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MORPHOLOGY AND PHYLOGENY OF NAKED AMOEBAE OF THE GENERA *THECAMOEBA* AND *STENAMOEBA* (THECAMOEBIDAE; AMOEBOZOA) FROM NATURAL BIOTOPES OF UKRAINE

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Background. The family Thecamoebidae includes the genera *Thecamoeba*, *Sappinia*, *Stenamoeba*, *Stratorugosa*, and *Thecochaos*, which are widely distributed in water bodies and terrestrial biotopes. Species identification of naked amoebae is impossible without a combination of morphological and genetic methods.

Material and Methods. Species were determined using modern light microscopy; genomic DNA was isolated using the guanidine isothiocyanate method, and phylogenetic analysis was performed using the MEGA 10.0 software application.

Results and Discussion. Based on morphological features and 18S rRNA gene sequences, we identified nine representatives of the genus *Thecamoeba* (*Thecamoeba striata* (OQ134483, OQ134482), *Thecamoeba quadrilineata* (ON398268, ON398269), *Thecamoeba sphaeronucleolus*, *Thecamoeba verrucosa*, *Thecamoeba terricola*, *Thecamoeba similis* (OL604178, OL604177), *Thecamoeba orbis*, *Thecamoeba hilla*, *Thecamoeba* sp. (MZ079371)) and two species of the genus *Stenamoeba* (*Stenamoeba stenopodia* (OP419588, OP375108), *Stenamoeba* sp.). Morphological characters are given for all species, taking into account molecular data. In the molecular cluster Discosea, Thecamoebidae is a monophyletic taxon that unites groups of amoebas from the genera *Thecamoeba*, *Sappinia*, *Thecochaos*, *Stenamoeba*. Usually, *Thecamoeba* and *Sappinia* together form a sister group to the genus *Thecochaos*.

Conclusions. *Thecamoeba*-like amoebae on the phylogenetic tree form three groups: striate (*Thecamoeba striata* (OQ134483, OQ134482, OR994897, OL423100) + *Thecamoeba vumurta* (OL423099)), rugose (*Thecamoeba similis* (OL604178, OL604177, PQ451966, AY294145) + *Thecamoeba foliovenanda* (MN544291, MN544293)), and striate (*Thecamoeba astrologa* (MW817155) + *Thecamoeba cosmophorea* (MH628647) + *Thecamoeba quadrilineata* (ON398269, ON398268) + *Thecamoeba* sp. (MZ079371)) and rugose (*Thecamoeba aesculea* (JN247436)) morphotypes. The group of amoebae of the genus *Stenamoeba* (*Stenamoeba stenopodia* (OP375108+OP419588), *Stenamoeba* sp. (PQ431550), *Stenamoeba dejonckheerei* (MT386405), *Stenamoeba berchidia* (KF547922), *Stenamoeba polymorpha* (KU955320)), which belong to the lingulate morphotype, have a basal position in the phylogenetic tree.

Keywords: naked amoebae, morphotype, *Thecamoeba*, *Stenamoeba*, phylogeny

INTRODUCTION

Naked amoebae are eukaryotic unicellular organisms that form lobed pseudopodia and subpseudopodia of various shapes during movement, with a smooth, uniform flow of the cell cytoplasm. Despite the small species diversity, these protists are quite widespread and numerous in marine and fresh water bodies, and terrestrial ecosystems. Naked amoebae are aquatic organisms; even soil inhabitants interact with capillary water which fills the space between soil particles. Among freshwater representatives, there are exclusively soil or obligate freshwater species, and species that occur in both types of ecotopes (Page & Siemensma, 1991). In soil ecosystems, Amoebozoa are a significant component of the carbon, nitrogen, and phosphorus cycle. Under favorable environmental conditions, naked amoebae multiply rapidly and become one of the dominant groups of protists in natural biocenoses, regulating the abundance of bacteria, fungi, algae, and other microorganisms (Anderson, 2007; Risse-Buhl *et al.*, 2015; Geisen *et al.*, 2018). Naked amoebae feed on various microorganisms, small protists, including naked amoebae, and are an important component in the flows of substances and energy in natural biotopes. Most representatives are free-living, although parasitic species are also found, causing diseases in animals and humans (keratitis, amoebiasis, diarrhea, amoebic meningoencephalitis, etc.) (Moran *et al.*, 2022; Stanley, 2003). The ability to form resting cysts has been described for some species.

Today, about 300 truly described species of naked amoebae are known. The number of works that provide reliable lists of these protists is small, and there are few diagnostic morphological features. Working with amoebae is possible only in laboratory conditions and is very difficult – it is necessary to isolate the amoebae in culture, which makes the research long-term and laborious. Identification of amoebae species is possible only with the involvement of modern research methods, such as modern light microscopy and molecular genetics. The only way to obtain reliable information about the species composition of naked amoebae in various natural biotopes is to study species isolated from remote locations and compare them with species found in regional faunas.

Modern phylogenetic systems of protists reveal the degree of similarity of species by comparing gene sequences (18S rRNA, actin, tubulin, HSP90) and confirm the reasonability of distinguishing groups of organisms in morphological classifications. In our studies, we use the modern system of eukaryotes by S. Adl and co-authors (Adl *et al.*, 2019). Based on phylogenetic analysis, two clades, Tevosa and Discosea, are distinguished within Amoebozoa. Tevosa, in turn, includes the molecular clusters Tubulinea and Evosea (Cutosea (exclusively marine amoebae) + Variosea). Naked amoebae are included in the molecular clusters Tubulinea, Discosea, and Variosea.

