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DIVERSITY AND DISTRIBUTION OF NAKED AMOEBAE IN WATER BODIES OF SUMY REGION (UKRAINE)

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Diversity and Distribution of Naked Amoebae in Water Bodies of Sumy Region (Ukraine). Patsyuk, M. K., Onyshchuk, I. P. — Taxonomy of naked amoebae and specifics of their distribution in water bodies of Sumy Region are presented. Our research identified 12 species of naked amoebae of 11 morphotypes. We established their ecological groups relative to abiotic aquatic factors: euryoxidic, stenooxidic, stenobiotic and those that survive in a wide range of organic matter content. According to the species composition, swamp and riparian species complexes of naked amoebae were identified. It was found that species complexes of amoeba are influenced by such factors as temperature, concentration of dissolved oxygen and organic compounds.
Key words: naked amoebae, morphotypes, species complexes of naked amoebae, water bodies of Sumy Region.

Introduction

Naked amoebae are single-celled eukaryotic organisms known for their fast reactions to environmental change. Free-living naked amoebae occur on the water-soil, water-animal, water-plant, water-air interfaces etc., feeding on bacteriae, single-celled fungi, algae and other protists, including amoebae (Rodriguez-Zaragoza, 1994).

Studying them is complicated by their small size and the enormity of their habitats. Species composition of amoebae depends on biotic and abiotic environmental factors, which dictate their distribution in the ecosystems. There are not enough data on the naked amoebae fauna in various conditions and geographically remote biotopes. The progress is made almost singularly by studying samples obtained from distant areas and comparing them to the species found in local faunas (Smirnov, 2008; Smirnov et al., 2011). Hence, we studied naked amoebae isolated from water bodies of Sumy Region of Ukraine (Vernitskiy & Patsyuk, 2017). Besides that, the research is of interest because amoebae remain mostly unstudied in the Region and in Ukraine.

Fig. 1. Sampling localities (Sumy Region, Ukraine).

Material and methods

Material was collected in 2016–2017 in various water bodies of Sumy Region. To study the species composition of naked amoebae we gathered 150 samples in 15 localities (fig. 1). The light microscopy, in particular, D.I.C. modification, yielded approximately five hundred specimens and 120 pictures.

Samples (water and disturbed bottom sediment) were manually put into glass containers of up to 500 ml volume and brought to the laboratory. Amoebae were isolated from samples of the upper layer of sediments and a small amount of water above it. They were cultured on Petri dishes of 100 mm diameter on non-nutrient agar (Page, 1988, 1991) at 20 °C.

Live protists in water droplets on slides were observed and photographed using light microscope Axio Imager MI (Centre for collective usage of scientific equipment "Animalia" of the I. I. Schmalhausen Institute of Zoology NAS of Ukraine) with differential interferential contrast.

It should be noted that a full range of approaches and methods (including ultrastructural and molecular biological) are currently used to fully identify naked amoebae. Therefore, our taxonomic identification does not pretend to unambiguity. In many faunal studies for naked amoebae identification morphological data were used. The identification of amoebae, started with sorting to morphotype (Smirnov & Goodkov, 1999; Smirnov, 2008). Then, their taxa were determined, if possible (Page, 1988, 1991; Smirnov & Goodkov, 2004; Smirnov et al., 2007, 2011; Smirnov, 2008).

We noted the temperature, concentration of dissolved oxygen and organic matter (after permanganate oxidation) at the sampling sites (Stroganov, 1980).

To compare fauna lists we used the Chekanovsky–Sørensen index. The clusters were bootstrapped, and the multidimensional analysis was carried out using PAST 1.18 software (Hammer, 2001).

Results and discussion

Twelve species of naked amoebae were found in water bodies in Sumy Region (fig. 2). The species belong to three classes, nine families and 10 genera. They are listed according to the accepted taxonomic system (Smirnov et al., 2011).

