

Reprint

# REPRODUCTIVE BIOLOGY OF INVERTEBRATES

Series edited by

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2000

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## VOLUME IX, PART C

Progress in Male Gamete Ultrastructure and Phylogeny

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# 1. CRUSTACEA-DECAPODA

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## I. ORDER EUPHAUSIACEA

Euphausiid sperm give little indication of the eucarid ground plan. They are ovoidal, lack appendages and have irregular central material which is considered to be chromatin (Jamieson, 1991b). This structure has been confirmed in a detailed description of the sperm of *Meganyctiphanes norvegica* (Fig.1) by Medina *et al.* (1998). If absence of arms were plesiomorphic for eucarids, the arms of most decapods would have to be regarded as having developed independently of those of phyllopods. This is further suggested by their absence from non-eucarid malacostracans. A proposed spermatological phylogeny of the Euphausiacea and other Eucarida by Medina *et al.* (1998) is shown in Fig. 1.

## II. ORDER DECAPODA

### A. Suborder Dendrobranchiata

Spermatozoal ultrastructure has been described for some members of six families of the superfamily Penaeoidea: Penaeidae, Sicyonidae, Atyidae, Aristeidae and Solenoceridae (see Table 1 for list of species investigated). A brief ultrastructural description of the sperm of *Sergestes arcticus* was also provided by Medina (1995a) to exemplify the superfamily Sergestoidea in support of general phylogenetic considerations.

#### 1. Penaeoidea

The Penaeoidea, which, with the Sergestoidea, form the Dendrobranchiata, were at one time grouped with the crangonid and palaemonid shrimps within the Natantia as opposed to the Reptantia which contained, *inter alia*, hermit crabs,

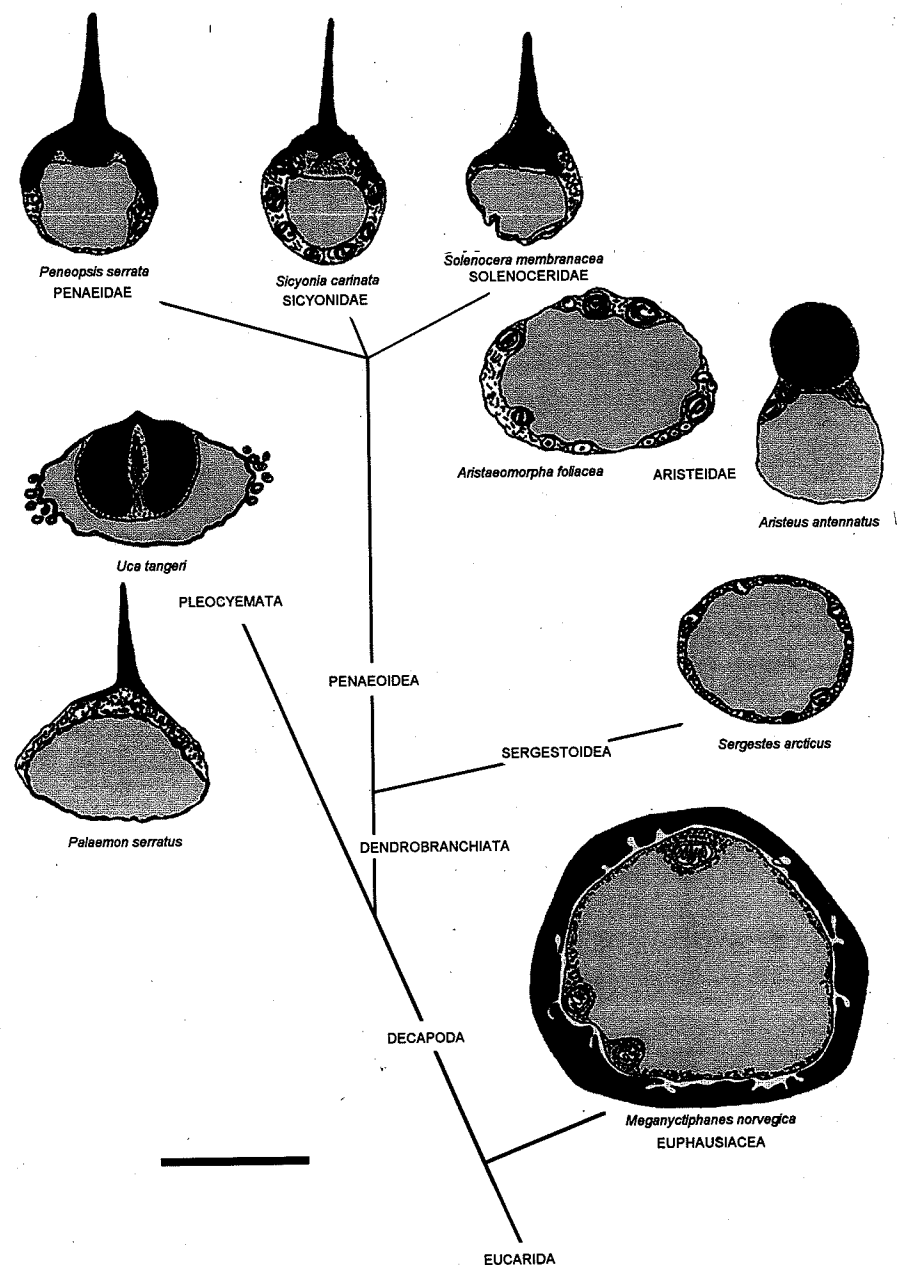


Fig. 1. A proposed spermatological phylogeny of the Euphausiacea and other Eucarida. From Medina *et al.* (1998). *Invertebrate Reproduction and Development*, 34, 65–68, fig. 2.

Table 1

List of Dendrobranchiata for which sperm ultrastructure is known. (Slightly modified from Medina, 1995a)

Superfamily and family	Species	Reference
<b>Penaeoidea</b>		
PENAEIDAE	<i>Parapeneus longirostris</i> (Lucas, 1846)	Medina, 1994
	<i>Peneopsis serrata</i> Bate, 1881	Medina <i>et al.</i> , 1994a
	<i>Penaeus aztecus</i> Ives, 1891	Clark <i>et al.</i> , 1973
	<i>Penaeus japonicus</i> Bate, 1888	Medina <i>et al.</i> , 1994b; Ogawa and Kakuda, 1987
	<i>Penaeus kerathurus</i> (Forskål, 1775)	Medina, <i>et al.</i> , 1994b
	<i>Penaeus setiferus</i> (Linnaeus, 1767)	Felgenhauer <i>et al.</i> , 1988; Krol <i>et al.</i> , 1992; Lu <i>et al.</i> , 1973
	<i>Penaeus vannamei</i> Boone, 1931	Dougherty and Dougherty, 1989; Krol <i>et al.</i> , 1992
	<i>Trachypeneus similis</i> (Smith, 1885)	Krol <i>et al.</i> , 1992
	<i>Sicyonia brevirostris</i> Stimpson, 1874	Brown <i>et al.</i> , 1977
	<i>Sicyonia carinata</i> (Brünnich, 1768)	Medina <i>et al.</i> , 1994a
SICYONIDAE	<i>Sicyonia ingentis</i> (Burkenroad, 1938)	Kleve, <i>et al.</i> , 1980; Shigekawa and Clark, 1986
ARISTEIDAE	<i>Aristaeomorpha foliacea</i> (Risso, 1827)	Medina, 1995b
	<i>Aristeus antennatus</i> (Risso, 1816)	Demestre <i>et al.</i> , 1993, 1997; Demestre and Fortuno, 1992; Medina, 1995b
SOLENOCERIDAE	<i>Solenocera membranacea</i> (Risso, 1816)	Medina, 1995a
<b>Sergestoidea</b>		
SERGESTIDAE	<i>Sergestes arcticus</i> Kroyer, 1855	Medina, 1995a

crayfish, lobsters and crabs. Penaeoids are now regarded as distinct from the suborder Eukyphida, containing the Procarididea and the Caridea, and the Euzygida, containing the Stenopodidea (Schram, 1986). Paraphyly of penaeid and eukyphid shrimps, as opposed to monophyly of the Natantia, appears to be indicated from rRNA studies (Abele, 1991). These authors, with considerable justification, retain the names Caridea for Eukyphida, and Stenopodidea for Euzygida and are followed here.

#### (a) Penaeidae

Within the Dendrobranchiata, penaeid sperm have been the most extensively studied in terms of the number of species examined. These include five *Penaeus* species (*P. setiferus*, *P. vannamei*, *P. aztecus*, *P. japonicus* and *P. kerathurus*), *Trachypeneus similis*, *Parapeneus longirostris* and *Peneopsis serrata* (Table 1). In gross

morphology, the penaeid spermatozoon basically consists of a subspheroidal or ovoid main body and a spike. The main body comprises the central nuclear region, a cytoplasmic band surrounding it posterolaterally, and the acrosomal cap, which overlies the nuclear region anteriorly and is prolonged into a tapering spike (Fig. 1). Both spike and acrosomal cap make up a membrane-bound acrosomal vesicle, with heterogeneous contents, which is directly invested by the plasma membrane. In particular, the spike morphology and substructure vary markedly from species to species. The whole acrosomal complex is completed with the subacrosomal region, which is quite simple in this family, merely containing a sparse flocculent material between the chromatin and acrosomal cap.

The sperm of *Parapeneus longirostris* and *Peneopsis serrata* (Fig. 1) have a central protuberance at the concave side of the acrosomal cap immediately opposite the spike. This supposed synapomorphy is consistent with the close phylogenetic proximity of the genera *Parapeneus* and *Peneopsis*, both grouped together by Burkenroad (1983) within the tribe Parapeneini, which also includes *Artemesia* and *Metapeneopsis*. Confirmation of such a structure in the latter genera would strengthen phylogenetic unity of this taxon.

As in all dendrobranchiate species whose spermatozoon has been ultrastructurally studied, the nuclear region of penaeid sperm consists of a non-membrane-bound, filamentous chromatin mass. Posterolaterally, the chromatin is surrounded by a band of cytoplasm which contains membrane lamellae, vesicles and mitochondria-like bodies, but lacks centrioles and microtubules. Within the Dendrobranchiata, the sperm of *Penaeus japonicus* are exceptional in that they exhibit several microtubule bundles in the cytoplasm (Medina *et al.*, 1994b). The microtubules appear in primary spermatocytes of *P. japonicus* and are retained through spermiogenesis to the mature spermatozoon. In other penaeid species (e.g. *Penaeus kerathurus*, *Parapeneus longirostris*), microtubules are absent from all spermatogenetic stages.

Recent molecular studies (Palumbi and Benzie, 1991) have revealed extensive genetic differences between species of *Penaeus* which have not been accompanied by substantial evolutionary morphological changes. As noted by Medina (1995a), this is congruent with the occurrence of diverse species-specific dissimilarities leading to different ultrastructure of sperm in the genus *Penaeus* and in general in the Penaeidae, and confirms the taxonomic validity of sperm morphology in the Dendrobranchiata.

#### (b) Sicyonidae

Ultrastructural data have been reported for three *Sicyonia* species: *S. brevirostris*, *S. carinata* (Fig. 1) and *S. ingentis* (Table 1). The ultrastructure of the spermatozoon is very similar in *S. ingentis* (Kleve *et al.*, 1980; Wikramanayake *et al.*, 1992) and *S. carinata* (Medina *et al.*, 1994a). In general, as in Penaeidae, the sperm consist of an acrosomal vesicle (formed by the spike and acrosomal cap), subacrosomal

region, and nuclear region surrounded by a cytoplasmic band. Anteriorly, the acrosome and plasma membranes are closely joined. As a taxonomically significant difference, the spike of *S. ingentis* is spiralled, whereas that of *S. carinata* is smooth. The plesiomorphies (1) absence of nuclear envelope and (2) perinuclear cytoplasmic band (containing small and large vesicles and lacking microtubules) are also found in this sperm type. Nevertheless, the highly elaborate subacrosomal region (comprising diverse distinct structures) (Kleve *et al.*, 1980; Medina *et al.*, 1994a) appear to be a clear synapomorphy and autapomorphy of the family Sicyonidae. Compared with the spermatozoa of the other dendrobranchiate families, the acrosomal vesicle shows the apomorphic character that the posterior membrane of the acrosomal cap is intricately folded in a ring of convoluted membrane pouches or digitations (Kleve *et al.*, 1980; Medina, 1995a; Medina *et al.*, 1994a).

Clark and co-workers have described morphological details and biochemical events of the acrosome reaction and penetration of the vitelline envelope in *Sicyonia ingentis* (Chen *et al.*, 1994; Clark *et al.*, 1981, 1984; Clark and Griffin, 1988; Griffin *et al.*, 1988; Griffin and Clark, 1990; Hertzler and Clark, 1993; Lindsay and Clark, 1992a, b; Wikramanayake *et al.*, 1992; Yudin *et al.*, 1979). These accounts investigate the role played by each of the spermatozoal components during fertilization and the biological significance of the acrosomal structures in dendrobranchiates.

#### (c) Aristeidae

The relatively high ultrastructural homogeneity found within the Penaeidae and Sicyonidae is not seen in the Aristeidae. Studies of *Aristeus antennatus* (Demestre *et al.*, 1993, 1997; Demestre and Fortuno, 1992; Medina, 1995b) and *Aristaeomorpha foliacea* (Medina, 1995b) indicate the existence in the family of at least two different ultrastructural sperm plans that are in turn discordant with the penaeid-sicyoniid assemblage. The *A. antennatus* (Fig. 1) sperm type exhibits diverse peculiarities in comparison with the other Dendrobranchiata. First, its spherical acrosome does not cap the nuclear region and lacks both spike and subacrosomal region; the inner arrangement of the acrosomal contents is complex and different from that of any other known dendrobranchiate spermatozoon. Secondly, the cytoplasm does not constitute a band around the filamentous chromatin mass but is accumulated in a collar between the acrosome and nuclear region, enclosing mitochondria-like vesicles and membrane lamellae. Consequently, most of the chromatin is bounded directly by the plasma membrane because the nuclear region is, as in all dendrobranchiates, not membrane-bound. Changes in the structure of the acrosome in the final stages of spermiogenesis are described by Demestre *et al.* (1997).

Medina (1995a) concurs with Demestre and Fortuno (1992) that the basic sperm structure of *Aristeus antennatus* resembles that of spiny lobsters, *Panulirus* spp. (Talbot and Summers, 1978), although with the highly significant absence of

the typically reptantian radial arms, which suggests parallelism rather than a close phylogenetic relationship.

The *Aristaeomorpha foliacea* sperm type (Fig. 1), lacking the acrosome, also differs from the dendrobranchiate unistellate spermatozoal morphology. It consists of a central nuclear region entirely surrounded by the plesiomorphic cytoplasmic band, which includes membrane lamellae, small peripheral vesicles and mitochondria-like bodies. Plesiomorphic features are also the absence of nuclear envelope, centrioles and microtubules.

#### (d) *Solenoceridae*

The spermatozoon of *Solenocera membranacea* (Fig. 1) is similar to that of Penaeidae in general morphology, though it shows conspicuous differences with regard to the other spiked dendrobranchiate sperm. The contents of the acrosomal vesicle are homogeneously electron-dense and the cap appears asymmetrical in sagittal sections, one of its lateral expansions projecting further than the other. Another distinctive feature of the *S. membranacea* spermatozoon is that the plasma membrane becomes separated from the anterior acrosome membrane, the intervening space being occupied by part of the cytoplasmic mass. The perinuclear cytoplasm is rather amorphous, though parallel lamellae and mitochondria-like bodies may be recognized. It is thick under the lateral edges of the acrosomal cap and grows thinner at the posterior part of the sperm. Anteriorly, it forms a thin band separating the scarce subacrosomal substance from the finely filamentous chromatin, a feature that recalls that observed in the penaeid *Parapeneus longirostris* (Medina, 1994).

#### (e) *Sergestidae*

The sperm of *Sergestes arcticus* (Fig. 1) are simple, spheroidal or slightly ellipsoidal cells which closely resemble those found in *Aristaeomorpha foliacea*. They consist of a central, non-membrane-bound nuclear region and surrounding cytoplasm. The finely filamentous chromatin mass is encircled by a thin cytoplasmic band that mainly contains densely-packed electron-clear vesicles and a few mitochondria-like bodies. Occasionally, the cytoplasm encloses lipid-like, highly osmiophilic inclusions. At some points, the cytoplasmic band may be interrupted, thus allowing a direct contact of the nucleoplasm with the plasma membrane. Acrosome, microtubules and centrioles are absent.

In eucarids, absence of the acrosome had been reported only in Euphausiacea (Jamieson, 1991b) and Stenopodidea (Felgenhauer and Abele, 1991). Indeed, there appear to be striking resemblances between the spermatozoa of *Sergestes arcticus*, *Aristaeomorpha foliacea* (Fig. 1) and euphausiid sp. (Jamieson, 1991b; Medina *et al.*, 1998) which very probably are indicative of a phylogenetic relationship. These are: (1) central nuclear region consisting of diffuse, finely

filamentous chromatin, (2) complete disruption of the nuclear envelope, (3) vesiculate, thin perinuclear cytoplasmic band, (4) absence of centrioles and microtubules, and (5) absence of acrosome. The question as to whether the acrosomeless and armless conditions of spermatozoa is plesiomorphic for the Dendrobranchiata has been raised by Jamieson (1991b) and Medina (1995a). The discussion by Medina (1995a) of this subject is reviewed below.

## 2. Phylogenetic relationships (Dendrobranchiata)

Medina (1995a) suggests as clear dendrobranchiate spermatozoal symplesiomorphies: (1) complete loss of the nuclear envelope, (2) filamentous chromatin, (3) absence of centrioles, (4) absence of radial (stellate) arms. The plesiomorphic perinuclear distribution of the cytoplasm does not occur in *Aristeus antennatus* (Fig. 1). In this species, the cytoplasm forms a collar between the acrosome and nuclear region. Whether the acrosome-less condition of *Aristaeomorpha foliacea* (Fig. 1) is an apomorphic character or is a plesiomorphy, is a matter that remains to be established when more data are available. Nonetheless, the finding of similar, acrosome-less sperm patterns in euphausiids (*Euphausia superba* Jamieson, 1991b; *Meganyctiphanes norvegica* Medina *et al.*, 1998; *Nyctiphanes australis*, this study), stenopids (*Stenopus hispidus*, Felgenhauer and Abele, 1991), sergestids (*Sergestes arcticus*) and aristeids (*Aristaeomorpha foliacea*) appears to point to its plesiomorphy. Although the loss of the acrosome is a repeated event throughout evolution of the crustacean sperm (Jamieson, 1989a), the assumption of sperm originally endowed with an acrosome would suppose the highly improbable independent loss of the acrosome in several separate lineages of the eucarid tree.

Jamieson (1991b) has suggested that "the malacostracan acrosome is a new development, in view of evidence that their acrosome originates from the endoplasmic reticulum and not, as is usual, from the Golgi." Several studies have demonstrated that the acrosomal structures in Decapoda derive from, or in association with, cisternae of the endoplasmic reticulum itself or of its specialized portion constituting the nuclear envelope (Anderson and Ellis, 1967; Arsenault, 1984; Arsenault *et al.*, 1979; Demestre *et al.*, 1993; Haley, 1986; Kaye *et al.*, 1961; Koehler, 1979; Langreth, 1969; McKnight and Hinsch, 1986; Medina, 1994; Medina and Rodriguez, 1992a; Moses, 1961b; Pearson and Walker, 1975; Pochon-Masson, 1968a, b; Reger, 1970; Shigekawa and Clark, 1986). Consequently, it can be said that the mechanisms involved in differentiation of the acrosomal structures are somehow plesiomorphic. It is not known whether similar mechanisms take place during spermiogenesis in euphausiids, sergestids, stenopids and acrosome-less aristeids (as they do, in fact, in *Aristeus antennatus*). If so, it is to be assumed that the capacity to build acrosomes with the involvement of endoplasmic reticulum membrane systems (irrespective of the appearance or not of a distinct acrosome in mature sperm) was present in ancestors of eucarids before separation of euphausiids and decapods. Such a widely

shared mechanism of acrosome formation is consistent with the occurrence of apparent homologies in the acrosomes of such distant taxa as the Penaeidae and the Brachyura (Medina and Rodriguez, 1992a). In contrast, the caridean spike, although it results in a sperm pattern closely resembling the penaeid-sicyoniid-solenocerid one, is deduced not to be phylogenetically related to the dendrobranchiate spike (Medina, 1994). Comparative sperm ultrastructural studies thus argue against a monophyletic "Natantia", as Burkenroad (1983) conjectured more than a decade ago.

Medina (1995a, and above) considers that if, as appears plausible, the absence of an acrosome is plesiomorphic for decapods, the spiked acrosome of dendrobranchiates would be a synapomorphy of the families Penaeidae, Sicyonidae and Solenoceridae, whereas the sperm of the Sergestidae and Aristeidae should be considered to be more primitive, that of *Aristeus antennatus* showing secondary (thus apomorphic) acquisition of the acrosome independent of the evolutionary line leading to the other acrosome-possessing dendrobranchiate spermatozoa. As euphausiid spermatozoa (Jamieson, 1991b) are similar to those of *Sergestes arcticus* and *Aristaeomorpha foliacea* (Fig. 1), occurrence of a plesiomorphic acrosome-less sperm is congruent with the statement of Burkenroad (1983) that the ancestors of the Decapoda were more euphausiid-like than the modern forms. According to this view, the primitive eucarids could have had euphausiid-like sperm, euphausiids, sergestids, aristeids and stenopids having retained this pattern. Among Aristeidae, some representatives (*A. antennatus*) might well have recreated a spheroidal acrosome with no ultrastructural resemblance to the acrosome of any of the other known dendrobranchiates, the sperm becoming arranged into a reptant-like pattern (although retaining the plesiomorphic absences of arms, microtubules and nuclear envelope, and therefore with no apparent direct phylogenetic relation to reptants) which represents an independent evolutionary line (Medina, 1995a; Medina *et al.*, 1998; see Fig. 1).

Spermiocladistic support for the conclusion (Abele, 1991; Felgenhauer and Abele, 1983) that the Caridea and Stenopodidea are not jointly monophyletic has yet to be obtained. Derivation of carids from primitive thalassinideans is not congruent with most recent observations on spermatozoal ultrastructure (Tudge, 1995a, b), unless important deviations (= apomorphies) from the reptant ground plan be assumed, namely the loss of the membrane-bound acrosome and of microtubule-containing radial arms, as well as the independent development of a non-membrane bound spike (Arsenault, 1984; Arsenault *et al.*, 1979; Dupré and Barros, 1983; Felgenhauer *et al.*, 1988; Koehler, 1979; Lynn and Clark, 1983a; Papathanassiou and King, 1984; Pochon-Masson, 1968b, 1969) that acts in a very distinct manner during fertilization (Lynn and Clark, 1983a; Barros *et al.*, 1986). These typically caridean characteristics confirm a sperm pattern that represents a fairly distinct, clearly identifiable evolutionary trend within the Decapoda (Medina, 1995a).

The occurrence of either a complete or a discontinuous double-membrane nuclear envelope, partially invested by the plasma membrane, as well as the occasional

presence of centrioles at the base of the acrosome, are shared by carids and reptantians, these features supporting a certain unity of both groups. However, the supposed reptantian origin of stenopodideans (Felgenhauer and Abele, 1983) is contraindicated by the ellipsoidal, armless and acrosome-less form of the spermatozoon of *Stenopus hispidus* (Felgenhauer and Abele, 1991), which is also characterized by having a lamellar body located against the plasma membrane at one side of the sperm cell (a structure that strongly reminiscent of the well-developed membrane system associated with proacrosomal vesicles in decapods). This sperm morphology would place the stenopodideans close to the euphausiids, hence suggesting an early separation of Stenopodidea from the reptantian-caridean stem just above the origin of the Dendrobranchiata and before appearance of the acrosome and of appendages in decapod spermatozoa (Medina, 1995a).

Medina (1995a), presented a tentative phylogenetic tree which attempted to reconcile the current knowledge of spermatozoal ultrastructure in eucarids with phyletic relationships suggested recently (Burkenroad, 1981; Felgenhauer and Abele, 1983; Jamieson, 1989a, 1991b; Kim and Abele, 1990; Schram, 1986). It is similar to Fig. 1, by Medina *et al.* (1998), except in inclusion of the Stenopodoidea and in lacking a diagram for the Euphausiacea. As in Fig. 1, separation of the Euphausiacea is followed by a node grouping the Decapoda, with two distinct evolutionary lines, one of which leads to Dendrobranchiata and the other to Pleocyemata. It is believed that the decapod sperm were originally devoid of an acrosome, a condition that was retained in Sergestidae as well as representatives of the family Aristeidae (*Aristaeomorpha foliacea*). However, another aristeid (*Aristeus antennatus*) has a spermatozoon possessing an apomorphic membrane-bound acrosome that resembles the sperm of *Panulirus* spp. owing, it is considered, to several parallelisms rather than to phylogenetically-based shared features. The three other families of the Dendrobranchiata have in common spermatozoa which share a synapomorphic membrane-bound acrosomal spike. From the node uniting these non-aristeid sperm, the first branch to emerge is represented by the spermatozoon of *Solenocera membranacea*, which shows an asymmetrical acrosomal cap and separation of the plasma and anterior acrosome membranes, allowing part of the cytoplasm to "leak" beyond the acrosomal cap. Finally, Sicyonidae and Penaeidae appear as two aligned groups, the sperm of which are easily distinguishable by the highly complicated, apomorphic subacrosomal region present in sicyoniids, in contrast to the simple one of penaeids. In the Penaeidae, two distinct sperm types have been recognized on the basis of the presence (*Parapeneus longirostris* and *Peneopsis serrata*) or absence (*Penaeus* spp.) of a central protuberance at the concave side of the acrosomal cap. This dendrobranchiate sperm phylogenetic arrangement is in agreement with the close interrelation that Burkenroad (1983) suggests between penaeids and sicyoniids. However, with our limited information, no spermatozoal evidence has been found to ally, as Burkenroad claims, aristeids and solenocerids. On the contrary, the spermatozoon of *S. membranacea* resembles the Penaeidae-Sicyonidae sperm rather than any of the known Aristeidae sperm types (Medina, 1995a).

Acrosome-less sperm, as seen in *Stenopus hispidus*, appear to represent the plesiomorphic condition in the Pleocyemata. A logical phylogenetic sequence would include a first offshoot leading to Stenopodidea in a scheme that is congruent with the phylogram of Felgenhauer and Abele (1983). However, another spermatologically plausible, albeit less probable, arrangement following the more recent cladograms of Schram (1986) and Kim and Abele (1990), would place the offshoot of Stenopodidea between the branches leading to Caridea and Reptantia (Medina, 1995a).

### B. Suborder Pleocyemata

We here follow the taxonomic synopsis of Bowman and Abele (1982) in placing all remaining decapods in the Pleocyemata.

#### 1. Caridea sensu lato

The infraorder Caridea sensu lato, as recognized by Bowman and Abele (1982), contains the infraorders Procarididea plus Caridea sensu Schram (1986). These two groups will be termed the 'procarideans' and 'carideans' here. Their sperm resemble those of dendrobranchiates but there are tendencies for the nucleus to become basally concave so that the sperm, with its anterior spike, takes on a tack-shape, and for development of cross-striated longitudinal fibres in the spike. Cross striation is, however, described for the spike of *Penaeus setiferus* by Felgenhauer *et al.* (1988) in the absence of fibres. Felgenhauer and Abele (1991) distinguish those carideans in which the spike is solid and contains cross-striated fibrils (e.g. *Palaemonetes*) from those in which the spike is tube-like with distinct electron-dense walls containing anastomosing radial fibrils (e.g. *Rhynchocinetes*, Dupré and Barros, 1983; *Procaris ascensionis*, Felgenhauer *et al.*, 1988).

The sperm of *Procaris ascensionis* has a typical tack or 'inverted umbrella' shape. It is said to differ from sperm of carideans in having fibrous ridges on the free margins of the cell body and in lacking periodic cross striations of the fibres which form the spike (Felgenhauer *et al.*, 1988). However, these striations are absent from some caridean sperm.

The spermatozoa of caridean shrimps have been described, or at least illustrated ultrastructurally, for the oplophoroid *Paratya australiensis* (Jamieson and Robertson, unpublished; this study) (Fig. 4A), *Atya margaritacea* and *Typhlatya rogersi* (Felgenhauer and Abele, 1991); the bresilioid *Rhynchocinetes typus* (Barros *et al.*, 1986); the palaemonoids *Palaemon elegans* (Pochon-Masson, 1969); *P. serratus* (Sellos and Le Gal, 1981) (Fig. 1); *Palaemonetes paludosus* (Koehler, 1979); *Palaemonetes kadiakensis* (Felgenhauer *et al.*, 1988; Felgenhauer and Abele, 1991); and *Macrobrachium australiense* (Butcher and Fielder, 1994), *M. rosenbergii* (Lynn and Clark, 1983a, b; Dougherty, 1987; Dougherty *et al.*, 1986; Harris and Sandifer, 1986); the crangonoids *Crangon septemspinosa* (Arsenault, 1984; Arsenault *et al.*,

1979, 1980); *C. vulgaris* (Pochon-Masson, 1968b); and the hippolytid *Hippolyte zostericola* (Felgenhauer and Abele, 1991).

