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Research Article

The community structure of Testate amoebae (Amoebozoa, Rhizaria) and their relation to water pH in reservoirs in Northeastern Bulgaria

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Abstract

The identification of variability in testate amoebae communities and their environmental dependencies makes it possible to clarify many issues related to the changes in natural and artificial ecosystems. The present study covers the benthic testate amoebae fauna of seven reservoirs: Brestovene, Beli Lom, Loznitsa, Kara Michal, Bogdantsi, Isperih, and Lipnik. Regression analysis and canonical correspondence analysis were performed to determine differences in testate amoebae communities between reservoirs. The obtained results show that in the Bogdantsi Reservoir, only two genera, *Centropyxis* and *Difflugia*, dominate, which, together with the established extremely low taxonomic diversity and abundance of testate amoebae, is an indication of the presence of strong eutrophication and unsuitable conditions in it. In the Loznitsa and Beli Lom reservoirs, dominance is distributed among a large number of genera, and a significantly larger number of species and individuals are found, which indicates the presence of a sustainable and suitable environment for development of testate amoebae. Our CCA results suggest that species distribution differs along the pH gradient. The species *Centropyxis aerophila, C. aerophila v. sphagnicola*, *Difflugia pristis*, and *Schonbornia viscicula* have a very positive correlation with pH, while *Cyclopyxis ambigua*, *Difflugia minuta*, and *D. elegans* have a negative correlation with pH.
Keywords: bioindicators, diversity, dominance, ecosystems.

Introduction

Environmental studies on testate amoebae worldwide are extremely popular and intense. Research in this field provides more accurate quantitative data on the presence, environment preferences and requirements of species, their distribution in different living environments and their role in natural ecosystems. These studies demonstrate the critical importance of establishing the structure of testate amoebae communities in different regions and biotopes, thus revealing the factors influencing the development of communities and allowing to anticipate the impact of environmental change on biodiversity.

The identification of variability in testate amoebae communities and their environmental dependencies makes it possible to clarify many issues related to biogeography of these organisms, as well as their role as bioindicators (Qin et al., 2009; Roe & Patterson, 2014; Arrieira et al., 2016; Ndayishimiye et al., 2021; Tran at al., 2021; Freitas et al., 2022). In this way testate amoebae are very important organisms for studying the role of environmental changes in the sustaining of biodiversity. The studies have shown that the abundance of each species of testate amoebae, and thus the structure of the communities, is controlled by a number of environmental factors, such as depth, dissolved oxygen, redox potential, pH, conductivity, temperature, eutrophication, salinity, metal and organic pollutant contamination, etc. (e.g. Patterson et al., 1985, 1996, 2002, 2012, 2013; Asioli et al., 1996; Dalby et al., 2000; Kumar & Patterson, 2000; Patterson & Kumar, 2000; Roe & Patterson, 2006, 2014; Escobar et al., 2008; Qin et al., 2009, 2013, 2024; Roe et al., 2010; Wall et al., 2010; Arrieira et al., 2015, 2016, 2017; Nasser et al., 2016; Tsyganov et al., 2019; Sysoev et al., 2024).

According to many authors, the taxonomic composition of the communities is influenced by the pH of the environment (Proctor & Maltby, 1998; Bobrov et al., 1999; Mitchell et al. 2000; Booth, 2002; Mitchell & Gilbert, 2004; Vincke et al., 2004; Opravilova & Hajek, 2006; Swindles & Roe, 2007; Escobar et al., 2008; Nguyen-Viet et al., 2008; Lorencova, 2009; Payne, 2010; Jassey et al., 2012; Tsyganov, 2012; Patterson et al., 2013; Mieczan & Adamczuk, 2015; Barnett et al., 2016; Arrieira et al., 2017; Siver et al., 2020). For example, Mieczan (2007a, b) found that the pH of the environment is in a positive correlation with the total number and the biomass of testate amoebae in biotopes, dominated by *Sphagnum*. Mieczan & Adamczuk (2015) and Song et al. (2018) point out that pH was one of the most important factors controlling distribution patterns in testate amoebae assemblages in mosses and peatlands. Arrieira et al. (2015) found that in the floodplain some species such as *Arcella discoides*, *A. mitrata* and *Euglypha acantophora* have a positive correlation with pH, while

Cucurbitella mespeliformis is in a negative correlation with it. Heger et al. (2016) conclude that litter pH significantly explained testate amoeba community structure in moss and soil in the Swiss Alps and that several species were either positively (e.g. *Trachelocorythion pulchellum*, *Tracheleuglypha dentata*, *Cyclopyxis eurystoma*, *Centropyxis aerophila*) or negatively (e.g. *Nebela tincta*, *Plagiopyxis callida*, *Phryganella acropodia*) correlated to litter pH.

