

Spermatozoal morphology in the “symmetrical” hermit crab, *Pylocheles (Bathycheles)* sp. (Crustacea, Decapoda, Anomura, Paguroidea, Pylochelidae)

Christopher C. TUDGE

Department of Invertebrate Zoology, National Museum of Natural History,
Smithsonian Institution, Washington D.C. 20560-0163 (USA)
tudge.christopher@nmnh.si.edu

David M. SCHELTINGA

Barrie G. M. JAMIESON

Department of Zoology and Entomology,
University of Queensland, Brisbane Qld 4072 (Australia)
dscheltinga@zoology.uq.edu.au
bjamieson@zoology.uq.edu.au

Tudge C. C., Scheltinga D. M. & Jamieson B. G. M. 2001. — Spermatozoal morphology in the “symmetrical” hermit crab, *Pylocheles (Bathycheles)* sp. (Crustacea, Decapoda, Anomura, Paguroidea, Pylochelidae). *Zoosystema* 23 (1) : 117-130.

ABSTRACT

Pylochelids, or the “symmetrical” hermit crabs are characterized by paired abdominal appendages (pleopods) on somites 2-6 of the calcified abdomen, in contrast to the asymmetrical pleopods, on an uncalcified abdomen, of the majority of hermit crabs in the superfamily Paguroidea. The spermatozoa of *Pylocheles (Bathycheles)* sp. are large cells (c. 14 µm wide) with a conspicuous depressed acrosome vesicle in the shape of a thick disk. A perforate (ring-like) operculum is positioned apically on the acrosome vesicle, and a wide, but shallow, perforatorial chamber penetrates the posterior end. Up to three short arms emanate from the cytoplasm of the sperm cell, but it is uncertain if these arms are microtubular in nature. The spermatozoal morphology provides no evidence for close affinities with any extant hermit crab family and indicates an isolated position for the Pylochelidae, though not incompatible with an origin from an ancestor shared with the Anomura.

KEY WORDS

Crustacea,
Decapoda,
Anomura,
Paguroidea,
Pylochelidae,
Pylocheles,
Spermatozoa,
ultrastructure,
symmetrical hermit crabs.

RÉSUMÉ

Morphologie des spermatozoïdes chez le pagure « symétrique », Pylocheles (Bathycheles) sp. (Crustacea, Decapoda, Anomura, Paguroidea, Pylochelidae).

Les Pylochelidés, aussi appelés pagures « symétriques », sont caractérisés par la présence d'appendices abdominaux (pléopodes) appariés sur les somites 2 à 6 de leur abdomen calcifié et symétrique, ce qui contraste avec les pléopodes asymétriques et l'abdomen non calcifié dont sont dotés la majorité des pagures de la superfamille Paguroidea. Les spermatozoïdes de *Pylocheles (Bathycheles) sp.* sont de grosses cellules (environ 14 µm de diamètre) avec une vésicule acrosomale évidente qui est déprimée et ressemble à un disque épais. Un opercule perforé (de forme annulaire) est en position apicale sur la vésicule acrosomale, dont la partie postérieure est creusée d'une cavité large, mais peu profonde, qui renferme le perforateur. Jusqu'à trois bras courts s'étendent à partir du cytoplasme du spermatozoïde, mais il n'est pas certain que ces bras soient de nature microtubulaire. La morphologie du spermatozoïde ne permet aucun rapprochement phylogénétique avec une famille existante de pagures, ce qui indique une position isolée pour les Pylochelidae mais n'exclut pas une origine remontant à un ancêtre commun aux Anomoures.

MOTS CLÉS

Crustacea,
Decapoda,
Anomura,
Paguroidea,
Pylochelidae,
Pylocheles,
Spermatozoa,
ultrastructure,
pagure symétrique.

INTRODUCTION

Members of the anomuran family Pylochelidae Bate, 1888 are often referred to as the "symmetrical" hermit crabs as they have paired pleopods on their calcified abdomen. This is in marked contrast to the asymmetrical, often soft, uncalcified abdomen of the majority of hermit crabs in the superfamily Paguroidea; with the exception of species in the diogenid genus *Cancellus* H. Milne Edwards, 1836, and members of the Lithodidae. The Pylochelidae currently contains 39 species, in seven genera, which are separated into six subfamilies (see Forest 1987a, b, for the most recent classification, dichotomous keys, and species list). Pylochelids have been collected extensively in the Indo-West Pacific region, from Madagascar to Japan and New Zealand, and several species (including the monotypic subfamily Mixtopagurinae) are known from the Caribbean and western Atlantic (Forest 1987a, b). The Indo-West Pacific is the most speciose region for this family and has been considered as the centre of their radiation and suggests a probable Tethyan origin for the group. These hermit crabs are primarily found in tropical waters but with

some representatives extending into temperate regions, and they have been collected from depths ranging from 30 m to 1570 m, but with the majority found at 200-500 m (Forest 1987a, b). The different genera within the Pylochelidae can easily be separated into groups according to the type of dwelling they use for habitation and protection. Unlike the majority of hermit crabs which utilize abandoned gastropod mollusc shells, the different groups of pylochelids are xylicolous (wood-dwellers; including hollow bamboo stems and corn cobs), petricolous (stone-dwellers), spongicolous (sponge-dwellers), and scaphipodicolous (tusk shell-dwellers). Although not exclusively found in these unusual habitations, it is their utilization of them that is characteristic of the family. It has been noted that many pylochelids may not be able to move around with their respective shelters and that they presumably leave periodically to forage for food or mates (Boas 1926; Forest 1987a).

The Pylochelidae is composed of several distinct lineages whose respective affinities are unclear, and is probably a polyphyletic or paraphyletic family (Forest 1987a; Richter & Scholtz 1994). Forest (1987b) suggests that the pylochelids are

closer to the Diogenidae, based on comparison of somatic morphology, with which they perhaps share an ancient common ancestor. At present, however, a special relationship between any lineage of pylochelids and any group of the Diogenidae is elusive. A basal (ancestral) position of the pylochelids, in relation to the other hermit crabs (Paguroidea), has received much support in the literature (Calman 1911; Makarov 1962; McLaughlin 1983 [as Pomatochelidae]; Martin & Abele 1986 [as Pomatochelidae]; Richter & Scholtz 1994). Makarov (1962) further suggested that the Pylochelidae still possess a number of characters relating them to the Thalassinidea, a view supported by an earlier observation that it was difficult to find characters which distinguished the two groups (Calman 1911).

Species in the genus *Pylocheles* A. Milne-Edwards, 1880 are generally xylicolous, but some have been reported from large scaphopod shells (Forest 1987a). *Pylocheles* is the type genus for the Pylochelidae, and the subfamily Pylochelinae Bate, 1888. The other five subfamilies are sufficiently distinct to possibly deserve separate familial rank (Forest pers. comm.). Richter & Scholtz (1994) placed *Pylocheles* (with the sister-taxon *Cheiroplatea* Bate, 1888) as the most basal line of the seven pylochelid genera in their phylogenetic analysis of hermit crab ancestry. This genus is, therefore, particularly pertinent to resolution of relationships of the family.

