

Distribution of *Thecamoeba aesculea* (Amoebozoa: Discosea) and molecular identification and description of its sibling species — *Thecamoeba sonatina* sp.n.

A.A. Shklyar*, Y.S. Mesentsev, E.D. Chikadze, A.V. Smirnov

Department of Invertebrate Zoology, Faculty of Biology, St. Petersburg University, 7/9 Universitetskaya Emb, St. Petersburg 199034 Russia.

* Corresponding author

Arseniy Shklyar: a.shklyar@spbu.ru; <https://orcid.org/0009-0003-2584-0225>

Yelisei Mesentsev: e.mesentsev@spbu.ru; <https://orcid.org/0000-0002-2541-1207>

Elizaveta Chikadze: st107864@student.spbu.ru; <https://orcid.org/0009-0006-4544-5013>

Alexey Smirnov: alexey.smirnov@spbu.ru; <https://orcid.org/0000-0002-9844-5344>

ABSTRACT: For a long time, the genus *Thecamoeba* was considered one of the few genera of naked lobose amoebae whose species could be reliably distinguished using light microscopy alone. However, molecular investigations have revealed genetic diversity within some “classical” species. Therefore, groups of sibling species within the genus *Thecamoeba* have been established. Amoebae in these groups can be reliably identified only based on molecular data. We studied three molecularly confirmed strains of *T. aesculea* isolated from geographically distant habitats and, using new data, refined the morphological characteristics of this species. In addition, we isolated and studied two cultures of amoebae that exhibit minor morphological differences in nuclear structure and locomotive form from *T. aesculea*. Analysis of molecular data revealed significant differences between these strains and other *T. aesculea* cultures, so we describe a new terrestrial species — *Thecamoeba sonatina* sp.n. Together, *T. aesculea* and *T. sonatina* sp.n. form a monophyletic group, which we designate as the “*T. aesculea* species group”. This is the fourth species group currently recognized within the genus *Thecamoeba*.

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KEY WORDS: 18S rRNA gene, free-living amoeba, distribution, phylogeny, systematics, Amoebozoa.

Распространение *Thecamoeba aesculea* (Амоебозоа: Discosea), молекулярная идентификация и описание ее родственного вида — *Thecamoeba sonatina* sp.n.

А.А. Шкляр*, Е.С. Мезенцев, Е.Д. Чикадзе, А.А. Смирнов

Кафедра зоологии беспозвоночных, Биологический факультет Санкт-Петербургского государственного университета, Университетская наб., 7/9, Санкт-Петербург 199034 Россия.

* Ответственный за переписку: a.shklyar@spbu.ru

РЕЗЮМЕ: Долгое время род *Thecamoeba* считался одним из немногих родов голых лобозных амёб, виды которого можно было достоверно различить используя только методы световой микроскопии. Однако широкое применение молекулярных методов выявило богатое генетическое разнообразие в пределах некоторых «классических»

видов. Поэтому были установлены группы близких видов внутри рода *Thecamoeba*. Амебы в этих группах могут быть достоверно идентифицированы только на основе молекулярных данных. Мы изучили три молекулярно подтвержденных штамма *T. aesculea*, выделенных из географически отдаленных местообитаний, и на основании новых данных уточнили морфологические характеристики этого вида. Кроме того, нами были изолированы и изучены две культуры амеб, которые демонстрируют незначительные морфологические различия в строении ядра и особенностях локомоторной формы от *T. aesculea*. Анализ молекулярных данных показал значительные отличия этих штаммов от остальных культур *T. aesculea*, поэтому мы описываем новый наземный вид — *Thecamoeba sonatina* sp.n. Вместе *T. aesculea* и *T. sonatina* sp.n. образуют монофилетическую группу, которую мы обозначаем как “группу видов *T. aesculea*”. Это четвертая видовая группа, в настоящее время установленная в роде *Thecamoeba*. Как цитировать эту работу: Shklyar A.A., Mesentsev Y.S., Chikadze E.D., Smirnov A.V. 2026. Distribution of *Thecamoeba aesculea* (Amoebozoa: Discosea) and molecular identification and description of its sibling species — *Thecamoeba sonatina* sp.n. // Invert. Zool. Vol.23. No.3. P.351–366, Suppl. Tables. doi: 10.15298/invertzool.23.3.01

КЛЮЧЕВЫЕ СЛОВА: ген 18S рРНК, свободноживущие амебы, распространение, филогения, систематика, Amoebozoa.

Introduction

Naked lobose amoebae of the genus *Thecamoeba* Fromentel, 1874 are widely distributed and can be isolated from terrestrial, freshwater, and marine habitats (Page, 1977; Smirnov, 1999a, b; Dyková *et al.*, 2008; Kudryavtsev, Hausmann, 2009; Mesentsev, Smirnov, 2019, 2021; Mesentsev *et al.*, 2020, 2022, 2023; Henderson *et al.*, 2024; Shklyar *et al.*, 2025; Mesentsev, 2025). These amoebae can often be isolated from moss, leaf litter, the top layer of soil, or from plant barks. They are relatively easy to distinguish from other amoebae by the characteristic folds or wrinkles on the cell surface. That is why they were once named “Shar-Pei of protists” (Howey, 2005). During locomotion, amoebae of the genus *Thecamoeba* have noticeable morphological features: the absence of pseudopodia and subpseudopodia, smooth and slightly wavy rounded or oval outlines elongated in the direction of movement, the presence of longitudinal dorsal ridges and/or irregular folds (Page, 1977; Rogerson, Patterson, 2000; Mesentsev, 2025). Cells show a smooth decrement in cell thickness from the posterior to the anterior end (Pussard, 1973). Based on these morphological traits, all *Thecamoeba* species belong to either the rugose or striate morphotype according to Smirnov and Goodkov (1999) and Smirnov and Brown (2004). In the modern system, the genus *Thecamoeba* belongs to Amoebozoa, class Discosea, subclass

Flabellinia, order Thecamoebida, family Thecamoebidae (Smirnov *et al.*, 2011). It occupies the same position in the system by Adl *et al.* (2019), except for the absence of ranks in the latter one.

For a long time, the genus *Thecamoeba* was considered one of the few genera of naked lobose amoebae whose species could be reliably distinguished using light microscopy only (Penard, 1905; Page, 1977, 1983, 1988, 1991; Bovee, 1985; Rogerson, Patterson, 2000). The main features for identifying *Thecamoeba* species were the characteristics of the locomotive form, the size of the moving cell and the nucleus, as well as the features of the intranuclear structures. However, recent studies have shown significant differences in 18S rRNA sequences between morphologically similar representatives of this genus, so, sibling species were described (Mesentsev *et al.*, 2022). Each group of morphologically similar species forms a separate monophyletic branch on the phylogenetic tree. In this regard, groups of sibling species were established as supraspecific taxonomic units (species groups *sensu* Mesentsev *et al.*, 2022). Members of *Thecamoeba* can be assigned to a species group from morphology, even based on single cell observations, while identification to species requires the use of molecular methods.

During our study of diversity of naked lobose amoebae in terrestrial habitats, we isolated five strains of *Thecamoeba* from various localities across Russia. By the morphological characters,

all these strains could be formally assigned to the species *T. aesculea*. However, careful observations on the structure of the nucleoli allowed us to divide these strains into two groups. This division was confirmed by the comparison of 18S rRNA gene sequences and phylogenetic analysis. Based on morphological and molecular data, we refined the diagnosis of *T. aesculea* and described a sibling species *Thecamoeba sonatina* sp.n.

Material and methods

COLLECTION AND CULTIVATION. Strains KP1TaL, Ta4, BDMTaL, KA1-4 and KMChPTaL were isolated from different terrestrial habitats (Suppl. Table 1). Strains KP1TaL and Ta4 were isolated from samples collected in the European part of Russia, in the Tula and Belgorod regions, respectively. Strain BDMTaL was isolated from samples collected near Lake Baikal, Irkutsk region, Russia. Strains KA1-4 and KMChPTaL were isolated from samples collected in the Far Eastern Federal District of Russia, in the Khabarovsk Krai and Kamchatka Krai, respectively.

To isolate amoebae, tiny pieces of sample material (0.1–0.5 g dry weight) were placed in sterile 60 mm Petri dishes filled with wMY agar (Spiegel *et al.*, 1995). To provide initial moisture, sample fragments were wetted with a drop of sterile PJ medium (Prescott, James, 1955). The inoculations were incubated under 12–14 °C and ambient laboratory light. Amoebae were detected after four weeks of incubation. The clonal cultures (strains) were established by excising small fragments of agar containing a single, well isolated amoeba cell (Page, 1988). These agar fragments were transferred to dishes with a fresh agar medium. Clonal cultures were maintained in 90 mm Petri dishes on the same medium and under the same conditions. Amoebae in culture fed on accompanying bacteria and fungi.

