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Foreword

The 13th International Conference on Copepoda was held from 16–21 July 2017, at Cabrillo Marine Aquarium (CMA) in Los Angeles, California, U.S.A. Approximately 150 participants from 29 countries convened at CMA to share their personal fascination and research interests on copepods. There was also a workshop on the Morphology and Systematics of Copepods from 10–14 July 2017, at the Scripps Institution of Oceanography in La Jolla, California, in conjunction with the conference.

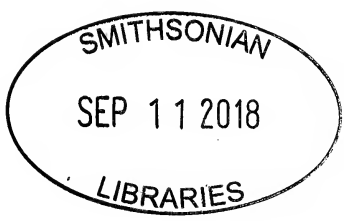
The scientific program consisted of 22 presentations in five symposia, 45 oral presentations in five contributed sessions, and 62 poster presentations. Three symposia were dedicated to Dr. Janet Bradford-Grieve, Dr. Janet W. Reid, and the late Prof. George W. Benz for their outstanding scientific contributions to marine planktonic, freshwater, and parasitic copepods, respectively. We would like to express our gratitude to Mike Schaadt (former Director of CMA), the local organizing committee (Dr. Julianne Kalman Passarelli, Dr. Danny Tang, Joey Pallares, Anthony Chan, Mildred Ronquillo, Ed Mastro, Caroline Brady, Bruno Passarelli, Shelly Moore, Prof. Ju-Shey Ho, Prof. Mark Ohman, and Dr. Regina Wetzer), the symposia organizers (Prof. Hans Dam, Prof. Mark Ohman, Prof. Rony Huys, Dr. Eduardo Suárez-Morales, and Dr. Leocadio Blanco-Bercial), and the staff and volunteers at CMA for their efforts and support. We also thank the conference sponsors, namely City of Los Angeles: Recreation and Parks, Port of Los Angeles, Friends of CMA, World Association of Copepodologists (WAC), Orange County Sanitation District, DoubleTree by Hilton San Pedro – Port of Los Angeles, Griffith Observatory, Battleship Iowa Museum, and California Science Center, for their generous patronage.

This issue contains selected papers on topics ranging from behavior, symbiosis, genetics, and stress tolerance that were presented at the conference. Furthermore, the Maxilliped Lecture presented by Dr. Eduardo Suárez-Morales (Past-President of WAC) during the mid-conference tour is provided. As co-editors of this issue, we are grateful to the reviewers for their efforts which improved the quality of the manuscripts. We also extend our appreciation to the Southern California Academy of Sciences for sponsoring the publication of this issue.

Danny Tang
Orange County Sanitation District, U.S.A.

Leocadio Blanco-Bercial
Bermuda Institute of Ocean Sciences, Bermuda

June 2018



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Monstrilloid Copepods: the Best of Three Worlds

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Abstract.—Monstrilloids are one of the most intriguing groups of copepods. Their complex life cycle represents the successful evolutionary outcome of dealing with three distinct kinds of habitat, viz., planktonic, benthic, and endoparasitic, each of which presents particular challenges that have been overcome by monstrilloids. These copepods combine a unique set of strategies and adaptations to complete their life cycle. The non-feeding planktonic adult phase lacks mouthparts and their antennules are fixed, thus limiting their swimming abilities but they compensate for this handicap by having powerful swimming legs and probably generate a very distinct hydrographic signal that may be useful in avoiding predators and allowing sexual recognition between adult males and females. Parasitizing exclusively on abundant, gregarious sessile or sedentary benthic organisms represents an advantage in that potential hosts can be found without the need for long-distance dispersal. The endoparasitic stages of monstrilloids are unique; after infection by an early planktonic nauplius, successive nauplioid stages feed on their own vitellum while developing feeding tubes to absorb nutrients from their hosts. They grow within the host's body as successive copepodite stages that are contained in a protective sheath. Preadult individuals exit through the host body wall causing significant host damage or death, behaving in these instances as parasitoids. The diversity of the group appears to be underestimated, and extensive geographic areas remain completely unknown for this group of copepods. More effort will be required to advance our knowledge of monstrilloid diversity and biology that are yet to be revealed.

In this contribution, I intend to explore the vicissitudes experienced by monstrilloid copepods in the three different types of habitat they frequent: the plankton, the benthos, and as endoparasites, the bodies of their hosts. Along the way I will also present an overview of the group. The name of this group is striking; are they really monsters? There are many kinds of monsters, but the basic concept implies the possession of unusual, extraordinary characters. Of course, many highly modified copepods would qualify as such in this concept. For the American zoologist James D. Dana (1849) monstrilloids were indeed monsters, and when he described the genus *Monstrilla* and emphasized their lack of mouthparts ("*...maxillis pedibusve non munitus*"), it was clear that he was impressed by these odd copepods. He may have asked himself how they feed or wondered about their life cycle, because for him this feature qualified as a monstrosity. This character in monstrilloids has been a source of puzzlement and doubt ever since, because it constitutes an obstacle to the study of evolutionary relationships. As Huys and Boxshall (1991) fully recognized, this lack of mouthparts makes any fruitful analysis of monstrilloid appendage

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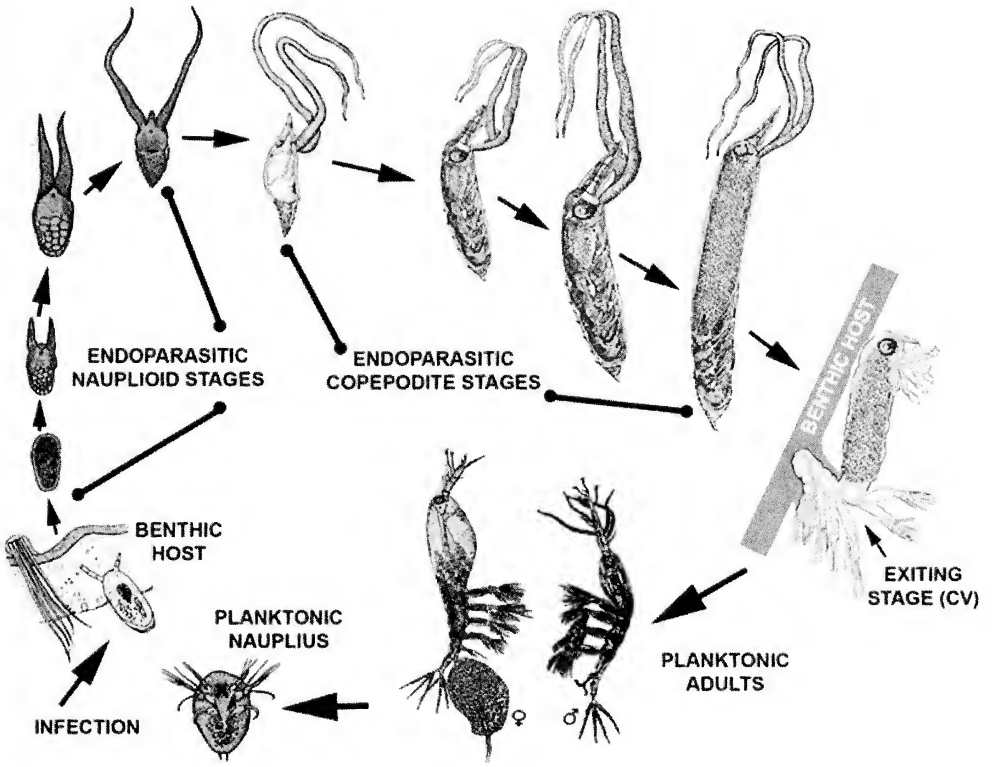

Fig. 1. Generalized life cycle of the Monstrilloida (based on Malaquin 1901; Suárez-Morales et al. 2014; Huys 2014).

homologies elusive. The ancestral links of these copepods remain hidden, a challenging mystery.

The Monstrilloida comprises a peculiar lineage of protelean parasitic copepods. They are endoparasitic in marine benthic invertebrates during their postnaupliar and copepodite stages but also have basically two free-living, planktonic phases, an infective naupliar stage and exclusively reproductive adult (Fig. 1; Malaquin 1901; Suárez-Morales et al. 2014). So, they are known mainly from adult individuals that are captured during zooplankton surveys in coastal environments (Suárez-Morales 2011).

As is true for many other highly modified copepods, their general morphology can be described oxymoronically as a model of complex simplicity. The lack of feeding appendages in monstrilloids has urged researchers to find new characters and explore their taxonomic value. In two essential publications, Grygier and Ohtsuka (1995, 2008) have contributed much to achieve this. The monstrilloids are currently represented by five valid genera: *Monstrilla* Dana, 1849, *Cymbasoma* Thompson, 1888, *Monstrillopsis* Sars, 1921, *Maemonstrilla* Grygier and Ohtsuka, 2008, and the recently described *Australomonstrillopsis* Suárez-Morales and McKinnon, 2014.

The main taxonomic characters used to identify monstrilloids include their body shape and proportions, the antennule length, the presence and development of the eyes, the position of the oral papilla, and the number of caudal setae, this last being one of the genus-defining features (Suárez-Morales 2011, 2015). Other characters are related to the

antennule setation pattern, with more than 30 named setal elements in males and females. Also, the structure and setation of the female fifth leg and the male genital complex are important. Cephalic cuticular features are also useful and some are reminiscent of their endoparasitic life, such as the paired scars of their feeding tubes that remain in the adults.

Early studies of the biology of these copepods by French researchers (e.g., Giard 1896; Malaquin 1901) revealed part of the monstrilloid life cycle, and Giard (1896) recognized its complexity as a zoological challenge, "*...L'ethologie des Copépodes de la famille des Monstrillidae est un problème qui a vainement exercé la sagacité des zoologistes....*" They have morphological, physiological, and behavioural adaptations to simultaneously thrive in all three of the above challenging environments. In this contribution I will provide some facts and ideas about the adaptive features they use to deal with the complications inherent to each of these ways of life.

Plankton

Sinking in the water column is one of the main problems that planktonic organisms face. Calanoid copepods, clearly the most successful group in the zooplankton (Bradford-Grieve et al. 2010) show effective adaptations to improve their buoyancy. These features include long, powerful antennules, remarkably well developed cephalic appendages armed with a number of extended setae, and a supply of lipids within the body (Visser and Jónasdóttir 1999; Schründer et al. 2014). Monstrilloids lack these advantages, but as we will see, they compensate for this with adaptive characters to survive in the plankton.

The monstrilloid antennules are usually equal to less than 45% of the total body length (i.e. combined length of the prosome and urosome). They are typically rigid, straight and anteriorly directed, with short muscles attached to a thick, diagonal band of cephalic muscles; they cannot be spread laterally. The antennules are 4-segmented in the females although some species have segments 3-4 or 2-4 fused as in several species of *Cymbasoma* (Suárez-Morales et al. 2006). In males, the antennules are 5-segmented, with a distal geniculation involving a single segment with a distinctive setation pattern (Huys et al. 2007).

The lack of mouthparts and antennules that are functional in locomotion highlights the role of thoracic leg propulsion in monstrilloids during their free-living planktonic phase. As with other planktonic copepods (Kiørboe 2011), adult monstrilloids have four pairs of biramous swimming legs. Both rami are always three-segmented, with a conservative ancestral armature (Huys and Boxshall 1991). These legs have a strong set of muscles and long, setulated setae, certainly an efficient gear for propelling themselves in the water column during their short planktonic phase. They have the necessary swimming power but with their rigidly fixed antennules how do they manage to navigate while seeking a mate in the three-dimensional pelagic realm?

Efficient swimming is a matter of decreased water resistance. Calanoid copepods show different kinds of displacements, including a gliding movement created mainly by mouthparts and antennae, and power swimming, in which the lateral sweep of the flexible antennules is strong and completed by that of the swimming legs (Jiang and Kiørboe 2011). This makes calanoids efficient swimmers indeed. Each of these two swimming modes has a differential hydrodynamic signature. Lacking the benefits of the large antennules and antennae, it is speculated that monstrilloids use their rigid, straight antennules as a form of streamlining to maintain an efficient, straight path during their displacements in the water column. They appear to be designed to obtain the best hydrodynamic advantage from the leg-based propulsion.

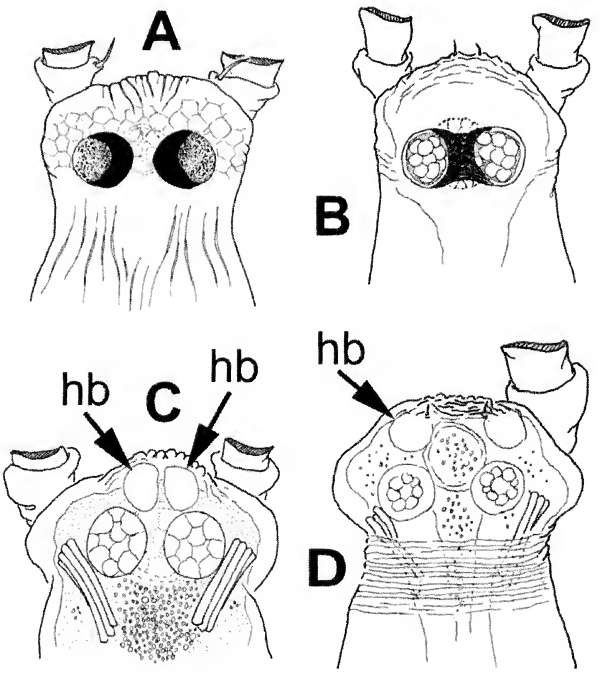

Fig. 2. Eye development in monstrilloid copepods and position of hyaline bodies (hb) with respect to ocelli. (A) Eyes with strongly pigmented inner half, without hb; (B) Eyes medially conjoined by pigmented area, without hb; (C) Eyes unpigmented, with hb on medial position; (D) Eyes unpigmented, with hb separate.

From the analysis of a series of video recordings taken by Dora Pilz (University of Miami) involving two monstrilloid species, including *Cymbasoma davisi* Suárez-Morales and Pilz, 2008, it was determined (pers. obs.) that monstrilloids display a weak hopping movement with a basically continuous straight trajectory that contrasts with the skillful swimming displayed by other copepods. A typical leg beat involves a rapid backward stroke of the four swimming legs in a 4-1 sequence (e.g., first swimming stroke of leg 4, last of leg 1), as described for other planktonic copepods (Jiang and Kiørboe 2011). A flap of the urosome can add strength to the leg movement. It is speculated that this kind of swimming creates a very different, probably weaker hydromechanical disturbance signal in the surrounding water than that generated by calanoids; this greatly reduced signal could represent an advantage to avoid alerting potential predators that are adapted to perceive planktonic copepods as prey.

As in many other copepods, the nauplius eye of monstrilloids consists of one median and two lateral ocelli, each within a pigment cup. The medial eye can be larger than the lateral ocelli cups or about the same size; in some cases, the eyes are small and inconspicuous, lacking pigmentation (Fig. 2C, D). The distances between the eyes, and the pigmentation pattern and intensity, are variable (Fig. 2). This kind of complete and strong eye development is comparable to that of holoplanktonic forms (i.e., calanoids) and presumably it constitutes an additional tool to survive in the plankton. In some species, a pair of lens-like "hyaline bodies" (hb in Fig. 2) is located near the lateral cups. Their function is unknown,

but they readily bring to my mind the cuticular lenses of pontellid calanoids, which are sensitive to light and polarization (Manor et al. 2009).

Finding a mate is essential for copepod reproduction. Detection of potential mates by pheromones has been widely documented, and distinctive hydromechanical signatures have also been reported (Strickler 1998; Kiørboe 2007, 2011). For the non-feeding adult monstrilloids with a very limited time in the plankton, finding a prospective mate in the shortest period possible is their priority. Their mating behaviour remains largely unknown, but it probably includes the tracking of unique hydromechanical cues produced by females or males, not primarily the tracing of pheromones. This set of adaptive behaviours and characters increases the chances of a successful planktonic stage.

The highly fecund free-spawning calanoids lack parental care (Titelman et al. 2007), whereas egg-carrying forms display a certain level of parental care. At certain conditions, some planktonic species show increased fecundity levels (Holste and Peck 2005). Monstrilloids lack egg sacs but they possess ventral ovigerous spines that comprise two slender, spiniform structures of variable length in different species. Eggs remain attached to these structures by a mucous substance. In *Maemonstrilla* the ovigerous spines are anteriorly directed, set close and parallel to the body axis. This peculiar pattern, together with the remarkably wide intercoxal sclerites of legs 1–4, enables these copepods to provide extended parental care by subthoracic brooding (Grygier and Ohtsuka 2008). This is yet another adaptation, not known in other planktonic copepods, to improve the survival of their offspring in the planktonic realm.

Mass aggregations of planktonic copepods have been reported for many reef-related species and are deemed as an adaptive behavior to enhance the likelihood of mate encounters (Titelman et al. 2007). Usually, monstrilloids are caught in numbers of 2 or 3 if at all, but they can swarm; in a Caribbean coral reef area more than 800 individuals of a single species were collected during one ordinary plankton trawl. They aggregate at dusk, thus contrasting with daylight swarming of planktonic copepods (Suárez-Morales 2001). This implies that monstrilloids probably avoid predators by undertaking vertical migration and remaining near the bottom, closer to their potential benthic hosts. Adaptive migratory patterns have been also proposed as planktonic copepod strategies to avoid predation in the water column (Pasternak et al. 2006).

Benthos

Monstrilloids are known as parasites of the benthic macrofauna, including several families of sessile and errant polychaetes, gastropod and bivalve molluscs, and even sponges (Huys et al. 2007; Suárez-Morales 2011) (Table 1). So, in this sense, the period in which monstrilloids live inside these benthic invertebrates, they are part of the hyperbenthic and epibenthic community (Gray and Elliott 2009), just as the epifauna and epiflora (i.e., symbiotic organisms living attached to the macrofauna), but inside the body.

The monstrilloid hosts tend to be sessile or sedentary and gregarious marine invertebrates, something that should favor maintenance of the local character of parasite faunas. Parasites are able to profoundly transform the community structure, behaviour, and reproduction of benthic invertebrates (Mouritsen and Poulin 2002), but the effect of monstrilloids on the population dynamics of their hosts has not been studied. It is likely that the monstrilloid life cycle takes advantage of various factors inherent to the benthic community: 1) the tendency of these benthic groups to have aggregate populations (Anderson 2008), and the concomitantly greater chance of larvae encountering the host

Table 1. Recorded hosts of the Monstrilloida.

Phylum/Class	Family	Species	Monstrilloid	Reference
Porifera	—	—	Monstrilloid	Huys et al. (2007)
Mollusca				
Gastropoda	Pyramidellidae	*Brachystomia scalaris* (McGillivray, 1843)	*Monstrilla helgolandica* Claus, 1863	Pelseneer (1914); Gallien (1934)
	Vermetidae		Monstrilloida	Huys et al. (2007)
	—	—	Monstrilloida	Boxshall and Halsey (2004)
Bivalvia	Mytilidae	*Perna perna* L., 1758	*Monstrilla* sp.	Suárez-Morales et al. (2010)
Annelida				
Polychaeta	Capitellidae	*Capitella capitata oculata* Hartman, 1961	*Monstrilla capitellicola* Hartman, 1961	Hartman (1961)
	Serpulidae	*Salmacina dysteri* (Huxley, 1855)	*Cymbasoma danae* (Malaquin, 1901) (as *Haemocera danae*)	Malaquin (1901)
		Filograna implexa Berkeley, 1835	*Monstrillopsis filogranarum* (Malaquin, 1901) (as *H. filogranarum*)	Malaquin (1901)
		Salmacina setosa Langerhans, 1884	*Cymbasoma roscovita* (Malaquin, 1901) (as *Haemocera roscovita*)	Malaquin (1901)
		Serpula vermicularis L., 1767	Monstrilloida	Huys and Boxshall (1991)
		S. dysteri and *F. implexa*	Monstrilloida	Nishi in Grygier (1995)
	Spionidae	*Polydora ciliata* (Johnston, 1938)	*Cymbasoma germanicum* (Timm, 1893)	Malaquin (1901)
		Dipolydora giardi (Mesnil, 1893)	*C. germanicum*	Giard (1895) (as *Thaumaleus* sp.); Malaquin (1901); Caullery and Mesnil (1914)
	Syllidae	*Exogone* Örsted, 1845	Monstrilloid	Caullery (1908)
		Syllis gracilis Grube, 1840	*Cymbasoma malaquini* (Caullery and Mesnil, 1914) (as *Haemocera*)	Caullery and Mesnil (1914)
		Haplosyllis sp.	*Monstrilla* sp.	Suárez-Morales et al. (2014)

within a reduced spatial scale; 2) monstrilloids are more frequently found as parasites of polychaetes, deemed as the most abundant group in the benthos (Dean 2008; Gray and Elliott 2009); 3) the sessile or sedentary (moving within one place, not fixed or with weak dispersal abilities) nature of their hosts enables monstrilloids to remain linked to basically the same host populations and also to suitable hydrographic conditions including tidal currents. As monstrilloids inhabit near-shore coastal habitats, including docks, they use retention areas that are locally generated by microscale dynamics to remain close to the benthic community and to their potential hosts (Suárez-Morales and Pilz 2008). There are relatively few records of monstrilloids in fully oceanic waters. Together with their weak dispersal abilities, their link to the benthic communities may also explain the presumed restricted distributional patterns of the Monstrilloida (Suárez-Morales 2011).

