

**TESTATE AMOEBAE (AMOEBOZOA, RHIZARIA) IN TERRESTRIAL MOSSES:
DIVERSITY AND COMMUNITIES STRUCTURE (“ZLATNI PYASATSI”
NATURAL PARK, NORTH-EAST BULGARIA)**

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ABSTRACT: *The specific composition of testate amoebae communities in the terrestrial mosses were studied in the “Zlatni Pyasatsi” Natural Park (North-East Bulgaria). Fifty eight taxa (including species, varieties and forms) belonging to 21 genera of testate amoebae were recorded. Testate amoebae communities are strongly dominated by the species Centropyxis aerophila v. sphagnicola, Plagiopyxis declivis, Euglypha rotunda, Centropyxis aerophila, Phryganella hemisphaerica, Cyclopyxis eurytoma, Trinema enchelys and Corythion dubium, which were found frequently and also presented with the highest relative abundance. The moss-dwelling testacean fauna of “Zlatni Pyasatsi” Natural Park was compared to other areas in Bulgaria. Interest from the ecological point of view represents finding in this study of species Cyphoderia loevis and Psammonobiotus linearis, which has not been reported as inhabitants of terrestrial mosses until now. This is first report for the establishment of the species outside of water biotope.*

KEYWORDS: Terrestrial Mosses, Testate Amoebae, Cyphoderia Loevis, Psammonobiotus Linearis

INTRODUCTION

Moss-dwelling testate amoebae inhabit peat bogs, rocky moss, soil moss and epiphytic moss and includes about 30% of the known testacean fauna [26]. In Sphagnum-dominated habitats testate amoebae are especially abundant and diverse. These organisms form decay resistant shell that can persist in peat for thousands of years [3, 41]. This and their cosmopolitan distribution and strong relationships between community composition and substrate moisture in peatland makes them useful in palaeoecological studies [7, 10] and testate amoebae have been widely studied in peatlands all over the world. By contrast, testate amoeba fauna in terrestrial mosses is characterized by relatively less taxonomic diversity and abundance [33]. However, the recent studies have shown that the richness of testate amoebae in terrestrial mosses may vary widely (from 9 to 88 species) [5, 6, 31, 32, 34, 45, 46, 47, 49, 50, 51]. Although there have been a number of studies on testate amoebae in terrestrial mosses the ecological preferences and biogeography of these organisms are still insufficiently explored.

The first data on the testate amoebae fauna inhabiting terrestrial mosses in Bulgaria is to be found in the work of Patev [39] "Contribution to the study of freshwater Rhizopoda in Bulgaria". The author reported for the finding of four species of testate amoebae in samples of mosses from the vicinity of the town Peshtera. Later Golemansky [23] investigated rhizopodic fauna in the epiphytic and soil mosses from different regions of Bulgaria and established 88 species of testate amoebae, sixteen of which are new to the Bulgarian fauna. Golemansky and Todorov [27] studied testate amoebae fauna in Vitosha Mountain and established 31 species in terrestrial mosses. The authors indicated that the hydrophilic soil

mosses are inhabited by a richer and more varied rhizopodic fauna compared to the epiphytic mosses. Studying the species composition and distribution of soil and moss testate amoebae fauna in different altitudinal zones of Pirin Mountain, Todorov [45] found in the aerophilic mosses 36 species belonging to 15 genera. Davidova [11, 12] investigated aquatic and moss testate amoebae fauna in the plateaus of Shumen and Provadia and established respectively 27 and 30 species in terrestrial mosses. Data on the testaceans living in mosses we find in the work of Golemansky et al. [28], which summarized the existing data on the taxonomic diversity and distribution of the rhizopods from the Western Rhodopes. In samples of soil moss authors established 88 species and 29 genera testate amoebae. The fauna of the epiphytic mosses is about four times poorer (25 species of 13 genera only). Investigating testate amoebae diversity and communities structure in epiphytic and soil mosses in the natural reserves of Uzunbudzhak and Silkosiya Davidova [13] found forty taxa (including species, varieties and forms) belonging to 15 genera and 10 families of testate amoebae, of which one variety is new to the Bulgarian fauna.

