

Ultrastructural observation of spermatogenesis in the free-living marine nematode *Parodontophora marisjaponici* (Nematoda: Araeolaimida: Axonolaimidae)

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Summary. Spermatogenesis in *Parodontophora marisjaponici*, described using TEM, is similar in basic features to that of the rhabditid nematodes. Sperm development includes formation of complexes of fibrous bodies (FB) and membranous organelles (MO), which appear in spermatocytes; the complexes dissociate in the spermatids; the immature sperm contains separate FB and MO; the mature spermatozoa are bipolar cells subdivided into pseudopod and a main cell body containing a nucleus without a nuclear envelope, and peripheral mitochondria and MO. However, *P. marisjaponici* has distinct peculiarities in morphology and differentiation of male gametes: i) tripartite FB-MO complexes that include mitochondria; and ii) fusion of initially separate MO into one giant cistern that in the mature spermatozoa opens to exterior *via* multiple pores. This type of sperm development and structure constitute the new pattern that is proposed as an apomorphy of the araeolaimid family Axonolaimidae. The study of spermatogenesis in other families inside the order Araeolaimida such as Comesomatidae will be fruitful for further analysis of sperm diversity in nematodes.

Key words: *Axonolaimus arcuatus*, centrioles, fibrous bodies, *Halalaimus dimorphus*, membranous organelles, pseudopod, sperm pattern.

Data on sperm structure and spermatogenesis of Metazoa are known to be informative to elucidate phylogenetic relations (Baccetti, 1985; Jamieson *et al.*, 1995; Liana & Witalinski, 2005; Pitnick *et al.*, 2008; Levron *et al.*, 2010; Dallai *et al.*, 2016; Bakhom *et al.*, 2017). Spermatozoon morphology and development have also been used for taxonomic and phylogenetic analyses of nematodes (Baccetti *et al.*, 1983; Justine & Jamieson, 1999; Justine, 2002; Yushin & Malakhov, 2004, 2014). Nematode spermatozoa represent an aberrant type of male gamete, in that they are characterised by the absence of an axoneme and an acrosome and their possession of unique organelles such as membranous organelles (MO) and fibrous bodies (FB) (Justine & Jamieson, 1999; Justine, 2002; Yushin & Malakhov, 2014). Most of the available information on the structure and development of nematode sperm was obtained from representatives of the extensive order Rhabditida belonging to the class Chromadorea (*sensu* De Ley & Blaxter (2002);

this classification will be used in the present paper). The relatively uniform sperm defined as the rhabditid pattern, are amoeboid bipolar cells with an anterior pseudopod and a posterior main cell body that contains a condensed nucleus, mitochondria, MO and FB (Justine & Jamieson, 1999; Justine, 2002; Yushin & Malakhov, 2014). The MO are derived from Golgi bodies and appear as a part of MO-FB complexes, which includes the prism-shaped paracrystalline FB composed of densely packed parallel filaments consisting of the unique cytoskeleton protein MSP (major sperm protein) (Justine, 2002; Chu & Shakes, 2013; Yushin *et al.*, 2016). The FB-MO complexes dissociate during the late stages of spermatogenesis into separate FB and MO. In immature spermatozoa MO are large (0.5–1.0 µm in diameter) vesicles with dense content and internal finger-like projections of the outer cell membrane (Justine, 2002). After sperm activation inside the female gonoduct the MO join to the plasmalemma of the sperm main cell body and

release their content into the uterus lumen. In the mature spermatozoon the empty MO resemble membranous sacs being continuous with the sperm plasmalemma. Sperm activation is also accompanied by the transformation of FB into the MSP-based cytoskeleton of a newly formed pseudopod (Justine & Jamieson, 1999; Justine, 2002). The pseudopod and amoeboid movement are typical characteristics of mature nematode sperm.

The nematode class Chromadorea besides Rhabditida includes a number of orders with a special structure of spermatozoa and different patterns of male gamete formation that may be compared with the plesiomorphic rhabditid pattern for phylogenetic conclusions (Justine, 2002; Yushin & Malakhov, 2014; Yushin *et al.*, 2018; Zograf *et al.*, 2016). However, data on some major groups of Chromadorea, such as the order Araeolaimida, are poor or absent. Until recently, the ultrastructure of the araeolaimid spermatozoa was presented only as a brief non-illustrated outline of sperm development in two species (Yushin & Malakhov, 2004).

The first detailed study of spermatozoa from the female gonoduct of the araeolaimid nematode *Axonolaimus arcuatus* (Axonolaimoidea, Axonolaimidae) showed an intriguing new pattern of sperm structure (Yushin & Gliznutsa, 2019). The mature spermatozoa from the uterus of *A. arcuatus* in general fit to the usual rhabditid style as the amoeboid cells subdivided into a pseudopod and a main cell body with nucleus and standard peripheral organelles, *i.e.* mitochondria and MO. However, the latter are not numerous individual pouches with pores but large membranous cisterns each opening to the exterior *via* multiple pores. Such extensive MO as in *A. arcuatus* show marked deviation from the rhabditid pattern and proposed as an apomorphic character for Araeolaimida-Axonolaimidae (Yushin & Gliznutsa, 2019).

Observations on the ultrastructure of the spermatozoon development in the axonolaimid nematodes are needed to improve understanding of the basic patterns and diversity of male gametes in Araeolaimida. The ultrastructural features of spermatogenesis in the axonolaimid nematode *Parodontophora marisjaponici* Platonova, 1971 (Araeolaimida: Axonolaimidae) have been studied in details through examination of developing spermatocytes, spermatids and spermatozoa. These new observations allow us to draw conclusions about spermatogenesis in Axonolaimidae, where the origin of the hypertrophic MO is the most intriguing event. New data also allow brief discussion of sperm patterns in Araeolaimida and Chromadorea.

MATERIAL AND METHODS

Gravid males and females of *P. marisjaponici* were extracted from samples of sandy mud on August 2001 at a depth of 8 m in the Vostok Bay, the Sea of Japan (42°89'39" N; 132°73'28" E). 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 specimens were fixed overnight at 4°C in 2.5% glutaraldehyde in 0.05 M cacodylate buffer with 21 mg ml⁻¹ NaCl, 0.5 mg ml⁻¹ MgCl₂, 1% dimethylsulphoxide (DMSO) 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 Reichert Ultracut E and Leica UC6 ultramicrotomes, stained with uranyl acetate and lead citrate, and examined with JEOL JEM 100B, Zeiss Sigma 300 VP and JEOL JEM 1010 electron microscopes.