LIGHT MICROSCOPY. Amoebae cultures were studied using a Leica M205C stereo microscope equipped with Rottermann contrast. Live cells of amoebae were examined and photographed on glass slides using Nikon Eclipse Ni microscope equipped with DIC, phase contrast and a Nikon DS-Ri2 camera powered with NIS-Elements BR software (Nikon, Japan). For the measuring and imaging of live cells in plastic Petri dishes filled with PJ medium, a DMI3000B microscope equipped with IMC (Integrated Modulation Contrast) optics and Leica DFC295 camera powered by Leica Application Suite (LAS) version 4.11.0 software. To increase the depth of focus, we applied z-stacking on locomotive amoebae, as described by Mesentsev *et al.* (2020).

DNA EXTRACTION, AMPLIFICATION AND SEQUENCING. For DNA isolation amoebae were collected from the surface of agar medium with tapered-

tip glass Pasteur pipette, transferred into 40-mm Petri dishes filled with fresh Millipore-filtered (0.22 µm) PJ medium, and left to starve for 10 days. Every day the medium was replaced with the fresh one. Then, dishes with cells were examined using an inverted Leica DMI 3000 microscope equipped with phase-contrast optics to check for the absence of visible food vacuoles inside cells, absence of fungal contamination, and other eukaryotes in the surrounding medium. Further, individual cells were collected and placed with 1–2 µl of the medium in 200-µl PCR tubes. The samples with starved amoebae were used for DNA isolation with Arcturus PicoPure DNA Extraction Kit (Thermo Fisher Scientific, Massachusetts). The purified DNA samples were amplified using Thermo Scientific Taq DNA Polymerase (Thermo Fisher Scientific, Massachusetts) and eukaryotic primers for amplification of the 18S rRNA gene: RibA (5'>ACCTGGTTGATCCTGCCAGT<3') primer, which is the second half of the original "Primer A" (Medlin *et al.*, 1988) and the reverse RibB (5'>TGATCCTTCTGCAGGTTACCTAC<3') primer (Pawlowski, 2000). The thermal cycle parameters were: initial denaturation (10 min at 95 °C) followed by 35 cycles of 30 sec at 94 °C, 1 min at 58 °C and 2 min at 72 °C, followed by 10 min at 72 °C for final extension. Obtained amplicon was purified in 1.5% agarose gel using the Cleanup Mini purification kit (Eurogene, Russia). Amplicons were sequenced using the ABI PRISM Big Dye Terminator Cycle Sequencing Kit (Thermo Fisher Scientific, Massachusetts) with the following primers: with s6F, s12.2, s12.2R, s14, and s20R primers for the 18S rRNA gene (Medlin *et al.*, 1988; Pawlowski, 2000; Adl *et al.*, 2014).

SEQUENCE ALIGNMENT AND PHYLOGENETIC ANALYSES. The resulting sequences were mounted into the Discosea alignment, which included all available named *Thecamoeba* sequences and a representative set of outgroups. Sequences were automatically aligned using the E-INS-i (Katoh *et al.*, 2005) method of MAFFT 7 (Katoh *et al.*, 2002) using the MAFFT online service (Katoh *et al.*, 2018). Nucleotide sequences were extracted from the databases of the National Center for Biotechnology Information (Benson *et al.*, 2013).

The phylogenetic analyses were performed using RAXML (Stamatakis, 2014) and MrBayes 3.2.6 (Ronquist, Huelsenbeck, 2003). For the maximum likelihood (ML) analysis, GTR + gamma model was applied. One hundred of the most parsimonious starting trees were built, and 1000 non-parametric bootstrap pseudoreplicates were performed on best-scoring ML tree. Bayesian analysis was performed on the same dataset with the site-heterogeneous GTR model with gamma distribution of rates across sites approximated by four categories. Trees were run as two separate chains (default heating parameters) for 10 million generations, by which time they had ceased converg-

ing (the final average standard deviation of the split frequencies was less than 0.01). The set of positions for the analysis was selected using Gblocks (Castresana, 2000; Talavera, Castresana 2007) as implemented in SeaView 4.0 (Gouy *et al.*, 2010). Sequence identity levels were calculated using the online tool “Ident and Sim” (Stothard, 2000). Comparisons were made based on separate pairwise sequence alignments using the common portion of both sequences. The system by Brimacombe (1991) and the secondary structure of *Tetrahymena thermophila* 18S rRNA (Lee, Gutell, 2012) were used as the references to identify helices and variable regions in the sequences of the 18S rRNA gene.

The sequences obtained in this study were deposited in GenBank under the accession numbers: strain KP1TaL — PX596654, Ta4 — PX596653, BDMTaL — PX596655, KA1-4 — PX596656, KMChPTaL — PX596657.

Results

LIGHT MICROSCOPY OF STRAINS KP1TaL, Ta4, BDMTaL. Amoebae of all three strains showed stronger adhesion and moved more actively on the plastic surface rather than on the glass slides. In locomotion, cells had a flattened shape and produced no pseudopodia or subpseudopodia (Fig. 1). The outlines of moving cells resembled a smooth oval, elongated in the direction of movement (Suppl. Table 2). The anterior part of the cell was rounded and extended; the posterior part gradually narrowed and was more tapered than the anterior one (Fig. 1A, D), so the cell could have ovoid outlines (Fig. 1F–J). Slowly moving cells were more expanded, with nearly circular outlines (Fig. 1B, I–J). The posterior end of the cell was usually raised above the substrate and had no differentiated uroidal structures. The lateral edges of the cell were flat and contained hyaloplasm (Fig. 1B, C, G, H and J). These lateral “lobes” extended almost to the posterior end of the cell or could be wider at the rear part, especially immediately after the amoeba began moving or changed direction. The dorsal surface of moving cells had pronounced uneven longitudinal folds. An anterolateral hyaline of hyaloplasm crescent was thickest at the anterior end and gradually tapering along lateral sides, it continued almost toward the posterior end of the cell.

Amoebae of all three strains had a single nucleus. It was almost spherical or ovoid (Suppl. Table 2). The nucleus had a well visible flexible

envelope, that could bend upon contact with other organelles or denser areas of the cytoplasm (Fig. 2). The nucleus typically contained a centrally located nucleolus, spherical or slightly irregularly rounded. Occasionally, in addition to the centrally located nucleolus, a small rounded body was observed in the karyoplasm (Fig. 2T). The nucleolus contained lacunae. The patterns of lacuna distribution within the nucleolus varied among cells. Often, the nucleolus contained numerous lacunae (less than 1 μm in diameter) that were irregularly arranged (Fig. 2A–C, I–J, Q–R). The larger lacunae tended to be in the central part of the nucleolus, while smaller ones were located closer to the periphery of the nucleolus. A less frequent, but consistently observed pattern involved a division of the nucleolus into two zones, peripheral and central (Fig. 2D–H, K, O, S–T). Typically, the central zone contained a few larger lacunae (approximately 1 μm or more in diameter), whereas the peripheral zone contained numerous smaller lacunae (less than 1 μm in diameter). The boundary between these zones was represented by a more or less regular layer of small lacunae. Rare deviations from these patterns of nucleolar organization were also observed. In some cases, the centrally located nucleolus exhibited a complex shape (Fig. 2P). In other cases, several large rounded bodies of the nucleolar material could be found in the karyoplasm (Fig. 2U), or the nucleus contained numerous small rounded bodies (Fig. 2V). The nucleolar material in such non-vesicular nucleoli did not contain lacunae. In addition, the cytoplasm contained food vacuoles filled with bacteria and fungal spores, and a contractile vacuole (Fig. 2W).

Amoebae at the final stages of cytokinesis were also observed in the Ta4 culture (Fig. 3). Cells were connected only by a small cytoplasmic bridge covered with folds. Each cell contained a nucleus with a remarkable nucleolar organization. The nucleolar material was arranged in a peripherally located network. The branches of this network had a thickness of 2–3 μm .

LIGHT MICROSCOPY OF STRAINS KA1-4, KMChPTaL. Amoebae of both strains rarely adhered to the glass, so on the glass slides they floated or weakly adhered to the surface and remained stationary. When cells were transferred in a liquid medium to a plastic Petri dish, most cells, after sinking to the bottom of the dish,