“Zlatni Pyasatsi” Natural Park was created for conservation and protection of valuable habitats, flora, fauna and specific landscapes, part of the European network Natura 2000 as a Special Area of Conservation. The aims of the present paper are to examine the diversity and the structure of the testacean communities of terrestrial mosses from the park and to compare the taxonomic composition and richness of testate amoebae inhabiting the park with those established in other regions in Bulgaria.

MATERIAL AND METHODS

Study Area

The "Zlatni Pyasatsi" Natural Park is located in the Northern Black Sea coast of eastern part of the Danubian Plain, 17 km northeast from Varna (43° 17' 0" N, 28° 02' 0" E) (Fig. 1). Parks average length is 9.2 km and average width – 1.2 km. The highest point is Chiplak Tepe (269.3 m a.s.l.), and the average altitude is 110 m. The total area of the park is 1324.7 hectares. The main elements of climate are strongly influenced by the breeze circulation and relief and climate characterized here as a specific and unique. The temperature regime is characterized by mild winters and warm summers. The average January temperature is about 1°C. The average monthly temperatures in summer are around 21-22°C. The humidity is relatively high – an average of 76%, as during different months of the year varies within narrow limits. The area is too poor as regards rainfalls – average annual rainfalls are 498 mm. Surface waters are formed by rainwaters in several dry ravines, a ravine with permanently running water and three larger naturally swampy areas. The natural vegetation are characterized by the development of tree communities dominated by *Carpinus orientalis*, *Quercus cerris*, *Quercus frainetto*, *Fraxinus oxycarpa*, *Abies pinsapo*, *Cedrus libani*, *Ulmus minor*, *Fraxinus americana*, *Fraxinus angustifolia* etc. [30].

Sampling and Testate Amoebae Analyses

Samples for testate amoebae analysis were taken in July 2011. Sampling sites were chosen, so that they covered most of the park and include rocky, soil, and epiphytic mosses.

The vegetation was sampled in 0.5/0.5 m plots. Thirty four moss samples were collected and transported in paper or nylon bags. In the laboratory the gathered moss tufts stayed for a

while in distilled water, after which they were ‘squeezed’ and washed in it. Then the water used for the soaking and washing was used, after a careful filtering and removing of vegetative moss particles, for establishing the taxonomic composition and the number of testate amoebae. All samples were preserved with 4% formaldehyde and stored in polyethylene bottles. The samples were counted under light microscope with magnification of 200-400x.

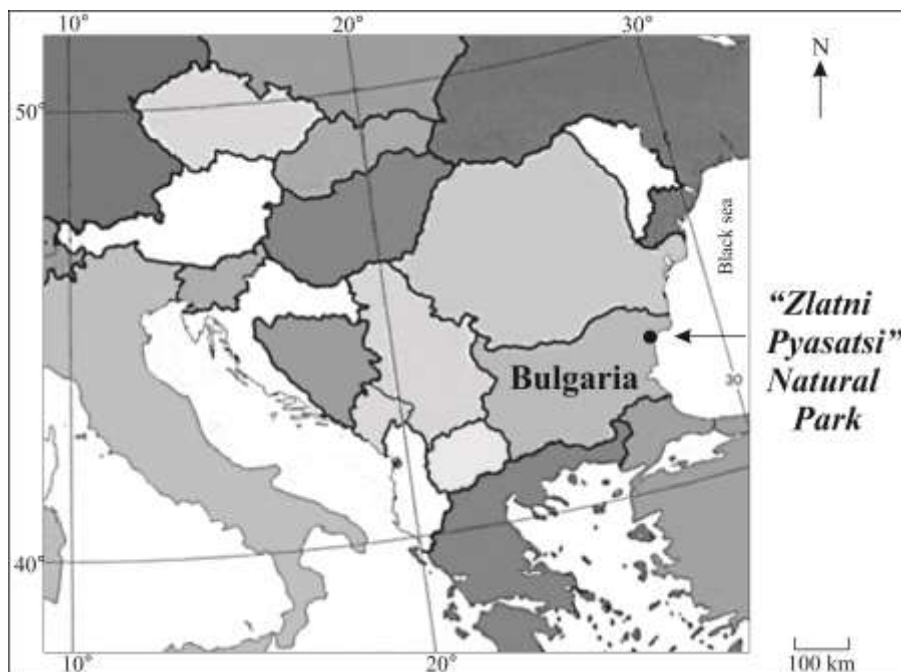


Figure 1. Location of the study site.

