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A new microsporidium *Paratuzetia kupermani* gen. et sp. n. (Microsporidia), a hyperparasite of the procercoid of the cestode *Khawia armeniaca* Chol. 1915 (Cestoda, Caryophyllidea)

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Summary

A new microsporidium *Paratuzetia kupermani* gen. et sp. n. (Microsporidia), the hyperparasite of the procercoid of the cestode *Khawia armeniaca* Chol. 1915 (Cestoda: Caryophyllidea), is described. Microsporidian development is monomorphic and occurs in direct contact with the cytoplasm of the host tegument. Division of cells of all life cycle stages is binary. Meronts possess diplokaryotic nuclear apparatus. Nuclei of sporogonial stages are single. Sporoblasts and spores develop in individual sporophorous vesicles. Lumen of the vesicles, 1 μm in diameter, is filled with wide short tubular structures. Spores are 2.0-2.5 × 1.2-1.4 μm in size, polar tube is anisophilar with 10-12 coils arranged in 2-3 layers in young spores and in a single layer in mature ones. Polaroplast consists of 2 parts, the posterior lamellar part surrounds the straight region of the polar tube and, in young spores, also its first coils.

Key words: microsporidia, cestode, procercoid, hyperparasite, *Khawia armeniaca, Paratuzetia kupermani*

Introduction

Several species of microsporidia have been described from adult cestodes. On the basis of their morphology and ultrastructure, they were attributed to the genera *Nosema* and *Unikaryon*, or else just mentioned without taxonomic reference (Kudo, 1924; Dissanaike, 1957; Mackiewicz, 1972; Subilia and Swiderski, 1984; Sene

et al., 1997). The only description of microsporidia from cestode larvae did not specify the systematic position of either parasite or its host (Guyenot et al., 1922).

In the course of a fine structural examination of the procercoid of *Khawia armeniaca*, parasitizing the body cavity of the annelid *Potamothrix hammonensis* (Oligochaeta, Tubificidae), developmental stages and spores of a new microsporidium were found in the tegument of the cestode. The present paper deals with the description of this hyperparasite.

Material and Methods

The oligochaetes *Potamothrix hammonensis* were collected in the waters of Sevan Lake (Armenia, Caucasus) at a depth of 1.5-5 m. Microsporidia-invaded procercoids of *Khawia armeniaca* found in the oligochaete body cavity were fixed with 2.5 % glutaraldehyde in 0.1 M cacodylate buffer at pH 7.2 for 1-2 days. Then the procercoids were washed 4 times for 20 minutes with the same buffer, postfixed in 1 % osmium tetroxide for 1 h and dehydrated in an ascending ethanol series. The samples were transferred to acetone and embedded in the Araldite resin. Semithin sections, stained with methylene blue, were examined under light microscope MFN-11. Ultrathin sections, stained with uranyl acetate and lead citrate, were examined using electron microscope JEOL 100C at an accelerating voltage of 80 kV.

Results

Since the microsporidium was found by chance during ultrastructural examination of the cestode procercoid, its description is done on the basis of EM observations only.

Description of *Paratuzetia kupermani* gen. et sp. n.

Host: procercoid of *Khawia armeniaca* Cholodkovsky, 1915 (Cestoda, Caryophyllidea) - parasite of the body cavity of the oligochaete *Potamothrix paravanicus* Poddubnaya, Pataridze (Oligochaeta), the intermediate host of this cestode. Adult cestode parasitizes the Sevan fish *Varicorhinus capoeta sevangi* (Filippi) (Pisces, Teleostei).

Localization is confined to tegument cells. Primary tegument of the procercoid functions as a secretory tissue. It consists of primary cytoplasmatic distal layer and underlying primary tegument cells, referred to as cytons. Distal cytoplasm is a thin syncytium filled with multiple electron-dense secretory granules, mitochondria, lipids and ribosomes, which initially concentrate in the primary cytons. Large cytons contain many free ribosomes, rough endoplasmstic reticulum (ER), the Golgi complex, mitochondria, lipids, autophagous vacuoles and electron-dense granules (Fig. 4, A) Undifferentiated cells underlie cytons in the parenchyma. In procercoids, microsporidia invade only primary tegument cells, the cytons, prevailing in this stage of cestode development.