Thecamoebidae Schaeffer, 1926 includes the genera *Thecamoeba* Fromentel, 1874, *Sappinia* Dangeard, 1896, *Stenamoeba* Smirnov, Nassonova, Chao et Cavalier-Smith, 2007, *Stratorugosa* Melton et Tekle, 2018, and *Thecochaos* Page, 1981. These genera have been studied to a various degree.

The genus *Thecamoeba* was established in 1874 by Fromentel (Fromentel, 1874). The first species of naked amoebae belonging to this genus were described in the late 19th and early 20th centuries (Carter, 1856; Greeff, 1866, 1891; Penard, 1902, 1905; Schaeffer, 1926; Lepsi, 1960). In 1971 and 1977, F. Page redescribed the species of the genus *Thecamoeba* on the basis of morphological characters and created laboratory cultures (Page, 1971, 1977).

The genus *Thecamoeba* includes 12 species that are supported by molecular data, which have a complex morphological and genetic structure and belong to the striate and rugose morphotypes. Representatives are characterized by a set of unique morphological features: a round, oval or elongated body shape; during locomotion, the amoeba does not form discrete pseudopodia and subpseudopodia; hyaline wrinkles or ridges are formed on the cell surface; differentiated uroid structures at the posterior end of the cell are absent; species differ in the specific structure of the nucleus, etc. (Page, 1988; Page & Siemensma, 1991). They have no floating forms and move in the water column in the form of wrinkled bubbles (balls), the latter resembling microscopic bent plastic bags, or “irregularly shaped pebbles” (Greeff, 1866).

The species were found in fresh and marine water bodies, soils, epiphytic and epilithic mosses and lichens (Patsyuk, 2023), thermal springs (Berrili *et al.*, 2021); they were also isolated from the intestines of the earthworm *Lumbricus terrestris* (Borovickova *et al.*, 2019), and as a host of the parasitic fungi *Acanlopage tetraceros* and *Cochlonema euryblastum* (Michel & Wylezich, 2005; Koehsler *et al.*, 2007; Michel *et al.*, 2014; 2015).

Today, identification of naked amoebae is possible only on the basis of morphological (modern light microscopy with the use of differential interference contrast) and molecular genetic data. In addition, data are available for some members of *Thecamoeba*, which were later redescribed and moved to other taxa of Amoebozoa (Cole *et al.*, 2010; Tekle *et al.*, 2016; Corsaro *et al.*, 2017).

The genus *Stenamoeba* was first established in 2007. Amoebae of this genus belong to the lingulate morphotype, have a flattened, elongated body shape, and do not form pseudopodia and subpseudopodia during movement. Species are known from water bodies, thermal springs, terrestrial biotopes, and from the internal organs of animals (Page, 1988; Murase & Frenzel, 2008; Dykova *et al.*, 2010; Dykova & Kostka, 2013; Geisen *et al.*, 2014; Peglar *et al.*, 2016; Borquez-Roman *et al.*, 2020). Today, the genus includes 8 species whose identification has been confirmed by molecular data (Dykova *et al.*, 2010; Geisen *et al.*, 2014; Peglar *et al.*, 2016; Borquez-Roman *et al.*, 2020).

The study of the morphological and genetic features of Thecamoebidae makes it possible to increase our understanding of the diversity and phylogeny of this group of naked amoebae and that of Amoebozoa as a whole, therefore the aim of this work was to study the morphology and phylogeny of naked amoebae of the genera *Thecamoeba* and *Stenamoeba*, which we isolated from natural biotopes.

MATERIALS AND METHODS

Sampling and identification of naked amoebae. Samples were taken from fresh and marine water bodies in Ukraine and other countries during 2014–2021 (**Table 1**). The samples included the surface layer of bottom soil and a small amount of the bottom water. Samples were collected in glass dishes of 500 mL and transported to the laboratory.

Table 1. **Sampling locations of naked amoebae**

No	Species of naked amoebae	Sampling points	Coordinates	Year of sampling
1	2	3	4	5
1.	*Thecamoeba striata*	a floodplain near Lemeshivka village, Kalynivka Raion, Vinnytsia Oblast	49°39′33.4″N 28°27′20.3″E	2017
		the Turiya River near Bakhiv village, Kovel Raion, Volyn Oblast	51°15′36.3″N 24°44′53.0″E	2014
		a floodplain near Dnipro city	48°29′32.0″N 34°51′22.8″E	2015–2016
		the Teteriv River near Zhytomyr city	50°14′33.9″N 28°39′06.2″E	2014
		the Kamenka River near Zhytomyr city	50°16′53.8″N 28°37′26.4″E	2014–2020
		Lake Synevyr, Zakarpattia Oblast	48°37′01.6″N 23°41′06.5″E	2018
		floodplain, Ivano-Frankivsk Oblast	48°51′01.6″N 24°49′16.0″E	2018
		the floodplain near Marianivka village, Kyiv Oblast	50°03′44.5″N 30°10′46.1″E	2015
		the Bug (Western Bug) near Kamianka-Buzka city, Lviv Oblast	50°06′51.2″N 24°21′06.5″E	2017–2018
		a floodplain near Velika Omeliana village, Rivne Oblast	50°36′14.4”N 26°05′10.8”E	2014
		the Dnipro River near Kherson city	46°37′56.6″N 32°37′49.5″E	2015
		the Sporka River near Nový Bor city, Czech Republic	50°45′19.1″N 14°32′54.9″E	2019
		the Warta River near Poznań city, Poland	52°24′38.3″N 16°56′30.6″E	2020
		Lake Geneva, Switzerland	46°12′25.2″N 6°09′31.4″E	2019
2.	*Thecamoeba quadrilineata*	the Teteriv River near Zhytomyr city	50°14′33.9″N 28°39′06.2″E	2014
		the Chobotivka River near Myropil village, Zhytomyr Oblast	50°06′24.5″N 27°41′51.1″E	2016
		the Dnipro River, Zaporizhzhia Oblast	47°50′31.2″N 35°05′26.6″E	2014
		the Opir River near Scole town, Lviv Oblast	49°02′08.7″N 23°31′15.4″E	2018
		the Inhulets River near Kherson city	46°42′40.8″N 32°47′50.9″E	2015
		the floodplain near Vöcklabruck city, Austria	47°59′51.5″N 13°39′01.1″E	2021
3.	*Thecamoeba sphaeronucleolus*	the Cherevakha River near Cherevakha village, Manevychi Raion, Volyn Oblast	51°16′56.0″N 25°26′24.4″E	2015
		the Psel River near Sumy city	50°54′23.7″N 34°48′14.3″E	2018
		the Prut River near Chernivtsi city	48°18′36.8″N 25°55′51.9″E	2016

