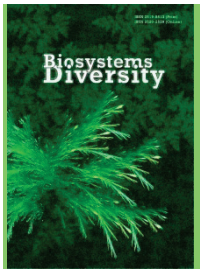

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New finds of naked amoebae

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Findings of naked amoebae in various habitats of Ukraine and other territories are presented. *Saccamoeba* sp., *Thecamoeba similis* (Greeff, 1891) Lepşi, 1960, *Vexillifera bacillipedes* Page, 1969, *Vannella lata* Page, 1988, *Acanthamoeba* sp., *Willaertia magna* De Jonckheere, Dive, Pussard & Vickerman, 1984 were identified based on morphological data and molecular genetic research methods. All these species of amoebae are characterized according to our own observations and measurements and the published data. The amoebae we found belong to three classes (Tubulinea, Discosea, Heterolobosea), six orders (Euamoebida, Thecamoebida, Dactylopodida, Vannellida, Centramoebida, Schizopyrenida), six families (Hartmannellidae, Thecamoebidae, Vexilliferidae, Vannellidae, Acanthamoebidae, Vahlkampfiidae) and six genera (*Saccamoeba*, *Thecamoeba*, *Vexillifera*, *Vannella*, *Acanthamoeba*, *Willaertia*). *W. magna* was the least common in water bodies of different regions of Ukraine; *Saccamoeba* sp., *V. bacillipedes* in the studied water bodies are either uncommon species or occupy the middle position in terms of frequency of occurrence In the water bodies of Ukraine *T. similis*, *V. lata*, *Acanthamoeba* sp. are both the least common and the most common species, or occupy the middle position in terms of frequency of occurrence. The following ecological groups of naked amoebae have been identified in relation to temperature: eurythermal, psychrophilic stenothermal, thermophilic stenothermal. In relation to the concentration of dissolved oxygen in water: euryoxidic and stenooxidic. Three species of amoebae can withstand either a wide range of values of organic substances dissolved in water, or belong to stenobiont species. The recorded amoebae belong to monotactic (*Saccamoeba* sp.), striate (*T. similis*), dactylopodial (*V. bacillipedes*), fan-shaped (*V. lata*), acanthopodial (*Acanthamoeba* sp.) and eruptive (*W. magna*) morphotypes.

Keywords: naked amoebae; morphotypes; fauna; water; soil; epiliths; epiphytes; Ukraine.

Introduction

Biodiversity of natural habitats plays a leading role in ecosystem processes. Aquatic and terrestrial microorganisms are the main components of life on Earth, in terms of biomass and diversity (Decaëns, 2010; Bar-On et al., 2018; Geisen et al., 2019). Natural habitats are teeming with life from viruses to eukaryotic microorganisms (bacteria, archaea, fungi, protists), including multicellular organisms. They form a complex system and control the functions of aquatic and terrestrial ecosystems (Geisen et al., 2019). In recent decades, much attention has been paid to the study of the role of protists in natural habitats. However, intensive research and study of these organisms in their natural biotopes has been unevenly conducted. Most researchers believe that true representation of the biodiversity of aquatic and terrestrial natural habitats cannot be achieved without taking into account protists (Wilkinson, 1998; Geisen et al., 2017; Geisen et al., 2018; Gao et al., 2019; Geisen et al., 2020).

Given the significant biomass of the protists in water bodies and soils, biodiversity in natural ecosystems would be different and significantly reduced without their existence (Orgiazzi et al., 2016). These animal-like organisms play a significant role in the dynamics of nutrients in natural habitats and as the main regulators of groups of animals and plants. Protists have an advantage over other inhabitants of water bodies and soils. They respond to the slightest influences of the environment (temperature, acidity, organic matter of water bodies and soils, soil composition, etc.). In addition, soils and water bodies are complex habitats but there is limited information on the specifics of the spread of protists within them (Geisen et al., 2020). Of great interest among this group of organisms are naked amoebae.

Naked amoebae are animal-like organisms characterized by an amoeboid method of locomotion. They inhabit various natural habitats, and a number of species are causative agents of opportunistic human infections. Some known representatives of the group are lobose, heterolobose and philose amoebae (Page, 1988, 1991).

During the period of research in 2013–2021 we identified 44 species of naked amoebae in freshwater water bodies, 23 species in soils, 13 species in epiphytic mosses and lichens and 10 species in epilithic habitats of Ukraine (Patsyuk, 2015, 2017, 2020; Patsyuk et al., 2019). We can say that the amoebae of the fauna of water bodies, soils, epiphytes and epiliths of Ukraine are similar. Specific species occur only in marine waters (Patsyuk, 2021). We are constantly recording both new species of naked amoebae and new locations for already known amoebae in Ukraine. However, it should be noted that there is a significant gap in studies of the distribution of amoebae in different natural habitats. Therefore, to address this issue, we have collected and analyzed material from remote locations in various natural habitats.

