continental shelf echinoid faunas: Gulf of Mexico, Red Sea (based on Roman, 1980), Spanish Mediterranean, Japan, New Zealand, and False Bay (South Africa). Histograms show the number of families in each fauna having 1, 2, 3, etc., species. For example, for Japan, 12 families have one species apiece, while only one family contains 12 species. See Table 1 for curve statistics, and the Faunal References.

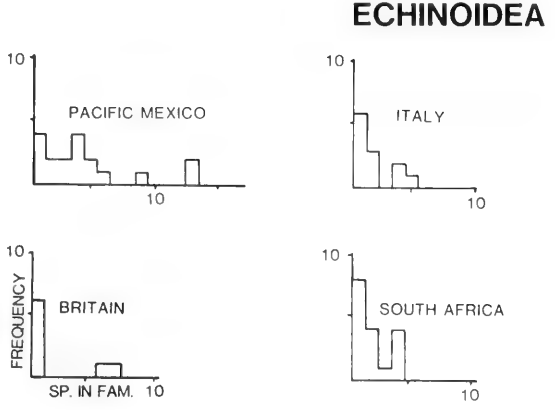


FIG. 6. Species-family frequency distributions in continental shelf echinoid faunas: Pacific Mexico, Italy, Britain, and South Africa

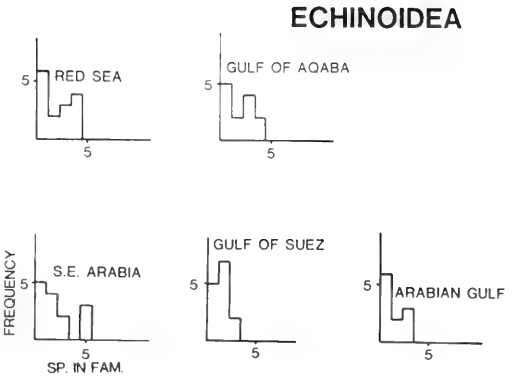


FIG. 5. Species-family frequency distributions in continental shelf echinoid faunas: Red Sea (based on Price, 1982), Gulf of Aqaba, Southeastern Arabia, Gulf of Suez, and Arabian Gulf.

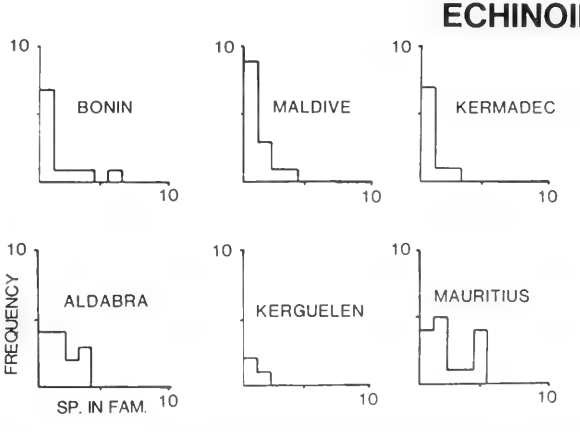


FIG. 7. Species-family frequency distributions in oceanic island echinoid faunas: Bonin, Maldives, Kermadec, Aldabra, Kerguelen, and Mauritius.

TABLE 1. Species-family frequency data for continental shelf and oceanic island echinoid faunas. See Fig. 2 for locations, and Figs. 4–9 for frequency distributions.

Location	Number of species	Number of families	% monospecific families	Species-family ratio
I. Continental shelf faunas				
Southeast Arabia	34	14	36	2.4
Britain	19	8	42	2.4
False Bay (South Africa)	7	5	43	1.4
Arabian Gulf	19	11	54	1.7
Gulf of Mexico	45	20	30	2.2
Gulf of Aqaba	29	13	38	2.2
Italy	25	12	50	2.1
Japan	95	28	43	3.4
Pacific Mexico	67	17	24	3.9
New Zealand	28	14	57	2.0
Red Sea	48	18	39	2.7
South Africa	35	17	47	2.0
Spanish Mediterranean	46	24	54	1.9
Gulf of Suez	25	14	36	1.8
II. Oceanic island faunas				
Aldabra	30	13	31	2.3
Ascension	10	8	75	1.3
Bermuda	13	10	70	1.3
Bonin	22	11	73	2.0
Easter	7	6	83	1.2
Faeroes	9	6	50	1.5
Hawaii	27	9	11	3.0
Iceland	11	9	78	1.2
Jan Mayen	3	2	50	1.5
Kerguelen	4	3	67	1.3
Kermadecs	11	9	67	1.3
Maldives	22	14	69	1.6
Mauritius	41	15	33	2.7
Pagan	17	8	50	2.1

elimination during regression—there will always be terrigenous intertidal and innershelf environments, for example. Therefore, taxa that penetrate shallow-water or intertidal environments would be less likely to suffer habitat loss than those confined to deeper-water environments on the shelf, and a supplementary approach to our island-mainland comparisons might be a tabulation of families having representatives in such nearshore habitats. However, there is a confounding factor that must be taken into consideration before this can be tested in the fossil record: taxa in these nearshore environments have biological attributes (e.g. broader environmental tolerances, dispersal capabilities, and geographical ranges than offshore taxa) that further enhance a clade's extinction-resistance (see Jackson, 1974; Jablonski, 1980, 1982; Jablonski & Valentine, 1981). Consequently, preferential survival of nearshore taxa across extinction boundaries might be for reasons other than the inevitable persistence of their habitat types during regression.

The species-level data present a somewhat different picture from the family level data. There are signficantly fewer species per family on islands relative to continental shelves for echinoids and bivalves, though not for gastropods. This raises the possiblity that, at least for some clades, families on islands may be *more* extinction-prone than they are on the mainland. However, it is not clear that differences in island-mainland species-family ratios ranging from 1:1.3 to 1:1.5 are sufficient to offset the advantages of widespread distribution at the family level among oceanic islands. The fact that some, but not all, clades have different taxonomic structures on islands vs. mainlands adds another level of complexity to the problem, and deserves further investigation.

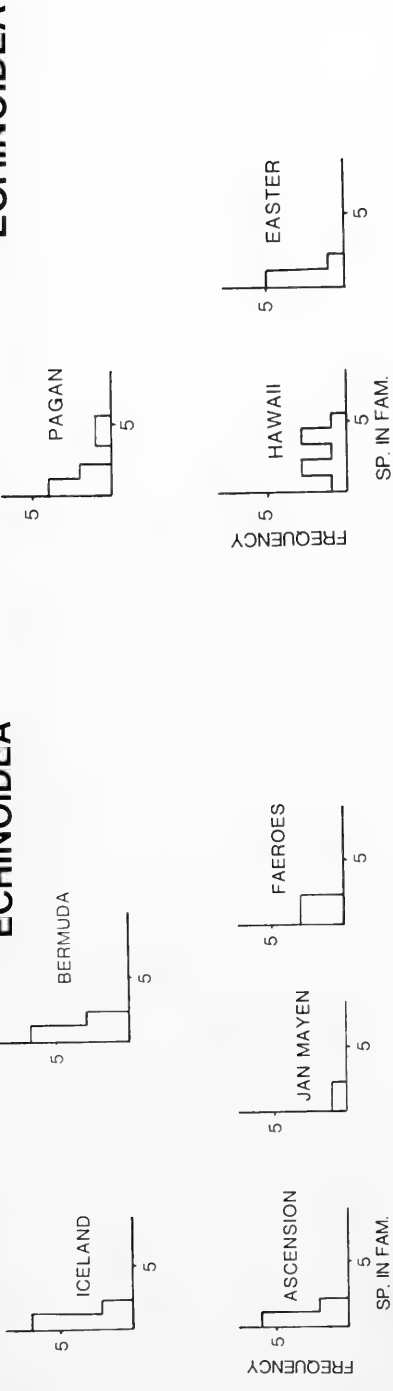


FIG. 8. Species-family frequency distributions in oceanic island echinoid faunas: Iceland, Bermuda, Ascension, Jan Mayen, and Faeroes.

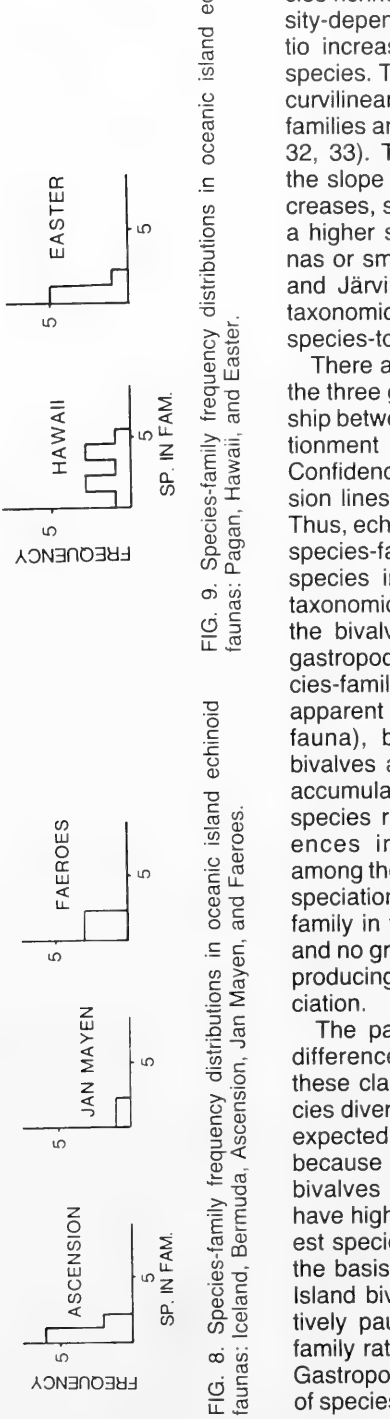


FIG. 9. Species-family frequency distributions in oceanic island echinoid faunas: Pagan, Hawaii, and Easter.

The basic factor governing species-family relationships appears simply to be local species richness. Species-family ratios are diversity-dependent, so that the species-family ratio increases monotonically with number of species. This is in itself a consequence of the curvilinear shape of the relationship between families and species in a fauna or clade (Figs. 32, 33). Though always a positive function, the slope of the species vs. family curve decreases, so that larger faunas or clades have a higher species-family ratio than small faunas or small clades. Simberloff (1970, 1978) and Järvinen (1982) discuss this aspect of taxonomic structure within clades as it affects species-to-genus ratios.

There are no significant differences among the three groups we examined in the relationship between species richness and the apportionment of these species among families. Confidence limits for the slopes of the regression lines (log-transformed data) all overlap. Thus, echinoids do not have exceptionally low species-family ratios, given the number of species in the class; at each locality their taxonomic structure simply conforms to that of the bivalves and gastropods. Similarly, the gastropods do not have inordinately high species-family ratios (despite, for example, the apparent outlier of 11.53 for the Hawaiian fauna), but fall on the line projected for bivalves and echinoids. Because the clades accumulate families at a similar proportion as species richness increases, no major differences in macroevolutionary processes among the three groups are indicated. A given speciation event is as likely to lead to a new family in the echinoids as in the gastropods, and no group is more adept than the others in producing new families in the course of speciation.

The pattern suggests, therefore, that the differences in taxonomic structure among these clades is a consequence of local species diversities. As a group, the echinoids are expected to have a low species-family ratio, because they have few species per locality; bivalves are intermediate and gastropods have highest species richness and thus highest species-family ratios. This pattern is also the basis of the island-mainland differences. Island bivalve and echinoid faunas are relatively paucispecific, and thus their species-family ratios are lower than on the mainland. Gastropod faunas have comparable numbers of species on islands and continental shelves,

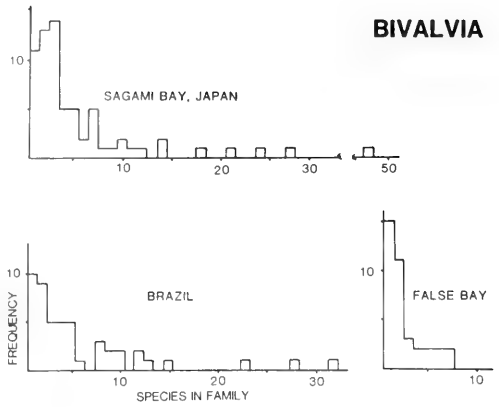


FIG. 10. Species-family frequency distributions in continental shelf bivalve faunas: Sagami Bay (Japan), Brazil, and False Bay (South Africa). See Table 2 for curve statistics, and the Faunal References.

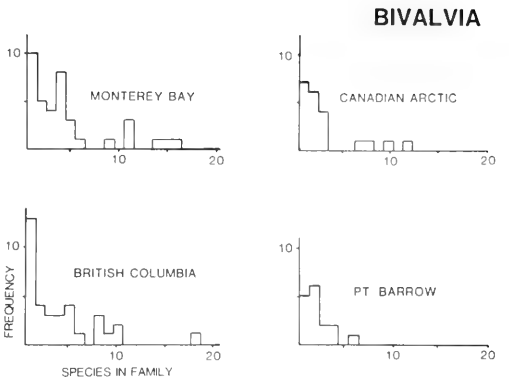


FIG. 11. Species-family frequency distributions in continental shelf bivalve faunas: Monterey Bay (California), Canadian Arctic, British Columbia, and Point Barrow (Alaska).

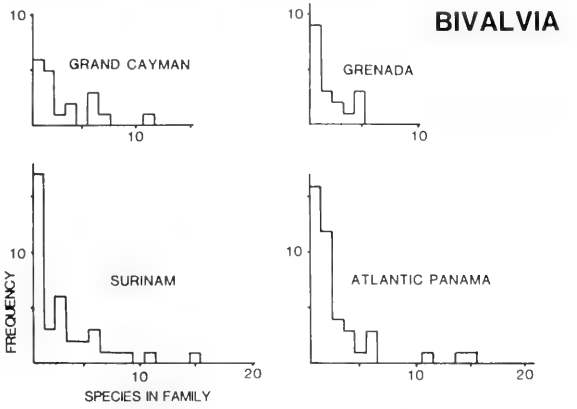


FIG. 12. Species-family frequency distributions in continental shelf bivalve faunas: Grand Cayman Island, Grenada, Surinam, and Atlantic Panama.

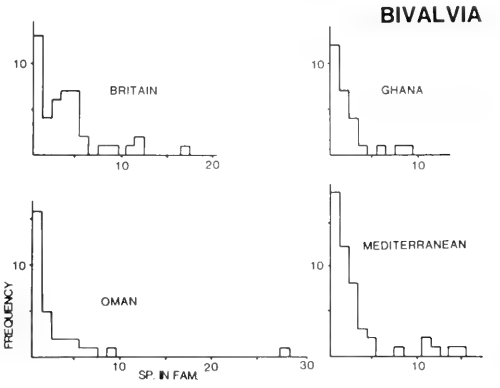


FIG. 13. Species-family frequency distributions in continental shelf bivalve faunas: Britain, Ghana, Oman, and the Mediterranean as a whole.

TABLE 2. Species-family frequency data for continental shelf and oceanic island bivalve faunas. See Fig. 2 for locations, and Figs. 10–18 for frequency distributions.

Location	Number of species	Number of families	% monospecific families	Species-family ratio
I. Continental shelf faunas				
Arctic Canada	68	21	33	3.2
British Columbia	121	35	37	3.5
Brazil	291	51	20	5.7
Britain	183	45	29	4.1
Grand Cayman Is.	73	20	30	3.6
False Bay (South Africa)	90	37	41	2.4
Ghana	61	27	44	2.3
Grenada	40	18	50	2.2
Jamaica	235	43	30	5.5
Gulf of Kutch	91	27	37	3.4
Mediterranean	149	49	37	3.0
Monterey Bay (California)	172	38	26	4.5
Aupourian Province (New Zealand)	264	48	27	5.5
Cookian Province (New Zealand)	152	40	20	3.8
Forsterian Province (New Zealand)	169	41	22	4.1
Oman	92	31	52	3.0
Atlantic Panama	138	42	36	3.3
Point Barrow (Alaska)	37	14	36	2.6
Sagami Bay (Japan)	393	66	17	6.0
Surinam	135	38	45	3.6
Texas	145	44	43	3.3
West Florida	155	38	32	4.1
II. Oceanic island faunas				
Aldabra	93	29	32	3.2
Ascension	22	15	67	1.5
Bermuda	122	39	41	3.1
Chatham	114	37	27	3.1
Cocos-Keeling	68	28	39	2.4
Easter	15	13	85	1.2
Faeroes	83	34	47	2.4
Fanning	52	23	43	2.3
Funafuti	79	32	62	2.5
Hawaii	139	39	26	3.6
Iceland	93	30	37	3.1
Jan Mayen	28	12	25	2.3
Kerguelen	29	16	50	1.8
Marquesas	11	8	75	1.4
Niue	6	5	80	1.2
Pagan	26	13	38	2.0

and as expected they lack significant differences in species-family ratios between the two habitats. It appears that what needs to be explained is not a source of evolutionary novelties that might maintain a high proportion of families to species in island faunas, but the reasons for such prolific within-habitat speciation in gastropods relative to bivalves and echinoids, or, from a different perspective, why bivalves are impoverished at the species level on tropical oceanic islands (possibly owing to lack of suitable habitats on atolls). Thus, the pattern of distribution of species-family ratios among clades could result from (a) differential extinction rates near an equilibrium (for example, differences in their ability to accommodate new species as they arrive), or (b) differential speciation rates in a situation far from equilibrium.

What, then, is the relationship between tax-

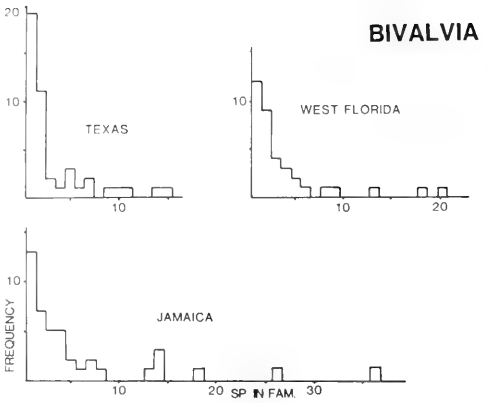


FIG. 14. Species-family frequency distributions in continental shelf bivalve faunas: Texas, West Florida, and Jamaica.

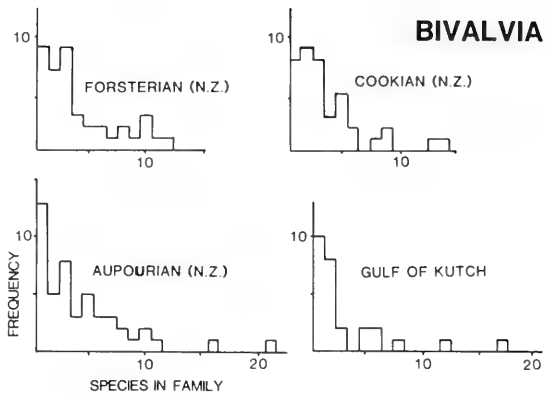


FIG. 15. Species-family frequency distributions in continental shelf bivalve faunas; Forsterian Province (New Zealand), Cookian Province (New Zealand), Aupourian Province (New Zealand), and Gulf of Kutch.

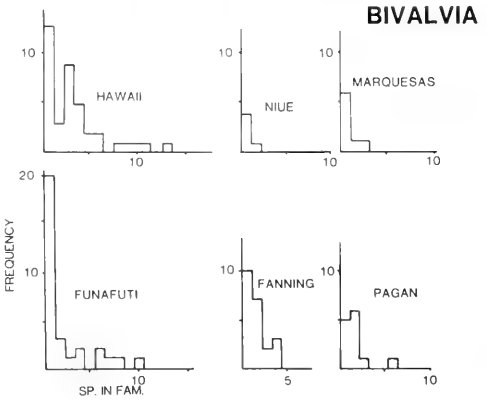


FIG. 16. Species-family frequency distributions in oceanic island bivalve faunas: Hawaii, Niue, Marquesas, Funafuti, Fanning, and Pagan.

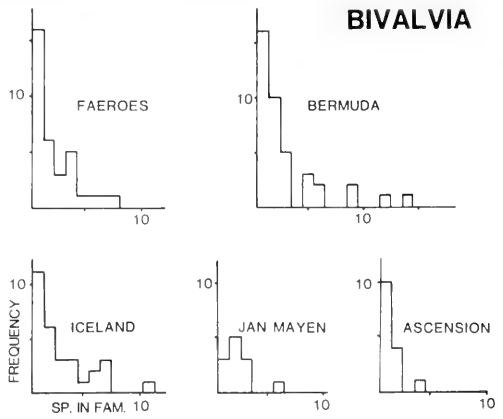


FIG. 17. Species-family frequency distributions in oceanic island bivalve faunas: Faeroes, Bermuda, Iceland, Jan Mayen, and Ascension.

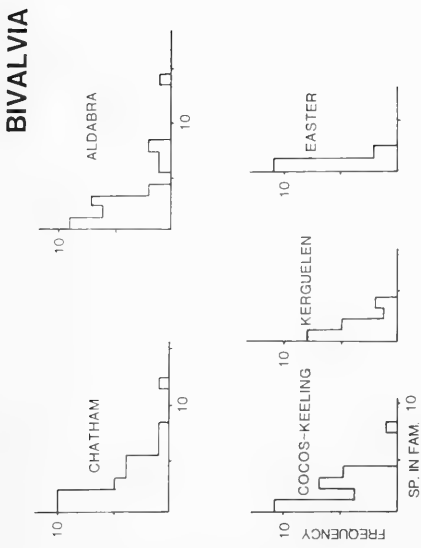


FIG. 18. Species-family frequency distributions in oceanic island bivalve faunas: Chatham, Aldabra, Cocos-Keeling, Kerguelen, and Easter.

onomic structure and extinction? It might be predicted that variations in species-family ratios determine susceptibility to mass extinction among clades: given the same percentage of species extinction, paucispecific clades are more likely than speciose clades to be reduced to such low species richnesses that random events will remove the last few surviving species. However, although this generalization may hold for extreme cases, it is not sufficient to explain patterns of extinction among clades during mass extinctions (Jablonski, in press). Many low-diversity clades exhibit impressive longevities, and high species richness does not always confer great extinction-resistance on a clade. For example, Ward & Signor (1983) found that speciose ammonite clades were among the shortest-lived. With respect to mass extinctions, Jablonski (in press b) found that paucispecific bivalve and gastropod genera had an equal, or even higher, probability of surviving the Cretaceous-Tertiary boundary than speciose genera (Fig. 34) and the literature shows that mass extinction events terminate both paucispecific and species-rich clades (e.g. both trilobites and productid brachiopods at the end of the Permian).

Taxonomic structure alone does not appear to be a major determinant of extinction or survival among clades during mass extinctions, probably because biological attributes that affect extinction and speciation rates are not randomly distributed among taxa but tend to covary. Consequently, clades that tend to exhibit high speciation rates tend to be extinction-prone as well, and clades that tend to resist speciation tend to have extinction-resistance species. For example, in bivalves and gastropods broad larval dispersal capability tends to reduce speciation rates and is generally accompanied by broad geographic ranges and high degree of environmental tolerances, which also impart extinction-resistance to those species. Conversely, species with low dispersal capabilities, and thus high speciation rates, also tend to have restricted geographic ranges and narrow environmental tolerances, and as predicted exhibit high extinction rates as well (Jackson, 1974; Jablonski, 1980, 1982; Hansen, 1980; Jablonski & Lutz, 1983). Therefore, the attributes that cause a clade to exhibit high speciation rates also impart upon it a volatility that may make it particularly vulnerable to environmental changes during mass extinction events (Stanley, 1979; Jablonski, in press b).

Biogeographic distributions may be far more important than species richness in determining a clade's survival or disapperance during mass extinctions. The few data available indicate that clades with representatives in more than one province have a greater probability of surviving mass extinction events than clades, however speciose, that are confined to a single province (e.g. Bretsky, 1973; Boucot, 1975; Jablonski, in press). Many authors have suggested that the tropical marine biota appear to suffer disproportionately during mass extinctions (e.g. Kauffman, 1979, Cavelier *et al.*, 1981; Boucot, 1983; Jablonski, 1984, in press b). If the tropics are subject to major perturbations during mass extinctions, this would further contribute to the lack of correlation between species richness and clade survival at those times. Widespread clades whose peak diversities are in the tropics would be more severely affected than clades in which diversity is evenly distributed with latitude or is highest outside the tropics. Clades restricted entirely to the tropics, which are often among the

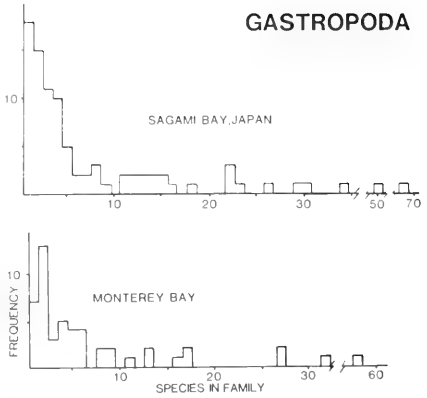


FIG. 19. Species-family frequency distributions in continental shelf gastropod faunas: Sagami Bay (Japan) and Monterey Bay (California). See Table 3 for curve statistics, and the Faunal References.

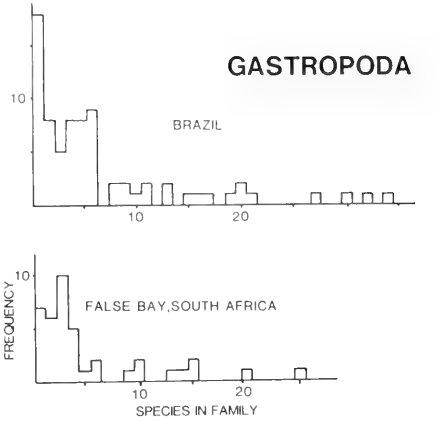


FIG. 20. Species-family frequency distributions in continental shelf gastropod faunas: Brazil and False Bay, South Africa.

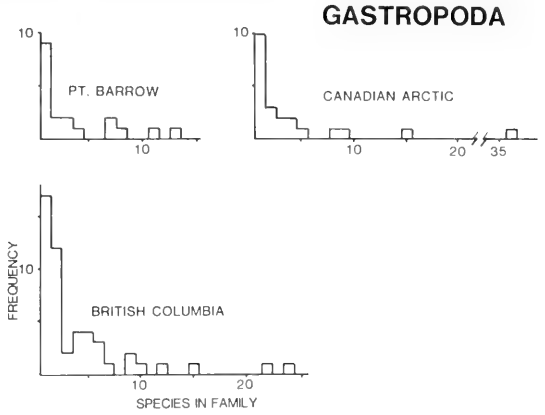


FIG. 21. Species-family frequency distributions in continental shelf gastropod faunas: Point Barrow (Alaska), the Canadian Arctic, and British Columbia.

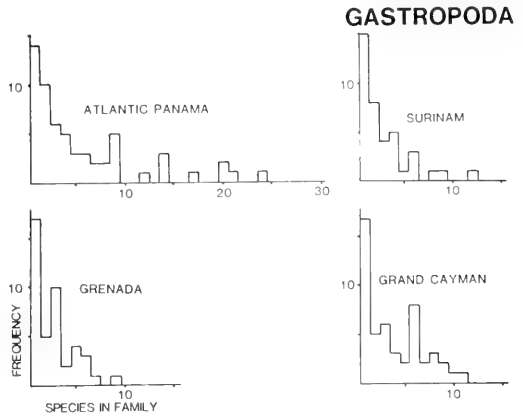


FIG. 22. Species-family frequency distributions in continental shelf gastropod faunas: Atlantic Panama, Surinam, Grenada, and Grand Cayman Island.

TABLE 3. Species-family frequency data for continental shelf and oceanic island gastropod faunas. See Fig. 2 for locations, and Figs. 19–31 for frequency distributions.

Location	Number of species	Number of families	% monospecific families	Species-family ratio
I. Continental shelf faunas				
Arctic Canada	103	22	45	4.7
British Columbia	209	50	34	4.1
Brazil	528	76	24	7.0
Britain	245	52	37	4.7
Grand Cayman Is.	204	51	33	4.0
False Bay (South Africa)	217	40	17	5.4
Ghana	99	39	44	2.5
Grenada	119	43	40	2.8
Jamaica	459	55	16	8.4
Mediterranean	214	62	27	3.4
Monterey Bay (California)	371	50	14	7.4
Aupourian Province (New Zealand)	843	112	25	7.5
Cookian Province (New Zealand)	353	89	36	4.0
Forsterian Province (New Zealand)	418	71	35	5.9
Oman	265	53	30	5.0
Atlantic Panama	389	65	29	6.0
Point Barrow (Alaska)	69	19	47	3.6
Sagami Bay (Japan)	696	89	20	7.8
Surinam	115	39	38	3.0
Texas	181	53	36	3.4
West Florida	166	52	31	3.2
II. Oceanic island faunas				
Aldabra	363	58	28	6.3
Ascension	50	30	70	1.6
Bermuda	192	51	25	3.8
Chatham	212	58	36	3.7
Cocos-Keeling	380	56	27	6.8
Easter	96	37	43	2.6
Faeroes	79	31	45	2.5
Fanning	380	59	25	6.4
Funafuti	245	64	31	4.6
Hawaii	693	61	16	11.3
Iceland	152	39	36	3.9
Jan Mayen	62	20	55	3.1
Kerguelen	84	28	46	3.0
Marquesas	125	27	33	4.6
Niue	199	33	36	6.0
Pagan	166	37	24	4.5

most speciose of all, would paradoxically be most extinction-prone. In simulating this latitudinal pattern of extinction in the modern biota, Jablonski (in press a) found that elimination of families restricted to the tropical islands in our survey resulted in an extinction of the magnitude of the Permo-Triassic, with clades ranked appropriately (that is, reef-building corals most severely reduced, echinoderm classes intermediate, and bivalves and gastopods least severely affected).

SUMMARY AND CONCLUSIONS

The taxonomic structure of the modern marine biota indicates that many families would be represented on oceanic islands following marine regression. Even if withdrawal of the seas from continental shelves completely eradicated the shelf benthos, 87% of the families would be represented on oceanic islands, which would not be subject to areal reduction during regression. However, for some taxa

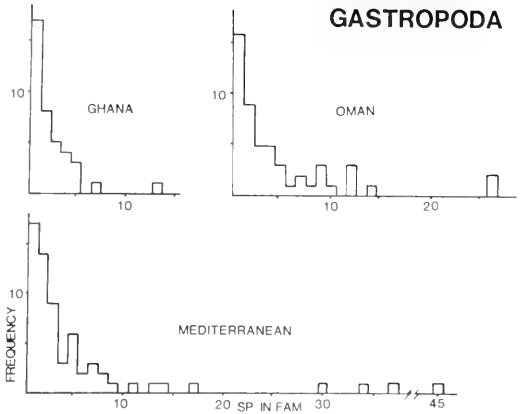


FIG. 23. Species-family frequency distributions in continental shelf gastropod faunas: Ghana, Oman, and the Mediterranean as a whole.

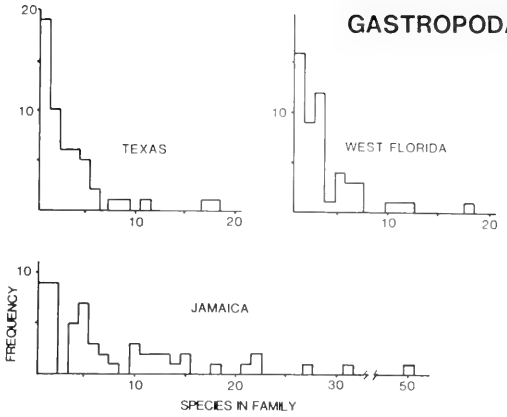


FIG. 24. Species-family frequency distributions in continental shelf gastropod faunas: Texas, West Florida, and Jamaica.

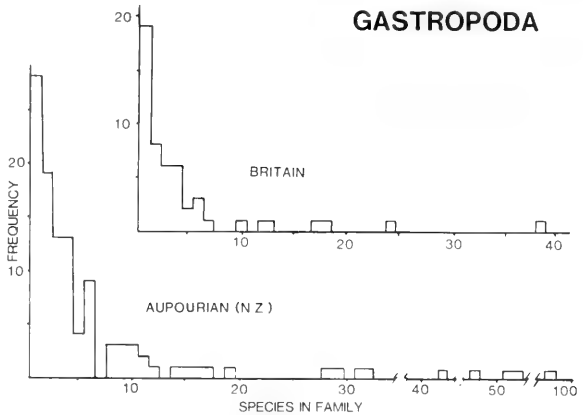


FIG. 25. Species-family frequency distributions in continental shelf gastropod faunas: Britain and the Aupourian Province (New Zealand).

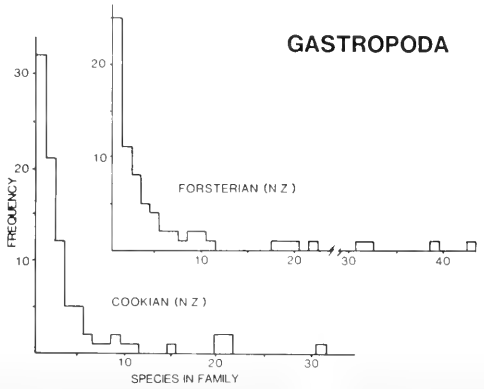


FIG. 26. Species-family frequency distributions in continental shelf gastropod faunas: Forsterian Province (New Zealand) and Cookian Province (New Zealand).

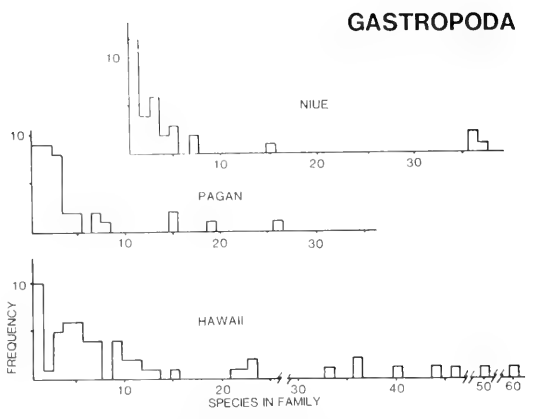


FIG. 27. Species-family frequency distributions in oceanic island gastropod faunas: Niue, Pagan, and Hawaii.

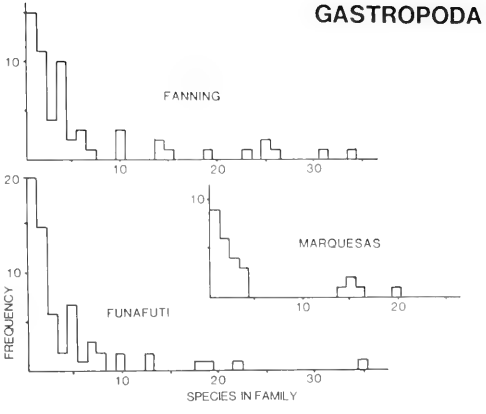


FIG. 28. Species-family frequency distributions in oceanic island gastropod faunas: Fanning, Funafuti, and Marquesas.

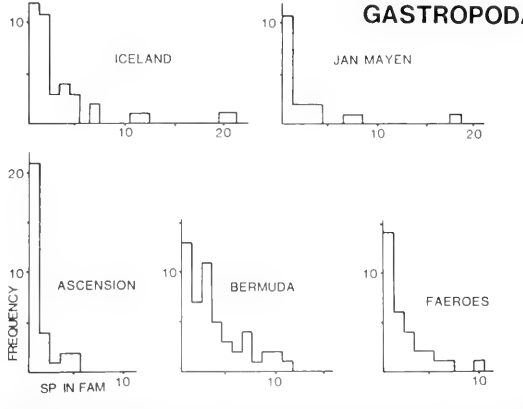


FIG. 29. Species-family frequency distributions in oceanic island gastropod faunas: Iceland, Jan Mayen, Ascension, Bermuda, and the Faeroes.

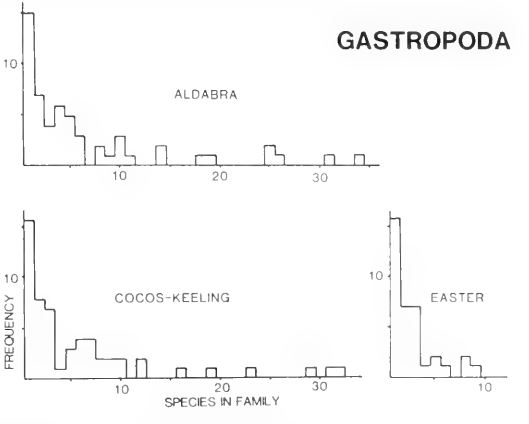


FIG. 30. Species-family frequency distributions in oceanic island gastropod faunas: Aldabra, Cocos-Keeling, and Easter.

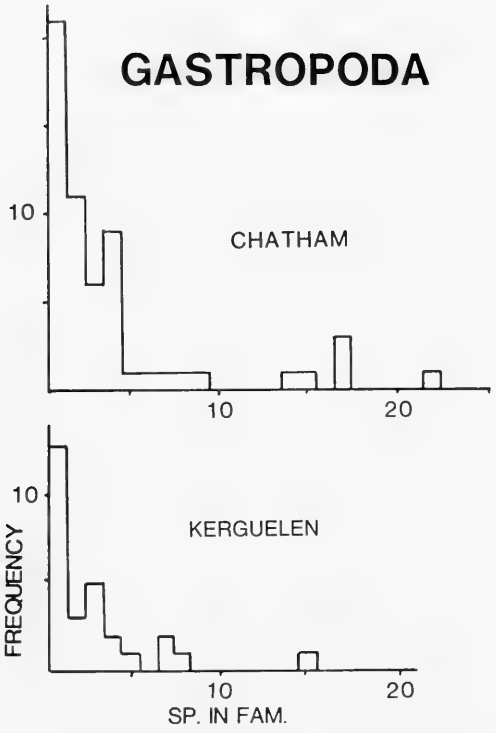


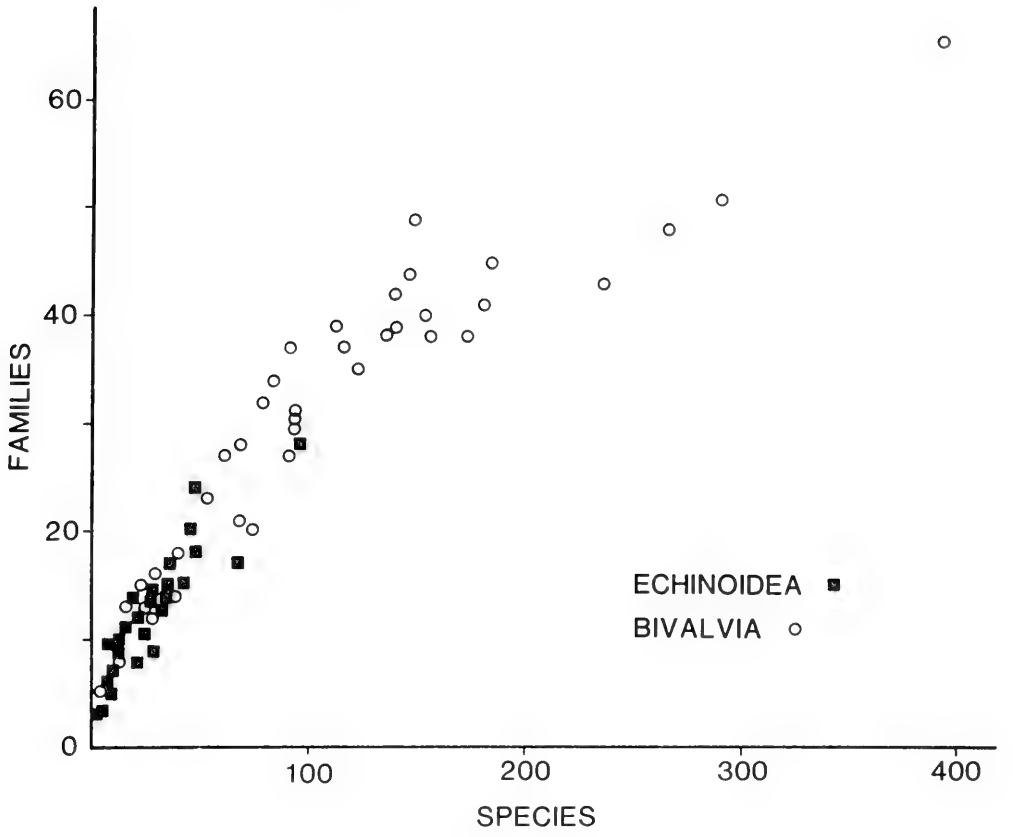
FIG. 31. Species-family frequency distributions in oceanic island gastropod faunas: Chatham and Kerguelen.

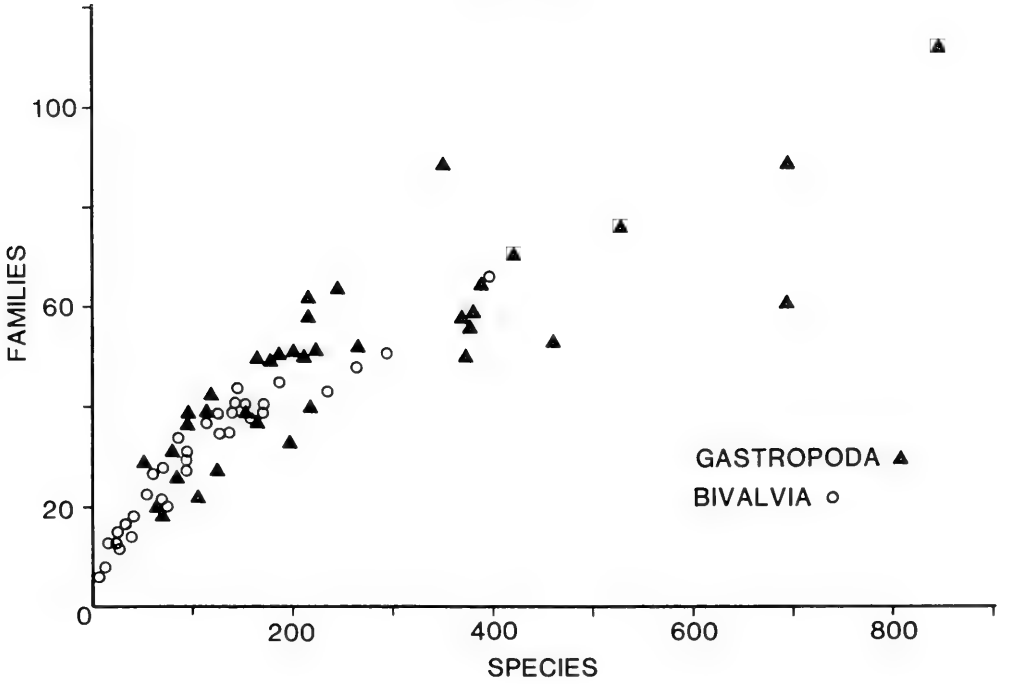
the security offered by these island refuges may be offset by differences in species-family ratios between mainlands and islands. For bivalves and echinoids—though not for gastropods—families on islands tend to have fewer species and thus may be less extinction-resistant than families on mainlands. Just as Simberloff (1970, 1978) found that species-genus and species-family ratios on islands commonly could be explained in terms of local species richness without recourse to competitive interactions, we found that species-family ratios within clades could be explained in terms of local species richness without recourse to different macroevolutionary processes among the three groups we examined.

It is not clear whether the differences in species-family ratios between mainland and island faunas are of sufficient magnitude to undermine the refugium effect. Taxonomic structure, e.g. species-family ratios, appears to be of secondary importance in determining survival during mass extinctions because the biological attributes that give rise to speciose clades tend to make those species extinction-prone. We suggest that taxonomic structure is more important during intervals of background extinction, though here, too, other biological properties obviously are also significant. In contrast, biogeographic patterns appear to have greater significance for survivorship during mass extinctions. Like Raup (1979b, 1982) we have used the taxonomic structure of the present-day biota to infer extinction patterns and probabilities in the fossil record. These results must be approached with caution, however, because species-family ratios have apparently changed since the Cambrian, and Recent taxa are on average the most speciose of the Phanerozoic (Valentine, 1969, 1970, 1973; Raup, 1975; Sepkoski *et al.*, 1982). Relative representation of clades on oceanic islands vs. mainlands thus may well have changed through time. Differences we observed in species-family ratios between island and mainland echinoids and bivalves could be a relatively recent phenomenon, or conversely, the island-mainland similarities between gastropod faunas may be a consequence of the explosive post-Paleozoic speciation in the tropical gastropods. The trend of increasing species-family ratios through the Phanerozoic has additional implications for both mass and background extinctions. For example, as Valentine (1974) points out, a given magnitude of familial extinction could result from an increasingly smaller quantity of species-level extinctions going back through the Phanerozoic. Finally, the observed decline in background extinction rates of Phanerozoic families (Raup & Sepkoski, 1981) need not reflect improvement in biological adaptation, but rather may simply be a function of increasing species-family ratios through time (Flessa & Jablonski, 1985).

FIG. 32. Number of families plotted against number of species in continental shelf and oceanic island faunas of echinoids and bivalves. See Table 1 for data.

FIG. 33. Number of families plotted against number of species in continental shelf and oceanic island faunas of bivalves and gastropods. See Table 1 for data.


FAMILIES
60
40
20
0
100
200
300
400
SPECIES
ECHINOIDEA
BIVALVIA


FAMILIES
100
60
20
0
200
400
600
800
SPECIES
GASTROPODA
BIVALVIA

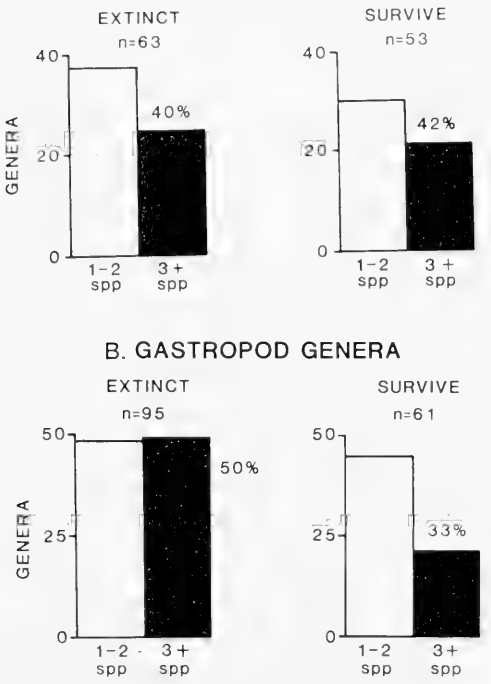


FIG. 34. Survivorship of species-rich and species-poor molluscan genera across the Cretaceous-Tertiary boundary. A, Gulf and Atlantic Coastal Plain bivalves; B, Gulf and Atlantic Coastal Plain gastropods. Note that species-rich genera are at least as well-represented among the victims of the extinction as among the survivors, suggesting that taxonomic structure does not play a major role in determining clade survival during mass extinction events. After Jablonski (in press b).

ACKNOWLEDGMENTS

We thank Susan M. Kidwell, Earl D. McCoy, and Geerat J. Vermeij for valuable comments and criticism. John D. Taylor and Geerat J. Vermeij provided unpublished faunal lists for the molluscan faunas of Aldabra and the Marianas, respectively, and advice and assistance with published sources came from David J. Bottjer (and the Hancock Library, University of Southern California), Philip W. Signor, and J. Wyatt Durham. Supported in part by NSF Grant EAR 81-21212.

REFERENCES CITED

ANDERSON, S., 1974, Patterns of faunal evolution. *Quarterly Review of Biology*, 49: 311–332.

BOUCOT, A. J., 1975, *Evolution and extinction rate controls*. Amsterdam: Elsevier, 427 p.

BOUCOT, A. J., 1983, Does evolution take place in an ecological vacuum? II. *Journal of Paleontology*, 57: 1–30.

BRETSKY, P. W., 1973, Evolutionary patterns in the Paleozoic Bivalvia: documentation and some theoretical considerations. *Geological Society of America, Bulletin, 84: 2079–2096.*

CAMPBELL, C. A. & VALENTINE, J. W., 1977, Comparability of modern and ancient faunal provinces. *Paleobiology*, 3: 49–57.

CAVELIER, C., CHATEAUNEUF, J. J., POMEROL, C., RABUSSIER, D., RENARD, M. & VERGNAUD-GRAZZINI, C., 1981, The geological events at the Eocene/Oligocene boundary. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 36: 223–248.

CONNOR, E. F. & McCOY, E. D., 1979, The statistics and biology of the species-area relationship. *American Naturalist*, 113: 791–833.

FISCHER, A. G. & ARTHUR, M. A., 1977, Secular variations in the pelagic realm. *Society of Economic Paleontologists and Mineralogists, Special Publication, 25: 19–50.*

FLESSA, K. W., 1975, Area, continental drift and mammalian diversity. *Paleobiology*, 1: 189–194.

FLESSA, K. W. & JABLONSKI, D., 1985, Declining Phanerozoic background extinction rates: effect of taxonomic structure? *Nature*, 313: 216–218.

FLESSA, K. W. & SEPKOSKI, J. J., Jr., 1978, On the relationship between Phanerozoic diversity and changes in habitable area. *Paleobiology*, 4: 359–366.

GILBERT, F. S., 1980, The equilibrium theory of island biogeography. *Journal of Biogeography*, 7: 209–235.

GOULD, S. J., 1976, Palaeontology plus ecology as palaeobiology. In: MAY, R. M., ed., *Theoretical ecology: principles and applications*. Saunders, Philadelphia, p. 218–236.

HANSEN, T. A., 1980, Influence of larval dispersal and geographic distribution on species longevity in neogastropods. *Paleobiology*, 6: 193–207.

HAQ, B. U., 1973, Transgression, climatic change and the diversity of calcareous nannoplankton, *Marine Geology*, 15: M25–M30.

JABLONSKI, D., 1980, Apparent versus real biotic effects of transgressions and regressions. *Paleobiology*, 6: 397–407.

JABLONSKI, D., 1982, Evolutionary rates and modes in Late Cretaceous gastropods: role of larval ecology. *Proceedings of the 3rd North American Paleontological Convention*, 1: 257–262.

JABLONSKI, D., in press a, Marine regressions and mass extinctions: a test using the modern biota.

In VALENTINE, J. W., ed., *Phanerozoic diversity patterns: profiles in macroevolution*. Princeton: Princeton, University Press.

JABLONSKI, D., in press b, Causes and consequences of mass extinctions: a comparative approach. In: ELLIOTT, D. K., ed., *Dynamics of extinction*. New York: Wiley.

JABLONSKI, D. & LUTZ, R. A., 1983, Larval ecology of marine benthic invertebrates: Paleobiological implications. *Biological Reviews*, 58: 21–89.

JABLONSKI, D. & VALENTINE, J. W., 1981, Onshore- offshore gradients in Recent eastern Pacific shelf faunas and their paleobiogeographic significance. In: SCUDDER, G. G. E. & REVEAL, J. L., ed., *Evolution today, Proceedings of the Second International Congress of Systematic and Evolutionary Biology*. Pittsburgh: Carnegie-Mellon University, p. 441–453.

JACKSON, J. B. C., 1974, Biogeographic consequences of eurytopy and stenotopy among marine bivalves and their evolutionary significance. *American Naturalist*, 108: 541–560.

JÄRVINEN, O., 1982, Species-to-genus ratios in biogeography: a historical note. *Journal of Biogeography* 9: 363–370.

KAUFFMAN, E. G., 1979, The ecology and biogeography of the Cretaceous-Tertiary extinction event. In: CHRISTENSEN, W. K. & BIRKELUND, T., ed., *Cretaceous-Tertiary Boundary Events Symposium*. University of Copenhagen, 2: 29–37.

McLAREN, D. J., 1983, Bolides and biostratigraphy. *Geological Society of America Bulletin*, 94: 313–324.

NEWELL, N. D., 1967, Revolutions in the history of life. *Geological Society of America Special Paper* 89: 63–91.

RAUP, D. M., 1975, Taxonomic diversity estimation using rarefaction. *Paleobiology*, 1: 333–342.

RAUP, D. M., 1979a, Biases in the fossil record of species and genera. *Bulletin of the Carnegie Museum of Natural History*, 13: 85–91.

RAUP, D. M., 1979b, Size of the Permo-Triassic bottleneck and its evolutionary implications. *Science*, 206: 217–218.

RAUP, D. M., 1982, Biogeographic extinction: a feasibility test. *Geological Society of America Special Paper*, 190: 277–281.

RAUP, D. M. & SEPKOSKI, J. J., Jr., 1982, Mass extinctions in the marine fossil record. *Science*, 215: 1501–1503.

SCHOPF, T. J. M., 1974, Permo-Triassic extinction: relation to seafloor spreading. *Journal of Geology*, 82: 129–143.

SCHOPF, T. J. M., 1979, The role of biogeographic provinces in regulating marine faunal diversity through geologic time. In: GRAY, J. & BOUCOT, A. J., ed., *Historical Biogeography, plate tectonics, and the changing environment*. Corvallis: Oregon State University Press, p. 449–457.

SEPKOSKI, J. J., Jr., BAMBACH, R. K., RAUP, D. M. & VALENTINE, J. W., 1981, Phanerozoic marine diversity and the fossil record. *Nature*, 293: 435–437.

SIMBERLOFF, D. S., 1970, Taxonomic diversity of island biotas. *Evolution*, 24: 23–47.

SIMBERLOFF, D. S., 1974, Permo-Triassic extinctions: effects of area on biotic equilibrium. *Journal of Geology*, 82: 267–274.

SIMBERLOFF, D. S., 1976, Species turnover and equilibrium island biogeography. *Science*, 194: 572–578.

SIMBERLOFF, D. S., 1978, Use of rarefaction and related methods in ecology. In: DICKSON, K. L., CAIRNS, J. Jr. & LIVINGSTON, R. J., ed., Biological data in water pollution assessment: quantitative and statistical analyses. *American Society of Testing and Materials, Special Technical Publication*, 652: 150–165.

SIMBERLOFF, D. S., 1981, Community effects of introduced species. In: NITECKI, M. N., ed., *Biotic crises in ecologic and evolutionary time*. New York: Plenum, p. 53–81.

SOKAL, R. R. & ROHLF, F. J., 1969, *Biometry*. San Francisco: Freeman, 776 p.

STANLEY, S. M., 1979, *Macroevolution*. San Francisco: Freeman, 332 p.

TAYLOR, D. W. & SOHL, N. F., 1962, An outline of gastropod classification. *Malacologia*, 1: 7–32.

VALENTINE, J. W., 1969, Patterns of taxonomic and ecological structure of the shelf benthos during Phanerozoic time. *Palaeontology*, 12: 684–709.

VALENTINE, J. W., 1970, How many invertebrate species? A new approximation. *Journal of Paleontology*, 44: 410–415.

VALENTINE, J. W., 1973, *Evolutionary paleoecology of the marine biosphere*. Englewood Cliffs, N. J.: Prentice-Hall, 511 p.

VALENTINE, J. W., 1974, Temporal bias in extinctions among taxonomic categories. *Journal of Paleontology*, 48: 549–552.

VALENTINE, J. W., FOIN, T. C. & PEART, D., 1978, A provincial model of Phanerozoic marine diversity. *Paleobiology*, 4: 55–66.

VERMEIJ, G. J., in press, An examination of environments and geographical areas containing modern survivors of extinction-ravaged taxa. In: ELLIOTT, D. K., ed., *Dynamics of extinction*. New York: Wiley.

WARD, P. D. & SIGNOR, P. W., III, 1983, Evolutionary tempo in Jurassic and Cretaceous ammonites. *Paleobiology*, 9: 183–198.

WILLIAMS, C. B., 1964, *Patterns in the balance of nature....* London: Academic Press, vii + 324 p.

WILLIS, J. C., 1922, *Age and area*. Cambridge: Cambridge University Press, 259 p.

WISE, K. P. & SCHOPF, T. J. M., 1981, Was marine faunal diversity in the Pleistocene affected by changes in sea level? *Paleobiology*, 7: 394–399.

FAUNAL REFERENCES

ABBOTT, R. T., 1958, The marine mollusks of Grand Cayman Island, British West Indies. *Academy of Natural Sciences of Philadelphia Monograph* 11: 138 p.

ABBOTT, R. T., 1974, *American seashells*. Ed. 2. New York: Van Nostrand Reinhold, 663 p.

ALTENA, C. O. VAN REGTEREN, 1971, The marine Mollusca of Suriname (Dutch Guiana) Holocene and Recent. Part II. Bivalvia and Scaphopoda. *Zoologische Verhandelingen (Leiden)*, 119: 100 p.

ALTENA, C. O. VAN REGTEREN, 1975, The marine Mollusca of Suriname (Dutch Guiana) Holocene and Recent. Part III. Gastropoda and Cephalopoda. *Zoologische Verhandelingen (Leiden)*, 139: 104 p.

ANDREWS, J., 1977, *Shells and shores of Texas*. Austin: University of Texas Press, 365 p.

BASSINDALE, R., 1961, On the marine fauna of Ghana. *Proceedings of the Zoological Society of London*, 137: 481–510.

BERNARD, F. R., 1970, A distributional checklist of the marine molluscs of British Columbia, based on faunistic surveys since 1950. *Syesis*, 3: 75–94.

BOSCH, D. & BOSCH, E., 1982, *Seashells of Oman*. London: Longman, 206 p.

CASO, M. E., 1961, *Estado actual de los conocimientos acerca de los equinodermos de Mexico*. Ph.D. dissertation, Departamento de Biologia, Universidad Nacional Autonoma, Mexico, 388 p.

CERNOHORSKY, W. O., 1970, The littoral marine molluscs of Niue Island. *Records of the Auckland Institute and Museum*, 7: 175–186.

CERNOHORSKY, W. O., 1971, *Marine shells of the Pacific*. Rev. ed. Sydney: Pacific Publications, 248 p.

CERNOHORSKY, W. O., 1972, *Marine shells of the Pacific*. Sydney: Pacific Publications, 2: 411 p.

CERNOHORSKY, W. O., 1978, *Tropical Pacific marine shells*. Sydney: Pacific Publications, 352 p.

CERNOHORSKY, W. O., 1978, Report on the molluscan fauna of the Lau Group, Fiji Islands. *Bulletin of the Royal Society of New Zealand*, 17: 39–52.

CHERBONNIER, G. & GUILLE, A., 1975, Echinodermes récoltés aux îles Kerguelen. *Bulletin du Museum National d'Histoire Naturelle (Paris), Zoologie*, 210: 603–630.

CLARK, A. M. & COURTMAN-STOCK, J., 1976, *The echinoderms of southern Africa*. London: British Museum (Natural History), 238 p.

CLARK, A. M. & ROWE, F. W. E., 1971, *Monograph of shallow-water Indo-West Pacific echinoderms*. London: British Museum (Natural History), 238 p.

CLARK, A. M. & SPENCER-DAVIES, P., 1965 (1966), Echinoderms of the Maldive Islands. *Annals and Magazine of Natural History*, ser. 13, 8: 597–612.

CLARK, H. L., 1942, The echinoderm fauna of Bermuda. *Bulletin of the Museum of Comparative Zoology, Harvard University*, 89: 367–391.

COLES, J., 1981, A check-list of the molluscs of the Cook Islands (Mollusca). *Poirieria*, 11(1): 7–11; 11(2): 17–21.

D'ANGELO, G. & GARGIULLO, S., 1978, *Guida alle Conchiglie Mediterranee*. Milano: Fabbri Editori, 223 p.

DAUTZENBERG, Ph. & BOUGE, J. L., 1933, Les mollusques testacés marins des etablissements français de l'Océanie. *Journal de Conchyliologie*, 77: 41–108, 145–326, 351–469.

DAY, J. H., FIELD, J. G. & PENRITH, M. J., 1970, The benthic fauna and fishes of False Bay, South Africa. *Transactions of the Royal Society of South Africa*, 39: 1–108.

DELL, R. K., 1960, Chatham Island marine Mollusca based upon the collections of the Chatham Islands Expedition, 1954. *Bulletin of the New Zealand Department of Scientific and Industrial Research*, 139: 141–157.

DELL, R. K., 1964, Marine Mollusca from Macquarie and Heard Islands. *Records of the Dominion Museum, Wellington, New Zealand*, 4: 267–301.

FELL, F. J., 1974, The echinoids of Easter Island (Rapa Nui). *Pacific Science*, 28: 147–158.

FELL, H. B., 1960, Archibenthal and littoral echinoderms of the Chatham Islands. *Bulletin of the New Zealand Department of Scientific and Industrial Research*, 139: 55–75.

FRETTER, V. & GRAHAM, A., 1962, *British prosobranch molluscs*. London: Ray Society, 755 p.

HEDLEY, C., 1899, The Mollusca of Funafuti. *Memoirs of the Australian Museum*, 3: 397–510, 547–565.

HEMMEN, J. & HEMMEN, C., 1979, Beitrag zur Kenntnis der Meeres Mollusken-Fauna der Karibischen See. Grenada. *Jahrbuch der Nassauischen vereins fur Naturkunde, Wiesbaden*, 104: 137–172.

HUMFREY, M., 1975, *Sea shells of the West Indies*. New York: Taplinger, 351 p.

KAY, E. A., 1971, The littoral marine molluscs of Fanning Island. *Pacific Science*, 25: 260–281.

KAY, E. A., 1979, *Hawaiian marine shells. Reef and shore fauna of Hawaii*, section 4: *Mollusca. Bernice Pauahi Bishop Museum Special Publication* 64(4): 653 p.

KAY, E. A. & SWITZER, M. F., 1974, Molluscan distribution patterns in Fanning Island lagoon and a comparison of the mollusks of the lagoon and the seaward reefs. *Pacific Science*, 28: 275–295.

KENSLEY, B., 1973, *Sea-shells of southern Africa-gastropods*. Capetown: Maskew Miller.

KUNDU, H. L., 1965, On the marine fauna of the

Gulf of Kutch. Part III. Pelecypoda. *Journal of the Bombay Natural History Society*, 62: 209–236.
KURODA, T., HABE, T. & OYAMA, K., 1971, *The sea shells of Sagami Bay*. Tokyo: Maruzen. xix + 739 + 489 + 51 p., 121 pl., 1 map.
LEMCHE, H., 1929, Gastropoda Opisthobranchia. *The zoology of the Faeroes*, 3(1, 53): 20 p.
LEMCHE, H., 1939, Gastropoda Opisthobranchia. *The zoology of Iceland*, 4(61): 54 p.
LIEBERKIND, I., 1929, Echinodermata. *The zoology of the Faeroes*, part 53: 20 p.
LUBINSKY, I., 1980, Marine bivalve molluscs of the Canadian central and eastern Arctic: faunal composition and zoogeography. *Canadian Bulletin of Fisheries and Aquatic Sciences*, 207: 111 p.
MacGINITIE, N., 1959, Marine Mollusca of Point Barrow, Alaska. *United States National Museum Proceedings*, 109: 59–208.
MACPHERSON, E., 1971, The marine molluscs of Canada: prosobranch gastropods, chitons and scaphopods. *National Museum of Canada Publications in Biological Oceanography*, 3: 149 p.
MADSEN, F. J., 1949, Marine Bivalvia. *The zoology of Iceland*, 4(63): 116 p.
MAES, V. O., 1967, The littoral marine mollusks of Cocos- Keeling Islands (Indian Ocean). *Proceedings of the Academy of Natural Sciences of Philadelphia*, 119: 93–217.
MARCUS, E., 1977, An annotated checklist of western Atlantic warm water opisthobranchs. *Journal of Molluscan Studies Supplement*, 4: 22 p.
McKNIGHT, D. G., 1968, Some echinoderms from the Kermadec Islands. *New Zealand Journal of Marine and Freshwater Research*, 2: 505–526.
McKNIGHT, D. G., 1969, An outline of the New Zealand shelf fauna. Benthos survey, station list, and distribution of the Echinoidea. *New Zealand Department of Scientific and Industrial Research Bulletin* 195(= *New Zealand Oceanographic Institute Memoir*, 47): 91 p.
McKNIGHT, D. G., 1972, Echinoderms collected by the Cook Islands eclipse expedition, 1965. *New Zealand Oceanographic Institute Research Bulletin* 1(3): 37–45.
MICHEL, C., 1974, Notes on marine biology studies made in Mauritius. *Bulletin of the Mauritius Institute*, 7(2): 287 p.
MORTENSEN, Th., 1927, *Handbook of the echinoderms of the British Isles*. London: Oxford University Press, 471 p.
MORTENSEN, Th., 1950, Echinoidea. *Report of the British, Australian and New Zealand Antarctic Research Expedition*, ser. B, 4: 287–310.
NISIYAMA, S., 1968, The echinoid fauna from Japan and adjacent regions. Part II. *Palaeontological Society of Japan Special Paper* 13: 491 p.
OLSSON, A. A. & McGINTY, T. L., 1958, Recent marine molluscs from the Caribbean coast of Panama with the description of some new genera and species. *Bulletins of American Paleontology* 39(177): 1–58.
PAWSON, D. L., 1978, The echinoderm fauna of Ascension Island, South Atlantic Ocean. *Smithsonian Contributions in Marine Science* 2: 31 p.
PERRY, L. M. & SCHWENGEL, J. S., 1955, *Marine shells of the western coast of Florida*. Ithaca: Paleontological Research Institution, 262 p.
PETERSEN, G. H., 1968, Marine Lamellibranchiata. *The zoology of the Faeroes*, 3(1, 55): 80 p.
POWELL, A. W. B., 1957, Mollusca of Kerguelen and Macquarie Islands. *Report of the British, Australian and New Zealand Antarctic Research Expedition*, ser. B, 6: 107–149.
POWELL, A. W. B., 1976, *Shells of New Zealand*. Ed. 5 revised. Christchurch: Whitcoulls, 154 p.
POWELL, A. W. B., 1979, *New Zealand Mollusca*. Auckland: Collins, 500 p.
PRICE, A. R. G., 1982, Echinoderms of Saudi Arabia. Comparison between echinoderm faunas of Arabian Gulf, SE Arabia, Red Sea and Gulf of Aqaba and Suez. *Fauna of Saudi Arabia*, 4: 3–21.
REHDER, H. A., 1968, The marine molluscan fauna of the Marquesas Islands. *Bulletin of the American Malacological Union*, 1968: 29–32.
REHDER, H. A., 1980, The marine mollusks of Easter Island (Isla de Pascua) and Sala y Gomez. *Smithsonian Contributions to Zoology*, 289: 167 p.
REHDER, H. A. & WILSON, B. R., 1975, New species of marine mollusks from Pitcairn Island and the Marquesas. *Smithsonian Contributions to Zoology*, 203: 16 p.
RIOS, E. C., 1970, *Coastal Brazilian seashells*. Fundacão Cidade do Rio Grande, Museu Oceanografico de Rio Grande, 225 p.
RODRIGUEZ, J., 1980, Echinoderms (except Holothuroidea) of the southern Mediterranean coast of Spain. In: JANGOUX, M., ed., *Echinoderms: present and past*. Rotterdam: A. A. Balkema, p. 127–131.
ROMAN, J., 1980, Une monographie des echinides de la Mer Rouge. Principaux résultats. In: JANGOUX, M., ed., *Echinoderms: present and past*. Rotterdam: A. A. Balkema, p. 133–136.
ROSEWATER, J., 1975, An annotated list of the marine mollusks of Ascension Island, South Atlantic Ocean. *Smithsonian Contributions to Zoology*, 189: 41 p.
SERAFY, D. K., 1979, Echinoids (Echinodermata: Echinoidea). *Memoirs of the Hourglass Cruises*, 5 (3): 120 p.
SHIGEI, M., 1970, Echinoids of the Bonin Islands, *Journal of the Faculty of Sciences of Tokyo University*, 12: 1–22.
SKJAEVELAND, S. H., 1973, Echinoderms of Jan Mayen Island. *Astarte*, 6: 69–74.
SLOAN, N. A., CLARK, A. M. & Taylor, J. D., 1979, The echinoderms of Aldabra and their habitats. *Bulletin of the British Museum (Natural History), Zoology* 37: 81–128.
SMITH, A. G. & GORDON, M., Jr., 1948, The marine mollusks and brachiopods of Monterey Bay,

California, and vicinity. *Proceedings of the California Academy of Sciences*, Ser: 4, 26: 147–241.

SNELI, J. A. & STEINNES, A., 1975, Marine Mollusca of Jan Mayen Island. *Astarte*, 8: 7–16.

SPARCK, R. & THORSON, G., 1931, Marine Gastropoda Prosobranchiata. *The Zoology of the Faeroes*, no. 52: 56 p.

TEBBLE, N., 1976, *British bivalve seashells*. Ed. 2. Edinburgh: Her Majesty's Stationery Office, 212 p.

THOMPSON, T. E., 1976, *Biology of opisthobranch molluscs*. London: Ray Society, 1: 207 p.

THORSON, G., 1941, Marine Gastropoda Prosobranchia. *The zoology of Iceland*, 4(60): 150 p.

TORTONESE, E., 1965, *Fauna d'Italia*. Vol. VI. *Echinodermata*. Bologna: Edizioni Calderini, 422 p.

VERMEIJ, G. J., KAY, E. A. & ELDREDGE, L. G., 1983, Molluscs of the northern Mariana Islands, with special reference to the selectivity of oceanic dispersal barriers. *Micronesica*, 19: 27– 55.

WALLER, T. R., 1973, The habits and habitats of some Bermudian marine mollusks. *Nautilus*, 87: 31–52.

MALACOLOGIA, 1986, 27(1): 67–81

EXTINCTION IN HAWAIIAN ACHATINELLINE SNAILS

Michael G. Hadfield

Pacific Biomedical Research Center,
University of Hawaii,
Honolulu, HI 96822, U.S.A.

ABSTRACT

Growth data point strongly to late maturity in the achatinellines, and information from both dissection and examination of age-frequency histograms supports the idea that annual fecundity is near one. The limited lifespan extrapolated suggests a reproductive life of perhaps six years; lifetime fecundity could thus be as low as 6, and seems certain not to exceed 24. This assemblage of traits predisposes populations of achatinellines to extinction in the face of predation, particularly of the selective, shell-collecting sort.

Key words: *Achatinella*; extinction; Hawaiian tree snails; life histories; mark-recapture studies; demography.

INTRODUCTION

That populations, varieties and species of the Hawaiian arboreal snails (subfamily Achatinellinae) are going extinct has been known for more than 120 years (Frick, 1856). In the 1880's the alarm was sounded by at least two important students of Hawaiian shells, D. D. Baldwin (1887) and J. T. Gulick (quoted in A. Gulick, 1932). Baldwin (1887: 56), speaking of the habitats of *Achatinella*, noted, "It is also generally supposed that these shells are becoming extinct by the ravages of cattle through our forests"; "Some of these hills have been denuded of woods, not only by cattle, but by the woodman's ax, and certain species are becoming rare"; and "The agencies now threatening the wholesale destruction of these little gems of the forest are the rats and mice, which have become very abundant in mountain forests, particularly where there are no cattle."

In later years, awareness of the loss of the unique tree snail fauna increased. Henshaw, writing on the *Partulina* of the island of Hawaii (in Pilsbry & Cooke, 1912–1914) noted the great declines of numbers of individuals in some areas and their disappearance in others. Bryan (1935) put it mildly, "Land snails were formerly more plentiful in certain parts of the islands than they are today." And Cooke (1941: 20), writing of *Achatinella apexfulva*, said, "It must have been a rather abundant lowland species then but, to my knowledge, no typical live specimens have been found in the last 40 or 50 years."

More recently Kondo (Ms., 1970) presented quantitative estimates of extinction in Hawaiian terrestrial gastropods (50% extinct) and succinctly outlined major causes for their disappearance. Hart (1975, 1978) repeated these figures in popular articles which had an important impact on public recognition of the problem.

The culmination of this growing record of the wholesale disappearance of species of a unique subfamily of snails, endemic to the Hawaiian Islands, has been preparation of evidence for and, finally, a federal declaration of "endangered status" for the remaining species of the genus *Achatinella* (Federal Register, 1981). The documents relative to the declaration cite 22 species (20, after Welch's revisions, 1942, 1954, 1958) of *Achatinella* as extinct, with the remaining 19 (or 17) species endangered. Species in the achatinelline genera *Partulina* and *Newcombia* are probably in no better condition. The partulinas of Hawaii island are probably gone, and no good, recent census exists for the islands of Molokai, Maui and Lanai. On all of these islands, habitat destruction alone has had a major impact.

Thus, extinction in the Hawaiian tree snails is an historical problem and continues today (Hadfield & Mountain, 1981). The goals of the

present account are not to further belabor this point, but rather to attempt first to clarify the magnitude and rate of the disappearance by asking, "How many snails *were* there?", and second to examine the causes of mass mortality in these snails. Both of these goals must be met by examining the literature, little of which was written to answer these particular questions. Subsequently, I present results of recent field studies on the achatinellines and analyze them with a goal of ascertaining what features of the life history of these gastropods have made them so vulnerable to the agents of extinction.

The systematics of the endemic Hawaiian subfamily Achatinellinae (Stylommatophora; Achatinellidae) are thoroughly reviewed in Pilsbry & Cooke (1912–1914) and elsewhere (Cooke & Kondo, 1960; Welch, 1938, 1942, 1954, 1958).

METHODS

Determination of primitive densities of achatinelline snails has depended almost entirely on extrapolating from collections. Only a single published attempt to estimate a population size for a Hawaiian tree snail species has been found; it is that of Henshaw for *Partulina confusa* (in Pilsbry and Cooke, 1912–1914). For other data I have used collecting notes of Baldwin, Gulick, Spalding and many others. As will be seen, comparing old collecting notes with recent experiences allows a very rough estimation of primitive snail densities.

Causes of extinction of Hawaiian tree snails have been cited since the time of the original notes on shell collecting in Hawaii and added to by nearly every student of these snails. These are summarized from the literature and reinterpreted in the light of the collecting notes and modern field studies.

Field methods used to study population sizes, growth rates, ages at maturity and other population characteristics were provided by Hadfield & Mountain (1981). These are mark-recapture studies which necessitate no sacrifice of living snails. Added to these are some general results of field surveys where 2 to 4 experienced workers hiked transects and counted all visible snails for noted time durations. To a lesser extent, information has been gleaned from assemblages of dead shells which accumulate on the ground beneath trees occupied by the achatinellines.

Data utilized in an earlier publication (Hadfield & Mountain, 1981) have been reanalyzed for some additional conclusions on life span and mortality.

Study sites include two areas in the Waianae Mountains of Oahu: Kanehoa Trail (see Hadfield & Mountain, 1981) and another ridge line in the northern third of the Waianae Mountains. Elevations lie between 600 and 800 m. Here the forests are a mixture of native trees and shrubs such as *Dodonaea* spp., *Metrosideros polymorpha*, *Dubautia* sp., *Scaevola gaudichaudiana*, *Osmanthus sandwichensis*, and *Alyxia olivaeformis*, and introduced forms including guava (*Psidium guajava*), silk oak (*Grevillea robusta*), *Stachytarpheta jamaicensis*, lantana (*Lantana camara*), etc. Snails seen in the Waianae sites belong to the species *Achatinella mustelina* (see Welch, 1938).

A second major study site has been established on the Nature Conservancy's Kamakou Preserve on Molokai. At this site, elevation about 1200 m, *Partulina redfieldii* occupies scattered, small ohia trees (*Metrosideros polymorpha*) which are semi-isolated from one another by broad expanses of grassland. This formerly dense mesic forest was opened up by the grazing activities of feral livestock beginning in the mid- to late 1800's. Occasionally, snails are seen here on the shrubs *Coprosma* sp. and *Wikstroemia* sp.

Data from the field studies have been analyzed as described in Hadfield & Mountain (1981) for growth rate, age at first reproduction and population density. Because growth rates for the Molokai snails were not the same for all sizes, as was seen in *Achatinella mustelina*, the von Bertalanffy growth equation (Fabens, 1965) was calculated for *Partulina redfieldii*. It provided a good fit for the data and allowed the size-frequency pattern to be transformed to reflect age frequencies.

Longevity of snails from both areas, Waianae and Molokai, was extrapolated from age-frequency distributions by determining the average sizes of year classes in the age frequency histograms and dividing the number of lipped (= maximum sized) shells by the average size/year class to estimate the expected number of year classes. The resulting number was added to the average age at lip development. Fecundity was estimated from

the literature and extrapolated from the histograms.

A Leslie Matrix (Searle, 1966) was used to assess the effects of fecundity and annual survivorship on the malthusian parameter, r_m, the intrinsic rate of increase of a population with a stable age distribution. Fecundity for each age (12 age classes) was entered as the top row of the matrix and annual survivorship as a diagonal across the next 11 rows (in a 12 $\times$ 12 matrix). A computer program evaluated the matrix to the 50th iteration and the dominant eigenvalue (λ) was determined as the arithmetic mean of the 12 values in the 50th iteration. The value of r_m is obtained as the natural log of λ (see Mertz, 1970, for a thorough discussion of these terms). The malthusian parameter usually proves to be the more useful estimator for comparing species with different life-history patterns. Briefly, r_m varies around zero, with positive values reflecting growing populations and negative values shrinking ones.

RESULTS AND DISCUSSION

1. How many snails were there?

Henshaw (in Pilsbry & Cooke, 1912–1914) estimated densities of *Partulina confusa* on the Waimea Plains (island of Hawaii) from his observations there in 1903. He referred to this population as an "isolated colony" living in about 150 pua trees (*Osmanthus sandwichensis*) growing within an area of about a "half a mile square." The trees were scattered, mostly separated, and 15 to 20 feet high. Henshaw stated (p. 97), "A rough estimate of the number of adult shells inhabiting this area when first visited is more than 75,000 shells, and it was possible to ride under the trees and from their trunks, leaves, and branches to pick shells literally by the handfuls. Cavities in the trunks and branches were usually packed with shells, mostly immature, from 50 to 75 being often found together."

Henshaw's estimate predicts 500 *adult* snails per tree! If the age frequency distribution of *P. confusa* was similar to those we have seen for *P. redfieldii* on Molokai in a somewhat similar setting, adult snails make up about 20–25% of the population. The total number of snails would thus have approached 300,000, equivalent to 2,000 per tree! Such figures seem impossible, but we shall never know. Henshaw reported that he gathered 1,100 adult shells from this spot in 6 hours, and his colleague, Mr. William Horner, did the same. Any place allowing the picking of 3 snails per minute was assuredly densely inhabited. Kondo (personal communication, July 1983) told this author that he visited the Waimea plain in 1946 and saw only dead shells of *P. confusa* covering the ground.

Other estimates of snail densities can only be roughly extrapolated from collecting records. Such values must be very coarse because it is usually impossible to determine an important series of parameters. Was the collector collecting only large, mature shells or all sizes seen? If only large shells were taken, was the collector choosing the more attractive shells only, or all seen? Was the collector alone, or did he have companions taking an equal number of shells? How hard did the collector search? Was he interested in cleaning out the spot or only obtaining what was easy to find? Did the collector climb the trees or in other ways obtain shells higher than his reach (and was he on horseback when reaching?)? Nonetheless, the following quotations give some idea of the enormous densities of achatinellines encountered by the early shell collectors.

J. T. Gulick, whose recorded collection was about 44,500 shells in about 3 years collecting (1851–53; see Clench, 1959), usually spoke of hundreds or thousands in each collecting lot (A. Gulick, 1932). He reported on riding into the northern Koolaus on July 28, 1853 with 10 others to a little valley and returning by four in the afternoon with over fourteen hundred shells (p. 120). On September 3, 1853, "Brother Thomas and I spent several hours in Punaluu Valley, where we procured over a hundred specimens of what I consider a new shell" (p. 124). On September 15, Gulick and two native men went into Waiawa Valley for most of a day and returned with 200 to 400 shells apiece (p. 125). Gulick also encouraged the Hawaiian residents of rural villages to collect for him, and he would periodically ride about the islands buying the shells, individual lots often "amounting to several thousands," and frequently filling his saddle bags with his purchases.

In 1852, Samuel and Henry Alexander traveled from Oahu to Lanai with a Mr. Bailey and collected "several thousand" on that small

island (M. C. Alexander, 1934). A note in the *Weekly Star*, a script journal of the Punahou School in Honolulu, tells of a picnic on March 7, 1853 when, " . . . into the woods back of sugarloaf . . . After dinner, we all dispersed about in the woods, for the purpose of procuring shells . . . the number procured that day was over four thousand." The same journal for March 16, 1853: "Last Saturday was rendered famous by a pretty general expedition to the mountains in search of shells. Over (2,000) two-thousand specimens were brought back alive by the hardy adventurers comprising about fourteen species of the genus *Achatinella*."

Baldwin (1887, 1900) gives a number of incredible collecting tallies. In the valleys on the eastern side of the Waianae Range of Oahu he could secure " . . . the shells very rapidly, often getting a hundred or more from a single tree," with the aid of a hook on a stick seven or eight feet long for pulling the branches of the trees downward. Four days collecting in the Waianae Range yielded over 2,000 shells. In one nine-day trip, Baldwin collected more than 3,500 shells; his daily yield varied from 152 to 864. Baldwin (1887) also reported a trip around Molokai when he collected 5,000 shells. And in Kohala, on the island of Hawaii, Baldwin reported (1887: 62), "during a recent visit to the locality, in a few minutes I collected several hundred specimens, picking them from trees and low bushes as rapidly as one would gather huckleberries from a prolific field."

Cooke (1903), reporting on *Achatinella multizonata* (= *A. bellula*) in Nuuanu Valley, collected 3,000 shells from a zone 100–400 yards wide and extending for about one mile on the northwestern side of the valley at elevations between 1,000 and 1,400 feet. These snails came from a number of semi-isolated populations within the described zone, suggesting fairly high local densities.

From Emerson (Ms., undated, post-1900), we learn of a single ahakea (*Bobea* sp.) tree yielding 3 score of a rare shell and a banana tree which yielded a score of *Achatinella bulimoides*. He also wrote of *A. viridans* so abundant on the midridge of Palolo Valley that they " . . . hung in clusters on the hoe vines" (probably *Dioscorea* sp.).

2. Modern notes

Using mark-recapture techniques, we estimated a resident population of *Achatinella mustelina* in four small trees in a 25 m^2 quadrate in the Waianae range to be about 210 animals (Hadfield & Mountain, 1981). The average number of snails, all sizes included, observed on any visit to this site was 44, or about 21% of the population. I will use this estimate of observed-to-actual snails to compare current densities with the older collecting records for the Waianaes cited above.

In the winter of 1982–1983, while conducting surveys under contract to the Army Corps of Engineers in the region of Makua Valley, three other experienced snail observers and I made the observations listed in Table 1. The figures provide some very rough estimates of density to compare with Baldwin's (1887) collecting data from the 1880's. Baldwin had a companion when he gathered 2,000 plus shells in four days; together they took about 500 shells per day. If we assume they took only larger shells (Baldwin, 1887, cautioned against collecting young or immature shells) and that they were not minutely searching out the snails, then they were collecting snails, steadily, at a rate of about 31 per person hour. Our rate, seen in Table 1, assuming 50% "large shells", varied from 1–7 shells per person hour of searching, an average of less than 4. If there is any validity to these calculations, then the best residual populations of *Achatinella mustelina* amount to about 12% of those seen 100 years ago in the Waianae range.

Another comparison may be made between our current study site for *Partulina redfieldii* on

TABLE 1. Transect sightings: *Achatinella mustelina* in the northern Waianae Range.

Date	No. searchers	Time	No. snails	Est. of total	No. large shells/person/hr*
Nov. 13 1982	4	2 hr	29	138	2
Dec. 18 1982	4	3 hr	161	770	7
Jan. 15 1983	2	2½ hr	48	229	5
Jan. 29 1983	4	1½ hr	15	71	1

*Estimated to be 50% of all snails seen, divided by number of observers and number of hours.

Molokai and the populations of *P. confusa* described by Henshaw as existing on the Waimea Plain of Hawaii in 1903. These locales share a common elevation, both are relatively wet, and both consist of isolated, low trees in a larger meadow at the time of observation. As noted above, Henshaw's estimates ran to 500 adult snails per tree. In our study area, we have counted the numbers of snails in seven small ohia trees. These densely foliated trees are about 1.5–4.5 m high and have crown diameters ranging from 2–3 m. We have actually counted 84 snails in these bushes, searching each extensively on each of three visits to the study site.

Counts of visible snails, expressed per tree, in the Kamakou meadow (Molokai) varied from 4 to 17; to some extent these numbers reflect the sizes of the trees. Data from three trees which have been sampled on three separate occasions are tabulated together with Weighted-Mean estimates of populations (see Begon, 1979, pp. 13–16) in Table 2. The estimates vary between 8 and 29 snails per tree; they are in close agreement with estimates achieved by a second method, the Peterson Estimate (Begon, 1979), which utilizes data from only the first recapture. To determine an estimator of the number of snails present from those seen, the weighted-mean determined population size for each tree was divided into the average number of snails seen per tree on each of three visits; the mean of these three values, 55.7%, was the estimator so derived. Thus for another tree, counted only once, with a visible snail population of 17, the total population is estimated to be 31. This is the upper limit to estimated population densities per tree in the Molokai study area.

The estimates of numbers of *Partulina confusa* in the trees of the Waimea Plain given by Henshaw are one to two orders of magnitude greater than those arrived at for *P. redfieldii*, above, in a similar habitat. Of course, we cannot guess if the populations on Molokai were ever as dense as those of Waimea.

From the foregoing, it is clear that the densities of achatinelline snails were once far greater than any seen today. While it would be possible to determine when whole habitats were eliminated for farming and logging, it is nearly impossible to learn just when the densities of still extant populations declined to their current levels. With such information, long-term rates of extinction could be calculated.

TABLE 2. *Partulina redfieldii*. Capture-recapture data and population estimates for three trees in the Kamakou study area, Molokai.

	Date		
	Aug. 5	Feb. 13	June 16
Tree A:			
No. captured (n_i)	15	12	11
No. previously marked (m_i)	—	6	7
No. released (r_i)	15	12	—
Total no. marked (M_i)	—	15	21
M_in_i	—	180	231
Tree B:			
n_i	4	8	6
m_i	—	4	5
r_i	4	8	—
M_i	—	4	8
M_in_i	—	32	48
Tree C:			
n_i	3	9	9
m_i	—	2	5
r_i	3	9	—
M_i	—	3	10
M_in_i	—	27	90

Population size, $\hat{N}_i = \frac{\Sigma M_i n_i}{(\Sigma m_i) + 1}$

$\hat{N}$-Tree A = 29; av. no. snails seen = 44.3% of estimated total
$\hat{N}$-Tree B = 8; av. no. snails seen = 75% of estimated total
$\hat{N}$-Tree C = 15; av. no. snails seen = 47.9% of estimated total

Average percent seen = 55.7%

3. Causes of mortality in achatinelline snails

For more than one hundred years, different authors have speculated on the causes of extinction in the Hawaiian tree snails (see Introduction above). The list of causes presented here includes factors cited by many authors in the past and also raises questions about causes of mortality perhaps not previously considered.

Habitat destruction is, of course, the single most important cause of population disappearance for any species. For the achatinellines this process may well have begun with the pre-historic landings of the Hawaiians.

Their clearing of lowland vegetation was probably responsible for unrecorded extinctions of low altitude populations or even species (Kirch, 1982). By the mid-1800's the achatinellines were snails of the mountain slopes and ridges, but Pilsbry wrote about 1912 (in Pilsbry & Cooke, 1912–1914: xxvi), "Once forests with Achatinellidae and Endodontidae shaded the plains far seaward from the lovely peak of Kaneohe, where now dead shells may be picked up in plowed fields, or gathered out of "pockets" in the rocks. It has been the same in the northwest. Forest-snails are found in the sand-dunes of Kahuku, now far from where living tree-snails exist." The shells of *Achatinella caesia littoralis*, an extinct subspecies, were found in the troughs of sand dunes near Kahuku, less than 100 feet from the sea. Pilsbry further noted, "Sixty years ago the Achatinellas were found in abundance at half the elevations now inhabited by them." (*ibid.*: xlix). Pilsbry attributed these losses to both disappearance of trees and to declining humidities brought on by the grazing out of underbrush by cattle and goats.

Much habitat went to cane and pineapple fields, some to reservoirs, and still more to forest cutting for logs. Reforestation, which might have provided a new home for relict achatinelline populations rarely included native species, and trees such as eucalyptus, ironwood and Norfolk Island pine, planted widely on Oahu, have never provided suitable habitats for the achatinellines. Emerson (Mss., undated) noted that a prime habitat for a distinct variant of *Achatinella rosea* was deeply submerged by a sugar plantation reservoir, probably in the late 1890's.

Accounts of total habitat destruction can be repeated for each of the islands. Farming occupies most of the suitable land on all the islands, and a drive from the coast to the upper elevations of Molokai takes one through extensive stands of the above-named exotic trees, supplemented with loblolly pines, Monterey cypress, and California coastal redwoods. This is not to say that achatinellines have not been seen on non-native vegetation. They have been reported on guava, lantana and the aboriginally introduced ti, banana and kukui. It is probable that lantana, reported in late 1800's publications to be locally infested with Achatinellas, proved unsuitable as a permanent substratum; at no location where lantana is currently abundant have I seen achatinellines on it except rarely.

A slightly more subtle form of habitat destruction was brought about by feral livestock, particularly goats and cattle. These browsers pushed forest destruction to much higher elevations than the farmers did, eating the undergrowth and lower tree branches, destroying seedlings, and probably transporting exotic plants ever higher into the native forests. Gulick, Frick, Baldwin, Emerson, Pilsbry and others spoke of the threat presented by cattle to native forests and their snail faunas.

Although habitat destruction accounts for a large percentage of the loss of achatinelline snails, there were still many square miles of forest left mostly intact, particularly at upper elevations. While it may be that these upper elevations never harbored the greatest of achatinelline densities, it is certain that they were rich country for the snails. These snails too have mostly disappeared, and their disappearance must be related to more selective agents of destruction. Perkins (1899) found only rats to be certain predators of tree snails, and he affirmed that native birds did not feed on them. The Polynesian rat had obviously been present a long time before Europeans were marveling at the great densities of tree snails in the Hawaiian Islands. However, the introduction of the European rats appears to have presented a major threat to Hawaii's endemic snails, a fact earlier recorded by Gulick (A. Gulick, 1932: 411), Baldwin (1887), and Perkins (1899). To this day, one can find ample evidence of their ravages wherever tree snails still persist. To my knowledge, no other predator crushes the shells in the manner of predating rats; in a recent survey of dead shells collected from the ground in a small area in the northern Waianae Range (see Fig. 1), 10% of the ground shells appeared to have been eaten by rats. If the conclusions of Atkinson (1977) are correct, the predator here is *Rattus rattus*, which became widespread on Oahu only in the 1870's.

There may have been other native snail predators. Frick (1856) wrote of a "centipede worm" that ate any tree snail unfortunate enough to land upon the ground; apparently the centipedes did not climb trees in search of prey. A terrestrial flatworm, *Geoplana septemlineata* (perhaps not native), is known to attack ground-living snails such as *Achatina fulica* (Mead, 1979), but I know of no evidence that the worms ascend trees to attack arboreal snails. *Geoplana* would, however, appear

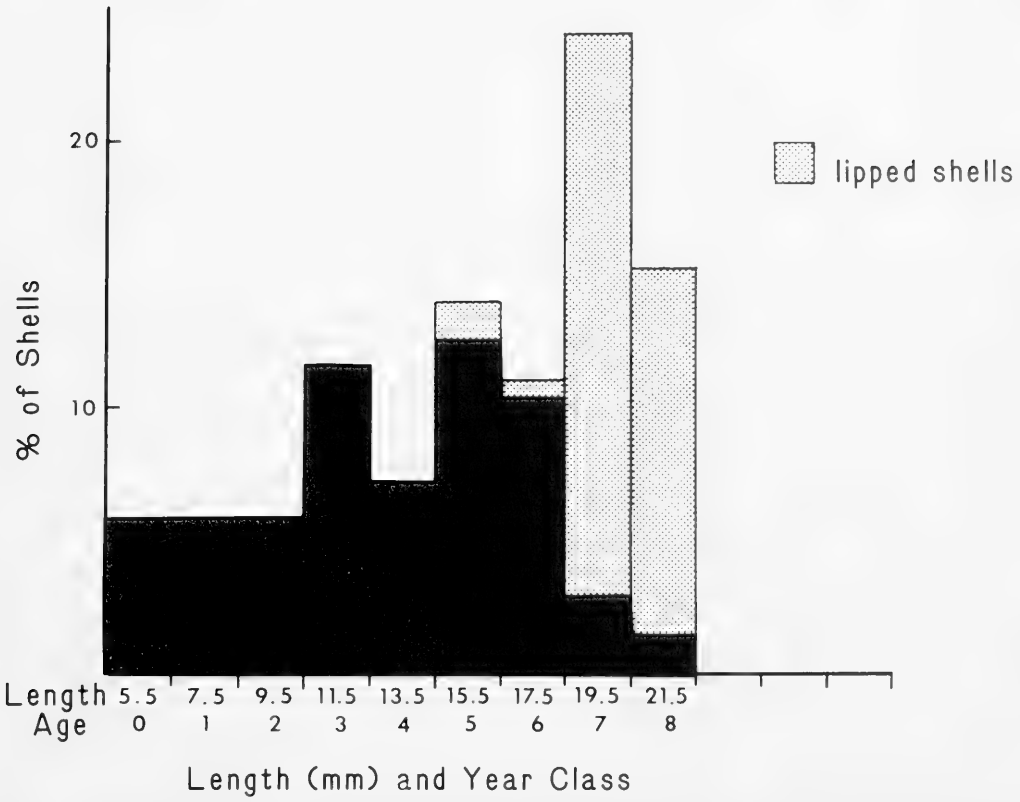


FIG. 1. *Achatinella mustelina*: size-, and extrapolated age-frequency distribution of dead shells collected at one time from a 5 × 5 m quadrate in the northern Waianae Range. "Lipped shells" are those with terminal growth; they include, at least in part, shells of older ages than others in the size/age class. Age-frequencies were extrapolated from data provided in Hadfield & Mountain (1981). n = 138.

to be a real danger to achatinellines blown or knocked to the ground. We have observed living *Geoplana septemlineata* in the forest litter at a place where Hurricane Iwa swept many living *Achatinella mustelina* to the ground in November 1982. We did not find evidence of unusually heavy mortality in these locales, however, and it appeared that the snails had rapidly ascended all nearby vegetation (personal observations made in December, 1982).

Introduced ants have been cited as a serious threat to Hawaii's native snails (Solem, 1976), but I know of no evidence to implicate them in predation on achatinellines.

While the Hawaiians were known to make occasional leis from the shells of tree snails, there can be little doubt that a predator of major importance, arriving in abundance in the 1800's and immediately commencing to depredate the achatinelline populations, was the European stock of *Homo sapiens*. How many shells were gathered as curiosities by itinerant voyagers we shall never know, but the recorded or semi-recorded collections are amazing in themselves. While many of the "missionary sons" were simply shell collecting, others such as J. T. Gulick seem to have had the motivations of a naturalist. These collectors scoured the islands from 1850 to 1900, and extensive collections were made even in the first decades of the twentieth century. How many did they collect? It is nearly impossible to determine, but the collecting notes cited above lend visions of tens of thousands of gastropods being frequently reduced to "shells." J. T. Gulick himself wrote to G. T. Romanes in 1888, "The collection was made during the years 1851–1853, when I visited all the districts of the Island of Oahu

in person and accompanied by troops of native assistants ransacked each valley." And after speaking of forest and snail destruction, Gulick concluded, "The collection is therefore not only unique but will always remain unique . . . " (A. Gulick, 1932: 411).

Emerson (Mss., undated) was of the certain opinion that the shell hunters were seriously reducing the densities of achatinelline populations. He tells of finding a dense population of an *Achatinella* species in Waiomau (Palolo) Valley and reporting its location to a friend. This was a grievous mistake, he noted, because the friend took a number of boys to the area, and, "The result was that the choice spot teaming with shells was ruthlessly plundered."

Kondo (1980) attributed extinctions to overcollecting; he reports for one locale, Kawaihalona Gulch, once rich in *Achatinella bulimoides*, "Collectors swarmed to Kawaihalona; *rosea* was soon gone forever." Massive collections were made by Gulick (Emerson, Ms., undated, recalls visiting Gulick's house as a child and seeing there, " . . . boxes and kegs of evidently unsorted shells."), Baldwin, Frick, Pease, Spalding, Meinecke (who collected more than 116,000; Kondo, Ms., 1980), and many others. How many populations might they have accounted for? Bryan (1935) wrote of "several collections made of upwards of 10,000 specimens each."

It must be remembered that the achatinelline snails are very slow-moving. They usually remain in the same tree for life, and named varieties were known from one small gulch, grove, or even a few trees. Predators as selective as human shell collectors could thus drastically affect such isolated populations with ease.

Probably the most serious modern predator of native Hawaiian snails is the introduced, North American gastropod *Euglandina rosea*. While it was introduced to Hawaii between 1955 and 1956 to prey on the African snail, *Achatina fulica*, it has become abundant in many areas far beyond the range of *A. fulica* (summarized by Mead, 1979). Correlated with the spread of *E. rosea* into the remaining habitats of *Achatinella* has been disappearance of the endemics (Mead, 1979; Van der Schalie, 1969). This problem is not unique to Hawaii. Clarke, Murray & Johnson (1984) have documented the disappearance of *Partula* on Moorea as populations of the introduced predator grow and expand there. *Euglandina rosea* was introduced to Moorea in 1977 where it spread rapidly to occupy nearly a third of the island by 1982. In that time, native populations of one *Partula* species had become extinct. The authors predict that by 1986 *E. rosea* will have spread throughout Moorea and eaten the remaining 7–9 partulid species endemic to that island.

The population of *Achatinella mustelina* that we had observed in the Waianae Range of Oahu for three years was found to have disappeared coincidently with the invasion of the area by large numbers of *E. rosea* (Hadfield & Mountain, 1981). Only dead shells of *A. vulpina* occurred in an area abundant in *E. rosea* in Halawa Valley, Oahu, in 1981 (unpublished, personal observations). It is, unfortunately, too late to learn if the dense populations of achatinellines originally present in Hawaii could have withstood the ravages of this snail; the marginal ones remaining obviously cannot.

Still, there seems to be another, yet unidentified, source of mortality in the Achatinellinae and possibly other terrestrial snails of Hawaii. In the Waianae Range of Oahu, wherever living populations of *Achatinella mustelina* persist, the ground is littered with dead shells. In May 1983, we scoured the ground in a 5 by 5 m quadrate containing about 50 small trees, bushes and shrubs with living *A. mustelina* on them. The result of this survey was the discovery of 161 dead shells, spanning the entire size range (Fig. 1) found elsewhere for this species (Hadfield & Mountain, 1981). Only 23 of the shells found were broken and thus, at least potentially, the prey of rats; the remainder were intact. Many of the large, lipped shells contained a small shell, that of the embryo they were brooding when they died. *Euglandina rosea* has not been seen in this area.

The number of living *A. mustelina* seen on the trees in the quadrate was 44. If our sighting efficiency was similar to that experienced elsewhere in these mountains (Hadfield & Mountain, 1981), this would represent a living resident population of 150 to 200 snails. The dead shells had obviously perished over some lengthy but unknown time period as their condition varied from fresh and glossy to thin, eroded and colorless.

There is no clear indication of what kills so many snails, but it is significant that the mortality is not size-dependent. In fact, the size-

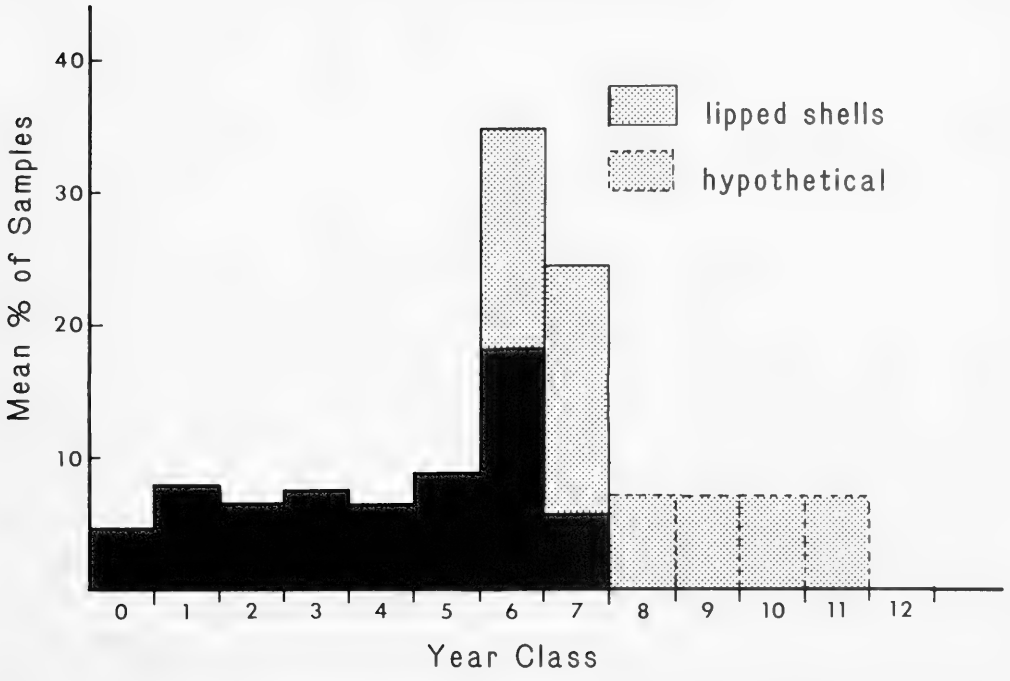


FIG. 2. *Achatinella mustelina*: age-frequency distribution. Size-frequency data from Hadfield & Mountain (1981), adjusted to reflect age-frequency. Solid black bars represent immature shells for which age estimates are considered good. Stippled bars with solid outlines represent lipped shells (i.e., those with maximum growth); the year classes at which they are located represent only the age at which maximum size was attained. "Hypothetical" bars are projected year classes. See text for explanation.

frequency distribution of the dead shells was similar to that of the live shells studied about 8 miles south of this location (see Hadfield & Mountain, 1981, and Fig. 2). Because it is not a characteristic of the study site for *Partulina redfieldii* on Molokai that dead shells are abundant on the ground, the observation may be taken as an indication that there is an additional mortality factor on Oahu. Still, some dead, but intact shells are present in the Kamakou meadow. In an effort to determine if the abundance of dead shells underlying the *Achatinella*-inhabited trees of Oahu was a recent occurrence, I talked with Dr. Yoshio Kondo, Malacologist Emeritus of the B. P. Bishop Museum, whose observations of Hawaiian terrestrial snails began in the 1930's. He informed me that this had, in his experience, always been so.

What kills so many snails so nonselectively? It could be *Geoplana septemlineata*, but this would require that relatively large numbers of snails are dislodged from the trees to the ground where the predators lurk. Furthermore, *Geoplana* has been found in the Molokai area. Predatory ants could be present, but I have not seen them in these areas. Introduced terrestrial snails, such as *Achatina fulica* and *Euglandina rosea* harbor dense populations of the nematode, *Angiostrongylus cantonensis* (see Mead, 1979), and this worm may have invaded the mountain trees. Yet *A. cantonensis* is generally dispersed through the food chain or infects snails through contact with rat feces, and it is difficult to envision its entry into the achatinellines (Alicata, 1965). Additionally, it has not been implicated in snail mortality.

Another possible explanation for the death of so many Achatinellas on Oahu is a microbial disease. Mead (1979: 85ff) discusses

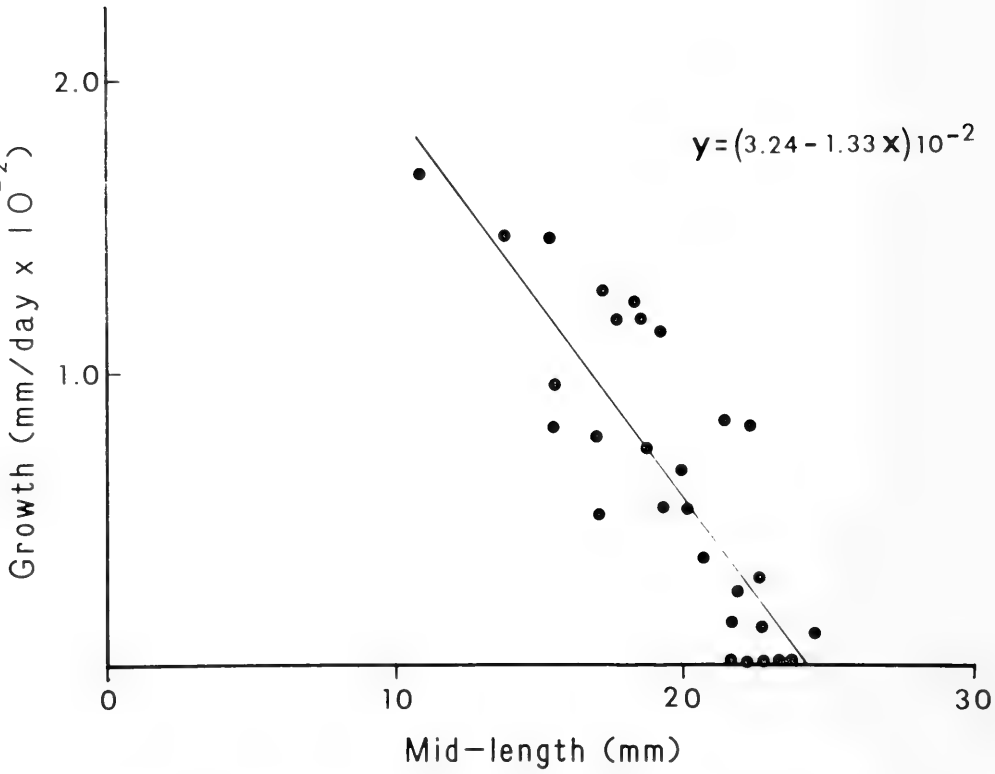


FIG. 3. *Partulina redfieldii*: growth rate vs. shell length. Shell length is plotted at the estimated mid-point of the mark-recapture interval for which the respective growth was recorded.

such a disease in Hawaiian populations of *Achatina fulica* and its spread to other exotic snails. These others (*Bradybaena similaris* and *Subulina octona*) have invaded the highest reaches of Oahu's mountains and could have transported such a disease. The current status of the remaining Achatinellas is too fragile to allow intense micropathological investigation of them.

4. What makes the achatinellines so vulnerable?

In order to understand the high rates of extinction in the achatinelline snails, as well as to determine means for circumventing further extinctions, life history characteristics of two species are being investigated through non-sacrificial field studies. Using mark-recapture techniques, we have estimated population densities, size-frequency distributions and size-related growth rates. From these data, age-frequency distributions, age at first reproduction, and lifespan have been extrapolated. Fecundity estimates are still very rough (Hadfield & Mountain, 1981) and may remain so. From published observations on numbers of embryos in dissected snails and our age-frequency distribution histograms, a very low fecundity—probably only one offspring per year—seems likely. Analysis of life-history traits for each of the species is detailed below.

Achatinella mustelina. Data for all life-history parameters utilized, except lifespan, have been published (Hadfield & Mountain, 1981). Lifespan has been estimated from size (age)-frequency distribution as follows. In Fig. 2, catch data for *A. mustelina* are plotted by year class. Animals that have not reached full shell growth, and thus whose size predicts age fairly accurately, are represented by black

bars. Because the snails form a thickened lip around the shell aperture and cease growing at or about the time of sexual maturity, those represented by solidly outlined, stippled bars are placed in the year class in which they stopped growing. They represent about 35% of all snails seen. The average size of the immature year classes is 7.3% of the total. If we assume very low mortality, the 35% of mature shells, divided by 7.3% per year class, would include nearly 5-year groups. Adding this on to the typical age at maturity, 6 years, we estimate a total number of year classes present at 11; the additional year classes are represented in Figure 2 by the "hypothetical" projection. This is admittedly a very coarse approximation, but is the only one currently available to us.

For *A. mustelina* we have estimated the following life table parameters: age at first reproduction, 6 years; longevity, 11 years; fecundity 1 (or up to 4) per year.

Partulina redfieldii: Growth rate was determined by regressing all mark-recapture obtained growth information against estimated lengths of shells at the mid-points of mark-recapture intervals (Fig. 3). The regression equation thus provides a measure of the change in growth rate with size. The regression data and size at birth (5.12 mm) were placed into the von Bertalanffy growth equation and solved for length at the end of each year to provide a year class-to-size-range table (Table 3). The size-frequency distribution for *Partulina redfieldii* in the Kamakou Preserve study area is shown in Figure 4, and the extrapolated age-frequency distribution in Figure 5.

TABLE 3. *Partulina redfieldii*: The distribution of sizes (= shell length) with age.

Age	Year class	Size range
0–1 yr	0	0–12.53 mm
1–2 yr	1	12.54–17.09 mm
2–3 yr	2	17.10–19.91 mm
3–4 yr	3	19.92–21.64 mm
4–5 yr	4	21.65–22.71 mm
5–6 yr	5	22.72–23.37 mm
> 6 yr	6+	> 23.38 mm

Data on the frequency of animals in immature year classes, where a steady mortality is indicated, were extrapolated as described above for *A. mustelina* to provide an estimate of the number of year classes in the mature snail population (dotted outlines in Fig. 5).

The summary of estimated life-history parameters for *P. redfieldii* thus includes: age at first reproduction, 4 years; longevity, 11 years; fecundity, 1 (estimated up to 4) per year.

The two achatinelline species studied both show the characteristics of late maturity and low fecundity, traits seemingly associated with evolution in a predator-free environment. Both traits may be related to the particularly large birth size in these snails. To understand the degree to which these life-history traits predispose such species to the threat of extinction with various sorts of new predators, *A. mustelina* and *P. redfieldii* were examined with reference to r_m, the malthusian parameter or intrinsic rate of population increase. The basic question is, to what level can annual survivorship fall—given the typical life-history characters described above—and the r_m value for the population remain positive?

For *A. mustelina*, with sexual maturity in the sixth year and annual fecundity of one until death after 11 years, survivorship must be greater than 0.825 annually for the population to remain stable or increasing (i.e., r_m 0). With fecundity estimated at 2 and 3 offspring per year, survivorship must still exceed 0.736 and 0.668, respectively, if the populations are not to decline.

Similar figures for *P. redfieldii*, with an earlier estimated age at reproductive maturity and thus, with a similar lifespan, a higher lifetime fecundity, are: for r_m to be greater than zero, with fecundities of 1 and 4, annual survivorships must exceed .734, and .573, respectively.

These requisite survivorship numbers are high. For small populations of *A. mustelina* such as those isolated in single trees, a mortality factor (predator, collector, disease, etc.) that removed 20%, that is just 6 of 30 snails present, for example, on a regular basis would cause the population to decline and presumably go extinct. Other manipulations of these data may approach a realistic situation. For instance, picture some relatively isolated and unique variety of *Achatinella* on Oahu about 1850 or 1860. Add a series of collectors who go back year after year and collect just half of all the mature shells present. For the population growth characteristic to remain positive, annual survivorship of all immature year classes must be at least 90%. If a size-

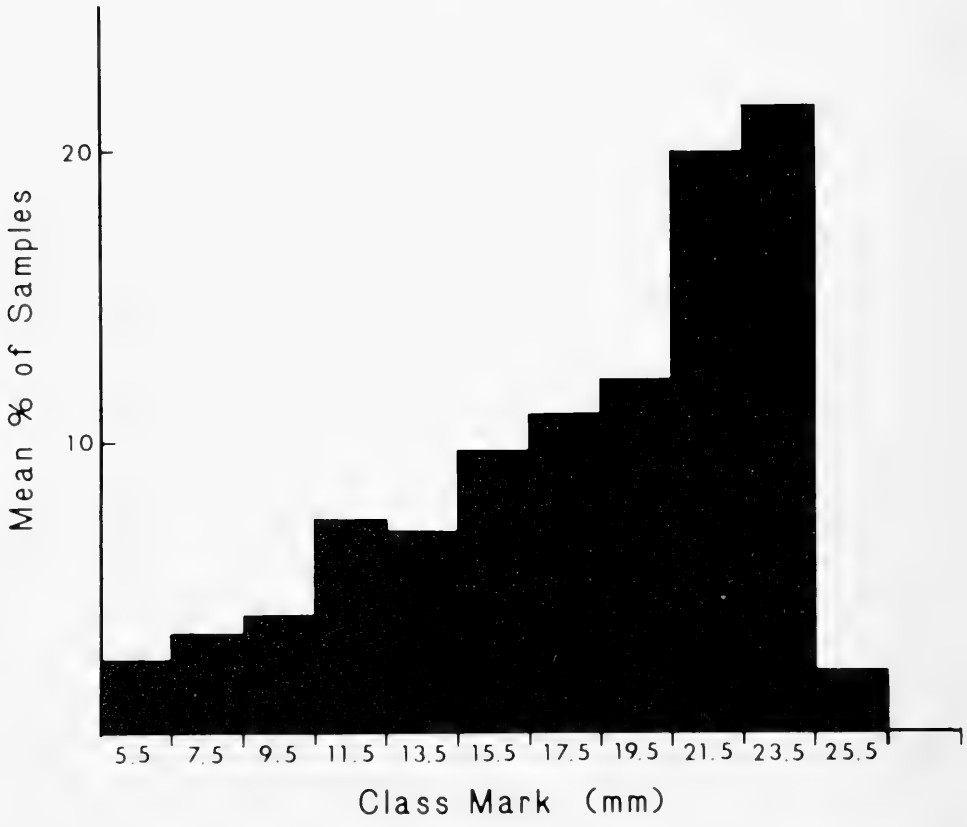


FIG. 4. *Partulina redfieldii*: size-frequency distribution. All animals measured on each of three occasions were sorted into 2 mm size classes, and, for each occasion, the percentage of snails represented by each class was determined. Presented here are the means of the percentage determinations for the three visits. Total snails seen was 100.

independent mortality factor is secondarily added, population extinction is almost guaranteed.

Did such repeated and selective collecting occur? The evidence is strong that it did. In addition to the references cited above relative to collections amassed by 19th century collectors, I sought indications of localized collecting pressures in the records of the B. P. Bishop Museum. Among many collections deposited there, I found one to be particularly useful due to the precision of its site records (that of I. Spalding). In the catalogue for this collection, I found that the collector returned each year from 1908 to 1916 to Wailupe Valley on southeastern Oahu. In each of these years, he collected from 30 to 413 specimens of *Achatinella fulgens* in that locale. Examination of the collection shows that these were mostly large, mature shells. The total taken in this period was 930. Additionally, at each visit to the valley the collector was accompanied by a shell-collecting friend whose take is not recorded. Similar records are frequent and seem significant in terms of the models presented above.

The calculations made here are based only on varying mortalities. They tend to show that *P. redfieldii*, due to its earlier reproductive onset is more robust than *A. mustelina*. This may be the reason for the survival of the small, isolated, one-tree populations of *P. redfieldii* which must have been in this state for a long time. There are, however, the indica-

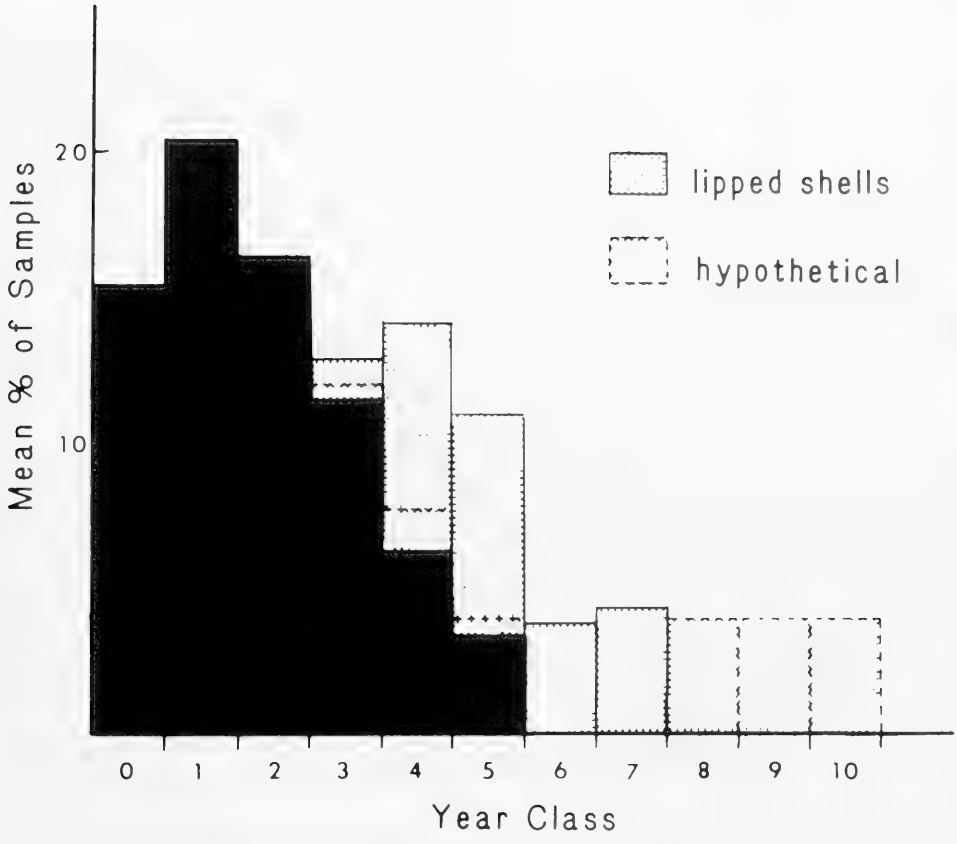


FIG. 5. *Partulina redfieldii*: age-frequency distribution of snails at the Molokai study site. The data in Fig. 4 were replotted in age classes predicted by the von Bertalanffy growth equation. Lipped shells are shown at the age at which terminal growth was reached. "Hypothetical" classes were roughly extrapolated from the shape of the distribution of the immature classes. The projected decline in the sizes of succeeding classes is shown by the horizontal dashed lines in year classes 3–5. There are no data to support a contention of zero mortality in adult snails, but the growth is so drawn to indicate maximum potential lifespan.

tions of lower unexplained mortality (i.e., whole, dead shells beneath the trees) in the *P. redfieldii* of Kamakou Preserve, Molokai.

None of these speculations include aspects of population density. To what low frequency can an achatinelline population fall before the probability of mating encounter falls too low for successful reproduction to occur? This is obviously an important question.

It is known that the destruction wrought on Hawaii's endemic achatinellines has also occurred in many other groups of native gastropods. Kondo (Ms., 1980) estimated at least 50% extinction for all of Hawaii's terrestrial snails. Most of Kauai's unique *Carelia* disappeared many generations ago; of about 200 species of endodontids formerly inhabiting the main islands, fewer than a dozen individuals have been seen in the last forty years. However, there are some groups of native and many exotic gastropods thriving in Hawaii. Minute tornatellinids were found covering all manner of weeds and crawling on the needles of *Casuarina* in the desolated hills above Kahuku, Oahu (personal observations, 1981). *Succinea* species are still abundant in

many Hawaiian mountain locales, and a few hardy *Auriculella* species persist in areas from which the achatinellines have disappeared. We know far too little about the life histories of these snails to single out the particular traits that favor survivorship. Certainly, any factor that increased fecundity would enhance the probability of population persistance. This may be the case with oviparous Auriculellas and other forms (Cooke & Kondo, 1960).

ACKNOWLEDGEMENTS

For encouraging and providing access and assistance to our field studies in the Kamakou Preserve of Molokai, I express my deepest thanks to Mr. Alan Holt and Mr. Ed Misaki of the Nature Conservancy. To the Nature Conservancy of Hawaii, I am especially indebted for being allowed to carry out the studies on Molokai.

Data collected in the Northern Waianae Range in 1982–1983 were obtained during survey work for the U.S. Army Corps of Engineers. I thank them for permission to utilize these data here.

Barbara Shank, Peter Galloway, Marilyn Dunlap, Douglas Stoner, Stephen Miller, Carl Christensen, Cynthia Hunter and Carol Hopper have all provided valuable field assistance.

Writing this paper would have been impossible without the support of: E. Alison Kay, who dug deeply into her historical files to aid me; Stephen Ralston, who advised me on data analysis and wrote the computer program for multiplying the Leslie Matrix; Yoshio Kondo, who gave me his time and with it his incredible store of knowledge of Hawaiian land snails; Carl Christensen, who provided access to the collections and records of the B. P. Bishop Museum and aimed me toward a number of references I might otherwise have missed; Stephen Miller, who helped digest the data and who ran the computer program over and over; and Susan Grau, who prepared the graphs for this paper. Comments and suggestions on the manuscript made by E. A. Kay, C. Christensen and A. Holt improved and clarified it. To all of the above, my utmost thanks.

REFERENCES CITED

ALEXANDER, M. C., 1934, *William Patterson Alexander in Kentucky, the Marquesas, and Hawaii*. Honolulu, privately printed, xvii + 516 p.

ALICATA, J. E., 1965, Biology and distribution of the rat lungworm, *Angiostrongylus cantonensis*, and its relation to eosinophilic meningitis and other neurological disorders of man and animals. In: DAWES, B., ed., *Advances in Parasitology*, Academic Press, New York, 3: 223–248.

ATKINSON, I. A. E., 1977, A reassessment of factors, particularly *Rattus rattus* L., that influenced the decline of endemic forest birds in the Hawaiian Islands. *Pacific Science*, 31: 109–133.

BALDWIN, D. D., 1887, The land shells of the Hawaiian Islands. *Hawaiian Annual*, 1887: 55–63.

BALDWIN, D. D., 1900, Land shell collecting on Oahu. *Hawaii's Young People*, 4(8): 239–243.

BEGON, M., 1979, *Investigating animal abundance: capture-recapture for biologists*. University Park Press, Baltimore, 97 p.

BRYAN, E. H., Jr., 1935, Hawaiian land shells. *Hawaiian Nature Notes* (Honolulu Star Bulletin, Ltd., Honolulu): 208–213.

CLARKE, B., MURRAY, J. & JOHNSON, M. S., 1984, The extinction of endemic species by a program of biological control. *Pacific Science*, 38: 97–104.

CLENCH, W. J., 1959, John T. Gulick's Hawaiian land shells. *Nautilus*, 72: 95–98.

COOKE, C. M., Jr., 1903. Distribution and variation of *Achatinella multizonata* from Nuuanu Valley. *Occasional Papers, Bernice Pauahi Bishop Museum*, 11: 65–76.

COOKE, C. M., Jr., 1941, Hawaiian land shells. *Paradise of the Pacific*, 53(12): 20–25.

COOKE, C. M., Jr. & KONDO, Y., 1960, Revision of Tornatellinidae and Achatinellidae (Gastropoda, Pulmonata). *Bernice Pauahi Bishop Museum Bulletin* 221: 303 p.

EMERSON, O. P. (Ms., undated, post-1900), The gay Achatinellidae and their habitats. (Bernice Pauahi Bishop Museum Library), 24 p.

FABENS, A. J., 1965, Properties and fitting of the von Bertalanffy growth curve. *Growth*, 28: 265–289.

FEDERAL REGISTER, 1981, Endangered and threatened wildlife and plants; listing the Hawaiian (Oahu) tree snails of the genus *Achatinella*, as endangered species. *F.R.*, 46(8): 3178–3181.

FRICK, D., 1856, Notes on Hawaiian terrestrial conchology. *Sandwich Islands Monthly Magazine*, 1: 137–140.

GULICK, A., 1932, *John T. Gulick evolutionist and missionary*. University of Chicago Press, Chicago, 556 p.

HADFIELD, M. G. & MOUNTAIN, B. S., 1981, A field study of a vanishing species, *Achatinella mustelina* (Gastropoda, Pulmonata), in the Wai-

anae Mountains of Oahu. *Pacific Science,* 34: 345–358.

HART, A., 1975, Living jewels imperiled. *Defenders*, 50(6): 482–486.

HART, A., 1978, The onslaught against Hawaii's tree snails. *Natural History*, 87(10): 46–57.

KIRCH, P. V., 1982, The impact of prehistoric Polynesians on the Hawaiian ecosystem. *Pacific Science*, 36: 1–14.

KONDO, Y. (Ms., 1970), *Colloquium on endangered species of Hawaii; extinct land molluscan species.* A report prepared for a meeting at the U.S. National Museum, 8 p.

KONDO, Y. (Ms., 1980), *Endangered land snails, Pacific.* A report prepared for the International Union for the Conservation of Nature and Natural Resources, 15 p.

MEAD, A., 1979, Economic malacology with particular reference to *Achatina fulica.* FRETTER, V. & PEAKE, J., eds., *Pulmonates*, 2B. Academic Press, London, 150 p.

MERTZ, D. B., 1970, Notes on methods used in life-history studies. In: CONNELL, J.H., MERTZ, D.B. & MURDOCK, W.W., eds., *Readings in ecology and ecological genetics*, Harper & Row, New York, p. 4–17.

PERKINS, R. C. L., 1899, Introduction In: SHARP, D., ed., *Fauna Hawaiiensis*, Cambridge University Press, Cambridge, I: xv-ccxxviii.

PILSBRY, H. A. & COOKE, C. M., 1912–1914. *Manual of conchology, structural and systematic*, ser. 2, vol. 22, Achatinellidae. *Academy of Natural Sciences of Philadelphia*, 428 p.

SCHALIE, H. VAN DER, 1969, Man meddles with nature—Hawaiian style. *The Biologist*, 51(4): 136–146.

SEARLE, S. R., 1966, *Matrix algebra for the biological sciences.* Wiley, New York, 296 p.

SOLEM, A., 1976, *Endodontoid land snails from the Pacific Islands.* Part I. *Family Endodontidae.* Field Museum Press, Chicago, 501 p.

WELCH, D'A., 1938, Distribution and variation of *Achatinella mustelina* Mighels in the Waianae Mountains, Oahu. *Bernice Pauahi Bishop Museum Bulletin*, 152: 164 p.

WELCH, D'A., 1942, Distribution and variation of the Hawaiian tree snail *Achatinella apexfulva* Dixon in the Koolau Range, Oahu. *Smithsonian Miscellaneous Collections,* 103(1): 1–236.

WELCH, D'A., 1954, Distribution and variation of the Hawaiian tree snail *Achatinella bulimoides* Swainson on the leeward and northern slopes of the Koolau Range, Oahu. *Proceedings of the Academy of Natural Sciences of Philadelphia*, 106: 63–107.

WELCH, D'A., 1958, Distribution and variation of the Hawaiian tree snail *Achatinella bulimoides* Swainson on the windward slope of the Koolau Range, Oahu. *Proceedings of the Academy of Natural Sciences of Philadelphia*, 110: 123–212.

MALACOLOGIA, 1986, 27(1): 83–96

ESTUDIO MORFOLOGICO DE LAS ESPICULAS DE *DORIOPSILLA AREOLATA* (GASTROPODA: NUDIBRANCHIA)

F.J. García, J.C. García & J.L. Cervera
Departamento de Zoología, Facultad de Biología, Universidad de Sevilla, Avenida Reina Mercedes s/n., Apartado 1.095, 41080 Sevilla, España

RESUMEN

La presencia de espículas en los Doridáceos constituye un importante mecanismo defensivo reforzado, frecuentemente, por la existencia de glándulas ácidas dispuestas por la superficie corporal. Se realiza un estudio detallado de la morfología de las espículas calcáreas de *Doriopsilla areolata* Bergh, así como de su disposición macroscópica en haces. Para ello, se ha considerado aisladamente varias regiones del cuerpo del animal, de las que se describe la morfología de los distintos tipos de espículas observadas en las mismas. Tales regiones son: región dorsal (de la que se describen también las espículas de las branquias y rinóforos), regiones laterales del cuerpo del animal y pie.

Palabras llaves: espículas; taxonomía; Gastropoda; Nudibranchia; *Doriopsilla*.

INTRODUCCION

La presencia de espículas calcáreas es frecuente en algunos grupos de opistobranquios (Acochlidiacea, Pleurobranchacea, Doridacea), una de cuyas funciones principales es probablemente la defensiva frente a posibles depredadores (Ros, 1976).

Numerosos son los trabajos que citan la presencia de espículas en las especies de opistobranquios descritas, pero pocos son los que lo hacen con cierta profundidad. Así, Alder & Hancock (1845–1855) describen la morfología general de las espículas de las especies por ellos tratadas y su localización en el cuerpo; además, realizan una discusión sobre la morfología de las espículas en Doridáceos, así como de su disposición corporal y de su formación y desarrollo. Vayssière (1901) describe también con detalle la morfología de las espículas y su disposición corporal.

Frecuentemente, la descripción de las espículas se hace refiriéndose al aspecto general que presentan, sin concretar la zona corporal a la que pertenecen, o si lo hacen es someramente (entre otros autores, Pruvot-Fol, 1953; Marcus & Marcus, 1967; Edmunds, 1968; Bouchet, 1977; Ballesteros & Ortea, 1980). En ocasiones se describe la disposición de las espículas al actuar como mecanismo protector de órganos sensoriales (Kress, 1981).

La abundancia de espículas que presenta la especie *Doriopsilla areolata* Bergh, así como su diversidad morfológica, comprobada en observaciones anteriores, nos hizo abordar el estudio morfológico de las mismas con el fin de contribuir, en primer lugar, a un mejor conocimiento de dicha especie, ya que sobre el aspecto tratado en este trabajo pocos son los datos que se han aportado hasta la fecha. En segundo lugar, el permitir realizar estudios encaminados a conocer con mayor precisión el papel que desempeñan en la defensa del animal. Y por último el presentar unos datos que permitan, al compararlos con los conseguidos en futuros trabajos dedicados también a las espículas de otras especies próximas desde el punto de vista sistemático a *D. areolata*, ver si puede considerarse la morfología y disposición de las espículas como criterio taxonómico.

MATERIAL Y METODO

Para la elaboración de este trabajo se han utilizado 3 ejemplares de *D. areolata* capturados en aguas atlánticas del Sur de España (El Portil, Huelva: 37° 12′ 40″ N, 7° 7′ 50″ W) en la zona mediolitoral y primeros niveles infralitorales. El tamaño de los ejemplares conservados era de 1.7–2.5 cm de longitud. La disposición de los haces de espículas se observó al microscopio esteroscópico, para lo

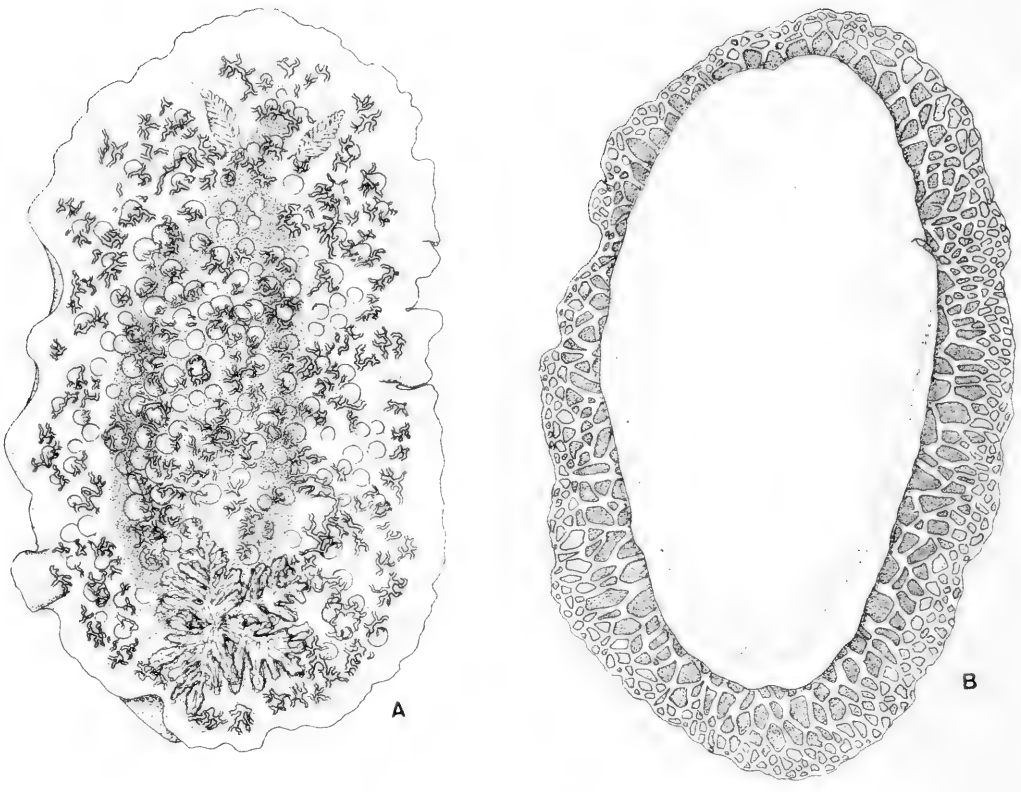


FIG. 1. Vista dorsal (A) y ventral (B) de un ejemplar de *Doriopsilla areolata*.

cual se retiró la capa externa del tegumento del animal para observar así, las espículas y su disposición con mayor claridad y detalle. Las espículas esferoidales se encontraban a "flor" de la superficie debido posiblemente al tiempo (más de dos años) que llevaban los ejemplares incluidos en los líquidos conservantes (glutaraldehido y tampón cacodilato), mientras que en ejemplares de capture e inclusión más reciente estas espículas aparecían cubiertas por el tegumento.

DISPOSICION GENERAL DE LAS ESPICULAS

El animal presenta por todo el cuerpo estructuras calcáreas dispuestas a modo de esqueleto, como se muestra en el corte transversal de un ejemplar, representado en la figura 2A. Mediante una observación más detallada se puede comprobar que las espículas se encuentran agrupadas de manera característica según las distintas regiones corporales.

En la pared dorsal del cuerpo aparecen reunidas en haces dispuestos paralelamente a la superficie (Fig. 2D). El conjunto de haces presenta un aspecto de red de luz romboidal, que deja entrever en dicha luz, algunas espículas que la atraviesan de manera aislada. Desde los nudos de dicha red y dispuestos perpendicularmente a la superficie corporal, se alzan haces de espículas que van a formar el esqueleto de los tubérculos dorsales del animal, repartidos por toda la superficie dorsal (Fig. 1A; Fig. 2B-D). En sus extremos se disponen espículas a modo de penacho, que pueden observarse por transparencia en el dorso del animal (Fig. 2B, C). Dispuestas por todo el tegumento y más superficialmente

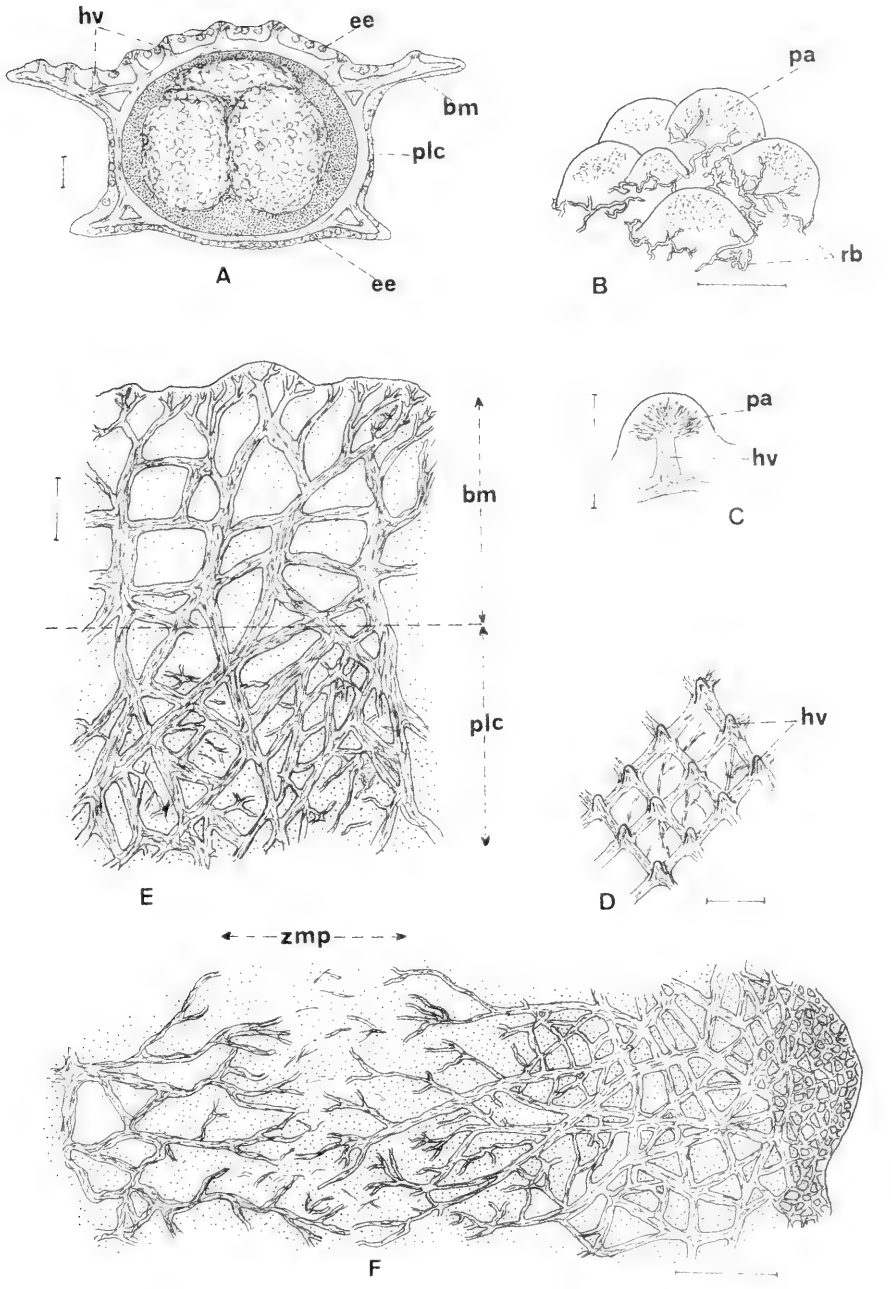


FIG. 2. A, corte transversal del cuerpo de un animal. B, detalle de los tubérculos dorsales del manto. C, corte longitudinal de un tubérculo dorsal del manto. D, detalle de la disposición de los haces de espículas en el dorso del animal (no se representan los penachos apicales de espículas). E, detalle de la disposición de las espículas en el borde del manto y pared lateral del cuerpo. F, disposición de los haces de espículas del pie; la disposición de las espículas en el lado izquierdo de la figura sólo se representa parcialmente. Las escalas indican 1 mm. bm, borde del manto; ee, espículas esferoidales; hv, haces verticales; pa, penacho apical; plc, pared lateral del cuerpo; rb, retículo blanquecino externo; zmp, zona media del pie.

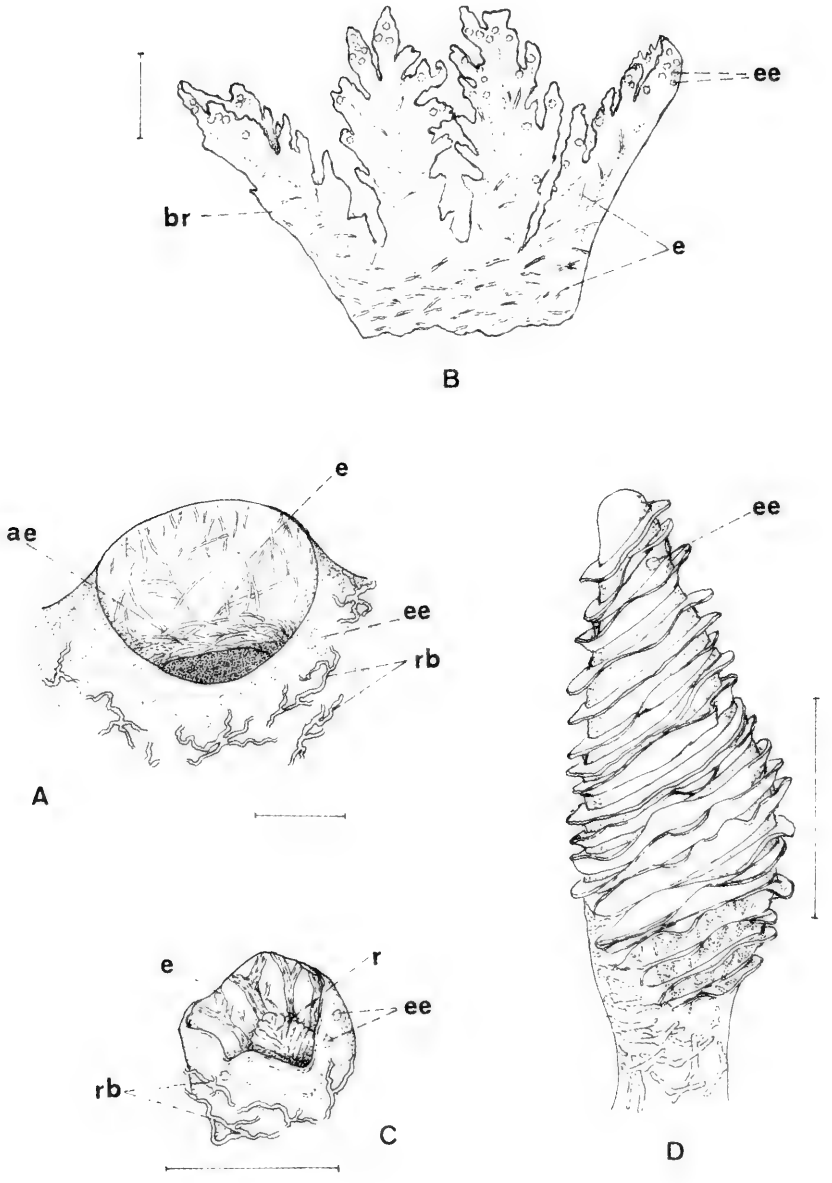


FIG. 3. A, disposición de las espículas del tegumento que bordea el orificio branquial; pueden observarse acúmulos de espículas dispuestas horizontalmente (para ello, las branquias no se ilustran). B, disposición de las espículas en las branquias. C, vaina rinofórica (el rinóforo se encuentra retraído). D, rinóforo. Las escalas indican 1 mm. ae, acúmulo basal de espículas; br, branquias; e, espículas; ee, espículas esferoidales; r, rinóforo; rb, retículo blanquecino externo.

que las anteriores, hay espículas esferoidales, que pueden estar de forma aislada o bien fusionadas entre sí formando grupos de número variable de ellas.

El tegumento que bordea el orificio por donde emergen las branquias, presenta espículas esferoidales iguales a las del resto del dorso. Más internamente, se distinguen espículas agrupadas en haces laxos dispuestos principalmente en posición vertical, junto con otros intercalados oblicuamente. La zona más inferior de esta porción tegumentaria está rodeada por espículas dispuestas horizontalmente a modo de anillo (Fig. 3A).

En la base de los penachos branquiales, las espículas de morfología alargada se encuentran dispuestas horizontalmente o ligeramente oblicuas. En los penachos propiamente dichos, estas espículas se hacen menos numerosas a la vez que se observan con mayor frecuencia las espículas esferoidales, las cuales son más numerosas en los extremos de aquéllos (Fig. 3B).

En la vaina rinofórica (Fig. 3C), las espículas están en haces dispuestos verticalmente, los cuales se ramifican al llegar a la zona más externa de dicha vaina. Hay espículas esferoidales aisladas, dispuestas por toda la superficie.

En los rinóforos (Fig. 3D), las espículas se encuentran en el tercio basal tanto en el tronco central como en las laminillas, en las cuales se disponen perpendicularmente al eje del rinóforo.

En el borde del manto, los haces abandonan la disposición que presentan en la zona central del dorso, para formar una red de organización menos geométrica (Fig. 1B; Fig. 2E). Los haces devienen más estrechos a medida que se aproximan a los bordes exteriores del manto a la vez que se ramifican más. Los haces verticales desaparecen también al aproximarse a los extremos del manto. Las espículas esféricas se hacen menos numerosas en la superficie inferior del borde del manto, así como en los lados del cuerpo del animal.

Los haces de espículas del borde del manto, al pasar a la zona lateral del cuerpo, se hacen menos densos, quedan las espículas dispuestas de una manera menos apretada y se observa mayor cantidad de espículas aisladas o agrupadas en haces de escaso número (Fig. 2E).

El pie, ventralmente, tiene una capa de espículas esferoidales agrupadas entre sí muy densamente (Fig. 2A). Más internamente, respecto a la capa anterior, están los haces de espículas alargadas, dispuestos transversalmente al eje longitudinal del animal. Estos haces son más estrechos que los indicados para las regiones dorsal y lateral del cuerpo. Se aprecia también gran cantidad de espículas aisladas, sobre todo en la zona media del pie (Fig. 2F). En la zona próxima a los bordes del pie, los haces se hacen más finos a la vez que aumentan sus ramificaciones.

DESCRIPCION MORFOLOGICA DE LAS ESPICULAS

1. Región dorsal

Repartidas por toda la superficie dorsal se aprecian espículas esferoidales (Fig. 4A–C), de superficie rugosa o espinosa, que pueden encontrarse aisladas o bien fusionadas entre sí. Estas estructuras presentan tamaños que oscilan, en los ejemplares observados, entre 75–90 μm en las encontradas por las zonas centrales del dorso del animal y entre 40–115 μm en las del borde del manto. Por debajo de estas espículas se disponen los haces de espículas descritos en el apartado anterior. Al observarlos al microscopio óptico se aprecia que la morfología de las espículas presenta algunas características según la región a la que pertenezcan.

En la zona central, las espículas son lineales, curvadas ligeramente por su centro, en cuyo caso pueden presentar una protuberancia en el área de curvatura (Fig. 4D-G); o bien estar curvadas de manera irregular (Fig. 4H-K). Por transparencia se observan líneas curvas concéntricas en los extremos, que se continúan siguiendo los ejes de las espículas, semejantes a las líneas de crecimiento de las escamas de los peces. Estas líneas curvas son menos perceptibles en las espículas con curvatura irregular y sobre todo en las de mayor tamaño. En los casos que presentan la protuberancia en la zona de curvatura, las prolongaciones longitudinales se dirigen a dicha protuberancia (Fig. 4G).

Ocasionalmente, se ven espículas ligeramente curvadas y con varios abultamientos repartidos por su superficie (Fig. 4L).

Los tamaños observados son muy variables. Así, entre las del primer tipo, los tamaños oscilan entre 200–640 μm y en las de curvatura irregular entre 115–385 μm.

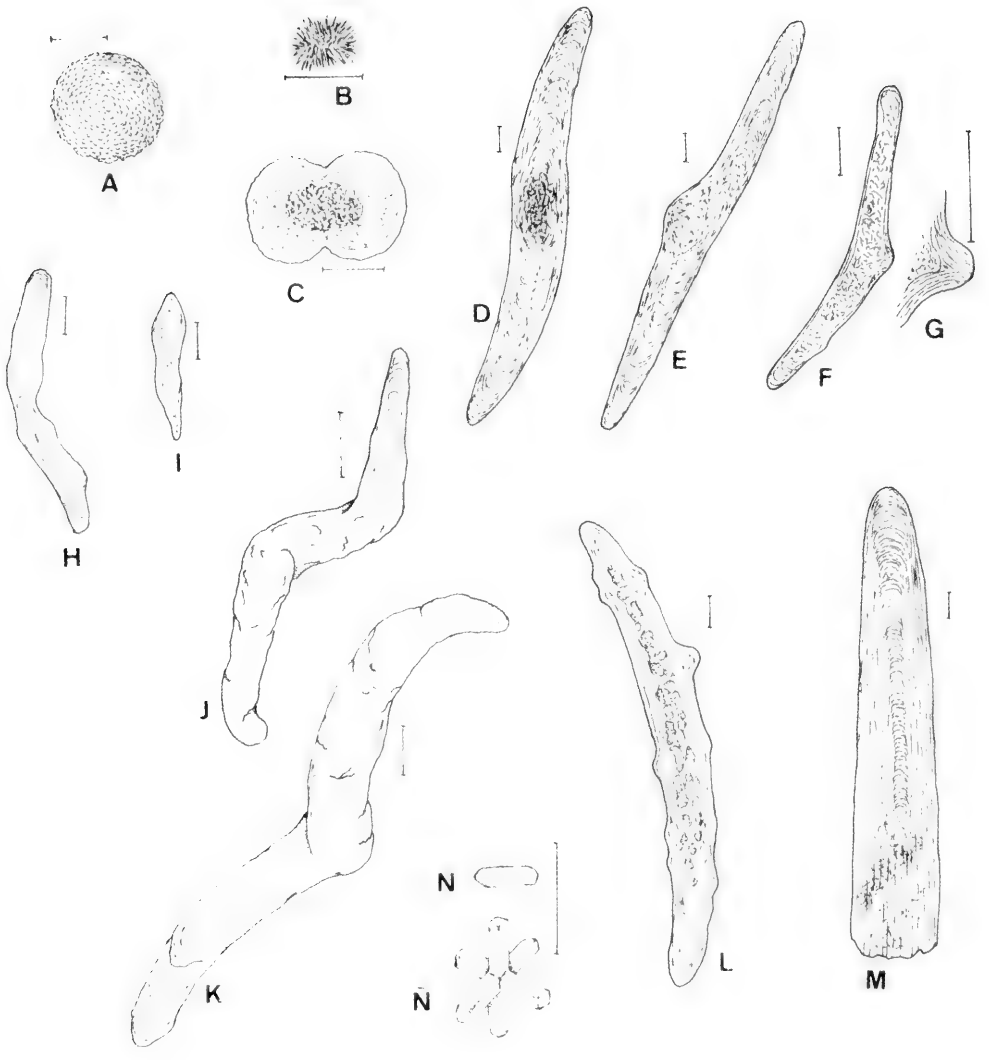


FIG. 4. Espículas de la zona central del dorso. Las escalas indican 30 μm, salvo en N y Ñ que indican 10 μm

Las espículas dispuestas verticalmente y dirigidas al interior de los tubérculos dorsales del animal son fusiformes, con uno de los extremos truncados y el otro redondeado (Fig. 4M). Las líneas concéntricas son también visibles por transparencia en el extremo redondeado, y continúan más o menos hacia el otro extremo. El tamaño de estas espículas varía considerablemente según el tamaño del tubérculo dorsal, e incluso dentro de un mismo tubérculo. Miden desde 57 μm hasta 580 μm.

En el borde del manto, las espículas alcanzan los tamaños mayores de todo el cuerpo (alcanzan 730 μm de longitud). La morfología es muy variable. Se distinguen: espículas fusiformes o ligeramente curvadas en el centro, con esta zona algo más ensanchada que el resto de la espícula (Fig. 5A); otras tienen forma de L con rugosidades por

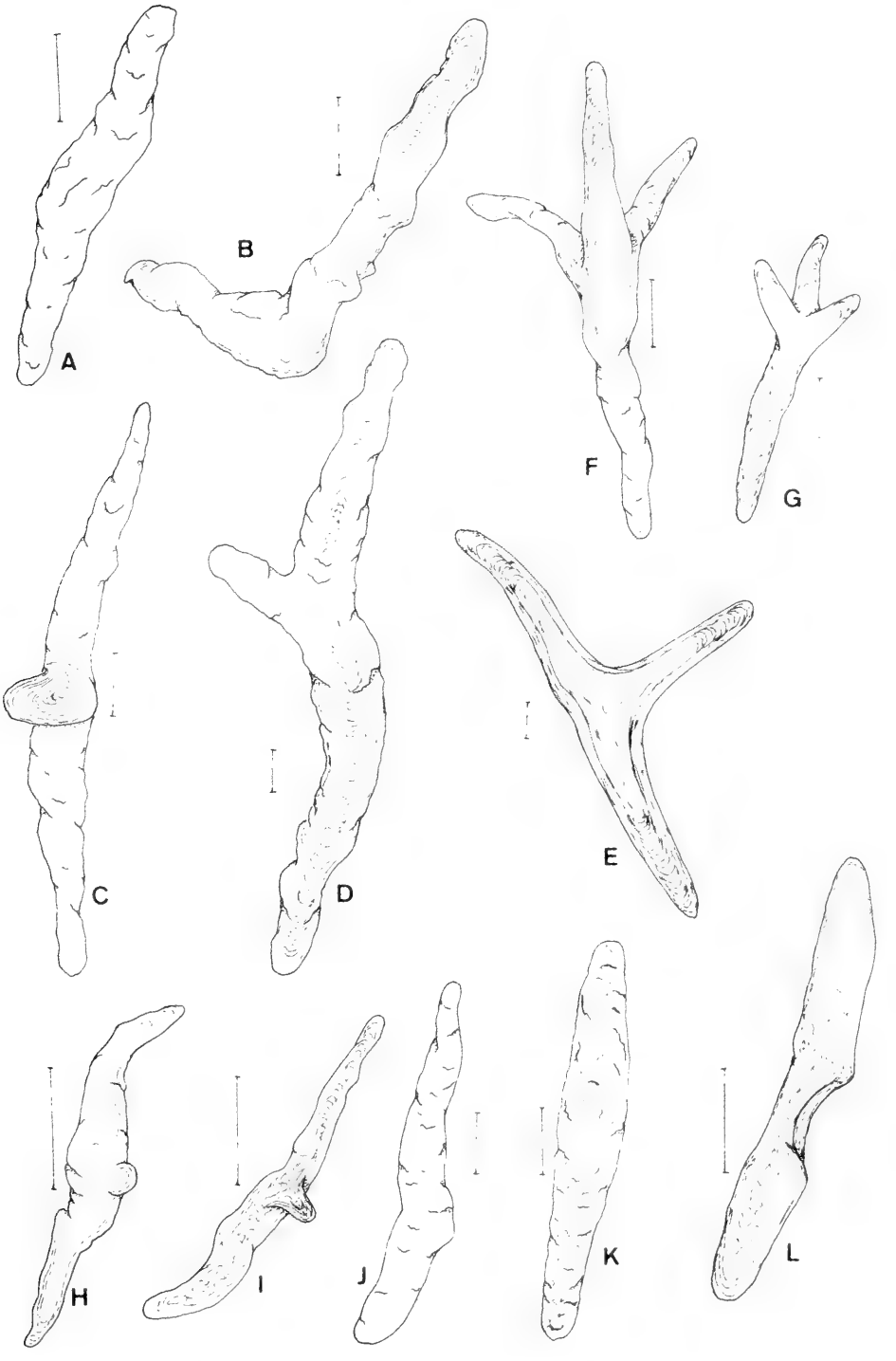


FIG. 5. A–G, espículas del borde del manto; H–L, espículas del tegumento que bordea el orificio branquial. Las escalas indican 50 μm.

su superficie (Fig. 5B); fusiformes con una protuberancia semejante a la descrita para la región central del dorso o a modo de espolón (Fig. 5C), que puede prolongarse para dar a la espícula un aspecto trirradiado (Fig. 5D, E); espículas tetrarradiadas, con un brazo más largo que los otros tres (Fig. 5F, G).

Se observan también espículas por toda la región dorsal, pero más frecuentemente en los bordes del manto, con aspecto de prisma rectangular, que pueden estar aisladas o bien agrupadas en forma de cruz o de hexactina (por analogía con las espículas de dicho nombre de las esponjas), con tamaños entre 10–35 μm (Fig. 4N, Ñ).

En el tegumento que bordea el orificio por donde emergen las branquias, las espículas, con frecuencia, presentan en el centro aproximadamente una protuberancia, uno de los extremos de la espícula suele estar curvado mientras que el otro se mantiene recto. Las líneas concéntricas se observan en los extremos y pueden llegar más o menos cerca de la zona central de la espícula. El tamaño de estas espículas es de 115–175 μm; se ha visto una espícula perteneciente a este tipo cuyo tamaño era de 307 μm (Fig. 5H-J). Espículas fusiformes con los extremos redondeados. Dentro de este modelo se distinguen tres tipos según el tamaño, oscilando éstos para cada grupo entre 20–30 μm, 75–95 μm y de 305–345 μm. Las líneas concéntricas no se observan en las mayores, mientras que en las pequeñas sólo se ven en los extremos (Fig. 5K).

Espículas fusiformes, o algo curvadas, que presentan en la zona central una depresión. El tamaño observado para este tipo alcanza aproximadamente 230 μm (Fig. 5L).

En las branquias el tamaño de las espículas esferoidales oscila aproximadamente entre 45–57 μm. Entre las espículas de morfología alargada se pueden distinguir varios tipos:

- Espículas con tamaño pequeño (10–15 μm), fusiformes.
- Espículas considerablemente mayores, entre las que se puede distinguir varios tipos: fusiformes con la zona central algo más ensanchada que el resto; esta zona queda además diferenciada de los extremos al presentar un estrechamiento a cada lado; las líneas curvas concéntricas se observan por las dos áreas laterales de la espícula y en el área central sólo se ven las líneas longitudinales que continúan desde las líneas curvas anteriores. El tamaño de la espícula alcanza aproximadamente 285 μm (Fig. 6A).

Espículas curvadas aproximadamente por el centro, con la superficie rugosa y las líneas curvas concéntricas visibles solamente en los extremos. La zona central, en algunos casos, está más ensanchada. Las espículas observadas que encajan en este tipo presentan tamaños entre 260–392 μm de longitud (Fig. 6B, C).

Espículas curvadas aproximadamente a dos tercios de distancia de uno de los extremos. De los dos brazos así formados, el más largo se mantiene recto mientras que el otro suele estar curvado. En la zona de curvatura hay una prominencia que sobresale más o menos. Las líneas curvas concéntricas sólo se observan en los extremos, el resto queda más o menos opaco según el tamaño de la espícula; en las de menor tamaño se ven mejor dichas líneas concéntricas y sus prolongaciones longitudinales. El tamaño de este tipo de espículas varía entre 150–270 μm de longitud (Fig. 6D-F).

Espículas fusiformes o curvadas por el centro; la zona central está dilatada con respecto al resto de la espícula y a veces sobresale de dicha zona una prominencia a cada lado. La superficie de la espícula es rugosa y las líneas curvas no son visibles. Los tamaños observados oscilan entre 120 y 190 μm (Fig. 6G, H).

En los rinóforos, en función del tamaño de las espículas encontradas, éstas se pueden reunir en dos grupos, espículas de tamaño menor de 25 μm y espículas mayores de 45 μm. Entre las primeras se distinguen varios tipos: pequeñas estructuras calcáreas esferoidales aisladas y a veces agrupadas de dos en dos, con tamaños aproximados entre 3 y 13 μm; espículas fusiformes de 7–11 μm de longitud, dispuestas de manera aislada o agrupadas en forma de estrella de seis puntas que llegan a alcanzar 23 μm (Fig. 6I), o en forma de cruz con tamaños entre 5 y 10 μm; espículas fusiformes (5–10 μm de longitud) con tubérculos dispuestos en su superficie (Fig. 6J).

Entre las espículas pertenecientes al segundo grupo se distinguen los modelos siguientes: espículas esferoidales, sobre todo en la zona próxima al vértice, de superficie rugosa, dispuestas aisladamente. Los tamaños observados en este tipo de espículas oscilan entre 45 y 75 μm (Fig. 6K); espículas con una curvatura aproximadamente

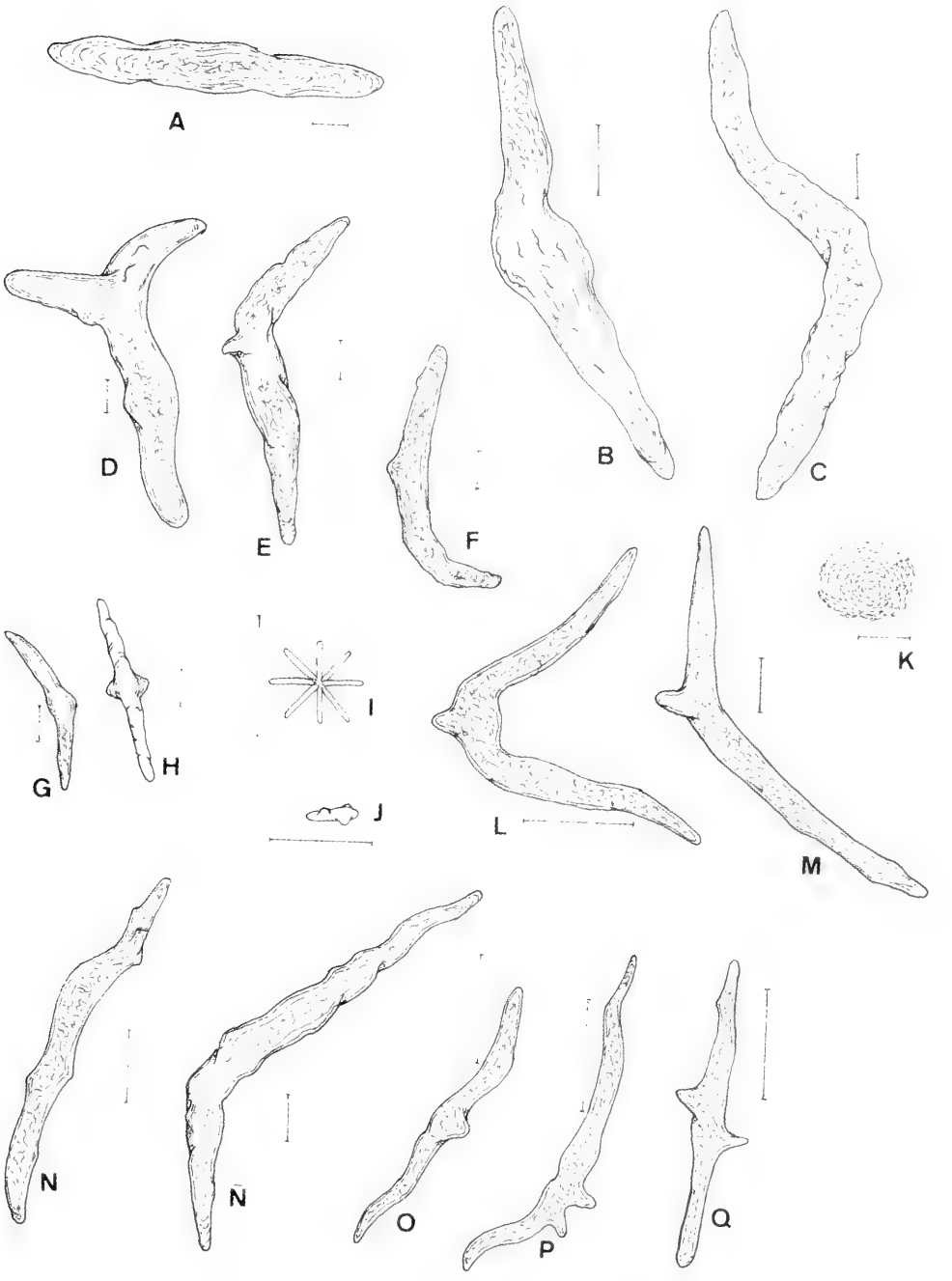


FIG. 6. A–H, espículas de las branquias; I–Q, espículas de los rinóforos. Las escalas indican 30 μm.

en la zona central, forma entre las dos semiespículas ángulos muy variables, en la zona de curvatura suele aparecer una prominencia (Fig. 6L, M); en ocasiones no aparece la prominencia y la zona de curvatura está desplazada hacia uno de los extremos, la

superficie es rugosa y las líneas concéntricas se observan sólo en los extremos de la espícula (Fig. 6N, Ñ). El tamaño de las espículas de estos últimos tipos oscila entre 75 y 305 μm.

Espículas con una prominencia central. A cada lado de dicha prominencia la espícula presenta una curvatura. El tamaño que alcanzan es de aproximadamente 75 μm (Fig. 6O). Presentan a veces dos prominencias dispuestas en el mismo lado de la espícula (Fig. 6P), u opuestamente (Fig. 6Q).

2. Región lateral

Al igual que en otras partes anteriormente citadas, se distinguen dos grupos de espículas atendiendo al tamaño.

Las de tamaño reducido son ovaladas, a veces con una depresión en cada extremo (Fig. 7A, B); o bien tienen los extremos agudos en cuyo caso la espícula es más estrecha que las anteriores. Los tamaños que alcanzan oscilan entre 7–19 μm y 7–11 μm, respectivamente. A veces se observan estas espículas agrupadas en forma de estrella de hasta doce puntas. Estas agrupaciones alcanzan tamaños entre 20 y 40 μm.

Entre las del segundo grupo (mayores de 20 μm) se distinguen los siguientes tipos:

Espículas esferoidales, en menor número que en otras zonas del cuerpo del animal, tienen la superficie rugosa. Los tamaños observados oscilan entre 20 y 35 μm (Fig. 7G). Espículas curvadas desde el centro, el cual suele ser algo más ancho que el resto de la espícula, mostrando además una prominencia por el lado convexo; próximos a los extremos suele haber otras protuberancias de menor tamaño que la central. Las líneas curvas concéntricas son visibles en los extremos. Los tamaños medidos de este tipo de espículas oscilan entre 92 y 153 μm (Fig. 7C, D).

Espículas semejantes al tipo anterior pero con los extremos que, bien vuelven a curvarse en sentido opuesto al seguido en el inicio de la curvatura, o bien no llegan a curvarse en sentido opuesto, pero sí lo hacen lo suficiente como para dar a la espícula un aspecto fusiforme con una curvatura central (Fig. 7E, F). En ninguna espícula de este tipo se han visto marcas concéntricas. El tamaño observado se encuentra entre 88 y 115 μm para las primeras, y entre 134 y 173 μm para las segundas.