Continued Table 1

1	2	3	4	5
4.	*Thecamoeba verrucosa*	the Uzh River near Korosten city, Zhytomyr Oblast	50°56′36.3″N 28°38′32.5″E	2015
		the river near Mala Vyska town, Kirovohrad Oblast	48°38′57.2″N 31°37′19.3″E	2017
		the Khorol River near Cherevki village, Myrhorod Raion, Poltava Oblast	50°07′51.2″N 33°43′09.4″E	2018
		the Dnipro River near Kherson city	46°37′33.5″N 32°37′15.0″E	2015
		a floodplain near Dresden city, Germany	51°03′15.8″N 13°43′54.5″E	2019
5.	*Thecamoeba terricola*	the Ubort River near Olevsk town, Zhytomyr Oblast	51°13′39.9″N 27°40′19.9″E 51°13′41.3″N 27°40′04.1″E	2017
		the Tereblia River near Synevyrska Polyana village, Zakarpattia Oblast	48°34′37.9″N 23°41′02.0″E	2018
		the Dnipro River near Kremenchuk city, Poltava Oblast	49°03′21.7″N 33°24′35.1″E	2014
		a lake near Schörfling am Attersee village, Austria	47°56′29.3″N 13°35′39.4″E	2021
6.	*Thecamoeba similis*	the Dniester River near Dubivtsi village, Ivano-Frankivsk Oblast	49°04′40.8″N 24°46′02.3″E	2018
		the Ager River, Vöcklabruck city, Austria	48°00′01.8″N 13°39′57.6″E	2021
7.	*Thecamoeba* sp.	a swamp near Kropyvnytskyi city	48°28′57.9″N 32°13′50.7″E	2017
		the Chorobra River near Zarichchia village, Korsun-Shevchenkivskyi Raion, Cherkasy Oblast	49°24′39.8″N 31°02′55.6″E	2014
8.	*Stenamoeba stenopodia*	the Shpikivka River near Shpikiv village, Tulchyn Raion, Vinnytsia Oblast	48°46′20.3″N 28°36′52.8″E	2017
		the Rudka River near Sytnytsia village, Volyn Oblast	51°00′24.9″N 25°40′30.9″E	2014
		the Gnilopyat River near Zhytomyr city	50°02′54.6″N 28°27′47.2″E	2017
		the Huiva River near Andrushivka village, Zhytomyr Oblast	50°01′35.3″N 28°59′41.0″E	2015
		the Southern Bug River near Mykolaiv city	46°59′10.9″N 31°58′38.8″E	2016
		the Dnipro River near Kremenchuk city, Poltava Oblast	49°03′58.2″N 33°23′41.1″E	2014
		the Sporka River near Nový Bor city, Czech Republic	50°45′19.1″N 14°32′54.9″E	2019
		a floodplain near Vöcklabruck city, Austria	47°59′51.5″N 13°39′01.1″E	2021
		the Warta River near Poznań city, Poland	52°24′38.3″N 16°56′30.6″E	2020

End of the Table 1

1	2	3	4	5
9.	*Thecamoeba orbis*	the Black Sea, Odesa Oblast	46°23′39.0″N 30°45′12.3″E	2019
	Thecamoeba hilla		46°23′31.3″N 30°45′12.3″E	
	Stenamoeba sp.		46°02′15.6″N 30°28′06.0″E	

Naked amoebae were propagated in laboratory conditions on non-nutrient agar-agar according to Page (Page, 1988; Page & Siemensma, 1991). The composition of the Prescott–James mineral medium included three basic solutions (each diluted in 100 mL of water).

Basic solution A	
$CaCl_2 \cdot 2H_2O$	0.433 g
KCl	0.162 g
Basic solution B	
K_2HPO_4	0.512 g
Basic solution C	
$MgSO_4 \cdot 7H_2O$	0.280 g

1 mL of stock solution was mixed with 997 mL of distilled water.

Species identification was carried out using a light microscope Axio Imager MI (Centre for collective usage of scientific equipment “Animalia” of I. I. Schmalhausen Institute of Zoology, NAS of Ukraine) with differential interferential contrast. The main morphological characters were the measurements of locomotor forms (we measured the cell width (B) as the distance perpendicular to the direction of movement at the widest part of the cell, and the cell length (L) as the distance between the front and rear ends of the moving cell; the ratio of the length to the width of the cell is L/B) (**Fig. 1**) (Page, 1988; Page & Siemensma, 1991), diameter of nucleus, and morphotype.

