

Microsporidia of Branchiopods from the Northern Regions of Ukraine[†]

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Four species of Microsporidia parasitizing Branchiopod hosts are described based on the light and ultrastructural data. *Microsporidium* sp. from the body cavity of *Lepidurus apus*, produced ovoid spores $3.5\pm0.4\times2.2\pm0.3\ \mu m$ in size. Mononuclear (asymmetrical) spores of *Larssonia hiberna* sp. n., measured $3.8\pm0.1\times2.0\pm0.2\ \mu m$, were found in fat tissues and hemocoel of *Daphnia magna*. Filamentous episporal surfacing inverted polaroplast and 8–9 coiled isofilar polar filament are characteristic features of this species. *Berwaldia singularis* Larsson and *Microsporidium stagnalis* sp. n. from *D. pulex* are presented. Ellipsoidal spores of *M. stagnalis*, $4.8\pm0.4\times2.4\pm0.3\ \mu m$ in sizes, formed a two-layered exospore, tripartite lamellar and tubular polaroplast, and the isofilar polar filament coiled in a two-layer spiral of 14 rings.

Introduction

The investigation of microsporidia is both of theoretical and practical interest. Because of their eurybiontic peculiarities, these crustaceans have various contacts with other hydrobionts. Thus, the species composition and host-specificity of microsporidia parasitizing them have to be refined.

More than 40 species of microsporidia of branchiopods have been registered up to date [6, 13]. Taking into account that about a half of them have been described more than half a century ago and much information is fragmentary in character and publications lack illustrations, it is not an easy task to identify the microsporidian species invading the branchiopods.

More than 90% of the microsporidia related to mentioned group of hosts were found in Europe, among which about one third were revealed in Russia [4]. Three cases of microsporidiosis of daphnia were registered in Latvia [1, 2, 29]. Microsporidia of branchiopods in the territory of Ukraine have never been under study.

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Methods

As study material, crustaceans were used that were collected in Kiev and Chernigov regions of Ukraine in 1991–1992. There were also used the collections (1972–1992) deposited at the Laboratory of Ecology and Toxicology of T. Shevchenko Kiev University.

Preparations of living spores were made according to the method described by Voronin and Issi [4]. The smears fixed with methanol were stained by Romanowski-Giemsa. For ultrastructural investigations, the infected individuals were maintained at an environmental temperature in 2.5% glutaraldehyde with phosphate buffer (pH 7.2) during 24 hours. After washing in a phosphate buffer and hour-and-half post-fixation in saturated osmium tetroxide at +4°C, the material was embedded in epoxy resins [28].

Results and Discussion

Microsporidia of tadpole shrimps (order Notostraca). The only species of microsporidia, *Nosema lepiduri* Vavra, 1960, was found in a tadpole shrimp species, *Lepidurus apus* L., and it was registered in Czechia [24]. The tape-shaped plasmodium of that microsporidium produced unicellulate sporoblasts that transform into oval spores $4.2 \times 2.3\text{--}2.4 \mu\text{m}$ in size. The taxonomic position of *N. lepidurus* needs to be clarified, since uninucleate stages are not characteristic for the genus *Nosema*.

In one tadpole shrimps individual that had the carapace about 1 cm in diameter, we detected the microsporidia forming ovoid sporal aggregations comprising the groups of 2, 4, 6, 10, and 14 spores. The invasion intensity was high. Besides tadpole shrimps, the larvae of dragonflies, aquatic beetles, caddis-flies, water bugs, and crustaceans (Phyllopoda, Cladocera, Copepoda, Ostracoda) inhabited that body of water. Microsporidia of the genus *Amblyospora* were registered in gnat larvae, *Aedes punctator* Kyrb [5].

Polysporous sporogony, which finishes with forming a variable number of ovoid spores, is characteristic of six genera of microsporidia. Among these, representatives of the genera *Duboscqia* and *Heterosporis* produce 8 and 16 spores, *Alfenia*, 4–8, *Geusia*, 6–8, *Lanatospora*, 6–16, and *Microsporidiopsis*, about 4 spores. None of the microsporidia registered by us can be identified as belonging to the genera listed above. Tentatively, until the new data on the ultrastructural and pre-sporal stages of its life cycle are obtained, we refer them to the group *Microsporidium* Sprague.

