

Spermatozoan ultrastructure and spermatogenesis in aplousobranch ascidians, with some phylogenetic considerations

Åke Franzén*

Swedish Museum of Natural History, Box 50007, S-104 05 Stockholm, Sweden

Date of final manuscript acceptance: December 10, 1991. Communicated by T. Fenchel, Helsingør

Abstract. Spermatozoa and some stages of spermatogenesis were studied for four species of aplousobranch ascidians. Spermatozoa of *Clavelina lepadiformis* (Müller) (family Clavelinidae) are plesiomorphous in that they have apical acrosomal vesicles and a moderately elongated, cylindrical nucleus. The elongated mitochondrion is twisted ca. 1 1/2 times around the nucleus. In their ultrastructural morphology, *C. lepadiformis* sperm conform in some respects to the least-derived ascidian sperm, those of the phlebobranch ascidian *Ciona intestinalis* L.; however, the sperm of *Clavelina lepadiformis* have two apomorphies not shared with those of *Ciona* sp.: (1) the mitochondrion of *Clavelina lepadiformis* is long and spiralled along the entire nucleus rather than being comparatively compact and not at all helical; (2) the mitochondrial cristae are elongated parallel to the long axis of the nucleus, whereas in *Ciona* sp. sperm the cristae are unmodified. In *Distaplia* sp., *Aplidium* sp. and *Synoicum pulmonaria* (Ellis and Solander) the spermatozoa are more derived and consist of a proximal cylindrical and a distal corkscrew-like part. The mitochondrion in *Distaplia* sp. and *Aplidium* sp. contains electron-dense material and extends in a long thread around the nucleus. In *S. pulmonaria* the mitochondrion surrounds the anterior part of the nucleus in mature spermatozoa, and an elongated, dense structure displaying fine striation is enclosed in the mitochondrion in late spermatids. The sperm ultrastructural morphology observed in this study is consistent with the majority view that clavelinids are closest to the ancestral ascidian but is also consistent with other conclusions, particularly that the Cionidae are closest to the stem ascidian.

Introduction

Electron microscope studies of tunicate sperm have been conducted for several ascidian species (Tuzet et al. 1972, 1974, Franzén 1976, Fukumoto 1979, 1981, 1983, 1984,

1985, 1986, Cloney and Abbott 1980, Cotelli et al. 1980, Villa 1981, Burighel et al. 1985). These investigations suggest that the sperm of solitary ascidians (orders Phlebobranchia and Stolidobranchia) are much more plesiomorphous than are sperm of the Aplousobranchia.

Ascidian spermatozoa characteristically lack a mid-piece. A single mitochondrion (lateral body) flanks the more or less elongated nucleus. In some solitary ascidians, e.g. *Ciona intestinalis* the mitochondrion is lost during "sperm reaction" (Lambert and Epel 1979). The question of whether an acrosome is present in ascidians has been a matter of debate. In various ascidians small vesicles at the spermatozoon apex have been referred to as an acrosome (Cloney and Abbott 1980, Cotelli et al. 1980, Fukumoto 1983, 1984). Woollacott (1977), in his study on *Ciona intestinalis* sperm, found no acrosomal vesicles, but Fukumoto (1984) has shown that sperm of this species do in fact possess small acrosomal vesicles. Schabtach and Ursprung (1965) reported an acrosome in the sperm of *Ascidia nigra* as a single electron-dense line of ca. 100 Å length. Cloney and Abbott (1980), discussing acrosomal structures in ascidians, believed that the structure reported for *A. nigra* was misinterpreted and that it was not an acrosome. In a review on morphological aspects of ascidian fertilization, Fukumoto (1990) states that ascidian spermatozoa have an acrosome, although a small one.

The tail of ascidian spermatozoa is a simple flagellum, with an axoneme displaying a 9 + 2 microtubular pattern.

The class Ascidiacea (ascidians) is sometimes divided into the orders Aplousobranchia, Phlebobranchia and Stolidobranchia. However, the ascidians are often divided into only two orders: (1) the Enterogona, including the suborders Aplousobranchiata and Phlebobranchiata; and (2) the Pleurogona, including the suborder Stolidobranchia. All aplousobranchs form compound colonies. Zooids of aplousobranchs may be enclosed in a common gelatinous tunic, or they may have individual tunics and form a loosely aggregated colony.

Most studies of ascidian sperm have been carried out on a number of species in the Phlebobranchia and

* Present address: Zoological Institute, Uppsala University, Box 561, S-751 22 Uppsala, Sweden

Stolidobranchia. Detailed studies on Aplousobranchia have been performed only for the family Didemnidae (Tuzet et al. 1972, 1974, Burighel et al. 1985). The Clavelinidae are one of the families within the order Aplousobranchia and are regarded as the least specialized ascidian group; information on spermiogenesis in this family is therefore of interest when considering theories about the structure of the ancestral ascidian sperm and may have implications for ascidian phylogeny. Holland (1989) suggests that some clavelinid with a distinct acrosome was probably ancestral to the solitary ascidians. Several light-microscopical and ultrastructural studies have shown that sperm morphology can be a useful indicator of phylogenetic relationships (Franzén 1970, 1977, Wirth 1984, Holland 1989, 1990, in press).

Materials and methods

Colonies of the ascidians *Clavelina lepadiformis* (Müller) and *Synoicum pulmonaria* (Ellis and Solander) were collected near the Klubban Biological Station off the west coast of Sweden during July and August in 1987 and 1988. Colonies of undetermined species of the genera *Distaplia* and *Aplidium* were collected from Moro Bay, California, USA in August 1986. Sexually mature individuals (blastozoids) were fixed for electron microscopy in cold (5°C) 3% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4). Following glutaraldehyde fixation the material was rinsed in cold 0.1 M sodium cacodylate buffer and postfixed for 1 h in 1% osmium tetroxide using the same buffer. Material for transmission electron microscopy (TEM) was dehydrated in a graded ethanol series and embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in a Jeol 100 S electron microscope.

Results

Clavelina lepadiformis (Figs. 1 and 2)

The mature spermatozoon of *Clavelina lepadiformis* is ca. 42 µm long. The sperm is composed of an elongated head region, about 6 µm long, and a tail flagellum, which is about 36 µm long and has a tapered end piece. The body of the head region is the nucleus, which is about 0.5 µm in diameter and contains a tightly coiled mass of electron-dense chromatin fibres limited by a nuclear envelope. Two distinct nuclear membranes could be seen in cross sections. The lumen of the nuclear envelope contained moderately dense material in some regions and in other regions had a low density equal to that of the background.

Examination of numerous grids with longitudinal sections of the anterior part of the nucleus revealed an acrosome-like structure present at the tip of the nucleus. This structure consisted of one or two flattened vesicles containing amorphous material (Fig. 1 A, B). The vesicles were ca. 300 × 20 nm (length × height) and were clearly separated from the nuclear envelope and the plasmalemma. The two membranes of the nuclear envelope were in close contact with each other at the tip of the nucleus and the chromatin was tightly connected with the nuclear envelope. Seen in sagittal section, the tip of the nucleus was wedge-shaped (Fig. 1 B).

Early spermatids contained a spherical nucleus with granular chromatin, several small mitochondria, numerous ribosomes, and some vesicles. During spermiogenesis the nucleus went through a process of elongation and chromatin condensation. Chromatin granules first condensed into coarse filaments which later become thinner and longer and were helically arranged. In mature spermatozoa the nucleus was compact and seemed to consist of dense chromatin. In lateral sagittal sections of late spermatids a distinct helical structure was seen, which appeared to be a tightly coiled mass of electron-dense chromatin fibres, limited by a nuclear envelope (Fig. 2 D). A number of microtubules, generally about ten, were seen in the cytoplasm near the nuclear envelope during elongation and condensation (Fig. 2 C, D). The microtubules generally were found between the nucleus and the ovoid mitochondrion, and in later stages they seemed to constitute an elongated helix.

