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VOLUME IX, PART C

Progress in Male Gamete Ultrastructure and Phylogeny

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4. LOPHOPHORATA

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I. INTRODUCTION

The oligomeric phyla are animals with three coelomic cavities in longitudinal sequence, the proto-, meso- and meta- coel, corresponding with three body divisions, the proto-, meso- and meta-some. Animals with this basic organization, though it may be obscured in the adult, range from lophophorates and echinoderms to hemichordates. The metacoel forms the main body cavity.

Included in the oligomeric phyla are forms which are undoubted deuterostomes and those which are intermediate in some features between protostomes and deuterostomes. Protostomes are typically animals in which the blastopore becomes the mouth, cleavage of the embryo is spiral and determinate; the mesoderm derives from a single cell, the 4d cell; and a coelom, if present, is schizocoelic, produced by splitting of the mesoderm. Major protostome phyla are the Platyhelminthes, Annelida, Mollusca and Arthropoda. Deuterostomes may be defined as animals in which, basically, the blastopore does not form the mouth, the anus typically arising at its site; cleavage is radial and indeterminate; and the mesoderm and enclosed coelom arise enterocoelically, by budding from the archenteron.

Three oligomeric phyla, Phoronida, Ectoprocta and Brachiopoda, comprising the superphylum Lophophorata of Valentine (1973, 1977) are structurally and developmentally on the border line between protostomes and deuterostomes. There has, therefore, been an extensive debate as to which group they belong to or on the validity of recognizing these two groups (references in Jamieson, 1991). The lophophore is a fan of ciliated feeding tentacles around the mouth.

II. PHORONIDA

Although the Phoronida are considered the most plesiomorphic lophophorates, they have complex sperm which show resemblances to those of the concentricycloid

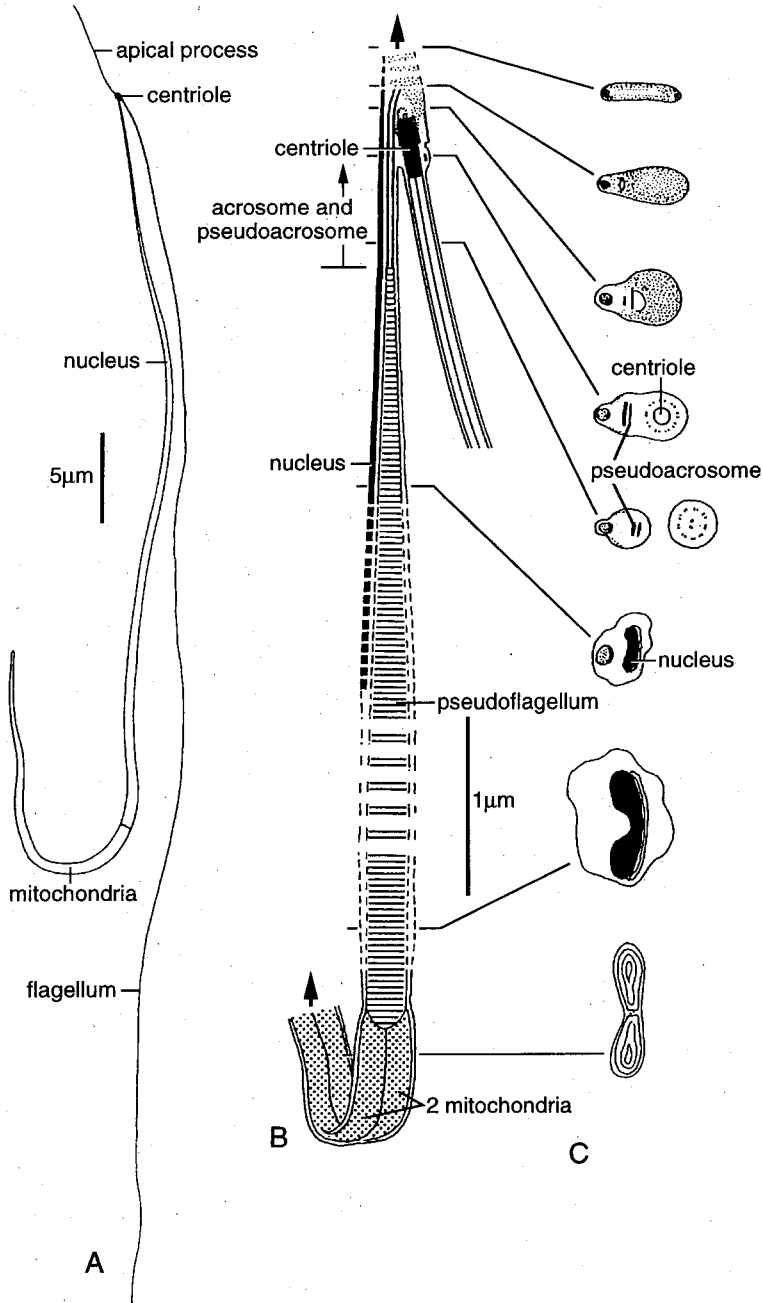
echinoderm *Xyloplax*, to the pterobranch hemichordate *Rhabdopleura*, and less obviously to those of some ectoprocts (Chapter 6, fig. 8).

