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## MORPHOTYPES IN NAKED AMOEBAS (PROTISTA): DISTRIBUTION IN WATER BODIES OF ZHYTOMYR AND VOLYN POLISSIA (UKRAINE) AND POSSIBLE ECOLOGICAL SIGNIFICANCE

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**Morphotypes in Naked Amoebas (Protista): Distribution in Water Bodies of Zhytomyr and Volyn Polissia (Ukraine) and Possible Ecological Significance.** Patsyuk, M. K. — The biotopic and seasonal distributions of the naked amoeba species related to the specific morphotypes were analyzed on the territory of Zhytomyr and Volyn Polissya. It was demonstrated that the polytactic and, to some extent, rugose and branched morphotypes of naked amoebas have the adaptive significance and could have emerged as a result of adaptation to characteristic conditions of oligotrophic lakes. In turn, the formation of lanceolate morphotype may be associated with the adaptation to low water temperatures, whereas the formation of flamellian morphotype may be an adaptation to high temperatures.

**Key words:** naked amoebas, morphotype, distribution, Polissia.

**Морфотипы голых амёб (Protista): распределение по водоёмам Житомирского и Волынского Полесья (Украина) и возможное экологическое значение.** Пацюк М. К. — Проанализированы биотопическое и сезонное распределения видов голых амёб, принадлежащих к определённым морфотипам, на территории Житомирского и Волынского Полесья. Показано, что политактический и в некоторой степени ругозный и разветвлённый морфотипы имеют адаптивное значение и могли сформироваться в результате адаптации к условиям, которые характерны для олиготрофных озёр. В свою очередь, формирование ланцетообразного морфотипа может быть связано с адаптацией к низким температурам воды, а фламелльного — к высоким.

**Ключевые слова:** голые амёбы, морфотип, распределение, Полесье.

### Introduction

It is the living material that is known to be suitable for identification of naked amoebas. Nowadays, the preliminary identification of naked amoebae morphotypes is also required.

Smirnov and Goodkov (1999) proposed to use the morphotype for amoeba identification and believed that the morphotype is the particular “concept of cell organization”, a set of attributes that describes the dynamically stable organization of amoeba locomotor form (general outlines, profile of cross-section, the presence of folds or ridges on the dorsal surface and structural features of uroid structures).

The authors recognized the following morphotypes of the naked amoebas: polytactic, monotactic, flamellian, flabellate, lens-like, striate, rugose, lingulate, lanceolate, fan-shaped, mayorellian, dactylopodial, acanthopodial, branched, and eruptive.

It is worth noting that under adverse or stressful environmental conditions certain amoeba species can change their morphotype. For example, such species as *Amoeba proteus* Leidy, 1878 demonstrates the polytactic morphotype under normal environmental conditions, whereas under changing conditions this amoeba can assume the temporary orthotactic morphotype (Grebecki, Grebecka, 1978). It is quite possible that the changes testify on the adaptive nature of morphotypes and can also reflect the physiological state of amoeba organism.

In this connection we believe that the morphotypes can serve as certain ecological characteristics of species, provided that those or other morphotypes have the adaptive value. To check this idea it is necessary to analyze the data about environmental conditions whereby the species with particular morphotypes dominate in the biotopes.

The issue on environmental significance of morphotypes in naked amoebas has not been previously discussed in the literature. Thus the present article focuses on the biotopic distribution, seasonal changes and compositions of naked amoebas' morphotypes in waterbodies of Zhytomyr and Volyn Polissia.

## Material and methods

The material was collected during 2009–2012 in the different waterbodies of Zhytomyr and Volyn Polissia. It should be noted that modern methods do not allow obtaining data about the abundance of amoebas. Therefore, we estimated only value ranges of the environmental factors, at which the certain naked amoeba species were observed. The dependence between various morphological types and certain environmental factors was analyzed using cluster analysis (Chekanowski-Sorensen's index).

For receiving materials the amoebas were cultivated on the non-nutrient agar by the Page's method (Page, 1988; Page, Siemansma, 1991).