Class Tubulinea Smirnov et al., 2005
Order Euamoebida Lepsi, 1960
Family Hartmannellidae Volkonsky, 1931
Genus *Saccamoeba* Frenzel, 1892
Saccamoeba sp.

Class Discosea Cavalier-Smith et al., 2004
Subclass Flabellinia Smirnov et al., 2005
Order Dactylopodida Smirnov et al., 2005
Family Vexilliferidae Page, 1987
Genus *Vexillifera* Schaeffer, 1926
Vexillifera sp.

Order Vannellida Smirnov et al., 2005
Family Vannellidae Bovee, 1970
Genus *Vannella* Bovee, 1965
Vannella* (cf) *lata Page, 1988

Order Himatismenida Page, 1987
Suborder *Tectiferina* Smirnov, Nassonova, Chao et Cavalier-Smith, 2011
Family Cochliopodiidae De Saedeleer, 1934
Genus *Cochliopodium* Hertwig et Lesser, 1874
Cochliopodium sp. (1)

Order Pellitida Smirnov, Nassonova, Chao et Cavalier-Smith, 2011
Family *Pellitidae* Smirnov et Kudryavtsev, 2005
Genus *Pellita* Smirnov et Kudryavtsev, 2005
Pellita digitata (Greef, 1866) Smirnov et Kudryavtsev, 2004

Subclass Longamoebia Smirnov, Nassonova, Chao et Cavalier-Smith, 2011
Order Dermamoebida Cavalier-Smith et al., 2004
Family Mayorellidae Schaeffer, 1926
Genus Mayorella Schaeffer, 1926
Mayorella vespertilioides Page, 1983
Mayorella sp.

Order Thecamoebida Smirnov, Nassonova, Chao et Cavalier-Smith, 2011
Family Thecamoebidae Schaeffer, 1926
Genus *Thecamoeba* Fromentel, 1874
Thecamoeba sphaeronucleolus Greef, 1891
Thecamoeba sp.
Genus *Stenamoeba* Smirnov et al., 2007
Stenamoeba stenopodia (Page, 1969) Smirnov et al., 2007