Cross-striations typical of, but not constant, for the spike of the caridean sperm are seen in that of *Macrobrachium rosenbergii* (Lynn and Clark, 1983a, b) and *M. australiense* (Butcher and Fielder, 1994); *Palaemonetes paludosus* and *Palaemon elegans* (Pochon-Masson, 1969). These elements of the spike continue into the cap-like expansion at its base lying on the nucleus. The caridean spike has been said not to be membrane bound and to be little more than a naked perforatorium of a secondarily simplified acrosome (Pochon-Masson, 1969). However, the same author also states that it is delimited by a simple membrane covered by the plasma membrane in *Palaemon*. A bounding membrane is said to be absent in *Crangon septemspinosa* by Arsenault *et al.* (1979). Cross-striations were not seen in the spike of *Crangon vulgaris* examined by Pochon-Masson (1968b) nor in *Paratya australiensis* (Jamieson and Robertson, unpublished; this study).

In *Paratya*, the nucleus is subspheroidal as in penaeids, but it is depressed in other carideans. It is ellipsoidal in *Palaemon elegans* (Pochon-Masson, 1969); oblong or oblate spheroidal (Arsenault *et al.*, 1979), roughly having the form of an ellipsoid with somewhat flattened free surface, in *Crangon septemspinosa*; while in *Palaemonetes paludosus* the nucleus has become inverted cup-shaped, giving the sperm, with its terminal spike, the approximate form of a tack (Koehler, 1979). Transition from an ovoid (plesiomorphic) to the concave (apomorphic) form occurs in spermiogenesis in *P. paludosus*. Persistence of the nuclear envelope appears usually to set carideans apart from penaeids, though some disruption of the envelope occurs in the *Palaemonetes paludosus* (see Koehler, 1979). In this species the envelope is said to be multilayered on the free, concave side but to be lost on the convex side nearest the spike, allowing the uncondensed chromatin to merge with the cytoplasm to form the so-called 'spermioplasm' as in *Sicyonia*; there are numerous PAS-positive vesicles, each with at least two membranes, embedded in the nucleus near its free, concave surface and originating by pinocytosis of the cell surface in the spermatid. Vesicles are normally present peripherally and mostly basal to the nucleus in caridean, as in penaeid sperm. They form a wide reticular zone around the base and sides of the nucleus in *Paratya australiensis*.

The sperm of *Rhynchocinetes typus*, described by Barros *et al.* (1986) from a scanning electron microscope examination, is of particular interest as it forms a link morphologically with the higher, non-natant decapods in having 11 coplanar radial arms in addition to the typical natantian terminal spike. Contact with the egg continues to be made by the terminal spike which exerts a lytic action. It remains to be determined whether the arms are homologous with those of higher decapods.

Mitochondria occur in the cytoplasmic collar of carid sperm but mostly lateral to the nucleus. Centrioles have been observed (generally absent from

dendrobranchiate sperm) between the spike and the nucleus, in the cytoplasmic 'collar' region, in several carids (e.g. *Crangon vulgaris*, Pochon-Masson, 1968b; *C. septemspinosa*, Arsenault *et al.*, 1979; *Palaemon elegans*, Pochon-Masson, 1969).

Origin of the acrosome during spermiogenesis from the Golgi apparatus is argued for *Crangon septemspinosa* by Arsenault *et al.* (1979), but generally in decapods a Golgi apparatus has not been reported and origin of the acrosome appears to be from vesicles derived from the endoplasmic reticulum.

## 2. Stenopodoidea

Sperm structure in this taxonomically problematic group has been examined, for *Stenopus hispidus*, by Felgenhauer and Abele (1991). The sperm of *S. hispidus*, were considered by Felgenhauer and Abele (1991) to resemble those of stomatopods as Burkenroad (1981) had suggested from a light microscope study of the sperm of *Stenopus cf. scutellus*. The spermatozoon of *S. hispidus* is a simple elliptical cell, ca. 7-10 µm in diameter, with a prominent lamellar body located on one side against the plasma membrane, and resembling that flanking the acrosome in brachyurans. No distinct acrosomal region or stellate appendages were present. Felgenhauer and Abele (1991) doubted, however, that the sperm were mature on the grounds that arms, typical of other reptants, were absent. The absence of an acrosome is a notable difference from stomatopod sperm and, with the ellipsoidal armless form, is considered (Jamieson, 1991b) to be a notable resemblance to euphausiid sperm of possible phylogenetic significance.

## C. Reptantia

Scholtz and Richter (1995) have argued for monophyly of the Reptantia and have developed a cladistic classification of the constituent groups. Although they did not give a data matrix or subject their data to parsimony analysis, we find their arguments cogent and, with some reservations (particularly with regard to the position of the Thalassinidia which we consider unresolved) we follow their taxonomic system and terminology, including the suspension of rank names such as infraorder. While we agree that naming of ranks is "fruitless" in so far as their equivalence is often questionable, a disadvantage of abandoning ranks is that the hierarchical nesting of names which is implicit when ranks are named (order, infraorder, etc.) is lost and can only be retrieved from an indented listing. The indented phylogenetic system of Scholtz and Richter (1995) is set out below. Only those ranks which were new in that publication are given author names here.

## Reptantia

### Polychelida

Eureptantia Scholtz and Richter 1995

Achelata Scholtz and Richter 1995

Macrochelata Scholtz and Richter 1995

### Homarida

Fractosternalia Scholtz and Richter 1995

### Astacida *incertae sedis*

Thalassinida (here given the more usual name Thalassinidea)

Meiura Scholtz and Richter 1995

Anomala (here termed Anomura *sensu strictu*)

Brachyura

Scholtz and Richter (1995) have argued that the Palinura is a paraphyletic taxon and have therefore abandoned it, recognizing the Polychelida (e.g. *Polycheles*) and the Achelata (e.g. *Palinurellus*, *Palinurus*, *Scyllarus*, and *Ibacus*) as distinct assemblages which do not make a monophyletic whole. They consider the Polychelida to be the sister group of all other reptantians, which they term 'Eureptantia'. The Achelata are seen as the sister group of the remaining eureptants, namely, in order of appearance in their phylogenetic tree, the Homarida, Astacida, Thalassinida, Anomala and Brachyura. Briefly, polychelids are defined by the unique presence of four pereopods with true chelae.

We cannot test the position of the Polychelida as none has been investigated for sperm ultrastructure. From a brief description by light microscopy the sperm are enigmatically said to be "similar to those of other reptantians" (Scholtz and Richter, 1995, after Andrews, 1911). Several species of the Achelata have been examined.

## D. Eureptantia

Eureptantians are defined by Scholtz and Richter (1995) on several synapomorphies, including the presence of scale-like teeth on the chelae or subchelae of the fifth pereopod.

### 1. Achelata

As their name suggests, achelates lack chelae (Scholtz and Richter, 1995). The ultrastructure of the spermatozoon of the spiny lobsters, *Panulirus argus* and *P. guttatus*, has been investigated by Talbot and Summers (1978), that of *Jasus novaehollandiae* by Jamieson (1991b); and Tudge *et al.*, (1998c) (Fig. 2) (family Palinuridae) and, in the family Scyllaridae, that of *Scyllarus chacei* by McKnight and Hinsch (1986), *Thenus orientalis* by Burton (1995, 1996) and *Scyllarus demani* and *Thenus indicus* by Burton (1996).



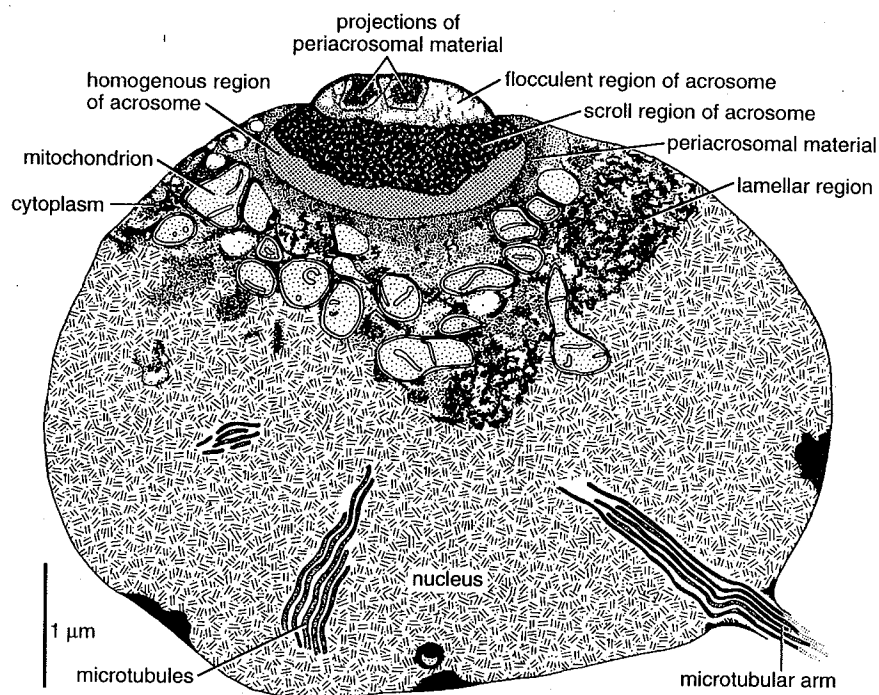


Fig. 2. *Jasus novaehollandiae*. Traced from transmission electron micrograph (TEM) of vertical section of spermatozoon. From Tudge *et al.* (1998c). *Journal of Morphology* **286**, 117–126.

Each *Panulirus* sperm is spherical and consists of a nucleus, lamellar region and, at one pole, the acrosome. The nucleus contains uncondensed, Feulgen-positive chromatin and is limited by an intact nuclear envelope which is very closely applied to the plasma membrane except where the nucleus abuts the acrosome and lamellar regions. A variable number (3–12) of spikes radiates from the nucleus. They are extensions of the nucleus and are bounded by its envelope. Microtubules span the nucleus and extend into the spikes. The chromatin is continuous with the lumen of the spike but does not extend into it. The spikes are stationary and the sperm is non-motile. The lamellar body, which lies at one side of the base of the acrosome and external to the nuclear envelope, contains numerous stacks of membranes and small mitochondria-like bodies (Talbot and Summers, 1978).

The acrosome vesicle (PAS-positive region) is lens shaped and is limited entirely by a membrane. It is structurally complex and is divisible into four discrete zones which are respectively, in posterior-anterior sequence, homogeneous; scrolled; crystalline; and flocculent. The homogeneous region forms an electron-dense cap situated in a depression in the nucleus and surrounding the scroll and part of the crystalline regions. The scroll region is electron dense with numerous

lucid channels which produce the distinctive scroll pattern. The crystalline region is dome-shaped and in section has a very regular grid arrangement of dense squares which in longitudinal section are seen to be vertical rods. The fourth, anteriormost, region contains a dispersed flocculent moderately dense material with coalesced beads or granules. The vesicle is surrounded by periacrosomal material which is flocculent near the base of the acrosome and filamentous at the apex. It includes electron-dense bundles of filaments which, in longitudinal sections, appear as dense cores in pockets formed between the acrosomal and plasma membranes. Microtubules and centrioles were sometimes seen in the basal part of the periacrosomal region (Talbot and Summers, 1978).

The spermatozoal ultrastructure of the spiny lobster, *Jasus novaehollandiae* (Fig. 2), is similar to that in other investigated palinurans and, in particular, the spermatozoa of *Panulirus* species. Shared characters include the globular nucleus penetrated by the bases of three or more microtubular arms, an anteriorly situated cytoplasmic zone with mitochondria and conspicuous lamellar bodies, a complex, four-zoned acrosomal vesicle (an additional crystalline region being present in *Panulirus*) with a homogeneous region, a scroll region, a flocculent region and a region of periacrosomal material which forms finger-like projections into the flocculent region. The related scyllarid slipper lobsters (*Scyllarus* and *Thenus*) possess spermatozoa with a similar acrosome morphology to *Jasus*, but the sperm cells are generally more flattened, numerous radiating acrosome fins are present, and the microtubular arms in *Scyllarus* are cytoplasmic in origin and not nuclear. Sperm morphology thus provides evidence in support of the hypothesis of two independent lines of evolution in the Palinuridae (Tudge *et al.*, 1998c).

The acrosome of *Scyllarus chacei* has electron-dense rays (40 in number) radiating from a dense disc which lies at the apex of the bell-shaped vesicle, under the plasma membrane, like the struts of an umbrella. Beneath these the acrosome contains homogeneous, scrolled and crystalline areas. The cytoplasmic area contains the lamellar complex, a few mitochondria and a large number of microtubules. The number of microtubular arms arising from the body of the sperm as extensions of the cytoplasm is not specified (McKnight and Hinsch, 1986). Burton (1995, 1996) describes the spermatids and spermatozoa of *Thenus orientalis*, *T. indicus* and *Scyllarus demani* and compares them to that of *S. chacei*.

Mature sperm of *Scyllarus demani* have the typical scyllarid format of spherical acrosome and highly convoluted nucleus. Acrosomal development of *S. demani* followed a similar pattern to *Thenus*, with structural differences of diagnostic significance occurring in the apex region. Within this region is the scroll material which consists of numerous coiled tubules underlying the radiating fins. The fin shape and structure are distinct with a fin membrane surrounding the inter-fin chamber, and the central striatum (McKnight and Hinsch, 1986) located between adjoining fin membranes. An acrosomal aperture is also present (Burton, 1996).

Burton (1996) recognizes the following intergeneric differences within the Scyllaridae. In *Thenus*, the filaments and microtubules are of nuclear origin, whereas in *Scyllarus demani* and *Scyllarus chacei* they originate in the cytoplasm. The acrosomal fin structure of *Scyllarus demani* and *S. chacei* (McKnight and Hinsch, 1986) is regular and thick walled with a central striatum, whereas in *Thenus* the fins are irregular, and the central striatum is absent. The acrosomal fin count in *Scyllarus demani* is less than that of *S. chacei*. The acrosomal aperture in *Scyllarus* is large, through which the scroll material may be seen. No acrosomal aperture was found in *Thenus*. The nuclear membrane of *Thenus* is less convoluted than that of *Scyllarus*, while its overall size is almost double. Within *Thenus* and *Scyllarus*, there is a reduction in spermatid size during development. In *Scyllarus chacei*, the average stage 1 spermatid size is 7  $\mu\text{m}$ , which reduces to 5  $\mu\text{m}$  for the mature sperm. Equivalent sizes for *S. demani* are 7  $\mu\text{m}$  and 6.5  $\mu\text{m}$  and *Thenus* are 9  $\mu\text{m}$  and 7  $\mu\text{m}$ .

Panulirid sperm conform to the general 'reptant' plan and are nearest to those of the astacids such as *Homarus* and *Nephrops*. The latter differ, however, in having a constant number (three) of spikes and in having a very elongate acrosomal vesicle with the periacrosomal material (percutor organ or perforatorium) extending up into the base of the vesicle. Presence of crystalline material in panulirid sperm (Talbot and Summers, 1978; McKnight and Hinsch, 1986) is an unusual condition for decapods, shared with nephropids, though with doubtful homology. In the absence of a basal invagination of the acrosome, the palinurid sperm differs conspicuously from sperm of astacids and the anomuran-brachyuran assemblage and Jamieson (1991b) considered that it did not appear that palinurids were near the ancestry of the latter assemblage, a view since endorsed in the phylogenetic tree presented by Scholtz and Richter (1995).

## 2. Macrochelata

Synapomorphies of the Macrochelata include hypertrophy of the first pereopod and its chela (Scholtz and Richter, 1995).

### E. Homarida

Monophyly of the Homarida is only weakly established and membership of the Enoplometopidae in the group is particularly uncertain (Scholtz and Richter, 1995). A spermatozoal apomorphy (Jamieson, 1991b) acknowledged by Scholtz and Richter (1995) is elongation of the acrosome but here, as in other features, *Enoplometopus*, with a rounded acrosome, is exceptional.

## 1. Enoplometopidae

*Enoplometopus occidentalis* (Haley, 1986) is exceptional for the investigated Nephropidae in its dome-shaped acrosome, wider rather than long, resembling that of the Astacidae. This supports exclusion of *Enoplometopus* from the Nephropidae by de Saint Laurent (1988), who placed it in a separate family, the Enoplometopidae, and superfamily, the Enoplometopoidea.

The sperm of *Enoplometopus occidentalis*, described by Haley (1986), who termed it an 'axiid', appeared to Jamieson (1991b) to be remarkably similar to that of the Astacidae and Parastacidae and to differ from that of the Nephropidae, in which it has also been placed, and from the paguroid-brachyuran assemblage, in the structure of the acrosome vesicle. This has the form of a thick-walled inverted cup, wider than long, enclosing a very spacious subacrosomal space in which there is finely granular material but no perforatorium. Centrioles at the base of the acrosome produce microtubules which extend between membranes of the lamellar region distally through the uncondensed nucleus as the cores of three radial arms. Decondensed nuclear material surrounds these microtubular cores at least in the bases of the arms. The nuclear and plasma membranes are fused except where the acrosome lies between them. Two types of mitochondrion-like structures are present. The first do not survive into early spermatids while the second form (apparently from membranes of the lamellar region, according to Haley (1986), but possibly in fact generating these) during spermiogenesis.

It is possible that enoplometopids are, alternative to their placement in the Homarida, the sister group of all other macrochelates (Scholtz and Richter, 1995). Either view is consistent with the apparently relatively plesiomorphic sperm ultrastructure.

## 2. Nephropidae

The two subfamilies of the Nephropidae have been examined for sperm ultrastructure: Nephropinae (*Nephrops norvegicus*, Chevaillier, 1965, 1966a, 1967b, c, 1969; Chevaillier and Maillet, 1965) and Homarinae (*Homarus americanus*, Talbot and Chanmanon, 1980a, b; *H. vulgaris*, Pochon-Masson, 1965b, c, 1968b). The acrosomal-nuclear complex is elongate in the Nephropidae in contrast with the compact and dome-shaped form in the Astacidae and Parastacidae (Fig. 3).

The spermatozoa of *Homarus americanus* (Talbot and Chanmanon, 1980b; Tsai and Talbot, 1993, 1994) and *H. vulgaris* (Pochon-Masson, 1965c, 1968b) conform with the gross ultrastructural pattern described for the Astacidae but differ, notably, in the pronounced elongation of the acrosome which projects as a cylinder and in the fundamentally different structure of the operculum (apical cap). Each sperm is 17 or 19  $\mu\text{m}$  long and consists of acrosome, subacrosomal region, collar containing various organelles, nucleus, and spikes (here three, each 20  $\mu\text{m}$  long in *H. vulgaris* and 38  $\mu\text{m}$  long in *H. americanus*) which are extensions of

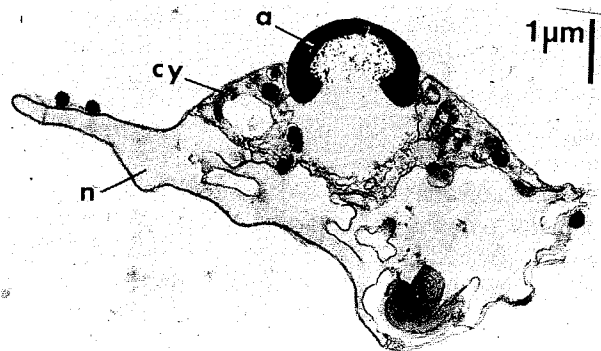


Fig. 3. *Cherax tenuimanus*. TEM of vertical section of spermatozoon. a, Acrosome; cy, cytoplasm; n, nucleus. From Jamieson, B.G.M. (1991b). *Memoirs of the Queensland Museum*, 31, 109–142.

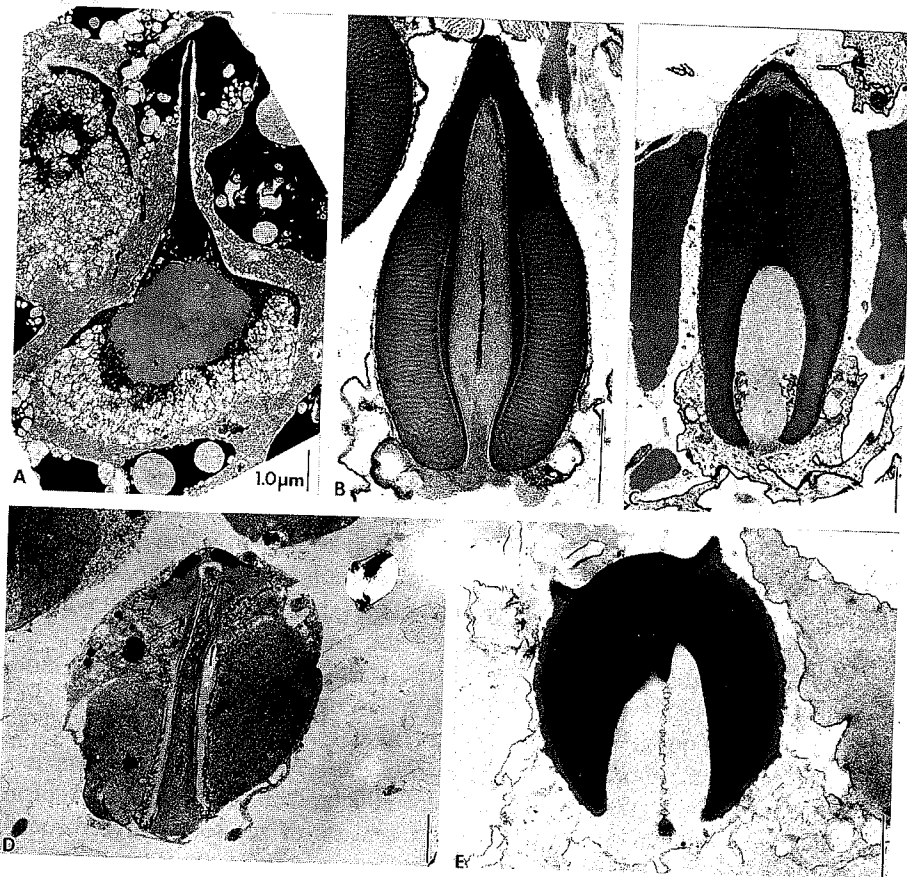


Fig. 4. TEM of vertical section of spermatozoa. A: *Paratya australiensis*, Caridea, Atyidae. B: *Eumunida sternomaculata*, Galatheoidea, Chirostylidae. C: *Coenobita brevimanus*, Paguroidea, Coenobitidae. D: *Axis glyptocercus*, Axioidea, Axiidae. E: *Hippa pacifica*, Hippoidea, Hippidae. (All original).

the nucleus. The acrosome is traversed throughout its length by a weakly PAS-positive electron-dense column, the inner acrosomal material, which widens at the ends to form a deep fossa enclosing the finely granular plug-like subacrosomal material, posteriorly, and a flange supporting an apical cap anteriorly. This column is surrounded by a wider zone, strongly PAS-positive and of moderate to low electron density, the outer acrosomal material (Talbot and Chanmanon, 1980a). The apical cap, which is weakly PAS-positive, has four concentric zones which, centripetally, are: (1) an external wide crystalline zone, (2) a narrow electron-dense crystalline zone, (3) a crystalline moderately electron-dense zone which is a cup-shaped extension of the central, inner acrosomal material (all three identical with the opercular sphincter in *H. vulgaris*, *sensu* Pochon-Masson, 1968b), and (4) the moderately dense contents of this cup (apical portion of central canal Pochon-Masson, 1968b) are continuous with the central column. The tip of the cap is deeply indented (Talbot and Chanmanon, 1980a). The acrosome is bounded by a single, tripartite membrane. The acrosome of *H. vulgaris* is almost identical but the central column is penetrated throughout its length by a narrow central canal (Pochon-Masson, 1968b).

The collar and region subjacent to the subacrosomal material, contain small mitochondria with poorly developed cristae and, centrally, a pair of centrioles. The subacrosomal material, which is more dense basally than elsewhere, and the collar are in direct continuity with the chromatin of the nucleus. The nucleus extends for a short distance as a 'cuff' around the base of the acrosome and is not delimited from the acrosome by a membrane. Elsewhere, though, it is bounded by a membrane which appears to be a product of the fusion of the nuclear envelope and the plasma membrane. This composite membrane projects outwards as the spikes or nuclear processes but the nuclear chromatin, which is granular or fibrillar and uncondensed, is said not to extend into them. The processes are traversed by microtubules ensheathed in and interwoven by sheet membranes. The microtubule-membrane complexes of the spikes converge in the region of the collar and interconnect to form (as in the Anomura, below) a three-sided vault which immediately underlies the base of the acrosome (Talbot and Chanmanon, 1980a).

The acrosome reaction of the sperm of *Homarus americanus* has been elegantly described by Talbot and Chanmanon (1980a) and Tsai and Talbot (1994) and corresponds closely to the report of Pochon-Masson (1965c, 1968b) for *H. vulgaris* (see also Brachyura, Pochon-Masson, 1968a). It has been shown, for *H. americanus*, that the subacrosomal material becomes the acrosomal filament (perforatorium in our terminology) and that the apical cap binds to the vitelline envelope and later contracts to eject the acrosomal filament (Tsai and Talbot, 1993, 1994).

The ultrastructure of the sperm of *Nephrops norvegicus* (Chevaillier and Maillet, 1965) is essentially similar to that in the Homarinae described above. There are again three nuclear processes containing a complex system of lamellae but remarkably,

unlike homarine sperm, the processes lack microtubules. Only the basal part of the spine contains lamellae and is Feulgen (DNA) positive. The acrosome ('capsule') is elongate and consists of a peripheral region and an axial baton. The baton is here interpreted as the homologue of the subacrosomal material or perforatorium in homarines, differing in being (like the entire acrosome) much more elongate. This is bounded by a space (here considered the equivalent of the central canal of *H. vulgaris*) surrounded by an inner fibrillar layer and, external to this, a homogeneous layer together probably equivalent to the inner acrosomal material (central column) in *Homarus*. It is proteinaceous and PAS negative. The peripheral region is clearly the homologue of the outer acrosomal region and, like it, is PAS-positive. A proteinaceous 'apical granule' is possibly the equivalent of the homarine apical cap (operculum).

### F. Fractosternalia

Members of the Fractosternalia, a new grouping of Scholtz and Richter (1995), have a divided sternum. It is the sister group of the Homarida and includes the Astacida, Thalassinidea and Meiura.

#### 1. Astacida

Monophyly of the Astacida (Astacidoidea and Parastacidoidea) has been the subject of dispute but a clear set of somatic and developmental synapomorphies has been presented by Scholtz and Richter (1995) which endorses the unequivocal claim for monophyly on the basis of sperm ultrastructure by Jamieson (1991b). Scholtz and Richter (1995) are uncertain whether the Astacida are the sister group of the Thalassinidea-Anomala (Anomura)-Brachyura assemblage or are exclusively the sister group of the Thalassinidea. We find no reason spermatologically to derive the Astacida from the Thalassinidea nor for deriving them above (Scholtz and Richter, 1995) rather than below the Homarida.

##### (a) Astacidae and Parastacidae

Ultrastructural studies of the Astacidea have been chiefly on the family Astacidae (*Astacus astacus* = *A. fluviatilis*, Pochon-Masson, 1968b; López-Camps *et al.*, 1981; *A. leptodactylus*—spermatocytes only—Eliakova and Goriachkina, 1966; *Cambaroides japonicus*, Kaye *et al.*, 1961; Yasuzumi *et al.*, 1961; Yasuzumi and Lee, 1966; *Cambarus* sp., Anderson and Ellis, 1967; *Pacifastacus leniusculus*, Dudenhausen and Talbot, 1979, 1982; *Procambarus clarkii*, Moses, 1961a, b; *P. leonensis*, Felgenhauer and Abele, 1991; *P. paeninsulanus*—chiefly Sertoli cells—Hinsch, 1992, 1993a, b). Those for Parastacidae are for *Cherax tenuimanus* (Beach and Talbot, 1987; Jamieson, 1991b) (Fig. 3) and *C. albidus* (Beach and Talbot, 1987).

The acrosome in all investigated astacids and parastacids is a dense inverted cup-shaped structure, crescentic in longitudinal section, with the opening towards the nucleus. It is wider rather than long, in contrast with nephropids (*Homarus*, *Nephrops*) in which, with the exception of *Enoplometopus*, it is greatly elongated. In *Astacus astacus*, the acrosome is differentiated into an apical operculum (Pochon-Masson, 1968b) or apical formation (López-Camps *et al.*, 1981) and a more basal, thick doughnut-like ring. No such apical differentiation is recognized in *Procambarus clarkii*, *P. leonensis*, *Cambarus* sp. and *Cambaroides japonicus* (Moses, 1961a; Felgenhauer and Abele, 1991; Anderson and Ellis, 1967; and Yasuzumi and Lee, 1966, respectively). In *Cherax albidus* (Parastacidae), some apical whorled material is present within the vesicle but is absent in *C. tenuimanus* (Beach and Talbot, 1987; Jamieson, 1991b) (Fig. 3). The mature acrosome of *Pacifastacus* is again differentiated as an apical cap consisting of whorled stacks of lamellae in addition to crystalline inner acrosomal material; and outer acrosomal material which is homogeneous except for a peripheral electron-dense band (Dudenhausen and Talbot, 1982). At maturity, in *Cambarus*, the crescent is embedded in dense material within the filamentous spermioplasm (Anderson and Ellis, 1967). It seems possible that the reported absence of an operculum in some species may be due to slight immaturity of the spermatozoon and that the internalized whorls of *Cherax albidus* represent an intermediate ontogenetic stage of the acrosome. The operculum of Astacida is very different in structure from that of meirans.