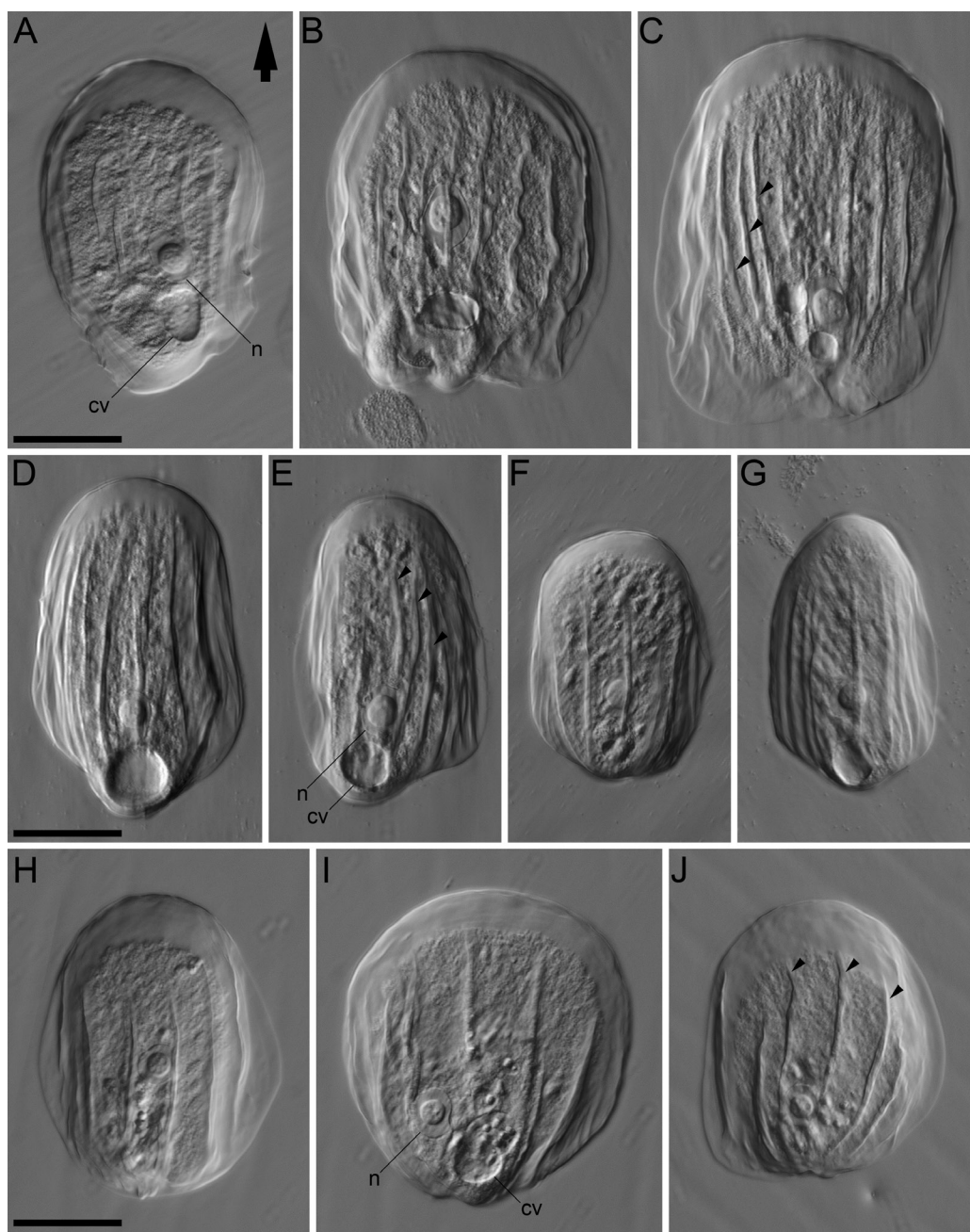


Fig. 1. Light microscopy of *Thecamoeba aesculea* cells, IMC, z-stacking. A–C — locomotive forms of strain KP1TaL amoebae; D–G — locomotive forms of strain Ta4 amoebae; H–J — locomotive forms of strain BDMTaL amoebae. Black arrowheads indicate dorsal ridges; black arrow shows the direction of cell movement (applicable to all illustrated amoebae). Abbreviations: cv — contractile vacuole; n — nucleus. Scale bars — 50 μm.

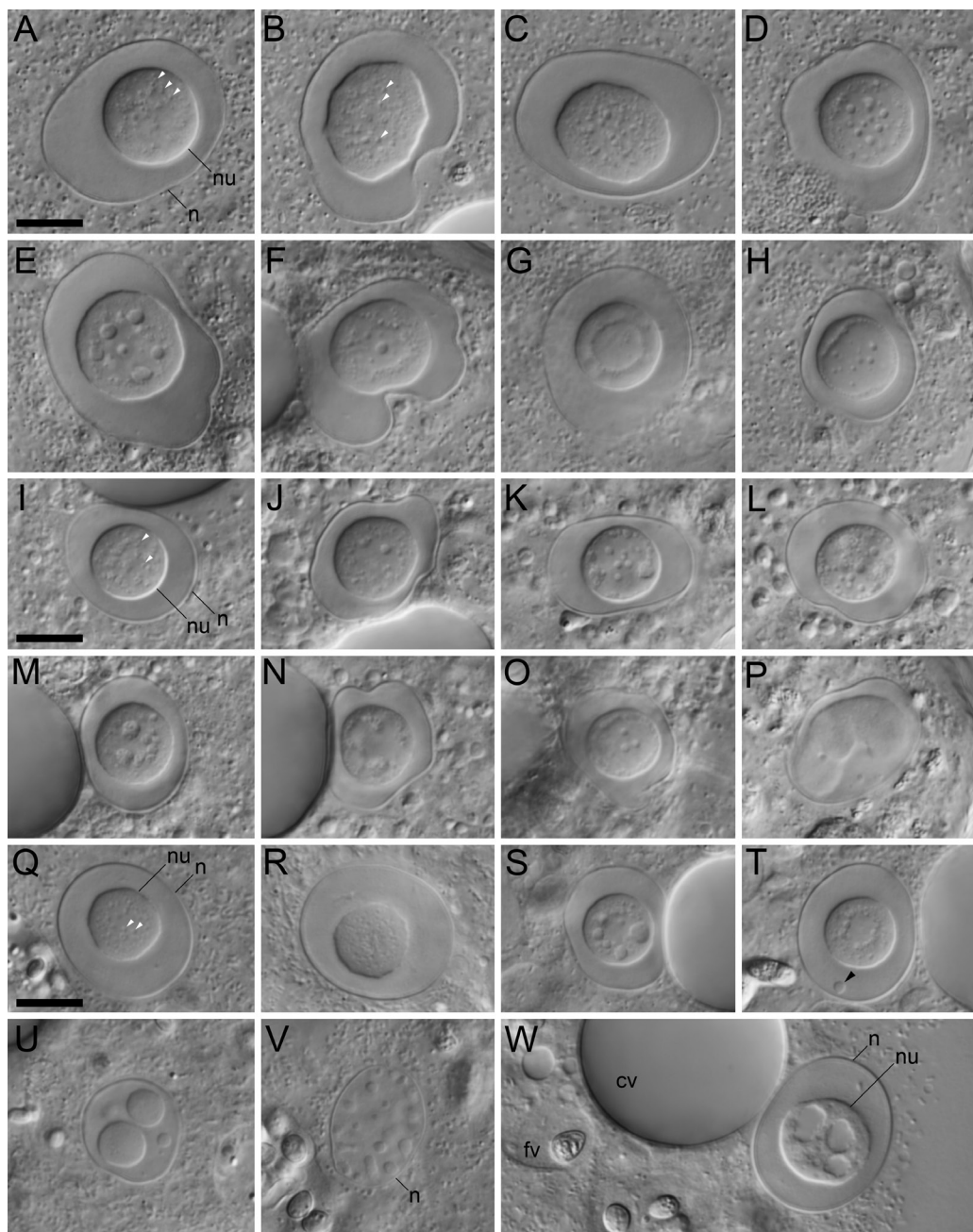


Fig. 2. Light microscopy of *Thecamoeba aesculea* nuclei, DIC. A–H — nuclei of strain KP1TaL amoebae; I–P — nuclei of strain Ta4 amoebae; Q–V — nuclei of strain BDMTaL amoebae; W — section of cytoplasm of BDMTaL amoebae. White arrowheads indicate small lacunae (less than 1 μm in diameter); black arrowhead indicate small rounded body in karyoplasm.

Abbreviations: cv — contractile vacuole; fv — food vacuole; n — nucleus; nu — nucleolus. Scale bars — 10 μm .

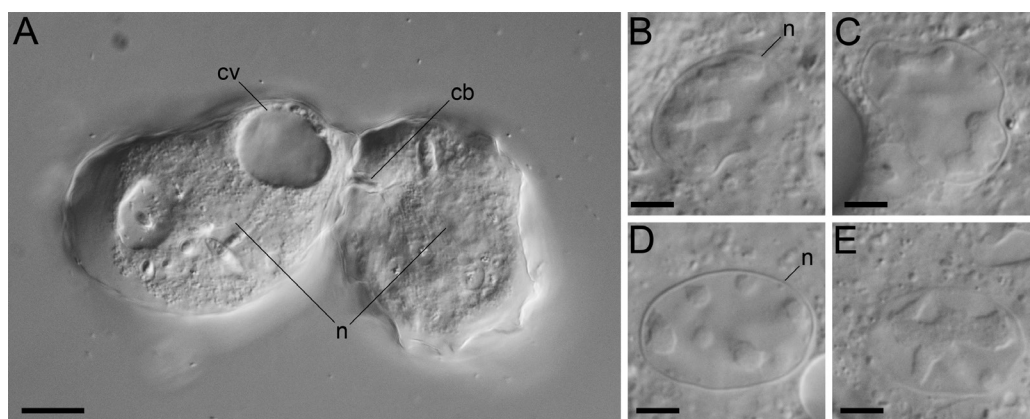


Fig. 3. Light microscopy of *Thecamoeba aesculea* strain Ta4 cell undergoing cytokinesis. A — The amoebae on the last stages of cytokinesis; B–E — The nuclei in the cells from A.

