Vestnik zoologii **32**(1-2): 108-115, 1998

УДК [593.195:595.771](438)

ULTRASTRUCTURE OF *NEOPEREZIA CHIRONOMI* (MICROSPORA, THELOHANIIDAE) FROM *CHIRONOMUS PLUMOSUS* LARVAE FOUND IN POLAND

I. WITA[1], M. O. OVCHARENKO[2], U. DZIESZUK[1]

[1]*Witold Stefanski Institute of Parasitology Polish Academy of Sciences, Pasteura 3, PO BOX 153 00–973 Warsaw, Poland*
[2]*Institute of Hydrobiology National Academy of Sciences Geroiv Stalingrada 12256210 Kyiv, Ukraine*

Accepted 25 July 1997

Ultrastructure of *Neoperezia chironomi* (Microspora, Thelohaniidae) from *Chironomus plumosus* Larvae Found in Poland. Wita I., Ovcharenko M. O., Dzieszuk U. — The microsporidium *Neoperezia chironomi* Issi and Voronin, 1979 a parasite of larvae of *Chironomus plumosus* L., is described based on ultrastructural characteristics. The earliest stages observed were diplokaryotic meronts. Sporogony is disporoblastic. The uninucleate sporoblasts and spores are enclosed in a sporophorous vesicle. Fixed and stained spores measure 4.0 (3.8–4.2) × 2.5 (2.3–2.8) μm. The polaroplast is lamellar. The polar filament is isofilar with 24–28 coils, arranged in 2–4 layers. The material from Poland is compared to the original description of *N. chironomi*.
Key words: Microsporidium, *Neoperezia chironomi, Chironomus plumosus,* larva, ultrastructure.

Ультраструктура *Neoperezia chironomi* (Microspora, Thelohaniidae), найденных в личинках *Chironomus plumosus* из Польши. Вита И., Овчаренко Н. А., Джешук У. — На основании данных ультраструктуры описана микроспоридия *N. chironomi* из водоемов Польши. Для описываемого вида характерны наличие диплокариотических меронтов и двуспоровая спорогония. Одноядерные споробласты и споры находились внутри спорофорного пузырька. Фиксированные споры имели размеры 4,0 (3,8–4,2) × 2,5 (2,3–2,8) мкм. Поляропласт пластинчатый. Полярный филамент изофиллярного типа в виде 2–4-слойной спирали, сложенной из 24–28 витков. Материал из Польши сравнивается с первоописанием *N. chironomi*.
Ключевые слова: микроспоридия, *Neoperezia chironomi, Chironomus plumosus,* личинка, ультраструктура.

Introduction. Microsporidian parasites of aquatic invertebrate hosts from Poland are practically unstudied. Three species of microsporidia from adults of *Nepa cinerea* L. (Heteroptera) were found in the north-west of Poland (Lipa, 1966): *Nosema bialovesianae* Lipa, 1966; *N. nepae* Lipa, 1966 and *Chapmanium nepae* (Lipa, 1966) Hazard and Oldacre, 1975. This paper presents the ultrastructural characteristics of a fourth microsporidum of aquatic invertebrates, found in Poland.

The monotypic genus *Neoperezia* Issi and Voronin, 1979 was described from the north-eastern region of Russia on the base of the light microscopical data (Issi, Voronin, 1979). Six years later the morphological characteristics of the genus were supplied by ultrastructural data (Issi, Voronin, 1985). Our investigation gives additional information on the ultrastructure and geographical distribution of *Neoperezia*.

Material and methods. A parasitized larva of *Chironomus plumosus* (Diptera, Chironomidae) was collected in September, 1987, in the littoral zone of Dgal lake from the north-east of Poland. Small samples of infected tissue were excised and fixed in 2.5% (v/v) glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.2) for 1–3 days. After washing in cacodylate buffer and postfixation in 2% (w/v) osmium tetroxide for 45 min. at 4°C, the pieces were washed, dehydrated and embedded in epon-araldite using standard procedures (Vávra, Maddox, 1976). Thin sections were cut on a LKB Ultratome III microtome and poststained in an aqueous solution of 1 % (w/v) uranyl acetate and Reynolds' lead citrate. The sections were viewed and micrographs taken with a Jeol 1200 electron microscope. The interpretation of the stages of the developmental cyclisased on ultrathin sections. Spore measurements were made on semithinsections (stained with toluidin blue solution) with an eye-piece micrometer at 1000 x.

