# Biotopic Distribution of Testate Amoebae (Protozoa: Arcellinida and Euglyphida) in Ovcharitsa Reservoir (Southeastern Bulgaria)

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#### Abstract:

The composition and structure of testate amoebae communities in different biotopes in Ovcharitsa Reservoir (Southeastern Bulgaria) was investigated. The taxonomic richness in littoral zone is quite similar to that in the benthal of the reservoir (44 species of 14 genera and 48 species of 15 genera, respectively). A lower diversity was established in the pelagial (18 species and 7 genera). Concerning the species composition in separate biotopes however some differences were found. Only 6 of all 75 established testate amoebae were found in all studied biotopes: *Centropyxis aculeata*, *C. aerophila*, *C. ecornis*, *C. hirsuta*, *Difflugia elegans and Phryganella hemisphaerica*. Only two of them – *Centropyxis aculeata* and *Phryganella hemisphaerica* were dominant species. These species, as well as *C. aerophila* were found to have a high frequency in all biotopes. Differences found are confirmed by the relatively low values of the coefficient of faunal similarity – between 15.8%-21.6% on a species level and 29.4%-61.1% on a genus level. Two species are new to the Bulgarian testacean fauna – *Schaudinnula arcelloides* (Awerintzew, 1907) and *Difflugiella sacculus* (Penard, 1902) Deflandre, 1953. Data about their morphometry, distribution and photos were presented.

Key words: Testate amoebae, Ovcharitsa Reservoir, biotopes, frequency, dominant structure

## Introduction

Testate amoebae are invariably found in a variety of freshwater reservoirs, where they play important roles and contribute to their biological characterization. Furthermore they react immediately to natural or anthropogenic changes in their habitat. These facts, as well as their considerable diversity, greater sensitivity to hydrological variations and availability of specific testate amoebae fauna inhabiting different biotopes, make them useful indicators of environmental changes (Moraczewski 1962, Schönborn 1962, 1966, Vikol 1992, McCarthy *et al.* 1995, Asioli *et al.* 1996, Burbidge and Schröder-Adams 1998, Manca *et al.* 1999, Patterson and Kumar 2000, Dalby *et al.* 2000, Scott *et al.* 2001, Patterson *et al.* 2002, Mazey and Tsyganov 2006).

Studies on the aquatic testate amoebae in some large reservoirs in Bulgaria until now show that the testacean fauna in them is varied as well as that the density and species diversity, dominant structure and frequency of occurrence of this group of organisms differ significantly in the separate biotopes (Golemansky and Todorov 1993, Davidova *et al.* 2008, Todorov *et al.* 2008, Davidova 2010).

The aim of this work is to establish the composition and the structure of the testate amoebae communities in different biotopes – littoral zone, benthal and pelagial in Ovcharitsa Reservoir, which is intended to provide technical water supply for one of the largest power plants in Bulgaria.

#### **Study Area**

Ovcharitsa Reservoir was built in 1966 on Ovcharitsa River, to the East of the town of Radnevo, in close proximity to the power plant Maritza Iztok-2. It is located at 42°15'25"N and 26°10'43"E. The reservoir is divided into two parts, warm and cold, which are not enclosed. The temperature in the warm lake often exceeds 30 °C. The reservoir has an open water area, low banks and shallows, covered by sparse hygrophyte vegetation. *Myriophyllum spicatum* is dominant in the aquatic vegetable communities from the littoral zone.

With its hydromorphological parameters Ovcharitsa belongs to medium deep, large, lowland reservoirs. Its total area is 6550 km², total volume is 45.8x106 m³ and the water mirror is with an area of 6.3 km² (Management plan for East Aegean Sea River Basin 2010-2015). The maximum depth measured at the present study was 10 m. Overall ecological condition of the reservoir's water is evaluated as moderately (Management plan for East Aegean Sea River Basin 2010-2015). The water in it never freezes and even winter temperature is around 18 °C.