A morphologically delimited midpiece was lacking, but a single elongated mitochondrion was laterally attached to the nucleus (Fig. 1 A, B). The mitochondrial cristae were mainly parallel with the long axis of the nucleus. In early stages there were several small ovoid or roughly spherical mitochondria scattered in the cytoplasm. During spermiogenesis the mitochondrial material accumulated into one large mitochondrion, which gradually transformed into an elongated mitochondrial rod, loosely twisted about 1½ times around the nucleus (Fig. 1 A). The mitochondrial rod was not exactly cylindrical but was ovoid or crescent-shaped when viewed in transverse sections. In the cytoplasm of spermatids there were numerous vesicles of varying size. In mature spermatozoa some of these vesicles seemed to be retained in the sparse cytoplasm surrounding the basal body of the flagellum. It is not clear whether there were one or two centrioles in early spermatids, but in late stages of spermatids and in mature spermatozoa only one centriole was present. This centriole was typically composed of nine triplets of microtubules embedded in an electron-dense matrix (Fig. 1 C, D). The centriole is the basal body of the tail flagellum, which displays the familiar 9 + 2 axonemal pattern. The main features of the sperm ultrastructure are given in Fig. 3 A.

Distaplia sp. (Figs. 3 B and 4)

The spermatozoon of *Distaplia* sp. consisted of an elongated head region, about 15 µm long, followed by a tail

