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Amoeba proteus Pallas, 1766 (Leidy, 1878) isolated from the natural biotopes of Ukraine (morphology and phylogenetic relationships)

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Original article

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Naked amoebas of the genus *Amoeba* Bory de Saint-Vincent, 1822 are large protists, which are among the favourite objects of biochemical and cytogenetic studies. However, they are rare in natural biotopes, and are least represented on molecular phylogenetic trees. I sequenced the 18S rRNA gene of the *Amoeba proteus* strain AP07 (ON907618), which presents the typical morphology of *A. proteus*. A phylogenetic analysis showed that *Amoeba proteus* AP07, found in the River Stokhid of the Volyn Region, is reliably grouped with the isolate *Amoeba proteus* (AJ314604) from the reservoirs of Switzerland. These isolates were placed in a sister group to representatives of the genus *Chaos* Linnaeus, 1767, which confirmed its position, as established earlier on the basis of morphological characteristics. All of them form a relatively well-supported clade, which corresponds to the family Amoebidae.

Key words: Amoebidae, distribution, SSU rRNA gene.

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Representatives of the family Amoebidae Ehrenberg, 1838 are the least common protists inhabiting marine and fresh waters and soils. Amoebidae are capable of forming resting stages (cysts), thus enabling them to survive adverse environmental conditions (Page 1988). The family includes the genera *Amoeba* Bory de Saint-Vincent, 1822, *Polychaos* Schaeffer, 1926, *Deuteramoeba* Page, 1987, *Chaos* Linnaeus, 1767, *Trichamoeba* Fromentel, 1874, *Hydramoeba* Reynolds & Looper, 1928, and *Parachaos* Willumsen, Siemensma & Suhr-Jessen, 1987. Although this group of protists is considered to be cosmopolitan, isolated finds of representatives of the mentioned genera are noted in the literature (Shaeffer 1926; Page & Baldock 1980; Pussard *et al.* 1980; Willumsen 1982; Page & Robson 1983; Bolivar *et al.* 2001; Mrva 2010/2011; Rogerson 2018).

In 1755, Rösel von Rosenhof observed and documented the first species of amoeba. He named the protist 'the little Proteus' (der kleine Proteus). In 1758, Linnaeus called Rosenhof's amoeba *Volvox chaos*, and later in 1767 he changed this name to *Chaos proteus*. In 1822, Vincent introduced a new name: 'amoeba'. *Amoeba proteus*, as a classical representative of the genus *Amoeba*, was first discovered by Pallas in 1766 and was named *Volvox proteus*. It was not until Leidy's work in 1878 that this species was adequately morphologically described and named *A. proteus*, with material that was later supplemented by Shaffer (Schaeffer 1916).

In my research, the species *Amoeba proteus* (Pallas, 1766) Leidy, 1878 was isolated from various freshwater bodies of Ukraine during 2019-2023 (Patsyuk 2014). *A. proteus* was found in the limnic

species complex of naked amoebae, characteristic of the Shatsky Lakes (Ukraine). A specific complex of conditions prevails in these reservoirs: the Shatsky Lakes are of a fluvioglacial origin and have a mainly sandy-muddy bottom, as well as calcium hydrocarbonate water with a low level of mineralisation. Some known strains of *A. proteus* have been isolated from water bodies in North America, from the Amazon River (Brazil), from a small water body in Bombay (Mumbai, India) and from reservoirs near the city of Naples (Italy) (Taylor 1925; Rao & Chatterjee 1974; Spear & Prescott 1980; Page & Siemensma 1991). *A. proteus*, which has been isolated only a few times from different natural biotopes, is described mainly by its morphological features. The genetic structure of the *A. proteus* species, as well as the area of its distribution, has remained unexplored.

There are relatively few recent and complete phylogenetic trees that have been constructed for amoeboid protists based on the 18S rRNA gene sequence. There are many trees for the amoeboid protists, but very few for Euamoebidae Lepsi, 1960. Amoeba species such as *Amoeba leningradensis* Page & Kalinina, 1984, *A. proteus*, *Chaos nobile* (Penard, 1902) Bovee & Jahn, 1973, and *Chaos carolinense* (Wilson, 1900) King & Jahn, 1948 were sequenced by I. Bolivar (Bolivar *et al.* 2001) for the first time and were grouped in a phylogenetic tree, i.e. the species of both genera were mixed on one tree.