The pylochelid specimen used in this study is a hitherto undescribed species of *Pylocheles* (subgenus *Bathycheles* Forest, 1987) from New Caledonia, with close affinities to *Pylocheles (Bathycheles) incisus* Forest, 1987. It is awaiting description by staff of the Muséum national d'Histoire naturelle, Paris. This specimen is the first member of the Pylochelidae to be investigated for spermatozoal morphology and the family is the second to last of 13 anomuran families (the last being the Aegliidae Dana, 1852) for which the sperm morphology of at least one representative has been described (Tudge 1992, 1995a, b, 1997a, b; Tudge & Jamieson 1991, 1996a, b; Tudge *et al.* 1998a, 1999; Jamieson & Tudge 2000, and references therein). The reproductive biology of the

Pylochelidae is unknown, but this study describes and illustrates the ultrastructure of the spermatozoon of this new species of *Pylocheles (Bathycheles)* and compares it with that of other investigated members of the Paguroidea and Anomura in general.

MATERIALS AND METHODS

The single male specimen of *Pylocheles (Bathycheles)* sp. was collected with two female specimens of another pylochelid, *Cheiroplatea pumicicola* Forest, 1987, from pumice strewn muddy bottom at 640-687 m depth (station CP1332) at Bligh Water, Fiji Archipelago (16°56'17"S, 178°07'86"E), South-West Pacific Ocean, on September 8, 1998, by the research vessel *Alis* (ORSTOM, Noumea).

The entire specimen was fixed in 3% glutaraldehyde in 0.2M phosphate buffer (pH = 7.2) for a minimum of two hours at 4 °C, and then transferred to Brisbane, Australia, where the gonads and vas deferens were removed and the remainder of the fixation process (outlined below) was carried out. The spermatozoa were viewed and photographed with an Olympus BH2 optical microscope equipped with Nomarski interference contrast optics, with an attached Olympus OM-2 camera.

After the initial glutaraldehyde fixation and first phosphate buffer wash, the fixation procedure for transmission electron microscopy was carried out in a Lynx-el. Microscopy Tissue Processor. Portions of the vas deferens were washed in phosphate buffer (three washes in 15 min.), postfixed in phosphate buffered 1% osmium tetroxide for 80 min.; similarly washed in buffer and dehydrated through ascending concentrations of ethanol (40-100%). After being infiltrated and embedded in Spurr's epoxy resin (Spurr 1969), thin sections (50-80 nm thick) were cut on a LKB 2128 UM IV microtome with a diamond knife. Sections were placed on carbon-stabilized colloidin-coated 200 µm mesh copper grids and stained in 6% aqueous uranyl acetate for 30 sec.; rinsed in distilled water; stained with

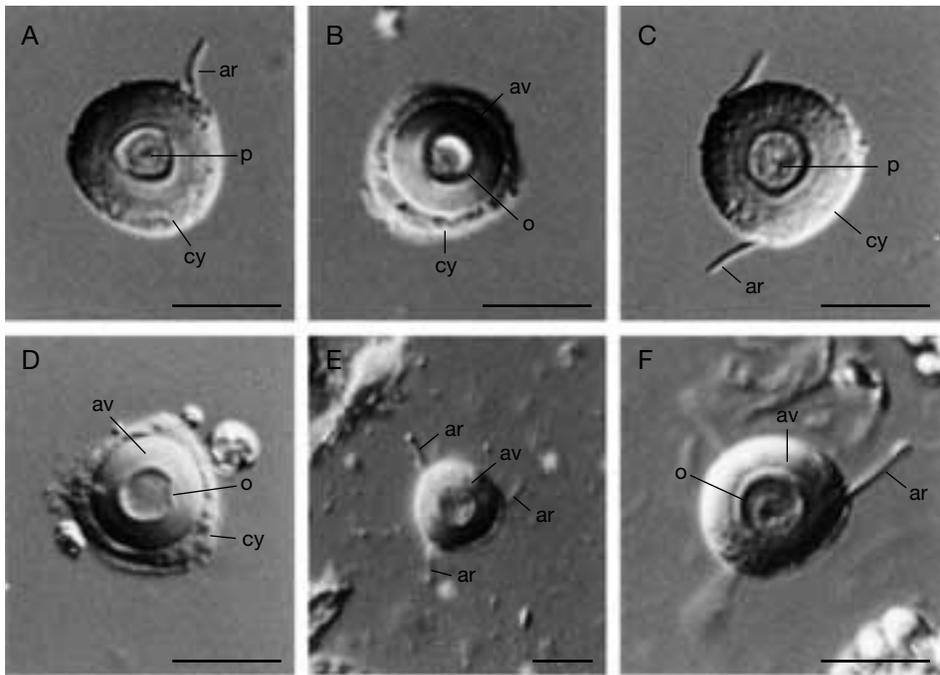


FIG. 1. — Light micrographs of spermatozoa of *Pylocheles (Bathycheles)* sp., view of spermatozoa; **A, C**, ventral; **B, D-F**, dorsal. Abbreviations: **ar**, arm; **av**, acrosome vesicle; **cy**, cytoplasm; **o**, operculum; **p**, perforatorial chamber. Scale bars: 10 μ m.

Reynold's lead citrate (Reynolds 1963) for four minutes and further stained in uranyl acetate for two minutes before a final rinse in distilled water (Daddow 1986). Micrographs were taken on an Hitachi 300 transmission electron microscope at 80 kV.

In all observations of the spermatozoa of *Pylocheles (Bathycheles)* sp., the acrosome is arbitrarily considered to occupy the dorsal or apical pole of the cell, while the cytoplasm and nucleus are considered ventral.

OBSERVATIONS

No spermatophores were observed, at either the light or transmission electron microscope (TEM) level, in the *Pylocheles (Bathycheles)* sp. specimen but observations of the related species *Pylocheles (Bathycheles) chacei* Forest, 1987 from preserved collections (Tudge, pers. obs. of USNM 265196) revealed large spherical sperma-

tophores clustered around the male gonopore. The spermatophores of this latter species varied in size and each contained many large spermatozoa which appear identical (using light microscopy) to those of *Pylocheles (Bathycheles)* sp. (Fig. 1), and are here assumed to be characteristic of the genus.

The free spermatozoa of both species are large cells (*c.* 14 μ m in width) with an obvious circular acrosome vesicle occupying most of the cell. When the sperm of *Pylocheles (Bathycheles)* sp. is viewed dorsally, a ring-like operculum is seen on top of the acrosome vesicle (Fig. 1B, D-F). When viewed ventrally the sperm cells have a granular area of cytoplasm and nucleus interrupted centrally by a circular perforatorial chamber (Fig. 1A, C). No more than three short arms (usually only one or two are visible) extend from blunt projections of the spermatozoal outline (Fig. 1). These blunt vertices are often apparent even when the accompanying arms are not visible.