Espículas algo curvadas, con los extremos redondeados y de superficie bastante lisa, aunque a veces se observan pequeñas prominencias; las líneas concéntricas y sus prolongaciones longitudinales son visibles. El tamaño oscila entre 175 y 253 μm (Fig. 7H).

Espículas semejantes al tipo anterior pero de superficie rugosa y curvaturas más irregulares. Según el tamaño se pueden reunir en dos grupos, 75–115 μm y 270–345 μm (Fig. 7I).

Espículas fusiformes con una prominencia central. No se ven líneas concéntricas a pesar de su transparencia, el tamaño aproximado es de 90 μm (Fig. 7J).

3. Pie

Por toda la superficie ventral del pie se encuentran dispuestas, muy densamente, espículas esferoidales de superficie espinosa, que pueden estar independientes o fusionadas unas con otras (Fig. 7K, L). Los tamaños observados varían entre 20 y 45 μm, aunque algunas llegan a las 75 μm.

Espículas de aspecto fusiforme con los extremos agudos (entre 15 y 20 μm), suelen estar aisladas o bien agrupadas a modo de estrella (Fig. 7M); en ocasiones estas espículas se encuentran atravesadas por pequeñas estructuras alargadas (Fig. 7N).

Espículas grandes (entre 345–460 μm), fusiformes, con los extremos redondeados; superficie rugosa y opaca (Fig. 7Ñ). A veces se observa en uno de los lados una depresión de la espícula; por los bordes de dicha depresión se observan las líneas curvas concéntricas (Fig. 7O). El tamaño de las espículas de este tipo varía entre 155–460 μm de longitud. Las mayores se encuentran en la zona central del pie; en los bordes de éste, las mayores observadas solo alcanzan 270 μm y tienen los dos extremos de diferente grosor (Fig. 7P).

Espículas con una curvatura en la zona central, se observan a veces, por dicha zona, líneas longitudinales paralelas a los bordes de la espícula. Los tamaños observados oscilan entre 125–385 μm de longitud (Fig. 7Q). En ocasiones se ven espículas semejantes a las anteriores pero de menor diámetro; estas espículas a veces están curvadas hasta formar aproximadamente un ángulo recto (Fig. 7R), e incluso se observan casos en los que se producen dos curvaturas a modo de zigzag (Fig. 7S), que da a la espícula un aspecto

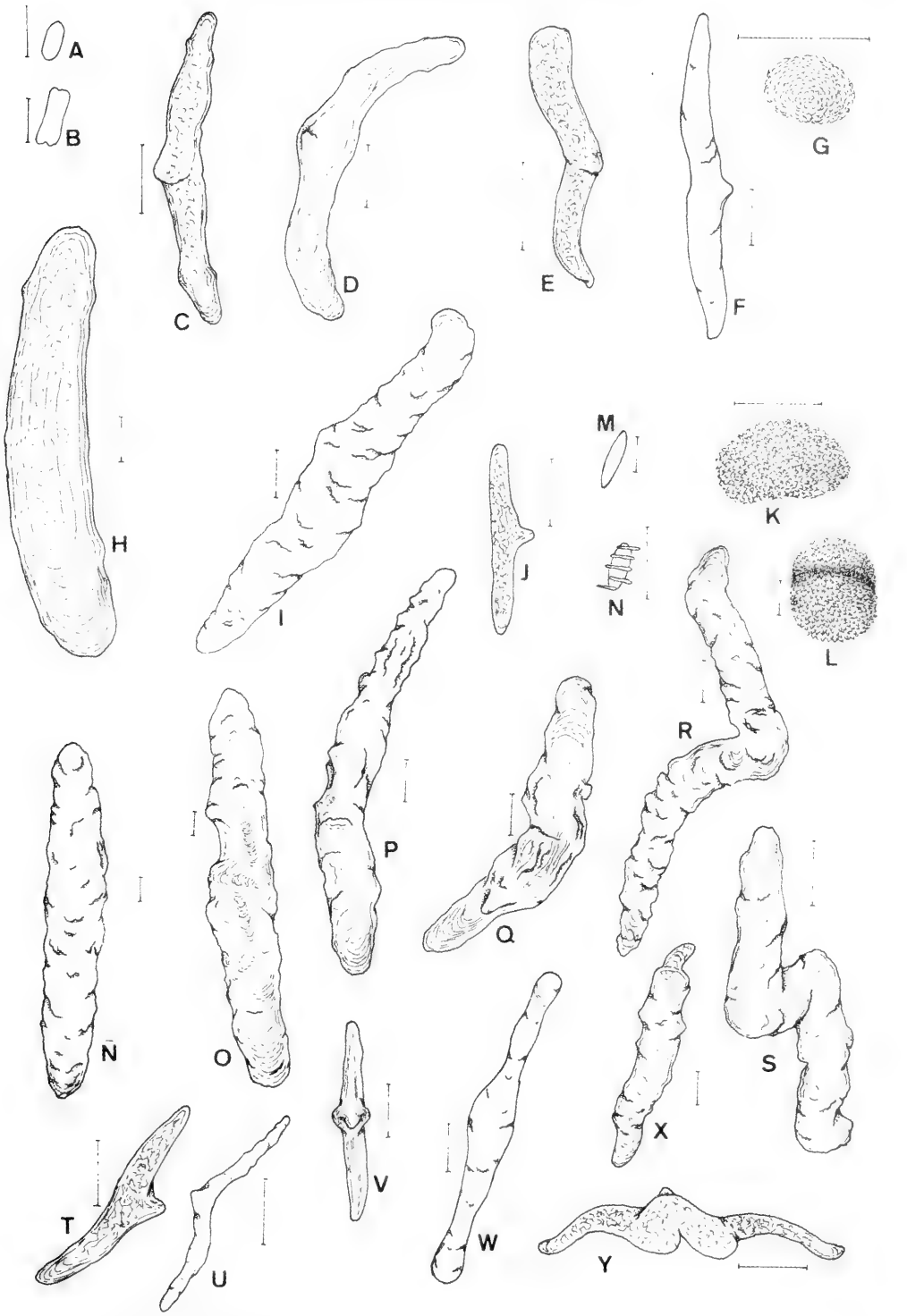


FIG. 7. A–J, espículas de la pared lateral del cuerpo; K–Y, espículas del pie. Las escalas indican 30 μm, salvo en A, B, M, N que indican 10 μm.

de S. Estos tipos de espículas alcanzan tamaños comprendidos entre 115–350 μm.

Espículas con una ligera curvatura central; en dicha zona presentan una prominencia más o menos manifiesta (Fig. 7T, U). Las espículas medidas alcanzan tamaños que oscilan entre 85–210 μm de longitud.

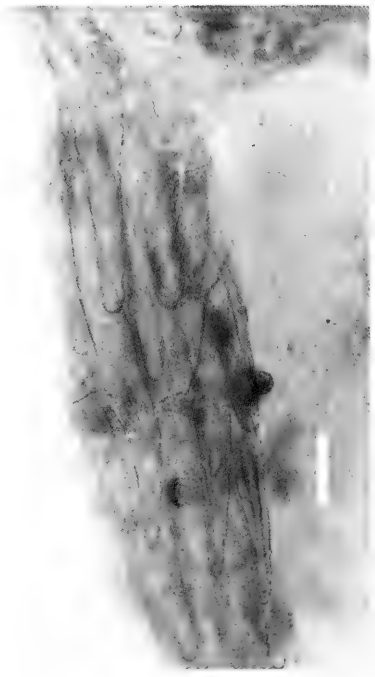
A veces se observan dos e incluso tres prominencias. En estos casos la espícula está menos curvada (Fig. 7V). El tamaño varía entre 90–200 μm, excepcionalmente llegan a 345 μm.

Esporádicamente se observan espículas con morfología que no encajan en los tipos anteriormente descritos, como son los representados en la figura 7Y–W.

DISCUSION

La existencia de espículas muy aparentes que se disponen irregularmente por todo el manto es señalada por Ros (1975) en *Doriopsilla pusilla*, lo cual hacen extensivo Ballesteros & Ortea (1980), a todas las *Doriopsilla*. Sin embargo y en base a nuestras observaciones, en *D. areolata* la disposición de las espi-´culas varía, según la región corporal considerada y dicha disposición se mantiene de un ejemplar a otro. Estudios futuros sobre la morfología de las espículas en otras especies de *Doriopsilla* podrán aclarar si existen o no importantes diferencias que permitan separar tales especies en base a la morfología de las espículas y por tanto, conocer si este carácter tiene una utilidad taxonómica.

Aunque en cada región corporal las espículas presentan peculiaridades, todos los tipos encontrados en el cuerpo del animal pueden incluirse en varios grupos en función del tamaño y de la morfología de las espículas. Se distinguen así: espículas de aspecto fusiforme y tamaños no superiores a 25 μm de longitud; espículas esferoidales, cuyos tamaños oscilan entre 7–90 μm; y espículas de morfología diversa, no esferoidal y



FOT. 2. Detalle de un haz de espículas del borde del manto. Se puede apreciar la disposición apretada de las espículas que lo integran. La escala corresponde a 100 μm.

FOT. 1. Detalle de las ramificaciónes de los haces de espículas del borde del manto. La escala corresponde a 200 μm.

tamaños mayores de 50 μm de longitud. Dentro de este tercer grupo, las espículas observadas se pueden considerar, de acuerdo a su morfología, en los cuatro tipos siguientes: 1) espículas fusiformes, sin prominencias muy sobresalientes; pueden ser algo curvadas y de superficie más o menos granulosa, pero se manifiesta una tendencia a adoptar un aspecto fusiforme; 2) espículas curvadas en mayor o menor grado y con una prominencia sobresaliente, la cual deviene como un simple mamelón o bien como una rama perfectamente diferenciada; 3) espículas con una o varias curvaturas muy pronunciadas con aspecto de L o S, sin presentar prominencias muy sobresalientes; 4) espículas tetrarradiadas; suelen tener uno de los radios más largo que el resto, el cual a veces constituye la prolongación de otro de ellos, al configurarse ambos en un mismo eje.

Las espículas fusiformes dispuestas en los tubérculos dorsales, debido al aspecto que presentan sus dos extremos (uno redondeado y el otro truncado y de superficie rugosa), así como el que las líneas curvas concéntricas sólo se encuentren dirigidas hacia el extremo redondeado, hace pensar que estas espículas no sean sino espículas rotas. No obstante no hemos observado ninguna espícula, en los tubérculos dorsales, que tengan los dos extremos semejantes y las líneas curvas concéntricas dirigidas a cada uno de ellos. Se podría considerar que mientras las demás espículas del cuerpo del animal presentan, en su formación, más de un sentido de crecimiento (lo que quedaría manifestado mediante las líneas concéntricas), en el caso de las espículas fusiformes de los tubérculos dorsales, sólo tuviese un sentido de crecimiento que sería el que indican las líneas concéntricas.

Pruvot-Fol (1952) hace referencia a las espículas de *D. areolata* ilustradas por Vayssière (1901, pl. 3, fig. 20), y al respecto señala: "Ils ont été représentés par Vayssière avec leur surface rendue rugueuse par de nombreux nodules ou tubercules, et partiellement avec des branches latérales. J'ai trouvé ces spicules de taille extrêmment différentes, mais toujours simples avec de rares nodules. . . ." Las espículas observadas por nosotros generalmente tienen la superficie menos rugosa que las representadas por Vayssière aunque la presencia de ramas laterales que indica este autor sí aparecen con frecuencia en nuestras observaciones. El hecho de que Pruvot-Fol encontrase las espículas simples y raramente con nódulos puede ser explicado si las observaciones que hubiese realizado se centrasen a la región dorsal del cuerpo del animal, ya que como se ilustra en la figura 4, es ésta la zona del cuerpo en la que gran parte de las espículas son simples o con nódulos, pero carecen de ramas laterales.

Pruvot-Fol (1952) ilustra el extremo de una espícula (p. 410, fig. 9), en el que aparece una serie de líneas curvas con el lado convexo dirigido hacia el extremo de la espícula y el cóncavo hacia el centro de la misma; y entre estas líneas curvas y la silueta de la espícula algunas líneas longitudinales. También señala para las espículas: " . . . surface striée longitudinalement, comme s'ils étaient entourés d'une sorte de gaine de fibrilles parallèles . . . A la cassure, on voit qu'ils sont formés de couches concentriques inégales. . . ." Estas dos precisiones realizadas por Pruvot-Fol podemos compararlas por una parte con las líneas curvas que indicamos en los extremos de muchas espículas observadas por nosotros, y por otra parte con las prolongaciones longitudinales de dichas líneas curvas.

Thompson (1975) considera la separación de los géneros *Dendrodoris* y *Doriopsilla* como inadecuada y por tanto que el segundo es sinónimo del primero. Considera además a *D. areolata* como especie posiblemente sinónima de *Dendrodoris miniata* (Alder & Hancock, 1864), aunque no explica las razones que le llevan a adoptar tal paralelismo. Para *D. miniata* indica " . . . The mantle was rather smooth and the general aspect of the body convex but with a lens it is possible to see numerous low soft papillae on the upper pallial surface." En *D. areolata*, por el contrario, aparecen numerosos tubérculos de tamaño considerable (sostenidos por acúmulos de espículas visibles por transparencia). Estimamos por tanto que en función del carácter indicado, *D. areolata* no debe considerarse como sinónima de *D. miniata*.

Ballesteros y Ortea (1980), describen en los ejemplares jóvenes de *D. areolata* un movimiento ondulatorio del borde del manto que no aprecian en los adultos, y consideran dicho comportamiento en los jóvenes como un mecanismo de advertir de su presencia a posibles enemigos, ya que su manto es de secreción ácida. La observación de los bordes del manto de un ejemplar joven (de 9 mm

de longitud) revela la inexistencia de espículas en el mismo, lo cual puede facilitar el movimiento ondulatorio a que se ha hecho alusión. Con la progresiva aparición y desarrollo de las espículas, los bordes del manto se hacen más rígidos y dificultan con ello su movimiento, como ocurre en los ejemplares adultos en los que los movimientos quedan muy restringidos.

AGRADECIMIENTOS

Agradecemos a J.I. Navas, la ayuda prestada en la elaboración de este trabajo.

REFERENCIAS CITADAS

ALDER, J. & HANCOCK, A., 1845–1855, *A monograph of the British nudibranchiate Mollusca*. Ray Society, London, parts 1–7.

BALLESTEROS, M. & ORTEA, J. A., 1980, Contribución al conocimiento de los Dendrodorididae (Moluscos: Opistobranquios: Doridáceos) del litoral Ibérico. I. *Publicaciones del Departamento de Zoología, Universidad de Barcelona*, 5: 25–37.

BOUCHET, P., 1977, Opisthobranches de profondeur de l'océan Atlantique: II. Notaspidea et Nudibranchiata. *Journal of Molluscan Studies*, 43: 28–66.

EDMUNDS, M., 1968, Opisthobranchiate Mollusca from Ghana. *Proceedings of the Malacological Society of London*, 38: 83–100.

KRESS, A., 1981, A scanning electron microscope study of notum structure in some dorid nudibranchs (Gastropoda: Opisthobranchia). *Journal of the Marine Biological Association of the United Kingdom,* 61: 177–191.

MARCUS, E. & MARCUS, E., 1967, American opisthobranch mollusks. *Studies in Tropical Oceanography, Miami*, 6: 1–256.

PRUVOT-FOL, A., 1952, Compléments à la connaissance anatomique de *Doriopsilla areolata* Bergh. *Bulletin de la Société Zoologique de France*, 77: 411–414.

PRUVOT-FOL, A., 1953, Étude de quelques opisthobranches de la côte Atlantique du Maroc et du Sénégal. *Travaux de l' Institut Scientifique Chérifien, Zoologie*, 5: 1–105, 3 pl.

ROS, J., 1975, Opistobranquios (Gastropoda: Euthyneura) del litoral ibérico. *Investigaciones Pesqueras*, 39: 269–372.

ROS, J., 1976, Sistemas de defensa en los Opistobranquios. *Oecologia Aquatica*, 2: 41–77.

THOMPSON, T. E., 1975, Dorid nudibranchs from eastern Australia (Gastropoda, Opisthobranchia). *Journal of Zoology, London*, 176: 477–517.

VAYSSIÈRE, A., 1901, Recherches zoologiques et anatomiques sur les Mollusques Opisthobranches du Golfe de Marseille (suite et fin). 3. *Annales du Museum d'Histoire Naturelle Marseille*, 6: 1–130, 7 pl.

A MORPHOLOGICAL STUDY OF THE SPICULES OF *DORIOPSILLA AREOLATA* (GASTROPODA: NUDIBRANCHIA)

F.J. García, J.C. García & J.L. Cervera

Doridacean spicules are important for defense; they accompany acid glands on the body surface. The morphology of the calcareous spicules of *Doriopsilla areolata* Bergh is studied and illustrated in detail. The spicules are shown to be aggregated in bundles of fibers. Different body surfaces are considered separately because the spicules differ from place to place. The dorsal region (including gills and rhinophores) and lateral regions of the body and foot each have several kinds of spicules.

MALACOLOGIA, 1986, 27(1): 97–106

HIGH GENETIC SIMILARITIES AND LOW HETEROZYGOSITIES IN LAND SNAILS OF THE GENUS *SAMOANA* FROM THE SOCIETY ISLANDS

Michael S. Johnson[1], James Murray[2] & Bryan Clarke[3]

ABSTRACT

Land snails of the genus *Samoana* from the Society Islands were examined for allozymic variation at 20 loci. The species *S. diaphana*, *S. attenuata*, *S. burchi*, and *S. annectens* form a tight group, with average genetic identities of 0.95. Combined with the results of earlier studies on the confamilial genus *Partula*, these findings indicate that speciation entailing little genic change at allozyme loci is common in the Partulidae. Unlike most species of *Partula*, however, these four species of *Samoana* have very low heterozygosities ($\bar{H} = 0.002$). In the single case in which two alleles at a locus were common in the same population, there was a marked deficit of heterozygotes, suggesting that self-fertilization may be common.

Comparison of allozymes of *Samoana* and *Partula* confirmed the distinctness of the genera, with genetic identities between them averaging approximately 0.25. Despite its current taxonomic placement, *S. jackieburchi* closely resembles *Partula*, and differs greatly from *Samoana*. The allozymes support conchological and other evidence that *S. jackieburchi* belongs in the genus *Partula*. It appears that *S. jackieburchi* is anatomically convergent with *Samoana*, and that genital anatomy is not infallible as a taxonomic character in pulmonate snails.

INTRODUCTION

Five species of the land snail genus *Samoana* have been described from the Society Islands, French Polynesia (Kondo, 1973, 1980). Unlike the closely related and co-occurring genus *Partula*, which has been the subject of detailed genetic and evolutionary studies (Crampton, 1916, 1925, 1932; Murray & Clarke, 1980), *Samoana* has received relatively little attention, due in part to the scarcity and inaccessibility of its populations. The work on *Partula* increases the interest in *Samoana* with respect to two questions. First, is *Samoana* similar to *Partula* in the genetic structure of its populations and in its mode of speciation? Second, do the two genera really represent distinct lineages?

The close similarity of the two genera is suggested by both *Samoana attenuata* and *S. diaphana* having been originally described as species of *Partula*. Indeed, these two species had been placed in separate subgenera within *Partula: S. attenuata* in *Partula sensu stricto* (Pilsbry, 1909–1910) and *S. diaphana* in *Leptopartula*, along with the conchologically similar *P. arguta* and *P. turgida* (Crampton & Cooke, 1953). The most obvious distinction between the two genera is in the structure of the male genitalia (Kondo, 1973): in *Samoana* the phallus is shorter and stouter, and the epiphallus is more distinct than in *Partula*.

This anatomical difference was the basis for recognizing the recently described *S. jackieburchi* as a member of the genus *Samoana* (Kondo, 1980). The shells of *S. jackieburchi* are indistinguishable from those of *P. otaheitana rubescens*, raising the question whether genital anatomy provides an adequate basis for distinguishing the two genera.

In an attempt to answer these questions about *Samoana*, we have examined electrophoretic patterns in the enzymes of all the species of *Samoana* from the Society Islands. We report here the results of these examinations, a comparison of *Samoana* and *Partula*, and a reappraisal of the taxonomic position of *S. jackieburchi*.

MATERIALS AND METHODS

Samples. Collections of adults and juveniles were taken from the islands of Tahiti, Moorea, Raiatea, and Huahine. More than

[1]Department of Zoology, University of Western Australia, Nedlands, Western Australia 6009, Australia.
[2]Department of Biology, University of Virginia, Charlottesville, Virginia 22901, U.S.A.
[3]Department of Genetics, University of Nottingham, Nottingham NG7 2RD, United Kingdom.

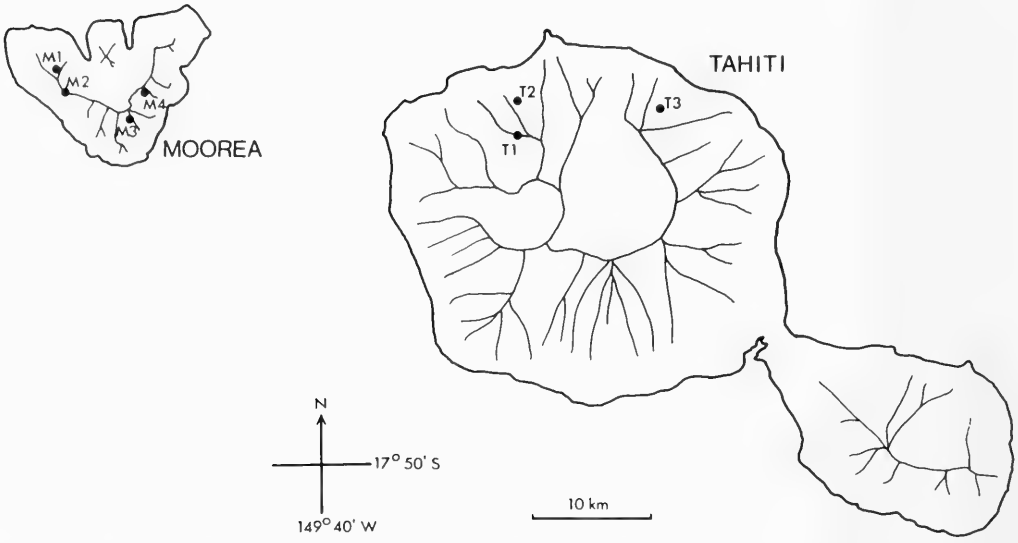


FIG. 1. Map of Tahiti and Moorea, showing collection sites. Lines indicate major mountain ridges. Locality codes: M1 = Faatoai; M2 = Uufau; M3 = Maatea; M4 = Hotutea; T1 = Fare Hamuta; T2 = Pirai; T3 = Tiarei.

one site was sampled in Tahiti and Moorea, as shown in Fig. 1. The collections provided samples of all the species of *Samoana* that have been recorded from the Society Islands:

S. attenuata (Pease), 48 individuals collected on three islands: Tahiti, from Pirai (N = 3) and Tiarei (N = 5) valleys; Moorea, from high in Faatoai Valley (N = 13) and from the pass at the head of Uufau Valley (N = 10); Raiatea, from Miti Miti Aute Rahi (N = 17). The species also occurs on the islands of Bora Bora and Tahaa.

S. annectens (Pease), 1 individual collected on Huahine.

S. burchi Kondo, 6 individuals collected on Tahiti, taken in a large area centering on Fare Hamuta on the Aorai Trail, at an elevation of approximately 800 m.

S. diaphana (Crampton & Cooke), 53 individuals collected on two islands: Moorea, from Faatoai (N = 20) and Uufau (N = 5) valleys, where it occurs sympatrically with *S. attenuata*, and from high in Maatea (N = 1) and Hotutea (N = 21) valleys, where *S. attenuata* is absent; Tahiti, from Fare Hamuta (N = 6), where it occurs sympatrically with *S. burchi*. This sample is notable, because *S. diaphana* has previously been reported only from Moorea (Kondo, 1973).

S. jackieburchi Kondo, 33 individuals from Tahiti, from Tiarei Valley.

These specimens were identified morphologically (see Kondo, 1973), and the identification of the Tahitian species was confirmed by Dr. Kondo. In the case of *S. jackieburchi*, identification required dissection to distinguish it from *P. otaheitana rubescens*. Our sample of 34 adults from Tiarei included a single *P. otaheitana*.

To increase our coverage of *Samoana*, a sample of 19 *S. conica* from Tutuila in Samoa (approx. 2000 km from Tahiti) was included. To allow comparisons between genera, data from 3 species of *Partula* were included: *P. otaheitana*, the species from which *S. jackieburchi* was recently removed (12 animals from Pirai Valley and 19 from Papehue Valley, Tahiti); *P. affinis*, until recently considered a subspecies of *P. otaheitana* (Kondo & Burch, 1979) (3 from Tiarei Valley, Tahiti); *P. gibba*, a more distant species of *Partula* (15 from Saipan). As a precaution, several specimens were dissected to confirm the presence of *Partula*-like genitalia in the Tahitian species of *Partula*. Allozymes in *P. otaheitana* and *P. gibba* have previously been compared with those in species of *Partula* from Moorea (Johnson *et al.*, 1977).

Morphology. The inclusion of the specimens from Fare Hamuta in *S. diaphana* was tested by examining both shells and genitalia from adults (*S. diaphana* is distinguished by an almost transparent, semi-globose shell; Crampton & Cooke, 1953; Kondo, 1973). In comparing the Moorean and Tahitian specimens, we recorded the weight (Wt), length (L), and width (W) of the shell, as described before (Murray & Clarke, 1980). Taking the shell as an approximate cone, an estimate of shell volume was obtained as $V = 1/12\pi W^2 L$. Only the adults from Moorea and Tahiti were included in these analyses, although juveniles of *S. diaphana* were also easily distinguished from the other species.

Comparisons of genital anatomy were based on the work of Kondo (1973), whose fig. 4 shows a distinctly inflated epiphallus for *S. diaphana* but not for the other species of *Samoana*. All of the adult *Samoana* from Moorea and Tahiti were examined for this characteristic.

Electrophoresis. Allozymic variation was examined by conventional starch-gel electrophoresis. The enzymes and procedures were those used by Johnson *et al.* (1977), except that a tris-maleate buffer (Selander *et al.*, 1971) was used for glutamate oxaloacetate transaminase (*Got-1* and *Got-2* loci), isocitrate dehydrogenase (*Idh-1* and *Idh-2*), and phosphoglucomutase (*Pgm-1* and *Pgm-2*), and a tris-EDTA-borate buffer (Selander *et al.*, 1971) was used for nucleoside phosphorylase (*Np*).

The enzymes represent 20 structural loci, each designated by an abbreviation of its encoded enzyme. Allelic designations indicate the electrophoretic mobilities of the corresponding allozymes relative to those of *P. gibba*. A homozygous stock of *P. gibba* is maintained as a standard in the laboratory. Negative mobilities refer to cathodally migrating allozymes. Although we do not have data from genetic crosses in *Samoana*, segregations of the allozymes in laboratory crosses of *Partula* are consistent with Mendelian inheritance (Johnson *et al.*, 1977, and unpublished).

Based on the 20 loci, unbiased estimates of genetic identity (Nei, 1978) were calculated between all pairs of species. The matrix of genetic identities was summarized by UPGMA clustering (Sneath & Sokal, 1973).

RESULTS

Morphology

The differences in size and shape among *S. diaphana*, *S. attenuata*, and *S. burchi* are clear from Fig. 2, in which the widths and lengths of the shells from adults are plotted. Although there is considerable variation in each species, *S. diaphana* is consistently smaller and, relatively, wider than its sympa-

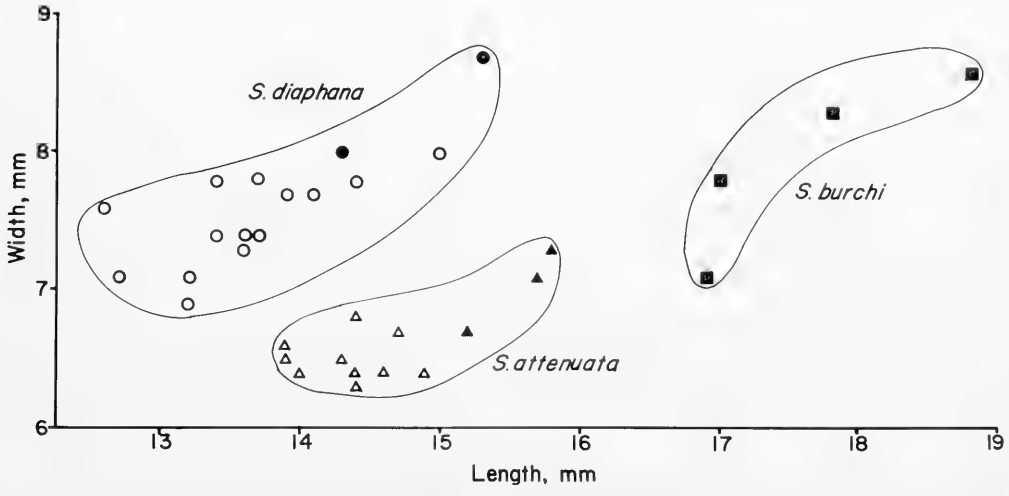


FIG. 2. Comparison of width and length of shells among three species of *Samoana*. Lines encompass conspecific specimens. Open symbols = specimens from Moorea; solid symbols = from Tahiti.

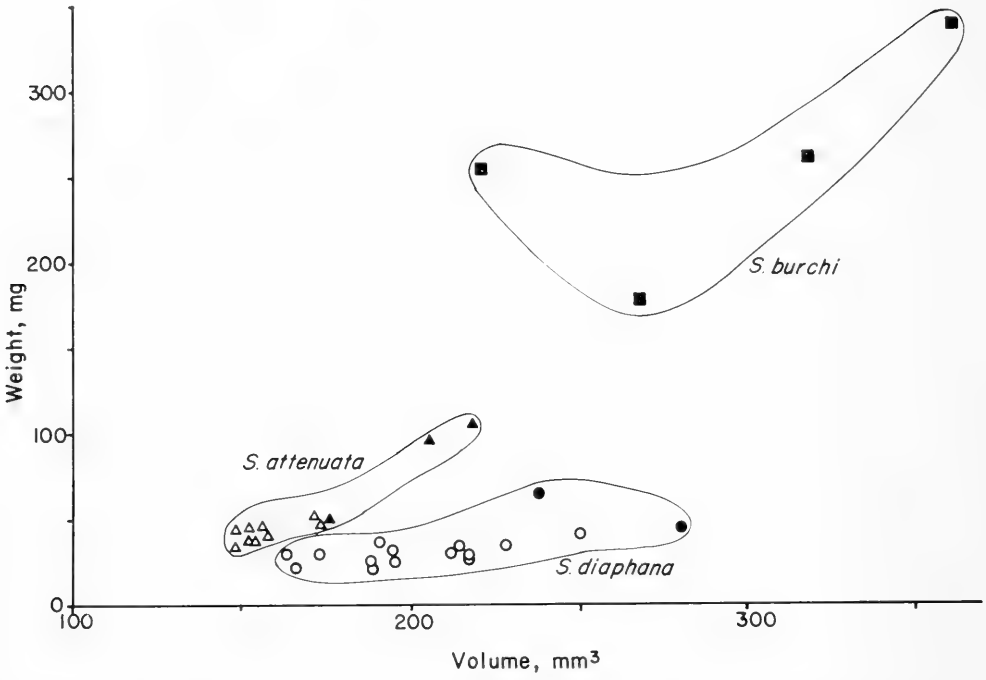


FIG. 3. Comparison of weight and "volume" of shells among three species of *Samoana*. Open symbols = from Moorea; solid = from Tahiti.

tric congeners. In addition, *S. diaphana* has a lighter, less robust, shell than *S. attenuata* and the even more heavily shelled *S. burchi* (Fig. 3). Although the shells of *S. diaphana* from Fare Hamuta are larger than most of those from Moorea, they clearly belong to the Moorean group.

The dissections confirm this placement. The two adult *S. diaphana* from Tahiti had an inflated epiphallus, as did the dissected specimens from each of the four Moorean populations. In contrast, none of the *S. burchi* or *S. attenuata* had an inflated epiphallus. Thus, the shells and genitalia were consistent in confirming the presence of *S. diaphana* on Tahiti.

Electrophoresis

With the exception of *S. jackieburchi*, the species of *Samoana* from the Society Islands have very similar allozymes. The four species that resemble each other (*S. diaphana*, *S. attenuata*, *S. burchi*, and *S. annectens*) will be considered before comparing them with the other species of *Samoana* and *Partula*. A striking feature of the four species is their low variability. In most samples, no heterozygotes were found at any of the 20 loci, and the highest average observed heterozygosity was 0.012 (Table 1). The only locus with two common alleles segregating in a population was *Pgi*, and the polymorphism occurred only in the sample of *S. diaphana* from Hotutea. Even in this case, however, there was a deficit of heterozygotes: the expectation according to the Hardy-Weinberg equilibrium was 9.0 heterozygotes, but only 3 were observed ($\chi^2_1 = 7.00$, $P < 0.01$).

In addition to the paucity of variation within populations, little allozymic divergence was detected between species. Of the 20 loci examined, 15 were identically monomorphic in all 4 species. Even among the 5 variable loci, the similarity between species was great (Table 1). Three of the variable loci (*Idh-2*, *Pgm-1*, and *Pgm-2*) had different alleles fixed in different populations. But these differences occurred *within* species. The greatest resemblance was found between *S. diaphana* and

TABLE 1. Allelic frequencies at loci which are variable in *Samoana* from the Society Islands (excluding *S. jackieburchi*). Loci for which the same allele occurred at a frequency of >0.95 in all populations are not shown, but are included in the calculation of average observed heterozygosity ($\bar{H}$). Locality codes: as in Fig. 1, except that R = Raiatea and H = Huahine.

		diaphana					*attenuata*					*burchi*	*annectens*
Locus	Allele	M1	M2	M3	M4	T1	M1	M2	T2	T3	R	T1	H
Sample size		20	5	1	21	6	13	10	3	5	17	6	1
Got-2	−1.00	—	—	—	—	—	—	—	—	.10	.03	—	—
	−1.40	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	.90	.97	1.00	1.00
Idh-2	1.00	1.00	1.00	1.00	1.00	1.00	—	—	1.00	1.00	—	1.00	1.00
	.50	—	—	—	—	—	1.00	1.00	—	—	1.00	—	—
Pgi	.95	1.00	1.00	1.00	.31	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	.50	—	—	—	.69	—	—	—	—	—	—	—	—
Pgm-1	1.13	1.00	—	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	1.04	—	1.00	—	—	—	—	—	—	—	—	—	—
Pgm-2	1.12	1.00	1.00	1.00	1.00	—	—	—	—	—	—	—	—
	1.09	—	—	—	—	1.00	—	—	1.00	—	—	1.00	—
	1.04	—	—	—	—	—	—	—	—	1.00	1.00	—	—
	.87	—	—	—	—	—	1.00	1.00	—	—	—	—	1.00
$\bar{H}$ (20 loci)		.002	.000	.000	.012	.000	.000	.000	.000	.010	.006	.000	.000

S. burchi from Fare Hamuta, which were identically homozygous for all 20 loci, giving a genetic identity of 1.00. The least resemblance between populations of different species was 0.86. The identities between species were not appreciably different from those between conspecific populations, which ranged from 0.89 to 1.00.

To simplify comparisons with the other species, allelic frequencies in *S. diaphana* and *S. attenuata* were taken as the averages among conspecific populations, weighted by sample size. The great genetic similarities between *S. diaphana*, *S. attenuata*, *S. burchi*, and *S. annectens*, and the lack of variation within them, set the group apart from other species (Table 2). With the exception of the homozygous *P. gibba*, all the other species examined had higher heterozygosities, ranging from 0.027 in *S. conica* to 0.135 in *S. jackieburchi* (Table 2). Furthermore, none showed a significant departure from Hardy-Weinberg equilibrium.

The pattern of genetic identities (Table 3) is well summarized by the phenogram in Fig. 4. *S. diaphana*, *S. attenuata*, *S. burchi*, and *S. annectens* form a very tight cluster, but this group has little similarity with either *S. conica* or *S. jackieburchi*. *S. conica* diverges strongly from all the other species. *S. jackieburchi*, however, falls clearly within the *Partula* cluster. Indeed, *S. jackieburchi* has an identity of 0.95 with *P. otaheitana* and 0.93 with *P. affinis*. The close similarity of *S. jackieburchi* to *P. otaheitana* also occurs in sympatry; the single specimen of *P. otaheitana rubescens* collected with the *S. jackieburchi* in the Tiarei sample is electrophoretically indistinguishable from it.

DISCUSSION

The close genetic identities among species of *Samoana* from the Society Islands are consistent with those previously reported for species of *Partula* on Moorea (Johnson *et al.*, 1977) and for *P. affinis* and *P. otaheitana* in the present study. Although they are by no means unique, such strong resemblances between reproductively isolated species are unusual (Thorpe, 1982). In the case of *Partula*, reproductive relationships have been established through extensive field and laboratory studies (Murray & Clarke, 1980), providing information that is not available for *Samoana*. Nevertheless, evidence for reproductive isolation in *Samoana* is provided by the distinctness of species in sympatry. Our samples of *S. diaphana* include two sites of sympatry with *S. attenuata* and one with *S. burchi*. In each case, *S. diaphana* has a distinctly less robust, smaller, and more globose shell than does *S. attenuata* or *S. burchi*. Similarly, although

TABLE 2. Allelic frequencies and average observed heterozygosity ($\bar{H}$) for 20 loci in species of *Samoana* and *Partula*. For loci not included in Table 1, *S. diaphana*, *S. burchi*, and *S. annectens* are identical to *S. attenuata*. *P. gibba* is homozygous for the 1.00 allele at each locus. *G6pd* and *Mdh-3* were monomorphic.

		Samoana			*Partula*	
Locus	Allele	*conica*	*attenuta*	*jackieburchi*	*otaheitana*	*affinis*
Sample size		19	48	33	31	3
Alph	1.02	.04	1.00	—	.02	—
	.95	—	—	1.00	.96	1.00
	.87	—	—	—	.02	—
	.55	.96	—	—	—	—
Got-1	1.70	.03	—	—	—	—
	1.50	.97	1.00	.21	.18	.17
	1.25	—	—	.13	—	—
	1.00	—	—	.31	.52	.83
	.80	—	—	—	—	—
	.71	—	—	.35	.28	—
	.55	—	—	—	.02	—
Got-2	−.60	—	.02	.25	—	—
	−1.00	—	—	.75	1.00	1.00
	−1.40	1.00	.98	—	—	—
Idh-1	1.06	1.00	—	—	—	—
	1.00	—	—	.60	.98	.50
	.89	—	1.00	.40	.02	.50
Idh-2	1.30	.08	—	—	—	—
	1.00	.92	.17	1.00	1.00	1.00
	.50	—	.83	—	—	—
Mdh-1	1.11	—	—	—	.37	.33
	1.00	1.00	—	.86	.61	.67
	.95	—	1.00	—	—	—
	.81	—	—	.14	.02	—
Mdh-2	1.50	1.00	—	—	.02	—
	1.00	—	—	1.00	.98	1.00
	.80	—	.99	—	—	—
	.70	—	.01	—	—	—
Mdh-4	.10	1.00	—	—	—	—
	1.00	—	1.00	1.00	1.00	1.00
Mpi	1.07	.13	—	—	—	—
	1.00	.87	—	.94	.82	1.00
	.92	—	—	.06	.18	—
	.86	—	1.00	—	—	—
Np	1.15	—	1.00	—	—	—
	1.00	1.00	—	—	—	—
	.84	—	—	1.00	.94	1.00
	.73	—	—	—	.06	—
Pep-2	1.13	1.00	—	—	—	—
	1.05	—	1.00	—	—	—
	1.00	—	—	1.00	1.00	1.00
Pep-4	1.03	—	1.00	—	—	—
	1.02	1.00	—	—	—	—
	1.00	—	—	1.00	.98	—
	.97	—	—	—	.02	1.00
Pep-6	1.29	1.00	—	—	—	—
	1.23	—	—	—	.25	—
	1.00	—	—	1.00	.66	1.00

Locus	Allele	*Samoana*			*Partula*	
		conica	*attenuta*	*jackieburchi*	*otaheitana*	*affinis*
	.85	—	1.00	—	.09	—
6Pgd	1.00	1.00	—	1.00	1.00	1.00
	.91	—	1.00	—	—	—
Pgi	1.42	.05	—	—	—	—
	1.00	—	—	.21	.74	—
	.95	.95	1.00	—	—	—
	.50	—	—	.79	.26	1.00
Pgm-1	1.13	1.00	1.00	—	—	—
	1.08	—	—	.03	.09	—
	1.04	—	—	—	—	—
	1.02	—	—	.97	.91	1.00
Pgm-2	1.09	—	.06	—	—	—
	1.04	.37	.46	1.00	1.00	1.00
	.95	.63	—	—	—	—
	.87	—	.48	—	—	—
Sod	1.60	1.00	—	—	—	—
	1.00	—	1.00	1.00	1.00	1.00
$\bar{H}$ (20 loci)		.027	.001	.135	.130	.067

Kondo (1973) considered the inflated epiphallus of his figured specimen of *S. diaphana* to be "a temporary non-reliable characteristic", our observations indicate that it does indeed consistently discriminate *S. diaphana* from the other species. This consistent association of characteristics which are presumably independent genetically and developmentally indicates that *S. diaphana* is a distinct entity, reproductively isolated from the other species. As the differences between *S. attenuata*, *S. burchi*, and *S. annectens* are for their shells only, and as these forms have not been found together, inferences on their reproductive isolation are much weaker. Even excluding comparisons between these three species, however, the very high allozymic similarities, despite reproductive isolation, are confirmed by the comparisons with *S. diaphana*.

Despite this similarity, the allozymes also provide further evidence for reproductive isolation between *S. diaphana* and *S. attenuata*. Where these two species occur together in Faatoai and Uufau valleys, they are fixed for alternate alleles at the *Idh-2* and *Pgm-2* loci (and at the *Pgm-1* locus in Uufau) (Table 1).

TABLE 3. Genetic identities among species of *Samoana* and *Partula*, based on 20 loci. For species represented by more than one population, allelic frequencies in the combined sample were used for interspecific comparisons.

	Samoana						*Partula*	
	con.	*dia.*	*att.*	*bur.*	*ann.*	*jac.*	*ota.*	*aff.*
S. conica	—							
S. diaphana	.35	.95						
S. attenuata	.34	.95	.90					
S. burchi	.36	.97	.96	—				
S. annectens	.37	.98	.99	.98	—			
S. jackieburchi	.28	.26	.25	.25	.26	—		
P. otaheitana	.27	.26	.24	.24	.25	.95	.90	
P. affinis	.27	.26	.25	.25	.26	.93	.90	
P. gibba	.31	.22	.19	.21	.22	.72	.74	.64

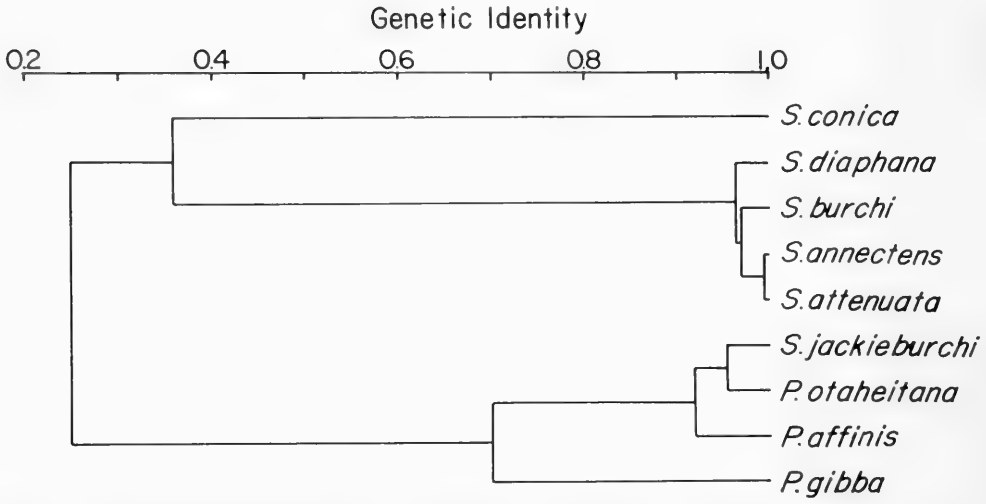


FIG. 4. Phenogram of genetic identities among species of *Samoana* and *Partula*, based on 20 loci.

The complete absence of heterozygotes at these loci demonstrates a lack of genetic exchange between the two species. Although such distinctness could also result from the coexistence of self-fertilizing strains (see later discussion), the finding of heterozygotes at some loci indicates that these species reproduce at least partly through outcrossing. Thus, the pattern of allozyme variation supports the existence of reproductive isolation, which was implied by the study of shells and genitalia. Nevertheless, *S. diaphana* and *S. burchi*, despite the reproductive isolation inferred from their conchological and anatomical differences are allozymically identical at all 20 loci where they occur together. Thus, although allozymes are locally useful in demonstrating reproductive isolation, they are not invariably so. Similarly, while genetic identities between species in many groups are usually below 0.85 (Thorpe, 1982), this is not true of either *Samoana* or *Partula*, suggesting that a genetic "yardstick" does not exist.

Although both genera have groups of species that closely resemble each other, the low heterozygosity in *Samoana* contrasts with the high values typical of most species of *Partula* (Table 2; Johnson *et al.*, 1977). Another difference between the genera is in terms of whether the genetic diversity of a species is concentrated within populations, or represented by differences between populations. Partitioning allozymic diversity into "within-population" and "between-population" components (Nei, 1973) shows different genetic patterns: in the Society Islands, only 2%, on average, of the intraspecific genic diversity in *Samoana* (excluding *S. jackieburchi*) occurs within populations, compared with 79% in *Partula* (unpublished data). Thus, although a single sample gives a good indication of allozymic diversity in the average species of *Partula*, several samples are required for species of *Samoana*.

As emphasized by Kondo (1973), species of *Samoana* are remarkable for their scarcity. In part, this may be an artifact of their relative inaccessibility, since they generally occur at higher altitudes than *Partula*. Our samples of *S. diaphana*, for example, increase the recorded sites for the species from 2 to 6 and suggest that it may be fairly continuously distributed in Moorea at high altitudes. Nevertheless, the area of high-altitude habitats is relatively small, and reduced population sizes may contribute to the low genetic variation in *Samoana* compared with the more widely distributed species of *Partula*. Despite their wide local distributions, the species of *Partula* are, almost without exception, endemic to single islands, whereas both *S. diaphana* and *S. attenuata* are found on more than one island. It seems likely that the species of *Partula* on Moorea represent a radiation within the island (Murray & Clarke, 1980; Johnson, Murray & Clarke, unpublished). In contrast, the occur-

rence of *S. diaphana* and *S. attenuata* on both Moorea and Tahiti implies interchanges between islands. Despite their apparent ability to disperse, the fact that alternate alleles are fixed in different populations of both *S. diaphana* and *S. attenuata* within islands indicates that local populations are relatively isolated.

Another possible reason for the low variation in *Samoana* is self-fertilization. A comparison of heterozygosities in outcrossing and self-fertilizing species of terrestrial pulmonates (Selander & Ochman, 1983) shows that heterozygosities as low as those found in *Samoana* are common in species that self-fertilize. No heterozygotes have been detected in *Partula gibba*, which reproduces largely, if not completely, by self-fertilization, whereas all the *Partula* species that are known to outcross are highly heterozygous (Johnson *et al.*, 1977; unpublished data). Although the low heterozygosities in *Samoana* may indicate selfing, the paucity of variation makes direct tests difficult. The large deficit of heterozygotes for *Pgi* in *S. diaphana* suggests that self-fertilization may be common, but extreme subdivision in an outcrossing population cannot be excluded. If the circumstantial evidence for selfing in Society Island *Samoana* proves to be correct, it clearly does not apply to the genus as a whole. Although not highly heterozygous, the sample of *S. conica* from Tutuila is in Hardy-Weinberg equilibrium for the *Mpi* and *Pgm-2* loci.

The contrasts with *S. conica*, in terms of both heterozygosity and genetic differences, emphasize the coherence of the *Samoana* species from the Society Islands. The close relationships among these species were also stressed by Kondo (1973) in his study of their reproductive anatomy. It was on anatomical grounds that *S. jackieburchi* was removed from *P. otaheitana* (Kondo, 1980). The electrophoretic data, however, do not support the placement of *S. jackieburchi* in *Samoana*. The *Partula* and *Samoana* from the Society Islands have very different sets of allozymes at 9 of 20 independent loci, and large differences at several others. They form two very distinct groups of species. The close clustering of species of *Partula* (Fig. 4) applies to all the 24 species that we have examined (unpublished data). The fact that *S. jackieburchi* most closely resembles the group of *Partula* species indicates clearly that it belongs in *Partula*.

This conclusion is supported by other evidence. The shells of *S. jackieburchi* and *P. otaheitana rubescens* are indistinguishable, and differ from Society Island species of *Samoana* in their size, shape, robustness, color, and direction of coiling (photographs in Kondo, 1980). *S. jackieburchi* also lacks the maculation of the mantle that occurs in *Samoana*, but is uncommon in *Partula*. Finally, a striking characteristic of *S. diaphana*, *S. attenuata*, *S. burchi*, and *S. annectens* is their very sticky mucus, a feature missing in *Partula*, and *S. jackieburchi*. Faced with the alternative of massive convergence of independent biochemical and morphological characters, Occam's razor requires the interpretation that it is the genital anatomy of *S. jackieburchi* which is convergent. This necessary interpretation has disturbing implications for pulmonate taxonomy, which often relies on genital anatomy. Our data indicate that genitalia are not infallible taxonomic characters. As in many cases of convergence, finer anatomical studies may show that the resemblance of *S. jackieburchi* to *Samoana* is superficial. In any event, that resemblance emphasizes the danger of reliance on any particular taxonomic character.

It is possible that the *Samoana*-like genitalia of *S. jackieburchi* do not delineate a separate species. The indistinguishable shells and allozymes of *S. jackieburchi* and *P. otaheitana rubescens* are consistent with an interpreation of these entities as a single species with polymorphic genitalia. Only detailed field and laboratory studies will settle the issue.

ACKNOWLEDGEMENTS

We thank Peter Kendrick and Jane Prince for assistance, and Dr. Yoshio Kondo for confirming our identifications of species of *Samoana*. Financial support was provided by the Australian Research Grants Scheme, the U.S.-Australian Cooperative Science Program (NSF: 2AS = 30), and the Science and Engineering Research Council.

LITERATURE CITED

CRAMPTON, H. E., 1916, Studies on the variation, distribution, and evolution of the genus *Partula*. The species inhabiting Tahiti. *Carnegie Institution of Washington Publications*, 228: 1–311.

CRAMPTON, H. E., 1925, Studies on the variation, distribution, and evolution of the genus *Partula*. The species of the Mariana Islands, Guam and Saipan. *Carnegie Institution of Washington Publications*, 228A: 1–116.

CRAMPTON, H. E., 1932, Studies of the variation, distribution, and evolution of the genus *Partula*. The species inhabiting Moorea. *Carnegie Institution of Washington Publications*, 310: 1–335.

CRAMPTON, H. E. & COOKE, C. M., 1953, New species of *Partula* from southeastern Polynesia. *Occasional Papers, Bernice Pauahi Bishop Museum*, 21: 135–159.

JOHNSON, M. S., CLARKE, B. & MURRAY, J., 1977, Genetic variation and reproductive isolation in *Partula*. *Evolution*, 31: 116–126.

KONDO, Y., 1973, *Samoana* of the Society Islands (Pulmonata: Partulidae). *Malacological Review*, 6: 19–33.

KONDO, Y., 1980, *Samoana jackieburchi*, new species (Gastropoda: Pulmonata: Partulidae). *Malacological Review*, 13: 25–32.

KONDO, Y. & BURCH, J. B., 1979, Extrusive genital anatomies and their internal postures in *Partula affinis* of Tahiti. *Malacological Review*, 12: 79–84.

MURRAY, J. & CLARKE, B., 1980, The genus *Partula* on Moorea: speciation in progress. *Proceedings of the Royal Society of London*, ser, B, 211: 83–117.

NEI, M., 1973, Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences*, U.S.A., 70: 3321–3323.

NEI, M., 1978, Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, 89: 583-590.

PILSBRY, H. A., 1909–1910, Family Partulidae. In: *Manual of Conchology*, ser. 2, 20: 155–336.

SELANDER, R. K. & OCHMAN, H., 1983, The genetic structure of populations as illustrated by molluscs. *Isozymes: Current Topics in Biological and Medical Research*, 10: 93–123.

SELANDER, R. K., SMITH, M. H., YANG, S. Y., JOHNSON, W. E. & GENTRY, J. B., 1971, Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse *Peromyscus polionotus*. *Studies in Genetics VI, University of Texas Publication* 7103: 49–90.

SNEATH, P. H. A. & SOKAL, R. R., 1973, *Numerical taxonomy; the principles and practise of numerical classification*. Freeman, San Francisco, xv + 573 p.

THORPE, J. P., The molecular clock hypothesis: biochemical evolution, genetic differentiation and systematics. *Annual Review of Ecology and Systematics*, 13: 139–168.

MALACOLOGIA, 1986, 27(1): 107–125

HYBRIDIZATION IN A UNIONID FAUNAL SUTURE ZONE

Pieter W. Kat[1]

Department of Earth & Planetary Sciences, The Johns Hopkins University, Baltimore, Maryland 21218, U.S.A.

ABSTRACT

Retreat of the Wisconsinan glaciers and expansion of unionid geographic ranges has resulted in re-establishment of contact between Interior Basin and northern Atlantic Slope species isolated by the Appalachian Mountains at the height of glaciation. One suture zone between these faunas occurs in the area around Lake Champlain, and molecular genetic, anatomical, and shell microstructural data indicate hybridization between species of *Anodonta* and *Lampsilis*. Additionally, introgression appears to occur over a wide geographic area. *Elliptio* populations around Lake Champlain exhibit no evidence of hybridization, but form a locally differentiated group when compared to northern Atlantic Slope *E. complanata*. Hybrid *Anodonta* and *Lampsilis* populations contain variant alleles not found among parental species. Probability of hybridization is proposed to be best predicted by similarity of glochidial hosts between unionid species, not necessarily by levels of electrophoretically determined genetic differentiation. Taxonomic implications of the data are discussed.

Key words: hybridization; zoogeography; genetics; Unionidae; Bivalvia.

INTRODUCTION

Hybrid zones have long been of special interest to evolutionary biologists. Introgressive hybridization (Anderson, 1949) can potentially enhance the level of genetic variation and thus the evolutionary flexibility of populations. For instance, Sage & Selander (1979) and Hunt & Selander (1973) observed increased levels of heterozygosity as well as unique alleles in hybrid populations of frogs and mice. Such unique alleles have been proposed to arise through increased mutation rates among hybrid populations (Thompson & Woodruff, 1978), or through intragenic recombination between the different parental alleles (Watt, 1972). Also, Anderson & Stebbins (1954) have proposed that hybridization can trigger episodes of innovative diversification, and certain species of plants are known to have had a hybrid origin (Grant, 1966; Lewis, 1966; Gallez & Gottlieb, 1982).

Hybrid zones are often established by changes in distribution of one or both of the taxa involved. Geographic ranges of all North American unionids have probably fluctuated to some extent during the repeated Quaternary glacial episodes, but such fluctuations are best documented for species of the northern Interior Basin and Atlantic Slope faunas (Simpson, 1896; Ortmann, 1913; Baker, 1920; Johnson, 1970, 1980; Clarke, 1973; Kat, 1982, 1983a, b, c; Smith, 1982; Kat & Davis, 1984). For example, fossil evidence has indicated that populations of at least three Interior Basin species were present on the Atlantic Slope about 200,000 years ago, but were subsequently eliminated by glaciers (Kat, 1983b). Contact between these faunas has recently been re-established by migration out of Wisconsinan refugia. One such suture zone (Remington, 1968) is located in the area around Lake Champlain. This lake has had a varied postglacial history including a saltwater phase and connections to both Interior Basin and Atlantic Slope drainages (Simpson, 1896; Clarke & Berg, 1959; Elson, 1969; Johnson, 1980; Smith, 1982). As a consequence of these historic connections and/or a more recent immigration route (via the Erie Canal system, which links Lake Erie, Lake Champlain, and the Hudson River), Lake Champlain and surrounding drainages contain species of both faunal regions (Smith, 1983). This area thus provides a natural experiment to determine the degree of genetic interaction between various members of these previously isolated faunas.

[1]Present address: Department of Malacology, National Museums of Kenya, P.O. Box 40658, Nairobi, Kenya.

Study of hybridization between Interior Basin and Atlantic Slope unionid species is of special interest for two reasons. First, because of patterns of Wisconsinan glaciation and the location of the hybrid zone, there is little uncertainity about its origin. This hybrid zone represents a postglacial secondary contact between taxa that existed in allopatry at the height of glaciation, and therefore can not be explained as differentiation within a continuous series of populations (Endler, 1977; White, 1978). Second, the Lake Champlain fauna allows study of the degree of interaction between species belonging to lineages that are diversifying at different rates, between species that differ in observed levels of heterozygosity and polymorphism, and between species that exhibit various levels of genetic differentiation.

Also included in this study is a genetic and morphological analysis of *Anodonta "cataracta" fragilis*. This taxon was thought by Clarke & Rick (1963) to represent an intergrade between *A. fragilis* from Newfoundland and northern Atlantic Slope *A. cataracta*. Previous studies, however, have indicated that Nova Scotian *A. "c." fragilis* are genetically distinct from *A. cataracta*, and that the taxon is more closely related to European than North American anodontines (Kat, 1983d, e; Kat & Davis, 1984). The taxonomic status of anodontines that resemble *A. fragilis* but occur outside Newfoundland is therefore still uncertain.

METHODS

Populations analysed in this study were collected from Lake Champlain and adjacent areas, as well as the Delmarva Peninsula, Nova Scotia, Michigan, and Wisconsin. The bivalves were maintained in aquaria for at least two weeks. Four individuals from each population were then relaxed (sodium nembutal) and fixed (10% formalin) in preparation for dissection. The remaining 20–25 individuals had wedges of tissue removed from the foot and viscera and these tissue samples were either homogenized and electrophoresed immediately or stored at -20° C for later analysis.

Starch-gel electrophoresis has been used with good success in a series of taxonomic analyses of the Unionidae (Davis *et al.*, 1981; Davis, 1983, 1984; Kat, 1983a, c, d; Kat & Davis, 1984). Methods for electrophoresis and enzyme staining were generally similar to those of Davis *et al.* (1981), and 15 loci of which at least eight were polymorphic among species were scored using the methods of Ayala *et al.* (1973). Nei's (1972) genetic distances were computer generated using a program written by Green (1979). This genetic distance matrix was then used in the multivariate analysis program NT-SYS (Rohlf *et al.*, 1972). Multidimensional scaling maximized goodness-of-fit of the regression of genetic distance and distance in three-dimensional space, and a minimum spanning tree was derived from these adjusted distances. The minimum spanning tree summarizes taxonomic relationships since distances between closely related taxonomic units are small, whereas those between distantly related taxonomic units are large. Distance between taxonomic units is here defined as a function of the observed Nei distances. Such multivariate analyses are especially useful in elucidation of relationships among taxa such as *Elliptio* that exhibit considerable polymorphism at a number of loci.

Fine detail of unionid stomach anatomy can be used as a taxonomic tool at a variety of taxonomic levels (Kat, 1983a, c, d). Techniques for dissection and illustration are discussed in Kat (1983d). Four individuals from each population were dissected and photographed to determine levels of intrapopulation variability.

Microstructure of conchiolin layers within the shell was examined with a scanning electron microscope. Previous studies (Kat, 1983a, e) reveal that the conchiolin layer is divisible into three distinct regions, of which the central, reticulate region in particular contains species-specific characters. In the past, patterns of resemblance among unionid taxa based on conchiolin layer microstructure have been highly compatible with patterns of resemblance suggested by electrophoretic data, and conchiolin layer microstructure was successfully used to discriminate among two races of *Elliptio complanata* and their hybrids (Kat, 1983a). Techniques for conchiolin layer preparation and microscopy are detailed in Kat (1983e).

RESULTS

A. Molecular genetics

Distributions of alleles among loci that best discriminate species of *Anodonta* and *Lamps-*

TABLE 1. Distribution of alleles among loci of *Anodonta* examined in this study. Loci that do not discriminate among species are not included.

		Species			
Enzyme	Allele	*A. cataracta*	*A. grandis* × *cataracta*	*A. grandis*	*A. fragilis*
PGM I	24	1.00	.26	.05	
	22		.74	.95	
	20				1.00
PGM II	32		.29		
	31				1.00
	30	1.00	.71		
	28			1.00	
LAP	34			.35	
	32		.52	.65	
	30	1.00	.48		
	28				1.00
MDH I	18				.50
	15	1.00	1.00	1.00	
	13				.50
MDH II	−9		.35	.55	
	−11	1.00	.65	.45	1.00
HEX	34		.20	.20	
	31	1.00	.72	.80	1.00
	28		.08		
MPI	26		1.00	.65	
	23				0 to .15
	20	.33 to .48		.35	.85 to 1.00
	18	.52 to .67			
ODH	15		.61	.72	
	9				1.00
	6	1.00	.39	.28	

ilis are presented in Tables 1 and 2. Similar data are not presented for populations of *Elliptio* because *Elliptio dilatata* from Wisconsin appears to possess only two relatively rare alleles not present among Atlantic Slope *E. complanata* examined to date: MDH I 14 and HEX 34. Also, the number of populations examined and the high levels of polymorphism characteristic of *Elliptio* would require a table of excessive proportions.

It is clear from Table 1 that the *Anodonta* population in Lake Champlain shares alleles characteristic of both Atlantic Slope *A. cataracta* and Interior Basin *A. grandis*. However, the Lake Champlain population also possesses alleles not present in either parental species (HEX 28 and PGM II 32), and is fixed for MPI 26. Over 15 loci examined, *A. cataracta* possesses 17 alleles, *A. grandis* 22, and *A. cataracta* × *grandis* 24: *A. grandis* shares 13 alleles with *A. cataracta* and 18 alleles with *A. cataracta* × *grandis*, while *A. cataracta* shares 14 alleles with *A. cataracta* × *grandis*. *A. "cataracta" fragilis*, however, is quite different from both *A. cataracta* and *A. grandis*. *A. "c." fragilis* has 18 alleles at the loci examined of which it shares 10 with *A. cataracta* and *A. grandis*. There is no evidence from electrophoresis to suggest any genetic exchange between *A. cataracta* and *A. fragilis*.

Table 2 presents allele frequencies for nine loci at which Atlantic Slope *Lampsilis radiata* differ from Interior Basin *L. siliquoidea*. Again, there is good evidence to support a hybrid

TABLE 2. Distribution of alleles among loci of *Lampsilis* examined in this study. Loci that do not discriminate among the parental species are not included.

		Species		
Enzyme	Allele	*L. radiata*	*L. radiata* × *siliquoidea*	*L. siliquoidea*
GPI	16	1.00	.90	.45
	10		.10	.55
PGM I	18	0 to .34		.20
	16	.62 to 1.00	.92	.80
	14		.08	
	12	.04 to .20		
PGM II	30		.05	.45
	28	.85 to 1.00	.95	.55
	26	.05 to .15		
LAP	34	.03 to .47	.35	.35
	32	.53 to 1.00	.50	.45
	30	.04 to .30		
	28		.15	.20
MPI	24	1.00	1.00	.70
	22			.30
6PGD	6	0 to .10		
	4	.70 to 1.00	.40	.45
	2	.04 to .30	.60	.55
G3PDH	11	0 to .13		
	9	0 to .60	.40	
	7	.40 to 1.00	.60	1.00
GPDH	32	1.00	.95	.70
	30		.05	.30
SOD II	− 7	1.00	.70	.08
	− 9		.30	.92

origin of the *Lampsilis* population in Lake Champlain, and this population also possesses an allele not present in either parental species (PGM I 14). *L. radiata* possesses a total of 26 alleles over the 15 loci examined, *L. siliquoidea* 25, and L. *radiata* × *siliquoidea* 26. *L. radiata* shares 19 alleles with *L. siliquoidea*, and 20 alleles with *L. radiata* x *siliquoidea*, while *L. siliquoidea* shares 24 alleles with the hybrid population.

In contrast to these examples of hybridization between species of *Anodonta* and *Lampsilis*, the population of *Elliptio* in Lake Champlain presents no evidence that it is of hybrid origin between Atlantic Slope *E. complanata* and Interior Basin *E. dilatata*. Rather, this population exhibits affinities to regional populations of *E. complanata* in Vermont and Maine.

Nei's (1972) genetic distances and similarities between all pairs of *Anodonta*, *Lampsilis*, and *Elliptio* populations examined are presented in Tables 3, 4, and 5, respectively. Table 3 indicates that *A. "cataracta" fragilis* is genetically almost invariant from Nova Scotia through Maine and Vermont, and is distantly related to *A. cataracta* and *A. grandis*. *A. cataracta* and *A. grandis* are genetically similar at a level of 0.649 ± .012, which is comparable to levels of similarity among other species in the *cataracta* clade such as *A. gibbosa* from Georgia (Kat, 1983d). *A. cataracta* × *grandis* exhibits intermediate levels of

TABLE 3. Genetic distances (above the diagonal) and similarities (below the diagonal between all pairs of *Anodonta* populations examined in this study. See Appendix for locations of the collection sites.

	Population										Species
	ME1	VT3	NS4	NS6	NS2	NJ2	DE2	NJ1	VT1	MI	
ME1	—	.001	.001	.001	.003	.544	.561	.568	.622	.585	*A. fragilis*
VT3	.999	—	.001	.001	.002	.542	.559	.566	.620	.583	*A. fragilis*
NS4	.999	.999	—	.001	.003	.541	.558	.564	.616	.538	*A. fragilis*
NS6	.999	.999	.999	—	.003	.541	.558	.564	.616	.538	*A. fragilis*
NS2	.998	.998	.999	.999	—	.542	.557	.563	.609	.581	*A. fragilis*
NJ2	.581	.582	.582	.582	.582	—	.003	.003	.222	.453	*A. cataracta*
DE2	.571	.572	.572	.572	.573	.999	—	.001	.224	.420	*A. cataracta*
NJ1	.567	.568	.569	.569	.570	.998	.999	—	.225	.423	*A. cataracta*
VT1	.537	.538	.540	.540	.544	.801	.800	.799	—	.124	hybrid
MI	.557	.558	.584	.584	.560	.636	.657	.655	.883	—	*A. grandis*

TABLE 4. Genetic distances (above the diagonal) and similarities (below the diagonal) between all pairs of *Lampsilis* populations examined. See Appendix for locations of collection sites.

	Population												Species
	NS7	NS4	NS1	NB	NS3	VT3	DE3	MD1	ME4	DE1	VT1	MI	
NS7	—	.014	.004	.009	.021	.022	.020	.023	.029	.051	.056	.209	*L. radiata*
NS4	.986	—	.011	.012	.033	.025	.024	.019	.031	.047	.052	.199	*L. radiata*
NS1	.999	.984	—	.011	.025	.026	.022	.027	.033	.059	.064	.212	*L. radiata*
NB	.991	.988	.989	—	.014	.018	.011	.011	.017	.029	.047	.218	*L. radiata*
NS3	.979	.968	.976	.986	—	.017	.003	.016	.014	.031	.052	.231	*L. radiata*
VT3	.978	.975	.974	.982	.983	—	.019	.016	.016	.027	.027	.204	*L. radiata*
DE3	.980	.977	.978	.989	.996	.981	—	.011	.010	.026	.043	.218	*L. radiata*
MD1	.977	.981	.974	.989	.984	.984	.989	—	.003	.012	.038	.208	*L. radiata*
MD4	.971	.970	.968	.983	.986	.984	.990	.995	—	.013	.037	.203	*L. radiata*
DE1	.950	.954	.943	.971	.970	.973	.974	.988	.987	—	.029	.220	*L. radiata*
VT1	.946	.950	.938	.954	.950	.973	.958	.963	.964	.971	—	.124	hybrid
MI	.811	.820	.809	.804	.794	.804	.812	.816	.802	.815	.881	—	*L. siliquoidea*

TABLE 5. Genetic distances (above the diagonal) and similarities (below the diagonal) between all pairs of *Elliptio* populations sampled in this study. See Appendix for locations of the collection sites.

	Population																Species
	VT1	VT3	ME1	VT2	MD4	MD2	MD3	PA	ME2	DE3	MD1	NJ2	NS8	NS7	NS5	WI	
VT1	—	.026	.046	.066	.041	.076	.059	.072	.052	.068	.041	.045	.052	.035	.033	.087	*E. complanata*
VT3	.976	—	.015	.072	.016	.059	.041	.089	.016	.057	.017	.012	.016	.015	.033	.044	*E. complanata*
ME1	.955	.985	—	.069	.016	.060	.048	.099	.025	.059	.023	.012	.024	.016	.040	.044	*E. complanata*
VT2	.936	.931	.932	—	.063	.088	.093	.078	.098	.101	.076	.076	.087	.063	.097	.124	*E. complanata*
MD4	.960	.984	.984	.939	—	.027	.023	.107	.035	.045	.022	.018	.028	.023	.053	.054	*E. complanata*
MD2	.927	.943	.942	.916	.973	—	.013	.144	.085	.035	.055	.057	.064	.064	.099	.072	*E. complanata*
MD3	.943	.960	.954	.911	.977	.987	—	.129	.064	.027	.040	.042	.044	.049	.073	.070	*E. complanata*
PA	.931	.915	.905	.925	.898	.866	.879	—	.089	.134	.089	.120	.126	.089	.094	.157	*E. complanata*
ME2	.949	.984	.975	.907	.965	.919	.938	.915	—	.067	.018	.019	.017	.020	.035	.053	*E. complanata*
DE3	.934	.944	.943	.904	.956	.966	.974	.874	.935	—	.043	.052	.052	.061	.086	.070	*E. complanata*
MD1	.960	.983	.977	.927	.978	.946	.960	.915	.982	.958	—	.017	.019	.014	.035	.059	*E. complanata*
NJ2	.956	.989	.988	.927	.982	.944	.959	.887	.981	.949	.983	—	.007	.015	.037	.051	*E. complanata*
NS8	.949	.984	.976	.917	.972	.938	.957	.882	.983	.949	.982	.993	—	.022	.037	.051	*E. complanata*
NS7	.966	.985	.984	.939	.977	.938	.953	.915	.980	.941	.987	.985	.979	—	.014	.046	*E. complanata*
NS5	.968	.967	.961	.907	.948	.906	.930	.910	.966	.917	.966	.964	.963	.986	—	.048	*E. complanata*
WI	.917	.957	.957	.883	.948	.930	.932	.855	.948	.932	.943	.951	.950	.955	.953	—	*E. dilatata*

similarity to both *A. grandis* (0.883) and *A. cataracta* (0.800).

Populations of *Lampsilis radiata* from the Delmarva Peninsula to Nova Scotia exhibit an average level of interpopulation similarity of 0.979 ± .012, and *L. radiata* and *L. siliquoidea* resemble each other at a level of 0.808 ± .007 (Table 4). This degree of resemblance is comparable to that observed among other species of the *radiata* clade such as southern Atlantic Slope *L. splendida* (Kat, 1983c). *L. radiata* × *siliquoidea* from Lake Champlain resembles *L. radiata* at a level of 0.954 ± .010 and *L. siliquoidea* at a level of 0.881: the higher degree of resemblance to *L. radiata* reflects greater similarity in the frequencies of shared alleles. Interestingly, Clarke & Berg (1959) also classified the Lake Champlain *Lampsilis* population as more *radiata*-like than *siliquoidea*-like based on conchological characters.

Populations of *Elliptio complanata* from the Delmarva Peninsula to Nova Scotia, Maine, and Vermont exhibit characteristically high levels of variability in genetic resemblance among populations, ranging from 0.993 to 0.866, with an average degree of resemblance of 0.950 ± .030 (Table 5). *E. complanata* resembles *E. dilatata* from Wisconsin at a level of 0.934 ± .028. This high level of resemblance among species within diversifying *Elliptio* clades is common (see Davis *et al.*, 1981; Davis, 1984). Table 5 indicates, however, that the Lake Champlain *Elliptio* population is most closely related to populations of *E. complanata* from Vermont, Maine, and Nova Scotia, and in fact exhibits less affinity with *E. dilatata* than other northeastern populations of *E. complanata*.

Minimum spanning trees based on genetic distances and connecting all populations of *Elliptio*, *Lampsilis*, and *Anodonta* are illustrated in Fig. 1. The distance measure between populations is a function (variable over each analysis) of the Nei genetic distances, and thus corresponds to taxonomic relatedness. Such distances are small, for example, among populations of *A. cataracta* and *A. "cataracta" fragilis*, but considerable between these species. *A. cataracta* ± *grandis* is shown to be almost equidistant between *A. cataracta* and *A. grandis*, while *L. radiata* × *siliquoidea* clusters considerably closer to *L. radiata* than *L. siliquoidea*. The minimum spanning tree between *Elliptio* populations generally connects geographically neighboring populations. Divergent populations within this group are those from Joes Pond, Vermont, and the Susquehanna River, Pennsylvania, both due to high frequencies of otherwise rare alleles at loci such as LAP and MPI.

Table 6 contains levels of observed heterozygosity and polymorphism for all populations of *Elliptio*, *Lampsilis*, and *Anodonta* examined. Populations of *Elliptio* exhibit characteristically high levels of H and P (average H = 0.139 ± .014; average P = 0.517 ± .054) except among peripheral populations in Nova Scotia (see Kat & Davis, 1984). Heterozygosity and polymorphism among populations of *Lampsilis* are characteristically lower than those observed among *Elliptio*, except in the case of *L. siliquoidea* from Michigan, which possesses the highest level of H and P thus far observed for any lampsiline population (see Kat, 1983c). *L. radiata* × *siliquoidea* from Lake Champlain is not more heterozygous than either parent (average H for central range populations of *L. radiata* = 0.058 ± .004), but exhibits a level of polymorphism equal to that of *L. siliquoidea*. Levels of heterozygosity for anodontine populations presented here are higher than those published earlier (Kat, 1983d) due to inclusion of loci with fixed heterozygosities (GPI for all species and MDH I for *A. "c." fragilis*). *A. cataracta* × *grandis* from Lake Champlain is considerably more heterozygous than either parent species.

B. Stomach anatomy

Fine detail of stomach anatomy can be used to discriminate between hybrids and parental species of the lampsilines and anodontines examined in this study. The *Elliptio* population in Lake Champlain (Fig. 2) is very similar in stomach anatomy to *E. complanata* from eastern Canada (see Kat, 1983a), as well as *E. dilatata* from Wisconsin (not figured). Stomach anatomy of *L. radiata* × *siliquoidea* from Lake Champlain (Fig. 3) is quite similar to that of *L. radiata* from eastern Canada (see Kat, 1983c) and the Delmarva Peninsula (Fig. 4), and also that of *L. siliquoidea* from Michigan (Fig. 3A). Differences are apparent, however, in the curvature of the minor typhlosole fold. *A. cataracta* × *grandis* in Lake Champlain (Fig. 5) also differs from *A. cataracta* from Virginia and *A. grandis* from Tennessee and Michigan in details of the minor typhlosole fold. This fold is gently rounded

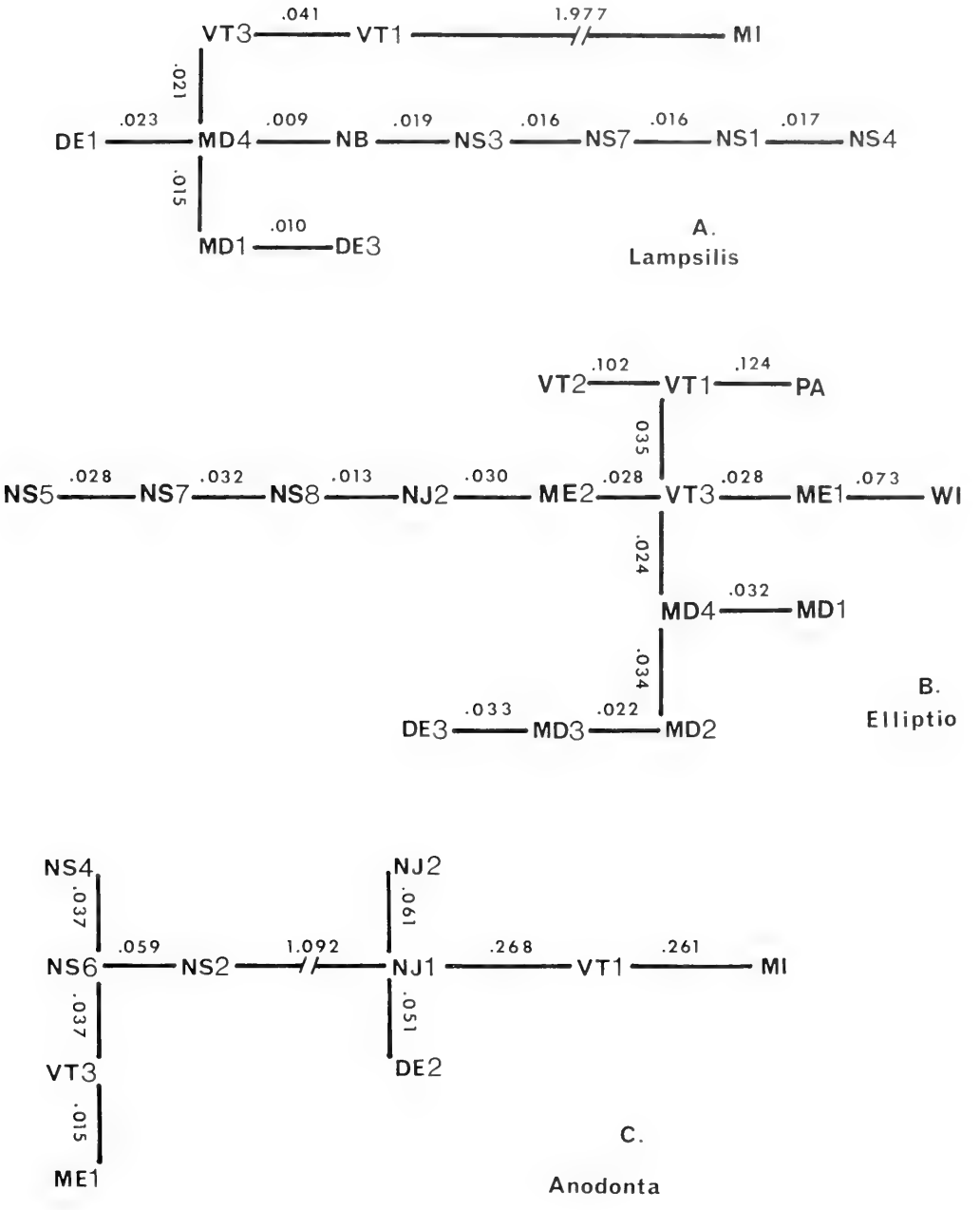


FIG. 1. Minimum spanning trees for all populations of *Lampsilis*, *Elliptio*, and *Anodonta*. Among the lampsilines, all populations except MI (*L. siliquoidea*) and VT1 (hybrid) are *L. radiata*. Among *Elliptio*, all populations except WI (*E. dilatata*) are *E. complanata*, although populations from Joes Pond, Vermont (VT2) and the Susquehanna River, Pennsylvania (PA) are relatively distant from other *E. complanata* populations. Among the anodontines, NS4, NS6, NS2, VT3, and ME1 are populations of *A. fragilis*, NJ2, NJ1, and DE2 are populations of *A. cataracta*, VT1 is a hybrid, and MI represents *A. grandis*. The distance measure between populations is a function of the Nei genetic distance

TABLE 6. Levels of heterozygosity (H) and polymorphism (P) for all species included in this study.

	H	P
Elliptio complanata	0.122 ± .037	0.499 ± .066
Elliptio dilatata	0.104	0.428
Lampsilis radiata	0.038 ± .023	0.305 ± .132
Lampsilis siliquoidea	0.113	0.600
L. radiata × *siliquoidea*	0.053	0.600
Anodonta cataracta	0.098 ± .003	0.142
Anodonta grandis	0.192	0.500
A. grandis × *cataracta*	0.256	0.570
Anodonta fragilis	0.148 ± .006	0.185 ± .039

in *A. cataracta* (see Kat, 1983d) but becomes more angular in *A. cataracta* × *grandis* (Fig. 5, 5A), and is V- or U-shaped in *A. grandis* (Fig. 5B, 5C). Overall stomach anatomy among anodontines of the *cataracta* group (*A. cataracta*, *A. grandis*, *A. gibbosa*) is quite similar (Kat, 1983d). *A. "cataracta" fragilis*, however, differs strongly from *A. cataracta* in stomach anatomy (Fig. 6, 6A) and there is no evidence from this character to suggest that *A. cataracta* and *A. fragilis* hybridize either in Nova Scotia or in New England.

C. Conchiolin layer microstructure

Conchiolin layers among unionids are composed of three parts: an upper, homogeneous region; a central, reticulate region that consists of a number of thin, usually vertically arranged lamellae that form chambers of various shapes and dimensions; and a lower, very thin homogeneous region (Kat, 1983e). Microstructure of conchiolin layers can discriminate among parental species and Lake Champlain hybrids of *Anodonta* and *Lamps-*

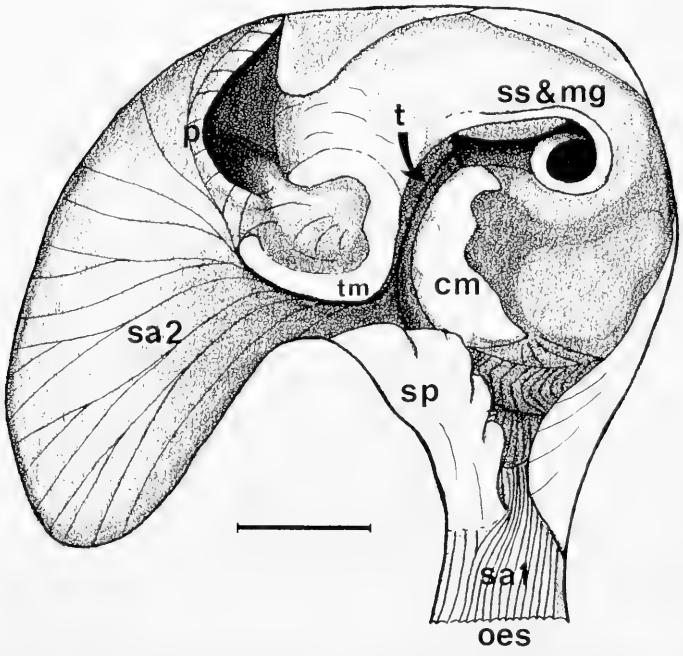


FIG. 2. Stomach floor of *Elliptio complanata* from Lake Champlain. Abbreviations: cm - conical mound, oes - oesophagus, p - sorting pouch, sa1 - sorting area 1, sa2 - sorting area 2, sp - sorting platform, ss & mg - style sac and midgut, t - major typhlosole, tm - minor typhlosole fold. Scale bar = 2 mm.

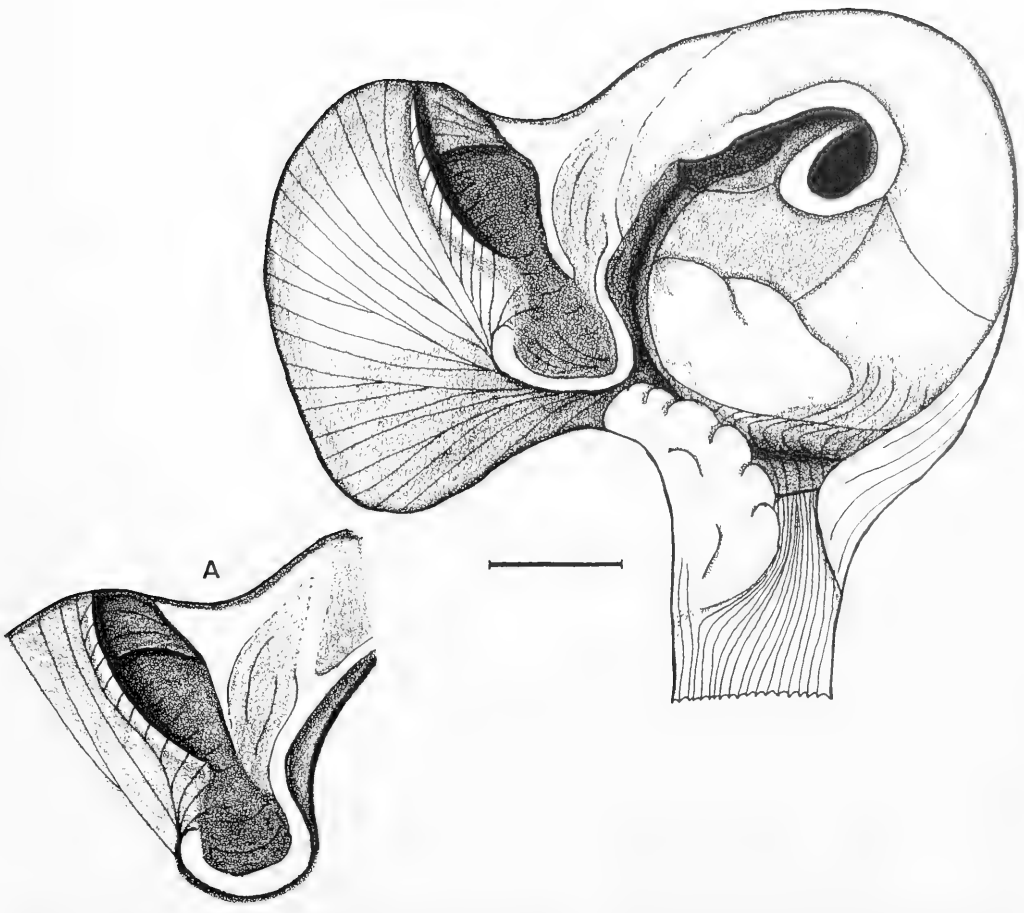


FIG. 3. Stomach floor of *Lampsilis radiata* × *siliquoidea* from Lake Champlain; inset A depicts the minor typhlosole of *L. siliquoidea* from Michigan. Scale bar = 2 mm; structures as in Fig. 2.

ilis, and suggests that Lake Champlain and surrounding areas are inhabited by a distinct subgroup of *Elliptio complanata*. *E. complanata* from Virginia and the southern Delmarva Peninsula are characterized by reticulate regions with short, widely-spaced lamellae that enclose triangular or rectangular chambers of various sizes (Kat, 1983a; Pl. 1:1). *E. complanata* on the northern Atlantic Slope possesses longer, straighter lamellae that enclose rather elongate, narrow chambers (Kat, 1983a; Pl. 1:2). *E. dilatata* from western North Carolina (Pl. 1:3), Wisconsin (Pl. 2:2), and western Ontario (Pl. 2:1) are characterized by a thin upper homogeneous region and long, vertical lamellae. *E. complanata* from Vermont (Pl. 1:4 and 1:5) and Lake Champlain (Pl. 1:6) all possess highly characteristic curved and striated lamellae that enclose variably shaped chambers. This particular conchiolin layer microstructure has not been observed in any other region of the geographic range of *E. complanata*, although similarly striated lamellae occur in a hybrid zone between races of *E. complanata* on the Delmarva Peninsula (Kat, 1983a).

Conchiolin layer microstructure of *Anodonta cataracta* (Pl. 2:4) consists of a thin upper homogeneous layer underlain by a poorly defined reticulate region composed of small, irregular chambers. *A. grandis* possesses a better defined reticulate region

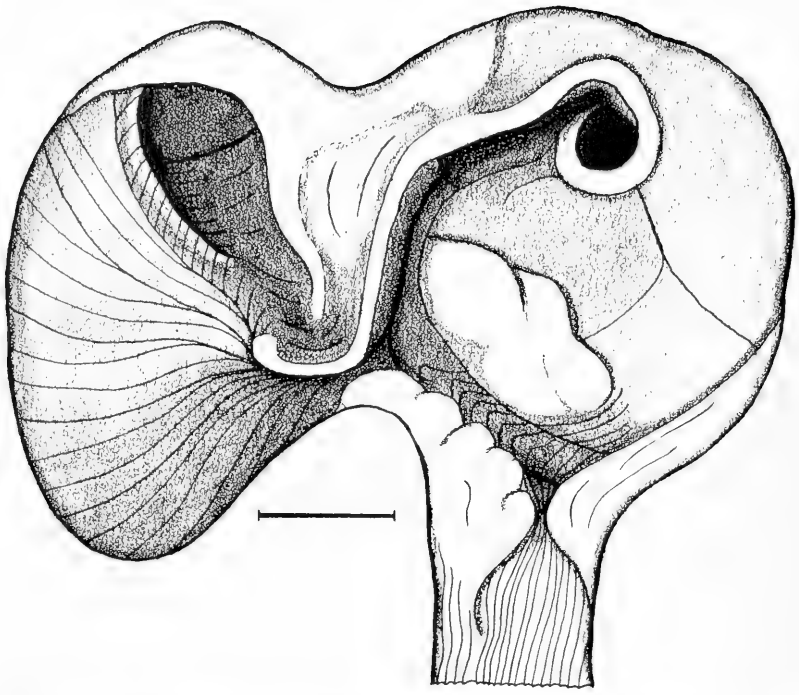


FIG. 4. Stomach floor of *Lampsilis radiata* from the Delmarva Peninsula (Andover Branch). Scale bar = 2 mm.

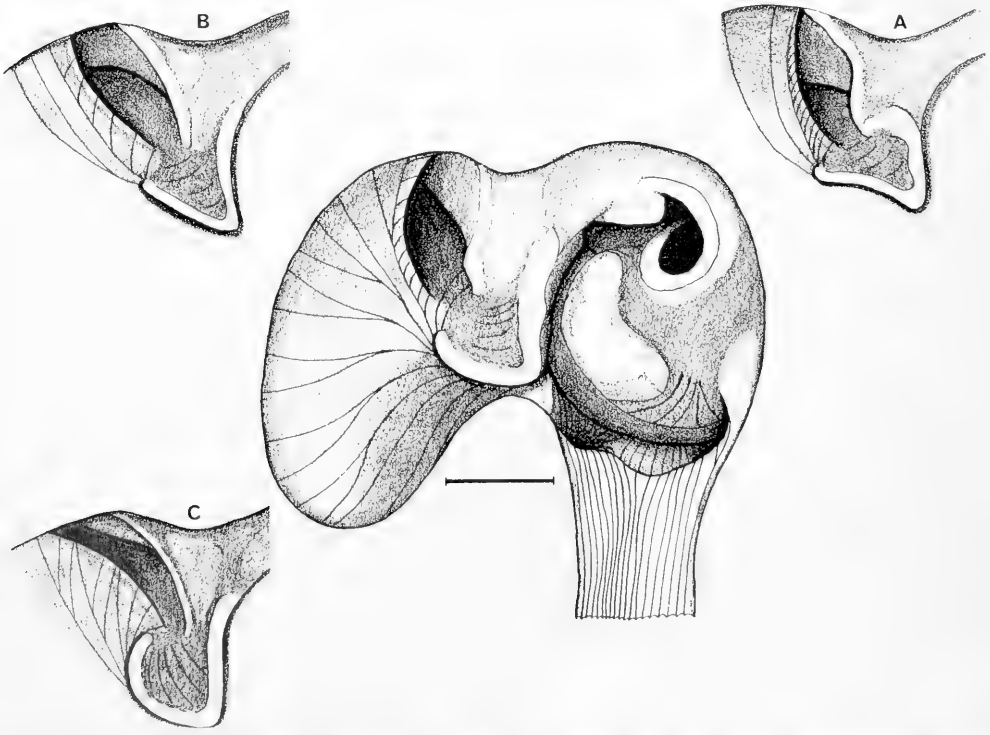


FIG. 5. Stomach floor of *Anodonta cataracta* x *grandis* from lake Champlain; inset A represents an extreme variant in the same population; inset B depicts the minor typhlosole fold of *A. grandis* from Tennessee; inset C shows the minor typhlosole fold of *A. grandis* from Michigan. Scale bar = 2 mm.

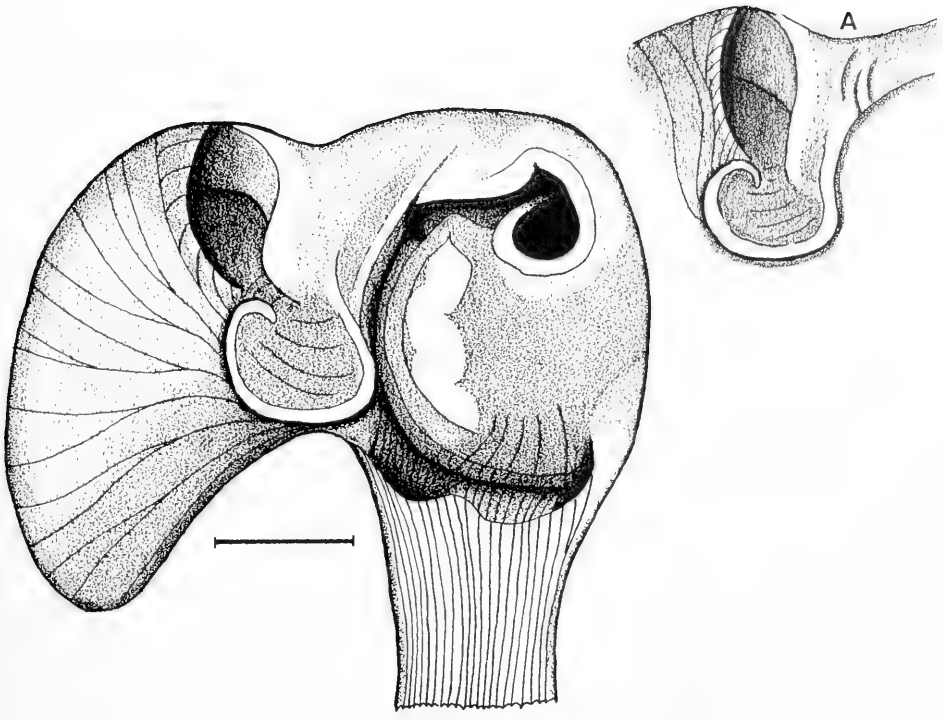


FIG. 6. Stomach floor of *Anodonta fragilis* from Maine (Lake St. George). Inset shows the minor typhlosole fold of *A. fragilis* from Nova Scotia (Placide Lake). Scale bar = 2 mm.

(Pl. 2:5), and *A. cataracta* × *grandis* possesses a reticulate region characterized by thick, roughly vertical lamellae that enclose variably sized and shaped chambers (Pl. 2:6). *A. "cataracta" fragilis* (Pl. 2:3) has a conchiolin layer microstructure very different from that of *A. cataracta*.

The conchiolin layer of *Lampsilis radiata* (Pl. 3:1, 3:2, 3:3, and 3:4) is characterized by a thick upper homogeneous region and a reticulate region composed of poorly defined, digitiform to blocky lamellae. *L. siliquoidea* (Pl. 3:6) also possesses a thick homogeneous region but has a reticulate region composed of densely packed, jagged lamellae. *L. radiata* × *siliquoidea* (Pl. 3:5) has a rather disorganized reticulate region composed of irregular, blocky lamellae.

DISCUSSION

Studies dealing with genetics of hybrid zones and dynamics of hybridization are numerous. Some of these studies indicate considerable genetic exchange within the hybrid zone and some, possibly asymmetrical, introgression elsewhere (e.g. Hunt & Selander, 1973; Avise & Smith, 1974; Patton *et al.*, 1979; Moran *et al.*, 1980; Hafner, 1982). Other studies report hybridization without any or much introgression beyond the often narrow hybrid zone (e.g. Nevo & Bar-El, 1976; McDonnell *et al.*, 1978; Sage & Selander, 1979; Barton *et al.*, 1983). While hybridization between *Anodonta grandis* and *A. cataracta* and *Lampsilis radiata* and *L. siliquoidea* is documented here, too few Interior Basin localities in particular were examined to be able to determine the extent of introgression. There is some evidence, however, that introgression takes place over a wide geographic area: Atlantic Slope *L. radiata* and *A. cataracta* exhibit a much higher frequency of fixed alleles among the 15 loci examined than do *A. grandis* and *L. siliquoidea* from Michigan, which both possess many "Atlantic Slope" alleles in low frequencies. Also, two of the loci

PLATE 1. Conchiolin layer microstructure of *Elliptio*. 1. Southern Atlantic Slope *E. complanata*; 2. Northern Atlantic Slope *E. complanata*; 3. *E. dilatata*; 4, 5. *E. complanata* from Vermont; 6. *E. complanata* from Lake Champlain. Scale bars = 10 µm.

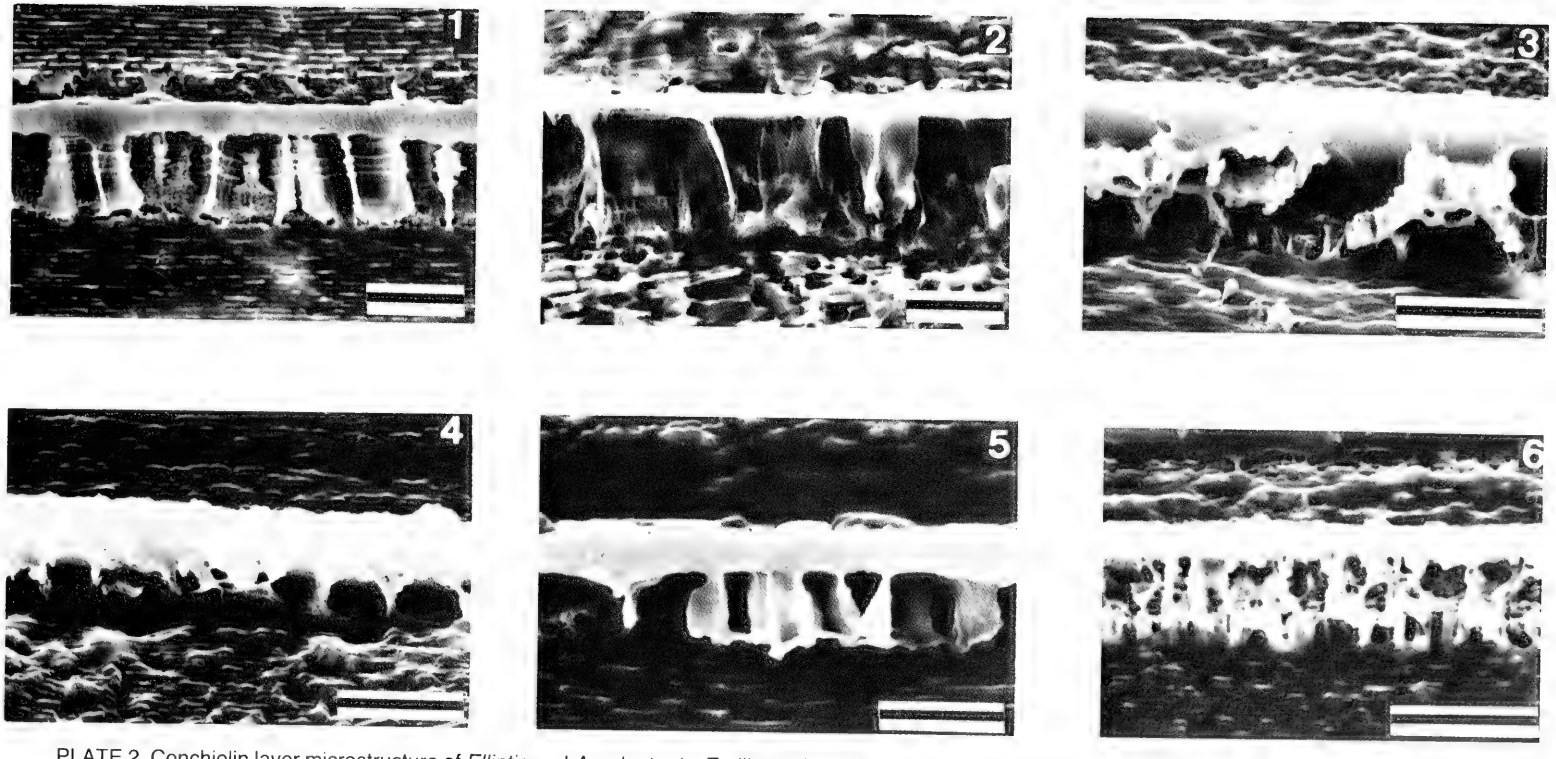


PLATE 2. Conchiolin layer microstructure of *Elliptio* and *Anodonta*. 1. *E. dilatata* from Wisconsin; 2. *E. dilatata* from Ontario; 3. *A. fragilis* from Nova Scotia; 4. *A. cataracta* from Pennsylvania; 5. *A. grandis* from Michigan; 6. *A cataracta* × *grandis* from Lake Champlain. Scale bars = 10μm.

PLATE 3. Conchiolin layer microstructure of *Lampsilis*. 1. *L. radiata* from Nova Scotia; 2. *L. radiata* from Maryland; 3. *L. radiata* from Nova Scotia; 4. *L. radiata* from Vermont, 5. *L. radiata* × *siliquoidea* from Lake Champlain; 6. *L. siliquoidea* from Michigan. Scale bars = 10μm.

surveyed possess soluble as well as mitochondrial forms: MDH and SOD (Davidson & Cortner, 1967; Beckman, 1973; Harris & Hopkinson, 1978). Since mitochondrial genes are exclusively inherited through maternal lines, alleles at these loci can be used to estimate relative parental contributions to hybrids and thus levels of introgression. Lake Champlain populations of *Anodonta* and *Lampsilis* contain 65% *cataracta* and 70% *radiata* alleles at these loci, for example, while Michigan populations contain 45% *cataracta* and 8% *radiata* alleles. If incidence of introgression is confirmed by genetic examination of "pure" populations of *A. grandis* and *Lampsilis siliquoidea* south of the maximal glacial advance, it would suggest that hybrids between these species are not inferior to their parents in terms of maladaptation to external environments and/or disruption of balanced gene complexes (Sage & Selander, 1979: Moore, 1977; see below). The population of *Elliptio* in Lake Champlain exhibits no evidence of a hybrid origin, and is genetically and morphologically related to populations of Atlantic Slope *E. complanata*. Conchiolin layer microstructure of populations of *E. complanata* from Lake Champlain and Vermont is different from that of other northern Atlantic Slope populations, but such divisions of *E. complanata* into locally differentiated populations have been observed before and are apparently characteristic of this species (Kat, 1983a; Kat & Davis, 1984). If *E. complanata* and *E. dilatata* hybridize, it probably occurs farther west than Lake Champlain, and the location of the hybrid zone could well reflect differences in routes of recolonization taken by *Elliptio* when compared with *Lampsilis* and *Anodonta*. For example, Interior Basin *Anodonta* and *Lampsilis* apparently followed retreating glaciers closely: subfossil *A. grandis* and *L. siliquoidea* occur in Lake Algonquin (12,000 to 10,000 B.P.) and Transitional (10,000 to 6,000 B.P.) sediments, respectively, in southwestern Ontario (Miller *et al.*, 1979), and subfossil *Lampsilis* (species undetermined) occur in 7,000 year old sediments around Lake Champlain (Elson, 1969). *E. complanata*, according to Clarke & Berg (1959), occurs westward to the upper great lakes (Ontario, Huron, Superior), and probably colonized this area via Lake Newbery, which inundated the present Finger Lakes basin in New York and drained into the Susquehanna River of the Atlantic Slope. Clarke & Berg (1959) also state that *E. dilatata* co-occurs with *E. complanata* in the St. Lawrence River drainage, but geographic ranges of these phenotypically variable species need to be confirmed with more reliable taxonomic methods than the conchological characters of previous authors.

Evolutionary biologists disagree on the stability of hybrid zones through time. Remington (1968) has argued, for example, that suture zones are ephemeral, leading either to fusion of parental gene pools or separation with perfection of reproductive isolating mechanisms. In contrast, others have indicated that zones of hybridization can be temporally stable and of ancient age (Short, 1972; Hunt & Selander, 1973; Sage & Selander, 1979). There can be little doubt that the hybrid zone between Interior Basin and Atlantic Slope *Anodonta* and *Lampsilis* is of postglacial origin, and the position of the hybrid zone in a repeatedly glaciated area implies that it will last only as long as the present interglacial stage. Evidence that a previous suture zone between these faunas was disrupted by glaciers is provided by the Fish House fossil assemblage near Camden, New Jersey (Kat, 1983b). Interestingly, this fauna contains no morphologic intermediates between sympatric *A. cataracta* and *A. grandis* to suggest that those species were then hybridizing. This lack of genetic interaction was proposed to have resulted from perfection of isolating mechanisms in the zone of sympatry. These adaptations were subsequently lost as sympatric populations were eliminated by glaciers and the geographic range of *A. grandis* restricted to refuges west of the Appalachian mountains. Reestablishment of contact between these species would thus again, at least initially, involve hybridization (Kat, 1983b).

Observations that alleles that are either rare or absent in parental populations occur in appreciable frequencies in hybrid zones are not uncommon. This phenomenon was first described from a land snail hybrid zone (Clarke, 1968) and since then, Hunt & Selander (1973) have found variant esterase alleles in a hybrid zone between semispecies of house mice, and Sage & Selander (1979) described unusual alleles at five of ten loci among hybrid frog populations. Other examples of rare alleles occurring within hybrid zones have been documented by Woodruff (1981) and Barton *et al.* (1983). In this study, the hybrid *Anodonta* population possesses

three variant alleles, while that of *Lampsilis* contains one. Hunt & Selander (1973) proposed that introgression modifies parental gene pools to relax incorporation against new alleles, and Stebbins (1971) suggested that minor alleles could be favored in low frequencies in the new genetic environment created by hybridization. More recently, Thompson & Woodruff (1978) and Woodruff *et al.* (1982) suggested that these new alleles might result from increased mutation rates among hybrids (as a consequence of heterozygosities involving dissimilar alleles), and Watt (1972) proposed that such new alleles could result from intragenic recombination between parental alleles. Such intragenic recombination has been proposed to explain patterns of allelic variability by various authors (Ohno *et al.*, 1969; Koehn & Eanes, 1976; Morgan & Strobeck, 1979; Tsuno, 1981), and Golding & Strobeck (1983) showed mathematically that sympatry of two previously isolated populations can increase the effective number of alleles maintained in the hybrid as well as parental populations. Whether these novel alleles do actually spread from hybrid zones to contribute to allelic diversity of parental populations has not been documented. In this study, increased levels of heterozygosity could be proposed to account for the appearance of variant alleles in the Lake Champlain population of *Anodonta* but not for that of *Lampsilis*, since levels of heterozygosity of the hybrid lampsiline population are comparable to, or lower than, those of its parent species.

Anodonta cataracta and *A. grandis*, and to a lesser extent *Lampsilis radiata* and *Lampsilis siliquoidea*, are genetically divergent species that apparently hybridize readily where their geographic ranges come into contact. The large and fertile (demibranchs of both hybrids were filled with glochidia at the time of collection) populations in Lake Champlain and elsewhere in the hybrid zone would appear to indicate that there is little selection against hybridization, although relative levels of fitness of hybrids and parents are not known. Unionids seem susceptible to accidental hybridization because of their external mode of fertilization, but could theoretically experience penalties for hybridization because of their complicated life cycle that includes an obligate parasitic stage. Parasitism involves genes controlling host recognition as well as genes involved with glochidial survival while on the host, and such gene complexes would appear to be highly species-specific (Bush, 1975a, b; Kat, 1984). Hybridization will likely disrupt adapted gene complexes, except possibly among unionid species possessing very generalized survival and host recognition genes. Such species are perhaps epitomized by *A. grandis*, which parasitizes over 30 fish hosts (Trdan & Hoeh, 1982), and to a lesser extent by *L. siliquoidea*, which parasitizes about 12 hosts (Trdan, 1981). Species like *E. complanata* and *A. implicata*, however, are only known to parasitize two and one hosts, respectively. Hybridization should be most strongly selected against among these more specialized unionids, unless both parental species parasitize the same host, an occurrence likely in the case of two *E. complanata* races that hybridize on the Delmarva Peninsula (Kat, 1983a). Similarity of hosts should therefore be a more reliable predictor of hybridization between related unionid species than levels of electrophoretically detected differentiation.

The data gathered in this study also have bearing on two taxonomic questions. First, Clarke & Berg (1959) suggested that in view of the width of the hybrid zone, *Lampsilis siliquoidea* should be reduced to the subspecies *L. radiata siliquoidea*. I disagree with this interpretation: *L. siliquoidea* from Michigan (which might constitute a periphery of the zone of introgression) already exhibits the same level of genetic divergence from *L. radiata* as do other recognized lampsiline species such as *L. splendida* from Georgia and *Lampsilis* sp. from Lake Waccamaw, North Carolina (Kat, 1983c). Also, stomach anatomy and conchiolin layer microstructure of these taxa are quite different. I suggest, therefore, that the taxon *L. radiata siliquoidea* be reserved to describe hybrid populations such as that in Lake Champlain, and that *L. siliquoidea* be used to describe Interior Basin populations outside the hybrid zone. Second, Clarke & Rick (1963) named *Anodonta cataracta fragilis* to describe phenotypic (umbonal sculpture) intergrades between *A. fragilis* from Newfoundland and *A. cataracta*. In this study and others (Kat, 1983d, e; Kat & Davis, 1984) genetic, anatomical, and conchiolin layer microstructural data suggest that all populations of *A. "cataracta" fragilis* are very distinct from *A. cataracta*, and that there is no evidence to suggest any hybridization between these taxa. I propose that, unless an

analysis of *A. fragilis* (based on a diversity of data) from Newfoundland (the type locality) reveals substantial differences from *A. "cataracta" fragilis* from Nova Scotia and northern New England, these taxa be considered synonymous and distinct from *A. cataracta*.

ACKNOWLEDGMENTS

Discussions with George Davis, Karl Kaufman, Gene Meyer, Blaire Van Valkenburgh, and Bob Wayne improved versions of the manuscript. I am indebted to Richard Trdan for collecting specimens from Wiggins Lake in Michigan, without which this paper could not have been written. Bill Manning greatly facilitated collecting in Lakes Memphremagog and Champlain. This study was supported, in part, by the National Science Foundation (DEB 78-01550 and DEB 81-18963), The Jessup Fund of the Academy of Natural Sciences, and a National Capital Shell Club scholarship.

REFERENCES CITED

ANDERSON, E., 1949, *Introgressive hybridization*. Wiley, New York, ix + 109 p.

ANDERSON, E. & STEBBINS, G. L., 1954, Hybridization as an evolutionary stimulus. *Evolution*, 8: 378–388.

AVISE, J. C. & SMITH, M. H., 1974, Biochemical genetics of sunfish. I. Geographic variation and subspecific intergradation in the bluegill, *Lepomis macrochirus*. *Evolution*, 28: 42–56.

AYALA, F. J., HEDGECOCK, D., ZUMWALT, G. S. & VALENTINE, J. W., 1973, Genetic variation in *Tridacna maxima*, an ecological analog of some unsuccessful evolutionary lineages. *Evolution*, 27: 177–191.

BAKER, F. C., 1920, The life of the Pleistocene or glacial period. . . . *University of Illinois Bulletin* 17(41): xiv + 476 p.

BARTON, N. H., HALLIDAY, R. B. & HEWITT, G. M., 1983, Rare electrophoretic variants in a hybrid zone. *Heredity*, 50: 139–146.

BECKMAN, G., 1973, Population studies in northern Sweden. IV. Polymorphism of superoxide dismutase. *Hereditas*, 73: 305–313.

BUSH, G. L., 1975a, Modes of animal speciation. *Annual Review of Ecology and Systematics*, 6: 339–364.

BUSH, G. L., 1975b, Sympatric speciation in phytophagous parasitic insects. In PRICE, P.M., ed., *Evolutionary strategies of parasitic insects*. Plenum Press, London.

CLARKE, A. H., Jr., 1973, The freshwater molluscs of the Canadian Interior Basin. *Malacologia*, 13: 1–509.

CLARKE, A. H., Jr. & BERG, C. O., 1959, The freshwater mussels of central New York. *Cornell University Agricultural Experiment Station Memoir* 367: 1–79.

CLARKE, A. H., Jr. & RICK, A. M., 1963, Supplementary records of Unionacea from Nova Scotia with a discussion of the identity of *Anodonta fragilis* Lamarck. *National Museums of Canada Bulletin*, 199: 15–27.

CLARKE, B. C., 1968, Balanced polymorphism and regional differentiation in land snails. In DRAKE, E. T., ed., *Evolution and environment*. Yale University Press, New Haven and London, p. 351–368.

DAVIDSON, R. G. & CORTNER, J. A., 1967, Mitochondrial malate dehydrogenase: a new genetic polymorphism in man. *Science*, 157: 1569–1570.

DAVIS, G. M., 1983, Relative roles of molecular genetics, anatomy, morphometrics and ecology in assessing relationships among North American Unionidae (Bivalvia). In OXFORD, G. S. & ROLLINSON, D., ed., *Protein polymorphism: adaptive and taxonomic significance*. Academic Press, London, p. 193–222.

DAVIS, G. M., 1984, Genetic relationships among some North American Unionidae (Bivalvia): sibling species, convergence, cladistic relationships. *Malacologia*, 25: 629–648.

DAVIS, G. M., HEARD, W. H., FULLER, S. L. H. & HESTERMAN, C., 1981, Molecular genetics and speciation in *Elliptio* and its relationships to other taxa of North American Unionidae (Bivalvia). *Biological Journal of the Linnean Society*, 15: 131–150.

ELSON, J. A., 1969, Late Quaternary marine submergence of Quebec. *Revue Géographique de Montréal*, 23: 247–258.

ENDLER, J. A., 1977, Geographic variation, speciation, and clines. *Monographs in Population Biology* (Princeton University Press), 10: ix + 246 p.

GALLEZ, G. P. & GOTTLIEB, L. D., 1982, Genetic evidence for the hybrid origin of the diploid plant *Stephanomeria diegensis*. *Evolution*, 36: 1158–1167.

GOLDING, G. B. & STROBECK, C., 1983, Increased number of alleles found in hybrid populations due to intragenic recombination. *Evolution*, 37: 17–29.

GRANT, V., 1966, The origin of a new species of *Gilia* in a hybridization experiment. *Genetics*, 54: 1189–1199.

GREEN, D. M., 1979, A BASIC computer program for calculating indices of genetic distance and similarity. *Journal of Heredity*, 70: 429–430.

HAFNER, J. C., 1982, Genetic interaction at a contact zone of *Urodema bilobatum* (Chiroptera: Phyllostomidae). *Evolution*, 36: 852–862.

HARRIS, H. & HOPKINSON, D. A., 1978, *Hand-*

book of enzyme electrophoresis in human genetics. North Holland Publishing Co., Amsterdam.
HUNT, W. G. & SELANDER, R. K., 1973, Biochemical genetics of hybridization in European house mice. *Heredity*, 31: 11–33.
JOHNSON, R. I., 1970, The systematics and zoogeography of the Unionidae (Mollusca: Bivalvia) of the southern Atlantic Slope region. *Bulletin of the Museum of Comparative Zoology*, 140: 263–450.
JOHNSON, R. I., 1980, Zoogeography of North American Unionidae (Mollusca: Bivalvia) north of the maximum Pleistocene glaciation. *Bulletin of the Museum of Comparative Zoology*, 149: 77–189.
KAT, P. W., 1982, The relationship between heterozygosity for enzyme loci and developmental homeostasis in peripheral populations of freshwater bivalves (Unionidae). *American Naturalist*, 119: 824–832.
KAT, P. W., 1983a, Patterns of electrophoretic and morphologic variability in a widely distributed unionid: an initial survey. *Netherlands Journal of Zoology*, 33: 21–40.
KAT, P. W., 1983b, Fossil evidence from Fish House clays for the origin and changes in species composition through time of the northern Atlantic Slope unionid fauna (Mollusca: Bivalvia). *Proceedings of the Academy of Natural Sciences of Philadelphia*, 135: 85–101.
KAT, P. W., 1983c, Morphologic divergence, genetics, and speciation among *Lampsilis* (Bivalvia: Unionidae). *Journal of Molluscan Studies*, 49: 133–145.
KAT, P. W., 1983d, Genetic and morphological divergence among nominal species of North American *Anodonta* (Bivalvia: Unionidae). *Malacologia*, 23: 361–374.
KAT, P. W., 1983e, Conchiolin layers among the Unionidae and Margaritiferidae (Bivalvia): microstructural characteristics and taxonomic implications. *Malacologia*, 24: 298–311.
KAT, P. W., 1984, Parasitism and the Unionacea (Bivalvia). *Biological Reviews*, 59: 189–207.
KAT, P. W. & DAVIS, G. M., 1984, Molecular genetics of peripheral populations of Nova Scotian Unionidae (Mollusca: Bivalvia). *Biological Journal of the Linnean Society*, 22: 157–185.
KOEHN, R. K. & EANES, W. F., 1976, An analysis of allelic diversity in natural populations of *Drosophila*: the correlation of rare alleles with heterozygosity. In KORBIN, S. & NEVO, E., ed., *Population genetics and ecology*. Academic Press, New York.
LEWIS, H., 1966, Speciation in flowering plants. *Science*, 152: 167–172.
McDONNELL, L. J., GARTSIDE, D. F. & LITTLEJOHN, M. J., 1978, Analysis of a narrow hybrid zone between two species of *Pseudophryne* (Anura: Leptodactylidae) in southeastern Australia. *Evolution*, 32: 602–612.
MILLER, B. B., KARROW, P. F. & KALAS, L. L., 1979, Late Quaternary mollusks from glacial Lake Algonquin, Nipissing, and Transitional sediments from southwestern Canada. *Quaternary Research*, 11: 93–112.
MOORE, W. S., 1977, An evaluation of narrow hybrid zones in vertebrates. *Quarterly Review of Biology*, 52: 263–277.
MORAN, C., WILKINSON, P. & SHAW, D. D., 1980, Allozyme variation across a narrow hybrid zone in the grasshopper, *Caledia captiva*. *Heredity*, 44: 69–81.
MORGAN, K. & STROBECK, C., 1979, Is intragenic recombination a factor in the maintenance of genetic variation in natural populations? *Nature*, 277: 383–384.
NEI, M., 1972, Genetic distance between populations. *American Naturalist*, 106: 283–291.
NEVO, E. & BAR-EL, H., 1976, Hybridization and speciation in fossorial mole rats. *Evolution*, 30: 831–840.
OHNO, S., STENIUS, C., CHRISTIAN, L. & SCHIPMANN, G., 1969, De novo mutation-like events observed at the 6PGD locus of the Japanese quail, and the principle of polymorphism breeding more polymorphism. *Biochemical Genetics*, 3: 417–428.
ORTMANN, A. E., 1913, The Alleghenian divide and its influence upon the freshwater fauna. *Proceedings of the American Philosophical Society*, 52: 287–390.
PATTON, J. L., HAFFNER, J. C., HAFFNER, M. S. & SMITH, M. F., 1979, Hybrid zones in *Thomomys bottae* pocket gophers: genetic, phenetic, and ecological concordance patterns. *Evolution*, 33: 860–867.
REMINGTON, C. L., 1968, Suture-zones of hybrid interaction between recently joined biotas. *Evolutionary Biology*, 2: 321–428.
ROHLF, E., KISHPAUGH, J. & KIRK, D., 1972, *NT-SYS: numerical taxonomy system of multivariate statistical programs*. State University of New York, Stony Brook, New York.
SAGE, D. E. & SELANDER, R. K., 1979, Hybridization between species of the *Rana pipiens* complex in central Texas. *Evolution*, 33: 1069–1088.
SHORT, L. L., 1972, Hybridization, taxonomy, and avian evolution. *Annals of the Missouri Botanical Garden*, 59: 447–453.
SIMPSON, C. T., 1896, On the Mississippi Valley Unionidae found in the St. Lawrence and Atlantic drainage areas. *American Naturalist*, 30: 379–384.
SMITH, D. G., 1982, The zoogeography of the freshwater mussels of the Taconic and southern Green Mountain region of northeastern North America (Mollusca: Pelecypoda: Unionacea). *Canadian Journal of Zoology*, 60: 261–267.
SMITH, D. G., 1983, Notes on Mississippi River basin Mollusca presently occurring in the Hudson River system. *Nautilus*, 97: 128–131.
THOMPSON, J. N. & WOODRUFF, R. C., 1978,

Mutator genes—pacemakers of evolution? *Nature*, 274: 317–321.

TRDAN, R. J., 1981, Reproductive biology of *L. radiata siliquoidea* (Pelecypoda: Unionidae). *American Midland Naturalist*, 106: 243–248.

TRDAN, R. J. & HOEH, W. R., 1982, Eurytopic host use by two congeneric species of freshwater mussel (Pelecypoda: Unionidae: *Anodonta*). *American Midland Naturalist*, 108: 381–388.

TSUNO, K., 1981, Studies on mutation at esterase loci in *Drosophila viridis*. I. Spontaneous mutation rates and newly arisen variants. *Japanese Journal of Genetics*, 56: 155–174.

WATT, W. B., 1972, Intragenic recombination as a source of population genetic variability. *American Naturalist*, 106: 737–753.

WHITE, M. J. D., 1978, *Modes of speciation*. Freeman, San Francisco, 455 p.

WOODRUFF, D. S., 1981, Toward a genodynamics of hybrid zones: studies of Australian frogs and West Indian land snails. In ATCHLEY, W. R. & WOODRUFF, D. S., ed., *Evolution and speciation*. Cambridge University Press, p. 171–197.

WOODRUFF, R. C., SLATKO, B. E. & THOMPSON, J. N., 1982, Factors affecting mutation rate in natural populations. In ASHBURNER, M., CARSON, H. L. & THOMPSON, J. N. eds, *Genetics and biology of* Drosophila, Vol. 3C, Academic Press, New York.

APPENDIX

Classification of species mentioned in the text, and location of the collection sites.

- Unionidae
  - Anodontinae
    - *Anodonta cataracta* Say
    - *Anodonta fragilis* Lamarck
    - *Anodonta gibbosa* Say
    - *Anodonta grandis* Say
  - Ambleminae
    - Lampsilini
      - *Lampsilis radiata* (Gmelin)
      - *Lampsilis siliquoidea* (Barnes)
      - *Lampsilis splendida* (Lea)
    - Pleurobemini
      - *Elliptio complanata* (Lightfoot)
      - *Elliptio dilatata* (Rafinesque)

DE1	Andover Branch, Millington, Kent Co., Delaware
DE2	Concord Pond, Sussex Co., Delaware
DE3	Deep Creek, Nanticoke Acres, Sussex Co., Delaware
MD1	Chester River, Millington, Kent Co., Maryland
MD2	Mason Branch, Queen Anne, Queen Annes Co., Maryland
MD3	Norwich Creek, Queen Anne, Talbot Co., Maryland
MD4	Sassafras River, Sassafras, Cecil Co., Maryland
ME1	Lake St. George, Waldo Co., Maine
ME2	Kennebec River, Somerset Co., Maine
MI	Wiggins Lake, Gladwin Co., Michigan
NB	French Lake, Oromocto, Sunbury Co., New Brunswick
NJ1	Delaware River, Burlington Co., New Jersey
NJ2	Swartswood Lake, Sussex Co., New Jersey
NS1	Lake Egmont, Cooks Brook, Halifax Co., Nova Scotia
NS2	First Lake O' Law, Baddeck, Victoria Co., Nova Scotia
NS3	Mattatall Lake, Wentworth Centre, Cumberland Co., Nova Scotia
NS4	Newville Lake, Halfway River East, Cumberland Co., Nova Scotia
NS5	Placide Lake, Havelock, Digby Co., Nova Scotia
NS6	Shaw Lake, Arichat, Isle Madame, Richmond Co., Nova Scotia
NS7	Grand Lake Shubenacadie, Grand Lake, Halifax Co., Nova Scotia
NS8	Sydney River, Sydney, Cape Breton Co., Nova Scotia
PA	Susquehanna River, Cumberland Co., Pennsylvania
VT1	Lake Champlain, South Hero, Grand Isle Co., Vermont
VT2	Joes Pond, Danville, Caledonia Co., Vermont
VT3	Lake Memphremagog, Newport, Orleans Co., Vermont
WI	St. Croix River, Hudson, St. Croix Co., Wisconsin

MALACOLOGIA, 1986, 27(1): 127–172

PHREATIC HYDROBIIDS (GASTROPODA: PROSOBRANCHIA) FROM THE EDWARDS (BALCONES FAULT ZONE) AQUIFER REGION, SOUTH-CENTRAL TEXAS

R. Hershler[1] & G. Longley

Edwards Aquifer Research and Data Center, Southwest Texas State University, San Marcos, Texas 78666-4615, U.S.A.

ABSTRACT

This paper provides a systematic analysis of phreatic hydrobiids from 23 localities in south-central Texas, including 14 artesian wells and four springs in the Edwards (Balcones Fault Zone) Aquifer. *Hauffenia micra* (Pilsbry & Ferriss) and *Horatia nugax* (Pilsbry & Ferriss) are redescribed as members of a new genus, and two additional new genera, seven new species and one new subspecies are also described (Table 2). Detailed morphological descriptions, provided for all taxa, emphasize characters of the shell, operculum, pallial cavity, digestive system, and reproductive system of both sexes. Two of the new genera are monotypic littoridinines having affinities with phreatic or epigean littoridinines from Mexico. The affinities of the third new genus, a hydrobiine which includes seven well-differentiated species, remain unclear. While all of the species are found in the Edwards (Balcones Fault Zone) Aquifer, at least four of the species are probably found in other aquifers of south-central Texas as well. With the description of seven new hydrobiid species, the rich and still poorly sampled troglobitic biota of the Edwards (Balcones Fault Zone) Aquifer now totals 39 troglobitic animal species, including four vertebrates.

Key words: Edwards (Balcones Fault Zone) Aquifer; south-central Texas; troglobitic fauna; Hydrobiidae; morphology; systematics.

INTRODUCTION

The Hydrobiidae (Gastropoda: Rissoacea) are a large family (over 100 genera and 1000 species; G. M. Davis, 1979) of dioecious, gill-breathing snails that have radiated into diverse fresh- and brackish-water habitats worldwide. Minute, unpigmented hydrobiids occupy groundwater habitats in numerous areas, with a large fauna occurring in karst regions of Europe (Vandel, 1965; Radoman, 1973), and lesser deployments occurring in North America (Morrison, 1949; Taylor, 1966), Mexico (Taylor, 1966; Hershler, 1984; 1985), Japan (Kuroda & Habe, 1958), and New Zealand (Ponder, 1966; Climo, 1974, 1977). Apart from the European deployment, little is known regarding the systematics and zoogeography of these taxa, in large part due the extremely small size (maximum shell dimension often $>$ 2 mm) of the snails and difficulties in sampling their habitat.

One of the poorer known phreatic hydrobiid faunas is that of Texas. *Horatia nugax* (Pilsbry & Ferriss, 1906) and *Hauffenia micra* (Pilsbry & Ferriss, 1906), described from river drift shells, have long been the sole described hydrobiids considered phreatic in Texas. As the anatomy of these taxa has not been studied, it is not known whether they are congeneric with any of the European phreatic hydrobiids (i.e., *Horatia s.s.* and *Hauffenia s.s.*) that they resemble in shell features. Later collections from a cave (Reddell, 1965), spring (Taylor, 1974), stream drift (Strecker, 1935; Hubricht, 1940; Fullington, 1978; J. R. Davis, 1983), and artesian wells (Karnei, 1978; Longley, 1975, 1978, 1981) provided possible additional records for these species as well as probable new taxa, pointing to the presence of a diverse phreatic snail fauna in south-central Texas.

Most of these collections have been from areas underlain by the Edwards (Balcones Fault Zone) Aquifer (Fig. 1). While phreatic faunas are known from several aquifers in Texas (Reddell, 1965, 1967, 1970; Reddell &

[1]Present address: Department of Invertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, DC 20560, U.S.A.

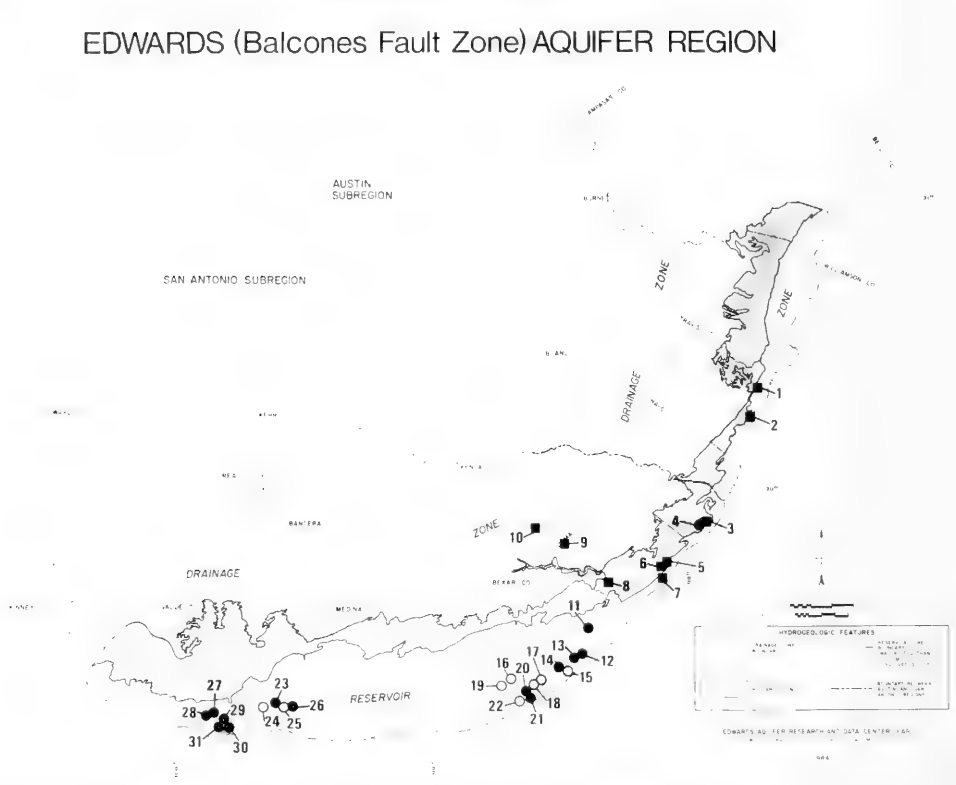


FIG. 1. Map of the Edwards (Balcones Fault Zone) Aquifer Region showing the 31 localities considered in this paper. Squares refer to springs or caves, while circles refer to artesian wells that have (filled) or have not (open) yielded hydrobiids. Locality numbers are as in Appendix 1.

Mitchell, 1969; Mitchell & Reddell, 1971), that of the Edwards is particularly rich, totalling 32 described animal species, including four vertebrates (Table 1). Twenty of these species, including nine amphipods, have been obtained from (and several are endemic to) the single well in the aquifer that has received continuous long-term sampling (Table 1), reflecting not only the high local diversity of the fauna, but also the probability that many more taxa await discovery elsewhere in the aquifer.

This diverse fauna poses a series of questions to the biologist. What are the origins of the various faunal elements? What roles have hydrologic factors such as the presence of groundwater divides played in effecting speciation in the aquifer? What is the food source for primary consumers in the deep artesian community, which has a surprisingly high trophic complexity (Longley, 1981)?

Study of the Edwards Aquifer hydrobiids is clearly necessary not only to help provide a clearer picture of this unique groundwater ecosystem, but also to further our understanding of the systematics, evolution, and adaptive radiation of the Hydrobiidae. This paper provides a systematic analysis of phreatic hydrobiids from 23 localities in south-central Texas, including 14 artesian wells and four springs in the Edwards Aquifer. We redescribe *Horatia nugax* and *Hauffenia micra* as members of a new genus and also describe two additional new genera, seven new species and one new subspecies. A classification of these taxa is given in Table 2. Detailed morphological descriptions are given for all taxa. The systematic relationships of the various taxa are assessed, with emphasis on comparisons with other phreatic hydrobiids that have been similarly studied.

The Edwards (Balcones Fault Zone) Aquifer. A brief description of the aquifer is necessary as a prelude to discussion below. For further details the reader is referred to Klemt *et al.* (1979). The Edwards (Balcones Fault Zone) Aquifer extends for 282 km (from

TABLE 1. Described biota of the Edwards Aquifer (from Reddell, 1965, 1967, 1970; Reddell & Mitchell, 1969; Longley, 1975, 1978; Bowman & Longley, 1976; Strenth, 1976; Young & Longley, 1976; Hart, 1978; Holsinger & Longley, 1980). Only troglobitic species found in the artesian zone of the aquifer are listed. Taxa marked with an asterisk have been collected from the artesian well at Southwest Texas State University.

Platyhelminthes
Kenkiidae
**Sphalloplana mohri* Hyman
Mollusca
Hydrobiidae
**Horatia nugax* (Pilsbry & Ferriss)
Hauffenia micra (Pilsbry & Ferriss)
Arthropoda
Cypridae
**Cypridopsis vidua obesa* Brady & Robertson
Cyclopidae
**Cyclops cavernarum* Ulrich
**Cyclops learii* Ulrich
Cyclops varicans rebellus Lilljeborg
Entocytheridae
Sphaeromicola moria Hart
Asellidae
**Lirceolus smithi* (Ulrich)
Asellus pilus Steeves
Asellus redelli Steeves
Cirolanidae
**Cirolanides texensis* Benedict
Monodellidae
**Monodella texana* Maguire
Crangonyctidae
**Stygobromus flagellatus* (Benedict)
**Stygobromus russelli* (Holsinger)
Stygobromus pecki (Holsinger)
Stygobromus balconis (Hubricht)
Stygobromus bifurcatus (Holsinger)
Hadziidae
**Texiweckelia texensis* (Holsinger)
**Texiweckelia insolita* Holsinger
**Texiweckelia samacos* Holsinger
**Allotexiweckelia hirsuta* Holsinger
Bogidiellidae
**Parabogidiella americana* Holsinger
Artesiidae
**Artesia subterranea* Holsinger
Sebidae
**Seborgia relicta* Holsinger
Palaemonidae
**Palaemonetes antrorum* Benedict
Palaemonetes holthuisi Strenth
Dytiscidae
**Hadeoporus texanus* Young & Longley
Chordata
Ambystomidae
**Typhlomolge rathbuni* Stejneger
Typhlomolge robusta Longley
Ictaluridae
Satan eurystomus Hubbs & Bailey
Trogloglanis pattersoni Eigenmann

Brackettville to north of Georgetown), paralleling the Balcones Escarpment and Fault Zone in south-central Texas, and varies in width from eight to 48 km (Fig. 1). The aquifer consists of a recharge and artesian (reservoir) zone. Recharge to the aquifer occurs largely by downward percolation from streams crossing areas where the Edwards outcrops (Fig. 1). To the south and east of the recharge zone the Edwards Formation dips downward (assuming artesian conditions), and the top of the formation is as much as 600 m beneath ground level in Bexar County (Klemt *et al.*, 1979). The Cretaceous Edwards limestone that comprises the aquifer is highly porous, due to the effects of solution and faulting. The aquifer is thought to have extensive water-filled caves and caverns (Pettit & George, 1956; Klemt *et al.*, 1979). Note that the Balcones Fault Zone has the highest density of caves of any physiographic region in Texas (Smith, 1971). As a result of this high secondary porosity, transmissivity is high in the aquifer, as seen in the occurrence of a large number of high capacity wells, some of which flow at ground level and discharge several thousand liters/second (Maclay & Small, 1976; Klemt *et al.*, 1979). Several groundwater divides are present in the aquifer, and smaller phreatic pools are also thought to have resulted from the intensive folding and fracturing in the bedrock (Holsinger & Longley, 1980). South and south-east of the "bad-water" line (reservoir boundary, Fig. 1), the Edwards water has sluggish circulation and is no longer of good quality, having $>$ 1000 mg/l total dissolved solids (Klemt *et al.*, 1979). Natural discharge of the aquifer occurs (or has occurred) at major springs near Uvalde (Leona Springs), San Antonio (San Pedro,

TABLE 2. Classification of phreatic hydrobiids from the Edwards (Balcones Fault Zone) Aquifer Region.

Family Hydrobiidae
Subfamily Hydrobiinae
Phreatodrobia micra (Pilsbry & Ferriss) n. gen.
Phreatodrobia nugax nugax (Pilsbry & Ferriss)
Phreatodrobia nugax inclinata n. subsp.
Phreatodrobia rotunda n. sp.
Phreatodrobia conica n. sp.
Phreatodrobia plana n. sp.
Phreatodrobia imitata n. sp.
Phreatodrobia punctata n. sp.
Subfamily Littoridininae
Balconorbis uvaldensis n. gen., n. sp.
Stygopyrgus bartonesus n. gen., n. sp.

San Antonio Springs), New Braunfels (Comal Springs), San Marcos (San Marcos Springs) and Austin (Barton Springs). Artificial discharge has also occurred in recent years through the hundreds of high capacity wells in the artesian zone. The Edwards (Balcones Fault Zone) Aquifer is separated from the Edwards (Plateau) Aquifer by a region where outcropped Edwards limestone has largely been eroded.

MATERIALS AND METHODS

Sampling and localities. The bulk of the material examined during this study was obtained by the well sampling program conducted by staff of the Edwards Aquifer Research and Data Center (EARDC) during 1976–1981. A total of 22 wells in the Edwards Aquifer were sampled often enough to either obtain troglobitic organisms or provide confidence that troglobites were absent in that area. Details for these wells regarding United States Geological Survey or Texas Board of Water Engineers well number, well owner, well depth, number of samples taken, and presence-absence of troglobitic fauna are given in Table 3.

Fine-mesh funnel (constricted or open) nets were attached to pipes from artesian wells using hose clamps (Fig. 2A). The collecting vessel at the end of the net was usually a 3.8 liter plastic jar, although occasionally a small section of 64 μm mesh netting material clamped to a section of polyvinylchloride (PVC) pipe (with screw-on cap) was used instead. All material collected was washed through netting attached to a plastic funnel and then transferred to 70% EtOH.

Groundwater outlets of four springs were sampled as follows. The "Pipe" (or "Diversion") orifice was sampled at San Marcos Springs. Developers of the spring capped this orifice (one of many feeding this spring) by cementing an old diving bell to the spring floor. A 29″ culvert pipe was then attached to the opening of the bell to divert the spring flow elsewhere. A 29″ diameter sampling net was placed at the end of the pipe to filter the water stream. At Barton Springs, sampling was done at the "Concession" Spring (Fig. 2B), which has been cemented over with several holes serving to release the spring flow.

TABLE 3. Data regarding 22 Edwards Aquifer wells that have been sampled.

Well no.	Owner	Depth (m)	Number of samples	Troglobites (+/−)
—	Southwest Texas State University	59	500+	+
AY-68-29-923	Longhorn Portland Cement Co. (#2)	143	36	+
AY-68-37-127	Brackenridge Zoo	124	45	+
AY-68-37-508	City Water Board (San Antonio) Artesian Station, Well 4	402	48	+
AY-68-36-918	Union Stockyards (#3)	412	8	+
AY-68-37-710	City Water Board (San Antonio) Mission Station	460	9	+
AY-68-43-115	J. H. Uptmore (#5)	227	10	+
AY-68-44-107	Lakeland City Water Co.	555	5	−
AY-68-44-215	City (Antonio) Public Service Board (#1)	358	22	+
AY-68-43-107	Rio Vista Farms	—	9	−
AY-68-43-608	Verstraeten Brothers	513	10+	+
AY-68-43-601	O. R. Mitchell	582	10+	+
AY-68-43-505	J. W. Watts	610	2	−
YP-69-43-103	King Farms	—	3	+
YP-69-43-801	D. C. Carnes	—	5	−
YP-69-50-? (=H-6-24)	C. Reagan	386	4	+
H-6-43	W. C. Reagan	373	5+	+
YP-69-50-109	R. Carnes	320	2	+
YP-69-50-105	R. K. Dunbar	288	2	+
H-5-135	S. Moerbe	98	2	+
H-5-158	G. Ligocky	286	10+	+
YP-69-50-501	Uvalde National Fish Hatchery	—	2	+

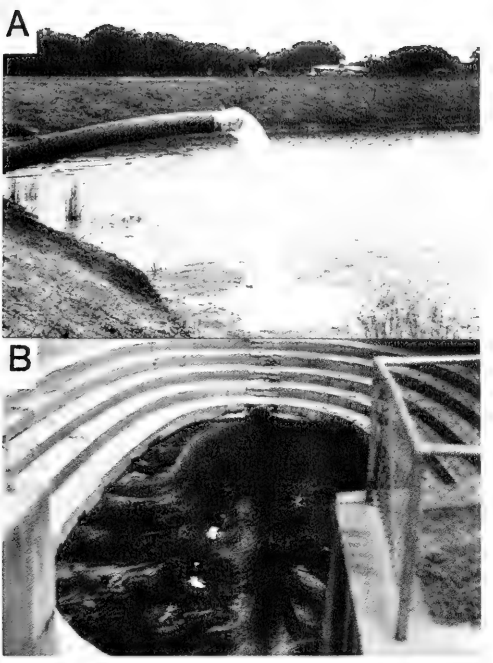


FIG. 2. Sampling nets placed on outlets of the G. Ligocky Well, Uvalde County (A) and Barton ("Concession") Springs, Travis County (B).

Three- and six-inch PVC pipes with attached irrigation "socks" or funnel netting (and "cap" type collection vessel) were wedged into these holes to sample the water stream. Similar techniques were used to sample the natural orifices at Comal (main spring) and Hueco Springs ("A", smaller; and "B", larger spring). Samples from springs were processed as above. Most of the wells and springs were sampled every 2–7 days during the sampling period.

Samples from caves were obtained by visual search for shells near permanent water and by sieving sediment with a fine hand sieve. Living snails were not obtained from any of these caves.

The location of the 31 localities considered in this paper are given in Fig. 1. Note that three of the localities (all caves) sampled are from areas not underlain by the Edwards (Balcones Fault Zone) Aquifer (see below). Locality data are given in Appendix 1.

Morphological study. Methods of morphological study largely follow those of G. M. Davis (1979) and Hershler (1985). Live material was available only for two species. Anatomical study centered on the pallial cavity (and contained structures), digestive system, and reproductive systems of both sexes. Usage of body surface references (left, right, dorsal, ventral) follows that of Fretter & Graham (1962). The nervous system was studied only in one species. Measurements of the length and width of the intestine coil in the pallial cavity refer to the maximum dimensions of the coil parallel and perpendicular to the length of the pallial cavity. To prepare snails for dissection, the calcified portion of the shell was first removed by placing individuals into concentrated Bouin's solution for 24 hours. Dissections were done using a Zeiss dissecting microscope (50X) fitted with an ocular micrometer, with specimens immersed in dilute Bouin's solution. Shells were measured using the same microscope (32X, 50X). Radulae were photographed using a scanning electron microscope. All radular data were obtained from these photographs. Shells were photographed using either a 35 mm camera attached to a Zeiss microscope, or the scanning electron microscope.

Deposition of type material. Holotypes and paratypes are deposited in the Academy of Natural Sciences of Philadelphia. Reference is made to catalog numbers (ANSP) assigned to this material. All other material examined is housed in the EARDC collection.

Taxonomic procedure. The higher level classification of the Hydrobiidae is currently confused, due to the high incidence of convergence and mosaic evolution within the family, and several classifications have recently been proposed (Taylor, 1966; Radoman, 1973; G. M. Davis *et al.*, 1982). Convergence in shell features is well documented in the Hydrobiidae (G. M. Davis, 1979) and higher-level classifications largely based on these features (i.e., Taylor, 1966) are likely to produce polyphyletic and artificial groupings. In this paper we follow the subfamilial breakdown used by G. M. Davis *et al.* (1982) in which the Hydrobiinae, Lithoglyphinae (see also Thompson, 1984), Littoridininae (see also Hershler, 1985), and Nymphophilinae are recognized. Note, however, that convergence may extend to key characters used in this classification: a spermathecal duct, diagnostic of the Littoridininae (G. M. Davis *et al.*, 1983), has apparently arisen numerous times among various rissoacean clades (Ponder, 1984). Further confusion has been added by the recent discovery of a genus having a hydrobiine-type fe-

TABLE 4. Generalized cusp formulae for the four radular tooth types.

Species	Central	Lateral	Inner marginal	Outer marginal
Phreatodrobia micra	5-1-5 / 1-1	5-1-6	21–23	13–16
Phreatodrobia nugax	5(6-8)-1-5(6,7) / 1(2)-1	5-1-6(7,8)	24–34	19–26
Phreatodrobia rotunda	6-1-5(6) / 1-1	5-1-5(6)	20–23	?
Phreatodrobia conica	6(7,8)-1-6(7,8)	7(8)-1-10(11)	21–26	15–17
Phreatodrobia plana	6(8)-1-6(7,8)	8(9)-1-11(12)	23	24–28
Phreatodrobia imitata	7(8)-1-7(8)	6(7)-1-6(7)	21–23	14–17
Phreatodrobia punctata	7(9)-1-7(8)	7(8,9)-1-10(11,12)	23–26	22–24
Stygopyrgus bartonensus	4(5)-1-4(5) / 1-1	4(5-7)-1-5(6)	22–25	15–17
Balconorbis uvaldensis	5(6)-1-4(5) / 1-1	6-1-6(7,8)	21–24	17–20

male reproductive system and a littoridinine-type penial gland (Giusti & Bodon, 1984; fig. 2B, G). While a search for morphological characters that identify clades within the Hydrobiidae must obviously be continued, we feel that the above classification is still the best available.

Only character-states unique to or diagnostic of the taxa concerned are emphasized in this paper. Radular data are presented in Table 4. Shell and other morphological data are presented in Appendices 2 and 3, respectively. The phenogram shown in Fig. 28 was generated using the CLUSTAN software package (developed by David Wishart of the Universities of St. Andrews and London), with Ward's method (error sum of squares) of clustering selected.

DESCRIPTION OF TAXA

Family HYDROBIIDAE
Subfamily Hydrobiinae
Phreatodrobia Hershler & Longley, new genus

Horatia Bourguignat, 1887 (in part): 47.
Hauffenia Pollonera, 1898 (in part): 3.

DIAGNOSIS. Shell (Figs. 3–6) minute (maximum dimension, < 2.5 mm), with four or fewer whorls, colorless, transparent, varying in shape from planispiral to trochoid to conical. Protoconch (Figs. 3K, L, S, 6F, 7A–F) with pitted microsculpture; teleoconch sculpture variable (Figs. 3–6, 8). Aperture slightly to highly flared. Operculum (Figs. 9, 10) nucleus typically sub-central; ventral surface smooth or with a central, knob-like process. Animal unpigmented and without eyespots (Figs. 11, 12). Pallial cavity typically slightly longer than wide; ctenidium absent, incomplete, or fully formed (Fig. 13A–G). Stomach chambers poorly distinguishable externally (Fig. 18). Intestine with loop(s) in roof of posterior portion of pallial cavity (Figs. 12, 13A–G), and sometimes on style sac (Fig. 18B, C). Central cusp of central and lateral teeth sometimes not enlarged relative to rest of cusp row (Figs. 16A–C, 17A–D); central teeth with (Figs. 14, 15A–E) or without (Figs. 15F–H, 16, 17A–D) basal cusps (projecting from the lateral angles). Radular cusps dagger-like in shape, sometimes highly numerous on central and lateral teeth. Pallial portion of prostate typically 50% of total prostate length (Figs. 19D, E). Penis simple, without lobes or specialized glands (Figs. 19A, C). Capsule gland usually large compared to albumen gland, with anterior end sometimes having a terminal bend or coil (Fig. 20A, B, H). Bursa copulatrix large relative to the seminal receptacle, with a straight or coiled duct, and largely or totally posterior to the albumen gland (Figs. 20, 21). Seminal receptacle and oviduct coil appressed to, or largely or totally posterior to albumen gland. Oviduct opens into the posterior or anterior end of the albumen gland.

FIG. 3. Shells of *Phreatodrobia nugax inclinata* (A–E, I)(Locality 11) and *P. micra* (F–H, J–L, M–T). Shell widths are as follows: A, B (1.33 mm, holotype); C (1.05 mm), D (1.04 mm), E (1.15 mm); F (1.10 mm), J (1.10 mm), N (1.16 mm) (syntypes, Locality 6); G (1.14 mm), K (0.945 mm), O (1.23 mm)(Locality 5A); H (0.8 mm), L (1.80 mm), P. (0.76 mm)(Locality 3); M (0.93 mm), Q (0.75 mm)(Locality 9); R (1.07 mm), S (0.89 mm), T (0.877 mm)(Locality 10). The scale bar next to I equals 0.1 mm.

REMARKS. The following features are also typical of the genus: a) the anus is positioned close to the mantle edge (Fig. 13); b) the kidney opening (Ro, Fig. 13A) is simple; c) the penis is three-four times as long as the snout (in preserved specimens), slender, with folds along the inner curvature, and coils on the right side of the "neck"; d) the gonads are simple and without lobes, and e) the anterior vas deferens exits from the mid-prostate just anterior to the end of the pallial cavity (Fig. 19D). *Phreatodrobia* is distinguished from *Horatia s.s.*, *Hauffenia s.s.*, and other European phreatic hydrobiines by its minute, fragile shell and simple penis, which lacks lobes and glandular swellings (see below). The minute shell with pitted apical microsculpture, simple penis, and lack of eyespots and body pigment distinguish *Phreatodrobia* from all other described North American hydrobiids. Apart from these features, the following shared character-states unite the morphologically diverse members of this genus: operculum nucleus central or near-central; pallial prostate relatively large; intestine with loop(s) in pallial roof; and bursa large relative to seminal receptacle and positioned partly or totally posterior to the albumen gland.

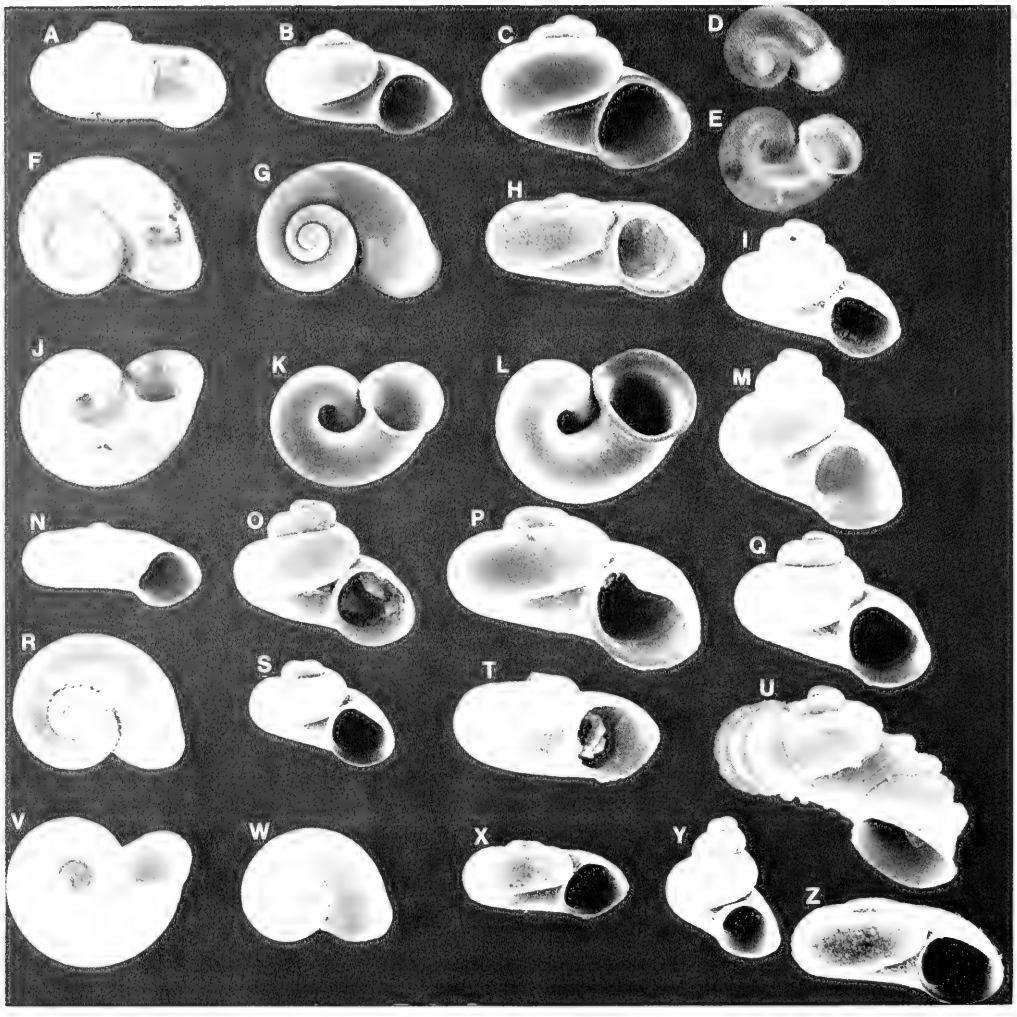


FIG. 4. Shells of *Phreatodrobia nugax nugax*. Shell widths are as follows: A, F, J (1.23 mm, holotype, Locality 6); B (1.71 mm), G (1.71 mm), K (1.6 mm)(Locality 4); C (1.55 mm), D (1.79 mm), E (1.79 mm), H (1.23 mm), L (1.53 mm)(Locality 3); I (1.07 mm, Locality 1); M (1.0 mm), Q (1.13 mm), U (1.47 mm), Y (1.09 mm), Z (0.89 mm)(Locality 2); N (1.57 mm), R (1.51 mm), V (1.64 mm)(Locality 8); O (1.53 mm)(Locality 10); P (1.08 mm)(Locality 24); S (0.85 mm), W (0.85 mm)(Localtiy 9); T (0.85 mm)(Locality 12); X (0.945 mm)(Locality 14).

TYPE-SPECIES. *Phreatodrobia micra* (Pilsbry & Ferriss, 1906).

DISTRIBUTION. Found throughout the Edwards (Balcones Fault Zone) Aquifer. Some species also probably live in other aquifers in south-central Texas (see below).

ETYMOLOGY. The generic name is derived from the Greek word *phreatos*, referring to the groundwater habitat shared by members of this taxon.

Phreatodrobia micra (Pilsbry & Ferriss)
Figs. 3F–H, J–T, 9F, G, 13B, 15A–C, 20D

Valvata micra Pilsbry & Ferriss, 1906: 172–173.

Horatia (Hauffenia) micra (Pilsbry & Ferriss). Pilsbry, 1916: 84

Hauffenia micra (Pilsbry & Ferriss). Burch, 1982: 30.

MATERIAL EXAMINED. HAYS COUNTY: San

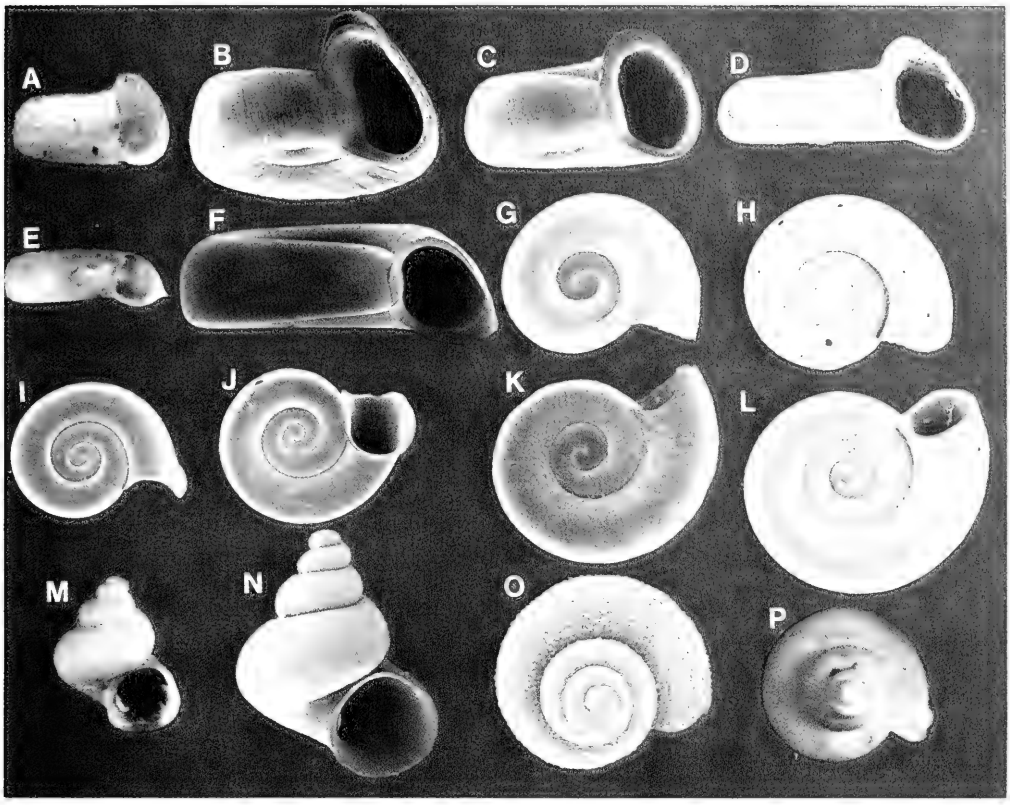


FIG. 5. Shells of *Phreatodrobia plana* (A–D, G, H, K, L), *P. rotunda* (E, F, I, J; Locality 3), and *P. conica* (M–P). Shell widths are as follows: A (0.822 mm, holotype), B (0.830 mm), C (0.77 mm), G (0.64 mm), K (0.74 mm)(Locality 3); D (1.14 mm), H (0.945 mm), L (1.12 mm)(Locality 8); E (2.26 mm, holotype, ANSP), F (1.99 mm), I (1.84 mm), J (2.06 mm); M (shell height, 1.61 mm, holotype, ANSP), N (shell height, 1.79 mm), O (1.29 mm)(Locality 5B); P (0.945 mm; Locality 5A).

Marcos Springs. COMAL COUNTY: Guadalupe River drift; Honey Creek Cave; Hueco Springs. KENDALL COUNTY: Century Caverns.

DIAGNOSIS. A small-sized species (shell width about 1.00 mm) with a planispiral, or near-planispiral shell, and a circular aperture that abuts against the penultimate whorl (Figs. 3F–H, J–T). Operculum (Figs. 9F, G) circular, with well-developed knob-like process on ventral surface; nucleus central. Ctenidium incomplete; osphradium fills large (39%) fraction of pallial cavity length (Fig. 13B). Central tooth of radula with single pair of basal cusps (Fig. 15C). Stomach almost twice as long as style sac; intestine with tight, U-shaped loop in pallial roof; long axis of loop at oblique angle to pallial cavity length (Fig. 13B). Ovary and testis occupy large proportion (50%) of digestive gland length. Albumen and capsule glands about equal in length (Fig. 20D). Oviduct opens into anterior end of albumen gland.

REMARKS. Distinctive features of this species include the typically planispiral shell with tubular whorls and simple aperture, circular operculum with well-developed process on the ventral surface, incomplete ctenidium, large-sized osphradium, and large-sized ovary and testis.

DESCRIPTION. The shell has 2.2–2.5 tubular whorls and averages 0.84–1.08 mm in width for the four populations studied. The protoconch has 1.25 whorls and has fairly

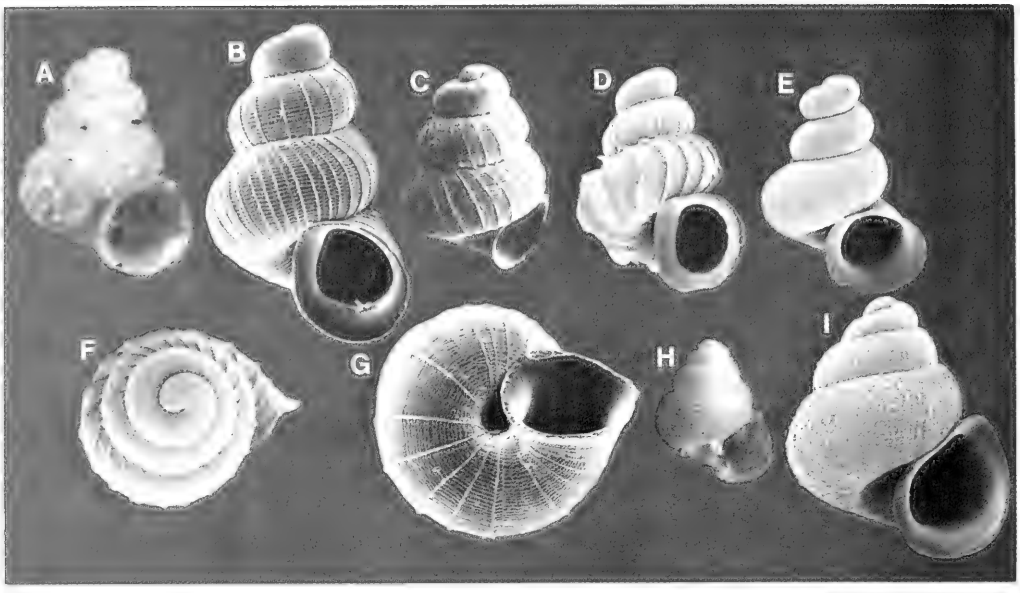


FIG. 6. Shells of *Phreatodrobia imitata* (A–G) and *P. punctata* (H, I; Locality 3). Shell heights are as follows: A (1.07 mm, holotype), B (1.05 mm), C (0.89 mm), D (1.05 mm), F (shell width, 0.75 mm), G (shell width, 0.75 mm)(Locality 20); E (1.0 mm; Locality 21); H (1.05 mm, holotype), I (1.15 mm).

large and deep pits (Fig. 3K, L, S). Axial growth lines are weakly developed on the teleoconch. Only the sample from Century Caverns includes shells with a slight spire projecting above the coil of the body whorl (Fig. 3R). A slight flaring of the aperture, particularly the inner lip, is seen in some specimens. The aperture is never free from the body whorl.

The description of operculum and anatomy is based on study of specimens from San Marcos Springs. The flat, thickened operculum has 2.5 whorls and is dark amber in color. The elevated knob-like process occupies a small portion of the operculum area and is composed of horny material (as is the rest of the operculum).

All specimens dissected had an incomplete ctenidium, consisting of an efferent branchial vessel (Ev) with a few stubby filaments at its anterior end (Fig. 13B). The filaments are much smaller, relative to pallial cavity width, than those of *Phreatodrobia nugax* (see below). A similar incomplete ctenidium was described for *Paluccia* Giusti & Pezzoli, a European hydrobiid (Giusti & Pezzoli, 1981). The large osphradium is typically positioned towards the anterior end of the incompletectenidium (Fig. 13B).

The central tooth of the radula is trapezoidal in shape. Note that the lateral angles are highly divergent (Fig. 15C). The stomach lacks a caecal appendix.

The ovary and testis consist of a solid, non-lobed mass. The vas deferens exits from the anterior end of the testis and consists of a few thickened coils (as in Fig. 18C) on the posterior half of the stomach. The prostate overlies the entire style sac and its anteriormost 44% is pallial. The penis is three-four times as long as the snout. The capsule gland opening is wide and slightly subterminal. The anterior portion of the capsule gland lacks a twist or coil. The tight coil of the anterior oviduct is appressed to the albumen gland and the seminal receptacle opens into the left side of the coil (Fig. 20D). The oviduct enters the albumen gland just after receiving the short duct from the bursa copulatrix.

HOLOTYPE. ANSP 91322 (cotypes) (Fig. 3F, J, N).

TYPE-LOCALITY. Drift debris of Guadalupe River about four miles above New Braunfels, Comal County (Fig. 1, Locality 6).