All the life cycle stages of the microsporidium develop in direct contact with the host cell cytoplasm, not inducing parasitophorous vacuole formation (Fig. 4, B, C).

Life cycle. The earliest developmental stages found on ultrathin sections are late diplokaryotic meronts (Fig. 1, A, B). These are either amoeboid cells, 2.2 × 2.0 μm in size, with one diplokaryon about 1.5 μm in diameter, or larger cells, 3.1 × 2.5 μm, with two diplokarya, each up to 1.2-1.4 μm in diameter, occupying most of the cell volume. Cytoplasm is filled with Golgi complex structures and tight rows of ER, densely incrusted with ribosomes. Numerous small granules are irregularly scattered at the cytoplasmic membrane. Meronts that have reached the length of 3.3 μm undergo binary division (Fig. 1, C, D). At the same time a continuous layer of electron-dense amorphous material forms outside on the surface of each cell, marking the transition to the next life cycle phase, the sporogony (Fig. 1, D).

Sporont, fully enclosed into the additional electron-dense sheath, deforms during fixation. The formation of the new layer (the future exospore), characteristic of sporogonial stages, is also accompanied by that of a thin membranous layer around each cell, almost adjacent to its surface (Fig. 2, A, B). We regard the latter as a sheath of an individual sporophorous vacuole. A sporont, with 2 single nuclei, gives rise to mononuclear sporoblasts.

Sporoblasts, 2.9 × 1.8 μm in size, look like gear-wheels on sections due to deformation. At the top of the protrusions, Golgi complex structures are found (Fig. 2, B). The membranous layer surrounds almost the whole cell. In the lumen between the membranoid and the cytoplasmatic membrane of the cell, scarce tubular structures can be seen.

Sporoblasts transform into **young spores**, initially retaining the gear-wheel shape. The electron-translucent layer of the future endospore begins to emerge between the cytoplasmatic membrane and the amorphous layer of the future exospore (Fig. 2, C-E). In the cytoplasm of the young spores, rudiments of the polar tube coils can be observed as single small vacuoles enclosed by a bilaminate membrane (Fig. 2, C). Complexes of small vesicular structures, belonging to the future polaroplast, can also be seen (Fig. 2, D).

Sheath of the parasite cell changes drastically during transition to sporogenesis. The cell is now covered with a dense epispore layer of tubular structures,

