

## REVIEW ARTICLE

# Comparative ultrastructure of the spermatozoa of the Majoidea (Crustacea, Decapoda, Brachyura) with new data on six species in five genera

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**Introduction**

The aim of the present work is to review and extend our knowledge of the ultrastructure of the spermatozoa of representatives of the Majoidea and to comment on its phylogenetic implications, with remarks on hymenosomatoids and cryptochirids. The use of brachyuran acrosome morphology for phylogenetic inference at the family level has recently been

**Abstract**

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Comparative ultrastructure of majoid spermatozoa belonging to 23 species, in 19 genera and five families, is considered, with new data on *Schizophrys aspera*; *S. rufescens* (Majidae, Majinae); *Camposcia retusa* (Inachidae); *Pyromaia tuberculata* (Inachoididae); and *Huenia heraldica* and *Menaethius monoceros* (Epiplutidae, Epiplutinae). The oregoniid *Chionoecetes opilio*, and inachids *Cyrtomaia furici*, *Platymaia rebierei*, *Macropodia longirostris* and *Inachus phalangium*, possibly with *Camposcia retusa*, but not *Podochela riisei*, appear to form a group. Within the inachids, *Macropodia* and *Inachus* are especially close. A domed central acrosome zone, seen in most inachid sperm, in majines (both *Schizophrys* species), in pisines (*Oxypleurodon orbiculatus* and *O. stuckiae*) and epiplutines (*Huenia heraldica* and *Menaethius monoceros*), appears to be an autapomorphy of these majoids. A peripheral acrosome zone is seen in the inachid *Grypacheus hyalinus*, two inachoidids (*P. tuberculata* and *Stenorhynchus seticornis*) and the majid *Maja squinado*. *Pyromaia tuberculata* differs from other inachoidids in having a slightly dome-shaped operculum. The mithracine *Macrocoeloma trispinosum* (Majidae) sperm more closely resembles Inachoididae, than Inachidae. Spermatologically, the family Majidae and the subfamily Majinae are not homogeneous. Spermatozoal ultrastructure does not support a majoid–hymenosomatid relationship and is equivocal with regard to the placement of Cryptochiridae in either the Thoracotremata or Heterotremata, the prominent operculum strongly differentiates cryptochirids from Majoidea.

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questioned in a valuable analysis of the congruence of spermatozoal characters with molecular (16S) phylogenetic trees in potamoid crabs as it appears to be strongly affected by small sample size and by convergent character evolution (Klaus and Brandis 2010). However, there are numerous examples of the utility of spermatozoal ultrastructure for inferring relationships in Animalia (Jamieson *et al.* 1995). As just two examples, of many, external to the Brachyura, strong endorsement of

‘spermiocladistics’ was the ratification from molecular biology by Abele *et al.* (1989) of placement of the Pentastomida in the Crustacea based on spermatozoal ultrastructure by Wingstrand (1972) confirmed by Storch and Jamieson (1992) and the erection of a higher platyhelminth taxon, the Trepanonemata, defined by a single central axonemal core (Ehlers 1985). Spermatozoal ultrastructure has contributed significantly to brachyuran phylogenetics and impacted the current hypotheses (Jamieson 1989a,b, 1990, 1991a,b, 1993a,b, 1994; Jamieson and Tudge 1990, 2000; Jamieson *et al.* 1993a,b,c,d, 1994, 1995, 1996, 1997, 1998; Guinot *et al.* 1994, 1997, 1998; Jamieson and Guinot 1996; Richer de Forges *et al.* 1997).

The Eubrachyura of Saint Laurent (1980) contains the Heterotremata, including the Majidae, and Thoracotremata of Guinot (1977, 1978) and therefore includes all Brachyura other than the Podotremata. Sperm morphology merits inclusion of the Thoracotremata, as a monophyletic assemblage, within the Heterotremata *sensu lato* (Jamieson 1991b, 1994; Jamieson *et al.* 1995; Jamieson and Tudge 2000), the Heterotremata then being synonymous with Eubrachyura. However, molecular analysis using nuclear protein-coding genes supported Heterotremata and Thoracotremata as being reciprocally monophyletic (Tsang *et al.* 2008). Chu *et al.* (2009a) in a phylogenetic analysis of protein-coding genes also find the Heterotremata and Thoracotremata to be monophyletic sister groups (see also Palacios-Theil *et al.* 2009), and until this issue is settled, we will therefore continue to use the descriptively valuable term Heterotremata.

For comparative purposes, a generalized brachyuran sperm is illustrated in Figure 1. The Eubrachyura has been defined by a spermatozoal synapomorphy: presence of the thickened ring (numbered 23 in Fig. 1) (Jamieson 1991b, 1994; Jamieson *et al.* 1995; Jamieson and Tudge 2000). Other spermatozoal synapomorphies of the Eubrachyura, although unambiguous, are less convincing. Multiplication of

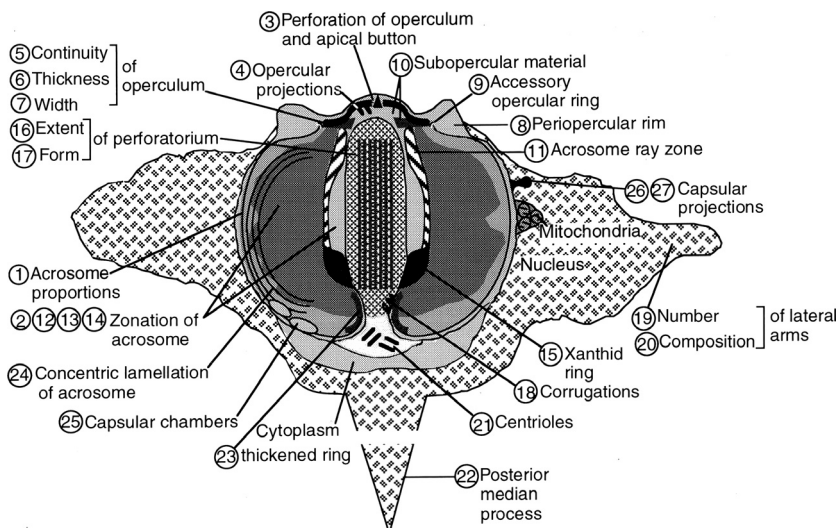
lateral radiating processes termed arms (19 and 20 in Fig. 1) from three, common to paguroids and podotremes, to several is a trend rather than a diagnostic basal apomorphy, there being three to many in majoids. Presence of a true acrosome ray zone (11 in Fig. 1) has been considered a basal synapomorphy of heterotremes, although often absent. It was ascertained for *Calocarcinus* (Trapeziidae), *Neodorippe* (Dorippidae), *Portunus* (Portunidae), *Pilodus* (Xanthidae) and *Potamonautes* (Potamonautidae) by Jamieson *et al.* (1995) and the epialtid *Menaethius monoceros* (then as a majid) (Jamieson 1991b, 1994), but its presence has not been confirmed in this and other majoids. Its use as a phylogenetic character is diminished by the difficulty of recognizing its fine detail unless the material is appropriately fixed. It is present, apparently homoplasiacally, in paguroids (Jamieson *et al.* 1995).

In morphocladistic analysis (Jamieson 1994; Jamieson *et al.* 1994, 1995; Jamieson and Tudge 2000), the Heterotremata *sensu lato* (including thoracotremes), that is, Eubrachyura, formed a monophyletic grouping whether or not non-spermatozoal characters were included. The sternal female pores constitute, as Guinot (1977, 1978) suggested, their non-spermatozoal synapomorphy. However, thoracotremes diverge from heterotremes in having sternal male openings.

When sperm ultrastructure alone was used in cladistic analysis, majoids 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; Jamieson *et al.* 1994, 1995) (see Phylogenetic considerations, below).

## Materials and Methods

The Majoidea Samouelle, 1819; previously Oxyrhyncha (see Garth 1958) or Majidae (see Griffin and Tranter 1986), is a superfamily with about 950 species distributed in 200 genera (Ng *et al.* 2008, 98), and its classification varies greatly



**Fig. 1**—A composite diagram of a brachyuran spermatozoon. Numerals refer to characters used in a previous cladistic analysis. No actual spermatozoon would possess all the character states shown. From Jamieson 1994 and Jamieson and Tudge 2000.

(Rathbun 1925; Garth 1958; Rice 1981; Griffin and Tranter 1986; Clark and Webber 1991). The list of Ng *et al.* (2008) included the five families quoted in Table 1, plus the Hymenosomatidae that is often elevated to superfamilial rank (see Guinot 2011b); the genus *Stenorhynchus*, usually placed in Inachidae, is here assigned to the Inachoididae, as proposed by Guinot (2012).

Except in ‘Materials’, the authorship of taxa is not given in this account and parentheses around author names refer to the source of information.

### Materials

Majidae, Majinae: *Schizophrys aspera* (H. Milne Edwards, 1834) from New Caledonia, collector B. Richer de Forges; *Schizophrys rufescens* Griffin and Tranter 1986 from North Stradbroke Island, Queensland, Australia, collector D. Harris.

Majidae, Inachinae: *Camposcia retusa* Latreille, 1829 from New Caledonia, collector B. Richer de Forges.

**Table 1** Ultrastructural investigations of sperm of the Majoidea

Majoidea		
Majidae		
Majinae	<i>Maja squinado</i>	Tudge and Justine (1994)
	<i>Maja brachydactyla</i>	Simeó <i>et al.</i> (2010)
	<i>Schizophrys aspera</i>	This study
	<i>Schizophrys rufescens</i>	This study
Mithracinae	<i>Macrocoeloma trispinosum</i>	Hinsch (1973)
	<i>Mithrax</i> sp.	Hinsch (1973)
Oregoniidae	<i>Chionoecetes opilio</i>	Beninger <i>et al.</i> (1988), Chiba <i>et al.</i> (1992)
Inachidae	<i>Camposcia retusa</i>	This study
	<i>Cyrtomaia furci</i>	Jamieson <i>et al.</i> (1998) and Jamieson and Tudge (2000)
	<i>Grypacheus hyalinus</i>	Jamieson <i>et al.</i> (1998) and Jamieson and Tudge (2000)
	<i>Inachus phalangium</i>	Rorandelli <i>et al.</i> (2008)
	<i>Macropodia longirostris</i>	Jamieson <i>et al.</i> (1998)
	<i>Platymaia rebierei</i>	Jamieson <i>et al.</i> (1998)
	<i>Podochela riisei</i>	Hinsch (1973)
Inachoididae	<i>Pyromaia tuberculata</i>	This study
	<i>Stenorhynchus seticornis</i>	Hinsch (1973)
Epiplatidae		
Epiplatinae	<i>Huenia heraldica</i>	This study
	<i>Menaethius monoceros</i>	Jamieson (1991b, 1994), this study
Pisinae	<i>Libinia dubia</i>	Hinsch (1973)
	<i>Libinia emarginata</i>	Hinsch (1969, 1971, 1973, 1986), Vaughn and Hinsch (1972) Hernandez <i>et al.</i> (1989), Murray <i>et al.</i> (1991)
	<i>Oxypleurodon</i> (as <i>Sphenocarcinus</i> )	Jamieson <i>et al.</i> (1998) and Jamieson and Tudge (2000)
	<i>orbiculatus</i>	
	<i>Oxypleurodon</i> (as <i>Sphenocarcinus</i> )	Jamieson <i>et al.</i> (1998) and Jamieson and
	<i>stuckiae</i>	Tudge (2000)
Tychinae	<i>Pitho lherminieri</i>	Hinsch (1973)

Inachoididae: *Pyromaia tuberculata* (Lockington, 1877) from Japan, collector T. Furota.