The sperm of *Phoronis pallida* (Fig. 1) is highly modified and consists of two limbs arranged as a narrow inverted V from the tip of which the acrosome protrudes. One limb is formed by the flagellum, emerging from a centriolar region. The other limb contains the nucleus, the hind-part of the acrosome, a 'pseudo-acrosome' and two mitochondria. The anterior part of the elongated, somewhat flattened and concave nucleus is modified as the pseudo-acrosome. The nucleus is kidney-shaped in cross section, with many dense granules in the concavity. The pseudo-acrosome seems to be composed of two components: a band-like nuclear process and a parallel ribbon-like structure of condensed material. It seems probable to the writer that at least the second band is a centriolar rootlet (see also *Xyloplax*). At the posterior end of the nucleus the two flattened mitochondria are attached as a ribbon-shaped structure. They appear to form by fusion of several small mitochondria of the spermatid and move posteriorly along the nuclear membrane from their origin near the centriole. A short, approximately 4 μm long, filament-like acrosome projects from the point of insertion of the normal '9+2' flagellum where it is in contact with the pseudo-acrosome. The acrosome continues backwards, alongside the pseudo-acrosome and anterior part of the nucleus, as a tubelike structure (Franzén and Ahlfors, 1980).

It is presumed that the sperm of *Phoronis pallida* are released in spermatophores, as in other species, which somehow reach the eggs (possibly by pseudocopulation) and that this entails sufficient modification of fertilization to account for the great modification of the sperm. In other species, fertilized eggs are brooded on the lophophore (Silén, 1954, 1972; Emig, 1977a,b; Barnes, 1980; Franzén and Ahlfors, 1980).

Forward dislocation of the centriole and a consequent, approximately parallel, course of nucleus and axoneme are seen also in pentastomids, branchiuran, and other maxillopod crustaceans, in the concentricycloid echinoderm *Xyloplax*, chaetognaths and acanthocephalans, but only in *Phoronis* and *Xyloplax* does the flagellum lose the cytoplasmic connection to the nucleus which exists in the spermatid so that the nucleus and the mitochondria lie in a lateral appendage. This dissociation of the axoneme also occurs in the pterobranch *Rhabdopleura* (Chapter 6, fig. 8). The centriole is, however, postnuclear in *Rhabdopleura* (Lester, 1988) and the appendage contains only the mitochondrion, while an acrosome is absent. Other hemichordates (the enteropneusts) have 'primitive' sperm. Branchiurans and the related pentastomids share with *Phoronis* the filamentous form of the acrosome.

Fig. 1. *Phoronis pallida* (Phoronida). A: Diagram of the entire spermatozoon. B: Diagram of spermatozoal components. Arrowheads indicate that the full extent of the acrosomal process and mitochondria are not shown. C: Cross sections at the levels indicated in B. From Jamieson, B.G.M. (1991), fig. 1.2, after Franzén, Å. and Ahlfors, K. (1980). *Journal of Submicroscopic Cytology*, 12, 585-597. figs. 11, 5 and 8.



The acrosome is also elongate, though otherwise distinctive, in *Xyloplax*. Continuation of the acrosome along the nucleus is restricted to *Phoronis*.

It has been argued (Jamieson, 1991) that the lateral appendage cannot parsimoniously be construed as plesiomorphic for hemichordates (*Rhabdopleura*) or echinoderms (*Xyloplax*) in view of the occurrence of 'primitive' sperm in enteropneusts, asteroids, ophiuroids, and crinoids. It was concluded that these spermatozoal similarities are chiefly dictated by the requirements of fertilization. It was, nevertheless, considered possible that development of the appendage and especially the close similarities of the sperm of *Xyloplax* and *Phoronis* represented the phenomenon of paramorphy (Jamieson, 1984) i.e. occurrence of a similar structure in response here to similar demands of fertilization biology, because of genetic (phylogenetic) relationship. Franzén and Ahlfors (1980) had attempted to relate the elongate acrosome to aspects of fertilization, notably the mode of penetration of the sperm into the egg and the structure of the egg envelope. Jamieson (1991) nevertheless recognized the possibility that the near-plesiosperm morphology of most echinoderm sperm was secondary and this remains worthy of consideration (see Chapter 5, Echinodermata).

III. BRYOZOA (ECTOPROCT POLYZOA)

A. Bryozoan Spermatozoa

The phylum Bryozoa is generally divided into the three classes Gymnolaemata, Stenolaemata and Phylactolaemata. Ultrastructural studies of spermiogenesis and spermatozoa have revealed some common morphological features, e.g., elongated midpiece and modified centriolar region. In several respects there are, however, differences in sperm morphology between the three classes (Franzén, 1998).

Ultrastructural studies on bryozoan sperm are listed in Table 1.

Table 1

TEM studies of spermatozoa of the Bryozoa

Taxon	Reference
Gymnolaemata	
<i>Bugula</i> sp.	Reger, 1971
<i>Cryptosula pallasiana</i>	Franzén, 1981
<i>Electra pilosa</i>	Franzén, 1981, 1998
<i>Flustra foliacea</i>	Franzén, 1976, 1981, 1983
<i>Membranipora membranacea</i>	Zimmer and Woollacott, 1974
<i>Triticella koreni</i>	Franzén, 1976
Stenolaemata	
<i>Tubulipora</i>	Franzén, 1984
Phylactolaemata	
<i>Plumatella fungosa</i>	Franzén, 1981, 1982

1. Gymnolaemata

The sperm of the gymnolaemate *Bugula*, described by Reger (1971), is virtually of the plesiosperm type, excepting its lack of a nuclear membrane and considerable elongation of the nucleus; the absence of an acrosome may well be secondary and it is possible that the oocytes lack outer envelopes requiring penetration by an acrosome. Loss of the acrosome has been ascribed to ready access of sperm to the oolemma in actinopterygian fish, because of the development of a micropyle (Jamieson, 1991). Mitochondria of gymnolaemate sperm are situated at the base of the flagellum around the basal centriole. From a micrograph it appears that the proximal centriole is at the side of and perpendicular to the basal body. The flagellum is of the '9+2' type.

