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Phylogenetic position of the amoeba genus *Deuteramoeba* (Amoebozoa, Tubulinea)

Oksana Kamyshatskaya[1], Elena Nassonova[2,1] and Alexey Smirnov[1]

[1] *Department of Invertebrate Zoology, Faculty of Biology, St. Petersburg State University, St. Petersburg, Russia*
[2] *Institute of Cytology, Russian Academy of Sciences, St. Petersburg, Russia*

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Summary

Amoebae of the family Amoebidae are among the largest amoebozoans, most widely known to non-specialists as they are popular objects for teaching and various demonstrations. At the same time, they are among the groups, which are the least represented in the molecular phylogenetic trees. Of seven genera comprising the family, members of only two - *Amoeba* and *Chaos* - are present in SSU rRNA gene trees and only one species of *Amoeba* - in multigene trees. We have sequenced SSU rRNA gene of the CCAP 1586/1 strain representing the type strain of the species *Deuteramoeba mycophaga*, thus adding the third genus to the phylogeny of this family. Phylogenetic analysis robustly placed it as a sister group to *Amoeba* plus *Chaos* clade, thus confirming its position deduced earlier from the morphological characters. We also observed structures that may evidence for the presence of the intranuclear parasite in this strain of *Deuteramoeba*.

Key words: Amoebozoa, Amoebidae, systematics, phylogeny

Introduction

Amoebae of the family Amoebidae are among the largest and the most remarkable amoebozoans populating freshwater and soil habitats. However, they are not among the most widely distributed species. While amoebae of the genera *Amoeba, Chaos* and *Polychaos* were isolated several or even many times from a variety of natural habitats (Page and Baldock, 1980; Page and Kalinina, 1984; Page, 1986; Smirnov and Goodkov, 1997, 1999; Goodkov et al., 1999; Bolivar et al., 2001), members of other four genera - *Deuteramoeba, Trichamoeba, Hydramoeba* and *Parachaos* were seen few times or even once only (Schaeffer, 1926; Page and Robson, 1983; Siemensma and Page, 1986; Pussard et al., 1980; Willumsen, 1982; Mrva, 2010/11). Four species belonging to the genera *Amoeba* and *Chaos* were sequenced by Bolivar et al. (2001) and grouped together in the phylogenetic tree; hence species of both genera were intermingled in the tree. Multigene data on *Amoeba proteus* were obtained by Kang et al. (2017), but in this tree no other species belonging to the family Amoebidae are present.

The genus *Deuteramoeba* was erected by Page (1986) to accommodate a species, first described as *Amoeba algonquinensis* by Baldock et al. (1983). The electron-microscopic study showed that this

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species, in contrast to those of the genus *Amoeba*, had amorphous but not filamentous glycocalix. At that time, this was a genus-level character; also later it was shown that filamentous and amorphous glycocalixes may be found in the same genus (Smirnov and Goodkov, 1997). The second species of this genus was first described as *Trichamoeba mycophaga* (Pussard et al., 1980) and transferred to the genus *Deuteramoeba* by Page (1988) because of its predominantly polypodial locomotive form. This species was isolated from soil (Pussard et al., 1980; Chakraborty and Old, 1986; Mrva, 2005, 2010/11; Page, 1988, 1991) and is cyst-forming; this maybe a specific adaptation to its soil lifestyle (Smirnov and Brown, 2004).

In the present study we have sequenced SSU rRNA gene of the CCAP 1586/1 strain representing the type strain of the species *Deuteramoeba mycophaga*. Phylogenetic analysis robustly placed it as a sister group to *Amoeba* plus *Chaos* clade, thus confirming its position deduced earlier from the morphological characters.

Material and methods

The strain CCAP 1586/1 was obtained from the Culture Collection of Algae and Protozoa (CCAP, nowadays it is a part of the UK National Culture Collection – UKNCC, Oban, Scotland, UK) in 2012 and maintained since that time on PJ medium (Prescott and James, 1955) and rice grains in 90 mm Petri dishes at +18 °C and non-regulated light. Amoebae were transferred to fresh medium monthly.