Description of *Microsporidium* sp.

Host: *Lepidurus apus* (Notostraca, Lepiduridae), an immature individual.

Localization: tissues in the protocephalic area.

Merogony: unknown.

Sporogony: finished by forming spores assembled as the groups incorporating an even number of spores, from 2 to 14.

Table

Comparative characteristics of the spores of the Microsporidia parasitizing crustaceans of the genus *Daphnia*

Microsporidia species	Species of the hosts	Light optic data			Ultrastructural peculiarities			Data sources
		Number of spores	Dimensions, μm	Shape	Exospore	Polaroplast	Polar tubule	
<i>Agglomerata cladocera</i>	<i>Daphnia magna</i>	8–16	2×3	×	×	×	×	[21]
<i>Baculea daphniae</i>	<i>D. pulex</i>	Not formed	2.8–3.0×0.2	Rod-shaped	1	Lamellate reduced	Isofilar, right	[17]
<i>Bervalda singularis</i>	<i>D. pulex</i>	Not formed	4.7×2.5	Oval	1	Lamellate	Isofilar, 15–18	[12]
<i>B. schaeferai</i>	<i>D. galeata</i>	Not formed	4.7×2.5	Ovoid	1	Lamellate	Isofilar, 15–17	[27]
<i>Duboscqia sidiae</i>	<i>D. magna</i> , <i>D. pulex</i>	16, sometimes 8	3.5×2.7	Oval	×	×	×	[31]
<i>Glugoides intestinalis</i>	<i>D. magna</i> , <i>D. pulex</i>	16 and more	1.1–1.7×2.4–2.7	Ovoid, kidney-shaped	2	Lamellate	Isofilar, 5–8	[15]
<i>Flabelliforma magnivora</i>	<i>D. magna</i>	4–16	2.3–3.0×4.1–4.9	Slightly pyriform	5	Lamellate	Isofilar, 14–17 (two layers)	[13]
<i>Curleya daphniae</i>	<i>D. pulex</i>	4	2.3×3.8, 2.6×4.6	Pyriform	3	Lamellate + chamber	Anisofilar, 3–4+2–5	[9]
<i>G. tetraspora</i>	<i>D. magna</i>	4	2.8–3.0×1.4–1.6	Pyriform	×	×	×	[7]

(continued)

Table

Comparative characteristics of the spores of the Microsporidia parasitizing crustaceans of the genus *Daphnia*

Microsporidia species	Species of the hosts	Light optic data			Ultrastructural peculiarities			Data sources
		Number of spores	Dimensions, μm	Shape	Exospore	Polaroplast	Polar tubule	
<i>G. vavriai</i>	<i>D. longispina</i>	4	5.5–6.0×2.0–2.8	Oval	x	x	x	[8]
<i>Gurleyea</i> sp.	<i>D. pulicaria</i>	4	3.6–4.2×2.6–3.1	Expended oval	x	x	x	[25]
<i>Larssonia obtusa</i>	<i>D. pulex</i>	Even, 4–32	4.3–4.6×2.6–3.0	Pyriform	2	Lamellate, inverted	Isofilar, 6–8	[1]
<i>Microsporidium acutum</i>	<i>D. pulex</i>	8	4.5–5.0×2.0–3.0	Sharpened	x	x	x	[20]
<i>M. elongatum</i>	<i>D. magna</i>	x	4.5×2.4	Ellipsoidal	x	x	x	[10]
<i>M. incurvatum</i>	<i>D. pulex</i>	x	5.0×2.0	Slightly curved	x	x	x	[20]
<i>M. obtusum</i>	<i>D. magna</i> , 2, 4, 6, 16 <i>D. tericulata</i>	3.0–4.0×1.5–2.0	Pyriform	x	x	x	x	[11, 19]
<i>Norlevinea daphniae</i>	<i>D. longispina</i>	4	5.5–6.0×2.7–3.0	Oblong ovoid	1	Vesicular	Anisofilar, 8	[26]
<i>Octospora bayeri</i>	<i>D. magna</i>	x	5.5–9.0×1.5–2.5	Falcate	x	x	x	[10]