The ultrastructural descriptions of spermatocytes, spermatids and spermatozoa are based on observations of five males and three females. The end of the spermatid stage is marked by detachment of the residual body; the spermatozoa from testes and activated spermatozoa from the uteri are termed as 'immature' and 'mature' spermatozoa respectively (Shepherd, 1981).

RESULTS

Spermatocytes and spermatids. The male reproductive system of *P. marisjaponici* comprises two opposite outstretched testes each as a simple epithelial tube filled with germ cells. The distal tip of the testis contains spermatogonia followed by growing spermatocytes. The region of meiosis marks the boundary between spermatocytes and spermatids. The prominent proximal part of the testis (seminal vesicle) is filled with immature sperm.

The spermatocytes are large cells each with a nucleus having a well developed nucleolus; the cytoplasm of spermatocytes contains numerous mitochondria, cisterns of RER, ribosomes, Golgi bodies and numerous vesicles with dense matter (Fig. 1A). These cytoplasmic characters reflect high synthetic activity of the cell resulting in formation of the tripartite organelle complexes each consisting of a mitochondrion and a paracrystalline body surrounded by a large vesicle (Figs 1B-C & 2).

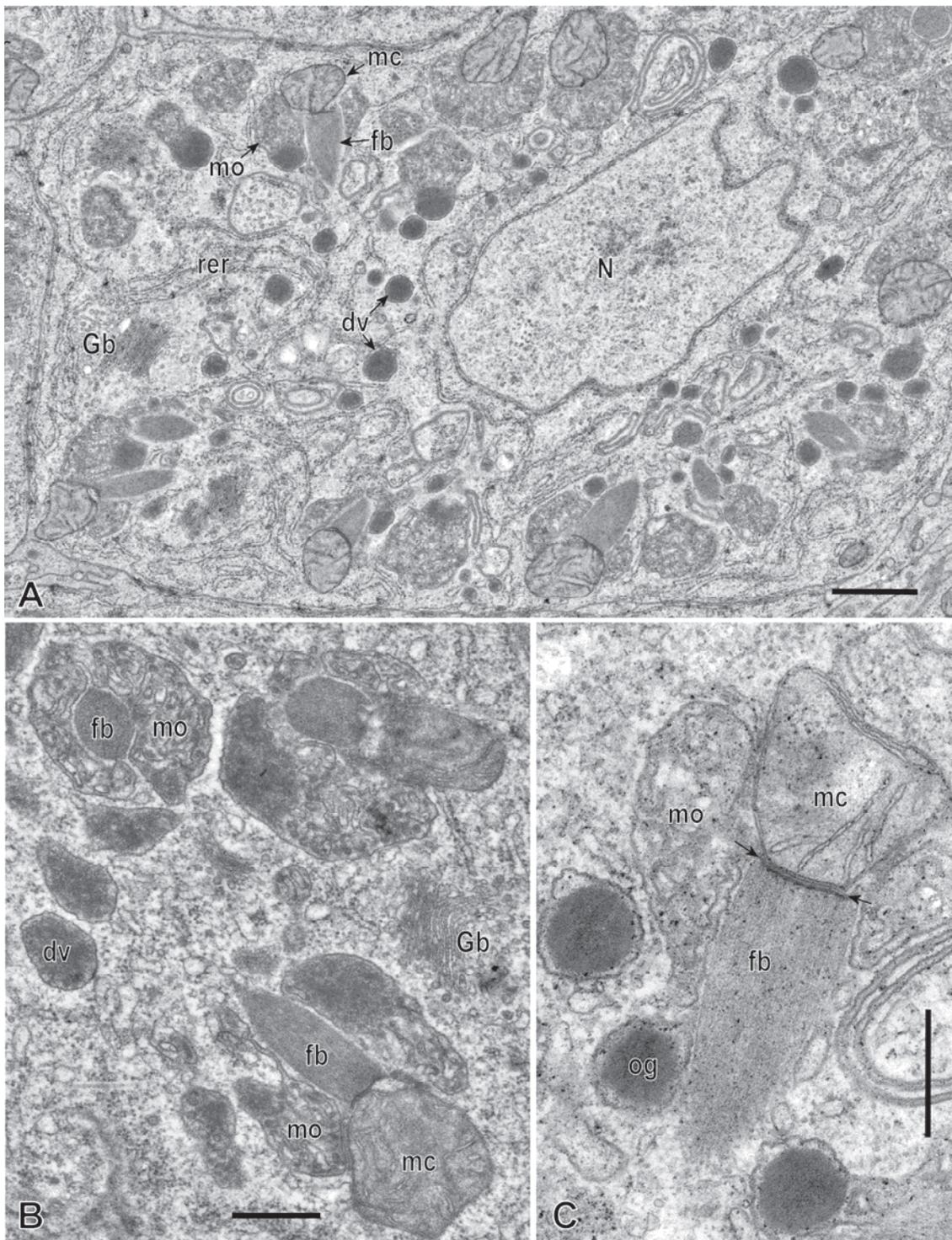


Fig. 1. *Parodontophora marisjaponici* spermatocytes, TEM. A: Early spermatocyte with newly formed tripartite organelle complexes containing mitochondrion (mc); fibrous body (fb) and membranous organelle (mo). B: Cross (upper left) and longitudinal (lower right) sections of the organelle complexes in the late spermatocyte. C: The late spermatocyte, longitudinal section of the organelle complex at higher magnification. Arrows mark the dense plate between the associated mitochondrion (mc) and fibrous body (fb). Abbreviations: dv, vesicles with dense matter; fb, fibrous body; Gb, Golgi body; mc, mitochondrion; mo, membranous organelle; N, nucleus; og, osmiophilic globules; rer, cisterns of RER. Scale bars: A = 1 μ m; B, C = 0.5 μ m.

