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REPRESENTATIVES OF THE GENUS *COCHLIOPODIUM* HERTWIG & LESSER, 1874 (AMOEBOZOA; DISCOSEA) IN THE NATURAL BIOTOPES OF UKRAINE

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Abstract. Amoebae species of the genus *Cochliopodium* are widespread in aquatic habitats and soils and exhibit limited morphological differentiation. Thus, the number of described species remains low. They are difficult to maintain in culture due to their nutritional and reproductive peculiarities. Today, accurate species identification requires modern light microscopy and DNA sequencing. GenBank contains a small number of 18S rRNA gene sequences of *Cochliopodium*. The aim of our study was to isolate species of naked amoebae from water bodies in Ukraine, identify them by morphological characteristics and 18S rRNA genome, and determine their phylogenetic position within Amoebozoa. From the waters of Ukraine, we identified the marine species *Cochliopodium gulosum* and the freshwater species *Cochliopodium actinophorum* (MZ079367), *Cochliopodium minus* (OK649264), and *Cochliopodium* sp. (MZ079368). They share morphological characteristics such as the locomotor form, the presence of extracellular integumentary structures of the tectum type, and a vesicular nucleus. In our analysis, naked amoebae belonging to the molecular clusters Tubulinea and Discosea form a clade within Amoebozoa. Within Tubulinea we recover species from the order Euamoebida, while within Discosea we recover Dactylopodida, Vannellida, Thecamoebida, Dermamoebida, Acanthamoebida, and Himatismenida. Representatives of the genera *Cochliopodium* and *Gocevia* are grouped in Himatismenida. One group is formed by sequences of the species *Cochliopodium minus* (OK649264, JQ271675, JQ271674), another group contains *Cochliopodium actinophorum* and *Cochliopodium* sp. (MZ079367, JF298250, MZ079368), and the third group, *Cochliopodium kieliense* and *Cochliopodium larifeili* (KJ569725, KJ569727, JF298253).

INTRODUCTION

Naked amoebae are the most abundant group of protists. Questions regarding their diversity, biogeography, and phylogeny remain open and poorly understood. Within the Amoebozoa group, naked amoebae belong to three molecular clusters: Tubulinea Smirnov et al., 2005, Discosea Cavalier-Smith et al., 2004, and Variosea Cavalier-Smith et al., 2004. Tubulinea includes representatives of amoeboid protists, which during movement form tubular, cylindrical or subcylindrical outgrowths of the cytoplasm (pseudopodia), with a polyaxial cytoplasmic flow. The flattened cells can change shape from monopodial to polypodial during movement. Flagellated stages are absent in the development cycle. Variosea includes organisms that differ in a wide variety of morphological features. Many representatives have a complex life cycle, including amoeboid, flagellated and fruiting stages. Discosea includes flattened amoebae that form various types of subpseudopodia during locomotion, with a polyaxial cytoplasmic flow. Flagellated stages are absent in the development cycle (Adl et al. 2012). Among naked amoebae, there are both free-living species and parasitic ones (e.g. *Acanthamoeba*, *Entamoeba*, *Thecamoeba*) (Moran et al. 2022; Page and Siemensma 1991). In nature, a small number of species form resting stages (cysts) (Page 1988).

Today, more than 250 species of naked amoebae have been described based on morphological and genetic characteristics. Between 2009–2024, we isolated more than 57 species from various natural biotopes in Ukraine (aquatic and terrestrial). Species identification was confirmed for 23 species based on the 18S rRNA gene, most of which were representatives of the Discosea class, which have a complex of unique morphological characteristics. The species of the genus *Cochliopodium* Hertwig & Lesser, 1874 were the most numerous in fresh and marine waters and soils (Patsyuk 2022, 2024). These amoebae have

