Ultrastructure of the spermatozoon of *Potamonautes perlatus sidneyii* (Heterotremata, Brachyura, Crustacea)

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Received 26 May 1992; accepted 7 August 1992

Features of the spermatozoon of *Potamonautes* which are general for heterotremes and endorse its inclusion in the Heterotremata are: extension of the subacrosomal chamber almost to the anterior apex of the sperm; presence of an acrosome ray zone; and presence of a thickened ring (though here vestigial) where the capsule surrounds the base of the subacrosomal chamber. A feature shared with 'higher' heterotremes is the restriction of cytoplasm to the periacrosomal region, the arms being nuclear only; and loss of a posterior median process, containing chromatin, which is present in raninids and majids. Peculiarities of *Potamonautes* relative to other heterotremes (and paguroid anomurans) are (i) the indistinct substructure of the acrosomal ray zone; (ii) absence of a recognizable inner acrosomal zone differentiated from this, and (iii) reduction of the thickened ring, the latter trend being taken to completion in grapsid Thoracotremata. Unknown in other Brachyura are (iv) a rim-like electron pale expansion around the operculum and (v) the elongate condition of at least one of the two centrioles. These five unusual features are regarded as apomorphies of *Potamonautes*.

Eienskappe van die sperms van *Potamonautes* wat algemeen is vir heterotreme en sy insluiting in die Heterotremata bevestig, is: uitbreiding van die subakrosomale kamer tot bykans die voorste (anterior) apeks van die sperm; teenwoordigheid van 'n akrosomale straalstreek; en die teenwoordigheid van 'n verdikte ring (hoewel vestigiaal) waar die kapsel die basis van die subakrosomaalkamer omring. 'n Eienskap wat gedeel word met 'hoër' heteroteme is die beperking van sitoplasma tot die periakrosomale streek, met die arms slegs kernagtig; en die verlies van 'n agterste (posterior) mediane uitsteeksel wat chromatien bevat en wat teenwoordig is by raninide en majide. Eienaardighede by *Potamonautes* in vergelyking met ander heterotreme (en paguroïede anomuras) is (i) die onduidelike substruktuur van die akrosomale straalstreek, (ii) afwesigheid van 'n herkenbare binneste akrosomale streek wat hiervan gedifferensieer is, en (iii) reduksie van die verdikte ring, laasgenoemde neiging word meer voltooid gevind by die grapside Thoracotremata. Wat onbekend is by ander Brachyura is (iv) 'n rifagtige uitbreiding rondom die operkulum en (v) die verlengde toestand van ten minste een van die twee sentriole. Hierdie vyf ongewone eienskappe word as apomorfieë van *Potamonautes* beskou.

Potamonautes MacLeay, 1838, is a genus of fresh-water crabs with more than a dozen subgenera and about 51 species, limited to Africa. *Potamonautes* was formerly placed, as a subgenus of *Potamon* Savigny, 1816, in the family Potamonidae Ortmann, 1896, in the subfamily Potamoninae. Bott (1955) raised *Potamonautes* to generic rank while retaining it in this subfamily. Guinot (1978: 214) placed *Potamonautes* in the family Potamidae s. lat. within the Heterotremata. Abele & Felgenhauer (1982) recognize the family Potamonautidae for this genus.

The ultrastructure of the spermatozoon has been shown to be of value in determining relationships within the Brachyura (Jamieson 1991). The discoidal acrosome and reduced arms of dromiid (*Dromidia, Petalomera)* sperm confirm dromiids as a group with no close relationship to other brachyurans. Phylogenetic heterogeneity of the Podotremata (*sensu* Guinot 1977, 1978) is supported by differences between dromiid and raninoid sperm and similarities (postnuclear tail) between *Ranina* and majids. The conventional oxystomate-oxyrhynch-cancrid-brachyrhynch subdivision of the Brachyura is not supported by sperm ultrastructure. As an example, dorippids and portunids, with similar sperm, are placeable in the Heterotremata of Guinot (1977, 1978), whereas the former classification (see Warner 1977) separates the two families in the Oxystomata and Brachyrhyncha, respectively. Familial characteristics of sperm are exemplified by the distinctive 'xanthid ring' basal around the perforatorium of xanthids. Thoracotremata of Guinot (1977, 1978) (exemplified by Mictyroidea, Grapsoidea and Ocypodoidea) are characterized, though with some exceptions, by presence of an apical opercular button, concentric lamination of the outer acrosome zone and modification of the xanthid ring (Jamieson 1991).