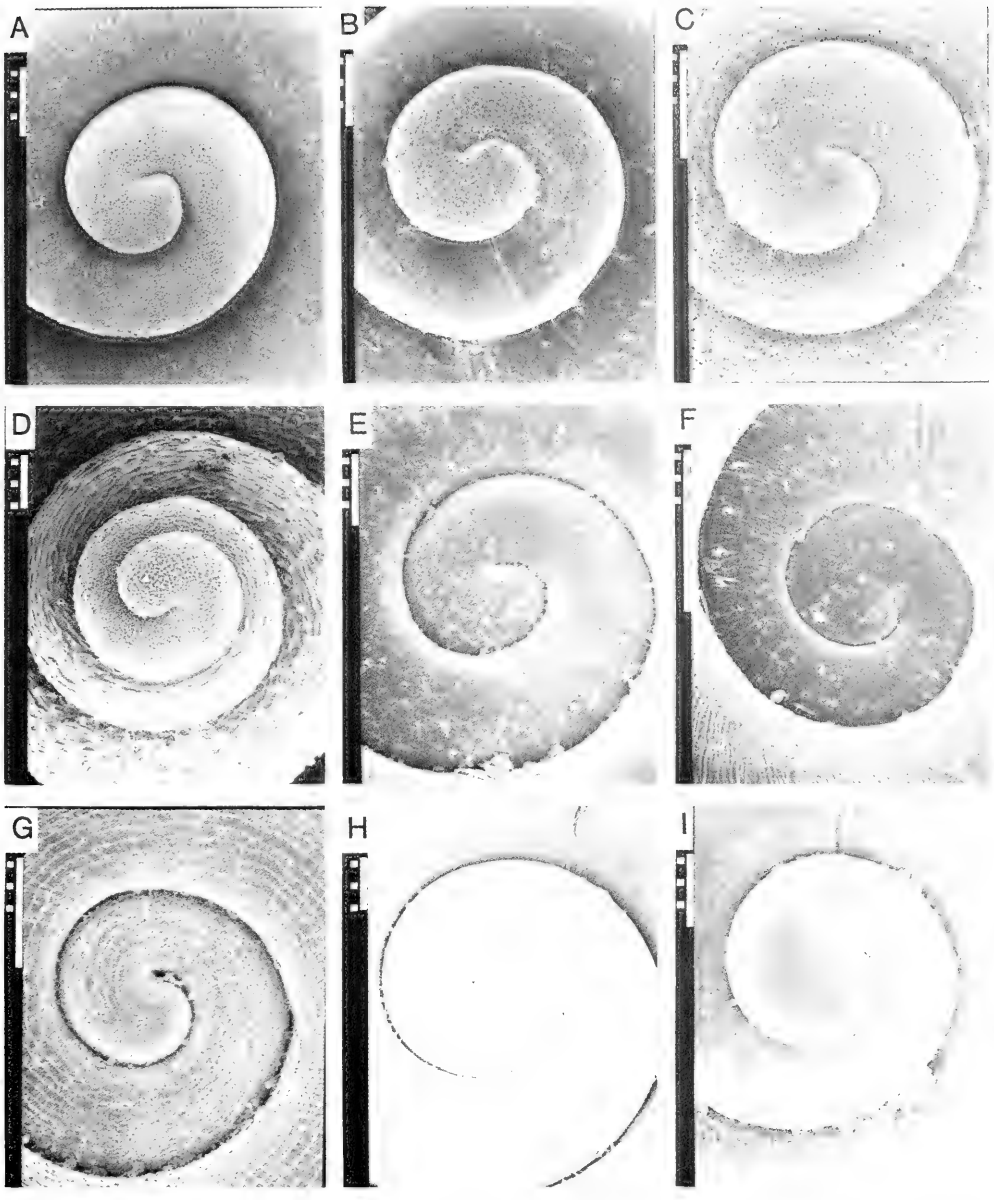


FIG. 7. Protoconchs of *Phreatodrobia nugax nugax* (A, B; Localities 3, 2), *P. punctata* (C, Locality 3), *P. conica* (D, Locality 5B), *P. rotunda* (E, Locality 3), *P. plana* (F, Locality 3), *Balconorbis uvaldensis* (G, Locality 30), "*Horatia*" sp. (H), and *Hauffenia subpiscinalis* (I). All scale bars equal 0.1 mm.

DISTRIBUTION. Edwards (Balcones Fault Zone) Aquifer, and (possibly) Cow Creek and Glen Rose Aquifers in Hays, Comal, and Kendall Counties (Fig. 1, Localities 3, 5, 6, 9, 10).

Phreatodrobia nugax (Pilsbry & Ferriss)
Figs. 3A–E, I, 4, 7A, B, 9A–E, 10–12, 13A, 14, 18A, 19A, B, D
Valvata micra nugax Pilsbry & Ferriss, 1906:

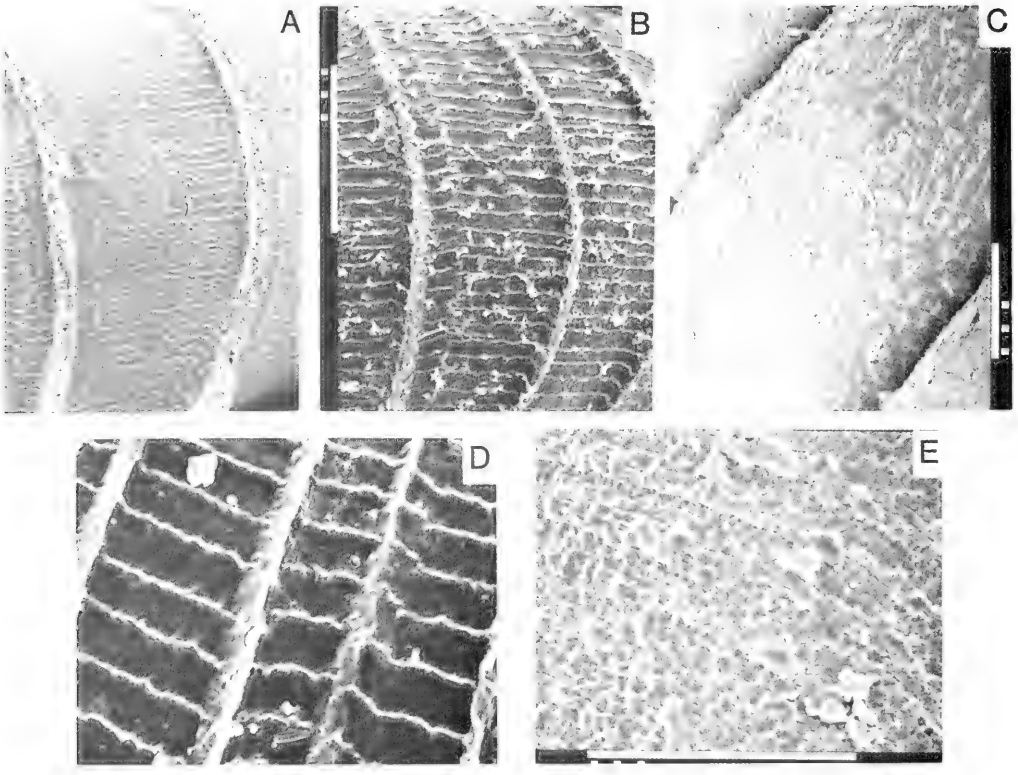


FIG. 8. SEM photographs of shell sculpture on *Phreatodrobia imitata* (A, B, D; Locality 20), *P. conica* (C; Locality 5B), and *P. punctata* shells (E, Locality 3). All scale bars equal 0.1 mm. The distance between spiral lines in D is 0.01 mm.

173.

Horatia (Hauffenia) micra nugax (Pilsbry & Ferriss). Pilsbry, 1916: 84.

Horatia nugax (Pilsbry & Ferriss). Taylor, 1975: 131.

MATERIAL EXAMINED. TRAVIS COUNTY: Salamander Cave; Barton Springs. HAYS COUNTY: San Marcos Springs; SWTSU Well. COMAL COUNTY: Guadalupe River drift; Natural Bridge Caverns; Honey Creek Cave. KENDALL COUNTY: Century Caverns. BEXAR COUNTY: Longhorn Portland Cement Company Well; Brackenridge Zoo Well; Union Stockyards Well. UVALDE COUNTY: W. C. Reagan Well.

DIAGNOSIS. A moderately large species (shell width about 1.3 mm), typically with a low trochoid shell (Figs. 3A–D, 4), but varying from near planispiral (Fig. 4H, N, X, Z) to low conical (Fig. 4Y). Aperture often free from penultimate whorl and highly flared; last 12% of body whorl highly thickened, imparting a white, opaque appearance to this part of shell (Fig. 4D, E). Operculum can have a central elevated thickening (Fig. 9B, D) on ventral surface. Ctenidium complete, with eight to 18 low filaments; osphradium length typically 25% of that of the pallial cavity (Fig. 13A). Central radular tooth with one or two basal cusps (Fig. 14). Stomach length almost twice that of the style sac (Fig. 18A); intestine with loose U-shaped loop in pallial roof; long axis of loop at oblique angle to pallial cavity length (Fig. 13A). Ovary and testis occupy large proportion (67%, 57%) of digestive gland length. Capsule gland with (Fig. 20A, B) or without terminal coil; oviduct opens into anterior end of albumen gland.

REMARKS. This species is distinguished by its low trochoid shell with thickened end of body whorl, complete ctenidium, and unusually large ovary. The capsule gland coil and

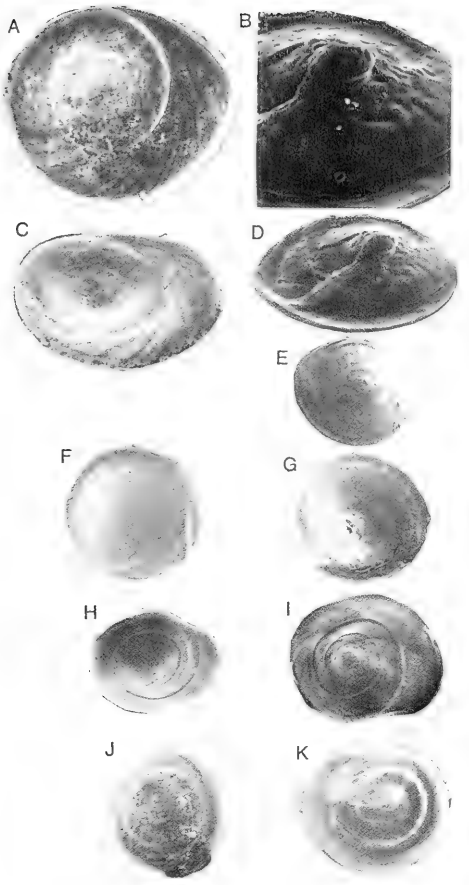


FIG. 9. Operculae of *Phreatodrobia nugax nugax* (A–E), *P. micra* (F, G; Locality 3), *P. rotunda* (H, I; Locality 3), and *P. imitata* (J, K; Locality 20). Operculum lengths are as follows: A (0.63 mm), C (0.57 mm)(Locality 3); B (ventral aspect, left-right distance, 0.29 mm), D (ventral aspect, 0.56 mm)(Locality 4); E (ventral aspect, 0.536 mm; Locality 24); F (0.29 mm), G (ventral aspect, 0.29 mm), H (0.536 mm), I (ventral aspect, 0.609 mm), J (0.29 mm), K (ventral aspect,, 0.314 mm).

second pair of basal cusps on the central radular tooth, seen in some populations of this species, are unique in the genus. Two subspecies are recognized on the basis of differences in shell morphology. Karnei (1978) described shells of this species (as Gastropod Genus No. 1) from Brackenridge Zoo Well. Longley (1975, 1981) incorrectly identified specimens of this species from the SWTSU Well as *P. micra*.

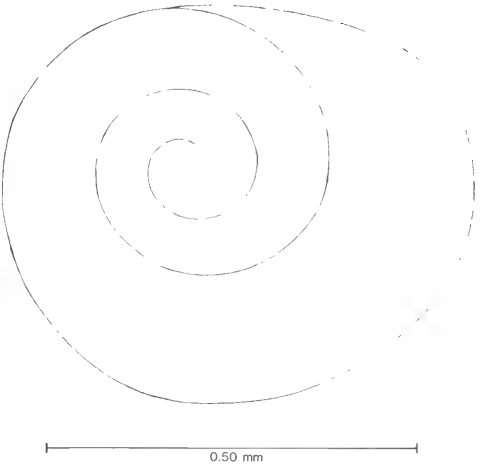


FIG. 10. Operculum of *Phreatodrobia nugax nugax* (Locality 3).

DESCRIPTION. The shell has 2.5–3.2 whorls and averages 0.93–1.71 mm in width. The protoconch, sometimes tilted (Fig. 3I), has 1.25–1.50 moderately pitted whorls (Fig. 7A, B). Axial growth lines are typically well developed on the teleoconch (Fig. 4), which may also have collabral costae (Fig. 4U). General shell form is highly variable in some populations. For the San Marcos Springs population, most individuals have the typical low trochoid shell (Fig. 4C), yet occasional

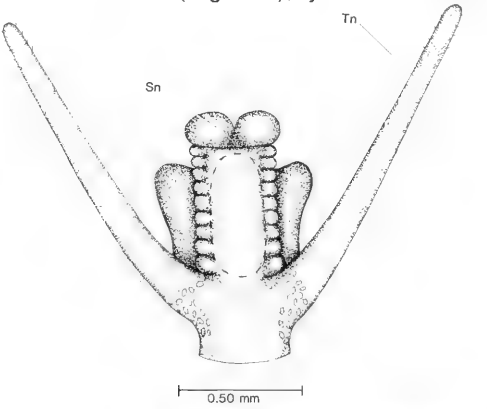


FIG. 11. Dorsal aspect of head of *Phreatodrobia nugax*. Note the presence of granules in the base of the tentacles (Tn), and the ciliation on the tentacle tips and along the outside edge of the base of the left tentacle. The dashed lines on the snout (Sn) indicate the position of the buccal mass. The anterior end of the foot is shown beneath the snout. Sn = snout; Tn = tentacle.

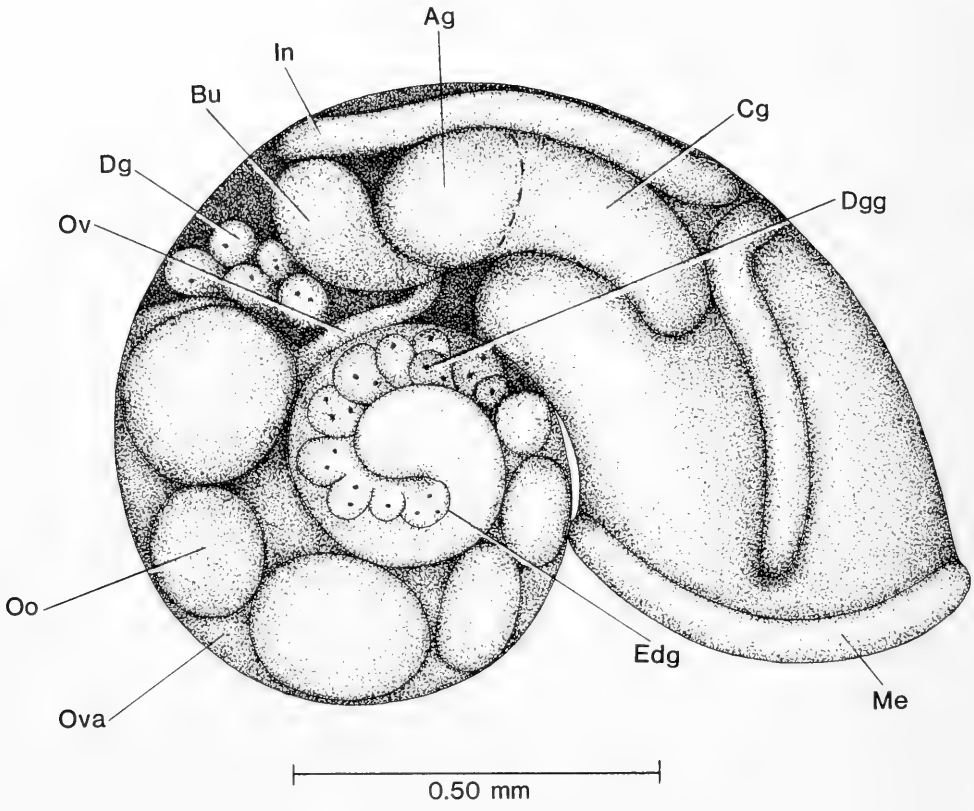


FIG. 12. Morphology of a female *Phreatodrobia nugax* (minus the head-foot), as seen from the right (and slightly dorsal) side. The kidney tissue is not shown. Ag = albumen gland; Bu = bursa copulatrix; Cg = capsule gland; Dg = digestive gland; Dgg = digestive gland granule; Edg = posterior end of digestive gland; In = intestine; Me = mantle edge; Oo = oocyte; Ov = oviduct; Ova = ovary.

smaller individuals with near-planispiral shells (Fig. 4H) were also found. Shell form in the Barton Springs population varies from near-planispiral to low trochoid, to low trochoid with costae, to low conical (Fig. 4M, Q, U, Y, Z). The aperture is moderately flared all around, with flaring most pronounced in large individuals. While typically wider than long and near-circular, aperture shape can be modified (especially in large-sized individuals from San Marcos Springs and the SWTSU Well) by a slight adapical notch or a pronounced abaxial fluting of the outer lip. The aperture is free from the body whorl in 0–70% of the samples from given populations, with populations with large-sized individuals having the highest incidence. The inner lip is especially thickened. The white thickening of the end of the body whorl was seen in all fresh specimens.

The operculum has 2.5 whorls (Fig. 10), with the nucleus positioned at about 43% of the operculum length, and varies from near-circular to ellipsoidal in shape (Fig. 9A, C, E). Only individuals from San Marcos Springs and the SWTSU Well typically have a well-developed thickening on the ventral opercular surface. In these populations the operculum has a low conical shape, with the process consisting of extra layers of material deposited at the apex (operculum nucleus) of the cone (which points into the foot). The process is less prominent and elevated than that of *P. micra* (compare Fig. 9B, D with Fig. 9G). In other populations the operculum is flatter

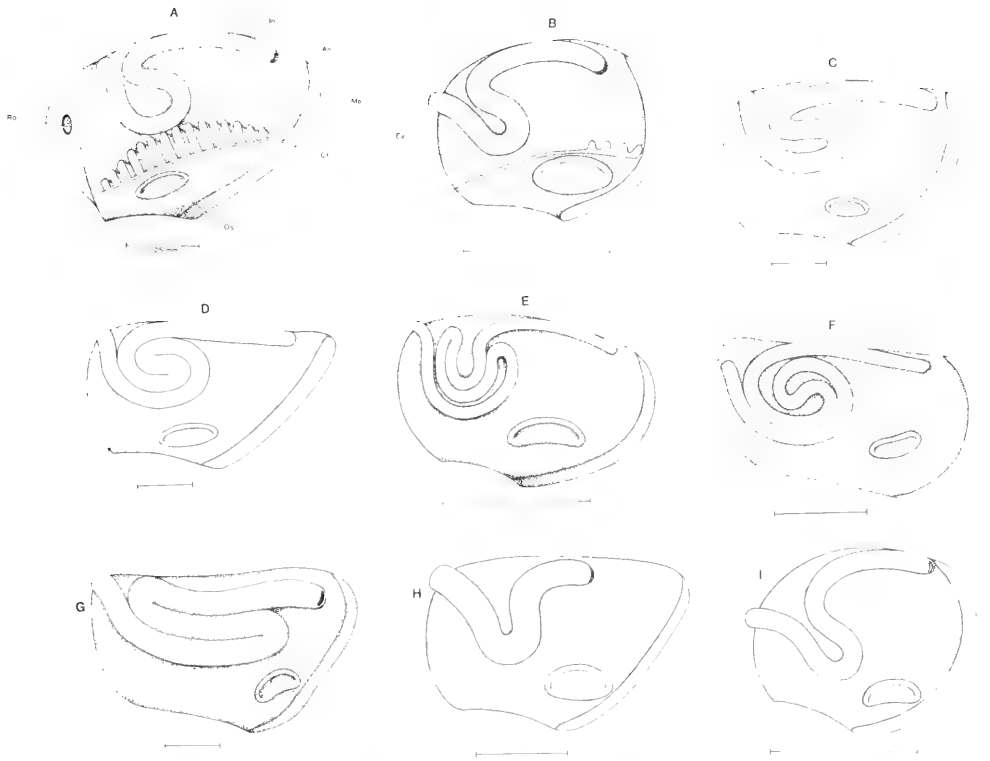


FIG. 13. Contents of the pallial cavity of *Phreatodrobia nugax* (A), *P. micra* (B), *P. conica* (C), *P. imitata* (D), *P. plana* (E), *P. punctata* (F), *P. rotunda* (G), *Balconorbis uvaldensis* (H), and *Stygopyrgus bartonensus* (I). The pallial roof has been slit along its length on the extreme right side and then folded over to the left and pinned. The pallial reproductive organs are not shown and the kidney is figured only in A. All scale bars equal 0.25 mm. An = anus; Ct = ctenidium; Ev = efferent branchial vessel; In = intestine; Me = mantle edge; Os = osphradium; Ro = renal opening.

and the process reduced or virtually absent (Fig. 9E).

Anatomical description is based on extensive study of material from San Marcos Springs and lesser study of populations from Barton Springs and wells owned by SWTSU (including live material), Longhorn Portland Cement Company, Union Stockyards, Brackenridge Zoo, and W. C. Reagan.

The snout (Fig. 11) is longer than wide, with folds along its sides. The pink-colored (haemoglobin) buccal mass is readily seen through the snout. The tentacles are elongate, rounded at the tips, moderately thickened relative to snout width, and held at 60–100 degrees to one another. Stiffened, elongate cilia fringe the tentacle tips. Four to five hypertrophied ciliary tufts line the outer side of the left tentacle near its base. White granules are clustered at the base of the tentacles and clear crystalline granules extend back along the "neck." The foot is long and slender, broad anteriorly and tapered posteriorly. The pedal glands consist of a single massive central gland flanked by seven to eight smaller glands on either side. Crystalline granules are found on the sides of the foot. The shell is carried with the coiling axis tilted about 30 degrees to the plane of the substrate. The intestine, gonad (ovary, white; testis, yellow), and pallial gonoduct (oviduct, white; prostate, green) are visible through the shell.

The ctenidium typically occupies 75% of the

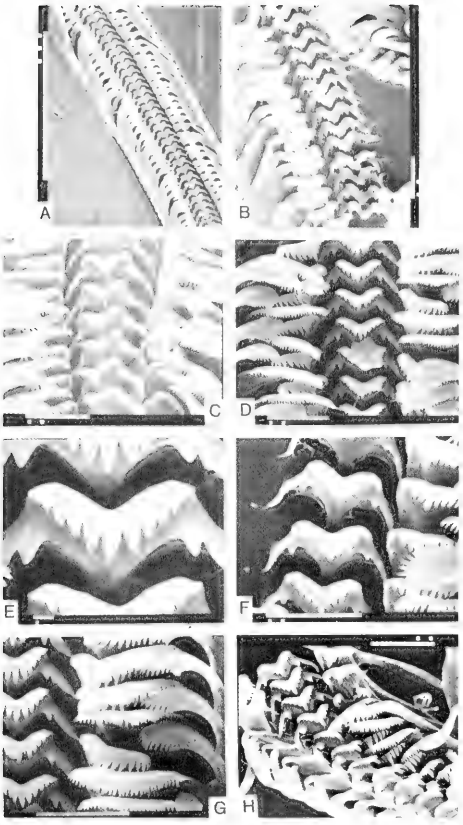


FIG. 14. Radulae of *P. nugax nugax* (A, C–H) and *P. nugax inclinata* (B). The localities are as follows: A, F (Loclocality 2), B (Locality 11), C (Locality 3), D, E, G (Locality 4), H (Locality 14). The scale bars equal 0.1 mm (A), 0.01 mm (B–D, F–H), or 0.001 mm (E).

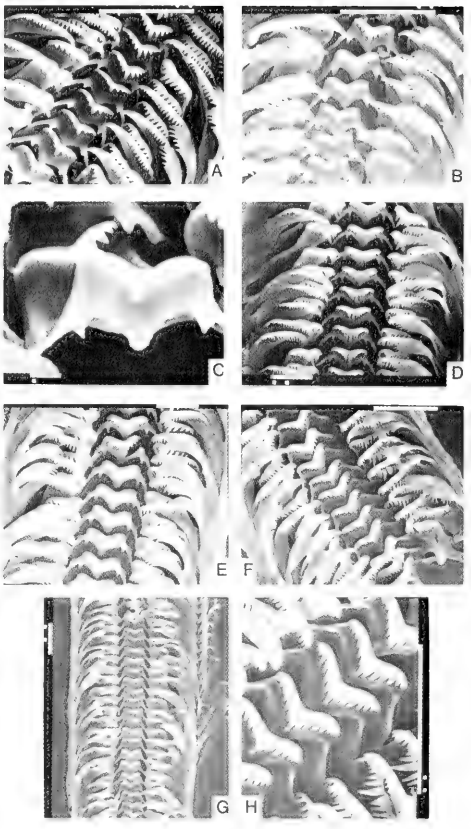


FIG. 15. Radulae of *Phreatodrobia micra* (A–C; Locality 3), *P. rotunda* (D, E; Locality 3) and *P. imitata* (F–H; Locality 20). Scale bars equal 0.01 mm (A, B, D–H) or 0.001 mm (C).

pallial cavity length (Fig. 13A). The ctenidial filaments are triangular in shape (when dissected out and laid flat), almost twice as long as wide, well-ciliated, and moderately thickened. The broadest part of the filament is positioned at almost two-thirds of the filament length. The osphradium occupies 22–28% of the pallial cavity length in all populations except that from the Union Stockyards Well (41%), which consists of very small-sized individuals. The osphradium is centered towards the posterior end of the ctenidium, with 20–30% of the ctenidium posterior to the end of the osphradium.

The central radular tooth is trapezoidal in shape with well developed lateral angles. A second pair of basal cusps on the central tooth was seen only in individuals from San Marcos Springs and the SWTSU Well. The inner marginal tooth is noteworthy for the large number of cusps it possesses (24–34) relative to other congeners. The stomach has a small caecal appendix (Fig. 18A). Note that the anterior arm of the U-shaped intestine loop in the pallial roof bends back posteriorly before turning anteriorly (Fig. 13A) (compare to simpler U-shaped loop in *P. micra*, Fig. 13B). The loop usually abuts against the longest gill filaments.

The testis (Fig. 19D) consists of simple lobes joining a central basal mass. The vas deferens exits at a point 25–30% back along the testis length. The seminal vesicle (Sv)

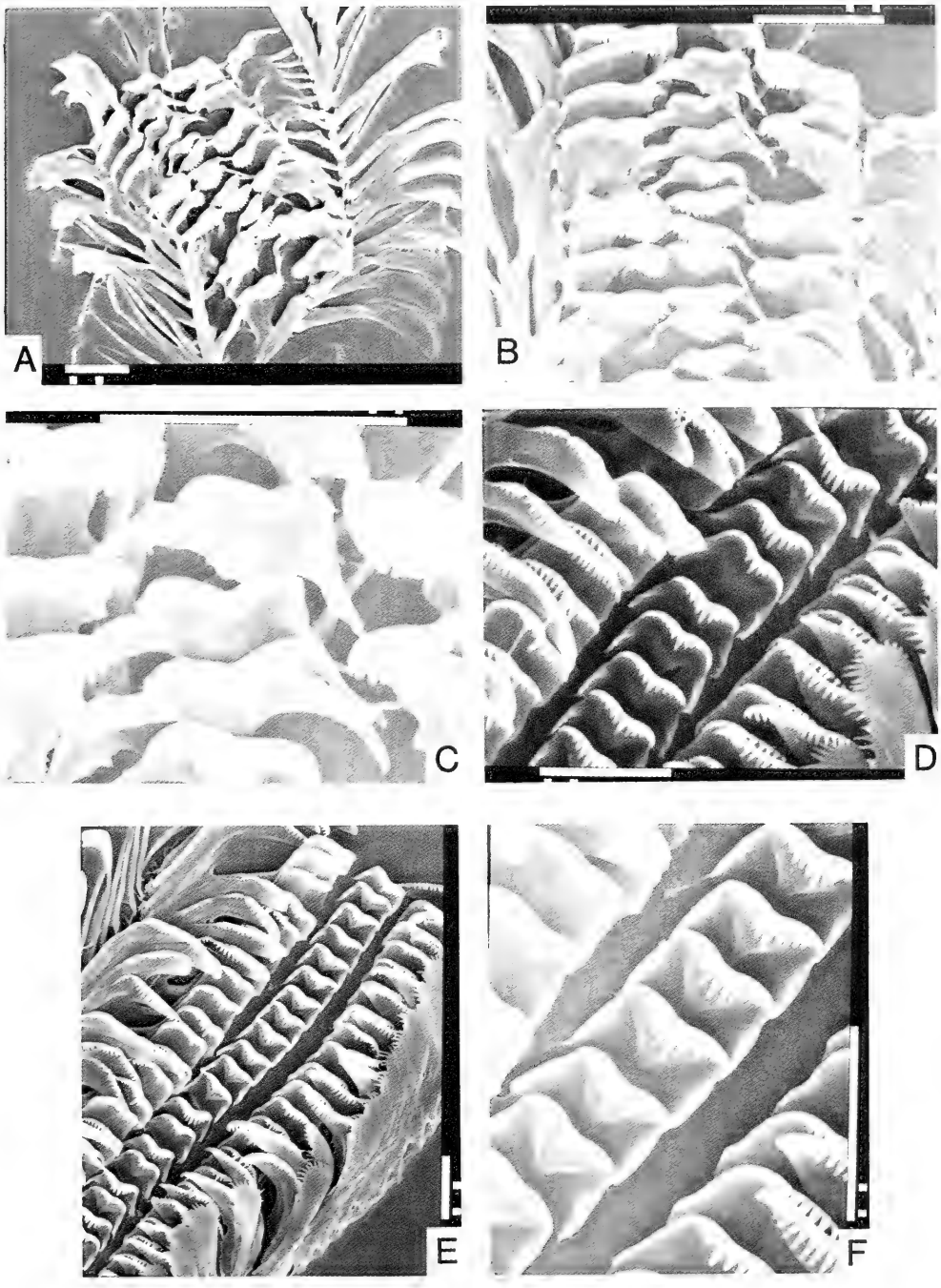


FIG. 16. Radulae of *Phreatodrobia plana* (A–C; Locality 3) and *P. conica* (D–F; Locality 5B). All scale bars equal 0.01 mm.

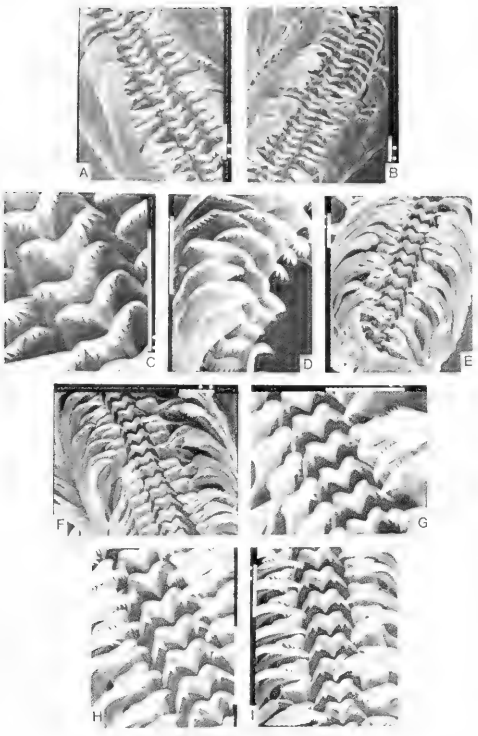


FIG. 17. Radulae of *Phreatodrobia punctata* (A–D), *Balconorbis uvaldensis* (E–G), and *Stygopyrgus bartonensus* (H, I). All scale bars equal 0.01 mm.

consists of a few, non-thickened coils largely hidden under the anterior portion of the testis. The anteriormost 52% of the prostate is pallial.

The penis (Fig. 19A, B) is about three times as long as the snout and has pronounced lobes along much of the inner curvature. The short penial filament lacks folds and has columnar epithelium (Co) along its sides. Long cilia are active along much of the length of the penial filament and extend onto the folds of the inner curvature. The penial folds have a glandular edge (not figured), and occasional glandular clusters (spherical bodies, Fig. 19A) are present along the penial length. The vas deferens undulates slightly in the penis.

The large ovary (Ova) lacks pronounced lobation and typically contains six to ten oocytes (Oo) of various sizes (Fig. 12). The anterior end of the ovary abuts against the stomach. The oviduct exits near the anterior end of the ovary and disappears beneath the bursa. The capsule gland is twice as long as the albumen gland. The ventral channel is narrow and has a pronounced lateral fold (not figured). The anterior end of the capsule gland terminates in a muscularized S-shaped coil (Fig. 20A) or simple twist (Fig. 20B) to the left side (populations from San Marcos and Barton Springs, SWTSU Well, Longhorn Portland Cement Company Well), or lacks such modifications and has a subterminal capsule gland opening (population from Union Stockyards Well). The development of the coil correlates with body size: larger females from Barton Springs, for instance, have the S-shaped coil while smaller individuals have a simple twist. The oviduct coil lies partly posterior to the albumen gland (Fig. 20A). The seminal receptacle opens into the end of the oviduct coil (right side) just posterior to the point where the short bursal duct joins the oviduct (Fig. 20C). The seminal receptacle always has a pink sheen as does the rather swollen oviduct coil, suggesting that the latter may also serve to store sperm. The bursa is flimsy in texture and was easily ruptured during dissection.

HOLOTYPE. ANSP 77574 (Fig. 4A, F, J).

TYPE-LOCALITY. Drift debris of Guadalupe River about four miles above New Braunfels, Comal County (Fig. 1, Locality 6).

DISTRIBUTION. Edwards (Balcones Fault Zone) Aquifer, and (possibly) Cow Creek and Glen Rose Aquifers in Travis, Hays, Comal, Kendall, Bexar, and Uvalde Counties (Fig. 1, Localities 1–4, 6, 8–12, 14, 26).

VARIATION. The large shell form variation seen in the populations from San Marcos and Barton Springs requires comment. Living specimens of the smaller, flat "form" from San Marcos Springs were always found encrusted with epigean epibionts whereas the more typical trochoid "form" was always found clean. A different habitat is suggested for these two "forms", with the former perhaps dwelling at or very near to the groundwater outlet. We do not know whether this dimorphism is phenotypic or genetic. The shells of living specimens of all Barton Springs "forms" were always clean. We suspect that this population is highly variable because it is of hybrid origin, with incompletely formed species having been brought into sympatry. More work is needed to sort out the systematics of this polytypic species.

DISCUSSION. *Phreatodrobia nugax* was originally described as a subspecies of *P.*

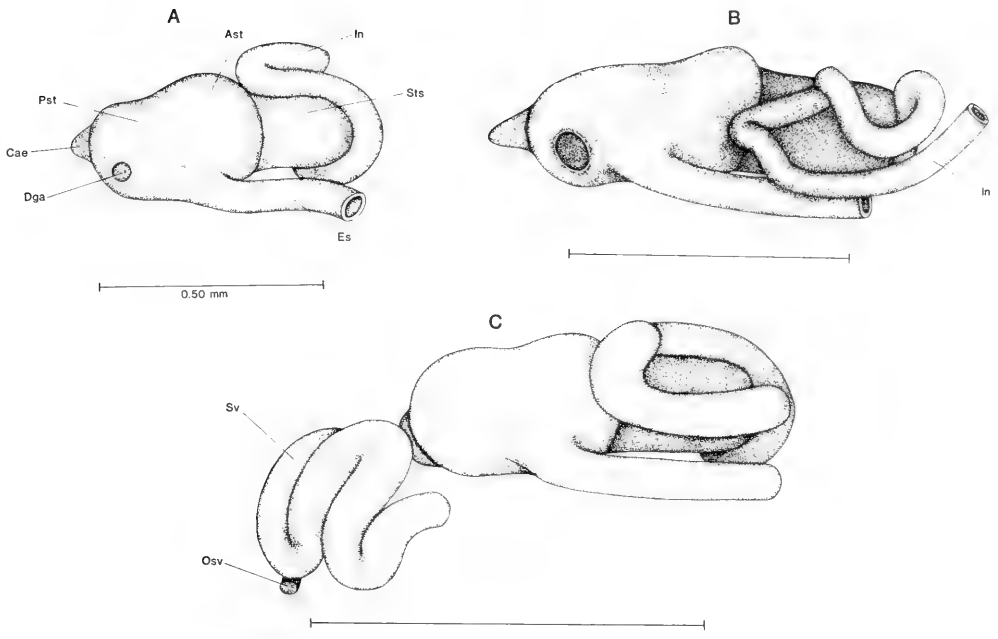


FIG. 18. Stomachs of *Phreatodrobia nugax* (A), *P. rotunda* (B), and *P. punctata* (C). Note the lack of external differentiation of the stomach chambers (Ast, Pst), and the coiling of the intestine (In) on the style sac (Sts) in B and C. The thickened coils of the seminal vesicle (Sv) of *P. punctata* are also figured in C. Ast = anterior stomach chamber; Cae = caecal appendix; Dga = digestive gland opening; Es = oesophagus; In = intestine; Osv = opening of seminal vesicle into testis; Pst = posterior stomach chamber; Sts = style sac; Sv = seminal vesicle.

micra and there has been some question as to whether these taxa are separate species (Fullington, 1978). We collected both species at three localities, two of which were caves from which only empty shells were obtained, the third of which was San Marcos Springs, from which living specimens of both species were obtained. As indicated above, there is more than sufficient anatomical evidence to merit separate species status for the San Marcos Springs populations. Note that at this locality the two species differ very significantly ($p < .001$, Mann-Whitney U Test) in size (shell width, data from Appendix 2). At the other two localities, Honey Creek Cave and Century Caverns, the size difference is somewhat less significant ($p < .05$). The shell shape difference between species is pronounced in samples from all three of these localities as well as for the type-specimens (which were also collected together at the same locality): compare Figs. 3F, J, N with 4A, F, J; 3R, S, T with 40; 3M, Q with 4S, W; and 3H, L, P with 4C, H, L. A shell height versus shell width plot for samples from given populations readily separates the two species (Fig. 22). Such pronounced differences in shell size and shape in sympatric populations suggests that two species exist at these localities (as well as in San Marcos Springs). It should be noted, however, that *P. nugax* can apparently converge on *P. micra* when found alone. Individuals from Union Stockyards Well have the very low spire, small size, and relatively large osphradium typical of *P. micra* (but have a complete ctenidium).

Phreatodrobia nugax nugax

MATERIAL EXAMINED. As for the species, excluding Longhorn Portland Cement Company Well.

DIAGNOSIS. Shell variable in size and form. Protoconch without tilt. Aperture rounded, free from or touching the penultimate whorl;

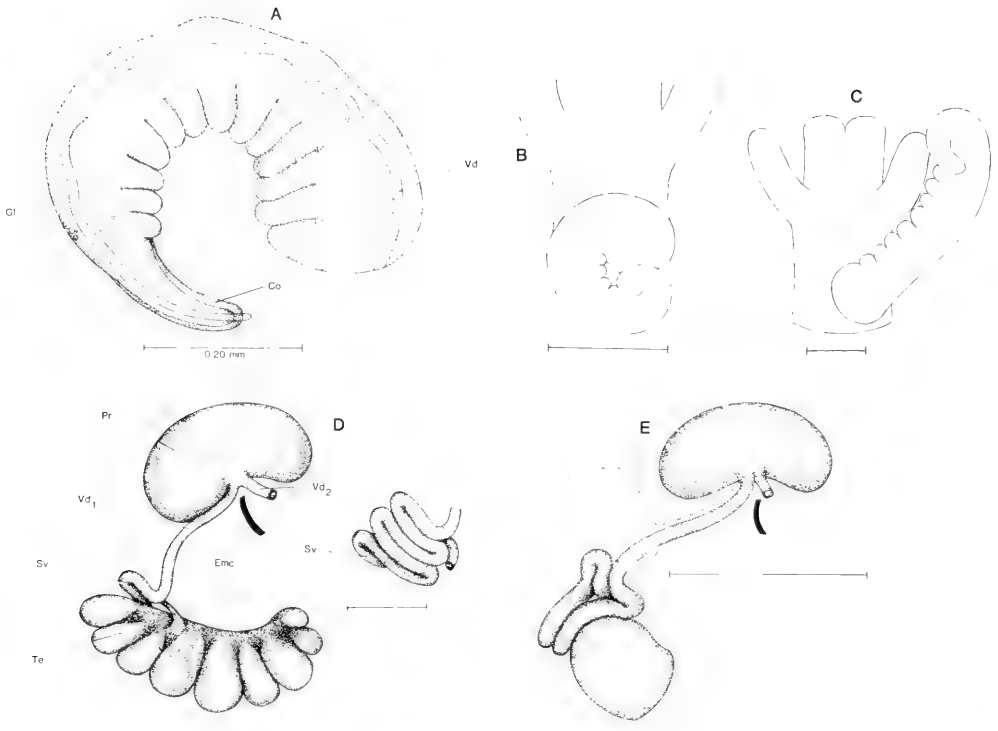


FIG. 19. Male reproductive morphology. A, B. Penis (dorsal aspect) of *Phreatodrobia nugax*. C. Penis (dorsal aspect) of *P. rotunda*. Note that the penis does not coil on the "neck" as in B. D. Testis (Te), Seminal vesicle (Sv), and prostate (Pr) of *P. nugax*. Note that about half of the prostate length is pallial. E. Testis (posterior portion not shown), seminal vesicle, and prostate of *P. plana*. The dashed lines indicate the position of the stomach. Co = columnar epithelium; Emc = posterior end of pallial cavity; Gl = glandular units; Pr = prostate; Sv = seminal vesicle; Te = testis; Vd = vas deferens; Vd_1 = vas deferens from seminal vesicle to prostate; Vd_2 = vas deferens from prostate to penis.

inner lip thickened and slightly to moderately flared. Apertural plane with or without slight tilt relative to coiling axis.

Phreatodrobia nugax inclinata Hershler & Longley, new subspecies

MATERIAL EXAMINED. BEXAR COUNTY: Longhorn Portland Cement Company Well.

DIAGNOSIS. Shell (Figs. 3A–E, I) only slightly wider than tall, of globose appearance, with protoconch tilted at 20° –30° relative to the teleoconch (Fig. 3I). Aperture fused to (not merely touching) the penultimate whorl; inner lip thin and flared only at fusion point. Aperture slightly angled anteriorly, apertural plane highly tilted (>30°) relative to shell axis.

REMARKS. The protoconch tilting and apertural peculiarities distinguish this subspecies from *P. n. nugax*. No anatomical differences were seen.

HOLOTYPE. ANSP 359089 (Fig. 3A, B).

PARATYPES. ANSP A10623D.

TYPE-LOCALITY. Longhorn Portland Cement Company Well (Fig. 1, Locality 11).

DISTRIBUTION. Thus far known only from the type-locality.

ETYMOLOGY. The subspecific epithet refers to the inclined or tilted position of the protoconch in this subspecies.

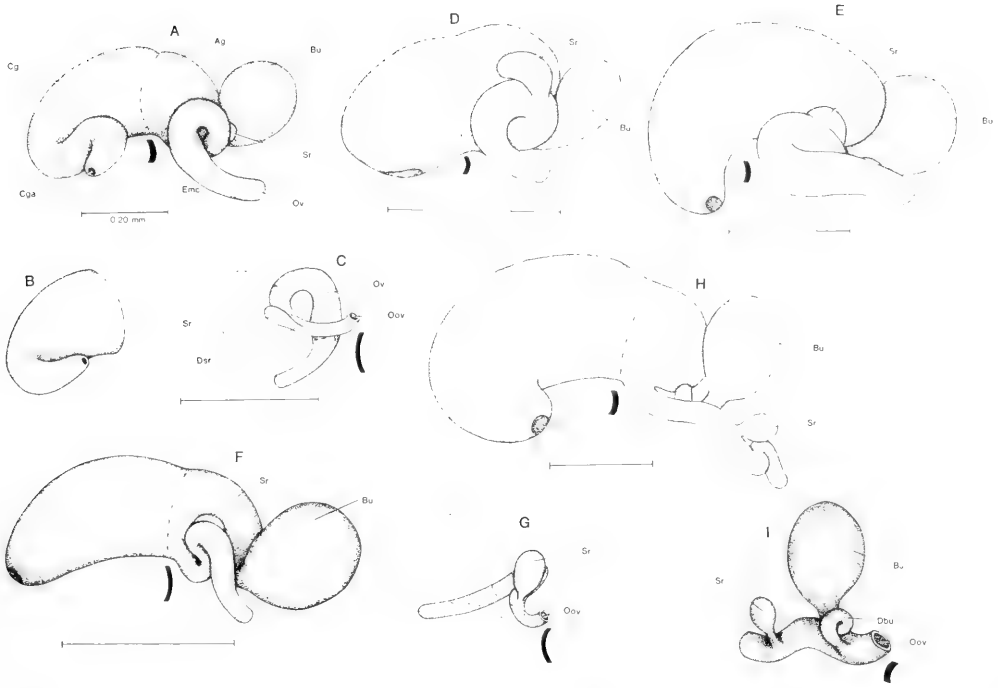


FIG. 20. Female reproductive morphology of *Phreatodrobia nugax* (A–C), *P. micra* (D), *P. conica* (E), *P. plana* (F, G), and *P. rotunda* (H, I). The left aspect is shown in A, B, D, E, F, and H; the right aspect is shown in all other figures. Only the anterior end of the capsule gland (Cg) is shown in B. Note the variation in coiling of the anterior end of the capsule gland (Cg) in *P. nugax* (A, B). All scale bars equal 0.2 mm. A and B have the same scale. Ag = albumen gland; Bu = bursa copulatrix; Cg = capsule gland; Cga = capsule gland opening; Dbu = duct of bursa copulatrix; Dsr = duct of seminal receptacle; Emc = posterior end of pallial cavity; Oov = opening of oviduct into pallial oviduct; Ov = oviduct; Sr = seminal receptacle.

Phreatodrobia rotunda Hershler & Longley, new species
Fig. 5E, F, I, J, 7E, 9H, I, 13G, 15D, E, 18B, 19C, 20H, I

MATERIAL EXAMINED. HAYS COUNTY: SWTSU Well; San Marcos Springs.

DIAGNOSIS. A large-sized species (shell width 2 mm). Shell (Fig. 5E, F, I, J) planispiral, with flattened base. Operculum (Fig. 9H, I) multi-whorled and striated. Ctenidium absent (Fig. 13G); osphradium short relative to pallial cavity length (13%). Central tooth of radula with a single pair of basal cusps (Fig. 15D, E). Style sac length 70% of that of stomach (Fig. 18B); intestine with complex coil on style sac (In, Fig. 18B) and narrow, elongate U-shaped loop in pallial roof; long axis of loop parallel to length of pallial cavity (Fig. 13G). Ovary and testis fill small portion (22%, 21%) of digestive gland length. Penis enlarged and without tight coil (Fig. 19C). Bursa duct coiled (Dbu, Fig. 20I); oviduct opens into anterior end of albumen gland.

REMARKS. This species is distinguished from other congeners by the following unique character-states: large planispiral shell, striated operculum, complex intestine coil on style sac, and very small-sized ovary and testis.

DESCRIPTION. Morphological description is based on study of material from San Marcos Springs. The shell has 3.0–3.8 whorls and varies from 1.83–2.16 mm in width. The protoconch, hidden (in apertural view) by rapidly expanding teleoconch whorls, has 1.25

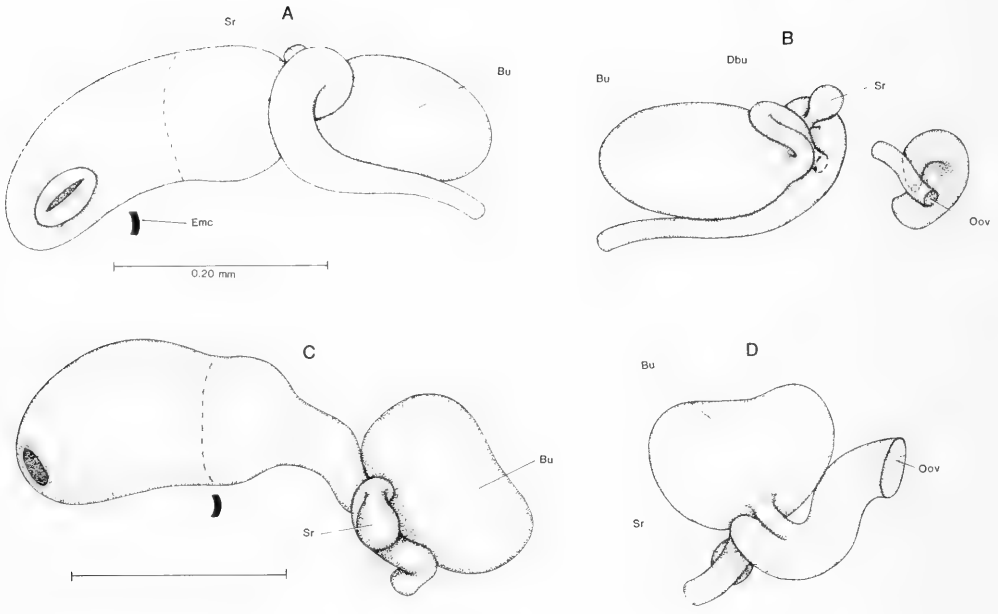


FIG. 21. Female reproductive morphology of *Phreatodrobia imitata* (A, B) and *P. punctata* (C, D). The left aspect is shown in A and C, and the right aspect in B and D. Note the muscularized capsule gland opening of *P. imitata* (A). All scale bars equal 0.2 mm. Bu = bursa copulatrix; Dbu = duct of bursa copulatrix; Emc = posterior end of pallial cavity; Oov = opening of oviduct into pallial oviduct; Sr = seminal receptacle.

whorls, the first whorl of which is marked by small pits, followed by a quarter whorl having strong, elevated growth lines (Fig. 7E). The teleoconch has fine growth lines. The apertural lip is relatively thin and appears broadly notched adapically when seen from above (Fig. 5I). The aperture is rounded and flared above and below. The inner lip is fused to the penultimate whorl. The outer lip is advanced relative to the remaining peristome, often angled or twisted (Fig. 5E), and is not flared. Note that the aperture does not extend above the penultimate whorl (Fig. 5E, F).

The operculum (Fig. 9H, I) is extremely thin, arched into a low cone, and has about five whorls. The ventral surface is smooth. The nucleus is positioned at about 44% of the operculum length. As for other *Phreatodrobia* that have a thin operculum, the operculum has a very light amber tint. Note the unusual operculum shape, with a sudden narrowing to the right in Fig. 9H. A large number of short, deep striations are arranged in rows which cross the growth lines at a high angle.

The central tooth of the radula is quite broad, as the lateral angles diverge at a high angle. The caecal appendix is enlarged compared to that of *P. nugax* (Fig. 18A, B). The intestine coil on the style sac sometimes extends onto the stomach. The U-shaped intestine loop in the pallial cavity roof is twice as long as wide and extends far into the anterior half of the pallial cavity (Fig. 13G).

The ovary and testis consist of a single solid mass. The vas deferens exits from the anterior end of the testis and has a few coils posterior to the stomach. The anteriormost 60% of the prostate is pallial. The penis is about four times as long as the snout and does not coil on the neck, but extends anteriorly (Fig. 19C). The capsule gland is more than twice as long as the albumen gland (Fig. 20H) and has a slight twist at its anterior end with a terminal opening. The section of oviduct anterior to the seminal receptacle is often swollen and has a pink sheen. The coiled bursa duct is shown in Fig. 20I. The seminal receptacle is positioned posterior to the bursa

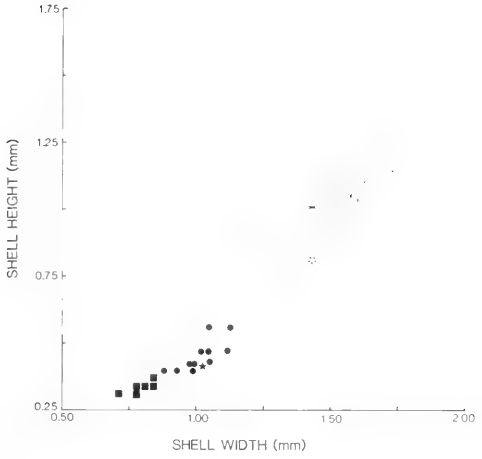


FIG. 22. Plot of shell height versus shell width for sympatric populations of *Phreatodrobia nugax* and *P. micra*. The populations represented are from San Marcos Springs (open square, *P. nugax*; filled square, *P. micra*) and Century Caverns (open circle, *P. nugax*; filled circle, *P. micra*). Type specimens for the two species are indicated by an open (*P. nugax*) or filled (*P. micra*) star. Note the clear separation between the species in the plot.

and albumen gland, and opens via a short duct anterior to the oviduct coil, and well posterior to the point where the bursa duct joins the oviduct (Fig. 20H, I).

HOLOTYPE. ANSP 359090 (Fig. 5E).

PARATYPES. ANSP A10623E.

TYPE-LOCALITY. San Marcos Springs (Fig. 1, Locality 3).

DISTRIBUTION. Edwards (Balcones Fault Zone) Aquifer in Hays County (Fig. 1, Localities 3, 4).

ETYMOLOGY. The epithet refers to the rounded outline of the shell of this species.

Phreatodrobia conica Hershler & Longley, new species
Figs. 5M–P, 7C, 8C, 13C, 16D–F, 20E

MATERIAL EXAMINED. COMAL COUNTY: Hueco (A, B) Springs; Honey Creek Cave. BEXAR COUNTY: City Water Board Artesian Station, Well 4.

DIAGNOSIS. Shell (Fig. 5M–P) large for genus (shell height 1.7 mm), conical, with a simple aperture and usually with a varix near the end of the body whorl (Fig. 5P). Teleoconch surface mottled with numerous short ridges. Ctenidium absent, osphradium filling 20% of the pallial cavity length (Fig. 13C). Central tooth of radula square-shaped and without basal cusps (Fig. 16D–F). Style sac length 79% of that of stomach; intestine loop in pallial roof U-shaped and with long axis parallel to pallial cavity length (Fig. 13C). Ovary and testis fill 30–40% of digestive gland length. Pallial portion of prostate relatively small; penis greatly elongate relative to snout. Oviduct enters anterior end of albumen gland.

REMARKS. Distinguishing features for this species include the conical shell with unusual sculpture pattern of small ridges, square-shaped central radular tooth, and small proportion of pallial prostate.

DESCRIPTION. The shell has 3.5–4.0 rounded whorls, a pronounced spire, and varies from 1.49–1.86 mm in height. The pits on the 1.5 protoconch whorls are well developed (Fig. 7C). Teleoconch sculpture consists of a large series of short ridges that are aligned at a slight angle to the whorl length. The ridges are occasionally elevated, particularly near the end of the body whorl, producing a mottled effect (Figs. 5O, 7C, 8C). Thickened axial growth lines are also present on the last half of the body whorl. Note that the ridges often converge and may be largely or totally absent in worn sections of the teleoconch (Fig. 5N). Seventy-three percent of adult shells from Hueco Springs (A, B) have a varix behind the aperture. The aperture is non-circular, thickened all around, and usually slightly separated from the penultimate whorl in adult shells.

Description of operculum and anatomy is based on study of specimens from Hueco (B) Springs. The operculum is flat, with about three whorls, and is slightly longer than wide. The nucleus is positioned at about 41% of the operculum length. Spiral growth lines are well developed on its surface.

The lateral angles of the central radular teeth project downwards instead of outwards, resulting in the unusual square shape of the tooth (Fig. 16D–F). Note that the lateral teeth sometimes have 10 or more cusps on one side of the central tooth (Fig. 16E). The stomach has a small caecal appendix.

The testis, unlobed as is the ovary, fills 42% of the digestive gland length. The seminal vesicle exits from the anterior end of the testis and coils posterior to the stomach. Only the anteriormost 33% of the prostate is pallial. The penis is seven-eight times as long as the

snout (preserved specimens) and has a somewhat thickened filament (not figured). The capsule gland is more than twice as long as the albumen gland and its anterior end bends back towards the end of the pallial cavity (Fig. 20E). The capsule gland opening is terminal. The tight oviduct coil is appressed against the posterior portion of the pallial oviduct, with the seminal receptacle opening into the right (inner) side of the coil. The short bursa duct joins the oviduct just before the opening into the albumen gland (not figured).

HOLOTYPE. ANSP 359086 (Fig. 5M).

PARATYPES. ANSP 359087/A10623B.

TYPE-LOCALITY. Hueco (B) Springs, Comal County (Fig. 1, Locality 6).

DISTRIBUTION. Edwards (Balcones Fault Zone) and possibly Cow Creek Aquifers in Comal and Bexar Counties (Fig. 1, Localities 6, 9, 13).

ETYMOLOGY. The epithet refers to the conical shell shape of this species.

Phreatodrobia plana Hershler & Longley, new species
Figs. 5A–D, G, H, K, L, 7F, 13E, 16A–C, 19E, 20F, G

MATERIAL EXAMINED. HAYS COUNTY: SWTSU Well, San Marcos Springs. COMAL COUNTY: Comal Springs; Natural Bridge Caverns.

DIAGNOSIS. A small-sized species with shell width ranging from 0.75–1.1 mm. Shell (Fig. 5A–D, G, H, K, L) planispiral, base flattened, aperture extends above penultimate whorl (Fig. 5A–C). Teleoconch sculpture consists of thickened, wrinkled collabral lines (Fig. 7F). Ctenidium absent, osphradium filling 33% of pallial cavity length (Fig. 13E). Central tooth of radula without basal cusps; lateral tooth lacking enlarged central cusp (Fig. 16A–C). Stomach length more than twice that of style sac (Fig. 19E); intestine coil in pallial roof complex (Fig. 13E). Ovary and testis fill 50–60% of digestive gland length. Oviduct enters anterior end of albumen gland (Fig. 20F).

REMARKS. Distinguishing features include the minute planispiral shell with adapically extended aperture, teleoconch sculpture consisting of thickened, wrinkled collabral lines, and the complex intestine coil in the pallial roof.

DESCRIPTION. The shell has 2.75–3.0 whorls and, while extremely small in the Comal and San Marcos Springs populations (shell width, <0.88 mm), often exceeds 1.00 mm in width in specimens from Natural Bridge Caverns. Note that the shells from Natural Bridge Caverns are considerably more flattened than those from San Marcos Springs. The whorls are rounded at the periphery and somewhat angled above and below. The protoconch is largely overlapped by the first teleoconch whorl (Fig. 7F). The first half whorl of the protoconch is covered with shallow pits which are joined by the thickened, wrinkled lines on the final protoconch whorl. Note how these thickened lines often converge on the teleoconch and appear plicate in places (Fig. 7F, upper left corner). These collabral lines are not well pronounced on the worn shells collected from Natural Bridge Caverns (Fig. 5D, H, L). The aperture is longer than wide, fused to the body whorl (Fig. 5B, C, D), and thickened all around. While extending above the penultimate whorl, the aperture does not extend below the flattened base. The lip is often fluted back ad- and abapically (appearing notched when seen from above and below), and the outer lip is bent adaxially in specimens from Natural Bridge Caverns (Fig. 5D) and Comal Springs (not figured).

Description of the operculum and anatomy is based on study of specimens from the SWTSU Well. The flat operculum is somewhat longer than wide, with about three whorls. The nucleus is positioned at about 40% of the operculum length. The lateral angles of the central radular tooth diverge at a high angle, producing an elongate trapezoidal shape for the tooth (Fig. 16B, C). Note the large number of cusps on the lateral tooth (often exceeding 20 for the entire row) and lack of enlarged central cusp. The stomach lacks a caecal appendix. The simple U-shaped intestine loop in the pallial roof, typical of *Phreatodrobia*, is modified in this species by addition of an extra, final loop outside of the preceding loop (Fig. 13E). Note that the long axes of the loops are oriented perpendicular to the pallial cavity length.

The testis and ovary are unlobed and fill 59% and 52% of the digestive gland length. The seminal vesicle exits from the anterior end of the testis and coils on the posterior part of the stomach (Fig. 19E). The anteriormost 47% of the prostate is pallial. The penis is about three times as long as the snout. The capsule gland is about twice as long as the albumen gland and has a terminal opening

(Fig. 20F). The oviduct coil is appressed to the left side of the albumen gland: note that the coil is counter-clockwise, not clockwise as is typical for the genus (Figs. 20A, D, E, 21A). The seminal receptacle opens into the right side of the oviduct coil just posterior to where the bursa duct enters (Fig. 20G).

HOLOTYPE. ANSP 359091 (Fig. 5A).

PARATYPES. ANSP A10623F.

TYPE-LOCALITY. San Marcos Springs, Hays County (Fig. 1, Locality 3).

DISTRIBUTION. Edwards (Balcones Fault Zone) and possibly Glen Rose Aquifers in Hays and Comal Counties (Fig. 1, Localities 3, 4, 8).

ETYMOLOGY. The epithet refers to the planispiral shell of this species.

Phreatodrobia imitata Hershler & Longley, new species
Figs. 6A–G, 8A, B, D, 9J, K, 13D, 15F–H, 21A, B

MATERIAL EXAMINED. BEXAR COUNTY: Verstraeten Well; O. R. Mitchell Well.

DIAGNOSIS. Shell (Fig. 6A–G, 8A, B, D) elongate-conical, height about 1 mm, with highly flared aperture. Teleoconch sculpture consisting of collabral costae and spiral lines. Ctenidium absent (Fig. 13D); osphradium filling 26% of pallial cavity length. Central tooth of radula without basal cusps (Fig. 15F–H). Style sac length two-thirds that of stomach; intestine coil in pallial cavity complex (Fig. 13D). Ovary and testis filling 30–40% of digestive gland length. Bursa duct coiled (Fig. 21B); oviduct entering posterior end of albumen gland; capsule gland opening with muscularized lip (Fig. 21A).

REMARKS. Distinguishing features of this species include the shell sculpture, consisting of collabral costae and spiral lines, complex intestine coil in pallial roof, and muscularized lip surrounding the capsule gland opening. Partial descriptions for this species were provided by Fullington (1978; for *Paludiscala* sp.) and Karnei (1978; for Gastropod Genus No. 2, Species 1 and 2) (both unpublished theses).

DESCRIPTION. The shell has 3.3–3.5 well-rounded whorls with deep sutures. Shell height averaged 1.01 mm for the Verstraeten Well sample and 1.03 mm for the O. R. Mitchell Well sample. The pits on the 1.0–1.25 protoconch whorls are well-developed (Fig. 6F). Spiral lines begin at the end of the protoconch and appear slightly wrinkled under high magnification (Fig. 8D). The lines cross the collabral costae (Figs. 6D, 8B). Low collabral ridges run between the costae and join the spiral lines (Fig. 8B, D). Costae are sometimes absent on the first 0.5–1.0 protoconch whorl (Fig. 6E). The costae are typically low (as in Figs. 6B, C, D, 8A, B): broad, lamelliform costae were not seen in the sample from O. R. Mitchell Well (n = 14) and were seen in only 10% of a sample from the Verstraeten Well (n = 32). Note that the lamelliform costae are not oriented perpendicular to the whorl surface, but curve (to the left in Fig. 6D). The aperture is rounded, moderately thickened, and highly flared, although flaring of the adapical lip is sometimes reduced (Fig. 6E). The aperture is sometimes loosened from the penultimate whorl. The umbilicus is open (Fig. 6G).

Anatomical description is based on study of material from Verstraeten Well. The thin operculum (Fig. 9J, K) is about as long as it is wide, with the nucleus positioned at about 40% of the operculum length. The ventral opercular surface is near smooth, with only a very small, low process (Fig. 9K).

The central tooth of the radula is trapezoidal in shape (Fig. 15F–H) and has especially narrow and elongate cusps. The stomach lacks a caecal appendix. The coil of the intestine in the pallial cavity roof (Fig. 13D) is modified by addition of an extra loop to the inside of the preceeding one. Note that the long axes of the loops are oriented parallel to the pallial cavity length.

The ovary and testis consist of a single solid mass and fill 34% and 28% of the digestive gland length. The vas deferens exits from the anterior end of the testis and coils posterior to the stomach. The anteriormost 45% of the prostate is pallial. The penis is only twice as long as the snout. The capsule gland is about one and a half times the length of the albumen gland (Fig. 21A). Note that a larger proportion of the capsule gland is pallial than typical for the genus. The narrow capsule gland opening is sub-terminal and surrounded by a muscular lip which measures 0.08 mm × 0.06 mm. The oviduct coil is partly posterior to the albumen gland. The short duct of the bursa loops back onto the right side of the bursa and joins the oviduct just before the opening into the posterior end of the albumen gland (Fig. 21B). Note that the bursa is oriented with its long axis parallel to the length of the pallial oviduct.

HOLOTYPE. ANSP 359088 (Fig. 6A).

PARATYPES. ANSP A10623C.

TYPE-LOCALITY. Verstraeten Well, Bexar County (Fig. 1, Locality 20).

DISTRIBUTION. Edwards Balcones Fault Zone) Aquifer in the Von Ormy Section of Bexar County (Fig. 1, Localities 20, 21).

ETYMOLOGY. The epithet refers to the convergence in shell form between this species and *Paludiscala* Taylor, 1966.

Phreatodrobia punctata Hershler & Longley, new species
Figs. 6H, I, 7C, 8E, 13F, 17A–D, 18C, 21C, D

MATERIAL EXAMINED. TRAVIS COUNTY: Barton Springs. HAYS COUNTY: San Marcos Springs.

DIAGNOSIS. A small-sized species, averaging 1.13 mm in shell height, with broadly conical shell (Fig. 6H, I) and flaring aperture. Teleoconch surface punctate (Figs. 6I, 7C, 8E). Ctenidium absent, osphradium filling 19% of pallial cavity length (Fig. 13F). Central tooth of radula almost square-shaped, without basal cusps (Fig. 17A–D). Central and lateral teeth without enlarged central cusp (Fig. 17A–D). Style sac length 63% of stomach length; intestine with loop on style sac and complex coil in pallial roof (Fig. 13F). Ovary and testis fill about 30% of digestive gland length. Oviduct enters posterior end of albumen gland (Fig. 21C).

REMARKS. This species is distinguished by its broadly conical shell with punctate teleoconch sculpture, and unusual morphology of the central and lateral radular teeth.

DESCRIPTION. The shell is one and a third times as long as wide, and has four moderately rounded whorls. The protoconch and teleoconch surfaces are covered with a series of deep pits surrounded by slight elevations, with sculptural relief more pronounced in the teleoconch. Note that the teleoconch sculpture is sometimes arranged into spiral and/or collabral rows (Fig. 6I, 7C). The aperture is moderately thickened, pyriform above, rounded below, and flared except where the inner lip fuses with the penultimate whorl (6I). Umbilicus present.

Description of operculum and anatomy is based on study of material from San Marcos Springs. The flat operculum is one and a half times as long as wide, with the nucleus positioned at about 41% of the operculum length. Spiral growth lines are well developed on the operculum surface. The ventral surface lacks a process or thickening.

Note that the lateral angles of the central tooth of the radula project down, imparting an almost square-shape to the tooth (Fig. 17C). The central and lateral radula teeth have as many as 18 and 22 cusps, respectively. The basal process of the central tooth is somewhat thickened. The stomach has a small caecal appendix (Fig. 18C). The looping of the intestine onto the right side of the style sac is shown in Fig. 18C. The intestine coil in the pallial roof has an extra loop to the inside of the previous loop with the long axes of the loops oriented parallel to the pallial cavity length. Note that the coil closely resembles that of *P. imitata* (compare Fig. 13D and F).

The ovary and testis, both without lobes, fill 32% and 35% of the pallial cavity length. The seminal vesicle exits from the anterior end of the testis and is composed of several thickened loops posterior to the stomach (Fig. 18C). The anteriormost 60% of the prostate is pallial. The capsule gland is over twice as long as the albumen gland, lacks an anterior twist or bend, and has a terminal opening. The oviduct coil is considerably posterior to the end of the albumen gland. The bursa is heart-shaped, and the short duct exits from the end of the shorter axis. The seminal receptacle enters the left side of the oviduct coil. Note that the oviduct widens greatly as it merges with the albumen gland.

HOLOTYPE. ANSP 359092 (Fig. 6H).

PARATYPES. ANSP A10623G.

TYPE-LOCALITY. San Marcos Springs, Hays County (Fig. 1, Locality 3).

DISTRIBUTION. Edwards (Balcones Fault Zone) Aquifer in Hays and Travis Counties (Fig. 1, Localities 2, 3).

ETYMOLOGY. The epithet refers to the punctate teleoconch sculpture characteristic of this species.

Subfamily Littoridininae
Balconorbis Hershler & Longley, new genus

DIAGNOSIS. Shell (Fig. 23A, B, E–H) minute (width about 1.0 mm), planispiral, transparent, colorless. Protoconch and teleoconch sculpture consisting of spiral lines (Fig. 7G). Operculum paucispiral with sub-central nucleus. Animal unpigmented and without eyespots. Pallial cavity longer than wide, ctenidium absent, osphradium filling 24% of

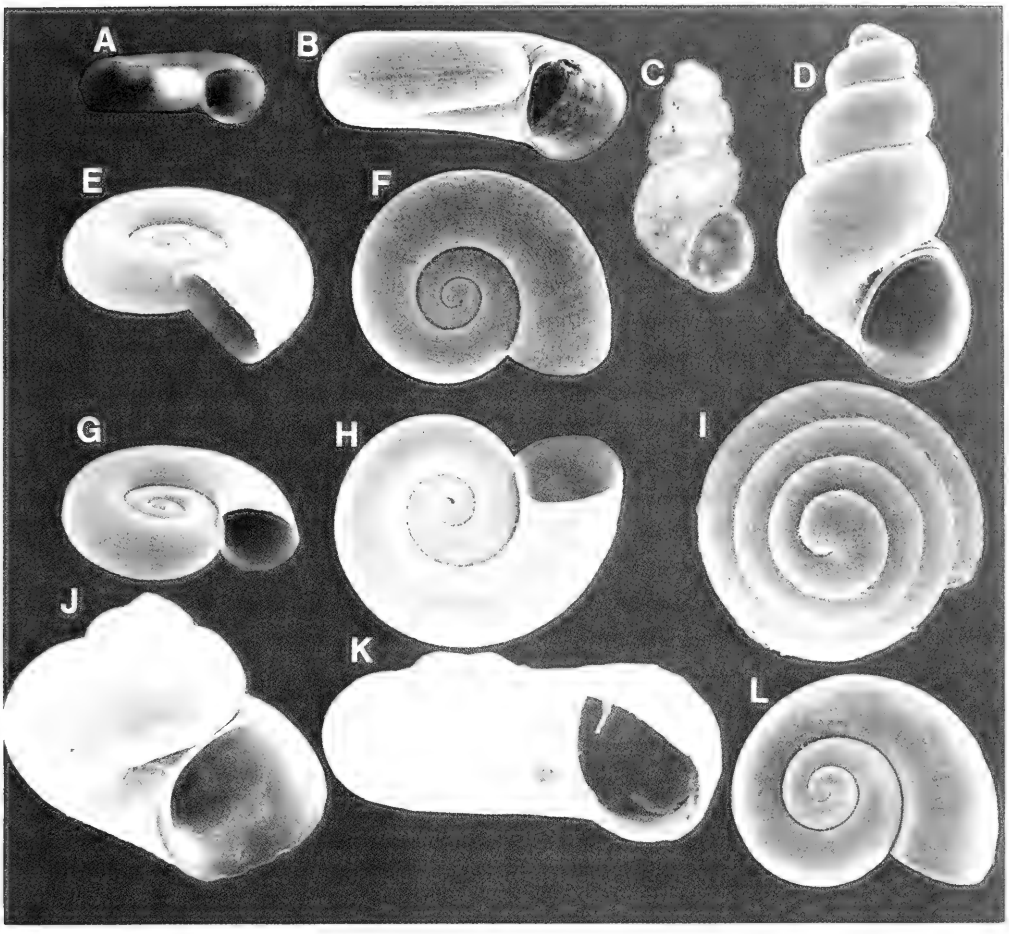


FIG. 23. Shells of *Balconorbis uvaldensis* (A, B, E–H; Locality 30), *Stygopyrgus bartonensis* (C, D, I; Locality 2), *Horatia klecakiana* (J), and "*Horatia*" sp. (K,L). Shell widths are as follows: A (1.02 mm, holotype), B (1.15 mm), C (shell height, 0.97 mm, holotype), D (shell height, 0.97 mm), E (0.932 mm), F (0.986 mm), G (0.803 mm), H (1.03 mm), I (0.513 mm), J (1.48 mm), K (1.6 mm), and L (1.83 mm).

pallial cavity length (Fig. 13H). Central tooth of radula (Fig. 17E–G) with single pair of basal cusps arising from lateral angles. Intestine with U-shaped loop in posterior portion of pallial roof; long axis of loop almost perpendicular to pallial cavity length (Fig. 13H). Anteriormost 47% of prostate pallial. Penis with single spherical lobe on outer curvature bearing a large apocrine gland (Fig. 24B). Capsule gland (Cg) with two tissue sections and terminal opening (Fig. 25B); posterior end of albumen gland coiled. Sperm pouches absent; sperm stored in anterior coil of oviduct. Spermathecal duct (Sd) issues from posterior end of pallial oviduct (where oviduct enters), joins capsule gland anteriorly.

REMARKS. The minute planispiral shell with spiral sculpture and absence of sperm pouches in the female reproductive system distinguish this genus from other littoridinines.

TYPE-SPECIES. *Balconorbis uvaldensis* Hershler & Longley, new species.

DISTRIBUTION. The Edwards (Balcones Fault Zone) Aquifer in Uvalde County.

ETYMOLOGY. The generic name is derived by combining Balcones, referring to the pres-

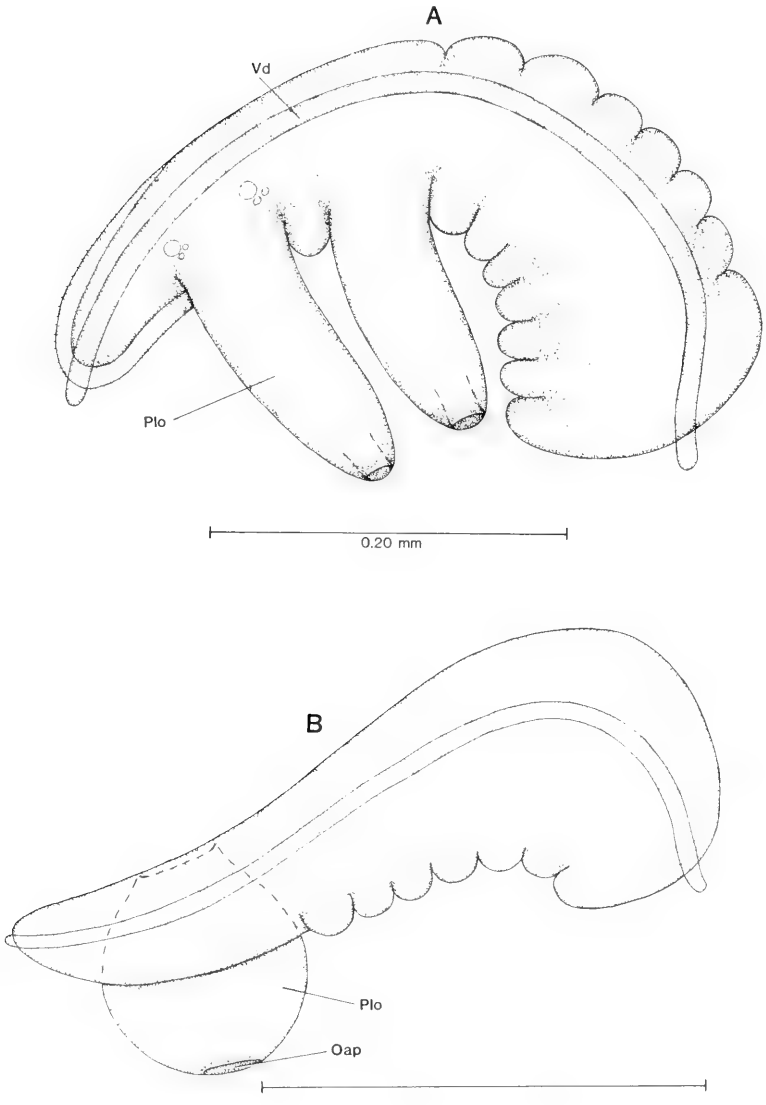


FIG. 24. Penes (dorsal aspect) of *Stygopyrgus bartonensis* (A) and *Balconorbis uvaldensis* (B). The dashed lines in the penial lobes (Plo) of *Stygopyrgus bartonensis* indicate the narrow distal ends of the ducts in the lobes. Note the lack of undulation of the vas deferens (Vd) in the penes. Both scale lines equal 0.20 mm. Oap = opening of apocrine gland; Plo = penial lobe; Vd = vas deferens.

ence of this genus in the Balcones Fault Zone region, with the Latin word *orbis*, referring to the circular outline of the shell.

Balconorbis uvaldensis Hershler & Longley, new species

Figs. 7G, 13H, 17E–G, 23A, B, E–H, 24B, 25B

MATERIAL EXAMINED. UVALDE COUNTY: King Farms Well; R. Carnes Well; R. K.

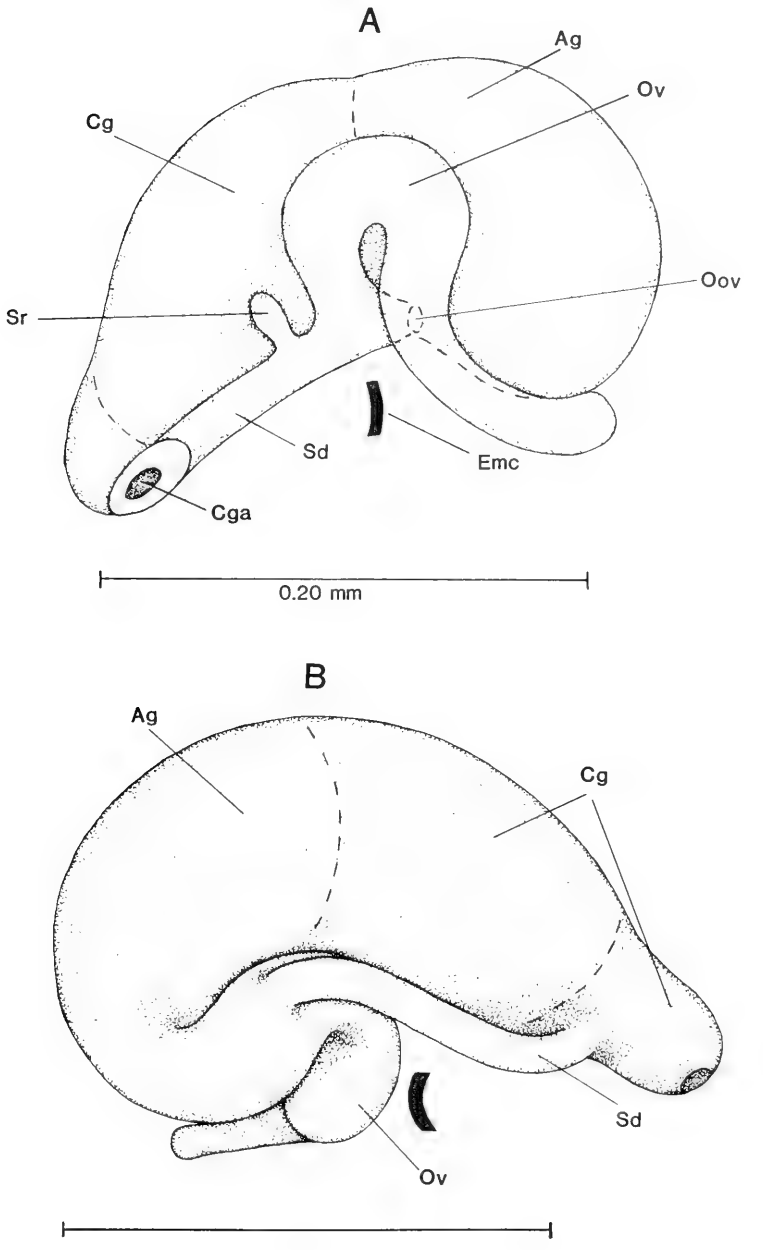


FIG. 25. Female reproductive morphology of *Stygopyrgus bartonensis* (A) and *Balconorbis uvaldensis* (B). The left aspect is shown in A and the right aspect in B. Note that both species lack a bursa copulatrix and have two tissue sections in the capsule gland (Cg), as indicated by dashed lines. Ag = albumen gland; Cg = capsule gland; Cga = capsule gland opening; Emc = posterior end of pallial cavity; Oov = opening of oviduct into pallial oviduct; Ov = oviduct; Sd = spermathecal duct; Sr = seminal receptacle.

Dunbar Well; S. Moerbe Well; G. Ligocky Well; Uvalde National Fish Hatchery Well.

DESCRIPTION. The shell has 2.75–3.0 tubular whorls and varies in width from 0.9 to 1.22 mm. Note that the spiral lines, which appear ragged on the protoconch (compared to the teleoconch lines), extend almost to the apex (Fig. 7G); and no other protoconch sculpture is present. The teleoconch also has numerous strong collabral growth lines which cross and often offset the spiral lines. The collabral lines are of highest relief near the aperture (Fig. 23G). The body whorl has about 50 spiral lines. The thin-lipped aperture is wider than long. The peristome is fairly straight while the rest of the lip is rounded.

The description of operculum and anatomy is based on study of specimens from G. Ligocky Well. The flat, thin operculum is wider than long, with about 2.5 whorls. The nucleus is positioned at 41% of the operculum length.

The central tooth of the radula is trapezoidal in shape with widely diverging lateral angles (Fig. 17E–G). Note that the cusps of the central teeth either come to a point (Fig. 17E, G) or are somewhat rounded (Fig. 17F). The anterior and posterior stomach chambers are well distinguished externally. The stomach lacks a caecal appendix. The anus, typically positioned near the mantle edge in hydrobiids, is about 30% back along the pallial cavity length (Fig. 13H).

The ovary and testis are without lobes. The vas deferens exits from the anterior end of the testis and consists of a few non-thickened coils posterior to the stomach. The penis is attached to and coils on the right side of the "neck", and is twice as long as the snout. Small folds extend for about two-thirds of the penis length from the base along the inner curvature. While attached to the right edge of the penis (about 0.08 mm from the tip), the penial lobe was folded under the penis (to the left side, as in Fig. 24B) in all but one specimen dissected. In that specimen the penial lobe simply projected to the right of the narrow attachment area. It is possible that the typical condition resulted from muscle contraction during fixation. The near-circular penial lobe measures about 0.10 mm across. The glandular opening is terminal and wide (Oap). The glandular lumen is fairly large and the gland is apocrine in type. The vas deferens does not coil in the penis.

The anterior end of the ovary abuts against the stomach. The coiled portion of the anterior oviduct has several swellings where sperm is presumably stored. Taking the coiled portion of the albumen gland into account, this gland is equal in length to the capsule gland. The anterior capsule gland section is about half the length of the posterior section and is clear, while the latter is white. Note the blunt anterior end of the capsule gland. The spermathecal duct (Sd) issues from the albumen gland just at the point where the former receives the oviduct. The spermathecal duct is largely ventral to the pallial oviduct and fairly thickened. It enters the capsule gland 0.04 mm from the terminal capsule gland opening.

HOLOTYPE. ANSP 359084 (Fig. 23A).

PARATYPES. ANSP 359085/A10623A.

TYPE-LOCALITY. G. Ligocky Well, Uvalde County (Fig. 1, Locality 30).

DISTRIBUTION. As for genus (Fig. 1, Localities 23, 27, 28, 29, 30, 31).

ETYMOLOGY. The epithet refers to the distribution of this species in Uvalde County.

Stygopyrgus Hershler & Longley, new genus

DIAGNOSIS. Shell (Fig. 23C, D, I) minute (about 1.0 mm in height), elongate-conic, transparent, colorless. Protoconch sculpture pitted (Fig. 23I); teleoconch sculpture consisting of spiral lines. Operculum paucispiral; nucleus positioned at 36% of operculum length. Pallial cavity about as wide as long, ctenidium absent, osphradium filling 25% of pallial cavity length (Fig. 13I). Central tooth of radula with one pair of basal cusps arising from prominent lateral angles. Intestine with U-shaped loop in pallial roof; long axis of loop perpendicular to pallial cavity length (Fig. 13I). Ovary and testis filling 33% and 60% of digestive gland length, with ovary covering posterior half of stomach. Half of prostate pallial; slender penis with two glandular lobes on inner curvature (Fig. 24A). Capsule gland with two tissue sections and subterminal, muscularized opening (Fig. 25A). Oviduct coil, spermathecal duct, and seminal receptacle appressed to left side of pallial oviduct; bursa absent. Oviduct and spermathecal duct open jointly into anterior end of albumen gland. Seminal receptacle opens into spermathecal duct; anterior end of spermathecal duct fused with capsule gland opening.

REMARKS. The minute, elongate-conical shell and unique configuration of the female reproductive system distinguish this genus from other Littoridininae.

TYPE-SPECIES. *Stygopyrgus bartonensis* Hershler & Longley, new species.

DISTRIBUTION. Thus far known only from Barton Springs, Travis County.

ETYMOLOGY. The generic name is derived from the Greek words *Stygos*, meaning lower world, and *pyrgos*, meaning tower, and refers to the phreatic habit and elongate shell of this taxon.

Stygopyrgus bartonensis Hershler & Longley, new species
Figs. 13I, 17H, I, 23C, D, I, 24A, 25A

MATERIAL EXAMINED: TRAVIS COUNTY: Barton Springs.

DESCRIPTION. The shell has 4.0–4.6 well-rounded whorls. Shell height varies from 0.97–1.3 mm. Note that the pitted microsculpture is best developed on the first half whorl of the protoconch (Fig. 23I). A total of about 20 fairly regularly spaced and pronounced spiral lines are found on the body whorl. Note that the lines are poorly developed to absent on the adapical third of the whorl. Collabral growth lines are also well developed on the teleoconch, although of lower relief than the spiral lines. The aperture is longer than wide, somewhat angled above, and touches the penultimate whorl. The lip is slightly thickened and does not flare. The umbilicus is chink-like.

The operculum is thin and flat. The central tooth of the radula is trapezoidal in shape, with diverging lateral angles (Fig. 17H, I). The dagger-like cusps of all four tooth types are elongate. The stomach lacks a caecal appendix. The anterior and posterior stomach chambers are well-distinguished externally.

Both gonads are without lobes. The seminal vesicle exits from the anterior end of the testis and consists of a few thickened coils posterior to the stomach. The penis is attached to and coils on the right side of the "neck," and is five times as long as the snout. The posterior half of the penis has deep folds (Fig. 24A). The two penial lobes, of similar appearance, are elongate (three times as long as wide), without folds, with a narrowed tip, and with a terminal pore through which glandular products are secreted. The glandular lumen is fairly large, filling about half to two-thirds of the penial lobe, and terminate distally in a narrow neck. The general appearance of the glandular lobe is similar to that described for *Mexipyrgus* Taylor, 1966 (Taylor, 1966; Hershler, 1985). A few glandular clusters are scattered throughout the penis and the penial folds have glandular edges. The small section of penis between the lobes and tip is lined (at the edges) with ciliated columnar epithelia. The vas deferens does not coil in the penis. Small spherical epibionts were seen clinging to the distal ends of the penial lobes.

The capsule gland is slightly larger than the albumen gland (Fig. 25A). The albumen gland is clear. The anterior section of the capsule gland narrows somewhat and is clear, while the much larger posterior section is yellow. The capsule gland opening is very slightly posterior to the anterior end of the gland. The U-shaped oviduct loop is centered at the junction between the albumen and capsule glands. The narrow posterior extension of the fused oviduct and spermathecal duct opens into the anterior end of the albumen gland on its left side. The seminal receptacle has a pink sheen and is less than 10% of the pallial oviduct length. Note that the seminal receptacle is positioned anterior to the end of the pallial cavity. The spermathecal duct joins the posterior side of the muscular capsule gland opening.

HOLOTYPE. ANSP 359093 (Fig. 23C).

PARATYPES. ANSP A10623H.

TYPE-LOCALITY. Barton ("Concession") Springs (Fig. 1, Locality 2).

DISTRIBUTION. As for genus.

ETYMOLOGY. The epithet refers to the type-locality.

DISCUSSION

Systematic relationships. A comparison among the nine species considered in this paper, as well as phreatic "*Orygoceras*" sp. (also known from south-central Texas), involving 40 binary-coded characters (72% from anatomy), is given in Appendix 4. A phenogram based on these data is shown in Fig. 26. As seen in the phenogram, the seven *Phreatodrobia* spp. form a cluster quite distinct from the remaining three species, all of which are monotypic littoridinines. The description of *Balconorbis* and *Stygopyrgus* brings the total of described phreatic littoridinine genera in the Western Hemisphere to four: *Paludiscala* and *Coahuilix* were previously described from (and considered en-

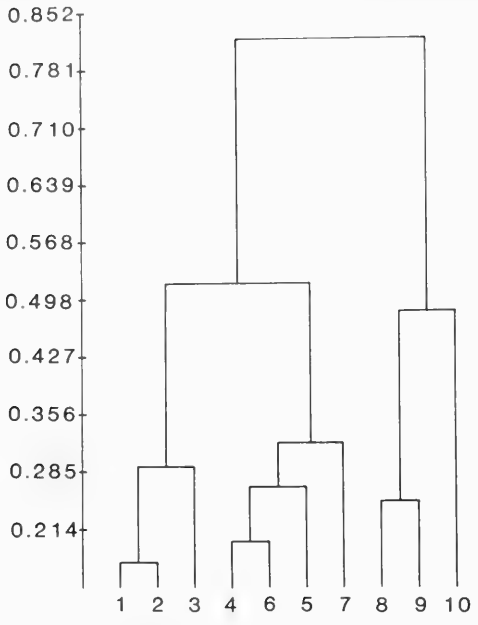


FIG. 26. Phenogram showing similarities among 10 species of phreatic Hydrobiidae from south-central Texas. The numbering of species is as in Appendix 4, which is also the source of the data used to generate the phenogram.

demic to) the Cuatro Ciénegas Basin in northern Mexico (Taylor, 1966; Hershler, 1984, 1985). A comparison among these taxa involving 16 morphological features is given in Table 5 and the anterior portion of their female reproductive systems is schematically diagrammed in Fig. 27. "*Orygoceras*" sp. is not included in this comparison as it has a number of unique character-states and probably represents an offshoot within the Littoridininae separate from the lineage(s) to which these other four genera belong to. The most similar pair among these four genera is *Paludiscala* and *Coahuilix*. Their penes are nearly identical as is the groundplan of their female reproductive systems, typified by the possession of a large-sized bursa, loss of the seminal receptacle (although a presumably secondarily-derived one is found in *Paludiscala*), and joint opening of the oviduct and sperm duct into the posterior section of the albumen gland (Fig. 27A, B). We believe that these two genera are closely related and belong to the same lineage within the Littoridininae.

The penis of *Balconorbis* is the same type as that of *Paludiscala* and *Coahuilix*, with a single spherical lobe on the outer curvature bearing an apocrine gland, and its female reproductive system (Fig. 27C) could represent a modification of that of the latter two genera involving loss of the bursa and resulting anterior extension of the sperm (now effectively a spermathecal) duct to join the capsule gland. Other character-states found in *Balconorbis*, notably the spiral protoconch microsculpture, could mitigate against a close phyletic relationship of this genus with those from Cuatro Ciénegas, although the value of this feature in separating higher taxa has recently been questioned (Hershler, 1985).

The mammiform penial glands and aspects of the female reproductive system (presence of a seminal receptacle and muscularized capsule gland opening, position of oviduct coil on left side of pallial oviduct) of *Stygopyrgus* readily distinguish this genus from the other three. *Stygopyrgus* may belong to a separate (from the above) littoridinine invasion of the phreatic habitat, perhaps from an ancestor belonging to the *Mexipyrgus-Durangonella* group (Hershler, 1984). Note that *Mexipyrgus* and *Durangonella* also have a muscularized anterior portion of the capsule gland and elongate, glandular (mammiform in *Mexipyrgus*) penial lobes. In conclusion, it appears that *Balconorbis* and *Stygopyrgus* may have close relatives among phreatic or epigean hydrobiids of the southwestern United States and northern Mexico.

Phreatodrobia nugax and *P. micra* were previously considered congeners of *Horatia s.s.* and *Hauffenia s.s.* from Europe. This is not an unrealistic possibility: such a situation exists for two Edwards Aquifer crustacean genera, *Monodella* Maguire and *Palaemonetes* Heller, both of which are considered to be of Tethyan origin and of brackish-water or marine ancestry (Stock, 1976; Strenth, 1976). The Hydrobiinae of Europe and North America also probably share a common, brackish-water ancestor (Johannson, 1956; G. M. Davis, 1979). A number of general similarities are seen between the above two sets of hydrobiid taxa. Note that *Horatia* and *Hauffenia* also have a low trochoid-planispiral shell (Binder, 1957, fig. 1; Pollonera, 1898, fig. 2); intestinal loop (simple) in the pallial roof (Boeters, 1974, fig. 3; Hershler, personal observations); bursa copulatrix positioned largely posterior to the albumen gland; oviduct coil located on the left

TABLE 5. Comparison of 16 morphological features among phreatic littoridinine genera of Texas and Mexico. Data regarding *Paludiscala* and *Coahuilix* are from Hershler (1985).

	Balconorbis	*Stygopyrgus*	*Paludiscala*	*Coahuilix*
1. Maximum shell size (height or width, mm)	1.22	1.30	2.60	1.37
2. Shell form	planispiral	elongate-conic	elongate-conic	planispiral
3. Protoconch sculpture	spiral lines	punctate	punctate	punctate
4. Teleoconch sculpture	spiral lines	spiral lines	collabral costae	absent
5. Ctenidium	absent	absent	present	present*
6. Intestine loop in pallial roof	present	present	absent	absent
7. Number of penial lobes	1	2	1	1
8. Position of penial lobe(s)	outer curvature of penis	inner	outer	outer
9. Penial gland type	apocrine	mammiform	apocrine	apocrine
10. Anterior oviduct coil	ventral to pallial oviduct	on left side of pallial oviduct	absent	present
11. Oviduct opens into	posterior tip of albumen gland	anterior end of albumen gland	posterior section of albumen gland	posterior section of albumen gland
12. Bursa copulatrix	absent	absent	present	present
13. Seminal receptacle	absent	present	present**	absent
14. Openings of spermathecal duct and capsule gland	fused	fused	fused	separate
15. Number of capsule gland tissue sections	2	2	3	2
16. Capsule gland opening	simple	muscularized	simple	muscularized

*Ctenidium absent in *C. hubbsi*, present in *C. landyei*.
**Seminal receptacle secondarily derived.

side of the pallial oviduct, and seminal receptacle opening into the oviduct coil (Radoman, 1966, fig. 8; Hershler, personal observations). Such general similarities also extend to the radula (see Fig. 27C, D for European genera) and protoconch microsculpture (Fig. 7I). The similarity of shell form, however, is superficial, the shell of the European genera (Fig. 23J) is larger, thicker, and more globose than that of any *Phreatodrobia*. Both *Horatia* and *Hauffenia* have penial lobes (Boeters, 1974, fig. 2; Giusti *et al.*, 1981, fig. 1), and the penial surface has complex glandular swellings (Hershler, personal observations), character-states not seen in any *Phreatodrobia*. No European hydrobiid has the thickened opercular process seen in the two *Phreatodrobia* spp. Other character-states seen in some *Phreatodrobia*, but not in any European hydrobiids, include a highly complex intestinal coil in the pallial roof, lack of basal cusps on the central tooth of the radula, and a coiled anterior end of the capsule gland. These differences suggest that *Phreatodrobia* represents a separate adaptive radiation meriting generic distinction from *Horatia*, *Hauffenia*, and other European hydrobiines.

Several other phreatic taxa are also known from North America, notably *Antrobia* Hubricht, *Fontigens* Pilsbry, *Antroselates* Hubricht, and "*Horatia*" (Hubricht, 1940). While none of these taxa has received detailed anatomical study, a limited comparison can be made with *Phreatodrobia*. *Fontigens*, while having a hydrobiine-type female reproductive system (Hershler, personal observations), is clearly separated from all other North American Hydrobiidae by its unique penis, which has two accessory glands fed by thickened ducts which run through the penis base to end blindly in the nuchal cavity. *Antroselates*, while lacking basal cusps on the central

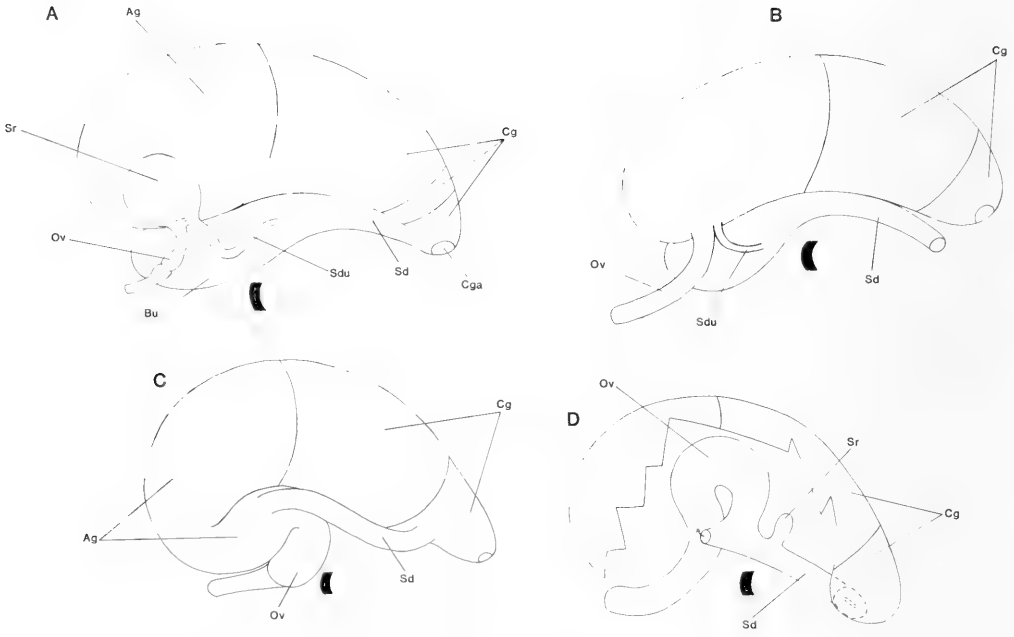


FIG. 27. Schematic representation of the female reproductive systems of *Paludiscala* (A), *Coahuilix* (B), *Balconorbis* (C), and *Stygopyrgus* (D). The right aspect is shown in all cases. In D, the arrow indicates the opening of the oviduct (Ov) into the pallial oviduct. Ag = albumen gland; Bu = bursa copulatrix; Cg = capsule gland; Cga = capsule gland opening; Ov = oviduct; Sd = spermathecal duct; Sdu = sperm duct; Sr = seminal receptacle.

tooth of the radula (Hubricht, 1963), as do some *Phreatodrobia*, is much larger (shell height, > 5 mm) and has an elongate, high-spired shell (Hubricht, 1963, pl. 8). "*Horatia*", collected from Manitou Cave in Alabama (Hubricht, 1940), has a near-planispiral shell (Figs. 23K, L) and an intestinal loop in the pallial roof (Hershler, personal observations), but differs from *Phreatodrobia* in having spiral lines on the protoconch (Fig. 7H). Note that the central teeth of the radula of both "*Horatia*" (Fig. 28A) and *Fontigens* (Fig. 28B) have short cusps, contrasting with the typically elongate cusps of *Phreatodrobia*. *Antrobia* has a hydrobiine female reproductive system, a slight intestinal loop in the pallial cavity, and a simple penis (Hubricht, 1971; Hershler, personal observations), yet differs from *Phreatodrobia* in having a thickened, globose, amnicolid-like shell (Hubricht, 1971, figs. 4–6) and spiral lines on the protoconch (Hershler, personal observations). On the basis of the limited data available, we conclude that while *Phreatodrobia* is probably not closely related to either *Fontingens* or *Antroselates*, it may belong to the same lineage as one or more of the remaining two taxa. It should also be pointed out that there are no known hydrobiines among epigean freshwater hydrobiids of North America.

As indicated in the phenogram, two pairs of *Phreatodrobia* spp. link closely: *P. micra* and *P. nugax* (0.175); and *P. conica* and *P. punctata* (0.200). *Phreatodrobia micra* and *P. nugax* share distinctive character-states that include presence of a ventral operculum process, an incomplete or complete ctenidium, and basal cusps on the central tooth of the radula. Note, however, that some populations of *P. nugax* have a smooth operculum. The shell similarity between these two species has been discussed above.

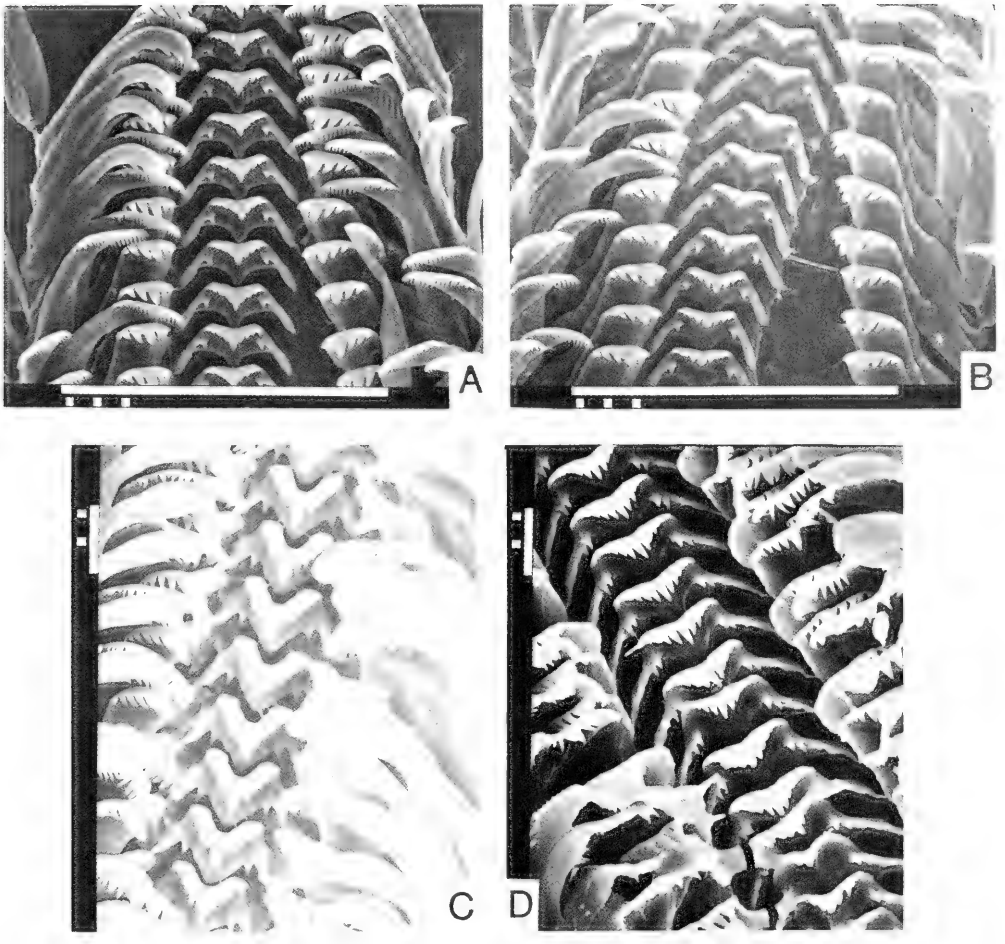


FIG. 28. Radulae of "*Horatia*" sp. (A), *Fontigens nickliniana* (B), *Horatia klecakiana* (C), and *Hauffenia subpiscinalis* (D). The scale bars in A and B equal 0.1 mm, while those in C and D equal 0.01 mm.

Phreatodrobia conica and *P. punctata* are distinguished from the above species pair by the following features: teleoconch sculpture consisting of low swellings or ridges; central tooth of radula square-shaped; lateral tooth of radula with numerous cusps; intestinal loop in pallial roof complex; and oviduct opening into posterior tip of albumen gland. The remaining three species, however, have a mosaic of character-states from the above two sets. *Phreatodrobia rotunda* has basal cusps on the central tooth of the radula, but has a complex pallial intestinal loop. *Phreatodrobia imitata* has a trapezoidal-shaped central tooth, but lacks basal cusps. The radula and pallial intestinal loop of *Phreatodrobia plana* are similar to those of *P. conica* and *P. punctata*, yet the organization of the bursa copulatrix complex in this species is near-identical to that of the other species pair.

Morphological diversity is marked within the genus, and a large number of unusual character-states are spread out among the various congeners. The morphological distinctiveness of the various species may explain the sympatry of five congeners in the aquifer beneath San Marcos Springs. Considering the mosaic pattern of character-state distribu-

tion among congeners, we feel that subgenera should not be recognized, although we suspect that *P. micra* and *P. nugax* are particularly closely related.

The problem of convergence. Several morphological features are probably particularly prone to convergence among phreatic hydrobiids. Blindness and lost body pigment are associated with invasion of the phreatic habitat (Culver, 1982) and have likely occurred in diverse hydrobiid lineages. Small body size, typical of phreatic hydrobiids, may also be associated with their purportedly food-poor environment (Culver, 1982). Several character-states are highly correlated with small body size, notably loss of the ctenidium, loss of sperm pouches, and looping of the intestine in the pallial roof. Again, shared possession of such character-states may not indicate phyletic affinity. If the systematist disregards all possible convergent character-states among these tiny snails, there may be rather few character-states from gross morphology remaining, as the anatomy of minute hydrobiids is rather simplified. Clarification of the systematic relationships of phreatic hydrobiids may only come when histological study of morphology is applied to the taxa in question. Not only does examination of tissue sections provide data on additional character-states, but it also can resolve whether given structures are homologous (a major concern when structures can be lost and regained), as convergence is unlikely to be precise at the cellular level. For examples of such studies applied to systematic relationships of rissoaceans, see Ponder (1984).

Distribution and habitat. The distribution of species (and subspecies) is shown in Fig. 29. Note that the fauna includes what may be locally endemic species as well as much more widespread species, a pattern also seen in the phreatic amphipod fauna of south-central Texas (Holsinger, 1967; Holsinger & Longley, 1980). While all species are found in the Edwards (Balcones Fault Zone) Aquifer, material collected from three localities in the drainage zone (Fig. 1, Localities 8–10) may not be from this aquifer. All three localities are wet caves, and all yielded only fresh shells. The source of water for the permanent streams in these caves is probably as follows: Natural Bridge Caverns (Glen Rose Formation), Honey Creek Cave (Cow Creek Formation), and Century Caverns (Lower Glen Rose Formation) (Knox, 1981; J. Knox, personal communication, 1984). Both the Glen Rose and Cow Creek are also Cretaceous limestone, but are members of the Trinity Group underlying the Edwards (Ashworth, 1983). While snail populations may not be living in the cave waters (the shells could have been washed in from elsewhere), it is still unlikely that the shells came from populations living in the Edwards (Balcones Fault Zone) Aquifer, given the sporadic occurrence of this aquifer in this region (Ashworth, 1983). It is therefore likely that the four species collected from these caves are found in aquifers other than the Edwards (Balcones Fault Zone). Note that Taylor (1974) collected living "*Horatia*" (possibly *P. micra* or *P. nugax*) from a spring in Real County, also in the drainage zone of the Edwards (Balcones Fault Zone) Aquifer. Also note that other invertebrate species (or sister species thereof) are found in the Edwards (Balcones Fault Zone) Aquifer as well as other aquifers in the Hill Country or Edwards Plateau (Mitchell & Reddell, 1971). All of the hydrobiid species may have considerably wider ranges than outlined above as the aquifers of south-central Texas have not been well sampled (see below).

The 14 artesian wells that yielded snails ranged in depth (beneath ground level) from 59–582 m (Table 2). All of these wells are tightly cased and there is no doubt that the snails were expelled from the deep artesian zone. Their habitat probably includes fractures, joints and caverns in the bedrock; and possibly, given the minute size of the snails, even interstices. Note, however, that snails were absent from eight wells in Bexar County (where three species occur), five of which yielded other troglobites (Table 2), a point which mitigates against common use of the interstitial habitat by the snails. It is likely that all or most of the species dwell in similar habitats in the recharge zone, where the aquifer is unconfined.

Faunal diversity in the Edwards (Balcones Fault Zone) Aquifer. The groundwater fauna of Texas has traditionally been sampled by collecting in wet caves. A tremendous effort has gone into such collecting and the aquatic fauna of caves in several physiographic regions of the state is well known (Mitchell & Reddell, 1971). Yet caves only offer a very small fraction of the total phreatic habitat. The deep artesian zone, for instance, is probably not accessible from caves. The recent application of sampling techniques involving plac-

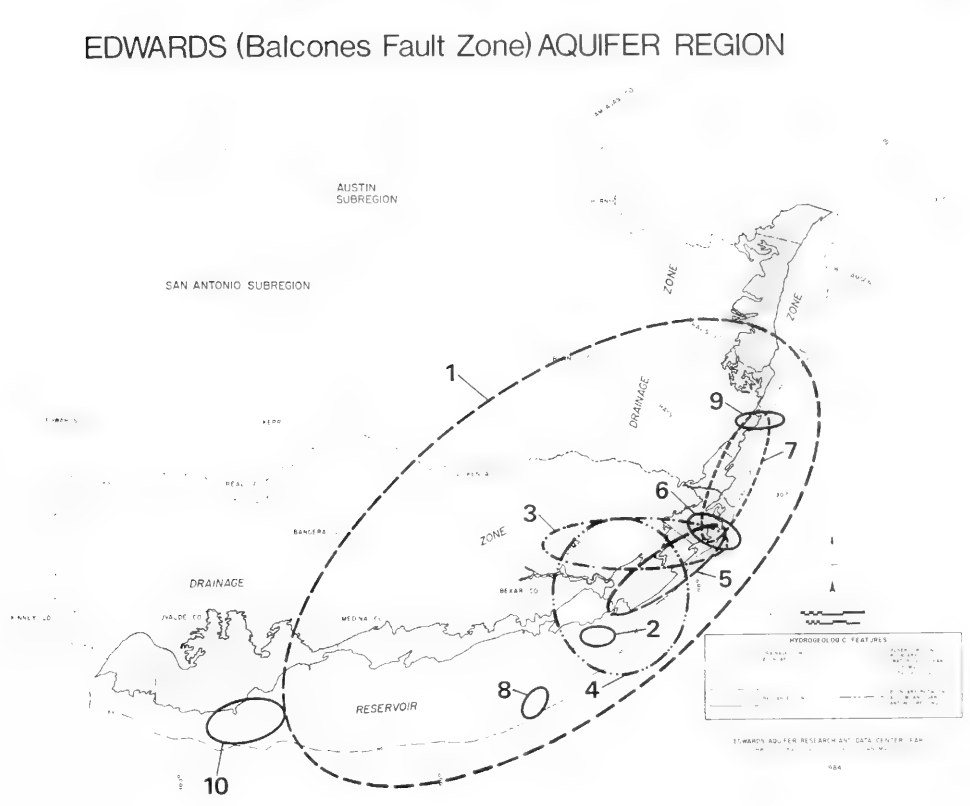


FIG. 29. Distribution of the 10 species and subspecies found in the Edwards (Balcones Fault Zone) Aquifer. 1 = *Phreatodrobia nugax nugax*; 2 = *P. n. inclinata*; 3 = *P. micra*; 4 = *P. conica*; 5 = *P. plana*; 6 = *P. rotunda*; 7 = *P. punctata*; 8 = *P. imitata*; 9 = *Stygopyrgus bartonensis*; 10 = *Balconorbis uvaldensis*.

ing nets over artesian well (for depths, see Table 2) or spring outlets has yielded many new species (see above) and demonstrated that a deep phreatic fauna does exist in some areas. Note that while the SWTSU Well (59 m deep) has yielded 20 troglobitic species, intensive collecting in the pool at the bottom of nearby Ezzell's Cave (in the same phreatic pool of the aquifer) only yielded nine species (J. D. Davis, 1979). With the description of the seven new species in this paper, the total described troglobitic fauna of the aquifer now totals 39 species. Collections from the localities considered in this paper have also yielded, apart from the snails, an additional 10–15 undescribed invertebrate species. Given the paucity of such sampling in most parts of the aquifer, a much larger number of species may yet await discovery. There is a large potential for discovery of additional new taxa in other aquifers of south-central Texas. The huge Edwards (Plateau) Aquifer, for instance, may contain a large number of sister taxa of the Edwards (Balcones Fault Zone) species, given that the two aquifers formed a single unit until at least the Miocene. Continued collecting in caves augmented by widespread application of the above sampling techniques will be necessary to more completely sample these and other aquifers in south-central Texas.

ACKNOWLEDGMENTS

The staff of the EARDC is thanked for their help with numerous aspects of the project. The senior author thanks the junior author for providing funds and facilities during an ex-

tended stay at the EARDC. We thank numerous individuals for allowing us to collect on their property. The following individuals lent material from either their personal or institutional collections: Drs. G. M. Davis, Academy of Natural Sciences of Philadelphia (*Phreatodrobia micra*, cotypes, ANSP 91322; *Phreatodrobia nugax*, holotype, ANSP 77574); A. Solem, Field Museum of Natural History (*Antrobia culveri*, holotype and paratypes, FMNH 164171, 164170/15); F. Giusti (*Horatia klecakiana*, *Hauffenia subpiscinalis*); and Mr. Leslie Hubricht ("*Horatia*", *Fontigens nickliniana*, *Phreatodrobia nugax* from Salamander Cave). Additional funding for the project came from grants to the senior author by the National Speleological Society and the United States Fish and Wildlife Service (Contract No. 14-16-0002-84-228, Amendment No. 1).

LITERATURE CITED

ASHWORTH, J. B., 1983, Ground-water availability of the Lower Cretaceous Formations in the Hill Country of south-central Texas. *Texas Department of Water Resources Report*, 273: 1–172.

BINDER, E., 1957, Note sur le genre *Horatia*. *Journal de Conchyliologie*, 97: 59–62.

BOETERS, H. D., 1974, *Horatia* Bourguignat, *Plagigeyeria* Tomlin und *Litthabitella* Boeters. *Archiv für Molluskenkunde*, 104: 85–92.

BOURGUIGNAT, J. R., 1887, *Etude sur les noms génériques des petits Paludinidées a opercule spirescent, suivie de la description d'un nouveau genre* Horatia. Tremblay, Paris, 56 p.

BOWMAN, T. E. & LONGLEY, G., 1976. Redescription and assignment to the new genus *Lirceolus* of the Texas troglobitic water slater, *Asellus smithi* (Ulrich) (Crustacea: Isopoda: Asellidae). *Proceedings of the Biological Society of Washington*, 88: 489–496.

BURCH, J. B., 1982, *Freshwater snails (Mollusca, Gastropoda) of North America*. Environmental Monitoring and Support Laboratory, Office of Research and Development, United States Environmental Protection Agency, 294 p.

CLIMO, F. M., 1974, Description and affinities of the subterranean molluscan fauna of New Zealand. *New Zealand Journal of Zoology*, 1: 247–284.

CLIMO, F. M., 1977, Notes on the New Zealand hydrobiid fauna (Mollusca: Gastropoda: Hydrobiidae). *Journal of the Royal Society of New Zealand*, 7: 67–77.

CULVER, D. C., 1982, *Cave life. Evolution and ecology*. Harvard, University Press, Cambridge, Mass., 189 p.

HERSHLER, R. & LONGLEY, G., in press, *Hadoceras taylori*, a new genus and species of phreatic Hydrobiidae (Gastropoda: Rissoacea) from south-central Texas. *Proceedings of the Biological Society of Washington*.

DAVIS, G. M., 1979, The origin and evolution of the gastropod family Pomatiopsidae, with emphasis on the Mekong River Triculinae. *Monograph of the Academy of Natural Sciences of Philadelphia*, 20: 1–120.

DAVIS, G. M., MAZURKIEWICZ, M. & MANDRACCHIA, M., 1982, *Spurwinkia*: morphology, systematics, and ecology of a new genus of North American marshland Hydrobiidae. *Proceedings of the Academy of Natural Sciences of Philadelphia*, 134: 143–177.

DAVIS, J. D., 1979, *A faunal survey of Ezell's Cave, Hays County, Texas*. M. S. Thesis, Southwest Texas State University, San Marcos, 96 p.

DAVIS, J. R., 1983, An additional record of living *Orygoceras* (Hydrobiidae) from Texas. *Nautilus*, 97: 112–113.

FRETTER, V. & GRAHAM, A., 1962. *British prosobranch molluscs*. Ray Society, London, xvi + 755 p.

FULLINGTON, R. W., 1978, *The Recent and fossil freshwater gastropod fauna of Texas*. Ph.D. Thesis, North Texas State University, Denton., 279 p.

GIUSTI, F. & PEZZOLI, E., 1981, Notulae malacologique XXV. Hydrobioidea nuove o poco conosciute dell'Italia appenninica (Gastropoda: Prosobranchia). *Archiv für Molluskenkunde*, 111: 207–222.

GIUSTI, F., PEZZOLI, E. & BODON, M., 1981, Notulae malacologique, XXVII. Primo contributo alla revisione del genre *Islamia* (Radoman, 1973) in Italia. *V Convegno Nazionale della Società Malacologica Italiana*: 49–71.

GIUSTI, F. & BODON, M., 1984, Notulae malacologique dell' Italia nord-occidentale. *Archiv für Molluskenkunde*, 114: 157–181.

HART, C. W., Jr., 1978, A new species of the genus *Sphaeromicola* (Ostracoda: Entocytheridae: Sphaeromicolinae) from Texas, with notes on relationships between European and North American species. *Proceedings of the Biological Society of Washington*, 91: 724–730.

HERSHLER, R., 1984, The hydrobiid snails (Gastropode: Rissoacea) of the Cuatro Ciénegas Basin: systematic relationships and ecology of a unique fauna. *Journal of the Arizona-Nevada Academy of Science*, 19: 61–76.

HERSHLER, R., 1985, Systematic revision of the Hydrobiidae (Gastropoda: Rissoacea) of the Cuatro Ciénegas Basin, Coahuila, Mexico. *Malacologia*, 26: 31–123.

HERSHLER, R. & LONGLEY, G., in press, *Hadoceras taylori*, a new genus and species of phreatic Hydrobiidae (Gastropoda: Rissoacea) from south-central Texas. *Proceedings of the Biological Society of Washington*, 99.

HOLSINGER, J. R., 1967, Systematics, speciation,

and distribution of the subterranean amphipod genus *Stygonectes* (Gammaridae). *United States National Museum Bulletin*, 259: 1–176.

HOLSINGER, J. R. & LONGLEY, G., 1980, The subterranean amphipod crustacean fauna of an artesian well in Texas. *Smithsonian Contributions to Zoology*, 308: 1–62.

HUBRICHT, L., 1940, A subterranean snail from an artesian well. *Nautilus*, 54: 34–35.

HUBRICHT, L., 1963, New species of Hydrobiidae. *Nautilus*. 76: 138–140.

HUBRICHT, L., 1971, New Hydrobiidae from Ozark caves. *Nautilus*, 84: 93–96.

JOHANNSON, J., 1956, Genital organs of two *Alvania* species and a comparison with related families (Moll. Pros.). *Arkiv för Zoologi*, 9: 377–387.

KARNEI, H. S., 1978, *A survey of the subterranean aquatic fauna of Bexar County, Texas*. M.S. Thesis, Southwest Texas State University, San Marcos, 116 p.

KLEMT, W. B., KNOWLES, T. R., ELDER, G. R. & SIEH, T. W., 1979, Ground-water resources and model applications for the Edwards (Balcones Fault Zone) Aquifer in the San Antonio Region, Texas. *Texas Department of Water Resources Report* 239: 1–88.

KNOX, J., 1981, Natural Bridge Caverns. *Texas Caver*, 26: 84–87.

KURODA, T. & HABE, T., 1958, Troglobiontic aquatic snails from Japan. *Venus*, 19: 183–196.

LONGLEY, G., 1975, *Environmental assessment Upper San Marcos River Watershed*. Report to the Soil Conservation Service, Temple, Texas, 367 p.

LONGLEY, G., 1978, Status of the Texas blind salamander. *Endangered Species Report 2, United States Fish and Wildlife Service*, p. 1–45.

LONGLEY, G., 1981, The Edwards Aquifer: earth's most diverse groundwater ecosystem? *International Journal of Speleology*, 11: 123–128.

MACLAY, R. W. & SMALL, T. A., 1976, Progress report on geology of the Edwards Aquifer, San Antonio area, Texas, and preliminary interpretation of borehole geophysical and laboratory data on carbonate rocks. *United States Department of the Interior, Geological Survey, Open File Report* 76–627, 65 p.

MITCHELL, R. W. & REDELL, J. R., 1971, The invertebrate fauna of Texas caves. In: LUNDELIU, E. L. & SLAUGHTER, B. H., (eds.), *Natural History of Texas Caves,* p. 35–90 Gulf Natural History, Dallas.

MORRISON, J. P. E., 1949, The cave snails of eastern North America. *Annual Report of the American Malacological Union for 1948:* 13–15.

PETTIT, B. M., Jr. & GEORGE, W. O., 1956, Ground-water resources of the San Antonio area, Texas. *Texas Board of Water Engineers, Bulletin* No. 5608, 1–2: 1–859.

PILSBRY, H. A., 1916, Note on *Valvata micra*. *Nautilus*, 30: 83–84.

PILSBRY, H. A. & FERRISS, J. H., 1906, Mollusca of the southwestern states. Part II. *Proceedings of the Academy of Natural Sciences of Philadelphia*, 58: 123–175.

POLLONERA, C., 1898, Intorno ad alcune Conchiglie del Friuli. *Bolletino Musei di Zoologia ed Anatomia comparata*, 13: 1–4.

PONDER, W. F., 1966, On a subterranean snail and a tornid from New Zealand. *Journal of the Malacological Society of Australia*, 10: 35–40.

PONDER, W. F., 1984, Review of the genera of the Barleeidae (Mollusca: Gastropoda: Rissoacea). *Records of the Australian Museum*, 35: 231–281.

RADOMAN, P., 1966, Die Gattungen *Pseudamnicola* und *Horatia*. *Archiv für Molluskenkunde*, 95: 243–253.

RADOMAN, P., 1973, New classification of fresh and brackish water Prosobranchia from the Balkans and Asia Minor. *Prirodnjacki Musej u Beogradu Posebna Izdanja*, 32: 1–30.

REDDELL, J. R., 1965, A checklist of the cave fauna of Texas. I. The Invertebrata (exclusive of Insecta). *Texas Journal of Science*, 17: 143–187.

REDDELL, J. R., 1967, A checklist of the cave fauna of Texas. III. Vertebrata. *Texas Journal of Science*, 19: 184–226.

REDDELL, J. R., 1970, A checklist of the cave fauna of Texas. IV. Additional records of Invertebrata (exclusive of Insecta). *Texas Journal of Science*, 21: 389–415.

REDDELL, J. R. & MITCHELL, R. W., 1969, A checklist and annotated bibliography of the subterranean aquatic fauna of Texas. *Texas Technological College, Water Resources Center (Lubbock), Special Report* 24: 1–48.

SMITH, A. R., 1971, Cave and karst regions of Texas. In LUNDELIUS, E. L. & SLAUGHTER, B. H. (eds.), *Natural history of Texas caves*, p. 1–14. Gulf Natural History, Dallas.

STOCK, J., 1976, A new genus and two new species of the crustacean order Thermosbaenacea from the West Indies. *Bijdragen tot de Dierkunde*, 46: 47–70.

STRECKER, J. K., 1935, Land and freshwater snails of Texas. *Transactions of the Texas Academy of Science*, 17: 5–44.

STRENTH, N. E., 1976, A review of the systematics and zoogeography of the freshwater species of *Palaemonetes* Heller (Crustacea: Decapoda) of North America. *Smithsonian Contributions to Zoology*, 228: 1–27.

TAYLOR, D. W., 1966, A remarkable snail fauna from Coahuila, Mexico. *Veliger*, 9: 152–228.

TAYLOR, D. W., 1974, The Tertiary gastropod *Orygoceras* found living. *Archiv für Molluskenkunde, 104*: 93–96.

TAYLOR, D. W., 1975, Index and bibliography of Late Cenozoic freshwater Mollusca of Western North America. *Museum of Paleontology, Univer-*

sity of Michigan, Papers on Paleontology, 10: 1–384.

THOMPSON, F. G., 1984, North American freshwater snail genera of the hydrobiid subfamily Lithoglyphinae. *Malacologia*, 25: 109–141.

VANDEL, A., 1965, Biospeleology. The biology of cavernicolous animals. *International Series of Monographs in Pure and Applied Zoology*, 22: 1–524.

YOUNG, F. N. & LONGLEY, G., 1976, A new subterranean aquatic beetle from Texas (Coleoptera: Dytiscidae-Hydroporinae). *Annals of the Entomological Society of America*, 69: 787–792.

APPENDIX 1. Data for all Texas localities considered in this paper. Numbers (in parentheses) refer to locations in Fig. 1. The collector and date(s) of collection are also given.

TRAVIS COUNTY: Salamander Cave (*1*), J. Reddell, 20 III 1966, 30°22′04″ N, 97°45′12″ W; Barton Springs (*2*), T. Spinelli, VI–VII 1982, R. Hershler, VI–VIII 1984, 30°16′ N, 97°47′ W.

HAYS COUNTY: San Marcos Springs (*3*), J. Davis, VIII–IX 1979, 29°54′ N, 97°56′ W; Artesian Well at Southwest Texas State University (*4*), G. Longley, IV–IX 1976, 29°53′24″ N, 97°56′08″ W.

COMAL COUNTY: Hueco Springs (A, B) (*5*), M. Brzozowski, VI–VII 1981, 29°46′ N, 98°08′ W; Guadalupe River drift (*6*), Pilsbry & Ferriss (1906), 29°45′30″ N, 98°08′30″ W; Comal Springs (main spring) (*7*), M. Brzozowski, VII–VIII 1981, 29°42′ N, 98°08′ W; Natural Bridge Caverns (River Styx at bottom of cave) (*8*), R. Hershler, 7 IX 1984, 29°41′22″ N, 98°20′30″ W; Honey Creek Cave (seeps feeding main spring from cave) (*9*), R. Hershler, 10 IX 1984, 29°50′ N, 98°30′ W.

KENDALL COUNTY: Century Caverns (stream at bottom of cave) (*10*), R. Hershler, 1 VIII 1984, 29°53′20″ N, 98°37′ W.

BEXAR COUNTY: Longhorn Portland Cement Co. Well (#2) (*11*), M. Brzozowski, II–IV 1981, 29°32′05″ N, 98°24 W; Brackenridge Zoo Well (*12*), City Water Board Artesian Station, Well 4 (*13*), M. Brzozowski, X 1980–VIII 1981, 29°25′48″ N, 98°26′14″ W; Union Stockyards Well (#3) (*14*), M. Brzozwski, 8–17 XIII 1980, 29°24′10″ N, 98°30′55″ W; City Water Board Mission Station Well (*15*), M. Brzozowski, 4–25 V 1981, 29°13′18″ N, 98°29′49″ W; J. H. Uptmore Well (#5) (*16*), M. Brzozowski, XII 1980–III 1981, 29°12′11″ N, 98°43′40″ W; Lackland City Water Co. Well (*17*), R. Rutland, VII–VIII 1979, 29°21′ N, 98°36′58″ W; City Public Service Board Well (#1) (*18*), R. Rutland, VII–IX 1979, 29°20′52″ N, 98°34′35″ W; Rio Vista Farms Well (*19*), M. Brzozowski, XII 1980–IV 1981, 29°20′23″ N, 98°44′38″ W; Verstraeten Brothers Well (*20*), H. Karnei, IV 1977–I 1978, 29°19′ N, 98°39′ W; O. R. Mitchell Well (*21*), H. Karnei, 14–23 VI 1977, R. C. Weidenfeld, 1–3 V 1980, 29°18′ N, 98°38′ W; J. W. Watts Well (*22*), M. Brzozowski, 5, 7 XI 1980, 29°17′38″ N, 98°41′20″ W.

UVALDE COUNTY: King Farms Well (*23*), M. Brzozowski, I–II 1981, 29°17′15″ N, 99°39′ W; D. C. Carnes Well (*24*), M. Brzozowski, I–II 1981, 29°16′37″ N, 99°40′51″ W; C. Reagan Well (*25*), M. Brzozowski, II– VIII 1981, 29°16′22″ N, 99°36′30″ W; W. C. Reagan Well (*26*), R. C. Weidenfeld, 1–25 III 1980, 29°16′06″ N, 99°34′ W; R. Carnes Well (*27*), M. Brzozowski, 2–16 II 1981, 29°13′59″ N, 99°51′52″ W; R. K. Dunbar Well (*28*), M. Brzozowski, 2–16 II 1981, 29°13′32″ N, 99°51′40″ W; S. Moerbe Well (*29*), M. Brzozowski, II–III 1981, 29°13′06″ N, 99°49′33″ W; G. Ligocky Well (*30*), R. C. Weidenfeld, I–VIII 1980, 29°11′37″ N, 99°48′38″ W; Uvalde National Fish Hatchery Well (*31*), R. C. Weidenfeld, I–IV 1980, 29°11′26″ N, 99°50′53″ W.

APPENDIX 2. Shell measurements. N = number of specimens, NW = number of whorls, PD = protoconch diameter, SH = shell height, SW = shell width, AH = aperture height, AW = aperture width, BW = height of body whorl. Locality numbers are those used in Fig. 1 and Appendix 1.

Species	Locality	N		NW	PD	SH	SW	AH	AW	BW
P. micra	6 (syntype)	1		2.5	—	0.419	1.02	0.419	0.434	0.419
	3	6	$\bar{x}$	2.43	—	0.336	0.791	0.300	0.307	0.336
			s	0.103	—	0.023	0.050	0.020	0.015	0.023
	5(B)	14	$\bar{x}$	2.49	—	0.471	1.08	0.416	0.454	0.454
	9	7	$\bar{x}$	2.21	—	0.353	0.840	0.300	0.353	0.316
			s	0.19	—	0.037	0.092	0.036	0.040	0.065
	10	11	$\bar{x}$	2.52	—	0.454	1.02	0.348	0.399	0.426
			s	0.048	—	0.039	0.088	0.036	0.046	0.032
P. nugax nugax	6 (holotype)	1		2.7	—	0.806	1.43	0.558	0.589	0.651
	1	1		2.7	0.317	0.837	1.05	0.372	0.434	0.651
	2	30	$\bar{x}$	3.17	0.362	1.11	1.14	0.570	0.605	0.868
			s	0.22	0.015	0.12	0.07	0.044	0.04	0.083
	3	20	$\bar{x}$	2.94	0.355	1.03	1.53	0.575	0.727	0.832
			s	0.11	0.025	0.13	0.12	0.068	0.058	0.097
	4	24	$\bar{x}$	2.83	0.377	0.935	1.51	0.532	0.695	0.766
			s	0.17	0.022	0.10	0.13	0.07	0.057	0.07
	8	16	$\bar{x}$	2.95	—	0.821	1.71	0.530	0.663	0.705
			s	0.17	—	0.094	0.108	0.040	0.039	0.075
	9	7	$\bar{x}$	2.5	—	0.613	0.933	0.360	0.425	0.493
			s	0.058	—	0.059	0.066	0.03	0.039	0.052
	10	6	$\bar{x}$	2.9	—	0.953	1.25	0.530	0.558	0.744
			s	0.2	—	0.13	0.20	0.059	0.068	0.098
	14	3	$\bar{x}$	2.47	0.342	0.440	0.01	0.350	0.456	0.394
			s	0.06	0.011	0.041	0.026	0.023	0.023	0.012
	26	2	$\bar{x}$	2.75	0.302	0.698	1.24	0.465	0.512	0.605
			s	—	0.007	0.022	—	—	0.022	0.066
P. nugax inclinata	11 (holotype)	1		3.4	0.376	1.18	1.33	0.496	0.620	0.899
	11	19	$\bar{x}$	3.07	0.360	1.11	1.21	0.456	0.606	0.828
			s	0.17	0.017	0.092	0.11	0.059	0.057	0.079
P. rotunda	3 (holotype)	1		3.6	0.455	0.837	2.26	0.837	0.837	—
	3	9	$\bar{x}$	3.42	0.392	0.769	2.01	0.769	0.741	—
			s	0.206	0.031	0.037	0.153	0.037	0.050	—
P. conica	5(B) (holotype)	1		3.75	0.36	1.61	1.21	0.744	0.729	1.22
	5(B)	5	$\bar{x}$	3.82	0.343	1.76	1.41	0.831	0.778	1.27
			s	0.26	0.01	0.057	0.041	0.01	0.021	0.015
	5(A)	6	$\bar{x}$	3.92	0.345	1.64	1.33	0.760	0.707	1.14
			s	0.12	0.01	0.061	0.057	0.015	0.021	0.037
P. plana	3 (holotype)	1		3.0	—	0.455	0.822	0.455	0.297	0.277
	3	8	$\bar{x}$	2.93	—	0.450	0.820	0.444	0.305	0.273
			s	0.113	—	0.041	0.037	0.042	0.028	0.030
	7	1		2.75	—	0.376	0.752	0.356	0.257	0.277
P. punctata	3 (holotype)	1		3.8	0.297	1.05	0.791	0.512	0.434	0.744
	3	15	$\bar{x}$	3.9	0.319	1.13	0.871	0.564	0.474	0.784
			s	0.15	0.015	0.081	0.055	0.050	0.035	0.056
P. imitata	20 (holotype)	1		3.5	0.36	1.07	0.81	0.43	0.47	0.713
	20	18	$\bar{x}$	3.5	0.36	1.01	0.747	0.388	0.403	0.663
			s	0.12	0.01	0.038	0.42	0.26	0.27	0.040
	21	4	$\bar{x}$	3.4	0.34	1.03	0.833	0.446	0.428	0.701
			s	0.12	0.026	0.020	0.08	0.034	0.03	0.015

APPENDIX 2 (Continued)

Species	Locality	N		NW	PD	SH	SW	AH	AW	BW
B. uvaldensis	30 (holotype)	1		2.75	—	0.434	1.02	0.31	0.372	0.326
	30	17	$\bar{x}$	2.81	—	0.428	1.08	0.347	0.397	0.375
			s	0.12	—	0.044	0.088	0.035	0.029	0.036
S. bartonensis	2 (holotype)	1		4.0	—	0.970	0.495	0.337	0.287	0.594
	2	5	$\bar{x}$	4.39	—	1.16	0.546	0.374	0.329	0.681
			s	0.26	—	0.11	0.023	0.015	0.021	0.050

APPENDIX 3A. Measurements of non-pallial organs and strucures. LST = stomach length, LSS = style sac length, LPO = pallial oviduct length, LBU = bursa length, LSR = seminal receptacle length, LOV = ovary length, LDG = digestive gland length, LPR = prostate length, LPP = pallial prostate length, LTS = testis length. Locality numbers refer to those used in Fig. 1 and Appendix 1.

Species (locality)		LST	LSS	LSS/LST	LPO	LBU	LSR	LOV	LOV/LDG	LPR	LPP	LPP/LPR	LTS	LTS/LDG
P. micra (3)	x̄	0.198	0.129	0.533	0.234	0.114	0.054	0.323	0.51	0.178	0.084	0.44	0.263	0.493
	s	—	0.01	0.057	0.052	0.01	0.021	0.448	0.12	—	0.007	—	0.69	0.14
	n	5	5	3	4	4	2	4	4	4	3	3	3	3
P. nugax (3)	x̄	0.370	0.200	0.564	0.455	0.23	0.095	0.97	0.67	0.44	0.198	0.52	0.574	053
	s	0.058	0.018	0.12	0.028	0.04	0.017	0.10	0.082	0.32	0.02	0.76	0.53	0.081
	n	6	5	5	4	4	5	5	6	4	3	3	4	4
P. rotunda (3)	x̄	0.430	0.277	0.7	0.461	0.188	0.079	0.489	0.22	0.396	0.238	0.60	0.436	0.21
	s	0.057	—	—	0.057	0.014	0.02	0.07	0.015	—	—	—	—	—
	n	3	1	1	3	2	3	3	3	1	1	1	1	1
P. conica (5B)	x̄	0.362	0.287	0.79	0.410	0.182	0.059	0.436	0.30	0.303	0.099	0.33	0.421	0.43
	s	0.042	0.034	0.09	0.057	0.015	—	0.073	0.11	0.025	0.016	0.07	0.86	0.014
	n	4	4	3	3	3	2	5	3	4	4	4	6	2
P. plana (3)	x̄	0.193	0.087	0.45	0.191	0.099	0.059	0.303	0.52	0.173	0.79	0.465	0.292	0.59
	s	0.01	0.01	0.058	0.01	—	—	0.061	0.11	0.03	—	0.07	0.025	0.75
	n	4	4	4	6	4	2	3	3	4	4	4	4	4
P. imitata (20)	x̄	0.23	0.18	0.67	0.246	0.16	0.04	0.213	0.342	0.28	0.125	0.45	0.28	0.405
	s	0.03	0.03	0.16	0.037	0.016	—	0.075	0.115	0.02	0.023	0.06	0.02	0.04
	n	6	6	6	8	4	4	5	5	3	3	2	3	2
P. punctata (3)	x̄	0.244	0.152	0.63	0.228	0.109	0.059	0.323	0.353	0.20	0.12	0.6	0.317	0.432
	s	0.03	0.011	0.12	0.011	0.042	—	0.037	0.049	—	—	—	0.034	0.01
	n	3	3	3	4	2	2	4	4	1	1	1	3	3
S. bartonensis (2)	x̄	0.193	0.191	0.80	0.311	—	0.030	0.261	0.334	0.139	0.069	0.50	0.426	
	s	0.019	0.03	0.067	0.05	—	0.014	0.077	0.091	—	—	—	0.13	0.14
	n	4	4	4	3		3	5	5	1	1	1	2	2
B. uvaldensis (30)	x̄	0.246	0.194	0.792	0.323	—	—	0.416	0.405	0.198	0.093	0.472	0.594	0.526
	s	0.041	0.029	0.048	0.02	—	—	0.054	0.037	0.024	0.01	0.051	0.139	0.083
	n	5	5	5	6	—	—	4	4	5	4	4	5	5

APPENDIX 3B. Measurements of pallial structures. LPC = pallial cavity length, LIC = intestine coil length, WIC = intestine coil width, LOS = osphradium length, LCT = ctenidium length, WCT = ctenidium width, NF = number of gill filaments, IMA = distance from anus to mantle edge. Locality numbers refer to those used in Fig. 1 and Appendix 1.

Species (locality)		LPC	LIC	WIC	LOS	LCT	WCT	NF	IMA	LIC/LPC	LOS/LPC
P. micra (3)	x̄	0.366	0.099	0.178	0.139	—	—	—	0.064	0.273	0.385
	s	0.057	0.014	0.028	—	—	—	—	0.019	0.033	0.064
	n	4	6	6	5	—	—	—	4	5	5
P.n. inclinata (11)	x̄	0.713	0.232	0.376	0.181	0.505	—	13.7	0.178	0.340	0.274
	s	0.054	0.07	0.056	0.031	0.014	—	1.42	—	0.13	0.037
	n	5	3	2	7	2	—	11	1	3	4
P.n. nugax (3)	x̄	0.754	0.289	0.319	0.183	0.578	0.119	14.4	0.178	0.40	0.25
	s	0.10	0.034	0.069	0.026	0.062	0.034	1.69	0.014	0.07	0.04
	n	13	7	7	12	9	5	11	5	7	12
(4)	x̄	0.792	0.218	0.356	0.174	0.590	0.178	13.0	0.198	0.31	0.222
	s	0.118	0.028	0.084	0.022	0.392	—	0.71	—	0.042	0.036
	n	5	2	2	5	4	1	5	1	2	5
(2)	x̄	0.729	0.396	—	0.158	0.560	—	12.4	0.139	0.54	0.235
	s	0.088	—	—	0.047	0.073	—	1.36	0.057	—	0.07
	n	5	1	—	5	5	—	11	4	1	2
(14)	x̄	0.382	0.139	0.198	0.153	0.322	0.079	11.0	0.109	0.32	0.41
	s	0.001	0.023	—	0.01	0.041	—	0.82	0.014	—	0.056
	n	3	3	1	4	3	1	4	2	1	4
(12)	x̄	0.455	0.265	0.257	0.119	—	—	8.5	0.119	0.44	0.26
	s	—	—	—	—	—	—	0.071	—	—	—
	n	1	1	1	1	—	—	2	1	1	1
(25)	x̄	0.653	0.168	0.244	0.191	0.614	—	9.0	0.158	0.247	0.28
	s	0.052	0.026	0.01	0.03	0.028	—	—	0.028	0.032	0.014
	n	3	4	4	3	3	—	1	3	3	2
P. rotunda (3)	x̄	1.08	0.644	0.337	0.139	—	—	—	0.119	0.64	0.13
	s	0.06	0.042	0.052	0.02	—	—	—	0.032	0.04	0.01
	n	5	2	3	3	—	—	—	5	2	3
P. conica (5B)	x̄	1.09	0.590	0.402	0.224	—	—	—	0.119	0.50	0.20
	s	0.073	0.082	0.042	0.063	—	—	—	0.028	0.07	0.05
	n	4	4	3	4	—	—	—	3	3	3
P. plana (3)	x̄	0.392	0.153	0.149	0.135	—	—	—	0.099	0.415	0.33
	s	0.063	0.03	0.017	0.025	—	—	—	—	0.15	0.059
	n	6	7	6	5	—	—	—	3	4	4
P. imitata (20)	x̄	0.600	0.33	0.247	0.155	—	—	—	0.125	0.36	0.26
	s	0.01	0.057	0.027	0.015	—	—	—	0.03	0.07	0.02
	n	6	6	6	6	—	—	—	3	4	6
P. punctata (3)	x̄	0.709	0.360	0.236	0.134	—	—	—	0.141	0.475	0.185
	s	0.068	0.03	0.053	0.01	—	—	—	0.012	0.02	0.017
	n	6	5	7	4	—	—	—	4	4	4
S. bartonensis (2)	x̄	0.302	0.094	0.198	0.089	—	—	—	0.079	0.270	0.246
	s	0.043	0.03	0.039	0.01	—	—	—	0.052	0.13	0.018
	n	5	4	5	5	—	—	—	3	4	5
B. uvaldensis (30)	x̄	0.671	0.172	0.232	0.160	—	—	—	0.210	0.26	0.24
	s	0.103	0.054	0.032	0.022	—	—	—	0.06	0.095	0.029
	n	8	6	6	8	—	—	—	5	6	8

APPENDIX 4. Comparison of 10 spp. of phreatic hydrobiids from south-central Texas involving 40 characters. OTU 1 = *Phreatodrobia micra*, 2 = *P. nugax*, 3 = *P. rotunda*, 4 = *P. conica*, 5 = *P. plana*, 6 = *P. punctata*, 7 = *P. imitata*, 8 = *Balconorbis uvaldensis*, 9 = *Stygopyrgus bartonensis*, 10 = *"Orygoceras"* sp. Data for *"Orygoceras"* sp. are from Hershler & Longley (in press).

Character	OTU									
	1	2	3	4	5	6	7	8	9	10
1. Maximum shell dimension >1.25 mm (0,1)	0	1	1	1	0	0	0	0	1	1
2. Shell form planispiral (0,1)	1	1	1	0	1	0	0	1	0	0
3. Shell form trochoid-low conic (0,1)	0	1	0	1	0	1	0	0	0	0
4. Shell form elongate-conic (0,1)	0	0	0	0	0	0	1	0	1	0
5. Shell uncoiled (0,1)	0	0	0	0	0	0	0	0	0	1
6. Protoconch microsculpture: spiral lines (0); punctate (1)	1	1	1	1	1	1	1	0	1	1
7. Teleoconch typically with spiral lines (0,1)	0	0	0	0	0	0	0	1	1	0
8. Teleoconch typically with irregular ridges (0,1)	0	0	0	1	1	1	0	0	0	0
9. Teleoconch typically with collabral costae and spiral threads (0,1)	0	0	0	0	0	0	1	0	0	0
10. Operculum concentric (0,1)	0	0	0	0	0	0	0	0	0	1
11. Operculum with ventral process (0,1)	1	1	0	0	0	0	0	0	0	1
12. Ctenidium (or vestige) present (0,1)	1	1	0	0	0	0	0	0	0	0
13. L osphradium/L pallial cavity typically >30% (0,1)	1	0	0	0	1	0	0	0	0	2
14. Number of intestinal loops in pallial cavity: 1 (0); 2 (1)	0	0	0	0	1	1	1	0	0	0
15. Long axis of loop(s): parallel to L pallial cavity (0); perpendicular (1)	1	1	0	1	0	0	0	1	1	1
16. Central radular tooth with basal cusps (0,1)	1	1	1	0	0	0	0	1	1	1
17. Central tooth shape: trapezoidal (0); square (1)	0	0	0	1	0	1	0	0	0	0
18. Lateral tooth with >20 cusps (0,1)	0	0	0	1	1	1	0	0	0	0
19. L style sac/L stomach >50% (0,1)	1	1	1	1	0	1	1	1	1	1
20. Intestine with loop on right side of style sac (0,1)	0	0	1	0	0	1	0	0	0	1
21. Pallial gonoducts more than four times as long as wide (0,1)	0	0	0	0	0	0	0	0	0	1
22. Penis lobed (0,1)	0	0	0	0	0	0	0	1	1	0
23. Penis with specialized glands (0,1)	0	0	0	0	0	0	0	1	1	0
24. L testis/L digestive gland >40% (0,1)	1	1	0	1	1	1	1	1	1	1
25. Seminal vesicle exits from anterior tip of testis (0,1)	1	0	1	1	1	1	1	1	1	1
26. Seminal vesicle coils on stomach (0,1)	1	0	0	0	1	0	0	0	0	0
27. L pallial prostate/L prostate >40% (0,1)	1	1	1	0	1	1	1	1	1	1
28. Vas deferens exits from prostate tip (0,1)	0	0	0	0	0	0	0	0	0	1
29. L ovary/L digestive gland typically >40% (0,1)	0	1	1	1	0	0	0	0	1	1
30. Sperm travels in female via: spermathecal duct (0); ventral gutter (1)	1	1	1	1	1	1	1	0	0	0
31. Capsule gland more than twice the length of albumen gland (0,1)	0	0	1	1	1	1	0	0	0	0
32. Anterior end of capsule gland typically coiled (0,1)	0	1	0	0	0	0	0	0	0	0
33. Capsule gland opening muscularized (0,1)	0	0	0	0	0	0	1	0	1	0
34. Capsule gland with >1 tissue section (0,1)	0	0	0	0	0	0	0	1	1	1

APPENDIX 4 (Continued)

Character	OTU									
	1	2	3	4	5	6	7	8	9	10
35. Posterior end of albumen gland coiled (0,1)	0	0	0	0	0	0	0	1	0	1
36. Albumen gland sac-like (0,1)	0	0	0	0	0	0	0	0	0	1
37. Oviduct coil positioned: on left side of albumen gland (0); posterior or ventral to gland (1)	0	0	1	0	0	1	0	1	0	0
38. Oviduct opens into posterior end of albumen gland (0,1)	0	0	0	0	0	1	1	1	0	1
39. Bursa present (0,1)	1	1	1	1	1	1	1	0	0	1
40. Seminal receptacle present (0,1)	1	1	1	1	1	1	1	0	1	0

Revised Ms. accepted 1 May 1985.

MALACOLOGIA, 1986, 27(1): 173–183

MODIFICATION AND EVALUATION OF BURCH AND CUADROS'S MEDIUM FOR THE MAINTENANCE OF THE TESTES OF A MARINE GASTROPOD[1]

Thomas C. Cheng & Eric J. Pearson

Marine Biomedical Research Program[2] and Department of Anatomy (Cell Biology), Medical University of South Carolina, P. O. Box 12559 (Fort Johnson), Charleston, South Carolina 29412, U.S.A.

ABSTRACT

Testes of *Ilyanassa obsoleta* were maintained in a modification of the medium originally devised by Burch & Cuadros (1965). The efficacy of the medium was assayed by monitoring oxygen utilization by testes and their ability to incorporate ^{3}H-thymidine. Scintillation counting and autoradiography were employed to quantify the uptake. It was determined that the testes could be effectively maintained for two weeks, the duration of the experiment. Control testes in isosmotic saline were not maintained as well as in the modified medium. The intent of this study was to devise a maintenance medium so that molecule(s) suspected of being responsible for parasitic castration associated with larval trematodes could be tested *in vitro*.

INTRODUCTION

Although the phenomenon of parasitic castration in molluscs due to larval digeneans has been recognized since the initial observation by McCrady (1873), the responsible mechanisms have not been studied except at the interpretive, descriptive level. In this laboratory we have been conducting experiments aimed at elucidating the chemical basis for parasitic castration. As one approach, we have been exposing molluscan gonads *in vitro* to molecules associated with species of larval trematodes known to cause castration. This has required devising media for the maintenance of molluscan gonads.

As one of our models for studying parasitic castration, we have been employing the intertidal gastropod *Ilyanassa obsoleta* (Say) which is known to be castrated by sporocysts of *Zoogonus lasius* (Olsson) (Cheng *et al.*, 1973; Sullivan *et al.*, 1985). Consequently, studies have been carried out to ascertain whether modifications of established media could be used to maintain the gonads of *I. obsoleta* for a satisfactory length of time.

As a result of an earlier study (Cheng *et al.*, 1984), we reported the efficacy of modifications of two established media for molluscan tissues for the maintenance of *I. obsoleta* gonads; specifically, that devised by Chernin (1963) for maintaining the heart of the freshwater pulmonate *Biomphalaria glabrata* (Say), and that devised by Tripp *et al.* (1966) for maintaining heart tissues and amoebocytes of the American oyster, *Crassostrea virginica* (Gmelin). Reported herein are our findings relative to the use of modifications of another medium, that of Burch & Cuadros (1965), originally designed for maintaining the gonads of a terrestrial gastropod, *Helix pomatia* Linn. and two freshwater species, *Biomphalaria glabrata* and *Pomatiopsis lapidaria* (Say).

MATERIALS AND METHODS

Testes

In view of the objective of this study, we only attempted to maintain the testes of *I. obsoleta in vitro* because these male gonads include considerably more dividing gametic cells than ovaries (our unpublished work).

All of the snails from which testes were removed were collected between January 1 and June 1, 1984, from the same intertidal mudflat in Clark Sound off Charleston Harbor,

[1]This research was supported by a grant (820536) from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Disease, and a contract (DE-A509-83ER60132) from the U.S. Department of Energy.
[2]World Health Organization Collaborating Centre on Molluscicides.

South Carolina. After ascertaining that these male snails were not infected with trematodes, specimens, each measuring 18 ± 3 mm in shell length, were sterilized externally by swabbing with 70% ethanol and their shells were gently crushed aseptically in a vice and the shell fragments were removed. Subsequently, the bright orange testis was carefully severed by dissection and placed in isosmotic saline (675 m 0sm) maintained at 4°C.

Prior to being placed in the maintenance medium being tested, each testis was passed through a series of six 10-min washes in sterile Petri dishes. The initial two washes were in isosmotic saline plus a high concentration of antibiotics (i.e., 1.5 × normal concentration of penicillin G, streptomycin sulfate, and Fungizone listed under "maintenance medium"). The subsequent four washes were in isosmotic saline plus the normal concentration of antibiotics.

Maintenance medium

As stated, the medium that we tested was a modification of that devised by Burch & Cuadros (1965). Specifically, we omitted the addition of crude snail extracts so that possible neurosecretory factors from the central nervous system or tentacles would not interfere with the results. The medium was rendered isosmotic (675 ± 10 m Osm) by adding 22 g of NaCl/liter. This amount of salt was arrived at by basing our calculation on the osmolality of the pooled hemolymph from 25 snails. Bacterial and fungal growths were inhibited by adding 800 IU of penicillin G/ml, 800 μg of streptomycin sulfate/ml, and 1% Fungizone. The final pH of the medium was adjusted to 7 with HCl or NaOH solutions.

A single testis was placed aseptically in each sterile 15-ml screw cap culture tube containing either 3 ml of isosmotic saline (control) or 3 ml of the maintenance medium (experimental). All cultures were incubated at 25°C on a slowly rotating rack (1/5 rotation/min). Forty-five testes were maintained in isosmotic saline and 45 in our modification of Burch & Cuadros's medium for each of the following time intervals: 0, 24, 96 hr, and 1 and 2 weeks. The viability of the maintained testes was assayed at each of the time intervals by measuring oxygen utilization by tissues. Thirty, additional testes were maintained in isosmotic saline (control) and 30 in the modified Burch & Cuadros's medium (experimental) for each of the same five time intervals and the viability of these tissues were determined by measuring the ability of the gametic cells to incorporate ^{3}H-thymidine by employing scintillation counting. In addition, 10 testes were maintained in the modified maintenance medium and in isosmotic saline for each of the five time intervals and their ability to incorporate ^{3}H-thymidine was ascertained by autoradiography. The detailed protocols followed in each of the three assay procedures are presented below.

Respirometry

Testes of the control and experimental groups employed in the respirometric assays at each of the five time intervals were pooled in 15-ml reaction vessels, 15 testes from each group per vessel. Each vessel contained 5 ml of fresh maintenance medium or isosmotic saline. This was done because preliminary studies had revealed that at least 15 testes per vessel were required for a reliable measurement of oxygen consumption.

A filter paper fan (Whatman No. 2) was placed in the center well of each reaction vessel. Also placed in the center well was 250 μl of 1M KOH to absorb the CO_2 given off by the respiring tissues. A model GRP-14 Gilson Differential Respirometer (Middleton, Wisconsin) was employed. After equilibration involving shaking (80 oscillations/min) for 60 min at 25°C, respirometric readings were recorded at 20 min intervals for 4 hr.

At the end of 4 hr, the 15 gonads comprising each group were placed on pre-weighed aluminum foil, dried for 2 days at 130°C, and weighed. Subsequently, the respirometric data were converted to μl O_2 consumed/g dry weight of tissue/20 min. The respirometric determinations were made in triplicate for both the experimental and control groups, 15 in each group, hence a total of 45 testes per category.

Exposure to ^{3}H-thymidine

Following maintenance in the medium (experimental) or saline (control) for 0, 24, 96 hr, and 1 and 2 weeks, the testes comprising each group were pooled in groups of ten in 3 ml of the maintenance medium or isosmotic saline each containing 5 μCi/ml of ^{3}H-thymidine (64 Ci/mmole, ICN, Irvine, California) in sterile vials and rotated for 3 hr at 100 rpm at 25°C on a rotating platform. Although

^{3}H-thymidine is taken up by cells within 15 min (Baserga & Malamud, 1969), we employed 3 hr of exposure to ensure that uptake throughout each testis had occurred. Subsequently, the testes were removed from the radioactive medium and washed six times in isosmotic saline before being processed for either scintillation counting or autoradiography.

Scintillation counting

Following maintenance and exposure to ^{3}H-thymidine, 30 testes comprising each of the five experimental groups and 30 comprising each of the five control groups were individually placed in scintillation vials (Fisher Scientific, Pittsburgh, Pennsylvania) and dried for 24 hr at 75°C. Subsequently, the tissues were homogenized by employing a glass homogenizer and the homogenates were digested with Scintigest (Fisher). Ten ml of Scintiverse (Fisher) were added to each digested sample and the solution was decolorized using hydrogen peroxide (0.25 ml/vial). Counts (decays detected by the photomultiplier)/min were obtained using a 2000 Series Packard Tri-Crab liquid scintillation spectrometer (Downers Grove, Illinois).

Autoradiography

After maintenance of the five time intervals and exposure to ^{3}H-thymidine, 10 testes of both the experimental and control groups at each of the five time intervals were individually placed on glass slides and macerated with a scapel. The macerated gonads were then smeared and air dried. The smears were fixed with Carnoy's fixative for 30 min and again were allowed to dry. Subsequently, the smears were rehydrated and dipped in liquid MTB_2 (Eastman Kodak, Rochester, New York) three times in 5 sec in the dark. The smears were then permitted to dry for 1 hr prior to being stored in a light-proof box for 7 days at 4°C. The smears were developed in D-19 developer (Kodak) and fixed in Kodak rapid fixer (Gude, 1968). The smears were then stained with hematoxylin and eosin.

The number of cells with labeled nuclei per 1000 cells and the number of silver grains associated with the nucleus of each labeled cell were counted under oil immersion. Preliminary studies had revealed that when fewer than 1000 cells were counted per slide, variability in the number of labeled cells in repeated counts was greater (± 28% of 500 cells counted, ± 14% of 1000 cells counted). To avoid biased sampling, all counts were made using a double blind protocol. Furthermore, only those cells with three or more silver grains associated with the nucleus were counted to avoid including cells labeled due to background exposure.

Statistical analysis

Data obtained from employing respirometry, scintillation counting and autoradiography at the stated time intervals were compared by employing analysis of variance. Specifically, the respirometric data were compared by using a split plot analysis computer program (Winer, 1977) and the scintillation and autoradiographic data were compared by use of a two-way factorial computer program (Winer, 1977). Comparison between the results obtained with the control (isosmotic saline) and experimental (modified Burch & Cuadros's) media at each time interval was performed with the least significant difference test (Winer, 1977).

RESULTS

Respirometry

The results of our respirometric determinations on the experimental and control groups of testes at the five time intervals are presented in Table 1. Note that the mean rate of O_2 consumption by the testes maintained in isosmotic saline declined with time, whereas the mean rate of O_2 consumption maintained in the modified medium remained relatively constant. After the second week the O_2 utilization for testes maintained in the modified medium was 6.494×10^{-1} µl O_2/gm dry weight/20 min, which is almost the same as that of 0 hr testes. On the other hand, the mean rate of O_2 utilization for testes maintained in saline for two weeks was essentially zero. This indicates that the control testes were no longer viable after two weeks.

A split-plot analysis of the respirometric data revealed that the difference in the means of testes maintained in the modified medium and saline at all of the time intervals was statistically significant ($p > 0.01$), with that of the experimental testes being higher (Table 2). In addition, this analysis revealed that

TABLE 1. Mean respiratory rates (in μl 0_2/gm dry weight/20 min) of testes maintained in modified Burch & Cuadros's medium or isosmotic saline at five time intervals.

	0 hr		24 hr		96 hr		1 week		2 weeks		
	Isosmotic saline	Modified medium	Isosmotic saline	Modified medium	Isosmotic saline	Modified medium	Isosmotic saline	Modified medium	Isosmotic saline	Modified medium	
Experiment 1 (n = 15/group)	0.6775	0.6062	0.5154	0.6653	0.3108	0.4674	0.2542	0.6778	0.1525[2]	1.0299	$\bar{IS}$[3] = 0.3211 $\bar{BC}$[4] = 0.6983 $\bar{B}_1$[5] = 0.5052
Experiment 2 (n = 15/group)	0.6416	0.8477	0.5211	0.4222	0.5384	0.7769	0.1038	0.2891	−0.3160[2]	0.4042	$\bar{IS}$ = 0.2978 $\bar{BC}$ = 0.5480 $\bar{B}$ 0.4229
Experiment 3 (n = 15/group)	0.3757	0.4358	0.4565	0.6376	0.6072	0.8653	0.2956	0.5784	0.0557	0.5142	$\bar{IS}$ + 0.3581 $\bar{BC}$ = 0.6063 $\bar{B}$ = 0.4822
	$\bar{X}_1$ = 0.5649	$\bar{X}_2$ = 0.6299	$\bar{X}_1$ = 0.4977	$\bar{X}_2$ = 0.5750	$\bar{X}_1$ = 0.4855	$\bar{X}_2$ = 0.7032	$\bar{X}_1$ = 0.2179	$\bar{X}_2$ = 0.5151	$\bar{X}_1$ = −0.1376[2]	$\bar{X}_2$ = 0.6496	
	$\bar{T}_1$ = 0.5974		$\bar{T}_2$[1] = 0.5364		$\bar{T}_3$[1] = 0.5943		$\bar{T}_4$[1] = 0.3665		$\bar{T}_5$[1] = 0.2559		

1. $\bar{T}$ = mean respiratory rate for time interval.
2. No oxygen consumption.
3. $\bar{IS}$ = mean respiratory rate in isosmotic saline.
4. $\bar{BC}$ = mean respiratory rate in modified Burch & Cuadros's medium
5. $\bar{B}$ = mean respiratory rate of the experimental and control groups throughout entire experiment.

TABLE 2. Split plot analysis of respirometric data comparing testes maintained in modified Burch & Cuadros's medium with testes maintained in isosmotic saline at 1, 24, 96 hr and 1 and 2 weeks.

	Sum of squares	Degrees of freedom	Mean square	F	Tail probability
Mean	6.62982	1	6.62982	111.08	0.0000
Trial	0.63606	2	0.01803	0.30	0.7473
Time	0.55586	4	0.13897	2.33	0.1437
Error	0.47750	8	0.05969		
Media	0.62583	1	0.62583	29.32	0.0006[1]
Media × trial	0.02363	2	0.01182	0.55	0.5955
Media × time	0.52225	4	0.13056	6.12	0.0148[2]
Error	0.17078	8	0.02135		

1. $p < 0.01$
2. $p < 0.05$

TABLE 3. Analysis of the mean respirometric rates (μl 0_2/gm dry weight/20 min) of testes maintained in modified Burch & Cuadros's medium and isosmotic saline. The differences between the rates in the modified medium and saline has been compared at each time interval by employing the least significant difference test.

Time interval	Mean respirometric rate in isosmotic saline	Mean respirometric rate modified medium	Difference	LSD[1]
0 hr	0.5649	0.6299	0.0650	< 0.2751
24 hr	0.4977	0.5750	0.0773	< 0.2751
96 hr	0.4855	0.7032	0.2177	< 0.2751
1 week	0.2179	0.5151	0.2972	>0.2751[2]
2 week	0.1376	0.6494	0.7873	>0.2751[2]

1. LSD = Least significant difference at which the null hypothesis can be accepted. The LSD is calculated from the equation $\bar{x} - \bar{y} > t_{(0.025)} \sqrt{\frac{2 \text{ MS error.}}{3}}$
2. The difference between the mean respirometric rates are statistically significant ($p < 0.05$, two tailed).

TABLE 4. Two-way factorial analysis of scintillation counting log-transformed data. The analysis was of the mean counts per minute of whole testes maintained in modified Burch & Cuadros's medium or isosmotic saline for 2, 24, 96 hr, 1 and 2 weeks. followed by exposure to 5 μCi/ml of ^{3}H-thymidine.

	Sum of squares	Degrees of freedom	Mean square	F	Tail probability
Mean	32290.278	1	32290.278	59451.5	0.0000[1]
Time	210.842	4	52.711	97.05	0.0000[1]
Media	8.413	1	7.413	13.65	0.0003[1]
Time × media	7.125	4	1.781	3.28	0.0119[2]
Error	156.423	288	0.543		

1. $p < 0.01$
2. $p < 0.05$

there is a statistically significant interaction between the groups of testes and the time intervals ($p > 0.05$). This indicated that the respirometric rates for the two groups of testes needed to be examined within each time period. As a result of employing the least significant difference test (Winer, 1971) to analyze the mean respirometric rate (pooled for the three trials) of the two groups of testes at each of the five time intervals it was found that the mean rate of the experimental testes was significantly higher than that of the control

TABLE 5. Analysis of log-transformed scintillation data. The mean counts per minute are of whole testes maintained in either modified Burch & Cuadros's medium or isosmotic saline followed by exposure to 5 μCi/ml of ^{3}H-thymidine. The difference between the experimental and control data are compared for each time period using the least significant difference test.

	Maintenance periods									
	0 hr		2 hr		96 hr		1 week		2 weeks	
	Isosmotic saline	Modified medium	Isosmotic saline	Modified medium	Isosmotic saline	Modified medium	Isosmotic saline	Modified medium	Isosmotic saline	Modified medium
Mean	11.801	11.576	10.526	11.132	10.289	10.463	9.791	10.257	8.863	9.420
Standard deviation	0.939	0.754	0.683	0.472	0.635	0.447	0.818	0.918	0.751	0.785
(Sample size) *n*	30	30	30	30	30	30	30	30	30	30
Comparison of difference of mean to LSD statistic[1]	0.225	< 0.373	0.606	> 0.373[2]	0.174	< 0.373	0.466	> 0.373[2]	0.557	>0.380[2]

[1]Least significant difference (LSD) test: $\bar{x} - y > t_{(0.025)} SP \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}$.
[2]Significantly different ($p < 0.05$, two tailed).

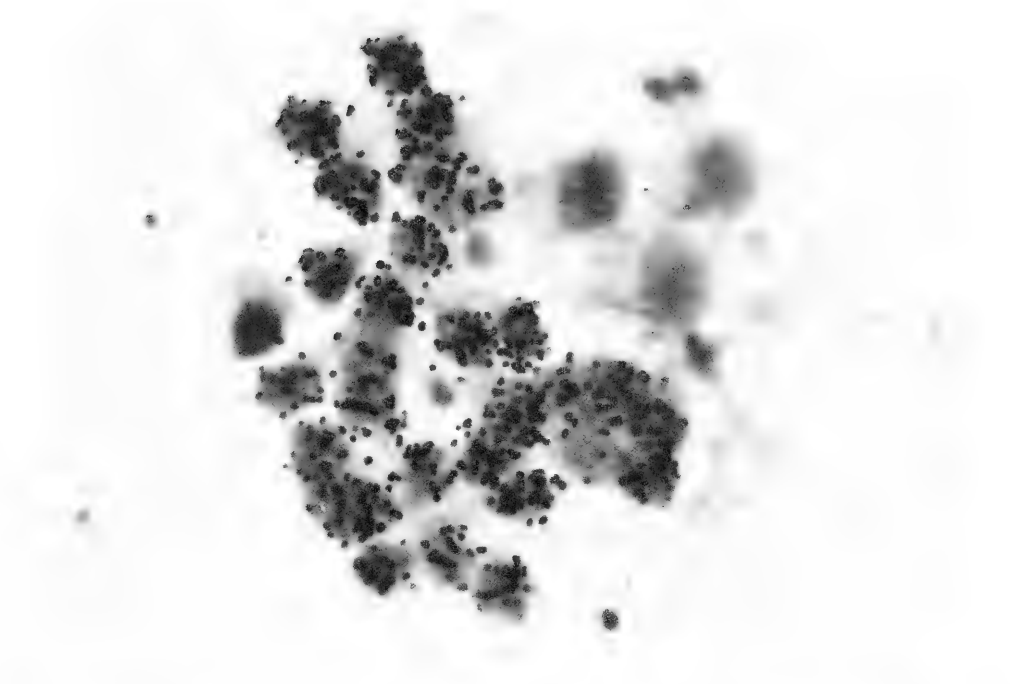


FIG. 1. Autoradiograph of a group of ^{3}H-thymidine labeled cells in testis of 0 hr *Ilyanassa obsoleta*. (See text for detailed description of methodology employed.)

testes at one and two weeks of maintenance ($p < 0.05$) but not at 0, 24, or 96 hr of maintenance ($p > .05$) (Table 3).

