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ORIGINAL PAPER

Spermatogenesis and the structure of the testes in Nemertodermatida

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Abstract The ultrastructure of the testes in two representatives of the enigmatic taxon Nemertodermatida was studied using transmission electron microscopy. *Nemertoderma westbladi* has paired testes, which are delineated by lining cells. Within each testis, different follicles, each surrounded by a membrane-like structure, are found. *Flagellophora apelti* has genuinely follicular testes, consisting of several follicles, each containing a certain stage of spermatogenesis. As the gametes are not enclosed by a structure that can be called a true gonad, the structure of the testes differs from most bilaterian animals, but resembles the organization of gametogenic areas of ctenophores. Each stage of spermatogenesis in *F. apelti* is described, enabling the inference of the origin of the structures seen in mature spermatozoa. The overall structure of the mature spermatozoa is similar in all nemertodermatids and unique within the Metazoa: an elongated head containing the nucleus; a middle piece containing an axoneme, mitochondrial derivatives and in *F. apelti* granular derivatives; and a flagellar tail.

Keywords Nemertodermatida · Acoelomorpha · Spermatogenesis · Ultrastructure · Germline · Bilateria

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Introduction

Nemertodermatida is a small taxon of marine organisms whose most striking feature is a statocyst containing (mostly) two statoliths. Members lack protonephridia, and their well-defined, sac-like gut has only a single opening—the mouth.

The most recent study on the phylogenetic position of Nemertodermatida, based on three independent datasets, places Nemertodermatida together with Acoela in a taxon Acoelomorpha, which shows a sister group relationship with *Xenoturbella*; the clade of Acoelomorpha + *Xenoturbella* is found within Deuterostomia as the sister group of the Ambulacraria (Philippe et al. 2011). However, the phylogenetic position of Nemertodermatida is far from settled. Originally considered to be part of the Acoela (Steinböck 1930), it was later seen as a separate taxon, Nemertodermatida, sister group of Acoela, with which it formed the taxon Acoelomorpha within the Plathelminthes (Ehlers 1985). Studies based on 18S small subunit rRNA and mitochondrial genes (Jondelius et al. 2002), on the nuclear protein coding myosin heavy chain type II (Ruiz-Trillo et al. 2002), on 18S rRNA and 28S rRNA genes (Wallberg et al. 2007) and on a combination of several molecular markers (Baguñà et al. 2008) all show a paraphyletic Acoelomorpha, with Nemertodermatida and Acoela appearing as separate branches at the base of the Bilateria. In a recent study, however, based on phylogenomic methods, Acoelomorpha is monophyletic and is the sister taxon of the Nephrozoa (all remaining Bilateria) (Hejnol et al. 2009; Edgecombe et al. 2011). Hejnol et al. (2009) also suggested a sister group relationship between Acoelomorpha and the enigmatic taxon *Xenoturbella*. Complete mitochondrial genome data of an acoel also support the view that the Acoelomorpha are the early

diverging extant lineage of Bilateria (Mwinyi et al. 2010). Although the exact phylogenetic position of Nemertodermatida is still debated, it is clear that they are one of the key taxa to understanding early bilaterian evolution and, more specifically, the evolution of early bilaterian morphology.

Studies on different aspects of the morphology of Nemertodermatida have already been conducted: on the parenchyma (Rieger et al. 1991), the nervous system (Raikova et al. 2000, 2004; Reuter et al. 2001), the mature sperm (Tyler and Rieger 1975, 1977; Hendelberg 1977; Lundin and Hendelberg 1998), the epidermis (Tyler and Rieger 1977; Lundin and Hendelberg 1995, 1996; Lundin 1997, 1998, 2001), the proboscis (Rieger et al. 1991) and the gut (Tyler and Rieger 1977). A taxonomic revision of the Nemertodermatida was made by Sterrer (1998). In order to resolve internal relationships within the Nemertodermatida, Lundin (2000) conducted a cladistical analysis using 72 morphological characters. A slightly altered version of this cladistical analysis was published by Lundin and Sterrer (2001). Although the adult body plan of Nemertodermatida is already relatively well documented, many aspects remain unknown, such as the ultrastructure of the testes and spermatogenesis. Lundin and Hendelberg (1998) described the spermiogenesis in *Meara stichopi* Westblad 1949 (Nemertodermatidae), but not the early stages, while Rieger et al. (1991) illustrated the position of the male and female germinative zone in *Flagellophora apelti* Faubel and Dörjes 1978.

Here, we give ultrastructural details on the morphology of the testis in *F. apelti* and in *Nemertoderma westbladi* Westblad 1937, two representatives of different nemertodermatidan subgroups. We describe the mature spermatozoon of both species in detail. For *F. apelti*, moreover, we describe the complete spermatogenesis. Our material of *N. westbladi* did not allow such a study for this species. The results of this study will allow a comparison with spermiogenesis and testis morphology in other taxa.