Morphological identifications of the testate amoebae are mainly based on the works by Deflandre [20, 21], Gauthier-Lievre and Thomas [28], Decloitre [22], Ogden and Hedley [36], Ogden [37], Ogden and Živcovic [38].

The frequency of occurrence of the species was calculated using the formula: $pF = m/n \times 100$, where m is the number of samples in which one species was found and n is the total number of samples. The percentages of testate amoebae in communities (relative abundances) was established by the formula $D = n_i/N \times 100$, where n_i is the number of individuals of each species and N – the total number of all individuals. To compare moss testate amoeba fauna in different regions in Bulgaria was calculated the index of Jaccard: $k = C/A + B - C \times 100$, where A is the number of species in the first biotope; B – the number of found species in the second biotope; C – the number of common species. Similarities of testate amoebae communities was carried out using the Euclidean distance measure [44].

RESULTS

Fifty eight taxa (including species, varieties and forms) belonging to 21 genera of testate amoebae were recorded in the present study. The list of observed taxa, their frequency of occurrence and relative abundance are given in Table 1.

Most of the testate amoebae belong to the genera *Centropyxis* (12 species) and *Euglypha* (12 species), which include 41.38% of the species. All of the remaining 19 genera were represented by 1-4 species.

The analysis of the frequency of occurrence has revealed that there are 10 constant species of testate amoebae in mosses. These include: *Centropyxis aerophila* v. *sphagnicola*, *Plagiopyxis declivis*, *Euglypha rotunda*, *Centropyxis aerophila*, *Phryganella hemisphaerica*, *Cyclopyxis eurystoma*, *Diffugia lucida*, *Trinema enchelys*, *T. penardi* and *T. lineare*. They represent 17.24% of all the species observed in this biotope. Common and characteristic components of testate amoebae communities of terrestrial mosses are and species *Microchlamys patella*, *Corythion dubium*, *Cyclopyxis kahli*, *Euglypha ciliata* f. *glabra*, *Assulina muscorum* and *Euglypha laevis*, which according to its frequency of occurrence belong to the group of incidental species (found in 25-50% of the samples) and represent 10.34% of all species. Forty-two species or 72.42% are accidental (found in less than 25% of the samples).

Considering the relative abundance of the genera it was established that genera *Euglypha* (21.94%), *Trinema* (19.38%), *Centropyxis* (17.69%), *Phryganella* (12.98%), *Plagiopyxis* (7.19%), *Cyclopyxis* (5.34%) and *Corythion* (5.00%), which include 89.52% of the specimens found in the mosses, have a dominant role. The remaining 14 genera comprise 10.48% of all found in this biotope specimens.

The high relative significance of the dominant genera is due to the abundant occurrence of only one or two species in the genus. These are *E. rotunda*, *T. enchelys*, *C. aerophila*, *C. aerophila* v. *sphagnicola*, *P. hemisphaerica*, *P. declivis*, which are dominants (with relative abundance > 5%) and *C. eurystoma* and *C. dubium*, belonging to the group of subdominants (with relative abundance 2-5%) (Table 1). To the latter group were included and species *D. lucida*, *T. penardi*, *T. lineare*, *E. ciliata* f. *glabra* and *A. muscorum*. Two species were recedent (with relative abundance 1-2%) and 43 species or 74.13% were subrecedent (with relative abundance < 1%).

DISCUSSION

The moss testate amoebae fauna of the “Zlatni Pyasatsi” Natural Park is strongly dominated by the eurybiont and cosmopolitan species: *C. aer.* v. *sphagnicola*, *P. declivis*, *E. rotunda*, *C. aerophila*, *P. hemisphaerica*, *C. eurystoma*, *T. enchelys* and *C. dubium*, which were found frequently and also presented with the highest relative abundance. Typical components of testate amoebae communities are and species *D. lucida*, *T. penardi*, *T. lineare*, *E. ciliata* f. *glabra* and *A. muscorum* established with high frequency, but with a relatively less number of specimens. The above-mentioned species represent 22.41% of the established species in the current studies, and comprise 86.13% of all found specimens.