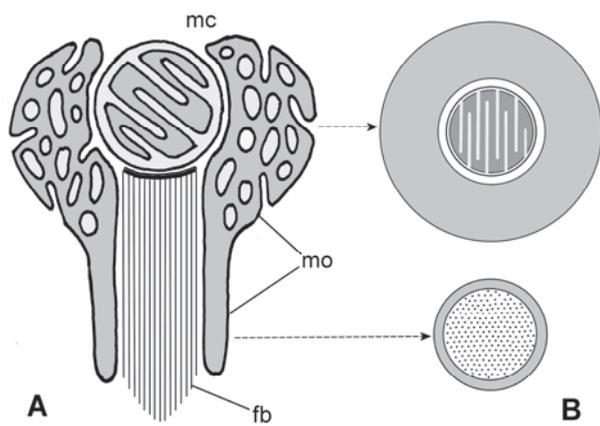


Fig. 2. Schematic explanation of components assembly in the tripartite organelle complexes from the spermatocytes of *Parodontophora marisjaponici*. A: Longitudinal section of the fully formed organelle complex containing mitochondrion (mc); fibrous body (fb) and membranous organelle (mo). B: Cross projections of the organelle complex at the levels marked by dotted arrows.

The paracrystalline bodies appear in the early spermatocytes as bundles of densely packed parallel fibres associated with mitochondria (Fig. 1A-C). The structure of the paracrystalline bodies is similar to the usual fibrous bodies (FB) of nematode sperm (Fig. 1B, C). The place of intimate contact of FB and mitochondrion is marked by characteristic dense plate between two components (Fig. 1C).

The large vesicles enveloping mitochondria and FB are derivatives of numerous Golgi bodies closely associating with organelle complexes and producing numerous vesicles and cisterns (Fig. 1A, B). The vesicles contain a system of internal membranes looking like invaginations of an outer membrane and may be identified as membranous organelles (MO) of typical nematode sperm (Fig. 1B, C).

The fully assembled organelle complex includes a mitochondrion, FB and MO arranged and orientated in uniform style (Figs 1A, C & 2). Mitochondrion and FB form the rod where the mitochondrion faces the periphery but the FB faces the centre of the cell (Fig. 1A). The MO envelops the central components forming a ring around the mitochondrion and FB (Figs 1B, C & 2). In the terminal spermatocytes the FB are about 1 μm long, and the diameter of the ring of MO reaches 1.5 μm at the mitochondrion level.

Numerous vesicles with dense matter appear in early spermatocytes (Fig. 1A), and later their content seem to accumulate as large osmiophilic globules inside the MO of the organelle complexes

(Fig. 1B, C). It must be mentioned that spermatocytes contain no mitochondria, bipartite FB-MO complexes as well as separate MO or FB outside the tripartite complexes.

The stages of meiosis have not been observed in the testes studied; the terminal spermatocytes adjoin with the spermatids, which have a condensing nucleus without nuclear envelope surrounded by organelle complexes (Fig. 3A). In the early spermatids complexes are individual components of the same structure and are radially orientated as in the late spermatocytes.

The majority of the spermatid zone is occupied by developing spermatids with concentric arrangement of the cell components. These are rounded cells 7-8 μm in diameter with a central condensed nucleus surrounded by pale space without organelles and mass of peripheral organelles (Fig. 3B). The centrioles of nine doublets (9×2) were detected at the nucleus (Fig. 4D).

The nuclear centre is surrounded by the transformed complex of organelles, which still consists of mitochondria, FB and MO (Fig. 3B, C). However, there is an increase in MO, which seem to be fused to form a general giant cistern enveloping all pairs of mitochondria and FB. The sections through the centre of the cell show MO as dividing into several compartments by mitochondria and FB that continue to be orientated towards peripheral and central cytoplasm, respectively (Fig. 3B, C). However, tangential sections at different levels show continuity of MO as the hypertrophied lamellar membranous system with cylindrical holes through, each containing a mitochondrion and a FB (Fig. 4A-C). The FB in spermatids increase up to 1.3 μm long and 0.8 μm wide; they start to detach from mitochondria and move to the centre of the cell (Fig. 3B, C). The united MO contains numerous osmiophilic globules inside (Figs 3B, C & 4A, B).

The concentric arrangement of the spermatid cytoplasm is enhanced by displacement of synthetic components (ribosomes, RER, Golgi bodies) to the periphery of the residual cytoplasm (Figs 3B, C & 4A). At the final development of the spermatid the residual cytoplasm is concentrated at one of the cell poles and detaches as the compact residual body. The cell forming after detachment of the residual body is considered here as the immature spermatozoon.

Spermatozoa. Newly formed immature spermatozoa are characterised by wide transparent cytoplasm that surrounds the central nucleus and includes free FB now extruded from MO (Fig. 5A), the MO and mitochondria surrounding the central cytoplasm being shifted to the cell periphery.

