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An Ultrastructural Study of Spermatozoa of the Majidae with Particular Reference to the Aberrant Spermatozoon of *Macropodia longirostris* (Crustacea, Decapoda, Brachyura)

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Abstract

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A total of 17 species, in 14 genera of majids have been examined for sperm ultrastructure. The present account describes the sperm of six of these species, in two subfamilies: Pisinae—*Sphenocarcinus orbiculatus* and *Sphenocarcinus stuckiae* and Inachinae—*Cyrtomaia furici*, *Grypacheus hyalinus*, *Platymaia rebierei* and *Macropodia longirostris*. *M. longirostris* has the only eubrachyuran sperm in which the acrosome is known to depart radically from a subspheroidal form. The acrosome is semilunar in shape and is bordered by a very thin layer of cytoplasm and an unusually uniform, narrow band of chromatin. The apical surface of the acrosome is almost flat, though slightly concave, whereas the posterior surface forms a hemisphere, and is almost completely occupied by the thin, centrally perforate, electron dense operculum. The bulk of the acrosome consists of a homogeneous, moderately electron dense outer acrosome zone. This surrounds a small inner acrosome zone internal to which is an ellipsoidal, pale perforatorium capped by a central acrosome zone. Majid sperm are distinguished by a flattened and/or centrally depressed operculum; a further characteristic is that the pointed perforatorium is relatively short and frequently does not reach the operculum. They vary *inter alia* with regard to presence or absence of a posterior median process and, apparently, of centrioles and of microtubules in the nuclear arms, and in the number of these arms. Perforation of the operculum, seen in the Pisinae, is not constant in the Inachinae. Spermatozoal ultrastructure offers no certain support for a close relationship of majids with parthenopids or hymenosomatids.

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Introduction

With the exception of the Xanthidae, the Majidae *sensu lato* is the most speciose brachyuran family, containing some 800 species assigned to more than 150 genera, the number of subfamilies varying greatly between different classifications. Majids, with parthenopids, constitute the Oxyrhyncha in the commonly used classification summarized by Warner (1977). Both families, together with the Xanthidae and several other families, belong to the Heterotremata in the classification of Guinot (1977, 1978). Guinot (1978) recognized the superfamily Majoidea Samouelle, 1819, with unspecified families.

Within the Majidae Samouelle, 1819, the number of subdivisions recognized has reached 50 (for a list see Manning & Holthuis 1981). However, Griffin and Tranter (1986) recognize only seven subfamilies for the Indo-Pacific as did Garth (1958) for the majids of the Pacific coast of America. For these authors, five subfamilies receive the same names: Oregoniinae, Inachinae, Pisinae, Majinae and Mithracinae, whereas two are named differently: the Tychinae in Griffin and Tranter are the Ophthalmiinae in Garth; and the Epialtinae in Griffin and Tranter are the Acanthonychinae in Garth.

From larval morphology, Ingle (1979) considers six subfamilies of Majidae: Oregoniinae, Acanthonychinae, Inachinae, Pisinae, Majinae and Ophthalmiinae, and he divides the Inachinae into two groups. For Rice (1988), the morphology of the megalopa supports conclusions based on zoeae and on adults and indicates monophyly of the majids in which, for instance, the inachines combine very primitive and advanced traits. On the basis of zoeal morphology, Clark and Webber (1991) do not distinguish more than four large families in the Majoidea: Oregoniidae, Macrocheiridae, Majidae and Inachidae, without indication of the subfamilies included in each.

Spermatozoal ultrastructure has been shown to provide characters of significance for phylogenetic analysis of the Brachyura (Jamieson 1994*b*). In the present study we describe the sperm of six species of majids for their interest *per se* and for the light which they may shed on majid interrelationships and taxonomy.

Prior to the present study, 11 species in nine genera of

majids had been examined for sperm ultrastructure. This study brings the number to 17 species, in 14 genera (see Table 1).

Material and methods

Material

The following new material is the subject of this paper:

Inachinae: *Cyrtomaia furici* Richer de Forges and Guinot, 1988, *Grypacheus hyalinus* (Alcock & Anderson, 1894) and *Platymaia rebierei* Guinot and Richer de Forges, 1985, from New Caledonia, collector B. Richer de Forges; *Macropodia longirostris* (Fabricius, 1775), from Banyuls-sur-Mer, France, collector Dr. C. C. Tudge.

Pisinae: *Sphenocarcinus orbiculatus* Guinot and Richer de Forges, 1985, and *Sphenocarcinus stuckiae* Guinot and Richer de Forges, 1985, from New Caledonia, collector B. Richer de Forges.

Histological procedures

The male reproductive material (usually both testes including the ducts of the vasa deferentia) was removed from fresh crab specimens and immediately fixed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH = 7.2) for a minimum of 2 h at 4°C, then posted to Brisbane at ambient temperature where the remainder of the fixation and embedding process for transmission electron microscopy was carried out.

The glutaraldehyde fixed gonad tissue was processed in the Zoology Department, The University of Queensland, by the standard fixation procedure (outlined below) for transmission electron microscopy. This was carried out in a Lynx -el. Microscopy Tissue Processor (Australian Biomedical Corporation, Mount Waverley, Victoria, Australia).

Portions of the testes (approximately 1 mm^3) were rinsed in phosphate buffer (three rinses, each of 15 min), postfixed in phosphate buffered 1% osmium tetroxide for 80 min; similarly rinsed in buffer and dehydrated through ascending concentrations of ethanol (20–100%). After being infiltrated and embedded in Spurr's epoxy resin (Spurr 1969), thin sections (500–800 Å 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 (according to Daddow 1986) in the following sequence: 30 s in Reynold's lead citrate, rinsed in distilled water, 1 min in 6% aqueous uranyl acetate, rinsed in distilled water, 30 s in Reynold's lead citrate, and a final rinse in distilled water. Micrographs were taken on an Hitachi H-300 transmission electron microscope at 80 kV and a JEOL 100-S transmission electron microscope at 60 kV. Light microscopic observations of glutaraldehyde fixed spermatozoa were made under Nomarski contrast using an Olympus BH2 microscope. Micrographs were taken with an Olympus OM-2 camera.

Results

This section details observations for the newly examined species. A comparison with other species is given in the Discussion.

For a comparative account and explanation of the various components of the brachyuran spermatozoon see previous work by Jamieson (Jamieson 1991*a,b*, 1994*a,b*; Jamieson *et al.* 1995). A diagram of its chief components is given by Jamieson (1994*b*).

General morphology

Each of the many spermatophores in the testes of *Sphenocarcinus orbiculatus* and *S. stuckiae* contain one to a maximum of five spermatozoa, while the spermatophores of *Cyrtomaia furici* and *Platymaia rebierei* each contain many spermatozoa. The spermatophores of *Macropodia longirostris* and *Grypacheus hyalinus* were not intact. With the exception of the semilunar form of the acrosome in *M. longirostris*, and features correlated with this, the spermatozoa (Figs 1–8) are typically brachyuran in gross morphology. An acrosome vesicle forms most of the volume of the spermatozoon. The acrosome is concentrically zoned but lacks the concentric lamellation seen in thoracotremes; it is capped apically by a dense operculum. The acrosome vesicle is centrally penetrated by a cylindrical perforatorial column. The nuclear material forms marginal projections or "arms". The spherical, or (excepting *M. longirostris*) only slightly depressed, form of the acrosome is typical of the Eubrachyura (Heterotremata + Thoracotremata). A chromatin-containing "posterior median process" of the nucleus is absent from the species examined here.

