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Unusual Polar Filament Structure in Two Microsporidia from Water Reservoirs with Radionuclide and Organic Pollution

by

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Summary. Two species of microsporidia with the unusual polar filament structure were found in *Cricotopus silvestris* and *Microtendipes pedellus* larvae which were collected near the zone of influence of the Chernobyl atomic power station (Ukraine) and from a high polluted pond in the Mazurian region of Poland. The first microsporidium had separate unikaryotic spores and was assigned to the family Unikaryonidae Sprague. The diameter of the middle coil of the triple-coiled polar filament of this microsporidium was larger than its two other coils. The observed polar filament was thus neither of isofilar nor of the classical anisofilar type. The second polysporoblastic microsporidium has unikaryotic spores and an uncoiled polar filament and was placed in the family Thelohaniidae Hazard and Oldacre. The rare single macrospores of this microsporidium have a double set of the polar filament complex. The relationship between ultrastructural features of microsporidian spores and water pollution is discussed.

Key words: Microspora, ultrastructure, midge larvae, water pollution.

Information about the relationship between water quality and morphological changes in intracellular protozoan parasites is not abundant. The majority of investigations were connected basically with seasonal and temperature influences [1, 8, 10, 13, 14]. The relation of water pollution and ultrastructure of a microsporidian spores is virtually uninvestigated.

In summer 1990, during investigation of the tissues of water invertebrates from the Ukrainian regions that had been subjected to the accident of the Chernobyl atomic power station, a microsporidium parasitizing the Malpighian tubules of a single chironomid larva was observed. The definition of the systematic status of

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this microsporidian parasite was difficult because of unusual construction of the spores. Later, unusual changes of spore construction were noted during parasitological investigations of the deer-farm water reservoirs in the Mazurian region of Poland in summer 1995. In both cases, teratological spore construction was analyzed in a hypothetical connection with the water pollution.