In all examined Astacidae and Parastacidae there is a large subacrosomal chamber. In *Cambaroides*, *Cambarus* and *Procambarus*, a plug-like mass of granular material with filamentous extensions fills the posterior opening of the acrosome. Thin beaded filaments, also shown for both *Cherax* species by Beach and Talbot (1987), extend into the central concavity from this basal material.

At full development an apical process, horn-like process of Yasuzumi and Lee (1966), or anterior acrosomal process of Anderson and Ellis (1967), which is possibly a derivative of the sustentacular cells (Moses, 1961a), emerges from the anterior region of the acrosome. This is clearly the structure questionably considered an acrosomal tubule in *Procambarus leonensis* by Felgenhauer and Abele (1991). As in most other Malacostraca, the acrosome does not appear to be a Golgi derivative, the hallmark of the acrosome in other animal groups. Dudenhausen and Talbot (1979) state that the proacrosomal vesicles, which fuse to form the acrosome, originate from the endoplasmic reticulum in *Pacifastacus*. Yasuzumi *et al.* (1961) state that the acrosome forms from granules in the spermatid similar to those found in the interzonal spindle region in the meiotic divisions.

The nucleus of the spermatozoon of *Astacus astacus* is a biconcave disc with major axis perpendicular to that of the gamete and with a sinuous outline. As in other decapods, the chromatin forms a fine, weakly osmiophile network of fibrils varying from 20 Å to 200 Å (Pochon-Masson, 1968b; Yasuzumi and Lee, 1966; Moses, 1961a). Occasional clear spaces contain microtubules. In the equatorial plane the nucleus is elongated to form the characteristic spikes (spines, arms or

pseudopodia). These number four in *Cambaroides* and *Procambarus clarkii* but exceed 20 in *P. leonensis* and five, six, or seven in *Cambarus viridis* (references in Moses, 1961a, b; Felgenhauer and Abele, 1991). Elsewhere, folds of the nuclear envelope surround mucoid digitations arising from the convoluted membranes in outer parts of the cell (Pochon-Masson, 1968b).

There is evidence for formation of lamellar material peripheral to the nucleus from the nuclear membrane, from smooth ER, and from mitochondria and for formation of the wall of the spines from the nuclear membrane and also from the convoluted membranes (Kaye *et al.*, 1961; Moses, 1961a, b; Eliakova and Goriachkina, 1966; Yasuzumi and Lee, 1966; Anderson and Ellis, 1967; Pochon-Masson, 1968b; Dudenhausen and Talbot, 1979). Yasuzumi and Lee (1966) have demonstrated that the convoluted membranes, especially surrounding the nuclear membranes, are the site of TTPase.

It is considered by Moses (1961b) and Anderson and Ellis (1967), for Astacidea, and by Talbot and Chanmanon (1980b), for *Homarus*, that the nuclear membrane becomes fused with the plasma membrane as a "tegument" containing "spermioplasm", admixed nucleoplasm and cytoplasm.

Microtubules, c. 200 Å (Pochon-Masson, 1968b), 220-310 Å (Yasuzumi and Lee, 1966) or c. 300 Å wide (Anderson and Ellis, 1967), with associated DNA, form several parallel bundles, some of which extend into the spines (Moses, 1961a, b; Anderson and Ellis, 1967; Pochon-Masson, 1968b), each of which contains, for instance, 30 evenly spaced microtubules in *Cambaroides* (Yasuzumi and Lee, 1966). The function of the microtubules has yet to be determined as the arms appear to be immotile.

Centrioles are said to be absent from the mature sperm of *Astacus astacus* by Pochon-Masson (1968b) and were observed to disintegrate by maturity in *Procambarus* (Moses, 1961a, b) and *Cambaroides* (Yasuzumi *et al.*, 1961) but persist in the mature sperm in *Cambarus* (Anderson and Ellis, 1967). No Golgi apparatus is known in spermatids or spermatozoa of crayfish but lamellar ER in the spermatid resembles this structure (Kaye *et al.*, 1961). In *A. astacus*, the sperm is not freed from a mucoid sphere until it reaches the external medium when, as in *Pacifastacus*, the spines unfold. The PAS-positive mucoid sheath is provided by the intercalary cells (Moses, 1961a).

## 2. Thalassinidea

The Thalassinidea (mud shrimps and mud lobsters), termed the 'Thalassinida' by Scholtz and Richter (1995) and usually regarded as an infraorder, are considered by some authors to be an important, pivotal group in the evolution and phylogeny of the other decapod infraorders (Burkenroad, 1963; Bauer, 1986). Thalassinideans have been linked with the anomurans on the basis of larval morphology (Gurney, 1942; MacDonald *et al.*, 1957), and adult somatic characters (Martin and Abele, 1986). In cladistic analysis of somatic characters by Poore (1994), they appear as

the sister group of the Anomura *sensu strictu*. However, Scholtz and Richter (1995) consider the Thalassinida, a term of Burkenroad (1981), to be the sister group of the Meiura (Anomura *sensu strictu*+Brachyura). They recognize the Thalassinida as monophyletic on putative synapomorphies of the cheliped (first pereopod), the sternite of thoracic segment 7; the form of the carapace; the narrow waist in the region of the first pleomer; and the habit of constructing complex vertical burrows.

Table 2

Thalassinidean taxa previously investigated for spermatozoal morphology

Superfamily and family	Species	Reference
<b>Axiioidea</b>		
AXIIDAE	<i>Axius glyptocercus</i>	Tudge, 1995a, b
CALOCARIDIDAE	<i>Calocaris macandreae</i>	Retzius, 1909 (light only)
<b>Callianassoidea</b>		
CALLIANASSIDAE	<i>Biffarius arenosus</i>	Tudge, 1995a, b (as <i>Callianassa arenosa</i> , light only)
	<i>Trypaea australiensis</i>	Tudge, 1995a, b (as <i>Callianassa</i> )
UPOGEBIIDAE	<i>Upogebia pusilla</i>	Koltzoff, 1906 (as <i>Gebia littoralis</i> ) (light only)
<b>Thalassinioidea</b>		
THALASSINIDAE	<i>Thalassina squamifera</i>	Tudge, 1995a, b

Six species of thalassinideans from five families across all three superfamilies have been investigated (see Table 2).

Reclassification of the infraorder Thalassinidea (Poore, 1994) has separated this group into three superfamilies, comprising 11 families, and has suggested that the Thalassinidea is a monophyletic group, with regard to the Anomura. Recent data from spermatozoal morphology would seem to vindicate this subdivision of the Thalassinidea, with *Axius glyptocercus* (Fig. 4D), *Thalassina squamifera* and *Trypaea australiensis* each showing its own distinctive spermatozoal ultrastructure (Tudge, 1995a, b). The overall sperm morphology for the Thalassinidea, though diverse, is sufficiently different from that of the Anomura *sensu strictu* to support the separation of the two based on evidence from somatic morphology (Martin and Abele, 1986; Poore, 1994; Scholtz and Richter, 1995).

The drawings of the calocarid, *Calocaris macandreae* (Retzius, 1909) and the upogebiid, *Upogebia pusilla* (Koltzoff, 1906), from early light microscope work show different sperm morphologies from those determined by transmission electron microscopy for *Thalassina* and *Trypaea*, but that of *C. macandreae* shows similarities to *Axius* (both members of the superfamily Axiioidea). The spermatozoa of *C. macandreae* are illustrated with four microtubular arms emerging from a spherical sperm cell which has apparently everted a columnar perforatorial chamber. This description approximates the sperm morphology seen in *Axius*

*glyptocercus* (like *Calocaris* from the superfamily Axioidea) or less so that seen in *Trypaea australiensis* (superfamily Callianassoidea). The spermatozoal morphology of *Thalassina squamifera*, on the other hand, is very different from any of the other investigated genera, even considering possible fixation anomalies (only formalin-fixed material has been studied). The large, horizontal, trilayered operculum, the ovoid acrosome vesicle with concentric zonation and the fact that the acrosome is posteriorly cupped by the cytoplasm and nucleus are all major differences from the previously studied thalassinideans. The spermatozoa of *Thalassina squamifera* possess an apparent thickened ring (at the base of the perforatorial chamber) which has previously been recorded in heterotreme and thoracotreme brachyurans (Jamieson, 1991b, 1994a, b; Jamieson *et al.*, 1995).

The variety of spermatozoal morphology across representatives of these five families appear to vindicate the separation of the infraorder into the three superfamilies (Poore, 1994). Burkenroad (1963) indicated in a dendrogram of eucarid evolution based on fossil evidence, that the thalassinoid lineage may be more ancient than either of the anomuran or brachyuran lineages. It is possible that the early Triassic thalassinideans were a diverse group of ancestral decapods which gave rise to the macruran (Astacidea, Homarida and Palinura), anomuran and brachyuran lineages.

### G. Anomura *sensu strictu*

Schram (1986) and Scholtz and Richter (1995) have abandoned the term 'Anomura' as this was originally formulated to include also the thalassinideans. They employ the name 'Anomala' of Boas (1880). As noted above, we prefer the term Anomura which is widely used for the restricted, non-thalassinidean taxon. An argument for this usage of the name Anomura is presented by McLaughlin and Holthuis (1985).

Once more the conclusion from sperm ultrastructure that the Anomura *sensu strictu* are the sister group of the Brachyura (Jamieson, 1991b, 1994a, b; Jamieson *et al.*, 1995; Tudge, 1997b) is endorsed by the somatic cladistic study of Scholtz and Richter (1995). Nevertheless, the position and monophyly of the Thalassinidea, placed by the latter authors below the Meiura, requires further scrutiny.

The Anomura (half crabs, porcelain crabs, mole crabs, king crabs and hermit crabs) is a very diverse taxon with 13 families spread across four major superfamilies. Their relationships to each other and to the other decapods is currently enigmatic and the subject of much investigation from a variety of evidence (McLaughlin, 1983b; Martin and Abele, 1986; Spears and Abele, 1988; Tudge, 1991, 1992, 1995a, b, 1997b; Cunningham *et al.*, 1992; Spears *et al.*, 1992; Richter and Scholtz, 1994; Scholtz and Richter, 1995). The total number of anomuran taxa for which the spermatozoal structure has been investigated, by light and transmission electron microscopy, is 61 species from 31 genera in 10 families (Table 3). The ultrastructure of anomuran spermatozoa is also briefly covered in several general reviews of crustacean gametes (Felgenhauer and Abele, 1991; Jamieson, 1991b; Krol *et al.*, 1992; Pochon-Masson, 1983).

Table 3

Anomuran taxa previously investigated for spermatozoal morphology

Superfamily and family	Species	Reference
<b>Galatheaidea</b>		
CHIROSTYLIDAE	<i>Eumunida sternomaculata</i>	Tudge, 1995a, b
	<i>Uroptychus</i> sp.	Tudge, 1995a, b
GALATHEIDAE	<i>Allogalathea elegans</i>	Jamieson, 1991b (as <i>Allogalathea</i> sp.); Tudge, 1995a, b
	<i>Cervimunida johnei</i>	Lohrmann and Rainieri, 1995
	<i>Galathea squamifera</i>	Koltzoff, 1906; Retzius, 1909 (light only)
	<i>Galathea strigosa</i>	Koltzoff, 1906 (light only)
	<i>Munida rugosa</i>	Koltzoff, 1906 (light only)
	<i>Munida</i> sp.	Tudge, 1995a, b
	<i>Munidopsis</i> sp.	Tudge, 1995a, b
	<i>Pleuroncodes planipes</i>	Hinsch, 1991
	<i>Aliaporcellana suluensis</i>	Tudge, 1995a, b; Tudge and Jamieson, 1996a
	<i>Petrolisthes armatus</i>	Tudge, 1995a, b; Tudge and Jamieson, 1996b
PORCELLANIDAE	<i>Petrolisthes lamarckii</i>	Jamieson, 1991b; Tudge, 1995a, b; Tudge and Jamieson, 1996b
	<i>Pisidia longicornis</i>	Retzius, 1909 (as <i>Porcellana longicornis</i> ); Tudge and Jamieson, 1996a
	<i>Polyonyx transversus</i>	Tudge, 1995a, b; Tudge and Jamieson, 1996b
<b>Hippoidea</b>		
HIPPIDAE	<i>Emerita analoga</i>	Vaughn, 1968a, b; Vaughn <i>et al.</i> , 1969; Vaughn and Locy, 1969; Vaughn and Thomson, 1972 (light only)
	<i>Emerita asiatica</i>	Subramoniam, 1977 (light only)
	<i>Emerita talpoida</i>	Pearse <i>et al.</i> , 1942; Barker and Austin, 1963
	<i>Hippa pacifica</i>	Tudge, 1995a, b
<b>Lomoidea</b>		
LOMIDAE	<i>Lomis hirta</i>	Tudge, 1995a, b; 1997a
<b>Paguroidea</b>		
COENOBITIDAE	<i>Birgus latro</i>	Jamieson, 1991b; Tudge and Jamieson, 1991; Tudge, 1992, 1995a, b
	<i>Coenobita brevipanus</i>	Tudge, 1995a, b
	<i>Coenobita clypeatus</i>	Hinsch, 1980a, b; Tudge, 1992
	<i>Coenobita perlatus</i>	Tudge, 1995a, b
	<i>Coenobita purpureus</i>	Tudge, 1995a, b
	<i>Coenobita rugosus</i>	Nath, 1942; Tudge, 1995a, b
	<i>Coenobita variabilis</i>	Tudge, 1992 (as <i>C. spinosus</i> ), 1995a, b

Table 3 (contd.)

Superfamily and family	Species	Reference
DIOGENIDAE	<i>Calcinus gaimardii</i>	Tudge, 1995a, b
	<i>Calcinus laevimanus</i>	Tudge, 1995a, b
	<i>Calcinus minutus</i>	Tudge, 1995a, b
	<i>Cancellus</i> sp.	Tudge, 1995a, b
	<i>Clibanarius corallinus</i>	Jamieson, 1991b; Tudge, 1992, 1995a, b
	<i>Clibanarius erythropus</i>	Koltzoff, 1906 (as <i>C. misanthropus</i> ); Tudge and Justine, 1994; Tudge, 1995a, b
	<i>Clibanarius longitarsus</i>	Nath, 1942; Dhillon, 1964, 1968; Tudge, 1995a, b
	✕ <i>Clibanarius nathi</i>	Nath, 1942 (light only)
	<i>Clibanarius taeniatus</i>	Tudge, 1992, 1995a, b
	<i>Clibanarius virescens</i>	Tudge, 1992, 1995a, b
	<i>Dardanus arrosor</i>	Koltzoff, 1906 (as <i>Pagurus striatus</i> ); Tudge, 1995a, b
	<i>Dardanus crassimanus</i>	Tudge, 1992, 1995a, b
	<i>Dardanus lagopodes</i>	Tudge, 1995a, b
	<i>Dardanus megistos</i>	Nath, 1942 (as <i>Pagurus punctulatus</i> ); Tudge, 1995a, b
	<i>Dardanus scutellatus</i>	Tudge, 1995a, b
	<i>Dardanus</i> sp.	Tudge, 1995a, b
	<i>Diogenes custos</i>	Tudge, 1992, 1995a, b
	<i>Diogenes gardineri</i>	Tudge, 1995a, b
	✕ <i>Diogenes miles</i>	Dhillon, 1968 (light only)
	<i>Diogenes pallescens</i>	Tudge, 1995a, b
	✕ <i>Paguristes oculatus</i>	Koltzoff, 1906 (as <i>P. maculatus</i> ) (light only)
	<i>Strigopagurus boreonotus</i>	Tudge, 1995a, b (as Gen. nov. sp. nov.), 1996
LITHODIDAE	<i>Hapalogaster dentata</i>	Goshima et al., 1995
	<i>Lithodes maja</i>	Retzius, 1909 (light only); Tudge et al., 1998a
	<i>Paralithodes camtschatica</i>	Marukawa, 1933 (light only)
PAGURIDAE	<i>Pagurus bernhardus</i>	Retzius, 1909; Pochon-Masson, 1963, 1965a, b, 1968a, b; Chevaillier, 1966a, b, 1967a-c, 1968 (all as <i>Eupagurus bernhardus</i> ); Tudge, 1995a, b
	<i>Pagurus chevreuxi</i>	Tudge, 1995a, b
	<i>Pagurus excavatus</i>	Koltzoff, 1906 (as <i>Eupagurus angulatus</i> ); Tudge, 1995a, b
	<i>Pagurus hirtimanus</i>	Tudge, 1995a, b
	<i>Pagurus prideaux</i>	Koltzoff, 1906 (as <i>Eupagurus prideauxii</i> ); Tudge, 1995a, b
	<i>Solitariopagurus therkayi</i>	Tudge, 1995a, b (as <i>Porcellanopagurus</i> sp.)
	<i>Xylopagurus caledonicus</i>	Tudge, 1995a, b (as <i>X.</i> sp.)
PARAPAGURIDAE	<i>Sympagurus</i> sp.	Tudge, 1995a, b (as <i>Parapagurus</i> sp.) (light only)
	<i>Sympagurus</i> sp.	Tudge, 1995a, b

## 1. Galatheoidea

The three investigated families, Chirostylidae, Galatheidae and Porcellanidae, in the superfamily Galatheoidea show particular spermatozoal morphologies which appear characteristic of each family. The spermatozoal morphology of the fourth family, the Aeglidae, is, at present, unknown.

### (a) Chirostylidae

The spermatozoal morphology of the investigated representatives, *Eumunida sternomaculata* (Fig. 4B) and *Uroptychus* sp., in the family Chirostylidae, is more similar to that of hermit crabs than to any other galatheid; particularly in the shape of the acrosome vesicle (spherical in *Uroptychus* and ovoid in *Eumunida*) and possibly the possession of an acrosome ray zone (Tudge, 1995a, b). Like the paguroids, both species have three prominent microtubular arms emanating from the cytoplasm. No microvillar projections are present in the perforatorial chamber but some perforatorial tubules occupy this position in *Eumunida*. Unlike the spermatozoa of the galatheids and porcellanids, the investigated chirostylids do not have the posterior perforatorial chamber wall folded into longitudinal grooves or septa.

### (b) Galatheidae

The spermatozoal morphology of the few investigated members of the Galatheidae is characterized by an elongate (sometimes fusiform) acrosome vesicle, division of the inner acrosome zone, posterior position of the outer acrosome zone and the presence of septa in the perforatorial chamber. In *Galathea squamifera* (Koltzoff, 1906; Retzius, 1909), *G. strigosa*, *Munida rugosa* (Koltzoff, 1906), *Pleuroncodes planipes* (Hinsch, 1991), *Allogalathea elegans* (Jamieson, 1991b; Tudge, 1995a, b), *Munida* sp., *Munidopsis* sp. (Tudge, 1995a, b) and *Cervimunida johni* (Lohrmann and Rainieri, 1995), the sperm cells are shown to have a superior acrosome vesicle that is attached to the nucleus by only a thin collar of cytoplasm. All the investigated galatheids, with the exception of *Pleuroncodes planipes*, have three microtubular arms emerging from the small cytoplasmic neck region. A spermatozoal feature that the galatheids (excepting *Cervimunida*?), *Munidopsis* and *Pleuroncodes*) share with the porcellanids is the folding of the posterior perforatorial chamber wall into grooves or septa. *Munidopsis* has a longitudinally arranged septum which 'hangs' into the perforatorial chamber, but it does not appear to be attached to the chamber wall. Peculiarities of the zonation of the acrosome vesicle in *Allogalathea*, *Munida*, *Munidopsis*, *Pleuroncodes* and possibly *Cervimunida*, which appear distinctive for galatheids are the restriction of the outer acrosome zone to the posterior half of the acrosome vesicle and the separation of the inner acrosome zone into two distinct regions.



(c) *Porcellanidae*

A particular suite of acrosome vesicle characters is shown in all the investigated members of the Porcellanidae; and consequently unites them (Tudge, 1995a, b; Tudge and Jamieson, 1996a, b). The acrosome vesicle is superior in relation to the cytoplasm (which generally forms a thin, neck-like region) and is capped by a broad, flat, centrally perforate operculum. Although it is often difficult to ascertain the exact number of microtubular arms in the spermatozoa of these porcellanids, it appears that there are four or more (definitely more than three) external microtubular arms in each of the investigated species. The wall of the perforatorial chamber in the spermatozoa of the investigated porcellanids is variously folded to form broad septa and the concentric zonation of the acrosome vesicle has three important features: the autapomorphic dense perforatorial cone and posterior perforatorial ring and the synapomorphic divided inner acrosome zone. A major difference in the overall sperm cell morphology divides the group. *Petrolisthes armatus* and *P. lamareckii* both exhibit unique acrosomal characters (a conspicuous opercular ridge and a tubular ring which encircles the perforatorial chamber at its midpoint) which, with the globular nuclear shape, separate them from the other porcellanid genera (Tudge and Jamieson, 1996b). The other three investigated genera, *Aliaporcellana*, *Pisidia* and *Polyonyx*, have an elongate spermatozoal morphology characterized by the possession of a central core of microtubules which extends the entire length of the sperm cell and splits, posteriorly, to form several microtubular arms (Tudge and Jamieson, 1996a, b). The cytoplasm and nucleus surround the microtubular core and form a thin veneer for most of its length. This basic division of the investigated Porcellanidae is supported by larval morphology (Sankolli, 1965; Van Dover *et al.*, 1982), adult somatic morphology (Haig, 1965) and gross spermatophore morphology (Tudge, 1995a; Tudge and Jamieson, 1996a, b).

## 2. Hippoidea

In the superfamily Hippoidea, only representatives from the family Hippidae have been investigated for spermatozoal morphology, the unstudied family being the Albuneidae.

(a) *Hippidae*

The spermatozoa observed in the genus *Emerita*, (Pearse *et al.*, 1942; Barker and Austin, 1963; Vaughn, 1968a, b; Vaughn *et al.*, 1969; Vaughn and Locy, 1969; Vaughn and Thomson, 1972; Subramoniam, 1977) have a more elongate acrosome vesicle than that described for *Hippa pacifica* (Tudge, 1995a, b) (Fig. 4E) and the number of recorded microtubular arms varies between three and nine (Pearse *et al.*, 1942; Barker and Austin, 1963; Vaughn *et al.*, 1969; Subramoniam, 1977). Generally,

the spermatozoal morphology of the genus *Emerita* resembles nephropid or homarid lobsters, in particular that of *Homarus americanus* (Talbot and Chanmanon, 1980a, b). The spermatozoa of *Hippa pacifica* are similar in morphology to *Emerita*, although the acrosome vesicle is much shorter and only three to five microtubular arms have been recorded (Tudge, 1995a, b). Though showing some spermatozoal characteristics which place them in the Anomura (microtubular arms and acrosome vesicle structure), it is difficult to ally them with any other anomuran superfamily.

## 3. Lomoidea

The superfamily Lomoidea contains the monospecific genus *Lomis* in the family Lomidae. The spermatozoa of *Lomis hirta* possess spermatozoal characters, such as microtubular arms (possibly three?) and an acrosome vesicle posteriorly penetrated by a perforatorial chamber, which justify its position in the Anomura. Its sperm morphology (Tudge, 1997a), is, however, distinct enough to warrant placement in its own family and superfamily (McLaughlin, 1983a). The small acrosome vesicle completely embedded in the cytoplasm has not been recorded for any other anomuran spermatozoon and is only approximated by that seen in some thalassinideans. The microtubular bundles, which appear to be concentrated in the cytoplasm, extend into the nucleus but it is not known whether these microtubular bundles (the bases of the microtubular arms) are cytoplasmic or nuclear in origin, in this crab. Although the sperm cells appear to have three vertices, only one or two microtubular arms have been seen on a single spermatozoon and it is not known if three microtubular arms is standard for *Lomis*. Another unique feature of the spermatozoa of *Lomis* is the discontinuous electron-dense operculum, which surrounds the entire acrosome vesicle and not only the apical pole, unlike all other recorded anomurans (Tudge, 1995a, 1997a). Richter and Scholtz (1994) consider the Lomoidea to be the sister group to the monophyletic Paguroidea.

## 4. Paguroidea

(a) *Coenobitidae*

The sperm of the two genera in the Coenobitidae, *Coenobita* (Fig. 4C) and *Birgus*, have been the subject of extensive ultrastructural study (see Table 3). The family appears to have a general sperm type that combines the following ultrastructural characters: (1) a large cylindrical to oblong-ovoid acrosomal vesicle with a conical operculum and a fairly deeply invaginated columnar perforatorium, which is not divided into a posterior bulb and anterior projection; (2) the posterior walls of the perforatorium extend into the lumen as long, microvillus-like projections; (3) the subopercular zone is divided into two distinct regions with the posteriormost region extending down the centre of the acrosomal vesicle to abut against the inner acrosome



zone; (4) most of the acrosome vesicle is composed of a conspicuous, alternatively light and dark banded acrosome ray zone; (5) three long microtubular arms emanate from the cytoplasmic region, between the nucleus and the acrosomal vesicle, and each arm splits to surround the base of the vesicle in a 'triad'; (6) mitochondria and membrane systems or lamellae are prominent components of the cytoplasm, and centrioles have been reported in the mature sperm of *C. clypeatus* (Hinsch, 1980a), *C. brevimanus* and *C. purpureus* (Tudge, 1995a, b).

The sperm of *Birgus latro* differs from the representatives of the genus *Coenobita* in that the acrosomal vesicle is more oblong-ovoid than cylindrical and the nuclear material is drawn out into filamentous extensions or arms (Tudge and Jamieson, 1991; Tudge, 1992, 1995a, b).

The monophyly of the Coenobitidae has never been disputed and morphological (Richter and Scholtz, 1994), ecological (Hartnoll, 1988) and spermatophoral (Tudge, 1991) evidence supports the spermatozoal unity of this group. The Coenobitidae is more often closely allied with the Diogenidae than with the Paguridae (MacDonald *et al.*, 1957; McLaughlin, 1983b; Martin and Abele, 1986; Cunningham *et al.*, 1992; Richter and Scholtz, 1994; Tudge, 1997b). Wolff (1961) stated that the Coenobitidae arose from the Diogenidae and the fact that some members of the Diogenidae are the only other hermit crabs, besides the semi-terrestrial coenobitids, to venture from sea water for any length of time (Hartnoll, 1988) adds evidence to this theory. Spermatologically, the investigated members of the Coenobitidae share many characters with certain members of the Diogenidae.

#### (b) Diogenidae

The investigated genera in the Diogenidae, *Calcinus*, *Cancellus*, *Clibanarius*, *Dardanus*, *Diogenes* and *Strigopagurus*, can be distinguished from each other by characteristic spermatozoal features. However, the presence of microvillar projections on the posterior wall of the perforatorial chamber, links them all (with the possible exception of *Cancellus* sp.).

The spermatozoa of *Calcinus gaimardii*, *C. laevimanus* and *C. minutus* exhibit an apomorphic feature in the splitting of the anterior end of the perforatorial chamber into two to many separate fingers (Tudge, 1995a, b).

The apparent absence of the microvillar projections in the perforatorial chamber of *Cancellus* sp. immediately sets this genus apart from the other diogenids (Tudge, 1995a, b). Although there are conspicuous longitudinally arranged perforatorial tubules present in the posterior region of the perforatorial chamber, it is difficult to ascertain whether they originate from the chamber walls and, therefore, could be termed microvillar projections. An important synapomorphy for *Cancellus* spermatozoa is the small central perforation in the operculum.

Transmission electron microscope investigations of the *Clibanarius* genus (Jamieson, 1991b; Tudge, 1992; Tudge and Justine, 1994; Tudge, 1995a, b) show the perforatorial chamber to have a bulbous posterior region and a thin anterior

projection. A distinctive apomorphy of *Clibanarius* spermatozoa is the presence of a dense perforatorial ring around the posterior perforatorial bulb, which has been observed in all members of the genus studied ultrastructurally. The size and shape of the dense perforatorial ring varies between the investigated species of *Clibanarius* but its position around the posterior perforatorial bulb is constant.

The spermatozoa of *Dardanus* genus are similar in size, shape and acrosome vesicle zonation to investigated members of the Coenobitidae. The spermatozoa of *Dardanus arrosor*, *D. crassimanus*, *D. lagopodes*, *D. scutellatus* and *Dardanus* sp. have most of the acrosome vesicle composed of an acrosome ray zone, a central inner acrosome zone which extends between the perforatorial chamber and the subopercular zone, and a pre-equatorial (except *D. scutellatus* and *Dardanus* sp.) columnar perforatorial chamber with microvillar projections (Tudge, 1992, 1995a, b). All of these spermatozoal characteristics are shared with the coenobitids and the only major ultrastructural differences between the dardanids and coenobitids are that the subopercular zone in the coenobitids is divided into separate regions and the posteriormost region extends down the centre of the acrosome vesicle before abutting the inner acrosome zone.

The spermatozoa of the genus *Diogenes* possess some of the characteristics of the coenobitid sperm-type, which include a large cylindrical acrosome vesicle, largely composed of an acrosome ray zone, and a perforatorial chamber with microvillar projections, although the perforatorial chamber does possess a slightly bulbous posterior region (Tudge, 1992, 1995a, b). An apomorphy for the genus *Diogenes* is that the inner acrosome zone is reduced posteriorly to a thin perforatorial covering, while anteriorly it is modified into a fibrillar acrosome core.

The light microscope observations of the spermatozoa of *Paguristes oculatus* by Koltzoff (1906) provide the only available data on this genus and are not detailed enough to allow ultrastructural comparisons with other investigated diogenid genera.