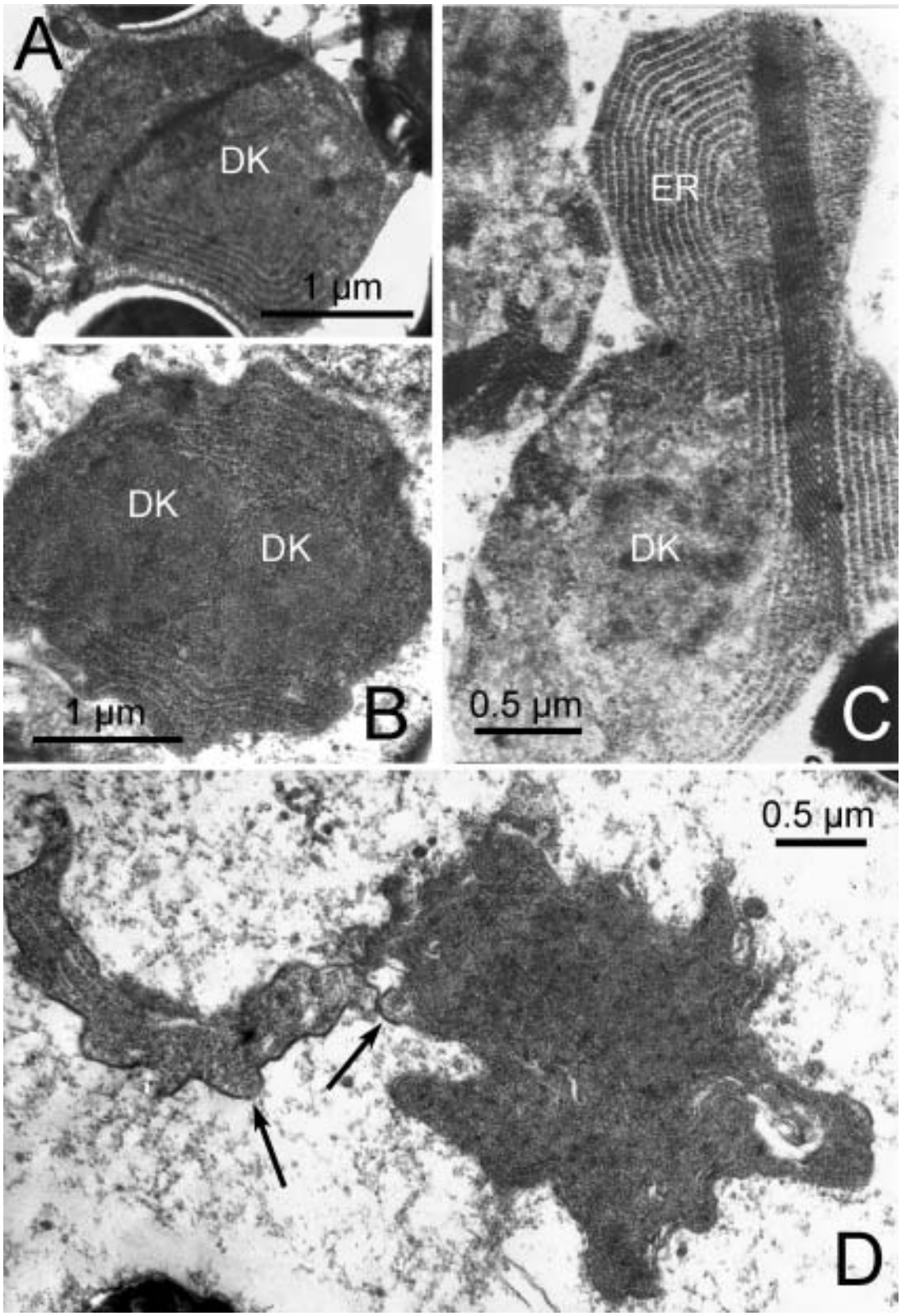

Fig. 1. Merogonial stages of *Paratuzetia kupermani*. A - a meront with one diplokaryon; B - a meront with two diplokarya, cytoplasmic membrane is covered with granules; C - binary division of meront; D - formation of an additional sheath, typical of sporont (*arrows*). *Abbreviations*: DK - diplokaryon, ER - endoplasmatic reticulum.

