

# ULTRASTRUCTURE AND PHYLOGENY OF CRUSTACEAN SPERMATOOZOA

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The flagellate spermatozoon of the Remipedia (*Speleonectes*), differs from the invertebrate 'primitive sperm' (aquasperm) only in lacking a mitochondrial midpiece and in containment in a spermatophore. A flagellum occurs elsewhere in Crustacea only in the Maxillopoda (Ascothoracica, Cirripedia, Branchiura, Mystacocarida) and in the related Pentastomida, only the Ascothoracica, of these, retaining the plesiomorphic basal flagellar insertion. Cephalocarid (*Hutchinsoniella*) sperm resembling those of remipedes but lacking the flagellum may represent the ground plan for the Phyllopoda, hitherto thought to be the simple, amoeba-like sperm seen in euphyllopods and conchostracans. The *Nebalia* sperm, lacking an acrosome and with microtubular arms, supports the phyllopod status of phyllocarids. Copepod sperm show no clear affinities with other groups, though the stellate acrosome-less sperm of the cyclopoid *Chondracanthus* resembles that of some branchiopods. Ostracod sperm include a filiform type performing undulatory waves by means of wing-like structures originating from the endoplasmic reticulum. In the Malacostraca, stomatopod (*Squilla*, *Oratosquilla*) sperm are ovoidal, lacking appendages, with acrosome (re-acquired?) and a perforatorium; absence of a nuclear membrane, and diffuse chromatin are decapod tendencies; unusual, doublet centrioles are a peracarid-decapod feature. The syncarid (*Anaspides tasmaniae*) sperm has a subacrosomal filament [perforatorium], exceptional for Crustacea in being coiled. A syncarid apomorphy is the cytoplasmic 'skirt', a plesiomorphy the condensed chromatin and persistent nuclear membrane. Peracarid monophyly is confirmed by presence, with the questionable exception of tanaids, of a cross striated pseudoflagellum (possibly a centriolar rootlet homologue) joining the main body at junction of acrosome and nucleus. Tanaid sperm, rounded, lacking appendages, with large acrosome and scattered mitochondria, seen also in syncarids and stomatopods, possibly indicate a basal rather than terminal or intercalated position of the tanaids in the Peracarida. Euphausiid and stenopodid sperm, ovoidal and lacking appendages, apparently lack an acrosome. Dendrobranchiate (penaeid), procaridean, caridean shrimps and prawns have sperm with a single acrosomal spike but rarely have arms analogous with those characteristic of decapods. Several spikes containing microtubules which traverse the nucleus and often contain chromatin are characteristic of Palinura (*Panulirus*, *Jasus*); Astacidea (Astacidae, Nephropidae); Thalassinidea; Anomura (Paguridae, Diogenidae, Coenobitidae); and Brachyura, though microtubules are reduced or absent above the 'oxyrhynchus'. The acrosome of Eubrachyura resembles that of paguroids, and especially in its subspheroidal shape *Pagurus* and *Clibanarius*, suggesting a paguroid-brachyuran (sister-group?) relationship while the thalassinid (*Callinassa*) acrosome differs greatly from that of the Astacidea-Anomura-Brachyura assemblage, contraindicating a thalassinid origin of the Brachyura. The discoidal acrosome and reduced arms of dromiid (*Dromidia*, *Petalomera*) sperm may be plesiomorphic conditions of a group with no close relationship to other brachyurans. Phylogenetic heterogeneity of the Podotremata is supported by differences between dromiid and raninoid sperm and similarities (postnuclear tail) between *Ranina* and majids. The conventional oxytommate-oxyrhynch-cancerid-brachyrynch subdivision of the Brachyura is not supported by sperm ultrastructure. Dorippids and portunids, with similar sperm, are placeable in the Heterotremata, whereas the former classification separates the two families in the Oxytommata and Brachyryncha, respectively. Familial characteristics of sperm are exemplified by the distinctive 'xanthid ring' basal around the perforatorium of xanthids. Thoracotremata (Mictyroidea, Grapsoidea and Ocypodoidea) appear to be typified by presence of an apical opercular button, concentric lamination of the outer acrosome zone and modification of the xanthid ring. □ *Crustacea, phylogeny, spermatozoa, ultrastructure*

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Spermiocladistics, the use of spermatozoal ultrastructure for reconstruction of phylogeny (Jamieson, 1987), has recently been vindicated (Abele *et al.*, 1989) from a parsimony analysis of RNA sequences which verified attribution of the Pentastomida to the Crustacea on the basis of sperm ultrastructure (Wingstrand, 1972). The present paper presents a preliminary survey of sperm ultrastructure in crabs (Brachyura) as a contribution, pending further descriptive work and a computer analysis, towards elucidation of the phylogeny of this group. A phylogenetic review of the sperm of the Crustacea, which includes new ultrastructural observations, will first be presented. A phylogenetic parsimony analysis derived solely from sperm ultrastructure of the type attempted by Jamieson *et al.* (1987) for Oligochaeta will be deferred pending accumulation of additional data.

### CRUSTACEAN SPERMATOZOA

A phylogenetic tree of the Crustacea based on the somatic cladistic analyses of Schram (1986) is given in Fig. 1. Other phylogenies significantly differing from this might have been used in this essentially heuristic survey (e.g. Bowman and Abele, 1982). The ultrastructure of spermatozoa of the included groups is indicated diagrammatically according to accounts published by authors cited in the text, below, for these taxa.

#### CLASS REMIPEDIA

The Remipedia are primitive, cavernicolous crustaceans only recently described (Yager, 1981) and placed at the base of the crustacean phylogenetic tree by Schram (1986). The body lacks tagmosis into thorax and abdomen. The head is small and the trunk is divided into many segments, each bearing biramous, paddle-like appendages. The single known species, *Speleonectes benjamini*, is hermaphrodite.

Spermatophores are produced each of which is about 38  $\mu\text{m}$  long with three to possibly six sperm (nuclei) individually located at the proximal end. Each sperm cell (Fig. 1) has three distinct regions: a large nucleus, an acrosomal complex and a flagellum. A flagellum is elsewhere seen in the Crustacea only in the Maxillopoda. The ovoid nuclei are approximately 8–9  $\mu\text{m}$  long and 5  $\mu\text{m}$  wide.

The inverted cup-shaped, electron dense acrosome is apical on the nucleus. An acrosomal rod penetrates much if not all of the length of the nucleus (as in cephalocarids). Several mitochondria

scattered in the cytoplasm are possibly eliminated by maturity. A flagellum, of the 9+2 pattern (origin unknown but presumably postnuclear), extends several times the length of the nucleus. Centrioles were not observed (Yager, 1989).

Jamieson (1987) drew parallels between the albeit aflagellate spermatozoon of the Cephalocarida and the flagellated aquasperm of the Xiphosura (with no implication of relationship) and suggested that this similarity, together with the flagellate condition of maxillopod sperm, seemed to suggest that ancestral crustaceans had a primitive sperm *sensu* Franzén (1956, 1970), the aquasperm, in the author's terminology, which in its least modified manifestation has been termed the plesiosperm (Jamieson, 1986). The remipedian sperm constitutes a remarkable validation of this view as it has the characters we might ascribe to a cephalocarid sperm if a flagellum were added; though mitochondria have not been seen in the cephalocarid. Yager (1989) appears correct in deducing that the rounded form of the nucleus in the remipedian sperm indicates that it is more plesiomorphic than that of the Ascothoracica (see below), hitherto thought to be the most plesiomorphic for the Crustacea, in which the nucleus is cylindrical.

Although the occurrence of a 'primitive' sperm in early evolution of the Crustacea can now confidently be asserted, presence of this (though somewhat more modified than the plesiosperm) in Remipedia does not obligatorily demand, nor does it contest, the status of most primitive crustacean taxon envisaged for the Remipedia by Schram (1986). Abele *et al.* (pers. comm.) have suggested from analysis of rRNA sequences that remipedes are phylogenetically allied to the copepod-cirripede section of the Maxillopoda and are nearer to the Copepoda.

#### CLASS MAXILLOPODA

Until discovery of remipedes, the most basic crustacean sperm, and still the least modified maxillopod sperm, (Grygier, 1980, 1981, 1982) was that of the starfish parasite *Dendrogaster* (Ascothoracica) (Fig. 1).

The anterolateral position of the acrosome in *Dendrogaster* is a notable modification, however; it consists of an empty vesicle overlain by an electron dense layer. The head is bullet-shaped, the midpiece, approximately as long but half as wide, has six or more swellings, possibly representing mitochondria; the post-

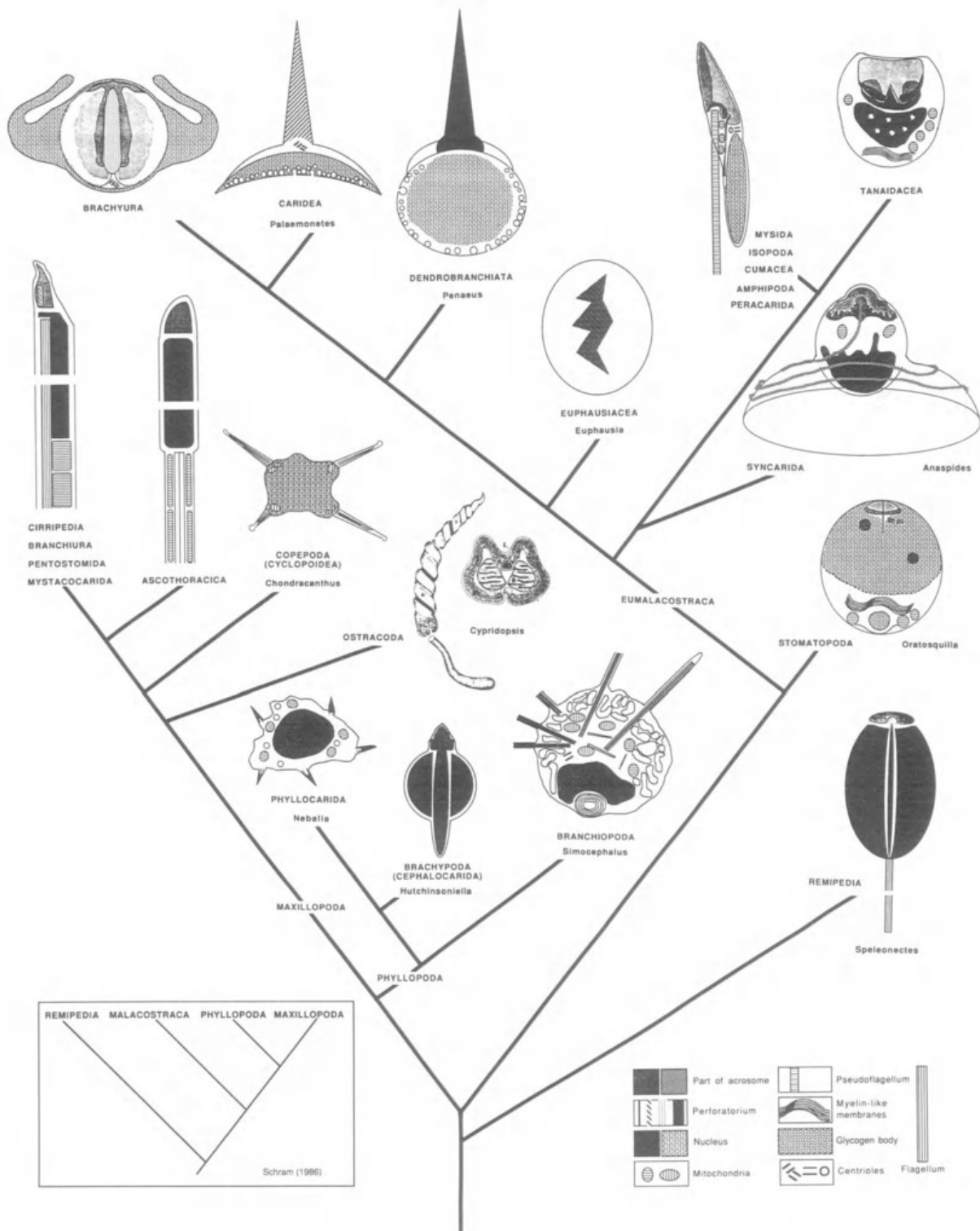


FIG. 1. Phylogeny of the chief groups of the Crustacea, based on Schram (1986; see inset) with diagram of spermatozoal ultrastructure after authors cited in the text. Original.

erior nuclear fossa houses the basal body of the 9+2 axoneme.