Material and methods

The material was collected in different regions of Ukraine (Fig. 1) in 2013–2021. Samples were taken from fresh water bodies, soils, epiphytic and epilithic habitats. To compare the composition of amoebae from remote locations, additional water samples were collected from water bodies in Austria, Poland and the Czech Republic. Freshwater samples, which included the surface layer of the bottom sediment and a minute amount of bottom water, were taken by hand in sterile glass vessels with a capacity of up to 500 mL. Soil samples were taken from the surface layer of soil (to the depth of 2 cm) in sterile zip-lock bags. To determine the species composition of the naked amoebae, 5 g of the studied soil was placed in a closed 150 mL flask filled with Prescott-James solution (Prescott & James, 1955) and left for 2 hours. The mixture was then shaken for 10 minutes and left for 30 minutes for sedimentation.

Samples of epiphytic mosses and lichens were taken at a height of 0.0, 1.0 and 1.5 m. The epilithic mosses and lichens were likewise sampled at a height of 0.0, 0.5 and 1.0 m. To determine the species composition of naked amoebae in epiphytes and epiliths per sample, 5 g of the studied substrate was placed in a closed 100 mL flask, filled with Prescott-

James solution (Prescott & James, 1955) and left for 2–3 hours to soak. The mixture was then shaken for 10 minutes.

The culturing of amoebae was performed as follows: 5 mL of sample (freshwater, soil, epiphytes and epiliths) was evenly distributed in a Petri dish with a diameter of 100 mm with non-nutrient agar (NNA) according to Page's method with the addition of rice grains (Page, 1991). Live protist cells were identified to species and photographed in water droplets on slides using an Axio Imager M1 light microscope (Center for collective usage of scientific equipment "Animalia" of the Schmalhausen Institute of Zoology NAS of Ukraine) with differential interference contrast. The main morphological features were the metric parameters of the motile forms (cell width (B), cell length (L), ratio of length to cell width (L/B)), diameter of the nucleus and cyst. Measurements were performed on intact cells or on photomicrographs. At least 50 amoebae from each strain were measured. Nuclei were measured in 50 amoebae from each strain. Cell measurements were performed using an eyepiece micrometer (×40).

Identification of amoebae was carried out in two stages. First their morphotype was determined, then Page's taxonomic identification key was used (Page, 1977, 1988, 1991), as well as other publications on the taxonomy of naked amoebae (Mulec et al., 2005; Niyyati et al., 2009; Corsaro et al., 2010). We noted the temperature, concentration of dissolved oxygen and organic matter (after permanganate oxidation) at the sampling sites (Nabyvanets et al., 2007).

"Pure" DNA is not produced in most cultures of amoebae because these contain other eukaryotes (such as fungi, animal-like organisms) that are eaten by amoebae. Therefore, before DNA isolation, amoebae were kept on NNA to purify them from other eukaryotic contaminants. We managed to clear only a few cultures of amoebae to clarify their taxonomic position. 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. Comparison of the obtained DNA sequences with GenBank data was performed using the BLAST program (NCBI) (https://blast.ncbi.nlm.nih.gov/Blast.cgi). In total, during the entire study period, we isolated DNA for 11 species of amoebae (numbers in the GenBank database for *Saccamoeba* sp. (3) – MZ079370, *Thecamoeba similis* (Greeff, 1891) Lepşi, 1960 – OL604177, OL604178, *Thecamoeba* sp. – MZ079371, *Vexillifera bacillipedes* Page, 1969 – OK649262, *Vannella lata* Page, 1988 – OL305063, OL305064, *Vannella* sp. – MZ079372, *Ripella* sp. – MZ079369, *Acanthamoeba* sp. – MZ079366, OK649261, *Cochliopodium actinophorum* Auerbach, 1856 – MZ079367, *Cochliopodium minus* Page, 1976 – OK649264, *Cochliopodium* sp. – MZ079368, *Willaertia magna* De Jonckheere, Dive, Pussard & Vickerman, 1984 – OK649263).

Fig. 1. Map of Ukraine
(the regions where the material was collected are indicated)

Results

We identified six species of naked amoebae. The amoeba system is given according to (Adl et al., 2012; 2019; Page, 1991).

