

Ultrastructure of spermatozoa in the free-living marine nematode *Monoposthia costata* (Chromadoria, Desmodorida)

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Summary. The spermatozoa from both male and female of the free-living marine nematode, *Monoposthia costata* (Desmodorida, Monoposthiidae), were studied using electron microscopy. The spermatozoa are cells, the surface of which is covered with numerous filopodia. Nuclei of the sperm cells are taper-shaped and composed of condensed chromatin without a nuclear envelope. There is a centrally located electron transparent halo filled with mitochondria. The peripheral cytoplasm is devoid of organelles and filled with filaments, which also strengthens the filopodia. The spermatozoa of *M. costata* exhibit the main ultrastructural features characteristic of nematodes: the absence of an acrosome, an axoneme and a nuclear envelope. However, in spermatozoa of *M. costata* and other desmodorid studied the membranous organelles were not found. In this respect sperm cells of *M. costata* differ from those in monhysterids and secernents, but resemble those of Chromadorida. It could be evidence of the phylogenetic closeness of these two orders of nematodes.

Key words: spermatogenesis, TEM, membranous organelles (MO), fibrous bodies (FB), filopodia, microtubules, MSP.

Different approaches to the problems of phylogeny of the phylum Nematoda generate questions concerning the correct positions of the groups. At the same time the application of alternative characteristics could be helpful. Spermatogenesis itself is a characteristics-rich process, while sperm structure is species-specific (Danilova, 1982).

The nematode spermatozoa are considered to represent an aberrant type of male gametes (Bacceti & Afzelius, 1976). Their characteristic features are the absence of an axoneme, an acrosome, and, with the exception of the members of the order Enoplida (Yushin & Malakhov, 1998), a nuclear envelope (Justine & Jamieson, 1999; Justine, 2002). The general pattern of nematode spermatozoon is a bipolar amoeboid cell subdivided into an anterior pseudopodium and a posterior main cell body (MCB). The MCB contains a condensed nucleus surrounded with mitochondria and so-called membranous organelles (MO). These unique organelles are characteristic of most nematode spermatozoa (Justine & Jamieson, 1999; Justine, 2002). MOs are derived from Golgi bodies and appear as parts of complexes with paracrystalline fibrous bodies

(FB). These MO-FB complexes dissociate during spermatogenesis into: *i*) separate MO, which move to the periphery, attach to the spermatozoon plasma membrane and open to the exterior *via* pores; and *ii*) free FB, which transform into the pseudopodium cytoskeleton during spermatozoon activation (Foor, 1983; Justine & Jamieson, 1999).

The main focus in the analysis of nematode spermatogenesis was the origin and fate of the unique male gametes structures, MO and FB. The results of investigations seem to justify this approach because of the peculiarity of the processes in different taxonomic groups. The investigations of spermatogenesis in nematodes have revealed several main types of the aberrant structures development that can characterize higher nematode taxa, including families and orders (Yushin & Malakhov, 2004).

For the representatives of the subclass Chromadoria (according to the classification by De Ley & Blaxter, 2002) the development of both types of the aberrant organelles is true for most of the representatives of the orders Monhysterida and Rhabditida (Yushin & Malakhov, 2004). Chromadorids represent two types of aberrant organelles developmental pathways: *i*) the presence