Epiplatidae, Epiplatinae: *Huenia heraldica* De Haan, 1839 and *Menaethius monoceros* (Latreille, 1825) 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. International samples were then posted to Brisbane, Australia, 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, 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, Ltd., Mount Waverley, Victoria, Australia).

Portions of the testis (approximately 1 mm<sup>3</sup>) 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 an LKB 2128 UM IV microtome with a diamond knife. Sections were placed on carbon-stabilized collodion-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, with a final rinse in distilled water. Micrographs were taken on a Hitachi H-300 transmission electron microscope at 80 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

### *The spermatozoa of the Majoidea*

*General structure.* Twenty-three species, in 19 genera and five families of majoids, have been examined for sperm ultrastructure (see Table 1).

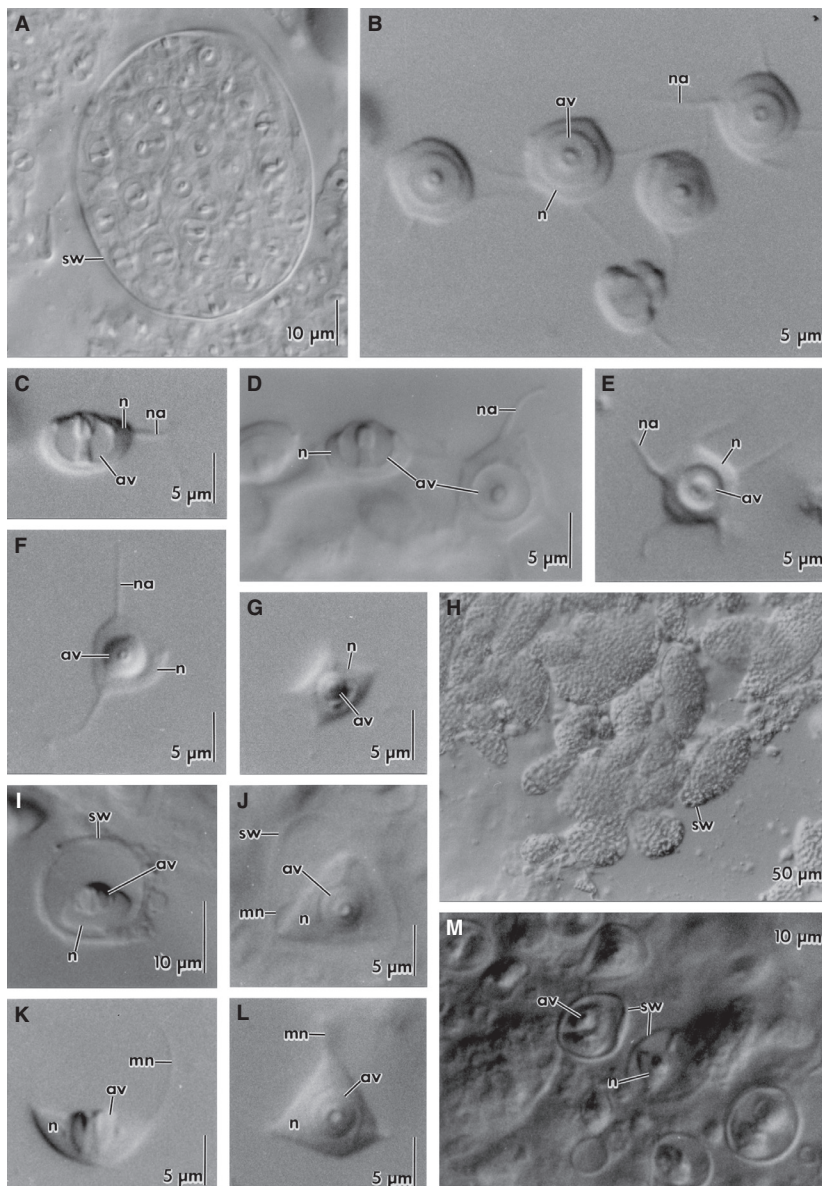
Majoid spermatozoa (Figs 2–9) are typically heterotreme in gross ultrastructure. The core of majoid spermatozoa consists of the concentrically zoned subspheroidal or (in the inachids, *Macropodia longirostris* and *Inachus phalangium*) semilunar acrosome, which is capped apically by a dense operculum. The acrosome is invested by a membrane underlain by a moderately electron-dense sheath, the capsule. Although concentrically zoned, the acrosome lacks the concentric lamellation seen in thoracotremes (24 in Fig. 1). The acrosome

vesicle is centrally penetrated by a cylindrical perforatorial column or chamber. A spherical, or only slightly depressed, form of the acrosome is typical of the Eubrachyura (Heterotremata + Thoracotremata). Unlike some Anomura, the acrosome does not project anteriorly from the body of the sperm. As in other brachyurans, the nucleus cups the acrosome, and a usually thin layer of cytoplasm invests the acrosome and intervenes between it and the nucleus. The sperm has marginal projections termed ‘arms’, which may contain chromatin and putative microtubules. A chromatin-containing ‘posterior median process’ of the nucleus, containing putative microtubules, is present in some (see below). In the cytoplasm, near the posterior end of the perforatorial chamber, centrioles may be visible. Failure in some species to demonstrate them does not necessarily imply absence. Cytoplasmic islets are usually

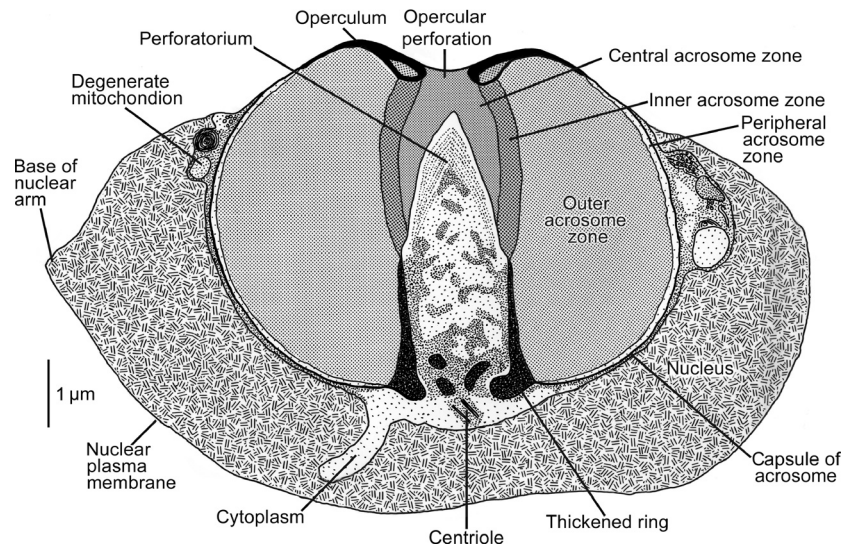
recognizable lateral to the acrosome and embedded in the chromatin; they may contain lamellae and bodies identifiable by homology with other crabs as degenerating mitochondria.

**Acrosome.** The dimensions of some acrosomal components in the majoids investigated in the present account 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 that opens into the columnar perforatorial chamber. A thickened ring, which is visible at the base of subacrosomal invagination in most heterotremes and many thoracotremes, is usually present.

The broad operculum of majoid sperm is highly unusual in being depressed centrally or (*Podocheila*, Hinsch 1973) at least flattened, whereas it is domed or conical in other



**Fig. 2**—Light micrographs. — **A–C.** *Schizophrys rufescens*. — **A.** Spermatophore containing many spermatozoa. — **B.** Apical view of spermatozoon. — **C.** Lateral view of a spermatozoon. — **D–E.** *Schizophrys aspera*. — **D.** Lateral and apical views of spermatozoon. — **E.** Apical view of a spermatozoon showing five arms. — **F.** *Camposcia retusa*. Apical view of a spermatozoon. — **G–H.** *Pyromaia tuberculata*. — **G.** Apical view of a spermatozoon showing four vertices. — **H.** Spermatophores containing many spermatozoa. — **I–J.** *Huenia heraldica*. — **I.** Lateral view of spermatophore containing one spermatozoon. — **J.** Apical view of spermatophore containing one spermatozoon. — **K–M.** *Menaethius monoceros*. — **K.** Lateral view of spermatozoa. — **L.** Apical view of spermatozoon. — **M.** Spermatophores containing one or two spermatozoa.



**Fig. 3**—*Schizophrys aspera*. Semi-diagrammatic longitudinal section of a spermatozoon, traced from a transmission electron micrograph.

Brachyura. The operculum is not only depressed centrally but is also perforate in *M. monoceros* (Jamieson 1991b, 1994; Figs 2L and 9A, in present study), *C. retusa* (Figs 2F and 6A), *H. heraldica* (Fig. 2J), *S. aspera* (Fig. 3), *P. tuberculata* (Fig. 7A) and *Maja brachydactyla* (Simeó et al. 2010; their Table 2) and in the pisines *Oxypleurodon orbiculatus*, *O. stuckiae* (Jamieson et al. 1998; Jamieson and Tudge 2000) and *Libinia emarginata* (Hinsch 1969, 1973). It is particularly strongly depressed centrally in the pisines so as to appear, in longitudinal section, inturned to penetrate into the central acrosome zone. It is also known to be perforate in *M. longirostris* but appears to be imperforate in *Cyrtomaia furci* and *Platymaia rebierei* (Jamieson et al. 1998). The circular central depression in the operculum of *Chionoecetes opilio*, demonstrated by scanning electron microscopy (SEM) by Chiba et al. (1992), may also be a perforation.

