

RESEARCH NOTE

Ultrastructural data on *Gurleya orchestiae* Ovcharenko et Kurandina, 1987 (Microsporidia, Gurleyidae)

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Abstract

Ultrastructural data of the sporulation stages of the microsporidium *Gurleya orchestiae*, infecting the muscles of talitrid amphipods *Orchestia* sp. (Crustacea, Amphipoda), are presented. The developmental stages of the parasite have isolated nuclei. Sporogonial reproduction is by rosette-like budding. Sporophorous vesicles contain 4, seldom 8 spores. The episporontal space contains granules and tubules. Spores are of oval shape, monokaryotic with lamellate bipartite polaroplast, double-layered exospore and isofilar polar filament, turned into 8–9 coils.

Key words

Microsporidia, *Gurleya orchestiae*, ultrastructure, *Orchestia* sp., Amphipoda

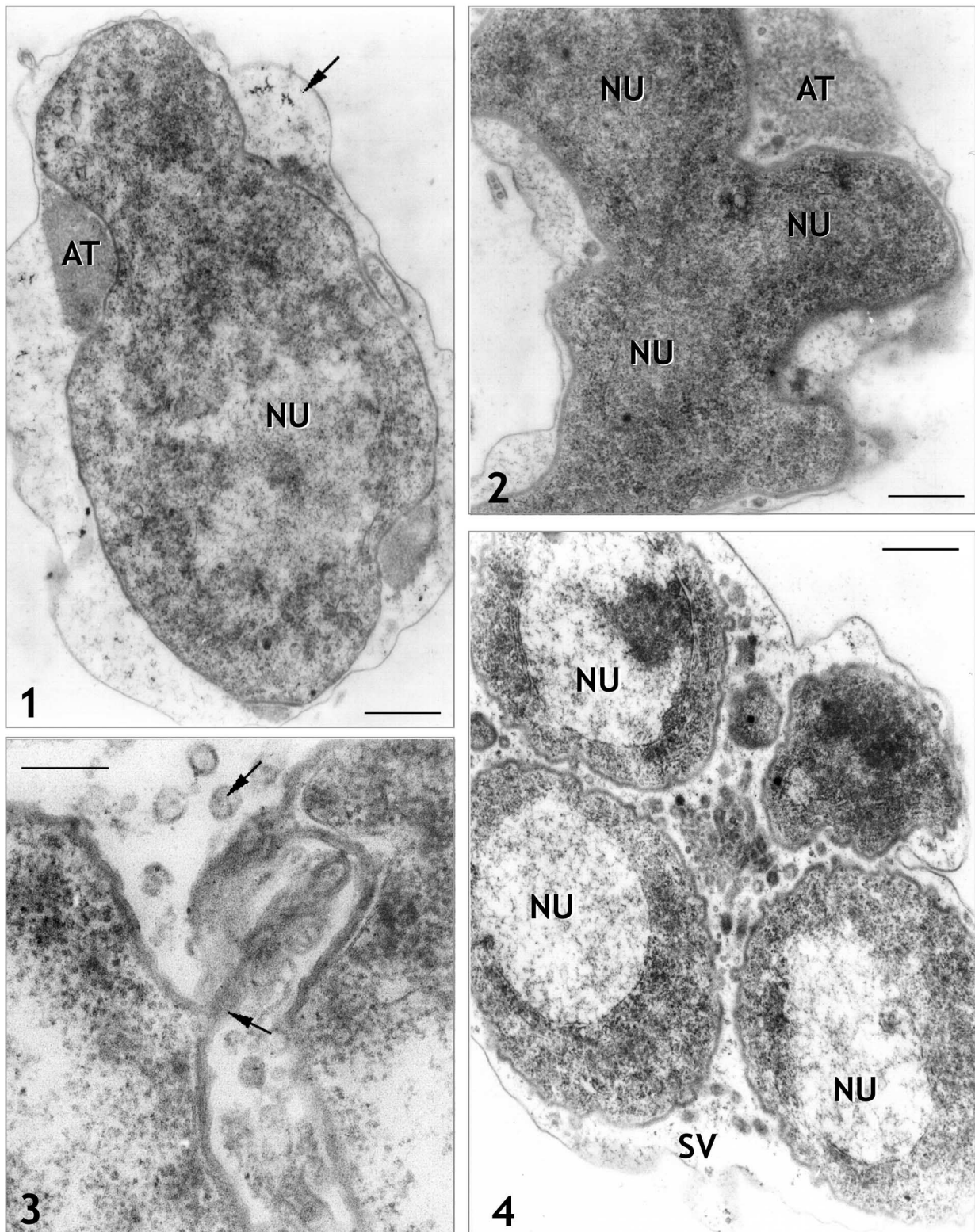
The tetrasporous microsporidium *Gurleya orchestiae* was described from the muscles of *Orchestia bottae* Edwards, 1840 in South Ukraine based on light microscopic data (Ovcharenko and Kurandina 1987). In August 1999, three infected amphipods of the same species were collected in the same region (46°35'N, 32°16'E), and electron microscopy preparations were made. The infected tissues were fixed in 2.5% glutaraldehyde solution for 1–3 days, postfixed in 2% osmium tetroxide for 1 h at 4°C and embedded in Epon-Araldite resin mixture as reported previously (Ovcharenko and Wita 2001a, b). Ultrathin sections were stained with uranyl acetate followed by lead citrate, and examined in a JEM 100B transmission electron microscope.