The sperm of other gymnolaemate bryozoans examined ultrastructurally are of an 'advanced' type. Absence of an acrosome, presence of two composite rodlike mitochondrial derivatives (reaching a length of 50 μm in *Flustra foliacea* and *Triticella koreni*) and of two variably developed granular rods alternating with these; and of a tubelike cell membrane around the axoneme are special features of gymnolaemate bryozoan sperm, including *Triticella*, *Flustra*, *Membranipora*, *Cryptosula*, and *Electra* (Zimmer and Woollacott, 1974; Franzén, 1976, 1977, 1981, 1983, 1998) (Figs. 2, 3). At the caudal end of the midpiece an annulus forms a boundary to the tail region. In the mature sperm of *F. foliacea*, a deep invagination of the plasma membrane is developed in connection with the annulus (Franzén, 1976, 1998).

Franzén (1998) has given a detailed description of spermiogenesis and the mature sperm of *Electra pilosa* (Fig. 2). The mature sperm consists of three slender components: the elongated head, the midpiece and the tail region. The head region measures approximately 120 μm in length and has the shape of a long, bent rod, tapering anteriorly. It is circular in cross-section and at its base has a diameter of approximately 0.5 μm . A longitudinally oriented basal body is inserted in an indentation in the posterior end of the nucleus. The nucleus contains almost homogeneous, densely electron-opaque material within a typical double nuclear membrane.

The midpiece consists of an axoneme issuing from the centriole and surrounded by two band-like mitochondria or mitochondrial derivatives and two thin, electron-dense, granular rods (from micrographs, better developed in the spermatid than the spermatozoon) alternating with the mitochondrial bands. The two mitochondrial derivatives have an orientation in a plane parallel to the two central singlets in the axoneme. The mitochondrial bands are of unequal size and structure. The larger one is provided with a row of lamellae of electron-opaque material, giving it a paracrystalline appearance. The smaller one is a thin band containing a few longitudinal mitochondrial cristae and has a double membrane. A small invagination of the plasma membrane at the posterior end of the midpiece forms an annulus.

The tail region consists of an axoneme surrounded by a thick cell membrane. There is a normal '9+2' axoneme. In the terminal part of the tail, the two central

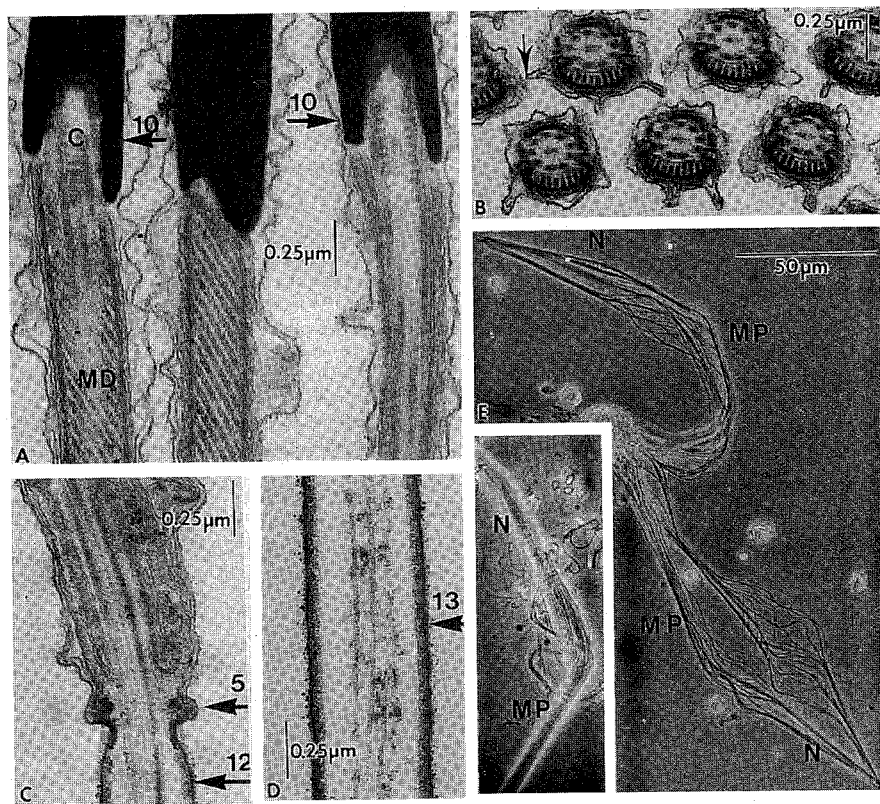


Fig. 2. *Electra pilosa* (Gymnolaemata, Bryozoa). A: Longitudinal section (LS) of nucleus and midpiece of spermatozoon. c, basal body = modified centriole; MD, mitochondrial derivative. B: Transverse section of a spermatozeugma through the midpiece region. Arrow indicates mucus between adjacent sperm. C: LS junction between midpiece and tail. D: LS terminal part of tail, showing thickened plasma membrane. E: Phase contrast image of a spermatozeugma in dissociation and (inset) the head region and part of the midpiece region of an intact spermatozeugma. MP, midpiece; N, nuclear region. Numbers 10, 5, 12 and 13, from original figures, indicate levels at which sections were obtained. After Franzén, Å. (1998), figs. 6, 17, 8, 9 and 15.

microtubules have disappeared and the nine peripheral components of the axoneme continue as single microtubules. The diameter of this part is about the same as in the main part of the tail. In the 'primitive' type of sperm (ect-aquasperm), the end part of the tail flagellum is often characterized by a distinctly smaller diameter.

Spermatozeugmata consist of a parallel aggregation of mature spermatozoa lacking a capsule, connected with each other by strings of mucus, and capable of active swimming (Franzén, 1998).

