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Pseudoparamoeba microlepis n. sp., *Korotnevella fousta* n. sp. (Amoebozoa, Dactylopodida), with notes on the evolution of scales among dactylopodid amoebae

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Abstract

The present paper describes two new species of lobose amoebae belonging to the family Paramoebidae within the order Dactylopodida: *Pseudoparamoeba microlepis* n. sp. and *Korotnevella fousta* n. sp. Among them, *P. microlepis* formally fits the diagnosis of the genus *Korotnevella*, because it has scales and lacks a parasome. At the same time its 18S rDNA gene sequence robustly groups with *Pseudoparamoeba pagei* and never branches among those of *Korotnevella* spp. *Korotnevella fousta* in 18S rDNA tree groups among other species of the genus *Korotnevella*. It has uniform scales with an elliptical basal plate and spine arising from its central part and surrounded by an "inverted skirt" structure. These findings show that the current differentiation between the genera *Korotnevella* and *Pseudoparamoeba* based on the presence of scales is not entirely valid. At the moment only molecular data can reliably differentiate these two genera.

Keywords: Amoebozoa; *Korotnevella*; *Pseudoparamoeba*; Phylogeny; Systematics; Ultrastructure

Introduction

The amoebae order Dactylopodida (Amoebozoa: Discosea: Flabellinia) was established by Smirnov et al. (2005) on the base of the results of the molecular phylogenetic analysis for a clade comprising the genera *Vexillifera*, *Neoparamoeba* and *Korotnevella*, which was first revealed in the 18S rDNA trees by Peglar et al. (2003) and further expanded by Mullen et al. (2005). It is supposed that this clade is morphologically well supported by the presence of non-furcating finger-shaped subpseudopodia (dactylopodia) shared by all its representatives and considered as synapomorphic character of all dactylopodids (Smirnov et al. 2005, 2011). Subpseudopodia of *Vexillifera* are longer and slender than those of other dactylopodid genera, and the amoebae itself are often considered as being of acanthopodial morphotype (Kudryavtsev et al. 2011; Smirnov and Brown 2004). This order now comprises six amoebae genera grouped in two families. The genera *Paramoeba, Neoparamoeba*, *Korotnevella*, *Pseudoparamoeba* and *Cunea* belong to the family Paramoebidae, while *Vexillifera* belong to the family Vexilliferidae (Kudryavtsev et al. 2011).

Traditionally, genera of Dactylopodida were differentiated from each other based on the structure of the cell coat

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and presence or absence of a kinetoplastid endosymbiont, the *Perkinsela amoebae*-like organism or PLO (Dyková et al. 2008), which was initially named as "parasome" or "Nebenkörper" (Schaudinn 1896). Representatives of two genera, *Paramoeba* and *Neoparamoeba*, both have parasomes. However, amoebae of the genus *Neoparamoeba* have amorphous glycocalyx or hair-like structures in their cell coat (Cann and Page 1982; Dyková et al. 1998, 2000, 2005, 2007; Page 1987), while those of the genus *Paramoeba* possess scales (Grell and Benwitz 1966, 1970; Kudryavtsev et al. 2011). Amoebae of the remaining four genera do not have parasomes. The main characteristic of the genus *Korotnevella* is the presence of scales in the cell coat (Goodkov 1988; Page 1981; O'Kelly et al. 2001; Pennick and Goodfellow 1975; Smirnov 1996–97, 1999; Udalov 2015). The only described species of the genus *Pseudoparamoeba*, *P. pagei*, has hexagonal glycostyles with a dome-shaped distal region (Page 1979b). The genus *Vexillifera* differs from others by its remarkable locomotive form with long and slender dactylopodia in combination with an amorphous glycocalyx, which may be covered with T-shaped or hexagonal glycostyles (Anderson 1994; Dyková et al. 2011; Mascaro et al. 1985; Page 1979a,c, 1983). Representatives of the recently described genus *Cunea* resemble *Vexillifera* in general appearance, but have an ability to adopt a monopodial locomotive form, which is not characteristic for vexilliferians; their cell coat contains glycostyles (Kudryavtsev and Pawlowski 2015).