Light-microscopic observations and photographing were performed in cultures, using Leica DMI3000 inverted microscope equipped with Phase contrast optics and on the glass object slides using upright Leica DM2500 microscope equipped with Phase contrast, DIC optics and Nikon DSFi-1 camera linked to the computer with NIS-Elements software. The size of the cells was measured in culture using inverted microscope, the size of the nucleus – on the object slides; special attention was paid to not pressing the amoebae with the coverslip to avoid deformation of the nucleus.

To obtain the SSU rRNA gene sequence, individual amoeba cells floating in the water were collected with tapered-tips glass Pasteur pipettes to the 60 mm Petri dishes filled with fresh Millipore-filtered (0.22 μm) medium. Amoebae were left in these dishes overnight, next day they were transferred to the dishes with fresh medium in the similar manner. After one more day, cells in 1-2 μl of the medium were placed in 200 μl PCR tubes and frozen under -20 °C. Right before the PCR, the tubes containing cells were defrozen under room temperature and frozen back for approximately 3 min; this cycle was repeated three times. After that, 48 μl of ready-made PCR mixture were added to the tubes.

The SSU rRNA gene was amplified by PCR using universal eukaryote primers RibA (5'> ac ctg gtt gat cct dcc agt <3') and RibB (5'> tga tcc atc tgc agg ttc acc tac <3') complementary to the 5' and 3' end of the gene (Medlin et al., 1988). Thermal cycle parameters were: initial denaturation (10 min at 95 °C) followed by 39 cycles of 30 s at 94 °C, 60 s at 50 °C and 120 s at 72 °C, followed by 10 min at 72 °C for final extension. Amplicons were purified using Cleanup mini Purification Kit (Eurogene) and sequenced using ABI-PRISM Big Dye Terminator Cycle Sequencing Kit. Besides amplification primers, additional primer F6 (5'> cng cgg taa ttc cag ctc <3') was used for sequencing.

Obtained sequences were added to the alignment containing all culture-derived sequences of Amoebida, a sample of other tubulinean sequences and some discosean sequences used as the outgroup. Sequences were aligned manually using SeaView 4.0 (Gouy et al., 2010). The phylogenetic analysis was performed using maximum likelihood method as implemented in PhyML program (Guindon and Gascuel, 2003) with GTR + γ + I model suggested by the program Modeltest (Posada and Crandall, 1998); 1471 sites were selected for the analysis. The number of invariant sites, alpha parameter and tree topology were optimized by PhyML, 100 bootstrap pseudoreplicates were performed. Bayesian analysis was performed on the same dataset using MrBayes 3.1.2, GTR model with γ - correction for intersite rate variation (8 categories) and the covarion model (Ronquist and Huelsenbeck, 2003). Trees were run as two separate chains (default heating parameters) for 10 million generations, by which time they had ceased converging (final average standard deviation of the split frequencies was less than 0.01). The quality of chains was estimated using built-in MrBayes tools and additionally - using the software Tracer 1.6 (Rambaut et al., 2014); based on the estimates by Tracer, the first 25 % of generations were discarded for burn-in.

Results and discussion

Light-microscopic observations show cells, fitting the previously published descriptions of this species (Old et al., 1985; Chakraborty and Old, 1986; Page, 1988, 1991; Mrva, 2010/11). Amoebae were polytactic, with relatively short and non-numerous pseudopodia (Fig. 1 A-B). Actively moving cells were nearly orthotactic (Fig. 1 C-E). Cells often formed a morulate uroid (Fig. 1 C, E-F); some cells had two or even three uroidal structures simultaneously, of those only one was functional while the others were at different stages of degradation. Sometimes cells in locomotion became rather flattened and even formed a sort of longitudinal dorsal ridges in the anterior part of the hyaloplasm (Fig. 1 D). The cytoplasm of the cell contained a lot of crystals. Some of them were truncate bipyramidal (Fig. 1 G), but the most of crystals should rather be recognized as truncate spindle-shaped, because they were rather smooth and had no pronounced edges. The abundance of crystals makes a lot of problems during observations as they obscure the internal structures and are highly refractive. Other inclusions were opaque granules of different size and vacuoles containing food remnants. Few cells contained large crystalloids of irregular form, sometimes they were comparable in size with the cell nucleus; such cells did not look healthy and were not actively moving.