### **Material and Methods**

Eighteen samples from the surface layer of the sediments, from the aquatic plants and the pelagial of the reservoir were collected in June 2010. The localities were chosen in order to sample as many types of environments and water depths as possible (Fig. 1). The materials were grouped into the following biotopes:

- **1. Littoral zone** washed water plants (*Myriophyllum sp.*, *Cladophora* sp.), stones, and benthic samples at a depth of 0.6-0.7 m 8 samples, N 1- 8;
- **2. Benthal** benthic samples at a depth from 5 to 10 m 8 samples, N 9- 16. Eckmann's grab was used for the collection of the benthic samples from the deep-water.
- 3. Pelagial -2 assembly plankton samples from the open surface of the reservoir were studied. They were collected with a plankton net made of bolting gauze with mesh sizes of 60  $\mu$ m and a mouth aperture of 20 cm.

Half of each sample was fixed with 4% formaldehyde. The other half was kept alive for investigation in vivo.

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 dominance was established on the basis of the relative significance of each species  $D = n_i/N \ x$  100, where  $n_i$  is the number of specimens of each species and N – the total number of all specimens. All species were divided into 4 groups: subrecedent – with relative significance < 1%; recedent – with relative significance 1-2%; subdominant – with relative significance 2-5%; dominant – with relative significance > 5%.

The similarity between testacean fauna in different biotopes of Ovcharitsa Rezervoir was calculated by Jaccard's similarity factor (k):  $k = C / A + B - C \times 100$ , where A is the number of species (genera) in the one biotope; B – the number of found species (genera) in the other biotope; C – the number of the common species.

Statistical analysis was performed using the computer program STATISTICA, version 6.0.

#### Results

# Composition and structure of testate amoebae communities

The list of taxa (a total 75), number of specimens, relative significance (D) and frequency of occurrence (pF) in testacean communities of studied biotopes in the reservoir are presented in Table 1. Two species (asterisked in Table 1) are new to the Bulgarian testacean fauna. The number of taxa and the relative significance of the genera, dominated in different biotopes are given in Fig. 2 and 3.

Forty-four species belonging to 14 genera were found in the littoral zone of Ovcharitsa Reservoir. The genera *Difflugia* (15 species) and *Centropyxis* (11) have the highest number of species accounting for 59.1% of the species in the biotope. As regards on the number of specimens genus *Centropyxis* (28.7%) has the highest relative significance followed by *Trinema* (20.0%), *Corythionella* (16.8%), *Difflugia* (15.7%) and *Phryganella* (5.0%). These 5 genera comprise 86.2% of all specimens found in this biotope. The high relative abundance of the genera *Trinema*, *Corythionella* and *Phryganella* is due to

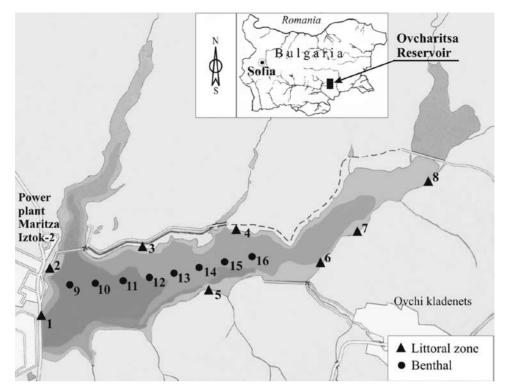


Fig. 1. Map, showing the location of Ovcharitsa Reservoir and study sites.

the presence of 1 species only, which occur with high population density, while the dominance of the genus *Difflugia* is due to the finding of a relatively large number of species.

Six dominant species (or 13.6% of all taxa) were found in this biotope, presented here in terms of their relative significance: *Trinema enchelys* (19.0%), *Corythionella georgiana* (16.8%), *Centropyxis hirsuta* (10.5%), *Centropyxis aculeata* (8.3%), *Difflugia minuta* (6.1%), *Phryganella hemisphaerica* (5.0%).