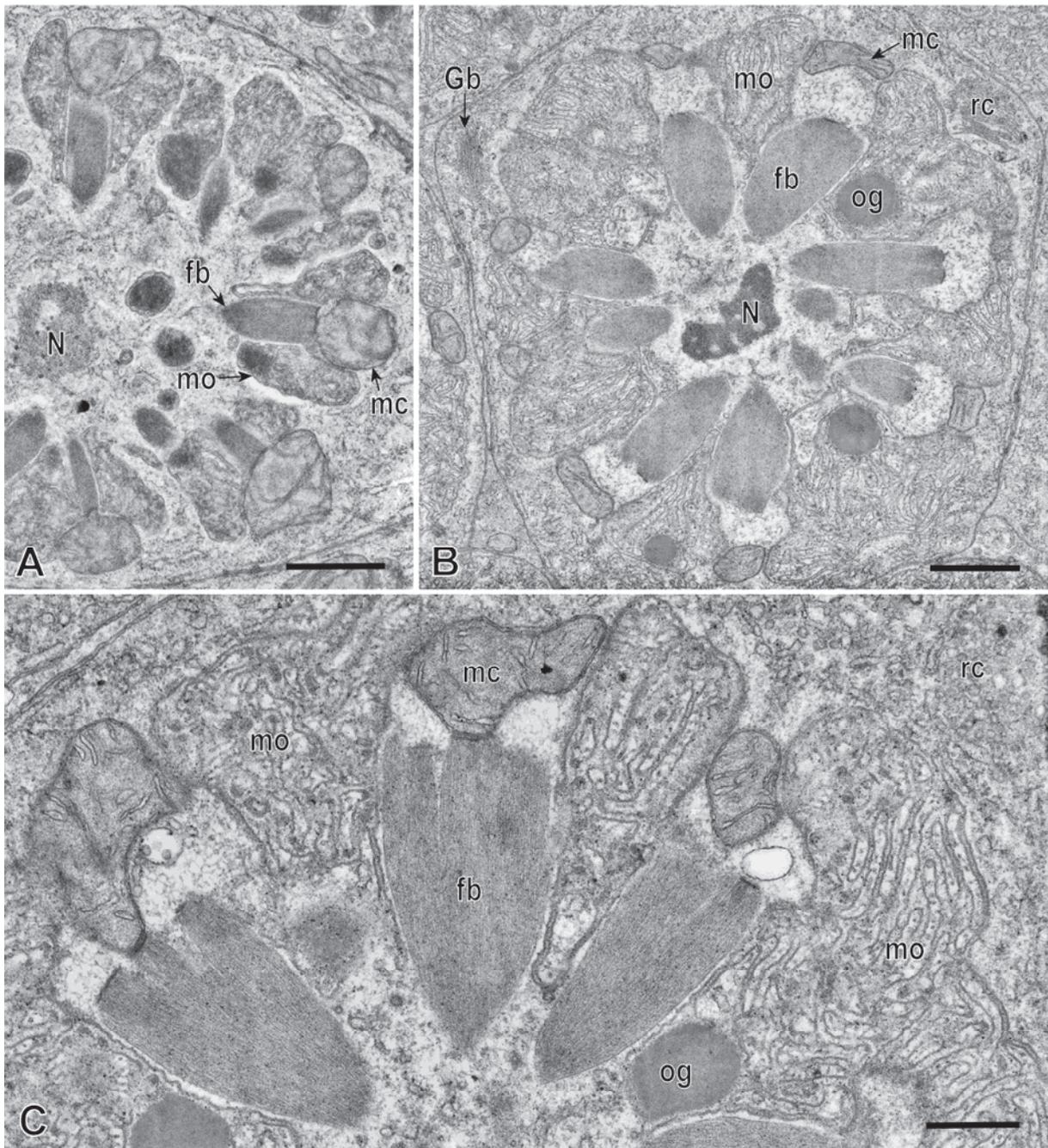


Fig. 3. *Parodontophora marisjaponici* spermatids, TEM. A: Early spermatid with separate organelle complexes. B: The developing spermatid, overview. C: High magnification of the part of the organelle complex in the spermatid; section runs through the centre of the cell. Abbreviations: fb, fibrous body; Gb, Golgi body; mc, mitochondrion; mo, membranous organelle; N, nucleus; og, osmiophilic globules; rc, residual cytoplasm. Scale bars: A, B = 1 μm ; C = 0.5 μm .

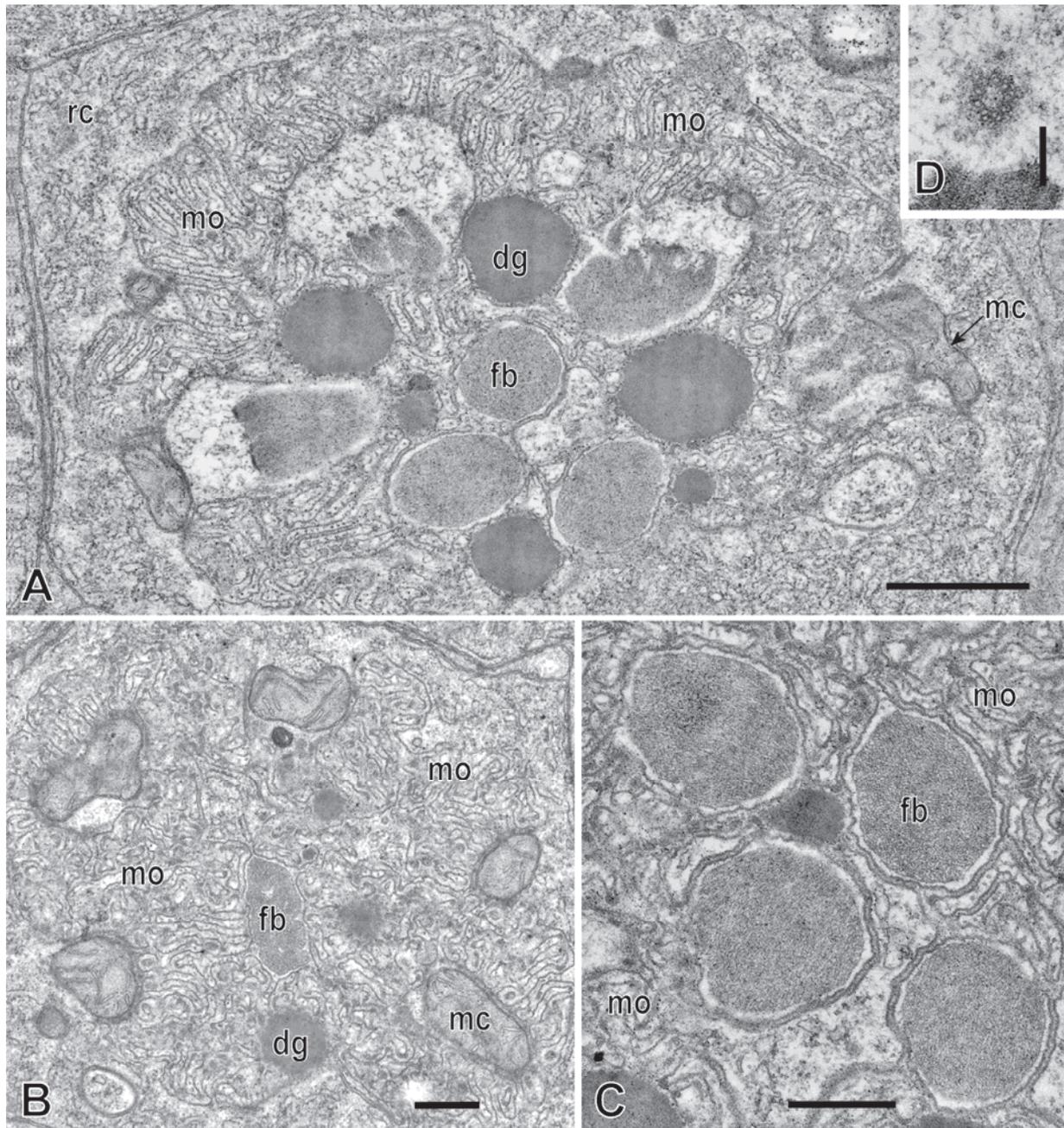


Fig. 4. *Parodontophora marisjaponici*, tangential sections of the spermatid periphery, TEM. A: Section showing continuity of membranous organelle. B: Section at the level of the peripheral mitochondria (mc) enveloped by continuous membranous organelle (mo). C: Cross sections of fibrous bodies (fb) enveloped by membranous organelle (mo). D: Cross section of the spermatid centriole. Abbreviations: fb, fibrous body; mc, mitochondrion; mo, membranous organelle; og, osmiophilic globules; rc, residual cytoplasm. Scale bars: A = 1 μm ; B, C = 0.5 μm . D = 0.2 μm .