The nucleus had the single central homogeneous nucleolus, often with a central lacuna (Fig. 1 H). The size of the nucleolus varied from 12 to 16 μm. Interestingly, some of the observed nuclei (Fig. 1 I-K) contained structures that could be interpreted as presence of an intra-nuclear parasite of amoebae, like *Paramicrosporidium* (Scheid, 2007; Michel et al., 2000, 2009a) or *Nucleophaga* (Michel et al., 2009b, 2012). We observed nuclei with a number of rounded bodies, 2-4 μm across, surrounding the nucleolus (Fig. 1 I-J). Each body had one or (rarely) two depressions or lacunas in the middle area when viewed with DIC optics. In the most of presumably infected cells these bodies surrounded the nucleolus forming a monolayer. These patterns are comparable to those of the developmental stages of *Paramicrosporidium* as illustrated by Schneid (2007) and Michel et al. (2009a). To the lesser extent they resemble *Nucleophaga* (Michel et al., 2009b, 2012). We also observed nuclei, where the nucleolus appeared to be hypertrophied and filled with the granular material (Fig. 1 K), which is comparable to the images of the sporogony in *Paramicrosporodium* (Schneid, 2007; Michel et al., 2000, 2009a). No other stages have been observed yet; should this observation be confirmed by the electron microscopy or molecular methods, this will expand the known range of hosts of these parasites.

It is noticeable that Page (1988 p. 60 Fig. 18, J) and Page (1991, p. 65, Fig. 20, h-i) showed the nucleus of *D. mycophaga* containing exactly the same structures as shown here (which is not surprising, given that those are the images of the same CCAP strain 1586/1). The depositor of this strain, according to CCAP record, was Old in the year 1984. In the formal description of this species, Chakraborty and Old (1986) noted that the studied culture was deposited with CCAP (without indication of the exact time). Neither Old et al. (1985) nor Chakraborty and Old (1986) who studied the ultrastructure of this amoeba species, found anything resembling these structures, neither in light microscopy nor in the electron-microscopic images of the nucleus. This suggests that either the culture probably got infected during its maintenance in CCAP but the putative infection was not recognized since this group of organisms was not known at that time, or a different strain rather than the one studied by Chakraborty and Old (1986) was deposited. Chakraborty and Old (1986) wrote that "amoebae were isolated from a sandy loam wheat field soil from Eyre Peninsular region of South Australia and from a red basaltic loam soil supporting a mixed eucalypt rainforest community, south of Burnie, Tasmania, Australia" (op. cit., p. 564); so, this presumes that more than one strain might have been available initially. Mrva (2010/11) reported isolates identified as *D. mycophaga* from several different habitats in Slovakia; however, he did not note any suspicious structures in the vesicular nucleus of these amoebae.

In the SSU phylogenetic tree, *Deuteramoeba mycophaga* robustly grouped with the other members of the family Amoebidae as a basal group for this family. This position is fully supported and evidences that *Deuteramoeba* is an independent genus within the family Amoebidae, as it was suggested from the morphological data (Page 1988). As in all other trees, members of the genera *Amoeba* and *Chaos* are intermingled in the tree, and their grouping has moderate support. Arrangement of the other clades in the tree corresponds to the generally recognized topology (Smirnov et al., 2005, 2011, 2017; Kang et al., 2017; Cavalier-Smith et al., 2016) and all these clades are highly supported. These data evidence once more that morphological

