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## 6. CHORDATA—HEMICHORDATA

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### I. INTRODUCTION

There have been no descriptions of hemichordate spermatozoa since the review of Jamieson (1991), on which this chapter is therefore based.

The phylum Chordata consists of the subphyla Hemichordata (acorn worms), Urochordata (tunicates or sea-squirts), Cephalochordata (lancelets), and Vertebrata (fish, amphibians, reptiles, birds and mammals). Hemichordates, urochordates and cephalochordates comprise the protochordates. Protochordates are rarely regarded as a formal taxonomic rank and in the absence of the vertebrates do not constitute a monophyletic group. Jefferies (1979, 1981) advocates inclusion of the extinct group Calcichordata in the Chordata.

The chordates are so-named because of the presence of an elastic dorsal rod, the notochord, but this cannot be said with certainty to be represented in hemichordates (Jamieson, 1991).

The validity of recognizing a monophyletic Chordata, and the interrelationships of its groups, are briefly discussed under Deuterostomes in Chapter 4, Section V, including the relationships of hemichordates as indicated from molecular studies (Holland *et al.*, 1991; Turbeville *et al.*, 1994; Halanych, 1995).

### II. SUBPHYLUM HEMICHORDATA

The hemichordates comprise a small group of worm-like marine animals possessing gill clefts. They contain two, or possibly three, extant classes: the Planctosphaeroidea, Enteropneusta, and the Pterobranchia. The Planctosphaeroidea is based on two planktonic specimens which may be larvae of an unknown type of hemichordate (Bullman, 1970). The extinct classes Graptolithina (Graptolites) and Acanthastida are also usually included.

The Enteropneusta (acorn worms) include *Saccoglossus* and *Balanoglossus* and have a row of paired gill slits. The Pterobranchia include *Cephalodiscus* which

is organized in aggregations and *Rhabdopleura* which forms stolons and is colonial. Pterobranchs differ from enteropneusts in having ciliated feeding tentacles, the lophophore, and in having, in *Cephalodiscus*, only one pair or, in *Rhabdopleura*, no gill slits. There is a widespread view that the 'notochord' or stomochord of hemichordates is not homologous with a true notochord and on this basis the Hemichordata is often excluded from the Chordata and raised to the rank of an independent, though unquestionably, deuterostomatous phylum. In view of the uniformity of gill skeleton structure with that of other chordates, and general chordate characteristics (below), evolution of gill slits is deduced to have been a monophyletic event and the subphylum is retained in the Chordata (Jamieson, 1991).

### III. CLASS ENTEROPNEUSTA

#### A. Introduction

Enteropneusts are worm-shaped marine animals mostly in burrows in shallow water but with representation near the Galapagos hydrothermal vent. They vary in length in different species from 2 cm to over 2 m. They have three body divisions conforming with the protosome, mesosome, and metasome of oligomerous phyla and here constituting a proboscis, collar and trunk (Fig. 1).

Notable chordate characters of enteropneusts are the paired pharyngeal gill slits, situated in the anterior part of the trunk, and the dorsal tubular nerve cord, in the collar. Elsewhere the nervous system resembles that of echinoderms in consisting of a sheet of nerve fibres and cells lying under the epidermis over the entire body. In some species the gill slits open into an atrium formed by lateral folds usually turned upwards to leave a long mid-dorsal opening. In other species each slit opens to a gill pouch. The gill slits, with a skeletal system resembling that in the cephalochordates, are not associated with actual gills and probably serve to filter off excess water from the material, often sand and mud, collected by mucus secreted by the proboscis and conveyed by cilia to the mouth. The mouth lies in a groove between the proboscis and collar. A short structure beneath the dorsal nerve cord

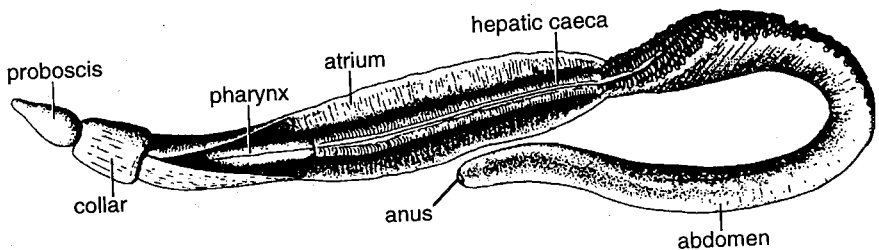


Fig. 1. *Balanoglossus*. An enteropneust, removed from its tube. From Young, J.Z. (1981), fig. 3.2. After van der Horst.

at the junction of the proboscis and trunk and forming a dorsal diverticulum of the pharynx constitutes the dubious notochord or stomochord and is associated with a skeletal plate (Fig. 2). Its vacuolated cells are reminiscent of those of a true notochord.