Endoparasitic

Monstrilloids are impressive parasites, and when they invade the host they condemn it. A free-swimming lecithotrophic naupliar stage is recognized as the infective stage (Grygier and Ohtsuka 1995). The antennae and claw-bearing mandibles are used to efficiently attach to and penetrate the host body wall (Fig. 1); the cephalic end of the nauplius penetrates first and then the antennae sway back and forth on the host integument to complete the invasion (Malaquin 1901).

Once inside the host, the endosymbiont nauplius starts to develop feeding tubes, but probably keep living on its vitellum for some time during 3-4 stages I call "nauplioid" herein. When the feeding tubes become functional and begin to extract fluids from the host, the larvae become truly endoparasitic. At these early stages the copepod is covered by a thin membrane around the body (Pelseneer 1914; Suárez-Morales et al. 2014: Fig. 9). It is likely that there are at least three copepodite stages and they can have 1-3 pairs of feeding tubes (Malaquin 1901; Caullery and Mesnil 1914; Suárez-Morales et al. 2014). Suárez-Morales et al. (2014: Fig. 9A) shows a CIII individual extracted from its polychaete host with the membrane still around its body. The same specimen (Suárez-Morales et al. 2014: Fig. 9B, C) shows two antero-ventral feeding filaments that are formed by tubes with small bulbous structures that are speculated to represent different molts. The bulbous structures lead to terminal pads that appear to be connected to the inner tissues of the host to extract its body fluids.

Usually, parasites found in the polychaete host are lodged along the main axis with their ventral surface facing the digestive system of the host, with the cephalic end pointing towards the posterior part of the host body. When more than one parasite is present they tend to lodge on opposed positions, both facing their ventral surface to the host digestive tube. Infection by monstrilloids can be detected as nodules on the mantle of molluscs (Suárez-Morales et al. 2010) or growing swellings of the body surface of polychaetes (Suárez-Morales et al. 2014). The effects of the parasite include intense haemocytic infiltration, swelling, and castration (Malaquin 1901; Suárez-Morales et al. 2010).

At the last juvenile phase, monstrilloids quite dramatically leave the host, breaking through its body wall by first exposing the urosome (Suárez-Morales et al. 2014: Fig. 11A, B) and then moving the cephalothorax until the legs and the antennules are completely withdrawn from the host body (Malaquin 1901). Caullery and Mesnil (1914) reported a different exiting sequence, with the middle of the body emerging first, followed by the cephalosome and finally the urosome. The final separation from the host is probably not immediate and the copepod probably remains partially attached for a while; the remains of

Table 2. Number of species of each monstrilloid genera reported from coastal waters of five continents.

	Cymbasoma	*Monstrilla*	*Monstrillopsis*	*Maemonstrilla*	*Australomonstrillopsis*	Total
Europe	15	14	5	—	—	34
Asia	14	13	2	9	—	38
America	20	20	9	—	—	49
Australia	25	1	3	4	1	34
Africa	4	7	—	1	—	12

the sac and the feeding tubes remain inside the host (Caullery and Mesnil 1914). According to Malaquin (1901), the host may recover after the parasite exits its body. In the mytilid mollusc *Perna perna*, the copepod does not kill the host (Suárez-Morales et al. 2010), but it does in other instances (Suárez-Morales et al. 2014). So, the boundary between being parasites and parasitoids is not quite clear in reference to monstrilloids; the outcome of the symbiosis may depend on the relative size of the host and also on the number of parasites in the individual, which is also related to their position and space arrangement within the host (Malaquin 1901). According to Malaquin (1901) and Caullery and Mesnil (1914), the sex of the parasite is determined by the number of individuals infecting a host. When 2-3 monstrilloids develop in the same host individual, they all develop into males. By contrast, females arise from hosts with a single parasite in the body.

Diversity and Distribution

Because of their morphological simplicity, incomplete descriptions, a long history of nomenclatural problems (Grygier 1994a; Suárez-Morales 2011; Grygier and Suárez-Morales submitted) and the difficulties in linking males and females of a particular species, the diversity of monstrilloids is far from being accurately known (Suárez-Morales 2011, 2015), and is certainly underestimated. Only a few years ago, just a couple of species of *Cymbasoma* were known from all of Australia but a recent revision of new material revealed a much higher diversity (i.e., 25 species) (Suárez-Morales and McKinnon 2014). Based on a revision of the available data and recent additions in 2017 (Suárez-Morales et al. 2017), up to 154 nominal species are recognized: 72 of *Cymbasoma*, 57 of *Monstrilla*, 14 of *Monstrillopsis*, 11 of *Maemonstrilla*, and 1 of *Australomonstrillopsis*. As it is likely that more undescribed species and probably new genera will result from ongoing surveys of the monstrilloid fauna from Australia, Canada, and Korea (Jeon et al. 2018), their true diversity is yet to be revealed.

How is this diversity distributed among the continents? Table 2 shows the distribution of the known diversity of the group. As stated by Suárez-Morales (2011), it is remarkable that several nominal species are reported in all the continents. This is a group of pseudo-cosmopolitan species, and many of their records are suspect as a result of the problems mentioned before. Here I show in parentheses the number of species that are actually known or are assumed to be subsumed under each of these names: *Cymbasoma rigidum* Thompson, 1888 (3), *C. longispinosum* (Bourne, 1890) (6), *Monstrilla grandis* Giesbrecht, 1891 (3-4?), *M. helgolandica* Claus, 1863 (2-3), and *Monstrillopsis dubia* (Scott, 1904) (4), but there are probably many more (Grygier 1994b; Suárez-Morales 2006; Üstün et al. 2014).

The genus *Cymbasoma* is slightly less diverse in Europe than in the Americas; it appears to be most diverse in Australia (Suárez-Morales and McKinnon 2016). A similar

situation is found for *Monstrilla*, which is less species-rich in European waters than it is in the Americas, where extensive areas (e.g., the South American Pacific coast) remain unexplored for this genus, without a single record. Knowledge of this genus is expanding in the Indo-Pacific region, mainly in Japan and Korea (Chang 2014; Jeon et al. 2018), but extensive unstudied areas still remain. Analysis of the Australian monstrilloids is still an ongoing project, and their numbers there could grow, especially for *Monstrilla*.

The less species-rich genera show a similar pattern. America has the highest number of species of *Monstrillopsis*, followed by Europe and Australia. *Monstrillopsis* tends to occur in temperate and cold latitudes, and only three species have been recorded from fully tropical areas (Suárez-Morales 2006; Suárez-Morales and McKinnon 2014). *Maemonstrilla* is largely restricted to the Indo-West Pacific. Most species have been found in Japanese coral reef areas and in Australian waters but some are known from India and Indonesia (Grygier and Ohtsuka 2008; Suárez-Morales and McKinnon 2014). *Australomonstrillopsis* is endemic to Australia (Suárez-Morales and McKinnon 2014). Africa is clearly a treasure-box of monstrilloid diversity yet to be opened.

Phylogeny

As a group associated with different types of habitats involving distinct life modes, monstrilloids have a unique mixture of characters that are shared with various other groups of copepods, and their phylogenetic relations within the Copepoda have been a matter of discussion for over a century. A common ancestor with the Phyllodicolidae, a cyclopoid family parasitic on polychaetes, was proposed by Gotto (1961). Later on, monstrilloids were positioned by Huys and Boxshall (1991) as a sister taxon of the order Siphonostomatoida. A phylogenetic analysis by Huys et al. (2007) suggested a common ectoparasitic ancestor for monstrilloids and caligiform taxa, with a host shift from pelagic vertebrates (teleosts) to sessile benthic invertebrates coincident with the divergence of these two lineages, and also resulted in the proposed demotion of monstrilloids to a family of the order Siphonostomatoida. To the contrary, a recent, comprehensive analysis of the copepod orders with upgraded molecular standards (Khodami et al. 2017) supports the status of the Monstrilloida as a monophyletic order forming a sister-group with the siphonostomatoids in a single clade. This is also coincident with a COI-based analysis performed by Su et al. (2016) for the Korean planktonic copepods. The phylogeny of the genera within the Monstrillidae has not been explored but it is hypothesized that, because of the presence of significant reductions (i.e., urosome segmentation, number of caudal setae, fifth leg armature), *Cymbasoma* could be revealed as the most derived genus and *Monstrilla* the most primitive, but a full analysis would be needed to support this.

There are several exclusive characters of the Monstrilloida including: 1) an infective nauplius vs. infective copepodites in other parasitic groups (Ho et al. 2003; Ohtsuka et al. 2018); 2) naupliar mouthpart structure (Grygier and Ohtsuka 1995); 3) the unique endoparasitic nauplioid/copepodite development pathway (Huys 2014; Suárez-Morales et al. 2014); and 4) distinctive leg development with early completion (at stage CIII) of the setal armature of legs 1-4, loss of one exopodal seta of leg 1 at CIV, and full development of leg 1 ENP at CIII (Suárez-Morales et al. 2014). Some other characters are shared with selected copepod taxa. For example, the lack of mouthparts and antennule structure and function in non-feeding adults are shared with members of the cyclopoid family Thaumatopsyllidae. Furthermore, the dual mode of parasitism (endo vs. ectoparasitic cycle) and the use of invertebrate benthic hosts are also shared by many siphonostomatoid and

cyclopoid taxa. Monstrilloids are clearly a compact, well defined but intriguing lineage still posing many, many questions we have not been able to solve.

In 1707, the Swiss diluvianist Johannes Scheuchzer published his *Complaints and Claims of the Fishes*, in which he gave voice to the fish; they claimed to be witnesses of the Universal Flood but also complained about the human misinterpretation of their fossils. So, I'm going to do the same here and speak out on behalf of monstrilloids: "*...we have been able to survive in three really harsh worlds and here we stand, probably against all odds. Today, with our raised rigid antennules, we claim for understanding and more research efforts from copepodologists. We, the monstrilloids claim our place in the world!!*"

Other copepod groups make similar requests, but there are so many aspects of monstrilloid ecology, biology, genetics, behavior, and taxonomy that we do not know or understand as yet (see Suárez-Morales 2011). There remains for us both an opportunity and a continuous challenge: research on this awesome group of crustaceans will always be a canvas on which we can keep spreading our science, our art, copepodology.

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Planktonic Phases in Symbiotic Copepods: a Review

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Abstract.—In symbiotic copepods, most naupliar stages are typically planktonic, playing a primary role in dispersal, while the first copepodid usually represents the infective stage. Later copepodid stages, including adults, are associated with host organisms. Many symbiotic copepods have abbreviated life cycles, with a reduced number of naupliar stages and two different feeding habits. These patterns are presumably related to distinct life cycles strategies. Exceptional cases are exemplified by members of the Monstrillidae and Thaumatopsyllidae, both of which are protelean parasites, with infective nauplii and non-feeding planktonic adults. In the Caligidae, the life cycle follows a generalized pattern, but adults of many species like *Caligus undulatus* seem to exhibit a dual mode of life involving host switching. Adults leaving the first host become temporarily planktonic before attaching to the final host. This dual mode of life is also found in adults of the Ergasilidae. Abbreviation of the planktonic phase is characteristic for some symbiotic taxa, thus suggesting that they have evolved to become highly efficient in locating and infecting new hosts without needing long-distance larval dispersal. The life cycle of copepods associated with zooplankters is also briefly reviewed. Zooplankters are clearly less used as hosts by copepods than benthic invertebrates. It is likely that symbiotic copepods dynamically utilize planktonic phases in their life cycle, thus maintaining the balance between dispersal, host location, reproduction, and predator-avoidance strategies.

Symbiotic copepods have one or more planktonic phases for dispersal, infection, host-switching, mating, and presumably, predator-avoidance (Kearn 2004; Huys 2014; Venmathi Maran et al. 2016). Usually, the primarily planktonic naupliar stages play a key role in dispersal. The first copepodid stage is infective, and the subsequent stages, including adults, then typically establish a symbiotic association with a host organism. In some taxa, adults are also a swimming stage for dispersal, mating, host-switching, and presumably predator-avoidance. In the Ergasilidae, adult females of some taxa seem to show a dual mode of life

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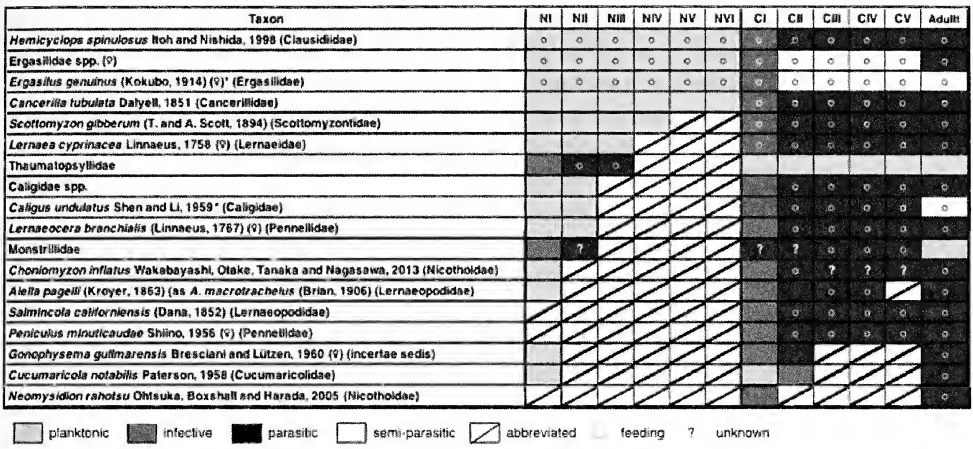

Fig. 1. Life cycle of representatives of symbiotic copepods. Note abbreviated naupliar stages. * represents only adults showing different pattern from those of other congeners. Based on: Paterson (1958); Kabata and Cousens (1973); Kawatow et al. (1980); Alston et al. (1996); Kearn (2004); Ohtsuka et al. (2004a, c, 2007, 2009); Boxshall (2005); Itoh and Nishida (2007, 2008); Dojiri et al. (2008); Suarez-Morales (2011); Ismail et al. (2013); Venmathi Maran et al. (2013, 2016); Huys (2014); Otake et al. (2016).

interfacing between hosts and the water column, while adult males are truly planktonic without any interaction with the host (Urawa et al. 1980a, b; Ohtsuka et al. 2004a). In the Caligidae, most species follow the above generalized life cycle, but adults of some species most likely show a dual life mode like some ergasilids (Ho and Lin 2004a; Venmathi Maran and Ohtsuka 2008; Suárez-Morales et al. 2012; Venmathi Maran et al. 2012a, b, 2016). In some species of the mesoparasitic family Pennellidae, pre-mated adult females and adult males are planktonic prior to mating in the water column (Kearn 2004; Ismail et al. 2013). Extremely aberrant life cycle types are found in the protelean life cycle of the Monstrillidae and Thaumatopsyllidae, in which the earliest naupliar stage is infective, and non-feeding adults remain truly planktonic during mating and dispersal (Malaquin 1901; Grygier and Ohtsuka 1995, 2008; Dojiri et al. 2008; Suárez-Morales 2011). The present paper briefly reviews the planktonic phases displayed by different groups of symbiotic copepods, and discusses their evolutionary and adaptive strategies.

Patterns of Planktonic Phases in Life Cycle

In general, symbiotic copepods primitively have six naupliar (NI–NVI) and six copepodid (CI–CVI) stages, the last of which corresponds to the adults, thus showing the maximum number of 12 developmental stages (Raibaut 1996; Boxshall 2005; Huys 2014). A typical example of such a complete life cycle in symbiotic copepods is displayed by the "*Saphirella*"-like forms (= *Hemicyclops* Boeck, 1873 and related genera) whose six naupliar stages actively feed (Fig. 1). Their first copepodid stage is frequently recorded as being abundant in coastal waters (Itoh and Nishida 1991), thus leading to the erroneous assumption that they are holoplanktonic during the entire life cycle (Gooding 1988; Itoh 2006). Their subsequent five copepodid stages are known to be loosely associated with benthic animals including ghost shrimps and polychaetes (Itoh and Nishida 2007, 2008).

The number of naupliar stages among symbiotic copepods is highly variable (i.e., 0, 1, 2, 3, 4 to 6), depending on the group and feeding types (Fig. 1). The planktotrophic

forms are characterized by the development of basal antennary and mandibular elements (Izawa 1986, 1987; Alston et al. 1996; Dojiri et al. 2008; Itoh and Nishida 2008), but these elements are absent from the lecithotrophic forms (Carton 1968; Kawatow et al. 1980; Urawa et al. 1980a; Izawa 1986, 1987; Grygier and Ohtsuka 1995, 2008; Ohtsuka et al. 2009). Lecithotrophic nauplii are common to most truly parasitic taxa belonging to the orders Cyclopoida (including "Poecilostomatoida") and Siphonostomatoida (Paterson 1958; Carton 1968; Izawa 1986, 1987; Raibaut 1996; Boxshall 2005; Ivanenko et al. 2007). Nauplii generally play a key role in dispersal, irrespective of their feeding strategy. Some species of the Nicothoidae (Paterson 1958; Boxshall 2005; Ohtsuka et al. 2007; Huys 2014) and Pennellidae (Boxshall 2005; Ismail et al. 2013; Huys 2014) are devoid of naupliar stages, and directly hatch as an infective copepodid. Remarkably, the aberrant families, Monstrillidae and Thaumatopsyllidae have infective nauplii (Fig. 1).

Copepodids of symbiotic copepods are usually feeding-stages, whose food items depend on a variety of hosts (Fig. 1). The permanent association with a host commences at the first, second or sixth (adult) copepodid stage (Boxshall 2005). In Caligidae and most other symbiotic families, the first copepodid is the infective stage. In the Ergasilidae, the first to fifth copepodid stages, adult males and pre-mated adult females are semi-planktonic, while postmated adult females infect fish hosts (Urawa et al. 1980b; Kearn 2004). The feeding habits of these semi-planktonic stages remain largely unknown. Kearn (2004) hypothesized that ergasilid copepodid stages and unmated adults feed on planktonic organisms. On the other hand, considering that the mouthparts of free-swimming copepodids resemble those of parasitic adult females, it may be inferred that they are able to feed on tissues and blood of any kind of fish host. This is indirectly supported by Alston et al. (1996), who obtained all copepodid stages of *Ergasilus briani* Markevich, 1933 in an experimental tank using the tench *Tinca tinca* (Linnaeus, 1758).

In some fish-parasitic families such as the Caligidae and Ergasilidae, adult females of some species are also free-swimming, and exhibit a dual mode of life involving switching between the host and the water column (Ho and Lin 2004a; Ohtsuka et al. 2004a, c; Venmathi Maran and Ohtsuka 2008; Venmathi Maran et al. 2012a, b, 2016; Suarez-Morales et al. 2012).

Some species of Pennellidae and Caligidae require both an intermediate and a definitive host (Bush et al. 2001; Kearn 2004; Hayward et al. 2011; Ismail et al. 2013; Huys 2014; Venmathi Maran et al. 2016). In these cases, adults are temporarily planktonic, actively searching for a new host.

Bizarre Life Cycle

In the Monstrillidae and Thaumatopsyllidae, the first nauplius is the infective stage, the subsequent stage(s) are endoparasitic, and adults are truly planktonic and non-feeding, lacking mouthparts (Suárez-Morales and Tovar 2004; Huys 2014; Suárez-Morales et al. 2014). The life cycles of these families have to a great extent been elucidated by Malaquin (1901), Caullery and Mesnil (1914), and more recently by Suárez-Morales and Tovar (2004), Dojiri et al. (2008), and Suárez-Morales et al. (2014). Differences between these two groups are found in the number of naupliar and copepodid stages in their life cycle. In the Monstrillidae, the life cycle consists of only one planktonic, infective lecithotrophic naupliar stage, at least 3 endoparasitic copepodid stages, and non-feeding planktonic adults (Fig. 1) (Malaquin 1901; Grygier and Ohtsuka 1995, 2008; Suárez-Morales 2011; Suárez-Morales et al. 2014). Huys (2014) regarded the early endoparasitc stages as naupliar

stages. From Malaquin's (1901) illustrations of the endoparasitic stages, two forms can be differentiated: sack-like bodies without and with paired absorptive processes. These may correspond to naupliar and copepodid stages, respectively. According to Malaquin's (1901) detailed observations, the naupliar body has vitelline tissues during these first endoparasitic stages and the feeding tubes are clearly underdeveloped and probably not functional. Therefore, it is likely that they do not feed on the host fluids until the individuals molt into successive copepodid stages in which the absorptive processes are fully developed. In contrast, thaumatopsyllids have one planktonic/infective and one or more endoparasitic naupliar stages, plus five or six non-feeding planktonic copepodid stages including adults (Fig. 1) (Suárez-Morales and Tovar 2004; Dojiri et al. 2008; Hendler and Dojiri 2009).