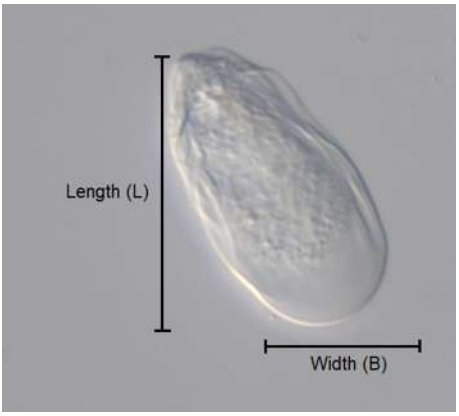

Fig. 1. Measurements of locomotor form of naked amoebaea

Isolation of DNA. Before DNA isolation, amoebae were incubated on starvation agar to eliminate eukaryotic contaminants. Genomic DNA was isolated using the guanidine isothiocyanate method (Maniatis *et al*., 1982). The 18S rRNA gene was 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 species. PCR was performed under the following conditions: initial denaturation at 95 °C for 10 min, 40 cycles (94 °C for 30 s, 50 °C for 60 s, 72 °C for 2 min 30 s), and final elongation for 10 minutes. Amplicons were purified using the CleanUp mini Purification Kit (Eurogene) and sequenced using the ABI-Prism Big Dye Terminator Cycle Sequencing Kit. The obtained DNA sequences were compared with GenBank data using the BLAST (NCBI) program (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The obtained sequences were automatically aligned using the Muscle algorithm implemented in the MEGA 10.0 software application. Based on the alignment, which consists of 18S rRNA sequences for representatives of the main groups Discosea and Tubulinea, a phylogenetic analysis was performed using the MEGA 10.0 software application. For phylogenetic analysis, both the sequences obtained by us and the sequences of other species of naked amoebae, which are available in the GenBank database, were used (**Table 2**).

Table 2. **DNA sequences used for phylogenetic analysis**

No	Species of naked amoebae	DNA sequence in GenBank
1	2	3
1.	*Thecamoeba foliovenanda*	MN544293
		MN544291
2.	*Thecamoeba similis*	OL604178
		OL604177
		PQ451966
		AY294145
3.	*Thecamoeba quadrilineata*	ON398269
		ON398268
4.	*Thecamoeba striata*	OQ134483
		OQ134482
		OR994897
		OL423100
5.	*Thecamoeba vumurta*	OL423099
6.	*Thecamoeba* sp.	MZ079371
7.	*Thecamoeba aesculea*	JN247436
8.	*Thecamoeba astrologa*	MW817155
9.	*Thecamoeba cosmophorea*	MH628647
10.	*Sappinia pedata*	EU004593
		PP647604
11.	*Sappinia diploidea*	DQ122380
		KP277502

Continued Table 2

1	2	3
12.	*Sappinia* sp.	KR069108
13.	*Sappinia platani*	EU881943
14.	*Thecochaos fibrillosum*	MW261337
15.	*Stenamoeba stenopodia*	OP375108
		OP419588
16.	*Stenamoeba* sp.	JQ271721
		PQ431550
17.	*Stenamoeba polymorpha*	KU955320
		PQ345829
18.	*Stenamoeba limacina*	GU810183
19.	*Stenamoeba berchidia*	KF547922
20.	*Stenamoeba dejonckheerei*	MT386405
21.	*Acanthamoeba castellanii*	M13435
22.	*Acanthamoeba lenticulata*	U94730
23.	*Acanthamoeba* sp.	MZ079366
		OK649261
24.	*Acanthamoeba polyphaga*	ON908497
		ON908496
25.	*Balamuthia mandrillaris*	AF019071
26.	*Pellita digitata*	KF705036
27.	*Pellita catalonica*	KF705039
28.	*Endostelium amerosporum*	KF705035
29.	*Endostelium crystalliferum*	KF705034
30.	*Paradermamoeba levis*	JN247435
31.	*Paradermamoeba valamo*	MW293873
32.	*Dermamoeba algensis*	AY294148
33.	*Mayorella* sp.	OP729930
34.	*Mayorella vespertilioides*	OP739500

End of the Table 2

1	2	3
35.	*Mayorella gemmifera*	EU719190
36.	*Ripella platypodia*	MF683472
37.	*Ripella tribonemae*	MF683608
38.	*Clydonella* sp.	AY183892
39.	*Vannella lata*	OL305064
		OL305063
40.	*Vannella aberdonica*	AY121853
41.	*Paramoeba eilhardi*	JN202441
42.	*Korotnevella stella*	ON398266
		ON398267
43.	*Vexillifera bacillipedes*	OK649262
		JQ271773
44.	*Vexillifera* sp.	LO049074
45.	*Saccamoeba limax*	OQ520144
		OP894078
46.	*Saccamoeba lacustris*	JN112797
47.	*Hartmannella cantabrigiensis*	AY294147
48.	*Amoeba proteus*	AJ314604
		ON907618
49.	*Chaos nobile*	AJ314606
50.	*Chaos carolinense*	AJ314607
51.	*Rhizamoeba saxonica*	EU719197
52.	*Leptomyxa reticulata*	AF293898
53.	*Echinamoeba exundans*	EU377601

Phylogenetic analysis was performed using the maximum likelihood method according to the Neighbor–Joining algorithm in the MEGA 10.0 software application. The optimal tree (GTR + I + G nucleotide substitution model) is shown. The constructed phylogeny was tested using bootstrap analysis (1000) (Kumar *et al.*, 2018).

RESULTS AND DISCUSSION

In total, we isolated nine species of naked amoebae of the genus *Thecamoeba* and two species of the genus *Stenamoeba* from natural biotopes. All identified species belong to the Amoebozoa Luhe, 1913, molecular cluster Discosea Cavalier-Smith *et al.*, 2004, family Thecamoebidae Schaeffer, 1926. For five species, 18S rRNA gene sequences were isolated, and species identification was confirmed.

