PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Phylogeny of the Brachyura with Particular Reference to the Podotremata: Evidence from a Review of Spermatozoal Ultrastructure (Crustacea, Decapoda)

B. G. M. Jamieson

Phil. Trans. R. Soc. Lond. B 1994 **345**, 373-393
doi: 10.1098/rstb.1994.0116

Phylogeny of the Brachyura with particular reference to the Podotremata: evidence from a review of spermatozoal ultrastructure (Crustacea, Decapoda)

B. G. M. JAMIESON

Zoology Department, University of Queensland, Brisbane 4072, Australia

SUMMARY

Parsimony analysis, whether using only spermatozoal characters or spermatozoal and non-spermatozoal characters, suggests that: (i) the Brachyura is a monophyletic taxon relative to the outgroup, three paguroids; (ii) the Podotremata is a monophyletic taxon and the sister-group of the heterotreme–thoracotreme assemblage; (iii) within the Podotremata, the Dromiidae appears paraphyletic as its clade includes *Paradynomene*; (iv) the Homolidae is a monophyletic family; (v) the Raninoidea and Cyclodorippoidea are sister groups within a monophyletic (unnamed) clade; (vi) *Latreillia* sp. forms a polytomy with Homolidae + Raninoidea–Cyclodorippoidea; and (vii) within the heterotreme–thoracotreme assemblage, the Thoracotremata is a monophyletic taxon but the Heterotremata *s. strict.* is a paraphyletic grouping. Analyses based on sperm data alone differ from the combined data in not including the Homolidae in the Archaeobrachyura (Homolidae + Raninoidea–Cyclodorippoidea), thus the Dromiidae–*Paradynomene* + Homolidae + *Latreillia* form a polytomous clade which is the sister-group of the Raninoidea + Cyclodorippoidea. Spermatozoal data also give majids the most basal position in the Heterotremata whereas for the combined data *Neodorippe* (a shell carrier) appears the least modified member of the heterotreme–thoracotreme assemblage. These findings are viewed in the perspective of other, notably molecular, studies. Spermatozoal synapomorphies are listed and illustrated.

1. INTRODUCTION

The use of spermatozoal ultrastructure for taxonomy and phylogeny is now widely accepted as it has proved effective in resolving hitherto intractable problems of taxonomic placement and relationship. A striking endorsement of its validity has been the recent ratification from molecular biology by Abele *et al.* (1989) of placement of the Pentastomida in the Crustacea from consideration of spermatozoal ultrastructure by Wingstrand (1972), confirmed by Storch & Jamieson (1992). There are many other examples of groups in which analysis of sperm ultrastructure has produced significant advances in phylogenetic reconstruction, for which only a few references can be mentioned here: anthozoan coelenterates (Schmidt & Zissler 1979); Platyhelminthes (Justine, many papers, e.g. 1991) and with the erection of a higher taxon, the Trepaxonemata, on sperm ultrastructure (Ehlers 1985); Nematoda (Baccetti *et al.* 1973); Polychaeta (Jamieson & Rouse 1989); Oligochaeta (Jamieson 1981, 1983; Jamieson *et al.* 1987); Clitellata (Ferraguti 1983); Mollusca (Healy, in many papers, e.g. 1988); insects and other Hexapoda (references in Jamieson 1987); Myriapoda (Baccetti 1978); Crustacea (Pochon-Masson *et al.* 1970; Jamieson 1991a); Chelicerata (Alberti 1990); Urochordata (Holland 1989); Echinodermata, protochordates and fish, including the erection of a new order, the Esociformes, (Jamieson 1991*b*); fish (Mattei 1991); Amphibia (Pugin-Rios 1980; Jamieson *et al.* 1992; Lee & Jamieson 1992); Amniota (Healy & Jamieson 1992; Jamieson & Healy 1992); Reptilia (Furieri 1970; Jamieson & Scheltinga 1993); Aves (Asa & Phillips 1987; Baccetti *et al.* 1991); Marsupalia (Temple-Smith 1987); Eutheria (Rouse & Robson 1986; Breed 1991); and many phyla (Franzén 1970; Afzelius 1979; Wirth 1991).

The principles of Hennigian phylogenetic systematics are discussed in many works (e.g. Ax 1984) and the application of computer procedures for phylogenetic analysis under the principle of parsimony is very clearly enunciated by Swofford (1993).

The internal relationships and classification of brachyuran crabs, and particularly of the Podotremata, are the subject of controversy. Guinot (1977, 1978, 1979, 1991; Guinot *et al.* 1994) divides the Brachyura into three sections mainly on the basis of the location of the male and female pores: the Podotremata, the Heterotremata and the Thoracotremata. Guinot (1978, p. 218) recognized that the coxal positions of male and female pores, with external fertilization, characterizing the podotremes, were symplesiomorphies.

Phil. Trans. R. Soc. Lond. B (1994) **345,** 373–393
Printed in Great Britain

The Podotremata *sensu* Guinot contain the Dromiacea and Archaeobrachyura. The Dromiacea consist of the Dromioidea and Homolodromioidea. The Archaeobrachyura contain the Homoloidea, Raninoidea, and Cyclodorippoidea = Tymoloidea (Guinot 1978, 1979). In other classifications the superfamily Homoloidea de Haan, 1839, which includes three families (Homolidae de Haan, 1839; Latreilliidae Stimpson, 1859; Poupiniidae Guinot 1991) are associated with or placed in the Dromiacea.

The Heterotremata and Thoracotremata share a sternal location of the female pores and development of a sternal vulva on sternite 6, in direct communication with the seminal receptacle, allowing for internal fertilization. The Thoracotremata differ in the constant sternal location of the male pores. While the Thoracotremata appeared to be a monophyletic group, the Heterotremata were suspected of being paraphyletic (Jamieson 1991*a*).

On the basis of larval morphology, some workers have excluded the Dromioidea from the Brachyura whereas Homoloidea and Raninidae are retained as early or pre-Brachyura (Williamson 1965, 1974; Rice 1970, 1980, 1981*a*,*b*, 1983; Rice & Provenzano 1970). Some features of the larvae of the Dromiidae are distinctly like those of anomurans, notably a shrimp-like shape, persistent uropods and functional third maxillipeds (Warner 1977; Williamson 1974). It was concluded (Rice 1981*a*,*b*, 1983) that the Dromioidea were close to the Anomura, that homolids arose near the base of the higher Brachyura but that apomorphic characters shared by the zoeae of raninids and higher brachyura, but not by homolids, indicate that homolids became separated from a primitive brachyuran line at an earlier stage than the raninids. The Dromiacea have been attributed an origin more primitive than most Anomura (*sensu strictu*), specifically from or near the Thalassinidea (Burkenroad 1963; Gurney 1942; Pike & Williamson 1960; Rice 1980, 1983; Williamson 1965, 1974; see also discussions in Stevcic 1971; Guinot 1979). However, other workers retained the Dromiacea (*sensu lato*) in the Brachyura, e.g. Balss (1957), Glaessner (1969; who nevertheless argued that they arose from within the Glypheoidea, a macruran group related to spiny lobsters, the Palinura), and Wright & Collins (1972). Recently, Williamson (1988, 1992) attempted to explain the dromiacean paradox by invoking horizontal gene transfer, giving anomuran larvae but brachyuran adults.

Nucleotide sequences of 18*S* ribosomal RNA support the exclusion of a mono- or poly-phyletic Dromiidae from the Brachyura, and their association with the Anomura, but inclusion of the Raninidae in the Brachyura (Spears & Abele 1988; Abele 1991; Spears *et al.* 1992); homolids were not considered.

The present study investigates monophyly versus paraphyly of the Podotremata (together with the contained Dromiacea and Archaeobrachyura, and the Heterotremata and Thoracotremata) using parsimony analysis. The characters employed are (i) spermatozoal only or (ii) spermatozoal and non-spermatozoal.

2. MATERIALS AND METHODS

(*a*) *Taxa included*

The taxa used as representatives of their various families and higher taxa, together with sources of data, are as follows.

(i) *Anomura, Paguroidea*

Paguridae: *Pagurus bernhardus* (C. C. Tudge, personal communication). Diogenidae: *Clibanarius taeniatus* (Tudge 1992). Coenobitidae: *Coenobita spinosa* (Tudge 1992).

(ii) *Podotremata*

Dromiidae: *Stimdromia* (= *Petalomera*) *lateralis* (Jamieson 1990; Guinot *et al.* 1994); *Dromidiopsis edwardsi* (Jamieson *et al.* 1994*e*). Dynomenidae: *Paradynomene tuberculata* (Jamieson *et al.* 1994*a*). Homolidae: *Paromola petterdi*, *Homola* sp. (Guinot *et al.* 1994); *Latreillopsis gracilipes* (Jamieson *et al.* 1994*d*). Latreilliidae: *Latreillia* sp. (B. G. M. Jamieson, D. Guinot & B. Richer de Forges, unpublished results). Cyclodorippidae: *Xeinostoma richeri* (Xeinostominae) and *Tymolus* sp. (Cyclodorippinae) (Jamieson *et al.* 1994*b*). Cymonomidae: *Cymonomus* sp. (Jamieson *et al.* 1994*b*). Raninidae: *Ranina ranina* (Raninae) (Jamieson 1989*b*); *Raninoides* sp.(Raninoidinae); *Lyreidus brevifrons* (Lyreidinae) (Jamieson *et al.* 1994*c*).

(iii) *Heterotremata*

Dorippidae: *Neodorippe astuta* (Jamieson & Tudge 1990). Majidae: *Menaetheus monoceros* (Jamieson 1991*a*); other majids (Hinsch 1973). Portunidae: *Portunus pelagicus* (Jamieson 1989*b*; Jamieson & Tudge 1990). Trapeziidae: *Calocarcinus africanus* (Jamieson *et al.* 1993). Potamonidae: *Potamonautes perlatus* (Jamieson 1993*a*). Xanthidae: *Etisus laevimanus*; *Pilodius areolatus* (Jamieson 1989*a*).

(iv) *Thoracotremata*

Mictyridae: *Mictyris longicarpa* (Jamieson 1991*a*). Ocypodidae: *Ocypode ceratophthalma*; *Uca dussumieri*; *Macrophthalmus crassipes* (Jamieson 1991*a*).

(*b*) *Comparative review of characters for parsimony analysis*

A review of the ultrastructure of the spermatozoa of the podotreme families, and comparison with the ultrastructure of other brachyuran groups and with anomurans yields the following suite of characters for cladistic analysis performed under the principle of parsimony using the PAUP program, version 3.0s of Swofford (1993). References to sources of data are given above and will be repeated or augmented in this account only where clarity demands. A diagram of a generalized brachyuran sperm on which the spermatozoal characters (1–27) are enumerated is shown in figure 1.

(i) *Spermatozoal characters*

Proportions of the acrosome (*character 1*)

Striking differences in the proportions of the spermatozoon, particularly the ratio of length to

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

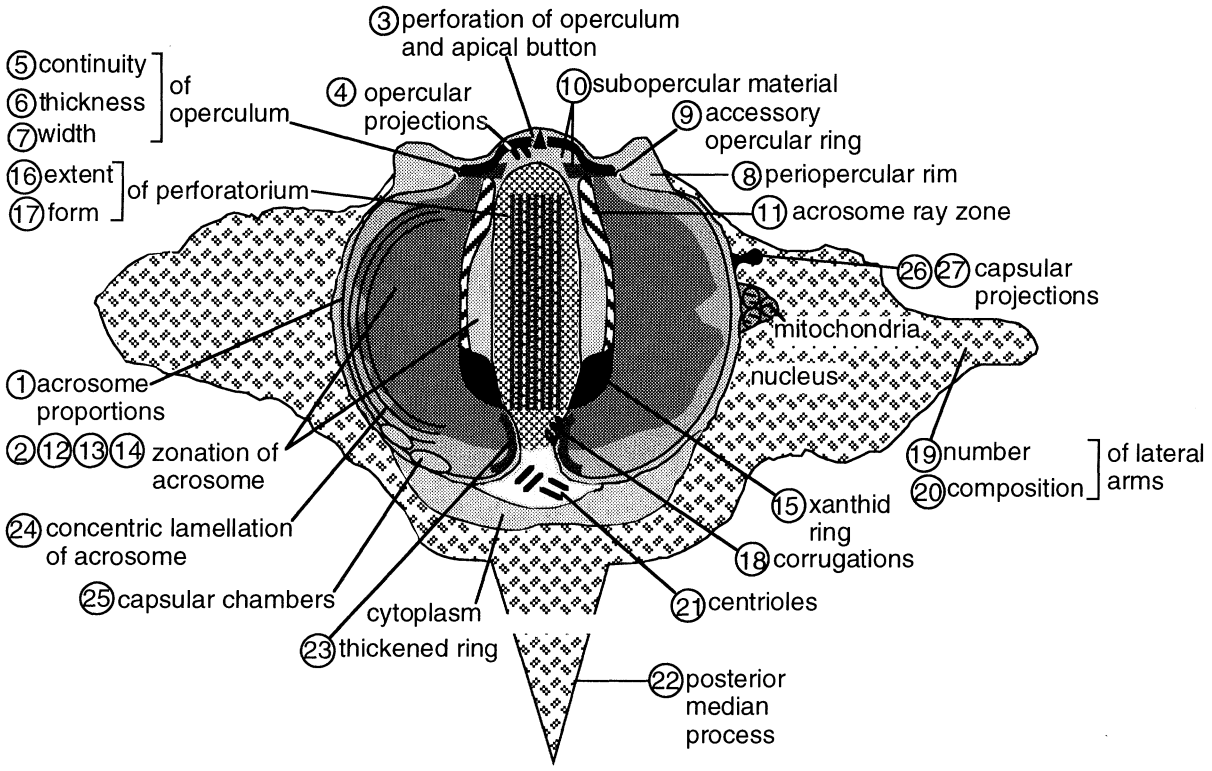


Figure 1. A composite diagram of a brachyuran spermatozoon showing the characters (numbered) used in the present analysis. No actual sperm would possess all of the character states shown.

width (L:W) of the acrosome, occur across the Brachyura. The acrosome is subspheroidal in the thoracotreme–heterotreme assemblage, with L:W ratios varying from 0.8–1.2. Though it is also subspheroidal (0.8) in *Ranina ranina* (figure 6*c*), it is more depressed in *Raninoides* sp. (figures 6*b* and 9*c*) (0.7) and is strongly depressed in the third raninoid investigated, *Lyreidus brevifrons* (figure 6*a*) (0.5). The acrosome reaches its strongest depression, in the investigated crabs, in the Dromiidae and the Dynomenidae, with a L:W ratio of 0.3 in *Stimdromia* (= *Petalomera*) *lateralis* (figure 5*a*), *Dromidiopsis edwardsi* (figures 5*b* and 9*a*) and *Paradynomene tuberculata* (figure 5*c*). The ratio is intermediate in homolids (0.4–0.6) and cyclodorippoids (0.5–0.6).

Zonation of the acrosome (*character 2*)

Zonation of the contents of the acrosome vesicle is predominantly horizontal in the dromiid *Stimdromia lateralis* (figure 5*a*), dynomenids, cyclodorippoids and homolids but is chiefly concentric around the perforatorium in the thoracotreme–heterotreme assemblage and in *Ranina ranina* (figure 6*c*). It is intermediate in *Dromidiopsis edwardsi* (figures 5*b* and 9*a*), *Latreillia* sp. (figures 5*e* and 9*e*), *Raninoides*, and in *Lyreidus brevifrons* (figure 6*a*). Horizontal zonation is probably partly at least correlated with depression of the acrosome and is not, therefore, a strictly independent character. Grouping of the various intermediate configurations in one character state is probably artificial but does not influence the topology of the consensus tree obtained as exclusion of this character has no effect on the overall topology.

Perforation of the operculum (*character 3*)

The operculum is an imperforate cap in paguroids, in most heterotremes (majids being one exception), in *Macrophthalmus* alone of the investigated thoracotremes (Jamieson 1991*a*) and, although apically very thin, in the cyclodorippoid *Cymonomus* sp. (figure 6*d*) (Jamieson *et al.* 1994*b*). It has a wide central perforation in dromiids (Jamieson 1994*e*), *Latreillia* (figures 5*e* and 9*e*), *Paradynomene* (figure 5*c*), homolids (figures 5*d* and 9*b*), the cyclodorippoids *Xeinostoma* sp. (figures 6*e* and 9*d*) and *Tymolus* sp. (figure 6*f*), raninoids (figures 6*a–c* and 9*c*), and majids (figure 7*a*). In most investigated thoracotremes a narrow orifice at its tip is plugged by an apical button although it is imperforate and lacks the button in *Macrophthalmus crassipes* (figure 8*b*).

Opercular projections (*character 4*)

Only the homolids have radial, inward, fingerlike projections of the operculum into the anterior region of the perforatorium (figures 5*d* and 9*b*). Irrespective of the results of parsimony analysis this is a clear autapomorphy for the Homolidae.

Opercular continuity with the capsule (*character 5*)

Only in the three examined raninoids (figures 6*a–c* and 9*c*) is the operculum continuous with or very poorly demarcated from the acrosome capsule.

Thickness of the operculum (*character 6*)

The operculum is particularly thin, and of similar form, in the cyclodorippoids *Xeinostoma richeri* (figures 5*e* and 9*d*) and *Tymolus* sp. (figure 6*f*). It is thicker in all other crabs or at least not as thin as in these two species.

Width of the operculum (*character 7*)

The operculum extends laterally for almost the whole width of the acrosome in the three cyclodorippoids (figures 6*d–f* and 9*d*) but is less extensive in other brachyurans.

Peripercular rim (*character 8*)

The peripercular rim is an electron pale rim encircling the periphery of the operculum and its underlying subopercular zone first seen in the sperm of *Potamonautes perlatus* (figures 7*d* and 9*f*) but since demonstrated in the xanthoids *Calocarcinus africanus* (figure 7*f*) and, although weakly developed, *Etisus laevimanus*. This rim is an expansion of the hyaline layer, usually considered to be the acrosomal membrane, which overlies the capsule. It has recently been demonstrated in hydrothermal vent bythograeids (Jamieson *et al.* 1995) but is unknown in other crabs.

Accessory opercular ring (*character 9*)

A sharply distinguished plaque, as seen in longitudinal section of the spermatozoon of *Calocarcinus africanus* (figure 7*f*), represents a ring (accessory ring) latero-posterior to the subopercular zone. The accessory ring also occurs in xanthids, e.g. *Etisus laevimanus*, *Pilodius areolatus* (figure 7*e*) (Jamieson *et al.* 1993) and in hydrothermal vent bythograeids (B. G. M. Jamieson, C. C. Tudge, D. Guinot & X. Segonzac, unpublished results).

Apical protuberance of subopercular material (*character 10*)

Apical protuberance of subopercular material through the perforation in the operculum is conspicuous in dromiids (figure 5*a*,*b*) and *Paradynomene* (figure 5*c*), is weakly developed in homolids (figures 5*d* and 9*b*), and is absent from other crabs though weak facultative protuberance occurs in *Tymolus* sp. (figure 6*f*).

Acrosome ray zone (*character 11*)

Acrosomal rays occur in heterotremes, e.g. xanthids (figure 7*e*) and portunids (figure 7*c*) (Jamieson 1989*b*; Jamieson & Tudge 1990). Similar rays are, however, visible in published micrographs of the sperm of the astacids, *Pacifastacus leniusculus* (Dudenhausen & Talbot 1979) and *Cambarus* sp. (Anderson & Ellis 1967); and are well known in the sperm of hermit crabs (e.g. Hinsch 1980; Tudge 1992; Tudge & Jamieson 1991), here being seen in *Coenobita*, although not in *Pagurus* or *Clibanarius*. The acrosome ray zone is very clearly developed in the coenobitid paguroid *Birgus latro*, in which it is a conspicuous region of large radiating tubules surrounding the central core of the acrosome (Tudge & Jamieson 1991). Acrosome rays are therefore possibly plesiomorphic for reptantians. Within the Brachyura, only the heterotremes show an acrosome ray zone comparable in location and structure with that of anomurans. The heterotreme zone is therefore distinguished here as a true acrosome ray zone. It is absent, it is deduced by loss, in thoracotremes. *Paradynomene tuberculata* (figure 5*c*) has an acrosomal zone the contents of which have the appearance of parallel dense lines separated by pale lines (tubules?), giving in longitudinal sagittal section a tortuous honey comb like or finger print like appearance. This was tentatively identified with the acrosome ray zone of other brachyuran sperm (Jamieson *et al.* 1994*a*). In the cyclodorippoid *Xeinostoma richeri* (figures 6*e* and 9*d*), a triangular zone, described for that species above, is questionably equivalent in structure to the acrosome ray zone of other brachyurans. Neither of these two examples is scored in the present parsimony analysis as a true acrosome zone. In *Cymonomus* sp. (figure 6*d*) and *Tymolus* sp. (figure 6*f*), no acrosomal ray structure is discernible. Absence of an acrosome ray zone is considered an homolid apomorphy probably homoplasic with absence in raninids (Jamieson *et al.* 1994*d*).