In these two groups, the presence of non-feeding planktonic adults implies very limited dispersal abilities. Development in free-living copepods generally takes 2-4 weeks from nauplii to copepodids, and 1-2 months or longer for adults (Hendler and Dojiri 2009). This duration is clearly abbreviated in the non-feeding adult stage, and more significantly affected in monstrillids than in thaumatopsyllids, because the former has a single planktonic instar (the adult) while the latter has 6 (copepodids I-V and adult). Furthermore, monstrillid adults can be locally abundant in the water column, particularly during nighttime or at dusk (Sale et al. 1976, 1978; Suárez-Morales 2001; Grygier and Ohtsuka 2008), thus suggesting an effective synchronized mating strategy to compensate for their short longevity. In contrast, the planktonic phase of the thaumatopsyllid *Caribeopsyllus amphiodiae* Ho, Dojiri, Hendler and Deets, 2003 seems to be ca. 3 days during the development from first to sixth copepodids, with adults surviving 3–27 days in culture (Hendler and Dojiri 2009).

Abbreviated Development

Reduction in the number of developmental stages, in particular naupliar instars, is common in symbiotic copepods in contrast to free-living taxa which typically display the ancestral complement of stages (i.e., six nauplii, six copepodids) (Raibaut 1996; Kearn 2004; Boxshall 2005; Huys 2014). The number and duration of these developmental stages appear to have been evolutionarily determined by feeding, predator-avoidance and reproductive strategies of symbiotic copepods, the life modes of host organisms, and other biological/environmental factors (see Hendler and Dojiri 2009). An extreme abbreviated case is found in the nicothoid *Neomysidion rahotsu* Ohtsuka, Boxshall and Harada, 2005 infecting mysids, in which only two stages are known: one copepodid stage for dispersal and infection, and the reproductive adult (Ohtsuka et al. 2005, 2007). The absence of entire naupliar stages seems to be related to the swarming behavior of the host mysid *Siriella okadai* Ii, 1964, occurring in surface waters at night (Ohtsuka et al. 2007), thus suggesting high local host availability for the infective stage during that time. The abbreviation of the copepodid stages is presumably due to the small size of the host organism. In fish-parasitic copepods, the full number of copepodid stages is common. Similarly, the ascidian endoparasite *Gonophysema gullmarensis* Bresciani and Lützen, 1960 shows highly abbreviated developmental stages, with one lecitrophic nauplius, one copepodid, an onychopodid (a reduced preadult larva), and adults (Bresciani and Lützen 1961).

Planktonic Adults of Caligidae and Ergasilidae

The elucidation of the caligid life cycle has been hampered by defining the correct number of developmental stages (Ho and Lin 2004a), but this dispute was settled recently

(Ohtsuka et al. 2009; Hamre et al. 2013; Venmathi Maran et al. 2013). The caligid development includes two naupliar, one copepodid (Fig. 2A–C), four chalimus stages, and the adult in the genus *Caligus* Müller, 1785 (Fig. 1), and two naupliar, one copepodid, two chalimi, two preadult stages, and the adult in *Lepeophtheirus* von Nordmann, 1832.

The occurrence of adults of the Caligidae in plankton samples has previously been attributed to their accidental detachment from the host fish (Ho and Lin 2004b; Venmathi Maran and Ohtsuka 2008; Venmathi Maran et al. 2016). Although some species like *Caligus undulatus* Shen and Li, 1959 frequently occur in the plankton, their hosts remain unknown (Venmathi Maran et al. 2016). Their ovigerous adult females were also found from plankton, but carried relatively fewer eggs per egg-string in comparison with congeners known from fish hosts (Venmathi Maran and Ohtsuka 2008; Ohtsuka et al. 2009). In *C. undulatus*, the number of eggs per string is at most 4, but in other congeners obtained from fish hosts off Japan and Taiwan, the number reaches up to 253 (Ohtsuka et al. 2009), thus implying that planktonic adults have limited access to food, possibly only during temporary contact/infection on fish. This behavior may be considered as an anti-predation strategy to avoid being consumed by cleaners, although it renders the parasites more vulnerable to predation by planktivorous fish (Venmathi Maran and Ohtsuka 2008; Venmathi Maran et al. 2016).

In three species of *Caligus*, host switching from wild intermediate fish to farmed definitive fish has been reported in Norway (Heuch et al. 2007), Australia (Hayward et al. 2008, 2009, 2011), and Japan (present study). In Norway, wild lumpfish *Cyclopterus lumpus* Linnaeus, 1758 seems to serve as an infection reservoir for *Caligus elongatus* von Nordmann, 1832 before it eventually infects farmed Atlantic salmon *Salmo salar* Linnaeus, 1758 (Heuch et al. 2007). In Australia, only adults of *Caligus chiastos* Lin and Ho, 2003 parasitize farmed southern bluefin tuna *Thunnus maccoyii* (Castelnau, 1872), and cause eye damage eventually leading to blindness (Hayward et al. 2008, 2009). Hayward et al. (2011) discovered that Degen's leatherjacket *Thamnaconus degeni* (Regan, 1903) residing outside the tuna cages serves as the wild intermediate host for chalimi development. In Japan, a similar phenomenon occurs in *Caligus sclerotinosus* Roubal, Armitage and Rohde, 1983 (Fig. 2D), a species that might have been introduced from Australia to Japan (Ho et al. 2004). It has spread among farms of red seabream *Pagrus major* (Temminck and Schlegel, 1843) in western Japan (Ho et al. 2004; Ohtsuka et al. 2010) and Korea (Venmathi Maran et al. 2012c). We document herein the prevalence and intensity of *C. sclerotinosus* on farmed *P. major* in Uwajima, Shikoku, Japan, from May 2006 to October 2007 (Fig. 3). In total, we examined 205 individuals of *P. major* and recovered 2,052 individuals of *C. sclerotinosus* from the hosts (mean standard length: 27.3 to 294.5 mm) (Fig. 3A). Among the caligids examined, only 3 chalimi (0.2%) were found on one occasion (December 2006) during the entire investigation period, and the remaining samples consisted primarily of adults (97.2%) and several unidentified, damaged individuals (2.6%) (Fig. 3B). Prevalence was constantly high, except for August 2006 (0%), reaching 100% in July 2006 and from December 2006 to October 2007, with mean intensity ranging between 1.4 (October 2006) and 26.3 (June 2007) (Fig. 3A). The intermediate host of *C. sclerotinosus* is still unknown. In these two cases, actively swimming adults searching for definitive hosts are regarded as a re-infective stage (Heuch et al. 2007). It is unknown whether such host switching is restricted to fish farms or not.

In the Ergasilidae, some species show a dual mode of life similar to that found in some Caligidae. Generally, copepodid stages I-V of representatives of *Ergasilus* von Nordmann, 1832 and related genera occur in the plankton, while the adult males die after mating

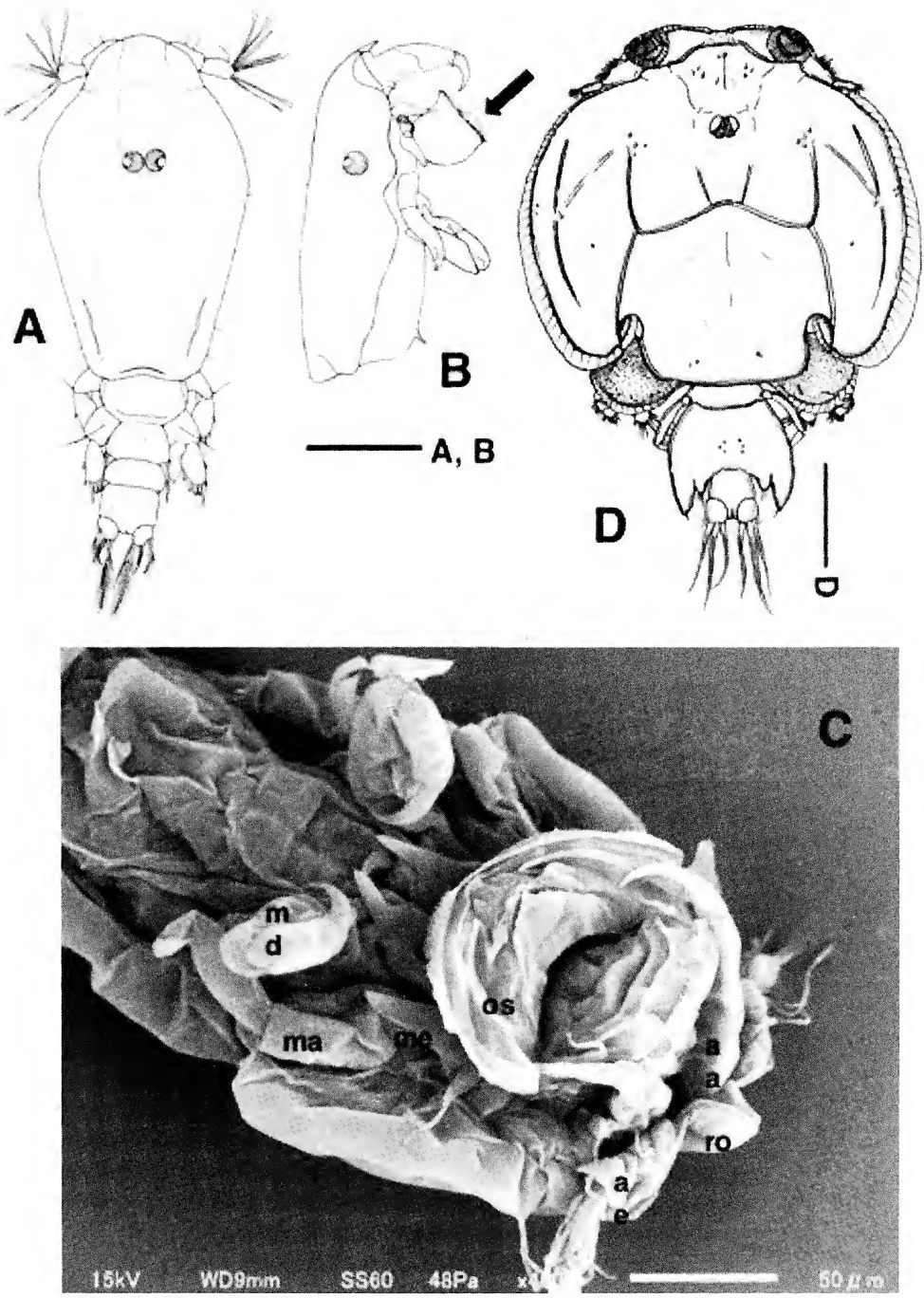

Fig. 2. *Caligus sclerotinosus* Roubal, Armitage and Rohde, 1983. (A) Copepodid I, dorsal view; (B) Cephalothorax of copepodid I, lateral view, with large oral cone indicated by arrow; (C) SEM micrograph of ventral view of cephalothorax of copepodid I. ae: antennule; aa: antenna; ma: maxilla; md: maxilliped; me: maxillule; os: oral sucker; ro: rostrum; (D) Adult male, dorsal view. Scale bars = 0.2 mm (A, B); 0.05 mm (C); 0.5 mm (D).

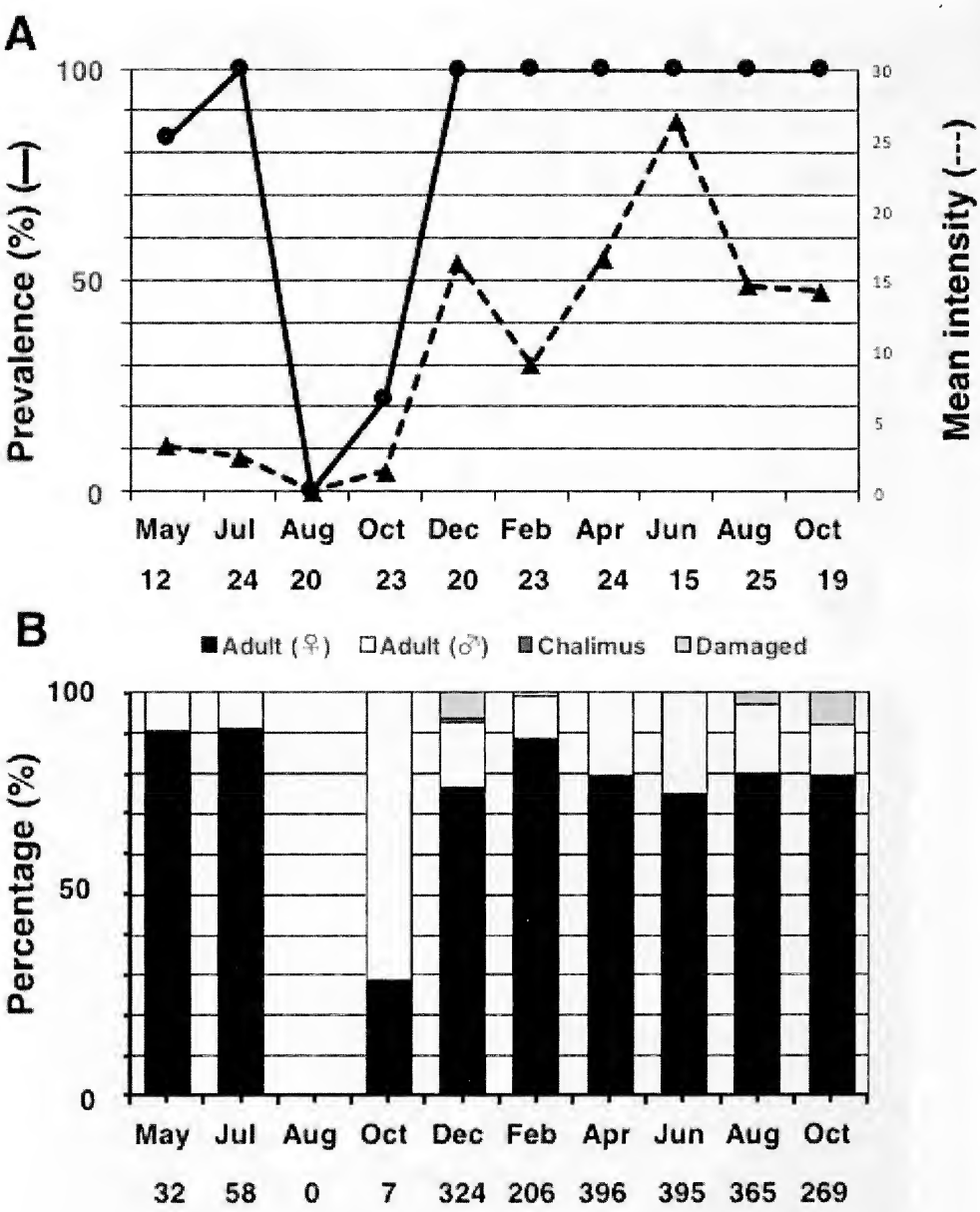

Fig. 3. Prevalence and mean intensity (A) and stage composition (B) of *Caligus sclerotinosus* Roubal, Armitage and Rohde, 1983 infecting farmed *Pagrus major* (Temminck and Schlegel, 1843) in Uwajima City, Japan, from May 2006 to October 2007. Numbers below months indicate total number of hosts collected and total number of parasites examined, respectively.

in the water column, and mated adult females become parasitic (Urawa et al. 1980a; Alston et al. 1996; Kearn 2004). However, the occurrence of ovigerous adult females of some ergasilids has been reported in plankton communities (Ohtsuka et al. 2004a, c). They have a relatively slender cephalothorax, not expanded like *Ergasilus lobus* Lin and Ho, 1998 [see Fig. 2A in Lin and Ho (1998)], and carry relatively fewer eggs in each sac com-

pared to other congeners permanently attached to host (Ohtsuka et al. 2004a), indirectly suggesting that these show a dual mode of life like that observed in *Caligus undulatus*. In Japanese waters, the enigmatic genus *Limnoncaea* Kokubo, 1914 was originally described from plankton samples (Kokubo 1914) and was later found to encompass species of two ergasilid genera, *Ergasilus* and *Thersitina* Norman, 1905, rendering it a junior subjective synonym of *Ergasilus* (Ohtsuka et al. 2004a, b, c).

Symbiotic Copepods on Plankters

Many copepods are symbiotically associated with phyto- or zooplanktonic organisms, and may complete their entire life cycle in planktonic communities. Associations between host plankters and symbiotic copepods are summarized in Table 1. Copepod symbioses in plankton communities are rare in comparison with those in benthic communities, partly because of the relatively lower diversity, shorter longevity, and smaller size (except for jellyfish and colonial forms like salps) of potential planktonic hosts (cf. Karplus 2014). Surprisingly, no record of the occurrence of symbiotic copepods has been reported from copepods and euphausiids in contrast to mysids (e.g., Mauchline 1998; Boxshall and Halsey 2004; Gómez-Gutiérrez et al. 2010). Two types of copepod symbionts on plankters can be recognized: (1) both naupliar and copepodid stages associated with the host (i.e., Miraciinae); (2) only copepodid stages, including adults, symbiotic on plankters (all other known cases). Although nauplii and copepodids of Miraciinae were thought to feed on *Trichodesmium* (Cyanobacteria) (Tokioka and Bieri 1966; Huys and Böttger-Schnack 1994; O'Neil and Roman 1994), gut content and stable isotope analyses (Eberl et al. 2007) have recently suggested that these copepods utilize the filamentous cyanobacteria as a habitat rather than a food source. All naupliar stages of a miraciinid *Macrosetella gracilis* (Dana, 1847) seem to be unable to swim (see Tokioka and Bieri 1966). Harpacticoids such as *Parathalestris cronii* (Krøyer, 1842) associated with floating macroalgae (Ingólfsson and Ólafsson 1997; Huys 2016) may also belong to the first type.

The complete life cycle of the truly parasitic species *Cardiodectes bellottii* (Richiardi, 1882) (as *C. medusaeus* (Wilson, 1908)) (Pennellidae) was described by Ho (1966) and Perkins (1983). The copepodid of *C. medusaeus* infects planktonic gastropod molluscs (i.e., Cavoliniidae and Janthinidae) as intermediate hosts, and then metamorphoses into the first chalimus stage (Ho 1966; Perkins 1983). After three molts of the chalimi on the hosts, adult females and males mate in the water, and then actively seek the definitive myctophid fish host. In this species, the planktonic phase comprises the copepodid and pre-copulatory adults. This species appears to lack a naupliar stage.

The life cycle of two nicothoids, *Hansenulus* Heron and Damkaer, 1986 and *Neomysidion* Ohtsuka, Boxshall and Harada, 2005, were unraveled by Heron and Damkaer (1986) and Ohtsuka et al. (2007), respectively. The planktonic/infective copepodid attaches to the mysid body surface, and finally lodges itself within the host marsupium involving either no or a few molts. Other genera and species of nicothoids were described from mysids by Hansen (1897).

Copepodids and adults of some species of the Macrochironidae are associated with medusae (Ohtsuka et al. 2015). However, the occurrence of host medusae in the plankton is seasonally limited for several months (Ohtsuka et al. 2012). In addition, free-swimming adults of some macrochironids have been described from the Indo-West Pacific (Wilson 1950; Browne and Kingsford 2005; Mulyadi 2005; Ohtsuka et al. 2012) and it is likely that during the absence of their main hosts, these copepods are able to utilize other host

Table 1. Copepods associated with planktonic organisms.

