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# Podotreme affinities of *Raninoides* sp. and *Lyreidus brevifrons*: evidence from spermatozoal ultrastructure (Crustacea: Brachyura: Raninoidea)

**Abstract** Spermatozoal synapomorphies which singly or collectively distinguish Raninoidea are: (1) the presence of single (*Ranina, Raninoides*) or multiple (*Lyreidus*) keel-like projections of the acrosomal capsule; (2) a very large, weakly electron-opaque peripheral acrosomal zone (*Ranina, Raninoides*) and an homologous large outer zone in *Lyreidus;* (3) poor differentiation of the operculum from the capsule (autapomorphy); (4) a very well developed, perforate subopercular zone, of variable form; (5) presence of unique inward longitudinal projections (septa or corrugations) in the wall of the subacrosomal chamber (autapomorphy). Shared, presumably synapomorphic characters of *Ranina* and *Raninoides* but not of *Lyreidus* within the Raninidae, are: (1) branching of some of the subacrosomal septa (unbranched in *Lyreidus*); (2) the subspheroidal form of the acrosome in *Ranina* with a length:width ratio (L:W) of 0.76, and, although slightly more depressed, in *Raninoides* (L:W 0.73), considered apomorphic relative to the more depressed form in *Lyreidus* (L:W 0.52); (3) single or multiple coiled perforatorial filaments (*Ranina, Raninoides*) contrasting with a capitate perforatorium with "amoeboid" head in *Lyreidus*; (4) division of the capsule wall to give one posterior (*Ranina*) or multiple enclaves, plesiomorphically (?) absent in *Lyreidus*. Similarities of *Lyreidus* with other podotremes include the capitate perforatorium, questionably related to the radiate spiked-wheel structure of homolids in which acrosome proportions are similar or less closely to the bilateral capitate perforatorium of dromiids and dynomenids, and basal capsular projections as in the dromiid *Stimdromia* (=*Petamolera*) *lateralis* and in cyclodorippoids. No spermatozoal synapomorphies support a sister-group relationship of raninoids and heterotreme-thoracotreme crabs.

## Introduction

The superfamily Raninoidea contains the single family Raninidae which is subdivided into several subfamilies. Prior to the present work, the spermatozoon of only a single species of the Raninidae had been described ultrastructurally, that of *Ranina ranina* (Linné, 1758) (Jamieson 1989), the sole member of the subfamily Ranininae (emend. Guinot 1993).

Features which the sperm of *Ranina ranina* share with "higher" Brachyura, above the Dromioidea and Homoloidea, were shown to include: the large subspheroidal acrosome, ensheathed by a thin layer of cytoplasm which is in turn cupped by the nucleus; extension of the nucleus as lateral arms and as a posterior median process (absent in the more advanced families, including portunids); extension of the cytoplasm into the basal region of each nuclear arm but not into the posterior process; and topographical equivalence and presumed homology of the various components of the acrosome, including differentiation of an apical operculum.

Because the sperm of the Dromiidae ("sponge crabs") were imperfectly known and those of the Dynomenidae and Homoloidea were unknown, it was not possible confidently to establish which of the above characters shared between Raninidae and "higher" crabs of the Oxyrhyncha–Cancridea–Brachyrhyncha assemblage (more appropriately termed the Heterotremata and Thoracotremata sensu Guinot 1978) were advanced states (synapomorphies) and

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which were states carried over from non-raninid ancestors (symplesiomorphies). The subspherical form of the acrosome of *Ranina ranina* was considered to be a synapomorphy of the Raninoidea with the Oxyrhyncha–Cancridea–Brachyrhyncha assemblage and to contraindicate inclusion of the Raninidae in the Dromiacea advocated by Stevcic (1973).

Although the sperm of *Ranina ranina* displayed characters which were unique for known brachyuran spermatozoa, it could only be surmised which of these might prove to be present in other raninid sperm and thus constitute synapomorphies diagnostic of the Raninoidea (autapomorphies). These unique characters of *R. ranina* included: penetration of the subacrosomal space anteriorly only to the equator of the acrosome and its conical form; differentiation within the subacrosomal material of a tortuous, filiform putative perforatorium; subdivision of the acrosome vesicle by division of the capsule to give a posterior acrosomal chamber and differentiation of the walls of this lining the subacrosomal chamber (infolded acrosome vesicle) as longitudinal pallisade-like corrugations. Absence of centrioles in *R. ranina* was considered an apomorphic homoplasy shared with some "brachygnathan" crabs in others of which, however, centrioles are plesiomorphically retained.

Spermatozoa of two further species of the Raninoidea are described in the present work: *Raninoides* sp., family Raninidae, subfamily Raninoidinae Lörenthey and Beurlen, 1929 (reinstated by Guinot 1993), and *Lyreidus brevifrons* Sakai, 1937. The genus *Lyreidus* de Haan, 1841, deserves separate subfamilial status within the same family (Guinot personal communication). These descriptions allow determination of spermatozoal synapomorphies between the genera *Ranina* and *Raninoides*, and comparison with the less similar sperm of *Lyreidus*. The study therefore permits a re-evaluation of spermatozoal synapomorphies within the Raninoidea and between these and other crabs.

## Materials and methods

Specimens of *Raninoides* sp. [currently being described in the Laboratoire de Zoologie (Arthropodes), Muséum National d'Histoire Naturelle, Paris] and *Lyreidus brevifrons* were collected by B. Richer de Forges during the "Bathus 1" cruise on the R. V. "Alis" in March 1993, at Stations DW653 (*Raninoides*) and CP656 (*Lyreidus*) on the east coast of New Caledonia. Portions of the testes and male ducts were fixed in 3% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.2) with 3% sucrose at 4 °C for 2 h, and were despatched in the fixative to Brisbane for further processing. On receipt in Brisbane they were washed in buffer, post-fixed for 80 min in similarly buffered 1% osmium tetroxide, washed in three 15 min changes of buffer, dehydrated through an ethanol series, and infiltrated and embedded in Spurr's epoxy resin. Sections were cut with diamond knives, on an LKB 2128 UM IV microtome. Thin sections, 50 to 80 nm thick, were collected on carbon-stabilized colloidin-coated 200-mesh copper grids, stained for 30 s in lead citrate, rinsed in distilled water, stained for 1 min in 6% aqueous uranyl acetate, rinsed in distilled water, and stained for a further 30 s in lead citrate, before final rinsing. Electron micrographs were taken on an Hitachi 300 electron microscope at 75 kV and a JEOL 100 at 60 kV.

## Results

### *Raninoides sp.*

*General morphology.* In the following account, some comparison will be made with the sperm of *Ranina ranina* first described by Jamieson (1989). The most conspicuous components of the spermatozoon of *Raninoides* sp. (refer to line drawing, Fig. 1, throughout) are an acrosome, arbitrarily considered to be "anterior", which has the form of a thick ellipse, and the large posterior nucleus which is continuous with three lateral projections or "arms" and a posterior median process.

*Acrosome.* The ellipsoidal core of the *Raninoides* sp. spermatozoon consists entirely of the complex acrosome (Fig. 2 A). The acrosome has a mean length to width ratio of 0.73. It is invested by a thin membrane, the acrosome membrane which, except where it covers the operculum, is underlain by a thin electron pale, almost hyaline, layer. This in turn surrounds an electron-dense sheath, the "capsule" (Fig. 2 A), which is interrupted at the apical ("anterior") pole and invaginated at the posterior pole. The mean length of the acrosome is 4.5 μm (range = 4.4 to 4.7 μm, $n$ = 3); the mean width is 6.2 μm (range = 5.7 to 6.5 μm, $n$ = 3).