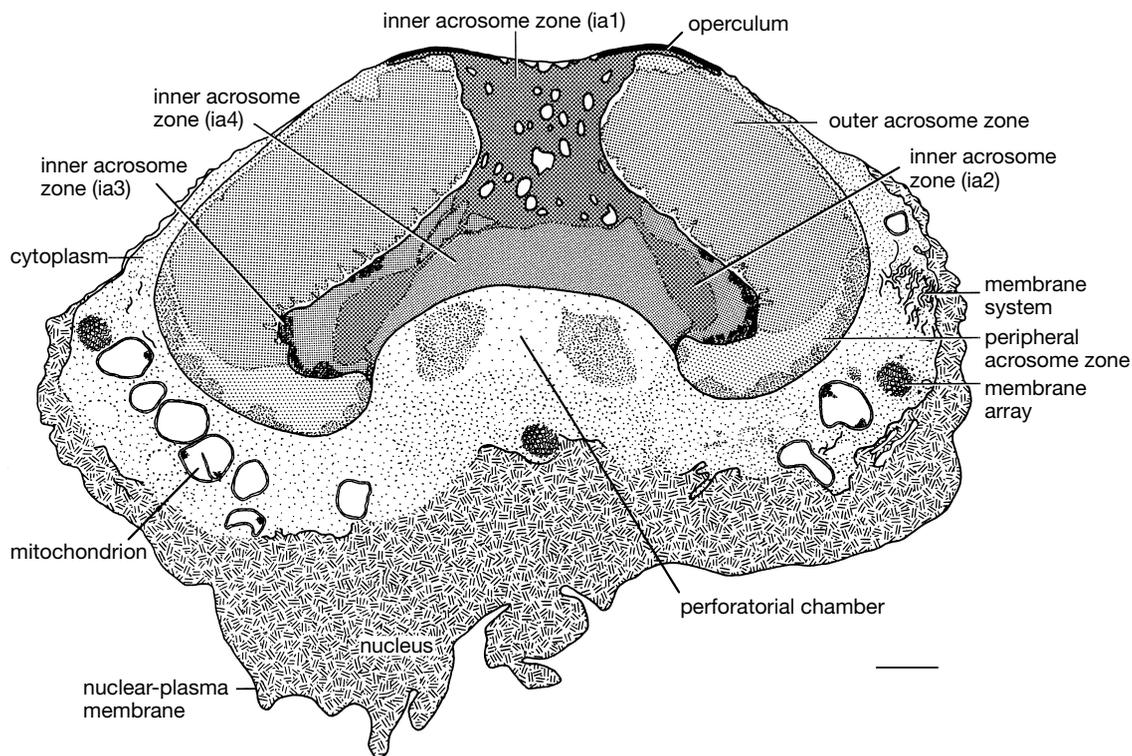


FIG. 2.— Semidiagrammatic representation of a longitudinal section of the spermatozoon of *Pylocheles* (*Bathychelès*) sp. Drawn from a micrograph. Scale bar: 1 μ m.

At the TEM level, the three components of the spermatozoa of *Pylocheles* (*Bathychelès*) sp. are clearly visible. A large, densely staining, depressed-ovoid acrosome vesicle occupies the apical pole of the cell, an amorphous, electron-lucent nucleus occupies the opposite pole (Figs 2; 3A, B). Heterogeneous cytoplasm intervenes between acrosome and nucleus and extends laterally around the acrosome vesicle (Figs 2; 3A, B). In longitudinal section (ls), through the mid-line of the cell, the spermatozoa are approximately 14.3 μ m in width and 11.3 μ m in length, including any projections of the nuclear material.

The acrosome vesicle, in ls, has an approximate width of 11 μ m and length of 6.6 μ m, giving it a length to width (l:w) ratio of 0.6. This depressed disk-shape is consistent throughout all of the spermatozoa recorded for this species. There is a shallow depression apically (opercular perfora-

tion) and a deeper and larger depression opposite this (perforatorial chamber). Both depressions are seen to be circular when viewed in transverse section (ts) by TEM (Fig. 4B, C) or by light microscopy (Fig. 1). The smaller, apical, depression is surrounded by a thin, extremely electron-dense ring, the operculum (Figs 3A, B; 4A). The inner diameter of the operculum is 1.6 μ m, and the outer diameter 5.7 μ m. The operculum is 0.2 μ m, or less, in thickness and is divided into an anterior (or upper) electron-dense zone and a less dense granular zone posterior to this (Fig. 4A). The latter zone is the more substantial of the two zones.

The contents of the acrosome vesicle are arranged in three concentric zones: an inner, an outer and a peripheral acrosome zone. Each of these zones is further subdivided into areas of differing appearance and electron density (Figs 2; 3A, B).

The inner acrosome zone extends through the centre of the acrosome vesicle from the opercular perforation, and the posterior boundary of the operculum, to surround the perforatorial chamber. It is thinnest at a distinct “waist” in the upper third of its length and broadest posteriorly around the perforatorial chamber. Four sub-regions are apparent:

1. the anterior half of the inner acrosome zone is strongly electron-dense but is irregularly interrupted by electron-lucent patches of various sizes, in which a coarse granularity is observed (Fig. 3A, B);
2. posterior to this “pock-marked” dense region, and appearing connected to it by tenuous filaments, is a ring of electron-dense material surrounding the perforatorial chamber (Figs 3A-C; 4B). This sub-region is of identical electron density to the anteriormost region (1) but contains no paler inclusions. In ls, this dense inner acrosome zone ring appears as a dark patch on each side of the perforatorial chamber (Fig. 3A);
3. lining the posterior periphery of the inner acrosome zone, and abutting the outer acrosome zone, is a thin region composed of a flocculent mix of electron-dense and electron-lucent lines. This heterogeneous region extends from the thin “waist” of the inner acrosome zone to the base of the perforatorial chamber and is thickest posteriorly (Figs 3A-C; 4B, C). Closer examination of this flocculent region reveals a fine patterned arrangement of parallel and anastomosing lines which become more randomly arranged toward the interior of the sperm cell (Fig. 3A, insert);
4. the remainder of the inner acrosome zone is a homogeneous, coarsely granular, region of moderate electron density which occupies the area closest to the perforatorial chamber and between the dense ring (region 2) and the flocculent region (3) (Figs 3A-C; 4B, C). Where it adjoins the more anterior “pock-marked” region (1), it forms an irregular boundary which sometimes exhibits a few electron-pale, tubular inclusions. It appears unlikely that these are continuous with the electron-pale patches of region one.

Exterior to the inner acrosome zone, and forming a thick, moderately electron-dense ring, is a zone

here termed the outer acrosome zone. It extends from the apical end of the acrosome vesicle (sub-adjacent to the operculum), posteriorly and somewhat laterally, to terminate short of the base of the acrosome vesicle (Figs 3A-C; 4B, C). At this point the peripheral acrosome zone intervenes between the operculum and the outer acrosome zone. The outer acrosome vesicle is homogeneously and densely granular with a thin, electron-pale strip bordering the inner edge, next to the inner acrosome zone. This electron-lucent strip becomes more irregular and breaks up into radiating strips posteriorly (below the thinner “waist” of the inner acrosome zone). Sometimes, at higher power, the outer acrosome zone is observed to be composed of very thin radiating lines of alternating dark and light bands. It is uncertain whether these fine bands are a permanent feature of the sperm cell, or an artifact of fixation, as they were not visible in some micrographs.

The peripheral acrosome zone extends around the periphery of the acrosome vesicle, exterior to the inner and outer acrosome zones, from the perforate operculum to the base of the perforatorial chamber. This zone is a homogeneously granular, yet electron-pale zone, with a posterior border of irregular, more electron-dense, patches (Figs 3A-C; 4B, C). Laterally the peripheral acrosome zone forms a thin veneer but expands into two more extensive areas apically (directly under the opercular ring) (Figs 3A; 4A) and posteriorly (surrounding the base of the perforatorial chamber) (Fig. 3A, B). The latter area is the more extensive of the two.

