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Dorippids are Heterotremata: evidence from ultrastructure of the spermatozoa of *Neodorippe astuta* (Dorippidae) and *Portunus pelagicus* (Portunidae) Brachyura: Decapoda

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Abstract. Ultrastructural comparison between the sperm of the dorippid crab *Neodorippe astuta* (Fabricius, 1793) and the portunid *Portunus pelagicus* (Linnaeus, 1766) from Queensland, Australia, supports placement of dorippids with portunids and their relatives in the heterotreme section of the Eubrachyura (the Heterotremata - Thoracotremata or the Oxyrhyncha - Cancridea - Brachygnatha assemblage) and not with *Ranina ranina* (in the Archaeobrachyura or the Oxystomata). Similarities between spermatozoa of *N. astuta* and of *P. pelagicus* (and other Eubrachyura) and *R. ranina* include: the large spherical, multi-layered, capsule-bound acrosome vesicle; the electron-dense operculum capping the vesicle; an invaginated core, or perforatorium; concentric zonation of the contents of the vesicle; a layer of cytoplasm, between the acrosome vesicle and the nucleus, which contains mitochondria (mostly degenerating) and lattice-like lamellar complexes or membrane remnants; a diffuse nucleus which is bounded externally by a combined nuclear and plasma membrane and cups the scanty cytoplasm and the large acrosome vesicle; and lateral arms into which the chromatin extends. Characteristic eubrachyuran features of the *N. astuta* sperm absent from *R. ranina* are the long perforatorium (short and conical with a unique subacrosomal chamber in *R. ranina*) extending almost to the operculum; presence in the perforatorium of longitudinally arranged convoluted tubules; a zone of acrosomal rays forming the outer part of an inner dense zone; the presence of a thickened ring surrounding the basal part of the perforatorium; and, basally, two centrioles (absent from *R. ranina* but also from some eubrachyurans). The sperm of *N. astuta* is more similar to that of *P. pelagicus* than to that of other investigated Brachyura. A heterotreme status of *N. astuta* is thus unequivocally supported. Both species lack the posterior median process seen in the nucleus of majids and *R. ranina*.

Introduction

The Dorippidae are currently placed in at least three discordant taxonomic settings. In the first, the Dorippidae, Calappidae and Leucosidae, are placed, with the Raninidae, in the Oxystomata (e.g. Warner 1977, after Glaessner 1969) (Table 1). Warner stated that the Dorippidae possess the characteristic elongated mouthparts of the Oxystomata and that the dorsal position of the posterior legs was a primitive feature that the family shared with the Raninidae. In the second classification, on the evidence of larval characters (Williamson 1965), the Dorippidae, Calappidae and Leucosidae are elevated to the Brachygnatha from the Oxystomata, which are suppressed, leaving the Raninidae uncertainly placed. In a third classification, based on the position of the male and female genital openings (Guinot 1977, 1978), dorippids (with the Calappoidea, Corystoidea, Portunoidea and Xanthoidea) are placed in the Heterotremata while raninids are relegated to the Archaeobrachyura, which with the Dromiacea constitute the Podotremata (Table 1). Isolation of the Raninidae had been independently suggested previously by various authors, for instance Števčić (1974), who recognized it as the sole member of the Oxystomata, and Bourne (1922), who abolished the Oxystomata and erected the new tribe, Gymnopleura, for raninids.

The Podotremata are here considered an artificial group based on symplesiomorphic location of male and female pores on the coxae. Saint-Laurent (1980a, b) rightly recognizes this group as a number of independent lineages, but follows Guinot in placing the dorippids in the Heterotremata.

Association of dorippids with portunids (*inter alia*) and separation from raninids is congruent with recent demonstration that the spermatozoa of *Ranina ranina* possess distinctive features which separate it from the Oxyrhyncha - Cancridea - Brachygnatha assemblage (Jamieson 1989b) or, in the alternative classification, the Eubrachyura (Heterotremata and Thoracotremata).