Results. Presporal stages were rare and mostly restricted to the last phase of sporogony. Merogonic stages (Figs. 1, 2) with diplokaryotic nuclei were observed in direct

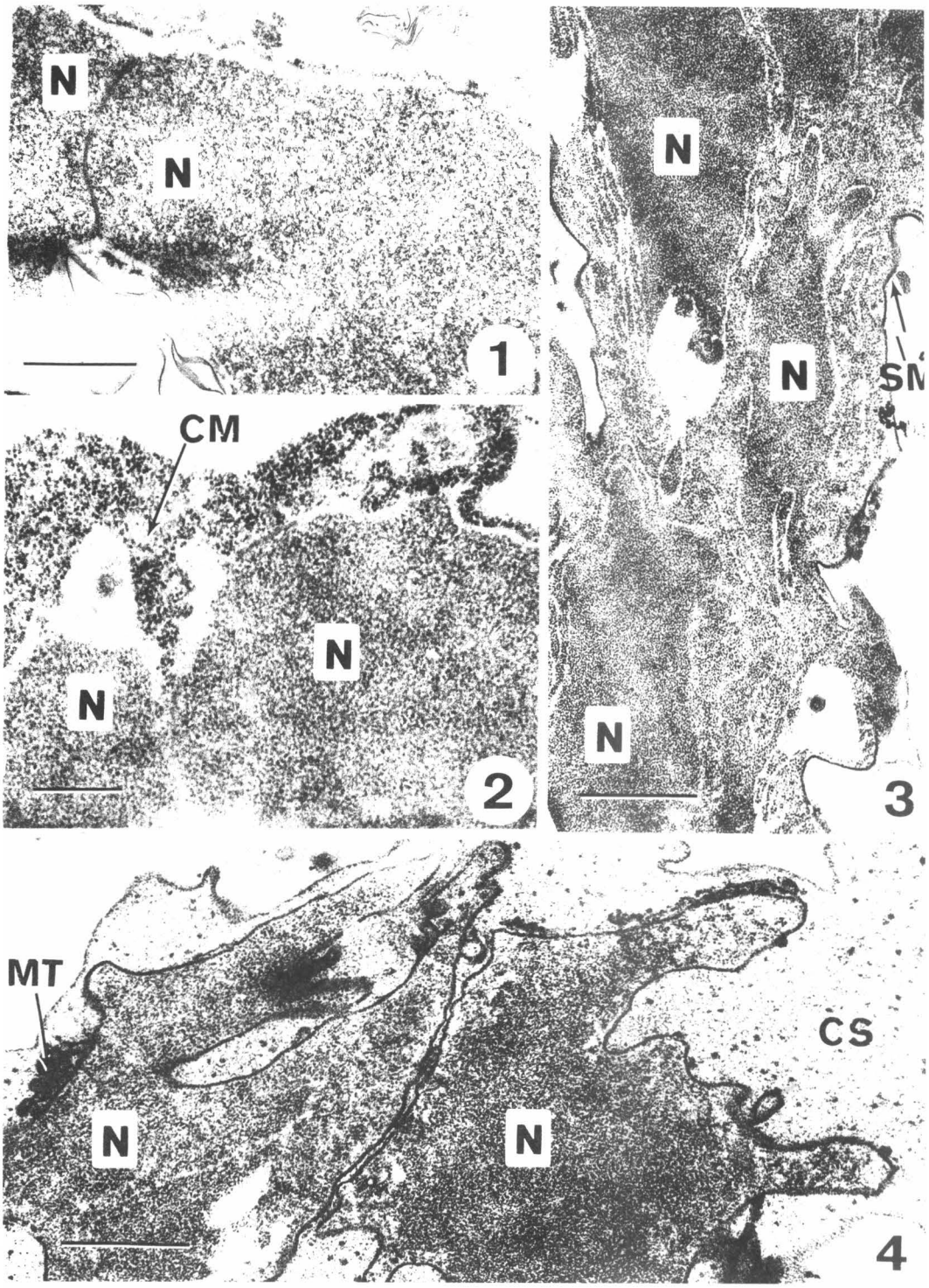
contact with the cytoplasm of the host cell. The late merogonial and early sporogonial stages had a uniform, granular cytoplasm with a great number of, mainly, free ribosomes.The last generation of merozoites transformed to sporonts (Figs. 3, 4). Each sporontformed two uninucleate sporoblasts, enclosed in the interfacsial envelope (Figs. 5–8). The episporontal space contained electron-dense granular inclusions, some thin fibrous, and other wide tubular (Figs. 4–6, 8, 9, 14–17). These inclusions were numerous in the early phase of sporogony, but disappeared successively during the maturation process of the spores. The sporophorous vesicle containing the mature diplospores had few aggregates of wide microtubules and the spores remained together due to the lamellar envelope of the sporophorous vesicle (Figs. 14–17, 20, 21). Probably the episporontal inclusions are used up in the formation of the wall of the spore and the layers of the sporophorous vesicle. Theinterfascial envelope formed long projections into the host cell cytoplasm (Figs. 6, 8, 9). At the end of sporogony this envelope became layered (Figs. 7, 11, 13–17).