Materials and methods

Specimens of *F. apelti* were extracted from sand samples collected with a Van Veen grab on the Belgian Continental Shelf at depths of 12–23 m in 2007 and 2008. Specimens of *N. westbladi* were extracted from mud samples collected around Essvik, Kristineberg, Sweden in 2008. The collected specimens of both species were fixed in a mixture of glutaraldehyde fixative and osmium fixative (Eisenman and Alfert 1982) for approximately 10 min at 4°C. The glutaraldehyde fixative was 4% glutaraldehyde in buffer a (100 mL: 0.2 M sodium cacodylate with 0.58 g NaCl and 11.97 g sucrose; pH 7.2), and the osmium fixative was 1% osmium tetroxide in buffer b (100 mL: 0.2 M sodium cacodylate with 3.48 g NaCl; pH 7.2). After fixation with the cocktail, the specimens were fixed in glutaraldehyde fixative for 1 h at 4°C and then post-fixed with osmium fixative for 1 h at 4°C. After rinsing in buffer b for 10 min at 4°C and rinsing for 5 min in double distilled water, they were dehydrated, using ethanol of increasing concentrations. After dehydration in an ethanol series, specimens of both species were subsequently infiltrated with a low-viscosity embedding medium (Spurr 1969) then polymerized at 70°C for 8 h. Semi-thin sections of 1 and 2 µm and ultra-thin sections of 60 nm were made on a Reichert-Jung Ultracut E or a Leica-Reichert Ultracut S (Leica, Vienna, Austria) equipped with a diamond knife. Specimens were sectioned semi-thin until the region of interest was reached, after which ultra-thin sections were made. Ultra-thin sections were post-stained with uranyl acetate (40 min at 20°C) and lead citrate (10 min at 20°C) and studied with a Jeol JEM-1010 transmission electron microscope (Jeol Ltd., Tokyo, Japan) operating at 60 kV. Photomicrographs were digitized using a Ditabis system (Pforzheim, Germany). Schematic figures were drawn from photomicrographs using Adobe Illustrator CS software.

Results

Structure of the testes

The single testis in *F. apelti* (Fig. 1a) is completely separated from the ovary. The testis is follicular: it comprises primary follicles, in which cells are densely packed. Although the cells in a follicle are lying closely together, no intercellular bridges could be observed. The different follicles are not grouped within a saccular structure but lie scattered in the posterior part of the body, spatially separated from each other. They are not surrounded by specialized cells, but lie adjacent to gut cells, stromal cells, and parenchymal cells. Within each follicle, only a single stage of spermatogenesis can be found. Different testicular follicles contain successive stages of spermatogenesis, those containing early stages (spermatogonia and spermatocytes) being found more anteriorly than those containing later stages (spermatids and mature spermatozoa).

The testes in *N. westbladi* (Fig. 1b) are paired, and situated dorso-laterally from the ovaries, partly enfolding them. The testes are clearly delimited by flattened lining cells which have a large nucleus with a prominent nucleolus; the cytoplasm of these lining cells is packed with networks of rough endoplasmatic reticulum. Within each testis, separate follicles can be found, in which cells of the same stages or spermatogenesis are lying closely together. Each follicle is surrounded by a membrane-like

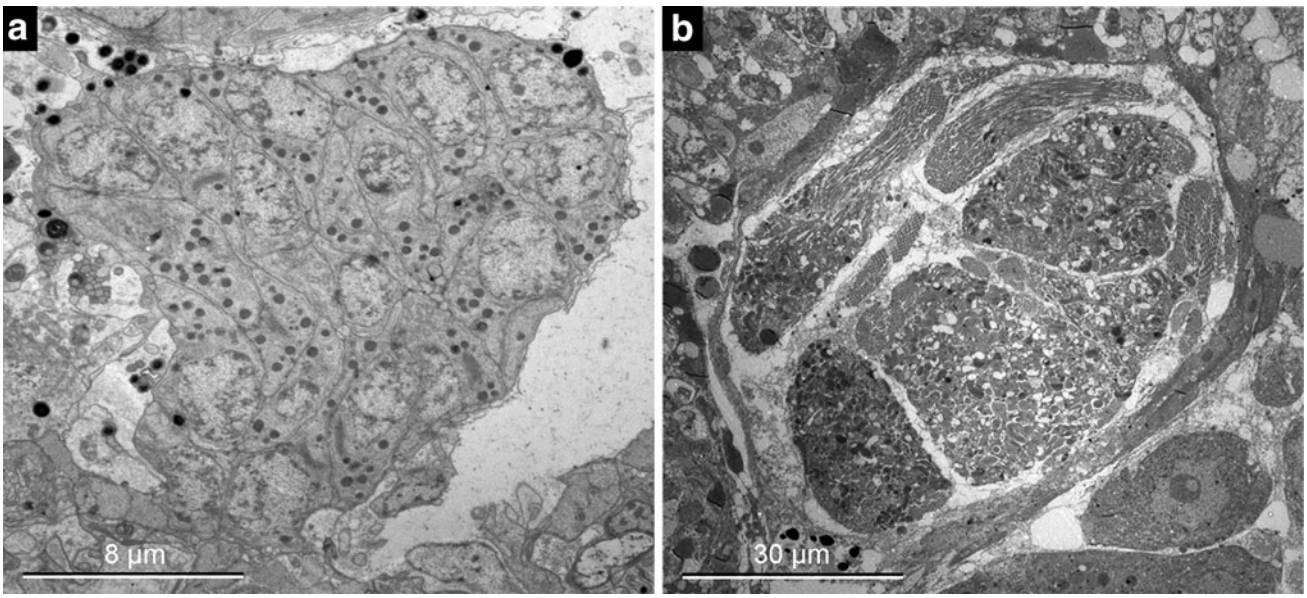

Fig. 1 Structure of the testes in Nemertodermatida. **a** TEM image of part of the testis in *Flagellophora apelti*: a testis follicle containing secondary spermatocytes; **b** TEM image of one of the two testes in *Nemertoderma westbladi*, the testis contains several delineated follicles

structure; which is presumably derived from the testes lining cells. Different stages of spermatogenesis can be found in neighboring follicles.