(continued)

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Comparative characteristics of the spores of the Microsporidia parasitizing crustaceans of the genus *Daphnia*

Microsporidia species	Species of the hosts	Light optic data			Ultrastructural peculiarities			Data sources
		Number of spores	Dimensions, μm	Shape	Exospore	Polaroplast	Polar tubule	
<i>Ordospora colligata</i>	<i>D. magna</i>	Not formed	1.3–2.3×2.3–3.7	Pear-shaped	1	Lamellate	Isofilar, 5–6	[14]
<i>Pleistophora daphniæ</i>	<i>D. pulex</i>	×	5.0–6.0×2.0–2.5	Ellipsoidal	×	×	×	[30]
<i>P. ellipsoidea</i>	<i>D. magna</i>	About 16	2.6–3.1×4.2–5.1	Oval, slightly pyriform	×	×	×	[29]
<i>Larssonia hiberna</i> sp. n.	<i>D. magna</i>	Even, 4–16	1.9–2.1×3.8–3.9	Slightly asymmetrical, pyriform (to ovoid)	3	Lamellate, inverted	Isofilar, 8–9	Our own data
<i>Microsporidium stagnalis</i>	<i>D. pulex</i>	Not formed	2.3–2.5×4.8–5.0	Ellipsoidal	2	Lamellate+tubular	Isofilar, 13–14	The same

Note. The quantity of spores is indicated for the species producing sporophorous vesicles. The base characteristics of the exospores are given (a number of layers is shown by the figures). For the spiral tubule, there are indicated its type, construction and number of coils in its helical part. The species-synonyms are not included in the Table. The lack of the data is designated by ×.

Spores ovoid, 3.5 ± 0.4 ($3.3\text{--}3.8$) $\times 2.2 \pm 0.3$ ($1.9\text{--}2.4$) μm , with a large obliquely disposed posterior vacuole. Mucocalyx absent. Stained spores approximately oval, $3.4 \pm 0.3 \times 2.0 \pm 0.3 \mu m$ in size.

Materials: preparations 0817 (aqueous) and 0818 (stained, permanent) from the sample 82-2, deposited in the collection of the Laboratory of Ecology and Toxicology of T. Shevchenko Kiev University.

Microsporidia of daphnia. Twenty-one species of microsporidia have been registered in hosts belonging to the genus *Daphnia*, 12 of these species are in need of additional studies to specify their taxonomic position (Table).

In January 1991, the polysporous microsporidia were detected in *D. magna* from the pond in the Kiev Zoo. These microsporidia were morphologically close to *Microsporidium obtusum* (Moniez, 1887) known also as *Microsporidium obtusa* Moniez, 1887, or *Plistophora obtusa* (Moniez, 1887). In due time *Simocephalus vetulus*, *Daphnia reticulata* [19, 20], *D. pulex*, *D. magna*, and *D. longispina* were indicated as its hosts [11]. Taking into account a narrow host-specificity of microsporidia encountered in *Daphnia*, as well as fragmentary descriptions made more than 100 years ago, we can suppose that the microorganism considered is in fact a species aggregate consisting of several separate taxa. A microsporidium identical with *M. obtusa* has been registered in Lithuania [1]. Based on the ultrastructure of the species under study and stages of its life cycle, the appropriateness of classifying *M. obtusa* in the separate genus *Larssonia* Vidtmann, Sokolova, 1994 is now proven. Showing a considerable similarity to *L. obtusa*, the microsporidia detected by us distinguished nonetheless by many characters. These are their shape, dimensions of spores, thickness of sporal envelopes, diameter of the polar tubule, character of inclusions in the sporophore vesicles, and host-specificity (see Table). Such essential distinctions substantiate the considering microsporidia detected by us as a new species, which is possibly identical with the taxon known as "*M. obtusa*" from *D. magna* [10].

Description of *Larssonia hiberna* sp. n.

Host: *D. magna* Straus (Cladocera, Daphnidae), female.

Localization: hemocoel and adipose tissues in the anterior body region.

Merogony: plasmodial cells containing 1–3 isolated nuclei were determined by us as merogony stages (Fig. 1, *a*).

