

## New data on sperm structure in mononchid nematodes (Enoplia, Mononchida)

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**Summary.** The spermatozoa from uteri of the freshwater nematode, *Mononchus niddensis* (Mononchida, Mononchidae), were studied using electron microscopy. These are amoeboid cells subdivided into small pseudopods attached to the uterus epithelium, and the main cell body which contains a nucleus and mitochondria. The membranous organelles (MO), a characteristic feature of many nematode spermatozoa, were not found in the spermatozoa of *M. niddensis*. These data differentiate *M. niddensis* from another mononchid so far studied, *Mylonchulus nainitalensis* (Baccetti *et al.*, 1983). The spermatozoa of the latter species have MO but lack mitochondria.

**Key words:** female reproductive system, membranous organelles, microtubules, mitochondria, *Mononchus niddensis*, nuclear envelope, ultrastructure.

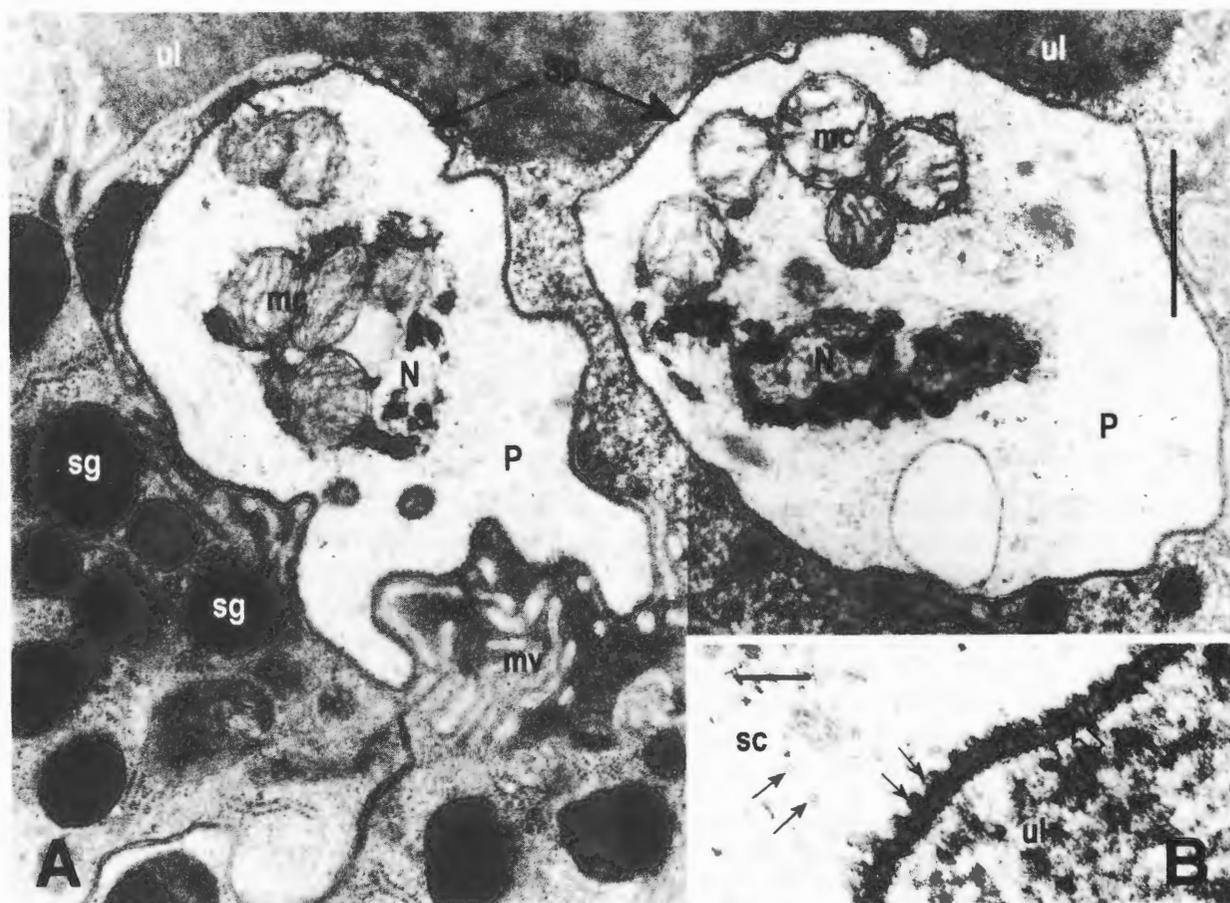
Unlike many other Metazoa, data on the sperm structure and spermatogenesis are rarely discussed in the analyses of the phylogenetic relationships in nematodes, probably due to the lack of studies on the relatively primitive aquatic nematodes from several important orders. Patterns of spermatozoon structure and development are discussed in detail in recently published reviews, which are based mainly on observations of parasitic and soil nematodes (Justine & Jamieson, 1999; Justine, 2002). Each pattern is distinguished clearly by the development of aberrant organelles, membranous organelles (MO) and fibrous bodies (FB), a characteristic feature found in many nematode spermatozoa (Justine & Jamieson, 1999; Yushin & Malakhov, 2004).