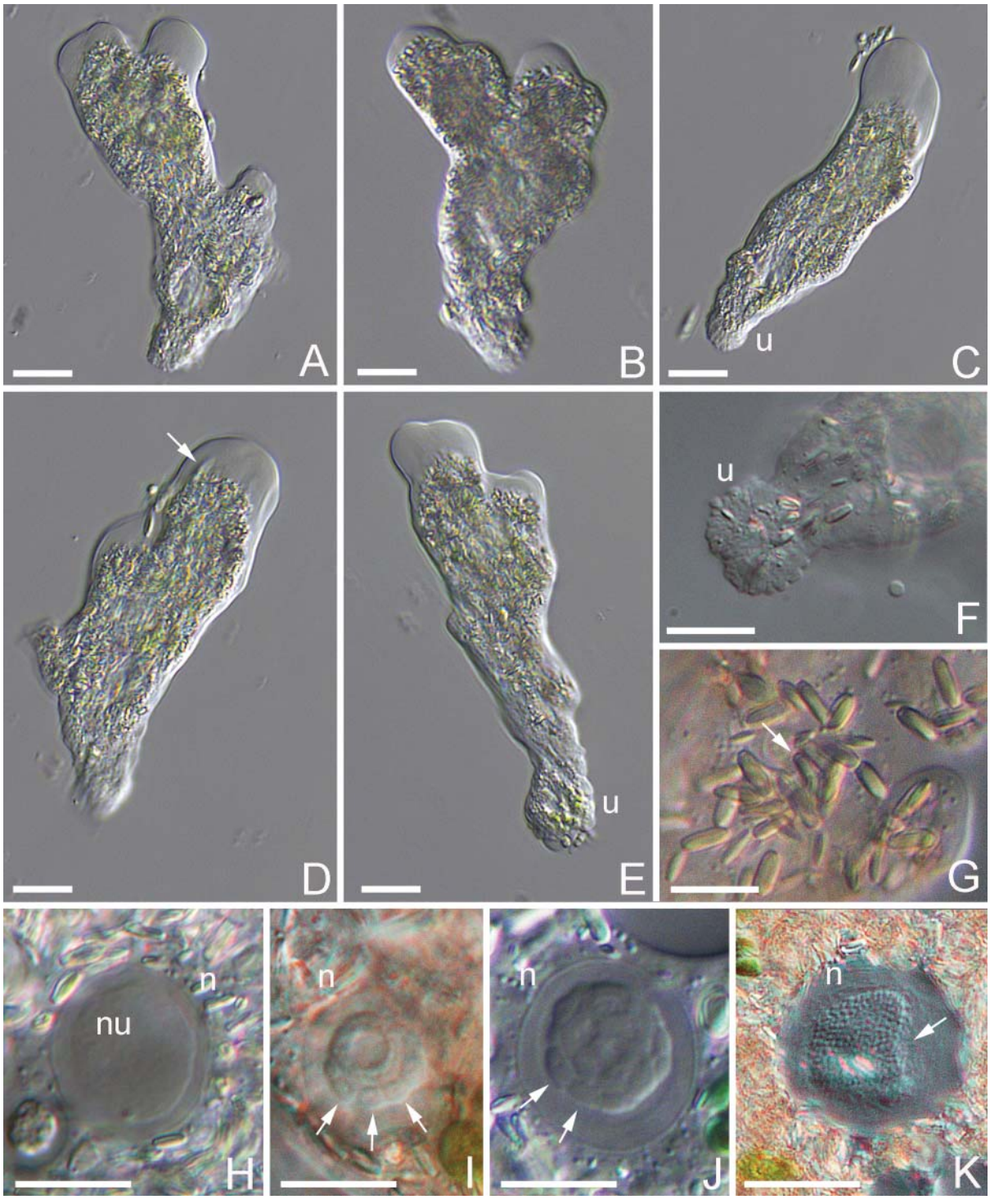

Fig. 1. Light microscopy of *Deuteramoeba mycophaga* CCAP 1586/1 strain. DIC. A-B – Polytactic locomotive form; C-D – orthotactic locomotive form, note tiny dorsal longitudinal ridges arrowed in D; E – elongate cell that just started to change the direction of movement; F – uroidal structures; G – crystals in the cytoplasm, of them the only one that can be considered as a truncate bipyramidal is *arrowed*; H – intact nucleus; I-K – nuclei, presumably infected with the parasite (several parasite cells are arrowed in I-J and the aggregation of granular material replacing the nucleolus in K). *Abbreviations*: u – uroid; n – nucleus; nu – nucleolus. Scale bars: 20 μm in A-E, 10 μm in F-K.