Multigene data for *A. proteus* was shown in the paper by Lahr (Lahr *et al.* 2013). Nonetheless, the isolation of a sufficient number of representatives of the genus *Amoeba* in nature and characterising their morphology and phylogenetic position remains the main task, which is necessary to clarify the taxonomic and phylogenetic position of this genus of amoeba. I sequenced the 18S rRNA gene of the *Amoeba proteus* strain AP07, whose morphology appears typical for the species *Amoeba proteus*. A phylogenetic analysis placed it robustly in a group with other representatives of Amoebidae, which confirms the position established earlier on the basis of its morphological features.

The purpose of this study was to establish new locations of the species *A. proteus* in the natural biotopes of Ukraine, with the involvement of morphological and molecular research methods.

Materials and Methods

The studied specimens of *A. proteus* were isolated from samples collected in the Stokhid River, Volyn Region of Ukraine (51°19′32.1″N 25°10′51.8″E; 51°23′28.8″N 25°12′17.0″E; 51°20′20.2″N 25°10′27.7″E). Specialists prefer to analyse the laboratory cultures of amoebae instead of the material obtained directly from nature, because finding and identifying the protists is not an easy task. A 5 ml freshwater sample was evenly distributed in a 100 mm Petri dish with non-nutrient agar (NNA), following Page's method (Page 1988). To obtain cultures for accumulation, the samples were sown and maintained on a Prescott James (PJ) medium supplemented with rice grains (Page 1988; Page & Siemensma 1991). The Prescott James medium had the following composition (Page 1988): from three stock solutions, each in 100 ml of glass-distilled water, 1 ml of each solution was combined with 997 ml of distilled water to make 1 l of the final dilution.

Stock solution A

$CaCl_2 \cdot 2H_2O$	0.433 g
KCl	0.162 g

Stock solution B

K_2HPO_4	0.512 g

Stock solution C

$MgSO_4 \cdot 7H_2O$	0.280 g

The cultures were cloned by transplanting single cells into a new dish with the medium and were then maintained at room temperature. After 8 days of incubation, the cells were examined using a Leica DM500 microscope. The identified amoebae were transferred (one cell at a time) to fresh medium using a Pasteur pipette with a narrowed end, and were multiplied again on non-nutrient agar for 5 days. The procedure was repeated 2-3 times. All the procedures were performed with sterile instruments and under sterile conditions. Next, the cells were collected with the minimum possible amount of medium and were placed in PCR tubes with a volume of 0.2 ml.

Species identification

Species identification and the production of microphotographs were carried out using the Axio Imager M1 light microscope ('Animalia' centre for the collective use of scientific instruments, I. I. Schmalhausen Institute of Zoology NAS of Ukraine), by depositing living cells in a drop of water on glass slides with the use of a differential interference contrast. The main morphological features were the following dimensions of the motile forms: cell width (B), as the distance measured perpendicular to the direction of movement in the widest part of the cell; cell length (L), as the distance between the anterior and posterior ends of the moving cell; the ratio of the cell length to width (L/B), and the nucleus and cyst diameter (Page & Siemensma 1991). The measure-

ments were made on intact cells or on the microphotographs, using an eyepiece micrometer (×40). Amoebae strains were identified by comparing the available original descriptions with data found in the works of F. Page (Page 1988; Page & Siemensma 1991). Live trophozoites (more than 50 naked amoebae cells) were observed and measured.

For the identification of naked amoebae, the traditional features for this group were used: morphology of the motile form taking into account the morphotype; morphology of the uroid and pseudopodia; nature of the movement of the cytoplasm; and the formation of a floating form.