The studies of Kumar & Patterson (2000), Patterson & Kumar (2000), Patterson et al. (2002, 2013), Arrieira et al. (2017) show the extreme importance of the variability of pH in lakes for the distribution of Arcellinida and for the formation of testate amoebae species communities in lacustrine habitats. Kumar & Patterson (2000) indicate that the species of the genus *Centropyxis* and *Difflugia protaeiformis* strains are indicative of contaminated substrates in higher pH lakes and that pH has dominant control on the distribution of this assemblage. Studying ecology of testate amoebae in peatlands of Central China Qin et al. (2013) establish a higher diversity of testate amoebae in alkaline lakes, pointing out strong correlations of the community with water pH. *Pontigulasia elisa*, *P. compressa* and *Lesquereusia modesta* were the most abundant taxa, making them indicators of higher pH (Qin et al., 2013). The researches by Siver et al. (2020) show that water depth and pH play an important role in the growth and occurrence of testate amoebae bearing siliceous plates in freshwater lakes and ponds along the east coast of North America. According to Qin et al. (2024) testate amoebae communities were strongly affected by the physicochemical properties of the lakes, especially water temperature and pH. The aims of the present work are to analyze the testate amoebae community structure and to investigate the relationship between testate amoebae populations and water pH in the sediments of seven reservoirs in Northeastern Bulgaria.

Material and methods

The present study covers the benthic testate amoebae fauna of seven reservoirs: Brestovene, Beli Lom, Loznitsa, Kara Michal, Bogdantsi, Isperih, and Lipnik located on the territory of the Razgrad District in Northeastern Bulgaria (Fig. 1). The studied reservoirs are used for fish farming, fishing, recreation and water sports, and are visited by many people. This, as well as the fact that they are surrounded by cultivated areas, shows that they are exposed to strong anthropogenic pressure. In the summer months, due to the drought, the water volume of the reservoirs decreases, the tributaries dry up and there is no inflow of fresh water. This is the reason for the intensification of putrefaction processes. The reservoirs are relatively small and, except of the Beli Lom Reservoir, are not subject to water quality monitoring by the institutions. Five benthic samples were collected per reservoir, from the coastal zone at a depth from 0.5 m – a total of 35 samples. At each sampling point, we measured pH using portable digital pH meter (Consort, Belgium). Data used in our analyses are means of the five samples for each reservoir (Table 1).

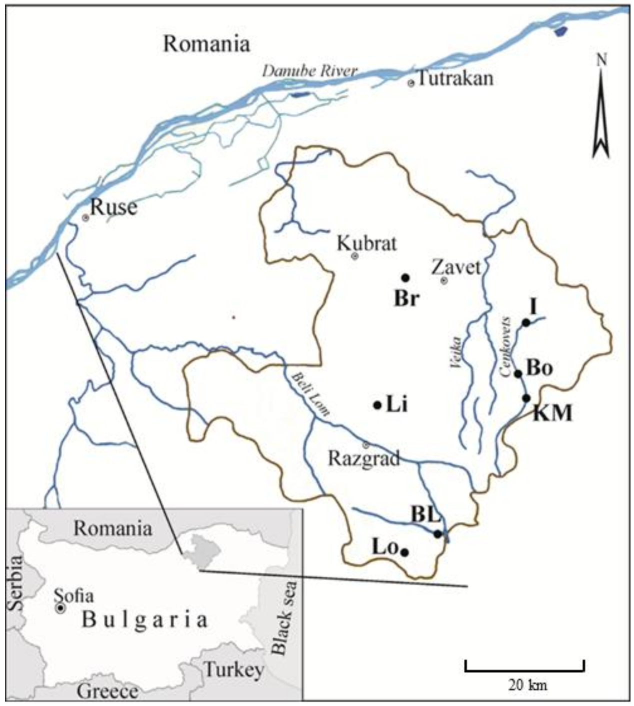

Figure 1. The map of the study region and the study reservoirs: Br - Brestovene, BL - Beli Lom, Lo - Loznitsa, KM - Kara Michal, Bo - Bogdantsi, I - Isperih, Li - Lipnik.