Thecamoeba striata Penard, 1890

The species belongs to the striate morphotype. Cell length 30–74 µm, cell width 18–38 µm, L/B ratio is 1.2–3.2. Diameter of nucleus is 6.2–10.0 µm. The sequences of the studied DNA samples in Genbank of the species *Thecamoeba striata* isolate THS19 and *Thecamoeba striata* isolate THS20 are OQ134482 and OQ134483, respectively.

Thecamoeba quadrilineata Carter, 1856

Amoebae of the striate morphotype. Cell length 38–80 µm, cell width – 20-38 µm, L/B ratio – 1.2–2.0. Single nucleus with a diameter of 8.0–11.5 µm. Sequences of the studied DNA samples in Genbank of the species *Thecamoeba quadrilineata* isolate THQD2 and *Thecamoeba quadrilineata* isolate THQA1 are ON398269 and ON398268, respectively.

Thecamoeba sphaeronucleolus Greef, 1891

Amoebae of the rugose morphotype. Cell length is 95–150 µm, cell width is 65–95 µm, L/B ratio is 1.3–2.0. The nucleus is 14–20 µm in diameter. In cultures, the presence of two-layer cysts (35–50 µm) with a well-defined internal granuloplasm was observed. Molecular data are missing in GenBank.

Thecamoeba verrucosa Ehrenberg, 1838

The species belongs to the rugose morphotype. Cell length is 110–180 µm, cell width is 70–90 µm, L/B ratio is 1.5–2.5. The single nucleus is 12–18 µm in diameter. In cultures, the amoeba formed cysts with a diameter of up to 25 µm. Molecular data are missing in GenBank.

Thecamoeba terricola Greeff,1866

Amoebae of the rugose morphotype. Cell length is 115–185 µm, cell width – 85–110 µm, L/B ratio is 1.4–1.8. The nucleus is elliptical in shape, with a diameter of 20–55 µm. Molecular data are missing in GenBank.

Thecamoeba similis Greeff, 1891

Amoebae of the striate morphotype. Cell length is 45–80 µm, cell width – 42–64 µm, L/B ratio is 0.9–1.8. The nucleus is round in shape, with a diameter of 8.2–12.8 µm. The sequences of the studied DNA samples in GenBank are *Thecamoeba similis* isolate Prut river and *Thecamoeba similis* isolate Baggersee Innsbruck (Baggersee Rossau) – OL604177 and OL604178, respectively.

Thecamoeba orbis Schaeffer, 1926

The amoeba belongs to the striate morphotype. Cell length is 12–22 µm, width is 10–18 µm, L/B ratio is 0.9–1.0. The nucleus is round in shape with a single central spherical nucleolus. The diameter of the nucleus is 3.1–4.5 µm. Molecular data are missing in GenBank.

Thecamoeba hilla Schaeffer, 1926

The amoeba belongs to the striate morphotype. Cell length is 36–74 µm, cell width is 30–46 µm, L/B ratio is 1.2–1.6. In the cytoplasm of the cell there is one elongated nucleus, the diameter of which is 7.0–7.8 µm. Molecular data are missing in GenBank.

Thecamoeba sp.

The amoeba belongs to the rugose morphotype. Cell length is 48–75 µm, cell width is 38–64 µm, L/B ratio is 1.2–1.8. Single nucleus, diameter 7.3–11.5 µm. The sequence of the studied DNA sample in GenBank of the species *Thecamoeba* sp. strain THS203 is MZ079371.

Stenamoeba stenopodia Page, 1969

The species belongs to the lingulate morphotype. The length of the locomotor form is 16–26 µm, the width is 6–8 µm, L/B ratio is 2.0–2.5. The diameter of the nucleus is 2.0–2.4 µm. The sequences of the studied DNA samples in GenBank of the species *Stenamoeba stenopodia* isolate UKSS7 and *Stenamoeba stenopodia* isolate POLSS7 are OP375108 and OP419588, respectively.

Stenamoeba sp.

The species belongs to the lingulate morphotype. Cell length is 18–25 µm, cell width – 6.0–6.8 µm, L/B ratio is 1.2–1.4. Diameter of nucleus is 1.4–2.0 µm. Molecular data are missing in GenBank.

In our previous studies, the identification of more than 20 species of naked amoebae was confirmed based on the sequenced 18S rRNA gene, and the results of phylogenetic analysis proved that the freshwater fauna of naked amoebae originated from the marine one, and the terrestrial one from the freshwater one (Patsyuk, 2023). The species of naked amoebae of the family Thecamoebidae are relatively easily cultivated in the laboratory, and we isolated a significant number of DNA sequences of these species (thus replenishing the GenBank database). We analyzed how the species of naked amoebae of the family Thecamoebidae are grouped on a common phylogenetic tree (**Fig. 3**) and a tree that includes the groups Discosea and Tubulinea (**Fig. 2**), thereby confirming the correctness of our research.

Thecamoeba striata (OQ134483) has one of the shortest sequences of the 18S rRNA gene (1620 nucleotides) among the representatives of the genera *Thecamoeba* and *Stenamoeba*, which we isolated during our own research, while the longest is found for the species *Thecamoeba similis* (OL604177) (1870 nucleotides). The average value of sequence polymorphism within one species of amoeba varies from 1.29 % to 5 %. No species of amoeba was represented by identical sequences of the 18S rRNA gene. Polymorphism of sequences within a species can arise due to point nucleotide substitutions, insertions, deletions. The value of sequence divergence was 1.34 % within *Thecamoeba similis*, 1.62 % within *Thecamoeba striata*. Sequence divergence ranged from 5.5 % to 17 % between different species of the genus *Thecamoeba*, from 1 % to 10 % between those of *Stenamoeba*.