Order Centramoebida Rogerson et Patterson, 2002

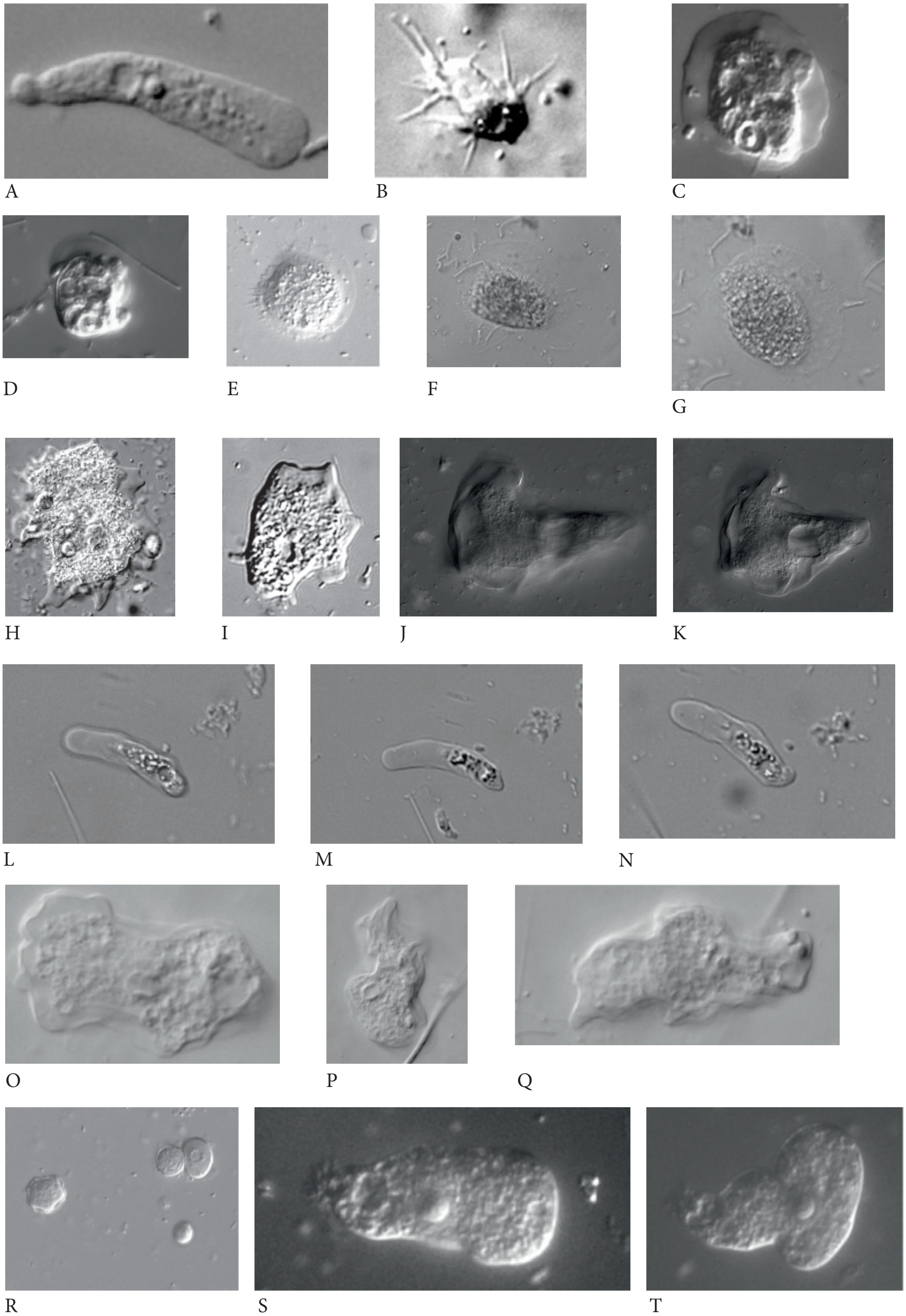

Fig. 2. Naked amoebae found in water bodies of the Sumy Region: A — *Saccamoeba* sp. ×1240; B — *Vexillifera* sp. ×1240; C, D — *Vannella lata* ×1240; E — *Cochliopodium* sp. ×1240; F, G — *Pellita digitata* ×1240; H — *Mayorella vespertilioides* ×1240; I — *Mayorella* sp. ×1240; J, K — *Thecamoeba sphaeronucleolus* ×1240; L, M, N — *Stenamoeba stenopodia* ×1240; O, P, Q — *Thecamoeba* sp. ×1240; R — *Acanthamoeba* sp. (cysts) ×1240. S, T — *Vahlkampfia* sp. ×1240.

Family Acanthamoebidae Sawyer et Griffin, 1975
Genus *Acanthamoeba* Valkonsky, 1931
Acanthamoeba sp.

Class Heterolobosea Page et Blanton, 1985
Family Vahlkampfiidae Jollos, 1917
Genus *Vahlkampfia* Chatton et Lalung-Bonnaire, 1912
Vahlkampfia sp.

Average sizes of the identified species: *Saccamoeba* sp. cell length 25–35 µm, length to breadth ratio (L/B) 1.5; *Vexillifera* sp. cell length 5.5–7.2 µm, breadth 3.3–6.9 µm, L/B 0.5–2.2; *Vannella lata* Page, 1988 cell breadth 30 µm, L/B 0.8; *Cochliopodium* sp. length of the locomotive form 35–38 µm, L/B 1–1.5; *Pellita digitata* Smirnov et Kudryavtsev, 2004 cell length 56–62 µm, L/B 1.0; *Mayorella vespertilioides* Page, 1983 cell length 68–70 µm, L/B 1.0–1.4; *Mayorella* sp. cell length 45.0–52.0 µm, L/B 2.0–2.8; *Thecamoeba sphaeronucleolus* Greef, 1891 cell length 110–135 µm, L/B 1.2; *Thecamoeba* sp. length 50–70 µm, L/B 2.0; *Stenamoeba stenopodia* Smirnov et al., 2007 cell length 8–10 µm, L/B 2.0–2.5; *Acanthamoeba* sp. length 6.2–8.3 µm, L/B 1.8–2.4; *Vahlkampfia* sp. cell length 45–50 µm, breadth 25–35 µm, L/B 1.5.

