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An ultrastructural study of the spermatozoa of *Eulalia* sp. (Phyllodocidae), *Lepidonotus* sp. (Polynoidae), *Lumbrineris* sp. (Lumbrineridae) and *Owenia fusiformis* (Oweniidae)

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ABSTRACT: The ultrastructure of the mature spermatozoa of four polychaetes is described: *Eulalia* sp. (Phyllodocidae), *Lepidonotus* sp. (Polynoidae), *Lumbrineris* sp. (Lumbrineridae) and *Owenia fusiformis* (Oweniidae). All the sperm show features typical of externally fertilizing sperm in having a rounded nucleus, a short unmodified midpiece, and a simple flagellum with a 9+2 axoneme. *Owenia fusiformis* and *Lepidonotus* sp. have a nuclear cone extending into the subacrosomal space that may act to present the inner acrosomal membrane to the egg during fertilization. The acrosome of *Lumbrineris* sp. is flattened and crenulated. The sperm of *Eulalia* sp. is unusual in having the four mitochondria of the midpiece ensheathed by a membrane. Comparisons are made with other polychaete sperm, and the use of sperm ultrastructure as a taxonomic tool within the Polychaeta is discussed.

INTRODUCTION

External fertilization is common amongst members of the Polychaeta. To date, the published ultrastructural studies of the sperm of polychaetes utilizing external fertilization include those on the chaetopterid *Chaetopterus pergamentaceus* (Anderson & Eckberg, 1983), the cirratulid *Cirriformia tentaculata* (Sawada, 1984), the amphinomid *Eurythoe complanata* (Rouse & Jamieson, 1987), the nereids *Nereis japonica* (Takashima & Takashima, 1963), *Nereis limbata* (Fallon & Austin, 1967), *Nereis virens* (Bass & Brafield, 1972), *Nereis irrorata* (Defretin & Wissocq, 1974), *Nereis diversicolor* (Bertout, 1976), *Perinereis brevicirrus* (Kubo & Sawada, 1977) and *Tylorrynchus heterochaetus* (Sato & Osanai, 1983), the opheliid *Travisia japonica* (Ochi et al., 1977), the sabellariids *Phragmatopoma lapidosa* (Eckelbarger, 1984) and *Phragmatopoma californica* (Kopp, 1985), the pectinariid *Cistenides okudai* (Sawada, 1984), the sabellids *Sabella penicillum* (Graebner & Kryvi, 1973a, b; Kryvi & Graebner, 1975) *Perkinsiana rubra* and *Pseudopotamilla reniformis* (Chughtai, 1986), the serpulids *Hydroides hexagonus* (Colwin & Colwin, 1961) and *Pomatoleios krausii* (Sawada, 1984) and the polygordiid *Polygordius lacteus* (Franzén, 1977).

These studies have demonstrated the variation in morphology of polychaete ectaquasperm (see Rouse & Jamieson, 1987). This study represents the first ultrastructural

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descriptions of sperm from the families Phyllodocidae (*Eulalia* sp.), Polynoidae (*Lepidonotus* sp.), Lumbrineridae (*Lumbrineris* sp.) and Oweniidae *Owenia fusiformis*. With further studies gamete morphology may prove useful as a taxonomic tool within families of polychaetes.

MATERIALS AND METHODS

Specimens of *Eulalia* sp., *Lepidonotus* sp., *Lumbrineris* sp. and *Owenia fusiformis* were placed in fixative for 15 min and then cut into pieces for further processing. In all cases initial fixation was achieved with 3 % glutaraldehyde, in 0.2 M sodium cacodylate buffer (pH 7.4) with 0.3 M sucrose added, at 4 ° C. Samples were post-fixed for 80 min in 1 % osmium tetroxide in 0.2 M sodium cacodylate buffer with 0.3 M sucrose; washed in buffer; dehydrated through an ethanol series; and infiltrated and embedded in Spurr's epoxy resin. All sections were cut, mainly with diamond knives, on an LKB 2128 UM IV microtome. Thin sections, 500–800 Å thick, were collected on carbon stabilized colloidin-coated 200 mesh copper grids. Sections were stained in either of two methods: (1) 40 min in a 10 % super-saturated solution of aqueous uranyl acetete, rinsed in distilled water followed by 20 min in Reynold's lead citrate and rinsed again before viewing; (2) a modification of the Daddow (1983) technique; 30 sec in Reynold's lead citrate, 1 min in 6 % aqueous uranyl acetate (after rinsing in distilled water) and a further 30 sec in lead citrate before final rinsing. All electron micrographs were taken on a Hitachi 300 electron microscope at 75 kV. *Lepidonotus* sp. was found under reef flat rubble at Heron Island, Great Barrier Reef. *Eulalia* sp. and *Lumbrineris* sp. were collected from intertidal algal turf at Hasting Point, New South Wales. These three species are undescribed. *Owenia fusiformis* was collected from intertidal sea grass beds at Cleveland, Queensland. Specimens of all species are lodged in the Australian Museum, Sydney with the following catalogue numbers; *Eulalia* sp. (AM.W 200615); *Lepidonotus* sp. (AM.W 200618); *Lumbrineris* sp. (AM.W 200617) and *Owenia fusiformis* (AM.W. 200620).