Amoebozoa Luhe, 1913
- Class Tubulinea Smirnov et al., 2005
  - Order Euamoebida Lepsi, 1960
    - Family Hartmannellidae (Volkonsky, 1931) Page, 1974
      - Genus *Saccamoeba* (Frenzel, 1892) Bovee, 1972
        - *Saccamoeba* sp.
- Class Discosea Cavalier-Smith et al., 2004
  - Order Thecamoebida Smirnov et Cavalier-Smith, 2008
    - Family Thecamoebidae (Schaeffer, 1926) Page, 1987
      - Genus *Thecamoeba* Fromentel, 1874
        - *Thecamoeba similis* (Greeff, 1891) Lepşi, 1960
  - Order Dactylopodida Smirnov et al., 2005
    - Family Vexilliferidae Page, 1987
      - Genus *Vexillifera* Schaeffer, 1926
        - *Vexillifera bacillipedes* Page, 1969
  - Order Vannellida Smirnov et al., 2005
    - Family Vannellidae (Bovee, 1970) Page, 1987
      - Genus *Vannella* Bovee, 1965
        - *Vannella lata* Page, 1988
  - Order Centramoebida (Rogerson & Patterson, 2002) Cavalier-Smith, 2004
    - Family Acanthamoebidae Sawyer & Griffin, 1975
      - Genus *Acanthamoeba* Volkonsky, 1931
        - *Acanthamoeba* sp.

Discoba Simpson in Hampl et al., 2009
- Class Heterolobosea Page & Blanton, 1985
  - Order Schizopyrenida Singh, 1952
    - Family Vahlkampfiidae Jollos, 1917
      - Genus *Willaertia* De Jonckheere, Dive, Pussard & Vickerman, 1984
        - *Willaertia magna* De Jonckheere, Dive, Pussard & Vickerman, 1984

We are the first to conduct research on the peculiarities of the distribution of naked amoebae in water bodies, soils, epiphytic and epilithic biotopes of Ukraine. There is no information in the literature on the ecology of naked amoebas.

All six amoebae were registered by us in water bodies of Ukraine (Table 1). According to the frequency of occurrence, these protists occupy different positions (Fig. 2). The least common was *W. magna* (17.7%); *Saccamoeba* sp. (19–44%), *V. bacillipedes* (14–43%) in the studied water bodies are either uncommon species or occupy the middle position in terms of frequency of occurrence. In the water bodies of Ukraine, amoebae species *T. similis* (9.8–69.0%), *V. lata* (6.7–65.5%), *Acanthamoeba* sp. (6.7–93.8%) are both the least common and the most common species, or occupy the middle position in terms of frequency of occurrence.

During sampling from water bodies, we recorded hydrophysical and hydrochemical parameters of the water (temperature, content of oxygen and organic substances dissolved in the water (by permanganate oxidability). We have established ranges of tolerance of amoebae to the main abiotic factors of the aquatic environment. Tolerance of living organisms to water temperature of water bodies can be assessed only by year-round observations. According to the results of seasonal studies in the species complex of naked amoebae in the Teteriv River, we found that *V. lata* can withstand water temperatures from +2 to +24 °C, *Acanthamoeba* sp. – from +6 to +22 °C, *V. bacillipedes* – from +12 to +22 °C, *W. magna* – from +12 to +22 °C, *Saccamoeba* sp. – from +2 to +6 °C. Accordingly, the species *V. lata* is eurythermal, *Saccamoeba* sp. is psychrophilic stenothermal, *Acanthamoeba* sp., *V. bacillipedes*, *W. magna*. are thermophilic stenothermal.

In relation to dissolved oxygen in water, two ecological groups of amoebae have been identified: euryoxidic and stenooxidic. The following amoebae belong to the euryoxidic group: *Saccamoeba* sp. (6.38–28.34 mg/L), *V. bacillipedes* (4.83–30.80 mg/L), *V. lata* (5.44–34.80 mg/L), *Acanthamoeba* sp. (5.44–28.43 mg/L), *W. magna* (6.38–28.06 mg/L). Stenooxidic include the amoeba *T. similis* (7.32–20.13 mg/L).

The following amoebae withstand high levels of organic matter dissolved in water, which are most favourable for their development: *V. lata*

(5.03–50.40 mg O_2/L), *V. bacillipedes* (9.63–50.40 mg O_2/L), *Acanthamoeba* sp. (9.64–50.01 mg O_2/L). *Saccamoeba* sp. prefers water bodies containing organic substances dissolved in water from 9.64 to 39.65 mg O_2/L; *T. similis* – from 11.15 to 39.18 mg O_2/L; *W. magna* – from 16.14 to 39.15 mg O_2/L.