*Strigopagurus boreonotus* has a spermatozoal ultrastructure which resembles that seen in the genus *Calcinus*, more than any other diogenid genus (Tudge, 1995a, b, 1996). The spherical acrosome vesicle penetrated by a large bulbous perforatorial chamber, with small microvillar projections, and the operculum and subopercular zone shape are similar to that seen in *Calcinus* species. An apomorphy of the spermatozoa of this species is the vesiculated or loculated appearance of the peripheral acrosome zone.

The morphological diversity, both adult and larval (Pike and Williamson, 1960), seen in the Diogenidae is reflected in the equally diverse spermatozoal (Tudge, 1995a, b) and spermatophore morphology (Tudge, 1991, 1995a). There appears to be no one spermatozoal character which unifies the group, but a suite of different characters is discernible which collectively unites the many genera.

(c) *Lithodidae*

In the family Lithodidae the members that have been examined for spermatozoal morphology are *Lithodes maja* by Retzius (1909) and Tudge *et al.* (1998a) (Fig. 5), *Paralithodes camtschatica* by Marukawa (1933) and *Hapalogaster dentata* by Goshima *et al.* (1995). The sperm of all three have an approximately spherical, concentrically zoned acrosome vesicle, capped by a centrally perforate operculum, penetrated by a perforatorial chamber, and posteriorly embedded in the cytoplasm. There is a large globular nucleus. Three conspicuous microtubular arms emerge from the cytoplasm below the acrosome vesicle. The spermatophores and spermatozoa of *L. maja* have been shown by Tudge *et al.* (1998a) to share many features with those of their nearest postulated relatives — members of the Paguridae (the genus *Pagurus* in particular). Spermatophore characters also link *L. maja* with pagurids: the presence of the accessory ampullae (synapomorphy, also with the parapagurids) and the

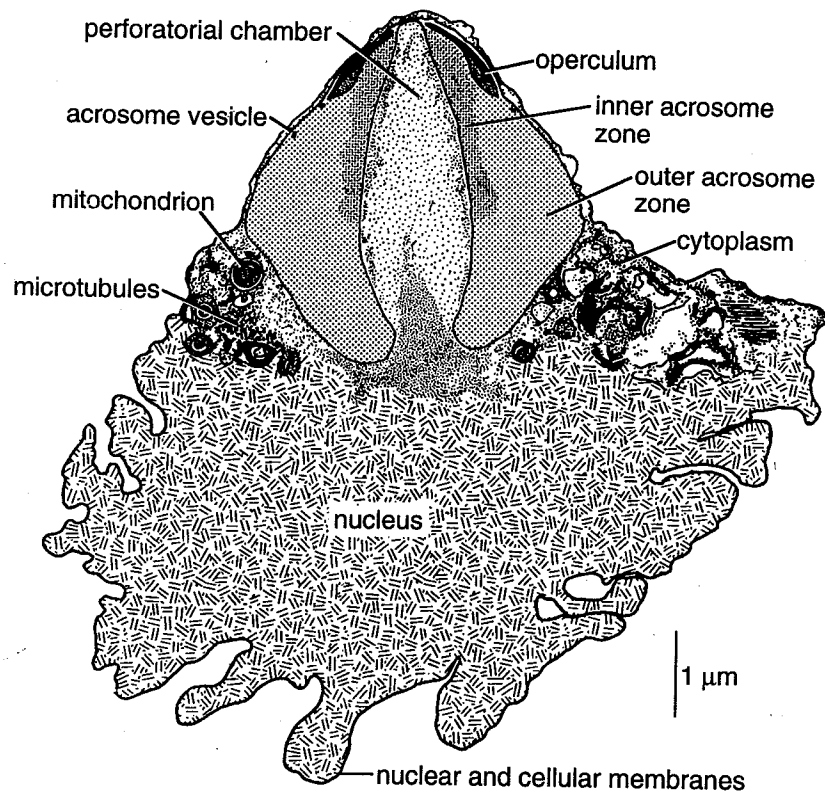


Fig. 5. *Lithodes maja*, Paguroidea, Lithodidae. Traced from TEM of vertical section of spermatozoon. From Tudge *et al.* (1998a). *Invertebrate Biology*, 117, 57–66.

homogeneous granular nature of the spermatophore wall. Spermatozoal characters include the simple concentric zonation of the acrosomal vesicle, the shape and differentiation of the operculum (synapomorphic with Paguridae), the electron-dense plume in the base of the perforatorial chamber (synapomorphy with *Pagurus*) and absence of microvillar projections in this chamber. Several features in which *L. maja* differs from species in the genus *Pagurus* include the central perforation of the operculum, the absence of reticulated acrosome zones and the possible presence of an irregular flange in some planes of the acrosomal vesicle (possible autapomorphy). This ultrastructural study for *L. maja* validates the molecular evidence (based on mitochondrial large-subunit rRNA similarity) that the lithodids have closer links with the genus *Pagurus*, within the Paguridae, than with other paguroideans (Cunningham *et al.*, 1992), a view supported by a recent morphological analysis (Richter and Scholtz, 1994).

(d) *Paguridae*

The spermatozoal morphology of the Paguridae has been well investigated at both the light and electron microscope level (see Table 3). Members of the family Paguridae are characterized by not possessing microvillar projections in the perforatorial chamber (an absence shared with the investigated representative from the Parapaguridae and the Lithodidae) and members of the genus *Pagurus* are further characterized by the presence of one or more reticulated acrosome zones in the acrosome vesicle (Tudge, 1995a, b). The sperm cell of *Pagurus hirtimanus* differs from those of *P. bernhardus*, *P. chevreuxi* and *P. prideaux* in having a large, elongate acrosome vesicle with an attenuated posterior region, as opposed to a small, ovoid acrosome vesicle. Minor differences in acrosome vesicle shape and ultrastructural zonation of the acrosome vesicle distinguish these latter European species.

The larval and adult morphological evidence for polyphyly (MacDonald *et al.*, 1957; Pike and Williamson, 1960; McLaughlin, 1974; Gore and Scotto, 1983; Ingle, 1985; Cunningham *et al.*, 1992; Richter and Scholtz, 1994) of the genus *Pagurus* is supported by spermatological evidence (Tudge 1995a, b, and references therein).

The spermatozoa of the genus *Solitariopagurus* share few characters with the representatives of the genus *Pagurus*, but the acrosome vesicle shape, perforatorial chamber shape and lack of microvillar projections in the perforatorial chamber confirm its placement in the Paguridae (Tudge, 1995a, b). The autapomorphic features of *Solitariopagurus* spermatozoa are an anterior perforatorial cone, a vesiculated, lacunar sheath encircling the posterior region of the perforatorial chamber and the folding of the perforatorial chamber walls into obvious septa.

The only notable spermatozoal feature which links *Xylopagurus* with the other members of the Paguridae is the lack of microvillar projections in the

perforatorial chamber (Tudge, 1995a, b). It is distinct in its acrosome vesicle zonation pattern and the occurrence of the cytoplasm, with microtubular arms, in the anterior part of the cell and not the posterior region (this feature is unique amongst the spermatozoa of all investigated paguroids).

#### (e) *Parapaguridae*

The spermatozoa of two representatives in the genus *Sympagurus*, from the family Parapaguridae, have been investigated at the light microscope level and using electron microscopy (Tudge, 1995a, b). The spermatozoa share several important spermatozoal and spermatophore features with representatives from the Paguridae. These are the possession of a reticulated acrosome zone in the acrosome vesicle, the shape of the perforatorial chamber, lack of microvillar projections in the perforatorial chamber and the presence of an accessory ampulla at the base of the main spermatophore ampulla. This latter feature is specifically shared with the genus *Pagurus* (Tudge, 1991, 1995a, 1997b) and all investigated members of the Lithodidae (Tudge *et al.*, 1998a). The extreme lengthening of the acrosome vesicle (autapomorphy) into a long cylinder is seen in *Sympagurus*, and separates these representatives from members of the Paguridae.

#### (f) *Pylochelidae*

Although the systematics and taxonomy of the family Pylochelidae have previously been investigated (Forest, 1987), their spermatozoal morphology is unknown. The pylochelids are considered the most primitive paguroid family (McLaughlin, 1983b; Martin and Abele, 1986) and have even been suggested to be directly descended from thalassinoid ancestors (Wolff, 1961).

### 5. Phylogenetic relationships (Anomura)

The use of spermatozoal ultrastructure in taxonomy and phylogeny is increasingly recognized for its value in investigating phylogenetic relationships in various animal groups. There are many phyla in which advances have been made in phylogenetic studies by using spermatozoal ultrastructure. Recent examples include studies in the Platyhelminthes (Ehlers, 1985; Justine, 1991); Polychaeta (Jamieson and Rouse, 1989); Oligochaeta (Jamieson, 1981, 1983); Mollusca (Healy, 1988); teleost fish (Jamieson, 1991c; Mattei, 1991); and Amniota (Healy and Jamieson, 1992; Jamieson and Healy, 1992).

Within the Crustacea, studies on spermatozoal ultrastructure in representatives of the Pentastomida (Wingstrand, 1972; Storch and Jamieson, 1992) have confirmed that the pentastomids are crustaceans; a view endorsed by molecular biology (Abele *et al.*, 1989). Similarly, spermatozoal ultrastructure has been shown by Jamieson and Tudge (1990) and Jamieson (1991b, 1994a, b) to support Guinot's re-

classification of the Brachyura (Guinot, 1977, 1978) into Podotremata, Heterotremata (*sensu lato*) and Thoracotremata, based on differential location of the male and female gonopores.

The phylogenetic significance of crustacean spermatozoa was recognized by Koltzoff (1906) and Wielgus (1973). Koltzoff (1906, p.424) was one of the first workers to construct a phylogeny of crustaceans based on spermatozoal structure at the light microscope level, in which he assigned the different sperm types studied a binomen consisting of the 'generic' name *Spermia* and a descriptive 'species' name.

The relationship of the Thalassinidea to the other decapod infraorders has long been the subject of debate. Thalassinideans are considered by some crustacean workers (Burkenroad, 1963; Bauer, 1986) to have important links with the other decapod infraorders and to have played an integral role in the evolution of the Decapoda. In a recent cladistic analysis of adult somatic characters the thalassinideans are depicted as the sister-group of the Anomura (=Anomala) (Poore, 1994).

The relationships between the families in the Anomura, and representatives within families, have been the subject of several investigations at the morphological and molecular level. Of note are the phylogenetic analyses of the constituent families of the Anomura (=Anomala) using morphological data (McLaughlin, 1983b; Martin and Abele, 1986; Richter and Scholtz, 1994; Scholtz and Richter, 1995) and the analyses of several members of the Anomura based on 18S rRNA nucleotide sequences by Spears and Abele (1988), Cunningham *et al.* (1992) and Spears *et al.* (1992).

A phylogenetic analysis of 42 species of anomuran crab (with three species of thalassinidean shrimp and six species of other decapods, variously used as the outgroup), using the computer program PAUP (Phylogenetic Analysis Using Parsimony) of Swofford (1991), was recently undertaken by Tudge (1995a, 1997b). The parsimony analysis procedure employed 26 spermatozoal characters and a further six characters from spermatophore morphology and was run using heuristic search strategies. The following relationships of the taxa were inferred from the trees produced. The Anomura do not form a monophyletic assemblage, as the lomoid, *Lomis*, is excluded from the anomuran clade whereas the thalassinid, *Thalassina*, is included. The synapomorphy joining the majority of the conventional anomuran taxa (*Lomis* excluded) is the cytoplasmic origin of the microtubular arms. When the palinurid and thalassinoid representatives are separately designated as outgroups the Astacidea and Brachyura jointly form a sister-group of the Anomura. The superfamilies Thalassinoidea, Paguroidea and Galatheaidea are not represented by monophyletic groups. In all analyses the anomuran families Coenobitidae and Porcellanidae each form a monophyletic group. The paguroid family Diogenidae is shown to be polyphyletic as the genera *Clibanarius* and *Cancellus* separate from a single clade containing the remaining diogenid genera. The families Paguridae and Parapaguridae form a monophyletic clade with the exception of the single *Solitariopagurus* species. The two representatives of the

family Chirostylidae (*Eumunida* and *Uroptychus*) fail to associate with the other investigated species in the Galatheoidea. The taxa in the family Galatheidæ are not a monophyletic assemblage. The only investigated hippoid, *Hippa*, is portrayed as the sister-group to the remainder of the anomuran taxa, with the exception of *Lomis* (Tudge, 1997a).

### H. Brachyura

The present account supplements but does not replace that of Jamieson *et al.* (1995) from which much further information may be obtained.

In previous analyses (Jamieson, 1991b, 1994a, b; Jamieson *et al.*, 1995) it has been shown that a classification, summarized by Warner (1977), which divides the Brachyura into five sections, the Dromiacea, Oxystomata, Oxyrhyncha, Cancrinea and Brachyrhyncha is not tenable from spermatozoal evidence. In contrast, the alternative classification, developed by Guinot (1977, 1978) in which the Brachyura are divided into three groups: the Podotremata (in turn divided into the Dromiacea and Archaeobranchyura), the Heterotremata and the Thoracotremata (Table 4) is largely supported.

Guinot's system is based on two apomorphies: location of female pores on the sternum of segment 6; and location of the male pores on the sternum of segment

Table 4  
Brachyuran classification of Guinot (1978)

Section	Sub-section	Superfamily	Sperm ultrastructure known
Podotremata	Dromiacea	Homolodromioidea	Homolodromiidae
		Dromioidea	Dromiidae
	Archaeobranchyura	Homoloidea	Homolidae
		Raninoidea	Raninidae
		Tymoloidea	Tymolidae
Eubranchyura#	Heterotremata	Dorippoidea	Dorippidae
		Calappoidea	Cancridae, Calappidae
		Portunoidea	Portunidae
		Xanthoidea	Xanthidae, Geryonidae
		Majoidea	Majidae
		Parthenopoidea	Parthenopidae
		Bellioidea	
		Leucosioidea	Leucosiidae
		Gecarcinoidea	
		Grapsoidea	Grapsoidea
	Thoracotremata	Mictyroidea	Mictyridae
		Pinnotheroidea	Pinnotheridae
		Hexapodoidea	Hexapodidae
		Ocypodoidea	Ocypodidae
		Hymenostomatoidea	Hymenosomatidae

#de Saint-Laurent (1980).

8; these contrast with a plesiomorphic location on the coxa of the corresponding ambulatory limb. The Thoracotremata possess both apomorphies; the Heterotremata have only the first, the male pores remaining plesiomorphically coxal, though in some families they have migrated to a coxosternal position (Palicidae, some xanthoids) or even a lateral sternal position (some portunids, e.g. *Callinectes*); the Podotremata, as the name suggests, have female and male pores on the coxae. Cladistic analysis (Jamieson, 1994a, b; Jamieson *et al.*, 1995) confirms the view previously proposed (Jamieson, 1991b) that recognition of the Heterotremata on a single apomorphy, the sternal female pores results in a paraphyletic group unless the Thoracotremata are included in it but that the Thoracotremata, so far as its sperm ultrastructure is known, is a monophyletic group.

The Thoracotremata show three synapomorphies: (1) concentric lamellation of the outer acrosome zone is present in five species, though varying in development in these and apparently absent in *Uca dussumieri* (Jamieson, 1991b) and *U. tangeri* (Medina and Rodriguez, 1992a), (2) the operculum has an apical button (not seen in *Macrophthalmus*); and (3) a differentiation of the acrosome contents which appears to be an extension of the basal ring ("xanthid ring" of Jamieson, 1989c) is present in at least the grapsids, the mictyrid, *Mictyris* and *Ocypode*, its homology being uncertain in *Uca* and *Macrophthalmus* (Jamieson, 1991b).

In cladistic studies (Jamieson, 1994a, b; Jamieson *et al.*, 1995), the Brachyura was found to be a monophyletic taxon relative to the anomuran outgroup, *Pagurus bernhardus* and *Clibanarius taeniatus*. Although the sperm of the Anomura and Brachyura are distinctive relative to other decapods, the Brachyura have only weak spermatozoal synapomorphies relative to anomurans despite forming a monophyletic brachyuran clade. Brachyuran monophyly is supported by shortening of the acrosome to a nearly spheroidal form; loss of corrugations of the wall of the perforatorial chamber, though these reappear in a different form in raninoids and cyclodorippoids; loss of microtubules from the lateral arms, a doubtful synapomorphy in view of their presence in at least some majids (Hinsch, 1973) and their sporadic demonstration in some other eubranchyurans (see below); and, somatically, development of a sella turcica and reduction of the uropods. Although spermatozoal support for a monophyletic Brachyura is weak, many constituent groups are, in contrast, strongly supported.

A generalized brachyuran spermatozoon, chiefly drawn from the Eubranchyura, is illustrated in Fig. 6. This shows the characters and some of their states which occur in the Brachyura and which are referred to in the following account. No sperm has all of the features shown, however. The nature and taxonomic distribution of some of these characters, with particular reference to the Eubranchyura, is briefly outlined here (for details, see Jamieson, 1994b; Jamieson *et al.*, 1995, 1996).

The operculum is an imperforate cap in most heterotremes. It is imperforate in *Macrophthalmus* alone of the investigated thoracotremes and also, though apically very thin, in a cyclodorippoid, *Cymonimus* sp. The operculum has a wide central



1991a, 1994a; Jamieson *et al.*, 1995) and in the gecarcinid *Cardisoma carnifex*, or is retained in a reduced form, as in the varunine grapsid *Varuna litterata*. The thickened ring is the sole basic synapomorphy of the Heterotremata + Thoracotremata, but is possibly found in *Thalassina squamifera*, alone, in the Thalassinoidea (Tudge, 1995a, b).

### 1. Dromiacea

The Dromiacea and the Archaeobrachyura constitute the Podotremata *sensu* Guinot. For a list of investigated podotremes, see Table 5. The Dromiacea as constituted by Guinot for the Dromiidae, Homolodromiidae, and Dynomenidae (Guinot, 1978, 1995) appears to form a monophyletic clade (Jamieson, 1994a, b; Jamieson *et al.*, 1995) defined by depression of the acrosome, well-developed protrusion of subopercular material through the operculum (a lesser protrusion occurs in homolids), and development of an anterolateral pale zone of the acrosome, but neither the constituent Dromiidae nor the Dynomenidae appear monophyletic spermatologically. It can thus be said that although there is distinctive dromiacean spermatozoal ground plan, sperm structure does not distinguish the constituent families Dromiidae, Homolodromiidae and Dynomenidae. This does not necessarily challenge definition of these families on the grounds of non-spermatozoal morphology (e.g. Guinot, 1995; McLay, 1993) and further analysis of non-spermatozoal characters is in progress to further ascertain the relationships of these families.

#### (a) Dromiidae

The dromiid spermatozoon, as exemplified by *Dromidiopsis edwardsi* (Jamieson *et al.*, 1993), *Stimdromia lateralis* (Jamieson, 1990) and *Dromidia antillensis* (Brown, 1966a; Felgenhauer and Abele, 1991), agrees with that of the Homolidae (Guinot *et al.*, 1994) and differs markedly from spermatozoa of other crabs (the raninid-heterotreme-thoracotreme assemblage) in the discoidal form of the acrosome and the capitate form of the perforatorium. Dromiids differ from homolids in the greater depression of the acrosome and the form of the head of the perforatorium, thus exhibiting a distinctive dromiid sperm type. The head is bilaterally prolonged in *Dromidiopsis edwardsi* and also shows bilateral symmetry, though this is less pronounced, in *S. lateralis*. In homolids the head of the perforatorium has the form of a horizontally disposed spiked wheel (Guinot *et al.*, 1994). Centrioles are unknown in dromiid sperm but are present in homolids. Nuclear arms in *Dromidiopsis edwardsi*, as in homolids, have the form of three small radial vertices. Dromiids, homolids, raninids, higher heterotremes and thoracotremes differ (homoplasiably?) from lower heterotremes, in lacking microtubules in the nuclear arms. Dromiid sperm lack the posterior median process of the nucleus (a term introduced by Hinsch, 1973) seen in homolids, some anomurans and lower

Table 5  
Ultrastructural studies of the spermatozoa of Podotremata

Superfamily, family and subfamily	Species	Reference
<b>Dromioidea</b>		
DROMIIDAE	<i>Cryptodromia antillensis</i>	Brown, 1966a, 1970; Felgenhauer and Abele, 1991 (both as <i>Dromidia antillensis</i> )
	<i>Dromidiopsis edwardsi</i>	Jamieson <i>et al.</i> , 1993; Jamieson, 1994a, b
	<i>Stimdromia lateralis</i>	Jamieson, 1990, 1991a, b, 1994a; Guinot <i>et al.</i> , 1994 (all as <i>Petalomera lateralis</i> )
	<i>Sphaerodromia lamellata</i>	Guinot <i>et al.</i> , 1998
DYNOMENIDAE	<i>Dynomene tanensis</i>	Jamieson <i>et al.</i> , 1995 (as <i>D. aff. devaneyi</i> ); Guinot <i>et al.</i> , 1998
	<i>Paradynomene tuberculata</i>	Jamieson <i>et al.</i> , 1993b; Jamieson, 1994a
<b>Homoloidea</b>		
HOMOLIDAE	<i>Homola ranunculus</i>	Guinot <i>et al.</i> , 1994
	<i>Homologenus levii</i>	Jamieson <i>et al.</i> , 1993c (as <i>Homologenus</i> sp.)
	<i>Homolomannia sibogae</i>	Jamieson <i>et al.</i> , 1993c
	<i>Latreillopsis gracilipes</i>	Jamieson <i>et al.</i> , 1993c (as <i>Latreillopsis</i> sp); Jamieson, 1994a, b
	<i>Dagnaudus petterdi</i>	Guinot <i>et al.</i> , 1994
	<i>Paromola bathyalis</i>	Guinot <i>et al.</i> , 1994 (as <i>Paromola petterdi</i> )
	<i>Paromolopsis boasi</i>	Jamieson <i>et al.</i> , 1993c
HOMOLODROMIIDAE	<i>Homolodromia kai</i>	Jamieson <i>et al.</i> , 1995; Guinot <i>et al.</i> , 1998
LATREILLIDAE	<i>Latreillia</i> sp.	Jamieson, 1994a, b
<b>Cyclodorippoidea</b>		
CYCLODORIPPIDAE	<i>Tymolus</i> sp.	Jamieson, 1994a; Jamieson <i>et al.</i> , 1994b
	<i>Xeinostoma richeri</i>	Jamieson, 1994a; Jamieson <i>et al.</i> , 1994b
CYMONOMIDAE	<i>Cymonomus</i> sp.	Jamieson, 1994a; Jamieson <i>et al.</i> , 1994b
<b>Raninoidea</b>		
RANINIDAE		
Lyreidinae	<i>Lyreidus brevifrons</i>	Jamieson, 1994a; Jamieson <i>et al.</i> , 1994a
Ranininae	<i>Ranina ranina</i>	Jamieson, 1989b, 1991a, b, 1994a; Guinot <i>et al.</i> , 1994
Raninoidinae	<i>Raninoides</i> sp.	Jamieson, 1994a, b; Jamieson <i>et al.</i> , 1994a
Notopodinae	<i>Cosmonotus</i> sp.	This study*

\* Material kindly supplied by B. Richer de Forges as part of a continuing collaboration between Jamieson, Guinot, Richer de Forges, Scheltinga and Tudge.

heterotremes. The sperm of *D. edwardsi* differs from other investigated dromiid sperm in the asymmetrical location of the opercular perforation relative to the longitudinal axis of the sperm; and in more complex zonation of the acrosome vesicle. The acrosome is deeply embedded in the nucleus in *D. edwardsi* whereas

in *S. lateralis* it is superficial on the nucleus. Both have an apical protuberance of subopercular material through the opercular perforation. The protuberance is less developed in homolids and is paralleled in hymenosomatids.

*Stimdromia lateralis* is diagnosed by the presence of capsular projections. *Dynomene tanensis* (Fig. 7), which computes as basal relative to these taxa (Jamieson *et al.*, 1995), appears to be unique in the Brachyura in having only two nuclear arms. *Paradynomene* and *Homolodromia* (Fig. 8) have a flange-like lateral extension of the lower acrosome zone, a striking similarity which computes as a synapomorphy.

Spermatologically *Sphaerodromia lamellata* appears closer to the dynomenid *Dynomene tanensis* than it is to the mutually paraphyletic *Dromidiopsis edwardsi* and *Stimdromia lateralis*. Neither the Dromiidae nor the Dynomenidae appear monophyletic spermatologically (Guinot *et al.*, 1998). In earlier cladistic analysis (Jamieson, 1994b; Jamieson *et al.*, 1995), the Dromiidae also computed as a paraphyletic group, though, again, part of a monophyletic dromiid-dynomenid-*Homolodromia* clade.

#### (b) Homolodromiidae

On morphological grounds, the Homolodromiidae is placed in a monotypic superfamily Homolodromioidea, within the Dromiacea, by Guinot (1978, 1995).

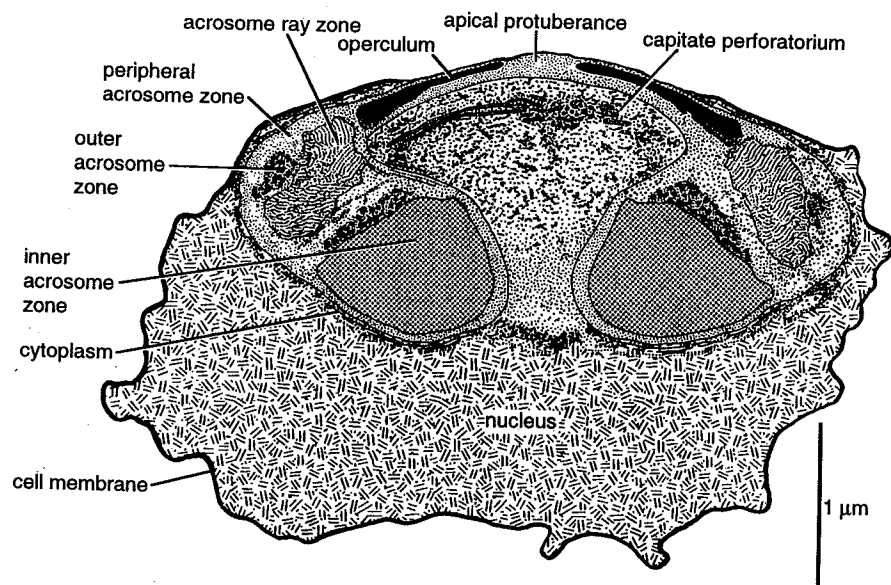


Fig. 7. *Dynomene tanensis*, Dromioidea, Dynomenidae. From Guinot *et al.* (1998). *Journal of Crustacean Biology*, 18, 78–94.

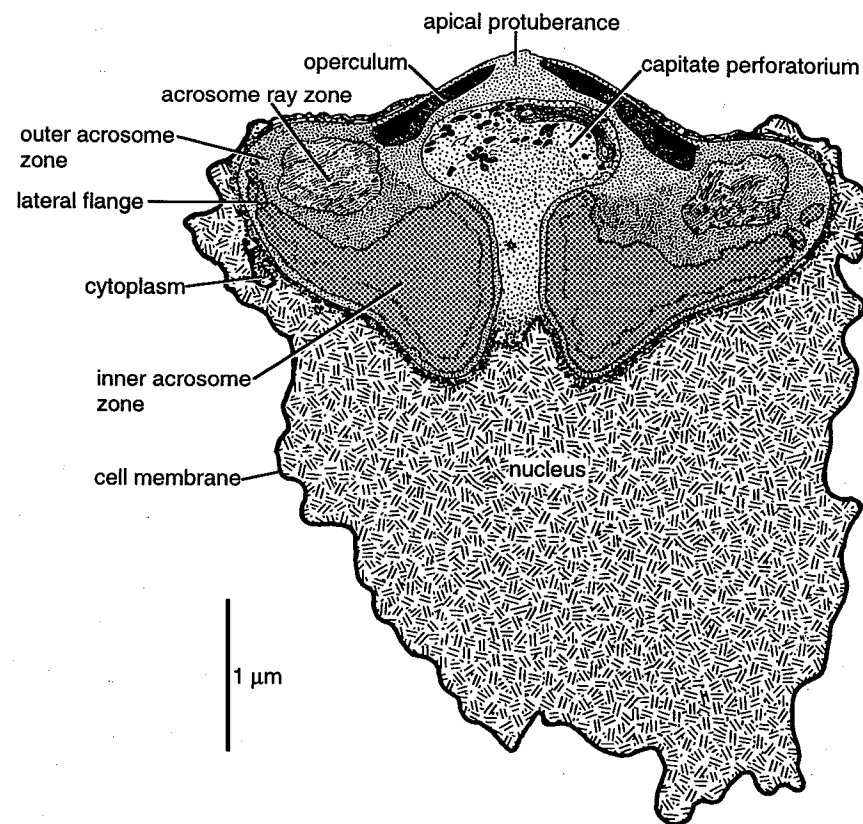


Fig. 8. *Homolodromia kai*, Dromioidea, Homolodromiidae. Traced from TEM of vertical section of spermatozoon. From Guinot *et al.* (1998). *Journal of Crustacean Biology*, 18, 78–94.

She lists a long series of characters in support of the contention that the Homolodromioidea represent the most primitive members of the Podotremata. Scholtz and Richter (1995) also supported the primitive status of the Homolodromiidae but went so far as to regard homolodromiids as the sister-group of all other Brachyura. However, they have since returned homolodromiids to the Dromiacea (oral statement in the 2nd European Crustacean Conference, Liège, Belgium, 1996) in which they were placed by Guinot.