There is light microscopical evidence that several bryozoan genera, e.g. *Caberea*, *Bowerbankia*, *Alcyonidium*, *Scrupocellaria*, have spermatozoa of the same type as *Electra* (Retzius, 1904; Franzén, 1956, 1998).

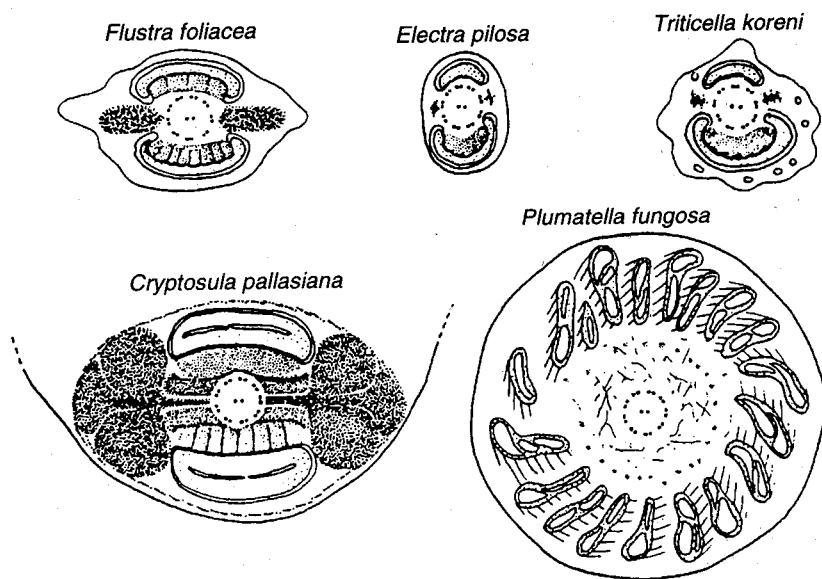


Fig. 3. Bryozoan spermatozoa. Drawings of the transverse sections of the mitochondrial midpiece regions in the Gymnolaemata: *Flustra foliacea*, *Electra pilosa* and *Triticella koreni*, and the Phylactolaemata: *Plumatella fungosa*. From Jamieson (1991), fig. 1.3, after Franzén, Å. (1981), fig. 2.

2. Stenolaemata

The Stenolaemata is a fossil group except for some Cyclostomata. In the cyclostomatous bryozoan *Tubulipora*, the midpiece contains four elongated rod-shaped mitochondria and four rods of granular, non-encapsulated electron-dense material surrounding the axoneme (Franzén, 1984, 1998). An acrosome is absent. There is thus a similarity between the sperm in the cyclostome *Tubulipora* and the gymnolaematous bryozoan sperm with two mitochondrial bands and two electron-dense rods. The absence of an acrosome in the Stenolaemata is deduced to be secondary, as in gymnolaemates. Sperm structure supports the hypothesis that Stenolaemata and Gymnolaemata are more closely related to each other than either of them is to the Phylactolaemata (Franzén, 1998). This conclusion is pertinent to the evidence from 18S rRNA sequencing that the Ectoprocta (Bryozoa) are not monophyletic (Mackey *et al.*, 1996).

3. Phylactolaemata

The sperm of the Phylactolaemata, examined in *Plumatella fungosa*, are also complex but differ greatly from those of the Gymnolaemata and Stenolaemata. The head contains a conical nucleus which is asymmetrically curved and terminates with a small acrosome, absent in the other two groups; a flagellar basal body is situated

in a posterior invagination of the nucleus; the elongated midpiece contains a double helix of many small mitochondria or mitochondrial derivatives (Fig. 3). The axoneme has the typical '9+2' structure and in the tail region it is surrounded, as in other bryozoans, by a spacious tube containing granular material. Spermiogenesis in the Phylactolaemata also differs greatly from that in the Gymnolaemata (Franzén, 1981, 1982, 1998).

B. Bryozoan Sperm Phylogeny

Franzén (1987) hypothesized that the ancestors of bryozoans had external fertilization with the release of sperm and eggs freely into the water and that the sperm were of the 'primitive' (ect-aquasperm) type. In view of new theories on fertilization biology in the early Bilateria (Buckland-Nicks and Scheltema, 1995), Franzén (1998) recognizes that an alternative hypothesis deserves consideration — namely that an ancestor of bryozoans had spermiogenesis, spermatozoa, and fertilization biology similar to that in Gymnolaemata and Stenolaemata. Early bryozoans might thus have had some kind of direct sperm transfer. Buckland-Nicks and Scheltema (1995) have recently discussed the hypothesis that early bilaterians had developed internal fertilization with direct sperm transfer. It is not at present possible to decide between these alternatives but it is probable that the structure of the gymno- and stenolaematous sperm is at least secondary in lacking the acrosome seen in sperm of the Phylactolaemata.

C. Fertilization Biology in Bryozoa

In *Bugula*, eggs are brooded in a special external chamber of the body wall, termed an 'ovicell' the single brooded embryo is nourished by placenta-like connections to the ovicell wall (Silén, 1966; Woollacott and Zimmer, 1972; Temkin, 1994).