At the anterior pole of the *Raninoides* sp. and *Ranina ranina* acrosome, there is a dense ring (Fig. 2 A) surrounding a central perforation. This ring is considered homologous with the operculum of other brachyuran and anomuran sperm. In both *Raninoides* sp. and *Ranina ranina*, it is poorly differentiated, consisting merely of a strongly electron-dense apical border of the capsule which is here at its thickest (Fig. 2 A); the mean total width of the operculum in *Raninoides* sp. is 4.1 μm (range = 4.0 to 4.2 μm, $n$ = 3).

The capsule and the overlying membrane sends an extension on each side into the thin layer of investing cytoplasm (Fig. 2 A). The acrosomal membrane is invaginated at the posterior pole where, with the capsule, it lines a complex subacrosomal chamber (Fig. 2 A, E). The subacrosomal chamber forms an anteriorly pointed ellipse and its anterior tip reaches almost to the anterior operculum.

The central axis of the acrosome formed by the subacrosomal chamber is surrounded by a moderately electron-dense layer (Fig. 2 A, D, E), which is clearly equivalent to the outer dense zone which surrounds the subacrosomal chamber in the sperm of *Ranina ranina*. This layer is replaced apically by a more dense, inverted, cup-shaped zone which extends to the apex of the sperm at the opercular perforation, a location which indicates homology with the anterior part of the inner dense zone of the *R. ranina* sperm and, despite its anomalous position, is here termed the inner acrosome zone. This cup-shaped layer is flanked by a narrow, more dense layer which has no equivalent in *R. ranina*. These two apical zones are surrounded by a zone, fan-shaped in longitudinal section of the sperm, which underlies and extends to the lateral limits of the operculum. The bulk of the contents of the acrosome, between the
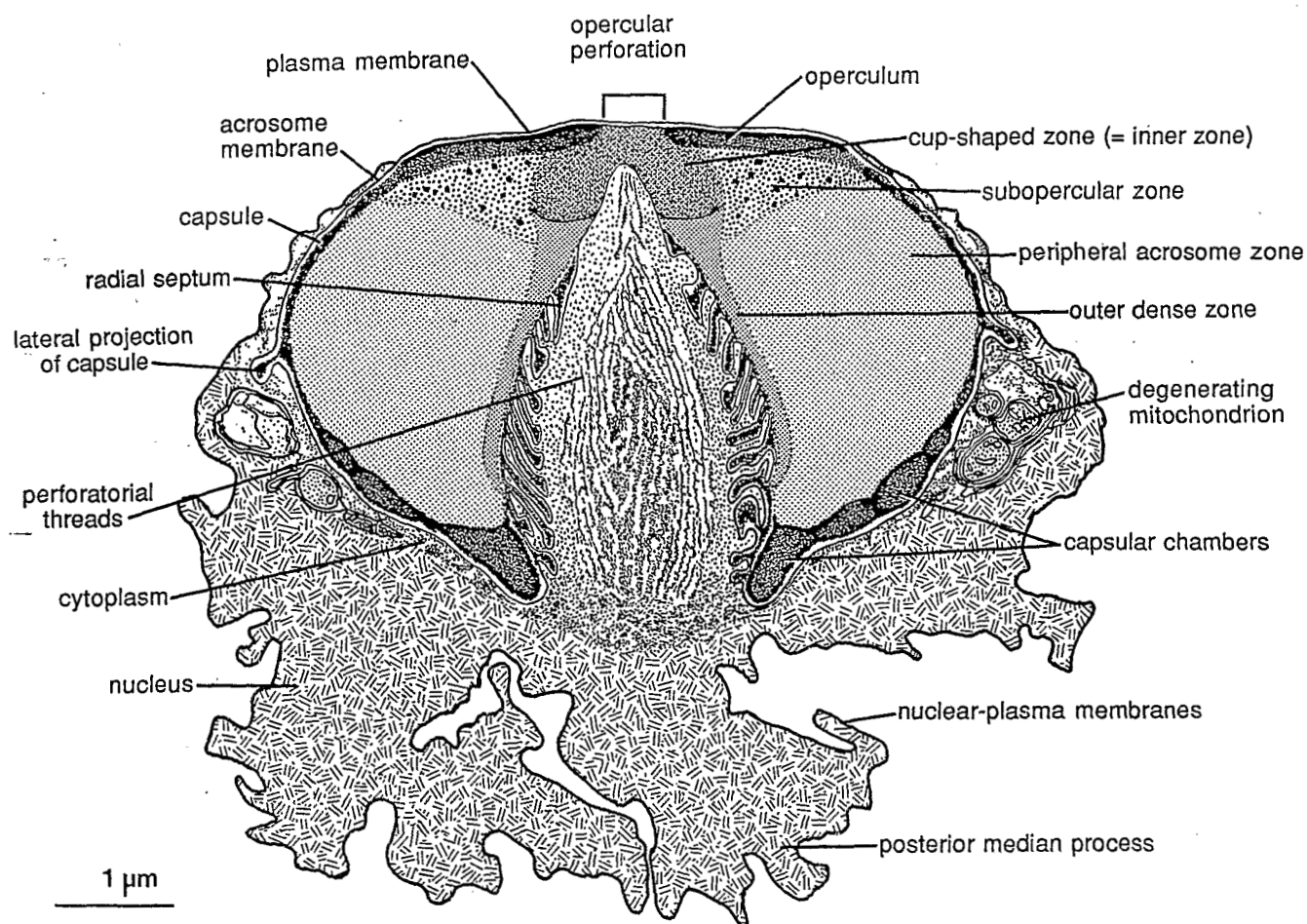
**Fig. 1** *Raninoides* sp. Line drawing of sagittal longitudinal section of spermatozoon prepared from tracings of transmission electron micrographs

subopercular zone, the inner acrosome zone and the capsule, is the most electron pale zone in the acrosome and constitutes the peripheral acrosomal zone ("peripheral contents", Jamieson 1989) of the acrosome vesicle (Fig. 2 A, C, D) recognized for *R. ranina*.

A discrete posterior chamber of the acrosome vesicle seen in *Ranina ranina* sperm is considered to be represented in *Raninoides* sp. by multiple small chambers with dense contents which fringe the peripheral acrosomal zone from the lateral limits of the subopercular zone to the base of the acrosome. These chambers, seen in longitudinal section in Fig. 2 A and transverse section in Fig. 2 D, are formed by division of the dense capsule. They are very small and scarcely recognizable anteriorly, but progressively enlarge posteriorly.

*Subacrosomal region.* At the posterior pole, the capsule is perforated by a narrow orifice which opens into the subacrosomal space which has the form of an anteriorly pointed ellipse. In cross section of the spermatozoon, the wall of the subacrosomal chamber is seen to consist of mod-

erately electron-dense radial septa which arise from a bounding ring and have their thickened free edges directed towards the core of the perforatorium (Fig. 2 D, F). The septa are mostly somewhat curved and vary in length from very short to a maximum of ~0.5 µm. Most are unbranched but some are dichotomously or trichotomously branched. They are embedded in a broad ring of less electron-dense material which is wider than the length of the septa and therefore more closely approaches the axis of the perforatorium, the pale core of which it directly bounds. Longitudinal sections of the sperm (Fig. 2 E) confirm that the septa are longitudinal laminae and are not merely villous projections.