In addition, we detected *T. similis*, *V. bacillipedes*, *V. lata*, *Acanthamoeba* sp., *W. magna* in the soils of Ukraine. *V. lata* and *W. magna* prefer grey forest soils; *V. bacillipedes* – dark chestnut chernozems, podzolized chernozems, weakly podzolic clayey sandgrounds, grey podzolized grounds, grey forest soils; *Acanthamoeba* sp. – common chernozems, podzolized chernozems, weakly podzolic clayey sandgrounds, grey podzolized grounds, grey forest soils; *T. similis* – podzolized chernozems, grey forest soils.

In grey forest soils the frequency of *V. bacillipedes* is 45%, *Acanthamoeba* sp. – 44%, *V. lata* – 15%, *W. magna* – 15%, *T. similis* – 0.6%. In grey podzolized grounds, the incidence of *V. bacillipedes* is 84%, *Acanthamoeba* sp. – 29%. In weakly podzolic clayey sandgrounds, the frequency of *Acanthamoeba* sp. is 48%, *V. bacillipedes* – 30%. In podzolized chernozems, the frequency of *Acanthamoeba* sp. is 61%, *V. bacillipedes* – 44%, *T. similis* – 25%. In common chernozems, the frequency of *Acanthamoeba* sp. is 100%. In dark chestnut chernozems, the frequency of *V. bacillipedes* is 100%. According to our data, the same species of naked amoebae in terms of frequency of occurrence in different types of soils are uncommon, or the most common, or occupy the middle position in terms of frequency of occurrence. *T. similis*, *V. lata*, *W. magna* are rare species in all types of soils studied.

In addition, we have found *V. bacillipedes* and *Acanthamoeba* sp. in epiphytic and epilithic biotopes of Ukraine. In epiphytic biotopes the frequency of these amoebae is 56% and 78%, respectively, in epilithic biotopes – 45% and 39%, respectively.

Table 1
Distribution of naked amoebae in different regions of Ukraine

No.	Species	Biotopes	Regions of Ukraine																					
			Vinnytsia	Volyn	Dnipropetrovsk	Zhytomyr	Zakarpattia	Zaporizhzhia	Ivano-Frankivsk	Kyiv	Kirovohrad	Lviv	Mykolaiv	Odesa	Poltava	Rivne	Sumy	Ternopil	Kharkiv	Kherson	Khmelnytsky	Cherkasy	Chernihiv	Chernivtsi
1.	*Saccamoeba* sp.	Water bodies	–	–	–	●	–	–	–	–	–	–	–	●	–	●	–	●	–	–	–	–	–	–
2.	*Thecamoeba similis* (Greeff, 1891) Lepşi, 1960	Water bodies	–	–	–	–	–	–	●	–	–	●	–	–	–	–	–	–	–	–	–	–	–	●
		soils	–	–	–	–	–	–	–	–	–	●	–	–	–	–	–	–	–	–	●	–	–	–
3.	*Vexillifera bacillipedes* Page, 1969	Water bodies	●	●	●	●	●	●	–	●	●	●	–	–	–	●	●	–	–	–	–	–	●	–
		soils	●	–	–	●	–	–	–	●	●		●	–	–	●	●	–	●	–	●	–	–	–
		epiphytes	●	–	–	●	–	–	–	●	–	●	●	–	–	●	●	–	–	–	–	–	–	–
		epiliths	–	–	–	●	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4.	*Vannella lata* Page, 1988	Water bodies	–	–	–	●	●	–	●	●	–	●	–	–	●	●	–	–	–	●	●	–	–	–
		soils	–	–	–	●	–	–	–	–	–	●	–	–	–	–	–	–	–	–	–	–	–	–
5.	*Acanthamoeba* sp.	Water bodies	●	●	●	●	●	–	●	–	●	●	–	●	–	–	●	●	–	●	●	●	●	–
		soils	●	–	–	●	–	–	–	●	–	–	–	–	–	–	●	–	●	–	●	–	–	–
		epiphytes	●	–	–	●	–	–	–	●	–	–	–	–	–	●	–	–	–	–	–	–	–	–
		epiliths	–	–	–	●	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
6.	*Willaertia magna* De Jonckheere, Dive, Pussard & Vickerman, 1984	Water bodies	–	–	–	●	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
		soils	●	–	–	●	–	–	–	●	–	–	–	–	–	–	–	–	–	–	–	–	–	–