a lens-like, rounded cell shape, sometimes elongated in length or width and a thick cell cover (tectum) that consists of polysaccharides which cover the cell membrane only from the dorsal side. The cytoplasm consists of granuloplasm and hyaloplasm. The hyaloplasm forms a flattened peripheral rim surrounding the central mass of granuloplasm. The anterior edge of the hyaloplasm is usually smooth; in some species it forms finger-like thin subpseudopodia. The granuloplasm of most members of the genus contains crystals of various shapes, digestive and contractile vacuoles, and inclusions (Bark 1973; Page 1986). In the dorsal part of the granuloplasm, a vesicular nucleus is located (multinucleate species are known). The posterior end of the amoeba cell (uroid) is usually of the adhesive type. Some species are characterized by the presence of a floating form (spherical, bell-shaped with small cytoplasmic outgrowths). These amoebae feed on bacteria, flagellates, diatoms, etc. (Bark 1973; Schaeffer 1926). In culture, some species can form cysts (Dykova et al. 1998; Page 1986; Yamaoka et al. 1984). The genus *Cochliopodium* was first established by Hertwig and Lesser (1874). The first representatives (*Cochliopodium actinophorum* and *Cochliopodium bilimbosum*) were described by Auerbach (1856), *Cochliopodium digitata* was discovered by Greeff (1866), and *Cochliopodium vestita* by Archer (1871). Until 2002, the order Himatismenida was included in the subclass Testacelobosia; then, Rogerson and Patterson (2002) included Himatismenida in the naked lobed amoebae as a taxon of uncertain systematic position. In modern amoeboid protist systems based on 18S rRNA gene sequences, members of Himatismenida are grouped within the Discosea molecular cluster (Cavalier-Smith et al. 2004; Tekle et al. 2013, 2014, 2022).

There are currently about 20 valid species of the genus *Cochliopodium*. Species identification based on the 18S rRNA gene has been confirmed for 12 species. GenBank contains the following 18S rRNA gene sequences of *Cochliopodiums*: *Cochliopodium kieliense* – 3 sequences (KJ569727, KJ569726, KJ569725); *Cochliopodium minutoidum* – 4 sequences (KJ569722, KJ569721, KJ569720, KJ569718); *Cochliopodium minus* – 22 sequences (KJ569717, KJ569716, KJ569715, KJ569714, KJ569713, KJ569709, KJ569708, KJ569704, KJ569703, KJ569702, KJ569701, KJ569700, AU785056, JF298257, KU215598, KU215597, OK649264, JQ271675, JQ271674, JQ271673, JQ271672, JQ271671); *Cochliopodium massiliensis* – 1 sequence (MK734144); *Cochliopodium pentatrifurcatum* – 1 sequence (KC247747); C*ochliopodium gallicum* – 5 sequences (MT975613, MT975612, MT975611, MT975610, MT975609); *Cochliopodium plurinucleolum* – 1 sequence (KJ569732); *Cochliopodium larifeili* – 4 sequences (JF298256, JF298255, JF298254, JF298253); *Cochliopodium arabianum* – 3 sequences (KP244686, KP244685, KP244684); *Cochliopodium spiniferum* – 1 sequence (AY775130); *Cohliopodium actinophorum* – 5 sequences (MZ079367, JF298251, JF298250, JF298249, JF298248); *Cochliopodium bilimbosum* – 1 sequence (JF298252). There are sequences of yet undescribed species of the genus *Cochliopodium* sp. (KC747718, MZ079368, KP719191, PV031683, PV031682, AY785057, PV031681, KF938513).

The goal of our study was to isolate species of naked amoebae of the genus *Cochliopodium* from natural biotopes of Ukraine, identify these protists by morphological and genetic characteristics, supplement the GenBank database with nucleotide DNA sequences of various species, and based on such data, determine the position of the species on the phylogenetic tree of Amoebozoa.

MATERIALS AND METHODS

Sample collection and culture of naked amoebae

Field studies were conducted during 2009–2024. Samples from fresh and marine water bodies (Table 1) were plated in 100 mm diameter Petri dishes on 1.5% non-nutritive agar-agar prepared on Prescott-James (PJ) mineral medium (Page 1988).