Table 1. Data for the investigated reservoirs.

Reservoirs	Coordinates (reservoir centers)	pH (mean value)
Brestovene	43°45'26.0"N 26°36'28.0"E	6.90
Beli Lom	43°23'09.9"N 26°40'57.5"E	6.82
Loznitsa	43°22'16.9"N 26°37'14.2"E	7.48
Kara Michal	43°35'20.7"N 26°50'23.7"E	7.36
Bogdantsi	43°37'25.4"N 26°49'45.0"E	7.55
Isperich	43°41'14.7"N 26°49'20.8"E	6.90
Lipnik	43°35'23.9"N 26°32'26.0"E	7.28

The frequency of occurrence of the particular species was calculated using the formula: pF = m/n x 100, where m is the number of samples in which one species was found and n is the total number of samples. Different species, depending on pF index, were divided into 3 categories as follows: constant - found in more than 50% of all samples; incidental – found in 25-50% of all samples; accidental – found in less than 25% of all samples. The relative abundance of each taxon was calculated as a percentage of the total count. The dominance was established on the basis of the relative abundance of each species D = n/N x 100, where n is the number of specimens of each species and N – the total number of all specimens. All species were divided into 4 groups: subrecedent – D < 1%; recedent – D between 1-2%; subdominant – D between 2-5%; dominant – D > 5%. Regression analysis and canonical correspondence analysis were performed to determine differences in testate amoebae communities between reservoirs. The data analysis was performed using STATISTICA and PAST program.

Results

Testate amoebae communities structure

The frequency of occurrence and the relative abundance of each species in the reservoirs are presented in Table 2. Analysis of the data showed the largest number of constant species – 5, in the benthal of the Loznitsa Reservoir. Ordered by decreasing frequency of occurrence these are *Centropyxis aerophila v. sphagnicola*, *Cyclopyxis eurystoma*, *Euglypha rotunda*, *Plagiopyxis declivis* and *Schonbornia viscicula*. Of the remaining 22 species, 9 are incidental, and 8 are rare, with a frequency of less than 25%, which belong to the group of accidental species. Four constant species – *C. aerophila v. sphagnicola*, *C. eurystoma*, *Difflugia gramen* and *P. declivis* were found in the Isperih Reservoir. Among the other testate amoebae, 2 are incidental and 11 are accidental. In each of the reservoirs: Brestovene, Beli Lom, Kara Michal, and Lipnik were found 2 constant species with distribution as follows: In the Brestovene Reservoir were found *C. eurystoma* and *Difflugia sarissa*; in the Beli Lom Reservoir – *Arcella hemisphaerica f. undulata* and *E. rotunda*; in the Kara Michal Reservoir – *Hyalosphaenia papilio*, found in all the samples examined, and *D. sarissa*; in the Lipnik Reservoir – *C. aerophila* and *C. aer. v. sphagnicola*. In all these reservoirs the greatest number is that of accidental species. In the Bogdantsi Reservoir all three species have a frequency of less than 50% and belong to the groups of incidental (1) and accidental (2) species. In the Brestovene and Bogdantsi reservoirs all established genera are dominant. In the Kara Michal, Isperih, and Lipnik reservoirs the number of dominant genera was also high and reached 75%, 80% and 83.33% respectively of the number of genera in the reservoir. In the Beli Lom and Loznitsa reservoirs, which are characterised by the greatest generic variety, the number of dominant genera was respectively 33.33% and 46.15% of all genera found in the reservoir.

A total of 10 genera are dominant in the testate amoebae communities in studied reservoirs, but their share in the formation of the communities in each reservoir is different. The representatives of the genera *Difflugia* and *Centropyxis* dominate the communities in the Bogdantsi, Isperih, and Lipnik reservoirs, as well as in the Brestovene and Kara Michal reservoirs (Fig. 2).

Table 2. Frequency of occurrence (pF) and relative abundance (D) of testate amoebae in the reservoirs.