Taxon	Host	Reference
Harpacticoida		
Microsetella Brady and Robertson, 1873	appendicularians (including discarded houses), chaetognaths	Ohtsuka and Kubo (1991); Ohtsuka et al. (1993); Steinberg et al. (1994); Huys (2016)
Miraciinae Dana, 1846 (*Distioculus* Huys and Böttger-Schnack, 1994, *Macrosetella* Scott, 1909, *Miracia* Dana, 1846, *Oculosetella* Dahl, 1895)	*Trichodesmium* Ehrenberg ex Gomont, 1892	Tokioka and Bieri (1966); Huys and Bottger-Schnack (1994); O'Neil and Roman (1994); Eberl et al. (2007); Huys (2016)
Nitokra medusaea Humes, 1953	*Aurelia* sp.	Humes (1953)
Parathalestris cronii (Krøyer, 1842)	floating macroalgae	Huys (2016)
Siphonostomatoida		
Cardiodectes bellottii (Richiardi, 1882) (as *C. medusaeus* (Wilson, 1908) or *C.* sp.)	gastropods (as intermediate hosts)	Ho (1966); Perkins (1983)
Hyalopontius Sars, 1909	unknown	Boxshall and Halsey (2004)
Nicothoidae Dana, 1852 (*Aspidoecia* Giard and Bonnier, 1889, *Hansenulus* Heron and Damkaer, 1986, *Mysidion* Hansen, 1897, *Neomysidion* Ohtsuka, Boxshall and Harada, 2005)	mysids	Hansen (1897); Bowman and Kornicker (1967); Heron and Damkaer (1986); Ohtsuka et al. (2005, 2007)
Pontoeciella Giesbrecht, 1895	unknown	Boxshall and Halsey (2004)
Ratania Giesbrecht, 1893	unknown	Boxshall and Halsey (2004)
Cyclopoida (formerly Poecilostomatoida)		
Macrochironidae Humes and Boxshall, 1996 (*Macrochiron* Barady, 1872, *Paramacrochiron* Sewell, 1949, *Pseudomacrochiron* Reddiah, 1969)	scyphomedusae, cubomedusae	Reddiah (1968, 1969); Humes (1970); Boxshall and Halsey (2004); Browne and Kingsford (2005); Ohtsuka et al. (2012, 2015)
Oncaeidae Giesbrecht, 1893 (*Oncaea* Philippi, 1843, *Triconia* Böttger-Schnack, 1999)	appendicularians (including discarded houses)	Alldredge (1972); Ohtsuka and Kubo (1991); Ohtsuka et al. (1993, 1996); Steinberg et al. (1994)
Pseudolubbockia dilatata Sars, 1909	*Aegina citrea* Eschscholtz, 1829	Gasca et al. (2007)
Sapphirina Thompson, 1829	thaliaceans	Heron (1973); Takahashi et al. (2013, 2015)

medusae and/or other planktonic organisms or even shift from a planktonic to a benthic mode of life, using benthic polyps (Ohtsuka et al. 2012).

Copepodid stages and adults of the shallow- and deep-water species of the Oncaeidae (e.g., *Oncaea* Philippi, 1843 and *Triconia* Böttger-Schnack, 1999) are associated with appendicularian houses, in particular discarded ones, on which phyto- and zooplankters still remain and are consumed by the copepods (Alldredge 1972; Ohtsuka and Kubo 1991; Ohtsuka et al. 1993, 1996; Steinberg et al. 1994). The naupliar stages of *Oncaea media* Giesbrecht, 1891 and *Monothula subtilis* (Giesbrecht, 1893) (as *Oncaea subtilis* Giesbrecht, 1893) were described by Malt (1982) based on specimens successfully reared in the laboratory using cultured phytoplankton. The ectinosomatid harpacticoid *Microstella* Brady and Robsetson, 1873 and some scolecitrichid genera of calanoid copepods are also associated with occupied, and in particular discarded, appendicularian houses (Ohtsuka and Kubo 1991; Ohtsuka et al. 1993; Steinberg et al. 1994). *Microsetella* is known as a parasite of chaetognaths (Huys 2016).

Copepodids and adults of *Sapphirina* Thompson, 1829 were found to be associated with thaliaceans (Heron 1973; Takahashi et al. 2013, 2015). Copepodids and adult females of *Sapphirina nigromaculata* Claus, 1863 were frequently attached to the chains of nurse and zooid stages of doliolids in the Kuroshio Extension (Takahashi et al. 2013). For *Sapphirina iris* Dana, 1849 there is evidence that the copepod can penetrate the salp body and remain lodged in the host while feeding on it (Gasca et al. 2015). According to Takahashi et al. (2013), the ingestion rate of *S. nigromaculata* on live doliolids corresponded to 29–37% of the copepod body carbon (5.1–6.4 μg C $ind^{-1}d^{-1}$), and the potential population ingestion rate of three dominant sapphirinids linearly increased with the size of the doliolid populations. Doliolids and salps attacked by sapphirinids were sometimes seriously damaged leading to death (Heron 1973; Takahashi et al. 2013), thus suggesting that this interaction can be regarded as predation or parasitoidism rather than commensalism or parasitism (Gasca et al. 2015).

The deep-sea cyclopoid *Pseudolubbockia dilatata* Sars, 1909 was recorded to be associated with the hydromedusa *Aegina citrea* Eschscholtz, 1829 off California using a ROV (Gasca et al. 2007). The presence of early copepodid stages and mated pairs of *P. dilatata* on the host suggests that they utilize it for refuge and mating, although the feeding habits of the symbiont were not determined (Gasca et al. 2007). Potential hosts of the few known holoplanktonic siphonostomatoid families (i.e., Megapontiidae, Pontoeciellidae, Ratanidae) are still unknown, although this order is generally regarded as symbiotic (Boxshall and Halsey 2004).

Interactions between Different Phases and Other Organisms

In addition to the morphological and behavioral differences between the planktonic and symbiotic phases of symbiotic copepods, it is worthwhile to note that some physiological and microbiological phenomena are also involved in the phase change.

Adults and probably chalimi of *Caligus fugu* (Yamaguti, 1936) infecting the toxic tetraodontid puffer are tolerant to its tetrodotoxin (TTX), and are able to accumulate it in their bodies, except for the ovaries and eggs (Ikeda et al. 2006). TTX-free eggs evidently produce TTX-free nauplii, suggesting that TTX accumulation in the adult body is by way of feeding on the toxic host tissues and blood. In the same species, different developmental stages exhibit different physiological reactions to TTX under a certain genetic mechanism, especially that concerning blocking of sodium channels (Chen and Chung 2014).

TTX-producing bacteria were found on the body of *C. fugu* (Venmathi Maran et al. 2007), but their actual interaction is still unknown.

Caligids have been regarded as pests in fish farms in many geographic regions (Ho and Lin 2004a; Johnson et al. 2004; Shinn et al. 2015). Economic losses caused by these pathogenic copepods on farmed Atlantic salmon *Salmo salar* Linnaeus, 1758 are estimated at US$480 million annually (Shinn et al. 2015). Usually, these copepods feed on the host skin, tissues and blood, causing physical damage and inflammation and finally leading to death (Nylund et al. 1993; Dojiri and Ho 2013). Secondary microbial infections occur via open wounds, thus enhancing the economic losses (Nylund et al. 1993; Dojiri and Ho 2013). Nylund et al. (1993) showed that infectious salmon anaemia (ISA) caused by *Aeromonas salmonicida* (Lehmann and Neumann, 1896) was horizontally transferred by *Lepeophtheirus salmonis* (Krøyer, 1837). Madinabeitia et al. (2009) also found the presence of the pathogenic bacterium *Lactococcus garvieae* (Collins, Farrow, Phillips and Kandler, 1984) on *Caligus longipedis* Bassett-Smith, 1898 infecting the striped jack *Pseudocaranx dentex* (Bloch and Schneider, 1801) suffering from lactococcosis in Japan. This study showed that the adults of *C. longipedis* carrying *L. garvieae* could be potential agents for the transmission of the bacteria between host fish. Therefore, the adults of some species of caligids may act as vectors of diseases among cultured fish. However, the microhabitats of these bacteria on and in the bodies of caligids remain unknown (Nylund et al. 1993). Pathogens likely spread by way of host switching of caligids, ergasilids, and other good swimmers carrying them.

Evolutionary and Adaptive Strategies of the Planktonic Phase in Symbiotic Copepods

Symbiotic copepods generally have one or more planktonic phases. However, the number and duration of developmental stages and feeding type of the planktonic phase vary between taxonomic groups (Fig. 1). Heterochrony is greatly important to determine developmental timing of reproductive and somatic gene expressions (Gould 1977), and has been considered an important mechanism in the evolution of the Copepoda (Huys and Boxshall 1991). Progenesis is conspicuous in some symbiotic copepods such as *Neomysidion* and the Thaumatopsyllidae.

What sort of evolutionary factors drive such different modes of life of symbiotic copepods? Hendler and Dojiri (2009) discussed the evolution of the bizarre thaumatopsyllids, and regarded predator avoidance as the most important factor. The endoparasitic metanauplii of *Caribeopsyllus amphiodiae* grow in the stomach of its ophiuroid host for 5 months contrasting with a very short duration of its planktonic phases. The microhabitat provides the metanauplii with refuge and sufficient food, while free-living nauplii are generally exposed to high mortality caused by predation (Hendler and Dojiri 2009).

The availability of hosts for symbiotic copepods may also be essential to determine the number and duration of the planktonic phase. In some cases, the aggregated distribution of host animals enhances infection efficiency of some ectoparasites due to their horizontal transfer (Brown and Brown 1986). Therefore, swarming hosts may represent a factor that accelerates the abbreviation of lecithotrophic naupliar stages for dispersal.

On the other hand, phylogenetic constraints are also remarkable in the evolution of symbiotic copepods. In the Caligidae, the number of developmental stages is so far known for 18 species, infecting a variety of coastal and oceanic fish, and has proven to be remarkably constant (8 stages in total in both *Caligus* and *Lepeophtheirus*) (Ohtsuka et al. 2009;

Madinabeitia and Nagasawa 2011; Hamre et al. 2013; Venmathi Maran et al. 2013; Huys 2014). The encounter and accessibility of ecologically diversified hosts for highly host-specific caligids seems to be greatly variable, but the number of developmental stages in the family appears nevertheless to be phylogenetically conservative. Some adaptive strategies may compensate for the conservative phylogenetic constrains. It may be possible that reproductive strategies of the symbionts are synchronized with the hosts' aggregating behavior, such as breeding and swarming. Such synchronization would enhance the infection efficiency and the life cycle flexibility, as caligids do in fish farms switching from single to multiple hosts.

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First Record of the Family Peltidiidae (Copepoda; Harpacticoida) from the Gulf of Mexico, with the Description of a New Species of *Peltidium*

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Abstract.—Female harpacticoid copepod specimens representing an undescribed species of *Peltidium* (Peltidiidae) were found from an unidentified species of *Sargassum* during a series of samplings carried out in 2014 in Tampa Bay, Florida, U.S.A. The new species, *Peltidium camilae*, is similar to *P. nichollsi* Geddes, 1968 and *P. lerneri* Geddes, 1968. These species share the female exopod of leg 5 with two inner and three apical setae, the second endopodal segment of leg 1 with three setae, and the third endopodal segment of legs 2-4 with three, five and four setae, respectively. *Peltidium camilae* n. sp. can be distinguished from *P. nichollsi* and *P. lerneri* by having a shorter endopod relative to the exopod on legs 2-4, apical claws on the terminal exopodal segment of leg 1 that are as long as the first exopodal segment of leg 1, and subequal middle and inner apical setae on the exopod of leg 5. This is the first record of the family Peltidiidae from the Gulf of Mexico.

The family Peltidiidae (Copepoda; Harpacticoida) contains mainly marine algal-dwellers able to cope with strong water flows over flat surfaces thanks to their flattened bodies (Boxshall and Halsey 2004; Song et al. 2015). The family is composed of eight genera (Boxshall and Halsey 2004), of which *Peltidium*, with 28 valid species, is the most speciose (Varela 2005; Wells 2007; Suárez-Morales and Jarquín-González 2013; Varela and Gómez 2013). Members of *Peltidium* have been recorded from all but the polar seas (Song et al. 2015). However, this group of harpacticoid copepods has not been reported from the Gulf of Mexico (Suárez-Morales et al. 2009; Song et al. 2015). As part of a field trip with students to learn about the marine biodiversity of the west coast of Florida, washings of an unidentified species of *Sargassum* C. Agardh, 1820 (Ochrophyta; Sargassaceae) were examined for harpacticoid copepods. Here we present a new species, *Peltidium camilae*, found in those macroalgal washings from Tampa Bay, Florida.

Materials and Methods

Samples of *Sargassum* sp. were collected at 1.5 m depth while SCUBA diving and were then placed in a resealable polyethylene bag. In the laboratory, several drops of formalin were added to the bag and then the content was shaken vigorously to detach the copepods from the macroalgae. The washings were poured through a 300 μm sieve, and then the sieve

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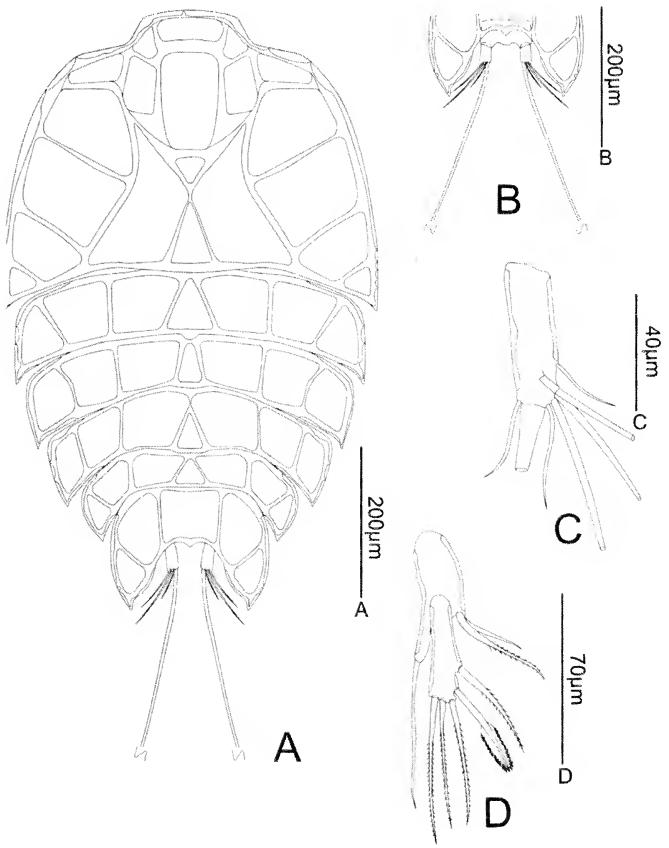

Fig. 1. *Peltidium camilae* n. sp., adult female. (A) Habitus, dorsal view; (B) Urosome showing posterior end of the genital double-somite, the anal somite, and caudal rami, ventral view; (C) Caudal ramus, dorsal view; (D) Leg 5.

contents were transferred to a petri dish filled with water from which copepods were manually separated from the debris at 60× magnification using a Wild M5 stereomicroscope. Observations and drawings of whole specimens and dissected appendages were made with the aid of a Leica CME microscope equipped with a drawing tube. The type material was deposited in the Florida International Crustacean Collection (FICC) at Florida International University (FIU). Abbreviations used in the text are: EXP, exopod; END, endopod; and P1-P5, legs 1-5.

Results

Peltidium camilae n. sp. (Figs. 1-3)

Type material. One ovigerous female holotype (HBG 8001) and one dissected, non-ovigerous female paratype (HBG 8002) preserved in 75% ethanol, February 14, 2014, ex *Sargassum* sp., 1.5 meters depth, col. C. Varela.

Type locality. Tampa Bay, Florida, U.S.A. (27°32'N, 82°44'W).

Description of adult female. Body (Fig. 1A) broad, dorsoventrally flattened, arched along longitudinal axis, strongly chitinized, and tapering posteriorly. Total body length

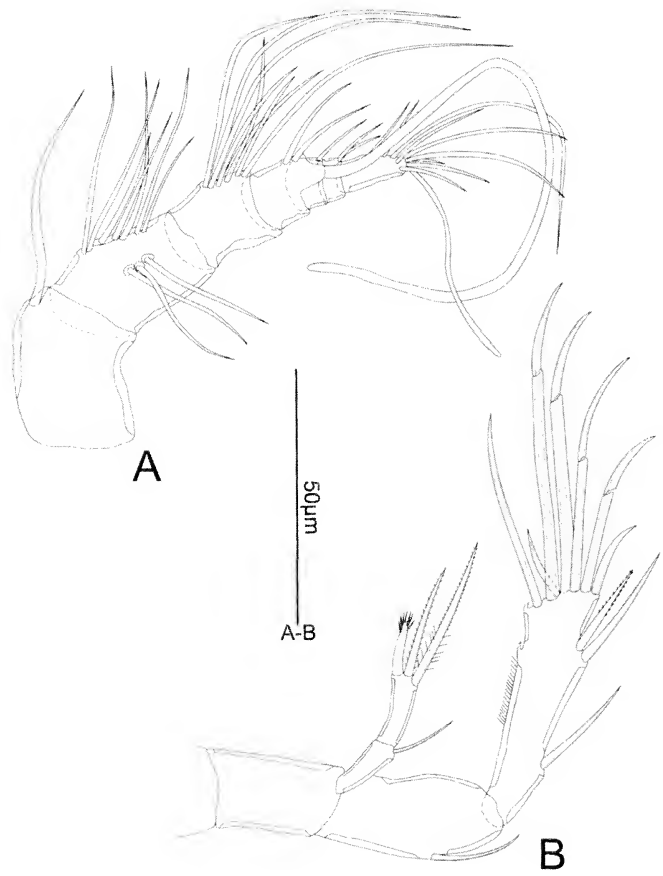

Fig. 2. *Peltidium camilae* n. sp., adult female. (A) Antennule; (B) Antenna.

measured from tip of rostrum to posterior margin of caudal rami ranging from 0.90 mm for the paratype to 1.05 mm for the holotype (mean = 0.98 mm); greatest width at posterior part of cephalothorax. Cephalothorax accounting for about half of body length; rostrum broad. Epimera of somites pointed and posteriorly directed. Urosome comprising P5-bearing somite, genital double-somite, and anal somite (Fig. 1B). Genital double-somite well developed, wider than long, with posterolateral corners reaching well beyond posterior margin of caudal rami. Each ramus (Fig. 1C) about 3 times as long as wide, slightly tapering distally, with 7 setae located on distal third; setae I and VI shortest, subequal in length.

Antennule (Fig. 2A) 7-segmented; segment 2 longest, segment 6 shortest. Armature formula: 1–[1], 2–[10], 3–[8],4[2+ae)], 5–[1], 6–[2], 7–[9+ae].

Antenna (Fig. 2B) with small coxa. Basis without abexopodal seta. Exopod 2-segmented, elongated; first segment with short, slender inner seta; second segment with 3 setae distally, of which smallest seta pectinate apically. Endopod 2-segmented; first segment shorter than second, with 1 inner seta; second segment with outer row of long spinules, 1 proximal seta, 1 spine and 1 seta subdistally along inner margin, and 7 apical elements, 4 of them geniculate.

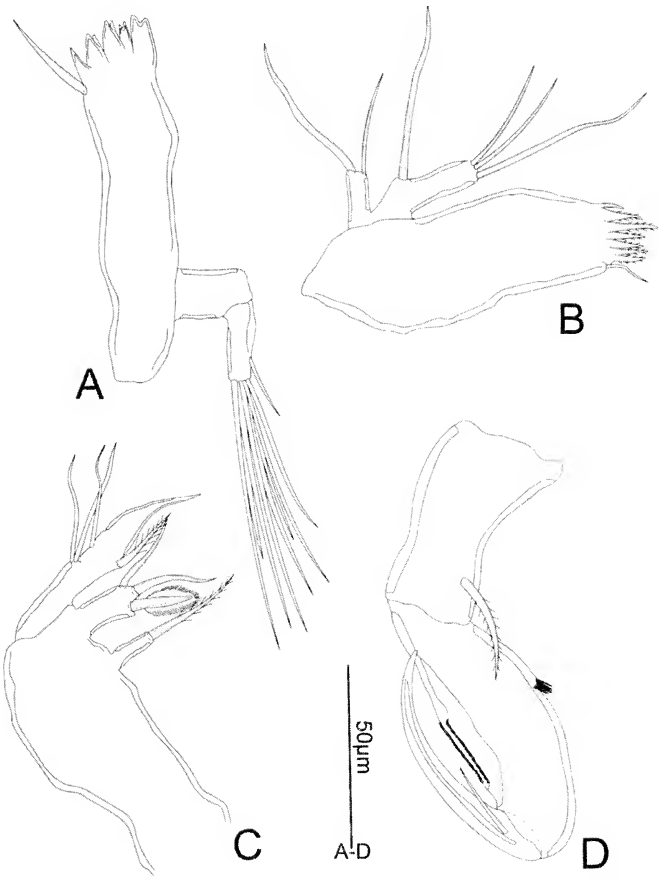

Fig. 3. *Peltidium camilae* n. sp., adult female. (A) Mandible; (B) Maxillule; (C) Maxilla; (D) Maxilliped.

Mandible (Fig. 3A) with long, slender coxa. Gnathobase with 1 stout seta and 4 bicuspidate and 1 monocuspidate teeth. Mandibular palp small, uniramous; basis unarmed; endopod 1-segmented with 1 lateral and 8 distal setae.

Maxillule (Fig. 3B) with 7 unarticulated spines and 1 short seta on praecoxal arthrite. Coxa and basis fused, with 3 distal setae. Endopod represented by 1 seta. Exopod 1-segmented, with 2 apical setae.

Maxilla (Fig. 3C) with robust syncoxa bearing 2 endites; proximal endite small, with 1 large apical seta; distal endite cylindrical, with 3 apical setae, 1 of them modified. Allobasis drawn into strong claw with 1 midventral seta. Endopod represented by 3 subequal setae.