The perforatorial chamber is a large, but shallow, invagination into the posterior region of the acrosome vesicle (Figs 2; 3). At its widest point it is 4.3 μm in diameter ($n = 6$), but extends into the acrosome vesicle for less than one third of the length of the latter. Light and TEM observations indicate that the perforatorial chamber has a circular outline when viewed in ts and that its contents are electron-pale, irregular patches of coarse granularity on an electron-lucent background (Fig. 4B, C). A distinct boundary, without any membrane, separates the perforatorial chamber contents from the subjacent cytoplasm (Fig. 3A).

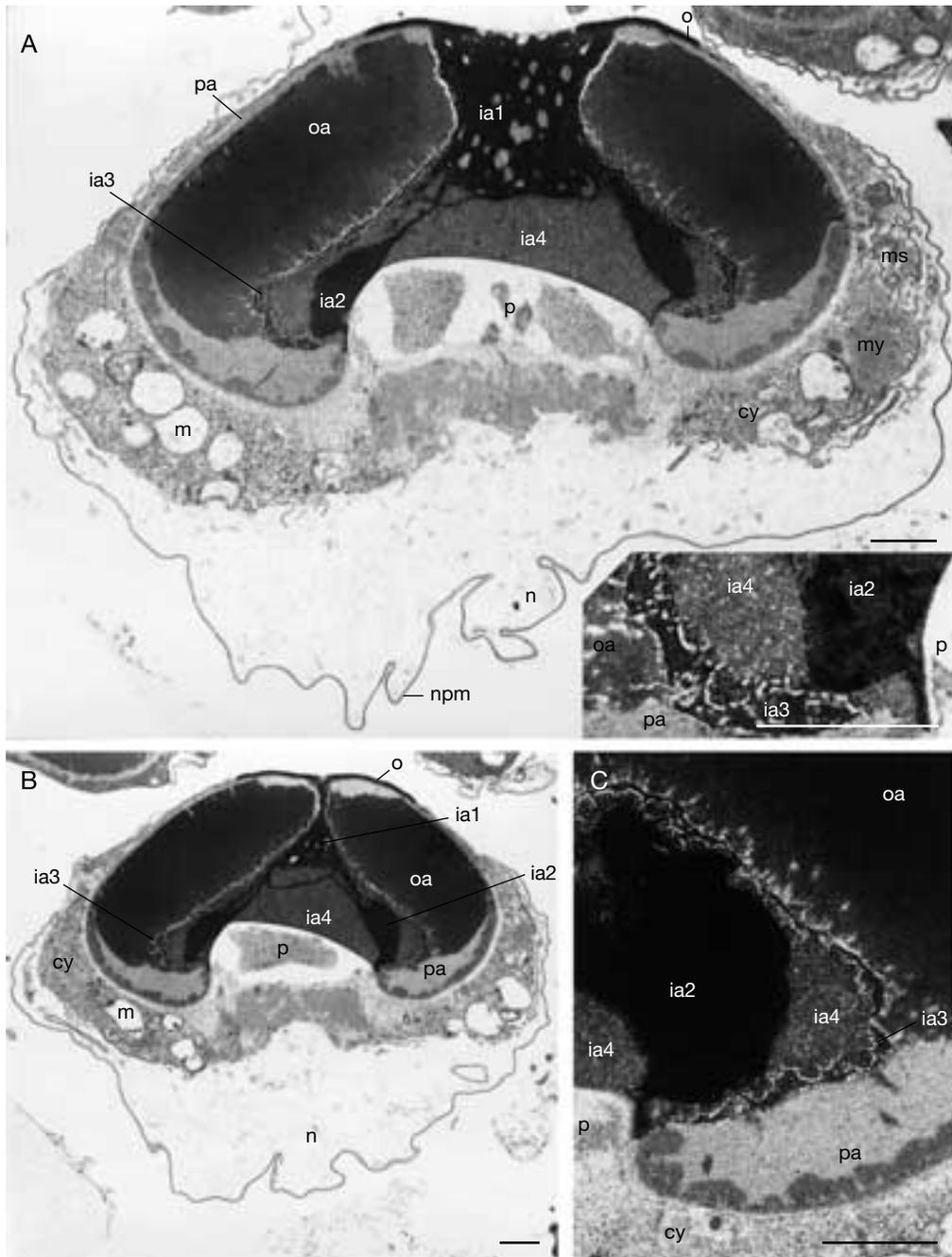


FIG. 3. — Transmission electron micrographs of spermatozoa of *Pylocheles* (*Bathychelès*) sp.; **A**, longitudinal section (ls) of spermatozoon. Insert: detail of flocculent region of inner acrosome zone; **B**, slightly oblique ls of spermatozoon; **C**, oblique section showing detail of three regions of the inner acrosome zone, the outer acrosome zone, and the peripheral acrosome zone. Abbreviations: **cy**, cytoplasm; **ia1**, inner acrosome zone 1; **ia2**, inner acrosome zone 2; **ia3**, inner acrosome zone 3; **ia4**, inner acrosome zone 4; **m**, mitochondrion; **ms**, membrane system; **my**, membrane array; **n**, nucleus; **npm**, nuclear-plasma membrane; **o**, operculum; **oa**, outer acrosome zone; **p**, perforatorial chamber; **pa**, peripheral acrosome zone. Scale bars: 1 μ m.