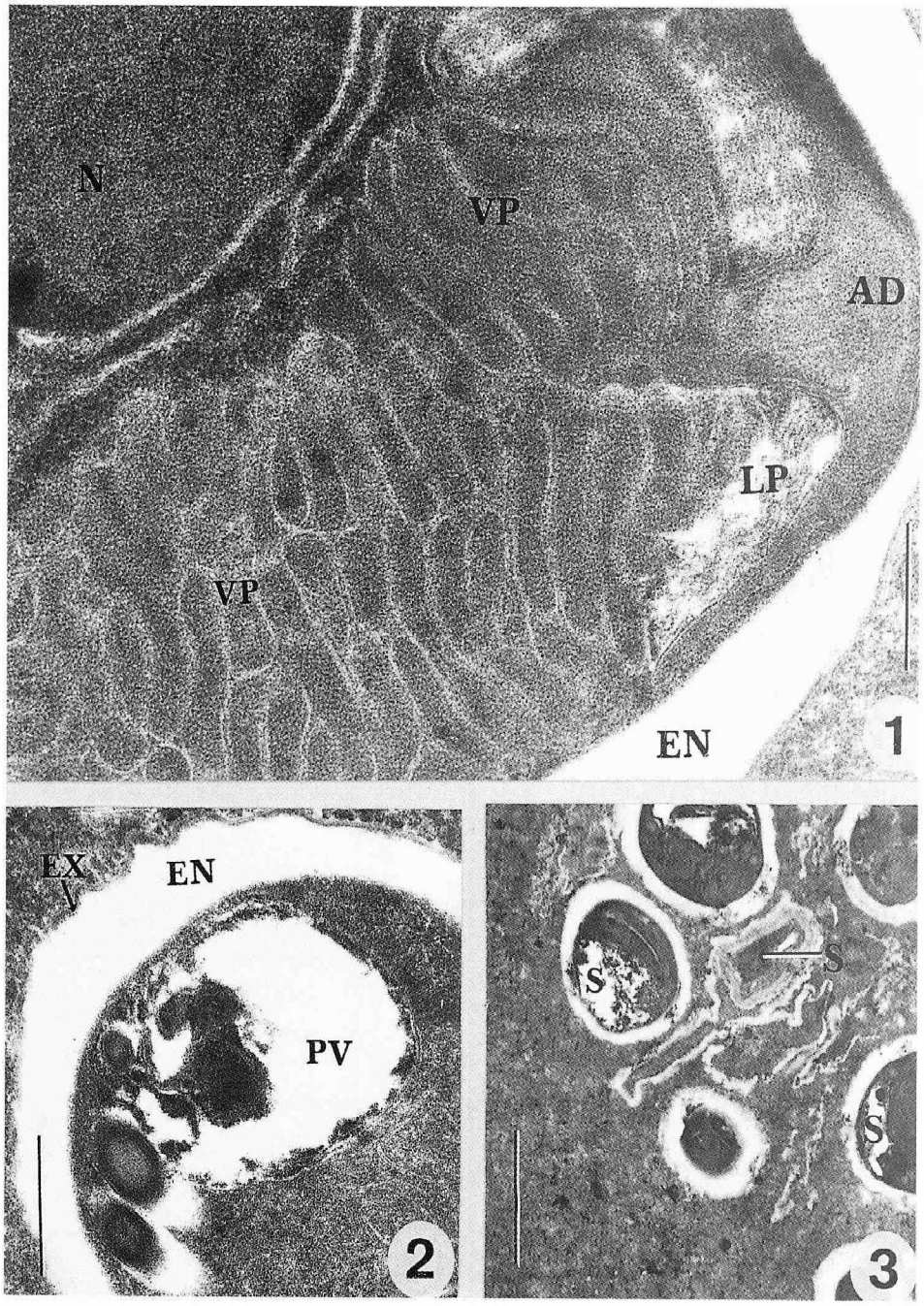
Material and Methods

The infected *Cricotopus silvestris* (Fabr.) larva was collected from a flood-lands pond of the Dnieper, 40 km north of Kiev in July 1990. The level of Sr^{90} contamination of molluscs conchas in the indicated reservoir has made more than 1.5 kBk/kg [7]. The larva of *Microtendipes pedellus* (De Geer) with hypertrophied white lobes of fat body was recorded in August 1995 in the small pond of deer-farm territory near Kosewo Górne (north-east region of Poland). For transmission electron microscopy, pieces of the infected segments were excised and fixed in a 2.5 % (v/v) glutaraldehyde in a 0.2 M sodium cacodylate buffer (pH 7.2) for 1–3 days. After washing in cacodylate buffer and postfixation in 2.0 % (w/v) osmium tetroxide in cacodylate buffer for 1 h at 4°C, the pieces were washed and dehydrated in an ascending series of ethanol to absolute acetone, and embedded in Epon-Araldite. Sections were stained using uranyl acetate and lead citrate [18]. For scanning electron microscopy, suspension of the spores was placed on slides, dehydrated, transferred to acetone, critical point dried and coated with gold. Photographs were taken with a Jeol 1200 electron microscope.