In the present investigation we compare the sperm of *Neodorippe astuta* with the spermatozoa of the Heterotremata exemplified by *Callinectes sapidus* (see Brown 1966a, b), *Carcinus maenas* (see Goudeau 1982), *Atergatis floridus* (see Jamieson 1989a, b), *Pinnixia* sp. (see Reger 1970), *Geryon* spp. (see Hinsch 1988), *Etisus laevi-*

Table 1. Dorippidae: alternative classifications

Section	Sub-section	Superfamily	Family	Source
Dromiacea		Dromioidea etc.[a]	Dromiidae etc.	Warner (1977)
Oxystomata		Dorippoidea	Dorippidae	
		Calappoidea	Calappidae, Leucosiidae	
		Raninoidea[b]	Raninidae	
Oxyrhyncha	(Brachygnatha)[c]		Majidae	
Cancridea		–[d]	Cancridae etc.	
Brachyrhyncha		Portunoidea	Portunidae etc.	
Podotremata	Dromiacea	Dromioidea etc.	Dromiidae etc.	Guinot (1978)
	Archaeobrachyura	Homoloidea	Homolidae, Latreilliidae	
		Raninoidea	Raninidae	
		Tymoloidea	Tymolidae	
Heterotremata	(Eubrachyura)[c]	Dorippoidea	Dorippidae etc	
		Portunoidea	Portunidae etc.	
Thoracotremata		Grapsoidea etc.	Grapsidae etc.	

[a] For brevity, other taxa are not listed
[b] Placed in Archaeobrachyura by Bowman and Abele (1982)
[c] Brachygnatha are limited to the Oxyrhyncha and Brachyrhyncha by Williamson (1965) and are considered by some workers (e.g. Barnes 1980) to be synonymous with the Brachyrhyncha alone
[d] No superfamily named
[e] Saint-Laurent (1980 b)

manus, Liagore rubromaculata, and *Pilodius areolatus* (see Jamieson 1989 c) and particularly *Portunus pelagicus* (see Jamieson 1989 b, and present study), and draw further comparisons with the sperm of *Ranina ranina.* The comparison will constitute a brief review of brachyuran sperm ultrastructure. The evidence will be used to assess the validity of a heterotreme, as opposed to an oxystomate, status for dorippids.

Materials and methods

Neodorippe astuta (Fabricius, 1793) was collected from Cabbage Tree Creek, S. E. Queensland (27'22" S; 153'00" E) in March 1989, and *Portunus pelagicus* (Linnaeus, 1766) from nearby Moreton Bay, in February 1988. Testis portions from both were fixed in 3% glutaraldehyde in 0.2 *M* phosphate buffer (pH 7.4), osmotically adjusted with 6% sucrose, at 4 °C, for 2 h, washed in the same buffer (3 washes in 15 min), postfixed in similarly buffered 1% osmium tetroxide for 80 min, dehydrated through an ascending series of ethanol, and then infiltrated and embedded in Spurr's epoxy resin (Spurr 1969). Thin sections (500 to 800 Å thick) were cut on a LKB 2128 UM IV microtome with diamond knives. Sections were placed on carbon-stabilized colloidin-coated 200 μm-mesh copper grids and stained in 6% aqueous uranyl acetate for 40 min, rinsed in distilled water, stained with lead citrate for 20 min, and further rinsed in distilled water. The sections were examined and micrographs taken on a Hitachi 300 transmission electron microscope (80 kV).

Results

General morphology

The general morphology of the spermatozoon of *Neodorippe astuta* is summarized in Fig. 1 A and may be compared with that of *Portunus pelagicus* (Fig. 1 B). The spermatozoon consists of a subspheroidal acrosome, the posterior two-thirds of which are embedded in a cup-like depression in an amorphous nucleus, leaving the operculum and apical portion free. A layer of cytoplasm intervenes between acrosome and nucleus (Fig. 2 A, B). The nucleus is drawn out to form two to several lateral arms. The thin layer of cytoplasm contains the remaining cell organelles: mitochondria, centrioles and lamellar structures. As in *P. pelagicus* (Fig. 1 B), no posterior median nuclear process is present.

The acrosome

The complex acrosome (Fig. 2 A) is slightly ellipsoidal, being larger in the anterior-posterior dimension (taking the operculum to be the anterior end). The acrosome vesicle has a mean length of 2.91 μm (range 2.66 to 3.16 μm; $n=7$) compared with a mean width of 2.57 μm (range 2.33 to 2.8 μm; $n=12$). These dimensions approximate those of the acrosome of *Portunus pelagicus* (see Jamieson 1989 b).

The operculum of the *Neodorippe astuta* acrosome is an electron-dense, gently convex, apical dome (Fig. 2 A, B). It has a mean width of 1.49 μm (range 1.37 to 1.63 μm), $n=7$, at its greatest, basal, width. The operculum of *Portunus pelagicus* is slightly more pointed at its apex and flatter in profile (Fig. 1 B).

