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NEW FINDS OF NAKED FILOSE AND LOBOSE AMOEBAE IN FRESHWATER BODIES AND SOILS OF UKRAINE

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In the natural biotopes of Ukraine, 12 species of naked amoebae were identified, of which seven species are lobose and five are filose. The molecular cluster Amoebozoa includes seven species of amoebae, Opisthokonta includes two species, and Rhizaria includes three species. The following species were recorded: Leptomyxa spec., Chaos nobile Penard, 1902, Chaos carolinense Wilson, 1900, Deuteramoeba algonquinensis Baldock, Rogerson & Berger, 1983, Polychaos annulatum Penard, 1902, Saccamoeba limna Bovee, 1972, Trichamoeba sinuosa Siemensma & Page, 1986, Nuclearia simplex Cienkowski, 1865, Nuclearia radians Greeff, 1869, Filoreta spec., Leptophrys spec., Vernalophrys algivore Gong et al., 2015. All protist species were infrequently encountered (ranging from 1.5% to 24%), except for Leptomyxa spec. (30%). Naked lobose amoebae belong to polytactic (C. nobile, C. carolinense, D. algonquinensis, P. annulatum), monopodial (S. limna, T. sinuosa) and branched (Leptomyxa spec.) morphotypes. Water temperature and dissolved oxygen concentration were measured at the sites where naked amoebae species were recorded. Accordingly, N. simplex, P. annulatum were recorded at a water temperature of +13 °C, at 10.42 mg/l dissolved oxygen in water; N. radians, V. algivore – +17 °C, 9.35 mg/l; Leptophrys spec. – +15 °C, 9.74 mg/l; C. carolinense – +15 °C, 9.85 mg/l; C. nobile, D. algonquinensis – +16 °C, – 8.95 mg/l; S. limna – +12 °C, 10.50 mg/l; T. sinuosa – +15 °C, 9.65 mg/l. The values of abiotic environmental factors at which soil amoebae species were recorded were established. Thus, Filoreta spec. and Leptomyxa spec. were found at soil temperatures of +12 °C and +10 °C and humidity levels of 90.34% and 94.45%, respectively. The filose amoeba Nuclearia simplex was identified through 18S rRNA gene sequencing (GenBank accession No. PV030488). This is the first report of these naked amoeba species in the fauna of Ukraine.

Key words: *naked lobose amoebae, naked filose amoebae, water bodies, soils, morphotypes, environmental factors.*

Пацюк М. К.

НОВІ ЗНАХІДКИ ГОЛИХ ФІЛОЗНИХ І ЛОБОЗНИХ АМЕБ У ПРІСНИХ ВОДОЙМАХ ТА ҐРУНТАХ УКРАЇНИ

У природних біотопах України ідентифіковано 12 видів голих амеб, з яких 7 видів – лобозних і 5 видів філозних. До молекулярного кластеру Amoebozoa належить сім видів амеб, до Opisthokonta – два види, до Rhizaria – три види. Це такі види: Leptomyxa spec., Chaos nobile Penard, 1902, Chaos carolinense Wilson, 1900, Deuteramoeba algonquinensis Baldock, Rogerson & Berger, 1983, Polychaos annulatum Penard, 1902, Saccamoeba limna Bovee, 1972, Trichamoeba sinuosa Siemensma & Page, 1986, Nuclearia simplex Cienkowski,

1865, Nuclearia radians Greeff, 1869, Filoreta spec., Leptophrys spec., Vernalophrys algivore Gong et al., 2015. Усі протисти виявилися малопоширеними (від 1,5 до 24 %), окрім Leptomyxa spec. (30 %). Голі лобозні амеби належать до політактичного (C. nobile, C. carolinense, D. algonquinensis, P. annulatum), моноподіального (S. limna, T. sinuosa) та розгалуженого (Leptomyxa spec.) морфотипів. Визначені температура води та концентрація розчиненого в воді кисню при яких реєструвалися види голих амеб. Відповідно, N. simplex, P. annulatum реєструвалися за температури води +13 °C, концентрації розчиненого в воді кисню 10,42 мг/л; N. radians, V. algivore – +17 °C, 9,35 мг/л; Leptophrys spec. –+15 °C, 9,74 мг/л; C. carolinense – +15 °C, 9,85 мг/л; C. nobile, D. algonquinensis – +16 °C, – 8,95 мг/л; S. limna – +12 °C, 10,50 мг/л; T. sinuosa – +15 °C, 9,65 мг/л. Встановлені значення абіотичних факторів середовища при яких реєструвалися ґрунтові види амеб. Так, Filoreta spec. та Leptomyxa spec. траплялися за температури ґрунтів +12 °C й +10 °C та вологості ґрунтів 90,34 % й 94,45 % відповідно. За геном 18S рРНК визначена видова ідентифікація філозної амеби Nuclearia simplex (послідовність ДНК у GenBank PV030488). Усі ідентифіковані види голих амеб виявилися новими для фауни України.