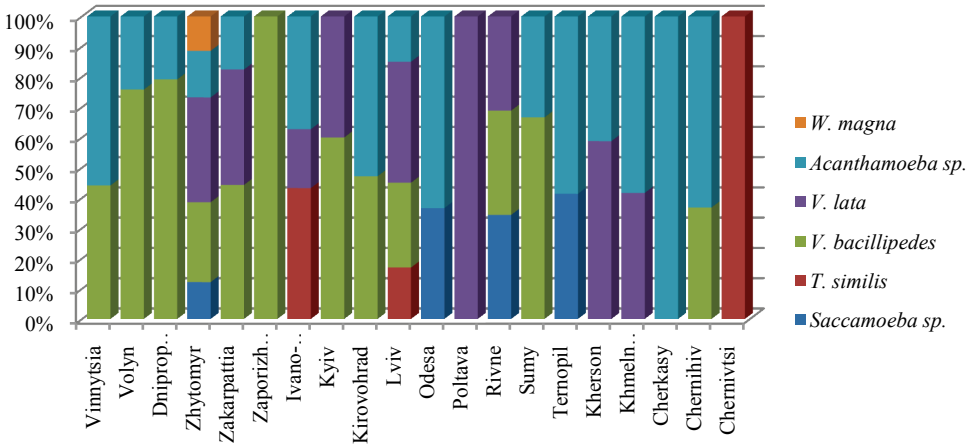

Fig. 2. Frequency of occurrence (%) of naked amoebae in water bodies of different regions of Ukraine

Discussion

For the naked amoebae, the scientific literature provides almost no characteristics, obtained by modern research methods. The following presents the morphological characteristics of six species of naked amoebae found in different habitats of Ukraine and abroad. All species are described on the basis of own observations and measurements and on literature data. The identification of species is also confirmed by molecular data.

Saccamoeba sp. (Fig. 3)

The amoeba belongs to the monotactic morphotype.

During the continuous movement, the amoeba acquires a polypodial shape. Its locomotion occurs in one direction under a constant current of the cytoplasm. The cytoplasm is divided into hyaloplasm and granuloplasm. Hyaloplasm forms temporary short pseudopodia, which quickly disappear during the movement of amoeba. Sometimes amoebae acquire a flattened shape. Monotactic "limax" amoebae change the direction of movement by slightly bending to the side. The hyaline cap at the anterior end of the amoeba can be seen only during the locomotion of the monotactic amoeba. Inclusions and cytoplasmic crystals are present in the granuloplasm. Contractile vacuoles are localized in the cytoplasm. Uroidal structures are of the tuberous type with small papillae.

The length of the monotactic trophozoite is 45–62 µm, the width is 10–12.5 µm, and the L/B ratio is 2.5–3.2.

The nucleus is of the vesicular type, 6.0–6.5 µm in diameter.

The formation of a floating form in cultures was not observed. In a drop of water, amoebae have a spherical shape with irregularities, without pseudopods.

In cultures, amoebae form bilayer cysts. The diameter of the cysts is 15–20 µm.

Locations. It was found in water bodies of Zhytomyr, Odessa, Rivne, Ternopil regions (Table 1); on the River Elbe near Ústí nad Labem, the Czech Republic.

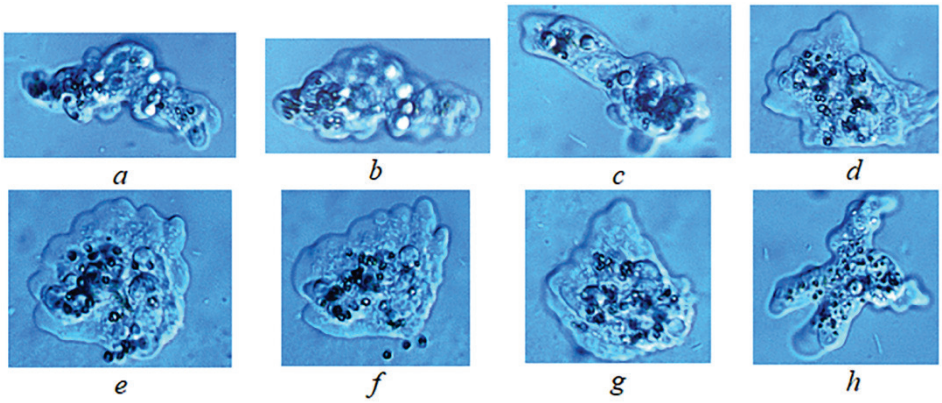

Fig. 3. Locomotor forms of *Saccamoeba* sp. (*a–h*)

Thecamoeba similis (Greeff, 1891) Lepşi, 1960 (Fig. 4)

The amoebae are of striate morphotype.