Maxilliped (Fig. 3D) subchelate. Syncoxa elongate, with concave outer margin and 1 outer subdistal seta. Basis slightly longer than syncoxa, with small outer patch of spinules and 2 inner longitudinal rows of small spinules. Endopodal claw slightly curved, almost as long as basis, with single proximal seta.

Leg 1 (Fig. 4A) with large coxa ornamented with row of small setules along inner and outer margins. Basis somewhat transversely elongated, with setules along entire inner margin and along proximal outer margin; inner seta reaching middle of second exopodal segment; outer seta about half length of inner seta. Exopod 3-segmented; first segment with

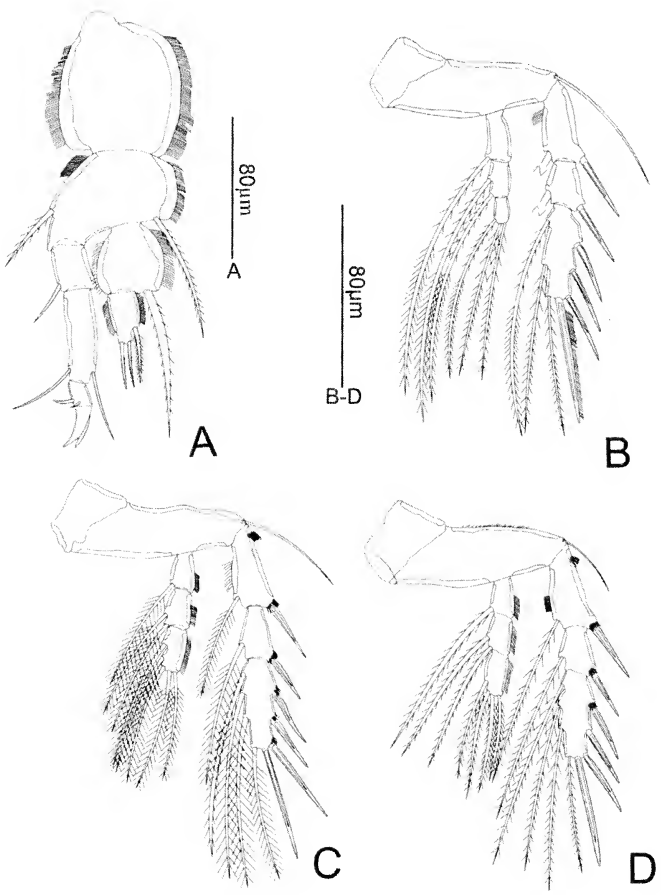

Fig. 4. *Peltidium camilae* n. sp., adult female. (A) Leg 1; (B) Leg 2; (C) Leg 3; (D) Leg 4.

outer spine; second segment longest, about 2 times as long as first segment, with outer subdistal spine and inner subdistal element; third segment with 2 subdistal and 2 distal claws. Endopod 2-segmented, shorter than exopod; first segment globose, with 1 long inner seta and row of short setules along inner and outer margins; second segment with 1 inner seta and 1 pinnate spine and 1 slender seta apically.

Legs 2 to 4 (Figs. 4B-D) with small coxa. Basis transversely elongated, with long outer seta. Rami 3-segmented. Endopod shorter than exopod. Armature of swimming legs 2-4 as in Table 1.

Table 1. Armature of swimming legs 2-4 (spines in Roman numerals, setae in Arabic) of *Peltidium camilae* n. sp. Sequence follows internal to external positions.

Leg	Exopod	Endopod
P2	1-I; 1-I; 2,1+I,III	1-0; 2-0; 1,2,0
P3	1-I; 1-I; 3,1+I,III	1-0; 2-0; 3,2,0
P4	1-I; 1-I; 3,1+I,III	1-0; 2-0; 2,2,0

Leg 5 (Fig. 1C) with distinct exopod and baseoendopod. Baseoendopod with outer basal seta issuing from long setophore; endopodal lobe with 2 unequal setae, 1 of them naked. Exopod slender, with 2 inner and 3 apical elements.

Male. Unknown.

Etymology. The species is named after the first author's daughter, Camila Varela Varona.

Remarks. *Peltidium camilae* n. sp. is attributed to Geddes' (1968) group B by the presence of five setiform elements on the P5 exopod, located either apically or on the inner edge. This group is composed of *P. angulatum* Thompson and Scott, 1903, *P. speciosum* Thompson and Scott, 1903, *P. perplexum* Thompson and Scott, 1903, *P. falcatum* Scott, 1909, *P. intermedium* Scott, 1909, *P. exiguum* Scott, 1909, *P. minutum* Scott, 1909, *P. hawaiiense* Pesta, 1935, *P. monardi* Pesta, 1935, *P. proximum* Nichols, 1941, *P. maldivianum* Sewell, 1940, *P. laudatum* Tanaka and Hue, 1966, *P. nichollsi* Geddes, 1968, *P. lerneri* Geddes, 1968, *P. quinquesetosum* Song and Yun, 1999, and *P. nayarit* Suárez-Morales and Jarquín-González, 2013. Amongst these, only three species are known from the Neotropical region. *Peltidium nichollsi* and *P. lerneri* were originally described from Exuma Cays, Bahamas (Geddes 1968), and *P. nayarit* is known from the state of Nayarit in the Eastern Tropical Pacific (Suárez-Morales and Jarquín-González 2013). For the distribution of the other species of the genus see Song et al. (2015) and Varela and Gómez (2013). *Peltidium camilae* n. sp. from the west coast of Florida, and the Bahamian species, *P. nichollsi* and *P. lerneri*, share three setae on P1 END2 and three, five and four setae, respectively, on the END3 of P2-P4.

Peltidium camilae n. sp. is more similar to *P. nichollsi* than to *P. lerneri* based on the shape of the setae on the second segment of the antennary exopod (with one strongly pinnate and two setiform elements in *P. nichollsi* and *P. camilae* n. sp., but with all elements setiform in *P. lerneri*), and the relative length of the P1 EXP1 (shorter than P1 EXP2 in *P. nichollsi* and *P. camilae* n. sp., but nearly as long as P1 EXP2 in *P. lerneri*). The new species can be further separated from the Bahamian species by the relative length of the END of P2-P4 (as long or slightly longer than the EXP in the Bahamian species, but shorter in the new species), relative length of the apical claws of the P1 EXP3 (as long as P1 EXP1 in the new species, longer than P1 EXP1 in *P. nichollsi*, and shorter than P1 EXP1 in *P. lerneri*), and relative length of the middle apical seta of the female P5 EXP (as long as the inner apical seta on P5 EXP in *P. camilae* n. sp., about half as long in *P. nichollsi*, and more than three times shorter in *P. lerneri*). Noticeable differences in the armature of the maxilliped were also detected, but they cannot be considered with confidence based on Geddes' (1968) descriptions of *P. nichollsi* and *P. lerneri*. For example, the absence of the seta on the maxillipedal syncoxa of *P. nichollsi* and *P. lerneri* is likely erroneous as this seta is present in other species of the genus including *P. camilae* n. sp. Also, the inner seta on the maxillipedal basis of *P. nichollsi* might actually be a long, slender spinule. Examination of type material of *P. nichollsi* and *P. lerneri* is needed to resolve this issue.

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Some Observations of Morphology and Behavior of a Hyperbenthic Misophrioid Copepod

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Abstract.—The locomotion, feeding, excretion, and oviposition of a member of the copepod family Misophriidae were observed based on a live specimen collected from a sandy bottom at a depth of 52 m off Nagannu Island, Okinawa, Japan. This species is related to *Arcticomisophria* Martínez Arbizu and Seifried, 1996 in the armature of leg 1, but the fifth leg is much more reduced. The combination of morphological characters strongly suggests that it represents an undescribed genus. The maxillipeds played a major role in attaching to the bottom and in crawling, while the antennae and mandibular palps were involved in slow swimming along the bottom. It fed on small-sized cultured phytoplankters, and excreted numerous fecal pellets. The female carried 4-5 eggs of 0.09 mm diameter that were loosely attached to the urosome. Nearly complete nuclear 18S and 28S rRNA gene sequences and a partial mitochondrial cytochrome *c* oxidase subunit 1 (CO1) gene sequence were obtained and are made available for future phylogenetic and systematic work.

The order Misophrioida is a compact podoplean order of the subclass Copepoda, and consists of three families accommodating 17 genera and 36 species (Walter and Boxshall 2017). The group exhibits numerous plesiomorphies in appendages, segmentation, and armature (Huys and Boxshall 1991; Boxshall and Halsey 2004). Misophrioids are exclusively distributed in deep/shallow hyperbenthic layers, the deep-sea, and anchialine caves, as free-living forms (Boxshall 1983; Boxshall and Iliffe 1986, 1987; Ohtsuka et al. 1992; Boxshall and Jaume 2000; Boxshall and Halsey 2004; Boxshall et al. 2014). Gurney (1933) established the order based on the body plan of adults and the peculiarly abbreviated life cycle, although Sars (1911) had formerly classified it as an aberrant family of another podoplean order, the Harpacticoida. Subsequently, Huys and Boxshall (1991) clearly recognized the Misophrioda as a robust taxon, although it is difficult to define by synapomorphies.

The biology and ecology of the Misophrioida have been poorly understood, mainly because of the difficulty of collection from their habitats, and their relatively low abundance

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in comparison with planktonic taxa such as the Calanoida and Cyclopoida. The only exception is the bathypelagic genus *Benthomisophria* Sars, 1909, in which the internal anatomy, post-naupliar development, specialized feeding organs, and feeding were well studied using deep-sea plankton samples (<4,000 m deep) (Boxshall and Roe 1980; Boxshall 1982, 1984). Sars (1911) observed the swimming behavior and the presence of an "ovisac" in the shallow-water hyperbenthic *Misophria pallida* Boeck, 1864. Gurney (1933) briefly described the naupliar and first copepodid stages of the species, and also confirmed the oviposition. The biological and ecological aspects of cavernicolous taxa remain unknown.

During our surveys on hyperbenthic copepods in subtropical regions of Japan, we found a live specimen of an undescribed misophrioid copepod (family Misophriidae) in a dredge sample collected from off Okinawa, southwestern Japan. The locomotion, feeding, excretion, and oviposition of the misophrioid were observed and are reported herein. In addition, we have succeeded in extracting and sequencing DNA of this misophrioid (Fig. 1), and the sequences were registered in the International Nucleotide Sequence Database Collaboration (INSDC) to be available for future phylogenetic and systematic work.

Materials and Methods

The live specimen of the undescribed taxon of the family Misophriidae (Fig. 1A) was collected from a depth of 52 m on sandy bottom off Nagannu Island, Okinawa (26°14.339′N, 127°32.280′E) during daytime (local time 13:52-14:17) on May 21, 2016. Sediment collections were carried out with a dredge (mouth 50 cm wide × 15 cm high; mesh size 5 mm) towed along the bottom twice by Hiroshima University's Training Research Vessel (TRV) *Toyoshio-maru*. Sediments were stirred up in sea-water, and the supernatant was filtered through a plankton net (mesh size 0.1 mm). The obtained specimen was kept in a lidded Tupperware container (200 mL) full of filtered sea-water at room temperature onboard and at ca. 20°C in the laboratory. It was adequately fed three species of cultured phytoplankters daily: diatom *Chaetoceros calcitrans* (Paulsen) Takano, 1968 (approximate cell size 6.8 μm × 4.8 μm); prasinophyte *Tetraselmis tetrathele* (West) Butcher, 1959 (14.8 μm × 9.2 μm); and eustigmatophyte *Nannochloropsis oculata* (Droop) Hibberd, 1981 (3.0 μm). Filtered sea-water was changed daily. Fecal pellets produced by the female were collected from the bottom of the Tupperware container with a fine pipette, fixed in 10% neutralized formalin-seawater, and measured.

The behavior of the live specimen was observed in a Costar cell plate and documented with a Sony Handycam HDR-CX550 video camera attached to an Olympus SZ-X7 dissecting microscope. The body and fecal pellets were measured with an Olympus DP-20 CCD camera attached to the same dissecting microscope. The misophrioid became moribund on June 6, 2016 (laboratory survival = 17 days), and was then fixed in 99.5% ethanol for genetic analysis.

After treatment for the genetic analysis described below, the specimen (exoskeleton only) was dissected and examined in lactophenol with a Nikon Optiphoto differential interference microscope. The urosome and appendages were mounted on two glass slides for morphological observation. The dissected specimen is deposited in the Kitakyushu Museum of Natural History and Human History, Japan (KMNH IvR 500959). Terminology used in descriptions follows Huys and Boxshall (1991).

Total DNA was extracted from the 99.5% ethanol preserved specimen using the DNeasy Blood and Tissue Kit (Qiagen, USA) following the manufacture's protocol with minor

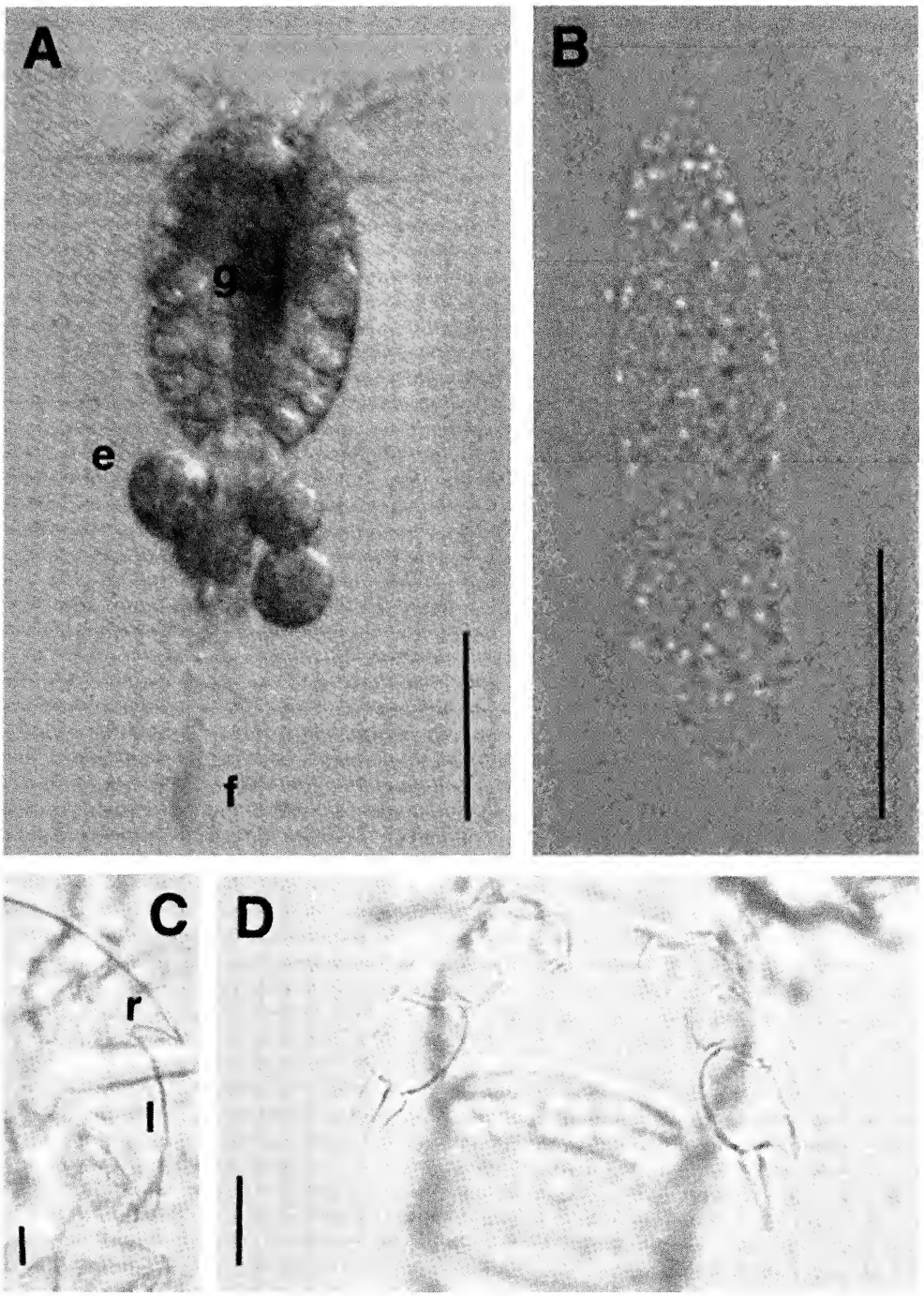

Fig. 1. Photographs of the ovigerous female of an undescribed genus of Misophriidae. (A) Habitus, dorsal view (e: egg mass, f: fecal pellet, g: gut full of cultured microalgae); (B) Fecal pellet fixed in 10% neutralized formalin-seawater; (C) Rostrum (r) and labrum (l), lateral view; (D) Leg 5, ventral view. Scale bars = 0.2 mm (A); 0.05 mm (B); 0.02 mm (C, D).

changes (elution volume = 100 µl). The whole body of the single specimen was put in a 1.5 ml microtube and protein was digested by a Proteinase K solution for 10 h at 56°C. After the protein digestion step, the chitinous exoskeleton was retrieved from the microtube and mounted as a morphological voucher specimen. Nearly complete sequences of nuclear 18S rRNA (18S) and 28S rRNA (28S) and mitochondrial cytochrome *c* oxidase subunit 1 (CO1) genes were PCR amplified. Primer sets for the PCR and Cycle sequencing (CS) reactions used in this study are shown in Table 1. The PCR reactions were performed using a T100 Thermal Cycler (Bio-Rad). The reaction solutions consisted of a 25 µl solution containing 0.5 µl KOD FX Neo (Toyobo, Japan), 12.5 µl of 2X PCR buffer for KOD FX Neo, 5 µl of dNTP mix, 1 µl of each primer (5 pmol), template DNA (2 µl for 18S and 1 µl for 28S and CO1), and 3 or 4 µl of sterilized distilled water. The PCR conditions consisted of an initial denaturation step at 95°C for 2 min, followed by 40 cycles of denaturation at 98°C for 10 s, annealing at 52°C (18S), 50°C (28S), or 45°C (CO1) for 30 s, extension at 68°C for 1 min 30 s (18S), 2 min (28S), or 1 min (CO1), and a final extension at 68°C for 5 min. The quantity and length of the PCR products were checked by 1% Agarose S (Nippon Gene, Japan) gel electrophoresis and stained with ethidium bromide. The products were purified for sequencing using a FastGene Gel/PCR Extraction Kit (Nippon Gene, Japan), according to the manufacture's protocol. Sequencing was performed by the Macrogen Japan Corp. (Tokyo) with the primer sets shown in Table 1. A homology search was performed by BLAST (Altschul et al. 1990, 1997) with blast program from the National Center for Biotechnology Information (NCBI, http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Results

Adult female. Body (Figs. 1A, 2A) cyclopiform, 0.52 mm in length, 0.23 mm in maximum width. Prosome (Figs. 1A, 2A) oval, about 1.5 times longer than wide, ca. 1.4 times as long as urosome; pediger 1 totally covered by carapace-like extension; pediger 4 posteriorly produced into paired lamellar expansions (Fig. 2B). Urosome (Fig. 2B) comprising pediger 5, genital double-somite, three free abdominal somites, and caudal rami; pediger 5 acutely produced posterolaterally; genital somite incompletely fused to first abdominal somite with suture clearly visible; gonopore covered by plate-like leg 6 with outer seta, small terminal spine, and minute subterminal prominence; copulatory pores paired, located at posterior one-third length; anal operculum weakly developed, fringed with spinular row dorsally.

Rostrum (Figs. 1C, 2A) forming triangular process, directed posteroventrally. Labrum (Figs. 1C, 2A) swollen ventrally. Antennule stretched out anterolaterally when alive (Fig. 1A) (terminal segments lost during DNA extraction). Antenna (Fig. 2C) consisting of coxa, basis, 3-segmented endopod, and 6-segmented exopod; coxa unarmed; basis with 2 setae distally; first endopod segment having 1 subterminal seta, second segment with 2 minute mid-lateral and 2 terminal setae, third segment with 6 setae distally; setal formula of exopod 0, 2, 1, 1, 1, 3 (based on suture lines on posterior surface). Mandible gnathobase (Fig. 2D) stout, bearing 8 teeth and 2 setae, with ventralmost tooth serrated terminally; palp (Fig. 2E) consisting of basis with 1 minute seta subdistally and patches of minute spinules; exopod incompletely 4-segmented, with setal formula 1, 1, 2, 2; endopod 2-segmented, with setal formula 2, 6. Maxilla (Fig. 2F) with praecoxal and coxal endites having 7, 3 and 3, 3 setae, respectively; basis fused to first endopod segment to form allobasis, with heavily chitinized process plus 3 setae; free endopod 3-segmented, with setal formula 2, 2, 4. Maxilliped basis (not figured) with 3 setae; endopod 5-segmented, with setal formula 2, 2, 2, 2, 4 (3 large + 1 small).

