The mature and immature spermatozoa of the free-living marine nematode *Daptonema* sp. (Nematoda: Monhysterida: Xyalidae)

Vladimir V. Yushin[1, 2], Vladimir V. Malakhov[2, 3] Myriam Claeys[4] and Wim Bert[4]

[1]National Scientific Centre of Marine Biology, Far Eastern Branch, Russian Academy of Sciences, Paltchevsky Street 17, 690041, Vladivostok, Russia
[2]Far Eastern Federal University, 690950, Vladivostok, Russia
[3]M.V. Lomonosov Moscow State University, Moscow, Russia
[4]Nematology Research Unit, Department of Biology, Ghent University, Belgium
e-mail: vvyushin@yandex.ru

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Summary. The immature spermatozoa stored in the testis and female postvulval uterine sac (PUS), and mature spermatozoa from the uterus of the free-living marine nematode *Daptonema* sp. (Monhysterida: Monhysterina: Xyalidae) were studied. The spermatozoa have a nucleus without a nuclear envelope. The central cytoplasm of the immature spermatozoa from the testis and the distal part of the PUS is occupied by a mass of pale fibrous bodies (FB) surrounded by mitochondria and osmiophilic membranous organelles (MO). The spermatozoa in the proximal half of PUS have a wide peripheral layer of electron-lucent filamentous cytoplasm. The uterus lumen contains an aggregation of mature spermatozoa of which the periphery is transformed into pseudopods. The FB replaced by a voluminous electron-lucent halo bounded by a continuous layer of MO, mitochondria and fibrous matter. The MO may be intact, but numerous MO are fused with the plasma membrane, having been transformed into transparent pouches, each one opening to the exterior *via* a pore. Ultrastructural data showed that the activation is regulated in the female when spermatozoa migrate toward the uterus and transform into amoeboid mature spermatozoa. In general, the spermatozoa of *Daptonema* sp. and some other Monhysterina closely resemble those of the taxa belonging to the order Rhabditida. However, the 'rhabditid' pattern of spermatozoon structure and development is most likely the plesiomorphic state in Rhabditida and close sister groups.

Key words: female gonoduct, fibrous bodies, major sperm protein, membranous organelles, Monhysterina, postvulval uterine sac, pseudopod, sperm activation, spermatogenesis, ultrastructure.

Spermatozoa usually display a large number of informative morphological characters and traits related to morphogenesis traits that can be analysed in the framework of metazoan taxonomy and phylogeny (Baccetti, 1985; Jamieson *et al.*, 1995; Reunov, 2005; Pitnick *et al.*, 2008). Also in the case of nematodes, spermatozoon features are phylogenetically informative, although available data are limited to well-studied groups and information for some key groups is missing entirely, making a successful comparative analysis of the phylum impossible at present (Justine & Jamieson, 1999; Justine, 2002; Yushin & Malakhov, 2004, 2014).

According De Ley & Blaxter (2002), the phylum Nematoda comprises two classes, Enoplea and Chromadorea. This classification will be followed in this paper. The structure and development of nematode sperm have been mainly studied in representatives of the diverse order Rhabditida, belonging to the class Chromadorea (Justine & Jamieson, 1999; Justine, 2002; Yushin *et al.*, 2016). Many species studied within Rhabditida produce similar spermatozoa of the 'rhabditid' pattern, which can be characterised as an amoeboid bipolar cell with an anterior pseudopod and posterior main cell body, which includes a nucleus (without nuclear envelope), mitochondria and unique 'membranous organelles' (MO) (Justine & Jamieson, 1999; Justine, 2002; Yushin & Malakhov, 2014). The MO develop as part of complexes with paracrystalline fibrous bodies (FB) composed of a unique

cytoskeleton protein MSP ('major sperm protein') (Justine, 2002; Chu & Shakes, 2013; Yushin *et al.*, 2016). The complexes of FB and MO ('FB-MO complexes') during late stages of spermatogenesis dissociate into separate FB and MO. After sperm activation inside the female gonoduct, MO join to the plasmalemma of the sperm's main cell body and release their content into the uterus lumen. The empty MO, appearing as membranous pouches continuous with the sperm plasmalemma, are retained as a stable feature of the mature sperm. Sperm activation is also accompanied by the transformation of FB into the MSP-based cytoskeleton of a newly formed pseudopod (Justine, 2002; Chu & Shakes 2013; Yushin *et al.*, 2016).

Surprisingly, also outside the order Rhabditida, sperm with structure and development closely similar to the 'rhabditid' pattern were detected, namely in the free-living marine monhysterid nematode *Sphaerolaimus hirsutus* Bastian, 1865 (Monhysterida, Monhysterina, Sphaerolaimidae) (Noury-Sraïry *et al.*, 1993; Justine & Jamieson, 1999; Justine, 2002). However, crucial final events of the spermiogenesis, *i.e.* the transformation of spermatozoon into final mature spermatozoa after activation, were not observed in this nematode.

In order to understand the basic pattern and diversity of male gametes in Chromadorea, additional ultrastructural studies of the spermatozoa of monhysterids belonging to Monhysterina is warranted. Furthermore, the spermatogenesis pattern is especially intriguing when the female gonoduct includes specialised storage areas for morphologically different spermatozoa. This is why a species from the genus *Daptonema* was selected for this study, in which females have a postvulval uterine sac (PUS) as a distinct part of the female gonoduct.

The main objective of the present study was to obtain ultrastructural information of immature spermatozoa from the testis, spermatozoa from the PUS and mature spermatozoa from the uterus of this species. These new and original data will enable the analysis of sperm storage in *Daptonema* and spermatogenesis patterns in the order Monhysterida.

MATERIAL AND METHODS

Gravid males and females from the genus *Daptonema* were extracted from samples of silty sand collected on 16 August 2001 at a depth of 1 m in the estuary of the Volchanka River flowing into Vostok Bay, the Sea of Japan. The nematodes were identified by Prof. N.P. Fadeeva (Far Eastern Federal University (FEFU), Vladivostok, Russia) as a new species, morphologically related to *D. setosum* (Bütschli, 1874). In the present paper, we entitle the studied population as '*Daptonema* sp. 2' to delineate current observations from previously published ultrastructural data on another (unidentified) species of the genus *Daptonema* (Justine & Jamieson, 1999; Justine, 2002). Preliminary data on mature spermatozoa of *Daptonema* sp. 2 are presented in the review by Yushin & Malakhov (2014).

Light microphotographs of whole mount preparations were taken with a Reichert Polyvar microscope. Before fixation for transmission electron microscopy (TEM), the head and tail of each animal were removed to facilitate the subsequent tissue fixation and embedding processes. The remaining bodies contained the testes (male specimens) and the uterus plus PUS (female specimens), both filled with spermatozoa.

The specimens were fixed overnight at 4°C in 2.5% glutaraldehyde in 0.05 M cacodylate buffer with 21 mg ml^{-1} NaCl, and then postfixed in 2% osmium tetroxide in the same buffer containing 23 mg ml^{-1} NaCl. The specimens were dehydrated in ethanol followed by isopropanol series and embedded in Spurr resin. Thin longitudinal sections were made with a diamond knife using Leica UC6 ultramicrotome, stained with uranyl acetate and lead citrate and examined with JEOL JEM 100S, JEOL JEM 1010, Zeiss Libra 120 and Zeiss Sigma 300 VP electron microscopes. Pictures taken with a JEOL JEM 1010 were digitized using a Ditabis system (Pforzheim, Germany).