Abbreviations: cb — cytoplasmic bridge; cv — contractile vacuole; n — nucleus. Scale bars: A — 20 μ m; B–E — 5 μ m.

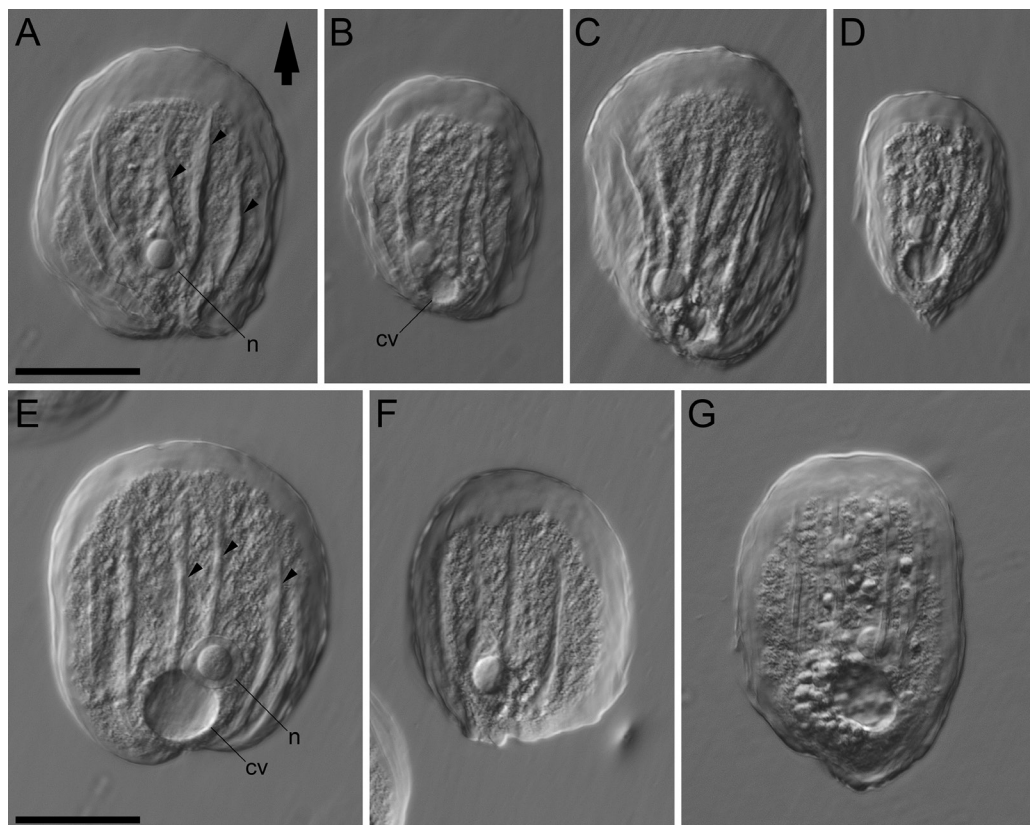


Fig. 4. Light microscopy of *Thecamoeba sonatina* sp.n. cells, IMC, z-stacking. A–D — locomotive forms of strain KA1-4 amoebae; E–G — locomotive forms of strain KMChPTaL amoebae. Black arrowheads indicate dorsal ridges; black arrow shows the direction of cell movement (applicable to all illustrated amoebae).

Abbreviations: cv — contractile vacuole; n — nucleus. Scale bars — 50 μ m.

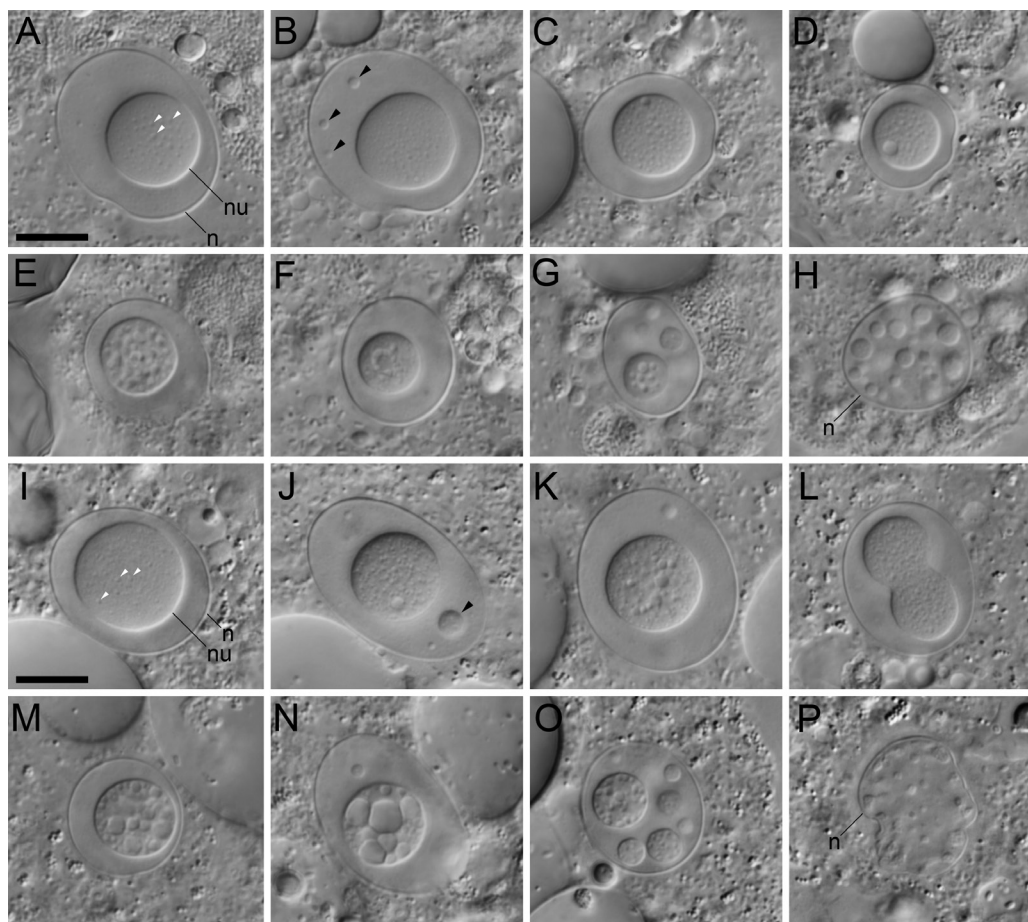


Fig. 5. Light microscopy of *Thecamoeba sonatina* sp.n. nuclei, DIC. A–H — nuclei of strain KA1-4 amoebae; I–P — nuclei of strain KMChPTaL amoebae. White arrowheads indicate small lacunae (less than 1 μm in diameter); black arrowheads indicate small rounded bodies in karyoplasm.

Abbreviations: cv — contractile vacuole; fv — food vacuole; n — nucleus; nu — nucleolus. Scale bars — 10 μm.

firmly adhered to the surface and soon started locomotion. During locomotion, amoebae had a flattened shape, moved as a whole, and did not form discrete pseudopodia or subpseudopodia (Fig. 4). The cells had a smooth and slightly wavy oval outline, elongated in the direction of movement (Suppl. Table 2). The anterior part of the cell was usually wider and rounded; the posterior part of the cell was narrower and sometimes smoothly tapered toward the posterior end, which could be rounded (Fig. 4B, C and F), slightly elongated (Fig. 4D and G), or, conversely, slightly constricted (Fig. 4A and E). The apex of the posterior end was usually raised above the substrate and did not have distinct uroidal structures. The cell surface was covered with folds,

which were primarily directed longitudinally or parallel to the cell edge. During locomotion, from four to seven uneven longitudinal ridges appeared on the dorsal side, converging to the posterior end. The hyaloplasm was widest at the anterior end of the moving cell and could occupy up to 1/4 of the total cell length. The hyaline zone, continued laterally, reaching almost the posterior end of the cell. Sometimes, on the lateral sides, the hyaline zone was wider and formed flattened lateral lobes.

Cells of both strains contained a contractile vacuole (Fig. 4) and numerous food vacuoles filled with fungal spores and bacteria. In addition, amoebae of these strains contained a single nucleus with flexible, well-visible envelope (Fig.

Table 1. Percentage identity levels of the 18S rRNA gene sequences (without intron; 1820nt) of different strains of the “*Thecamoeba aesculea* species group” (lower left corner). Percentage identity levels of the S956 intron sequences (439nt) of different strains of *T. aesculea*; *T. sonatina* sp.n. lacks the s956 intron (upper right corner).