The cell surface is moderately wrinkled, smooths over during movement. There are dorsal folds of the cytoplasm that converge to the posterior end of the cell. Sickle-shaped hyaloplasm surrounds the anterior and lateral parts of the cell.

A large contractile vacuole is localized in the posterior part of the cell, the diameter of the vacuole is 12.0–12.5 µm. Numerous well-rounded inclusions with a halo of transparent cytoplasm are present in the cytoplasm of the cell.

Uroidal structures are absent.

The length of the amoeba is 45–80 µm, the width is 42–64 µm, and the L/B ratio is 0.9–1.8.

The nucleus is round in shape with a diameter of 8.2–12.8 µm.

The formation of cysts in cultures was not observed.

Differential diagnosis: it can be distinguished from other species by the metric parameters of cell and the distribution of many nucleoli in the periphery of the nucleus (Page, 1991).

Locations. Europe, India, the Netherlands (Page, 1991). We found it in water bodies of Ivano-Frankivsk, Lviv, Chernivtsi regions; soils of Lviv and Khmelnytsky regions (Table 1); Lake Bager, Austria.

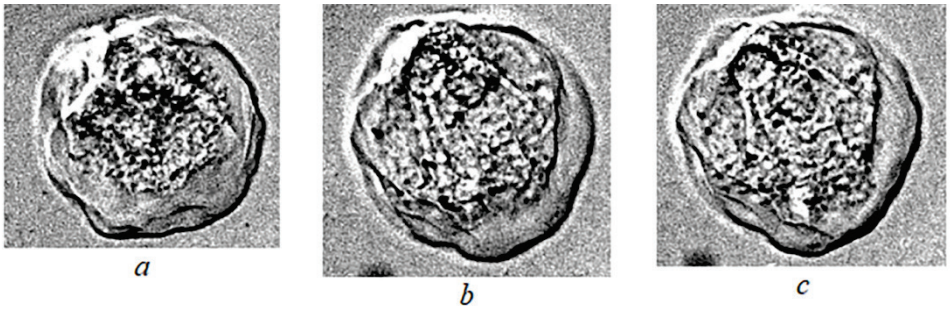

Fig. 4. Locomotor forms of *Thecamoeba similis* (*a–c*)

Vexillifera bacillipedes Page, 1969 (Fig. 5)

This species was identified to the genus in our previous studies and was referred to as *Vexillifera* sp. (Patsyuk, 2013, 2020; Patsyuk et al., 2019).

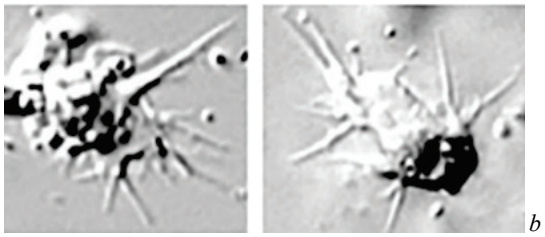

Fig. 5. Locomotor forms of *Vexillifera bacillipedes* (*a*, *b*)

The amoeba is of the dactylopodial morphotype.

The motile forms have a triangular shape with an extended front edge of the cell. The frontal hyaloplasm occupies about 1/5 of the whole cell, forming numerous short subpseudopodia (dactylopodia), the length of which does not exceed ½ cell length. Some subpseudopodia may be located laterally. The granuloplasm of the cell contains contractile vacuoles (1–2), which are concentrated closer to the posterior end of the cell.

Uroidal structures are absent. The posterior end of the cell is round or triangular.

The amoeba is 8–22 long, 5–12 µm wide, and its L/B ratio is 1.0–2.6.

The nucleus, 2.5–2.8 µm in diameter, is usually located near the border of hyaloplasm and granuloplasm.

In our cultures, amoebae did not form cysts and floating forms.

Differential diagnosis: it differs from other species in size of cell and nucleus; triangular shape of the cell has a wide front end. Subseudopodia are either absent or 6 in number, elongated, protrude from the anterior edge of the cell (Page, 1991).