Observations on the protists and photomicrographs were made using the light microscope Axio Imager M1 equipped with the differential interference contrast in the Centre of scientific devices collective usage "Animalia" of the Shmalhausen Institute of Zoology, Kyiv.

Identification of amoebas was made in two stages: first the identification of their morphotype following special studies (Smirnov, Goodkov, 1999; Smirnov, Brown, 2004; Smirnov, 2008); after that, if the data allowed, the keys to taxa of Page (Page, 1988; Page, Siemansma, 1991) together with the later publications on naked amoebas (Smirnov, Goodkov, 1994; Smirnov et al., 2007, 2011; Smirnov, 2008) were used.

Samples for the research of seasonal changes in naked amoeba populations were collected 3 times per month during the 2009–2010 years in the Kamyanka River in Zhytomyr neighborhood.

The following parameters of water were defined: the temperature, the content of oxygen and dissolved organic matter by permanganate oxidability — the amount of oxygen spent for the oxidation of dissolved organic matter (Stroganov, Buzinova, 1980).

## Results and discussion

According to our previous data (Patsyuk, 2012; Patsyuk, Dovgal, 2012), 40 naked amoeba species belonging to 13 morphotypes occurred in different waters of Zhytomyr and Volyn Polissia. Data on their distribution by types of waters are shown in the table 1, where the presence of certain species morphotype is marked with "+", and the absence — with "–".

Amoebas of the flamellian, fan-shaped, mayorellian, and dactylopodial morphotypes were found in all waterbody types (table 1), so it was not possible to evaluate their ecological significance.

As shown in the table 1, the largest numbers of naked amoeba species of monotactic morphotype (5 species) were observed in rivers and floodplains; those of mayorellian morphotype were in rivers and lakes (4 species), and those of dactylopodial morphotype were in floodplains (5 species).

The amoebas of the lingulate morphotype were recorded in bogs and floodplains; the lens-like morphotype in rivers, bogs, floodplains and lakes and the lanceolate morphotype in rivers and floodplains. As for the eruptive amoeba morphotype, the representatives were characteristic for all natural biotopes excepting lakes. It should be noted that the amoebas of lingulate morphotype were not found in rivers and canals, lens-like morphotype were absent in canals, whereas the representatives of lanceolate morphotype were absent in bogs, canals and lakes.

The species of amoebas representing the polytactic morphotype occurred only in lakes, the two species of rugose morphotype occurred in floodplains and lakes, whereas two amoeba species of branched morphotype were found both in bogs and lakes.

In order to establish the nature of the naked amoeba morphotypes distribution we calculated the indexes of faunal similarity between the sets of naked amoeba morphotypes from different modes of waterbodies (table 2, fig. 1).

As illustrated (fig. 1), there are two clusters of waterbodies clearly separated by morphotype compositions. One of them includes the lakes, whereas the other one integrates the land reclamation canals, bogs, rivers and floodplains. The probabilities of these two clusters based on the Bootstrap-based analysis were 100 % for the first cluster and 53 % for the second cluster.

Apparently, it is the presence of amoeba species of polytactic and partly rugose and branched morphotypes that primarily determines the originality of the lake cluster.

It is known that the morphotype of naked amoebas may also reflect the physiological state of their cell and depend on the environmental conditions. In this regard, information on adaptive and, therefore, ecological significance of morphotypes can be obtained *in vivo*,

Table 1. Distribution of naked amoeba morphotypes in the different waterbodies of Ukrainian Polissia

Таблица 1. Распределение морфотипов голых амёб по типам водоёмов Украинского Полесья