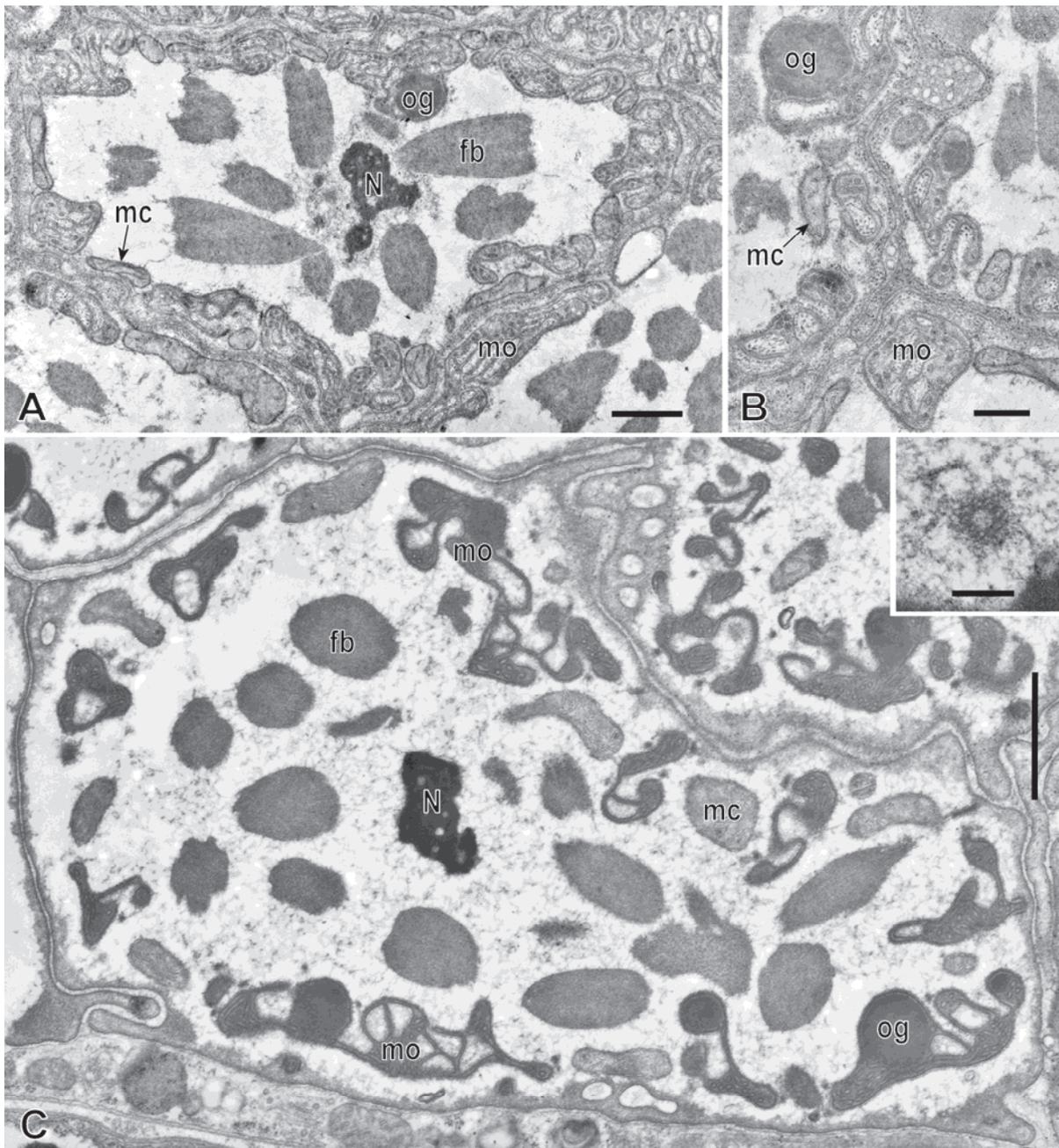


Fig. 5. *Parodontophora marisjaponici* immature spermatozoa, TEM. A and B: The newly formed immature spermatozoon: overview (A); periphery of three neighbouring spermatozoa (B). C: Fully formed immature spermatozoon, overview. Insert: the cross section of the immature sperm centriole. Abbreviations: fb, fibrous body; mc, mitochondrion; mo, membranous organelle; N, nucleus; og, osmiophilic globules. Scale bars: A, C = 1 μm ; B = 0.5 μm ; insert in C = 0.2 μm .