These species were reported by many authors as frequent and constant components of testate amoebae fauna of terrestrial mosses in different parts of the world. Smith [43] investigated the testate amoebae fauna on the moss *Sanionia uncinata* and reported that the most abundant are *D. lucida* and *A. muscorum*. Comparing communities from the coastal lowlands on Devon Island, Canadian Arctic Beyens et al. [5] indicated that the dry terrestrial moss habitats are characterized by *A. muscorum* – *C. dubium* assemblages, and soil mosses had a soil fauna association of *Plagiopyxis callida* – *P. declivis*. Török [47] examined six species of terrestrial

mosses in Hungary and indicated that *T. penardi*, is a characteristic species in *Cirriphyllum tommasinii*. The dominant testacean species in mosses, according to Török [47] are also *C. aerophila* v. *sphagnicola*, *P. declivis*, *C. dubium*, *E. rotunda*, *T. enchelys*, *T. lineare* and *E. laevis*. In the southeastern Alps in Italy, in the altitudinal range from 1000-

Table 1. List of taxa, frequency of occurrence (pF) and relative abundance (D) in the studied mosses of “Zlatni Pyasatsi” Natural Park.

Taxa	pF	D
Arcella arenaria v. compressa Chardez, 1974	20.58	0.30
A. arenaria v. sphagnicola Deflandre, 1928	2.94	0.04
A. catinus Penard, 1890	5.88	0.13
A. discoides Ehrenberg, 1843	2.94	0.02
Assulina muscorum Greeff, 1888	26.47	2.05
Centropyxis aerophila Deflandre, 1929	73.53	9.22
C. aerophila v. sphagnicola Deflandre, 1929	91.18	7.85
C. cassis (Wallich, 1864) Deflandre, 1929	5.88	0.04
C. deflandriana Bonnet, 1959	2.94	0.02
C. elongata (Penard, 1890) Thomas, 1959	5.88	0.11
C. globulosa Bonnet & Thomas, 1955	2.94	0.02
C. laevigata Penard, 1890	2.94	0.04
C. minuta Deflandre, 1929	2.94	0.02
C. orbicularis Deflandre, 1929	5.88	0.09
C. platystoma (Penard, 1890) Deflandre, 1929	11.76	0.17
C. sylvatica (Deflandre, 1929) Bonnet & Thomas, 1955	2.94	0.11
Corythion dubium Taranek, 1881	38.24	4.50
C. orbicularis (Penard, 1910) Iudina, 1996	5.88	0.50
Cyclopyxis eurystoma Deflandre, 1929	64.71	4.76
C. kahli Deflandre, 1929	29.41	0.58
Cyphoderia loevis Penard, 1902	2.94	0.21
Difflugia lucida Penard, 1890	64.71	3.24
D. pristis Penard, 1902	5.88	0.04
D. pulex Penard, 1902	5.88	0.21
Diffugiella horrida Schönborn, 1965	5.88	0.92
D. oviformis Bonnet & Thomas, 1955	8.82	0.36
D. pusilla Playfair, 1918	2.94	0.04
Euglypha acanthophora Ehrenberg, 1841) Perty, 1849	2.94	0.06
E. ciliata (Ehrenberg, 1848) Leidy, 1878	20.59	1.22
E. ciliata f. glabra Wailes, 1915	29.41	2.45
E. compressa Carter, 1864	11.76	0.47
E. compressa f. glabra Wailes, 1915	11.76	0.20
E. filifera Penard, 1890	5.88	0.06
E. filifera v. cylindracea Playfair, 1918	2.94	3.12
E. laevis (Ehrenberg, 1845) Perty, 1849	26.47	0.60
E. polylepis Bonnet, 1960	2.94	0.09
E. rotunda Wailes & Penard, 1911	76.47	13.17
E. strigosa f. glabra Wailes, 1898	5.88	0.02
E. tuberculata Dujardin, 1841	2.94	0.50
Heleopera petricola Leidy, 1879	2.94	0.02