Scintillation counting

The raw data collected were as counts per minute (cpm) per gonad. These were not converted to cpm/g dry weight because accurate weighing of individual dried testis was rendered questionable because of the small weights of the tissue and its hygroscopic nature.

The data were log-transformed for statistical analysis in order to achieve homogeneity of variances of the mean cpm at the five time intervals. One assumption of the F-test (Winer, 1971) that was performed as part of the two-way factorial analysis was that the standard deviations were all estimates of the same "true" standard deviation. Thus, the data were log-transformed so that the standard deviations were normalized.

As a result of the two-way factorial analysis of the log-transformed data, the differences between the data collected at the five time intervals and the differences between the data associated with the experimental and control groups were ascertained to be statistically significant ($p < 0.01$) (Table 4). In other words, the average cpm for testes maintained in the modified medium and in saline at all of the time intervals were also significantly different. Furthermore, interaction between the media data and the time interval data was significant ($p < 0.05$), thus indicating that a least significant difference test (Winer, 1971) was necessary to compare the mean cpm obtained from the two groups of testes at each time interval. As a result, the mean cpm for testes maintained in the modified medium was determined to be significantly higher than the mean cpm for testes maintained in saline at 24 hr, and 1 and 2 week time periods ($p < 0.05$, two-tailed) (Table 5). This permits the conclusion that the testes maintained in the modified medium were incorporating more ^{3}H-thymidine. It is noted, however, that the difference between ^{3}H-thymidine uptake by testes maintained in the modified medium and saline was not significant at 96 hr. This, in our opinion, is an artifact.

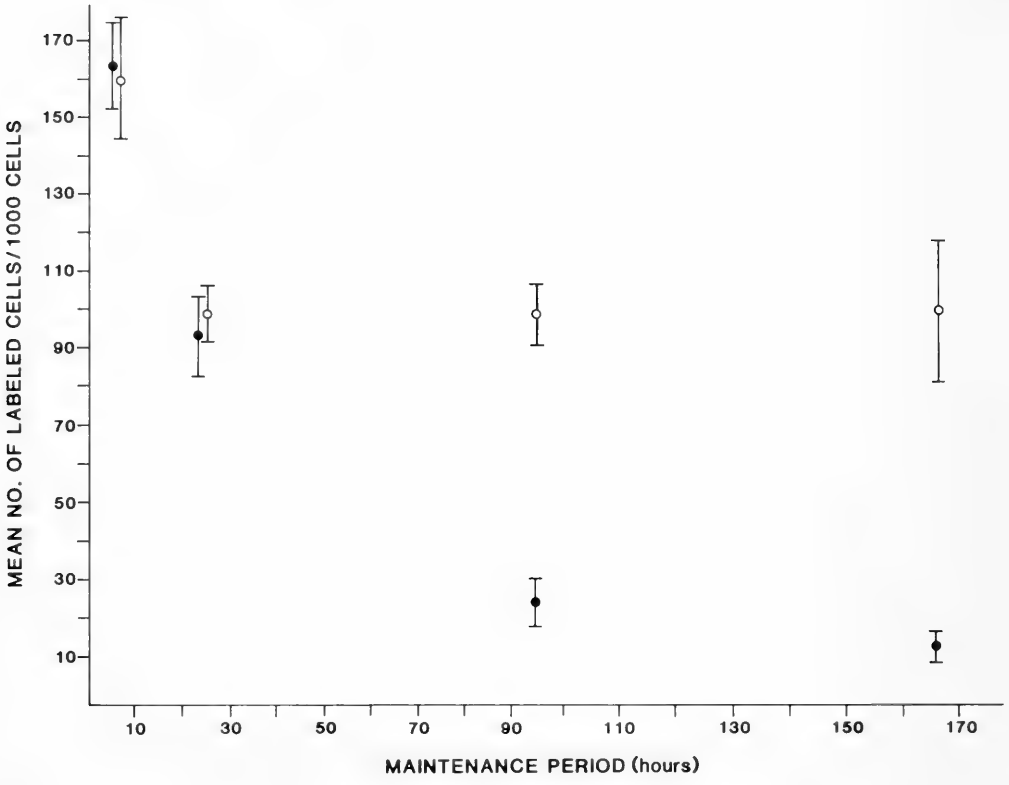


FIG. 2. Mean number of labeled cells/1000 cells determined by counting smears of testes maintained in either modified Burch & Cuadros's medium (○) or isosmotic saline (●) for the time periods indicated. Vertical lines represent 1 standard deviation.

Autoradiography

Smears made from testes that were freshly dissected (0 hr) or maintained for 24 hr include numerous cells that were heavily labeled (Fig. 1). As depicted in Fig. 2, the number of labeled cells/1000 cells decreased somewhat between the 0 and 24 hr maintenance periods in both the experimental and control groups. At 96 hr and 1 week, however, the labeling index for testes maintained in isosmotic saline fell while that for testes maintained in the modified medium remained constant (Fig. 2). This is interpreted to mean that the experimental testes maintained for 96 hr and 1 week incorporated more ^{3}H-thymidine than the control testes at the same time intervals.

By designating those cells with 10 or mor silver grains associated with their nuclei as being heavily labeled, the percent of heavily labeled cells in testes maintained in saline declined dramatically at 96 hr (as did the total number of labeled cells), whereas the percentage in testes maintained in the modified medium remained at a fairly constant level (Fig. 3).

A two-way factorial analysis (Winer, 1971) performed on the cell labeling index data (i.e., total number of labeled cells/1000 cells) for cells maintained in the modified medium and saline for 0, 24, 96 hr and 1 week revealed that the differences in the labeling indices (combining the values for both groups of testes) for each time period, and the difference in indices between the two groups of testes (combined across all time periods) was statistically significant ($p<0.01$) (Table 6). Also, there was a statistically significant interaction between the two groups of testes and the time

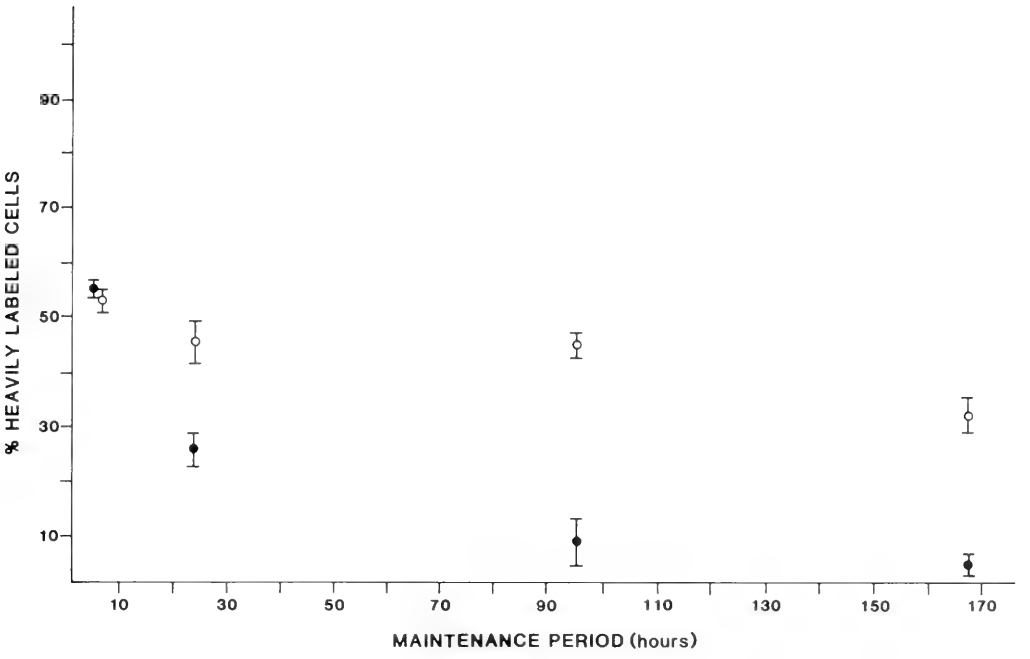


FIG. 3. Mean number of heavily labeled cells (with 10 or more silver grains associated with the nucleus), determined by counting smears of testes maintained in either Burch & Caudros's medium (○) or isosmotic saline (●) for the time periods indicated. Vertical lines represent 1 standard deviation.

periods, indicating that further analysis was necessary to compare the indices of the two groups of testes at each time interval. Application of the least significant difference test revealed that the labeling indices for the experimental testes maintained for 96 hr and 1 week were significantly higher than those for the control testes maintained for the same time periods ($p > 0.05$, two tailed) (Table 7).

The labeling indices for experimental testes maintained for 96 hr and 1 week were almost identical; however, the standard deviation of the 1 week value was large in comparison to that of the 96 hr value.

The results of our statistical analyses per-

TABLE 6. Two-way factorial analysis of autoradiographic data. Shown are the mean number of labeled cells/1000 cells for testes maintained in isosmotic saline or modified Burch & Cuadros's medium for 0, 24, 96 hr and 1 week followed by exposure to 5 μCi/ml of ^{3}H-thymidine.

	Sum of squares	Degrees of freedom	Mean square	F	Tail probability
Mean	673259.37	1	673259.37	549.18	0.0000[1]
Time	142220.00	3	47406.67	38.67	0.0000[1]
Media	28492.71	1	28492.71	23.24	0.0000[1]
Time × media	27536.18	3	9178.73	7.49	0.0002[1]
Error	87041.96	71	1225.94		

[1] Statistically significant ($p < 0.001$).

TABLE 7. Analysis of mean number of labeled cells/1000 cells in testes maintained in modified Burch & Cuadros's medium and isosmotic saline and exposed to ^{3}H-thymidine. The difference between the experimental and control groups is compared at each time period using the least significant difference test (LSD).

	0 hr		24 hr		96 hr		1 week	
	Isosmotic saline	Modified medium	Isosmotic saline	Modified medium	Isosmotic saline	Modified medium	Isosmotic saline	Modified medium
Mean no. of labeled cells/1000 cells	163.2	159.5	90.3	95.6	22.2	94.7	17.8	95.7
Standard deviation	32.47	48.59	36.37	25.43	17.28	23.41	12.41	57.10
Sample size (n)	10	10	10	10	10	10	10	10
Comparison of difference of means to LSD statistic	3.7	<31.22	5.3	<31.22	72.5	>31.22	97.9	>32.08[1]

[1] Statistically significant ($p < 0.05$).

mit the conclusion that testes maintained in the modified medium for 96 hr and 1 week incorporated more ^{3}H-thymidine than did testes maintained in saline for the same time periods.

DISCUSSION

Several maintenance media have been devised for molluscan cells and/or tissues (see Malek & Cheng, 1974, for review). In this study, the testes of *I. obsoleta* were maintained *in vitro* in a modification of the medium originally devised by Burch & Cuadros (1965).

By employing three assay methods (respirometry, scintillation spectrometry, autoradiography), we have demonstrated that our modification of Burch and Cuadros's medium can be employed to maintain testes of *I. obsoleta* for 2 weeks, the duration of the study. Specifically, we have demonstrated that testes maintained in the modified medium revealed significantly higher O_2 utilization than did testes maintained in isosmotic saline at 1 and 2 weeks. Furthermore, the mean respirometric rate for testes maintained in the modified medium remained fairly constant (ranging from 0.5151 to 0.7032 μl O_2/gm dry weight/20 min) at all of the time intervals (0, 24, 96 hr, 1 and 2 weeks), whereas the mean respirometric rate of testes maintained in isosmotic saline fell precipitously after 96 hr (from 0.4855 to 0.1376 μl O_2/gm dry weight/20 min).

Our quantitative autoradiographic data revealed that *I. obsoleta* testes maintained in the modified medium took up ^{3}H-thymidine at about the same level as at 24 and 96 hr although there was a larger variance at one week. In contrast, uptake of ^{3}H-thymidine by testes maintained in saline dropped dramatically after 24 hr.

Finally, our scintillation counting studies revealed that testes maintained in the modified medium for 24 hr, 1 and 2 weeks took up greater amounts of ^{3}H-thymidine than did testes maintained in saline. The only discrepancy between our respirometric and autoradiographic results and our scintillation counting results rests with our findings that there was no difference in the uptake of ^{3}H-thymidine by the experimental and control groups of testes at 96 hr. This is believed to be an artifact.

In an earlier study, Cheng *et al.* (1984) compared the efficacy of modifications of the media devised by Chernin (1963) and Tripp *et al.* (1966) for maintaining gonads of *I. obsoleta*. It was found that these two media could be employed to maintain ovaries and testes of *I. obsoleta* for up to 48 hr. In view of the results reported herein, our modification of Burch & Cuadros's medium is more efficacious for maintaining the testes of *I. obsoleta* and consequently is currently being employed in *in vitro* tests for the detection of a molecule from sporocysts of the trematode *Zoogonus lasius* responsible for parasitic castration of *I. obsoleta*.

ACKNOWLEDGEMENTS

We thank Dr. Rebecca G. Knapp, Department of Biometry, Medical University of South Carolina, for her assistance in the statistical analysis of our data and J. Bradford Cheng for preparing the photomicrograph.

REFERENCES CITED

BASERGA, R. & MALAMUD, D., 1969, *Modern methods in experimental pathology: autoradiography: techniques and applications*. Harper and Row, New York, 281 p.

BURCH, J. B. & CUADROS, C., 1965, A culture medium for snail cells and tissues. *Nature*, 206: 637–638.

CHENG, T. C., HOWLAND, K. H. & SULLIVAN, J. T., 1984, Comparison of the efficacy of two maintenance media for gonads of *Ilyanassa obsoleta* (Mollusca: Gastropoda). *Transactions of the American Microscopical Society*, 103: 249–262.

CHENG, T. C., SULLIVAN, J. T. & HARRIS, K. R., 1973, Parasitic castration of the marine prosobranch gastropod *Nassarius obsoletus* by sporocysts of *Zoogonus rubellus* (Trematoda): histopathology. *Journal of Invertebrate Pathology*, 21: 183–190.

CHERNIN, E., 1963, Observations on hearts explanted in vitro from the snail *Australorbis glabratus*. *Journal of Parasitology*, 49: 353–364.

GUDE, W. D., 1968, *Autoradiographic techniques: localization of radioisotopes in biological material*. Prentice-Hall, Englewood Cliffs, New Jersey, 113 p.

MALEK, E. A. & CHENG, T. C., 1974, *Medical and economic malacology*. Academic Press, New York, 398 p.

McCRADY, J., 1873, Observations on the food and the reproductive organs of *Ostrea virginica*, with some account of *Bucephalus cuculus* nov. spec. *Proceedings of the Boston Society of Natural History*, 16: 170–192.

SULLIVAN, J. T., CHENG, T. C. & HOWLAND, K. H., 1985, Studies on parasitic castration: castration of *Ilyanassa obsoleta* (Mollusca: Gastropoda) by several marine trematodes. *Transactions of the American Microscopical Society*, 104: 154–171.

TRIPP, M. R., BISIGNANI, L. A. & KENNY, M. T., Oyster amoebocytes *in vitro*. *Journal of Invertebrate Pathology*, 8: 137–140.

WINER, B. J., 1971, *Statistical principles in experimental design*. McGraw-Hill, New York, 907 p.

MALACOLOGIA, 1986, 27(1): 185–202

THE REPRODUCTIVE CYCLES AND GLOCHIDIA OF FRESH-WATER MUSSELS (BIVALVIA: HYRIIDAE) OF THE MACLEAY RIVER, NORTHERN NEW SOUTH WALES, AUSTRALIA

H. A. Jones, R. D. Simpson & C. L. Humphrey

Department of Zoology, University of New England, Armidale, NSW 2351, Australia

ABSTRACT

An investigation of the reproductive biology of five fresh-water mussel species, *Cucumerunio novaehollandiae*, *Hyridella australis*, *H. depressa*, *Hyridella* sp. and *Alathyria profuga* was undertaken in the Macleay River, northern New South Wales. Gametogenesis was studied in detail for *C. novaehollandiae* but only the cycle of larval production was described for the other species. In *C. novaehollandiae* gametogenesis occurred throughout the year. Ripe oocytes and spermatozoa were abundant in the ovaries and testes from January until August. The breeding season was highly synchronized and occurred in April, although it is possible that a second breeding season occurred during August in the upper reaches of the river. The reproductive cycle of the downstream populations lagged behind the cycle of the upstream populations of *C. novaehollandiae*. Spawning was associated with the occurrence of floods, and the resulting drop in water temperature might possibly be an important exogenous factor influencing spawning. The brooding period extended over nine weeks. Glochidial release proceeded from mid-May to the end of July in the upper reaches of the river and from June until August further downstream.

The breeding season in *Hyridella australis*, a repetitive breeder, occurred from spring to autumn but was observed only during spring and summer in *H. depressa* and *Hyridella* sp. *H. australis* produced three broods during the breeding season. The brooding period was from eight to eleven weeks depending on water temperature. Glochidia were released throughout most of the year with peak release periods in November, February and May. Females of *A. profuga* were gravid in mid-summer when most individuals were collected.

The glochidia of *Cucumerunio novaehollandiae*, *Hyridella australis* and *H. depressa* are described. Except for *H. depressa*, these are much smaller than the known glochidia of other Australian species and also differ markedly in shape.

INTRODUCTION

The fresh-water Unionacea have highly specialized life cycles. Among the Hyriidae, the eggs are moved into specialized portions of the inner gills (marsupia) where they develop into a hooked larval stage (the glochidium). Mature glochidia are released into the water where they spend some time attached to a vertebrate host. This is generally a fish, although tadpoles (Seshaiya, 1941; Walker, 1981) and a salamander (Howard, 1951) have also been shown to be host species.

General reproductive patterns are well known for both North American unionaceans (Lefevre & Curtis, 1910, 1912; Coker *et al.*, 1921; van der Schalie, 1938; Pennak, 1953; Clarke & Berg, 1959) and European unionaceans (Bloomer, 1935, 1946; Negus, 1966; Tudorancea, 1969, 1972; Wood, 1974; Haukioja & Hakala, 1978; Dartnall & Walkey, 1979). Detailed life histories providing information on gametogenesis, breeding seasons, periods of glochidial release, fish hosts and the duration of the parasitic period are unavailable for most species. The reproductive biology of *Margaritifera margaritifera*, however, is well known in both Europe and North America (Murphy, 1942; Roscoe & Redelings, 1964; Wood, 1974; Smith, 1976, 1979; Bauer, 1979). Trdan (1981) determined the breeding season, period of glochidial development and fish hosts for *Lampsilis radiata siliquoidea* but ignored gametogenesis. Reproductive cycles of unionaceans, including gametogenesis, have been determined in both temperate (Matteson, 1948; van der Schalie & van der Schalie, 1963; Stein, 1969; Yokley, 1972; Giusti *et al.*, 1975; Heard, 1975; Zale & Neves, 1982) and tropical (Lomte & Nagabhushanam, 1969; Ghosh & Ghose, 1972;

Nagabhushanam & Lohgaonker, 1978; Dudgeon & Morton, 1983; Humphrey, 1984) regions.

Despite the numerous morphological descriptions of glochidia in the literature (Surber, 1912; Coker *et al.*, 1921; Clarke & Berg, 1959), few of these enable identification of glochidia at the species level (Rand & Wiles, 1982). The type of glochidium (*i.e.* hooked, hookless and axehead types) is constant for the genus and in some cases the shape is also characteristic (Lefevre & Curtis, 1910). Identification of glochidia at species level, especially conspecifics, is often more difficult (Porter & Horn, 1980) but it has been achieved by using scanning electron microscopy (Rand & Wiles, 1982) and analysing glochidial morphometrics (Wiles, 1975).

Little is known of the reproductive biology of the fresh-water mussels from the Australasian region. There is, at present, only one comprehensive study of the reproductive biology of an Australian mussel and this is a tropical species (Humphrey, 1984). Fish hosts have been found for several species (Percival, 1931; Hiscock, 1951; Atkins, 1979; Walker, 1981; Humphrey, 1984). The available data indicate that the glochidia of Australian fresh-water mussels are nonspecific parasites of fish (Atkins, 1979; Walker, 1981; Humphrey, 1984). The glochidia of less than half of Australia's 17 species of fresh-water mussels have been described (McMichael & Hiscock, 1958; Atkins, 1979; Walker, 1981) although there are several unpublished records (K. F. Walker, personal communication).

The aim of the present study was to investigate reproductive strategies of warm-temperate mussels in the Macleay River, New South Wales. (Other workers are currently studying reproduction of fresh-water mussels in the Murray River.) Five and possibly six species occur in the Macleay River system although one, *Velesunio ambiguus*, is found only in the tablelands section of the Apsley River (Fig. 1). *Cucumerunio novaehollandiae* (Gray) is ubiquitous throughout the river and for this reason was chosen for a detailed investigation of its reproductive cycle, including gametogenesis, breeding season and the period of glochidial release. Upstream and downstream populations were chosen for a comparison of the reproductive cycle in different parts of the river. Less detailed study was made of the reproductive cycles of the four other hyriid species occurring in the river—*Hyridella australis* (L.), *Hyridella depressa* (L.), *Hyridella* sp. and *Alathyria profuga* (Gould).

MATERIALS AND METHODS

The study area

The Macleay River is situated in northern New South Wales, with its source in the New England Tablelands. Three major tributaries, the Apsley, Chandler and Muddy Rivers drain the central catchment area via a system of deep gorges from which the river emerges near its junction with the Georges River (Fig. 1). From here, the river flows more or less directly to the sea 220 km downstream.

The Macleay is a bicarbonate river, with soft waters of low salinity and turbidity. The chemical characteristics of the river at Turner's Flat (mean values) were as follows: Calcium 9.48 mgl^{-1}, bicarbonate 55.51 mgl^{-1}, pH 7.7, hardness 46.0 mgl^{-1}, chlorine 12.40 mgl^{-1}, conductivity 143 μScm^{-1} and salinity 62 mgl^{-1} T.D.S. (N.S.W. Water Conservation and Irrigation Commission; Australian Water Resources Council, 1976).

Discharge is seasonal but variable and the highest discharge rates occur during the months from January to June with a minor peak in the spring (Fig. 2). During the study period water temperatures rose to a peak of 27°C in mid-summer and began falling during March, reaching a minimum of 11°C in June (Fig. 3). This was typical of previous years. Little difference in temperature was apparent between upstream and downstream parts of the river except in May when the water temperature of the lower reaches was 2°C higher than upstream.

Collections and species identifications

Sampling of the fresh-water mussel populations was confined to stretches of the Macleay River below Georges River since the rugged terrain and inaccessible nature of the central gorge system precluded sampling above this point. The river was regularly sampled at two stations: at Honeymoon Bend (station 1), approximately 160 km above the tidal limit and at Toorooka (station 4), 50 km above the tidal limit. Infrequent sampling was carried out at two other stations (2 and 3) along the river (Fig. 1).

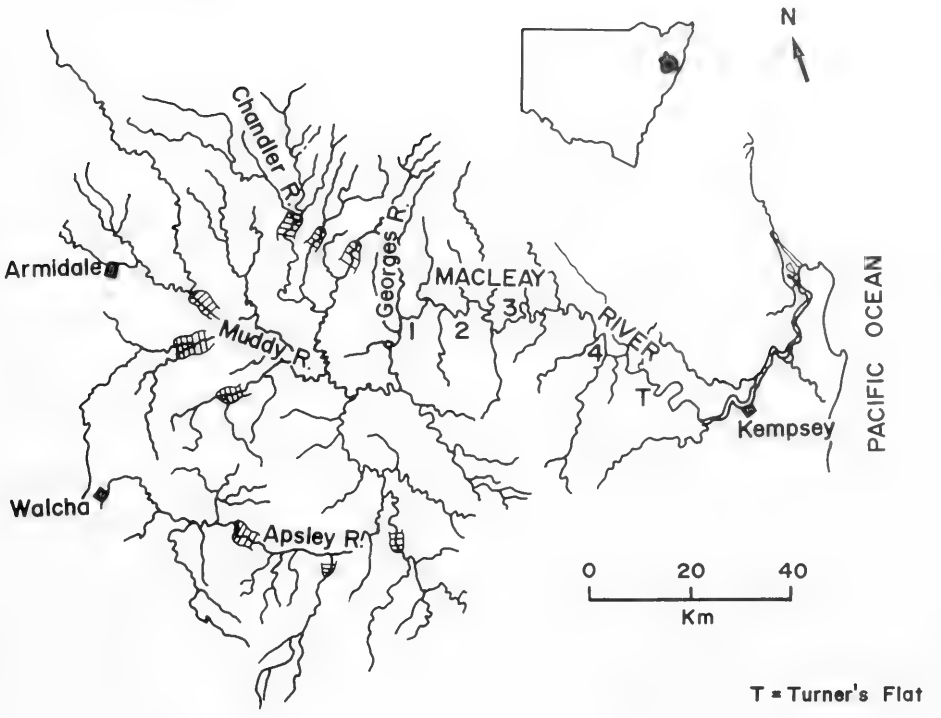


FIG.1. The Macleay River, showing the locations of the sampling stations (1, 2, 3, 4). Hatching indicates waterfalls at the top of the central gorge system.

Species identifications were based on McMichael & Hiscock (1958) and shell collections held by the Australian Museum (Sydney). We are confident about the identifications made of four species that occur in the main stem Macleay River. These species are *Cucumerunio novaehollandiae*, *Hyridella australis*, *H. depressa* and *Alathyria profuga*. (The occurrence of *A. profuga* in the Macleay River drainage is a new distributional record.) There is a likelihood, moreover, that a fifth species occurs although uncommonly; some shells collected during the present study match closely, published descriptions (McMichael & Hiscock, 1958) and museum identifications of *H. drapeta*. However, the glochidia (found in only one of the specimens collected from the Macleay River) do not match published descriptions of the glochidia of this species (Atkins, 1979). In morphology they are similar to the glochidia of *H. depressa*, although from the scant data available they are smaller (see Table 1). More collections will need to be made to determine whether these shells are merely ecophenotypic variants of *H. depressa* or whether in fact they represent individuals of an undescribed species of *Hyridella*. For now, individuals of this type are referred to as *Hyridella* sp.

The abundances of the five species occurring in the main stem Macleay River were, in decreasing order, *Cucumerunio novaehollandiae*, *Hyridella australis*, *H. depressa*, *A. profuga* and *Hyridella* sp. Specimens were collected monthly from July 1982 to July 1983 by snorkelling or by hand. Supplementary collections were made in January 1985. Care was taken to process mussels quickly after collection since gravid females are known to abort the larvae from the marsupia when under stress (Lefevre & Curtis, 1910, 1912; Hiscock, 1951). Animals were either packed

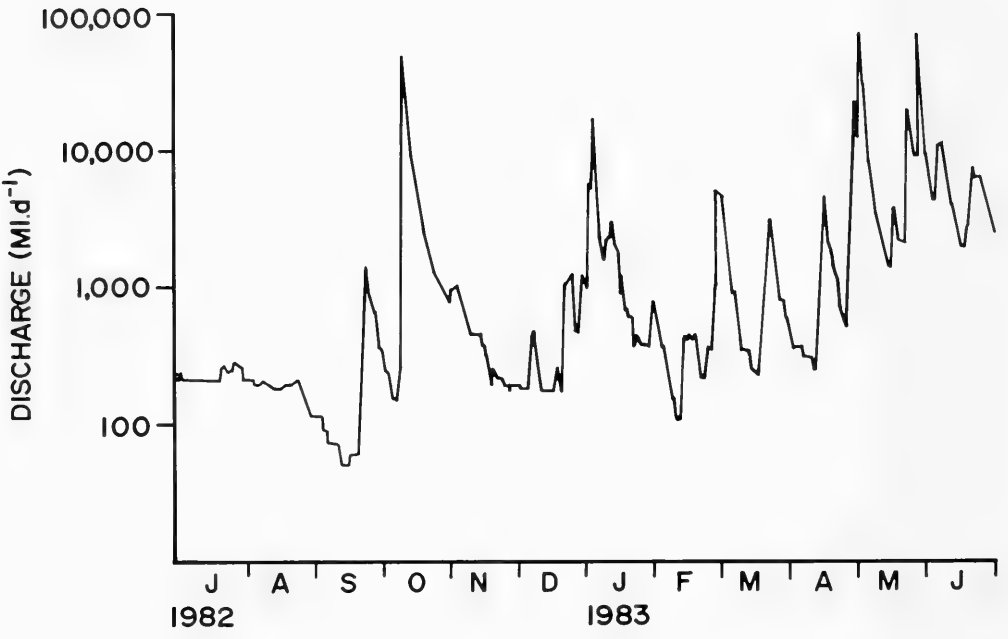


FIG. 2. Daily discharge of the Macleay River during the study period. (Based on data supplied by the New South Wales Water Conservation and Irrigation Commission).

in ice or fixed in 10% formalin made up from river water. Approximately 50 *C. novaehollandiae* were collected monthly from each of these stations, but the other species were collected in similar numbers from station 1 only. Only *C. novaehollandiae* was abundant at station 4.

Examination of gonads

Adult *C. novaehollandiae* ranging from 100–160 mm in length were sectioned at 6 μm through the central region of the visceral mass and stained with Masson's trichrome or Mayer's haematoxylin and counterstained with eosin. Quantitative data of the stages of spermatogenesis were obtained from microscopical examination of stained sections through the central region of the visceral mass. In each of five individuals from each month, ten acini were selected at random and the proportion of each cell type, along a line through each acinus, was calculated using an ocular micrometer.

Oocyte sizes were measured with an ocular micrometer from visceral smears of 10 individuals per month and seasonal changes in the mean oocyte size determined. 4500 oocytes were measured. The number of oocytes (sample size) required to give a representative mean oocyte size in each individual was determined by plotting the means against sample size (Fig. 4) until the mean value ceased to fluctuate (Elliott, 1977).

The inner demibranchs of the gills of females from all four species were examined to determine gravidity. Small portions from gravid gills were removed and examined under the microscope so that the stage of development of the larvae could be determined. Four stages were recognized:

Stage I. Marsupium empty and undeveloped.

Stage II. Eggs or embryos present in the marsupia. Embryos included all stages of development from zygotes to individuals in which the larval shell had not formed.

Stage III. Glochidia present in the marsupia. Glochidia were characterized by the development of the adductor muscle and the larval shell. This included individuals in which hooks were unformed or rudimentary to fully developed larvae, free of their vitelline membrane.

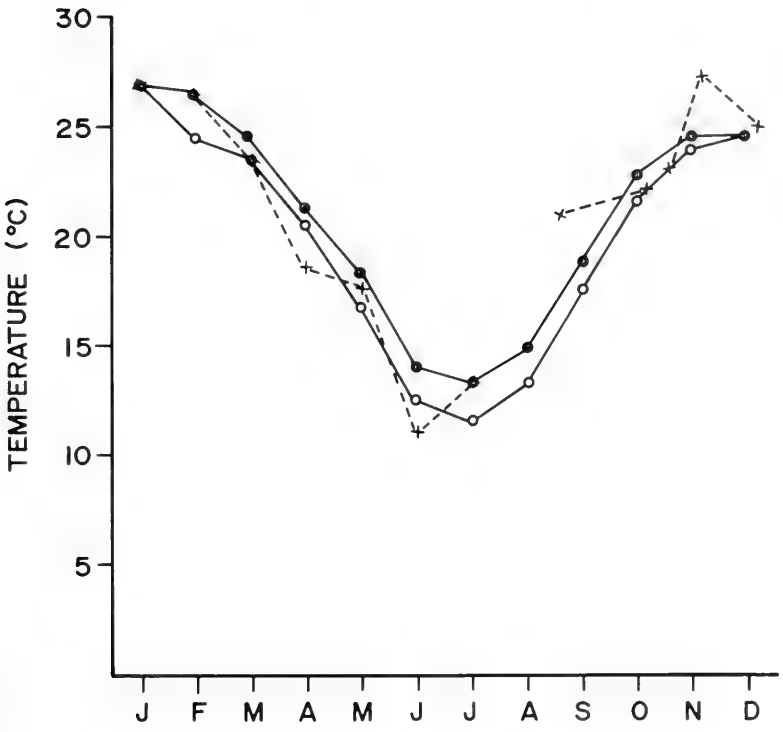


FIG. 3. Monthly water temperatures for the Macleay River, showing readings taken during the study period (broken line) and the mean monthly water temperatures for Georges River junction (closed circles) and Turner's Flat (open circles) since 1976. (Data supplied by the New South Wales Water Conservation Irrigation Commission)

Stage IV. Post-glochidial release phase. Some glochidia may be retained in the marsupium, which often has a bubbly appearance immediately after glochidial release. The water tubes are clearly visible in the inner demibranchs of *C. novaehollandiae* that have recently released glochidia. The dorsal two-thirds of the marsupia in *H. australis* retains a mass of tissue after the glochidia have been released, which imparts a false impression of gravidity. Aborted glochidia could be distinguished from those normally released at stage IV only if they were not mature glochidia.

The stages of glochidial development were further subdivided into early glochidia, intermediate glochidia and mature glochidia after Heard (1975). Dimensions of length (maximum valve diameter in the hinge plane), hinge length and height (depth from hinge to hook) of mature glochidia were measured. References made to glochidial dimensions are in the order of height × length.

Definition of some terms is needed here. The breeding season, for animals that fertilize externally, spans the coincident periods of spawning of eggs and spermatozoa (Giese, 1959; Simpson, 1977). For those animals that do not fertilize externally, spawning and fertilization can be two separate events. The breeding season of fresh-water mussels is when the spermatozoa are spawned by the males to fertilize ova being moved into the gill chambers. The gestation period then follows and refers to the time elapsing between the movement of the oocytes into the marsupia until the development of mature glochidia. The gestation period is part of the total brooding period, which spans the time of placement of oocytes in the marsupia to the release of glochidia.

TABLE 1. Morphometric data for glochidia of the five species of fresh-water mussels found in the Macleay River.

Species	n*	Mean length ± SD (μm)	Mean height ± SD (μm)	Hinge length ± SD (μm)	Ht/Lth ratio (%)	Hinge/Lth ratio (%)
C. novaehollandiae	50	52.2 ± 0.6	64.1 ± 0.2	35	116	64
H. australis	50	73.9 ± 0.5	94.7 ± 0.3	40	128	68
H. depressa[1]	50	253 ± 5	244 ± 5	152 ± 6	97	60
Hyridella sp.[1]	20	239 ± 4	233 ± 4	136 ± 2	97	57
A. profuga[1]	20	239 ± 5	204 ± 2	165 ± 4	85	69
A. profuga[2]	—	245	200	—	82	—
H. drapeta[3]	—	330 ± 10	230 ± 10	248	71	75

*From five individuals (*C. novaehollandiae*, *H. australis* and *H. depressa*) and one individual each (*Hyridella* sp. and *A. profuga*).
[1]Measurements made from preserved material.
[2]From McMichael & Hiscock (1958).
[3]From Atkins (1979). Hinge length is estimated from an illustration.

RESULTS

Spermatogenic cycle

The pattern of spermatogenesis in male *Cucumerunio novaehollandiae* from the upstream station between July 1982 and July 1983 was determined (Fig. 5). Spermatogenesis occurred throughout the year but at reduced tempo during the colder months of June and July as indicated by the reduced numbers of cells in the earlier spermatogenic stages (Table 2). The period between August and November was a recovery period in which unspawned gametes from the previous season were either resorbed or released and during which a build-up of spermatogonia and sperm-morulae occurred (Fig. 6). Typical spermatogenesis was almost completely absent during this time, most of the activity being directed towards the production of sperm-morulae. An increase in the tempo of spermatogenesis occurred from late November until late April during which intensive production of spermatozoa and enlarging of the acini occurred (Figs. 7–8). This phase was also characterized by large clusters of spermatids that were absent in the months prior to November.

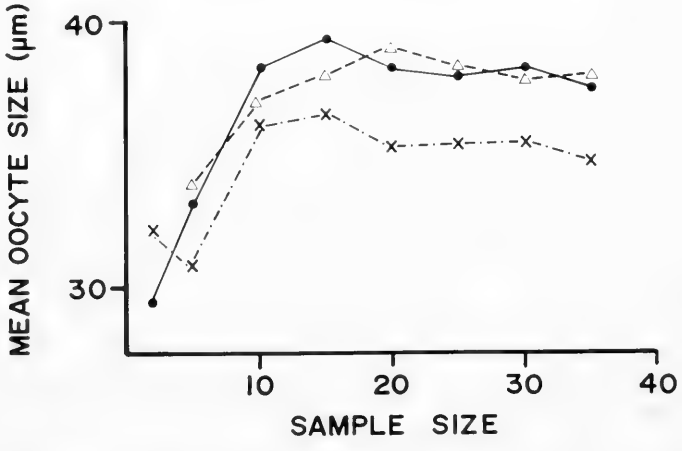


FIG. 4. Relationship between number of oocytes measured (sample size) and mean oocyte size in *Cucumerunio novaehollandiae* from the Macleay River: 26 January 1983, Honeymoon Bend (triangles); 26 March 1983, Toorooka (crosses); 15 July 1983, Toorooka (closed circles).

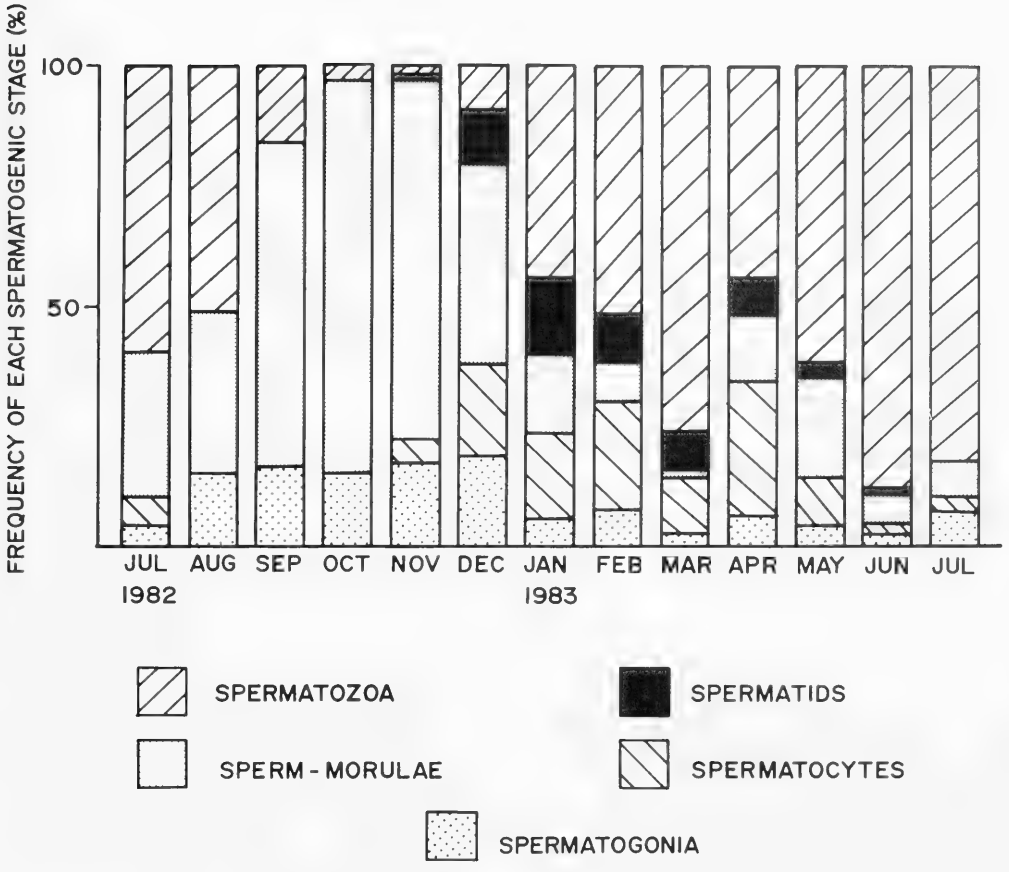


FIG. 5. Monthly changes in the proportion of the spermatogenic stages represented in the testes of *Cucumerunio novaehollandiae* from the upper reaches of the Macleay River.

Spawning occurred in the period of late March and April but was incomplete and many spermatozoa remained in the acini. After spawning, spermatogenesis continued from the remaining spermatogenic cells and the acini were again packed with spermatozoa in June and July (Fig. 9). Hence, three phases of spermatogenesis were recognized:

1) a recovery phase characterized by nests of spermatogonia and a high proportion of sperm-morulae;

2) an active phase characterized by nests of primary spermatocytes, secondary spermatocytes and spermatids. Spermatozoa were present in the lumen of the acini, and

3) a maturation phase in which spermatozoa were abundant but spermatogenic activity was reduced.

Female cycle

The following description of seasonal oocyte production is for females from the upper reaches of the river. Both developing oocytes, connected to the follicle walls by a stalk, and mature oocytes were absent from the ovary between August and November. Mature oocytes remaining from the previous reproductive period were resorbed early during this period (Fig. 10). Nutritive granules were prolific along the follicle walls which, by late October, were thickened with oogonia (Fig. 11).

TABLE 2. Monthly descriptions of spermatogenesis in *Cucumerunio novaehollandiae*.

JULY: Both spent and mature individuals were present. The acini of mature individuals were large and closely spaced. The acini were filled with mature spermatozoa and very few of the earlier spermatogenic stages were present. The acini of spent individuals were small and widely spaced. Few spermatozoa were present in the lumen of the acini and spermatogonia and sperm-morulae were abundant.

AUGUST: Acini were slightly reduced in size when compared with individuals from July and fewer spermatozoa were present. Acini walls were thicker and there were numerous clusters of spermatogonia. Sperm-morulae were common.

SEPTEMBER: Acini were small, widely spaced and completely filled with sperm-morulae. Nests of spermatogonia were common and very few spermatozoa were present.

OCTOBER: Very little change from the previous month.

NOVEMBER: Clusters of primary spermatocytes appeared in some individuals and many of the sperm-morulae appeared to be metamorphosing into clumps of spermatozoa.

DECEMBER: Spermatozoa began to build up in the acini lumina and clusters of spermatocytes and spermatids were abundant. Sperm-morulae still dominated the acini.

JANUARY: In all individuals, spermatozoa were abundant and large clusters of spermatids in various stages of metamorphosis into spermatozoa were present. Spermatocytes were very common but there were fewer sperm-morulae.

FEBRUARY: Acini were large and closely spaced. Spermatogenesis was slightly more advanced than the previous month.

MARCH: Acini were densely packed with spermatozoa with peripheral bands of spermatocytes and spermatids.

APRIL: Acini were reduced in size and contained fewer spermatozoa than in March. The incidence of sperm-morulae increased.

MAY: Little change from April.

JUNE: Acini were again filled with spermatozoa but very few earlier spermatogenic stages were present.

JULY: Little change from the previous month except for the appearance of bands of spermatogonia and sperm-morulae around the periphery of acini.

Rapid growth of primary oocytes owing to vitellogenesis occurred from November (Fig. 12) and continued until the end of March when the acini were packed with mature oocytes. A sharp decrease in mean oocyte diameter occurred between March and April, coinciding with the movement of eggs into the marsupia. Following spawning, there was a second rapid build-up in the numbers of mature oocytes and acini were again packed with oocytes by mid-July (Fig. 13). Oogenesis in the downstream population lagged behind the upstream population and spawning did not occur until late-April or early-May (Fig. 14). Hence, three phases could be recognized in the oogenic cycle:

1) a recovery period from the end of the previous breeding season until late October (upstream) or December (downstream). Ripe oocytes remaining from the previous reproductive period were resorbed or passed out of the ovaries and the follicle walls thickened owing to a build-up of oogonia and nutrient reserves;

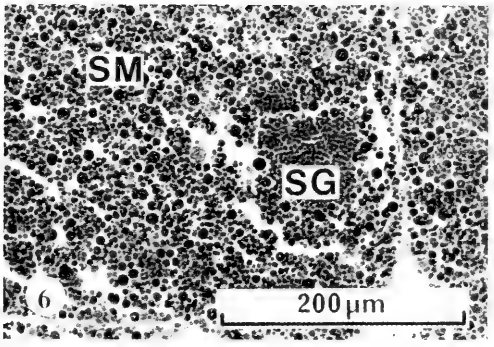
2) a growth phase from late October until about the end of March (upstream) and from December until about May (downstream). During this phase vitellogenesis occurred and the acini became swollen as they filled with primary oocytes, and

3) a spawning phase from late March (upstream) until early May (downstream).

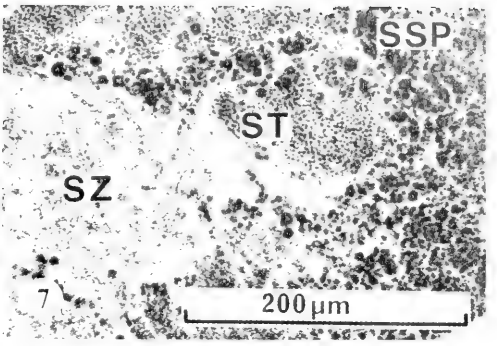
Description of the glochidia

Mature glochidia of all species were collected, although these were found in the marsupia of only one female each of *A. profuga* and *Hyridella* sp. The glochidial morphometrics of these species are summarized in Table 1.

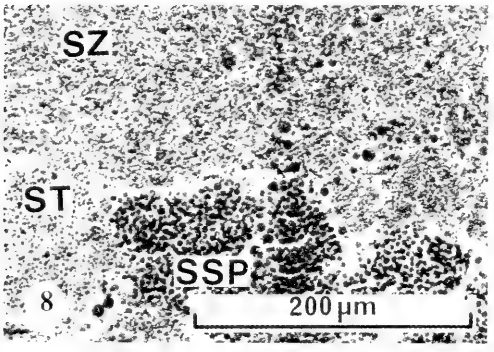
The glochidia of *C. novaehollandiae* (Figs. 15-17) are extremely small (64 × 55 μm), globose and of suboval outline. Two short hooks are present on the inside of each shell valve. Scanning electron microscopy has revealed a convoluted surface structure of the shell valves (K. F. Walker, personal communication). Glochidia of *H. australis* (Figs. 18–20) are small in comparison with other hyriids, measuring 95 × 74 μm. The glochidia are slightly elongate, subtriangular and the shell valves are perforated by small pores. The larvae are double-hooked and the teeth are recurved and set on a common base. The



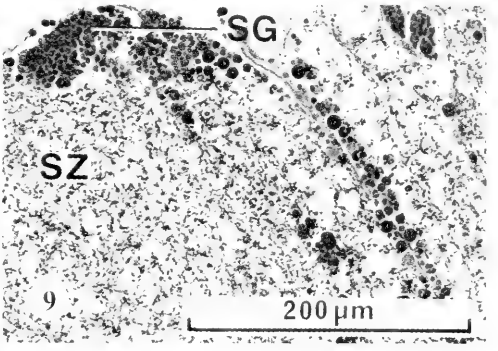
6



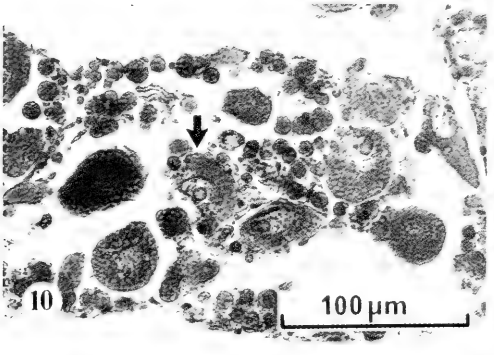
7



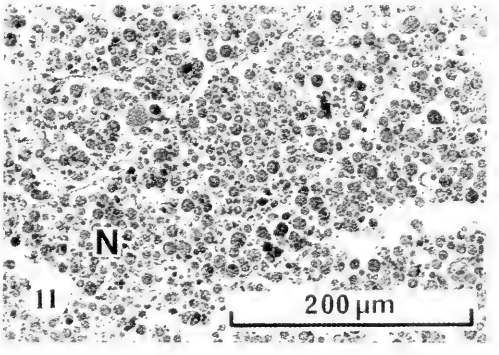
8



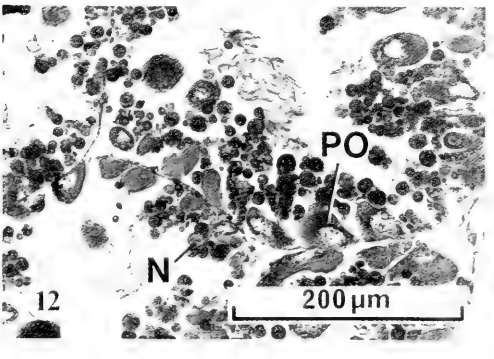
9



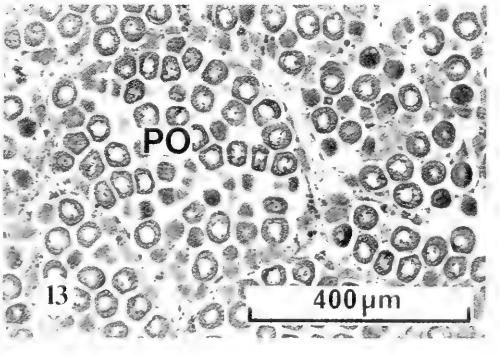
10



11



12



13

glochidia of *H. depressa* (Figs. 21–25) are large (244 × 253 μm), subtriangular and possess a single, bifurcated hook set slightly off-centre on the ventral surface of each valve. The glochidia of *Hyridella* sp. also possess a bifurcated hook; they are very similar in morphology to *H. depressa* but are slightly smaller (233 × 239 μm). The glochidia of *A. profuga* compare well with published descriptions for this species (McMichael & Hiscock, 1958) (see Table 1).

Brooding period

Cucumerunio novaehollandiae

C. novaehollandiae is a winter breeder with a highly seasonal cycle (Figs. 26–27). The brooding period extends from April to August. The reproductive cycle of downstream populations, however, lags slightly behind that of the populations upstream (Table 3).

When collected on 9 May 1983, immediately after a major flood (Fig. 2), most of the females from the downstream population had just moved eggs into their marsupia. Five weeks later these were at an intermediate glochidial stage and mature glochidia were being released in mid-July, 9 weeks after spawning. Mussels from the upstream station were releasing glochidia during mid-May and had finished by mid-July. On the basis of a similar progression in development, females in the upstream population probably spawned in late March or early April.

Hyridella australis

The reproductive activity in this species extended throughout most of the year except for the coldest month, July (Fig. 28). Most of the November collection from the upstream population aborted their larvae. Consequently, it was not possible to accurately separate females with mature glochidia in their gills (stage III) from those which had released their larvae (stage IV).

There were at least two, probably three, brooding periods during 1982/1983, indicated by the high proportion of females carrying glochidia at different times of the year. The four week intervals between sampling precluded accurate assessment of the time elapsing between fertilization and glochidial release. However, the gestation period appears to have been about eight weeks during the summer months when the water temperature was in the vicinity of 27°C, and about 11 weeks during the autumn when the water temperature was lower (11°C). Breeding periods must have occurred between 26 November and 22 December 1982, and again between 28 February and 26 March in 1983, that is, approximately 13 to 14 weeks apart.

One of us (C.L.H.) has observed the release of glochidia of *H. australis* while diving in clean river conditions in January 1985. Glochidia are extruded from the exhalent siphon of mature females in a wormlike conglutinate. The conglutinate, approximately 4 cm in length, is tan coloured and bears white, transversely striated bands along one side. Laboratory examination revealed that the tanned material is composed entirely of mature glochidia bound together in a mucous matrix with the white striations comprising a loosely binding tissue. Distinct, rhythmical pumping actions of the exhalent siphon were noted that caused the wormlike mass to wave and fall about the siphon where it was posteriorly inserted or attached. Discharged conglutinates were also found lying free and intact on the sediments adjacent to adult females.

Hyridella depressa, Hyridella sp. and *Alathyria profuga*

Scant data were obtained for the brooding periods of these species (Table 4). However, *H. depressa* and *Hyridella* sp. were gravid during the spring and summer. It is possible that both species breed more than once per year, as in *H. australis*. Females of *A. profuga* bearing glochidia were present only in mid-

FIGS. 6–13. Stages of gonadal activity in *Cucumerunio novaehollandiae* collected from the upper reaches of the Macleay River. FIG. 6. Sperm-morulae in an inactive testis, 26 October 1982. FIG. 7. Maturing testis, 26 February 1982. FIG. 8. Mature testis immediately prior to spawning, 26 March 1983. FIG. 9. Mature spermatozoa in a testis, 15 July 1983. FIG. 10. Deteriorating oocytes (arrow) from an ovary, 27 July 1982. FIG. 11. Ovary in the resting phase and filled with nutrient matter, 26 October 1982. FIG. 12. Early oogenesis, 26 November 1982. FIG. 13. Mature ovary, 15 July 1983, N. nutrient granules; PO, primary oocytes; SG. spermatogonia; SM, sperm-morulae; SSP, secondary spermatocytes; ST, spermatids; SZ, spermatozoa.

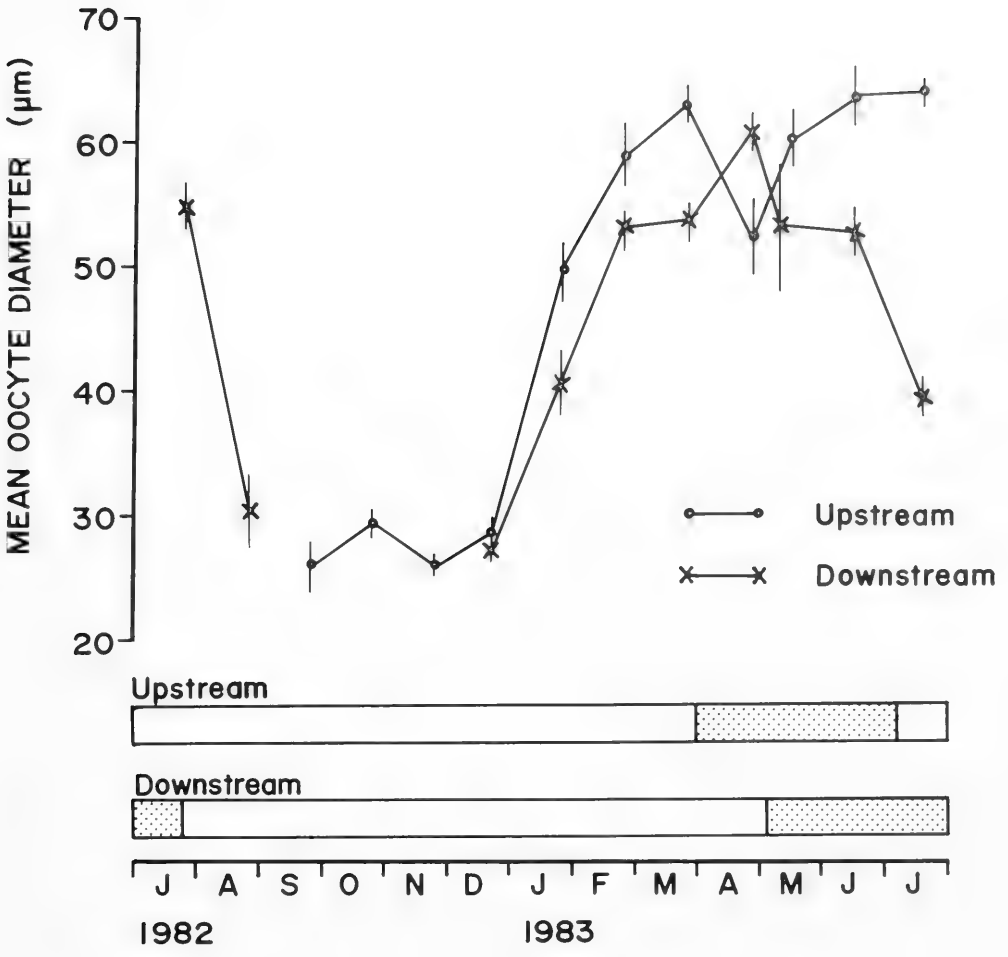


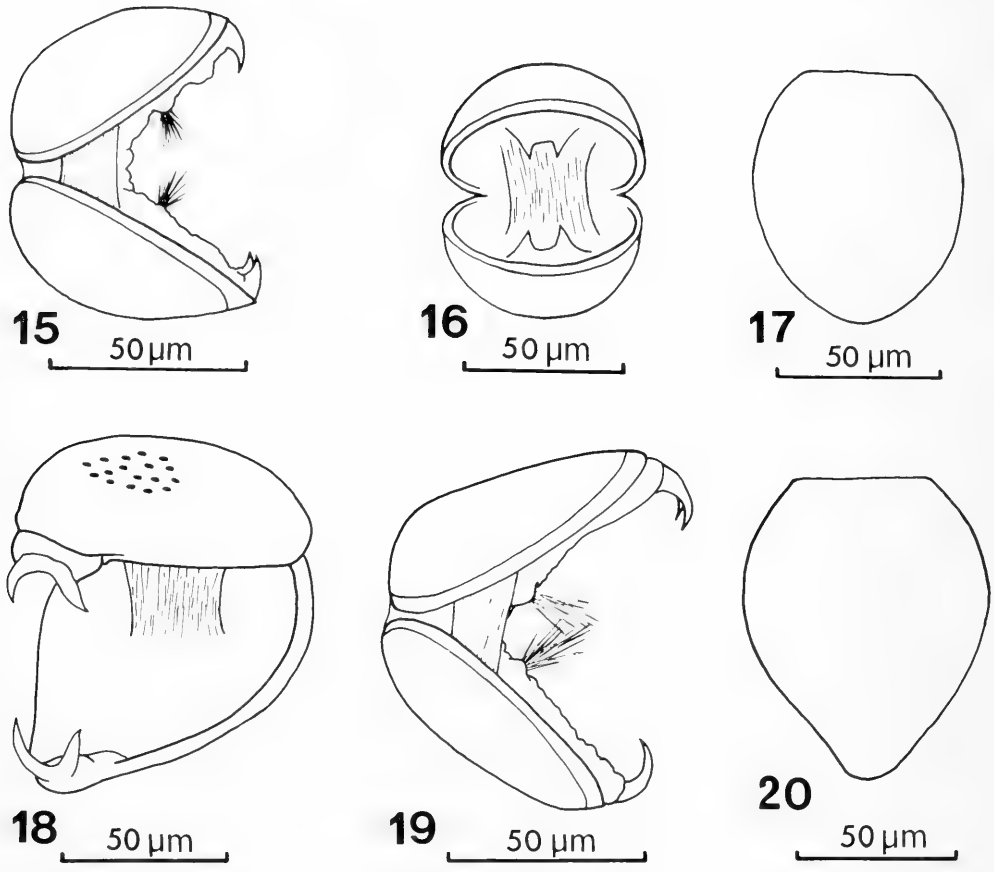
FIG. 14. Seasonal variation in primary oocyte size of *Cucumerunio novaehollandiae*. The bars are equal to 1 standard error of the mean. Brooding periods (stippled areas) are shown for upstream and downstream populations.

-summer, although very few specimens were collected at other times of the year.

DISCUSSION

Gametogenesis occurred throughout most of the year in *Cucumerunio novaehollandiae*, with peak activity during the hottest months of the year. This is typical of unionaceans from temperate climates (van der Schalie & van der Schalie, 1963; Yokley, 1972; Giusti *et al.*, 1975; Heard, 1975; Smith, 1979; Zale & Neves, 1982). Mature ova were produced during the summer and autumn, coinciding with the peak in spermiogenesis.

A highly synchronized breeding season occurred during the autumn. However, males only partially spawned. This could reduce the risk of mistiming the release of gametes by the sexes. In other temperate fresh-water mussels both males and females spawn at the same time (Zale & Neves, 1982). Giusti *et al.* (1975), however, noted that not all male *Anodonta cygnea* spawned at the same time and *Elliptio complanatus* males release spermatozoa over a time span of about one

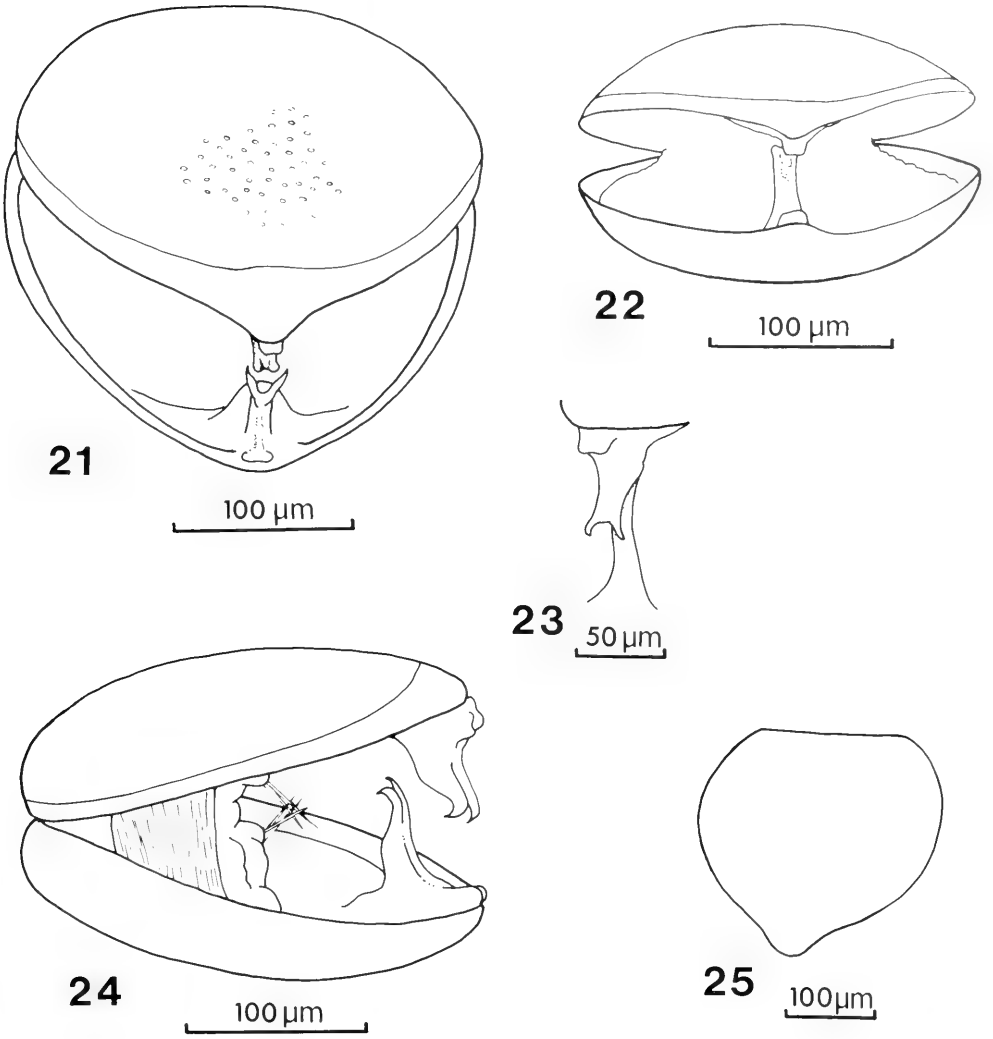


FIGS. 15–17. Glochidium of *Cucumerunio novaehollandiae*. FIG. 15. Lateral view. FIG. 16. Ventral view. FIG. 17. Outline of the shell valves. FIGS. 18-20. Glochidium of *Hyridella australis*. FIG. 18. Oblique ventral view. FIG. 19. Lateral view. FIG. 20. Outline of the shell valves.

month, which overlaps the period when females are likely to be receptive to fertilization (Matteson, 1948).

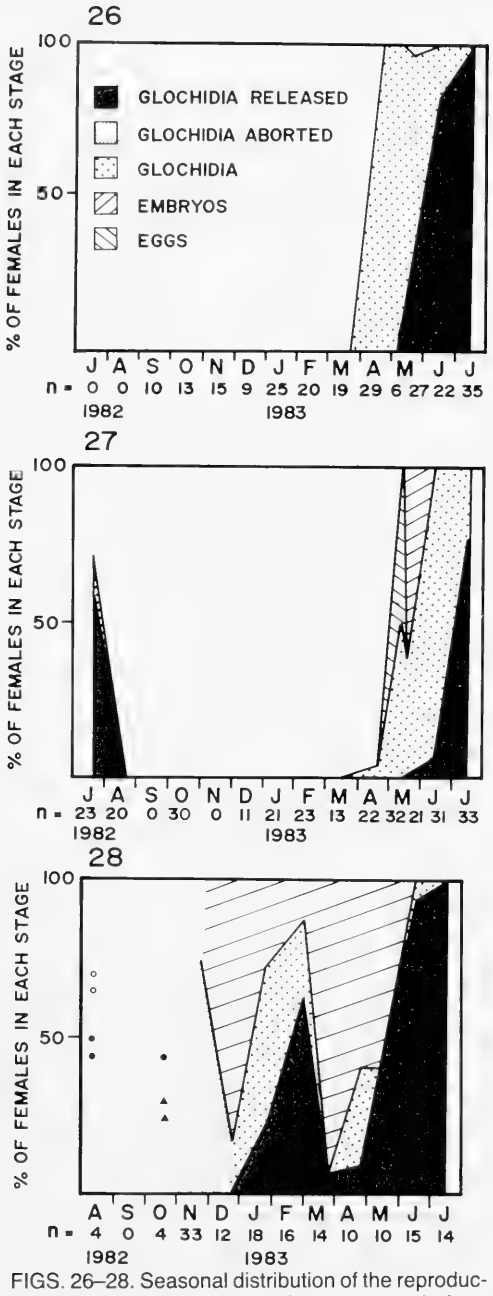
In contrast to *C. novaehollandiae*, other members of the southeastern Australian fresh-water mussel fauna may have much broader breeding seasons. Atkins (1979), in a study of a coastal Victorian stream, found *Hyridella drapeta* glochidia on fish throughout the year, with peak infections during the spring. Similarly, Hiscock (1951) found *Velesunio ambiguus* glochidia on fish throughout the year except between May and September, when no fish were examined. In agreement with Hiscock's data, Walker (1981) found that glochidia may be present in the marsupia of *Velesunio ambiguus* throughout the year, although two peaks in gravidity were recognized; one in spring and the other during late summer/early autumn. The results of the present study indicate that *H. australis*, *H. depressa* and *Hyridella* sp. breed throughout much of the warmer part of the year. This also appears to be the case for the New Zealand species, *H. menziesi* (Percival, 1931) and prolonged breeding seasons may well prove to be characteristic of the genus *Hyridella*.

A series of broadly synchronized reproduc-



FIGS. 21–25. Glochidium of *Hyridella depressa*. FIG. 21. Oblique view. FIG. 22. Ventral view. FIG. 23. Lateral view of the forked teeth and the protuberances at their bases. FIG. 24. Structure of the teeth. FIG. 25. Outline of the shell valves.

tive cycles, such as occurs in *H. australis*, have been infrequently reported for freshwater mussels from temperate regions (Allen, 1924, cited by Heard, 1975) but asynchronous, repetitive breeding cycles occur throughout the year in some tropical unionaceans (Kenmuir, 1981b; Humphrey, 1984). More detailed investigations of the reproductive biology of temperate zone unionaceans may find repetitive breeding to be more widespread than presently indicated; especially since some tachytictic species such as *Unio* spp. have the potential to produce many broods per year (Dudgeon & Morton, 1983). There are, however, several cases of freshwater mussels breeding twice per year (Lefevre & Curtis, 1910, 1912; Ortmann, 1912; Wood, 1974; Heard, 1975). Successive build-ups of large numbers of mature gametes indicated that *C. novaehollandiae* may breed



FIGS. 26–28. Seasonal distribution of the reproductive stages in the gills of freshwater mussels from the Macleay River. FIG. 26. *Cucumerunio novaehollandiae*, upstream population. FIG. 27. *C. novaehollandiae*, downstream population. FIG. 28. *Hyridella australis*, upstream population. For individual animals, open circles = non-gravid females; closed circles = embryos in marsupia; closed triangles = glochidia in marsupia; open triangles = non-gravid but glochidia recently released.

TABLE 3. Proportion of *Cucumerunio novaehollandiae* with different stages of developing larvae in gills throughout the Macleay River, 9 May 1983.

Stage of development	Station 1	2	3	4
I	0.0	5.3	5.0	0.0
II	0.0	0.0	0.0	50.0
III Early glochidia	0.0	94.7	0.0	0.0
Intermediate glochidia	100.0	0.0	95.0	50.0
Mature glochidia	0.0	0.0	0.0	0.0

twice per year: once in autumn and again in late winter/early spring. This pattern only occurred in the upper reaches of the river. Similarly Porter & Horn (1980) detected area variation in the reproductive cycle of a North American mussel in Lake Waccamaw. Also, variation in reproductive patterns within the one species of fresh-water mussel has been previously shown between rivers (Bauer, 1979) and at different latitudes (Matteson, 1948; Smith, 1976; Kenmuir, 1981a).

Sperm-morulae are of widespread occurrence in the Bivalvia (Bloomer, 1935, 1936, 1939; Coe & Turner, 1938; Ropes & Stickney, 1965; van der Schlie & Locke, 1941), including the Hyriidae (Heard, 1975; Peredo & Parada, 1984; Humphrey, 1984). Sperm-morulae occurred seasonally in *C. novaehollandiae* and were absent during the period of spermiogenesis. Seasonality of occurrence of sperm-morulae has also been described for other bivalves (Heard, 1975; Smith, 1979). Bloomer (1946) inferred that sperm-morulae metamorphosed into spermatozoa in *Anodonta cygnea* from the observation that sperm-morulae disappeared prior to the appearance of spermatozoa. This was also the case for *C. novaehollandiae* in which the process of atypical spermatogenesis was observed to the stage where the sperm-morulae consisted of elongate spermatids immediately prior to active spermatogenesis. Coe & Turner (1938) thought that sperm-morulae in *Mya arenaria* underwent cytolysis but there was no evidence of this in *C. novaehollandiae*.

Loosanoff & Davis (1952) and Sastry (1963) have demonstrated from their experiments with marine bivalves the importance of temperature as an activator of spawning. Environmental cues responsible for initiating

TABLE 4. Miscellaneous brooding records for individuals of the minor species found in the Macleay River.

Date	State of marsupia								
	Embryos			Glochidia			Empty		
	*Hd**	*Hs**	*Ap**	*Hd*	*Hs*	*Ap*	*Hd*	*Hs*	*Ap*
3 Aug. 1982	1	—	—	—	—	—	—	—	—
26 Oct. 1982	6	2	—	3	—	—	5	—	—
7 Nov. 1982	—	—	—	—	—	1	—	—	—
26 Nov. 1982	2	—	—	—	—	—	—	—	—
22 Dec. 1982	4	—	—	—	—	—	—	—	1
22 Jan. 1983	—	—	—	—	—	—	—	—	2
26 Feb. 1983	2	—	—	—	—	—	—	—	—
26 Mar. 1983	—	—	—	—	—	—	2	—	—
26 Apr. 1983	—	—	—	—	—	—	1	—	1
16 May 1983	—	—	—	—	—	—	1	—	—
1/2 Jan. 1985	11	—	12	6	—	3	—	1	9
16 Jan. 1985	4	—	6	5	—	2	6	3	6

**Hd* = *Hyridella depressa*; *Hs* = *Hyridella* sp.; *Ap* = *Alathyria profuga*

spawning in fresh-water mussels have not been experimentally identified but numerous authors have brought attention to the correlation between breeding season and water temperature (Harms, 1909; Tudorancea, 1969; Yokley, 1972; Kenmuir, 1981b; Zale & Neves, 1982). Reproductive activity in *H. australis* may well be limited by low temperatures since gravid females were absent during the coldest months of the year. Similarly, low water temperatures were found to reduce breeding activity in *Velesunio angasi*, a tropical northern Australian species (Humphrey, 1984). Conversely, spawning in *C. novaehollandiae* appears to be related to falling water temperatures. Spawning in this species took place immediately after a flood which resulted in a sudden drop in water temperature. Sudden temperature changes brought about by the Danube high floods were thought to initiate breeding in *Unio tumidus* (Tudorancea, 1969, 1972) and a similar mechanism may be responsible for the highly synchronous breeding season in *C. novaehollandiae*.

The duration of the brooding periods in *C. novaehollandiae* and *H. australis* are comparable with many temperate unionaceans. Organogenesis is completed in two weeks and glochidia are mature in one month in *Elliptio complanatus* (Matteson, 1948). Development took two months in *Anodonta cygnea* (Wood, 1974; Giusti *et al.*, 1975), one month for *Unio tumidus* (Tudorancea, 1969) and *Margaritifera margaritifera* (Smith, 1976) and seven to eight weeks for four species of fresh-water mussels from the Upper Tennessee River drainage, U.S.A. (Zale & Neves, 1982). Yokley (1972) found that the brooding period in *Pleurobema cordatum* took four to six weeks depending on water temperature. Water temperature also affects the gestation period in *M. margaritifera* (Smith, 1976). The results of the present study suggest that the gestation periods of *H. australis* and *C. novaehollandiae* are similarly influenced by water temperature.

Glochidial release by way of worm-like conglutinates such as occurs in *H. australis* has not previously been reported in hyriid unionaceans, although this mode of release is common to members of several genera of North American unionids (Kat, 1984). The larval conglutinates reported in unionids resemble various vermiform food items of the fish host and thereby enhance the likelihood of host contact (Chamberlain, 1934; Kat, 1984). At this early stage of investigation the appearance and rhythmical waving action of the conglutinates of *H. australis* suggest a similar mimicry of host food items as is displayed by the North American species.

Of the species examined in the present study, only the glochidia of *H. australis* and *A. profuga* have been previously described (McMichael & Hiscock, 1958). However, the descriptions by McMichael & Hiscock for the glochidia of *H. australis* match in size and general outline those for the glochidia of *H. depressa*, described here for the first time. In the present study, identifications of adults

were carefully checked and therefore it appears that McMichael & Hiscock incorrectly assigned a description of *H. depressa* glochidia to *H. australis*.

The glochidia of the Australian hyriids can no longer be viewed as a group which vary only slightly in size and shape (Atkins, 1979; Walker, 1981). *C. novaehollandiae* and *H. australis* produce much smaller glochidia than any other Australian fresh-water mussel. Indeed, in relation to other unionaceans the glochidia of *C. novaehollandiae* are amongst the smallest known, exceeded only by *M. margaritifera* (47 μm diameter) (Roscoe & Redelings, 1964) and *Margaritana* (= *Margaritifera*) *monodonta* (50 × 52 μm) (Lefevre & Curtis, 1912). Moreover, the morphology of the hooks of *H. australis* and *C. novaehollandiae* differ markedly from previous descriptions of glochidia of Australian fresh-water mussels. Typically, the Hyriidae possess a single, curved hook on each valve which may, or may not, have a forked point (Parodiz & Bonetto, 1963). These hooks, however, have been greatly modified in *H. australis* and *C. novaehollandiae*. The glochidia of *H. australis* bear a protruding double-hook on each valve. *H. glenelgis* also produces double-hooked glochidia (K. F. Walker, personal communication). In *C. novaehollandiae* the glochidia possess a pair of short, recurved hooks on each valve. Further, finer details of the hook morphology of *H. depressa* glochidia show clear differences between this species and its congeners.

H. australis, *H. depressa* and *H. drapeta* have almost identical geographical ranges and possess only slight conchological and anatomical differences (McMichael & Hiscock, 1958). The distinctive characteristics of the glochidia of each (namely, size and shape of the shell and structure of the hooks) are strong evidence that these mussels are separate species and not ecophenotypic variants of the one species as hinted at by Walker (1981). On the contrary, the limited evidence presented in this study suggests that the *Hyridella* complex may need to be subdivided even further. The hooks of *H. depressa* and *H. australis* appear to be modifications of a single hook in which a forked point has evolved. In *H. australis* this is more advanced. Even in *C. novaehollandiae* it appears as though the two hooks have evolved from a single hook through to the stage where they are now almost separate.

ACKNOWLEDGEMENTS

Thanks are due Dr. K. F. Walker who provided information on fresh-water mussels of southeastern Australia. We are grateful also for the services of Ms. L. Bridges (illustrations), Mr. R. Hobbs and Ms. L. Keogh (photography).

REFERENCES CITED

ATKINS, L. G., 1979, Observations of the glochidial stage of the fresh-water mussel *Hyridella (Hyridella) drapeta* (Iredale) (Mollusca: Pelecypoda). *Australian Journal of Marine and Freshwater Research*, 30: 411–416.

AUSTRALIAN WATER RESOURCES COUNCIL, 1976 ["1975"], *Review of Australia's water resources*. Department of Natural Resources, Australian Water Resources Council, Canberra: Australian Government Publishing Service, 170 p.

BAUER, G., 1979, Untersuchungen zur Fortpflanzungsbiologie der Flussperlmuschel (*Margaritana margaritifera*) im Fichtelgebirge. *Archiv für Hydrobiologie*, 85: 152–165.

BLOOMER, H. H., 1935, A further note on the sex of *Anodonta cygnea* L. *Proceedings of the Malacological Society of London*, 21: 304–321.

BLOOMER, H. H., 1936, A note on the sex of *Anodonta anatina*. *Proceedings of the Malacological Society of London*, 22: 129–134.

BLOOMER, H. H., 1939, A note on the sex of *Pseudanodonta* Bourguignat and *Anodonta* Lamarck. *Proceedings of the Malacological Society of London*, 23: 285–297.

BLOOMER, H. H., 1946, The seasonal production of spermatozoa and other notes on the biology of *Anodonta cygnea* (L.). *Proceedings of the Malacological Society of London*, 27: 62–68.

CHAMBERLAIN, T. K., 1934, The glochidial conglutinates of the Arkansas Fanshell, *Cyprogenia aberti* (Conrad). *Biological Bulletin*, 66: 55–61.

CLARKE, A. H. & BERG, C. O., 1959, The freshwater mussels of central New York. *Memoirs of the Cornell University Experimental Station*, 367: 1–79.

COE, W. R. & TURNER, H. J., 1938, Development of the gonads and gametes in the soft-shell clam (*Mya arenaria*). *Journal of Morphology*, 62: 91–111.

COKER, R. E., SHIRA, A. F., CLARK, H. W. & HOWARD, A. D., 1921, Natural history and propagation of freshwater mussels. *Bulletin of the United States Bureau of Fisheries*, 37: 77–181.

DARTNALL, H. J. G. & WALKEY, M., 1979, The distribution of glochidia of the swan mussel, *Anodonta cygnea* (Mollusca) on the three-spined

stickleback *Gasterosteus aculeatus* (Pisces). *Journal of Zoology (London)*, 189: 31–37.

DUDGEON, D. & MORTON, B., 1983, The population dynamics and sexual strategy of *Anodonta woodiana* (Bivalvia: Unionacea) in Plover Cove Reservoir, Hong Kong. *Journal of Zoology (London)*, 201: 161–183.

ELLIOTT, J. M., 1977, Some methods for the statistical analysis of samples of benthic invertebrates. *Freshwater Biological Association, Scientific Publications*, No. 25: 1–144.

GHOSH, C. & GHOSE, K. C., 1972, Reproductive system and gonadal activities in *Lamellidens marginalis* (Simpson, 1900). *Veliger*, 14: 283–288.

GIESE, A. C., 1959, Comparative physiology: annual reproductive cycles of marine invertebrates. *Annual Review of Physiology*, 21: 547–576.

GIUSTI, F. L., CASTAGNOLO, L., FARINA, M. & RENZONI, A., 1975, The reproductive cycle and glochidium of *Anodonta cygnea*. L. from Lago Trasimeno (central Italy). *Monitore Zoologico Italiano*, 9: 99–118.

HARMS, W., 1909, Postembryonale Entwicklungsgeschichte der Unioniden. *Zoologische Jahrbücher, Abteilung für Anatomie und Ontogenie der Tiere*, 28: 325–386.

HAUKIOJA, E. & HAKALA, T., 1978, Life history evolution in *Anodonta piscinalis* (Mollusca, Pelecypoda). *Oecologia*, 35: 253–266.

HEARD, W. H., 1975, Sexuality and other aspects of reproduction in *Anodonta* (Pelecypoda: Unionidae). *Malacologia*, 15: 81–103.

HISCOCK, I. D., 1951, A note on the life history of the Australian freshwater mussel, *Hyridella australis* Lam. *Transactions of the Royal Society of South Australia*, 74: 146–148.

HOWARD, A. D., 1951, A river mussel parasitic on a salamander. *Natural History Miscellanea, Chicago*, No. 77: 1–6.

HUMPHREY, C. L., 1984, Biology and ecology of the freshwater mussel *Velesunio angasi* (Bivalvia: Hyriidae) in the Magela Creek, Alligator Rivers Region, Northern Territory. Ph.D. Thesis, University of New England, Armidale, 479 p.

KAT, P. W., 1984, Parasitism and the Unionacea (Bivalvia). *Biological Reviews*, 59: 189–207.

KENMUIR, D. H. S., 1981a, Seasonal breeding activity in freshwater mussels (Lamellibranchiata: Unionacea) in Lake Kariba and Lake McIlwaine. *Transactions of the Zimbabwe Science Association*, 60(4): 18–23.

KENMUIR, D. H. S., 1981b, Repetitive spawning behaviour in two species of freshwater mussels (Lamellibranchiata: Unionacea) In Lake Kariba. *Transactions of the Zimbabwe Science Association*, 60(4): 49–56.

LEFEVRE, G. & CURTIS, W. C., 1910, Reproduction and parasitism in the Unionidae. *Journal of Experimental Zoology*, 9: 79–115.

LEFEVRE, G. & CURTIS, W. C., 1912, Studies on the reproduction and artificial propagation of freshwater mussels. *Bulletin of the United States Bureau of Fisheries*, 30: 105–201.

LOMTE, V. S. & NAGABHUSHANAM, R., 1969, Reproductive cycle in the freshwater mussel *Parreysia corrugata*. *Marathwada University Journal of Science*, 8: 113–118.

LOOSANOFF, V. L. & DAVIS, H. C., 1952, Temperature requirements for maturation of gonads of northern oysters. *Biological Bulletin (Woods Hole, Massachusetts)*, 103: 80–96.

MATTESON, M. R., 1948, Life history of *Elliptio complanatus* (Dillwyn, 1817). *American Midland Naturalist*, 40: 690–723.

McMICHAEL, D. F. & HISCOCK, I. D., 1958, A monograph of the freshwater mussels (Mollusca: Pelecypoda) of the Australian region. *Australian Journal of Marine and Freshwater Research*, 9: 372–508.

MURPHY, G., 1942, Relationship of the freshwater mussel to trout in the Truckee River. *California Fish and Game*, 28: 89–102.

NAGABHUSHANAM, R. & LOHGAONKER, A. L., 1978, Seasonal reproductive cycle in the mussel, *Lamellidens corrianus*. *Hydrobiologia*, 61: 9–14.

NEGUS, C. L., 1966, A quantitative study of growth and production of unionid mussels in the River Thames at Reading. *Journal of Animal Ecology*, 35: 513–532.

ORTMANN, A. E., 1912, Notes upon the families and genera of the naiades. *Annals of the Carnegie Museum*, 8: 222–365.

PARODIZ, J. J. & BONETTO, A. A., 1963, Taxonomy and zoogeographic relationships of the South American naiades (Pelecypoda: Unionacea and Mutelacea). *Malacologia*, 1: 179–213.

PENNAK, R. W., 1953, *Freshwater invertebrates of the United States*. Ronald Press, New York, p. 694–707.

PERCIVAL, E., 1931, A note on the life history of *Diplodon lutulentus* Gould. *Transactions and Proceedings of the New Zealand Institute*, 62: 86–91.

PEREDO, S. & PARADA, E., 1984, Gonadal organization and gametogenesis in the fresh-water mussel *Diplodon chilensis chilensis* (Mollusca: Bivalvia). *Veliger*, 27: 126–133.

PORTER, H. J. & HORN, K. J., 1980, Freshwater mussel glochidia from Lake Waccamaw, Columbus County, North Carolina. *American Malacological Union Bulletin*, 1980: 13–17.

RAND, T. G. & WILES, M., 1982, Species differentiation of the glochidia of *Anodonta cataracta* Say, 1817 and *Anodonta implicata* Say, 1829 (Mollusca: Unionidae) by scanning electron microscopy. *Canadian Journal of Zoology*, 60: 1722–1727.

ROPES, J. W. & STICKNEY, A. P., 1965, Reproductive cycle of *Mya arenaria* in New England. *Biological Bulletin (Woods Hole, Massachusetts)*, 128: 315–327.

ROSCOE, E. J. & REDELINGS, S., 1964, The ecology of the freshwater pearl mussel,

Margaritifera margaritifera (L.). *Sterkiana*, 16: 19–32.

SASTRY, A. N., 1963, Reproduction of the bay scallop. *Aequipecten irradians* Lamarck. Influence of temperature on maturation and spawning. *Biological Bulletin (Woods Hole, Massachusetts)*, 125: 146–153.

SCHALIE, H. van der, 1938, The naiad fauna of the Huron River, in south-eastern Michigan. *University of Michigan Museum of Zoology Miscellaneous Publications*, 40: 1–83, 12 pl., map.

SCHALIE, H. van der & LOCKE, F., 1941, Hermaphroditism in *Anodonta grandis*, a freshwater mussel. *Occasional Papers of the Museum of Zoology, University of Michigan*, 432: 1–7.

SCHALIE, H. van der & SCHALIE, van der, A., 1963, The distribution, ecology and life history of the mussel, *Actinonaias ellipsiformis* (Conrad), in Michigan. *Occasional Papers of the Museum of Zoology, University of Michigan*, 663:1–17.

SESHAIYA, R. V., 1941, Tadpoles as hosts for the glochidia of the freshwater mussel. *Current Science*, 10: 535–536.

SIMPSON, R. D., 1977, The reproduction of some littoral molluscs from Macquarie Island (sub-Antarctic). *Marine Biology*, 44: 125–142.

SMITH, D. G., 1976, Notes on the biology of *Margaritifera margaritifera* (Lin.) in central Massachussetts. *American Midland Naturalist* 96: 252–256.

SMITH, D. G., 1979, Sexual characteristics of *Margaritifera margaritifera* (Linnaeus) populations in central New England. *Veliger*, 21: 381–383.

STEIN, C. B., 1969, Gonad development in the three-ridge naiad *Amblema plicata* (Say, 1817). *Annual Report of the American Malacological Union*, 1969: 30.

SURBER, T., 1912, Identification of the glochidia of freshwater mussels. *United States Bureau of Fisheries Document*, No. 771: 1–10, 3 pl.

TRDAN, R. J., 1981, Reproductive biology of *Lampsilis radiata siliquoidea* (Pelecypoda: Unionidae). *American Midland Naturalist*, 106: 243–248.

TUDORANCEA, C., 1969, Comparison of the populations of *Unio tumidus* Philipsson from the complex of Crapina-Jijila marshes. *Ekologia Polska., A*, 17: 185–204.

TUDORANCEA, C., 1972, Studies on Unionidae populations from the Crapina-Jijila complex of pools (Danube zone) liable to inundation. *Hydrobiologia*, 39: 527–561.

WALKER, K. F., 1981, The ecology of freshwater mussels in the River Murray. *Australian Water Resources Council Technical Paper*, No. 63.

WILES, M., 1975, The glochidia of certain Unionidae (Mollusca) in Nova Scotia and their fish hosts. *Canadian Journal of Zoology*, 53: 33–41.

WOOD, E. M., 1974, Development and morphology of the glochidium larva of *Anodonta cygnea* (Mollusca: Bivalvia). *Journal of Zoology (London)*, 173: 1–13.

YOKLEY, P., 1972, Life history of *Pleurobema cordatum* (Rafinesque, 1820) (Bivalvia: Unionacea). *Malacologia*, 11: 351–364.

ZALE, A. V. & NEVES, R. J., 1982, Reproductive biology of four freshwater mussel species (Mollusca: Unionidae) in Virginia. *Freshwater Invertebrate Biology*, 1: 17–28.

The Division of Mollusks, Department of Invertebrate Zoology, National Museums of Natural History, Smithsonian Institution announces the availability of two fellowships to be awarded to graduate students of systematic malacology:

1. Rosewater Fellow Award (up to $500)
2. Smithsonian—COA Fellow Award (up to $1,000)

These awards are to help support students for short term research visits to the collections and libraries of the Division of Mollusks, National Museum of Natural History and are to be used for systematic studies of Mollusca. Funds can help cover travel, subsistence, and research costs [xerox, postage, etc.]. Interested students should submit a 1-page proposal, a budget with indication of matching funding, if available, and a supporting letter from their faculty advisors. Deadline for applications is March 1, 1986. Awards will be announced on April 1, 1986.

INSTRUCTIONS FOR AUTHORS

1. MALACOLOGIA publishes original research on the Mollusca that is of high quality and of broad international interest. Papers combining synthesis with innovation are particularly desired. While publishing symposia from time to time, MALACOLOGIA encourages submission of single manuscripts on diverse topics. Papers of local geographical or systematic interest should be submitted elsewhere, as should papers whose primary thrust is physiology or biochemistry. Nearly all branches of malacology are represented on the pages of MALACOLOGIA.

2. Manuscripts submitted for publication are received with the tacit understanding that they have not been submitted or published elsewhere in whole or in part.

3. Manuscripts may be in English, French, German or Spanish. Papers in languages other than English must include a translation of the Abstract in English. Authors desiring to have their abstracts published in other languages must provide the translations (complete with main titles). Include all foreign accents. Both American and British spellings are allowed.

4. Unless indicated otherwise below, contributors should follow the recommendations in the *Council of Biology Editors (CBE) Style Manual* (ed. 5, 1983) available for U.S. $24.00 from CBE, 9650 Rockville Pike, Bethesda, MD 20814, U.S.A.

5. Be brief.

6. Manuscripts must be typed on one side of good quality white paper, double-spaced throughout (including the references, tables and figure captions), and with ample margins. Tables and figure captions should be typed on separate pages and put at the end of the manuscript. Make the hierarchy of headings within the text simple and consistent. Avoid internal page references (which have to be added in page proof).

7. Choose a running title (a shortened version of the main title) of fewer than 50 letters and spaces.

8. Provide a concise and informative Abstract summarizing not only contents but results. A separate summary generally is superfluous.

9. Supply between five and eight key (topic) words to go at the end of the Abstract.

10. Use the metric system throughout. Micron should be abbreviated μm.

11. Illustrations are printed either in one column or the full width of a page of the journal, so plan accordingly. The maximum size of a printed figure is 13.5 × 20.0 cm (preferably not as tall as this so that the caption does not have to be on the opposite page).

12. Drawings and lettering must be dark black on white, blue tracing, or blue-lined paper. Lines, stippling, letters and numbers should be thick enough to allow reduction by ½ or ⅓. Letters and numbers should be at least 3 mm high after reduction. Several drawings or photographs may be grouped together to fit a page. Photographs are to be high contrast. High contrast is especially important for histological photographs.

13. All illustrations are to be numbered sequentially as figures (not grouped as plates or as lettered subseries), and are to be arranged as closely as possible to the order in which they are first cited in the text. Each figure must be cited in the text.

14. Scale lines are required for all nondiagrammatic figures, and should be convenient lengths (e.g. "200 μm," not "163 μm"). Magnifications in captions are not acceptable.

15. All illustrations should be mounted, numbered, labeled or lettered, i.e. ready for the printer.

16. A caption should summarize what is shown in an illustration, and should not duplicate information given in the text. Each lettered abbreviation labeling an individual feature in a figure must either be explained in each caption (listed alphabetically), or be grouped in one alphabetic sequence after the Methods section. Use the latter method if

many abbreviations are repeated on different figures.

17. Tables are to be used sparingly, and vertical lines not at all.

18. References cited in the text must be in the Literature Cited section and *vice versa*. Follow a recent issue of MALACOLOGIA for bibliographic style, noting especially that serials are cited unabbreviated. Supply pagination for books. Supply information on plates, etc., only if they are not included in the pagination.

19. In systematic papers, synonymies should not give complete citations but should relate by author, date and page to the Literature Cited section.

20. For systematic papers, all new type-species must be deposited in museums where they may be studied by other scientists. Likewise MALACOLOGIA requires that voucher specimens upon which a paper is based be deposited in a museum where they may eventually be reidentified.

21. Submit each manuscript in triplicate. The second and third copies can be reproductions.

REPRINTS AND PAGE COSTS

22. When 100 or more reprints are ordered, an author receives 25 additional copies free. Reprints must be ordered at the time proof is returned to the Editorial Office. Later orders cannot be considered. For each authors' change in page proof, the cost is U.S. $3.00 or more.

23. When an article is 10 or more printed pages long, MALACOLOGIA requests that an author pay part of the publication costs.

SUBSCRIPTION COSTS

24. For Vol. 27, personal subscriptions are U.S. $17.00 and institutional subscriptions are U.S. $27.00. For information on Vol. 28, address inquiries to the Subscription Office.

VOL. 27, NO. 1 MALACOLOGIA 1986

CONTENTS

AMERICAN MALACOLOGICAL UNION SYMPOSIUM PROCEEDINGS
MOLLUSCAN EXTINCTIONS IN THE GEOLOGIC PAST
AND AT THE PRESENT TIME

Organized by Geerat J. Vermeij
9 August 1983. Seattle, Washington, U.S.A.

VOL. 27, NO. 2 1986

MALACOLOGIA

International Journal of Malacology

Revista Internacional de Malacologia

Journal International de Malacologie

Международный Журнал Малакологии

Internationale Malakologische Zeitschrift

MALACOLOGIA

Editors-in-Chief:

GEORGE M. DAVIS

ROBERT ROBERTSON

Editorial and Subscription Offices:

Department of Malacology
The Academy of Natural Sciences of Philadelphia
Nineteenth Street and the Parkway
Philadelphia, Pennsylvania 19103, U.S.A.

MALACOLOGIA is published by the INSTITUTE OF MALACOLOGY, the Sponsor Members of which (also serving as editors) are:

KENNETH J. BOSS, *President-Elect*
Museum of Comparative Zoology
Cambridge, Massachusetts

JOHN B. BURCH

MELBOURNE R. CARRIKER, *President*
University of Delaware, Lewes

GEORGE M. DAVIS
Secretary and Treasurer

PETER JUNG, *Participating Member*
Naturhistorisches Museum, Basel, Switzerland

OLIVER E. PAGET, *Participating Member*
Naturhistorisches Museum, Wien, Austria

ROBERT ROBERTSON

CLYDE F. E. ROPER
Smithsonian Institution
Washington, D.C.

W. D. RUSSELL-HUNTER, *President-Elect*
Syracuse University, New York

NORMAN F. SOHL
United States Geological Survey
Washington, D.C.

SHI-KUEI WU
University of Colorado Museum, Boulder

J FRANCIS ALLEN, *Emerita*
Environmental Protection Agency
Washington, D.C.

ELMER G. BERRY, *Emeritus*
Germantown, Maryland

1986

MALACOLOGIA, 1986, 27(2): 203–241

NEW CALEDONIAN CHAROPID LAND SNAILS. I. REVISION OF THE GENUS *PARARHYTIDA* (GASTROPODA: CHAROPIDAE)

Peter Mordan[1] & Simon Tillier[2]

ABSTRACT

Six species of the charopid genus *Pararhytida*, three of them previously undescribed, are recognised in a taxonomic revision based on material from 72 sites.

Pararhytida is endemic to New Caledonia, being found in most areas of primary forest on the mainland, as well as the Belep Islands. It appears to be absent from the Loyalty Islands and earlier records from the Isle of Pines are not confirmed. Whereas the largest species, *P. dictyodes*, occurs throughout the mainland, the remaining species are more restricted in distribution.

The occurrence of spermatophores in the Charopidae is recorded for the first time.

Key words: Charopidae; *Pararhytida*; taxonomy; New Caledonia.

INTRODUCTION

The endemic New Caledonian charopid genus *Pararhytida*, previously revised by Franc (1956) and Solem (1961) on a purely conchological basis, is remarkable in several respects:

1. One species reaches 37 mm in shell diameter, exceeding the size of any other known endodontoid. 2. Part of the dorsal surface of the tail is thickened to form a pseudo-operculum, a structure analogous to the operculum of prosobranchs and some lower pulmonates, and known elsewhere only in the related New Caledonian genus *Rhytidopsis* Ancey. 3. Sperm is exchanged in a horny spermatophore, which in some species is strikingly similar in morphology to that of helicarionid snails. Although previously unrecorded in endodontoids, the occurence of a horny spermatophore is common in New Caledonian charopids, but only in *Pararhytida* is it formed of a fusiform body prolonged as a thin, denticulate tail.

Six species of *Pararhytida* are recognised in the present paper, three of them being described as new: *Pararhytida dictyodes* (Pfeiffer), the type species; *P. mouensis* (Crosse); *P. marteli* (Dautzenberg); *P. phacoides* n.sp., *P. pyrosticta* n.sp. and *P. thyrophora* n.sp. One of the four species recognised by both Franc (1956) and Solem (1961), *P. dictyonina* (Euthyme), is synonymised with *P. mouensis*. Species belonging to *Micromphalia* Ancey, 1882, and *Plesiopsis* Ancey, 1888, treated by both Franc and Solem as subgenera of *Pararhytida*, are excluded from the genus. *Tropidotropis gudei* Preston, 1907, considered by Solem (1961) to be a synonym of *T. trichocoma* (Crosse), is a juvenile *Pararhytida mouensis*.

This study is based on material collected at 72 sites in New Caledonia (Table 1; Fig. 1). Except where otherwise stated, specimens are from the Muséum national d'Histoire naturelle, Paris (MNHN), but some are also from the Field Museum of Natural History, Chicago (FMNH). All the relevant type material is housed in either the MNHN or the British Museum (Natural History), London (BMNH) and has been examined.

DISTRIBUTION AND ECOLOGY

Pararhytida is found in almost all the mainland areas of New Caledonia where primary forest remains, as well as the Belep Islands, but is apparently absent from the Loyalty Islands. We do not record *Pararhytida* from the Isle of Pines, although both Crosse (1894) and Franc (1956) mention *P. dictyodes* from there; this may be due to insufficient collecting by us.

[1]Department of Zoology, British Museum (Natural History), London SW7 5BD, England.
[2]Laboratoire de Biologie des Invertébrés marins et Malacologie, Muséum national d'Histoire naturelle, 55 rue Buffon, F-75005 Paris, France.

TABLE 1. List of sampling stations.

5. Le Cresson, 164° 18′ 36″ E; 20° 29° 00″ S. 100 m, dry forest on calcareous outcrop. Rainfall 1200 mm. A. & S. Tillier coll., 30.vi.1979. *P. dictyodes:* 3a + 23s. P. Mordan, A. & S. Tillier coll., 29.i.1981. *P. dictyodes:* 1s + 1 juv. a + 1 juv. s. Probably *idem.* FMNH 159259, L. Price coll. *P. dictyodes:* 17a + 1s.

6. Grottes de Koumac, 164° 20′ 27″ E; 20° 31′ 52″ S. 80 m, dry forest on calcareous outcrop. Rainfall 1200 mm. P. Bouchet coll., 14–15.vi.1978. *P. dictyodes:* 11s + 1 juv. a + 5 juv. or broken. A. & S. Tillier coll. 30.vi.1979. *P. dictyodes:* 3s + 3 juv. s + 2 juv. a. P. Mordan, A. & S. Tillier coll., 29.i.1981. *P. dictyodes:* 6s + 2 juv. s.

7. Mandjelia, 14° 30′ 06″ E; 20° 22′ 29″ S. 400 m, 5 km N of sawmill, rainforest. Rainfall 1900 mm. A & S. Tillier coll., 2.vii.1979. *P. dictyodes:* 2a.

9. Ruisseau de l'Etoile du Nord (Oue Paoulou), 164° 20′ 27″ E; 20° 34′ 48″ S. 150 m, dry forest, probably on a calcareous outcrop. Rainfall 1200 mm. A & S. Tillier coll., 30.vi.1979. *P. dictyodes:* 2s.

12. Mt. Taom, 164° 34′ 45″ E; 20° 46′ 55″ S. 900 m, altitude rainforest in a thalweg, on ultrabasic rock. Rainfall 2500 mm. A. & S. Tillier coll. 30.vi.1979. *P. dictyodes:* 5s + 1 juv. s. + 1 juv a. (SEM).

14. Momies de la Fatenaoué, S side Mt. Tende, 164° 43′ 22″ E; 20° 52′ 36″ S. 100–200 m, dry forest. Rainfall 1250 mm. A. & S. Tillier coll. 4.vii.1979. *P. dictyodes:* 2s.

16. Plateau de Tango, track to Bobeitio, 164° 00′ 27″ E; 20° 58′ 29″ S. 300–350 m, rainforest. Rainfall 1800 mm. P. Bouchet coll. 24.xii.1978. *P. dictyodes:* 1s + 1 juv. s + 2 broken + 1 juv. a.

18. Goipin, 165° 16′ 30″ E; 21° 13′ 19″ S. 50–150 m, rainforest. P. Bouchet coll. 6.v.1979. *P. dictyodes:* 5s.

19. Forêt Plate, 165° 06′ 23″ E; 21° 08′ 57″ S. 540 m, NE slope Mt. Paéoua, rainforest. Rainfall 1841 mm. P. Bouchet, A. & S. Tillier coll. 15.vii.1979. *P. dictyodes:* 1s + 1 juv. s + 1 broken.

20. Mt. Paéoua, 165° 05′ 27″ E; 21° 10′ 48″ S. 950–1000 m, altitude rainforest. Rainfall 3000 mm. A. & S. Tillier coll. 5.vii.1979. *P. dictyodes:* 2a + 3 juv. a + 3 juv. s + 1 broken.

25. Adio, vallée sèche, 165° 14′ 46″ E; 21° 14′ 44″ S. 180 m, dry forest. P. Bouchet coll. 6.v.1979. *P. dictyodes:* 6s + 2 juv. + 1 broken. L. Price coll. 7. xi.1967. FMNH 159309. *P. dictyodes:* 9a + 1 juv. a.

36. Mt. Vulcain, Gallieni mine, 166° 20′ 55″ E; 21° 54′ 33″ S. 700–900 m, maquis. Rainfall 3500 mm. P. Bouchet coll. 5.xii.1978. *P. dictyodes:* 1 juv. s.

37. Mt. Dzumac, 166° 27′ 19″ E; 22° 02′ 30″ S. 950–1000 m, NW of summit, rainforest. Rainfall 3000 mm. P. Bouchet & S. Tillier coll. 4.vi.1979. *P. mouensis:* 1a. *P. dictyodes:* 1 juv. s.

43. Rivière Bleue, 166° 39′ 25″ E; 22° 05′ 47″ S. 160 m, right side of the river, rainforest. Rainfall 2700 mm. P. Bouchet coll. 6.i.1979. *P. mouensis:* 1a. P. Mordan & S. Tillier coll. 29.ii.1981. *P. mouensis:* 3a + 3s.

47. Mt. Guemba, 166° 56′ 10″ E; 22° 10′ 22″ S. 450 m, rainforest. Rainfall 3200 mm. P. Bouchet coll. 10.vi.1978. *P. mouensis:* 1a. *P. marteli:* 1a.

48. Touaourou (St.-Gabriel), 166° 58′ 00″ E; 22° 12′ 00″ S. 10–30 m, rainforest on uplifted coral reef. Rainfall 3000 mm. A. Warén coll. 30.vii.1979 (road to Ni mine, 200 m from main road). *P. marteli:* 1a + 7s + 6s juv. or broken; P. Bouchet coll. 29.v.1978, 8.xii.1978 and 19.vii.1979: *P. marteli:* 4a + 3 a juv. + 9s + 6s juv. or broken.

49. Right side of Kuebeni River, 167° 00′ 07″ E; 22° 16′ 23″ S. 50–80 m, rainforest on ultrabasic rocks. Rainfall 2500 mm. P. Bouchet coll. 12.iv.1979. *P. marteli:* 3a + 7 juv. a + 7s.

50. Goro, 167° 00′ 21″ E; 22° 18′ 57″ S. 30 m, rainforest on ultrabasic rock. Rainfall 1900 mm. P. Bouchet coll. 3.ix.1978. *P. marteli:* 1a.

57. Ile Pott (Belep Islands), S plateau, 163° 16′ 00″ E; 19° 35′ 27″ S. 100–150 m, maquis. Rainfall 1250 mm. P. Bouchet & C. Cherel coll. 27.viii.1978. *P. thyrophora:* 3s.

58. Ile Art (Belep Islands), N plateau, 163° 24′ E; 19° 42′ S. 200–250 m, maquis. Rainfall 1250 mm. P. Bouchet & A. Warén coll. 20.viii.1978. *P. thyrophora:* 13a + 50s + 48 juv. (29a) + 1 broken.

65. Mt. Nindo (near Poum), 164° 10′ 41″ E; 20° 17′ 47″ S. 70 m, gallery forest. Rainfall 1250 mm. P. Bouchet coll. 20.viii.1978. *P. dictyodes:* 3a + 2 juv. a.

66. Col d'Amos, 164° 25′ 20″ E; 20° 18′ 52″ S. 200 m, rainforest. Rainfall 1600 mm. P. Mordan, A. & S. Tillier coll. 31.i.1981. *P. dictyodes:* 17a + 6s + 6s juv. or broken.

69. Mandjelia, 164° 30′ 06″ E; 20° 22′ 29″ S. 550 m, below the sawmill, rainforest. Rainfall 1800 mm. A. & S. Tillier coll. 30.vi.1979. *P. dictyodes:* 1s.

70. Station de Djavel, 164° 23′ 36″ E; 20° 24′ 33″ S. 50 m, secondary dry vegetation. Rainfall 2000 mm. A. & S. Tillier coll. 30.vi.1978. *P. dictyodes:* 4s.

71. Nehoue valley, 164 °16′ 00″ E; 20° 26′ 18″ S. 50 m, dry forest. Rainfall 1300 mm. P. Mordan, A. & S. Tillier coll. 1.ii.1981. *P. dictyodes:* 2a + 11s + 3 juv. (1a).

72. Mt. Ignambi, 164° 36′ E; 20° 27′ S. 850–950 m, rainforest. Rainfall 3000 mm. P. Bouchet coll. 27.xii.1978. *P. pyrosticta:* 1a + 1 juv. a.

74. Colnett, 164° 44′ 24″ E; 20° 29′ 34″ S. 20 m, track to the cascade. Rainfall 4000 mm. P. Mordan & S. Tillier coll. ii.1981. *P. dictyodes:* 1 juv. s.

79. E. slope of Mt. Panié, 164° 48′ 43″ E; 20° 35′ 27″ S. 280 m, rainforest. Rainfall 5100 mm. P. Bouchet & C. Cherel coll. 14.viii.1978. *P. dictyodes:* 1a + 1 juv. a + 1s + 2 juv. s.

80. E. slope of Mt. Panié, 164° 48′ 22″ E; 20° 35′ 54″ S. 580 m, rainforest. Rainfall 5700 mm. P. Bouchet & C. Cherel coll. 14.viii.1978. *P. dictyodes:* 1a + 1 juv. a + 1 juv. s. L. Price coll., 500–700 m, 4. xi.1967. FMNH 159344. *P. dictyodes:* 2a. 900–970 m, rainforest. Rainfall 6400 mm. P. Bouchet & C. Cherel coll. 14.viii.1978. *P. dictyodes:* 2a.

83. Thiem, 165° 06′ 23″ E; 20° 45′ 43″ S. 10–50 m, rainforest. Rainfall 2500 mm. P. Bouchet

coll. 25.xii.1978. *P. dictyodes:* 2a + 12s + 6 juv. a.

84. S. slope of Mt. Tchingou, 165° 00′ 00″ E; 20° 54′ 27″ S. 900–1000 m, rainforest. Rainfall 3000 mm. P. Bouchet, A. & S. Tillier coll. vii.1979. *P. pyrosticta:* 1a + 1s. 1250 m, rainforest. Rainfall 3500 mm. P. Bouchet, A. & S. Tillier coll. vii. 1979. *P. pyrosticta:* 6a + 2s + 6 juv. a + 5 juv. s.

86. N side of Amoa River, 10 km up the valley, 165° 12′ 12″ E; 20° 58′ 00″ S. 20 m, rainforest. Rainfall 2500 mm. P. Bouchet, A. & S. Tillier coll. 12.vii.1978. *P. dictyodes:* 1a. P. Mordan, A. & S. Tillier coll. 18.i.1981. *P. dictyodes:* 1a.

88. S side Mt. Koniambo, 164° 49′ 25″ E; 21° 02′ 00″ S. 600 m, maquis. Rainfall 1500 mm. P. Mordan, A. & S. Tillier coll. 28.i.1981. *P. dictyodes:* 1s.

89. N side of Tchamba River, 5 km up the valley, 165° 19′ 52″ E; 21° 01′ 51″ S. 100 m, forest. Rainfall 2500 mm. P. Mordan, A. & S. Tillier coll. 17.i.1981. *P. dictyodes:* 2s.

91. Mt. Aoupinié, 165° 18′ 00″ E; 21° 10′ 09″ S. 600 m, track above the sawmill, above Goa tribe, rainforest. Rainfall 2700 mm. P. Mordan, A. & S. Tillier coll. 16.i.1981. *P. dictyodes:* 5a + 1 juv. a + 1s + 1 juv. s. *P. pyrosticta:* 1a.

92. Mt. Aoupinié, 165° 15′ 42″ E; 21° 10′ 42″ S. 1000 m, summit area, altitude rainforest. Rainfall 3500 mm. P. Mordan, A. & S. Tillier coll. 16.i.1981. *P. pyrosticta:* 1 juv. a + 2 juv. s.

94. Moneo, 165° 29′ 31″ E; 21° 09′ 36″ S. 10–50 m, rainforest. Rainfall 2500 mm. P. Bouchet coll. 15.v.1978. *P. dictyodes:* 6s + 5 juv. s.

97. Mt. Boulinda, 165° 08′ 57″ E; 21° 14′ 44″ S. 980–1020 m, between Petit and Grand Boulinda, altitude rainforest. Rainfall 3000 mm. A. & S. Tillier coll. 6.vii.1979. *P. dictyodes:* 1s + 1 juv. s. *P. phacoides:* 3a + 1s (broken).

98. Adio caves, 165° 14′ 18″ E; 21° 15′ 36″ S. 180 m, forest on a calcareous outcrop. Rainfall 1400 mm. P. Bouchet & C. Cherel coll. 20.viii.1978. *P. dictyodes:* 1a + 11s + 1 juv. s.

110. S slope of Mt. Table Unio, 165° 45′ 55″ E; 21° 33′ 36″ S. 850–950 m, rainforest. Rainfall 2400 mm. S. Tillier coll. 7.v.1979. *P. dictyodes:* 4a + 1s.

114. Mt. Rembai, 165° 50′ 13″ E; 21° 34′ 54″ S. 800–850 m, N crest, rainforest. Rainfall 2400 mm. S. Tillier coll. 8.vi.1979. *P. dictyodes:* 1a + 2s + 1 juv. a + 1 juv. s.

115. Mt. Canala, 165° 55′ 48″ E; 21° 35′ 00″ S. 900–1050 m, rainforest. Rainfall 2800 mm. P. Bouchet coll. 21.i.1979. *P. dictyodes* 2a.

116. N side Col d'Amieu, 165° 48′ 08″ E; 21° 36′ 00″ S. 400–500 m, W of the Maison Forestière, rainforest. Rainfall 1800 mm. P. Bouchet coll. 18.xi.1978; S. Tillier coll. 7.v.1979. *P. dictyodes:* 2a + 1s + 1 juv. s.

117. Plateau de Dogny, 165° 52′ 33″ E; 21° 36′ 26″ S. 950 m, rainforest. Rainfall 2600 mm. P. Bouchet coll. 1.i.1979. *P. dictyodes:* 1s broken + 1 juv. s.

118. Mt. Nakada, 166° 02′ 26″ E; 21° 38′ 37″ S. 850 m, rainforest. Rainfall 2600 mm. S. Tillier coll. 19.vi.1979. *P. dictyodes:* 6a + 1s + 2 juv. a. *P. phacoides:* 1s + 1 juv. a + 1 juv. s.

119. Mt. Nakada, 166° 03′ 08″ E; 21° 38′ 57″ S. 500 m, above the sawmill, rainforest. Rainfall 2000 mm. S. Tillier coll. vi.1979. *P. dictyodes:* 2a.

123. Mt. Do, 165° 59′ 11″ E; 21° 45′ 30″ S. 950 m, rainforest. Rainfall 2600 mm. P. Bouchet coll. 16.iv.1979. *P. dictyodes:* 1a + 2s + 1 juv. s.

125. Mt. Humboldt, 166° 23′ 29″ E; 21° 53′ 28″ S. 1150 m, crest leading to Mt. Vulcain, rainforest. Rainfall 4500 mm. S. Tillier coll. 22.ii.1981. *P. mouensis:* 1 juv. a.

128. Col de la Ouinné, between Mt. Dzumac and Mt. Ouin, 166° 27′ 54″ E; 22° 01′ 18″ S. 850 m, rainforest. Rainfall 3000 mm. P. Mordan, A. & S. Tillier coll. 25.i.1981. *P. mouensis:* 1a.

130. Mt. Mou, 166° 20′ 34″ E; 22° 03′ 55″ S. 1200 m, altitude rainforest. Rainfall 3400 mm. P. Bouchet & C. Cherel coll. 9.viii.1978. *P. mouensis:* 1 juv. s.

131. Mt. Mou, 166° 19′ 46″ E; 22° 04′ 28″ S. 370–450 m, E of sanatorium, rainforest. Rainfall 1800 mm. P. Bouchet & C. Cherel coll. 5.viii.1978. *P. dictyodes:* 1s + 1 juv. a. P. Mordan, A. & S. Tillier coll. 23.i.1981. *P. dictyodes:* 3s + 2 broken. A. & B. Solem coll. 23.i.1962. FMNH 135440. *P. dictyodes:* 3s.

136. Montagne des Sources, 166° 35′ 56″ E; 22° 07′ 32″ S. 875 m, W slope, rainforest. Rainfall 3500 mm. P. Bouchet, S. Tillier & A. Warén coll. 3.v.1979. *P. mouensis:* 1a + 1s + 2s broken.

140. Mt. Koghi, 166° 30′ 21″ E; 22° 10′ 35″ S. 480–520 m, rainforest. Rainfall 2000 mm. P. Mordan, A. & S. Tillier coll. 10.i.1981. *P. mouensis:* 1a.

142. Col de Mouirange, 166° 39′ 00″ E; 22° 12′ 00″ S. 180–250 m, rainforest. Rainfall 1800 mm. P. Mordan, A. & S. Tillier coll. 11.i.1981. *P. dictyodes:* 1s broken + 1 juv. s. *P. mouensis:* 3a + 3s + 1 juv. a.

143. Col. de Mouirange, 166° 40′ 14″ E; 22° 13′ 19″ S. 200 m, rainforest. Rainfall 1800 mm. S. Tillier coll. 5.vi.1979. *P. dictyodes:* 2s broken.

146. Lac en Y, 166° 55′ 42″ E; 22° 15′ 36″ S. 250 m, maquis. Rainfall 3200 mm. P. Bouchet & S. Tillier coll. 25.vi.1979. *P. dictyodes:* 1 juv. a.

150. Prony, Baie Est, 166° 54′ 11″ E; 22° 22′ 23″ S. 150 m, rainforest. Rainfall 2700 mm. P. Bouchet coll. 10.vi.1978. *P. mouensis:* 1s.

179. Forêt Nord, 166° 53′ 00″ E; 22° 17′ 00″ S. 220–250 m, rainforest. Rainfall 3000 mm. P. Mordan, A. & S. Tillier coll. 24.i.1981. *P. mouensis:* 2a + 3s + 1 broken.

181. Mt. Ningua, 166° 09′ 25″ E; 21° 45′ 17″ S. 950 m, rainforest. Rainfall 2800 mm. S. Tillier coll. vi. 1979. *P. dictyodes:* 1 juv. a + 1s + 1 juv. s. 700 m, rainforest. P. Lespes coll. 10.viii.1979. *P. dictyodes:* 1s (broken).

183. 6 km E of Ouegoa, 140 m. Rainfall 1600 mm. L. Price coll. 1.xi.1967. FMNH 159246 & 159228. *P. dictyodes:* 8a + 2s + 4 juv. a.

184. Bac de la Ouaieme, 0 m, rainforest. Rainfall 3000 mm. P. Bouchet & C. Cherel coll. 12.viii.1978. *P. dictyodes:* 1a + 4s + 2 juv. s.

185. Near Thiem, 100 m, rainforest. Rainfall 2800 mm. L. Price coll. 15.x.1967. FMNH 159219. *P. dictyodes:* 1a.

TABLE 1 (Continued)

185. Near Thiem, 100 m, rainforest. Rainfall 2800 mm. L. Price coll. 15.x.1967. FMNH 159219. *P. dictyodes:* 1a.

186. N side of Tiwaka River, near Ouagap, 100 m, rainforest. Rainfall 3200 mm. L. Price coll. 11.x.1967. FMNH 159247. *P. dictyodes:* 3a + 1 juv. a.

187. S side of Amoa River, 4 km up the valley, 20 m, rainforest. Rainfall 2800 mm. A. Warén coll. 8.viii.1978. *P. dictyodes:* 3s + 2 juv. s.

188. N side of Tiwaka River, 13 km up the valley, 100 m, forest. Rainfall 2500 mm. P. Mordan, A. & S. Tillier coll. 17.i.1981. *P. dictyodes:* 7s + 1 juv. s + 2 broken.

189. E side Col des Roussettes, 400 m, rainforest. Rainfall 1700 mm. L. Price coll. FMNH 159325. *P. dictyodes:* 2a.

190. Col d'Amieu, 530 m, rainforest. Rainfall 1800 mm. L. Price coll. 1967. *P. dictyodes:* 10a + 2 juv. a.

191. Dothio-Nakey, 400 m, rainforest. Rainfall 2000 mm. L. Price coll. 21.x.1967. FMNH 159341. *P. dictyodes:* 2a.

192. Near Mt. Ouénarou, 200 m, rainforest. Rainfall 2300 mm. L. Price coll. 15.xi.1967. FMNH 159239. *P. dictyodes:* 3a. *P. mouensis:* 3a.

193. Forêt Cachée, 250 m, valley of the Creek Pernod, rainforest. Rainfall 2700 mm. McKee coll. 29.vi.1978. *P. dictyodes:* 1s.

194. Faux Bon Secours, 300 m, rainforest. Rainfall 3000 mm. McKee coll. 17.iii.1980. *P. dictyodes:* 1s.

195. 8 km S of Yaté, 30 m, rainforest. Rainfall 3000 mm. L. Price coll. 19.xi.1967. FMNH 159238 *P. marteli:* 10a + 7 juv. a.

Pararhytida was collected from forest with rainfall ranging from 1200 mm to more than 6000 mm a year. It is absent from very dry environments, and also from high-altitude rainforest ("forêts à mousses"). It appears that with the exception of *P. dictyodes*, which is found throughout the entire rainfall range of the genus, each species is restricted to a part of this range.

Living *Pararhytida* is always found at ground level, resting in the leaf litter. Dead and rotting palm sheaths are a particularly favoured resting site; the snails are never found associated with logs, a site occupied by a number of other New Caledonian charopid genera.

ANATOMY AND MORPHOLOGY

Shell

The shell of *Pararhytida* is very large for a charopid, ranging from 13.5 mm to 37 mm in diameter. No other endodontoid is known to reach such a large size (Solem, 1961; 1976; 1983). The shell is rather flat (H/D ranging from 0.44 to 0.65, with a mean of 0.56), and carinated. The umbilicus is open and rather small, from 0.055 to 0.15 (mean 0.088) the shell diameter. The adult shells have from 5.5 whorls in small species, to 6.9 whorls in the largest (*P. dictyodes*).

The aperture of adult shells is slightly expanded, but not deflected as in many endodontoids. As the shell takes on the adult characters (in its last 0.2 of a whorl), the position of maximum apertural height is displaced outwards from the columellar extremity of its basal border to the middle of the latter.

Shell sculpture consists of oblique radial ridges, which are too dense and faint to be accurately counted. The apical whorls of large *P. dictyodes* have only coarse radial ridges, whereas the apical whorls of smaller species additionally show traces of very faint spiral sculpturing (Fig. 2A). In New Caledonian charopids the distinction between groups having radial apical sculpture and those having spiral apical sculpture is much less well defined than was stated by Solem (1961).

The colour pattern consists primarily of reddish-brown flammules radiating outwards from the suture, on a light beige background. The flammules are generally well defined only near the suture; further away they are interrupted by pale zones and spotted by almost white, oval specks. Young shells of *P. mouensis* and *P. pyrosticta* are known to have periostracal hairs along the carina (Fig. 2B) which, as shown by Preston's original description of *Tropidotropis gudei*, resemble those of *Tropidotropis*.

Foot and pseudo-operculum

The foot of *Pararhytida* is aulacopod. Its most striking feature is the presence of a pseudo-operculum on the tail (Solem, Tillier & Mordan, 1984), formed from a dorsal epidermal thickening. It occupies the same position as the prosobranch operculum, and completely occludes the shell aperture when the foot is retracted. It is not horny as in prosobranchs. Surprisingly, neither Fischer (1875) nor Starmühlner (1970), both of whom dissected *P. dictyodes*, mentioned this feature. A similar structure is found in the New Caledonian charopid genus *Rhytidopsis*, but

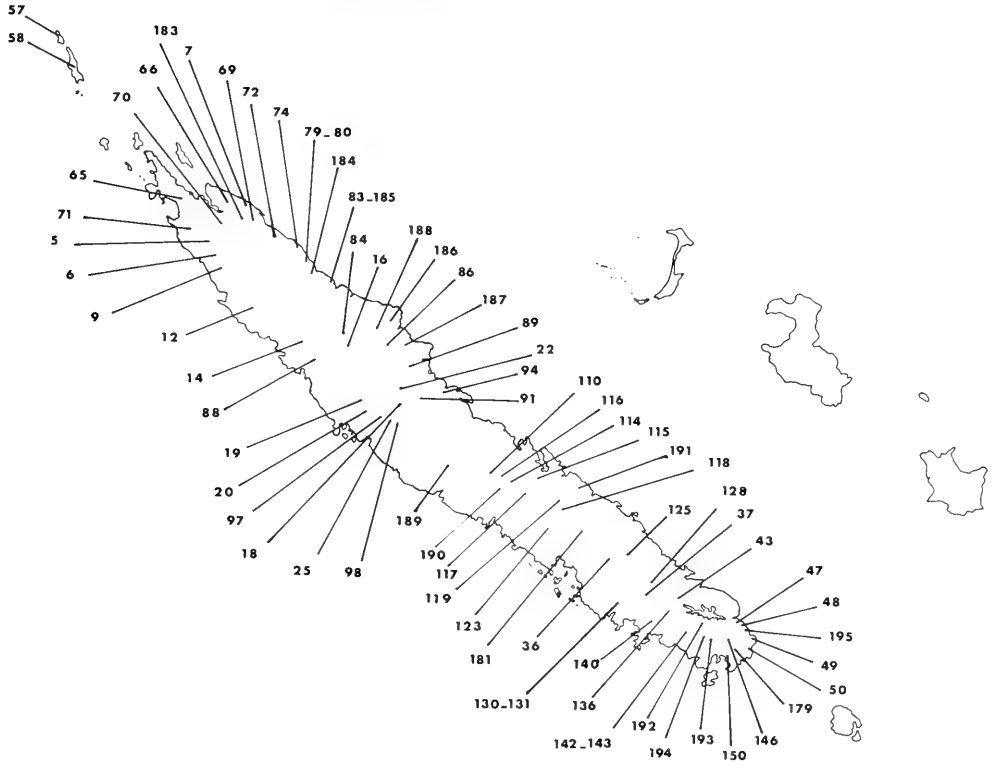


FIG. 1. Map of collecting stations (listed in Table 1).

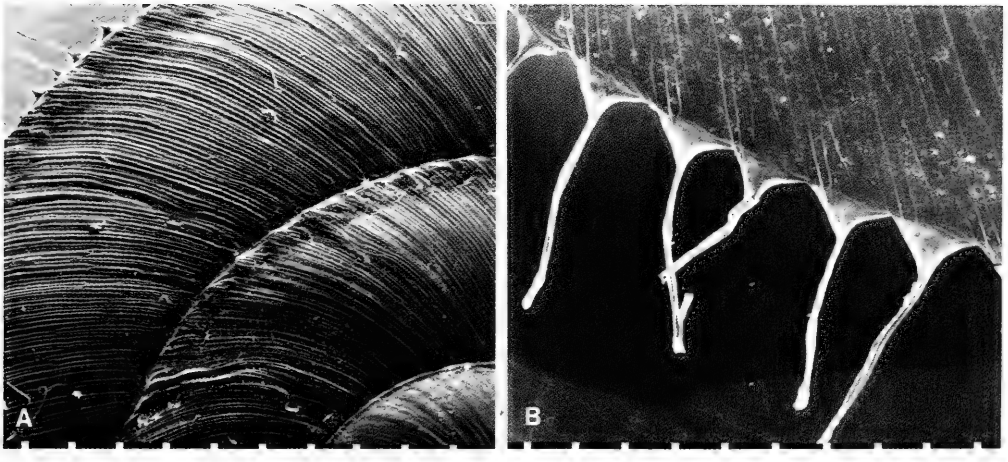


FIG. 2. Shell of juvenile *P. pyrosticta* n. sp., Tchingou, sta. 84. A. Surface sculpture, scale 300 μm. B. Carinal hairs, scale 100 μm.

TABLE 2. Number and size of radular teeth in *Pararhytida*.

Species	No. specs.	Number of teeth			Width of tooth in mm			
			Per side		Central		Lateral	
		Per row	Marg.	Lat.	Mesocone	Whole tooth	Mesocone	Whole tooth
dictyodes	11 (1 juv.)	79–93	23–30	14–19	12.5–22.5	20.5–38.5	15.5–28.0	23–45
mouensis	4	65–75	20–26	11–14	12–16.5	26–32.5	16.5–22	30–39
marteli	1	67	21	12	13	26.5	20.5	30
phacoides	2 (1 juv.)	69	24	10	12.5	26	13	30.5
pyrosticta	2	67–71	22–23	11–12	9.5–11	20–20.5	12–13.5	22.5–23
thyrophora	1	75	22	15	8.5	25	16	30

is known from no other stylommatophoran. The supposed function of the pseudo-operculum in efficiently blocking the shell aperture implies a novel pattern of foot retraction in the Stylommatophora: instead of being proximal to the mantle border inside the shell cavity when retracted, the tail of *Pararhytida* remains distal to the border. Furthermore, its tip has to be retracted before its dorsal side, such that only the pseudo-operculum remains exposed in the fully retracted animal.

Jaw and radula

The jaw is thin, arcuate and smooth. It is not very rigid and is easily dissolved by sodium hypochlorite solution. Its size appears to vary roughly in proportion to that of the animal.

The overall pattern of radular anatomy in *Pararhytida* is relatively uniform, and conforms well to the standard charopid pattern described by Solem (1983: 34). The central tooth is tricuspid, and smaller and narrower than the adjacent laterals. The lateral teeth are also tricuspid, but asymmetrical in that the endocone is often slightly higher than the ectocone, and typically rather narrower. The transition from laterals to marginals is gradual, occuring over two to three teeth, thus making precise counting of the numbers of laterals and marginals impossible. The marginal teeth are tricuspid and have a highly characteristic shape: the mesocone is normally broad and blunt; the endocone is of equal height to, or slightly shorter than the mesocone, and strongly falciform, pointing in towards the mesocone; the ectocone is sharp, conical, and with its tip normally well below the top of the tooth.

The anterior surfaces of the central and lateral teeth bear a characteristic group of shallow pits and grooves (Fig. 14) which presumably form part of the inter-row support mechanism (Solem, 1972), and articulate with the basal plate of the tooth in front. Table 2 lists the ranges of tooth number and size for the material examined. It is clear from the data for *P. dictyodes*, where a relatively large number of specimens have been examined, that there can be great intra-specific variation. Also, the table demonstrates that there is considerable size overlap between the various species. There were no obvious differences seen in the radulae of sympatric species pairs: *P. mouensis* and *P. marteli* from sta. 47 had remarkably similar radulae, in both tooth number and shape, and sympatric populations of *P. dictyodes* and *P. mouensis* (sta. 192) had normal radulae for their species. Indeed, the most extreme forms of *P. dictyodes* were found in allopatric situations. From the limited information available, there would thus appear to be no evidence for any form of character displacement in the radular morphology of *Pararhytida*.

Visceral mass

In *Pararhytida* the total length of the visceral mass varies from 3.5 to 5.5 whorls, but intraspecific variation never exceeds one whorl. Its length is not directly proportional to the length of the coiled shell since the species with the most whorls (*P. dictyodes*) has the shortest visceral mass (Table 3).

The length of the lung varies from 0.5 to 1.2

TABLE 3. Length in whorls of various parts of the visceral mass.

Sta.	Visc. mass	Lung	Top of stomach	Stomach	Upper spire
Pararhytida dictyodes					
7	3.6	0.75			
20	4	0.66	1		2.33
25	3.3	0.75	1		1.55
25	3.35	0.75	1.1	0.9	1.5
79	4.5	0.75	1.3	1(0.25)	2.4
80	5.25	0.8	1.25	1.1(0.25)	3.2
83	4	0.75	1		2.25
86	3.25	0.6	0.75	0.66	1.9
91	3.25	0.55	1.2	1	1.5
110	4.4	0.75	1.15	1	2.5
115	3.75	0.75	1.2	1(0.25)	1.9
118	4.25	0.6	1.15	1?(0.25)	2.5
123	4.2	0.7	1.2	1?(0.25)	2.3
184	3.6	0.75	1.3		1.55
185	3.5	0.75	1.3		1.45
186	3.45	0.8	0.9		1.75
189	3.75	0.5	1		2.25
190	3.75	0.75	1	0.75	2
191	?	0.75	1		?
192	4	0.8	1	?(0.3)	2.2
Pararhytida mouensis					
43		1.2	1.2		
47	5.2	0.95	1.25		3
128	4.5	0.95	1.1		2.55
136	4.3	1.15	1.1		2.05
141		1.1			
179	4.2	0.9	1.3		2
192	4	1	12		1.8
Pararhytida marteli					
47	5.4?	1.05	0.9		
48	5.6	1.3	1		3.3
48		1.2	1.1		
195	5.5?	0.9	1.1		3.5?
Pararhytida phacoides					
97	4.2	0.9	1.2		2.1
118		0.6(juv.)			
Pararhytida pyrosticta					
72	4.75	0.65	1.1	1(0.2)	3
84	4.9?	0.85	1.15		2.9
84	5.1?	0.75	1.15	1	3.2
91	4?	0.75	1.25		2?
Pararhytida thyrophora					
58	5.4?	0.6	1.4	1.3(0.2)	3.4?

whorls above the pallial border. Within a species, maximum variation is 0.4 whorls. The stomach and crop, which generally occupy an entire whorl, have their distal extremity normally between 0.9 and 1.2 whorls above the lung, although exceptionally it may lie further down (0.75 whorls in some *P. dictyodes*), or up (1.4 whorls in *P. thyrophora*). The upper part of the visceral mass, which includes the digestive gland and hermaphrodite gland, varies in length between 1.5 and 3.5 whorls, and almost as much variation may be found within a single species (Table 3).

Internally the digestive tract shows only faint oesophageal ridges, and no crop ridges or typhlosole.

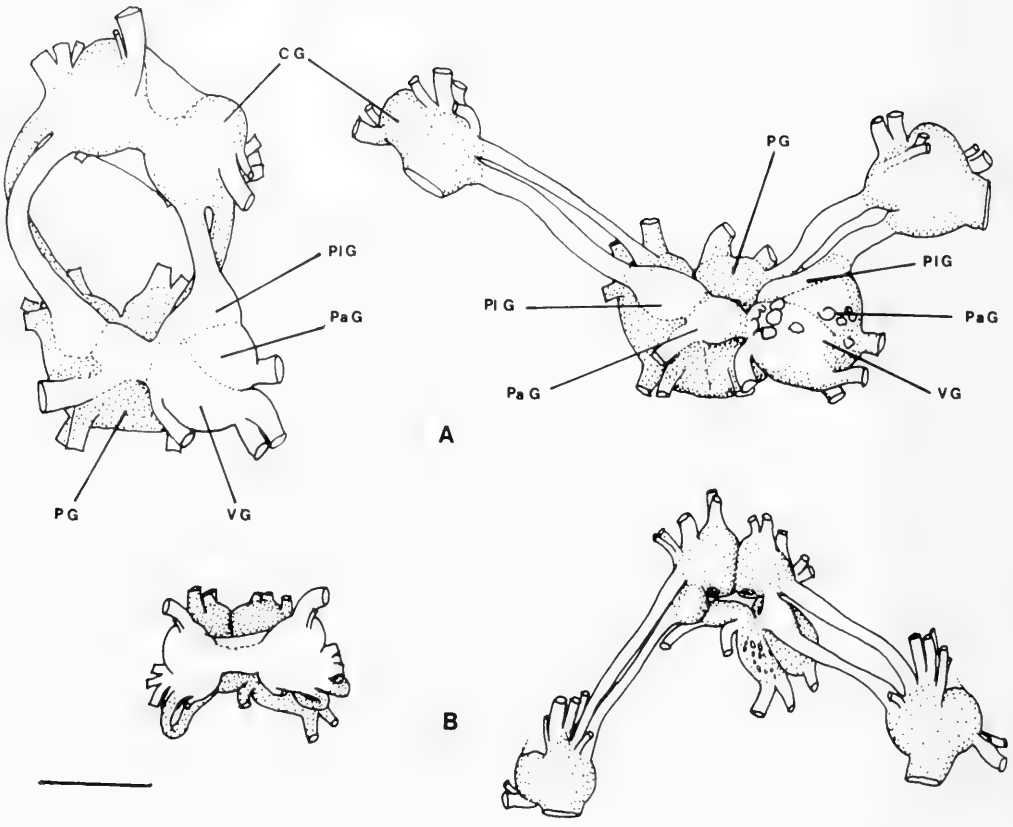


FIG. 3. Central nervous system of *Pararhytida*. A. *P. dictyodes*, Col d'Amos, sta. 66; B. *P. marteli*, S of Yaté, sta. 195. Scale line both 1 mm. CG, cerebral ganglion; PaG, parietal ganglion; PG, pedal ganglion; PIG, pleural ganglion; VG, visceral ganglion.

Central nervous system

The central nervous system has been described by Fischer (1875) and Starmühlner (1970), but with so little accuracy that no useful comparisons can be made. In particular, Fischer's figure misled Bargmann (1930) to erroneous conclusions concerning the pattern of compaction of the visceral chain: the visceral ganglion is not, in fact, fused with the left parietal ganglion. Within *Pararhytida*, the arrangement of the central nervous system seems to relate primarily to size:
1. In the four smallest species the cerebral commissure and the lateral connectives are relatively long (Fig. 3B). 2. In the large *P. dictyodes*, the cerebral commissure is short, and the lateral connectives shorter, particularly on the right side (Fig. 3A); the left parietal ganglion is attached to the left pleural ganglion, and the visceral, right parietal, and right pleural ganglia form a single mass in which individual ganglia are barely distinguishable. 3. In species of intermediate size (*P. mouensis*), the arrangement of the central nervous system is also intermediate.

In all species the ganglia of the visceral chain are adpressed, and displaced to the right to such an extent in small species that the left parietal ganglion is close to the median plane; in the large *P. dictyodes* it actually lies in the median plane. Additionally, in small species, the right parietal ganglion has moved to a position underneath the right pleural (Fig. 3A).

Pulmonary complex

The kidney is U-shaped (Fig. 4), and varies from almost one-half to a little less than

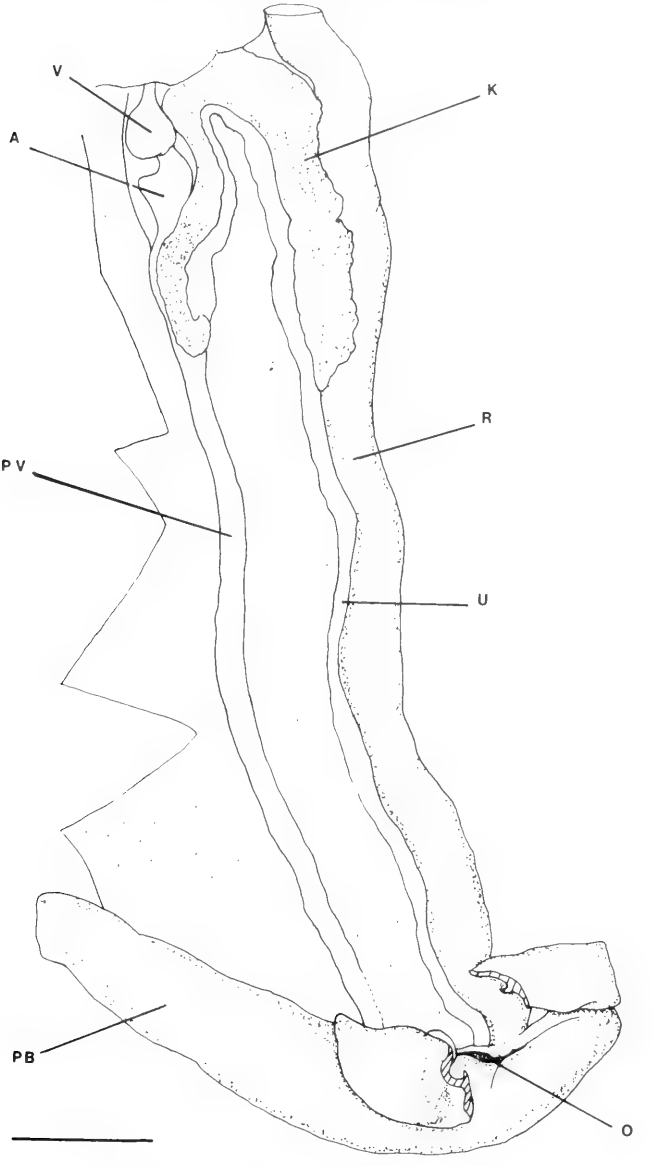


FIG. 4. Pallial complex of *Pararhytida dictyodes*, Le Cresson, sta. 5. Scale line 5 mm. A, auricle; K, kidney; O, anus and ureter opening; PB, pallial border; PV, pulmonary vein; R, rectum; U, ureter; V, ventricle.

one-third the length of the lung. The two arms of the kidney are subequal in *P. phacoides* and *P. pyrosticta*; the rectal arm is slightly longer than the cardiac arm in *P. thyrophora* and *P. dictyodes*, and is about 1.5 times the length of the cardiac arm in *P. mouensis* and *P. marteli.*

Only the principal pulmonary vein can be clearly distinguished on the lung roof, which does not have any other obvious venation. The rectum and ureter open contiguously on the dorsal side of the pneumostome, and the opening is protected ventrally by a small lappet of the mantle border.

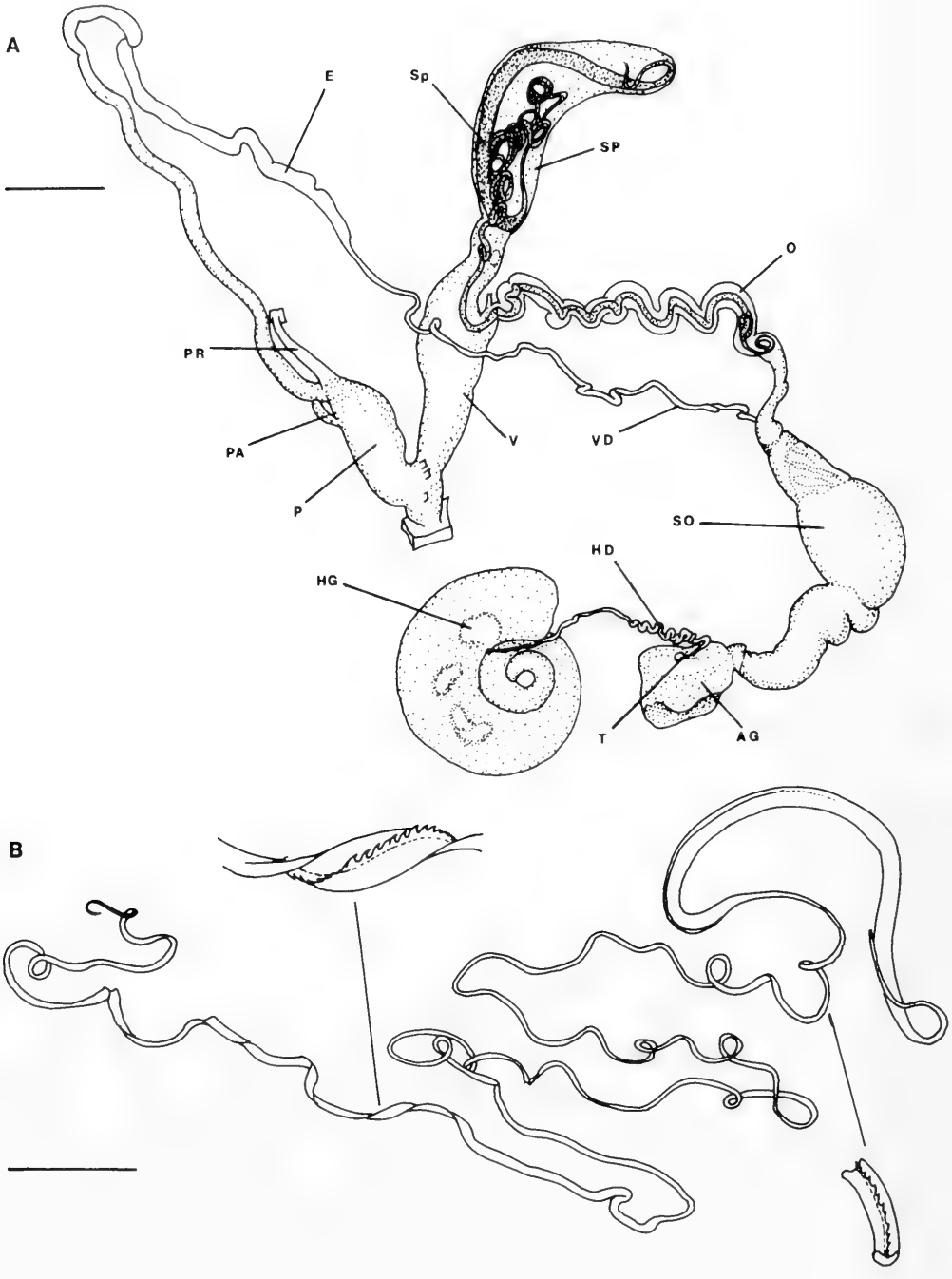


FIG. 5. A, genital apparatus of *Pararhytida dictyodes*, showing position of spermatophore, Mt. Table Unio, sta. 110. B, spermatophore. Scale lines both 5 mm. AG, albumen gland; E, epiphallus; HD, hermaphrodite duct; HG, hermaphrodite gland; O, oviduct; P, penis; PA, penial appendix; PR, penial retractor; SO, spermoviduct; SP, spermatheca; Sp, spermatophore; T, talon; V, vagina; VD, vas deferens.

Genital apparatus

Hermaphrodite and female portion (Fig. 5A)

The hermaphrodite gland is composed of between three and seven lobes, and their number can vary considerably within a species (from three to six in *P. dictyodes*, and from four to seven in *P. mouensis*). The two extremities of the hermaphrodite duct are thin and almost straight, but the median portion is thickened and convoluted, forming a seminal vesicle (*sensu* Bayne, 1973). Its distal end opens into the stalk of a spherical talon, whose intraspecific variation in size may be as great as that within the entire genus (½ or slightly more).

The size of the albumen gland is also variable, apparently depending primarily on the degree of maturity. When fully developed, it occupies most of the space between the top of the kidney and the crop, as in most charopids (Solem, 1983). The length of the free oviduct varies from one-half to about twice the spermoviduct length. It is usually contorted, and maintained in this state by connective tissue attached to a branch of the retractor muscles, which inserts in the angle between the oviduct and spermathecal stalk. Its wall is thick and internally bears coarse, longitudinal ribs.

The spermatheca is always short, its top never reaching as far as the carrefour region. It is adpressed to the columellar side of the spermoviduct. The head varies from pear-shaped to elongate, and shape appears fairly constant within species. The spermathecal stalk is thick-walled, with longitudinal internal ribbing. Generally it contains a curious structure resembling a small, hard, hemispherical knob, housed in a horseshoe-shaped ridge (e.g. Fig. 19D). The position of this structure varies, even within species, from just above the oviducal opening to the top of the spermathecal stalk. Its function is unknown.

The vagina is long, and in all but one species contains a large, transverse ridge. *P. mouensis* has only a vaginal constriction which, from its position, may well be homologous with the ridge (Fig. 28B). This species additionally possesses one or two vaginal pouches or appendices close to the constriction. In all species the atrium is very short.

Penial complex (Fig. 5A)

This comprises a short, fusiform penis with a subapical (possibly glandular) appendix, and a long, thin epiphallus. The penis retractor muscle is inserted at the penis/epiphallus junction, and originates about one-fifth the way up the inner lung wall.

The internal ornamentation of the penis (Fig. 19) consists basically of two main longitudinal pilasters: one interrupted by the opening of the penial appendix, and often bifurcated at its upper part; the second situated on the opposite side, and extending further up the penis. The upper part of the second pilaster may be prominent, forming a kind of verge, and may also be prolonged as a transverse ridge running between the opening of the epiphallus and the opening of the penial appendix. The space between these two pilasters is often occupied by a number of less-prominent secondary pilasters. This pattern may be altered in various ways: all pilasters may be equally developed, or so reduced that only an apical verge may be distinguished within the penis. In some cases the epiphallic pore is prominent, or surrounded by a circular ridge, reminiscent of the ring pilaster of typical charopids (Solem, 1983). A few millimeters above the pore, the wall of the epiphallus bears numerous ridges; further up only three ridges are normally distinguishable, two of which are larger than the third. The junction of the epiphallus and vas deferens does not show any particular morphological differentiation, being marked only by the termination of the epiphallic ridges and by a diminution in the diameter of the duct.

Spermatophore (Fig. 5B)

Although previously undescribed in charopids, the occurrence of a horny spermatophore appears to be the rule rather than the exception in New Caledonian members of this family (Tillier, unpublished). In *Pararhytida* the spermatophore is always elongate-fusiform in shape and bears a longitudinal ridge. Complete spermatophores have only been recorded from within the spermatheca, when the spermatophore pore has been directed towards the oviduct. In some species it is prolonged by a long tail which runs down the spermathecal stalk and then up the free oviduct, reaching as far as the spermoviduct

(Figs. 5A, 27D, 37A). When such a tail is developed, the spermatophore ridge extends to its extremity, where it becomes finely denticulated. The function of this tail is most probably to transport the sperm from the spermathecal head to the spermoviduct.

TAXONOMIC CRITERIA

This section considers the rationale upon which the taxonomic decisions taken in this paper were made. The basic criterion used to decide whether two forms belong to different species was the absence of intermediate forms, which we suppose reflects the absence of gene flow. Two situations may arise: 1. The two forms are sympatric. In such a situation specific distinction has been easy as there is normally a large morphological gap between taxa. Such situations have allowed us to gauge the degree of interspecific character difference expected within the group. 2. The two forms are allopatric. If the morphological gap is smaller than that between sympatric (more strictly syntopic) species, then we have considered the forms conspecific. When the gap is larger, as has happened frequently, one can either separate the two forms as distinct species, or integrate them into a coherent pattern of intraspecific geographic variation.

An example will illustrate this last case. There are greater character differences between some northern and southern *P. dictyodes* than between sympatric *P. dictyodes* and *P. phacoides.* However, we have considered the former to be conspecific because in this case not only does each character vary along a geographical cline, but also the morphoclines themselves vary independently, and we have interpreted this as evidence of continuous gene flow. The alternative situation would be one in which there is congruence between morphoclinal discontinuities, suggesting areas where gene flow is absent or severely restricted.

Where any major doubt has remained, our decision has been to lump rather than split. Particularly in the cases of *P. dictyodes* and *P. mouensis*, however, we cannot exclude the possibility that our taxonomy has been too conservative; had there been less material of either species more taxa would probably have been recognised.

The result is that in *Pararhytida* no single character seems to allow species recognition throughout the genus: species are defined in terms of character combinations, in relation to geographical position. In all cases intrapopulation variation is much lower than that between allopatric populations; that is, variation is mainly geographic.

Multivariate analysis of clinal variation in both conchological and anatomical characters of *Pararhytida* will form the subject of a further paper.

SYSTEMATIC REVIEW

Genus *Pararhytida* Ancey, 1882

Type species: *Helix dictyodes* Pfeiffer, 1847 (by subsequent designation of Pilsbry, 1894: 52).

Diagnosis

Pararhytida differs from all known charopid genera, other than *Rhytidopsis*, by its pseudo-operculum and by the presence of a transverse ridge, sometimes developed into a foliated appendage, within the vagina. It differs from *Rhytidopsis* by: 1. Its mode of life in the leaf litter (*Rhytidopsis* is arboreal); 2. The simplicity of the transverse vaginal ridge, which is considerably more complex in *Rhytidopsis;* 3. The shape of the penis and the long, thin epiphallus; and 4. Its large, flattened carinated shell, and weak shell sculpture. (The anatomy and biology of *Rhytidopsis* will be the subject of a further paper.)

Micromphalia Ancey, 1882 and *Plesiopsis* Ancey, 1888 were considered by both Franc (1956) and Solem (1961) to be subgenera of *Pararhytida.* As they possess neither a pseudo-operculum nor any kind of peculiar vaginal structure, they are here removed from *Pararhytida.*

Pararhytida dictyodes (Pfeiffer, 1847) (Figs. 4–20)

Helix dictyodes Pfeiffer, 1847: 111 (New Guinea). Gassies, 1863: 241, pl. 1, fig. 19; Fischer, 1875: 273.

Helix dictyoides [sic] Pfeiffer. Reeve, 1852: pl. 80, species 423.

Trochomorpha dictyodes (Pfeiffer). Crosse, 1894: 241, pl. 8, fig. 3.

Pararhytida dictyodes (Pfeiffer). Dautzenberg, 1923: 140.
Pararhytida (Pararhytida) dictyodes (Pfeiffer). Franc, 1956: 136; Solem, 1961: 467; Starmühlner, 1970: 302, figs. 14-18.

Lectotype (here designated): New Guinea (in error), Lieutenant Ince, Cuming Collection. BMNH Reg. no. 1981262 (Fig. 6). Dimensions (mm): Shell height 16.1. Shell diameter 27.1. Aperture height 10.6. Aperture diameter 13.9. Umbilicus width 2.8. Whorls 6.25.

Paralectotypes: 2 specimens from above lot. BMNH Reg. no. 1981263. Dimensions (mm): Shell heights 14.5, 15.2. Shell diameters 26.2, 26.8. Aperture heights 10.0, 10.5. Aperture diameters 13.3, 13.8. Umbilicus widths 2.4, 2.5. Whorls 6.4, 6.1.

Other material: Sta. 7(2), sta. 9(2), sta. 12(7), sta. 14(2), sta. 16(5), sta. 18(5), sta. 19(3), sta. 20(9), sta. 25(19), sta. 36(1), sta. 37(1), sta. 65(5), sta. 66(29), sta. 69(1), sta. 70(4), sta. 71(16), sta. 74(1), sta. 79(5), sta. 80(7), sta. 83(20), sta. 86(2), sta. 88(1), sta. 89(2), 91(8), sta. 94(11), sta. 97(2), sta. 98(13), sta. 110(5), sta. 114(5), sta. 115(2), sta. 116(4), sta. 117(2), sta. 118(9), sta. 119(2), sta. 123(4), sta. 131(10), sta. 142(2), sta. 143(2), sta. 146(1), sta. 181(4), sta. 183(14), sta. 184(7), sta. 185(1), sta. 186(4), sta. 187(5), sta. 188(10), sta. 189(2), sta. 190(12), sta. 191(2), sta. 192(3), 193(1), sta. 194(1).

Preserved material: 5, 6, 7, 12, 16, 20, 25, 65, 66, 71, 79, 80, 83, 86, 91, 98, 110, 114, 115, 116, 118, 119, 123, 131, 146, 181, 183, 184, 185, 186, 189, 190, 191, 192.

Distribution

P. dictyodes occurs principally on the mainland; it is absent from the Belep Islands, and its presence on the Isle of Pines (Crosse, 1894) has not been confirmed by recent collections. It appears to be absent from the coastal areas along the W coast, and also from the extreme SE. It is, however, the most widely distributed species of *Pararhytida*, tolerating the greatest range of altitude and rainfall (Table 1).

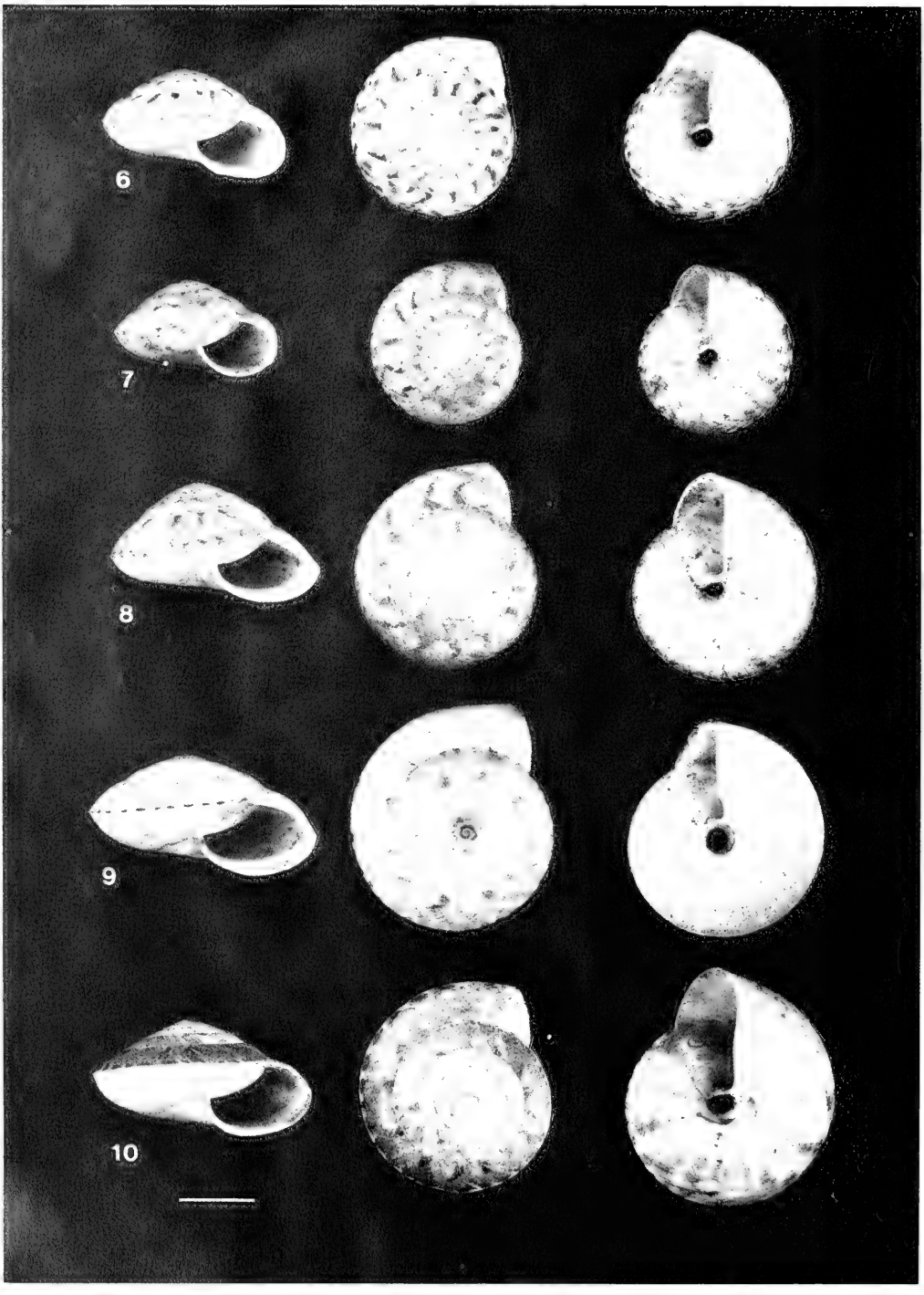
Shell

The shell of *P. dictyodes* is generally larger than in other species, ranging from 21 × 11.1 mm to 36.8 × 21.2 mm. There are from 5.6 to 6.9 whorls (mean 6.24, s.d. 0.19) in adult shells. Its dimensions overlap only with those of *P. mouensis* from the extreme SE (Mt. Guemba, sta. 47), where *P. dictyodes* does not occur. The only shell character allowing constant specific recognition, even in juveniles, is the relative flatness of the shell apex.

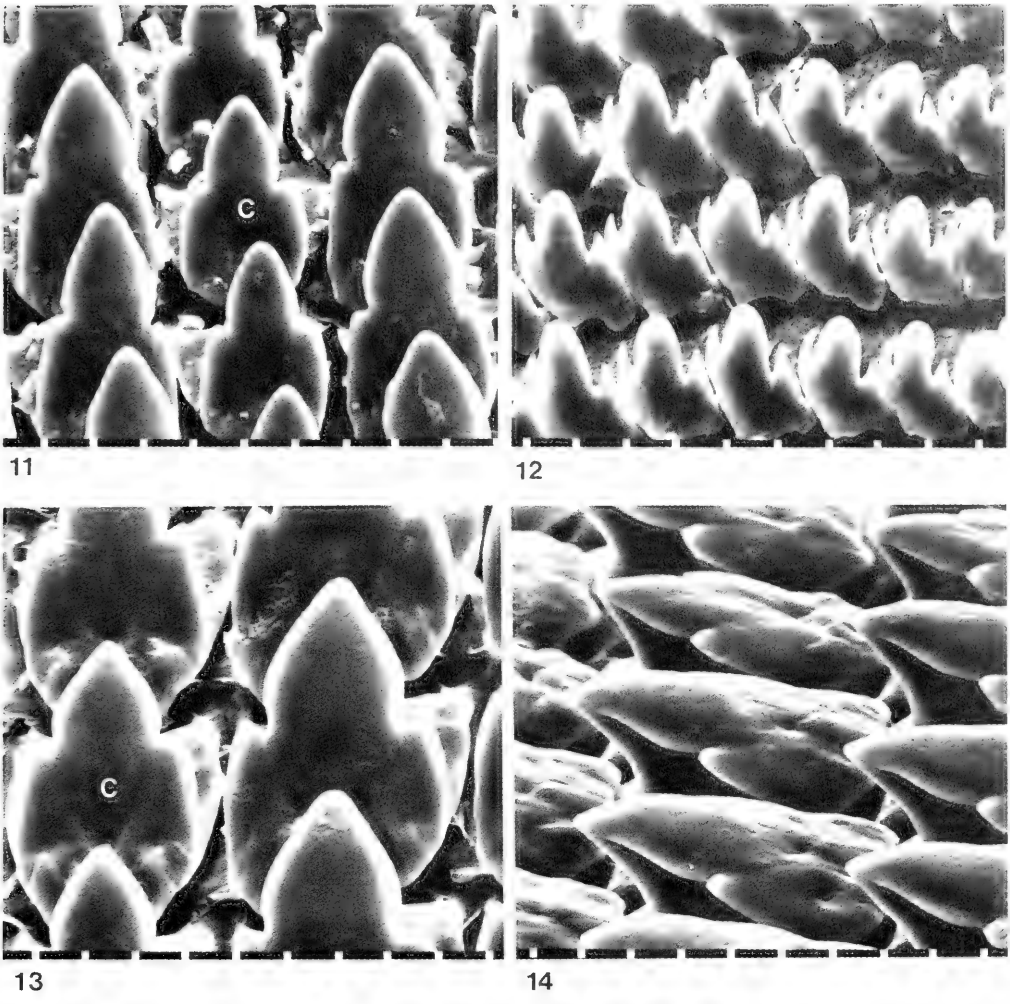
Geographic variation in shell dimensions is considerable and will form the subject of a further paper. The shell is relatively small in the extreme N (Fig. 7), and in some localities on the NW side of the mainland (Mt. Koniambo, sta. 88; Plateau de Tango, sta. 16). Its size increases southeastwards, and approaching the isolated massifs along the northwestern coast (eastern coast: stas. 83 (Fig. 8), 86, 89, 185, 186, 187, 188; northwestern massifs: 12, 20, 97). The maximum size is reached SE of the Houailou valley (around stas. 110, 114 (Fig. 9), 189). In the SE plains (stas. 142, 192, 193, 194 (Fig. 10)) size again decreases slightly.

Radula (Figs. 11–14)

One juvenile and eleven adult radulae, from a wide geographical range of sites, were examined with a stereoscan electron microscope. The species shows considerable variation in tooth size, overlapping at the lower end of the range with the five other species (Table 2). The number of teeth per row, however, always exceeded that of other *Pararhytida* species. Overall tooth shape varies particularly in respect of the central tooth, which appeared to be especially narrow at stations on the NE coast (stas. 66, 80, 184); Fig. 11 shows the normal central and lateral dentition of *P. dictyodes*. There was little difference between the central and lateral teeth of immature and adult individuals at sta. 91, although the marginals of the former were markedly narrower (Fig. 12). A radula with quite exceptionally large teeth (Figs. 13, 14) was recorded from Mt. Paeoua, but other similarly isolated mountain sites nearby (stas. 91, 189) did not show any tendency towards size increase. Specimens of *P. dictyodes* occurring sympatrically with *P. pyrosticta* (sta. 91), *P. phacoides* (sta. 118), and *P. mouensis* (sta. 192) all appear to have quite normally sized teeth, and in the last case (sta. 192) the two co-occurring species have radular teeth of almost identical size.



FIGS. 6–10. 6. *Helix dictyodes* Pfeiffer, lectotype, BMNH 1981262. New Guinea [*sic*], leg. Lieutenant Ince, Cuming Collection. 7. *P. dictyodes*, Grottes de Koumac, sta. 6. 8. *P. dictyodes*, Thiem, sta. 83. 9. *P. dictyodes*, Rembai, sta. 114. 10. *P. dictyodes*, Faux Bon Secours, sta. 194. Scale line all 10 mm.



FIGS. 11–14. 11. Central and lateral teeth, *P. dictyodes*, Tiwaka, sta. 186. 12. Marginal teeth, *P. dictyodes*, Aoupinié, sta. 91. 13. Central and lateral teeth, *P. dictyodes*, Mt. Paéoua, sta. 20. 14. Side view of lateral teeth, *P. dictyodes*, Mt. Paéoua, sta. 20. Scale divisions all 10 μm.

Pulmonary complex

The length of the lung (Fig. 4) is normally about 0.75 whorls. It never exceeds 0.8 whorls, and is shorter (0.5–0.6 whorls) in the wet stations between the valleys of the Amoa and Houailou rivers (stas. 86, 91, 189). The rectal arm of the kidney is slightly longer than the cardiac arm, and its length is ca. ⅓ the lung length.

Genital apparatus (Figs. 5, 15–20)

As with the shell and other organ systems, the genital apparatus of *P. dictyodes* shows considerable geographic variation. However, each part of the genital system shows independent clinal variation.

Hermaphrodite gland (Figs. 5, 15–18): In the northern part of the mainland (stas. 5, 7, 65, 66, 71, 79, 80, 183, 184; Figs. 15A, B, C)

FIG. 15. Genital apparatus of *Pararhytida dictyodes*. A, sta. 5; B. sta. 184; C. sta. 80; D. sta. 83. Scale line all 5 mm. AG, albumen gland; E, epiphallus; HD, hermaphrodite duct; HG, hermaphrodite gland; O, oviduct; P, penis; PA, penial appendix; PR, penial retractor; SO, spermoviduct; SP, spermatheca; T, talon; V, vagina; VD, vas deferens.