Testacea	Brestovene		Beli Lom		Loznitsa		Kara Michal		Bogdantsi		Isperich		Lipnik	
	pF	D	pF	D	pF	D	pF	D	pF	D	pF	D	pF	D
***Arcella** discoides* Ehrenberg, 1843	–	–	–	–	–	–	–	–	–	–	–	–	20	3,12
A.disc. v. scutelliformis Playfair, 1918	–	–	20	2,79	40	1,53	–	–	–	–	–	–	20	3,12
A.disc. v. foveosa Playfair, 1918	–	–	20	1,39	–	–	–	–	–	–	–	–	–	–
A.hemisphaerica Perty, 1852	–	–	40	3,47	–	–	20	2,78	–	–	–	–	–	–
A.hem. f. undulata Deflandre, 1928	–	–	60	19,4	–	–	–	–	–	–	–	–	–	–
A.hem.v. intermedia f. undulata Deflandre, 1928	–	–	20	17,4	–	–	–	–	–	–	–	–	–	–
***Centropyxis** aculeata* Ehrenberg, 1838	20	5,00	40	1,39	40	1,53	–	–	–	–	–	–	–	–
C. aerophila Deflandre, 1929	–	–	20	0,69	40	3,82	–	–	–	–	20	1,85	60	9,38
C. aer. v. sphagnicola Deflandre, 1929	–	–	20	0,69	60	9,16	20	2,78	–	–	80	9,88	60	34,4
C. constricta Ehrenberg, 1841, Penard, 1890	–	–	–	–	–	–	–	–	20	25	–	–	–	–
C. deflandriana Bonnet, 1959	–	–	–	–	–	–	–	–	–	–	40	3,09	–	–
C. ecornis Ehrenberg, 1841	–	–	–	–	–	–	40	5,56	–	–	–	–	–	–
C. hirsuta Deflandre, 1929	–	–	–	–	20	0,77	20	2,78	20	25	–	–	20	3,12
C. platystoma Penard, 1890	–	–	–	–	20	1,53	–	–	–	–	20	1,22	–	–
C. sylvatica Deflandre, 1929	–	–	–	–	20	0,77	–	–	–	–	20	0,62	–	–
***Corythion** pullchelum* Penard, 1890	–	–	–	–	20	0,77	–	–	–	–	–	–	–	–
***Cyclopyxis** ambigua* Bonnet & Thomas,1960	–	–	–	–	–	–	–	–	–	–	40	6,17	–	–
C. eurystoma Deflandre, 1929	80	30,0	–	–	60	18,3	20	5,56	–	–	80	5,56	40	6,24
C. kahli Deflandre, 1929	–	–	40	1,39	40	1,53	–	–	–	–	–	–	–	–

***Difflugia** acuminata* Ehrenberg, 1838	–	–	–	–	–	–	–	–	–	–	20	0,62	–	–
D. ampullula Playfair, 1918	–	–	–	–	–	–	20	2,78	–	–	–	–	–	–
D. bryophila (Penard, 1902) Jung, 1942	–	–	20	0,69	–	–	–	–	–	–	–	–	–	–
D. decloitrei Godeanu, 1972	–	–	20	0,69	–	–	–	–	–	–	–	–	20	3,12
D. elegans Penard, 1890	20	5,00	–	–	–	–	–	–	–	–	–	–	20	3,12
D. glans Penard, 1902	–	–	20	0,69	–	–	–	–	–	–	–	–	20	3,12
D. globularis (Wallich, 1864) Leidy, 1877	20	5,00	–	–	–	–	–	–	–	–	20	0,62	20	9,38
D. globulosa Dujardin, 1837	–	–	40	1,39	–	–	–	–	–	–	–	–	–	–
D. gramen Penard, 1902	–	–	20	0,69	–	–	20	5,56	40	50	80	61,1	–	–
D. lacustris (Penard, 1899) Ogden, 1983	–	–	20	2,08	–	–	20	2,78	–	–	20	0,62	–	–
D. lobostoma Leidy, 1879	–	–	20	0,69	–	–	–	–	–	–	20	0,62	–	–
D. minuta Rampi, 1950	40	15,0	20	0,69	–	–	20	2,78	–	–	–	–	–	–
D. penardi Hopkinson, 1909	–	–	–	–	–	–	–	–	–	–	20	0,62	–	–
D. pristis Penard, 1902	–	–	–	–	–	–	40	5,56	–	–	–	–	20	3,12
D. pulex Penard, 1902	–	–	20	1,39	–	–	20	2,78	–	–	–	–	–	–
D. sarrisa Li Sun Taï, 1931	60	20,0	20	1,39	–	–	60	13,8	–	–	–	–	20	3,12
D. schurmanni van Oye, 1932	–	–	–	–	20	0,77	–	–	–	–	20	1,85	–	–
D. tenuis (Penard, 1890) Ogden, 1983	–	–	20	0,69	–	–	–	–	–	–	–	–	–	–
***Difflugiella** oviformis* Bonnet &Thomas, 1955	–	–	20	2,08	40	4,58	–	–	–	–	–	–	–	–
***Euglypha** rotunda* Wailes, Penard, 1911	–	–	60	2,08	60	14,5	–	–	–	–	–	–	–	–
***Hyalosphaenia** papilio* Leidy, 1874	–	–	–	–	–	–	100	30,6	–	–	–	–	–	–
H. punctata Penard, 1891	–	–	20	0,69	–	–	–	–	–	–	–	–	–	–
***Microchlamys** patella* (Clap, Lachmann, 1885) Cockerell, 1911	20	10,0	40	1,39	40	3,05	–	–	–	–	–	–	–	–
***Phryganella** acropodia* (Hertwig & Lesser, 1874) Hopkinson, 1909	–	–	–	–	20	0,77	–	–	–	–	–	–	–	–
P. hemisphaerica Penard, 1902	–	–	–	–	40	2,29	–	–	–	–	20	0,62	20	3,12