The naked amoebae of the family Thecamoebidae form one of the major groups on the phylogenetic tree of eukaryotes, in particular Amoebozoa (**Fig. 2**, **3**). Phylogenetic analysis shows that within Discosea, Thecamoebidae is a monophyletic taxon that unites the genera *Thecamoeba*, *Sappinia*, *Thecochaos*, and *Stenamoeba*. All four genera also form separate phylogenetic groups. *Thecamoeba* species group (*Thecamoeba foliovenanda* (MN544293) + (((*Thecamoeba similis* (OL604178) + *Thecamoeba similis* (OL604177)) + (*Thecamoeba quadrilineata* (ON398268) + *Thecamoeba quadrilineata* (ON398269))))) + *Sappinia* (((*Sappinia* pedata (EU004593) + *Sappinia pedata* (PP647604)) + *Sappinia diploidea* (KP277502)) + *Sappinia platani* (EU881943)) is sister to the genus *Thecochaos*, which includes only one species, *Thecochaos fibrillosum*. The group of species of the genus *Stenamoeba* (((*Stenamoeba stenopodia* (OP375108) + *Stenamoeba stenopodia* (OP419588)) + *Stenamoeba* sp. (JQ271721)) + (((*Stenamoeba polymorpha* (PQ345829) + *Stenamoeba polymorpha* (KU955320)) + *Stenamoeba limacina* (GU810183))) occupies a basal position in relation to the genera *Thecamoeba*, *Sappinia* and *Thecochaos*. In addition to Thecamoebidae, the Discosea molecular cluster also includes other heterogeneous groups of naked amoebae. The Acanthopodida

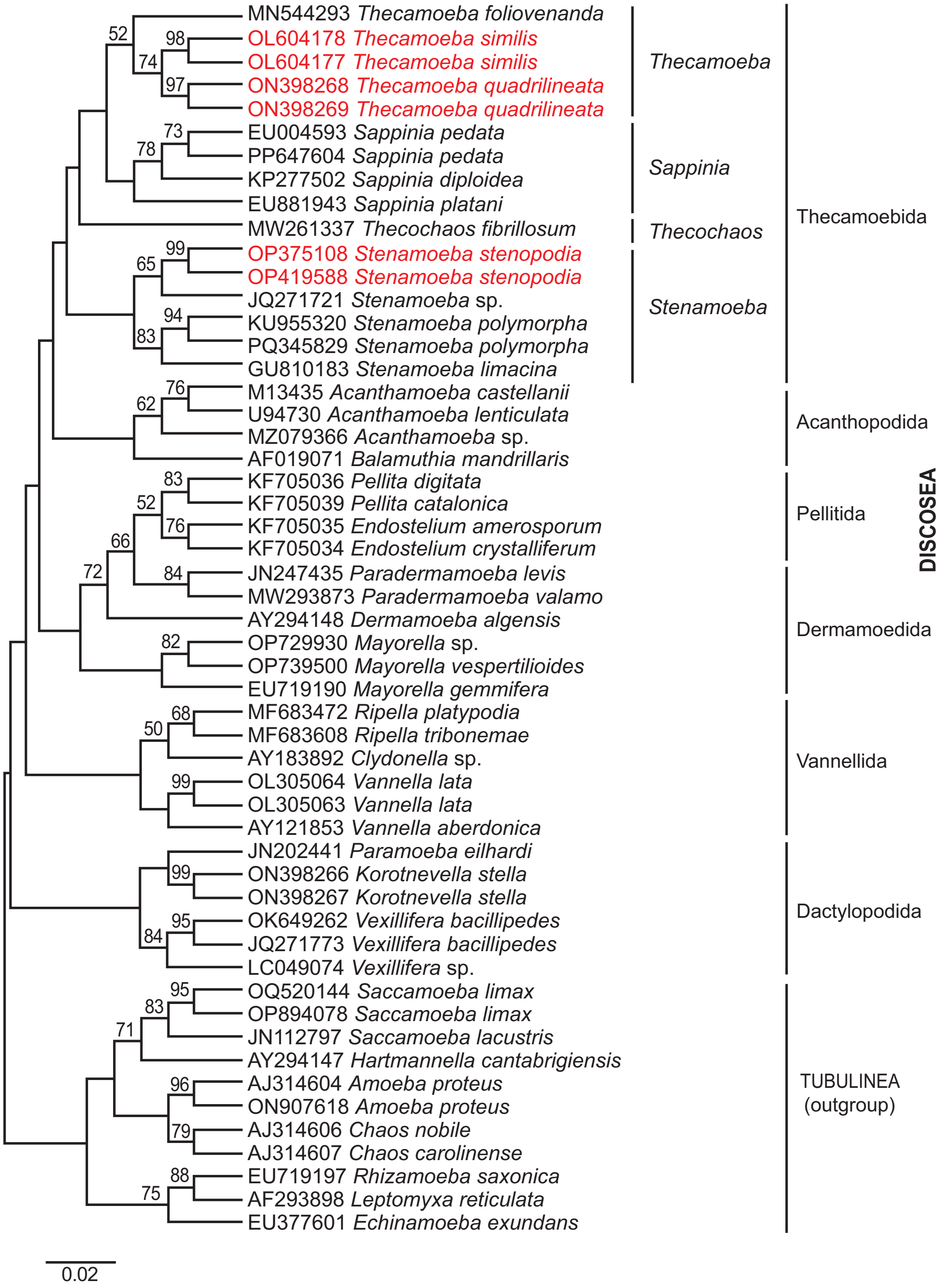

Fig. 2. Phylogenetic tree of naked amoebae, based on 18S rRNA gene sequences, showing the position of the family Thecamoebidae within the Discosea molecular cluster. The scale bar indicates the equivalence of distances between sequences