The core of the perforatorium in cross section has the appearance of being filled by many fine electron-dense threads coiled in a single whorl (Fig. 2 D), but in longitudinal section (Fig. 2 A, E) these threads appear to be orientated longitudinally, extending to the narrow tip of the perforatorium shortly beneath the opercular perforation. The apparent threads are therefore very delicate longitudinal laminae which form a roll with its long axis in the long axis of the sperm (see "Discussion – Synapomorphies of *Raninoides* with *Ranina*"). The ring from which the septa arise may be the equivalent of the more posterior part of the inner acrosome zone of the *Ranina ranina* sperm and it is continuous with the similarly named inverted cup-shaped zone beneath the opercular perforation.

*Cytoplasm.* As in *Ranina ranina*, the spermatozoal cytoplasm in *Raninoides* sp. is limited to a thin layer surrounding the capsule of the acrosome and extending into one or more of the lateral arms (Fig. 2 A, D). In *Raninoides* sp. the cytoplasm is unevenly distributed, it and its contained mitochondria being more developed at one of the three arms than at the other arms. As in *Ranina ranina*, there is no extension of cytoplasm into the posterior median process. Again, as in *R. ranina*, the cytoplasmic prolongations in the arms have numerous, often contiguous, subspheroidal mitochondria with few, slender cristae (Fig. 2 A, D) and, although degenerating mitochondria are present, some appear intact and possibly remain functional until fertilization. Stacked membranes of a lamellar complex are present in the periphery of the cytoplasm (Fig. 2 A, D).

A large structure, not observed in *Ranina ranina*, and resembling a myelin-body is present in the cytoplasm on one side of the acrosome (Fig. 2 D), as illustrated for *Carcinus maenas* (Chevaillier 1967; Pochon-Masson 1968) and there termed the "lamellar system", and in *Portunus pelagicus* (see Jamieson 1989). This appears to be derived from mitochondrial membranes.

*Nucleus.* As in *Ranina ranina*, the nuclear material is located in the three lateral arms, in the median posterior projection and in the confluence of these as a narrow zone around the acrosome and its cytoplasmic sheath. The nuclear material is extremely attenuated over approximately the upper third of the acrosome (Fig. 2 A), and does not extend over the opercular region at the apex of the latter. As in *R. ranina*, no nuclear membrane is apparent between the cytoplasmic sheath and the nuclear material. In the region of the capsular flange there is mixing of cytoplasm and electron-dense DNA fibrils and mitochondria are in contact with the fibrils (Fig. 2 A). The thick dense membrane externally limiting the nucleus is interpreted as the combined nuclear and plasma membranes, here termed the cell membrane. In *Raninoides* sp., as in *Ranina ranina* and all other brachyurans, the chromatin consists of electron-dense filaments in a pale matrix.

*Lyreidus brevifrons*

*General morphology.* The spermatozoon of *Lyreidus brevifrons* (refer to line drawing, Fig. 3, throughout) accords with that of *Raninoides* sp. (and *Ranina ranina*) in its chief components but differs, among other respects, in the more depressed form of the acrosome, which has a mean length to width ratio of only 0.52, in having a capitate perforatorium, in lacking a discrete peripheral acrosomal zone, in the configuration of the subacrosomal corrugations, and in lacking posterior acrosomal chambers. In the following account the components of the sperm are, with a few exceptions, described only where they differ from those of *Raninoides* sp. In other regards, the above account for *Raninoides* sp. may also be applied to *L. brevifrons*.

*Acrosome and subacrosomal region.* The acrosome of *Lyreidus brevifrons* does not form a symmetrical ellipsoid. In longitudinal section, the curvature of its basal surface is less than that of its almost semicircular anterior surface (Fig. 4 A, B). The mean length of the acrosome is 2.6 μm (range = 2.2 to -3.0 μm, *n* = 7); the mean width is 5.0 μm (range = 4.5 to 5.7 μm, *n* = 7).

As in the other two raninids, the operculum in *Lyreidus brevifrons* is poorly differentiated, consisting merely of a strongly electron-dense apical border of the capsule which is here at its thickest (Fig. 4 B); as in the other two species, the operculum appears virtually continuous with the dense material of the capsule; the mean total width of the operculum is 2.7 μm (range = 2.4 to 3.2 μm, *n* = 9).

Whereas in *Raninoides* sp. and *Ranina ranina* the capsule and the overlying membrane sends an extension on each side into the thin layer of investing cytoplasm, in *Lyreidus brevifrons* ≈10 low, rounded projections are visible on each side in longitudinal section of the sperm (Fig. 4 B). In transverse section of the spermatozoon (Fig. 4 C), the projections appear more numerous and extend all round the margin of the acrosome. As their appearance is similar in longitudinal and transverse sections, the projections presumably represent oblique, possibly spiral ridges. At the base of the acrosome they are continuous with longitudinal corrugations, numbering in the order of 20, which line the subacrosomal chamber. In *L. brevifrons* (Fig. 4 E, F), the corrugations appear as low, unbranched clavate profiles in transverse section of the chamber and as longitudinal ridges in longitudinal section, extending to shortly above the equator of the acrosome. In contrast, in *Ranina ranina* these corrugations line only the posterior half of the subacrosomal chamber, which is subequatorial in the acrosome and often branch, while in *Raninoides* sp. they form high, simple to trifoliate septa extending the length of the bipolar chamber.