A thick electron-dense envelope, the capsule, invests the acrosome region, excepting the operculum (Fig. 2 A, C–E, I), as in *Portunus pelagicus* (see Jamieson 1989 b, and herein), *Callinectes sapidus* (see Brown 1966 b), *Eriocheir japonicus* (see Yasuzumi 1960), *Altergatis floridus* (see Jamieson 1989 c) and all other investigated Brachyura.

The core of the acrosome vesicle in *Neodorippe astuta* is occupied by a large subacrosomal space, the contents of which comprise the putative perforatorium (Fig. 2 A,

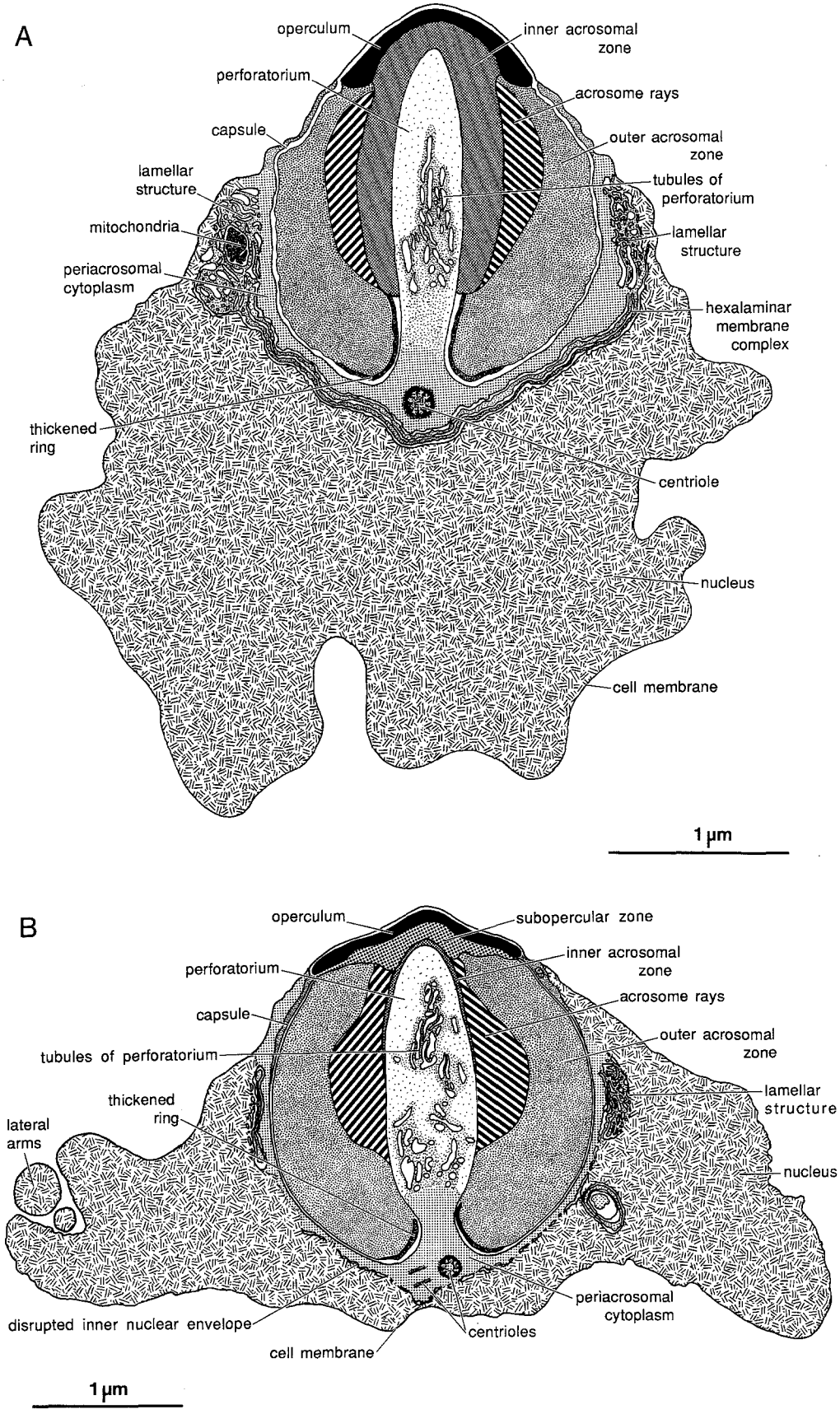
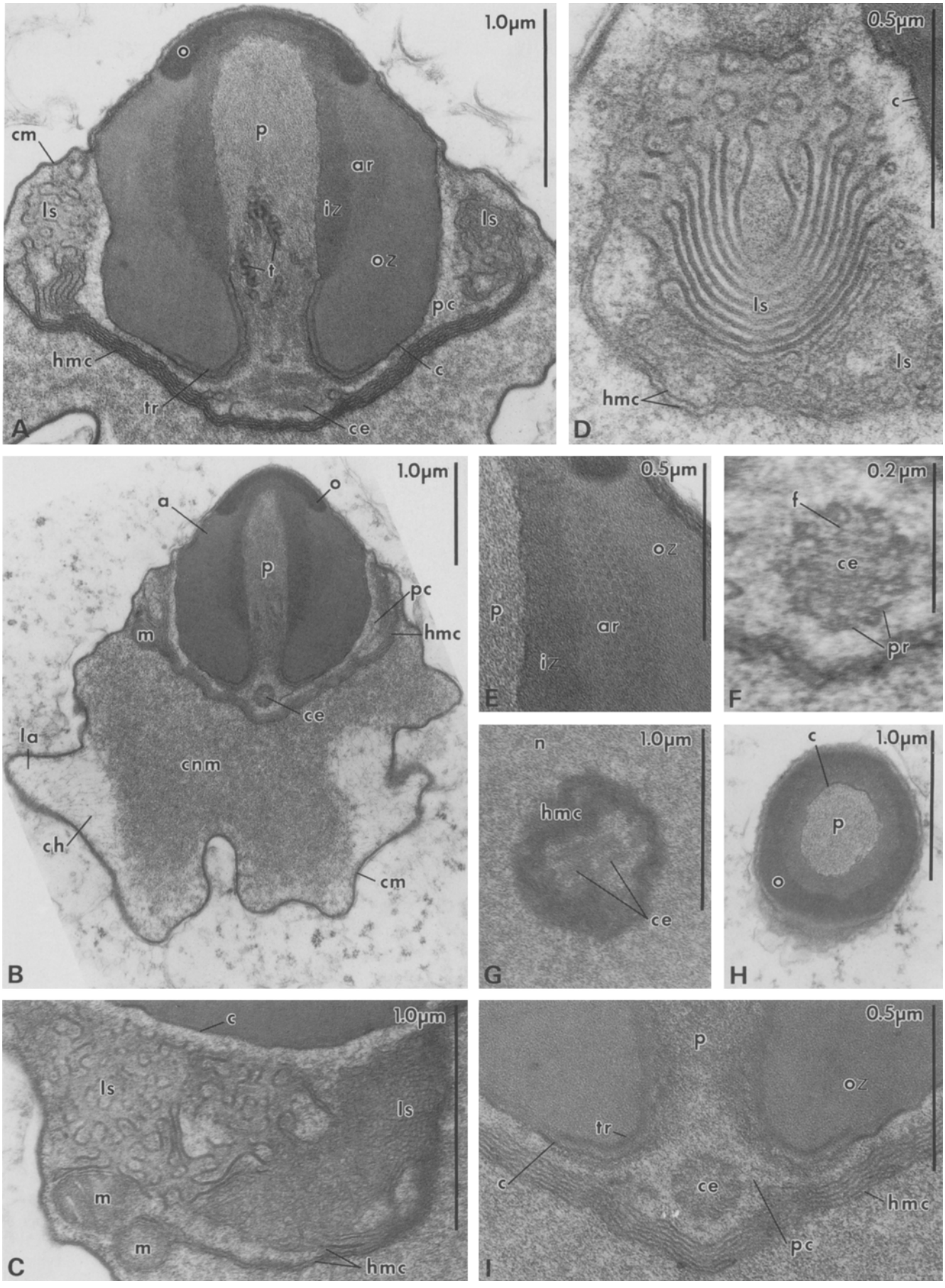