The shape and dimensions of the spores were similar to those of *G. orchestiae* as originally described (Ovcharenko and Kurandina 1987). The parasite infects the cytoplasm of the muscle cells. No hypertrophy of infected muscles was observed. The earliest stage observed were rounded uninucleate sporonts (Fig. 1). Their cytoplasm had a weakly developed endoplasmic reticulum. Uni- and tetranucleate sporonts were enclosed within fragile sporophorous vesicle containing granules and aggregated fine tubules (Figs 1 and 2). Episporontal material was a product of exospore production (Fig. 3).

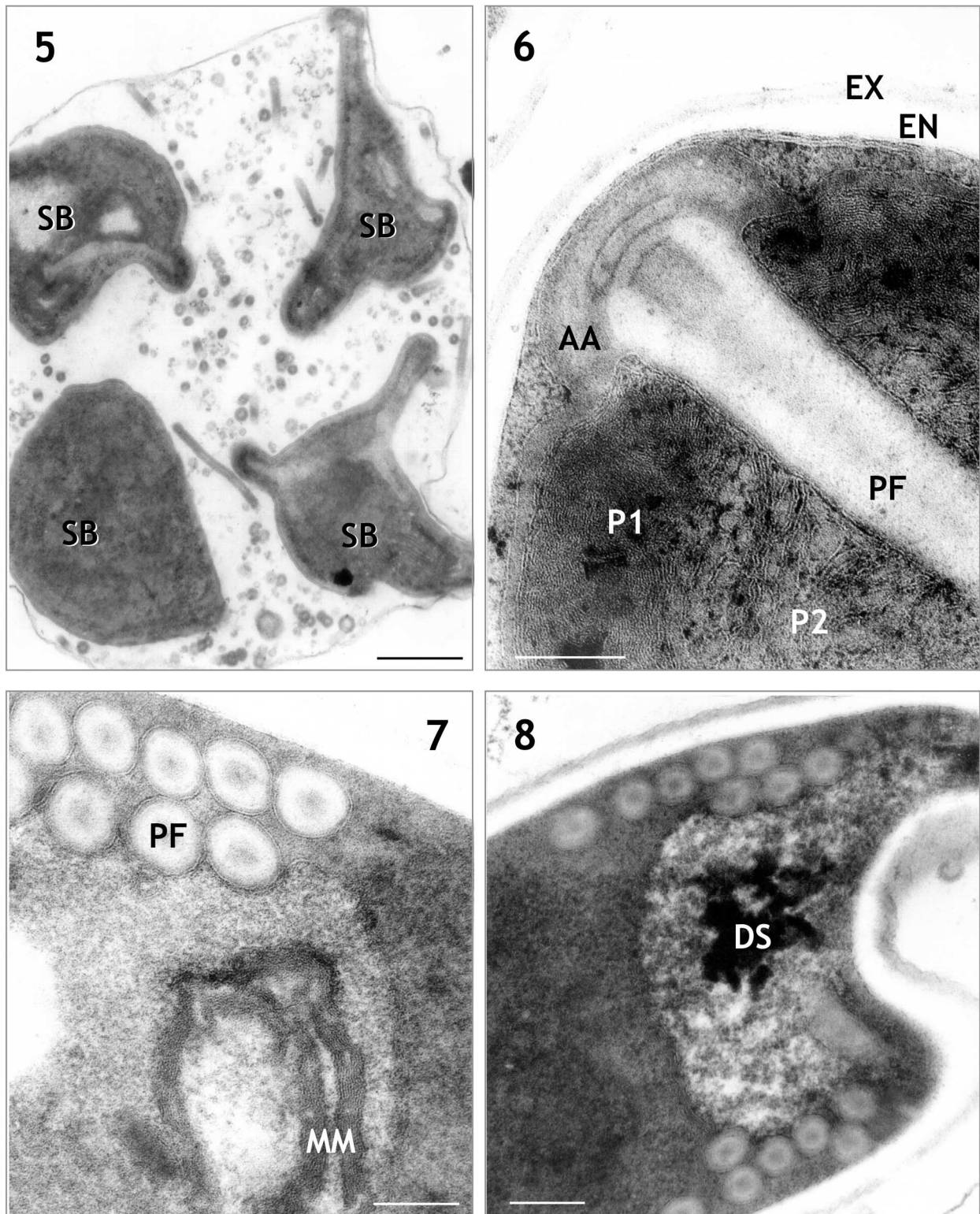
During the development of the sporoblasts episporontal inclusions transform into tubules (Figs 3–5). Episporontal tubules and the exospore have an identical structure. Each rosette-like budding plasmodium produces four uninucleate sporoblasts (Figs 2 and 4). The cytoplasm of the sporoblasts contains numerous ribosomes and a weakly developed endoplasmic reticulum (Fig. 4). When the sporoblasts matured the differentiation of the spore organelles could be followed. The shape of the future spore became more regular, and the cytoplasm grew denser (Fig. 5). Generally, each sporophorous vesicle contained four spores, but in addition octosporous vesicles containing spores measuring about $4.2 \times 2.6 \mu\text{m}$ were rarely observed.