*Lyreidus brevifrons* differs fundamentally from the other two raninids in the form and fine structure of the contents of the subacrosomal chamber. These contents are here considered to constitute the perforatorium. The corrugations project into its substance from the capsular wall of the chamber. The perforatorium lacks longitudinal fibrillar elements tentatively identified as perforatorial in the other two species, and differs greatly in widening anteriorly to give an irregular capitate form (Fig. 4 B). Unlike the smooth, bilaterally symmetrical head of dromiid sperm
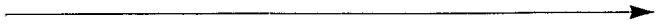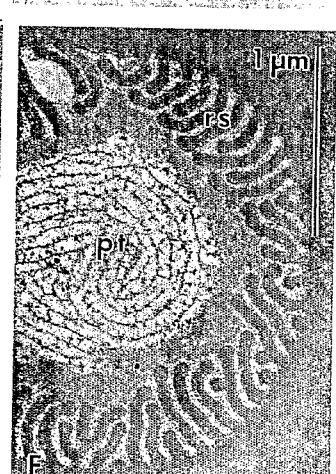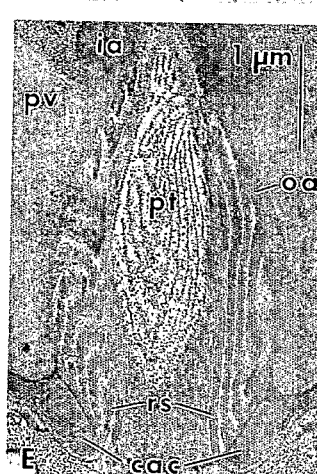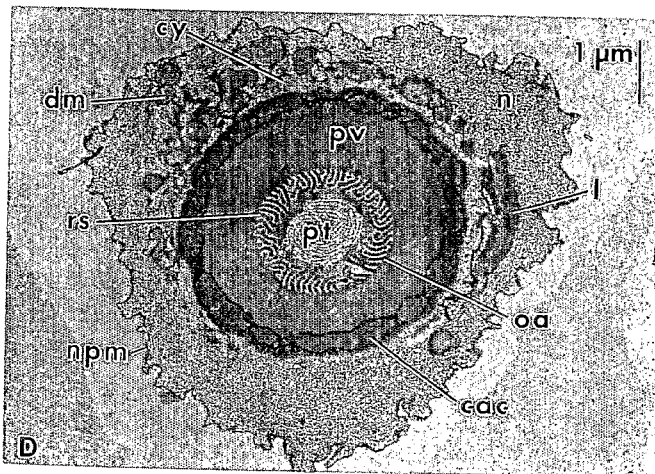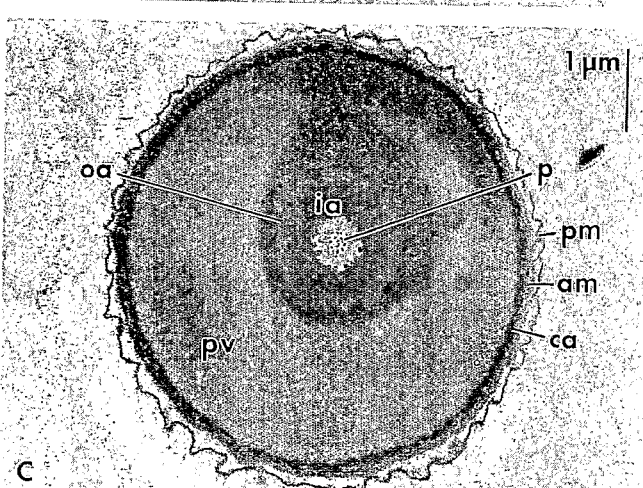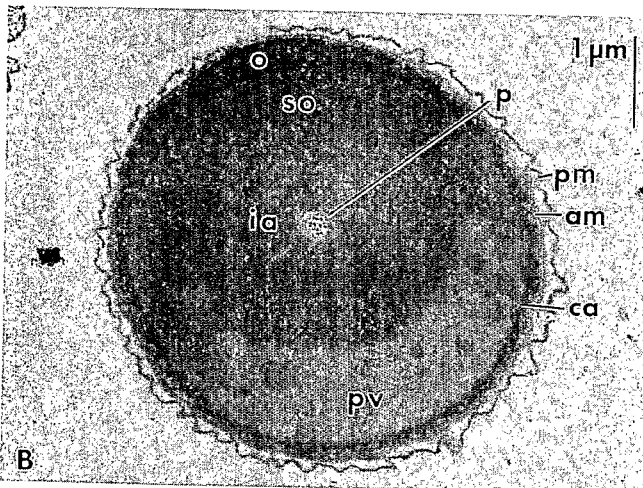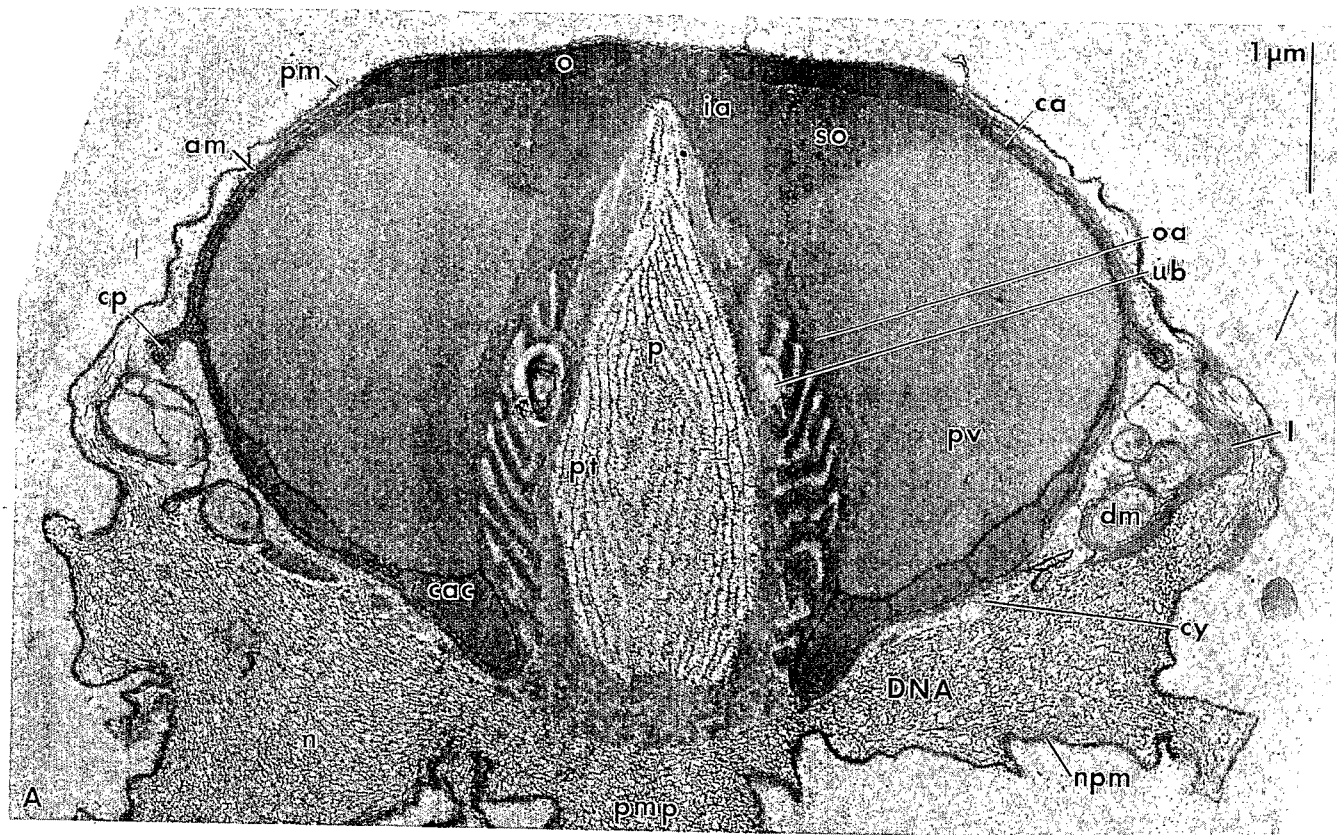
**Fig. 2** *Raninoides* sp. Transmission electron micrographs of spermatozoon. **A** Sagittal longitudinal section (LS); **B** slightly oblique transverse section (TS) through perforatorium near tip; **C** TS slightly more posterior than **B**; **D** TS through radial septa lining perforatorium; **E** LS through perforatorium showing septa; **F** TS showing detail of radial septa lining perforatorium (*am* acrosome membrane; *ca* capsule; *cac* capsular chamber; *cp* external capsular projection; *cy* cytoplasm; *dm* mitochondrion; *DNA* DNA fibrils; *ia* inner dense zone of acrosome; *l* part of lamellar complex; *n* nucleus; *npm* combined nuclear and plasma membranes; *o* operculum; *oa* outer dense zone; *p* perforatorium; *pm* plasma membrane; *pt* perforatorial threads; *pv* peripheral contents of acrosome vesicle; *pmp* posterior median process; *rs* radial septa; *so* subopercular zone; *ub* unidentified body)
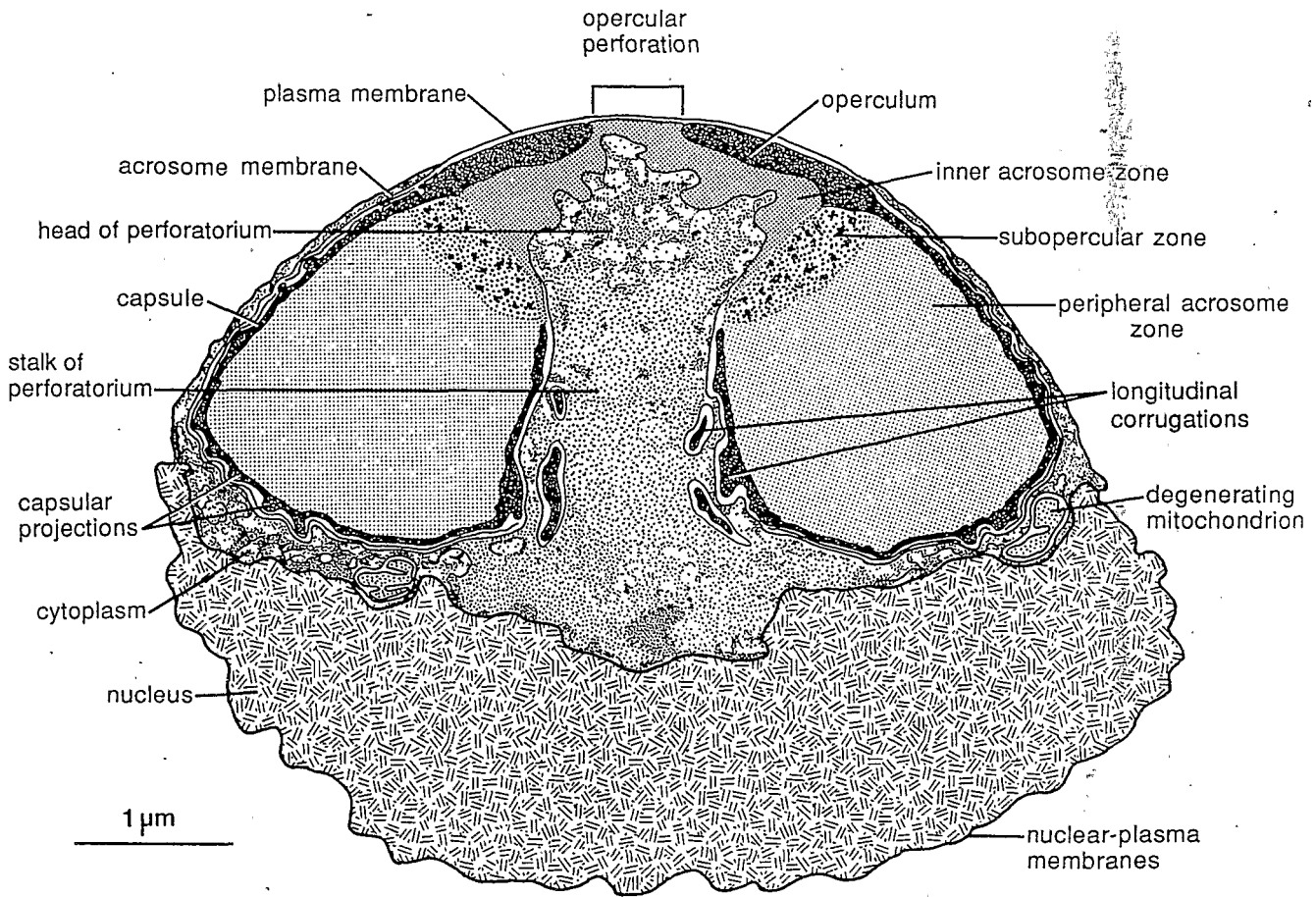
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Fig. 3 *Lyreidus brevifrons*. Line drawing of sagittal longitudinal section of spermatozoon prepared from tracings of transmission electron micrographs