Plagiopyxis *declivis* Thomas, 1955	–	–	20	0,69	60	8,4	20	2,78	–	–	60	4,32	40	12,5
P. intermedia Bonnet, 1959	–	–	–	–	20	0,77	–	–	–	–	–	–	–	–
P. minuta Bonnet, 1959	–	–	–	–	–	–	–	–	–	–	20	0,62	–	–
Pontigulasia *rhumbleri* Hopkinson, 1909	–	–	20	0,69	–	–	–	–	–	–	–	–	–	–
Schonbornia *viscicula* Decloitre, 1964	20	5,00	–	–	60	3,82	40	5,56	–	–	–	–	–	–
Tracheleuglypha *acola* Bonnet & Thomas, 1955	–	–	20	15,3	40	3,05	–	–	–	–	–	–	–	–
Trinema *enchelys* (Ehrenberg, 1838) Leidy, 1878	20	5,00	20	14,6	20	2,29	40	5,56	–	–	–	–	–	–
T. lineare Penard, 1890	–	–	20	2,79	40	16	–	–	–	–	–	–	–	–

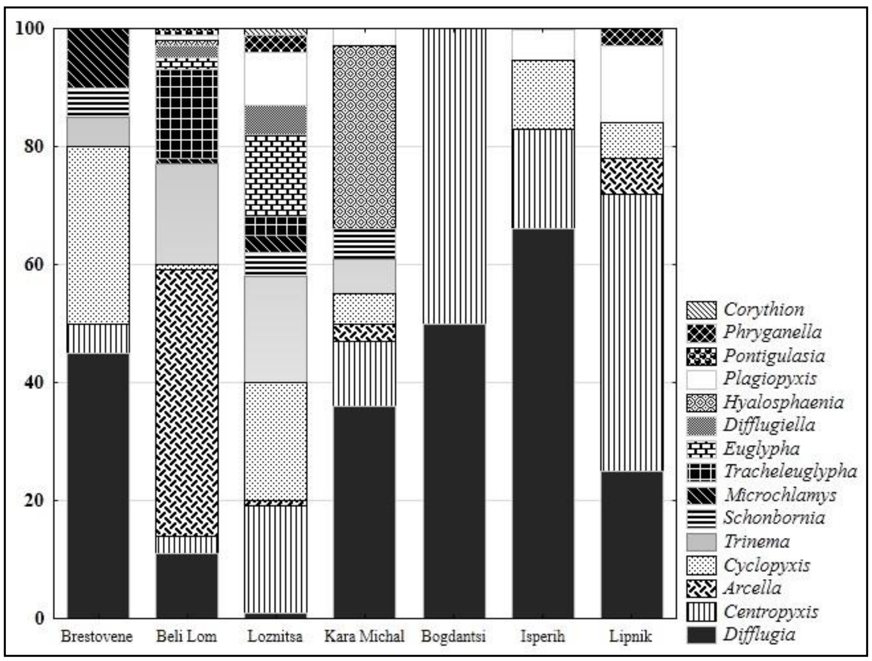

Figure 2. Relative abundance of the genera of testate amoebae in reservoirs.