The distribution of naked amoebae, their species diversity, occurrence of findings are determined by such abiotic aquatic factors as temperature, water pH, concentration of dissolved oxygen and organic matter (Patcyuk & Dovgal, 2012; Patsyuk, 2012, 2013, 2014 a–d). Thus when studying the amoeba fauna of water bodies in Sumy Region we registered the hydrophysical and hydrochemical parameters of the habitats. We evaluated the ranges of habitat characteristics under which several naked amoebae species were found (table 1). Notably, since the sampling was carried out in spring and summer (2017), the water temperature ranged within +18 to +22 °C on average.

Hence, the identified amoebae tolerate typical for these protists values of abiotic aquatic parameters (temperature, concentrations of dissolved oxygen and organic matter), established in earlier studies (table 2) (Patcyuk & Dovgal, 2012; Patsyuk, 2012, 2013, 2014 a–d). We compared the identified species with the established ecological groups of naked amoebae (Patsyuk, 2013) in regards to their environment. One found species was eurioxidic (*V. lata* was recorded at 5.4 mg/l oxygen), three were stenooxidic (*Vexillifera* sp. was found at 10.2 mg/l oxygen, *S. stenopodia* at 12.00 mg/l, *Cochliopodium* sp. at 8.10 mg/l). Three

Table 1. Hydrochemical parameters of water bodies of Sumy Region, where naked amoebae were recorded

No.	Naked amoeba morphotypes	Naked amoeba species	Dissolved oxygen, mg/l	Dissolved organic matter, mg O_2/l
1	monotactic	*Saccamoeba* sp.	6.32	21.00
2	dactylopodial	*Vexillifera* sp.	10.2	28.00
3	fan-shaped	*V. lata*	5.44	32.50
4	lens-like	*Cochliopodium* sp.	8.10	28.00
5	flamellian	*P. digitata*	5.42	20.05
6	mayorellian	*M. vespertilioides*	10.20	24.45
		Mayorella sp.	20.04	11.13
7	rugose	*T. sphaeronucleolus*	6.32	21.00
8	striate	*Thecamoeba* sp.	11.02	12.08
9	lingulate	*S. stenopodia*	12.00	28.00
10	acanthopodial	*Acanthamoeba* sp.	10.20	25.00
11	eruptive	*Vahlkampfia* sp.	25.05	50.02

Table 2. Tolerance of various naked amoebae to main abiotic factors (Patsyuk, 2013)

No.	Species	Temperature, °C	Dissolved oxygen, mg/l	Dissolved organic matter, mg O_2/l
1	*S. stagnicola*	–	4.52–17.21	2.43–30.52
2	*Saccamoeba* sp. (1)	–	1.37–18.32	5.57–38.03
3	*K. stella*	3–26	4.50–31.94	9.03–50.01
4	*Korotnevella* sp. (2)	–	1.37–11.05	6.00–48.50
5	*Vexillifera* sp.	–	3.05–18.04	1.32–50.01
6	*Cochliopodium* sp. (1)	3–26	3.05–17.84	2.17–50.21
7	*V. lata*	3–26	2.35–30.05	2.84–50.01
8	*Ripella* sp.	–	5.28–31.94	4.21–56.50
9	*M. cantabrigiensis*	4–26	3.05–17.84	5.60–50.01
10	*Mayorella* sp. (1)	–	4.35–31.94	3.25–28.53
11	*T. striata*	3–26	3.05–28.02	2.17–50.01
12	*S. stenopodia*	–	4.85–15.32	2.54–37.12
13	*Flamella* sp.	–	3.04–18.04	7.81–50.38
14	*Vahlkampfia* sp. (1)	3–26	2.35–24.02	2.17–50.01
15	*Vahlkampfia* sp. (2)	–	2.35–24.02	3.15–38.03