Ключові слова: *голі лобозні амеби, голі філозні амеби, водойми, ґрунти, морфотипи, фактори середовища.*

Today, naked lobose amoebae belong to three molecular clusters: Tubulinea Smirnov et al., 2005 (amoebae with tubular, cylindrical in cross-section pseudopodia, monoaxial cytoplasmic flow, no flagellated forms in the life cycle), Discosea Cavalier-Smith et al., 2004 (flattened naked amoebae with polyaxial cytoplasmic flow, no tubular pseudopodia, simple life cycle, no flagellated forms) and Variosea Cavalier-Smith et al., 2004 (flattened naked amoebae, reticulate or flagellated plasmodia, different patterns of cytoplasmic flow, complex life cycles include flagellated stages) [1, 19]. Naked filose amoebae have two life forms – flattened (substrate) and radial (floating). In the first case, the cell is spread out on the substrate, forms pseudopodia and moves actively, in the second case, it is rounded, with filopodia directed in different directions. Both forms have been described for some representatives of filose amoebae [1].

Despite the high abundance of naked amoebae in marine and freshwater bodies, soils [2, 12], a small number of described species are known. Most protistologists believe that naked amoebae are cosmopolitan organisms due to their small cell size, ability to form resting cyst stages, ability to spread by water currents and simple agamous cycle [4, 5, 12, 19]. Nowadays, though, it has been proven that the distribution of naked amoebae species is limited by abiotic environmental factors [6, 7]. The data on the distribution of naked amoebae is limited. Difficulties in research are associated with the complexity of identifying naked amoebae, which is possible only with a combination of modern light microscopy (DIC contrast) and molecular genetic methods (gene sequencing). The only way to obtain reliable data on the fauna of naked amoebae is to study samples from separate locations and compare them with already known species that are identified in the local faunas [19].

MATERIALS AND METHODOLOGY

Sampling was carried out in freshwater bodies and soils of Ukraine during 2023-2024. A total of 450 field samples were examined and analyzed (Table 1). Naked amoebae were propagated on non-nutritious agar-agar according to the method of F. Page [12]. Species identification was carried out using an Axio Imager M1 light microscope with differential interference contrast.

Also, water samples were taken to determine the concentration of dissolved oxygen in water according to Winkler [11]. At the sampling site, water temperature was measured with a calibrated water mercury thermometer with a graduation value of 0.1-0.5 °C [9].

Soil temperature was determined using a soil thermometer; actual soil acidity was determined using a standard method [8, 20]; pH was determined using a 150-M laboratory pH meter. Soil moisture was determined using a weight method [8, 20]. The frequency of occurrence of species was determined as the proportion of samples in which the species was found out of the total number of samples studied [3, 18], since modern studies do not allow obtaining data on the abundance of amoeba species.

Table 1

Sites and sampled material to study naked amoebas

№	Location	Sample characteristics	Number of studied samples
1.	Lake Lucimer, Shatsk National Nature Park	surface layer of bottom soil and a small amount of bottom water	58
2.	Lake Svitiaz, Shatsk National Nature Park	surface layer of bottom soil and a small amount of bottom water	57
3.	Lake Synevyr, Zakarpattia region	surface layer of bottom soil and a small amount of bottom water	50
4.	Tysa River, Zakarpattia region	surface layer of bottom soil and a small amount of bottom water	55
5.	Styr River, Volyn region	surface layer of bottom soil and a small amount of bottom water	50
6.	Horyn River, Ternopil region	surface layer of bottom soil and a small amount of bottom water	50
7.	Limnytsia River, Ivano-Frankivsk region	surface layer of bottom soil and a small amount of bottom water	50
8.	soil, Zhytomyr region	surface soil layer up to 5 cm, forest zone	40
9.	soil, Lviv region	surface soil layer up to 5 cm, forest zone	40