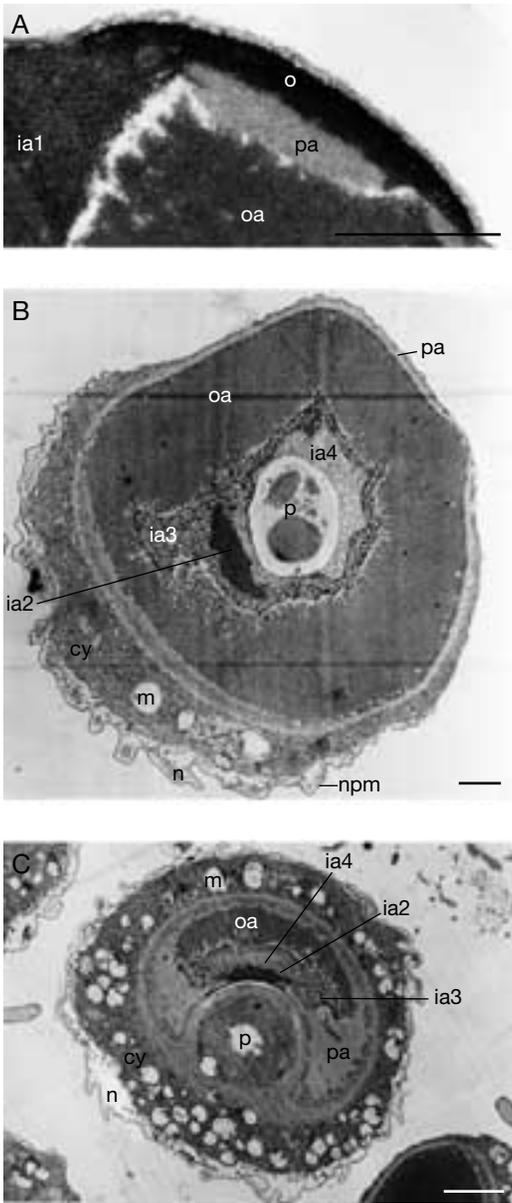


FIG. 4. — Transmission electron micrographs of spermatozoa of *Pylocheles* (*Bathycheltes*) sp.; **A**, detail of one side of the opercular ring; **B**, transverse section (ts) through the perforatorial chamber and base of the acrosome vesicle; **C**, slightly oblique ts through base of the acrosome vesicle and cytoplasm. Abbreviations: **cy**, cytoplasm; **ia1**, inner acrosome zone 1; **ia2**, inner acrosome zone 2; **ia3**, inner acrosome zone 3; **ia4**, inner acrosome zone 4; **m**, mitochondrion; **n**, nucleus; **npm**, nuclear-plasma membrane; **o**, operculum; **oa**, outer acrosome zone; **p**, perforatorial chamber; **pa**, peripheral acrosome zone. Scale bars: A, B, 1 μ m; C, 2 μ m.

The extensive cytoplasm forms an enveloping layer around the acrosome vesicle except for the region immediately surrounding the apical operculum. The cytoplasm is thickest posterolaterally, attenuating anteriorly and directly below the perforatorial chamber (Figs 2; 3A, B; 4B, C). It is composed of a heterogeneous assemblage of moderately electron-dense organelles and other subcellular structures. These organelles include many spherical, acristate or sparsely cristate, electron-lucent mitochondria (Figs 3A, B; 4B, C; 5A-C) (concentrated in a postero-lateral ring); irregular membrane systems, including the occasional whorl or scroll (Figs 3A; 4B, C; 5B, C); bundles each consisting of lattice-like arrangements of membranes (Figs 3A; 5); and various granules and vesicles resembling rough endoplasmic reticulum (Figs 3A, B; 4B; 5A-C). In both ts and ls of the spermatozoal arms, their contents consist of a fairly homogeneous array of moderately electron-dense granules, without any indication of the microtubules usual in anomuran sperm arms; nor were microtubules visible in the cytoplasm. The arms originate from within the cytoplasm and extend away from the main body of the cell and are surrounded by the nuclear membrane, but their composition is unlike that of cytoplasm or nuclear material. The membrane lattice in some sections appears as 0.02 μ m electron-dense rings, surrounded by a paler ring 0.06 μ m in diameter, giving a spurious appearance of microtubules (Fig. 5). In some (longitudinal?) sections they appear as wavy double membranes.

As previously described, the nuclear material is an amorphous, posteriorly directed plume of electron-lucent matrix, broken occasionally by small patches of more electron-dense granules. A thin veneer of nuclear material also extends anteriorly and laterally to cover most of the cytoplasm in this region of the sperm cell (Figs 2; 3A). Rare micrographs showed a more electron-dense nuclear material. Irrespective of the appearance of the nuclear contents, this organelle is always bounded by a thick, electron-dense membrane, constituted by both the nuclear and plasma membranes (Figs 3A, B; 4C; 5A, C).

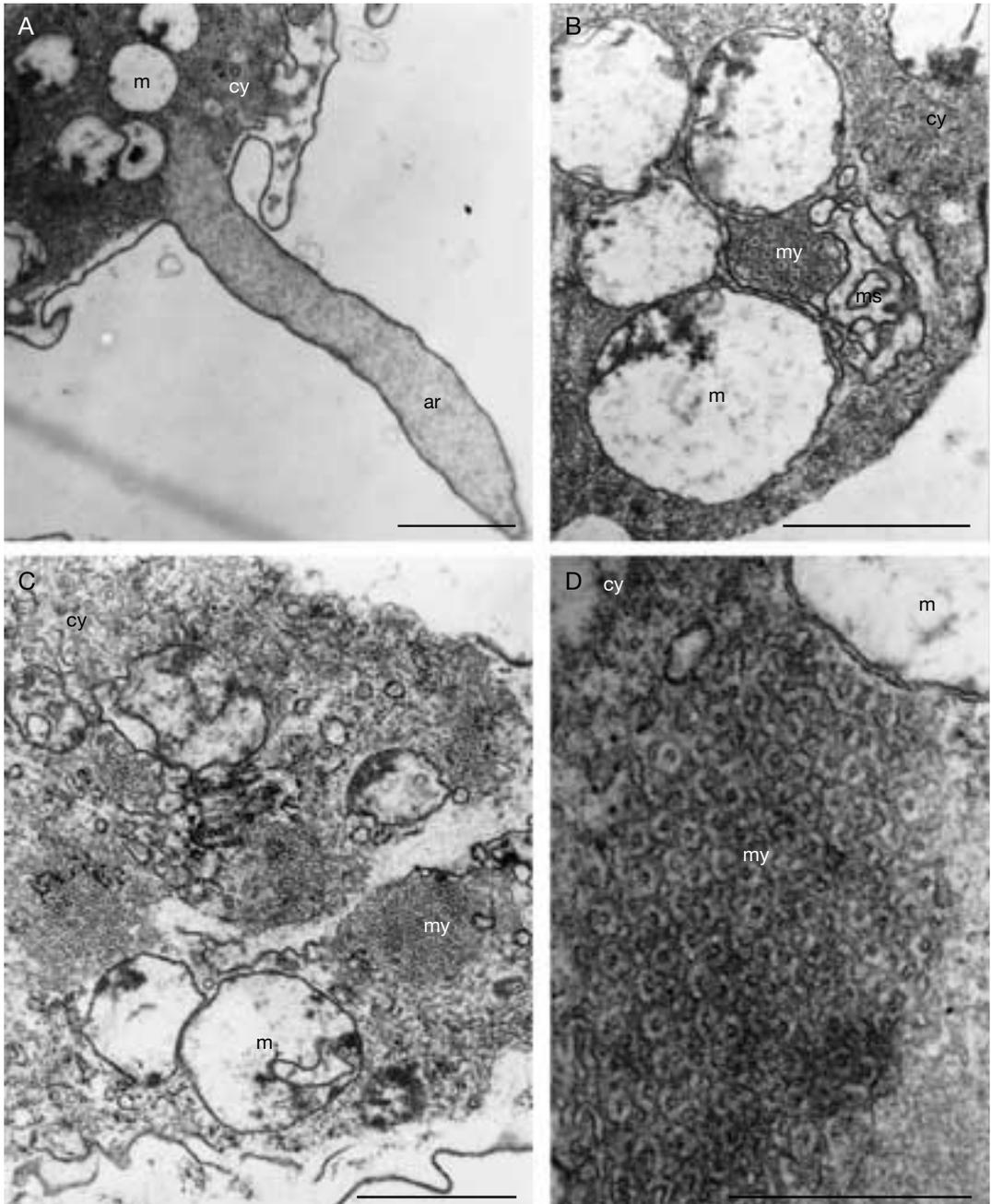


FIG. 5. — Transmission electron micrographs of spermatozoa of *Pylocheles* (*Bathychelès*) sp.; **A**, Is of arm emanating from cytoplasm. Note: lack of visible microtubules; **B**, **C**, detail of cytoplasm showing membrane arrays, mitochondria, and membrane systems; **D**, high power view of lattice-like membrane array. Abbreviations: **ar**, arm; **cy**, cytoplasm; **m**, mitochondrion; **ms**, membrane system; **my**, membrane array. Scale bars: A-C, 1 μ m; D, 0.5 μ m.