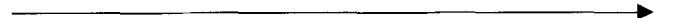
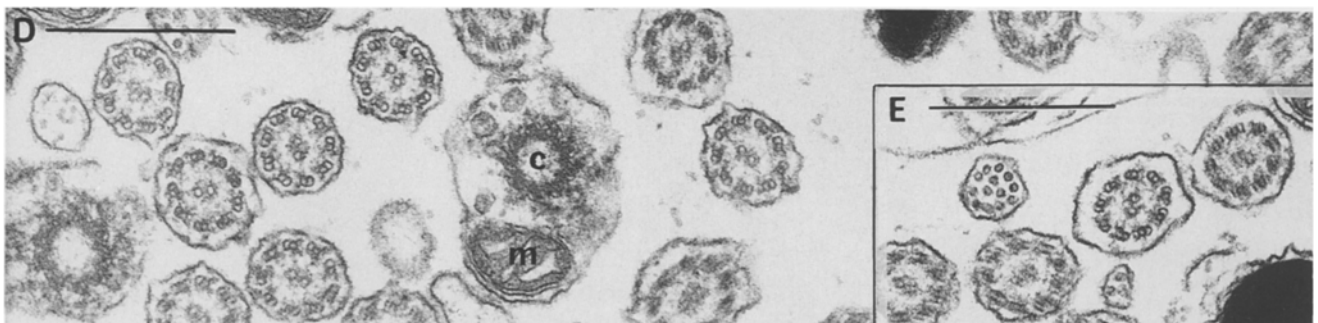
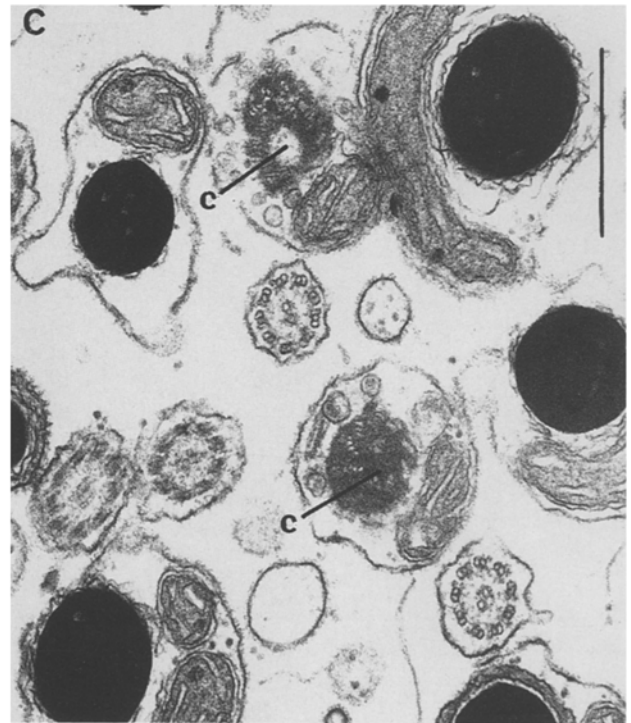
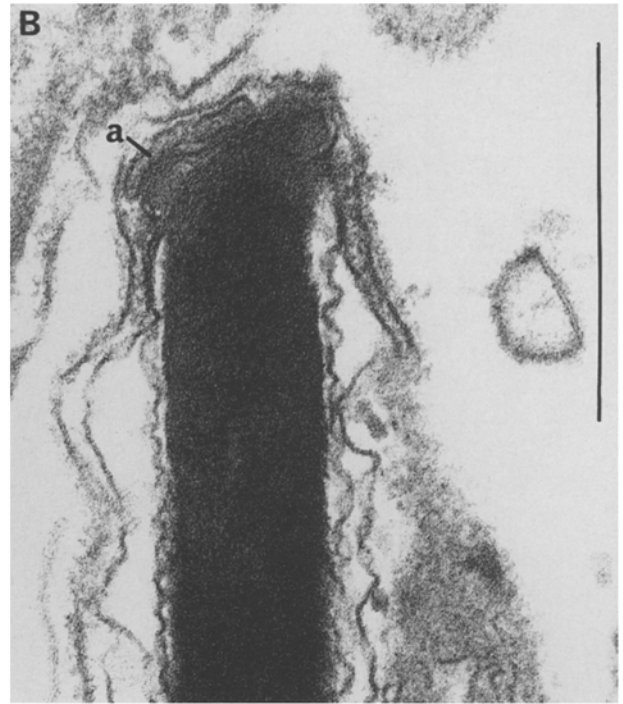
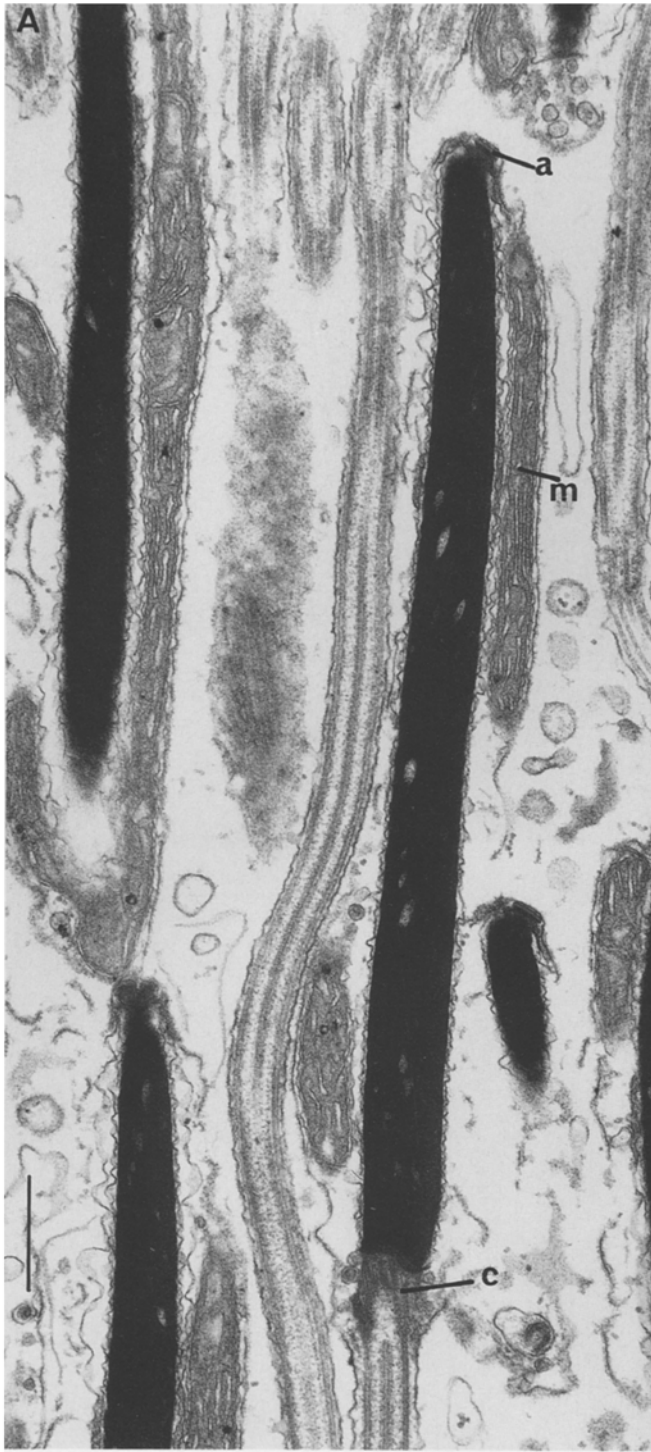
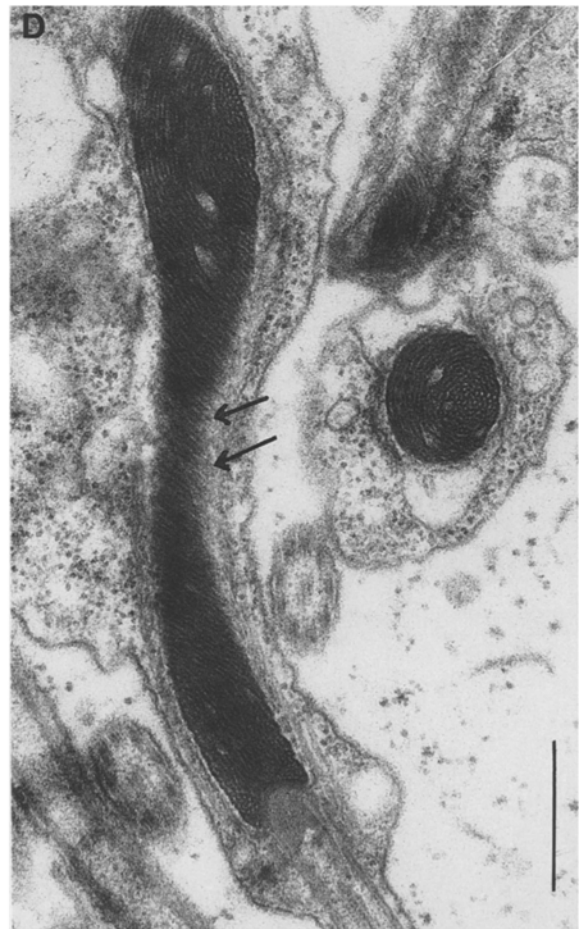
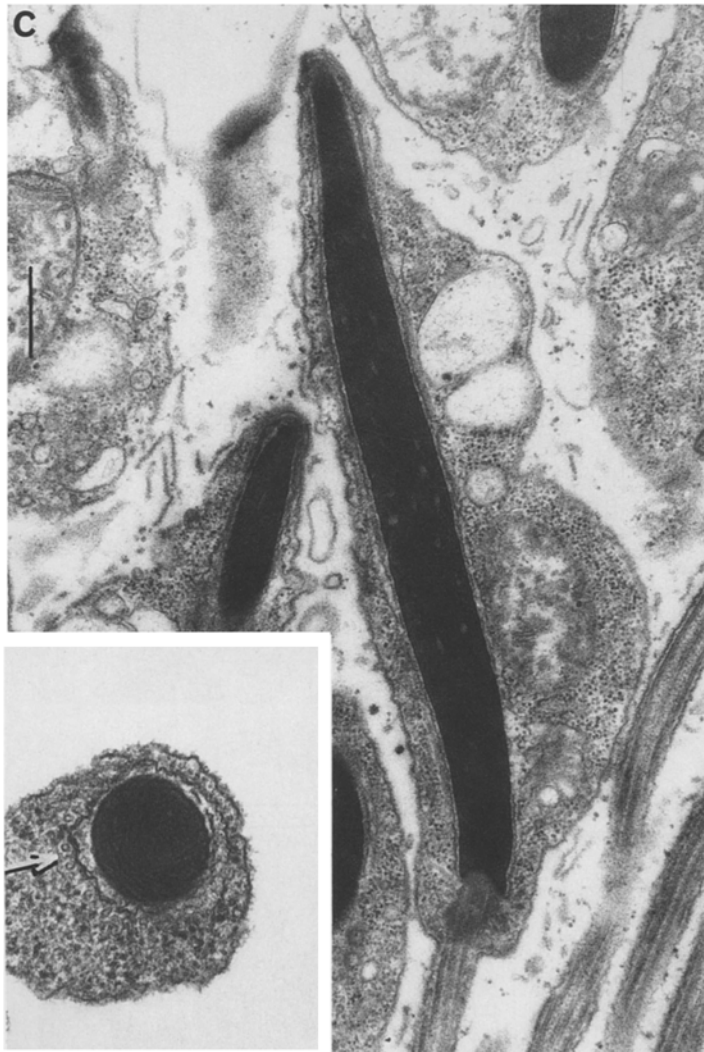
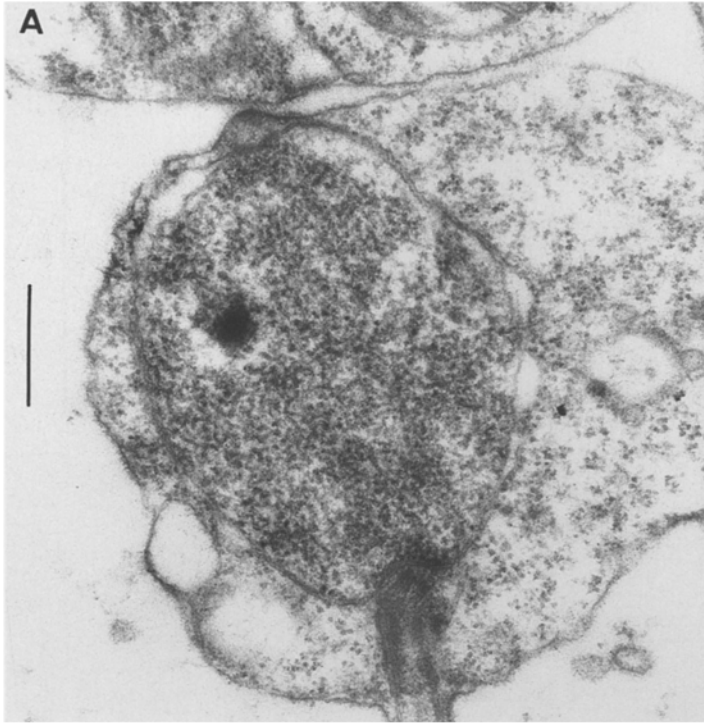