Outer acrosome zone (*character 12*)

The junction of an outer acrosome zone and a more lateral peripheral acrosome zone in the Xanthidae *s. lat.* (figure 7*e*) (Jamieson 1989*a*, 1991; Jamieson *et al.* 1993) and in *Calocarcinus africanus* (figure 7*f*) (Jamieson *et al.* 1993) is very irregular, having a ragged appearance. This state has also been observed in bythograeids (B. G. M. Jamieson, C. C. Tudge, D. Guinot & X. Segonzac, unpublished results) but is unknown in other crabs.

Anterolateral pale zone of the acrosome (*character 13*)

An anterolateral electron pale zone of the acrosome is characteristic of the two examined dromiids, *Stimdromia lateralis* (figure 5*a*) and *Dromidiopsis edwardsi* (figures 5*b* and 9*a*), and of the dynomenid *Paradynomene tuberculata* (figure 5*c*). It is absent, at least in this form, from other crabs.

Lateral flange-like extension of lower dense zone (*character 14*)

There is a lateral, discontinuous flange-like extension of the lower dense zone of the acrosome in *Paradynomene tuberculata* (figure 5*c*) and *Cymonomus* sp. (figure 6*d*).

Xanthid ring (*character 15*)

A notable autapomorphy of the Xanthidae (figure 7*e*) and Panopaeidae is differentiation of the posterior region of the inner dense zone surrounding the perforatorium as a prominent strongly electron dense ring, the 'xanthid ring' (Jamieson 1989*a*, 1993*a*,*b*). A structure which was deduced to be a slightly modified form of this occurs in *Calocarcinus* (figure 7*f*) (Jamieson *et al.* 1993). A differentiation of the acrosome contents which was considered to be an extension of the basal xanthid ring (see, however, apparent homoplasic development in the parsimony analysis, below) is present in at least the grapsids, in *Mictyris longicarpus* (figure 8*d*), and the ocypodid *Ocypode ceratophthalma* (figure 8*c*), its homology being uncertain in the ocypodids *Uca* (figure 8*a*) and *Macrophthalmus* (figure 8*b*) (Jamieson 1991*a*). No other brachyuran families are known to possess the xanthid ring.

Extent of the perforatorium (*character 16*)

In all investigated brachyuran sperm, excepting

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

those of *Ranina ranina* (figure 6*c*), the perforatorium extends from the posterior pole of the acrosome to shortly below the anteriorly situated operculum, a condition described as pre-equatorial for this character. In *Ranina ranina* (figure 6*c*), as in the paguroid *Coenobita spinosa*, the anterior limit of the conical perforatorial chamber is shortly postequatorial.

Form of the anterior end of the perforatorium (*character 17*)

In the thoracotreme–heterotreme assemblage (figures 7 and 8) and in cyclodorippoids (figure 5*e–f*), the anterior end of the perforatorium is rounded or pointed. The perforatorial chamber in *Ranina* (figure 6*c*), in which identification of perforatorial material is in question, is pointed. In contrast, the anterior end or head of the perforatorium in dromiids (figures 5*a*,*b* and 9*a*), homolids (figures 5*d* and 9*b*) and *Paradynomene* (figure 5*c*) is capitate. It is also classifiable as capitate in *Lyreidus brevifrons* (figure 6*a*) in which the expanded head has an 'amoeboid' outline. In the dromiids *Stimdromia lateralis* (figure 5*a*) and *Dromidiopsis edwardsi* (figures 5*b* and 9*a*) the capitate head is bilaterally symmetrical, being longer in one axis than that at right angles. In all eight examined homolids (see *Paromola petterdi* and *Latreillopsis gracilipes* (figures 5*d* and 9*b*)) the head of the perforatorium has long radial projections, numbering approximately 12. *L. gracilipes* (figures 5*d* and 9*b*) is distinctive in the great length of the rays which extend almost to the base of the acrosome.

Complications of the inner wall of the perforatorial chamber (*character 18*)

The spermatozoa of the cyclodorippoids *Xeinostoma richeri* (figures 6*e* and 9*d*), *Tymolus* sp. (figure 6*f*), and *Cymonomus* sp. (figure 6*d*) have slender dense filaments extending into the perforatorium from its walls, their bases associated with corrugations of its basal wall. In *X. richeri* (figures 6*e* and 9*d*) and *Tymolus* (figure 6*f*) the corrugations are regular semicircular to nearly circular evaginations of the acrosome membrane which lines the perforatorial chamber. In raninoids the wall of the perforatorium has corrugations which differ from those of cyclodorippoids in being invaginations. These are simple in *Lyreidus brevifrons* (figure 6*a*) but are simple to trifoliate in *Ranina ranina* (figure 6*c*) and *Raninoides* (figures 6*b* and 9*c*). In the remaining podotreme crabs and in the thoracotreme–heterotreme assemblage the wall of the perforatorial chamber is simple. In paguroids, *Pagurus bernhardus* has simple evaginations while *Clibanarius taeniatus* (figure 5*f*) and *Coenobita* have only filaments.

Lateral arms (*characters 19*)

The plesiomorphic number of lateral arms in brachyuran spermatozoa appears to be three, a number which also occurs in the majority of investigated Anomura including the three taxa used as the outgroup in the parsimony analysis. This number is seen in the Homolidae, Cyclodorippoidea, Raninoidea, and in *Dromidiopsis edwardsi* but is not constant for dromiids, as arms are apparently absent in *Stimdromia lateralis*. They are also absent in *Paradynomene tuberculata*. The number in *Latreillia* has not been determined. Several lateral arms are present in members of the thoracotreme–heterotreme assemblage, including those studied here, but three are illustrated by scanning electron microscopy for the leucosiid *Iliacantha subglobosa* by Felgenhauer & Abele (1991). The number in the majid investigated, *Menaethius monoceros*, is not known with certainty but Hinsch (1969) reports three nuclear arms in *Libinia emarginata*.

Composition of the lateral arms (*character 20*)

The composition of the lateral arms, where present, varies significantly in the anomuran–brachyuran assemblage. The arms are purely microtubular in *Coenobita*. They contain microtubules and some chromatin in *Pagurus* and *Clibanarius taeniatus* (figure 5*f*). Both types seem to occur in majids. They contain only chromatin, with no microtubules, in other investigated crabs.

Centrioles (*character 21*)

Centrioles have been demonstrated in the sperm of homolids (though yet to be seen in *Homola* sp.), cyclodorippoids (*Xeinostoma* and *Tymolus*), some heterotremes, e.g. most investigated majids, *Calocarcinus*, *Neodorippe*, *Portunus* and *Potamonautes*. In *Potamonautes* they are elongated. They appear to be absent from raninoids, some majids, xanthoids, and thoracotremes. Centrioles have not been seen in *Latreillia*, *Paradynomene* and *Cymonomus* but absence from these requires confirmation. In paguroid sperm, centrioles have been observed in *Pagurus bernhardus* but not in *Clibanarius taeniatus* or *Coenobita*. Uncertainty as to the occurrence of centrioles in some brachyuran sperm led to exclusion of this character from the parsimony analyses. Inclusion did not, however, alter the topology of the consensus tree shown in figure 2*b*.

Posterior median process of the nucleus (*character 22*)

The nucleus extends posteriorly as a long median process in *Pagurus*, but not the other two paguroids, in *Latreillia*, homolids, all three raninoids and cyclodorippoids and has been observed by Hinsch (1969) in majids. The process is absent in dromiids, *Paradynomene*, in non-majid heterotremes and all thoracotremes.

Thickened ring (*character 23*)

A short, curved lamina which is adpressed to the inner surface of the acrosomal capsule where the latter invaginates to form the subacrosomal chamber is termed the thickened ring. It is present only in heterotremes and thoracotremes (figures 7 and 8), although it is reduced in *Potamonautes* (figures 7*d* and 9*f*), and is lost in grapsid thoracotremes (Jamieson 1991*a*, 1993*a*).

Concentric lamellation of the acrosome (*character 24*)

Concentric lamellation of the outer acrosome zone is characteristic of thoracotremes, although varying in development, but is apparently absent in *Uca dussumieri* (figure 8*a*). It is not seen in other brachyuran sperm nor in anomurans (Jamieson 1991*a*).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Capsular chambers (*character 25*)

In *Ranina ranina* (figure 6*c*), the capsule of the acrosome divides posteriorly to isolate a posterior chamber of the acrosome vesicle. The multiple enclaves in the capsule, of which the largest is posterior, in *Raninoides* (figures 6*b*, 9*c*) were considered homologous, and synapomorphic, with the posterior chamber of *Ranina* by Jamieson *et al.* (1994*c*) and it was suggested that the absence of chambers in *Lyreidus* (figures 6*a*) may well be plesiomorphic. They are absent from the sperm of all non-raninoid crabs.

Capsular projections (*character 26 and 27*)

In *Raninoides* (figures 6*b* and 9*c*) and *Ranina* (figure 6*c*) the capsule and the overlying membrane sends an extension on each side into the thin layer of investing cytoplasm and may be termed the capsular flange (character 27). In *Lyreidus brevifrons* (figure 6*a*) approximately ten low, rounded projections are visible on each side in longitudinal section of the sperm and appear to extend all round the margin of the acrosome, possibly as spiral ridges (character 26). At the base of the acrosome in this species they are continuous with longitudinal corrugations which line the subacrosomal chamber.

External projections of the acrosome are also present in cyclodorippoids. In *Xeinostoma richeri* (figures 6*e* and 9*d*) the lower layer of the operculum extends into a rounded evagination (representing a circum-acrosomal flange) of the acrosome membrane around which the upper limit of the cytoplasm extends and below which the upper limit of the nucleus abuts. This structure is not included in the parsimony analysis as homology with that flange in raninoids is very doubtful and it is otherwise known only for *X. richeri* (figures 6*e* and 9*d*). In *X. richeri*, in addition to this large evagination of the acrosome membrane, there are several smaller, somewhat irregular evaginations from its vicinity to the posterior limit of the acrosome which are included under character 21. No periacrosomal flange occurs in the other two investigated cyclodorippoids, *Tymolus* sp. (figure 6*f*) and *Cymonomus* sp. (figure 6*d*), but some small evaginations are present. The only other crab in which capsular projections are known is *Stimdromia lateralis* (figure 5*a*).

(ii) *Non-spermatozoal characters* (Guinot, personal communication)

Sternal location of genital pores (*character 28*)

The evolutionary system of classification of the Brachuyra developed by Guinot (1977, 1978) is based on two apomorphies: location of female pores on the sternum of segment 6; and location of the male pores on the sternum of segment 8; these contrast with a plesiomorphic location, as in anomurans and other non-brachyuran reptantians, 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, although 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. The purely coxal location has been coded as ancestral in the present analysis and location of only the female pores on the sternum as state 1 in relation to location of male and female pores on sterna, coded as 2 in an ordered character.

Presence of spermathecae (*character 29*)

Spermathecae, formed by contributions from two somites, are present in podotremes, where they are associated with external fertilization of the eggs. They are absent in this form from anomurans, heterotremes and thoracotremes.

Reduction of pereiopod 5 (*character 30*)

P5 is reduced in both length and width in paguroids, podotremes (excepting Raninidae and poupinids) and in *Neodorippe* but not in other heterotreme and thoracotreme families.

P5 dorsal or subdorsal (*character 31*)

P5 is approximately in the same plane as the other pereiopods in paguroids, most heterotremes and thoracotremes but its origin is more dorsal in *Neodorippe* and in podotremes, excepting Raninidae.

Subchelate or chelate modification of P5 (*character 32*)

The propodus and dactylus of P5 form a subchela or a chela in paguroids, podotremes and *Neodorippe* but not in other heterotremes or thoracotremes. Podotreme exceptions are the Raninoidea and Poupinidae and some latreilliids which have a simple dactylus and no corresponding modification of the propodus. Modification is weak in *Paradynomene tuberculata*, *Xeinostoma* sp. and *Cymonomus* sp.

Sella turcica (*character 33*)

The sella turcica is present in all brachyurans. It is not seen in anomurans.

Presence and degree of development of uropods (*character 34*)

Uropods are present in paguroids, but are vestigial in dromiids and *Paradynomene*. They are absent from the remaining crabs.

(*c*) *Character coding employed*

(i) *Spermatozoal characters*

(1) Acrosome length:width: 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0.

(2) Zonation of the contents of the acrosome vesicle

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

predominantly: 0, horizontal; 1, concentric; 2, intermediate.
(3) Operculum: 0, imperforate; 1, perforate, open; 2, perforate, closed with apical button.
(4) 0, Opercular projections into subopercular material absent; 1, present.
(5) Operculum: 0, discontinuous with capsule; 1, continuous with capsule.
(6) Operculum: 0, moderately thick; 1, very thin double lamina.
(7) Operculum width: 0, not extremely wide; 1, extremely wide 1.
(8) Periopercular rim: 0, absent; 1, weak; 2, well developed.
(9) Accessory opercular ring: 0, absent; 1, present.
(10) Subopercular protuberance through operculum: 0, absent; 1, weak; 2, well developed.
(11) True acrosome ray zone: 0, absent; 1, present; 2, lost.
(12) Outer acrosome zone border with peripheral zone: 0, not ragged; 1, ragged.
(13) Anterolateral pale zone of acrosome contents: 0, absent; 1, present.
(14) Flangelike peripheral extension of lower acrosome zone: 0, absent; 1, present.
(15) Xanthid ring: 0, absent; 1, present; 2, modified and short; 3, modified and elongate.
(16) Subacrosomal chamber or perforatorium: 0, postequatorial; 1, extending preequatorially.
(17) Head of perforatorium: 0, non-capitate; 1, amoeboid; 2, spiked wheel; 3, bilateral.
(18) Corrugations of wall of perforatorial chamber: 0, absent; 1, simple invaginations; 2, branched invaginations; 3, invaginations with filaments; 4, filaments only; 5, evaginations only.
(19) Lateral arms: 0, absent; 1, three; 2, several.
(20) Lateral arms: 0, absent; 1, microtubular with chromatin; 2, nuclear only; 3, microtubular only.
(21) Centrioles: 0, absent; 1, present; 2, elongate. (Excluded.)
(22) Posterior median process of nucleus: 0, absent; 1, present.
(23) Thickened ring: 0, absent; 1, present.
(24) Concentric lamellae: 0, absent; 1, present.
(25) Capsular chambers: 0, absent; 1, one chamber; 2, several.
(26) Capsular projections: 0, absent; 1, present.
(27) Capsular flange: 0, absent; 1, present.

(ii) *Non-spermatozoal characters*

(28) Genital pores: 0, all coxal; 1, female sternal; 2, male and female sternal.
(29) Separate spermatheca: 0, absent; 1, present.
(30) P5, reduction of: 0, absent; 1, present.
(31) P5 dorsal or subdorsal origin: 0, absent; 1, present.
(32) P5 subcheliform or cheliform modification: 0, absent; 1, weak; 2, strong.
(33) Sella turcica: 0, absent; 1, present.
(34) Uropods: 0, present; 1, vestigial; 2, absent.

(*d*) *Data matrix*

taxon	1111111111222222222233333 12345678901234567890123456789012 34
Stimdromia lateralis	30100000020010013000000000100111211
Paromola petterdi	60110000010000012012110000000111212
Calocarcinus africanus	81000002101100210022101000010000 12
Dromidiopsis edwardsi	32100000020010013012000000000111211
Paradynomene tuberculata	30100000020011013000?0000000111111
Latreillopsis gracilipes	60110000010000012012110000000111212
Raninoides sp.	72101000000000001021201002110111012
Lyreidus brevifrons	52101000000000001111201000100111012
Xeinostoma richeri	50100110000000001031211000100111112
Cymonomus sp.	60000010000001010312?1000100111112
Tymolus sp.	60100110000000001031211000100111212
Etisus laevimanus	91000001101100110022001000010000 12
Neodorippe astuta	B10000000010000100220010000101121 2
Portunus pelagicus	A10000000010000100221010000100001 2
Mictyris longicarpa	C12000000020003100220011000200001 2
Ocypode ceratophthalma	91200000002000310022001100020000 12
Uca dussumieri	91200000002000010022001000020000 12
Macrophthalmus crassipes	A10000000020000100220011000200001 2
Pilodius areolatus	91000000101100110022001000010000 12
Ranina ranina	81101000000000000212110011101010 12
Homola sp.	50110000010000012012?1000000111212
Majids	A11000000010000100?111100001000012
Potamonautes perlatus	91000002001000010022201000010000 12
Latreillia sp.	62100000000000130?2?1000000111212
Pagurus bernhardus	F10000000000000105111100000001020 0
Clibanarius taeniatus	C10000000000000104110000000001020 0
Coenobita spinosa	Q10000000010000004130000000001020 0

(*e*) *Branch-and-bound search settings*

Initial upper bound: unknown (compute via stepwise). Addition sequence: furthest. Branches having maximum length zero collapsed to yield polytomies. Topological constraints not enforced. Trees are unrooted. Multi-state taxa interpreted as polymorphism. Ordered characters: 1, 11, 25, 32, 34. All other characters unordered. Outgroup = three

paguroid species: *Pagurus bernhardus*, *Clibanarius taeniatus*, *Coenobita spinosa*.

(*f*) *Heuristic searching*

A search with identical setting but with characters 1, 8, 11, 25 and 32 ordered and 34 irreversible was performed, thus differing only in the treatment of 8, the periopercular rim, and 34, uropods. Because of the great length of time required for branch and bound, the search was heuristic. To anticipate, this gave an identical 50% majority rule tree topology to that obtained in the above branch and bound search. It will therefore be referred to only where it clarifies character transformations. Various character deletions were also attempted and will be referred to where relevant. In addition, a further analysis was made with the character status of the branch and bound search but with no scaling (see Podotremes, below).

(*g*) *Bootstrap method with heuristic search*

Bootstrapping uses the methodology of Felsenstein (1985; see brief review by Trueman 1993) which aims to assign confidence limits to nodes on a tree. In essence it randomly resamples characters to form data sets having the same number of characters as the original, irrespective of repeats, then searches these sets for the most parsimonious trees and counts the number of times in which nodes occurring on the original tree also appear in the replicates. Clearly, as repeats of characters may randomly occur, only those parts of the tree which are insensitive to such weighting will appear frequently in the replicates. In PAUP the weights are allowed to take on any non-negative value and the total of all weights is not constrained. Bootstrapping has been adversely criticized by Trueman (1993) who argues that nodes which recur frequently because they are supported by large numbers of characters will be supported by bootstraping while nodes supported by only one or a few characters will not recur often and may not be supported, even though there is no evidence to contradict their existence. He concludes that as a measure of confidence of the tree as a whole, bootstrapping is quite useless. It is, indeed, curious that recourse to a method which for n characters is potentially capable of representing and therefore weighting a given character n times while effectively giving all other characters zero weight is considered desirable, notwithstanding the low probability of random occurrence of such a weighting or of its occurring in further replications. The low bootstrap (confidence) values often encountered for groupings considered reliable in terms of characters from diverse sources extraneous to the matrix used, and from inspection of the matrix, is itself a measure of the large numbers of trees obtained by random character sampling which are in conflict with the 'true' tree or, at least, with the most parsimonious consensus tree obtained by normal searching.

As it has become customary to apply bootstrap analysis, it is employed here, noting the above caveats and therefore with reservation. Although branch and bound searching is desirable in bootstrap analysis, only the faster heuristic analysis was applied as this took 51 h even when applied to a reduced number of taxa.

In the bootstrap analysis 1000 replications were made, with the following options applied. Addition sequence: simple; one tree held at each step during stepwise addition; tree-bisection-reconnection (TBR) branch-swapping performed; MULPARS option in effect; steepest descent option not in effect; branches having maximum length zero collapsed to yield polytomies; topological constraints not enforced; trees unrooted; multi-state taxa interpreted as polymorphism; character-state optimization: accelerated transformation (ACCTRAN). The outgroup was restricted to *Pagurus bernhardus*. The following taxa were deleted: *Paromola petterdi*; *Calocarcinus africanus*; *Raninoides* sp.; *Xeinostoma richeri*; majids; *Latreillia* sp.; *Clibanarius taeniatus*; and *Coenobita spinosa*. Characters were unordered excepting 1, 11, 25 which was, however, uninformative because of deletion of *Raninoides*, 32, 34. They were scaled to each contribute a score of 1 irrespective of the number of states. (Slower runs using unscaled characters gave very similar trees.)

(*h*) *Skewedness test*

It has been suggested that the skewedness of the distribution of all trees from a given data set provides a measure of its information content. If the distribution is symmetrical the data are random and the most parsimonious tree obtained is not (or is only coincidentally) a representation of the phylogenetic history of the taxa concerned (Huelsenbeck 1991). A table based on DNA nucleotides which contains the critical values of a constant $g1$, reflecting skewedness, has been provided (Hillis & Huelsenbeck 1992). To the writer, the fear that data may be random seems unwarranted in relation to complex morphological characters (whether spermatozoal or not). Indeed, it is not easy to envisage characters across a range of taxa which would not have some congruence with any actual groups and it is clear, from inspection that characters (correctly character states) which are intuitively observed as recognizable attributes and are therefore chosen for parsimony analysis rarely show the 'noise' which would be attributable to character states randomly distributed through the matrix. An example of such non-randomness would be the occurrence of vertebrae in a survey of the animal kingdom or of the thickened ring in crab sperm. This confidence may well not apply to nucleotide substitutions which, theoretically at least, and particularly in non-coding sequences or in most third base positions which in coding sequences are 'silent' with regard to amino acid changes, are presumed to randomly mutate.