The sporoblasts measured approximately 4 μm in diameter (Fig. 6). They were uninucleate. The cytoplasm contained the polar filament primordia (Figs. 5–8). Theyformnear electron dense globular resudial body presumed to be Golgi origin (Jensen, Wellings, 1972, Takvorian, Cali, 1994). The homogenous electron dense substance separated these primordia from the cytoplasm of the sporoblast (Figs. 6, 8). Around the central opaque axis of the polar filamentwas formed the clear layer and the homogenousenvelope (Fig. 7). The numerous membrane-limited microcisterns were visible between the coils of the polar filament of immature spores (Figs. 10, 11).

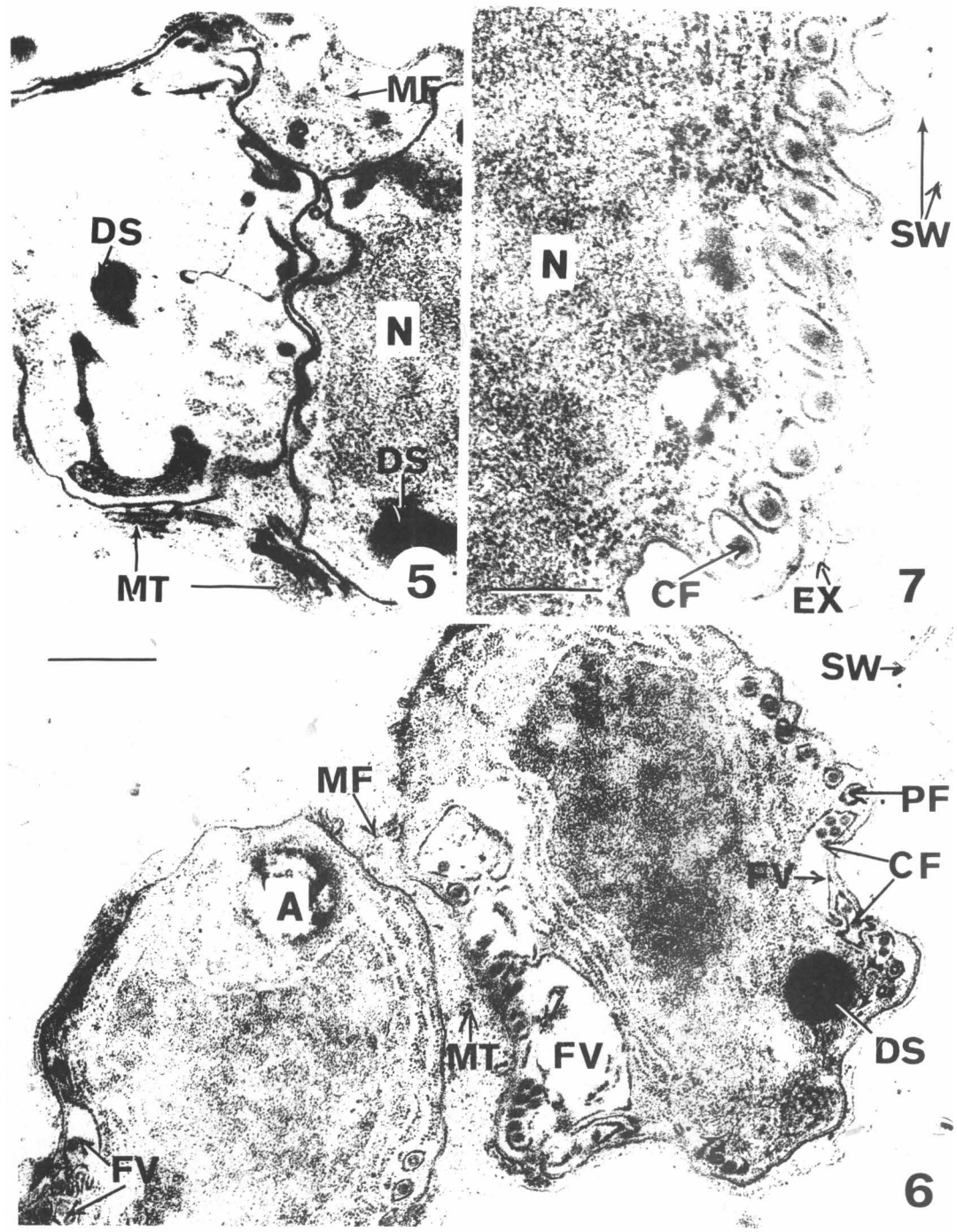
Mature and immature spores occurred in groups of two (Figs. 14, 16, 20, 21). Fixed spores are oval or slightly pyriform with a single nucleus (Figs. 10–13). Theymeasured 4.0 (3.8–4.2) × 2.5 (2.3–2.8) μm in the stained semithin sections (Tabl.1). Young spores (Figs. 9–11) have a spherical nucleus and a posterior vacuole containing electron-dense material. The mature spores have an opaque cytoplasm;the distinct membrane-lined posterior vacuole was filled with dense heterogeneous inclusions (Figs. 12, 13). The spore wall had the normal three components: an internal plasma membrane, a structureless endospore130 (125–140) nm thick, and a 24–28 nm thick slightly wrinkled electron-dense exospore. In a large number of mature spores the spore wall was deformed in certain areas (Figs 12, 13). The polaroplast had two regions with regularly arranged lamellae. In the anterior region the lamellae were closely packed and condensed (Fig. 12). The posterior region, occupying about 3/4 of the length of the polaroplast, contained almost parallel rows of thin lamellae (Fig. 13). The polar filament was isofilar with 24–28, 120 (100–130)nm wide coils in 2–4 layers close to the spore wall and attached to the anchoring disc by an, up to 280 nm wide, pa-dike attachment section (Fig. 12). The angle of tilt of the anterior filament coil to the long axis of the spore was 35–40°. The large nucleus, 1.5–2.0 μm indiameter, was located in the central part of the spore (Figs. 10, 11, 13). The space around the nucleus is filled with a great number of ribosomes, in spirally arranged rows.