Locations. Europe (Page, 1991). We found it in water bodies of Vinnytsia, Volyn, Dnipropetrovsk, Zhytomyr, Zaporizhzhia, Kyiv, Kirovohrad, Lviv, Rivne, Sumy, Zakarpattia, Chernihiv regions; soils of Vinnytsia, Zhytomyr, Kyiv, Kirovohrad, Mykolaiv, Rivne, Sumy, Kharkiv, Khmelnytsky regions; epiphytic biotopes of Vinnytsia, Zhytomyr, Kyiv, Lviv, Mykolaiv, Rivne and Sumy regions; epilithic biotopes of Zhytomyr region (Table 1); the river Elbe near Ústí nad Labem, Czech Republic; lake near the village of Schörfling am Attersee, Austria.

In previous studies, *V. bacillipedes* was marked as a stenoxidic species in relation to oxygen dissolved in the water (Patsyuk, 2013). Long-term studies allow us to classify this species as a euryoxidic.

Vannella lata Page, 1988 (Fig. 6)

The amoeba belongs to the fan-shaped morphotype.

Trophozoites of semicircular shape with a wide hyaline zone on the sides of the cell. Flattened hyaloplasm, usually crescent-shaped, occupies

almost half the length of the cell. Width is greater than length. Longitudinal ridges and folds are absent. There are up to 4 contractile vacuoles in the cytoplasm of the cell, these can be localized in any part of the granuloplasm. The cytoplasm does not form pseudopodia or subpseudopodia.

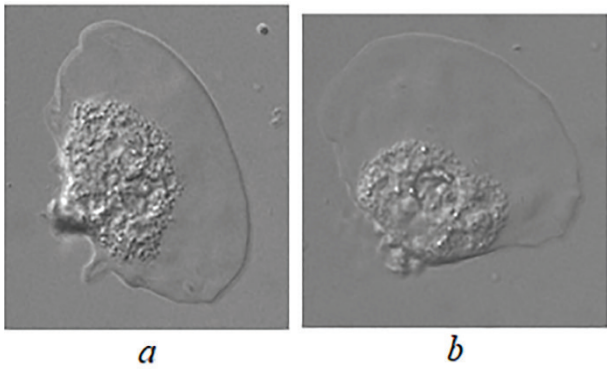

Fig. 6. Locomotor forms of *Vannella lata* (*a*, *b*)

Uroidal structures are absent. The posterior end of the cell is rounded.

The length of amoeba is 40–58 µm, width 50–64 µm, L/B ratio 0.8–1.0.

There is a vesicular nucleus with a diameter of 5.8–8.0 µm.

Amoebae form floating forms of radial type, the cytoplasm has a central granuloplasm with 6–8 narrow sharp long hyaline pseudopodia.

The formation of cysts was not seen in culture.

Differential diagnosis: it differs from other species of the genus *Vannella* by a fan-shaped cell shape, which is twice as large in width compared to length, a floating form with long pointed pseudopodia, that are three times the length of the diameter (Page, 1991).

Locations. Cosmopolitan (Page, 1988, 1991). We found it in water bodies of Zhytomyr, Ivano-Frankivsk, Kyiv, Lviv, Poltava, Rivne, Zakarpattia and Kherson and Khmelnytsky regions; soils of Zhytomyr and Lviv regions (Table 1); the River Warta near Poznan, Poland.

Acanthamoeba sp. (Fig. 7)

This amoeba belongs to the acanthopodial morphotype.

The amoebae are of triangular shape. The anterior end of the cell is wide. The frontal zone of the hyaloplasm is clearly expressed, convex, forms short outgrowths, the acanthopodia. The granuloplasm contains a contractile vacuole, which is located near the posterior edge of the cell.

The uroid is adhesive, carries a bundle of thin filaments.

The length of the amoeba is 12–18 µm, the width is 4.5–6.5 µm, and the L/B ratio is 1.5. The diameter of nucleus is 1.5–1.7 µm, concentrated at the anterior edge of the cell.

The formation of a floating form was not observed.

In culture, spherical cysts with a two-layer shell were formed, single or in clusters. The ectocyst is uneven, thin, barely in contact with the endocyst. The latter is spherical, uneven, and often forms 5–6 shovel-like rays, which are in contact with the ectocyst (in the form of a star). The average diameter of the cysts is 14.2 µm.

Most members of the genus *Acanthamoeba* are parasitic species (Mulec et al., 2005).

We isolated it from the mantle cavity of the mollusk *Viviparus viviparus* Linnaeus, 1758 (Patsyuk, 2017).