RESULTS AND DISCUSSION

During 2009-2022, we conducted studies of the species composition of naked amoebae in fresh and marine water bodies, soils, epiphytic and epilithic mosses and lichens of Ukraine, and also studied the reaction of these protists to abiotic environmental factors (water temperature; concentrations of dissolved oxygen and organic matter in water; water salinity; temperature, acidity and humidity of soils) [13-15, 17]. As a result of the studies, 44 freshwater species, 12 marine species and 23 species from terrestrial biotopes were identified. Ecological groups of these protists in relation to environmental factors were also identified. During 2015-2020, we identified 10 species of naked filose amoebae in various types of water bodies in Ukraine [16]. We continue to study the species composition of naked amoebae in various natural biotopes of Ukraine, including from remote locations, where we constantly find new species.

We have identified new species of naked amoebae in freshwater and soils of Ukraine. According to the modern system of Eukaryotes, the molecular cluster Amoebozoa of Ukraine includes seven species of amoebae while Opisthokonta includes two species, and Rhizaria includes three species [1]. The list of species is given below.

Amoebozoa Luhe, 1913
Tubulinea Smirnov et al., 2005
Leptomyxida Pussard & Pons, 1976
Leptomyxidae Pussard & Pons, 1976
Leptomyxa Goodey, 1915
Leptomyxa spec.
Euamoebida Lepsi, 1960
Amoebidae Ehrenberg, 1838
Chaos Linneaus, 1767
Chaos nobile Penard, 1902
Chaos carolinense Wilson, 1900
Deuteramoeba Page, 1987
Deuteramoeba algonquinensis Baldock, Rogerson & Berger, 1983
Polychaos Schaeffer, 1926
Polychaos annulatum Penard, 1902
Hartmannellidae Volkonsky, 1931
Saccamoeba Frenzel, 1892
Saccamoeba limna Bovee, 1972
Trichamoeba Fromentel, 1874
Trichamoeba sinuosa Siemensma & Page, 1986

Opisthokonta Cavalier-Smith, 1987
Cristidiscoidea Cavalier-Smith, 2009
Nucleariida Cavalier-Smith, 1993
Nucleariidae Cann & Page, 1979
Nuclearia Cienkowsky, 1865
Nuclearia simplex Cienkowski, 1865
Nuclearia radians Greeff, 1869
Rhizaria Cavalier-Smith, 2002
Proteomyxidea Lankester, 1885
Reticulosida Cavalier-Smith, 2003
Filoretidae Cavalier-Smith and Bass, 2009
Filoreta Cavalier-Smith & Bass, 2009
Filoreta spec.
Vampyrellida West, 1901
Vampyrellidea Cavalier-Smith, 2018
Leptophrys Hertwig & Lesser, 1874
Leptophrys spec.
Vernalophrys Gong, Patterson and Hu, 2015
Vernalophrys algivore Gong et al., 2015

***Nuclearia simplex* Cienkowski, 1865**

A naked filose amoeba that forms non-granular, non-anastomosing pseudopodia. The body is usually spherical in shape. During movement, it acquires an elongated or fusiform shape. It usually forms two bundles of filopodia that are directed forward in the direction of movement. The amoeba moves by "folding" the filopodia: the pseudopodia attaches to the substrate with its end and bends in the middle. At the bend point, a drop of hyaloplasm is formed, into which the remains of the pseudopodia are drawn from both ends. The cell is pulled forward and the drop of hyaloplasm that has formed merges with the cytoplasm of the cell. New filopodia are formed and the process is repeated.

Under the cell membrane and filopodia, there is a cortical layer of microfilaments [10].

A single round nucleus is located in the center of the cell cytoplasm. In *N. simplex*, a capsule of fibrillar material has been found surrounding the nucleus [10].

The cell length is 25 to 27 μm.

The amoeba is polyphagous, phagocytizes small food objects such as detritus, bacteria, unicellular algae [10].