The structure of immature and mature spermatozoa of three males and three females were examined for the present paper, in which the terminology of Shepherd (1981) for stages of spermatogenesis is employed.

RESULTS

The males of *Daptonema* sp. 2 have two reflexed outstretched testes each terminating with dilated seminal vesicle filled with spermatozoa. The promonodelphic female reproductive system consists of one anterior branch, which includes an outstretched ovary, oviduct and uterus; the posterior branch is reduced to a PUS (Fig. 1A, B). The seminal vesicles of the testes contained uniform spermatozoa stored before ejaculation (Fig. 2A). The female PUS and dilated uterus were filled with spermatozoa of clearly different structure (Figs 1A & 2B-D).

Immature spermatozoa from testis. The immature spermatozoa are densely packed in the seminal

vesicle; they are unpolarised cells of variable shape and uniform in internal structure (Figs 2A & 3A).

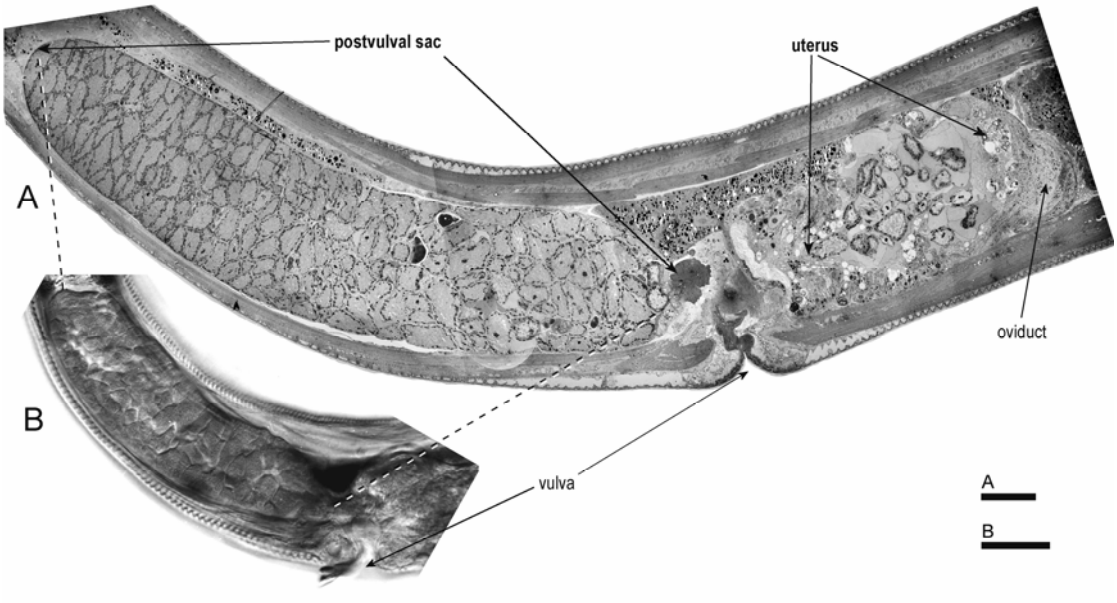

Fig. 1. *Daptonema* sp. 2. A: Part of the longitudinal section through a female showing uterus and postvulval uterine sac filled with spermatozoa, TEM. B: Postvulval uterine sac, wholemount preparation, interference contrast. Scale bars: A = 20 μm; B = 50 μm.

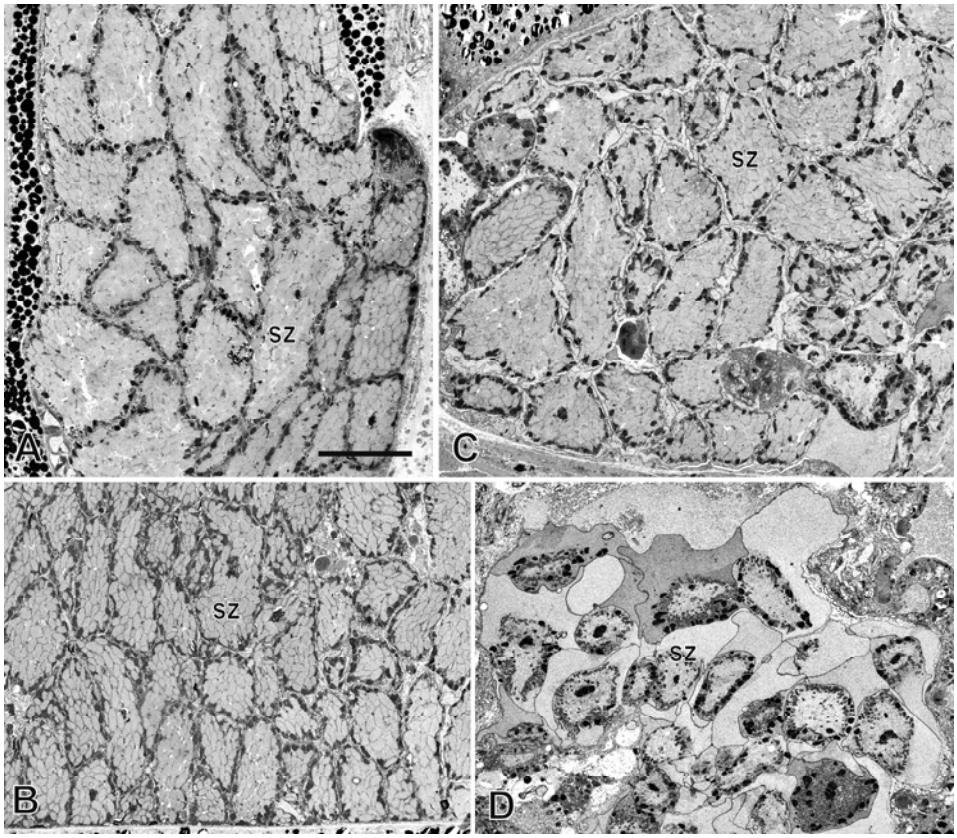

Fig. 2. *Daptonema* sp. 2, TEM. Four low magnification overviews of the clusters of spermatozoa (sz) storing in male (A) and female (B-D) gonoducts are assembled using common magnification marked on (A) by scale bar 10 μm.

A: The immature spermatozoa from the seminal vesicle. B & C: The immature spermatozoa from the postvulval uterine sac (B, proximal tip; C, distal part). D: The mature (activated) spermatozoa in uterus.

The size of cells is about 10-12 μm in diameter. When elongated, the spermatozoa are often relatively narrow but are up to 20 μm in length. The central nucleus lacks a nuclear envelope; the densely packed nuclear chromatin has an irregular outline and is covered by less dense amorphous particles (Fig. 3B).

The voluminous sperm cytoplasm surrounding the nucleus is filled with moderately dense oval or spindle-shaped bodies of about 2 × 1 μm in size (Figs 3A, B & 4A). These bodies appear filamentous in structure and most likely correspond to fibrous bodies (FB) known from the immature spermatozoa of many other nematodes (Justine, 2002). The electron-lucent FB have clear cut outlines due to thin dense surface coat and dense 10 nm thick fibres filling the narrow space between FB (Figs 3B & 4A-C).