Spermatogenesis in *Flagellophora apelti*

The spermatogonium (Fig. 2a)

Typical of these cells is the high nucleo-cytoplasmic ratio. The nucleus has a nucleolus and small clumps of heterochromatin. The cytoplasm contains ribosomes, a Golgi complex, mitochondria and a pair of centrioles. The centrioles are arranged perpendicularly, forming a diplosome, which is located in proximity to the nucleus.

The primary spermatocyte (Fig. 2b)

Primary spermatocytes have a relatively undifferentiated nucleus and cytoplasm. The nucleus has a round to ovoid shape and takes up a large part of the cell. It contains few clumps of heterochromatin and synaptonemal complexes, the latter indicating that the cells are in prophase I (Fig. 2b, *arrowhead*). The cytoplasm contains mitochondria, ribosomes, a Golgi complex, some swollen endoplasmatic reticulum and a pair of centrioles close to the nucleus. There are approximately 16–20 primary spermatocytes in a follicle.

The secondary spermatocyte (Fig. 2c, d)

Secondary spermatocytes have nuclei that still have a round-ovoid shape but contain more heterochromatin than nuclei of primary spermatocytes. The heterochromatin is more condensed than in earlier stages. As in the primary spermatocytes, several small mitochondria, ribosomes, swollen endoplasmatic reticulum, a Golgi complex, and a perpendicularly organized pair of centrioles (Fig. 2c) can be found. The large Golgi complex and the pair of centrioles are located in proximity to the nucleus. In the cytoplasm of the secondary spermatocyte, there are also vesicles containing large, electron-dense granules. In this stage, each vesicle contains one electron-dense granule; in some of the granules, more electron-dense structures can be found. A follicle of secondary spermatocytes contains usually between 32 and 40 cells.

The spermatid (Fig. 3)

Elongation of the cell starts after the second meiotic division and its round-ovoid shape is becoming more elongated during the spermatid stage. The round nucleus, containing scattered clumps of heterochromatin in the early stages (Fig. 3a), changes shape to an electron-dense structure (Fig. 3b, c), which will elongate. Various organelles can be found in the cytoplasm: mitochondria, a large Golgi complex, ribosomes and swollen endoplasmatic reticulum. The Golgi complex is found in the vicinity of the nucleus. The electron-dense granules are more grouped in this early spermatid stage; the multiple vesicles each containing a single granule fuse so that in the late spermatid, only one clearly delimited vesicle can be found, containing multiple granules (Fig. 3a, c, black arrowhead). The mitochondrion starts to elongate: the