There is no endostyle but the pharynx possesses a ventral ciliated groove. The anterior part of the intestine bears numerous hepatic caeca. In contrast with vertebrates, the blood is said to flow forwards in the dorsal blood vessel and backwards in the ventral vessel. The dorsal vessel expands into a heartlike sinus, surrounded by a muscular pericardium, differing in its dorsal location from a

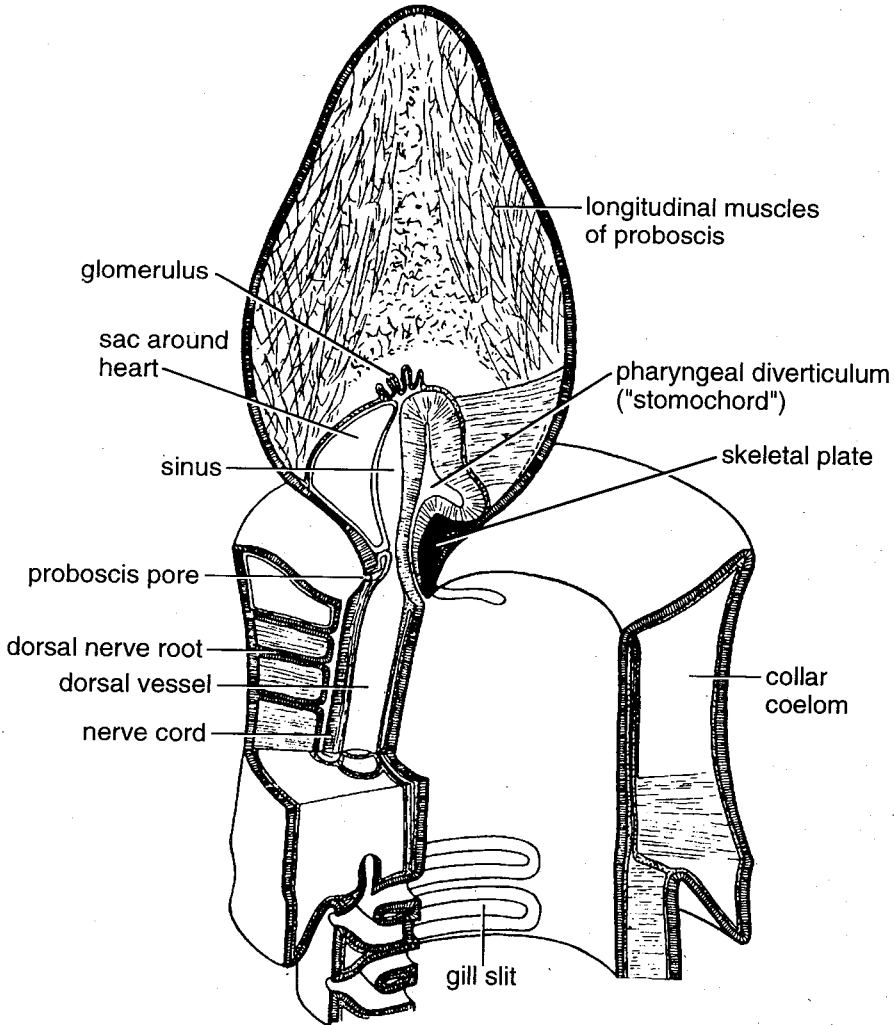


Fig. 2. *Balanoglossus*. Diagrammatic section of the anterior end. From Young, J.Z. (1981), fig. 3.5. After Spengel.

vertebrate heart. The front of the sinus forms a series of glomeruli which are covered by a region of the proboscis coelom which is specialized to form excretory cells. The entire body surface is ciliated. A postanal region is present in some species during embryonic development. The tornaria larva has many features of echinoderm larvae (Young, 1981; Nelson, 1984; Jamieson, 1991).

## B. Spermatozoa of the Enteropneusta

### 1. Sperm literature

Knowledge of enteropneust sperm was, until recently, limited to description of the acrosome of *Saccoglossus kowalevskii* and its reaction during fertilization (Colwin *et al.*, 1957; Colwin and Colwin, 1963, 1967), with some additional data on sperm structure by Afzelius (1979), augmented by Jamieson (1991) from light microscope observations in these papers and from scrutiny of micrographs presented there. Franzén *et al.* (1985) have given a detailed description of the sperm of *Saxipendium coronatum*, the 'spaghetti worm' of the Galapagos hydrothermal vent, living at a depth of 2,478 m.

### 2. *Saccoglossus* sperm

The spermatozoon is of the so-called 'primitive' type, that is an ect-aquasperm of the pelsiosperm (round-headed) type.