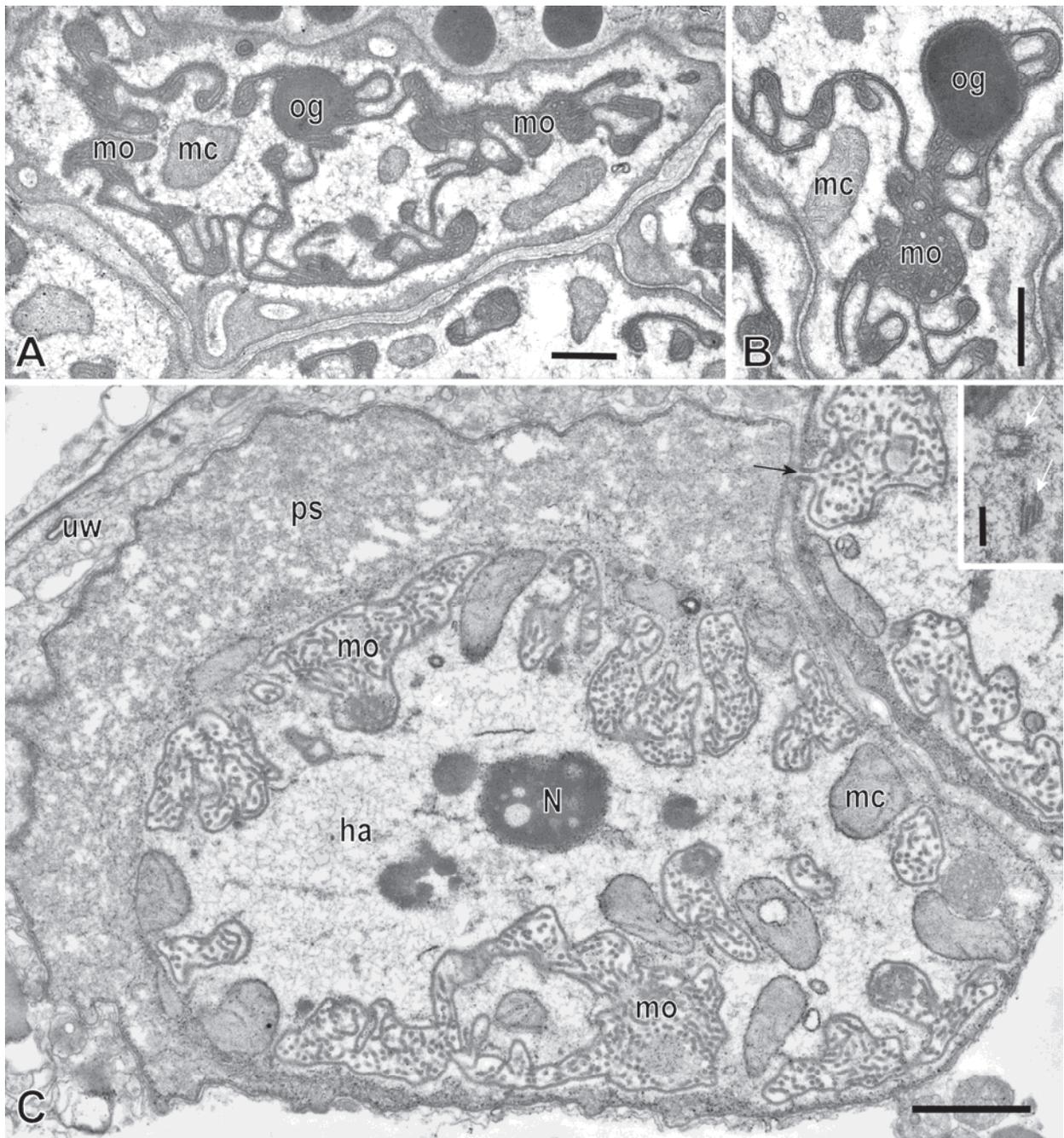


Fig. 6. *Parodontophora marisjaponici* spermatozoa, TEM. A and B: Immature spermatozoa, (A) the tangential sections through the cell periphery showing continuity of membranous organelle; (B) membranous organelle structure at higher magnification. C: Mature spermatozoon in uterus, overview; black arrow marked duct of membranous organelle. Insert: Oblique and tangential sections of the pair of centrioles (white arrows) in the mature spermatozoon. Abbreviations: ha, halo; mc, mitochondrion; mo, membranous organelle; N, nucleus; og, osmiophilic globules; ps, pseudopod; uw, uterus wall. Scale bars: A, B = 0.5 μm ; C = 1 μm ; insert in C = 0.2 μm .

The lamellar structure of MO in immature spermatozoa is transformed into tubular membranous elements crossing internal content of moderate density (Fig. 5B). The osmiophilic globules persist inside the transformed MO.

The finally formed immature spermatozoa are densely packed in the seminal vesicle; they are unpolarised cells of variable shape and uniform in internal structure (Fig. 5C). The cells may be only approximately evaluated as 7-8 μm in size.

The central nucleus has an irregular outline and lacks a nuclear envelope. The centrioles of nine doublets (9×2) were detected near the nucleus (Fig. 5C, insert). The central cytoplasm of the spermatozoon is transparent and contains FB, which now are randomly orientated. The MO and mitochondria occupy the cell periphery; the MO became a complicated labyrinth-like system of cisterns united by thin bridges (Figs 5C & 6A, B). The cisterns of MO are filled with osmiophilic matter crossed by tubular membranous elements. Osmiophilic globules are stored in dilated parts of the cisterns (Figs 5C & 6A, B). As was observed in spermatids, mitochondria on the sections disrupt the membranous net; however, observations of the tangential sections shows continuity of MO as one hypertrophied cistern enveloping mitochondria (Fig. 6A, B).

The female reproductive system of *P. marisjaponici* consists of two branches; each includes an outstretched ovary, oviduct and uterus. The uterus lumen of the females observed was filled with fertilised eggs with eggshell. On the thin sections observed with TEM the spermatozoa were found in the uterus lumen at the distal tip adjoining the oviduct. These are mature (activated) spermatozoa that have irregular outlines and may be estimated only approximately as 7-8 μm in size.

The mature spermatozoa are polarised cells each subdivided into a pseudopod and a main cell body with concentric arrangement of the cell components (Fig. 6C). The central nucleus is surrounded by a broad transparent halo where FB are no longer present; a pair of centrioles were detected near the nucleus (Fig. 6C and insert).

The cell periphery is occupied by layer of organelles, *i.e.* mitochondria and large dilated MO (Figs 6C & 7A-D). As it was shown in the previous stages, the sections through the cell centre show mitochondria disrupting MO into separate compartment (Fig. 6C). However, examination of the tangential sections shows continuity of MO, which possibly comprises one giant net-like cistern enveloping numerous mitochondria (Fig. 7A, B).

The structure of MO in the mature spermatozoon changes drastically. The MO transforms into the dilated cistern with transparent space (Fig. 7B, D). Numerous tubular invaginations of the outer membrane cross the internal space of MO. The dense content of the MO disappears but numerous osmiophilic globules are still stored inside together with groups of small transparent vesicles (Fig. 7A-D).

Transparency of MO coincides with the formation of 0.2 μm long ducts that open to the exterior *via* pores 0.15 μm in diameter (Figs 6C & 7C). These

ducts are numerous throughout the entire MO and, as a result, several ducts of one branch of MO may be easily detected on one section (Fig. 7D).

The pseudopods of the mature spermatozoa have irregular contours, contain no organelles and are filled with fibrous material that also form a submembranous layer in the main cell body (Fig. 6C). The surface of the spermatozoon is smooth and bears no filopodia.

DISCUSSION

The structure of the mature spermatozoa of *P. marisjaponici* is in general agreement with spermatozoa previously described in the nematode *Axonolaimus arcuatus* belonging to the same family Axonolaimidae of the order Araeolaimida (Yushin & Gliznutsa, 2019). In general, these are clearly polarised cells subdivided into a pseudopod and a main cell body that contains a central nucleus without nuclear envelope surrounded by a layer of peripheral organelles, *i.e.* mitochondria and MO. In both cases, the nucleus and peripheral organelles are separated by wide space (halo) that is arranged as the characteristic concentric structure of the main cell body.