H. sylvatica Penard, 1890	8.82	0.09
Microchlamys patella (Clap. & Lach., 1885) Cockerell, 1911	44.12	1.66
Microcorycia flava (Greeff, 1866) Cockerell, 1911	2.94	0.02
Nebela collaris (Ehrenb., 1848) sensu Kosakyan & Gomaa, 2012	2.94	0.04
Paraquadrulla irregularis (Wallich, 1863)	8.82	0.21
Phryganella hemisphaerica Penard, 1902	67.65	12.98
Plagiopyxis declivis Thomas, 1955	79.41	6.70
P. minuta Bonnet, 1959	11.76	0.50
Psammonobiotus linearis Golemansky, 1970	2.94	0.36
Pseudodifflugia compressa Schulze, 1874	2.94	0.02
P. fascicularis Penard, 1902	2.94	0.02
Trachelocorythion pulchellum Bonnet, 1979	8.82	0.06
Tracheleuglypha acolla Bonnet & Thomas, 1955	17.65	0.32
T. dentata Deflandre, 1938	2.94	0.06
Trinema complanatum Penard, 1890	14.71	0.20
T. enchelys (Ehrenberg, 1838) Leidy, 1878	61.76	13.19
T. lineare Penard, 1890	52.94	3.59
T. penardi Thomas & Chardez, 1958	55.88	2.41

2200 m a.s.l., Mitchell et al. [32] established that the most frequent taxa on the forest moss *Hylocomium splendens* included *A. muscorum*, *C. aerophila*, *C. dubium*, *E. ciliata*, *E. laevis*, *Phryganella acropodia*, and *T. enchelys*. On Île de la Possession of the sub-Antarctic, the characteristic taxa for dry mosses are *C. dubium*, *A. muscorum*, *C. aerophila*, *C. aerophila* v. *sphagnicola*, *E. ciliata* f. *glabra* [50]. The most frequently occurring species in different species of mosses on King George Island (South Shetland Islands, Antarctic Peninsula) are *T. lineare*, *C. dubium*, and *C. aerophila* [31]. These similarities are not surprising, and illustrate the cosmopolitan distribution of many moss-dwelling testate amoebae from the same habitat type.

Taxa indicative of drier conditions such as *Arcella arenaria*, *Trigonopyxis arcuata* and *Bullinularia indica*, reported by some authors as common inhabitants of terrestrial mosses [4, 23, 41, 43] in the present research were not found. The reason for their absence in the studied mosses probably is relatively high and constant humidity in the park throughout the year.

Species richness of moss inhabiting testate amoebae in the “Zlatni Pyasatsi” Natural Park is higher compared to the other areas from Bulgaria (Table 2). The reasons for this may be on the one hand considerably larger number of investigated samples, and the other – some differences in environmental conditions defining the specific character of each area. The Jaccard index indicates that

Table 2. Species richness of testate amoebae in terrestrial mosses – comparison with other Bulgarian areas