species tolerated a wide range of dissolved organic content, measured with permanganate oxidation (*Vexillifera* sp. was found at 28.00 mg O_2/l, *V. lata* at 32.50 mg O_2/l, *Cochliopodium* sp. at 28.00 mg O_2/l), *S. stenopodia* was stenobiontic (tolerating dissolved organic matter levels of 28.00 mg O_2/l).

We analyzed the specific morphotype features of naked amoebae in the water bodies of Sumy Region. All in all, we identified 11 morphotypes of naked amoebae: monotactic, dactylopodial, fan-shaped, lens-like, flamellian, mayorellian, rugose, striate, lingulate, acanthopodial, eruptive. Amoebae of the monotactic morphotype were found at 6.32 mg/l oxygen and 21.00 mg O_2/l organic matter, respectively; dactylopodial, 10.20 mg/l and 28.00 mg O_2/l; fan-shaped, 5.44 mg/l and 32.50 mg O_2/l; lens-like, 8.10 mg/l and 28.00 mg O_2/l; flamellian, 5.42 mg/l and 20.05 mg O_2/l; mayorellian, 10.20 mg/l and 20.04 mg/l and 24.45 mg O_2/l and 11.13 mg O_2/l; rugose, 6.32 mg/l and 21.00 mg O_2/l; striate, 11.02 mg/l and 12.08 mg O_2/l; lingulate — 12.00 mg/l and 28.00 mg O_2/l; acanthopodial, 10.20 mg/l and 25.00 mg O_2/l; eruptive, 25.05 mg/l and 50.02 mg O_2/l (table 1). Of course, it is entirely

Table 3. Naked amoebae distribution in the water bodies of Sumy Region

No.	Amoeba species	Water body		
		River	Bog	Riparian basin
1	*Saccamoeba* sp.	+	–	–
2	*Vexillifera* sp.	+	+	+
3	*V. lata*	+	–	+
4	*Cochliopodium* sp.	+	–	+
5	*P. digitata*	+	–	–
6	*M. vespertilioides*	+	–	+
7	*Mayorella* sp.	–	–	+
8	*T. sphaeronucleolus*	+	–	–
9	*Thecamoeba* sp.	–	+	–
10	*S. stenopodia*	–	+	+
11	*Acanthamoeba* sp.	+	–	–
12	*Vahlkampfia* sp.	+	–	–
Total		9	3	6

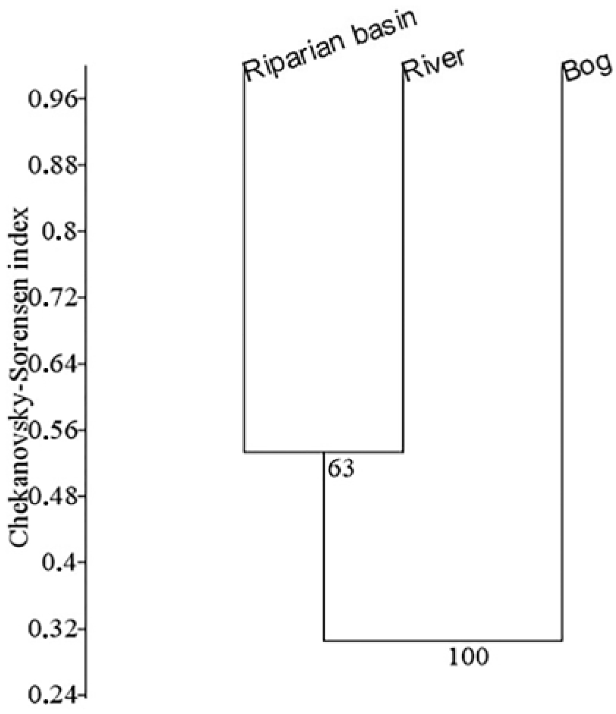

Fig. 3. Similarity of naked amoebae species complexes, according to the Chekanovsky–Sørensen index (cluster probability shown as % at the nodes, bootstrap 1000).