or the radial "spiked wheel" of homolids, the head of the perforatorium in the sperm of *L. brevifrons* appears irregularly stellate or amoeboid in transverse section (Fig. 4 D). Whether its irregular form indicates that its lobes are mobile in the unreacted spermatozoon is unknown.

No equivalent of the outer dense zone (as distinct from the capsular layer), which surrounds the subacrosomal chamber in the sperm of *Ranina ranina* and which is apparently weakly developed in *Raninoides* sp., can be recognized in *Lyreidus brevifrons*. However, a dense layer in which the lobes of the perforatorial head are embedded and which extends to the opercular perforation is identifiable with the inner acrosome zone of those genera. This apical, inner dense zone is surrounded by an anterolateral pale zone which also underlies the lateral extremity of the operculum (Fig. 4 B). This is clearly identifiable with the subopercular zone of the other two raninid genera in which, however, it extends to the inner limit of the operculum.

Between the subopercular zone and the capsule is the largest zone in the acrosome (Fig. 4 B, C) which constitutes the peripheral acrosomal zone ("peripheral contents", Jamieson 1989) recognized for *Ranina ranina* and *Rani-*

*noides* sp. In those genera, however, it is the palest zone in the acrosome whereas in *Lyreidus brevifrons*, although only moderately electron-dense, it is darker than the subopercular zone.

*Lyreidus brevifrons* has no equivalent of the discrete posterior chamber of the acrosome vesicle seen in *Ranina ranina* sperm and represented in *Raninoides* sp. by multiple small chambers, and in both of which genera it is formed by subdivision of the capsule.

At the posterior pole the capsule is perforated by a narrower orifice which opens into the subacrosomal space. The corrugations already referred to vary in length from very short to a maximum of ~0.2 µm. They are not embedded in a broad ring of less electron-dense material as in *Raninoides* sp., but pale material intervenes between some of them (Fig. 4 C, E, F).