DNA isolation

In most amoebae, the 'pure' DNA cannot be isolated, because the amoeba cultures also contain other eukaryotes (for example, fungi and animal-like organisms), on which they feed. Therefore, before DNA isolation, the amoebae were kept on starved agar to clean them from eukaryotic contaminants. I was able to obtain 'pure' DNA sequences from only one naked amoeba culture. Genomic DNA was isolated using the guanidine isothiocyanate method (Maniatis *et al*. 1982). The 18S rRNA gene was then amplified using the universal eukaryotic primers RibA 5'-ACCTGGTTGATCCTGCCAGT-3' and RibB 5'-TGATCCTTCTGCAGGTTCACCTAC-3' (Medlin *et al*. 1988). The same sequencing primers were used for each strain. Basic conditions for the PCR were: an initial denaturation at 95°C for 10 minutes; 40 cycles of 94°C for 30 seconds; 50°C for 60 seconds; 72°C for 30 seconds; and 10 minutes final elongation. Amplicons were sequenced using the ABI Prism Big Dye Terminator Cycle Sequencing Kit. The resulting sequences were automatically aligned using the Muscle algorithm, implemented in MEGA 10.0. The comparison of the obtained DNA sequences with GenBank data was carried out using the BLAST program (NCBI) (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Based on the alignment, which consisted of the sequences of the 18S rRNA gene for representatives of the genera *Chaos* and *Amoeba*, a phylogenetic analysis was performed using the MEGA 10.0 program (Kumar *et al.* 2018). In the analysis, I used the sequences obtained in the present study and the sequences for other species of naked amoebae that are available in the GenBank database. The evolutionary history was inferred using the Neighbour-Joining method (Saitou & Nei 1987). The optimal tree is shown (GTR+I+G model of nucleotide substitution). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein 1985). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al.* 2004). This analysis involved 7 nucleotide sequences. There were 2043 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar *et al.* 2018). The outgroup is naked amoeba of the genus *Saccamoeba* (*Saccamoeba limax* (AF293902), *Saccamoeba lacustris* strain SL-2 (GQ221845), *Saccamoeba* sp. W187G (JQ271720)), which belong to a different family (Fig. 2).

Results and Discussion

Morphology

Most of the studied amoebae had a polytactic morphotype, with 4-6 well-defined cylindrical pseudopodia of different sizes and a small hyaline cap at each end (Fig. 1). There were also specimens of amoebae with a wide hyaline cap. Usually, all pseudopodia do not move at the same time, but there was always a dominant pseudopodium that changed the direction of movement of the amoeba. Under bright light, the amoebas changed their cell shape to orthotactic, i.e. elongated, wide (due to a decrease in the length), and without the hyaline caps on the pseudopodia. During locomotion, the dorsal and lateral folds of the cytoplasm were visible.

Crystals and other inclusions were present in the cytoplasm, and the contractile vacuole was located, as a rule, near the caudal end of the cell.

In the orthotactic forms, the caudal part of the cell formed a morular or nodular uroid.

The cell length was 210-340 µm, the width was 50-115 µm and the L/B ratio was 10-22.

The nucleus was of a vesicular type, disk-shaped, with a diameter of 29-34 µm.

Cyst formation was not observed in these cultures.

The main morphological features that confirmed that the naked amoebas I identified belonged to the species *A. proteus* were the following: a large size and polypodial cell shape; during movement in the polypodial forms, a larger pseudopodium was always dominant, at the end of which a hyaline cap was visible; the polytactic amoebae often changed their form to orthotactic; uroid was of a morular or nodular type; and the nucleus was often deformed as if it was wrinkled, or was sometimes 'twisted'.

There was a thin layer of ectoplasm, which lacked granules, under the plasma membrane of the amoeba. The ectoplasm surrounded the granular endoplasm.