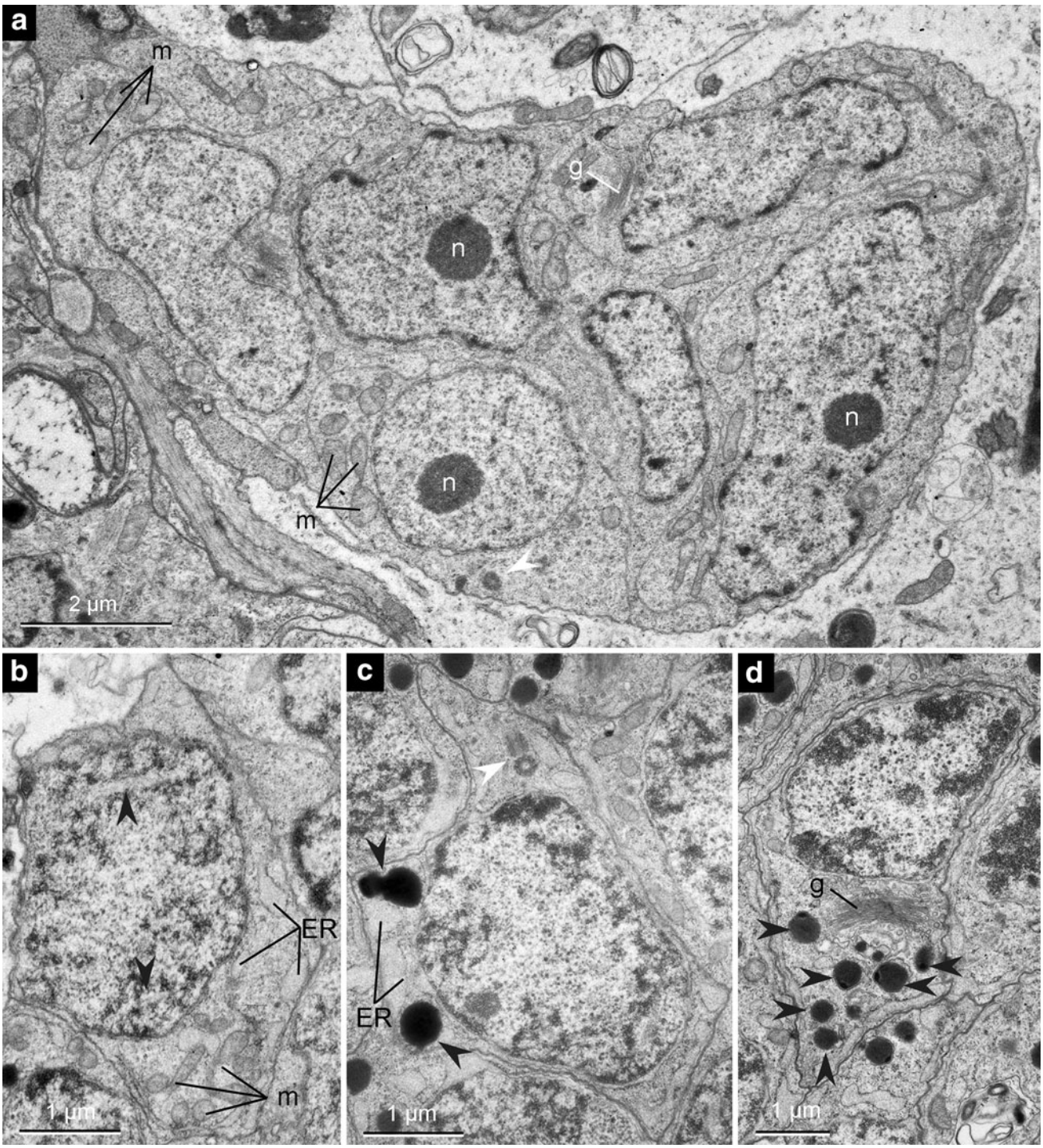

Fig. 2 Early stages of spermatogenesis in *F. apelti*. **a** Spermatogonia. A nucleolus (*n*) can be seen in the nucleus. The cytoplasm contains ribosomes, Golgi complexes (*g*), a pair of centrioles (*white arrowhead*) and mitochondria (*m*). **b** Primary spermatocyte. The large nucleus contains scattered clumps of heterochromatin and synaptonemal complexes (*arrowhead*). The cytoplasm contains Golgi complexes, mitochondria (*m*), ribosomes and swollen endoplasmatic reticulum (*ER*). **c**, **d** Secondary spermatocytes. The large nucleus contains scattered clumps of heterochromatin. Centrioles (*white arrowhead*), electron-dense granules (*black arrowheads*), a Golgi complex (*g*), mitochondria, ribosomes, and swollen endoplasmatic reticulum (*ER*) are all found in the cytoplasm

shape changes from ovoid to more elongated (Fig. 3a, d, white arrowhead). In most cases, a single axoneme grows outwards within the elongating spermatid from a centriolar region that remains near the nucleus (Fig. 3b). Aberrant spermatids with two axonemes were observed, but we never found more than one aberrant spermatid