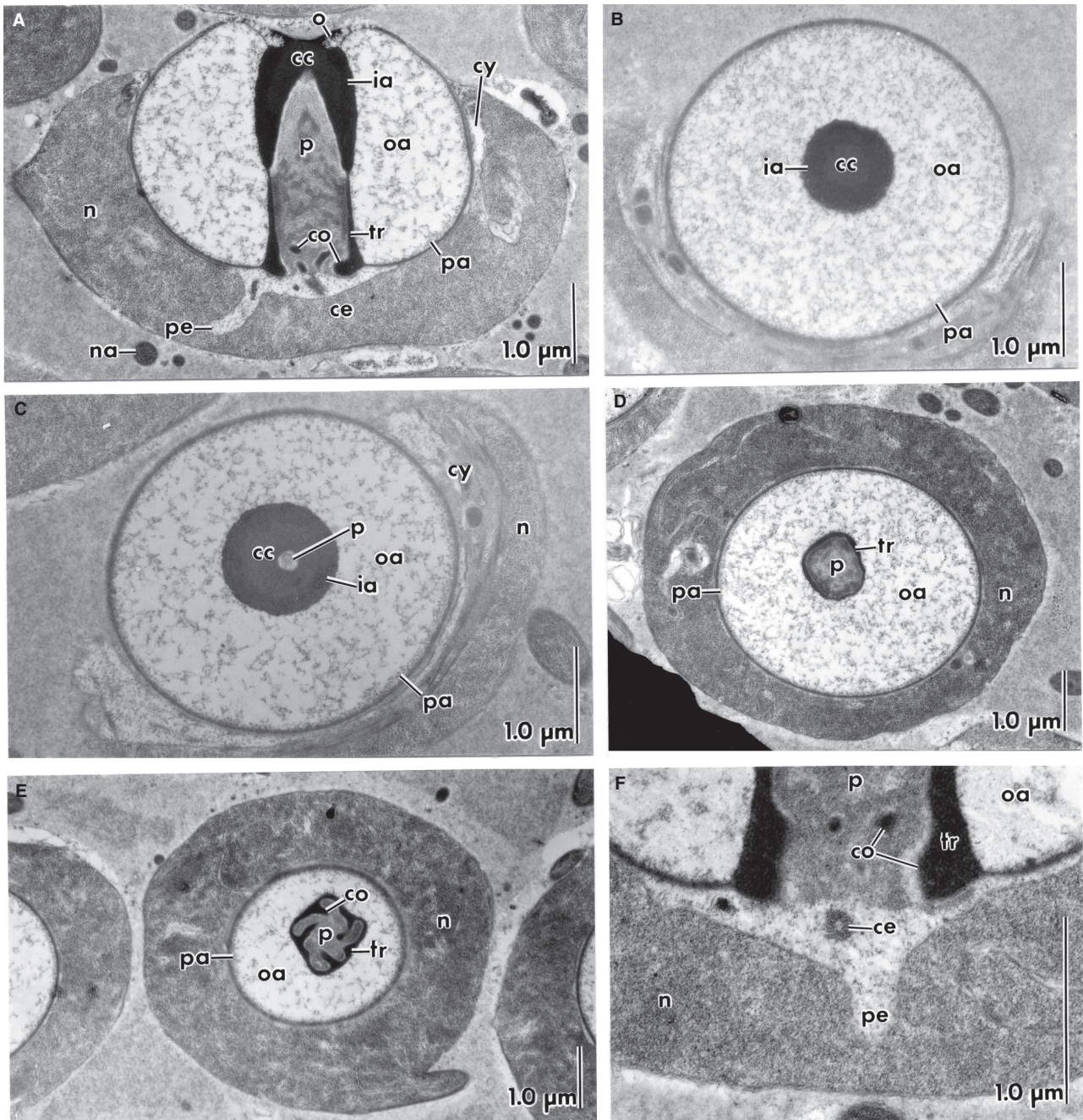
The central, subopercular axis of the acrosome is formed by the perforatorial chamber, with the contained perforatorium. This has been shown to penetrate the egg membranes in *Libinia emarginata* (Hinsch 1971). A feature of majoid sperm is the squat, approximately rhombohedroidal shape of the perforatorial chamber, having a pointed spatulate shape in longitudinal section (16 and 17 in Fig. 1). This form of the perforatorial chamber is exemplified by the majid mithracine *Macrocoeloma trispinosum* (Hinsch 1973), the oregoniid *C. opilio* (Beninger et al. 1988), the inachdids *Podochela riisei* (Hinsch 1973), *P. rebierei*, *C. furci*, *M. longirostris* (Jamieson et al. 1998; Jamieson and Tudge 2000), *C. retusa* (Fig. 6A) and *I. phalangium* (Rorandelli et al. 2008), the majids *Maja squinado* (Tudge and Justine 1994), *S. aspera* (Figs 3 and 4A) and *S. rufescens* (Fig. 5A), the inachoidids *P. tuberculata* (Fig. 7H) and *Stenorhynchus seticornis* (Hinsch 1973), the epialtines *H. heraldica* (Fig. 8A) and *M. monoceros* (Fig. 9A), the pisines *O. orbiculatus* and *O. stuckiae* (Jamieson et al. 1998; Jamieson and Tudge 2000), and the tychine *Pitho lher-*

*mineri* (Hinsch 1973). It is thus observed in all majoid families and subfamilies examined for sperm ultrastructure (Table 1). The report of a ‘spiked-wheel’ formation at the head of the perforatorial chamber (Simeó et al. 2010; their Table 2) appears to be a lapse, as this structure is limited in the Brachyura to the sperm of the Homolidae (Jamieson et al. 1993b). The apex of the perforatorial chamber usually does not extend to the anteriormost limit of the operculum. However, in the mature sperm of *M. brachydactyla*, a button-like expansion of the tip of the perforatorial chamber projects into the operculum (Simeó et al. 2010) but possibly indicates a partial acrosome reaction. It is not homologous with the apical button characteristic of thoracotremes (3 in Fig. 1), which is separate from the perforatorial chamber.

The acrosome was first demonstrated to contain actin in *M. squinado* (Tudge and Justine 1994). Subsequently, the perforatorial chamber was shown to contain actin basally in *I. phalangium* (Rorandelli et al. 2008) and *M. brachydactyla* (Simeó et al. 2010).

The perforatorial chamber can be constricted near its base by several to many inward projections or folds of its wall and of the adjacent acrosome vesicle, forming longitudinal corrugations: four in *C. furci*, five in *O. stuckiae* (Jamieson et al. 1998), *S. aspera* (Fig. 4E) and *S. rufescens* (Fig. 5F) or many in *M. longirostris* (Jamieson et al. 1998); the arrangement being present, but number undetermined in *P. rebierei*, *O. orbiculatus*, *Grypacheus hyalinus*, *C. retusa* (Fig. 6F), *P. tuberculata* (Fig. 7E) and *M. monoceros* (Fig. 9D). They have not been observed in *H. heraldica* (Fig. 8A,G).

*Zonation of the acrosome.* The axial acrosomal material between the perforatorial chamber and the operculum or the central perforation of the latter is termed the central acrosome zone in majoids. The perforatorial chamber and the central acrosome zone are surrounded by a moderately electron-



**Fig. 4**—Transmission electron micrographs of *Schizophrys aspera* spermatozoa. — **A**. Longitudinal section. — **B–E**. Transverse sections through the acrosome vesicle, (**B**) at the level of just below the operculum, (**C**) the anterior portion of the perforatorial chamber, (**D**) the mid portion of the perforatorial chamber and (**E**) the base of the perforatorial chamber showing the inward extensions of the perforatorial wall. — **F**. Longitudinal section of the posterior portion of the spermatozoon showing the centriole and the posterior extension of the cytoplasm into the nucleus. cc, central acrosome zone; ce, centriole; co, corrugations (longitudinal incomplete septa)/extensions of the perforatorial chamber wall; cy, cytoplasm; ia, inner acrosome zone; n, nucleus; na, nuclear arm; o, operculum; oa, outer acrosome zone; p, perforatorial chamber; pa, peripheral acrosome zone; pe, posterior extension of cytoplasm; tr, thickened ring.

dense layer, the inner acrosome zone that extends from the operculum almost to the posterior end of the acrosome, reaching and often continuous with the thickened ring. The acro-

some ray zone, seen in some heterotreme sperm, has only questionably been noted (in *M. monoceros*). An outer acrosome zone surrounds the inner acrosome zone and the base of

the perforatorial chamber, being several times wider than the inner zone. This outer zone extends to the convex margin of the acrosome, being bounded by the capsule in *M. longirostris* or is surrounded by a further, peripheral acrosome zone in *M. squinado* (Tudge and Justine 1994), *C. furici*, *G. hyalinus*, *P. rebierei*, *O. orbiculatus* and *O. stuckiae* (Jamieson et al. 1998) and the inachoidids *P. tuberculata* (Fig. 7A–H) and *Stenorhynchus seticornis* (Hinsch 1973). This description holds, with some variation in proportions, in five of the species investigated in the present study: *S. aspera* (Figs 3 and 4A–F), *S. rufescens* (Fig. 5A–H), *C. retusa* (Fig. 6A–G), *H. heraldica* (Fig. 8A–G) and *M. monoceros* (Fig. 9A–E), but the central acrosome zone is not differentiated from the inner acrosome zone in *P. tuberculata* (Fig. 7A,B,H) nor, from a single micrograph, in *C. opilio* (Fig. 3C of Beninger et al. 1988). The peripheral acrosome zone is weakly developed in *S. aspera* and *S. rufescens* but is strongly developed, although always much narrower than the outer acrosome zone, in *C. retusa*, *P. tuberculata*, *H. heraldica* and *M. monoceros*.

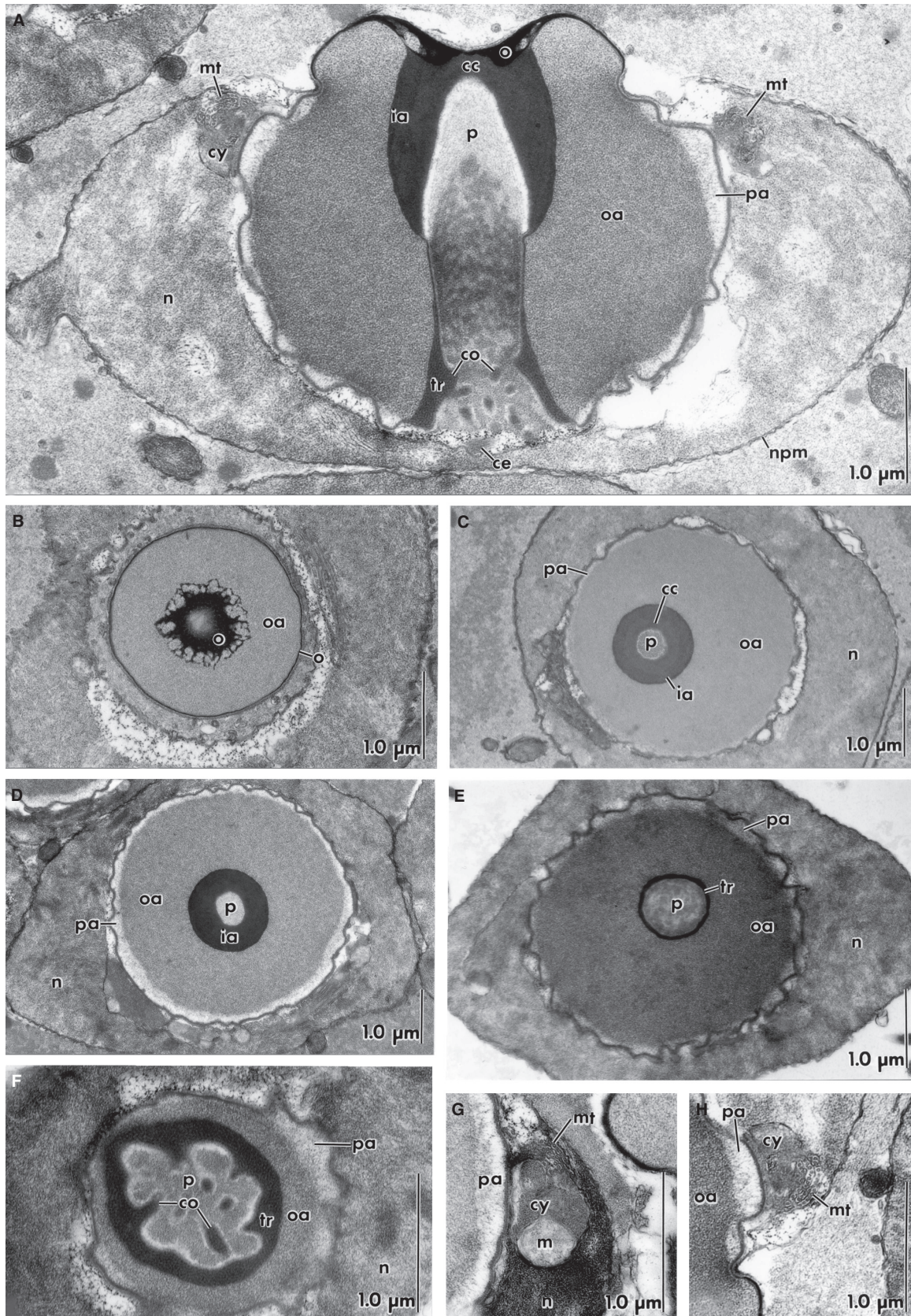
There is no periopercular rim in majoids in the sense of a non-nuclear structure (8 in Fig. 1) but Rorandelli et al. (2008) use this term for a nuclear rim in *I. phalangium*. In *M. brachydactyla*, the three concentric layers are again recognized, named internal, intermediate and external acrosomal layers by Simeó et al. (2010), but it is here suggested that the internal zone is actually the central zone, the intermediate zone is the outer acrosome zone and the external zone is the peripheral zone.