As in other brachyurans, the nucleus consists of uncondensed, fibrous chromatin which cups the acrosome and a thin layer of cytoplasm invests the acrosome and intervenes between it and the nucleus. In the cytoplasm at the posterior end of the perforatorial chamber centrioles may be visible. Failure to demonstrate centrioles does not necessarily exclude their presence. Cytoplasmic islets are usually recognizable lateral to the acrosome and embedded in the

Table 1. *Ultrastructural descriptions of spermatozoa of the family Majidae*

Majinae	*Maja squinado*	Tudge and Justine 1994
Oregoniinae	*Chionoecetes opilio*	Beninger *et al.* 1988; Chiba *et al.* 1992
Inachinae	*Cyrtomaia furici*	This study
	Grypacheus hyalinus	This study
	Macropodia longirostris	This study
	Platymaia rebierei	This study
	Podochela riisei	Hinsch 1973
	Podochela gracilipes	Hinsch 1973
	Stenorhynchus seticornis	Hinsch 1973
Pisinae	*Libinia dubia*	Hinsch 1973
	Libinia emarginata	Hinsch 1969, 1971, 1973, 1986; Vaughn and Hinsch 1972; Hernandez *et al.* 1989; Perez *et al.* 1986
	Sphenocarcinus orbiculatus	This study
	Sphenocarcinus stuckiae	This study
Mithracinae	*Macrocoeloma trispinosum*	Hinsch 1973
	Mithrax sp.	Hinsch 1973
Acanthonychinae (Epialtinae)	*Menaethius monoceros*	Jamieson 1991*a,b*, 1994*a,b*; Jamieson *et al.* 1995
Ophthalmiinae (Tychinae)	*Pitho lherminieri*	Hinsch 1973

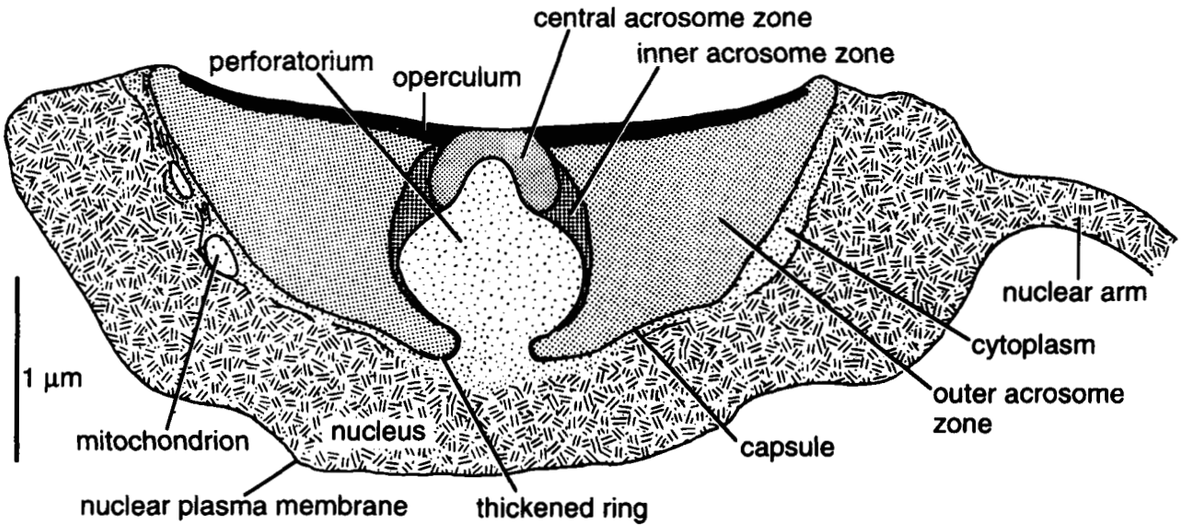


Fig. 1. Macropodia longirostris. Semi-diagrammatic longitudinal section of a spermatozoa, traced from a transmission electron micrograph.

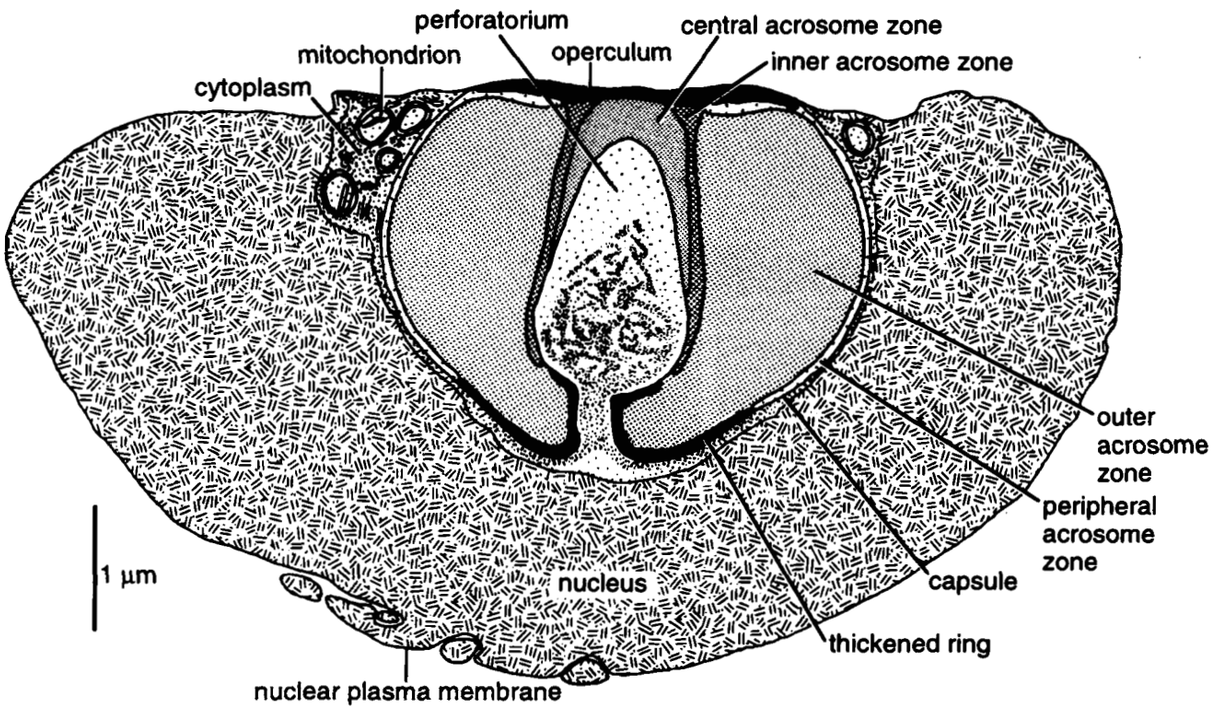


Fig. 2. Cyrtomaia furici. Semi-diagrammatic longitudinal section of a spermatozoa, traced from a transmission electron micrograph.

chromatin; they contain lamellae and bodies identifiable by homology with other crabs as degenerating mitochondria.

The sperm of *M. longirostris* is the only known eubrachyuran sperm in which the acrosome departs radically from a subspheroidal form. Its acrosome is semilunar in shape (Figs 1, 4A,E–G).

Acrosome

The acrosome of *Macropodia longirostris* will chiefly be described after those of subspheroidal form. The core of these spermatozoa consists entirely of the concentrically zoned subspheroidal acrosome which is capped by, and includes, the opercular complex (Figs 2, 3, 5A, 6A,E, 7A, 8). The acrosome is invested by an acrosomal membrane underlain by a moderately electron dense sheath, the "capsule" (Figs 1–3, 4G, 5A, 6A,E, 7A, 8). The dimensions of some acrosomal components in the investigated majids are given in Table 2.