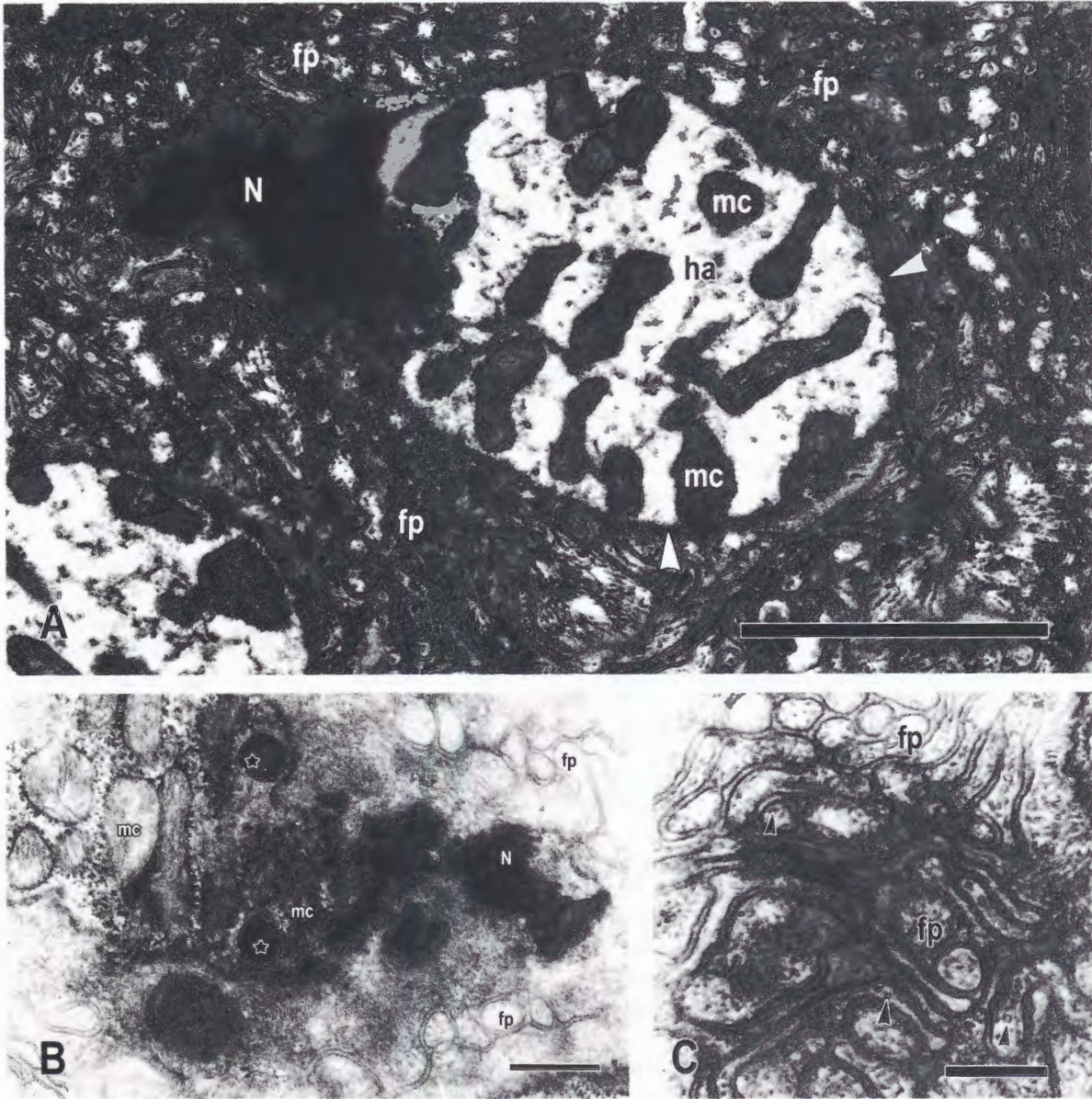


Fig. 1. *Monoposthia costata*, immature spermatozoa from testes. A: Spermatozoon from the testis, general view. Taper-shaped nucleus (N) is devoid of nuclear envelope. Electron transparent halo (ha) is filled with mitochondria (mc) and surrounded with the electron-dense layer (arrowheads). Numerous filopodia (fp) cover surface of the spermatozoon. B: Posterior part of the spermatozoon at high magnification. Nucleus (N) is shifted to the periphery of the cell. Mitochondria (mc) are randomly scattered in the central part of the spermatozoon. There are a few electron dense bodies (asterisk) in between mitochondria. The surface of the sell is covered with filopodia (fp). C: Section through the filopodia (fp), note microtubule like fibers (arrowheads) fill filopodia. Scale bars: A = 2 μm ; B = 1 μm ; C = 0.5 μm .

of just FB; and *ii*) the absence of the both types of aberrant structures (Yushin & Malakhov, 2004). The investigated spermatogenesis of desmodorid *Metachromadora itoi* (representative of the family Desmodoridae) revealed the absence of MO with the presence of FB (Yushin & Coomans, 2005). Such a deviation in the structure of male gametes in desmodorids, as a submicroscopic characteristic, should attract the attention of nematologists and scientists dealing with studies on sperm evolution in other multicellular animals. The data on the sperm structure in another species of the order Desmodorida, *Monoposthia costata* (Bastian, 1865) (family Monoposthiidae), would provide the opportunity to evaluate accurately the significance of the cytological characteristic for the developing natural system of the order Desmodorida and the phylum of nematodes in general.