The flagellate condition of the ascothoracian sperm is elsewhere restricted (apart from the Remipedia) to the related maxillopod groups Mystacocarida (Brown and Metz, 1967) (Fig. 2G, H), Branchiura, and the related pentastomids, (Wingstrand, 1972; Abele *et al.*, 1989) and Cirripedia (Turquier and Pochon-Masson, 1969, 1971; Munn and Barnes, 1970a, b; Pochon-Masson *et al.*, 1970; Kubo *et al.*, 1979; Healy and Anderson, 1990) (Fig. 1, 2K). These maxillopods, with the exception of the Ascothoracica, are unified by the synapomorphic origin of the flagellum at the anterior end of the nucleus. The Ascothoracica, with their postnuclear axoneme, appear to be an isolated relict, preserved through the adoption of parasitism, that arose near the base of the Maxillopoda.

#### COPEPODA

Copepods, usually placed in the Maxillopoda, have a wide variety of aflagellate sperm (Coste *et al.*, 1979; Pochon-Masson and Gharagozoulou-van Ginneken, 1979; Rousset *et al.*, 1978; Brown, 1970; Raymond *et al.*, 1974; Manier *et al.*, 1978).

Their form is very variable. In *Tisbe holothuriae* (Harpacticoida) (Fig. 2I) the spermatozoon is elongate with definite head and neck (Pochon-Masson *et al.*, 1970; Pochon-Masson

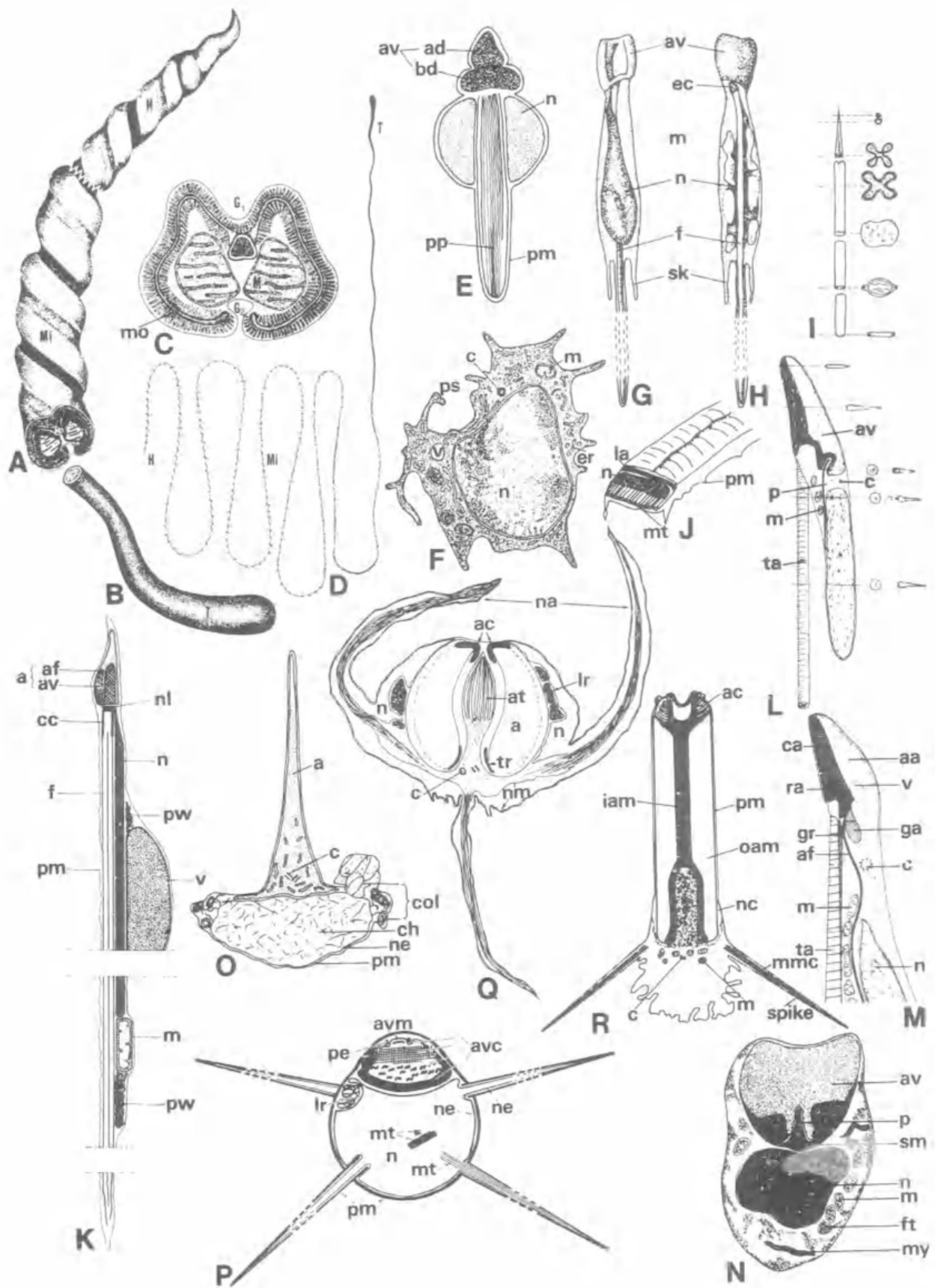
and Gharagozoulou-van Ginneken, 1977, 1978, 1979); in *Chondracanthus angustatus* (Cyclopoida) the form is star-shaped (Rousset *et al.*, 1978). In the Calanoida, *Calanus hyperboreus* has a discoidal spermatozoon (Brown, 1970) while in *Calanus finmarchicus* it is oblong (Raymont *et al.*, 1974). Caligoida have a globular spermatozoon in *Neobranchia cygniformis* (Manier *et al.*, 1978) whereas in *Lernanthropus kroyeri* (Fig. 2J) it has the form of an elongate spindle with sinuous contours (Coste *et al.*, 1979). Mitochondria are represented in all groups except the Caligoida. Only in *Neobranchia cygniformis* do centrioles persist into the mature spermatozoon. Microtubules are present only in elongate sperm (*Lernanthropus kroyeri*) or in those with spine-like protuberances (*Chondracanthus angustatus*). The acrosome varies from a twisted point on the tip of the nucleus in *Tisbe holothuriae* to merely a dense plate on the nucleus in *L. kroyeri* or, possibly, a group of vesicles in *Neobranchia cygniformis*.

The sperm of the cyclopoid *Chondracanthus angustatus* (Fig. 1) deserve special mention as they show remarkable similarities to those of the Branchiopoda and Phyllocarida. The mature (spermatophoral) spermatozoon of *C. angustatus* consists of a globular region with irregular contours from which arise three or four ribbon-like arms, with axial microtubules, which give the gamete a stellate appearance. Mitochondria

FIG. 2. Ultrastructure of the spermatozoa of some major groups of the Crustacea. From Jamieson (1987) after various authors. A-D, Ostracod, *Cypridopsis*. A, head and middle part. B, Tail. C, Enlarged cross section from middle region. D, Complete sperm (from Reger, 1970a). E, Cephalocarid, *Hutchinsoniella macrantha* (from micrographs by Brown and Metz, 1967). F, Branchiopod, *Polyartemia forcipata* (from Wingstrand, 1978). G, H, Mystacocarid, *Derocheilocaris typicus* (after Brown and Metz, 1967). I, Copepod, Harpacticoid, *Tisbe holothuriae* (after Pochon-Masson and Gharagozoulou van-Ginneken, 1977). J, Copepod, Siphonostomid, *Lernanthropus kroyeri*, mid-region of sperm (from Coste *et al.*, 1979). K, Cirripede, Generalized diagram (from Pochon-Masson *et al.*, 1970). L, Isopod, *Armadillium vulgare* (from Reger *et al.*, 1979). M, Isopod (from Cotelli *et al.*, 1976). N, Tanaid, *Tanais cavolinii* (from Cotelli and Lora Lamia Donin, 1980). O, Crangonid shrimp, *Crangon vulgaris* (from Pochon-Masson 1968b). P, Decapod, Palinura, *Panulirus argus* (from Talbot and Summers, 1978). Q, Decapod, Brachyura, Generalized oxyrhynch sperm (from Hinsch, 1973). R, Decapod, Astacidea, *Homarus americanus* (Talbot and Chanmanon, 1980a).

Abbreviations. a= acrosome; aa= amorphous part of acrosome; ac= apical cap; ad= anterior disc; af= acrosomal filament (perforatorium); at= acrosomal tubule; av= acrosomal vesicle; avc= contents of acrosomal vesicle; avm= acrosome vesicle membrane; bd= basal disc; c= centriole; col= collar; ec= electron dense core; f= flagellum; ft= foamy texture; G1, G2= grooves on surface of sperm; ga= granular part of acrosome; gr= groove; H= head; la= laminar acrosome; iam= inner acrosomal material; lr= lamellar region; m= mitochondrion; Mi= middle part of sperm; mmc= microtubule membrane complex; mo= membranous organelle; mt= microtubules; my= myelin figure; n= nucleus; na= nuclear arms; nc= nuclear cuff; ne= nuclear envelope; nl= nuclear lamella; nm= nuclear membrane; oam= outer acrosomal membrane; p= perforatorium; pe= periacrosomal material; pm= plasma membrane; pp= posterior projection; ps= pseudopodium; pw= periflagellar wall; ra= reniform part of acrosome; sk= skirtlike structure; sm= subacrosomal material; T= tail part of sperm; ta= cross striated tail-like appendage; tr= thickened ring; v= vesicle.





are grouped at the bases of the arms. The swollen portion of the sperm is entirely occupied by nucleoplasm. A nuclear envelope is absent. The chromatin is finely granular and homogeneous. Centrioles and acrosome are absent. As in phyllocarids and branchiopods, an acrosome is absent; numerous vesicles produced in the late spermatid from the nuclear membrane are not considered to be acrosomal (Rousset *et al.*, 1978).

#### OSTRACODA

Ostracods, regarded from somatic morphology as derived from the base of the Maxillopoda (Fig. 1), have aflagellate filiform sperm performing undulatory waves generated by peculiar membranous organelles (Tétart, 1967; Reger, 1970a; Reger and Florendo, 1969a, b) (Fig. 2 A-D), the contractile bands of Gupta (1968) or wing-like structures of Zissler (1966, 1969). Recently Wingstrand (1988) has described non-filiform sperm in ostracods.

#### CLASS PHYLLOPODA

The classification of Schram (1986) which places the Branchiopoda, Brachypoda (Cephalocarida) and Phyllocarida in an enlarged Phyllopoda is observed here.

#### CEPHALOCARIDA

The cephalocaridan sperm was described by Brown and Metz (1967) for *Hutchinsoniella macrantha* (Figs. 1, 2E). Before discovery of remipedes, cephalocarids were generally regarded as the most plesiomorph crustaceans on general anatomy, including (Paulus, 1979) that of the ommatidia.

Although the sperm is acentriolar and aflagellate, and mitochondria have not been observed, it has an anterior pointed acrosome and a rounded nucleus perforated by a rod which is interpreted as equivalent to the perforatorium of *Limulus* by Baccetti (1979). This, with flagellation of maxillopod sperm, suggested (Jamieson, 1987) that ancestral crustaceans had a primitive sperm *sensu* Franzén (1956, 1970), a fact since demonstrated by Yager for remipedes. Because of the absence of a flagellum, cephalocarid sperm are more derived than those of remipedes.

#### BRANCHIOPODA

The branchiopods, widely regarded (Siewing, 1963) as a basal group for the Crustacea have profoundly modified sperm, supporting the advanced position given to the group by Schram

(1986). Wingstrand (1978) concludes from an exemplary study of the astounding variety and bizarre forms of branchiopod sperm (Berard, 1974; Brown, 1969; Delavault and Berard, 1974; Garreau de Loubresse, 1967) that the ancestral branchiopods must have had simple, amoeba-like sperm of the type seen in euphyllipods and conchostracans. There is, however, no suggestion that this amoeboid form seen, for instance, in *Polyartemia forcipatus* (Fig. 2F), represents a primitive sperm type for the Crustacea as a whole and a flagellated form of the cephalocarid sperm may reasonably be envisaged as ancestral in the Phyllopoda.