At the posterior pole of the acrosome the capsule is interrupted, as in all brachyurans, by invagination of the acrosome membrane and capsule as an orifice which opens into the columnar subacrosomal (perforatorial) chamber (Figs 1–

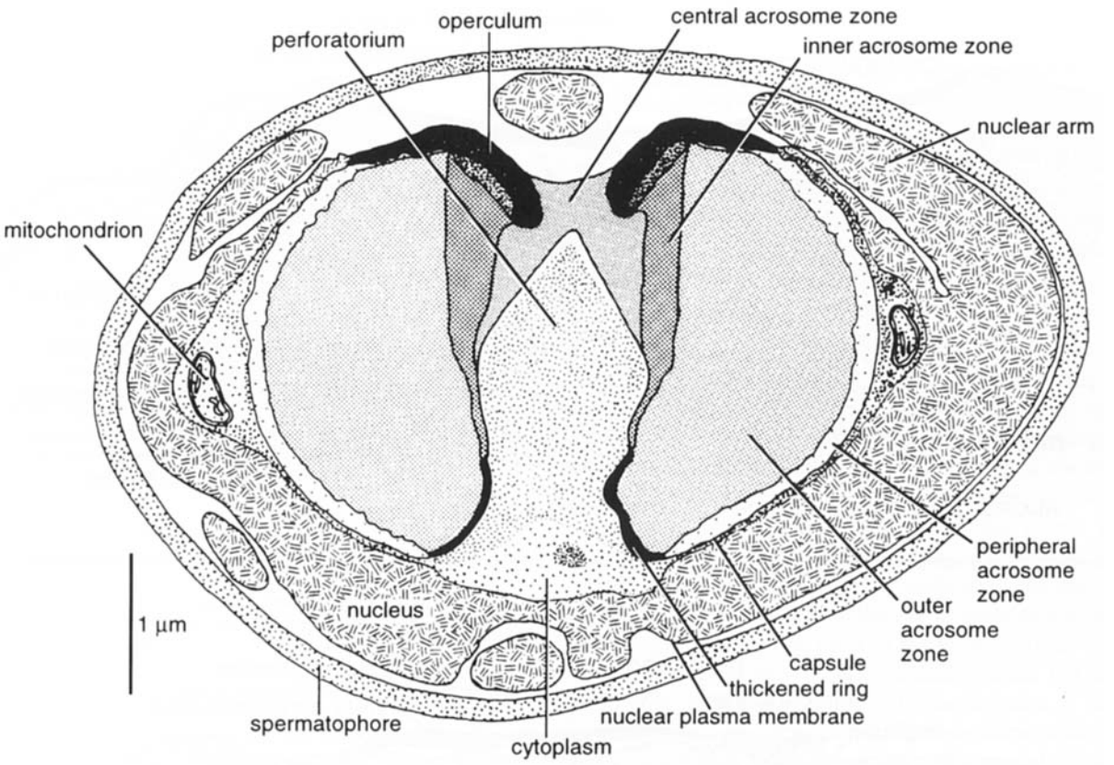


Fig. 3. Sphenocarcinus orbiculatus. Semi-diagrammatic longitudinal section of a spermatozoa, traced from a transmission electron micrograph.

3, 4A,E,F, 5A, 6A,D,G, 7A, 8). A "thickened ring" which is visible at the base of subacrosomal invagination in most heterotremes and many thoracotremes (see Discussion) is present (Figs 1–3, 4A,D, 5A,C,E, 6A,D,E, 7A,F, 8). The thickened ring is well developed, compared with other Brachyura, in *Cyrtomaia furici* (Fig. 5A), *Platymaia rebierei* (Figs 6A,D), *Sphenocarcinus orbiculatus* (Fig. 8) and *S. stuckiae* (Figs 7A,F), has "normal" development in *Grypacheus hyalinus* (Fig. 6E) but is only weakly developed in *Macropodia longirostris* (Figs 4A,D).

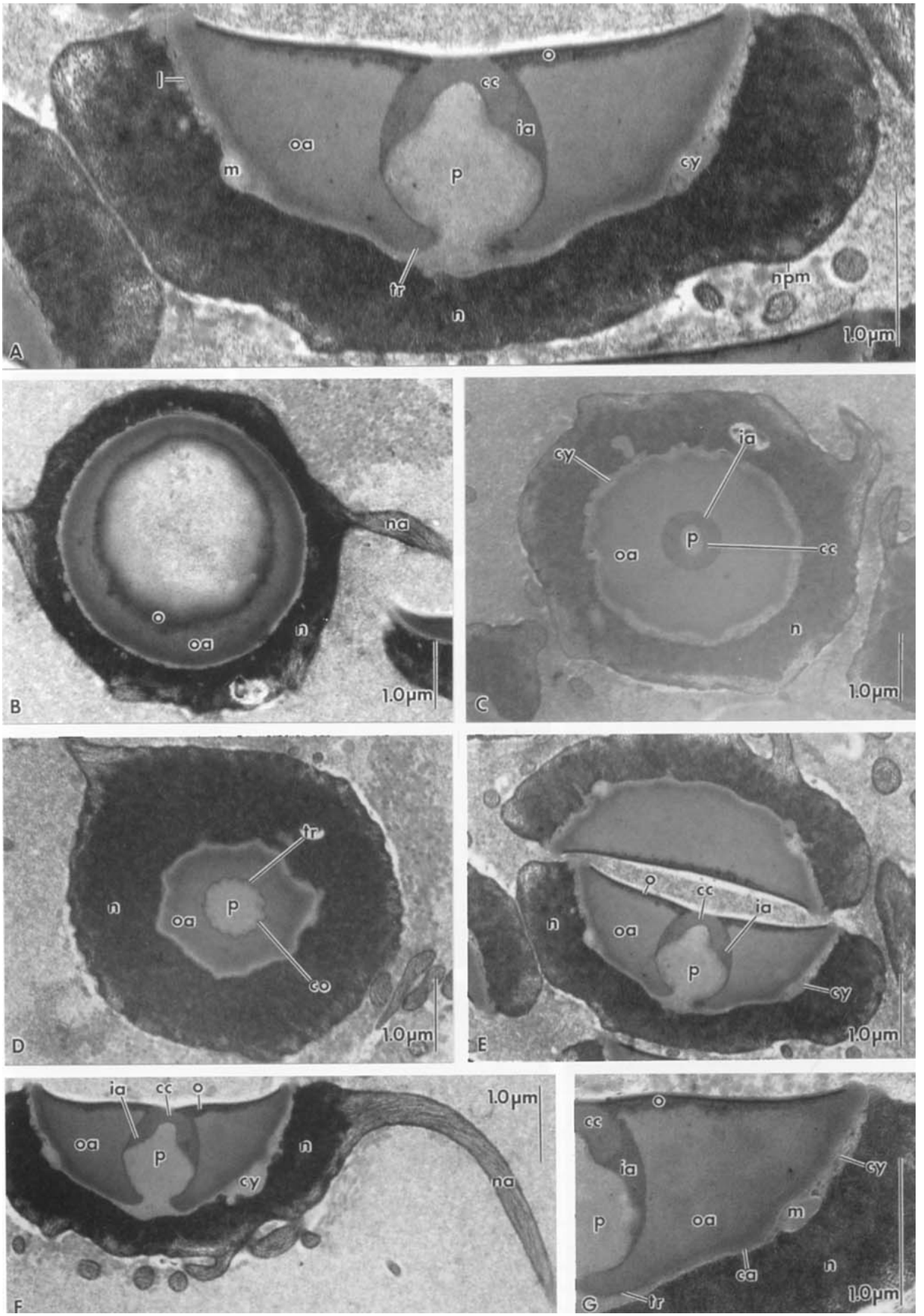
The central subacrosomal axis of the acrosome is formed by the perforatorial chamber, the contents of which constitute the perforatorium. A frequent feature of majid sperm is the pointed, somewhat squat shape of the perforatorium and, more significantly, the fact that its apex often does not extend to the anteriormost limit of the operculum. The perforatorial chamber is widest just posterior to its equator and is circular in transverse section for most of its length. Near its base the chamber is constricted by several-to-many inward projections or folds of its wall and of the adjacent acrosome vesicle, forming longitudinal corrugations: four in *Cyrtomaia furici* (Fig. 5E), five in *Sphenocarcinus stuckiae* (Fig. 7F) or many in *Macropodia longirostris* (Fig. 4D); the arrangement being undetermined in *Platymaia rebierei*, *Sphenocarcinus orbiculatus* and *Grypacheus hyalinus*.

The axial acrosomal material between the perforatorium and the operculum or the central perforation of the latter is here termed the central acrosome zone (Figs 1–3, 4A,C,E–G, 5A,F,G,I, 6A,C,E, 7A,C,D, 8). The perforatorium and the central acrosome zone are surrounded by a moderately electron dense layer, the inner acrosome zone (Figs 1–3, 4A,C,E–G, 5A,F–H,I, 6A,C,F, 7A,C–E, 8) which extends from the operculum almost to the posterior end of the acrosome, reaching the thickened ring. No acrosome ray zone, seen in many heterotreme sperm, is recognizable. There is no xanthid ring or modification of this.

