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ELECTRON MICROSCOPICAL STUDY OF SPERMATOZOA OF THE CESTODE *DILEPIS UNDULA* (CYCLOPHYLLIDEA, DILEPIDIDAE)

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Electron Microscopical Study of Spermatozoa of the Cestode *Dilepis undula* (Cyclophyllidea, Dilepididae). Swiderski Z., Salamatin R. V., Tkach V. V. — The fine structure of spermatozoon of the dilepidid cestode *Dilepis undula* is described. The main structural elements of the sperm cell are: 1) plasma membrane and underlying single row of cortical microtubules; 2) a single axoneme of the unique platyhelminth 9+″1″ type; 3) periaxonemal sheath composed of the crystalline-like material; 4) elongated electron-dense nucleus; 5) crested body. The similarities and differences between the ultrastructure of spermatozoa of *D. undula* and that of some other cestodes, are briefly discussed.

Key words: spermatozoa, ultrastructure, Cestoda, Dilepididae, *Dilepis undula*.

Электронно-микроскопическое исследование сперматозоидов цестоды *Dilepis undula* (Cyclophyllidea, Dilepididae). Свидерский З., Саламатин Р. В., Ткач В. В. — Описано тонкое строение сперматозоидов *Dilepis undula*. Основными структурными элементами сперматозоида являются: 1) плазматическая мембрана с подлежащим одним рядом кортикальных микротрубочек; 2) единственная аксонема, устроенная по типу 9+″1″, уникальному для плоских червей; 3) цилиндрический периаксонемальный "футляр", образованный из кристаллоподобного материала; 4) удлиненное электронно-плотное ядро и 5) гребневидное тело. Кратко обсуждены сходства и различия в структуре сперматозоидов у *D. undula* и некоторых других цестод.

Ключевые слова: сперматозоиды, ультраструктура, Cestoda, Dilepididae, *Dilepis undula*.

Inroduction

Ultrastructural characteristics of spermatozoa provide important criteria for the systematics and analysis of the phylogenetic relationships among cestodes (Swiderski, 1968, 1970; Euzet et al., 1981; Justine, 1991, 1995, 1998; Bâ, Marchand, 1995).

Up to now, data on the spermatozoa ultrastructure of about 70 cestode species belonging to all 12 recognised orders of Eucestoda, are available in the literature (see Justine, 1991, 1998; Bâ, Marchand, 1995). For more detailed analysis, however, spermatozoa of more cestode taxa should be studied. According to our knowledge, ultrastructure of spermatozoa of only one dilepidid species, *Molluscotaenia crassiscolex*, has been examined so far (Swiderski, Tkach, 1996). Miquel and Marchand (1997) in their paper on the spermatozoa of *Dipylidium caninum* noted that it was the first study on the representative of Dilepididae. However, in their later paper (Miquel, Marchand, 1998) the authors corrected this systematic allocation of *D. caninum* which, according to the widely accepted classification (Jones, 1994), belongs to the family Dipylidiidae. The aim of the present study is to describe the fine structure of spermatozoon of the cestode *Dilepis undula* which is a characteristic representative of the family Dilepididae and a type species of the genus *Dilepis*.

Material and methods

Adult specimens of *Dilepis undula* (Schrank, 1788), containing mature and pregravid proglottids, were removed from the intestine of a fieldfare (*Turdus pilaris*), from the vicinity of Kyiv, Ukraine. The proglottids were quickly rinsed in saline, cut into small pieces and fixed in 4% glutaraldehyde in sodium cacodylate buffer (pH 7.4). After washing in the same buffer and postfixation for 2 h in 1% OsO_4, they were dehydrated in an ethanol series, infiltrated with propylene oxide and embedded in Spurr's resin. Ultrathin sections, mounted on uncoated copper grids, double stained with lead citrate and uranyl acetate, were examined under a JEM-1200EX electron microscope operated at an accelerateing voltage of 80 kV.