Two other basic components of immature spermatozoa are mitochondria and membranous organelles (MO) arranged as a layer in the thin peripheral cytoplasm surrounding the mass of FB (Figs 3A, B & 4A-C). The MO are vesicles with strongly osmiophilic content (Fig. 4A, C). The MO have a characteristic bottle-like shape, consisting of a spheric body and wide and prominent bulge (Fig. 4B-E). The spheric part of MO comprises finger-like invaginations of the outer membrane, while the bulges have no membranous components inside. The spheric part of MO is *ca* 0.6 μm in diameter, the total length of the organelles including the bulge is 1.5 μm. The MO are regularly interspersed by elongated mitochondria (Fig. 4B, C & E). The space of peripheral cytoplasm between organelles contains bundles of 15 nm thick tubules, which surround MO and mitochondria (Fig. 4D, E). The spermatozoon plasmalemma is smooth and only a 10-20 nm wide transparent space separates neighbouring cells (Fig. 4C, E).

Spermatozoa from postvulval uterine sac. The conspicuous PUS is 250-300 μm long and up to 50-70 μm wide in the inseminated females (Fig. 1A, B). The PUS is separated from the body cavity by thin epithelial wall and filled with densely packed spermatozoa (Figs 1A & 2B, C). The spermatozoa from the distal half (blind tip) of PUS are identical in size and morphology to those accumulating in the seminal vesicles of males and therefore may be considered as the 'immature spermatozoa' (Figs 2B & 5A). The sperm cytoplasm is filled with electron-lucent FB separated by narrow spaces, which are filled with dense fibres (Fig. 5A). The peripheral cytoplasm contains mitochondria and MO with osmiophilic content.

The spermatozoa in the proximal half of PUS, connecting to vulva, are also similar to immature spermatozoa but they appear slightly dilated, reaching 12-14 μm in size (Figs 1A & 2C). The nucleus of the spermatozoa is surrounded by a mass of FB retaining the oval contours and dimensions of the immature spermatozoa (Figs 5B & 6A). The intact electron-dense MO and mitochondria together form a border around the central mass of FB; however, peripheral organelles are now separated from the sperm plasma membrane by a distinct layer of electron-lucent filamentous cytoplasm (Figs 5B & 6A, B). The tubules surround the organelles and they appear in the peripheral cytoplasm and below the plasma membrane of spermatozoon (Fig. 6B). A narrow transparent space separates spermatozoa.

Mature spermatozoa. The uterus lumen is filled by mature (activated) spermatozoa (Fig. 1A). These spermatozoa bear prominent pseudopods and are morphologically different compared to the immature spermatozoa found in testes and PUS (Figs 2D & 7A). The main cell body of these spermatozoa contains cellular components arranged concentrically; the cell body is more or less uniform in size (8-10 μm) and regular in shape (Fig. 7A). The periphery of spermatozoa now forms prominent pseudopods, 5-10 μm in length, and these considerably increase total cell dimensions (Figs 2D & 7A). As the pseudopods protrude in different directions, they do not provide distinct polarity to the sperm cells.

In the mature spermatozoon, FB around nucleus are no longer detected; they are replaced by a voluminous electron-lucent halo surrounded by peripheral layer of MO, mitochondria and fibrous matter (Fig. 7A, B). Part of the MO forming this layer look intact and retain their osmiophilic content and characteristic bottle-like shape (Figs 7A, B & 8A). However, numerous MO appearing as transparent pouches with a system of internal membranes were also observed in each spermatozoon (Figs 7A & 8A). These MO are fused with the plasma membrane, each opening to the exterior *via* a pore.

The filamentous content of the sperm cytoplasm is diverse. The central halo is evenly filled with 10 nm thick dense fibres (Figs 7A, B & 8A, B). The 15 nm thick tubules are associated with peripheral organelles and are especially abundant at the base of pseudopods (Figs 7B & 8A, B). The pseudopods contain fibrous matter, while individual filaments were unresolved.

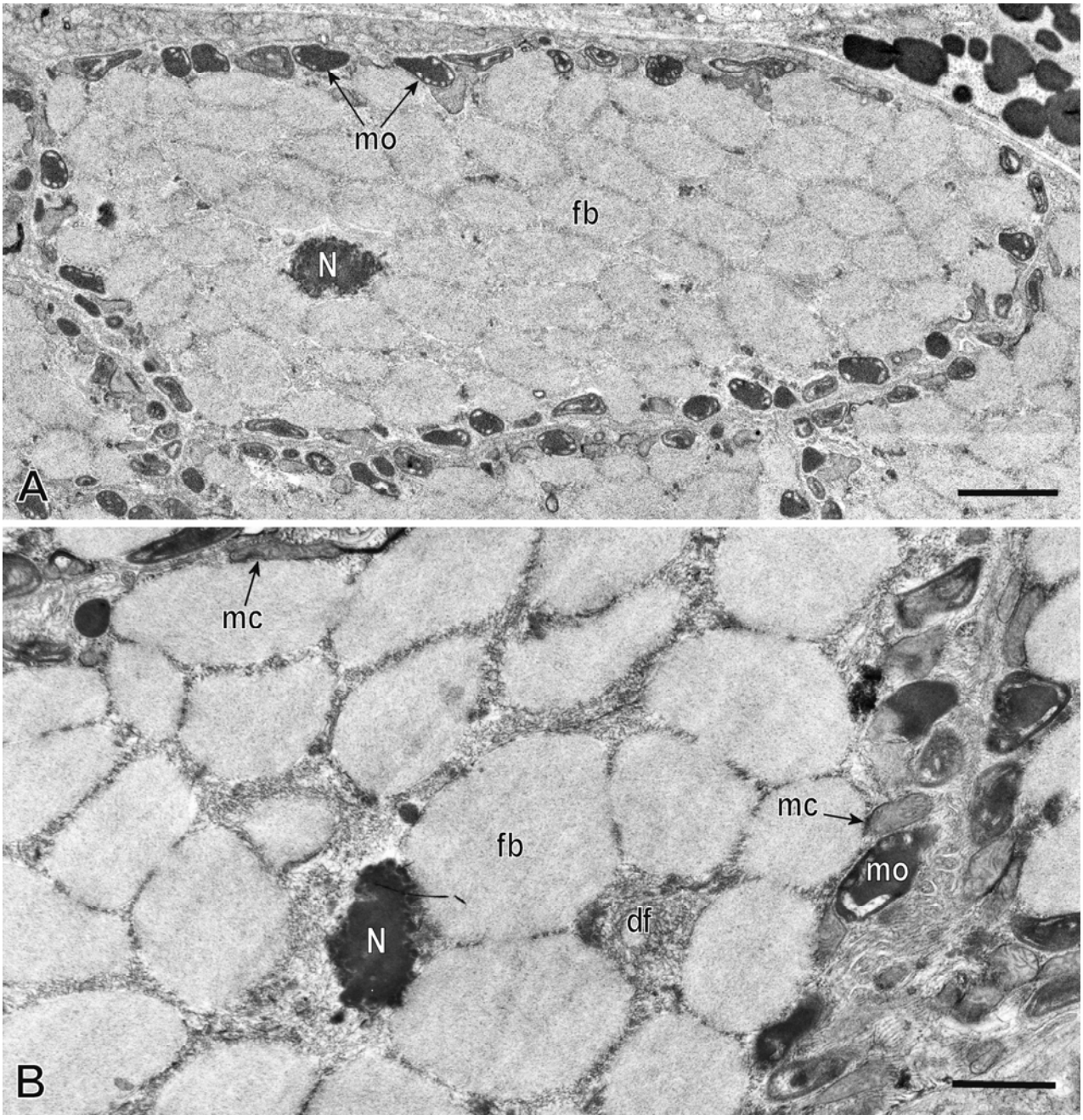

Fig. 3. *Daptonema* sp. 2, immature spermatozoa from testis, TEM. A: General view of the immature spermatozoon. B: The immature spermatozoon at higher magnification. Abbreviations: df – dense fibres; fb – fibrous body; mc – mitochondria; mo – membranous organelles; N – nucleus. Scale bars: A = 2 μm; B = 1 μm.