Location	Number of samples	Number of species	k* (%)	The frequent species	Author (s)
Vitosha Mountain	14	31	23.61	<i>Arcella arenaria</i> , <i>Centropyxis aerophila</i> , <i>Centropyxis cassis</i> , <i>Cyclopyxis eurystoma</i>	Golemansky & Todorov [27]
Pirin Mountain	16	35	32.86	<i>C. aerophila</i> , <i>C. sylvatica</i> , <i>Trinema lineare</i> , <i>Phryganella acropodia</i> , <i>Euglypha rotunda</i> , <i>E. laevis</i>	Todorov [45]
Strandzha Mountain	14	40	46.27	<i>C. aerophila</i> , <i>C. aerophila</i> v. <i>sphagnicola</i> , <i>Phryganella hemisphaerica</i> , <i>E. rotunda</i> , <i>Corythion dubium</i> , <i>Euglypha ciliata</i> , <i>Trinema enchelys</i> , <i>Assulina muscorum</i> , <i>C. eurystoma</i> , <i>C. kahli</i> , <i>T. lineare</i>	Davidova [13]
Shumen plateau	17	27	32.81	<i>E. rotunda</i> , <i>T. enchelys</i> , <i>T. lineare</i> , <i>C. eurystoma</i> , <i>C. aerophila</i> , <i>C. aerophila</i> v. <i>sphagnicola</i> , <i>C. dubium</i>	Davidova [12]
Provadia plateau	10	30	39.68	<i>E. rotunda</i> , <i>C. eurystoma</i> , <i>C. aerophila</i> , <i>C. aerophila</i> v. <i>sphagnicola</i> , <i>T. lineare</i> , <i>C. dubium</i> , <i>T. enchelys</i>	Davidova [11]
“Zlatni Pjasatsi” Natural Park	34	58	-	<i>C. aerophila</i> v. <i>sphagnicola</i> , <i>Plagiopyxis declivis</i> , <i>E. rotunda</i> , <i>C. aerophila</i> , <i>P. hemisphaerica</i> , <i>C. eurystoma</i> , <i>Diffflugia lucida</i> , <i>T. enchelys</i> , <i>T. penardi</i> , <i>T. lineare</i> , <i>Microchlamys patella</i> , <i>C. dubium</i> , <i>C. kahli</i> , <i>Euglypha ciliata</i> f. <i>glabra</i> , <i>A. muscorum</i> , <i>E. laevis</i>	Present study

*Index of Jaccard - coefficient of similarity with the fauna of “Zlatni Pjasatsi” Natural Park

The composition of the testate amoebae fauna on different areas from Bulgaria is rather different too. In qualitatively regard, more significant (but not a big) similarity exists between testate amoebae of investigated by us park with these of the Strandzha Mountain – the coefficients of faunal similarity on a species level is 46.27.

Analysing the structure of the testacean communities in the mosses, were found that the frequent in the terrestrial mosses in different areas from Bulgaria are species, mainly belonging to the genera *Euglypha*, *Trinema* and *Centropyxis*. The species *C. aerophila*, *C. eurystoma*, *T. lineare*, *E. rotunda*, *E. laevis*, *C. aerophila* v. *sphagnicola*, *P. hemisphaerica*, *C. dubium*, *T. enchelys*, *A. muscorum* and *C. kahli*, are established with high frequency both in the current research and in terrestrial mosses in one, several or all other investigated areas in Bulgaria (Table 2). However, their share in the formation of communities is different. The species *C. eurystoma*, *T. lineare*, *E. rotunda*, *C. dubium*, and *T. enchelys* are constant taxa both in mosses of Zlatni Pjasatsi, and in those of Shumen and Provadia Plateaus [11, 12], but their frequency of occurrence is significantly higher in samples of Shumen and Provadia plateaus (Fig. 2). The species *P. hemisphaerica*, *D. lucida* and *P. declivis*, which were established in the present study with exceptionally high frequency, in the mosses from Shumen and Provadia plateaus are presented rarely or in general are not found. With similar high frequency in all three areas are established *C. aerophila*, *C. aerophila* v. *sphagnicola* and *T. penardi*, and the species *A. muscorum* and *C. kahli* were found with a similar, but relatively low frequency in the compared areas. The observed differences and established values of the coefficient of similarity between the testate amoebae fauna of compared areas are due to some specific environmental conditions in different areas, determining the development of population of these species.

Interest from the ecological point of view represents finding in this study of species *Cyphoderia loevis* and *Psammonobiotus linearis*, which has not been reported as inhabitants of terrestrial mosses until now.