Results

Mature spermatozoa of *D. undula* are strongly elongated, filiform cells, tapered at both extremities and lacking mitochondria. They measure about 300–350 μm in length and their maximum diameter, in the nucleus containing region, is 0,5–0,6 μm.

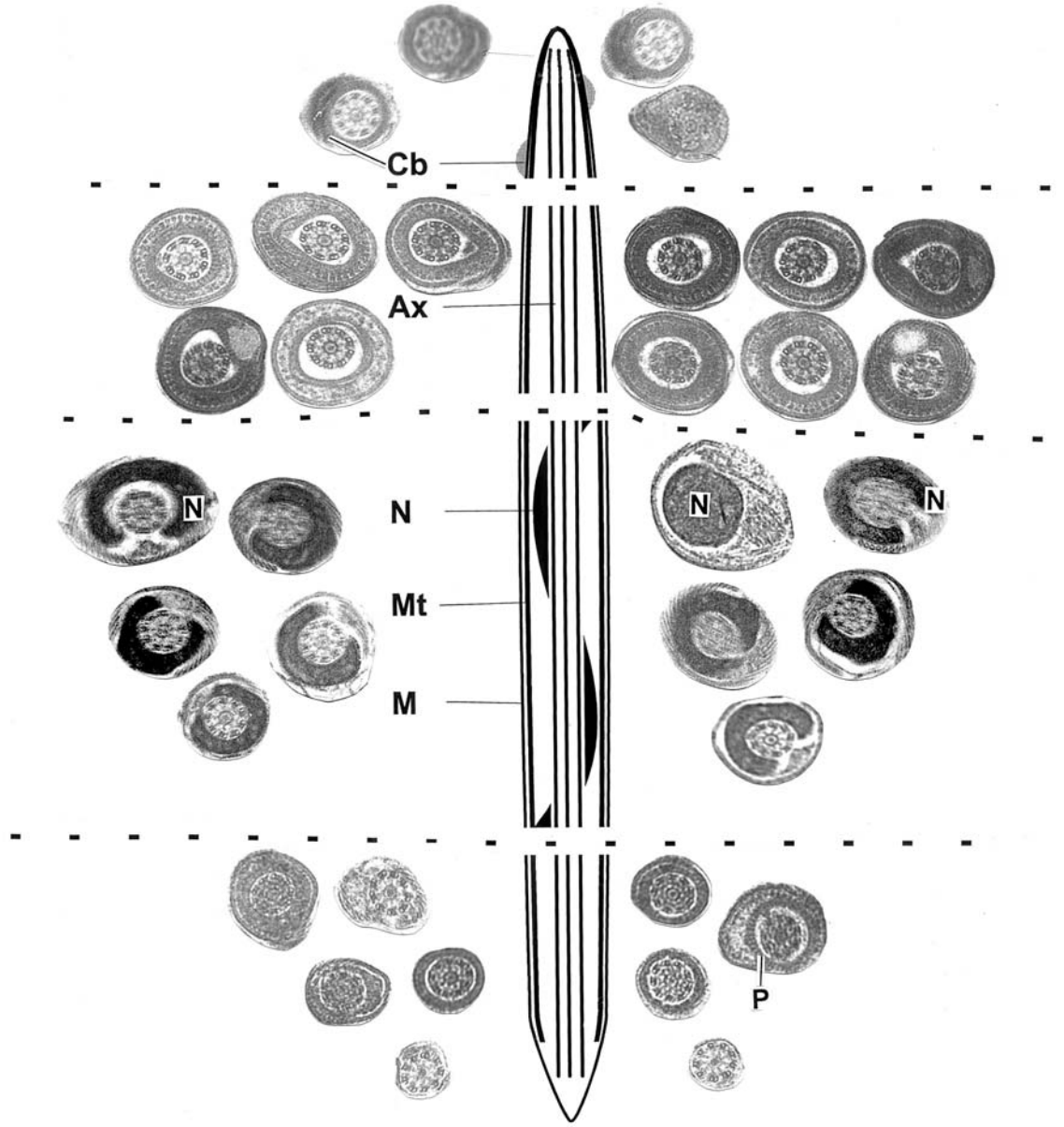


Fig. 1. Diagram and corresponding reconstruction of the spermatozoon of *D. undula* from the representative cross-sections of consecutive regions along longitudinal axis of sperm body.
Abbreviations to all figures: Ax — axoneme; c — core; Cb — crested body; cm — central matrix; CU — central core unit of the axoneme; cx — cortex; DA — dynein arms; DPT — double peripheral tubules; M — plasma membrane; Mt — microtubules; N — nucleus; P — periaxonemal sheath; s — spokes; sf A — subfiber A or tubule A; sf B — subfiber B or tubule B.

Рис. 1. Диаграмма и соответствующая реконструкция сперматозоида *D. undula,* составленная из поперечных срезов участков, последовательно расположенных вдоль продольной оси сперматозоида.