The distribution of all trees is obtained in the PAUP program from exhaustive searching. Because of the inordinate time taken for this an approximate

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

estimate of skewedness was obtained by invoking the random trees option. The reliability of the estimate of $g1$ would clearly increase with increasing numbers of random trees. No attempt was made to make a thorough analysis of the relationship of tree numbers to stability of the value of $g1$ but a preliminary survey was made taking 10, 100, 1000, and 1 000 000 trees (see table 1). Characters were all unordered and unscaled and the full set of taxa was used.

3. RESULTS AND DISCUSSION

(*a*) *Spermatozoal characters only*

Branch and bound searching, using options listed above, for spermatozoal characters only gave 72 shortest and equally parsimonious trees, using the outgroup method. Characters 21, 29–34 were excluded. Character-state optimization was by accelerated transformation (ACCTRAN). Tree length = 37 558; consistency index (CI) = 0.741; homoplasy index (HI) = 0.281; retention index (RI) = 0.882; rescaled consistency index (RC) = 0.653. The 50% majority rule consensus tree of the 72 trees is shown in figure 2*a*. Clades are supported by 100% of trees unless otherwise indicated.

(*b*) *Spermatozoal and non-spermatozoal characters*

(i) *Branch and bound searching*

Branch and bound searching for combined spermatozoal and non-spermatozoal characters gave 36 shortest and equally parsimonious trees. Character 21 was excluded. Character-state optimization was by accelerated transformation (ACCTRAN). The tree length = 48 247; CI = 0.701; HI = 0.316; RI = 0.891; RC = 0.625. The 50% majority rule consensus tree is shown in figure 2*b*. Clades are supported by 100% of trees unless otherwise indicated. The unambiguous apomorphic changes on the nodes of this tree are given in figure 10.

(ii) *Heuristic bootstrap searching*

For the reduced set of taxa, the bootstrap method with heuristic search for 1000 replications gave a 50% majority rule consensus tree of length 45 216; CI = 0.719; HI = 0.281; CI (excluding uninformative characters) = 0.680; HI (excluding uninformative characters) = 0.320; RI = 0.857; RC = 0.616.

The bootstrap results will chiefly be discussed in the section on the main parsimony analyses below but some observations on bootstrap values are appropriate here. Irrespective of the validity or otherwise of bootstrap analysis, it is widely agreed that bootstrap values are conservative estimates of significance. This is clearly the case in the present analysis as clades with incontrovertibly closely related taxa reveal (figure 3*a*). Thus the two xanthids *Etisus laevimanus* and *Pilodius areolatus* associate in 97% of trees and the podotreme clade in 87%. However, other groups which are indisputably monophyletic have low bootstrap values, notably the clade composed of *Latreillopsis gracilipes* and *Homola* sp., with a value of only 63%. The validity of bootstrapping in terms of gross topology, irrespective of the questionable value of bootstrap values on the respective nodes, appears to be indicated by the virtual identity of the bootstrap tree and that obtained by branch and bound searching for the same 19 taxa. The only different clade in the branch and bound 50% majority rule consensus tree is shown in figure 3*b*.

(iii) *Skewedness test*

The distribution of 1 million random trees for 26 taxa and 33 characters is given in figure 4. The $g1$ value obtained for one sample of 1 million random trees of −0.476 750 indicates that the data are not random in terms of the table given by Hillis & Huelsenbeck (1992) for binary and four state characters and that the matrix therefore has phylogenetic signal. In table 1 it is seen that the value of $g1$ for 1 million trees is closely approximated by samples of 10 000 trees, commonly taken as an appropriate sample size, but that, as expected, samples of 100 and 10 trees give increasingly unreliable estimates of $g1$. The asymptotic nature of the values from 10 000 to 1 million suggest that these numbers approximate closely to the actual but uncalculated $g1$ value for all trees.

(*c*) *Conclusions from spermatozoal and combined data with branch and bound searching*

Parsimony analysis using the branch and bound option, whether using only spermatozoal characters or

Table 1. *Values of g1 for varying numbers of random trees*

number of random trees	mean	s.d.	$g1$	$g2$
10	214.300 000	4.196 427	−0.202 655	−1.511 010
100	207.520 000	10.137 534	−0.593 975	0.651 119
1000	208.320 000	9.735 276	−0.506 447	0.212 825
	208.500 000	10.052 064	−0.504 116	0.271 684
10 000	208.527 300	9.697 982	−0.414 515	0.083 662
	208.597 000	9.819 277	−0.470 237	0.120 878
	208.330 600	9.936 393	−0.465 377	0.121 493
	208.596 000	9.954 978	−0.475 676	0.148 266
	208.608 900	9.951 720	−0.507 428	0.177 716
1 000 000	208.527 943	9.852 468	−0.476 750	0.206 812

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

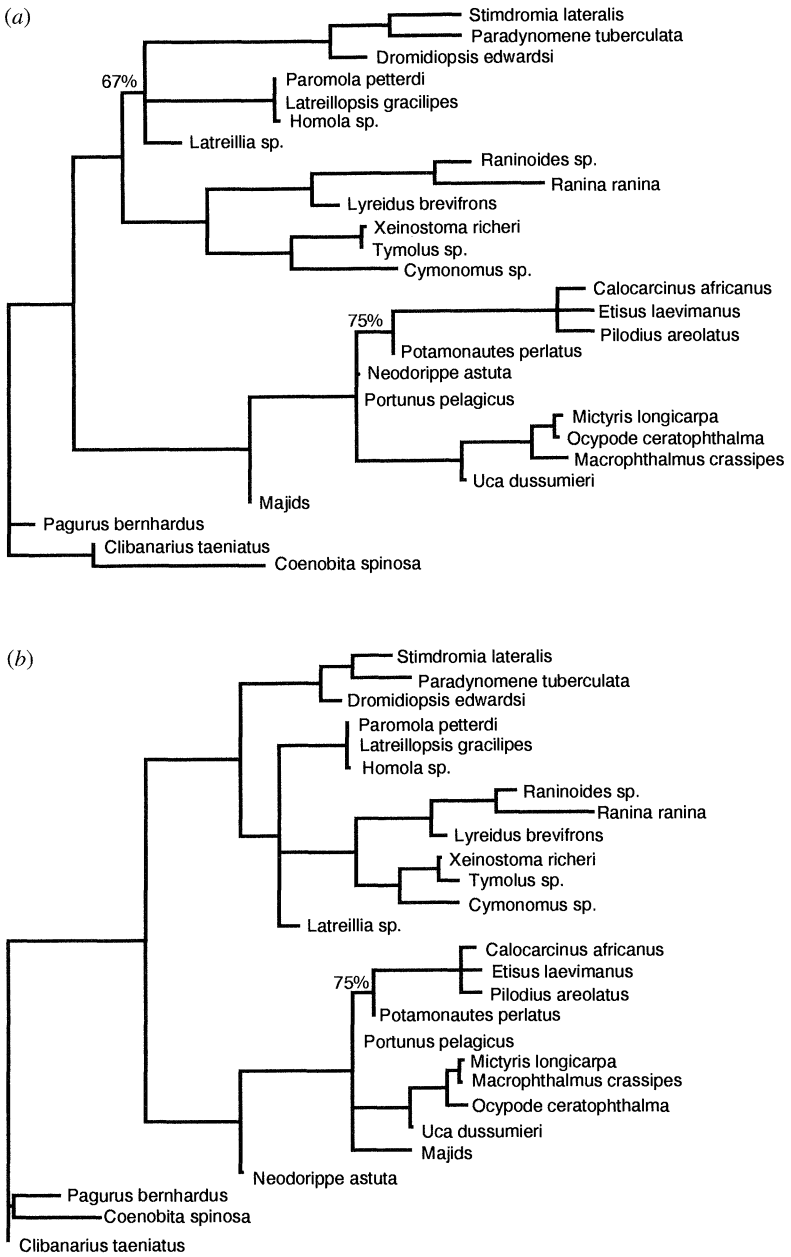


Figure 2. (*a*) Branch and bound 50% majority rule consensus tree of 72 shortest and equally parsimonious trees, for spermatozoal characters only, using the outgroup method. Characters 21, 29–34 excluded. Character-state optimization: ACCTRAN. Tree length = 37 558; CI = 0.741; HI = 0.281; RI = 0.882; RC = 0.653. Clades are supported by 100% of trees unless otherwise indicated. (*b*) Branch and bound 50% majority rule consensus tree of 36 shortest and equally parsimonious trees for combined spermatozoal and non-spermatozoal characters. Unrooted tree(s) rooted using outgroup method. Character 21 excluded. Character-state optimization: ACCTRAN. Tree length = 48 247; CI = 0.701; HI = 0.316; RI = 0.891; RC = 0.625. Clades are supported by 100% of trees unless otherwise indicated.

spermatozoal and non-spermatozoal characters, permits the following conclusions. Reference will also be made to the bootstrap analysis. The close agreement for the smaller data set of bootstrap analysis and branch and bound searching indicates that boot strapping is not ineffective in finding a meaningful topology, as would be expected in a process which samples a large number of the characters. This does not, however, validate it as a method for determining the confidence which may be attached to individual branches.

(i) *Brachyura*

The Brachyura is a monophyletic taxon relative to the outgroup, the three (questionably monophyletic) paguroids. Six synapomorphies relative to the anomurans (character number appended in brackets) of varying cogency indicate monophyly. Shortening of the acrosome to a spherical condition (1). Loss of corrugations of the walls of the perforatorial chamber (18) does not give a monothetic character in view of presence in cyclodorippoids and raninoids. Loss of microtubules from the lateral arms (20) is a doubtful

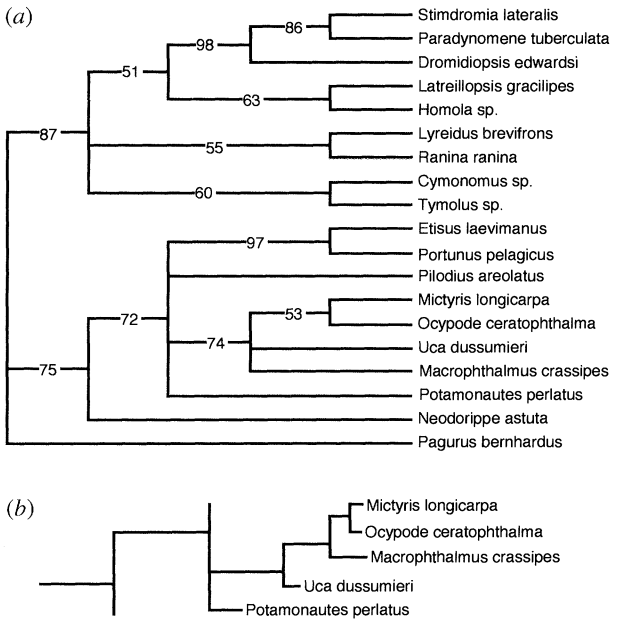


Figure 3. (*a*) 50% majority rule consensus tree obtained by the bootstrap method with heuristic search for 1000 replications. Tree length = 45 216; CI = 0.719; HI = 0.281; CI excluding uninformative characters = 0.680; HI excluding uninformative characters = 0.320; RI = 0.857; RC = 0.616. (*b*) The sole clade, from branch and bound searching, which differed in small details from the bootstrap analysis for the same data. Tree length = 43 216; CI = 0.752; HI = 0.248; CI excluding uninformative characters = 0.716; HI excluding uninformative characters = 0.284; RI = 0.879; RC = 0.661.

synapomorphy in view of their presence in at least some majids. Of the non-spermatozoal characters the unique development of the sella turcica appears to convincingly support brachyuran monophyly (including podotremes). The other putative synapomorphies are (31) dorsal movement of the base of pereiopods 5 (P5) (an ambiguous change, reversed in heterotremes above *Neodorippe*; exclusion of this character does not alter the topology of the consensus tree shown in figure 2*b*) and loss of the uropods present in anomurans (34). Uropods are present, if vestigial, in some podotremes and redevelopment seems unlikely though conceivable genetically. When this character is run 'irreversible', so that uropods once lost cannot be regained, a reasonable progression is seen, from a vestigial condition in basal brachyurans, retained in the dromiid–*Paradynomene* assemblage, to loss in the ancestor of the other represented podotremes (Archaeobrachyura) and a homoplasic loss in that of the heterotreme–thoracotreme assemblage. The sella turcica is thus the only striking synapomorphy supporting brachyuran monophyly. Many groups within the Brachyura are, in contrast, strongly supported.

In the heuristic search with no scaling of characters, clades obtained agreed with the previous branch and bound and heuristic analyses with the exception that the homolids formed a polytomy with the dromiid–*Paradynomene*, cyclodorippoid–raninoid and *Latreillia* clades. Thus scaling gave better resolution than was obtained with unscaled characters.

In the bootstrap analysis (figure 3*a*) and the branch and bound search for the smaller taxon sample, the three paguroid, podotreme, and heterotreme–thoracotreme clades form a trichotomy. In view of dichotomous resolution of the relationships of these clades in the larger analysis, this lack of resolution cannot be considered to negate the monophyly of Brachyura obtained in the latter analysis.

(ii) *Podotremata*

The sperm of the two investigated dromiids, of *Paradynomene*, an homolid, *Latreillia* (and one paguroid, *Clibanarius*) are illustrated semidiagrammatically in figure 5. Transmission electron micrographs of the sperm of a dromiid (*Dromidiopsis*), an homolid (*Latreillopsis*), a raninoid (*Raninoides*) and a cyclodorippoid (*Xeinostoma*) are given in figure 9.

The Podotremata is a monophyletic taxon and the sister-group of the heterotreme-thoracotreme assemblage (figure 2*a*,*b*). Its synapomorphies are (1) depression of the acrosome; (2) development of a predominantly horizontal zonation of the acrosome compared with the concentric zonation of paguroids and heterotremes. The bilateral capitate perforatorial

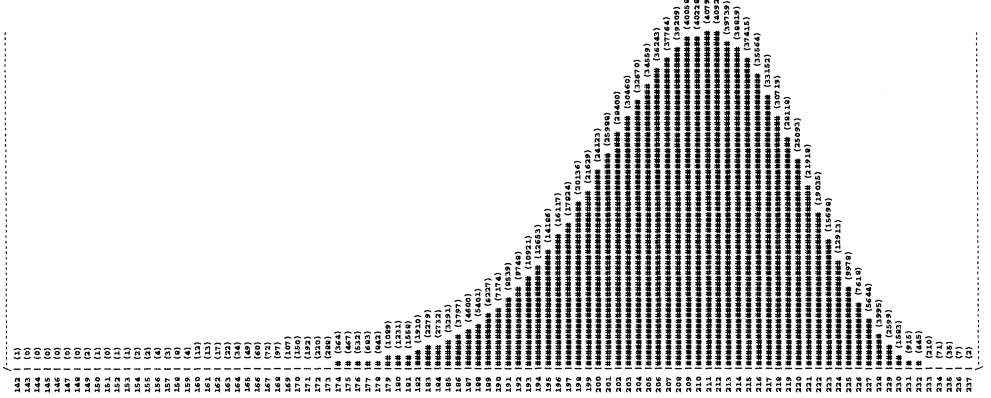


Figure 4. Skewed frequency distribution of 1 million random trees for 26 taxa and 33 characters; all unordered and unscaled. Mean = 208.527 943; s.d. = 9.852 468. Heuristic searching. The value of $g1$ is −0.476 750, indicating strong phylogenetic signal; $g2$ = 0.206 812.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

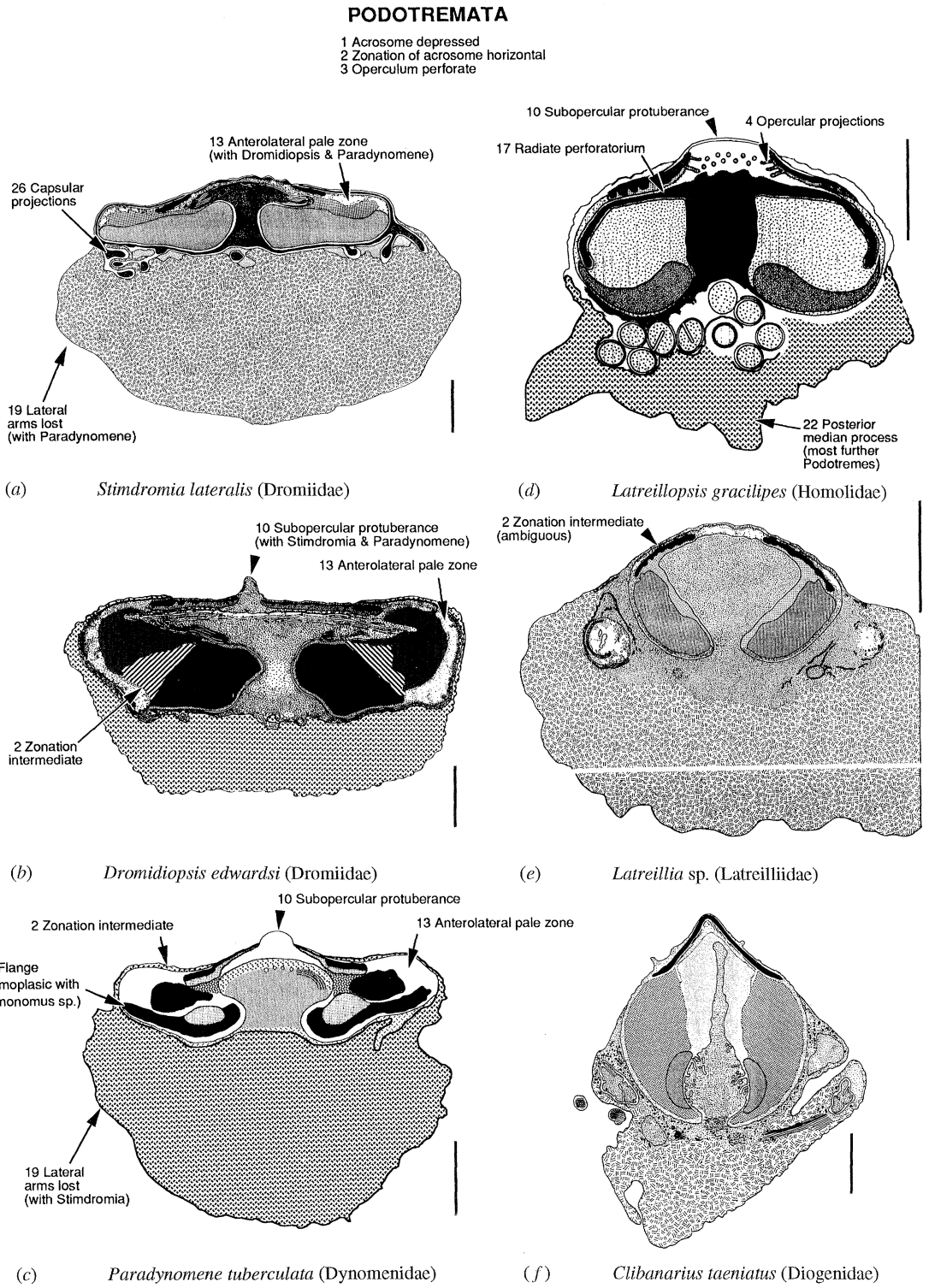


Figure 5. Drawings of spermatozoa of some podotremes and an anomuran used in this analysis. (*a*) *Stimdromia lateralis* (Dromiidae); (*b*) *Dromidiopsis edwardsi* (Dromiidae); (*c*) *Paradynomene tuberculata* (Dynomenidae); (*d*) *Latreillopsis gracilipes* (Homolidae); (*e*) *Latreillia* sp. (Latreilliidae); (*f*) *Clibanarius taeniatus* (Anomura, Diogenidae). The chief apomorphies are indicated but see text for a detailed explanation. The section for *Stimdromia* is not precisely sagittal; in micrographs which are sagittal, perforation of the operculum is seen. Scale bar 1 μm. Sources as listed in § 2.

head (17), seen in dromiids and *Paradynomene* computes as an ambiguous synapomorphy of the Podotremata (developing from the simple, non-capitate form in paguroids and ancestral crabs) to be lost in some members. This character is treated as 'unordered' in the full branch and bound parsimony analysis as the various forms of the capitate perforatorium do not necessarily form a series. However, if it is treated as 'ordered' no change in the topology of the consensus tree shown in figure 2*b* occurs. This is true also, if an additional character in which the capitate condition, irrespective of its various

forms, is included as a single apomorphy. In some additional runs a simple perforatorium computed as basic for podotremes.