In *Membranipora membranacea* and *Electra pilosa*, sperm are tightly organized in aggregate spermatozeugmata (references in Franzén, 1998). The simultaneously hermaphroditic zooids of *M. membranacea* colonies spawn primary oocytes and spermatozeugmata (aggregates of 32 or 64 spermatozoa) into ambient sea water (Temkin, 1994). Eggs are released through the inter-tentacular organ (ITO) whereas spermatozeugmata are spawned through the tips of the two distomedial tentacles. Fertilized eggs undergo planktotrophic development to form long-lived cyphonautes larvae. A single sperm fuses with primary oocytes during, or shortly after, ovulation. Activation does not immediately follow sperm-egg fusion, but occurs after oocytes are spawned through the ITO. The period between sperm-egg fusion and egg activation may last up to four days. Internal sperm-egg fusion does not preclude cross-fertilization in *M. membranacea*, because spawned spermatozeugmata enter maternal coeloms through ITOs after being drawn into lophophores. The ITO actively regulates the entrance of spermatozeugmata and the release of oocytes by the closure

of the distal pore. The ITO does not act as a filter to prevent self-fertilization, so that the paternal colony may also function as the maternal colony. Self-fertilization may be reduced in *M. membranacea* by increasing sperm dispersal away from the paternal colony, which is accomplished by the bending of the distomedial tentacles such that they release spermatzeugmata into the exhalant feeding current of the colony (Temkin, 1994). The capture of spawned spermatzeugmata in the lophophore and concentration of sperm and eggs within the maternal coelom followed by (internal) fertilization may lead to nearly 100 per cent success in fertilizing the eggs (Temkin, 1994), in contrast to gamete interaction occurring in the ambient water where dilution may limit fertilization success.

The existence of modified sperm in many, and probably most, bryozoan species is correlated by Franzén (1976) with such modified fertilization biology. Peculiar undulating movements of the swimming sperm of *Flustra* and *Triticella* are also related by Franzén (1976) to the absence of radial symmetry of the sperm.

IV. BRACHIOPODA

A. Introduction

Brachiopods are grouped with ectoproct Bryozoa and Phoronida in the superphylum Lophophorata. Embryological features, such as the tripartite (oligomeric) coelom, the monociliated epidermis of the lophophore and the indeterminate development have been considered to suggest close affinity with deuterostomes (Zimmer, 1973; Rieger, 1976; Storch and Welsch, 1976). However, the molecular configuration of their ribosomal RNA, while supporting placement of brachiopods in the Lophophorata, suggests a relationship with protostomes (Ishikawa, 1977). Analysis of 18S rRNA indicates that phoronids and brachiopods form a monophyletic clade, and that ectoprocts but not entoprocts are protostomes (Mackey *et al.*, 1996). Monophyly of the Brachiopoda (Inarticulata and Articulata) has been questioned (Valentine, 1973, 1977) but appears to be supported by the very full fossil record of the brachiopods (Williams and Rowell, 1965).

B. Ultrastructure of Brachiopod Spermatozoa

The brachiopods listed in Table 2 have been investigated for spermatozoal ultrastructure.

All known brachiopod sperm are ect- or ent-aquasperm approximating to the plesiosperm *sensu* Jamieson (Fig. 4).

Table 2

TEM studies of spermatozoa of the Brachiopoda

Taxon	Reference
Inarticulata	
<i>Crania anomala</i>	Afzelius and Ferraguti, 1978
<i>Discinisca tenuis</i>	Hodgson and Reunov, 1994
<i>Lingula anatina</i>	Chuang, 1983
<i>Lingula unguis</i>	Sawada, 1973
Articulata	
<i>Kraussina rubra</i>	Hodgson and Reunov, 1994
<i>Terebratulula vitrea</i>	Bozzo and Bargalló, 1985; Bozzo, 1988
<i>Terebratulina caputserpentis</i>	Afzelius and Ferraguti, 1978

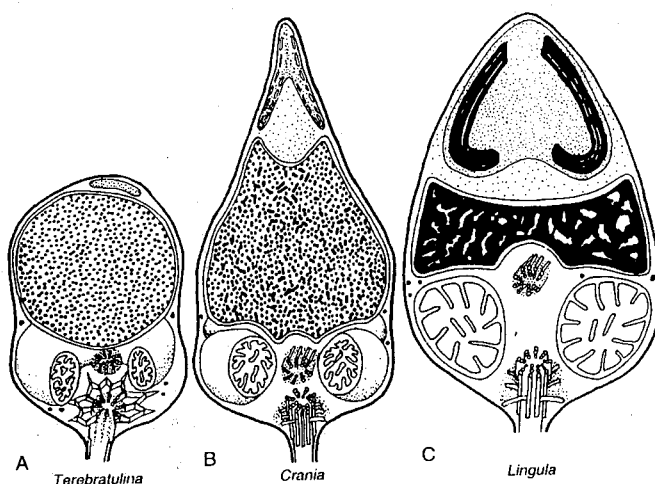


Fig. 4. Semidiagrammatic drawings of the spermatozoa of brachiopods. A: The articulate, *Terebratulina caputserpentis*. B and C: The inarticulates, *Crania anomala* and *Lingula anatina*. From Jamieson (1991), fig. 1.4, A and B, after Afzelius, B.A., and Ferraguti, M. (1978). *Journal of Ultrastructure Research*, 63, 308–315, figs. 11,12. C, after Chuang, S.H. (1983), fig. 8.

1. Inarticulata

The sperm of *Lingula unguis* (Sawada, 1973) *L. anatina* (Chuang, 1983) and *Crania anomala* (Afzelius and Ferraguti, 1978) are little modified from the plesiosperm condition from which they differ in having well-developed periacrosomal or subacrosomal material and, in *L. unguis*, in inflation of the acrosome vesicle. *Lingula* is considered to be a survivor of the most primitive group in the Inarticulata.