Сокращения ко всем иллюстрациям: Ax — аксонема; c — центральный электронно-плотный цилиндр сердцевинной части аксонемы; Cb — гребневидное тело; cm — центральный матрикс; CU — сердцевинная часть аксонемы; cx — кортекс; DA — динеиновые выросты; DPT — двойные периферические микротрубочки (дублеты); M — плазматическая мембрана; Mt — кортикальные микротрубочки; N — ядро; P — цилиндрический периаксонемальный “футляр”; s — “спицы”; соединяющие периферические дублеты с сердцевинной частью аксонемы; sf A — микротрубочка “A” в дублете; sf B — микротрубочка “B” в дублете.

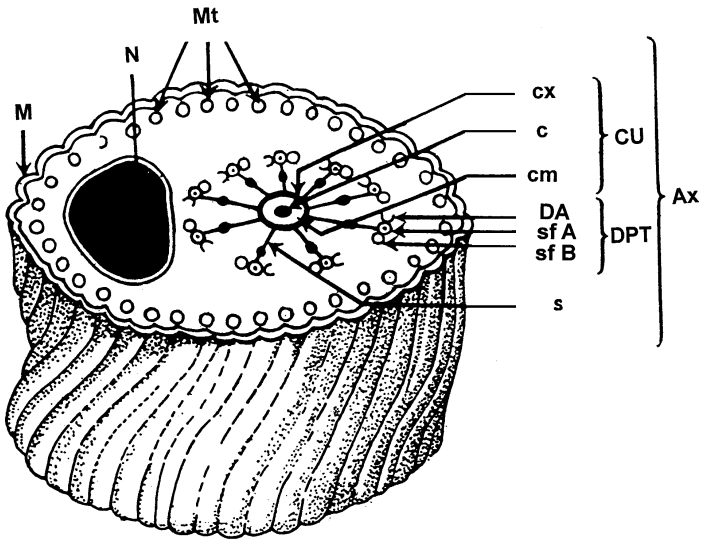


Fig. 2. A three-dimentional diagram of the nucleus containing part of the spermatozoon. Note a complex structure of the 9+"1" axoneme of platyhelmynth type.

Рис. 2. Трехмерная диаграмма ядросодержащего участка сперматозоида. Важно отметить сложную структуру аксонемы, устроенной по типу 9+"1", характерному для плоских червей.