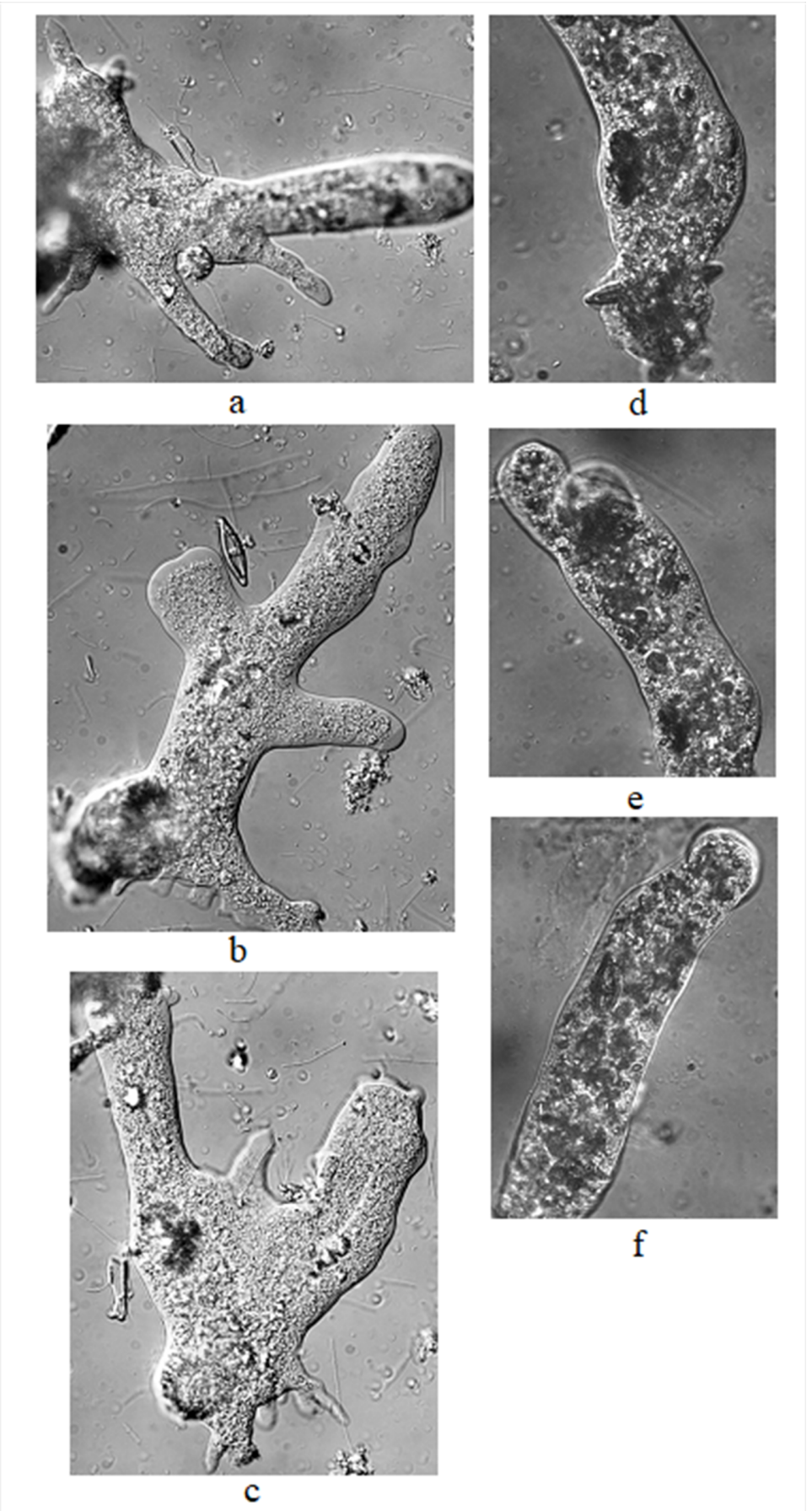

Fig. 1. *Amoeba proteus* Pallas, 1766 (Leidy, 1878). ×1240 (own photos) a-c – polytactic amoebae, d-f – monopodial amoebae.

The latter consisted of an outer strong plasmagel and an inner liquid plasmosol, which exhibited flow movements. The plasmagel could turn into plasmosol. In the actively moving protist, the plasmosol flowed quickly in the direction of movement of the amoeba, while the plasmagel was almost always at rest.

In actively moving amoebas, the plasmosol had the form of a long tube with a gradual expansion at the rear end of the cell. The plasmosol occupied almost the entire volume of the cell; it was a liquid that contained a large number of small crystals and granules (the diameter of the crystals according to my data was from 0.18 to 3.5 μm, while that of the granules was up to 5.5 μm). The shapes varied from oval and rectangular to hexagonal. The granules or crystals in the plasmosol moved in a straight line until they reached the end of the plasmagel of the pseudopodium. The plasmagel was relatively homogeneous. Plasmagel particles of various sizes and shapes could often be seen attached to the nucleus.