The Prescott-James (PJ) mineral medium of the following three main solutions was prepared (each diluted with 100 mL water):

Main solution A	
$CaCl_2*2H_2O$	0.433 g
KCl	0.162 g
Main solution B	
K_2HPO_4	0.512 g
Main solution C	
$MgSO_{4*}7H_2O$	0.280 g

Naked amoebae were maintained in the laboratory at room temperature, under unregulated lighting. Each Petri dish was inspected every eight days for several months. The detected amoebae were transferred, one cell at a time, to Petri dishes with new medium, using a fine Pasteur pipette. Rice grains were added to each culture to maintain the amoebae strains.

Live amoebae were observed under a modern light microscope Zeiss Axio Imager MI (Germany) with differential interference contrast on temporary slides. Amoeba cells were measured using an ocular micrometer. Up to 20 cells of one amoeba species were measured from each culture. Species were determined by morphological features (cell size, nuclear size, cytoplasmic flow

Table 1. Location of species of naked amoebae of the genus *Cochliopodium* in fresh and marine waters of Ukraine.

No	Species	Sampling location	Coordinates	Year of sampling
1.	*Cochliopodium actinophorum*	Teteriv River near Zhytomyr city	50°14′33.9″N 28°39′06.2″E	2009 2013 2022
		Kamenka River near Zhytomyr city	50°16′53.8″N 28°37′26.4″E	2012 2023
		Dnipro River near Kherson city	46°37′33.5″N 32°37′15.0″E	2015
			46°37′56.6″N 32°37′49.5″E	2021
		Gnilopyat River near Zhytomyr city	50°02′54.6″N 28°27′47.2″E	2010 2024
		Dniester River in Ivano-Frankivsk Oblast	48°82′80.7″N 25°34′93.0″E	2018
		Inhul River near Mykolaiv city	46°58′40.9″N 32°00′00.1″E	2016
		Desna River in Chernihiv Oblast	51°92′55.1″N 33°23′98.″E	2018
2.	*Cochliopodium minus*	Stokhid River in Volyn Oblast	51°13′54.9″N 25°39′96.6″E	2014
3.	*Cochliopodium* sp.	Ikva River in Rivne Oblast	49°96′56.0″N 25°22′92.0″E	2017
4.	*Cochliopodium gulosum*	Black Sea, Odesa Oblast	46°23′39.0″N 30°45′12.3″E	2019
			46°23′31.3″N 30°45′12.3″E	2019
			46°02′15.6″N 30°28′06.0″E	2019

pattern, presence/absence of uroid structures, formation of a floating form, presence/absence of pseudopodia (subpseudopodia)).

Isolation of DNA

Genomic DNA was isolated by 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 polymerase chain reaction included the following steps: initial denaturation at 95 °C for 10 minutes, 40 cycles (94 °C for 30 seconds, 50 °C for 60 seconds, 72 °C for 2 minutes 30 seconds) and final elongation (72 °C 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 (performed bidirectional sequencing).

The obtained DNA sequences were compared with GenBank data using the BLAST (NCBI) program (https://blast.ncbi.nlm.nih.gov/Blast.cgii). The obtained sequences were automatically aligned (editing errors were removed) using the Muscle algorithm implemented in the MEGA 10.0 program. The divergence of sequences between species of the genus *Cochliopodium* was calculated using the Kimura 2-parameter (K2P) model in the MEGA 10.0.

Phylogenetic analysis was performed using the MEGA 10.0 program. For phylogenetic analysis, we used our own DNA sequences of *Cochliopodium minus* (OK649264), *Cochliopodium actinophorum* (MZ079367), *Cochliopodium* sp. (MZ079368), as well as DNA from the same species available in the GenBank database to confirm the accuracy of the study (Table 2).

Phylogenetic analysis was performed using the maximum likelihood method in MEGA 10.0. The optimal tree (GTR + I + G nucleotide substitution model) is shown. The constructed phylogeny was tested using bootstrap analysis (1000) (Darriba et al. 2012; Kumar et al. 2016; Saitou and Nei 1987; Tamura et al. 2004).

