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Relationship of Homolidae and Dromiidae: Evidence from Spermatozoal Ultrastructure (Crustacea, Decapoda)

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Abstract

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The homolid spermatozoon, as exemplified by *Homola* sp., *Paromola* sp. and *Paromola petterdi*, differs markedly from spermatozoa of crabs of the Heterotremata–Thoracotremata assemblage but agrees with the sperm of dromiids, in the strongly anteroposteriorly depressed acrosome (apomorphy?) and the capitate form of the perforatorium (a major synapomorphy seen nowhere else in the Crustacea). These similarities support inclusion of the Dromiidae and Homolidae in a single grouping, the Podotremata. The homolid perforatorium differs from that of dromiids in the autapomorphic spiked-wheel form of the anterior expansion. Homolid spermatozoa show nuclear arms symplesiomorphic of all investigated crabs (small or questionably sometimes absent in Dromiidae), and corresponding loss of purely microtubular arms seen in other reptants. Homolid sperm agree with those of dromiids (synapomorphy?), raninids, higher heterotremes and thoracotremes (homoplasies?) but differ from lower heterotremes, in lacking microtubules in the nuclear arms. A posterior median process of the nucleus in homolids, not seen in dromiids, is shared with anomurans and lower heterotremes. No features in the ultrastructure of homolid or dromiid sperm have been detected which associate them exclusively with either the Raninidae or the heterotreme and thoracotreme Brachyura.

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Introduction

Guinot (1977, 1978, 1979, 1991) divides the Brachyura (crabs) into three sections mainly on the basis of the location of the male and female pores: the Podotremata (containing the Dromiacea), the Heterotremata and the Thoracotremata. The coxal positions of male and female pores and isolation of the spermathecae from the oviducts, with external fertilization, characterizing the podotremes, were considered by Guinot (1978, p. 218) to be an ensemble of plesiomorphies (symplesiomorphies). Jamieson (1990, p. 126) considered that this symplesiomorphic definition left the validity of the Podotremata in some doubt. The Podotremata diagnosed by Guinot (1978, 1979) contain not only the Dromioidea and Homolodromioidea (both comprising the restricted subsection Dromiacea) but also the Homoloidea, Raninoidea, and Cyclodorippoidea (formerly Tymoloidea), all three comprising a subsection Archaeobrachyura which Guinot, at that time, admitted was a grade. The superfamily Homoloidea de Haan, 1839, which included three families (Homolidae de Haan, 1839; Latreilliidae Stimpson, 1858; Poupiniidae Guinot, 1991) had long been associated with the Dromiacea and many workers subordinated the Homoloidea in the Dromiacea. The Heterotremata and Thoracotremata share a synapomorphy in the sternal location of the female pores and development of the spermatheca as a sternal vulva on sternite 6 allowing for internal fertilization. The Thoracotremata are further apomorphic in the constant sternal location of the male pores.

In transferring the Homoloidea to the Archaeobrachyura, Guinot (1979) listed morphological characters of the adult, notably the absence of uropods, features of the thoracic sternum and the axial thoracic skeleton, which separated the Homoloidea from the Dromiacea. Based on larval morphology, Williamson (1965, 1974) and Rice (1980, 1981*a*,*b*) excluded the Dromioidea from the Brachyura while the Homoloidea were retained. However, a polyphyletic origin of the Brachyura was found unacceptable to Balss (1957) and to paleontologists (e.g. Glaessner 1969; Wright & Collins 1972) who retain the Dromiacea in the Brachyura.

With regard to wider dromiacean (*sensu lato*) and brachyuran relationships, it has been debated whether dromiaceans arose at the base of all crabs, from within the macrurans, or from basal anomurans. Glaessner (1969) considered there to be good palaeontological evidence that the Dromiacea (*sensu lato*, including homolids) arose from within the Glypheoidea, a macruran group related to spiny lobsters (Palinura). It has been acknowledged that specialized features of the zoea larvae of the Dromiidae are not brachyuran and the larvae have been considered distinctly like those of anomurans, having a