Fig. 1. *Neodorippe astuta* (A) and *Portunus pelagicus* (B). Semidiagrammatic longitudinal sections of spermatozoa, based on tracings of micrographs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B, H). It extends as a large, column-like invagination from the periacrosomal cytoplasm at the base of the acrosome to a position below but within the convex dome of the apical cap. The subopercular zone or subcap zone, present as a granular zone between the operculum and the perforatorium of *Portunus pelagicus* (Fig. 1 B), is absent from *N. astuta* (Fig. 1 A). In *N. astuta,* this area contains material of similar density and appearance to that in the inner acrosomal zone and is therefore considered continuous with the latter (Fig. 2 A, B).

Surrounding the perforatorium, occurring as layers or rings, are three major concentric zones (Figs. 1 A, 2 A, B). The innermost is the inner acrosomal zone, which adheres as a finely granular, inverted cup to the anterior three-fourths of the perforatorial column. The comparable inner acrosomal zone of *Portunus pelagicus* is relatively reduced and covers only the anterior half of the perforatorium as a thin envelope (Fig. 1 B). External to the inner acrosmal zone, but still included as part of this zone, is an area of radiating rays or tubules (Fig. 2 A, E). Like the inner component, the acrosomal ray zone does not fully envelope the perforatorium, but extends from the base of the operculum to the thickened ring at the base of the vesicle in *Neodorippe astuta* (Figs. 1 A, 2 A, B, I), but ends well anterior of this in *P. pelagicus* (Fig. 1 B) as in *Callinectes sapidus* (see Brown 1966 b).

The remainder of the acrosome vesicle, exterior to the perforatorium and acrosome rays, but bounded by the capsule, is a homogeneous zone of varying electron density (Fig. 2 A, B, E). This outer acrosomal zone is present in many Brachyura, including *Portunus pelagicus* (Fig. 1 B) (see "Discussion").

Subacrosomal region

In *Neodorippe astuta* (Figs. 1 A, 2 A, B), as in *Portunus pelagicus* (Fig. 1 B), the subacrosomal region consists of the longitudinally orientated perforatorium, which extends from the base of the acrosome vesicle to a subterminal position beneath the operculum. It forms a long invaginated core and appears continuous with the cytoplasmic region, in the vicinity of the centrioles (Fig. 2 A, I). Within the perforatorium there is an assemblage of perforatorial tubules (Fig. 2 A, B). These tubules, irregular in form and wider than typical 24 nm microtubules, appear hollow in cross-section and have a predominantly anterior-posterior orientation. They are more numerous near the base of the perforatorium and extend nearly to the apex of the column in both species.

Cytoplasm

A residual layer of cytoplasm persists between the nuclear cup and the acrosome vesicle. In *Neodorippe astuta,* this cytoplasm is separated from the nuclear material by a hexalaminar membrane complex (Fig. 2 A, B, I). In *Portunus pelagicus,* only a disrupted double membrane separates the much reduced residual cytoplasm from the nucleus (Fig. 1 B). The *N. astuta* hexalaminar membrane remains intact for most of its length, but peripherally subdivides into convoluted lamellar structures (Fig. 2 A, C, D). In some cases the lamellar structures appear regularly stacked and are reminiscent of a Golgi apparatus (Fig. 2 D).

The cytoplasm is therefore held in a membrane-bound cup formed by the nucleus and encompassing the posterior half of the acrosome vesicle. The lamellar structures and other cytoplasmic organelles are displaced to the rim of this cup. They include intact and degenerating mitochondria (Fig. 2 C) and sometimes microtubules. The disrupted mitochondrial membranes appear to contribute some of the lamellae. In the majority of spermatozoa seen, the inner (anterior) four membranes of the hexalaminar membrane complex are continuous with the lamellar structures (Fig. 2 A, C, D). The remaining outer (posterior) double membrane (possibly the inner nuclear membrane) is substantially intact and encompasses the residual cytoplasm completely. The lamellar structures are considerably smaller and less numerous in *Portunus pelagicus* than in *Neodorippe astuta,* although similarly contiguous with the disrupted nuclear envelope (Fig. 1 B).

Centrioles

Two centrioles are present in the cytoplasm (Fig. 2 A, F, G), directly below the perforatorium. This position is identical with that of *Portunus pelagicus* (Fig. 1 B). The centrioles appear to be randomly orientated to one another, contrasting with the mutually perpendicular arrangement in *P. pelagicus.* In *Neodorippe astuta,* and less certainly demonstrable in *P. pelagicus,* each centriole consists of nine doublets, each of which has a short centrally directed "foot" and a lateral arm (Fig. 2 I; see "Discussion").

Nucleus

The nucleus envelops the posterior half of the acrosome vesicle and is separated from the residual cytoplasm by

Fig. 2. *Neodorippe astuta.* Transmission electron micrographs of spermatozoon. (A) Longitudinal section (LS) of acrosome vesicle, showing conspicuous acrosome rays and hexalaminar membrane complex. (B) LS of entire sperm cell. (C) Transverse section (TS) through lamellar structure, showing apparent connections between hexalaminar membrane complex, lamellar structure and mitochondria. (D) LS through a lamellar structure resembling a Golgi complex. (E) LS showing details of acrosome rays from (A); individual rays appear as tubules in cross-section. (F) TS of a centriole showing its doublet construction, each doublet with a lateral arm and a radial "foot". (G) TS through the two centrioles surrounded by fused hexalaminar membrane. (H) TS apically through acrosome vesicle, showing zonation. (I) LS through base of acrosome vesicle, showing thickened ring, hexalaminar membrane complex and one centriole. a: acrosome; ar: acrosome rays; c: capsule; ce: centrioles; ch: chromatin strands; cm: cell membrane; cnm: condensed nuclear material; f: foot; hmc: hexalaminar membrane complex; iz: inner acrosomal zone; la: lateral arms; ls: lamellar structure; m: mitochondria; n: nucleus; o: operculum; oz: outer acrosomal zone; p: perforatorium; pc: periacrosomal cytoplasm; pr: projection or arm; t: tubules of perforatorium; tr: thickened ring