Thin-walled uninucleate spores in the squash preparations were of oval shape, measuring $4.3 \pm 0.6 \times 2.7 \pm 0.7 \mu\text{m}$. The spores were enclosed in a thin fragile sporophorous vesicle. The spore wall exhibited the three classic layers: an internal plasma membrane (Figs 3 and 11), a structureless endospore 70 nm wide, and a 35 nm wide layered exospore (Figs 6–8). The exospore was composed of two distinct parts: an internal electron-dense layer, and a membrane-bound external layer of

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Figs 1–4. Sporogonial development of *Gurleya orchestiae*. **1.** Early sporont with a single nucleus (NU) and weakly developed endoplasmic reticulum. The episporontal space contains granules and aggregates of fine tubules (AT). The sporont is surrounded by the fragile envelope of the sporophorous vesicle (arrowed). **2.** Rosette-like budding sporogonial plasmodium with separate nuclei, and dense cytoplasm. Granular and tubular (AT) episporontal inclusions are visible. **3.** Part of a sectioned sporophorous vesicle. Episporontal inclusions are involved in the exospore production (arrowed). **4.** Four sporoblasts with separate nuclei and weakly developed endoplasmic reticulum inside a sporophorous vesicle (SV) containing inclusions. Scale bars: 1 = 0.7 μm , 2 and 4 = 0.5 μm , 3 = 0.3 μm



Figs 5–8. Late sporogony and ultrastructure of mature spores. **5.** Transversely sectioned fragile sporophorous vesicle containing four late sporoblasts (SB) and tubular inclusions. **6.** Longitudinal section of the anterior part of a mature spore with typically constructed anchoring apparatus (AA), basal part of the polar filament (PF) and lamellar polaroplast. The polar filament passes through the narrow lamellar (P1) and wide lamellar (P2) regions of polaroplast. The layered exospore (EX) and the electron-transparent endospore (EN) are visible. **Figs 7 and 8.** Longitudinal sections of the posterior part of the spore. The polar filament is coiled with 8–9 turns, arranged in two rows. Multimembranous (MM) and dense formless structures (DS) are visible inside the spongy posterior vacuoles. Scale bars: 5 = 0.6 μm , 6 = 1.7 μm , 7 = 1.6 μm , 8 = 0.2 μm

low electron density (Fig. 6). The 160–180 nm wide isofilar polar filament was attached to a typically constructed anchoring apparatus (Fig. 6). It passed backwards through the polaroplast before it turned to the spore wall. The polar filament was coiled with 8–9 turns, arranged in two rows in the posterior part of the spore (Figs 7 and 8). The polaroplast had two regions with regularly arranged, closely packed lamellae, formed by unit membrane folds: anteriorly narrow, posteriorly wider (Fig. 6). The posterior vacuole was never well fixed, but could be traced as membrane delimited spongy area at the posterior pole of the spores (Figs 7 and 8). Tightly packed multimembranes and dense formless structures were observed inside posterior vacuoles (Figs 7 and 8).

The obtained data demonstrate that the investigated microsporidium is cytologically similar to species of the genus *Gurleya* described from amphipods and trichopterans (Larsson 1995, Wita *et al.* 1999). Two ultrastructurally studied species – *G. daphniae* and *G. dorisnae*, infecting cladocerans and copepods – have anisofilar polar filament and vesicular posterior polaroplast (Friedrich *et al.* 1996, Voronin 1996). The achieved data confirm the suggested heterogeneous character of the genus *Gurleya*.

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