Fig. 4 *Lyreidus brevifrons*. A Light micrograph of lateral view of sperm. B–F Transmission electron micrographs of spermatozoon. B sagittal LS: C TS showing projections from wall of perforatorial chamber into perforatorium, 3 nuclear arms are also visible; D slightly oblique TS of "amoeboid" head of perforatorium; E oblique section of base of perforatorium showing projections of wall; F parasagittal section of base of perforatorium showing that projections of wall are longitudinal septa and that they form a series with basal capsular projections (*arrowhead*) (*a* acrosome; *anm* anterior nuclear membrane: *co* corrugations; *la* lateral arm; *ph* head of perforatorium: further abbreviations as in legend to Fig. 2)
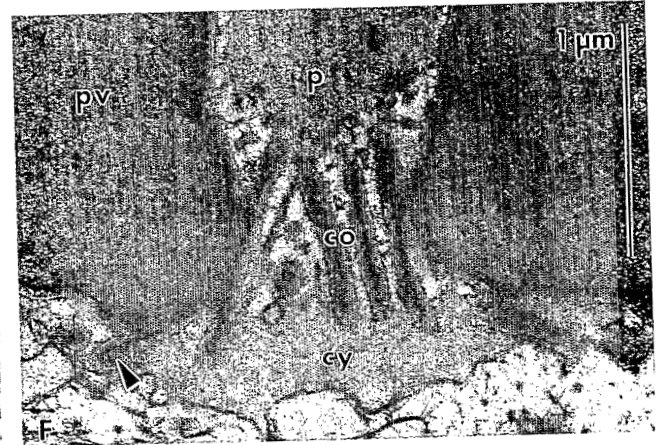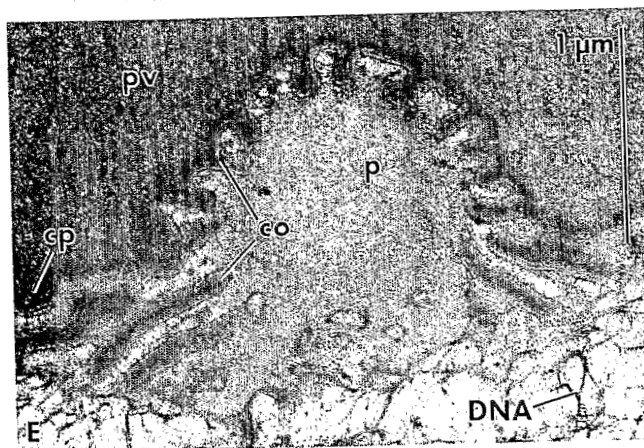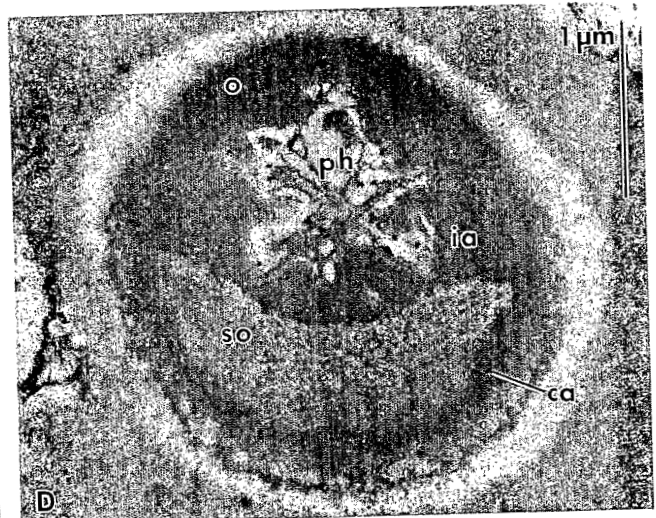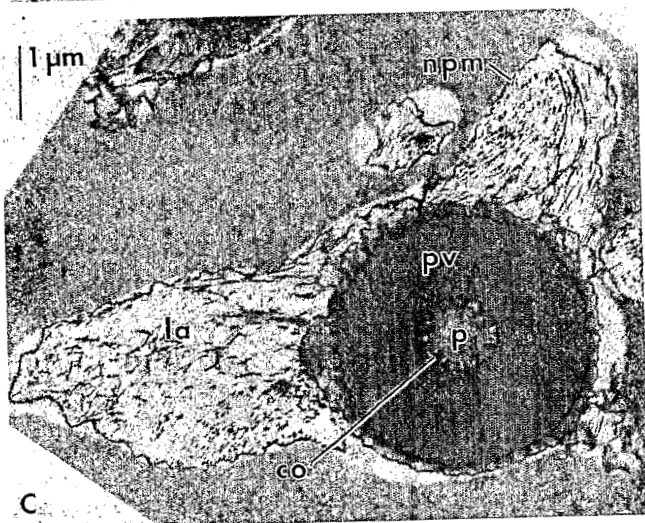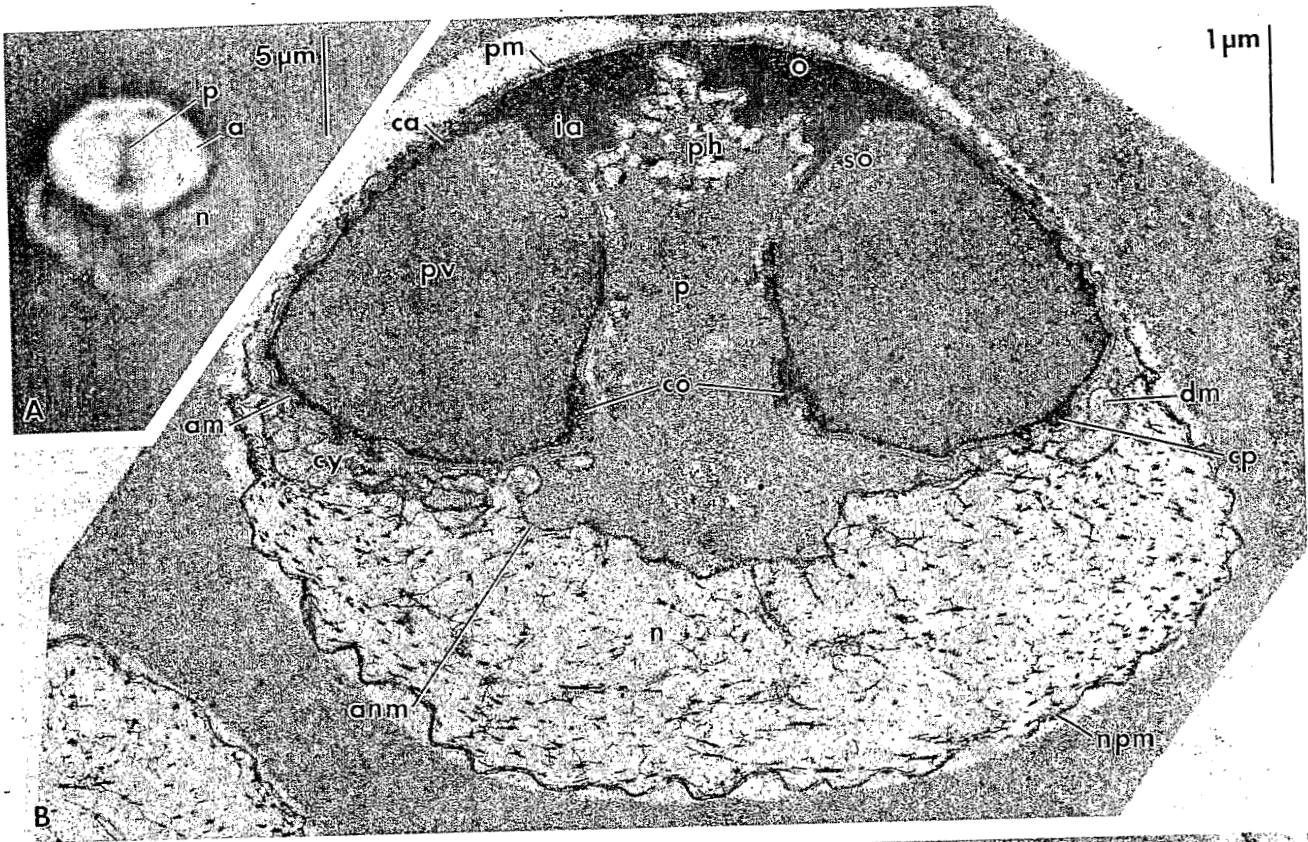
*Cytoplasm.* As in *Ranina ranina* and *Raninoides* sp., spermatozoal cytoplasm is limited to a thin layer surrounding the capsule of the acrosome. This bulges but does not greatly extend into the lateral arms (Fig. 4C). Few recognizable mitochondria are visible and all are high degenerate. Contorted membranes which are probably derived from mitochondria are present. Although these membranes may be considered to constitute a lamellar system, no discrete unilateral myelin-like body such as that seen in the cytoplasm on one side of the acrosome in *Raninoides* sp. is present.

*Nucleus.* As in *Ranina ranina*, the nuclear material is located in the three lateral arms (Fig. 4C), in the short median posterior process and in the confluence of these as a narrow zone around the acrosome and its cytoplasmic sheath. The median posterior process is sometimes longer than the remainder of the sperm in *Lyreidus brevifrons*. It and the lateral arms appear, from their variable profiles, to be very labile in form and are presumably amoeboid in activity.

Unlike the other two genera, the nuclear material extends only a little if at all anterior to the base of the acrosome (Fig. 4A, B). It differs further in being separated by a nuclear membrane from the cytoplasm. The membrane is occasionally perforate and, though dense, is not multilaminate. There is therefore none of the mixing of cytoplasm and electron-dense DNA fibrils seen in *Raninoides* sp. A thick dense membrane externally limiting the nucleus is again interpreted as the combined nuclear and plasma membranes and the chromatin consists of electron-dense filaments in a pale matrix.