If homolodromiids are the most primitive dromiaceans, it is difficult, to evaluate the relatively advanced position which *Homolodromia* (Fig. 8) appears to occupy in spermiocladistic analysis (Jamieson *et al.*, 1995) relative to other dromiaceans and the similarity of the sperm of *Homolodromia* to that of *Paradynomene*. It is noteworthy that Guinot (1978) stated that in some regards it is the dynomenids which seem closer to the Homolodromiidae than do the Dromiidae.



The Homolodromiidae have a unique combination of morphological characters, though mostly plesiomorphic. These are, *inter alia*, fusion of the ophthalmic segment to the anterior carapace (in *Homolodromia*); the soft branchiostegite; endophragmal skeleton with anastomoses; abdominal pleura developed; and retention of abdominal pleopods in the male on segments 3 to 5. Occurrence of uropods which are not dorsal and are represented by small lobes on the abdominal segment 6 appears to be a homolodromiid synapomorphy (Guinot, 1995).

In terms of the ultrastructural characters used in spermiocladistic analyses (Jamieson *et al.*, 1995), the spermatozoon of *Homolodromia kai* has the following characteristics. The ratio of length to width of the acrosome is 0.4; zonation of the acrosome is predominantly horizontal; the operculum is perforate and lacks opercular projections diagnostic of homolids; the operculum is not continuous with the acrosomal capsule, and, in contrast with raninoids, it is moderately thick and is of moderate width, not thin and occupying much of the width of the acrosome as in cyclodorippoids; there is no periopercular rim nor an accessory opercular ring; protrusion of subopercular material through the operculum is well developed; a true acrosome ray zone of the type seen in paguroids, some other anomurans and in brachyurans of the Heterotremata *sensu stricto*, is absent although the possibly homologous 'finger-print' like zone is present; the ragged outer acrosomal zone and the xanthid ring, typical of xanthids and some of their relatives, are absent; an anterior pale zone of the acrosome, seen also in *Stimdromia*, *Dromidiopsis*, *Dynomene* and *Paradynomene*, is present; the subacrosomal chamber extends pre-equatorially in the acrosome as in all investigated species excepting *Ranina ranina*; the head of the putative perforatorium is bilaterally symmetrical, as in *Stimdromia*, *Dromidiopsis* and *Paradynomene*; corrugations of the wall of the perforatorial chamber, a thickened ring, concentric lamellae, capsular chambers, projections and flanges are absent. Nuclear arms and a definite posterior median process are not demonstrable. Thus, *Homolodromia* displays a mixture of dromiid and dynomenid spermatozoal features.

The bilateral perforatorial head is seen in dromiids (*Dromidiopsis edwardsi* and *Stimdromia lateralis*); in the two investigated dynomenids, *Paradynomene tuberculata* and *Dynomene tanensis* (Fig. 7), and in *Homolodromia kai*, it contrasts with that of homolid sperm which has the form of a horizontally disposed spiked wheel (Jamieson, 1994b; Jamieson *et al.*, 1993c). *Paradynomene* and *Homolodromia* (Fig. 8) have a striking synapomorphy, as noted above. *Paradynomene* is distinguished (ambiguously) by slight lengthening of the acrosome, whereas *Homolodromia* shows no individual apomorphy; in the spermatozoal analysis, it is distinguished from *Paradynomene* only by its slightly more depressed acrosome.

## 2. Archaeobrachyura

The Archaeobrachyura of Guinot (1978) contain the raninoids, homolids and cyclodorippoids (tymoloids). From cladistic analysis (Jamieson, 1994a, b; Jamieson *et al.*, 1995) the single spermatozoal synapomorphy for the Archaeobrachyura is weak: the presence of a posterior median process. It is, however, reinforced by the somatic character, loss of the uropods (Jamieson, 1994a). The grouping Archaeobrachyura is not, however, supported in purely spermatozoal analysis (Jamieson *et al.*, 1995) in which *Latreillia* and the homolids group with the Dromiacea (Dromiidae, Dynomenidae and Homolodromiidae) and not with the raninoid+cyclodorippoid assemblage.

### (a) Homoloidea

Spermatozoal ultrastructure has been examined in seven species of the Homolidae: *Homola ranunculus*, *Paromola bathyalis* and *Dagnaudus* (= *Paromola*) *petterdi* (Guinot *et al.*, 1994) and *Homologenus levii*, *Latreillopsis gracilipes* (Fig. 9), *Homolomannia sibogae*, and *Paromolopsis boasi* (Jamieson *et al.*, 1993c).

From spermatozoal ultrastructure, the Homolidae is a convincingly monophyletic entity (Jamieson *et al.*, 1993c, 1995; Jamieson, 1994b). The synapomorphies of homolid spermatozoa are striking and have so far proved constant; they are as follows. The 'spiked-wheel' form of the anterior expansion of the perforatorium, in which slender pointed processes radiate from a central axis, is restricted to the Homolidae for which it is, thus, an autapomorphy. The presence of numerous radial extensions of the acrosomal operculum into the perforatorium is a further autapomorphy of the homolids (Jamieson *et al.*, 1993c) seen in no other brachyurans. Projection of subacrosomal material into the opercular perforation occurs but is weaker than the strong protrusion which occurs in dromiaceans. The radial spikes, approximately 12 in number, extend far laterally. They are supported by fibrous cores which radiate from the central core of the perforatorium. The spikes are much longer in *Latreillopsis gracilipes* (Fig. 9) than in the other species, curving around the inner aspect of the vesicle almost to its base.

### (b) Latreilliidae

The Latreilliidae were placed in the Archaeobrachyura by Guinot (1978). The position of *Latreillia* sp., the sperm of which was described ultrastructurally by Jamieson (1994b) (Fig. 10), is equivocal in cladistic analyses (Jamieson, 1994b; Jamieson *et al.*, 1995). If spermatozoal ultrastructure alone is considered it forms a polytomy: *Latreillia*, Homolidae, Dromiacea (Dromiidae, Dynomenidae, Homolodromiidae). It is noteworthy that when, in purely spermatological analysis, the homolids lose their morphological archaeobrachyuran position and group with



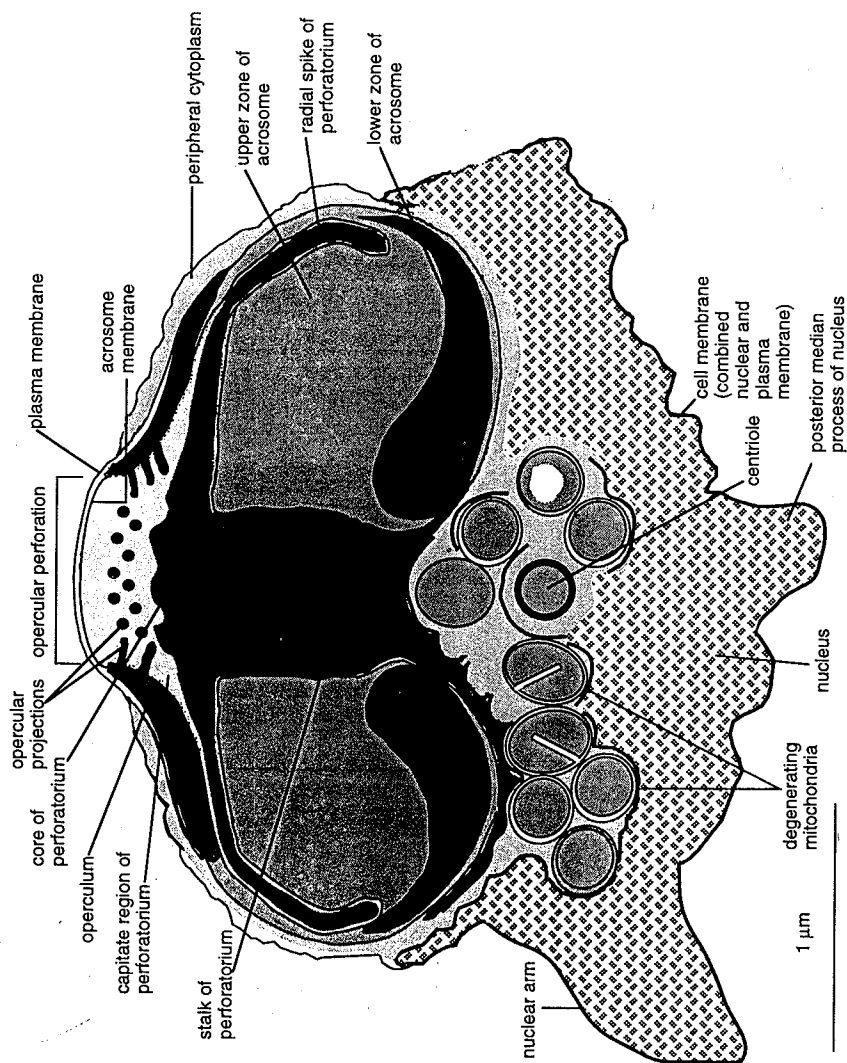


Fig. 9. *Latreilopsis gracilipes*, Homoloidea, Homoloidea. Traced from TEM of vertical section of spermatozoan. After Jamieson, B.G.M. (1994b). *Philosophical Transactions of the Royal Society of London B*, 345, 373–393.

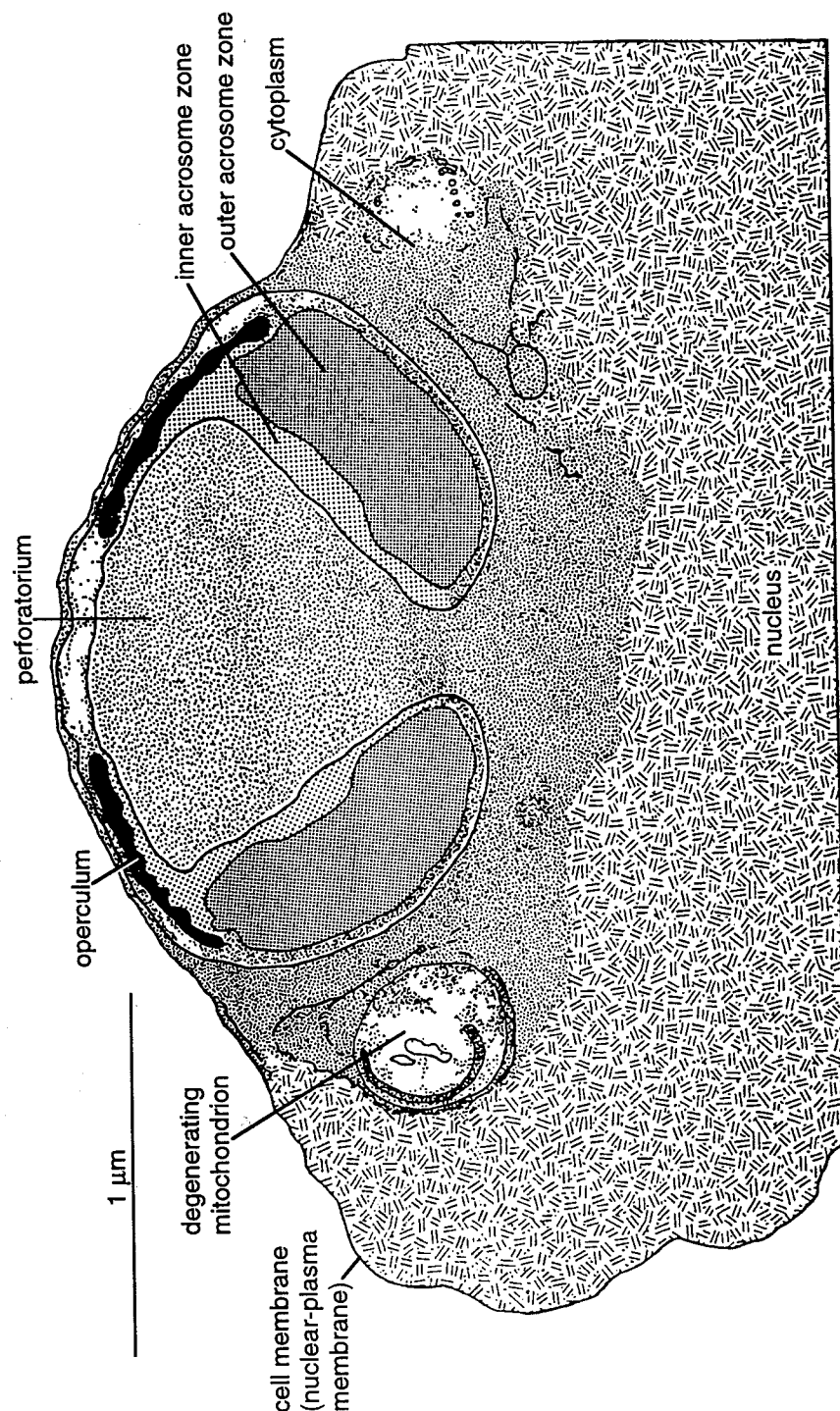


Fig. 10. *Latreillia* sp., Homoloidea, Latreillidae. Traced from TEM of vertical section of spermatozoan. After Jamieson, B.G.M. (1994b). *Philosophical Transactions of the Royal Society of London B*, 345, 373–393.

the dromiaceans, *Latreillia* does the same. This supports an association with homolids, with which they are strongly linked morphologically in the superfamily Homoloidea which also contains the Poupiniidae (Guinot, 1978; Guinot and Richer de Forges, 1995).

A dubious apomorphy of *Latreillia* is development, homoplasically with *Dromidiopsis*, of a condition of the acrosome vesicle contents intermediate between the horizontally zoned and concentric conditions (Jamieson *et al.*, 1995).

*Latreillia* is unique outside the Dromiacea in having a bilaterally symmetrical capitate perforatorium, though the condition is weakly developed. This computes as retention of a plesiomorphic condition which was itself a synapomorphy of the Podotremata but other interpretations are plausible, including homoplastic development of the bilateral condition. It is possible that the sperm of *Latreillia* is secondarily simplified. If not, there is much in its ultrastructure which would qualify it to be considered the most plesiomorphic podotreme spermatozoon.

### (c) *Raninoidea*

Raninoids, with dorippoids and calappoids, constitute the spermatologically heterogeneous and clearly polyphyletic Oxystomata (Warner, 1977). They are referred to the Archaeobrachyura by Guinot (1978). Spermatologically, the Raninoidea and Cyclodorippoidea form a monophyletic (unnamed) clade.

Spermatozoal ultrastructure has been investigated in *Ranina ranina* (Jamieson, 1989b), in the subfamily Ranininae, *Raninoides* sp. (Jamieson *et al.*, 1994a), in the subfamily Raninoidinae (reinstated by Guinot, 1993), *Lyreidus brevifrons* (Jamieson *et al.*, 1994a), in the subfamily Lyreidinae of Guinot (1993) and *Cosmonotus* sp. in the Notopodinae (Fig. 11A) (this study).

These raninoids, as a group, are well defined spermatologically by virtual continuity of the operculum with the capsule and alteration of the zonation of the acrosome vesicle to a condition intermediate between horizontal and concentric, with development of a concentric condition in *Ranina ranina* and *Cosmonotus*. The intermediate condition is homoplastic with *Dromidiopsis* and *Latreillia* (Jamieson, 1994a, b; Jamieson *et al.*, 1995).

*Ranina* (Jamieson, 1989b) and *Raninoides* (Jamieson *et al.*, 1994a) share strong synapomorphies: development of posterior capsular chambers, one in *Ranina* increasing to several in *Raninoides*; and the remarkable lateral flange on the capsule. These are not seen in *Lyreidus* though multiple capsular projections, perhaps equivalent to the single flange, are present. A single poorly developed flange is, however present in *Cosmonotus* sp., in which although capsular chambers are absent, there are numerous vesicle-like enclaves in the cytoplasm directly adjacent to and sometimes interrupting the capsule. Branched septum-like corrugations of the wall of the perforatorial chamber have possibly developed from the unbranched form basal to the raninoid-cyclodorippoid clade and persistent in *Lyreidus*. Corrugations are, however, absent in *Cosmonotus* (Fig. 11A).

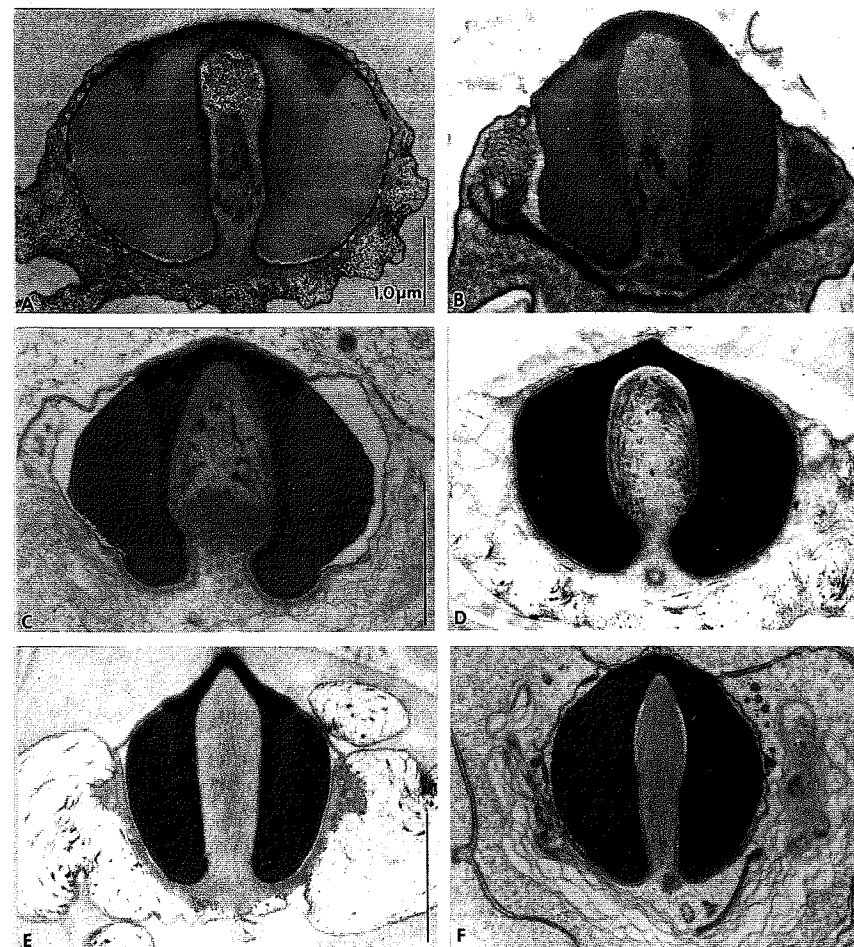


Fig. 11. TEM of spermatozoa in vertical section. A: *Cosmonotus* sp., Raninoidea, Raninidae. B: *Neodorippe astuta* (cf. *callida*), Dorippoidea, Dorippidae, Dorippinae. C: *Ethusina indica*, Dorippidae, Ethusinae. D: *Retropluma* sp., Retroplumidae. E: *Calappa* aff. *gallus*, Calappoidea, Calappidae. F: *Mursia microspina*, Calappoidea, Calappidae. (All original).

There is a strong trend towards a subspheroidal form of the acrosome, most developed in *Ranina* in which zonation becomes concentric; and in which the perforatorium, apparently secondarily, becomes only postequatorial. In *Lyreidus*, the acrosome becomes secondarily depressed; and an 'amoeboid' form of the head of the perforatorium is seen as development of a capitate condition independently of that in dromiids and homolids (Jamieson, 1994a, b; Jamieson *et al.*, 1995). *Raninoides* and *Cosmonotus* have a depressed subspherical acrosome.

In *Cosmonotus* (Fig. 11A), there are no less than four concentric acrosome zones around the perforatorial chamber: a narrow dense, inner zone; a fusiform, less dense outer zone; a parenthesis less but still moderately dense third zone, and a large, pale peripheral zone. As in *Raninoides*, there is a wide subopercular zone. The perforatorium has a clavate expansion anteriorly, which resembles the head of the perforatorium in *Lyreidus* in substructure but differs from the latter in having a rounded and not 'amoeboid' outline. The posterior median process is represented by a single or bifid process.

#### (d) Cyclodorippoidea

The spermatozoa of two genera and species of Cyclodorippidae, *Xeinostoma richeri* (Xeinostominae) and *Tymolus* sp. (Cyclodorippinae), and one species of *Cymonomus* sp. (Cymonomidae) (Fig. 12) were described by Jamieson *et al.* (1994b).

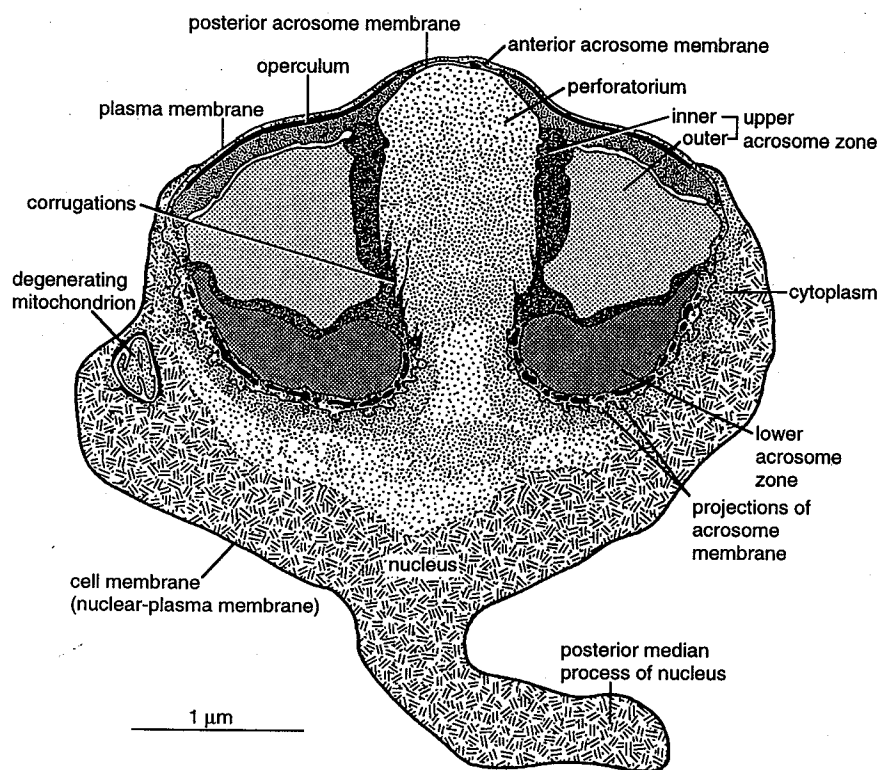


Fig. 12. *Cymonomus* sp., Cyclodorippoidea, Cymonomidae. Traced from TEM of vertical section of spermatozoon. After Jamieson, B.G.M. (1994b). *Philosophical Transactions of the Royal Society of London B*, 345, 373–393.

In spermiocladistic analysis (Jamieson, 1994b; Jamieson *et al.*, 1995), the Cyclodorippoidea form the sister group of the Raninoidea. The sperm is anteroposteriorly depressed, with a mean ratio of length to width 0.5:0.6. The distinctive cyclodorippoid feature is that the operculum extends to the lateral limits of the acrosome (autapomorphy of cyclodorippoids). The contents of the acrosome vesicle have two major horizontal zones, with a dense lower zone, as in homolids and dynomenids, including a dense lower (posterior) zone. The perforatorium is very wide (0.3 width of acrosome), anteriorly rounded, is not capitate, and lacks radiate projections. A periacrosomal flange (*Xeinostoma richeri*) and smaller evaginations of the acrosome membrane (or capsule?) (*X. richeri*, less distinctly *Cymonomus* sp. and *Tymolus* sp.) are reminiscent of the single acrosomal flange of *Ranina* and *Raninoides* sperm and the multiple keels of the *Lyreidus* sperm. Slender dense filaments extend into the perforatorium from its walls, their bases associated with corrugations of its basal wall. Development of these filaments computes as an ambiguous synapomorphy. The nucleus, cupping the acrosome and cytoplasm, has a well-developed posterior median process as in homolid and raninoid sperm. The nuclear arms lack microtubules. The cytoplasm forms a narrow postacrosomal band extending anteriorly as far as the operculum, associated with few, degenerate mitochondria. The non-capitate form of the perforatorium differs from the capitate condition in dromiids, the related dynomenids, and homolodromiids and from homolids and, though homoplastic, the raninoid *Lyreidus*.

A synapomorphy of *Xeinostoma* and *Tymolus* is the extreme thinness of the operculum, a possible synapomorphy of the Xeinostominae. *Xeinostoma* is apomorphic in further depression of the acrosome.

Alone in the investigated podotremes, *Cymonomus* (Fig. 12) is apomorphic in losing the opercular perforation, though this requires confirmation from further material. This autapomorphy supports erection of a separate family Cymonomidae (Tavares, 1994). It appears to have developed the flange-like extension of the lower acrosome zone independently of *Paradynomene* and *Homolodromia* but the similarity is striking and cyclodorippoid relationships require further investigation.

A similarity (homoplasy?) to *Paradynomene* and *Homolodromia* is the discontinuous flange-like peripheral continuation of the lower zone of the acrosome contents in *Cymonomus* sp.

#### I. Eubrachyura

The Eubrachyura of de Saint Laurent (1980) contains the Heterotremata and Thoracotremata of Guinot (1977) and therefore includes all Brachyura other than the Podotremata. Jamieson (1991b, 1994b; Jamieson *et al.*, 1995) has argued for inclusion of the Thoracotremata, as a monophyletic assemblage, within the Heterotremata *sensu lato*, the latter taxon being synonymous with Eubrachyura. The Eubrachyura is defined by a convincing synapomorphy, presence of the thickened ring (Jamieson, 1991b, 1994b; Jamieson *et al.*, 1995). Other spermatozoal

synapomorphies, although unambiguous, are less convincing. Multiplication of lateral arms from three, common to paguroids and podotremes, to several is a trend rather than a diagnostic basal apomorphy as it results from polymorphism, there being three in at least some majids as in the leucosiid *Iliacantha subglobosa* (Felgenhauer and Abele, 1991). Presence of a true acrosome ray zone has been considered a basal synapomorphy of heterotremes but is seen, apparently homoplasically, in paguroids, and increasing numbers of heterotreme sperm which lack evident acrosome rays are being found.

### 1. Heterotremata

Cladistically (Jamieson, 1994a, b; Jamieson *et al.*, 1995), the Heterotremata *sensu lato* form a grouping whether or not non-spermatozoal characters are included. The sternal female pores constitute, as Guinot (1978, 1993) suggested, their non-spermatozoal synapomorphy.

#### (a) Dorippidae

Characteristic eubranchyuran features of the sperm of *Neodorippe astuta* (possibly *N. callida*), described by Jamieson and Tudge (1990) (Fig. 11B), and *Ethusina indica* (this study) (Fig. 11C) are the perforatorium extending almost to the operculum; presence in the perforatorium of convoluted tubules; a zone of acrosomal rays forming the outer part of an inner dense zone; the presence of a thickened ring surrounding the basal part of the perforatorium; and, basally, two centrioles. An acrosome ray zone, peripheral to the inner acrosome zone, in *N. astuta* is apparently absent, or at most very poorly developed, in *E. indica*.

When somatic and spermatozoal characters were combined, *Neodorippe astuta* formed the plesiomorphic sister-group of all other included enbranchyuran crabs (Jamieson, 1994a, b; Jamieson *et al.*, 1995). The sole (ambiguous) spermatozoal apomorphy was found to be very slight elongation of the acrosome beyond a spheroidal shape. However, in *Ethusina indica* (Fig. 11C), the acrosome is slightly wider than long and the perforatorial column is elliptical and not stoutly baton-shaped, unlike *N. astuta*. Furthermore, a multilaminar membrane, which is strongly developed between the cytoplasm and nucleus in *N. astuta*, is absent or in a state of disruption in *E. indica*. This difference in the membranes possibly represents a difference in maturity of the sperm, however.

It is noteworthy, in view of their relatively plesiomorphic spermatozoal ultrastructure, that dorippids exhibit carrying behaviour, like most dromiids, *Neodorippe callida* attaching to leaves. There are, however, no spermatozoal apomorphies distinguishing the remaining crabs of the Heterotremata *sensu lato* from *Neodorippe*, though somatic synapomorphies are loss of subcheliform development of pereopods 5 (P5) (and also P4).