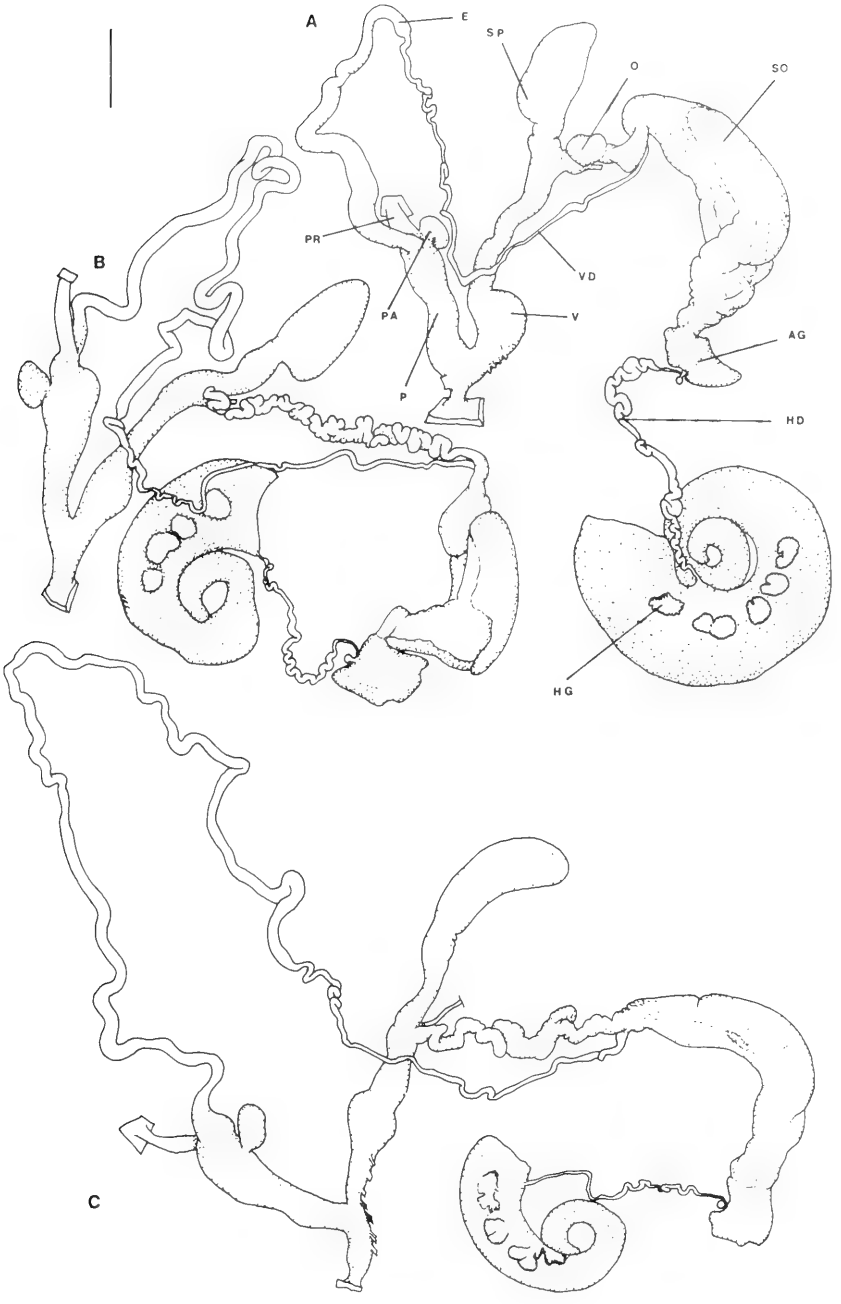


FIG. 16. Genital apparatus of *Pararhytida dictyodes*. A, sta. 20; B. sta. 91; C. sta. 25. Scale line all 5 mm. AG, albumen gland; E, epiphallus; HD, hermaphrodite duct; HG, hermaphrodite gland; O, oviduct; P, penis; PA, penial appendix; PR, penial retractor; SO, spermoviduct; SP, spermatheca; V, vagina; VD, vas deferens.

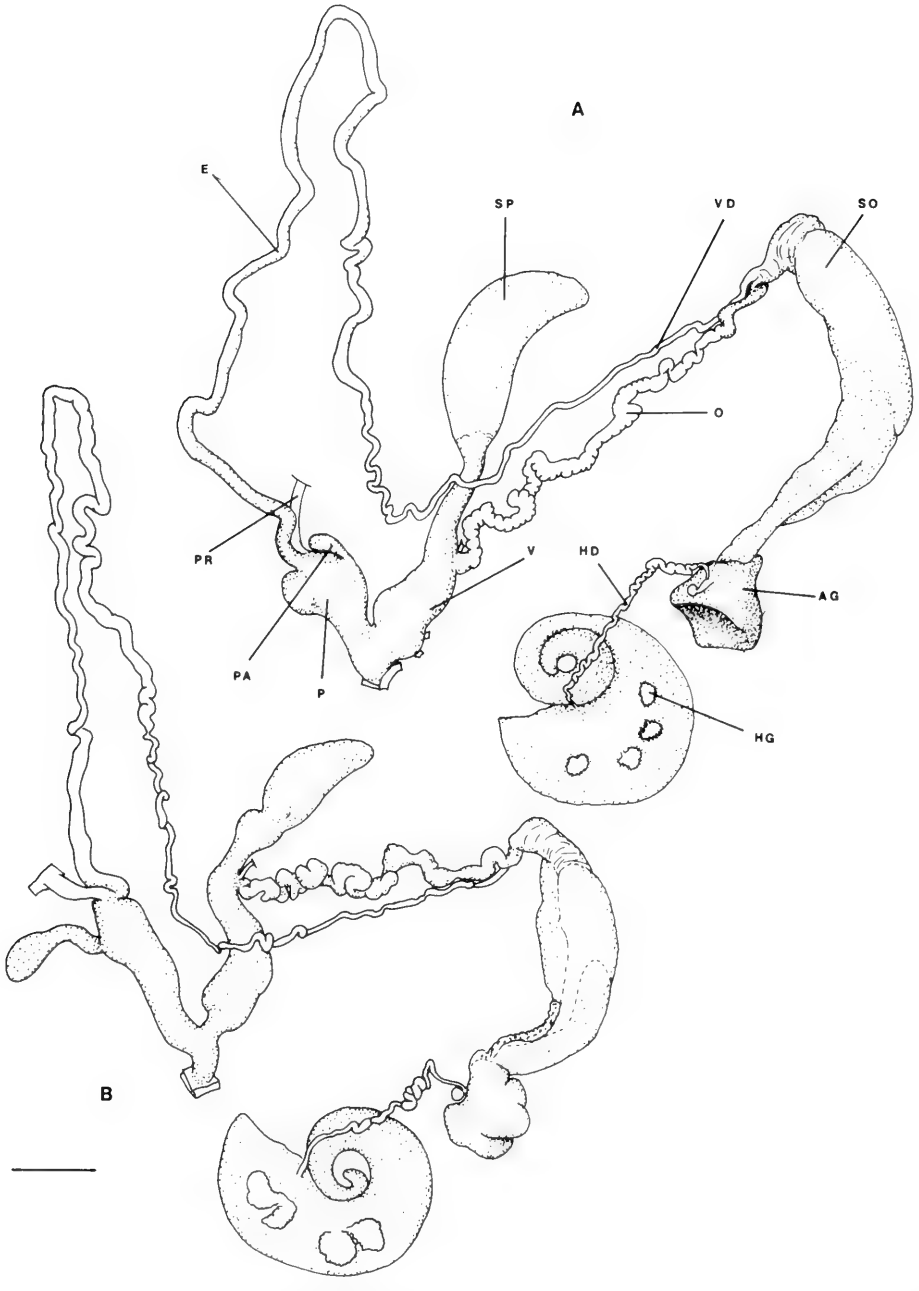


FIG. 17. Genital apparatus of *Pararhytida dictyodes*. A, sta. 115; B. sta. 189. Scale line both 5 mm. AG, albumen gland; E, epiphallus; HD, hermaphrodite duct; HG, hermaphrodite gland; O, oviduct; P, penis; PA, penial appendix; PR, penial retractor; SO, spermoviduct; SP, spermatheca; V, vagina; VD, vas deferens.

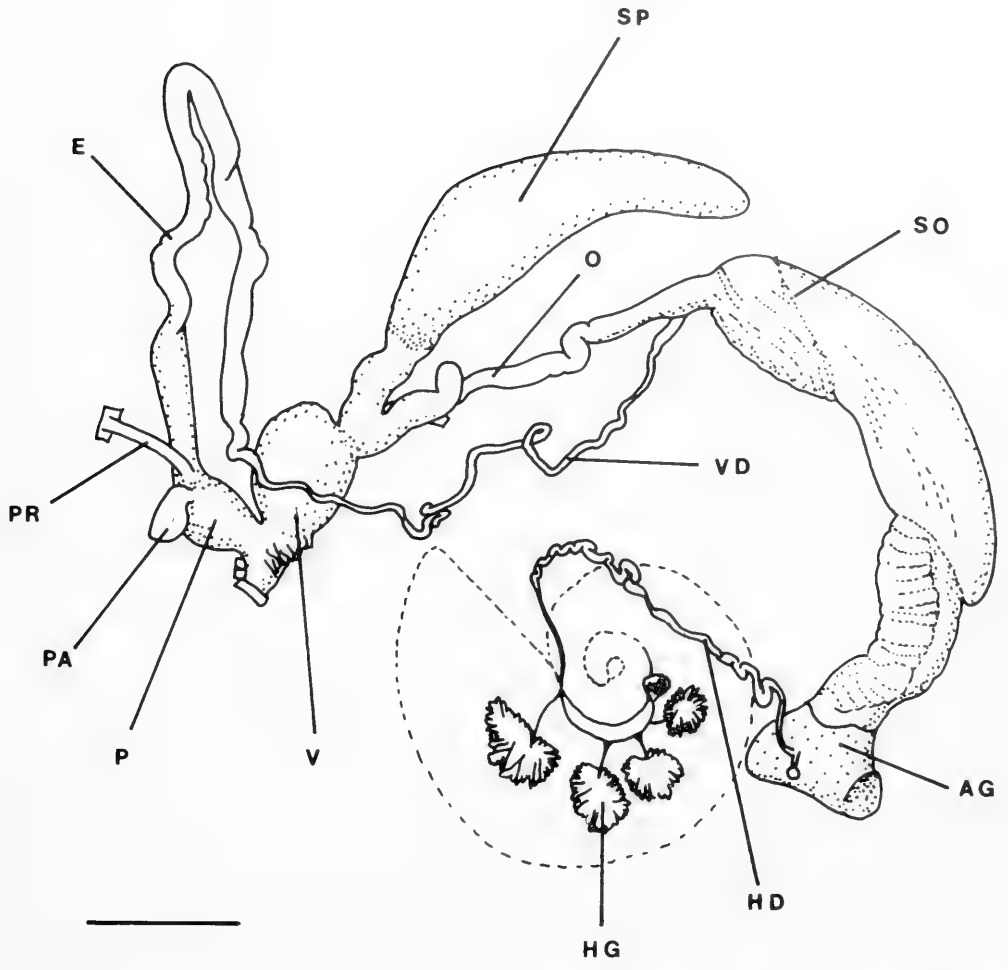


FIG. 18. Genital apparatus of *Pararhytida dictyodes*, sta. 192. Scale line 5 mm. AG, albumen gland; E, epiphallus; HD, hermaphrodite duct; HG, hermaphrodite gland; O, oviduct; P, penis; PA, penial appendix; PR, penial retractor; SO, spermoviduct; SP, spermatheca; V, vagina; VD, vas deferens.

it comprises five lobes, but along the E coast there is a progressive tendency for the second and third lobes (that is, from the top of the stomach) to fuse: at Thiem (sta. 83, Fig. 15D) they are almost fused and further southeastwards (stas. 25, 86, 91, 185, 186; Figs. 16 B, C) fusion is complete. In the central western massif of the Paéoua (sta. 20) the hermaphrodite gland has five (or possibly six) lobes; this may simply be an effect of increased size (the snails are larger than at sta. 25, the nearest from which we have preserved material), or it may be that the number of lobes remains constantly five along the NW coast (there is no preserved material between stas. 6 and 20). Further southeastwards, between the Col des Roussettes, the Col d'Amieu, and Mt. Do, the number of lobes is reduced to three by the fusion of the upper two lobes (stas. 110, 123, 189, 190; Fig. 17B), but eastwards and southwards it becomes four again (stas. 115, 118, 191, 192; Figs. 17A, 18).

Albumen gland and spermoviduct: size variation in the albumen gland, talon and spermoviduct was not analysed because this appears to depend principally on the state of maturity of the animal.

Free oviduct: generally this is longer than the spermatheca when uncoiled; when coiled it is compacted along the spermatheca stalk. It does, however, show enormous geographical variation in length, by up to about four times. It tends to be shorter in the NE valleys (stas. 86, 185, 186), and reaches its minimum on the central Mt. Paéoua (sta. 20, Fig. 16A). It seems that the reduction in length of the free oviduct from N to S is gradual, whereas the increase in length southeastwards is rather abrupt between stas. 20 (Fig. 16A) and 86 on the one hand (short oviduct) and sta. 91 on the other (long oviduct). The oviducal length is intermediate at sta. 25 (Fig. 16C). In the S plains (sta. 192, Fig. 18) the oviduct is again shorter than further N.

Spermatheca and vagina: the head of the spermatheca is thin-walled and smooth. It is always elongate, and almost constant in shape. Its size is more or less uniform within each of two large geographical regions: it is smaller north of the Col d'Amieu (sta. 190; circa 1 cm in length), and larger S and E of this station (circa 1.5 cm in length). In the S plains (sta. 192) it is again reduced to about the same size as S of the Col d'Amieu.

The spermathecal stalk and vagina form a single functional unit, as shown by the absence of any discontinuity in internal ornamentation at the level of oviduct insertion (Figs. 19, 20). The total length of this unit is almost constant over the entire range of the species, being shorter only in the S plains (sta. 192, Fig. 20D), but the relative size of stalk and vagina exhibit considerable geographic variation. The stalk is longer than the vagina in the NW region (stas. 65, 66, 70, 71, 184), and shorter on the E slope of Mt. Panié (stas. 79, 80). The stalk continues to reduce in relative length southeastwards (stas. 83, 185, 186), reaching a minimum at stas. 20 and 86. Its relative length again increases southeastwards of these stations, and from the Col d'Amieu southeastwards the stalk length is equal to or slightly greater than that of the vagina.

The level of insertion of the transverse vaginal ridge is generally at the mid-point of the length of the vagina, but varies between the lower quarter at Mt. Paéoua (sta. 20, Fig. 20A) and the upper extremity of the vagina at Mt. Ouénarou (sta. 192, Fig. 20D). This variation in the position of the ridge is probably also geographic, since it lies below the middle of the vagina in all stations around Mt. Paéoua (stas. 25, 86, 91, 115, 189, 190; Figs. 20B, C), that is, in central New Caledonia.

The transverse ridge in the vagina is never perfectly symmetrical, tending to be more expanded on the penial side (Fig. 19A). In Thiem (sta. 83), on Mt. Paéoua (sta. 20, Fig. 20A) and in the southern Mt. Ouénarou (sta. 192, Fig. 20D), this trend is developed to a point where the transverse ridge becomes a foliated appendage hanging in the vagina, attached only along a small portion of the vaginal circumference. Along the NW coast at least, this particular character-state is developed along a cline: an intermediate condition can be observed in stations along the northwestern coast around Thiem (stas. 80, 86, 184, 185; Figs. 19 B, C).

The position of the knob inside the spermathecal stalk varies independently from the length of the latter: in N populations (southwards to stas. 20 and 86), it is at about the same level or just above the oviducal opening; from stas. 25 and 91 southeastwards it is at the upper extremity of the stalk (Figs. 19, 20).

Penial complex: externally, the general trend is reduction in absolute and relative length of the penis proper from N (Fig. 15) to S (Fig. 18). In N New Caledonia, the eastern slope of Mt. Panié excepted, the penis is longer than the vagina (stas. 5, 7, 65, 66, 71, 183, 184; Figs. 15A, B). Further S the penis is generally slightly shorter than the vagina (Fig. 16). On the eastern slope of Mt. Panié (stas. 79, 80; Fig. 15C), in the Amoa and Tiwaka valleys (stas. 86, 186), and on Mt. Paéoua (sta. 20; Fig. 16A), the penis becomes much shorter than the vagina, although this is due more to a relative increase in the length of the vagina than to a shortening of the penis. From stas. 91 and 189 southeastwards the absolute length of the penis regularly decreases in correlation with the vaginal length (Fig. 17B). Penial shape also varies in relation to internal characters. In N stations (stas. 79 and 80 excepted) the two principal pilasters are particularly prominent, and the longer one apically inflated, even forming a verge in stas. 65, 66 and 183 (Fig. 19A). Correlatively the head of the penis becomes inflated (stas. 5, 7, 65, 66, 71, 83, 183, 184, 185; Fig. 15A, B, D). Further S, and on Mt. Panié, the apical part of the principal pilaster is weaker or even lacking and the two pilasters less prominent, resulting in a more fusiform penis (stas. 20, 25, 79, 80, 86, 91, 110, 186, 189; Figs. 15C and 19B,

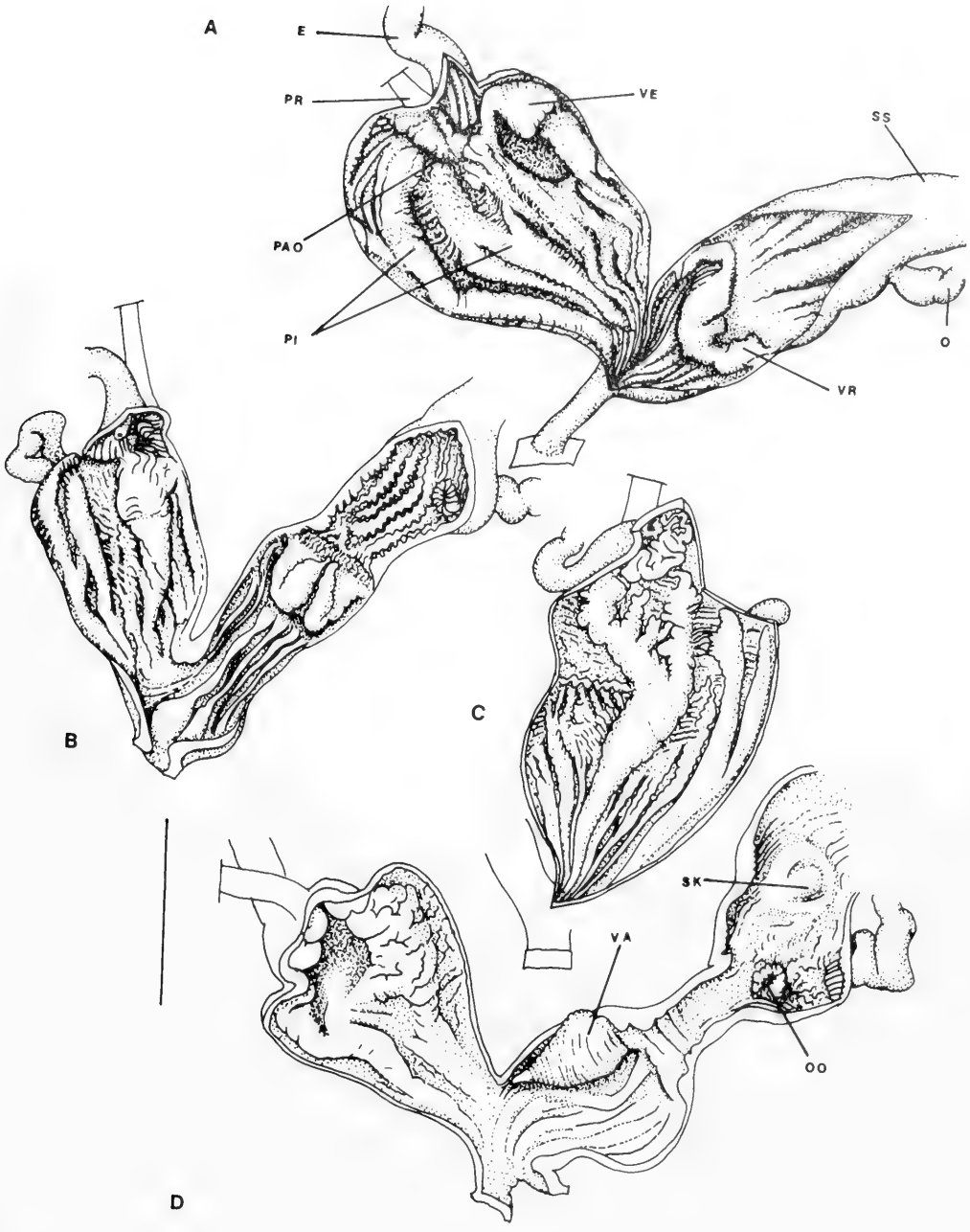


FIG. 19. Genital apparatus of *Pararhytida dictyodes*. A, sta. 66; B, sta. 80; C, sta. 184, D, sta. 83. Scale lines all 5 mm. E, epiphallus; O, oviduct; OO, oviducal opening; PAO, penial appendix opening; PI, penial pilasters; PR, penial retractor; SK, spermathecal knob; SS, spermathecal stalk; VA, vaginal appendix; VE, verge; VR, vaginal ridge.

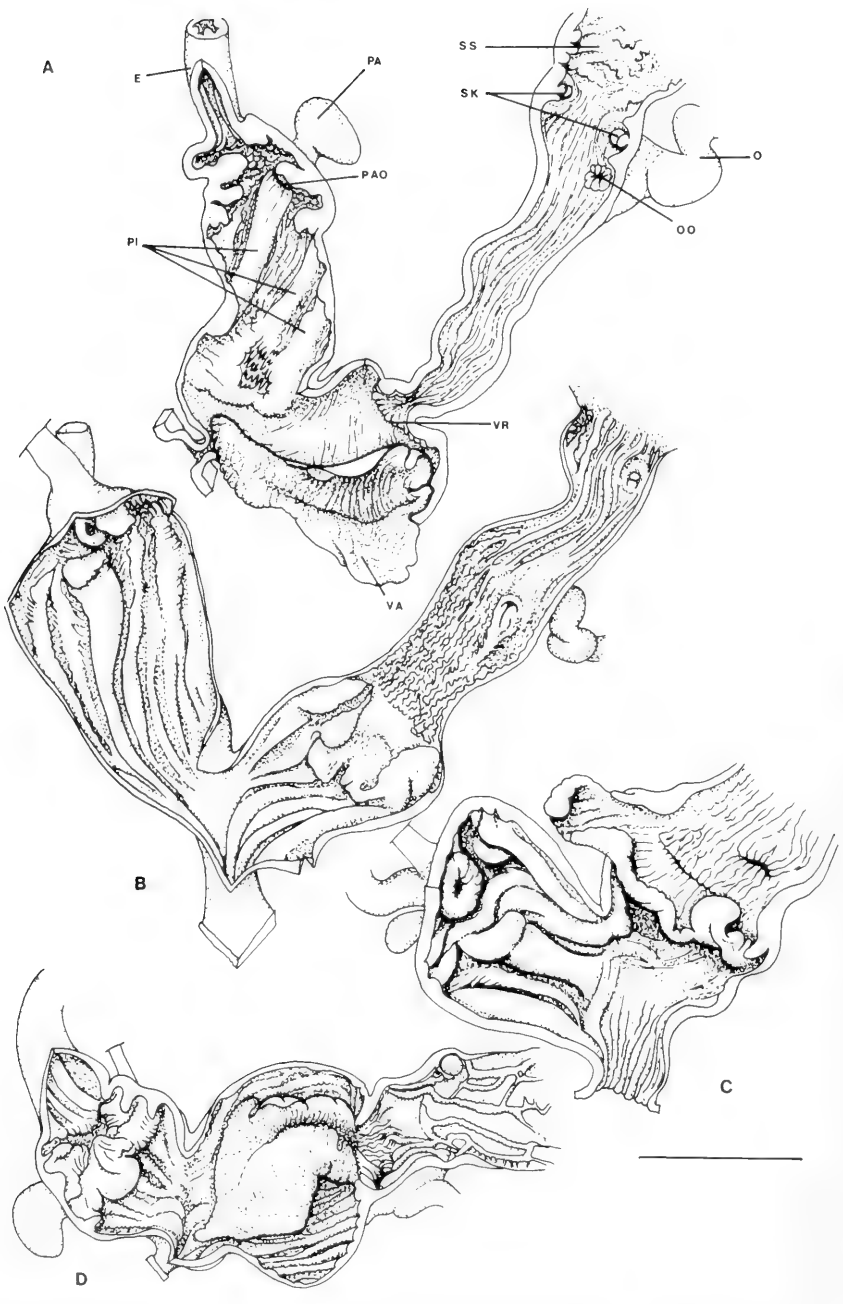


FIG. 20. Genital apparatus of *Pararhytida dictyodes*. A, sta. 20; B, sta. 91; C, sta. 191; D, sta. 192. Scale lines all 5 mm. E, epiphallus; O, oviduct; OO, oviducal opening; PAO, penial appendix opening; PI, penial pilasters; PR, penial retractor; SK, spermathecal knob (cut in 20A); SS, spermathecal stalk; VA, vaginal appendage; VR, vaginal ridge.

16A and 20A, 6B and 20B). S and E of Col d'Amieu (stas. 115, 118, 123, 190, 191; Fig. 20C) the pilasters are thicker, stouter, and more regular. The longer principal pilaster is prolonged transversely between the opening of the penial appendix and the epiphallic pore, and one of the secondary pilasters is apically inflated. This internal morphology produces a penis which externally resembles that from the N stations, but which results from a different internal pilaster structure (Figs. 19A, 20C). In the southernmost station (192) the penis has only numerous more-or-less equal pilasters that abut apically onto a large transverse ridge (Fig. 20D).

Spermatophore: In contrast to most other genital characters, the size and shape of the spermatophore of *P. dictyodes* seems remarkably constant. It is horny, and comprises a fusiform body, and a thin tail at least five times the body length. The distal part of the tail is thicker than the proximal part (Fig. 5B), becoming thinner again towards its extremity, which is perforated. It bears a denticulate ridge which originates on the distal part of the spermatophore body.

Discussion

P. dictyodes is easily recognised by its large size and flat apical whorls. Anatomically, it is the only *Pararhytida* with: 1. A lung never exceeding 0.8 whorls in length, and typically shorter. 2. A spermathecal head of the size and shape described above. Other characters are so variable that they cannot be considered diagnostic.

The problem lies not so much in the recognition of *P. dictyodes* as defined here, but rather to be sure that all the populations studied belong to a single species. However, even if geographic forms could be recognised, the fact that characters appear to vary independently suggests that gene flow is indeed taking place.

Pararhytida mouensis (Crosse, 1868)
(Figs. 21, 24, 25, 27–29)

Helix mouensis Crosse, 1868: 152, pl. 8, fig. 5 (Mt. Mou).
Helix dictyonina Euthyme, 1885: 257 (Noumea).
Helix dictyonina var. *globulosa* Euthyme, 1885: 256.
Trochomorpha dictyonina (Euthyme). Crosse, 1894: 243, pl. 8, fig. 4; Dautzenberg, 1906: 258, pl. 8, figs. 4–6.
Pararhytida dictyonina (Euthyme). Dautzenberg, 1923: 140.
Charopa (Tropidotropis) gudei Preston, 1907: 220, fig. 7 (New Caledonia).
Pararhytida (Pararhytida) mouensis (Crosse). Franc, 1956: 137; Solem, 1961: 467.
Pararhytida (Pararhytida) dictyonina (Euthyme). Franc, 1956: 137; Solem, 1961: 467.

Lectotype of *H. mouensis* Crosse: MNHN. Mont Mou, Marie Colln. (Fig. 21). Dimensions (mm): Shell height 8.6. Shell diameter 17.3. Aperture height 6.4. Aperture diameter 8.2. Umbilicus width 2.7. Whorls 5.2.

Other type material: Lectotype (BMNH Reg. no. 1907.5.20.106) and paralectotype (BMNH Reg. no. 1923.2.20.7) of *C. gudei* Preston, New Caledonia, ex Preston. Lectotype and 5 paralectotypes (MNHN) of *H. dictyonina* Euthyme, New Caledonia (Fig. 22).

Other material: sta. 37(1), sta. 43(7), sta. 47(1), sta. 125(1), sta. 128(1), sta. 130(1), 136(4), sta. 140(1), sta. 142(7), sta. 150(1), sta. 179(6), sta. 192(3).

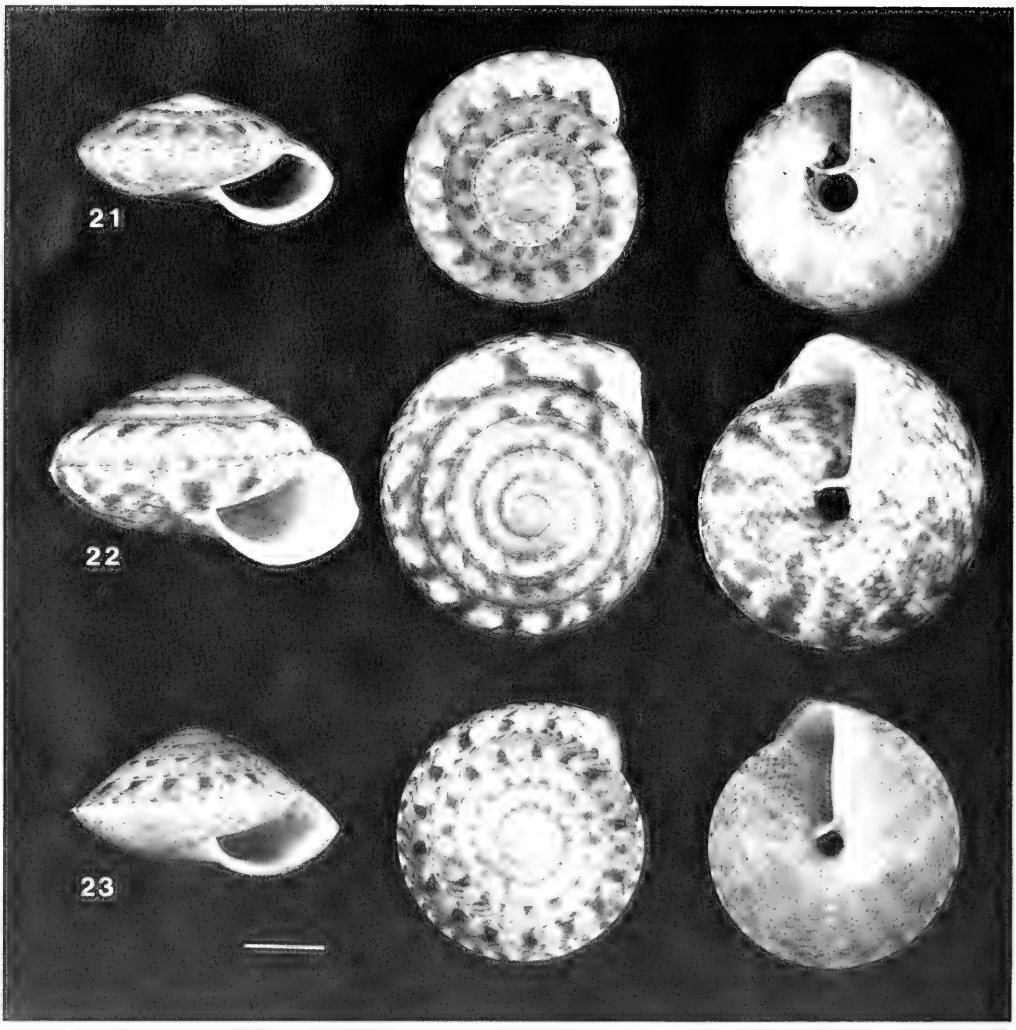
Preserved material: 37, 43, 47, 125, 128, 136, 140, 142, 179, 192.

Distribution

P. mouensis is restricted to the SE mainland of New Caledonia, from Mt. Humboldt (sta. 125) southeastward. We did not collect it in the coastal lowlands NW of Noumea, nor between Yaté and Goro (stas. 48, 49, 50, 195). It was found at stations with rainfall ranging from 1800 mm (Col de Mouirange, sta. 142) to 4500 mm (Mt. Humboldt, sta. 125). It occurs sympatrically with *P. dictyodes* at low altitudes in the S plains (stas. 142 and 192), and with *P. marteli* on Mt. Guemba (sta. 47).

Shell (Figs. 21, 22)

The shell varies in size from 18.9 mm × 10.5 mm to 24.9 mm × 15.1 mm. These extremes are found only at the edges of the range, the smallest in the W Mt. Koghi (sta. 140) and Mt. Mou (the type locality), and the largest on the eastern Mt. Guemba (sta. 47). The species is generally intermediate in size between the larger *P. dictyodes* and the remaining four smaller species. In contrast to *P. dictyodes*, the suture is moderately im-



FIGS. 21–23. 21. *Helix mouensis* Crosse, lectotype, MNHN. Mt. Mou, leg. Marie, 1868. 22. *Helix dictyonina* Euthyme, lectotype, MNHN. Noumea, Jousseaume Collection. 23. *Trochomorpha (Videna) marteli* Dautzenberg, lectotype, MNHN. New Caledonia. Scale line all 5 mm.

pressed. The shell is dome-shaped, quite unlike that of any other *Pararhytida*. The adult whorl count is broadly related to shell diameter, from 5.6 whorls in Mt. Koghi (sta. 140, D = 18.9 mm) to 6.25 whorls in Mt. Guemba (sta. 47, D = 24.9 mm) and 6.4 in Col de Mouirange (sta. 142, D ca. 22 mm); mean whorl count is 6.08 (s.d. 0.26).

Radula (Figs. 24, 25)

The radulae of four specimens were examined. The size and shape of individual central and lateral teeth are close to those of *P. dictyodes*, although appearing slightly shorter and broader, and show a considerable degree of variation (Table 2). The marginal teeth are in general shorter and narrower than in *P. dictyodes*, and both these and the laterals are typically fewer. At two of the four sites *P. mouensis* is sympatric with other species: with *P. marteli* at sta. 47, and with *P. dictyodes* at sta. 192. However there was as much radular variation between the two sympatric sites as between allopatric situations, and at sta. 47 tooth size and number

were almost identical in *P. marteli* and *P. mouensis.*

Pulmonary complex

The pulmonary cavity occupies the last 0.9 to 1.2 whorls of the visceral coil, but we did not have enough preserved material to distinguish any clear geographic pattern of variation. The rectal arm of the kidney is only slightly longer than the cardiac arm in northern stations (near Mt. Dzumac, sta. 128), but about 1.5 times longer in the southern plains (stas. 43, 47, 142, 179, 192).

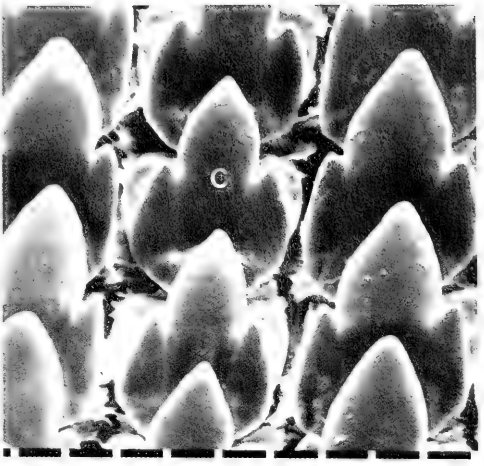
Genital apparatus

The number of lobes in the hermaphrodite gland increases from N to S, from four close to Mt. Dzumac (sta. 128), to five on the Montagne des Sources (sta. 136) and Mt. Ouénarou (sta. 192), six in the Rivière Bleue valley (sta. 43) and Mt. Guemba (sta. 47), and finally seven in the Col de Mouirange (sta. 142) (Fig. 27).

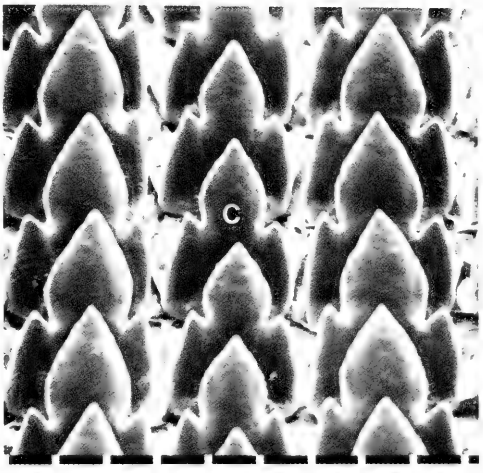
The relative length of the free oviduct also increases from N to S: it is shorter than the spermathecal length in northern stations (stas. 43 and 128; Figs. 27A, B), about the same length at station 136, and distinctly shorter at the other stations (sta. 47, 142, 179, 192; Figs. 27C, D).

The spermatheca has a highly characteristic shape, with a very long head of a constant diameter that is greater than the diameter of the short spermathecal stalk (Fig. 27).

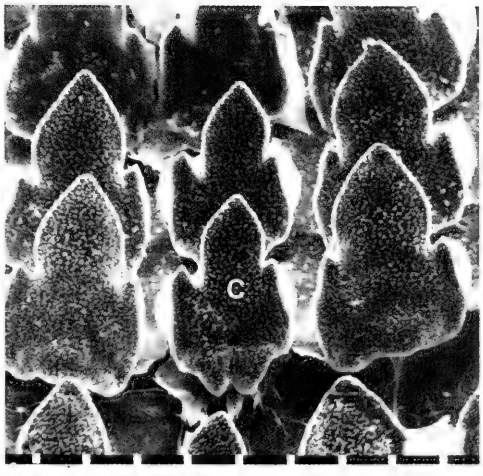
The vagina does not possess a well-developed transverse ridge or internal appendage as do all other species (Fig. 28), but at most shows a thickening of the wall and an interruption in its longitudinal internal ridges. From their position, these ridges are considered homologous with those of other species; when present they are located below the mid-point of the vagina, except at sta. 128 (Fig. 28A). Although an internal ridge is lacking, the vagina of *P. mouensis* does possess one or two outgrowths of the vaginal wall which form pouch-like structures. These are not visible externally except as a thicken-



24



25



26

FIGS. 24–26. 24. Central and lateral teeth, *P. mouensis*, Mt. Ouénarou, sta. 192. 25. Central and lateral teeth, *P. mouensis*, Col de la Ouinné, sta. 128. 26. Central and lateral teeth, *P. marteli*, Mt. Guemba, sta. 47. Scale divisions all 10 μm.

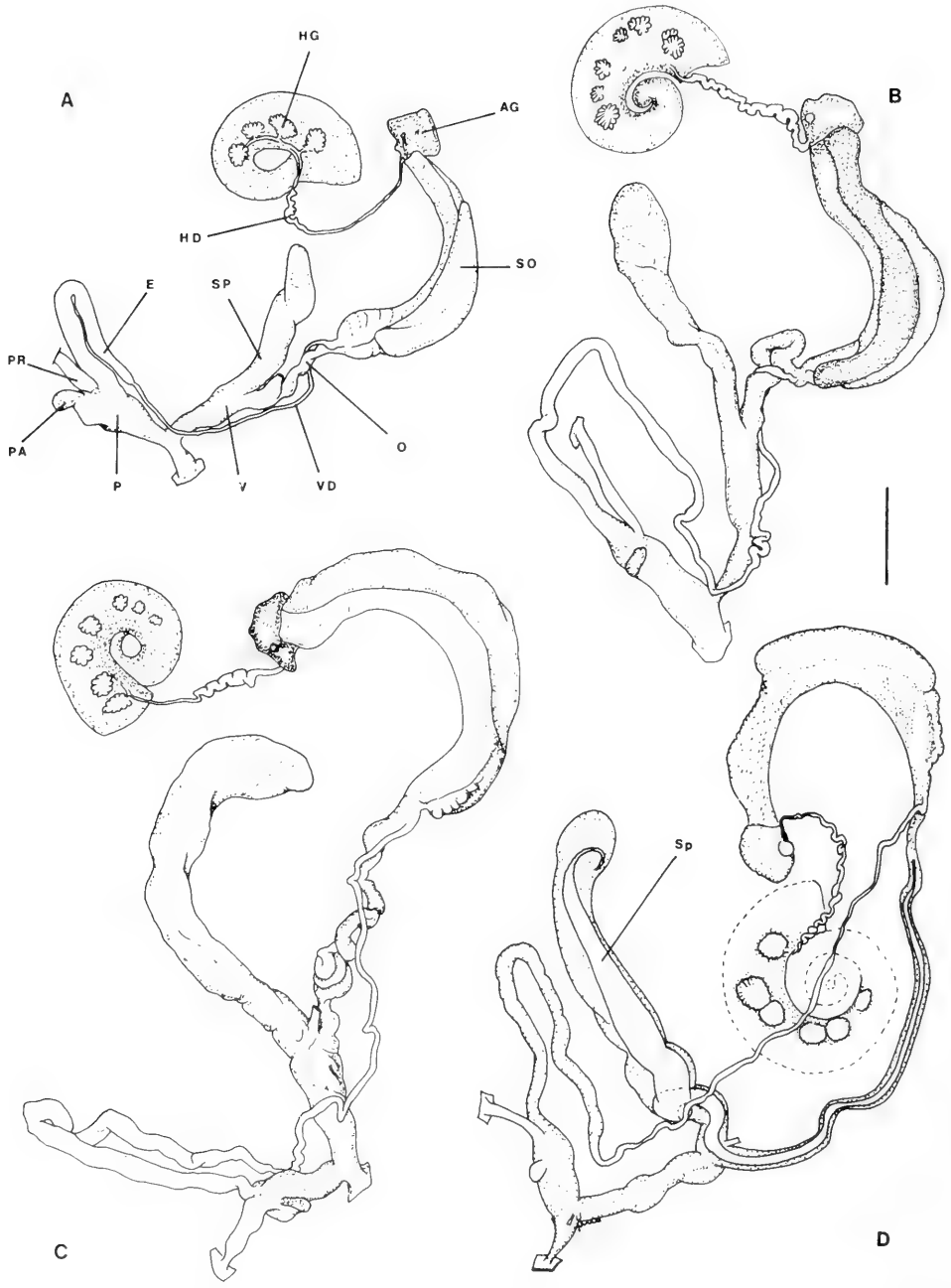


FIG. 27. Genital apparatus of *Pararhytida mouensis*. A, sta. 128. B, sta. 43. C, sta. 142. D, sta. 47, showing position of spermatophore. Scale line all 5 mm. AG, albumen gland; E, epiphallus; HD, hermaphrodite duct; HG, hermaphrodite gland; O, oviduct; P, penis; PA, penial appendix; PR, penial retractor; SO, spermoviduct; SP, spermatheca; Sp, spermatophore; V, vagina; VD, vas deferens.

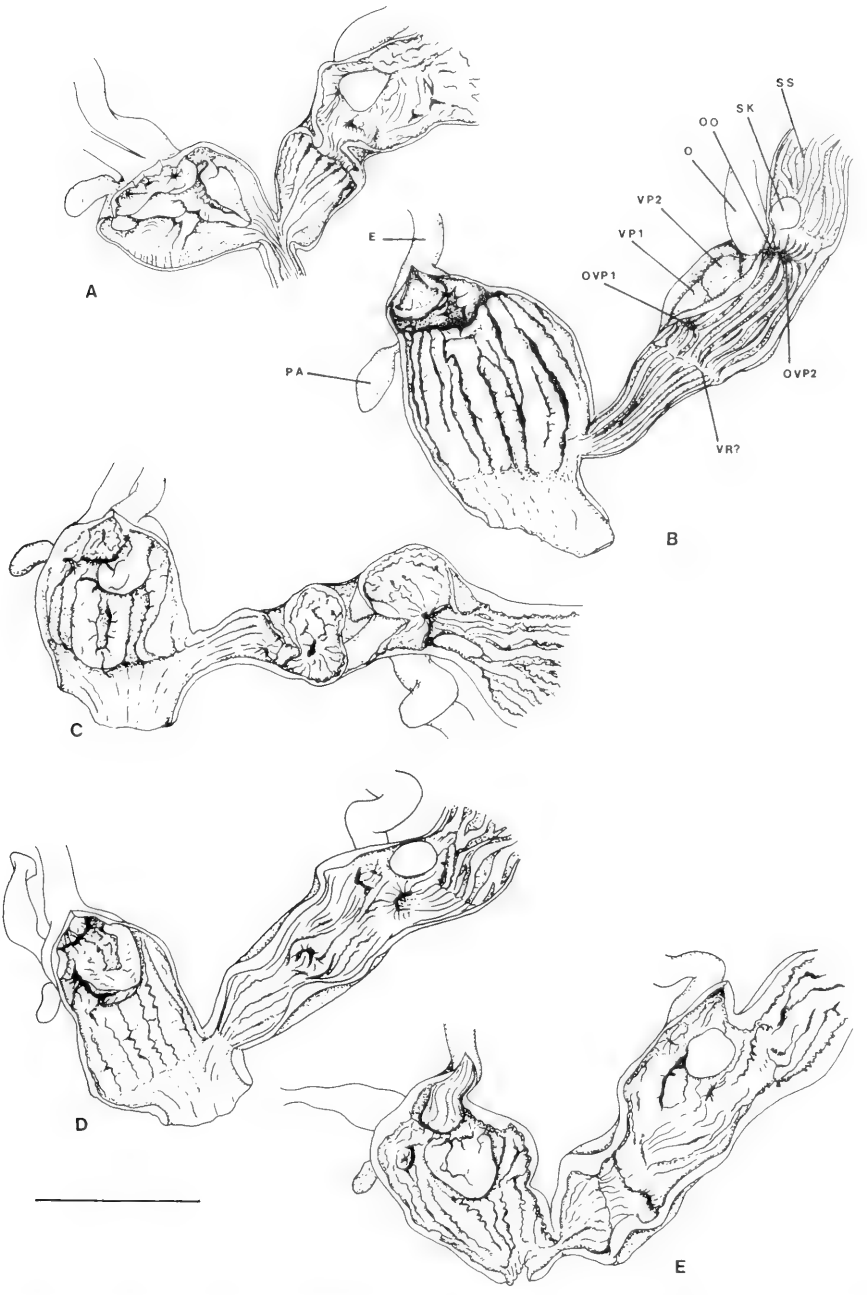


FIG. 28. Internal genital morphology of *Pararhytida mouensis*. A, sta. 128. B, sta. 43. C, sta. 48, showing the interior of the two vaginal pouches, but the vagina unopened within the sheath that surrounds the pouches. D, sta. 136. E, sta. 142. Scale lines all 5 mm. E, epiphallus; O, oviduct; OO, oviducal opening; OVP1, OVP2, openings of the vaginal pouches; PA, penial appendix; SK, spermathecal knob; SS, spermathecal stalk; VP1, VP2, vaginal pouches; VR, vaginal ridge.

ing of the vagina, being tightly bound in connective tissue, but are clearly visible when the vagina is opened (Figs. 28B, C). Their number and position vary geographically. In southern and central stations (stas. 43, 136, 179; Fig. 28B) the lower pouch lies just above the transverse ridge, and the upper pouch just beside the oviducal opening. In the NE (Mt. Guemba, sta. 47) only the upper pouch is present; this pattern probably arose through the loss of the lower pouch, which is less well developed in sta. 179 than in stas. 43 and 136. In the N (sta. 128, Fig. 28B) there is also a single upper vaginal pouch, but here it probably results from the fusion of the two pouches, since at the intermediate stations (142, 192) the two pouches are contiguous beside the oviducal opening (Fig. 28E). The knob in the spermathecal stalk is always situated just above the oviducal opening.

The penis is nearly constant in size, and is of a regular fusiform shape (Fig. 27). The epiphallus is a little more than twice as long as the penis, except in the northernmost station (128) where it is shorter (Fig. 27A). The internal ornamentation is typically formed of numerous short pilasters, and a verge originating from the upper end of the pilaster zone (Figs. 28A, C–E). This pattern may be modified, even within a population, by loss of the verge or weakening of the pilasters, the latter arrangement (Fig. 28B) permitting recognition of the homology of pilasters and verge between *P. mouensis* and *P. dictyodes*.

Spermatophore: these or fragments were only found at three stations (47, 136, 142); here the shape is fusiform, with no clear delimitation between body and tail (Fig. 29). A ridge runs the entire length of the spermatophore, appearing smooth at low magnifications, but minutely serrate at high. At Mt. Guemba (sta. 47), the spermatophore is much larger and more elongate than at the other stations. The size is probably related to the larger size of the animals there, but the change in shape is more problematic. At sta. 47 a spermatophore was found *in situ*, with its body in the spermathecal head, and the tail lying within the free oviduct (Fig. 27D).

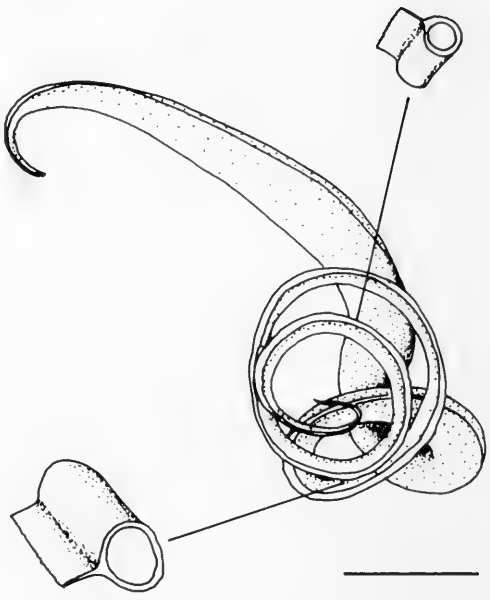


FIG. 29. Spermatophore of *Pararhytida mouensis*, sta. 47. Scale line 1 mm.

Discussion

P. mouensis is generally smaller than *P. dictyodes* and larger than the other four species of *Pararhytida*. It overlaps in size with *P. dictyodes* only at Mt. Guemba (sta. 47) where *dictyodes* has not been recorded, and with the other four species in the chain of Mt. Mou and Mt. Koghi (type locality and sta. 140), where similarly none of the small species is found. Conchologically it can be distinguished from *P. dictyodes* by its more impressed suture (especially on the first whorl), and from the small species by its rounded profile. It is the only species without a vaginal ridge or appendage, and possessing vaginal pouches. Although we were unable to collect topotypic material of *P. mouensis*, we are in little doubt that it is only the westernmost form of what has previously been called *P. dictyonina*, from which it differs in whorl number but not in rate of whorl increase. Furthermore, the shells of *P. dictyonina* become flatter at stations approaching those where typical *P. mouensis* occurs. The attribution of animals from Mt. Guemba to *P. mouensis* is more questionable, since their shells more closely resemble those of *P. dictyodes*. However, these animals are closer in anatomy to *P. mouensis* than to any other species, and we have no reason to preclude the possibility of a cline existing between Mt. Guemba and the remaining stations. Moreover, it seems

unlikely that the observed differences in spermatophore morphology between Mt. Guemba specimens and other *P. mouensis* are sufficient to prevent successful copulation. The anatomical change may result from sympatry with the smaller *P. marteli* at Mt. Guemba, and with the larger *P. dictyodes* over the rest of its range.

Pararhytida marteli (Dautzenberg, 1906)
(Figs. 23, 26, 30)

Trochomorpha (Videna) marteli Dautzenberg, 1906: 257, pl. 8, figs. 7–9 (New Caledonia).
Pararhytida (Pararhytida) marteli (Dautzenberg). Franc, 1956: 138; Solem, 1961: 467.

Lectotype (here designated): New Caledonia, leg. Martel, MNHN. (Fig. 23). Dimensions (mm): Shell height 9.8. Shell diameter 17.9. Aperture height 6.8. Aperture diameter 8.7. Umbilicus width 1.7. Whorls 5.9.

Other material: sta. 47(1), sta. 48(36), sta. 49(20), sta. 50(1), sta. 195(17).

Preserved material: 47, 48, 49, 50, 195.

Distribution

P. marteli has been recorded only from the extreme SE of New Caledonia, from Yaté (close to sta. 47) to Goro (sta. 50). However, we did not collect it from the Kouakoué, NW of sta. 47, and cannot be sure that it does not occur there. It was typically collected from stations with high rainfall, from 1900 mm to 3000 mm a year.

Shell (Fig. 23)

The shell is sharply carinated, domed above and shallowly rounded below, ranging in size from 16.1 × 8.8 mm to 20 × 10.9 mm. The carina is situated about half way up the palatal wall. Adult shells have from 5.2 to 6.2 whorls (mean 5.59; s.d. 0.2).

Radula

Only one radula was examined, from a specimen collected at Mt. Guemba (sta. 47, Fig. 26). The teeth are generally similar in shape, size, and number to *P. phacoides* (Table 2), and also to *P. mouensis* with which it is sympatric at sta. 47, although in the former case the lateral mesocones of *P. phacoides* are significantly smaller.

Pulmonary complex

The length of the lung varies between 0.9 and 1.3 whorls, as in *P. mouensis* (the stomach and crop have the usual length of one whorl, but the upper part of the visceral coil is longer than usual, extending between 3.3 and 3.6 whorls). The rectal arm of the kidney is about 1.5 times longer than the cardiac arm, and occupies less than 1/3 of the lung length.

Genital apparatus

The hermaphrodite gland has five lobes at stas. 47, 49 and 195 (Figs. 30A, C), but only four at Touaourou (sta. 48). Externally the genitalia resemble, in reduced form, those of *P. mouensis* (Figs. 27, 30). From N (sta. 47) to S (sta. 48 southward) the relative length of the free oviduct varies from slightly longer than the spermatheca to about half the length of the latter. Internally the only constant differences from the arrangement found in *P. mouensis* are the presence of a vaginal appendage and the absence of a vaginal pouch in *P. marteli*; the appendage is attached at the mid-point of the vagina (Figs. 30C, D).

Spermatophore: One complete spermatophore was found at Mt. Guemba (sta. 47; Fig. 30E). It has no tail and is smaller and stouter than in *P. mouensis*. Its longitudinal ridge is denticulate only at the open end.

Discussion

The shell of *P. mouensis* is thicker and more rounded than that of *P. marteli*. Although the anatomical differences between these two species are slight, their distinctness is confirmed at Mt. Guemba where they occur sympatrically.

Distinguishing the shells from those of *P. phacoides*, *P. pyrosticta* and *P. thyrophora* is more difficult: those of *P. marteli* are intermediate in shape between the more rounded *P. thyrophora* and the flatter *P. phacoides* and *P. pyrosticta*. In these last two species, the carina is situated higher on the whorl contour,

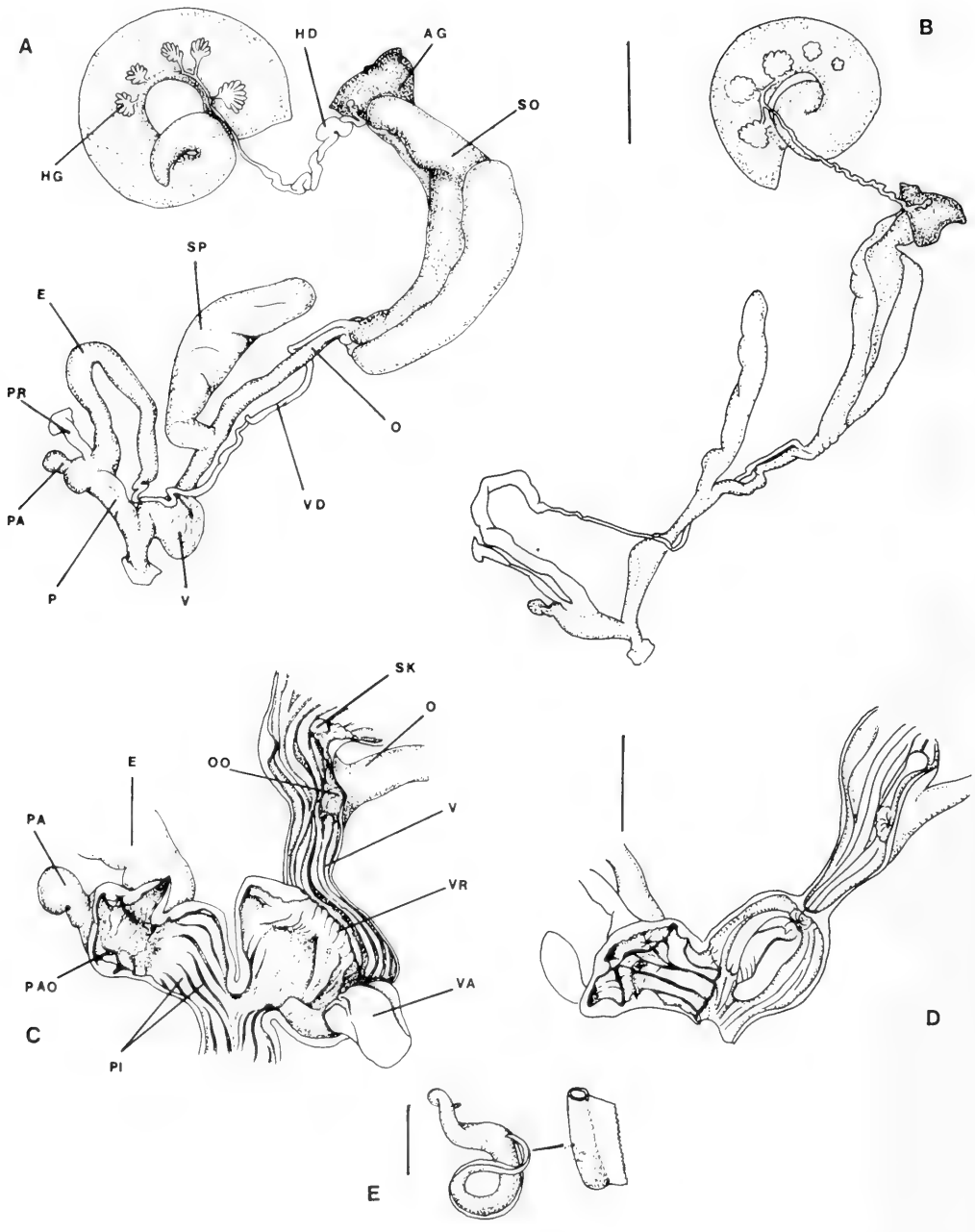


FIG. 30. Genital apparatus of *Pararhytida marteli*. A and C, sta. 195. B, External morphology, sta. 49. D, internal morphology, sta. 48. E, spermatophore, sta. 47. Scale lines A and B both 5 mm. C–E both 2.5 mm. AG, albumen gland; E, epiphallus; HD, hermaphrodite duct; HG, hermaphrodite gland; O, oviduct; OO, oviducal opening; P, penis; PA, penial appendix; PAO, penial appendix opening; PI, penial pilasters; PR, penial retractor; SK, spermathecal knob; SO, spermoviduct; SP, spermatheca; V, vagina; VA, vaginal appendage; VD, vas deferens; VR, vaginal ridge.

and they more closely resemble juveniles of *P. dictyodes.*

Pararhytida phacoides Mordan & Tillier, n. sp.
(Figs. 31, 34, 37)

Holotype: Mt. Boulinda, 980–1020 m, between Petit and Grand Boulinda, altitude rainforest. Coll. A. & S. Tillier, 6.vii.1979 (sta. 97), MNHN (Fig. 31). Dimensions (mm): Shell height 10.1. Shell diameter 19.4. Aperture height 7.3. Aperture diameter 9.9. Umbilicus width 1.7. Whorls 5.6.

Paratypes (preserved): 2, as above, MNHN.

Other material (preserved): sta. 118 (2 + 1 shell), 97 (1 broken shell).

Etymology: lens-like. Greek: phacos, a lentil.

Distribution

The two stations where this species was collected (stas. 97 and 118) probably represent the N and S limits of its distribution: to the N it is replaced by *P. pyrosticta* and to the S by *P. mouensis.* At both stations rainfall is high (3000 mm at sta. 97 and 2600 mm at sta. 118), and the distributions of the small *Pararhytida* species suggest that this relationship is not chance. *P. phacoides* is probably endemic to very wet rainforests on the mountains of southern central New Caledonia.

Shell (Fig. 31)

Only three adult shells were collected, ranging from 19.7 to 21.5 mm in width, from 9.7 to 10.6 mm in height, with an umbilicus of *circa* 2 mm in diameter. They have from 5.3 to 5.7 (mean 5.5, s.d. 0.18) whorls, and are sharply carinated, with the carina on the upper part of the palatal wall. The aperture is only slightly expanded. The upper surface of the initial whorls is convex, giving the shells a slightly more conical shape than in juvenile *P. dictyodes.* The border of the umbilicus of shells from Mt. Nakada is slightly shouldered.

Radula

Two specimens were examined from the only known localities for this species (stas. 97 and 118); the radula from sta. 118 was juvenile and was not measured. The radula from sta. 97 (Fig. 34) is similar to that of *P. marteli*, except that the marginal teeth tended to be broader, whilst the centrals and laterals are slightly narrower and more pointed.

Pulmonary complex

The lung occupies the final 0.9 whorl in the fully adult preserved specimen (sta. 97). The arms of the kidney are almost equal in length, which is about ⅓ of the total lung length. The pulmonary complex of *P. phacoides* is closer in anatomy to that of *P. dictyodes* than to either *P. mouensis* or *P. marteli.*

Genital apparatus

In both internal and external anatomy, the genitalia of *P. phacoides* resemble a reduced version of the genital apparatus of *P. dictyodes* (Figs. 37A, B). The hermaphrodite gland of the only specimen in which it was observed had four lobes. The only significant difference is in the shape of the spermathecal head, which is triangular and relatively shorter than in *P. dictyodes.* However, this shape may simply have been due to the presence of a spermatophore.

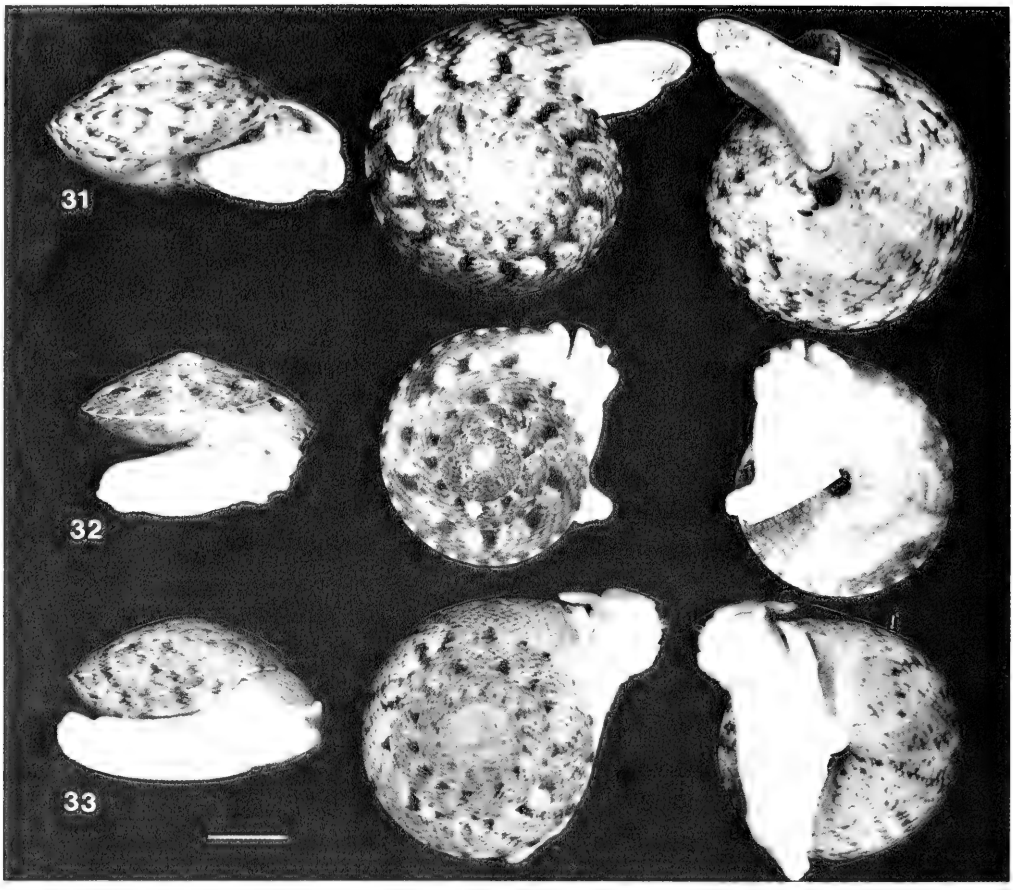
Spermatophore: this found at Mt. Boulinda is identical in shape and arrangement with those of *P. dictyodes*, and is only slightly smaller (Fig. 37C).

Discussion

If *P. phacoides* had not been found sympatrically with *P. dictyodes* at both stations where it was collected, separation from the latter would have been extremely difficult. It resembles closely a young *P. dictyodes.* There is, however, a slight difference in convexity of the early whorls, and also in rate of whorl increase, and maturity is reached at less than six whorls. In shell characters *P. phacoides* is simlar to *P. pyrosticta*, but the latter is slightly smaller for the same whorl count. Anatomical differences between *P. phacoides*, *P. pyrosticta* and *P. thyrophora* will be considered later.

Pararhytida pyrosticta Mordan & Tillier, n. sp.
(Figs. 2, 32, 35, 38)

Pararhytida marteli (Dautzenberg). Dautzenberg, 1923: 140.



FIGS. 31–33. 31. *Pararhytida phacoides* n. sp., holotype, MNHN. Mt. Boulinda, sta. 97. 32. *Pararhytida pyrosticta* n. sp., holotype, MNHN. Mt. Tchingou, sta. 84. 33. *Pararhytida thyrophora* n. sp., holotype, MNHN. Ile Art, sta. 58. Scale line all 5 mm.

Holotype: S slope of Mt. Tchingou, 1250 m, rainforest. Coll. P. Bouchet, A. & S. Tillier, vii.1979 (sta. 84), MNHN (Fig. 32). Dimensions (mm): Shell height 8.0. Shell diameter 16.5. Aperture height 5.8. Aperture diameter 8.1. Umbilicus width 1.8. Whorls 5.1.

Paratypes (preserved): 5 + 6 juveniles, as above, MNHN (7 shells).

Other material: sta. 72(2), sta. 91(1), sta. 92(3).

Preserved material: 72, 84, 91, 92.

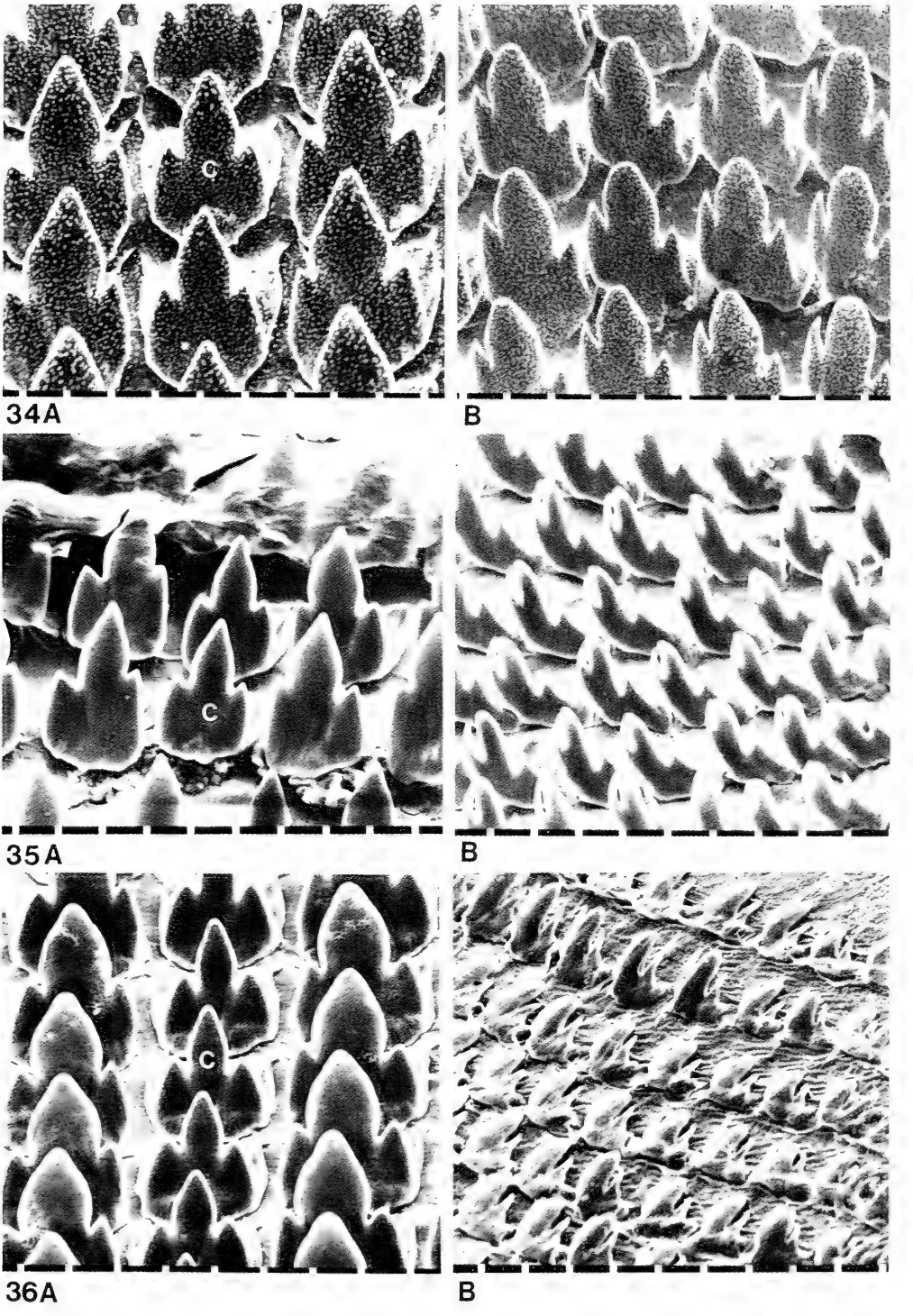
Etymology: with flame-like dots. Greek: pyr, fire; stictus, spotted.

Distribution

P. pyrosticta is restricted to very wet rainforests on the mountains of mainland New Caledonia N of the Houailou valley. This area corresponds to the northernmost part of the central chain together with the eastern slopes and summit areas of the Panié massif. At the stations from which it was collected rainfall ranges from 2500 to 3500 mm per annum.

Shell (Fig. 32)

The shell is similar to that of *P. phacoides*, being only slightly smaller and with fewer



FIGS. 34–36. 34A, central and lateral teeth; B, marginals. *Pararhytida phacoides* n. sp., Boulinda, sta. 97. 35A, central and lateral teeth; B, marginals. *Pararhytida pyrosticta* n. sp., Mt. Ignambi, sta. 72. 36A, central and lateral teeth; B, marginals. *Pararhytida thyrophora* n. sp., Ile Art, sta. 58. Scale divisions all 10 μm.

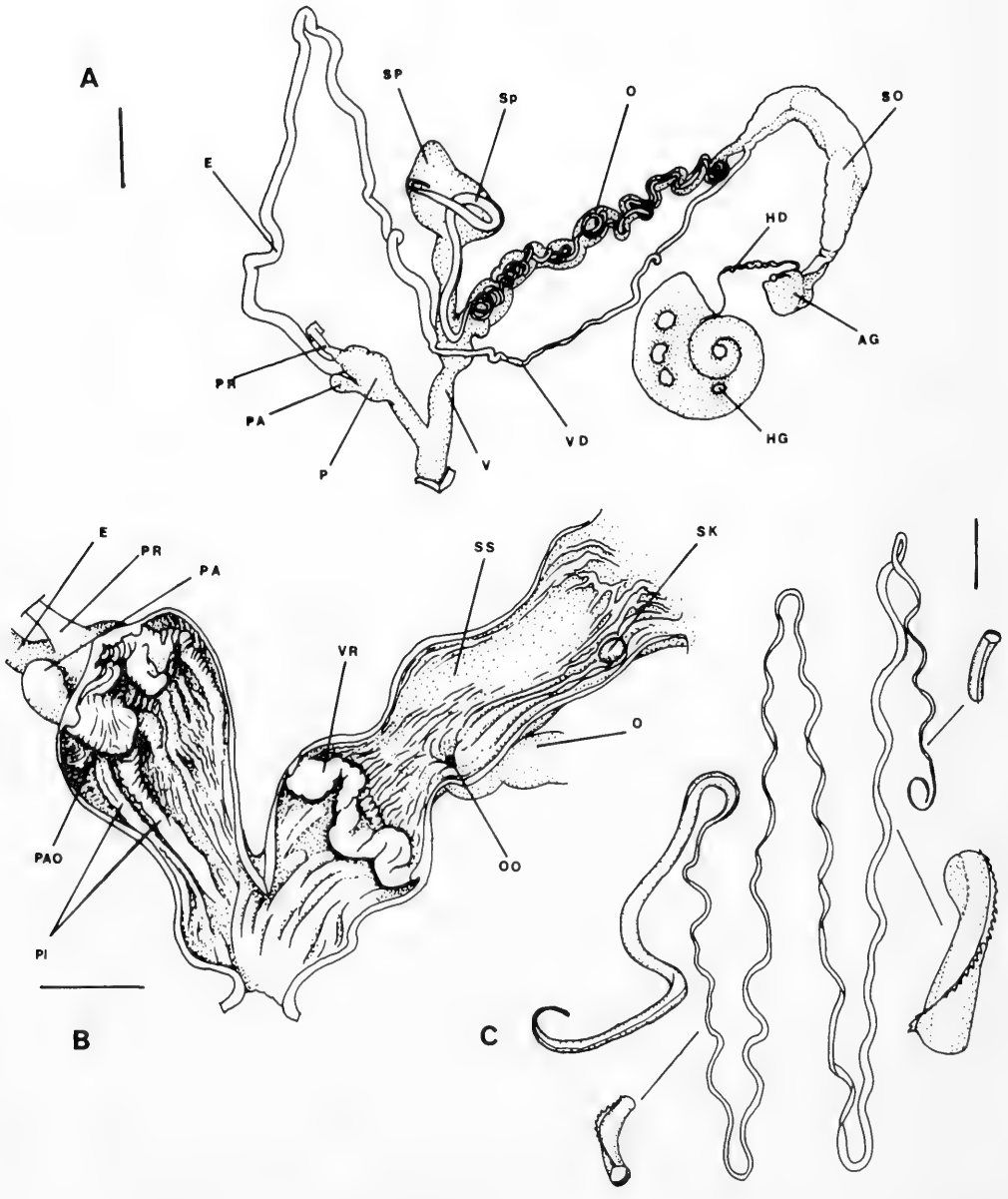


FIG. 37. Genital apparatus of *Pararhytida phacoides* n. sp., sta. 97. A, External morphology, indicating position of spermatophore. Scale line 5 mm. B, internal morphology. Scale line 2.5 mm. C, spermatophore. Scale line 2.5 mm. AG, albumen gland; E, epiphallus; HD, hermaphrodite duct; HG, hermaphrodite gland; O, oviduct; OO, oviducal opening; P, penis; PA, penial appendix; PAO, penial appendix opening; PI, penial pilasters; PR, penial retractor; SK, spermathecal knob; SO, spermoviduct; SP, spermatheca; Sp, spermatophore; SS, spermathecal stalk; V, vagina; VD, vas deferens; VR, vaginal ridge.

whorls when adult. Shells measure from 14.7 to 18 mm in diameter and from 7.3 to 8.4 mm in height, with an umbilicus from 1 to 1.9 mm in diameter. They have from 5 to 5.3 whorls on Mt. Ignambi (sta. 72) and Mt. Aoupinié (stas. 91, 92), but only between 4.7 and 5.1 whorls on Mt. Tchingou (sta. 84). Mean adult whorl count is 4.99 (s.d. 0.23).

Radula

Two radulae were examined (stas. 72 and 84, Fig. 35) and in both, the lateral teeth were distinctly narrower and sharper than in any other *Pararhytida* species. The mesocone of the central tooth also is narrow in comparison with species other than *P. thyrophora.* The number of teeth (Table 2) is about average for the smaller taxa.

Pulmonary complex

The lung cavity extends back 0.6 whorls on northern Mt. Ignambi (sta. 72), 0.75 whorls on the southern Aoupinié (stas. 91 and 92), and 0.8 to 0.9 whorls on the northwestern Mt. Tchingou (sta. 84). The rectal arm of the kidney is slightly longer than the cardiac arm; its absolute length is nearly constant, and thus it occupies more than ⅓ of the lung length in Mt. Ignambi, but less than ⅓ in Mt. Tchingou.

Genital apparatus

Externally, the genital apparatus of *P. pyrosticta* is characterised by a penis that is markedly shorter than the vagina, and by the size and shape of the spermatheca which has an ovoid head and a short, thick stalk (Fig. 38B).

Internally the penis has at most equally weak pilasters, and a small subapical verge in Mt. Tchingou specimens. All dissected specimens possessed an elongated vaginal appendage which is inserted above the midpoint of the vagina in Mt. Ignambi and Mt. Aoupinié specimens, and below it in specimens from Mt. Tchingou. The spermathecal knob is located just above the oviducal opening (Figs. 38C, D). In all specimens dissected the hermaphrodite gland has five lobes. The free oviduct is shorter at Mt. Tchingou than at the other two mountains.

Spermatophore: One complete spermatophore and some fragments were found in the Mt. Tchingou specimens. The complete spermatophore is very elongate, but without a differentiated tail, as in *P. mouensis* (Fig. 38A). The ridge is finely denticulated and extends around the imperforate extremity.

Discussion

P. pyrosticta looks externally very like *P. phacoides*, being only slightly smaller. In terms of genital anatomy the two species differ principally in spermatophore morphology, spermathecal shape, oviducal length, and internal penial and vaginal ornamentation.

Pararhytida thyrophora Mordan & Tillier, n. sp.
(Figs. 33, 36, 39)

Holotype: Ile Art, Belep Islands, N plateau. Coll. P. Bouchet and A. Warén, 7.vii.1979 (sta. 58). MNHN (Fig. 33). Dimensions (mm): Shell height 9.6. Shell diameter 18.3. Aperture height 6.7. Aperture diameter 9.1. Umbilicus width 1.7. Whorls 5.2.

Paratypes (preserved): 10 + 25 juveniles, as above, MNHN (+ 69 shells); 3 specs. BMNH Reg. no. 1984101; 3 specs., The Australian Museum, Sydney.

Other material: sta. 57(3).

Etymology: door-bearing. Greek: thyra, a door.

Distribution

P. thyrophora is known only from the Belep islands of Art and Pott. It was collected in dry, high maquis, where rainfall averages 1250 mm per year.

Shell (Fig. 33)

The shell ranges in size from 17 × 9 mm to 22.5 × 12.1 mm, with the umbilicus about 1 mm in diameter. The upper surface of the shell is more convex than in either *P. phacoides* or *P. pyrosticta*, but less than in some *P. mouensis.* The aperture is more rounded in adults than in juveniles, and the carina is situated about half way up the palatal wall. The number of whorls in the adult varies from 5.3 to 6.2 (mean 5.65; s.d. 0.19).

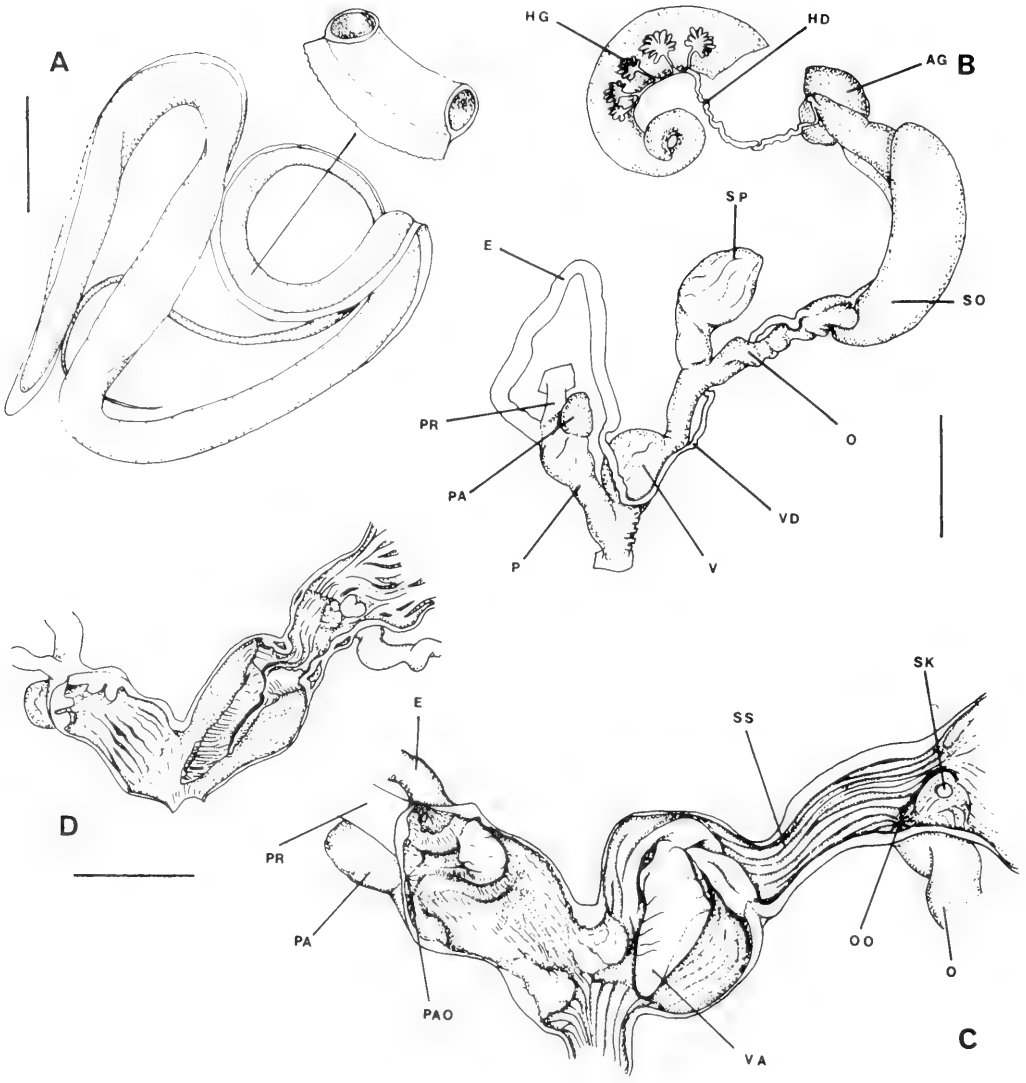


FIG. 38. Genital apparatus of *Pararhytida pyrosticta* n. sp., stas. 84 and 91. A, spermatophore, sta. 84, scale line 1.25 mm. B, external morphology, sta. 84, scale line 5 mm. C (sta. 84) and D (sta. 91), internal morphology, scale lines both 2.5 mm. AG, albumen gland; E, epiphallus; HD, hermaphrodite duct; HG, hermaphrodite gland; O, oviduct; OO, oviducal opening; P, penis; PA, penial appendix; PAO, penial appendix opening; PR, penial retractor; SK, spermathecal knob; SO, spermoviduct; SP, spermatheca; SS, spermathecal stalk; V, vagina; VA, vaginal appendage VD, vas deferens.

Radula

A single radula was examined from Ile Art (sta. 58, Fig. 36). It was characterised by the narrowness of the central tooth mesocone relative to the width of the entire tooth and relative to the mesocone of the laterals. The size and shape of the lateral teeth were normal for the small species of *Pararhytida*, but the number of teeth in each row (75) was higher.

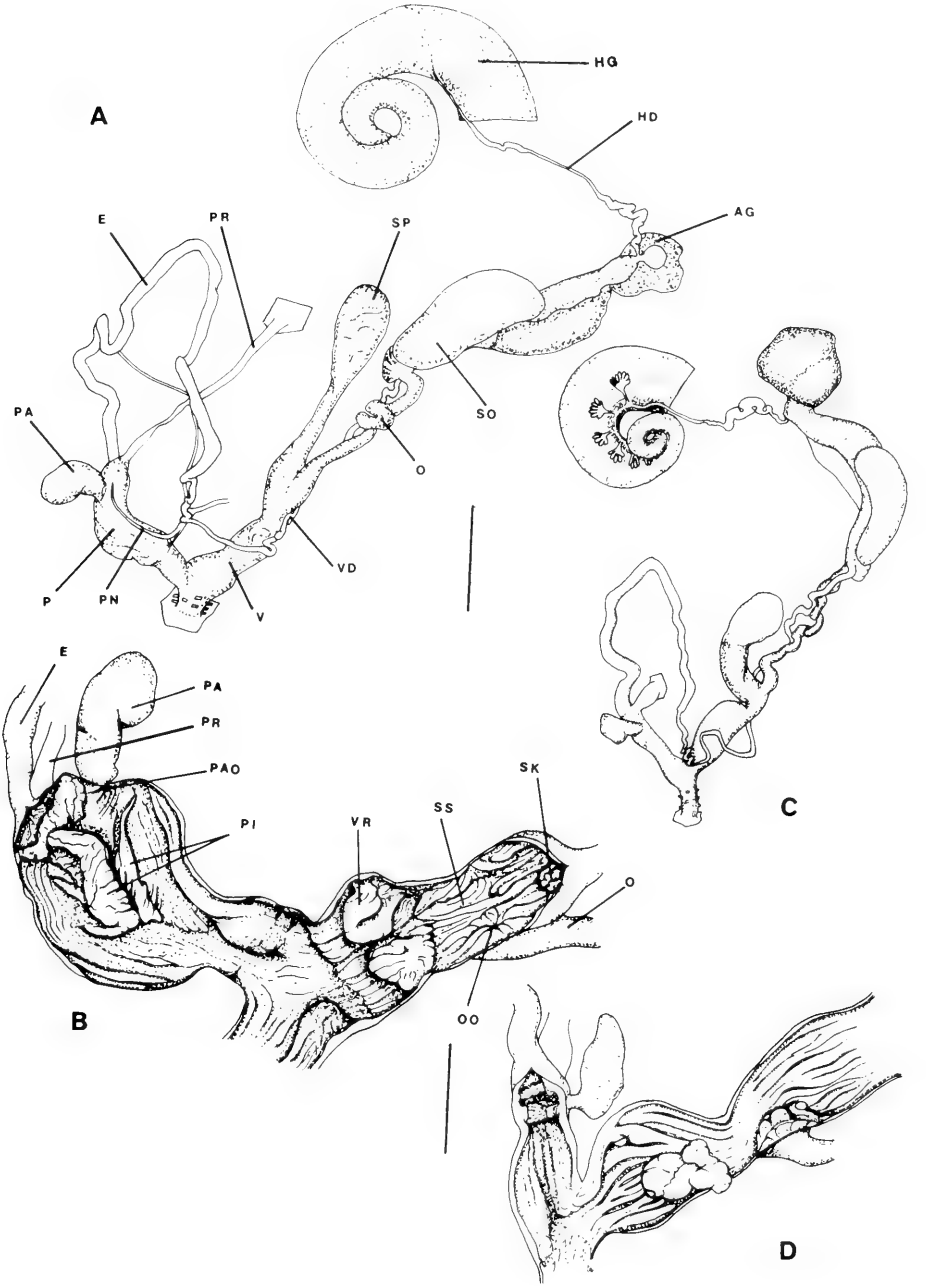


FIG. 39. Genital apparatus of *Pararhytida thyrophora* n. sp., stas. 57 and 58. A (sta. 58) and C (sta. 57), external morphology, scale lines both 5 mm. B (sta. 58) and D (sta. 57), internal morphology, scale lines both 2.5 mm. AG, albumen gland; E, epiphallus; HD, hermaphrodite duct; HG, hermaphrodite gland; O, oviduct; OO, oviducal opening; P, penis; PA, penial appendix; PAO, penial appendix opening; PI, penial pilasters; PN, penial nerve; PR, penial retractor; SK, spermathecal knob; SO, spermoviduct; SP, spermatheca; SS, spermathecal stalk; V, vagina; VD, vas deferens; VR, vaginal ridge.

Pulmonary complex

The lung cavity extends about 0.6 whorls in the dissected specimens (sta. 58). The rectal arm of the kidney is only slightly longer than the cardiac arm, and its length approaches one half of the lung length; only *P. dictyodes* has such a short lung, and no other species of *Pararhytida* has such a proportionally long kidney.

Genital apparatus

The penis is nearly as long as the vagina, and the free oviduct longer than the spermatheca (Figs. 39A, C). The shape of the latter is characteristic, with a pear-shaped head and a relatively thin stalk which is longer than the head. We were unable to count the lobes of the hermaphrodite gland accurately, but there were probably five or six.

Internally the penis has weak pilasters and a subapical verge at sta. 58 but not at sta. 57 (Figs. 39B, D), although in both cases the animals were fully mature. The vagina has an irregular transverse ridge which is almost developed into a full appendage. It is weaker than the appendage of *P. pyrosticta*, but better developed than in *P. phacoides.* It appears to be composed of two parts which partially interlock and occlude the vagina, and is situated at about the middle of the vaginal length. The spermathecal knob lies well above the oviducal opening, at about the mid-point of the spermathecal stalk. No spermatophore was found.

Discussion

The shell is more rounded in shape than any other small species, some *P. mouensis* excepted; in this last species, however, the shell is much thicker. In comparison with *P. marteli* the carination is less sharp and the apex more shallowly domed. Anatomically, the length and proportions of the pulmonary complex, and the shape of the spermatheca, allow easy species recognition. The insular situation of *P. thyrophora* makes the possibility of any significant gene flow with the mainland, and thus with other species of *Pararhytida*, seem highly unlikely.

ACKNOWLEDGEMENTS

We are extremely grateful to Dr. Alan Solem of the Field Museum of Natural History, Chicago, for the loan of material, and to Anne Thompson for assistance with the stereoscan photomicrography.

REFERENCES

ANCEY, C. F., 1882, Classification des formes helicoides de la Nouvelle-Calédonie. *Le Naturaliste*, 4: 85–87.

ANCEY, C. F., 1888, Nouvelles contributions malacologiques. *Bulletin de la Société Malacologique de France*, 5: 341–376.

BARGMANN, H. E., 1930, The morphology of the central nervous system in the Gastropoda Pulmonata. *Journal of the Linnean Society of London, Zoology*, 37: 1–59.

BAYNE, C. J., 1973, Physiology of the pulmonate reproductive tract: location of spermatozoa in isolated, self-fertilising succinid snails (with a discussion of pulmonate tract terminology). *Veliger*, 16: 169–175.

CROSSE, H., 1868, Description d'espèces terrestres inédites provenant de la Nouvelle Calédonie. *Journal de Conchyliologie,* 16: 146–164.

CROSSE, H., 1894, Faune malacologique terrestre et fluviatile de la Nouvelle-Calédonie et ses dépendances. *Journal de Conchyliologie*, 42: 161–473.

DAUTZENBERG, P., 1906, Description d'une nouvelle espèce terrestre Néo-Calédonienne. *Journal de Conchyliologie*, 54: 257–259.

DAUTZENBERG, P., 1923, Mollusques terrestres de la Nouvelle-Calédonie et des Îles Loyalty. *In*: SARASIN, F. & ROUX, J., *Nova Caledonia.* A. Zoologie, 3: 135–156.

EUTHYME, 1885, Description de quelques mollusques exotiques nouveaux. *Bulletin de la Société Malacologique de France*, 2: 237–260.

FISCHER, P., 1875, Note sur l'anatomie de l'*Helix dictyodes*, Pfeiffer. *Journal de Conchyliologie*, 23: 273–276, pl. 14, figs. 3–6.

FRANC, A., 1956, Mollusques terrestres et fluviatiles de l'Archipel Néo-Calédonien. *Mémoires du Muséum National d'Histoire Naturelle*, n.s., A, Zoologie, 13: 1–200, 24 pl.

GASSIES, J.-B., 1863, Faune conchyliologique terrestre et fluvio-lacustre de la Nouvelle-Calédonie. *Actes de la Société Linnéenne de Bordeaux*, 24: 211–330, 8 pl.

PFEIFFER, L., 1847, Descriptions of 38 new species of land-shells in the collection of Hugh Cuming. *Proceedings of the Zoological Society of London*, "1846": 109–116.

PILSBRY, H. A., 1894, Guide to the study of Helices. *Manual of Conchology.* Ser. 2: *Pulmonata*, 9: 366 p., 71 pl. Academy of Natural Sciences, Philadelphia.

PRESTON, H. B., 1907, Descriptions of nine new species of land-shells from New Caledonia. *Annals and Magazine of Natural History*, ser. 7, 19: 217–221.

REEVE, L. A., 1851–4, Monograph of the genus *Helix*. *Conchologia Iconica*, vol. 7. Reeve, London.

SOLEM, A., 1961, New Caledonian land and freshwater snails. An annotated check list. *Fieldiana Zoology*, 41: 419–501.

SOLEM, A., 1972, Malacological applications of scanning electron microscopy. II. Radular structure and functioning. *Veliger*, 14: 327–336.

SOLEM, A., 1976, *Endodontoid land snails from Pacific islands (Mollusca: Pulmonata: Sigmurethra)*. Part I. *Family Endodontidae*, 508 p. Field Museum of Natural History, Chicago.

SOLEM, A., 1983, *Endodontoid land snails from Pacific Islands (Mollusca: Pulmonata: Sigmurethra)*. Part II. *Families Punctidae and Charopidae, Zoogeography*, 336 p. Field Museum of Natural History, Chicago.

SOLEM, A., TILLIER, S. & MORDAN, P., 1984, Pseudo-operculate pulmonate land snails from New Caledonia. *Veliger*, 27: 193–199.

STARMÜHLNER, F., 1970, Ergebnisse der österreichischen Neukaledonien-Expedition 1965. Terrestrische Gastropoda I (excl. Veronicellidae und Athoracophoridae). *Annalen des Naturhistorischen Museums in Wien*, 74: 289–324.

Revised Ms. accepted 4 October 1985

MALACOLOGIA, 1986, 27(2): 243–247

GENETIC STUDIES OF BIPHALLIC *BIOMPHALARIA GLABRATA*

Charles S. Richards[1,2] & Dennis J. Minchella[3]

ABSTRACT

A biphallic *Biomphalaria glabrata* was observed with preputium, verge and vas deferens on the right as well as the left side. The snail was allowed to reproduce by self-fertilization and its offspring were followed for several generations. Despite selection, the biphallic condition continued to occur in about ¼ of the snails. The original mutant snail was mated in series to four normal albinos of different stocks of *B. glabrata*. Each cross resulted in some biphallic F_1s, and by selection some lines were derived showing essentially 100% frequency of biphallic snails. A dominant factor with low penetrance is postulated to be involved in the biphallic trait.

Key words: *Biomphalaria glabrata*; biphallic snails; genetics, molluscan; *Schistosoma mansoni*; selection.

INTRODUCTION

Genetic studies on *Biomphalaria glabrata* (Say) concerned with variations in patterns of susceptibility for infection with different strains of *Schistosoma mansoni* have involved snail crosses. Although pigmentation variations in *B. glabrata* have provided good genetic markers for crossing experiments (Richards, 1984) and competition studies (Minchella & LoVerde, 1983), other visible characters, particularly any demonstrating linkage with susceptibility factors, would be valuable.

Modifications of the reproductive system of *B. glabrata*, visible *in vivo*, have been described (Richards, 1974). Eversion of the preputium even in isolated snails varies in frequency in different *B. glabrata* stocks, apparently involving heritable factors. Abnormalities such as double, triple, or forked preputia also occur more frequently in some stocks. Eversion and abnormality of the preputium, associated with swollen tentacles, is inherited as a simple recessive character (Richards, 1974).

The genetic basis of the biphallic trait in *B. glabrata* was studied using selection via self-fertilization and crossing experiments. Snails were also exposed to two strains of *S. mansoni* to determine if the biphallic trait was associated with susceptibility factors.

MATERIALS AND METHODS

B. glabrata stocks and *S. mansoni* strains used have been described (Fletcher *et al.*, 1981; Richards, 1984). Snails isolated as juveniles were reared singly in 400 ml beakers and fed romaine lettuce. Juveniles were exposed individually to 5 miracidia per snail. Adults (determined by onset of egg-laying) were exposed to 25 miracidia per snail.

Snails of various ages, both fresh and preserved, were dissected in order to study the morphology of the biphallic trait. However, during the genetic studies, the presence or absence of a developing preputium on the right side was determined *in vivo* by examination with a binocular dissecting microscope.

During selection for the biphallic trait, only those snails expressing the trait were allowed to contribute to the next generation via selfing. In crossing experiments two snails were maintained together in a 400 ml beaker for one week, and then reisolated.

RESULTS

Morphology

Snails expressing the biphallic trait were dissected. The male genitalia on the right side developed from the external opening inward.

[1]Biomedical Research Institute, 12111 Parklawn Drive, Rockville, Maryland 20852, U.S.A.
[2]Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20014, U.S.A.
[3]Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907, U.S.A.

TABLE 1. Frequencies of biphallic snails in descendents of snail 831 following four serial crosses with normal albino stock snails (percentage in parentheses). The F_2 and F_3 generations were products of selection and self-fertilization.

Normal parent	F_1	F_2	F_3
243432....24	9/65 (13.8)	76/188 (40.4)	
4XR	7/69 (10.1)	85/312 (27.2)	
21539	4/32 (12.5)		
4132	6/14 (42.8)	15/75 (20)	41/100 (42)

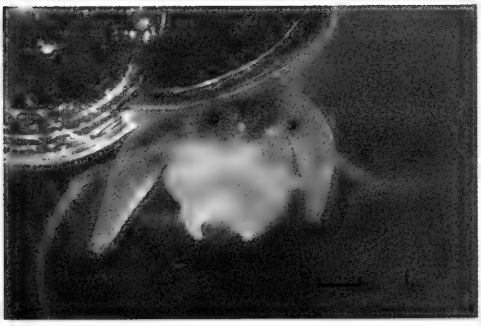


FIG. 1. Photomicrograph of biphallic snail 831, showing everted preputia. Scale line = 1 mm.

In adult snails the preputium, verge, verge sac and vas deferens appeared normal, but no connection with the rest of the reproductive system was observed in any of the dissected snails. The vas deferens terminated posteriad with a closed unattached end. Since the snail stock in which the biphallic condition occurred has the preputium (or preputia) everted most of the time, the biphallic condition was readily apparent in adult snails (Fig. 1).

Occurrence of biphallic snails in descendents of snail #83

After mating with snail 13142-10-1-684117, parent 13141-121116 produced hybrid F_1 #8 which appeared normal as did its offspring 83 produced by selfing. (For genealogical details of these snail stocks see Richards, 1984.) Three of four offspring of 83 by selfing isolated as juveniles survived. One of these (831) was biphallic, the other two (832 and 834) normal. All three snails produced progenies by selfing including some biphallic snails. Descendents of snail 831 were followed for four generations by self-fertilization of isolated snails and with selection for the biphallic condition in each generation. Snail 831 produced 5/28 (17.9%) biphallic offspring; the five biphallic snails produced 34/185 (18.4%) biphallic offspring; the third generation yielded 65/370 (17.6%) biphallic snails; the fourth generation 121/426 (28.4%). In succeeding generations there was no consistent indication of higher frequencies.

Crossing experiments

The original biphallic snail (831) after selfing was mated in series to four albino snails of different stocks. Since snail 831 was black-eyed with coalesced mantle pigment, albino snails were used in the crossing experiments so that it could be determined whether the progenies were hybrids or the products of self-fertilization. The results are shown in Table 1. Each albino parent produced some F_1s with the biphallic trait and these snails were selfed to produce future generations.

In the first cross F_1 #1 produced 22/71 (31%) biphallic F_2s by selfing. F_1 #1 was then backcrossed to one of the offspring by pre-cross selfing of the albino parent (1221124-1) (Fig. 2). This albino produced 20/72 (27.8%) hybrid biphallic offspring. The offspring of F_1 #1 by selfing were followed through several generations, including lines of three pigment phenotypes: black-eyed snails with coalesced mantle pigment ($c^bc^bS^dS^d$), black-eyed snails with spotted mantle (c^bc^bSS) and albino snails (cc—) (Richards, 1984). In all three pigment phenotypes selection resulted in lines showing high biphallic frequencies. One line (1-19-56) was maintained since it demonstrated a pattern of *S. mansoni* susceptibility not previously observed in our snail stocks: juvenile susceptible/adult nonsusceptible to both PR-1 and PR-2 (Richards, 1984).

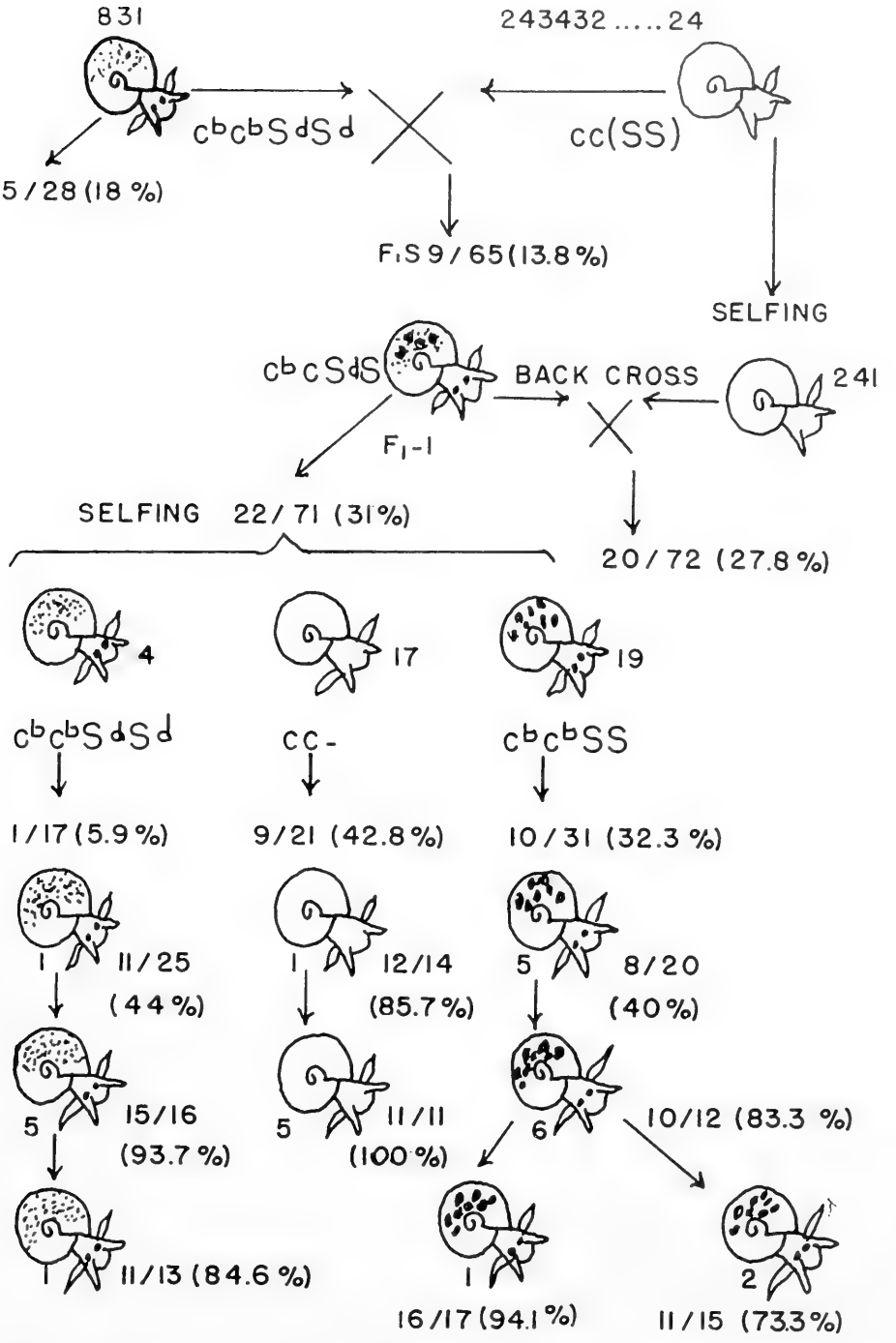


FIG. 2. Diagram showing frequencies of biphallic snails; in three lines from F_1 #1, by pre-backcross selfing, and from a backcross between F_1 #1 and a normal stock snail.

Susceptibility to *S. mansoni*

The parental stocks from which snail 83 was derived differed in their susceptibilities for *S. mansoni* strains PR-2 and PR-T-13. Snail stock 13141 was susceptible to PR-T-13 but not to PR-2, while stock 13142-10-1-6 was susceptible to PR-2 but not PR-T-13. Snail 831 was exposed as an adult to PR-T-13 with negative results, snail 832 to PR-T-13 with positive results, and snail 834 to PR-2 with positive results. Each of these snails by selfing produced lines giving consistent susceptibility results: lines 831 and 834 were PR-2 susceptible and PR-T-13 nonsusceptible, while line 832 was PR-2 nonsusceptible and PR-T-13 susceptible.

DISCUSSION

The results presented suggest that the biphallic condition is inherited. The first snail in which this was observed, 831, involved snail lines that had been followed as individuals reared in isolation and reproducing by selfing through many generations, except when controlled crosses were made. The biphallic condition had never been observed before in our snail stocks. A mutation is thus suggested. Three sibling snails (831, 832, 834) all produced progenies including biphallic snails. This could result either from a mutation in their parent snail (83), or in interbreeding of the three snails before isolation. In studies on size of *B. glabrata* at maturity (Richards & Merritt, 1975) onset of egg-laying had been observed at snail diameters as small as 5 mm. Snail stocks involved in studies reported here were relatively small. If snails 831, 832, and 834 mated before isolation, a mutation in 831 might explain the results. *S. mansoni* susceptibility results, however, suggested that the three snails had not interbred, since each gave rise to a snail line showing a consistent susceptibility pattern. Snails 831 and 832 differed in susceptibility to PR-T-13 and gave rise to lines showing reverse susceptibilities: 831 susceptible to PR-2, nonsusceptible to PR-T-13; 832 susceptible to PR-T-13, nonsusceptible to PR-2. From these results it is concluded that a mutation for the biphallic condition occurred in the parent snail (83).

The descendents of biphallic snail 831 were followed for several generations by isolating biphallic individuals and allowing them to reproduce by self-fertilization. Although frequencies of the biphallic condition increased from 17.9% to 28.4%, they continued to vary unpredictably.

When biphallic snail 831 was mated with normal stock snails, some hybrid F_1s of the normal parents were biphallic, suggesting involvement of a dominant factor with low penetrance. When descendents of the first cross were followed through several generations, with selection, several lines were derived showing consistently high biphallic frequencies. One line had a stable frequency of 100% biphallic individuals indicating that the low penetrance may be improved by selection.

The genetics of the biphallic condition are not simple. The biphallic trait did not show an association with susceptibility to the two strains of *S. mansoni* tested, and variation in expression of the trait makes it a relatively poor genetic marker. The biphallic condition may be regulated by a dominant factor with low penetrance which can be improved by selection. This study contributes to our understanding of the genetics of *Biomphalaria*. However additional genetic studies will be required in order to investigate rationally the interactions between *Biomphalaria* and its schistosome parasites.

ACKNOWLEDGMENTS

The technical assistance of Mr. Paul C. Shade is gratefully acknowledged. These studies were funded in part by Office of Naval Research Contract N1.N00014-78-C-0081.

LITERATURE CITED

FLETCHER, M., LoVERDE, P.T. & RICHARDS, C.S., 1981, *Schistosoma mansoni*: electrophoretic characterization of strains selected for different levels of infectivity to snails. *Experimental Parasitology*, 52: 362–370.

MINCHELLA, D.J. & LoVERDE, P.T., 1983, Laboratory comparison of the relative success of *Biomphalaria glabrata* stocks which are susceptible and insusceptible to infection with *Schistosoma mansoni*. *Parasitology*, 86: 335–344.

RICHARDS, C.S., 1974, Everted preputium and swollen tentacles in *Biomphalaria glabrata*: genetic studies. *Journal of Invertebrate Pathology*, 24: 159–164.

RICHARDS, C.S., 1984, Influence of snail age on genetic variations in susceptibility of *Biomphalaria glabrata* for infection with *Schistosoma mansoni*. *Malacologia*, 25: 493–502.

RICHARDS, C.S. & MERRITT, J.W., 1975, Variation in size of *Biomphalaria glabrata* at maturity. *Veliger*, 17: 393–395.

Revised Ms. accepted 13 September 1984

MALACOLOGIA, 1986, 27(2): 249–263

FACTORS REGULATING OVIPOSITION IN *BULINUS TROPICUS* IN SNAIL-CONDITIONED WATER

M. Asghar Chaudhry & Elfed Morgan

Department of Zoology and Comparative Physiology, University of Birmingham, P.O. Box 363, Birmingham, B15 2TT, United Kingdom

ABSTRACT

Ageing of the culture medium results in inhibition of oviposition in *Bulinus tropicus*, but the effect does not seem to be due to changes in the oxygen tension or ionic composition of the rearing water. Medium conditioned by closely related species also inhibits egg laying but that of taxonomically more remote snails has no such effect. Three week old snail-conditioned water retains its inhibitory property after dialysis but artificial media of similar inorganic ion concentration, with or without lettuce extract are not inhibitory. The results are consistent with the suggestion that some inhibitory compound is produced by the snails themselves but conspecific faecal homogenates do not have this inhibitory property. Bioassay of different fractions of the culture medium, separated by ultra-filtration, suggests that the inhibitory compound has a molecular weight of less than 500.

Key words: *Bulinus tropicus*; oviposition; inhibition; closed cultures.

INTRODUCTION

A progressive decline in the oviposition of freshwater snails maintained in closed cultures, and its subsequent acceleration on replacement of the medium has been reported by various workers for a number of different snail species (Wright, 1960; van der Steen, 1967; Thomas, 1973; Kits & ter Maat, 1982). The onset of oviposition is often immediate and predictable, and it is likely that some change in the composition of the holding water is involved. Various factors have been implicated. For example, Mooij-Vogelaar & van der Steen (1973) have suggested that inhibited oviposition in fresh water pulmonate snails may be due to the depletion of dissolved oxygen in unchanged culture water, a point of view shared by Kits & ter Maat (1982). Alternatively the depletion of calcium or the accumulation of excretory ammonia may limit the oviposition of *Biomphalaria glabrata* (Thomas, 1973; Thomas & Benjamin, 1974a, b and Thomas *et al.*, 1974), and indirect evidence for the release of an oviposition-inhibiting substance appears frequently in the literature. For instance Wright (1960) showed that growth and oviposition in *Bulinus forskalii* was inhibited by conditioned water from a crowded conspecific culture but the inhibitory effect was lost if the same water was allowed to stand over activated charcoal for 24 hours. An inverse relationship between the densities of adult and young snails has also been found under field conditions in *Lymnaea elodes* (Eisenberg, 1966) and Wright (1960) suggested that under crowded conditions fecundity was inhibited by some chemical substance released by snails. Essentially similar results have been reported by Berrie & Visser (1963) and Levy *et al.* (1973) and recent studies on a terrestrial pulmonate, *Theba pisana* have also revealed a negative feedback effect on growth and oviposition consistent with the release of an inhibitory substance under crowded conditions (Lazaridou-Dimitriadou & Daguzan, 1981). Berrie & Visser (1963) and Levy *et al.* (1973) were able to isolate inhibitory compounds from water containing crowded populations of *Biomphalaria sudanica* and *Fossaria cubensis* respectively, and Gazinelli *et al.* (1970) have suggested that snail faeces may be the source of a similar inhibitory substance in *B. glabrata.*

However, as Thomas (1973) has pointed out, the snail-conditioned environment is a complex mixture of both organic and inorganic molecules originating from the snails themselves and from their decaying plant food and the source of such an inhibitory substance is difficult to predict. Indeed it may be argued that the case for the existence of an inhibitory compound *per se* is far from

definitive (Thomas, 1973; Thomas, Lough & Lodge, 1975). Both promoting and inhibiting effects have been reported on the growth of undivided snails (Thomas, Goldsworthy & Aram, 1975; Thomas & Benjamin, 1974b) and bioassay of fractions separated by ultrafiltration techniques suggests that several growth factors may be involved (Thomas, Goldsworthy & Aram, 1975).

The present work investigates the factors regulating oviposition in *Bulinus tropicus* (Krauss) maintained in different culture media in the laboratory, and the results of a preliminary attempt to isolate the inhibitor using ultrafiltration membranes are described.

MATERIAL AND CULTURE METHODS

Bulinus tropicus was selected from laboratory stock in the Department of Zoology and Comparative Physiology at the University of Birmingham, derived from samples provided by Dr. C.A. Wright at the British Museum (Natural History). The snails were kept in shallow plastic trays (35 + 24 + 5 cm deep) containing fish-conditioned water, i.e. water taken from 15 litre tanks containing five juvenile fishes of the species *Cichlasoma nigrofasciatum*, the water in these holding tanks being replenished periodically with Birmingham tap water, dechlorinated by standing.

The snails were maintained under a LD 12:12 lighting regimen in a controlled environment room at 24 ± 1°C, the trays being covered with glass plates to reduce evaporation, and the animals fed daily on dried scalded lettuce given in excess.

For experimental purposes the snails were transferred to enamelled pie dishes (24 + 14 + 5 cm deep) covered with perforated cling film, with 5 snails to 400 ml of fish-conditioned water unless otherwise stated. All experiments were carried out in the snail culture room where the animals had been kept for over 30 generations. Other experimental details are indicated where relevant in the text.

STUDIES ON THE BEHAVIOUR OF SNAILS IN UNCHANGED MEDIUM

The locomotor activity, feeding and egg laying behaviour of *B. tropicus* maintained in closed cultures all decrease with time in unchanged media. To investigate these changes in behaviour 15 snails were transferred to 900 ml of fish-conditioned water in a small perspex tank and their feeding and locomotor activity recorded by direct observation for a period of 8 min each morning, afternoon and evening for three weeks, using the scoring method described by Chaudhry & Morgan (1983). The relationship between egg laying and water change was investigated in another experiment in which the oviposition rate of snails kept in an unchanged medium was compared with that of those for which the water was changed weekly. Six replicate dishes (5 snails per 400 ml) were divided into two equal groups and the numbers of eggs produced recorded over a five week period. In one group the water was changed just once, at the end of the third week of the experiment, while that of the other, control, snails was replaced each week with fish-conditioned water.

Results and Discussion

The mean numbers of animals feeding or actively crawling were higher during the first week of the experiment (Fig. 1) but both activities declined gradually during weeks two and three in unchanged cultures. By the end of this period nearly all the animals were found to be inactive on the sides or bottom of the dish. Control animals, for which the water was changed each week showed no such progressive decline in feeding behaviour and locomotor activity. Egg laying was similarly attenuated (Fig. 2). During the initial week of the experiment the rate of oviposition was the same in both experimental and control groups but where the water remained unchanged egg laying declined rapidly during the second and third weeks. Replacing the culture water resulted in a significant increase in fecundity which again declined when the water was unchanged. In contrast, changing the culture water each week during the five week period resulted in an almost constant rate of oviposition.

These changes in behaviour are predictable and presumably are precipitated by differences in some physical or chemical properties of fish-conditioned water and the snail culture medium it replaces. Both water bodies had equilibrated to room temperature in the snail room where the animals were kept, and

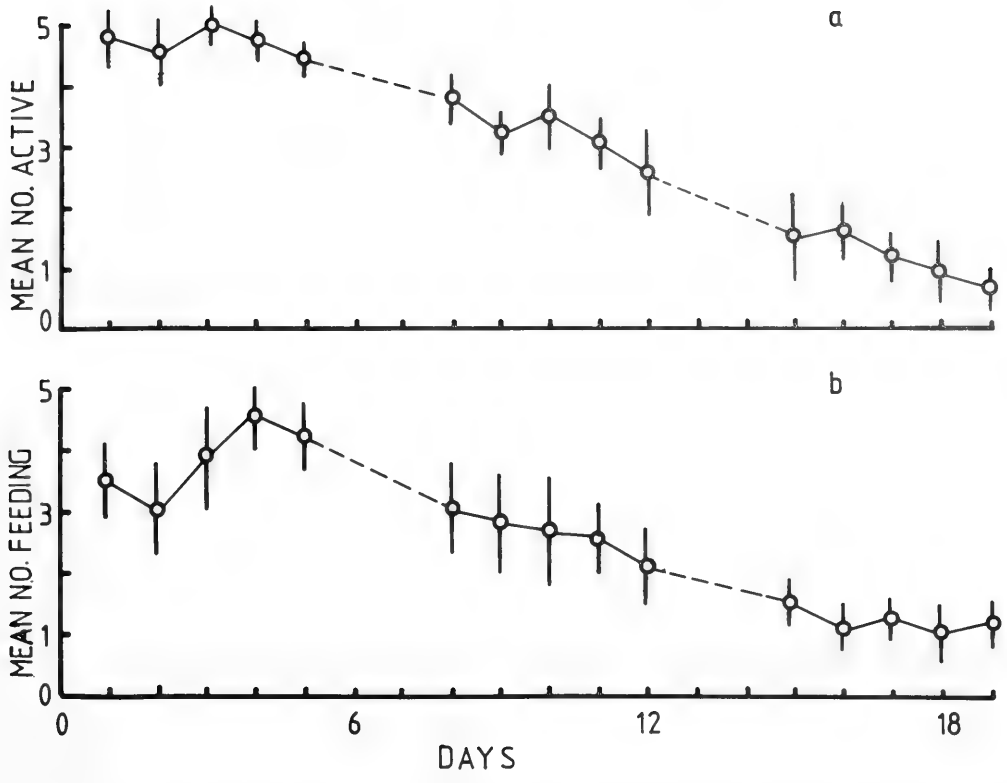


FIG. 1. The locomotor and feeding activity (a and b respectively) of 15 *B. tropicus* snails recorded daily (Monday to Friday) for a period of three weeks in unchanged cultures. The points indicate mean values obtained from three experiments, and the vertical bars show the standard error values (98% confidence limits = 2 × S.E.).

although the temperature was not measured during the experiments it is unlikely that changes in this parameter were responsible for the changes in behaviour recorded.

The snails themselves will modify their medium in many ways. Oxygen and essential minerals will be removed from the water, while the products of metabolic waste will accumulate. Other compounds of organic and inorganic nature will be introduced into the medium in the food, and the results described above could equally be attributed to a depletion of resources or to the accumulation of some inhibitory factor in the medium.

These possibilities have been investigated in a series of experiments in which changes in the rate of oviposition have been used as a bioassay.

IS INHIBITION DUE TO REDUCED OXYGEN TENSION OR CHANGES IN INORGANIC ION CONCENTRATIONS?

The possibility that the results described above (Fig. 2) could be due to changes in oxygen tension was investigated in an experiment in which 30 actively laying snails were divided into two groups in two lots of three dishes each containing five snails. In all experimental dishes the water remained unchanged during the first three weeks, after which time it was replaced with fish-conditioned water, but in one group of three it was aerated by bubbling with compressed air throughout the experiment. The oxygen level of the culture water was deter-

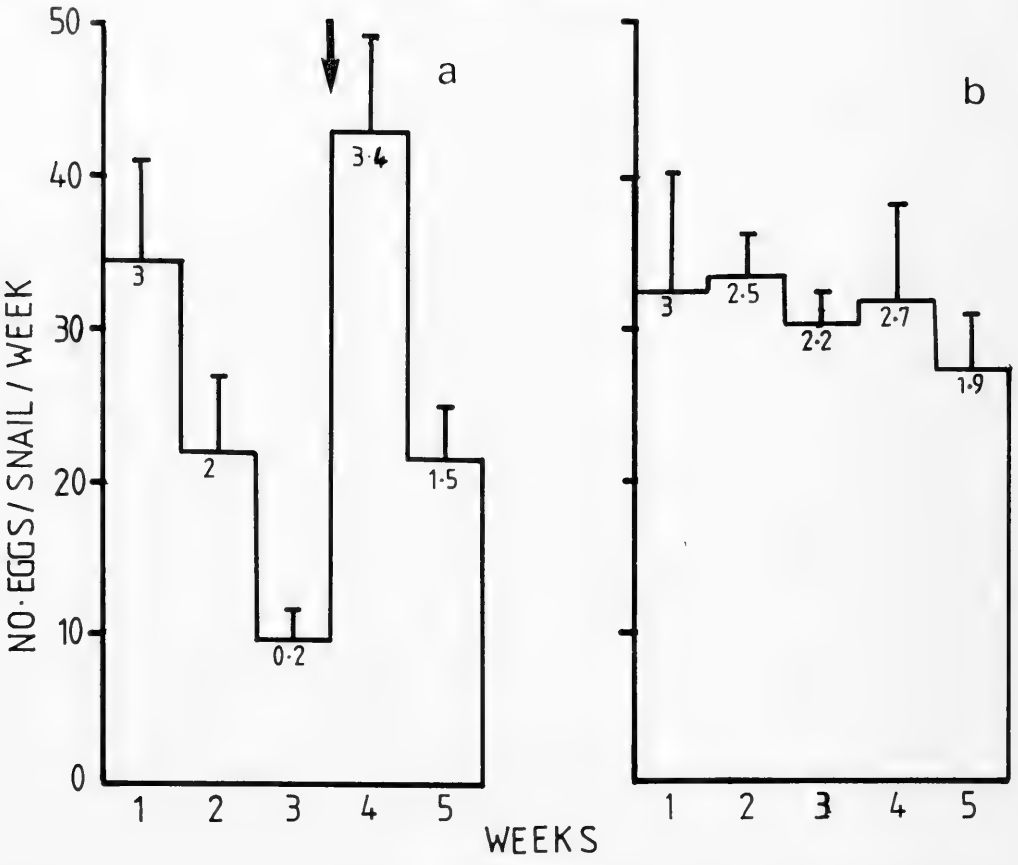


FIG. 2. The oviposition of two groups of 15 *B. tropicus* snails. In (a) the water was replaced completely at the beginning of the 4th week as indicated by the arrow, while in (b) the water was changed weekly. Figures at the column heads indicate the total number of egg masses per snail, and the vertical bars indicate the standard error values derived from three experiments.

mined weekly, 2 ml water samples being taken from each dish and analysed with a Radiometer (PHM71) electrode calibrated with water of known PO_2 at 24°C. The number of egg masses produced in each dish was recorded daily.

In another series of experiments the possible influence of changes in inorganic ions was investigated. The ionic composition of the culture media was ascertained by analysing 15 ml aliquots removed from the experimental dishes. The water sample was replaced with an equal volume of medium taken from an equivalent dish of similar age and snail density. Preliminary analysis using conventional spectrophotometric and volumetric techniques (see A.O.A.C. handbook, 1975) showed the main cations present in snail-conditioned water to be Na^+, K^+, Mg^{++}, Ca^{++}, and NH_2^+, other elemental ions being present only in trace quantities, while the major anions were found to be Cl^-, NO_3^- and HCO_3^-. Changes in the concentration of one or more of these ions could, in theory, cause a decrease in the oviposition rate of *B. tropicus*, and this possibility has been investigated in three different series of experiments.

In the first of these three replicate dishes were set up, each containing 5 reproductive *B. tropicus* snails in 400 ml of fish-conditioned water, and the pH, ammonia and mineral content of the water were recorded at the beginning of the experiment, and each week

over a period of three weeks, the ammonia concentration being determined by the A.O.A.C. (1975) method while the metallic cations were measured by atomic absorption spectrophotometry. The pH was measured using a Radiometer (Copenhagen) pH meter.

No attempt was made to monitor changes in anion content during the conditioning period. Instead the possible involvement of free anions in the regulation of oviposition was investigated in two series of experiments involving the bioassay of artificially prepared media, and of dialysed snail-conditioned water. In the first of these, artificial media were prepared by dissolving known weights of NaCl, MgO, $Ca(NO_3)_2$ and NH_4NO_3 in deionised water to produce the ionic equivalent of fish-conditioned water or of three week old snail-conditioned water. A sample of dried, scalded lettuce was macerated and added to 400 ml of the latter medium which was kept for three weeks at 27°C to allow for the possible introduction of other ions into the culture through the decay of uneaten food. A further sample of lettuce infusion was made up using fish-conditioned water. Five groups of snails were set up, each with three dishes of 5 snails in 400 ml of fish-conditioned water. The water of all snails was replaced with new fish-conditioned water at the end of each of the first two weeks of the experiments. At the end of the third week the water in two groups of snails was replaced with artificial three week old snail conditioned water or with fish-conditioned water, in both of which partially homogenized lettuce had been allowed to stand for 3 weeks. Two further groups were transferred to artificial media containing the ionic equivalents of three week old snail-conditioned water alone, or of unused fish-conditioned water. Between weeks four and five the water of these four groups remained unchanged. The fifth group served as a control and its medium was replaced with natural fish-conditioned water at the end of each week throughout the experiment. Oviposition was noted daily for each group.

In the third series of experiments, 15 mature snails were maintained in 900 ml of fish-conditioned water which was circulated (20 ml min^{-1}) through a dialysis tube immersed in a bath of fish-conditioned water. This was changed daily. Two similar populations served as controls. In one of these the water was replaced weekly while in the other it remained unchanged throughout the experiment. Each group consisted of three replicates, and the concentrations of the major inorganic cations and the pH of the dialysed culture medium were monitored weekly to confirm the efficacy of the dialysis.

Results and Discussion

In the present investigation (Fig. 3a) the level of oxygen in the undisturbed medium was considerably reduced during the three week conditioning period. Nevertheless the depletion of oxygen *per se* does not seem to have limited the oviposition of *B. tropicus* in the above experiments. Egg laying was similarly inhibited in the parallel group of snails in which the oxygen tension of the culture water was maintained at a constant level, or even increased slightly by bubbling with compressed air (Fig. 3b). Replacing the culture water at the end of the third week resulted in a marked increase in oviposition rate in both groups.

The ionic composition of the undisturbed media altered gradually during the conditioning period (Table 1). The concentrations of Na^+, K^+, MG^{++} and NH_4^+ increased progressively, presumably introduced into the system in food, or as the byproducts of metabolism, while the Ca^{++} content decreased significantly ($P < 0.001$, Anova) from 0.43 to 0.14 m mol l^{-1} over the three weeks. Similarly the pH of the snail-conditioned water decreased significantly during this period ($P < 0.01$). It is unlikely that changes in these cations are responsible for the observed decrease in oviposition. Transfer to artificial environments equivalent to three week old snail-conditioned water with or without lettuce extract did not inhibit oviposition in actively laying *B. tropicus* (Fig. 4), and the laying rates in these media were comparable to those in which the water was replaced at the end of week 3 with fish-conditioned water or an artificial medium of similar ionic composition. The decrease in oviposition seen during the fifth week of this experiment is clearly different from the almost complete inhibition which follows transfer to snail-conditioned water (e.g. compare Fig. 4 with Fig. 2a, Fig. 3c, d) and probably reflects a depletion of physiological resources.

Furthermore, the oviposition of snails kept in dialysed culture media showed, over the first three weeks of the experiments, an exponential decrease characteristic of snails kept in closed culture (Fig. 5). Changing the

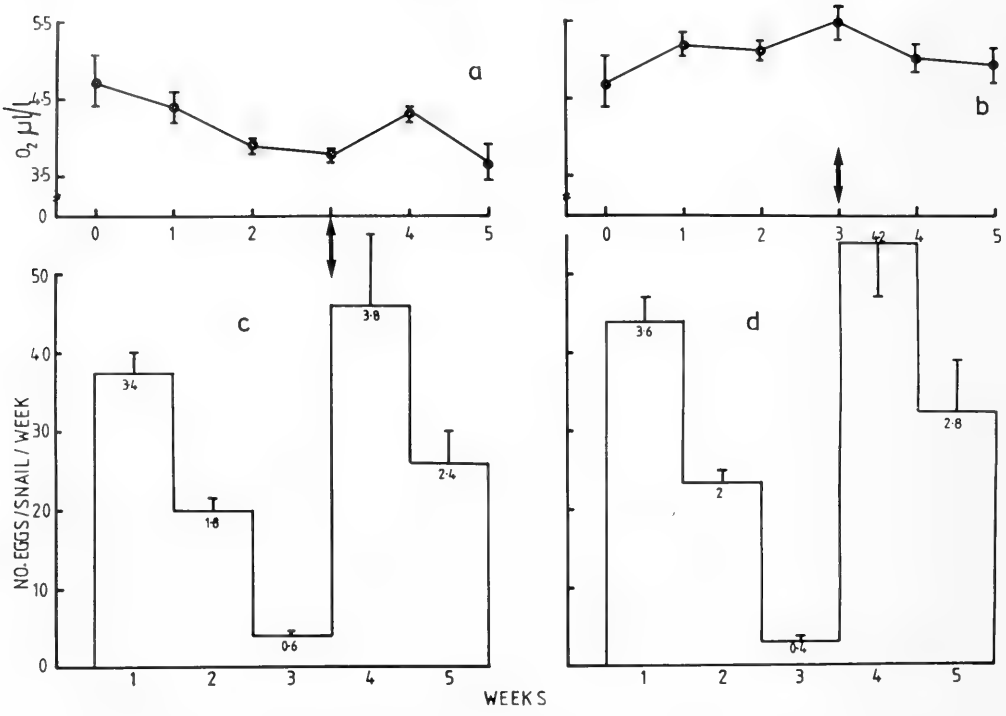


FIG. 3. Oxygen levels (a and b) and the oviposition rate (c and d) of *B. tropicus* cultures in non-aerated and aerated water respectively. The arrows indicate the time of replacement of both culture media with fish-conditioned water. Otherwise legend as for Fig. 2.

TABLE 1. Ionic concentration of unchanged *B. tropicus* culture media (5 snails per 400 ml of fish-conditioned water), measured at the start of the experiment, and at weekly intervals thereafter.

Week	N^+ (m. mol/l)	K^+ (m. mol/)	Ca^{++} (m. mol/l)	Mg^{++} (m. mol/l)	NH_4^+ (ppm)	pH
0	0.3 ± 0.02	0.045 ± 0.07	0.44 ± 0.03	0.07 ± 0.01	0.09 ± 0.06	7.3 ± 0.05
1	0.48 ± 0.02	0.47 ± 0.14	0.40 ± 0.06	0.11 ± 0.02	2.01 ± 0.27	7.03 ± 0.05
2	0.426 ± 0.60	0.60 ± 0.04	0.32 ± 0.08	0.16 ± 0.03	3.48 ± 0.36	7.06 ± 0.15
3	0.79 ± 0.10	1.35 ± 0.30	0.14 ± 0.09	0.19 ± 0.04	4.14 ± 0.27	6.8 ± 0.20

water of the dialysed group at the end of the third week increased their oviposition rate, while control snails for which the culture water was replaced weekly remained equally fecund throughout. Unlike unchanged culture media the concentration of the major cations and the pH of the dialysed water remained constant during the experiment (Table 2).

The anion composition of the water will also change during the conditioning of unchanged culture media but again the inhibition of egg laying cannot be attributable to these changes, at least in so far as the major inorganic anions are concerned. Like the cations the anions equilibrate when dialysed against fish-conditioned water, but as may be seen from Fig. 5, oviposition still declined under these conditions. On the other hand transfer to artificial media enriched with chloride and nitrate did not impair egg laying (Fig. 4e, d).

It would appear therefore that inhibition is effected by the accumulation of some other substance, possibly of organic nature, in the culture medium. As plant food extract was found to be without effect it is likely that this compound is produced by the snails themselves.

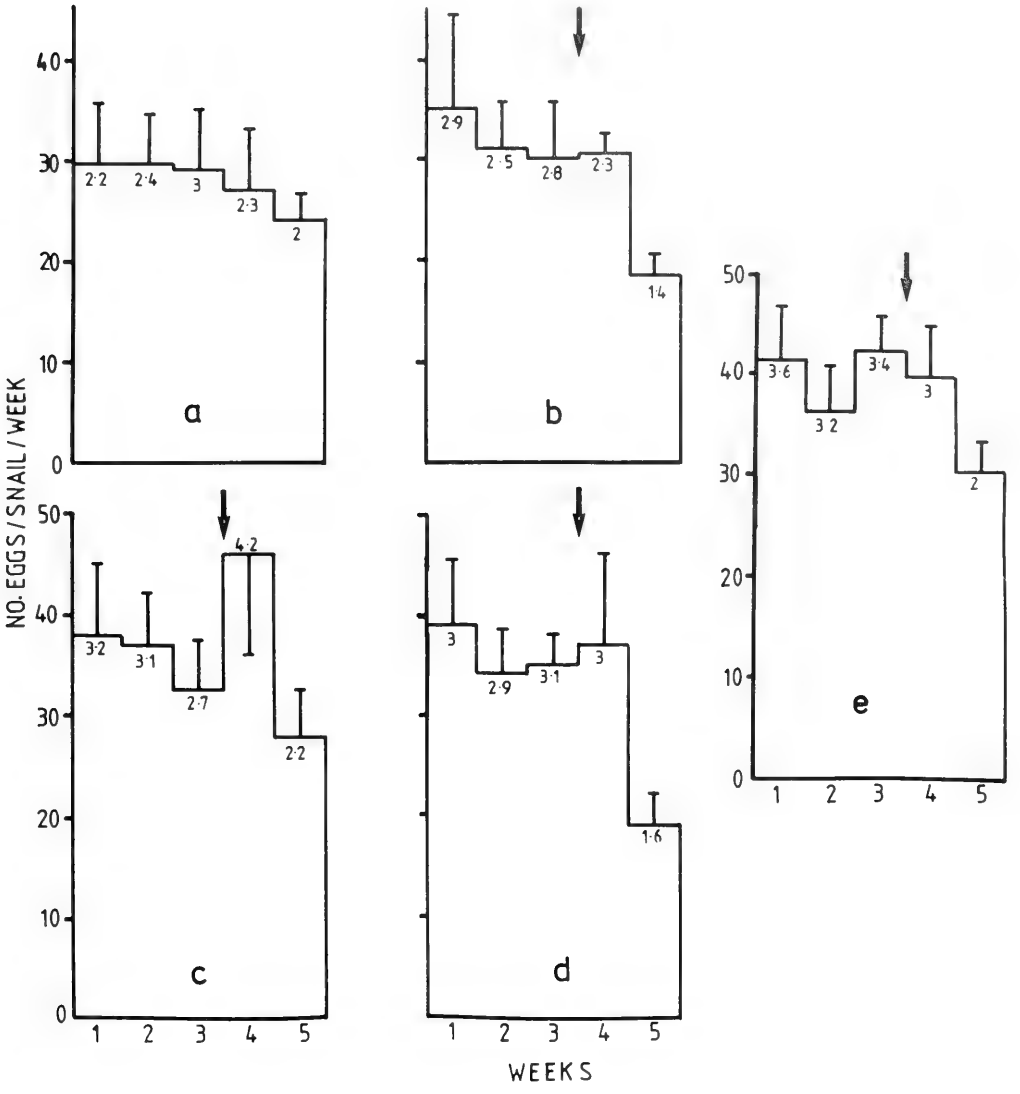


FIG. 4. The oviposition rate of five groups, each of 15 *B. tropicus* snails in different culture regimens. Fig. 4a shows the results obtained with a control group of snails for which the water was replaced with new fish-conditioned water each week throughout the experiment. Other groups were transferred at the end of week three to artificial media equivalent to three week old snail-conditioned water, with or without lettuce extract (c and d respectively), or to artificial fish-conditioned water similarly lacking (b) or containing (e) lettuce extract. The times of transfer of the snails to the different experimental media are indicated by the arrows. Otherwise legend as for Fig. 2.

SPECIES SPECIFICITY OF CONDITIONED MEDIA

The specificity of inhibition with regard to egg laying in *B. tropicus* was investigated using water conditioned by mature specimens of three different fresh-water pulmonates, *Bulinus globosus*, *Biomphalaria glabrata*, *Physa* sp. and by the prosobranch *Melanoides tuberculata*, each species being

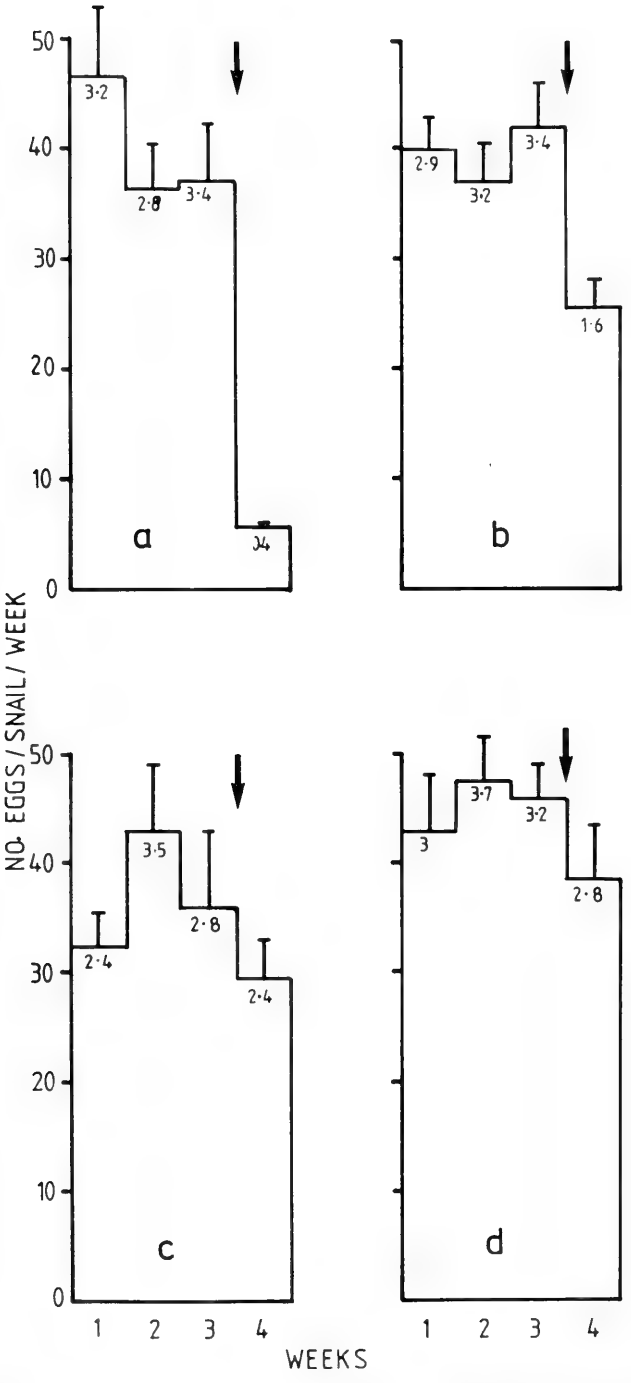


FIG. 5. The oviposition rate of 4 groups, each of 15 *B. tropicus* snails, after transfer to media conditioned by heterospecific snails. In each group the water was replaced weekly with fish-conditioned water for the first three weeks, after which the water was replaced with media conditioned by *Bulinus globosus*, *Physa* sp., *Biomphalaria glabrata* and *Melanoides tuberculata* (a to d respectively). The time of transfer is indicated by the arrows. Otherwise legend as for Fig. 2.

TABLE 2. Ionic concentrations of dialysed culture media of *B. tropicus* (15 snails/900 ml of fish-conditioned water) recorded at the start of the experiment and at weekly intervals thereafter.

Week	Na^+ (m. mol/l)	K^+ (m. mol/l)	Ca^{++} (m. mol/l)	Mg^{++} (m. mol/l)	NH_4^+ (ppm)	pH
0	0.3	0.04	0.44	0.08	0.09	6.9
1	0.24	0.22	0.45	0.10	0.25	6.7
2	0.32	0.20	0.42	0.10	0.29	6.8
3	0.25	0.23	0.41	0.10	0.31	6.5

maintained at a density of five snails in 400 ml of water for a period of three weeks. Four groups of *B. tropicus*, selected randomly from laboratory stock were transferred, five to a dish containing 400 ml of fish-conditioned water, and their media replaced weekly for the first three weeks. At the beginning of the fourth week each group was transferred to one of the heterospecifically conditioned media described above, the oviposition rates of each group being recorded weekly. All snails were fed dried, scalded lettuce *ad libitum* during the conditioning and experimental periods, and each experiment was replicated three times.

Results and Discussion

The inhibitory properties of heterospecifically-conditioned media appear to be related to the taxonomic affinities of the species involved. Media conditioned by *Bulinus globosus* and *Physa* sp. significantly reduced the oviposition rate of *B. tropicus* (Fig. 6), the latter being rather less effective (Anova, $P < 0.01$ and < 0.05 respectively). Media conditioned by *Biomphalaria glabrata* lowered the oviposition rate of *B. tropicus* but not significantly so, and water conditioned by *Melanoides tuberculata* also failed to inhibit. Inhibitory secretions have been postulated to account for the success of *Helisoma duryi* in competition with other helminthologically important snails (see references in Madsen, 1982), and for other fresh-water pulmonates (e.g. Levy *et al.*, 1973) and the results obtained here support this hypothesis. Madsen (1979a, b), investigating the competition between *Helisoma duryi* and *Biomphalaria* sp. in the laboratory, found many unhatched egg masses in older aquaria, but as the effect showed no species specificity, he concluded that the factors responsible may have originated from the food or metabolic waste. He subsequently attributed the success of *Helisoma* to direct interaction (Madsen, 1982), but did observe inhibitory effects in newly established aquaria with low densities of adult snails, conditions which may have approximated to those of the present experiments.

FRACTIONATION OF SNAIL-CONDITIONED MEDIUM

An attempt was made to separate the inhibitory fraction by filtration and ultra-filtration techniques. In a preliminary experiment three groups of actively laying snails were maintained, 5 per 400 ml of fish-conditioned water for three weeks, and the medium was changed weekly. The faeces were then removed by filtration, homogenized and made up to 400 ml with fish-conditioned water for bioassay. Three week snail-conditioned water from which the faeces had been filtered was similarly bioassayed while in another experiment 3 further groups of 5 snails were kept in unchanged culture for 5 weeks and the medium bubbled over activated charcoal for 5 min at the end of each week when oviposition was recorded.

In a further series of experiments three-week conditioned medium was concentrated by evaporation at room temperature and filtered through Whatman No. 1 filter paper, and Gelman metrical autoclave and Millipore 0.22 μm membranes to remove faecal material and bacteria respectively.

Further fractionation was achieved by ultrafiltration using Millipore Pellicon (PT series) and Amicon Diaflo membranes in magnetically stirred cells pressurized with nitrogen to 10 p.s.i.

The active fraction was subjected to high pressure liquid chromatography (HPLC) when one litre of three-week snail-conditioned water was concentrated by freeze-drying after the initial filtration. The

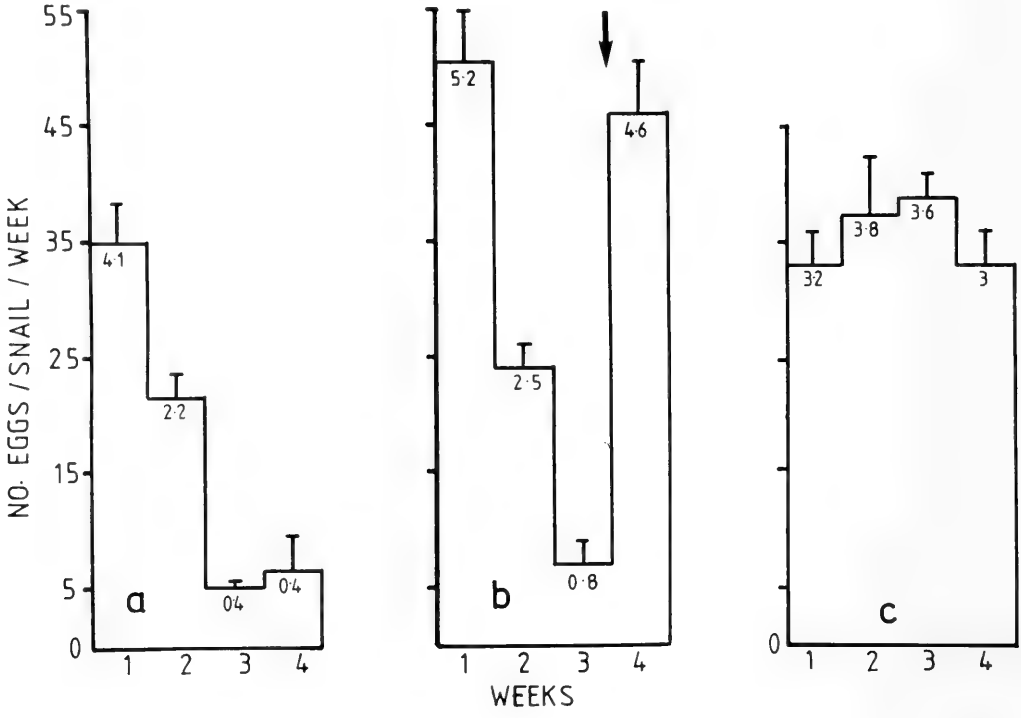


FIG. 6. The oviposition rate of *B. tropicus* in unchanged culture medium (a), in dialysed medium replaced with fish-conditioned water when indicated by the arrow (b), and replaced weekly with fish-conditioned water (c). Otherwise legend as for Fig. 2.

residue was dissolved in 25 ml of deionized water before filtering through an Amicon UM-05 membrane with a 500 MW cut off, the filtrate being again freeze-dried and redissolved in 4 ml of distilled water to obtain a stock solution. Part of this solution was diluted with fish-conditioned water to obtain a stock solution and the remainder subjected to HPLC analysis. The chromatography column (25 + 4.5 mm) was packed with 'Spherisorb 50 DS' and the sample spun at 1.5 ml min^{-1} at 3000 p.s.i. and at room temperature. The eluents were scanned with a uv detector (Spectromonitor III) at 210 nm and the peaks recorded at 500 mm/h. Polar and non-polar fractions were eluted with distilled water and 30% methyl cyanide (acetonitrile) respectively and the two fractions bioassayed as before. The solvent in the latter fraction was rotary-evaporated at 34°C and freeze-dried prior to assay.

Results and Discussion

Faecal homogenates are without effect on the oviposition of *B. tropicus* (Fig. 7). Faecal homogenates have been shown to both accelerate and retard the growth of *Biomphalaria glabrata*, the effect being determined by the diet of the donor snail (Thomas, Lough & Lodge, 1975), and Gazinelli *et al.* (1970) have reported an active component in the faeces of this species which inhibited the uptake of ^{59}Fe, and presumably growth. Oviposition in *B. glabrata* however, is unaffected by faecal material (Thomas, Lough & Lodge, 1975).

The oviposition rate of snails transferred to faecal homogenate is not significantly lower than in the first three weeks of the experiment when the water was replaced weekly with fish-conditioned water alone. In contrast the oviposition of snails transferred to three week

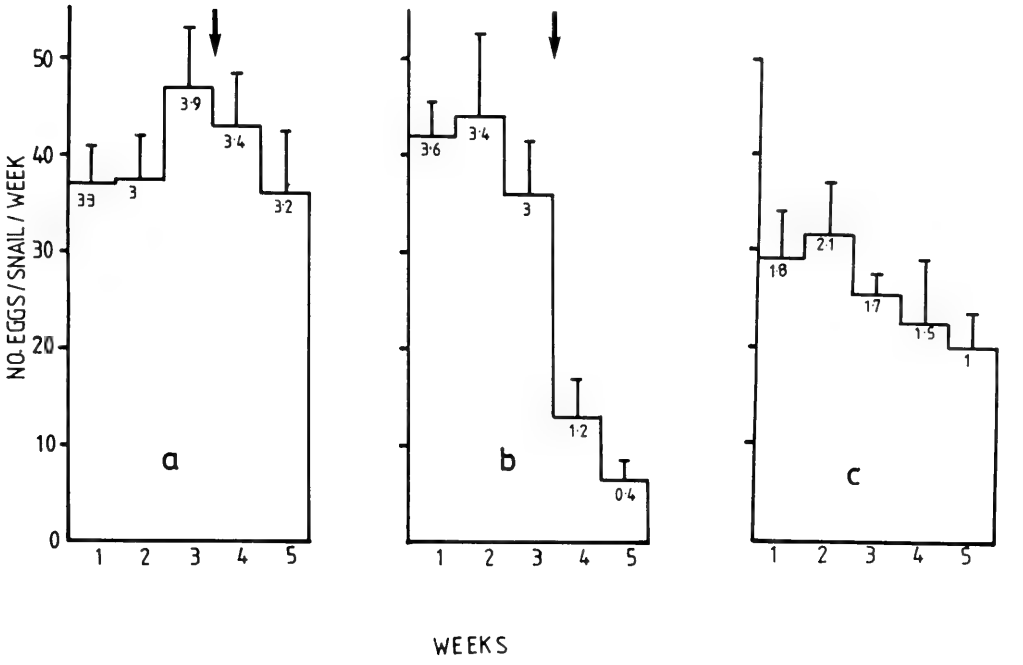


FIG. 7. The oviposition rate of *B. tropicus* following transfer of snails to experimental media containing faecal homogenates of conspecific snails (a) and three week conspecific conditioned water (b). The water was replaced with fish-conditioned water at the end of each week for the first two weeks of each experiment and with the experimental medium at the end of week three, as indicated by the arrows. In Fig. 7c the water remained unchanged but was filtered each week through a column of activated charcoal after bubbling with compressed air. Otherwise legend as for Fig. 2.

old snail-conditioned water from which the faeces had been removed is considerably reduced. However, the inhibitor appears to be removed from the medium by passing over activated charcoal (Fig. 7c). Snails kept in medium bubbled each week over activated charcoal show a slight decrease in weekly oviposition rate, but this is not statistically significant (Anova $P > 0.5$) and the rapid attenuation of egg laying during the second and third weeks, which is characteristic of snails kept in unchanged media, is not evident. Similar results have been obtained by Wright (1960) with *B. forskalii*.

Bioassays performed on media fractionated by ultrafiltration indicate that the inhibitor is of low molecular weight. The patterns of oviposition in groups of five actively laying assay snails on being transferred to the various experimental media are seen in Fig. 8, together with the results of a further assay performed on snail-conditioned water filtered through a preliminary filter of 0.22 μm pore diameter.

The oviposition of assay snails was inhibited by each filtrate fraction down to and including that obtained with membranes having a 5×10^2 MW cut off, whereas the resuspended retentates of these membranes were ineffective.

On subfractionation of the 5×10^2 MW filtrate by HPLC analysis, two major peaks become evident (Fig. 9) representing the polar and non-polar fractions respectively. Each peak is a compound one, being made up of a number of sub-fractions, indicating the presence of a number of different types of molecule, but detailed interpretation of the second peak is complicated by the presence of the methyl cyanide elutant which forms the predominant component.

The results of a preliminary bioassay of the polar and non-polar fractions were inconclusive. The inhibitory potency of the whole $5 \times$

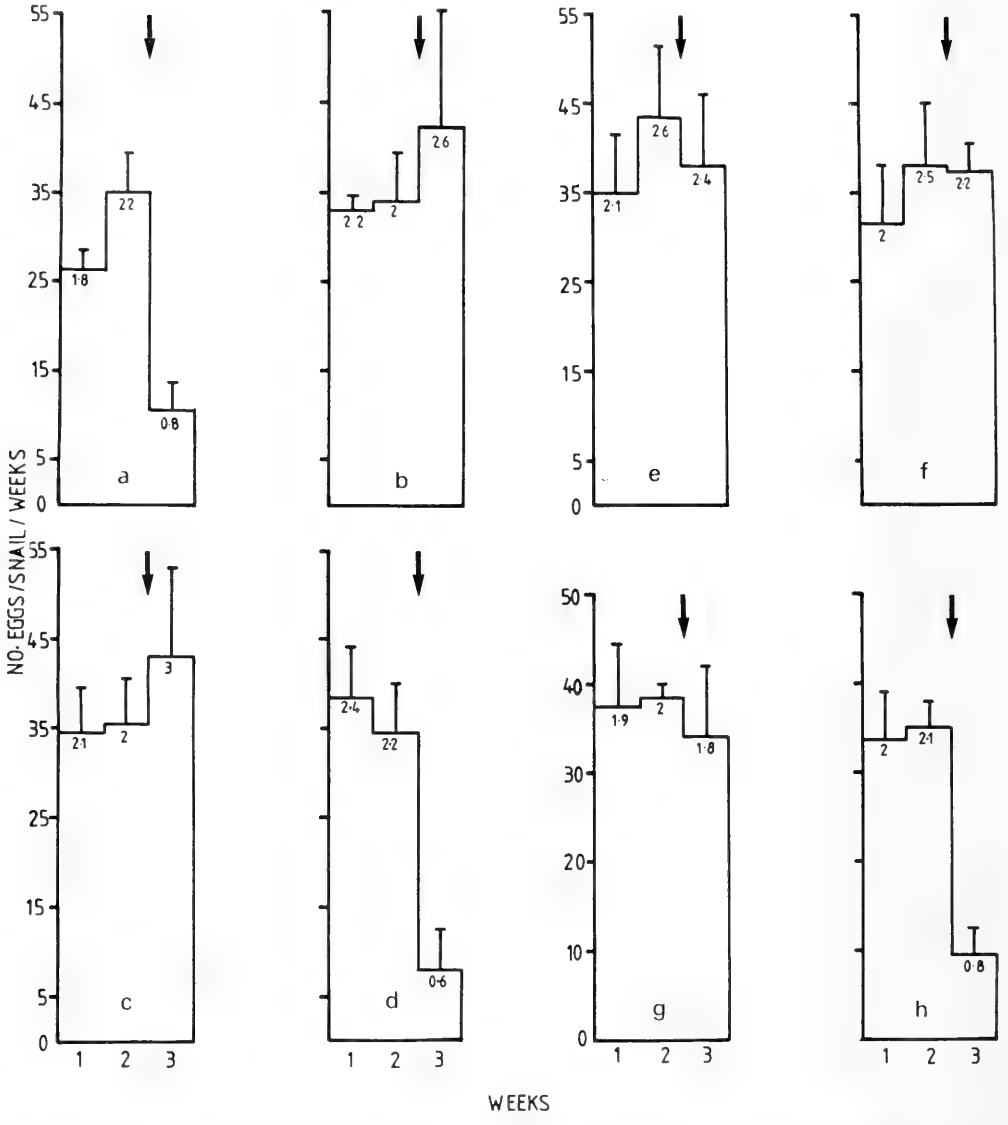


FIG. 8. The oviposition rate of *B. tropicus* after transfer to water containing different fractions of three week old snail-conditioned water, separated by ultra-filtration. Histograms a to d show the results obtained with 0.22 μm filtrate; 100,000 MW retentate; 10,000 MW retentate, and 10,000 MW filtrate respectively. The results of similar experiments involving the 5,000 M retentate; 1,000 MW retentate; 500 MW retentate and 500 MW filtrate are shown in e to h respectively. Otherwise legend as for Fig. 2.

10^2 MW fraction was unaffected by the freeze-drying process, and the polar fraction of the 5×10^2 MW filtrate again appeared to have retained its inhibitory potency. The non-polar fraction however was found to be toxic to the assaying animals, all of which died during the first 24 hours after transfer to the test medium.

GENERAL DISCUSSION

The inhibitory effects of snail-conditioned media on the oviposition of *B. tropicus* described here are similar to those reported by previous authors for other fresh-water pulmonate species (Chernin & Michelson,

FIG. 9. HPLC spectrum of the 500 MW filtrate fraction. Two consecutive runs are shown, and the arrows indicate where the polar and non-polar fractions were separated prior to bioassay.

1957a, b; Wright, 1960; Berrie & Visser, 1963; Gazinelli *et al.*, 1970; Levy *et al.*, 1973; Madsen, 1979a, b). Although the ionic composition of the medium altered gradually during the conditioning period it is unlikely that changes of an inorganic nature are responsible. Egg laying was inhibited even when the oxygen tension was held constant (Fig. 3), and the transfer of snails to artificial media containing the major inorganic ions in the concentrations found in three week old snail conditioned water had no observed inhibitory effect (Fig. 4). Trace elements and the major anions were not measured routinely, but the specific nature of the inhibition (Fig. 6) and the inhibitory effects of dialysed media are difficult to explain in terms of changes in these factors. Instead a compound of organic nature is implicated, and it seems likely that this may be produced by the snails themselves as fish-conditioned water containing lettuce alone (Fig. 4) had no inhibitory effect. Similar inhibitory factors have been reported for terrestrial pulmonates (e.g. Cameron & Carter, 1979; Dan & Bailey, 1982).

Where snail pheromones or growth-modulating compounds have been isolated from snail-conditioned media they have been found to be made up of relatively small molecules and the inhibitor of egg laying in *B. tropicus* appears to be typical in this respect. In *Biomphalaria sudanica* an inhibitor of somatic growth has been identified as a dimethyl ester of formula $C_{18}H_{32}O_7$, with a molecular weight of 360 (Berrie & Visser, 1963), whereas the alarm pheromones produced by the opisthobranch *Navanax* have been shown to be methyl ketones of even smaller molecular weights (Sleeper & Fenical, 1977; Sleeper, Paul & Fenical, 1980). The growth-promoting substances present in *B. glabrata*-conditioned water are similarly small molecules, within the 5×10^2–10^4 MW range, and Thomas, Goldsworthy & Aram (1975) have suggested that polypeptides and/or glyco or lipo proteins may be implicated. The inhibitory activity recorded with *B. tropicus*-conditioned water after passing through a 5×10^2 ultrafiltration membrane indicates that the size of the inhibitor molecule in the present experiments may be of the same order of magnitude and as such might be expected to pass through the viscin dialysis membrane used in some of the above experiments. Snail-conditioned water thus dialysed retains its inhibitory properties however, and there are a number of possible explanations for this apparent paradox. In the dialysis experiments the media were unpressurized and it is conceivable that pressures comparable to those used in ultrafiltration would be required to force the larger organic molecules through the viscin tubing. An alternative possibility is that the inhibitor may be present in a charged form, and is retained within the dialysis membrane by the osmotic gradient and indeed HPLC analysis of the 500 MW filtrate indicates that the inhibitor is present in the polarized fraction. As in *Navanax*, the crude extract contains more than one compound (Sleeper & Fenical, 1977; Sleeper, Paul & Fenical, 1980) but the relative potency of the different subfractions has yet to be investigated for *B. tropicus*.

REFERENCES CITED

A.O.A.C., 1975, *Official Methods of Analysis of the Association of Official Analytical Chemists.* Ed. 12, 1904 p. Washington, D.C.

BERRIE, A.D. & VISSER, S.A., 1963, Investigation of growth-inhibiting substance affecting a natural population of freshwater snails. *Physiological Zoology*, 36: 167–173.

CAMERON, R.A.D. & CARTER, M.A., 1979, Intra- and interspecific effects of population density on growth and activity in some helicid land snails (Gastropoda: Pulmonata). *Journal of Animal Ecology*, 48: 237–246.

CHAUDHRY, M.A. & MORGAN, E., 1983, Circadian variation in the behaviour and physiology of *Bulinus tropicus* (Gastropoda: Pulmonata). *Canadian Journal of Zoology*, 61: 909–914.

CHERNIN, E. & MICHELSON, E.H., 1957a, Studies on the biological control of *Schistosoma*-bearing snails. III. The effects of population density on growth and fecundity in *Australorbis glabratus*. *American Journal of Hygiene*, 65: 57–70.

CHERNIN, E. & MICHELSON, E.H., 1957b, Studies on the biological control of *Schistosoma*-bearing snails. IV. Further observations on the effect of crowding on growth and fecundity of *Australorbis glabratus*. *American Journal of Hygiene*, 65: 71–80.

DAN, N.A. & BAILEY, S.E.R., 1982, Growth, mortality and feeding rates of the snail *Helix aspersa* at different population densities in the laboratory, and the depression of activity of helicid snails by other individuals or their mucus. *Journal of Molluscan Studies*, 48: 257–265.

EISENBERG, R.M., 1966, The regulation of density in a natural population of the pond snail, *Lymnaea elodes*. *Ecology*, 47: 889–906.

GAZINELLI, G., ROMALHO-PINOT, F.J., PELLEGRINO, J. & GILBERT, G., 1970, Uptake of ^{59}Fe as a tool for study of the crowding effect in *Biomphalaria glabrata*. *American Journal of Tropical Medicine and Hygiene*, 19: 1034–1037.

KITS, K.S. & MAAT, A. ter, 1982, Neurophysiology of the peptidergic caudo-dorsal cells in *Lymnaea stagnalis*. In: *Proceedings of the International Minisymposium on Molluscan Neuroendocrinology*. Free University Amsterdam. Netherlands, August 16–20, p. 60–68.

LAZARIDOU-DIMITRIADOU, M. & DAGUZAN, J., 1981, Effects of crowding on growth, mortality rate and reproduction of *Theba pisana* (Gastropoda: Pulmonata). *Malacologia*, 20: 195–204.

LEVY, M.G., TUNIS, M. & ISSERHOFF, H., 1973, Population control in snails by natural inhibitors. *Nature*, 241: 65–66.

MADSEN, H., 1979a, Further laboratory studies on the interspecific competition between *Helisoma duryi* (Wetherby) and the intermediate hosts of *Schistosoma mansoni* Sambon: *Biomphalaria alexandrina* (Ehrenberg) and *B. camerunensis* (Boettger). *Hydrobiologia*, 66: 181–192.

MADSEN, H., 1979b, Preliminary observations on the role of conditioning and mechanical interference with egg masses and juveniles in the competitive relationships between *Helisoma duryi* (Wetherby) and the intermediate host of *Schistosoma mansoni* Sambon: *Biomphalaria camerunesis* (Boettger). *Hydrobiologia*, 67: 207–214.

MADSEN, H., 1982, Development of egg masses and growth of newly hatched snails of some species of intermediate hosts of Schistosomiasis in water conditioned by *Helisoma duryi* (Wetherby) (Pulmonata: Planorbidae). *Malacologia*, 22: 427–434.

MOOIJ-VOGELAAR, J.W. & STEEN, W.J. van der, 1973, Effects of density on feeding and growth in pond snail *Lymnaea stagnalis* (L.). *Proceedings Koninklijk Nederlandse Akademie van Wetenschappen*, ser. C, 76: 47–60.

SLEEPER, H.L. & FENICAL, W., 1977, Navenones A-C; trail-breaking alarm pheromones from the marine opisthobranch *Navanax inermis*. *Journal of the American Chemical Society*, 99: 2367–2368.

SLEEPER, H.L., PAUL, V.J. & FENICAL, W., 1980, Alarm pheromones from the marine opisthobranch *Navanax inermis*. *Journal of Chemical Ecology*, 6: 57–70.

STEEN, W.J. van der, 1967, The influence of environmental factors on the oviposition of *Lymnaea stagnalis* (L.) under the laboratory conditions. *Archives Néerlandaise Zoologie*, 17: 403–468.

THOMAS, J.D., 1973, Schistosomiasis and the control of molluscan hosts of human schistosomes with particular reference to self-regulatory mechanisms. In DAWES, B., ed., *Advances in Parasitology*. Academic Press, London, 11: 307–394.

THOMAS, J.D. & BENJAMIN, M., 1974a, The effects of population density on growth and reproduction of *Biomphalaria glabrata* (Say). *Journal of Animal Ecology*, 43: 31–50.

THOMAS, J.D. & BENJAMIN, M., 1974b, Effects of numbers, biomass and conditioning time on growth and natality rates of *Biomphalaria glabrata* (Say), the snail host of *Schistosoma mansoni* Sambon. *Journal of Applied Ecology*, 11: 832–840.

THOMAS, J.D., BENJAMIN, M., LOUGH, A. & ARAM, R.H., 1974, The effect of calcium in the external environment on the growth and natality rates of *Biomphalaria glabrata* (Say). *Journal of Animal Ecology*, 43: 839–860.

THOMAS, J.D., GOLDSWORTHY, G.J. & ARAM, R.H., 1975, Studies on the chemical ecology of snails. The effect of chemical conditioning by adult snails on the growth of juvenile snails. *Journal of Animal Ecology*, 44: 1–27.

THOMAS, J.D., LOUGH, A.S. & LODGE, R.W., 1975, The chemical ecology of *Biomphalaria*

glabrata (Say), the snail host of *Schistosoma mansoni* Sambon. The search for factors in media conditioned by snails which inhibit their growth and reproduction. *Journal of Applied Ecology*, 12: 421–436.

WRIGHT, C.A., 1960, The crowding phenomenon in laboratory colonies of freshwater snails. *Annals of Tropical Medicine and Parasitology*, 54: 224–232.

Revised Ms. accepted 14 March 1985

MALACOLOGIA, 1986, 27(2): 265–269

EFFECTS OF LONG-TERM EXPOSURE TO LOW CONCENTRATIONS OF MOLLUSCICIDES ON A FRESH-WATER SNAIL, *INDOPLANORBIS EXUSTUS*, A VECTOR OF SCHISTOSOMIASIS

B.D. Parashar & K.M. Rao

Department of Entomology, Defence Research & Development Establishment, Tansen Road, Gwalior 474002, India.

ABSTRACT

This paper deals with the long-term effects of low concentrations (0.01, 0.05, 0.10, and 0.15 mg/l) of four molluscicides, namely Santobrite, copper sulphate, Yurimin, and Bayluscide on the fresh-water snail *Indoplanorbis exustus*, vector of schistosomiasis. The results show that Santobrite is less effective at 0.15 mg/l, and ineffective at the other concentrations against immature, young mature, and adult stages of the snail. Yurimin is effective against immature and adult *I. exustus* at 0.05, 0.10 and 0.15 mg/l, and against the young mature stage at 0.15 mg/l. Copper sulphate is effective at all four concentrations against immature and adult *I. exustus* and at concentrations of 0.05, 0.10 and 0.15 mg/l against the young mature stage. Bayluscide is highly effective at all concentrations against all snail stages. Use of low concentrations of molluscicides for snail control, keeping in view their toxicity to non-target organisms, is discussed.

INTRODUCTION

Indoplanorbis exustus (Deshayes) is a fresh-water planorbid gastropod, the vector of *Schistosoma indicum, S. nasale*, and *S. spindale*, the causative agents of schistosomiasis among horses, mules, sheep, goats, camels and cattle of economic and agricultural significance (Malek & Cheng, 1974). Synthetic molluscicides are used as one of the major components of integrated pest management for the control of fresh-water snails.

Two methods of application of molluscicides are currently in use for snail control, *i.e.* use of high concentrations for the short-term, and low concentrations for long-term. If continuous molluscicidal treatment of snail habitats can be satisfactorily implemented by use of low concentrations for long periods, multiple attack points in the trematode life cycle against both parasite and snail could be achieved. Whereas, short-term applications of high concentrations of molluscicides kill the existing snails with immediate lethal effects on biota of the environment, prolonged applications of molluscicides at low concentrations would cause minimal ecological disturbance in addition to snail control.

The present study has been undertaken so as to work out the susceptibility of immature, young mature and adult stages of the snail *I. exustus* to low concentrations of four molluscicides. This study has an important bearing on the control of snails by working out effective concentrations of molluscicides that may be needed for the control of this species in nature.