The acrosome (Fig. 3) resembles that of echinoderms in the presence of an almost isodiametric acrosome vesicle with well-developed periacrosomal material whereas this material, if present, is mainly subacrosomal in most phyla with plesiosperm. The acrosome in *Saccoglossus* only slightly indents the nucleus and a distinct excavation for subacrosomal material is not developed. This contrasts with echinoderms (see Chapter 5) in which, in all but echinoids and the concentricycloid *Xyloplax*, the acrosome is embedded in a deep anterior excavation of the nucleus.

The external boundary of the membrane-bound acrosome vesicle (Fig. 3) is the plasma membrane and its inner boundary is the nuclear envelope. Periacrosomal material surround the vesicle except at the apex. Posteriorly (proximally), the acrosomal region intrudes into a shallow depression in the nucleus and anteriorly it protrudes beyond the spherical nuclear outline. The periacrosomal material is dense and finely granular, thick around the sides of the vesicle, forming a surrounding annulus, but forming only a thin sheet on the adnuclear side. The acrosome vesicle is bounded by a well-developed membrane. The vesicle is nearly filled by a large dense acrosomal granule and is lined, except at the apex, by a thin, finely granular, layer of material. An apical space separates the acrosomal granule from the unlined apical region of the membrane. Where the lining layer is present it adjoins the granule except that a ring-shaped space separates them around the sides of the

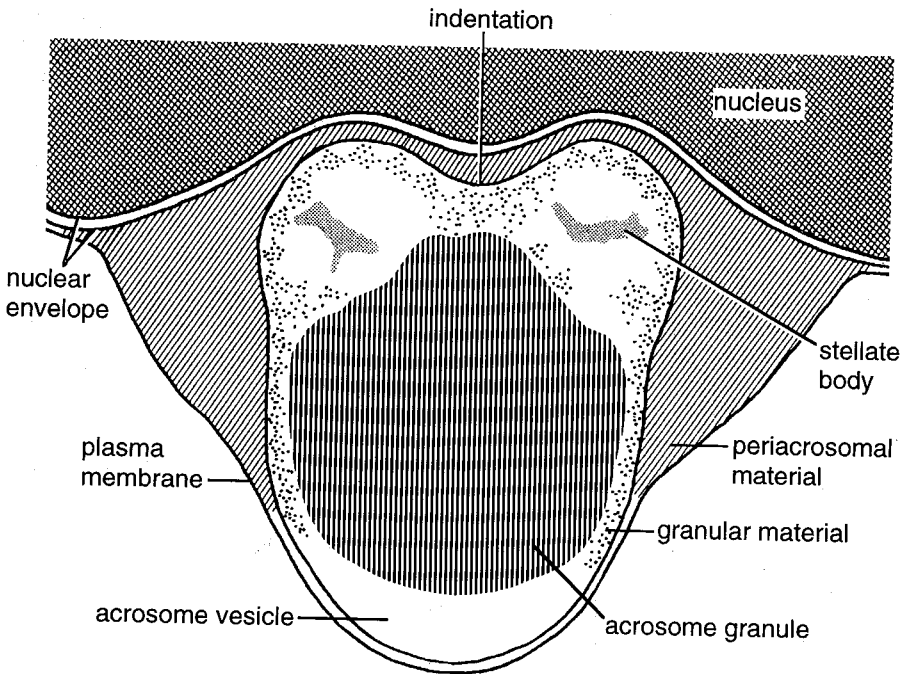


Fig. 3. *Saccoglossus kowalevskii*. Diagram of median longitudinal section of unactivated acrosomal region. Relabelled from Colwin, A. L. and Colwin, L. H. (1963), fig. 1.

adnuclear end of the vesicle; about a dozen irregularly stellate bodies are arranged in a circle within this space. The base of the vesicle is indented by a single shallow axial invagination. The plasma membrane, the acrosomal membrane and the inner and outer nuclear membranes are tripartite, or unit, membranes.

The acrosome reaction and penetration of the egg have been elegantly investigated by Colwin *et al.* (1957) and Colwin and Colwin (1963, 1967) and are summarized in Fig. 4.

The almost spherical nucleus is slightly indented anteriorly by the acrosome and posteriorly in the vicinity of the centrioles.

Around the centrioles are the mitochondria, constituting with it a somewhat flattened compact midpiece; in longitudinal sections the mitochondria are seen as rounded, internally cristate structures on each side; several discrete structures are described for the midpiece of the reacted sperm and this presumably indicates the existence of a number of separate mitochondria as is characteristic of ect-aquasperm.