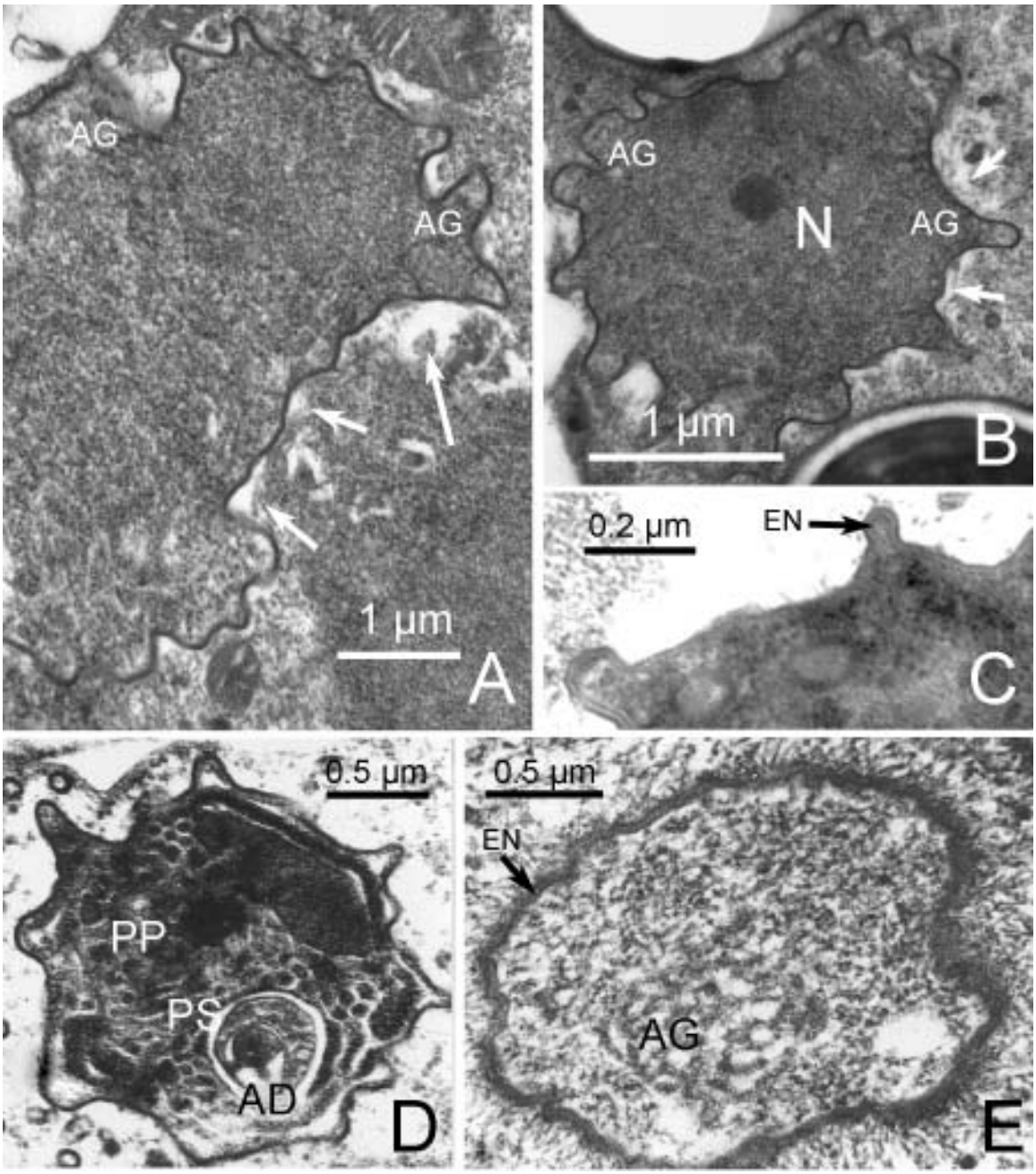

Fig. 2. Sporogonial stages of *Paratuzetia kupermani*. A - a monokaryotic sporont, sporophorous vesicle formation begins (*arrows*); B - a sporoblast, deformed by fixation; in its projections Golgi structures are seen; C - a region of a young spore with nascent polar tube and endospore; D - a transverse section of the anterior pole of a young spore with nascent anchoring disc, polar sac and anterior vesicular part of the polaroplast; E - a young spore with its lumen filled with Golgi structures and its endospore surrounded with epispore structures. *Abbreviations*: AG - Golgi complex, EN - endospore, N - nucleus, AD - anchoring disc, PP - anterior region of polaroplast, PS - polar sac.

perpendicular to the cell surface, which is surrounded by a thin membranous layer of the sporophorous vesicle wall (Fig. 2, E).

The wall of the young spore has a most peculiar structure (Fig. 3). Epispore layer, limited by the membranoid, reaches its maximal development and a height of 0.5-1.0 μm (Fig. 3, A-C). This epispore layer is represented by elongated vesicles (or broad short tubules), filled with electron-dense material arranged along the axis. At several regions, this layer forms long

protrusions into the host cell cytoplasm towards the neighbour spores (Fig.3, A-D). At the periphery of these protrusions, a branching tubular-vesicular network develops as their continuation, the diameter of tubules being 50-70 nm (Figs 3, D; 4, E). This tubular network, typical of most microsporidian species, is very often located between the parasite cells and host cell mitochondria (Fig. 3, D). Young spores are 2.2 × 2.5 μm in size.

Mature spores have regular ovoid shape and a size (without the tubular cover) of 2.0-2.5 × 1.2-1.4 μm, rarely 2.8 × 1.3 μm. Presumably, fixation of both young and mature spores was always unsuccessful due to the presence of the sporophorous vesicle wall and the epispore layer. Therefore, description of inner spore structure relies on fragmentary good pictures of spores from different sections. The spore has a mushroom-shaped anchoring disc with a diameter of about 0.3 μm. An isofilar polar tube has 10-12 (rarely, more) coils and tapers towards its distal end. In the young spores, coils are often arranged in 2-3 layers, whereas in mature spores, always in one layer. Diameter of anterior coils is about 100 nm, that of posterior ones, about 70 nm. The polar sac covers the polaroplast approximately by 1/3 of spore length (Fig. 3, E). Lamellar polaroplast is concentrated along the straight region of the polar tube, and sometimes, as tubular structures, even around its first coils (Fig. 3, E). The anterior part of the lamellar polaroplast bears a vesicular structure situated between the lamellae and the polar sac and covering the polaroplast like an umbrella. A single nucleus, 0.5 μm in diameter, is located centrally in the spore. On the posterior pole of the young spores the posterosome, the Golgi complex derivative, is present. In mature spores there is a large posterior vacuole, up to 0.8 μm in diameter, behind the posterosome.