MATERIALS AND METHODS

Snails were drawn from standard laboratory cultures of *I. exustus*. At the start of the experiment, they were grouped into the following categories:

1. Immature 3–6 mm diameter,
2. Young mature 9–12 mm diameter,
3. Adult Above 13 mm diameter.

Four chemicals, namely Santobrite (sodium pentachloro-phenate), copper sulphate, Yurimin (3,5-dibromo4-hydroxy-nitrobenzene) and Bayluscide (2-aminoethanol salt of 2′,5-dichloro-nitrosalicylanilide) were chosen for the evaluation of their toxicity at low concentrations against three stages of snails. The low concentrations employed for study were 0.01, 0.05, 0.10 and 0.15 mg/litre.

For each concentration of molluscicide, 20

snails were exposed in a glass container (28 cm diameter) containing 5 litres of dechlorinated tap water for 24 hr. After this period, snails were transferred to another glass container having the same concentration of molluscicide in a similar amount of dechlorinated tap water. Five replications were employed so as to minimize variation in mortality rates of snails. For each stage of snail, different experimental sets were conducted. Dead snails were removed from the container so as to prevent fouling of the environment. Spinach leaves were supplied as food for these snails *ad libitum*. This experiment was continued till a last snail survived. Controls were also kept under similar conditions. All these studies were carried out in a controlled environment room (temperature 30 ± 2°C; R.H. 70% ± 5). The lighting regimen maintained during experimentation was equal periods of light and dark (LD 12:12) in a 24 hr cycle.

The data on exposure period and resultant percent mortality were subjected to probit analysis for the determination of LD_{90} (duration for 90% mortality of snails) as per method of Finney (1971). In the present study, the concentration which brought about 50% or more reduction in the LD_{90} value of experimental snails as compared to controls was considered an effective concentration.

RESULTS

The mean survival time of immature, young mature and adult stages of *I. exustus* at 0.01, 0.05, 0.10 and 0.15 mg/l concentrations of Santobrite, copper sulphate, Yurimin and Bayluscide are illustrated in Figs. 1, 2 and 3, respectively.

The LD_{90} values for control snails of immature, young mature, and adult categories were 188.9, 150.1 and 101.2 days, respectively. For each concentration of molluscicides, percent reduction in LD_{90} value of experimental snails as compared to control values was worked out. These values were shown as relative toxicity for various molluscicides in Table 1.

Santobrite was not effective at all the concentrations against three stages of the snail. Yurimin is effective against immature and adult *I. exustus* at 0.05, 0.10 and 0.15 mg/l concentrations, while against young mature snails only 0.15 mg/l concentration was effective. Copper sulphate is effective at all concentrations ranging from 0.01 mg/l–0.15 mg/l against immature and adult stages, but in the case of young mature 0.05–0.15 mg/l concentrations were effective. Bayluscide is effective against all the stages of *I. exustus* at all concentrations used. Out of three stages of snail, the adult stage has been observed to be most susceptible and the young mature stage as the most resistant stage of the snail to molluscicides.

It has been observed during experimentation that none of the three stages of the snails tried to leave the container containing a molluscicidal solution.

DISCUSSION

The present study indicates the ineffectiveness of Santobrite at 0.01–0.15 mg/l and Yurimin at 0.01–0.10 mg/l concentrations for snail control. Copper sulphate appeared to be a promising compound at 0.05–0.15 mg/l concentrations against the most resistant stage of the snail, *i.e.* the young mature stage (Table 1, Figs. 1, 2, and 3), the LD_{90} being 44.5–60.0 days. Walker & Cardarelli (1975) estimated the LD_{90} for *Biomphalaria glabrata* between 0.025–0.030 ppm/day when the exposure was extended for 60 days. Upatham & Christae (1975) noted that E-51, a slow release formulation of copper sulphate at 1.25 ppm available copper, gave LT_{100} of 19 days against St. Lucian strain of *B. glabrata*, while 0.63 ppm resulted LT_{100} of 11 days against *Lymnaea cubensis*. Cardarelli (1977) reported 58%, 94% and 100% mortality of *B. glabrata* in 60 days at 0.013, 0.065, and 0.13 ppm concentration of copper sulphate, respectively, with control mortality of 4%.

The relative toxicity of Bayluscide was higher than other molluscicides (Table 1) against all stages of snails, as is also apparent from their mean survival time at different concentrations (Figs. 1, 2 and 3). Its lower value of LD_{90} (4.72–74.06 days) than the control (150 days) against the most resistant stage of snail shows its effectiveness at all concentrations (0.01–0.15 mg/l). Long-term exposure of *B. glabrata* to 0.05 ppm concentration of Bayluscide resulted in 32, 94, and 100% mortality in 30, 60, and 120 days, respectively, while exposure to 0.1 ppm effected 62 and 100% mortality in 5 and 30 days, respectively (Cardarelli, 1977).

This study reveals that out of four

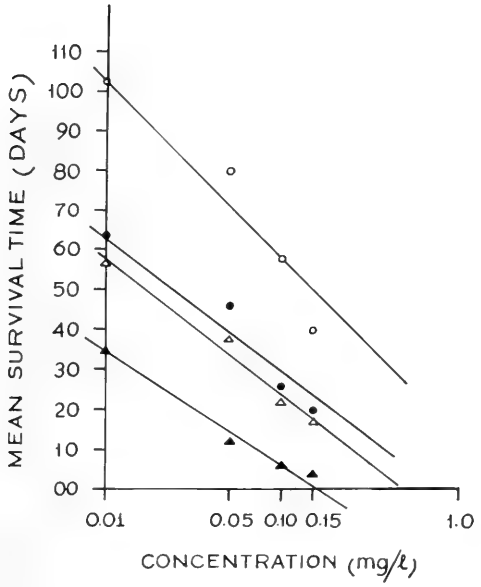


FIG. 1. Mean survival time of immature stage of snail, *Indoplanorbis exustus*, at four concentrations of molluscicides, viz. Santobrite (○), Yurimin (●), copper sulphate (△) and Bayluscide (▲).

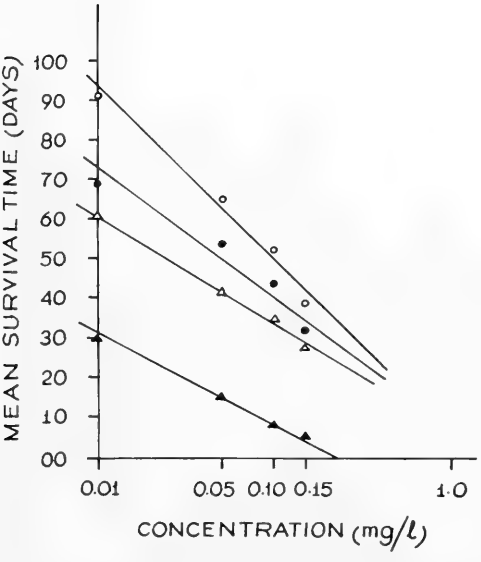


FIG. 2. Mean survival time of young mature stage of snail, *Indoplanorbis exustus*, at four concentrations of molluscicides, viz. Santobrite (○), Yurimin (●), copper sulphate (△) and Bayluscide (▲).

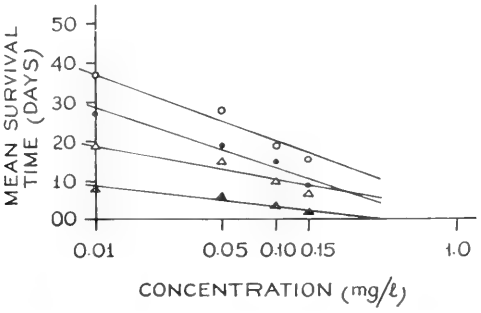


FIG. 3. Mean survival time of adult stage of snail, *Indoplanorbis exustus*, at four concentrations of molluscicides, viz. Santobrite (○), Yurimin (●), copper sulphate (△) and Bayluscide (▲).

molluscicides, Bayluscide proves to be the most toxic to all stages of *I. exustus* at low concentrations, being followed by copper sulphate and Yurimin. However, Santobrite is least toxic (Table 1, Figs. 1, 2 and 3). It has also been observed that out of three stages of the snail, the adult is the most susceptible and the young mature stage is most resistant.

The less percent reduction in LD_{90} values of the most resistant stage of *I. exustus* as compared to the controls, even at 0.15 mg/l of, Santobrite suggests that relatively higher concentrations of this chemical would be required for achieving considerable effect on snail mortality. However, it may be harmful to other organisms at higher concentrations. Besides, it has been reported to be strongly piscicidal (WHO, 1965) and herbicidal (WHO, 1980) in activity and potentially dangerous to operators (Blair, 1961). Its acute oral toxicity against rats varies from 40–250 mg/kg (LD_{50}) (WHO, 1980).

The effective low concentrations of Yurimin (0.10 and 0.15 mg/l) against the most resistant stage of *I. exustus* are enough lower to cause serious damage to the non-target biota as evident by its toxicity to animals and plants. The fish LC_{50} varies from 0.16–0.83 mg/l. The acute oral mammalian toxicity of Yurimin is also low (LD_{50} = 168 mg/kg) in mice). No apparent toxic hazards to human beings or vegetation were reported. It does not exert herbicidal activity (WHO, 1973).

The low concentrations at which copper sulphate is effective against young mature snails (0.05–0.15 mg/l) are safer for non-target organisms. Copper sulphate is toxic to fishes but only somewhat at higher concentrations (WHO, 1965). Its acute oral LD_{50} for

TABLE 1. Relative toxicity of molluscicides to three stages of *I. exustus* based on percent reduction in LD_{90} value as compared to control.

Molluscicide	Concentration (mg/l)	Relative toxicity		
		3–6 mm (dia.)	9–12 mm (dia.)	13 + mm (dia.)
Santobrite	0.01	18.90	6.52	26.18
	0.05	36.58	25.45	42.00
	0.10	41.68	33.11	50.53
	0.15	64.72	44.60	68.75
Yurimin	0.01	32.08	25.52	42.20
	0.05	51.91	30.38	57.09
	0.10	63.54	51.39	65.36
	0.15	72.77	66.00	79.10
Copper sulphate	0.01	55.48	45.61	68.22
	0.05	67.88	59.99	77.22
	0.10	76.39	62.17	85.56
	0.15	81.46	70.33	88.84
Bayluscide	0.01	59.89	50.66	83.39
	0.05	92.78	88.85	95.31
	0.10	96.34	94.30	97.36
	0.15	97.96	96.86	98.80

rat is 300 mg/kg (WHO, 1980). It also exerts some herbicidal activity (WHO, 1980).

Bayluscide has the advantage over other molluscicides of being extremely effective against all stages of snails at very low concentrations (0.01–0.15 mg/l) and at the same time it is not toxic to humans and has limited biocidal effect. However, fish proved to be extremely susceptible to it (LC_{50} = 0.05–0.30 ppm) (Malek & Cheng, 1974). The oral LD_{50} for rats has been reported to be over 5 g/kg of body weight (WHO, 1980). Human beings show no toxic symptoms at 30 mg/kg administered as a single oral dose. Regarding phytotoxicity of Bayluscide, Abdallah & Nasr (1961) found that it is harmless to crops at dosages very much higher than those used for snail control. It also does not exert any herbicidal activity (Malek & Cheng, 1974).

At higher concentrations snails detect ions of molluscicides and react in many instances by leaving the water, thus escaping the toxic effects of molluscicides (Etges, 1963). However, this avoidance behaviour is not exhibited at low concentrations (Frick & De Jimenez, 1964). The concentrations used in the present study are too low to be detected by snails. It is also apparent from the behaviour of snails during this study, since they do not react to molluscicide exposure by leaving the experimental containers.

It can be surmised from this study that 0.15, 0.05–0.15 and 0.01–0.15 mg/l concentrations of Yurimin, copper sulphate, and Bayluscide respectively may be used for the control of *I. exustus* since these are effective concentrations and also safer as regards their toxicity to non-target organisms.

ACKNOWLEDGEMENT

The authors are indebted to Dr. P.K. Ramachandran, Director, Defence Research & Development Establishment, Gwalior, India, for his keen interest and constant encouragement.

REFERENCES CITED

ABDALLAH, A. & NASR, T.S., 1961, Evaluation of a new molluscicide, Bayer, 73. *Journal of Egyptian Medical Association*; 44: 160–170.

BLAIR, D.M., 1961, Dangers in using and handling sodium pentachlorophenate as a molluscicide. *Bulletin of the World Health Organisation,* 25: 597–601.

CARDARELLI, N.F., 1977, *Controlled release molluscicides.* University of Akron, Akron, Ohio, 133 p.

ETGES, F.J., 1963, Effects of some molluscicidal chemicals on chemokinesis in *Australorbis*

glabratus. American Journal of Tropical Medicine and Hygiene, 12: 701–714, 1 fig.

FINNEY, D.J., 1971, *Probit analysis.* Cambridge University Press, London, 333 p.

FRICK, L.P. & JIMENEZ, W.A. de, 1964, Molluscicidal qualities of three organotin compounds revealed by 6 h and 24 h exposure against representative stages and sizes of *Australorbis glabratus. Bulletin of the World Health Organisation*, 31: 420–431.

MALEK, E.A. & CHENG, T.C., 1974, *Medical and economic malacology.* Academic Press, New York and London, 398 p.

UPATHAM, E.S. & CHRISTAE, J.D., 1975, Laboratory trials of copper and TBTO slow release compounds. *Controlled Release of Molluscicides Newsletter.* Univ. Akron, Akron, Ohio.

WALKER, K.E. & CARDARELLI, N.F., 1975, Slow release copper toxicant compositions. *U.S. Patent. App. Ser.* No. 557, 051.

WORLD HEALTH ORGANIZATION, 1965, Snail control in the prevention of bilharziasis. *World Health Organization Monograph Series*, 5: 255.

WORLD HEALTH ORGANIZATION, 1973, Schistosomiasis control. *Technical Report Series, World Health Organization*, 515: 46 p.

WORLD HEALTH ORGANIZATION, 1980, Epidemiology and control of schistosomiasis. *Technical Report Series, World Health Organization*, 643: 63 p.

Revised Ms. accepted 9 July 1985

MALACOLOGIA, 1986, 27(2): 271–280

THE TAXONOMIC STATUS OF *PHILOMYCUS TOGATUS* (PULMONATA: PHILOMYCIDAE): A MORPHOLOGICAL AND ELECTROPHORETIC COMPARISON WITH *PHILOMYCUS CAROLINIANUS*

H. Lee Fairbanks

Pennsylvania State University, Beaver Campus, Monaca, PA 15061, U.S.A.

ABSTRACT

The past and present taxonomic status of *Philomycus togatus* (Gould) is reviewed. Morphological comparisons between *P. togatus* and *P. carolinianus* (Bosc) from three localities demonstrated clear and consistent differences in mantle pattern, color of the foot margin, and color of the mucus. Reproductive system differences include shape and size of the penis and length and thickness of the penial sheath. Electrophoretic comparisons of seven enzyme systems revealed 13 loci. *P. carolinianus* was monomorphic at all loci. *P. togatus* was polymorphic at two loci; however, no heterozygotes were found. Between species differences existed at four loci, with each species fixed for alternative alleles, only one of eleven alleles was found in both species. The genetic distance (Nei's (1978)D) between species was .432.

Key words: Philomycidae; *Philomycus*; taxonomy; morphology; electrophoresis; genetic distance.

INTRODUCTION

Gould (1841) described *Limax togata*, noting that "It is very probable that the great development of the shield . . . may entitle this animal to be regarded as a new genus." A. Binney (1842, 1851) placed *L. togata* in the synonymy of *Tebennophorus caroliniensis* A. Binney (1842). Later, Gould (1862: 182) stated "*Limax togata* is *Tebennophorus caroliniensis* Binney." W. G. Binney (1878) also placed *L. togata* in the synonymy of *T. caroliniensis*. No further mention of *Limax togata* was made until Pilsbry (1948) included it (as well as *T. caroliniensis*) in the synonymy of *Philomycus carolinianus* (Bosc, 1802), considering both as part of *P. c. flexuolaris* (Rafinesque, 1820). However, Hubricht (1951) elevated *P. flexuolaris* to specific rank and later (1956) identified slugs from Shenandoah National Park, Virginia as *Philomycus carolinianus togatus*. In addition, he placed *P. c. collinus* Hubricht, 1951 in the synonymy of *P. c. togatus* (1956). Hubricht (1968) identified slugs from Kentucky as *Philomycus togatus*, thus becoming the first author to return the taxon to the species level. There have been several additional identifications of slugs as *P. togatus* (e.g. Grimm, 1971; Hubricht, 1971, 1973, 1977; MacNamara & Harman, 1975; Kearney & Gilbert, 1978), including fig. 405f in Pilsbry's 1948 monograph (Hubricht, 1951).

Very little is known about the biology of the Philomycidae in the United States of America. Webb (1970), in a study of *Philomycus carolinianus*, noted that mating was reciprocal, a preformed spermatophore was not involved (also noted by Kugler, 1965), and the dart was used to " . . . form a definite wound. . . . " Tompa (1980) also studied *P. carolinianus* and noted that the slug " . . . does not lose or detach the dart during mating. . . . " Ikeda (1937) studied *P. bilineatus* (now *Meghimatium bilineatus*; see Pilsbry, 1948) in Japan. He noted that *M. bilineatus* reproductive activity is " . . . definitely cyclic, . . . ", that " . . . such definite cycle of sexual activity is accompanied by certain definite changes in reproductive organs, . . . ", and that the ovotestis, albumen gland and penis seem to undergo the greatest cyclic changes in size.

Despite the taxonomic changes no data have been published to substantiate the redesignation of *Philomycus togatus* as a distinct species. In light of this, a comparative morphological study of specimens of *P. carolinianus* and specimens identified as *P. togatus* was initiated. In addition, because it has been shown that electrophoresis of proteins can be useful in the detection of cryptic

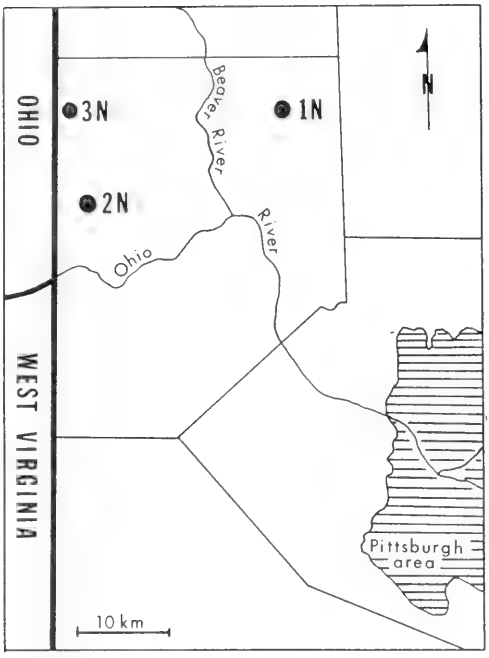


FIG. 1. Collection sites in Beaver County, Pennsylvania. 1N = Brush Creek; 2N = State Game Area 173; 3N = State Game Area 285.

species of gastropods (e.g. Chambers, 1978; Murphy, 1978), an electrophoretic analysis of some tissue proteins was included in the study.

MATERIALS AND METHODS

Specimens of *Philomycus carolinianus* and *P. togatus* were collected from three localities in western Pennsylvania (Fig. 1). Site selection was based upon availability of old growth forest on public land. Voucher specimens from each locality have been deposited in The Academy of Natural Sciences of Philadelphia (ANSP) as noted below. Locality descriptions are as follows:

1N—Brush Creek County Park; on slopes ca. 10 m above creek; elevation ca. 290 m; Marion Township, Beaver County, Pa.; 40°47′50″N; 80°14′14″W. Collection area = ca. 40 m^2. Species collected *P. carolinianus* (18 specimens); ANSP A10655; HLF 374, 445.

2N—State Game Area 173; along banks of McLaughlin Run; elevation ca. 340 m; Ohio Township, Beaver County, Pa.; 40°40′19″N; 80°28′23″W. Collection area = ca. 400 m^2. Species collected *P. carolinianus* (10 specimens); ANSP A10651; HLF 441. *P. togatus* (11 specimens); ANSP A10652; HLF 438.

3N—State Game Area 285; along banks of small creek ca. 0.6 km E of Ohio State line; elevation ca. 295 m; Darlington Township, Beaver County, Pa.; 40°47′22″N; 80°30′15″ W. Collection area = 400 m^2. Species collected *P. carolinianus* (5 specimens, not found at the time of the electrophoretic tests); ANSP A10653; HLF 436. *P. togatus* (19 specimens); ANSP 358186, A10205, A10654; HLF 437. Because the holotype of *Limax togata* Gould, 1841, is not extant, the identification of *Philomycus togatus* was corroborated by L. Hubricht (personal communication, 16 February 1983). Identification of *P. carolinianus* was accomplished using the description given by Pilsbry (1948).

For the morphological comparisons similarly-sized slugs were selected. Following the examination of external characteristics, the slugs were drowned in distilled water. The specimens were dissected in water, and the jaws, radulae, and reproductive systems were removed. The dissected bodies were preserved in 70% ethanol. Some reproductive systems were stained, cleared and mounted on glass slides using Gregg's (1959) method with the following modifications: the tissues were left in hematoxylin for 3 min and the acid-alcohol destaining solution for 30 sec. Some radulae and jaws were mounted in Permount on glass slides.

The tissue samples for electrophoretic analysis were obtained by cutting off the posterior 5 mm of each slug. The slugs were then killed by freezing and saved for morphological comparisons. The tissue sample was prepared for electrophoresis using the procedures of Brussard & McCracken (1974) and modified as described by Selander & Hudson (1976). The samples were stored at −85°C until the tests were conducted. A Buchler Vertical Gel Apparatus (catalog number 3-1072) with a Gelman regulated power supply (model 38520) was used for electrophoresis. All tests were conducted in a refrigerator at 4°C. Voltage was set at 250 volts, and current flow ranged from 47–49 milliamperes during each of the 5 hr tests. The gels (52 g of starch in 400 ml of buffer) were made using Electrostarch (Madison, Wis.), lot number 392.

The following enzyme systems were assayed:

(1) Stain formulae from Siciliano & Shaw (1978)
   a. Glucose-6-phosphate dehydrogenase = G-6-PD
   b. Glutamate oxalacetate transaminase = GOT
   c. Isocitrate dehydrogenase = IDH
   d. Malic enzyme = ME
   e. 6-phosphogluconate dehydrogenase = 6-PGD
   f. Phosphoglucomutase = PGM

(2) Stain formula from Selander et al. (1971)
   Leucine aminopeptidase = LAP

The buffer system used for all stains was Tris-Versene-Borate pH 8.0 (Siciliano & Shaw, 1978).

Gels were scored as described by Chambers (1978) except that the electromorph at a given locus most common among all specimens was designated with the superscript 100. Overlapping tests were run to ensure that the electromorphs on different gels were scored correctly. All presumed alleles were assumed to be autosomal and codominant.

Genetic distance (D) was calculated using Nei's (1978) method for small samples.

RESULTS

Morphology

Specimens used for the external morphological studies and in the electrophoretic tests were collected between 29 June 1982 and 28 September 1982.

External examination of the bodies of the slugs (Fig. 2) revealed three obvious and consistent differences between species. All of the specimens of *Philomycus carolinianus* had a double row of black spots (one row on each side of a black-brown antero-posterior stripe) on the mid-dorsal surface of the mantle (Fig. 2c, d). All had a cream-white foot edge, and produced milky-white mucus when handled. The specimens of *P. togatus* had one broad or two narrow gray-black stripes, had an orange-red foot edge, and produced orange mucus when handled. There was an antero-posterior gray-black stripe on each side of the mantle in both species.

Collections of specimens for dissection were made between 16 May and 21 May 1985, the dissections were conducted on 23 May 1985. Eight specimens of *Philomycus carolinianus*, three each from localities 1N and 2N and two from locality 3N (avg. wt. = 3.2 g, range = 2.4 g–4.1 g, s.d. = .62) and six specimens of *P. togatus*, three each from localities 2N and 3N (avg. wt. = 3.3 g, range = 2.5 g–4.1 g, s.d. = .61) were dissected. The only obvious difference between the reproductive systems of these two species (Fig. 3) was in the appearance of the penis. Measurements (Table 1) show that the penis of *P. carolinianus* is shorter, larger in diameter (at the origin), and tapers more rapidly to the distal end than the penis of *P. togatus.* Longitudinal sections of the two penises (Fig. 4) revealed obvious differences in penis shape and length of the penial sheath, i.e. the penial sheath in *P. carolinianus* was nearly as long as the penis whereas in *P. togatus* it was approximately 75% as long as the penis. The remaining organs of the reproductive systems were similar in the two species.

Comparisons of the jaws provided no diagnostic characters; they were all typical of the genus (Pilsbry, 1948: 750). The same was true of the radulae.

Electrophoresis

Thirteen loci were detected among the seven enzyme systems assayed. Eight loci, G-6-PD 1, G-6-PD 2, IDH 2, 6-PGD 1, GOT 2, PGM 1, ME 1, and ME 2, were monomorphic with the electromorphs for these loci the same in both species.

The frequency distribution of the 11 alleles (electromorphs) detected for the loci polymorphic within species or with different alleles in each species is shown in Table 2. Only one of these alleles was found in both species. The *Philomycus carolinianus* populations were monomorphic for these loci whereas each population of *P. togatus* had at least one polymorphic locus. No heterozygotes were detected.

Table 3 shows the genetic distance estimates (D) between the various populations for both species. The interspecies values averaged .411. The interpopulation value for *Philomycus togatus* was .013.

DISCUSSION

Gould's 1841 description of *Limax togata*: ". . . and the shield extends quite back to the

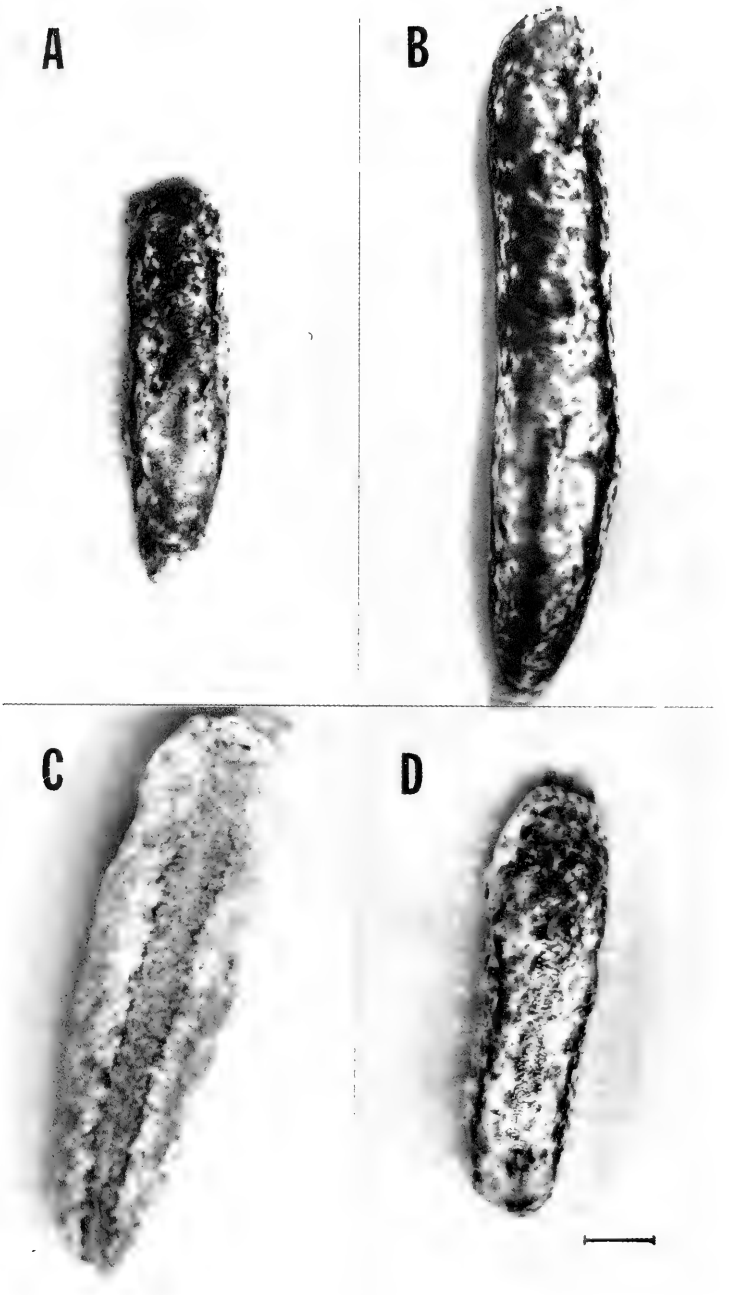


FIG. 2. Mantle patterns: A–B, *Philomycus togatus*; C–D, *Philomycus carolinianus*. Scale bar 5 mm. A 5 mm piece of tail has been removed from C and D.

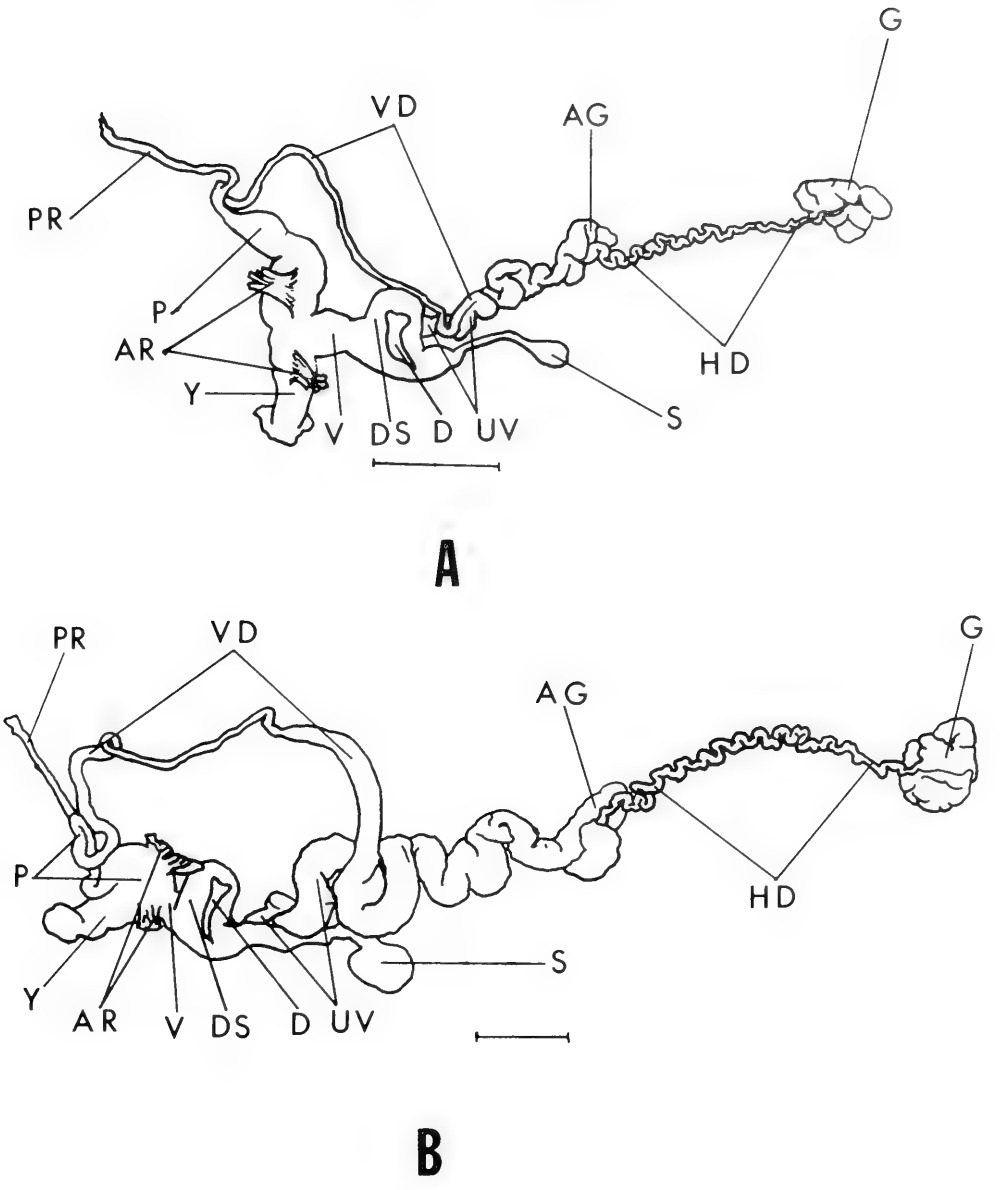


FIG. 3. Genitalia: A, *Philomycus togatus*; B, *Philomycus carolinianus*. Scale bars 5 mm. AG, albumen gland; AR, accessory retractor muscles; D, dart; DS, dart sac; G, gonad; HD, hermaphroditic duct; P, penis; PR, penial retractor muscle; S, spermatheca; UV, free oviduct; V, vagina; VD, vas deferens; Y, atrium.

extremity of the animal, enveloping the whole animal except the head . . . " indicates quite clearly that the slug was a philomycid. The description of the mantle color pattern, " . . . its margin is light fawn-color, the back is dark purplish slate color, and the sides are mottled with the two colors . . . " implies a broad antero-posterior band on the dorsal surface without the double row of black spots as in *Philomycus carolinianus*. Examination of a

TABLE 1. Penial measurements. Measurements obtained using an ocular micrometer; all measurements in mm. Numbers represent mean, range, and standard deviation. L = length; A = diameter proximal end; B diameter midpoint; C = diameter distal end.

	P. carolinianus			*P. togatus*		
N	8			6		
Penis						
L	7.5	6.0–11.0	1.49	9.9	8.0–12.0	1.32
A	3.2	2.8– 3.5	.26	2.5	2.0– 2.8	.29
B	1.3	0.8– 1.6	.26	1.6	1.2– 2.0	.29
C	0.6	0.5– 1.0	.30	0.6	0.5– 0.7	.05

total of 63 specimens of both species (33 *P. carolinianus* and 30 *P. togatus*) substantiates the constancy of this difference.

Chichester & Getz (1973) noted that the color of the mucus should be recorded when one is gathering data concerning a species of slug. Unfortunately, few descriptions have included such data and therefore comparisons are difficult. For *Philomycus carolinianus* these data have been recorded (*ibid.*) and they substantiate the difference between the above species and *P. togatus* as noted in this study.

Among the species of *Philomycus* only *P. rushi* Clapp, 1920, a small (15–20 mm) slug, has been described as having orange or red in the sides of the foot. Pilsbry (1948) synonymized *P. rushi* with *Pallifera ohioensis* (Sterki). There are two large *Pallifera* (*P. varia* and *P. ragsdalei*) with red or orange in the foot margins. However, *Philomycus togatus* has a dart sac and dart and therefore must remain in the genus *Philomycus.* This makes it the only known species in the genus recorded as having orange foot margins.

Chichester & Getz (1968) noted that in most species of terrestrial slugs " . . . the distal genitalia are specifically diagnostic." The examination of specimens of *Philomycus togatus* and *P. carolinianus*, in the same stages of the reproductive cycle, demonstrated significant differences in the terminal genitalia (Fig. 4), *i.e.* differences in size and shape of the penis. The length and thickness of the penial sheath provided additional differences. Although Pilsbry (1948) noted an extension of the atrium covering the proximal portion of the penis in *P. carolinianus*, Kugler (1965) called it a penial sheath. No within species differences and no intergradation between species was observed.

Available biological data demonstrate that cross-fertilization (amphimixis) occurs within populations of *Philomycus carolinianus* (Kugler, 1965; Tompa, 1980; Webb, 1970). McCracken & Selander (1980) using electrophoretic data have noted "The agreement between observed and expected proportions of heterozygotes in our genetically variable populations of slugs indicates that they, too, are largely if not completely amphimictic. These species are . . . *Philomycus carolinianus* and three unidentified species of *Philomycus*. . . . " In this study both populations of *P. carolinianus* were monomorphic at all loci studied. Foltz *et al.* (1982) have noted that a possible explanation for monogenicity among cross-fertilizing species is that " . . . they are outcrossers that have lost all heterozygosity as a consequence of founder effects and genetic drift in small populations." In view of the lack of data supporting self-fertilization in this species this explanation seems most probable for the variation observed.

There are no published data available concerning the biology or genetic variation of *Philomycus togatus.* One might assume that the biology of *P. togatus* would be similar to that of *P. carolinianus.* However, this investigation detected two polymorphic loci in *P. togatus*, but no heterozygotes. In population 3N the probability of not detecting a heterozygote is highly significant (.0002). That is, it is highly unlikely, based upon the sample size, that no heterozygotes would be observed. The absence of heterozygotes in polymorphic populations implies some sort of automictic reproduction. Ikeda (1937) stated that *Philomycus bilineatus* " . . . in isolation reproduces by self-fertilization . . . " Nicklas and Hoffman (1981) concluded that *Deroceras laeve* reproduces by apomictic parthenogenesis. Foltz *et al.* (1982) con-

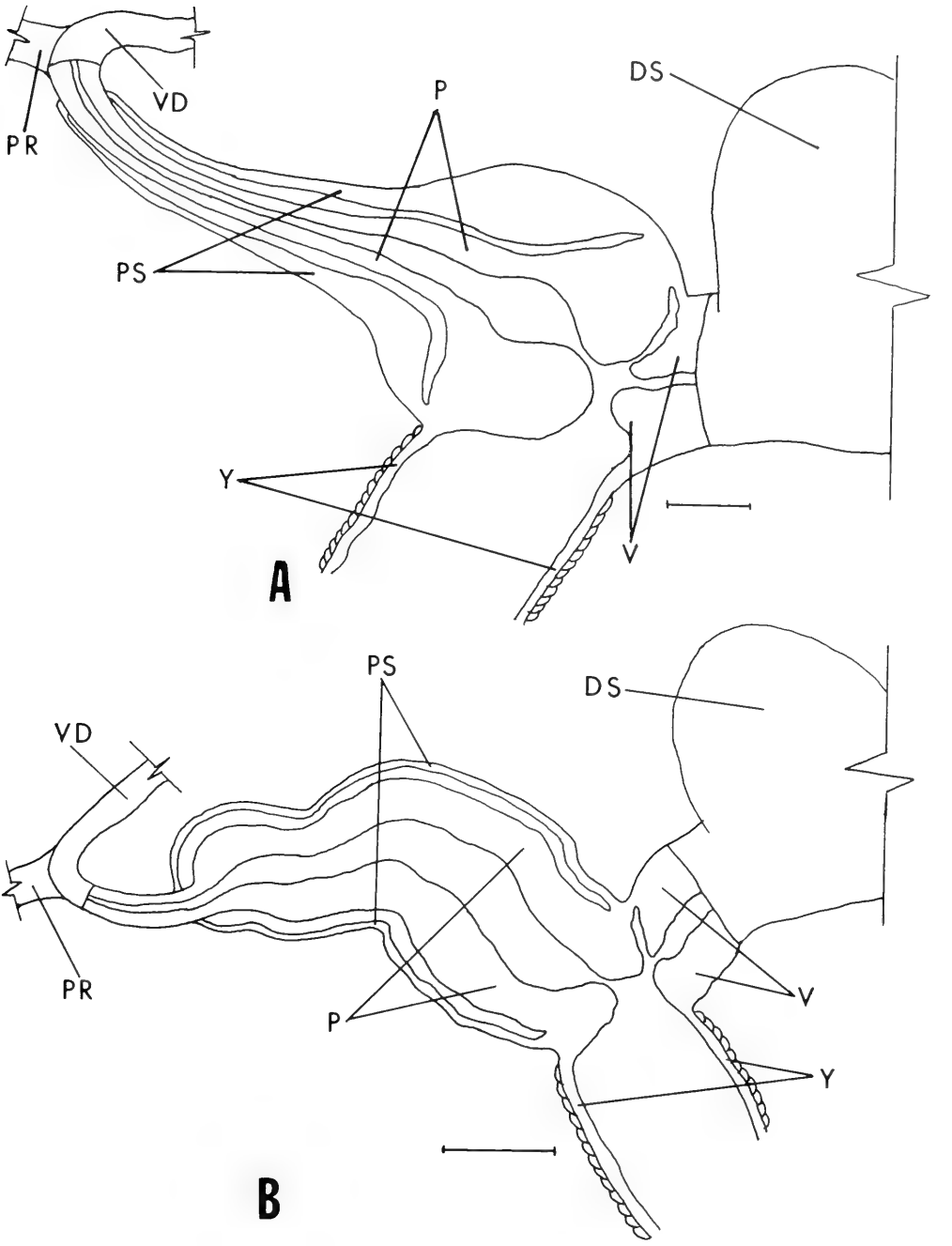


FIG. 4. Longitudinal section of the penises: A, *Philomycus carolinianus*; B, *Philomycus togatus*. Scale bars 1 mm. DS, dart sac; P, penis; PR, penial retractor muscle; PS, penial sheath; V, vagina; VD, vas deferens; Y, atrium.

TABLE 2. Allelic frequency distribution at loci polymorphic within species and at loci with different alleles in each species.

		Philomycus togatus		*Philomycus carolinianus*	
Site code		3N	2N	2N	1N
Number of specimens		10	5	5	10
Locus	Allele				
IDH 1	103	1.00	.40	—	—
	100	—	.60	1.00	1.00
6-PGD 2	107	.70	.60	—	—
	105	.30	.40	—	—
	100	—	—	1.00	1.00
GOT 1	100	1.00	1.00	—	—
	96	—	—	1.00	1.00
LAP 1	103	—	—	1.00	1.00
	100	1.00	1.00	—	—
LAP 2	100	1.00	1.00	—	—
	98	—	—	1.00	1.00
Number of loci polymorphic		1	2	0	0
Observed heterozygosity		0	0	—	—
Probability of not detecting a heterozygote		.0002	.051	—	—

TABLE 3. Genetic distances (Nei, 1978) between populations.

	Site code	*Philomycus togatus*		*Philomycus carolinianus*	
Site code		3N	2N	2N	1N
P. togatus	3N	—	.013	.433	.433
P. togatus	2N		–	.389	.389
P. carolinianus	2N			—	.000
P. carolinianus	1N				—

cluded that three species of arionid slugs were reproducing by self-fertilization. Thus, automixis does occur in slugs. Self-fertilization would seem to be the most likely explanation for the observed lack of heterozygotes in *P. togatus*. However, the small sample sizes necessitate only a tentative conclusion.

Electrophoretic comparisons of specimens from locality 2N, in which *Philomycus togatus* and *P. carolinianus* are sympatric, show that of eleven alleles only one is found in both species (Table 2). The genetic distance between these species in this locality was .389. The interpopulation genetic distance estimate for *P. togatus* (.013) was comparable to intraspecific distances between populations in two species of *Physa* (≦ .012) (Buth & Suloway, 1983) and within each of three species of *Crepidula* (≦ .097) (Hoagland, 1984). Both of these authors used Nei's coefficient. The overall genetic distance estimate between *P. togatus* and *P. carolinianus* was .432, similar to that between two species of *Physa* (.45) (Buth & Suloway, 1983), and between several species of *Sphincterochila* (.372—.399) (Nevo *et al.*, 1983). Davis (1983) has noted that for some Bivalvia there is high probability that two taxa are distinct species of D ≧ .222, which is well below the estimates above.

Philomycus togatus and *P. carolinianus*

were found in sympatry in two localities, 2N and 3N. In both of these areas these species can be collected from the same log. Both species have been collected grouped together under the same piece of loose bark. In spite of the opportunity for cross-fertilization between these species, the data demonstrate that this does not occur. It is clear that the morphological and electrophoretic data support the earlier redesignation (Hubricht, 1968) of *Philomycus togatus* as a distinct species.

LITERATURE CITED

BINNEY, A., 1842, Binney on the naked air-breathing Mollusca. *Boston Journal of Natural History*, 4: 171.

BINNEY, A., 1851, *The terrestrial air-breathing mollusks of the United States*. Gould, A.A., ed., 2: 20.

BINNEY, W.G., 1878, *The terrestrial air-breathing mollusks of the United States*. 5: 182, Published in *Bulletin of the Museum of Comparative Zoology, Harvard*, vol. 4.

BOSC, L.A.G., 1802, *Histoire naturelle des vers contenant leur description et leurs moeurs*. 1: 80.

BRUSSARD, P.F. & McCRACKEN, G.F., 1974, Allozymic variation in a North American colony of *Cepaea nemoralis*. *Heredity*, 33: 98–101.

BUTH, D.G. & SULOWAY, J.J., 1983, Biochemical genetics of the snail genus *Physa*: a comparison of populations of two species. *Malacologia*, 23: 351–359.

CHAMBERS, S.M., 1978, An electrophoretically detected sibling species of *Goniobasis floridensis* (Mesogastropoda: Pleuroceridae). *Malacologia*, 17: 157–162.

CHICHESTER, L.F. & GETZ, L.L., 1968, Terrestrial slugs. *The Biologist*, 50: 148–166.

CHICHESTER, L.F. & GETZ, L.L., 1973, The terrestrial slugs of northeastern North America. *Sterkiana*, 51: 11–42.

CLAPP, W.F., 1920, The shell of *Philomycus carolinianus* (Bosc). *Nautilus*, 33: 83–89.

DAVIS, G.M., 1983, Relative roles of molecular genetics, anatomy, morphometrics and ecology in assessing relationships among North American Unionidae (Bivalvia). In OXFORD, G.S. & ROLLINSON, D., ed., *Systematics Association Special Volume* 24: 193–222.

FOLTZ, D.W., OCHMAN, H., JONES, J.S., EVANGELISTI, S.M. & SELANDER, R.K., 1982, Genetic population structure and breeding systems in arionid slugs (Mollusca: Pulmonata), *Biological Journal of the Linnean Society*, 17: 225–241.

GOULD, A.A., 1841, *Report on the invertebrates of Massachusetts, comprising, the Mollusca, Crustacea, Annelida and Radiata*, vol. 3.

GOULD, A.A., 1862, *Otia Conchologica: Descriptions of shells and Mollusks from 1839 to 1862*. Boston, 256 p.

GREGG, W.O., 1959, A technique for preparing *in toto* mounts of Molluscan anatomical dissections. *The American Malacological Union Annual Report for 1958*, 25: 39.

GRIMM, R.W., 1971, Annotated checklist of the land snails of Maryland and the District of Columbia. *Sterkiana*, 41: 51–57.

HOAGLAND, K.E., 1984, Use of molecular genetics to distinguish species of the gastropod genus *Crepidula* (Prosobranchia: Calyptraeidae). *Malacologia*, 25: 607–628.

HUBRICHT, L., 1951, The Limacidae and Philomycidae of Pittsylvania County, Virginia. *Nautilus*, 65: 20–22.

HUBRICHT, L., 1956, Land snails of Shenandoah National Park. *Nautilus*, 70: 15–16.

HUBRICHT, L., 1968. The land snails of Kentucky. *Sterkiana*, 32: 1–6.

HUBRICHT, L., 1971, The land snails of Virginia. *Sterkiana*, 42: 41–45.

HUBRICHT, L., 1973, The land snails of Tennessee. *Sterkiana*, 49: 11–17.

HUBRICHT, L., 1977, The land snails of Mississippi. *Sterkiana*, 68: 1–4.

IKEDA, K., 1937, Cytogenetic studies on the self-fertilization of *Philomycus bilineatus* Benson. *Journal of Science of Hiroshima University*, ser. B, div. 1, 5: 67–123.

KEARNEY, S.R. & GILBERT, F.F., 1978, Terrestrial gastropods from the Himsworth Game Preserve, Ontario, and their significance in *Parelaphostrongylus tenius* transmission. *Canadian Journal of Zoology*, 56: 688–694.

KUGLER, O.E., 1965, A morphological and histochemical study of the reproductive system of the slug, *Philomycus carolinianus* (Bosc). *Journal of Morphology*, 116: 117–132.

MacNAMARA, M.C. & HARMAN, W.N., 1975, Further studies on the Mollusca of the Otsego Lake Area. *Nautilus*, 89: 87–90.

McCRACKEN, G.F. & SELANDER, R.K., 1980, Self-fertilization and monogenic strains in natural populations of terrestrial slugs. *Proceedings of the National Academy of Science*, 77: 684–688.

MURPHY, P.G., 1978, *Collisella austrodigitalis* sp. nov.: a sibling species of limpet (Acmaeidae) discovered by electrophoresis. *Biological Bulletin*, 155: 193–206.

NEVO, E., BAR-EL, C. & BAR, Z., 1983, Genetic diversity, climatic selection and speciation of *Spincterochila* landsnails in Israel. *Biological Journal of the Linnean Society*, 19: 339–373.

NEI, M., 1978, Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, 89: 585–590.

NICKLAS, N.C. & HOFFMAN, R.J., 1981, Apomictic parthenogenesis in a hermaphroditic terrestrial slug, *Deroceras laeve* (Muller). *Biological Bulletin*, 36: 80–85.

PILSBRY, H.A., 1948, *Land Mollusca of North America (North of Mexico)*. Vol. 2, part 2. Mono-

graph 3, Academy of Natural Sciences of Philadelphia, p. 750–759.

RAFINESQUE, C.S., 1820, *Annals of nature or annual synopsis of new genera and species of animals and plants discovered in North America*, p. 10.

SELANDER, R.K. & HUDSON, R.O., 1976, Animal population structure under close inbreeding: the land snail *Rumina* in southern France. *American Naturalist*, 110: 697–718.

SELANDER, R.K., SMITH, M.H., YANG, S.Y., JOHNSON, W.E. & GENTRY, J.B., 1971, Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse (*Peromyscus polionotus*), Studies in Genetics VI. *University of Texas Publicaton* 7102: 49–90.

SICILIANO, M.J. & SHAW, C.B., 1978, Separation and visualization of enzymes on gels. In SMITH, I., ed., *Chromatographic and electrophoretic techniques*, 2, ed. 4, p. 187–216, Heineman Medical, London.

TOMPA, A.S., 1980, The ultrastructure and mineralogy of the dart from *Philomycus carolinianus* (Pulmonata: Gastropoda) with a brief survey of the occurrence of darts in land snails. *Veliger* 23: 35–42.

WEBB, G.R., 1970, Observations on the sexology of *Philomycus carolinianus* (Bosc). *Gastropodia*, 1(7): 62–65.

Revised Ms. accepted 22 August 1985

MALACOLOGIA, 1986, 27(2): 281–290

MODIFICATION OF PREDATORY SNAIL CHEMOTAXIS BY SUBSTANCES IN BIVALVE PREY ODORS

Dan Rittschof[1] & Anne B. Brown[2]

College of Marine Studies
University of Delaware
Lewes, DE 19958, U.S.A.

ABSTRACT

Effects of bivalve odors were tested on chemotactically stimulated movements in newly hatched predatory marine gastropods (*Urosalpinx cinerea*). Odors from *Mytilus edulis* (shown previously to contain chemosuppressant activity, Williams *et al.*, 1983) go well as odors from *Geukensia demissa*, *Mercenaria mercenaria*, *Mulinia lateralis*, and *Tagelus plebeius* contained substances that suppress chemotactic responses of newly hatched snails to purified barnacle odors.

In addition we: 1) demonstrated that mussel suppressants are molecules of less than 1000 Daltons, 2) showed suppression is by a non-competitive mechanism, 3) used laboratory chemicals identified as components of mussel odors and determined that ammonium ion enhances chemotactic responses to barnacle odor. The other components of mussel odor that could be identified with certainty did not affect snail responses to barnacle odor.

INTRODUCTION

Newly hatched predatory snails, *Urosalpinx cinerea*, are attracted specifically to barnacles (Rittschof *et al.*, 1983) by peptides (Rittschof *et al.*, 1984a) present in odors associated with intact prey. Prey odors are the assemblage of volatile and non-volatile substances that result from soaking living prey in sea water. Snails move in a directed manner that is determined by a combination of flow and odor (Rittschof *et al.*, 1983; Brown & Rittschof, 1984). Although odors from mussels, *Mytilus edulis*, and oysters, *Crassostrea virginica*, are not in themselves attractive to newly hatched snails (Rittschof *et al.*, 1983), consumption of these prey by them results in development of a chemotactic response to their odors (Wood, 1968). Odor components involved in the later response are substances similar in chromatographic properties to barnacle odor peptides.

In the laboratory, odors from *M. edulis* and *C. virginica* interfere with responses of newly hatched *U. cinerea* to attractant from barnacles (Williams *et al.*, 1983). Williams *et al.* (1983) demonstrated that chemotaxis to attractive prey odors is suppressed by both long- and short-term exposure of juvenile snails to oyster or mussel odor. Mixtures of relatively high concentrations of oyster or mussel odor with odor from barnacles evoke lower percentages of chemotaxis by the snails, than does barnacle odor alone. Williams *et al.* (1983) observed that combinations of dilute attractant and suppressant occasionally result in enhancement of the chemotactic response of the snails (an increase in the percentage of snails responding to mixtures when compared to the percentage of snails responding to just barnacle odor). The method used to generate suppressant odors in the Williams study was similar to techniques used with barnacle prey to obtain stimulants of *U. cinerea* and similar to the technique used with mussels to generate odors attractive to lobsters (Derby & Atema, 1981).

Subsequently, Rittschof *et al.* (1984b) found that newly hatched *Urosalpinx cinerea* incubated as encapsulated embryos in mussel or oyster odors until hatching are more sensitive to barnacle odor than are snails that developed either in control sea water or in the presence of barnacle odor. Increased sensi-

[1]Present address: Duke University Marine Laboratory, Pivers Island, Beaufort, NC 28516, U.S.A.
[2]Present address: Aquatic Terrestrial Research Inc., 1230A W. Second Street, Los Angeles, CA 90026, U.S.A.

tivity of snails to barnacle odors after exposure to odors containing suppressant substances suggests that snails have mechanisms for compensating to chemical interference.

Further chemical characterization of substances that suppress predatory snail chemotaxis was performed in the present investigation. Specifically, we: 1) confirmed that suppressant substances are released by bivalves, 2) demonstrated that mussel suppressants are molecules of fewer than 1000 Daltons, 3) used laboratory chemicals identified as components of mussel odors and determined that ammonium ion enhances chemotactic responses to barnacle odor. The other components that could be identified with certainty did not affect snail responses to barnacle odor.

MATERIALS AND METHODS

Biological materials

Capsules containing embryonic *Urosalpinx cinerea* (Say) were collected from both breakwaters of Delaware Bay during July and September and maintained in the laboratory (Rittschof *et al.*, 1984a). Capsules were cleaned of debris and maintained in aerated sea water at 22 ± 2°C at approximately 1000 capsules liter^{-1}. Snails were used in assays one to five days after hatching.

Five species [*Mytilus edulis* Linné, *Geukensia demissa* (Dillwyn), *Mercenaria mercenaria* (Linné), *Tagelus plebeius* (Lightfoot) and *Mulinia lateralis* (Say)] of living, pumping bivalves were used to generate test odors. Blue mussels, *M. edulis*, and ribbed mussels, *G. demissa*, were collected from the mouth of the Broadkill River, Lewes, Delaware. Mussels were cleaned of most fouling organisms by brushing; however, mussels fouled with bryozoans or barnacles were not used. Hard clams, *M. mercenaria*, and stout razor clams, *T. plebeius*, were collected from Savages Ditch, Indian River Bay, Delaware. Razor clams were bound loosely with rubber bands to prevent gaping. Adult mud clams, *M. lateralis*, were raised in the laboratory from larvae (Brown, 1984).

Prey odors were generated by exposing 50 g fresh weight of live, pumping bivalves per liter of sea water for four hours. This was done in 4.5 liter aquaria with aged sea water (Rittschof *et al.*, 1983; Williams *et al.*, 1983). After four hr of exposure with aeration, bivalves were removed and the sea water filtered through 1, 0.45, and 0.22 micron filters, and either used immediately, or frozen in 50 ml portions in a dry ice/methanol bath and stored at −20°C until used.

Biological assays

Snail creeping assays (Rittschof et al., 1983) used homogeneous solutions containing aged sea water (sea water control). Sea water plus an amount of fractionated barnacle odor sufficient to elicit response from approximately 50% of the snails (stimulus control) or the same amount of stimulus and a dilution of bivalve (or fractionated) bivalve odor. Using an amount of stimulus (barnacle odor) sufficient to elicit a 50% response enabled detection of enhancement (significantly greater response) or suppression (significantly reduced response) with the same assay. In the assay, snails were exposed to a flow of homogeneously mixed solution. Snails creeping upstream 1 or more cm/10 min were scored as responding. Snails that did not creep 1 cm upstream were counted as not responding. Stimulus for snail creeping was pressure dialyzed barnacle odor <10,000 D and >1000 D that was purified and concentrated several thousand-fold (Rittschof *et al.*, 1984a). Potential suppressants were diluted between 1:1 and 1:50 with sea water. At least three dilutions of suppressant odor, a stimulus control and a sea water control were included in each assay series. Potential suppressants were also tested for stimulus activity in the absence of barnacle odor by dilution with aged sea water (Rittschof *et al.*, 1983; Williams *et al.*, 1983).

In a separate series of assays with mussel suppressant, the level of suppressant was held constant in the presence of a 50-fold range of dilution of stimulus. The concentration of suppressant used was that necessary to inhibit responses ($P < 0.01$) by snails to the 50% response level of attractant. Dilutions of attractant tested were × (standard stimulus level in all except this series of experiments), 4×, 2×, 0.30×, and 0.20×.

Fractionation of mussel suppressant

A series of experiments was conducted to determine basic molecular characteristics of suppressant(s) from mussels. In all experiments sea water was subjected to the same

treatment and tested in suppressant assays as a control.

Molecular sizing

In order to compare stimulus and suppressants on the basis of molecular size, mussel odor was fractionated in size ranges of <500, <1000, 1000–10,000 and 10,000–1000,000 Daltons by pressure dialysis (Amicon UM05, UM2, YM10, and YM100 membranes). After dialysis each fraction was diluted with filtered sea water (<1000 Daltons) to the pre-dialysis volume. The same dilution of filtered sea water was used in stimulus and sea water controls.

Adsorption chromatography

As another basis of comparison with stimulus, mussel odor was fractionated by passage of sea water containing suppressant through Amberlite XAD-7. Stimulus from barnacle odor can be extracted and concentrated from sea water with Amberlite XAD-7 resin. Other potential adsorbents were also tested. These included: Amberlite XAD-2 (Rohm and Haas Inc.), Sep-Pak C_{18} cartridges, and Sep-Pak silica cartridges (Waters Corp.). Amberlite resins were extensively washed as recommended by Jolley (1981) prior to use. Adsorbents were washed with ten volumes of distilled water. Adsorbed material was eluted with 100% Liquid Chromatography grade methanol. Methanol was removed under vacuum and remaining material reconstituted with sea water to its pretreatment volume.

In three series of experiments the pH of mussel odor was lowered to 2 with concentrated reagent grade HCl to increase adsorption of organic compounds (Jolley, 1981). The XAD-7 column was washed with ten volumes of pH 2 water. Elution of adsorbed material was with 90% methanol .01 M NaOH. After elution, the pH was adjusted back to the initial pH of 7.65—7.75 with concentrated NaOH.

Final experiments tested commercially purchased primary amines [ammonium chloride (Fisher), tryptophane, and L-dopamine (Sigma)] that were detected in the size fraction (< 1000 Daltons) of mussel odor with suppressant activity. These compounds were tested in dilution series that bracketed the range that they occurred in suppressant. Concentrations and identities of these compounds were determined by High Performance Liquid Chromatography.

Amino acid analysis

Mussel suppressant was tested for presence of amino acids, other primary amines, and carbohydrate. Primary amines were derivitized with o-pthaldialdehyde, separated by high performance liquid chromatography on an Altex 25 cm, 5 μm particle size, octadecylsilane column, detected fluorometrically and identified by co-migration with standards (Lindroth & Mopper, 1979; Mopper & Lindroth, 1982).

Data analysis

The Log Likelihood Statistic (G, Sokal & Rohlf, 1981) was used to test for suppressant activity (Williams *et al.*, 1983). In addition, Probit Analysis (Finney, 1969) was used to determine concentration of test solutions that were effective in inhibiting responses by 50% (EC_{50}). EC_{50}s were determined by a BASIC computer program (Lieberman, 1983) modified from Applesoft BASIC to MBASIC.

RESULTS

Bivalve suppressants

Prior study of bivalve odor potency has shown that pumping activity is important for odor production (Blake, 1960; Williams *et al.*, 1983). Accordingly, tests comparing bivalve odor potency used only preparations where bivalves were actively ventilating. Odors from all five bivalve prey contained highly significant ($p << 0.01$ by G statistic) suppressant activity (Fig. 1A-E). Probit estimates (Lieberman, 1983) of odor dilutions effective for reducing responses to 50% of control ranged from 1:50 for *Mytilus edulis* odor to 1:12 for *Mulinia lateralis* odor. There were no significant differences in potencies of suppressants produced by the 5 bivalves tested ($p > .05$).

Mytilus edulis suppressant

Suppressant from blue mussels (Fig. 1A) was chosen for further study. In addition to being the only common bivalve consumed by oyster drills at Delaware Bay breakwaters, blue mussels pumped predictably and could be obtained readily in quantity and in an unfouled condition. First, the effects of various stimulus concentrations were tested. Then, molecular size and adsorptive proper-

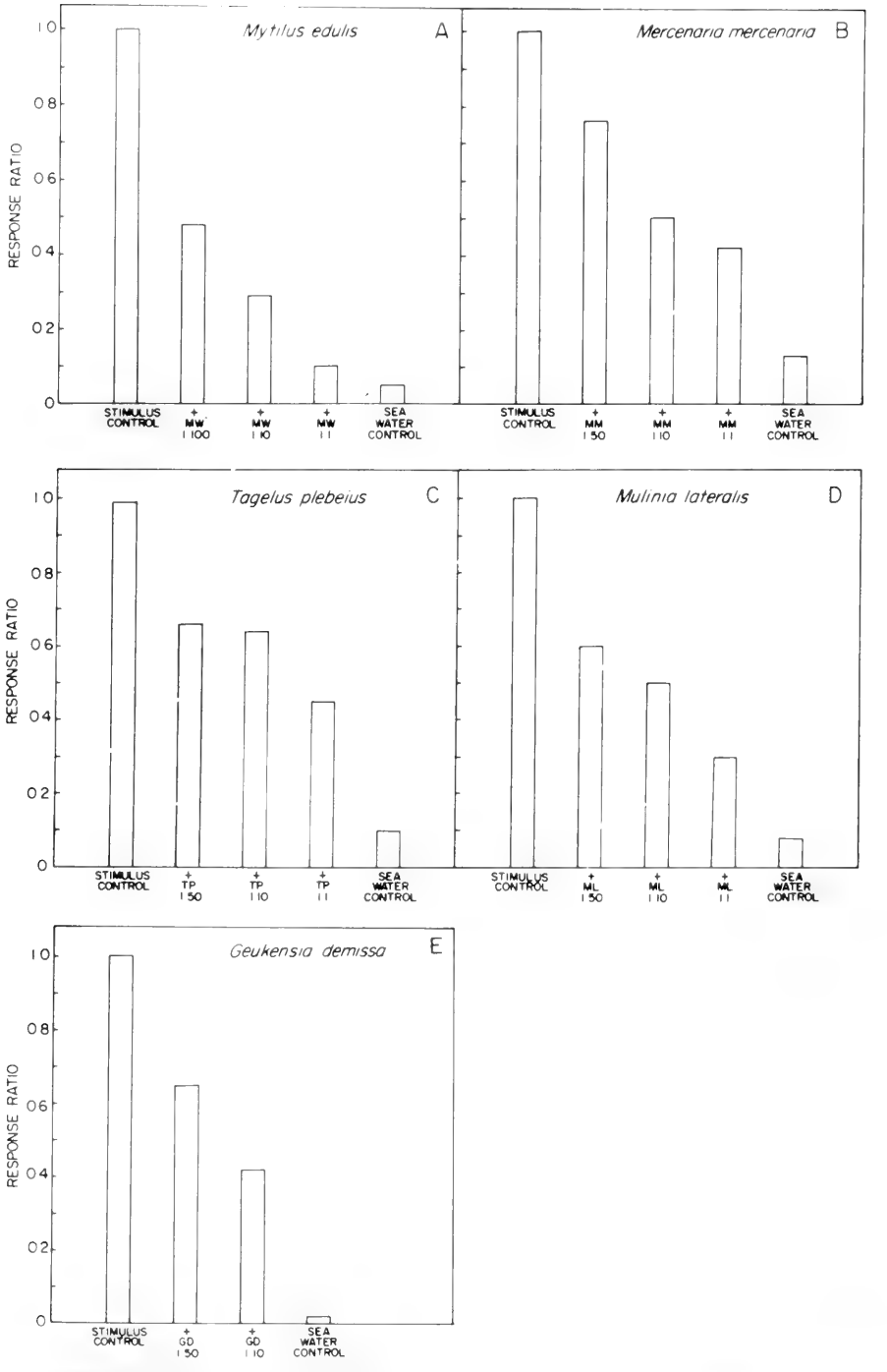


FIG. 1. Suppressant activity in odor preparations from five bivalve species. Because of variability in response of different batches of snails, the ratio of the response to stimulus control to stimulus + dilutions of each bivalve odor has been presented. Dilutions tested, suppressant odor : sea water are presented below each bar. Numbers of snails tested per bar ranged from 110 to 60. Responses of snails to stimulus in sea water varied from 40 to 60%.

TABLE 1. Response of snails to attractant in the absence and in the presence of suppressant (1:15 dilution) from blue mussels.

Dilution of attractant	Without suppressant		With suppressant	
	Number tested	Percentage responding	Number tested	Percentage responding
4×*	87	67	71	25
2×	53	50	56	18
1×	137	39	142	18
0.3×	52	31	55	20
0.2×	64	22	81	17

*× is the concentration of stimulant used in all other experiments.

ties were examined, enabling comparison of suppressant with known stimulant substances (Rittschof *et al.*, 1984a).

Effect of modifying stimulus concentration

The mechanism of action (competitive vs. non-competitive) was tested by determining the effect of varying stimulus concentration. Attractant concentration was varied 20-fold and tested with the same concentration of suppressant from mussels. In the absence of suppressant there was a consistent, positive, dilution versus response relationship (Overall $G = 37.2$ 4 d.f. $p << 0.001$) (Table 1). In the presence of suppressant, the response to stimulus was reduced to the same level in all dilutions of stimulus (Table 1). Responses by snails in the presence of suppressant were low and statistically the same through a 20-fold range of stimulus concentration (Overall $g = 2.1$ 4 d.f. $p > 0.05$).

Size fractionation of mussel inhibitor

Cascade pressure dialysis was employed to estimate the molecular size of substances in mussel water with suppressant activity. Suppressant substances passed readily through membranes with nominal molecular exclusions of 100,000, 10,000, and 1,000 Daltons. The original suppressant activity was detected in the fraction of substances with molecular weights of less than 1000 Daltons. There was no evidence of substances greater than 1000 Daltons with suppressant activity (Fig. 2). Next, suppressant was subjected to pressure dialysis with a 500 Dalton cutoff membrane. Suppressant activity was retained above the membrane. The fraction containing substances passing through the membrane facilitated snails' responses to attractant (Fig. 2).

Affinity of suppressant for adsorbents

Mussel suppressant at normal ionic strength and pH of sea water had little or no affinity for Amberlite XAD-2, XAD-4, Amberline XAD-7, Waters Sep-Pak Silica or Waters Sep-Pak reversed phase Silica. In each case the eluate (undiluted and diluted 1:10) was as potent as the starting material. As observed occasionally in molecular sizing experiments, low dilutions of suppressant containing eluate (1:50 to 1:100) periodically facilitated the response to stimulus.

Amberlite XAD-7 is effective at removing substances such as humic acids form sea water if the pH is lowered to 2.0 during the adsorption process (Jolley, 1981). A portion of the suppressant was removed by lowering the pH to 2.0 prior to passage through XAD-7 (Table 2). Attempts to recover suppressant adsorbed on the resin by elution with 90% methanol 0.01 N sodium hydroxide were unsuccessful. The basic methanol eluates of columns, whether they were control or experimental, were slightly inhibitory. This was the case even after removal of methanol and neutralization of pH.

Information about size and adsorptive properties was used to formulate additional chemical and biological tests of mussel odor and its components. The data indicate suppressants are relatively small hydrophilic compounds. Two common classes of small hydrophilic compounds are primary amines (amino acids, ammonium and other organic amines) and sugars. Chemical tests determined the types and concentrations of potential suppressant

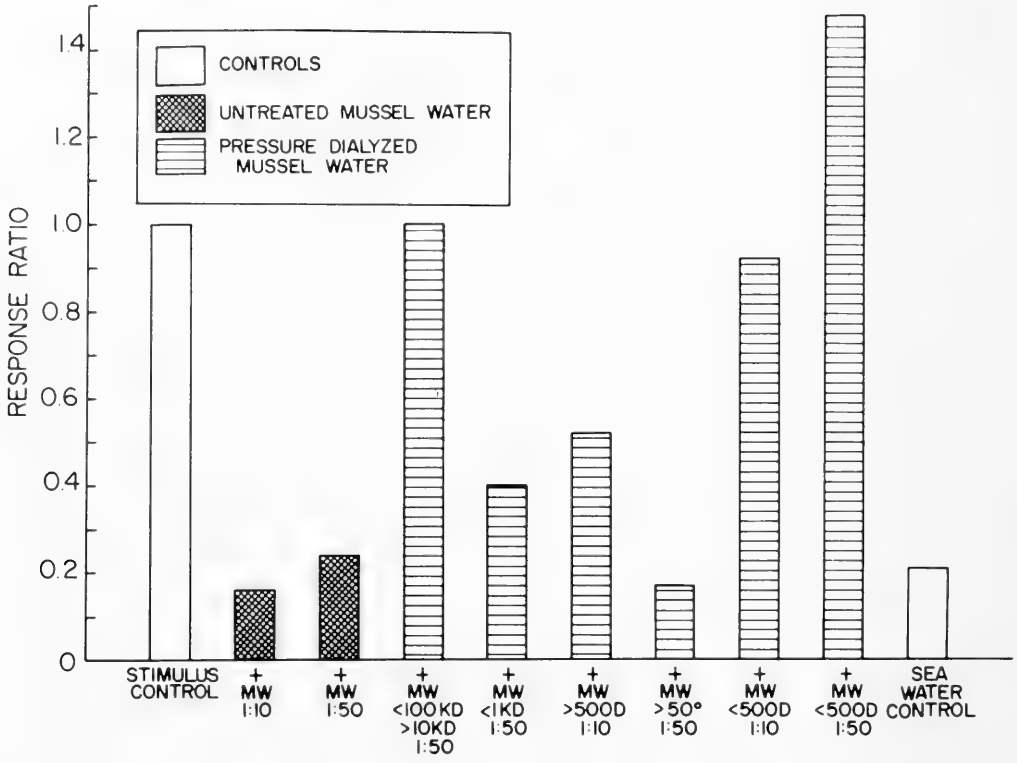


FIG. 2. Size fractionation of suppressant from *Mytilus edulis*. Stimulus was mixed in 1 part sea water <1000 Dalton and 49 parts unfiltered sea water. Dilution of inhibitor fractions was in a similar fashion. Inhibitory activity resided in the <1000 Dalton fraction and in the fraction <1000 Daltons and >500 Daltons. The fraction less than 500 Daltons enhanced response percentages.

substances, and biological assays tested, the effectiveness of these compounds as suppressants at the concentrations at which they occurred in active suppressant preparations. Suppressant preparation for these experiments was first passed through XAD-7 resin and 10,000 and 1,000 Dalton membranes prior to chemical and biological determinations.

HPLC analysis of primary amines in sea water and mussel suppressant indicated elevated levels of L-dopamine, tryptophane and ammonium in the suppressant preparation. Comparison of peak areas with standards of known concentration indicated that there were approximately 0.6 μM levels of L-Dopamine, 0.2 μM tryptophane and approximately 3 μM ammonium. Bioassays were used to test the activity of 2 micromolar to .2 micromolar tryptophane, 1.33 to .13 micromolar L-Dopamine and 30 to 3 micromolar ammonium. Analysis of reagent grade tryptophane showed 5 to 10% contamination with dopamine. After each assay, water from the most concentrated dilution of each compound tested was analyzed by HPLC. In each case, the resultant peaks demonstrated that concentrations of the added compounds were approximately ten times greater than the mussel odor fraction with 100% suppressant activity. Biological assays indicated that ammonium and L-DOPA had insignificant levels of inhibitory activity. Tryptophane significantly inhibited responses of snails at three of the four dilutions tested. However, compared to suppression of a comparable dilution of mussel water, inhibition was slight (Table 3). Additions of ammonium at levels approaching those found in suppressant enhanced responses significantly (Table 3).

TABLE 2. Removal of suppressant activity from sea water by adsorption onto Amberlite XAD-7 resin at pH 2.0.

Group or fraction*	Number tested	Percent response	Comparison	G	Sig
Experiment One					
Stimulus control[a]	72	50	—	—	—
+MW 1:15[b]	83	22	vs cont	12.5	<0.005
Effluent[c]	73	36	vs cont	2.5	ns
			vs +MW	4.1	<0.05
Experiment Two					
Stimulus control	74	51	—	—	—
+MW 1:15	82	26	vs cont	9.9	<0.005
Effluent	83	37	vs cont	3.1	ns
			vs +MW	2.6	ns
Experiment Three					
Stimulus control	90	47	—	—	—
+MW 1:15	85	24	vs cont	10.3	<0.005
Eluate[d]	81	37	vs cont	1.6	ns
			vs +MW	3.5	ns
Experiment Four					
Stimulus control	99	38	—	—	—
Eluate	111	37	vs cont	0.1	ns
Eluate control[e]	103	27	vs cont	3.4	ns
			vs eluate	2.1	ns

[a]Stimulus control: sea water whose pH was lowered to 2.0 with HCl and then returned to 7.7 with NaOH was diluted 1:15 with untreated sea water; then stimulus was added.

[b]+MW: The pH of mussel water was lowered to 2.0, then returned to 7.7. This was then diluted 1:15 with untreated sea water and stimulus was added.

[c]Effluent: Mussel water at pH 2.0 was passed through an XAD-7 column. The pH was then returned to normal and the solution mixed with untreated sea water and stimulus as described above.

[d]Eluate: The XAD-7 column from above([c]) was rinsed with pH 2.0 distilled water, then eluted with 90% methanol in 0.01 N NaOH. This material was rotary evaporated to near dryness, reconstituted with sea water to 0.5 the original volume, and tested for inhibitory activity.

[e]Eluate control: Identical to Eluate ([d]) except the material originally passed through the column (as in [c]) was sea water, not mussel water.

DISCUSSION

Substances that suppress chemotactic responses of newly hatched oyster drills to barnacle attractant occur in all five species of bivalve tested. Thus the suppressant phenomenon reported by Williams *et al.* (1983) appears common to odors of many bivalves. Within the limits of discrimination of the biological assay, suppressant potencies in all odors were similar. Cascade dialysis experiments showed that suppressant activity present in blue mussel odor is associated with molecules of less than 1000 Daltons. Little confidence can be placed upon the size of the suppressant based upon its retention by the 500 Dalton membrance because charged substances of less than 500 Daltons are often retained. The passage of facilitory activity through the membrane is, however, strong evidence that facilitation and suppression are due to different substances. Experiments with organic adsorbents indicate that at least a fraction of the suppressant activity is associated with hydrophilic, possibly charged substances. Ammonium, tryptophane and L-Dopa were present in relatively high concentrations in fractionated mussel odor with potent suppressant activity. L-Dopa, a neurotransmitter in a variety of vertebrates and invertebrates, is also a major building block of mussel periostracum and byssus (Waite & Tanzer, 1981). Thus, it is not surprising to find L-Dopa in high concentration in mussel odor. When tested for suppressant activity, ammonium, L-Dopa and tryptophane were ineffective at concentrations similar to

TABLE 3. Tests of primary amines for suppressant activity.

Substance*	Concentration	Number tested	Percentage responding	G vs. cont	Sig. (p)
Stimulus control	Standard	69	56	—	—
+ 1:10 MW	—	78	13	32.9	<<0.005
+ Ammonium	3.0 × 10–5	55	62	0.4	ns
+ Ammonium	1.5 × 10–5	87	65	1.3	ns
+ Ammonium	3.0 × 10–6	61	77	6.1	<0.05
No stimulus	—	54	2		
+ Ammonium	3 × 10–5m	67	3		
+ Ammonium	3 × 10–6M	40	4		
Stimulus control	Standard	52	87	—	—
+ 1:10 MW	—	89	17	69.6	<<0.005
+ Tryptophane	2 × 10–6M	56	52	15.6	<0.005
+ Tryptophane	1 × 10–6M	46	89	0.2	ns
+ Tryptophane	2 × 10–7M	56	66	6.2	<0.005
+ Tryptophane	2 × 10–8M	59	71	3.9	<0.05
No stimulus	—	64	2		
Stimulus control	Standard	71	52	—	—
+ 1:10 MW	—	81	17	20.8	<0.005
+ L-DOPA	1.3 × 10–6M	55	44	0.9	ns
+ L-DOPA	6.6 × 10–7	46	63	1.4	ns
+ L-DOPA	1.3 × 10–7	71	52	0	ns
No stimulus	—	77	4		
+ L-DOPA	1.3 × –6M	36	8		
+ L-DOPA	1.3 × –7	29	8		

*For each substance the concentration approximately equivalent to the concentration of that substance in a 1:10 dilution of inhibitor is underlined.

those in mussel suppressant. Ammonium at 3 $\times$ 10^{-6} M facilitated responses to attractant.

The general occurrence of suppressant in odors of all bivalves tested suggests that interference with drill chemoreception may be due to products common to bivalve metabolism. Interference is not competitive with stimulus because inhibition cannot be eliminated by increasing attractant concentration. Suppressants are markedly different chemically from stimulus. Stimulants are proteinaceous (Blake, 1961; Rittschof *et al.*, 1984a). Suppressants have a molecular size that corresponds at their largest size to tripeptides and disaccharides and at their smallest to inorganic ions. The resistance of suppressants to extraction procedures known to isolate stimulus (Rittschof *et al.*, 1984a) supports the view that they are markedly different from stimulants. Sugars and amino sugars would be resistant to these extraction procedures and are thus additional candidates as suppressants.

Although substances produced by bivalves interfere with chemotactic responses of newly hatched drills, larger drills that have consumed bivalve prey, including oysters, mussels, clams and scallops, can locate all of these prey from a distance (Haskin, 1950; Blake, 1961; Wood, 1968; Pratt, 1974; Ordzie & Garofalo, 1980). Work in progress demonstrates that the stimulants in at least *Crassostrea virginica* odor are similar chemically to stimulants from barnacles. Thus, conditioning of drills to bivalve prey odors may include sensitization to stimulant substances and desensitization to suppressants. The occurrence of desensitization to suppressants is supported by the observation that exposure of snail embryos to bivalve odors results in increased responsiveness of the snails to barnacle attractant upon hatching (Rittschof *et al.*, 1984b). Purification and identification of all active odor components will assist interpretation of these interactions.

Facilitation (Williams *et al.*, 1983; Rittschof *et al.*, 1983) of drill responses to stimulus can be attributed to presence of ammonium. Ammonium was reported to be attractive to *Urosalpinx cinerea* by Blake (1961) and was suggested as a non-specific attractant by Wood (1968). However, Pratt (1974) showed that ammonium at levels corresponding to those of prey effluents was not attractive to *U.*

cinerea and noted that his results disagreed with the opinions of Blake and Wood. Our finding that ammonium is not attractive in itself but has the capacity to facilitate responses in concert with low levels of specific stimulants resolves this conflict.

The well-documented ability of drills to locate prey with relatively high metabolic rates [Haskin, 1950; (either smaller individuals or higher oxygen consumption) Blake, 1962] may be explained by the facilitation of chemotaxis by ammonium. Drills may be directed to prey by the combination of stimulant and ammonium. If the mechanisms of generation of ammonium and stimulus are different (for example, slow release and hydrolysis of attractant and metabolic production of ammonium) then the ratio of ammonium to stimulus would be higher in metabolically active prey.

Ammonium ion concentrations may be used by drills to determine distances to prey. Brown & Rittschof (1984) demonstrated that drills respond to combinations of flow and stimulus concentrations predictably. Ammonium ion could serve to modulate responses by providing distance information. Diffusion would result in relatively rapid attenuation of the ammonium signal with little concomitant change in the more slowly diffusing peptide attractant signal. Thus, the presence of ammonium with attractant could facilitate a response because it indicates prey are relatively close-by. The possibility exists that ammonium could function in a similar fashion for other organisms, lobsters, for example (Derby & Atema, 1981). In the case of the latter, the existence of specific and sensitive ammonium receptors has been demonstrated (Derby & Atema, 1982a).

Bivalve odors contain a mixture of low molecular weight substances that can have both negative and positive effects on chemoreceptive responses of *Urosalpinx cinerea*. Negative effects of bivalve odors (specifically of *M. edulis*) on chemotaxis have been observed previously for *U. cinerea* (Williams *et al.*, 1983) and for *Asterias forbesii* (Davis, 1975). Many reports indicate positive chemotactic responses to these types of odors especially after ingestive experience. These reports include responses by gastropods (Haskin, 1950; Chew & Eisler, 1958; Wood, 1968), echinoderms (Castilla, 1972), flatworms (Ferrero *et al.*, 1976) and crustaceans (Derby & Atema, 1980, 1981, 1982a, b). In the case of drills, it appears that the observed response is dependent upon the concentration of each effector. At high odor concentrations the noncompetitive nature of suppressant activity dominates. Suppressant effectiveness diminishes rapidly with dilution. Even our most potent suppressant solutions cannot be diluted more than 100 fold without total loss of activity. In comparison, attractant from barnacles generated from a similar biomass can easily be diluted 500 fold and retain detectable activity (Rittschof *et al.*, 1983, 1984a). As suppressant activity is diluted, facilitation of response to stimulus by ammonium is manifested. Although chemical detection of prey by snails is obviously complex, understanding the nature of the chemicals involved provides a basis for future hypotheses.

The ability to detect levels of stimulus in natural waters containing very high concentrations of mussels and barnacles (Rittschof *et al.*, 1984b) using newly hatched drills suggests that bivalve suppressants are not functioning as defenses at distances of several meters. They may be effective however over short distances (centimeters). Laboratory evidence of attractiveness of mixtures containing bivalve odors (Rittschof *et al.*, 1983), and the evidence presented here of general production of suppressant activity by bivalves, many of which experience little if any muricid gastropod predation, support the hypothesis that suppressants are not specific antipredator substances. However, the interplay of these natural suppressants with stimulants and enhancing substances provides the basis for continued study and dissection of a complex rheotactic and chemotactic guidance system.

ACKNOWLEDGEMENTS

We thank R. Shepherd, K. Mopper, M.R. Carriker, C. Merrill, D. Keiber, C. Griffith, D. Einholf and J. Deschamps for critical advice and assistance. Sea Grant #NA83AA-D-0017.

LITERATURE CITED

BLAKE, J.W., 1960, Oxygen consumption of bivalve prey and their attractiveness to the gastropod, *Urosalpinx cinerea*. *Limnology and Oceanography*, 5: 273–280.

BLAKE, J.W., 1961, Preliminary characterization of oyster metabolites attractive to the predatory

gastropod, *Urosalpinx cinerea*. Ph.D. thesis, University of North Carolina, Chapel Hill, 46 p.

BROWN, A.B., 1984, Development of *Mulinia lateralis* (Say) in response to estrogen supplements. Masters thesis, University of Delaware, 114 p.

BROWN, B.B. & RITTSCHOF, D., 1984, Integration of flow and chemical cue by predatory snails. *Marine Behavior and Physiology*, 11: 75–93.

CASTILLA, J.C., 1972, Responses of *Asterias rubens* to bivalve prey in a Y-maze. *Marine Biology*, 12: 222–228.

CHEW, K.K. & EISLER, E., 1958, A preliminary study of the feeding habits of the Japanese oyster drill, *Ocinebra japonica*. *Journal of the Fisheries Research Board of Canada*, 15: 529–535.

DAVIS, S., 1975, Chemoreception in the starfish, *Asterias forbesi*. Masters thesis, University of Delaware, 95 p.

DERBY, C.D. & ATEMA, J., 1980, Induced host odor attraction in the pea crab *Pinnotheres maculatus*. *Biological Bulletin*, 158: 26–33.

DERBY, C.D. & ATEMA, J., 1981, Selective improvement in responses to prey odors by the lobster, *Homarus americanus* following feeding experience. *Journal of Chemical Ecology*, 7: 1073–1080.

DERBY, C.D. & ATEMA, J., 1982a, Chemosensitivity of walking legs of the lobster *Homarus americanus*: neurophysiological response spectrum and thresholds. *Journal of Experimental Biology*, 98: 303–315.

DERBY, C.D. & ATEMA, J., 1982b, The function of chemo- and mechanoreceptors in lobster (*Homarus americanus*) feeding behaviour. *Journal of Experimental Biology*, 98: 317–327.

FERRERO, E., TONGIORGI, P., GALLENI, L., SALGHETTI, U. & SALVADEGO, P., 1976, Chemical attraction of *Stylochus mediterraneus* Galleni (Turbellaria: Pollycladia) toward its prey *Mytilus galloprovincialis* L. *Marine Biology Letters*, 1: 213–224.

FINNEY, D.J., 1971, *Probit analysis*. Cambridge University Press, London, England, 333 p.

HASKIN, H.H., 1950, The selection of food by the common oyster drill. *Proceedings of the National Shellfisheries Association*, 1950: 62–68.

JOLLEY, R.L., 1981, Concentrating organics in water for biological testing. *Environmental Science and Technology*, 158: 874–880.

LIEBERMAN, H.R., 1983, Estimating LD50 using the probit technique: A BASIC computer program. *Drug and Chemical Toxicology*, 6: 111–116.

LINDROTH, P. & MOPPER, K., 1979, High performance liquid chromatographic determination of amino acids by precolumn derivitization with opthaldialdehyde. *Analytical Chemistry*, 51: 1667–1674.

MOPPER, K. & LINDROTH, P., 1982, Diel and depth variations in dissolved free amino acids and ammonium in the Baltic Sea determined by shipboard HPLC analysis. *Limnology and Oceanography*, 27: 336–347.

ORDZIE, C.J. & GAROFALO, G.C., 1980, Predation, attack success and attraction to the bay scallop, *Argopecten irradians* (Lamarck) by the oyster drill, *Urosalpinx cinerea* (Say). *Journal of Experimental Marine Biology and Ecology*, 191: 199–209.

PRATT, D.M., 1974, Attraction to prey and stimulus to attack in the predatory gastropod *Urosalpinx cinerea*. *Marine Biology*, 27: 37–45.

RITTSCHOF, D., WILLIAMS, L.G., BROWN, B. & CARRIKER, M.R., 1983, Chemical attraction of newly hatched oyster drills. *Biological Bulletin*, 164: 493–505.

RITTSCHOF, D., WILLIAMS, L.G. & SHEPHERD, R.G., 1984a, Concentration and preliminary characterization of a snail attractant from sea water. *Journal of Chemical Ecology*, 10: 63–79.

RITTSCHOF, D., KEIBER, D. & MERRILL, C.L., 1984b, Modification of response thresholds of newly hatched snails by odor exposure during development. *Chemical Senses*, 9: 181–192.

SOKAL, R.R. & ROHLF, F.J., 1981, *Biometry*. Freeman, San Francisco, U.S.A., 829 p.

WAITE, J.H. & TANZER, M.L., 1981, Polyphenolic substances of *Mytilus edulis:* Novel adhesive containing L.-DOPA and hydroxyproline. *Science*, 212: 1038–1040.

WILLIAMS, L.G., RITTSCHOF, D., BROWN, B. & CARRIKER, M.R., 1983, Chemotaxis of oyster drills *Urosalpinx cinerea* to competing prey odors. *Biological Bulletin*, 164: 536–548.

WOOD, L., 1968, Physiological and ecological aspects of prey selection by the marine gastropod *Urosalpinx cinerea* (Prosobranchia: Muricidae). *Malacologia*, 6: 267–320.

Revised Ms. accepted 18 March 1985

MALACOLOGIA, 1986, 27(2): 291–298

ANOMALIES IN NATICID PREDATORY BEHAVIOR: A CRITIQUE AND EXPERIMENTAL OBSERVATIONS

Jennifer A. Kitchell[1], Christofer H. Boggs[2], James A. Rice[3], James F. Kitchell[4], Antoni Hoffman[5] & Jordi Martinell[6]

ABSTRACT

Reports of multiply bored prey of naticid gastropods, a rare but persistent occurrence in the fossil record dating from the Late Cretaceous, represent potentially serious problems for the study of naticid prey selection in the fossil record. We report controlled laboratory trials wherein multiple complete naticid boreholes were produced on single, live *Terebra dislocata* as a result of active escape behavior by the prey. These results are compared with a fossil assemblage characterized by a high frequency of multiple complete boreholes. Passive mechanisms such as interruption of predatory action and mechanical abrasion of incomplete boreholes can also result in apparent multiply bored prey in fossil assemblages. Generalizations that naticids frequently consume prey without drilling or that naticids frequently bore empty shells are unsubstantiated.

Key words: predator-prey interactions; naticid gastropods; prey selection; phenomenon of multiple boreholes; laboratory trials; fossil assemblages.

INTRODUCTION

Multiply bored prey of drilling predators present a potential enigma. Previous experimental work (Kitchell *et al.*, 1981) demonstrated that a naticid gastropod predator, *Polinices duplicatus* (Say), behaviorally selects prey in accord with an energy maximization principle. These results are significant in that they permit predictions of predator-prey interactions on an evolutionary as well as an ecological time scale (Kitchell *et al.*, 1981; Kitchell, 1982), and provide the necessary framework for studies of predator-prey coevolution (DeAngelis *et al.*, 1984; Kitchell, 1983). Antithetical to these results are (1) reports of occasional multiply bored prey (e.g. Hoffman *et al.*, 1974; Stanton & Nelson, 1980), (2) allegations that naticid predators bore dead or empty shells (e.g. Hoffman *et al.*, 1974; Stanton & Nelson, 1980), and (3) reports that naticids do not bore all of their prey (e.g. Taylor *et al.*, 1980).

Allegations (1) and (2) raise serious doubts about the application of foraging theory to the prey choice behavior of naticids; allegation (3) raises serious doubts about the importance of prey handling time (*i.e.*, drilling time) in this predator-prey interaction. Our purpose in this paper is to assess these allegations. We present laboratory data on the production of multiply bored prey, comparison with fossil assemblages, and reassessment of several older studies whose results have been uncritically perpetuated in the literature.

In general, individual prey of naticid gastropods exhibit a single borehole. Single, completed boreholes signify successful predation and are significantly more common than single, incomplete (i.e. imperforate) boreholes that evidence unsuccessful or interrupted predation. Occasionally, a pair or series of boreholes are observed, consisting entirely of incomplete boreholes (Fig. 1A) or of a single complete borehole and one (Figure 1B) or several (Figure 1C) incomplete boreholes. In an earlier study (Kitchell *et al.*, 1981), we used the term "multiple boreholes" in reference to boreholes of this type. Such a pair or series evidences a chronological sequence of unsuccessful predation attempts, followed ultimately by successful predation. These "multiple boreholes" may be of equal diameter

[1]Museum of Paleontology, University of Michigan, Ann Arbor, Michigan 48109, U.S.A.
[2]National Marine Fisheries Service, Honolulu, Hawaii 96812, U.S.A.
[3]Department of Zoology, North Carolina State University, Raleigh, North Carolina 27695, U.S.A.
[4]Department of Zoology, University of Wisconsin, Madison, Wisconsin 53706, U.S.A.
[5]Polish Academy of Sciences, Warsaw, Poland.
[6]Departamento de Paleontologia, Facultad de Geologia de la Universidad de Barcelona, Barcelona, Spain.

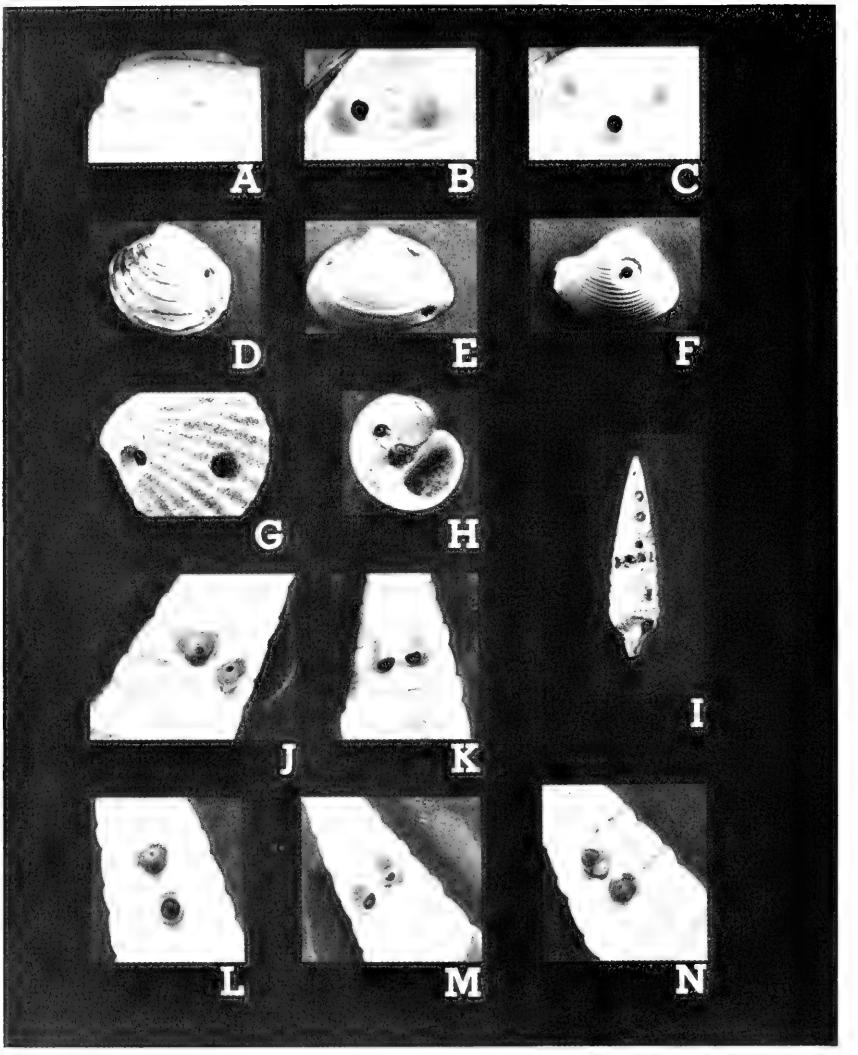


FIG. 1. A. Multiple incomplete boreholes resulting from observed active escape behavior by prey. Prey, *Mercenaria mercenaria*; predator, *Polinices duplicatus*. Outer borehole diameters range from 3.2 mm for the most shallow borehole to 3.7 mm for the two deep boreholes. B. Single incomplete borehole, followed by observed complete borehole penetration (r:R ratio 0.47) and prey consumption. Prey, *Mercenaria mercenaria*; predator, *Polinices duplicatus*. C. Multiple interruption of drilling by observed prey escape behavior, followed by successful drilling of complete borehole (r:R ratio 0.49). Prey, *Mercenaria mercenaria*; predator, *Polinices duplicatus*. D. Two naticid boreholes (one complete, r:R ratio 0.5), one nonfunctional (r:R ratio 0.28) in *Venus multilamella*; Pliocene, l'Emporda deposits, Spain. E. Two boreholes in *Corbula gibba*, Pliocene, l'Emporda deposits, Spain. F. Typical naticid borehole in *Corbula gibba* illustrating pronounced conchiolin layer; Pliocene, l'Emporda deposits, Spain. G. Two complete boreholes in *Venericardia granulata*, Jackson Bluff Formation, Miocene, Leon Co., Florida (r:R ratios 0.51 and 0.62). H. Multiple naticid boreholes in the naticid *Euspira rectilabrum*, Late Cretaceous, Ripley Formation, Coon Creek, McNairy Co., Tennessee (USGS Loc. 16951); specimen maximum diameter 3.45 mm; outer borehole diameters 0.64 mm, 0.62 mm, 0.5 mm; r:R ratios 0.58, 0.83, and 0.55, respectively. I. Multiple nonfunctional and incomplete boreholes in *Strioterebrum monidum*, Miocene (?), lower Gatun Formation; USNM 646047. Height 23.5 mm; see Woodring, 1970, pl. 64, figs. 3, 4. J, K, M, N. Observed nonfunctional boreholes resulting from multiple predation attempts and prey escapes; prey, *Terebra dislocata*; predator, *Polinices duplicatus*. L. Observed nonfunctional borehole and complete borehole; prey, *Terebra dislocata*; predator, *Polinices duplicatus*.

(Fig. 1A-C), indicating repeated attempts by the same or similarly-sized predator, or of unequal diameters evidencing repeated attempts by different predators. The "multiply bored prey" referred to in the present study are categorically distinct from those described above. In exceptional instances, a pair or series of naticid boreholes, each of which completely perforates the shell, is encountered in a single prey. These exceptional cases are a major focus of this study.

Multiply bored prey, in which more than one borehole is complete, are extremely rare, whereas the other category of multiple boreholes is merely uncommon. We introduce the term "nonfunctional borehole" to refer specifically to a borehole that has completely perforated the prey's shell but is nonfunctional in that the opening of the inner borehole is not sufficiently large for insertion of the proboscis. A nonfunctional borehole evidences that boring of the prey is nearly complete, but feeding has not yet begun. Typical complete naticid boreholes, for example, have ratios of inner borehole diameter (r) to outer borehole diameter (R) of >0.5 (see Kitchell *et al.*, 1981). Nonfunctional boreholes typically have r:R ratios <0.5 (see below). The significance of this lack of functionality has not been appreciated.

FOSSIL EVIDENCE OF MULTIPLY BORED PREY

Carriker & Yochelson (1968) reviewed Recent predatory gastropod boreholes and provided one example of multiply bored prey (their pl. 2, fig. 8). However, only one of the boreholes is functionally complete; the second borehole is perforate but appears nonfunctional (r:R = 0.2 in one dimension). Hoffman et al. (1974) reported examining 20,000 Middle Miocene specimens (Korytnica clays, Poland) of potential prey of both naticid and muricid drilling gastropods, and provide one unambiguous example of a multiply bored naticid in which three boreholes are complete (their pl. 2, fig. 3). One of us (Martinell), examining 4,200 Pliocene specimens (l'Emporda, Spain), observed 6 gastropods each with two naticid boreholes, and two bivalves with multiple naticid boreholes. The bivalve examples are given in our Fig. 1D, E. One of the boreholes in *Venus multilamella* (Fig. 1D) is functionally incomplete. The senior author has obtained metric data on 14,000 specimens (assemblages range in age from Late Cretaceous-Recent) of potential naticid prey, and has found 12 examples of multiply bored prey (e.g. Fig. 1G, H). It is of some interest that one of these multiply bored prey is naticid (*Euspira rectilabrum*) from the Late Cretaceous Ripley Formation (USNM #16951). The significance is two-fold: first, it evidences the relative antiquity of multiply bored prey. Until recently, the oldest documented naticid boreholes of any type were of Albian age (Sohl, 1969; Taylor *et al.*, 1980; but see Fursich & Jablonski, 1984, and Newton, 1983, for Triassic reports of naticid boreholes). Secondly, Vermeij & Dudley (1982) recently reported finding no evidence of any cannibalistic interactions in these same Late Cretaceous assemblages. This specimen, however, documents that both cannibalism and multiply bored prey range at least from the Late Cretaceous to the Recent.

ALTERNATIVE EXPLANATIONS OF MULTIPLE COMPLETE BOREHOLES

Multiple complete boreholes are evidence of several possibilities regarding naticid predator-prey interactions:

(1) The prey was simultaneously bored by more than one predator. Although this behavior is common among drilling muricids, none who has observed naticid predators in experimental or field settings has reported multiple, simultaneous predatory attacks, a finding consistent with the naticid behavior of enveloping the prey in the mesopodium. We discount this possibility.

(2) The shell of the bored prey was bored again after the prey had been consumed by a naticid predator. This is the current explanation provided in Stanton & Nelson (1980) who concluded that "the presence of some shells with two or three borings would suggest that naticids bored whatever shell they encountered, whether it was alive or dead, and apparent prey may have been only dead empty shells" (p. 127). Similarly, Hoffman *et al.* (1974) reported that " . . . naticids do not distinguish between the alive and dead specimens and they bore each shell they find, sometimes those previously bored" (p. 252). The latter study concluded that "this indicates that the object of attack was selected by the naticids rather at random and without any preference." Such a premise is not logically sound. Selection should rapidly discriminate against any naticid that expended time and energy, both of which are substantial in this

TABLE 1. Borehole number and type on *Strioterebrum monidum* of the Bowden Formation, Jamaica, and Gatun Formation, Panama. USNM and USGS locality numbers. Height refers to actual specimen height; all specimens are not complete.

Collection locality #	Height specimen (mm)	No. of boreholes	Borehole type: nonfunctional	No. of incomplete	Complete
135283	17.0	8	0	7	1
135283	18.3	4	1	3	0
135283	15.5	2	0	1	1
135283	14.9	1	0	0	1
135283	16.1	3	0	3	0
135283	14.2	4	0	4	0
135283	19.8	11	0	10	1
646046	17.0	15	3	12	0
2580	13.8	5	0	5	0
2580	9.2	7	2	4	1
23741	18.3	4	0	4	0
23741	17.8	9	2	5	2
23741	15.6	6	1	4	1
23741	15.0	4	1	3	0
23741	15.5	9	0	8	1
23741	10.1	2	2	0	0
23741	13.4	6	1	3	2
23741	13.2	3	0	3	0
23741	11.6	1	0	0	1
23741	13.1	1	0	1	0
23741	8.3	2	0	1	1
23741	3.6	3	0	3	0

system (see Kitchell *et al.*, 1981), in boring empty shells. Secondly, such a premise has no empirical support. No researcher working in experimental settings (e.g. Edwards & Heubner, 1977; Kitchell *et al.*, 1981) has reported the selection and boring of empty shells, despite the potential, i.e., naticids maintained in experimental chambers with both live prey and empty shells. Thirdly, if this were the case, one would expect many more multiply bored prey simply because empty shells are relatively common.

(3) Another mechanism that would produce apparently multiply bored prey is abrasion of the remaining shell material in an incomplete borehole. In our laboratory studies, incomplete boreholes are observed to result from active escape behaviors of the prey. In the field, stochastic interruptions of the predatory interaction during drilling would also result in an incomplete borehole. Incomplete boreholes may "weather out" producing a prey which is only apparently multiply bored. This is a logical possibility, particularly for species of *Corbula* (Fig. 1F) whose inner and conchiolin layers are frequently sloughed off. Incomplete boreholes may readily become "complete".

(4) A bored prey may escape consumption passively by accidental interruption of the predation process after boring but prior to feeding. We find this a satisfactory explanation for the rare occurrence of multiple complete boreholes in such sessile or extremely sluggish prey as corbulid bivalves (Ziegelmeier, 1954; Hoffman *et al.*, 1974) or scaphopods (cf. Reyment, 1966; Hoffman *et al.*, 1974).

(5) Lastly, a bored prey may escape predation actively after boring is completed but before feeding is initiated. We will now substantiate this explanation for multiple complete boreholes in mobile prey.

EXPERIMENTAL PRODUCTION OF MULTIPLE BOREHOLES

Fossil Example. To our knowledge, there is one exceptional species of naticid prey in the fossil record characterized by an extremely high frequency of multiple complete boreholes. This is *Strioterebrum monidum*, first described by Woodring (1970) from collections of the Miocene Bowden Formation, Jamaica, and Gatun Formation, Panama (Pliocene? N. Sohl, personal communication, 1983). Woodring remarked that "the extraor-

TABLE 2. Results of predation experiments on *Terebra*. Brackets indicate same predator individual-same prey individual interaction over time. Subscripts a, b, c refer to chronological series of predation events.

Observation	Predator diam. (mm)	Prey height (mm)	r:R ratio	Borehole type
Prey escaped	16.2	31.1	0.21	Nonfunctional
Prey escaped	16.4	32.2	0.07	Nonfunctional
Prey consumed	16.4	32.2	0.60	Complete
Prey escaped	16.9	21.0	0.35	Nonfunctional[a]
Prey escaped	16.9	21.0	0.45	Nonfunctional[b]
Prey consumed	16.9	21.0	0.58	Complete[c]
Prey escaped	16.9	29.7	0.18	Nonfunctional
Prey consumed	16.9	32.7	0.59	Complete
Prey escaped	18.5	22.1	0.12	Nonfunctional[a]
Prey escaped	18.5	22.1	0.43	Nonfunctional[b]
Prey consumed	18.5	22.1	0.48	Complete[c]
Prey escaped	18.5	28.6	0.24	Nonfunctional
Prey escaped	21.7	29.0	0.10	Nonfunctional
Prey escaped	21.7	30.9	0.08	Nonfunctional
Prey escaped	23.0	24.7	0.09	Nonfunctional[a]
Prey escaped	23.0	24.7	NA	Incomplete[b]
Prey consumed	23.0	28.4	0.59	Complete
Prey escaped	23.0	29.9	NA	Incomplete[a]
Prey escaped	23.0	29.9	0.45	Nonfunctional[b]
Prey escaped	23.0	31.2	0.21	Nonfunctional[a]
Prey escaped	23.0	31.2	0.09	Nonfunctional[b]
Prey escaped	23.2	20.7	0.13	Nonfunctional
Prey escaped	23.2	29.1	0.21	Nonfunctional[a]
Prey escaped	23.2	29.1	0.21	Nonfunctional[b]
Prey consumed	23.2	28.9	0.60	Complete
Prey escaped	23.2	34.2	NA	Incomplete
Prey escaped	24.8	31.2	0.14	Nonfunctional
Prey escaped	24.8	33.5	0.15	Nonfunctional
Prey escaped	26.2	25.7	0.22	Nonfunctional
Prey escaped	27.2	16.3	0.44	Nonfunctional
Prey escaped	27.2	29.5	0.09	Nonfunctional
Prey escaped	30.6	29.2	NA	Incomplete[a]
Prey escaped	30.6	29.2	0.26	Nonfunctional[b]
Prey consumed	30.6	29.2	0.49	Complete

dinary number of bore holes, made by an unknown predator, in some shells of this species from Jamaica and Panama, 1,000 kilometers apart, is noteworthy." The senior author has more recently examined these exceptional assemblages (USNM #369347, #646047, USGS #135283). The number of boreholes per specimen, as well as the type of borehole and its diameter, are given in Table 1. The total number of boreholes per individual ranged from 1 to 15. Boreholes were of the complete, incomplete, and nonfunctional types (Fig. 1L). Outer borehole diameters, a measure of predator size (see Kitchell *et al.*, 1981), ranged from 0.5 to 1.7 mm.

Materials and Methods. In search of an explanation for these multiply bored gastropods, we attempted an experiment. Live *Terebra* (*T. dislocata* (Say); see Abbott, 1974; T. Bratcher, personal communication), a species similar in morphology to *S. monidum*, and live co-occurring *Polinices duplicatus*, a naticid, were collected in the Gulf of Mexico near Panacea, Florida. We maintained live prey and predators in plexiglass experimental chambers submerged in a 400 l Instant Ocean aquarium. Chambers contained 3–4 cm of fine sand. Temperature was maintained at 20°C. Photoperiod approximated a normal day-night cycle with seasonal variation (see Kitchell *et al.*, 1981, and Boggs *et al.*, 1984, for additional description of general laboratory conditions and observational methods). The *Terebra* ranged in size (height) from 16.3 to 48.8 mm and were placed in chambers with the predators. The predators ranged in size (maximum dimension) from 16.2 to 27.2 mm.

Predators and prey were monitored regularly, and all predation attacks and prey escapes were recorded. At periodic intervals, prey were briefly removed from their chambers in order to assess the number, location, size, and type of boreholes. Consumed prey were removed from the system; live prey were returned to the system for continued exposure to predation. It is of interest that although only naticid predation occurred, not all subsequent incomplete boreholes were characterized by a central boss, a condition that closely resembles the fossil assemblage.

Results. A number of live perforated *Terebra* were observed in the chambers (Table 2). In several cases, live *Terebra* had multiple perforations. These results conclusively demonstrate that *Terebra* is capable of escaping naticid predators, yet escape is frequently not initiated until the borehole has perforated the shell (Fig. 1J, K, L, M, N). In all these cases, the prey successfully escaped before the predator could enlarge the inner diameter of the borehole for feeding. For example, a 18.5 mm predator was confined with a 22.1 mm prey. The first borehole was perforate but nonfunctional; the ratio of the inner borehole diameter (r) to the outer borehole diameter (R) was 0.12. The prey had escaped before the predator was able to complete the requisite inner borehole dimension and was alive and active. We later observed a second functionally incomplete borehole in the same individual; the r:R ratio was 0.43. Again, the prey was alive, although the predator had achieved a greater degree of opening of the second inner borehole diameter. We then observed a third predation attempt, which was successful. The r:R ratio in this case was 0.48. In other examples, initial boreholes were incomplete, indicating prey escape occurred before perforation.

The size range of bored (i.e. selected) prey, evidenced by a complete or incomplete borehole, ranged from 16.3 to 33.5 mm, indicating that predation attempts were confined to the smaller available prey sizes. Such size selection of prey would be predicted given the size range of predators (Kitchell *et al.*, 1981).

Discussion. Outer borehole diameter is a function of predator size, and does not significantly change dimensions over the duration of drilling. Inner borehole diameter, however, is obviously smallest at the time of initial perforation. Carriker & Van Zandt (1972) observed the formation of the inner borehole by muricid drilling gastropods: "As the diameter of the break at the bottom of the borehole approaches the diameter of the tip of the proboscis, the snail attempts to force the proboscis through the opening." Such attempted forcing of the proboscis may be the stimulus which leads to *Terebra*'s escape, so frequently coincident with perforation. Carriker & Van Zandt continued: "This testing is repeated at the beginning of each rasping period, and sometimes at its termination, until the hole is large enough to admit the proboscis. Hole boring is then discontinued, and the snail begins feeding." As is evident from the number of prey successfully preyed upon by the predator, not all *Terebra* escape attempts are successful. It is noteworthy, however, that in all consumed prey, the final borehole was a typical complete borehole: the ratios of outer to inner borehole diameters were normal ($\geqq 0.5$).

These results indicate that multiple complete boreholes evidence successful prey escape, rather than "predation" on an empty shell. Prey such as *Terebra* can bear the scars of multiple predation attempts, including perforated boreholes, and yet be living; only complete boreholes with normal r:R ratios show mortality by predation.

We conclude that multiple complete boreholes on single prey individuals are (i) in general, extremely rare; (ii) when frequent, usually associated with highly mobile prey. Mobility does not exclusively refer to gastropod prey. In laboratory trials using the bivalves *Mya arenaria* and *Mercenaria mercenaria* as prey, we have also observed active prey escape behavior that disrupted drilling as illustrated in Fig. 1A, B, C; (iii) in addition, we have suggested several passive mechanisms that may result in apparent multiply bored prey.

DO NATICIDS CONSUME PREY WITHOUT DRILLING?

Although we cannot deny that some naticids consume some prey without drilling [e.g. Schneider's (1981) report on *Ensis* predation; also Vermeij, 1980], we question any such generalization. In particular, such generalizations cannot be made reliably by reference to either Medcof & Thurber (1958) or Edwards (1975), as has been the case. In the latter study of beach assemblages, for example, Edwards (1975) correctly reported the proportion of bored (naticid) prey to total or

available prey, a statement that was subsequently misinterpreted (see below) to represent the ratio of prey that naticids drill to those prey that naticids consume but do not drill. Edwards (1975), referring to this total beach assemblage, reported that "the overall value was 3/4ths bored". This proportion refers to the percentage of total shells collected by Edwards that are bored, *i.e.* 3/4 of all shells are bored. This statement was misconstrued apparently to read that of all naticid prey, 3/4ths are bored and eaten and 1/4th are not bored but eaten. Taylor *et al.* (1980: 397), in reviewing naticid predation, reported, citing Edwards (1975), that "in some Recent species such as *Polynices duplicatus* . . . about 25% of the prey are not bored." Unfortunately, subsequent papers have cited this reference as the source for the statement that naticids do not drill all of their prey.

The Medcof & Thurber (1958) situation is somewhat different. Their Table V summarizes their results: 4,428 *Mya arenaria* were planted in 3 experimental plots with the naticid, *Lunatia heros*. Twelve days later, the plots were censused, and only 1,244 live *Mya* were recovered. One hundred and sixty eight empty, articulated *Mya* were also recovered. Of these, 88 were not drilled and 80 were drilled. This ratio of not drilled:drilled represents 52.4%. Remarkable as it may seem, this observation led Medcof & Thurber to conclude that "drills kill more than half their prey without boring their shells." Moreover, another 13 *Mya* had incomplete boreholes. These numbers (88 + 13/168) compute to 61.1%, representing the proportion of recovered, empty shells lacking complete boreholes. Medcof & Thurber concluded that "an acceptable explanation is that about 60% of the time greater clam drills destroy soft-shell clams without perforating their shells" (p. 1366). The authors assigned all recovered but empty shells within the study plots to naticid predation. A more parsimonious explanation in the absence of any direct evidence is that *Mya* experienced a high mortality rate after being transported to the experimental plots. In our laboratory studies, for example, we routinely keep prey in holding chambers separate from the predators, yet prey die and rapidly decompose, leaving an unbored, empty articulated shell. Similarly, prey within predation chambers with *Polinices duplicatus* have been observed to die, gape, and decompose without the predator taking any part in the process. Thirdly, in chambers holding bivalves, naticids, and crabs, we have observed a crab successfully interrupt naticid drilling and consume the vulnerable bivalve.

To summarize, in laboratory studies of normal naticid predation, the evidence preponderantly indicates that naticids drill shells of prey they consume (*e.g.* Edwards & Huebner, 1977; Kitchell *et al.*, 1981, Ansell 1982; Boggs *et al.*, 1984). Moreover, naticids drill live prey even when recently dead or moribund prey are available. We have mechanically opened holes in *M. mercenaria*, for example, without harming the prey, and yet predators have not utilized these ready-made holes. We also have observed predators to initiate a new borehole even when an incomplete borehole, produced by our deliberate interruptions of drilling, was available (Kitchell *et al.*, 1981).

CONCLUSIONS

Multiple complete boreholes in mobile prey can be due to prey escape, as evidenced experimentally for *Terebra*, and can also result incidentally from the weathering, abrasion, or sloughing off of shell material associated with incomplete boreholes. Nonfunctional boreholes evidence prey selection behavior but do not evidence mortality by naticid predation. The incidence of mortality by predation must be based on complete boreholes. Multiple complete boreholes do not pose a problem for models of prey selection. We conclude that naticid predators can readily distinguish live prey from empty shells, and that drilling is a highly stereotypic behavioral response of naticid gastropods to selected prey.

REFERENCES

ABBOTT, R.T., 1974, *American seashells*. Ed. 2. Van Nostrand Reinhold, New York, 663 p., 24 pl.

ANSELL, A.D., 1982, Experimental studies of a benthic predator-prey relationship. III. *Malacologia*, 22: 367–375.

BOGGS, C.H., RICE, J.A., KITCHELL, J.A. & KITCHELL, J.F., 1984, Predation at a snail's pace: what's time to a gastropod? *Oecologia*, 62: 13–17.

CARRIKER, M.R. & VAN ZANDT, D., 1972, Predatory behavior of a shell-boring muricid gastropod. In WINN, H.E. & OLLA, B.L., ed., *Behavior of marine animals*, 1: 157–244. Plenum Press, New York.

CARRIKER, M.R. & YOCHELSON, E.L., 1968, Recent gastropod boreholes and Ordovician cylindrical borings. *United States Geological Survey Professional Paper*, 593-B: 1–23.

DeANGELIS, D.L., KITCHELL, J.A., POST, W.M. & TRAVIS, C.C., 1984, A model of naticid gastropod predator-prey coevolution. *Lecture Notes in Biomathematics*, 54: 120–136.

EDWARDS, D.C., 1975, Preferred prey of *Polinices duplicatus* in Cape Cod inlets. *Bulletin of the American Malacological Union*, 40: 17–20.

EDWARDS, D.C. & HUEBNER, J.D., 1977. Feeding and growth rates of *Polinices duplicatus* preying on *Mya arenaria* at Barnstable Harbor, Massachusetts. *Ecology*, 58: 1218–1236.

FURSICH, F.T. & JABLONSKI, D., 1984. Late Triassic naticid drillholes: carnivorous gastropods gain a major adaptation but fail to radiate. *Science,* 224: 78–80.

HOFFMAN, A., PISERA, A. & RYSZKIEWICZ, M., 1974, Predation by muricid and naticid gastropods on the Lower Tortonian mollusks from the Korytnica clays. *Acta Geologica Polonica*, 24: 249–260.

KITCHELL, J.A., 1982, Coevolution in a predator-prey system. *Proceedings of Third North American Paleontological Convention*, 2: 301–305.

KITCHELL, J.A., 1983, An evolutionary model of predator-mediated divergence and coexistence. *Geological Society of America Abstracts with Programs*, 15: 614.

KITCHELL, J.A., BOGGS, C.H., KITCHELL, J.F. & RICE, J.A., 1981, Prey selection by naticid gastropods: experimental tests and application to the fossil record. *Paleobiology*, 7: 533–552.

MEDCOF, J.C. & THURBER, L.W., 1958, Trial control of the greater clam drill (*Lunatia heros*) by manual collection. *Journal of the Fisheries Research Board of Canada*, 15: 1355–1369.

NEWTON, C.R., 1983, Triassic origin of shell-boring gastropods. *Geological Society of America Abstracts with Programs*, 15: 652–653.

REYMENT, R.A., 1966, Preliminary observations on gastropod predation in the western Niger delta. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 6: 45–59.

SCHNEIDER, D., 1981, Escape response of an infaunal clam *Ensis directus* Conrad 1843, to a predatory snail, *Polinices duplicatus* Say 1822. *Veliger*, 24: 371–372.

SOHL, N.F., 1969, The fossil record of shell boring by snails. *American Zoologist*, 9: 725–734.

STANTON, R.J., Jr. & NELSON, P.C., 1980. Reconstruction of the trophic web in paleontology: community structure in the Stone City Formation (Middle Eocene, Texas). *Journal of Paleontology*, 54: 118–135.

TAYLOR, J.D., MORRIS, N.J. & TAYLOR, C.N., 1980, Food specialization and the evolution of predatory prosobranch gastropods. *Palaeontology*, 23: 375–409.

VERMEIJ, G.J., 1980, Drilling predation on bivalves in Guam: some paleoecological implications. *Malacologia,* 19: 329–334.

VERMEIJ, G.J. & DUDLEY, E.C., 1982, Shell repair and drilling in some gastropods from the Ripley Formation (Upper Cretaceous) of the southeastern U.S.A. *Cretaceous Research*, 3: 397–403.

WOODRING, W.P., 1970, Geology and paleontology of Canal Zone and adjoining parts of Panama; description of Tertiary mollusks (Gastropods: Eulimidae, Marginellidae to Helminthoglyptidae). [*United States*] *Geological Survey Professional Paper* 306-D: iii + 452 p., pl. 48–66.

ZIEGELMEIER, E., 1954, Beobachtungen über der Nahrungswerwerb bei der Naticidae *Lunatia nitida* Donovan (Gastropoda: Prosobranchia) *Helgoländer wissenschaftliche Meeresuntersuchungen* 5: 1–33.

Revised Ms. accepted 18 January 1985.

MALACOLOGIA, 1986, 27(2): 299–305

ALGAL GARDENS AND HERBIVORY IN A SCAVENGING SANDY-BEACH NASSARIID WHELK

S.A. Harris[1], F.M. da Silva[1], J.J. Bolton[2] & A.C. Brown[1]*

Departments of Zoology[1] and Botany[2], University of Cape Town, South Africa 7700

ABSTRACT

The shells of living *Bullia digitalis* (Dillwyn), a nassariid whelk common on the sandy beaches of the west and south coasts of South Africa, are colonised by a variety of algae. Some of these are typical of rocky shores but the most consistent invader is a green, filamentous, boring alga (Chlorophyta), the morphology of which corresponds to *Eugomontia sacculata* Kornm. Behavioural observations indicate that some or all of these algae supplement the predominantly carnivorous diet of the whelk. The gut contents and digestive glands of the animals are frequently green and it is demonstrated that this is due to the presence of chlorophyll *a*. Cellulolytic symbiotic bacteria, as well as α-amylase, cellulase and laminarinase activity, are shown to be present in the gut. It is concluded that *B. digitalis* ingests and utilises green algal material growing on its shell and thus plays a more complex role than previously thought in the sandy-beach food web.

INTRODUCTION

Bullia digitalis (Dillwyn) is a nassariid whelk that is characteristic of high-energy sandy beaches along the west and south coasts of southern Africa. Our knowledge of this and other species of the genus has been reviewed by Brown (1982). The whelk is essentially a scavenger of washed-up animal matter, although it will turn predator on occasion. The supply of carrion to the beaches in question is highly erratic, however, and tends to be seasonal, while predation appears to be relatively uncommon. Thus, although the animal can consume food up to one third of its own tissue weight in a single meal (Brown, 1961), it seemed unlikely that carrion and prey could supply all its requirements. Colclough & Brown (1984) therefore investigated the possibility of the animal making use of dissolved organic matter in the surrounding sea water to supplement its diet. The results proved positive but it is clear that this source of nutrition could not by itself supply the needs of the whelk for an extended period. The possibility that the animals eat stranded plant material has never been completely rejected, as some members of the Nassariidae are known to graze plants (Kilburn & Rippey, 1982) and *Nassarius obsoletus* has been shown to be an obligate omnivore (Curtis & Hurd, 1979). Nevertheless, this possibility has not been confirmed in the field, nor has algal material offered to captive whelks in the laboratory ever been eaten.

Omnivory is not well documented among the Neogastropoda, with the exception of the Nassariidae. It has not been reported for the *Bullia* group. Of relevance to nutrition is the presence or absence of a crystalline style and gastric shield, structures which may also have systematic and evolutionary significance. Yonge (1930) considered that "the crystalline style of Mollusca and a carnivorous habit cannot normally co-exist" and indeed among Neogastropoda crystalline styles appear to be virtually confined to the omnivorous Nassariidae (Kato & Kubomura, 1955; Curtis & Hurd, 1979). Dissection of the gut of *Bullia digitalis* and of *B. rhodostoma* and serial sections of the former species (H. du Preez, S.A. Harris & A.C. Brown, unpubl.) have failed to reveal the presence of a style, although it is possible that a style is only transitorily present or shows daily cycling, as has been demonstrated in *Nassarius obsoletus* (Curtis, 1980).

Recently, da Silva & Brown (1984) have reported the consistent presence of algae associated with the shells of living *B. digitalis* and have described behaviour suggesting

*From whom reprints may be obtained.

FIG. 1. Light micrograph of a shell fragment of *Bullia digitalis* (× 200), showing branching filaments of the green alga *Eugomontia* embedded within it.

that the animal periodically crops this "garden" with its long, mobile proboscis and ingests the algal material. Contributory circumstantial evidence is that the gut contents and digestive glands are frequently green.

The aims of the present work were to establish the nature of the alga or algae associated with the shell, to discover whether the green colour of the digestive system is due to ingested chlorophyll and to assess the digestive capabilities of the animal with regard to utilising algal material.

THE ALGAL GARDEN

Examination of intact shells and shell fragments of *Bullia digitalis* by light microscopy revealed the consistent presence of an extensive growth of a green filamentous, boring alga embedded within the outer layer of the shell (Fig. 1). Contact between the alga and the exterior was maintained through numerous small holes, some 7 μm in diameter (Fig. 2). It was found possible to isolate the alga from the shell by treatment with dilute HCl. It consists of uniseriate filaments displaying an irregular but predominantly opposite pattern of branching. The diameter of the algal filaments is 5 to 7 μm. The vegetative features thus correspond to those of *Eugomontia sacculata* Kornmann, a boring species found in both living and dead shells in temperate regions in both hemispheres (Kornmann, 1960; Wilkinson & Burrows, 1972a, b; South & Adams, 1976).

Scanning electron microscopy of shell fragments fixed in 2% gluteraldehyde, critical point dried after dehydration and coated with gold-palladium, showed that the algal filaments bore only into the outer prismatic layer and ramify throughout the crystalline matrix of this layer (Fig. 3).

Culture of the alga was attempted by keeping shell fragments in an enriched seawater medium (ES of Provasoli, 1968) at 15°C and a photoperiod of 16 hr at a light intensity of 50 to 60 $\mu Em^{-2}.s^{-1}$. This proved successful in that after some weeks isolated patches of the alga were observed growing on the bottom of the Petri dish, presumably resulting from released spores. Sporangia were not observed

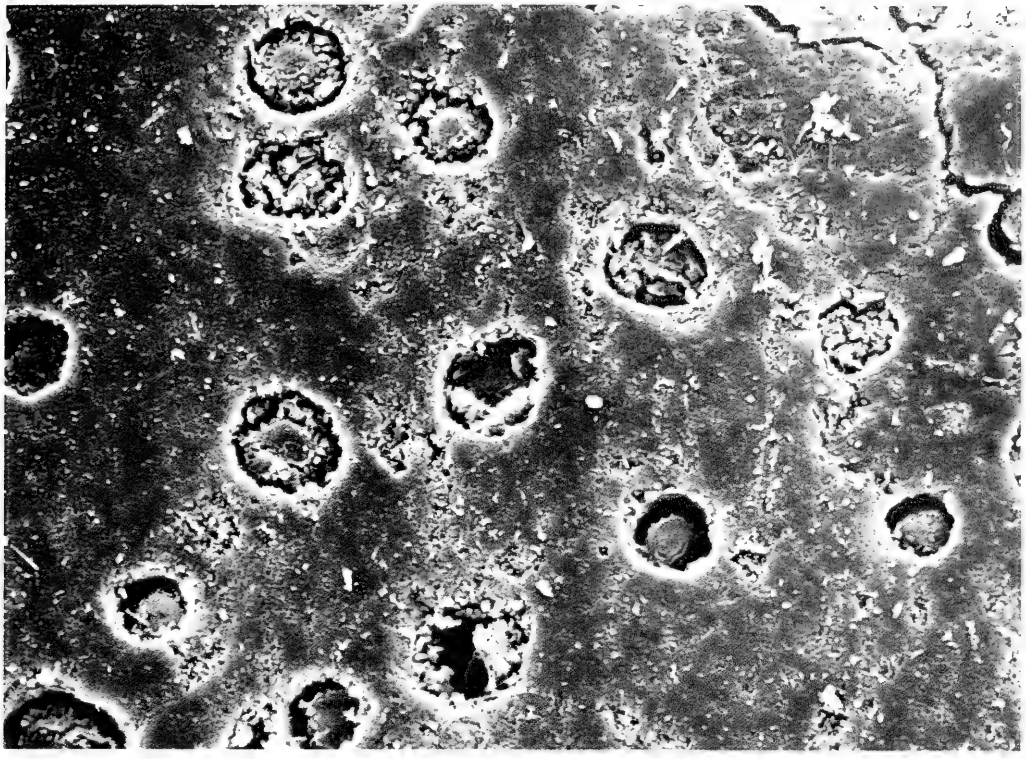


FIG. 2. Scanning electron micrograph of the shell surface of *B. digitalis*, with holes caused by the boring alga (×1670). The tips of the filaments are situated in the holes, just below the surface.

on the shell surface due to dense growth of other algae which flourished in the culture medium. These algae, which were initially present on the shell in low numbers, were typically rocky-shore forms comprising species of *Ectocarpus*, *Enteromorpha* and *Ulva*. Their possible use as food cannot be ignored.

DIGESTIVE GLAND CHLOROPHYLL ANALYSIS

The gut contents and digestive glands of *B. digitalis* vary considerably in colour. They are frequently brown or grey and are bright blue after the animals have been feeding on the siphonophore *Physalia*. The green colour already referred to is also common, particularly in individuals from the west coast during winter.

Green digestive glands from four whelks from the west coast were each homogenised in 12 ml 90% acetone and the extracts ultrasonified for 30 min in the dark on ice. The samples were centrifuged at 9,000 rpm for 15 mins at 15°C and the supernatant analysed on a spectrophotometer linked to a Spectroprinter. The four samples gave very similar results, showing absorbance peaks in the 660–663 nm and 400–410 nm regions. These maxima correspond to those of chlorophyll *a* (Bogorad, 1962; Round, 1973) and we conclude that the green colour observed is indeed due to chlorophyll resulting from the ingestion of green algal material.

CARBOHYDRASES

Material and methods: To test for cellulolytic bacteria in the gut of *B. digitalis*, a modification of the method of Teather & Wood (1982) was employed. The whole gut, without digestive gland, was homogenised in 600 μl sea water and the crude supernatant subjected to a dilution series. 10 to 20 μl of the diluted bacterial suspension were then plated onto a growing agar medium (GAM) (2%

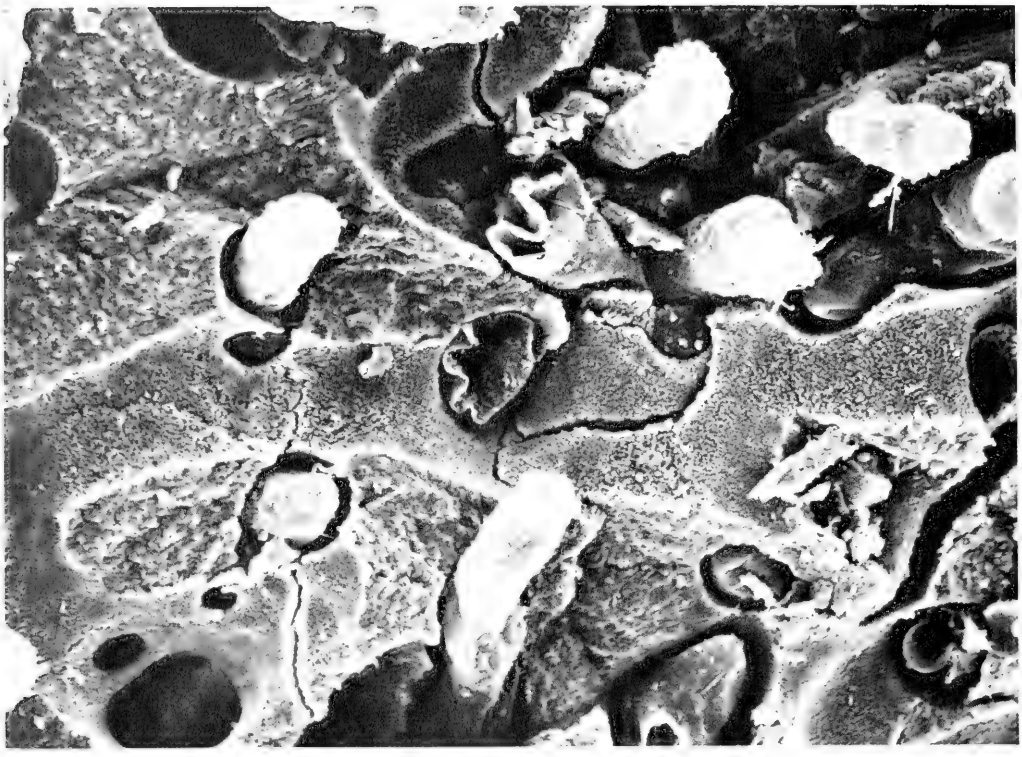


FIG. 3. Algal filaments ramifying through the crystalline matrix of the outer prismatic layer of the shell of *B. digitalis* (SEM ×1670).

TABLE 1. The carbohydrase activities obtained from the Nelson-Somogyi enzyme assays (n = 10).

Substrate	Enzyme	Enzyme activity (mg glucose·mg protein^{-1}·hour^{-1}) $\bar{X} \pm$ S.D.	% Total activity
Glycogen	α-amylase	0.4256 ± 0.0922	39.31
Starch	α-amylase	0.4113 ± 0.1016	37.99
CMC	cellulase	0.1511 ± 0.0634	13.96
Laminarin	laminarinase	0.0946 ± 0.0822	8.74

agar, 0.5% peptone and 0.1% yeast extract). Colonies of pure bacterial isolates were stabbed onto a final plate medium consisting of 2.5% w/v agar containing 0.1% w/v peptone and 0.01% carboxymethyl cellulose. The plates were incubated at 23°C for four days and then flooded with an aqueous solution of Congo Red for 15 mins. This stain reacts with β-D-glucans in the CmC substrate, providing a rapid and sensitive assay for bacterial strains possessing β-D-glucanohydrolases (Teather & Wood, 1982). The Congo Red was then poured off and the visualised zones of β-D-glucans hydrolysis stabilised by flooding with 1N HCL for 15 mins; this changes the dye colour to blue and inhibits further enzyme activity. Results were recorded photographically. Scanning electron micrographs were prepared both of bacteria from the colonies growing in culture and of the bacteria *in situ* in the animal's gut.

In a further series of experiments, homo-

genised whole guts were centrifuged in phosphate buffer (pH 7) and the supernatant subjected to cellulase, α-amylase and laminarinase determinations, using the Nelson-Somogyi colorimetric method (Nelson, 1944; Somogyi, 1952). Attempts to first eliminate enzyme-producing bacteria with β-mercaptoethanol or Ampicillin had to be abandoned, as both interfered with the assay. In order to obtain comparative results, each sample was analysed for total protein content, using the Folin Ciocalteus (2N) reagent and following the method of Lowry *et al.* (1951). Crystalline bovine albumin was used as a standard. Results of the enzyme analysis were expressed in mg glucose evolved. mg protein^{-1}. hour^{-1}.

Results: Isolated bacterial colonies appeared on the GAM plates within one to three days. About 10% of the bacteria were cellulolytic, as was clear from the visualised zones of β-D-glucans. The bacteria were of the agarose-eating type, identifiable by depressions in the agar. SEM examination showed the pure bacterial isolates to be rod-like, while those photographed in the gut were predominantly coccoid. These could, however, be the same bacteria under different conditions.

Results of the carbohydrase assays are given in Table 1. All three activities tested were positive, at levels indicated by the amount of reducing sugar evolved from each substrate. Thus α-amylase activity (0.837 mg glucose.mg protein^{-1}.hr^{-1}) accounted for some 77% of the total activity measured, while cellulase and laminarinase accounted for only 14% and 9% respectively.

DISCUSSION

Among marine invertebrates, the hydrolytic capacity of the carbohydrases is generally greater with respect to reserve carbohydrates (starch, glycogen, laminarin) than to structural carbohydrates (cellulose and chitin) (Elyakova *et al.*, 1981). The present study supports this with regard to α-amylase and cellulase but not with regard to laminarinase. It is difficult to compare our work quantitatively with other published data, because of the variety of techniques employed, but qualitative comparisons can be made.

Stone & Morton (1958) studied the distribution of carbohydrases in molluscs having a wide range of feeding habits. They found α-amylase activity to be present among both herbivores and carnivores, in accordance with the ability to digest starch and glycogen respectively. There was minimal cellulase activity but high levels of laminarinase in the carnivorous whelks, a finding confirmed for *Nassarius reticulatus* by Kristensen (1972). Slight hydrolysis of alginic acid and alginate was also detected in this species, although the animal apparently never feeds on brown algae.

It was suggested by Stone & Morton (1958) that, far from being strictly functional, cellulases may form part of the basic digestive enzyme system in the Mollusca, and this concept has been supported by subsequent work. Yokoe & Yasumasu (1964) proposed that the distribution of cellulases in invertebrates generally is more closely correlated with phylogeny than with feeding habits, while Gianfreda *et al.* (1979) were unable to explain the significance of the β-1, 4-glucanase (cellulase), found in most carnivorous molluscs examined, on functional grounds. They inclined to the view that it simply represents an evolutionary remnant from an originally herbivorous stock. This view is supported by Agnisola *et al.* (1981). The presence of these carbohydrases in *Bullia digitalis* is thus no proof, by itself, that the animal ingests plant material; on the other hand, it is a good indication that the whelk can utilise such material if it is ingested.

Production of cellulase by symbiotic bacteria in the gut is well known among the Mollusca. In most cases it is not certain, however, whether the host animal also produces such enzymes, although this is clearly the case in marine borers. Morton (1978) has suggested that in these animals the bacteria provide the enzymes for the initial breakdown and that this is followed by digestion within the cells of the animal's digestive diverticula, using enzymes produced by the animal. In the present work, the presence of cellulose-digesting bacteria in *Bullia digitalis* has been demonstrated but whether the animal itself also produces such enzymes is not clear, nor is it known to what extent digestion may be intra-cellular.

However this may be, the presence of chlorophyll in the digestive system, together with enzyme activity appropriate to the digestion of algal material, leads to the firm conclusion that the whelk can and does supplement its predominantly carnivorous diet with algae. It thus plays a more complex role

than previously thought in the sandy-beach food web.

Less certain is the source of this algal material. Macrophytic algae do not occur on high-energy sandy beaches (Brown, 1964) and there is no hint, after nearly three decades of observation, that *Bullia* eats stranded plant material. The only alternative would appear to be an algal "garden" growing on the shell itself. Cropping of such a garden has been witnessed (da Silva & Brown, 1984) and subsequently confirmed but whether it is the embedded *Eugomontia* or other green algae growing on the shell which are being ingested is not certain. The boring alga grew only very slowly in culture and according to Kornmann (1960) only the reproductive structures of *Eugomontia sacculata* protrude from the shell. It is thus questionable whether the small amount of material made available could be of any importance in the diet of the whelk or account for the large amounts of chlorophyll found in the digestive system. The same applies to the non-boring algae discovered on the shell, for these were very sparse until they were cultured.

It may, however, be that field conditions, such as the buffetting of the shell by waves and sand, stimulate algal growth or even that the whelk itself encourages the growth of the algae in some way. Since the whelk commonly buries itself just below the sand surface (Brown, 1982) burial is unlikely to adversely effect algal growth, as light is known to penetrate several centimeters through sand, many algae flourishing while buried in sand for long periods (Bally *et al.*, 1984).

ACKNOWLEDGEMENTS

Mr. Klaus Schultes and Miss Jean Harris assisted with the preparation of scanning electron micrographs, while Mr. Neville Eden prepared the photographs for publication. Miss Cathy Roberts gave advice on the bacterial section of the work. We are indebted also to Dr. Robert Robertson for notes on the extent of herbivory and the occurrence of crystalline styles in neogastropods. The project was supported by a postgraduate CSIR grant made available to the first author.

REFERENCES CITED

AGNISOLA, C., SALVADORE, S. & SCARDI, V., 1981, On the occurrence of cellulolytic activity in the digestive gland of some marine carnivorous molluscs. *Comparative Biochemistry and Physiology*, 70B: 521–526.

BALLY, R., McQUAID, C.D. & BROWN, A.C., 1984, Shores of mixed sand and rock: an unexplored marine ecosystem. *South African Journal of Science*, 80: 500–503.

BOGORAD, L., 1962, Chlorophylls. In LEWIN, R.A. (ed.), *Physiology and biochemistry of Algae*, Academic Press, p. 395–408.

BROWN, A.C., 1961, Physiological-ecological studies on two sandy-beach Gastropoda from South Africa: *Bullia digitalis* Meuschen and *Bullia laevissima* (Gmelin). *Zeitschrift für Morphologie und Ökologie der Tiere*, 49: 629–657.

BROWN, A.C., 1964, Food relationships on the intertidal sandy beaches of the Cape Peninsula. *South African Journal of Science*, 60: 35–41.

BROWN, A.C., 1982, The biology of sandy-beach whelks of the genus *Bullia* (Nassariidae). *Oceanography and Marine Biology Annual Review*, 20: 309–361.

COLCLOUGH, J.H. & BROWN, A.C., 1984, Uptake of dissolved organic matter by a marine whelk. *Transactions of the Royal Society of South Africa*, 45: 169–176.

CURTIS, L.A., 1980, Daily cycling of the crystalline style in the omnivorous, deposit-feeding estuarine snail *Ilyanassa obsoleta*. *Marine Biology*, 59: 137–140.

CURTIS, L.A. & HURD, L.E., 1979, On the broad nutritional requirements of the mud snail, *Ilyanassa* (*Nassarius*) *obsoleta* (Say), and its polytrophic role in the food web. *Journal of Experimental Marine Biology and Ecology,* 41: 289–297.

DA SILVA, F.M. & BROWN, A.C., 1984, The gardens of the sandy-beach whelk *Bullia digitalis* (Dillwyn). *Journal of Molluscan Studies,* 50: 64–65.

ELYAKOVA, L.A., SHEVCHENKO, N.M. & AVAEVA, S.M., 1981, A comparative study of carbohydrase activities in marine invertebrates. *Comparative Biochemistry and Physiology*, 69B: 905–908.

GIANFREDA, L., IMPERATO, A., PALESCANDOLO, R. & SCARDI, V., 1979, Distribution of β-1,4-glucanase and β-glucosidase activities among marine molluscs with different feeding habits. *Comparative Biochemistry and Physiology*, 63B: 345–348.

KATO, K. & KUBOMURA, K., 1955, Uric acid and guanine excretion from the crystalline style sac in some gastropods. *Science Reports of the Saitama University*, ser. B, 2: 21–34.

KILBURN, R. & RIPPEY, E., 1982, *Sea shells of southern Africa*. Macmillan, South Africa, 247 p.

KORNMANN, V.P., 1960, Die heterogene Gattung *Gomontia*. II: Der fädige Anteil, *Eugomontia sacculata* (nov. gen., nov. spec.). *Helgoländer wissenschaftliche Meeresuntersuchungen*, 6: 229–238.

KRISTENSEN, J.H., 1972, Carbohydrases of some

marine invertebrates with notes on their food and on the natural occurrence of the carbohydrates studied. *Marine Biology*, 14: 130–142.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J., 1951, Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, 193: 265–275.

MORTON, B., 1978, Feeding and digestion in shipworms. *Oceanography and Marine Biology Annual Review*, 16: 107–144.

NELSON, N., 1944, A photometric adaptation of the Somogyi method for the determination of glucose. *Journal of Biological Chemistry*, 169: 375–380.

PROVASOLI, L., 1968, Media and prospects for culturation of marine algae. WATONABE, A. & HATTORI, A. (ed.), In *Cultures and collections of Algae*. Japanese Society of Plant Physiology, p. 47–49.

ROUND, F.E., 1973, *The biology of the Algae*. Arnold, London, 278 p.

SOMOGYI, M., 1952, Notes on sugar determination. *Journal of Biological Chemistry*, 195: 19–23.

SOUTH, G.R. & ADAMS, N., 1976, The marine algae of the Kaikaura coast. *National Museum of New Zealand Miscellaneous Series*, 1: 67 p.

STONE, B.A. & MORTON, J.E., 1958, The distribution of cellulases and related enzymes in Mollusca. *Proceedings of the Malacological Society of London*, 33: 127–141.

TEATHER, R.M. & WOOD, P.J., 1982, Use of Congo Red-polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from the bovine rumen. *Applied Environmental Microbiology*, 43: 777–780.

WILKINSON, M. & BURROWS, E.M., 1972a, The distribution of marine shell-boring green algae. *Journal of the Marine Biological Association of the United Kingdom*, 52: 59–65.

WILKINSON, M. & BURROWS, E.M., 1972b, An experimental taxonomic study of the Algae confused under the name *Gomontia polyrhiza*. *Journal of the Marine Biological Association of the United Kingdom*, 52: 49–57.

YOKOE, Y. & YASUMASU, I., 1964, The distribution of cellulase in invertebrates. *Comparative Biochemistry and Physiology*, 13: 323-338.

YONGE, C.M., 1930, The crystalline style of the Mollusca and a carnivorous habit cannot normally co-exist. *Nature*, 125: 444–445.

Revised Ms. accepted 18 March 1985

MALACOLOGIA, 1986, 27(2): 307–311

THE AUTOTOMY ESCAPE RESPONSE OF THE TERRESTRIAL SLUG *PROPHYSAON FOLIOLATUM* (PULMONATA: ARIONIDAE)

I. Deyrup-Olsen, A. W. Martin & R.T. Paine

Department of Zoology, University of Washington, Seattle, Washington 98195, U.S.A.

ABSTRACT

The autotomy escape response of the terrestrial slug *Prophysaon foliolatum* (Gould, 1851) is mediated by a peripheral reflex mechanism, with sensory components restricted to the region directly involved in autotomy (autotomy zone and autotomy section of the foot). The response has two major components: (1) Muscle contraction severs the posterior autotomy section of the foot from the rest of the body. (2) Mucus is secreted differentially, with sticky, yellow mucus over the anterior body, and relatively non-viscous, colorless mucus over the autotomy section.

The autotomy section is packed with glycogen cells. If food is lacking, the stored nutrients are utilized by the slug. The autotomy section offers a significant reward to a natural predator, such as the carabid beetle *Scaphinotus angusticollis*.

Key words: *Prophysaon foliolatum*; Pulmonata; autotomy; escape response; mucus; glycogen cells.

INTRODUCTION

Since the early observations of Raymond (1890), Pilsbry & Vanatta (1898), and others it has been known that terrestrial slugs of the genus *Prophysaon* can autotomize the "tail" (posterior section of the foot). Observations of *Prophysaon andersoni* were reported by Hand & Ingram (1950), who noted that there is considerable variation between individuals in responsiveness to stimulation of the tail by cutting or puncture, and that the tail section can be regenerated. They demonstrated that attack by predators normally occurring with *Prophysaon* (the carabid beetle *Scaphinotus* sp.; the snail *Haplotrema minimum*) could initiate autotomy. They suggested that autotomy in *Prophysaon* is a mechanism of defense. Indeed, a wide variety of molluscs are known to effect defensive autotomy (literature reviewed by Stasek, 1967), and the process is known to occur in some arthropods, vertebrates, and other animals as well. Autotomy of the tail in lizards in response to attack by predators has been studied fairly extensively in relation to energetics and adaptive strategies (Vitt *et al.*, 1977; Ballinger, 1981). In contrast, little is known of the physiology of the process in molluscs. Accordingly, we have studied structural and functional aspects of autotomy in *Prophysaon foliolatum* (Gould, 1851; Arionidae), a slug in which the response is readily and reproducibly elicited.

METHODS AND MATERIALS

Slugs were collected on Tatoosh Island, off the northwest coast of Washington State, in summer and autumn. The animals were maintained in the laboratory at 10°C, in plastic boxes on moist paper towels with food (lettuce, potatoes, carrots, or yams) present at all times. Eggs laid in September or October were allowed to hatch and the young were reared to maturity. Both animals collected in the field and those reared in the laboratory were used in the investigation; no systematic differences were noted between these two groups.

Experiments were carried out with two major objectives: (1) Investigation of the nature and localization of stimuli which elicited the autotomy response, and the general site of integration of the response (whether peripheral or central); (2) Description of the structure and composition, with respect to stored nutrients, of the autotomized part of the foot. Observations of autotomy were made both on intact slugs and on an *in vitro* preparation of the posterior body wall (Deyrup-Olsen & Martin, 1982). The latter was prepared as follows. The body was rapidly severed about halfway between the anterior and posterior margins of the mantle and the cerebral ganglia were destroyed. The viscera were then withdrawn from the posterior portion of the body, leaving a sac of epidermis and underlying muscle

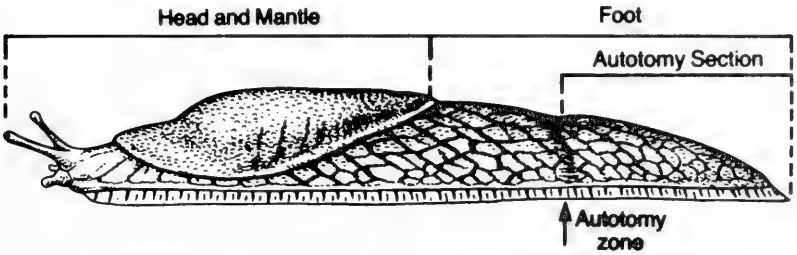
terminated by the autotomy section (AS; this part of the body was termed a "tail" by earlier workers). The sac, or posterior chamber, was then attached to a glass manometer tube, while a fine plastic tube, threaded through the manometer tube to the lower end of the chamber, served as a portal of entry of injected materials. The chamber was filled with Ringer solution (slightly modified from Roach, 1963, in accord with our measurements of the composition of *Prophysaon* blood; the osmotic pressure was increased by 10%, and the Ca concentration raised from 3 mM to 5mM). The preparation could then be stimulated mechanically or electrically, or subjected to the action of a variety of agents, especially neurotropic agents known to function in gastropod physiology.

Stimuli tested were as follows: (1) Electrical pulses delivered from a Grass Instruments Stimulator Model S4, at varied strength and frequency up to 10 V and 20 Hz. (2) Injections into the intact slug or the posterior chamber of Ringer solution containing 1 to 10 μmol of one of the gastropod neurotransmitters: acetylcholine, 5-hydroxytryptamine, dopamine, *gamma*-aminobutyric acid, noradrenaline, FMRFamide (L-phenylalanyl-L-methionyl-L-arginyl-L-phenylalaninamide), octopamine, or histamine; and neural blocking agents: atropine sulfate and hexamethonium bromide (block acetylcholine receptors), propanolol and phentolamine (block adrenergic receptors), and cyproheptidine (blocks 5-hydroxytryptamine receptors in some systems). All test substances were obtained from Sigma Chemical Company, St. Louis. (3) Mechanical stimuli: In applying this type of stimulation, we attempted to simulate the attack on *Prophysaon foliolatum* by the carabid beetle, *Scaphinotus angusticollis*. The beetles were collected in the same area as the *Prophysaon* individuals, and were placed in closed containers with individual slugs for observation of the interaction between them. The acts of predation by the beetle were highly variable, although a beetle generally attacked a slug within seconds to an hour of access to it. The attacks consisted of several to scores of mandibular bites delivered at first at apparently random sites on the slug's body. The bites were shallow and were never observed to draw blood. Although in half of the 10 trials of slug-beetle pairs the beetle failed to cause autotomy within a 12 to 24 hour observation period, in 5 cases the slug autotomized. In each test in which the beetle succeeded in causing the response, it was biting and often tugging on the autotomy section or at the autotomy zone. The beetle seized the AS and held it against its mouth for several hours; at the end of this time the beetle had ingested the core tissue and only a limp sac of muscle and epidermis remained. Meantime, the slug withdrew from the field of action, or was removed by the experimenter. Basing our technique on these observations, we stimulated the body wall with repeated shallow pinches with a pair of iris forceps, a type of stimulation referred to as "beetle" in this paper. Because Hand & Ingram (1950) used puncture and cutting of the AS in *Prophysaon andersoni*, we also tested the effect of application of stimulation with a surgical towel clamp with sharp points, penetrating the body wall and causing loss of a small amount of blood. This type of stimulation will be referred to as "penetrating." The significance of differences between the effects of different stimuli was assessed with the *t* test.

Direct and microscopic observations were made of the mucus output of the body wall resulting from effective stimulation. The observations were supplemented with study of fresh sections of body tissue stained for glycogen (Lugol's reagent) and of histological preparations (Bouin's fixation, paraffin sections, hematoxylin-eosin staining). The mass and water content of the AS, relative to the body as a whole, were measured (Mettler analytical balance; drying at 105°C to constant weight). Chemical analyses of the AS, following freeze-thawing, included tests for glucose (glucose kit, Boeringer Mannheim; based on glucose oxidase oxidation of glucose, with peroxidase conversion of the chromogen 2,2'-azino-di(3-ethylbenzthiazoline)-6-sulfonate to the form absorbing at 575 nm); glycogen (Murat & Serfaty, 1974); galactogen (hydrolysis followed by tests for galactose with a kit supplied by Boeringer Mannheim (based on β-galactose dehydrogenase oxidation, and measurement of NADH absorbance at 356 nm); and soluble protein (Lowry *et al.*, 1951).

RESULTS

In a typical autotomy response, induced by *Scaphinotus* or by our technique of beetle stimulation, the sequence of events was fairly stereotyped. If the attack was anterior to the



Stimuli causing autotomy of whole animal (W.) or posterior chamber (P.C.)		
	"Beetle"	Penetrating
Anterior to autotomy zone	No autotomy	No autotomy
Autotomy zone	11±4 (8) W.	——
Autotomy section	43±25 (n=14) w. 43±25 (n=7) p.c.	9±5 (n=6) w. 2±1 (n=6) p.c.

FIG. 1. Number of stimuli, of varying type and site of application, resulting in autotomy in *Prophysaon foliolatum*. Autotomy occurred only when stimuli were applied to the autotomy zone or autotomy section; only the whole animal, with beetle type stimulation, was tested at the autotomy zone. Stimuli were more effective at the autotomy zone than the autotomy section ($P < 0.05$). Penetrating stimuli were more effective than beetle stimulation in the whole animal ($P < 0.05$) and the posterior chamber ($P < 0.01$). The whole animal and posterior chamber did not differ in sensitivity of the autotomy section to beetle stimuli, but penetrating stimuli were significantly less effective ($P < 0.05$) in the whole animal than in the posterior chamber.

AS, a golden (or yellow-green), sticky mucus was secreted at the site of attack. Attack on the AS itself elicited locally a colorless, thin secretion. We noted that a beetle, if biting the anterior part of the body, frequently withdrew and wiped its mandibles vigorously on the substrate, clearing them of adherent mucus. After a highly variable number of pinches, if these were delivered at or posterior to the autotomy zone (junction between the anterior body and the AS), the following events took place: the autotomy zone contracted sharply; the AS appeared to swell slightly, as if engorged with blood; and the anterior body secreted copiously its characteristic yellow mucus. Autotomy was then completed rapidly, in less than 2 to about 5 sec. Following autotomy, the AS remained in place; we never observed the crawling movement of the AS described by Hand & Ingram in *Prophysaon andersoni*. However, in our experiments a slight swaying motion of the AS was noted and its surface displayed shallow, puckering movements.

Among the stimuli tested, only mechanical stimuli resulted in autotomy. Electrical and chemical stimuli, although applied in widely ranging intensity and at varying sites, on or in the body, never caused autotomy. The *in vitro* preparation responded to mechanical stimulation in a manner indistinguishable from the responses of the intact animal, indicating that the head ganglia were not involved directly in the reflex. Furthermore, in no case did autotomy result from stimulation of the anterior region of the body. The most sensitive region for triggering the response was the autotomy zone, while repeated stimulation of the AS itself also induced the response. Mechanical stimulation involving penetration of the body wall was significantly more effective than the superficial stimulation characteristic of beetle bites. These results are summarized in Fig. 1. Although stimulation anterior to the autotomy zone did not induce autotomy, in some instances it appeared to accelerate the response when stimuli were shifted to the AS. In addition, with penetrating stimulation of the AS, more stimuli were required in the intact animal than in the *in vitro* system. Thus, the anterior part of the body does exert some control over the AS.

We used the *in vitro* posterior chamber preparation to investigate whether administration of neurotropic agents (neurotransmitters and blocking agents) could accelerate or otherwise alter the autotomy response. In no case was acceleration seen, and the only

agent which appeared to have any effect, 5-hydroxytryptamine (1 to 10 μmol/test), gave inconsistent results, ranging from no significant effect (8/13 trials) to pronounced inhibition (5/13 trials) of the response. Injections of agents into the anterior hemocoel or AS of intact slugs also were, generally, without effect. However, a single agent—atropine sulfate—infiltrated into the AS (2.5 μmol in 0.25 ml slug Ringer solution) totally suppressed the autotomy response. The results indicate that the reflex involves a cholinergic mechanism (muscarinic; hexamethonium, a nicotinic blocking agent, was without effect). In addition, it could be concluded that the neuromuscular mechanism for autotomy is not readily accessible to chemical influences from the anterior body nor, in general, from the AS itself.

The differences between the mucus produced by the AS and that produced by the rest of the body were associated with differences between the epithelial mucus cells in the two regions. On the mantle and back to the autotomy zone very large yellow cells were present; their dense distribution around the mantle margin gave this region a golden color. On effective stimulation they released elliptical vesicles which burst rapidly and released a dense yellow mucus characterized by many granules. The AS and foot sole also had mucus cells but their secretions were released in relatively stable vesicles and appeared thin, non-granular, and colorless. This mucus was similar to that of the arionid slug *Ariolimax columbianus* (Deyrup-Olsen *et al.*, 1983).

The AS, ranging from 200 to 500 mg wet weight, comprised 11% (sd ± 2, n = 11) of the total body weight of adult (reproductively capable) slugs. It is made up chiefly of spherical cells (diameter 50 to 70 μm); these stain deep red with iodine-potassium iodide solution in the characteristic reaction for glycogen. We conclude that they are glycogen cells, widely distributed storage elements of gastropod connective tissue (Joosse & Geraerts, 1983). The cells are embedded in a meshwork of muscle cells and connective tissue. In animals starved for 13 days the glycogen cells appeared to be depleted.

The tissue making up the AS had relatively high contents of water (89.4%, sd ± 2.3, n = 6, of total tissue weight), soluble protein (13.5%, sd ± 2.5, n = 6, of dry weight) and glycogen (17.5%, sd ± 3.6, n = 5, of dry weight), and no traces were found of galactogen, a storage carbohydrate used by gastropods in reproduction. Thus the central core of the AS provides a good source of water, protein, and carbohydrate to a predator such as *Scaphinotus angusticollis* (the weights of these beetles varied, averaging about 100 mg).

Paired blood vessels run longitudinally through the AS and injections of colored materials (e.g., India Ink) into the anterior hemocoel resulted in rapid coloration of the AS. Thus, there is effective blood circulation between the AS and the anterior body—of obvious importance in the supply of nutrients for storage in the AS. However, autotomy did not result in significant loss of blood from the anterior body, which was rapidly and effectively sealed off at the autotomy zone by contraction of its sphincter muscle (Hand & Ingram, 1950).

In accord with the report by Hand & Ingram (1950), the slugs proved able to regenerate the AS in a few weeks. In our laboratory conditions the process of autotomy and regeneration was repeated 3 successive times in 8 slugs, and perhaps could have occurred further had it appeared useful to extend the observations.

Young *Prophysaon foliolatum* individuals, within a day or two of hatching (body size less than 10 mg, compared with the adult range of about 2 to 10 g) showed fully coordinated autotomy responses with temporal characteristics similar to those of the adult.

DISCUSSION

The escape reaction of *Prophysaon foliolatum* is complex, involving diverse structural and physiological mechanisms. Maintenance of stored water and nutrients in the AS, which offers a significant reward to predators such as carabid beetles, must depend on coordination of metabolism and circulation. The neuromuscular reflex severing the AS from the body as a whole is entirely peripheral, and is triggered specifically by receptors located within the autotomy zone and the AS. This confirms the work of others, indicating that the sensory zone for autotomy in a variety of molluscan species is localized in the autotomized part (Stasek, 1967). Information from these sensitive areas in *Prophysaon* is also transmitted anteriorly, since mucus secretion over the body as a whole acompanies autotomy. The yellow color and dense, sticky

quality of the anterior mucus may tend to divert the attack by predators from the anterior body towards the dispensable AS.

Whereas the slug's behavior—moving away when attacked—indicates that the anterior ganglia (brain) register events occuring in the AS, the brain appears to be unnecessary for autotomy. Such decentralization of important neuromuscular functions is well known in molluscan physiology, as in the highly coordinated movements of the isolated foot in slugs (Deyrup-Olsen & Martin, 1982) and of severed tentacles in cephalopods (Lucas, cited by Stasek, 1967). The response depends on cholinergic neurons. The quality of stimulation—penetrating or shallow—affected the speed of the response, but our work with neurotropic agents offered no clues as to the nature of this differentiation. Indeed, it was surprising that the autotomy response appeared to be so refractory to such substances which, in our tests, frequently caused strong stimulation of the general body musculature and mucus cells. Overall, it may be concluded that the autotomy response is a highly organized and stable mechanism of defense. Its persistence in the genus *Prophysaon*, despite its presumed high cost, suggests that the response contributes significantly to survival of slugs in nature.

ACKNOWLEDGEMENTS

We thank colleagues in the Department of Zoology, University of Washington, for the following assistance: J. S. Edwards, for identification of the carabid beetle; E. Plisetskaya, for glycogen analyses; P. M. Brunner, for histology.

LITERATURE CITED

BALLINGER, R. E., 1981, Can predator defense be tributive or toxins non-toxic? *American Naturalist*, 117: 794–795.

DEYRUP-OLSEN, I., LUCHTEL, D. L. & MARTIN, A. W., 1963, Components of mucus of terrestrial slugs (Gastropoda). *American Journal of Physiology*, 235: R448–452.

DEYRUP-OLSEN, I. & MARTIN, A. W., 1982, Surface exudations in terrestrial slugs. *Comparative Biochemistry and Physiology, C, Comparative Pharmacology*, 72: 45–51.

HAND, C. & INGRAM, W. M., 1950, Natural history observations on *Prophysaon andersoni* (J.G. Cooper), with special reference to amputation. *Bulletin of the Southern California Academy of Sciences*, 49: 15–28.

JOOSSE, J. & GERAERTS, W. P. M., 1983, in SALEUDDIN, A. S. M. & WILBUR, K. M., ed., *The Mollusca*, 4: 317–406. Academic Press.

LOWRY, O. H., ROSEBOUGH, N. R., FARR, L. A. & RANDALL, R. J., 1951, Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, 193: 265–275.

MURAT, J. C. & SERFATY, A., 1974, Simple enzymatic determination of polysaccharide (glycogen) content of animal tissues. *Clinical Chemistry*, 20: 1576–1577.

PILSBRY, H. A. & VANATTA, E. G., 1898, Revision of the North American slugs: *Binneya*, *Hemphillia*, *Hesperarion*, *Prophysaon* and *Anadenulus*. *Proceedings of the Academy of Natural Sciences of Philadelphia*, 50: 219–248.

RAYMOND, W. J., 1890, Why does *Prophysaon* shed its tail? *Nautilus*, 4: 1.

ROACH, D. K., 1963, Analysis of haemolymph of *Arion ater* L. *Journal of Experimental Biology*, 40: 612–623.

STASEK, R., 1967, Autotomy in the Mollusca. *Occasional Papers of the California Academy of Sciences*, 61: 1–44.

VITT, L. J., CONGDON, J. D. & DICKSON, N. A., 1977, Adaptive strategies and energetics of tail autotomy in lizards. *Ecology*, 58: 326–337.

Revised Ms. accepted 15 July 1985.

MALACOLOGIA, 1986, 27(2): 313–321

POLYMORPHISM IN A LABORATORY POPULATION OF *BIOMPHALARIA GLABRATA* FROM A SEASONALLY DRYING HABITAT IN NORTH-EAST BRAZIL

O.S. Pieri[1] & J.D. Thomas

School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG, United Kingdom

ABSTRACT

Quantitative studies of a laboratory population of *Biomphalaria glabrata* (Say), originating from a seasonally drying habitat in NE Brazil, revealed that the snails were polymorphic with respect to morphology, behavior and diapause. The various morphs can be placed in a series on the basis of discontinuous variates, such as apertural lamellae, and continuous variates, such as shell weight and surface area of the shell aperture.

Statistical analysis revealed that lamellate snails (both diapausing and resident) tend to have relatively heavier, flatter shells, with smaller, flatter apertures, which are more deflected to the left than is the case with resident non-lamellate snails. Snails with lamellae, particularly those with six lamellae, were more prone to emigrate from the water than those without lamellae. All the various sets of lamellae tend to occur at constant relative distances from the aperture, possibly because post-lamellate growth is programmed to stop, either after a fixed time, or after a certain amount of growth has occurred.

It is postulated that the polymorphisms exhibited by the snails are adaptive, and should be taken into account when designing control measures against these snails, which act as hosts of *Schistosoma mansoni*.

INTRODUCTION

It is known that populations of *Biomphalaria glabrata* (Say) from seasonally drying habitats, such as those occurring in NE Brazil, may be polymorphic with respect to morphology, behaviour and dormancy (Paraense, 1957; Richards, 1963, 1964, 1967; Etges & Gilbertson, 1966; Michelson & Mota, 1982). Although, according to Richards (1968), the morphological features in the polymorphic series are controlled by multi-factorial inheritance, there is also evidence that environmental factors may be involved in triggering gene expression. However, as these have not been identified a research programme has been initiated in this laboratory to rectify this deficiency in our knowledge. As a prerequisite for such a study it is necessary to provide a sound, quantitative description of the various morphological types and elucidate the relationships between these and the propensity to emigrate from the water or undergo diapause.

The work described in the present paper aims to provide more precise information regarding the following questions: (i) to what extent are morphological features such as deflection of the shell aperture to the left, thickening of the shell and similar features correlated with the presence of apertural lamellae? (ii) to what extent are morphological features, such as the presence of varying numbers of apertural lamellae or their absence correlated with behavioural and physiological responses such as emigration from the water or diapause and (iii) what are the costs and benefits of these polymorphic features to the snails?

MATERIALS AND METHODS

I—Pre-treatment

The experimental snail colony, which was initiated by seeding an aquarium containing 30 litres of aerated, filtered tapwater (Thomas, 1973) with four lamellate (4 ± 1 mm shell diameter) *B. glabrata* from Touros, NE

[1]Present address: Departamento de Biologia, Instituto Oswaldo Cruz, CP-926 Rio de Janeiro, Brazil.