The centrioles appear to include a proximal centriole perpendicular to the distal centriole which forms the basal body. The flagellum is continuous with, and in the same axis as, the basal body. Afzelius (1979) illustrates the anchoring apparatus, consisting of a 9-pointed skewed star formed by lamellae radiating from the distal

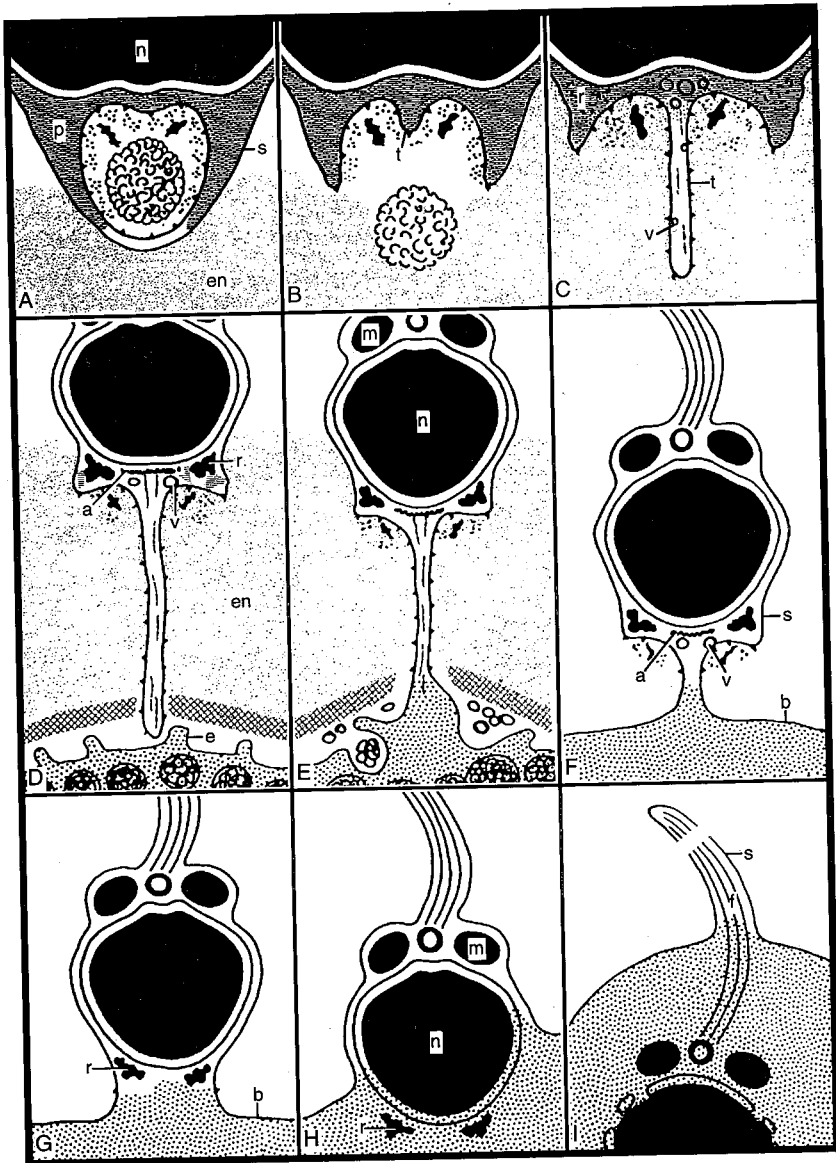


Fig. 4. *Saccoglossus kowalevskii*. Diagrams of the acrosome reaction and association of the spermatozoon with egg and blastomere. Small triangles indicate surface of acrosome membrane initially facing into acrosome vesicle but subsequently (after insertion into plasma membrane) facing external medium. A-E: Sperm-egg association: confrontation, sperm activation, and membrane fusion with envelope-enclosed egg. (Subsequent states of sperm-egg association resemble those shown in F-I except that the egg envelope (en) would be present). A: acrosomal region of unactivated sperm cell; except apically, periacrosomal material (p) surrounds the membrane bound acrosome vesicle. B-D: Sperm activation:



centriole to the plasmalemma. Each of these primary processes branches into two secondary processes which in turn give tertiary processes. The structure of the axoneme has not been described but, from micrographs, is probably of the '9+2' pattern.

### 3. *Saxipendium* sperm

The sperm of *Saxipendium coronatum* (Figs. 5, 6), described by Franzén *et al.* (1985) is about 29  $\mu\text{m}$  long. The head is pyramidal, 3.3  $\mu\text{m}$  in diameter at the base and 3  $\mu\text{m}$  long, including an apical acrosome. A flattened midpiece is closely applied to the base of the head region. A long flagellum emerges from the centre of the midpiece.