The endospore is up to 100 nm thick. A distinct tubular epispore, typical of young spores, disappears in mature ones, and the thin membranous layer becomes separated from the spore wall with a considerable distance. There are thin short filamentous offshoots on the exospore surface similar to the fibrils described in the microsporidium *Trichotuzetia* (Vavra et al., 1997).

On the basis of the data available, it is difficult to judge about the origin of the membranoid, bounding tubular structures on the cell surface of the merogonial stages. Absence of ribosomes makes doubtful the host cell ER participation in its formation. At the same time, ultrathin sections show that these membranes are not tightly associated with the spore surface, as they are retained on the sections where spores are lost (Fig. 3, D).Yet, taking into account close relation of the microsporidium described with the Tuzetiidae family relatives, parasitic derivation of the membranoid appears more probable.

Pathogenic impact on the host cell. Ultrathin sections of infected cells demonstrate 2-4 times hypertrophy of the cell and its nucleus, the loss of secretory granules, Golgi complex structures, ribosomes and ER (Fig. 4, A-C). This makes host cell cytoplasm electron-translucent as compared to the electron-dense one of uninfected cells. Tubular-vesicular structures are not abundant in the cytoplasm but are mainly located between maturing spores and host cell mitochondria. Electron-translucent zones around the maturing spores do not form, either. Altogether, these observations testify in favour of moderate pathogenicity of this parasite.

Pathogenic impact on the host organism. During microsporidian infection the outer layer of the cestode tegument is destroyed, which results in disfunction of secretion normally aimed at overcoming the defence reaction of the host, the oligochaete. As a result, the number of microsporidia-infected procercoids decreases. Presence of empty (exfilamented) spores on the sections allows one to suggest the possibility of invasion of new tegument cells of the same host or of other procercoids by spores discharging from neighbouring cells or procercoids.

Location: Caucasus, Armenia, Sevan Lake.

Discussion

Though several microsporidian species are known to parasitize adult cestodes, only one microsporidian hyperparsite has been reported from cestode larvae. Therefore, there are no data on the specificity of microsporidia to certain life stages of their cestode hosts.

The first review on microsporidia from three species of adult cestodes was given by Kudo (1924). Microsporidia, referred to as *Gen. inc. helminthophthorus*, were found in parenchyma, gonads and eggs of *Taenia bacillaris, T. denticulata* and *T. expansa* in Germany and France (Labbe, 1899; Moniez, 1922). According to the former author, microsporidian spores were ovoid and had 4.2-5.9 × 1.7-2.5 μm in size; according to the latter, the spores were 5 × 2.5 μm in size. Both authors noted that invasion of ovaries with microsporidia suppressed development of this organ, thus preventing transovarial transmission of microsporidia. A microsporidium named *Nosema helminthorum* was later described from mature segment of the cestode *Moniezia expansa* (Dissanaike, 1957).

Examination of ultrastructural organisation of this microsporidian (Canning and Gunn, 1984) revealed both diplokaryotic and mononuclear forms in its life cycle, which does not match the diagnosis of the *Nosema* genus. The number of nuclei in mature spores was not determined. Unfortunately, the authors did not