An outer acrosome zone (Figs 1–3, 4A–G, 5A–C,E–I, 6A,C–F, 7A,C–F, 8) surrounds the inner acrosome zone and the base of the perforatorial chamber, being several times wider than the inner zone. It is uniform in structure and moderately electron dense, though paler than the inner acrosome zone. This outer zone extends to the convex margin of the acrosome, being bounded by the capsule in *Macropodia longirostris* (Figs 1, 4A–G), but is surrounded by a further, pale peripheral acrosome zone in *Cyrtomaia furici*, *Grypacheus hyalinus*, *Platymaia rebierei*, *Sphenocarcinus orbiculatus* and *S. stuckiae* (Figs 2, 3, 5A,F–H, 6A,C–F, 7A,C–F, 8).

The operculum is flattened (not domed as in other Brachyura) in all species, with or without a central depression. It is perforate in *Sphenocarcinus orbiculatus* (Figs 3, 8), *S. stuckiae* (Fig. 7A), *Grypacheus hyalinus* (Fig. 6E) and *Macropodia longirostris* (Figs 1, 4A,E–G) but appears to be imperforate in *Cyrtomaia furici* (Figs 2, 5A)

Fig. 4. Macropodia longirostris.—*A–G.* Transmission electron micrographs of spermatozoa.—*A.* Longitudinal section (LS) showing its semilunar shape.—*B–D.* Transverse sections (TSs) through the acrosome vesicle (*B*) at the level of the operculum, (*C*) the anterior portion of the perforatorium, and (*D*) the base of the perforatorium showing the corrugated perforatorial wall.—*E.* LS of two "conjugated" spermatozoa.—*F.* LS showing a nuclear arm.—*G.* LS showing cytoplasm.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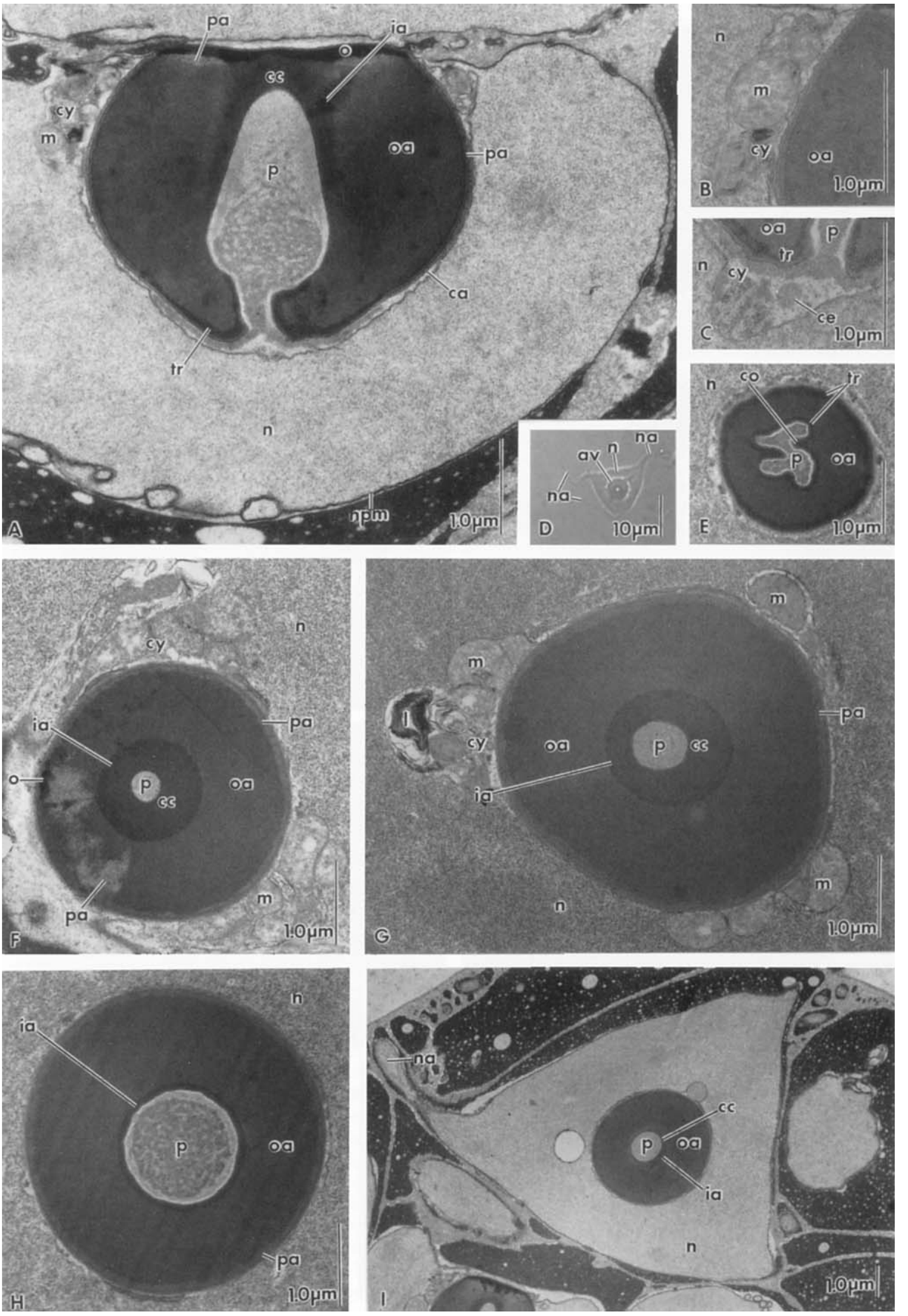
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and *Platymaia rebierei* (Figs 6A,C). The perforate operculum of *Sphenocarcinus orbiculatus* (Figs 3, 8) and *S. stuckiae* (Fig. 7A) is centrally depressed and penetrates into the central acrosome zone. That of *Grypacheus hyalinus* (Fig. 6E) is also centrally depressed but does not penetrate the central zone. Dimensions of the opercula are given in Table 2.

No accessory ring present lateral to the operculum in xanthoids and, differently orientated, in thoracotremes, is present in majids, nor is there an "opercular overhang". There is no trace of a periopercular rim in any of the examined majid species.

The acrosome of *Macropodia longirostris* is semilunar in shape and is bordered by a very thin layer of cytoplasm and an unusually uniform band of chromatin which is a little more than half the thickness of the acrosome (Figs 1, 4A–G). The apical surface of the acrosome, is almost flat, though slightly concave, whereas the posterior surface forms a hemisphere. The thin, centrally perforate, electron dense operculum almost completely occupies the anterior surface. The bulk of the acrosome consists of a homogeneous, moderately electron dense outer acrosome zone. Internal to this, in succession, is the dense inner acrosome zone and, within this, the pale perforatorium, the narrow base of which is continuous with the posterior cytoplasm. The inner acrosome zone is widest anterolaterally to the perforatorium. A concavo–convex central acrosome zone lies anterior to the perforatorium, separating it from the operculum and surrounded by the inner acrosome zone. Two sperm may be "conjugated" by their anterior faces (Fig. 4E). This appears to be a random occurrence as most of the sperm in the spermatophore are separate. It remains possible, nevertheless, that the semilunar shape is functionally adaptive to close packaging in some part of the male or female reproductive systems. Alternatively, the broad opercular face of the sperm may be an adaptation for sperm–egg contact.

Cytoplasm

The cytoplasm in the sperm of all investigated majid species forms a thin layer, of irregular thickness, ensheathing the whole of the acrosome excepting its opercular region (Figs 1–3, 4A,E–G, 5A,B, 6A,C,E, 7A, 8). Laterally it expands, sometimes far, into the nuclear material as islets which contain putative mitochondrial remnants and groups of membranes (Figs 4A,G, 5B,F,G, 6C,E, 7A, 8). The periacrosomal cytoplasm is continuous with a mass lying at the posterior pole of the perforatorial chamber. The material within the posterior perforatorial chamber may also be regarded as cytoplasm. No cytoplasm extends into the nuclear arms and microtubules are not present.