In *Lingula anatina*, the acrosome vesicle occupies almost half the length of the head and midpiece (Fig. 4C). The vesicle is of the inflated type and is pyriform with a posterior concavity. Its anteroposterior length is about 1.2 μm and the widest

transverse diameter about 1.1 μm (Chuang, 1983), compared with 1.5 μm and 1 μm for that of *L. unguis* (Sawada, 1973). Its moderately dense, finely granular contents are enclosed by four to six closely apposed, more dense rings. These rings are open at the anterior and posterior ends, total about 300 Å in thickness and vary in longitudinal extent. In *L. unguis* the rings have a thickness of 800 Å and a height of 0.8 μm (Sawada, 1973) and do not appear to be interrupted. A periacrosomal space is present in both species. In *L. anatina* it is filled with sparse granular substance and is narrow though widened in the posterior axial region to 0.25 μm , and may contain occasional oval dense-cored vesicles with a longer diameter of 0.2 μm .

In both *Lingula* species, the nucleus is virtually crescentic in longitudinal section because of broad excavation by the acrosome, though described as cylindroid by Chuang (1983). In *L. anatina* it is 1.5–1.7 μm across, concave anteriorly, convex laterally; posteriorly it has a deep central concavity housing the proximal centriole and five to seven peripheral concavities for the mitochondria; seven mitochondria are illustrated. The nuclear membrane is dense and finely corrugated. The very dense nucleoprotein strands, as in *L. unguis*, are closely packed except for one or two clear spaces. The nucleoplasm is finely granular (Chuang, 1983).

In *Lingula anatina* the proximal centriole, perpendicular to the distal centriole (basal body) as in *L. unguis*, is about 0.2 μm long; the distal centriole, 0.6–0.7 μm long and about 0.7 μm wide, has a satellite in the form of radiating tubules extending to the plasma membrane. The oval to subspherical mitochondria, up to 1 μm in diameter, widely encircle the centrioles; the intervening cytoplasm has finely granular material, some ribosomes and occasional larger putative glycogen granules (Chuang, 1983).

The sperm tail in *Lingula anatina*, about 50 μm long and proximally 0.3 μm wide, has a wavy outline indicating a ridged surface. In both species, it has the '9+2' pattern of microtubules. The doublets are reduced to nine singlets in the tapering endpiece (Chuang, 1983).

In the sperm of *Crania anomala* (Fig. 4B), the acrosome is large and fairly complex but smaller than that of *Lingula*. The acrosome vesicle forms a hollow cone projecting about 1 μm in front of the nucleus and basally 0.7 μm wide. The inner and outer margins are periodically banded owing to the presence, on the vesicular side of each, of regularly spaced radially orientated longitudinal lamellae. The central axis of the acrosome vesicle, in front of the basal invagination, contains material which is electron-dense and homogeneous. The material in the large subacrosomal space is fairly homogeneous. The nucleus is rounded though indented by the subacrosomal material and posteriorly by the mitochondria. The chromatin is fairly uniformly granulated, with 300 Å strands.

The midpiece contains four or five rounded, approximately 1 μm mitochondria in a circle around the central axis which is occupied by the two mutually perpendicular centrioles. The distal centriole (basal body) is longitudinal and is connected by simple, unbranched arms to the plasma membrane as an anchoring fibre apparatus which resembles that illustrated for *Lingula anatina* by Chuang (1983) more closely

than it does the more classical satellite system of *Terebratulina* (see below). The sperm tail is about 50 μm long and is of the '9+2' pattern. It projects from the side of the midpiece or, normally, from its posterior end (Afzelius and Ferraguti, 1978).

The spermatozoon of *Discinisca tenuis* has a similar morphology to that of the other investigated inarticulate brachiopods. The head is about 1.9 μm long and is composed of a nucleus capped by an acrosome which is pyriform in shape. The nucleus, which is about 1.5 μm in mid-diameter, has a broad anterior invagination as well as a smaller posterior fossa and in addition the base of the nucleus is indented laterally to accommodate the mitochondria. The posterior portion of the acrosome is housed in the anterior nuclear invagination, with subacrosomal material which has a periodic substructure separating nucleus and acrosome. The acrosome, which is about 0.6 μm long and 0.9 μm in width at its base, is differentiated internally with an electron-lucent centre and electron-dense outer region.

The midpiece consists of a ring of four or five large spheroidal mitochondria with well-developed lamellar cristae. The mitochondria are closely adpressed to the base of the nucleus. The proximal and distal centrioles, are orthogonal in their arrangement, are located in the centre of the mitochondrial ring, the proximal centriole being partially housed in the posterior fossa. From the distal centriole nine branched satellite projections arise which bifurcate before being inserted onto a slightly thickened marginal ring. Some glycogen is present within the midpiece (Hodgson and Reunov, 1994).

2. Articulata

The sperm of *Terebratulina caputserpentis* (Fig. 4A), described by Afzelius and Ferraguti (1978), and reviewed by Chuang (1983), of *Terebratula vitrea* (Bozzo and Bargalló, 1985; Bozzo, 1988), and of *Kraussina rubra* (Hodgson and Reunov, 1994) are again of the plesiosperm type, with rounded nucleus but in other respects differ from those of the Inarticulata. Those of the first two species are internally fertilizing and are ent-aquasperm. It is suggested that *K. rubra* has external fertilization (Hodgson and Reunov, 1994).

The *Terebratulina* acrosome is only a thin disc at the anterior end, about 0.1 μm high by 0.6 μm wide. The subacrosomal space is very narrow, with no subacrosomal material. The nucleus, 1.5 μm wide, is spherical and unindented. As a major difference from *Crania*, the midpiece has a single, ring-shaped mitochondrion (here considered a paramorphy with echinoderms and cephalochordates). Furthermore, the two centrioles are orthogonal in the long axis of the sperm. The distal centriole has a satellite apparatus consisting of nine rays each of which has two branches which fuse with their neighbours to form a star-like structure; only doublets have been seen. The tail is 50 μm long, with a '9+2' axoneme. It and the midpiece is sometimes not in line with the head (Afzelius and Ferraguti, 1978).