Results

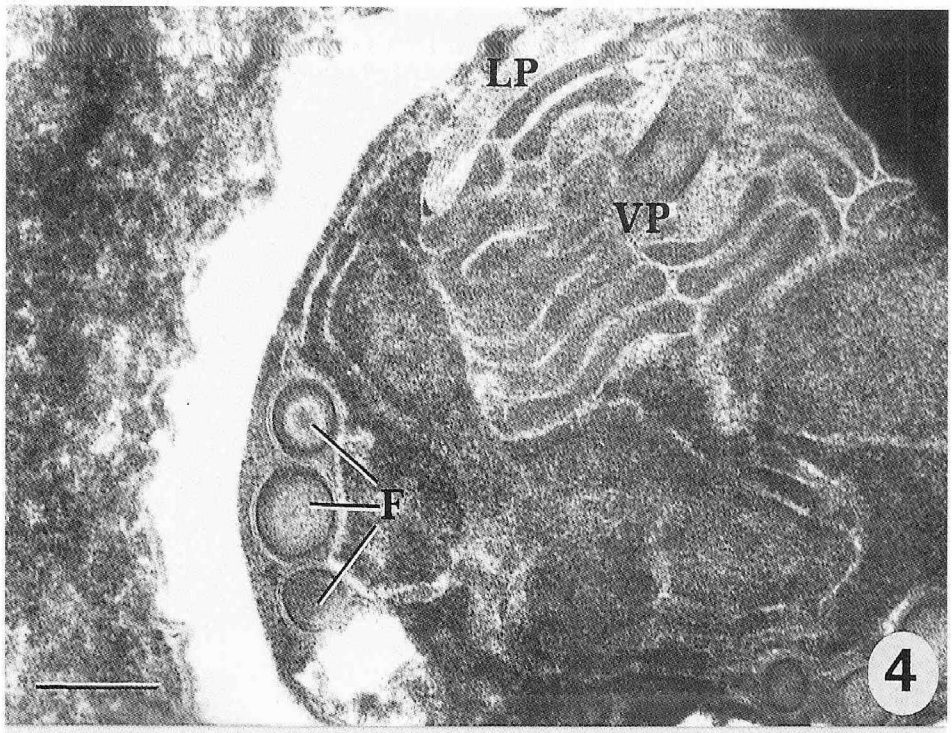
The fixed single monokaryotic spores of the microsporidium from the Malpighian tubules of the *Cricotopus silvestris* larva were broadly oval and measured 2.7 (2.4–3.2) × 1.9 (1.2–2.1) μm (Fig. 3). Their tripartite envelope was composed of a plasmalemma, a thick translucent endospore and internal double layered exospore 15–34 nm wide (Figs. 1–5). The endospore measured 60–100 nm at the anterior pole and about 240 nm over the rest of the spores. The polaroplast consisted of the two parts (Figs. 1, 4). The anterior lamellar part of polaroplast occupied about 1/8 of their length. The vesicular polaroplast consisted of flattened vesicles 45 nm in transverse section. The polar filament was neither the isofilar nor anisofilar type, 234 (209–270) nm wide where it attached to the anchoring disc at the anterior pole of the spore (Fig. 1). The second coil of three coiled polar filament had the largest diameter, measuring 189 (182–191) nm (Figs. 4, 5). The first and third of coils were narrower, 134 (133–136) nm and 117 (113–124) nm. The transversely cut filament exhibited some concentric layers of varying electron density (Fig. 5). The membrane lined posterior vacuole contained electron-dense inclusions (Fig. 2).

The fixed spores of microsporidium from the fat body cells of the larva of *Microtendipes pedellus* with a single nucleus were thin-walled, ovocylindrical and measured 0.9 (0.8–1.0) x 2.3 (1.9–2.5) μm (Figs. 6, 7). The spore coat consisted of a plasmalemma, a thin endospore and a *Thelohania*-like layered exospore (Figs. 6, 8). The polaroplast was composed of a closely packed narrow lamellar and posterior tubular parts (Fig. 6). The uncoiled isofilar polar filament was about 100 nm wide. This polysporoblastic microsporidium generated sporophorous vesicles containing tubular inclusions and 4, 6 or 8 spores. Unusual solitaire macrospores with a



Figs 1–5. Spores of microsporidium from *Cricotopus silvestris* larva.
Fig.1. The anterior part of the spore. The polaroplast consists two parts : lamellar (LP) and vesicular (VP). The umbrella shaped anchoring disc (AD) covered the lamellar polaroplast (bar 0.2 μm).
Fig. 2. Posterior part of the spore with membrane lined posterior vacuole (PV), thick endospore (EN) and *Thelohania*-like structure of exospore (EX) (bar 0.3 μm).
Fig. 3. Spores (S) in a Malpighian tubule wall (bar 0.9 μm).

Abbreviations: AD — anchoring disc, EN — endospore, EX — exospore, F — polar filament, PL — plasmalemma, VP — vesicular polaroplast, LP — lamellar polaroplast, TP —tubular polaroplast, N — nucleus, S — spore, MS — macrospore, PV — posterior vacuole.