The acrosome vesicle is rounded with a slightly flattened or concave adnuclear face and has a thin limiting membrane. The contents of the vesicle may appear to be differentiated into an anterior electron-dense part and a posterior more electron-lucent part, possibly as a result of an incipient acrosome reaction during processing. Periacrosomal material surrounds the vesicle but at the apex the acrosome membrane lies close to the sperm plasma membrane.

The nucleus is electron-dense with small scattered areas of low density. Its anterior surface has a ring-shaped depression around the base of the acrosome and the posterior face is indented by the centriolar fossa. The ring-shaped depression is interrupted by four extended elevations, which by SEM are seen as ridges, radiating from the acrosomal region.

The mitochondria, the centriolar region and the anchoring fibre (satellite) apparatus comprise the midpiece. A number of small mitochondria are present in the late spermatid but it was not clear, owing to difficulties of fixation, whether these are reduced to four or five mitochondria or a single annular mitochondrion. The latter view is favoured but Franzén (1956), by light microscopy, demonstrated a few spherical mitochondria in the enteropneusts, *Glossobalanus sarniensis* and *Protoglossus* sp. The posterior part of the midpiece regularly shows vacuoles which appear to have released their contents and therefore appear by SEM as craters.

The proximal centriole lies in the centriolar fossa (Fig. 5) at right angles to, but in the same axis as, the distal centriole (basal body). The posterior part of the distal

(contd.)

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acrosome membrane, now continuous with sperm plasma membrane (s), forms acrosomal tubule (t) and everts, externalizing acrosome contents; periacrosomal material transforms into ring (r), vesicles (v), adnuclear sheet (a), etc., within the tubule. D: Tubule makes contact with egg plasma membrane (e). E: Early zygote established by sperm-egg membrane fusion. F-I: Sperm-blastomere association; internal sperm organelles, such as nucleus (n), mitochondria (m), and fibrils of flagellar axis (f), progressively intermingle with cytoplasm of denuded blastomere; blastomere plasma membrane (b) is continuous with sperm plasma membrane. (The stages of sperm activation and membrane fusion which precede this intermingling resemble those shown in A-E, except that the egg envelope would be absent from the denuded blastomere). From Colwin, A. L. and Colwin, L. H. (1967), fig. 1.

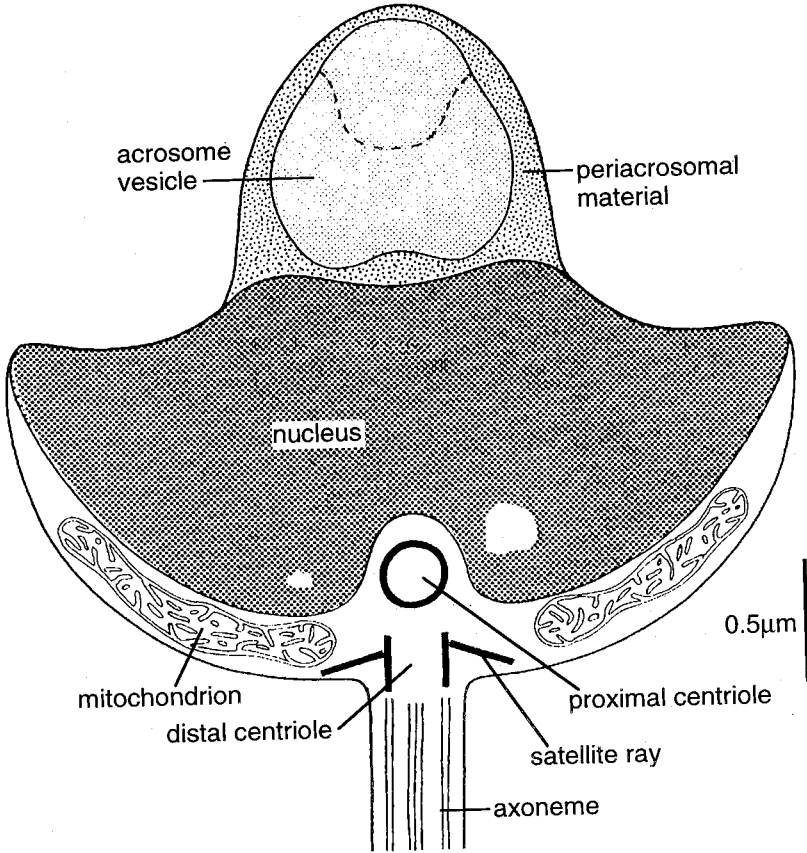


Fig. 5. *Saxipendium coronatum*. Diagram of the mature spermatozoon. (Scale bar = 0.5  $\mu\text{m}$ ). From Jamieson (1991), fig. 2.5, after Franzén, Å, Woodwick, K. H. and Sensenbaugh, T. (1985), fig. 12.

centriole gives rise to satellite rays; these branch into secondary fibres which join the plasmalemma at electron-dense thickenings.