RESULTS

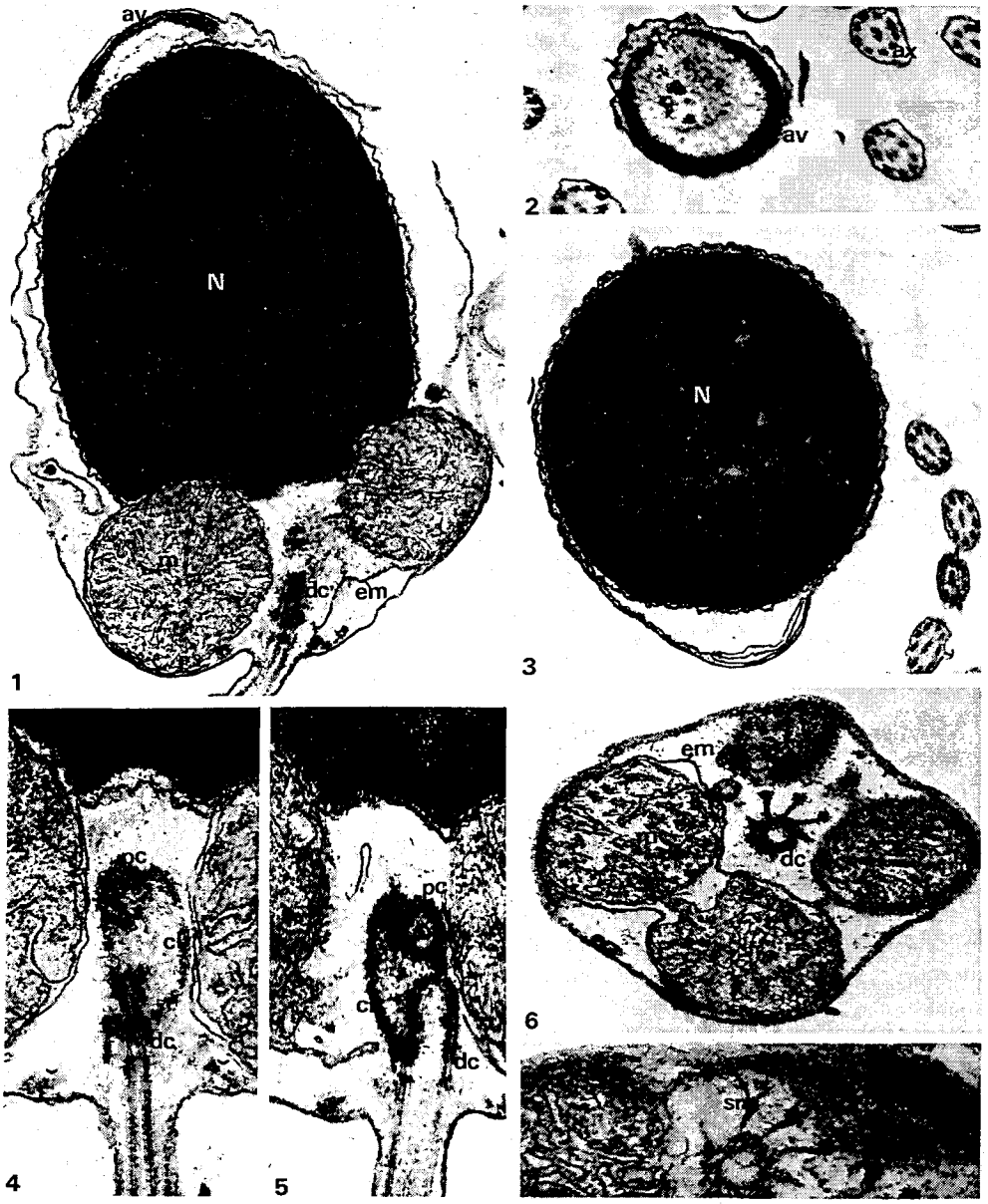
Eulalia sp. (Phyllodocidae)

The spermatozoon is 40 µm long, the head being 3.6 µm long (Fig. 1). The acrosome consists of a single vesicle with contents of uniform electron density. The shape of the acrosome is that of an inverted dish, flattened anteriorly (Fig. 1). In transverse section the acrosome appears as a circular ring (Fig. 2). Little sub-acrosomal material is evident.