Species, strain	<i>T. aesculea</i> JN247436	<i>T. aesculea</i> strain Ta4	<i>T. aesculea</i> strain KP1TaL	<i>T. aesculea</i> strain BDMTaL	<i>T. sonatina</i> sp.n. strain KMChPTaL	<i>T. sonatina</i> sp.n. strain KA1-4
<i>T. aesculea</i> JN247436	—	99,77	99,77	95,67	N/A	N/A
<i>T. aesculea</i> strain Ta4	98,91	—	100	95,9	N/A	N/A
<i>T. aesculea</i> strain KP1TaL	98,8	99,39	—	95,9	N/A	N/A
<i>T. aesculea</i> strain BDMTaL	98,68	99,76	99,53	—	N/A	N/A
<i>T. sonatina</i> sp.n. strain KMChPTaL	95,25	94,87	94,98	94,7	—	N/A
<i>T. sonatina</i> sp.n. strain KA1-4	95,23	94,93	95,04	94,76	99,95	—
<i>T. oculus</i> JN247436	74,6	73,18	72,83	72,98	72,62	72,62

5). The nucleus usually had a single centrally located, rounded nucleolus (Fig. 5A–F, I, M–N). The surface of the nucleolus was always smooth. The centrally located nucleolus always contained multiple lacunae. Several distinct patterns of lacuna organization were observed. In the vast majority of cases, the nucleolus contained numerous small lacunae (<1 µm in diameter) that were evenly distributed in the nucleolus (Fig. 5A–D, I–J). In addition to these small lacunae, larger lacunae (>1 µm in diameter) could sometimes be present (Fig. 5C–D, J–K). Occasionally, alongside the centrally located rounded nucleolus, other much smaller rounded bodies were observed in the karyoplasm (Fig. 5B, J). Nucleoli with small lacunae could also exhibit shapes other than rounded. In some cases, the nucleus contained several large rounded bodies with lacunae, distributed across the karyoplasm (Fig. 5G, O). Occasionally, we observed amoebae with nuclei containing multiple rounded bodies or a peripheral network; in both cases, there were no lacunae (Fig. 5H, P).

MOLECULAR PHYLOGENY. The 18S rRNA gene sequences were obtained for all studied strains (Suppl. Table 3). The obtained fragments varied in length, but each included

a section from the beginning of the 6th to the end of the 41st helix, thus contained the V1–V8 variable regions. Strains KP1TaL and Ta4 had nearly identical sequences with sub-1% differences (Table 1). They were also almost identical to the *T. aesculea* JN247436 sequence of the type strain, including the first-group intron described by Corsaro (2023). Differences from JN247436 sequence were primarily single-nucleotide substitutions and deletions. The greatest number of such differences occurred at the end of the *T. aesculea* JN247436 sequence. Since the other sequences were identical to each other in this region, and the sequence of *T. aesculea* JN247436 was identical throughout the alignment except for this region, the differences are most likely due to deterioration of the sequencing in this part of the *T. aesculea* JN247436 sequence.

The 18S rRNA gene sequence of the strain BDM differs from the two aforementioned strains and *T. aesculea* JN247436 by approximately two percent. In addition to single substitutions and insertions, there are two regions where longer fragments with differences are noticeable, and changes in the secondary structure of the molecule can be expected in these regions. These two regions were located in the distal portions of

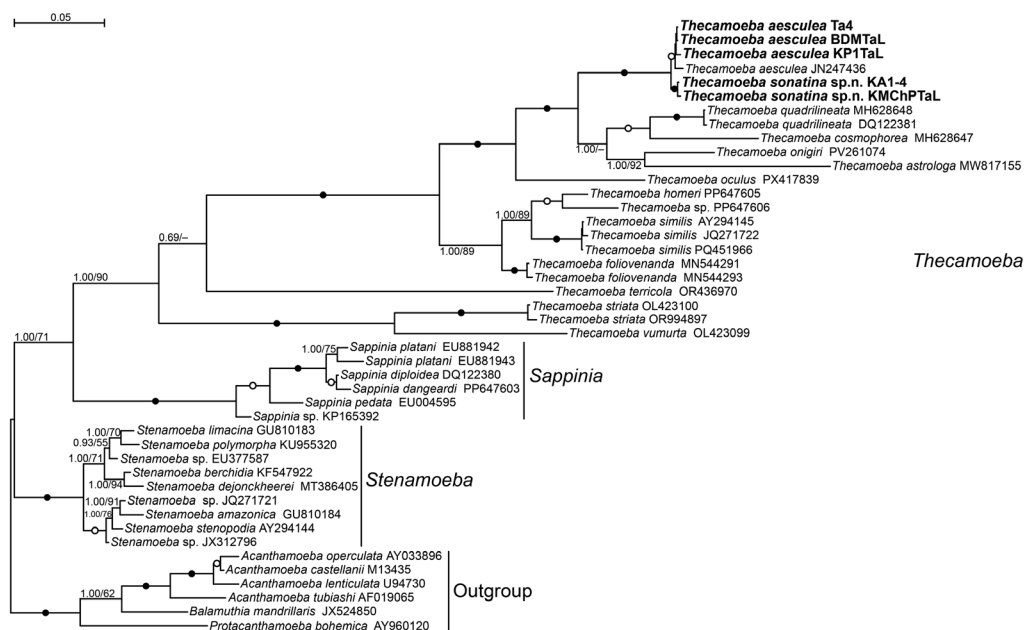


Fig. 6. Molecular phylogenetic tree based on 38 named 18S rRNA gene sequences belonging to Thecamoebida and some species of Acanthopodida used as outgroup. 1604 sites used in the analysis. Node supports indicated as PP/BS values; black circles mark fully supported nodes (1.0/100 support). Supports less than 0.5/50 are not indicated.

helices P6 and P9.3 of the intron (the regions were determined according to the scheme of intron S956 predicted secondary structure provided in Corsaro, 2023). Except the intron, the obtained sequence of the BDM strain did not almost differ from the strains and *T. aesculea* (Table 1).

The 18S rRNA gene sequences of strains KA1-4 and KMChPTaL differ by only one nucleotide. The main difference between the sequences of these two strains and those of the other strains studied is the absence of the S956 intron. The differences between these sequences and those of the other strains studied, excluding introns, are approximately 5%.

The sequences of all studied strains and *T. aesculea* JN247436, form a single, highly supported clade, which divides into two branches (Fig. 6). The first branch contains sequences from *T. aesculea* JN247436 and strains KP1TaL, Ta4, and BDMTaL; sequences from strains Ka1-4 and KMChPTaL form the second branch. This common clade is sister to the clade comprising members of the “*T. quadrilineata* species group”. However, their union has low bootstrap support. *T. oculus* occupies a more basal position to this union. Otherwise, the tree topology is similar to

the results of phylogenetic analysis from other studies (Henderson *et al.*, 2024; Mesentsev *et al.*, 2025; Shklyar *et al.*, 2025).

Taxonomy

Thecamoeba sonatina Shklyar et Mesentsev
sp.n.
Figs 4–5.

DIAGNOSIS. Cells flattened, rounded or slightly elongated during locomotion, no differentiated uroidal structures. Pronounced dorsal and lateral longitudinal folds. The length in locomotion 78–141 μm , breadth 53–143 μm , L/B ratio 0.9–1.8. The single nucleus 14–27 μm in maximal dimension. The nucleus contains a nucleolus 8–15 μm in maximal dimension, with numerous small lacunae located regularly.

DIFFERENCES FROM CLOSELY RELATED SPECIES. *Thecamoeba sonatina* sp.n. can be distinguished from *T. aesculea* by the regular arrangement of lacunae within the nucleolus. Unlike *T. aesculea*, *Thecamoeba sonatina* sp.n. does not have intron s956 in 18S rRNA gene. *Thecamoeba sonatina* sp.n. can be distinguished from *T. sphaeronucleolus* by the homogeneous structure of its nucleolus — a single rounded nucleolus with lacunae, rather than a rounded nucleolus composed of multiple granules.