The present study examines the ultrastructure of the spermatozoa of the heterotreme *Potamonautes* for its interest *per se* and with a view to providing evidence of the relationships of the genus within the heterotreme-thoracotreme assemblage.

Materials and Methods

Specimens of *Potamonautes perlatus sidneyii* were kindly collected by Mr. Martin Hill from the vicinity of Grahamstown, South Africa, in July 1990. Small portions of testes were fixed in 3% glutaraldehyde in 0,1 mol.dm^{-3} phosphate buffer (pH 7,4), with 6% sucrose, at 4°C, washed in buffer, post-fixed for 80 min in similarly buffered 1% osmium tetroxide, washed in buffer, dehydrated through an ascending ethanol series, and infiltrated and embedded in Spurr's epoxyresin. Thin sections were cut with a diamond knife on an LKB 2128 UM IV ultratome, collected on carbon stabilized colloidin-coated 200 mesh copper grids, and stained by the Daddow method: for 0,5 min in Reynolds' lead citrate, 1 min in 6% aqueous uranyl acetate and a further 0,5 min in lead citrate, before rinsing in distilled water. Specimens were examined with a Hitachi 300 and a Jeol 100S transmission electron microscope operated at 80kV and 60kV respectively.

Results

To minimize repetition of data from *Potamonautes* in the Discussion, some comparison with archaeobrachyuran and other heterotreme crabs will be made in the results. Comparison with thoracotremes will chiefly be reserved to the Discussion.

General morphology

The spermatozoon of *Potamonautes perlatus sidneyii* is illustrated from transmission electron microscopy in a line drawing (Figure 1) and in micrographs (Figures 2 and 3).

Each of the many spermatophores in the testes of *Potamonautes perlatus sidneyii* typically contains a single spermatozoon. The spermatozoon (refer to line drawing, Figure 1, throughout) is spheroidal but slightly depressed anteroposteriorly and, like all decapod sperm, lacks a flagellum (Figure 2A). This simple form is modified by the presence of broadly based lateral projections or 'arms'. A plesiomorphic, chromatin-containing 'posterior median process', seen in *Ranina* and some majids, is absent. As is usual for brachyurans, the nucleus consists of diffuse, fibrous chromatin. and forms a cup surrounding the acrosome, a thin layer of cytoplasm intervening between nucleus and acrosome. The cytoplasm contains sparse mitochondria (Figure 3B), some convoluted membranous (lamellar) structures of probable mitochondrial origin (Figure 2A) and, basal to the perforatorium, the two centrioles (Figures 2C–E).

Acrosome

The subspheroidal core of the *Potamonautes perlatus* spermatozoon consists entirely of the complex acrosome (Figures 2A, 3A). This is composed of a number of components which seem identifiable with, and are presumably homologous with, those described for other higher brachyuran sperm (see review by Jamieson 1991).

The acrosome (Figures 2A, 3A) is invested by an acrosomal membrane underlain by an electron dense sheath, the 'capsule'. The length of the acrosome, from the apex of the operculum to the base of the capsule, is 2,7–3,0 µm (mean of 5 = 2,8 µm); the width is 2,9–3,2 µm (mean of 5 = 3,1 µm). The acrosomal membrane is separated by a very thin layer from the capsule and, like the capsule, is invaginated to cover a columnar subacrosomal chamber (Figure 2A) the contents of which are the perforatorium. The anterior tip of this chamber extends almost to the anterior apex of the sperm. As in other crabs, this central, subacrosomal axis of the acrosome is surrounded by a wide electron dense sheath, but here no inner acrosomal zone can be distinguished with certainty from the acrosome ray zone (Figures 2A, 3A). The ray zone is wide and outwardly convex but, though large, is identified with difficulty as substructuring to give the microtubule-like rays (optimally exemplified by the anomuran *Birgus*) is indistinct. In the absence of a distinct inner acrosomal zone, axial relative to the ray zone, an electron dense prominent annulus, the 'xanthid ring' seen in xanthids is absent (see Jamieson 1991).