Table 6

Ultrastructural studies of the spermatozoa of Heterotremata *sensu strictu*

Superfamily, family and subfamily	Species	Reference
<b>Dorippoidea</b>		
DORIPPIDAE		
Dorippinae	<i>Neodorippe</i> c.f. <i>callida</i>	Jamieson and Tudge, 1990; Jamieson, 1991a, b, 1994a (all as <i>N. astuta</i> )
Ethusinae	<i>Ethusina indica</i>	This study*
RETROPLUMIDAE	<i>Retropluma</i> sp.	This study*
<b>Majoidea</b>		
MAJIDAE		
Majinae	<i>Maja squinado</i>	Tudge and Justine, 1994
Oregoniinae	<i>Chionoecetes opilio</i>	Beninger <i>et al.</i> , 1988; Chiba <i>et al.</i> , 1992
Inachinae	<i>Cyrtomaia furci</i>	This study* and Jamieson <i>et al.</i> , 1998
	<i>Grypaeus hyalinus</i>	This study* and Jamieson <i>et al.</i> , 1998
	<i>Macropodia longirostris</i>	This study* and Jamieson <i>et al.</i> , 1998
	<i>Platymaia rebierei</i>	This study* and Jamieson <i>et al.</i> , 1998
	<i>Podochela risei</i>	Hinsch, 1973
	<i>Stenorhynchus seticornis</i>	Hinsch, 1973
Pisinae	<i>Hyastenus diacanthus</i>	This study and Jamieson <i>et al.</i> , 1998
Mithracinae	<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>Macrocoeloma trispinosum</i>	Hinsch, 1973
	<i>Mithrax</i> sp.	Hinsch, 1973
Acanthonychinae	<i>Menaethius monoceros</i>	Jamieson, 1991b, 1994a
	<i>Sphenocarcinus orbiculatus</i>	This study* and Jamieson <i>et al.</i> , 1998
	<i>Sphenocarcinus stuckiae</i>	This study* and Jamieson <i>et al.</i> , 1998
Ophthalmiinae	<i>Pitho lherminieri</i>	Hinsch, 1973
<b>Calappoidea</b>		
CALAPPIDAE		
	<i>Calappa hepatica</i>	Jamieson, 1991b
	<i>Calappa</i> aff. <i>gallus</i>	This study*
	<i>Mursia microspina</i>	This study*
<b>Corystoidea</b>		
CORYSTIDAE		
CANCRIDAE		
	<i>Corystes cassivelaunus</i>	Jamieson <i>et al.</i> , 1997
	<i>Cancer borealis</i>	Langreth, 1965, 1969
	<i>Cancer irroratus</i>	Langreth, 1965, 1969
	<i>Cancer magister</i>	Langreth, 1965, 1969
	<i>Cancer pagurus</i>	Pochon-Masson, 1968a; Tudge <i>et al.</i> , 1994; Tudge and Justine, 1994; Jamieson <i>et al.</i> , 1997
	<i>Cancer productus</i>	Langreth, 1965, 1969
	<i>Plateipistoma nanum</i>	Jamieson <i>et al.</i> , 1997
<b>Portunoidea</b>		
Portuniinae	<i>Portunus pelagicus</i>	Jamieson, 1989b, 1991b; Jamieson and Tudge, 1990; El-Sherief, 1991; Guinot <i>et al.</i> , 1994; Jamieson, 1994a

(contd.)

Table 6 (contd.)

Superfamily, family and subfamily	Species	Reference
	<i>Callinectes sapidus</i>	Brown, 1966a, b; Felgenhauer and Abele, 1991
	<i>Xaiva</i> sp.	This study*
Caphyrinae	<i>Caphyra laevis</i>	Jamieson, 1991b, 1994a
	<i>Caphyra rotundifrons</i>	Jamieson, 1991b, 1994a
Carcininae	<i>Carcinus maenas</i>	Pochon-Masson, 1962, 1968a; Chevaillier, 1966a, 1967b, 1969; Pearson and Walker, 1975; Goudeau, 1982; Reger <i>et al.</i> , 1984
Polybiinae	<i>Ovalipes ocellatus</i>	Hinsch, 1986
	<i>Ovalipes mollerii</i>	This study*
Podophthalminae	<i>Podophthalmus vigil</i>	This study
<b>Cryptochiroidea</b>		
CRYPTOCHIRIDAE		
(= HAPALOCARCINIDAE)	<i>Cryptochirus coralliodytes</i>	This study
	<i>Hapalocarcinus marsupialis</i>	This study*
<b>Xanthoidea</b>		
XANTHIDAE		
	<i>Atergatis floridus</i>	Jamieson, 1989a, 1989c, 1991b; Jamieson <i>et al.</i> , 1993a
	<i>Etisus laevimanus</i>	Jamieson, 1989c, 1991b
	<i>Pilodius areolatus</i>	Jamieson, 1989c, 1991b, 1994a
	<i>Liagore rubromaculata</i>	Jamieson, 1989c, 1991b
EUMEDONIDAE	<i>Eumedonus granulatus</i>	This study*
	<i>Harrovia albolineata</i>	This study*
PANOPEIDAE	<i>Eurypanopeus depressus</i>	Felgenhauer and Abele, 1991; This study
	<i>Eurytium limosum</i>	Felgenhauer and Abele, 1991
	<i>Panopeus obesus</i>	This study
MENIPPIDAE	<i>Menippe mercenaria</i>	Brown, 1966a
	<i>Eriphia sebana</i>	Jamieson, unpublished
PILUMNIDAE	<i>Pilumnus semilanus</i>	This study
TRAPEZIIDAE	<i>Trapezia cymodoce</i>	Jamieson, 1993a
	<i>Tetralia fulva</i>	This study
	<i>Tetralia nigrolineata</i>	This study
TRAPEZIIDAE(?)	<i>Calocarcinus africanus</i>	Jamieson <i>et al.</i> , 1993a, Jamieson, 1994a
	(Bythograeidae?)	
GERYONIDAE	<i>Geryon fenneri</i>	Hinsch, 1988
	<i>Geryon quinquedens</i>	Hinsch, 1988
BYTHOGRAEIDAE	<i>Austino-graea alayseae</i>	Tudge <i>et al.</i> (1998b)
	<i>Bythograea thermidron</i>	Tudge <i>et al.</i> (1998b)
	<i>Segonzacia mesatlantica</i>	Tudge <i>et al.</i> (1998b)
GONEPLACIDAE	<i>Australocarcinus riparius</i>	Jamieson and Guinot, 1996
	<i>Carcinoplax microphthalmus</i>	This study*
	<i>Ceratoplax</i> sp.	This study
	<i>Goneplacid</i> sp.	This study*
HEXAPODIDAE	<i>Hexaplax megalops</i>	This study
<b>Parthenopoidea</b>		
PARTHENOPIIDAE		
	<i>Parthenopidae</i> sp.	This study*
	<i>Heterocrypta granulata</i>	Hinsch, 1973

(contd.)

Table 5 (contd.)

Superfamily, family and subfamily	Species	Reference
	<i>Parthenope serratus</i>	Hinsch, 1973
<b>Potamoidea</b>		
POTAMIDAE		
	<i>Potamon fluviatile</i>	Tudge and Justine, 1994; Guinot <i>et al.</i> , 1997
	<i>Potamon ibericum</i>	Guinot <i>et al.</i> , 1997
POTAMONAUTIDAE	<i>Potamonautes perlatus</i>	Jamieson, 1993b, 1994a, b
	<i>sidneyi</i>	
SUNDATHELPUSIDAE	<i>Holthuisiana transversa</i>	This study (Fig. 14F)
<b>Leucosoidea</b>		
LEUCOSIIDAE		
Leucosiinae	<i>Iliacantha subglubosa</i>	Felgenhauer and Abele, 1991
Philyrinae	<i>Philyra laevis</i>	This study
	<i>Randallia serenei</i>	This study*
<b>Hymenosomatoidea</b>		
HYMENOSOMATIDAE		
	<i>Odiomaris (=Halocarcinus)</i>	Richer de Forges <i>et al.</i> , 1997
	<i>pilosus</i>	
	<i>Odiomaris estuarius</i>	Richer de Forges <i>et al.</i> , 1997
	<i>Elemena vesca</i>	This study*

\* Material kindly supplied by B. Richer de Forges as part of a continuing collaboration between Jamieson, Guinot, Richer de Forges, Scheltinga and Tudge.

## (b) Majidae

Majids, with parthenopids, constitute the Oxyrhyncha in the classification summarized by Warner (1977). Both are heterotremes in the classification of Guinot (1977, 1978).

When sperm ultrastructure alone was used in cladistic analysis, 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, 1994a, b; Jamieson *et al.*, 1995).

Some 17 species, in 15 genera of majids, have been examined for sperm ultrastructure (see Table 6). The sperm of *Menaethius monoceros*, described by Jamieson (1991b) and those described, notably by Hinsch (1973), are characterized by a broad operculum which is highly unusual in being depressed centrally or (Podocheila, Hinsch, 1973), at least flattened. In *Menaethius monoceros* (Jamieson, 1991b, 1994b), *Sphenocarcinus orbiculatus* (Fig. 13C) and *S. stuckiae* (Fig. 13D) (this study), the operculum is not only depressed centrally but is also perforate. If a vertical section of a spermatozoon is not precisely median it is possible for the central perforation of the operculum to be missed. It is therefore possible that the circular central depression in the operculum of *Chionoecetes opilio*, demonstrated by scanning electron microscopy (SEM) by Chiba *et al.* (1992) but not confirmed in a thin section, is in fact a perforation. The operculum also appears to be imperforate in *Cyrtomaia furici* (Fig. 13A) and *Platymaia rebieri* (this study).

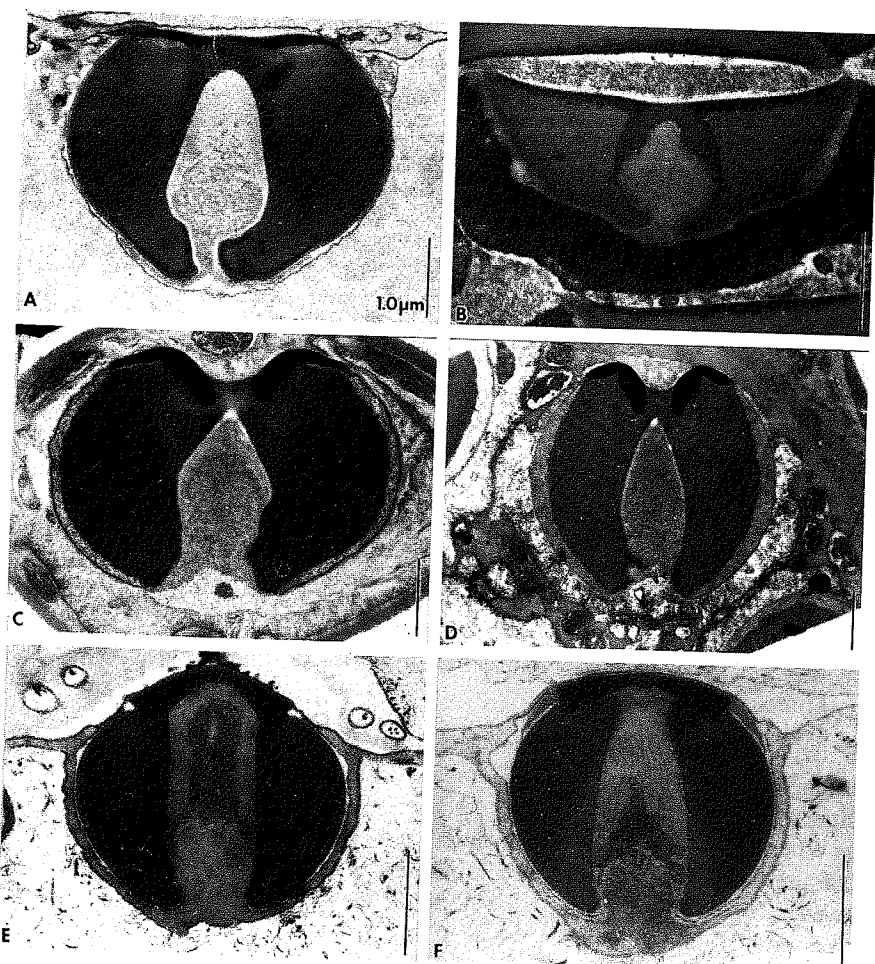


Fig. 13. TEM of spermatozoa in vertical section. A–D. Majidae. A: *Cyrtomaia furici*, Inachinae. B: *Macropodia longirostris*, Inachinae. C: *Sphenocarcinus orbiculatus*, D: *Sphenocarcinus stuckiae*, Acanthonychinae. E and F. Cryptochiridae (= Hapalocarcinidae). E: *Cryptochirus coralliodytes*. F: *Hapalocarcinus marsupialis*. (All original).

A further feature of majid sperm is the squat, pointed approximately rhombohedroidal shape of the perforatorial column. As a third feature, there may be a posterior median extension of the nucleus, in addition to the nuclear arms. This posterior median extension is also present in raninids, in which, as in the majid *Pitho* (Hinsch, 1973) it is particularly well developed. 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* (Jamieson, 1991b). It is not described for *Chionoecetes opilio* by Chiba *et al.* (1992) but appears to be weakly developed in one sperm illustrated by SEM while absent in another, seeming to confirm its lability. It has not been demonstrated in *Cyrtomaia furici*, *Macropodia longirostris*, *Platymaia rebierei*, or the two *Sphenocarcinus* species in the present study. Parsimony analysis (Jamieson *et al.*, 1995) suggests that the posterior median process of majids may have developed independently of that of podotremes.

The sperm of *Cyrtomaia furici*, and at least *Sphenocarcinus orbiculatus*, have a triradiate form, with an arm at each vertex, a condition which is here considered plesiomorphic for the Meiura (Anomala + Brachyura of Scholtz and Richter, 1995). Several arms are demonstrable in *Macropodia longirostris* but it remains to be determined whether three are larger than the others.

Chiba *et al.* (1992) demonstrate 4 to 10, with a mean value of 7 lateral arms, by SEM, and a well-developed lamellar complex and degenerate mitochondria adjacent to the nucleus, by TEM, for *Chionoecetes opilio*. Strong development of microtubules in the lateral arms, demonstrated by Hinsch (1973), is regarded as a plesiomorphic condition (Jamieson, 1991b) further supporting a basal position for majids as microtubules, while present in anomuran lateral arms (Tudge, 1992, 1995a, b) are reduced or absent from most eubranchyurans. Microtubules have been observed in the reacting spermatozoa of the portunid *Carcinus maenas* (Pochon-Masson, 1968b), the immature sperm cells of *Cancer* species (Langreth, 1969) and the mature spermatozoa of the xanthoid, *Pilumnus semilanatus* (Fig. 14B) and the eumedonids, *Eumedonius granulatus* (Fig. 15E) and *Harrovia albolineata* (Fig. 15F) (this study). However, microtubules were not seen in *Menaethius monoceros* by Jamieson (1991b), or in other majids investigated by the authors, or in *Chionoecetes opilio* by Chiba *et al.* (1992). The state of maturity and fixation of sperm may well effect the visibility of microtubules.

Hinsch (1973) attributes a very similar form, relative to 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. However, from a study of the megalopa, Rice (1981, 1983) regards majids as a monophyletic group quite 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) to regard parthenopids as highly evolved products of a lineage including portunids and geryonids. Guinot (1978) considers that majids are highly evolved heterotremes, citing the observation of Bouvier (1940, p. 307) that the nervous system is condensed, but adds that without doubt there exist majids which are primitive and others which are very advanced.

The posterior process, occurring also in the 'outgroup' Paguroidea, is seen as a plesiomorphy retained paraphyletically in raninoids and majids to be apomorphically lost in higher crabs (Jamieson, 1991b, 1994b; Jamieson *et al.*, 1995). The basal position of Majidae, from a purely spermatological viewpoint,



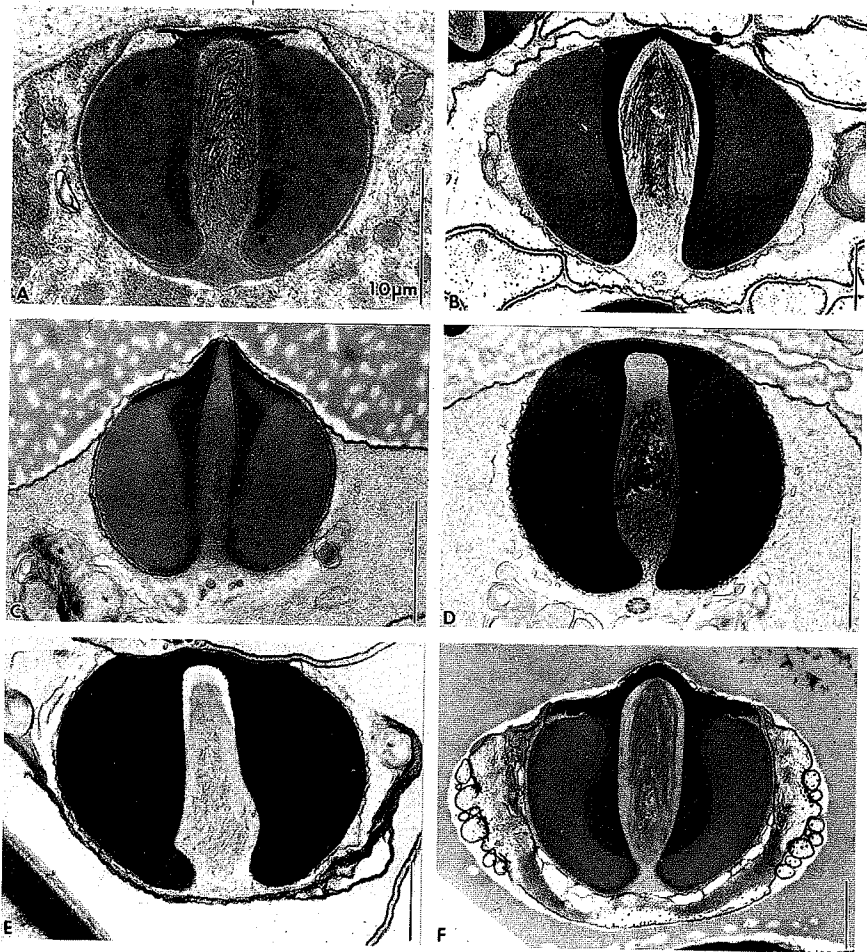


Fig. 14. TEM of spermatozoa in vertical section. A: *Eurypanopeus depressus*, Panopeidae. B: *Pilumnus semilanatus*, Pilumnidae. C: *Tetralia fulva*. D: *Tetralia nigrolineata*, Trapeziidae. E: Parthenopidae sp. F: *Holthuisiana transversa*, Sundathelphusidae. (All original).

corresponds with the basal position attributed to them by Rice (1981) from zoeal morphology.

As a symplesiomorphy, centrioles are present in majids (Chiba *et al.*, 1992; Hinsch, 1973; Jamieson, 1991b), including *Cyrtomaia furci*, *Platymaia rebierei*, *Macropodia longirostris* and *Sphenocarcinus orbiculatus* (present study), as *inter alia* in parthenopids (Hinsch, 1973), portunids, dorippids, and *Macrophthalmus* but not, for instance, xanthids (Jamieson, 1991b, 1994b; Jamieson *et al.*, 1995).

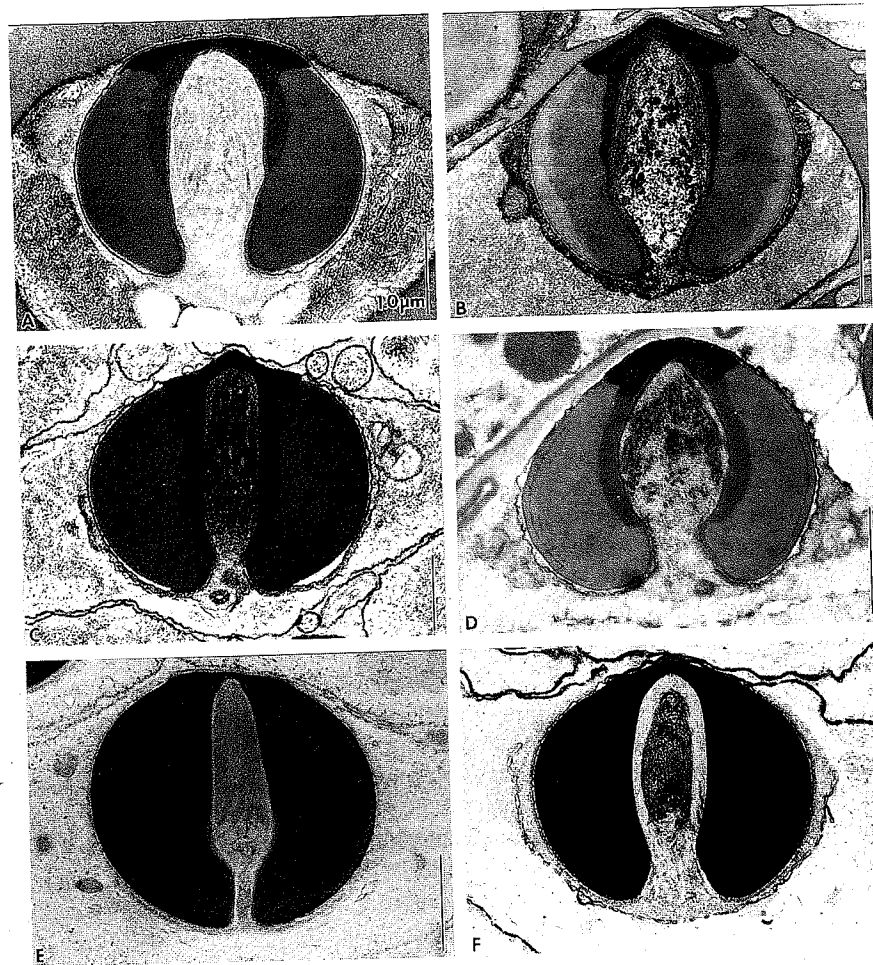


Fig. 15. TEM of spermatozoa in vertical section. A: *Xaiva* sp., Portuninae. B: *Caphyra rotundifrons*, Caphyrinae. C: *Ovalipes mollerii*, Polybiinae. D: *Podophthalmus vigil*, Podophthalminae. E: *Eumedonus granulosus*, Eumedonidae. F: *Harrovia albolineata*, Eumedonidae; as in *E. granulosus*, microtubules are visible, in the nucleus, to the right of the acrosome. (All original).

The sperm of *Macropodia longirostris* deserves special mention as it is the only eubrachyuran sperm in which the acrosome is known to depart radically from a subspheroidal form (Fig. 13B). The acrosome 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. The anterior surface of the acrosome is almost flat, though slightly concave, whereas the posterior surface forms a semicircle. The anterior surface is almost completely occupied by the thin, centrally perforate, electron-dense operculum. 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 perforatorium, the narrow base of which is continuous with the posterior cytoplasm and the anterior and anterolateral aspects of which are covered by and project into a dense inner acrosome zone. 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.

#### (c) *Parthenopidae*

The sperm of the parthenopid *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 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 with majids.

The spermatozoon of *Parthenopidae* sp. (Fig. 14E) also has a wide, thin operculum. This consists of two layers separated by a considerable hiatus. The perforatorial column tapers anteriorly but bulges near its posterior end, the bulge involving the anterior part of the thickened ring. A thin inner acrosomal zone is surrounded by an equally thin reticulate zone (perhaps equivalent to an acrosome ray zone), both being widest near their posterior ends. The bulk of the acrosome is occupied by the homogeneous outer acrosome zone. There are two, mutually perpendicular centrioles in the cytoplasm posterior to the perforatorial column. These show the usual malacostracan feature of nine doublets (Jamieson, 1987, 1989a), although some of the doublets seem to be accompanied by an indistinct third microtubule. The spermatozoon is not triradiate and no posterior median process is evident.

#### (d) *Eumedonidae*

The spermatozoon of *Eumedonus granulosus* (Fig. 15E) and *Harrovia albolineata* (Fig. 15F) show basic eubranchyuran features but also features which are unusual. In midvertical section of the sperm, the perforatorial column in *E. granulosus* appears dagger-shaped, with anteriorly converging straight sides and a narrow base whereas in *Harrovia albolineata* it forms an elongate, almost parallel-sided ellipse. The basal invagination in *E. granulosus* (less so in *H. albolineata*) is unusual in being irregular in cross section, being approximately X-shaped owing to alternation of dense inward projections of the acrosome vesicle and evaginations of the perforatorial chamber, usually each four in number. The basal perforatorial invagination is wider in *H. albolineata* and the few evaginations of the perforatorial

chamber are less regular. Departure from a circular cross section of the perforatorial chamber is also seen in the goneplacid sp. in which it is triradiate. The bulk of the eumedonid acrosome vesicle is occupied by the outer acrosome zone which is moderately and homogeneously electron-dense. A much narrower anteriorly widening inner acrosome zone extends from the subposterior region of the perforatorial column to the operculum and is divisible into dense posterior and anterior less dense moieties. This horizontal division of the inner acrosome zone and presence of only two concentric zones, the inner and outer zones, is distinctive. The anterior zone is much less well defined and is smaller in *H. albolineata* than in *E. granulosus*. No acrosome rays are demonstrable although their presence in the anterior zone in *H. albolineata* cannot be completely ruled out. A centriole, presumably one of a pair, is observable in the cytoplasm posterior to the invagination of the perforatorial chamber in *E. granulosus* and has, at most, doublets. The operculum in all three species is imperforate although its posterior face is depleted centrally by intrusion of the perforatorium. The operculum is unusually small in *E. granulosus*. In both species the nuclear arms are highly unusual for eubranchyurans (see also majids, Hinsch (1973) and *Pilumnus semilanatus*, this study) in possessing an internal 'rope' of microtubules which extends from deep in the chromatin into the arms. There appear to be several arms but no posterior median process has been demonstrated.

#### (e) *Retroplumidae*

The sperm of *Retropluma* sp. (Fig. 11D) lacks distinctive features relative to the basic eubranchyuran pattern. The perforatorial column has a lemon-shaped outline, with a stout base within the thickened ring, and an apical protrusion which abuts on a narrow axial core of the operculum which is less dense than the surrounding, strongly electron-dense moderately wide portion of the operculum. The dense part is thus interrupted but not truly perforate (Fig. 11D). The operculum has three layers: a thick outer layer, a moderately thick layer beneath this, and a small wedge of material which might be considered a subopercular layer. The bulk of the contents of the acrosome vesicle consists of the homogeneous moderately electron-dense outer acrosome zone. The anterior half of the more dense, narrow, anteriorly widening inner acrosome zone is differentiated as a distinct acrosome ray zone, with oblique, posterolaterally directed rays. This anterior restriction of the acrosome ray zone resembles the condition in *Trapezia cymodoce*. The cytoplasm in the base of the perforatorial chamber houses two short centrioles and is continuous as a moderately thin layer around the acrosome as far as the operculum. As is usual in brachyurans, it has extensions into the chromatin which contain various inclusions, notably degenerate mitochondria. A thin double membrane separates cytoplasm and nucleus and contrasts with the multilaminar membrane of the questionably related *Neodorippe*. The sperm is not triradiate and the external shape of the nucleus is irregular, with long but indefinitely shaped chromatin-filled arms lacking microtubules. No posterior median process is apparent.



(f) *Calappidae*

The general morphology of the acrosomes of *Calappa hepatica* and *C. aff. gallus* (Fig. 11E) are reminiscent in some respects of the majid *Menaethius*, including the relatively straight, anteriorly divergent inner margins to the outer acrosome zone, the approximately rhombohedroidal perforatorial column and the well-developed thickened ring, but the operculum differs notably from majids in being pointed apically and unlike some majids, is imperforate (Jamieson, 1991a, b; this study). No acrosome ray zone is discernible. The sperm are not triradiate and no posterior median process is demonstrable. In *C. hepatica*, there is a single pale lamina lining the inner surface of the capsule. In at least *C. hepatica* postperforatorial centrioles are present.

The spermatozoon of *Mursia microspina* (Fig. 11F) is somewhat different. The perforatorial chamber is an elongate ellipsoid, with or without a straight-sided posterior moiety, and the inner acrosome zone is a very indistinct narrow electron-dense sheath around this. The inner margins of the large outer acrosome zone are not therefore straight and anteriorly divergent but follow the ellipsoidal shape of the inner zone and perforatorium. Two short mutually perpendicular postperforatorial centrioles are present.

The Calappidae is regarded from zoeal morphology as a fairly advanced family which may be near the ancestry of the Cancridae, Corystidae and Atelecyclidae (Rice, 1981).

(g) *Cancridae and Corystidae*

The Cancridea constitute one of the five major subdivisions of the Brachyura in the system summarized by Warner (1977). The Cancridae were retained with the Corystidae in a superfamily Corystoidea, within the Heterotremata, by Guinot (1978). The spermatozoon of *Cancer pagurus*, the type species of the genus *Cancer*, has been briefly mentioned by Pochon-Masson (1968a) and four additional *Cancer* species have been used in a combined account of spermiogenesis (chiefly of *C. borealis*) by Langreth (1965, 1969). Tudge *et al.* (1994) and Tudge and Justine (1994) briefly described the ultrastructure of the sperm of *C. pagurus* in the course of immunofluorescence studies of actin and tubulin distribution in this cell. The ultrastructure of the spermatozoa of *Cancer pagurus* and *Platopistoma nanum* and a corystid, *Corystes cassivelaunus* (Fig. 16), the type and only species of the genus *Corystes*, has been described by Jamieson *et al.* (1997). A combination of characters, not individually unique, possessed by *Corystes cassivelaunus*, and the cancrids defines a corystoid-type of spermatozoon: the basally bulbous, anteriorly narrowing perforatorium, the extent of this almost to the plasma membrane through a widely perforate operculum, and the simple inner acrosome zone, lacking an acrosome ray zone. The sperm of the cancrids closely resemble each other, that of the corystid differing, for instance, in the less pointed, and less

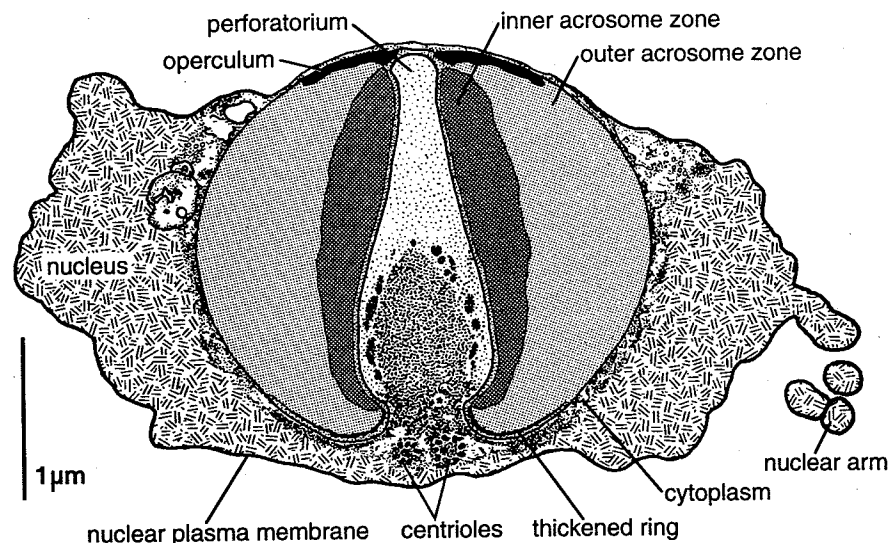


Fig. 16. *Corystes cassivelaunus*, Corystidae. Traced from TEM of vertical section of spermatozoon. From Jamieson *et al.* (1997). *Helgoländer Meeresuntersuchungen*, 51, 83–93.

tapered, form of the perforatorium. This relative uniformity of spermatozoal ultrastructure in the cancelrid+corystid assemblage so far investigated supports inclusion of the two families in the superfamily Corystoidea by Guinot (1978).