Table 1. List of PCR and cycle sequencing (CS) primers used in this study.

Target gene	Primer name	Reaction	Sequence (5' to 3')	Direction	Source
18S rRNA	Euk18SF	PCR & CS	ACCTGGTTGATCCTGCCAG	Forward	Moon-van der Staay et al. (2000)
	Euk18SR	PCR & CS	TGATCCTTCYGCAGGTTCAC	Reverse	Moon-van der Staay et al. (2000)
	18SF2	CS	CCTGAGAAACGGCTRCCACAT	Forward	Yamaguchi and Endo (2003)
28S rRNA	28S-01	PCR & CS	GACTACCCCCTGAATTTAAGCAT	Forward	Kim et al. (2000)
	CS632	PCR & CS	CGATGAAGAACGCAGC	Forward	Schlötterer et al. (1994)
	28jj	PCR & CS	AGTAGGGTAAAACTAACCT	Reverse	Hillis and Dixon (1991)
	28S_18R	CS	CAGGCATAGTTCACCATCTTTC	Reverse	This study
	28S_24R	CS	ACATGGAACCCTTCTCCAC	Reverse	This study
	28S_32R	CS	AGAGCACTGGGCAGAATTC	Reverse	This study
	28S-42F	CS	GAGTTTGACTGGGGCGGTA	Forward	This study
COI	LCO1490	PCR & CS	GGTCAACAAATCATAAAGATATTGG	Forward	Folmer et al. (1994)
	HCO2198	PCR & CS	TAAACTTCAGGGTGACCAAAAAATCA	Reverse	Folmer et al. (1994)

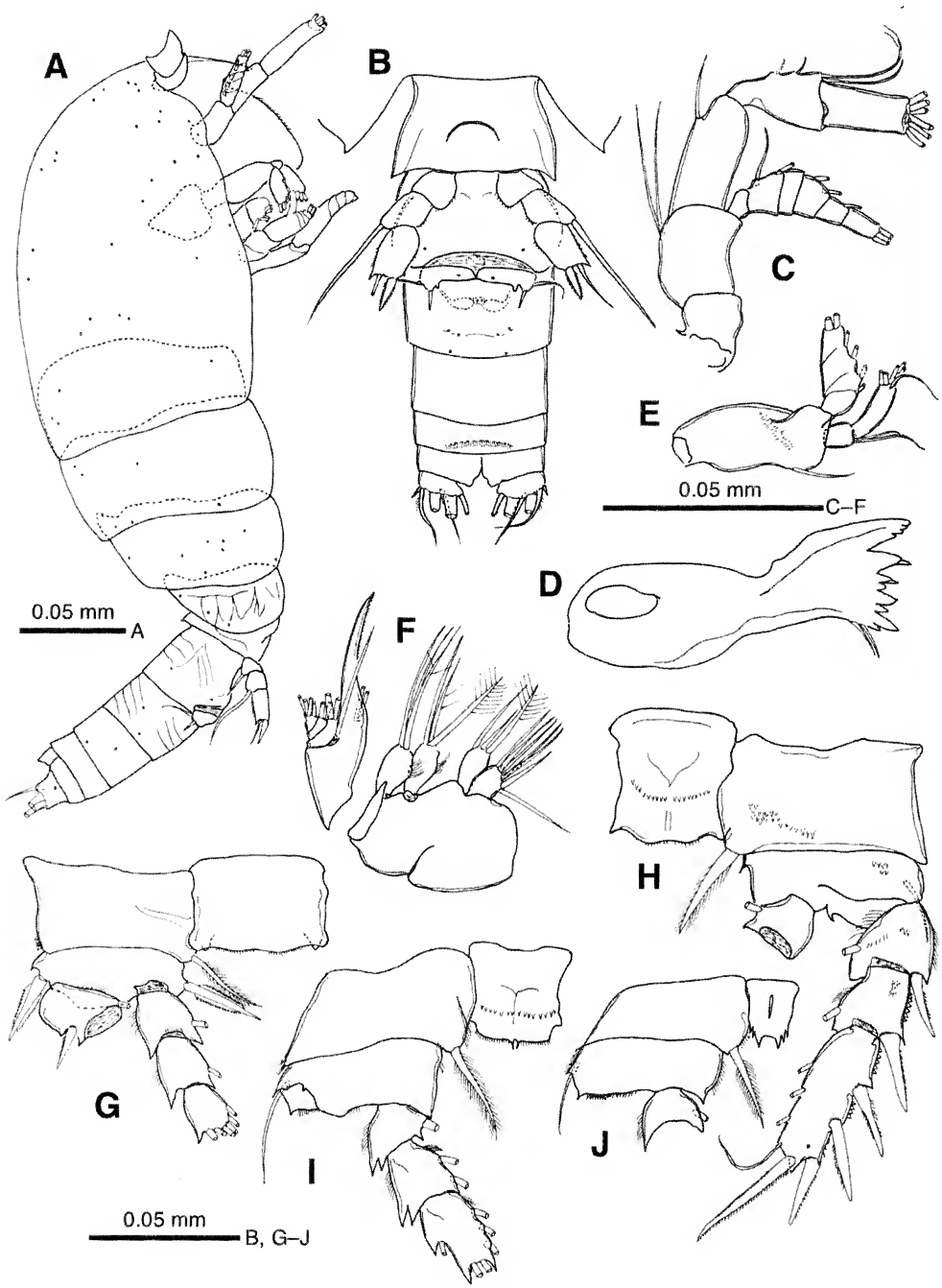

Fig. 2. Ovigerous female of an undescribed genus of Misophriidae. (A) Habitus, lateral view; (B) Prosomal end and urosome, ventral view; (C) Antenna; (D) Mandibular gnathobase; (E) Mandibular palp; (F) Maxilla; (G) Leg 1; (H) Leg 2; (I) Leg 3; (J) Leg 4.

Many segments of legs 1-4 lost during treatment for DNA extraction, but some diagnostic features confirmed. Leg 1 (Fig. 2G) with spiniform element on each side of basis; left endopod 3-segmented with single seta present on inner margin of second endopodal segment. Leg 2 (Fig. 2H) with middle spinular row on intercoxal sclerite; inner proximal margin of basis with tiny, acutely-pointed process; exopod 3-segmented, with setal formula I-1; I-1; III,I,4. Leg 3 (Fig. 2I) intercoxal sclerite with middle spinular row and 2 tiny, acutely-pointed processes on distal margin; endopod 3-segmented, with setal formula 0-1; 0-2; 1,2,3; outer distal corners of first and second endopodal segments each sharply produced into 2 processes. Leg 4 (Fig. 2J) with narrow intercoxal sclerite having 3 pairs of small acute processes distally.

Leg 5 (Figs. 1D, 2B) uniramous, symmetrical; protopod with single seta at outer distal corner; exopod 2-segmented, with first segment bearing long outer seta reaching beyond terminal seta on succeeding segment, and second segment having fine seta and spine distally (corresponding to elements "e" and "f", respectively, sensu Boxshall and Jaume (2000)) and triangular process on outer distal corner.

Two types of locomotory behavior were observed: (1) crawling over the bottom with frequent intermittent stops, and (2) free-swimming in a continuous and smooth manner across the water column. In the first locomotory type, the antennae and mandibular palps beat rapidly and legs 1-4 were held in an anteriorly-directed position as the copepod moved slowly along the bottom with the ventral side of the body down. The aforementioned head appendages ceased movement during intermittent stops. The maxillipeds did not firmly grasp the bottom during this behavior. This was confirmed by the observation that when it started climbing the vertical wall of a cell, it sometimes slowly fell without maxillipedal grasping, indicating a weak attachment. However, successful vertical climbs were also observed. Mean speed determined between two successive stops was 2.91 $\pm$ 0.71 (SD) mm per second (N = 17). Free swimming in the water column typically commenced suddenly, after a period of crawling along the bottom. After a short period of free swimming in the water column, the female returned to the bottom and resumed crawling motions.

The misophrioid copepod frequently remained stationary, clinging to the bottom using the maxillipeds and first legs. Attachment seemed to be mainly secured using the tips of the maxillipedal setae, although the anterodistal surface of leg 1 also touched the surface of the bottom supporting the copepod in position. While stationary, the antennules, antennae and mandibular palps were stretched out laterally, and were not involved in attachment. The body was flexed slightly ventrally at the prosome/urosome articulation. In this position, feeding seemed to take place, with the antennae and mandibular palps rapidly beating.

Feeding of the misophrioid copepod on cultured phytoplankters was indirectly confirmed by the excretion of numerous fecal pellets (Fig. 1B) and by its survival for 17 days in the laboratory. The fecal pellets were elongate, oval, 112.6-154.2 μm long and 32.7-38.7 μm wide (average $\pm$ standard deviation = 134.1 $\pm$ 15.8 μm, 34.8 $\pm$ 1.9 μm, N = 10). Pellet contents were tinged brownish green, indicating that the copepod had fed on the cultured phytoplankters. The approximate volume of fecal pellets produced was 8.5×10^4 μm^3, assuming they were uniformly oval in shape.

Oviposition took place twice during incubation. On the fifth day after collection, the first oviposition event was observed. The female produced 5 eggs carried on the urosome (Fig. 1A). The eggs seemed to be loosely attached to the copepod body, and were not enveloped by a sac-like structure, as seen in some calanoids (see Boxshall and Jaume 2000).

The eggs were ca. 0.09 mm in diameter (Fig. 1A). Two days later all the eggs were detached from the copepod. Hatching of the detached eggs was not confirmed, because they were lost during treatment. On the 10th day of incubation, the second oviposition event was recorded. The female produced 4 eggs. All the eggs had detached from the copepod two days after oviposition. Hatching was unsuccessful.

Three nucleotide sequences were obtained: 28S (Accession no. LC320121, 3496 bp); 18S (Accession no. LC320120, 1718 bp); and CO1 (Accession no. LC320122, 658 bp).

Discussion

The present specimen is assigned to an undescribed genus of the family Misophriidae based on the following features: (1) the carapace-like extension covering the first pedigerous somite; (2) the reduction in segmentation (6-segmented) and setation (0, 2, 1, 1, 1, 3) of the antennary exopod; and (3) the absence of an intercoxal sclerite on the fifth legs (see Boxshall and Jaume 2000). A close relationship between this species and the genus *Arcticomisophria* Martínez Arbizu and Seifried, 1996 is suggested by the presence of: (1) a spiniform outer element on the basis of leg 1; (2) a single inner seta on the second endopod segment of leg 1 (see Jaume and Boxshall 1997; Martínez Arbizu and Jaume 1999). *Arcticomisophria* comprises two species, *A. bathylaptevensis* Martínez Arbizu and Seifried, 1996 and *A. hispida* Jaume and Boxshall, 1997 which share the above-mentioned diagnostic features. However, leg 5 has more plesiomorphic segmentation and setation in *Arcticomisophria* than in the present misophriid specimen. Among genera of the Misophriidae, *Arcticomisophria* exhibits the most primitive state of leg 5 (ANCESTOR in Fig. 3). The fifth leg of the present undescribed genus is much more reduced than that of *Arcticomisophria* in both segmentation and setation (Fig. 3), and resembles to some extent those of species of other misophriid genera such as, *Misophria* Boeck, 1865, *Misophriella* Boxshall, 1983, *Misophriopsis* Boxshall, 1983 and *Stygomisophria* Ohtsuka, Huys, Boxshall and Itô, 1992, based on either one of these two characters: (1) no separation is present between the coxa and basis, resulting in an undivided protopod; or (2) the endopod is absent. Loss of the subdivision of the protopod seems to have occurred convergently in the Misophriidae. The present female specimen shares some apomorphic character states with some of these genera, but we consider that its formal taxonomic classification should be postponed pending the description of all the appendages from newly collected specimens of both sexes.

The locomotory behavior of a misophrioid copepod had been only briefly observed before, in a shallow-water hyperbenthic species *Misophria pallida* by Sars (1911). It was characterized by a combination of rapid rotation of the antennae and oral appendages (possibly the mandibular palps) and powerful strokes of the legs and urosome along the bottom (Sars 1911). In the misophrioid examined in the present study, a similar motion pattern was observed, but power strokes of the legs were not observed during the slow swimming behavior along the bottom.

The crawling behavior of the present specimen using the maxillipeds is similar to that reported for the siphonostomatoid *Aphotopontius mammillatus* Humes, 1987 (Heptner and Ivanenko 2002), although the latter lacks oral appendages for swimming. In *A. mammillatus*, Heptner and Ivanenko (2002) distinguished between crawling and walking by differences in the position of the maxillipeds. In the present misophrioid, only crawling was observed.

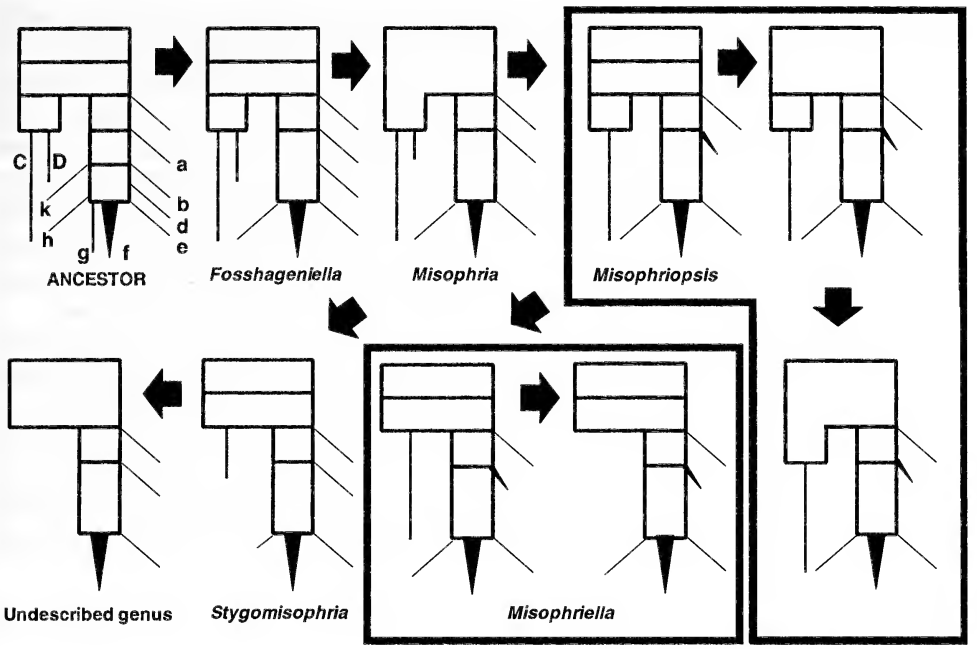

Fig. 3. Schematic illustrations showing segmentation and setation patterns of leg 5 of the adult female of some representatives of the family Misophriidae. Hypothetical ancestor based on *Arcticomisophria hispida* described by Jaume and Boxshall (1997), but elements e and f missing in their original description. Arrows indicate possible derivations of segmentation and setation (modified from Huys and Boxshall (1991)). Elements a, b, e, f, h, k, C, and D as identified by Boxshall and Jaume (2000).

The feeding habits of the deep-sea genus *Benthomisophria* were well studied by Boxshall and Roe (1980). Copepodids and adults of *Benthomisophria palliata* Sars, 1909 gorged on zooplankters such as small copepods, chaetognaths, cnidarians, and radiolarians, at depths of 2,500 m to 4,000 m (Boxshall and Roe 1980). Their prosomes occasionally became grossly expanded due to the volume of gut contents, suggesting an opportunistic feeding strategy of the copepod in the nutritionally poor deep ocean (Boxshall and Roe 1980). The gut content analysis of *Misophriella schminkei* Martínez Arbizu and Jaume, 1999 collected from hyperbenthic waters in Antarctica revealed that it may be a predator or a scavenger, feeding on cyclopoid copepods (Martínez Arbizu and Jaume 1999).

In contrast, the misophrioid examined in the present study survived only on cultured phytoplankters. The size of the phytoplankters was more than 3 μm, implying that the misophrioid employs suspension feeding, as observed in small particle-feeding calanoids (e.g., Paffenhöfer et al. 1982). The volume of fecal pellets produced by the adult female feeding on small cultured phytoplankters was ca. 8.5×10^4 μm^3, which is larger than those of *Oithona* spp. (of nearly equal prosome length), but within the range exhibited by small calanoids such as *Paracalanus* Boeck, 1865 and *Pseudocalanus* Boeck, 1872 (Uye and Kaname 1994; Mauchline 1998). This might not be the only feeding mode of the misophrioid since the heavily chitinized claw present on the maxillary basis (Fig. 2F) suggests "chopsticks mode" carnivory (Boxshall 1985) could occur as well.

Gurney's (1933) brief observations on the oviposition and developmental stages of *M. pallida* were of pivotal importance in understanding the biology of misophrioids. Adult

females of *M. pallida* carry two to four eggs loosely attached to the genital double-somite (Sars 1911; Gurney 1933). The female of the undescribed misophrioid also carried four to five eggs on its genital double-somite. Retention of eggs by adult females is quite frequent among podoplean copepods (Huys and Boxshall 1991). The unique characteristic of these misophrioids is the low number of relatively large-sized eggs in each clutch. Egg size has never been reported for misophrioids, but can be measured at about 0.09 mm in diameter based on an illustration of *M. pallida* by Sars (1911, Plate I). In the present specimen, the egg diameter was very similar to that of *M. pallida*. The interval between two consecutive clutches was four days in the present study, at around 20°C. All eggs were detached from the adult soon after oviposition, but it is uncertain whether this is normal in the wild.

The hatching naupliar stage was briefly illustrated by Gurney (1933). It was non-feeding, lacking masticatory blades on the mandible (Gurney 1933). The naupliar stage metamorphosed directly into the first copepodid stage, indicating a considerably abbreviated life cycle for this misophrioid (Gurney 1933). Such a naupliar abbreviation is commonly seen in siphonostomatoid copepods such the Caligidae and Nicothidae, in which zero to two naupliar stages were found (Ohtsuka et al. 2005, 2007, 2009; Venmathi Maran et al. 2013; Otake et al. 2016). The number of copepodid stages was the typical six in *B. palliata* (Boxshall and Roe 1980).

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Phylogenetic Study of Dioecious and Parthenogenetic Populations of *Canthocamptus staphylinus* (Crustacea, Copepoda, Harpacticoida)

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Abstract.—The phylogenetic relationships of four dioecious populations and one parthenogenetic population of the harpacticoid *Canthocamptus staphylinus* (Jurine, 1820) were studied. Analysis of the mtCOI gene revealed two main clades as a phylogenetic tree and a network of haplotypes: a clade with Fennoscandian populations in Lake Pääjärvi (Finland) and Lake Vänern (Sweden), and a second clade with populations in Lake Võrtsjärv (Estonia), Orlov Pond in Saint Petersburg (Russia), and the type locality of the species in Lake Geneva (Switzerland). The parthenogenetic population of *C. staphylinus* showed the smallest nucleotide and haplotype polymorphisms and could have evolved as a reaction to the changing environmental conditions following the Last Glacial Maximum, 20K YBP.

The harpacticoid copepod *Canthocamptus staphylinus* (Jurine, 1820) is one of the most widely distributed freshwater harpacticoid species in the Palearctic region (Lang 1948; Borutzky 1952; Fefilova 2015). Normally, it is a stenothermal and psychrophilic form and, according to different sources, inhabits waterbodies with temperatures ranging from 10° to 19°C (Sarvala 1979a). As part of the life cycle, adult *C. staphylinus* rests encysted in the bottom mud. Discovery of the species in different types of water bodies — from small spring pools to large lakes and rivers — indicates its high environmental plasticity. Individuals are both geographically and ecologically variable in several morphological characteristics, including the structure of the fifth pair of thoracal legs, numbers of spinules on the anal operculum, form of the spermatophore, and the development of the aesthetask borne on the fourth segment of the antennule (Lang 1948; Borutzky 1952; Fefilova 2015). This variability suggests that the forms described as *C. staphylinus* in the literature may actually represent a group of closely related species.

Breeding of harpacticoid copepods is commonly dioecious. They possess clear sexual dimorphism — females are usually larger than males and have different morphologies of antennules and swimming legs (Borutzky 1952; Huys and Boxshall 1991; Suárez-Morales 2015). However, parthenogenetic reproduction has been verified in a few harpacticoid species. The first reports were for *Elaphoidella bidens* (Schmeil, 1894) and *Epactophanes richardi* Mrazek, 1893, both from the family Canthocamptidae. For *C. staphylinus* a parthenogenetic life cycle was proved to occur in the stock living in the oligotrophic Finnish Lake Pääjärvi. There are both field data (males comprised only 0.28% of adults) and laboratory observations (unmated females isolated as nauplii or copepodids and reared individually in the laboratory, produced viable offspring) (Sarvala 1979a). In

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Table 1. *Canthocamptus* spp. sampling sites.