Naked amoeba morphotypes	Naked amoeba species	Waterbody types				
		River	Bog	Canal	Floodplain	Lake
Polytactic	<i>Amoeba proteus</i> Leidy, 1878	-	-	-	-	+
	<i>Polychaos</i> sp.	-	-	-	-	+
Total		0	0	0	0	2
Monotactic	<i>Deuteroamoeba mycophaga</i> Pussard, Alabouvette et Pons, 1980	+	-	-	+	-
	<i>Saccamoeba stagnicola</i> Page, 1974	+	-	-	+	-
	<i>Saccamoeba wakulla</i> Bovee, 1972	-	-	-	-	+
	<i>Saccamoeba</i> sp. (1)	+	+	+	+	+
	<i>Saccamoeba</i> sp. (2)	+	+	-	+	-
	<i>Saccamoeba</i> sp. (3)	+	-	-	+	+
Total		5	2	1	5	3
Flamellian	<i>Flamella</i> sp.	+	+	+	+	-
	<i>Pellita digitata</i> (Greef, 1866)	-	-	-	-	+
	Smirnov et Kudryavtsev, 2004	-	-	-	-	+
Total		1	1	1	1	1
Lens-like	<i>Cochliopodium</i> sp. (1)	+	+	-	+	-
	<i>Cochliopodium</i> sp. (2)	-	-	-	-	+
Total		1	1	0	1	1
Striate	<i>Thecamoeba striata</i> Penard, 1890	+	+	+	+	-
	<i>Thecamoeba quadrilineata</i> Carter, 1856	-	-	-	+	-
Total		1	1	1	2	0
Rugose	<i>Thecamoeba verrucosa</i> Ehrenberg, 1838	-	-	-	+	-
	<i>Thecamoeba sphaeronucleolus</i> Greef, 1891	-	-	-	-	+
Total		0	0	0	1	1
Lingulate	<i>Stenamoeba stenopodia</i> (Page, 1969) Smirnov et al., 2007	-	+	-	+	-
	<i>Stenamoeba</i> sp.	-	-	-	-	+
Total		0	1	0	1	1
Lanceolate	<i>Paradermamoeba valamo</i> Smirnov et Goodkov, 1993	+	-	-	+	-
	<i>Paradermamoeba levis</i> Smirnov et Goodkov, 1994	+	-	-	+	-
Total		2	0	0	2	0
Fan-shaped	<i>Ripella platypodia</i> (Glaeser, 1912) Smirnov, Nassonova, Chao et Cavalier-Smith, 2007	-	-	-	-	+
	<i>Ripella</i> sp.	-	+	-	-	-
	<i>Vannella lata</i> Page, 1988	+	+	+	+	-
Total		1	2	1	1	1
Mayorellian	<i>Mayorella viridis</i> Leidy, 1874	-	-	-	-	+
	<i>Mayorella cantabrigiensis</i> Page, 1983	+	+	-	+	-
	<i>Mayorella vespertilioides</i> Page, 1983	+	-	-	+	+
	<i>Mayorella penardi</i> Page, 1972	-	-	-	-	+
	<i>Mayorella</i> sp. (1)	+	+	+	+	+
Total		4	2	1	3	4
Dactylopodial	<i>Korotnevella stella</i> Schaeffer, 1926	+	-	-	+	-
	<i>Korotnevella diskophora</i> Smirnov, 1999	-	-	-	+	-
	<i>Korotnevella</i> sp.(1)	-	-	-	+	+
	<i>Korotnevella</i> sp.(2)	-	-	-	+	+
	<i>Vexillifera</i> sp.	+	+	+	+	+
Total		2	1	1	5	3
Branched	<i>Rhizamoeba</i> sp. (1)	-	+	-	-	-
	<i>Rhizamoeba</i> sp. (2)	-	-	-	-	+
Total		0	1	0	0	1
Eruptive	<i>Vahlkampfia</i> sp. (1)	+	+	+	+	-
	<i>Vahlkampfia</i> sp. (2)	+	+	+	+	-
	<i>Willaertia</i> sp.	+	-	-	-	-
Total		3	2	2	2	0