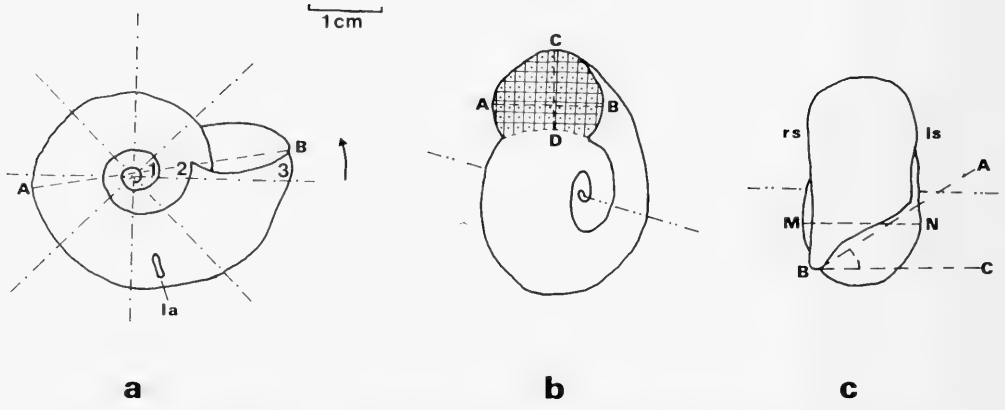


FIG. 1. Schematic representation of the measurements taken of the shell of *Biomphalaria glabrata*. The shells were drawn in three different positions using a dissecting microscope at 12× with a camera lucida. See text for detailed explanation.

(a). Shell drawn from the left side allowing the counting of the number of whorls and measurements of the shell diameter (line AB).

(b). Shell drawn with a front view of the aperture allowing measurements of its surface area as well as height and width (lines AB and CD, respectively).

(c). Shell drawn at a vertical position allowing measurements of the shell aperture deflection (angle ABC) and of the shell height (line MN).

Notes: (i) On (a) above the radial lines (–·–) are placed at 45 degrees from each other; the horizontal radius indicates the limits of each whorl, numbered anticlockwise 1, 2 and 3. See Mandahl-Barth (1962). (ii) The axis of volution, not seen on (a), is represented as (–···–) on (b) and (c). (iii) The position of one of the palatal lamellae (lamella III) is indicated by Ia on (a). (iv) On (c) the right side of the shell is indicated by rs and the left one, by ls.

Brazil, was maintained at a temperature of 26 ± 1°C and a constant photoperiod of 12 hr light, 12 hr dark. Fresh lettuce was provided as food, care being taken to ensure that some always remained uneaten. Tygan mesh barriers were used to prevent snails from leaving the aquarium.

Sampling of the population began approximately seven months after the founder snails were introduced. By this time it had been observed that considerable numbers of snails from a population of approximately 600–800 were beginning to emigrate from the water and enter into diapause on the sides of the tank. Preliminary observations revealed that most of these were in the 2.1 to 6.0 mm shell diameter range. For example the result of one census showed that the average number of emigrants in the 2.1–4.0 mm, 4.1–6.0 mm and 6.1–8.0 mm shell diameter range were 105.6, 89.8 and 13.7 per month respectively; none of the emigrants was smaller than 2.1 mm shell diameter. It was, therefore, decided to sample from within the following size categories, 3.1–3.5, 3.6–4.0, 4.1–4.5 and 4.6–5.0 mm shell diameter, for the reasons given below. Firstly, most of the lamellate snails were in this range. Secondly, it has been observed by Paraense (1957) and Richards (1963) that snails larger than 5 mm shell diameter may resorb the lamellae. Approximately 20 snails were taken as representatives of each size category from both emigrating, diapausing and resident, non-diapausing snails at three day intervals over a period of five weeks. This meant that all the diapausing snails were roughly in the same phase and had not suffered much loss of organic reserves (von Brand *et al.*, 1957). The following criteria were used in selecting representative snails. Firstly, only snails outside the water, with their bodies retracted at least one eighth of the way into the body whorl of the shell, were selected as examples of diapausing snails. Secondly, only snails that were actually moving or feeding below the water surface were selected as being representative resident snails. Approximately equal numbers of both categories were selected at the same time.

Immediately after collection the resident snails were immersed in water at a temperature of approximately 70°C for 15–30 sec, with the shell aperture slightly above the

water surface and then completely immersed for 1–2 sec. This treatment made it possible to withdraw the entire body with the aid of a gentle, steady pull from a fine forceps (Paraense, personal communication). Diapausing snails were treated in the same way after immersion in water at 26 ± 1°C had forced them to re-emerge from their shells.

II—Measurements

Both the shells and bodies of the snails were placed in individually numbered vials and dried at 110°C until a constant weight had been achieved, using a Sartorius analytical balance with a readability of 0.01 g. Shell measurements were made from drawings (12 × magnification) on 2 mm graph paper with the aid of a camera lucida attached to a dissecting microscope (Brown, 1980).

The conventions used for shell measurements are illustrated in Fig. 1 a-c and described below:

(i) Shell diameter. This was measured on the left side of the shell, along a straight line running from the extreme outer edge of the aperture through the center of the spire.

(ii) Number of whorls. These were counted as described by Mandahl-Barth (1957, 1962) (Fig. 1a). Accurate measurements, to the nearest eighth of a whorl, were obtained with the help of tracing paper divided into eight equal segments by radii superimposed on the left side of the shell. For example, in Fig. 1a the aperture is estimated at three whorls from the base of the embryonic shell and the lamellate set, at 2/8 of a whorl from the aperture.

(iii) Aperture surface area. As the aperture margin (peristome) is in one plane its surface area was calculated as follows. The shell was placed under the microscope so that the image of the peristome was in the same plane as the 2 × 2 mm graph paper. Accurate positioning was facilitated by using the largest magnification (50 ×). The measurements were taken as described by Batchelet (1975) using the grid formed by the 2 × 2 mm graph paper (Fig. 1b). By counting the squares whose centres fell within the limits of the peristome at 12 × magnification it was possible to estimate the area. For example, in Fig. 1b a total of 66 squares was counted giving an area of $66 \times 0.03 = 2.0$ mm^2.

(iv) Maximum height and width of shell aperture. A straight line was first drawn through the intersections between the body whorl and the left and right lips. The maximum length was a line drawn parallel to it to give the maximum distance between the right and left lips (AB in Fig. 1a). The aperture width given by line CD (Fig. 1b) was the maximum distance between the outer lip and the junction of the aperture plane with the body whorl. This junction was delineated with the aid of a small glass slide placed at the aperture to delimit the peristome plane.

(v) Deflection of the aperture. This was measured with the shell axis and aperture plane positioned horizontally and vertically respectively (Fig. 1c). One side of the angle is given by the line AB, drawn over the peristome contour and the other by the line BC drawn parallel to the shell axis. In the example given the aperture is deflected 33° to the left.

(vi) Shell height. This was the maximum height along a line parallel to the shell axis (MN in Fig. 1c).

RESULTS

The forms of the shell apertures of lamellate and non-lamellate snails are illustrated in Fig. 2. The convention used for identifying the lamellae shows that, although the numbers may vary, their distribution conforms to a basic pattern. By far the commonest categories of lamellate shell were those with four, five or six lamellae (Figs. 2, c-f). Other patterns such as those with only one large parietal lamella (Fig. 2b) or two lamellae (e.g., lamellae I and V) were found in only 10.7% and 13.3% of diapausing and resident, lamellate snails respectively.

Table 1, based on data from 77 diapausing and 82 resident snails (3.1–5.0 mm shell diameter), gives the number of snails with non-lamellate shells and also those with lamellate shells of various kinds. These data were used as a basis for testing several null hypotheses by means of contingency tables. The results of these statistical analyses in

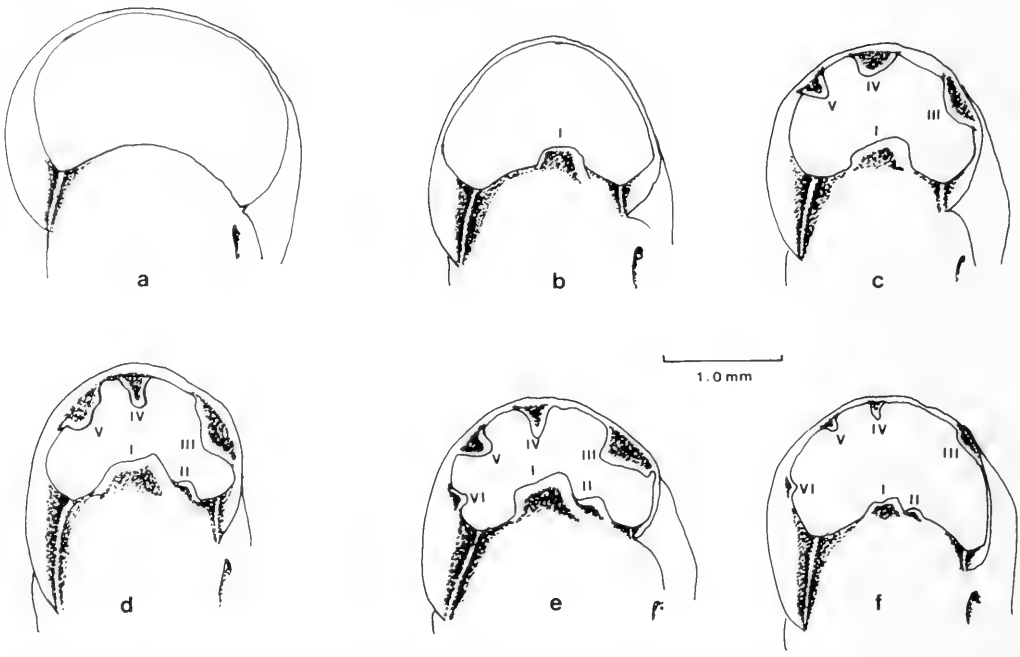


FIG. 2. Apertural lamellae of *Biomphalaria glabrata* with shell diameters ranging from 4.5 to 5.0 mm. Individual lamellae were numbered according to Paraense & Deslandes (1956).
(a). Non-lamellate shell.
(b). Shell aperture with one parietal lamella (I).
(c). Shell aperture with one parietal (I) and three palatal (III, IV and V) lamellae.
(d). Shell aperture with two parietal (I and II), and three palatal (III, IV and V) lamellae.
(e). Shell aperture with two parietal (I and II), and four palatal (III, IV, V and VI) lamellae.
(f). As in (e) but with lamellae relatively smaller.

TABLE 1. Number of diapausing and resident *B. glabrata* of different size categories ranging from 3.1 mm to 5.0 mm of shell diameter with or without apertural lamellae. Different types of lamellae set are schematically represented in Fig. 2. A, 3.1 mm–3.5 mm shell diameter; B, 3.6 mm–4.0 mm shell diameter; C, 4.1 mm–4.5 mm shell diameter; D, 4.6 mm–5.0 mm shell diameter.

	DIAPAUSING					RESIDENT				
LAMELLAE FORMATION	A	B	C	D	TOTAL	A	B	C	D	TOTAL
Six lamellae set	12	10	14	7	43	2	3	3	4	12
Five lamellae set	5	6	4	2	17	2	2	1	1	6
Four lamellae set	0	2	1	4	7	2	0	0	6	8
Others	2	2	3	1	8	0	0	2	2	4
Total lamellates	19	20	22	14	75	6	5	6	13	30
Nonlamellates	2	0	0	0	2	19	10	11	12	52
Total	21	20	22	14	77	25	15	17	25	82

Table 2 make it possible to make the following statements.

(i) The results in Table 2a reveal a significant association between the two classifications (lamellae formation and the tendency to diapause). There is strong evidence, therefore, that the formation of lamellae is correlated with the tendency to diapause. Thus, 97.4% of diapausing snails were lamellate compared with 34.1% of resident snails.

(ii) Table 2b shows that there is no signif-

TABLE 2. Chi-square evaluation of various aspects of the data in Table 1. Numbers in parentheses indicate the expected values according to the null hypothesis. ρ, probability; χ^2, Chi-square; ν, degrees of freedom.

A. Lamellae formation vs. tendency for diapause

Lamellae formation	Tendency for diapause		Chi-square evaluation
	Diapausing	Resident	
Lamellates	75 (50.8)	30 (54.2)	$\chi^2 = 65.50$
			$\nu = 1$
Nonlamellates	2 (26.2)	52 (27.8)	$\rho < 0.05$

B. Lamellae formation vs. shell diameter

Shell diameter	Lamellae formation		Chi-square evaluation
	Lamellates	Nonlamellates	
3.1–3.5 mm	25 (30.4)	21 (15.6)	$\chi^2 = 4.01$
3.6–4.0 mm	25 (23.1)	10 (11.9)	$\nu = 3$
4.1–4.5 mm	28 (25.8)	11 (13.2)	$\rho > 0.05$
4.6–5.0 mm	27 (24.4)	12 (12.6)	

C. Type of lamellae vs. tendency for diapause

Type of lamellae	Tendency for diapause		Chi-square evaluation
	Diapausing	Resident	
Six lamellae set	43 (39.3)	12 (15.7)	$\chi^2 = 5.94$
Five lamellae set	17 (16.4)	6 (6.6)	$\nu = 3$
Four lamellae set	7 (10.7)	8 (4.3)	$\rho > 0.05$
Others	8 (8.6)	4 (3.4)	

D. Predominance of a given type of lamellae among diapausing snails

Six lamellae set	Five lamellae set	Four lamellae set	Others	Chi-square evaluation
43	17	7	8	$\chi^2 = 45.1$
				$\nu = 3$
(18.7)	(18.7)	(18.7)	(18.7)	$\rho < 0.05$

E. Predominance of a given type of lamellae among resident snails

Six lamellae set	Five lamellae set	Four lamellae set	Others	Chi-square evaluation
12	6	8	4	$\chi^2 = 4.90$
				$\nu = 3$
(7.5)	(7.5)	(7.5)	(7.5)	$\rho > 0.05$

icant association between the two classifications (lamellae formation and shell diameter in the 3.1–5.0 mm range). It can be concluded that the ability to form lamellae may be shown by snails at any size within the 3.1 to 5.0 mm shell diameter range.

(iii) Neither was there a significant association between the classification in Table 2c (type of lamellae and tendency to

diapause). It can be concluded, therefore, that there are no differences between diapausing and resident snails in the relative proportion of the various sets of lamellae.

(iv) Table 2d shows that, among diapausing snails, the six-lamellate type is significantly predominant over others.

(v) In contrast, there was no significant difference between the proportion of the various lamellate types among resident snails (Table 2e).

Morphometric studies were carried out on the 30 resident non-lamellate snails as well as on 30 randomly selected snails among both the 75 resident lamellates and the 50 diapausing lamellates. The mean values of the various measurements and their derived ratios are given in Table 3. Possible differences between the three categories were evaluated statistically by means of a one-way analysis of variance. Tukey's Multiple Comparison Test was used for assessing pairwise differences in cases where the F-ratios obtained were higher than expected according to the null hypothesis (Meyers & Grossen, 1974). The results can be summarized as follows:

(i) There were no significant differences between the mean shell diameter, the mean ratios of number of whorls/shell diameter or the mean ratios of body dry weight/shell diameter of snails in the three categories studied.

(ii) The shell dry weight/shell diameter ratios were significantly greater in both diapausing and resident lamellate snails than in resident non-lamellate snails. However, there were no significant differences between this ratio in diapausing and resident lamellate snails.

(iii) The following ratios were significantly less in both diapausing and resident lamellate snails than in resident non-lamellate snails: apertural surface area/shell diameter, apertural surface area/body weight, height/width of shell aperture, shell height/shell diameter. However, there were no significant differences between any of these ratios in diapausing and resident lamellate snails.

(iv) The deflection of the shell aperture to the left was significantly greater in both diapausing and resident lamellate snails than in resident, non-lamellate snails but there were no significant differences between this measurement in the case of snails in the first two categories.

The values of coefficients of variation (standard deviation as a percentage of the mean) in Table 3 indicate considerable variation in the measurements. This is particularly the case with the ratios involving dry weight measurements. However, there is nothing to suggest that any category of snails is more variable than another.

Measurements of the distance between the lamellae and the shell aperture revealed that the lamellae were situated 1/8, 2/8 or some other fraction of a whorl from the aperture in 50 (66.7%), 17 (22.7%) and 8 (10.6%), respectively, of the 75 diapausing lamellate snails examined. Resident lamellate snails resembled them in this respect. Thus, of the 30 such snails examined 22 (73.3%), 5 (16.7%) and 3 (10.0%) had the lamellae situated 1/8, 2/8 or some other fraction of a whorl, respectively, from the aperture. Chi-square tests (one-way classification) showed that the location of the lamellae 1/8 of a whorl from the aperture was significantly predominant over the other two categories for both diapausing lamellates (χ^2 : 8.33; $P<0.01$) and resident lamellates (χ^2 : 6.53; $P<0.05$).

During the course of this study other observations were made on the behaviour of the snails which are relevant to the polymorphisms. Firstly, after the snails have started to diapause, their bodies are generally retracted an appreciable distance into the body whorl of the shell. Secondly, after snails have entered diapause it proved difficult to reverse the process in the short term. Thus, even after being returned to the water repeatedly they persisted in leaving the water to diapause on the sides of the aquarium.

DISCUSSION

The results show that the *B. glabrata* population studied was polymorphic, although it had started from four lamellate individuals originating from a seasonally drying habitat in Touros, NE Brazil. The various morphs can be placed in a series, on the basis of discontinuous variates, such as apertural lamellae and continuous variates, such as shell weight, surface area of aperture, etc. At the end of the range are the morphs with a full set of six lamellae and relatively heavier, flatter shells

TABLE 3. Shell measurements and derived ratios (mean and standard deviation) of diapausing lamellate (DL), resident lamellate (RL) and resident non-lamellate (RN) *B. glabrata*. F-ratios were obtained through One-way Analysis of Variance comparing differences in each of the various shell measurements and ratios among the three groups of snails (two degrees of freedom for the between-group variance and 87 degrees of freedom for the within-group variance). Significance of pairwise differences was evaluated through Tukey's Multiple Comparison Test (Meyers & Grossen, 1974). Values for coefficient of variation are given in parenthesis. HSD, Tukey's range statistics.

Shell measurements and ratios	Diapausing lamellates	Resident lamellates	Resident non-lamellates	F-ratio ($F_{2,87}$)	Tukey's HSD	Significant pairwise differences ($p < 0.05$)
Shell diameter (mm)	4.1 ± 0.5 (12.1)	4.2 ± 0.5 (11.9)	4.0 ± 0.7 (17.5)	1.38	—	—
Number of whorls up to the aperture/shell diameter ratio (mm^{-1})	0.86 ± 0.08 (8.3)	0.84 ± 0.08 (9.5)	0.87 ± 0.09 (10.3)	0.54	—	—
Shell dry weight/ shell diameter ratio ($mg \times mm^{-1}$)	1.29 ± 0.30 (23.1)	1.19 ± 0.31 (26.1)	0.84 ± 0.31 (36.9)	17.33***	0.195	DL vs. RN and RL vs. RN
Body dry weight/ shell dry weight ratio ($mg \times mm^{-1}$)	0.30 ± 0.10 (33.3)	0.32 ± 0.11 (34.4)	0.28 ± 0.07 (25.0)	1.27	—	—
Aperture surface area/ shell diameter ratio (mm)	0.76 ± 0.12 (15.8)	0.76 ± 0.08 (10.5)	0.85 ± 0.16 (18.8)	4.71*	0.08	DL vs. RN and RL vs. RN
Aperture surface area/ body dry weight ratio ($mm^{-2} \times mg^{-1}$)	2.62 ± 0.55 (20.9)	2.56 ± 0.97 (37.4)	3.12 ± 0.72 (23.0)	4.71*	0.48	DL vs. RN and RL vs. RN
Aperture height/ aperture width ratio	1.29 ± 0.20 (15.5)	1.36 ± 0.24 (17.6)	1.67 ± 0.15 (8.9)	28.74***	0.12	DL vs. RN and RL vs. RN
Shell height/shell diameter ratio	0.43 ± 0.03 (6.9)	0.44 ± 0.04 (9.1)	0.49 ± 0.04 (8.1)	28.03***	0.02	DL vs. RN and RL vs. RN
Aperture deflection (degrees)	31.6 ± 5.04 (15.9)	29.5 ± 4.56 (15.4)	25.3 ± 4.36 (17.2)	13.57***	2.94	DL vs. RN and RL vs. RN

(*) Significant at the $\alpha = 0.05$ level; (***) significant at the $\alpha = 0.001$ level.

with smaller, flatter apertures which tend to be more deflected to the left than in other morphs. There was a definite tendency for this morph and others with smaller number of lamellae to emigrate from the water and enter into diapause. The emigrating, diapausing, lamellate snails differed from the resident lamellate in having proportionately more individuals with a fully developed set of six lamellae. At the other end of the scale are the snails with non-lamellate, relatively lighter, higher shells with larger, higher apertures which are less deflected to the left than in other forms. These results provide quantitative support for the observations made by Paraense (1957) and Richards (1963, 1964, 1967, 1968) on lamellate *B. glabrata* collected from various areas.

Intermediate morphs can be distinguished by the number of lamellae. Thus, some snails have only one lamella to a set whereas others may have two, four or five. It is likely that these are distinct morphs rather than transitional stages in the development of a full six lamellate set for the following reasons. Firstly, each set of lamellae occurred in approximately the same proportion in all the size categories of snails examined. Secondly, all the lamellae observed in sets of five or less were as thick and well formed as those in sets of six. In fact only three out of 105 lamellate shells examined had rudimentary or incompletely developed lamellae. Two of these were six lamellate sets and one had four lamellae.

The appearence of lamellae is only one of several changes that occur during the growth and development of lamellate snails. Certain of these, such as shell thickening, a reduction in the size of the aperture and a change in its shape appear to be synchronized with the appearence of the lamellae, and it would be of interest to ascertain whether the greater relative weight of lamellate shells is due mainly to

their post-lamellate growth. It was surprising to find that all the sets of lamellae occurred at an almost constant relative distance of 1/8 to 2/8 of a whorl from the aperture. A likely explanation is that somatic post-lamellate growth of the shell is programmed to stop either after a fixed time interval or after a certain amount of growth has occurred. It can be postulated that lamellate snails emerge from the water and undergo diapause only after growth has slowed down or stopped. However, some lamellate snails appear to remain in the water. Two explanations can be advanced to account for this phenomenon. First, they may be transient stages which have not yet emigrated because growth has not yet stopped. Alternatively, they may enter a state of diapause or reduced activity while still in the water thus representing another survival strategy. These hypotheses could be tested by monitoring individual snails during the transition from the non-lamellate to the lamellate, diapausing stage. Growth stoppage has also been observed in *B. glabrata* following a period of unusual shell growth leading to the formation of circular thickenings, called ribs, around the aperture (Richards, 1963). Snails entering diapause may deposit an epiphragm of mucous material near the aperture as reported by Paraense (1957) and Richards (1963, 1964, 1967).

It follows that these morphological and behavioural changes must be accompanied by changes in the physiology and biochemistry of the snail. However, practically nothing is known about the molecular nature of the latter although they are manifested by changes in the colour of the hepatopancreas, the rate of shell deposition and growth as the snails enter the lamellate phase (Richards, 1963). As might be expected the rate of oxygen consumption declines as snails enter the diapausing or aestivating phase (von Brand *et al.*, 1948; Magalhaes-Neto, 1953; von Brand *et al.*, 1957; Heeg, 1977).

The sequential series of events that lead to different morphs (or phases), dispersion and a state of dormancy described for lamellate *B. glabrata* have also been noted in other molluscan species, including *Biomphalaria schrammi* by Paraense & Deslandes (1956) in Brazil, *Biomphalaria pfeifferi gaudi* by McCullogh (1958) in Africa, four varieties of *Biomphalaria* in Africa by Mandahl-Barth (1957), 21 species of freshwater molluscs including members of the subfamilies Planorbinae, Helisomatinae, Segmentininae, Planorbulinae, Plesiophysinae by Richards (1963) and *Ferrissia wautieri* by Richardot (1977a, b). The behavioural responses shown by the lamellate snails such as emigration from the water and dormancy are also characteristic of a large number of non-lamellate pulmonates including those which serve as hosts for schistosomiasis. Various terms have been used to describe the state of dormancy which these snails enter into, such as anhydrobiosis (Stiglingh & van Eeden, 1977) or aestivation (Brown, 1980). As pointed out by the latter author, this strategy is central to the ecology of many freshwater snails including snail hosts of schistosomiasis.

The seasonally drying aquatic habitats encountered in many areas of NE Brazil (Barbosa & Olivier, 1958; Barbosa, 1962) appear to favour the mixed strategies involving the formation of lamellate, emigrating, diapausing morphs as well as others. They, therefore, appear to resemble plants which also live in seasonal environments with unpredictable levels of future adversity, in their strategy for survival. Thus, seed and dormancy polymorphisms are common among fugitive or weedy species such as *Rumex crispus* and *Xanthium* spp. (Cavers & Harper, 1966; Harper, 1977), while leaf polymorphism is frequently encountered in desert plants. As pointed out by Harper (1977) polymorphism is advantageous because it gives a degree of buffering against sudden selective forces that favour one or other morph. This helps to explain why snails, like weeds, are often difficult to control or eradicate by the application of chemicals and biological methods.

ACKNOWLEDGEMENTS

We express our gratitude to the following: UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases for providing financial support, to Dr. W.L. Paraense for providing snail material, to Professor J. Maynard-Smith for providing facilities in the School of Biology, University of Sussex, to Mr. M.J. Stenning for technical assistance, to Dr P.R. Sterry and Dr R.L. Patience for reading the manuscript critically.

REFERENCES

BARBOSA, F.S., 1962, Aspects of the ecology of the intermediate hosts of *Schistosoma mansoni*

interfering with the transmission of bilharziasis in North-Eastern Brazil. *In* WOLSTENHOLME, G.E.W. & O'CONNOR, M. (ed.), *CIBA Foundation Symposium on Bilharziasis*, Churchill, London, p. 23–35.

BARBOSA, F.S. & OLIVIER, L., 1958, Studies on the snail vectors of bilharziasis mansoni in North-Eastern Brazil. *Bulletin of the World Health Organization*, 18: 895–908.

BATSCHELET, E., 1975, *Introduction to mathematics for life scientists*. Springer-Verlag, Berlin, 513 p.

BRAND, T. von, NOLAN, M.O. & MANN, E.R., 1948, Observations on the respiration of *Australorbis glabratus* and some other aquatic snails. *Biological Bulletin*, 95: 199–213.

BRAND, T. von, McMAHON, P. & NOLAN, M.O., 1957, Physiological observations on starvation and dessication of the snail *Australorbis glabratus*. *Biological Bulletin*, 113: 89–102.

BROWN, D.S., 1980, *Freshwater snails of Africa and their medical importance*. Taylor & Francis, London, 487 p.

CAVERS, P.B. & HARPER, J.L., 1966, Germination polymorphism in *Rumex crispus* and *Rumex obtusifolius*. *Journal of Ecology*, 54: 367–382.

ETGES, F.J. & GILBERTSON, D.E., 1966, Repellent action of some chemical molluscicides on schistosome vector snails. *American Journal of Tropical Medicine and Hygiene*, 15: 618–624.

HARPER, J.L., 1977, *Population biology of plants*. Academic Press, London, 892 p.

HEEG, J., 1977, Oxygen consumption and the use of metabolic reserves during starvation and aestivation in *Bulinus (Physopsis) africanus* (Pulmonata: Planorbidae). *Malacologia*, 16: 549–560.

MAGALHAES-NETO, B., 1953, Acao da dessecacao e do jejum sobre a respiracao do *Australorbis glabratus*. *Publicacoes Avulsas do Instituto Aggeu Magalhaes*, 2: 5–10.

MANDAHL-BARTH, G., 1957, Intermediate hosts of *Schistosoma*, African *Biomphalaria* and *Bulinus*: I. *Bulletin of the World Health Organization*, 16: 1103–1163.

MANDAHL-BARTH, G., 1962, Key to the identification of East and Central African freshwater snails of medical and veterinary importance. *Bulletin of the World Health Organization*, 27: 135–150.

McCULLOUGH, F.S., 1958, The internal lamellae in the shell of *Biomphalaria pfeifferi gaudi* (Ranson) from Ghana, West Africa. *Journal de Conchyliologie*, 97: 171–179.

MEYERS, L.S. & GROSSEN, N.E., 1974, *Behavior research: theory, procedure and design*. Freeman, San Francisco, 355 p.

MICHELSON, E.H. & MOTA, E., 1982, Malacological observations bearing on the epidemiology of schistosomiasis in a rural Bahian community. *Revista do Instituto de Medicina Tropical de São Paulo*, 24: 75-82.

PARAENSE, W.L., 1957, Apertural lamellae in *Australorbis glabratus*. *Proceedings of the Malacological Society of London*, 32: 175–179.

PARAENSE, W.L. & DESLANDES, N., 1956, Observations on *Australorbis janeirensis* (Clessin, 1884). *Revista Brasileira de Biologia*, 16: 81–102.

RICHARDOT, M., 1977a, Ecological factors inducing aestivation in the freshwater limpet *Ferrissia wautieri* (Basommatophora: Ancylidae). I—Oxygen content, organic matter content and pH of the water. *Malacological Review*, 10: 7–13.

RICHARDOT, M., 1977b, Ecological factors inducing aestivation in the freshwater limpet *Ferrissia wautieri* (Basommatophora: Ancylidae), II—Photoperiod, light intensity and water temperature. *Malacological Review*, 10: 15–30.

RICHARDS, C.S., 1963, Apertural lamellae, epiphragms and aestivation of planorbid mollusks. *American Journal of Tropical Medicine and Hygiene*, 12: 254–263.

RICHARDS, C.S., 1964, Apertural lamellae as supporting structures in *Australorbis glabratus*. *Nautilus*, 78: 57–60.

RICHARDS, C.S., 1967, Estivation of *Biomphalaria glabrata* (Basommatophora : Planorbidae), associated characteristics and relation to infection with *Schistosoma mansoni*. *American Journal of Tropical Medicine and Hygiene*, 16: 797–802.

RICHARDS, C.S., 1968, Aestivation of *Biomphalaria glabrata* (Basommatophora: Planorbidae). Genetic Studies. *Malacologia*, 7: 109–116.

STIGLINGH, I. & EEDEN, J.A. van, 1977, Population fluctuations and ecology of *Bulinus tropicus* (Mollusca: Basommatophora). *Wetenskaplike Bydraes van die PU vir CHO Reeks B: Natuurwetenskappe*, 87:1–37.

THOMAS, J.D., 1973, Schistosomiasis and the control of mulluscan host of human schistosomes with particular reference to possible self-regulatory mechanisms. *In* DAWES, B. (ed.), *Advances in Parasitology*. Academic Press, London, 11: 307–399.

MALACOLOGIA, 1986, 27(2): 323–339

GENETIC VARIATION IN SEVEN WOOD-BORING TEREDINID AND PHOLADID BIVALVES WITH DIFFERENT PATTERNS OF LIFE HISTORY AND DISPERSAL

K. Elaine Hoagland

Center for Marine and Environmental Studies, Lehigh University, Bethlehem, PA 18015, U.S.A.

ABSTRACT

Intrapopulation genetic variation and genetic distance between populations were investigated for ten populations of seven teredinid and pholadid species in the bivalve superfamily Pholadacea. Genetic variation as measured by allozyme heterozygosity, percent polymorphic loci, and number of alleles per locus tended to be higher in species with planktotrophic larvae, lower in species with brooded larvae. Two introduced populations were genetically impoverished, perhaps due to founder effects and bottlenecks. Most of the 20 loci resolved for all species showed heterozygote deficiency, as has been reported for many marine bivalves. Wahlund effects and inbreeding are not sufficient to explain heterozygote deficiency; natural selection and null alleles may also be causes. Species both with and without planktonic larvae show heterozygote deficiences. Substantial genetic differentiation exists between populations of one species with planktonic larvae. Patterns of genetic variation could not be correlated with latitude or local environmental factors. The data suggest that breeding structure and larval dispersal influence intra- and interpopulation allozyme variation.

Key words: shipworm; Teredinidae; Pholadidae; Pholadacea; allozymes; heterozygosity; genetic variation; brooding; planktotrophy; dispersal; heterozygote deficiency.

INTRODUCTION

Since the advent of electrophoresis as a tool in population genetics, many researchers have sought to describe patterns in genetic variation at the population and species levels. Some have related genetic variation to heterogeneity or uncertainty of the environment (Levinton, 1973; Selander & Kaufman, 1973), although Johnson (1976) demonstrated the ambiguity of many such studies. High genetic variability has been correlated with geographical range and colonization ability (Nevo, 1978). Species with dispersive larvae are assumed to be outbreeders and good colonizers, hence more variable genetically at the population level, but less genetically differentiated between populations, than species that brood their young or otherwise lack a dispersive larval stage (Crisp, 1978; Ament, 1979; Hamrick *et al.*, 1979). Most of the data supporting these ideas involve several populations of one or two species (Koehn *et al.*, 1976; Ayala & Valentine, 1974; Lavie & Nevo, 1986). Most molluscan data sets include only a few enzyme systems that are usually highly polymorphic (Levinton, 1975; Berger, 1977; Wilkins and O'Regan, 1980; Beaumont *et al.*, 1980; Badino & Sella, 1980).

The type of larval development in bivalves is related to the ability of the young to disperse; hence it bears a relationship to geographic range, population size, and genetic structure of populations. The positive correlation of genetic variation with these three factors has been supported theoretically and empirically (Soulé, 1976; Snyder & Gooch, 1973), but contradictory evidence was cited by Valentine (1976). Few data are available that delineate genetic patterns in similar species with different types of larval development and different levels of fecundity (Lavie & Nevo, 1986).

This paper reports the electrophoretic analysis of a broad range of enzyme systems for ten populations of seven species of wood-boring teredinid and pholadid bivalves within one superfamily, the Pholadacea. Two populations are introduced; the effects of introduction on population genetic structure was investigated. The genetic distance between populations was compared for two species and related to typical interspecific values. The other purpose of the work was to compare heterozygosity in populations of species with

different life histories and modes of dispersal. Each species has a characteristic development pattern, ranging from completely planktonic to completely brooded development with the release of pediveliger young. Some species follow an intermediate path, with a brooded period followed by a planktonic period. Levels of fecundity are proportional to the amount of time spent in the plankton. The species are otherwise ecologically similar, spending their adult lives burrowing in wood. Some of the species chosen for analysis are congeners. Several species were taken from the same geographical area, so that the degree of environmental variation (e.g., seasonal temperatures and salinities; variation in food and exposure to predators) would not be a major factor explaining the observed differences in genetic variation among taxa.

METHODS

Populations Studied

The populations of Pholadacea that were examined are described in Table 1. The density of each species in the white pine collecting panels indicates the strength of the age-class settlement over the time period of the submergence of the panels. The accuracy of the estimate is lower for species lacking a planktonic larval stage, such as *Teredo bartschi*. Such species are expected to have highly clumped distributions (Hoagland *et al.*, 1981).

The salinity at all sites is seasonally variable. The yearly salinity ranges are based on my observations in the case of the New Jersey sites, and data from laboratories at localities cited in Table 1 for the other sites. All sites are estuarine except Millstone, Connecticut, and Virginia Key, Florida, which have approximately full oceanic salinity for most of the year.

Teredo bartschi is a tropical to subtropical species that was accidentally introduced into the thermal effluents of two nuclear power plants in the cold temperate zone (Hoagland & Turner, 1980). The Fort Pierce, Florida, population is within the natural distribution of the species. The New Jersey and Connecticut populations had been established for seven and five years, respectively.

Life History

Data on the life history of the teredinids from New Jersey were obtained between 1976 and 1981 by submerging wood panels for one to twelve month periods, then retrieving them and submerging new panels monthly at 20 stations in Barnegat Bay (Hoagland, 1983a). In the laboratory, the shipworms were dissected from the wood, identified, measured, and examined for larvae in the gills. Other life historical and ecological data were available in the literature (Turner & Johnson, 1971; Hoagland & Turner, 1981). Temperature and salinity tolerances were determined in the laboratory; details of these experiments are reported elsewhere (Hoagland, 1983b, 1986).

Electrophoresis

Specimens were dissected from the wood while alive, identified, and either electrophoresed immediately or frozen in Tris tissue buffer until they were subjected to electrophoretic analysis. Voucher specimens are on deposit at The Academy of Natural Sciences of Philadelphia (ANSP); catalogue numbers are in Table. 1. Horizontal starch-gel electrophoresis followed by staining for specific enzymes was employed to study 23 enzyme systems. The general methods of Ayala *et al.* (1973) were applied to mollusks as amended by Dillon & Davis (1980) and Davis *et al.* (1981). Starch gels (13%) were prepared using 33.5 g of Electrostarch and 250 ml of one of four gel buffers: 1) tris citrate, pH 6.0; 2) tris NaOH borate (Poulik), tray buffer pH 7.6 and gel buffer pH 8.9; 3) tris-EDTA-borate (TEB) pH 8.0; and 4) TEB, pH 9.1. Four enzyme systems were run on TEB of pH 9.1 but with tray buffer of pH 8. Enzymes assayed and electrophoretic conditions are reported in Table 2.

Five wicks of No. 3 Whatman filter paper were saturated with homogenized tissue, blotted, and applied, one wick from each individual, to each gel. Five gels were run concurrently; each was then analyzed for three enzyme systems. Each individual was investigated for all enzyme systems over a 2-day period.

Six individuals from a reference population and 25 individuals from the population being tested were run on a single gel. Runs were repeated if the relative position of the reference population was unclear or if results were

TABLE 1. Populations of Pholadacea used in this study.

Species	Population	No. of genomes sampled	Approx. density*	Yearly salinity range (‰)	Comments
Teredo bartschi	Blue Hole, intracoastal waterway near Harbor Branch Laboratory, Ft. Pierce, Florida	98	10^1–10^2	10–39	Panels submerged May 2, 1980 removed Nov. 20, 1980 in mangrove area. ANSP A9100.
Teredo bartschi	Oyster Creek nuclear generating station, Oyster Creek and Forked River, New Jersey	200	10^2–10^3	7–30	Panels submerged from docks and removed in 1979 and 1980. ANSP A8717A-E.
Teredo bartschi	Millstone nuclear generating station, Millstone Point, Niantic Bay, Waterford, Conn.	40	10^0–10^1	28–33	Panels submerged from docks June 17 and removed Nov. 7, 1980. ANSP A8693, A8726C.
Teredo navalis	Oyster Creek nuclear generating station, Oyster Creek and Forked River, New Jersey	180	10^1	7–30	Panels submerged from docks and removed in 1980. ANSP A8362.
Teredo navalis	Millstone nuclear generating station, Millstone Point, Niantic Bay, Waterford, Conn.	102	10^1	28–33	Panels submerged from docks June 17 and removed Nov. 7, 1980. ANSP A8726A & B.
Bankia gouldi	Oyster Creek and Forked River, New Jersey	214	10^1	7–30	Panels submerged from docks and removed in 1980. ANSP A7927E.
Bankia fimbriatula	Rocky Point, 1 mi. S. of "Crossroads" where Intracoastal Waterway meets St. Lucie River, near Hobe Sound, Florida	100	10^0–10^1	~10–30	Dead red mangrove collected Oct. 22, 1979. ANSP A8680A.
Lyrodus floridanus	Rosenstiel School, U. Miami, Bear Cut, Virginia Key, Florida	104	10^1	~31–35	From pine panels submerged for 6 months from a dock. Collected Oct. 29, 1980. ANSP A8680B & C.
Lyrodus bipartitus	Little Jim Creek, Ft. Pierce Inlet, near Ft. Pierce, Florida	130	10^1–12^2	10–39	Panels submerged May 2 and removed Nov. 20, 1980, exposed in red mangrove area. ANSP A8726D.
Martesia striata	Rocky Point, 1 mi. S. of "Crossroads" where Intracoastal Waterway meets St. Lucie River, near Hobe Sound, Florida.	124	10^2	~10–30	From dead red mangrove collected Oct. 22, 1980. ANSP 353444.

*Order of magnitude estimate per wood volume equivalent to a test panel 2 × 9 × 21 cm.

otherwise ambiguous. Although a sample size of at least 50 individuals per population was sought for each enzyme locus, it was not always reached because a few species were rare. The sample size also varied among enzyme systems for a single population when the zymograms could not be resolved for all individuals. After preliminary results showed no differences in allelic patterns due to tissue type, entire animals minus brooded larvae were homogenized. All specimens were 4 to 12 months old and most were female.

Gels were scored as described in Ayala *et al.* (1973). The alleles of each locus were

TABLE 2. Enzymes assayed, buffers, current, voltage, and duration of electrophoresis.

Enzyme	No. loci	Gel & tray buffer	Current/ voltage	Run time (hr)
Acid phosphatase (AcPh)	1	TC6	35 MA	3.5
Adenylate kinase (Adkin)	1*	Poulik	35 MA	3.0
Aldehyde oxidase (AO)	2*	TEB 9	350 V	4.5
Aspartate amino transferase (AAT)	1	TEB 9	350 V	4.5
Esterase NA (EST NA)	3*	TEB 9/8	35 MA	2.0
Glucose-6-phosphate dehydrogenase (G6PDH)	1*	TC 6	35 MA	2.5
Glucose-phosphate isomerase (GPI = PGI)	2*	TC 6	35 MA	2.0
Glutamate dehydrogenase (GDH)	1*	Poulik	35 MA	3.0
Glyceraldehyde-3-phosphate dehydrogenase (G3PDH)	1*	TEB 8	35 MA	3.5
α-Glycerophosphate dehydrogenase (α GPDH)	1*	Poulik	35 MA	3.0
Hexokinase (HEX)	1	Poulik	35 MA	3.0
Isocitrate dehydrogenase (IsDH)	2	TEB 8	35 MA	3.5
Lactate dehydrogenase (LDH)	1	TEB 8	35 MA	3.5
Leucine amino peptidase (LAP)	1	TC 6	35 MA	2.0
Mannose-6-phosphate isomerase (MPI)	1	TEB 9/8	35 MA	2.0
NAD-dependent malate dehydrogenase (NAD-MDH)	2*	TC 6	35 MA	3.5
Peptidase G (Pep G)	3*	TEB 8	35 MA	3.5
Phosphoglucomutase (PGM)	1	TC 6	35 MA	2.0
		TEB 9	350 V	4.5
6-phosphogluconate dehydrogenase (6-PGDH)	1	Poulik	35 MA	3.0
Sorbitol dehydrogenase (SoDH)	1	Poulik	35 MA	3.0
Superoxide dismutase (SOD)	2*	TEB 9/8	35 MA	2.0
Triosephosphate isomerase (TPI)	1*	TEB 8	35 MA	3.5
Xanthine dehydrogenase (XDH)	1	Poulik	35 MA	3.0

*Locus with missing data. (If there are multiple loci, the upper locus has missing data, except for Pep G, where the lowest locus has missing data.)

identified by the distance, in mm, that they migrated with respect to the most common allele of a reference population. *Teredo bartschi* from Oyster Creek, New Jersey, was the reference population because it was abundant and nearly monomorphic. Assignment of electrophoretic patterns to loci and consequent interpretations were made with the aid of data collected on the same enzyme systems for mollusks (Davis *et al.*, 1981) and other organisms (Lewontin, 1974).

Calculations were made of the allele frequencies at each locus, the average number of alleles per locus (A), the percent polymorphic loci (P), and Nei's genetic distance (D) between populations. A locus was scored as polymorphic if the frequency of the most common allele was $<.99$. Heterozygosity of each enzyme locus for each population (h) was predicted from the Hardy-Weinberg equilibrium. Heterozygosity was also calculated directly ($h_{obs.}$). A heterozygote deficiency index was calculated as $(h_{obs.}-h_{pred.})/h_{pred.}$. The average individual heterozygosity per locus was calculated, again using both the Hardy-Weinberg assumptions ($H_{pred.}$) and the direct method ($H_{obs.}$), by averaging the respective h values over all loci.

RESULTS

Genetic variation

The allele frequencies for each population are in the Appendix. Of the 32 loci resolved, 20 yielded consistent results for all populations and were used in the analysis of genetic variation. Fourteen loci were polymorphic in at least one species. At 5 loci (AcPh, SOD, LDH, 6-PGDH and IsDH), one allele was fixed in each of the seven species. Only for XDH

TABLE 3. Pholadacea. Average heterozygote deficiencies.

	Mean ± S.E. het. def.	No. loci polymorphic	Total no. loci lacking heterozygotes
T.b. Conn.	−1.00	2	2
T.b. N.J.	−0.50	2	0
T.b. Fla.	−0.54 ± .18	7	3
L.b. Fla.	−0.51 ± .11	10	1
T.n. Conn.	−0.36 ± .08	10	0
T.n. N.J.	−0.67 ± .09	11	1
L.f. Fla.	−0.42 ± .15	9	3
B.f. Fla.	−0.38 ± .12	11	1
B.g. N.J.	−0.48 ± .08	14	0
M.s. Fla.	−0.43 ± .12	13	3

was the same allele fixed in all the species. Regardless of the species, genetic variation was consistently higher at some loci (eg., GPI, PGM, MPI) than others.

The heterozygote deficiencies (Table 3) were large at most loci. Lack of heterozygotes occurred at three loci in three populations, but all three populations also exhibit loci in Hardy-Weinberg equilibrium (d ≃ O). Loci lacking heterozygotes are not the same in each population. Heterozygote deficiencies ranged widely among populations of one species. The introduced population of *Teredo bartschi* from Millstone, Connecticut, had only two polymorphic loci, and both had complete lack of heterozygotes.

Table 4 summarizes commonly-used indices of genetic variation for the 10 populations. The values of P and A for the Millstone population of *Teredo bartschi* may be underestimated because only 20 specimens were obtained over a 2-year period. Means of the various indices for the Pholadacea were calculated with and without the two introduced populations. In comparing *Teredo bartschi* with other taxa, the latter values should be used. The values for the recently-introduced *T. bartschi* are probably altered by founder effects and are not representative of natural populations (Hoagland, 1983b).

Interpopulation variation is assessed using Nei's genetic distance (D), Table 5. The relationships do not change using other indices. Differences between populations are considerably smaller than those between species. The genetic distance between populations of *Teredo navalis* with planktonic larvae is substantial at 0.29. In fact, the genetic distance between the populations of *T. bartschi* is much less (0.08 to 0.11).

Ecology

Table 6 reviews life historical and ecological information based on field and laboratory work (Turner, 1966; Turner & Johnson, 1971; Hoagland, 1983b, 1986). The most important difference among the species is the type of larval development. Two species, *Teredo bartschi* and *Lyrodus bipartitus*, brood the young in the gill to the pediveliger stage; they produce relatively few yolky eggs. *Teredo navalis* and *L. floridanus* also brood the young, but release them in the straight hinge veliger stage, after which they undergo several more weeks of planktotrophic development. The other three species are completely planktonic; fertilization of up to a million eggs per reproductive event is external, or could involve pseudocopulation, transfer of sperm using the siphons.

Despite these differences, all shipworms can be classified as opportunistic (Turner, 1973), because they colonize wood, an ephemeral substrate and food resource that they themselves destroy. Although the peaks in settlement activity of sympatric species do not coincide, most natural wood collected in Florida contained numerous co-existing species. *Lyrodus massa*, *L. floridanus*, *L. bipartitus*, *Teredo furcifera*, *T. bartschi*, and *Martesia striata* were found together in mangrove wood from the Ft. Pierce Inlet, Florida, although *L. massa* and *T. furcifera* were rare. Most predators on the shipworms that I observed, including nereid polychaetes, flatworms, and protozoa, appeared to be nonselective. All 7 species of shipworms have the same trophic position, eating both wood and algae. I have reared adults and larvae of 4 of the 7 species on the same diet.

TABLE 4. Genetic variation in 10 populations of Pholadacea, 20 loci.

Population	Percent polymorphic loci, P*	Average no. of alleles per locus A (S.E.)	Avg. individual observed heterozygosity (S.E.)	Avg. individual predicted heterozygosity (S.E.)
Brooders				
T. bartschi Conn.	10	1.15 (.11)	0 (–)	0.04 (0.03)
T. bartschi N.J.	10	1.15 (.11)	0.004 (0.003)	0.01 (0.01)
T. bartschi Fla.	35	1.35 (.11)	0.008 (0.004)	0.05 (0.03)
L. bipartitus	45	1.95 (.26)	0.082 (0.025)	0.18 (0.05)
Mixed development				
T. navalis Conn.	55	2.20 (.34)	0.150 (0.042)	0.24 (0.06)
T. navalis N.J.	55	2.10 (.32)	0.069 (0.024)	0.21 (0.05)
L. floridanus	45	1.55 (.15)	0.042 (0.020)	0.09 (0.04)
Planktonic larvae				
B. fimbriatula	55	1.90 (.25)	0.138 (0.038)	0.24 (0.05)
B. gouldi	70	2.40 (.28)	0.133 (0.033)	0.27 (0.05)
M. striata	65	2.30 (.29)	0.146 (0.049)	0.25 (0.06)
Mean (S.E.), 10 pops.	44 (07)	1.81 (0.15)	0.077 (0.020)	0.16 (0.03)
Mean (S.E.), excluding introduced populations	53 (04)	1.97 (0.13)	0.096 (0.019)	0.19 (0.03)

*A locus is considered polymorphic if the frequency of the most common allele is less than .99.

TABLE 5. Pholadacea. Nei's Distance Matrix.

	T.b. Fla.	*T.b.* Conn.	*T.n.* N.J.	*T.n.* Conn.	*L.f.* Fla.	*L.b.* Fla.	*B.g.* N.J.	*B.f.* Fla.	*M.s.* Fla.
T.b. N.J.	0.11	0.10	0.67	0.88	0.60	0.94	0.95	0.71	1.23
T.b. Fla.		0.08	0.63	0.85	0.57	0.99	1.09	0.89	1.27
T.b. Conn.			0.69	0.82	0.58	1.04	1.07	0.86	1.24
T.n. N.J.				0.29	0.82	0.92	0.82	0.85	1.23
T.n. Conn.					0.98	0.95	0.81	1.09	1.06
L.f. Fla.						0.84	0.90	0.75	1.23
L.b. Fla.							1.07	0.78	1.62
B.g. N.J.								0.48	0.98
B.f. Fla.									1.53

All the species of this study have been found as adults in driftwood. Brooding females are able to reach new sites while carrying larvae. Species such as *Teredo bartschi* and *Lyrodus bipartitus* that brood young during all months of the year are very likely to colonize as adults with larvae. *Teredo bartschi* living in a New Jersey heated effluent retained larvae in the gills through the winter months, although successful settlement did not occur between December and April. Larvae of *T. bartschi* have survived for at least two weeks in the laboratory without growth when either food or temperature was inadequate for metamorphosis. This phenomenon has been reported for many teredinids (Turner & Johnson, 1971). All populations used in this study lived in water 0.5 to 2 m deep and were exposed to seasonal changes in temperature and salinity (Tables 1, 6). The environment is seasonally variable with regard to temperature in Connecticut, salinity in Florida, and both in New Jersey.

Field observations suggest that in New Jersey, adult *Teredo bartschi* and *T. navalis* rarely live more than 18 months. *Bankia gouldi* often lives a full 2 years or longer. Specimens of *T. bartschi* have been found brooding larvae only 4 weeks after they have come in contact with wood, while the other two New Jersey species live in the wood for a minimum of 5 weeks before reaching maturity

TABLE 6. Comparative life history and ecology of seven species of Pholadacea.

	T. bartschi (N.J.)	*T. navalis* (N.J.)	*L. bipartitus* (Fla.)	*L. floridanus* (Fla.)	*B. fimbriatula* (Fla.)	*B. gouldi* (N.J.)	*M. striata* (Fla.)
Breeding season	May–Nov.	June–Oct.	All year	All year	Unknown	June–Aug.	Unknown
Eggs per reproductive event	10^3–10^4	10^4–10^5	10^3–10^4	10^4–10^5	10^6 or more	10^6 or more	10^6 or more
Brooded larval stage (weeks)	3–4	about 2	3–4	about 2	0	0	0
Planktonic larval stage (weeks)	0	about 2	0	about 2	about 4	about 4	about 4
Pediveliger stage (days)	0–14	unknown	unknown	unknown	unknown	unknown	about 16
Year to year variation in population density per test panel	10^3	10^2	10^2	unknown	unknown	10^2	unknown
Known species range	Worldwide warm water	Worldwide cold water	W. Atlantic warm water	W. Atlantic warm water	Worldwide warm water	E. Coast N. & S. America temperate	Worldwide warm water
Salinity tolerance[1] (‰)	7–60	7–45+	unknown	unknown	unknown	7–45+	unknown
Temperature tolerance[1] (°C)	11–35	0–30	unknown	unknown	unknown	0–30	unknown
Field temperatures (°C)	2–31[2]	0–31	12–33	16–31	14–31	0–31	14–31

[1]Survival of more than 50% of 100 individuals after 10 days.
[2]When the power plant in Oyster Creek is not operating, the temperature can drop to 0°C.

as females. Some populations of *Teredo bartschi* and *Lyrodus bipartitus* are highly skewed toward individuals brooding young (as high as 90%). Sex ratios are close to 60% female in *B. gouldi*, *B. fimbriatula*, and *T. navalis*. Although most males are smaller than most females, the largest individuals of all species tend to be male. This fact coupled with the sex ratio bias towards females suggest that pholadaceans undergo alternating sexuality.

The life history characters of the subtropical larviparous species *Teredo bartschi* and *Lyrodus bipartitus*, including shorter generation time, more generations per year, and shorter lifespan, imply a higher population turnover rate than in the species with planktonic larval development. The potential for inbreeding is also greater in the larviparous species because the pediveliger young from one brood often settle together (and with the parents) on the same substratum (Turner & Johnson, 1971; Hoagland & Turner, 1981).

The histories of the two introduced populations of *Teredo bartschi* are quite distinct. The New Jersey population, founded in late 1973 or 1974, underwent a severe bottleneck in 1976 when the thermal effluent ceased temporarily during winter (Hoagland & Turner, 1980). After rebounding in 1978, another bottleneck and recovery occurred in 1980, when the thermal effluent was absent from January 5 to July 17. Fluctuations of the native *T. navalis* and *Bankia gouldi* were less severe, but the population of *B. gouldi* has been low (about 10 per panel) since the introduction of *T. bartschi*, compared with the 100 to 1,000 per panel in 1971–1973. The Connecticut population of *T. bartschi* has not suffered dramatic bottlenecks but has remained small (fewer than 10 per panel). The native Florida population of *T. bartschi* is patchy and unpredictable in time and space. A few specimens were collected in panels submerged in August, 1979, and retrieved in October, 1979, near Ft. Pierce, while panels submerged at the same locality in May, 1980, and retrieved in November, 1980, contained hundreds of specimens. Panels submerged less than a mile away contained no *T. bartschi*, and none were found in the Miami area. The distribution of *Lyrodus bipartitus* is more extensive and predictable; the species was found in abundance (200/panel) at both localities and in both years. *Bankia fimbriatula* was rare where it was collected, while *Martesia striata* was very common.

DISCUSSION

Patterns of genetic variation at the various enzyme loci

It is reasonable to ask whether methodology is relevant to the interpretation of genetic variation. Sequential electrophoresis and isoelectric focusing have revealed more genetic variation at some loci than simple starch-gel electrophoresis (Ramshaw *et al.*, 1979). However, there is no relationship between the amount of genetic variation uncovered by these newer techniques and the species (Hamrick *et al.*, 1979) or the biochemical function of the enzymes (Jones, 1980). No systematic bias is expected from using the starch-gel method.

The P values in Table 4 are similar to those summarized by Selander (1976) for marine invertebrates (0.587) and for all invertebrates (0.467). Selander's heterozygosity of 0.147 for marine invertebrates and 0.083 for marine snails compare well with the 0.096 for the 7 pholadacean species, excluding the 2 introduced populations.

Electrophoretic results obtained by Cole & Turner (1978) for pholadaceans are not directly comparable with the data reported here. Their H values were higher, and their P values inconsistent with mine. They analyzed a different set of enzymes for each species and obtained results across all 5 species for only 6 highly polymorphic loci of the 22 examined, accounting for their high H values. A similar explanation might account for the high average H value (0.367) calculated by Wilkins (1975) for 12 marine bivalves. Simon & Archie (1985) demonstrated the necessity of using the same enzymes in any comparison of H values across taxa. However, Archie (1985) also pointed out that certain statistical tests to detect differences in heterozygosity between samples are invalid when the same set of (non-random) loci are used. Generally, low values of $\bar{H}$ and loci fewer than 40 make statistical comparisons difficult.

The amount of genetic variation in the pholadaceans was found to differ in a characteristic way for particular genetic loci. The esterases, peptidases, GPI, PGM, LAP, and MPI were highly polymorphic, as has been found for many organisms (Hamrick *et al.*, 1979; Badino & Sella, 1980). Sarich (1977) claimed that the highly polymorphic enzymes accumulated substitutions at a rate ten times that of the less polymorphic enzymes. He

proposed that rapidly-substituting loci should be more polymorphic than slowly-substituting loci at any instant even if polymorphism is due to neutral allele substitutions. But natural selection on individual enzymes could be responsible for maintaining polymorphism in enzymes whose substrates are variable (Gillespie & Langley, 1974), or in enzymes that participate in regulatory reactions (Johnson, 1976).

The heterozygote deficiencies in Table 3 are large. They show no relationship with species-specific life history patterns or the metabolic functions of the enzymes. The lack of heterozygotes at two loci in the Millstone population of *Teredo bartschi* could be due to separate introductions, or simply to small sample size. No species shows a pattern of complete absence of heterozygotes, decreasing the possibility that obligate self-fertilization or parthenogenesis occurs (Selander & Hudson, 1976). No marine bivalves are known to undergo parthenogenesis. In shipworms, plenty of male gametes are present in all populations studied. Circumstantial evidence for self-fertilization has been reported in shipworms (Eckelbarger & Reish, 1972).

Large heterozygote deficiences have been reported for numerous marine invertebrates, including bivalves. Zouros & Foltz (1984) reviewed the literature and mathematically analyzed the relationship of differential selection in different parts of the life cycle and differential reproduction of heterozygote individuals to heterozygote deficiency. They concluded that, to achieve levels of heterozygote deficiency reported in the literature, selection differentials would have to be very high. Beaumont (1982) and others have suggested a combination of factors could be operating, including inbreeding, selection on juvenile stages, and a Wahlund Effect, although in lab experiments Beaumont *et al.* (1983) implicated selection alone. Inbreeding alone cannot explain the heterozygote deficiencies seen for teredinids, because within a population some loci lack heterozygotes while others are in Hardy-Weinberg equilibrium.

Population subdivision models of heterozygote deficiency have been suggested by Tracey *et al.* (1975). Because many marine populations with large heterozygote deficiency are founded by pelagic larvae, they proposed temporary subpopulations and fine-scale patchiness to explain the genetic structure. Johnson & Black (1984), studying a limpet with planktonic development, suggested that heterozygote deficiency at all 7 loci was due not to temporal variation, but to binomial sampling variance among small local breeding groups plus mixing of larvae on a local scale.

In teredinids, species both with and without planktonic larval dispersal show heterozygote deficiency. Temporal and small-scale local genetic variation could be important for all species of teredinids. Shipworm larvae can travel within an estuary or along a coastline before settlement. It is likely that even pediveligers move far enough to encounter temperature and salinity regimes, with concommitant changes in food and predators, different from those of the parents. But adults also disperse in small groups within driftwood or boats. The extent of the Wahlund Effect may depend upon the life history characteristics of the species, but it could operate in all species.

The presence of null alleles is a possible explanation of heterozygote deficiency, one that is difficult to verify without breeding experiments. Thus, natural selection, null alleles, inbreeding, sampling error, and Wahlund Effects can all contribute to heterozygote deficiency; the values in Table 3 suggest that more than one factor is involved. Mis-identification of specimens can lead to artificially high heterozygote deficiencies if there are loci with fixed alternate alleles in the confounded species. The only species that was difficult to identify was *Lyrodus floridanus*, which is easily confused with *L. pedicellatus* when not brooding young. Since many animals were brooding, and no specimens of *L. pedicellatus* were identified in the material, it is unlikely that those species were confused. Morphologically similar species have been identified electrophoretically; in fact I have found undescribed species of *Crepidula* via electrophoretic analysis (Hoagland, 1984a). In such cases, certain specimens show a consistent pattern; they are fixed for alternate alleles at one or more loci. This situation has not been found in the data set reported upon here.

Correlation of genetic variation with life history and other possible factors

Comparing the genetic variation (Table 4) with life history (Table 6), an imperfect but suggestive trend of greater genetic variation with longer larval life in the plankton can be seen (Fig. 1). If means are calculated sepa-

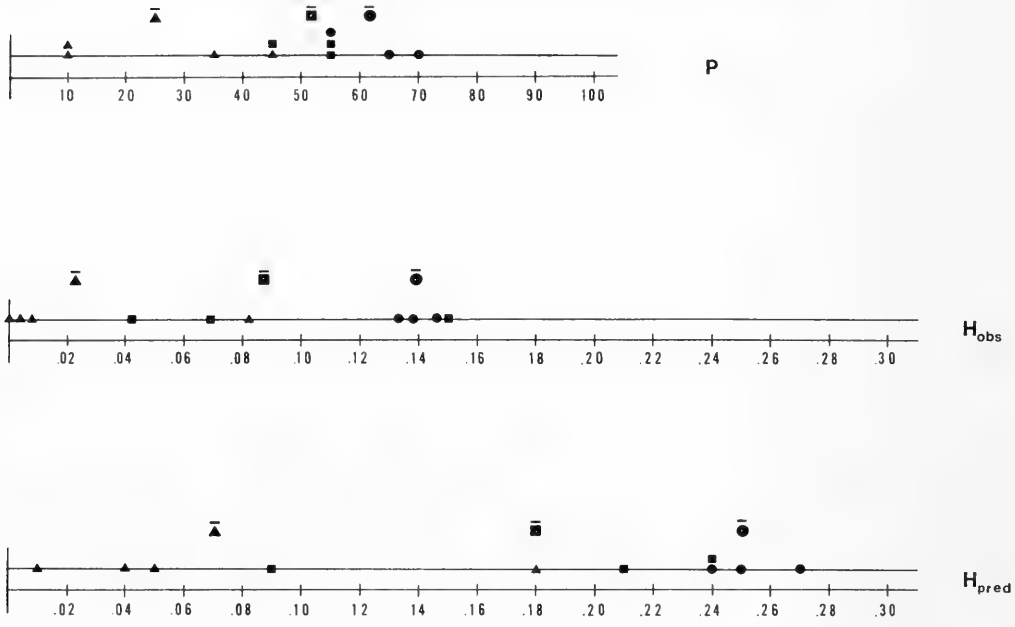


FIG. 1. The values of predicted heterozygosity ($H_{pred.}$), observed heterozygosity ($H_{obs.}$) and percent polymorphic loci (P) for populations of species with the three types of larval development. ▲ = brooded development, release of pediveliger. • = planktonic development, release of eggs and sperm. ■ = mixed brooded and planktonic development, release of straight-hinge larvae. The original data are in Table 4. Averages of data for each larval type are indicated by the symbols with bars above.

rately for the introduced populations, the natural populations of larviparous species, the populations with mixed development, and those with pure planktonic development, these life history categories representing increasing opportunity for dispersal and outbreeding fall in the order of increasing heterozygosity (Table 7). The data suggest that the level of heterozygosity and genetic polymorphism in general may be related to life history characteristics. If so, this could be an example of population structure influencing the level of polymorphism.

Battaglia *et al.* (1978), working with six species of crustaceans, also found that some oviparous species had higher heterozygosities than a brooded species. Unfortunately, information on the type of larval development was not given for all six species. Nevo *et al.* (1984) and Snyder & Gooch (1973) reported a correlation between species heterozygosity and dispersal. Hamrick *et al.* (1979) noticed higher variation in widespread outcrossing and wind-pollinated plants, relative to regionally-distributed plants. They also found that fecundity was positively correlated with genetic variation. Fecundity is positively correlated with both the length of planktonic life and heterozygosity in Pholadacea (Table 6). Lavie & Nevo (1986) attributed higher heterozygosity in *Cerithium scabridum* than in *C. rupestre* to greater niche-width in the former species due to its high intertidal position. However, *C. scabridum* also has higher fecundity, correlated with planktonic larvae lacking in *C. rupestre*. Dispersal rather than niche-width at any one locality might be responsible for the high heterozygosity of populations of *C. scabridum*.

Soulé (1976) suggested that life cycle heterogeneity should be reflected in high genetic variation. If so, planktonic developers should have higher genetic variability than brooders, and this is so. But we might also expect the species with mixed brooded-planktonic development like *Teredo navalis* to have the highest genetic variation; this is not so.

Animals with pelagic larvae are expected to have similar gene frequencies throughout their ranges (Soulé, 1976), yet I found considerable genetic distance between two populations of *T. navalis* (Table 5). Perhaps it is

TABLE 7. Pholadacea. Heterozygosity values averaged over populations with the same type of larval development. Number of populations in parentheses. Data from Table 6.

	H observed	H predicted	P	A
Introduced brooders (2)	.002	.025	10	1.15
Brooders, natural populations (2)	.045	.115	40	2.08
Mixed strategy (3)	.087	.180	52	1.95
Planktotrophic species (3)	.139	.253	63	2.20

due to the patchy distribution of wood substratum in the Northwestern Atlantic, which effectively isolates populations despite the pelagic larvae. Burton (1983) also made the point that substantial genetic differentiation has been observed between populations of marine invertebrates supposed to have high dispersal capabilities.

The proximal cause of greater intrapopulation genetic diversity in populations of species with planktonic larvae has been cited as the larger gene pool (Selander, 1976). The data for pholadaceans are in agreement. The smaller population size of *Bankia fimbriatula* may explain its lower genetic variation compared with *B. gouldi*. Fuller & Lester (1980) showed that heterozygosity was proportional to effective population size as well as to the potential for immigration; Motro & Thomson (1982) showed a large effect of repeated bottlenecks on level of heterozygosity. The high percentage of animals brooding eggs in some populations suggests that *Teredo bartschi* might be a simultaneous hermaphrodite, which would influence the effective population size. Richards *et al.* (1980) found hermaphrodite gonads in a large percentage of *T. bartschi*, unlike *Bankia gouldi* and *T. navalis*, which are usually either male or female. Even if selfing is rare and alternating sexuality is the rule as in *T. navalis* and *B. gouldi* (Hoagland, 1984b), the population structure of *T. bartschi* in which offspring (males) settle on the same wood as parents (females) leads to inbreeding. Dispersal of adults in wood does occur regularly, but large groups of closely-related individuals move together in the same piece of wood. Pediveligers of *T. bartschi* are capable of swimming and crawling before metamorphosis, but field studies in Barnegat Bay indicate that most settle near the parents.

The pholadacean data show that species with brooded development such as *Teredo bartschi* can be widely distributed and have low genetic variability within and between populations yet have isolation of populations and high potential for inbreeding, compared with the planktonic developers. Similarly, Davis *et al.* (1981) found that some widespread, phenotypically uniform species of freshwater mussels (Unionidae) have low genetic variation within and between isolated populations. Selander & Kaufman (1975) and Selander & Hudson (1976) found monomorphism yet good colonization ability in the widespread introduced self-fertilizing land snail *Rumina decollata*; McCracken & Selander (1980) found the same pattern in selfing populations of introduced slugs.

Teredo bartschi fits the "general purpose genotype" model of Selander & Hudson (1976) for species with broad physiological tolerances despite reduced genetic variability. There are no differences in breadth of tolerance in the 10 populations of shipworms that can be related to levels of genetic variation. *T. bartschi* of New Jersey has as broad a tolerance for temperature and salinity as the Florida population (Hoagland, 1986).

There is no detectable relationship between the level of genetic variation and latitude of the pholadacean populations. The three sympatric species of New Jersey differed much more in levels of genetic variation than did the congeners *Bankia gouldi* and *B. fimbriatula*, which are temperate and tropical respectively, but which have similar patterns of larval development and dispersal. Redfield *et al.* (1980) also found no correlation between latitude and genetic variation.

Nelson and Hedgecock (1980) implicated a heterogeneous diet in levels of enzyme heterozygosity. No differences in diet or parasite infection are known that correlate with enzyme polymorphism in the Pholadacea. The anatomical data for the six Teredinidae of this study (Turner, 1966) reveal a similarity in the size of the gill versus the caecum compared with some other teredinid genera, suggesting a similar diet.

Populations introduced in thermal effluents

Nevo *et al.* (1977) found lower heterozygosity for barnacles in a thermal effluent than at a control station. Such a trend is not evident in my data except as due to founder effects and bottlenecking (Table 7). *Teredo navalis* from a thermal effluent actually had a higher heterozygosity than a population from just outside such an effluent (Table 4). There is some evidence that enzymes such as αGPDH in invertebrates have greater variability in thermally variable environments (Johnson, 1976). The thermal effluent of a nuclear power plant is actually a variable environment because of frequent plant outages. Genetic similarity of the introduced populations of *T. bartschi* (Table 5) could confirm the identity of the species but not the pathway of introduction. It is quite likely that the introductions were independently derived from the Floridian or other natural populations.

CONCLUSION

The ecological requirements of the pholadaceans of this study are similar. All face a temporally unstable environment over more than one generation. Some species show high polymorphism within populations, while others are nearly monomorphic as determined by electrophoresis of enzymes. The sympatry of some of the species with differing levels of population heterozygosity is due in part to factors other than the physical environment. The degree of genetic variability is related to the breeding structure and type of larval development and dispersal of the species. Species of Pholadacea with completely planktonic larval development have higher genetic variability, higher fecundity, more constant population size, and probably more outbreeding than do larviparous species. Larviparous species are more likely to be monomorphic through inbreeding and local bottlenecks; their distribution is patchy. I hypothesize that constraints of life history and population structure affect the genetic structure (i.e., level of polymorphism) of populations.

ACKNOWLEDGMENTS

This work was supported by U.S. Nuclear Regulatory Commission contract AT-04-76-347 and a Fleischmann Foundation grant to the Wetlands Institute, Stone Harbor, New Jersey. Laboratory facilities for electrophoretic work were made available by G. M. Davis at The Academy of Natural Sciences of Philadelphia. D. Mook of the Harbor Branch Laboratory, Fort Pierce, Florida, and D. Moore of the Rosenstiel School of Atmospheric and Planetary Science, University of Miami, helped collect specimens. M. Kessen and D. Morgan of the Millstone Environmental Laboratory, Northeast Utilities, made it possible for me to collect specimens near their facility. L. Crocket and J. Selman assisted in laboratory and field work. R. Turner assisted in field work and in identification of specimens; without the foundation of knowledge she has built for the Teredinidae, this work would not have been possible. The manuscript was improved by the criticisms of A. J. Cain, G. M. Davis, M. Itzkowitz, and J. J. Murray.

LITERATURE CITED

AMENT, A., 1979, Geographic variation in relation to life history in three species of the marine gastropod genus *Crepidula*: growth rates of newly hatched larvae and juveniles. *In* STANCYK, S., ed., *Reproductive Ecology of Marine Invertebrates*, p. 61–76. University of South Carolina Press, Columbia, S.C.

ARCHIE, J. W., 1985, Statistical analysis of heterozygosity: data-independent sample comparisons. *Evolution*, 39: 623–637.

AYALA, F. J., HEDGECOCK, D., ZUMWALT, G. S. & VALENTINE, J. W., 1973, Genetic variation in *Tridacna maxima*, an ecological analog of some unsuccessful evolutionary lineages. *Evolution*, 27: 177–191.

AYALA, F. J. & VALENTINE, J. W., 1974, Genetic variability in the cosmopolitan deep-water ophiuran *Ophiomusium lymani*. *Marine Biology*, 27: 51–57.

BADINO, G. & SELLA, G., 1980, Phosphoglucose isomerase variability in sympatric populations of Mediterranean species of *Patella* (Gastropoda, Prosobranchia). *Marine Ecology—Progress Series*, 2: 315–320.

BATTAGLIA, B., BISOL, P.M. & FAVA, G., 1978, Genetic variability in relation to the environment in some marine invertebrates. *In* BATTAGLIA, B. & BEARDMORE, J., ed., *Marine organisms: genetics, ecology and evolution*, p. 53–70. Plenum, N.Y.

BEAUMONT, A. R., 1982, Variations in heterozygosity at two loci between year classes of a population of *Chlamys opercularis* (L.) from a

Scottish sea-loch. *Marine Biology Letters*, 3: 25–34.

BEAUMONT, A. R., BEVERIDGE, C. M. & BUDD, M. D., 1983, Selection and heterozygosity within single families of the mussel *Mytilus edulis* (L.). *Marine Biology Letters*, 4: 151–162.

BEAUMONT, A. R., DAY, T. R. & GADE, G., 1980, Genetic variation at the octopine dehydrogenase locus in the adductor muscle of *Cerastoderma edulis* (L.) and 6 other bivalve species. *Marine Biology Letters*, 1: 137–148.

BERGER, E. M., 1977, Gene-enzyme variation in three sympatric species of *Littorina*. II. The Roscoff population, with a note on the origin of North American *L. littorea*. *Biological Bulletin*, 153: 255–264.

BURTON, R. S., 1983, Protein polymorphisms and genetic differentiation of marine invertebrate populations. *Marine Biology Letters*, 4: 193–206.

COLE, T. & TURNER, R. D., 1978, Genetic relations of deep-sea wood-borers. *American Malacological Union Bulletin* for 1977: 19–25.

CRISP, D. J., 1978, Genetic consequences of different reproductive strategies in marine invertebrates. *In* BATTAGLIA, B. & BEARDMORE, J., ed., *Marine organisms: genetics, ecology, and evolution*, p. 257–274. Plenum, N.Y.

DAVIS, G. M., HEARD, W., FULLER, S. L. H. & HESTERMANN, C., 1981, Molecular genetics and speciation in *Elliptio*, and its relationship to other taxa of North American Unionidae (Bivalvia). *Biological Journal Linnean Society*, 15: 131–150.

DILLON, R.T. & DAVIS, G. M., 1980, The *Goniobasis* of southern Virginia and northwestern North Carolina: electrophoretic and shell morphometric relationships. *Malacologia*, 20: 83–98.

ECKELBARGER, K. J. & REISH, D. J., 1972, A first report of self-fertilization in the wood-boring family Teredinidae (Mollusca; Bivalvia). *Bulletin of the Southern California Academy of Sciences*, 71: 48–50.

FULLER, B. & LESTER, L. J., 1980, Correlations of allozymic variation with habitat parameters using the grass shrimp, *Palaemonetes pugio*. *Evolution*, 34: 1099–1104.

GILLESPIE, J. H. & LANGLEY, C. H., 1974, A general model to account for enzyme variation in natural populations. *Genetics*, 76: 837–848.

HAMRICK, J. L., LINHART, Y. B. & MITTON, J. B., 1979, Relationships between life history characteristics and electrophoretically detectable genetic variation in plants. *Annual Reviews of Ecology and Systematics*, 10: 173–200.

HOAGLAND, K. E., 1983a, Ecological studies of wood-boring bivalves and fouling organisms in the vicinity of the Oyster Creek Nuclear Generating Station. Final Report, Sept. 1, 1976— Dec. 31, 1982. NTIS # NUREG/CR-3446, 173 p.

HOAGLAND, K. E., 1983b, Life history characteristics and physiological tolerances of *Teredo bartschi*, a shipworm introduced into two temperate zone nuclear power plant effluents. *In* SENGUPTA, S. & LEE, S. S., ed., *Waste heat management and utilization*, p. 609–622. Hemisphere Publishing, N.Y.

HOAGLAND, K. E., 1984a, Use of molecular genetics to distinguish species of the gastropod genus *Crepidula* (Prosobranchia: Calyptraeidae). *Malacologia*, 25: 607–628.

HOAGLAND, K. E., 1984b, Use of the terms protandry, protogyny, and hermaphroditism in malacology. *American Malacological Bulletin*, 3: 85–88.

HOAGLAND, K. E., 1986, Effects of temperature, salinity, and substratum on larvae of the shipworms *Teredo bartschi* Clapp and *T. navalis* Linnaeus (Bivalvia: Teredinidae). *American Malacological Bulletin*, 4: 89–99.

HOAGLAND, K. E., CROCKET, L. & TURNER, R. D., 1981, Ecological studies of wood-boring bivalves in the vicinity of Oyster Creek Nuclear Generating Station, Sept. 1, 1979—Feb. 28, 1980. Report to the U.S. Nuclear Regulatory Commission. NTIS # NUREG/CR-1517, 65 p.

HOAGLAND, K. E. & TURNER, R. D., 1980, Range extensions of teredinids (shipworms) and polychaetes in the vicinity of a temperate-zone nuclear generating station. *Marine Biology*, 58: 55–64.

HOAGLAND, K. E. & TURNER, R. D., 1981, Evolution and adaptive radiation of wood-boring bivalves (Pholadacea). *Malacologia*, 21: 111–148.

JOHNSON, G. B., 1976, Genetic polymorphism and enzyme function. *In* AYALA, F. J., ed., *Evolution*, p. 46–59. Sinauer, Sunderland, Mass.

JOHNSON, M. S. & BLACK, R., 1984, The Wahlund effect and the geographical scale of variation in the intertidal limpet *Siphonaria* sp. *Marine Biology*, 79: 295–302.

JONES, J. S., 1980, How much genetic variation? *Nature*, 288: 10–11.

KOEHN, R. K., MILKMAN, R. & MITTON, J. B., 1976, Population genetics of marine pelecypods. IV. Selection, migration, and genetic differentiation in the blue mussel *Mytilus edulis*. *Evolution*, 30: 2–32.

LAVIE, B. & NEVO, E., 1986, Genetic diversity of marine gastropods: contrasting strategies of *Cerithium rupestre* and *C. scabridum* in the Mediterranean Sea. *Marine Ecology—Progress Series*, 28: 99–103.

LEVINTON, J., 1973, Genetic variation in a gradient of environmental variability: marine Bivalvia. *Science*, 180: 75–76.

LEVINTON, J. S., 1975, Levels of genetic polymorphism at two enzyme encoding loci in eight species of the genus *Macoma* (Mollusca: Bivalvia). *Marine Biology*, 33: 41–47.

LEWONTIN, R. C., 1974, *The genetic basis of evolutionary change*. Columbia University Press, N.Y., 346 p.

McCRACKEN, G. F. & SELANDER, R. K., 1980, Self-fertilization and monogenic strains in natural populations of terrestrial slugs. *Proceedings of the National Academy of Sciences*, U.S.A., 77: 684–688.

MOTRO, U. & THOMSON, G., 1982, On heterozygosity and the effective size of populations subject to size changes. *Evolution*, 36: 1059–1066.

NELSON, K. & HEDGECOCK, D., 1980, Enzyme polymorphism and adaptive stragegy in the decapod Crustacea. *American Naturalist*, 116: 238–280.

NEVO, E., 1978, Genetic variation in natural populations: patterns and theory. *Theoretical Population Biology*, 13: 121–177.

NEVO, E., BEILS, A. & BEN-SHLOMO, R., 1984, The evolutionary significance of genetic diversity: ecological, demographic and life history correlates. *In* MANI, G. S., ed., *Evolutionary dynamics of genetic diversity*, *lecture notes in biomathematics*, 53: 13–213. Springer-Verlag, Berlin.

NEVO, E., SHIMONY, T. & LIBNI, M., 1977, Thermal selection of allozyme polymorphism in barnacles. *Nature*, 267: 699–701.

RAMSHAW, J. A. M., COYNE, J. A. & LEWONTIN, R. C., 1979, The sensitivity of gel electrophoresis as a detector of genetic variation. *Genetics*, 93: 1019–1037.

REDFIELD, J. A., HEDGECOCK, D., NELSON, K. & SALINI, J. P., 1980, Low heterozygosity in tropical marine crustaceans of Australia and the trophic stability hypothesis. *Marine Biology Letters*, 1: 303–313.

RICHARDS, B. R., BELMORE, C. I., HILLMAN, R. E. & MACIOLEK, N. J., 1980, Woodborer study associated with the Oyster Creek generating station. Annual Report for the period Dec. 1, 1978— Nov. 30, 1979, to Jersey Central Power and Light Co., Feb. 29, 1980. 18 p. and 3 appendices.

SARICH, V. M., 1977, Rates, sample sizes, and the neutrality hypothesis for electrophoresis in evolutionary studies. *Nature*, 265: 24–28.

SELANDER, R. K., 1976, Genic variation in natural population. *In* AYALA, F. J., ed., *Molecular evolutions*, p. 21–45. Sinauer, Sunderland, Mass.

SELANDER, R. K. & HUDSON, R. O., 1976, Animal population structure under close inbreeding: the land snail *Rumina* in southern France. *American Naturalist*, 110: 695–718.

SELANDER, R. K. & KAUFMAN, D. W., 1973, Genic variability and strategies of adaptation in animals. *Proceedings of the National Academy of Sciences*, U.S.A., 70: 1875–1877.

SELANDER, R. K. & KAUFMAN, D. W., 1975, Genetic population structure and breeding systems. *In* MARKERT, C. L., ed., *Isozymes IV: genetics and evolution*, p. 27–48. Academic Press, N.Y.

SIMON, C. & ARCHIE, J., 1985, An empirical demonstration of the lability of heterozygosity estimates. *Evolution*, 39: 463–466.

SNYDER, J. P. & GOOCH, J. L., 1973, Genetic differentiation in *Littorina saxatilis* (Gastropoda). *Marine Biology*, 22: 177–182.

SOULÉ, M., 1976, Allozyme variation: its determinants in space and time. *In* AYALA, F. J., ed., *Molecular evolution*, p. 60–77. Sinauer, Sunderland, Mass.

TRACEY, M. L., NELSON, K., HEDGECOCK, D., SHLESER, R. A. & PRESSICK, M. L., 1975, Biochemical genetics of lobsters: genetic variation and the structure of American lobster (*Homarus americanus*) populations. *Journal of the Fisheries Research Board of Canada*, 32: 2091–2101.

TURNER, R. D., 1966, *A survey and illustrated catalogue of the Teredinidae*. Museum of Comparative Zoology, Harvard University, Cambridge, Mass., 265 p.

TURNER, R. D., 1973, Wood-boring bivalves, opportunistic species in the deep sea. *Science*, 180: 1377–1379.

TURNER, R. D. & JOHNSON, A. C., 1971, Biology of marine wood-boring molluscs. *In* JONES, E.B.G. & ELTRINGHAM, S.K., ed., *Marine borers, Fungi, and fouling organisms of wood*, p. 259–301. Organization for Economic Cooperation and Development, Paris.

VALENTINE, J. W., 1976, Genetic strategies of adaptation. *In* AYALA, F. J., ed., *Molecular evolution*, p. 78–94. Sinauer, Sunderland, Mass.

WILKINS, N. P., 1975, Genic variability in marine Bivalvia: implications and applications in molluscan mariculture. *Proceedings of the 10th European Symposium on Marine Biology*, 1: 549–563.

WILKINS, N. P. & O'REGAN, D., 1980, Genetic variation in sympatric sibling species of *Littorina*. *Veliger*, 22: 355–359.

ZOUROS, E. & FOLTZ, D. W., 1984, Possible explanations of heterozygote deficiency in bivalve molluscs. *Malacologia*, 5: 583–591.

APPENDIX. Allele frequencies for ten populations of Pholadacea. *T.b.* = *Teredo bartschi*; *T.n.* = *Teredo navalis*; *L.f.* = *Lyrodus floridanus*; *L.b.* = *Lyrodus bipartitus*; *B.g.* = *Bankia gouldi*; *B.f.* = *Bankia fimbriatula*; *M.s.* = *Martesia striata*. Localities as in Table 1.

		Populations									
Locus	Allele	*T.b.* N.J.	*T.b.* Conn.	*T.b.* Fla.	*T.n.* N.J.	*T.n.* Conn.	*L.f.* Miami	*L.b.* Ft. Pierce	*B.g.* N.J.	*B.f.* Fla.	*M.s.* Fla.
AcPh	98	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
	100	1.00	1.00	1.00	0.00	1.00	0.00	0.09	1.00	0.00	1.00
	103	0.00	0.00	0.00	0.00	0.00	1.00	0.91	0.00	1.00	0.00
AO I	85	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
	97	0.00	0.42	0.00	0.00	0.73	0.43	0.00	0.29	0.00	0.00
	100	1.00	0.58	1.00	1.00	0.27	0.57	0.82	0.71	0.85	0.00
	102	0.00	0.00	0.00	0.00	0.00	0.00	0.18	0.00	0.15	0.00
AAT	78	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08
	80	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.22
	82	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.70
	93	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.86	0.05	0.00
	95	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.69	0.00
	97	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.26	0.00
	99	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
	100	1.00	1.00	1.00	1.00	1.00	0.00	0.00	0.00	0.00	0.00
EST NA I	93	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.98
	95	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02
	98	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	100	0.96	1.00	0.98	0.96	0.50	0 96	1.00	0.16	0.00	0.00
	102	0.02	0.00	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.00
	103	0.00	0.00	0.00	0.04	0.00	0.04	0.00	0.84	1.00	0.00
	104	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
EST NA II	98	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.68
	100	0.96	0.13	0.00	0.00	0.00	0.00	0.90	0.74	1.00	0.32
	101	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	102	0.04	0.79	1.00	0.84	0.45	0.04	0.05	0.26	0.00	0.00
	103	0.00	0.00	0.00	0.08	0.00	0.96	0.00	0.00	0.00	0.00
	105	0.00	0.00	0.00	0.08	0.55	0.00	0.00	0.00	0.00	0.00
GPI I	100	1.00	1.00	0.47	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	102	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.30	0.05
	103	0.00	0.00	0.53	0.00	0.00	0.00	0.63	0.00	0.00	0.00
	105	0.00	0.00	0.00	0.51	0.19	0.20	0.37	0.57	0.70	0.50
	107	0.00	0.00	0.00	0.34	0.74	0.76	0.00	0.02	0.00	0.35
	109	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.07
	111	0.00	0.00	0.00	0.06	0.07	0.00	0.00	0.36	0.00	0.03
	115	0.00	0.00	0.00	0.09	0.00	0.00	0.00	0.05	0.00	0.00
HEX	91	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.90
	94	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10
	95	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00
	97	0.00	0.00	0.02	0.73	0.94	0.04	1.00	0.68	0.35	0.00
	100	1.00	1.00	0.98	0.27	0.02	0.96	0.00	0.25	0.65	0.00
	102	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00
IsDH I	94	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
	100	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00
IsDH II	95	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
	97	0.00	0.00	0.00	0.00	0.00	0.03	0.73	0.00	0.00	0.00
	100	1.00	1.00	1.00	0.92	0.00	0.97	0.27	0.00	0.54	0.00
	102	0.00	0.00	0.00	0.08	1.00	0.00	0.00	0.00	0.00	0.00
	103	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.46	0.00
	null	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00

APPENDIX (Continued)

Locus	Allele	Populations									
		T.b. N.J.	*T.b.* Conn.	*T.b.* Fla.	*T.n.* N.J.	*T.n.* Conn.	*L.f.* Miami	*L.b.* Ft. Pierce	*B.g.* N.J.	*B.f.* Fla.	*M.s.* Fla.
LDH	96	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
	97	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
	100	1.00	1.00	1.00	0.00	0.00	1.00	0.00	0.00	0.25	0.00
	101	0.00	0.00	0.00	1.00	1.00	0.00	0.00	1.00	0.75	0.00
LAP	96	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.07	0.00	0.00
	99	0.00	0.00	0.00	0.03	0.01	0.00	0.00	0.00	0.00	0.00
	100	1.00	0.00	0.09	0.64	0.34	0.00	0.00	0.51	0.56	0.00
	101	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.00
	102	0.00	1.00	0.00	0.10	0.15	0.02	0.28	0.26	0.44	0.13
	103	0.00	0.00	0.91	0.00	0.01	0.00	0.00	0.00	0.00	0.00
	104	0.00	0.00	0.00	0.12	0.48	0.98	0.50	0.16	0.00	0.00
	105	0.00	0.00	0.00	0.04	0.01	0.00	0.09	0.00	0.00	0.28
	106	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.09
	108	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.50
MPI	92	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.09	0.15	0.00
	94	0.00	0.00	0.00	0.19	0.02	0.70	0.36	0.66	0.65	0.00
	96	0.00	0.00	0.00	0.25	0.73	0.00	0.08	0.14	0.00	0.00
	98	0.00	0.00	0.00	0.45	0.25	0.23	0.38	0.11	0.20	0.11
	100	1.00	1.00	0.98	0.11	0.00	0.07	0.18	0.00	0.00	0.16
	102	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.73
MDH I	95	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.98	0.00	0.00
	97	0.00	0.00	0.00	1.00	1.00	0.00	0.03	0.00	0.00	0.00
	100	1.00	1.00	1.00	0.00	0.00	1.00	0.00	0.02	1.00	0.00
	101	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	103	0.00	0.00	0.00	0.00	0.00	0.00	0.91	0.00	0.00	0.00
	104	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.98
	105	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00
	109	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02
Pep G II	98	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	100	1.00	1.00	0.96	0.25	0.36	0.00	0.00	0.03	0.21	0.00
	103	0.00	0.00	0.00	0.00	0.02	0.00	0.08	0.50	0.69	0.54
	105	0.00	0.00	0.00	0.00	0.58	1.00	0.82	0.16	0.06	0.28
	106	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00
	107	0.00	0.00	0.00	0.75	0.04	0.00	0.00	0.31	0.04	0.12
	109	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.06
Pep G III	90	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.09	0.00	0.00
	93	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.11	0.00	0.00
	95	0.00	0.00	0.00	0.00	0.28	0.00	0.00	0.14	0.00	0.10
	98	0.00	0.00	0.00	0.24	0.20	0.00	0.00	0.66	0.00	0.45
	100	1.00	1.00	1.00	0.76	0.38	1.00	0.00	0.00	0.00	0.37
	102	0.00	0.00	0.00	0.00	0.12	0.00	0.00	0.00	0.00	0.00
	103	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.88	0.00
	104	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08
	105	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.00
PGM	95	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02
	97	0.00	0.00	0.00	0.05	0.08	1.00	0.00	0.09	0.08	0.11
	100	1.00	1.00	1.00	0.11	0.32	0.00	0.85	0.09	0.50	0.09
	102	0.00	0.00	0.00	0.69	0.56	0.00	0.13	0.75	0.08	0.78
	103	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00
	104	0.00	0.00	0.00	0.15	0.04	0.00	0.00	0.07	0.30	0.00
	106	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00

APPENDIX (Continued)

Locus	Allele	Populations									
		T.b. N.J.	*T.b.* Conn.	*T.b.* Fla.	*T.n.* N.J.	*T.n.* Conn.	*L.f.* Miami	*L.b.* Ft. Pierce	*B.g.* N.J.	*B.f.* Fla.	*M.s.* Fla.
6-PGD	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00	0.92
	101	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	0.00
	105	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08
SoDH	94	0.00	0.00	0.00	0.86	1 00	0.06	1.00	0.03	1.00	0.00
	98	0.00	0.00	0.00	0.14	0.00	0.94	0.00	0.83	0.00	0.80
	100	1.00	1.00	1.00	0.00	0.00	0.00	0.00	0.14	0.00	0.20
SOD I	95	0.00	0.00	0.00	1.00	1.00	0.00	0.00	0.00	0.00	0.00
	100	1.00	1.00	0.98	0.00	0.00	1.00	1.00	0.96	1.00	0.00
	102	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.04	0.00	1.00
	106	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
XDH	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Revised Ms. accepted 10 April 1986

MALACOLOGIA, 1986, 27(2): 341–355

ENVIRONMENT AND DIVERSITIES OF FOREST SNAIL FAUNAS FROM COASTAL BRITISH COLUMBIA

R. A. D. Cameron

Department of Extramural Studies, University of Birmingham, P. O. Box 363, Birmingham B15 2TT United Kingdom

ABSTRACT

The snail faunas of 38 lowland forest sites from Vancouver Island, the lower Frazer Valley and the Chilliwack Valley, British Columbia, are described and related to environmental variation between sites.

Characters of the litter and soil, and the associated vegetation appear to explain most of the variation in diversity and abundance of snails between sites. Sites with mor litter, usually dominated by Douglas Fir, and with dry soils, have impoverished faunas in which only five species are found frequently. Sites with mull litter and damper soils, in which Western Red Cedar and various hardwoods are important, are much richer, with 14 species occurring frequently, and 26 species recorded overall.

Successional status and disturbance by logging or burning have lesser effects, involving only a few species, except where disturbance is very recent.

The faunas are discussed in a regional context, and compared with those from temperate forests elsewhere. Diversity is greater than in most other coniferous forests, but less than in the richest deciduous forests. These differences are accompanied by differences in the range of morphology of snails shown, a study on which is forthcoming.

INTRODUCTION

Studies on the land molluscs of the Pacific North-West of North America have been concerned with taxonomy and geographical distribution (Henderson, 1929, 1936; Pilsbry, 1939–1948; Branson, 1977) and ecological information is scanty, except for some introduced species (Rollo & Wellington, 1975; Roth & Pearce, 1984).

The forests of the coastal Pacific North-West occur in an area with a mild oceanic climate favourable to snails. This climate is generally similar to that of areas originally occupied by temperate deciduous forests in western Europe, except that rainfall is more seasonal, summers being drier (Waring & Franklin, 1979). This pattern of precipitation may explain the predominance of conifers. The climate, the infrequency of natural fires, and the quality of litter produced by some of the dominant species may account for the unusually high botanical diversity for coniferous forest (Franklin & Dyrness, 1973).

This study surveys snail faunas from forests on a variety of soils and in different successional states. It examines environmental correlations with diversity, and compares the diversity of snails found with that found in other temperate forest regions. A later paper (Cameron, in preparation) will discuss the range of morphological types within these faunas, and compare it with the ranges found elsewhere, as an indication of the extent to which similar niches are occupied.

METHODS

Sampling of molluscs

Sample sites were confined to areas of relatively uniform vegetation within a 30 × 30 m square. Within this, molluscs were hand-collected for one hour, paying particular attention to logs, rocks and tree-trunks. Litter was collected in small amounts from all over the site, to a total volume of about 5 litres. This litter was bagged, returned to the laboratory, and passed through a coarse mesh sieve to remove large debris, any snails seen being removed. The residue was oven-dried for 24 hr and immersed in water. Floating material was removed, re-dried and passed through a graded series of sieves. Material passing through the smallest mesh (0.5 mm), was

discarded, and the remaining material searched for snails under bright lights.

This method reliably extracts intact shells (Cameron & Morgan-Huws, 1975; Cameron, 1982). Snails alive at the time of sampling dry out, and air in all intact shells causes them to float. Slugs are lost in this process, and since searching in dry weather is also inadequate (Wäreborn, 1969), slug records are not analysed in detail.

Site descriptions

Records were made at each site of the presence or absence of all canopy tree species, and of shrub and field-layer species chosen for conspicuousness, frequent occurrence and ease of identification. Since each site was not searched exhaustively, recorded absence may include rarity or unusual inconspicuousness. For presentation and analysis all species recorded in less than one fifth of the sites for which full records were made have been omitted.

Dominance in the canopy was also noted. At some sites, two or more species were recorded as co-dominant.

Litter was recorded as mor, mull or intermediate, the latter category including moders, and cases where the litter showed great variation within the site (Klinka *et al.*, 1981). Soil moisture was ranked subjectively by feel on a five point scale, later condensed to three categories for analysis. There was virtually no precipitation during the sample period (August 14–27, 1984).

Timing of major disturbance, such as logging or crown fires, was estimated on the sizes of the dominant trees. For analysis, disturbance has been reduced to three categories: disturbed in last 40 years, disturbed between 40 and 100 years ago, any disturbance more than 100 years ago. Many sites in the last category had trees 300 or more years old. Lesser disturbances, such as ground fires, could have escaped detection.

NOMENCLATURE AND IDENTIFICATION OF MOLLUSCS

With the few exceptions noted below, nomenclature follows Branson (1977). The great majority of specimens presented no difficulty in identification; Pilsbry (1948) and Pearce (in preparation) were used as aids.

Retinella electrina and *R. binneyana occidentalis* are recorded in the region (Henderson, 1929; Pilsbry, 1948). Their status seems somewhat unclear, and in the past they have been ascribed to European species now placed in the genus *Nesovitrea* (Waldén, 1966). All specimens found in this study have been ascribed to *binneyana occidentalis*, and I have followed Waldén in using the genus *Nesovitrea*.

Branson (1975) refers specimens from the Olympic Peninsula, Washington, which resemble *Striatura pugetensis* to *Radiodiscus hubrichti*. This ascription is rejected by Solem (1977) on the basis of shell microsculpture, and all shells of this type have been identified as *S. pugetensis*.

A large species of *Vertigo* was found at sites 27 and 28 near Lake Horne, Vancouver Island. The specimens, which are clearly distinct from the *V. columbiana* and *V. andrusiana* found elsewhere, have been provisionally identified as *V. rowelli*, which is, however, not recorded north of Oregon (Pilsbry, 1948).

Haplotrema sportella was generally easily distinguished from *H. vancouverense* by small size (11–16 mm diameter), the marked convex depression of the upper surface of the last whorl at the mouth, and by pronounced radial ribbing. At site 7, where *H. vancouverense* was not found, it retained the shell characters, but was much larger (19–22 mm diameter), overlapping the range of *H. vancouverense*.

Specimens of all species collected are held by the author.

SAMPLE SITES AND THEIR CHARACTERISTICS

Fig. 1 shows the distribution of sample sites, which range from the west coast of Vancouver Island to the Chilliwack Valley. All sites are below 350 m above sea level and lie in the Coastal Western Hemlock and Coastal Douglas Fir zones of Krajina (1969), although those in the extreme W are in the Sitka Spruce zone differentiated by Franklin & Dyrness (1973). Precipitation ranges from 1200 to 3000 mm annually (Anon., 1982); most lie in the 1600–2000 mm range characteristic of the transition between the Hemlock and Douglas Fir zones (Krajina, 1969).

The sites are in no sense a random selection of stands. They were chosen to give a range of successional and edaphic status,

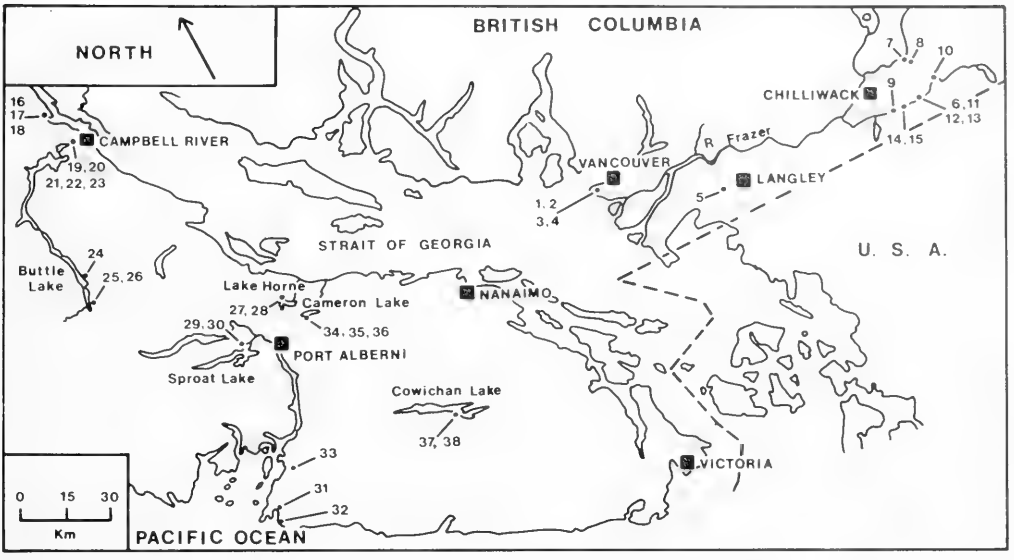


FIG. 1. Map of the lower Frazer valley and south Vancouver Island, showing the distribution of sample sites.

and in particular to include examples of bottomlands and limestone substrate on which mull litter might be found.

Four sites out of the 38 sampled had either suffered gross recent disturbances or were without full canopy cover. Two, sites 6 and 13, were on limestone crags with scrub by roadsides in the Chilliwack Valley, one, site 23, was on a grassy bluff partly overhung by Douglas Fir, and the last, site 24, was in a stand of large Douglas Fir under which there had been an intense ground fire in the last twelve months. These sites are omitted from the vegetation analysis, and their snail faunas are considered separately in some analyses. Full environmental data for the remaining 34 sites are given in Appendix A.

Given the limited and partly subjective nature of the environmental data, sophisticated statistical analysis has not been attempted. Table 1 shows strong associations between mor litter, dry soils and dominance of Douglas Fir, between intermediate litter and soil moisture and dominance by Red Alder (sometimes with Douglas Fir or Sitka Spruce), and between mull litter, damp soil and dominance by a number of combinations of canopy species, always including Western Red Cedar, Large-leaved Maple or Cottonwoods.

Associations between recorded species have been estimated using Fager's Index (Southwood, 1966). Fig. 2 shows these associations as a dendrogram. Two groups of loosely associated species can be identified. Fig. 3 shows the association of each species (and of canopy dominants) with litter type. Members of one group (A) associate with mor litter, those in the other (C) with mull litter. In all but two cases, associations with intermediate litter are intermediate between those with mor and mull. The two exceptions, occurrence of Western Hemlock and dominance by Red Alder, relate to disturbance (Table 2). Red Alder is a characteristic pioneer, while Western Hemlock is the presumed climax dominant (Krajina, 1969; Franklin & Dyrness, 1973). Their associations are concordant with their successional status.

These patterns of site characteristics are concordant with the pattern of plant associations in the region (Krajina, 1969; Franklin & Dyrness, 1973). Moisture and nutrient gradients are the prime determinants of community composition, with Douglas Fir and *Gaultheria shallon* characterizing dry, oligotrophic sites, and Cedar and *Polystichum munitum* the damper and more enriched ones.

Most sites investigated have some degree of disturbance and even those not obviously logged are likely to have been subject to occasional wildfires (Franklin & Hemstrom, 1981). Mueller-Dombois (1965) shows that the understory and field layer species compositions associated with particular edaphic conditions are not destroyed by logging, and the association of Cedar, Large Leaved Ma-

TABLE 1. Numbers of sites with particular combinations of soil, litter and canopy dominants. For Dominants, A = Douglas Fir, Sitka Spruce and any combination of those with Red Alder. Alder = Dominated by Red Alder alone. B = any combination of Dominants which includes Western Red Cedar, Large-leaved Maple or Cottonwoods.

	Litter				Litter				Soil		
Soil	Mor	Int	Mull	Dominants	Mor	Int.	Mull	Dominants	Dry	Int.	Damp
Dry	6	0	0	A	8	5	0	A	6	5	2
Int.	1	5	2	Alder	0	4	1	Alder	0	2	3
Damp	1	4	15	B	0	0	16	B	0	1	15

ple and Cottonwoods with favourable edaphic conditions is widespread (Franklin & Dyrness, 1973; Franklin & Hemstrom, 1981).

On this basis the sites have been allocated to categories by litter type for analysis of snail faunas but some attention is given to disturbance and moisture regime in particular instances.

THE MOLLUSCAN FAUNA

Appendix B gives the numbers of each snail species found at each site and the presence or absence of slugs. Twenty-six species of snail and 7 of slug were recorded in the study. Numbers are based on specimens alive at time of sampling, and obviously fresh shells. Old, badly worn shells (usually broken) are excluded. They constituted only a small proportion of finds.

Tables 3 and 4 show the relationships of snail diversity and numbers to litter type for the 34 sites in the main series. Since neither numbers of species nor of individuals are normally distributed, medians and ranges are used in preference to means and standard errors.

Sites with mor litter have fewest species, and fewest individuals overall and species by species, while those with mull litter have the most. Five species occur in more than half the mor sites, while 14 do so in the mull. No species is characteristic of either mor or intermediate litter, but 10 species are found only in mull sites. Differences in median number of species in each litter type are highly significant, both overall (Kruskall-Wallis test, $p < 0.001$) and in paired comparisons (Mann-Whitney test, $p<0.001$ in each case).

Similarities between snail faunas at each site are estimated using Sorensen's Index (Southwood, 1966). Fig. 4 shows relationships between sites as a dendrogram, and Fig. 5 orders similarities with a typical mor and a typical mull site. Associations of sites related closely to litter type, and by inference, to the associated vegetation. Sites closely associated with those of a different litter type have either the highest (mor and intermediate) or lowest (mull) diversities for their type.

Figs. 3 and 4 include the four aberrant sites. The sites from limestone crags (6 and 13) associate completely with the mull sites, showing no reduction in diversity. The other two (23 and 24) are more isolated. Site 7 also stands apart, though clearly closest to the mull series to which it belongs.

A few species show signs of association with environmental factors other than litter

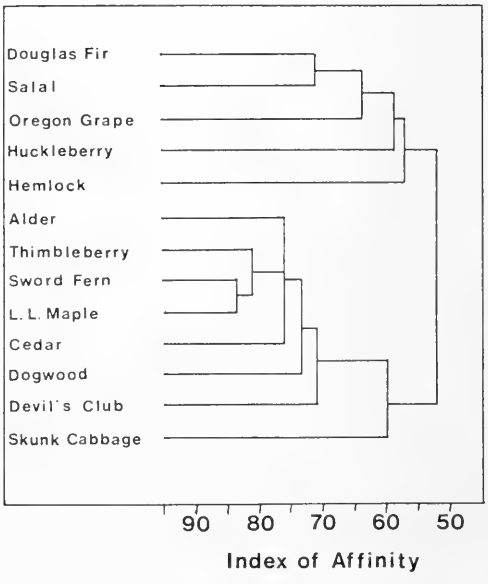


FIG. 2. Dendrogram of associations between plant species, links made with closest member of each group. Scientific names of plants are given in Appendix A.

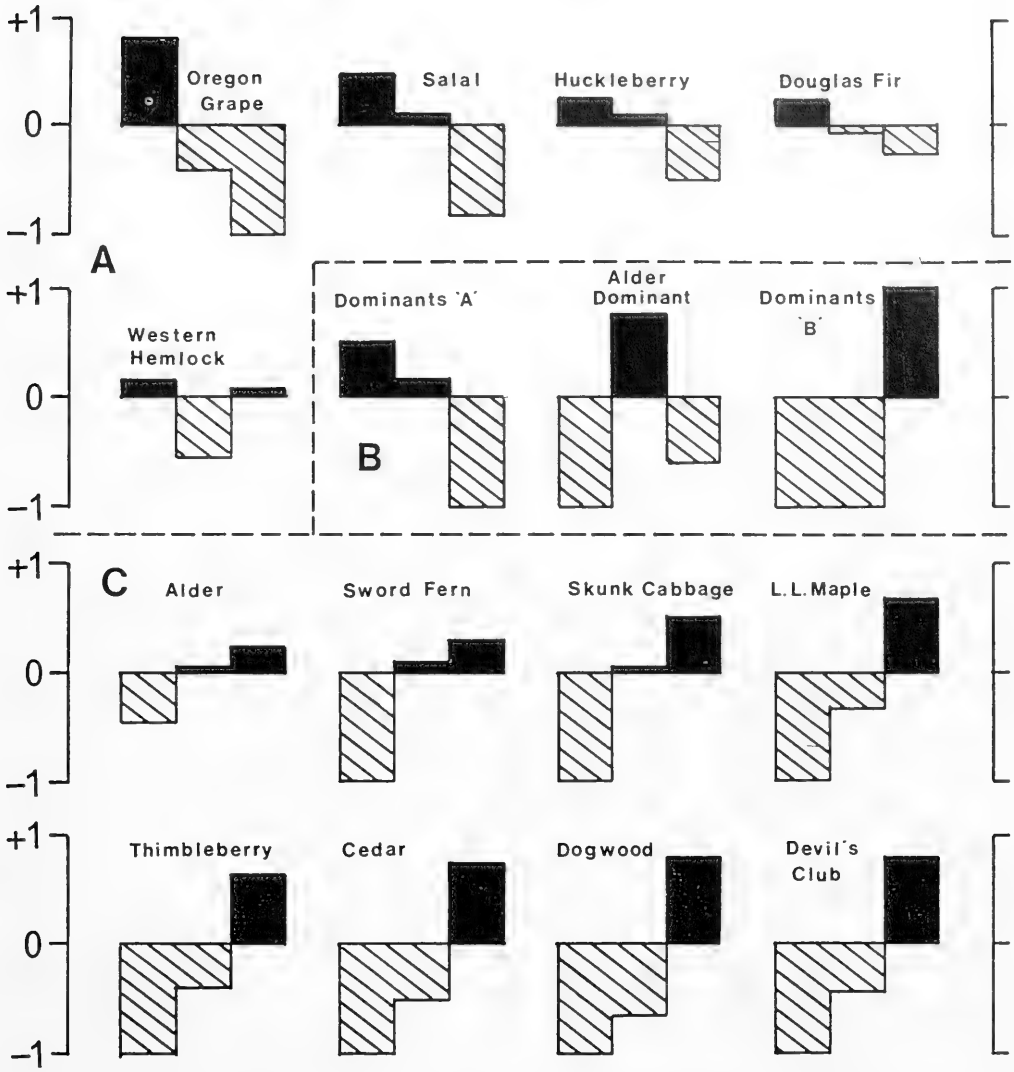


FIG. 3. Frequency distributions of plant species occurrence and dominance as a proportion of maximum deviation from random expectation. Section A: occurrences associated with mor litter: Section B: Canopy dominance by 'A,' Douglas Fir, Sitka Spruce or either with Alder; Alder: Red Alder alone. 'B,' any combination including Cedar, Maple or Cottonwood. Section C: occurrences associated with mull litter. The left column for each diagram indicates the association with mor litter, the centre column the association with intermediate litter, and the right association with mull litter.

type. *Triodopsis germana* shows a stronger association with damp soil than with mull litter, while *Carychium occidentale* and to a lesser extent *Monadenia fidelis* occur less often in the more recently disturbed sites, even within the mull litter series. *Microphysula cookei* was found only at one site in the main series, but at three of the disturbed sites. These sites have in common the presence of exposed limestone rock, which, however, also occurs at site 28 in which *M. cookei* was not found.

A number of other species occur at only a few sites. More samples would be needed to determine any geographical or habitat patterns, although the restriciton of *Allogona townsendiana* to the vicinity of the Chilliwack Valley is in accord with its previously known

TABLE 2. Numbers of sites containing Alder and Hemlock, classified by time since last major disturbance.

	Disturbance occurred			
Sites with	A less than 40 yr ago	B 40–100 yr ago	C More than 100 yr ago	χ^2 A+B:C
Alder	4	10	8	4.63 $P < 0.05$
Hemlock	0	1	8	7.40 $P < 0.01$
Total sites	5	12	17	

TABLE 3. Medians and ranges for numbers of species and individuals of snails found in sites with different litter types, and the number of species occurring in given frequency ranges.

		No. spp. per site		No. individs. per site		No. spp. occurring in			
	n	Median	Range	Median	Range	100%	50%–99%	<50%	Total
Mor	8	5.5	5–7	28	17–55	3	2	4	9
Intermediate	9	10.0	7–11	57	22–170	4	6	6	16
Mull	17	15.0	11–17	151	67–226	6	8	12	26

distribution in British Columbia (Pilsbry, 1948).

Amongst the slugs, *Ariolimax columbianus* was recorded at nearly all sites, and was usually abundant. The introduced *Arion ater* was found in the Chilliwack Valley, and at sites close to Vancouver. *Deroceras laeve* shows a pronounced association with damp soil. Other species were recorded too infrequently to interpret.

DISCUSSION

The regional context

None of the species recorded here, with the exception of *Vertigo rowelli* (see above), are outside their previously known range. Many other species are known from the region. Many of these are characteristic of higher altitudes, of wetlands, or of open habitats. (Henderson, 1929, 1936; Pilsbry, 1948; Branson, 1977). In open and disturbed sites there are a number of introduced species, of which only *Arion ater* appears to have penetrated the more natural sites studied here, although *Vallonia pulchella* is also recorded from one site (Branson, 1977; Rollo & Wellington, 1975; Roth & Pearce, 1984). *Cionella lubrica* was found at four sites. Although native, it is frequently associated with disturbance, and may have been spread locally by human activity (Roth & Pearce, 1984).

The faunas described here are very similar to those recorded by Myers (1972) in a small but detailed study in the Western Cascades of Washington, and to those recorded by Branson (1977) on the Olympic Peninsula, if comparisons are restricted to forested sites below 500 m above sea level. Pearce (in preparation) records a similar set of species from Thurston County, Puget Sound, Washington.

Some species found here have wide geographical ranges, with some being Holarctic (Pilsbry, 1948; Kerney & Cameron, 1979). There are some affinities with faunas from the mountain belt, and with those from Albertan prairie parkland to the east of the continental divide (Karlin, 1961; Platt, 1980; Boag & Wishart, 1982; Van Es & Boag, 1981). Both in Alberta and further south, there are some species, especially *Discus cronkhitei*, *Zonitoides arboreus* and *Vitrina alaskana* which are both abundant and widespread, but which are rare and restricted in the Pacific North-West, at least at lower altitudes (this study; Branson, 1977).

Diversity, abundance and the environment

The results of this study are in agreement with the generally observed pattern of high

TABLE 4. Frequency of occurrence and median numbers (where present) for each species of snail in sites classified by litter type. A = species occurring in all or nearly all sites; B = species occurring in all litter types, but least frequent in mor; C = species absent from sites with mor litter; D = species found only in sites with mull litter.

	Mor n = 8		Intermediate n = 9		Mull n = 17		
	% occurrence	Median no. where present	% occurrence	Median no. where present	% occurrence	Median no. where present	Comments
A							
Haplotrema vancouverense	100	3	100	3	94	5	Not at Popkum
Pristiloma lansingi	100	4	100	6	100	17	
Striatura pugetensis	100	6	100	11	100	25	
Punctum randolphii	87	6	89	10	100	11	
B							
Vespericola columbiana	50	2.5	100	3	100	6	
Haplotrema sportella	37	2	89	3	88	6	
Nesovitrea binneyana	37	2	67	2.5	100	4	
Planogyra clappi	37	2	56	6	100	11	
Columella edentula	25	1	56	2	94	4	
C							
Vertigo columbiana	0	—	89	3	88	4	
Triodopsis germana	0	—	33	2	71	3	
Euconulus fulvus	0	—	22	1	76	3	
Carychium occidentale	0	—	22	26	59	22	
Monadenia fidelis	0	—	11	1	82	3	
Zonitoides arboreus	0	—	11	1	29	1	
Pristiloma stearnsi	0	—	11	2	41	6	Vancouver Island only
D							
Punctum conspectum	0	—	0	—	35	4	
Discus cronkhitei	0	—	0	—	18	6	Cameron Lake V.I. only
Cionella lubrica	0	—	0	—	18	2	
Allogona townsendiana	0	—	0	—	12	2	Chilliwack only
Pristiloma johnsoni	0	—	0	—	12	2	Vancouver Island only
Vertigo andrusiana	0	—	0	—	12	7.5	Vancouver Island only
Vertigo cf. *rowelli*	0	—	0	—	12	2.5	Lake Horne, V.I. only
Microphysula cookei	0	—	0	—	6	10	Also in disturbed sites
Vitrina alaskana	0	—	0	—	6	5	Popkum
Vallonia pulchella	0	—	0	—	6	3	Popkum

diversity and abundance associating with damp and nutrient-rich substrates in temperate forests (Burch, 1955; Wäreborn, 1969; Cameron, 1973, Coney *et al.*, 1982). The faunas on mor and intermediate litter are merely impoverished versions of those found on mull. In some other studies of temperate forest, where moisture and litter type are not so closely associated, a few species are characteristic of more oligotrophic sites (Cameron, 1973; Coney *et al.*, 1982).

Soil, litter and vegetational characters are usually strongly associated, and even sophisticated multivariate analysis cannot certainly determine causal relationships with the snail fauna. Soil moisture and calcium availability can affect snails directly (Boycott, 1934; Wäreborn, 1969, 1982). Vegetation may have less direct effects, through the creation of shelter, through variation in the quantity and availability of calcium salts in the litter, and through the effects of litter chemistry on soil structure and content (Wäreborn, 1969, 1979; Coney *et al.*, 1982).

No chemical analyses of litter were carried out in this study. Myers (1972) gives some data on litter under pure stands of Cedar, Red Alder and Douglas Fir. As in this study, snail faunas were most diverse and abundant under Cedar, and least under Douglas Fir, and this correlates with moisture content and chemical composition.

Coniferous forests are often reported as having very impoverished and sparse mollus-

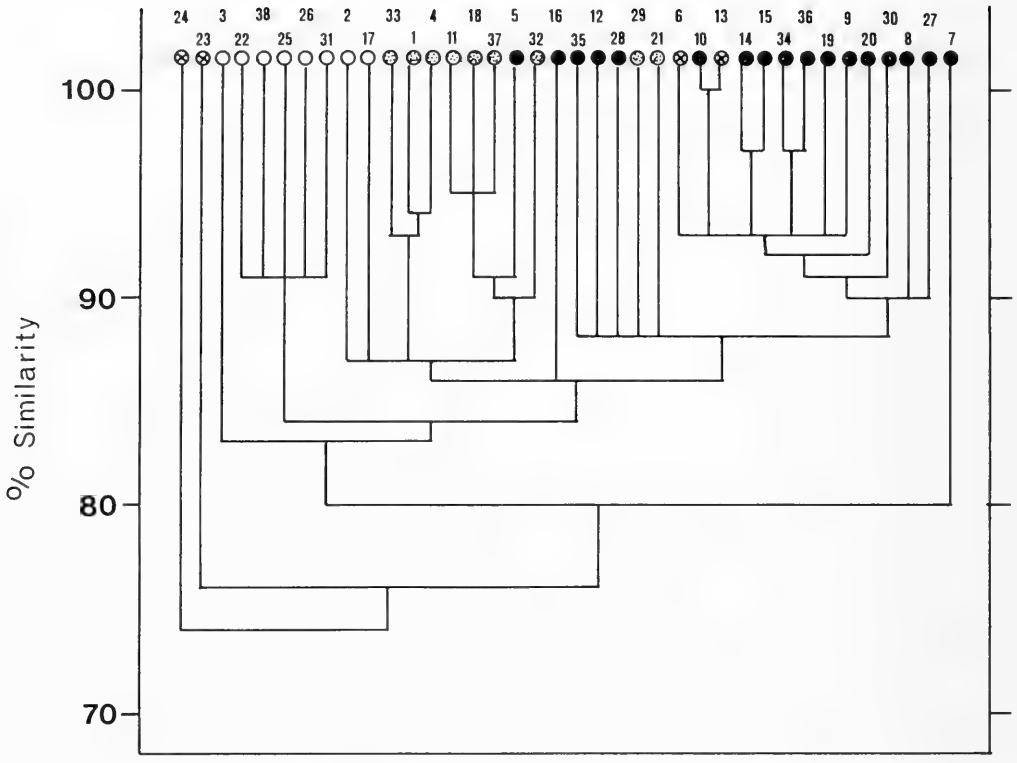


FIG. 4. Dendrogram of affinities between the snail faunas at each site, links made with the closest member of each group. Open circles = sites with mor litter; stippled circles = sites with intermediate litter; black circles = sites with mull litter; crossed circles = disturbed or open sites.

can faunas. Many coniferous forests have mor litter and podsolized soils, and often occur in climatic regimes less favourable to snails than temperate hardwood forests. In both European and North American studies, conifer stands, especially of *Pinus* or *Picea* on podsols or peat, are very impoverished, and may hold only one or two species (Bless, 1977; Matzke, 1965; Favre, 1927; Burch, 1956; Karlin, 1961), but where soil and litter conditions are more favourable, rich faunas are found, often far exceeding those of oligotrophic hardwood stands (Favre, 1927; Frömming, 1958; Burch, 1956).

Many coniferous forests studied are also unstable, being successional regrowth following logging or fire; young stands have a particularly dense canopy, which may temporarily extinguish the ground flora. Second growth conifer stands in the central Russian plain have much lower densities and diversities of snails than mature old-growth forests with a more open canopy (Shikov, 1984).

Some caution is necessary in interpreting diversity. In nearly all the studies quoted above, low diversity is associated with low density, and many species have highly aggregated distributions even within the confines of a single site. The chances of failing to detect a species clearly increase as densities fall. Karlin (1961) noted that although coniferous forests in the mountains of Colorado and New Mexico had very few species, successional stands of Aspen within them had diverse faunas. He suggests that the complete suite of species is present throughout, but increases dramatically in density during an Aspen-dominated successional episode. Boag & Wishart (1982) found that a pure Spruce stand supported about the same number of species as did deciduous and mixed stands, but that densities were lower. Each site was visited on several occasions, reducing the effect of sample-size or density on records of occurrence.

In the sites studied here, canopy was never

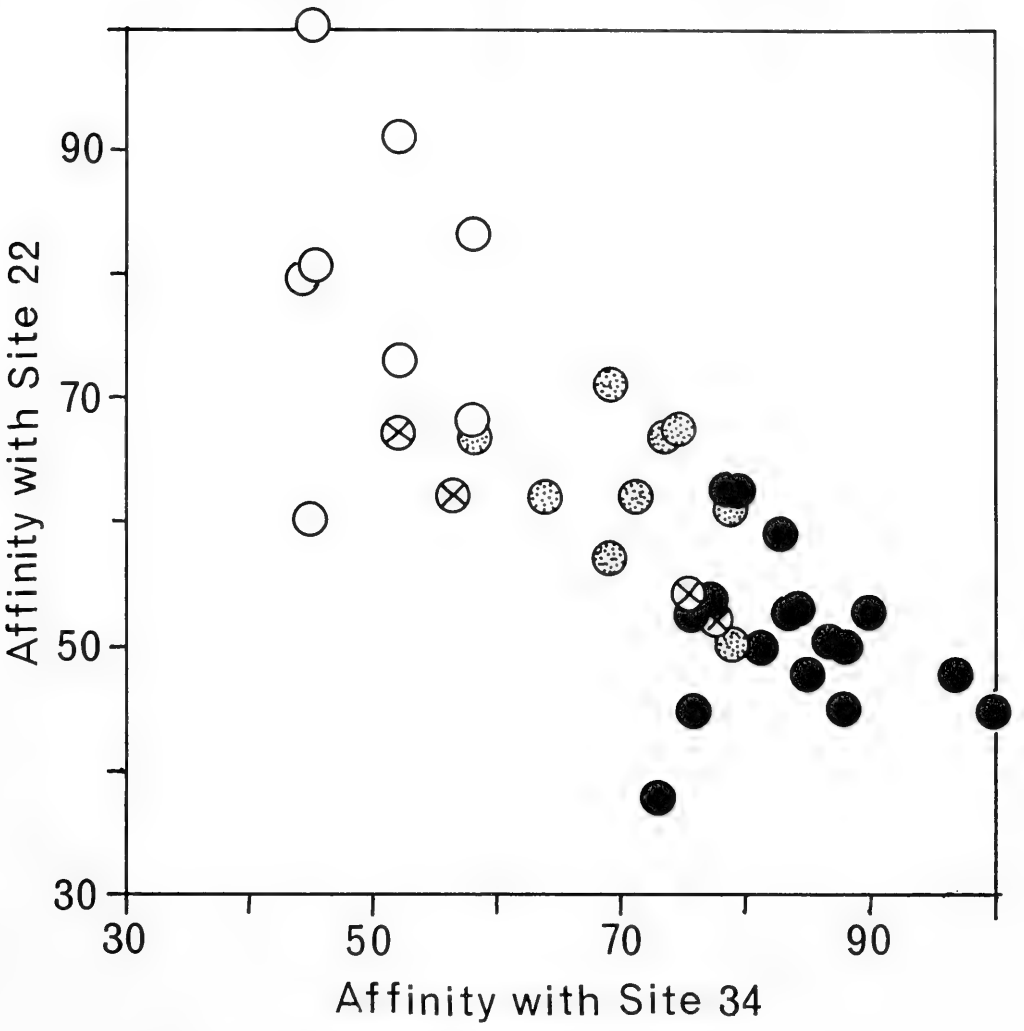


FIG. 5. The scatter of snail faunas from each site ordered on their affinities with that of site 22 (mor) and site 34 (mull). Open circles = mor litter, stippled circles = intermediate litter, black circles = mull litter, circles with cross = open and disturbed sites.

so dense as to eliminate the ground flora, and Douglas Fir litter is less acidic and nutrient deficient than that of some Pines and Spruces. The total array of species recorded in mor litter is less than that recorded for any single undisturbed mull site, and there seems no reason to doubt a genuine difference in diversity between the two types.

Most of the sites in this study are disturbed to some degree, and some, dominated by hardwoods, are at quite early stages in the protracted succession to dominance by Hemlock (Franklin & Dyrness, 1973). As is the case with plants (Mueller-Dombois, 1965), only a few snail species appear to be affected by successional state independently of soil and litter conditions. European temperate forests (nearly all of which have been heavily managed for many centuries) also support large and diverse snail faunas, and forests with appropriate soil and litter characters only have a depauperate snail fauna if they have originated in isolated plantations or from succession on previously open, agricultural land (Boycott, 1934; Paul, 1978; Cameron, 1973; Cameron, Down & Pannett, 1980). A large number of species survive routine forestry operations.

The upper limit of diversity

Allowing a margin of error for species missed in sampling, the upper limit on snail species in a single site in this study could be put at around 20–22 species (observed maximum 17) and for the forest fauna in this biogeoclimatic zone of about 30 (26 observed). Inspection of Branson (1977) and Pearce (in preparation) suggests that *Triodopsis devia*, *Megomphix hemphilli*, *Nesovitrea electrina*, *Hawaiia miniscula* and *Vertigo modesta* might be found in forests of coastal British Columbia, since they have been found in similar habitats further south. This level of diversity is high for coniferous forest, and perhaps reflects the peculiarly favourable climatic regime of the Pacific North-West compared with other areas where conifers are naturally dominant (Waring & Franklin, 1979).

More southerly hardwood and mixed forests with favourable soil conditions in N. America have richer faunas, at least in the Appalachian ranges. Coney *et al.* (1982) record 57 species from 37 forest sites in Tennessee, and Branson & Batch (1970) 45 species in a forest series in Kentucky, with 30 species at the richest site. Other North American studies reporting lower diversities are generally from sub-optimal habitats or have used sampling methods inappropriate for producing a full faunal list.

European forest faunas at comparable or more northerly latitudes are also generally more diverse where soil and rock conditions are good. Even the rather dry, rock-free and disturbed woods of the English South Downs, where the dominant Beech *Fagus sylvatica* produces a rather harsh and slowly decomposing litter, have a recorded total of 34 species, and a maximum of 24 at any one site (Cameron, 1973). Individual woodland sites with 30–45 species of snail are known from a number of European countries (Cameron, 1978; Long, 1969; Körnig, 1966; Favre, 1927; Waldén, 1981).

None of these temperate forest faunas reach the levels of diversity recorded in certain gully woodlands in the North Island of New Zealand (Solem, Climo & Roscoe, 1981; Solem & Climo, 1985; Solem, 1984) where the total forest fauna is in excess of 80 species, and the richest individual sites may contain 60 or more species. Solem (1984) gives a survey of diversity levels in land snails. The causes of such differences in diversity, however, can only be elucidated by detailed studies of niches available and occupied in different regions. The snail fauna of the forests of the Pacific North-West differ from those of other temperate forests not only in diversity, but also in the range of morphologies present; a discussion of these differences is in preparation.

ACKNOWLEDGEMENTS

This study was made possible by the award of a British Ecological Society Travelling Fellowship, and by support from the University of Birmingham. I thank Professor G. G. E. Scudder for generously making facilities available at the Department of Zoology, University of British Columbia, Dr. Tom Carefoot and Dr. Sandra Millen for advice and assistance, Dr. Judith Myers and Dr. Jamie Smith for help and hospitality, and Professor A. J. Cain for access to literature and for his critical reading of the manuscript. Mr T. Pearce helped with the identification of difficult specimens, and with discussion of the results.

LITERATURE CITED

ANONYMOUS, 1982, *Canadian Climatic Normals Volume 3: Precipitation 1951–1980.* Environment Canada: Ottawa.

BLESS, R., 1977, Die Schneckenfauna des Kottenforstes bei Bonn (Mollusca: Gastropoda). *Decheniana* (Bonn), 130: 77–100.

BOAG, D.A. & WISHART, W.D., 1982, Distribution and abundance of terrestrial gastropods on a winter range of bighorn sheep in south western Alberta. *Canadian Journal of Zoology*, 60: 2633–2640.

BOYCOTT, A.E., 1934, The habitats of land Mollusca in Britain. *Journal of Ecology*, 22: 1–38.

BRANSON, B.A., 1975, *Radiodiscus hubrichti* (Pulmonata: Endodontidae) new species from the Olympic Peninsula, Washington. *Nautilus*, 89: 47–48.

BRANSON, B.A., 1977, Freshwater and terrestrial Mollusca of the Olympic Peninsula, Washington. *Veliger*, 19: 310–330.

BRANSON, B.A. & BATCH, D.L., 1970. An ecological study of valley forest gastropods in a mixed mesophytic situation in northern Kentucky. *Veliger*, 12: 333–350.

BURCH, J.B., 1955, Some ecological factors of the soil affecting the distribution and abundance of land snails in eastern Virginia. *Nautilus*, 69: 62–69.

BURCH, J.B., 1956, Distribution of land snails in

plant associations in eastern Virginia. *Nautilus*, 70: 60–64.

CAMERON, R.A.D., 1973, Some woodland mollusc faunas from southern England. *Malacologia*, 14: 355–370.

CAMERON, R.A.D., 1978, Terrestrial snail faunas of the Malham area. *Field Studies*, 4: 715–728.

CAMERON, R.A.D., 1982, Life histories, density and biomass in a woodland snail community. *Journal of Molluscan Studies*, 48: 159–166.

CAMERON, R.A.D., DOWN, K. & PANNETT, D.J., 1980. Historical and environmental influences on hedgerow snail faunas. *Biological Journal of the Linnean Society*, 13: 75–88.

CAMERON, R.A.D. & MORGAN-HUWS, D.I., 1975, Snail faunas in the early stages of a chalk grassland succession. *Biological Journal of the Linnean Society*, 7: 215–229.

CONEY, C.C., TARPLEY, W.A., WARDEN, J.C. & NAGEL, J.W., 1982, Ecological studies of land snails in the Hiwassee River Basin of Tennessee, USA. *Malacological Review*, 15: 69–106.

FAVRE, J., 1927, Les mollusques post-glaciaires et actuels du Bassin du Genève. *Mémoires de la Société de Physique et d'Histoire Naturelle de Genève*, 40: 171–434.

FRANKLIN, J.F. & DYRNESS, C.T., 1973, Natural vegetation of Oregon and Washington. *U.S. Forest Service General Technical Report PNW-8*.

FRANKLIN, J.F. & HEMSTROM, M.A., 1981, Aspects of succession in the coniferous forests of the Pacific North West. *In* WEST, D.C., SHUGART, H.H. & BOTKIN, D.B., (ed.), *Forest Succession, Concepts and Application*. Springer Verlag, New York.

FRÖMMING, E., 1958, Schnecken im Nadelholzwald. *Biologisches Zentrallblatt*, 1: 54–63.

HENDERSON, J., 1929, The non-marine Mollusca of Oregon and Washington. *University of Colorado Studies*, 17: 47–190.

HENDERSON, J., 1936, The non-marine Mollusca of Oregon and Washington—supplement. *University of Colorado Studies*, 23: 251–280.

KARLIN, E.J., 1961, Ecological relationships between vegetation and the distribution of land snails in Montana, Colorado and New Mexico. *American Midland Naturalist*, 65: 60–66.

KERNEY, M.P. & CAMERON, R.A.D., 1979, *Field-Guide to the land snails of Britain and north-west Europe*. London: Collins.

KLINKA, K., GREEN, R.N., TROWBRIDGE, R.L. & LOWE, L.E., 1981, Taxonomic classification of humus forms in ecosystems of British Columbia. *Land Management Report 8*, Ministry of Forests, Province of British Columbia.

KÖRNIG, G., 1966. Die Molluskengesellschaften des mitteldeutschen Hügellandes. *Malakologische Abhandlungen Staatliches Museum für Tierkunde in Dresden*, 2: 1–112.

KRAJINA, V.J., 1969, Ecology of forest trees in British Columbia. *Ecology of Western North America*, 2: 1–146.

LONG, D.C., 1969, A small bog in Cranham Wood. *Conchologist's Newsletter*, 30: 111–113.

MATZKE, M., 1965, Die Molluskenfauna in den Forsten und Waldern bei Lichtenstein am Fusse des Erzgebirges. *Malakologische Abhandlungen Staatliches Museum für Tierkunde in Dresden*, 1: 139–157.

MUELLER-DOMBOIS, D., 1965, Initial stages of secondary succession in the coastal Douglas-Fir and Western Hemlock zones. *Ecology of western North America*, 1: 38–41.

MYERS, L.D., 1972, Primary and secondary influencing agents on gastropod populations of three habitats in Washington State. *Sterkiana*, 47: 39–45.

PAUL, C.R.C., 1978, The ecology of Mollusca in ancient woodland. 3: Frequency of occurrence in West Cambridgeshire woods. *Journal of Conchology*, 29: 295–300.

PILSBRY, H.A., 1939, Land Mollusca of North America (north of Mexico). *Academy of Natural Sciences of Philadelphia Monographs*, 3, 1(1): 573 p.

PILSBRY, H.A., 1940, Land Mollusca of North America (north of Mexico). *Academy of Natural Sciences of Philadelphia Monographs*, 3, 1(2): 418 p.

PILSBRY, H.A., 1946, Land Mollusca of North America (north of Mexico). *Academy of Natural Sciences of Philadelphia Monographs*, 3, 2(1): 520 p.

PILSBRY, H.A., 1948, Land Mollusca of North America (North of Mexico). *Academy of Natural Sciences of Philadelphia Monographs*, 3, 2(2): 593 p.

PLATT, T.R., 1980, Observations on the terrestrial gastropods in the vicinity of Jasper, Alberta (Canada). *Nautilus*, 94: 18–21.

ROLLO, C.D. & WELLINGTON, W., 1975, Terrestrial slugs in the vicinity of Vancouver, British Columbia. *Nautilus*, 89: 107–115.

ROTH, B. & PEARCE, T.A., 1984, *Vitrea contracta* (Westerlund) and other introduced land mollusks in Lynnwood, Washington. *Veliger*, 27: 90–92.

SHIKOV, E.V., 1984, Effects of land use changes on the land mollusc fauna in the central portion of the Russian plain. *In*: SOLEM, A. & VAN BRUGGEN, A.C. (ed.), *World-wide snails*. Brill, Leiden.

SOLEM, A., 1977, *Radiodiscus hubrichti* Branson, 1975, a synonym of *Striatura (S.) pugetensis* (Dall, 1895) (Pulmonata: Zonitidae). *Nautilus*, 91: 146–148.

SOLEM, A., 1984, A world model of land snail diversity and abundance. *In* SOLEM, A. & VAN BRUGGEN, A.C., (ed.) *World-wide snails*. Brill, Leiden.

SOLEM, A. & CLIMO, F.M., 1985, Structure and habitat correlations of sympatric New Zealand land snail species. *Malacologia*, 26: 1–30.

SOLEM, A., CLIMO, F.M. & ROSCOE, D.J., 1981, Sympatric species diversity of New Zealand land

snails. *New Zealand Journal of Zoology*, 8: 453–485.
SOUTHWOOD, T.R.E., 1966, *Ecological methods*. Methuen, London.
VAN ES, D.A. & BOAG, J., 1981, Terrestrial molluscs of Central Alberta. *Canadian Field Naturalist*, 95: 75–79.
WALDÉN, H., 1966, Zur Frage der Taxonomie, Nomenklatur und Ökologie von *Nesovitrea hammonis* (Strom) und *petronella* (L. Pfeiffer). *Archiv für Molluskenkunde*, 95: 161–195.
WALDÉN, H., 1981, Communities and diversities of land molluscs in Scandinavian woodlands, I. High diversity communities in taluses and boulder slopes in S.W. Sweden. *Journal of Conchology*, 30: 351–372.
WÄREBORN, I., 1969, Land molluscs and their environments in an oligotrophic area in southern Sweden. *Oikos*, 20: 461–479.
WÄREBORN, I., 1979, Reproduction of two species of land snails in relation to calcium salts in the foerna layer. *Malacologia*, 18: 177–180.
WÄREBORN, I., 1982, *Environments and molluscs in a non-calcareous forest area in Southern Sweden*. Ph.D. Thesis. Lund University.
WARING, R.H. & FRANKLIN, J.F., 1979, Evergreen coniferous forests of the Pacific Northwest. *Science*, 204: 1380–1386.

APPENDIX A. Details of environmental data on collecting sites. * = gross recent disturbance or open site. <40 = disturbed in last 40 years. <100 disturbed between 40 and 100 years ago. 100+ = disturbed, if at all, more than 100 years ago.
Abbreviations and scientific names of plant species:

Al	Red Alder, *Alnus rubra*	Hm	Western hemlock, *Tsuga heterophylla*
Ar	Arbutus, *Arbutus menziesii*	Ma	Large Leaved Maple. *Acer macrophyllum*
Cd	Western Red Cedar, *Thuja plicata*	Or	Oregon Grape *Mahonia nervosa*
Ct	Cottonwood, *Populus trichocarpa*	Sa	Salal *Gaultheria shallon*
De	Devil's Club, *Oplopanax horridus*	Sk	Sitka Spruce *Picea sitchensis*
Dg	Dogwoods. *Cornus nuttalli* and *C. stolonifera*	Sw	Sword Fern *Polystichum munitum*
Df	Douglas Fir, *Pseudotsuga menziesii*	Th	Thimbleberry *Rubus parviflorus*
Hk	Red Huckleberry *Vaccinium parvifolium*	Wi	Willows *Salix* spp.

Location	Site No.	Litter	Moisture	Disturbance	Dominance	Species occurrence												
						Sa	Or	Hk	Sw	De	Th	Sk	Hm	Df	Al	Ma	Dg	Cd
UBC Endowment Lands	1	Int	Damp	<100	Al			+	+		+	+			+	+		+
	2	Mor	Int	<100	Df, Al	+		+						+	+			
	3	Mor	Dry	<100	Df	+	+	+						+				
	4	Int	Damp	<100	Al			+	+						+			
Frazer and Chilliwack Valleys	5	Mull	Int	<100	Al			+	+		+				+	+	+	
	6	Mull	Damp	*		Too disturbed to record, see text.												
	7	Mull	Damp	< 40	Ct.Wi.						+				+	+	+	+
	8	Mull	Damp	100+	Cd.Ma.				+	+	+				+	+	+	+
	9	Mull	Damp	100+	Cd.Ct.				+		+		+		+	+	+	+
	10	Mull	Damp	<100	Cd.Ma.				+	+	+		+			+		+
	11	Int	Int	< 40	Df.		+			+				+				
	12	Mull	Int	< 40	Ma.Df.				+	+	+			+	+	+	+	
	13	Mull	Damp	*		Too disturbed to record, see text												
	14	Mull	Damp	100+	Ct.Ma.				+	+	+				+	+	+	+
	15	Mull	Damp	100+	Ct.Ma.				+	+	+				+	+	+	+
Menzies Bay, Campbell River	16	Mull	Damp	<100	Wi.Al.				+	+	+				+			
	17	Mor	Dry	100+	Df.	+	+	+						+				
	18	Int	Int	100+	Df.			+	+					+		+		
Elk Falls, Campbell River	19	Mull	Damp	100+	Cd.Df.				+	+	+	+	+	+	+	+		+
	20	Mull	Damp	100+	Cd.Df.Ma.				+	+	+	+	+	+		+		+
	21	Imt	Int	100+	Df.	+			+					+				
	22	Mor	Dry	100+	Df.	+	+						+	+				
	23	*	Dry	*		Too disturbed to record, see text												
Buttle Lake	24	*	Dry	*	Df.	Too disturbed to record, see text												
	25	Mor	Dry	100+	Df.	+	+	+					+	+				
	26	Mor	Dry	100+	Df.	+	+	+					+	+				
Lake Horne	27	Mull	Damp	<100	Df.Cd.Ma.	+		+	+					+	+	+		
	28	Mull	Damp	<100	Ma.Al.Ar.				+		+				+	+	+	+
Sproat Lake	29	Int	Int	<100	Df.	+			+					+	+	+		
	30	Mull	Damp	100+	Ma.Ct.				+	+	+	+		+	+	+	+	
Bamfield	31	Mor	Damp	< 40	Sk.Al.										+			
	32	Int	Damp	100+	Sk.Al.	+		+	+		+	+			+			
	33	Int	Damp	< 40	Al.				+		+				+		+	
Cameron Lake	34	Mull	Damp	100+	Cd.Df.				+	+	+	+	+	+		+	+	+
	35	Mull	Damp	100+	Cd.			+	+	+	+	+	+	+		+		+
	36	Mull	Damp	100+	Ma.Cd.Wi.						+	+			+		+	
Cowichan Lake	37	Int	Int	<100	Al.	+			+				+	+	+	+		+
	38	Mor	Dry	<100	Df.	+	+								+	+		

APPENDIX B. Numbers of snails, and presence or absence of slugs at each site. Sites arranged by litter type.

	Mor litter sites								Intermediate litter sites								
	2	3	17	22	25	26	31	38	1	4	11	18	21	29	32	33	37
Snails																	
Allogona townsendiana																	
Carychium occidentale														2	50		
Cionella lubrica																	
Columella edentula	1	1							1			2		1	9		2
Discus cronkhitei																	
Euconulus fulvus													1	1			
Haplotrema sportella	2	2	1						5	3	2	2		4	3	3	2
Haplotrema vancouverense	3	2	8	3	2	3	17	6	2	3	3	5	4	7	2	2	5
Monadenia fidelis													1				
Microphysula cookei																	
Nesovitrea binneyana			1	2				2			2	3	6	2	1		7
Planogyra clappi			2		2	2					2	3	6	2	1		7
Punctum conspectum																	
Punctum randolphii	6		2	2	5	8	7	10	12	8	25	6	3		41	5	55
Pristiloma johnsoni																	
Pristiloma lansingi	4	2	25	20	3	4	4	5	4	5	6	32	21	5	45	4	6
Pristiloma stearnsi																	2
Striatura pugetensis	6	10	12	28	5	6	2	5	8	3	35	25	10	4	11	4	18
Triodopsis germana									2	2						1	
Vallonia pulchella																	
Vespericola columbiana	2					1	3	4	5	6	1	1	4	2	3	3	3
Vertigo andrusiana																	
Vertigo columbiana									1	2	17	3	4	3	6		2
Vertigo rowelli																	
Vitrina alaskana																	
Zonitroides arboreus													1				
No. of species	7	5	7	5	5	6	5	6	9	8	9	10	11	11	10	7	11
No. of individuals	24	17	51	55	17	24	33	32	40	31	97	86	57	33	170	22	112
Slugs																	
Ariolimax columbianus		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Arion ater		+	+						+								
Deroceras laeve							+		+	+					+	+	
Prophysaon andersoni									+								
Prophysaon foliolatum		+							+		+				+	+	
Prophysaon vanattae			+								+						
Hemphilla glandulosa							+								+	+	

Mull litter sites																	Recently disturbed and open sites			
5	7	8	9	10	12	14	15	16	19	20	27	28	30	34	35	36	6	13	23	24
	2	2																		
		29				15	37		37	64	54		14	12	12	10				
			18					2				2							6	
	4	2	2	13	1	5	2	3	9	9	4	12	3	4	8	3	8	7		
														6	2	8				
	16	3	1	13	3	6	11		3		2	4	1	2		4	4	4		3
10	27	6	14	5	8	14	9	4			3	2	10	3	5	4	13	13		
7		6	3	2	2	3	5	2	9	5	8	11	6	2	7	2	8	4	3	6
1	3	1	3	2	4	2	3		3	2			3	3	3	2	1	1		
				10													6	2		10
4	36	3	16	3	2	10	8	3	4	1	3	9	10	4	7	4	3	2	1	4
4	36	3	16	3	2	10	8	3	4	1	3	9	10	4	7	4	3	2	1	4
	4		4	5		1		1							4			3	14	
5	15	12	9	23	18	25	19	8	8	11	10	16	6	10	12	9	53	21	1	1
												2	2							
14	11	28	9	32	8	33	40	55	24	15	17	25	12	12	25	10	18	21	1	
4											5	16	8	5	7	6				
25	14	16	27	26	6	43	35	45	25	15	23	27	15	28	38	18	14	12	12	28
	1		4			3	5	7	3	3	7	19	2	3		2				
	3																			
3	7	6	6	3	7	6	8	3	7	6	2	8	4	9	6	4	16	5	1	9
									10					5						
3		2	4	32		5	3	6	4	7	4	10	2	6	13	3	8	7		
											1	4								
	5																2			
		1							2				1		1	2			1	
11	16	15	14	14	11	15	14	14	14	12	15	17	17	17	16	16	14	14	10	8
78	186	121	52	226	67	209	216	158	149	145	151	183	114	119	167	92	168	154	41	62
+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
	+	+	+	+																
					+	+	+	+				+		+	+	+	+	+		
														+	+		+	+		

Revised Ms. accepted 10 April 1986

MALACOLOGIA, 1986, 27(2): 357–374

MICROGEOGRAPHIC VARIATION IN THE BANDED SPRING SNAIL (HYDROBIIDAE: *MEXIPYRGUS*) FROM THE CUATRO CIÉNEGAS BASIN, COAHUILA, MÉXICO

Robert Hershler

Department of Invertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, DC 20560, U.S.A.

W.L. Minckley

Department of Zoology, Arizona State University, Tempe, Arizona 85287, U.S.A.

ABSTRACT

Microgeographic variation is documented among 16 populations of the aquatic snail *Mexipyrgus churinceanus* Taylor from El Mojarral spring system in Cuatro Ciénegas, northern México. Patterns of variation among these snails include a steep cline linking two nominal species, and a gentler one tending to link one of these with a third nominal species from an adjacent spring system.

The form of these clines is apparently explained by the presence or absence of gene flow barriers in the spring system, although alternative explanations are discussed. A review of ideas on the origin of aquatic habitats in the basin suitable for *Mexipyrgus* suggests that the clines represent secondary intergradation, with divergence having occurred in allopatric populations isolated by desiccation of a barrial lake(s) or fragmentation of a massive, continuous spring, and secondary contact made possible by reintegration of habitats. The results, suggesting genetic exchange among distinctive populations having restricted distributions, support an earlier contention that these populations comprise a single, polytypic species.

Key words: Hydrobiidae; Cuatro Ciénegas; springs; morphology; variation; clines.

INTRODUCTION

Aquatic prosobranch snails of the genus *Mexipyrgus* Taylor, 1966 (Gastropoda: Hydrobiidae), endemic to the desert valley of Cuatro Ciénegas, northeastern México, provide outstanding examples of localized morphological differentiation. Distinctive populations are distributed among spring-fed pools and stream outflows of springs in this small, 1200 km^2, endorheic, intermontane basin (Taylor, 1966; Taylor & Minckley, 1966; Hershler, 1984, 1985). Two contrasting opinions have been put forward regarding their taxonomic status: Taylor (1966) described six species from seven collecting sites; and Hershler (1985) reduced the genus to monotypy (*Mexipyrgus churinceanus* Taylor), based on principal component analysis of data from more than 30 localities. Systematic problems associated with allopatric populations (Mayr, 1963; Wiley, 1981) are thus epitomized by these animals.

While spring-fed pools in the basin are insular in nature, some have outflows interconnecting with other outflows or spring pools, providing possible contact zones between populations. The snail is habitat-specific, occurring only where there are ample and invariable sources of water, gentle or negligible currents, warm and constant temperature, and substrate consisting of an accumulation of soft, flocculent sediments (Taylor, 1966; Hershler, 1984, 1985). Physically connected places that provide continuous habitat of this type are rare. Discovery of a zone of syntopy would be of great value in providing a measure of evolutionary divergence among distinctive stocks.

One interconnected series of large springs and outflows in an area locally known as El Mojarral contains type localities of two nominal species of *Mexipyrgus* (*sensu* Taylor, 1966). Evidence was sought of syntopy, allotopy, and/or intergradation involving these as well as a third nominal species from an adjacent spring system. We here describe

microgeographic variation of snails in the Mojarral area, comparing patterns of morphology in a relatively continuous habitat with those in one with apparent barriers to gene flow. Opportunity also is taken to report additional hydrographic descriptions and interpretations which pertain to origins, age, and evolutionary history of the basin and its unique biota.

STUDY AREA, MATERIALS, AND METHODS

Description of the area. Average annual rainfall of less than 200 mm (Vivo Escoto, 1964), coupled with high temperatures and evaporation rates, disallows other than storm-induced runoff, and permanent aquatic habitats in the Cuatro Ciénegas Basin are fed exclusively by springs (Minckley, 1969). Water rises near ends of bajadas with considerable force, its artesian nature indicating an origin at high elevation such as from precipitation on the eroded, north-plunging Sierra de San Marcos anticline. Massive Cretaceous limestones of surrounding mountains (Baker, 1971) are characterized by permeable strata and solution channels through which water may pass until forced upward by obstructing faults of basin margins.

Minckley (1969) presented a simplistic explanation of origin and succession in most present aquatic habitats of the basin, beginning with development of solution channels. Such channels presumably foundered as the basin was dewatered to form pits (pozos) that expanded by lateral collapse to form lake-springs or limnocrenes (lagunas). Further slumping of banks produced increased surface area and heterogeneity, followed by vegetation invasion and development of marshlands (ciénegas). Surface streams were further proposed to represent foundered subterranean waterways. Downflow, dune-impounded or otherwise formed lentic habitats developed into terminal, shallow, variably mineralized barrial (basin floor) lakes. With climatic changes toward greater aridity (Van Devender, 1976, 1977; Wells, 1978; Axelrod, 1979) the successional sequence ends in formation of extensive playas, wet only after periods of rainfall.

El Mojarral. A series of springs and marshlands comprising El Mojarral is about 11.5 km SW of the town of Cuatro Ciénegas (Minckley, 1969; Fig. 2). The drainage (Fig. 1) includes three major spring pools: Mojarral West Laguna; "Middle spring;" and Mojarral East Laguna. Extensive ciénegas lie between and adjacent to pools, and a number of smaller springs, streams, and marshes are upslope (south) of the major part of the system. Drainage of El Mojarral is N and E toward an eastern sump. A surface outflow of Mojarral West enters "Middle spring," whose surface outflow in turn (and in part) enters Mojarral East. There also are underwater outflows (black dots in Fig. 1); one from Mojarral West Laguna is via a large, tubular vent in the eastern end. Underwater inflow (open circles in Fig. 1) and outflows in "Middle spring" are similarly large and tube-like, and a number of smaller springs rise from the floor of the western end of Mojarral East. The surface outflow of Mojarral East receives several small, surface distributaries of the Río Mesquites (arrows in Fig. 1), and ultimately joins that river, the largest in the basin (not included in Fig. 1).

Spring inflows to the Mojarral system are thermal at 30 to 35° C and characterized by hard water of crystal clarity. Depths of pools range from less than 1.0 to 4.7 m. They are unshaded except locally by banks; sunlight penetrates to the bottoms. All may have common groundwater sources or are linked by subterranean conduits, given their linear alignment, proximity, and similarities in water quality. Slight temperature decreases downflow may thus represent cooling of groundwater in travel from its source, or may indicate multiple sources. Reductions in EDTA water hardness downflow from Mojarral West Laguna (to Mojarral East Laguna) indicated by Minckley & Cole (1968) (1254 *vs.* 1208 mg/l) were probably not significant. Values in the two springs were actually or essentially the same in 1966 and 1968 (1234 mg/l in each and 1236 *vs.* 1249, respectively; Arnold, 1972; Minckley, unpublished data). Attempts to trace subterranean flow from Mojarral West to "Middle spring" by copious application of fluorescein dye in 1968 and 1970 failed (Arnold, 1972; Minckley, unpublished data). Dye was not detected by eye or through use of activated charcoal collectors examined under strong ultraviolet light at various, presumably appropriate times following repeated dye application. Discharge volumes are great and dye dilution was either too high or subterranean distances and complexities far greater than anticipated.

Spring pools have little substrate diversity,

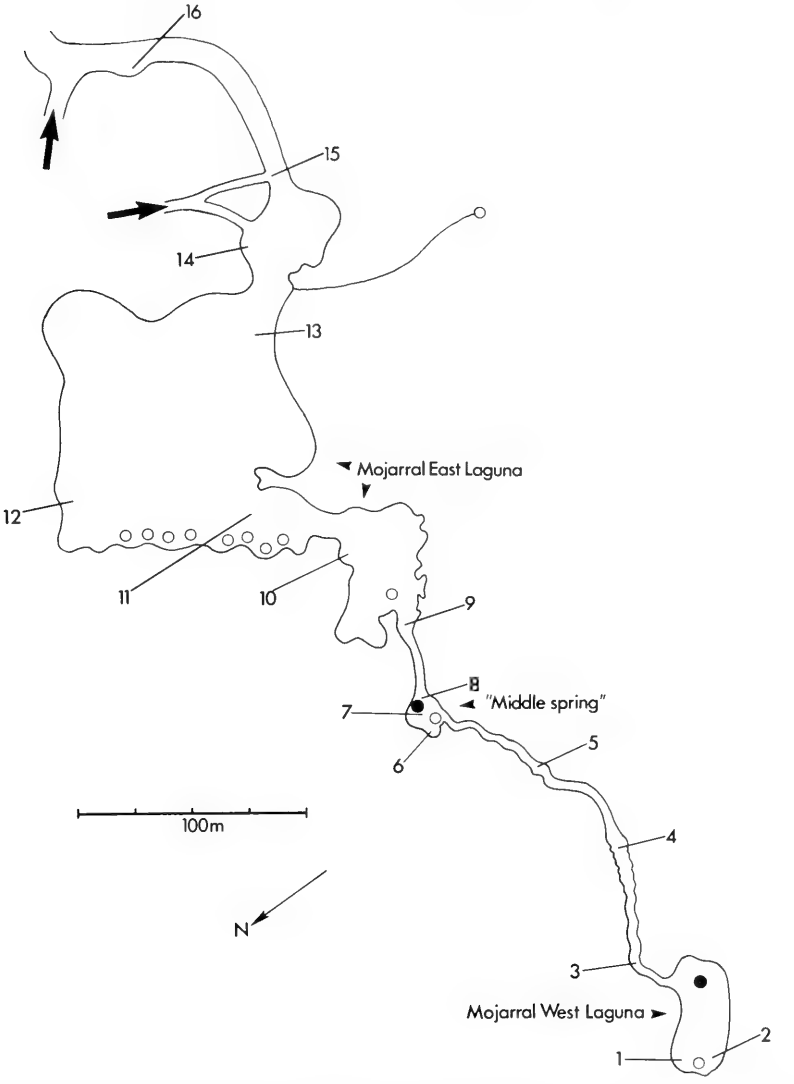


FIG. 1. Map of El Mojarral drainage showing the location of 16 sampling points. Open and filled circles indicate spring orifices and underwater outflows, respectively. The large arrows to the E indicate inflowing branches of the Río Mesquites.

consisting predominantly of flocculent, organic, copropelic sediment less than 1.0 cm to greater than 0.5 m deep that overlies firmer layers of bits of travertine and snail shell. Exposed shell and travertine fragments armor areas where currents remove copropelic materials. Local stands of waterlily (*Nymphaea ampla* [Salisb.]) vegetate some bottoms, sparse beds of sedges are in shallows, and stony travertine deposits comprise shorelines and bottoms adjacent to inflows.

Nominal taxa. Three nominal species of *Mexipyrgus* are in the Mojarral area, capsule descriptions for which are as follows (modified from Taylor, 1966).

1) *Mexipyrgus mojarralis* Taylor. Shell (Fig. 3A-F) small, 4.5–5.0 mm high; spiral sculpture well-developed on body whorl; periostracal bands few (two to four) in number, with thickened subsutural band usually

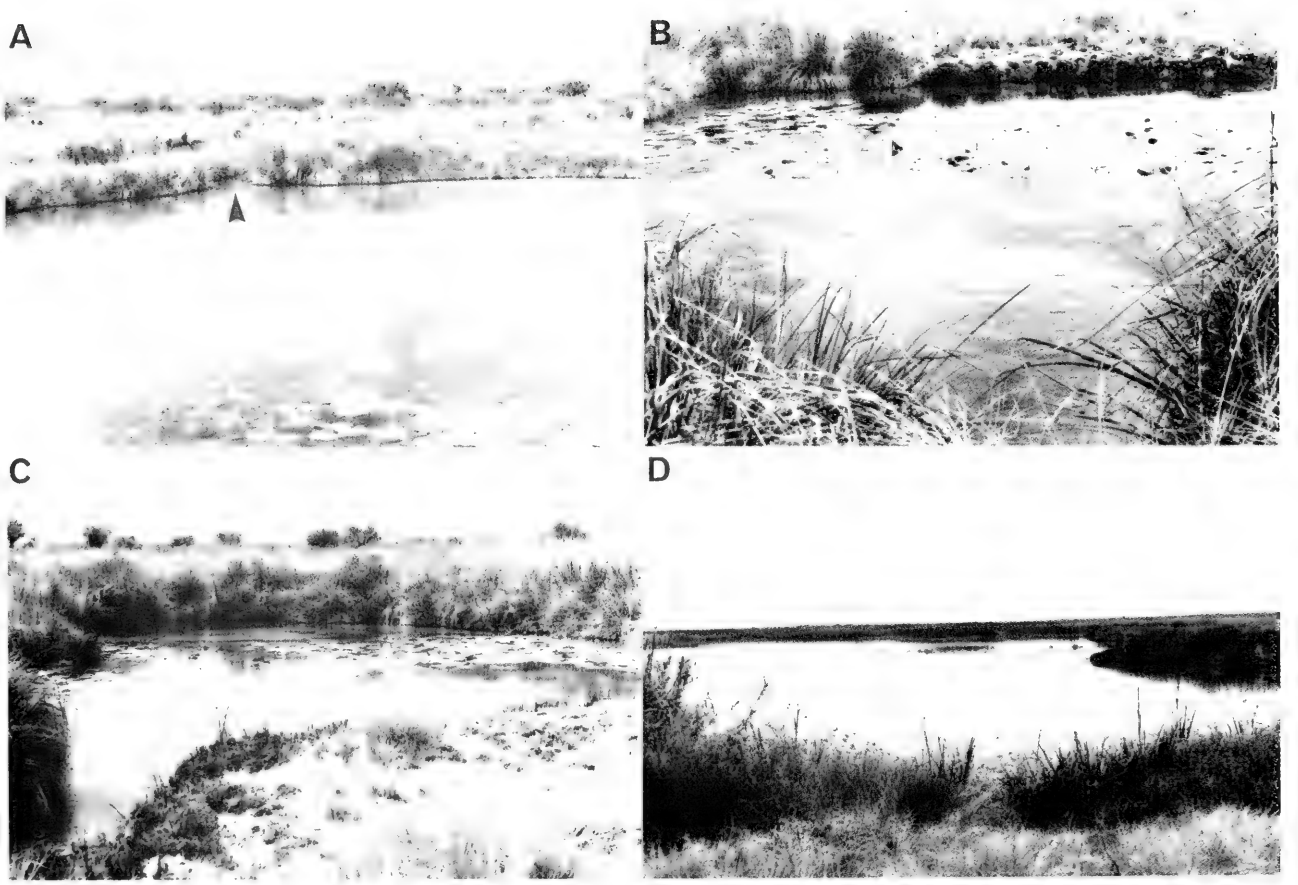


FIG. 2. El Mojarral spring pools. A. SE portion of Mojarral West Laguna. Note the waterlily stand in the foreground and underwater outflow tube to the right. The arrow indicates the surface outflow. B. Northern half of "Middle spring." Note the waterlily stand crossing the spring. C. "Middle spring" viewed from the S. Riparian vegetation in the foreground has been largely removed by a recent fire. The surface outflow is in the lower left. Again note the extensive waterlily stand. D. Mojarral East Laguna, viewed from the N.

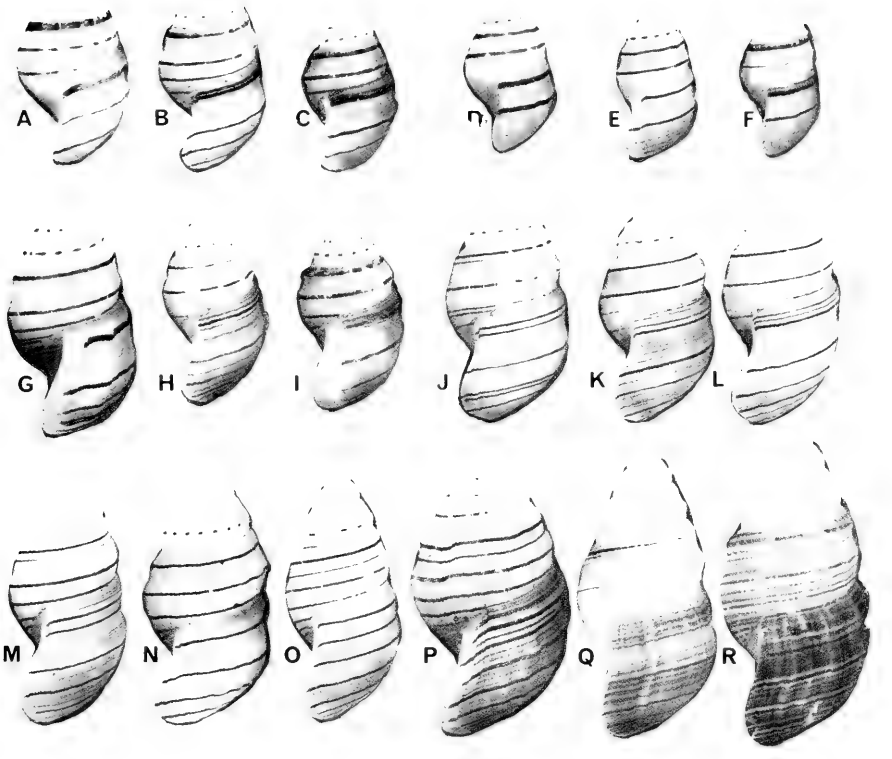


FIG. 3. Photographs of shells of *Mexipyrgus churinceanus* from El Mojarral. The shells are from localities 2 (A-C), 6 (D-F), 9 (G-I), 12 (J-L), 14 (M-O), and 16 (P-R). Shell "A" is 4.89 mm in height and the other photos are printed to the same enlargement.

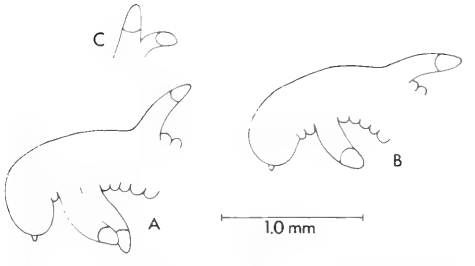


FIG 4. Penial variation seen among *Mexipyrgus churinceanus* from El Mojarral. Note the variation in number of lobes on the inner curvature (A, B) and occasional presence (C) of an additional lobe on the outer curvature.

present; penis with a single (not two as implied in Taylor, 1966) lobe on the inner curvature (Fig. 4B). Type locality, Mojarral West Laguna.

2) *M. multilineatus* Taylor. Shell (Fig. 3J-O) intermediate in size, 5.0–5.1 mm high; spiral sculpture poorly defined on body whorl; up to 20 bands present, with thickened subsutural band absent; penis with one or two lobes on inner curvature (Fig. 4). Type locality, Mojarral East Laguna.

3) *M. lugoi* Taylor. Shell (Fig. 3P-R) large, 7.3 mm high; spiral sculpture on body whorl poorly developed; 25–35 bands present, with thick-

TABLE 1. Collection sites for this study, numbered as in Fig. 1, with collection dates and USNM (National Museum of Natural History) catalog numbers for samples in parentheses.

1. Mojarral West Laguna, 16 m S of N end, right offshore of E side, 3 cm deep (3/11/81) (850287).
2. Mojarral West Laguna, 4 m S of NW corner, right offshore, 1 m deep (3/19/81) (850285).
3. Stream from Mojarral West Laguna, 20 m downstream from spring (12/12/81) (850281).
4. Stream from Mojarral West Laguna, 80 m downstream from spring (12/12/81) (850288).
5. Stream from Mojarral West Laguna, 42 m above entrance to "Middle spring" (12/12/81) (850276).
6. "Middle spring," 1 m S of N corner, 5 m W of E side, 2 m deep (12/14/81) (850277).
7. "Middle spring," 8 m S of N tip, 6 m W of E side, in *Nymphaea* "reef," 7 cm deep (12/14/81) (850286).
8. "Middle spring," 1 m N of S tip, 3 m E of W side, 1 m deep (12/14/81) (850282).
9. Mojarral East Laguna, western lobe, at inflow from "Middle spring" (12/14/81) (850279).
10. Mojarral East Laguna, western lobe, 13 m NW of connection to eastern lobe, 0.7 m deep (12/14/81) (850284).
11. Mojarral East Laguna, eastern lobe, 53 m SE of connection to western lobe, 1 m deep (3/18/81) (850274).
12. Mojarral East Laguna, eastern lobe, 11 m S of NE corner, 7 m offshore (2/14/81) (850278).
13. Mojarral East Laguna, eastern lobe, 40 m N of SW corner, right offshore (3/17/81) (850273).
14. Stream from Mojarral East Laguna, 30 m downstream from spring, east side, near inflow from first arm of the Río Mesquites (12/14/81) (850283).
15. Stream from Mojarral East Laguna, at inflow of the second arm of the Río Mesquites (12/14/81) (850275).
16. Stream from the Mojarral East Laguna, shallow pooled area, N side of stream, just above inflow of third arm of the Río Mesquites (12/14/81) (850280).

ened subsutural band absent; penis with two lobes on inner curvature (Fig. 4A). Type locality, Río Mesquites, one to two km upflow from confluence with outflow of Mojarral East Laguna.