Sporogony is completed by forming the spores assembled together as the groups composed of an even number of spores, from 4 to 16. Envelope of sporogonal vesicles unsteady. Episporontal cavity contains microgranular fibrillar and complexly ornamented inclusions (Fig. 1, *b*) converting into microtubular stretched structures forming the foam-like layer (Fig. 1, *b*, *c*). As the spores mature, the thickness and structure of this covering change and the whole spore takes a shape of a narrow ornamental design containing filamentous microgranular formations (Fig. 1, *a*).

Spores uninucleate, somewhat asymmetrical, and, depending on their location in the preparations, pyriform to ovoid in shape (Fig. 1, *a*). Rear vacuole occupying about 1/5 of the spore length. The living spores 3.8 ± 0.1 ($3.8\text{--}3.9$) $\times 2.0 \pm 0.2$ ($1.9\text{--}2.1$) μm , the stained spores $3.8 \pm 0.2 \times 2.5 \pm 0.2 \mu m$ in sizes, and the glutaraldehyde-fixed spores $3.3\text{--}4.0 \times 1.9\text{--}2.4 \mu m$. Sometimes the macrospores $5.0\text{--}6.5$

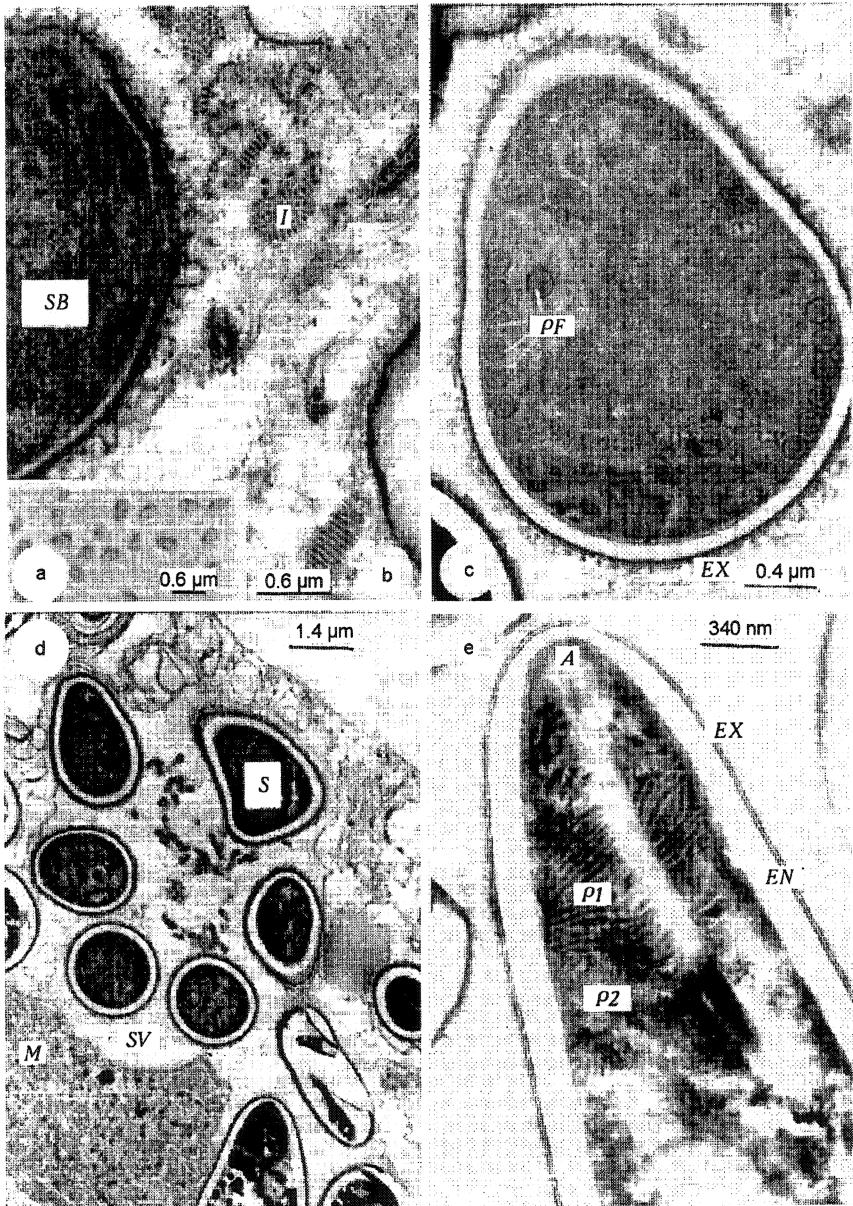


Fig. 1. Sporogony and spores of *Larssonia hiberna* sp. n. *a*, living spores under the light microscope; *b*, late sporoblast (SB) within the sporophorous vesicle contained inclusions (I); *c*, young spore with isofilar polar tubule (PF) and laminated exospore (EX) covered with fibrillar structures; *d*, mature spores (S) within the sporophorous vesicle (SV) with the inclusions. It is seen a part of merogonial plasmodium (M); *e*, longitudinal section through the fore part of the mature spore with its laminated exospore (EX) covered with the microfibrillar formations, endospore (EN), anchor disk (A), and two laminated zones of the polaroplast (P1, P2).

$\times 2.2$ – $2.9 \mu\text{m}$ in size and also teratospores may be encountered. Vesicles in sporophores reach $7.5 \mu\text{m}$ in diameter. Exospore 42.7 – $50.0 \mu\text{m}$ in diameter, laminate, with two electron-dense layers separated by an electron-transparent area (Fig. 1, e). Endospore from 110 to 180 nm thick. Protoplast lamellate, inverted (Fig. 1, e). Polar tubule isofilar, 125 – 170 nm in diameter, helical, with 8 – 9 coils (Fig. 1, c).