As in other heterotremes, the inner zone of the acrosome (here the putative acrosome ray zone) is surrounded by a broad, inwardly concave layer, the outer acrosomal zone (Figures 2A, 3A). In *Potamonautes perlatus* as in heterotremes excepting xanthids this zone is the only additional concentric layer and continues to the capsule.

At the anterior pole of the *Potamonautes perlatus* acrosome, as in all other brachyurans and paguroids with the doubtful exception of *Pagurus bernhardus* (see Chevaillier

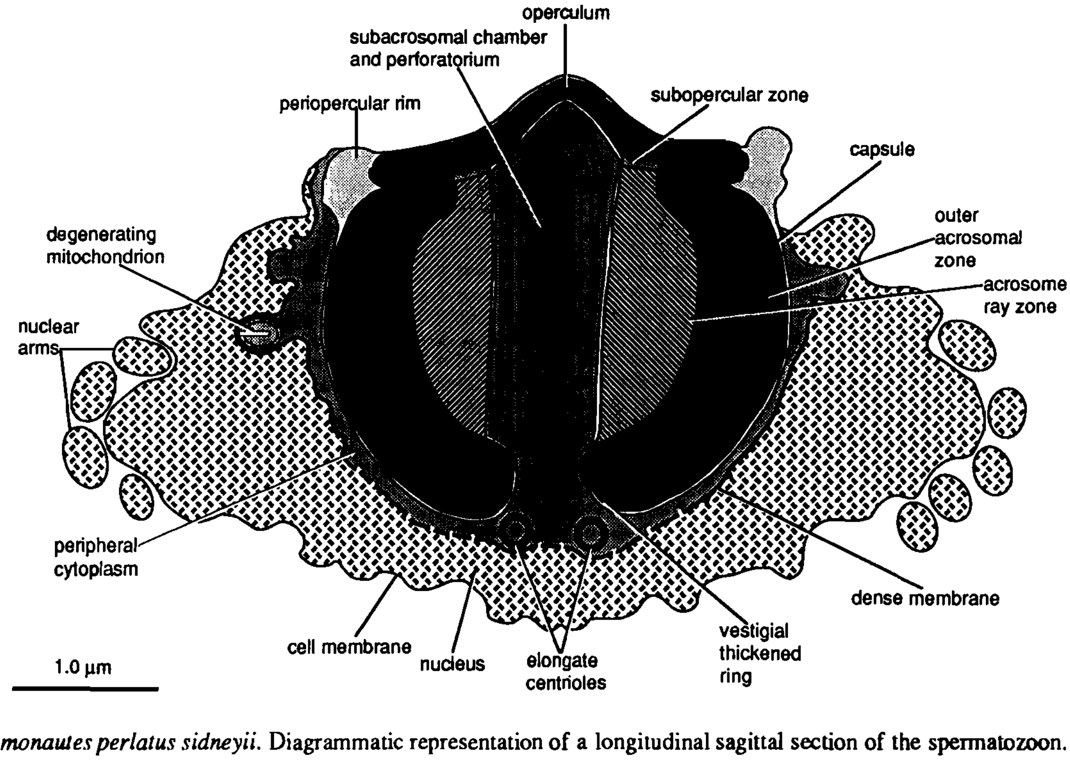

Figure 1 *Potamonautes perlatus sidneyii.* Diagrammatic representation of a longitudinal sagittal section of the spermatozoon.