However, the structure of the communities is different in the Beli Lom and Loznitsa reservoirs. In the benthal of the Beli Lom Reservoir with the highest relative abundance were the species of the genus *Arcella* (45%), followed by genera *Trinema* (17%) and *Tracheleuglypha* (15%), whereas in the Loznitsa Reservoir with a large number of specimens were genera *Cyclopyxis* (20%), *Centropyxis* (18%), *Trinema* (18%), *Euglypha* (14%), *Plagiopyxis* (9%) and *Difflugiella* (5%). A specific feature of the Kara Michal Reservoir is the significant share of the genus *Hyalosphaenia* (31%), which, however, is due to the development of only one species – *Hyalosphaenia papilio*.

The highest number of dominant species - 8 was found in the Kara Michal Reservoir, which comprise up to 77.9% of the total number of found specimens. The remaining 8 species belonged to the group of subdominants. 5 dominant species were found in the sediments of each of the Loznitsa and Lipnik reservoirs, which accounted for 66.4% and 71.9% respectively of the established specimens in the reservoirs. The distribution of the remaining species in the Loznitsa Reservoir was: 7 subdominant, 4 recedent and 6 subrecedent, while in Lipnik all the other 9 species were subdominant. In the Beli Lom and Isperih reservoirs were identified four dominant species, to which belonged respectively 66.7% and 82.7% of the specimens found in the reservoir. Of the remaining species in the Beli Lom Reservoir 6 were subdominant, 7 – recedent, and 12 – subrecedent, while in the Isperih Reservoir 2 species were subdominant, 3 – recedent, and 8 – subrecedent. In the Brestovene Reservoir and the Bogdantsi Reservoir all identified species were dominant.

Relationships between testate amoebae communities and water pH

The data of the regression analysis showed that a relation between water pH in the reservoirs and the species richness of testacea is not established (Fig. 3). The Canonical correspondence analysis also showed that the water pH was not significant in affecting the testate amoebae communities (CCA permutation test, $p>0.05$) (Fig. 4). But the ordination map suggests that species distribution differs along the pH gradient. The species *C. aerophila*, *C. aerophila v. sphagnicola*, *Difflugia pristis* and *S. viscicula* have a very positive correlation with pH, while *Cyclopyxis ambigua*, *Difflugia minuta*, and *D. elegans* have a negative correlation with pH.

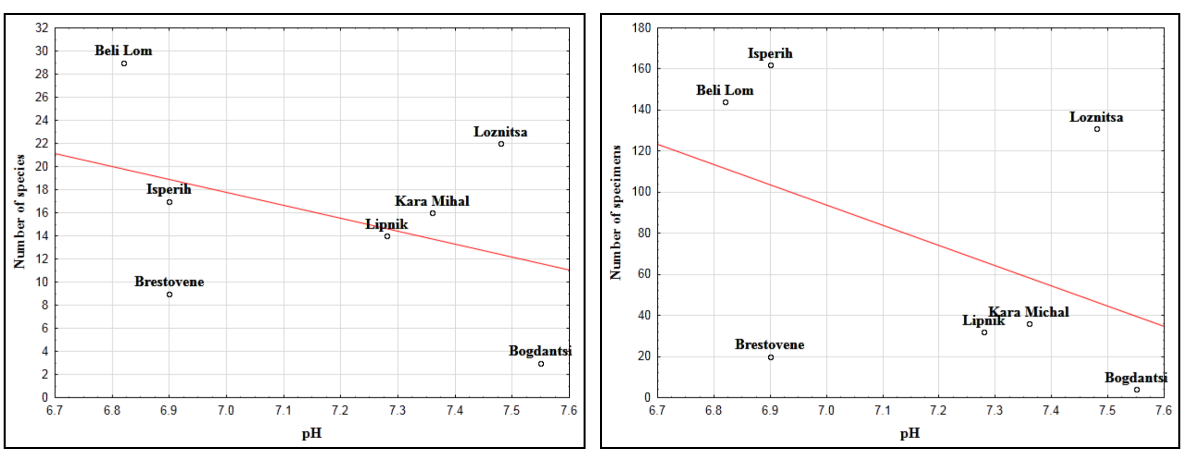

Figure 3. Regression analysis showing the relationship between the number of species and number of specimens of testate amoebae and water pH.