Material: Gapentotype (preparations 0803, aqueous; 0804, stained, permanent) and electronograms (6642–6649) of the sample 75–1 deposited in the collection of the Laboratory of Ecology and Toxicology of T. Shevchenko Kiev University.

Another species of polysporal microsporidia forming 8 , 12 , 16 and more unicellular spores was found by us on 11 September 1991 in the upper part of a pond near Yurovka village (12 km south of Kiev) in *D. pulex*. Microsporidia *Parathelohania detinovae* Kiloczyckiy, 1997 affected the *Anopheles maculipennis* Mg [5] have been also registered in this sample. The ultrastructure of spores found in *D. pulex* featured a unique structure of the polaroplast consisted of three zones (narrow-lamellate, broad-lamellate, and tubular ones) (Fig. 2, e). A similar structure is known for the *Pleistophore*-like microsporidia isolated from silkworm *Bombyx mori* L. [22]; however, Larsson and Yan pointed out that electron microphotographs might have been interpreted erroneously [16]. The polaroplast constituted of inverted lamellate and tubular parts is one of the basal characters of the genus *Agglomerata* [16]. The lamellate zones in our microsporidia were of the classical structural type as distinct from the inverted polaroplast type in *Agglomerata*. Besides, the exospore in *Agglomerata* has five layers with clear borders between them, and the episporontal space contains well-contoured inclusions [16]. The spores in microsporidia of *D. pulex* had a two-layered exospore with weakly distinguished interlaminar borders; neither episporontal inclusions nor vesicles in the sporophores were found (Fig. 2, c, e).

Taking into account the lacking data on the ultrastructure of early developmental stages and unique structural features of the extrusion apparatus, we believe that it is appropriate to include the analyzed microsporidia as a new species into the group *Microsporidium*.

Description of *Microsporidium stagnalis* sp.n.

Host: *Daphnia pulex* de Geer (Cladocera, Daphnididae), female.

Localization: hematocoel and adipose tissue.

Merogony: unknown.

Sporogony. In the process of sporogony, the groups containing 8 , 12 , 16 and more spores are formed. The presence of sporophoral vesicles is not proven. Sporoblasts of the early generation in the cross-section approximately round-shaped (rotundate), the late sporoblasts stellate (Fig. 2, c).