group includes amoebae of the acanthopodial morphotype: *Acanthamoeba castellanii* (M13435) + *Acanthamoeba lenticulate* (U94730) + *Acanthamoeba* sp. (MZ079366) + *Balamuthia mandrillaris* (AF019071). The group Pellitida includes amoeba of the flamellian morphotype: *Pellita digitata* (KF705036) + *Pellita catalonica* (KF705039) + *Endostelium amerosporum* (KF705035) + *Endostelium crystalliferum* (KF705034). Dermamoebida unites the species of lanceolate amoeba (*Paradermamoeba levis* (JN247435) + *Paradermamoeba valamo* (MW293873) + *Dermamoeba algensis* (AY294148)) and mayorellian (*Mayorella* sp. (OP729930) + *Mayorella vespertilioides* (OP739500) + *Mayorella gemmifera* (EU719190)) morphotypes. Fan-shaped amoebae form the Vannellida group: *Ripella platypodia* (MF683472) + *Ripella tribonemae* (MF683608) + *Clydonella* sp. (AY183892) + *Vannella lata* (OL305064) + *Vannella lata* (OL305063) + *Vannella aberdonica* (AY121853). Dactylopodida is represented by *Paramoeba eilharia* (JN202441) + *Korotnevella stella* (ON398266) + *Korotnevella stella* (ON398267) + *Vexillifera bacillipedes* (OK649262) + *Vexillifera bacillipedes* (JQ271773) + *Vexillifera* sp. (LC049074), which belong to the dactylopodial morphotype. The same pattern is shown by the phylogenetic trees of naked amoebae, devoted to the phylogeny of Amoebozoa, by other authors (Cavalier-Smith *et al.*, 2016; Tekle *et al.*, 2016).

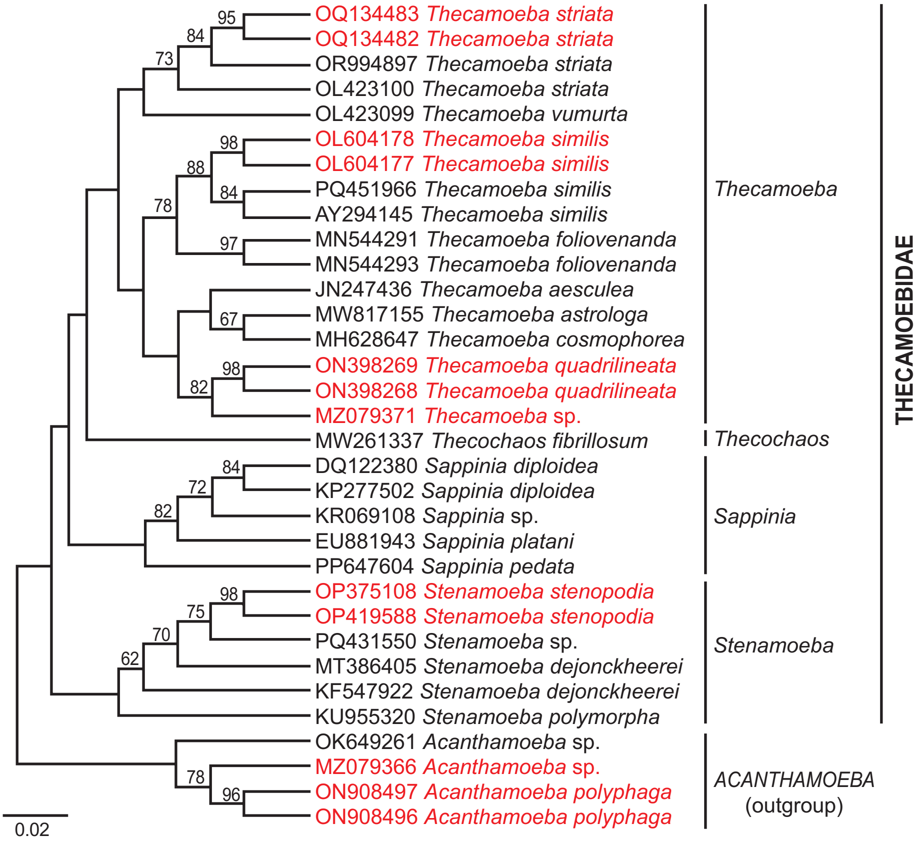

Fig. 3. Phylogenetic tree of naked amoebae, based on 18S rRNA gene sequences of members of the family Thecamoebidae (original sequences are highlighted in red). The scale bar indicates equivalence of distance between sequences

The genus *Thecamoeba* forms three groups on the phylogenetic tree (**Fig. 3**). The first group includes the species *Thecamoeba striata* (OQ134483, OQ134482, OR994897, OL423100) and *Thecamoeba vumurta* (OL423099), which belong to the striate morphotype. Amoebae of this morphotype have an oval body shape with several almost parallel dorsal ridges. The species have a single nucleus, with nucleolar material located peripherally in it. The second group unites thecamoebae of the rugose morphotype, which have an elongated cell shape with numerous dorsal ridges and folds. Lenticular nucleoli are located on the periphery of the nucleus. These are the following species: *Thecamoeba similis* (OL604178, OL604177, PQ451966, AY294145) and *Thecamoeba foliovenanda* (MN544291, MN544293). The third group includes amoebae of the striate (*Thecamoeba astrologa* (MW817155) + *Thecamoeba cosmophorea* (MH628647) + *Thecamoeba quadrilineata* (ON398269, ON398268) + *Thecamoeba* sp. (MZ079371)) and rugose (*Thecamoeba aesculea* (JN247436)) morphotypes. In these species, the nucleus is of the vesicular type, the nucleoli are located either in the center or on the periphery of the nucleus. This division refutes the previously existing assumption that the species are grouped based on morphological characters of naked amoebae (Bovee & Jahn, 1966; Page, 1977). By the 18S rRNA gene sequences, the species of naked amoebae are well distinguished from each other.