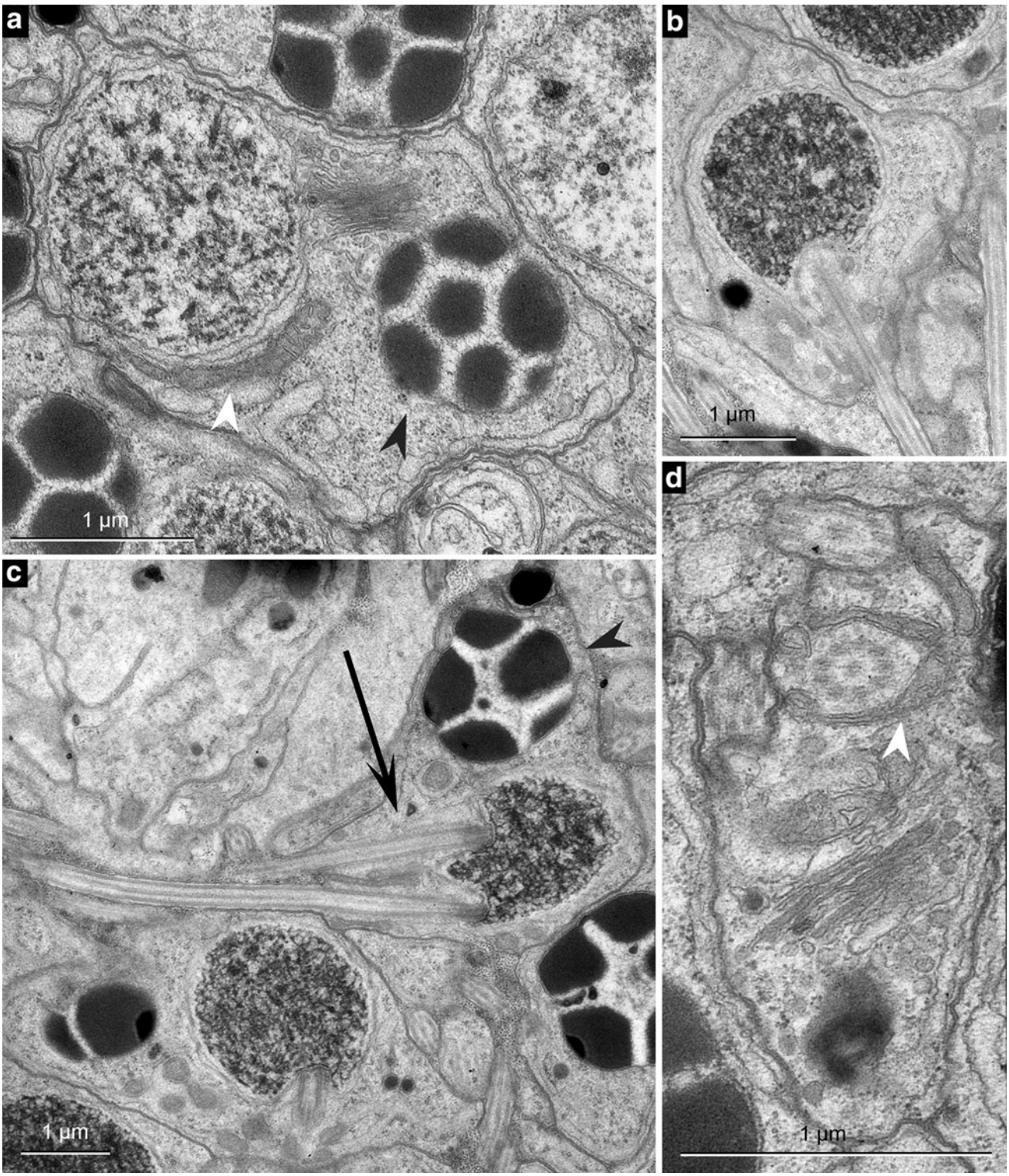

Fig. 3 Spermatids in *F. apelti*. **a** Early spermatid. The nucleus contains scattered clumps of heterochromatin, the mitochondria (*white arrowhead*) are more elongated, the electron-dense granules are grouped (*black arrowhead*) and the endoplasmatic reticulum is swollen. The Golgi complex near the nucleus should be noted. **b** The nucleus in a later spermatid stage is more electron-dense and a single axoneme grows outwards from a centriolar region that remains near the nucleus. **c** Aberrant spermatid with two axonemes (*arrow*). The grouped electron-dense granules (*black arrowhead*) should be noted. **d** Section of a spermatid where the elongated shape of the mitochondrion is visible (*white arrowhead*)

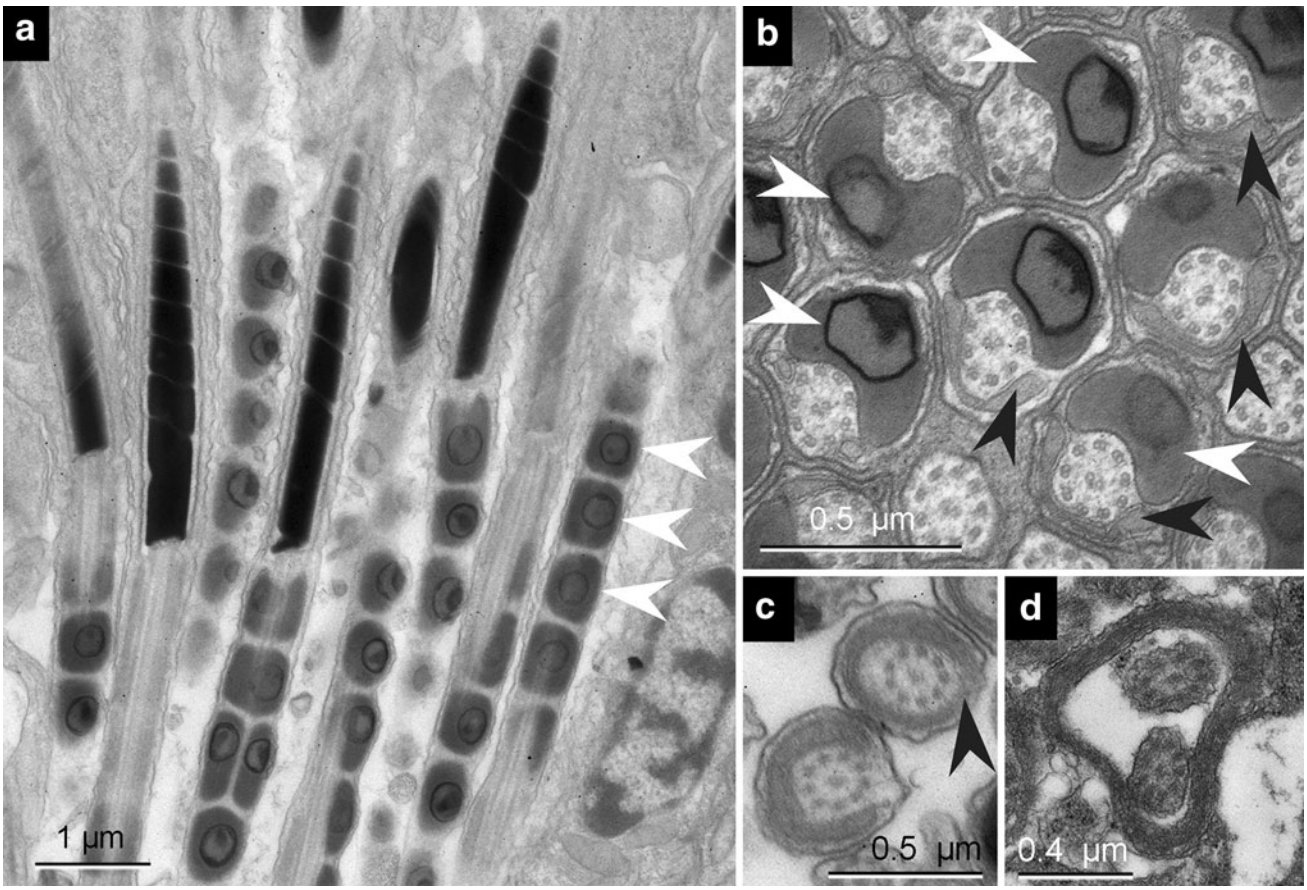

Fig. 4 Mature spermatozoa in the testis of *F. apelti* (**a**–**c**) and *N. westbladi* (**d**). **a** Longitudinal sections of mature sperm in *F. apelti*. The nucleus with a single helical groove is localized in the head. The middle piece contains an axial filament, mitochondrial derivatives and granular derivatives (*white arrowheads*). **b** Transverse sections of the mature sperm cell of *F. apelti,* including mitochondrial derivatives (*black arrowheads*) and granular derivatives (*white arrowheads*). **c** The mitochondrial derivatives at the posterior end of the middle piece of the sperm cell in *F. apelti* have a tubular shape (*black arrowhead*). **d** Middle piece of an aberrant spermatozoon in *N. westbladi*. Two axial filaments are surrounded by one mitochondrial derivative

(Fig. 3c) per follicle. Follicles of spermatids contain approximately 64–80 cells.