Despite the numerous studies on testate amoebae fauna in different parts of the world, the species *Cyphoderia loevis* has been found relatively rare and according to its ecological preferences is defined as aquatic species. *C. loevis* was described for first time by Penard [40] from the sediment of Lake Lemane (Geneve), at a depth of 20-40 m. Later it was found in samples from periphyton and benthos of freshwater reservoirs [1, 2, 8], as well as in the plankton and benthos of some rivers [29, 48]. In Bulgaria the species has been found in samples of benthos and aquatic vegetation from the littoral zone in two quarry lakes of Sofia district [42], in the benthos of Ticha Reservoir [17], in the benthos and phytal of Rabisha Reservoir [14] as well as in the benthos and phytal of the rivers Karaagach and Fakijska [15]. Single specimens of the species have been identified in samples of wet

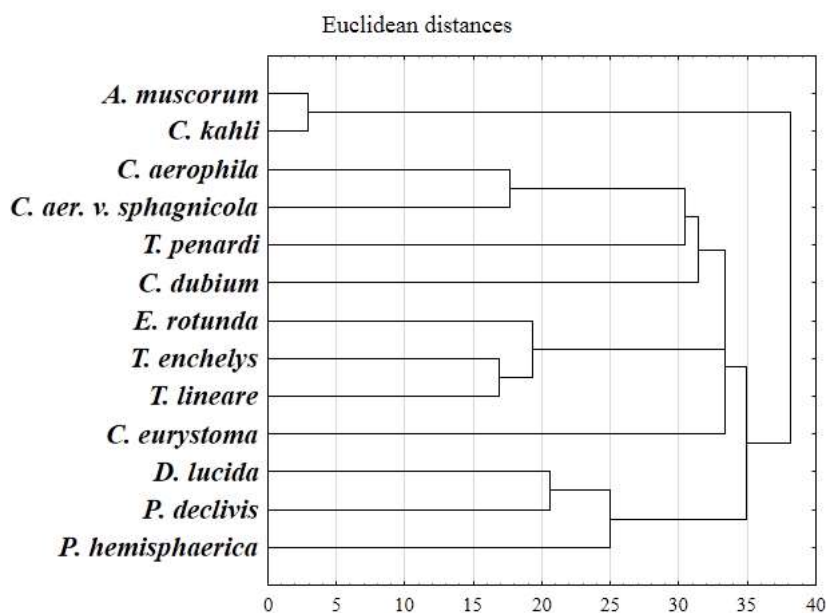


Figure 2. Cluster dendrogram, showing the similarity of testate amoebae communities in terrestrial mosses in “Zlatni Pjasatsi” Natural Park, Shumen Plateau and Provadia Plateau.

Mosses from the river banks [15]. During the present investigation *C. loevis* was observed in the samples of rocky mosses.

Psammonobiotus linearis one of the nine known *Psammonobiotus* species was originally described from the Black Sea by Golemansky [24]. Later it was observed in the interstitial water of the supralittoral sand beaches of the Polish coast of the Baltic Sea [25] and of the Bay of Biscay region,

French Atlantic [9]. For first time Nicholls & MacIsaac [35] established *P. linearis* from a freshwater habitat. The authors found this species in beach sand samples at the eastern end of Lake Ontario and in Rondeau Bay, Lake Erie (Great Lakes, Canada). Considering its widespread occurrence at marine, brackish and freshwater beach localities, its tolerance to changes of salinity in the interstitial habitat, *P. linearis* has been designated as an euryhaline psammobiont.

In recent years, however, the species was established in different artificial and natural freshwater reservoirs in Bulgaria. In the benthal and in the phytal of Rabisha Reservoir it was both the most frequently occurring and also had the greatest population density [14]. With a relatively high frequency and abundance was found in sediments both in the medium deep, large Ovcharitsa Reservoir and in the coastal shallow lake Durankulak [16, 18]. A few specimens of this species were also found in the samples of wet mosses from River Rezovska [15]. During the present investigation *P. linearis* was observed in the soil mosses.

CONCLUSIONS

The moss-dwelling testacean fauna of “Zlatni Pyasatsi” Natural Park is rich and varied. Testate amoebae communities are dominated by the species: *C. aerophila* v. *sphagnicola*, *P.*

declivis, *E. rotunda*, *C. aerophila*, *P. hemisphaerica*, *C. eurystoma*, *T. enchelys* and *C. dubium*. This is first report for the establishment of *C. loevis* and *P. linearis* outside of water biotope. The finding them in varied biotops gives us the reason to consider that these two species have more wide ecological plasticity and distribution, can adapt well to different habitats and can be assigned to the cosmopolitan eurybionts.

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