MATERIAL AND METHODS

Adult males and females of *M. costata* were collected from silty sand at 1 m depth at the Vostok Marine Biological Station of the Institute of Marine Biology (Great Peter Bay, the Sea of Japan). Live males were cut into pieces each containing a whole testis. Females were cut at the

head and tail regions to obtain a piece containing ovaries and uteri. These specimens were fixed for transmission electron microscopy (TEM) at 4°C in 2.5% glutaraldehyde in 0.05 M cacodylate buffer containing 21 mg ml⁻¹ NaCl and then postfixed in 2% osmium tetroxide in the same buffer containing 23 mg ml⁻¹ NaCl. Postfixation was followed by *en bloc* staining in a 1% solution of uranyl acetate in distilled water; then the specimens were dehydrated in an ethanol series followed by an acetone series and embedded in Spurr resin (Spurr, Sigma). Thin sections were cut with a Reichert Ultracut E ultratome, stained with lead citrate, and subsequently examined with a JEOL JEM 100S electron microscope. The reproductive system of five males and females were examined for the present paper.

RESULTS

The immature spermatozoa are elongated cells that exceed 8.5–9.5 µm length and 3.5–5 µm width with the periphery covered with numerous filopodia (Figs 1A, 4A). Each filopodium is about 0.05 – 0.1 µm thick (Fig. 1C). The taper-shaped nucleus without nuclear envelopment is situated in a posterior part of the cell (Fig. 1A, B). It is about

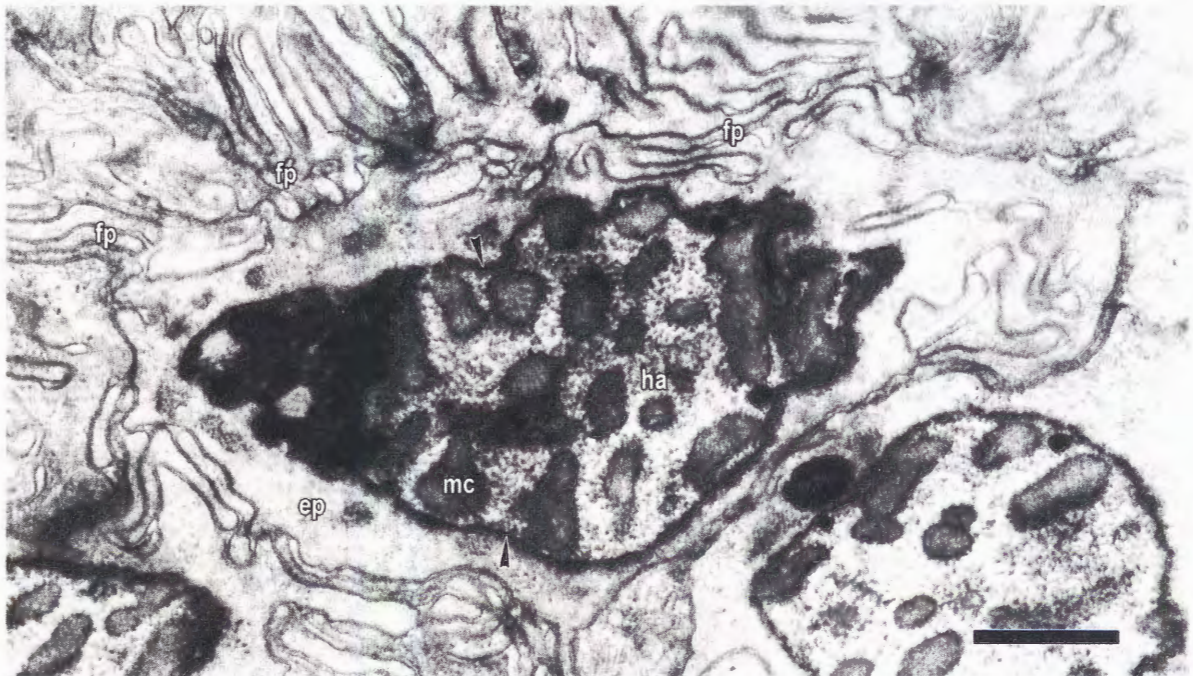


Fig. 2. *Monoposthia costata*. Mature spermatozoon from the spermatheca. Posteriorly located nucleus (N) devoid of nuclear envelope. Halo (ha) is filled with mitochondria (mc) and surrounded with electron dense material (arrowheads). Surface of the cell forms numerous filopodia (fp). ep – ectoplasm. Scale bar: 2 µm.