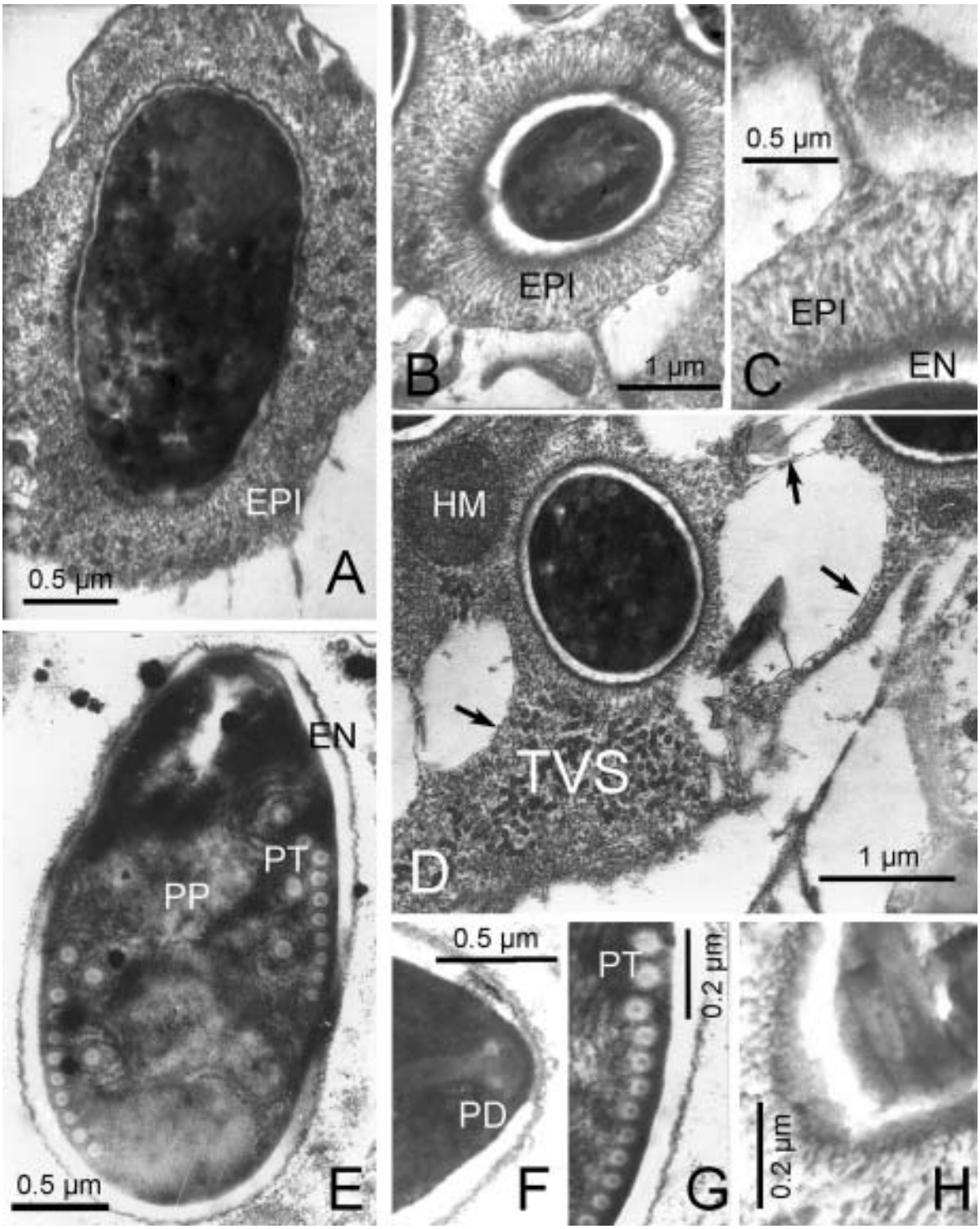

Fig. 3. Spore structure of *Paratuzetia kupermani*. A - a young spore with a tubular network emerging on the periphery of its epispore; B - maturing spore; C - a part of the epispore layer, tubular structures and secreted material along their axis can be seen; D - region of the host cell: tubular-vesicular network and the membranous sheath of the sporophorous vesicle at the place where the spores previously were (*arrows*); E - a longitudinal section of a young spore; F - anterior pole of the spore with the anchoring disc; G - polar tube; H - posterior pole of the mature spore with its wall, covered with fibrils. *Abbreviations*: EPI - epispore; HM - host cell mitochondria; PT - polar tube; TVS - tubular-vesicular network. For other abbreviations see Fig. 2.

