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New data on the ultrastructure of *Paradermamoeba levis* (Amoebozoa, Discosea, Dermamoebida): Cytoplasmic MTOCs are found among Dermamoebida

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Abstract

In this detailed electron microscopic study of *Paradermamoeba levis* Smirnov et Goodkov 1994 (phylum Amoebozoa, class Discosea, subclass Longamoebia, order Dermamoebida, family Dermamoebidae) based on numerous fixations and studies of a large number of cells we provide the first comprehensive description of the ultrastructure of this species. *P. levis* possesses cytoplasmic microtubule-organizing centres (MTOCs) associated with dictyosomes of the Golgi complex. This finding adds evidence to our earlier suggestion that the presence of cytoplasmic MTOCs is a synapomorphy of the phylogenetic lineages forming the subclass Longamoebia. The so-called "supernumerary nucleus" of *P. levis* noted in the initial description was found to be not an individual structure but an outgrowth of the cell nucleus containing its own nucleolus. Enigmatic trichocyst-like bodies were noted in all studied strains, originating from different geographic locations. This proves that these bodies are integral parts of the cell structure, not an occasional property of the type strain. *P. levis* is now reliably recorded from several European locations (North-Western Russia, Croatia, Switzerland, UK) and Far East of Russia.

Keywords: Amoebozoa; Cell coat; *Paradermamoeba*; Ultrastructure

Introduction

The genus *Paradermamoeba* was established by Smirnov and Goodkov (1993) to accommodate the species *P. valamo*, isolated from freshwater sediments of a shallow lake at Valamo Island (Ladoga Lake, North-West Russia). This remarkable amoeba of lanceolate morphotype (Smirnov and Goodkov 1999; Smirnov and Brown 2004) possesses a thick (ca. 520 nm) glycocalyx consisting of a layer of tightly packed helical glycostyles. In electron-microscopic images each helix consists of 7–7.5 turns and is terminated with a funnel-shaped structure, pentagonal in cross-section (Smirnov and Goodkov 1993, 1994, 2004). Another species of the genus *Paradermamoeba*, *P. levis*, isolated from the same location, was subsequently described (Smirnov and Goodkov 1994). These two species share the same general characters, however *P. levis* is about half the size of *P. valamo*, the flattened lateral extensions of the cell body are much less pronounced in this species and the thickness of the cell coat reaches only 220 nm; each helix in *P. levis* consists of 4 turns only. In the type strain of *P. levis* a considerable