RESULTS

During research in fresh and marine waters of Ukraine, we isolated the following species of naked amoebae of the genus *Cochliopodium*:

Cochliopodium actinophorum Auerbach, 1856 (Figure 1a).

The locomotor form is round, oval with a narrow peripheral hyaline border. Subpseudopodia are absent.

Table 2. DNA sequences of various amoeboid protists that were used in phylogenetic analysis (sequences obtained in the current study are highlighted in bold with an asterisk).

No	Species of naked amoebae	DNA sequence number in GenBank
1.	*Amoeba proteus*	ON907618
2.	*Amoeba proteus*	AJ314604
3.	*Chaos nobile*	AJ314606
4.	*Chaos carolinense*	AJ314607
5.	*Saccamoeba limax*	EU869301
6.	*Saccamoeba limax*	OQ520144
7.	*Saccamoeba lacustris*	GQ221845
8.	*Vexillifera bacillipedes*	HQ687485
9.	*Vexillifera bacillipedes*	HQ687484
10.	*Vexillifera bacillipedes*	OK649262
11.	*Korotnevella stella*	AY686573
12.	*Vannella* sp.	MZ079372
13.	*Vannella lata*	OL305063
14.	*Vannella lata*	OL305064
15.	*Ripella* sp.	MZ079369
16.	*Stenamoeba stenopodia*	OP375108
17.	*Platyamoeba stenopodia*	AY294144
18.	*Thecamoeba* sp.	MZ079371
19.	*Thecamoeba quadrilineata*	DQ122381
20.	*Thecamoeba quadrilineata*	ON398268
21.	*Thecamoeba similis*	OL597873
22.	*Mayorella* sp.	OZ243098
23.	*Mayorella* sp.	OP729930
24.	*Acanthamoeba* sp.	MZ079366
25.	*Acanthamoeba* sp.	OK649261
26.	***Cochliopodium minus***	**OK649264***
27.	*Cochliopodium minus*	JQ271675
28.	*Cochliopodium minus*	JQ271674
29.	***Cochliopodium actinophorum***	**MZ079367***
30.	*Cochliopodium actinophorum*	JF298250
31.	***Cochliopodium* sp.**	**MZ079368***
32.	*Cochliopodium kieliense*	KJ569727
33.	*Cochliopodium kieliense*	KJ569725
34.	*Cochliopodium larifeili*	JF298253
35.	*Gocevia fonbrunei*	JF694281
36.	*Vahlkampfia* sp.	MT739329
37.	*Vahlkampfia avara*	PQ819802
38.	*Paravahlkampfia* sp.	PV873343

The central granuloplasm is shifted to the posterior end of the cell. Irregularities are formed on the lateral areas of the hyaloplasm. The granuloplasm contains granules and digestive vacuoles. An adhesive uroid is present at the posterior end of the cell. The cell length (L) is 28–50 µm, width (B) is 32–76 µm, the L/B ratio is 0.6–1.1. The nucleus is of the vesicular type with a diameter of 7.8–13.3 µm.

Sequence of the studied DNA sample in Genbank: MZ079367.

Biotopes: freshwater bodies.

Cochliopodium minus Page, 1976 (Figure 1b).

The locomotor form is round, sometimes fan shaped. Hyaloplasm surrounds granuloplasm. Part of the frontal hyaloplasm forms conical, finger-like subpseudopodia, which during movement gradually move to the back of the cell. Granuloplasm contains transparent vesicles, crystals, granules, and digestive vacuoles. The back end of the cell (uroid) is smooth.

The cell length is 18–74 µm, width is 18–75 µm, the L/B ratio is 0.8. The nucleus is of the vesicular type with a diameter of 7–9 µm.

Sequence of the studied DNA sample in Genbank: OK649264.

Biotopes: freshwater bodies.

Cochliopodium gulosum Schaeffer, 1926

Amoebae are oval and slightly elongated. The central mass of the granuloplasm is surrounded by a hyaline border. Digestive vacuoles and round granules are often observed in the granuloplasm. The anterior edge of the hyaline border forms short finger-like subpseudopodia. The uroid is absent. The posterior end of the cell is smooth.