No.	Country, area	Locality	Coordinates	Number of individuals	Abbreviations
1	Geneva, Switzerland	Lake Geneva	46°27′N, 06°31′E	8	Geneva
2	Hämeenlinna, Finland	Lake Pääjärvi	61°04′N, 25°08′E	11	Paajarvi
3	Estonia	Lake Võrtsjärv	57°40′N, 26°40′E	5	Vortsjarv
4	St. Petersburg, Russia	Orlov Pond	59°51′N, 30°02′E	10	SPB
5	Sweden	Lake Vänern	58°55′N, 13°30′E	9	Vanern
6	Syktyvkar, Komi Republic, Russia	A pond in the Botanical Garden	61°40′N, 50°49′E	1	*C. microstaphylinus*

addition, parthenogenesis has been suspected on the basis of the scarcity of males in some other *Elaphoidella* species: *E. leruthi* Chappuis, 1937 and *E. elaphoides* (Chappuis, 1924) in the northernmost part of their range (Chappuis 1955), and *E. grandidieri* (Guerne and Richard, 1893).

For different types of invertebrate animals, a parthenogenetic life style serves as an adaptation to unfavorable environmental conditions (Clark and Bowen 1976; Glesener and Tilman 1978; Grebelnyi 1996; Hebert and Finston 2001; Hebert et al. 2007; Weeks et al. 2008). Asexual or parthenogenetic organisms occur more often at higher latitudes and altitudes, on islands and in environments variously classified as marginal, stressful, transient or disturbed (Cuelar 1977; Glesner and Tilman 1978; Bell 1982; Lynch 1984; Suomalainen et al. 1987). Although clonal reproduction is expected to be reproductively advantageous (Maynard-Smith 1978; Bell 1982), obligately asexual organisms are short-lived on geologic time scales and have been regarded as evolutionary dead ends (Bell 1982; Lorenzo-Carballa et al. 2012.). However, in the short term, switching to an asexual mode can help organisms to exploit a narrow spectrum of environmental resources more efficiently than could genetically diverse sexual populations (the "demographic balance" hypothesis, Gets 2001; Haag and Ebert 2004). Molecular responses to environmental changes are modulated by phenotypic plasticity (physiological acclimatization) and genetic adaptation (genetic evolution through natural selection under new environmental conditions) and may play important roles in the persistence of the species (Smolina 2015). To reveal the phylogenetic relationships among parthenogenetic and dioecious populations of *C. staphylinus* we studied the genetic population structure of this species across a wide geographic area using mitochondrial DNA sequence data.

Material and Methods

Samples were collected from 2015 to 2016 from four dioecious populations of *C. staphylinus* (including the type locality of the species in Lake Geneva, Switzerland) and one parthenogenetic population in Lake Pääjärvi. In addition, a population of *Canthocamptus microstaphylinus* Wolf, 1905 was sampled in 2015 in Syktyvkar, Komi Republic, Russia and used as a group for comparison with closely related species. Samples from the Orlov pond and Lake Geneva were collected by Natalia M. Sukhikh in November 2010 (Table 1, Fig. 1). All samples were collected close to the shore with a hydrobiological

Fig. 1. Sample locations of specimens of *Canthocamptus* spp. examined in this study. Locality names are listed in Table 1.

100-μm mesh hand net. Organisms in these samples were preserved in 96% alcohol solution. Morphological identification of species was performed using an ES Bimam R 13-1 Microscope (Russia), and Micromed MC-4-Zoom Led stereoscopic microscope (Russia) according to the descriptions by Lang (1948).

A 611 base pair (bp) fragment of the mitochondrial cytochrome oxidase I (mtCOI) gene was sequenced from 44 adult copepods. Genomic DNA was extracted from copepods preserved in 96% ethanol following protocols outlined in Walsh et al. (1991). Specifically, the bodies of each crustacean were added to 5% Chelex-100 (Sigma-Aldrich, St Louis, MO, USA) solution in bidistilled water. Samples were spun for 30 s at 12,000 rpm. The mixture was incubated at 90°C for 30 min, then spun for 30 s at 12,000 rpm. Samples were incubated at 90°C for 15 min and spun for 15 s at 12,000 rpm. Then samples were stored at −20°C and amplified through PCR. Universal primers LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′TAAACTTCAGGGTGACCAAAAAATCA-3′) (Folmer et al. 1994) were used for amplification of COI by polymerase chain reaction (PCR). PCR started with DNA denaturing at 95°C (60 s), followed by 35 cycles of 30 s denaturing at 95°C, 30 s annealing at 50°C, and 50 s extension at 72°C, and then a final extension at 72°C for 7 min (Lee 1999). The product was purified with a Qiaquick PCR purification kit and sequenced in an ABI Prism 310 automated sequencer. To avoid mistakes in sequences we used bi-directional sequencing

Table 2. Percent genetic distances for the six populations of *Canthocamptus* spp. based on mtCOI sequences.

Population	Pääjärvi	Vänern	SPB	Võrtsjärv	Geneva
Pääjärvi					
Vänern	2.5				
SPB	22.5	22.1			
Võrtsjärv	23.5	23.7	3.1		
Geneva	23.4	23.1	2.1	1.2	
C. microstaphylinus	25.5	27.0	26.8	26.2	26.6

with above mentioned primers. In case of double peaks or ambiguous base calls each sequence was compared with obtained chromatograms, a signal that exceeded the other was taken into account. All processing was done at the Center for Collective Use "Molecular Biology" of the Institute of Biology, part of the Komi Scientific Center of the Ural Branch of the Russian Academy of Science.

Nucleotide sequences were aligned with the algorithm CrustalW and corrected manually (due to several shifts of nucleotide motifs after alignment) using the program package Geneious (version 7.0.6.) (Kearse 2012). Phylogenetic trees were also constructed in Geneious using a Maximum Likelihood method with a high level of the bootstrap coefficient (1000 replications). A median network of haplotypes was constructed with Network 4.6.1.3 (Bandelt 1999). Statistical analysis of the DNA polymorphism was performed in DNAsp 5.10 (Librado 2009). All sequences of *C. staphylinus* were registered in GenBank under the accession numbers KP974713–KP974719 and MG209708-MG209737. In addition, a sequence of *C. microstaphylinus* (accession number KP974734.1) sampled and sequenced according to methods described above was included in the phylogenetic analysis. For comparison, three further sequences obtained from GenBank were added: *C. staphylinus* from Northern Germany (MF077881.1), *Canthocamptus coreensis* Chang, 2002 (KT030277.1) and *Elaphoidella humphreysi* Karanovic, 2006 (JN039173).

Results

A total of 43 *C. staphylinus* females were sequenced for the mtCOI gene. Males were observed in all populations except the parthenogenetic one in Pääjärvi. Moreover, some of the females in Pääjärvi were bearing egg sacs but had no spermatophores attached to their bodies, suggesting that mating had not taken place for these individuals.

Phylogenetic analysis revealed two strongly separated clades with maximum pairwise divergences of 23.7% between geographically separate populations (Fig. 2, Table 2). All clades were strongly supported by bootstrap percentages and were divided mostly according to the populations' geographical locations. The clade I was common for three populations of the species *C. staphylinus*: the type locality in Lake Geneva, Orlov Pond in Saint Petersburg and Lake Võrtsjärv. These three populations formed three main groups that roughly but not completely followed their geographical origin with genetic divergences between them varying from 1.2% to 3.1%. Two sequences of *C. staphylinus* from Orlov pond were placed in the group for Lake Geneva sequences, while two sequences of the species from Lake Geneva fell into groups for Lake Võrtsjärv and Orlov pond. The clade II included two subclades, consisting of the parthenogenetic population from Lake Pääjärvi and the dioecious population from Lake Vänern together with the sequence of

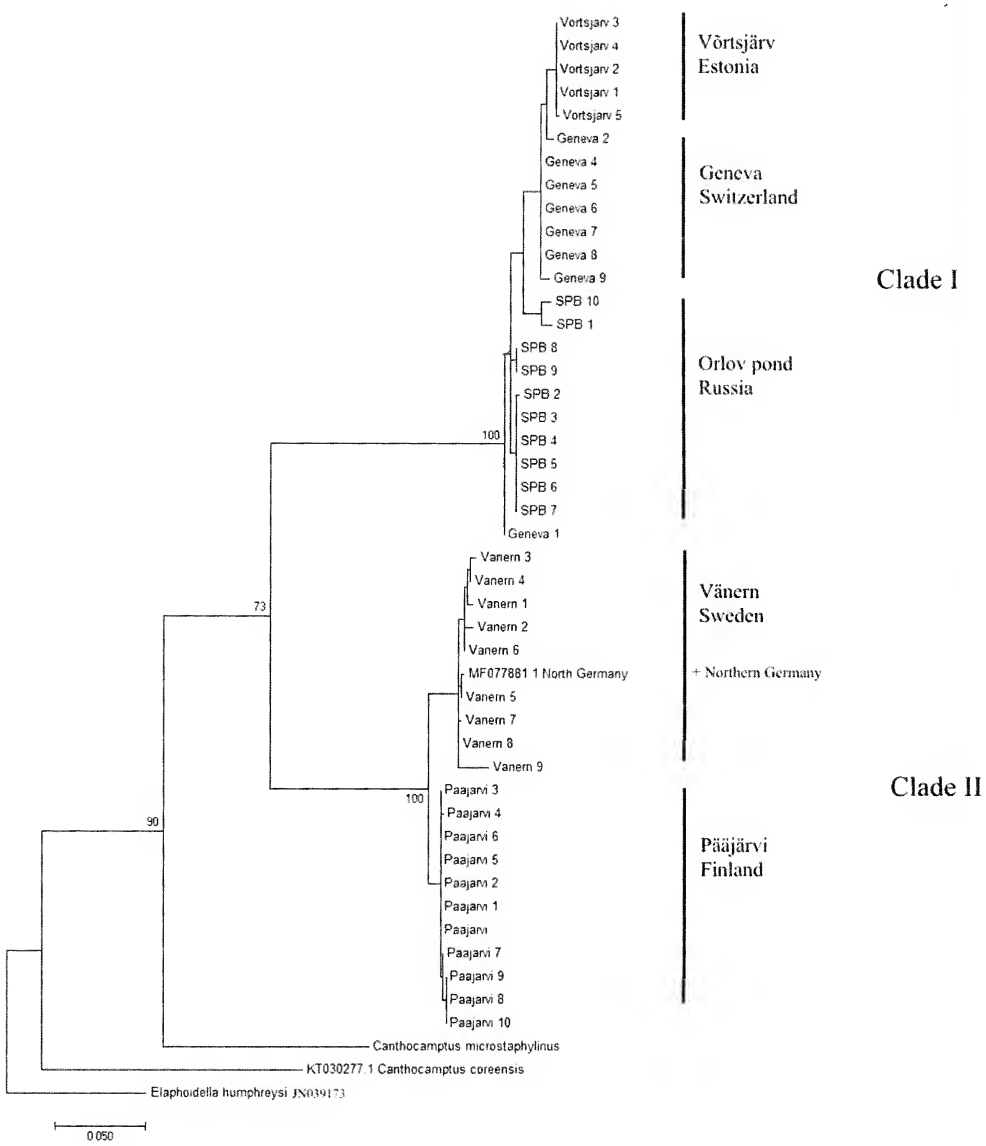

Fig. 2. Phylogenetic tree of the relationship between *Canthocamptus staphylinus* populations based on data from mitochondrial cytochrome oxidase subunit I (COI) region (611 bp) by the maximum likelihood method. Numbers beside nodes indicate bootstrap values. As an outgroup, a sequence of *Elaphoidella humphreysi* from NCBI was used (accession number JN039173). From GenBank, a sequence of *C. staphylinus* from northern Germany (accession number MF077881), a sequence of *Canthocamptus microstaphylinus* (accession number KP974734.1), and a sequence of *Canthocamptus coreensis* (accession number KT030277.1) were also included.

C. staphylinus sampled in Northern Germany with genetic divergence of 2.5% between them.

The COI haplotype network formed two separate clades similar to those distinct in the phylogenetic tree (Fig. 3). The total number of haplotypes was 22. The *C. staphylinus* clade II consisted of the parthenogenetic population from Pääjärvi and

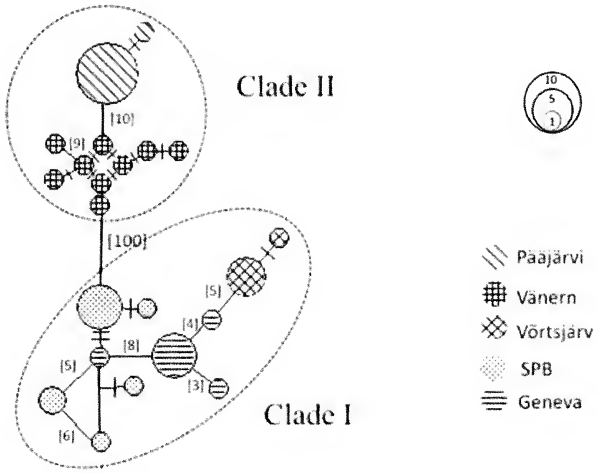

Fig. 3. Haplotype network based on statistical parsimony, representing genealogical relationships between mtCOI haplotypes in sexual and parthenogenetic populations of *Canthocamptus staphylinus*. Numbers in square brackets and hash marks represent mutations between haplotypes. The size of the circles correlates with haplotype frequency. The dashed circles in the network indicate the three main clades with a significant geographic association (see the main text).

the dioecious population from Vänern. The most frequent haplotype 1 in the haplotype network belonged to the parthenogenetic population. The clade I was formed of the *C. staphylinus* populations from Lake Geneva (the species type locality), Orlov Pond in Saint Petersburg and Lake Võrtsjärv. The latter clade held a central position in the haplotype network, with all other haplotypes extending away from it. Numbers of mutations were 100 between the first and the second clades.

Analyses of the sequence polymorphism of the populations allocated to *C. staphylinus* are summarized in Table 3. Among 43 obtained sequences, there were 22 haplotypes, and 135 polymorphic sites with 147 mutations. General haplotype and nucleotide diversities were 0.920 and 0.1067, respectively. The haplotype and nucleotide diversities observed in the parthenogenetic population were lower than in the dioecious populations. The smallest numbers of polymorphic sites and mutations were detected in populations from Lake Pääjärvi and Lake Võrtsjärv.

Table 3. Polymorphism of the COI gene from *Canthocamptus staphylinus* populations. n — number of sequences, N — Number of haplotypes, Hd — Haplotype diversity, L — length of sequences, Pi — Nucleotide diversity, S — Number of polymorphic (segregating) sites, Eta — number of mutations. The parthenogenetic population is indicated in bold.

Population	n	N	Hd	L	Pi	S	Eta
Pääjärvi	**11**	**2**	**0.182**	**611**	**0.00033**	**1**	**1**
Vänern	9	9	1.000	611	0.00884	18	18
SPB	10	5	0.756	611	0.01118	20	20
Võrtsjärv	5	2	0.400	611	0.00066	1	1
Geneva	8	4	0.643	611	0.00702	17	17

Discussion

The demographic advantage of obligate parthenogenesis for aquatic invertebrates is the possibility of accelerating the life cycle during a short period of favorable conditions (Lynch and Gabriel 1983; Hebert and Hann 1986). In copepods, parthenogenetic reproduction has remained poorly known, reflecting that it is a rarity in nature and uncommon for this taxon. As was mentioned above, in a few species of harpacticoids of the family Canthocamptidae parthenogenesis has been suspected because males are rarely found, and in some of those species laboratory experiments show that females can reproduce by themselves without males (Sarvala 1979a; Dole-Oliver et al. 2000; Guterrez-Aguirre et al. 2011). Across the distributional range of *C. staphylinus*, only one parthenogenetic population is known, that from Lake Pääjärvi (and possibly another population at 80 km distance from it). All other populations studied, including those of the present work, were dioecious with males present in every population (Smyly 1957; Sarvala 1979a; Dole-Oliver et al. 2000; Fefilova 2015). The parthenogenetic population of *E. richardi* was also described from the Scandinavian region. Moreover, *E. richardi* var. *angulatus* Kulhavi, 1957 hatched out from parthenogenetic eggs, was able to develop and reproduce sexually with sperm transfer from males to females (Lang 1935; Borutzky 1952).

Many species of copepods have female-biased adult sex ratios (Kiørboe 2006). A variety of factors have been suggested to influence the sex determination in this taxon: temperature (Katona 1970); food (Irigoien et al. 2000); population density (Heinle 1969) and pressure (Vacquier and Belser 1965). For example, in *Calanus* spp. food quantity and quality have a significant effect on the seasonally changing population sex ratio, supporting the existence of a strong environmental influence on sex determination (Svensen and Tande 1999; Irigoien et al. 2000; Miller et al. 2005; Gusmão 2009).

Our analysis of genetic population structure reveals a broad range of genetic divergence (1.2–23.7%) among populations of *C. staphylinus* (Fig. 2, Table 2). The genetic divergence between clades of haplotypes also was large and generated a haplotype network with its branches containing many mutations between populations (Fig. 3). Interestingly, the genetic divergence between the two clades of *C. staphylinus* was similar to that between *C. staphylinus* and closely related but separate species of *C. microstaphylinus* (more than 20%). This suggests the existence of a complex of at least two cryptic species among the *C. staphylinus* populations. This complex would include a "northern" species with populations from Finland, Sweden and Northern Germany, and a "continental" species with populations from Russia, Estonia and Switzerland (Fig. 2).

Similar unexpectedly wide genetic variability revealing the existence of several cryptic forms has been found in other studies of molecular-genetic population structure of harpacticoids. For example, in a study of *Nannopus palustris* Brady, 1880 genetic distances of mtDNA reached up to 78% between populations and allowed to separate several cryptic forms (Garlitzka et al. 2012). According to studies on phylogenetic and phylogeographic structure among populations of *Tigriopus californicus* (Baker, 1912) divergences of mtDNA often exceeded 20% (Burton et al. 2007; Willet and Ladner 2009). High levels of intra- and interspecific divergence seem to be widespread phenomena among members of the order Harpacticoida (Schizas et al. 1999; Easton et al. 2010). In freshwater copepod species, the observed molecular genetic divergence can be the result of founder effects accompanied by limited gene flow between populations, even those in adjacent habitats (Bucklin 1998).

The pattern of population structure which is observed in *C. staphylinus* is defined by the present-day gene flow as well as the historical processes. The clade which consists of the

Pääjärvi and Vänern populations (Fig. 2) suggests that they have a common history. Indeed, during the development of the Baltic Sea after the last Ice Age, the basins of Pääjärvi and Vänern were both parts of the Yoldia Sea stage (10300–9500 years BP; Björck 1995), the water of which was fresh for most of the time. While Lake Pääjärvi was already isolated from the Yoldia Sea (Okko 1969), Lake Vänern became separated slightly later, at about 9500 years BP, just at the transition to the following Baltic freshwater stage, the Ancylus Lake. Hence, the animal populations of these lakes would have close relationships.

The unexpected clear distinction between the Swedish and Finnish populations on one hand, and the geographically rather close Võrtsjärv and Saint Petersburg populations on the other hand, indicating long isolation of these populations, also becomes understandable on the basis of existing data on the geological history of the area. The continental ice started to retreat from Estonia around 13000 years BP and had disappeared by 11000 years BP (Moora et al. 2002). There was an Ice Võrtsjärv up to 12200 years BP, partly dammed by ice, followed by Big or Ancient Võrtsjärv during 12200–7500 or 7000 years BP, after which there has been the contemporary lake. Võrtsjärv was thus from the beginning separated from the successive stages of the Baltic basin. The same applies to most of the Saint Petersburg region. The distance between Võrtsjärv and Saint Petersburg is not so great, around 700 km, and resting stages in the form of cysts might allow more efficient dispersal than is the rule in harpacticoids.

The similarity of the populations from Võrtsjärv and Saint Petersburg with that from Lake Geneva (Figs. 2 and 3) is more difficult to explain on the basis of existing geological information. The overlapping structure of the distribution of sequences in the phylogenetic tree and the haplotype network together with relatively small genetic differences in spite of considerable geographical distances suggests that these populations might have an ancient history of dispersal long before than the last Ice Age. The relationships between these harpacticoid populations represent a similar problem as the present-day distributions of the so-called "glacial relicts" in and around the Baltic Sea, which have been much discussed relative to the improving knowledge of the geological history (Segerstråle 1982). Even in case of the "glacial relicts" the explanations have remained speculative.

A detailed analysis of the distribution patterns of dioecious and parthenogenetic forms of insects was carried out by Suomalainen and collaborators (Suomalainen and Saura 1973; Suomalainen et al. 1976, 1987). It proved that the invasion of polyploid populations (which are common for asexual organisms like hermaphrodites and parthenogens) occurred as a result of the Quaternary Glaciations. These polyploid insect populations are supposed to have originated from diploid populations that survived to the end of the glaciation in the Central European mountains. Moreover, some diploid populations are still capable of parthenogenetic reproduction (Grebelnyi 1996).