Apical perforation of the spermatozoal operculum (3) is a synapomorphy of podotremes, the same condition in majids being independent (homoplasic). The distinct spermatheca is a major podotreme synapomorphy (autapomorphy) as its constitution, involving two somites, is unlikely to have occurred more than once. Monophyly of the Podotremata is supported in the bootstrap analysis at 87% (figure 3*a*). This finding does not exclude, nor does it support, the possibility that some supposed dromiids, notably *Hypoconcha* (see Spears *et al.* 1992), have been missclassified and may be closer phylogenetically to anomurans that they are to other brachyurans.

(iii) *Dromiidae*

The Dromiidae have been elusive of definition as they appear in this analysis to be paraphyletic. *Paradynomene*, the only dynomenid available for spermatological studies, groups more closely with *Stimdromia* than does *Dromidiopsis*. Spermatozoa of these taxa are illustrated in figure 5*a–c*. Synapomorphies of the three taxa are: (1) further depression of the acrosome; (13) presence of an anterolateral pale zone of the acrosome; (10) subopercular protuberance through operculum; and (34) ambiguous, presence of vestigial uropods. This clade is supported at 98% in the bootstrap analysis. The sole apomorphy of *Dromidiopsis* is (2) the zonation of the acrosome intermediate between the horizontal and concentric conditions. *Stimdromia lateralis* (figure 5*a*) is diagnosed by (26) presence of capsular projections.

(iv) *Dynomenidae*

Paradynomene tuberculata (figure 5*c*) is a somewhat atypical representative of the Dynomenidae. Apomorphies are (32) reduction in the subcheliform development of P5 (exclusion of this character does not alter the topology of the consensus tree shown in figure 2*b*) and (14), ambiguous, a discontinuous, flange-like development of the lower acrosome zone, also seen, apparently homoplasically, in *Dromidiopsis*. It remains to be seen whether these features occur in other dynomenid sperm.

(v) *Homolidae*

The Homolidae (figure 5*d*) is a monophyletic family (figure 2*a*,*b*). They have two very convincing autapomorphies: (17) development of the radiate perforatorium, apparently from a basic bilateral or (in some runs) simple, condition of podotremes; and (4) small digitiform projections from the operculum into the subopercular material. Subopercular protuberance through the operculum (10) has developed independently of the dromiid–*Paradynomene* clade.

(vi) *Raninoidea and Cyclodorippoidea*

The Raninoidea (figure 6*a–c*) and Cyclodorippoidea (figure 6*d–f*) form a monophyletic (unnamed) clade (figure 2*a*,*b*). They show no strong synapomorphies, however. Synapomorphies are: (32) reduction of the subcheliform development of P5 and three ambiguous changes: (17) reversal from a bilateral to a non-capitate condition of the perforatorium; (18) loss of corrugations of the wall of the perforatorial chamber (to be reversed in raninoids); and (26) development of outward projections of the capsule.

(vii) *Raninoidea*

Raninoids (figure 6*a–c*) are well defined spermatologically by (5) virtual continuity of the operculum with the capsule. Less striking, because it is ambiguous, is (2) alteration of the zonation of the acrosome vesicle to an intermediate condition. Loss of the subcheliform or cheliform modification of P5 (32) is attributable to the burrowing habit.

(viii) Ranina *and* Raninoides

These two genera share strong synapomorphies: (25) development of posterior capsular chambers, one in *Ranina* (figure 6*c*) increasing to several in *Raninoides* (figure 6*b*); and (27) the remarkable lateral flange on the capsule. An ambiguous change, not shown in some runs, is (18) development of branched septum-like corrugations of the wall of the perforatorial chamber from the unbranched form basal to the raninoid–cyclodorippoid clade and persistent in *Lyreidus*. There is also a strong trend (1) towards a subspheroidal form of the acrosome, most developed in *Ranina* in which (2) zonation becomes concentric and (16) the perforatorium, apparently secondarily, becomes only postequatorial. In *Lyreidus* (figure 6*a*), (1) the acrosome becomes secondarily depressed; and (17) the 'amoeboid' form of the head of the perforatorium is seen as development of a capitate condition independently of that in dromiids and homolids. The affinities of the Raninoidea are further discussed in § 3*e*.

(ix) *Cyclodorippoidea*

The three cyclodorippoids (figure 6*d–f*) are well defined by (7) the extreme width of the operculum relative to the acrosome. As an ambiguous change, (18) corrugations of the wall of the perforatorial chamber are invaginations with filaments. A synapomorphy of *Xeinostoma* (figure 6*e*) and *Tymolus* (figure 6*f*) is (6) the extreme thinness of the operculum. *Xeinostoma* is apomorphic in (1) further depression of the acrosome. *Tymolus* redevelops (32) strong subcheliform development of P5. *Cymonomus* (figure 6*d*) is apomorphic for all investigated podotremes in (3) losing the opercular perforation. It appears to have developed (14) the flange-like extension of the lower acrosome zone independently of *Dromidiopsis* but the similarity is striking and cyclodorippoid relationships require further investigation.

(x) *Latreilliidae*

The position of *Latreillia* sp. (figure 5*e*) is equivocal, forming a polytomy either with Homolidae + Dromiidae – *Paradynomene* (sperm only) or with Homolidae + Raninoidea – Cyclodorippoidea with the combined data set. Many spermatozoa of this

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

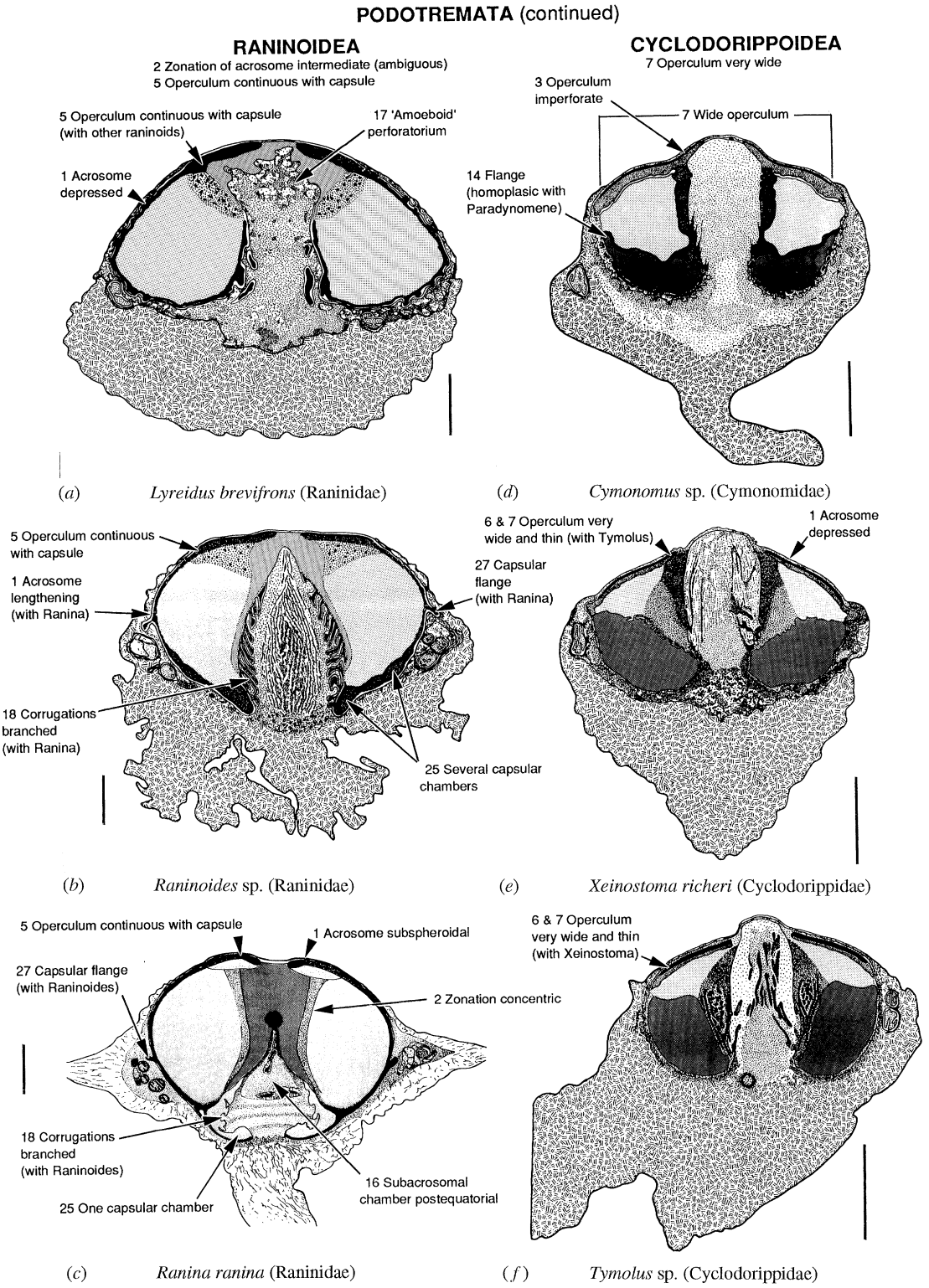


Figure 6. Drawings of spermatozoa of further podotremes used in this analysis. (*a*) *Lyreidus brevifrons* (Raninidae, Lyreidinae); (*b*) *Raninoides* sp.(Raninidae, Raninoidinae); (*c*) *Ranina ranina* (Raninidae, Raninae); (*d*) *Cymonomus* sp. (Cymonomidae); (*e*) *Xeinostoma richeri* (Cyclodorippidae, Xeinostominae); (*f*) *Tymolus* sp. (Cyclodorippidae, Cyclodorippinae). The chief apomorphies are indicated but see text for a detailed explanation. Scale bar 1 µm. Sources as listed in § 2.

species appeared malformed and although the spermatozoon illustrated appears normal further investigation of *Latreillia* is required. The sole detected apomorphy is ambiguous: (2) independent development of an intermediate condition of the vesicle contents from the horizontally zoned condition.

(xi) *Heterotremata and Thoracotremata*

Within the heterotreme–thoracotreme assemblage, the Thoracotremata (figure 8) is a monophyletic taxon but the Heterotremata *sensu strictu* (figure 7) is a paraphyletic grouping (figure 2*a*,*b*). The combined Heterotremata–Thoracotremata, which may be

termed the Heterotremata *sensu lato*, is defined by a convincing synapomorphy, (23) presence of the thickened ring. Other spermatozoal synapomorphies, although unambiguous, are less compelling. (19) Multiplication of lateral arms from three, common to paguroids and podotremes, to several is a trend rather than a diagnostic basal apomorphy as it results from polymorphism, there being three in at least some majids as in the leucosiid *Iliacantha subglobosa* (Felgenhauer & Abele 1991). Presence of a true acrosome ray zone (11) appears to be a synapomorphy but is seen, apparently homoplasically, in paguroids. The Heterotremata *sensu lato* forms a grouping whether or not non-spermatozoal characters are included (figure 2*a*,*b*) but (28) the sternal female pores constitute, as Guinot suggested, their non-spermatozoal synapomorphy. This clade is supported in the bootstrap analysis but only at 75% (figure 3*a*), clearly an example of the conservative estimates given by bootstrapping. Within the heterotremes, *Potamonautes* (figure 7*d*), Calo*carcinus* (figure 7*f*) and the two xanthids are unified by (8) a periopercular rim,

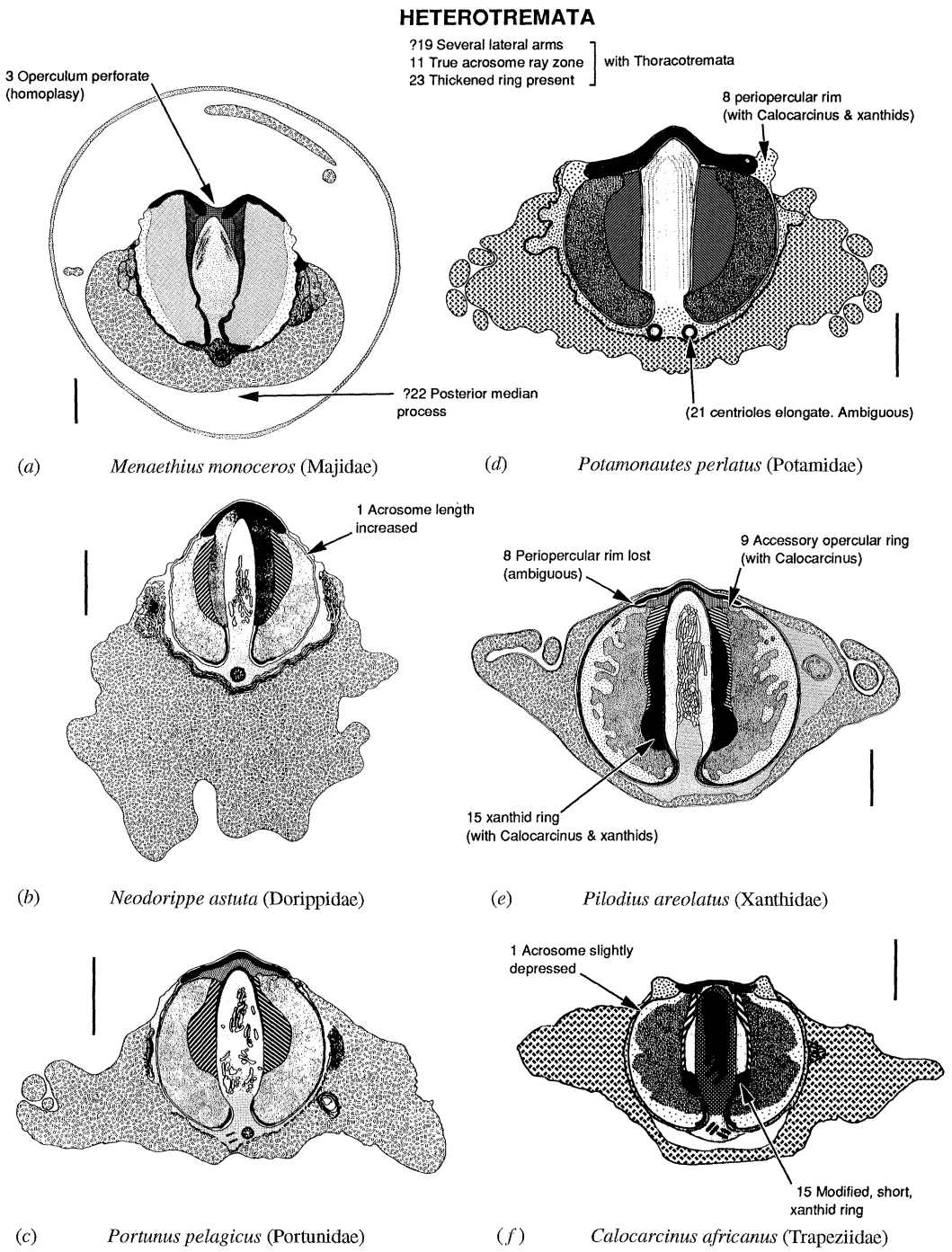


Figure 7. Drawings of spermatozoa of Heterotremata used in this analysis. (*a*) *Menaetheus monoceros* (Majidae); (*b*) *Neodorippe astuta* (Dorippidae); (*c*) *Portunus pelagicus* (Portunidae); (*d*) *Potamonautes perlatus* (Potamidae); (*e*) *Pilodius areolatus* (Xanthidae); (*f*) *Calocarcinus africanus* (Trapeziidae). The chief apomorphies are indicated but see text for a detailed explanation. Scale bar 1 μm. Sources as listed in § 2.

remaining well developed in *Potamonautes* (figure 7*d*) and *Calocarcinus* (figure 7*f*), becoming weak in the xanthid *Etisus*, and lost in *Pilodius* (figure 7*e*) but this character is ambiguous. In the heuristic search, when the character is treated as ordered, it is unambiguous, being represented weakly in the ancestor of this clade and in *Etisus*, developing from this state to well developed in *Calocarcinus* and *Potamonautes*, and being lost in *Pilodius*.

The Thoracotremata (figure 8*a–d*) selected are monophyletic on the basis of two unambiguous characters: (11) loss of the acrosome ray zone and (28) movement of the male pores (in addition to the female pores) onto the sternum. Development of the characteristic apical button in the perforatorium (3) appears ambiguous owing to its alternative absence or loss in *Macrophthalmus* (figure 8*b*). A more detailed investigation of thoracotremes might resolve the issue of whether the button is basic to thoracotremes. In view of the close relationship generally recognized between *Macrophthalmus* and *Ocypode* (figure 9*c*), it seems likely that the absence in the former is due to loss of a basic thoracotreme condition. The concentric lamellae (24) appear to be a development, not seen in *Uca* (figure 9*a*), basal to the higher thoracotremes, *Mictyris* (figure 8*d*), *Ocypode* (figure 8*c*) and *Macrophthalmus* (figure 8*d*). It must be stressed, however, that this study is not aimed at elucidating internal relationships of the heterotreme–thoracotreme assemblage of which many investigated taxa are omitted from this study. An interesting outcome of the analysis is that the 'modified xanthid ring', which has been recognized as a characteristic of some thoracotreme sperm and considered to suggest derivation of thoracotremes from a xanthid stock (Jamieson 1991*a*), computes as an entirely independent development not related to the xanthid structure.

(*d*) *Incongruity between combined and exclusively spermatozoal data*

Notwithstanding the remarkable degree of resolution which spermatozoal data, considered in isolation, have provided there are a few incongruities between the phylograms which they yield and those produced from the combined data set. A major difference in the analysis based on spermatozoal ultrastructure only, is that the Homolidae group, not with the Raninoidea–Cyclodorippoidea, but with the Dromiidae–Paradynomene + Homolidae + *Latreillia* clade. This clade is the sister-group of the Raninoidea +

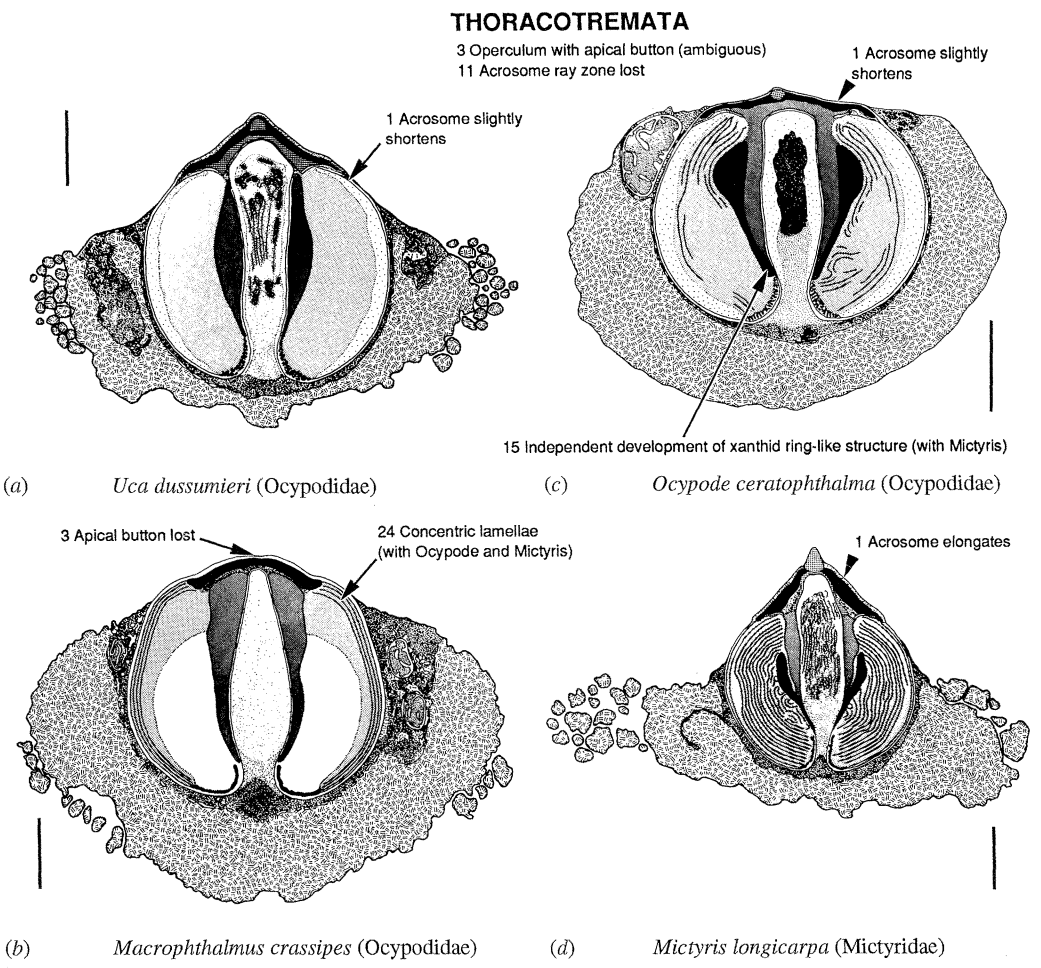


Figure 8. Drawings of spermatozoa of Thoracotremata used in this analysis. (*a*) *Uca dussumieri*; (*b*) *Macrophthalmus crassipes*; (*c*) *Ocypode ceratophthalma* (all Ocypodidae); (*d*) *Mictyris longicarpa* (Mictyridae). The chief apomorphies are indicated but see text for a detailed explanation. Scale bar 1 μm. Sources as listed in § 2.

paraphyletic Cyclodorippoidea. The unambiguous spermatozoal synapomorphy linking homolids with this sister-group in the combined analysis, (22) possession of a posterior median process, is not strong, especially in view of the occurrence of a posterior extension of the nucleus in some paguroids, though this is doubtfully homologous. However, linkage of homoloids with raninoids, confirming the Archaeobrachyura as a monophyletic group is preferred as it is considered that more reliance can be placed on the combined data set than on spermatozoal data alone. Grouping of homolids with the dromiid–*Paradynomene* assemblage in the bootstrap analysis (figure 3*a*) appears to be attributable to the smaller sample of taxa as the smaller branch and bound analysis shows the same divergence from the full analysis. The same applies to the trichotomy of the cyclodorippoids with raninoids and the remaining podotremes while cyclodorippoids group with raninoids in the larger analysis.