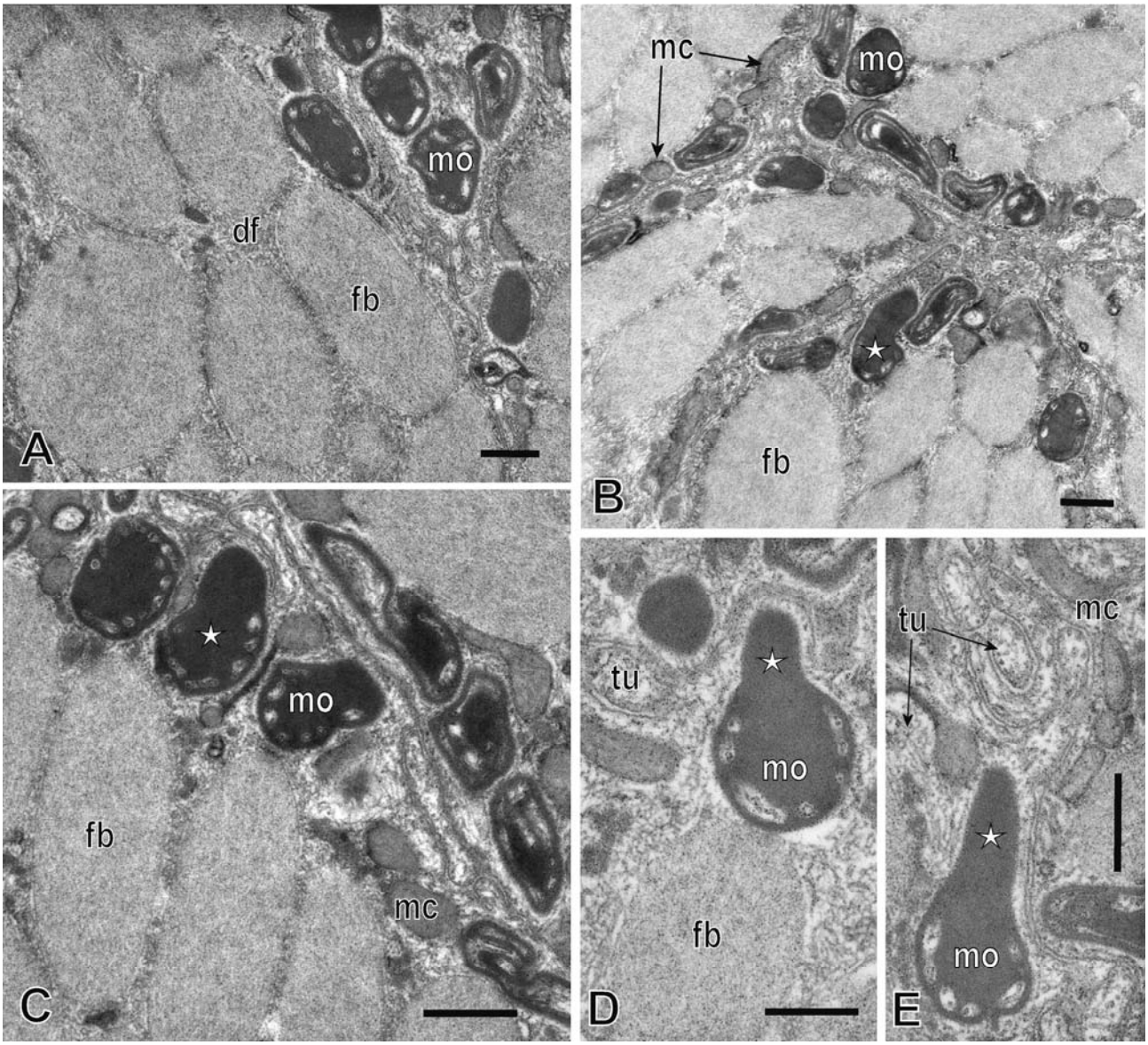

Fig. 4. *Daptonema* sp. 2, immature spermatozoa from testis, TEM. A: Fibrous bodies (fb) at the spermatozoon periphery. B: Peripheral cytoplasm of several neighbouring spermatozoa. Asterisk marks the membranous organelle with a bulge. C: Peripheral cytoplasm of two neighbouring spermatozoa at higher magnification. Asterisk marks the membranous organelle with a bulge. D and E: The bottle-shaped membranous organelles (mo) with a bulge (asterisk) orientated to the plasma membrane of spermatozoon. Note tubules (tu) surrounding the peripheral organelles. Abbreviations: df – dense fibres; fb – fibrous body; mc – mitochondria; mo – membranous organelles; tu – tubules. Scale bars: 0.5 μm.