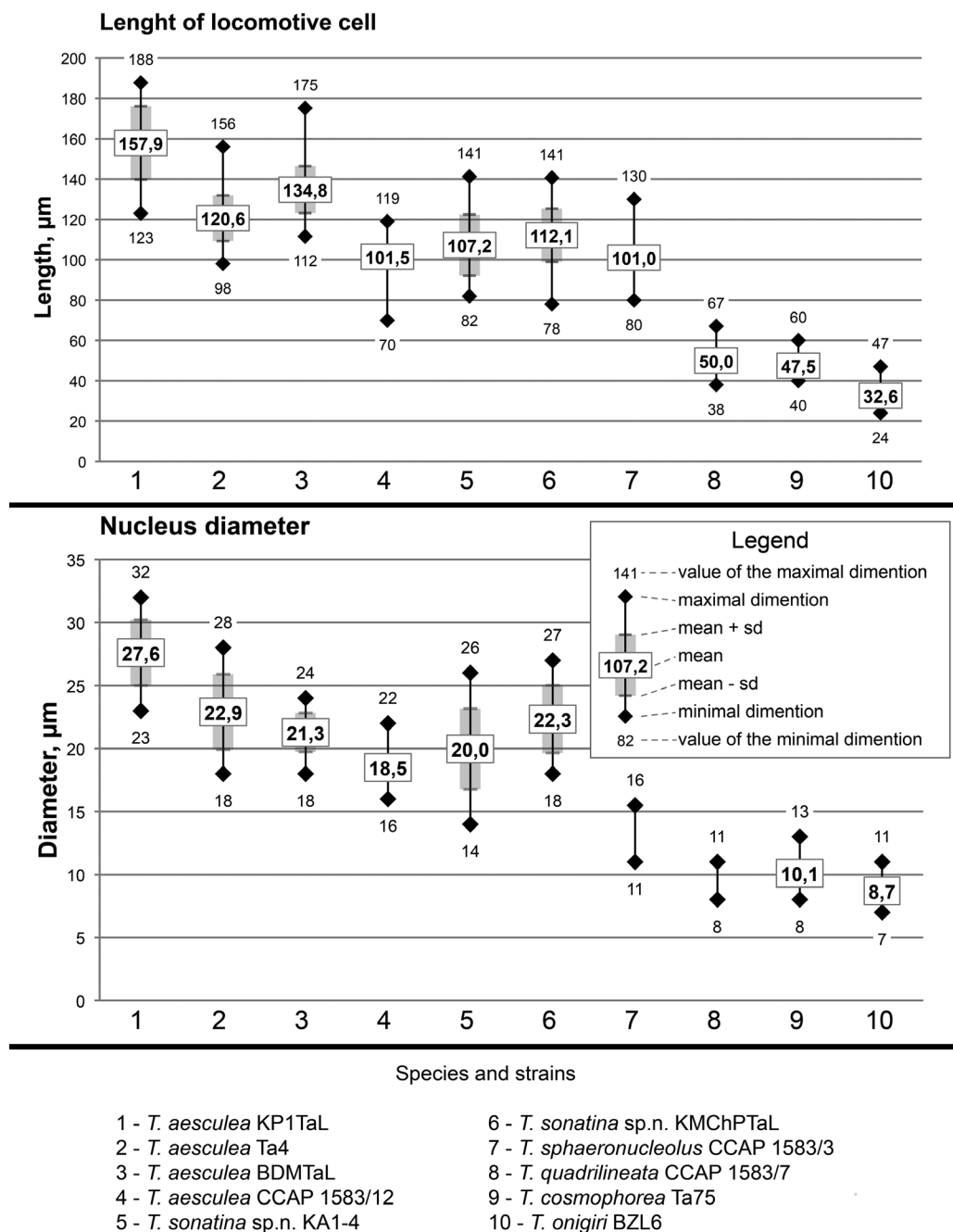


Fig. 7. Morphometric data on freshwater and terrestrial *Thecamoeba* species with vesicular nucleus. For strains 4, 7–10, no standard deviation can be calculated from published data; for strains 7 and 8, there are no published data on the mean nuclear diameter.

ETYMOLOGY. The name refers to the Latinized Italian adjective *sonatina*, meaning “a small sonata”. It alludes to the fact that in *T. sonatina* sp.n. the 18S rRNA gene sequence lacks the intron that is present in all known isolates of its sibling species, *T. aesculea*.

KNOWN STRAINS. KA1-4 (type strain) and KMChPTaL.

MOLECULAR DATA. GenBank accession numbers: 18S rRNA gene from KA1-4 strain (type strain) — PX596656; KMChPTaL strain — PX596657.

TYPE MATERIAL. Holotype (circled cell), amoeba of the strain KA1-4 on the slide (Heidenhain’s iron hematoxylin-stained permanent preparation) is deposited with the collection of slides of the Laboratory of Cytology of Unicellular Organisms, Institute of Cytology RAS, under the number 1097. All other amoebae on the slide are from the same clonal culture.

ZOOBANK REGISTRATION. Present work: 4B6544F4-A892-4334-9627-F7894C50BDBD.

Thecamoeba sonatina sp.n.: 5F423E29-9BB1-4398-A7B1-31593A91BC3B.

Thecamoeba aesculea Kudryavtsev et
Hausmann, 2009 **emend.**
Figs 1–3.

DIAGNOSIS. Cells rounded or slightly elongated during locomotion with a flattened shape, no differentiated uroidal structures. Pronounced dorsal and lateral longitudinal folds. The length in locomotion 70–188 µm, breadth 56–138 µm, L/B ratio 0.8–1.9. The single nucleus 16–32 µm in maximal dimension. The nucleus contains a nucleolus 8–18 µm in maximal dimension, with numerous small lacunae located irregularly.

DIFFERENCES FROM CLOSELY RELATED SPECIES. *Thecamoeba aesculea* can be distinguished from *T. sonatina* sp.n. by the irregular arrangement of lacunae within the nucleolus. Unlike *Thecamoeba sonatina* sp.n., *T. aesculea* have intron s956 in 18S rRNA gene. *Thecamoeba aesculea* can be distinguished from *T. sphaeronucleolus* by the homogeneous structure of its nucleolus – a single rounded nucleolus with lacunae, rather than a rounded nucleolus composed of multiple granules.

KNOWN STRAINS. Type strain CCAP 1583/12 (Kudryavtsev, Hausmann, 2009) — now lost, KP1TaL, Ta4, BDMTaL.

MOLECULAR DATA. 18S rRNA gene from CCAP 1583/12 (type strain) — JN247436.1 (Smirnov *et al.*, 2011); KP1TaL strain — PX596654; Ta4 strain — PX596653; BDMTaL strain — PX596655.

Discussion

The flattened cell shape without discrete pseudopodia or subpseudopodia, smooth oval

outlines, gradual decrement in the cell thickness from the posterior to the anterior end, as well as the presence of longitudinal dorsal folds allow all strains to be identified as members of the genus *Thecamoeba* (Page, 1988; Mesentsev, 2025). Amoebae of studied strains demonstrate traits of striate or rugose morphotype under different condition (Smirnov, Goodkov, 1999; Smirnov, Brown, 2004). In active locomotion (especially on plastic substrate) they have elongated oval outlines and a few almost parallel longitudinal dorsal ridges, so they could be assigned to the striate morphotype. When moving more slowly, they have rounded outlines, wider uneven frontal edge and uneven folds on the dorsal surface so they could be recognized as belonging to the rugose morphotype.

The vesicular nucleus, observed in all studied strains, is a characteristic of five terrestrial and freshwater species of the genus *Thecamoeba* (Mesentsev, 2025). The studied strains differ from *T. quadrilineata*, *T. cosmophorea* and *T. onigiri* being considerably larger in size (Fig. 7; Suppl. Table 2). The species *T. sphaeronucleolus* has similar size, but its nucleolus was described as granular, with non-homogeneous texture (Greeff, 1891; Page, 1971, 1977; Mesentsev, 2025). Based on the morphological characters (nuclear structure and the size of the locomotive form), the studied strains are most similar to *T. aesculea* (Kudryavtsev, Hausmann, 2009; Mesentsev *et al.*, 2025). However, according to the original description, amoebae of *T. aesculea* show characters of the rugose morphotype, whereas the studied amoebae during active locomotion are rather belong to the striate morphotype. We suggest that this difference may be a result of slightly different observation conditions. Images of the locomotive form of the type strain of *T. aesculea* were obtained from amoebae moving on the glass slide (Kudryavtsev, Hausmann, 2009, figs 4–9), while our images were taken from amoebae, moving in plastic Petri dishes (Figs 1 and 4). On glass slides, amoebae of our strains adopted a shape, more similar to *T. aesculea* and became more rugose rather than on the plastic surface. All examined strains differed from the type strain of *T. aesculea* by having slightly larger size, but it may also be due to the different degrees of spreading of the cell body over the substrate on the object glass and on the plastic of the Petri dish.

Studied strains can be divided into two groups based on the nuclear morphology: the first group includes strains KP1TaL, Ta4, and BDMTaL, while the other one includes strains KA1-4 and KMChPTaL. In amoebae of the first group, the nucleolus usually had small, but variable-sized unevenly distributed lacunae. Larger lacunae were located closer to the center of the nucleolus and had more space between each other. The space between the large lacunae was usually almost homogeneous. The peripheral zone of the nucleolus had numerous smaller lacunae. An extreme example of this distribution of lacunae of varying sizes is a visual separation of the nucleus into a clearly defined central zone with a few larger lacunae or none at all, and a peripheral zone with numerous small nucleoli, many of which mark the boundaries of these zones (Fig. 2F, G, O, and T). The nucleoli of cells in the second group of strains also typically contained numerous lacunae, which were either small and nearly similar or can vary several times in size. However, unlike amoebae of the first group, in these strains lacunae are always evenly distributed and there are no smooth homogeneous zones inside the nucleolus.

Rare variants of nucleolar organisation could be associated with a pre- or post-mitotic period. For example, in the strain Ta4 we observed an amoeba undergoing cytokinesis (Fig. 3). The nuclei of this amoeba contained nucleolar material arranged as a network. This pattern of nucleolar organisation is markedly different from the usual one, represented by a centrally located rounded nucleolus. Changes in the morphology of the nucleus during the cell division and karyokinesis in particular have been noted for *T. terricola* (Mesentsev *et al.*, 2023) and have been shown stepwise in the amoeba identified as *T. sphaeronucleolus* (Houssay, Prenant, 1970). The changes in the nucleolus organisation in postmitotic nuclei described for *T. sphaeronucleolus* are consistent with the patterns we observed.