#### PHYLLOCARIDA

Phyllocarid (*Nebalia*) sperm have no polarity; no acrosome; and possess pseudopodia-like lobes; and 20–30 spines, each supported by nine small tubules. The large number of spines is considered by Jespersen (1979) to indicate that they are not modified flagella. Although this may well be correct, it may be noted that larger numbers of modified axonemes occur in each sperm of catenulid turbellarians. Phyllocarid sperm were considered nearest to those of branchiopods by Jamieson (1989c) who noted that Lauterbach (1975), on other grounds, had suggested a branchiopod origin for phyllocarids and hence the Malacostraca. I concur here with Schram (1986) in excluding the Phyllocarida from the Malacostraca and allying them with the former Branchiopoda and Cephalocarida in the Phyllopoda. However, spermatological evidence appears to support a sister-group relationship between phyllocarids and branchiopods, with cephalocarids as the sister-group of the phyllocarid-branchiopod assemblage (Fig. 1) contrary to the sister-group relationship of cephalocarids and branchiopods recognized by Schram for extant forms.

#### CLASS MALACOSTRACA

##### STOMATOPODA

The sperm of *Squilla mantis* has been described by Cotelli and Lora Lamia Donin (1983), that of *Oratosquilla stephensoni* by Jamieson (1989c) and that of *Gonodactylus bredinii* by Felgenhauer and Abele (1990). Each stomatopod sperm (Fig. 1), aflagellate and obovoid, is surrounded by an electron dense coat. A spermatophore is absent. In contrast, spermatophores are present in eucarids, peracarids (isopods, amphipods and mysidaceans) and copepods. The

discus-shaped acrosome vesicle, is penetrated and underlain by a straight, slender acrosome rod (perforatorium) ensheathed, below the vesicle, in subacrosomal material. Feulgen-positive granular material, indicating chromatin, fills most of the length of the cell but there is no certain nuclear membrane. Two centrioles, consisting of doublets each with a radial 'foot' as in decapods and peracarids, occur near the acrosome and like it are embedded in the chromatin. Myelin-like membranes are associated with degenerating mitochondria in the posterior region of the cell. Thiéry-positive granules are aggregated as a glycogen body posteriorly in the cell. Stomatopods resemble decapods in their diffuse sperm chromatin but are placed below the syncarid-peracarid-decapod assemblage.

If, as is generally agreed, syncarids originated from the malacostracan stem above the departure of the Hoplocarida but at the base of the Eumalacostraca (Brooks, 1969; Jespersen, 1983), the development in stomatopods of a diffuse nucleus and disappearance of a discrete nuclear membrane must be considered parallelisms (not synapomorphies) with these conditions in decapods. The nuclear membrane tends to be disrupted in dendrobranchiate shrimps and prawns (Talbot and Summers, 1978), is usually intact in procarideans and carideans, and is usually disrupted in Anomura and Brachyura.

#### SYNCARIDA

Each spermatozoon of the syncarid *Anaspides tasmaniae* (Fig. 1), described by Jespersen (1983), is surrounded by a capsule (coat) as in stomatopods, and, as in the latter, a spermatophore is absent. A very elongate subacrosomal filament (perforatorium) bypasses the nucleus as in isopods, amphipods and cumaceans, rather than penetrating it as in stomatopods. In *Anaspides* the perforatorium makes a posteriorly widening spiral of 3-4 turns, a remarkable convergence to the condition in the xiphosuran *Limulus*. As in stomatopods, subacrosomal material forms a sheath around the filament. Posteriorly the filament forms, with the peripheral cytoplasm, a membranous skirt, not seen in other crustacean sperm, which gives the sperm the form of a bell. An axoneme is absent at all stages. The nucleus is condensed with a persistent envelope.

In the phylogram (Fig. 1) somatic evidence for the position of the syncarids at the base of the eumalacostracans has been accepted. Similarities of syncarids with most peracarids are the presence of a perforatorium (itself a plesiomor-

phy) which, as questionable synapomorphies, (1) is filiform and (2) bypasses the nucleus. The caridoid escape reaction (Dahl, 1983) unites syncarids, eucarids and peracarids.

#### PERACARIDA

Monophyly of peracarids has been denied by Wattling (1981) who considers that they consist of three independent lineages from a syncarid-like ancestor, the mysidaceans; the amphipods; and an isopod-tanaid-cumacean assemblage. From sperm ultrastructure this is clearly incorrect. Thus, in mysidaceans, amphipods, isopods (Fig. 2L, M) and Cumacea each sperm consists of two convergent linear components: the main body of the sperm, containing the nucleus and capped by the acrosome, and joining this anteriorly, a transversely striated tail-like but non-flagellar structure (possibly a centriolar rootlet homologue) (references in Cotelli *et al.*, 1976; Reger *et al.*, 1970; Reger *et al.*, 1979; Fain-Maurel *et al.*, 1975a,b). This highly peculiar morphology is unlikely to have originated more than once.

Tanaid sperm, rounded, lacking appendages, with large acrosome and scattered mitochondria (Cotelli and Lora Lamia Donin, 1980) (Fig. 2N), seen also in syncarids and stomatopods, possibly indicate a basal rather than terminal or intercalated position of the tanaids in the Peracarida but these may represent apomorphies related to the specialized fertilization biology of tanaeids, with fertilization in a tube. Presence of the perforatorium in non-tanaid peracarids may be a plesiomorphy or a reacquisition. It has been suggested, however, that the gamete described by Cotelli and Lora Lamia Donin (1980) is in fact a spermatid and that mature tanaid sperm conform, by light microscopy, to the typical peracarid structure (Siegs, pers. comm.). This observation, if verified, would unite all peracarids as a monophyletic entity.

#### EUCARIDA

##### ORDER EUPHAUSTACEA

Euphausiid sperm, ovoidal and lacking appendages, and with irregular central material which may be chromatin (Jamieson, unpublished) (Fig. 1), but otherwise virtually unknown, give little indication of the eucarid ground plan. If lack 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.

## ORDER DECAPODA

Since completion of the draft of this review, a review of decapod sperm by Felgenhauer and Abele (in press) has been made available to me through the kindness of the authors. Brief references to species which they investigated is made in the following account of decapod sperm.

## SUBORDER DENDROBRANCHIATA

## SUPERFAMILY 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, 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 penaid and eukyphid shrimps, as opposed to monophyly of the Natantia, appears to be indicated from rRNA studies by Abele *et al.* (pers. comm.). These authors, with considerable justification, retain the names Caridea for Eukyphida, and Stenopodidea for Euzygida and are followed here.

Although it does not establish (nor does it contraindicate) its monophyly, the old group Natantia is characterized by uniformity of gross spermatozoal ultrastructure. Similarities include division of the spermatozoon into three regions: acrosomal spike, cytoplasmic collar and nucleus. However, some claims made by Talbot and Summers (1978) and Kleve *et al.* (1980) for characteristics uniting natantian sperm (absence of centrioles, dissolution of the nuclear envelope with confluence of nucleoplasm and cytoplasm to form spermioplasm) and supposedly distinguishing them from 'reptant sperm' are unreliable, being typical of the penaeids but not of carids, though some disruption of the nuclear envelope occurs in the carid *Palaemonetes*. The single spike, giving what is paradoxically but conveniently called the 'unistellate' condition, distinguishes 'natantian' sperm from the 'multistellate' sperm (with more than one spike or arm) of the Astacidean-Palinuran-Decapod assemblage. The distinction goes deeper as the natantian spike is acrosomal in function, contains actin and undergoes a  $\text{Ca}^{++}$  dependent reaction (*Penaeus aztecus*, *P. setiferus*, Brown *et al.*, 1976; *Sicyonia ingentis*, Clark *et al.*, 1981, Clark and Griffin, 1988; *S. brevirostris*,

Kleve and Clark, 1976, Brown *et al.*, 1977). Fluorescein labelled anti-actin indicates that it is the spike which contains actin, and therefore functions like 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, 1983a). The multiple spikes of non-natant decapods are not acrosomal and contain either cords of microtubules or extensions of the nucleus or both.

Sperm ultrastructure has been described for the penaeids *Penaeus aztecus*, Clark *et al.*, 1973 (Fig. 1); *P. japonicus*, Ogawa and Kakuda, 1987; *P. setiferus*, Lu *et al.*, 1973; Felgenhauer, Abele and Kim, 1988; Felgenhauer and Abele, 1990; *Sicyonia brevirostris*, Brown *et al.*, 1977; and *S. ingentis*, Kleve *et al.*, 1980, Shigekawa *et al.*, 1980, Shigekawa and Clark, 1986, Clark *et al.*, 1981, Clark and Griffin, 1988.

The spermatozoon of *Sicyonia ingentis* well exemplifies penaeid sperm though the acrosome (spike) region is more elaborate than in *Penaeus*. The sperm is composed of a spherical mainbody which is partially encompassed by a morphologically complex cap region (acrosomal complex) from which extends the single spike. The mainbody houses an uncondensed Feulgen-positive nuclear region which is surrounded posteriorly and laterally by a cytoplasmic layer. A single layer of 0.06  $\mu\text{m}$  vesicles lines the periphery of this layer; the bounding membranes of the vesicles are apposed to and appear to fuse with the plasma membrane. Large, 0.7  $\mu\text{m}$  vesicles containing whorled membranous and granular material extend from the inner surface of the cytoplasmic layer into the central fibrillar region. A nuclear membrane is also absent in the sperm of *Penaeus setiferus* (Lu *et al.*, 1973). In *Sicyonia* the nucleus is separated from the cap-like acrosomal complex by a dense plate and a highly organized crystalline lattice which is composed of geometric 350 Å squares. The cap region consists, in posterior-anterior sequence, of the dense plate; the crystalline lattice; convoluted membrane pouches surrounding these; a central granular core immediately anterior to the lattice and medial to the pouches; spherical bodies (voids in the core substance); an electron



dense saucer-shaped plate embedded in the centre of the cap and with 12–15 petaloid radiating extensions; and a large anterior granule. The anterior granule gives RNA-ase stable red fluorescence with acridine orange staining. It is conical, with its concave posterior surface applied to the saucer-shaped plate. The spike, which is helicoidal and approximately 6 µm long, extends from the anterior end of the granule. Cap and spike are bound by a double membrane formed by fusion of the plasma membrane and the convoluted pouch membrane. The pouches and anterior granule, which are PAS-positive, and the spike are considered to comprise the acrosome (Kleve *et al.*, 1980).

Although the nucleus is typically subspheroidal in penaeids, it is shown to be considerably depressed antero-posteriorly in *Penaeus japonica* by Ogawa and Kakuda (1987).

#### SUBORDER PLEOCYEMATA

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

#### INFRAORDER CARIDEA *s.lat.*

The Infraorder Caridea *s.lat.*, as recognized by Bowman and Abele (1982) contains the infraorders Procarididea and 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 (1990) distinguish those carideans in which the spike is solid and contains cross striated fibrils (e.g. *Palaemonetes*) from those in which the spike is tubelike 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 *sensu stricto* 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, in prep.; *Atya margaritacea* and *Typhlatya rogersi*, Felgenhauer and Abele, 1990; the bresilioid *Rhynchocinetes typus*, Barros *et al.*, 1986; the palaemonoids *Palaemon elegans*, Pochon-Masson, 1969; *P. serratus*, Sellos and Le Gal, 1981; *Palaemonetes paludosus*, Koehler, 1979 (Fig. 1); *Palaemonetes kadiakensis*, Felgenhauer *et al.*, 1988, Felgenhauer and Abele, 1990; and *Macrobrachium rosenbergii*, Lynn and Clark, 1983a, b, Dougherty, 1987, Dougherty *et al.*, 1986, Harris and Sandifer, 1986; and the crangonoids *Crangon septemspinosa*, Arsenault *et al.*, 1979, 1980, Arsenault, 1984; *C. vulgaris*, Pochon-Masson, 1968b (Fig. 20); and the hippolytid *Hippolyte zostericola*, Felgenhauer and Abele, 1990.

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; *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 (1979). Cross striations were not seen in the spike of *Crangon vulgaris* examined by Pochon-Masson (1968b) nor in *Paratya australiensis*, (Jamieson and Robertson, in prep.).

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), having the form roughly 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 *Palaemonetes*.

*monetes paludosus*, (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 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 peripheral 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 australensis*.