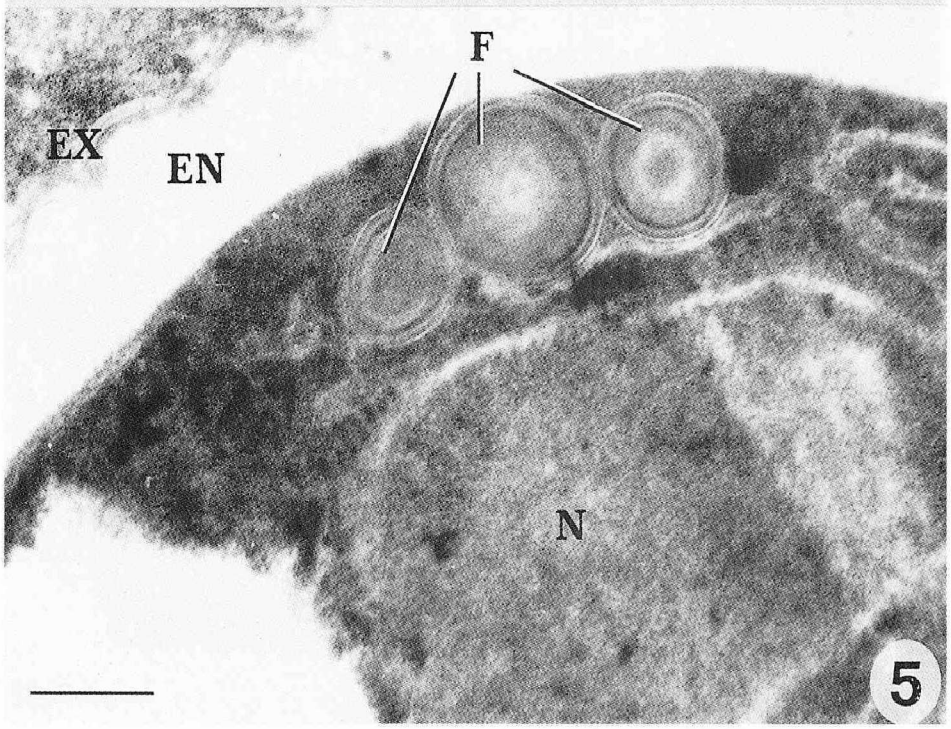
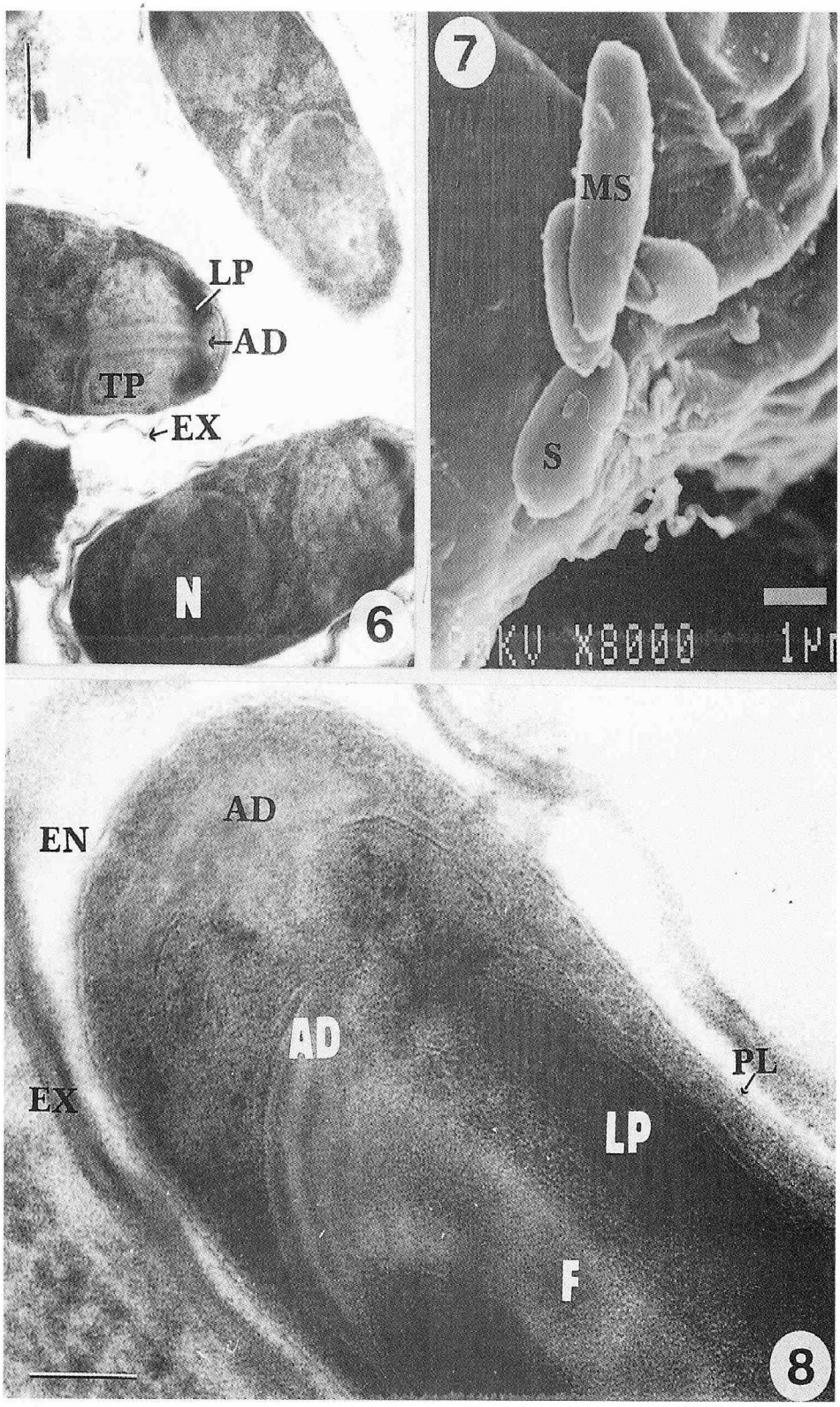