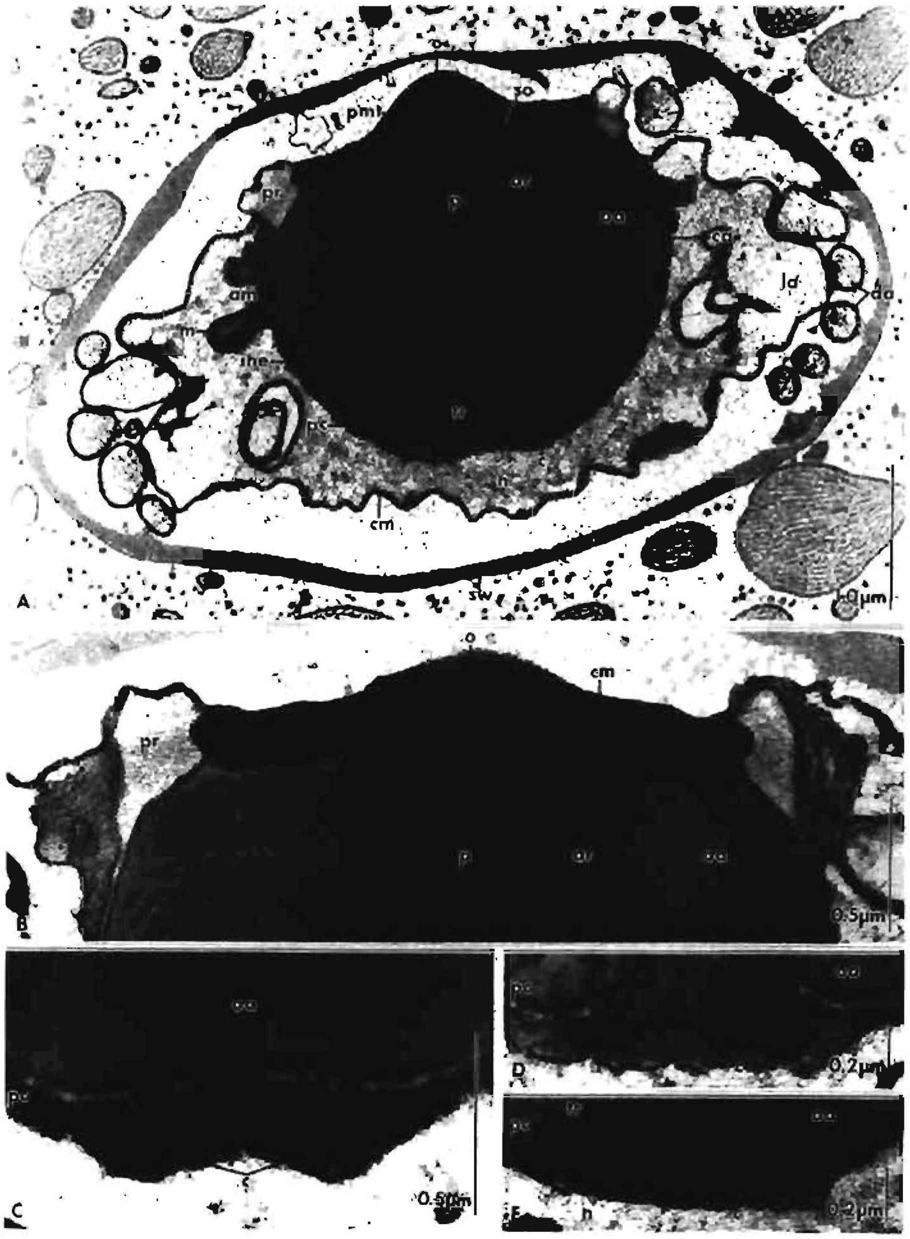

Figure 2 *Potamonautes perlatus sidneyi*. Transmission electron micrographs of the spermatozoon. A. Longitudinal sagittal section of spermatozoon in its spermatophore. B. Detail of the operculum and periopercular rim. C. Longitudinal sagittal section showing the two centrioles in cross section. D and E. Longitudinal sections at right angles to C, showing a centriole in longitudinal section. Note centriolar elongation which is unknown in other brachyurans. Abbreviations: am = acrosome membrane; ar = acrosome ray zone; c = centrioles; ca = capsule; cm = cell membrane; da = divarications of arms; ine = dense inner nuclear envelope; la = base of lateral arm; m = degenerating mitochondrion; n = nucleus; o = operculum; oa = outer acrosomal zone; p = perforatorium; pc = vestigial periacrosomal cytoplasm; pmt = microtubules of perforatorium; pr = periopercular rim; so = subopercular zone; sw = wall of spermatophore; tr = thickened ring.

1968), there is a dense caplike structure, the operculum (Figure 2A, B), which here has a width of 2,1–2,5 μm (mean of 5 = 2,3 μm). As in *Portunus*, the operculum is imperforate whereas in *Ranina* and the majid *Menaetheus monoceros* it is perforate (see Jamieson 1991) (it is also perforate but is closed by an apical button in most of the investigated thoracotremes, see Discussion).