The cell length is 92–100 µm, width is 58–80 µm, the L/B ratio is 1.0–1.1. The nucleus is of the vesicular type with a diameter of 9.5–14.5 µm.

DNA was not isolated for *Cochliopodium gulosum* because clonal cultures of this species could not be established, so the amoeba was studied using light microscopy by morphological characteristics.

Biotopes: marine water bodies.

Cochliopodium sp. (Figure 1c).

The locomotor form is oval, usually elongated in width. The central granuloplasm is fully surrounded by a hyaline border. The granuloplasm contains many small granules, digestive vacuoles, and crystal-like inclusions. The anterior edge of the hyaloplasm is smooth, it does not form subpseudopodia. The posterior end of the cell is smooth, uroid structures are absent. In old cultures, cysts (12–18 µm) are formed.

The cell length is 30–55 µm, width is 35–80 µm, the L/B ratio is 0.5–1.0. The nucleus is of the vesicular type with a diameter of 8.0–12.0 µm.

Sequence of the studied DNA sample in Genbank: MZ079368.

We sequenced three 18S rRNA gene sequences from three members of the genus *Cochliopodium*. The longest sequence is from *Cochliopodium actinophorum* (1637 bp), the shortest is from *Cochliopodium minus* (640 bp), and *Cochliopodium* sp. is 1164 bp. This difference in sequence length arises from the absence/presence of most variable regions. The G/C pair content in the obtained sequences is quite common: *Cochliopo-*

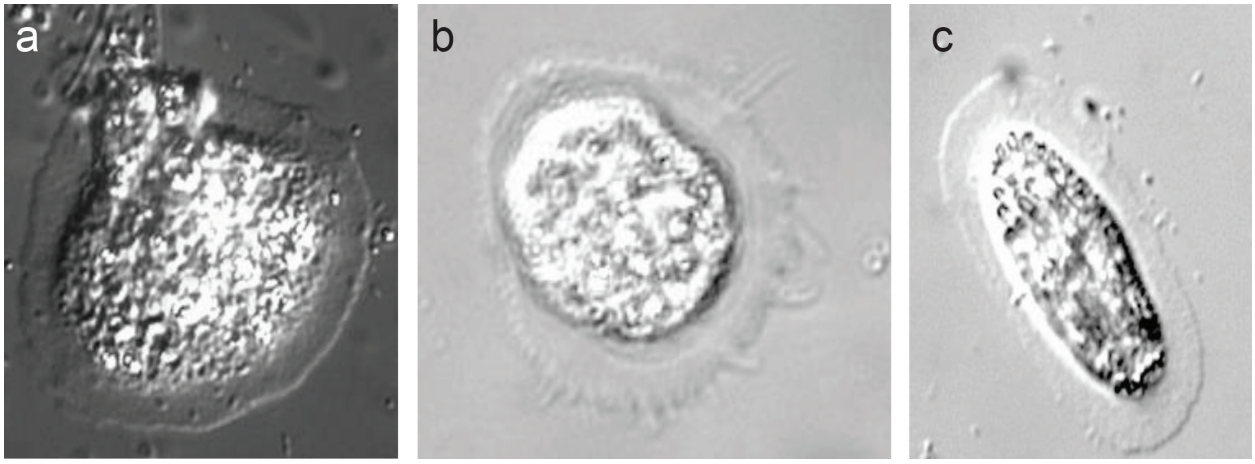

Figure 1. Species of naked amoebae of the genus *Cochliopodium* from fresh and marine waters of Ukraine: a – *Cochliopodium actinophorum*; b – *Cochliopodium minus*; c – *Cochliopodium* sp. (own photo; ×1240).

dium actinophorum (62.8%), *Cochliopodium minus* (74%), and *Cochliopodium* sp. (65.8%).