Discussion. Disporous species of microsporidia Hith sporophorous vesicle, have been found in 7 genera of invertebrate hosts (Tabl. 2). Microsporidia of the genus *Evlachovaia* arecharacterized by two types of sporogony (Issi, 1986); while representatives of the genus *Holobispora*, described from Copepodahosts, have spores with drop-shaped electron-dense secretions (Voronin, 1986).

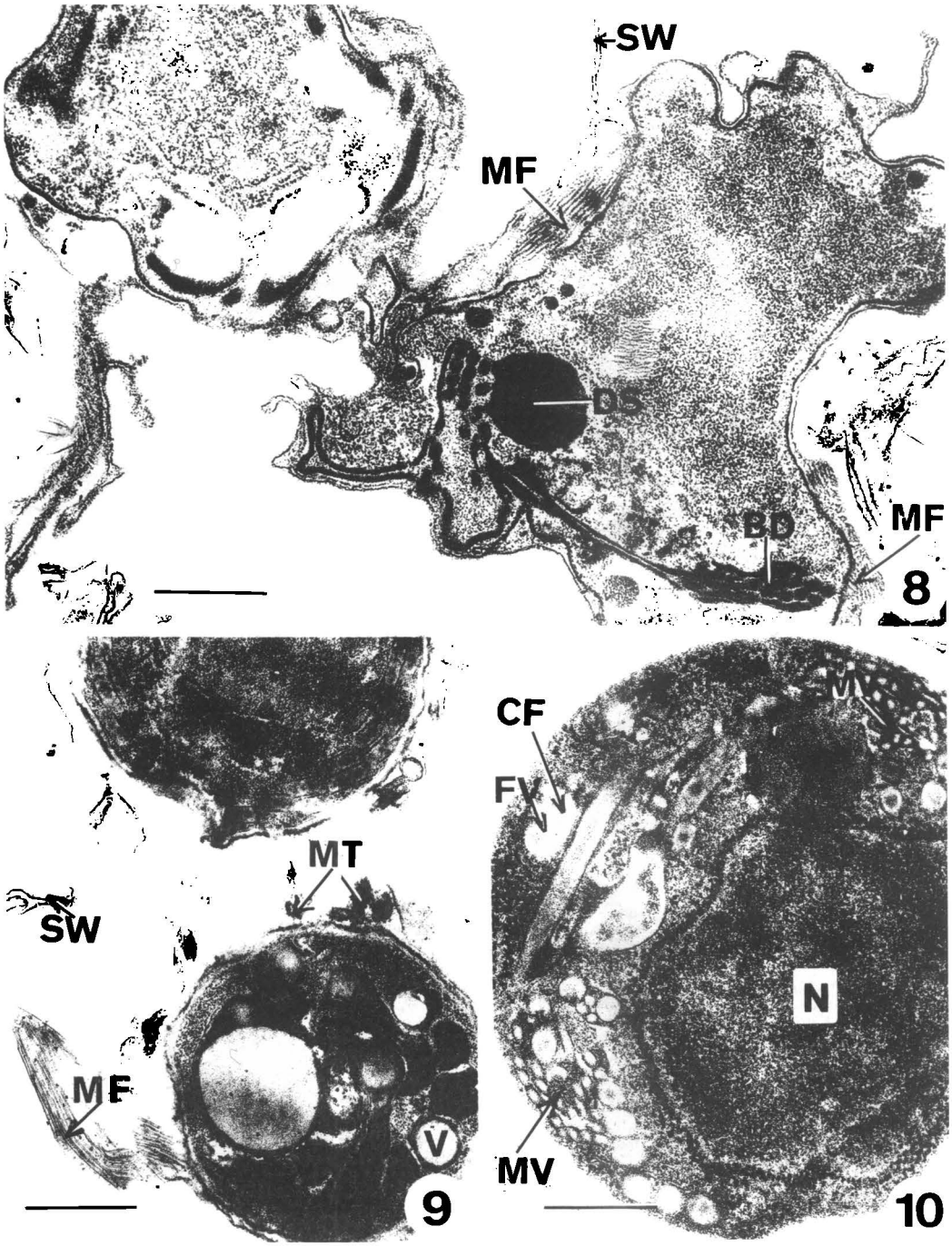
The layered envelope of the sporophorous vesicle is characteristic for the genera *Berwaldia* and *Neoperezia*. Microsporidia of the genus *Berwaldia* do not have diplokaryotic development and their sporophorous vesicle is attached spot-wide to the exospore (Larsson, 1981). *Abelspora portucalensis* Azevedo, 1987, the single species of the genus, lacks the cementing substance of the spores and forms the envelope of the sporophorous vesicle as an uniform, not layered, structure (Azevedo, 1987; Larsson, 1988).