Distribution. North America, Europe, Japan. Freshwater species [12].

Sampling site. Lake Lucimer (Shatsk National Nature Park).

***Nuclearia radians* Greeff, 1869**

Cells are always spherical in shape, with radiating, fibrous, unbranched pseudopodia. The cytoplasm is granular, there are digestive vacuoles, which may contain algae residues. Contractile vacuoles with a diameter of up to 3 μm. The cell has a jelly-like membrane of densely packed bacteria. The length of the pseudopodia exceeds the diameter of the cell. The pseudopodia are formed from the hyaline zone of the cell and narrow towards the terminal end. A slow-moving amoeba.

The nucleus is spherical in shape, with a diameter of 5 to 7.5 μm.

The cell diameter is 20 to 42 μm.

Distribution. Europe. Freshwater species [12].

Sampling site. Lake Synevyr (Zakarpattia region).

***Leptophrys* spec.**

The naked filose amoeba is fan-shaped, and when moving it spreads along the substrate and flattens. As a result of locomotion, sticky ends are formed, which are stretched and retracted again. Colorless pseudopodia are formed at the edges of the cell from the hyaline cytoplasm. Filopodia are long, thin, tapered, unbranched. The formation of a large number of pseudopodia can indicate the direction of movement of the amoeba. They move slowly by retracting their sticky posterior ends. The color of the central body of the cell ranges from light yellow to orange, depending on the food source. Numerous contractile vacuoles and refractile granules are visible in the cytoplasm of the cell.

Polynuclear (up to 3.0 μm).

Sampling site. Styr River (Volyn region).

***Filoreta* spec.**

Cells of various shapes, from round to fusiform. Large thin cytoplasmic threads connect the cells to each other. The flow of cytoplasm is always bidirectional. The cytoplasmic threads are devoid of granules in one group of cells, while they are present in the other. As a rule, they are mononuclear.

Sampling site. Soil, forest zone (Lviv region).

***Vernalophrys algivore* Gong et al., 2015**

Naked filose amoeba is fan-shaped with thin, tapered, unbranched filopodia that arise from the hyaline layer of the cytoplasm. Filopodia can reach a length of up to 45 μm. The cortical cytoplasm is transparent, the endoplasm contains inclusions and numerous vacuoles; small refractive granules are concentrated under the cell surface. Cell movement is slow.

Length of the trophozoites is 12-28 μm.

In cultures, the amoeba formed cysts with a diameter of 25 to 30 μm.

Sampling site. Lake Synevyr (Zakarpattia region).

***Chaos nobile* Penard, 1902**

Polypodial polynuclear amoebae. Wide tubular pseudopodia of granulo- and hyaloplasm are present, usually formed in the anterior part of the cell, and participate in movement. Cells have lateral wrinkles and several small thick pseudopodia. Uroid of morular or bulb-shaped type.

The diameter of the biconvex or rounded nuclei is from 15 to 20 μm.

Cyst formation in cultures was not observed.

The length of the amoeba is 250-450 μm.

Distribution. North America, Europe. Freshwater species [12].

Sampling site. Horyn River (Ternopil region).

***Chaos carolinense* Wilson, 1900**

Polypodial naked lobose amoeba with a lot of elliptical nuclei. In the cellular cytoplasm there are crystals of bipyramidal or flat shape. During rapid movement, the amoebae acquire an orthotactic shape.

The diameter of the nuclei is from 25 to 30 μm. The length of the amoeba is 450-650 μm.

Distribution. North America, Netherlands, Switzerland. Freshwater species [12].

Sampling site. Tysa River (Zakarpattia region).

***Deuteramoeba algonquinensis* Baldock, Rogerson & Berger, 1983**

Amoeba of polytactic morphotype. During rapid locomotion, a large dominant pseudopodium is formed. Hyaline caps that form on pseudopodia are often filled with granuloplasm. In the cellular cytoplasm, there are numerous bipyramidal crystals, inclusions. Uroid of morular type.

The nucleus is spherical in shape, with a diameter of 10-15 μm.

The length of the cell is 85 to 150 μm.

Distribution. North America. Freshwater species [12].

Місцезнаходження. Horyn River (Ternopil region).