The nucleus is ellipsoidal in longitudinal section with indentations posteriorly where it meets the mitochondria (Figs 1, 4, 5). In transverse section the nucleus is circular (Fig.

Abbreviations used in Figures

a	acrosome	m	mitochondrion
ap	anchoring plate	N	nucleus
ax	axoneme	n	nuclear membrane
dc	distal centriole	pc	proximal centriole
dr	dense ring	r	anchoring ray
f	centriolar fossa	sr	satellite ray
l	centriolar link		



Figures 1–7. *Eulalia* sp. (Phyllodocidae) spermatozoon. Fig. 1. Longitudinal section through mature spermatozoon. × 26 000. Fig. 2. Transverse section through an acrosome and tails of adjacent spermatozoa. Note 9 + 2 organization of axoneme. × 28 000. Fig. 3. Transverse section through nucleus of mature spermatozoon. × 28 000. Fig. 4. Longitudinal section through midpiece. Note centriolar link. × 39 000. Fig. 5. Longitudinal section through midpiece. × 39 000. Fig. 6. Transverse section through midpiece. Note extramitochondrial membrane and satellite rays from distal centriole. × 28 000. Fig. 7. Branching satellite rays from the distal centriole. × 39 000

3). The nuclear chromatin shows uniform condensation with a coarse granular appearance. Posteriorly, four large spherical mitochondria abut and indent the nucleus. The mitochondria show many prominent cristae and extend laterally beyond the nucleus (Figs 1, 4, 5). An extra membrane, possibly, a nuclear membrane, ensheaths the mitochondria (Figs 1, 6).

There are two centrioles, posterior to the nucleus, surrounded by the mitochondria. They are arranged with the proximal centriole lying centrally across the vertical axis of the spermatozoon and the distal centriole tilted at approximately 30° to the vertical axis (Figs 1, 4, 5). There is a prominent electron dense bridge connecting the two centrioles. The bridge connects the base of the distal centriole to one side of the proximal centriole (Figs 4, 5). A satellite complex originates from the distal centriole and links it to the plasma membrane (Figs 1, 4, 5, 6, 7). The satellite complex is composed of branching rays extending from each triplet of the centriole to the plasma membrane (Figs 4, 5, 6, 7). The axoneme, arising from the distal centriole, has a 9+2 arrangement of microtubules (Fig. 2).

Lepidonotus sp. (Polynoidae).

The spermatozoon has a head length of 3.6 μm (Fig. 8). The acrosome, semispherical, is deeply invaginated with a thickened posterior rim (Figs 8, 10). The contents of the acrosome vesicle are of uniform electron density and in transverse section the acrosome is a circular shape (Figs 9, 10). No organized sub-acrosomal material is evident; however, some flocculent material lies between the inner acrosomal and nuclear membranes (Fig. 8).

The nucleus is ellipsoidal in longitudinal section, narrowing anteriorly, and circular in transverse section. There is also a prominent conical projection occupying the space created by the acrosome invagination (Figs 8, 10, 11). The nucleus is uniformly dense though some intra-nuclear vesicles of low electron density are apparent. The nuclear membrane is loosely applied to the nucleus (Fig. 8). Five spherical mitochondria lie at the base of the nucleus (Figs 8, 13). They have prominent cristae and are closely applied to the nucleus (Figs 11, 12, 13). The centriolar complex is enclosed by the mitochondria.

Two centrioles are present, the distal of which lies on the central vertical axis of the spermatozoon. The proximal centriole lies on the central axis at approximately 30° to the distal centriole (Fig. 12). A satellite complex is present with the rays branching before fusing with the plasma membrane. Each ray sends a branch that joins the plasma membrane at a 45° angle (Fig. 12). The rays also bifircate and fan out and form a dense ring (Figs 11 inset, 14). A link is also visible from the proximal centriole to a fossa in the