The sperm of *M. longirostris* was formerly the only eubranchyuran sperm in which the acrosome was known to depart radically from a subspheroidal form (Jamieson et al. 1998; Jamieson and Tudge 2000). However, the sperm of *I. phalangium* is closely similar, supporting their close phylogenetic relationship (Rorandelli et al. 2008). In both species, the acrosome is semilunar in shape and its anterior surface is slightly concave, whereas the posterior surface forms a hemisphere. The anterior surface is almost completely occupied by the thin, centrally perforate, electron-dense operculum, surrounded by the narrow periopercular rim. This rim has been shown to support a glycocalyx in *I. phalangium*, which is lost on entry into the female ducts and may be a general feature of brachyuran sperm although yet to be described in other species (Rorandelli et al. 2008). The bulk of the acrosome consists of a homogeneous, moderately electron-dense zone. This surrounds a vertically ellipsoidal axial core, which consists of the pale perforatorial chamber, the narrow base of which is continuous with the posterior cytoplasm. The anterior and anterolateral aspects of the chamber are capped by, and project into, a dense inner acrosome zone lying immediately under the operculum. The configuration of acrosome zones is identical in the two species. In *M. longirostris*, two sperm may be ‘conjugated’ by their anterior faces but most of the sperm in the spermatophore are separate. It remains possible that the semilunar shape is functionally adaptive to close packaging in some

part of the male or female reproductive systems (Jamieson et al. 1998). The gross ultrastructure of oregoniid sperm, known only for *C. opilio* (Beninger et al. 1988; their Fig. 3C), appears to be intermediate between that of these two inachids (*Inachus*, *Macropodia*) and those majoids with subspheroidal acrosomes. In the oregoniids, the operculum extends horizontally for most of the width of the acrosome, and the greater part of its width, centrally, is depressed; the acrosome thus forms a truncated sphere approaching a semilunar form. Above the short, spatulate perforatorial chamber, there is a dense acrosome zone that is not visibly differentiated into a central and an inner zone. The outer acrosome zone is wide and lacks a peripheral zone. The nucleus is moderately wide and (Fig. 3D of Beninger et al.) is seen to extend into a nuclear arm.

**Thickened ring.** The thickened ring (23 in Fig. 1) is attributed to ‘typical Oxyrhyncha sperm’ and specifically to *Mithrax* sp. by Hinsch (1973). It is well developed, compared with other, in *C. opilio* (Beninger et al. 1988; Chiba et al. 1992), *C. furici*, *P. rebierei*, *O. orbiculatus*, *O. stuckiae* (Jamieson et al. 1998), *S. aspera* (Figs 3 and 4A,D–F), *S. rufescens* (Fig. 5A,E,F) and *P. tuberculata* (Fig. 7A,B,D,E,G,H), is moderately developed in *G. hyalinus* (Jamieson et al. 1998; Jamieson and Tudge 2000), *C. retusa* (Fig. 6A,E,F,G) and *M. brachydactyla* (Simeó et al. 2010) but is only weakly developed in *M. longirostris* (Jamieson et al. 1998; Jamieson and Tudge 2000) and is not differentiated in the related *I. phalangium* (Rorandelli et al. 2008). It is weakly differentiated in *H. heraldica* (Fig. 8A,D,G).

**Nucleus.** The form of the nucleus is highly distinctive in the two newly examined epialtines, *H. heraldica* (Fig. 8A,D,G) and *M. monoceros* (Fig. 9A–E). In these, it is strongly electron-dense, appears drawn out laterally, is shallow longitudinally and is separated in places from the acrosome by an unusually wide band of cytoplasm. In the Inachidae, *M. longirostris* (Jamieson et al. 1998) and *I. phalangium* (Rorandelli et al. 2008) also resemble each other: they have a narrow, highly condensed layer of chromatin, which cups the semilunar acrosome. In *I. phalangium* further, less-condensed chromatin borders this layer and extends into the nuclear arms. In the majines *S. aspera* (Figs 3 and 4A–F) and *S. rufescens* (Fig. 5A–E), the nucleus is wide lateral to the acrosome, whereas in the other majine *M. brachydactyla*, it forms a fairly narrow zone around the acrosome and extending into the arms (Simeó et al. 2010). In the majid mithracine *M. trispinosum*, it also forms a narrow zone (Hinsch 1973). The nuclear material is copious in the inachids *C. retusa* (Fig. 6A), *C. furici* and *G. hyalinus*, *P. rebierei* (Jamieson et al. 1998; Jamieson and Tudge 2000) and *Podochela riisei* (Hinsch 1973) and in the inachoidids *P. tuberculata* (Fig. 7A) and *Stenorhynchus seticornis* (Hinsch 1973), although narrower in *S. seticornis*. It is moderately wide in *C. opilio* (Beninger et al. 1988), *Libinia emarginata*, *L. dubia* and *O. orbiculatus* and *O. stuckiae* (Jamieson et al. 1998; Jamieson and Tudge 2000).





**Posterior median process.** In majoid sperm, in addition to lateral arms, there may be a posterior median extension of the spermatozoon, which may contain nuclear material with or without microtubules (22 in Fig. 1). The process has been observed in some profiles of the sperm of *H. heraldica* and *M. monoceros* but, in these species, it contains no nuclear material (present study). This extension is well developed in raninids, in which it contains nuclear material, as in the majoids *L. emarginata*, *P. lherminieri* and supposedly in *M. trispinosum* and *Stenorhynchus seticornis* (Hinsch 1973). The constancy of this process in majoids is questionable but apparent absence in some cases may be due to fixation and/or facultative withdrawal in life, or to the plane of section, as it is variably in evidence in *M. monoceros* (Jamieson 1991b) and in an SEM micrograph of *C. opilio* by Chiba et al. (1992), thus seeming to confirm its lability. It has not been demonstrated in *C. furici*, *M. longirostris*, *P. rebierei* or the two *Oxypleurodon* species (Jamieson and Tudge 2000) and has not been observed in *M. brachydactyla* (Simeó et al. 2010) or in *S. rufescens* and *C. retusa* (present study). A posterior nuclear process, occurring also in the ‘outgroup’ Paguroidea, is seen as a plesiomorphy retained paraphyletically in raninoids and majoids, being apomorphically lost in higher crabs (Jamieson 1991a,b; Jamieson et al. 1995).

**Lateral arms.** The sperm of the inachid *C. furici*, and at least the pisine *O. orbiculatus*, has a triradiate form, with a nuclear arm at each vertex, a condition considered by Jamieson and Tudge (2000) to be plesiomorphic for the Meiura (Anomala + Brachyura) of Scholtz and Richter (1995). Three arms (or at least three prominent vertices) are also seen in *C. retusa* (Fig. 2F), *H. heraldica* (Fig. 2J) and *M. monoceros* (Fig. 2L). Several arms were demonstrable in *M. longirostris* but it remained to be determined whether three were larger than the others. Four or five arms are present in *S. aspera* (Fig. 2D,E) and *S. rufescens* (Fig. 2B) and, indistinctly, in *P. tuberculata* (Fig. 2G) and in *Stenorhynchus seticornis* (Hinsch 1973). Four arms are demonstrated in the comprehensive account of *I. phalangium* (Rorandelli et al. 2008) and, although occasionally with only three, in *M. brachydactyla* (Simeó et al. 2010). In *C. opilio*, there are 4–10 processes radiating from the nuclear body, with a mean value of 7 (Chiba et al. 1992); the processes contain chromatin but, like the nucleus, lack microtubules (Beninger et al. 1988; Chiba et al. 1992).