Locations: we found it in water bodies of Vinnytsia, Volyn, Dnipropetrovsk, Zhytomyr, Ivano-Frankivsk, Kirovohrad, Lviv, Odesa, Sumy, Ternopil, Zakarpattia, Kherson, Khmelnytsky, Cherkasy, Chernihiv regions; soils of Vinnytsia, Zhytomyr, Kyiv, Sumy, Kharkiv, Khmelnytsky regions; epiphytic biotopes of Vinnytsia, Zhytomyr, Kyiv and Rivne regions; epilithic biotopes of Zhytomyr region (Table 1); on the River Elbe near Ústí nad Labem, Czech Republic.

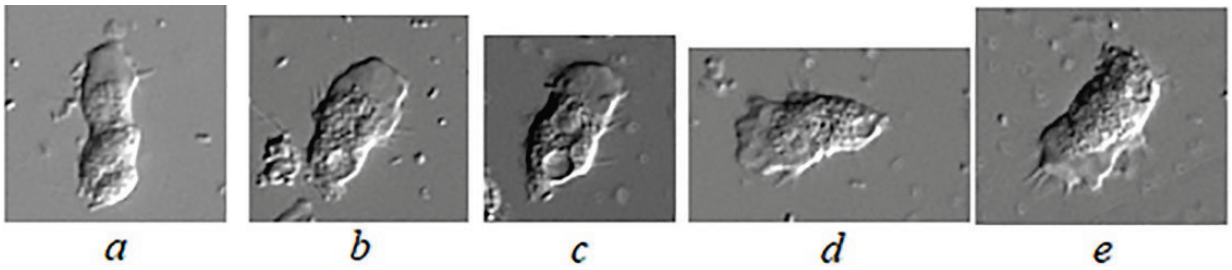

Fig. 7. Locomotor forms of *Acanthamoeba* sp. (*a*–*e*)

Willaertia magna De Jonckheere, Dive, Pussard & Vickerman, 1984 (Fig. 8)

The amoeba belongs to the eruptive morphotype.

The motile form of the amoeba is elongated, the movement is due to the formation of slow wavy, eruptive hemispherical bulges of the hyaloplasm at the front end of the body of the amoeba. Such bulges also occur on the lateral part of the cell, and the amoeba can change direction. The anterior zone of the hyaloplasm forms a hyaline cap. Granuloplasm contains vacuole, inclusions and nucleus (or nuclei).

Uroid is of convex shape with thin threads, separated from the main part of the cell by a constriction.

The amoeba is 98–124 µm long, 32–42 µm wide, and its L/B ratio is 3.2–4.4.

The diameter of nucleus is 12–20 µm. The nucleus is concentrated in the center of the cell and moves along with the cytoplasmic current.

The formation of cysts in cultures was not observed.

Differential diagnosis: it differs from other representatives of heterolobose amoebae by the nature of the movement of the cytoplasm, cell size, structure of the nuclear apparatus and cysts. Cysts are spherical or polygonal, with a thick inner wall and a delicate porous outer layer. There are 5 to 14 pores. Large cysts are multinucleate (Page, 1988, 1991). Phylogenetic analysis shows that *W. magna* is closest to the genus *Naegleria* Alexeieff, 1912 (Hasni et al., 2019).

Locations: we found this species in water bodies in Zhytomyr city, and in soils of Vinnytsia, Zhytomyr and Kyiv regions (Table 1).

Fig. 8. Locomotor form of *Willaertia magna*

Conclusions

Thus, the amoebae we found belong to three classes, six orders, six families and six genera. Using morphological features and molecular data, two species of amoebae are identified to the genus level (*Saccamoeba* sp., *Acanthamoeba* sp.), other four amoebae to species level (*T. similis*,

V. bacillipedes, *V. lata*, *W. magna*). In addition to water bodies, soils, epiphytes and epiliths of Ukraine, the amoebae we mentioned also occurred in water bodies in Austria, Poland and the Czech Republic. The following ecological groups of naked amoebae have been identified. In relation to temperature: eurythermal (*V. lata*), psychrophilic stenothermal (*Saccamoeba* sp.), thermophilic stenothermal (*V. bacillipedes*, *Acanthamoeba* sp., *W. magna*). In relation to the concentration of dissolved oxygen in water: euryoxidic (*Saccamoeba* sp., *V. bacillipedes*, *V. lata*, *Acanthamoeba* sp., *W. magna*), stenooxidic (*T. similis*). *V. lata*, *V. bacillipedes*, *Acanthamoeba* sp. can withstand a wide range of values of organic substances dissolved in water. Stenobiont species include *Saccamoeba* sp., *T. similis*, *W. magna*. The identified amoebae belong to the monotactic, striate, dactylopodial, fan-shaped, acanthopodial and eruptive morphotypes.

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