The head of the spermatozoon of *Kraussina rubra* is about 2 μm long and is composed of a cylindrical nucleus, 1.9 μm long \times 1.1 μm diameter, anterior to

which is a disc-shaped acrosome, 0.5 μm in diameter. The nucleus is rounded anteriorly, and posteriorly there is a small fossa. The midpiece has a single ring-shaped mitochondrion which has well-developed lamellar cristae. The mitochondrion surrounds the centrioles, the longitudinal axis of which lie almost in line with each other. From the distal centriole nine satellite arms connect to the cell membrane. These arms are in turn linked to one another (Hodgson and Reunov, 1994).

During spermatogenesis in *Kraussina rubra*, as in *Discinisca tenuis*, each spermatogonium and spermatocyte develops a flagellum which appears to be absorbed prior to cell division. In *D. tenuis*, proacrosomal vesicles form within spermatogonia whereas acrosome formation in *K. rubra* does not commence until spermiogenesis. In the early spermatid of both species, a single acrosomal vesicle forms in the presumptive posterior region of the cell and during spermiogenesis the developing acrosome migrates anteriorly where it assumes its mature form (Hodgson and Reunov, 1994).

Chuang (1983) lists several features of the sperm of *Terebratulina caputserpentis* which he considers adaptations to internal fertilization: reduction in the size and differentiation of the acrosome; reduction in the subacrosomal space; changes in the shape and condensation of the nucleus; reduction in number of the mitochondria and fusion as a single annulus; parallel rather than mutually perpendicular centrioles; and increased complexity of the centriolar anchoring apparatus. All of these features, or trends, also occur in ect-aquasperm in other groups and none of them can confidently be attributed to the ent-aquasperm mode of fertilization.

C. Brachiopod Sperm Phylogeny

Hodgson and Reunov (1994) note that despite the fact that both *Discinisca tenuis* (Inarticulata) and *Kraussina rubra* (Articulata) have aquasperm, they show clear differences in morphology. The spermatozoon of *D. tenuis* more closely resembles that of *Crania anomala* (Afzelius and Ferraguti, 1978), *Lingula unguis* (Sawada, 1973), and *L. anatina* (Chuang, 1983), all inarticulates. Similarities between *D. tenuis* and these other inarticulates include: a nucleus which has a broad anterior invagination; a relatively large acrosome which is differentiated internally; a midpiece consisting of several mitochondria surrounding orthogonally arranged centrioles. By contrast, the sperm of *K. rubra* (Articulata) is very similar to its fellow articulates *Terebratulina caputserpentis* (Afzelius and Ferraguti, 1978) and *Terebratula vitrea* (Bozzo and Bargalló, 1985; Bozzo, 1988). In both these articulates, the sperm nucleus does not have an anterior invagination and the acrosome is small and disc-like. In addition, the midpiece has a single ring-shaped mitochondrion (Afzelius and Ferraguti, 1978); the longitudinal axis of the proximal centriole is almost in line with that of the distal centriole, the latter being surrounded by a complex organization of satellite fibres.

The large acrosome and separate mitochondria in the Inarticulata, and the converse in the Articulata, appear to be supported from light microscope investigations

of other species (references in Afzelius and Ferraguti, 1978). These differences in the sperm are presumably phylogenetically determined but the writer is doubtful (Jamieson, 1991) that they are sufficient to support the view (Valentine, 1973, 1977) that the two classes are not cophyletic. Afzelius and Ferraguti (1978) point out that the sperm of the inarticulate *Crania*, are very similar to those of lower protostomes (see, for instance, aquasperm sperm of polychaetes) and, correspondingly, the coelom of inarticulates is shizocoelic (Williams and Rowell, 1965) and that, in contrast, the sperm of the articulate, *Terebratulina*, shows a mixture of features of the sperm of lower deuterostomes and protostomes. They state that *Terebratulina* resembles lower deuterostomes in the complex centriolar satellite apparatus and fusion of the mitochondria in a ring but differs in the small subacrosomal space and conclude that protobrachiopods were close to the branching point between the protostome and deuterostome lines, with the ancestors of the articulates closer to the deuterostome branch; or that embryological and spermatozoal similarities of articulates and deuterostomes occurred independently and in parallel. Hodgson and Reunov (1994) support this view. However, Jamieson (1991), while agreeing to the basal position of brachiopods relative to the deuterostomes considers that the sperm of *Lingula*, and even that of *Crania*, is closer to that of deuterostomes than it is to that of *Terebratulina*.

D. Fertilization Biology in a Terebratellid

New information is available on the fertilization biology of the terebratellid brachiopod, *Calloria inconspicua* (Chuang, 1996). Mature males shed spermatozoa or fragments of their testes. Each female studied spawned several times within the observation period of 12 days, releasing either a single developmental stage or a mixture of several developmental stages, including occasional fragments of the ovaries. The ova, 54–192 μm in diameter, varied widely in size even in an individual spawn. Ova were fertilized either outside or inside the mantle cavity. Those released into the mantle cavity, if not spawned immediately, were brooded for varying periods in the space between the brachial membrane and the cirri. The follicle cells are lost after fertilization; the fertilization membrane persists during the embryonic stage; the coeloblastula has one layer of blastomeres, and gastrulation is by invagination (Chuang, 1996). It seems possible that shedding of gonad fragments and undersized gametes were abnormalities owing to culture conditions.

V. DEUTEROSTOMES

Deuterostomes, proper, are the hemichordates, echinoderms, ascidians cephalochordates and vertebrates. The pterobranch hemichordates are the only deuterostomes which retain the lophophore. This is borne on the mesosome of the tripartite, oligomeric body; pterobranchs thus retain a body form which may be attributed to the earliest deuterostomes. From animals with this form it is reasonable to derive the enteropneusts, echinoderms and ascidians (Jamieson, 1991).