In the mature sperm of *Cancer borealis* illustrated by Langreth (1965), the large, dense operculum is crater-like and centrally perforate but Jamieson (1991b), summarizing Langreth's work, considered that, as the pointed tip of the perforatorium protrudes through it, perforation of the operculum might indicate that the acrosome reaction has commenced. A similar condition was, however, shown for *C. pagurus* by Jamieson *et al.* (1997) (see also Tudge *et al.*, 1994; Tudge and Justine, 1994). Jamieson *et al.* (1997) therefore concluded that the slightly protuberant perforatorium may be a normal condition of cancelrid sperm while noting that penetration of the 'cap' (operculum) appears to occur only at maturity (Langreth, 1965) and presumably indicates readiness for reaction.

Other features of *Cancer* sperm demonstrated by Langreth (1965) and reviewed by Jamieson (1991b), using current terminology, were an inner dense zone differentiated externally as an acrosome ray zone (presence of the acrosome ray zone was not confirmed); a large, electron-pale, outer acrosome zone; a conspicuous thickened ring in continuity with the thinner capsule; presence of chromatin throughout the length of the rather short arms; absence of a posterior median process; and a shape of the perforatorial column, similar to that reported here, widest at its posterior fourth and tapering almost straight to a pointed tip.

Tudge *et al.* (1994) recognized a very narrow peripheral acrosome zone, in addition to the inner and outer acrosome zones. However, although a pale layer was

observed under the capsule by Jamieson *et al.* (1997) it was continuous with the thickened ring and was not distinguished as a peripheral acrosome zone comparable with the wide layer seen, for instance, in xanthid sperm.

The combination of perforation of the operculum and absence of an acrosome ray zone (at least, in a clearly recognizable form) in *Cancer* sperm are features of the Potamidae which possibly indicate that the Potamidae, modified for a freshwater existence, is related to the cancrinid+corystid assemblage. Some elongation of the centrioles, apparent at least in *Corystes*, may be a further link with potamids in which they are greatly elongated. The coenospermial spermatophores (more than one spermatozoon per spermatophore) of cancrinoids are a notable difference from the cleistospermia (one spermatozoon, only, per spermatophore) of potamids but the latter is probably an apomorphic modification for fertilization biology (Jamieson *et al.*, 1997).

#### (h) Goneplacidae and Hexapodidae

The superfamilial status of these two families is uncertain. The spermatozoon of the freshwater crab *Australocarcinus riparius*, the first goneplacid to be examined for sperm ultrastructure (Jamieson and Guinot, 1996), is clearly heterotreme. However, absence of a recognizable acrosome ray zone is not a general heterotreme feature, though constant for thoracotremes. This zone is also unrecognizable in potamids, corystoids and some species of other families. The operculum has an almost flat base and a low triangular anterior surface. Only a very thin layer of the outer, anterior surface is strongly electron-dense. The much thicker lower zone may be termed the subopercular zone. There is a convex ring at the posterior end of the inner acrosome zone which is somewhat similar to the xanthid ring, characteristic of the Xanthidae and Panopeidae, but homology is doubtful and it is not seen in other investigated goneplacid sperm. Other typical xanthid features such as the accessory opercular ring and the opercular overhang are absent from the sperm of goneplacids and relationship with xanthoids cannot be considered to be supported spermatologically. The remainder of the contents of the vesicle in *A. riparius* consists of an homogenous outer acrosome zone. No special similarities to the sperm of potamids, which similarly have an obligatory freshwater existence at all stages of the life cycle, are apparent. In producing more than one spermatozoon per spermatophore, *A. riparius*, having marine relatives, may be less evolved along the path of lecithotrophy than are the potamids, lacking marine cofamilials (Jamieson and Guinot, 1996).

The sperm of the goneplacids *Carcinoplax microphthalmus* (Fig. 17A), *Ceratoplax* sp. (Fig. 17B), goneplacid sp. (Fig. 17C) and the hexapodid *Hexaplax megalops* (Fig. 17D) (this study), differ from that of *Australocarcinus riparius* in lacking the xanthid-ring-like structure. This difference is consistent with the view of Guinot (personal communication) that *Australocarcinus* belongs to a different lineage from *Carcinoplax*, on the one hand, and *Ceratoplax* on the other. Furthermore,

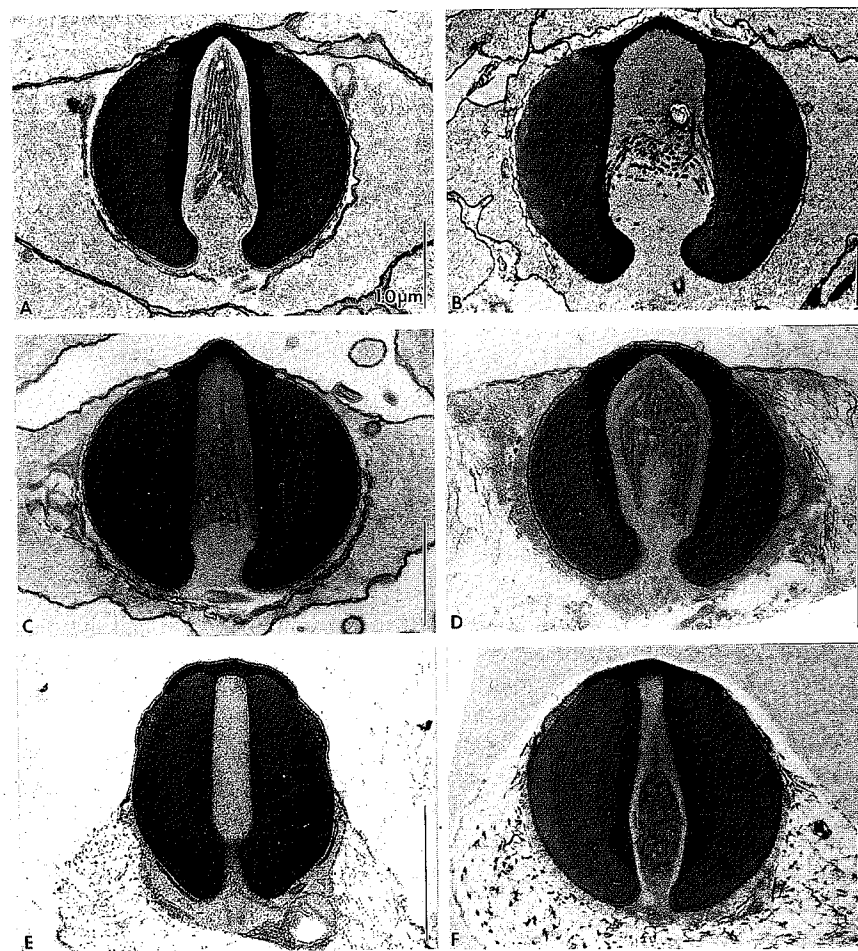


Fig. 17. TEM of spermatozoa in vertical section. A: *Carcinoplax microphthalmus*, Goneplacidae. B: *Ceratoplax* sp., Goneplacidae. C: Goneplacid sp., Goneplacidae. D: *Hexaplax megalops*, Hexapodidae. E: *Philyra laevis*, Leucosiidae, Philyrinae. F: *Randallia serenei*, Leucosiidae, Philyrinae. (All original).

these species differ from *Australocarcinus* in that the perforatorial chamber is not as conspicuously widened posteriorly (*Carcinoplax microphthalmus*; goneplacid sp.) or is widest near its anterior end (*Hexaplax megalops*) and the operculum is not as wide transversely. A further difference in, at least, *Carcinoplax microphthalmus* is the presence of acrosome rays, in the anterior half of the inner acrosome zone. Rays are also possibly present in an anterolateral differentiation of the inner acrosome zone in goneplacid sp. Postperforatorial centrioles are present in *Carcinoplax microphthalmus*, *Hexaplax* sp. and goneplacid sp.

The spermatozoon of *Hexaplex megalops* (Fig. 17D) has a narrow peripheral acrosome zone lined along its inner face by a single lamella resembling one of the concentric lamellae of thoracotremes, in addition to the inner and outer acrosome zones, and a periopercular rim.

The posterior perforatorial invagination is trifurcate in cross section in goneplacid sp. A multilaminar membrane between cytoplasm and nucleus is observable only in goneplacid sp. where it is disorganized and forms whorls around putative mitochondrial remnants. The sperm of all these goneplacid and hexapodid species have multiple nuclear arms and are not triradiate.

#### (i) Portunidae

The ultrastructure of sperm of *Carcinus maenas* was described by Pochon-Masson (1968a). Subsequent investigations of portunid sperm were made by El-Sherief (1991), Jamieson (1991b), Jamieson and Tudge (1990), and Li (1995). Portunids have a relatively generalized eubrachyuran sperm, that of *Portunus pelagicus* showing no apomorphies beyond those of basal heterotremes (Jamieson *et al.*, 1995).

The sperm of *Xaiva* sp., examined here (Fig. 15A), has a general portunid facies but differs, *inter alia*, in the less pointed operculum and the fact that this is less dense, or possibly perforate, at its apex. Acrosome rays, well-developed in *Portunus pelagicus*, are not apparent. As in the latter species, two short centrioles are present.

*Caphyra laevis*, *C. rotundifrons* (Fig. 15B) and *Portunus pelagicus* have sperm with the typical portunid ellipsoidal perforatorium. Remarkable intrageneric uniformity is seen in *Caphyra*. A sister-group relationship of *C. laevis* living in colonies of the soft coral *Xenia*, and *C. rotundifrons*, living in tufts of the turtle weed, *Chlorodesmis*, on coral reefs (here Heron Island, Great Barrier Reef, Australia) is suspected (Jamieson, 1991b).

*Podophthalmus vigil*, in the separate portunid subfamily Podophthalminae, has a sperm (Fig. 15D) which differs from that of *Portunus* in the less pointed operculum and the apparent absence of acrosome rays in a zone which is narrower than that in *Portunus*. As in other portunids, two centrioles are present.

The sperm of the polybiin *Ovalipes mollerii* briefly described here (Fig. 15C), resembles that of *Ovalipes ocellatus*, described by Hinsch (1986). A notable similarity is a peripheral acrosome zone, though, probably because of a difference in processing, this is faintly demarcated in *O. mollerii*. The almost straight-sided form of the perforatorium is reminiscent of the Xanthidae and there is a faint density similar to the accessory opercular ring of the latter but the basal xanthid ring is absent, and two conspicuous but not elongate centrioles are present.

#### (j) Potamonautidae

Peculiarities (putative apomorphies) of *Potamonautes*, exemplified by *P. perlatus sidneyi*, relative to other heterotremes are: (1) the indistinct substructure of the acrosomal ray zone; (2) absence of a recognizable inner acrosomal zone differentiated from this, and (3) reduction of the thickened ring, the latter trend being taken to completion in grapsid Thoracotremata. Unusual features are: (4) a rim-like electron-pale expansion around the operculum, and (5) the elongate condition of at least one of the two centrioles (Jamieson, 1993b). The periopercular rim is an expansion of the hyaline layer, usually considered to be the acrosomal membrane, which overlies the capsule. First demonstrated in the sperm of *Potamonautes perlatus*, it was later shown in the xanthoids *Calocarcinus africanus* (Jamieson *et al.*, 1993a) and, though weakly developed, *Etisus laevimanus* (Jamieson, 1994b). It is now known to be well developed in the bythograeids *Austinograea alayseae*, *Bythograea thermydron* and (weakly) *Segonzacia mesatlantica* (Tudge *et al.*, 1998b), in the xanthids *Eurypanopeus depressus* (Fig. 14A) (this study) and (weakly) *Eurytium limosum* (this study) and also weakly developed in the gecarcinid *Cardisoma carnifex* (Jamieson *et al.*, 1996). Elongation of the centrioles is elsewhere seen in the Potamidae (see below), congruent with their close relationship to Potamonautidae.

#### (k) Potamidae

The sperm of *Potamon fluviatile* and *P. ibericum* (Fig. 18) (described by Guinot *et al.*, 1997) (for *P. fluviatile*, see also Tudge and Justine, 1994), are virtually indistinguishable and do not support separate subgeneric rank (*Potamon* and *Pontipotamon*, respectively). Synapomorphic with the sperm of the South African freshwater crab *Potamonautes perlatus sidneyi*, are the elongation of the two centrioles and disposition of the centrioles almost parallel to each other, unknown elsewhere in the Brachyura; and reduction of the thickened ring (homoplastic with grapsids and gecarcinids). Other, probably synapomorphic, similarities of *Potamon* and *Potamonautes* include the wide inner acrosome zone; absence of a definite acrosome ray zone (homoplastic in other families) and the cleistospermial spermatophores. Further similarities, of questionable polarity, are the simple, not multilaminar, nuclear membrane and the tendency of the nuclear arms to wrap around the nucleus. Differences of *Potamon* from *Potamonautes*, which possibly support their present generic separation and give weak support to their former separate familial placement in the Potamidae and Potamonautidae respectively, are perforation of the operculum and the weak, rather than strong, development of a periopercular rim. Absence in *Potamon* and *Potamonautes* of an accessory opercular ring and a xanthid ring separates them from xanthids. No close affinities with other heterotreme families are seen but their assignment to the Heterotremata is not in doubt. Their sperm lack two of the distinctive features of thoracotreme sperm

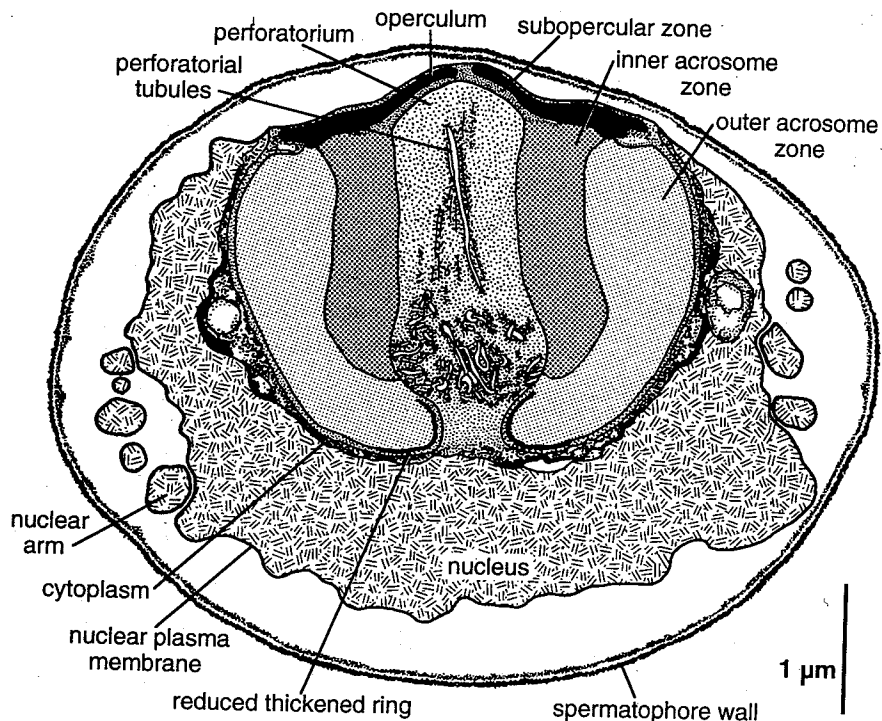


Fig. 18. *Potamon ibericum*, Potamidae. Traced from TEM of vertical section of spermatozoon. From Guinot et al. (1997). *Journal of Zoology, London*, 241, 229–244.

(apical button on the operculum and concentric lamellation of the outer acrosome zone). No clear correlates of sperm structure with a freshwater existence are recognizable but reduction of the thickened ring possibly relates to peculiarities of the acrosome reaction. However, the production of spermatophores with single spermatozoa (cleistospemia) is possibly a device to prevent polyspermy and wastage of the small number of lecithotrophic eggs produced in potamids (Guinot et al., 1997).

#### (l) Geryonidae

The sperm of *Geryon fenneri* and *G. quinquedens*, as described by Hinsch (1988), are unspecialized heterotreme sperm. They have lost the posterior nuclear process of the raninoids but lack the xanthid ring (see below). They were placed, only provisionally, in the Xanthoidea by Guinot (1978) who stressed their uncertain origins.

#### (m) Cryptochiridae (=Haplocarcinidae)

Two species of the coral gall family, Cryptochiridae, have been examined for sperm ultrastructure, *Cryptochirus coralliodytes* (Fig. 13E) and *Haplocarcinus marsupialis* (Fig. 13F; this study). They 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. This extends far on each side, for a distance approximately equivalent to the width of the acrosome, and in the longitudinal section of the spermatozoon has the appearance of a broad epaulette. A similarity and apparent synapomorphy with xanthids and bythograeids, with *Calocarcinus* but not *Trapezia*, is the highly distinctive oblique accessory opercular ring. A thin reticular zone immediately surrounding the posterior half of the perforatorium column may be the equivalent of an acrosome ray zone; it resembles that in the Parthenopidae species but there it surrounds a thin inner acrosome zone. A periopercular rim is moderately developed in *C. coralliodytes*. Its equivalent in *H. marsupialis* is filled with a moderately electron-dense wedge of material which abuts, above the accessory opercular ring, 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 has an apical button resembling that of thoracotremes, but probably homoplastic with these, which is not recognizable in *C. coralliodytes*.

#### (n) Leucosiidae

The sperm of the leucosiids *Iliacantha subglobosa* (Felgenhauer and Abele, 1991) and *Randallia serenei* (Fig. 17F) are triradiate, with three well-developed arms, a feature which appears plesiomorphic for brachyurans. In contrast, the sperm of *Philyra laevis* has an amorphous cross section, with an undetermined number of long, broad, contorted nuclear arms which do not show a radial arrangement. In other respects, the sperm of *P. laevis* (Fig. 17E) is markedly different from that of *R. serenei*.

The sperm of *Randallia serenei* (Fig. 17F) is enigmatic in that it possesses a structure, anterior to the dense ring, which strongly resembles, though it may be homoplastic with, a xanthid ring (but see also *Australocarcinus*). *Randallia serenei* also has concentric lamellations of the acrosome contents which resemble the 'onion ring' lamellation of thoracotreme sperm, notably, in having a tendency to form 'wavelets' or 'finger-print' patterns, like that in *Cardisoma* or, less closely, *Mictyris*. What, if any, phylogenetic significance attaches to these apparently conflicting morphological resemblances is uncertain. They may well be homoplastic similarities nevertheless reflecting a common eubrachyuran gene pool. The domed, bluntly pointed, moderately wide, imperforate operculum resembles that of portunids but zonation of the acrosome is more complex. The 'xanthid ring' is continuous anteriorly with an inner acrosome zone which anteriorly, near the perforatorium

displays acrosome rays. This zone, like the ring, is strongly electron-dense. It is widest at the equator of the sperm, tapering in an arc to the operculum and the anterior end of the ring. It is surrounded by a less dense outer acrosome zone which, like it, is narrow relative to the wide zone of concentric lamellations. External to the latter zone is a less dense, peripheral acrosome zone, separated from it by a smooth arc, the junction lacking the 'ragged' form seen in xanthid sperm. On each side of the perforatorial column, abutting the operculum, a subcircular zone resembling the peripheral zone in electron density, intervenes between the lamellations and the operculum. This distinctive *Randallia* sperm also has a peculiar type of periopercular rim which is unusual in extending down the acrosome for about one third of its height and in containing dense granular material.

The sperm of *Philyra laevis* (Fig. 17E) differs, *inter alia*, in its amorphous outline; in the rounded oblong rather than spheroidal form of the acrosome (a condition known elsewhere in *Neodorippe astuta*, Jamieson and Tudge, 1990; Jamieson, 1991a, b, 1994a); in the narrow almost tubular perforatorial chamber; in the unusually wide zone of cytoplasm which extends posteriorly from the equator of the acrosome; and in the much less complex zonation of the acrosome. A notable resemblance and presumed synapomorphy is the presence of an extensive periopercular rim which, as in *Randallia*, closely follows the anterolateral curve of the acrosome and has granular contents. The acrosome contents show remarkably little differentiation, consisting almost entirely of an homogeneous moderately electron-dense outer acrosome zone with, ensheathing the perforatorial chamber, a very thin and poorly defined, more dense inner acrosome zone. There is no 'xanthid ring', although a barely perceptible thickening of the inner zone is detectable immediately anterior to the thickened ring. There is no trace of concentric lamellation. The material of this thickening in the inner acrosome zone shows a few oblique striations which may be the equivalent of acrosome rays. There is a pair of mutually perpendicular centrioles of normal length in the cytoplasm behind the perforatorial invagination; a few degenerate mitochondria and lamellae are present in the cytoplasm. No definite membrane separates the cytoplasm from the nucleus.

#### (o) Xanthidae

A eubranchyuran feature of xanthid sperm, not seen in podotremes, is modification of the capsule around the base of the perforatorium as a thickened ring (Jamieson, 1989c).

A notable xanthid autapomorphy is differentiation of the posterior region of the inner dense zone surrounding the perforatorium as a prominent strongly electron dense ring, the 'xanthid ring', shown for four xanthid genera, examined by Jamieson (1989c, 1991b). A funnel-like structure, seen in the acrosome of sperm of the Thoracotremata, was considered by Jamieson (1991b) to be derived from this, and

was hence termed the "modified xanthid ring". It was therefore considered to suggest origin of thoracotremes from the Xanthoidea or their immediate ancestors. Rice (1981, 1983) saw primitive xanthids as ancestors of what are here termed 'heterotreme' and 'thoracotreme' brachyurans (excepting the majids, which, it is here concurred, seem more basal). Martin (1988) considered that the Xanthidae "may lie at or near the stem of the higher eubranchyurans", thereby giving xanthids a higher position though, like Rice, recognizing their pivotal position in generation of further families. However, cladistic analysis has suggested that the structure in thoracotremes originated independently of the xanthid ring (Jamieson, 1994b; Jamieson *et al.*, 1995) and has not resolved the position of xanthids relative to thoracotremes.

#### (p) Pilumnidae

Some reference to the sperm of *Pilumnus semilanatus* (Fig. 14B) has been made above. Although a xanthoid, its sperm differs notably from those of the Xanthidae in lacking a xanthid ring, opercular overhang, accessory opercular ring and peripheral acrosome zone. Unlike examined xanthid sperm, centrioles are present.

#### (q) Trapeziidae

*Tetralia fulva* (Fig. 14C) and *Tetralia nigrolineata* (Fig. 14D) have sperm which differ more than is expected within a family. The operculum in *T. fulva* is pointed and apically perforate whereas that in *T. nigrolineata* is only slightly domed and imperforate. They also show little similarity, beyond general heterotreme sperm characters, to the sperm of *Trapezia cymodoce* described by Jamieson (1993a). Like the latter species, they have centrioles and lack the xanthid features of xanthid ring, opercular overhang, accessory opercular ring and peripheral acrosome zone.

#### (r) Hymenosomatidae

The spermatozoa of *Odiomaris pilosus* have the components typical of eubranchyuran sperm but differ significantly from all other investigated eubranchyurans (including the Majoidea and the Thoracotremata with which they are sometimes associated) in at least nine characteristics: 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 the longitudinal section as the inner zone is narrow and of doubtful homology; 7. the thin, putative inner acrosome zone is anteriorly almost septate owing to several longitudinal corrugations; 8. basally

there is a unique 'fringe zone'; 9. the acrosome, including the epiopercular dome, is longer than wide. From a purely spermatological viewpoint, *Odiomaris*, and provisionally the Hymenosomatidae, are thus not identifiable with the Thoracotremata *s. strict.* and it seems possible that they have acquired the thoracotreme condition of the genital pores independently of the Thoracotremata. Without recourse to unsubstantiated hypotheses relating to influence of possible altered fertilization biology on sperm structure, it is difficult to accommodate the hymenosomatids in any of the three brachyuran divisions — Podotremata, Heterotremata, and Thoracotremata. Consideration has to be given to placing hymenosomatids in a fourth, new, division of the Brachyura. Additional studies on somatic morphology are needed to test this hypothesis (Richer de Forges *et al.*, 1997).

The sperm of *Elemena vesca* (Fig. 19A; this study) is identical in its chief characteristics to that of *Odiomaris*.

## 2. Thoracotremata

The Thoracotremata are defined by displacement of the female and male pores from the coxae to the sterna of segments 6 and 8 respectively, whereas only the female pores are sternal in the Heterotremata *sensu strictu* Guinot (1977, 1978). The thoracotreme condition frees the ambulatory limbs from a reproductive function (Rice, 1981). Spermatozoal ultrastructure has been described for two grapsids, *Grapsus albolineatus* and *Sesarma* (now *Parasesarma*) *erythrodactyla*; the mictyrid *Mictyris longicarpus*; the ocypodids *Ocypode ceratophthalma* and *Uca dussumieri*; and the macrophthalmid *Macrophthalmus crassipes* by Jamieson (1991b). All of these sperm show general eubrachyuran ultrastructure but thoracotreme synapomorphies are apparent, as is endorsed by cladistic analysis (Jamieson, 1994b). Reger (1970) examined spermatozoal ultrastructure in *Pinnixa* sp. (Pinnotheridae). Jamieson *et al.* (1996) examined two further thoracotremes: *Cardisoma carnifex*, endorsing the thoracotreme synapomorphies, and *Varuna litterata*, largely lacking them (see Grapsidae, below). Other thoracotremes are added in the present study (see Table 7).

Monophyly of the Thoracotremata is based on three putative spermatozoal synapomorphies (Jamieson, 1994b; Jamieson *et al.*, 1995), in addition to location of the genital pores on the sternum (Guinot, 1978). The first is loss of the acrosome ray zone in the acrosome, its presence being characteristic, though inconstant, of heterotremes. The second apparent synapomorphy is the unique development of an apical button, filling a central perforation of the operculum, which is characteristic of thoracotremes. This was, however, an ambiguous character in a recent parsimony analysis (Jamieson *et al.*, 1995; see Ocypodidae, below). A third characteristic and, it is deduced, synapomorphic feature of thoracotreme sperm is presence of concentric lamellae in the acrosome, though these are not seen in *Uca*. Indication (Jamieson *et al.*, 1995) that it is a synapomorphy only of higher thoracotremes may be spurious as this 'onion ring' lamellation is seen *inter alia*, not only in *Mictyris longicarpus*,

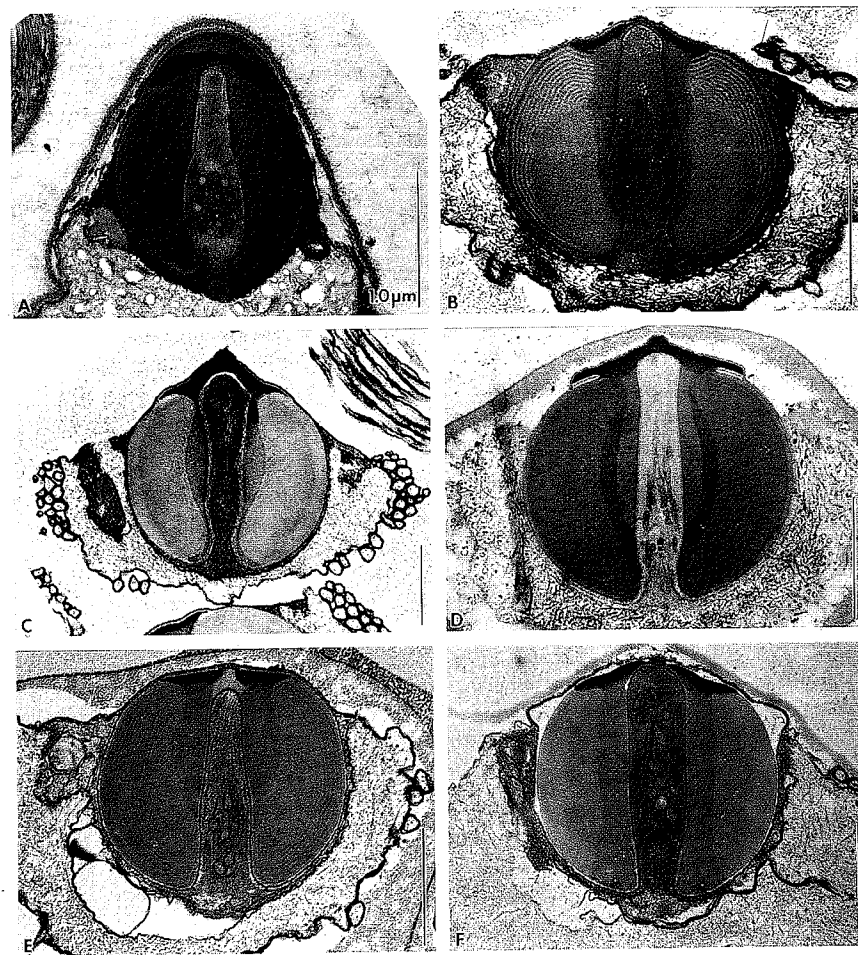


Fig. 19. TEM of spermatozoa in vertical section. A: *Elemena vesca*, Hymenosomatidae. B: *Macrophthalmus crassipes*, Ocypodidae, Macrophthalminae. C: *Uca dussumieri*, Ocypodinae. D: *Grapsus albolineatus*, Grapsidae, Grapsinae. E: *Cyclograpsus punctatus*, Grapsidae, Cyclograpsinae. F: *Sesarma catenata*, Grapsidae, Sesarminae. (All original).