Fig. 4. Part of mature spore with three coiled polar filament (F), lamellar (LP) and vesicular (VP) polaroplast (bar 0.3 μm).

Fig. 5. Lateral part of the spore. Structure of polar filament (F), endospore (EN) and exospore (EX) are visible (bar 0.2 μm).



Figs 6–8. Spores of microsporidium from *Microtendipes pedellus*. (Abbreviations as in Figs 1–5)
Fig. 6. Spores with a narrow lamellar (LP) and tubular (TP) polaroplast (bar 0.6 μm).
Fig. 7. Normal (S) and teratological (MS) spores.
Fig. 8. The anterior part of the teratological macrospore with a double set of anchoring disc (AD) (bar 0.2 μm).

double set of polar filaments and polaroplast complexes were simultaneously produced (Figs. 7, 8).

Discussion

It is now well known that the polar filament is not uniformly constructed in all microsporidia, and two different morphological types have been described [11, 17]. An isofilar polar filament is uniformly thick from the attachment section to the tip. Abruptly constricted at some point, an anisofilar polar filament has a wide anterior and a narrow posterior portion. In a typical anisofilar filament both the wide and narrow parts are coiled and inadequately structured. In the microsporidium from the Malpighian tubules of the *Cricotropus silvestris* larva, the anterior and posterior coils of three coiled polar filament were narrow and the middle coil was broad but adequately structured (Figs. 4, 5). In this case, the extruded polar filament was like a short sleeve with a broadening in the middle. Such construction could be interpreted as a teratological change of isofilar polar filament bounding due to radionuclide pollution or as a new type of polar filament structure. The way of output of sporoplasm from an extruded polar filament by the latter interpretation would be incomprehensible.

The generation of a double set of polar filaments in the microsporidium in the infected fat body cells of the *Microtendipes pedellus* larva is probably related to the organic pollution of the water reservoir and an example of teratological macrospore generation. The rare macrospores were generated equally with a classical structured spores.

The definition of the systematic status of these microsporidia is difficult because their rarity of occurrence and shortage of material. Uninucleate spores with an unknown type of polar filament have been described from some microsporidian genera [2, 3, 4]. The genus which most closely resembles the microsporidium we found in the *Cricotropus silvestris* larva is *Unikaryon*. This genus includes monokaryotic microsporidia of invertebrates which do not form parasitophorous vacuoles [1, 16], but these microsporidia have an isofilar polar filament. Probably the microsporidium we found can by assigned to the family Unikaryonidae on the grounds of the apansporoblastic uninucleate sporogony [15].

The isofilar uncoiled polar filament is characteristic for a number of microsporidia with rod-shaped spores like *Baculea, Cylindrospora, Helmichia, Pernivesicula* and *Scipionospora* [5, 6, 9, 10, 12], but the microsporidium from the fat body of *Microtendipes pedellus* had another polaroplast ultrastructure consisting of narrow closely packed lamellar and tubular parts. Previously this microsporidium was placed in the family Thelohaniidae based on octosporoblastic sporogony and features of exospore construction [11, 15].

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