Spores: ellipsoidal, uninucleate, with a somewhat narrowed anterior pole, 4.8 ± 0.4 (4.8 – 5.0) $\times 2.4 \pm 0.3$ (2.3 – $2.5 \mu\text{m}$ (Fig. 2, a). Rear vacuole occupying only about $1/3$ of the length of live spores. Spores stained by Romanovski-Giemsa 3.8 – 4.4×2.2 – $3.2 \mu\text{m}$, and 2.8 – $3.3 \times 1.7 \mu\text{m}$ after glutaraldehyde fixation. Exospore two-layered, with an indistinct border between the layers (Fig. 2, e). Exospore 29 – 40 nm thick; endospore 114 – 160 nm thick. Polaroplast occupying $2/3$ of spore's

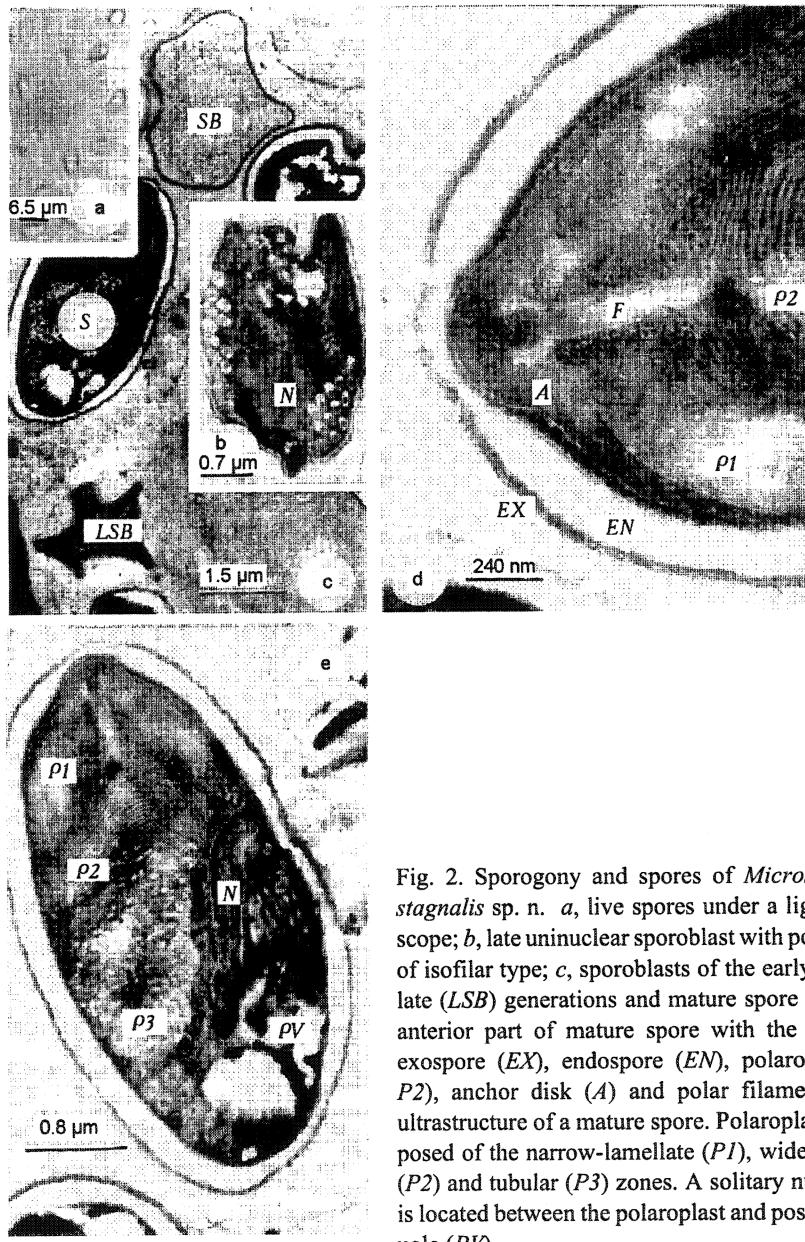


Fig. 2. Sporogony and spores of *Microsporidium stagnalis* sp. n. *a*, live spores under a light microscope; *b*, late uninuclear sporoblast with polar tubule of isofilar type; *c*, sporoblasts of the early (*SB*) and late (*LSB*) generations and mature spore (*S*); *d*, the anterior part of mature spore with the laminated exospore (*EX*), endospore (*EN*), polaroplast (*P1*, *P2*), anchor disk (*A*) and polar filament (*F*); *e*, ultrastructure of a mature spore. Polaroplast is composed of the narrow-lamellate (*P1*), wide-lamellate (*P2*) and tubular (*P3*) zones. A solitary nucleus (*N*) is located between the polaroplast and posterior vacuole (*PV*).

length. Upper part of the polaroplast (almost 1/2 of the spore volume) lamellate, with two sections of plates densely and loosely placed in layers; lower part tubular (Fig. 2, *d*, *e*).