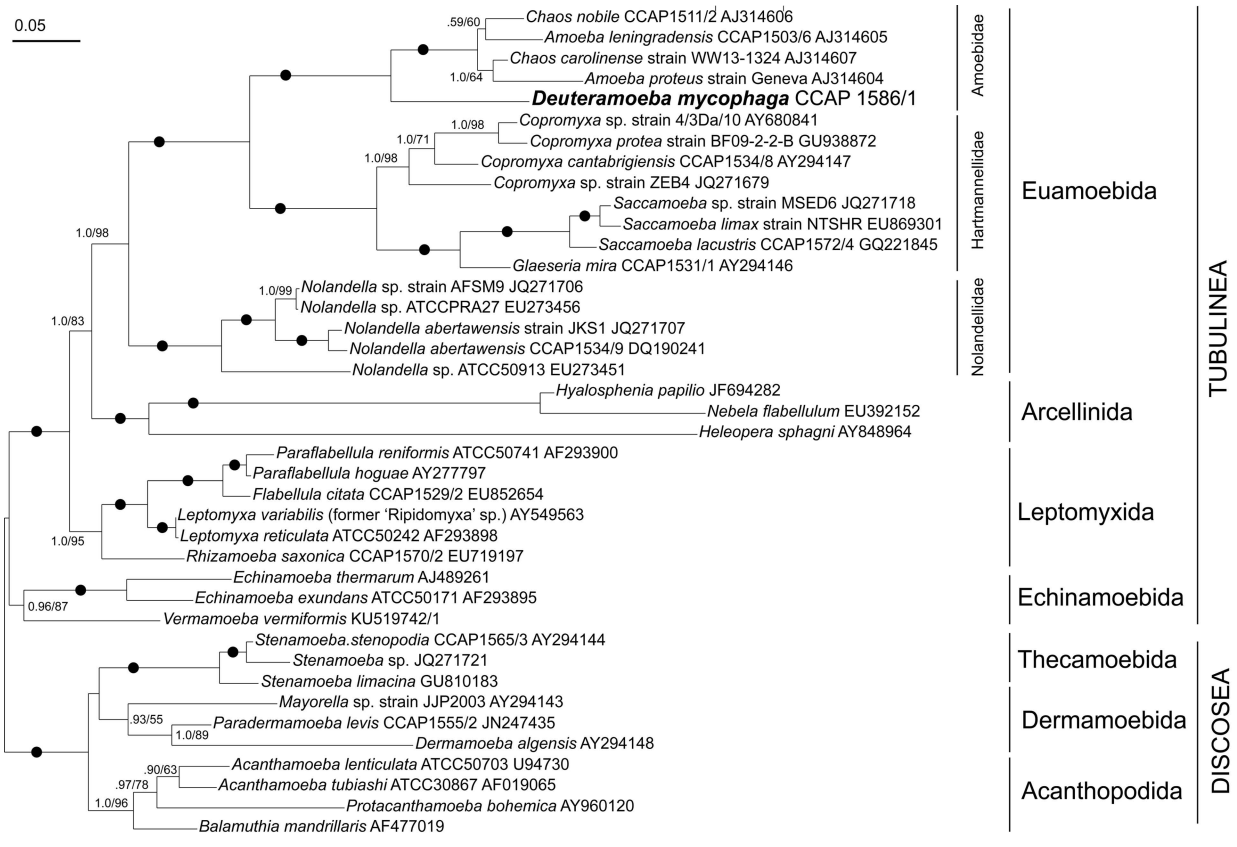

Fig. 2. Phylogenetic tree based on SSU rRNA gene, showing the position of *Deuteramoeba mycophaga*. 1477 sites; MrBayes (GTR+ γ model, 25 rate categories) and PhyML (GTR + γ + I model). Supports indicated as PP/BS; black dotes mean full (1.0/100) support of branches.

systematics of amoebae, when it concerns species with remarkable morphological characters, remains in many cases valid and that the "proteus-like" organization of the cell (large cells forming discrete tubular pseudopodia) may represent a valid taxonomic character that probably unifies the family Amoebidae (Bovee, 1985; Page, 1986, 1987).

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References

Baldock B.M., Rogerson A. and Berger J. 1983. A new species of fresh-water amoeba: *Amoeba algonquinensis* n. sp. (Gymnamoebia: Amoebidae). Trans. Amer. Microsc. Soc. 102, 113–21.

Bolivar I., Fahrni J.F., Smirnov A. and Pawlowski J. 2001. SSU rRNA-based phylogenetic position of the genera *Amoeba* and *Chaos* (Lobosea, Gymnamoebia): the origin of gymnamoebae revisited. Mol. Biol. Evol. 18, 2306–2314.