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

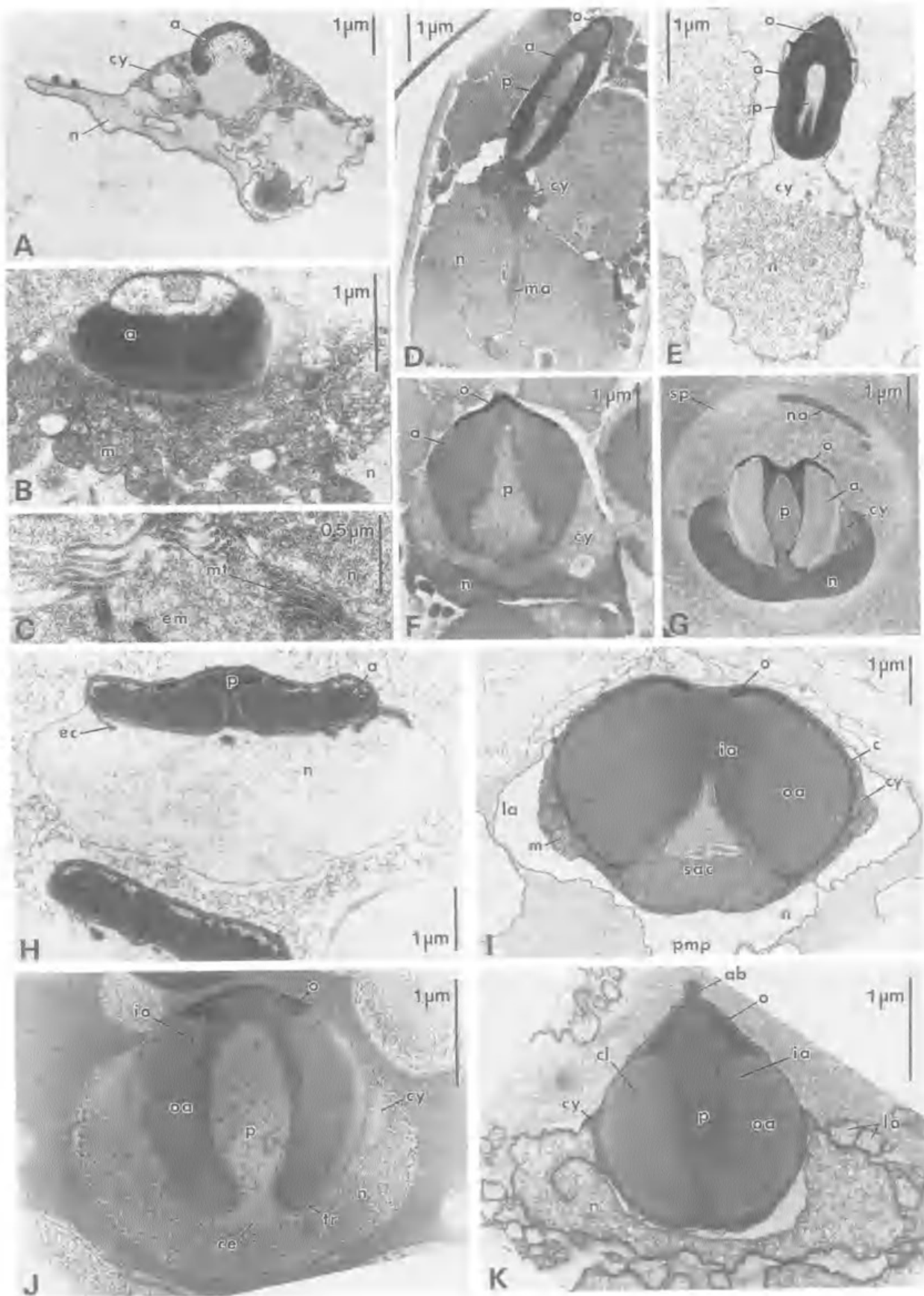
#### INFRAORDER STENOPODOIDEA

Sperm structure in this taxonomically problematic group has been examined, for *Stenopus hispidus*, by Felgenhauer and Abele (1990). The sperm of *S. hispidus*, were considered by Felgenhauer and Abele (1990) to resemble those of stomatopods as Burkenroad (1981) had suggested from a light microscope study of the sperm of *S. 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 (1990) 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 here seen as a notable resemblance to euphausiid sperm of possible phylogenetic significance.

#### INFRAORDER ASTACIDEA

Ultrastructural studies of the Astacidea include the families Astacidae (*Astacus astacus* = *A. fluviatilis*), Pochon-Masson, 1968b; López-Camps *et al.*, 1981; *A. leptodactylus* — spermatoocytes 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, 1979a, 1982; *Procambarus clarkii*, Moses, 1961a, b; *P. leonensis*, Felgenhauer and Abele, 1990; Nephropidae, subfamily Nephropinae (*Nephrops norvegicus*, Chevaillier, 1965; Chevaillier and Maillet, 1965; Chevaillier, 1966b, 1967a, 1967b, 1968); subfamily Homarinae (*Homarus americanus*, Talbot and Chanmanon, 1980a (Fig. 2R), 1980b; *H. vulgaris*, Pochon-Masson, 1965b, 1965c, 1968a; Enoplometopidae (*Enoplometopus occidentalis*, Haley, 1986) and Parastacidae (*Cherax tenuimanus*, Beach and Talbot, 1987, Jamieson, un-

FIG. 3. Micrographs of the ultrastructure of the sperm of some decapods. A, a parastacid, *Cherax tenuimanus*. B and C, a palinurid, *Jaşus novaehollandiae*. C, microtubular arm of *J. novaehollandiae*. D, a galatheid, *Allogalatea* sp. E, a porcellanid, *Petrolisthes lamarckii*. F, diogenid, *Clibanarius corallinus*. G, a majid, *Menaethius monoceros*. H, a dromiid, *Petalomera lateralis*. I, a raninid, *Ranina ranina*. J, a portunid, *Caphyra rotundifrons*. K, a mictyrid, *Mictyris longicarpus*. All original. Abbreviations: a= acrosome; ab= apical button; c= capsule; ce= centriole; cl= concentric lamellae; cy= cytoplasm; ec= extensions of capsule; em= extra-cellular matrix; ia= inner acrosome zone; la= lateral arms; m= mitochondria; ma= microtubular arm; mt= microtubules; n= nucleus; na= nuclear arm; o= operculum; oa= outer acrosome zone; p= perforatorium; pmp= posterior median process; sac= subacrosomal chamber; sp= spermatophore; tr= thickened ring.





published) and *C. albidus*, Beach and Talbot, 1987).

The acrosomal-nuclear complex is elongate in the Nephropidae (Figs 2R, 4) but compact and dome-shaped in the Astacidae and Parastacidae (Figs 3A, 4). *Enoplometopus* is exceptional for the investigated Nephropidae in its dome-shaped acrosome, wider than long (Fig. 4), 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.

#### ASTACIDAE AND PARASTACIDAE

Sperm ultrastructure of astacids and parastacids indicates combined monophyly of the two families. 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, 1990). 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; Eliakova and Goriachkina, 1966; Yasuzumi and Lee, 1966; Anderson and Ellis, 1967; Pochon-Masson, 1968b; Moses, 1969a, b; 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 (1980a), for Homarus,

that the nuclear membrane becomes fused with the plasma membrane as a 'tegument' containing 'spermioplasm', admixed nucleoplasm and cytoplasm.

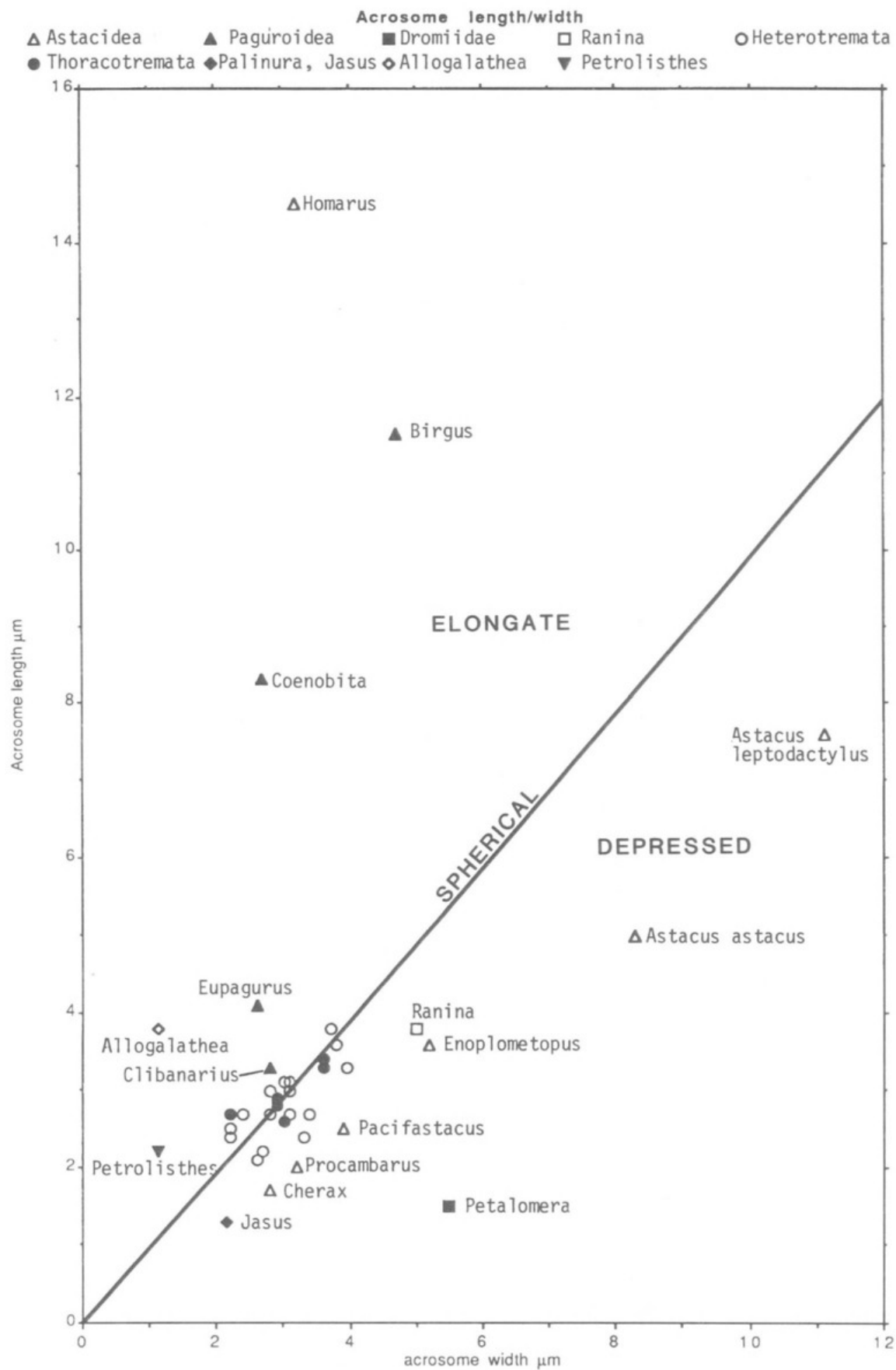
Microtubules, c. 200 Å (Pochon-Masson, 1968b), 220–310 Å (Yasuzumi and Lee, 1966) or c. 300 Å wide (Anderson and Ellis, 1967), with associated DNA, form several parallel bundles some of which extend into the spines (Moses, 1961a, b; Anderson and Ellis, 1967; Pochon-Masson, 1968b), each of which contains, for instance, 30 evenly spaced microtubules in *Cambaroides* (Yasuzumi and Lee, 1966). The microtubules probably are responsible for movement of the spines which has been observed in crustacean sperm (Pochon-Masson, 1968b).

Centrioles are said to be absent from the mature sperm of *A. 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).

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 than long, in contrast with nephropids (*Homarus*, *Nephrops*) in which, with the exception of *Enoplometopus*, it is greatly elongated (Fig. 4). 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, 1990; Anderson and Ellis, 1967; Yasuzumi and Lee, 1966, respectively). In *Cherax albidus* (Parastacidae) some apical whorled material is present within the vesicle but is absent in *C. tenuimanus* (present study; Beach and Talbot, 1987) (Fig. 3A). 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 periph-

FIG. 4. Plot of acrosome length against width for various reptants. Standard deviations for each species are not shown but are small.





eral electron dense band (Dudenhause 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.

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 (1990). As in most other Malacostraca, the acrosome does not appear to be a Golgi derivative, the hall-mark of the acrosome in other animal groups. Dudenhause and Talbot (1979) state that the proacrosomal vesicles, which fuse to form the acrosome, originate from the ER 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.

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).

#### NEPHROPIDAE

The spermatozoa of *Homarus americanus* (Talbot and Chanmanon, 1980a) (Fig. 2R) and *H. vulgaris* (Pochon-Masson, 1965c, 1968b) conform with the gross ultrastructural pattern described for the Astacidae but differ, chiefly, in the pronounced elongation of the acrosome (Fig. 4) which projects as a cylinder. 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 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) which 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, contains 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 axiid, below) a three-sided vault the apex of which immediately underlies the base of the acrosome (Talbot and Chanmanon, 1980a).