1.2-1.5 μm in its widest region and 1.5 μm high. Right under the nucleus in the bulk of electron-transparent cytoplasm the number of mitochondria and membranous shreds are situated (Figs 1B, 2A). The mitochondria differ in form from elongated to roundish and may reach a considerable size of over 1 μm . A thin electron-dense layer about 0.06-0.08 μm surrounds the central electron-transparent part of the cytoplasm (Fig. 1A). The peripheral electron-dense cytoplasm lacks organelles and is filled with filaments of about 28-34 nm in cross section (Fig. 1A, C). The filopodia are also filled with filaments (Fig. 1C). Some of the sperm cells seen on the longitudinal section of the nematode body contain a few osmiophilic rounded bodies up to 0.5 μm in diam. (Fig. 1B).

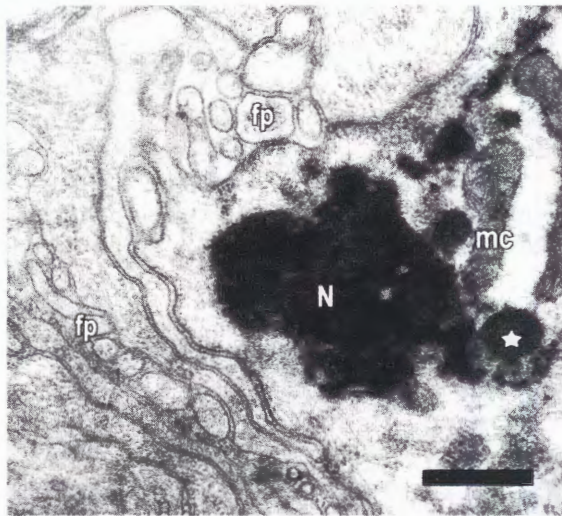


Fig. 3. *Monoposthia costata*. Fragment of mature spermatozoon. Filopodia (fp) is filled with microtubule like fibers. mc – mitochondria; N – nucleus; asterisk – electron dense body. Scale bars: 0.5 μm .

The mature sperm cells are located in a spermatheca, a specialized region of the female reproductive system. The nucleus of the spermatozoon from the female reproductive system is taper-shaped and shifted from the centre of the cell to the periphery (Figs 2, 4B). It is about 1.2-1.5 μm in its widest region and 1.5 μm high and devoid of nuclear envelope. Chromatin condensation is similar to that of spermatozoa from the seminal vesicle. A centrally located electron transparent halo is filled with mitochondria 0.9-2.3 μm length. A few round-shaped electron dense bodies could be found in between mitochondria (Fig. 3). Thin electron

dense layer (0.08-0.1 μm thick) surrounds the central part of the spermatozoon including mitochondria and dense bodies (Fig. 2). The surface of the spermatozoon is covered with numerous filopodia (0.2-0.4 μm in diam.) of different length and shape. Ectoplasm of the sperm cell and filopodia are filled with filaments of about 28-30 nm in diameter (Fig. 3).

Spermatozoa of *Monoposthia costata* are conventional for nematodes in having no acrosome, axoneme or nuclear envelope.

DISCUSSION

The ultrastructure of sperm cells of a representative of the order Desmodorida, *Monoposthia costata* has been investigated. The cells represent the typical pattern of nematode spermatozoa and are devoid of an acrosome, an axoneme and a nuclear envelope. In contrast with the most nematode sperms studied, spermatozoa of *M. costata* are also devoid of aberrant organelles, membranous organelles and fibrous bodies, unique organelles described for most of the rhabditids; in addition, mature spermatozoa from the females of *M. costata* are devoid of a pseudopodium.

The absence of aberrant organelles is shown to occur in a number of species of the subclass Chromadoria. In this context free FBs are well developed and possibly have the same form as FBs of other nematodes while the MO are totally absent. This pattern was described in Rhabditida (infraorder Tylenchomorpha, superfamily Tylenchoidea) and free-living marine nematodes from the orders Chromadorida and Desmodorida (Shepherd *et al.*, 1973; Goldstein & Triantaphyllou, 1980; Shepherd & Clark, 1983; Cares & Baldwin, 1995; Endo *et al.*, 1998; Yushin & Zograf, 2002; Zograf & Yushin, 2004; Zograf *et al.*, 2004; Yushin & Coomans, 2005). FBs are described as electron-dense bodies consisting of either paracrystalline fibers or filamentous material. We observed that the round electron-dense bodies in the cytoplasm of the spermatozoa of *M. costata* differed morphologically from FBs of other nematodes studied. Moreover, these bodies remain intact in mature spermatozoa form the uterus of females. So we presume that spermatozoa of *M. costata* are devoid of both MO and FB.