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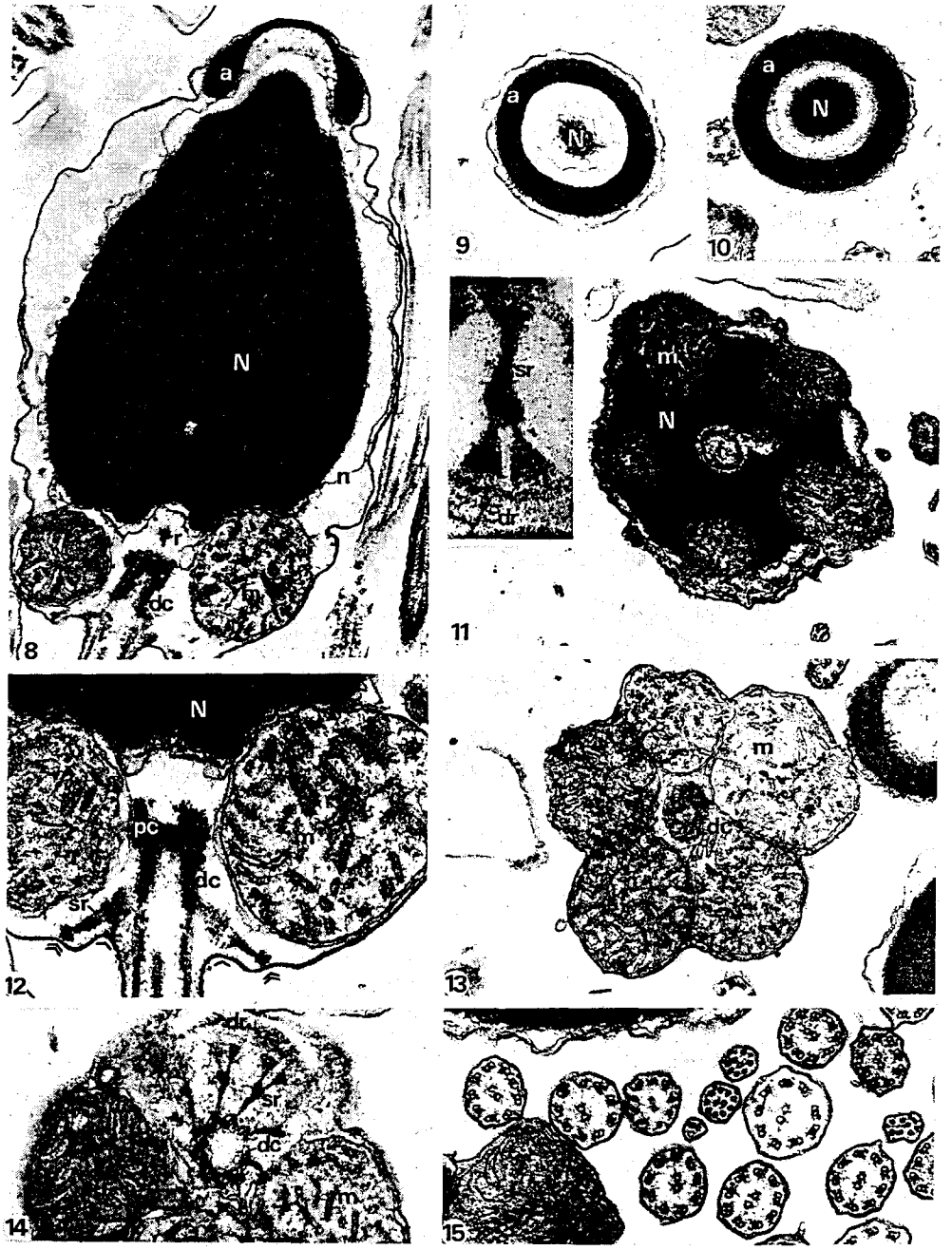
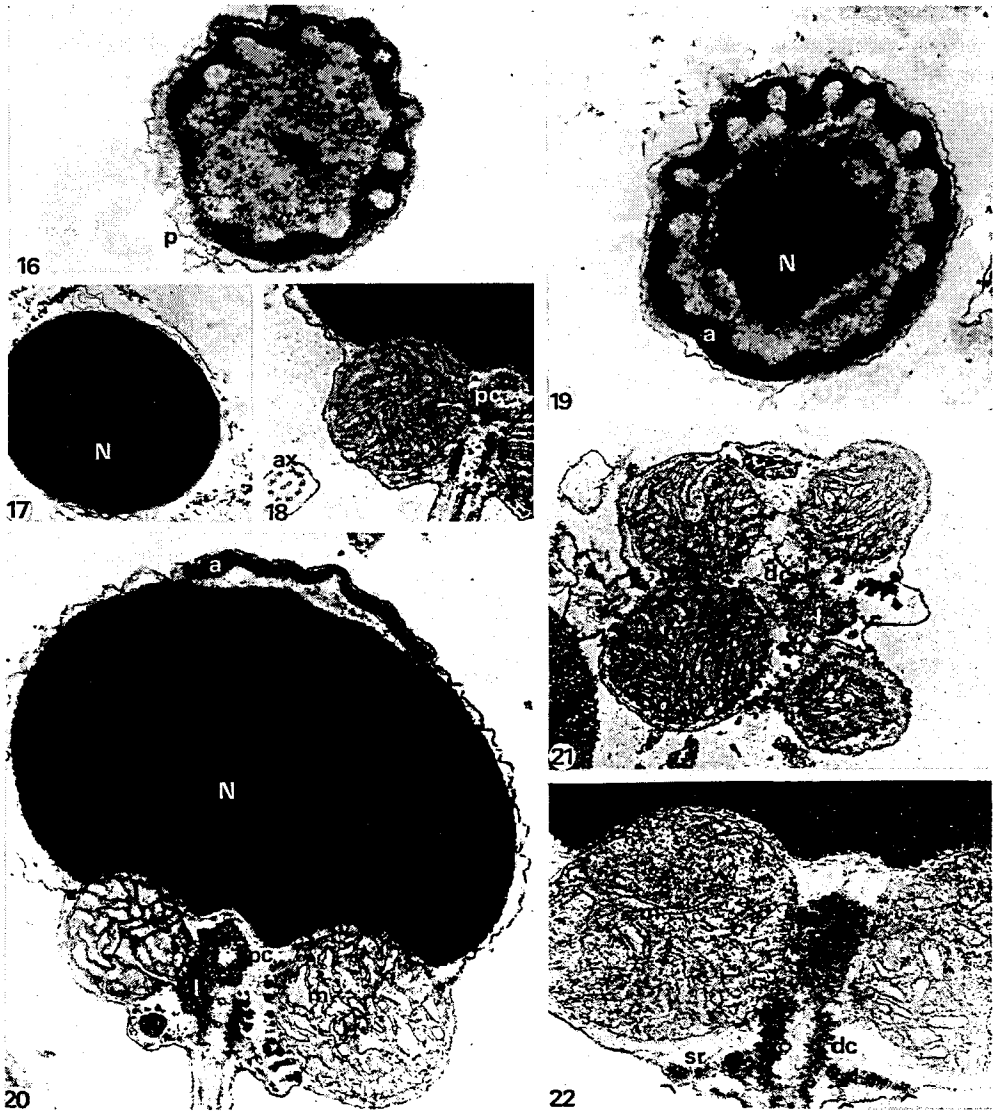
Figures 8–15. *Lepidonotus* sp. (Polynoidae) spermatozoon. Fig. 8. Longitudinal section through mature spermatozoon. Note loosely applied nuclear membrane. × 24 500. Fig. 9. Transverse section through an acrosome and the tip of the nuclear cone. × 24 500. Fig. 10. Transverse section through base of acrosome and the nuclear cone. × 24 500. Fig. 11. Transverse section through the base of the nucleus. Note fossa for centriolar ray. × 24 500. Inset. Detail of one satellite ray showing striated bifircations fusing to dense ring. × 57 000. Fig. 12. Longitudinal section through midpiece. Note satellite rays and the connections to the plasma membrane (arrows, double arrows indicate dense ring). × 24 500. Fig. 13. Transverse section through midpiece. Note distal centriole. × 24 500. Fig. 14. Branching satellite rays from the distal centriole, fusing to form the dense ring. × 37 000. Fig. 15. Transverse sections of sperm tails. × 37 000