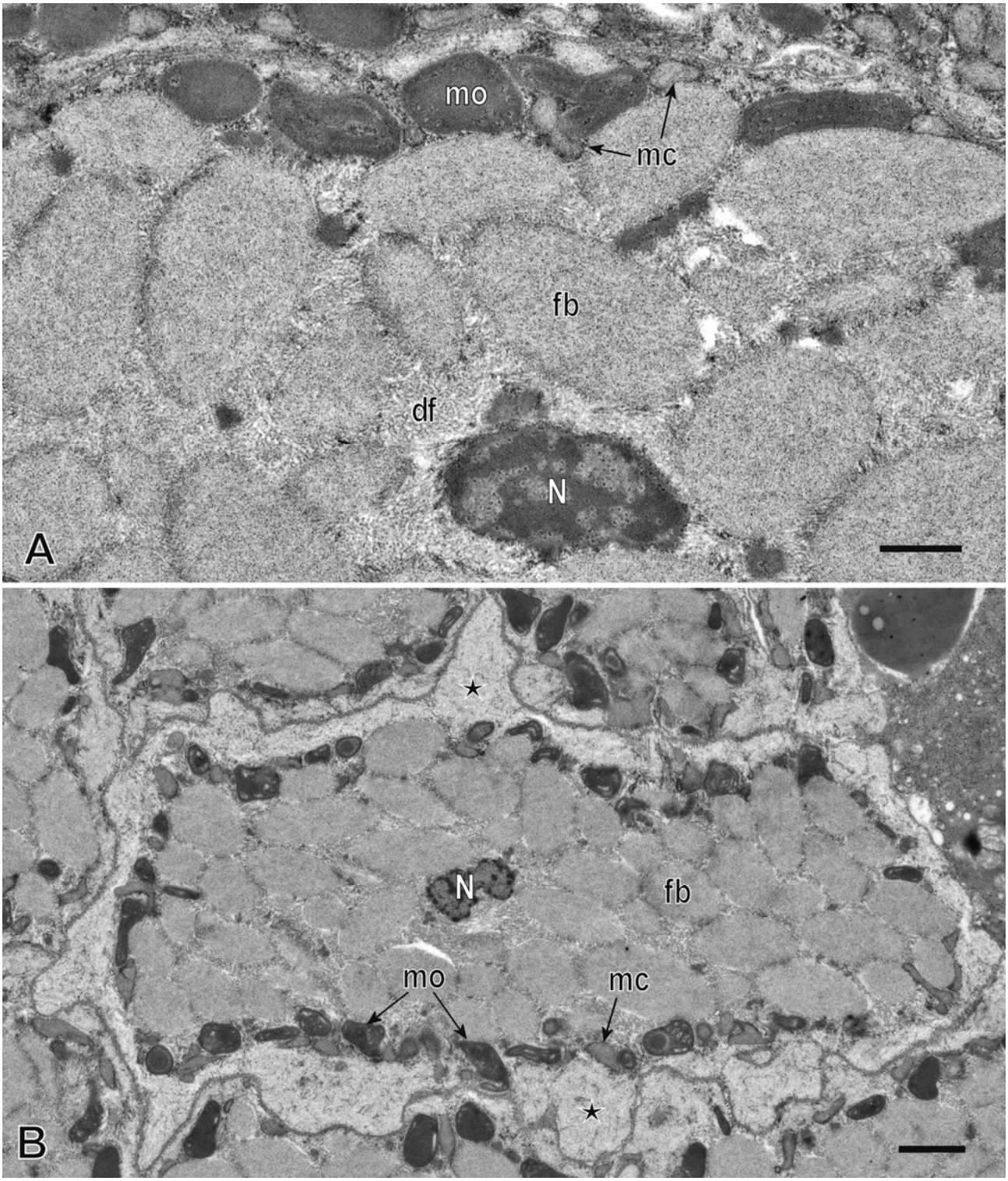

Fig. 5. *Daptonema* sp. 2, immature spermatozoa from the postvulval uterine sac, TEM. A: High magnification of the immature sperm from distal part of the postvulval uterine sac, note identity to the spermatozoa from testis (Fig. 3). B: general view of immature spermatozoon from the proximal part of the postrvulval uterine sac; asterisks mark dilated peripheral cytoplasm of the spermatozoon. Abbreviations: df – dense fibres; fb – fibrous body; mc – mitochondria; mo – membranous organelles; N – nucleus. Scale bars: A = 0.5 μm; B = 1 μm.

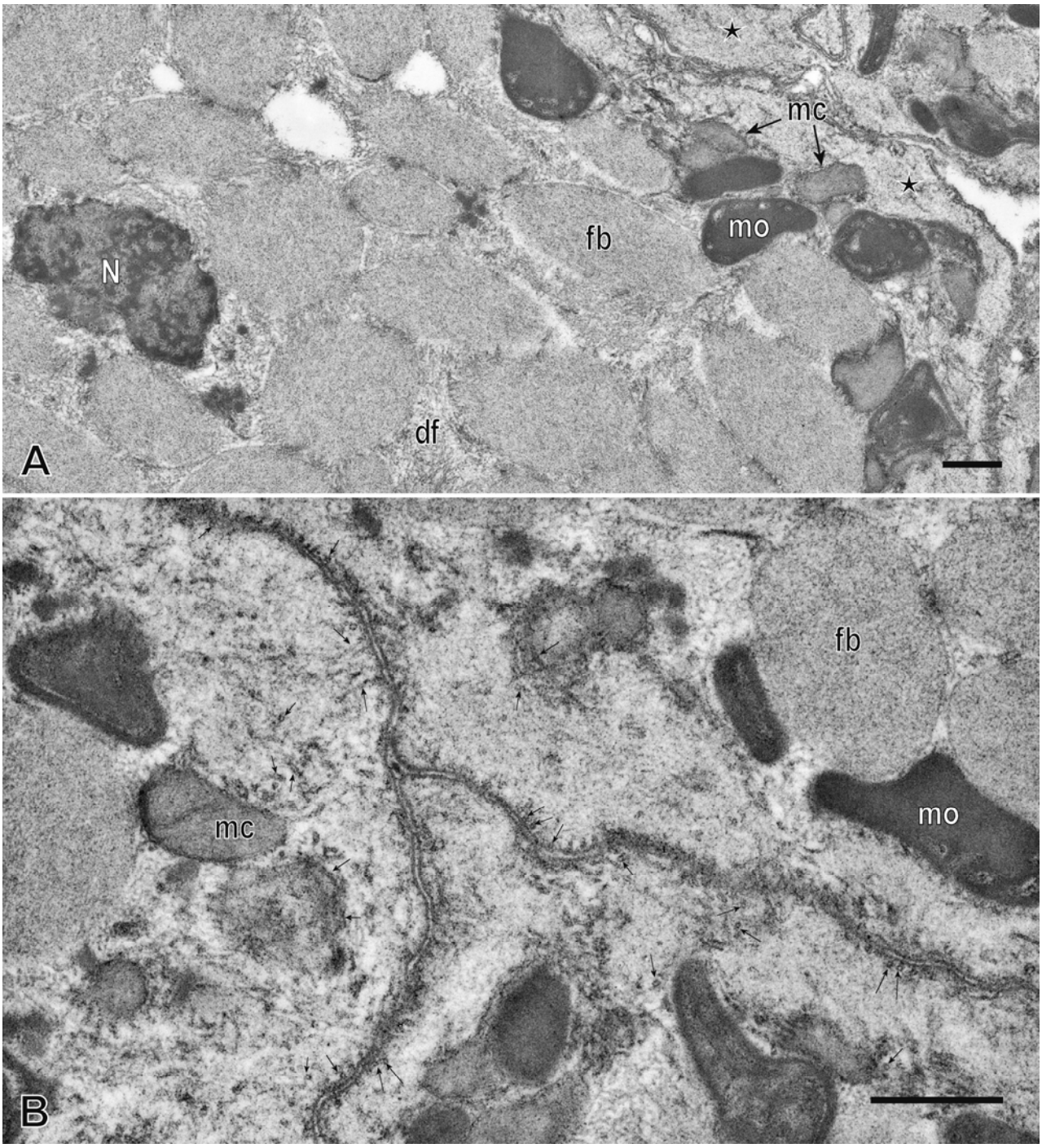

Fig. 6. *Daptonema* sp. 2, immature spermatozoa from the distal part of the postvulval uterine sac, TEM. A: Higher magnification of the spermatozoon showing details of structure; asterisks mark dilated peripheral cytoplasm of spermatozoon. B. High magnification of the peripheral cytoplasm of three merging spermatozoa; small arrows shows tubules in the peripheral cytoplasm and under spermatozoon plasma membrane. Abbreviations: df – dense fibres; fb – fibrous body; mc – mitochondria; mo – membranous organelles; N – nucleus. Scale bars: 0.5 μm.