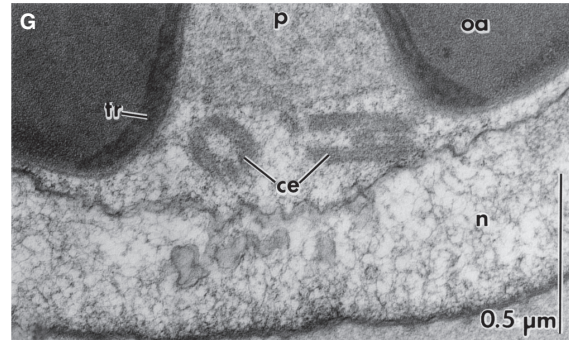
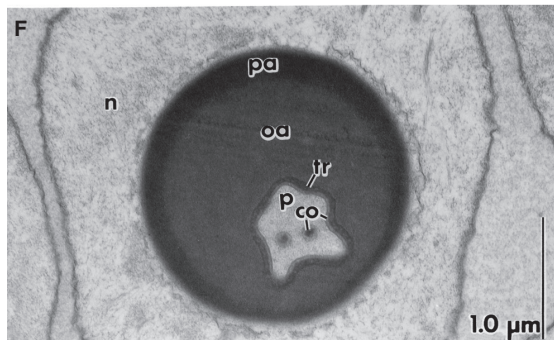
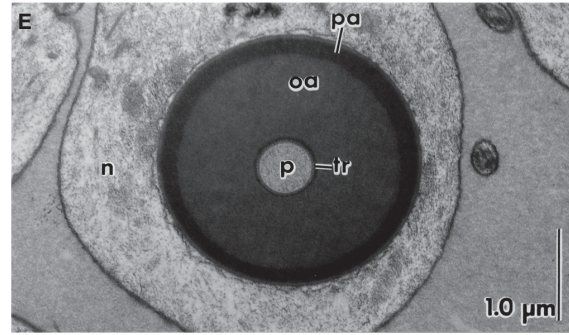
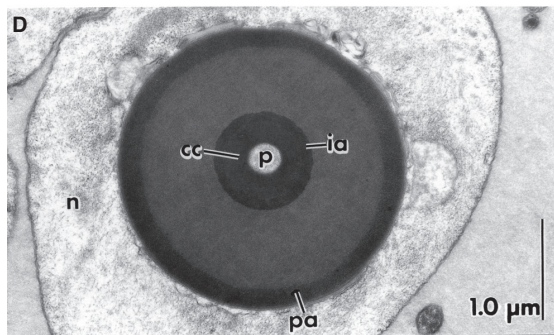
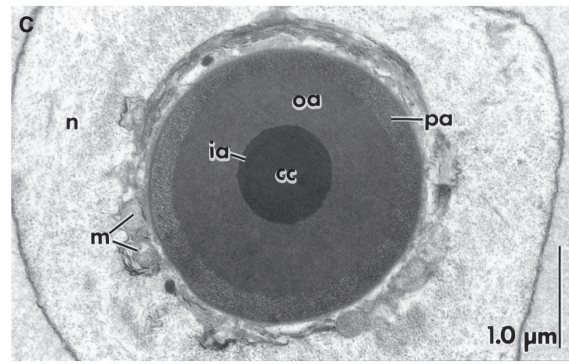
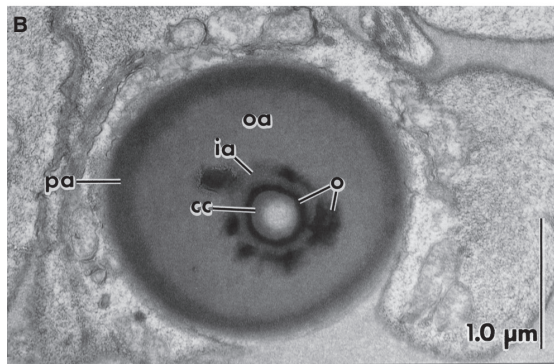
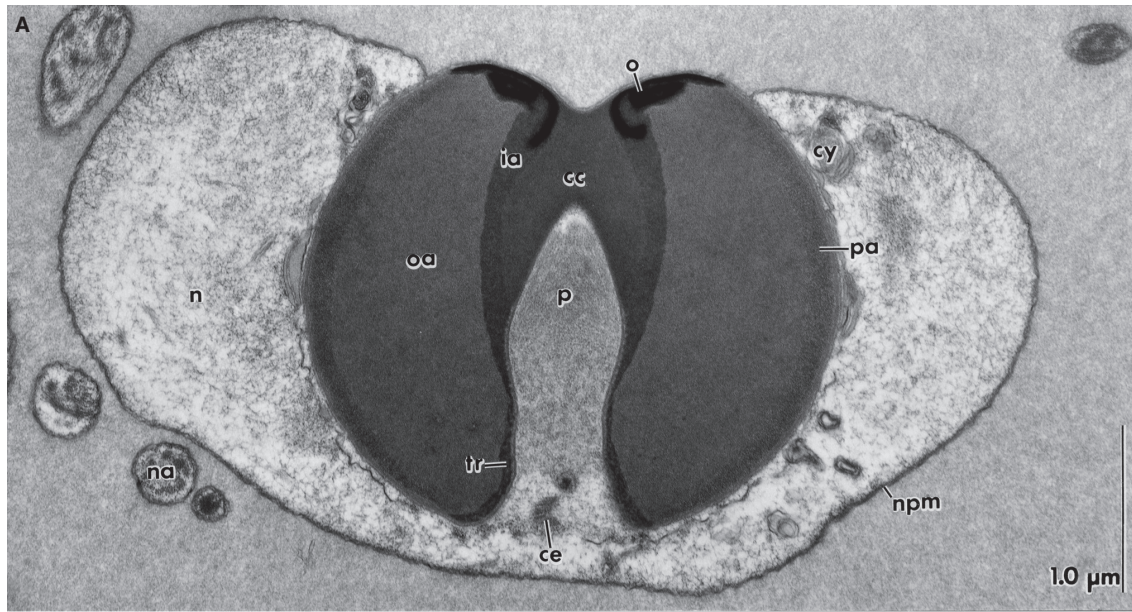
Strong development of microtubules in the lateral arms, which was demonstrated by Hinsch (1973) in *L. emarginata*,

is regarded as a plesiomorphic condition for Brachyura (Jamieson 1991b), further supporting a basal position for majoids as microtubules, also present in anomuran lateral arms (Tudge 1992, 1995a,b, 2009), are reduced or absent from most eubrachiurans. Hinsch (1969) states that in *L. emarginata* and *M. trispinosum*, nuclear material forms the three lateral arms and posterior median process and that a core of microtubules of centriolar origin extends the length of these processes. The ‘fibrils’ in the arms arise as microtubules in association with the centrioles and pass through a ‘pore’ in the nuclear membrane and lie within the nucleoplasm of the arms. Tudge and Justine (1994) also reported, for *M. squinado*, that bundles of microtubules encircle the acrosome vesicle and emerge as discrete arms via the nuclear material; they detected alpha and beta tubulin in a pattern consistent with the arrangement of external microtubular arms and internal bundles of cytoplasmic microtubules and intense actin fluorescence in the entire acrosome and extruded perforatorial column. Perez et al. (1986) did not observe microtubules by electron microscopy although they demonstrated the existence of contractile proteins (myosin, actin) and tubulin within the DNA-containing nucleocytoplasmic compartments of *Libinia* sperm.

Putative microtubules have been reported in the reacting spermatozoa of many other eubrachiurans (see references in Jamieson and Tudge 2000). They are present in the nuclear arms and the cytoplasm in *H. heraldica* (Fig. 8B,C,E) and have been observed in the cytoplasm only in *S. rufescens* (Fig. 5A,G,H). They were not seen in the nuclear arms of *M. monoceros* (Jamieson 1991b), or in other majoids investigated by Jamieson and Tudge (2000), nor in *C. opilio* (Chiba et al. 1992) or *P. tuberculata* (present study). It is uncertain whether the three short arms of *Podochela* (Hinsch 1973) contain nuclear material. The state of maturity and fixation of sperm may well affect the visibility of microtubules. As stated by Chiba et al. (1992), the functional significance of the nuclear processes or arms in brachyuran spermatozoa is still obscure, but Simeó and colleagues (Simeó et al. 2010) have suggested a role in elicitation of the acrosome reaction at egg attachment via large surface area ion transport.

**Centrioles.** As a symplesiomorphy, centrioles (labelled 21 in Fig. 1) are present in majoids (Hinsch 1973; Jamieson 1991b, 1994; Chiba et al. 1992; Jamieson et al. 1995; Jamieson and Tudge 2000) including *C. furici*, *P. rebierei* and *O. orbiculatus* (Jamieson et al. 1998; Jamieson and Tudge 2000), as *inter alia*

**Fig. 5**—Transmission electron micrographs of *Schizophrys rufescens* spermatozoa. — **A**. Longitudinal section. — **B–F**. Transverse sections through the acrosome vesicle, (**B**) at the level of the operculum, (**C**) the anterior portion of the perforatorial chamber, (**D**) the anterior/mid portion of the perforatorial chamber, (**E**) the mid/posterior portion of the perforatorial chamber and (**F**) the base of the perforatorial chamber showing the extensions of the perforatorial wall. — **G–H**. Sections through the cytoplasm showing microtubules. cc, central acrosome zone; ce, centriole; co, corrugations (longitudinal incomplete septa)/extensions of the perforatorial chamber wall; cy, cytoplasm; ia, inner acrosome zone; m, degenerating mitochondrion; mt, microtubules; n, nucleus; o, operculum; oa, outer acrosome zone; p, perforatorial chamber; pa, peripheral acrosome zone; tr, thickened ring.



in parthenopoids (Hinsch 1973; as Parthenopidae), portunoids, dorippoids and the ocypodoid *Macrophthalmus* but not, for instance, xanthoids (Jamieson 1991b; Jamieson et al. 1995). Two centrioles are present in *L. emarginata*, *L. dubia* and, it is implied, in *M. trispinosum*, *Stenorhynchus seticornis* and *Pitho lherminieri* (Hinsch 1973), *M. squinado* (Tudge and Justine 1994), *C. retusa* (Fig. 6G), *P. tuberculata* (Fig. 7G) and *H. heraldica* (Fig. 8D). The subacrosomal portion of the cytoplasm in *C. opilio* (Chiba et al. 1992) ‘occasionally’ contained centrioles. Observation of only a single centriole in *S. aspera* (Figs 3 and 4A,F) and *S. rufescens* (Fig. 5A) does not appear to be an artefact of the plane of section. A centriole is illustrated for *I. phalangium* (Rorandelli et al. 2008) but, although none was seen in *M. longirostris* (Jamieson et al. 1998) and *M. monoceros* (present study), its absence requires confirmation.

**Spermatophores.** Some additional data on spermatophores are now available. In *S. rufescens* (Fig. 2A) and *P. tuberculata* (Fig. 2H), there are many spermatozoa per spermatophore. No spermatophores have been observed in *C. retusa* in which the sperm are grouped together in a large mass. In *M. monoceros*, there is usually only one spermatozoon (Fig. 2M) or few to several spermatozoa (Fig. 9A), with a maximum of approximately six, present in a spermatophore. That of *H. heraldica* contains only one spermatozoon (Figs 2I and 8E,G). There are as many as 70 per spermatophore in *I. phalangium* (Rorandelli et al. 2008).

## Discussion

The comparative morphology of majoid sperm has been treated above and this discussion will deal with phylogenetic considerations.