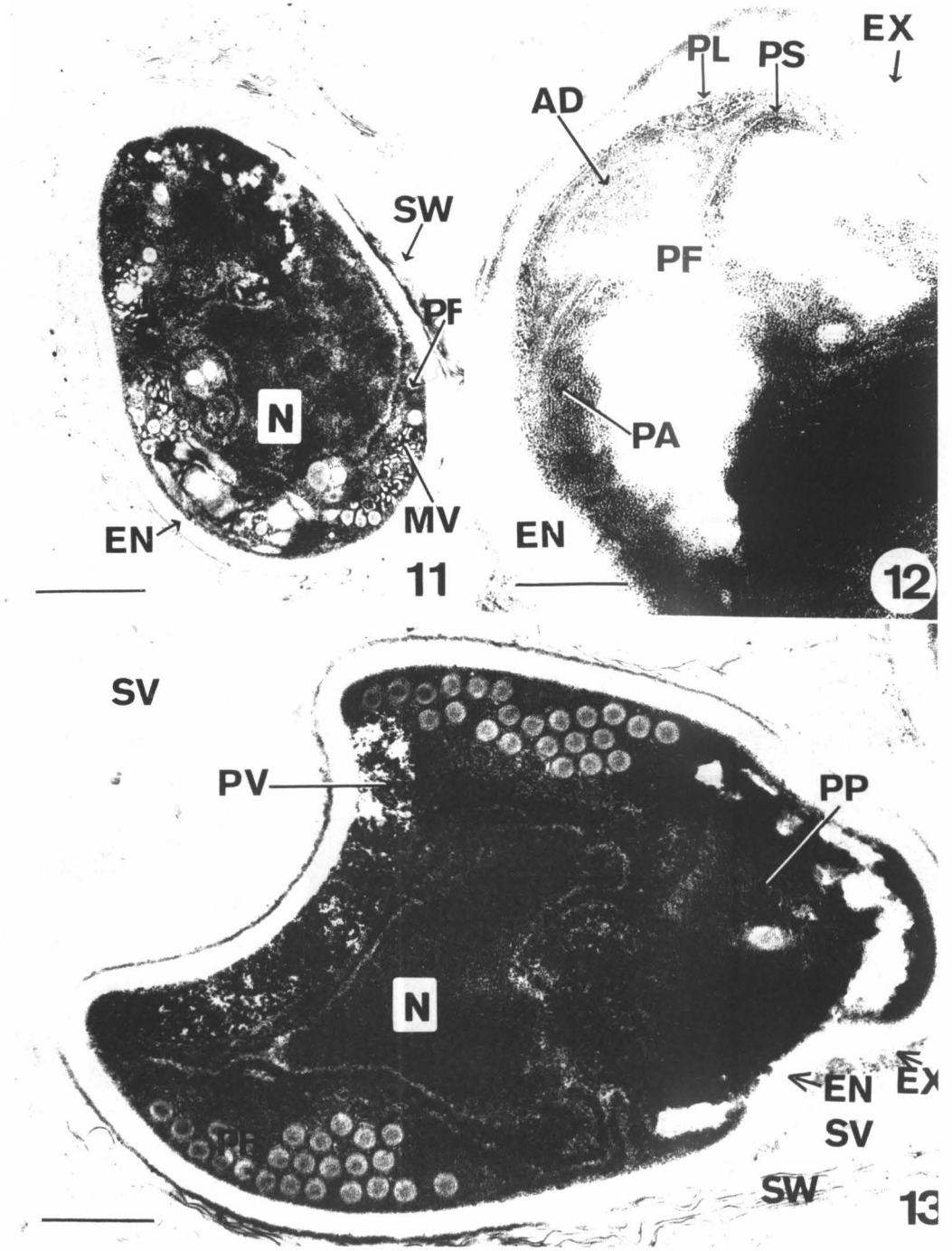
Figs. 1–4. Electron micrographs of early developmental stages of we found *Neoperezia:* 1 – meront with diplokaryotic nuclei (N) (bar, 0,9 μm); 2 – cytoplasm of meront (CM) contains numerous ribosomes (bar, 0,3 μm); 3 – phase of transformationofthe sporogonial plasmodium; thickening of their envelope (SM) (bar, 1,5 μm); 4 – early binucleate sporont; forming of a tubular inclusions (MT) appear in a cytoplasm (CS) (bar, 0,8 μm).