Stenamoeba group of amoebas includes 18S rRNA gene sequences of the following species: *Stenamoeba stenopodia* (OP375108 + OP419588) + *Stenamoeba* sp. (PQ431550) + *Stenamoeba dejonckheerei* (MT386405) + *Stenamoeba berchidia* (KF547922) + *Stenamoeba polymorpha* (KU955320). These species belong to the lingulate morphotype (elongated cell shape, longitudinal hyaline folds/ridges absent) and occupy a basal position in the Thecamoebidae branch.

CONCLUSION

The family Thecamoebidae includes species of the genera *Thecamoeba*, *Thecochaos*, *Stenamoeba*, *Sappinia*, and *Stratorugosa*. Identification of species is possible on the basis of morphological characters and gene sequencing. There are species that are similar in morphological characters, but clearly differ in molecular data. We have isolated the following species from natural biotopes: *Thecamoeba sphaeronucleolus*, *Thecamoeba verrucosa*, *Thecamoeba terricola*, *Thecamoeba orbis*, *Thecamoeba hilla*, *Thecamoeba* sp. (MZ079371), *Thecamoeba striata* (OQ134483, OQ134482), *Thecamoeba quadrilineata* (ON398268, ON398269), *Thecamoeba similis* (OL604178, OL604177), *Stenamoeba stenopodia* (OP419588, OP375108), *Stenamoeba* sp. Phylogenetic analysis shows that the family Thecamoebidae is a monophyletic taxon, *Thecochaos fibrillosum* is sister to the genera *Thecamoeba* and *Sappinia*, which form a common group in the tree. *Stenamoeba*-like naked amoebae occupy a basal position on the branch of Thecamoebidae. Overall, the Discosea molecular cluster includes naked amoebae species from the groups Thecamoebida, Acanthopodida, Pellitida, Dermamoebida, Vannellida, and Dactylopodida.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: the author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Animal Rights: this article does not contain any studies with animals.

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МОРФОЛОГІЯ І ФІЛОГЕНІЯ ГОЛИХ АМЕБ РОДІВ *THECAMOEBA* ТА *STENAMOEBA* (THECAMOEBIDAE; AMOEBOZOA) З ПРИРОДНИХ БІОТОПІВ УКРАЇНИ

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Вступ. Родина Thecamoebadae включає роди *Thecamoeba*, *Sappinia*, *Stenamoeba*, *Stratorugosa*, *Thecochaos*, які поширені у водоймах і наземних біотопах. Видова ідентифікація голих амеб неможлива без поєднання морфологічних і генетичних методів.

Матеріал і методи. Види визначали за допомогою сучасної світлової мікроскопії, геномну ДНК виділено гуанідинізотіоціанатним методом, філогенетичний аналіз проведено у програмі MEGA 10.0.

Результати й обговорення. На основі морфологічних ознак і послідовностей гена 18S рРНК ми ідентифікували 9 представників роду *Thecamoeba* (*Thecamoeba striata* Penard, 1890 (OQ134483, OQ134482), *Thecamoeba quadrilineata* Carter, 1856 (ON398268, ON398269), *Thecamoeba sphaeronucleolus* Greef, 1891, *Thecamoeba verrucosa* Ehrenberg, 1838, *Thecamoeba terricola* Greeff,1866, *Thecamoeba similis* Greeff, 1891 (OL604178, OL604177), *Thecamoeba orbis* Schaeffer, 1926, *Thecamoeba hilla* Schaeffer, 1926, *Thecamoeba* sp. (MZ079371)) та 2 види роду *Stenamoeba* (*Stenamoeba stenopodia* Page, 1969 (OP419588, OP375108), *Stenamoeba* sp.). Для усіх видів наведено морфологічні ознаки з урахуванням молекулярних даних. У молекулярному кластері Discosea родина Thecamoebidae є монофілетичним таксоном і об'єднує групи амеб з родів *Thecamoeba*, *Sappinia*, *Thecachaos*, *Stenamoeba*. Зазвичай, *Thecamoeba* й *Sappinia* утворюють спільну групу, яка є сестринською щодо роду *Thecochaos*.

Висновки. *Thecamoeba*-подібні амеби на філогенетичному дереві утворюють три групи: стріатного (*Thecamoeba striata* (OQ134483, OQ134482, OR994897, OL423100) + *Thecamoeba vumurta* (OL423099)), ругозного (*Thecamoeba similis* (OL604178, OL604177, PQ451966, AY294145) + *Thecamoeba foliovenanda* (MN544291, MN544293)) та стріатного (*Thecamoeba astrologa* (MW817155) + *Thecamoeba cosmophorea* (MH628647) + *Thecamoeba quadrilineata* (ON398269, ON398268) + *Thecamoeba* sp. (MZ079371)) й ругозного (*Thecamoeba aesculea* (JN247436)) морфотипів. Група амеб роду *Stenamoeba* (*Stenamoeba stenopodia* (OP375108+OP419588), *Stenamoeba* sp. (PQ431550), *Stenamoeba dejonckheerei* (MT386405), *Stenamoeba berchidia* (KF547922), *Stenamoeba polymorpha* (KU955320)), які належать до язикоподібного морфотипу, на філогенетичному дереві займає базальне положення.

Ключові слова: голі амеби, морфотип, *Thecamoeba*, *Stenamoeba*, філогенія

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