A further difference of exclusively spermatozoal data (figure 2*a*) from the combined data (figure 2*b*), is that majids appear the least modified members of the heterotreme–thoracotreme assemblage, usurping *Neodorippe* (notably, a shell carrier, like some dromiids). Again, the combined data are probably more reliable but a firm decision between these alternatives cannot be made here in a study the chief aim of which is resolution of podotreme relationships. It must await incorporation of additional heterotremes. Finally, *Latreillia* changes its relationships from a trichotomy with the Homolidae and Raninoidea–Cyclodorippoidea to a trichotomy with the Homolidae and Dromiidae–*Paradynomene* clades. As only the *Latreillia* sperm showed developmental abnormalities, solution of this problem requires further material.

(*e*) *Concluding remarks*

It will be useful here to summarize the findings of this study and to add some comments on them. The parsimony analysis, whether using only spermatozoal characters or spermatozoal and non-spermatozoal characters, leads to the following conclusions.

The Brachyura is a monophyletic taxon relative to the outgroup, three paguroids.

The Podotremata appears to be a monophyletic taxon and the sister-group of the heterotreme–thoracotreme assemblage. However, this cannot be stated without reservation as there is molecular evidence (Spears *et al.* 1992) that Dromiidae and consequently the Podotremata are not monophyletic and that raninoids are more closely related to the heterotreme–thoracotreme assemblage than they are to other podotrematous crabs. Jamieson (1990) advocated exclusion of the Raninoidea from the Podotremata on the grounds that the sperm of *Ranina ranina* shared a number of synapomorphies (e.g. spherical, embedded, complexly zoned acrosome) with the so-called Oxystomata–Oxyrhyncha–Cancridea–Brachygnatha (i.e. heterotreme–thoracotreme) assemblage of crabs. In the present analysis, based on a much larger sample of taxa, the spherical acrosome emerges as a plesiomorphic state for the Brachyura *s. lat.* and raninoids group within the Podotremata, thus weakening the link between raninoids and the higher (heterotreme–thoracotreme) crabs. From 18*S* rRNA analysis (Spears *et al.* 1992), however, the raninoids, exemplified by *Raninoides louisianensis* and *Ranilia muricata*, grouped strongly with a Brachyura which excluded dromiids. Those authors stated, with reason, that 'the exclusion of dromiids from the Brachyura is consistent with the findings of Jamieson (1990)'. In the latter work exclusion of dromiaceans from the Brachyura was considered on the grounds that if brevity (or absence) of the arms in dromiids were plesiomorphic the Dromiacea might be more basal even than the anomurans which have well developed arms. This appeared to be supported by the non-brachyuran larvae of dromiids. However, the alternative possibility that dromiids were monophyletic with true crabs was not excluded. In the present study, the apparent absence of arms in *Stimdromia lateralis* and *Paradynomene tuberculata* is seen as a secondary condition relative to plesiomorphic presence. Thus, from spermatozoal ultrastructure inclusion of raninoids with dromiids in the Podotremata is supported, if weakly. In view of the molecular evidence of Spears *et al.* (1992), however, it is clear that inclusion of raninoids in the Podotremata (as a clade as opposed to a grade) cannot be considered settled despite the convincing nature of spermatozoal evidence in other areas of the phylogenetic analysis.

In the present study, the Dromiidae appears paraphyletic as its clade includes *Paradynomene*. From their 18*S* rRNA study, Spears *et al.* (1992) also found little support for the Dromiidae as a monophyletic group. Despite a rigorous investigation aimed at minimizing systematic error which might result from failure of the parsimony method to detect high levels of homoplasy, the two dromiids included in their analysis never formed a clade. In a bootstrap analysis the dromiid *Hypoconcha arcuata* grouped with a hermit crab while *Dromidia antillensis* formed their sister taxon. Examination of the spermatozoa of *Hypoconcha* would be very desirable. From the molecular and spermatological evidence, considered separately, the Dromiidae as at present constituted are therefore only monophyletic if (i) they include the Dynomenidae or (ii) they include at least the Paguroidea. The first is doubtfully and the second is certainly not an acceptable position, at least from the standpoint of nomenclature, and it is clear that the Dromiidae and Dynomenidae and their relationships with the Anomura require further elucidation.

The Homolidae emege from this study as a convincingly monophyletic family with strong and unique synapomorphies.

The Raninoidea and Cyclodorippoidea are sister groups within a monophyletic (unnamed) clade. *Latreillia* sp., the sole representative in this study of the Latreiliidae forms a polytomy with Homolidae + Raninoidea–Cyclodorippoidea and its affinities remain uncertain. Within the heterotreme–thoracotreme assemblage, the Thoracotremata is a

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

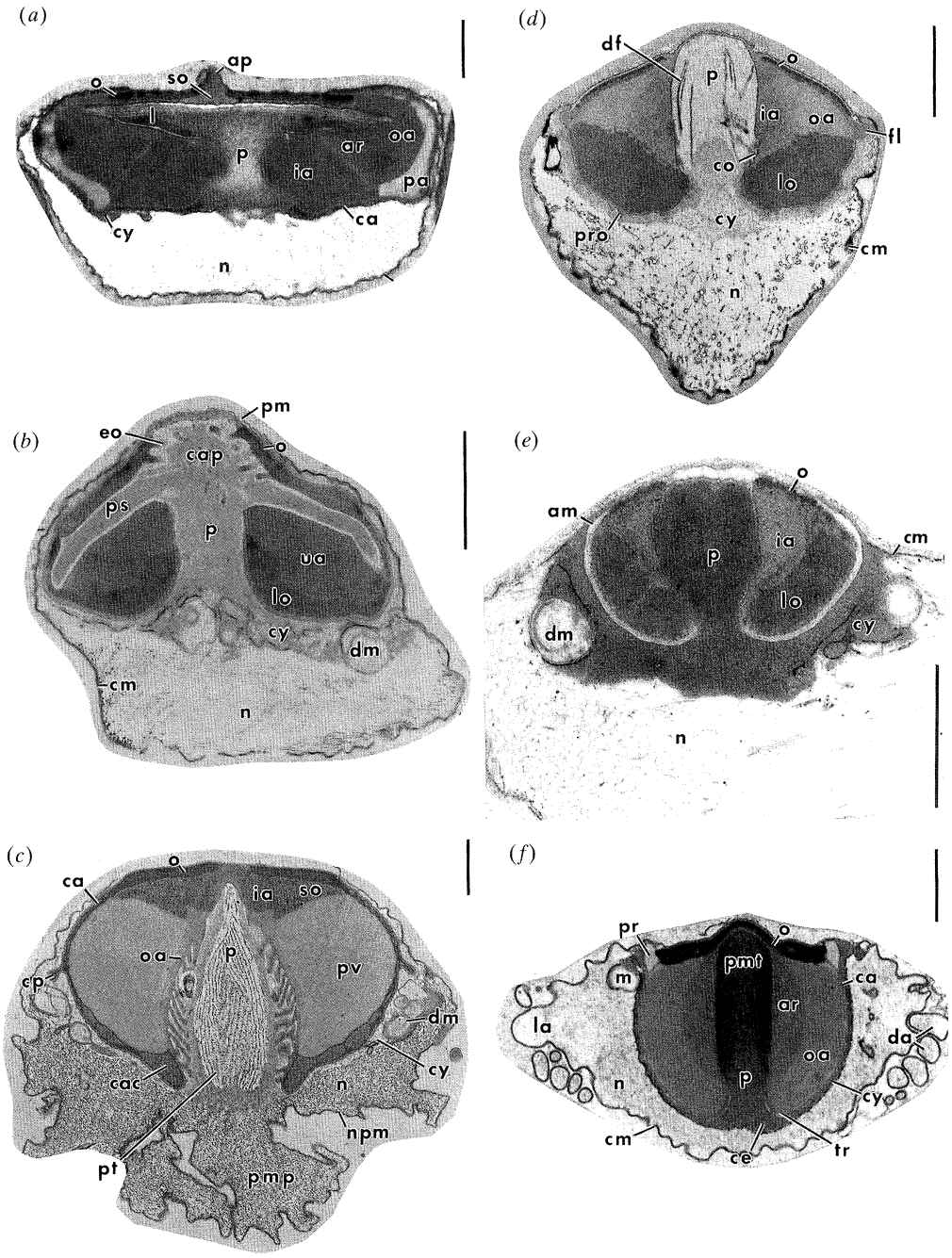


Figure 9. Transmission electron micrographs of longitudinal sagittal sections of representative podotreme sperm: (*a*) a dromiid (*Dromidiopsis edwardsi*); (*b*) an homolid (*Latreillopsis gracilipes*); (*c*) a raninoid (*Raninoides* sp.); (*d*) a cyclodorippoid (*Xeinostoma richeri*); (*e*) a latreilliid (*Latreillia* sp.); and (*f*) a heterotreme (*Potamonautes perlatus*). am; acrosome membrane, ap; apical protuberance, ar; acrosome ray zone, ca; capsule, cac; capsular chambers, cap, capitate region of perforatorium, ce; centriole, cm; cell membrane, co; corrugations, cp; capsular projection, cy; cytoplasm, da; divarications of arms, df; dense filament, dm; degenerate mitochondrion, eo, extensions of operculum, fl; periacrosomal flange, ia; inner acrosome zone, l; lamellae, la; lateral arm, lo; lower acrosome zone, m; mitochondrion, n; nucleus, npm; nuclear-plasma membrane, o; operculum, oa; outer acrosome zone, p; perforatorium, pa; anterolateral pale zone, pmp; posterior median process, pmt; microtubules of perforatorium, pr; periopercular rim, pro; projections of acrosome membrane, ps; perforatorial spike, pt; perforatorial tubules, pv; peripheral acrosome zone, so; subopercular zone, tr; thickened ring, ua; upper acrosome zone.

monophyletic taxon but the Heterotremata *s. strict.* is a paraphyletic grouping. Analyses based on sperm data alone differ from the combined data in not including the Homolidae in the Archaeobrachyura (Homolidae + Raninoidea–Cyclodorippoidea), thus the Dromiidae–*Paradynomene* + Homolidae + *Latreillia* form a polytomous clade which is the sister-group of the Raninoidea + Cyclodorippoidea. Spermatozoal data also give majids the most basal position in the Heterotremata whereas for the combined data *Neodorippe* appears the least modified member of the heterotreme–thoracotreme assemblage. It is of interest

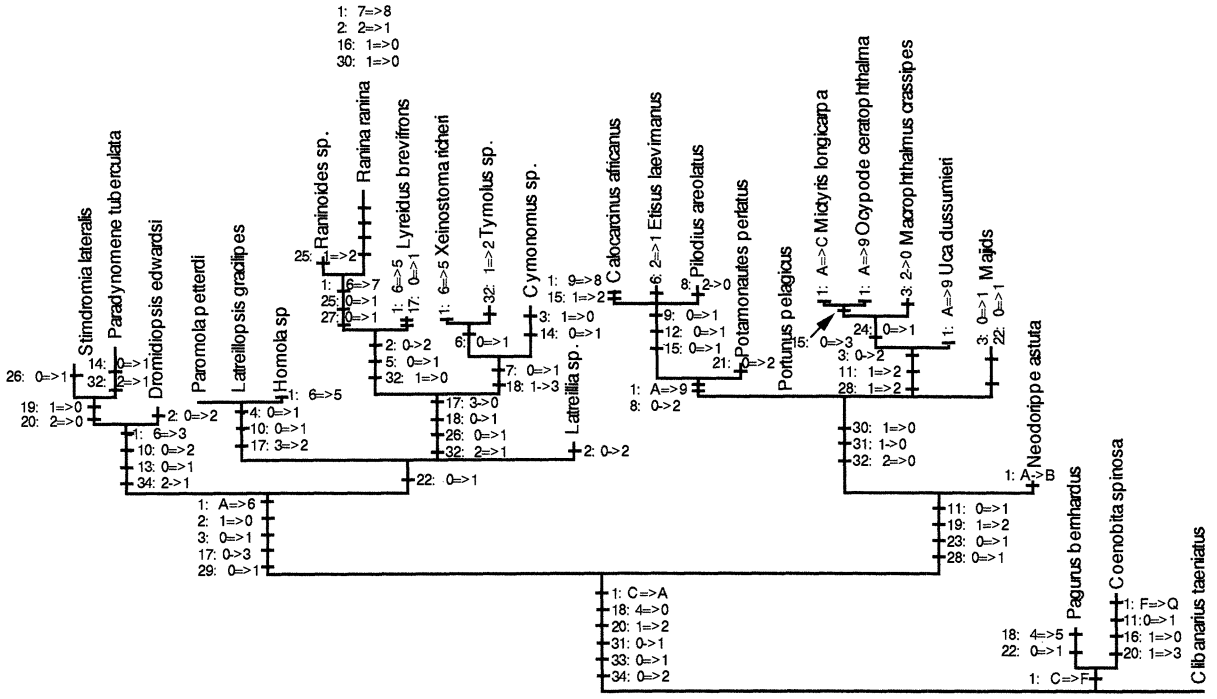


Figure 10. Unambiguous apomorphic changes on the nodes of the phylogram for combined spermatozoal and non-spermatozoal data which is shown in figure 2*b*.

that *Neodorippe* shares the habit of carrying a shell with some dromiids and the possibility exists that it has retained this mode from a podotrematous ancestor.

I am grateful to Dr Bertrand Richer de Forges for originally collecting much of the material used in this study and to Dr Danièle Guinot, for her constant advice and information. Mr Chris Tudge is thanked for his careful reading of the manuscript and for drawing many of the illustrations. Mrs L. Daddow, Mr D. Scheltinga and Mr C. Tudge gave excellent technical assistance. Two anonymous reviewers made constructive comments. This work was made possible by Australian Research Council funding.

REFERENCES

Abele, L.G. 1991 Comparison of morphological and molecular phylogeny of the Decapoda. *Mem. Qld Mus.* **31**, 101–108.

Abele, L.G., Kim, W. & Felgenhauer, B.E. 1989 Molecular evidence for inclusion of the phylum Pentastomida in the Crustacea. *Molec. biol. Evol.* **6**, 685–691.

Afzelius, B.A. 1979 Sperm structure in relation to phylogeny in the lower Metazoa. In *The spermatozoon: maturation, motility, surface properties and comparative aspects* (ed. D. W. Fawcett & J. M. Bedford), pp. 243–251. Baltimore: Urban and Schwarzenburg.

Alberti, G. 1990 Comparative spermatology of Araneae. *Acta Zool. Fennica* **190**, 17–34.

Anderson, W.A. & Ellis, R.A. 1967 Cytodifferentiation of the crayfish spermatozoon: acrosome formation, transformation of mitochondria and development of microtubules. *Zeitschr. Zellforsch. Mikrosk. Anat.* **77**, 80–94.

Asa, C.S. & Phillips, D.M. 1987 Ultrastructure of avian spermatozoa: a short review. In *New horizons in sperm cell research* (ed. H. Mohri), pp. 365–373. Tokyo/New York: Japan Sci. Soc. Press, Gordon and Breach Scientific Publications.

Ax, P. 1984 *Das Phylogenetische Systeme*. Stuttgart: Gustav Fischer.

Baccetti, B. 1978 The evolution of myriapod spermatozoa. *Abh. Ver. Naturwiss. Ver. Hamburg* **21/22**, 203–217.

Baccetti, B., Burrini, A.G. & Falchetti, E. 1991 Spermatozoa and relationships in Palaeognath birds. *Biol. Cell* **71**, 209–216.

Baccetti, B., Dallai, R., Grimaldi de Zio, S. & Marinari, A. 1973 The evolution of the nematode spermatozoon. *Gamete Res.* **8**, 309–323.

Balss, H. 1957 Decapoda. VIII. Systematik. In *Klassen und Ordnungen des Tierreichs* (ed. H. G. Bronns), pp. 1505–1672, fig. 1131–1199. Leipzig and Heidelberg: Winter, Funfter Band, I. Abteilung, 7. Buch, 12. Lief.

Breed, W.G. 1991 Variation in sperm head morphology of the Murinae (Family Muridae): some taxonomic implications. In *Comparative spermatology 20 years after* (ed. B. Baccetti), pp. 937–939. New York: Raven Press.

Burkenroad, M.D. 1963 The evolution of the Eucarida (Crustacea, Eumalacostraca), in relation to the fossil record. *Tulane Stud. Geol* **2**(1), 3–16.

Dudenhausen, E. & Talbot, P. 1979 Spermiogenesis in the crayfish, *Pacifastacus leniusculus*. *J. Cell Biol.* **83**, 225a.

Ehlers, U. 1985 Phylogenetic relationships within the Platyhelminthes. In *The Origin and relationships of lower invertebrates* (ed. S. C. Morris, J. D. George, R. Gibson & H. M. Platt), pp. 143–158. Oxford: Systematics Association. Clarendon Press.

Felgenhauer, B.E. & Abele, L.G. 1991 Morphological diversity of decapod spermatozoa. In *Crustacean sexual biology* (ed. J. Martin and R. Bauer), pp. 322–341. New York: Columbia University Press.

Felsenstein, J.S. 1985 Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Ferraguti, M. 1983 Annelida–Clitellata. In *Reproductive biology of invertebrates*, II. *Spermatogenesis and sperm function* (ed. K. G. Adiyodi & R. G. Adiyodi), pp.343–376. New York: John Wiley and Sons.

Franzén, Å. 1970 Phylogenetic aspects of the morphology of spermatozoa and spermiogenesis. In *Comparative spermatology* (ed. B. Baccetti), pp. 29–46. New York: Academic Press; and Rome: Accademia Nazionale dei Lincei.

Furieri, P. 1970 Sperm morphology of some reptiles: Squamata and Chelonia. In *Comparative spermatology* (ed. B. Baccetti), pp. 115–131. New York: Academic Press; and Rome: Accademia Nazionale dei Lincei.

Glaessner, M.F. 1969 Decapoda. In *Treatise on invertebrate paleontology*, part R, *Arthropoda* (ed. R. C. Moore), pp. 400–532. Boulder, Colorado: The Geological Society of America, Inc.

Guinot, D. 1977 Propositions pour une nouvelle classification des crustacés décapodes brachyoures. *C.r. Acad. Sci.*, Paris **D 285**, 1049–1052.

Guinot, D. 1978 Principes d'une classification évolutive des crustacés décapodes brachyoures. *Bull. Biol. France Belgique* **112**, 211–292.

Guinot, D. 1979 Données nouvelles sur la morphologie, la phylogenèse et la taxonomie des Crustacés Décapodes Brachyoures. *Mém. Mus. natn. Hist. nat., Paris* (A) Zool. **112**, 1–354.

Guinot, D. 1991 Etablissement de la famille des Poupiniidae pour *Poupinia hirsuta* gen. nov., sp. nov. de Polynésie (Crustacea Decapoda Brachyura Homoloidea). *Bull. Mus. natn. Hist. nat.*, Paris (A) **12** (3–4) 1990 (1991), 577–606.

Guinot, D., Jamieson, B.G.M. & Richer de Forges, B. 1994 Relationship of Homolidae and Dromiidae: evidence from spermatozoal ultrastructure (Crustacea, Decapoda). *Acta Zool.* **74**. (In the press.)

Gurney, R. 1942 Larvae of decapod Crustacea. Ray Soc. London, **129**, 1–306.

Healy, J.M. 1988 Sperm morphology and its systematic importance in the Gastropoda. *Malacol. Rev. (Suppl.)* **4**, 251–266.

Hillis, D.M. & Huelsenbeck, J.P. 1992 Signal, noise, and reliability in molecular phylogenetic analyses. *J. Hered.* **83**, 189–195.

Hinsch, G.W. 1969 Microtubules in the sperm of the spider crab, *Libinia emerginata* L. *J. Ultrastruct. Res.* **29**, 525–534.

Hinsch, G.W. 1973 Sperm structure of *Oxyrhyncha*. *Can. J. Zool.* **51**, 421–426.

Hinsch, G.W. 1980 Spermiogenesis in *Coenobita clypeatus* I. Sperm structure. *Int. J. Invert. Reprod.* **2**, 189–198.

Holland, L.Z. 1989 Fine structure of spermatids and sperm of *Dolioletta gegenbauri* and *Doliolum nationalis* (Tunicata: Thaliacea): implications for tunicate phylogeny. *Mar. Biol.* **101**, 83–95.