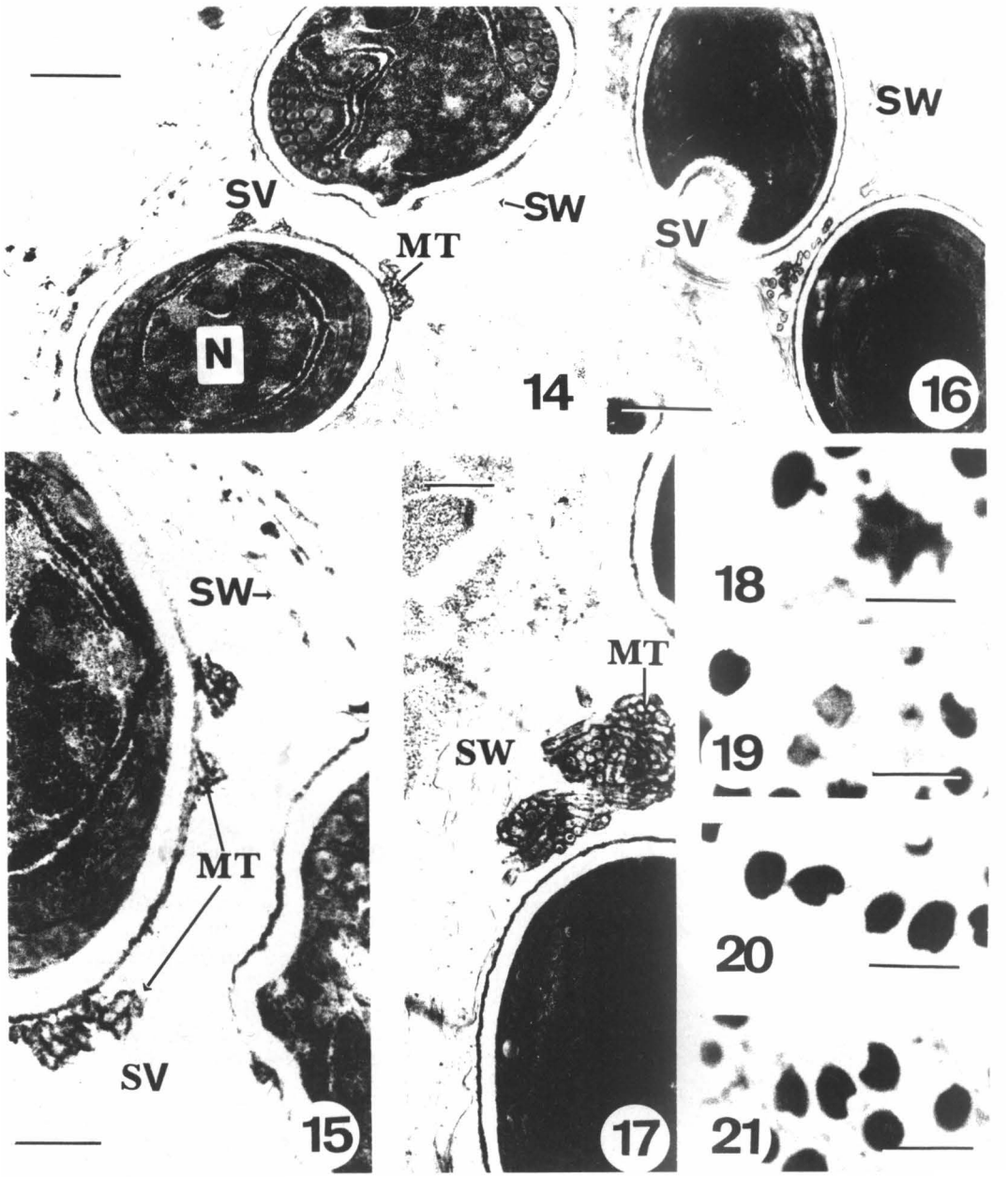
Figs. 5–7. Electron micrographs of sporogonial stages: 5 – two sporoblasts with electron-dense resudial body (DS); thin fibrous (MF), tubular (MT) and granular inclusions in the sporophorous vesicle (bar, 0,4 μm); 6 – late sporoblasts with avacuole-shaped polar filamentprimordia(FV), early anchoring apparatus (A) and polar filaments (PF); cor of polar filament (CF), round electron dense substance (DS), tubular and fibrillar inclusions and envelope of sporophorous vesicle (SW) are visible (bar. 0,5 μm); 7 – genesis of the exospore (EX) from the fine granulated substance within the sporophorous vesicle (SW) (bar, 0,4 μm).