Fig. 1. *Clavelina lepadiformis*. TEM micrographs of the spermatozoon. Scale bars = 0.5 µm. (A) Approximately longitudinally sectioned spermatozoa displaying elongated, cylindrical nucleus and elongated mitochondrion (m), which forms about one turn around the nucleus. The anterior part of the flagellum is also seen in one sperm. a: aerosomal vesicle; c: centriole. (B) Longitudinal section of anterior part of head region. The putative acrosome is seen between the nuclear envelope and the plasmalemma. The plasmalemma is covered by a glycocalyx. (C) Transverse sections at different levels showing nucleus, mitochondrion, centriole and tail flagellum. (D) Transverse sections of flagella and two centrioles. (E) Transverse sections of flagella, including the end piece of one flagellum





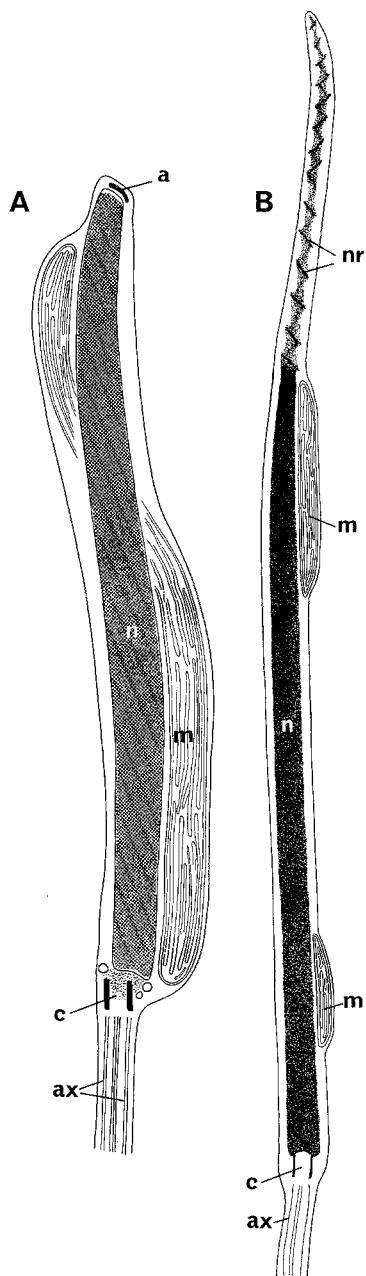


Fig. 3. *Clavelina lepadiformis* (A) and *Distoplia* sp. (B). Schematic diagrams of the spermatozoa. In the diagram of the more derived spermatozoon (*Distoplia* sp.) no acrosome is shown although this only means that, in this species, no acrosomal vesicles were observed in the present study. a: acrosomal vesicle; n: nucleus; m: mitochondrion; c: centriole; ax: axoneme; nr: nuclear ridge

Fig. 2. *Clavelina lepadiformis*. TEM micrographs of spermatids. Scale bars = 0.5 μ m. (A) Early spermatid connected by cytoplasmic bridges to other spermatids. The nucleus is shown in early stage of condensation. (B) Longitudinal section of a spermatid at a fibrous stage of nuclear condensation. (C) Longitudinal section of a spermatid with a condensed nucleus. Microtubules partly surround the nucleus. The inset shows the nucleus with surrounding microtubules (arrow), in transverse section. (D) Oblique longitudinal section showing the helical arrangement of the nuclear material (arrows)

(Fig. 3B). During spermiogenesis the nucleus underwent the conventional process of chromatin condensation and elongation. In late spermatids, where chromatin condensation was beginning, there was a single, large mitochondrion, apparently formed by the fusion of several small mitochondria present in earlier stages. Simultaneously with nuclear elongation, the mitochondrion began to transform into an elongated structure surrounding the anterior part of the nucleus. In mature spermatozoa the mitochondrion formed a thread-like structure around the anterior part of the nucleus. During later stages of spermatid differentiation the mitochondrial cristae became elongated parallel to the nucleus. As the mitochondrion grew longer a long dense strand appeared in the matrix on the opposite side of the nucleus.

The nucleus of the mature spermatozoon consisted of two parts: (1) a proximal part which was approximately cylindrical at its base but slightly tapered anteriorly, and (2) a distal part (about 4 μ m long) possessing a helical nuclear ridge. The helical ridge gave the anterior part of the nucleus a corkscrew-like appearance, whereas the posterior part was a solid rod.

In its posterior part the mitochondrion consisted only of dense material which formed an extended helix in close association with the nucleus (Fig. 4B, D).

Synoicum pulmonaria (Figs. 5 and 6)

In early spermatid stages the nuclei first displayed an irregular shape but soon became spherical. The chromatin was coarsely granular. As chromatin condensation started, dense strands or lamellae were formed. The lamellae formed by the condensing chromatin became helically arranged and tightly packed. Finally, the nuclear content became uniformly electron-dense. During nuclear condensation the nuclear envelope appeared inflated, forming perinuclear cisternae. During a later stage of nuclear elongation and condensation the anterior part (about 9 μ m) of the nucleus acquired a helical ridge or keel. In the nearly mature sperm the nucleus was about 23 μ m long, with a maximum diameter of 0.4 μ m in the proximal region near the centriole. A slight depression which housed the centriolar fossa was formed at the posterior end of the nucleus.

In spermatocytes and in very early spermatids there were several small mitochondria. When nuclear condensation began the mitochondria seemed to fuse, and at one mid-spermatid stage there was only one large mitochondrion, which had an ovoid shape and was situated close to the nucleus at a level where the helical ridge started (Fig. 5B). As sperm maturation continued the mitochondrion changed its shape, becoming anteriorly elongated by forming an anterior extension which was in close contact with the nuclear ridge (Fig. 5, 6).

The mature spermatozoon of *Synoicum pulmonaria* consisted of an elongated, tapering nucleus followed by a flagellum. The nucleus consisted of two parts: (1) a proximal part, which was principally cylindrical but tapered in the anterior direction; and (2) a distal part (up to 9 μ m long) with a helical nuclear ridge. The nuclear region was