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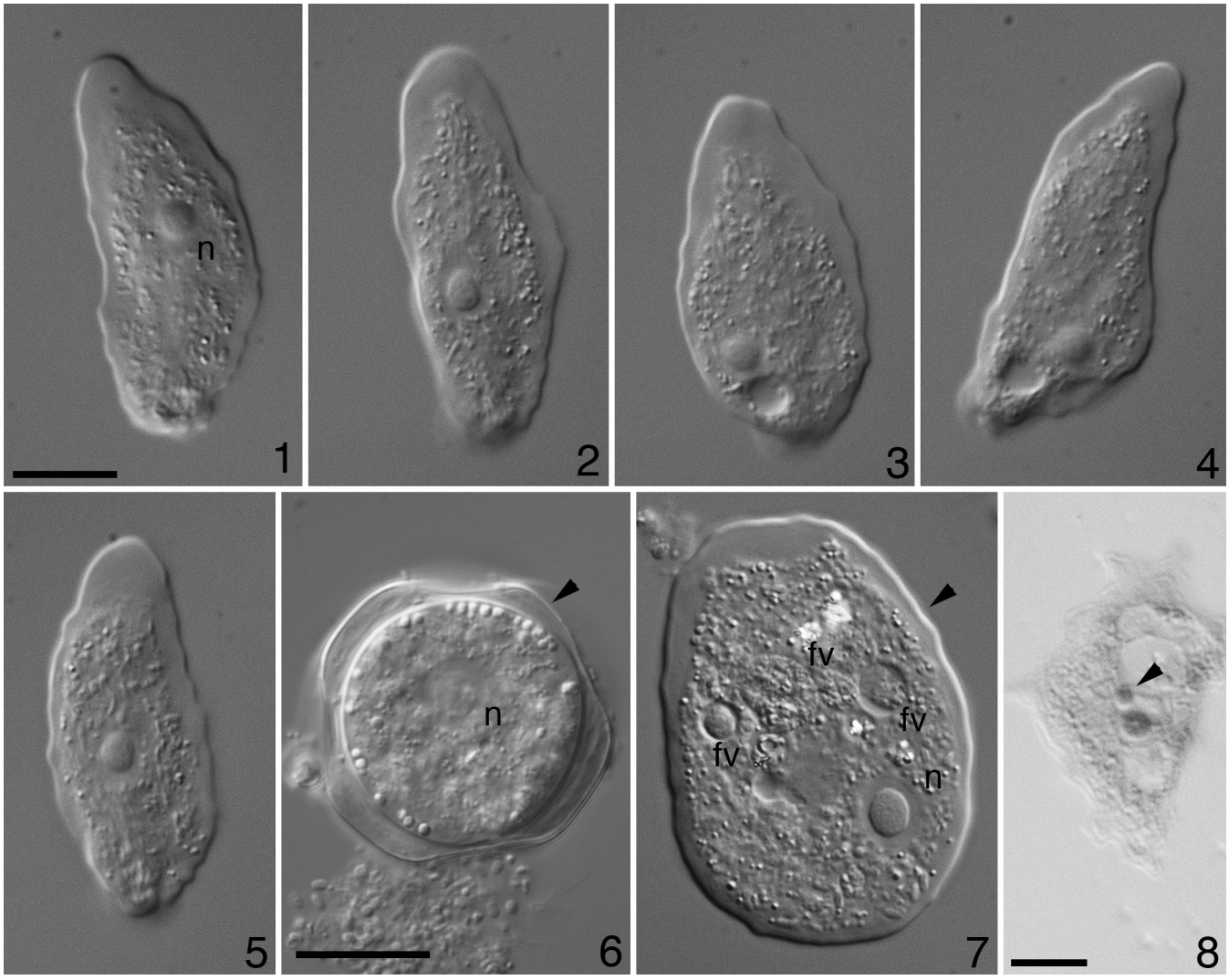
proportion of binucleate cells with closely apposed nuclei were noted; one nucleus was often smaller than the other one and considered as "supernumerary" (Smirnov and Goodkov 1994). This species is cyst-forming, while in *P. valamo* cysts were never noted (Smirnov and Goodkov 2004). At the time of description the genus *Paradermamoeba* was classified within the family Thecamoebidae Schaeffer, 1926 within the subclass Gymnamoebia of the class Lobosea (Page 1987). In the modern system it is placed in the family Dermamoebidae Cavalier-Smith et Smirnov, 2011, order Dermamoebida, subclass Longamoebia, class Discosea, subphylum Lobosa, phylum Amoebozoa (Smirnov et al. 2011a,b).

The thick cell coat of amoebae of genus *Paradermamoeba* makes it difficult to fix them for electron microscopy. In the initial description (Smirnov and Goodkov 1993, 1994) it was possible to provide data only on the cell coat. Further Smirnov and Goodkov (2004) applied Triton X-100 treatment to achieve better fixation of *P. valamo* and *P. levis*. This method allowed them to obtain data on their internal structure; remarkable points were enigmatic trichocyst-like bodies and relatively small size and number of dictyosomes in the cytoplasm. However, the fixation quality in this study was lower than that achieved in the present paper; all *P. levis* cells studied were uninucleate.

For the present study we re-isolated *P. levis* from Valamo Island (type location) and obtained new strains of *P. levis* from the river Krka (Croatia). We found a fixation protocol allowing better preservation of the cytoplasm and performed electron-microscopic investigation of uninucleate cells and cells possessing supernumerary nucleus. This study provides the first comprehensive description of the ultrastructure of *P. levis* and contributes to the knowledge on the biogeography of *Paradermamoeba*.



Figs 1–8. Light microscopy images of *Paradermamoeba levis*. (1–5) Sample of the locomotive forms on a glass surface. (6) Cyst; the outer layer of the cyst envelope (arrowhead) is separated from the inner one in several areas. (7) Cell pressed with the coverslip to show the nucleus (n), food vacuoles (fv) and glycocalyx layer (arrowhead). (8) Stained preparation (holotype) photographed using DIC to show the nucleus with supernumerary nucleolus (arrowhead). Scale bar is 10 μm.