## Discussion

### Synapomorphies within the Raninoidea

The present study of the spermatozoa of *Ranoides* sp. and *Lyreidus brevifrons*, coupled with the study of the spermatozoon of *Ranina ranina* (by Jamieson 1989), has established several synapomorphies which singly or collectively distinguish the Raninoidea (Table 1). These synapomorphies are enumerated below:

(1) The presence of single (*Ranina ranina*, *Raninoides* sp.) or multiple (*Lyreidus brevifrons*) keel-like projections of the acrosomal capsule. The lateral capsular projection observed in *Ranina ranina* is found also in *Raninoides* sp. and therefore emerges as a remarkable synapomorphy of the two. The multiple projections (keels?) in *L. brevifrons* may represent a precursory plesiomorphic condition for raninids, as the multiple basal projections of the capsule in the dromiid *Stimdromia* (=*Petalomera*) *lateralis* (see Jamieson 1990) and in cyclodorippoids (Jamieson et al. 1994c) are reminiscent of these and possibly symplesiomorphic with them.

(2) The very large, weakly electron-opaque peripheral acrosomal zone is highly characteristic and synapomorphic

of the sperm of *Ranina ranina* and *Raninoides* sp. The large outer zone in *Lyreidus brevifrons* is presumably homologous but, in the absence of a concentric inner acrosome zone in this species, has not been termed the peripheral zone.

(3) The poor differentiation of the operculum from the capsule is peculiar to raninids and is possibly synapomorphic and autapomorphic.

(4) A very well developed subopercular zone, of variable form. Its form differs in the three genera, being fan-shaped in longitudinal section in *Raninoides* sp., a depressed ring in *Ranina ranina*, and somewhat intermediate in *Lyreidus brevifrons*. The subopercular zone in dromiids (Jamieson et al. 1993), dynomenids (Jamieson et al. 1994a) and homolids (Guinot et al. 1994; Jamieson et al. 1994b) differs in being continuous across the anterior pole of the acrosome.

(5) A most striking synapomorphy of the three raninids is the presence of inward longitudinal projections, with similar fine structure, in the wall of the subacrosomal chamber. In *Ranina ranina* these take the form of low corrugations and are limited to a posterior subdivision of the chamber. In *Raninoides* sp. no such subdivision of the chamber occurs and the projections form longitudinal radial septa throughout the length of the chamber. In *Lyreidus brevifrons* they appear as low, unbranched clavate profiles in transverse section of the chamber and as longitudinal ridges in longitudinal section, extending to shortly above the equator of the acrosome. Corrugations of the wall of the perforatorial chamber also occur in cyclodorippoids but are there of a different form (Jamieson et al. 1994c).

### Synapomorphies of *Raninoides* with *Ranina*

Shared, presumably synapomorphic characters of *Ranina ranina* and *Raninoides* sp. but not of *Lyreidus brevifrons* within the Raninoidea, are:

(1) Branching of some of the subacrosomal septa. In both species the septa are uni- to trifoliate. In *Lyreidus brevifrons* they have the simplest form, being unbranched, consistent with a plesiomorphic status for this genus, and link with the external keels.

(2) The subspheroidal form of the acrosome in *Ranina ranina* and, though slightly more depressed, *Raninoides* sp., is unique within the Brachyura. However, if raninids are basal relative to the higher crabs (the heterotreme–thoracotreme assemblage) as suggested by Jamieson (1989) from initial raninid sperm data and by Spears et al. (1992) from 18S rRNA sequences, the subspheroidal form might be considered an intermediate step to the spheroidal condition in the higher crabs. However, the present study failed to find support in the form of synapomorphies for a sister-group status for raninids relative to the heterotreme–thoracotreme assemblage. Spherical acrosomes are not a uniquely heterotreme–thoracotreme feature, as they also occur in some paguroids (Tudge 1992).

The subspheroidal form of the acrosome in *Ranina ranina* and *Raninoides* sp., could conceivably have devel-

**Table 1** Comparison of the spermatozoa of *Ranina ranina*, *Raninoides* sp., *Lyreidus brevifrons* and other brachyurans

| Characteristic | Dromiidae Dynomenidae Homolidae Cyclodorippoidea | *Ranina ranina* | *Raninoides* sp. | *Lyreidus brevifrons* | Eubrachyura (Heterotremata–Thoracotremata) |
|---|---|---|---|---|---|
| Acrosome | discoidal | subspheroidal | subspheroidal | discoidal | spheroidal |
| Capsular flanges | absent; or basal in *Stimdromia lateralis* and Cyclodorippoidea | anterolateral, single | anterolateral, single | many anterolateral to posterior | absent |
| Peripheral vesicular contents | narrow | large | large | not differentiated | narrow |
| Subopercular zone | apical, not perforate; prominent; absent in Cyclodorippoidea | large, perforate | large, perforate | moderately large, perforate | narrow, perforate in majids, calappids and cancrids; imperforate in higher crabs |
| Operculum well defined from capsule | yes | no | no | no | yes |
| Perforatorium | capitate in dromiids, dynomenids and homolids; not capitate in Cyclodorippoidea; in all not coiled and without longitudinal tubules | not capitate; contains coiled filament | not capitate; contains coiled maze of fine filaments | irregular capitate head | not capitate; contains longitudinal tubules |
| Posterior acrosomal chamber | absent | single | multiple | absent | absent |
| Longitudinal corrugations on wall subacrosomal chamber | absent or (Cyclodorippoidea) of a different form | basal invaginations | invaginations extend length of perforatorium | subequatorial invaginations | absent |
| Mitochondria | few, degenerate | numerous simple, well developed to degenerate | numerous simple, well developed to degenerate | numerous simple, well developed to degenerate | few, highly degenerate |

oped via a moderately depressed from (as seen in *Lyreidus brevifrons*) from the depressed form seen in modern non-raninid podotremes and presumably present in a common ancestor of dromiids, dynomenids, homolids and raninids (Guinot et al. 1994). The ratio of length to width of the acrosome (L:W) in cyclodorippoids overlaps that in raninids. The mean L:W of the acrosome is 0.76 in *Ranina ranina*; 0.73 in *Raninoides* sp.; 0.52 in *L. brevifrons*; 0.26 and 0.34 in the dromiids *Stimdromia lateralis* and *Dromidiopsis edwardsi*, respectively; 0.34 in the dynomenid *Paradynomene tuberculata*; and varies from 0.39 to 0.56 in seven homolid species, and from 0.54 to 0.63 in three cyclodorippoid species, *Xeinostoma richeri*, *Cymonomus* sp. and *Tymolus* sp. (Jamieson et al. 1993, 1994a–c). Thus, the dromiids and their putative sister-group the dynomenids have the most depressed acrosomes in terms of mean L:W ratios (0.26 to 0.34), while the ratios for the three raninids (0.53 to 0.76) overlap with homolids (means=0.49 to 0.57) and cyclodorippoids (means=0.54 to 0.63). From these data it seems possible that a depressed acrosome is a synapomorphy of the Podotremata and simultaneously a

symplesiomorphy of its members. Whether the increase in anteroposterior length in raninids is synapomorphic or merely homoplasic with homolids is not at present determinable, but may be resolved by a parsimony analysis when data from further taxa become available.