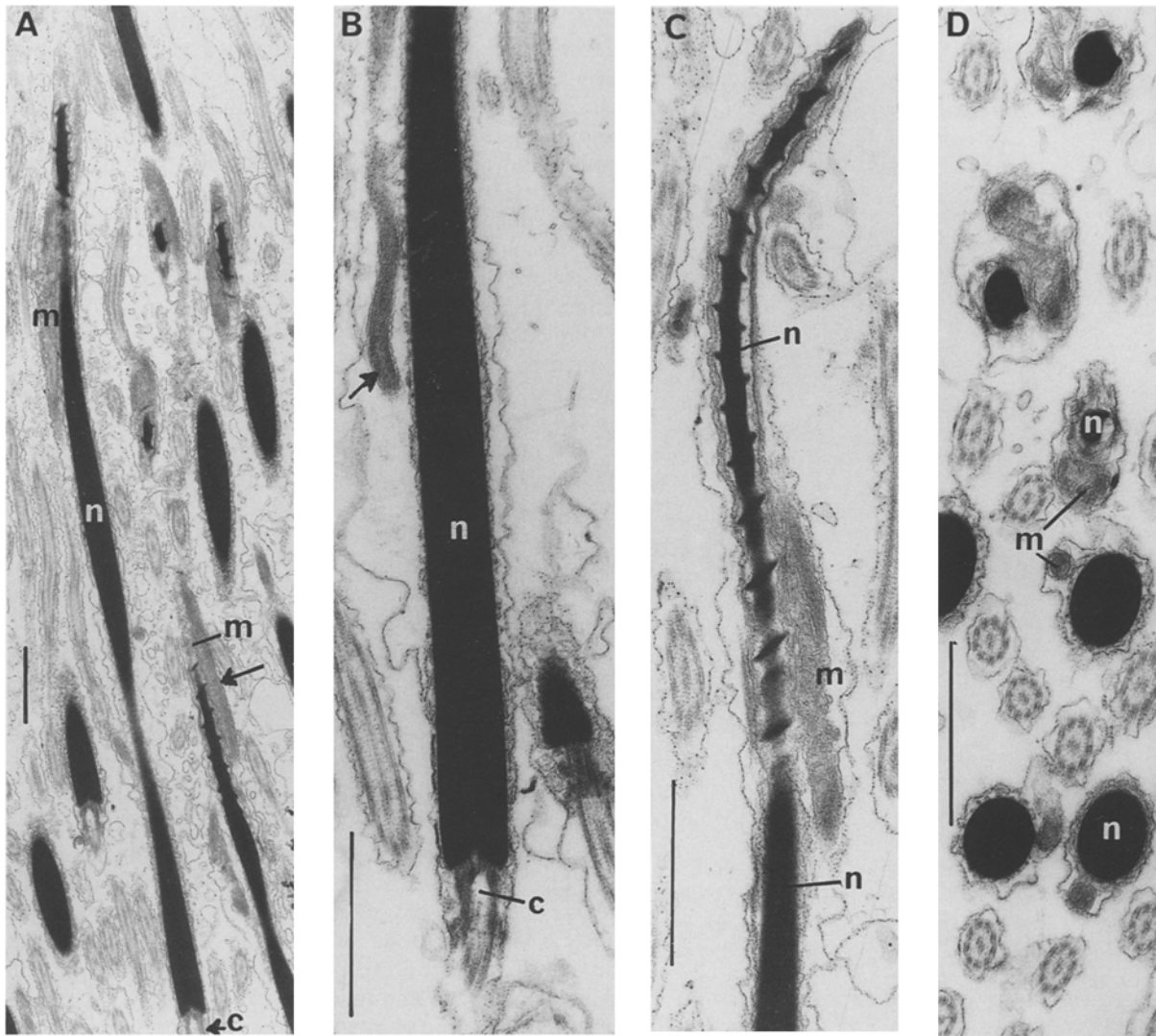


Fig. 4. *Distaplia* sp. TEM Micrographs of some late spermatids and mature spermatozoa. Scale bars = 0.5 μ m. (A) Survey micrographs of late spermatids and mature spermatozoa, showing a very elongated nucleus (n) with a helical ridge in the anterior part. m: mitochondrion; c: centriole. (B) Approximately longitudinal section of the proximal part of the nucleus. Arrow indicates mitochondrion.

(C) Approximately longitudinal section of the anterior part of a nucleus with a helical ridge. An electron-dense structure, probably a mitochondrial derivative, is seen next to the nucleus. (D) Transverse sections, at different levels, of late spermatids or mature spermatozoa

about 23 μ m long, and the flagellum about 52 μ m long. As in other ascidians, there was no typical mitochondrial midpiece, but at one mid-spermatid stage the mitochondrion formed a lateral body in close contact with the middle part of the nucleus; this mitochondrial mass had an anterior extension in close contact with the nuclear ridge. Light microscopical observations indicated that the lateral body was reduced in late stages of sperm maturation, and the mitochondrion was restricted to the anterior part of the nucleus with the nuclear ridge.

Aplidium sp. (Fig. 7)

The nucleus of the spermatozoon was elongated and cylindrical. A very shallow basal invagination contained the centriole and the proximal portion of the axoneme. In

the elongating spermatid a row of microtubules surrounded the nucleus (Fig. 7); these microtubules appeared between the nucleus and the mitochondrion at the beginning of mitochondrion elongation. In mature spermatozoa a single elongated mitochondrion was laterally attached to the nucleus, forming an elongated helix around the nucleus. Like the sperm of other ascidians, that of *Aplidium* sp. had a single centriole. It was not possible to determine the microtubular patterns of the centriole, but radiating dense structures indicated the presence of some type of anchoring fibre apparatus.

Discussion

The chordate subphylum Tunicata is currently divided into three classes: Ascidiacea, Appendicularia (= Lar-

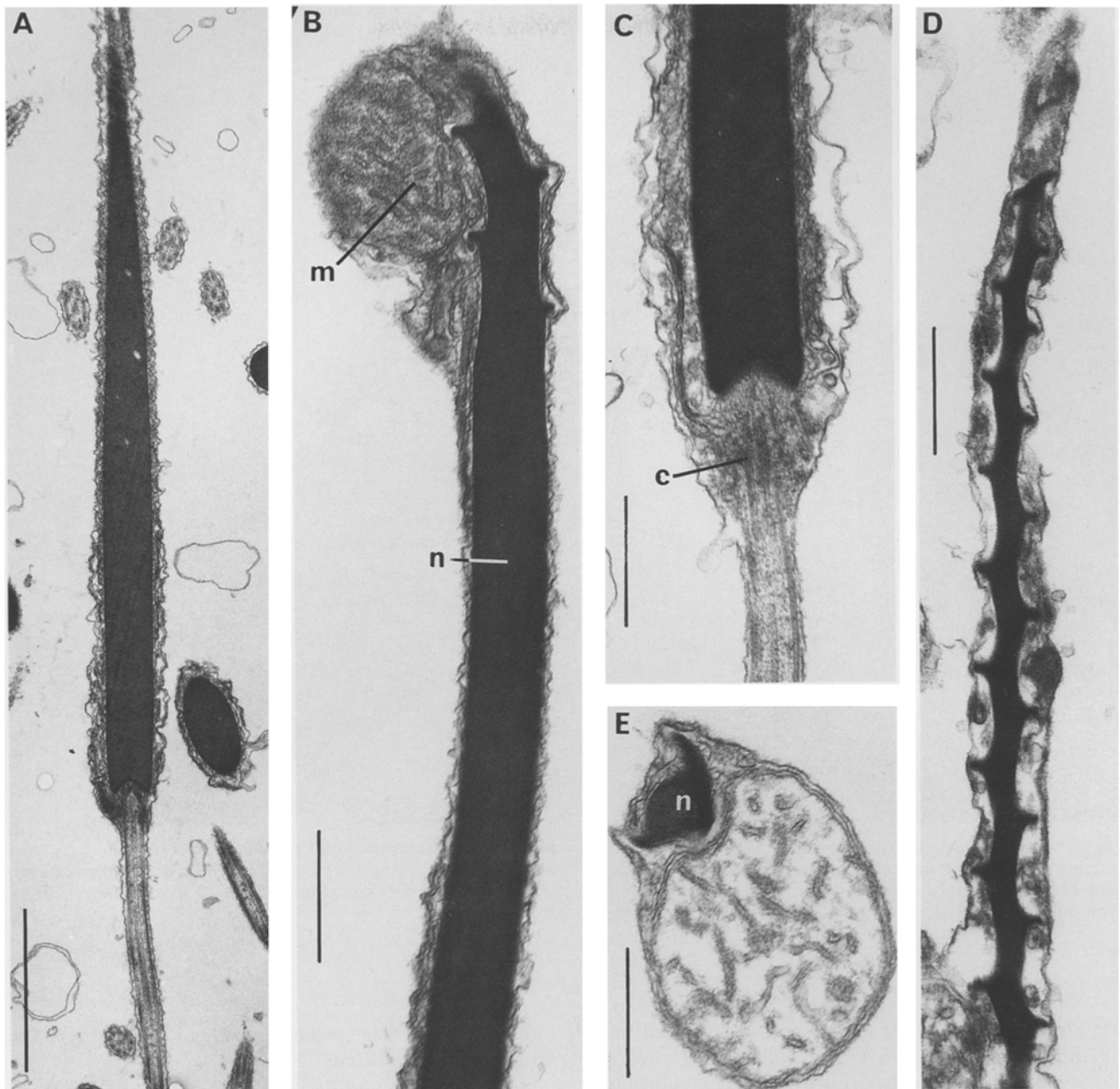


Fig. 5. *Synoicum pulmonaria*. TEM micrographs of some stages of spermiogenesis. Scale bars=0.5 μ m. (A) Survey micrograph of a longitudinally sectioned late spermatid showing the proximal part of the nucleus and the proximal part of the tail flagellum. (B) Longitudinal section of part of the nucleus (n) showing the two first turns of the nuclear ridge and the mitochondrial body (m). (C)

Longitudinal section of late spermatid showing part of the nucleus and the junction between nucleus and tail region. c: centriole. (D) Longitudinal section of the anterior part of the nuclear region with a helical ridge. (E) Transverse section of nuclear region at the top, where the mitochondrial body is

vacea) and Thaliacea. Most authors are of the opinion that the subphylum is monophyletic (Garstang 1928, Brien 1948, Young 1950), but there is no general agreement about the phylogenetic relations within the Tunicata.