A subopercular zone is not identifiable with certainty. A lower tier to the operculum is here regarded as opercular rather than subopercular. An insignificant differentiation below the operculum and around the anterior end of the subacrosomal chamber (Figures 1, 2A, B) is tentatively interpreted as the subopercular zone but nothing as pronounced as that in portunids exists. In *Potamonautes perlatus*, as in portunids and thoracotremes, the operculum and subopercular zone extend far laterally, covering much of the outer acrosomal zone. In contrast the operculum is limited in *Calappa* to the width of the ray zone (see Jamieson 1991).

At the opposite, posterior, pole the capsule is perforated by invagination of the acrosome membrane and capsule as a narrow orifice which opens into the columnar subacrosomal chamber. A thickening of the capsule which forms a 'thickened ring' on each side of the subacrosomal invagination in other heterotremes is poorly if at all developed, a reduction taken to completion in grapsid thoracotremes (Figure 2A).

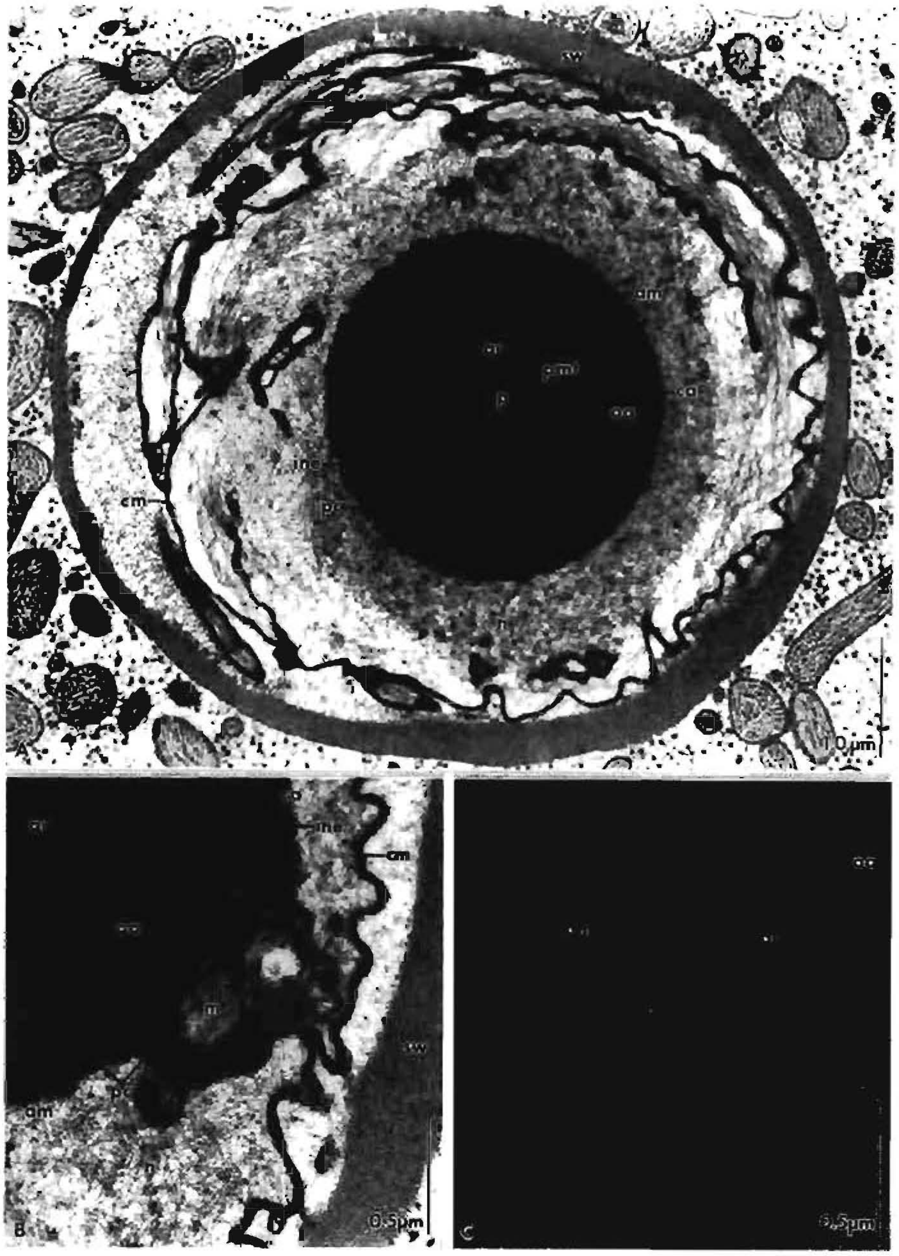

Figure 3 *Potamonautes perlatus sidneyii*. Transmission electron micrographs of the spermatozoon. A. Cross section shortly below the equator of the acrosome. B. Detail of the cytoplasmic dilatation, showing degenerating mitochondria. C. Transverse section of the perforatorium, in the subacrosomal chamber, showing the circlet of microtubules. Abbreviations: am = acrosome membrane; ar = acrosome ray zone; ca = capsule; cm = cell membrane; ine = dense inner nuclear envelope; m = degenerating mitochondrion; oa = outer acrosomal zone; p = perforatorium; pc = vestigial periacrosomal cytoplasm; pmt = microtubules of perforatorium; sw = wall of spermatophore.

Periopercular rim

A unique feature of the spermatozoon of *Potamonautes perlatus* is the presence of an electron pale rim encircling the periphery of the operculum and its underlying subopercular zone (Figures 2A, B). This rim is an expansion of the hyaline layer, usually considered to be the acrosomal membrane, which overlies the capsule. The rim extends for a considerable distance down the periphery of the outer acrosomal zone. In most longitudinal sections (Left side of Figure 1, 2B) it is seen to be flanked by an expansion of the periacrosomal cytoplasm.

Subacrosomal region

In *Potamonautes perlatus*, as in other heterotreme (and thoracotreme) Brachyura the subacrosomal material consists of a highly differentiated stout perforatorium which extends from the posterior perforation in the capsule to the vicinity of the operculum at the apex of the acrosome (Figure 2A, 3A). The perforatorium in *Potamonautes* is a stout cylinder, parallel-sided or slightly widening anteriad, with a broad pointed tip and a posterior stalk constricted at the thickened ring. In cross section, the perforatorium is seen to contain, as in xanthids and portunids (Jamieson 1991) many circular profiles resembling microtubules. It is peculiar, however, in distribution of these microtubules in a circlet, often several microtubules thick, around an electron pale core (Figure 3C).