The nucleus, contractile vacuole and digestive vacuole were located in the plasmosol (in a liquid and mobile environment). The nucleus sometimes flowed toward the tip of the extended pseudopodium. In relation to the rear end of the cell, the nucleus gradually moved forward or backward. The nucleus in *A. proteus* was of the vesicular type. The diameter was 29-32 μm. The number of chromosomes in *A. proteus* reached 500 or more (Ord 1973).

Contractile vacuoles, as a rule, float in the plasmosol, are sometimes attached to the plasmagel and are transported to the rear end of the cell. Metcalf believed that in *A. proteus*, the contractile vacuole performs the function of an excretory organ, and the granules that surround the contractile vacuole have a functional significance in the excretion processes (Metcalf 1910). In active individuals, the vacuole is located closer to the posterior part of the cell. I observed the presence of crystals in the vacuoles. At the same time, the vacuoles moved freely in the plasmosol and remained intact. The crystals were stored in vacuoles during the continuous movement of the amoeba. After a certain period of time, the shrinking vacuole approached the plasmalemma (a temporary pore appeared) and slowly expanded, inflated and burst. A new contractile vacuole then appeared in its original position, closer to the uroid. The granules, crystals and vacuoles were arranged randomly in the plasmagel. The plasmagel turned into plasmosol and vice versa; however, I did not observe changes in the vacuoles.

During movement in *A. proteus*, wide smooth tubular pseudopodia are formed. They can occur in different parts of the cell, in different directions, only to retract and disappear later, while others are formed in their place. During the formation of a new pseudopodium, the plasmagel on the inner surface softens, which leads to a decrease in its thickness and strength. A protrusion in the form of a tubercle is formed. The hyaline cap is formed by the release of fluid from the plasmosol through the plasmagel, with the fluid accumulating at the tip of the pseudopodia under the plasmolemma. The plasmosol flows out and a jelly-like protrusion forms on the inner surface of the plasmagel. The diameter of the cap changes during the locomotion of the amoeba: sometimes it is half as thick as the diameter of the pseudopodium; while sometimes it is thin, barely noticeable or completely absent. The hyaline cap does not contain granules. Very rarely, the thin wall of the plasmagel breaks, the plasmosol flows into the hyaline cap and very small granules occur in the latter.

When the amoeba moves slowly, the pseudopodium expands, and the liquid part of the plasmosol gradually and continuously passes through the plasmagel into the hyaline cap and spreads laterally, forming a liquid layer between the plasmagel and the plasmalemma. As the pseudopodium expands, the plasmagel layer stretches and tends to become thinner. A change in the speed of movement is caused by a change in the structure of the plasmagel, which is adjacent to the hyaline cap. If the plasmagel becomes thinner, weaker and porous, it stretches more easily, allowing liquid from the plasmosol to pass through better, and accelerating the speed of movement. It should be noted that in monopodial forms, there is a fountain-like movement of the protoplasm, as a result of which the ectoplasmic gel begins to contract almost immediately after its formation at the front end of the body of the amoeba. The documented speed of movement of *A. proteus* has been 4-5 mm/s (Cameron *et al*. 2007). In my studies, the speed of movement of the amoebas was 5-5.5 mm/s.

The floating forms of *A. proteus* have fixed pseudopodia. Such amoebas roll over in one direction, then in another.

The uroid is of a morular or nodular type. It is formed after the outflow of the main mass of cytoplasm, with which the amoeba was attached, from the surface of the substrate.

The polypodial cells with dominant pseudopodia were measured. The length of the amoeba cell was 210-340 μm, width was 50-115 μm and the L/B ratio was10-22. It is known that the cell size depends on the level of amoeba nutrition: hungry amoebae that have just been fed accelerate the process of cyst formation (Taylor 1924).

Compared to other species of the genus *Amoeba*, the species *A. proteus* differs in terms of the average cell size and its granular disk-like, sometimes biconcave nucleus (Page & Siemensma 1991).