Centrioles

Centrioles are demonstrable, posterior to the perforatorial chamber, in *Cyrtomaia furici* (Fig. 5C), *Platymaia rebierei* (Fig. 6D), *Macropodia longirostris*, *Sphenocarcinus stuckiae* (Fig. 7A) and *S. orbiculatus* (Fig. 8). They have yet to be observed in *Grypacheus hyalinus*.

Nucleus

In all species, as in other brachyurans, the nuclear material is located in the cup-shaped structure around both the acrosome and its cytoplasmic sheath and in the lateral arms (Figs 1–3, 4A–F, 5A,D,I, 6A,B, 7A–D, 8).

Only traces of the nuclear envelope remain between the nucleus and cytoplasm, in contrast to the pronounced multilaminar membranes in some brachyurans. The external surface of the cell is bounded by a membrane which may represent fused nuclear and plasma membranes— the nuclear plasma membrane (Figs 1–3, 4A, 5A, 6A, 7A, 8). The general chromatin consists of a diffuse network of electron dense filaments in a pale matrix as in other brachyurans, or, in *Macropodia longirostris*, appears densely packed.

Nuclear arms

The sperm of *Cyrtomaia furici* (Figs 5D,I), *Platymaia rebierei* (Fig. 6B), *Sphenocarcinus stuckiae* (Fig. 7B) and *S. orbiculatus*, have a triradiate form, with three arms, one at each vertex. Arms are visible in *Macropodia longirostris* but the number was indeterminable (Figs 4B,F).

Posterior median process

A chromatin-containing "posterior median process" of the nucleus has not been observed in the new majid material. Small posterior irregularities sometimes visible in *Cyrtomaia furici* (Fig. 5A) are possibly the equivalent of a modified posterior median process.

Discussion

Majid sperm conform to the general organization of brachyuran and, specifically, eubrachyuran sperm (Jamieson 1991*b*). Features considered to distinguish eubrachyuran sperm from those of podotremes (Jamieson 1991*b*, 1994*a*, *b*; Jamieson *et al.* 1995) are as follows: the acrosome is not significantly depressed; zonation of the acrosome is concentric, often being horizontal in podotremes; continuity of the operculum with the acrosomal capsule, and presence of capsular chambers, and of flange-like outward projections of the capsule, characteristic of raninoids, are not seen; the perforatorium narrows apically and, unlike that of podotremes, is

Fig. 5. Cyrtomaia furici.—A–C, E–I. Transmission electron micrographs of spermatozoa. *D.* Light micrograph.—*A.* Longitudinal section (LS).—*B.* LS showing cytoplasm.—*C.* LS showing centriole.—*D.* Light micrograph of a spermatozoon showing three arms.—*E.* Transverse section (TS) through the acrosome vesicle at the level of the base of the perforatorium showing the corrugated perforatorial wall.—*F–H.* TSs through the acrosome vesicle (*F*) at the level of just below the operculum (slightly oblique), (*G*) the anterior portion of the perforatorium, and (*H*) the mid/posterior portion of the perforatorium.—*I.* TS showing its triradiate form.

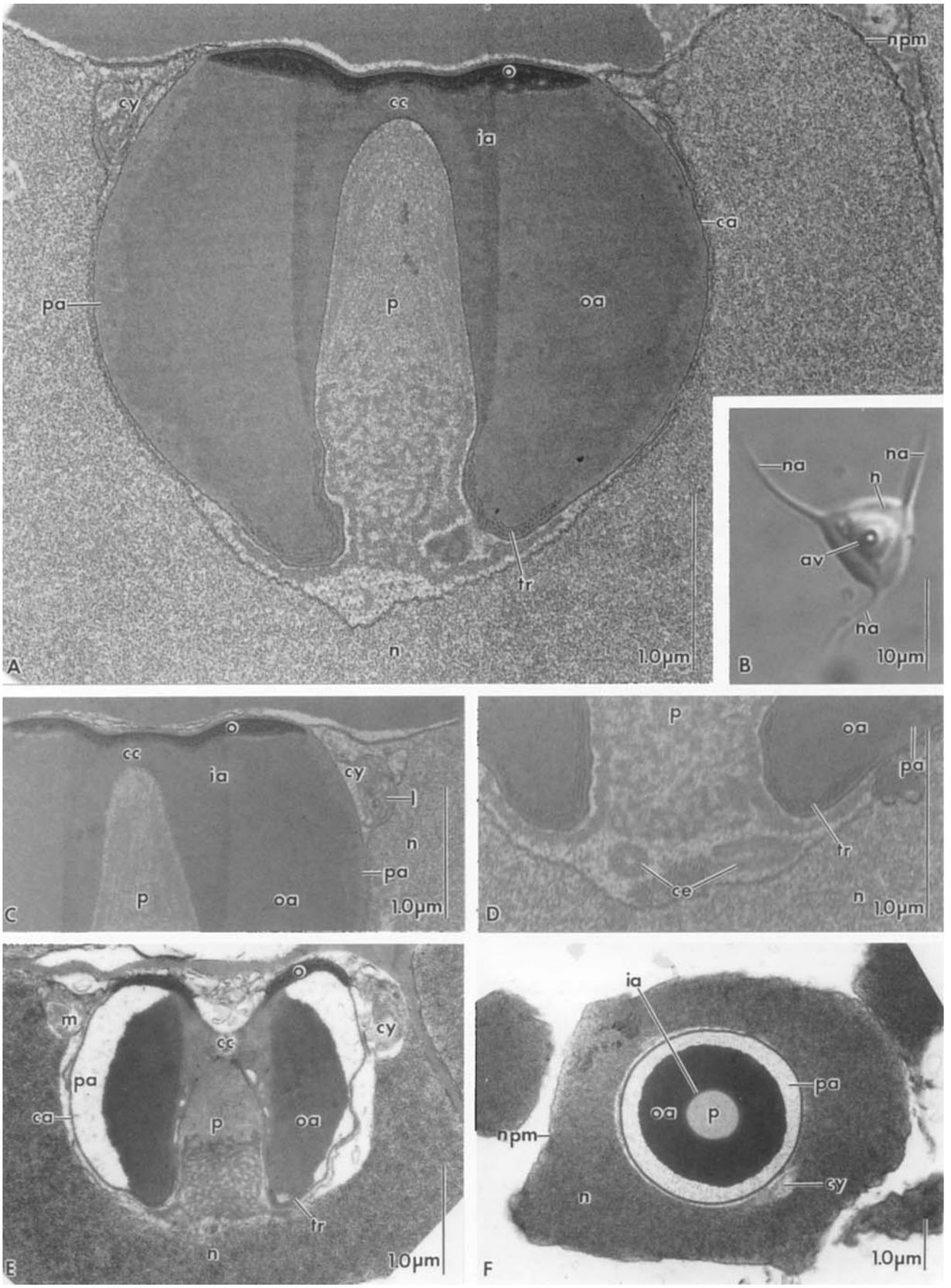
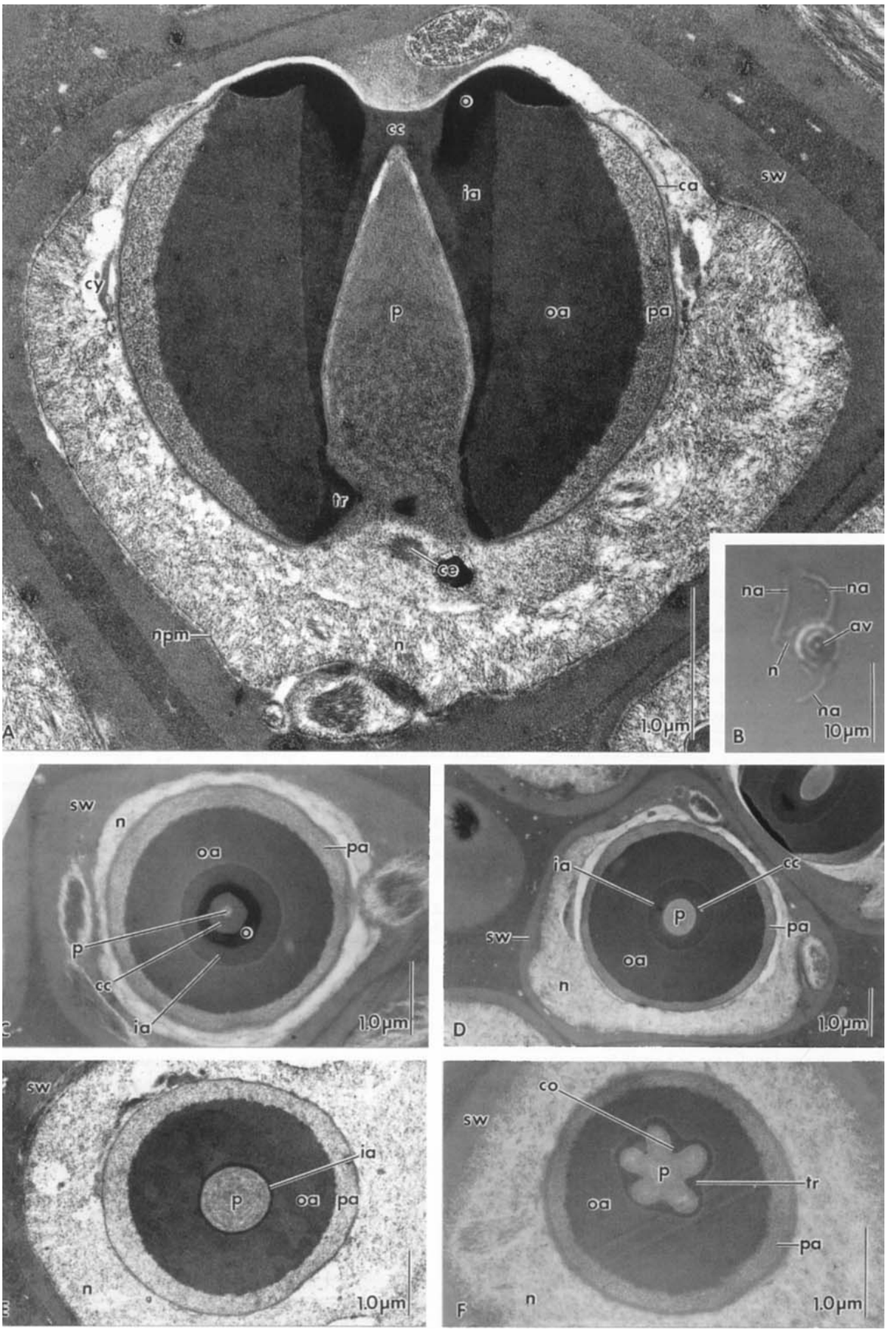