#### 4. Fertilization biology in enteropneusts

Fertilization in *Saccoglossus* is external (Colwin *et al.*, 1957; Colwin and Colwin, 1963, 1967) and this is suspected for *Saxipendium*, the small eggs of which, observed in the female, suggest indirect development, presumably via a tornaria as in other enteropneusts (Franzén *et al.*, 1985).

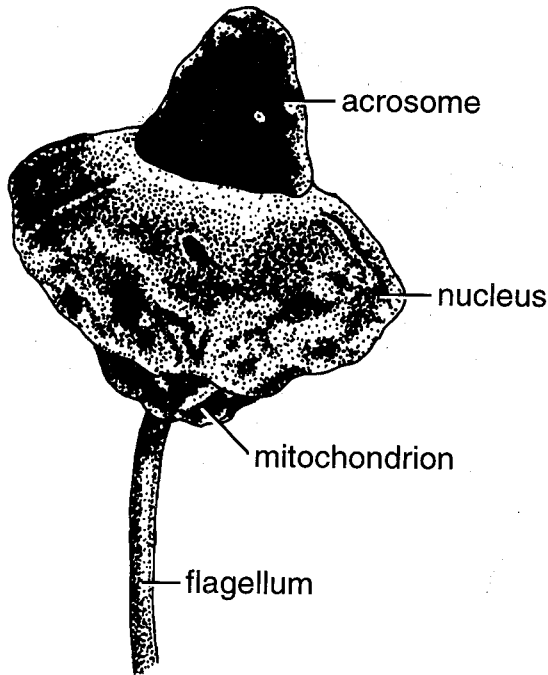


Fig. 6. *Saxipendium coronatum*. External appearance of a spermatozoon. From Jamieson, B.G.M. (1991), fig. 2.6, drawn from an SEM micrograph of Franzén, Å, Woodwick, K. H., and Sensenbaugh, T. (1985), fig. 15.

### 5. Summary of enteropneust sperm

Jamieson (1991) has summarized accounts of the two species examined ultrastructurally. The acrosome is subspheroidal but sufficiently depressed on the adnuclear face to appear dome-shaped. It rests in a slight depression of the nucleus and is surrounded by a thick layer of periacrosomal material. The nucleus is electron-dense; it is subspheroidal in *Saccoglossus*, though scalloped posteriorly by the mitochondria, but has a hemispherical-bicornuate longitudinal section in *Saxipendium*. There are a few rounded mitochondria; it is uncertain whether these are fused into a single annular mass in *Saxipendium* in which they do not intrude on the nucleus. A proximal centriole is present, perpendicular to the distal centriole. An 'anchoring apparatus' of dichotomous satellite rays connects the basal body to the plasma membrane. The flagellum arises centrally from the midpiece.

## IV. CLASS PTEROBRANCHIA

### A. Introduction

Pterobranchs are colonial or aggregated sedentary marine lophophorates. An external cuticular skeleton is present and they are therefore known as fossils, from the Lower Ordovician to Eocene (Nelson, 1984).

There are two orders, the Cephalodisca and Rhabdopleurida.

### B. Order Cephalodisca

Zooids of the Cephalodisca are free, produced by budding, but do not form true colonies though aggregated in a many-chambered gelatinous house. Living forms have four to nine pairs of arms with tentacles. Extant genera are *Atubaria* (one species, near Japan) and *Cephalodiscus* (mostly Antarctic).

Each zooid of *Cephalodiscus* (Fig. 7) has a proboscis, collar, and trunk, each possessing one or more coelomic cavities.

The collar is prolonged into a number of ciliated feeding arms, the lophophore. The single pair of gill slits serves for egress of water drawn in by the tentacles in feeding. The intestine is reflected so that the anus is near the mouth. A thickening of the roof of the pharynx corresponds exactly with the stomochord of enteropneusts and again contains vacuolated cells. The nervous system does not form a hollow tube but the blood system resembles that of the enteropneust *Balanoglossus*. The larva somewhat resembles that of ectoprocts but is derivable from the same plan as echinoderm larvae (Young, 1981).

It is unfortunate, in view of the relevance of cephalodiscans to hypotheses of the origin of echinoderms, that the sperm of *Cephalodiscus* have not been examined ultrastructurally.