Investigations on sperm ultrastructure in *Oikopleura dioica* have shown that appendicularian sperm in some respects resembles the ancestral or primitive sperm of the Metazoa (Flood and Afzelius 1978, Franzén 1956). Appendicularian sperm have a small, rounded nucleus, a

midpiece containing an elongated mitochondrion, and an apical acrosomal vesicle. The acrosome undergoes a typical acrosomal reaction upon contact with the egg (Holland et al. 1988). Appendicularian sperm apparently have more plesiomorphous characters than do other tunicate sperms. Holland (1988, 1989, 1990) believes this to indicate that the appendicularians are closely related to the stem tunicate.

In the Ascidiacea, the least-derived sperm have to date been known to occur among solitary ascidians. These

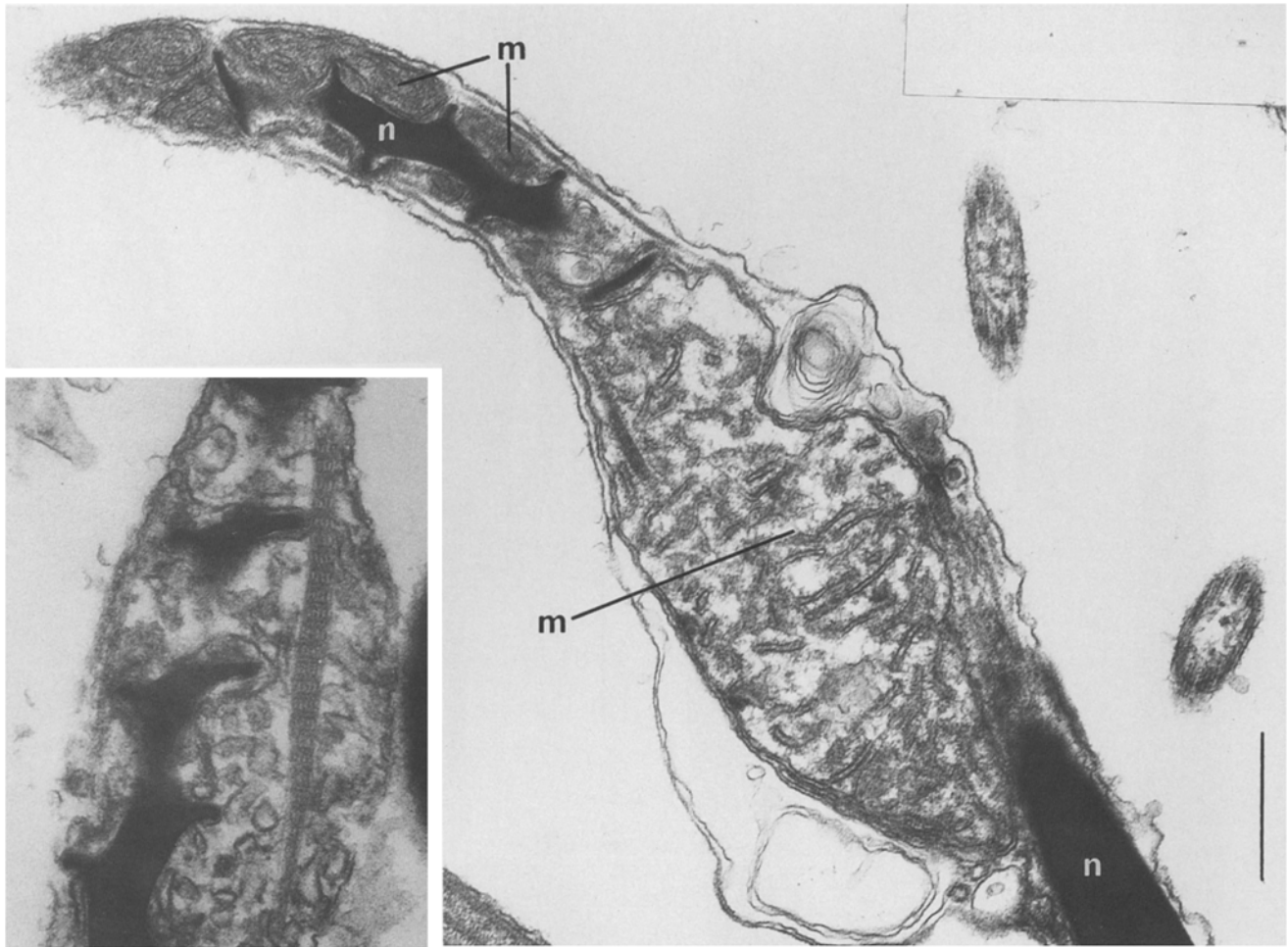


Fig. 6. *Synoicum pulmonaria*. Approximately longitudinal section of anterior part of sperm nucleus (n). A mitochondrial mass (m) is present as a lateral body at the junction between the proximal cylindrical and the distal helical part of the nucleus. From this

lateral body a mitochondrial extension proceeds anteriorly, surrounding the helical part of the nucleus. The inset shows an elongated cross-striated structure close to the mitochondrion. Scale bar = 0.5 μm

sperm are characterized by a more or less elongated nucleus and the absence of a typical midpiece. A single, ovoid or elongated mitochondrion is laterally attached to the nucleus. In several species a minute acrosomal vesicle, or acrosomal homologue, has been demonstrated at the apex of the nucleus. Generally, only a careful examination of serial sections in a transmission electron microscope can reveal these small structures.

Observations made in previous studies on sperm structure and spermiogenesis in ascidians conflict in some respects with general ideas about ascidian phylogeny and relationships. The solitary ascidians are generally regarded to be highly evolved. This conclusion is based mainly on anatomical characteristics, especially the structure of the pharynx. The present investigation shows that sperm ultrastructure and spermiogenesis in the aplousobranch ascidian *Clavelina lepadiformis* conform to the general pattern unique to the solitary ascidians. However, the *C. lepadiformis* sperm exhibits apomorphies not shared with *Ciona* sp. Most important among these are the elongated mitochondrion spiraled about the entire nucleus and the

mitochondrial cristae, which are elongated parallel to the long axis of the nucleus.

Clavelina lepadiformis is a member of the ascidian family Clavelinidae, subfamily Clavelininae, in the order Aplousobranchia. The genus *Distaplia* also belongs to the family Clavelinidae, but to the subfamily Holozoinae. In addition, the order Aplousobranchia includes the families Didemnidae and Polydinidae. Berrill (1936) considers *Ciona intestinalis* to be the most primitive species of the Ascidiacea. Kott (1969) states that in *Clavelina lepadiformis* the close relationship of the Clavelinidae with the primitive ascidian is clearly demonstrated. Hawkins et al. (1983) have performed atomic absorption and electron spin resonance analysis on 50 ascidian species and concluded that the family Cionidae belongs to the Aplousobranchia, aplousobranchs being closest to the stem ascidians.