shrimp-like shape, persistent uropods, and functional third maxillipeds (Warner 1977; Williamson 1974). More precisely, the Dromiacea have been attributed an origin near, or from, the Thalassinidea, and therefore at a level more primitive than most Anomura (*sensu strictu*) (Burkenroad 1963; Gurney 1942; Pike & Williamson 1960; Rice 1980, 1983; Williamson 1965, 1974; see also discussions in Stevcic 1971; Guinot 1979). Both Dromiacea (specifically dynomenids) and Thalassinidea first appear in the fossil record at the beginning of the Jurassic (Glaessner 1969). A large number of fossils attributable to the Homolidae are known since the mid-Jurassic with a conspicuous radiation in the Cretaceous; the known fossil genera virtually disappeared in the Tertiary. The appearance of homolids is thus earlier than the Cretaceous origin (e.g. Dorippidae) of heterotrematous and thoracotrematous brachyurans.

As to relationships of homolids and raninids, the interpretation of homolid relationships from larval morphology and ontogeny has been somewhat equivocal (Williamson 1988) but has tended to endorse an origin of homolids near the base of the Heterotremata–Thoracotremata–raninid assemblage. Thus, on the basis of ontogenetic criteria, Williamson (1965, 1974) recognizes profound differences considered to separate Homolidae and Dromiacea and corresponding with a very ancient bifurcation: homolid larvae are primitive and at a level equivalent to that of anomuran larvae but have particular characters suggesting that they represent a pre-brachyuran stock; the Dromiidae can be excluded from the Brachyura. Rice (1970, 1980) and Rice & Provenzano (1970) expressed similar views: homolid and raninid larvae present similarities which suggest that they belong to a pre-brachyuran stock. 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. For Rice (1980, p. 298, fig. 9) 'the modern larval condition in the homolids, raninids and the higher Brachyura have all evolved from a more primitive homolid which possessed larval characters common to all three'. Finally, Williamson (1988, 1992) explained the dromiacean paradox by invoking horizontal gene transfer, giving anomuran larvae but brachyuran adults.

Nucleotide sequences of 18S ribosomal RNA support the exclusion of the Dromiidae from the Brachyura and inclusion of the Raninidae in the Brachyura (Spears & Abele 1988; Abele 1991; Spears *et al.* 1993). In the latter work the Dromiidae appear paraphyletic; *Hypoconcha* is the sister-taxon of the Anomura (*Clibanarius*) while *Dromia* is at the base of the raninid–heterotreme assemblage.

In the present study we describe the spermatozoal ultrastructure of three homolid species, collected off New Caledonia, in an attempt to elucidate homolid relationships: *Homola* sp. and *Paromola* sp., and a new genus to receive *Paromola* (formerly *Latreillopsis*) *petterdi* (Grant 1905), all three taxa described by Guinot and Richer de Forges (in press). Sperm of a dromiid, a raninid and a heterotreme are illustrated for comparative purposes.

Materials and Methods

Specimens of the three homolid species were collected by B. Richer de Forges during the BERYX 11 Cruise on the R.V. 'Alis' (13–23 October 1992), South of New Caledonia on the guyots of the Norfolk Ridge. 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 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, 50–80 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, 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.

Specimens of *Ranina ranina* (Linné, 1758) were collected from Heron Island, Great Barrier Reef, in October 1984 and of *Portunus pelagicus* (Linné, 1758) from Moreton Bay, in February 1988. Specimens of *Petalomera lateralis* (Gray, 1831) were collected from One Tree Island, Great Barrier Reef, in December 1988, all localities in Queensland, Australia (for ultrastructural procedures see Jamieson 1989*a* and 1990, respectively).

Results

General

A generalized homolid sperm is illustrated semidiagrammatically in Fig. 1. The bulk of the homolid spermatozoon consists of an ellipsoidal acrosome bordered posteriorly by the irregular nucleus. A thick zone of cytoplasm, containing degenerating mitochondria and tortuous membranes intervenes between the acrosome and nucleus. The longitudinal axis of the spermatozoon is occupied by a wide cylindrical, anteriorly widening column, identified as a perforatorium, which is capitate anteriorly by virtue of lateral expansion near its tip. The expansion does not form a continuous flange but is subdivided into laterally directed horizontal spikes, radiating in the form of a spiked-wheel, or the ribs of an umbrella, and contained within the anterior material of the acrosome. A low dome-shaped dense layer, with a wide apical interruption, covers the anterior limit of the perforatorium and its spikes and extends laterally over much of the anterior aspect of the acrosome vesicle; this layer is identifiable with the operculum of the sperm of anomurans, dromiids, raninids and higher crabs. It is covered by the general acrosome membrane and the plasma membrane of the sperm cell.