The absence of MO and FB in spermatozoa of nematodes was described for three representatives of the order Chromadorida from two different families (Yushin & Coomans, 2000; Yushin & Zograf, 2004). At the same time other chromadorids studied have been shown to have FBs (Yushin & Zograf, 2002; Zograf & Yushin, 2004). Thus, the absence of FBs

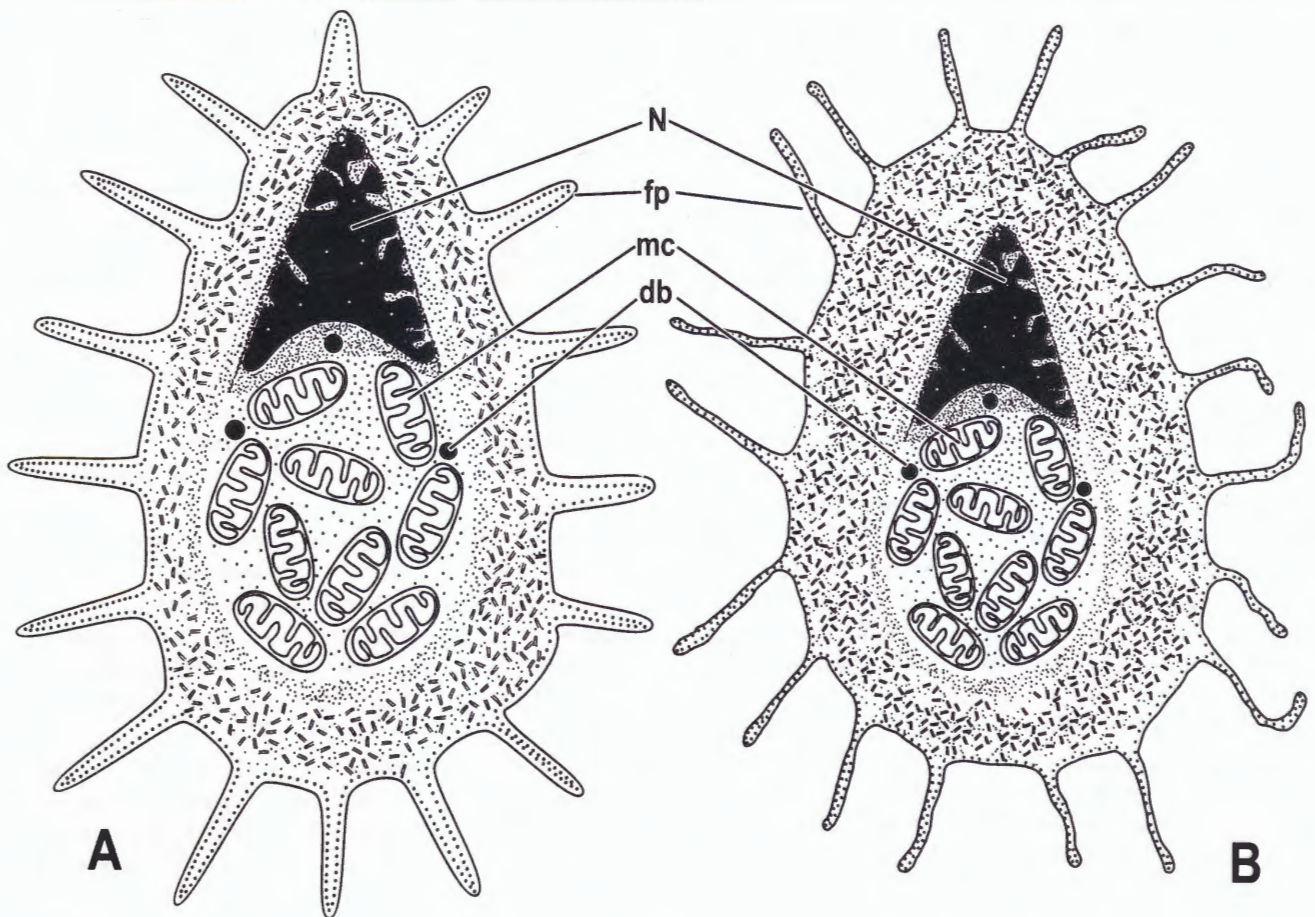


Fig. 4. *Monoposthia costata*, schematic representation of the spermatozoon structure. A: Immature spermatozoon from testis. B: Mature spermatozoon from spermatheca. Electron-transparent halo (halo) underlies the nucleus (N) and is filled with mitochondria (mc). The surface of the cell is covered with numerous filopodia.

is proposed to be a result of a secondary reduction (Yushin & Coomans, 2000). To date, the only representative of the order Desmodorida studied, *Metachromadora itoi*, has been shown to possess FBs (Yushin & Coomans, 2005). We may expect that the meaning of whether FBs are present or absent will be discovered when a wider range of representatives of the order Desmodorida is studied.