Huelsenbeck, J.P. 1991 Tree-length distribution skewdness: an indicator of phylogenetic information. *Syst. Zool.* **40** (3), 257–270.

Jamieson, B.G.M. 1981 *The ultrastructure of the Oligochaeta*. London, New York: Academic Press.

Jamieson B.G.M. 1983 Spermatozoal ultrastructure: evolution and congruence with a holomorphological phylogeny of the Oligochaeta (Annelida). *Zool. Scripta* **12**, 107–114.

Jamieson, B.G.M. 1987 *The ultrastructure and phylogeny of insect spermatozoa*. Cambridge University Press.

Jamieson, B.G.M. 1989*a* The ultrastructure of the spermatozoa of four species of xanthid crabs (Crustacea, Brachyura, Xanthidae). *J. Submicrosc. Cytol. Pathol.* **21**, 579–586.

Jamieson, B.G.M. 1989*b* Ultrastructural comparison of the spermatozoa of *Ranina ranina* (Oxystomata) and of *Portunus pelagicus* (Brachygnatha) (Crustacea, Brachyura). *Zoomorphology* **109**, 103–111.

Jamieson, B.G.M. 1990 The ultrastructure of the spermatozoa of *Petalomera lateralis* (Gray) (Crustacea, Brachyura, Dromiacea) and its phylogenetic significance. *Invert. Reprod. Devel.* **17**, 39–45. 17.

Jamieson, B.G.M. 1991*a* Ultrastructure and phylogeny of crustacean spermatozoa. *Mem. Qld Mus.* **31**, 109–142.

Jamieson, B.G.M. 1991*b* *Fish evolution and systematics: evidence from spermatozoa*. Cambridge University Press.

Jamieson, B.G.M. 1993*a* Ultrastructure of the spermatozoon of *Potamonautes perlatus sidneyi* (Heterotremata, Brachyura, Crustacea). *S. Afr. J. Zool.* **28**(1), 40–45.

Jamieson, B.G.M. 1993*b* Spermatological evidence for the taxonomic status of *Trapezia* (Heterotremata, Brachyura, Crustacea). *Mem. Qld Mus.* **33**, 225–234.

Jamieson, B. G. M., Guinot, D. & Richer de Forges, B. 1993 The spermatozoon of *Calocarcinus africanus* (Heterotremata, Brachyura, Crustacea): ultrastructural synapomorphies with xanthid sperm. *Invert. Reprod. Devel.* **24**, 189–196.

Jamieson, B.G.M., Guinot, D. & Richer de Forges, B. 1994*a* The ultrastructure of the spermatozoon of *Paradynomene tuberculata* Sakai, 1963, (Crustacea, Brachyura, Dynomenidae): synapomorphies with dromiid sperm. *Helgol. Meeresunters* **47**(3), 311–322.

Jamieson, B.G.M., Guinot, D. & Richer de Forges, B. 1994*b* Relationships of the Cyclodorippoidea Ortmann: evidence from spermatozoal ultrastructure in the genera *Xeinostoma*, *Tymolus* and *Cymonomus* (Crustacea, Decapoda). *Invert. Reprod. Devel.* (Submitted.)

Jamieson, B.G.M., Guinot, D. & Richer de Forges, B. 1994*c* Podotreme affinities of *Raninoides* sp. and *Lyreidus brevifrons*: evidence from spermatozoal ultrastructure (Crustacea, Brachyura, Raninoidea). *Mar. Biol.* (In the press.)

Jamieson, B.G.M., Guinot, D. & Richer de Forges, B. 1994*d* Spermatozoal ultrastructure in four genera of Homolidae (Crustacea, Decapoda): exemplified by *Homologenus* sp., *Latreillopsis* sp., *Homolomannia sibo*gae and *Paramolopsis boasi*. *Helgol. Meeresunters* **47**(3), 323–334.

Jamieson B.G.M., Erséus, C. & Ferraguti, M. 1987 Parsimony analysis of the phylogeny of some Oligochaeta (Annelida) using spermatozoal ultrastructure. *Cladistics* **3**, 141–155.

Jamieson, B.G.M., Lee, M.S.Y. & Long, K. 1992 Ultrastructure of the spermatozoon of the internally fertilizing frog *Ascaphus truei* (Ascaphidae, Anura, Amphibia) confirms its primitive status. *Herpetologica* **49**, 52–65.

Jamieson B.G.M. & Rouse G.W. 1989 The spermatozoa of the Polychaeta (Annelida): an ultrastructural review. *Biol. Rev.* **64**, 93–157.

Jamieson, B.G.M. & Scheltinga, D.M. 1993 The ultrastructure of spermatozoa of *Nangura spinosa* (Scincidae, Reptilia). *Mem. Qld Mus.* **34**(1), 169–179.

Jamieson, B.G.M. & Tudge, C.C. 1990 Dorippids are Heterotremata: evidence from ultrastructure of the spermatozoa of *Neodorippe astuta* (Dorippidae) and *Portunus pelagicus* (Portunidae) Brachyura: Decapoda. *Mar. Biol.* **106**, 347–354.

Jamieson, B.G.M., Tudge, C.C. & Scheltinga, D.M. 1994*e* The ultrastructure of the spermatozoon of *Dromidiopsis edwardsi* Rathbun, 1919 (Crustacea, Brachyura, Dromiidae): confirmation of a dromiid sperm type. *Aust. J. Zool.* **41**, 537–538.

Justine, J.L. 1991 Phylogeny of parasitic Platyhelminthes: a critical study of synapomorphies proposed on the basis of the ultrastructure of spermiogenesis and spermatozoa. *Can. J. Zool.* **69**, 1421–1440.

Lee, M.S.Y. & Jamieson, B.G.M. 1992 The ultrastructure of the spermatozoa of three species of myobatrachid frogs (Anura, Amphibia) with phylogenetic considerations. *Acta Zool.* (Stockholm) 73, 213–222.

Mattei, X. 1991 Spermatozoon ultrastructure and its systematic implications in fishes. *Can. J. Zool.* **69**, 3038–3055.

Pike, R.B. & Williamson, D.I. 1960 Larvae of Decapod Crustacea of the families Dromiidae and Homolidae from the Bay of Naples. *Pubbl. Staz. Zool. Napoli* **31**, 553–563.

Pochon-Masson, J., Bocquet-Vedrine, J. & Turquier, Y.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

1970 Contribution a l'étude du spermatozoïde des crustacés cirripedes. In *Comparative spermatology* (ed. B. Baccetti), pp. 205–219. New York: Academic Press; and Rome: Accademia Nazionale dei Lincei.

Pugin-Rios, E. 1980 Étude comparative sur la structure du spermatozoïde des Amphibiens Anoures. Comportement des gamètes lors de la fécondation. Thèse, L'Université de Rennes.

Rice, A.L. 1970 Decapod crustacean larvae collected during the International Indian Ocean Expedition. Families Raninidae and Homolidae. *Bull. Brit. Mus. nat. Hist.* **21**, 1–24, figs 1–9.

Rice, A.L. 1980 Crab zoeal morphology and its bearing on classification of the Brachyura. *Trans. Zool. Soc. Lond.* **35**, 271–424.

Rice, A.L. 1981*a* Crab zoeae and brachyuran classification: a re-appraisal. *Bull. Brit. Mus. (nat. Hist.)*, Zool. **40**(5), 287–296.

Rice A.L. 1981*b* The megalopa stage in brachyuran crabs. The Podotremata Guinot. *J. nat. Hist.* **15**, 1003–1011.

Rice, A.L. 1983 Zoeal evidence for brachyuran phylogeny. In *Crustacean phylogeny* (ed. F. R. Schram), pp. 313–329. Rotterdam: Balkema.

Rice, A.L. & Provenzano, A.J. 1970 The larval stages of *Homola barbata* (Fabricius) (Crustacea, Decapoda, Homolidae) reared in the laboratory. Biological Results of the University of Miami, Deep-Sea Expeditions, 55. (*Bull. Mar. Sci.* **20**(2)), 446–471.

Rouse, G.W. & Robson, S.K. 1986 An ultrastructural study of megachiropteran (Mammalia: Chiroptera) spermatozoa: implications for chiropteran phylogeny. *J. Submicr. Cytol.* **18**, 137–152.

Schmidt, H. & Zissler, D. 1979 Die Spermien der Anthozoen und ihre phylogenetische Bedeutung. *Zoologica* (Stuttgart) **44**, 1–98.

Spears, T. & Abele, L.G. 1988 Molecular phylogeny of brachyuran crustaceans based on 18S rRNA nucleotide sequences. *Am. Zool.* **28**, 2A (Abstract).

Spears, T., Abele, L.G. & Kim, W. 1992 The monophyly of brachyuran crabs: a phylogenetic study based on 18S rRNA. *Syst. Biol.* **41**(4), 446–461.

Stevcic, Z. 1971 The main features of brachyuran evolution. *Syst. Zool.* **20**, 331–340.

Storch, V. & Jamieson, B.G.M. 1992 Further spermatological evidence for the phylogenetic relationships of the pentastomida (Tongue worms). *Int. J. Parasit.* **22**, 95–108.

Swofford, D. 1993 PAUP: Phylogenetic analysis using parsimony, version 3.0s. Computer program distributed by the Illinois Natural History Survey, Champaign, Illinois.

Temple-Smith, P. 1987 Sperm structure and marsupial phylogeny. In *Possums and opossums: studies in evolution* (ed. M. Archer), pp. 171–193. Sydney: Surrey Beatty & Sons and the Royal Zoological Society of New South Wales.

Trueman, J.W.H. 1993 Randomization confounded: a response to Carpenter. *Cladistics*, **9**(1), 101–109.

Tudge, C.C. 1992 Comparative ultrastructure of hermit crab spermatozoa (Decapoda: Anomura: Paguroidea). *J. Crust. Biol.* **12**(3), 397–409.

Tudge, C.C. & Jamieson, B.G.M 1991 Ultrastructure of the mature spermatozoon of the Coconut Crab *Birgus latro* (L.) (Coenobitidae, Paguroidea, Decapoda). *Mar. Biol.* **108**, 395–402.

Warner, G.F. 1977 *The biology of crabs*. London: Elek Science.

Williamson, D.I. 1965 Some larval stages of three Australian crabs belonging to the families Homolidae and Raninidae, and observations on the affinities of these families (Crustacea: Decapoda). *Aust. J. Mar. Freshw. Res.* **16**, 369–398.

Williamson, D.I. 1974 Larval characters and the origin of crabs (Crustacea, Decapoda, Brachyura). *Thalassia Jugosl.* **10**, 401–414.

Williamson, D.I. 1988 Evolutionary trends in larval form. *Symp. Zool. Soc. Lond.* **59**, 11–25.

Williamson, D.I. 1992 Larvae and Evolution: Toward a New Zoology. London: Chapman and Hall.

Wingstrand, K.G. 1972 Comparative spermatology of a pentastomid, *Raillietiella hemidactyli* and a branchiuran crustacean, *Argulus foliaceus*, with a discussion of pentastomid relationships. *Kong. Dan. Vid. Selsk. Biol. Skrift.* **19**, 1–72.

Wirth, U. 1991 Cladistic analysis of sperm characters. In *Comparative spermatology 20 years after* (ed. B. Baccetti), vol. 75, pp. 1025–1029. New York: Serono Publications from Raven Press.

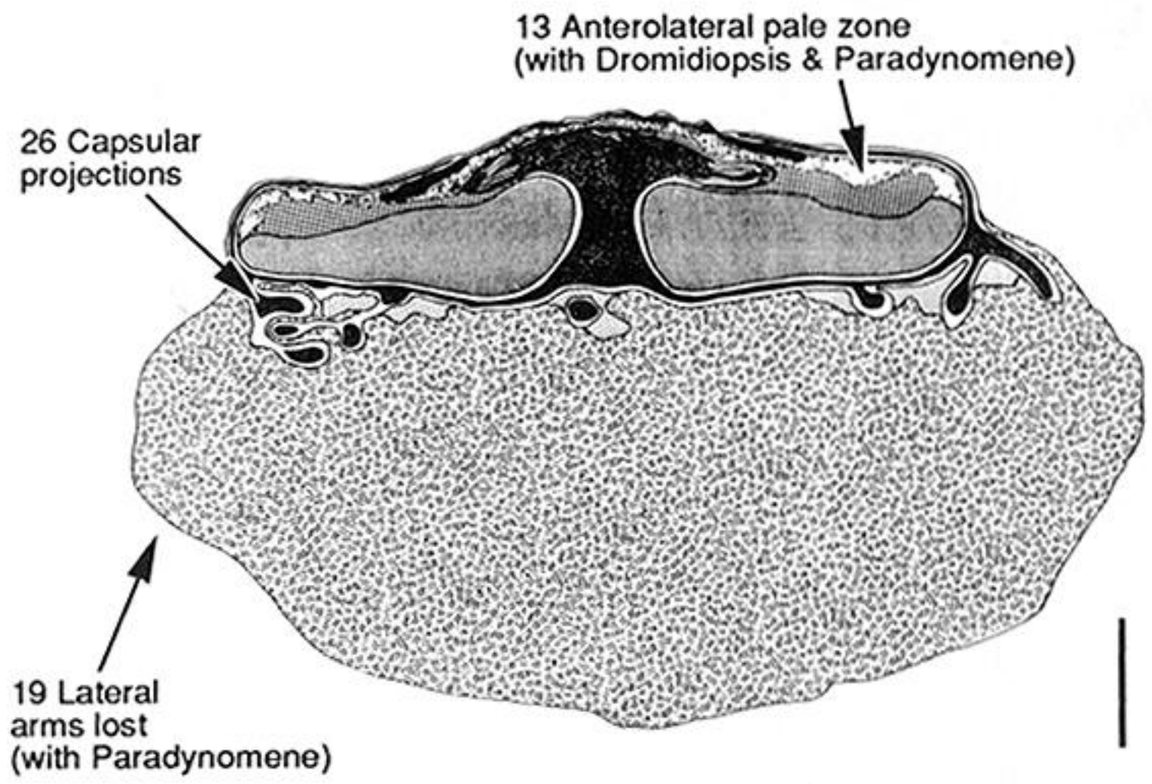
Wright, C.W. & Collins, J.S.H. 1972 British Cretaceous crabs. In *Paleontographical Society monographs*. pp. 114. London: Paleontographical Society.

(*Received 18 January 1994; accepted 22 February 1994*)

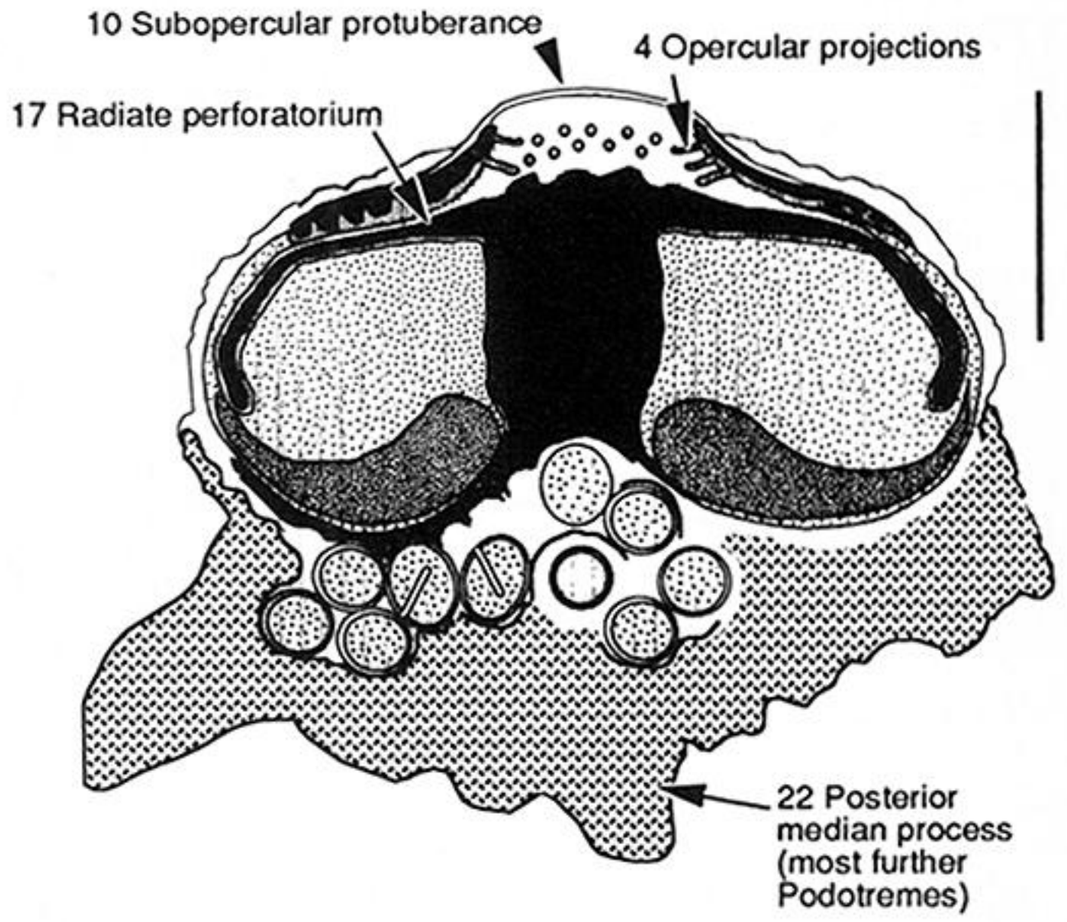
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PODOTREMATA

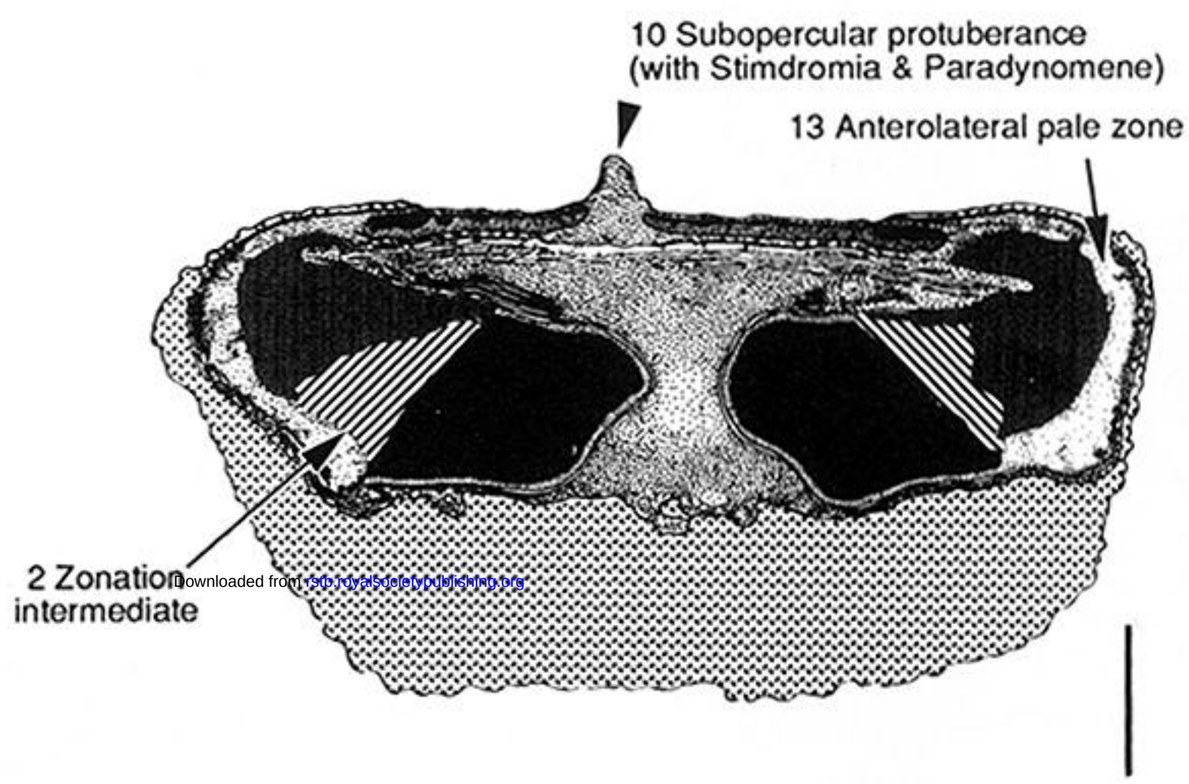
1 Acrosome depressed
2 Zonation of acrosome horizontal
3 Operculum perforate