Molecular data confirm the division of the studied strains into two groups based on the morphology of the nucleolar material. Strains within each group are almost identical in the 18S rRNA gene sequence and differ by about 5% between the groups. Strains KP1TaL, Ta4, and BDMTaL have almost identical sequences of this gene to those of *T. aesculea* (Smirnov *et al.*, 2011). Differences in the shape of motile

cells between the studied strains and the original description of *T. aesculea* are most likely due to differences in substrates, as discussed above. A detailed comparison of the organization of the nucleolar material in the type culture of *T. aesculea* amoebae based on literature data is nearly impossible, as detailed photographs of the nucleus were not provided by Kudryavtsev and Hausmann (2009). However, the nucleolus of *T. aesculea* has been described as “non-homogeneous”, and an image of the ultrastructure of the nuclear components also shows that the nucleolus is divided into a more homogeneous peripheral zone and a heterogeneous central zone with large electron-transparent spaces. Amoebae from the second group, in addition to differences in the 18S rRNA gene sequence, also lack an intron, which has been described in detail in *T. aesculea* (Corsaro, 2023). The intron sequence is almost identical between this species and members of the first group, except the strain BDMTaL.

The amoebae of the studied strains are consistent with the diagnosis of *T. aesculea* (Kudryavtsev, Hausmann, 2009). However, given subtle but remarkable morphological differences in the nucleolar organization, as well as sequence similarity, the strains of the first group were identified as belonging to the species *T. aesculea*, while the strains of the second group belong to the new species *Thecamoeba sonatina* sp.n. New morphological data obtained during this study allow us to refine the diagnosis of *T. aesculea*, more accurately reflecting the differences between the new species and the current one.

DISTRIBUTION AND IDENTIFICATION OF AMOEBAE OF THE GENUS *Thecamoeba*. The two studied species have tiny morphological differences, primarily in the relative positions of lacunae of varying sizes within the nucleolus. However, even these minor differences allowed the strains to be separated into two groups early in the study. This separation was later confirmed by comparing 18S rRNA gene sequences. Therefore, these morphological features can be considered species-specific rather than strain-specific, and can also be used to separate species. On the other hand, the described morphological differences are not always clearly evident and can only be detected using high-resolution light microscopy and after careful observation by experienced and



Fig. 8. Map showing the finding sites of members of *T. aesculea* species group. A — *T. aesculea* CCAP 1583/12; B — *T. aesculea*, strain KP1TaL; C — *T. aesculea*, strain Ta4; D — *T. aesculea*, strain BDMTaL; E — *T. sonatina* sp.n., strain KA1-4; F — *T. sonatina* sp.n., strain KMChPTaL.

knowledgeable microscopist. Single cells of different species may exhibit similar patterns of nucleolar material organization, so reliably identifying the species based on a single cell without establishing a clonal culture may not always be possible. Therefore, the use of molecular methods is practically obligatory for reliable species identification among *Thecamoeba*.

Strong morphological similarities allow the species *T. aesculea* and *T. sonatina* sp.n. to be grouped together in the “*T. aesculea* species group”. These species form a clade on the phylogenetic tree, as do other groups of sibling species in *Thecamoeba* (Mesentsev *et al.*, 2022). The use of the term “species group” rather than “species” may be preferable in case an isolate cannot be accurately identified using the molecular methods.

The three studied strains of *T. aesculea* were isolated in the Tula, Belgorod, and Irkutsk regions. Including the type strain isolated in St. Petersburg, four *T. aesculea* isolates are currently known (Fig. 8, A–D). All of them were obtained from terrestrial habitats and identified molecularly. Both *T. sonatina* sp.n. strains were isolated from terrestrial habitats in the Khabarovsk and Kamchatka territories and were also identified molecularly (Fig. 8, E and F). Despite the large geographic distance between the sites of *T. aesculea* isolates, the sequences of their 18S rRNA gene, including the intron, have a high

level of identity (about 99%). This may indicate a high degree of stability of this gene or intensive dispersal of these amoebae, leading to the emergence of the same lineage in geographically distant regions. The same considerations concern the species *T. sonatina* sp.n. Its isolates were obtained from two locations in north-eastern Eurasia, along the western Pacific coast.

A high degree of similarity, and sometimes complete identity, of 18S rRNA gene sequences among *Thecamoeba* strains isolated from distant locations was previously noted in *T. similis*, *T. striata*, *T. foliovenanda*, and *T. quadrilineata* (Kamyshatskaya *et al.*, 2018; Mesentsev *et al.*, 2024, 2025). The obtained data confirm that the traditional morphological features previously used for species identification may be unstable, while the 18S rRNA gene sequence proved to be more reliable, and therefore convenient for identification.

The collection site of strain BDMTaL, isolated from Kamchatka is rather distant from other locations from which other three strains of *T. aesculea* were isolated. We noted that strain BDMTaL has minor structural differences in the intron sequence and minor differences in the 18S rRNA gene sequence compared to other strains of *T. aesculea*. These differences indicate that the intron sequence may evolve faster than the 18S rRNA gene (Table 1).

Compliance with ethical standards

CONFLICT OF INTEREST: The authors declare that they have no conflict of interest.

Supplementary data. The following tables are available online.

Supplementary Table 1. Substrate types and collection localities of the examined samples.

Supplementary Table 2. Morphometric data of studied strains and freshwater and terrestrial *Thecamoeba* species with vesicular nucleus type strains (mean values are given in brackets).

Supplementary Table 3. Data on 18S rRNA gene sequences obtained in this study, including sequence length, nucleotide composition, and variable regions covered.

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References

- Adl S.M., Bass D., Lane Ch.E., Lukeš J., Schoch C.L., Smirnov A., Agatha S., Berney C., Brown M.W., Burki F., Cárdenas P., Čepička I., Chistyakova L., del Campo J., Dunthorn M., Edvardsen B., Eglit Y., Guillou L., Hampl V., Heiss A.A., Hoppenrath M., James T.Y., Karnkowska A., Karpov S., Kim E., Kolisko M., Kudryavtsev A., Lahr D.J.G., Lara E., Le Gall L., Lynn D.H., Mann D.G., Massana R., Mitchell E.A.D., Morrow Ch., Park J.S., Pawłowski J.W., Powell M.J., Richter D.J., Rueckert S., Shadwick L., Shimano S., Spiegel F.W., Torruella G., Youssef N., Zlatogursky V., Zhang Q. 2019. Revisions to the classification, nomenclature, and diversity of Eukaryotes // *J. Eukaryot. Microbiol.* Vol.66. No.1. P.4–119. <https://doi.org/10.1111/jeu.12691>
- Adl S.M., Habura A., Eglit Y. 2014. Amplification primers of SSU rDNA for soil protists // *Soil Biol. Biochem.* Vol.69. P.328–342. <https://doi.org/10.1016/j.soilbio.2013.10.024>
- Benson D.A., Cavanaugh M., Clark K., Karsch-Mizrachi I., Lipman D.J., Ostell J., Sayers E.W. 2013. GenBank // *Nucleic Acids Res.* Vol.41. No.D1. P.D36–D42. <https://doi.org/10.1093/nar/gkx1094>
- Bovee E.C. 1985. *Class Lobosea* Carpenter, 1861 // J.J. Lee, S.H. Hutner, E.C. Bovee (eds.) *An illustrated guide to the Protozoa*. Lawrence: Society of Protozoologists. P.158–188.
- Brimacombe R. 1991. RNA-protein interactions in the *Escherichia coli* ribosome // *Biochimie.* Vol.73. No.7–8. P.927–936. [https://doi.org/10.1016/0300-9084\(91\)90134-M](https://doi.org/10.1016/0300-9084(91)90134-M)
- Castresana J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis // *Mol. Biol. Evol.* Vol.17. No.4. P.540–552. <https://doi.org/10.1093/oxfordjournals.molbev.a026334>
- Corsaro D. 2023. Characterisation of a group I intron in the 18S rDNA of *Thecamoeba* (Amoebozoa; Discosea) // *J. Biomed. Res. Environ. Sc.* Vol.4. No.5. P.849–852. <https://doi.org/10.37871/jbres1742>
- Dyková I., Fiala I., Dvořáková H., Pecková H. 2008. Living together: The marine amoeba *Thecamoeba hilla* Schaeffer, 1926 and its endosymbiont *Labyrinthula* sp. // *Eur. J. Protistol.* Vol.44. No.4. P.308–316. <https://doi.org/10.1016/j.ejop.2008.04.001>
- Gouy M., Guindon S., Gascuel O. 2010. SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building // *Mol. Biol. Evol.* Vol.27. No.2. P.221–224. <https://doi.org/10.1093/molbev/msp259>
- Henderson T.C., Garcia-Gimeno L., Beasley Jr. C.E., Fry N.W., Bess J., Brown M.W. 2024. High above the rest: standing behaviors in the amoebae of *Sappinia* and *Thecamoeba*. // *Eur. J. Protistol.* Vol.94. Art.126082. <https://doi.org/10.1016/j.ejop.2024.126082>
- Houssay D., Prenant M. 1970. “*Thecamoeba sphaeronucleolus*.” Données physiologiques et étude morphologique en microscopie optique et électronique. // *Arch. Protistenkd.* Vol.112. P.228–251.
- Howey R.L. 2005. *Thecamoeba*: The Shar-Pei of the Protists // *Micscape Microscopy Magazine.* Art.112.
- Katoh K., Kuma K.I., Toh H., Miyata T. 2005. MAFFT version 5: Improvement in accuracy of multiple sequence alignment // *Nucleic Acids Res.* Vol.33. No.2. P.511–518. <https://doi.org/10.1093/nar/gki198>
- Katoh K., Misawa K., Kuma K.I., Miyata T. 2002. MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform // *Nucleic Acids Res.* Vol.30. No.14. P.3059–3066. <https://doi.org/10.1093/nar/gkf436>
- Katoh K., Rozewicki J., Yamada K.D. 2018. MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization // *Brief. Bioinform.* Vol.20. No.4. P.1160–1166. <https://doi.org/10.1093/bib/bbx108>
- Kudryavtsev A., Hausmann K. 2009. *Thecamoeba aesculea* n. sp. (Amoebozoa, Thecamoebidae), a terrestrial amoeba with affinities to *Th. sphaeronucleolus* (Greeff, 1891) // *Acta Protozool.* Vol.48. No.2. P.91–96.
- Lee J.C., Gutell R.R. 2012. A Comparison of the crystal structures of Eukaryotic and Bacterial SSU ribosomal RNAs reveals common structural features in the hypervariable regions // *PLoS One.* Vol.7. No.5. Art.e38203. <https://doi.org/10.1371/journal.pone.0038203>
- Medlin L., Elwood H.J., Stickel S., Sogin M.L. 1988. The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions // *Gene.* Vol.71. No.2. P.491–499. [https://doi.org/10.1016/0378-1119\(88\)90066-2](https://doi.org/10.1016/0378-1119(88)90066-2)
- Mesentsev Y. 2025. The genus *Thecamoeba* (Amoebozoa; Discosea; Thecamoebida): diversity and systematics of “amoebae with a pellicle” // *Protistology.* Vol.19.