Fourteen (31.8%) of all 44 found species had an occurrence of more than 50% and appeared to be constant species – *Centropyxis aculeata* (87.5%), *C. aerophila* (87.5%), *Cyclopyxis eurystoma* (87.5%), *Plagiopyxis declivis* (87.5%), *Phryganella hemisphaerica* (75.0%), *Trinema enchelys* (75.0%), *Difflugia lucida* (62.5%), *D. sarissa* (62.5%), *Centropyxis aerophila v. sphagnicola* (50.0%), *C. hirsuta* (50.0%), *C. marsupiformis* (50.0%), *Difflugia glans* (50.0%), *D. pulex* (50.0%), *Pseudodifflugia gracilis* (50.0%) (Table 1).

The species richness among samples varied between 9 and 23 species. A significant number of species was established in the station 8 (23 species), as well as in stations 6 and 7 (respectively 17 and 19 species) (Fig. 1). In these places the reservoir

shores were colonized by submerged vegetation and algae, which constitute a favorable habitat for testate amoebae and explain the greater taxonomic diversity in them. The close correlation between vegetation and testate amoebae diversity has already been demonstrated (SMITH 1992, DABÉS and VELHO 2001, LEDEGANCK *et al.* 2002, LANSAC-TÔHA *et al.* 2008, TODOROV *et al.* 2008).

A total of 48 taxa, belonging to 15 genera were found in the benthal of Ovcharitsa Reservoir. The genera Difflugia (25 species; 45.6% relative significance) and Centropyxis (7: 21.8%) are characterised by the highest species diversity and abundance. They include 66.7% of the species and 67.4 % of the specimens, established in the biotope. Dominant, but with significantly lower abundance is also the genus Phryganella (6.1%) (Fig. 2, 3). This dominance is due to the presence of only one species – *Phryganella* hemisphaerica. No representatives of the genus Trinema were found in this biotope. The dominant species are 4 (8.3% of all established taxa): Difflugia manicata (13.6%), Centropyxis aculeata (10.9%), Phryganella hemisphaerica (6.1%) and Difflugia pulex (5.4%).

Of all 48 species found in this biotope, 9 (18.8%) are constant – *Difflugia pulex* (87.5%), *Centropyxis aculeata* (75.0%), *Difflugia manicata* (75.0%),

**Table 1.** List of taxa, number of specimens (N), relative significance (D) and frequency of occurrence (pF) in the studied biotopes of Ovcharitsa Reservoir.