The mature spermatozoon (Figs. 4a, b, c and 5a)

The mature spermatozoon in *F. apelti* is divided into three regions: a head, a middle piece and a tail.

The head is tipped with an acrosome (Fig. 5a) and contains the electron-dense nucleus, which shows a single helical groove along its entire length. The nucleus is approximately 6 µm long and has a diameter of 0.3 µm. The helical groove has a depth of 0.12 µm; there are 4 revolutions per µm.

The middle piece measures approximately 5.5 µm and contains the basal body where the axoneme is attached, the axoneme itself, mitochondrial derivatives (Fig. 4b, c, *black arrowheads*), and 8–10 electron-dense granular derivates (Fig. 4a, b, *white arrowheads*). Peripheral fibers are found on the outside of the microtubule doublets in the basal region of the axoneme. The granular derivatives are 0.4–0.6 µm long and 0.35–0.4 µm wide and partly surround the axoneme. They contain an ovoid substructure, which is more electron-dense. In cross sections, the granular derivatives are bean-shaped and cover about half of the section; the other half is covered by the axoneme and the mitochondrial derivative. The mitochondrial derivatives are elongated and flattened. They occur at the same level as the granular derivatives, but extend more at the distal end.

The single axoneme has a $9 \times 2 + 2$ pattern, and its free part extends as a flagellar tail. Toward the distal tip, the $9 \times 2 + 2$ structure disintegrates.

Mature spermatozoon in N. westbladi (Figs. 4d and 5b)

Figure 5b gives a schematic overview of the mature sperm in *N. westbladi.* Normal spermatozoa in *N. westbladi* have an elongated, electron-dense nucleus, a middle piece containing an axial filament surrounded by a tubular

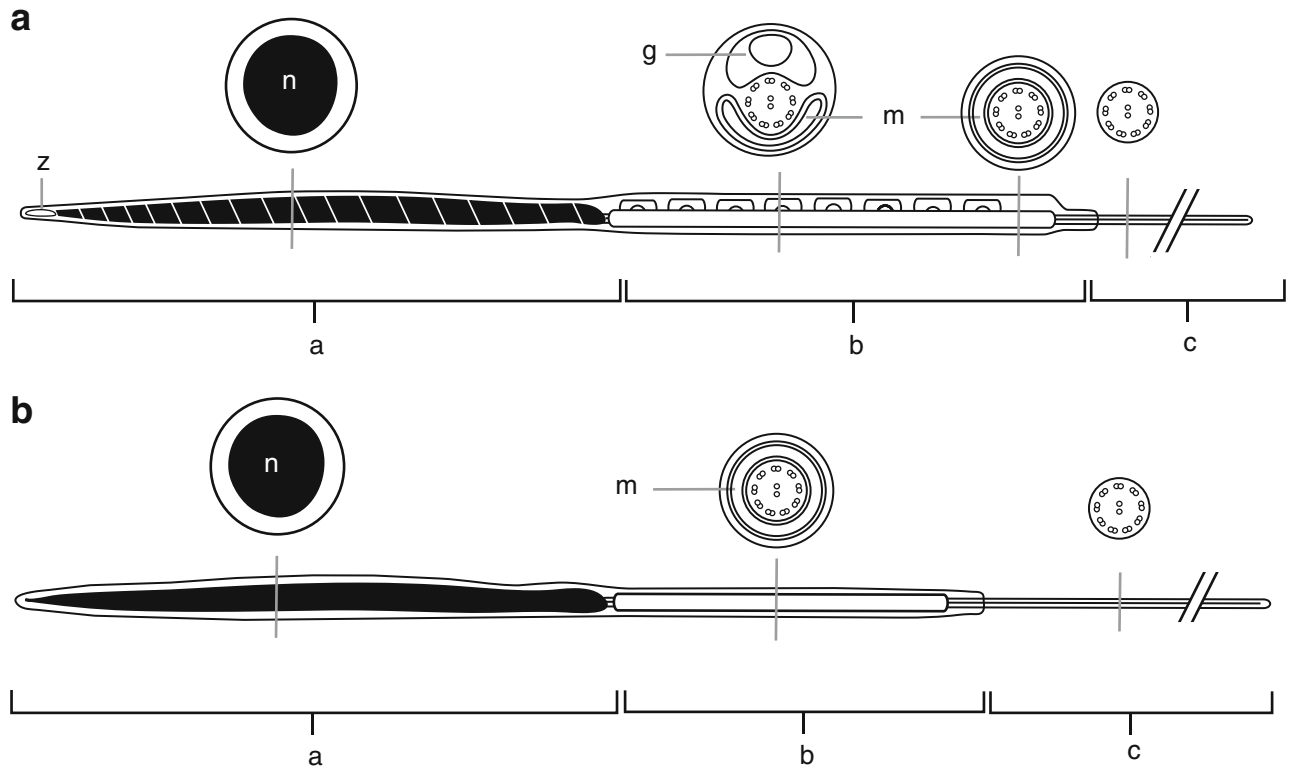

Fig. 5 Schematic representation of the mature sperm in *Flagellophora apelti* (**a**) and *Nemertoderma westbladi* (**b**). *a* = head, *b* = middle piece, *c* = tail, *g* = granular derivative, *m* = mitochondrial derivative, *n* = nucleus, *z* = acrosome

mitochondrial derivative and a tail (free axial filament). We occasionally found aberrant spermatozoa with two axonemes instead of one in their middle piece and tail, surrounded by a single mitochondrial derivative (Fig. 4d).