Cytoplasm

A thin layer of spermatozoal cytoplasm surrounds the acrosomal capsule (Figures 2A, 3A). As in portunids, dorippids, trapeziids, and xanthids (Jamieson 1991, 1992), the cytoplasm does not extend into the arms. In majids, in contrast, the cytoplasm extends into the lateral arms together with contained microtubules which are not discernible in the other heterotremes mentioned. In heterotremes and thoracotremes the cytoplasm is enlarged anterolaterally and asymmetrically peripheral to the capsule and contains degenerating mitochondria but in *Potamonautes* the cytoplasm is even more reduced in amount than is usual (Figure 2A). A few degenerating mitochondria are nevertheless present (Figures 2A, 3B). The postacrosomal cytoplasm typical of brachyurans is minimal in *Potamonautes*, being virtually restricted to that surrounding the two, parallel centrioles (Figure 2A). Occurrence of centrioles is a plesiomorphic condition seen in majids, parthenopids, calappids, portunids, dorippids, trapeziids and *Macrophthalmus* but absent in xanthids and most thoracotremes (see Jamieson 1991). However, *Potamonautes* is unique in elongation of the centrioles (observable in only one centriole in each thin section) (Figures 2D, E). The two centrioles are mutually parallel (Figure 2C).

Convoluted membranes forming a lamellar structure, typical of eubrachyuran sperm, are represented in *Potamonautes* only by convolutions of the dense membrane (see below) around dilatations of the periacrosomal cytoplasm (Figure 2A).

Nucleus

In *Potamonautes*, as in other brachyurans, the nuclear material is located in the lateral arms and their branches and in the cup-shaped structure around both the acrosome and its cytoplasmic sheath (Figures 2A, 3A). The chromatin is separated from the operculum by the unique peri-opercular rim (Figures 2A, B). Here, as in portunids and xanthids, the envelope (here termed the dense membrane) between the chromatin and the basal cytoplasm is not recognizably multilayered, though clearly developed and electron dense, and shows frequent interruptions (Figures 2A, 3A). In contrast in some eubrachyurans, for instance dorippids, and trapeziids it forms a hexalaminar or multilaminar membrane. Unlike other eubrachyurans, in which the nuclear membrane ends anteriorly at the lamellar structures, the dense membrane in *Potamonautes* extends far anteriorly, flanking cytoplasm which borders the peri-opercular rim (Figure 2A). The external, basal surface of the cell is bounded by a dense membrane which may represent fused nuclear and plasma membranes, here termed the cell membrane (Figure 2A, 3A). The general chromatin consists of electron dense filaments in a pale matrix as in other brachyurans (see Discussion).