The large order Mononchida, which includes free-living freshwater and soil nematodes, is represented in most reviews only by the observation of sperm structure in *Mylonchulus nainitalensis*, Mylonchulidae (Baccetti *et al.*, 1983). Spermatozoa of *M. nainitalensis* were described as bipolar cells each with a solid nucleus without a nuclear envelope and prominent cytoplasm filled with numerous 'membranous vesicles' (Baccetti *et al.*, 1983). The latter are termed as MO in the recent reviews (Justine & Jamieson, 1999; Justine, 2002). A striking and definitive feature of the *M.*

*nainitalensis* spermatozoa is the total absence of mitochondria. Our ultrastructural observations of the female reproductive system in the mononchid *Mononchus niddensis* Skwarra, 1921 (Mononchidae) allow us to revise opinions concerning the mononchid spermatozoa.

### MATERIAL AND METHODS

Gravid females of *M. niddensis* were collected in sand of the rill running from the pond at the Vostok Marine Biological Station of the Institute of Marine Biology (Vostok Bay, Sea of Japan). Animals were cut at the head and tail regions to obtain a piece containing ovaries and uteri with fertilized eggs. These specimens were fixed for transmission electron microscopy at 4°C in 2.5% glutaraldehyde in 0.05 M cacodylate buffer and then postfixed in 2% osmium tetroxide in the same buffer. Postfixation was followed by *en bloc* staining for 2 h in 1% solution of uranyl acetate in distilled water and then the specimens were dehydrated in ethanol and embedded in Spurr resin. Thin sections were stained with lead citrate and examined with a JEOL JEM 100B electron microscope. The uteri of three individuals of *M. niddensis* were studied.



**Fig. 1.** *Monochus niddensis*, mature spermatozoa from uterus, transmission electron microscopy. A: Two spermatozoa (Sp) are attached to the uterus wall by pseudopods (P), opposite parts of spermatozoa are exposed to the uterus lumen (ul). Reticulate nucleus (N) devoid of a nuclear envelope is surrounded by transparent cytoplasm, which contains a cluster of spheric mitochondria (mc). Note microvilli (mv) and secretory granules (sg) of the uterus epithelial cells. B: High magnification of the spermatozoon periphery. The sperm cytoplasm (sc) contains fibres with microtubule-like profiles (thin arrows); surface of the spermatozoon is covered by extracellular layer (hollow arrow), ul – uterus lumen. (Scale bar: A – 1  $\mu$ m; B – 0.1  $\mu$ m).

## RESULTS

Unusual cells, which were identified as spermatozoa, were found in the distal parts of uteri. These are small (about 4  $\mu$ m in diameter) amoeboid cells with distinct polarity (Fig. 1A). Usually they are subdivided into small pseudopods attached to the surface of the microvillous epithelium of the uterus and the main cell body (MCB) faced to the uterus lumen. The MCB contains a nucleus with highly condensed chromatin of reticulate structure without a nuclear envelope. A cluster of spheric mitochondria 0.6  $\mu$ m in diameter is present at the cell pole opposite to the pseudopod. The cytoplasm of the sperm is transparent; it contains sparse microtubule-like fibres (MLF) and occasional bundles of filaments

(Fig. 1A, B). The MLF are 13–16 nm thick and have profiles resembling microtubules in cross sections (Fig. 1B). The MLF also underlie the sperm plasmalemma as parallel arrays. An 18 nm thick extracellular layer of dense filamentous material covers the sperm surface.

The MO or other components usual for nematode spermatozoa were not detected in the sperm cytoplasm. This conclusion was verified by analysis of several spermatozoa by serial thin sectioning.