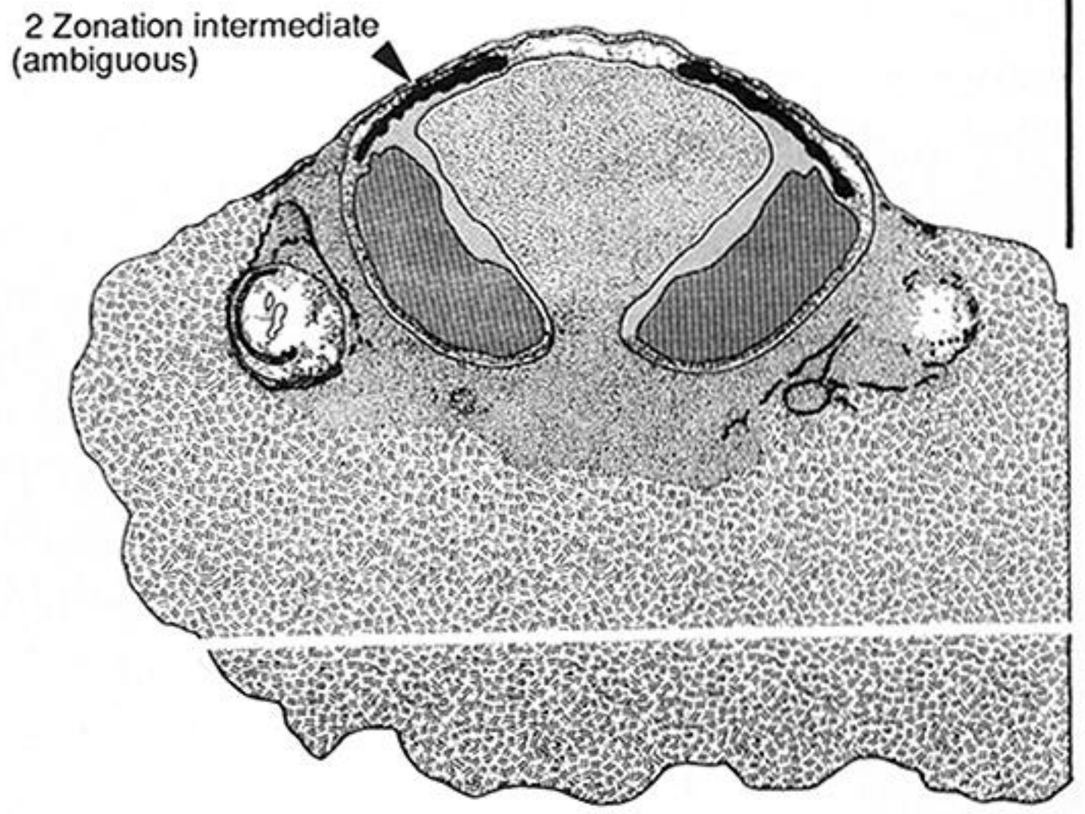
(*a*) *Stimdromia lateralis* (Dromiidae)



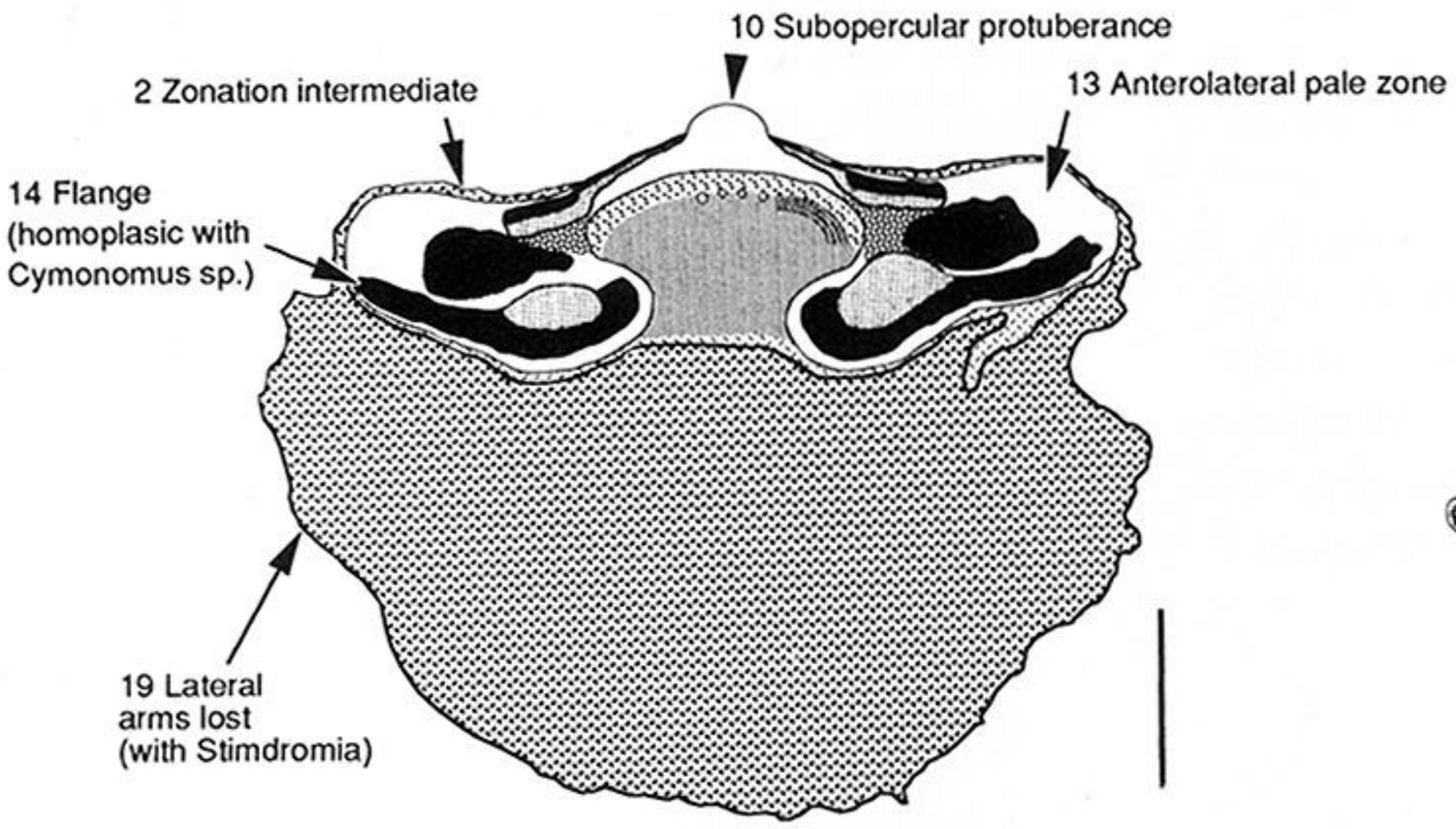
(*d*) *Latreillopsis gracilipes* (Homolidae)



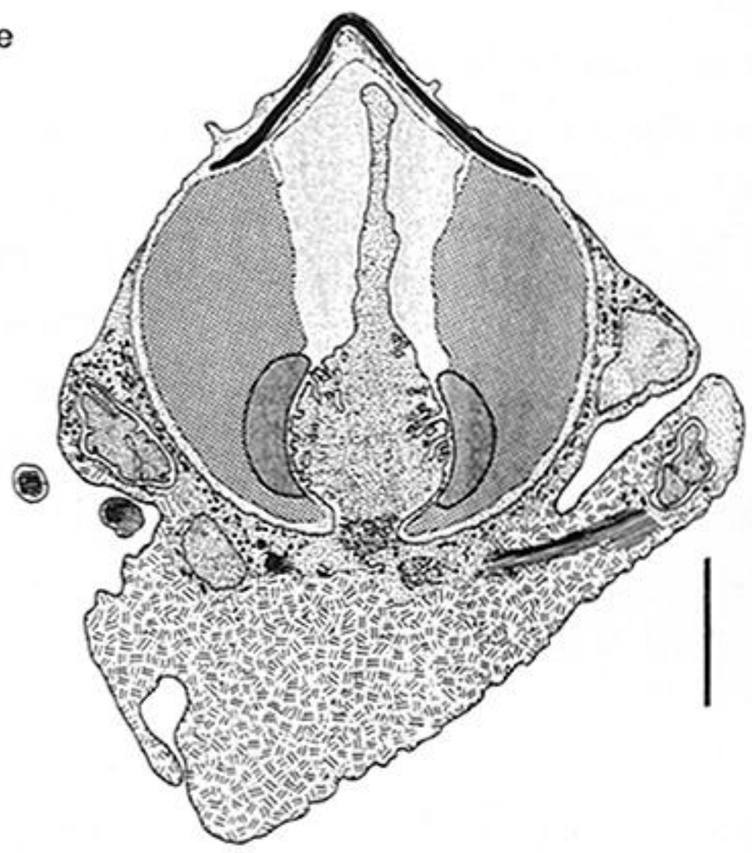
(*b*) *Dromidiopsis edwardsi* (Dromiidae)



(*e*) *Latreillia* sp. (Latreilliidae)



(*c*) *Paradynomene tuberculata* (Dynomenidae)

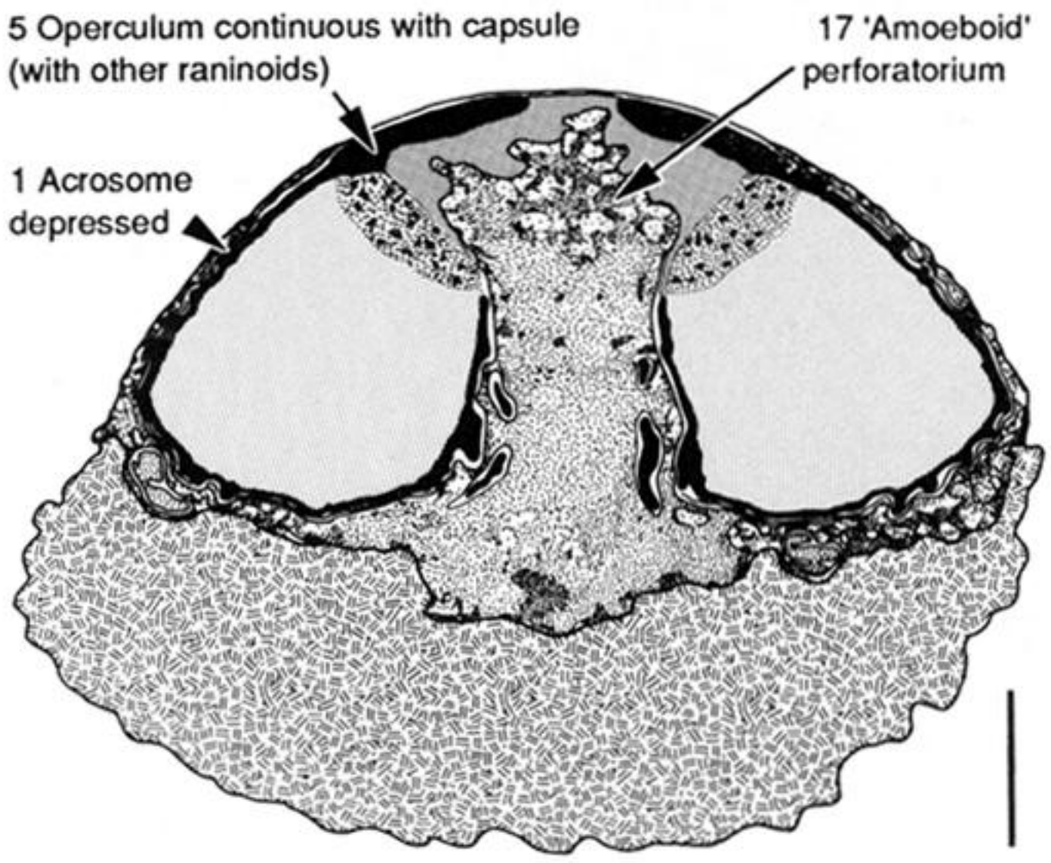


(*f*) *Clibanarius taeniatus* (Diogenidae)

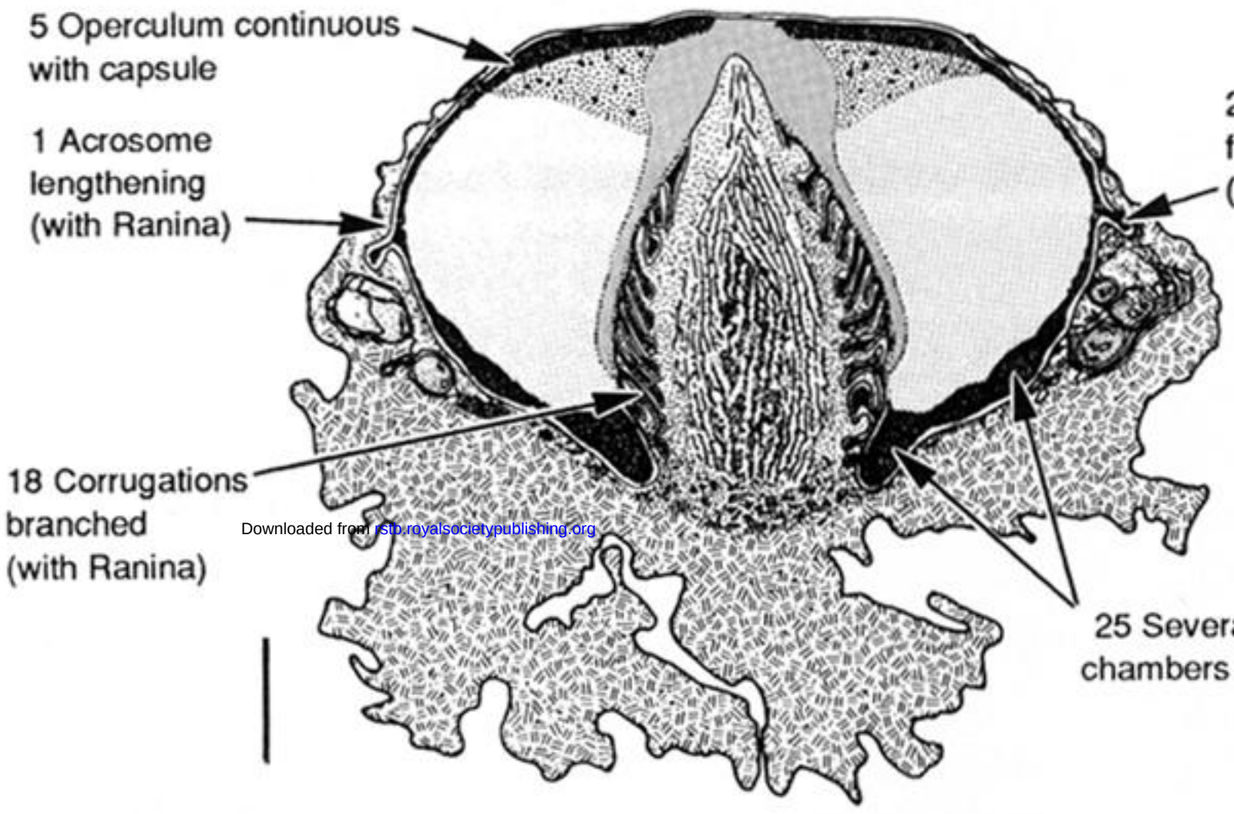
Figure 5. Drawings of spermatozoa of some podotremes and an anomuran used in this analysis. (*a*) *Stimdromia lateralis* (Dromiidae); (*b*) *Dromidiopsis edwardsi* (Dromiidae); (*c*) *Paradynomene tuberculata* (Dynomenidae); (*d*) *Latreillopsis gracilipes* (Homolidae); (*e*) *Latreillia* sp. (Latreilliidae); (*f*) *Clibanarius taeniatus* (Anomura, Diogenidae). The chief apomorphies are indicated but see text for a detailed explanation. The section for *Stimdromia* is not precisely sagittal; in micrographs which are sagittal, perforation of the operculum is seen. Scale bar 1 μm. Sources as listed in § 2.

RANINOIDEA

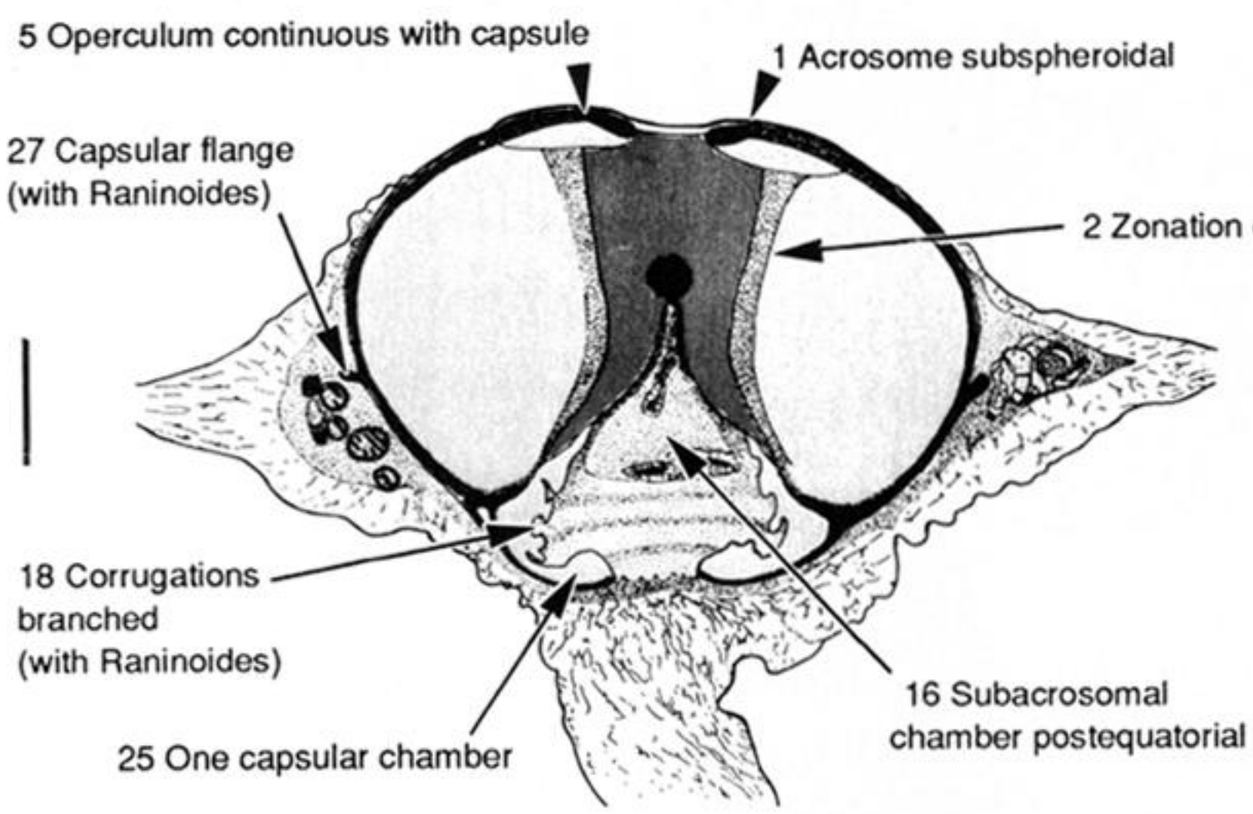
2 Zonation of acrosome intermediate (ambiguous)
5 Operculum continuous with capsule



(*a*) *Lyreidus brevifrons* (Raninidae)



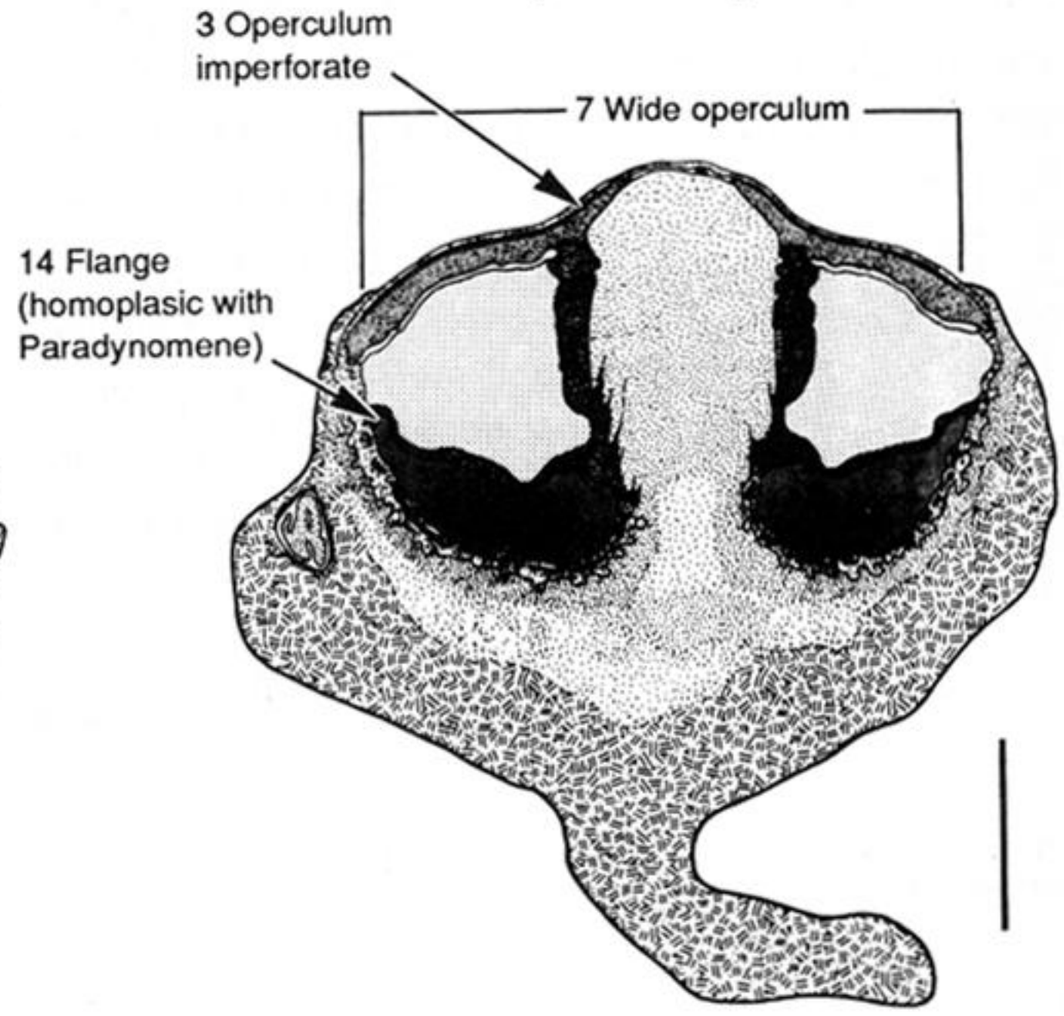
(*b*) *Raninoides* sp. (Raninidae)