Acrosome

The acrosome is a thick disc, domed centrally at its free, polar surface (Figs 1, 2A, 3A, B, 4A, B, 5A). Dimensions of the acrosome, width and anteroposterior length are, respectively: 3.96–4.92 μm and 2.09–2.69 μm, ratio width : length 1.93, mean of 7 (*Homola* sp.); 3.79–4.67 μm and 1.85–2.31 μm, ratio width : length 2.03, mean of 3 (*Paromola* sp.); 3.46–3.68 μm and 1.97–2.09 μm, ratio width : length 1.78, mean of 3

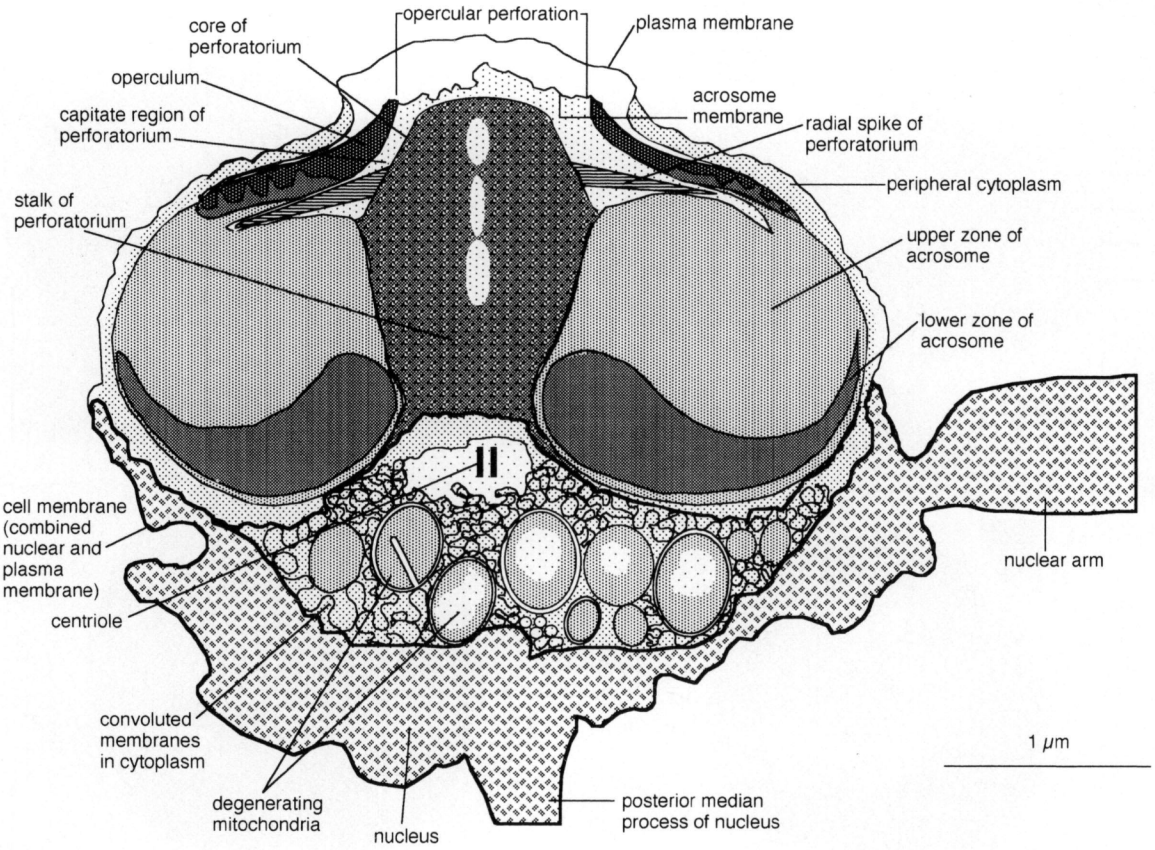

Fig. 1. Generalized homolid sperm, based on *Homola* sp., *Paromola* sp. and *Paromola petterdi* (semidiagrammatic).