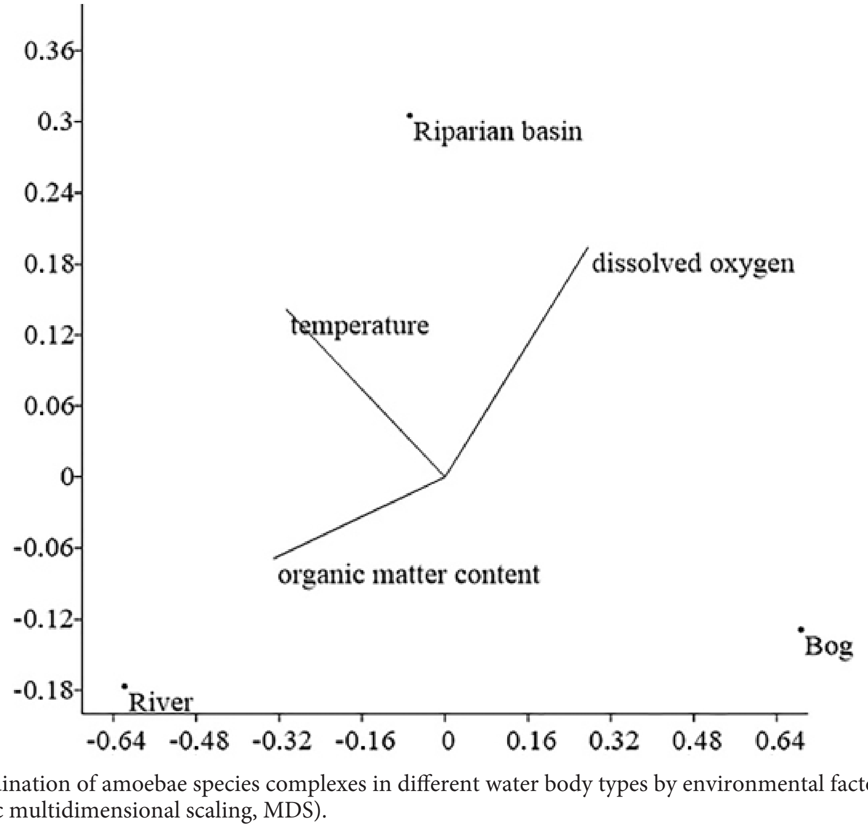

Fig. 4. Ordination of amoebae species complexes in different water body types by environmental factors (non-parametric multidimensional scaling, MDS).

possible for a species of a given morphotype to be found in different conditions. However, the affinity of several morphotypes to certain combinations of environmental conditions is confirmed by the higher quantities of amoebae in what probably are their optimal habitats.

Since naked amoebae are cosmopolitan species, found in benthos and periphyton of marine and freshwater ecosystems, present in almost all biotopes, and a staple of food chains, we tried to analyze their species composition in various water bodies of Sumy Region. We used the classification of continental waters, accepted in hydrobiology (Konstantinov, 1986). The protist distribution in various water bodies of the Region is presented in table 3.

Analysis of the water body type (river, bog, riparian basin) and the corresponding diversity of amoebae allowed us to determine characteristics of amoebae, typical of waters of different types.

The rivers of Sumy Region were richer in amoebae (nine species) than the riparian basins (six species) and bogs (three species).

Strictly freshwater species in rivers of Sumy Region are *Saccamoeba* sp., *P. digitata, T. sphaeronucleolus, Acanthamoeba* sp. and *Vahlkampfia* sp. In the bogs we found *Thecamoeba* sp., in riparian basins *Mayorella* sp.