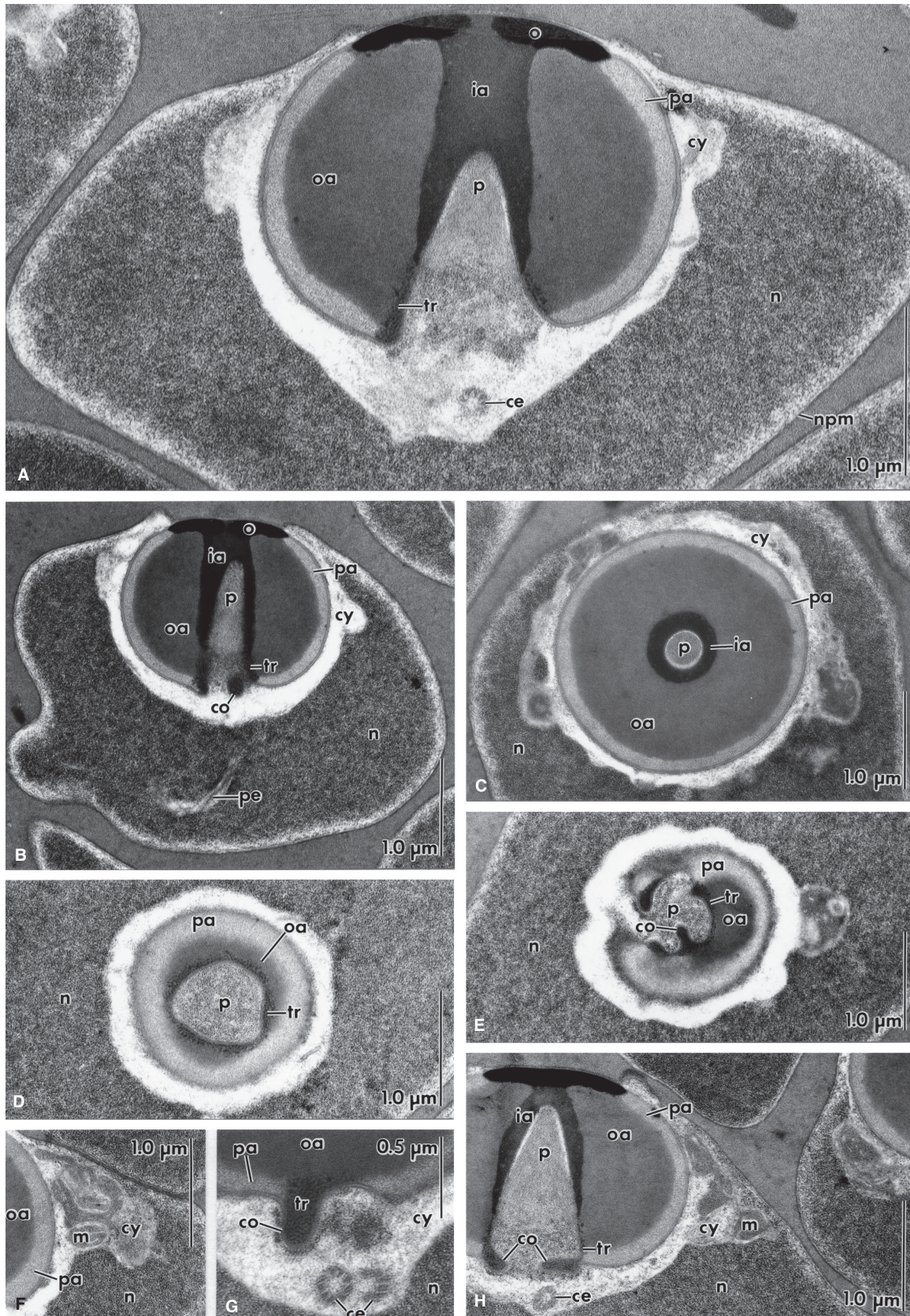
There has been some discordance among phylogenies of the Brachyura based on molecular and other data, partly due to differences in the taxa and genetic sequences sampled; but where non-majoid families have been sampled with the Majoidea (see below), the latter group has usually emerged as the most basal of the Eubrachyura. It should be noted that most of the molecular and larval phylogenies for the majoids are based on relatively small taxon sets from the Americas and not from the Old World majoid fauna (the centre of majoid biodiversity is in the Pacific). This will have implications for current comparisons with spermatozoal data, and their inferred phylogenies, from the mostly Pacific taxa. From cla-

distic analysis of spermatozoal characters of a wide range of brachyuran families, with anomuran outgroups (Jamieson 1994; Jamieson et al. 1995), ‘majids’, that is, majoids (represented by the generalized Oxyrhyncha of Hinsch (1973) and the epialtine *M. monoceros*) were found to be the most basal eubrachyurans. However, when sperm data were combined with non-spermatozoal characters, dorippids [represented by *Neodorippe callida* (as *N. astuta*)] appeared to be more basal. Porter et al. (2005) found *Maja* basal to the examined Eubrachyura, using 16S mtDNA, 18S and 28S rRNA, and the histone H3 gene. The basal position of Majoidea, from a purely spermatological viewpoint, corresponds with the basal position also attributed to them by Rice (1981, 1983), as Majidae, from zoeal larval morphology.

On the basis of characters of foregut ossicles, Brösing et al. (2006) found the Majidae (represented only by *Hyas* and *Libinia*) to be the most basal eubrachyurans; the analysis was unusual in giving the Raninoidea the most advanced position. From analysis of small subunit nuclear ribosomal RNA, Ah Yong et al. (2007) found either the Majoidea or the Dorippoidea (including Dorippidae and Ethusidae) to be the least derived eubrachyurans but the single minimal length topology showed the epialtid *Menaethius* (*M. monoceros*) and the majid *Schizophrys* (*S. aspera*), the only majoids sampled, to be jointly the most basal eubrachyurans of 25 brachyuran families. While they questioned some results from sperm ultrastructure, the tree of Ah Yong et al. (2007; their Fig. 4) placed thoracotreme genera as an advanced group within the heterotreme assemblage and recognized the Majoidea as the most basal heterotremes, both arrangements previously deduced from sperm ultrastructure (Jamieson 1991b; Jamieson et al. 1995). However, Chu et al. (2009b), using protein-coding genes (NaK, GAPDH and enolase), give the Majoidea an advanced position in the Heterotremata. The majority view is that majoids are the most basal eubrachyurans.

From larval morphology of majoids only, Marques and Pohle (1998) found that the Oregoniinae (*Hyas* + *Chionoecetes*) formed a basal monophyletic group but, contrary to established ideas for the monophyly of Inachinae, *Macrocheira* was basal to the Oregoniinae. *Macrocheira* is traditionally classified in the Inachidae (Ng et al. 2008) but is a somewhat aberrant member of that family being considered the ‘most basal’ genus of all Majoidea by Clark and Webber (1991) based on larval features. Other taxa in the analysis of Marques and Pohle (1998) did not form monophyletic groupings based on classical subfamilial assignments. In a further analysis of larval

**Fig. 6**—Transmission electron micrographs of *Camposcia retusa* spermatozoa. — **A**. Longitudinal section. — **B–F**. Transverse sections through the acrosome vesicle, (**B**) at the level of the operculum, (**C**) at the level of just below the operculum, (**D**) the anterior portion of the perforatorial chamber, (**E**) the mid portion of the perforatorial chamber and (**F**) the base of the perforatorial chamber showing the extensions of the perforatorial wall. — **G**. LS of the posterior portion of the spermatozoa showing the two centrioles. cc, central acrosome zone; ce, centriole; co, corrugations (longitudinal incomplete septa)/extensions of the perforatorial chamber wall; cy, cytoplasm; ia, inner acrosome zone; m, degenerating mitochondrion; mt, microtubules; mn, microtubular-nuclear arm; n, nucleus; o, operculum; oa, outer acrosome zone; p, perforatorial chamber; pa, peripheral acrosome zone; pe, posterior extension of cytoplasm; sw, spermatophore wall; tr, thickened ring.



characters, in this case combined with molecular data for 16S, CO1 and 28S, for 37 majoid species, Hultgren and Stachowicz (2008), found a monophyletic Oregoniidae branching close to the base of the tree (Fig. 10) (in fact forming an unresolved basal assemblage with Majidae and an inachid + mithracid clade). They also found a close phylogenetic association among the investigated Epialtidae, Pisidae, Tychiidae and part of the Mithracidae, and some support for monophyly of the Inachidae and Majidae. However, some majoid families were not monophyletic (Mithracidae, Pisidae, Epialtidae) and these authors suggested that adult morphological characters traditionally used to classify majoids into different families may be subject to convergence.

Although spermatozoal ultrastructure has contributed convincingly to brachyuran phylogenetics (see above), the paucity of data for many majoid taxa suggests that a cladistic analysis would be premature. Therefore, this discussion will be confined to an investigation of relationships and trends that appear discernible from comparative ultrastructure of majoid species examined to date and will be related to the molecular and larval phylogeny of Hultgren and Stachowicz (2008).

The flattened subspheroidal form of the acrosome and wide depressed operculum of the oregoniid (*C. opilio*) sperm is a departure from the plesiomorphic eubrachyuran condition of a spheroidal acrosome with domed operculum computed by Jamieson *et al.* (1995). The *Chionoecetes* condition closely resembles that in the inachids *C. furci* and *P. rebierei*. The latter two species therefore form a morphological link between the oregoniid and the semilunar condition in the inachids *M. longirostris* and *I. phalangium*. These four inachids may thus represent a subgroup within the Inachidae in which *Macropodia* and *Inachus* are especially close. Of the remaining examined inachids, *C. retusa* (Fig. 6A) shows some apical flattening of the acrosome and broadening of the operculum that might merit its inclusion in this subgroup. The acrosome of the inachid *P. riisei* is more nearly subspheroidal, with no opercular depression, and would not support inclusion in the subgroup, a finding in agreement with investigations based on external morphology (Guinot 2012). Most inachid sperm, when well fixed, display a clearly defined, domed central acrosome zone capping the perforatorial chamber: *Camposcia retusa* (Fig. 6A–D), *C. furci*, *G. hyalinus*, *I. phalangium*, *M. longirostris* and *P. rebierei*. This zone is absent in the inachoidid *P. tuberculata* (Fig. 7A–C) and apparently in *S. seticornis*; and

presence is doubtful in *Podochela riisei* (micrographs in Hinsch 1973). Placement of *Stenorhynchus* in the Inachoididae by Guinot (2012), instead of the Inachidae, is supported by the absence of the central acrosome zone usually present in inachids. Apparent absence of this zone in *Podochela* is also atypical of inachids. This genus is traditionally included in Inachidae (Ng *et al.* 2008), but is shown to be distinct from typical inachids such as *I. phalangium* by Guinot (2012).