Analysis of mtCOI gene reveals that the parthenogenetic population of *C. staphylinus* is a separate subclade on the phylogenetic tree and includes two separate haplotypes with only one mutation between the distinct sequences (Figs. 2 and 3). In addition, the smallest values of haplotype and nucleotide diversity were observed for this population (Table 3). These facts support the suggestion that the parthenogenetic population of *C. staphylinus* has a clonal genetic structure. Usually, in sexual reproduction, mitochondrial genes are inherited exclusively from the mother (Travis 2000). The mtCOI gene shows rapid evolutionary rates, lack of introns and genetic recombination and allows to trace maternal lineage far back in time (Meyer 1993). In view of this, the clonal genetic structure from the observed parthenogenetic population points out that the males' genetic information was restricted during several generations.

The small genetic distance between the Vänern and Pääjärvi populations suggests that parthenogenesis in *C. staphylinus* in Pääjärvi might be of relatively recent origin. This idea was mentioned previously with statements of restricted distribution and high embryonic mortality in the parthenogenetic population (Sarvala 1979a). In some species of millipedes, parthenogenetic and dioecious populations were also mixed in one clade of the phylogenetic tree (Short and Vahtera 2017), suggesting that parthenogenesis may have evolved more recently as a specific adaptation to certain environmental conditions without changing the structure of nucleotide sequences.

As a rather big and fast-growing harpacticoid, *C. staphylinus* has relatively high food requirements, and thus prefers eutrophic environments (Sarvala 1979a,b; Hämäläinen and Karjalainen 1996). However, Lake Pääjärvi is oligotrophic, and food seems to be a limiting factor for the species. Under such conditions, the parthenogenetic mode of reproduction should give a selective advantage. Females of *C. staphylinus* from the parthenogenetic population of Lake Pääjärvi can copulate if males are present, but only few males are produced (Sarvala 1979a). Likewise, even other populations of *C. staphylinus* are often strongly female-biased (Lilljeborg 1902; Bevercombe 1973; Young 1974), or the numbers of males decrease during cold periods (Donner 1928; Smyly 1957).

Clearly, further studies of other populations of *C. staphylinus* (dioecious and possibly parthenogenetic), and possibly other species of this genus are needed to clarify both the taxonomy of this complex of species and the phenomenon of parthenogenesis in Canthocamptidae.

Conclusions

1) The harpacticoid species *C. staphylinus* shows a high level of genetic divergence between populations.
2) The parthenogenetic population of *C. staphylinus* from Lake Pääjärvi was genetically similar to the dioecious population from Lake Vänern, suggesting a relatively recent origin of parthenogenesis in this taxon.
3) The parthenogenetic population showed the smallest nucleotide and haplotype diversity, suggesting a clonal genetic structure in the population through the line of maternal inheritance of mitochondrial DNA.
4) The "northern" and "continental" clades of *C. staphylinus* can likely be considered as a complex of cryptic species.

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The Evolution of the Thermal Niche Across Locally Adapted Populations of the Copepod *Tigriopus californicus*

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Thermal performance is a key component of fitness particularly for ectotherms living in thermally variable environments. Local adaptation can occur within populations of a species that inhabit regions with divergent thermal conditions, but this adaptation may result in trade-offs in other measures of fitness. If these trade-offs affect other aspects of thermal performance, several different patterns are possible (Huey and Kingsolver 1993). One potential pattern from a trade-off is a shift in the thermal niche, meaning that an organism that can handle a new range of higher temperatures can no longer handle colder temperatures as well. A second type of pattern is a generalist/specialist trade-off whereby populations may have broader thermal niches but lower fitness at optimal temperatures [i.e. "a jack-of-all-trades is a master of none" (Huey and Hertz 1984)]. Another possibility is that increased investment associated with local thermal adaptation (i.e. high temperature tolerance) may result in trade-offs in non-thermally dependent traits (Angilletta et al. 2003). The nature and structure of these trade-offs could determine the degree to which organisms will be able to respond to a changing climate.

The copepod *Tigriopus californicus* (Baker, 1912) has become an important system in which to study the evolution of local adaptation to the thermal environment. Geographically distinct populations of this copepod occur in upper intertidal pools along the Pacific coast from central Baja Mexico to Alaska. These populations often show high degrees of genetic divergence from one another indicating that levels of gene flow between populations can be very limited over long periods of time (Burton 1997; Edmands 2001; Willett and Ladner 2009). There is also a clear latitudinal gradient in high temperature survival that is suggestive of local thermal adaptation for this species (Willett 2010; Kelly et al. 2012; Leong et al. 2018). This latitudinal gradient for high temperature tolerance has been seen for nauplii and copepodids as well as adults (Tangwancharoen and Burton 2014).

Local thermal adaptation in *T. californicus* is also suggested by studies of fitness components and competitive fitness under non-extreme temperatures. Hong and Shurin (2015) examined 15 populations of *T. californicus* from Vancouver Island, BC, Canada, to southern California (CA) for a set of life history traits that contribute to fitness under four different temperature conditions (from 15°C to 30°C). They estimated the net fitness effect of these traits by calculating an intrinsic population growth rate (r) and found a consistent shift in the thermal niche from south to north and also higher r in the northern populations. Willett (2010) also found that for comparisons across a set of moderate temperatures there was a flip in competitive fitness between pairs of southern and central CA *T. californicus* populations. Central CA populations outcompeted southern populations at 16°C while the opposite pattern was observed in a fluctuating environment with an average temperature of 24°C (a 20°C to 28°C daily cycle). Combined these results suggest that

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Table 1. Sample sizes of thermal stress assays for populations of *Tigriopus californicus*. Numbers indicate replicates with each replicate having 10 males and 10 females. Populations are arranged from the most southern (SRQ) to the most northern (FHL).

Population	37°	38°	39°	-2°
SRQ	5	10	8	23
BR	13	14	4	31
SD	8	8	-	16
CAT	7	10	4	21
AB	13	22	-	35
SC	9	8	-	17
BB	12	8	-	20
FHL	8	4	-	12

northern populations may achieve higher net fitness at intermediate temperatures but have lowered survival at high temperatures.

Differences in cold tolerance have also been observed across *T. californicus* populations for several different measures of function/survival at low temperatures (Wallace et al. 2014). *T. californicus* showed surprisingly high levels of cold tolerance with recovery from exposure to -2°C for all tested populations and recovery from freezing in the most northern populations. One central CA *T. californicus* population showed an unexpectedly low level of tolerance more comparable to southern CA populations than populations further north (in Washington State and British Columbia, Canada). When compared to results from high and moderate temperatures for other central CA populations this finding could suggest that this population has a narrowed thermal niche and could be more of a thermal specialist. However, the populations used by Wallace et al. (2014) were not the same as those used in other studies of this species, so we do not know both high and low temperature tolerance for these same populations that would enable us to get a full picture of the width of the thermal niche and how it changes across populations. In this study we explore both high and low temperature tolerance for a set of eight populations of *T. californicus* to gain a better understanding of the evolution of the thermal tolerance breadth and gain insights into the thermal niche width for this species.

The eight populations used in this study were selected for temperature assays because they span a wide portion of the range of this species and have recently been targeted for a comparative genomics study (Barreto et al. in prep.). These populations stretch from San Roque in Baja California Sur, Mexico (SRQ, 27°11′12″N, 114°23′52″W) to Friday Harbor in WA, USA ((FHL, 48°32′47″N, 123°0′35″W). The other populations from south to north were the California, USA populations of Bird Rock (BR, 32°48′54″N, 117°16′23″W), San Diego (SD: 32°44′44″N, 117°15′18″W), Catalina Island (CAT, 33°26.8′N, 118°28.6′W), Abalone Cove (AB, 33°44′16″N, 118°22′31″W), Santa Cruz (SC, 36°56′58″N, 122°02′49″W), and Bodega Bay (BB, 38°19′4″N, 123°4′23″W). Copepods were maintained in the laboratory at 20°C with a 12 hr:12 hr Light:Dark cycle for at least 1 yr before conducting the high temperature assays and 2 yr before conducting the cold tolerance assays.

Acute, high temperature stress tolerance assays were done by measuring survival 3 d after a 1 hr exposure to the stressful temperatures of 37°C, 38°C, or 39°C as described in Willett (2010). Sets of 10 males and 10 females were done for each population and the number of replicates for each temperature treatment is shown in Table 1. Not all populations were

tested at 39°C if high levels of mortality were observed for that population at 38°C. Survival of copepods under heat stress was modeled as binomial using a generalized linear model (function glm in R version 3.3.0; R Core Team 2016).

For the chill coma recovery assays, a modified version of the assay from Wallace et al. (2014) was used. Ten male and ten female copepods from a target population were placed in 10 mL of instant ocean seawater in a 50 mL centrifuge tube. Tubes were then placed in a chilled water bath at -2°C (containing a 50% ethylene gycol mixture) for 20 min. With this temperature exposure all copepods exhibited a chill coma phenotype wherein the copepods fell to the bottom and were immobile. Tubes were removed from the water bath and copepods were transferred to petri dishes where the seawater was allowed to return to room temperature while monitoring the recovery of copepods to an active state. Dishes were checked at roughly 2-min intervals to determine the number of copepods that had recovered and were swimming. Very limited mortality was observed for this cold stress exposure and copepods had largely all recovered by the time the plates had reached 14°C about 15 min after transferring them to the petri dishes. The time for recovery of 50% of copepods was used as our measure of chill coma recovery and analyzed as a generalized linear model in R.

The -2°C temperature was chosen to enable comparison to previous results (Wallace et al. 2014) but it is likely to be more environmentally realistic for the northern populations than the southern populations. Temperature data from nearby weather stations suggested that central CA locations may experience a small number of days below freezing while locations to the north in WA, and British Columbia, Canada experience more than 38 freezing days per year (Wallace et al. 2014) and southern CA locations experiencing no days below freezing. We looked at data from different weather stations near the populations used in this study and found a similar pattern. Over the last 12 yr the most northern FHL population had an average extreme low of -7.7°C, while the central CA locations SC and BB had values of -2°C and 0.5°C respectively, while locations near the southern CA populations of CAT and SD had average extreme lows of 4.7°C and 3.6°C respectively (data from NOAA at www.ncdc.noaa.gov/cdo-web/). The connection between pool temperature and nearby air temperatures is not direct and can be complicated by pool volume, substrate color, tidal timing, and other pool-specific environment factors but measured pool temperatures are more variable than nearby surface ocean temperatures (Kelly et al. 2012; Leong et al. 2018).

Copepods exposed to an acute, one-hour heat stress showed a general pattern of higher tolerance at lower latitudes (Fig. 1). The best fit model to these data includes the factors latitude, sex, and heat shock temperature. All main effects are significant (latitude $P = 0.001$, sex (males) $P = 0.01$, temperature $P<0.001$). Males have significantly lower acute stress tolerance than females, consistent with previous studies (Willett 2010). The southernmost population (SRQ) from central Baja California, Mexico showed much higher tolerance with some copepods surviving the 39°C exposure similar to previous results for this and nearby populations (Kelly et al. 2012; Pereira et al. 2017). There are some examples of regional variation that contrast with the general latitudinal pattern. In this dataset, the SD population has lower thermal tolerance than neighboring populations, a trend that has also been observed in previous studies for acute, high temperature assays (Willett 2010; Pereira et al. 2014). It is possible these local deviations from the latitudinal pattern could reflect finer scale differences in thermal adaptation.

Chill coma recovery time also followed a latitudinal gradient in which northern populations showed faster recovery than the southern populations, as would be expected

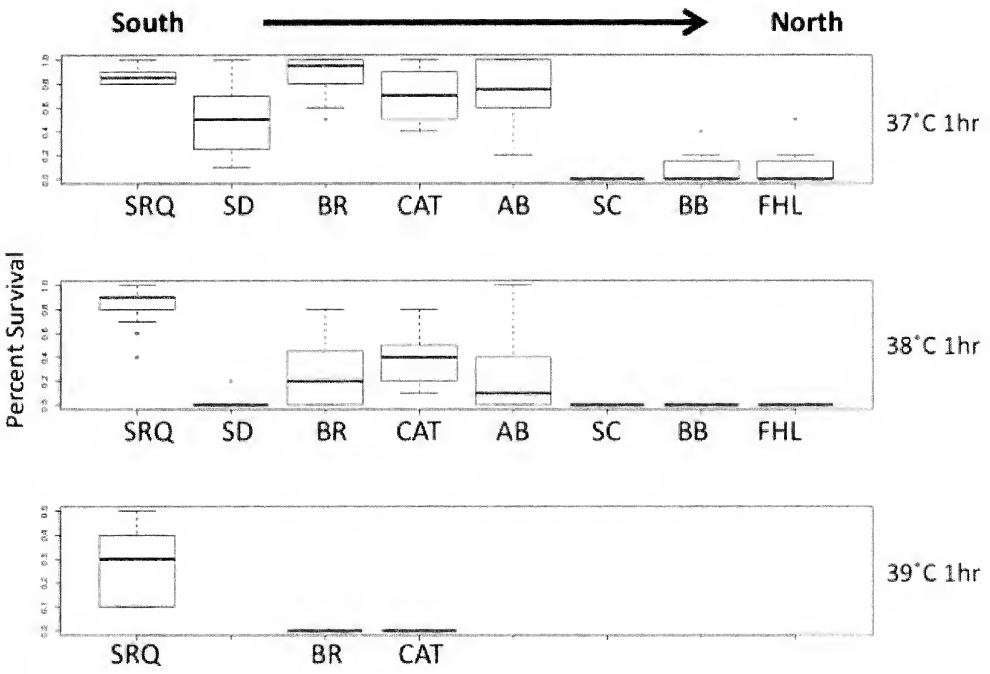

Fig. 1. High temperature acute stress assays for populations of *Tigriopus californicus*. Copepods were tested at three different temperatures (37°C top, 38°C middle, and 39°C bottom panel) for 1 hr and survival was measured 3 d after heat shock. Populations are arranged from south (on the left) to north (on the right). Plots show combined data for males and females. Box plots for each population/temperature combination depict median (bold line), first and third quartiles (box), largest non-outlier values (whiskers), and outliers (dots).

with local adaptation (Fig. 2). Both latitude and sex are significant factors in analyses of these data ($P<0.001$ for both) with males showing less thermal tolerance at colder temperatures (i.e. longer recovery times). Two populations of interest in comparison to the Wallace et al. (2014) results are the SC and BB populations from central CA. They showed an intermediate level of tolerance that falls between the recovery times of the more northern and southern populations. In the Wallace et al. study, the central CA population of Hopkins Marine Station (just south of SC) showed a cold temperature tolerance much more similar to a southern CA population proximate to the SD population included in our study. It is unclear why this Hopkins population showed this lower cold tolerance in the previous study and whether it also has correspondingly higher high temperature survival. In contrast, the results from our study suggest an intermediate both cold and hot temperature tolerance for the central CA populations with both increased cold tolerance and decreased heat tolerance in comparison to more southern populations but slightly decreased cold tolerance in comparison to the FHL population to the north.

Using a combination of measures of high and low thermal performance from the same populations, we can make stronger inferences about changes in the width of the thermal tolerance range among populations and how performance at extreme temperatures relates to performance at temperatures closer to optimal temperatures. Overall the results of our study are most consistent with a niche shift with an increase in high temperature tolerance and a decrease in cold temperature tolerance at lower latitudes and the opposite pattern

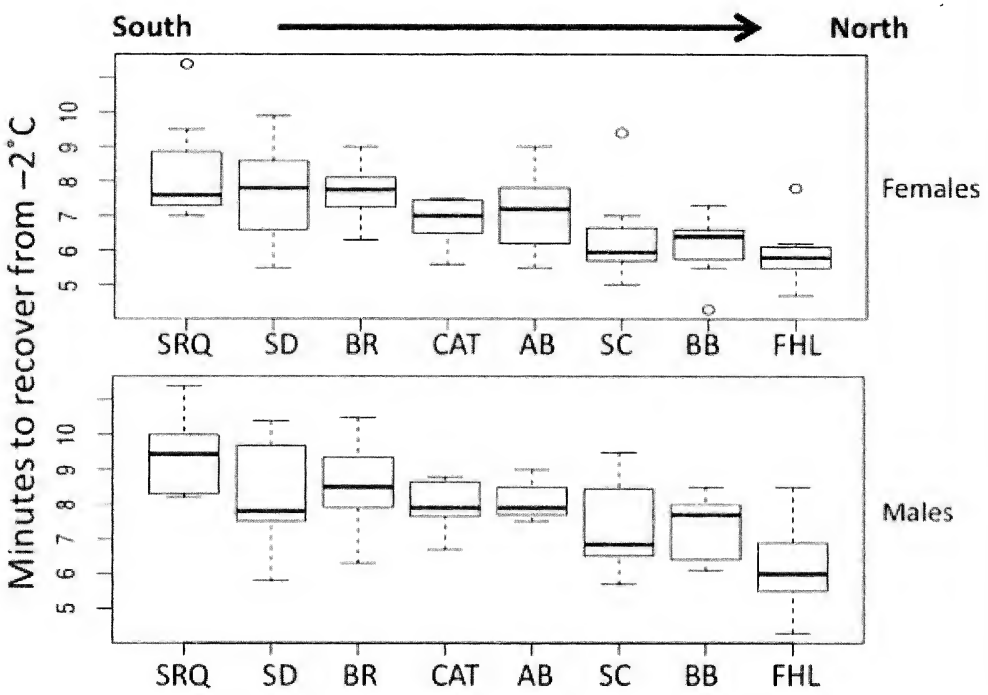

Fig. 2. Chill coma recovery for populations of *Tigriopus californicus*. The number of minutes for half of the copepods within an assay to recover from a -2°C exposure of 20 min is shown. Populations are arranged from south (on the left) to north (on the right). Results from females are shown on the top and males are on the bottom. Boxplots are as described in Fig. 1.

at higher latitudes. A niche shift was also suggested by the results of Hong and Shurin (2015) for testing over a range of moderate temperatures. If the central CA populations had shown narrower tolerance ranges this could have suggested that these populations were behaving more as thermal specialists (particularly when associated with the higher competitive fitness these populations show at intermediate temperatures in comparison to southern CA population; Willett 2010).

Combined with the results from previous studies, there is some evidence for not only a niche shift but also an increase in combined measures of fitness for more northern populations (Willett 2010; Hong and Shurin 2015). Hong and Shurin (2015) found a pattern of a niche shift towards lower temperatures with increasing latitude and also increasing composite fitness for the northern populations (as measured by the population growth rate r). As mentioned above, Willett (2010) found higher competitive fitness for central CA populations in competitive fitness assays. The increase in fitness with latitude is consistent with these populations showing a pattern of counter-gradient variation, perhaps due to stronger selection for shorter development due to a shorter growing season at more northern latitudes (Yamahira et al. 2007; Gardiner and Munday 2010; Hong and Shurin 2015). Faster development for northern populations has also been described by Edmands and Harrison (2003). Although niche width does not appear to decrease with increasing fitness for northern populations (which would have suggested a generalist/specialist trade-off), there may be trade-offs with survival and fecundity associated with the overall higher estimates of population growth rate for these higher latitude populations (Hong and Shurin

2015). Other studies of trade-offs in *T. californicus* have found no evidence for fitness trade-offs associated with laboratory selection for high temperature tolerance (Kelly et al. 2013) but potential trade-offs when there are joint salinity and high temperature stresses (Kelly et al. 2016; Leong et al. 2018).

Given the low levels of gene flow among populations of *T. californicus*, if the environment becomes less suitable due to future climate change and conditions begin to exceed their thermal limits, individual populations continued survival would require evolutionary adaptation as it would be less likely that immigrants from more tolerant populations will arrive to rescue populations by introducing more tolerant alleles. Kelly et al. (2012) suggest that there is limited potential for selection to improve heat tolerance in *T. californicus* over relatively short time periods for any single population. The results from the current study suggest that all of the populations show roughly similar thermal niche widths but horizontal shifts from south to north and this is likely to mean that sensitivity to future change will depend on specific scenarios of environmental change for each location. If higher temperature stresses become more common, copepod fitness could be negatively impacted as conditions in the field have been found to approach the lethal temperatures measured in these high temperature assays (Kelly at el. 2012; Leong et al. 2018). Further complications in predicting future responses of *T. californicus* populations to changing temperatures could also stem from potential differences in the physiological mechanism underlying response to thermal variation across populations. Even populations with similar phenotypic responses to thermal stress can show dramatically different patterns of gene expression (Lima and Willett 2017) suggesting differences in how these populations respond at the physiological level. Therefore, these populations may respond differently to selection and have different abilities to adapt to changing environments.

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CONTENTS

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