the membrane complex. Close to the acrosome vesicle, the chromatin forms a homogeneous dense mass but, in *Neodorippe astuta,* as it extends into the lateral arms, the chromatin is progressively less condensed and forms loosely packed electron-dense strands or filaments (Fig. 2B). These chromatin filaments or DNA strands are further reduced in density towards the tips of the arms. In *Portunus pelagicus,* the entire nuclear material has this decondensed appearance.

As in *Portunus pelagicus* (Fig. 1B), the thick, dense outer cell membrane in *Neodorippe astuta* (Fig. 2B) is interpreted to be a combination of the nuclear membrane and the plasma membrane.

Discussion

The general morphology of the mature spermatozoon of *Neodorippe astuta* is typical of the Brachyura. These have a subspheroidal, multi-zoned acrosome vesicle (in dromiids depressed), capped by an electron-dense "apical cap" or operculum. This acrosome, enveloped in scanty cytoplasm, resides in a cup formed by the nucleus. The nucleus forms 2 to 10 radiating arms with the notable exceptions of investigated Dromiidae, which have only stubby radial arms in *Dromidia antillensis* (see Brown 1966a), or appear to lack nuclear extensions in *Petalomera lateralis* (see Jamieson 1990). Dromiids differ further from other examined brachyuran sperm in having a discoidal acrosome and a unique capitate perforatorium. The spermatozoal morphology here described for *N. astuta* and *Portunus pelagicus* is similar, with minor variations, to that of the mature spermatozoa of all Eubrachyura studied to date (Jamieson 1989b).

The spermatozoon is enclosed within a thick, complex, surface membrane, generally agreed to be a fusion of the plasmalemmal and nuclear membranes. This appears as a single, electron-dense layer and only fortuitous fixation allow the separate layers to be elucidated (Hinsch 1969). Langreth (1969) considered that the fusion of two nuclear membranes and the cell membrane made it a tripartite surface membrane, while Reger (1970) stated that a pentalaminar nuclear membrane and a plasma membrane composed the complex cell membrane.

In most of the Brachyuran sperm described to date, the nuclear material has been described as homogeneous electron-dense chromatin filaments in a pale matrix (Brown 1966a, b, Jamieson 1989b). In *Neodorippe astuta,* the chromatin assumes this loose filamentous form only in the arms. It remains dense and granular directly below the acrosome vesicle. A similar arrangement of nuclear material has been reported for *Geryon* spp. by Hinsch (1988).

The boundary between nucleoplasm and cytoplasm is often indistinct in the mature sperm. This is due to the varying breakdown of the inner nuclear membrane. In *Cancer* spp. (see Langreth 1969), the nuclear membrane disintegrates, mixing the nucleoplasm and cytoplasm to form a spermioplasm. Similarly, Brown (1966b) found no clearly identifiable nuclear envelope separating the two regions in *Callinectes sapidus.* In *Portunus pelagicus* a much disrupted nuclear membrane persists (Jamieson 1989b). *Neodorippe astuta* appears unique, in studies to date, in possessing a fully formed hexalaminar membrane complex, the posterior membrane probably being the inner nuclear membrane. This membrane complex cups the basal half of the acrosome vesicle and thin cytoplasmic layer. At its rim, the complex disrupts to form a ring of conspicuous lamellar structures. These lamellae have been described for all brachyuran sperm studied, but variously named and derived (Jamieson 1989b).

Chevaillier (1967) has described "systemes lamellaires" in the sperm of the macruran *Nephrops norvegicus* and the anomuran *Eupagurus bernhardus.* Pochon-Masson (1968) derived the membrane complex of *Carcinus maenas* from dilated ergastoplasmic cisternae and nuclear envelope proliferations. Langreth (1969) found that in *Cancer* spp. the membrane complex is derived from endoplasmic reticulum. In *Libinia emarginata,* Hinsch (1969) described the complex as apparently deriving from remnants of cytoplasmic organelles, including mitochondria. Reger (1970) recognized membraneous organelles in the membrane complex of *Pinnixia* sp. composed of pentalaminar membranes, and derives them from a fusion of nuclear and endoplasmic reticulum membranes.