The acrosome reaction of the *H. americanus* sperm has been elegantly described by Talbot

and Chanmanon (1980b) and corresponds closely to the report of Pochon-Masson (1965c, 1968b) for *H. vulgaris* (see also Brachyura, Pochon-Masson, 1968a) but cannot be described here.

The ultrastructure of the sperm of the Subfamily Nephropinae, exemplified by *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 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).

#### ENOPLOMETOPIDAE

As indicated above, the sperm of *Enoplometopus occidentalis*, described by Haley (1986), who termed it an axiid, appears to the writer 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 struc-

tures are present. The first do not survive into early spermatids while the second form (apparently from membranes of the lamellar region according to Haley but possibly in fact generating these) during spermiogenesis.

#### INFRAORDER THALASSINIDEA

The Thalassinidea contains seven families of which only two families have representatives which have been investigated for sperm ultrastructure: *Callinassa australiensis* (Callinassidae) and *Thalassinia anomala* (Thalassinidae) (Tudge, pers. comm.). *C. australiensis* has a spherical sperm with four radiating microtubular arms; a small, flat acrosome; the remainder of the sperm body being composed of nuclear and cytoplasmic material. *T. anomala* has a morphologically different sperm being more oblong in shape and possessing a larger acrosome vesicle capped by an operculum with three horizontal layers. The acrosome vesicle is anterior to the cytoplasmic region, from which several microtubular arms originate, and a small nuclear region is present posteriorly.

#### INFRAORDER PALINURA

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 (in prep.) (Fig. 3B,C) (Palinuridae) and that of *Scyllarus chacei* (Scyllaridae) by McKnight and Hinsch (1986).

Each *Panulirus* sperm (Fig. 2P) 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.

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 acrosome of *Scyllarus chacei* is unique in investigated Crustacea in having 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 nuclear membrane is folded and irregular and the chromatin diffuse. 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).

Panulirid sperm conform to the general 'rep-tant' 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. Possession of crystalline material (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-brachyurid assemblage and it would not appear that palin-

urids are near the ancestry of the latter assemblage.

INFRAORDER ANOMURA (*s. strict.* Anomala *s.* Schram, 1986)

The Anomura contain 13 families. Sperm morphology at the light and electron microscope level has been carried out on representatives from six of these: within the Paguroidea, the Diogenidae (*Clibanarius longitarsis*, Dhillon, 1964, 1968; *Clibanarius taeniatulus*, *Clibanarius virescens*, Tudge, unpubl.; *Clibanarius coral-linus*, Jamieson, in prep. (Fig. 3F); *Dardanus* sp., and *Diogenes* sp., Tudge, unpubl.); the Coenobitidae (*Coenobita clypeatus*, Hinsch, 1980 a, b; *Coenobita spinosus*, Tudge, unpubl., and *Birgus latro*, Tudge and Jamieson, 1991); and the Paguridae (*Pagurus* (= *Eupagurus*) *bernhardus*, Pochon-Masson, 1963; Chevallier, 1966, 1967, 1968, 1970); in the Galatheididae, *Allogalathea* sp. (Jamieson, in prep.) (Fig. 3D); in the Porcellanidae, *Petrolisthes lamarckii* (Jamieson, in prep.) (Fig. 3E) and in the Hippidae, *Emerita talpoida*, Pearse *et al.*, 1942; Barker and Austin, 1963; *E. analoga*, Vaughn, 1968a, b; Vaughn *et al.*, 1969; Vaughn and Locy, 1969; Vaughn and Thomson, 1972; and *E. asiatica*, Subramoniam, 1977).

Most of the anomurans have sperm morphology characterised by an elongate to oblate, complex acrosome projecting anteriorly to the nuclear material and capped by an electron-dense, domed or conical operculum; and three long microtubular arms (possibly more in the Hippidae), radiating from the cytoplasmic region anterior to the nucleus; and diffuse chromatin. *Clibanarius* spp. and *Pagurus bernhardus*, are exceptional only in having a shorter, more ovoid acrosome.

A scatter diagram showing the proportions of the acrosomes (length: width) in various reptants, including anomurans, is given in Fig. 4.

#### BRACHYURA

In the present study of brachyuran spermatozoal ultrastructure it is proposed to investigate the validity of two conflicting classifications of the Brachyura. The first, which has been summarized by Warner (1977) and is the more familiar to most workers, divides the Brachyura into five sections, the Dromiacea, Oxystomata, Oxyrhyncha, Cancridea and Brachyrhyncha. This classification, with included families for which sperm ultrastructure is known, is shown in Table 1.

Footnotes in the Table allude to the alternative



TABLE 1. Ultrastructural investigations of spermatozoa of the Brachyura.

Higher taxon & Family	Species	Sperm ultrastructure
<b>Dromiacea</b> <sup>1</sup>		
Dromiidae	<i>Dromidia antillensis</i> Stimpson <i>Petalomera lateralis</i> (Gray)	Brown (1966a, 1970); Felgenhauer and Abele (1990) Jamieson (1990)
<b>Oxystomata</b>		
Raninidae <sup>2</sup>	<i>Ranina ranina</i> (Linnaeus)	Jamieson (1989b)
<b>Oxyrhyncha</b>		
Majidae <sup>3</sup>	<i>Chionoecetes opilio</i> (Fabricius) <i>Libinia dubia</i> Milne Edwards <i>Libinia emarginata</i> Linnaeus	Beninger <i>et al.</i> (1988) Hinsch (1973) Hinsch (1969, 1971, 1973, 1986); Vaughn and Hinsch (1972); Hernandez <i>et al.</i> (1989)
	<i>Macrocoeloma trispinosum</i> (Latreille) <i>Menaethius monoceros</i> (Latreille) <i>Mithrax</i> sp. Latreille <i>Pitho lherminieri</i> Rathbun <i>Podochela gracilipes</i> Stimpson <i>Podochela risei</i> Stimpson <i>Stenorhynchus seticornis</i> Lamarck <i>Heterocrypta granulata</i> (Gibbes) <i>Parthenope serratus</i> (H. Milne Edwards)	Hinsch (1973) Present study Hinsch (1973) Hinsch (1973) Hinsch (1973) Hinsch (1973) Hinsch (1973) Hinsch (1973) Hinsch (1973)
<b>Parthenopidae</b> <sup>3</sup>		
<b>Cancridea</b>		
Cancridae <sup>3</sup>	<i>Cancer borealis</i> Stimpson <i>Cancer irroratus</i> Say <i>Cancer magister</i> Dana <i>Cancer pagurus</i> Linnaeus <i>Cancer productus</i> Randall	Langreth (1965, 1969) Langreth (1965, 1969) Langreth (1965, 1969) Pochon-Masson (1968a) Langreth (1965, 1969)
<b>Brachyrhyncha</b>		
Portunidae <sup>3</sup>	<i>Callinectes sapidus</i> Rathbun <i>Portunus pelagicus</i> (Linnaeus) <i>Carcinus maenas</i> (Linnaeus)	Brown (1966a,b); Felgenhauer and Abele (1990) Jamieson (1989b, 1990); Jamieson and Tudge (1990) Chevaillier (1966b, 1967, 1969); Goudea (1982); Pearson and Walker (1975); Pochon-Masson (1962 [spermiogenesis only], 1965, 1968b); Reger <i>et al.</i> (1984)
	<i>Ovalipes ocellatus</i> <i>Caphyra laevis</i> (A. Milne Edwards) <i>Caphyra rotundifrons</i> (A. Milne Edwards)	Hinsch (1986) Present study Present study
Dorippidae <sup>3</sup>	<i>Neodorippe astuta</i> (Fabricius)	Jamieson and Tudge (1990)
Calappidae <sup>3</sup>	<i>Calappa hepatica</i> Alcock	Present study
Xanthidae <sup>3</sup>	<i>Menippe mercenaria</i> (Say) <i>Atergatis floridus</i> (Linnaeus) <i>Liagore rubromaculata</i> De Haan <i>Etisus laevimanus</i> Randall <i>Pilodius areolatus</i> (Milne-Edwards) <i>Eurypanopeus depressus</i> (Smith, 1869) <i>Eurytium limosum</i> (Say, 1818) <i>Iliacantha subglobosa</i> (Stimpson, 1871)	Brown (1966a) Jamieson (1989a, 1989c) Jamieson (1989a) Jamieson (1989a) Jamieson (1989a) Felgenhauer and Abele (1990) Felgenhauer and Abele (1990) Felgenhauer and Abele (1990)
Leucosiidae <sup>3</sup>	<i>Pinnixia</i> sp. White	Reger (1970c)
Pinnotheridae <sup>4</sup>	<i>Eriocheir japonicus</i> De Haan	Du <i>et al.</i> (1987); Yasuzumi (1960)
Grapsidae <sup>4</sup>	<i>Grapsus albolineatus</i> Lamarck <i>Sesarma erythroductyla</i> Hess <i>Sesarma reticulatum</i> (Say)	Present study Present study Felgenhauer and Abele (1990)
Geryonidae <sup>4</sup>	<i>Geryon fenneri</i> Manning & Holthuis <i>Geryon quinquedens</i> Smith	Hinsch (1988) Hinsch (1988)
Mictyridae <sup>4</sup>	<i>Mictyris longicarpus</i> Latreille	Present study
Ocypodidae <sup>4</sup>	<i>Ocypoda ceratophthalma</i> Ortmann <i>Uca dussumieri</i> H. Milne Edwards	Present study Present study
Macrophthalminae <sup>4</sup>	<i>Macrophthalmus crassipes</i> H. Milne Edwards	Present study

1, 2, 3, 4 Attributions in the alternative system of Guinot (1978) are: <sup>1</sup>Podotremata, Dromiacea <sup>2</sup>Podotremata, Archaeobrachyura  
<sup>3</sup>Heterotremata <sup>4</sup>Thoracotremata.

TABLE 2. Brachyuran classification of Guinot (1978).

Section	Sub-section	Superfamily	Sperm ultrastructure known
Podotremata	Dromiacea	Homolodromoidea Dromioidea	Dromiidae*
	Archaeobrachyura	Homoloidea Raninoidea Tymoloidea	Raninidae*
Eubrachyura <sup>a</sup>	Heterotremata	Dorippoidea	Dorippidae*
		Calappoidea	Cancridae, Calappidae*
		Portunoidea	Portunidae*
		Xanthoidea	Xanthidae*, Geryonidae
		Majoidea	Majidae*
		Parthenopoidea	Parthenopidae
		Bellioidea	
		Leucosioidea	Leucosiidae
Thoracotremata	Gecarcinoidea	Grapsoidea	Grapsidae*
		Mictyroidea	Mictyridae*
		Pinnotheroidea	Pinnotheridae
		Hexapodoidea	
		Ocypodoidea	Ocypodidae*
		Hymenostomatoidea	
*Jamieson, Jamieson and Tudge.			
<sup>a</sup> De Saint-Laurent (1980b).			

classification, developed by Guinot (1977, 1978) in which the Brachyura are divided into three groups: the Podotremata (in turn divided into the Dromiacea and Archaeobrachyura), the Heterotremata and the Thoracotremata (Table 2).

It will be shown that Guinot's classification, though requiring modification, is more congruent with sperm ultrastructure than is that presented by Warner. Guinot's system is based on two, and only two, apomorphies: location of female pores on the sternum of segment 6; and location of the male pores on the sternum of segment 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. Although this classification is better supported by sperm ultrastructure, recognition of the Heterotremata on a single apomorphy, the sternal female pores, might not be expected to give a robust group though more confidence might be attached to the Thoracotre-

mata based on the apomorphic, sternal location of female and male pores. Even if acquisition of sternal female pores were a unique, monophyletic event, the Heterotremata must be paraphyletic if its descendants (Thoracotremata) are not included in it as a subset. In Fig. 5 paraphyly of the Heterotremata and monophyly of the included Thoracotremata is indicated.

This caveat does not, however, undermine the terminal group, the Thoracotremata and this group is supported by spermatozoal apomorphies. The six species of the Thoracotremata examined here (Fig. 8) show three synapomorphies (Fig. 5): (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*; (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, 1989a) is present in at least the grapsids, the mictyrid and *Ocypoda*, its homology being uncertain in *Uca* and *Macrophthalmus*.