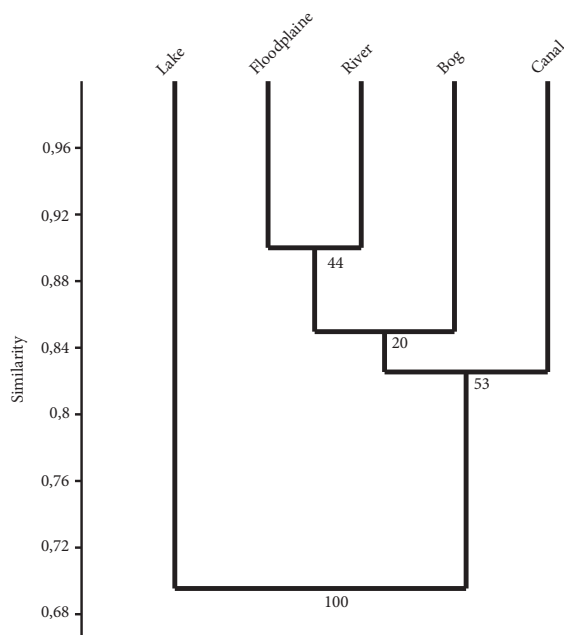


Fig. 1. The similarity between morphotype compositions in different types of waterbodies based on the Chekanowski-Sorensen's index (the probabilities of clusters (in %) based on Bootstrap analysis with 1000 repositions are marked in the nodes of the dendrogram).

Рис. 1. Сходство состава морфотипов голых амёб по индексу Чекановского-Съёренсена (в узлах дендрограммы указаны вероятности кластеров (%) по результатам бутстреп-анализа при 1000 перестановок).

by tracking the seasonal distribution of certain morphotypes with simultaneous detection of changes in environmental factors.

The results on morphotype seasonal distributions in the Kamyanka River are presented in table 3. As table 3 suggests, 9 naked amoeba morphotypes have been identified during period of observations: monotactic, flamellian, lens-like, striate, lanceolate, fan-shaped, mayorellian, dactylopodial and eruptive.

The seasonal changes of some environmental factors are shown in table 4.

The amoebas of monotactic, flamellian, lens-like, striate, fan-shaped, mayorellian, dactylopodial and eruptive morphotypes were present in April, June and July. The amoebas of lens-like, striate, mayorellian and eruptive morphotypes dominated in December; lens-like, lanceolate, fan-shaped and eruptive morphotypes were more numerous in January, whereas monotactic, lens-like, lanceolate and eruptive morphotypes in February (table 3).

It should be mentioned that the maximum species richness (2 species) of amoebas of monotactic morphotype was observed in April, May, August and September; of lanceolate morphotype in January, February and March; of mayorellian morphotype in April, August

Table 2. The values of Chekanowski-Sorensen's index between lists of naked amoeba morphotypes in the different modes of waterbodies

Таблица 2. Индексы фаунистического сходства между комплексами морфотипов голых амёб водоёмов разных типов (Индекс Чекановского-Съёренсена)

Waterbody types	River	Bog	Canal	Floodplain	Lake
River	1	0.84	0.88	0.90	0.63
Bog	–	1	0.82	0.86	0.80
Canal	–	–	1	0.78	0.59
Floodplain	–	–	–	1	0.76
Lake	–	–	–	–	1

Table 3. Naked amoeba morphotypes observed in the Kamyanka River (Zhytomyr) in different seasons

Таблица 3. Сезонное распределение морфотипов голых амёб в р. Каменка (Житомир)

Naked amoeba morphotypes	Months											
	01	02	03	04	05	06	07	08	09	10	11	12
Monotactic		+	+	+	+	+	+	+	+	+	+	
Flamellian				+		+	+					
Lens-like	+	+	+	+	+	+	+	+	+	+	+	+
Striate				+	+	+	+	+	+	+	+	+
Lanceolate	+	+	+									
Fan-shaped	+		+	+	+	+	+	+	+	+		
Mayorellian				+	+	+	+	+	+	+		+
Dactylopodial				+	+	+	+	+	+	+	+	
Eruptive	+	+	+	+	+	+	+	+	+		+	+
Total	4	4	5	8	7	8	8	7	7	6	5	4

and September; of dactylopodial morphotype in April, May, June, July, August and September; and of eruptive morphotype in April, May, July and August. The naked amoebas of monotactic, striate, fan-shaped, mayorellian, dactylopodial and eruptive morphotypes were observed in all seasons (table 3).