	Biotopes									
Taxa		Littoral zone			Benthal			Pelagial		
	N	D	pF	N	D	pF	N	D	pF	
Arcella discoides Ehrenberg, 1843	1	0.18	12.5	-	-	-	-	-	-	
A. hemisphaerica Perty, 1852	_	-	-	_	-	ı	1	1.25	50.0	
Centropyxis aculeata (Ehrenberg, 1830) Stein, 1857	45	8.32	87.5	16	10.88	75.0	15	18.75	100.0	
C. aerophila Deflandre, 1929	23	4.26	87.5	5	3.41	50.0	5	6.25	100.0	
C. aerophila v. sphagnicola Deflandre, 1929	10	1.85	50.0	-	-	-	-	-	-	
C. cassis (Wallich, 1864) Deflandre, 1929	-	-	-	1	0.68	12.5	-	-	-	
C. constricta (Ehrenberg, 1841) Deflandre, 1929	1	0.18	12.5	-	-	-	-	-	-	
C. deflandriana Bonnet, 1959	1	0.18	12.5	-	-	-	-	-	-	
C. delicatula Penard, 1902	1	0.18	12.5	1	0.68	12.5	-	-	-	
C. ecornis (Ehrenberg, 1841) Leidy, 1879	4	0.74	37.5	5	3.41	50.0	5	6.25	50.0	
C. hirsuta Deflandre, 1929	57	10.54	50.0	1	0.68	12.5	2	2.50	50.0	
C. marsupiformis (Wallich, 1864) Deflandre, 1929	10	1.85	50.0	3	2.04	37.5	-	-	-	
C. platystoma (Penard, 1890) Deflandre, 1929	1	0.18	12.5	-	-	-	-	-	-	
C. sylvatica (Deflandre, 1929) Bonnet & Thomas, 1955	2	0.37	25.0	-	-	-	1	1.25	50.0	
Corythionella georgiana Nicholls, 2005	91	16.82	37.5	3	2.04	25.0	_	-	-	
Cyclopyxis aplanata Deflandre, 1929	1	0.18	12.5	_	-	-	_	-	_	
C. eurystoma Deflandre, 1929		4.08	87.5	4	2.72	50.0	_	-	-	
C. kahli Deflandre, 1929		0.18	12.5	-	_	-	_	-	-	
Difflugia ampullula Playfair, 1918		0.74	25.0	3	2.04	37.5	-	-	-	
D. angulostoma G. – Lievre et Thomas, 1958		-	-	1	0.68	12.5	-	-	-	
D. balcanica Ogden & Zivkovic, 1983	1	0.18	12.5	1	0.68	12.5	-	-	-	
D. bicornis Penard, 1890	-	-	-	1	0.68	12.5	2	2.50	50.0	
D. brevicolla Cash, 1909	_	-	-	1	0.68	12.5	-	-	-	
D. bryophila (Penard, 1902) Jung, 1942	-	-	-	1	0.68	12.5	-	-	-	
D. capreolata Penard, 1902	_	-	-	1	0.68	12.5	-	-	-	
D. corona Wallich, 1864	-	-	-	1	0.68	12.5	2	2.50	50.0	
D. decloitrei Godeanu, 1972	1	0.18	12.5	-	-	-	-	-	-	
D. dificilis ecornis Chardez, 1956	1	0.18	12.5	-	-	-	-	-	-	
D. distenda Ogden, 1983	-	-	-	1	0.68	12.5	-	-	-	
D. dragana Ogden & Zivkovic, 1983	-	-	-	1	0.68	12.5	-	-	-	
D. elegans Penard, 1890	2	0.37	25.0	1	0.68	12.5	2	2.50	50.0	
D. glans Penard, 1902		0.92	50.0	-	-	-	1	1.25	50.0	
D. globularis (Wallich, 1864) Leidy, 1877		-	-	2	1.36	12.5	-	-	-	
D. globulosa Dujardin, 1837		0.18	12.5	-	-	-	1	1.25	50.0	
D. gramen Penard, 1902		-	-	5	3.41	50.0	-	-	-	
D. lacustris (Penard, 1899) Ogden, 1983	-	-	-	1	0.68	12.5	-	-	-	
D. lanceolata Penard, 1890	-	-	-	1	0.68	12.5	-	-	-	
D. levanderi Playfair, 1918	-	-	-	3	2.04	12.5	-	-	-	

Table 1. Continued.