In molecular phylogenetic trees species belonging to the genera *Paramoeba* and *Neoparamoeba* form a well-supported clade but are mixed inside this clade; it was even suggested to merge these genera (Feehan et al. 2013; Kudryavtsev et al. 2011). Amoebae of the genus *Korotnevella* form a separate clade in the 18S rDNA trees (Mullen et al. 2005; Peglar et al. 2003). The genus *Cunea* represents an independent lineage within Dactylopodida (Kudryavtsev and Pawlowski 2015). All species of the genus *Vexillifera* form a robust clade, usually basal to all other dactylopodids, except for an ATCC strain 50883 (deposited as "*Vexillifera armata*"), which always forms an independent clade with *Pseudoparamoeba pagei* (Dyková et al. 2011; Mullen et al. 2005). Based on the results of phylogenetic analyses, Kudryavtsev et al. (2011) transferred the genera *Neoparamoeba* and *Pseudoparamoeba* to the family Paramoebidae; with this change the family Vexilliferidae remained monotypic. Kudryavtsev and Pawlowski (2015) added the newly described genus *Cunea* to the family Paramoebidae, so it now contains five genera of amoebae.

This paper describes a new species, *Pseudoparamoeba microlepis* n. sp., which resembles *Korotnevella* in morphology (it has scales in the cell coat, no parasome and a *Korotnevella*-like locomotive form). At the same time its 18S rDNA gene sequence strongly supports a clade with *Pseudoparamoeba pagei* and "*Vexillifera armata*" ATCC 50883 and never branches among *Korotnevella* species. Another new species described here is *Korotnevella fousta* n. sp. with scales similar to those of *K. diskophora*. The diagnoses and potential relations of the genera *Pseudoparamoeba* and *Korotnevella* are discussed.

Material and Methods

Isolation and culturing

The sample containing *Pseudoparamoeba microlepis* n. sp. was taken from a freshwater pond in Krka National Park, Gradina, Croatia (N43°54.308′, E15°58.632′) in August, 2013. The sample containing *Korotnevella fousta* n. sp. was collected from the freshwater lake at Sredniy Island, the Chupa Inlet, Kandalaksha Bay, the White Sea (N66°17.233′ E 33°38.422′) in August 8, 2012.

Samples were transported to the laboratory and inoculated in 90 mm Petri dishes with 0.025% wheat grass (WG) (Weizengras, Sanatur GmbH, D-78224 Singen) extract made with Prescott-James (PJ) medium (Prescott and James 1955). Dishes were examined with a phase-contrast inverted Nikon Eclipse TS 100 microscope after a week of incubation. Individual cells were collected using a tapered Pasteur pipette, washed in fresh sterile medium and transferred to sterile 60 mm dishes filled with the same medium to obtain clonal cultures. Both strains were maintained solely on bacteria. Cultures were maintained at +15 °C under room light.

Light microscopy

Live cells were observed and photographed either in cultures, in plastic Petri dishes, using a phase-contrast inverted Leica DMI3000B microscope (63× objective) or on the glass object slides using a Leica DM 2500 microscope (100× oil immersion objective) equipped with DIC. Measurements of trophozoites and cysts were taken from micrographs made with an inverted Leica DMI3000B microscope.

Transmission electron microscopy

For transmission electron microscopy cells were fixed in Petri dishes at +4 °C with 2.5% glutaraldehyde in 0.05 M cacodylate buffer (pH 7.4) for 40 min, and then post-fixed with 1% osmium tetroxide in the same buffer for 1 h. Amoebae were washed with a buffer at room temperature three times between fixation steps and prior to dehydration, 5 min each time. After osmium postfixation amoebae were scraped away from the substratum, concentrated by centrifugation at 1200 r.p.m. and embedded in 2% agar. Pieces of agar containing amoebae were cut out and dehydrated in a graded ethanol series followed by acetone, and embedded in Epon 812 resin (Fluka). Sections were made using a Leica EM UC6 ultramicrotome with glass knife. Sections were double-stained with 2% uranyl acetate in 70% ethanol for 15 min and Reynolds'