Among the aplousobranchs, spermiogenesis has until now been studied only in the Didemnidae. Spermiogenesis in this family has been found to follow the same pattern as in solitary ascidians, but the mature spermatozoa

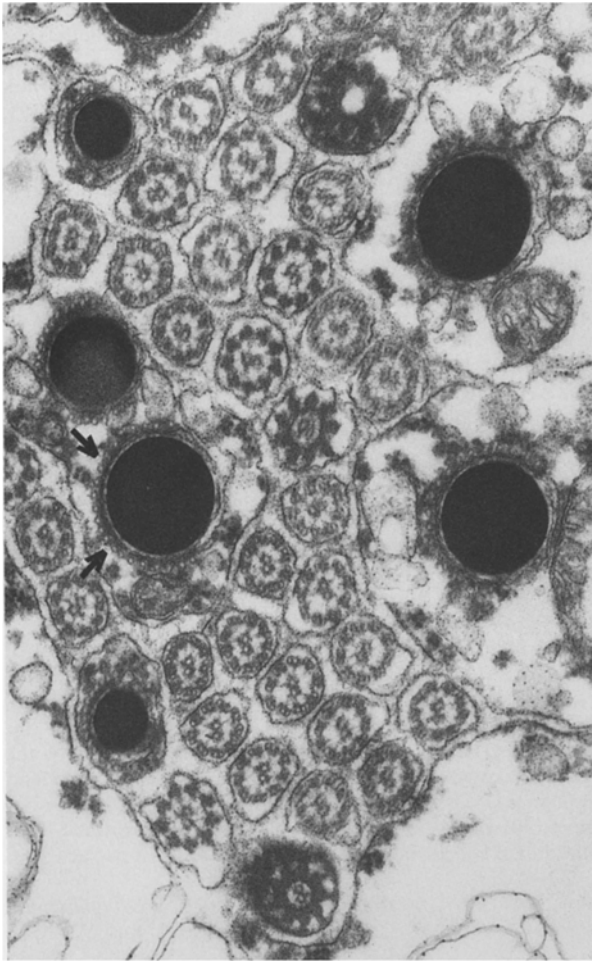


Fig. 7. *Aplidium* sp. Transverse section of late spermatids. Microtubules surround the nuclear cover (arrows). Radial spokes of an anchoring apparatus surround the centriole and the proximal part of the axoneme

have several derived characteristics. The spermatozoa of the didemnids *Lissoclinum perforatum* and *Diplosoma listerianum* have a complex groove in the plasmalemma; this groove twists once or twice around the nucleus (Burighel et al. 1985). The mitochondrion also spirals around the nucleus and extends along the entire length of the head region. The mitochondrial cristae are arranged parallel to the long axis of the mitochondrion in *L. perforatum*. In *D. listerianum* the mitochondrial cristae form a more complex pattern. No acrosomal structures could be observed in the two species studied by Burighel et al. (1985). The didemnid *Trididemnum cereum* has been studied by Tuzet et al. (1974), whose micrographs show that the mature sperm nucleus is elongated and surrounded by a helical structure along its entire length. This spiral structure makes more than 20 turns, and the authors considered it to be derived from the nucleus. Holland (1988) suggested that this structure in the *T. cereum* sperm was a mitochondrion with a shape similar to those she described in the sperm of salps. However, Holland (personal communication) later had the opportunity to ex-

amine the sperm of this species and found that the spiral structure was part of the nucleus, as Tuzet et al. (1974) had suggested; this structure is similar to that described in the present paper for *Distaplia* sp. In *T. cereum*, a rather modified mitochondrion is also twisted about the nucleus, and was evidently not fixed in the material which Tuzet and co-workers studied (Holland personal communication). Tuzet et al. (1974) also described an acrosome in the sperm of this species but did not present convincing micrographs of an acrosomal structure.

It may be concluded that in the family Didemnidae the sperm are derived in several respects: the nucleus is very elongated, and the mitochondrion is an elongated structure which exhibits a derived internal morphology and twists around the nucleus. No acrosomal vesicles have been convincingly demonstrated.

It is reasonable to suppose that sperm ultrastructure of *Clavelina lepadiformis* and in the solitary ascidian *Ciona intestinalis* is representative of the ancestral ascidian. Both within the family Clavelinidae (e.g. the genus *Distaplia*) and in other aplousobranch families, more derived sperms have also evolved. The most important finding is, however, that the primitive aplousobranch *C. lepadiformis* also shows primitive features in its sperm morphology.

It is possible, but not very likely, that the species *Clavelina lepadiformis* is unique in having retained plesiomorphic sperm resembling those of solitary ascidians. Fukumoto (1985) reported that the sperm of *Clavelina huntsmani*, *Aplidium californicum* and *Distaplia occidentalis* have apical acrosomal vesicles, although he did not present any description or micrographs. These short notes by Fukumoto thus were not entirely convincing, and nothing was mentioned about the general morphology of the sperm in *C. huntsmani* or the other aplousobranchs. Holland (1990) reported that no acrosomal vesicles were present in *C. oblonga*.

The small vesicles at the nuclear apex of ascidian sperm are not quite equivalent to the typical acrosome in plesiomorphic metazoan sperm. However, among lower metazoans, in the Cnidaria, the typical acrosome is not present but is represented by a number of electron-dense acrosomal vesicles. In the primitive scyphozoan medusa *Nausithoe* sp., Afzelius and Franzén (1971) described this type of acrosomal vesicle. The ascidian egg is enclosed in a non-cellular chorion, or egg membrane, which is surrounded by a layer of vacuolated follicle cells. Test cells are present in the perivitelline space. It seems obvious that these cellular and extracellular vestments present a barrier to the successful interaction of ascidian sperm and egg. With regard to this it is notable that the ascidian sperm possesses an acrosome which is only slightly developed morphologically. Holland (1988, 1989, 1990) has discussed the correlation between elaborate egg vestments and the reduced acrosomes in ascidians. In one study, Holland (1988) argues that the streamlined shape of ascidian sperm may have evolved in association with the egg vestments. Franzén (1983) has suggested that in some animal groups (e.g. bivalves) there is a correlation between the evolution of an elongated sperm nucleus and large, yolk-rich eggs. Elongated nuclei are found in sperm

which in all other respects are primitive or show pleiomorphous characteristics.

The theory of a general correlation between sperm morphology and fertilization biology, presented by Franzén (1956, 1970), has been supported by many sub-microscopical studies and for most animal groups. Animals shedding eggs and sperm in seawater have primitive sperm, while animals displaying internal fertilization usually have sperm with an elongated head and a modified midpiece in which the mitochondria are often enlarged and elongated. However, there are some exceptions to the rule regarding this correlation, and an important one is found among the tunicates (Franzén 1958). As Holland (1990) states, the rule is valid for appendicularians, salps, doliolids, and pyrosomes but not for ascidians. Appendicularians exhibit external fertilization, and Flood and Afzelius (1978) showed that the sperm of *Oikopleura dioica* has much in common with the primitive type. Holland et al. (1988) found that the acrosomal vesicle in *O. dioica* sperm is the only one among tunicates that undergoes an acrosomal reaction typical for invertebrates.

Among ascidians, solitary species display external fertilization, whereas fertilization in colonial species takes place in the atrial cavity or the oviduct. Sperm of the colonial species *Clavelina lepadiformis* have some important characteristics in common with those of the solitary ascidians, especially *Ciona intestinalis*. The explanation for the occurrence of a type of modified sperm in solitary ascidians and *C. lepadiformis* may be that it evolved in connection with the evolution of internal fertilization among ancestral ascidians. This sperm type was then retained by solitary ascidians, which secondarily developed external fertilization. The same, or very similar, ideas concerning sperm evolution in ascidians have also been proposed by Holland (1989, 1990).