	Biotopes								
Taxa	Littoral zone		Benthal			Pelagial			
	N	D	pF	N	D	pF	N	D	pF
D. lobostoma Leidy, 1879	-	-	-	2	1.36	12.5	1	1.25	50.0
D. lobostoma f. multilobata (Leidy, 1879)					_		1	1.25	50.0
GLievre & Thomas, 1958	-	-	-	-	_	-	1	1.25	30.0
D. lucida Penard, 1890	10	1.85	62.5	-	-	-	-	-	-
D. manicata Penard, 1902	-	-	-	20	13.61	75.0	-	-	-
D. mica Frenzel, 1892	-	-	-	1	0.68	12.5	-	-	-
D. minuta Rampi, 1950	33	6.10	12.5	1	0.68	12.5	-	-	-
D. penardi Hopkinson, 1909	2	0.37	12.5	-	-	-	-	-	-
D. pristis Penard, 1902	3	0.56	12.5	4	2.72	37.5	-	-	-
D. pulex Penard, 1902	6	1.11	50.0	8	5.44	87.5	-	-	-
D. sarissa Li Sun Taï, 1931	8	1.49	62.5	4	2.72	25.0	-	-	-
D. serbica Ogden & Zivkovic, 1983	-	-	-	1	0.68	12.5	-	-	-
D. tenuis (Penard, 1890) Ogden, 1983	5	0.92	12.5	-	-	-	-	-	-
D. tricornis Ogden, 1983	3	0.56	25.0	-	-	-	-	-	-
D. viscidula Penard, 1902	-	-	-	1	0.68	12.5	-	-	-
Difflugiella angusta Schönborn, 1965		-	-	2	1.36	12.5	-	-	-
D. oviformis Bonnet &Thomas, 1955	4	0.74	37.5	-	-	-	3	3.75	50.0
*D. sacculus (Penard, 1902) Deflandre, 1953		0.37	12.5	-	-	-	-	-	-
D. pusilla Playfair, 1918		-	_	3	2.04	12.5	-	-	-
D. vulgaris (France, 1913) Grospietsch, 1964		-	-	-	-	-	1	1.25	50.0
Euglypha acanthophora (Ehrenberg, 1841) Perty, 1849		-	-	-	-	-	1	1.25	50.0
E. rotunda Wailes, Penard, 1911		1.30	12.5	2	1.36	12.5	-	-	-
E. tuberculata Dujardin, 1841		-	-	3	2.04	25.0	-	-	-
Hyalosphaenia subflava Cash, 1909		0.18	12.5	_	-	_	-	-	_
Microchlamys patella (Claparede & Lachmann, 1885) Cockerell, 1911	9	1.67	37.5	2	1.36	25.0	-	-	_
Pentagonia shablensis Todorov & Golemansky, 1998	-	-	-	1	0.68	12.5	-	-	_
Phryganella hemisphaerica Penard, 1902		5.00	75.0	9	6.12	75.0	33	41.25	100.0
Plagiopyxis declivis Thomas, 1955	13	2.41	87.5	3	2.04	25.0	-	-	-
Pl. minuta Bonnet, 1959	1	0.18	12.5	-	-	-	_	-	_
Psammonobiotus linearis Golemansky, 1970	-	-	_	4	2.72	25.0	_	-	-
Pseudodifflugia compressa Schulze, 1874		0.18	12.5	6	4.08	50.0	_	_	_
P. gracilis Schlumberger, 1845		2.03	50.0	2	1.36	25.0	_	-	_
*Schaudinnula arcelloides Awerintzew, 1907		-	-	1	0.68	12.5	_	-	_
Tracheleuglypha acolla Bonnet & Thomas, 1955		0.18	12.5	-	-	-	_	-	_
T. dentata Deflandre, 1938		_		2	1.36	12.5	_	-	_
Trinema enchelys (Ehrenberg, 1838) Leidy, 1878		19.04	75.0	_	-		3	3.75	50.0
T. lineare Penard, 1890	103	0.92	12.5	_	_	_	-	-	-
Zivkovicia compressa (Carter, 1864) Ogden, 1987	-	-	-	1	0.68	12.5	_	-	_
Total species/genera 75/18		44/14	l		48/15			18/7	

<sup>\*</sup> New testate amoebae for Bulgarian fauna.

Phryganella hemisphaerica (75.0%), Centropyxis aerophila (50.0%), C. ecornis (50.0%), Cyclopyxis eurystoma (50.0%), Difflugia gramen (50.0%), Pseudodifflugia compressa (50.0%) (Table 1).

The maximum number of species was registered at the stations 9 and 10 (21 species). The species richness on the other stations varied from 6 to 11 species (Fig. 1). The probable reason for this is that stations 9 and 10 are the closest to the 'warm pool' and the water temperature in them is relatively high throughout the year.