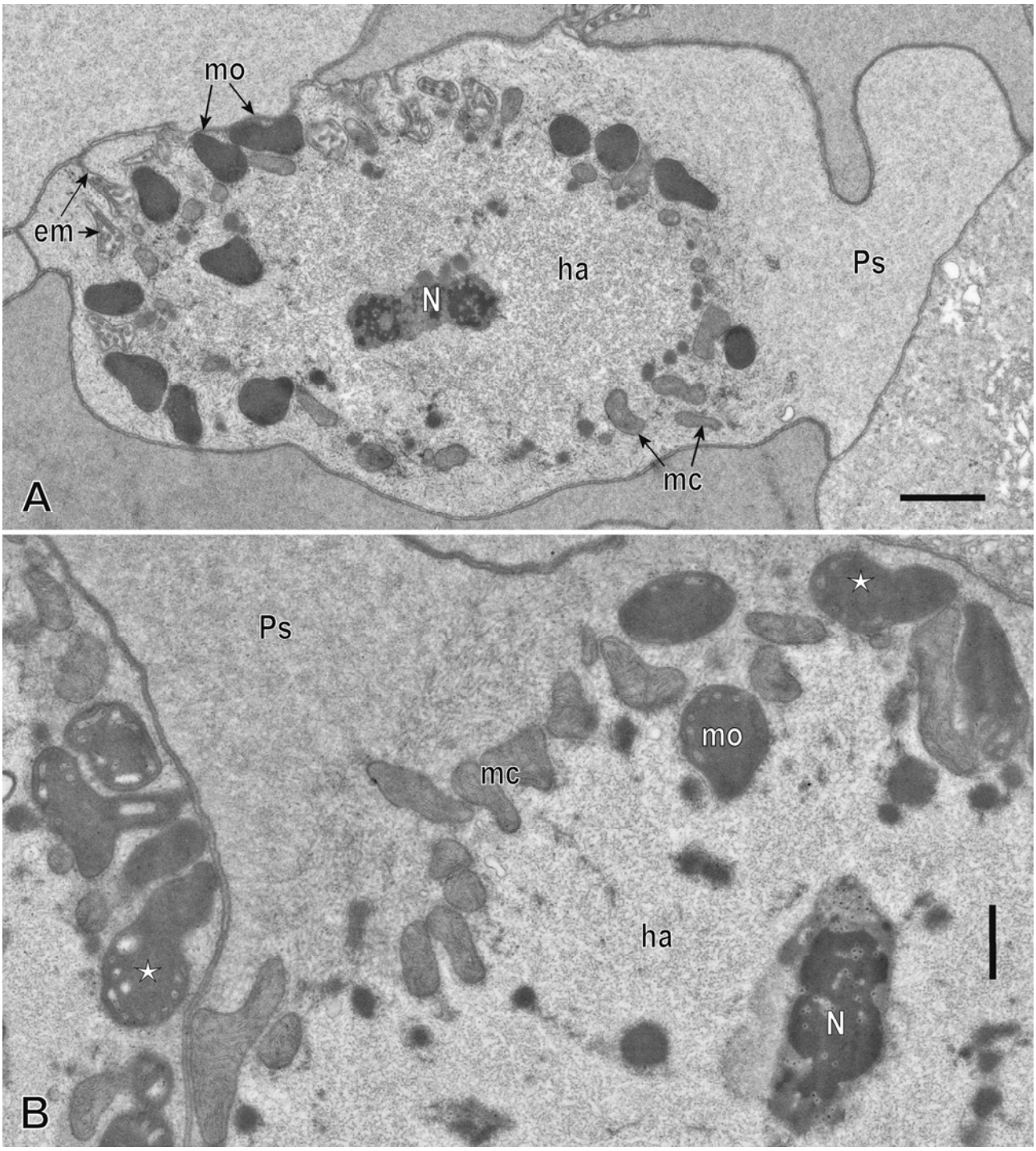

Fig. 7. *Daptonema* sp. 2, mature spermatozoa from uterus, TEM. A: General view of spermatozoon. B: Higher magnification of spermatozoon; asterisks – intact bottle-shaped membranous organelles. Abbreviations: em – empty membranous organelles; ha – halo; mc – mitochondria; mo – membranous organelles; N – nucleus; Ps – Pseudopod. Scale bars: A= 1 μm; B = 0.5 μm.