middle of the base of the nucleus (Fig. 8). The axoneme arising from the distal centriole is of a 9+2 arrangement of microtubules (Fig. 15). The microtubules shown a breakdown of organization at the posterior end of the tail (Fig. 15).





Figures 16–22. *Lumbrineris* sp. (Lumbrineridae) spermatozoon. Fig. 16. Transverse section through acrosomal region. Note crenulated shape of the acrosome vesicle. × 23 400. Fig. 17. Transverse section through the nucleus. × 11 700. Fig. 18. Longitudinal section through midpiece. Note proximal centriole in nuclear fossa and the 9 + 2 organization of microtubules in adjacent sperm tail. × 23 400. Fig. 19. Transverse section through base of acrosome and top of the nucleus. × 23 400. Fig. 20. Longitudinal section through mature spermatozoon. × 23 400. Fig. 21. Transverse section through midpiece. Note the centriolar link on one side of the distal centriole. × 23 400. Fig. 22. Longitudinal section through midpiece. Note satellite rays and the connections to the plasma membrane (arrows). × 35 000

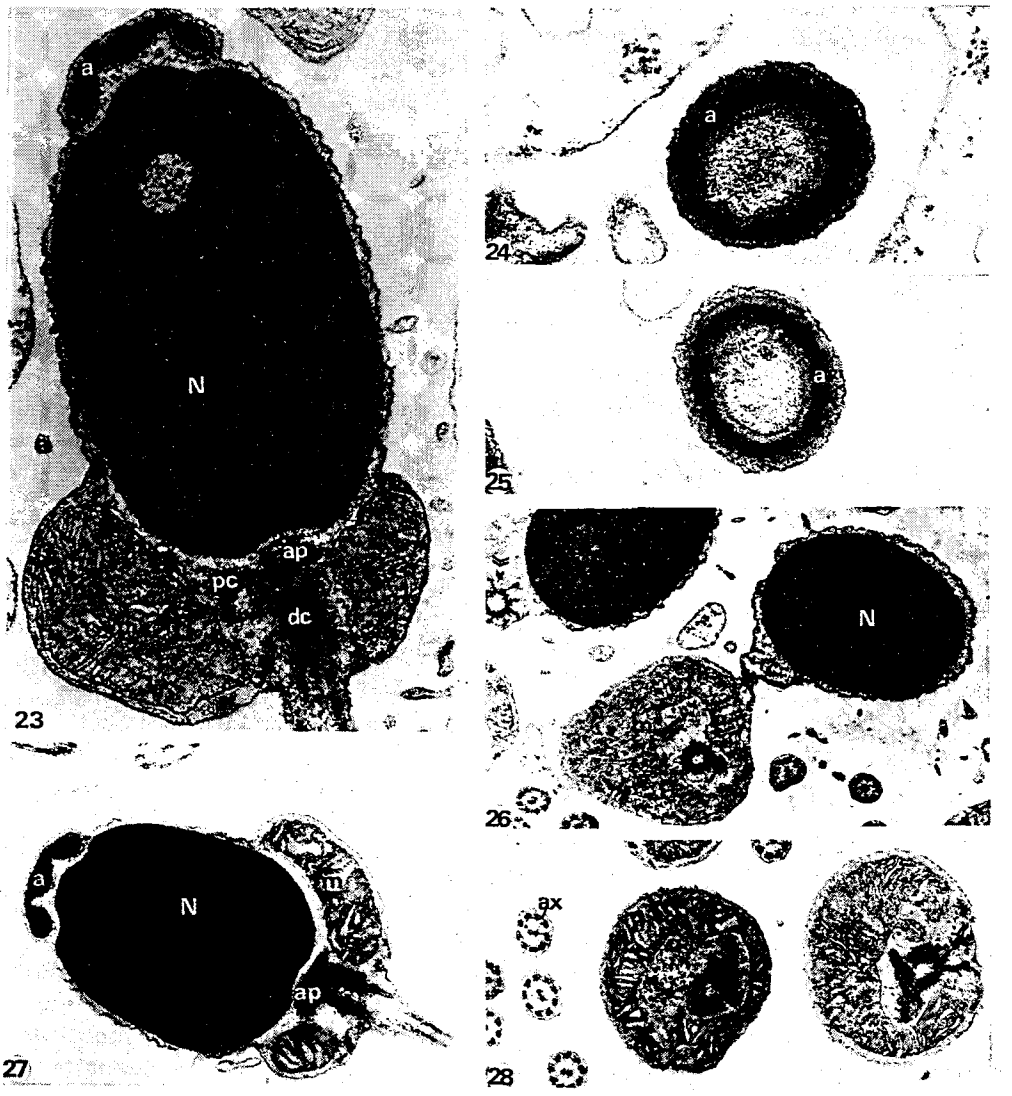
Lumbrineris sp. (Lumbrineridae)

The sperm head is 3 μm long. A single acrosome vesicle caps the anterior end of the nucleus. The acrosome has the shape of a crenulated dish to which the plasma membrane is closely applied (Fig. 20). The acrosome contents are differentiated into an outer electron transparent layer and an inner electron dense layer (Fig. 16). Sub-acrosomal material is present as a flocculent material dispersed between the nucleus and the acrosome (Figs 16, 19). The nucleus is spherical and flattened in the anterior/posterior plane and shows marked electron density with a granular appearance (Figs 17, 20). In transverse section the nucleus is circular (Fig. 17). Posteriorly, four large mitochondria, with prominent cristae, abut and indent the nucleus (Figs 18, 20, 21). Two centrioles are present in the mature spermatozoon of *Lumbrineris* sp. They are oriented at 90° to each other, with the distal centriole lying along the central vertical axis of the spermatozoon, and linked by an amorphous centriolar bridge of moderate electron density (Figs 18, 20, 22). This extends from one side of the proximal centriole down the length of the distal centriole. The proximal centriole lies in a semi-cylindrical fossa at the centre of the base of the nucleus and shows some connection to the thickened nuclear membrane in this region (Figs 18, 20). The distal centriole forms the anchor for the axoneme and distinct satellite fibrils branch and extend laterally from this centriole, fusing with the plasma membrane (Figs 21, 22). The axoneme is of a 9+2 arrangement of microtubules with the central singlets arising distal the centriolar apparatus (Fig. 18).