### C. Order Rhabdopleurida

#### 1. Introduction

Zooids are attached and form true colonies. The zooids of the genus *Rhabdopleura* have two tentacular arms. The coenecium or colony of *Rhabdopleura* is made up of a series of translucent tubes, each with a regular annulated pattern. A stolon running through the bases of all the tubes and interconnecting all the zooids of the colony is the site of asexual budding of new zooids. The zooid, approximately 1 mm long, is divided into three regions. The oral shield (also termed the 'protosome', 'cephalic shield', 'buccal shield' or 'proboscis') is a disc-shaped organ used in forward locomotion and in secretion of the coenecium. It contains an unpaired coelomic cavity, the protocoel. The collar region (mesosome) contains the paired mesocoels which surround the pharynx. This region bears the two tentaculate arms

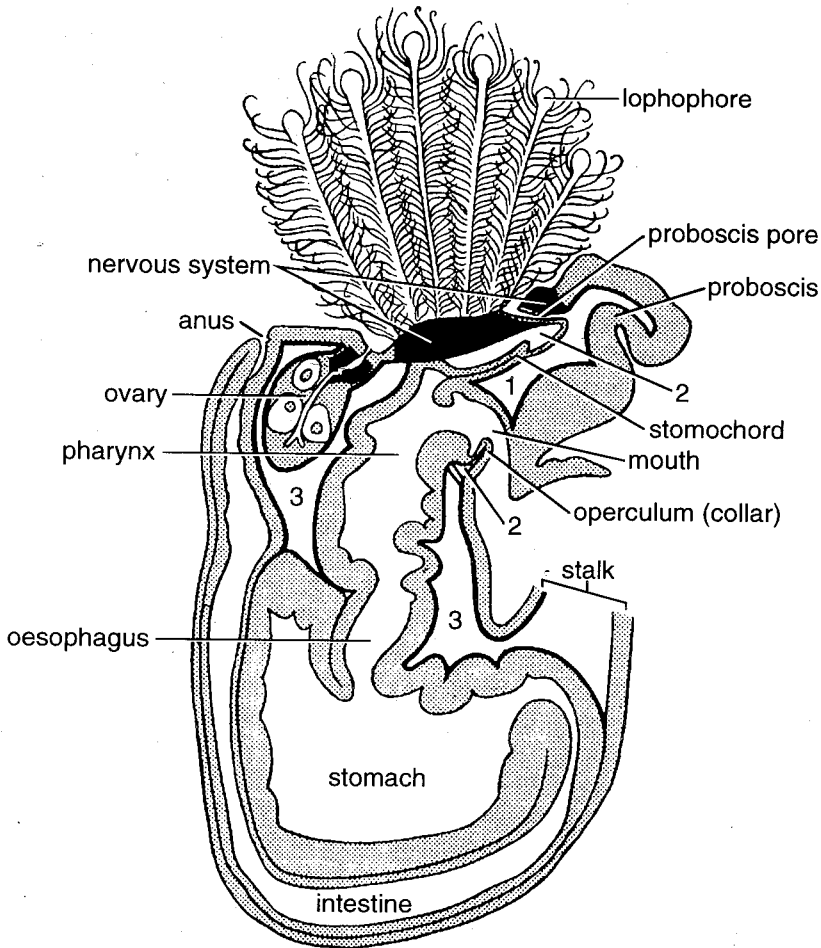


Fig. 7. *Cephalodiscus*. Longitudinal median section. 1-3 denote the three coelomic cavities. From Young, J. Z. (1981), fig. 3.12. After Harmer.

and the oral lamellae (two ventral projections, each with a ciliated groove used to transport particles collected by the arms into the mouth). The trunk sac or metasome encloses the recurved digestive tract, the single gonad and the paired metacoels (Lester, 1988, and references therein). The nervous system appears very primitive, with cell bodies and fibres confined within the epithelial layer. The short-lived larva has cilia not in bands (Young, 1981). They occur in the Atlantic, Pacific, and Antarctic.

## 2. Anatomy of the gonads

In the pterobranch *Rhabdopleura normani*, the sexes are separate among the zooids of a colony, but a given colony may contain females and males. In zooids of either sex, the single gonad is associated with a large haemal sinus in the trunk sac and is displaced laterally (to the right or to the left). The wall of the gonad is composed of three layers: an outer metasomal peritoneum, an internal lining of germinal epithelium, and an intervening genital haemal sinus. The mature gametes lie in the lumen within the gonad (Lester, 1988).

## 3. *Rhabdopleura normani* sperm

The ultrastructure of the sperm of *Rhabdopleura normani*, a species living in shallow water around Bermuda, has been briefly described by Lester (1988) in the account of gonads and of larval development. A diagram by Jamieson (1991) based on Lester's description is given in Fig. 8 where comparison is made with other lophorate-derived groups.