Discussion

Structure of the testes

Gonads can be defined in different ways. According to the definition of Schmidt-Rhaesa (2007), gonads are organs bordered by epithelia and surrounding the gametes. This definition excludes cases in which gametes are present between epithelia or between parenchymal cells (Schmidt-Rhaesa 2007).

As is the case for most other Bilateria, Nemertodermatida are generally known to have lining cells around the gonads. Tyler and Rieger (1977) found a clear epithelium delimiting the gonads in *N. westbladi,* which they considered an advanced character. In the cladistical analysis by Lundin and Sterrer (2001), all studied species are considered to have testes lined by cells (for *Ascoparia neglecta* Sterrer 1998 and *Ascoparia secunda* Sterrer 1998 no data were available). The testes in *M. stichopi* consist of individual follicles surrounded by flattened follicle wall cells (Lundin and Hendelberg 1998). It is possible that these flattened follicle wall cells have microvilli-like protrusions, which have a nutritive function (Lundin and Hendelberg 1998). The follicles in *M. stichopi* are found in two rows in the anterior part of the body.

However, in this study, we observed clear differences in the lining of the testes between the species studied. In *N. westbladi*, the testes are paired and clearly delimited by cells. Within each testis, the follicles are surrounded by presumable remnants of the testis lining cells. The origin of the lining cells of the testis in unknown, and therefore, it is not possible to state if this species has true gonads in the sense of Schmidt-Rhaesa (2007). *F. apelti* has an unpaired, follicular testis in the posterior part of the body; each follicle is densely packed and encompassed by surrounding gut and stromal cells, but no lining cells unite the individual follicles. Consequently, *F. apelti* lacks true testes. Nevertheless, for clarity's sake, we used the term "testes" in this paper.

In animals at a more basal phylogenetic position, such as cnidarians and ctenophores, gonads are not separate organs, and some authors prefer to call these concentrations of gametes "gametogenic areas" (Schmidt-Rhaesa 2007). The germ cells in cnidarians are generally found in interstitial

positions of the body tissue which, without the germ cells or before they arise, exhibit no reproductive specialization (Campbell 1974). Ctenophora also lack true lining cells around their testes. The testicular compartments in this taxon are delineated by processes of surrounding cells, and each of the compartments contains cells differentiating synchronously (Pianka 1974; Hernandez-Nicaise 1991). In these aspects, the testes structure of Ctenophora is comparable to what we observed in *F. apelti*. But while the testicular follicles in Ctenophora are lying concentrated in the vicinity of the meridional canal, the follicles in *F. apelti* are lying scattered in the posterior part of the body.

A "true" gonad is known for most Bilateria, except some species of Catenulida (Platyhelminthes), Acoela and *Xenoturbella* (Schmidt-Rhaesa 2007). No delimited gonads or organs associated with germ cell development have been found so far in *Xenoturbella bocki* Westblad 1949. Instead, spermatid clusters occur in the parenchymal or gastrodermal tissues (Obst et al. 2011).

Detailed studies of the ultrastructure of the testes and the organization of the different stages of spermatogenesis within the testes of species of Acoela are scarce. The male gonad of *Isodiametra pulchra* Smith and Bush (1991) was studied in detail, and this species has paired, compact, non-follicular testes where the early stages of spermatogenesis occur on the outer, dorsal periphery and the later stages on the inner, ventral side (Boone et al. 2011). No clear lining cells surround the testes. The structure of the testes in species of Acoela varies greatly: testes can be saccular or asaccular, mixed with the ovary or separate, follicular or non-follicular, compact or diffuse (Rieger et al. 1991). Further ultrastructural research on Acoela is needed to determine whether there are similarities in the structure of the gonads in Acoela and Nemertodermatida.

Spermatogenesis

The complete process of spermatogenesis in a species of Nemertodermatida has never been described. Lundin and Hendelberg (1998) did give a detailed description of spermiogenesis in *M. stichopi*; however, they did not cover the early stages of spermatogenesis nor mention the positions of developmental stages within the testes.

In both *M. stichopi* and *F. apelti*, the nucleus transforms from a large, heterogeneously electron-dense appearance, to an elongated and homogeneously electron-dense shape. In *M. stichopi*, two mitochondria start to elongate and coil around the axoneme during the spermatid stage. In *F. apelti*, we observed an elongation but no coiling. Lundin and Hendelberg (1998) noted the presence of vacuoles filled with granules or tiny vesicles of medium electron density in spermatids of *M. stichopi*, which disappear during spermiogenesis. In *F. apelti*, electron-dense granules appear during the secondary spermatocyte stage, and these granules group during the spermatid stage. In the mature spermatozoon, they are found as granular derivatives.

While spermatogonia and primary spermatocytes are similar in acoelans (Boone et al. 2011) and nemertodermatids, the first differences appear in secondary spermatocytes, with the appearance of species-specific granules in the cytoplasm. Spermatids also differ between the two taxa, as the major morphological changes toward the mature spermatozoon take place. In acoelans, accessory microtubules appear, the nucleus starts to elongate, different granules and ovoid-shaped mitochondria can be found (Raikova and Justine 1994, 1999; Raikova et al. 1997; Boone et al. 2011). The two flagella start their incorporation; their orientation is inverted. So the overall morphology and morphogenesis of spermatids in Acoela is very different from those of *F. apelti*. In *Xenoturbella bocki*, no studies of spermatogenesis have yet been conducted.