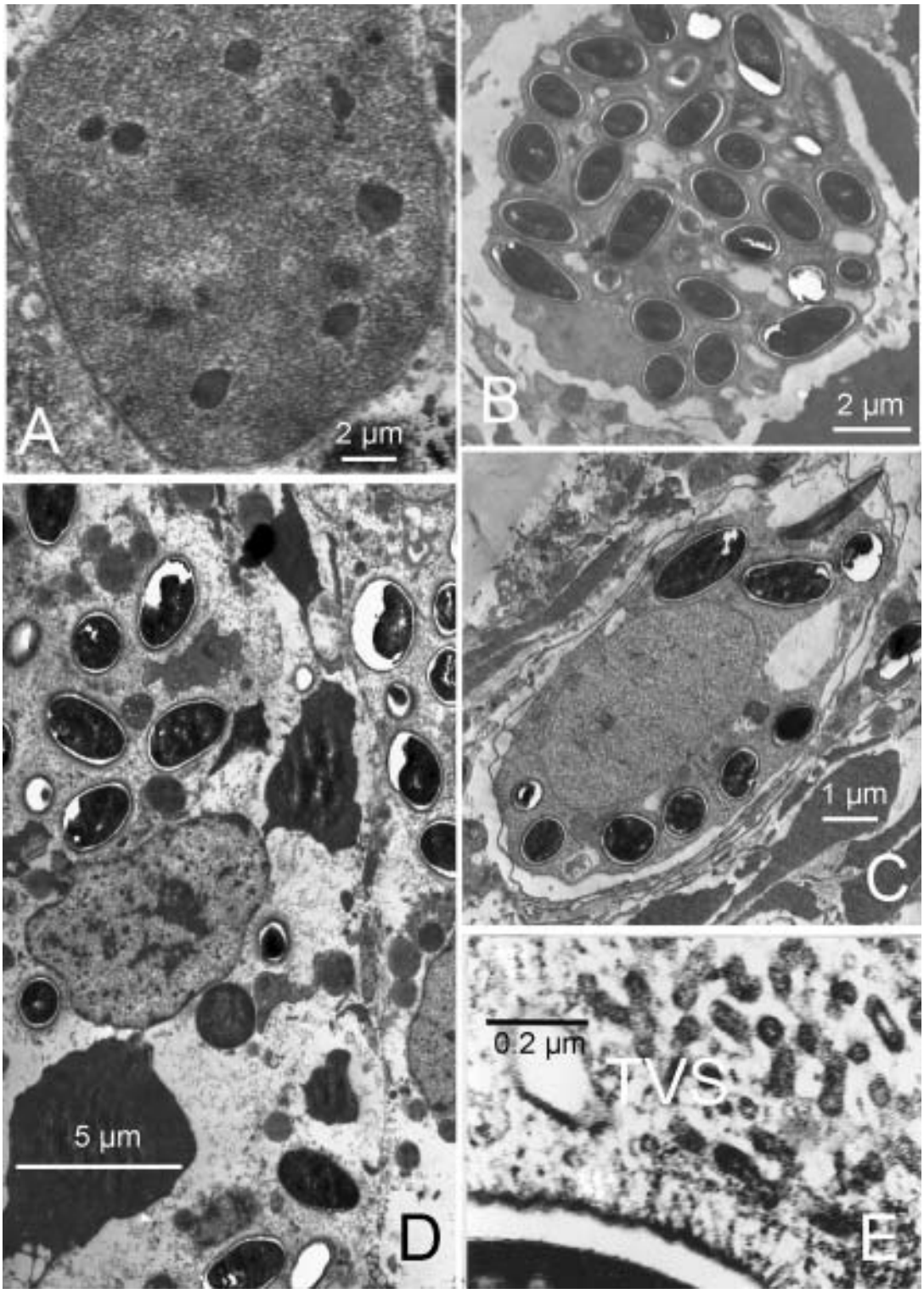

Fig. 4. Pathology of the cestode tegument cells, caused by the microsporidium *Paratuzetia kupermani*. A - microsporidia-free cyton (primary tegument cell), with electron-dense cytoplasm filled with small and large secretory granules and ribosomes; B and C - invaded cytons, cytoplasm lacks secretory granules but retains mitochondria; D - primary tegument: infected cells have electron-translucent cytoplasm and larger size as compared to non-infected cells; E - host cell cytoplasm with a branching tubular-vesicular network, formed by the parasite. For abbreviations see Fig. 2.

define the systematic position of this species in view of the new data about it being a dimorphic form.