Specimens examined and methodology. Specimens of *Mexipyrgus* were collected during March and December 1981 from 16 sampling points along a transect through the El Mojarral system (Fig. 1). Precise locality data are in Table 1. At each locale 100–300 individuals were secured by repeated sweeps of a fine-meshed hand sieve through soft sediments within a randomly chosen area not exceeding 4.0 m^2. Snails were relaxed in the field using menthol crystals, fixed in dilute formalin, and preserved in 70% ethanol.

A series of adults, recognized by completion and thickening of the inner shell lip, was chosen from each sample; empty shells were excluded. Relaxed snails were readily sexed by noting presence or absence of the penis. The following features, including all those used in diagnoses of nominal species (excepting shell sculptural pattern), were scored, counted or measured (numbers per sample in parentheses) as follows: presence or absence of banding on the outer shell lip (100); presence or absence of a thickened subsutural band (relative to other shell bands) on the outer lip (50 banded shells); number of bands on the outer lip (50 banded shells); shell length (15 females); and number of lobes on inner and outer curvature of the penis, expressed as an "inner-outer" formula (25 males). In addition, the following shell parameters were measured or counted for 15 individuals of each sex from five of the 16 sampling points (2, 8, 12, 14, and 16; Fig. 1): shell height (SH), shell width (SW), length of body whorl (LBW), aperture height (AH), aperture width (AW), and number of whorls (WLS). Measurements were made at 25× using a Wild M-5 dissecting microscope fitted with an ocular micrometer. All shell bands distinctive at 25× were counted.

Descriptive statistics were generated using the computer-mediated SAS program, while ANOVA and Tukey HSD multiple range test ($p = .05$) were performed using SYSTAT (Wilkinson, 1984). CLUSTAN (Wishart, 1978) was used to extract principal components from the correlation matrix of shell morphometry data, with separate analyses for males and females.

RESULTS

Summary statistics are in Tables 2, 4, and 5, with frequencies or mean values of several characters plotted by collecting station in Fig. 5. Results of Tukey HSD Test for multiple comparisons among means of shell heights are in Table 3.

Several kinds of variation were evident

TABLE 2. Summary data for morphologic features scored or measured for samples from 16 localities. The numbers of individuals used are indicated in parentheses.

Locality	Mean adult shell length (♀, n = 15)	Frequency of banded shells (n = 100)	Mean number of bands on the shell (n = 50)	Frequency of shells with a thickened subsutural band (n = 50)	Frequency of males with the following penial formulae (n = 25): 1–1	2–1	Other
1	4.10	99	4.10	78	96	—	4(0–1) (n = 26)
2	4.81	100	4.38	96	96	—	4(0–1) (n = 26)
3	4.71	100	5.76	86	100	—	—
4	5.08	100	6.20	88	100	—	—
5	4.89	96	5.72	94	100	—	—
6	3.96	95	5.12	86	100	—	—
7	4.34	83 (n = 113)	6.66	90	96	—	4(0–1)
8	5.16	73	7.74	60	96	4	— (n = 26)
9	5.70	68	10.0	56	94	3	3(1–2) (n = 33)
10	5.23	41	13.0	0	91	3	3(1–2); 3(2–2) (n = 34)
11	5.28	25	12.1	2	85	6	6(0–1); 3(1–2) (n = 33)
12	5.97	15	8.74	0	83	17	— (n = 30)
13	6.48	19	13.9 (n = 42)	5 (n = 42)	61	36	3(0–1)
14	6.38	50	17.7	2	60	32	4(0–0); 4(1–2)
15	7.30	66	20.8 (n = 38)	3 (n = 38)	32	52	4(0–0); 4(2–0); 8(2–2)
16	7.29	88	22.9 (n = 32)	17 (n = 35)	16	84	—

TABLE 3. Results of the Tukey HSD multiple comparison test among means for shell height. The means (station numbers in parentheses) are ranked by magnitude on the left, and groups of stations containing means that do not differ significantly (p = .05) from one another are indicated to the right.

3.96 (6)	6, 1
4.10 (1)	1, 7
4.34 (7)	7, 3
4.71 (3)	3, 2, 5
4.81 (2)	2, 5, 4, 8, 10
4.89 (5)	4, 8, 10, 11
5.08 (4)	11, 9
5.16 (8)	9, 12
5.23 (10)	14, 13
5.28 (11)	16, 15
5.70 (9)	
5.97 (12)	
6.38 (14)	
6.48 (13)	
7.29 (16)	
7.30 (15)	

(Fig. 5). Size (SH), although variable and even differing significantly among sampling points within the relatively small Mojarral West (Table 3), showed a general pattern of gradual increase downflow, with overlap especially common among adjacent, upflow sampling points. Note that significant breaks in size occurred at sampling point 9 and others downflow (Table 3). Numbers of shell bands increased downflow in a similar fashion. Overlap was again pronounced upflow, with, for instance, no significant differences among any pairs of samples from points 1–8 (Tukey HSD Test, $p > 0.05$). Variation in subsutural banding involved a steep cline, with high frequencies of thickened banding upflow declining sharply to near-zero frequencies downflow (Table 2). Frequency of thickened banding for sampling points 8 and 9 (58%, pooled data) differed significantly with either pooled data from upflow points 1–7 (88%) or downflow sampling points 10–16 (3%) (separate Chi-square tests with continuity corrections, $p < .001$ for both comparisons).

Snails from not only Mojarral West (sampling (points 1 and 2; Fig. 3A-C), but also its outflow points 3–5) and the two northernmost points in "Middle spring" (points 6, 7; Fig. 3D-F) are clearly referable to *Mexipyrgus mojarralis*. Small size (SH less than 5.1 mm), few shell bands (fewer than 7), high frequency of thickened subsutural banding (greater than 78%), well-developed spiral sculpture on the body whorl, and a "1-1" penial type are characteristic (Table 2). Snails from the next two downflow sampling points (8, 9; Fig. 3G-I) were intermediate between *M. mojarralis* and *M. multilineatus*. Non-thickened subsutural banding, rarely seen in upflow *M. mojarralis*, was common, and a few individuals had the "2-1" penial type not seen

TABLE 4. Shell measurements for females from five localities. For all samples, n = 15. For explanation of abbreviations see p. 362.

Locality		Parameter					
		SH	SW	LBW	AH	AW	WLS
2	$\bar{x}$	4.80	2.65	3.22	1.79	1.60	6.13
	s	0.167	0.101	0.124	0.087	0.061	0.160
8	$\bar{x}$	5.16	2.67	3.37	1.93	1.63	6.43
	s	0.456	0.221	0.261	0.178	0.102	0.506
12	$\bar{x}$	5.97	3.00	3.90	2.17	1.87	6.42
	s	0.464	0.143	0.220	0.123	0.101	0.376
14	$\bar{x}$	6.38	3.05	3.93	2.19	1.87	6.87
	s	0.346	0.133	0.209	0.125	0.067	0.229
16	$\bar{x}$	7.29	3.67	4.64	2.65	2.25	6.95
	s	0.361	0.190	0.230	0.172	0.101	0.254

TABLE 5. Shell measurements for males from five localities. For all samples, n = 15. For explanation of abbreviations see p. 362.

Locality		Parameter					
		SH	SW	LBW	AH	AW	WLS
2	$\bar{x}$	4.14	2.19	2.81	1.63	1.41	6.00
	s	0.210	0.094	0.108	0.057	0.042	0.267
8	$\bar{x}$	4.61	2.42	3.10	1.85	1.56	6.17
	s	0.207	0.191	0.152	0.119	0.106	0.244
12	$\bar{x}$	5.67	2.71	3.82	2.19	1.83	6.43
	s	0.243	0.061	0.098	0.102	0.056	0.200
14	$\bar{x}$	5.82	2.78	3.82	2.15	1.86	6.63
	s	0.262	0.118	0.156	0.128	0.078	0.160
16	$\bar{x}$	6.28	3.17	4.11	2.52	2.09	6.54
	s	0.276	0.092	0.180	0.128	0.091	0.229

upflow. Widespread character discordance occurred (*i.e.*, individuals with *M. mojarralis* sculpture, yet *M. multilineatus* banding). Separation of snails from these points on the basis of subsutural banding type yielded groups (thickened banding, mean SH = 5.35 mm, N = 21; non-thickened, 5.47 and 20 respectively) that did not differ in size (t-test, $p > 0.2$), further indicating that syntopic taxa were not present.

Snails from sampling points 10–13 resembled *Mexipyrgus multilineatus*, but trends among these points and those further downflow involving increasing size, number of bands, and frequencies of "2-1" penial type, indicated apparent gradation toward a more *M. lugoi*-like snail (Fig. 3J-R). Note that snails from point 16 remained transitional in morphology in that 100% occurrence of the "2-1" penial type (typical of *M. lugoi*, *fide* Taylor, 1966, and Hershler, 1985) was not present. Again, syntopic taxa were not recognizable at any point.

Principal components analysis was used to gauge distinctiveness of populations referable to the three nominal species (sampling points 2 and 12 as respective topotypes of *Mexipyrgus mojarralis* and *M. multilineatus* and snails from sampling point 16 as a population trending strongly toward *M. lugoi*) as well as two populations from intermediate geographic positions (points 8 and 14). Scores for the first two principal components are in Fig. 6 (females) and Fig. 7 (males). Topotypes of *Mexipyrgus mojarralis* (sampling point 2) and *M. multilineatus* (point 12) are broadly separated, especially males (Fig. 7), while an intermediate state of specimens

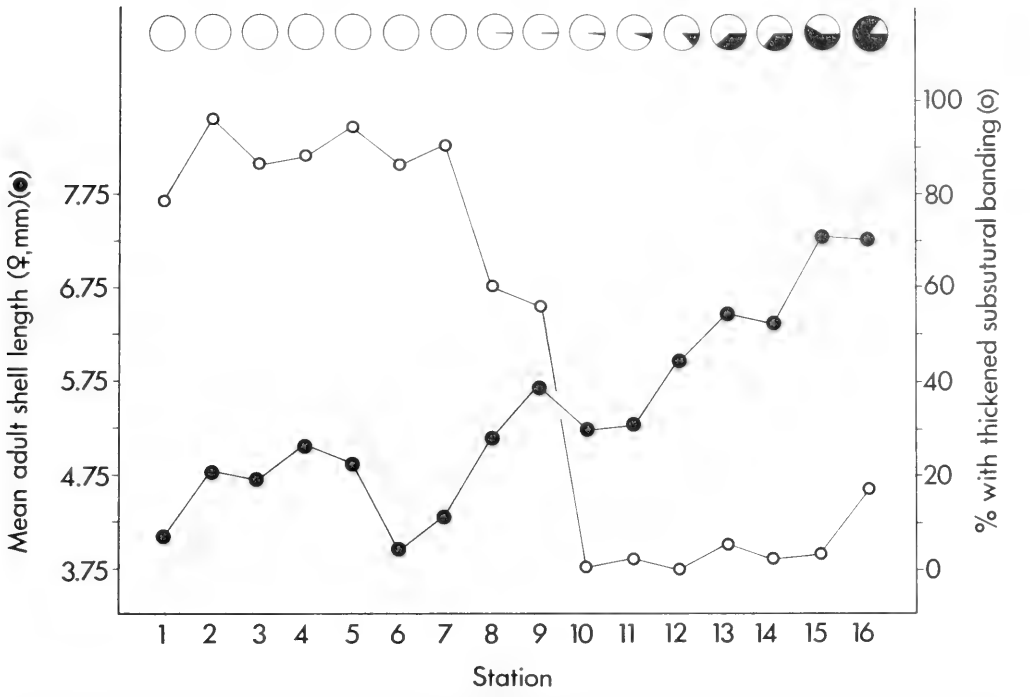


FIG. 5. Variation among localities of mean value of shell length, frequency (%) of occurrence of a thickened subsutural band, and penial lobation (circles above top of plot). Variation in penial lobation is expressed as relative frequency of "1-1" (light) versus "2-1" (dark) types.

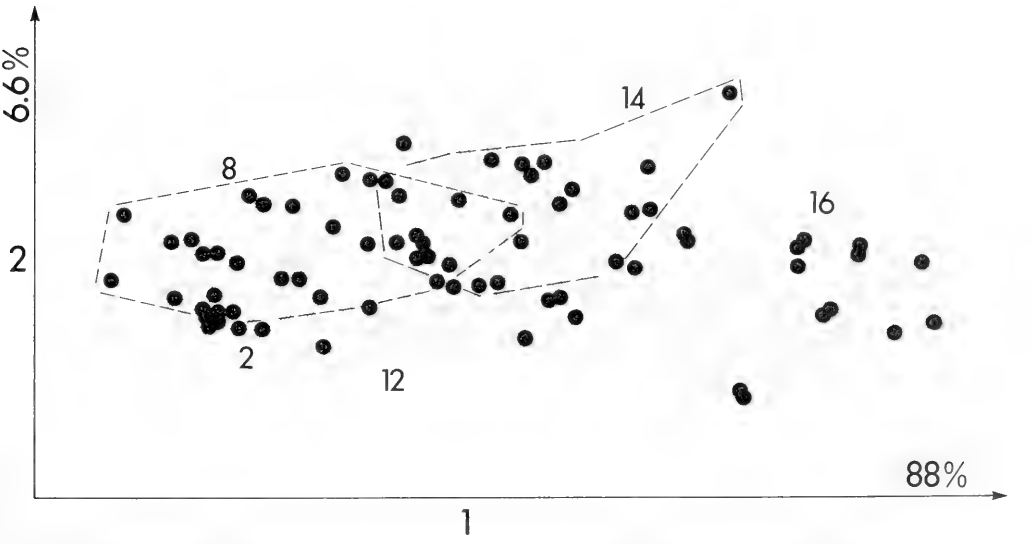


FIG. 6. Plot of scores of first two principal components extracted from the female shell data set.

from point 8 is clearly defined. Variability in the last sample, expressed as mean coefficient of variation for the six shell parameters (males, 5.74; females, 7.51), also is elevated relative to that seen in animals from sampling points 2 (4.03, 3.74) and 12 (3.31, 5.86).

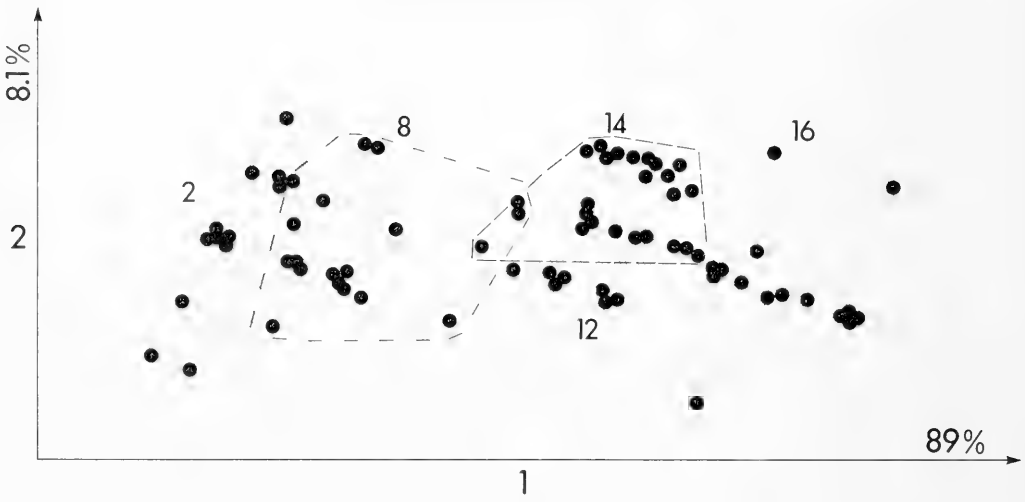


FIG. 7. Plot of scores of first two principal components extracted from the male shell data set.

While topotypes of *Mexipyrgus multilineatus* are less separated from the population trending toward *M. lugoi* (sampling point 16), neither sex from sampling point 14 occupied a morphologically intermediate position, nor did they exhibit elevated variation relative to the other two (locality 14, males 4.22, females 4.62; locality 16, males 4.10, females 4.96; data for locality 12 given above). Separation is largely along the first principal component, which accounts for 88–89% of total variation and is clearly related to size given high positive coefficients of all shell parameters (Table 6). The major role of size or size-dependent shape also is indicated by significant correlations ($p < .05$) between shell height and the other five parameters (males, 78% of possible comparisons significant; females, 92%). Distinctiveness of the *M. lugoi*-like population in this light is largely attributable to the significant increases in size among downflow populations (Table 3).

DISCUSSION AND CONCLUSIONS

We conclude that upstream to downstream genetic exchange among populations in the Mojarral system is evident: 1) we were unable to demonstrate syntopy between forms; 2) there was an absence of absolute barriers that would promote allotopy and *Mexipyrgus* was almost continuously distributed along a transect of 16 sampling points; and 3) there seems to be ample evidence for clinal intergradation of morphological characters along geographic and habitat gradients.

Clinal variation. Clinal variation among interfertile populations is not surprising (Endler, 1977), especially in a sedentary, ovoviviparous animal that lacks broadcast gametes or larvae. However, the two patterns that exist, a steep cline linking *Mexipyrgus mojarralis* with *M. multilineatus* and a more gentle one that tends to connect *M. multilineatus* and *M. lugoi*, require further explanation.

Steep clines predictably occur in areas having reduced (Endler, 1977) or punctuated gene flow, rather than consistent temporal and/or spatial passage of genetic material. The zone of steepness in the Mojarral system, at the southern end of "Middle spring" and entrance into Mojarral East (sampling points 8, 9), occurs coincident with a major habitat discontinuity. Soft substrate is essentially absent in the lowermost 60 m of surface stream from Mojarral West Laguna as well as from the virtual entirety of stream connecting "Middle spring" and Mojarral East. In both cases water flows swiftly over bare travertine. Only unidirectional and perhaps infrequent gene flow is therefore likely, with snails occasionally displaced downstream by currents or drifted with algal mats (at least as young), floating due to accumulation of photosynthetic gasses (Arnold, 1972). Any possible continuity of soft bottom type is further interrupted within "Middle spring" by steep (near vertical) slope of a travertine or travertine-armored, *Nymphaea*-covered reef, that largely sepa-

TABLE 6. Principal components analysis of shell parameters.

Parameter	Females		Males	
	PCI	PCII	PCI	PCII
Shell height	0.428	0.047	0.425	0.038
Shell width	0.420	−0.308	0.417	−0.242
Length of body whorl	0.395	0.076	0.424	−0.089
Aperture height	0.421	−0.231	0.418	−0.263
Aperture width	0.419	−0.322	0.425	−0.166
Number of whorls	0.362	0.860	0.331	0.914

rates the spring into two halves (Fig. 8). This may either represent part of a collapsed roof of the spring or an accumulated travertine postdating such an event. Transport may also occur through subsurface channels possibly connecting at least Mojarral West with "Middle spring" and perhaps Mojarral East. Substrate and other conditions in underground conduits are unknown.

Contrasting lack of evident habitat discontinuity provides an apparent explanation for the uniform cline trending from *Mexipyrgus multilineatus* toward *M. lugoi*. Although much of the western portion of Mojarral East Laguna is floored by travertine, pockets of copropelic sediments are present and bottoms of the eastern two-thirds and the laguna outflow are almost continuously of copropel. The laguna is relatively large, and most currents are from wave action despite a net linear flow from west to east. Such should allow multidirectional active and passive dispersal, and if gene flow occurs it should produce a gradual (net) pattern of influence in the same direction, as was observed in morphology.

Although these explanations appear reasonable, other alternatives exist that merit some speculation. One viable option relates to changes in selection pressure, which can also effect clinal variation (Endler, 1977). In Cuatro Ciénegas, differential predation pressure by a molluscivorous form of the polymorphic fish *Cichlasoma minckleyi* Kornfield & Taylor[1] which feeds heavily on hydrobiids (Taylor & Minckley, 1966; Sage & Selander, 1975; Kornfield *et al.*, 1982), has already been proposed as a major evolutionary force (Vermeij & Covich, 1978). *Cichlasoma minckleyi* is a visual predator, fanning aside soft substrates to expose and eat *Mexipyrgus*, and foraging in vegetation and over hard bottoms in search of other molluscan prey (*e.g. Mexithauma quadripaludium* Taylor and *Nymphophilus minckleyi* Taylor). Decreased incidence of periostracal banding on *Mexipyrgus* in Mojarral East (Table 2, 15–18%) compared to upstream sampling points (77–100%) may indicate that unbanded snails appear more cryptic on or in the light-colored sediments in shallow water. Sediments in Mojarral West are comparably light, but the spring is smaller and deeper, with more shading from banks and travertine ledges that may impart a selective advantage to a more heavily banded shell. A significant increase in shell size at the northern end of Mojarral East Laguna could also reflect an increased selective pressure, with increased size reflecting adaptation affording resistance to a crushing predator (Vermeij & Covich, 1978).

Predation by fishes could also influence population sizes of snails, which in turn might be reflected in temporal changes such as stunting or other density dependent factors. Possibly density dependent influences on sculpture, periostracal banding, *etc.*, are not apparent. No quantitative data on *Mexipyrgus* populations other than a maximum density of 49,000 individuals/m^2 (Hershler, 1985) are available. Differential abundance of the molluscivorous form of cichlid relative to another (detritivorous) form, or changes in absolute abundance (and thus influence) of the predator due to variations in year class strength, might also be significant factors in such a system. As for snails, no adequately quantitive data exist on population size of the basin's fishes (see, however, Minckley, 1984). Hershler's (1985) comment that temporal variations in snail size occur at a given locality was based on casual observation and

[1]Minckley (1984) perceived the Cuatro Ciénegas cichlids to comprise a flock of distinct species rather than a single, polytypic form; the history of the discovery of, and research on, this fascinating problem was reviewed and further discussed by Williams *et al.* (1984).

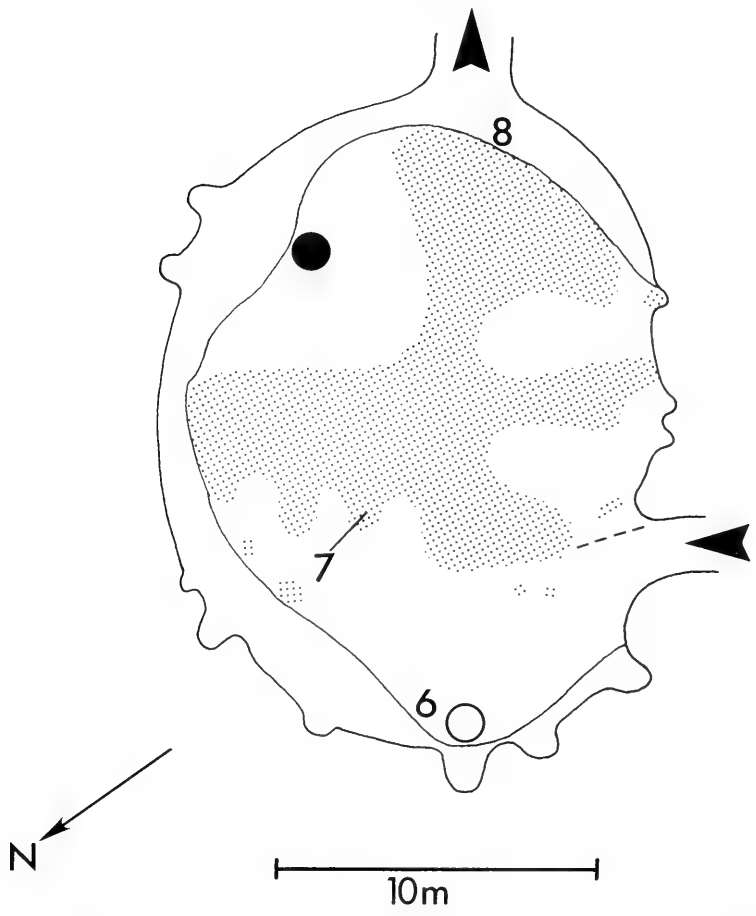


FIG. 8. Map of the "Middle spring." The location of sampling points 6-8 is shown, as are the positions of the large spring orifice (open circle), underwater outflow tube (closed circle), surface inflow and outflow streams (arrows), and extent of the elevated *Nymphaea* reef (stippled). The area in between the outline of the spring and inner line continuing around most of the spring circumference is bare travertine. The N edge of the reef, as well as the area just E of the stream inflow (indicated by a dashed line) are elevated above the bottom of the spring.

has not yet been tested. Another possibility is that sizes of *Mexipyrgus* in Cuatro Ciénegas relate to habitat conditions. The unusually small shell of *M. mojarralis*, for example, could reflect warm water, and the larger shell of *multilineatus* a phenotypic response to cooler or slightly varying water temperatures. Retention of juvenile characters, small size relative to other populations, and various anomalies have been demonstrated in warm-spring fishes (Hubbs, 1959; Miller, 1961; Deacon & Minckley, 1974; Hubbs *et al.*, 1974). However, there seems little correlation among water temperatures and shell size within the Cuatro Ciénegas basin, e.g. *Mexipyrgus* from Laguna Escobeda, one of the warmest large springs, are not exceptionally small relative to animals from cooler habitats such as Laguna Tio Candido, and are larger than specimens from slightly cooler Mojarral East (Taylor, 1966; Hershler, 1985, fig. 37, table 48). Further, *Nymphophilus minckleyi* from three diverse habitats do not differ significantly in size (warm, Mojarral East; cool, Laguna Tío Candido; variable, Río Mesquites; Hershler, 1985, table 3).

Primary or secondary intergradation? An answer to the question of primary vs. secondary intergradation necessitates reexamination of ideas on origins of aquatic habitats now

occupied by *Mexipyrgus*. The following scenario was presented for evolution of large laguna systems:

> "Development of these complex lake-springs begins with sinkhole formation. Subsequently, in a actively-flowing aquifer-system, foundering and possible dissolution of the banks produce a linear, tortuous channel. In systems on the barrial, far from the mountain fronts, continuing headwater foundering probably produces elongate channels similar to that occupied by the Río Mesquites" (Minckley, 1969: 18–19).

Minckley (unpublished data) is now convinced that aggradation may have played as great an alternative role in formation of present-day habitats as do processes of chemical dissolution, foundering, and erosion. Travertine deposits, often masked by accumulation of evaporites or overgrowth of halophytes in moist areas, are substantially more extensive throughout parts of the basin fed by mineralized water than before realized. Included are linings for waterways that grow to enclose flowing streams, broad cones that elevate springs above surrounding terrain, and travertine shields downslope from outflows. These structures are most readily identified in places desiccated by lowering of water table due to canalization (Minckley, 1969, 1978; S. Contreras-Balderas, 1984) or natural processes. Similar fossil to modern deposits have been described for Miocene to Recent springs of the Verde Formation, Arizona (summarized by Donchin, 1983). Cole & Batchelder (1969) documented incipient roofing of a spring outflow by travertine. Minckley (1973) described isolation of an Arizona spring by formation of a travertine dam, as illustrated by Hendrickson & Minckley (1985, fig. 20). Travertine deposition may be chemical due to changes in pH, physical through release of pressure or evaporation, biogenic through algal activity, or a combination of all three processes (Bathurst, 1975; Hardie *et al.*, 1978).

In Cuatro Ciénegas and elsewhere, migration of spring sources and shifts in outflows have obviously occurred through irregular travertine impoundment. As discharge volumes vary, so do spatial relations of deposition. Springs break out to flow from bases of travertine tubes, domes, and shields so that source migrations may occur in essentially any direction. Downslope movement of spring sources that might be expected with discharge decrease, for example, might be countered by travertine accumulation, so that sources remain near mountain fronts despite lowering of the basin floor due to dewatering (see below).

Origin of the molluscan fauna of such a system is problematical. Did the progenitor(s) of *Mexipyrgus* populations achieve a wide distribution through active, upflow dispersal over hard bottoms? Was an ancestral form widely distributed in an intermontane lake, then left as relicts as inflowing springs were isolated by dropping water level? Does the present spring complex represent remnants from a formerly massive, single outflow, fragmented by travertine deposition or other factors? Or, did an ancestor(s) arrive and become distributed within the area by passive means, such as in mud on the feet of waterbirds?

Mexipyrgus is so remarkably restricted to soft sediments that the first query seems readily rejected. Further, other hydrobiids in the basin that commonly occur on hard surfaces, *e.g. Mexithauma quadripaludium* and *Nymphophilus minckleyi* (Taylor, 1966; Hershler, 1984, 1985), show little intraspecific variation attributable to isolation. They presumably disperse (or dispersed in the past, assuming interconnection of now isolated habitats) at rates adequate to maintain panmixia.

Intermontane lakes were common in northern México during wetter periods of the Pleistocene (Miller, 1981). Some filled to top their basin walls or were captured by headward erosion of adjacent streams (Strain, 1966, 1971), while others had maximum levels controlled by climatic factors since they occupy basins that remain closed today. Highest stages of such lakes corresponded to an exceedingly wet period 22,500–15,000 years before present (ybp) (Wendorf, 1961). Minckley (1969) reported no evidence in the Cuatro Ciénegas basin for high level lacustrine conditions, *e.g.* wave-cut terraces or beachlines. He favored presence of lower level lakes on the barrial due to persistent (or periodic) drainage through deep, antecedent channels breaching surrounding mountains. Permanent shoreline marks are not necessarily formed in lakes of short duration or when water onlaps fine-grained sediments of bajadas or basin floors.

Perhaps stabilized dunes in the far western and eastern parts of the Cuatro Ciénegas basin (Minckley, 1969) are mute testimony to presence of such a lake(s). Barrial lake conditions were directly indicated in cores drilled by Mexican government workers (verbally reported to Minckley [unpublished data] as intended for petroleum exploration) in the late 1960s. Sediments in three cores drawn from the basin floor 2–3 km southwest of the village of Cuatro Ciénegas were uniformly saturated with water, malodorous, and variably light in color, with only minor interbedding of dark, apparently organic material. Soft, light-colored sediments appeared as alternating crystalline evaporites and dune sands. Other harder, stony materials were apparent marls, showing varve-like banding as in lacustrine beds deposited below depth of wave action. Thicknesses and depths of various layers below surface could not be ascertained, no conglomerate or travertine was seen, and the drillers did not encounter bedrock at maximum depths of 400–600 m.

In contrast, only minor indications of lake sediments appeared in the areas of present springs on or adjacent to bajadas of surrounding mountains. Four holes drilled by Mexican workers within 1.2 km of Sierra de San Marcos yielded fine-grained (calcareous) spring sediments, organic materials, and travertines, interbedded with angular fanglomerates typical of bajada surfaces (Minckley, unpublished data). Again, no depths or thicknesses of sediment layers could be determined, but drillers encountered limestone bedrock at 75 to 250 m. In that same area, Meyer (1972, 1973) documented a sequence of springs, ciénegas, travertine deposits, and adjacent grasslands similar to those existing today in sediments and pollens of two cores (6.2 and 13.9 m long). Varve-like banding was noted in short segments of his cores, but no extensive lake deposits were penetrated. The same evidences of little or no change in aquatic and/or terrestrial habitats and an absence of lake beds were in four additional cores 2.7 to 7.9 m long, taken at the same time at nearby places (Minckley, unpublished data). Radiocarbon dates near the bottom of Meyer's (1973) longest core indicated an age of ca. 30,000 ybp, providing an estimate of minimum age for springs to have been undisturbed by lacustrine inundation in the Cuatro Ciénegas basin.

Water chemistry of an intermontane lake might have been amenable for *Mexipyrgus* or its ancestor(s) at a time of high inflow volume or external drainage. Present lakes on the floor of the Cuatro Ciénegas basin are almost certainly too saline and fluctuant in chemistry (Minckley & Cole, 1968; Arnold, 1972) to support the taxon. Climatic conditions might also have been more moderate, allowing for at least seasonal dispersal, yet the region has been at a temperate latitude (Dickinson, 1981) and presumably experienced thermal variations for millenia. Soft bottoms would have been available, or springs may have entered the bottom without or with minimal travertine formation due to hypolimnetic conditions.

Evidence of hydrobiid snails associated with lacustrine habitats of western North America is not uncommon. A possibility thus exists that the progenitor(s) of *Mexipyrgus* attained Cuatro Ciénegas springs through intralacustrine dispersal. In one example, however, an abundance of fossil and subfossil *Tryonia protea* (Gould) and lesser numbers of *Fontelicella longinqua* (Gould) around the Salton Sea, California (Gregg & Taylor, 1965; Taylor, 1981), most probably result from erosional reworking of the Mio-Pliocene Bouse Formation (J.J. Landye, unpublished data). Populations of the former persist in only a few thermal springs (Taylor, 1981). Spring-inhabiting *Fontelicella* spp. (determined by Landye, in Donchin, 1983) were apparently restricted to the vicinity of groundwater inflows in the Mio-Pleistocene Verde Lake, Arizona (Donchin, 1983). Hydrobiids of the genera *Durangonella* Morrison, 1945 and *Tryonia* Stimpson, 1865 nonetheless inhabit lakes, springs, marshlands in and near the Cuatro Ciénegas basin. The former was thought related to *Mexipyrgus* by Hershler (1985), an opinion modified by Hershler & Thompson (1986), who demonstrated a nearer relationship between *Mexipyrgus* and *Tryonia*.

The third alternative, a massive spring that now exists as a series of isolated sources, is perhaps more tenable than the presence of major lake(s) and requires less rationalization of habitats. Cretaceous limestones of Texas of the same origins and cavernous qualities of those in northern México (Baker, 1971) provide aquifers for massive springs (Smith, 1971; Brune, 1981), individuals of which now or in the recent past discharge(d) water volumes surely equivalent to total output of the Cuatro Ciénegas basin. Further, discharges of Cuatro Ciénegas springs must have been greater in times of more precipitation and less evapotranspiration. Higher volumes would

tend to move zones of travertine deposition downflow and resist sealing, diversion, and impoundment of outflows, further maintaining thermal and chemical constancy requisite to *Mexipyrgus* evolution. Fragmentation of such a spring could be piecemeal or systematic, and if the latter could help explain trends in morphology indicated by Hershler (1985: 104) for extant *Mexipyrgus* stocks.

Lastly, passive dispersal of *Mexipyrgus* or its ancestor(s) to and/or within the Cuatro Ciénegas basin cannot be precluded. Waterbirds are abundantly attracted to aquatic habitats in an otherwise hostile desert (Urban, 1959; A. Contreras-Balderas, 1984), and bird movements are evident from place to place within the basin. Founding of new populations by a few individual snails in this manner, if such were documentable, could explain confusing distributions of some morphological types (Hershler, 1985) in the basin. Transport by movements of waterbirds could further explain occurrence of *Mexipyrgus* and other snails in isolated habitats where no access is obvious. On the other hand, if passive dispersal resulted in substantial gene flow, differentiation in *Mexipyrgus* should be suppressed or negated. We cannot further assess this mechanism as a factor in the origin and evolution of the present fauna.

The unlikelihood that *Mexipyrgus* disperses upflow over hard bottoms, as evidenced by its ecology (Taylor, 1966; Hershler, 1984, 1985), seems to preclude possibilities that intergradation in the Mojarral system is primary in nature. Divergence of allopatric populations isolated by desiccation of a barrial lake(s) or progressive fragmentation of a large, continuous spring, seem a viable alternative hypothesis for the origin of differentiation, with reintegration of habitats resulting in secondary contact and intergradation. This does not preclude the possibility for passive dispersal as a contributing factor.

Systematics. Taylor's (1966: 189) suspicion that "divergence of the various populations of *Mexipyrgus* may have had nothing to do with reproductive isolation, except through geographic separation" is confirmed, at least in the case of the Mojarral area. Our results clearly demonstrate genetic exchange between two nominal species (*M. mojarralis* and *M. multilineatus*) in the Mojarral system, and provide strong indications of intergradation between *M. multilineatus* of Mojarral East and *M. lugoi* of the Río Mesquites. When one applies the biological species concept, synonymization of nominal *Mexipyrgus* to a single, polytypic species, *M. churinceanus*, is supported (Hershler, 1985).

The three nominal taxa do, however, conform to the concept of subspecies in that they present arrays of populations with distinctive features and restricted distributions. We stress that differences among these snails are not mere correlates of a downstream trend toward increased size. Number of shell bands, for instance, does not significantly correlate with shell height at any of the 16 sampling points, although an apparently spurious correlation ($r = .91$) occurs when comparing means for these characters among points. As mentioned above, subsutural banding does not appear directly related to size, nor does penial lobation, as seen by a lack of size differences among groups of males having "1-1" (mean shell height, 5.71 mm, $n = 21$) or "2-1" (5.89, 9) penial types (pooled data from sampling points 13 and 14; t-test, $p > .05$).

Similar morphological characters involving shell sizes, sculpture, and banding pattern have apparently been independently derived in separated populations of *Mexipyrgus* (Hershler, 1985). Or, do characters in common among populations reflect historical processes and events that we do not yet understand? We have not yet devised a way to separate historic vs. derived conditions or states. Even more enigmatic is the question as to why *Mexipyrgus* shows such differentiation while populations of other genera are almost monotypic in the basin. The amount of time required for (or available for) differentiation of the kind seen in Cuatro Ciénegas *Mexipyrgus* also is poorly understood and in debate. Taylor (1966) suggested serious consideration that habitats of the area may have existed since the middle or early Tertiary, and pointed out that *Mexipyrgus* shared characters with *Tryonia* (late Oligocene or early Miocene to Recent) and *Pyrgophorus* Ancey, 1888 (early Pliocene to Recent). Hershler (1985) considered the possibility that differentiation could have been far more rapid, perhaps within the later Pleistocene. There is little doubt that basin and range topography formed in northern México in early Middle Tertiary (Minckley *et al.*, 1986), and that potentials for hydrobiid snail habitat date to that time and before.

Documentation that hybridization occurs between distinct phenotypes of Cuatro

Ciénegas *Mexipyrgus* presents the further possibility of a complex history of differentiation involving genetic exchange as means of disseminating distinctive variation among populations. Perhaps characters or character sets assort independently in hybrids where they are fixed by selection or by chance. If repeated separation and reintegration of aquatic habitats has in fact occurred, as implied by either the presence of a single, large, travertine-mediated spring outflow fragmented into subsystems or by presence of numerous, temporally-separated barrial lakes amenable for dispersal of *Mexipyrgus*, such may be the case. We cannot answer these questions with the current data set or within the framework of the present paper. The "natural laboratory" of Cuatro Ciénegas springs (Taylor & Minckley, 1966) seems to provide an inexhaustible supply of problems to be explored.

ACKNOWLEDGEMENTS

We thank the Mexican government for providing the necessary permits for collecting freshwater snails in their country. Computing facilities were provided by the University of Florida. Mr. Victor Krantz photographed the shells and Mrs. Molly Ryan (USNM) helped prepare the illustrations. Mr. J. Landye shared with us his ideas concerning snail evolution in Cuatro Ciénegas. Landye as well as Drs. J.A. Endler, M.G. Harasewych and two anonymous reviewers provided useful criticism of the manuscript.

LITERATURE CITED

ARNOLD, E.T., 1972, *Behavioral ecology of pupfishes (Cyprinodontidae, genus* Cyprinodon*) from northern Mexico*. Unpublished Ph.D. dissertation, Arizona State University, Tempe, AZ, U.S.A., x + 128 pp.

AXELROD, D.I., 1979, Age and origin of Sonoran Desert vegetation. *Occasional Papers of the California Academy of Science*, 132: 1–74.

BAKER, C.L., 1971, Geologic reconnaissance in the eastern cordillera of Mexico. *Special Paper of the Geological Society of America*, 131: i–x, 1–83, 20 pls.

BATHURST, R., 1975, Carbonate sedimentology, carbonate sediments, and their diagenesis. *Developments in Sedimentology*, 12: 1–658. Elsevier, Amsterdam, Netherlands.

BRUNE, G., 1981, *Springs of Texas*, vol. 1. Branch-Smith, Inc., Fort Worth, TX, U.S.A., 584 pp.

COLE, G.A. & BATCHELDER, G.L., 1969, Dynamics of an Arizona travertine-forming stream. *Journal of the Arizona Academy of Science*, 5: 271–283.

CONTRERAS-BALDERAS, A.J., 1984, Birds from Cuatro Cienegas, Coahuila, Mexico. *Journal of the Arizona-Nevada Academy of Science*, 19: 77–80.

CONTRERAS-BALDERAS, S., 1984, Environmental impacts in Cuatro Ciénegas, Coahuila, México: a commentary. *Journal of the Arizona-Nevada Academy of Science*, 19: 85–88.

DEACON, J.E. & MINCKLEY, W.L., 1974, Desert fishes. *In* BROWN, G.W., Jr. ed., *Desert biology*, 11: 385–488. Academic Press, New York, NY, U.S.A.

DICKINSON, W.R., 1981, Plate tectonics and the continental margin of California. *In* ERNST, W.R., ed., *The geotectonic development of California*, pp. 1–28. Prentice Hall, Inc., Englewood Cliffs, NJ, U.S.A.

DONCHIN, J.H., 1983, Stratigraphy and sedimentary environments of the Miocene-Pliocene Verde Formation in the southeastern Verde Valley, Yavapai County, Arizona. Unpublished M.S. thesis, Northern Arizona University, Flagstaff, AZ, U.S.A., xvi + 182 pp., 2 pls.

ENDLER, J.A., 1977, *Geographic variation, speciation, and clines*. Princeton University Press, Princeton, NJ, U.S.A., ix + 246 pp.

GREGG, W.O. & TAYLOR, D.W., 1965, *Fontelicella* (Prosobranchia: Hydrobiidae), a new genus of West American freshwater snails. *Malacologia*, 3: 103–110.

HARDIE, L.A., SMOOT, J.P. & EUGSTER, H.P., 1978, Saline lakes and their deposits: a sedimentological approach. *In* MATTER, A. & TUCKER, M.E. eds., Modern and ancient lake sediments. *International Association of Sedimentology, Spec. Bull.* No. 2, pp. 7–41. Blackwell Science Publication, London, England.

HENDRICKSON, D.A. & MINCKLEY, W.L., 1985, Ciénegas, vanishing climax communities of the American Southwest. *Desert Plants* (Special Issue), 6("1984"): 131–175, front and back cover pl.

HERSHLER, R., 1984, The hydrobiid snails (Gastropoda: Rissoacea) of the Cuatro Ciénegas Basin: systematic relationships and ecology of a unique fauna. *Journal of the Arizona-Nevada Academy of Science*, 19: 61–76.

HERSHLER, R., 1985, Systematic revision of the Hydrobiidae (Gastropoda: Rissoacea) of the Cuatro Ciénegas Basin, Coahuila, México. *Malacologia*, 26: 31–123.

HERSHLER, R. & THOMPSON, F.G., 1986, North American Hydrobiidae (Gastropoda: Rissoacea): redescription and systematic relationships of *Tryonia* Stimpson, 1865, and *Pyrgulopsis* Call and Pilsbry, 1886. Submitted to *Nautilus*.

HUBBS, C., 1959, High incidence of vertebral de-

formities in two natural populations of fishes inhabiting warm springs. *Ecology*, 40: 154–155.

HUBBS, C.L., MILLER, R.R. & HUBBS, L.C., 1974, Hydrographic history and relict fishes of the north-central Great Basin. *Memoirs of the California Academy of Science*, 7: 1–259.

KORNFIELD, I., SMITH, D.C., GAGNON, P.S. & TAYLOR, J.N., 1982, The cichlid fish of Cuatro Ciénegas, México: direct evidence of conspecificity among distinct trophic morphs. *Evolution,* 36: 658–664.

MAYR, E., 1963, *Animal species and evolution.* Belknap Press, Harvard University Press, Cambridge, MA, U.S.A., xiv + 797 pp.

MEYER, E.R., 1972, *Late-Quaternary paleoecology of the Cuatro Cienegas basin, Coahuila, México.* Unpublished Ph.D. dissertation, Arizona State University, Tempe, AZ, U.S.A., x + 74 pp.

MEYER, E.R., 1973, Late-Quaternary paleoecology of the Cuatro Ciénegas basin, Coahuila, México. *Ecology*, 54: 982–995.

MILLER, R.R., 1961, Speciation rates in some freshwater fishes of western North America. *In* BLAIR, W.F., ed., *Vertebrate speciation*, pp. 537–560. University of Texas Press, Austin, TX, U.S.A.

MILLER, R.R., 1981, Coevolution of desert and pupfishes (genus *Cyprinodon*) in the American Southwest. *In* NAIMAN, R.J. & SOLTZ D.L., eds., *Fishes in North American deserts.* Wiley, New York, NY, U.S.A.

MINCKLEY, W.L., 1969, Environments of the Bolson of Cuatro Ciénegas, Coahuila, México. *University of Texas at El Paso Science Series*, 2: 1–65.

MINCKLEY, W.L., 1973, *Fishes of Arizona.* Arizona Game Fish Department, Phoenix, AZ, U.S.A., xvi + 293 pp.

MINCKLEY, W.L., 1978, Endemic fishes of the Cuatro Ciénegas basin, northern Coahuila, México. *In* WAUER, R.H. & RISKIND, D.H., eds., Symposium on the biological resources of the Chihuahuan Desert region, United States and Mexico. *U.S. National Park Service Transactions and Proceedings Series*, No. 3 (1977), U.S. Government Printing Office, Washington, DC, U.S.A.

MINCKLEY, W.L., 1984, Cuatro Ciénegas fishes: research review and a local test of diversity versus habitat size. *Journal of the Arizona-Nevada Academy of Science*, 19: 13–21.

MINCKLEY, W.L. & COLE, G.A., 1968, Preliminary limnologic information on waters of the Cuatro Ciénegas Basin, Coahuila, México. *Southwestern Naturalist*, 13: 421–431.

MINCKLEY, W.L., HENDRICKSON, D.A. & BOND, C.E., 1986, Geography of western North American freshwater fishes: description and relationships to intracontinental tectonism. *In* HOCUTT, C.H. & WILEY, E.O., eds., *Zoogeography of North American freshwater fishes.* Wiley, New York, NY, U.S.A.

SAGE, R.D. & SELANDER, R.K., 1975, Trophic radiation through polymorphism in cichlid fishes. *Proceedings of the National Academy of Sciences*, 72: 4669–4773.

SMITH, A.R., 1971, Cave and karst regions of Texas. *In* LUNDELIUS, E.L. & SLAUGHTER, B.H., eds., *Natural history of Texas caves*, pp. 1–14 Gulf Natural History, Dallas, TX, U.S.A.

STRAIN, W.S., 1966, Blancan mammalian fauna and Pleistocene formations, Hudspeth County, Texas. *Bulletin of the Texas Memorial Museum*, 10: 1–55.

STRAIN, W.S., 1971, Late Cenozoic bolson integration in the Chihuahua Tectonic Belt. *In* Symposium in honor of Professor Ronald K. Deford. *West Texas Geological Society Publication* 71-59, pp. 167–173. Midland, TX, U.S.A.

TAYLOR, D.W., 1966, A remarkable snail fauna from Coahuila, Mexico. *Veliger*, 9: 152–228.

TAYLOR, D.W., 1981, Freshwater mollusks of California: a distributional checklist. *California Fish and Game*, 67: 140–163.

TAYLOR, D.W. & MINCKLEY, W.L., 1966, New world for biologists. *Pacific Discovery*, 19: 18–22.

URBAN, E.K., 1959, Birds from Coahuila, Mexico. *Publications of the Museum of Natural History, University of Kansas*, 11: 443–516.

VAN DEVENDER, T.R., 1976, The biota of the hot deserts of North America during the last glaciation: the packrat midden record. *American Quaternary Association Abstracts for 1976 meeting*, pp. 62–67.

VAN DEVENDER, T.R., 1977, Holocene woodlands in Southwestern deserts. *Science*, 198: 189–192.

VERMEIJ, G.J. & COVICH, A.P., 1978. Coevolution of freshwater gastropods and their predators. *American Naturalist*, 112: 833–843.

VIVO ESCOTO, J.A., 1964, Weather and climate of Mexico and Central America. *In* WEST, R.C., ed., *Handbook of Middle American Indians*, Volume I, *Natural Environments and Early Cultures*, pp. 187–215. University of Texas Press, Austin, TX, U.S.A.

WELLS, P.V., 1978, Post-glacial origin of the present Chihuahuan Desert less than 11,500 years ago. *In* WAUER, R.H. & RISKIND, D.H., eds., Symposium on the biological resources of the Chihuahuan Desert region, United States and Mexico. *U.S. National Park Service Transactions and Proceedings* Series, No. 3 (1977), U.S. Government Printing Office, Washington, DC, U.S.A.

WENDORF, F., 1961, An interpretation of late Pleistocene environments of the Llano Estacado. *In Paleoecology of the Llano Estacado*, pp. 115–133. Fort Burgwin Research Center, Museum of New Mexico Press, Albuquerque, NM, U.S.A.

WILEY, E.O., 1981, *Phylogenetics, the theory and practice of phylogenetic systematics.* Wiley, New York, NY, U.S.A., xiv + 439 pp.

WILKINSON, L., 1984, *SYSTAT, the system for statistics (version 2)*. Systat, Inc., Evanston, ILL, U.S.A.

WILLIAMS, J.E., BOWMAN, D.B., BROOKS, J.E., ECHELLE, A.A., EDWARDS, R.J., HENDRICKSON, D.A. & LANDYE, J.J., 1986, Endangered aquatic ecosystems in North American deserts, with a list of vanishing fishes of the region. *Journal of the Arizona-Nevada Academy of Science*, 20("1985"): 1–62, frontispiece.

WISHART, D., 1978, *Clustan user manual (third edition)*. University College London, London, Great Britain, 175 pp.

Revised Ms. accepted 9 June 1986

MALACOLOGIA, 1986, 27(2): 375–402

A. MYRA KEEN (1905–1986):

A. MYRA KEEN (1905–1986): A BRIEF BIOGRAPHY AND MALACOLOGICAL EVALUATION[1]

Robert Robertson

Department of Malacology, Academy of Natural Sciences, Nineteenth and the Parkway, Philadelphia, PA 19103, U.S.A.

Introduction

Professor (Angeline) Myra Keen, who has been called "one of the great giants of American malacology" (Abbott, 1986),[2] died at the age of 80 on January 4, 1986, after a long and courageous bout with cancer. During most of her career she considered herself an invertebrate paleontologist first and a malacologist second. She contributed greatly to both fields. Professor Keen was successful as a teacher, advisor, compiler, researcher, author, scholar, editorial referee, expert on zoological nomenclature, artist, photographer, curator, public speaker, and creator of exhibits. She had an excellent international reputation among her peers, and because several of her books have strong appeal to shell collectors she was famous among them too.

Youth

Professor Keen, who in later years preferred to be called A. Myra Keen or simply Myra Keen (hereafter shortened to "Myra"), was born on May 23, 1905, in Colorado Springs, Colorado, U.S.A. She was an only child, and her upbringing was rural: her parents farmed and raised cattle on a ranch about twenty miles south of Colorado Springs. When only four years old she rode a burro, and when she was thirteen she bought her own horse; she fancied being a rodeo queen! It was on the ranch that the young Myra became interested in the natural sciences. First she showed an interest in birds, then insects[3]. Myra first went to school in a one-room building housing eight grades. After graduating, her liking for birds took her to Colorado College. Initially she intended to become a naturalist, but she ended up majoring in psychology because she did not like dissecting cats, seeing insects in their death throes in cyanide jars, or the smell of formalin. Myra received her A.B. from Colorado College in 1930[4].

Graduate Schools

Myra then moved to California, where she first saw the ocean that was later to become of such interest to her. She went to Stanford University with a scholarship and received her M.A in psychology in 1931. Thence she went to the University of California, at Berkeley, where she was awarded a Ph.D. in psychology in 1934. Her thesis was on "Children's reasoning in psychological and physical causation." She had some biology and geology courses in college, but was largely self-taught in malacology, the field to which she would devote much of her life. The training in statistics that she had as an adjunct to psychology was, though, of later use.

Early years at Stanford University

Myra graduated during the Depression, and could not find a job in psychology. She and her mother moved to the coast at Monterey, where living was inexpensive. Myra made a little money by sewing, and her father kept sending some of the proceeds from a Colorado chicken farm. By chance, Myra one day saw and bought some seashells in a Berkeley curio shop. She became fascinated by them. Shortly

[1]There are extensive papers and other memorabilia from Dr. Keen in the Smithsonian Institution Archives, Washington, D.C. These were not consulted during preparation of this paper. Any more extensive biography should make use of these archives.

[2]The quotations in the remainder of this paper either are from references cited on p. 401, or are Myra's sayings recalled by this author.

[3]Keen, Angeline, 1928, Photographing insects afield. *Photo-Era Magazine*, 61(2): 76–78 (August). In 1936, she also published a photograph of cliff swallow nests in *National Geographic Magazine*, 69(4): 522 (April). Her early photos no doubt were published elsewhere too.

[4]In 1930, when her affiliation was still "Colorado College," Myra published "Growth curves and IQ's, as determined by testing large families" (*School and Society*, 32(831): 737–742 [29 Nov.]).

afterwards, she learned that Ida Shepard (Mrs. Tom Shaw) Oldroyd (1856–1940) was working on shells at Stanford University and wanted a helper. (Mrs. Oldroyd is best remembered for her books on the marine shells of the Puget Sound area (1924) and of the entire west coast of North America (1924–1927)). Myra hastened to volunteer, and in the same year that she received her Ph.D. (1934) she began working at Stanford University, where she was to remain for the rest of her long and active career.

Mrs. Oldroyd, who proved to be no teacher, first had Myra identify and curate land shells. Myra, however, soon came under the influence of Dr. Hubert Gregory Schenck (1897–1960), a Stanford paleontologist whom she found "very stimulating" (Myra used the word "very" sparingly); "from then on I had somebody who could give me the academic guidance that I needed." Schenck steered her interests into paleontology, but first they worked together on a project quantifying marine faunal provinces on the western coast of North America, using as a basis Recent marine mollusks. Myra was appointed Curator of Paleontology in 1936 (a post created just for her and that she retained until 1957). She had become Mrs. Oldroyd's successor. The year 1937 was a banner one: Myra received her first stipend from Stanford!

Many malacologists influenced Myra in her formative years. Among these were the great collectors Emery Perkins Chace (1882–1980) and Elsie Margaret (Herbst) Chace (1885–1975). Junius Henderson (1865–1937) was a visiting scientist (and amateur malacologist) at Stanford in 1934, and taught Myra the use of some of the books in her field. John Quincy Burch (1894–1974), a book and specimen shell dealer, was editor of the mimeographed *Minutes of the Conchological Club of Southern California*—which Myra used as an outlet for some of her early work. Dr. Fred Baker (1854–1938), whom she visited in San Diego in 1936, was another important influence, as was Dr. Paul Bartsch (1871–1960), whom she came to know when she spent a month at the United States National Museum (Smithsonian Institution), Washington, D.C., in 1940. These insights proved useful to Myra as she worked and corresponded with the individuals involved.

World War II

Myra Keen and Dr. Eliot Blackwelder, Chairman of the Department of Geology,

FIG. 1. Prof. A. Myra Keen. Pacific Grove, California. June 20, 1969. Photo by Robert Robertson.

were the only ones left to teach geology during one war year. Myra was the first woman to teach in the department.

Professorship

Belatedly, Myra was appointed Assistant Professor of Paleontology and Research Associate in Geology in 1954. In 1955, a formal course on the Mollusca taught by Myra was introduced as part of the advanced paleontology program at Stanford. She became Curator of Malacology in 1957. In 1960, she became Associate Professor with tenure, and in 1965, at age 60, full Professor. Myra was then one of the three women professors in the sciences at Stanford. She also taught popular for-credit courses in advanced paleontology, biological oceanography, and curatorial methods.

Students

It was Myra who was the main force in training students in malacology at Stanford. She advised more than a dozen advanced degree candidates in geology and biology. Her stu-

FIG. 2. Prof. A. Myra Keen with four of her students. From left to right: Judith T. Smith, Eugene V. Coan, A. Myra Keen, Robert Robertson, and James H. McLean. Pacific Grove, California. June 20, 1969. Photo by Rudolf Stohler.

dents who have gone on to publish on mollusks are (with their main research interests):

Eugene Victor Coan (1943-) [systematics of Recent mollusks, especially bivalves, in the eastern Pacific; zoogeography; nomenclature; history of malacology].

Carole Stentz Hickman (1942–) [Tertiary mollusk faunas; systematics; archaeogastropods; radulae; functional morphology].

Cortez William Hoskins [Recent Cuban molluscan biofacies; petroleum geology].

James Hamilton McLean (1936-) [systematics of Recent eastern Pacific marine mollusks; Archaeogastropoda; hydrothermal vent limpets; Recent Monoplacophora].

David Nicol (1915–) [bivalves, etc.; evolution; ecology; paleontology; systematics; marine zoogeography].

Robert Robertson (1934-) [systematics and natural history of marine gastropods].

Judith Terry Smith (1940–) [systematics, zoogeography and evolution of Cymatiidae; giant pectens; Cenozoic marine mollusks of the eastern Pacific].

Lee Anderson Smith (no kin of Judith) [paleontology and systematics of Clavagellidae; Gulf of Mexico Pleistocene].

Frances Joan Estelle Wagner [Canadian Quaternary marine Mollusca].

Myra always tried to teach her students to think and write clearly, to describe shells well, and to be meticulous in bibliographic and nomenclatural matters.

In her course on *Curatorial methods in paleontology*, Myra would give each student a mixture of heavy and fragile junk shells to pack as if for mailing. She then proceeded to climb on a chair and to hurl the packages one by one onto the floor. Few students passed this test!

Field work

In 1935, in connection with her zoogeographic work with Schenck, Myra and her parents drove to Neah Bay, northwestern Washington, where she began to collect shells at about every degree of latitude south to northern California.

Myra collected in southern California in 1936 (Mission Bay at San Diego), and in the Gulf of California (twice south to Jalisco), Mexico, in 1941, 1956, 1960 (twice), and 1965. On one of these trips she noted "very makeshift conditions" (another "very"!). She was also impressed by the great rise and fall of tides at Puerto Peñasco.

Research interests

Myra's research interests ranged widely: from marine molluscan Cenozoic paleontology, neontology, and zoogeography of western North America, to marine molluscan (especially bivalve) systematics, to zoological nomenclature, to the Recent marine mollusk fauna of tropical West America (the Panamic Province). Above all else, Myra liked unscrambling difficult ("thorny") nomenclatural problems, and writing good synonymies.

Myra was also taxon-oriented: she was particularly interested in the systematics of the Cardiidae, Vermetidae, Muricidae—especially the subfamily Typhinae, and *Berthelinia* (a bivalved gastropod).

Myra's first paper (1936a) concerned a new genus of cardiids (*Clinocardium*). One of her last papers (1980f) reviewed the systematics of the whole family.

The Vermetidae were a challenge; Myra's systematics helped to put them in order (1960h, 1961c, 1982c, d, 1983c; Keen & Hadfield, 1985; Keen & Morton, 1960).

Myra's interest in the Typhinae derived from discovery of a Neogene *Typhis* in California—a new stratigraphic and geographic record (1944a; Keen & Campbell, 1964).

In a small boat, looking through a clump of seaweed that a diver had brought up, Myra discovered living *Berthelinia* on its algal host *Caulerpa* in the Gulf of California, Mexico (1960g; Keen & A.G. Smith, 1961). Previously, this bivalved gastropod had been known only from European fossils and live-collected specimens from the western and central Pacific and Australia.

Publications

Myra was the author of 14 books (including different editions), and 64 malacological papers three or more pages long, and 128 shorter papers and abstracts (see the accompanying bibliography); unrevised reprints and book reviews are not counted. Myra also published three papers on Foraminifera and one on Brachiopoda.

Myra's first book was *An abridged check list and bibliography of* [Recent] *west North American marine Mollusca* (1937g), which supplemented William Healey Dall's (1845–1927) *Summary of the marine shell-bearing mollusks of the northwest coast of America. . . .* (1921). Myra attempted to ascertain the latitudinal range end points of each species, information needed for her zoogeographic work with Schenck.

An *Illustrated* [dichotomous] *key to west North American* [marine] *pelecypod genera* then received Myra's attention. In 1939, she published this in collaboration with Donald Leslie Frizzell (1906–1972); there was a 1953 revision. A companion booklet on gastropods, co-authored with John C. Pearson, appeared in 1952 and was revised in 1958. The two works were revised and combined in *Marine molluscan genera of western North America: an illustrated key* (Keen, 1963b). This was further revised in a second edition, co-authored with Eugene V. Coan, published in 1974.

Schenck & Keen's *California fossils for the field geologist* was issued in preliminary form in 1940(b) and published by Stanford in 1950. Myra co-authored with Herdis Bentson a *Check list of California Tertiary marine Mollusca (1944).*

The beginnings of Myra's greatest book are of interest, told here mainly in her own words. In the middle 1950's, Harry J. Bauer, "a wealthy man in southern California, kept clamoring for a book [on Recent Panamic marine mollusks], and John [Q.] Burch told him that I could do [such] a book. . . . So [Bauer] wrote to me and wanted me to do the book, and I said 'Nothing doing. I'm not interested in doing books.' So he kept after me, until finally I said, 'Well, I'll get a group of students together as a committee, and I'll supervise them. That's all I can do.' So [Bauer] sent money to start the project . . . , and I got my committee together and got them started. One was going to do the cowries, and another was going to do another group. It looked as though we were going fine. Then the committee all fell apart. One man died and another moved away, and there was nobody left but me. We had accepted the money for it, so I was stuck with doing the book. . . . The actual writing . . . took about six months. Of course it took a little longer than that to get the information together [beforehand], and a year or so to get the manuscript into shape for the [Stanford] press to publish it." The result was *Sea shells of tropical west America; marine mollusks from Lower California to Colombia* (ed. 1, 1958d). The second edition (1971c), subsidized by Dwight Willard Taylor (1932-), was greatly revised and enlarged; the southern limit was extended to Peru, and nudibranchs and cephalopods were included; in all, some 3,325 species were treated. This

is Myra's largest and undoubtedly greatest work. It remains the prime reference on eastern Pacific mollusks, and it has had the beneficial effect of stimulating further research.

Myra devoted much time and effort to revising Cenozoic mollusk families for the encyclopedic *Treatise on Invertebrate Paleontology*. Only her archaeogastropod (1960j) and bivalve (1969d) family treatments have been published. It is most regrettable that her work on certain mesogastropod, neogastropod, and opisthobranch families remains unpublished (some of this work has been circulated in manuscript form).

One additional useful nomenclature paper by Myra is published posthumously here (p. 403).

Myra named 3 families, 7 subfamilies, 7 genera, 5 subgenera, 69 species, and 2 subspecies, a total of 93 taxa (see the accompanying alphabetical list), all of them marine mollusks.

Curation

Myra spent many years at Stanford University adding to, cataloging, and systematically arranging the Cenozoic mollusk collection. She enjoyed "trying to make sense out of the things that had been described and putting them in an orderly fashion." As curator, Myra "was proud that the . . . specimens [are] not only well arranged but also identified. She acquired shells from all over the world in return for identifying duplicate lots."

In 1961, Myra believed that the Stanford mollusk collections "rank[ed] among the half-dozen largest university collections in the [United States]." According to Solem (1975), the Recent collection ranked fourteenth among all museum and university collections in the country (judged by the numbers of cataloged lots). Judith T. Smith (1978) has published on the *Primary types in the Stanford paleontological* [and neontological] *type collection*, a very useful compilation and key to Stanford malacological publications.

Myra planned, and with amateur help put together, Stanford's "Conchology Museum" (one room in the Geology Department), one of the nicest such exhibits that I have seen anywhere.

Memberships and elected positions

Myra belonged to numerous paleontological and malacological organizations, only a few of which are mentioned here. She was President of the American Malacological Union [AMU] in 1948. Myra presided over the fourteenth national annual meeting, which was held in Pittsburgh, Pennsylvania; there were 41 attendees. Also in 1948, Myra was one of the chief organizers and Acting Chairman of the Pacific Division of AMU; she was Chairman in 1964. In 1949, Myra was Chairman of the Pacific Coast Section and Fellow of the Paleontological Society. In 1970, she was President of the Western Society of Malacologists [WSM], the organization replacing the Pacific Division of AMU. She was an Honorary Life Member of both AMU and WSM. Myra was also Chairman of the Committee on Nomenclature of the Society of Systematic Zoology.

Honors and other responsibilities

Myra received many awards, and only a few of them are mentioned here. In 1929, as an undergraduate, she was elected to Phi Beta Kappa. Myra was on the Editorial Boards of the *Veliger* since 1960, and of *Malacologia* since its beginning in 1962. In 1964, she was awarded a prestigious John Simon Guggenheim Fellowship (see below under European travels).

Myra was honored publicly by Emperor Hirohito of Japan when he requested a meeting with her during his state visit to the United States in 1975 (Keen, 1985). She talked with him about the molluscan faunas of Japan and northwestern North America, and they exchanged gifts.

In 1979, Myra was the first woman to receive the Fellows Medal from the California Academy of Sciences. In 1984, she received a citation from the Board of Trustees of Colorado College, her alma mater, in recognition of her personal and scholarly accomplishments.

European travels

Myra spent nearly all her adult life in California. She did, though, visit Europe four times, searching for, studying, and photographing type specimens. In 1958, she worked for three weeks at the British Museum (Natural History) [BMNH], London, after which she wanted to make a return visit. Her wish was fulfilled. During parts of 1964 and 1965, when she was on sabbatical leave from Stanford, her Guggenheim Fellowship enabled her to visit Europe for the second and third times. She saw some of the marine

laboratories and worked further at BMNH and some other museums. On the continent, she ranged from Copenhagen to Amsterdam. In 1967 she visited BMNH for another three weeks. Myra published four papers on West American mollusk types at BMNH (1966a, c, e, 1968c).

Professional shortcomings

Myra was more a compiler than a researcher, and she considered nomenclature to be all important. On occasions, she would even decide the outcome of a taxonomic problem from nomenclatural considerations alone. In distinguishing taxa, she often used single characters, and she named some new species distinguished zoogeographically rather than morphologically (such as cognate taxa on the eastern and western coasts of tropical America). Myra never studied radulae or anatomy, her reasons being that she was a paleontologist with a primary interest in bivalves. In her paper on refereeing manuscripts (1978b) she hardly mentioned "value as a contribution to science," i.e. the vital matter of whether a manuscript has substance.

Personality, beliefs and quotations

Myra was the gentlest, most serene person I have known. She also had a marvelous faculty to put at ease anyone with her, and to bring out the best in a person. Myra was shy, but sure of her convictions. She was a pacifist, a nonmilitant feminist, and a conservationist. In 1964, she joined the Religious Society of Friends ("Quakers").

Some quotations that may help to reveal more of Myra the person are:

Values: "People are more important than shells any day."

To a proud amateur with an uninteresting shell: "My, that is a shell!"

On marriage: "I was never particularly adverse to the idea of marriage. I expected to marry some day if the right person came along, but I was never out searching. I was too interested in what I was doing."

On excesses: "One should not have an appetite one cannot control which is likely to lead to excesses. One should avoid excesses of any kind."

On the United States space program: "There are many greater needs here on earth."

Classical music "feeds the spirit. Music continues to be one of the joys of my life." (She particularly liked Brahms. She also liked poetry referring to seashells and to the sea.)

To an A+ student (jokingly): "You haven't left any room for improvement."

Last years and legacy

Myra became Professor of Paleontology Emeritus and Curator of Malacology Emeritus in 1970, and she continued to teach at Stanford until 1972. Then she went into retirement, first in her modest home in nearby Palo Alto, and then in Friends House, a retirement community in Santa Rosa, California, where she spent her last two years.

Myra was one of the world's foremost experts on the systematics of Cenozoic marine mollusks, a well-deserved reputation gained during her 38-year career at Stanford. She attracted and welcomed numerous visitors (providing they did not smoke). She also corresponded extensively, keeping in close touch with ex-students and other friends. Myra did all she could to help amateur shell collectors. Last but not least, she made Stanford a malacological center.

Among the legacies left by versatile Myra are many publications notable for their nomenclatural precision, a series of malacological books found very useful by professionals and amateurs alike, and a dozen or more devoted students—some of whom presently are privileged to try following and going beyond her scholarly footsteps. Myra also left an excellently curated research collection. Unfortunately, she could not prevail upon the Stanford administration to have a continuing professorship or curatorship of malacology at Stanford. Sadly, following Myra's retirement, the entire Stanford collection of fossil and Recent mollusks and other groups was sent on permanent loan to the California Academy of Sciences, San Francisco.

Myra was greatly admired by her students and the host of associates and colleagues who came to know her gentle and gracious ways. Myra's closest relatives are some loving cousins. Her students and associates are her "children."

Acknowledgments

The following colleagues helped greatly by providing information and by criticizing drafts of the manuscript: Dr. Arthur E. Bogan, Dr.

Eugene V. Coan, Jean M. Crabtree, Dr. George M. Davis, Helen DuShane, Dr. Kenneth C. Emberton, Jr., Sandra M. Gardner, Virginia Orr Maes, Dr. James H. McLean, Ellen J. Moore, and Dr. Judith Terry Smith. I alone, though, am responsible for the selection and interpretation of facts.

A. MYRA KEEN (1905–1986): LIST OF MOLLUSCAN TAXA

Eugene Coan

Research Associate,
Department of Invertebrate Zoology,
California Academy of Sciences,
Golden Gate Park,
San Francisco, CA 94118, U.S.A.

Full references are given in the Bibliography beginning on p. 388. Citations to type species of the new generic units, to the genera that are the basis of the new family-level taxa, and to homonyms that she renamed and their preoccupying taxa are not provided.[1]

ABBREVIATIONS: AMNH—American Museum of Natural History [New York] CAS—California Academy of Sciences [San Francisco]; CASPTC—California Academy of Sciences Paleontological Type Collection; LACM—Los Angeles County Museum of Natural History; LSJU—[Leland] Stanford [Junior] University; OD—original designation; SBMNH—Santa Barbara Museum of Natural History; SUPTC-Stanford University Paleontological [and neontological] Type Collection; UCMP—University of California Museum of Paleontology [Berkeley].

LIST OF TAXA

americana, Leptomya—Keen, 1958c: 246, 254, 255, pl. 30, figs. 9, 10, pl. 31, figs. 3, 5, 6. East side of Punta Alegre, San Miguel Bay, Panama; Robert Van Vleck Anderson, 1913. Holotype—SUPTC 8504 [at CAS]. Remarks—Synonym of *L. ecuadoriana* Soot-Ryen, 1957, according to Keen (1971c: 259).

amictoideum, Cymatium (Gutturnium)—Keen, 1971c: 505, 506, fig. 954. 27 to 55 m off the northwestern end of San José Island, Panama Bay; R. G. Shaver. Holotype—SUPTC 10043 [at CAS].

anchuela, Mitrella (Mitrella)—Keen, 1943b: 48, 57, pl. 4, fig. 12. About twelve miles northeast of Bakersfield, Kern County, California; lowermost part of Round Mountain silt, Temblor formation; lower to middle Miocene; LSJU loc. 2121. Holotype—SUPTC 7539 [at CAS]. Remarks—See Addicott (1970: 87, pl. 9, figs. 9, 10, 21, 22).

anomioides, Plicatula—Keen, 1958c: 241–242, 255, pl. 31, figs. 4, 7, 8. Guaymas, Sonora, Mexico, on breakwater in front of Miramar Hotel. Holotype—SUPTC 8500 [at CAS].

(Arctopratulum), Nemocardium—Keen, 1954d: 11–14. Type species (OD)—*N.* (*A.*) *griphus* Keen, 1954 [which see].

Aspellinae—Keen, 1971a: 296, a subfamily based on *Aspella* Mörch, 1877. Remarks—The validity of this subfamily remains in dispute (Vokes, 1975: 122–123).

Axinopsida—Keen & Chavan, in Chavan, 1951: 211, new name for *Axinopsis* Sars, 1878, *non* Tate, 1868.

bakhanstranum, Epitonium (Nitidiscala)—Keen, 1962f: 179. Salt Works, Carmen Island, Gulf of California. Holotype—CASPTC 4763. Remarks—Synonym of *Nitidiscala hindsii* (Carpenter, 1857), according to DuShane (1974: 34), who later elevated *Nitidiscala* to full generic status.

belvederica, Berthelinia (Edenttellina) chloris—Keen & Smith, 1961: 51, 53–61, pl. 5, lower fig., text figs. 18, 19, 21–24, 27–32. Puerto Ballandra Bay, about 10 miles northeast of La Paz, Baja California [Sur]; A. G. Smith, 4 October 1960. Holotype—CASPTC 12317. Remarks—Synonym of *B.* (*E.*) *chloris* (Dall, 1918), according to Keen (1971c: 817).

Bernardinidae—Keen, 1963b: 91, a new family based on *Bernardina* Dall, 1910. Remarks—This family may belong in the Cyamiacea rather than in the Arcticacea, where it was originally placed (Coan, 1984: 228).

berryana, Grippina—Keen, 1971c: 269, 270, fig. 693. Bahía Salinas, Isla Carmen, Gulf of California, in 5 to 9 m. Holotype—SUPTC 10040 [at CAS].

[1]Type localities are given more or less as in the original publications. It is beyond the scope of this paper to correct geological age data, etc.

berryi, Pitar (Pitar)—Keen, 1971c: 167, 168, fig. 397. Off La Cruz, Banderas Bay, Jalisco, Mexico, depth 18 to 37 m. Holotype—SUPTC 10038 [at CAS].

birchi, Nucula (Ennucula)—Keen, 1943b: 41, 55, pl. 3, figs. 9–12. About twelve miles northeast of Bakersfield, Kern County, California; lowermost part of Round Mountain silt, Temblor formation; lower to middle Miocene; LSJU loc. 2121. Holotype—SUPTC 7527 [at CAS].

bravoensis, Turbonilla (Pyrgiscus)—Keen, 1943b: 51–52, 57, 58, pl. 4, figs. 20, 26, 27. About twelve miles northeast of Bakersfield, Kern County, California; lower part of Round Mountain silt, Temblor formation; lower to middle Miocene; LSJU loc. 2121. Holotype—SUPTC 7546 [at CAS]. Remarks—See Addicott (1970: 146, pl. 21, figs. 34–36).

Cabralista—Keen, 1969d: 651, new name for *Cabralia* Böhm, 1899, *non* Moore, 1886.

caulerpae, Mitrella—Keen, 1971c: 589, 590, fig. 1232. Puerto Ballandra, about 10 miles northeast of La Paz, [Baja California Sur], in sand among *Caulerpa* holdfasts; A. G. Smith, 1960. Holotype—CAS 13632 [missing].

Chionista—Keen, 1958c: 242–243. Type species (OD)—*Venus fluctifraga* Sowerby, 1853.

cistula, Lasaea—Keen, 1938a: 22, 24–26, 32, pl. 2, figs. 7–9. Moss Beach, Half Moon Bay, San Mateo County, California. Holotype—SUPTC 6048 [at CAS].

clarki, Typhis (Typhisopsis)—Keen & Campbell, 1964: 48–50, pl. 9, figs. 15, 19, 23. Venado Island, Panama Bay, Walter D. Clark, March 1946. Holotype—SUPTC 9724 [at CAS].

Clinocardium—Keen, 1936a: 119–120. Type species (OD)—*Cardium nuttallii* Conrad, 1837.

coani, Tellina (Angulus)—Keen, 1971c: 211, 212, fig. 512. Candelero Bay, near La Paz, Baja California [Sur]. Holotype—SUPTC 10039 [at CAS]. Remarks—See Gemmell *et al.* (1983).

conchita, Balcis—Keen, 1943b: 43, 57, pl. 4, fig. 5. About twelve miles northeast of Bakersfield, Kern County, California; lowermost part of Round Mountain silt, Temblor formation; lower to middle Miocene; LSJU loc. 2121. Holotype—SUPTC 7538 [at CAS]. Remarks—See Addicott (1970: 57–58, pl. 20, figs. 1, 32).

cultrata, Adrana—Keen, 1958c: 240–241. Seven miles west of Champerico, Guatemala, in 14 fathoms (25 meters). Holotype—CASPTC 9155.

cultrata, Amerycina—Keen, 1971c: 135, 136, fig. 310. Off Isla Partida, Espíritu Santo Island, near La Paz, Baja California [Sur], in 5 to 33 m. Holotype—SUPTC 10037 [at CAS].

decoris, Phyllonotus peratus—Keen, 1960d: 107–108, pl. 10, figs. 4, 5, 7. West Mexican coast near the Guatemalan border; depth about 15 fathoms. Shrimp boat. Holotype—SUPTC 8753 [at CAS]. Remarks—Synonym of *P. peratus*, according to Keen (1971c: 517).

devexa, Episcynia—Keen, 1946: 9–11, pl. 1, figs. 1–4. Scorpion Harbor, Santa Cruz Island, Santa Barbara County, California, in 2 to 3 fathoms. Holotype—SUPTC 7907 [at CAS].

Distichotyphis—Keen & Campbell, 1964: 56. Type species (OD)—*D. vemae* Keen & Campbell, 1964 [which see].

durhami, Ferminoscala—Keen, 1943b: 46, 58, pl. 4, fig. 31. About twelve miles northeast of Bakersfield, Kern County, California; lowermost part of Round Mountain silt, Temblor formation; lower to middle Miocene; LSJU loc. 2121. Holotype—SUPTC 7534 [at CAS]. Remarks—*Scalina durhami* (Keen), according to Addicott (1970: 56–57, pl. 3, figs. 21, 24).

electilis, Moniliopsis—Keen, 1943b: 49, 57, pl. 4, fig. 15. About twelve miles northeast of Bakersfield, Kern County, California; lowermost part of Round Mountain silt, Temblor formation; lower to middle Miocene; LSJU loc. 2121. Holotype—SUPTC 7540 [at CAS]. Remarks—*Ophiodermella electilis* (Keen), according to Addicott (1970: 135).

elytrum, Macoma (Psammacoma)—Keen, 1958c: 244, 254, pl. 30, fig. 14. Southsouthwest of Maldonado Point, [Oaxaca,] Mexico. Holotype—CASPTC 10503.

erythrostigma, Siphonochelus (Siphonochelus)—Keen & Campbell, 1964: 51–52, pl. 10, figs. 27, 31, 35. Moreton Bay area off Brisbane, Queensland, Australia by Mr. Wicks. Holotype—SUPTC 9732 [at CAS].

(Eualetes), Tripsycha—Keen, 1971a: 296. Type species (OD)—*Vermetus centiquadratus* Valenciennes, 1846.

fastigata, Nuculana (Saccella)—Keen, 1958c: 240, 255, pl. 31, figs. 1, 2. Off Ballenas Bay, [Puntarenas Prov.,] Gulf of Nicoya, Costa Rica. . . . 35 fathoms (64 meters). Holotype—CASPTC 9149.

fayae, Anachis (?Costoanachis)—Keen, 1971c: 578, 579, fig. 1178. Guaymas,

Sonora, Mexico. Holotype—SBMNH 33316 [not SBMNH 12658 as originally published].

fayae, Pterotyphis (Tripterotyphis)—Keen & Campbell, 1964: 54–56, pl. 11, figs. 39, 40, 43, 44, text fig. 1. Barra de Navidad, Jalisco, Mexico; Faye Howard & Gale Sphon. January 7–11, 1962. Holotype—SBMNH 15999.

ghanaense, Dendropoma—Keen & Morton, 1960: 44–45, 48–51, pl. 4, figs. 7, 8, text figs. 14–19, 33. Dixcove, 10 to 15 miles west of Takoradi, Gold Coast (i.e. Ghana), West Africa. R. Bassindale, 1953. Holotype—SUPTC 8751 [at CAS] [in a cluster] [not SUPTC 8754 as originally published].

gluma, Volvulella—Keen, 1943b: 54–55, 57, pl. 4, fig. 10. About twelve miles northeast of Bakersfield, Kern County, California; lowermost part of Round Mountain silt, Temblor formation; lower to middle Miocene; Robert T. White; LSJU loc. 2641. Holotype—SUPTC 7550 [at CAS]. Remarks—See Addicott (1970: 141, pl. 20, figs. 3, 4).

gnomon, Hastula—Keen, 1943b: 47, 57, pl. 4, fig. 11. About twelve miles northeast of Bakersfield, Kern County, California; lowermost part of Round Mountain silt, Temblor formation; lower to middle Miocene; Donald Birch; LSJU loc. 2121. Holotype—SUPTC 7536 [at CAS]. Remarks—See Addicott (1970: 127, pl. 17, figs. 23–26).

griphus, Nemocardium (Arctopratulum)—Keen, 1954d: 12–14, 24, pl. 1 figs. 12, 14, 17, text figs. 3–4. Middle fork of Wishkah River, 14 mi. N. of Aberdeen, Grays Harbor Co., Washington; Astoria formation; middle [to late] Miocene; H. Hannibal, 1912; LSJU loc. NP-243. Holotype—SUPTC 8295 [at CAS].

Halistylinae—Keen, 1958d: 260, new subfamily [not indicated as new] based on *Halistylus* Dall, 1890.

hannibali, Clinocardium—Keen, 1954d: 18–19, 21, 24, pl. 1, fig. 16, text fig. 9. Chehalis and Summit Sts., Aberdeen, Washington. . . . Montesano formation, upper Miocene-lower Pliocene; Harold Hannibal, 1912; LSJU loc. NP-235. Holotype—SUPTC 8302 [at CAS].

helenae, Nassarina (Cigclirina)—Keen, 1971c: 592, 594–595, fig. 1247. Guaymas, [Sonora, Mexico;] 45 m. Holotype—SUPTC 10047 [at CAS].

Homalopomatinae—Keen, 1960j: 270, new subfamily based on *Homalopoma* Carpenter, 1864.

imperialis, Typhis (Typhina)—Keen & Campbell, 1964: 46–48, pl. 8, figs. 1–4. Off Tosa, Japan in approximately 200 m. Holotype—Teramachi Collection, Kyoto, Japan.

(Indotyphis), Laevityphis—Keen, 1944: 55, 59–63. Type species (OD)—*Typhis bantamensis* Oostingh, 1933.

insolida, Similivenus—Keen, 1954a: 218, new name for *Venus solida* Deshayes, 1825, *non V. solida* Schroeter, 1802. Acy [Oise], France; Paris Basin Eocene. Holotype—École des Mines, Paris.

ischnon, Olivella—Keen, 1943b: 50, 57, pl. 4, figs. 3, 4. About twelve miles northeast of Bakersfield, Kern County, California; lowermost part of Round Mountain silt, Temblor formation; lower to middle Miocene; Robert T. White; LSJU loc. 2641. Holotype—SUPTC 7542 [at CAS]. Remarks—*O.* (*Olivella*) *ischnon* Keen, according to Addicott (1970: 121–122, pl. 17, figs. 9, 13).

Isoarcidae—Keen, 1969d: 241, new family based on *Isoarca* Münster, 1842.

(Isorobitella), Orobitella—Keen, 1962k: 323–326. Type species (OD)—*O.* (*I.*) *singularis* Keen, 1962 [which see].

jayana, Cancellaria (Narona)—Keen, 1958c: 249–250, 254, pl. 30, fig. 5. Panama Bay, about 1 mile off entrance to Panama Canal, depth about 10 fathoms. Walter D. Clark, 1944. Holotype—SUPTC 8502 [at CAS]. Remarks—*C. (Bivetia) jayana*, according to Keen (1971c: 651).

judithae, Liocerithium—Keen, 1971c: 410–412, fig. 517. Angel de la Guarda Island, Gulf of California. Holotype—SUPTC 10042 [at CAS].

Laevicardiinae—Keen, 1936b: 367, a new subfamily [not indicated as new] based on *Laevicardium* Swainson, 1840.

lampada, Typhis (Talityphis)—Keen, 1943b: 53–54, 56, pl. 3, figs. 14, 19, 23. About twelve miles northeast of Bakersfield, Kern County, California; lowermost part of Round Mountain silt, Temblor formation; lower to middle Miocene; Donald Birch; LSJU loc. 2121. Holotype—SUPTC 7548 [at CAS]. Remarks—See also Keen (1939b) and Addicott (1970: 83, pl. 8, fig. 17, pl. 9, figs. 5, 18).

lens, Teinostoma (Teinostoma?)—Keen, 1943b: 51, 57, pl. 4, figs. 7–9. About twelve miles northeast of Bakersfield, Kern County, California; lowermost part of Round Mountain silt, Temblor formation; lower to middle Miocene; LSJU loc. 2121. Holotype—SUPTC 7545 [at CAS]. Remarks—*Vitrinella (Vitrinellops) lens* (Keen), according to Addicott (1970: 46, pl. 2, figs. 4, 5, 10).

loismartinae, Cylichna?—Keen, 1943b: 44,

57, pl. 4, figs. 16, 18. About twelve miles northeast of Bakersfield, Kern County, California; lowermost part of Round Mountain silt, Temblor formation; lower to middle Miocene; LSJU loc. 2121. Holotype—SUPTC 7532 [at CAS]. Remarks—See Addicott (1970: 139–140, pl. 20, figs. 7, 17, 26).

ludbrookae, Laevityphis (Laevityphis)—Keen & Campbell, 1964: 52–53, pl. 10, figs. 33, 34, 36, a new name for *Typhis tripterus* Tate, 1888, *non T. tripterus* Grateloup, 1833. Adelaide Bore, S[outh] A[ustralia]; clayey green sand, 62 to 63 m level; upper Eocene. Holotype—University of Adelaide, Geology T 453B.

macleani, Decipifus—Keen, 1971c: 586, 588, fig. 1224. Puertecitos, Baja Calif. Norte; J. H. McLean, 1962. Holotype—LACM 1266.

mamillatum, Stephopoma—Morton & Keen, 1960: 28–35, pl. 1, figs. 1, 2, text figs. 1, 2, 5–7, 13, 14. Off Gorée, Sénégal; 50 m. Holotype—Paris Museum [in a cluster].

marchadi, Dendropoma—Keen & Morton, 1960: 37–41, 46–49, 51, pl. 2, figs. 1–3, text figs. 1–5, 23–25, 33. Gorée, Sénégal, in lower intertidal zone. Holotype—Paris Museum [in a cluster].

mariposa, Turbonilla (Pyrgolampros)—Keen, 1943b: 52, 57, 58, pl. 4, figs. 19, 25. About twelve miles northeast of Bakersfield, Kern County, California; lowermost part of Round Mountain silt, Temblor formation; lower to middle Miocene; LSJU loc. 2121. Holotype—SUPTC 7547 [at CAS]. Remarks—See Addicott (1970: 146–147, pl. 21, figs. 17, 26, 40, 42).

medialis, Episcynia—Keen, 1971c: 381, 383, fig. 352. Off Cabo Haro, Guaymas, [Sonora,] Mexico, in 18 m. Holotype—SUPTC 10041 [at CAS].

menuda, Lucinisca—Keen, 1943b: 40–41, 56, pl. 3, figs. 15, 16. About twelve miles northeast of Bakersfield, Kern County, California; lowermost part of Round Mountain silt, Temblor formation; lower to middle Miocene; LSJU loc. 2121. Robert T. White. Holotype—SUPTC 7526 [at CAS].

nipponensis, Siphonochelus (Siphonochelus)—Keen & Campbell, 1964: 50–52, pl. 10, figs. 25, 29. Off Tosa, Japan, in excess of 200 m. Holotype—Teramachi Collection, Kyoto, Japan.

nipponica, Lasaea—Keen, 1938a: 22, 24, 26–28, text fig. 14. Watonoha, Rikuzen, north-east Matsusima, Japan. Holotype—SUPTC 6049 [at CAS].

olssoni, Typhis (Talityphis)—Keen, 1943b: 54, new name for *Typhis (Talityphis) costaricensis* Olsson, 1942, *non T. linguiferus costaricensis* Olsson, 1922. Quebrada Peñitas, Burica Peninsula, [Puntarenas Prov.,] Costa Rica; Pliocene. Holotype—Paleontological Research Institution 4064.

oregonensis, Crassinella—Keen, 1938a: 31–32, pl. 2, figs. 11, 12. See also Keen (1939a: 252). South Slough, near highway bridge, Coos Bay, Oregon; 1 to 2 fathoms . . . M. Keen. Holotype—SUPTC 6052 [at CAS]. Remarks—This was based on a stray valve of *C. lunulata* (Conrad, 1834) brought to Coos Bay with a shipment of oysters for mariculture (Coan, 1979: 4–5).

perata, Nassarina (Cigclirina)—Keen, 1971c: 592, 594, 595, fig. 1248. Puerto Videra, Chiapas, Mexico, 37 to 45 m. Holotype—LACM [1464].

peratus, Phyllonotus—Keen, 1960d: 105–107, pl. 10, fig. 6. 14 mi. SE of Judas Point, [Puntarenas Prov.,] Costa Rica; depth 42 fathoms; March 1, 1938, mud and shell bottom. CAS loc 17974. Holotype—CASPTC 7780.

perplexa, Aspella (Dermomurex)—Keen, 1958c: 248–249, 254, pl. 30, figs. 11–13. Perlas Islands, Panama. Walter D. Clark, 1943. Holotype—SUPTC 8496 [at CAS]. Remarks—Synonym of *A. (D.) indentata* (Carpenter, 1857), according to Keen (1971c: 527).

personatum, Crucibulum—Keen, 1958c: 247–248, 254, pl. 30, figs. 6–8. Panama, James Zetek. Holotype—SUPTC 8498 [at CAS]. Remarks—*C. (Crucibulum) personatum* Keen, according to Keen (1971c: 462, 463, fig. 824).

pomeyroli, Granocardium (Ethmocardium)—Keen, 1954d: 8–9, 24, pl. 1, figs. 2–4, text figs. 1, 2. Coal-bearing beds in area of Moméa tribe, New Caledonia. Upper Cretaceous. R. Pomeyrol, 1951. Holotype—SUPTC 8287 [at CAS].

praeblandum, Clinocardium—Keen, 1954d: 15–16, 21, 24, pl. 1, figs. 1, 6, text figs. 5, 6. West end of Las Trampas Ridge near Walnut Creek, Contra Costa Co., California. Briones formation; lower upper Miocene. Bruce L. Clark. Holotype—UCMP 14836.

precursor, Typhis (Talityphis)—Keen & Campbell, 1964: 49–50, pl. 9, figs. 14, 18, 21, 22. 6 km west of Puerto Colombia, Depto. Atlántico, Colombia; Las Perdices shales, upper Oligocene (possibly lower Miocene); Max Steineke; UC loc. S-8012. Holotype—UCMP 15083.

pristinum, Clinocardium—Keen, 1954d: 16–18, 21, 24, pl. 1, figs. 9, 15, text figs. 7, 8. Southwest part of Shell Ridge, near Walnut Creek, Contra Costa Co., California; San Pablo group, probably Neroly formation; upper Miocene. Holotype—UCMP 14838.

Protocardiinae—Keen, 1951a: 7, new subfamily based on *Protocardia* Beyrich, 1845.

Pteropsellinae—Keen, 1969d: 605–606, new subfamily name based on *Pteropsella* Vokes, 1956 [replaces Pteropsinae Dall, 1894, which was based on a junior homonym].

Ptychomyidae—Keen, 1969d: 655, new family based on *Ptychomya* Agassiz, 1842.

rotundomontana, Chrysallida—Keen, 1943b: 43–44, 58, pl. 4, fig. 28. About twelve miles northeast of Bakersfield, Kern County, California; lowermost part of Round Mountain silt, Temblor formation; lower to middle Miocene; LSJU loc. 2121. Holotype—SUPTC 7531 [at CAS]. Remarks—*Odostomia (Besla) rotundomontana* (Keen), according to Addicott (1970: 143, pl. 21, figs. 29–31).

Samarangiinae—Keen, 1969d: 679, new subfamily based on *Samarangia* Dall, 1902.

scandix, Syrnola—Keen, 1943b: 50–51, 58, pl. 4, figs. 24, 29, 30. Twelve miles northeast of Bakersfield, Kern County, California; lowermost part of Round Mountain silt, Temblor formation; lower to middle Miocene; LSJU loc. 2121. Holotype—SUPTC 7544 [at CAS]. Remarks—Synonym of *Pyramidella (Syrnola) ochsneri* (Anderson & Martin, 1914), according to Addicott (1970: 142, pl. 2, figs. 4–6).

schencki, Laevityphis (Laevityphis)—Keen & Campbell, 1964: 50, 53–54, pl. 9, figs. 16, 20. Puerto Colombia, Dept. Atlántico, Colombia; Las Perdices shales; upper Oligocene (possibly lower Miocene); Hubert G. Schenck, ca. 1933. Holotype—SUPTC 9723 [at CAS].

singularis, Orobitella (Isorobitella)—Keen, 1962k: 323–326, figs. 4, 5. Bahía de San Quintín, Baja California Norte. . . , Mexico, mud flats on northeast part of bay; J. L. Barnard and P. T. Beaudette, April, 1961. Holotype—SUPTC 9518 [at CAS].

(Temblornia), Bornia—Keen, 1943b: 38–39, 55, pl. 3, figs. 6, 7. Type species (OD)—*Donax triangulata* Anderson & Martin, 1914.

temblorensis, Cylichna—Keen, 1943b: 44–45, 57, pl. 4, figs. 13, 14. About twelve miles northeast of Bakersfield, Kern County, California; lowermost part of Round Mountain silt, Temblor formation; lower to middle Miocene; LSJU loc. 2121. Holotype—SUPTC 7533 [at CAS]. Remarks—See Addicott (1970: 140, pl. 20, figs. 10, 11, 18, 25).

teramachii, Typhis (Typhina)—Keen & Campbell, 1964: 48, pl. 8, figs. 9–11. Off Kii, Japan, in more than 100 m. Holotype—Teramachi Collection, Kyoto, Japan.

tholia, Dendropoma—Keen & Morton, 1960: 41–48, 51, pl. 3, figs. 4–6, text figs. 6–13, 20–22, 33. Inhaca Island, off Lorenço Marques, Mozambique . . . in lower balanoid zone. William Macnae, May, 1953. Holotype—SUPTC 8750 [at CAS] [in a cluster] [not SUPTC 8753, as originally published].

Tripsycha—Keen, 1961c: 196. Type species (OD)—*Vermetus tripsycha* Pilsbry & Lowe, 1932.

vemae, Distichotyphis—Keen & Campbell, 1964: 54, 56–57; pl. 11, figs. 45–47. Off the Panama-Costa Rica coast, in 1016 fathoms (uncorrected) = 1892 meters depth; Nov. 30, 1958; Vema Station. V-15-60. Holotype—AMNH 110459

Ventricolaria—Keen, 1954a: 218. Type species (OD)—*Venus rigida* Dillwyn, 1817.

verruculastra, Semele—Keen, 1966d: 32–33. Hannibal Bank, Panama, in 64–73 meters; March, 1938; Sta. 224; CAS loc. 17996. Holotype—CAS 036679, *ex* CASPTC 9256. Remarks—Synonym of *S. formosa* (Sowerby, 1833), according to Coan (1983).

vespera, Nassarina (Nassarina)—Keen, 1971c: 592, 594; fig. 1246. Port Parker, [Bahía Santa Elena, Guanacaste Prov.,] Costa Rica, 27 m. Holotype—CASPTC 13635 [missing].

watsonae, Anachis—Keen, 1943b: 42–43, 57, pl. 4, figs. 1, 2. About twelve miles northeast of Bakersfield, Kern County, California; lowermost part of Round Mountain silt, Temblor formation; lower to middle Miocene; LSJU loc. 2121. Holotype—SUPTC 7530 [at CAS]. Remarks—*A. (Costoanachis) watsonae* Keen, according to Addicott (1970: 86, pl. 9, figs. 11, 12).

whitei, Ferminoscala—Keen, 1943b: 46, 58, pl. 4, figs. 32, 33. About twelve miles northeast of Bakersfield, Kern County, California; lowermost part of Round Mountain silt, Temblor formation; lower to middle Miocene; Robert T. White; LSJU loc. 2121. Holotype—SUPTC 7535 [at CAS]. Remarks—*Scalina whitei* (Keen), according to Addicott (1970: 57, pl. 3, figs. 20, 25–28).

A. MYRA KEEN (1905–1986): MALACOLOGICAL BIBLIOGRAPHY[1,2,3]

Eugene Coan

This Bibliography contains references to works by Dr. A. Myra Keen on the Mollusca and closely related topics, including papers cited in the preceding List of Molluscan Taxa. References to her publications on such other subjects as history, ornithology, photography, psychology, and religion are not included, nor are her published photographs of insects and birds. Her full-scale book reviews are included, but not short publication notices.

When Myra Keen first began publishing photographs and articles about photography in the 1920s, she went under the name Angeline Keen. Later, as she came to prefer her middle name, a transition began—from Angeline M. Keen, through A. Myra Keen, and finally to simply Myra Keen.

In addition to the published sections on bivalves and archaeogastropods that she prepared for the *Treatise on Invertebrate Paleontology*, Dr. Keen revised several mesogastropod, neogastropod and opisthobranch groups that were not published. However, many of the manuscripts have been circulated among interested workers.

Still unpublished is Dr. Keen's treatment of the Vermetidae of the Miocene of the Dominican Republic, co-authored with Peter Jung (see Keen, 1982c, abstract).

In the following citations, volume, bulletin, monograph, memoir, minutes, and special paper numbers are listed in bold face; series numbers (in parentheses) precede volume numbers; issue numbers (in parentheses) follow volume numbers; supplementary information, such as secondary methods of listing volumes, part numbers, and parenthetical statements, are given in brackets. Plates are listed, but not text figures, maps, charts, or tables.

Exact dates of publication are given when possible. The dates of works published by Stanford University Press have been verified through press records. In some cases, these differ from those given by other authors.

ICZN-International Commission on Zoological Nomenclature; Opinions are rulings by ICZN.

CHAVAN, André [*Axinopsida* Keen & Chavan in]

1951, Dénominations superspécifiques de mollusques modifiées ou nouvelles. *Bulletin de la Société Géologique de France*, (6) **1** (*Compte Rendu Sommaire des Séances*, 11–12): 210–212 (July).

KEEN, Angeline Myra

1936a, A new pelecypod genus of the family Cardiidae [*Clinocardium*]. *Transactions of the San Diego Society of Natural History*, **8**(17): 119–120 (12 March).

1936b, Revision of cardiid pelecypods [abst.]. *Proceedings of the Geological Society of America*, **1935:** 367 (June).

1936c, Edgar Allan Poe's conchological text. *Nautilus*, **50**(2): 42–44 (29 Oct.).

1937a, [Review of] "Pyramidellidae from Siogama Bay, northeast Honsyu, Japan," by S. Nomura. *Nautilus*, **50**(3): 108 (29 Jan.).

1937b, Nomenclatural units of the pelecypod family Cardiidae. *Bulletin du Musée royal d'Histoire naturelle de Belgique*, **13**(7): 22 pp. (March).

1937c, Status of west American molluscan names [abst.]. *Proceedings of the Geological Society of America*, **1936**: 385 (July, as "June").

1937d, Percentage method of correlation [abst.]. *Proceedings of the Geological Society of America*, **1936**: 390–391 (July, as "June").

1937e, Statistical methods applied to paleontology [abst.] *Proceedings of the Geological Society of America*, **1936**: 396 (July, as "June").

1937f, Abridged check-list of western North American marine Mollusca [abst.]. *Proceedings of the Geological Society of America,* **1936**: 397 (July, as "June").

1937g, *An abridged check list and bibliography of west North American marine Mollusca*. Stanford, Calif. (Stanford University Press) & London (Oxford University Press), 87 pp. (28 Sept.) [Supplement: Keen, 1956b and 1982e].

1938a, New pelecypod species of the genera *Lasaea* and *Crassinella*. *Proceedings of the Malacological Society of London*, **23**(1): 18–32, pl. 2 (16 March) [see Keen (1939a)].

[1]Many untitled extracts of letters from Myra Keen concerning nomenclature were published in the mimeographed *Minutes of the Conchological Club of Southern California.* Only formally titled papers are listed here. All are well enumerated in *Minutes* 169: 18–20 (1957) and 199: 7 (1960). ED.

[2]Myra Keen published numerous notes and comments in the *Bulletin of Zoological Nomenclature*; only those mentioning mollusks are listed here. ED.

[3]The bibliographic style is more or less that preferred by Dr. Keen.

1938b, West American Cardiidae [abst.]. *Proceedings of the Geological Society of America*, **1937**: 295 (June).

1939a, New pelecypod species of the genera *Lasaea* and *Crassinella* [corrections]. *Proceedings of the Malacological Society of London*, **23**(4): 252 (15 March).

1939b, New *Typhis* from the California Miocene [abst.]. *Bulletin of the Geological Society of America*, **50**(12)[2]: 1972 (1 Dec.).

1940a, The percentage method of stratigraphic dating. *Proceedings of the Sixth Pacific Science Congress (1939) of the Pacific Science Association*, **2**: 659–663 (Fall).

1940b, Molluscan species common to western North America and Japan. *Proceedings of the Sixth Pacific Science Congress (1939) of the Pacific Science Association*, **3**: 479–483 (Fall).

1942a, A statistical analysis of the percentage of living species of mollusks in the Vaqueros formation. Pp. 35–37 [59–61], *in:* Hubert G. SCHENCK & T. CHILDS, "Significance of *Lepidocyclina (Lepidocyclina) californica*, new species, in the Vaqueros formation (Tertiary), California." *Stanford University Publications, University Series, Geological Sciences*, **3**(2): 59 pp. [25–83], 4 pls. (7 July).

1942b, Viability of a marine snail [*Nerita scabricosta*]. *Nautilus* **56**(1): 34–35 (23 July).

1943a, A report on the Stanford University conchological collection. *Minutes of the Conchological Club of Southern California*, **24**: 5–8 (June).

1943b, New mollusks from the Round Mountain silt (Temblor) Miocene of California. *Transactions of the San Diego Society of Natural History*, **10**(2): 25–58, pls. 3, 4 (30 Dec.).

1944, Catalogue and revision of the gastropod subfamily Typhinae. *Journal of Paleontology*, **18**(1): 50–72 (28 Jan.).

1945a, Information about the International Zoological Commission [sic]. *Minutes of the Conchological Club of Southern California*, **46**: 5–6 (March).

1945b, List of shells collected in vicinity of Oro Bay, New Guinea, by Lt. Col. Hubert G. Schenck and associates. *Minutes of the Conchological Club of Southern California*, **49**: 36–38 (June).

1946, A new gastropod of the genus *Episcynia* Mörch. *Nautilus*, **60**(1): 8–11, pl. 1, figs. 1–4 (30 Aug.).

1947a, *Purpura* and *Thais*. *Minutes of the Conchological Club of Southern California*, **70**: 1–2 (June).

1947b, Exhibit of rare shells received at Stanford University during the war [abst]. *American Malacological Union News Bulletin and Annual Report*, **1947**: 16–17 (Dec.).

1949a, History of marine malacology on the Pacific coast of North America [abst.]. *American Malacological Union News Bulletin and Annual Report*, **1948**: 9–10 (March).

1949b, Some techniques for mounting specimens in the museum [abstr.]. *American Malacological Union News Bulletin and Annual Report*, **1948**: 17–18 (March).

1949c, The Pacific Division of the American Malacological Union. *American Malacological Union News Bulletin and Annual Report*, **1948**: 21 (March).

1949d, Note on *Axinopsis*. *Minutes of the Conchological Club of Southern California*, **94**: 1 (Oct.).

1949e, Phylogeny of the genus *Nemocardium* [abst.]. *American Malacological Union News Bulletin and Annual Report*, **1949**: 20 (Nov.).

1949f, The Japanese pearl culture industry [abst.] *American Malacological Union News Bulletin and Annual Report*, **1949**: 21 (Nov.) [see also Keen 1963g)].

1949g, Notes on west American species of "Vermetidae" [abst.]. *American Malacological Union News Bulletin and Annual Report*, **1949**: 23 (Nov.).

1950a, Notes on the history of *Nemocardium* (family Cardiidae). *Journal de Conchyliologie* **90[(4)43]** (1): 23–29 (15 Jan.).

1950b, Species of the cardiid genus *Clinocardium* [abst.]. *American Malacological Union News Bulletin and Annual Report*, **1950**: 22 (Dec.).

1951a, Outline of a proposed classification of the pelecypod family Cardiidae. *Minutes of the Conchological Club of Southern California*, **111**: 6–8 (July) [correction in Minutes **112**: 12 (Aug.)].

1951b, Veneridae of western North America. *Minutes of the Conchological Club of Southern California,* **111**: 9 (July) [unsigned].

1951c, The molluscan names in Renier's "Tavole." *Nautilus*, **65**(1): 8–15 (27 Aug.) [see also Keen 1954c)].

1951d, Outline of a proposed classification of the pelecypod family Veneridae. *Minutes of the Conchological Club of Southern California*, **113**: 2–11 [1–10] (Sept.).

1952a, Support for Dr. L.R. Cox's proposal for the rejection of the "Prodromo" and "Prospetto della classe dei vermi" of Renier as not having been duly "published" as required by the "Règles." Z.N.(S.) 432. *Bulletin of Zoological Nomenclature*, **6**(10): 312 (29 Aug.).

1952b, West American Tertiary molluscan faunas [abst.]. *American Malacological Union Annual Report*, **1952**: 31 (Dec.).

1953a, Three regional check lists of interest to paleontologists. *Journal of Paleontology*, **27**(1): 159 (16 Jan.).

1953b, Errata for Keen & Pearson. *Minutes of the Conchological Club of Southern California*, **126**: 1 (Feb.) [see Keen & Pearson (1952)].

1953c, A progress report on some revisions for the 'Treatise on Invertebrate Paleontology' [abst.]. *American Malacological Union Annual Report*, **1953**: 8–9 (31 Dec.).

1953d, A critique of Dr. J. Brookes Knight's paper, 'Primitive fossil gastropods and their bearing on gastropod classification' [abst.]. *American Malacological Union Annual Report*, **1953**: 24 (31 Dec.).

1954a, *Ventricolaria* and *Similivenus insolida*, new names in the pelecypod family Veneridae. *Journal of Paleontology*, **28**(2): 217–218 (29 April) [reprinted in Keen, 1954b].

1954b, Nomenclatural notes on the pelecypod family Veneridae. *Minutes of the Conchological Club of Southern California*, **139**: 50–55 (June) [partly a reprint of Keen, 1954a].

1954c, Application for a ruling that works credited to S. A. Renier as of the dates 1804 and 1807 were not published within the meaning of Article 25 of the "Règles." Z.N.(S.) 688. *Bulletin of Zoological Nomenclature* **9**(9): 257–262 (22 Oct.) [see Opinions 316, 17 Dec. 1954, and 427, 26 Oct. 1956].

1954d, Five new species and a new subgenus in the pelecypod family Cardiidae. *Bulletins of American Paleontology*, **35**(153): 307–330 [1–24], pl. 29 [pl. 1] (20 Dec.).

1954e, The brackish-water Cardiacea [abst.]. *American Malacological Union Annual Report,* **1954**: 26–27 (Dec.).

1955a, Request for the suppression of the generic name "*Jumala*" Friele, 1882 (class Gastropoda) as a name calculated to give offence on religious grounds. Z.N.(S.) 307. *Bulletin of Zoological Nomenclature*, **11**(2): 61–65 (31 Jan.) [see Opinion 469, 31 May 1957].

1955b, A few minor pelecypod groups revised for the "Treatise on Invertebrate Paleontology" [abst.]. *American Malacological Union Annual Reports*, **1955** [Bulletin **22**]: 28 (31 Dec.).

1955c, Some rare books in the Stanford conchological library [abst.]. *American Malacological Union Annual Reports*, **1955** [Bulletin **22**]: 29 (31 Dec.).

1956a, Comment on a paper by David Nicol [Arctic bivalves]. *Nautilus* **69**(4): 139–140 (10 May).

1956b, *Supplement to "An abridged check list . . . "; papers on west American marine Mollusca, published during the years 1937 to 1956.* 13 pp. Los Angeles Calif. (J. Q. Burch) (Aug.) [mimeographed; reprinted: Keen (1982e)].

1956c, Nomenclatural problems in the Archaeogastropoda [abst.]. *American Malacological Union . . . Annual Report*, **1956** [Bulletin **31**]: 6–7 (31 Dec.).

1958a, [Comment concerning specific names ending in "*-i*" and "*-ii.*"]. *Bulletin of Zoological Nomenclature*, **15**(20–21): 684 (18 April).

1958b, Mode of citation to be adopted for citing an author's name subsequent to the original proposal of a zoological name. Z.N.(S.) 1331. *Bulletin of Zoological Nomenclature* **15**(23–24): 749–750 (9 May).

1958c, New mollusks from tropical west America. *Bulletins of American Paleontology*, **38**(172): 235–255, pls. 30, 31 (23 May) [correction sheet supplied Sept. 1958].

1958d, *Sea shells of tropical west America; marine mollusks from Lower California to Columbia*, ed. 1, Stanford, Calif. (Stanford University Press), xii + 624 pp.; 10 pls. (5 Dec.) [pp. 624–626, with additional errata, included in March 1959 binding; reprinted in 1960 with errata corrected in text].

1959a, Cleaning shells with a vibratool [abst.]. *American Malacological Union . . . Annual Reports*, **1958** [Bulletin **25**]: 34 (1 Jan.).

1959b, The new draft of the International Rules of Zoological Nomenclature [abst.]. *American Malacological Union . . . Annual Reports*, **1958** [Bulletin **25**]: 40 (1 Jan.).

1959c, [Review of] "Manuel de Paléontologie Animale," by L. Moret. *Science*, **129**(3357): 1217–1218 (1 May).

1959d, Some side notes on "Seashells of Tropical West America" [Anomiidae; Muricidae]. *Veliger*, **2**(1): 1–3, pl. 1 (1 July).

1959e, Some type designations in Anomiacea. *Minutes of the Conchological Club of Southern California*, **191**: 22 (Sept.).

1960a, [Review of] "Molluscs," by John E. Morton. *Veliger*, **2**(3): 68–69 (1 Jan.).

1960b, The Molluscan Section, British Museum of Natural History [abst.]. *American Malacological Union . . . Annual Reports*, **1959** [Bulletin **26**]: 36–37 (1 Jan.).

1960c, The London Colloquium on Taxonomy, and its consequences [abst.]. *American Malacological Union . . . Annual Reports*, **1959** [Bulletin **26**]: 40–41 (1 Jan.).

1960d, New *Phyllonotus* from the eastern Pacific. *Nautilus*, **73**(3): 103–109, pl. 10 (25 Jan.).

1960e, Some range extensions for Panamic province Mollusca. *Minutes of the Conchological Club of Southern California*, **194**: 19–19A (Jan.).

1960f, [Review of] "Pectinidae of the eastern Pacific," by Gilbert Grau. *Veliger*, **2**(4): 100–101 (1 April).

1960g, A bivalve gastropod. *Nature*, **186** (4722): 406–407 (30 April).

1960h, Vermetid gastropods and marine intertidal zonation. *Veliger*, **3**(1): 1–2 (1 July).

1960i, The riddle of the bivalved gastropod. *Veliger*, **3**(1): 28–30 (1 July).

1960j, [Discussions of gastropods with Cenozoic type species]. *In* J. Brookes KNIGHT, L. R. COX, A. Myra KEEN *et al.*, eds., "Part **I** [Gastropoda], Mollusca **1**": 351 pp., *in* Raymond C. MOORE, ed., *Treatise on Invertebrate Paleontology*. Lawrence, Kansas (Geological Society of America & University of Kansas) (about 15 Aug.) [Keen contributions: pp. 226–231 [Fissurellacea]; 233–236 [Patellacea, Cocculinacea]; 246–247, 249–262, 263–267, 268–274 [Trochacea]; 275–289 [Neritacea], some groups in cooperation with other workers; see p. 171 for explanation].

1960k, [Review of] "Seashore life of Japan," by K. Baba. *Veliger*, **3**(2): 53–54 (1 Oct.).

1960l, Pelecypoda fossils. *McGraw-Hill Encyclopedia of Science and Technology*, **9**: 613–614 (Oct.).

1961a, High-lights of a collecting trip [Gulf of California]. *Veliger*, **3**(3): 79 (1 Jan.).

1961b, [Review of] "Gastropoda Euthyneura," by A. Zilch. *Veliger*, **3**(3): 88 (1 Jan.).

1961c, A proposed reclassification of the gastropod family Vermetidae. *British Museum (Natural History), Bulletin (Zoology),* **7**(3): 181–214 pls. 54, 55 (24 Feb.).

1961d, [Reviews of] "Indo-Pacific Mollusca," ed. by R. T. Abbott; "Symposium on edible mollusks," by G. D. Waugh *et al. Veliger* **3**(4): 115–116 (1 April).

1961e, What is *Anatina anatina*? *Veliger*, **4**(1): 9–12 (1 July).

1961f, [Reviews of] "Colored illustrations of the shells of Japan," by T. Habe; and "The molluscan shells," by K. Oyama. *Veliger*, **4**(2): 117–118 (1 Oct.).

1961g, A molluscan paradox: the bivalved gastropod. *The Shelletter of Shells and Their Neighbors*, **1**(7): 1–2 (Oct.).

1961h, Comments on the proposal to place the generic name *Gari* Schumacher, 1817, on the Official List unemended. Z.N.(S.) 1461. *Bulletin of Zoological Nomenclature*, **18**(5): 300 (10 Nov.) [see Opinion 910, 5 June 1970].

1961i, Comments on the proposed use of the Plenary Powers to suppress the generic name *Cerastes* Laurenti, 1768. Z.N.(S.) 724. *Bulletin of Zoological Nomenclature*, **18**(6): 354 (17 Nov.) [see Opinion 661, 26 April 1963].

1961j, Comment on the proposed validation of *Panopea* Ménard de la Groye, 1807. Z.N.(S.) 1049. *Bulletin of Zoological Nomenclature*, **18**(6): 376 (17 Nov.) [petition revived by the ICZN Secretary, *Bulletin of Zoological Nomenclature*, **40**(2): 179–183, 1983].

1961k, What is *Anatina anatina*? [abst.]. *American Malacological Union . . . Annual Reports*, **1961** [Bulletin **28**]: 36 (1 Dec). [see Keen (1961e)].

1961l, Oddities among the Pelecypoda [abst.]. *American Malacological Union . . . Annual Reports*, **1961** [Bulletin **28**]: 38–39 (1 Dec.)

1961m, Malacology at Stanford. *School of Mineral Sciences Newsletter, Stanford University, December 1961*: 1 (Dec.).

1962a, Reinstatement of the specific name *Macoma inquinata* (Deshayes). *Veliger*, **4**(3): 161 (1 Jan.).

1962b, On the systematic place of *Cypraea mus*. *Veliger*, **4**(3): 161 (1 Jan.).

1962c, [Review of] "The giant African snail," by Albert R. Mead. *Science*, **135**(3502): 427 (9 Feb.).

1962d, Shallow-water marine research, Gulf of California. *Proceedings of the First National Coastal and Shallow Water Research Conference (Oct. 1961)*: 668–669 (Feb.).

1962e, Comment on the proposed designation of a type-species for *Clathurella* Carpenter, 1857. Z.N.(S.) 518. *Bulletin of Zoological Nomenclature*, **19**(2): 99 (23 March) [see Opinion 666, 12 July 1963].

1962f, Nomenclatural notes on some west American mollusks, with proposal of a new species name [*Apolymetis, Psammotreta, Tresus, Schizothaerus*, Epitoniidae, Oken]. *Veliger*, **4**(4): 178–180 (1 April).

1962g, [Review of] "Fossils: An introduction to prehistoric life," by William H. Matthew, III. *Veliger*, **5**(1): 59 (1 July).

1962h, [Review of] "Sea shells of the world," by R. T. Abbott. *Veliger*, **5**(1): 61 (1 July).

1962i, Comments on the proposed use of the Plenary Powers to suppress the generic name *Pupa* Röding, 1798. Z.N.(S.) 581. *Bulletin of Zoological Nomenclature*, **19**(5): 260 (10 Sept.).

1962j, Comments on a paper by R. T. Abbott [terms for type specimens]. *Veliger*, **5**(2): 95 (1 Oct.).

1962k, A new west Mexican subgenus and new species of Montacutidae (Mollusca: Pelecypoda), with a list of Mollusca from Bahía de San Quintin. *Pacific Naturalist* **3**(9): 321–328 (16 Oct.).

1962l, Small pelecypods: How to identify them [abst.]. *American Malacological Union . . . Annual Reports*, **1962** [*Bulletin* **29**]: 29 (1 Dec.).

1962m, Before Linnaeus [abst.]. *American Malacological Union . . . Annual Reports*, **1962** [*Bulletin* **29**]: 30 (1 Dec.) [see also Keen, 1983a, d)].

1963a, [Review of] "British prosobranch molluscs," by V. Fretter & A. Graham. *Science*, **139**(3550): 102 (11 Jan.).

1963b, *Marine molluscan genera of western North America: An illustrated key*. Stanford, Calif. (Stanford University Press) [vi +] 126 pp. (14 Feb.) ["with the assistance of Eugene Coan"].

1963c, Comments on the proposed validation of Mörch's 1852–1853 catalogue. *Bulletin of Zoological Nomenclature*, **20**(3): 164 (26 April) [see Opinion 714, 26 Nov. 1964].

1963d, Comment on the name of the type-species of *Xenophora*. Z.N.(S.) 1483. *Bulletin of Zoological Nomenclature*, **20**(3): 164 (26 April) [see Opinion 715, 31 Dec. 1964].

1963e, [Review of] "Fossils: A guide to prehistoric life," by F. H. T. Rhodes, H. Zim & P. R. Shaffer. *Veliger*, **6**(1): 54 (1 July).

1963f, Paleontological hoaxes [abst.]. *American Malacological Union . . . Annual Reports*, **1963** [*Bulletin* **30**]: 36 (1 Dec.) [see Keen (1977c)].

1963g, Japanese pearl culture [abst.]. *American Malacological Union . . . Annual Reports*, **1963** [Bulletin **30**]: 36–37 (1 Dec.) [see also Keen (1949f)].

1964a, *Conus gloriamaris*. *Veliger*, **6**(3): 172 (1 January).

1964b, Species tentatively identified [*Neritopsis radula*]. *Hawaiian Shell News*, **12**(7) [ser. **53**]: 6 (May).

1964c, A quantitative analysis of molluscan collections from Isla Espíritu Santo, Baja California, Mexico. *Proceedings of the California Academy of Sciences*, (4)**30**(9): 175–206 (1 July).

1964d, [Review of] "Fossils in America," by J. E. Rawson. *Veliger*, **7**(1): 58–59 (1 July).

1964e, *Purpura*, *Ocenebra*, and *Muricanthus* (Gastropoda): Request for clarification of status. Z.N.(S.) 1621. *Bulletin of Zoological Nomenclature*, **21**(3): 235–239 (7 Aug.) [see Opinion 886, 24 Oct. 1969].

1964f, *Nana* Schumacher, 1817 (Gastropoda): Proposed suppression under the Plenary Powers. Z.N.(S.) 1622. *Bulletin of Zoological Nomenclature*, **21**(4): 303–304 (16 Oct.) [see Opinion 793, 5 June 1970].

1964g, Some nomenclatural problems [abst.]. *American Malacological Union . . . Annual Reports*, **1964** [Bulletin **31**]: 50. (1 Dec.) [concerning Keen (1964e, f)].

1964h, Comment on the proposed emendation under the Plenary Powers to *Cavolinia* of *Cavolina* Abildgaard, 1791. Z.N.(S.) 1103. *Bulletin of Zoological Nomenclature*, **21**(6): 414 (31 Dec.) [see Opinion 883, 12 May 1969].

1964i, Six misidentified type-species in the superfamily Muricacea (Gastropoda). Z.N.(S.) 1623. *Bulletin of Zoological Nomenclature*, **21**(6): 422–428 (31 Dec.) [see Opinion 911, 5 June 1970].

1965a, [Review of] "Van Nostrand's standard catalog of shells," by J. L. Wagner & R. T. Abbott. *Veliger*, **7**(4): 255 (1 April).

1965b, [Review of] "Shelling in the Sea of Cortez," by Paul E. Violette. *Veliger*, **8**(1): 43 (1 July).

1965c, Search for tropical west American molluscan types in some European museums [abst.]. *American Malacological Union . . . Annual Reports*, **1965** [*Bulletin* **32**]: 49–50 (1 Dec.).

1965d, Some contrasting seashores, Pacific and Atlantic [abst.]. *American Malacological Union . . . Annual Reports*, **1965** [*Bulletin* **32**]: 53 (1 Dec.).

1966a, West American mollusk types at the British Museum (Natural History); I. T. A. Conrad and the Nuttall Collection. *Veliger*, **8**(3): 167–172 (1 Jan.).

1966b, [Review of] "A study on the Olividae of the China coast," by Lou Tze-Kong. *Veliger*, **8**(3): 205 (1 Jan.).

1966c, West American mollusk types in the British Museum (Natural History); II. Species described by R. B. Hinds. *Veliger*, **8**(4): 265–275, pls. 46, 47 (1 April) [corrections by editor: *Veliger*, **9**(1): 87 (1 July)].

1966d, Mörch's west Central American molluscan types with proposal of a new name for a species of *Semele*. *Occasional Papers of the California Academy of Sciences*, **59**: 33 pp. (30 June).

1966e, West American mollusk types at the British Museum (Natural History); III. Alcide d'Orbigny's South American collection. *Veliger*, **9**(1): 1–7, pl. 1 (1 July).

1966f, [Reviews of] "Illustrations to 'Catalogue of the collection of Mazatlan shells' by Philip P. Carpenter," by D. C. Brann; "Neogene mollusks from northwestern Ecuador," by A. A. Olsson; "A survey and illustrated catalogue of the Teredinidae . . . ," by R. D. Turner; and "Catalogue of the Paleocene and Eocene Mollusca of the southern and eastern United States," by K. v. W. Palmer & D. C. Brann. *Veliger*, **9**(1): 88–89 (1 July).

1966g, [Reviews of] "British bivalve sea shells," by N. Tebble; and "Mattheva, a proposed new class of Mollusks," by E. L. Yochelson. *Veliger*, **9**(2): 253 (1 Oct.).

1966h, Comment on the request for action on the name *Voluta mitra* Linnaeus, 1758 (Gastropoda). Z.N.(S.) 1728. *Bulletin of Zoological Nomenclature*, **23**(4): 146 (14

Oct.) [see Keen, 1967b; Opinion 885, 24 Oct. 1969].

1966i, *Tectarius* (Mollusca: Gastropoda): Request for validation in its accustomed sense. Z.N.(S.) 1754. *Bulletin of Zoological Nomenclature*, **23**(4): 179–180 (14 Oct.) [see Opinion 871, 28 Feb. 1969].

1966j, *Hippella* Moerch (Mollusca: Pelecypoda): Request for suppression under the plenary powers. Z.N.(S.) 1755. *Bulletin of Zoological Nomenclature*, **23**(4): 181–182 (14 Oct.) [see Opinion 872, 28 Feb. 1969].

1966k, Some notes on the molluscan collections at the University of Copenhagen [abst.]. *American Malacological Union . . . Annual Reports*, **1966** [*Bulletin* **33**]: 73 (1 Dec.).

1967a, [Review of] "Shell collecting. An illustrated history," by S. P. Dance. *Veliger*, **9**(3): 357 (1 Jan.).

1967b, Comment on the proposed validation of *Voluta episcopalis* Linnaeus, 1758. Z.N.(S.) 1728. *Bulletin of Zoological Nomenclature*, **24**(1): 9 (6 March) [see Opinion 885, 24 Oct. 1969].

1967c, Published in synonymy—published as a synonym. *Veliger*, **9**(4): 444 (1 April).

1967d, [Reviews of] "The molluscan families Speightiidae and Turridae . . . ," by A. W. B. Powell; "Shell structure of patelloid and bellerophontoid gastropods," by C. MacClintock; and "How to clean sea shells," by E. Bergeron. *Veliger* **10**(1): 90 (1 July).

1967e, Support for the proposed addition to the Official List of *Biradiolites* d'Orbigny, 1850, and *Durania* Douvillé, 1908. Z.N.(S.) 1765. *Bulletin of Zoological Nomenclature*, **24**(4): 209 (20 Sept.) [see Opinion 891, 24 Oct. 1969].

1968a, [Reviews of] "Van Nostrand's standard catalog of shells" [ed. 2], by J. L. Wagner & R. T. Abbott; and "Chitons and gastropods . . . from the western Pacific Islands," by H. S. Ladd. *Veliger*, **10**(3): 295 (1 Jan.).

1968b, Rediscovery of a lost species, *Columbella procera* Sowerby, 1832 [abst.]. *American Malacological Union . . . Annual Reports*, **1967** [*Bulletin* **34**]: 68 (20 March).

1968c, West American mollusk types at the British Museum (Natural History); IV. Carpenter's Mazatlan collection. *Veliger*, **10**(4): 389–439, pls. 55–59 (1 April).

1968d, [Review of] "Marine botany: An introduction," by E. Y. Dawson. *Veliger*, **11**(1): 83 (1 July).

1968e, Cenozoic invertebrate paleontology, western United States. P. 1334, *in*: Raymond C. MOORE *et al.*, "Developments, trends, and outlooks in paleontology." *Journal of Paleontology*, **42**(6): 1327–1377 (16 Dec.).

1969a, Problems and pitfalls in searching type specimens [abst.]. *Echo (Western Society of Malacologists)* **1**: 9 (20 March).

1969b, An overlooked subgenus and species from Panama [*Alora gouldii*]. *Veliger*, **11**(4): 439 (1 April).

1969c, *Laternula* living on the Pacific coast? *Veliger*, **11**(4): 439 (1 April).

1969d, [Discussions of various families of bivalves]. *In*: L. R. COX *et al.*, eds., "Part **N** [Bivalvia], Mollusca **6**," Vols. **1** & **2**: xxxviii + 952 pp. *In*: R. C. MOORE, ed., *Treatise on Invertebrate Paleontology*. Lawrence, Kansas (Geological Society of America & University of Kansas) (Nov.). [Keen contributions: **1**: 230–231 [Nuculidae], 241 [Isoarcidae], 269–270 [Philobryidae] **2**: 537 [Chlamydoconchacea], 583–639 [Cardiacea, Tridacnacea, Mactracea, Solenacea, Tellinacea], 643–658 [Dreissenacea, Arcticacea, Glossacea], 664–702 [Corbiculacea, Veneracea, Myacea, Gastrochaenacea, Hiatellacea], 843–859 [Pandoracea, Poromyacea, Clavagellacea], some groups in cooperation with other workers].

1970a, Memorial to Joseph John Graham (1909–1967). *Proceedings of the Geological Society of America* **1967**: 211–213 (Jan.).

1970b, New processes in scientific illustration [abst.]. *Echo (Western Society of Malacologists)*, **2:** 29 (9 March).

1970c, Future work needed [abst.] [fauna of the Gulf of California]. *Echo (Western Society of Malacologists)*, **2**: 39 (9 March).

1970d, Comments on the proposed ruling on works on New Zealand Mollusca by R. S. Allan and H. J. Finlay. Z.N.(S.) 1868. *Bulletin of Zoological Nomenclature*, **26**(5/6): 184 (7 April).

1970e, [Review of] "Stratigraphy and paleontology of the marine Neogene formations of the Coalinga region, California," by O. S. Adegoke. *Journal of Paleontology*, **44**(4): 793–794 (23 July).

1970f, Comment on a nomenclatural matter in Mitridae. *Veliger*, **13**(2): 202 (1 Oct.).

1970g, Western marine mollusks. Pp. 51–53, *in*: A. H. CLARKE, ed., "Proceedings of the American Malacological Union Symposium on the Rare and Endangered Mollusks of North America." *Malacologia*, **10**(1): 1–56, 2 pls. (14 Nov.).

1971a, Two new supraspecific taxa in the Gastropoda [*Eualetes*; Aspellinae]. *Veliger*, **13**(3): 296 (1 Jan.).

1971b, [Reviews of] "The systematics and biology of abyssal and hadal Bivalvia," by J. Knudsen; and "Coastal Brazilian sea

shells," by E. C. Rios. *Veliger*, **14**(1): 135 (1 July).

1971c, *Sea shells of tropical west America; marine mollusks from Baja California to Peru*, ed. 2. Stanford, Calif. (Stanford University Press), xiv + 1064 pp., 22 pls. (1 Sept.) [reprinted in April 1984 with only 12 pls.] ["with the assistance of James H. McLean"; additions and corrections: Keen & Coan, 1975a].

1971d, Sea shells of tropical West America, a revised edition [abst.]. *Echo (Western Society of Malacologists)*, **4**: 23–24 (27 Dec.).

1971e, A review of the Muricacea [abst.]. *Echo (Western Society of Malacologists)*, **4**: 35–36 (27 Dec.).

1972a, A taxonomic note [Art. 23-b of the ICZN Code]. *Veliger*, **14**(4): 440–441 (1 April).

1972b, [Review of] "Fossil mollusks of coastal Oregon," by E. J. Moore. *Veliger*, **14**(4): 445 (1 April).

1972c, [Review of] "The sea shells of Sagami Bay . . . ," by T. Kuroda, T. Habe & K. Oyama. *Veliger*, **15**(2): 163 (1 Oct.).

1973a, Suggested generic allocations for some Japanese molluscan species [Cardiidae, Vermetidae, Marginellidae]. *Tohoku University, Science Reports* (2) (Geology), special vol. **6**: 1–6 (25 Feb.) [Marginellidae by Eugene V. Coan & Barry Roth].

1973b, Geologic history of the Pelecypoda (Bivalvia) [abst.]. *Echo (Western Society of Malacologists)*, **5**: 31–32 (5 March).

1973c, Some nomenclatural problems in Sacoglossa. *Veliger*, **16**(2): 238 (1 Oct.).

1973d, Comments on the problem of the type species of *Lucina* (Mollusca: Pelecypoda). Z.N.(S.) 2001. *Bulletin of Zoological Nomenclature*, **30**(2): 75–76 (10 Oct.). [see also Keen & Abbott (1972) and Opinion 1095, 1 Nov. 1977].

1974a, Re *Laura* Trinchese, 1872 (Gastropoda: Opisthobranchia). *Veliger*, **16**(4): 426 (1 April).

1974b, Taxonomic problems in the Sacoglossa [abst.]. *Echo (Western Society of Malacologists)*, **6**: 20–23 (3 April).

1974c, Memorial to John Q(uincy) Burch (1894–1974). *Annual Report of the Western Society of Malacologists*, **7**: 8–9 (12 Nov.) [unsigned].

1974d, Excerpts from and comments on: "Stanford contributions to malacology—an evaluation and appreciation" by Dr. S. Stillman Berry (originally presented to the Stanford meeting of the American Malacological Union, Pacific Division, July 15, 1955). *Annual Report of the Western Society of Malacologists*, **7**: 18–19 (12 Nov.).

1974e, Sidelights on some malacologists, [Tryon, Kossuth, R. v V. Anderson, Willett, Rafinesque]. *Annual Report of the Western Society of Malacologists*, **7**: 37–40 (12 Nov.).

1975a, [Review of] "Revision of Matajiro Yokoyama's type Mollusca from the Tertiary and Quaternary of the Kanto area," by K. Oyama. *Veliger*, **17**(3): 322 (1 Jan.).

1975b, On some west American species of *Calliostoma*. *Veliger*, **17**(4): 413–414 (1 April).

1975c, [Review of] "Molluscan phylogeny: The paleontological viewpoint," by B. Runnegar & J. Pojeta, Jr. *Veliger*, **17**(4): 421 (1 April).

1976a, Pacific outposts of the Tertiary Caribbean Province [abst.]. *Bulletin of the American Malacological Union*, **42** [for **1975**]: 46 (30 Jan.).

1976b, Another check-list plan? [abst.]. *Bulletin of the American Malacological Union*, **42** [for **1975**]: 66 (30 Jan.).

1976c, The letters a curator receives! [abst.]. *Annual Report of the Western Society of Malacologists*, **9**: 48–49 (12 Oct.).

1977a, Comment on "A review of the Eratoidae" by Crawford N. Cate. *Veliger*, **19**(4): 446–448 (1 April).

1977b, [Reviews of] "Bathyal gastropods of the family Turridae . . . ," by C. S. Hickman; "Brazilian marine mollusk iconography," by E. C. Rios; and "*Murex* shells of the world," by G. E. Radwin & A. D'Attilio. *Veliger*, **19**(4): 455–456 (1 April).

1977c, Paleontological hoaxes. *Natural History*, **86**(5): 24, 26, 30 (May).

1977d, A new sea-floor oasis. *Veliger*, **20**(2): 179–180 (1 Oct.).

1977e, Telegraphic style versus normal style. *Veliger* **20**(2): 187 (1 Oct.).

1977f, A deep-water paradox [abst.]. *Annual Report of the Western Society of Malacologists*, **10**: 10 (14 Dec.).

1977g, Guidelines for writers and readers: A workshop [abst.]. *Annual Report of the Western Society of Malacologists,* **10**: 11–12 (14 Dec.).

1978a, [Review of] "Shells of New Zealand," by A. W. B. Powell. *Veliger*, **20**(3): 306 (1 Jan.).

1978b, The role of the editorial referee. *Veliger*, **20**(4): 387–390 (1 April).

1978c, [List of errata for plate legends]. 2 pp. inserted in copies of reprint edition of I. S. Oldroyd, *The marine shells of the west coast of North America*," 4 vols. Stanford, Calif. (Stanford University Press) (19 April).

1978d, [Review of] "Marine shells of southern California," by J. H. McLean. *Veliger* **21**(1): 151 (1 July).

1979a, Phylogeny of the pelecypod family Cardiidae [abst.]. *Annual Report of the*

Western Society of Malacologists, **11**: 11 (9 Jan.).
1979b, [Review of] "A new monoplacophoran limpet from the Continental Shelf off southern California," by J. H. McLean. *Veliger*, **22**(2): 212 (1 Oct.).
1980a, [Review of] "Bivalve mollusks of the western Beaufort Sea," by F. R. Bernard. *Veliger*, **22**(3): 208 (1 Jan.).
1980b, *Spiroglyphus* and *Stoa*, taxonomic problems in the Vermetidae. *Veliger*, **22**(4): 388–391 (1 April).
1980c, Note on a Panamic province cone. *Veliger*, **22**(4): 404 (1 April).
1980d, [Notice of] Bulletin of the Institute of Malacology, Tokyo. *Veliger*, **23**(1): 107–108 (1 July).
1980e, Memorial to Hubert Gregory Schenck, 1897–1960. *Memorials of the Geological Society of America*, **10**: 5 pp. (July) [preprint: March 1980].
1980f, The pelecypod family Cardiidae: A taxonomic summary. *Tulane Studies in Geology and Paleontology*, **16**(1–2): 1–40, 13 pls. (17 Sept.).
1980g, *Pseudocardia* Conrad, 1866, a disregarded name in Carditidae. *Tulane Studies in Geology and Paleontology*, **16**(1–2): 41–44 (17 Sept.).
1980h, *Siphonium*, an over-used name in Mollusca. *Festivus (San Diego Shell Club)*, **12**(10): 125–126 (Oct.).
1981a, [Review of] "Catalogo dei molluschi conchiferi viventi nel Mediterraneo," by P. Piani; and "An outline of classification of living shelled marine mollusks," by K. C. Vaught. *Veliger*, **23**(3): 287–288 (1 Jan.).
1981b, [Review of] "Intertidal invertebrates of California," by R. H. Morris, D. P. Abbott & E. C. Haderlie. *Veliger*, **23**(4): 381 (1 April).
1981c, *Siphonium*, an over-used name in Mollusca [abst.]. *Annual Report of the Western Society of Malacologists*, **13**: 10 (29 June) [see Keen (1980h)].
1981d, In memoriam: Emery P. Chace (1882–1980). *Annual Report of the Western Society of Malacologists*, **13**[1980]: 19–20 (29 June).
1982a, A footnote to the history of malacology in the United States [American Association of Conchologists]. *Bulletin of the American Malacological Union*, **1981**: iv–v (Feb.).
1982b, [Review of] "James Graham Cooper—Pioneer western naturalist," by E. V. Coan. *Veliger*, **25**(1): 90 (1 July).
1982c, Fossil Vermetidae from the Miocene of the Dominican Republic. *Annual Report of the Western Society of Malacologists*, **14**: 15 (13 July).
1982d, Vermetidae of the Gulf of California, Mexico. *Annual Report of the Western Society of Malcologists*, **14**: 16 (13 July).
1982e, Supplement on "An abridged checklist": Papers on West American marine Mollusca, published during the years 1937 to 1956. *Festivus (San Diego Shell Club)*, **14**(9): 107–116 (9 Sept.) [reprint of Keen (1956b)].
1983a, On Linnaeus' bookshelf. *Festivus (San Diego Shell Club)*, **15**(1): 5–15 (Jan.).
1983b, Dr. Rudolf Stohler and *The Veliger*. *Hawaiian Shell News*, **31**(7)[(n.s.)**283**]: 9–10 (July).
1983c, Vermetidae of the tropical eastern Pacific. *Annual Report of the Western Society of Malacologists*, **15**: 10 (30 Aug.).
1983d, On Linnaeus' bookshelf [abst.]. *Annual Report of the Western Society of Malacologists* **15**: 15 (30 Aug.) [see Keen (1983a)].
1984, [Review of] "Illustration of the types named by S. Stillman Berry in his 'Leaflets in Malacology,'" by C. M. Hertz. *Veliger*, **27**(2): 244 (5 Oct.).
1985, Myra Keen and the Emperor of Japan. *Hawaiian Shell News*, **33**(1) [(n.s.)**301**]: 1, 8 (Jan.).
1986, see pp. 403–404 herein.

KEEN, Angeline Myra; & Robert Tucker ABBOTT
1972, Problem of the type species of *Lucina* (Mollusca: Pelecypoda). Z.N.(S.) 2001. *Bulletin of Zoological Nomenclature*, **29**(3): 158–161 (30 Nov.) [see Keen (1973d) and Opinion 1095, 1 Nov. 1977].

KEEN, Angeline Myra; & Herdis BENTSON
1940, Check list of California Tertiary marine Mollusca [abst.]. *Bulletin of the Geological Society of America*, **51**(12)[2]: 1972–1973 (1 Dec.).
1944, Check list of California Tertiary marine Mollusca. *Special Papers of the Geological Society of America*, **56**: viii + 280 pp. (30 Aug.).

KEEN, Angeline Myra; & G. Bruce CAMPBELL
1964, Ten new species of Typhinae (Gastropoda: Muricidae). *Veliger*, **7**(1): 46–57, pls. 8–11 (1 July).

KEEN, Angeline Myra; & Eugene Victor COAN
1963, See Keen (1963b).
1969, *Realia* Baird, 1850 (Gastropoda): Request for suppression under the Plenary Powers. Z.N.(S.) 1878. *Bulletin of Zoological Nomenclature*, **26**(2): 99–104 (8 Aug.) [see Opinion 973, 31 Dec. 1971].
1974, *Marine molluscan genera of western North America; an illustrated key*, ed. 2. Stanford, Calif. (Stanford University Press) vii + 208 pp. (2 May).
1975a, "Sea shells of tropical West America": Additions and corrections to 1975. *Occasional Paper of the Western Society of Malacologists*, **1**: 66 pp. (22 June).
1975b. Notice concerning Occasional Paper 1 of the Western Society of Malacologists.

Annual Report of the Western Society of Malacologists **8**: 5–6 (1 Nov.) [unsigned; more "additions and corrections"].

KEEN, Angeline Myra; Eugene Victor COAN; & Barry ROTH
1973, See Keen (1973a).

KEEN, Angeline Myra; & Charlotte L. DOTY
1942, An annotated check list of the gastropods of Cape Arago, Oregon. *Oregon State Monographs, Studies in Zoology*, **3**: 16 pp. (15 May).

KEEN, Angeline Myra; & Donald Leslie FRIZZELL
1939, *Illustrated key to West North American pelecypod genera*. Stanford, Calif. (Stanford University Press) 28 pp. (20 Feb.) [reprinted in 1946].
1953, *Illustrated key to west North American pelecypod genera*, revised ed. Stanford, Calif. (Stanford University Press), 32 pp. (2 Feb.) [superseded by Keen (1963b) and Keen & Coan (1974)].

KEEN, Angeline Myra; & Michael G. HADFIELD
1985. *Spiroglyphus* Daudin, 1800 and *Stoa* de Serres, 1855 (Mollusca, Gastropoda, Vermetidae): Proposed suppression of two equivocal generic names. Z.N.(S.) 2340. *Bulletin of Zoological Nomenclature*, **42**(1): 46–49 (2 April) [no Opinion published yet].

KEEN, Angeline Myra; & James H. McLEAN
1971, See Keen (1971c).

KEEN, Angeline Myra; & John Edward MORTON
1960, Some new African species of *Dendropoma* (Vermetidae: Mesogastropoda). *Proceedings of the Malacological Society of London*, **34**(1): 36–51, pls. 2–4 (April).

KEEN, Angeline Myra; & Siemon William MULLER
1948, See Schenk & McMasters (1948).
1951, Objection to the proposed acceptance of "*Gryphaea angulata*" Lamarck, 1819, as the type species of the genus "*Gryphaea*" Lamarck, 1819 (Class Pelecypoda): Comment on proposal submitted by M. Gilbert Ranson. Z.N.(S.) 365. *Bulletin of Zoological Nomenclature*, **2**(11): 332 (28 Sept.) [see Opinion 338, 17 March 1955].
1952, Proposed use of the Plenary Powers to suppress the generic name "*Acmea*" Hartmann, 1821, and to validate the generic names "*Acmaea*" Eschscholtz, 1833, and "*Truncatella*" Risso, 1826 (class Gastropoda). Z.N.(S.) 27. *Bulletin of Zoological Nomenclature*, **9**(4–5): 130 (30 Dec.).
1953, Comment on the question of the species to be accepted as the type species of a nominal genus, the name of which was published prior to 1st January, 1931, in the synonymy of a genus. *Bulletin of Zoological Nomenclature*, **10**(10–11): 342 (24 July).
1956, See Schenk & McMasters (1956).
1959, See Schenk & McMasters (1959).

KEEN, Angeline Myra; & John C. PEARSON
1952, *Illustrated key to west North American gastropod genera*. Stanford, Calif. (Stanford University Press), 39 pp. (19 June) [offset errata sheet issued 11 Feb. 1953; see Keen (1953b); volume reprinted in Oct. 1958] [superseded by Keen (1963b) and Keen & Coan (1974)].

KEEN, Angeline Myra; Norman John SILBERLING; & Benjamin Markham PAGE
1971, [Memorial to] Siemon William Muller (1900–1970). *Bulletin of the American Association of Petroleum Geologists*, **55**(1): 133–134 (Jan.).

KEEN, Angeline Myra; & Allyn Goodwin SMITH
1961, West American species of the bivalved gastropod genus *Berthelinia*. *Proceedings of the California Academy of Sciences*, (4)**30**(2): 47–66, pl. 5 (20 March).

KEEN, Angeline Myra; & Thomas F. THOMPSON
1946, Notes on the Gatun formation (Miocene), Panama Canal Zone [abst.]. *Bulletin of the Geological Society of America*, **57**(12)[2]: 1260 (Dec.).

MORTON, John Edward; & Angeline Myra KEEN
1960, A new species of *Stephopoma* (Siliquariidae: Mesogastropoda) from the eastern Atlantic Ocean. *Proceedings of the Malacological Society of London*, **34**(1): 27–35, 1 pl. (April).

PAGE, Benjamin Markham; Norman John SILBERLING; & Angeline Myra KEEN
1975, Memorial to Siemon W. Muller, 1900–1970. *Memorials of the Geological Society of America*, **4**: 142–146.

SCHENCK, Hubert Gregory; & Angeline Myra KEEN
1936a, Bathymetric distribution of marine Pelecypoda [abst.]. *Proceedings of the Geological Society of America*, **1935**: 367–368 (June).
1936b, West American marine molluscan provinces [abst.]. *Proceedings of the Geological Society of America*, **1935**: 413–414 (June).
1936c, Marine molluscan provinces of western North America. *Proceedings of the American Philosophical Society*, **76**(6): 921–938 (Dec.).
1937, An index-method for comparing molluscan faunules. *Proceedings of the American Philosophical Society*, **77**(2): 161–182 (26 Feb.).
1940a, Biometrical analysis of molluscan assemblages. Pp. 379–392, 2 pls., *in* "Contribution à l'étude de la répartition actuelle et passée des organismes dans la zone néritique." *Mémoires de la Société de Biogéographie*, **7**: 436 pp., 9 pls. (25 May).
1940b, *California fossils for the field geologist* (preliminary edition). Stanford, Calif.

(Stanford University) 86 pp., 56 pls. (25 June) [not by Stanford University Press].

1941, Renaming primary homonyms after generic reallocation [abst.]. *Bulletin of the Geological Society of America,* **52**(12) [2]: 1983 (1 Dec.).

1942, Renaming primary homonyms after generic reallocation. *Journal of Paleontology*, **16**(6): 779–780 (9 Nov.).

1950, *California fossils for the field geologist*, ed. 1. Stanford, Calif. (Stanford University Press), 88 pp., 56 pls. (12 Sept.) [reprinted June 1955].

SCHENK, Edward Theodore; & John Herbert McMASTERS

1948, *Procedure in Taxonomy*, revised ed. Stanford, Calif. (Stanford University Press), vii + 93 pp. (apparently early 1948) [revised by Angeline Myra Keen & Siemon William Muller].

1956, *Procedure in taxonomy*, ed. 3. Stanford, Calif. (Stanford University Press), vii + 119 pp. (20 Sept.) [revised by Angeline Myra Keen & Siemon William Muller].

1959, *Procedure in taxonomy*, ed. 3. 2nd printing ("with substantial additions"). Stanford, Calif. (Stanford University Press), vii + 149 pp. (5 Nov.) [revised by Angeline Myra Keen & Siemon William Muller].

ACKNOWLEDGMENTS

I thank the following persons for providing information, advice, materials, or assistance: Bill Carver, Robert Hollywood, Ellen J. Moore, Robert Robertson, Suzie Ruggles, and Bob and Marie Schutz.

INDEX OF SPECIFIC KEY WORDS IN TITLES AND CONTENTS OF MOLLUSCAN PAPERS

Singly-authored papers are indicated by years and letters only; co-authored papers are indicated by "K" and the last name(s) of the other author(s), years, and letters (where these are needed). Book reviews are omitted.

OTHER LITERATURE CITED OR CONSULTED

[ABBOTT, R.T.], 1986, A. Myra Keen—1905–1986. *Nautilus*, 100(1): 38 (31 Jan.).

ABBOTT, R.T. & YOUNG, M.E., eds., 1973, *American malacologists . . .* , ed. 1 [only ed.]. American Malacologists, Falls Church, Virginia, iv + 494 pp.

ADDICOTT, W.O., 1970, Miocene gastropods and biostratigraphy of the Kern River area, California. [United States] *Geological Survey Professional Paper*, 642: 174 pp., 21 pls.

AMERICAN MEN AND WOMEN OF SCIENCE, 1976. Bowker, New York and London, ed. 13, 3: 2267.

ANONYMOUS, 1958, Sea shells of tropical west America—a new book by Myra Keen for the amateur sea shell collector. [Stanford University] *Mineral Sciences Newsletter*, p. 9 (Dec.).

ANONYMOUS, 1986a, A. Myra Keen, Stanford professor, 79, authority on seashells, mollusks. *San Jose Mercury News*, 135(8): 7-B (8 Jan.).

ANONYMOUS, 1986b, Dr. A. Myra Keen 1906 [sic]–1986. *Hawaiian Shell News*, 33(3): 12 (March).

COAN, E.V., 1979, Recent eastern Pacific species of the crassatellid bivalve genus *Crassinella*. *Veliger*, 22(1): 1–11, 4 pls. (1 July).

COAN [E.V.], 1983a, *Transcript of oral history of Myra Keen*. Tapes in Smithsonian Institution Archives; copies of tape and transcript in American Malacological Union Archives at Academy of Natural Sciences of Philadelphia (taped Sept.).

COAN, E.V., 1983b, A *Semele* story (Bivalvia: Semelidae). *Nautilus*, 97(4): 132–134 (28 Oct.).

COAN, E.V., 1984, The Bernardinidae of the eastern Pacific (Mollusca: Bivalvia). *Veliger*, 27(2): 227–237, 3 pls. (5 Oct.).

COAN, E.V., 1986a, Myra Keen, 1905–1986: an appreciation. *The Festivus* (San Diego Shell Club), 18(2): 14–15 (14 Feb.).

COAN, E. [V.], 1986b, A. Myra Keen (1905–1986). *Veliger*, 29(1): 2 (1 July).

DuSHANE, H., 1974, The Panamic-Galapagan Epitoniidae. *Veliger*, 16 (Suppl.): 84 pp., 15 pls. (31 May).

EVITT, W.R., INGLE, J.C., Jr. & KRAUSKOPF, W.B., 1986, Memorial resolution: A. Myra Keen 1905–1986. *Stanford University Campus Report*, May 21, pp. 14–15.

GEMMELL, J., MYERS, B.W. & HERTZ, C.M., 1983, Observations on *Tellina coani* Keen, 1971. *The Festivus* (San Diego Shell Club), 15(10): 103–104 (13 Oct.).

GIBBONS, A., 1979, Stanford professor receives prestigious science award. *Peninsula Times Tribune*, 1(200): B-1 (20 Nov.).

GREEN, L., 1975a, Shell expert to meet Hirohito. *Palo Alto Times*, 83(242): 13 (9 Oct.).

GREEN, L., 1975b, Meeting an emperor, seated 'like a queen.' *Palo Alto Times*, 83(243): 1–2 (10 Oct.).

GREEN, L., 1984, Myra Keen pulls up roots after a life devoted to science. *Peninsula Times Tribune*, 6(44): C-1, C-6 (13 Feb.).

HOWARD, F.B., 1970, Myra Keen and the singing snails. *The Tabulata* (Santa Barbara Shell Club), 3(3): 3–8 (1 July).

JOHNSTON, T., 1986, Myra Keen, world-renowned expert on seashells, dies at 80. *Stanford University Campus Report*, 18(14): 4 (8 Jan.).

KING, C.L., 1983, Stanford geologist became first lady. *Stanford Earth Scientist Section of the Stanford Observer*, October: 7–8.

KREISLER-BOMBEN, K. von, 1984, Myra Keen, Pp. 44–45 in: So, who's retired? *Stanford Magazine*, 12(4): 42–47 (Winter).

MOORE, E.J., 1986, [untitled obituary]. *Shells and Sea Life*, 18(1): 5 (Jan.).

MOORE, G.W., 1986, A[ngeline] Myra Keen. *Geotimes*, 31(4): 26.

PALO ALTO FRIENDS MEETING, 1986, *Memorial minute for Myra Keen*, First month 1986, 2 pp.

SCHUTZ, R. & RUTH, S., 1983, "There is a Beyond we're living in . . . " Interviews with Myra Keen. . . . *Friends Bulletin*, 51(7): 111–115 (April).

SMITH, J.T., 1978, Primary types in the Stanford paleontological [and neontological] type collection *Bulletins of American Paleontology*, 72(300): 313–552 (14 March).

SMITH, J.T., 1986, A. Myra Keen (1905–1986). *Veliger*, 28(4): 463–464 (1 April).

SOLEM, A., 1975, The Recent mollusk collection resources of North America. A report to the Association of Systematics Collections. *Veliger*, 18(2): 222–236 (1 Oct.).

STANFORD [UNIVERSITY] FACULTY, 1980 ed., p. 13.

TIMES TRIBUNE STAFF, 1986, A. Myra Keen, seashell expert, former Stanford professor, dies. *Peninsula Times Tribune*, 8(8): C-6 (8 Jan.).

VOKES, E.H., 1975, Cenozoic Muricidae of the western Atlantic region. Part VI—*Aspella* and *Dermomurex*. *Tulane Studies in Geology and Paleontology*, 11(3): 121–162, 7 pls. (5 Feb.).

LIST OF 40 TAXA NAMED IN HONOR OF DR. A. MYRA KEEN

Mainly after Howard (1970)

Unless otherwise indicated, the taxa are molluscan.

Trachycardium keeni Glibert, 1936
Septifer keeni Nomura, 1936
Chattonia trigonata keeni Chavan, 1939
Alvania (Willettia) keenae Gordon, 1939
Anomalina keenae Martin, 1943 (Foraminifera)
Glycymeris keenae Willett, 1944
Ocenebra keenae Bormann, 1946
Permopora keenae Elias, 1947 (Bryozoa)
Rissoina keenae Smith & Gordon, 1948
Schizothaerus keenae Kuroda & Habe, 1950
Bornia (Temblornia) keenae Marks, 1951
Keenaea Habe, 1951
Teinostoma myrae Pilsbry & Olsson, 1952
Venericardia keenae Verastegui, 1953
Ensis myrae Berry, 1953
Pyrina keenae Hamilton, 1956 (Echinoidea)
Angaria keenae Von der Osten, 1957
Chione keenae Soot-Ryen, 1957
Stephopoma myrakeenae Olsson & McGinty, 1958
Nomaeopelta myrae Berry, 1959
Eocypraea (Apiocypraea?) keenae Woodring, 1959
Trivia myrae Campbell, 1961
Periploma keenae Rogers, 1962
Glyphostoma myrakeenae Olsson, 1964
Transenpitar keenae Fischer-Piette & Testud, 1968
Typhis (Rugotyphis) keenae Gutmann, 1969
Cinclidotyphis myrae DuShane, 1969
Mitrolumna keenae Emerson & D'Attilio, 1969
Clinocardium myrae Adegoke, 1969
Scissurella keenae McLean, 1969
Murexiella keenae E. Vokes, 1970
Aspella myrakenae Emerson & D'Attilio, 1970
Calliostoma keenae McLean, 1970
Glyphostoma myrae Shasky, 1971
Callucina keenae Chavan in Moore, 1971
Primovula myrakeenae Azuma & Cate, 1971
Littorina keenae Rosewater, 1978
Myrakeenini Harry, 1985 (new tribe)
Myrakeena Harry, 1985 (new genus)
Tritonia myrakeenae Bertsch & Mozqueira, 1986

MALACOLOGIA, 1986, 27(2): 403–404

SOME IMPORTANT SOURCES FOR SUBSEQUENT DESIGNATIONS OF THE TYPE SPECIES OF MOLLUSCAN GENERA[1]

A. Myra Keen
(posthumous)
Stanford University

MONTFORT, D. de, 1810, *Conchyliologie systématique*. Paris, 2: 676 pp.

SCHMIDT, F.C., 1818, *Versuch über die beste Einrichtung zur Aufstellung, Behandlung und Aufbewahrung der verschieden Naturkörper und Geganstände der Kunst vorzüglich der Conchylien-Sammlungen*. . . . Gotha, 8 + 252 pp. See WINCKWORTH, R., 1944, *Proceedings of the Malacological Society of London*, 26(1): 23–24.

FLEMING, J., 1818, 1822, Conchology. Mollusca. Supplement to eds. 4–6 of *Encyclopaedia Britannica*, 3: 284–314; 5: 567–577. See WINCKWORTH, R., 1929, *Proceedings of the Malacological Society of London*, 18(5–6): 224–228, 263.

CHILDREN, J.G., 1822–1824?, Lamarck's genera of shells. *Quarterly Journal of Science*, 14: 64–86; 14: 298–322; 15: 23–52; 15: 216–258; 16: 49–79; 16: 241–264. Also reprint, 177 pp. See KENNARD, A.S., SALISBURY, A.E. & WOODWARD, B.B., 1931, *Smithsonian Miscellaneous Collections*, 82(17): 1–40.

ANTON, H.E., 1838, *Verzeichniss der Conchylien*. . . . Halle, xvi + 110 pp. Date corrected from 1839 following statement on p. 110 and CERNOHORSKY, W.O., 1978, *Veliger*, 20(3): 299.

HERRMANNSEN, A.N., 1846, 1849, 1852, *Indicis generum malacozoorum primordia*. . . . Kassel, 2 vols., xxvii + 637 + xxix–xlii + 717 + v + 140 pp.

GRAY, J.E., 1847, A list of the genera of Recent Mollusca, their synonyma and types. *Proceedings of the Zoological Society of London*, 15: 129–219.

WOODWARD, S.P., 1851–1856, *A manual of the Mollusca*. . . . [ed. 1]. London, pp. xviii + 486 + 24 pp.

STOLICZKA, F., 1867–1868, Cretaceous fauna of southern India. The Gastropoda. *Memoirs of the Geological Survey of India; Palaeontologia Indica*, [ser. 1], 2: 498 pp., 28 pl.

TATE, R., 1868, *Appendix to the Manual of Mollusca of S.P. Woodward*. . . . [in ed. 2], London, 86 pp.

STOLICZKA, F., 1870–1871, Cretaceous fauna of southern India. The Pelecypoda, with a review of all known genera of this class, fossil and Recent. *Memoirs of the Geological Survey of India: Paleontologia Indica*, [ser. 1], 3: xxii + 1–537 pp.

KOBELT, W., 1876–1881, *Illustrirtes Conchylienbuch*. Nürnberg, 2 vols., xvi + 392 pp. 112 pl. Dates from REHDER, H.A., 1952, *Nautilus*, 66(2): 59–60.

BUCQUOY, E., DAUTZENBERG, P. & DOLLFUS, G., 1882–1898, *Les Mollusques marins du Roussillon*. Paris, 2 vols., 570 + 884 pp.

COSSMANN, [A.E.M.], 1886–1891, Catalogue [illustré] des coquilles fossiles de l'Éocène des environs de Paris. . . . [fasc. 1–5]. *Annales de la Société Royale Malacologique de Belgique*, 21: 17–186; 22: 3–214; 23: 3–324; 24: 3–381; 26: 3–163.

PILSBRY, H.A. *et al.*, 1888–1898, *Manual of Conchology*, ser. 1, 10(2): 161 to 17: 348. Conchological Section, Academy of Natural Sciences of Philadelphia.

DALL, W.H., 1890–1903, Contributions to the Tertiary fauna of Florida. . . . *Transactions of the Wagner Free Institute of Science of Philadelphia*, 3(1–6): 1654 pp.

SACCO, F., 1890–1904, *I molluschi dei terreni terziarii del Piemonte e della Liguria*. . . . [continuation of work started by L. Bellardi]. Turin, parts 6–30. Later parts only. Also published in *Memorie della Reale Accademia delle Scienze di Torino*.

COSSMANN, [A.E.M.], 1895–1925, *Essais de paléoconchologie comparée*. Paris, livraisons 1–13: 159 + 179 + 201 + 293 + 215 + 151 + 261 + 248 + 215 + 292 + 388 + 348 + 345 pp.

DALL, W.H., 1899–1903, [Synopses of Leptonacea, Solenidae, Tellinidae, Cardiidae, Lucinacea, Veneridae, Astartidae]. *Proceedings of the United States National Museum*, 21: 873–897; 22: 107–112; 23: 285–326; 23: 381–392; 23: 779–833; 26: 335–412; 26: 933–951.

SUTER, H., 1913, *Manual of the New Zealand Mollusca*. Wellington, xxiii + 1120 pp.

WOODRING, W.P., 1925, 1928, Miocene mollusks from Bowden, Jamaica [Part I], Pelecypods and scaphopods. Part II, Gastropods and discussion of results. *Carnegie Institution of Washington*

[1]Based on a work sheet given to students in Dr. Keen's malacology course in the mid-1950s. It deserves wider dissemination. ED.

Publication 366: v + 222 pp.; 385: vii + 564 pp.

KNIGHT, J.B., 1941, Paleozoic gastropod genotypes. *Geological Society of America Special Papers* 32: vi + 510 pp.

MOORE, R.C., ed., 1960, *Treatise on invertebrate paleontology*. Part I. Mollusca 1. Mollusca—general features; Scaphopoda; Monoplacophora; Gastropoda—general features; Archaegastropoda and some (mainly Paleozoic) Caenogastropoda and Opisthobranchia. Geological Society of America and University of Kansas Press, xxiii + 351 pp.

MOORE, R.C., ed., 1969–1971. *Treatise on invertebrate paleontology*. Part N. Mollusca 6. Bivalvia. Geological Society of America and University of Kansas, 3 vols., xxxviii + ii + iv + 1224 pp.

INDEX TO TAXA IN VOLUME 27

An asterisk (*) denotes a new taxon. The articles on Dr. A. Myra Keen (pp. 379–408) are not indexed here (see index of key words in the titles and contents of her molluscan papers, pp. 402–404).

AWARDS FOR STUDY AT
The Academy of Natural Sciences of Philadelphia

The Academy of Natural Sciences of Philadelphia, through its Jessup and McHenry funds, makes available each year a limited number of awards to support students pursuing natural history studies at the Academy. These awards are primarily intended to assist predoctoral and immediate postdoctoral students. Awards usually include a stipend to help defray living expenses, and support for travel to and from the Academy. Application deadlines are 1 March and 1 October each year. Further information may be obtained by writing to: Chairman, Jessup-McHenry Award Committee, Academy of Natural Sciences of Philadelphia, 19th and the Parkway, Philadelphia, Pennsylvania 19103, U.S.A.

INSTRUCTIONS FOR AUTHORS

1. MALACOLOGIA publishes original research on the Mollusca that is of high quality and of broad international interest. Papers combining synthesis with innovation are particularly desired. While publishing symposia from time to time, MALACOLOGIA encourages submission of single manuscripts on diverse topics. Papers of local geographical or systematic interest should be submitted elsewhere, as should papers whose primary thrust is physiology or biochemistry. Nearly all branches of malacology are represented on the pages of MALACOLOGIA.

2. Manuscripts submitted for publication are received with the tacit understanding that they have not been submitted or published elsewhere in whole or in part.

3. Manuscripts may be in English, French, German or Spanish. Papers in languages other than English must include a translation of the Abstract in English. Authors desiring to have their abstracts published in other languages must provide the translations (complete with main titles). Include all foreign accents. Both American and British spellings are allowed.

4. Unless indicated otherwise below, contributors should follow the recommendations in the *Council of Biology Editors (CBE) Style Manual* (ed. 5, 1983) available for U.S. $24.00 from CBE, 9650 Rockville Pike, Bethesda, MD 20814, U.S.A.

5. Be brief.

6. Manuscripts must be typed on one side of good quality white paper, double-spaced throughout (including the references, tables and figure captions), and with ample margins. Tables and figure captions should be typed on separate pages and put at the end of the manuscript. Make the hierarchy of headings within the text simple and consistent. Avoid internal page references (which have to be added in page proof).

7. Choose a running title (a shortened version of the main title) of fewer than 50 letters and spaces.

8. Provide a concise and informative Abstract summarizing not only contents but results. A separate summary generally is superfluous.

9. Supply between five and eight key (topic) words to go at the end of the Abstract.

10. Use the metric system throughout. Micron should be abbreviated μm.

11. Illustrations are printed either in one column or the full width of a page of the journal, so plan accordingly. The maximum size of a printed figure is 13.5 × 20.0 cm (preferably not as tall as this so that the caption does not have to be on the opposite page).

12. Drawings and lettering must be dark black on white, blue tracing, or blue-lined paper. Lines, stippling, letters and numbers should be thick enough to allow reduction by ½ or ⅓. Letters and numbers should be at least 3 mm high after reduction. Several drawings or photographs may be grouped together to fit a page. Photographs are to be high contrast. High contrast is especially important for histological photographs.

13. All illustrations are to be numbered sequentially as figures (not grouped as plates or as lettered subseries), and are to be arranged as closely as possible to the order in which they are first cited in the text. Each figure must be cited in the text.

14. Scale lines are required for all nondiagrammatic figures, and should be convenient lengths (e.g., "200 μm," not "163 μm"). Magnifications in captions are not acceptable.

15. All illustrations should be mounted, numbered, labeled or lettered, i.e. ready for the printer.

16. A caption should summarize what is shown in an illustration, and should not duplicate information given in the text. Each lettered abbreviation labeling an individual feature in a figure must either be explained in each caption (listed alphabetically), or be grouped in one alphabetic sequence after the Methods section. Use the latter method if many abbreviations are repeated on different figures.

17. Tables are to be used sparingly, and vertical lines not at all.

18. References cited in the text must be in the Literature Cited section and *vice versa*. Follow a recent issue of MALACOLOGIA for bibliographic style, noting especially that serials are cited unabbreviated. Supply pagination for books. Supply information on plates, etc., only if they are not included in the pagination.

19. In systematic papers, synonymies should not give complete citations but should relate by author, date and page to the Literature Cited section.

20. For systematic papers, all new type-specimens must be deposited in museums where they may be studied by other scientists. Likewise MALACOLOGIA requires that voucher specimens upon which a paper is based be deposited in a museum where they may eventually be reidentified.

21. Submit each manuscript in triplicate. The second and third copies can be reproductions.

REPRINTS AND PAGE COSTS

22. When 100 or more reprints are ordered, an author receives 25 additional copies free. Reprints must be ordered at the time proof is returned to the Editorial Office. Later orders cannot be considered. For each authors' change in page proof, the cost is U.S. $3.00 or more.

23. When an article is 10 or more printed pages long, MALACOLOGIA requests that an author pay part of the publication costs.

SUBSCRIPTION COSTS

24. For Vol. 28, personal subscriptions are U.S. $17.00 and institutional subscriptions are U.S. $27.00. Address inquiries to the Subscription Office.

VOL. 27, NO. 2 MALACOLOGIA 1986

CONTENTS