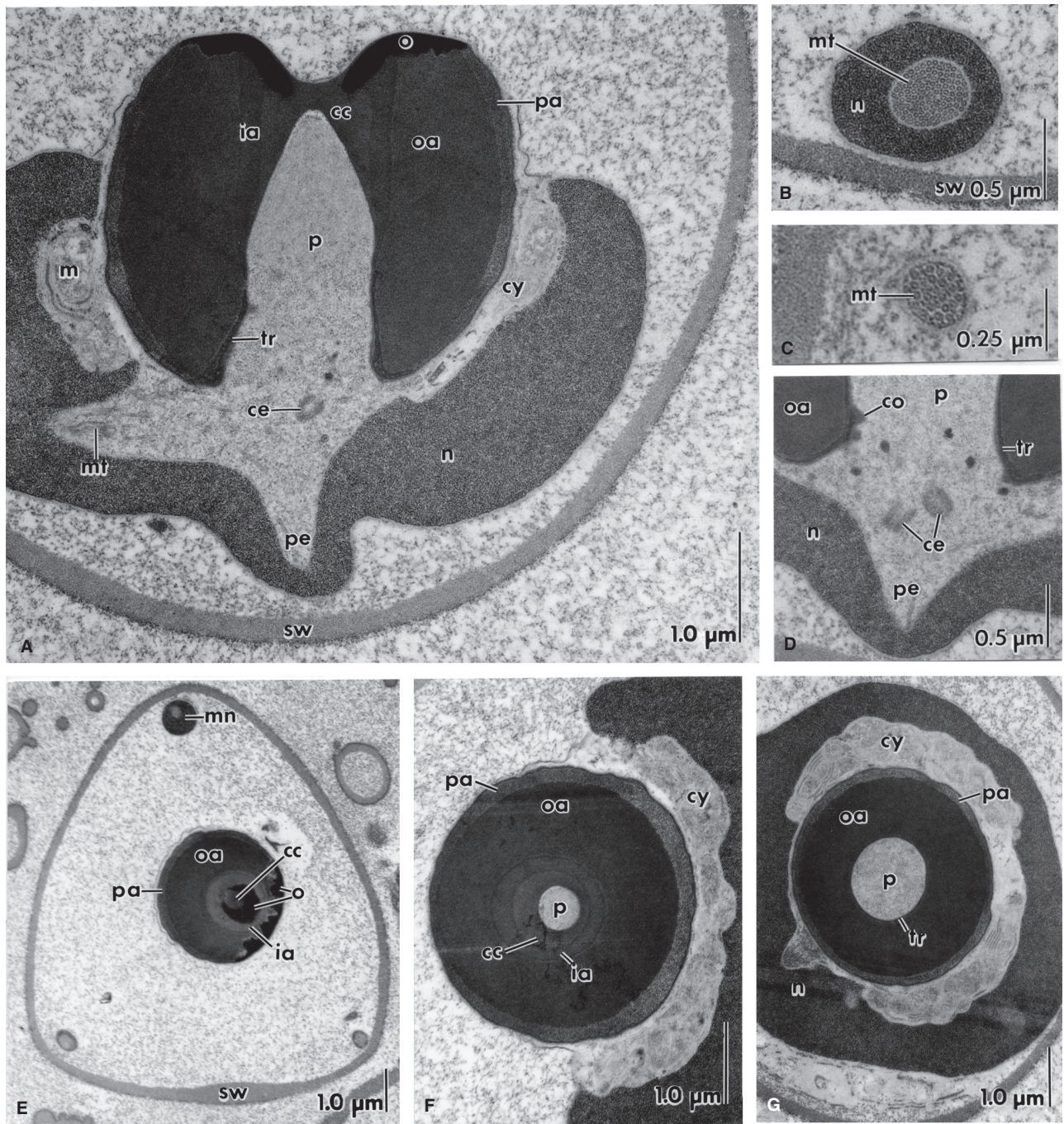
The central acrosome zone is also seen in both *Schizophrys* species (Figs 3–5), in the pisines *O. orbiculatus* and *O. stuckiae* and the epialtines *H. heraldica* (Fig. 8A,F) and *M. monoceros* (Fig. 9A,B,E) and appears to be an autapomorphy of these majoids. It has not been seen in the tychine *P. lherminieri* and is not described for the majine *M. squinado* (Tudge and Justine 1994).

Although *G. hyalinus* has a broad operculum and some flattening, the presence of a peripheral acrosome zone is a resemblance to the two Inachoididae *P. tuberculata* and, particularly, *S. seticornis* but also to *M. squinado*. *Pyromaia tuberculata*, although also possessing a peripheral acrosome zone, is distinct from the other inachoidids in having a slightly dome-shaped operculum. Oregoniid–inachid relationships are unresolved in the phylogeny of Hultgren and Stachowicz (2008) where the Inachoididae is not represented.

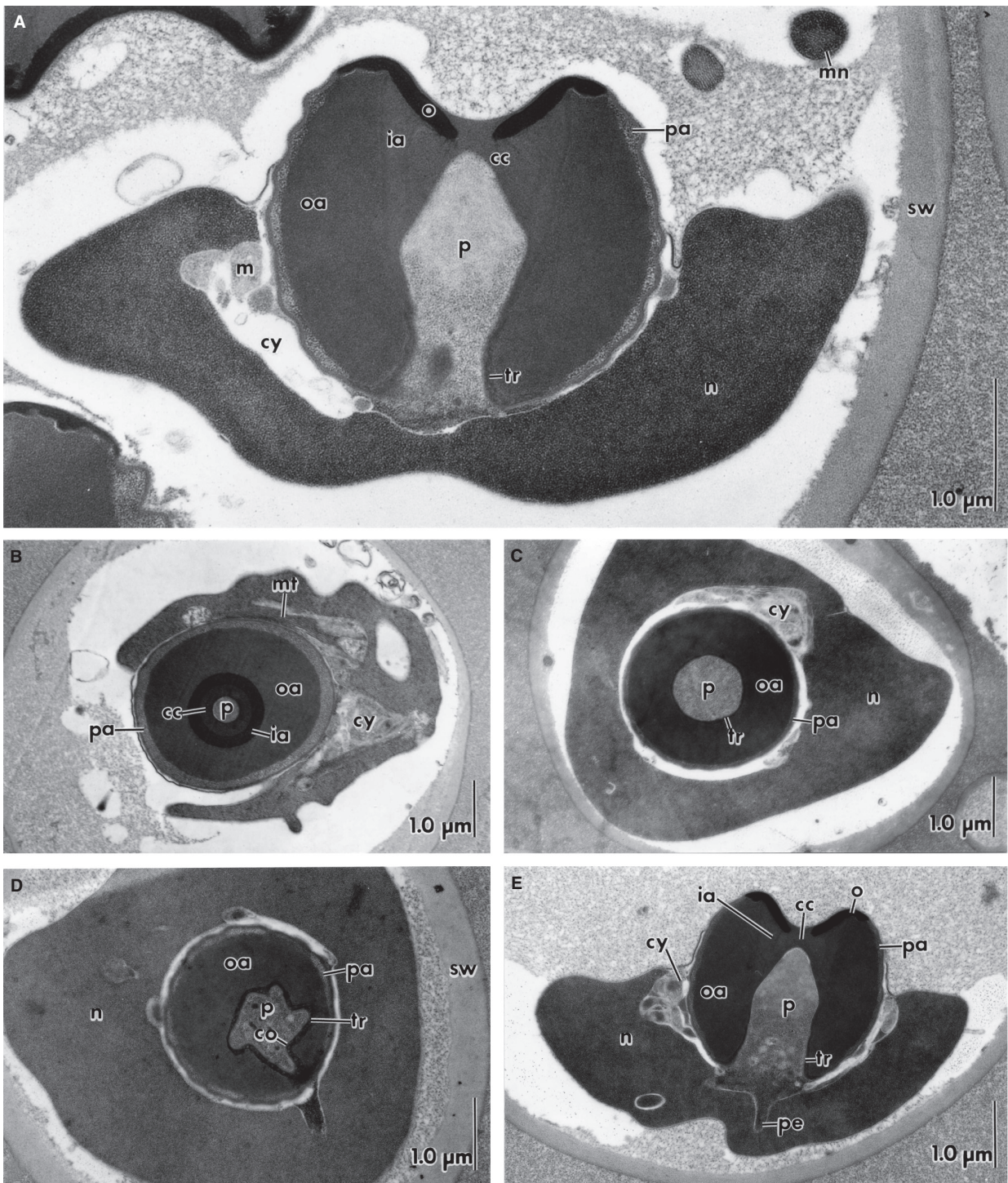
Hultgren and Stachowicz (2008) found Inachidae and part of Mithracidae (here regarded as a subfamily of the Majidae, see Table 1) to be sister taxa. From a micrograph by Hinsch (1973), the sperm of the mithracine *M. trispinosum* more closely resembles those of the Inachoididae, including the albeit indistinct presence of a peripheral acrosome zone, than those of the Inachidae. It also, however, shows similarities to *M. brachydactyla*, which is here interpreted as having a peripheral acrosome zone. Similarity of *M. trispinosum* to the majine *S. aspera* (Figs 3 and 4), lacking a peripheral acrosome zone, and *S. rufescens* (Fig. 5) is not strong. *S. rufescens* exhibited a rudimentary peripheral zone but this may be the product of partial acrosome reaction. From the small sample of Majidae known for spermatozoal ultrastructure (the Majinae *M. brachydactyla*, *S. aspera*, *S. rufescens* and the mithracine *M. trispinosum*), the family does not appear homogeneous. Based on CO1 and 18S sequences, Sotelo *et al.* (2009) found *Schizophrys* to be basal to *Maja*.

The phylogeny of Hultgren and Stachowicz (2008) gives *Libinia*, in a highly polyphyletic Pisidae, an advanced position, but the phylogenetic position of *Libinia* within the Majoidea is

**Fig. 7**—Transmission electron micrographs of *Pyromaia tuberculata* spermatozoa. — **A–B**. Longitudinal sections. — **C–E**. Transverse sections through the acrosome vesicle, (**C**) at the anterior portion of the perforatorial chamber, (**D**) the mid/posterior portion of the perforatorial chamber and (**E**) the base of the perforatorial chamber showing the extensions of the perforatorial wall. — **F**. Section through the cytoplasm. — **G**. Longitudinal section of the posterior portion of the spermatozoa showing the two centrioles. — **H**. Longitudinal section of the spermatozoa showing the extensions of the perforatorial wall. ce, centriole; co, corrugations (longitudinal incomplete septa)/extensions of the perforatorial chamber wall; cy, cytoplasm; ia, inner acrosome zone; m, degenerating mitochondrion; n, nucleus; npm, nuclear plasma membrane; o, operculum; oa, outer acrosome zone; p, perforatorial chamber; pa, peripheral acrosome zone; pe, posterior extension of cytoplasm; tr, thickened ring.



**Fig. 8**—Transmission electron micrographs of *Huenia heraldica* spermatozoa. — **A**. Longitudinal section. — **B–C**. Transverse sections through an arm. — **D**. LS of the posterior portion of the spermatozoa showing the two centrioles. — **E–G**. Transverse sections through the acrosome vesicle, (**E**) at the level of the operculum, (**F**) the anterior portion of the perforatorial chamber, (**G**) the mid portion of the perforatorial chamber. cc, central acrosome zone; ce, centriole; co, corrugations (longitudinal incomplete septa)/extensions of the perforatorial chamber wall; cy, cytoplasm; ia, inner acrosome zone; m, degenerating mitochondrion; mn, microtubular-nuclear arm; mt, microtubules; n, nucleus; o, operculum; oa, outer acrosome zone; p, perforatorial chamber; pa, peripheral acrosome zone; pe, posterior extension of cytoplasm; sw, spermatophore wall; tr, thickened ring.



**Fig. 9**—Transmission electron micrographs of *Menaethius monoceros* spermatozoa. — **A**. Longitudinal section. — **B–D**. Transverse sections through the acrosome vesicle, (**B**) the anterior portion of the perforatorial chamber, (**C**) the mid portion of the perforatorial chamber, (**D**) the base of the perforatorial chamber showing the inward extensions of the perforatorial wall. — **E**. Longitudinal section of a spermatozoon showing a posterior extension of the cytoplasm into the nucleus. cc, central acrosome zone; co, corrugations (longitudinal incomplete septa)/extensions of the perforatorial chamber wall; cy, cytoplasm; ia, inner acrosome zone; m, degenerating mitochondrion; mt, microtubules; n, nucleus; o, operculum; oa, outer acrosome zone; p, perforatorial chamber; pa, peripheral acrosome zone; pe, posterior extension of cytoplasm; sw, spermatophore wall; tr, thickened ring.