(*Paromola petterdi*). This albeit small sample indicates that the acrosome is less depressed in *Paromola petterdi* than in the other two species.

The acrosome vesicle (Fig. 2A, *Homola* sp.; Figs 3A, B, *Paromola* sp.; Figs 4A, B, *Paromola petterdi*; Fig. 5A, *Homola* sp.) is bounded by a generally thin acrosomal membrane which is most clearly distinguished as a crenulate dense membrane anterior to the tip of the perforatorium. The operculum appears to be continuous with, or is at least closely contiguous with, this anterior region of the membrane, which it circumscribes. A thin moderately pale layer underlies the membrane where it bounds the acrosome vesicle, extends from the posterior limit of the operculum, around the sides and posterior face of the acrosome vesicle and is invaginated posteriorly along the posterolateral walls of the perforatorium. This pale layer may be equivalent to the capsule observed in the acrosomes of other crab sperm.

The bulk of the contents of the acrosome vesicle form an inflated ring surrounding the axial perforatorial chamber. The substance of the ring (Fig. 2A, *Homola* sp.; Figs 3A, B, *Paromola* sp.; Figs 4A, B, *Paromola petterdi*; Fig. 5A, *Homola* sp.) is subdivided into an upper, large moderately electron-dense zone, constituting most of its thickness, and a lower strongly electron dense zone which in vertical section is approximately crescent shaped with the concavity anterior. These zones are seen in transverse section in Figs 2D–F (*Homola* sp.); Figs 3D, E (*Paromola* sp.); and Fig. 4C (*Paromola petterdi*).

The upper, paler zone is directly overlain by the spikes of the perforatorium or, between these, by material resembling it in density. It is uncertain whether this overlying material is part of the upper acrosomal zone or is to be regarded as a separate subopercular zone or is, indeed, part of the operculum. Above the level of the spikes this zone is covered by and seems continuous with the dense material of the operculum. In all three species the operculum extends dense extensions into the substance of the perforatorium in the vicinity of the base of the spikes (Fig. 2A, B, *Homola* sp.). These extensions are numerous and are arranged radially.

The centre of the acrosome vesicle is penetrated by a stout vertical column of dense material which widens subapically in a capitate configuration, as seen in vertical section, composed of the radiating spines (Fig. 2A, *Homola* sp.; Figs 3A, B, *Paromola* sp.; Figs 4A, B, *Paromola petterdi*; Fig. 5A, *Homola* sp.), the whole constituting the putative perforatorium. Its stalk is circular in cross-section (Figs 2D, E, *Homola* sp.; Fig. 3D, *Paromola* sp.; Fig. 4C, *Paromola petterdi*). The transverse head of this capitate structure and the surrounding operculum occupy and account for the dome-shaped summit of the acrosome. The dense material of which the stalk and head of the perforatorium is composed is not homogeneous though forming a continuum, in *Homola* sp. (Figs 2A, 5A) and *Paromola petterdi* (Figs 4A, B); a central anteriorly tapering core, filling the entire perforatorial chamber in its posterior half, is moderately electron dense whereas the base of the spike is electron pale. In *Paromola* sp. the entire perforatorium, stalk and spikes, is moderately dense, though some suggestion of a central core may be visible (Figs 3A, B).