***Polychaos annulatum* Penard, 1902**

Amoeba of polytactic morphotype with short, blunt, digitate pseudopodia. When moving rapidly, they become monopodial. The speed and direction of movement are constantly changing. In the cellular cytoplasm, there are crystals of bipyramidal or irregular shape, one contractile vacuole. Uroid of bulbous type.

The length of amoeba is 250-300 μm.

The nucleus has a diameter of 15 to 20 μm.

Distribution. Lake Lucimer (Shatsk National Nature Park).

***Saccamoeba limna* Bovee, 1972**

A monopodial amoeba. The body is usually wider and thicker at the anterior end of the cell with a well-defined hyaline cap that disappears during the movement of the amoeba. The cellular cytoplasm has a large number of small bipyramidal crystals and a contractile vacuole, which is localized mainly near the uroid. The components of the cytoplasm move with the cytoplasmic current during the locomotion of the amoeba.

The posterior end of the cell forms a hyaline bulb-shaped uroid with short filaments.

The length of amoeba is 110-115 μm.

The nucleus is of vesicular type, 8 μm in diameter.

Distribution. North America. Freshwater species [12].

Sampling site. Lake Svitiaz (Shatsk National Nature Park).

***Leptomyxa* spec.**

Slow-moving amoebae. The branched form of the trophozoite forms pseudopodia of various shapes: from long narrow to expanded short digitate. Thus, elongated tubular pseudopodia are scattered in all directions. In cultures, the amoebae did not change their body shape to monopodial. The formation of small contractile vacuoles was observed in different parts of the cytoplasm.

Amoebae are mononuclear. The nucleus is of the vesicular type, with a diameter of 3.5 to 4.0 μm.

The length of amoeba is 50-85 μm.

Sampling site. Soil, forest zone (Zhytomyr region).

***Trichamoeba sinuosa* Siemensma & Page, 1986**

Amoeba of monopodial morphotype. During rapid locomotion, it acquires a zigzag shape. The anterior end of the cell is wide. There are many bipyramidal crystals in the cytoplasm. The contractile vacuole is large, formed as a result of the fusion of small ones, and is concentrated closer to the posterior end of the cell, between the nucleus and the uroid. Amoebae constantly move in one direction. They can form short hemispherical pseudopodia only to change the direction of movement. The movement of amoebas is slow, non-eruptive, the shape of the anterior part of the cell changes, curving alternately to the right and left. The hyaline cap was not formed, since it was filled with granuloplasm.

Uroid of the bulbous type with short villi.

Large nucleus with a diameter of 15 to 25 μm.

The length of the amoeba is 150-280 μm.

Distribution. Netherlands, Denmark. Freshwater species [12].

Sampling site. Limnytsia River (Ivano-Frankivsk region).

Seven species of naked lobose and five species of naked filose amoebae were new to the fauna of Ukraine. Ten species of naked amoebae were found in water bodies, and two species in soils. All found amoebae are rare in natural biotopes (from 1.5 to 24%), except for *Leptomyxa* spec. (30%) (Fig. 1).

The studied species were recorded under the following abiotic factors of the aquatic environment: *Nuclearia simplex*, *Polychaos annulatum* at water temperature +13 °C, concentration of dissolved oxygen in water 10.42 mg/l; *Nuclearia radians*, *Vernalophrys algivore* at water temperature +17 °C, concentration of dissolved oxygen in water 9.35 mg/l; *Leptophrys* spec. at water temperature +15 °C, concentration of dissolved oxygen in water 9.74 mg/l; *Chaos carolinense* at water temperature +15 °C, concentration of dissolved oxygen in water 9.85 mg/l; *Chaos nobile*, *Deuteramoeba algonquinensis* at water temperature +16 °C, concentration of dissolved oxygen in water 8.95 mg/l; *Saccamoeba limna* at water temperature +12 °C, concentration of dissolved oxygen in water 10.50 mg/l; *Trichamoeba sinuosa* at a water temperature of +15 °C, a concentration of dissolved oxygen in water of 9.65 mg/l. *Filoreta* spec. and *Leptomyxa* spec. occurred in soils of Ukraine at temperatures of +12 °C and +10 °C and humidity of 90.34% and 94.45%, respectively.