According to our data, only the amoebas of lanceolate morphotype prefer cold season (found in January, February and early March); the species of this morphotype in other seasons were not registered. Moreover, we have repeatedly found the amoebas of this morphotype in winter in other waters. Amoebas of this morphotype occurred at the temperature from + 3 °C to + 6 °C, content of oxygen dissolved in the water from 3.48 mg/L to 8.35 mg/L and content of organic substances dissolved in the water (by permanganate oxidability) from 10.54 mg O<sub>2</sub>/L to 36.21 mg O<sub>2</sub>/L (table 4). It should be noted, that the temperature range in the Kamyanka River for the investigation period varied from + 3 °C to + 26 °C, content of oxygen dissolved in the water amounted from 3.05 mg/L to 17.31 mg/L, and content of organic substances dissolved in the water varied from 2.17 mg O<sub>2</sub>/L to 50.01 mg O<sub>2</sub>/L (table 4).

Vice versa, the amoebas of flamellian morphotype were found only in the warm seasons. Amoebas of this morphotype were found at the temperature of water from +16 °C to + 26 °C, content of oxygen dissolved in the water from 3.05 mg/L to 12.45 mg/L and the permanganate oxidability from 28.98 mg O<sub>2</sub>/L to 50.01 mg O<sub>2</sub>/L (table 4).

Thus the amoebas of lanceolate and flamellian morphotypes prefer certain seasons of the year and these morphotypes may be associated with adaptations to such factor as water temperature.

We can assume that the morphotype composition depends on the hydrochemical and tropical peculiarities of Lakes of Shatsk, as well as on the bottom soil composition and

Table 4. Hydrochemical parameter values in the Kamyanka River (Zhytomyr) during the 2009–2010 years

Таблица 4. Значение гидрохимических показателей р. Каменка (Житомир) в течение 2009–2010 гг.

Month	Temperature, °C	Content of oxygen dissolved in the water, mg/L	Permanganate oxidability, mg O <sub>2</sub> /L
January	+4	8.35	30.84
February	+3	3.48	10.54
March	+6	3.81	36.21
April	+16	12.45	38.03
May	+20	17.31	13.28
June	+26	3.05	50.01
July	+24	10.54	28.98
August	+24	12.69	28.26
September	+14	17.21	13.29
October	+10	9.45	9.54
November	+3	11.85	9.03
December	+4	9.56	2.17

character. Lakes of Shatsk have the fluvioglacial origin, with mostly sandy-muddy bottom. The water is calcium-hydrocarbonate with low level of mineralization. The temperature range for the research period varied from +4 °C to +20 °C, content of the dissolved oxygen in the water from 3.05 mg/L to 12.08 mg/L, content of the dissolved in the water organic substances (by permanganate oxidability) from 3.54 mg O<sub>2</sub>/L to 25.12 mg O<sub>2</sub>/L. Thus the polytactic and to some extent rugose and branched morphotypes might have been formed as a result of adaptation to the mentioned conditions.

Thus, according to our observations, the polytactic and, to some extent rugose and branched morphotypes of naked amoebas have the adaptive value and could emerge as a result of adaptation to conditions that are characteristic of oligotrophic lakes. In turn, the formation of lanceolate morphotype may be associated with adaptation to low water temperatures whereas the flamellian type may be an adaptation to high temperatures.

However, it is not likely that particular amoeba morphotypes are strictly specific to certain environmental conditions. It should be noted that the finds of species belonging to a certain morphotype in other conditions are quite possible. However, the connection between particular morphotypes and particular sets of environmental conditions indicates that the species of such morphotypes are more numerous under such conditions which are probably close to optimal.

The received information allows defining a set of environmental factors to which the species of particular morphotypes (polytactic, rugose, branched, lanceolate, and flamellian) may be adapted. In the future this may be the basis for a special study of the ecological importance of morphotypes, using experimental and laboratory methods.

Thus, further studies of adaptive values of morphotypes are quite perspective and allow to get the new information about autecology and physiology of naked amoebas.

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