The function of the brachyuran-anomuran lattice-like membrane complex is unknown. Microtubules (Langreth 1965, 1969) and mitochondria (Langreth 1969, Reger 1970, Hinsch 1973, 1988) are attributed to the complex in some crabs and Pearson and Walker (1975) have shown that the complex does not show any cytochrome *c*-oxidase activity unless there are still recognisable mitochondrial fragments associated with it. Goudeau (1982) has suggested that this prominent membrane system in *Carcinus maenas* may play an important role in the acrosome reaction by linking the acrosome to the nucleus and assisting in pulling the nuclear material through the vitelline envelopes.

Mitochondria, although few, can be readily identified in the mature sperm of *Neodorippe astuta,* as in *Ranina ranina* (see Jamieson 1989b). In contrast, in *Cancer* spp. (see Langreth 1969), *Libinia emarginata* (see Hinsch 1969), *Pinnixia* sp. (see Reger 1970), *Macrocoeloma trispinosum* (see Hinsch 1973), *Carcinus maenas* (see Pearson and Walker 1975), *Geryon* spp. (see Hinsch 1988), *Portunus pelagicus* (see Jamieson 1989b), and *Atergatis floridus* (see Jamieson 1989c), mitochondria are associated with the lamellar complex in the spermatid but remain only as degenerate remnants in the mature sperm. In *L. emarginata* these mitochondrial remnants contribute to the membrane complex on degeneration (Hinsch 1969), but Langreth (1969) considered that, in *Cancer* spp., the appearance of mitochondria caught up in the membrane complex whorls is fortuitous as most mitochondria are lost as cytoplasm is sloughed to nurse cells.

Microtubules were reported to be present in the lamellar complex of the spermatid of *Cancer* spp. (see Langreth 1969), but were no longer seen when the spermatozoa was mature. Similarly, a few, rare microtubules are seen in the lamellae of *Neodorippe astuta.*

Centrioles persist in the mature sperm of most of the Brachyura studied (Pochon-Masson 1962, Hinsch 1969,

1986, 1988, Langreth 1969, Reger 1970, Pearson and Walker 1975, Goudeau 1982), but are absent from the four xanthid species *Atergatis floridus, Pilodius areolatus, Etisus laevimanus* and *Liagore rubromaculata* (see Jamieson 1989c), as in *Ranina ranina* (see Jamieson 1989b) and *Petalomera lateralis* (see Jamieson 1990). Triplet centrioles have been reported for *Carcinus maenas* (see Pochon-Masson 1968; p. 36) and *Cancer* spp. (see Langreth 1969; p. 590) but we are unable to confirm the existence of triplets in micrographs in these or any other works on brachyuran sperm. In contrast, doublets were seen in the majid *Heterocrypta granulata* (see Hinsch 1973), in *Neodorippe astuta* and, less certainly, in *Portunus pelagicus* (present study). The structure of the doublet centriole of *N. astuta* resembles that described by Cotelli et al. (1975, 1976) for peracarids: a short radial "foot" is directed centrally and an arm or "bud" extends laterally from each doublet. This doublet centriolar structure may be an autapomorphy of the Malacostraca (Jamieson 1989a).

A consistent feature of all investigated eubrachyuran sperm is the spherical, concentrically layered acrosome vesicle with an anterior apical cap or operculum and the posterior perforatorial invagination, all contained within an electron-dense capsule. Interspecific homologies can be drawn between the various layers or zones, although differences occur in the extent of each. The invaginated, longitudinal perforatorium containing convoluted but chiefly longitudinal tubules has been variously named in previous papers (see Jamieson 1989b for list of synonyms) and forms the core of the vesicle.

The "thickened ring" occurs as an electron-dense collar surrounding the base of the perforatorium, subjacent to the capsule in all described brachyuran sperm, excepting the oxystomate (archaeobrachyuran) *Ranina ranina* (see Jamieson 1989b) and dromiids (Jamieson 1990).

Immediately anterior to the perforatorium and forming a very dense cap of varying form, is the operculum or apical cap (Brown 1966a). The operculum is generally in the form of an inverted bowl, with the exception of the oxyrhynch crabs (Hinsch 1973) and *Ranina ranina* (see Jamieson 1989b) in which an apical perforation is present, forming a ring. In *Callinectes sapidus* (see Brown 1966a, b) and *Portunus pelagicus* (see Jamieson 1989b), the centre of the dome projects distally as a pointed cone. The presence of a "subcap" zone (Brown 1966a, b) or subopercular zone (Jamieson 1989b), directly beneath the operculum is variable.