There is now little support for the contention of Løvtrup (1977) that echinoderms are further from chordates than are the Mollusca. However, phylogenetic analysis of molecular sequences has been equivocal in this respect. An early study of 18S ribosomal RNA base sequences divorced the echinoderms from chordates, showing them as the sister-group of the Annelida (Field *et al.*, 1988) (molluscs were not considered). In contrast, another study, probably more reliable as more taxa, including molluscs, were used, showed echinoderms as the sister-group of the chordates (Lake, 1990). More recently, Halanych (1995) has confirmed, from 18S sequences, the traditional view that pterobranchs are most closely related to the enteropneust hemichordates and indicates that hemichordates are more closely related to echinoderms than to chordates. His findings suggest that ciliated gill slits and the dorsal hollow nerve chord are plesiomorphic features of the Deuterostomia. Previously, Holland *et al.* (1991) showed from 18S sequencing that the enteropneust *Saccoglossus cambrensis* and vertebrates share a common ancestor not shared by echinoderms. Turbeville *et al.* (1994) found that 18S sequencing failed to support a monophyletic Chordata; the urochordates formed the sister-taxon to the hemichordates, and together this clade plus the echinoderms formed the sister-taxon to the cephalochordates plus craniates. Parsimony analysis of morphological plus molecular characters supported both monophyly of echinoderms plus enteropneust hemichordates and a sister-group relationship of this clade to chordates. Neighbour-joining, Fitch-Margoliash, and maximum-likelihood analyses supported a chordate lineage that was the sister-group to an echinoderm-plus-hemichordate lineage.

A phylogenetic analyses of 18S rRNA sequences by Mackey *et al.* (1996) has indicated that (1) entoprocts and lophophorates have spiralian, protostomatous affinities, (2) Ectoprocta (Bryozoa) and Endoprocta are not sister-taxa, (3) phoronids and brachiopods form a monophyletic clade, and (4) neither Ectoprocta nor Annelida appear to be monophyletic. It suggests that deuterostomous and pseudocoelomate features may have arisen at least twice in evolutionary history and supports a Spiralia-Radialia-based classification rather than one based on the Protostomia-Deuterostomia concept (Mackey *et al.*, 1996). Molecular analysis has, thus, so far failed to clarify deuterostome phylogeny.

It is presumed that the enteropneusts and chordates emerged from a rich and varied sessile hemichordate fauna (including graptolites, pterobranchs and acanthastids) which is known to have occurred in the lower Ordovician (references in Clark, 1964). Unity of enteropneusts with pterobranchs is underlined by the shared presence of gill clefts (pharyngotremy) in extant pterobranchs, albeit a single pair in *Cephalodiscus* and none in *Rhabdopleura*. It is conjectured that enteropneusts have lost the lophophore in acquiring a worm-like burrowing body. The origins of the echinoderms are obscure but close similarities between the enteropneust tornaria larva, the bipinnaria larva of asteroids and the auricularia of holothurians, support close relationship of hemichordates and echinoderms (Clark, 1964).

Clark (1964) argues for derivation of echinoderms from a pterobranch-like ancestor (after Grobben) rather than a dipleurula-like ancestor (as proposed by

MacBride) but points out that the dipleurula is essentially the same as a pterobranch with paired lophophores. Similarities of the coelom, and in other regards, between hemichordates and echinoderms are considered by Clark (1964) to be too great to be regarded as convergence. The fact that echinoderms are undoubtedly highly modified animals, far removed from any common ancestor between them and hemichordates, implies that the hemichordates (or, at least, the pterobranchs in these) bear the closest resemblance to that ancestor. From these ancestral hemichordates arose the enteropneusts. The pterobranchs are, thus, confirmed as animals with a structure closest to that envisaged for the earliest deuterostomes. Indeed, the pterobranch epistome, used in locomotion, is considered to be equivalent to the attachment pit of crinoid and asteroid larvae and the madreporic vesicle of echinoderms to be homologous with the cardio-pericardial vesicle of pterobranchs (Clark, 1964). Clark considers holothurian organization to approach that of unsegmented coelomate deuterostomatous worms, whereas that of the other echinoderms does not. Asteroids approach the echinoids much more closely than holothuroids in the functional attributes of the metacoel. However, these are not the internal relationships of the echinoderm classes which are suggested by molecular sequencing in which crinoids are the sister-group of all other classes, asteroids are not close to echinoids, and holothuroids, with echinoids, form an advanced clade (Littlewood *et al.*, 1997, see Chapter 5).

The relationship between tunicates and the Chordata *sensu strictu* is supported by overwhelming evidence of the close and fundamental similarities between the ascidian tadpole larva and chordates (Berrill, 1955; Clark, 1964) and by the identical mode of development of the unique pharyngotremy of the 'protochordates' (enteropneusts, tunicates and cephalochordates) despite enormous hypertrophy of the pharynx and multiplication and subdivision of gill slits in tunicates. Presence (though whether by interpolation is highly debatable) of that larval stage, the ascidian tadpole, which underlines the chordate affinities of the tunicates, nevertheless obscures the origin of tunicates from oligomeric deuterostome ancestors, though a *Cephalodiscus*-like ancestor is envisaged. It is noteworthy that before chordates developed the segmented musculature associated with the internal skeleton they retained in their ontogeny the three coelomic cavities of the oligomeric ancestors.

Evolution of lophophorate and deuterostome sperm is discussed in chapter 8, Section VI. The spermatozoa of the Echinodermata are the subject of Chapter 5.

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