Owenia fusiformis (Oweniidae)

The spermatozoon head is 2.7 μm long. The acrosomé lies at the anterior end of the nucleus. The single vesicle has the shape of an inverted bowl with a thickened rim (Figs 23, 27). The acrosome contents show two distinct regions of electron density. A layer of moderate electron density lines both the inner and outer acrosomal membranes. The thickened rim of the acrosome vesicle is occupied by a region of marked electron density (Figs 23, 24, 25, 27). The boundary between the two regions shows distinct undulations which in favourable sections show a distinct helical arrangement (Figs 24, 25). Some scattered material occupies the sub-acrosomal space. This material also appears to extend down the side of the nucleus to the midpiece (Figs 23, 26, 27). The nucleus is ellipsoid with a slight invagination posteriorly, occupied by part of the anchoring apparatus. Anteriorly, there is a ridge upon which the acrosome vesicle rests (Figs 23, 27). The nucleus shows uniform chromatin condensation with nuclear vacuoles occasionally visible (Figs 26, 27). There is a single asymmetric mitochondrion in the midpiece. It is circular in transverse section, shows prominent cristae and forms a ring around the centriolar apparatus (Figs 23, 26, 27, 28). The mitochondrion is asymmetric in that it extends anteriorly on only one side of the nucleus. In favourable sections the mitochondrion can be seen to wrap around up to half of the nucleus (Figs 23, 27). There appears to be no consistent orientation of this extension in relation to the position of the centrioles. The proximal centriole lies at 90° to the vertical axis of the spermatozoon and slightly to one side of the distal centriole (Fig. 23). Next to the proximal centriole and above the distal centriole is a dense plate. The dense plate appears to link the two centrioles and occupies the slight fossa at the posterior end of the nucleus (Figs 23, 27). The distal

centriole, lying along the central vertical axis of the spermatozoon, has satellite fibrils that branch and link to the plasma membrane forming a circular ring (Fig. 28). The axoneme arising from the distal centriole is of a 9+2 organization of microtubules.



Figures 23–28. *Owenia fusiformis.* (Oweniidae) spermatozoon. Fig. 23. Longitudinal section through mature spermatozoon. × 35 000. Fig. 24. Transverse section through the acrosome. Fig. 25. Transverse section through base of acrosome. × 46 800. Fig. 26. Transverse section through midpiece. Note the single mitochondrion and the centriolar rays branching (arrows). × 17 500. Fig. 27. Longitudinal section through mature spermatozoon. × 17 500. Fig. 28. Transverse section through midpiece. Note the centriolar rays fusing with the plasma membrane (arrows) and the 9 + 2 organization of microtubules in adjacent sperm tail. × 23 400

DISCUSSION

The four sperm described here exhibit a common structural pattern in having a cap-like acrosome, a sub-spherical nucleus, a small number of cristate mitochondria and two centrioles, the distal (posterior) of which forms the basal body of the axoneme which has a 9+2 arrangement of microtubules. This morphology is generally seen in animals where the gametes are released into the surrounding water for fertilization to take place (Franzén, 1982). Rouse & Jamieson (1987) named the sperm of externally fertilizing animals ect-aquasperm, removing the phylogenetic implications of the term 'primitive sperm' (Retzius, 1904; Franzén, 1956). The variability of ect-aquasperm, within the structural parameters outlined above is exemplified in the sperm described here.

The flattened crenulate shape of the acrosome of *Lumbrineris* sp. is a modification not seen in other ect-aquasperm, where the tendency is for the formation of a subacrosomal space. It is distinctly different from those sperm described in the closely related family Onuphidae e.g. *Hyalinoecia tubicola* (Cotelli & Lora Lamia Donin, 1975). The fertilization reaction involved in presenting such a large area of acrosome to the egg is worthy of study. The functional significance of the crenulated shape of the acrosome is possibly similar to that proposed for the many invaginations of the inner acrosomal membrane of *Sabella penicillum* sperm (Kryvi & Graebner, 1975). These authors suggest this allows a better distribution of lytic enzymes. The complex substructuring of the acrosome vesicle of *Owenia fusiformis* shows similarities to the ect-aquasperm acrosome of *Crania anomala*, a brachiopod (Afzelius & Ferraguti, 1978). The functional significance of this structuring has yet to be elucidated. The acrosomes of the sperm of *Eulalia* sp. and *Lepidonotus* sp. are simple and show no substructuring. The fertilization reactions of these species may be less complex than those with more complex sperm and probably relates to the vitelline layers of their respective eggs.

The structure of the acrosome of ect-aquasperm is widely variable between families of polychaetes. Within families where more than one species has been studied some consistency may be found. Nereids show marked similarities of acrosome structure (Takashima & Takashima, 1963; Fallon & Austin, 1967; Defretin & Wissocq, 1974; Bertout, 1976; Kubo & Sawada, 1977) with the exception of *Tylorrynchus heterochaetus* (Sato & Osanai, 1983), a brackish water species. Sabellariids studied to date (Eckelbarger, 1984; Kopp, 1985) share a unique acrosomal structure. However there are exceptions e.g. the ect-aquasperm of the serpulid *Hydroides hexagonus* (Colwin & Colwin, 1961) is very different from those of other serpulids e.g. *Pomatoleios krausii* (Sawada, 1984) and resembles that of the sabellids *Sabella penicillum* (Graebner & Kryvi, 1973a, b) and *Psuedopotamilla reniformis* (Chughtai, 1986). This does not indicate that *Hydroides, Sabella* and *Psuedopotamilla* are closely related. It suggests they have a similar reproductive method, probably in the way their gametes fuse.