The pelagial of the reservoir was characterized with a lower diversity (18 taxa and 7 genera). The genera Difflugia (7 species) and Centropyxis (5) had the highest diversity and predominated in testacean communities (Fig. 2). They constituted 66.7% of all found species. Concerning the number of specimens with the highest relative significance is the genus Phryganella (41.3%), followed by the genera Centropyxis (35.0%) and Difflugia (12.5%) (Fig. 3). To these three genera belong 88.8% of all specimens found in this biotope. Here, as well as in the other biotopes, the dominant role of the genus *Phryganella* is due to the presence of one species – Phryganella hemisphaerica with a significant number of specimens and the dominance of the genus Difflugia – to the presence of several species with single specimens. Four species (22.2% of all taxa) were found to predominate in the biotope: Phryganella hemisphaerica (41.3%), Centropyxis aculeata (18.8%), C. aerophila (6.3%) and *C. ecornis* (6.3%) (Table 1).

## Ecological notes on the new species to the Bulgarian testacean fauna

Schaudinnula arcelloides (AWERINTZEW, 1907) (Fig. 4-6)

First, AWERINTZEW (1907) described Schaudinnula arcelloides in fresh water samples from the Island Waigatsch (Arctic Ocean, Russia, Europe) (after Schönborn 1965). Later, investigating the testate amoebae fauna of Masurian Lakes (Poland), Schönborn (1965) found it in the sediment of eutrophic lake Mikolajskie. In our study one specimen of the species was found in benthic sample. The shell morphology and dimensions of our individual correspond well with Schönborn's (1965) description.

Measurements: See Table 2.

Difflugiella sacculus (Penard, 1902) Deflandre, 1953 (Fig. 7)

This species was observed and described for the first time by Penard (1902) in the samples of Lake Leman as *Cryptodifflugia sacculus*. The author found a large number of specimens in different types of algae and indicates that the species shows special preference to them. Later Deflandre (1953) studied in detail the genera *Cryptodifflugia* and *Difflugiella* and relying mainly circular cross-section of the shell of *Cryptodifflugia sacculus*, assigned it to a genus *Difflugiella*. Grospietsch (1964) also confirmed that. In the present investigation only two specimens of the species were found in samples of the littoral zone. Their morphological features and shell dimensions agree well to the original and Grospietsch's (1964) description of species.

**Measurements:** See Table 2.

#### **Discussion**

The testacean communities in Ovcharitsa Reservoir are mainly composed of aquatic species with cosmopolitan distribution. The genera Difflugia, Centropyxis, Trinema, Corythionella, Phryganella and Difflugiella predominate in the studied reservoir. The genus Difflugia was found to have the greatest number of species in the separate biotopes. In the benthal this number exceeds about three times the number of species of the genus Centropyxis. Considering the number of specimens found, however, that distribution of the genera is retained only in the benthal, while in the other biotopes some differences were established. In the littoral zone of the reservoir, genera Centropyxis and Trinema tend to increase their predominance. In the pelagial about 42% of all counted specimens are of a genus Phryganella, which were represented by one species only, and other 35% belong to the genus Centropyxis.

The dominant complex in the communities is characterized by the presence mainly of *Centropyxis* and *Difflugia* species. The dominant species of the genus *Centropyxis* are 4 but only one of them dominates in all studied biotopes, while those of the genus *Difflugia* are 3, as none of them is found in the three biotopes (Fig. 8). The taxonomic richness of thecamoebian fauna in littoral zone is quite similar to that in the benthal of the reservoir (44 species of 14 genera and 48 species of 15 genera, respectively). A lower diversity was established in the pelagial (18 species and 7 genera). Concerning

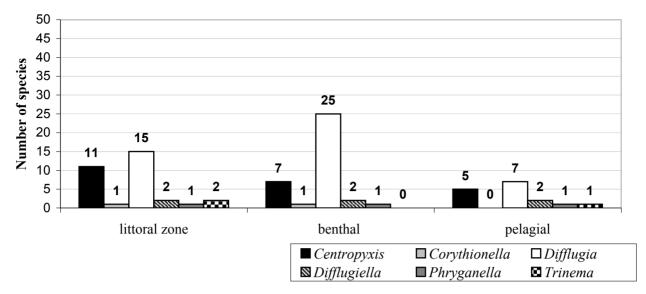


Fig. 2. Number of taxa of the genera, dominated in different biotopes.