Figs. 8–10. Electron micrographs of immature spores: 8 – section which appears to be through the posterior end of an immature spore, Microfibrillar inclusions (MF) within the sporophorous vesicle (SW) are represented (bar. 0.5 μm); 9 – immature diplospore; forming of layered wall of sporophorous vesicle (SW); inclusions (WF, MT); phase of morphogenesis of the anterior part of the extrusion apparatus from vacuoles (V) (bar, 0,9 μm); 10 – transversely sectioned immature spore witha microvesicles (MV) (bar, 0,4 μm).



Figs. 11–13. Ultrastructure of the spores: 11 – longitudinally sectioned immature spore with a single nucleus (N), electron lucent endospore (EN), polar filament (PF) and microvesicles (MV); the sporophorous vesicle wall (SW) is layered (bar, 0.8 μm); 12 – The anterior part of the spore;.details of the anterior polaroplast (PA), polar filament (PF), anchoring disc (AD), polar sac (PS); the spore wall consists from a plasmalemma (PL), endospore (EN) and exospore (EX) (bar, 0,2 μm); 13 – the posterior part of the spore with a nucleus (N), coiled part of polar filament (PF), posterior polaroplast (PP) and posterior vacuole (PV); the layered wall (SW) of sporophorous vesicle (SV) is showed (bar, 0,5 μm).



Figs. 14–21. Transformation of a sporophorous vesicle wall (SW) during maturation of spores: thin walled vesicle (SV) of immature spores (14, 15) becomes layered when containing mature spores (16, 17); the aggregates of the tubular inclusions (MT) are represented (bars, fig. 14 – 0,8 μm, 15–16 – 0,4 μm, 16 – 1.5 μm, 17 – 0,7 μnm). Light microscopical data of the sporonts and spores: tetranucleate spogonial plasmodium (18) matured in the sporoblasts (19), containing diplospores enclosed in the sporophorous vesicle (20–21) (scale bars: 5,9 μm).

The presence of diplokaryotic stages was found in the genera *Neoperezia* and *Issia*. All stages of the developmental cycle of representatives of the genus *Issia* are diplokaryotic (Issi, 1986). The microsporidium we found, with monokaryotic spores, can be assigned with highprobability to the monotypic genus *Neoperezia* Issi et Voronin, 1979, known from Russia. In their description, the genus *Neoperezia* is characterized by having diplokaryotic merogony, and the formation, at the end of development, of monokaryotic diplospores in a lamellar, sporophorous vesicle (Issi, Voronin, 1979). The type species, *Neoperezia chironomi,* has diplospores associated with a fine structure

Table 1. Differences in characteristics between *Neoperezia chironomi* from difference area