The spermatozoa of *Lepidonotus* sp. and *Owenia fusiformis* have a nuclear cone, a feature also seen in the sperm of *Tylorrynchus heterochaetus* (Sato & Osanai, 1983) and *Hydroides hexagonus* (Colwin & Colwin, 1961). It may act in place of the subacrosomal space in supporting and presenting a particular area of the inner acrosomal membrane to the oolemma on fertilization.

The spermatozoon of *Lumbrineris* sp. is little different from the primitive state (Afzelius, 1979) of two centrioles, lying along the central axis of the spermatozoon,

perpendicular to each other. *Owenia fusiformis* has the proximal centriole perpendicular to the distal centriole but lying to one side of it. This is also a feature of the sperm of *Phragmatopoma lapidosa* (Eckelbarger, 1984) and *Hyalinoecia tubicola* (Cotelli & Lora Lamia Donin, 1975). However the presence of an anchoring plate is unique to *O. fusiformis*. The sperm of the polynoid *Lepidonotus* sp. has both centrioles on the same central axis. However, rather than being perpendicular, the proximal centriole lies at 30° to the distal centriole. *Hydroides hexagonus* (Colwin & Colwin, 1961), and *Chitinopoma serrula* (Franzén, 1982) have the same morphology in this regard. This may be a character for their respective families, independently evolved. Links between the proximal centriole and the central fossa at the base of the nucleus were shown for *Lepidonotus* sp. and *Eurythoe complanata* (Rouse & Jamieson, 1987), amply demonstrating the anchoring role of the centrioles for the flagellum. *Eulalia* sp. and *Owenia fusiformis* had a displaced anchoring apparatus. A similar offset nature is visible in the spermatozoon of *Phragmatopoma lapidosa* (Eckelbarger, 1984). The latter author maintains that this is to offset the effect of long curved acrosome. The effect on motility of this tilted tail complex would be marked and comparisons of *Eulalia* sp. and *Owenia fusiformis* spermatozoa with those of other polychaetes would be interesting.

The existence of branched satellite rays in the anchoring apparatus of polychaete sperm has been shown for the first time in this study. This is easily demonstrated in *Eulalia* sp., *Owenia fusiformis, Lumbrineris* sp. and is exemplified in *Lepidonotus* sp. In the sperm of *Lepidonotus* sp. the rays are clearly striated. It is apparent that this satellite structure very rarely preserves well for ultrastructural examination.

The spermatozoon of *Owenia fusiformis* had the unusual feature, shared, in the Polychaeta, with *Chaetopterus pergamentaceus* (Anderson & Eckberg, 1983) and *Nereis virens* (Bass & Brafield, 1972) of a single mitochondrion around the anchoring apparatus. This also occurs in the ect-aquasperm of species in several other invertebrate phyla e.g. the anthozoan *Metridium* sp. (Afzelius, 1979), the echiurid *Ikedosoma gogoshimense* (Sawada et al., 1975), the articulate brachiopod *Terebratulina caput-serpentis* (Afzelius & Ferraguti, 1978) and is a feature of echinoderm sperm (see Jamieson, 1985 for review). Little phylogenetic significance can be attached to this feature as it has evidently evolved several times. Within the Polychaeta this also seems to be the case. The families where a single mitochondrion forms the midpiece (Chaetopteridae, Nereidae and Oweniidae) are distantly related. Also the sperm of *Chaetopterus variopedatus* Chaetopteridae (Harley & Jamieson, in preparation) and several nereid species e.g. *Perinereis brevicirrus* (Kubo & Sawada, 1977) have several mitochondria in the midpiece. Gardiner (1978) reported (but did not show micrographs) that the sperm of *O. fusiformis* had "a few mitochondria in a very short midpiece", rather than the single mitochondrion reported here. This may represent intra-specific variation or alternatively the taxonomy of *O. fusiformis*, a "cosmopolitan" species, may need revising.

Eulalia sp. possesses a highly unusual feature in having its mitochondria ensheathed by an extra-mitochondrial membrane. It is known that during spermiogenesis numerous smaller mitochondria fuse to form the final number (usually four) in the mature sperm (Baccetti & Afzelius, 1976). An ultrastructural study of spermiogenesis in *Eulalia* sp. would be of value to determine the origin of the extra-mitochondrial membrane.

CONCLUSIONS

The establishment of relationships between polychaete families or orders with the use of sperm ultrastructure is unlikely; however, some potential exists for its use within families. It appears that considerable variation in reproductive method can exist under the term "external fertilization" and this is reflected in the variation of ect-aquasperm structure. Further study is needed to determine the extent of this variation, and hence its value taxonomically, at the intrafamily level.

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