Acknowledgements. This study was carried out at the Swedish Museum of Natural History and at Klubban Biological Station, Uppsala University. I am grateful to H. Malmikumpu for excellent technical assistance in the preparation and sectioning of material for electron microscopy, and to C. Hammar and J. Ibanez-Arellano for preparing the photographic material. I thank Dr. K. H. Woodwick, California State University, Fresno, for help and generous support during my stay in California. Dr. L. Z. Holland gave valuable suggestions on the manuscript, which I hereby thankfully acknowledge. This study was supported by a Swedish Natural Science Research Council (NFR) grant.

Literature cited

- Afzelius, B. A., Franzén, Å. (1971). The spermatozoon of the jellyfish *Nausithoe*. *J. Ultrastruct. Res.* 37: 186–199
- Berrill, N. J. (1936). Studies in tunicate development. V. Evolution and classification. *Phil. Trans. R. Soc. (Ser. B)* 226: 43–70
- Brien, P. (1948). Embranchement des tuniciers. *Traité Zool.* 11: 553–894
- Burighel, P., Martinucci, G. B., Magri, F. (1985). Unusual structures in the spermatozoa of the ascidians *Lissoclinum perforatum* and *Diplosoma listerianum* (Didemniidae). *Cell Tissue Res.* 241: 513–521
- Cloney, R. A., Abbott, L. C. (1980). The spermatozoa of ascidians: acrosome and nuclear envelope. *Cell Tissue Res.* 206: 261–270
- Cotelli, R., De Santis, R., Rosati, F., Monroy, A. (1980). Acrosome differentiation in the spermatogenesis of *Ciona intestinalis*. *Dev. Growth Differentiation* 22: 561–569
- Flood, P. R., Afzelius, B. A. (1978). The spermatozoon of *Oikopleura dioica* Fol (Larvacea, Tunicata). *Cell Tissue Res.* 191: 27–37
- Franzén, Å. (1956). On spermiogenesis, morphology of the spermatozoon and biology of fertilization among invertebrates. *Zool. Bidr. Upps.* 31: 354–482
- Franzén, Å. (1958). On sperm morphology and acrosome filament formation in some Annelida, Echiuroidea, and Tunicata. *Zool. Bidr. Uppsala* 33: 1–28
- Franzén, Å. (1970). Phylogenetic aspects of the morphology of spermatozoa and spermiogenesis. In: Baccetti, B. (ed.) *Comparative spermatology*. Academic Press, New York, p. 29–46
- Franzén, Å. (1976). The fine structure of spermatid differentiation in a tunicate, *Corella parallelogramma* (Muller). *ZOON* 4: 115–120
- Franzén, Å. (1977). Sperm structure with regard to fertilization biology and phylogenetics. *Verh. dt. zool. Ges.* 1977: 123–138
- Franzén, Å. (1983). Ultrastructural studies of spermatozoa in three bivalve species with notes on evolution of elongated sperm nucleus in primitive spermatozoa. *Gamete Res.* 7: 199–214
- Fukumoto, M. (1979). Tube-like structures in mitochondria of tunicate (*Pyura vittata*) spermatids. *J. Ultrastruct. Res.* 68: 1–5
- Fukumoto, M. (1981). The spermatozoa and spermiogenesis of *Perophora formosana* (Ascidia) with special reference to the striated apical structure and the filamentous structures in the mitochondrion. *J. Ultrastruct. Res.* 77: 37–53
- Fukumoto, M. (1983). Fine structure and differentiation of the acrosome-like structure in solitary ascidians, *Pyura haustor* and *Styela plicata*. *Dev. Growth Differentiation* 25: 503–515
- Fukumoto, M. (1984). Fertilization in ascidians: acrosome fragmentation in *Ciona intestinalis* spermatozoa. *J. Ultrastruct. Res.* 87: 252–262
- Fukumoto, M. (1985). Acrosome differentiation in *Molgula manhattensis* (Ascidacea, Tunicata). *J. Ultrastruct. Res.* 92: 158–166
- Fukumoto, M. (1986). The acrosome in ascidians I. Pleurogona. *Int. J. Invert. Reprod. Dev.* 10: 335–346
- Fukumoto, M. (1990). Morphological aspects of ascidian fertilization. *Zool. Sci.* 7: 989–998
- Garstang, W. (1928). The morphology of the Tunicata, and its bearing on the phylogeny of the Chordata. *Q. Jl. microsc. Sci.* 72: 51–187
- Hawkins, C. L., Kott, P., Parry, D. L., Swinehart, J. H. (1983). Vanadium content and oxidation state related to ascidian phylogeny. *Comp. Biochem. Physiol.* 76 B: 555–558
- Holland, L. Z. (1988). Spermatogenesis in the salps *Thalia democratica* and *Cyclosalpa affinis* (Tunicata: Thaliacea): an electron microscope study. *J. Morph.* 198: 189–204
- Holland, L. Z. (1989). Fine structure of spermatids and sperm of *Doliolletta gegenbauri* and *Doliolum nationalis* (Tunicata: Thaliacea): implications for tunicate phylogeny. *Mar. Biol.* 101: 83–95
- Holland, L. Z. (1990). Spermatogenesis in *Pyrosoma atlanticum* (Tunicata: Thaliacea: Pyrosomatida): implications for tunicate phylogeny. *Mar. Biol.* 105: 451–470
- Holland, L. Z. (in press). The phylogenetic significance of tunicate sperm morphology. In: Baccetti, B. (ed.) *Sixth International Congress of Spermatology*. Siena, Italy
- Holland, L. Z., Gorsky, G., Fenaux, R. (1988). Fertilization in *Oikopleura dioica* (Tunicata, Appendicularia): acrosome reaction, cortical reaction and sperm-egg fusion. *Zoomorphology* 108: 229–243
- Kott, P. (1969). Antarctic Ascidiacea. *Antarctic Res. Ser.* 13: 1–239
- Lambert, C. C., Epel, D. (1979). Calcium-mediated mitochondrial movement in ascidian sperm during fertilization. *Devl Biol.* 69: 296–304
- Schabtach, E., Ursprung, H. (1965). The fine structure of the sperm of a tunicate, *Ascidia nigra*. *J. exp. Zool.* 159: 357–366

- Tuzet, O., Bogaraze, D., Lafargue, F. (1972). Recherches ultrastructurales sur la spermiogenèse de *Diplosoma listerianum* (Milne-Edwards, 1841) et *Lissoclinum pseudoleptoclinum* (Von Drasche, 1883) ascidies, composées, aplousobranches. *Annls Sci. nat. (sér. Zool.)* (12th series) 14: 177–190
- Tuzet, O., Bogaraze, D., Lafargue, F. (1974). La spermatogenèse de *Polysyncratum lacazei*, 1872 et *Trididemnum cereum* Giard 1872 (Ascidies composées, Aplousobranches). *Bull. biol. Fr. Belg.* 108: 151–167
- Villa, L. (1981). An electron microscope study of spermiogenesis and spermatozoa of *Molgula impura* and *Styela plicata* (Ascidiacea, Tunicata). *Acta Embryol. Morph. exp. (N.S.)* 2: 69–85
- Wirth, U. (1984). Die Struktur der Metazoen-Spermien und ihre Bedeutung für die Phylogenetik. *Verh. naturw. Ver. Hamb.* 27: 295–362
- Woollacott, R. M. (1977). Spermatozoa of *Ciona intestinalis* and analysis of ascidian fertilization. *J. Morph.* 152: 77–88
- Young, J. Z. (1950). *The life of vertebrates*. Clarendon Press, Oxford