In contrast to spermatozoal support for at least the thoracotrema assemblage, the Dromiacea-Oxystomata-Oxyrhyncha-Cancridae-Brachyrhyncha classification (henceforth D-B classification) is

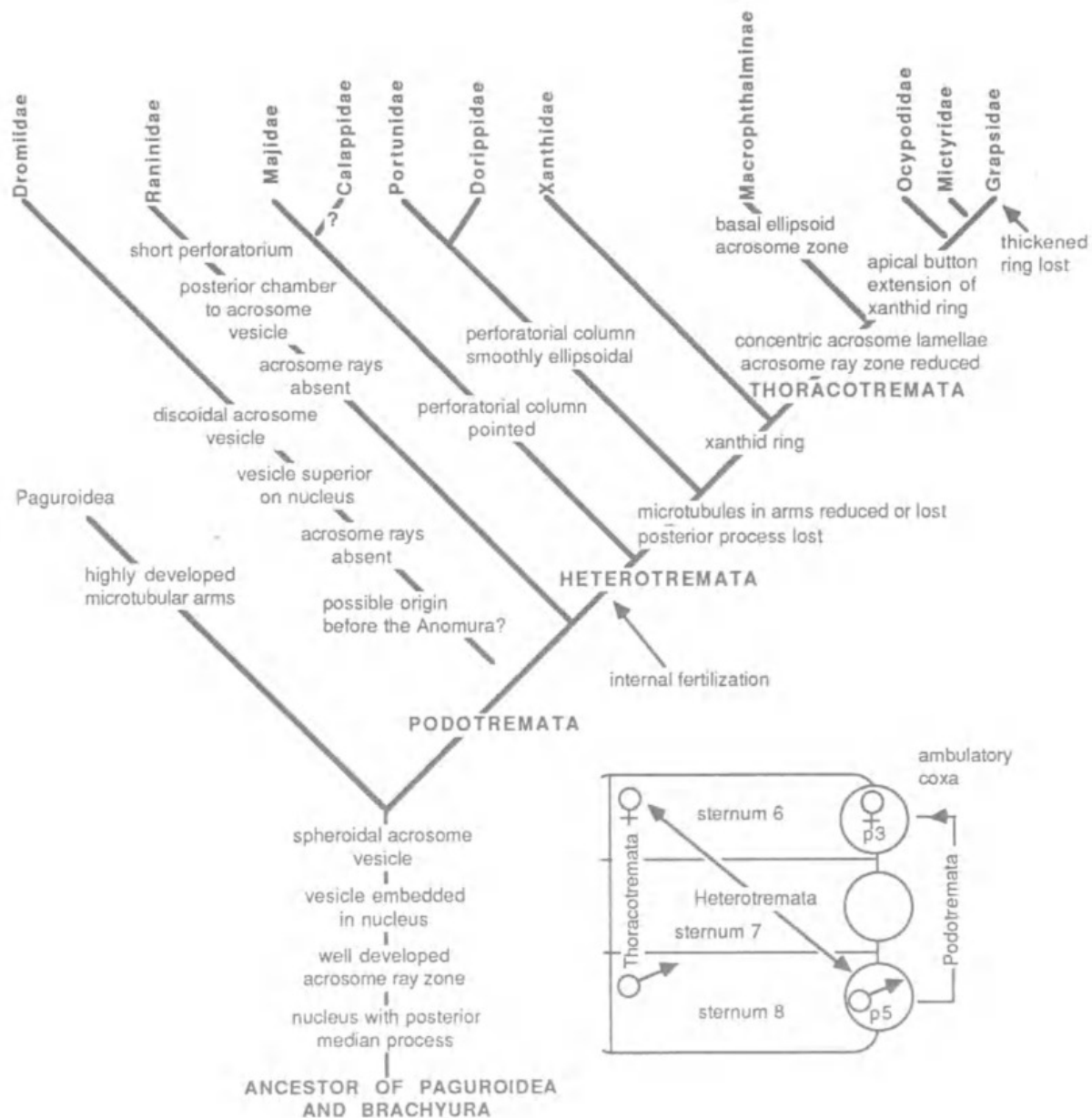


FIG. 5. Tentative, heuristic phylogeny of the Brachyura derived from consideration of the ultrastructure of spermatozoa superimposed on a phylogeny deduced from the classification of Guinot (1977, 1978) which is indicated at bottom right. The phylogeny is limited to the families investigated in the present study and, while it shows perceived trends in spermatozoal anatomy, may be expected to be modified when further taxa are examined.

refuted by the very close, and distinctive, similarity of the sperm of portunids (*Portunus*, *Calinectes*, *Carcinus*, *Caplyra*) with those of the Dorippidae, exemplified by *Neodorippe*, (Fig. 7A). In the D-B system dorippids are placed with raninoids in the Oxystomata while portunids are far removed, in the Brachyrhyncha. The sperm of *Ranina*, described by Jamieson (1989b) (Figs 3I, 6B) is radically different from that of *Neodorippe*. The heterogeneity of the Oxysto-

mata and Brachyrhyncha appears to be endorsed from studies of larval stages (Rice, 1980; Wear and Fielder, 1985).

It might alternatively be argued that the Thoracotremata do not have their origin in the Heterotremata and that the two are independent, monophyletic groups originating from a common ancestor. This view has been espoused by De Saint-Laurent (1980) but the overlap in zoeal morphology demonstrated by Rice (1981), with that

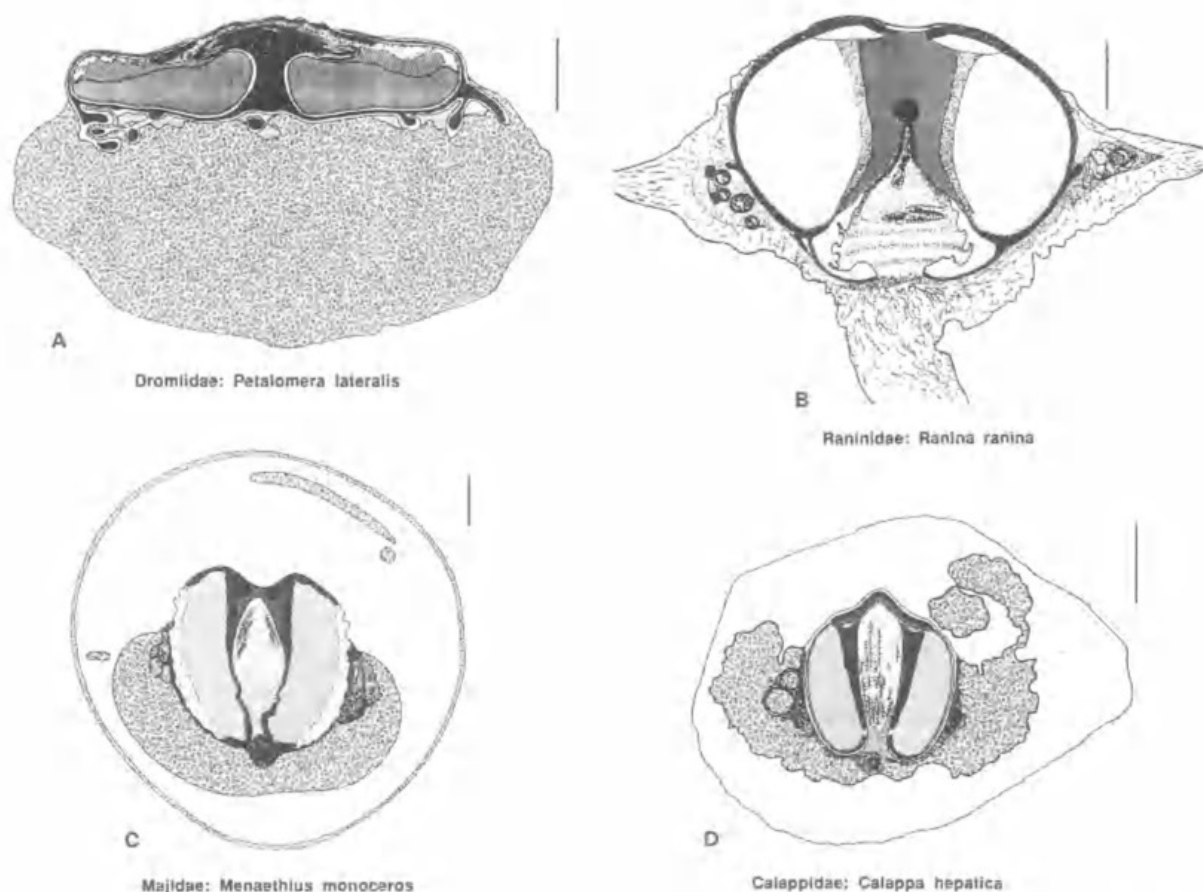


FIG. 6. A–D, Semi-diagrammatic longitudinal sections of spermatozoa from selected brachyuran families. A and B, Podotremata; C and D, Heterotremata. Traced from micrographs. Scale bars = 1 µm.

in spermatozoal ultrastructure suggests that thoroacotremes arose from paraphyletic heterotremes as proposed above.

A systematic account of brachyuran sperm now follows.

#### DROMIACEA

**Dromiidae.** The dromiid sperm, as exemplified by *Petalomera lateralis* (Figs 3H, 6A) and *Dromidia antillensis*, differs markedly from spermatozoa of other crabs (the Oxystomata–Oxyrhyncha–Cancridea–Brachygnatha {O–C–B} assemblage or the raninoid–heterotreme–thoracotreme assemblage) in the discoid, relatively undifferentiated acrosome capping, but not embedded in the nucleus (plesiomorphies); the capitate form of the perforatorium and the composition of this (autapomorphies); the greater, apomorphic, reduction of cytoplasm and organelles, including mitochondria and centrioles; and the absence (*Petalomera*) or brevity (*Dromidia*) of nuclear arms. In view of some similarities of the acrosome to those of Eu-

brachyura (non-dromiaceans) suggestive of relationship, brevity of arms may be secondary by reduction. Presence of well developed nuclear arms is a synapomorphy of all investigated non-dromiid brachyurans and of the *Palinura*, *Astacidea* and *Anomura* while absence is a symplesiomorphy of other Malacostraca. Absence from dromiid sperm of a posterior median process of the nucleus, which is present in *Pagurus* (Pochon-Masson, 1968a), *Ranina* (Jamieson, 1989b) and majids (Hinsch, 1973; there attributed to a generalized oxyrhynch, Fig. 2Q), is presumably an apomorphic loss, if dromiids are indeed brachyurans. If brevity of lateral arms in dromiids were plesiomorphic, the Dromiacea might be derived from early decapods before evolution of the *Palinura*–*Astacidea*–*Anomura*–*Brachyura* assemblage as suggested (Rice, 1983; Wear and Fielder, 1985) by their non-brachyuran, anomuran type larvae. If dromiids are monophyletic with true crabs, zoeal morphology would demand a basal position in the *Brachyura*.



## RANINOIDEA

Raninoids, with dorippoids and calappoids, constitute the spermatologically heterogeneous and clearly polyphyletic Oxystomata (Warner, 1977) or the Archaeobranchyura, containing only raninoids, homoloids and tymoloids, of Guinot (1978). Homoloids and tymoloid sperm are unknown ultrastructurally and therefore the archaeobranchyuran grouping cannot be fully tested spermatologically. Nevertheless, the sperm of *Ranina ranina* (Figs 3I, 6B) is sufficiently similar to those of eubranchyurans and different from those of dromiids to suggest that the Podotremata (Dromiacea and Archaeobranchyura) is a paraphyletic assemblage. Several features are shared between the sperm of *R. ranina* and those of the Eubranchyura. These were previously considered to be synapomorphies (Jamieson, 1989b) but may be plesiomorphies carried over from similar morphology known for anomurans and particularly paguroids: the large spherical, multi-layered, capsule-bound acrosome vesicle (contrast the disc-shaped acrosome of dromiids); the electron dense operculum capping the vesicle; an invaginated core, or perforatorium; concentric zonation of the contents of the vesicle; a layer of cytoplasm, between the acrosome vesicle and the nucleus, which contains mitochondria (mostly degenerating) and lattice-like lamellar complexes or membrane remnants; a diffuse nucleus which is bounded externally by a combined nuclear and plasma membrane and cups the scanty cytoplasm and the large acrosome vesicle; and lateral arms into which the chromatin extends. These arms contain microtubules in 'oxyrhynchs' (Hinsch, 1973) and anomurans (Tudge, unpublished) but microtubules are reduced or absent at maturity in the arms of higher crabs though also shown for the portunid *Carcinus maenas* by Pochon-Masson (1968b). Significant differences of the *Ranina* sperm from those of the O-C-B, including *Portunus*, are: anterior termination of the subacrosomal space at the equator of the acrosome and its conical form (plesiomorphy or raninoid apomorphy?), in the latter assemblage reaching the operculum; differentiation within the subacrosomal material of a coiled, filiform putative perforatorium (plesiomorphy, or apomorphic homoplasy with Anaspidacea?) whereas the entire subacrosomal contents in the O-C-B form a stout perforatorial column; subdivision from the acrosome vesicle in *Ranina* of a posterior acrosomal chamber; and differentiation of the walls of this, lining the subacrosomal chamber, as

longitudinal corrugations (raninoid autapomorphies) (Jamieson, 1989b). A further supposed difference, plesiomorphic persistence in *Ranina* of numerous well developed, simple mitochondria in contrast to a stated degeneration, with greater development of a myelin-like lamellar complex, in the O-C-B can now be less certainly maintained as apparently intact mitochondria are demonstrated in the present work for *Macrophthalmus crassipes*. The posterior median process seen in the nucleus of *R. ranina* is also seen in *Pagurus* (Pochon-Masson, 1968a), suggesting that raninoids are plesiomorphic in this respect, and in majids (Hinsch, 1973). This possibly supports origin of majids from the base of the Eubranchyura advocated by Rice (1983). Sperm ultrastructure is consistent with the view that the Raninoidea are the plesiomorphic sister-group of the Oxyrhyncha-Cancridae-Brachyrhyncha assemblage or of the Heterotremata-Thoracotremata assemblage.