Mature spermatozoon

Tyler and Rieger (1975, 1977) studied the sperm of *Nemertoderma* sp. A, which was later identified as *F. apelti* (Sterrer 1998). They described the general structure of the sperm cell: an elongated nucleus in the head, a middle piece containing an axial filament and mitochondria, and a flagellum with $9 \times 2 + 2$ arrangement of the microtubules extending as a tail. Tyler and Rieger (1975) described six to eight crescent-shaped bodies with vesicular structures inside the middle piece as "presumably mitochondrial derivatives". However, our study shows that the mitochondrial derivatives in the middle piece are not the crescent-shaped bodies, but thin and elongated structures in which the inner and outer membranes as well as the remnants of the cristae can be discerned. We observed the initiation of the elongation process of the mitochondria during the spermatid stage and found no indication that the mitochondria give rise to the crescent-shaped bodies that Tyler and Rieger (1975) call "mitochondrial derivatives". The structures that Tyler and Rieger (1975) presume to be mitochondrial derivatives are in fact granular derivatives. In the spermatid stages, several granules can be found within one lined vesicle and in some of these granules more electron-dense structures can be found. This is also the case in the crescent-shaped bodies in the mature spermatozoa, so most likely those structures are derived from the granule-filled vacuoles in the spermatid. We found eight to ten granular derivatives, while Tyler and Rieger (1975) counted six to eight; the dimensions of the mature spermatozoa also differ slightly. Sterrer (1998) studied sperm of *F. apelti* using light microscopy, and he detected that the number in the segments of the middle piece (granular derivatives) and the proportions of the mature sperm cells

varied depending on the sampling site where the specimens were found; we join this view in order to clarify the differences between our measurement and those of Tyler and Rieger (1975).

The mature sperm of several species of Nemertodermatida has been studied ultrastructurally: *M. stichopi* (Hendelberg 1977; Lundin and Hendelberg 1998), *Nemertoderma bathycola* (Hendelberg 1977), *N. westbladi* (Hendelberg 1977) and *F. apelti* (Tyler and Rieger 1975, 1977, this study). The general structure of the sperm cells is similar for all the species studied: nemertodermatids have filiform sperm with an elongated head containing the nucleus, a middle piece containing an axoneme and mitochondria, and a tail made up of a flagellum with $9 \times 2 + 2$ arrangement of the microtubules. However, the detailed morphology of each sperm part appears to be species-specific. The head in *F. apelti* contains an acrosome, while no acrosome was found in *M. stichopi* (Lundin and Hendelberg 1998) or *N. westbladi* (Tyler and Rieger 1977). In *N. bathycola,* the presence of an acrosome is unclear (Hendelberg 1977). The electron-dense and elongated nucleus has a spiral groove in *F. apelti* and a tapering corkshrew-like proximal end in *M. stichopi.* The middle piece contains an axoneme and mitochondria in all the studied species. In *M. stichopi*, the mitochondrial derivatives are elongated tubes, which are spirally coiled around the axial filament (Lundin and Hendelberg 1998). In *N. westbladi* and *N. bathycola*, the mitochondrial derivatives are tubes that surround the axial filament (Hendelberg 1977; Tyler and Rieger 1977). In *F. apelti*, the mitochondrial derivatives are flattened and elongated. We note that at the distal end of the middle piece in *F. apelti* (Fig. 4c), the mitochondrial derivative also has a similar shape to that of *N. westbladi* and *N. bathycola*: a tube surrounding the axial filament. In all species, the $9 \times 2 + 2$ type axoneme extends as a flagellar tail. In both *F. apelti* and *M. stichopi*, the nucleus and mitochondria start to elongate during the spermatid stage. Granular vesicles can be found in the spermatids of both species, as well as in the mature spermatozoon of *F. apelti*, but not in the mature spermatozoon of *M. stichopi*.

We found some aberrant spermatids (Fig. 3c) in *F. apelti* and aberrant spermatozoa in *N. westbladi* (Fig. 4d) that had two axonemes. Two axonemes were also found in some spermatids of *M. stichopi* (Lundin and Hendelberg 1998) and some mature spermatozoa of *F. apelti* (Tyler and Rieger 1977). Lundin and Hendelberg (1998) attribute the occurrence of biflagellar spermatids and spermatozoa to folding of the sections. As the spermatid with two axonemes in our Fig. 3c is sectioned longitudinally, we consider this highly unlikely. Tyler and Rieger (1977) ascribed phylogenetic importance to the occurrence of biflagellate sperm: they remark the resemblance of the aberrant nemertodermatid sperm to acoel sperm with $9 + 2$ axonemes, indicating a tie between Acoela and Nemertodermatida (Tyler and Rieger 1977). We assume that aberrant biflagellar sperm are the consequence of failure in the transfer of centrioles during the division of the secondary spermatocyte.

The sperm of acoelan species is considerably different from that of nemertodermatid species (see also Hendelberg 1977). Acoelan species have filiform spermatozoa with an elongated nucleus, but apart from that, their morphology is quite different. Acoel sperm always have two flagella, which are inverted and incorporated into the sperm cell (Hendelberg 1977), while nemertodermatid sperm only have one flagellum, which is neither incorporated nor inverted. Furthermore, the cytoplasmic region of sperm of acoelan species contains accessory microtubules and the mitochondria are not elongated or coiled, but keep their ovoid shape.

The mature sperm of *Xenoturbella* is round-headed, uniflagellate, and lacks a middle piece; it resembles the assumed bilaterian primary condition. However, as *Xenoturbella* is probably free-spawning (Obst et al. 2011), its sperm morphology is probably related to this method of fertilization (Franzén 1956), which makes it less useful for inferring phylogenetic relationships.

Lundin and Hendelberg (1998) indicated that the mature sperm in Nemertodermatida resemble the type that Franzén (1956) denotes as "modified from the primitive metazoan sperm type", and their unique morphology probably reflects an internal fertilization.

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