PRIVATE Indications	*Neoperezia chironomi* type species	*N. chironomi* original data
Location of collection	Russia (Leningrad region)	Poland (Mazurian region)
Size of fixed spores, μm	4.1–5.2 × 3.1–3.5	3.8–4.2 × 2.3–2.8
Diameter of polar filament, nm	140 – 180	100 – 130
Number of coils of polar filament	22 – 27	24 – 28
Thickness of endospore, nm	220	125 – 140
Spore-cementing substance	small-granular	tubular granular, fibrillar in the immature, and rarely tubular, or absent, in the mature spores

Table 2. Some data of bisporous microsporidia parasitizing invertebrate hosts

Genus	A	B	C	D	E	Author(s), year
Abelspora	–	1	–	1	U	Azevedo, 1987
Berwaldia	–	1	+	1	L	Larsson, 1981
Evalachovaia	?	1	–	2	?	Voronin & Issi, 1986 in Issi, 1986
Holobispora	?	1	+	1	?	Voronin, 1986
Issia	+	2	–	1	U	Weiser, 1977
Neoperezia	+	1	+	1	L	Issi & Voronin, 1979
Telomyxa	–	1	+	1	U	Léger & Hesse, 1910
We found Microsporidium	+	1	+	1	L	Original data

Notes: A – presence (+) or absence (–) of diplokarya in the presporal stages; B – number of nuclei in the spore; C – presence (+) or absence (–) of cementing substance; D – monomorphic (1) or dimorphic (2) development; E – structure of sporophorous vesicle wall (U – uniform; L – layered).

substance. In comparison with the description of *N. chironomi* by Issi, Voronin (1979), the fine structure of the microsporidium we found shows some differences (Tabl. 1). The differences are found in the width of the endospore, diameter of the polar filament, size of the spores and structure of the spore-cementing substance. It wasfound that *Neoperezia*from Poland has a thinner endospore, a longer and thinner polar filament and smaller spores. The comparatively thinner envelope of spores of the microsporidium we found leads to its distortion (Figs. 13, 16). The cementing substance, gluing spores of the described microsporidium together, is electron-lucent, or, perhaps, absent in the mature spores and is represented byheterogeneous inclusions in the sporoblasts.

The lack of comparative light-microscopical datamakes for some difficulties for the precise comparison of the present species withthe type species, but available ultrastructural data provide sufficient grounds for defining described microsporidium as a *Neoperezia chironomi* .

Azevedo C. Fine structure of the microsporidian Abelspora portucalensis gen. n., sp. n. (Microsporida) parasite of the hepatopancreas of Carcinus maenas (Crustacea, Decapoda) // J. Invertebr. Pathol. – 1987. – **49**. – P. 83–92.

Issi I. V., Voronin V. N. The contemporary state of the problems on bispore genera of microsporidians. // Parazytologia. – 1979. – **13**. – P. 150–158 (in Russian).

Issi I. V., Voronin V. N. Ultrathin structure of Microsporidia Neoperezia chironomi on the stages of sporogony // Cytologia. – 1985. – **27**. – P. 142–147 (in Russian).

Issi I. V. Microsporidia as a phylum of parasitic protozoa // Protozoologia (Leningrad). – 1986. – **10**. – P. 1–136 (in Russian).

Jensen H., Wellings S. R.. Development of the polar filament polaroplast complex in a microsporidian parasite // J. Protozool. – 1972. – **19**. – P. 297–305.

Larsson R.. A new microsporidium Berwaldia singularis gen. et sp. nov. from Daphnia pulex and a survey of microsporidia described from Cladocera // Parasitology. – 1981. – **83**. – P. 325–342.

Larsson R.. Identification of Microsporidian genera (Protozoa, Microspora) – a Guide with Comments on the Taxonomy // Arch. Protistenkd. – 1988. – **136**. – P. 1–37.

Lipa J. J. Miscellaneous Observations on Protozoan Infections of Nepa cinerea Linnaeus Including Descriptions of Two Previously Unknown Species of Microsporidia, Nosema bialovesianae sp. n. and Thelohania nepae sp. n. // J. Invertebr. Pathol. – 1966. – **8**. – P. 158–166.

Takvorian P. M., Cali A. Enzyme Histochemical Identification of the Golgi Apparatus in the Microsporidian, Glugea stephani // J. Eukaryotic Microbiol. – 1994. – **41**. – P. 635–648.

Vàvra J.,Maddox J. V. Methods in Microsporidiology. / Bulla L. A., Cheng T. C. (eds). Comparative Pathobiology. – New York : Plenum Press, 1976. – Vol. 1. – P. 281–319. .

Voronin V. N. The microsporidia of crustaceans // Protozoology (Leningrad). – 1986. – **10**. – P. 137–166 (in Russian).