Representatives of the family Amoebidae Ehrenberg, 1838 are rather large protists that inhabit freshwater and soil biotopes. These heterotrophic organisms are widespread (Page & Siemensma 1991), although I found a small number of amoeba representatives from the genera *Deuteramoeba* Page, 1987 and *Polychaos* Schaeffer, 1926 (Patsyuk 2014). The protists are supported in ecosystems and play a fundamental role in the biological control of bacteria, as well as other protozoa and fungi. *A. proteus* is known from the natural biotopes of North America and Europe (Page & Siemensma 1991). I found the species in Peremut, Luky and Chorne Lakes (in the territory of Shatsky National Nature Park) (Patsyuk 2014) and in the Stokhid River, Volyn Region of Ukraine. I encountered this species in the Stokhid River at a concentration of oxygen dissolved in the water of 12.08 mg/l and a concentration of organic substances dissolved in the water (according to the permanganate index) of 25.14 mg O_2/l.

Phylogenetic relationships

The 18S rRNA gene sequence was obtained for the species *Amoeba proteus* isolate AP07 (ON907618), isolated from the Stokhid River (Volyn Region, Ukraine) (GenBank Accession Number ON907618). According to the phylogenetic analysis, the described *A. proteus* reliably groups with *A. proteus*, the sequence of which was deposited in GenBank under Number AJ314604.

In the systematics of naked amoebae, *A. proteus* belongs to the family Amoebidae (Page & Siemensma 1991). According to the modern system of Eukaryotes, the protist belongs to the molecular cluster Tubulinea Smirnov *et al*., 2005, which includes naked and testate lobose amoebae with tubular cylindrical pseudopodia and a monoaxial cytoplasmic flow (Cavalier-Smith *et al*. 2016). Molecular phylogenetic data shows that representatives of the genus *Saccamoeba* Frenzel, 1892 group well with representatives of the genera *Deuteramoeba* Page, 1987 and *Amoeba*, which belong to the Tubulinea group. In turn, Tubulinea and Discosea Cavalier-Smith *et al*., 2004, according to a molecular phylogenetic analysis, form a separate group Lobosa Carpenter, 1861 as part of Amoebozoa Luhe, 1913 (Bolivar *et al.* 2001; Dykova *et al*. 2008).

The sequence of the 18S rRNA gene of the *A. proteus* AP07 I obtained from the Stokhid River in the Volyn Region is 100% similar to the sequence of *A. proteus* deposited in GenBank under Number AJ314604, which was found in the reservoirs of Switzerland (Fig. 2). This group of amoebae is a sister to members of the genus *Chaos*, with a high level of support. With the species *Chaos nobile* CCAP 1511/2, the similarity is only 92 %, and the latter is grouped with *Chaos carolinense* JG10.98 and *Chaos carolinense* WW-13-324 and *Amoeba*