*Ocypode ceratophthalma* and *Macrophthalmus crassipes* but appears to be foreshadowed in some heterotremes, being indicated by Brown (1966b) for the portunid *Callinectes sapidus*. It is otherwise absent from the sperm of non-thoracotreme brachyurans and anomurans (Jamieson, 1991b). Further parsimony analysis (Jamieson *et al.*, 1995) supports monophyly of the Thoracotremata on the basis of two unambiguous characters: loss of the acrosome ray zone and movement of the male pores (following that of the female pores basic to heterotremes) onto the sternum.



Table 7

Ultrastructural studies of the spermatozoa of Thoracotremata

Superfamily, family and subfamily	Species	Reference
<b>Gecarcinoidea</b>		
GECARCINIDAE	<i>Cardisoma carnifex</i>	Jamieson <i>et al.</i> , 1996
<b>Mictyroidea</b>		
MICTYRIDAE	<i>Mictyris longicarpus</i>	Jamieson, 1993a, 1994a
<b>Pinnotheroidea</b>		
PINNOTHERIDAE	<i>Pinnixa</i> sp.	Reger, 1970 (as <i>Pinnixia</i> )
<b>Ocypodoidea</b>		
OCYPODIDAE		
Macrophthalminae	<i>Macrophthalmus crassipes</i>	Jamieson, 1991b, 1994b
Ocypodinae	<i>Ocypode ceratophthalma</i>	Jamieson, 1991b, 1994b
	<i>Uca dussumieri</i>	Jamieson, 1991b, 1994b
	<i>Uca polita</i>	This study
	<i>Uca pugilator</i>	This study
	<i>Uca tangeri</i>	Medina, 1992; Medina and Rodriguez, 1992a, b
<b>Grapsoidae</b>		
GRAPSIDAE		
Grapsinae	<i>Grapsus albolineatus</i>	Jamieson, 1991b
Cyclograpsinae	<i>Cyclograpsus punctatus</i>	This study
Varuninae	<i>Varuna litterata</i>	Jamieson <i>et al.</i> , 1996
	<i>Eriocheir japonicus</i>	Yasuzumi, 1960
	<i>Eriocheir sinensis</i>	Du <i>et al.</i> , 1987, 1993
Sesarminae	<i>Parasesarma erythrodictyla</i>	Jamieson, 1991b (as <i>Sesarma</i> )
	<i>Sesarma catenata</i>	This study
	<i>Sesarma cinereum</i>	This study
	<i>Sesarma haematocheir</i>	Honma <i>et al.</i> , 1992
	<i>Sesarma reticulatum</i>	Felgenhauer and Abele, 1991; This study

Another feature of thoracotreme sperm is the "modified xanthid ring". In cladistic analyses it computes as an entirely independent development not related to the xanthid structure (Jamieson, 1994b; Jamieson *et al.*, 1995). Nevertheless, its derivation from the xanthid ring remains plausible.

The acrosome ray zone, so well developed in paguroids, such as *Birgus latro* (Tudge and Jamieson, 1991), as in crabs (portunids, dorippids and xanthids), is so reduced in the Thoracotremata as to be unrecognizable.

Thus, although the Heterotremata *sensu strictu* appear to be a paraphyletic assemblage, and as such to be a grade rather than a clade, three albeit inconstant synapomorphies within the Thoracotremata suggest that the species examined to date, at least, form a monophyletic group (Jamieson, 1991b; Jamieson *et al.*, 1995, 1996).

## (a) Ocypodidae

**Ocypodinae.** *Ocypode ceratophthalma* has a classical thoracotreme spermatozoon with the putative synapomorphies of apical button in a perforate operculum, 'onion ring' lamellation and no acrosome ray zone. *Uca dussumieri* (Fig. 19C) lacks the concentric lamellation (Jamieson, 1991b; Jamieson *et al.*, 1995) as apparently does *Uca tangeri* (Medina, 1992; Medina and Rodriguez, 1992a, b).

**Macrophthalminae.** Distinctive features of the sperm of *Macrophthalmus* (Fig. 19B) relative to other thoracotremes are the absence of the apical button and presence of a large posterior ellipsoidal, almost spheroidal, acrosome zone peripheral to the inner dense zone and abutting on the thickened ring though extending pre-equatorially. No certain equivalent of the xanthid ring is seen though it is not inconceivable that this zone is a great enlargement of this ring. A further peculiarity of *Macrophthalmus* is that the perforatorial column tapers uniformly from approximately its posterior fourth to a rounded apical point whereas in the other five examined ocypodids the apex of the column is broad (though as always much narrower than the length of the column) and is flattened or gently convex. Jamieson (1991b) placed *Macrophthalmus* below the ocypodids, mictyrid and grapsids as this was intuitively considered more parsimonious than assuming that it is derived above this assemblage by loss of the apical button. From zoeae, Rice (1981) recognizes the Macrophthalminae as a subfamily, less advanced than the Ocypodinae, in the family Ocypodidae, the latter possibly derived from grapsids. The higher status for the grapsids in the spermatozoal phylogeny takes into account loss of the thickened ring which is present from majids to ocypodids.

In view of the close, supposedly intrafamilial, relationship generally recognized between *Ocypode*, which has an apical button, filling a central perforation of the operculum, so characteristic of thoracotremes, and *Macrophthalmus*, which lacks this condition, it was considered likely (Jamieson *et al.*, 1995) that the absence of the apical button in *Macrophthalmus*, as in *Varuna litterata* (Jamieson *et al.*, 1996, and below), was due to loss of a basic thoracotreme condition. In the recent parsimony analysis (Jamieson *et al.*, 1995), it remained uncertain that the apical button and opercular perforation were basic thoracotreme synapomorphies owing to their alternative absence or loss in *Macrophthalmus*. A more detailed investigation of thoracotremes might resolve the issue of whether the button is basic to thoracotremes. In view of the close relationship generally recognized between *Macrophthalmus* and *Ocypode*, it nevertheless seems likely that the absence in *Macrophthalmus* is due to loss of a basic thoracotreme condition.

## (b) Mictyridae

*Mictyris longicarpus*, as indicated above, has a classical thoracotreme sperm, with apical button in a perforate operculum, 'onion ring' lamellation, which is particularly well developed, and no acrosome ray zone (Jamieson, 1991b, 1994b; Jamieson *et al.*, 1995).

(c) *Pinnotheridae*

Generally the structure of the sperm of *Pinnixia* sp. (sic for *Pinnixa* sp.), described by Reger (1970), is not inconsistent with that presented here for thoracotremes but concentric lamellation of the acrosome, if present, is not preserved by the techniques employed. An apical interruption of the opercular density in the sperm of *Pinnixa*, from the spermatheca, possibly corresponds with a button. In *Pinnixa*, a poorly defined zone external to the innermost dense zone may be equivalent to the 'modified xanthid ring' typical of thoracotremes.

(d) *Gecarcinidae*

*Cardisoma carnifex* is terrestrial, like the grapsid *Sesarma haematocheir*. Despite its terrestrial mode of life, *C. carnifex* returns to the sea to breed and its sperm (Fig. 20) displays all of the diagnostic features of thoracotreme sperm: absence (clearly loss) of the acrosome ray zone; presence of an apical button filling an opercular perforation; and concentric lamellation of the outer acrosome zone. To these may be added the near-horizontal accessory opercular ring. *Cardisoma carnifex* groups with thoracotremes in cladistic analysis (Jamieson *et al.*, 1996).

(e) *Grapsidae*

As shown for *Grapsus albolineatus* (Jamieson, 1994b; Jamieson *et al.*, 1995; and this study) (Fig. 19D), the grapsines *Sesarma* (now *Parasesarma*) *erythrodictyla*, *S. catenata* (Fig. 19F), and *Cyclograpsus punctatus* (Fig. 19E) (this study) (but not varunines) have added their own synapomorphy, loss of the thickened ring, to the putative thoracotreme synapomorphies. Presence of the ring is a basal synapomorphy of the Eubrachyura *sensu de Saint Laurent*, i.e. the Heterotremata *sensu lato* (Jamieson, 1994b) or Heterotremata + Thoracotremata *sensu* (Guinot, 1977, 1978), including other thoracotremes. This absence occurs homoplasically with grapsines in the heterotreme *Potamonautus perlatus* (Jamieson, 1993b, 1994b). Honma *et al.* (1992) claim to have observed acrosome rays in the Japanese land crab, *Sesarma haematocheir*, but these are not apparent in their micrographs. They do not state that the operculum (acrosomal cap) is perforate but a large central orifice is seen in scanning electron micrographs of opercula. As usual in brachyuran sperm, they observed mitochondria with poorly developed cristae. The outer membranes of some of these were continuous with the lamellar complex, and centrioles were present, as has been observed in other eubrachyurans.

The grapsid *Varuna litterata* is a euryhaline, often freshwater species, though returning to marine (or estuarine) conditions to breed. Its sperm (Fig. 21) shows only a negative state, absence of acrosome rays (other than a questionable presence of concentric lamellae) which would place it in the Thoracotremata. It does not display the grapsid synapomorphy, loss of the thickened ring (Jamieson *et al.*,

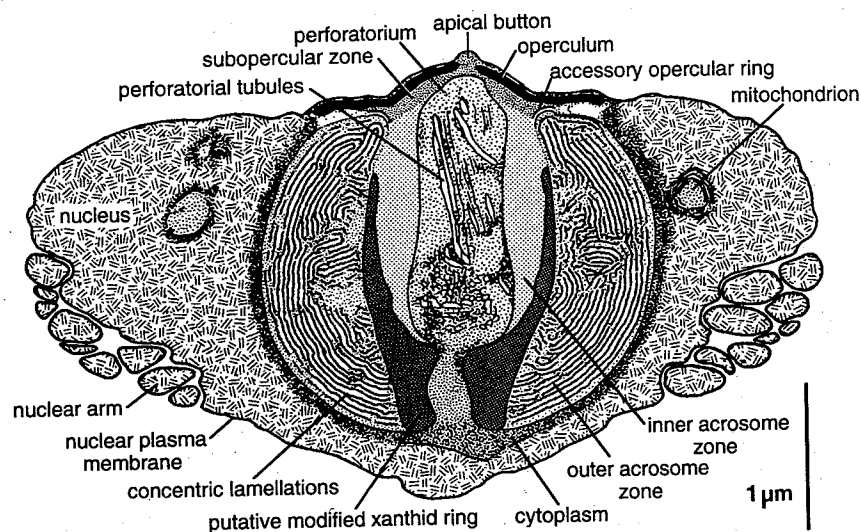


Fig. 20. *Cardisoma carnifex*, Gecarcinidae. Traced from TEM of vertical section of spermatozoon. From Jamieson *et al.* (1996). *Invertebrate Reproduction and Development*, 29, 111–126.

1996). Although, *V. litterata* falls outside the Thoracotremata if only spermatozoal characters are considered in cladistic analysis (Jamieson *et al.*, 1996), it is included in a monophyletic Thoracotremata when somatic characters are added. It seems likely that absence of the thoracotreme spermatozoal synapomorphies of an apical button in an opercular perforation; concentric lamellation of the outer acrosome zone, and the accessory opercular ring is secondary.

### III. CYTOSKELETAL PROTEINS IN CRUSTACEAN SPERMATOZOA

Investigations into the presence and role of cytoskeletal proteins in the spermatozoa of invertebrates are limited in number and taxonomic scope. Tilney pioneered much of the work on actin in the spermatozoa of echinoderms, in particular the holothurian genus *Thyone* (Tilney *et al.*, 1973; Tilney, 1975, 1976a, b; Tilney and Inoué, 1982); and actin filaments in the sperm of the bivalve, *Mytilus* and the horse-shoe crab, *Limulus* (Tilney, 1975). Several species of decapod crustaceans have been the subjects of spermatozoal cytoskeletal protein investigations. Among the prawns and shrimps the genera *Penaeus*, *Sicyonia* (Brown *et al.*, 1976; Griffin *et al.*, 1988) and *Rhynchocinetes* (Pérez *et al.*, 1991) have been studied, as also the lobsters, *Jasus* (Dupré and Schatten, 1990, 1993), and *Homarus* (Tsai and Talbot, 1994), and the crayfish, *Orconectes* (Dupré and Schatten, 1990, 1993). The hermit crabs in the genera *Clibanarius* and *Diogenes* (Tudge and Justine, 1994; Tudge, 1995a) have been investigated. The majid crabs, *Libinia* (Perez *et al.*, 1986; Hernandez *et al.*, 1989) and *Maja* (Tudge and Justine, 1994; Tudge, 1995a), the cancrid crab, *Cancer*



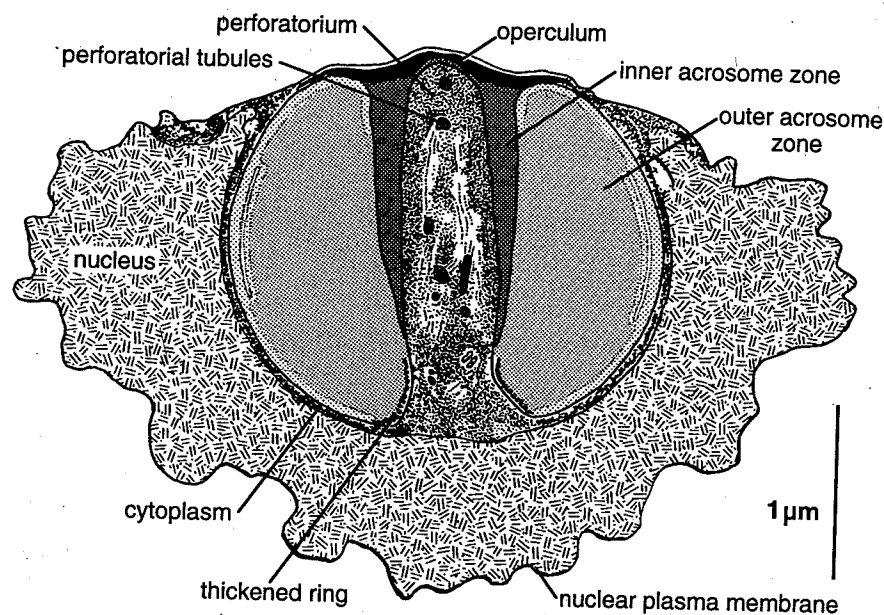


Fig. 21. *Varuna litterata*, Grapsidae, Varuninae. Traced from TEM of vertical section of spermatozoon. From Jamieson *et al.* (1996). *Invertebrate Reproduction and Development*, 29, 111–126.

(Tudge *et al.*, 1994; Tudge and Justine, 1994; Tudge, 1995a) and the potamid crab, *Potamon* (Tudge and Justine, 1994; Tudge, 1995a) are the brachyuran representatives which have been investigated.

### A. Actin

The cytoskeletal protein actin was identified in the spike (or acrosomal filament) of the shrimp, *Penaeus aztecus*, *P. setiferus* and *Sicyonia brevirostris* by Brown *et al.* (1976) while in *Rhyncocinetes typus* (Pérez *et al.*, 1991) actin was reported to be restricted to the rays and radial spines in the reacted spermatozoa. Conversely, Griffin *et al.* (1988) could not identify actin in the acrosomal filament of *Sicyonia ingentis*. Tsai and Talbot (1994) reported that actin was not present in the apical cap of the spermatozoa of the lobster *Homarus americanus*, but two unspecified proteins were involved in a contractile reaction observed in the acrosome of the spermatozoa. The acrosome vesicles in the spermatozoa of *Jasus frontalis* and *Orconectes propincus* were shown to contain actin, in the form of filaments, as revealed by the Rhodamine-Phalloidin fluorescent stain. In *Jasus*, the actin fluoresced in a cog-shaped pattern within the acrosome vesicle, while in *Orconectes* the actin was indicated by a lens-shaped region within the acrosome vesicle (Dupré and Schatten, 1993). Actin observed at the posterior end of the acrosome vesicle of the spermatozoa of the hermit crab, *Clibanarius erythropus*, correlates with the cytoplasmic region of the

sperm cell (Tudge and Justine, 1994; Tudge, 1995a) and could well indicate G-actin in association with cytoplasmic elements such as membrane systems around the mitochondria and the bases of the microtubular arms. In the spermatozoa of another species of hermit crab, *Diogenes gardineri*, actin was restricted to the opercular end of the acrosome vesicle (Tudge, 1995a) which appeared to be undergoing acrosome reaction. The actin in the spermatozoa of the spider crab, *Maja squinado*, is extensive, but is restricted to the acrosome vesicle and to elements of the extruded perforatorial column. In reacted spermatozoa, the everted perforatorial column labels positively for actin near its anteriormost tip (Tudge and Justine, 1994; Tudge, 1995a). As in the reacted spermatozoa of *M. squinado*, actin was identified in the everted perforatorial column (or 'acrosomal filament tip') of *Libinia emarginata* (Perez *et al.*, 1986; Hernandez *et al.*, 1989). It is believed that G-actin polymerizes to F-actin in the everting perforatorial column and that this process is an integral part of the acrosome reaction in *L. emarginata* (Hernandez *et al.*, 1989), *Cancer pagurus* (Tudge *et al.*, 1994) and many other invertebrates (Tilney *et al.*, 1973; Tilney, 1975; Tilney and Inoué, 1982). Actin appears in two distinct concentric rings in the unreacted spermatozoa of the brachyuran crab *Cancer pagurus*, with additional presence in the perforatorial projection and subacrosomal region in the reacted spermatozoa (Tudge *et al.*, 1994; Tudge and Justine, 1994; Tudge, 1995a). The two actin rings probably correspond to the conspicuous internal zonation of the acrosome vesicle seen at the transmission electron microscope level for *C. pagurus* and other *Cancer* species (Langreth, 1965, 1969); although no obvious actin filaments are visible within the acrosome vesicle. The pattern of actin in the spermatozoa of the freshwater crab, *Potamon fluviatile*, is associated with the opercular or anteriormost pole of the acrosome vesicle (Tudge and Justine, 1994; Tudge, 1995a) and suggests some form of reaction of the operculum.

### B. Actin-associated Proteins (e.g. $\alpha$ -Actinin, Spectrin and Tropomyosin)

Weak fluorescence was seen in the spermatozoa of the hermit crabs *Clibanarius erythropus* and *Diogenes gardineri*, for all the three abovementioned proteins in the former species and only for  $\alpha$ -actinin in the latter species (Tudge, 1995a). In *Diogenes gardineri*, the pattern of  $\alpha$ -actinin mirrored that for actin at the opercular end of the acrosome vesicle. In the spermatozoa of the cancrid crab, *Cancer pagurus*,  $\alpha$ -actinin, spectrin and tropomyosin all showed positive (although weakly) in the cytoplasmic region below the acrosome vesicle (Tudge *et al.*, 1994; Tudge, 1995a).

### C. Tubulins

The presence of a tubulin-like protein was identified in the rays and spines of the unfolded (=reacted) spermatozoa of the shrimp *Rhyncocinetes typus* by Perez *et al.* (1991). The three prominent microtubular arms of hermit crab spermatozoa (Tudge, 1992, 1995a, b) and majid crab spermatozoa (Hinsch, 1969, 1973) are composed

of bundles of microtubules made up of the standard arrangement of  $\alpha$  and  $\beta$  tubulin monomers, combined into heterodimers which form linear polymers. The microtubular bundles in the spermatozoa pass through the cytoplasmic region and emerge as discrete arms. These microtubular arms in the spermatozoa of the hermit crab, *Clibanarius erythropus*, label clearly with anti- $\alpha$ -tubulin to indicate the presence of tubulin (Tudge and Justine, 1994; Tudge, 1995a). The pattern of fluorescence indicating tubulins in the spermatozoa of the spider crab *Maja squinado* occurred in a ring or band around the spermatozoon approximately level with the opercular region (Tudge and Justine, 1994; Tudge, 1995a). This band of tubulin is consistent with the position of the microtubular bundles which occur in the cytoplasmic collar, found around the acrosome vesicle at its anteriormost end. Tubulins have previously been labelled with indirect immunofluorescence techniques in the spermatozoa of another spider crab, *Libinia emarginata* (Perez *et al.*, 1986), where they were similarly shown to occur in the cytoplasmic region and in the lateral extensions or arms. No significant fluorescence for tubulin was obtained in the spermatozoa of the brachyuran crabs, *Cancer pagurus* and *Potamon fluviatile*, when using labelled anti- $\alpha$ -tubulin and anti- $\beta$ -tubulin (Tudge *et al.*, 1994; Tudge and Justine, 1994; Tudge, 1995a). This result is to be expected as very few or no microtubules occur in the mature spermatozoa of brachyuran crustaceans (Jamieson, 1991b).

In conclusion, it is seen that actin is present in the spermatozoa of several species of decapod crabs and that in some shrimps and prawns, in the 'macrurans' *Jasus frontalis*, *Orconectes propincus*, and in the crabs *Libinia emarginata*, *Maja squinado* and *Cancer pagurus* it appears extensively in the acrosome vesicle (acrosomal filament) and perforatorial column. The fact that actin is present in these regions of unreacted and reacted spermatozoa seems to indicate that this cytoskeletal protein plays an important role in the acrosome reaction and subsequent fertilization events. Tubulin is obviously present in the crabs with microtubular arms and microtubular bundles associated with the spermatozoon. Tubulin is the essential building block of microtubules which are necessary for construction and function of the axoneme or flagellum in flagellate spermatozoa. In the immotile spermatozoa of decapod crabs microtubules have a different role. The bundles of microtubules are not arranged in the standard '9+2' arrangement seen in most axonemes, but are loosely aggregated (Hinsch, 1969, 1973; Jamieson, 1991b; Tudge and Jamieson, 1991, 1996a, b; Tudge, 1992, 1995a). Each tubule lacks the linking dynein arms needed for parallel gliding motion and the subsequent undulating swimming action. Motility has yet to be recorded for the microtubular arms of macruran, anomuran and brachyuran crab spermatozoa.

## IV. ACROSOME REACTION

### A. Dendrobranchiata

As noted previously (Jamieson, 1991b), the 'natantian' spike is acrosomal in function, contains actin and undergoes a  $\text{Ca}^{2+}$  dependent reaction (*Penaeus aztecus*, *P. setiferus*, Brown *et al.*, 1976; *Sicyonia ingentis*, Clark *et al.*, 1981, Clark and Griffin, 1988; *S. brevirostris*, Brown *et al.*, 1977). Fluorescein-labelled anti-actin indicates that it is the spike which contains actin, and therefore functions as an acrosome filament (perforatorium). Acridine orange and PAS-positive response of the amorphous cap from which the spike arises suggest that the cap is at least analogous to an acrosome vesicle (Brown *et al.*, 1976). In *Macrobrachium rosenbergii*, although the sperm first attaches to the egg by its wide base, the spike bends within 15 seconds and penetrates the egg investment from which the sperm base is released (Lynn and Clark, 1983b). The multiple spikes of non-natant decapods are not acrosomal and contain either cords of microtubules or extensions of the nucleus or both.

Detailed investigations of the acrosome reaction in natant decapods (shrimps and prawns) are effectively limited to the dendrobranchiate sicyonid, *Sicyonia ingentis* and cover the general morphology of the acrosome reaction (Shigekawa *et al.*, 1980; Anchordoguy *et al.*, 1988; Clark and Griffin, 1988), the ultrastructural changes in the sperm cell during the reaction (Clark *et al.*, 1981; Griffin *et al.*, 1988; Wikramanayake *et al.*, 1992; Chen *et al.*, 1994) and physiological changes and chemical mediators of the cellular reaction (Clark and Griffin, 1988; Griffin and Clark, 1990; Lindsay and Clark, 1992a, b; Wikramanayake *et al.*, 1992; Chen *et al.*, 1994).

The acrosome reaction of *Sicyonia ingentis* is a biphasic reaction consisting of acrosomal exocytosis followed by acrosome filament formation (Clark *et al.*, 1981; Clark and Griffin, 1988). During exocytosis the single spike of the sperm cell retracts and the outer membrane breaks down (probably associated with microfilament depolymerization). Acrosome filament formation involves the anterior extension of a 10  $\mu\text{m}$  spike composed of tubular structures (apparently not actin or tubulin) which has lateral 'petals' at its tip. The filament is thought to originate from a crystalline lattice at its base (Clark and Griffin, 1988).

### B. Homarida

Within the macrurous decapods (Homarida and Palinura) only the homarids, *Homarus vulgaris* and *H. americanus* have been studied to elucidate the changes associated with the acrosome reaction in the sperm cells. Ultrastructure of the acrosome reaction of *H. vulgaris* was first described by Pochon-Masson (1965c, 1968b) while the same process in its American counterpart, *H. americanus*, was studied later by Talbot and Chanmanon (1980b) and Talbot (1991). A more detailed

analysis of the ultrastructural changes to the acrosome of sperm cells of *H. americanus* using modern video microscopy and immuno-cytochemistry revealed the speed, complexity and cytochemical makeup of the acrosome reaction and the involved organelles (Tsai and Talbot, 1993, 1994).

The homarid sperm acrosome reaction is very rapid (approximately one to two seconds) and occurs in two steps, eversion of the acrosome contents and ejection of the subacrosomal material (Talbot, 1991). A normal acrosome reaction proceeds as follows: the operculum (apical cap) binds with the egg and initiates the reaction; the operculum rotates outwards and posteriorly down the acrosome vesicle; the acrosome contents swell and evert anteriorly, a distance of 14–18  $\mu\text{m}$ , to meet the oolemma (effectively bridging the vitelline envelope); the operculum contracts around the base of the acrosome vesicle forcing the cytoplasm, nucleus and microtubular arms into the space left by the acrosome contents eversion and projects them into the egg; the everted acrosome contents and operculum remain on the surface of the egg (Talbot and Chanmanon, 1980b; Talbot, 1991; Tsai and Talbot, 1993, 1994).

### C. Anomura

The acrosome reaction in the Anomura is relatively undocumented, with the exception of some light microscopy (e.g. Koltzoff, 1906; Retzius, 1909; Pochon-Masson, 1968b; Tudge and Justine, 1994; Tudge, 1995a), but it superficially appears to be a similar process to that seen in the above homarids. The similar reaction in some brachyuran representatives has been well illustrated in the literature (see below).

### D. Brachyura

The acrosome reaction has been documented in the spermatozoa of several brachyuran crustaceans including *Callinectes sapidus* (Brown, 1966a, b), *Libinia emarginata* (Hinsch, 1971; Hernandez *et al.*, 1989), *Carcinus maenas* (Pochon-Masson, 1968a; Goudeau, 1982), *Eriocheir sinensis* (Du *et al.*, 1987, 1993), *Uca tangeri* (Medina, 1992; Medina and Rodriguez, 1992b), and *Maja squinado* (Tudge and Justine, 1994; Tudge, 1995a). Some light microscope observations of the acrosome reaction in fluorescent-labelled *Cancer pagurus* and *Potamon fluviatile* is also illustrated in Tudge *et al.* (1994), Tudge and Justine (1994) and Tudge (1995a).

The brachyuran acrosome reaction has been described as consisting of two (Medina and Rodriguez, 1992b) or four (Du *et al.*, 1987) separate phases. Principally, some initial changes to the acrosome vesicle contents (which include perforation of the operculum, dissolution and eversion of the vesicle contents) and then the extension of an acrosome filament or tubule. During the early stages of the acrosome reaction, in some brachyuran spermatozoa, the dense operculum, after

penetration by the perforatorial column, 'peels back' onto the outer surface of the acrosome vesicle (Du *et al.*, 1987; Medina and Rodriguez, 1992b). At the same time, the outer contents of the acrosome vesicle protrude through the opercular area and begin to dissolve, often leaving only the convoluted capsular membrane. The perforatorial column then extends anteriorly, at least its own length, as the acrosome filament and is seen to drag the thickened ring, cytoplasmic membranes and the nucleus with it. The penetrating acrosome filament is seen as a mechanism for getting the nuclear material through the egg chorion (Du *et al.*, 1987).

### ACKNOWLEDGEMENTS

The constant advice and encouragement of Professor Danièle Guinot and the incomparable collections of Dr. Bertrand Richer de Forges who fixed material on board ship in difficult conditions are gratefully acknowledged. We are also much indebted to Professor Larry Abele for collecting and embedding material and to Professor Wendell Patten for kindly donating specimens. Much of the material was collected and processed with the financial support of the Australian Research Council. Mrs L. Daddow and Mr D. M. Scheltinga gave invaluable support and excellent technical assistance in all aspects of electron microscopy.

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