The spermatozoon (Fig. 8) is filiform and uniflagellate. No acrosome has been observed. The elongate, conical nucleus is 3.6  $\mu\text{m}$  long and 0.5  $\mu\text{m}$  wide. A midpiece appendix, 0.24  $\mu\text{m}$  wide and at least 4  $\mu\text{m}$  long, containing a mitochondrial filament, joins the base of the 'head piece' near the flagellar basal body. It therefore forms a free appendage paralleling the flagellum which is of the '9+2' type (Lester, 1988). The mitochondrion filament is presumably a single mitochondrial derivative as there is a single mitochondrion in the spermatid.

## 4. Fertilization biology in *Rhabdopleura*

The method of sperm release in *Rhabdopleura* is unknown. Presumably the males are broadcast spawners and the sperm are released through the anterior gonoduct into the surrounding sea water while the zooid projects from the ostium of its tube. The derived condition of the sperm is tentatively related to the altered method of fertilization and brood protection. Females brood 200  $\mu\text{m}$  eggs and embryos in their distinctive, basally coiled tubes. The yolky eggs undergo radial cleavage and develop into ciliated, lecithotrophic, oblong larvae (400  $\mu\text{m}$  in length). The apparent absence of an acrosome is attributed to the absence of an egg envelope, eliminating the need to enzymatically degrade a path to contact the oolemma (Lester, 1988). It would appear that in the terminology of Rouse and Jamieson (1987), the sperm are ent-aquasperm.

## 5. Sperm and hemichordate phylogeny

The enteropneust (*Saccoglossus* and *Saxipendium*) spermatozoon has much the appearance which might be attributed to a precursor of the echinoderms; it is an ect-

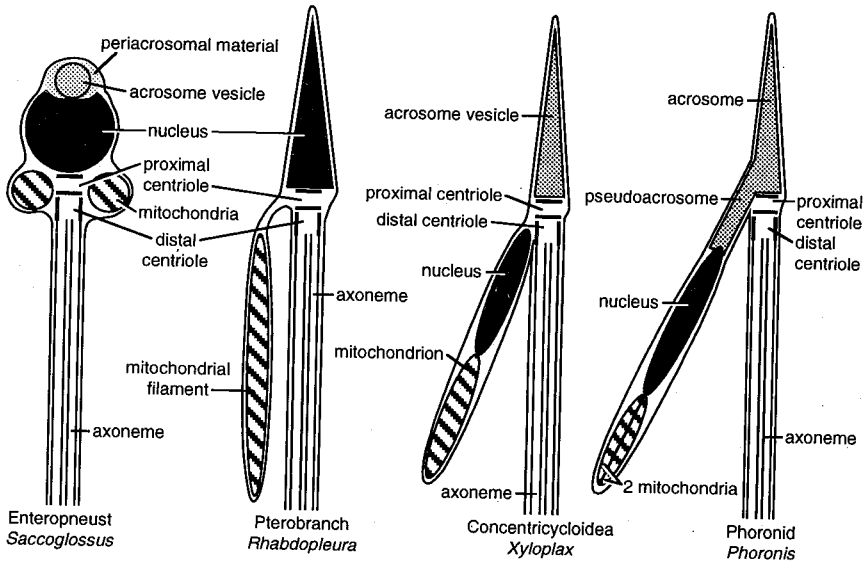


Fig. 8. Highly schematic comparison of the spermatozoon of the pterobranch *Rhabdopleura normani* with that of an enteropneust (*Saccoglossus*), a concentricycloid echinoderm (*Xyloplax*) and a phoronid (*Phoronis*). Location of the mitochondrial material in an appendage of the head is shared by *Rhabdopleura*, *Xyloplax* and *Phoronis*, while *Xyloplax* and *Rhabdopleura* further resemble each other in location of the nucleus in the appendage. These similarities are possibly symparomorphies, parallel developments by virtue of relationship. However, the possibility that complex sperm are basic to the groups represented and that plesiosperm are secondary deserves consideration. From Jamieson (1991), fig. 2.8, after authors cited.

aquasperm distinguished like that of echinoderms (the echinosperm) in having an inflated acrosome enveloped in periacrosomal material, whereas the basic aquasperm of the Metazoa (the hypothetical plesiosperm) is attributed a depressed acrosome with, at most, subacrosomal material. In *Saxipendium*, a further resemblance to the echinosperm may be the uncertainly demonstrated annular mitochondrion. It would not seem unreasonable to suggest that, in their spermatozoa, enteropneusts have retained features of a common ancestral stock of echinoderms and hemichordates, whereas echinoderms have developed the echinosperm as a more apomorphic form of the plesiosperm. However, *Rhabdopleura* which, as a member of the Pterobranchia, might be expected to have sperm near the ground plan for echinoderm sperm has highly modified sperm. For further discussion, see Chapter 5, and Chapter 8, Section VI, in which an alternative view that the echinosperm is a secondary simplification for broadcast spawning in secondarily radiate animals is given some consideration.

## ACKNOWLEDGEMENTS

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