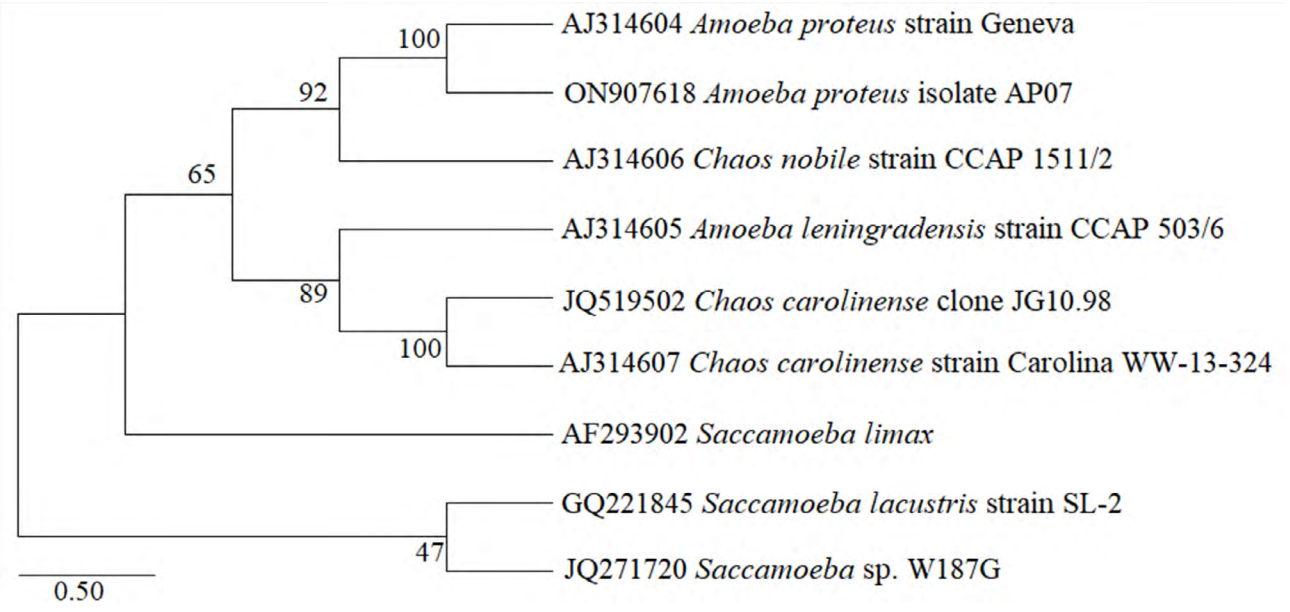

Fig. 2. Fragment of a phylogenetic tree built on the basis of 18S rRNA gene sequences for representatives of the genera *Amoeba* and *Chaos*. The scale bar shows the distance equivalence between sequences. Outgroup – naked amoeba of the genus *Saccamoeba*.

leningradensis CCAP 503/6 with a similarity of 89%. As in the known phylogenetic trees (Cavalier-Smith *et al.* 2016; Lahr *et al.* 2013), representatives of the genera *Amoeba* and *Chaos* are mixed; however, the morphological taxonomy of the naked amoebae remains true. Considering the species of naked amoebae from the genera *Amoeba* and *Chaos* with good morphological features, such as large cells with tubular cylindrical pseudopodia, the presence of hyaline caps at the ends of the pseudopodia, a change in the shape of the body to a monopodial one during rapid locomotion, morular-type uroid and the presence of bipyramidal crystals in the cytoplasm of the cell, may represent real taxonomic features that unite them in the Amoebidae family. The only morphological difference between *Amoeba* and *Chaos* is the number of nuclei: *Amoeba* are always uninucleate, while *Chaos* are both uninucleate and multinucleate. Research shows that the taxonomic position of the 'proteus-type' amoeba species is becoming complex, and the characteristics that can be used to classify these organisms are becoming more shadowed. Thus, to establish the taxonomic position of the naked amoebae of the family Amoebidae, it is necessary to take into account the molecular genetic and morphological features of the species.

The analysed DNA sequences of two naked amoeba isolates *A. proteus* AP07 (ON907618) and *A. proteus* (AJ314604) were closely related to an isolate of the genus *Chaos* (*Chaos nobile* CCAP 1511/2). This group is closely grouped with the isolates of *Amoeba leningradensis* CCAP 503/6, *Chaos carolinense* JG10.98 and *Chaos carolinense* WW-13-324. Together, all of them form a relatively well-supported clade, which corresponds to the family Amoebidae.

Conclusions

Data on the biogeography of naked amoebas is scarce, despite their wide distribution in various natural biotopes. This is due to the difficulty of species identification. Since I isolated *A. proteus* in a small number of samples, and there is practically no data on its distribution in the well-studied European fauna, this can serve as an argument against the hypothesis of the cosmopolitanism of naked amoebas. Studies of remote locations may provide an opportunity to expand our understanding of the diversity of naked amoebas, including *A. proteus*.

Author Contributions

Research concept and design, Collection and assembly of data, Data analysis and interpretation, Writing the article, Critical revision of the article, Final approval of article – M. Patsyuk.

Conflicts of Interest

The author declares no conflict of interest.