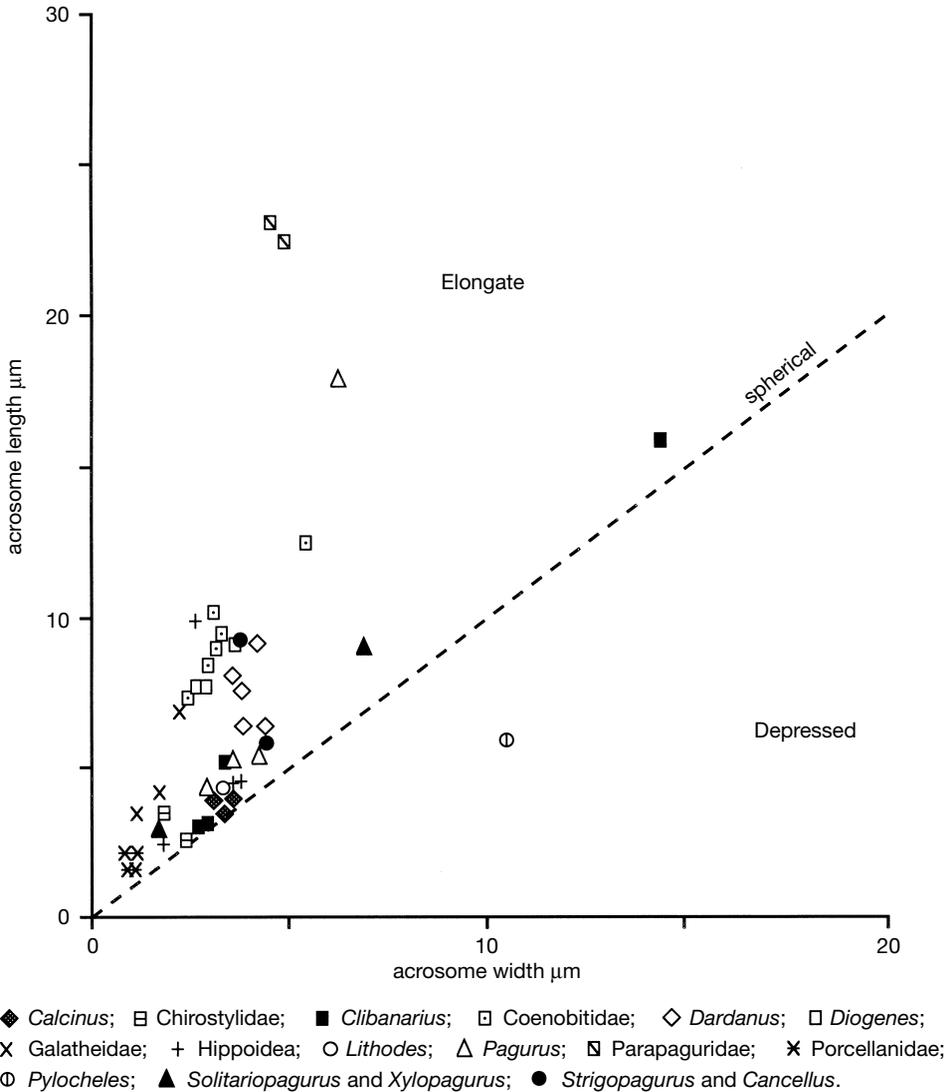


FIG. 6. — Graph of acrosome vesicle length to width (l:w) ratios in selected Anomura with the position of *Pylocheles* (*Bathychelès*) sp. shown, as indicated.

DISCUSSION

The spermatozoal morphology exhibited by *Pylocheles* (*Bathychelès*) sp. is unlike any anomuran spermatozoa studied to date, and in particular, has no counterpart in any investigated paguroid (hermit crab) (Tudge & Jamieson 1991; Tudge 1992, 1995a, b; Tudge *et al.* 1998a, and refe-

rences therein). Despite this uniqueness, the spermatozoa do exhibit certain characters which are shared by other paguroids, and indeed, by many anomurans (Tudge 1997b). These include a complex concentrically zoned acrosome vesicle, capped by an electron-dense operculum and posteriorly penetrated by a perforatorial chamber, and three or more arms emanating from the cyto-

plasm of the sperm cell. The depressed-disk shape of the acrosome vesicle in *Pylocheles* (*Bathycheles*) sp. spermatozoa is unique in the Anomura investigated to date. A spherical to oblong-ovoid or cylindrical acrosome vesicle shape predominates in the Anomura and l:w ratios of approximately 1.0 or more have been recorded in all investigated anomuran species (Tudge 1995a, 1997b). *Pylocheles* (*Bathycheles*) sp. with a l:w ratio of 0.6 falls into the depressed acrosome category (Fig. 6) and shares this "depressed shape" with a few thoracotreme and heterotreme brachyurans, all investigated podotreme brachyurans, and some astacid, palinurid and enoplometopid crayfish and lobsters (Jamieson 1991: fig. 4). Although significantly depressed, the internal acrosome zonation is still concentric and does not approach the markedly horizontal acrosome zonation characteristic of most investigated podotremate Brachyura (Jamieson *et al.* 1995; Jamieson & Tudge 2000, and references therein).

Another character shared with these primitive, podotreme brachyurans (as well as a few heterotremes) is the centrally perforate operculum (Jamieson 1989, 1990, 1991, 1994; Jamieson *et al.* 1993a-c, 1994a, b, 1995; Guinot *et al.* 1994, 1998). This characteristic opercular feature is also found in the astacideans *Pacifastacus* Bott, 1950 and *Homarus* Weber, 1795, the thalassinidean *Axius* Leach, 1815, and all investigated porcellanid anomurans, but has been recorded in only two other paguroid anomurans, the diogenid *Cancellus* (Tudge 1995a, b, 1997a, b) and *Lithodes* Latreille, 1806 (Tudge *et al.* 1998a). It is apparent that the centrally perforate operculum has independently developed in the spermatozoa of many decapod crustaceans (Jamieson & Tudge 2000).

The arrangement of the four distinct sub-regions within the inner acrosome zone in the spermatozoa of *Pylocheles* (*Bathycheles*) sp. is unknown in other decapods. Similar areas, equivalent to individual sub-regions, have been observed in the anomurans, *Aliaporcellana* Nakasone & Miyake, 1969, *Pisidia* Leach, 1820, *Polyonyx* Stimpson, 1858 (Tudge 1995a, b; Tudge & Jamieson 1996a, b), *Birgus* Leach, 1815 (Tudge & Jamie-

son 1991; Tudge 1995a), *Clibanarius* Dana, 1852 species (Tudge 1992, 1995a, b), podotreme brachyurans *Ranina* Lamarck, 1801 (Jamieson 1989) and *Tymolus* Stimpson, 1858 (Jamieson *et al.* 1994a, b).