## MAJIDAE AND PARTHENOPIDAE

Majids and parthenopids constitute the Oxyrhyncha in the classification summarized by Warner (1977). Both are heterotremes in the classification of Guinot (1977, 1978). Some 10 species, in 6 genera of majids have been examined for sperm ultrastructure (Table 1), of which *Menaethius monoceros* is illustrated here (Figs 3G, 6C). The sperm of this species 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 *M. monoceros* the operculum is not only depressed centrally but is also perforate (Figs 3G, 6C). A further feature of majid sperm is the squat, pointed approximately rhombohedroidal shape of the perforatorial column. As a third feature, there is a posterior median extension of the nucleus, in addition to the nuclear arms, which is also present in *Ranina ranina*, 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 *Monaethius monoceros*. Strong development of microtubules in the arms, demonstrated by Hinsch (1973) is here regarded as a plesiomorphic condition further supporting a basal position for majids as microtubules are reduced or absent from 'higher' crabs. 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 (1988) 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) to regard parthenopids as highly evolved products of a lineage including portunids and geryonids. Guinot (1978) notes that the unity of majids is demonstrated by interruption of the sternal sutures (4/5-7/8). With condensation of the nervous system, she considers this to indicate that majids are advanced heterotremes. The posterior process, occurring also in the 'outgroup' Paguroidea, is here seen as a plesiomorphy retained paraphyletically in raninoids and majids to be apomorphically lost in higher crabs (Fig. 5). Therefore parsimony favours a more basal position of majids in the phylogeny (Fig. 5) from a purely spermatological viewpoint, as advocated by Rice (1981) from zoeal morphology.

As a symplesiomorphy, centrioles are present in majids, as *inter alia* in parthenopids, portunids, dorippids, and *Macrophthalmus* but not in, for instance, xanthids (Hinsch, 1973; present study).

As parthenopid sperm have not been examined in the present study it is not possible to adjudicate the position of this family spermatologically. *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 wide, thin, very 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 insufficient grounds for recognizing a particular relationship with majids.

#### CALAPPIDAE

Spermatozoal evidence is insufficient for placement of the calappids of which only *Calappa hepatica* has been examined (Fig. 6D). The general morphology of the acrosome is 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. Placement near the majids in the phylogram (Fig. 5) merely indicates, therefore, a 'nearest neighbour' in terms of general gestalt. Investigation of the sperm of additional calappids, with other families, may yet contribute to resolution of the phylogenetic position of this family. It is regarded from zoeal morphology as a fairly advanced family which may be near the ancestry of the Cancridae, Corystidae and Atelecyclidae (Rice, 1981).

#### CANCRIDAE

Cancrid sperm have not been investigated in the present work but that of *Cancer pagurus* 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) (Table 1). Some discussion of these is warranted as the Cancridea constitute one of the five major subdivisions of the Brachyura in the system summarized by Warner (1977). The Cancridae are placed with the Corystidae in a restricted superfamily Corystoidea by Guinot (1978) (Table 2).

In the mature sperm of *C. borealis* illustrated by Langreth (1965), the large, dense operculum is craterlike and centrally perforate but as the pointed tip of the perforatorium protrudes through it, perforation of the operculum may indicate that the acrosome reaction has commenced. This is supported by mention by Langreth of penetration of the 'cap' only at maturity. Otherwise the sperm is portunid-like, with, in the terminology of the present work, an inner dense zone differentiated externally as an acrosome ray zone and surrounded by the large, electron pale, outer zone. A conspicuous thickened ring is present in continuity with the thinner but distinctly developed, similarly electron dense capsule. DNA is present throughout the length of the rather short arms. No posterior median process is present. The shape of the perforatorial column, widest at its posterior forth and tapering almost straight to a pointed tip differs from the more bulbous form in portunids.

Although little can be stated with certainty as to similarity and relationships with other families, this sperm is at a similar morphological level to those of portunids and does not in itself

support recognition of a separate higher category for cancrids.

#### PORTUNIDAE AND DORIPPIDAE

The sperm of *Caphyra laevis* *C. rotundifrons* (Fig. 3J) and *Portunus pelagicus* (Fig. 7B) show the typical portunid ellipsoidal perforatorium. The sperm of *Carcinus maenas* has been described by Pochon-Masson (1968a). 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) is to be suspected. Each species mimics the colour of its host species.

Ultrastructural comparison between the sperm of the dorippid crab *Neodorippe astuta* (Fig. 7A) and the portunid *Portunus pelagicus* (Fig. 7B) has been shown by Jamieson and Tudge (1990) to support placement of dorippids with portunids and their relatives in the heterotreme section of the Eubranchyura and not, as in Table 1, with *Ranina ranina* (in the Archaeobranchyura or the Oxysomata). Characteristic eubranchyuran features of the *N. astuta* sperm (absent from *R. ranina*) are the long perforatorium (short and conical with a unique subacrosomal chamber in *R. ranina*) extending almost to the operculum; presence in the perforatorium of longitudinally arranged 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 (absent from *R. ranina* but also from some eubranchyurans). The sperm of *N. astuta* is more similar to those of portunids (*P. pelagicus*, *Caphyra laevis* and *C. rotundifrons*, present account; *Carcinus maenas*, Pochon-Masson, 1968a; and *Ovalipes ocellatus*, Hinsch, 1986) than to that of other investigated Brachyura. A smoothly rounded (bulbous) ellipsoidal perforatorial column (more slender in *C. maenas* and *Neodorippe*), well developed acrosome ray zone, and persistence of centrioles characterizes portunids and dorippids; general similarity of gestalt is apparent although difficult to quantify (Fig. 7A, B). Spermatologically dorippids and portunids thus appear to form a monophyletic group within the Heterotremata, though this does not in itself validate the Heterotremata. It has been shown above (see also Fig. 5) that the Heterotremata form a paraphyletic group unless their descendants (the Thoracotremata) are included as a subset.

#### GERYONIDAE

The sperm of *Geryon fenneri* and *G. quinquedens*, 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), though placed in the Xanthoidea by Guinot (1978).

#### LEUCOSIIDAE

Little can be said of the sperm of the leucosid *Iliacantha subglobosa* illustrated by Felgenhauer and Abele (1990) beyond the fact that it is a strongly triradiate sperm, with 3 well developed arms, a feature which appears plesiomorphic for heterotremes.

#### XANTHIDAE

Features of xanthid sperm, illustrated in Fig. 7C for *Pilodius areolatus*, which are seen in other higher Eubranchyura and in raninoids (albeit some of them symplesiomorphies include: the large subspheroidal acrosome (a similarity of the raninoid+eubranchyuran assemblage contrasting with the disc-shaped dromioid acrosome); enclosure of the acrosome by a thin layer of cytoplasm which is in turn cupped by the nucleus; extension of the nucleus as lateral arms; presence of cytoplasm (here vestigial) in the basal region of each nuclear arm; absence of the posterior median process (presence being a paguroid-raninoid-majid feature, loss of which is here seen as an apomorphy), and topographical equivalence and presumed homology of components of the acrosome, viz. the electron dense capsule; inner and outer dense zones, surrounding the longitudinal axis; peripheral vesicular contents; an apical operculum; subopercular- or subcap-zone; and basally open subacrosomal chamber enclosing perforatorial material. Eubranchyuran features of xanthids, not seen in raninoids, include: anterior termination of the subacrosomal space and enclosed perforatorium at the base of the operculum (contrasting with termination at the equator of the acrosome in raninoids); modification of the capsule around the base of the perforatorium as a thickened ring; absence of longitudinal corrugations lining the subacrosomal chamber (presence is a raninoid autapomorphy); and degeneration of all mitochondria (some apparently persisting in raninoids) (Jamieson, 1989b).

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

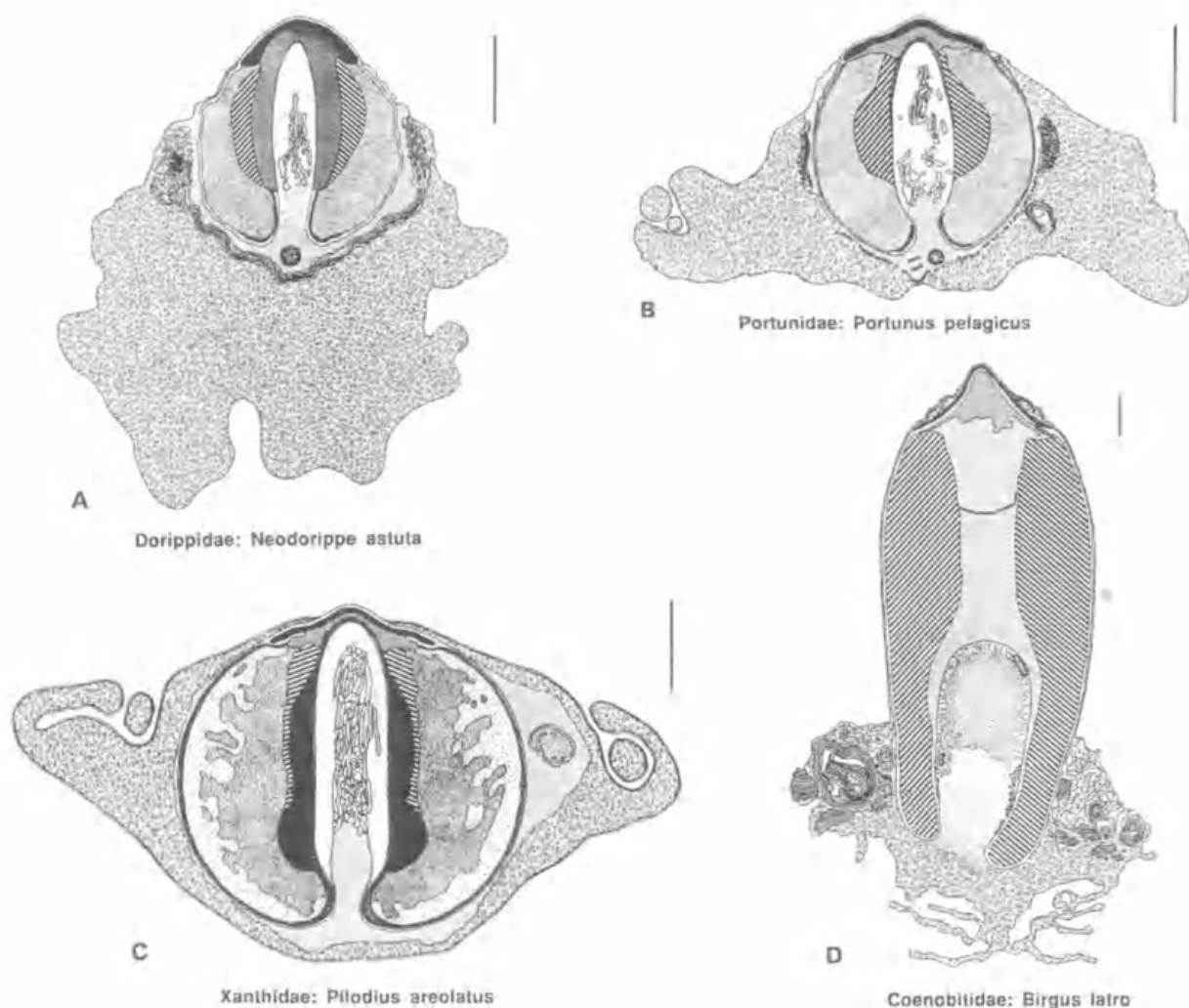


FIG. 7. A–C, Semi-diagrammatic longitudinal sections of spermatozoa from selected brachyuran families. Heterotremata. D, Semi-diagrammatic longitudinal section of coconut crab spermatozoon. Anomura. Traced from micrographs. Scale bars = 1 µm.

ring', shown for all four species, in separate xanthid genera, examined by Jamieson (1989a). What is here considered to be an elaboration of this ring, to form a funnel-like structure, is seen in the Thoracotremata and suggests origin of the latter 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). Christensen (1988) considered that the Xanthidae 'may lie at or near the stem of the higher eubrachyurans', thereby giving xanthids a higher position though, like Rice, recognizing their pivotal position in generation of further families. I have inclined to the higher position for the xanthids (Fig. 5) rather

than postulate that the xanthid ring has been lost in the portunid-dorippid branch.