References

Bolivar I., Fahrni J.F., Smirnov A., 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. https://doi.org/10.1093/oxfordjournals.molbev.a003777

Cameron I., Rinaldi R.A., Kirby G., Davidson D. 2007. *Amoeba proteus* display sawalking form of locomotion. Cell Biol. Int. **31:** 759-762. https://doi.org/10.1016/j.cellbi.2007.01.009

Cavalier-Smith T., Chao E. E., Lewis R. 2016. 187-gene phylogeny of protozoan phylum Amoebozoa reveals a new class (Cutosea) of deep-branching, ultrastructurally unique, enveloped marine Lobosa and clarifies amoeba evolution. Mol. Phylogenet. Evol. **99**: 275-296. https://doi.org/10.1016/j.ympev.2016.03.023

Dykova I., Kostka M., Peckova H. 2008. Morphology and SSU rDNA-based Phylogeny of a New Strain of *Saccamoeba* sp. (*Saccamoeba* Frenzel, 1892, Amoebozoa). Acta Protozool. **47**: 397-405.

Felsenstein J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. J. Evol. **39**: 783-791. https://doi.org/10.2307/2408678

Kumar S., Stecher G., Li M., Knyaz C., Tamura K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Mol. Biol. Evol. **35**: 1547-1549. https://doi.org/10.1093/molbev/msy096

Lahr D. J. G., Grant J. R., Katz L. A. 2013. Multigene phylogenetic reconstruction of the Tubulinea (Amoebozoa) corroborates four of the six major lineages, while additionally revealing that shell composition does not predict phylogeny in the Arcellinida. Protist. 164:323-339. https://doi.org/10.1016/j.protis.2013.02.003

Maniatis T., Fritsch E.F., Sambrook J. 1982. Molecular cloning, a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 545.

Medlin L., Elwood H.J., Stickel S., Sogin M.L. 1988. The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. Gene. **71**: 491-499. https://doi.org/10.1016/0378-1119(88)90066-2

Metcalf M.M. 1910. Studies on Amoeba. Jour. Exp. Zool. **9**: 301-331.

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

Ord M.J. 1973. Chemical mutagens, in The biology of *Amoeba*. New York: Acad. Press., 349-370.

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

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

Page F.C. 1988. A New Key to Freshwater and Soil Gymnamoebea. Freshwater Biological Association, Ambleside, Cumbria, UK, 122.

Page F.C., Siemensma F.J. 1991. Nackte Rhizopoda und Heliozoea (Protozoenfauna Band 2). Gustav Fischer Verlag, Stuttgart, New York, 3-170.

Patsyuk M.K. 2014. Morphotypes in Naked Amoebas (Protista): Distribution in Water Bodies of Zhytomyr and Volyn Polissia (Ukraine) and Possible Ecological Significance. Vestnik zoologii. **48**: 547-552. https://doi.org/10.2478/vzoo-2014-0065

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

Rao M.V.N., Chatterjee S. 1974. Regulation of nuclear DNA synthesis in Amoeba interspecific hybrids. Exp. Cell. Res. **88**: 371-374.

Rogerson A. 2018. A Half-century of Research on Free-living Amoebae (1965–2017): Review of Biogeographic, Ecological and Physiological Studies. Acta Protozool. **7**: 1-28. https://doi.org/10.4467/16890027AP.18.001.8395

Saitou N., Nei M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4**: 406-425. https://doi.org/10.1093/oxfordjournals.molbev.a040454

Schaeffer A.A. 1916. Notes on the specific and other characters of *Amoeba proteus* Pallas (Leidy), *A. discoides* spec. nov. and *A. dubia* spec. nov. Arch. Protistenk. **37**: 204-228.

Schaeffer A.A. 1926. Taxonomy of the amoebas. Pap. Dept. Mar. Biol., Carnegie Inst. of Washington. **24**: 1-116.

Spear B.B., Prescott D.M. 1980. Nuclear DNA in normal and refed *Amoeba proteus*. Exp. cell Res. **130**: 387-412.

Tamura K., Nei M., Kumar S. 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proc. Natl. Acad. Sci. USA**. 101**: 11030-11035. https://doi.org/10.1073/pnas.0404206101

Taylor M. 1924. *Amoeba proteus*: Some New Observations on its Nucleus, Life-history, and Culture. J. Cell. Sci. **s2-69**: 119-149. https://doi.org/10.1242/jcs.s2-69.273.119

Taylor M. 1925. *Amoeba proteus*: some new observation on its nucleus, life-history, and culture. Quart. J. Microsc. Sci. **69**: 119-143.

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