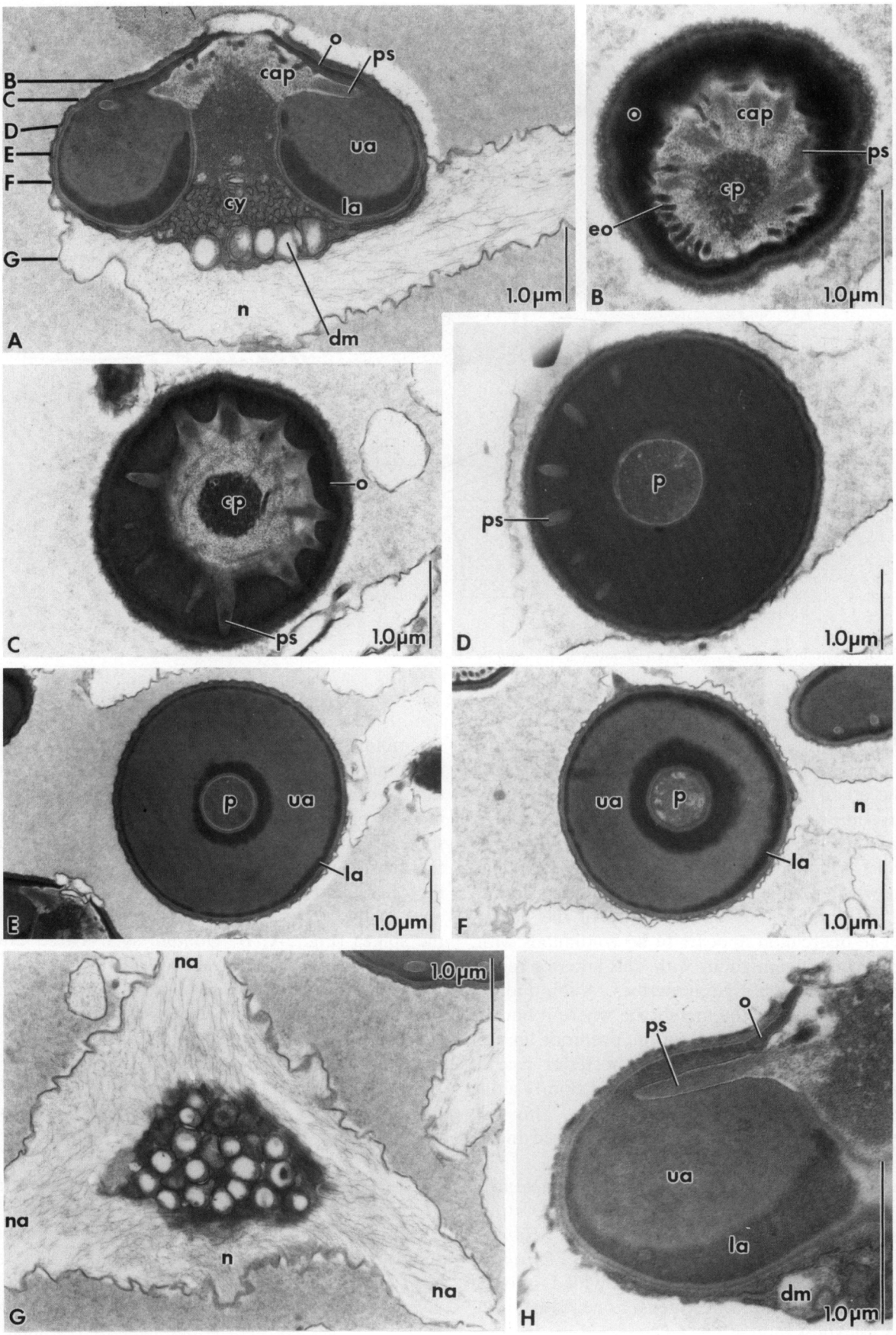

Fig. 2. Transmission electron micrographs of spermatozoa of *Homola* sp.—*A*. Sagittal longitudinal section slightly to one side of the apical hiatus in the operculum. Capital letters indicate planes of sectioning in subsequent illustrations bearing those letters.—*B*. Oblique transverse section (TS) through the operculum and supporting rays of radial spikes of perforatorium.—*C*, *D*. TS acrosome showing radial spikes of perforatorium.—*E*. TS acrosome through base of perforatorium.—*F*. TS acrosome through anterior extension of cytoplasm.—*G*. TS nucleus, at junction with cytoplasm, showing triradiate form.—*H*. Detail of LS acrosome showing perforatorial spike.