Analyzing species composition of the waters of the studied Region, one should note sporadic occurrence of most species. The most frequently found are *Vexillifera* sp. (6 WB), *V. lata* (5 WB), *Cochliopodium* sp. (5 WB), which is 40 % and 33 %, respectively. *T. sphaeronucleolus* and *P. digitata* were recorded only once (6 %), and so can be considered regionally rare.

Cluster analysis using the Chekanovsky–Sørensen index showed that the highest faunistic similarity was observed in rivers and riparian basins (0.53), and in riparian basins and bogs (0.44), while the species composition of rivers and bogs were most dissimilar (0.17).

According to the analysis (fig. 3), naked amoebae complexes formed two clusters. One of them included rivers and riparian basins and the other one only bogs. Bootstrap analysis (1000 permutations) showed the 63 % and 100 % probabilities of cluster existence, respectively. Species composition of naked amoebae in a certain water body probably to a large extent depends on the distance to a river, where the protist fauna is richest. Species composition of amoebae is different only in bogs with unusual environmental characteristics. Bog waters in the studied area are generally weakly acidic with low content of organic matter, confirmed by low permanganate oxidation compared to rivers and riparian basins.

The diversity of naked amoebae in the studied Region is influenced by hydrophysical and hydrochemical habitat parameters. Species complex of amoebae in rivers (and riparian basins) tends to the highest content of organic matter and highest temperature (fig. 4). The complex includes *V. lata*, *Vexillifera* sp. and *Cochliopodium* sp., which according to our previous research tolerate high content of dissolved organic matter, and *V. lata* and *Cochliopodium* sp. are eurythermal (Patcyuk, 2013). Species composition of amoebae in bogs is shaped by low dissolved organic matter and lower temperatures. As to the dissolved oxygen, our research showed that it only weakly influences species complexes of bogs, rivers and riparian basins. We have not finished studying the distribution of naked amoebae in Ukraine, so the paucity of the species we found so far could be just a statistical fluke since we only surveyed a few water bodies; this will be addressed in further research.

Thus, it is highly probable that the above listed amoebae species are typical for the Sumy Region, which should be thoroughly checked in further faunistic studies.

As to morphotypes, the dactylopodial amoebae occur in all kinds of water bodies in the Sumy Region according to our data. The fan-shaped, lens-like, and mayorellian morphotypes are recorded in rivers and riparian basins. The rugose, monopodial, flamellian, acanthopodial and eruptive morphotypes are common in rivers. The striate morphotype is common in bogs; lingulate amoebae are usual in bogs and riparian basins. Supposedly, the distribution depends on hydrochemical and trophic parameters of the Sumy Region water bodies. The data allowed us identify the habitat parameters that could induce specific morphotypes of naked amoebae as adaptive mechanisms.

Conclusion

Twelve species of naked amoebae belonging to ten morphotypes were identified in water bodies of Sumy Region. Euryoxidic (*V. lata*), stenooxidic (*Vexillifera* sp., *S. stenopodia, Cochliopodium* sp.), and stenobiontic (*S. stenopodia*) ecological preferences were observed. *Vexillifera* sp., *V. lata, Cochliopodium* sp. tolerates wide range of permanganate oxidation.

Nine amoeba species in the Sumy Region were found in rivers, six were identified in riparian basins, and only three were recorded in bogs. The species composition of naked amoebae showed two species complexes: bog and riparian (including species mostly found in the rivers and in drainage basins). The species complexes of rivers, riparian basins and bogs were influenced by hydrophysical and hydrochemical factors of the habitat. Species composition of the naked amoebae of rivers and riparian basins is linked to higher temperature and higher organic matter content than the bog species complex.

Certain morphotypes tend to occur under specific environmental parameters. This is confirmed by higher numbers of species of the morphotypes in these conditions which likely are nearly optimal for them.

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