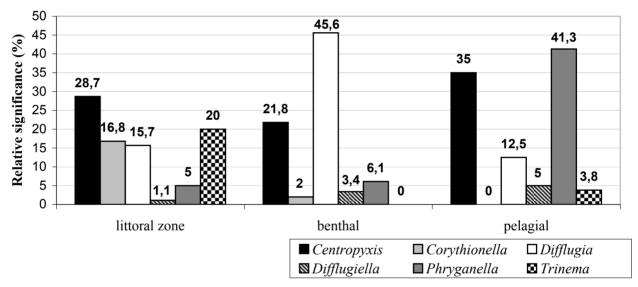
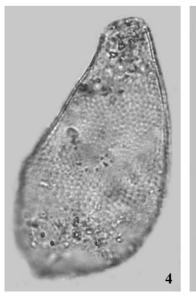
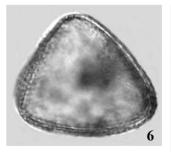


Fig. 3. Relative significance of the genera, dominated in different biotopes.







Figs. 4-6. Schaudinnula arcelloides. 4 – oblique lateral view, showing general test form, outline and composition; 5 – lateral view; 6 – cross-section of the shell. Scale bars – 10 μm.



Fig. 7. Difflugiella sacculus – lateral view. Scale bar – 5 μm.

the species composition in different biotopes, however, some differences were found, which leads to the conclusion that each biotope is characterized by specific testate amoebae fauna. On the one hand this is evident in the comparatively low coefficients of faunal similarity – between 15.8%-21.6% on a species level and 29.4%-61.1% on a genus level (Table 3). On the other hand it is confirmed having in mind the structure of testate amoebae communities. Only two species – *Centropyxis aculeata* and *Phryganella hemisphaerica* of the 10 dominant species predominate in all three studied biotopes (Fig. 8). The two species, indicated above, as well as *C. aerophila* are only established with a high frequency in all biotopes. These three species, are frequently reported

in the literature as being dominant taxa in different environments (McCarthy et al. 1995, Scott et al. 2001, Davidova 2005, 2008, Golemansky et al. 2006, Mazey and Tsyganov 2008, Todorov and Golemansky 2000). Only 6 of all 75 established testate amoebae were found in all studied biotopes: Centropyxis aculeata, C. aerophila, C. ecornis, C. hirsuta, Difflugia elegans and Phryganella hemisphaerica. Other 23 species were present in 2 of the studied biotopes, and about 62% (46 taxa) of all established testaceans were represented in one biotope only.

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Table 2. Measurements (in µm) of the new testate amoebae for Bulgarian fauna according to different authors.

Species	Authors	Length of shell	Breadth of shell	Depth of shell	Diameter of shell	Diameter of aperture	n
Schaudinnula	Schönborn, 1965	98-105	40	-	-	-	-
arcelloides	Present study	100	56	47	-	16	1
Difflugiella sacculus	Penard, 1902	17-26	-	-	-	-	-
	Grospietsch, 1964	16-30	-	-	15-22	-	-
	Present study	25-27,5	-	-	12-13	4-5	2

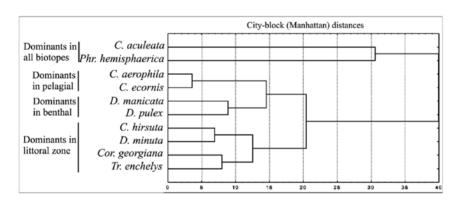


Fig. 8. Cluster dendrogram, showing the dominant species in biotopes.

Table 3. Similarity (k) between the testacean fauna in different biotopes of Ovcharitsa Rezervoir

Species level Genus level	Littoral zone	Benthal	Pelagial		
Littoral zone		30.0%	21.6%		
Benthal	61.1%		15.8%		
Pelagial	50.0%	29.4%			

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