An enigmatic feature observed in the spermatozoa of *Pylocheles* (*Bathycheles*) sp. is the absence of visible microtubules within the cytoplasm of the cell or in the external arms (their absence via a fixation anomaly is also a possibility). In all other anomurans investigated for sperm ultrastructure the three or more microtubular arms are composed of clearly visible bundles of microtubules, which often can be linked directly with the same structures within the cytoplasm (Tudge & Jamieson 1991; Tudge 1992, 1995a, b; Tudge *et al.* 1998a, b; Jamieson & Tudge 2000, and references therein).

In *Pylocheles* (*Bathycheles*) sp. sperm, the cytoplasm has complex and often geometrically patterned, lattice-like membrane arrays (Fig. 5B-D). Similar structured membrane systems, and annulate lamellae, have been recorded from the cytoplasm of sperm cells of other decapods such as *Enoplometopus* A. Milne-Edwards, 1862 (Haley 1986), *Birgus* (Tudge & Jamieson 1991; Tudge 1995a) and *Australocarcinus* Davie, 1988 (Jamieson & Guinot 1996) and often with associated microtubules. Microtubule and lamellar membrane associations were also recorded in *Homarus* by Talbot & Chanmanon (1980), but they were in the nuclear material and arms. Closer examination of the lattice-like membrane arrays of *Pylocheles* (*Bathycheles*) sp. sperm reveals that although not containing visible microtubules, they superficially resemble the microtubule/macrotubule structures observed in the extracellular matrix surrounding the spermatozoa in the palinurid lobster, *Jasus* Parker, 1884 (Tudge *et al.* 1998b).

What does the ultrastructure of the spermatozoa of *Pylocheles* (*Bathycheles*) reveal about the relationship of this genus, representing the family Pylochelidae, to other hermit crabs (Paguroidea), the Anomura, or decapods in general? The spermatozoa of *Pylocheles* (*Bathycheles*) sp. exhibit no apomorphies that necessitates recognizing a rela-

tionship with any anomuran family. In fact, the unusual arrangement of the sub-regions of the inner acrosome zone and the unique depressed acrosome vesicle support the statements and/or trees of McLaughlin (1983), Forest (1987a, b), Richter & Scholtz (1994) and McLaughlin & Lemaitre (1997) which indicate that the Pylochelidae derived as an independent lineage from basal paguroid (possibly even basal anomuran) stock. The statement by Makarov (1962: 115) that the pylochelids are “*the most primitive family of the Paguridea*” is difficult to reconcile on spermatozoal ultrastructure data alone, but may be a reflection of the high degree of retention of plesiomorphic morphological characters (Calman 1911; McLaughlin 1983; Martin & Abele 1986; Richter & Scholtz 1994; McLaughlin & Lemaitre 1997). We find no spermatological support for a close link between *Pylocheles* (*Bathycheles*) sp. and members of the Thalassinidea (Tudge 1995a, b; Jamieson & Tudge 2000; and unpublished data) contrary to the previous suggestions of Calman (1911) and Makarov (1962) based on morphological evidence.

In summary, the spermatozoa of *Pylocheles* (*Bathycheles*) sp., currently representing the family Pylochelidae, exhibit apomorphic ultrastructural characters which support derivation independent of paguroid stock, but without showing any closer affinities to the spermatozoa of any extant hermit crab group. In fact, differences between the spermatozoa of *Pylocheles* (*Bathycheles*) sp. and that of studied anomurans is as great as those seen between the spermatozoa of primitive podotreme brachyurans and the more derived heterotremes. Further research on the ultrastructure of spermatozoa in the Pylochelidae is needed to confirm whether the observations made here for *Pylocheles* (*Bathycheles*) sp. are general for the genus and for the Pylochelidae as a whole.

Acknowledgements

Dr B. Richer de Forges, and the crew of the R/V *Alis* (IRD, Nouméa) are thanked for the collection and initial fixation of *Pylocheles* (*Bathycheles*) sp. The expert assistance of L. Daddow and

T. Gorringe (Department of Zoology and Entomology, The University of Queensland, Australia) with aspects of electron microscopy and photography (respectively) is heartily acknowledged. Dr K. Fauchald (Department of Invertebrate Zoology, NMNH, Smithsonian Institution, USA) is thanked for supporting access to departmental facilities by CCT. This research was initiated under the auspices of an ARC Australian Postdoctoral Research Fellowship, 1996-1999, to CCT and a current ARC Large Grant to BGMJ.

REFERENCES

- BOAS J. E. V. 1926. — Zur Kenntnis symmetrischer Paguriden. *Det Kongelige Danske Videnskabernes Selskab Skrifter* 6: 3-52.
- CALMAN W. T. 1911. — *The Life of Crustacea*. Methuen and Co. Ltd, London, 289 p.
- DADDOW L. Y. M. 1986. — An abbreviated method of the double lead stain technique. *Journal of Submicroscopic Cytology* 18: 221-224.
- FOREST J. 1987a. — Ethology and distribution of Pylochelidae (Crustacea Decapoda Coenobitoidea). *Bulletin of Marine Science* 41: 309-321.
- FOREST J. 1987b. — Les Pylochelidae ou “pagures symétriques” (Crustacea Décapoda Coenobitoidea), in Résultat de la Campagne MUSORSTOM. I & II: Philippines. Vol. 3. *Mémoires du Muséum national d'Histoire naturelle* 137 (A): 1-254.
- GUINOT D., JAMIESON B. G. M. & RICHER DE FORGES B. 1994. — Relationship of Homolidae and Dromiidae: evidence from spermatozoal ultrastructure (Crustacea, Decapoda). *Acta Zoologica*, Stockholm 75: 255-267.
- GUINOT D., JAMIESON B. G. M., RICHER DE FORGES B. & TUDGE C. C. 1998. — Comparative spermatozoal ultrastructure of the three dromiacean families exemplified by *Homolodromia kai* (Homolodromiidae), *Sphaerodromia lamellata* (Dromiidae), and *Dynomene tanensis* (Dynomeneidae) (Podotremata: Brachyura). *Journal of Crustacean Biology* 18: 78-94.
- HALEY S. R. 1986. — Ultrastructure of spermatogenesis in the Hawaiian red lobster, *Enoplometopus occidentalis* (Randall). *Journal of Morphology* 190: 81-92.
- JAMIESON B. G. M. 1989. — Ultrastructural comparison of the spermatozoa of *Ranina ranina* (Oxystomata) and of other crabs exemplified by *Portunus pelagicus* (Brachygnatha) (Crustacea, Brachyura). *Zoomorphology* 109: 103-111.
- JAMIESON B. G. M. 1990. — The ultrastructure of the spermatozoa of *Petalomera lateralis* (Gray) (Crus-