In the tree we constructed, the group Himatismenida Page, 1987 is formed by species of the genera *Cochliopodium* and *Gocevia* Valkanov, 1932. We attempted to analyze the phylogenetic relationships of species within the genus *Cochliopodium* and possible phylogenetic relationships of *Cochliopodium* representatives with other groups of naked amoebae. The cluster is formed by freshwater species (*Cochliopodium minus* (OK649264, JQ271675, JQ271674), *Cochliopodium actinophorum* (MZ079367, JF298250), *Cochliopodium* sp. (MZ079368), *Cochliopodium kieliense* (KJ569725, KJ569727), *Cochliopodium larifeili* (JF298253)) with a sufficiently high boost support (from 50 to 98%) (Figure 2).

The first group is formed by three sequences of the species (*Cochliopodium minus* (OK649264, JQ271675, JQ271674), which are grouped together with a sufficiently high bootstrap support (85–96%). The OK649264 sequence is sister to the JQ271675 + JQ271674 sequence group. The second group is formed by the *Cochliopodium actinophorum* (MZ079367 + JF298250) and *Cochliopodium* sp. (MZ079368) sequences (91–92%). The first and second groups of 18S rRNA gene sequences of different species of the genus *Cochliopodium* are sister to each other. The third group consists of two sequences of *Cochliopodium kieliense* (KJ569725 + KJ569727) and a sequence of the species *Cochliopodium larifeili* (JF298253) (63–98%). The latter is sister to the two sequences mentioned above. The third group of sequences is sister to the first and second. In addition, a separate branch is formed on the phylogenetic tree by a representative of the genus *Gocevia*, which is sister to the species of the genus *Cochliopodium* and belongs to the order Himatismenida (Figure 2).

None of the amoebae species identified by us was represented by identical 18S rRNA gene sequences. The average value of sequence polymorphism within an amoebae species varies from 2.5 to 4%. The value of sequence divergence was 2.7% within the species *Cochliopodium minus,* 4% within *Cochliopodium actinophorum*, and 3.4% within *Cochliopodium* sp. Sequence divergence between different species of the genus *Cochliopodium* varied from 4 to 19%.

According to the 18S rRNA gene sequence data, there is a genetic difference between representatives of the genus *Cochliopodium* and other genera within the Discosea group. For example, the distance between the genes *Cochliopodium minus* (OK649264) and *Acanthamoeba* sp. (OK649261) and *Acanthamoeba* sp. (MZ079366) is 0.178 and 0.151, respectively. The species *Cochliopodium actinophorum* (MZ079367) is even more distant from *Mayorella* sp. (OP729930) – 0.212.

Also, within Discosea, the species of naked amoebae belonging to the following orders group together: Dactylopodida Smirnov et al., 2005 (*Vexillifera bacillipedes* (HQ687485, HQ687484, OK649262) + *Korotnevella stella* (AY686573)); Vannellida Smirnov et al., 2005 (*Vannella* sp. (MZ079372) + *Vannella lata* (OL305063, OL305064) + *Ripella* sp. (MZ079369)); Thecamoebida Smirnov et Cavalier-Smith, 2008 (*Stenamoeba stenopodia* (OP375108) + *Platyamoeba stenopodia* (AY294144) + *Thecamoeba* sp. (MZ079371) + *Thecamoeba quadrilineata* (DQ122381, ON398268) + *Thecamoeba similis* (OL597873)); Dermamoebida Cavalier-Smith, 2004 (*Mayorella* sp. (OZ243098, OP729930)); Acanthamoebida Page, 1976 (*Acanthamoeba* sp. (MZ079366, OK649261).

The Tubulinea group is formed by the species of amoebae belonging to the order Euamoebida Lepsi, 1960 (*Amoeba proteus* (ON907618, AJ314604) + *Chaos nobile* (AJ314606) + *Chaos carolinense* (AJ314607) + *Saccamoeba limax* (EU869301, OQ520144) + *Saccamoeba lacustris* (GQ221845)).