Fig. 6. Platymaia rebierei.—*A, C, D.* Transmission electron micrographs of spermatozoa. *B.* Light micrograph.—*A.* Longitudinal section (LS).—*B.* Light micrograph of a spermatozoon showing three arms.—*C.* LS showing cytoplasm.—*D.* LS showing two perpendicular centrioles. *Grypacheus hyalinus.*—*E, F.* Transmission electron micrographs of spermatozoa.—*E.* LS.—*F.* TS through the equator.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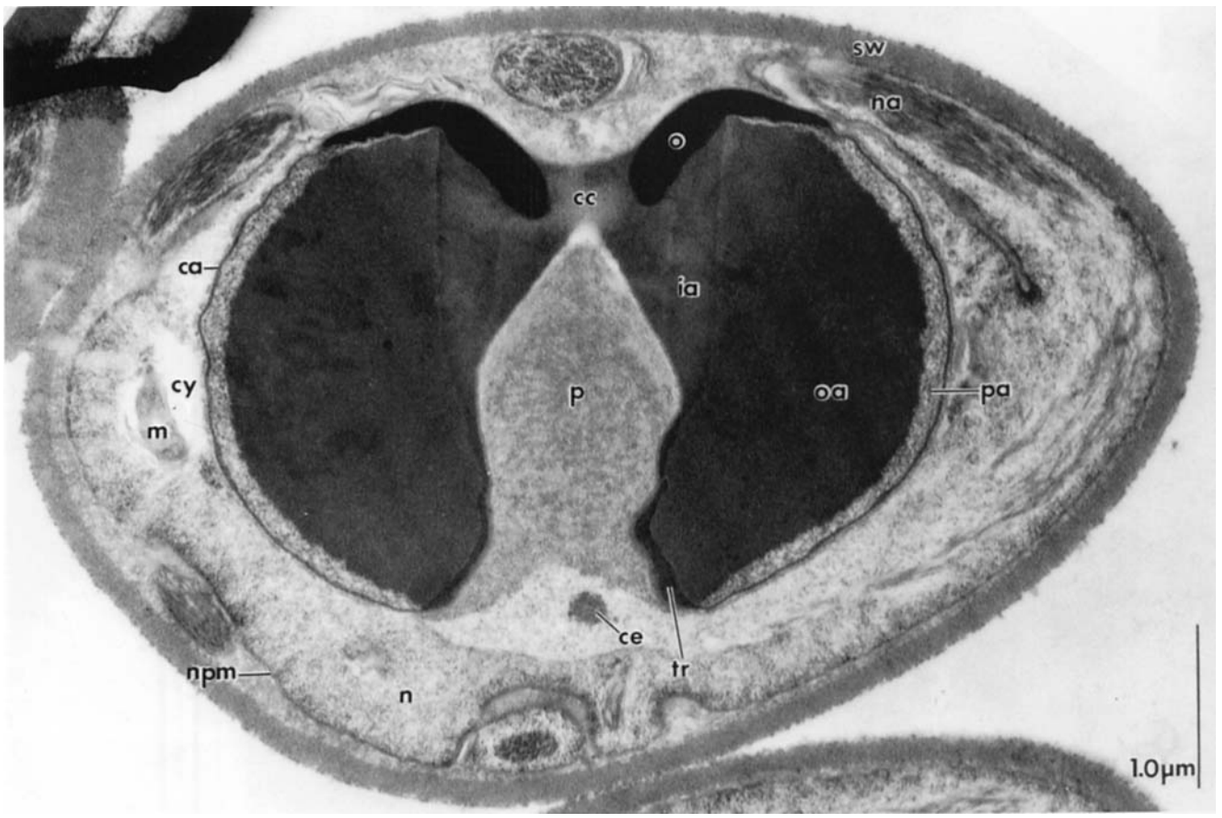

Fig. 8. Sphenocarcinus orbiculatus. Transmission electron micrograph. Longitudinal section (LS) of a spermatozoon in a spermatophore.

Table 2. *Acrosome dimensions in majids (this study)*

Species	Acrosome mean greatest length μm, *n*, SD	Acrosome mean greatest width μm, *n*, SD	Acrosome length:width ratio	Operculum mean greatest width μm, *n*, SD
Inachinae				
Cyrtomaia furici	3.23, 3, 0.15	3.87, 3, 0.15	0.83	2.53, 3, 0.12
Grypacheus hyalinus	2.65, 4, 0.26	3.65, 4, 0.86	0.73	2.70, 4, 0.34
Macropodia longirostris	1.38, 8, 0.05	3.64, 8, 0.22	0.38	3.29, 8, 0.19
Platymaia rebierei	2.86, 6, 0.18	3.86, 6, 0.30	0.74	2.42, 6, 0.16
Pisinae				
Sphenocarcinus orbiculatus	3.55, 2, 0.57	4.55, 2, 0.04	0.78	3.15, 2, 0.07
Sphenocarcinus stuckiae	3.20, 5, 0.39	3.82, 5, 0.14	0.84	2.37, 5, 0.10

never capitate or radiate; the opercular projections into the subopercular material, seen in homolids, are absent; an anterolateral pale zone, and a flange-like lower zone of the acrosome, seen in *Paradynomene* and *Homolodromia* (Jamieson *et al.* 1995), are absent; and the perforatorial chamber lacks the corrugations (inward projections) seen in raninoids. Majid sperm differ from this definition, however, in a tendency to depression of the acrosome, taken to its extreme in the semilunar acrosome of *Macropodia longirostris*, and in having inward projections of the perforatorial chamber. The presence in majids of numerous projections as in podotremes appears to endorse the basal position of majids demonstrated in cladistic analyses (Jamieson 1994*a*; Jamieson *et al.* 1995). Nevertheless, lesser numbers of projections occur in some members of other eubrachyuran families (unpublished observations) and they are well developed, though distinctive, in hymenosomatids.