Bovee E.C. 1985. Class Lobosea Carpenter. 1861. In: An Illustrated Guide to the Protozoa (Eds. Lee J.J., Hutner S.H. and Bovee E.C.). Allen Press, Lawrence, pp. 158–211.

Cavalier-Smith T., Chao E.E. and Lewis R. 2016. 18S-gene phylogeny of protozoan phylum Amoebozoa reveals a new class (Cutosea) of deep-branching, ultrastructurally unique, enveloped

marine Lobosa and clarifies amoeba evolution. Mol. Phylogen. Evol. 99, 275–296.

Chakraborty S. and Old K.M. 1986. Ultrastructure and description of a fungus-feeding amoeba *Trichamoeba mycophaga* n. sp. (Amoebidae, Amoebea), from Australia. J. Protozool. 33, 564–569.

Gouy M., Guindon S. and Gascuel O. 2010. SeaView Version 4: A multiplatform graphical user interface for sequence alignment and phylogenetic tree building. Mol. Biol. Evol. 27, 221–224.

Goodkov A.V., Smirnov A.V. and Skovorodkin I.N. 1999. Study of a rediscovered large freshwater multinucleate amoeba *Chaos illinoisense* (Kudo, 1950). Protistology 1, 55–61.

Guindon S. and Gascuel O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52, 696-704.

Kang S., Tice A.K., Spiegel F.W., Silberman J.D., Pánek T., Čepička I., Kostka M.,Kosakyan A., Alcântara D.M.C., Roger A.J., Shadwick L.L., Smirnov A., Kudryavtsev A., Lahr D.J.G. and Brown M.W. 2017. Between a pod and a hard test: the deep evolution of amoebae. Mol. Biol. Evol. 34, 2258–2270.

Medlin L., Elwood H.J., Stickel S. and Sogin M.L. 1988. The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. Gene 71, 491–499.

Michel R., Schmid E.N., Böker T., Hager D.G., Müller K-D., Hoffmann R. and Seitz H.M. 2000. *Vannella* sp. harboring Microsporidia-like organisms isolated from the contact lens and inflamed eye of a female keratitis patient. Parasitol. Res. 86, 514–520.

Michel R., Müller K-D. and Hauröder B. 2009a. A novel microsporidian endoparasite replicating within the nucleus of *Saccamoeba limax* isolated from a pond. Endocytobios Cell. Res. 19, 120–126.

Michel R., Hauröder B. and Zöller L. 2009b. Isolation of the amoeba *Thecamoeba quadrilineata* harboring intranuclear spore forming endoparasites considered as fungus-like organisms. Acta Protozool. 48, 41–49.

Michel R., Müller K-D., Schmid E.N., Theegarten N.N., Hauröder B. and Corsaro D. 2012. Isolation of *Thecamoeba terricola* from bark of *Platanus occidentalis* harbouring spore-forming eukaryotic endo- parasites with intranuclear development. Endocytobios Cell. Res. 22, 37–42.

Mrva M. 2005. Rhizopods (Rhizopoda). In: Fauna of Devínska Kobyla (Eds.: Majzlan O. et al.). APOP, Bratislava. pp. 17–19 (in Slovak).

Mrva M. 2010/11. Morphological studies on two rare soil amoebae *Deuteramoeba algonquinensis* and *D. mycophaga* (Gymnamoebia, Amoebidae). Protistology. 6, 284–289.

Old K.M., Chakraborty S. and Gibbs R. 1985. Fine structure of a new mycophagous amoeba and its feeding on *Cochliobolus sativus*. Soil Biol. Biochem. 17, 645–655.

Page F.C. and Baldock B.M. 1980. *Polychaos fasciculatum* (Penard 1902) (Amoebida): a light-and electron-microscopical study. Arch. Protistenkd. 123, 221–235.

Page F.C. 1981. A light- and electron-microscopical study of *Protacanthamoeba caledonica* n. sp., type-species of *Protacanthamoeba* n.g. (Amoebida, Acanthamoebidae). J. Protozool. 28, 70–78.

Page F.C. 1986. The genera and possible relationships of the family Amoebidae, with special attention to comparative ultrastructure. Protistologica. 22, 301–316.