The remainder of the acrosome vesicle is composed of the inner and outer acrosomal zones. In eubrachyurans, the inner zone is composed of two distinct layers. The innermost, enveloping the perforatorium, appears finely granular and electron-dense. Immediately exterior to this layer is the very distinct acrosomal ray zone. Generally forming a concentric ring level with the equator of the acrosome vesicle, this ring is composed of electron-dense radiating tubules. This combined inner acrosomal zone has been variously named by different authors (see Jamieson 1989b) and appears to be a consistent component of decapod brachyuran and anomuran acrosomes. The zone of acrosomal rays, although very prominent in *Portunus pelagicus* (see Jamieson 1989b), *Callinectes sapidus* (see Brown 1966a, b), and *Neodorippe astuta* (present study), is reduced in *Atergatis floridus* (see Jamieson 1989c), *Cancer* spp. (see Langreth 1969), *Pinnixia* sp. (see Reger 1970) and the oxyrhynch crabs (Hinsch 1969, 1973). The exteriormost region of the vesicle is filled with an homogeneous, moderately electron-dense material which reaches the capsule. Both *A. floridus* (see Jamieson 1989c) and *Geryon* spp. (see Hinsch 1988) have a further peripheral layer of similar but less electron-dense granular material abutting the capsule.

The above comparison of the spermatozoa of *Neodorippe astuta* with that of *Portunus pelagicus* and other brachyurans allows us to assess the validity of placement of dorippids with *Ranina ranina* (in the Archaeobrachyura or the Oxystomata) or with portunids and their relatives in the heterotreme section of the Eubrachyura (the Heterotremata-Thoracotremata or the Oxyrhyncha - Cancridea - Brachygnatha assemblage).

Similarities between spermatozoa of *Neodorippe astuta* and of *Portunus pelagicus* (and other Eubrachyura) and *Ranina ranina* include: the large spherical, multilayered, capsule-bound acrosome vesicle; the electron-dense operculum capping the vesicle; an invaginated core, or perforatorium; concentric zonation of the contents of the vesicle; a layer of cytoplasm, between the acrosome vesicle and the nucleus, which contains mitochondria (mostly degenerating) and lattice-like lamellar complexes or membrane remnants; a diffuse nucleus which is bounded externally by a combined nuclear and plasma membrane and cups the scanty cytoplasm and the large acrosome vesicle; and lateral arms into which the chromatin extends.

Characteristic eubrachyuran features of the *Neodorippe astuta* sperm absent from *Ranina ranina* are the long perforatorium (short and conical with a unique subacrosomal chamber in *R. ranina*) extending almost to the operculum; presence in the perforatorium of longitudinally arranged convoluted tubules; a zone of acrosomal rays forming the outer part of an inner dense zone; the presence of a thickened ring surrounding the basal part of the perforatorium; and, basally, two centrioles (absent from *R. ranina* but also from some eubrachyurans). The sperm of *N. astuta* is more similar to that of *Portunus pelagicus* than to that of other investigated Brachyura, although differences are noted below. A heterotreme status of *N. astuta* is thus unequivocally supported. Both species lack the posterior median process seen in the nucleus of majids and *R. ranina*. The median process may be a synapomorphy between the Archaeobrachyura, exemplified by *R. ranina,* and majids, as "lower" Heterotremata, which has been apomorphically lost in "higher" heterotremes and the Thoracotremata. Its absence from the Dromiacea, as in Anomura, is clearly plesiomorphic.

The operculum of *Neodorippe astuta* is a more rounded dome and lacks the central apical point of *Portunus pelagicus*. The distinct subopercular zone of *P. pelagicus* appears to be absent from *N. astuta,* in which it is replaced with a continuation of the inner acrosome zone. This forms an extensive cylinder, capping much of the

perforatorial column. The inner acrosome zone is reduced in *P. pelagicus* to a thin apical cap. Although the lamellar complex occurs in both genera, it is a far more extensive structure in *N. astuta,* with its unique hexalaminar membrane. The acrosome of *P. pelagicus* is also more evenly rounded than that of *N. astuta.* The change in density and form of the chromatin between the body of the nucleus and the lateral arms in *N. astuta,* although not unique (see Hinsch 1988), is pronounced and differs markedly from the homogeneous, paler, filamentous matrix in *P. pelagicus.*

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