#### THORACOTREMATA

Rice (1981) observes that migration of the female and male pores from the coxae to the sterna of segments 6 and 8 respectively, typifying the Thoracotremata, frees the ambulatory limbs from a reproductive function. The sperm of the Thoracotremata are here examined for two grapsids, *Grapsus albolineatus* (Fig. 8A) and *Sesarma erythrodactyla* (Fig. 8B); the mictyrid *Mictyris longicarpus* (Figs 3K, 8C); the ocypodids *Ocypoda ceratophthalma* (Fig. 8D) and *Uca dussumieri* (Fig. 8E); and the macrophthalmid *Macrophthalmus crassipes* (Fig. 8F). All of these sperm show general eubrachyuran ultra-



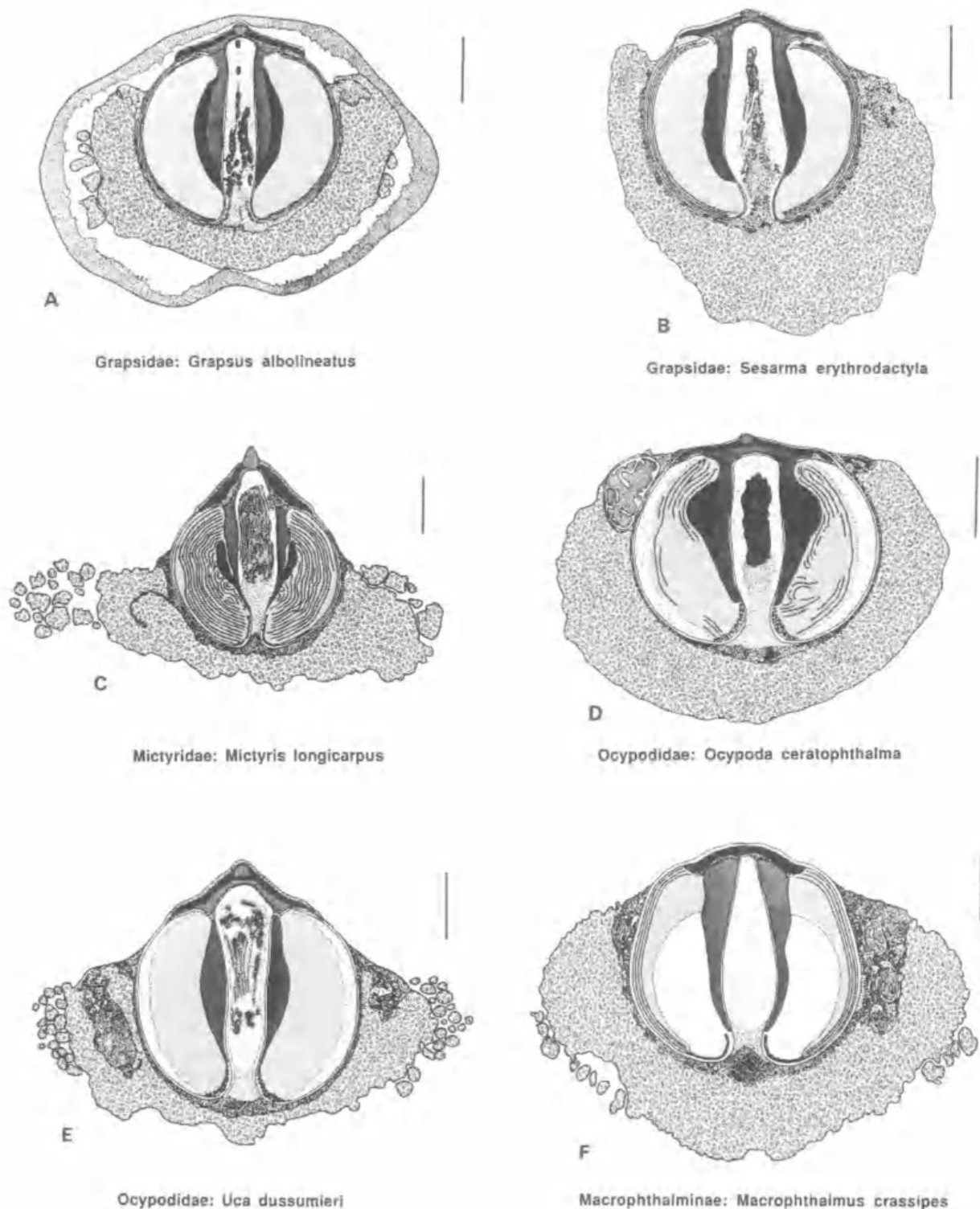


FIG. 8. A–F, Semi-diagrammatic longitudinal sections of spermatozoa from selected brachyuran families, Thoracotremata. Traced from micrographs. Scale bars = 1  $\mu$ m.

structure but, as noted above, thoracotremate synapomorphies are apparent.

The first of these synapomorphies, concentric lamellation of the outer acrosome zone is present

in five of these six species, though varying in development but apparently is absent in *Uca dussumieri*. It reaches its greatest development in *Mictyris longicarpus*. This lamellation is fore-

shadowed in some heterotremes, being indicated by Brown (1966b) for the portunid *Callinectes sapidus*.

The electron dense operculum is interrupted apically by a well defined vesicle which may be ellipsoidal or (*M. longicarpus*) pointed, which I have termed the apical button. This is not seen in *Macrophthalmus* which appears distinctive in other respects.

The differentiation of the acrosome contents which appears to be an extension of the basal ring ('xanthid ring' of Jamieson, 1989a) is present in at least the grapsids, the mictyrid and *Ocypoda*, its occurrence and homology being uncertain in *Uca* and *Macrophthalmus*.

Both grapsids, *Grapsus albalineus* and *Sesarma erythrodactyla*, are clearly apomorphic in absence of the so-called thickened ring which surrounds the base of the perforatorial column in all examined families, from majids to mictyrids in the phylogeny (Fig. 5). In grapsids the capsule of the acrosome, of which the thickened ring is a specialization in other taxa, is nevertheless intact.

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

Distinctive features of the sperm of *Macrophthalmus* 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 species the apex of the column is broad (though as always much narrower than the length of the column) and is flattened or gently convex. In the phylogram (Fig. 5) *Macrophthalmus* has been placed below the ocypodids, mictyrid and grapsids as this is 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 (Fig. 5)

takes into account loss of the thickened ring which is present from majids to ocypodids.

The sperm of the Pinnotheridae (*Pinnixia* sp.) and Geryonidae (*Geryon fenneri* and *G. quinquedens*, considered heterotremes, above) are known only from the literature (Reger, 1970b; Hinsch, 1988) and differences in fixation and staining protocols and mode of illustration relative to those employed in the present study make it difficult to draw comparisons with thoracotreme sperm described here. Generally their structure 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 button appears definitely to be absent in spermathecal sperm of *Geryon* while an apical interruption of the opercular density in that of *Pinnixia*, also from the spermatheca, possibly corresponds with a button. In *Pinnixia* a poorly defined zone external to the innermost dense zone may be equivalent to the extended xanthid ring typical of thoracotremes.

Thus although the Heterotremata *sensu stricto* 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 here, at least, form a monophyletic group.

## CONCLUSIONS

Occurrence in *Speleonectes* of a flagellate spermatozoon approaching in structure the invertebrate 'primitive sperm' (aquasperm) is consistent with the supposedly primitive status of the Remipedia but does not rule out an alternative placement with the ascothoracican through cirripedian section, also with flagellated sperm, of the Maxillopoda.

Cephalocarid (*Hutchinsoniella*) sperm resembling those of remipedes but lacking the flagellum may represent the ground plan for the Phyllopoda, hitherto thought to be the simple, amoeba-like sperm seen in euphyllopods and conchostracans. The *Nebalia* sperm, lacking an acrosome and with microtubular arms, supports the phyllopod status of phyllocarids. Nevertheless, the possibility exists 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. If so, one of the objections to relating phyllocarids to Malacostraca would be lost.

Copepod sperm show no clear affinities with

other groups, though the stellate acrosome-less sperm of the cyclopoid *Chondracanthus* resembles that of some branchiopods. Ostracod sperm include a filiform type performing undulatory waves by means of wing-like structures originating from the endoplasmic reticulum.

In the Malacostraca, stomatopod (*Squilla*, *Oratosquilla*, *Gonodactylus*) sperm are ovoidal, lacking appendages, with acrosome and a perforatorium; absence of a nuclear membrane, and diffuse chromatin are decapod tendencies; unusual, doublet centrioles are a peracarid-decapod feature. The syncarid (*Anaspides tasmaniae*) sperm has a subacrosomal filament [perforatorium], exceptional for Crustacea in being coiled. A syncarid apomorphy is the cytoplasmic 'skirt', a plesiomorphy the condensed chromatin and persistent nuclear membrane. Peracarid monophyly is confirmed by presence, with the questionable exception of tanaids, of a cross striated pseudoflagellum (possibly a centriolar rootlet homologue) joining the mainbody at junction of acrosome and nucleus. Tanaid sperm, rounded, lacking appendages, with large acrosome and scattered mitochondria, seen also in syncarids and stomatopods, possibly indicate a basal rather than terminal or intercalated position of the tanaids in the Peracarida.

Euphausiid sperm, ovoidal and lacking appendages, are insufficiently known to contribute to determination of the eucarid ground plan. Pending confirmation, they and the stenopodideans appear unique in the Malacostraca (phyllocarids excluded) in lacking the acrosome. Dendrobranchiate (penaeid) and procaridean and caridean shrimps and prawns have sperm with a single acrosomal spike but rarely have arms analogous with those characteristic of decapods. Spermatologically, the unistellate condition affords some support for the concept of the Natantia. It is difficult to envisage the spike as a symplesiomorphy of a paraphyletic Natantia which was replaced by the replantian acrosome. However, paraphyly of the Natantia is indicated in parsimony analysis of 18S rRNA sequences by Kim and Abele (1990).

Several spikes containing microtubules which traverse and often contain chromatin are characteristic of Palinura (*Panulirus*, *Jasus*); Astacidea (Astacidae, Nephropidae); Thalassinidea; Anomura (Paguridae, Diogenidae, Coenobitidae); and Brachyura, though microtubules are reduced or absent above the 'oxyrhynch'. The acrosome of Eubrachyura resembles that of paguroids, and especially in its subspheroidal

shape *Pagurus* and *Clibanarius*, suggesting a paguroid-brachyuran (sister-group?) relationship while the thalassinid (*Callinassa*) acrosome differs greatly from that of the Astacidea-Anomura-Brachyura assemblage, contra-indicating a thalassinid origin of the Brachyura.

The discoidal acrosome and reduced arms of dromiid (*Dromidia*, *Petalomera*) sperm may be plesiomorphic conditions of a group with no close relationship to other brachyurans. Phylogenetic heterogeneity of the Podotremata is supported by differences between dromiid and raninoid sperm and similarities (postnuclear tail) between *Rahina* and majids. The conventional oxytomete-oxyrhynch-cancerid-brachyrhynch subdivision of the Brachyura is not supported by sperm ultrastructure. Dorippids and portunids, with similar sperm, are placeable in the Heterotremata, whereas the former classification separates the two families in the Oxytomete and Brachyrhyncha, respectively, but the Heterotremata *sensu* Guinot is a paraphyletic assemblage, representing a grade typified by migration of the female pores onto the sternum, unless it is enlarged to include the thoracotremes. In contrast examined Thoracotremata (Mictyroidea, Grapsoidea and Ocypodoidea) appear to form a monophyletic group typified by presence of an apical opercular button, concentric lamination of the outer acrosome zone and modification of the xanthid ring, though none of these three characters is sufficiently constant to allow a monothetic definition of the group.

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