The mature spermatozoa of the two axonolaimids in general follow the plesiomorphic rhabditid pattern of spermatozoa outlined in the introduction and well known for a variety of representatives of the order Rhabditida and Monhysterida (Noury-Sraïry *et al.*, 1993; Justine, 2002; Giblin-Davis *et al.*, 2010; Yushin & Malakhov, 2004, 2014; Zograf, 2014; Slos *et al.*, 2015; Yushin *et al.*, 2016, 2018; Yushin & Gliznutsa, 2019). However, mature spermatozoa in both species demonstrate a clear deviation in structure of MO that is unusual for nematodes. These MO are remarkably large membranous cisterns each opening to the exterior *via* multiple pores.

Preliminary non-illustrated data on sperm development in *P. marisjaponici* revealed some key events, which also suggested the rhabditid pattern of spermatogenesis, *i.e.* MO-FB complexes appearing in spermatocytes and dissociating into separate organelles in spermatids and immature spermatozoa (Yushin & Malakhov, 2004). These authors also mentioned that each FB-MO complex includes a mitochondrion. In the activated sperm from the uterus, MO join to plasmalemma and FB transform into the pseudopod cytoskeleton.

Our detailed observations of spermatocytes in *P. marisjaponici* reveal formation of the tripartite organelle complexes each consisting of a mitochondrion, a paracrystalline body (FB) and MO

with internal system of membranes. Mitochondria are joined to FB by characteristic dense plate forming the bipartite rods each enveloped by ring-shaped MO. In the nematode spermatogenous cells mitochondria usually are free components never associating with other organelles (Justine, 2002; Yushin & Malakhov, 2014).

In *A. arcuatus* the large ramified MO were interpreted as a result of fusion of previously individual MO during sperm development (Yushin & Gliznitsa, 2019). Our observations directly confirm this hypothesis. In the spermatids of *P. marisjaponici* fusing MO are transformed into a giant cistern, which

envelopes all mitochondria and FB. This sole MO now has characteristic lamellar arrangement of internal membranes. In the late spermatids mitochondria and FB dissociate and the latter move out of MO toward the centre of the cell. In the immature sperm, the giant MO transforms into labyrinth-like system of cisterns united by thin bridges. The lamellar internal arrangement of membranes now transformed into tubular membranous elements is reminiscent of finger-like projections of the outer membrane of usual MO (Justine, 2002; Yushin & Malakhov, 2014). In contrast to the spermatids the content of MO in the immature spermatozoa becomes strongly osmiophilic.

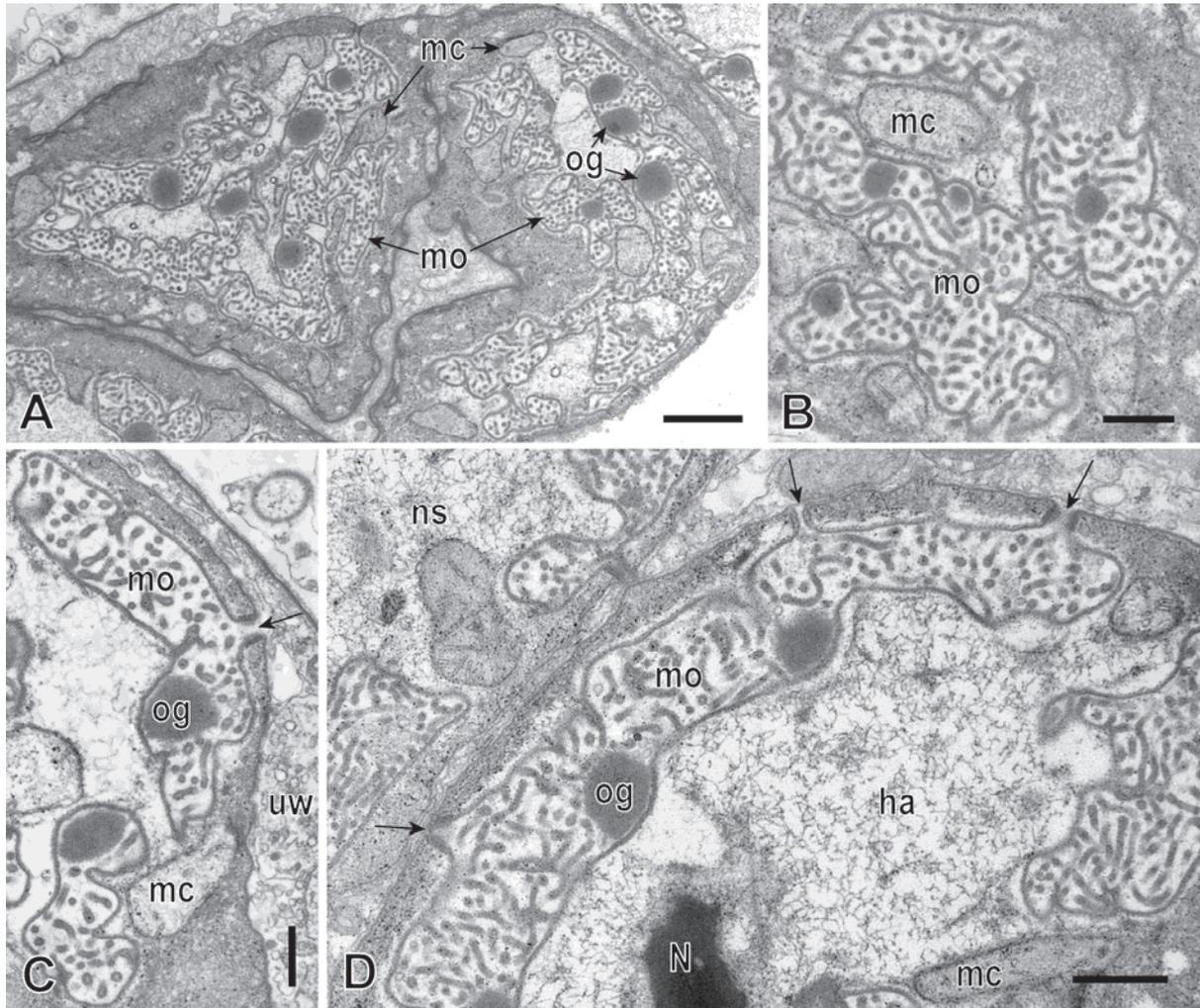


Fig. 7. *Parodontophora marisjaponici* mature spermatozoa, TEM. A: Tangential sections through the cell periphery of two spermatozoa showing continuity of membranous organelle. B: Higher magnification of tangential section showing continuity of membranous organelle (mo) and position of mitochondria (mc). C: The sperm periphery at higher magnification, the cistern of membranous organelle opens through the duct (arrow) to exterior. D: Section demonstrating the extensive cistern of the MO that opens to exterior by at least three ducts (arrows). Abbreviations: ha, halo; mc, mitochondrion; mo, membranous organelle; N, nucleus; ns, neighboring spermatozoon; og, osmiophilic globules; uw, uterus wall. Scale bars: A = 1 μ m; B-D = 0.5 μ m.