Page F.C. 1987. The classification of "naked" amoebae (Phylum Rhizopoda). Arch. Protistenkd. 133, 199–217.

Page F.C. 1988. A new key to freshwater and soil Gymnamoebae. Freshwater Biol. Ass., Ambleside.

Page F.C. 1991. Nackte Rhizopoda. In: Nackte Rhizopoda und Heliozoea (Eds.: Page F.C. and Siemensma F.J.). G. Fischer, Stuttgart–New York. pp. 1–170.

Page F.C. and Robson E.A. 1983. Fine-structure and taxonomic position of *Hydramoeba hydroxena* (Entz, 1912). Protistologica. 19, 41–50.

Page F.C. and Kalinina L.V. 1984. *Amoeba leningradensis* n. sp. (Amoebidae): a taxonomic study incorporating morphological and physiological aspects. Arch. Protistenkd. 128, 37–53.

Posada D. and Crandall K.A. 1998. Model-test: testing the model of DNA substitution. Bioinformatics. 14, 817–818.

Prescott D.M. and James T.W. 1955. Culturing of *Amoeba proteus* on *Tetrahymena*. Exp. Cell. Res. 81, 256–258.

Pussard M., Alabouvette C., Lemaitre I. and Pons R. 1980. Une nouvelle amibe mycophage endogee *Cashia mycophaga* n. sp. (Hartmanellidae, Amoebida). Protistologica. 16, 443–451.

Rambaut A., Suchard M.A., Xie D. and Drummond A.J. 2014. Tracer v1.6, Available from http://beast.bio.ed.ac.uk/Trace.

Ronquist F. and Huelsenbeck J.P. 2003. Mrbayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics. 19, 1572–1574.

Schaeffer A.A. 1926. Taxonomy of the amoebas.

Pap. Dept. Mar. Biol., Carnegie Inst. of Washington. 24, 1–116.

Scheid P. 2007. Mechanism of intrusion of a microsporidian-like organism into the nucleus of host amoebae (*Vannella* sp.) isolated from a keratitis patient. Parasitol. Res. 101, 1097–1102.

Siemensma F.J. and Page F.C. 1986. A light-and electron microscopic study of *Trichamoeba sinuosa* n. sp. (Amoebida) with a re-diagnosis of the genus. Protistologica. 22, 117–125.

Smirnov A.V. and Goodkov A.V. 1997. Description of a large, multinucleate lobose amoeba *Chaos glabrum* n. sp. (Lobosea, Amoebidae), with notes on the diagnosis of the genus *Chaos*. Acta Protozool. 36, 227–233.

Smirnov A.V. and Goodkov A.V. 1998. Study of *Polychaos annulatum* Penard, 1902 comb. nov. (Gymnamoebia, Amoebidae) with taxonomical analysis of *Polychaos fasciculatum*-like species. Eur. J. Protistol. 34, 1–9.

Smirnov A.V. and Brown S. 2004. Guide to the methods of study and identification of soil gymnamoebae. Protistology. 3, 148–190.

Smirnov A., Nassonova E., Berney C., Fahrni J., Bolivar I. and Pawlowski J. 2005. Molecular phylogeny and classification of the lobose amoebae. Protist 2, 129–142.

Smirnov A., Chao E., Nassonova E. and Cavalier-Smith T. 2011. A revised classification of naked lobose amoebae (Amoebozoa: Lobosa). Protist. 162, 545–570.

Smirnov A., Nassonova E., Geisen S., Bonkowski M., Kudryavtsev A., Berney C., Glotova A., Bondarenko N., Dyková I., Mrva M., Fahrni J. and Pawlowski J. 2017. Phylogeny and systematics of leptomyxid amoebae (Amoebozoa, Tubulinea, Leptomyxida). Protist. 168, 220–252.

Willumsen N. B. S. 1982. *Chaos zoochlorellae* sp. nov. (Gymnamoebia, Amoebidae) from a Danish freshwater pond. J. Nat. Hist. 16, 803-813.

Address for correspondence: Oksana Kamyshatskaya. Department of Invertebrate Zoology, Faculty of Biology, St. Petersburg State University, Universitetskaya nab. 7/9, 199034 St. Petersburg, Russia;
e-mail: *oksana.kamyshatskaya@gmail.com*