Discussion

The analysis of the data showed that a favorable dominant structure of the testate amoebae fauna was identified only in the Beli Lom and Loznitsa reservoirs. In these reservoirs the specimens were divided among all species and the number of subrecedent species exceeded that of the dominant ones. The relative abundance of the dominant species had roughly the same values. A similar dominant structure was observed in the Isperih Reservoir, but the dominance there was primarily linked to one species – *D. gramen*, whose relative significance considerably exceeded that of the other dominant species. In the other reservoirs, the dominant structure was extremely unfavorable. In the benthos of Kara Michal and Lipnik reservoirs only dominant and subdominant species were found, while in Brestovene and Bogdantsi reservoirs all the species were dominants.

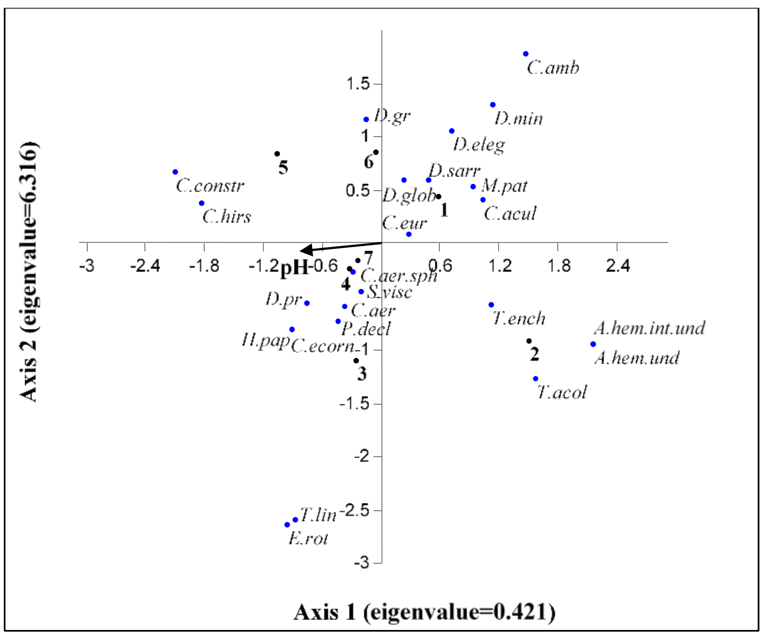

Figure 4. Canonical correspondence analysis scores of the pH and dominant testate amoebae. For full names of the taxa, see Table 2.

The comparison between the complexes of dominant species in individual reservoirs showed some difference between them. Some of the species were relatively widespread, they were present in most reservoirs, but were dominant only in 1, 2 or 3 of them. Such species are *C. aerophila* which was dominant in the Lipnik Reservoir, *Centropyxis hirsuta* – dominant in the Bogdantsi Reservoir, *D. sarrisa* – dominant in the Brestovene and Kara Michal reservoirs, *P. declivis* – in the Loznitsa and Lipnik reservoirs, *C. aer. v. sphagnicola* – in the Loznitsa, Isperih, and Lipnik reservoirs, *D. gramen* – in the Kara Michal, Bogdantsi, and Isperih reservoirs, and *T. enchelys* – in the Brestovene, Beli Lom, and Kara Michal reservoirs. It is important to note that 6 of the identified 24 dominant species, or 25%, were found only in a single reservoir. They were dominant and presented with a significant number of specimens. These were *A. hem. f. undulata* and *A. hem. v. intermedia f. undulata* in the Beli Lom Reservoir, *C. ecornis* and *H. papilio* in the Kara Michal Reservoir, *C. ambigua* in the Isperih Reservoir and *Centropyxis constricta* in the Bogdantsi Reservoir. Only one species – *C. eurystoma* was dominant in all 5 reservoirs, in which it was identified. The remaining 10 dominant species were relatively rare, present only in some of the reservoirs, and in one or two of them they were dominant. In the majority of the reservoirs: Brestovene, Kara Michal, Bogdantsi, Isperih, and Lipnik, a high relative share of the genera *Centropyxis* and *Difflugia* is observed, which indicates the presence in them of organic /industrial or natural/ pollution of the environment. A similar dependence has been indicated for other reservoirs in different parts of the world. A number of studies on the ecology of testate amoebae in lakes have found that most species of *Difflugia* and *Centropyxis* are more abundant in mesotrophic to eutrophic conditions or at high water pH (Asioli et al., 1996; Patterson et al., 1996; Kumar & Patterson, 2000; Beyens & Meisterfeld, 2001; Patterson et al., 2013; Macumber et al., 2014; Roe & Patterson, 2014; Heger et al., 2016).