(3) A single coiled thread was observed in the perforatorial chamber of *Ranina ranina* and was tentatively interpreted as a filamentous perforatorium. In *Raninoides* sp., fine threadlike profiles interpreted as numerous delicate enrolled lamellae fill the core of the perforatorium. Coiling or enrolling of perforatorial elements is therefore a questionable synapomorphy of these two raninids. No close homology with the capitate, stellate perforatorium of *Lyreidus brevifrons* is recognizable. Instead, the capitate form of the perforatorium in *L. brevifrons* is reminiscent of dromiids and dynomenids, in which it is bilaterally symmetrical, and is particularly reminiscent of the radiate spiked-wheel structure of homolids in which the proportions of the acrosome are similar (L:W=0.55 in *Paromola petterdi* compared with 0.52 in *L. brevifrons*). Determination of whether or not the capitate form in *L. brevifrons* is

homoplasic or truly synapomorphic with that in other podotremes awaits investigation by parsimony analysis.

(4) In Ranina ranina, the capsule 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 sp. are here considered homologous and synapomorphic with the posterior chamber of Ranina ranina. The absence of these in Lyreidus brevifrons may well be plesiomorphic.

(5) The absence of an appreciable membrane between the nucleus and cytoplasm in Ranina ranina and Raninoides sp. is a striking difference from sperm of the heterotreme–thoracotreme assemblage, in which the membrane persists and is often multilaminar, although it may be perforate-or grossly disrupted (Libinia emarginata, Hinsch 1969; Jamieson 1991). However, a dense membrane is present in Lyreidus brevifrons. In non-raninid podotremes the membrane is absent (e.g. Homolomannia, Jamieson et al. 1994 b) or present (e.g. Paromola petterdi, Guinot et al. 1994). It is absent also in anomurans such as Birgus latro (Tudge and Jamieson 1991). The presence of a membrane around the entire nucleus can reasonably be construed as plesiomorphic. In view of the absence of a membrane anterior to the nucleus in the L. brevifrons sperm, which on the whole appears plesiomorphic relative to the sperm of Ranina ranina and Raninoides sp., such absence cannot be interpreted as a synapomorphy of non-raninid podotremes with raninids.

Comparison of Raninoidea with other podotremes

In contrast to the absence of synapomorphies exclusive to raninoids and heterotremes (see following subsection), a number of similarities of the sperm of Raninoidea with those of other podotremes have emerged from this study, but it is difficult in some cases to establish whether they are synapomorphic, homoplasic or symplesiomorphic, although it is hoped that a parsimony analysis will resolve this problem when more data are available. These similarities include the following: (1) The capitate perforatorium of Lyreidus brevifrons, which is questionably related to the radiate spiked-wheel structure of homolids (in which acrosome proportions are similar) or less closely related to the bilateral capitate perforatorium of dromiids and dynomenids. (2) The basal capsular projections as in the dromiid Stimdromia lateralis and in cyclodorippoids. (3) The persistence of mitochondria which are less degenerate than in other brachyurans, except, possibly, the cyclodorippoids. Mitochondria are better developed in the sperm of Ranina ranina and Raninoides sp. than in the heterotreme–thoracotreme assemblage, but are nevertheless degenerate as in these; their persistence must be regarded as plesiomorphic; they are highly degenerate in L. brevifrons as in homolids. (4) The thin cytoplasmic layer (restricted to the base of the acrosome in the dromiid Stimdromia lateralis (Jamieson 1990). (5) The presence of nuclear arms [absent in S. lateralis (Jamieson 1990) and the dynomenid Paradynomene tuberculata (Jamieson et al. 1994 a)]. (6) The presence of

a posterior median process (possibly, however, homoplasic with anomurans). (7) The perforate condition of the operculum, with a single apical opening, which appears to be an apomorphy of podotremes but also occurs (homplasically or by retention?) in lower heterotremes.

Comparison of raninoids with heterotreme–thoracotreme crabs

Although there are several similarities of raninoid sperm with those of the heterotreme–thoracotreme assemblage, none of the similarities appears to be synapomorphic and therefore indicative of a close, sister-taxon relationship. Several features shared between raninoids and heterotreme–thoracotreme crabs which can reasonably be considered symplesiomorphies are also present in at least some non-raninoid podotremes: (1) Enclosure of the acrosome by a thin layer of cytoplasm. (2) Cupping (minimal in Lyreidus brevifrons) of the cytoplasm and acrosome by the nucleus. (3) The presence of lateral nuclear arms; the number of lateral arms in brachyuran sperm varies from 3, as in the raninoids, in the majids Libinia emarginata, Macrocoeloma trispinosum, Mithrax sp., and Pitho iherminieri, the portunid Carcinus maenas and cancrid (Cancer) species, to 4 or 5 in the majid Stenorhynchus seticornis and 8 morphologically somewhat pleudopodium-like arms (with some variation) in Callinectes sapidus and Portunus pelagicus (see Jamieson 1989); there are also 3 arms in anomurans, suggesting that 3 is a symplesiomorphic number for the anomuran–brachyuran assemblage. (4) The presence of a posterior median process and extension of the cytoplasm into the bases of the nuclear arms (majid heterotremes); possibly, however, a homoplasy of these heterotremes with podotremes. (5) The electron-dense capsule is a symplesiomorphy of raninoids and higher crabs, being seen also in anomurans and, although differing (secondarily?) in structure, in non-raninoid podotremes. (6) A concentric arrangement of zones of the acrosome vesicle is an anomuran and heterotreme–thoracotreme symplesiomorphy (also seen in nephropids, e.g. Pochon-Masson 1965), the predominantly horizontal arrangement in non-raninoid podotremes being apparently a synapomorphy of these (the arrangement in Lyreidus brevifrons is equivocal). As noted in the previous subsection, the perforate condition of the operculum, seen in lower heterotreme (e.g. majid and cancrid) sperm could be a retention of a podotreme apomorphy or a homoplasy. The subequatorial perforatorial chamber in Ranina ranina was thought potentially autapomorphic of raninoids (Jamieson 1989), but this is refuted by the more extensive, bipolar chamber in Raninoides sp. and the capitate form in L. brevifrons. The perforatorial chamber may display either condition (subequatorial or bipolar) in anomurans (Tudge 1992) and its bipolar form in Raninoides sp. cannot be considered synapomorphic of raninoids and heterotreme–thoracotreme crabs. Absence of microtubules from the nuclear arms in raninoids is apparently homoplasic with higher heterotreme–thoracotremes as they are present in majids (Hinsch 1969). The raninoid

sperm lack a thickening of the capsule as a "thickened ring" on each side of the posterior perforation seen in heterotreme–thoracotreme sperm (Brown 1966a, b; Hinsch 1973; Jamieson 1989). Absence of centrioles in the raninoids appears to be a homoplasy shared with some heterotreme–thoracotreme crabs, in others of which, however, centrioles are plesiomorphically retained.

Thus, the ultrastructure of the sperm of *Raninoides* sp. reveals significant similarities with that of *Ranina ranina* but fewer with *Lyreidus brevifrons*. Some similarities of *L. brevifrons* with dromioids and homoloids are seen, including the capitate perforatorium, the homolid-like proportions of the acrosome, and the presence of capsular projections or keels. However, one or all of these may be homoplasic. The lack of exclusive synapomorphies with the heterotreme–thoracotreme assemblage cannot necessarily be considered to refute the basal relationship of raninoids relative to a number of thoracotremes indicated by 18S rRNA sequences (Spears et al. 1992), although offering no support for this relationship. When additional taxa are studied, cladistic analysis of spermatozoal features should enable further resolution of the relationships of Raninoidea.

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