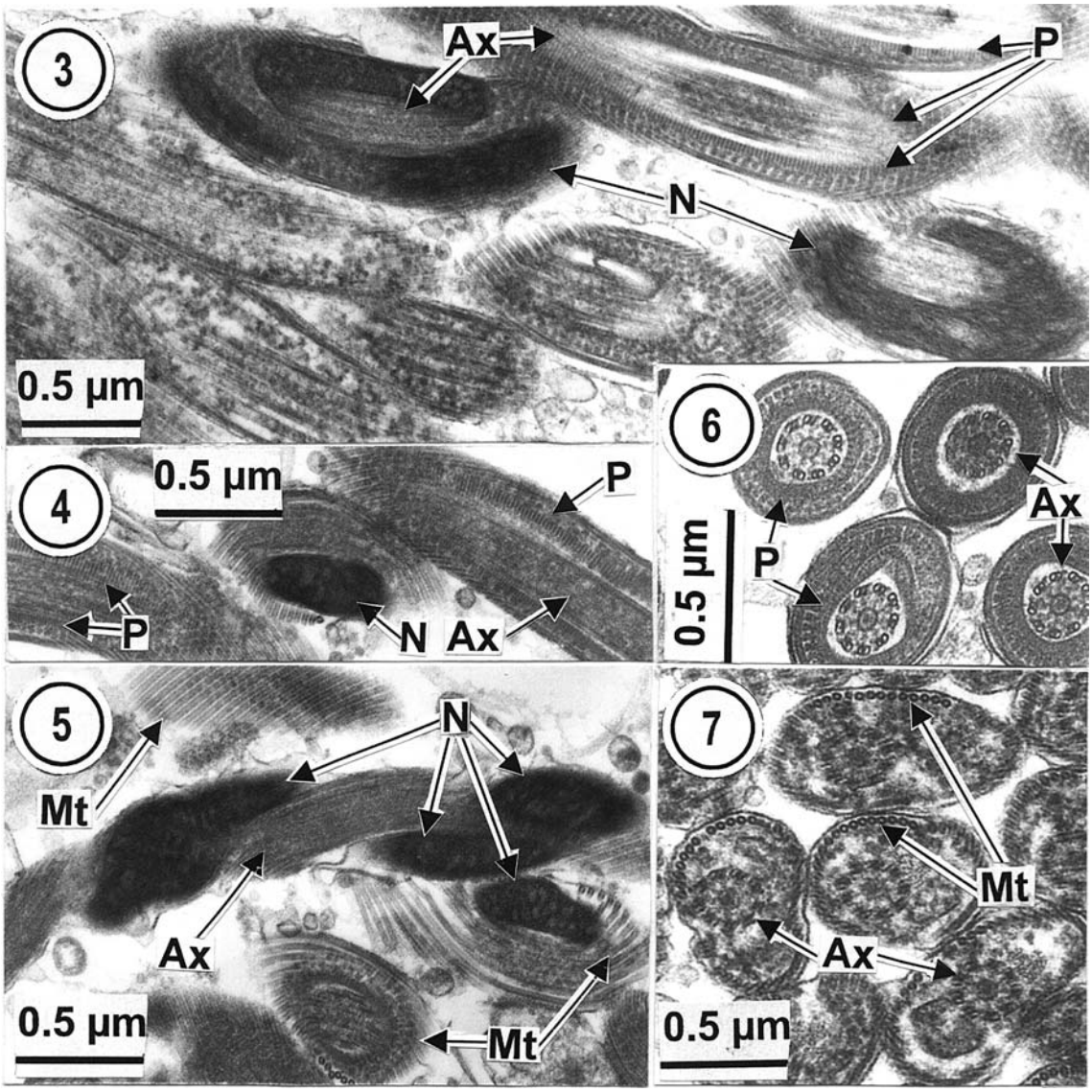
Main structural components of the sperm cell are: (1) plasma membrane and underlying single row of cortical microtubules; (2) a single axoneme of the unique platyhelminth 9+″1″ type; (3) periaxonemal sheath composed of the crystalline-like material; (4) elongated electron-dense nucleus; (5) crested body (Mokhtar-Maamouri, Swiderski, 1975).

Smooth plasma membrane is of uniform thickness. Cortical microtubules, situated under the plasma membrane (figs 2, 6, 7) are spiralled at variable angle (between 35–45°) to the spermatozoon axis.