The absence of FB does not mean the absence of the major sperm protein (MSP) and other proteins of the cytoskeleton of nematode spermatozoa; it means only the absence of the stage of the condensation of the cytoskeleton proteins into FB (Yushin & Zograf, 2004). The presence of so-called microtubule-like fibers (MLF) has been shown for a number of species (Shepherd & Clark, 1983; Poinar & Hess-Poinar, 1993; Turpeenniemi, 1998; Yushin & Zograf, 2004; Zograf *et al.*, 2004). The authors differentiate these filaments from true microtubules, known in aberrant spermatozoa of

many Metazoa (Bacetti & Afzelius, 1976), on the base of their thickness. Microtubules have a characteristic diameter of 24-25 nm, while the diameter of MLF of nematode spermatozoa is less than 24 nm and sometimes does not exceed 16 nm. It is very likely that MLF are assembled from the MSP-based filaments, while tubulins are scanty or absent in nematode spermatozoa (Justine & Jamieson, 1999). We found filaments of about 28-34 nm in diam. in spermatozoa of *M. costata*. It was shown that a few filaments of MSP may form microfibrils more than 24 nm thick (Stewart *et al.*, 1994). So, it is possible that filopodia of *M. costata* spermatozoa are strengthened with MSP. However, final conclusions on the composition of filaments will be valid only when definitive immunocytochemical analysis has been carried out.

Nematode sperm cells usually show a distinct polarity, which is a subdivision of the cell into the main cell body and the pseudopodium. The spermatozoa of *M. costata*, in both the testis and uterus, preserve an inner polarity that is expressed

in the dislodged position of the nucleus and subnuclear transparent halo filled with mitochondria and dense bodies. However, the spermatozoa of *M. costata*, in both the testis and uterus, lack an outer polarity. Unpolarized spermatozoa have been observed in the representatives of several very distant nematode taxa (Baccetti *et al.*, 1983; Van de Velde *et al.*, 1991; Takahashi *et al.*, 1994; Cares & Baldwin, 1995; Yushin & Malakhov, 1999; Yushin & Zograf, 2004; Zograf *et al.*, 2004), so this character cannot be used fruitfully for phylogenetic implications.

Filopodium formation at different stages of sperm development has been observed in several nematodes including chromadorids, desmodorid and tylenchids (Baccetti *et al.*, 1983; Riemann, 1983; Shepherd *et al.*, 1973; Shepherd & Clark, 1976; 1983; Shakes & Ward, 1989; Poinar & Hess-Poinar, 1993; Cares & Baldwin, 1995; Endo *et al.*, 1998; Yushin & Coomans, 2000; Yushin, 2003; Yushin *et al.*, 2002; Yushin & Zograf, 2004; Zograf & Yushin, 2004; Zograf *et al.*, 2004). In most cases transformation of the filopodia into large pseudopodia takes place after activation of the spermatozoa (Yushin & Zograf, 2004; Zograf & Yushin, 2004; Zograf *et al.*, 2004; Yushin & Coomans, 2005). It seems that the numerous short filopodia covering the surface of the spermatozoa of *M. costata* are an intrinsic feature, they show no tendency to change considerably after copulation. In this respect, the sperm filopodia of the desmodorid resemble the filopodia of the tylenchids and chromadorids.

The data obtained in the present study shows the close relationships between Desmodorida and Chromadorida. The two patterns of spermatozoon structure are represented in these groups. It is hard to define which model is more widely represented in nematodes of these groups, the random sample of species of the subclass Chromadoria that have been investigated predominately shows the absence of MO, while the absence and the presence of FB are approximately equal in frequency. However, the final conclusions should be made only after the investigation of the spermatozoon structure of representatives of the remaining orders of the subclass: Desmoscolecida, Araeolaimida, Plectida (according the system by De Ley and Blaxter, 2002).