- tacea, Brachyura, Dromiacea) and its phylogenetic significance. *Invertebrate Reproduction and Development* 17: 39-45.
- JAMIESON B. G. M. 1991. — Ultrastructure and phylogeny of crustacean spermatozoa. *Memoirs of the Queensland Museum* 31: 109-142.
- JAMIESON B. G. M. 1994. — Phylogeny of the Brachyura with particular reference to the Podotremata: evidence from a review of spermatozoal ultrastructure (Crustacea, Decapoda). *Philosophical Transactions of the Royal Society of London* 345 (B): 373-393.
- JAMIESON B. G. M. & GUINOT D. 1996. — Ultrastructure of the spermatozoon of *Australocarcinus riparius* (Crustacea: Brachyura: Goneplacidae: Trogloplacinae). *Memoirs of the Queensland Museum* 39: 289-296.
- JAMIESON B. G. M. & TUDGE C. C. 2000. — Crustacea-Decapoda, in JAMIESON B. G. M. (ed.), *Reproductive Biology of Invertebrates*. Vol. IX, part C: *Progress in Male Gamete Ultrastructure and Phylogeny*. John Wiley & Sons, Chichester: 1-95.
- JAMIESON B. G. M., GUINOT D. & RICHER DE FORGES B. 1993a. — The ultrastructure of the spermatozoon of *Paradynomene tuberculata* Sakai, 1963 (Crustacea, Brachyura, Dynomenidae): synapomorphies with dromiid sperm. *Helgoländer Meeresuntersuchungen* 47: 311-322.
- JAMIESON B. G. M., GUINOT D. & RICHER DE FORGES B. 1993b. — Spermatozoal ultrastructure in four genera of Homolidae (Crustacea, Decapoda): exemplified by *Homologenus* sp., *Latreillopsis* sp., *Homolomannia sibogae* and *Paromolopsis boasi*. *Helgoländer Meeresuntersuchungen* 47: 323-334.
- JAMIESON B. G. M., TUDGE C. C. & SCHELTINGA D. M. 1993c. — The ultrastructure of the spermatozoon of *Dromidiopsis edwardsi* Rathbun, 1919 (Crustacea: Brachyura: Dromiidae): confirmation of a dromiid sperm type. *Australian Journal of Zoology* 41: 537-548.
- JAMIESON B. G. M., GUINOT D. & RICHER DE FORGES B. 1994a. — Relationships of the Cyclodorippoidea Ortmann: evidence from spermatozoal ultrastructure in the genera *Xeinostoma*, *Tymolus* and *Cymonomus* (Crustacea, Decapoda). *Invertebrate Reproduction and Development* 26: 153-164.
- JAMIESON B. G. M., GUINOT D. & RICHER DE FORGES B. 1994b. — Podotreme affinities of *Raninoides* sp. and *Lyreidus brevifrons*: evidence from spermatozoal ultrastructure (Crustacea: Brachyura: Raninoidea). *Marine Biology* 120: 239-249.
- JAMIESON B. G. M., GUINOT D. & RICHER DE FORGES B. 1995. — Phylogeny of the Brachyura (Crustacea, Decapoda): Evidence from spermatozoal ultrastructure, in JAMIESON B. G. M., AUSIO J. & JUSTINE J.-L. (eds), *Advances in spermatozoal phylogeny and taxonomy. Mémoires du Muséum national d'Histoire naturelle* 166: 265-283.
- MAKAROV V. V. 1962. — *Fauna of U.S.S.R. Crustacea*. Vol. 10 (3): *Anomura*. Israel Program for Scientific Translations, Jerusalem, 283 p. [English translation of Makarov 1938].
- MARTIN J. W. & ABELE L. G. 1986. — Phylogenetic relationships of the genus *Aegla* (Decapoda: Anomura: Aegliidae), with comments on anomuran phylogeny. *Journal of Crustacean Biology* 6: 576-616.
- MCLAUGHLIN P. A. 1983. — Hermit crabs - are they really polyphyletic? *Journal of Crustacean Biology* 3: 608-621.
- MCLAUGHLIN P. A. & LEMAITRE R. 1997. — Carcinization in the Anomura - fact or fiction? I: Evidence from adult morphology. *Contributions to Zoology* 67: 79-123.
- REYNOLDS E. S. 1963. — The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *Journal of Cell Biology* 17: 208-212.
- RICHTER S. & SCHOLTZ G. 1994. — Morphological evidence for a hermit crab ancestry of lithodids (Crustacea, Decapoda, Anomala, Paguroidea). *Zoologischer Anzeiger* 233: 187-210.
- SPURR A. R. 1969. — A low viscosity epoxy-resin embedding medium for electron microscopy. *Journal of Ultrastructure Research* 26: 31-43.
- TALBOT P. & CHANMANON P. 1980. — The structure of sperm from the lobster, *Homarus americanus*. *Journal of Ultrastructure Research* 70: 275-286.
- TUDGE C. C. 1992. — Comparative ultrastructure of hermit crab spermatozoa (Decapoda: Anomura: Paguroidea). *Journal of Crustacean Biology* 12: 397-409.
- TUDGE C. C. 1995a. — *The Ultrastructure and Phylogeny of Anomuran Crab Spermatozoa*. Ph.D. Thesis, Zoology Department, The University of Queensland, Brisbane, Australia, 346 p.
- TUDGE C. C. 1995b. — Ultrastructure and phylogeny of the spermatozoa of the infraorders Thalassinidea and Anomura (Decapoda, Crustacea), in JAMIESON B. G. M., AUSIO J. & JUSTINE J.-L. (eds), *Advances in spermatozoal phylogeny and taxonomy. Mémoires du Muséum national d'Histoire naturelle* 166: 251-263.
- TUDGE C. C. 1997a. — Spermatological evidence supports the taxonomic placement of the Australian endemic Hairy Stone Crab, *Lomis hirta* (Decapoda: Anomura: Lomidae). *Memoirs of the Museum of Victoria* 56: 235-244.
- TUDGE C. C. 1997b. — Phylogeny of the Anomura (Decapoda, Crustacea): Spermatozoa and spermatophore morphological evidence. *Contributions to Zoology* 67: 125-141.
- TUDGE C. C. & JAMIESON B. G. M. 1991. — Ultrastructure of the mature spermatozoon of the coconut crab *Birgus latro* (Coenobitidae: Paguroidea: Decapoda). *Marine Biology* 108: 395-402.

- TUDGE C. C. & JAMIESON B. G. M. 1996a. — Spermatophore and spermatozoal morphology in the Porcellanidae. I: *Aliaporcellana suluensis* and *Pisida longicornis* (Decapoda, Anomura, Porcellanidae). *Crustacean Research* 25: 73-85.
- TUDGE C. C. & JAMIESON B. G. M. 1996b. — Spermatophore and spermatozoal morphology in the Porcellanidae. II: the genera *Petrolisthes* and *Polyonyx* (Decapoda, Anomura, Porcellanidae). *Journal of Crustacean Biology* 16: 535-546.
- TUDGE C. C., JAMIESON B. G. M., SANDBERG L. & ERSÉUS C. 1998a. — Ultrastructure of the mature spermatozoon of the king crab *Lithodes maja* (Lithodidae, Anomura, Decapoda): further confirmation of a lithodid-pagurid relationship. *Invertebrate Biology* 117: 57-66.
- TUDGE C. C., SCHELTINGA D. M. & JAMIESON B. G. M. 1998b. — Spermatozoal ultrastructure in the spiny lobster *Jasus novaehollandiae* Holthuis, 1963 (Palinuridae, Palinura, Decapoda). *Journal of Morphology* 236: 117-126.
- TUDGE C. C., SCHELTINGA D. M. & JAMIESON B. G. M. 1999. — Spermatozoal ultrastructure in the Hippoidea (Anomura, Decapoda). *Journal of Submicroscopic Cytology and Pathology* 31: 1-13.

*Submitted on 11 April 2000;
accepted on 3 October 2000.*