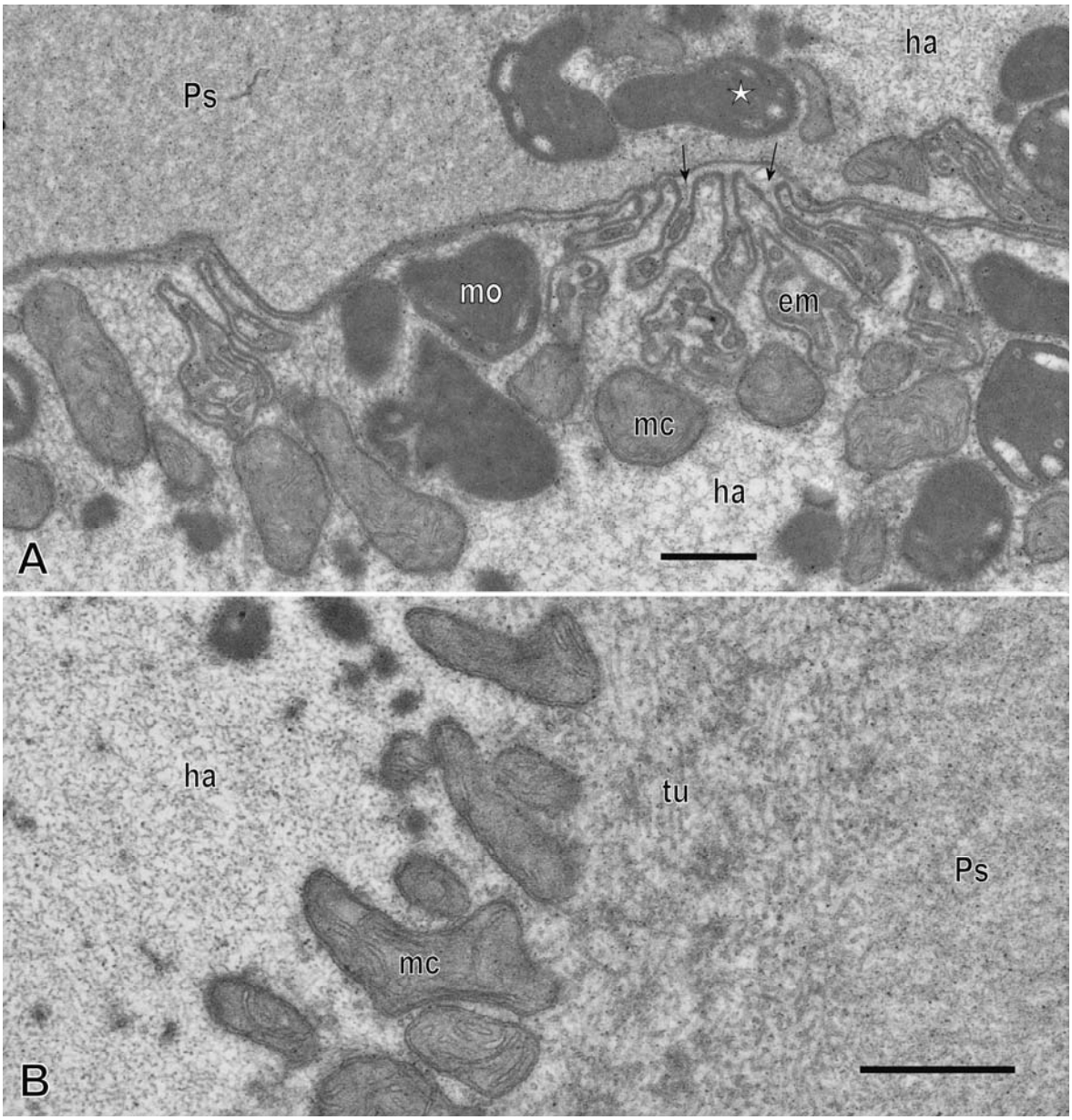

Fig. 8. *Daptonema* sp. 2, mature spermatozoa from uterus, TEM, high magnifications. A: Periphery of the spermatozoon with organelles (arrows – pores of empty membranous organelles; asterisk – intact bottle-shaped membranous organelle). B: Central halo (ha) filled with dense fibres is separated from peripheral filamentous pseudopod by a layer of mitochondria. Note area of tubules (tu) between mitochondria and filamentous content of the pseudopod. Abbreviations: em – empty membranous organelles; ha – halo; mc – mitochondria; mo – membranous organelles; Ps – Pseudopod; tu – tubules. Scale bars: 0.5 μm.

DISCUSSION

In the nematode taxa with monodelphic females the presence or absence of a PUS together with its size and proportions are used as informative characters in species descriptions and diagnoses (Lorenzen, 1977; Abebe *et al.*, 2006; Schmidt-Rhaesa, 2014). In many cases, the PUS develops as a spacious part of the female gonoduct, which contains spermatozoa (Lorenzen, 1977; Vincx & Coomans, 1983; Peralta & Peña-Santiago, 1996; Abebe *et al.*, 2006; Zhuo *et al.*, 2010; Adeldoost *et al.*, 2015; Tsalolikhin, 2017; Ahmad *et al.*, 2018;

Pedram *et al.*, 2018; Slos *et al.*, 2018). In such cases, it is obviously assumed that the PUS has a sperm storage function comparable to the spermathecae or seminal receptacles.

Among monodelphic females of the monhysterid family Xyalidae, the PUS is characteristic of several species from the large genus *Daptonema* (Lorenzen, 1977; Coomans & Abebe, 2006; Fonseca & Bezerra, 2014). Some species of *Daptonema* have a voluminous and easily detectable PUS containing a mass of spermatozoa (Lorenzen, 1977; Vincx & Coomans, 1983; Tsalolikhin, 2017). *Daptonema* sp. 2 presented in this paper is one of the species whose females have a PUS, which functions as the sperm storage analogous to spermatheca (Figs 1 & 2B-C). The densely-packed spermatozoa from the PUS may be considered as immature spermatozoa, as their structure is nearly identical to the spermatozoa from the seminal vesicles of males. Furthermore, it seems that the immature spermatozoa can persist in the PUS as inactive cells for a long time, with their further activation regulated by the female. This practice of conserving immature (non-activated) spermatozoa in uteri or spermathecae is also known for several other nematodes, as detected by light and electron microscopic observations (Riemann, 1983; Adamson & Van Waerebeke, 1985; Yushin, 2003; Zograf *et al.*, 2004; Yushin & Coomans, 2005).

Sperm structure and development of monhysterid nematodes were studied for the first time in *Sphaerolaimus hirsutus* (Monhysterina, Sphaerolaimidae), revealing a 'rhabditid' pattern of spermatogenesis (Noury-Sraïry *et al.*, 1993; Justine & Jamieson, 1999; Justine, 2002). In *S. hirsutus*, spermatocytes produce FB-MO complexes that dissociate in the spermatids to form separate MO and FB. In the immature spermatozoa from the testis, FB dissolve, but separate MO concentrate together with mitochondria in a spheric layer surrounding a wide transparent space (halo) with a centrally-located nucleus. The early transformation of FB into cytoskeleton components of immature spermatozoa is not a regular feature for rhabditids. Nevertheless, this is known for several taxa and may be considered as a variation of the basic rhabditid pattern (Wolf *et al.*, 1978; Shakes & Ward 1989; Ugwunna & Foor, 1982; Wright & Sommerville, 1985; Yushin *et al.*, 2007; Zograf, 2014; Slos *et al.*, 2015).

The spermatozoon structure data of *Daptonema* sp. 2 (Monhysterina, Xyalidae) also shows a close similarity to the rhabditid pattern. The immature spermatozoa from the testis retain FB as a central mass, enclosed by a sphere compiled from intact MO and mitochondria. The spermatozoa of this type were not only observed in testis, but also in the distal part of the female PUS. Specific to the spermatozoa in *Daptonema* sp. 2, the bottle-like MO have an enormously prominent bulge, unlike the usual knob-like projection that marks the pole of MO joining to the sperm plasmalemma for fusion and pore formation during final spermatozoon maturation (Justine, 2002; Yushin & Malakhov, 2014).

The filamentous content of cell periphery in the immature spermatozoa in *S. hirsutus* is interpreted as derived from dissolved FB (Noury-Sraïry *et al.*, 1993). In *Daptonema* sp. 2, the immature spermatozoa observed in the proximal portion of the PUS still retain FB which partially dissolve to develop fibrous peripheral cytoplasm reminiscent of that of *S. hirsutus* spermatozoa (compare Fig. 5B from Noury-Sraïry *et al.*, 1993 and Figs 5B & 6A, B in the present paper). This amoeboid pseudopod-like periphery seems to reflect minor amoeboid motility and shows early activation of spermatozoa in the testis of *S. hirsutus* or in the PUS of *Daptonema* sp. 2.

In the terminal spermatozoa in the testis of *S. hirsutus*, FB have transformed into the central halo and filamentous periphery separating MO from the plasma membrane. The final events of differentiation of spermatozoa, characteristic of mature spermatozoa from the female gonoduct, were not observed in *S. hirsutus* (Noury-Sraïry *et al.*, 1993). The pictures from the review of Yushin & Malakhov (2014) and further observations presented in this paper show a radical transformation of the spermatozoa of *Daptonema* sp. 2 as a result of activation in the female gonoduct.

In *Daptonema* sp. 2, the early process of maturation, *i.e.* formation of amoeboid periphery, is already instigated in the PUS and seems to be critical for the later appearance of the spermatozoa in the uterus. Finally, the spermatozoa from the uterus demonstrate details associated with maturity: the absence of FB, the cytoplasm around the nucleus having become an electron-lucent halo surrounded by sphere of MO and mitochondria, and the presence of prominent pseudopods with filamentous content. Two types of MO were observed in the spermatozoa of *Daptonema* sp 2, *i.e.* intact bottle-shaped MO with osmiophilic content, and empty MO appearing as pouches opening to exterior *via* pores. These pouches are very characteristic of activated spermatozoa in diverse nematode taxa and they are considered a key marker of maturity (Justine, 2002; Yushin & Malakhov, 2014). The presence of intact MO, albeit with the majority open and empty, has also previously been observed in

activated spermatozoa of some nematodes from diverse orders (Yushin & Malakhov, 1994; Justine & Jamieson, 1999; Geldziler *et al.*, 2006; Yushin *et al.*, 2011; Yushin & Ryss, 2011; Lak *et al.*, 2015).