Some structures seen in other eubrachyurans are absent from majids. Thus the periopercular rim seen, *inter alia*, in

Fig. 7. Sphenocarcinus stuckiae.—*A*, *C–F*. Transmission electron micrographs of spermatozoa. *B*. Light micrograph.—*A*. Longitudinal section (LS) of a spermatozoon in a spermatophore.—*B*. Light micrograph of a spermatozoon showing three arms.—*C–F*. Transverse sections (TSs) through the acrosome vesicle (*C*) at the level of the operculum, (*D*) the anterior portion of the perforatorium, (*E*) the mid/posterior portion of the perforatorium, and (*F*) the base of the perforatorium showing the corrugated perforatorial wall forming five robust incomplete septa.

Potamonautes, is absent; xanthoid features such as the xanthid ring surrounding the base of the perforatorial chamber, the accessory opercular ring, and the opercular overhang are absent, though a peripheral acrosome zone, typical of xanthids, is present in some species (all except *Macropodia longirostris*, in this study), differing in not having the ragged inner margin seen in xanthids. Features characterizing the Thoracotremata are absent: the apical button and concentric lamellae in the outer acrosome zone. An acrosome ray zone is absent as also noted in the spermatozoa of potamids (Guinot *et al.* 1997) and corystoids (Jamieson *et al.* 1997).

The Majidae, whether regarded as a family or a group of families, appear to be unified by the apomorphically flattened and/or centrally depressed form of the operculum. Furthermore, the perforatorium is frequently short, not extending anteriorly as far as the operculum, with the correlated presence of a central acrosome zone between the tip of the perforatorium and the operculum. This zone ensheathes the perforatorium to varying extents. The perforatorium is also short in the sperm of *Ranina ranina* but there it ends postequatorially; it extends preequatorially in other raninoids. This brevity of the perforatorium appears to be characteristic of majids, though within a species some sperm may have a longer perforatorium approaching the operculum. Whether, where individual variation occurs, the longer perforatorium reflects greater maturity and/or an incipient acrosome reaction is unknown. Such variation during the reaction from a preequatorial to an opercular extent of the perforatorium is seen in micrographs of *Maja squinado* by Tudge and Justine (1994). That the short form of the perforatorium is plesiomorphic or apomorphic has not been established. Only the long condition is known for the sperm of *Libinia emarginata* and *Pitho lherminieri*, from micrographs by Hinsch (1969, 1973, respectively).

The heterogeneity of the Majidae which is evident in subdivision into several subfamilies might be expected to be reflected in spermatozoal ultrastructure. Nevertheless, the sperm of the two species of the Pisinae examined here, *Sphenocarcinus stuckiae* and *S. orbiculatus*, and that of the inachine *Grypacheus hyalinus* are closely similar to the sperm of the acanthonychine *Menaethius monoceros* (see Jamieson 1991*b*, 1994*a,b*; Jamieson *et al.* 1995). They are characterized by an inturned perforate operculum and a rhombohedroidal, pointed perforatorium. *Sphenocarcinus*, sometimes subsumed in *Rochinia*, was removed from the Acanthonychinae (= Epialtinae) and placed in the Pisinae by Griffin and Tranter (1986). However, on the basis of zoeal character of *Rochinia carpenteri*, Clark and Webber (1991) propose that *Rochinia* should be included in the family Inachidae, in a new subfamily (unnamed). Spermatozoal ultrastructure clearly indicates a close relationship of *Sphenocarcinus* with *Menaethius*. It remains to be determined whether *Sphenocarcinus* should be returned to the Acanthonychinae or, depending on the sperm structure of the type genus of the Pisinae, kept within the Pisinae. The sperm of a further pisine, *Libinia* resembles that of *Sphenocarcinus* though apparently differing in having a well developed posterior median process and many sperm per spermatophore. These similarities of some pisine, inachine and acanthonychine sperm would, if species have been correctly assigned to their subfamilies, endorse monophyly of this section of the Majidae. Monophyly of the family Majidae is further indicated by much overlap in spermatozoal characters between the different subfamilies of the family as a whole. It is possible that additional subfamilial patterns and trends will emerge when further species are examined.

Although the Inachinae appear less constant in sperm structure than the albeit small sample from other subfamilies, they show a tendency to flattening of the operculum and of the acrosome seen in *Cyrtomaia furici* and taken to an extreme in the semilunar spermatozoon of *Macropodia longirostris*. In both species the nucleus forms an unusually regular, narrow band. This depression of the acrosome is apparent in five of the six inachines which have been examined; thus the ratio of length to width of the acrosome is as follows: *Podochela* spp. 1.0 (spheroidal); *Cyrtomaia furici* 0.8; *Grypacheus hyalinus*, *Platymaia rebierei* and *Stenorhynchus seticornis* 0.7; and *Macropodia longirostris* 0.4 (see references in Table 1). Although depression of the acrosome is a podotreme feature, it is probable that depression is an independent apomorphy in majids.

In the pisines *Libinia emarginata* (see Hinsch 1969), *Sphenocarcinus orbiculatus* and *S. stuckiae*, the inachine *Grypacheus hyalinus* (this study) and the acanthonychine *Menaethius monoceros* described by Jamieson (1991*a,b*, 1994*b*), the operculum is not only perforate but is also depressed centrally. In *Macropodia longirostris* the operculum is perforated but not centrally depressed. It is possible that the circular central depression in the operculum of the oregoniine *Chionoecetes opilio* demonstrated by scanning electron microscopy by Chiba *et al.* (1992), but not confirmed by transmission electron microscopy, is also a perforation. The operculum appears to be imperforate in the inachines *Cyrtomaia furici* and *Platymaia rebierei* (this study), *Pitho lherminieri*, *Podochela* spp., *Stenorhynchus seticornis* and *Macrocoeloma trispinosum* (references in Table 1). However, demonstration of perforation of the operculum is difficult, particularly from published micrographs, as longitudinal sections must be median. Furthermore, slight acrosome reaction will result in perforation of opercula which in the resting condition are imperforate.

A peripheral acrosome zone is known in the acanthonychine *Menaethius monoceros*, the pisines *Libinia emarginata*, *L. dubia*, *Sphenocarcinus stuckiae* and *S. orbiculatus*, the mithracine *Macrocoeloma trispinosum* and in the inachines *Cyrtomaia furici*, *Grypacheus hyalinus*, *Platymaia rebierei* and *Stenorhynchus seticornis* though absent from *Macropodia longirostris* and *Podochela* spp., the ophthalmine *Pitho lherminieri* and the oregogoniine *Chionoecetes opilio* (references in Table 1).

The thickened ring, a eubrachyuran autapomorphy (known probably homoplasically in one anomuran), is well developed in the inachines *Cyrtomaia furici*, *Grypacheus hyalinus*, *Platymaia rebierei*, *Podochela* spp., and *Stenorhynchus seticornis*, the mithracine *Macrocoeloma trispinosum*, the majine *Maja squinado*, the acanthonychine *Menaethius monoceros*, the pisines *Sphenocarcinus orbiculatus*, *S. stuckiae*, *Libinia emarginata*, the ophthalmiine *Pitho lherminieri* and the oregoniine *Chionoecetes opilio* but is weakly developed in the inachine *Macropodia longirostris* (references in Table 1). It does not therefore show a clear subfamilial pattern.