In this study no significant correlation was found between the water pH and species richness and composition of the testate amoebae communities in different reservoirs. This is in agreement with results obtained by Kurina et al. (2010). Examining the population of testate amoebae from the bogs of Western Siberia the authors conclude that the composition of testacea communities and the shell density in the samples did not depend on the pH. Exploring correlations between the morphological structure of the benthic testate amoebae community and environmental variables along the depth gradient in a deep freshwater lake - Valdayskoe Lake Sysoev et al. (2024) did not find any correlation between pH and body size. But our results contrast with the conclusions from some other authors. Investigating ecology of testate amoebae in subtropical Florida lakes Escobar et al. (2008) showed that alkalinity and pH are the water variables that most influence the relative abundance of species. Patterson et al. (2013) indicate that there is a strong link between arcellacean faunas and pH levels based on the analysis of 22 samples in James and Granite lakes. These differences can be explained by the fact that those compared by Escobar et al. (2008) and Patterson et al. (2013) lakes differ significantly from each other in terms of pH. Florida lakes have pH values from 4.6 to 8.7, and Northeastern Ontario lakes have a significant pH gradient - from higher pH environments in the northern basin of James and Granite lakes /6.33-6.7/ to very low pH in the southern basin of James Lake /2.1-2.69/. The studied by as reservoirs have very close pH values /from 6.82 to 7.55/. On the other hand, however, Lorencova (2009), studying the testacean fauna inhabiting the sediments of 5 acidified to different degrees lakes in the Sumava Mts, where the pH varies within relatively narrow limits - from 4.5 to 5.8, concludes that the pH value of the water may have some affect on thecamoebian assemblages. However, the author examines the effect of this factor not on its own, but in combination with other factors such as trophy, abundance of phytoplankton and type of sediment. Moreover, Lorencova (2009) does not indicate whether the established relationship is significant. Qin et al. (2013) and Siver et al. (2020) found that both pH and water depth represent significant controls of testate amoebae community composition and the abundance of idiosomes of siliceous shells in sediments. Qin et al. (2024) showed that the testate amoebae community composition in lake sediments of the Qinghai-Tibet Plateau is significantly correlated with pH, but species richness is negatively correlated with water pH.

The our CCA results suggests some links between species and pH. Heger et al. (2016) also found that *C. aerophila* positively correlated with pH, and Barnett et al. (2016) indicated that *D. pristis* is positively correlative to pH. Regarding the latter species, Payne (2010) obtains completely different results – he notes that *D. pristis* type are negatively correlated with pH. Studying peatland testate amoeba communities Payne (2010) also indicates that *Trinema lineare* and *Euglypha rotunda* are positively correlated with pH. In our studies, however, there is only a very weak positive correlation of these species with pH (Fig. 4). Barnett et al. (2016) found that *E. rotunda* showed a negative relationship with pH. These differences may be explained by the fact that the two studies treated completely different habitats – Payne (2010) studied peatland testate amoeba communities, while Barnett et al. (2016) studied salt-marsh. The species discussed above have a cosmopolitan distribution and develop in different habitats. These species appear to exhibit different pH preferences, depending on the particular habitat, as well as in relation to the influence of other environmental factors. Arrieira et al. (2015) made a similar conclusion and indicated that local factors were responsible for temporal variation as a preponderant structuring factor in the testate amoebae community.

Conclusion

In the Bogdantsi Reservoir only two genera – *Centropyxis* and *Difflugia*, dominate, which, together with the established extremely low taxonomic diversity and abundance of testate amoebae, is an indication of the presence of strong eutrophication and unsuitable conditions in it. In the Loznitsa and Beli Lom reservoirs dominance is distributed among a large number of genera, a significantly larger number of species and individuals are found, which indicates the presence of a sustainable and suitable environment for development of testate amoebae. In this study no significant correlation was found between the water pH and species richness of the testate amoebae communities in different reservoirs. Our CCA results suggest that species distribution differs along the pH gradient.

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