Discussion

Features of the spermatozoon of *Potamonautes perlatus* which are general for heterotremes are: extension of the subacrosomal chamber almost to the anterior apex of the sperm whereas it reaches only to the approximate equator of the acrosome in the archaeobrachyuran *Ranina*; presence of an acrosomal ray zone (albeit with indistinct substructure) flanked by an outer acrosomal zone; presence of a thickened ring (though weakly developed in *Potamonautes*) where the capsule surrounds the base of the subacrosomal chamber, and absence of the apical button which occurs above a central perforation of the operculum in most investigated thoracotremes. A feature shared with 'higher' heterotremes is the restriction of cytoplasm to the periacrosomal region; unlike majids, it does not extend into the arms. Peculiarities of *Potamonautes* relative to other heterotremes (and paguroid anomurans) are (i) the indistinct substructure of the acrosomal ray zone; (ii) absence of a recognizable inner acrosomal zone differentiated from this, and (iii) reduction of the thickened ring, the latter trend being taken to completion in grapsid Thoracotremata (see Jamieson 1991). Unknown in other Brachyura are (iv) a rim-like electron pale expansion around the operculum and (v) the elongate condition of at least one of the two centrioles. These five unusual features are regarded as apomorphies of *Potamonautes* and are consistent with morphological specializations, including major specializations of genital pleopods. The sperm of *Potamonautes perlatus* thus confirms its heterotreme status and endorses separation from thoracotremes though showing a (homoplasic?) approach to some thoracotremes (reduction of the thickened ring) on the basis of the arrangement of the genital pores (Guinot 1977, 1978).

Acknowledgements

I am deeply grateful to Dr Danièle Guinot for providing information on the taxonomic status of *Potamonautes*. I am also indebted to Mrs L. Daddow, Mr C. Tudge and Mr D. Scheltinga for technical assistance, and further to Mr Tudge for commenting on the manuscript. Dr A.N. Hodgson and Dr Robin Cross kindly provided facilities in the Zoology Department and Electron microscope Unit, Rhodes University. The computer-generated line drawing is by the author. This work was made possible by support from the Department of Zoology and Entomology, Rhodes University, and Australian Research Council funding.

References

ABELE, L.G. & FELGENHAUER, B.E. 1982. Eucarida. In: Synopsis and Classification of Living Organisms, pp.296–326. (Ed.) S.B. Parker. McGraw-Hill Book Co. Inc., New York.

BOTT, R. 1955. Die Süsswasserkrabben von Afrika (Crust. Decap.) und ihre Stammesgeschichte. *Annls Mus. r. Congo belge* C-Zool., ser. 3 1(3): 209–349.

CHEVAILLIER, P. 1968. Étude cytochimique ultrastructurale des nucléoprotéines dans le spermatozoide du pagure *Eupagurus bernhardus* L. *J. microsc. (Paris)* 7: 107–114.

GUINOT, D. 1977. Propositions pour une nouvelle classification des crustacés décapodes brachyoures. *C.R. hebd. Séanc. Acad. Sci.*, Paris D285: 1049–1052.

GUINOT, D. 1978. Principes d'une classification évolutive des crustacés décapodes brachyoures. *Bull. biol, Fr Belg*. 112: 211–292.

JAMIESON, B.G.M. 1991. Ultrastructure and phylogeny of crustacean spermatozoa. *Mem. Qd Mus*. 31: 109–142.

JAMIESON, B.G.M. 1992. Spermatological evidence for the taxonomic status of *Trapezia* (Heterotremata, Brachyura, Crustacea). *Mem. Qd Mus*. (In press).

ORTMANN, A.E. 1896. Carcinologische Studien. *Zool. Jahrb. Syst*. 10: 358–372.

WARNER, G.F. 1977. The biology of crabs. Elek Science, London.