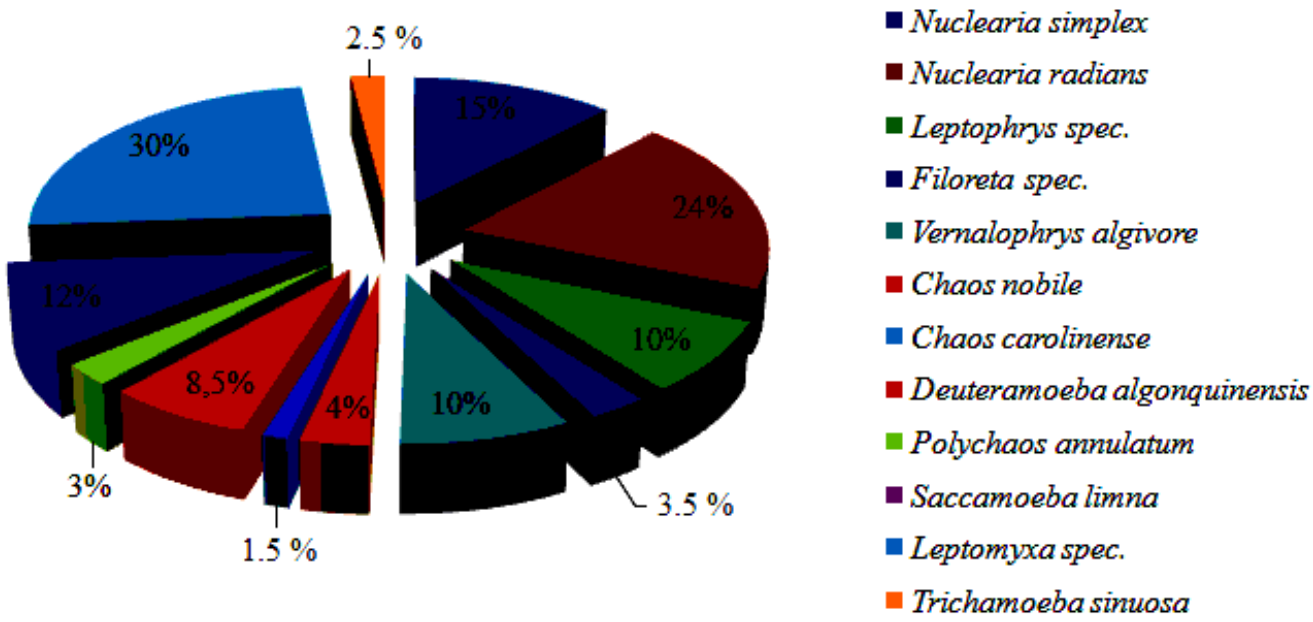

Fig. 1. Frequency of occurrence of naked amoebae in natural biotopes of Ukraine (in %)

CONCLUSIONS

Thus, in the natural biotopes (freshwater bodies, soils) of Ukraine, we found 12 species of naked amoebae, of which 7 species are lobose (*Leptomyxa* spec., *Chaos nobile*, *Chaos carolinense*, *Deuteramoeba algonquinensis*, *Polychaos annulatum*, *Saccamoeba limna*, *Trichamoeba sinuosa*) and 5 species are filose

(*Nuclearia simplex*, *Nuclearia radians*, *Filoreta* spec., *Leptophrys* spec., *Vernalophrys algivore*). All these species were rare, except for *Leptomyxa* spec. Naked lobose amoebae belong to the polytactic (*Chaos nobile*, *Chaos carolinense*, *Deuteramoeba algonquinensis*, *Polychaos annulatum*), monopodial (*Saccamoeba limna*, *Trichamoeba sinuosa*) and branched (*Leptomyxa* spec.) morphotypes. Abiotic environmental factors were determined, under which species of naked amoebae were recorded. Thus, the temperature of the studied water bodies ranged from +12 °C and +17 °C, the content of dissolved oxygen in water was 8.95–10.50 mg/l; soil temperature ranged from +10 °C to +12 °C; soil humidity was 90.34–94.45%. The species identification of the filose amoeba *Nuclearia simplex* was confirmed using the 18S rRNA gene (DNA sequence in GenBank PV030488). All detected species of naked amoebae are new to the fauna of Ukraine.

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