A microsporidium with spores 2-2.5 × 1.5 μm in size was once found in Italy, in an undetermined cestode larva (related, in the opinion of Kudo (1924), to *Ligula colubri blumenbachii* Cobbold) parasitizing the grass snake *Tropidonotus natrix* (Guyenot et al., 1922).

Brief information regarding findings of microsporidia in cestodes in North America is drawn from an overview on caryophyllid cestodes (Mackiewicz, 1972). According to the author, a microsporidium, identified by V. Sprague as *Nosema* sp., disturbed the spermatogenesis process in cases of an intensive invasion of many body regions and testicles of *Glaridacris confuses*. Unidentified microsporidian species causing light infection were also found in cestodes *Hunterella nodulosa, Khawia iowensis* and *Isoglaridacris folius* in Tennessee.

An unidentified microsporidium is also mentioned as a parasite of genital ducts and testicles of the cestode *Oochoristica agamae* (Subilia and Swiderski, 1984).

The most complete information is available on the microsporidium *Unikaryon nomimoscolexi* (Sene et al., 1997) from *Nomimoscolex* sp. (Cestoda, Proteocephalidea), parasitizing in the gut of the widehead catfish *Clarotes laticeps* (Pisces, Teleostei) in Senegal (Western Africa). This mononuclear microsporidium invades parenchymal cells. Sporogony proceeds without sporophorous vesicle formation. Average measurements of ovoid spores on ultrathin sections are 3.43 × 1.51 μm. Isofilar polar tube forms 6-8 coils.

Summarising the above, microsporidia of cestodes are an insufficiently studied group of parasites. The parasites found are either identified as members of *Nosema* and *Unikaryon* genera or just mentioned as "microsporidia". According to the modern data (Canning and Gunn, 1984), such a prevailing parasite of cestodes as *Nosema helminthorum* might even not belong to the *Nosema* genus at all.

Microsporidia with spores enveloped in individual sporophorous vacuoles are referred to the family Tuzetiidae (Larsson, 1983). Representatives of two genera from this family, *Tuzetia* and *Trichotuzetia*, have spores with an epispore coating very similar to that of the microsporidium described in this work. No microsporidium from this family has been previously reported from cestodes.

Comparison shows that the form described here is very distinct from the known genera of the Tuzetiidae family. Presence of diplokaryotic meronts distinguishes it from representatives of the monokaryotic genera *Tuzetia* and *Trichotuzetia*, while episporal structures in the sporophorous vesicle lumen and absence of a multilayered exospore make it different from the *Alfvenia* species. It does not fit the criteria of the *Janacekia* genus due to binary division of all the developmental stages and absence of multinuclear forms, and it cannot be included into *Nelliemelba* because of presence of diplokaryotic meronts and isofilar polar tube. These significant distinctions allow us to consider the microsporidium described as a new species of a new genus.

Diagnosis of the genus *Paratuzetia* gen. n.

Monomorphic form with diplokaryotic meronts and mononuclear sporogonial stages. No multinuclear plasmodia formed. Binary division of cells. Sporogonial stages in individual sporophorous vacuoles with lumen entirely filled with wide tubular structures. Spores with isofilar polar tube, polaroplast, consisting of two parts, and large posterior vacuole. Development in direct contact with host cell.

Type species: *Paratuzetia kupermani* sp. n. from a cestode larva.

Etymology. The name of the genus *Paratuzetia* reflects the presence of individual sporophorous vacuoles around (="para-" in Greek) sporogonial stages and the similarity of the epispore cover with that of the microsporidia from the genera *Tuzetia* and *Trichotuzetia.*

Diagnosis of the species *Paratuzetia kupermani* sp. n.

Spores ovoid, 2.0-2.5 × 1.2-1.4 μm in size, with large posterior vacuole. Polar tube, with 10-12 coils, arranged in 2-3 layers in young spores, and in one layer, in mature spores. Epispore tubular layer up to 1 μm in height.

Type host: larva of cestode *Khawia armeniaca* Cholodkovsky, 1915 (Cestoda, Caryophyllidea) from oligochaete *Potamothrix paravanicus.*

Etymology. The species is named in honour of B.I. Kuperman, who supervised the research on cestode ultrastructure performed by the first author of the present paper.

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