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REFERENCES

- BACCETTI, B. & AFZELIUS, B.A. 1976. The Biology of the Sperm Cell. Ed. S. Krager. Basel. 254 pp.
- BACCETTI, B., DALLAI, R., GRIMALDI DE ZIO, S. & MARINARI, A. 1983. The evolution of nematode spermatozoon. *Gamete Research* 8: 309-323.
- CARES, J.E. & BALDWIN, J.G. 1995. Comparative fine structure of sperm of *Heterodera schachtii* and *Punctodera chaloensis*, with phylogenetic implications for Heteroderinae (Nemata: Heteroderidae). *Canadian Journal of Zoology* 73: 309-320.
- DANILOVA, L.V. [Spermatogonia, spermatocytes, spermatids]. In: *Sovremennye problemi spermatogeneza*. pp. 25-62. Moskva. Nauka.
- DE LEY, P. & BLAXTER, M. 2002. Systematic Position and Phylogeny. In: *The Biology of Nematodes* (D.L. Lee Ed.). pp. 1-30. London, NY. Taylor and Francis.
- ENDO, B. Y., ZUNKE, U. & WERGIN, W. P. 1998. Spermatogenesis in the lesion nematode, *Pratylenchus penetrans* (Nemata, Pratylenchidae). *Journal of Helminthological Society of Washington* 65: 227-242.
- FOOR, W.E. 1983. Nematoda. In: *Reproductive biology of Invertebrates*. Volume II: *Spermatogenesis and Sperm Function* (K.G. Adioydi & R.G. Adioydi Eds.). pp. 221-256. Chichester. John Wiley and Sons.
- GOLDSTEIN, P. & TRIANTAPHYLLOU, A.C. 1980. The ultrastructure of sperm development in the plant-parasitic nematode *Meloidogyne hapla*. *Journal of Ultrastructure Research* 71: 143-153.
- JUSTINE, J.-L. & JAMIESON, B.G.M. 1999. Nematoda. In: *Reproductive biology of Invertebrates*. Volume IX, part B. (B.G.M. Jamieson Ed.). pp. 183-266. New Delhi. Oxford & IBH.
- JUSTINE, J.-L. 2002. Male and Female Gametes and Fertilization. In: *The biology of Nematodes* (D.L. Lee Ed.). pp 73-119. London, NY. Taylor & Francis.
- POINAR, G.O. & HESS-POINAR, R.T. 1993. The fine structure of *Gastromermis* sp. (Nematoda: Mermitidae) sperm. *Journal of Submicroscopic Cytology and Pathology* 25: 417-431.
- RIEMANN, F. 1983. Observations on spermatozoa in aquatic nematodes. *Systematic Association Special Volumes* 22: 85-93.
- SHAKES, D.C. & WARD, S. 1989. Mutations that disrupt the morphogenesis and localization of a sperm-specific organelle in *Caenorhabditis elegans*. *Developmental Biology* 134: 307-316.