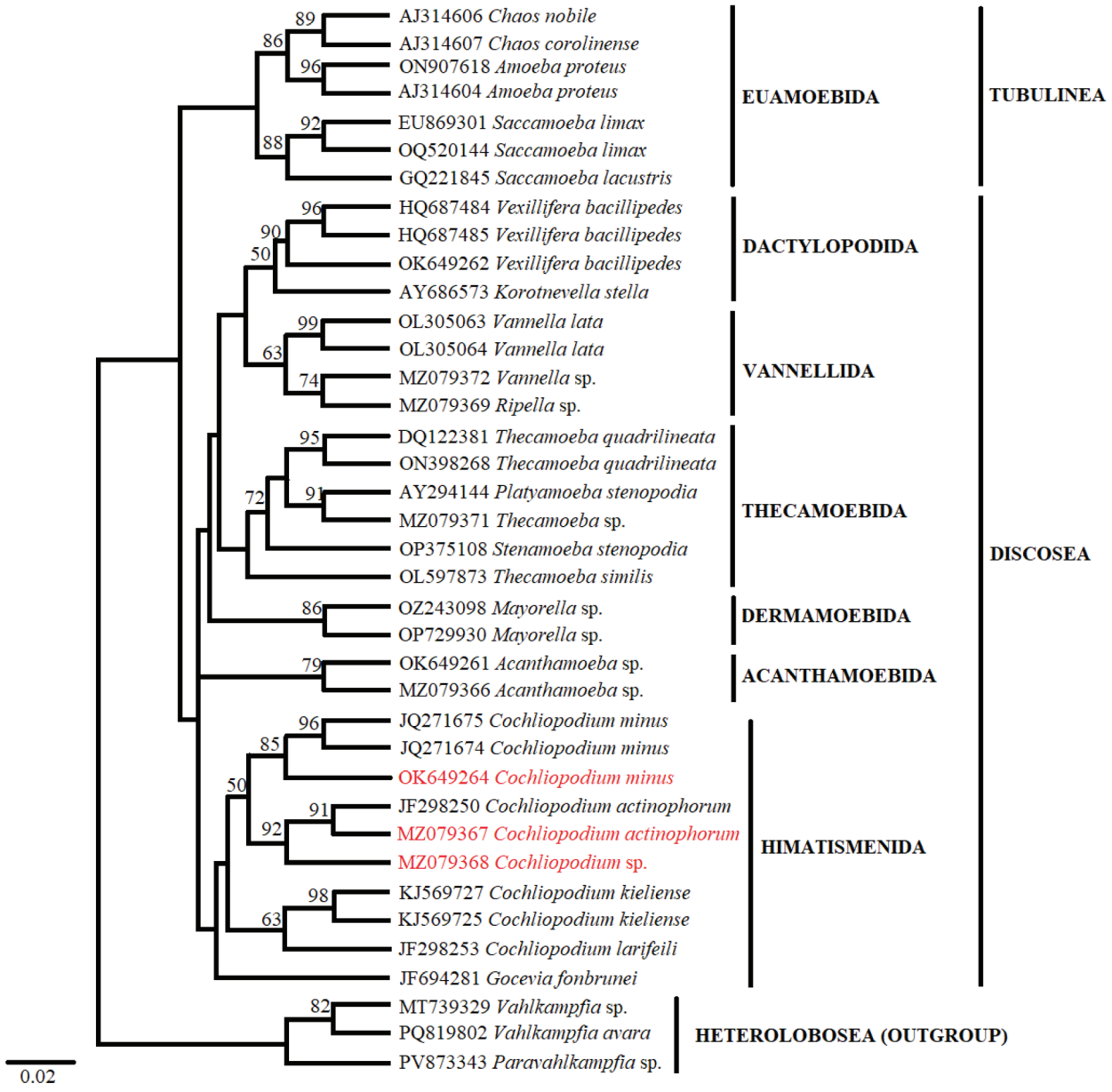

Figure 2. Position of species of naked amoebae of the genus *Cochliopodium* on the phylogenetic tree of Amoebozoa (scale bar indicates equivalence of distance between sequences). Bootstrap support values below 50 are not indicated. Original sequences are highlighted in red.