## DISCUSSION

Our observations on *M. niddensis*, which are only the second attempt to describe the pattern of mononchid spermatozoa, differ from previous data on another mononchid, *M. nainitalensis* (Baccetti *et al.*, 1983). The sperm of *M. niddensis* are

relatively simple and lack MO, which are a usual feature of the nematode spermatozoa and were found in *M. nainitalensis*. The absence of MO in spermatozoa is known for nematode species belonging to very distant orders: Dorylaimida, Chromadorida and Tylenchida (Justine & Jamieson, 1999; Yushin & Malakhov, 2004). As a result of our observations on *M. niddensis* one more order has to be included into this list, the order Mononchida.

The absence of mitochondria in eukaryotic cells is a unique feature. It is indicated, for example, in aberrant spermatozoa of some Metazoa including nematodes from the orders Mononchida and Dioctophymida (Justine & Jamieson, 1999). But unlike the sperm of the mononchid *M. nainitalensis*, which lack mitochondria (Baccetti *et al.*, 1983), the sperm of *M. niddensis* have many unequivocal mitochondria of usual structure.

Thus, observations of two mononchid spermatozoa result in description of two very different patterns of sperm structure: i) spermatozoa with MO and without mitochondria, *M. nainitalensis* (Baccetti *et al.*, 1983); ii) spermatozoa with mitochondria but without MO, *M. niddensis* (present study). These striking differences in spermatozoan internal structure in two mononchids may be explained in part by position of two species in two different families of the order Mononchida, Mylonchulidae and Mononchidae respectively. In any case the spermatozoa of mononchids show well identified diversity, which may be used for phylogenetic analysis of the order.

The presence of microtubules was reported by Baccetti *et al.* (1983) for the spermatozoa of *M. nainitalensis*. This coincides with our observations on *M. niddensis* where MLF fill the cytoplasm of spermatozoa. But unlike true tubulin-containing microtubules, which have diameter 24 nm, the MLF thickness does not exceed 16 nm. This precludes identification of the MLF of nematode

spermatozoa with microtubules (Turpeenniemi, 1998; Yushin & Zograf, 2004). It is very likely that the MLF are assembled from the major sperm protein (MSP) which is the prevalent protein of the sperm cytoskeleton while tubulins are scanty or absent in nematode spermatozoa (Justine, 2002).

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## REFERENCES

- Baccetti, B., Dallai, R., Grimaldi de Zio, S. & Marinari, A. 1983. The evolution of the nematode spermatozoon. *Gamete Research* 8: 309-323.
- Justine, J.-L. 2002. Male and female gametes and fertilization. In: *The Biology of Nematodes*. (D.L. Lee. Ed.). pp. 73-119. London and New York, Taylor & Francis.
- Justine, J.-L., & Jamieson, B.G.M. 1999. Nematoda. In: *Reproductive Biology of Invertebrates*. Vol. IX, part B. (B.G.M. Jamieson. Ed.). pp. 183-266. New Delhi, Oxford & IBH.
- Turpeenniemi, T.A. 1998. Ultrastructure of spermatozoa in the nematode *Halalaimus dimorphus* (Nemata: Oxystominidae). *Journal of Nematology* 30: 391-403.
- Yushin, V.V., & Malakhov, V.V. 2003. Spermatogenesis and nematode phylogeny. *Nematology Monographs and Perspectives* 2: 655-665.
- Yushin, V.V., Zograf, J.K. 2004. Ultrastructure of spermatozoa in the free-living marine nematode *Paracanthochus macrodon* (Nematoda, Chromadorida). *Invertebrate Reproduction and Development* 45: 59-67.

**Юшин В.В.** Новые данные о структуре спермиев у нематод-мононхид (Euplia, Mononchida).

**Резюме.** Сперматозоиды из маток пресноводных нематод *Mononchus niddensis* (Mononchida, Mononchidae) были исследованы с помощью электронного микроскопа. Это амебоидные клетки, подразделяющиеся на псевдоподию, прикрепленную к стенке матки, и основное тело, содержащее ядро и митохондрии. Мембранные органеллы (МО), характерная особенность спермиев многих нематод, у *M. niddensis* обнаружены не были. Эти данные отличают *M. niddensis* от другого изученного в этом отношении вида мононхид - *Mylonchulus nainitalensis* (Baccetti *et al.*, 1983), сперматозоиды которого имеют МО, но не имеют митохондрий.

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