The axoneme is 0.15–0.18 μm in diameter (figs 6, 7). It contains a central core unit (0.05–0.06 μm) and nine peripheral doublets of microtubules (figs 1, 2, 6). The central core unit consists of an electron-dense core and two cylindrical areas around it composed of: (a) an electron-lucent matrix, and (b) an electron-dense cortex. The peripheral doublet unit of axoneme is composed of: (1) subfiber A or tubule A connected by spoke with the central region through an electron-dense region which partially occlude the lumen of the tubule A; (2) two dyneine arms attached to tubule A; and (3) subfiber B or tubule B (figs 1, 2, 6). The spokes connecting the peripheral doublets with the central region, sometimes appear on the cross-section with different prominence, which confirms their helical arrangement around the central core unit. The periaxonemal sheath has striated appearance on the sections. It is composed of crystalline-like material and consists of moderately electron-dense radial rods separated by electron-lucent spaces of the same thickness (figs 3–6). Numerous granules of moderately electron-dense material occur in the middle and posterior regions of the spermatozoa.

An electron-dense elongated nucleus forming a loose spiral around the axoneme (figs 1, 3, 5), is changing its shape and thickness, when observed on cross-sections at different levels. It begins as a cylindrical structure, initially round on cross-sections, and progressively flattens towards the posterior end, becoming crescent-shaped on the cross-sections (fig. 2). The periodicity of the nuclear spiral varies along the nucleus length. The nucleus occupies relatively short part of the spermatozoon length. Externally, the spermatozoon shows near its anterior end a single, short, helical crested body, associated with cortical microtubules.

Cross-sections through the spermatozoon, near its anterior and posterior tips, are much smaller in diameter and contain only the axoneme surrounded by the plasma membrane (fig. 1).



Figs 3–7. Details of supermatozoal ultrastructure. Figs 3–5: oblique sections through the nuclear part showing arrangement of microtubiules at different angles to the axoneme, spiral pattern of nucleus and periaxonemal sheath composed of the crystalline-like material. Figs 6–7: cross-sections through the anuclear parts. Note: a single row of cortical microtubules and striated appearance of the periaxonemal sheath.

Рис. 3–7. Детали ультраструктуры сперматозоида. Рис. 3–5: срезы через ядросодержащую часть, демонстрирующие расположение микротрубочек под различным углом к аксонеме, спиральную форму ядра, а также периаксонемальный "футляр", состоящий из кристаллоподобного вещества. Рис. 6–7: поперечные срезы через безъядерную часть. Важно отметить наличие одного ряда кортикальных микротрубочек и исчерченность периаксонемального "футляра".

Discussion

An apical cone and a posterior electron-dense material, described in spermatozoa of some other cyclophyllidean cestodes (Justine, 1998; Miquel, Marchand, 1998), were not observed in two dilepidids examined so far (Swiderski, Tkach, 1996; present study); in both cases the fully mature spermatozoa stored in the seminal receptacle, were studied.

In *D. undula,* only one crested body was observed, while 1 to 6 crest-like bodies were reported in other cestodes (for references see Miquel, Marchand, 1997, 1998). The main

difference between two dilepidids, examined so far, is the presence of the periaxonemal sheath in *D. undula* (present study) and its absence in *Molluscotaenia crassiscolex* (Swiderski, Tkach, 1996).

The fine structure of the spermatozoon of *D. undula* is similar to that described in other cyclophyllideans (Swiderski, 1970; Euzet et al., 1981; Justine, 1991, 1995, 1998; Bâ, Marchand, 1995) and is characterised by the presence of a single axoneme. This type of the spermatozoon organisation is observed in the evolutionary more advanced groups of cestodes. It represents probably a result of the secondary reduction in the number of axonemes from the ancestral 2-axoneme type which is observed in more primitive Pseudophyllidea, Tetrarhynchidea and Proteocephalidea (Euzet et al., 1981).

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