- SHEPHERD, A. M. & CLARK, S. A. 1983. Spermatogenesis and sperm structure in some *Meloidogyne* species (Heteroderoidea, Meloidogynidae) and a comparison with those in some cyst nematodes (Heteroderoidea, Heteroderidae). *Revue de Nématologie* 6: 17-32.
- SHEPHERD, A. M., CLARK, S. A. & KEMPTON, A. 1973. Spermatogenesis and sperm ultrastructure in some cyst nematodes, *Heterodera* spp. *Nematologica* 19: 551-560.
- SHEPHERD, A.M. & CLARK, S.A. 1976. Spermatogenesis and the ultrastructure of the sperm and male reproductive tract of *Aphelenchoides blastophorus* (Nematoda: Tylenchida, Aphelenchina). *Nematologica* 22: 1-9.
- STEWART, M., KING, K.L. & ROBERTS, T.M. 1994. The motile major sperm protein (MSP) of *Ascaris suum* forms filaments constructed from two helical subfilaments. *Journal of Molecular Biology* 243: 60-71.
- TAKAHASHI, Y., GOTO, C. & KITA, K.K. 1994. Ultrastructural study of *Trichinella spiralis* with emphasis on adult male reproductive organs. *Journal of Helminthology* 68: 353-358.
- TURPEENNIEMI, T.A. 1998. Ultrastructure of spermatozoa in the nematode *Halalaimus dimorphus* (Nemata: Oxystominidae). *Journal of Nematology* 30: 391-403.
- VAN DE VELDE, M.-C., COOMANS, A., VAN RANST, L., KRUGER, J.C.D. & CLAEYS, M. 1991. Ultrastructure of sperm cells in the female gonoduct of *Xiphinema*. *Tissue and Cell* 23: 881-891.
- YUSHIN, V. V. & COOMANS, A. 2000. Ultrastructure of sperm development in the free-living marine nematodes of the family Chromadoridae (Chromadorida: Chromadorina). *Nematology* 2: 285-296.
- YUSHIN, V.V. & MALAKHOV, V. V. 1999. [Spermatozoa of nematodes of the Enoplida order have a nuclear membrane]. *Doklady Akademii Nauk* 367: 718-720.
- YUSHIN, V.V. & ZOGRAF, J.K. 2002. [Electron microscope study of the spermatogenesis in a free-living marine nematode *Neochromadora poecilosoma* (Chromadorida, Chromadoridae)]. *Biologiya Morya* 28: 47-52.
- YUSHIN, V.V. & ZOGRAF, J.K. 2004. Ultrastructure of spermatozoa in the free-living marine nematode *Paracanthonus macrodon* (Nematoda, Chromadorida). *Invertebrate Reproduction and Development* 45: 59-67.
- YUSHIN, V.V. 2003. Ultrastructure of spermatozoa in the free-living marine nematode of the family Selachinematidae (Chromadorida: Cyatholaimina). *Russian Journal of Nematology* 11: 81-90.
- YUSHIN, V.V. & COOMANS, A. 2005. Ultrastructure of sperm development in the free-living marine nematode *Metachromadora itoi* (Chromadorida, Desmodorida). *Acta Zoologica* 86: 255-265.
- YUSHIN, V.V. & MALAKHOV, V.V. 1998. Ultrastructure of sperm development in the free-living marine nematode *Enoplus anisospiculus* (Enoplida: Enoplidae). *Fundamental and Applied Nematology* 21: 213-225.
- YUSHIN, V.V., COOMANS, A. & MALAKHOV V.V. 2002. Ultrastructure of spermatogenesis in free-living marine nematode *Pontonema vulgare* (Enoplida, Oncholaimidae). *Canadian Journal of Zoology* 80: 1371-1382.
- YUSHIN, V.V. & MALAKHOV, V.V. 2004. Spermatogenesis and nematode phylogeny. In: *Proceedings of the Fourth International Congress of Nematology*, 8-13 June 2002, Tenerife, Spain. Nematology Monographs and Perspectives, vol. 2. (R. Cook & D.J. Hunt Eds.). pp. 655-665. The Netherlands. Brill.
- ZOGRAF, J.K. & YUSHIN, V.V. 2004. [Electron microscopic study of spermatogenesis in a free-living marine nematode *Paracyatholaimus pugettensis* (Chromadorida, Cyatholaimidae)]. *Biologiya Morya* 30: 455-461.
- ZOGRAF, J. K., YUSHIN, V. V. & MALAKHOV, V. V. 2004. Ultrastructure of spermatogenesis in the free-living nematode *Halichoanolaimus sonorus* (Chromadorida, Selachinematidae). *Nematology* 6: 797-809.

Зограф Ю.К., Астахова А.А., Юшин В.В. Ультраструктура сперматозоидов свободноживущей морской нематоды *Monoposthia costata* (Chromadorida, Desmodorida).

Резюме. С использованием метода электронной микроскопии было изучено строение сперматозоидов из семенников и матки свободноживущей морской нематоды *Monoposthia costata* (Desmodorida, Monoposthiidae). Поверхность сперматозоидов покрыта многочисленными филоподиями, в то время как конусовидное ядро образовано сильноконденсированным хроматином и лишено ядерной оболочки. Под ядром располагается электронно-прозрачное гало, в котором располагаются митохондрии. Периферическая цитоплазма лишена органелл и заполнена филаментами. В целом сперматозоиды *M. costata* характеризуются общими для всех сперматозоидов нематод чертами: отсутствием акросомы, аксонемы и ядерной оболочки. Несмотря на это, в сперматозоидах *M. costata* и других десмодорид, изученных к настоящему времени, не были обнаружены типичные для большинства нематод мембранные органеллы. В этом отношении сперматозоиды *M. costata* отличаются от спермиев ранее изученных монхистерид и сецернентий, но напоминают таковые хромадорид, что может служить одним из подтверждений филогенетической близости отрядов Chromadorida и Desmodorida.