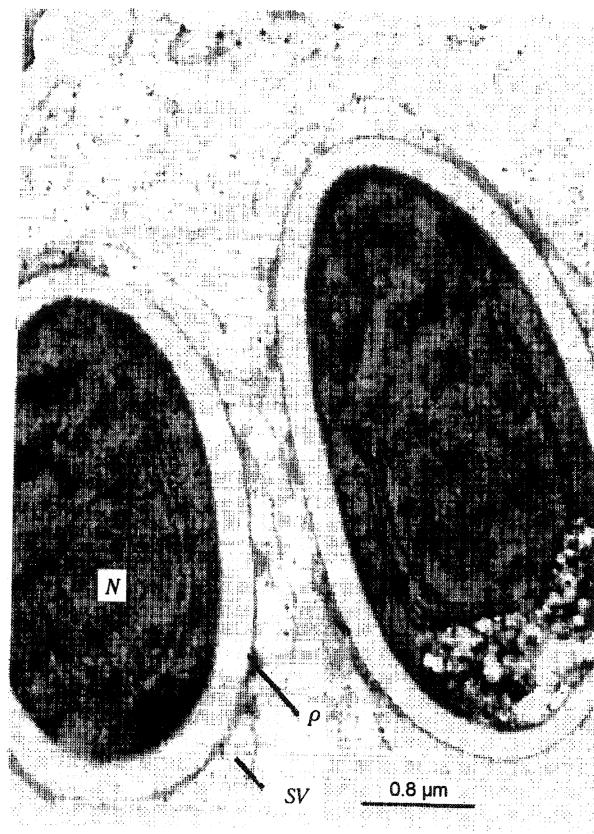


Fig. 3. Spores of *Berwoldia singularis* Larsson within: the sporophores vesicles (*S*) with exospore (*P*), which has regular thickenings, and solitary nucleus (*N*).

Anchor disk covering about 1/2 of the lamellate part of polaroplast (Fig. 2, *d*). Polar tubule isofilious, helical, its 14 coils arranged in two rows (9+5) at an angle of 45% to the spore's longitudinal axis (Fig. 2, *b, e*). Polar tubule 88–100 nm in diameter.

Material: Gapentotype (preparations 0854, stained, constant) and electronograms (6859–6864) of the sample 75-1 deposited in the collection of the Laboratory of Ecology and Toxicology of T. Shevchenko Kiev University.

The microsporidia referred to the species *Berwoldia singularis* Larsson, 1981 were found by us in May 1992, in the water body located near Kruglik village (Kiev region).

Description of *Berwoldia singularis* Larsson, 1981.

Host: *Daphnia pulex* de Geer (Cladocera, Daphniidae), female.

Localization: adipose body, hematocoel.

Merogony: unknown.

The sporogony is completed by forming the spores assembled as irregular groups. These are disintegrated in the water into the spores situated separately or in pairs.

Spores $5.0 \pm 0.1(2.4-2.6) \mu\text{m}$, elongate-ovate; vacuole-like zone located laterally. Spores stained by Romanowski-Giemsa oval in shape, $3.9 \pm 0.3 \times 2.4 \pm 0.3 \mu\text{m}$; spores fixed by glutaraldehyde $3.8 \times 1.9 \mu\text{m}$. Exospore up to 10 nm ; endospore up to 170 nm . Polaroplast laminar, formed by densely and loosely arranged plates (Fig. 3). Isofilar polar tubule up to 120 nm , coiled in 15–16 turns.

Materials: light microscopy preparations 0832–0833 and electron microscopic images 7323–7325 of the sample 92-2 deposited in the collection of the Laboratory of Ecology and Toxicology of T. Shevchenko Kiev University.

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