Taking into account both the detailed study of spermatogenesis in the sphaerolaimid *S. hirsutus* (Noury-Sraïry *et al.*, 1993) and the observation of immature and mature spermatozoa in the xyalid *Daptonema* sp. 2 (Yushin & Malakhov, 2014 and the present paper), the spermatogenesis pattern in the monhysterids from the suborder Monhysterina can be ascertained. In general, all events coincide well with the characteristic rhabditid pattern of spermatogenesis. However, the widespread presence of a nearly uniform rhabditid pattern of spermatozoa displayed by a variety of distant taxa within Rhabditida (Spiruromorpha, Ascaridomorpha, Panagrolaimomorpha, Tylenchomorpha, Diplogasteromorpha, Rhabditomorpha, and Myolaimina), and now identified in Monhysterida, suggests that this pattern must be considered as the plesiomorphic condition of Rhabditida and close sister groups. Correspondingly, various deviations from the rhabditid pattern described in several taxa of rhabditids and in the monhysterids Linhomoeina may be considered as apomorphies (Justine, 2002; Yushin, 2007, 2008; Giblin-Davis *et al.*, 2010; Yushin & Malakhov, 2014; Zograf, 2014; Slos *et al.*, 2015). Earlier ultrastructural data on spermatozoa from the testes of some representatives of Xyalidae have already shown some similarities with the rhabditid pattern, although these observations were too incomplete to allow for consistent analysis or well-based conclusions (Nicholas & Stewart, 1997; Justine & Jamieson, 1999; Justine, 2002).

The spermatozoon structure and development in Linhomoeina, another suborder of the order Monhysterida, have been studied in details in two species of the family Linhomoeidae, *Paralinhomoeus* sp. and *Terschellingia glabricutis* (Yushin, 2007, 2008). The immature spermatozoa of both species contain a nucleus without nuclear envelope, mitochondria and many paracrystalline FB composed of characteristic parallel fibres. These spermatozoa lack MO, whereas FB of normal structure appear as separate organelles in spermatocytes free of membranous components. The noticeable total absence of MO at all stages of spermatogenesis separates Linhomoeina from their monhysterid relatives in the suborder Monhysterina (Sphaerolaimidae and Xyalidae). This difference in male gametes may be considered as a potential character for the diagnosis of two suborders of Monhysterida.

Spermatozoa with well-developed FB, never associated with MO, were found outside Monhysterida in a variety of nematodes from the orders Chromadorida, Desmodorida and Rhabditida (Justine, 2002; Yushin & Zograf, 2002; Yushin, 2003; Zograf *et al.*, 2004; Zograf & Yushin, 2004; Yushin & Coomans, 2005). These spermatozoa present one more pattern of spermatogenesis and sperm structure characteristic of the nematode subclass Chromadorea (Justine, 2002; Yushin & Malakhov, 2004, 2014).

As reported by Noury-Sraïry *et al.* (1993) the cytoplasm of spermatids and spermatozoa in *S. hirsutus* contains two types of specific fibres. The central cytoplasm adjacent to the nucleus is filled with "twisted filaments" about 12 nm in diameter, while the periphery contains numerous "small membranous tubules", 20-30 nm in diameter, associated with a layer of organelles.

Similar cytoskeleton components with the same localisations are observed in the spermatozoa of *Daptonema* sp. 2. In the immature spermatozoa, dense 10 nm thick fibres fill the space between FB, while a peripheral layer of MO and mitochondria is associated with 15 nm thick tubules that are also detected beneath the plasma membrane. In the mature spermatozoa, FB are completely replaced by dense fibres of the central halo, while peripheral organelles are associated with tubules. The mature spermatozoa of *Daptonema* sp. 2 have prominent pseudopods filled with filamentous matter while individual filaments were unresolved. Stratification of the fibre content is clearly visible on sections of the border between halo and pseudopod (Fig. 8B).

It is very likely that cytoskeleton fibres, including tubules, in the spermatozoa of both monhysterid nematodes, *S. hirsutus* and *Daptonema* sp. 2, essentially contain MSP (Noury-Sraïry *et al.*, 1993). The tubules have been observed in spermatozoa of variety of distantly related taxa in both classes of the phylum Nematoda, Enoplea and Chromadorea; they have a diameter ranging from 13 to 20 nm, as reported by different authors (Yushin, 2010; Zograf *et al.*, 2016), and 15 nm in *Daptonema* sp. 2. These tubules cannot be identified as classic tubulin-containing microtubules, which have a normal diameter 24-25 nm (Chaaban & Brouhard, 2017) and which absent in nematode spermatozoa where the prevalent cytoskeleton protein responsible for amoeboid movement is MSP (Justine, 2002; Yushin *et al.*, 2016).

CONCLUSION

The prominent postvulval uterine sac of *Daptonema* sp. 2 contains inactive immature spermatozoa, and can be considered a sperm storage organ, analogous to spermatheca. Our results show clear evidence of the migration of spermatozoa into the uterus and their transformation after activation into mature spermatozoa bearing prominent pseudopods and capable of fertilisation.

Our analysis of spermatogenesis and sperm structure in two Monhysterina species, *S. hirsutus* and *Daptonema* sp. 2, shows the similarity of spermatogenesis features and events with several taxa of Rhabditida, another taxa of Chromadorea. This 'rhabditid' pattern must be considered as the plesiomorphic situation of the Rhabditida and sister groups, and, correspondingly, various deviations from this 'rhabditid' pattern can be considered as apomorphies. For example, the disappearance of MO in combination with the retention of FB known in several rhabditid taxa and in the monhysterids Linhomoeina must be considered as an apomorphic trait.

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V.V. Yushin, V.V. Malakhov, M. Claeys and W. Bert. Незрелые и зрелые сперматозоиды свободноживущей морской нематоды *Daptonema* sp. (Nematoda: Monhysterida: Xyalidae).

Резюме. Изучены незрелые сперматозоиды из семенников и поствульварного маточного мешка (ПММ) свободноживущей морской нематоды *Daptonema* sp. (Monhysterida: Monhysterina: Xyalidae). Ядро сперматозоидов лишено ядерной оболочки. В центральной цитоплазме незрелых сперматозоидов из семенника и дистальной части ПММ расположена масса волокнистых тел (ВТ), окруженная митохондриями и осмиофильными мембранными органеллами (МО). В сперматозоидах из проксимальной части ПММ развивается периферическая электронно-светлая волокнистая цитоплазма. Просвет матки заполняет масса зрелых сперматозоидов, периферическая цитоплазма которых трансформирована в псевдоподии. Вместо ВТ в центральной части развивается электронно-светлое гало, окружённое слоем, сформированным МО, митохондриями и волокнистым материалом. МО могут оставаться интактными, однако множество из них присоединяется к плазмалемме, трансформируясь в прозрачные мешочки, открытые наружу порами. Данные по ультраструктуре показали, что активация сперматозоидов регулируется во время их миграции в матку, где они трансформируются в амебоидные зрелые сперматозоиды. В целом сперматозоиды *Daptonema* sp. и некоторых других Monhysterina сходны со сперматозоидами таксонов, входящих в отряд Rhabditida. Однако «рабдитидный» паттерн строения и развития сперматозоидов должен рассматриваться как плезиоморфный для Rhabditida и близких к ним групп.