The outgroup is represented by the species of heterolobose naked amoebae (*Vahlkampfia* sp. (MT739329) + *Vahlkampfia avara* (PQ819802) + *Paravahlkampfia* sp. (PV873343)) of the Discoba group.

DISCUSSION

Light microscopy does not provide reliable data for the identification of naked amoebae. The species diversity of naked amoebae is much higher than currently described. Therefore, in studying the phylogenetic relationships between different representatives of amoeboid protists, in addition to morphological features, molecular genetic data, namely, nucleotide sequences of various genes, are of particular value. For phylogenetic analysis, it is best to use 18S rRNA gene sequences, which allow for the analysis of phylogenetic relationships at different taxonomic levels, both species and genus, as well as at the level of phyla and kingdoms (Hillis et al. 1996; Sims et al. 2002; Schlegel 1991).

The analysis of the DNA sequences obtained by us for the three species of *Cochliopodium* from Ukraine shows that the genus *Cochliopodium* is monophyletic and is placed within the Discosea group. The mutual arrangement of the main branches within the tree generally corresponds to the results obtained earlier by various authors (Cavalier-Smith et al. 2004; Peglar et al. 2003).

Amoebozoa forms a single clade within which the Tubulinea and Discosea groups are distinguished. The differences in the tree obtained by us can be explained by the fact that we used both complete and partial sequences of the 18S rRNA gene for different representatives of the Amoebozoa in the phylogenetic analysis.

The genus *Cochliopodium* is sister to Acanthamoeba, but this position is not supported by the bootstrap value for this group. The topology of the "*Cochliopodium*" branch of the phylogenetic tree is constant. At the top of the branch are the species groups *Cochliopodium minus* (OK649264 + JQ271675 + JQ271674), *Cochliopodium actiniphorum* (JF298250 + MZ079367) and *Cochliopodium* sp. (MZ079368). At the base of the branch is the species group *Cochliopodium kieliense* (KJ569727 + KJ569725), which is grouped together with the species *Cochliopodium larifeili* (JF298253). In addition, the results of molecular phylogenetic analysis are in good agreement with the morphological characters of the genus *Cochliopodium,* such as the presence of a tectum, which is represented by carbohydrate scales and has a single structural plan (Page and Siemensma 1991). The structure of the scale is a species-specific feature and is constant within a morphological species. *Cochliopodium minus* often forms short subpseudopodia during movement, while all other species have the "classical" locomotor form, which is characteristic of the genus *Cochliopodium*.

During the study of water bodies in Ukraine, we isolated four *Cochliopodium* species (*C. actinophorum*, *C. minus*, *C. gulosum*, and *Cochliopodium* sp.), of which three were sequenced (C. *actinophorum* – MZ079367, *C. minus* – OK649264, and *Cochliopodium* sp. – MZ079368, MZ079368). *Cochliopodium gulosum* was studied morphologically, since it was not possible to establish clonal cultures of this species in laboratory conditions. In a phylogenetic tree constructed using the maximum likelihood method based on 38 18S rRNA gene sequences from different species of naked amoebae, representatives of the genus *Cochliopodium* form a separate cluster within the Amoebozoa (at the base of the molecular group Discosea). In our tree, the *Cochliopodium* group is sister to the Acanthamoeba, but this position is not supported by the bootstrap value for this grouping. The results of the phylogenetic analysis are consistent with the morphological data – the peculiarities of the locomotor form of amoebae, the vesicular nucleus, all representatives of the genus *Cochliopodium* have extracellular covering structures (tectum), which have a common structural plan for all species of the genus. On the phylogenetic tree of Amoebozoa, in the molecular cluster Discosea, in addition to Himatismenida, the species of naked amoebae belonging to the orders Dactylopodida, Vannellida, Thecamoebida, Dermamoebida, and Acanthamoebida are grouped. In the molecular cluster Tubulinea, the species belonging to the order Euamoebida are grouped.

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