- No.2. P.71–101. <https://doi.org/10.21685/1680-0826-2025-19-2-1>
- Mesentsev Y., Bondarenko N., Nassonova E., Smirnov A. 2021. *Vannella primobolina* n. sp. – an unusual species of the genus *Vannella* (Amoebozoa, Discosea, Vannelida) with pronounced dorsal ridges and folds // Eur. J. Protistol. Vol.77. Art.125757. <https://doi.org/10.1016/j.ejop.2020.125757>
- Mesentsev Y., Kamyshatskaya O., Nassonova E., Smirnov A. 2022. *Thecamoeba vumurta* n. sp. (Amoebozoa, Discosea, Thecamoebida) from freshwater pond sediment – a sibling species of *T. striata* (Penard, 1890) Schaeffer, 1926 // Eur. J. Protistol. Vol.83. Art.125866. <https://doi.org/10.1016/j.ejop.2022.125866>
- Mesentsev Y., Kamyshatskaya O., Smirnov A. 2020. *Thecamoeba foliovenanda* n. sp. (Amoebozoa, Discosea, Thecamoebida) – One more case of sibling species among amoebae of the genus *Thecamoeba* // Eur. J. Protistol. Vol.76. Art.125716. <https://doi.org/10.1016/j.ejop.2020.125716>
- Mesentsev Y., Shklyar A., Smirnov A. 2025. Re-isolation of *Thecamoeba similis* (Amoebozoa, Thecamoebida) and molecular signatures of clades, forming the “*T. similis* species group” // Protistology. Vol.19. No.2. P.123–140. <https://doi.org/10.21685/1680-0826-2025-19-2-4>
- Mesentsev Y.S., Smirnov A.V. 2019. *Thecamoeba cosmophorea* n. sp. (Amoebozoa, Discosea, Thecamoebida) — An example of sibling species within the genus *Thecamoeba* // Eur. J. Protistol. Vol.67. P.132–141. <https://doi.org/10.1016/j.ejop.2018.12.003>
- Mesentsev Y., Smirnov A. 2021. *Thecamoeba astrologa* n. sp. – a new species of the genus *Thecamoeba* (Amoebozoa, Discosea, Thecamoebida) with an unusually polymorphic nuclear structure // Eur. J. Protistol. Vol.81. Art.125837. <https://doi.org/10.1016/j.ejop.2021.125837>
- Mesentsev Y., Surkova A., Kamyshatskaya O., Nassonova E., Smirnov A. 2023. Re-isolation and reevaluation of boundaries of *Thecamoeba terricola* Greeff, 1886 (Amoebozoa, Discosea, Thecamoebida), with description of *Thecamoeba vicaria* n. sp. // Eur. J. Protistol. Vol.91. Art.126030. <https://doi.org/10.1016/j.ejop.2023.126030>
- Page F.C. 1971. A comparative study of five fresh-water and marine species of Thecamoebidae // Trans. Am. Microsc. Soc. Vol.90. P.157.
- Page F.C. 1977. The genus *Thecamoeba* (Protozoa, Gymnamoebia) Species distinctions, locomotive morphology, and protozoan prey // J. Nat. Hist. Vol.11. P.25–63.
- Page F.C. 1983. Marine Gymnamoebae. ITE. Cambridge. 54 p.
- Page F.C. 1988. A new key to freshwater and soil gymnamoebae. FBA. Ambleside. 122 p.
- Page F.C. 1991. Nackte Rhizopoda // D.D. Matthes (ed.). Protozoenfauna. Bd.2. Nackte Rhizopoda und Heliozoa. Stuttgart, New York: Gustav Fischer Verlag. S.75–84.
- Pawlowski J. 2000. Introduction to the molecular systematics of foraminifera // Micropaleontology. Vol.46. P.1–12.
- Penard E. 1905. Observations sur les Amibes a pellicule // Arch. Protistenkd. Vol.6. P.175–206.
- Prescott D.M., James T.W. 1955. Culturing of *Amoeba proteus* on *Tetrahymena* // Exp. Cell Res. Vol.8. No.1. P.256–258. [https://doi.org/10.1016/0014-4827\(55\)90067-7](https://doi.org/10.1016/0014-4827(55)90067-7)
- Pussard M. 1973. Description d’une amibe de type flabel-lulien, *Pessonella marginata* n. g. n. sp. (Mayorellidae, Amoebaeae) // Protistologica. Vol.9. P.175–185.
- Rogerson A., Patterson D.J. 2000. The naked ramicristate amoebae (Gymnamoebae) // J.J. Lee, G.F. Leedale, P. Bradbury (eds.). An Illustrated Guide to the Protozoa. Lawrence: Blackwell Pub. P.1023–1052.
- Ronquist F., Huelsenbeck J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models // Bioinformatics. Vol.19. No.2. P.1572–1574. <https://doi.org/10.1093/bioinformatics/btg180>
- Shklyar A.A., Mesentsev Y.S., Chikadze E.D., Smirnov A.V. 2025. *Thecamoeba onigiri* n. sp. (Amoebozoa, Discosea, Thecamoebida) — one more species of the genus *Thecamoeba* with polymorphic nuclear structure // Protistology. Vol.19. No.1. P.56–65. <https://doi.org/10.21685/1680-0826-2025-19-1-5>
- Smirnov A.V. 1999a. An illustrated survey of gymnamoebae isolated from anaerobic sediments of the Nivå Bay (The Sound) (Rhizopoda, Lobosea) // Ophelia. Vol.50. No.2. P.113–148. <https://doi.org/10.1080/00785326.1999.10409392>
- Smirnov A.V. 1999b. Re-description of *Thecamoeba munda* Schaeffer 1926 (Gymnamoebia, Thecamoebidae), isolated from the Baltic Sea // Eur. J. Protistol. Vol.35. No.1. P.66–69. [https://doi.org/10.1016/s0932-4739\(99\)80023-6](https://doi.org/10.1016/s0932-4739(99)80023-6)
- Smirnov A.V., Brown S. 2004. Guide to the methods of study and identification of soil gymnamoebae // Protistology. Vol.3. P.148–190.
- Smirnov A.V., Chao E., Nassonova E.S., Cavalier-Smith T. 2011. A revised classification of naked lobose amoebae (Amoebozoa: Lobosa) // Protist. Vol.162. P.545–570. <https://doi.org/10.1016/j.protis.2011.04.004>
- Smirnov A.V., Goodkov A.V. 1999. An Illustrated list of basic morphotypes of Gymnamoebia (Rhizopoda, Lobosea) // Protistology. Vol.1. P.20–29.
- Spiegel F.W., Moore D.L., Feldman J. 1995. *Tychosporium acutostipes*, a new protostelid which modifies the concept of the Protosteliidae // Mycologia. Vol.87. P.265–270. <https://doi.org/10.2307/3760912>
- Stamatakis A. 2014. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies // Bioinformatics. Vol.30. P.1312–1313. <https://doi.org/10.1093/bioinformatics/btu033>
- Stothard P. 2000. The Sequence Manipulation Suite: JavaScript programs for analyzing and formatting protein and DNA sequences // Biotechniques. Vol.28. No.6. P.1102–1104. <https://doi.org/10.2144/00286ir01>
- Talavera G., Castresana J. 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments // Syst. Biol. Vol.56. P.564–577. <https://doi.org/10.1080/10635150701472164>

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