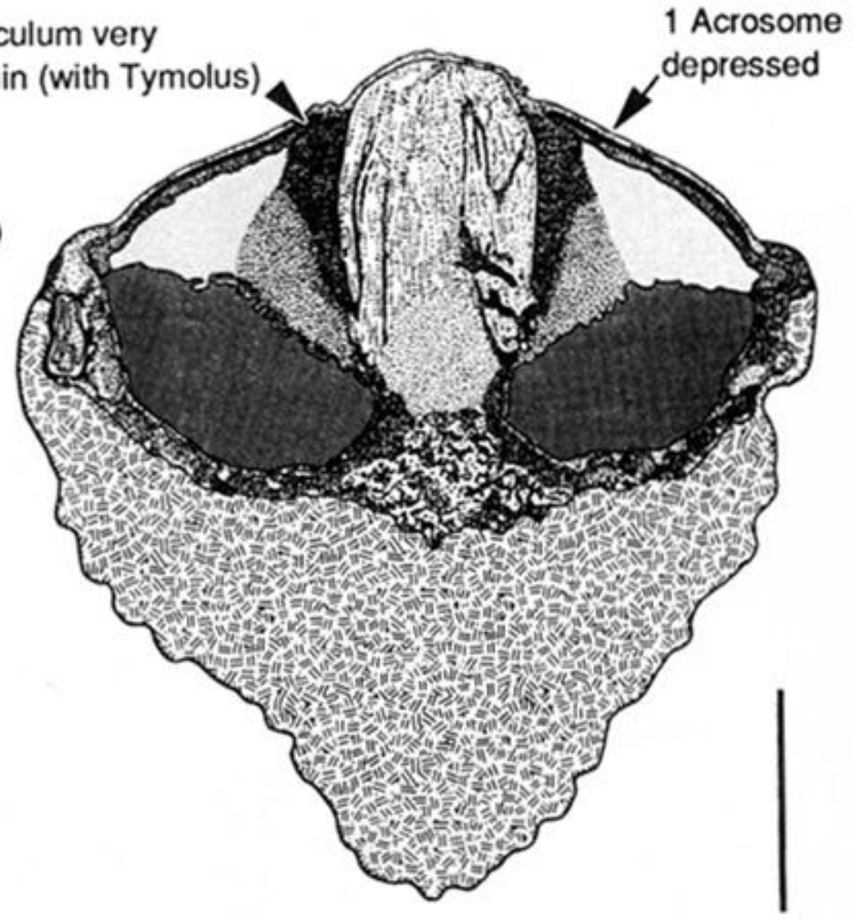
(*c*) *Ranina ranina* (Raninidae)

CYCLODORIPPOIDEA

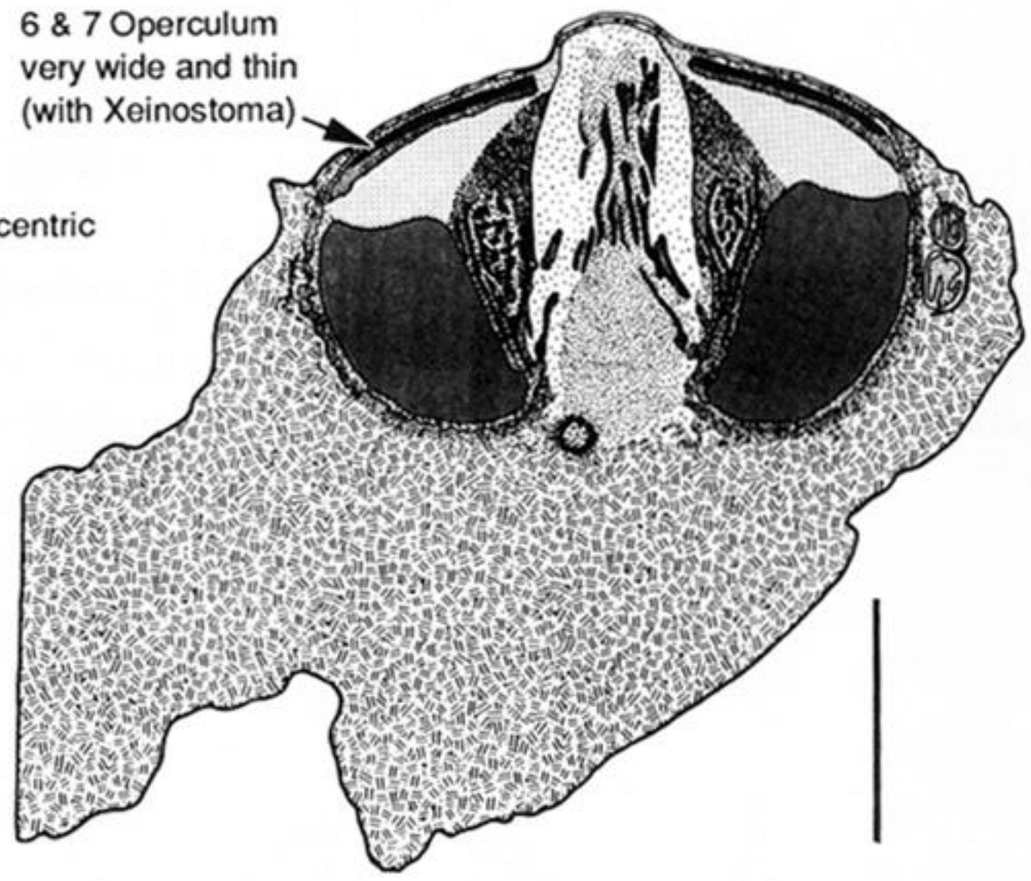
7 Operculum very wide



(*d*) *Cymonomus* sp. (Cymonomidae)



(*e*) *Xeinostoma richeri* (Cyclodorippidae)



(*f*) *Tymolus* sp. (Cyclodorippidae)

Figure 6. Drawings of spermatozoa of further podotremes used in this analysis. (*a*) *Lyreidus brevifrons* (Raninidae, Lyreidinae); (*b*) *Raninoides* sp.(Raninidae, Raninoidinae); (*c*) *Ranina ranina* (Raninidae, Raninae); (*d*) *Cymonomus* sp. (Cymonomidae); (*e*) *Xeinostoma richeri* (Cyclodorippidae, Xeinostominae); (*f*) *Tymolus* sp. (Cyclodorippidae, Cyclodorippinae). The chief apomorphies are indicated but see text for a detailed explanation. Scale bar 1 µm. Sources as listed in § 2.

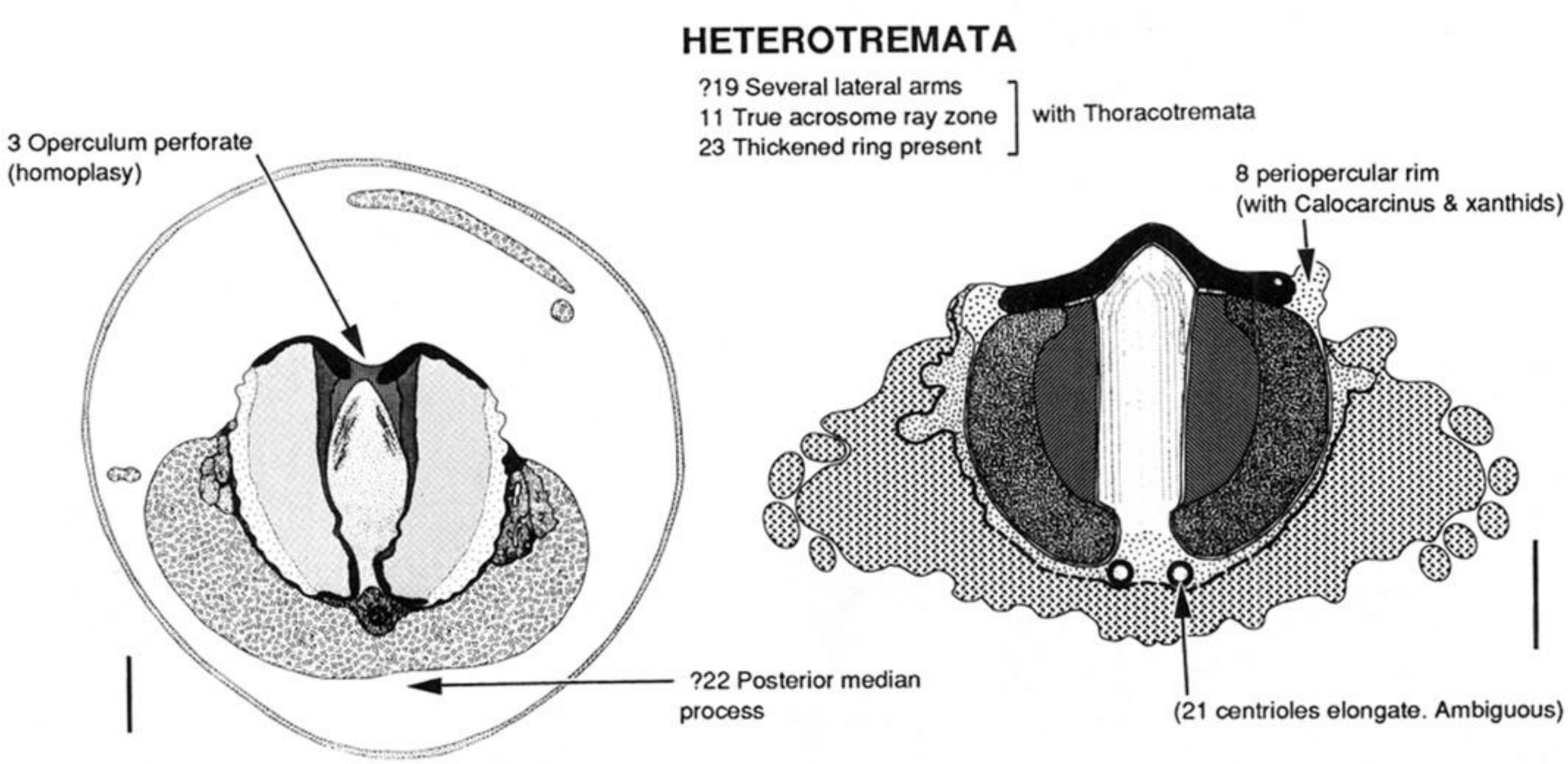
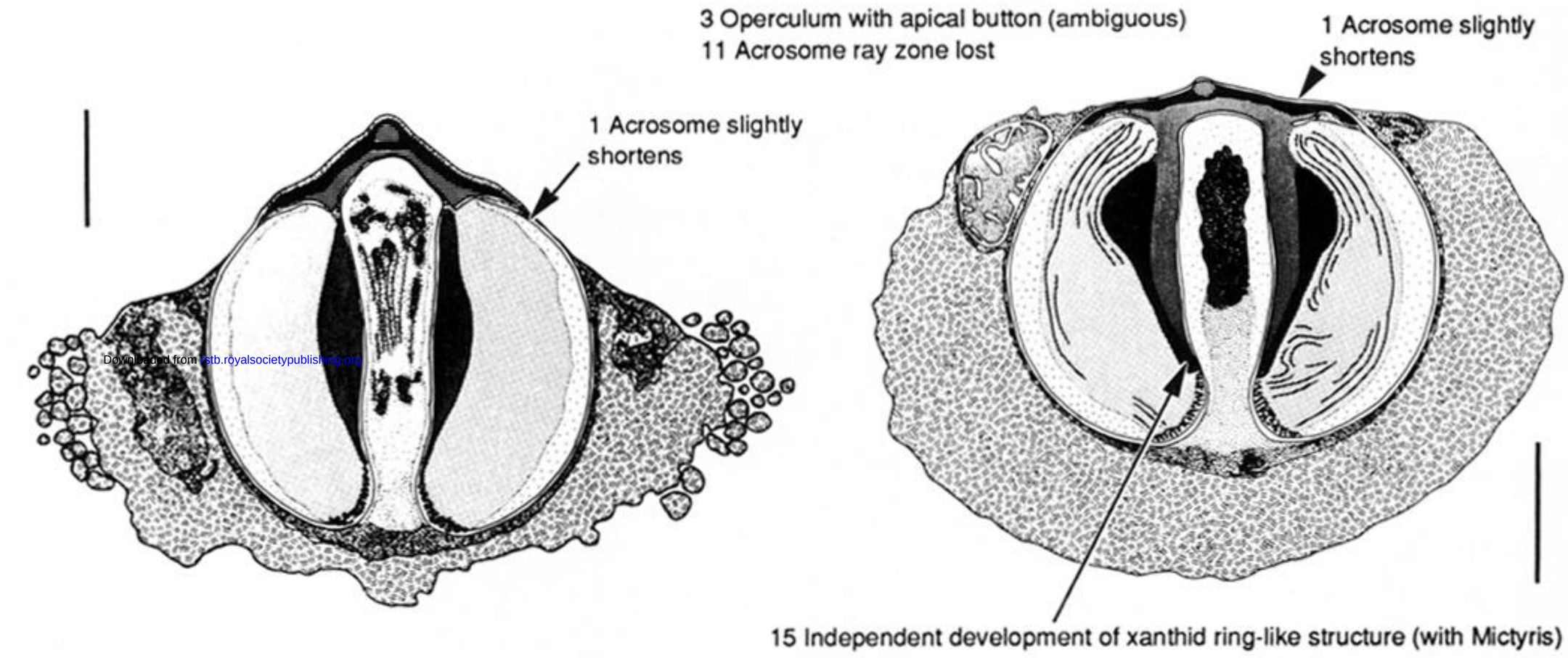


Figure 7. Drawings of spermatozoa of Heterotremata used in this analysis. (*a*) *Menaetheus monoceros* (Majidae); (*b*) *Neodorippe astuta* (Dorippidae); (*c*) *Portunus pelagicus* (Portunidae); (*d*) *Potamonautes perlatus* (Potamidae); (*e*) *Pilodius areolatus* (Xanthidae); (*f*) *Calocarcinus africanus* (Trapeziidae). The chief apomorphies are indicated but see text for a detailed explanation. Scale bar 1 µm. Sources as listed in § 2.



(*a*) *Uca dussumieri* (Ocypodidae)

(*c*) *Ocypode ceratophthalma* (Ocypodidae)

(*b*) *Macrophthalmus crassipes* (Ocypodidae)

(*d*) *Mictyris longicarpa* (Mictyridae)

igure 8. Drawings of spermatozoa of Thoracotremata used in this analysis. (*a*) *Uca dussumieri*; (*b*) *Macrophthalmus assipes*; (*c*) *Ocypode ceratophthalma* (all Ocypodidae); (*d*) *Mictyris longicarpa* (Mictyridae). The chief apomorphies re indicated but see text for a detailed explanation. Scale bar 1 µm. Sources as listed in § 2.

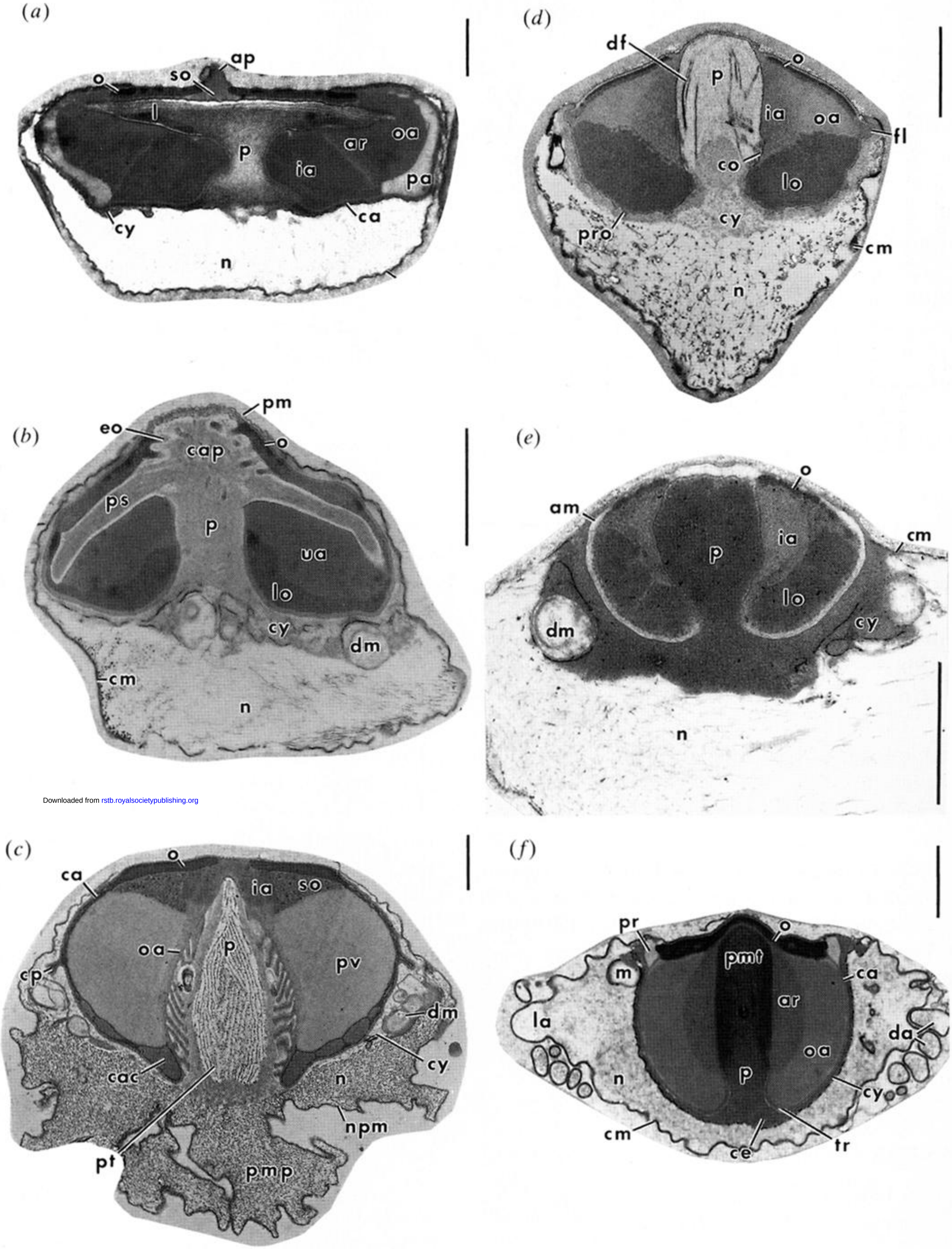


Figure 9. Transmission electron micrographs of longitudinal sagittal sections of representative podotreme sperm: (*a*) a dromiid (*Dromidiopsis edwardsi*); (*b*) an homolid (*Latreillopsis gracilipes*); (*c*) a raninoid (*Raninoides* sp.); (*d*) a cyclodorippoid (*Xeinostoma richeri*); (*e*) a latreilliid (*Latreillia* sp.); and (*f*) a heterotreme (*Potamonautes perlatus*). am; acrosome membrane, ap; apical protuberance, ar; acrosome ray zone, ca; capsule, cac; capsular chambers, cap, capitate region of perforatorium, ce; centriole, cm; cell membrane, co; corrugations, cp; capsular projection, cy; cytoplasm, da; divarications of arms, df; dense filament, dm; degenerate mitochondrion, eo, extensions of operculum, fl; periacrosomal flange, ia; inner acrosome zone, l; lamellae, la; lateral arm, lo; lower acrosome zone, m; mitochondrion, n; nucleus, npm; nuclear-plasma membrane, o; operculum, oa; outer acrosome zone, p; perforatorium, pa; anterolateral pale zone, pmp; posterior median process, pmt; microtubules of perforatorium, pr; peripercular rim, pro; projections of acrosome membrane, ps; perforatorial spike, pt; perforatorial tubules, pv; peripheral acrosome zone, so; subopercular zone, tr; thickened ring, ua; upper acrosome zone.