**Table 2** Acrosome dimensions in majoids (present study)

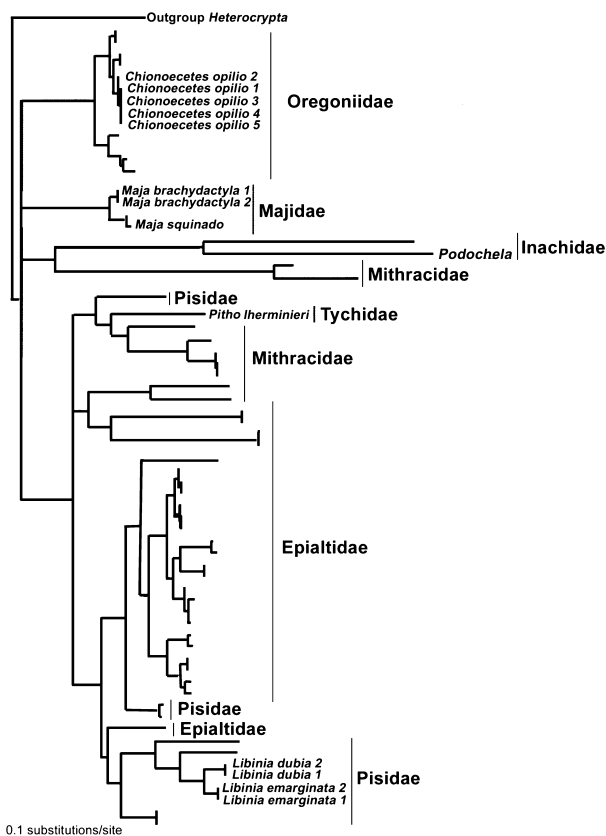
Species	Acrosome mean greatest length $\mu\text{m}$ (n, SD)	Acrosome mean greatest width $\mu\text{m}$ (n, SD)	Ratio length: width acrosome	Operculum mean greatest width $\mu\text{m}$ (n, SD)
<b>Majidae</b>				
<i>Schizophrys aspera</i>	3.36 (2, 0.30)	4.22 (2, 0.11)	0.80	2.50 (2, 0.10)
<i>Schizophrys rufescens</i>	3.81 (13, 0.19)	4.50 (14, 0.27)	0.85	2.41 (14, 0.21)
<b>Inachidae</b>				
<i>Camposcia retusa</i>	2.55 (8, 0.16)	3.11 (8, 0.15)	0.82	1.56 (8, 0.07)
<b>Inachoididae</b>				
<i>Pyromaia tuberculata</i>	1.85 (11, 0.13)	2.45 (11, 0.15)	0.76	1.33 (11, 0.08)
<b>Epialtidae</b>				
<i>Huenia heraldica</i>	2.98 (11, 0.30)	3.65 (11, 0.26)	0.82	2.45 (12, 0.25)
<i>Menaethius monoceros</i>	2.97 (12, 0.38)	3.35 (12, 0.33)	0.89	2.49 (11, 0.41)

not determinable from spermatozoal descriptions. In cross sections of the sperm of *L. emarginata* (Hinsch 1969, 1986; Vaughn and Hinsch 1972) and *L. dubia* (Hinsch 1973), it can be seen that there is a peripheral acrosome zone, although

sometimes indistinct. It is also illustrated for *Macrocoeloma* and *Stenorhynchus* (Hinsch 1973). From the generalized ‘oxyrhynch’ sperm, which has a globular acrosome, is it not apparent that a central acrosome zone is present.

The Dorippidae and Hymenosomatidae (Guinot and Richer de Forges 1997) have been found in various phylogenies to be adjacent to the Majoidea and require some discussion here.

Dorippidae were found to consist of two main lineages from analysis of 16S rDNA gene sequences of five species (Fan *et al.* 2004) but a more complete phylogenetic tree inferred from three mitochondrial genes (16S and 12S rRNA, and COI) by Sin *et al.* (2009) showed several distinct lineages concurring with multiple groupings based on the overall carapace morphology and structure of the first gonopods (the first and second abdominal pleopods in males are modified into two pairs of gonopods for reproduction). From morphology, the superfamily Dorippoidea (Dorippidae and Ethusidae, in the past considered as two subfamilies of Dorippidae), is considered to be basal in the Heterotremata, like the Majoidea, but the precise relationships between the two superfamilies remain unknown (see Guinot 2011b; 46–48). It is suggested (p. 48 in the latter work) that hymenosomatids, majoids and dorippids had a common ancestor, and that hymenosomatids and majoids subsequently diverged (hence the morphological and molecular similarities between hymenosomatids and the basal majoids such as inachoidids and inachids) while dorippids retained these ancestral relations. The sperm of the investigated dorippids, *Neodorippe callida* (as *N. astuta*) (Jamieson and Tudge 1990, 2002; Jamieson 1991a,b, 1994) and *Ethusina indica* (Jamieson and Tudge 2000) have characteristic eubranchyuran features. Differences of dorippid sperm from the usual majoid condition are perforatorial chamber extending almost to the operculum; presence, at least in *N. callida*, of an acrosome ray zone; absence of the central acrosome zone; and prominence of the operculum. In *E. indica*, the acrosome is slightly wider than long, and the perforatorial column is ellipti-



**Fig. 10**—Phylogenetic tree of the Majoidea, shown as a Bayesian consensus tree based on larval characters combined with molecular data for 16S, CO1 and 28S, adapted from Hultgren and Stachowicz (2008). Only those species common to the present study are named.



cal and not stoutly baton-shaped, unlike *N. callida*. Furthermore, a multilaminar membrane, seen in *N. callida*, is absent or in a state of disruption in *E. indica* (Jamieson and Tudge 1990, 2000). Only the sperm of *N. callida* has been included in a cladistic analysis and which of the two species is the more basal has not been determined. When somatic and spermatozoal characters were combined, *Neodorippe* formed the sister group of all other included eubrachiurans. As previously noted when sperm ultrastructure alone was used in cladistic analysis, majoids appeared to be the most basal and plesiomorphic family of the investigated Eubrachiura (Jamieson 1994; Jamieson et al. 1994, 1995; Jamieson and Tudge 2000).

The spermatozoa of the Hymenosomatidae, studied in two species of *Odiomaris*, *O. pilosus* and *O. estuarius* and in *Elamena vesca* (Richer de Forges et al. 1997; Jamieson and Tudge 2000), differ in at least nine major characteristics from those of all other investigated brachiuran taxa (Richer de Forges et al. 1997; Jamieson and Tudge 2000). The combination of spermatozoal characters, collectively and often individually, is so markedly distinctive from that of the families with which the Hymenosomatidae has traditionally been associated, with either the heterotreme Majoidea or thoracotremes such as Varunidae, Ocypodidae and Gecarcinidae (Jamieson et al. 1995), that Richer de Forges et al. (1997) recognized a ‘hymenosomatid type of spermatozoon’. A ‘majid–hymenosomatid’ relationship was not supported by spermatozoal ultrastructure, because the two families differed in the nine distinctive characters of the Hymenosomatidae (Jamieson and Tudge 2000). One of these hymenosomatid characters, the almost septate condition of the inner acrosome zone, is, exceptionally, approached in the inachid *C. furci* but could not be considered a convincing synapomorphy between the two families (Jamieson et al. 1998). The highly developed projection of the acrosome from the nucleus in hymenosomatid spermatozoa recalls the totally emergent acrosome of podotremes. A less pronounced emergence is seen in Anomura (Jamieson and Tudge 2000), dorippids (Jamieson and Tudge 1990, 2000) and majoids (Jamieson et al. 1998; Jamieson and Tudge 2000) and may represent the plesiomorphic condition in Brachiura. This acrosome emergence is apomorphically increased in podotremes and may also have occurred independently in the hymenosomatid spermatozoon, while thoracotremes and most heterotremes have completely withdrawn the acrosome into the nucleus and cytoplasm. Taxonomically, hymenosomatids may be grouped, at least provisionally, in the superfamily Hymenosomatoidea in close proximity to the Majoidea and Dorippoidea (Guinot 2011a, b). In the minimal length topology of Ah Yong et al. (2007) based on small subunit nuclear ribosomal RNA sequences, the Dorippidae and Hymenosomatidae were sister groups immediately above the majoids represented by the epialtid *Menaethius* and the majid *Schizophrys*.

Some mention should be made of the Cryptochiridae (the sole family of the superfamily Cryptochiroidea). This family

has been traditionally regarded as thoracotreme because of its sternal male gonopores. The thoracotreme status of the Cryptochiridae was supported by molecular analyses using 16S rDNA sequence data of *Haplocarcinus marsupialis* by Wetzer et al. (2009) who concluded that cryptochirids were ‘highly modified grapsids’. Earlier, the family Cryptochiridae had, however, provisionally been removed from the Thoracotremata by Guinot and Richer de Forges (1997). Several works (Gurney 1942; Fize 1956; Wear and Fielder 1985) recognize numerous similarities in zoeal features between Cryptochiroidea and Hymenosomatoidea (as well as with Pinnotheroidea), which all present traits that are unique to Brachiura and may be those of ancient groups (Guinot 2011b). Relationships of Cryptochiridae therefore require reappraisal (see Ng et al. 2008).

From sperm ultrastructure, Cryptochiridae was included in the Heterotremata *sensu stricto* by Jamieson and Tudge (2000) who described the sperm of *Cryptochirus coralliodytes* and *H. marsupialis*. The sperm of the two species share a striking synapomorphy, which is an autapomorphy of the family, the presence of a collar-like lateral extension of the cytoplasm around the operculum with the appearance of a broad epaulette. A similarity and putative synapomorphy with the heterotreme xanthids and bythograeids is the highly distinctive oblique orientation of the accessory opercular ring (9 in Fig. 1). A thin reticular zone immediately surrounding the posterior half of the perforatorial column may be the equivalent of a heterotreme acrosome ray zone. A periopercular rim (8 in Fig. 1) is moderately developed in *C. coralliodytes*. Its equivalent in *H. marsupialis* is filled with a moderately electron-dense wedge of material, which abuts on the rim of the dense operculum. There are many slender nuclear arms in *C. coralliodytes* but these have not been seen in *H. marsupialis*. The operculum of the latter species is strongly protuberant and has an apical button resembling that of thoracotreme sperm although this is not recognizable in the domed but less protuberant operculum of *C. coralliodytes*. Thus, sperm ultrastructure is somewhat equivocal with regard to placement of these cryptochirids in the Thoracotremata or Heterotremata but the prominent operculum strongly differentiates them from the Majoidea. The Hymenosomatidae are distinguished by the emergent acrosome, its strong protuberance being unique in the Brachiura. Thus, the Cryptochiroidea and Hymenosomatoidea (each with only one family) are similar in having male sternal gonopores (as the thoracotremes), sperm features that are equivocal with regard to their placement within Brachiura, and larval traits that are unique to Brachiura (see Rice 1983; for example). Larval data (e.g. Wear and Fielder 1985) support a close relationship of Cryptochiroidea with Hymenosomatoidea, the latter being commonly regarded as close to Majoidea (Ng et al. 2008). All these similarities, perhaps due to convergence, first recognized by Rice (1983) and later Ng et al. (2008), do not permit for the moment a reasonable hypothesis on the phylogeny of these families.

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