A triradiate form, with an arm at each vertex, is here considered plesiomorphic for the Meiura *sensu* Scholtz and

Richter (1995) (Anomala + Brachyura). Three arms are seen in the acanthonychine *Menaethius monoceros*, and the inachines *Cyrtomaia furici*, *Platymaia rebierei* and *Podochela* spp., the pisines *Sphenocarcinus orbiculatus*, *S. stuckiae*, *Libinia emarginata* and *L. dubia*, the mithracines *Macrocoeloma trispinosum* and *Mithrax* sp., and the ophthalmine *Pitho lherminieri* (references in Table 1). For *Chionoecetes opilio* Chiba *et al.* (1992) demonstrated four to ten, with a mean value of seven lateral arms, by scanning electron microscopy, and a well developed lamellar complex and degenerate mitochondria adjacent to the nucleus, by transmission electron microscopy and in *Stenorhynchus seticornis* there are four or five arms (Hinsch 1973). Numbers are unknown in *Grypacheus hyalinus*, *Macropodia longirostris* and *Maja squinado*.

Presence of microtubules in the arms has been demonstrated for *Libinia emarginata*, *L. dubia*, *Macrocoeloma trispinosum* and *Mithrax* sp., by Hinsch (1973), and *Maja squinado* by Tudge and Justine (1994) but is unknown in other majids. Development of microtubules in the lateral arms of "oxyrhynchs", demonstrated by Hinsch (1973) is regarded as a plesiomorphic condition (Jamieson 1991*b*) further supporting a basal position for majids as microtubules are present in anomalans (Tudge 1992, 1995*a,b*) but are reduced or absent in most eubrachyurans. However, the state of maturity and fixation of sperm may well affect the visibility of microtubules. Microtubules have also been observed in the reacting spermatozoa of the portunid *Carcinus maenas* (see Pochon-Masson 1968), the immature sperm cells of *Cancer* species (Langreth 1969) and the mature spermatozoa of the xanthoid, *Pilumnus semilanatus* and the eumedonids, *Eumedonus granulosus* and *Harrovia albolineata* (our unpublished data).

There is a posterior median extension of the nucleus in *Libinia dubia*, *L. emarginata* and especially well developed in *Pitho lherminieri* (see Hinsch 1973), in addition to the nuclear arms. The constancy of this process in majids is questionable but apparent absence may be due to fixation and/or facultative withdrawal in life as it is variably in evidence in *Menaethius monoceros*. The posterior median process, occurring also in some podotremes, including *Ranina ranina*, and in Anomura, may have developed homoplasically in majids to be lost in higher crabs (Jamieson 1991*b*, 1994*a*; Jamieson *et al.* 1995).

As a symplesiomorphy, centrioles are present in majids in cytoplasm basal to the perforatorial chamber (Chiba *et al.* 1992; Hinsch 1973; Jamieson 1991*b*). They are demonstrable in *Cyrtomaia furici*, *Platymaia rebierei*, *Macropodia longirostris*, *Sphenocarcinus stuckiae* and *S. orbiculatus*, as in *Menaethius monoceros*, *Podochela* spp., *Stenorhynchus seticornis*, *Maja squinado*, *Libinia emarginata*, *Chionoecetes opilio* and *Pitho lherminieri* (references in Table 1). They have yet to be observed in *Grypacheus hyalinus* or *Macrocoeloma trispinosum*. They are present *inter alia* in parthenopids (Hinsch 1973), portunids, dorippids, and *Macrophthalmus* but not, for instance, xanthids (Jamieson 1991*b*, 1994*a*; Jamieson *et al.* 1995).

Parthenopids and hymenosomatids have been considered to be closely related to majids, the three families constituting the Oxyrhyncha Latreille (1803; see Balss 1957). Hinsch (1973) attributes a very similar form, relative to that of majid sperm, to the parthenopids *Parthenope serratus* and *Heterocrypta granulata* (though with different layering of the acrosome contents) and sees the posterior process as a basic "oxyrhynch" character. The sperm of *Heterocrypta* is distinguished from other crabs, including *Parthenope*, in the unusually large amount of cytoplasm between the nucleus and the acrosome. From the micrographs by Hinsch (1973) both parthenopid genera have a broad but thin, slightly convex operculum perhaps more like opercula of majids than other families and the perforatorial column, in *Parthenope*, at least, is approximately rhombohedroidal, but these are insubstantial grounds for recognizing a particular relationship of parthenopids with majids. A major difference is the presence in parthenopids of an acrosome ray zone.

The spermatozoon of a parthenopid species examined by the authors (in preparation) also has a wide, thin operculum. This consists of two layers separated by a considerable hiatus. The perforatorial column is not rhombohedroidal but tapers anteriorly and bulges near its posterior end, the bulge involving the anterior part of the thickened ring. Unlike what appears to be the majid ground plan, the perforatorium closely approaches the operculum and there is no central acrosome zone. A thin reticulate zone (perhaps equivalent to an acrosome ray zone) is present. There are two, mutually perpendicular centrioles. The spermatozoon is not triradiate and no posterior median process is evident. In micrographs of *Parthenope serratus* and *Heterocrypta granulata* by Hinsch (1973), the perforatorium does not appear to reach the operculum, a feature resembling majids. However, in *P. serratus*, at least, as in our parthenopid species, an acrosome ray zone, unknown in majids, is well developed. Centrioles have been demonstrated for *Heterocrypta*. Resemblance of parthenopid spermatozoa to those of majids is therefore equivocal.

From a study of the megalopa, Rice (1981, 1983, 1988) regards majids as a monophyletic group distinct from the remaining Brachyura and states that there is no justification for retaining them with parthenopids in the Oxyrhyncha. In contrast to the basal position of majids, studies of the zoea led Rice (1981, 1983, 1988) to regard parthenopids as highly evolved products of a lineage including portunids and geryonids. Guinot (1978) notes that the unity of majids is demonstrated by interruption of the sternal sutures (4/5–7/8). With condensation of the nervous system (Bouvier 1940), she considers this to indicate that majids are advanced heterotremes but adds that without doubt there exist majids which are primitive and others which are very advanced. Spermatozoal ultrastructure, we have seen above, is at most tenuously similar between majids and parthenopids and cannot be considered to offer particular support for a close relationship.

A putative majid–hymenosomatids relationship is not supported by sperm ultrastructure. The hymenosomatid sperm differ from those of the Majidae in nine of their ten major features: (1) presence of an epiopercular dome; (2) separation of all but the central region of the operculum from the remainder of the acrosome by an infra-opercular rim; (3) the fact that the acrosome is smaller in volume than the nucleus; (4) the acrosome is strongly emergent from the nucleus, being surrounded only basally by nuclear material; (5) the cytoplasmic sheath, ending anteriorly with the nucleus, is also basal; (6) division of the acrosome contents into an inner and outer acrosome zone is scarcely apparent in longitudinal section as the inner zone is narrow and of doubtful

homology; (7) basally there is a unique "fringe zone"; (8) the acrosome, including the epinuclear dome, is longer than it is wide. (9) The unique helical and posterolateral disposition of the nuclear arms. A possible similarity is that the inner acrosome zone in hymenosomatids is anteriorly almost septate owing to several longitudinal corrugations. Longitudinal incomplete septa (corrugations) are also known for some majids (see above).

When sperm ultrastructure alone was used in cladistic analysis (parthenopids and hymenosomatids were not included) majids appeared to be the most basal and plesiomorphic family of the investigated Eubrachyura. However, when somatic characters were added the Dorippidae occupied this position (Jamieson 1994*a*; Jamieson *et al.* 1995). A basal origin of Majidae, from a purely spermatological viewpoint, corresponds with the basal position attributed to them by Rice (1981) from zoeal morphology.

Abbreviations used in the figures

av	acrosome
ca	capsule
cc	central acrosome zone
ce	centriole
co	corrugations (longitudinal incomplete septa)
cy	cytoplasm
ia	inner acrosome zone
l	lamellae
m	degenerating mitochondrion
n	nucleus
na	nuclear arm
npm	nuclear plasma membrane
o	operculum
oa	outer acrosome zone
p	perforatorium
pa	peripheral acrosome zone
sw	spermatophore wall
tr	thickened ring

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