Finally, in the mature spermatozoa of *P. marisjaponici* and *A. arcuatus* the MO is represented by a giant dilated cistern with numerous pores, reflecting the original state of MO as the separate organelles first appeared in spermatocytes (Yushin & Gliznutsa, 2019). The pseudopod cytoskeleton in the mature spermatozoa of two axonolaimids is the derivative of FB formed in the spermatocytes together with MO and stored in the immature spermatozoa up to their activation.

In the nematode orders of the class Chromadorea the plesiomorphic rhabditid pattern of spermatozoa was found, apart from the order Rhabditida only in monhysterids (Monhysterida-Sphaerolaimoidea) (Noury-Sraïry *et al.*, 1993; Justine & Jamieson, 1999; Justine, 2002; Yushin *et al.*, 2018). The diversity of the sperm patterns in Chromadorea is poor and, as well as the nearly uniform rhabditid pattern, includes reduction of MO or even of all specific organelles (Yushin & Comans, 2000; Justine, 2002; Yushin & Malakhov, 2004, 2014; Yushin *et al.*, 2016, 2017; Zograf *et al.*, 2016). Only one strong deviation with totally unique pattern was described for the rhabditid *Aspiculuris tetraptera* (Oxyuridomorpha) where spermatogenesis includes fusion of mitochondria with the haploid nucleus into the remarkable axial rod (Lee & Anya, 1967).

Spermatogenesis of the axonolaimid *P. marisjaponici* includes formation of tripartite FB-MO complexes with mitochondria (spermatocytes), fusion of MO into giant cistern with lamellar internal membranes (spermatids), and transformation of MO into labyrinth-like system of osmiophilic cisterns (immature spermatozoa). As a result, the mature spermatozoa in two axonolaimids have distinctive MO as the extensive cistern with multiple pores (Yushin & Gliznutsa, 2019). The sole MO developing from fusion of numerous MO has never been observed before in the chromadorean spermatozoa; the ontogeny and structure of these spermatozoa present deep deviation from the plesiomorphic rhabditid pattern and we propose this character as an apomorphy for Araeolaimida-Axonolaimidae (Yushin & Gliznutsa, 2019).

The initial tripartite complexes that includes FB, MO and mitochondria also are unusual components never described before in spermatogenous cells of nematodes and may be considered as additional character of apomorphy of axonolaimids.

Brief non-illustrated description of sperm development in the araeolaimid nematode *Sabatieria palmaris* presenting another family of the order, Comesomatidae, includes information on typical paracrystalline FB that appear inside the sole giant MO formed in the spermatids by fusion of

numerous MO (Yushin & Malakhov, 2004). This hypertrophied FB-MO complex persists in spermatozoa and may be preliminary compared with the analogous complex described in *P. marisjaponici*. This remarkable evidence of similarity may be of important value for discussion of general 'araeolaimid' pattern of spermatogenesis and spermatozoa. However, new detailed study of the comesomatid spermatozoa is necessary for fruitful comparative analysis.

It must be mentioned that in another class of nematodes, Enoplea, the giant cavity with internal tubular processes and characteristic pores opening to exterior were described in the immature spermatozoa of the free-living marine nematode *Halalaimus dimorphus* (Enoplida, Oxystominidae) (Turpeenniemi, 1998). Descriptions and electron micrographs presented in this paper apparently show a sole hypertrophied MO located posteriorly to the nucleus. This specific MO of *H. dimorphus* may be considered only as convergent in origin when compared with analogous sperm components from such a distant taxon as Araeolaomida. However, araeolaimids and enoplids in this case demonstrate remarkable plasticity for parallel evolution of nematode spermatozoa.

CONCLUSION

The araeolaimid nematodes from the family Axonolaimidae show two distinct peculiarities in morphology and differentiation of male gametes: i) tripartite FB-MO complexes that appear in spermatocytes and include mitochondria; and ii) fusion of initially separate MO into one giant cistern that in the mature spermatozoa opens to the exterior *via* multiple pores. This type of sperm development and structure has never been detected in nematodes from the class Chromadorea and we propose it as an apomorphy of the family Axonolaimidae. The study of spermatogenesis in other families inside the order Araeolaimida such as Comesomatidae will be fruitful for further analysis of sperm diversity in Chromadorea.

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В.В. Юшин и Л.А. Глизнаца. Ультраструктурное исследование сперматогенеза у свободноживущей морской нематоды *Parodontophora marisjaponici* (Nematoda: Araeolaimida: Axonolaimidae).

Резюме. Сперматогенез у *Parodontophora marisjaponici* по основным признакам сходен с таковым у рабдитидных нематод. Развитие сперматозоидов включает образование комплексов волокнистых тел (ВТ) и мембранных органелл (МО), которые появляются в сперматоцитах; комплексы диссоциируют в сперматидях; незрелые сперматозоиды содержат отдельные ВТ и МО, зрелые сперматозоиды представляют собой биполярные клетки, разделенные на псевдоподию и главное тело клетки, содержащее ядро без ядерной оболочки, периферические митохондрии и МО. В морфологии и дифференцировке мужских гамет *P. marisjaponici* имеет ряд особенностей: i) трехкомпонентные комплексы ВТ-МО, которые включают митохондрии; ii) слияние первоначально отдельных МО в одну гигантскую цистерну, которая в зрелых сперматозоидах открывается наружу через многочисленные поры. Такой тип развития и строения сперматозоидов представляет собой уникальный паттерн сперматогенеза нематод и его следует рассматривать в качестве апоморфного признака семейства ареолаймид Axonolaimidae. Изучение сперматогенеза в других семействах отряда Araeolaimida, таких как Comesomatidae, будет полезным для дальнейшего анализа разнообразия сперматозоидов у нематод.