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Ecology of Communities of Testate Amoebae (Amoebozoa, Rhizaria) Associated with Terrestrial Bryophytes in Zlatni Pyasatsi Natural Park, North-eastern Bulgaria

***Rositsa Davidova*[1]*, Anna Ganeva*[2]*, Maria Boycheva*[1]**

[1] Faculty of Natural Sciences, University of Shumen "Ep. K. Preslavsky", 115 Universitetska Street, 9700 Shumen, Bulgaria; E-mail: davidova_sh@yahoo.com
[2] Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2 Gagarin Street, 1113 Sofia, Bulgaria

Abstract: Species richness and structure of communities of testate amoebae associated with particular species of bryophytes (*Amblystegium serpens*, *Brachytheciastrum velutinum*, *Radula complanata*, *Homalothecium sericeum*, *Homalothecium lutescens*, *Hypnum cupressiforme* and *Scorpiuruim circinatum*) from Zlatni Pyasatsi Natural Park, NE Bulgaria, were studied. Bryophytes growing on different type of substratum (soil, rocky and epiphytic) and with different degree of moisture (wet, moist and dry) were examined. Comparisons of species numbers of testate amoebae between particular bryophytes did not show statistically significant differences. Species richness in soil bryophytes was significantly higher in comparison with those from rocky and epiphytic bryophytes. Results demonstrate that the community structure of testate amoebae varies greatly between bryophyte species and is determined by the type of substratum, on which bryophytes develop, and by the degree of its moisture.

Key words: testacean communities, richness, distribution, moisture, type of substratum

Introduction

Data on the diversity and structure of the testacean communities in bryophytes (except *Sphagnum* species) in different parts of the world could be found in the works by Beyens *et al.* (1986, 1990), Török (1993), Beyens, Chardez (1994), Todorov, Golemansky (1996), Todorov (1998), Van Kerckvoorde *et al.* (2000), Mitchell *et al.* (2004), Nguyen-Viet *et al.* (2004) and others. These studies indicated that communities developing in these conditions include mostly cosmopolitan and ubiquitous taxa that can also be seen in soils, in peat and in freshwater reservoirs. However, the species richness and abundance of each taxon in bryophytes vary widely (from nine to 88 species) and knowledge of the ecological preferences and biogeography of testate amoebae inhabiting bryophytes is important.

The ecology of most testacean living in mosses is still rather poorly studied although there are a number of recent studies, in different regions of the world (Charman, Warner 1997, Bobrov *et al.* 1999, Mitchell *et al.* 2000, Booth 2002, Lamentowicz, Mitchell 2005, Mieczan 2007a, b, Lamentowicz *et al.* 2010, 2013, Jassey *et al.* 2013). These studies showed that the richness and the structure of communities are strongly related to the environmenthal variables, such as moisture conditions, pH, chemical parameters, light, and food availability. Some investigations described the distribution of testate amoebae with respect to individual moss species (Jassey *et al.* 2011a, b). However, they reveal mainly the structure of testate amoeba communities in *Sphagnum*-dominated peatlands.

There are some publications regarding the environmental preferences of testate amoebae in bryophytes. Vincke *et al.* (2004, 2006) investigated bryophyte-dwelling testate amoebae from the region of Ile de la Possession (Crozet Archipelago, sub-

Antarctica) and from South Georgia and found that moisture played a key role in determining the distribution pattern of testacean communities. Nguyen-Viet *et al*. (2007) studied the relationships between testate amoebae communities and heavy metal concentrations in bryophyes and suggested that testate amoebae were sensitive to and might be good bioindicators of heavy metal pollution, especially lead. Meyer *et al*. (2010, 2012) showed that atmospheric pollutions had a negative impact on testate amoebae communities and that simple parameters, such as total biomass or total abundance of testate amoebae living in "bryophyte-testate amoebae" microsystems, were not only good indicators of atmospheric pollution but also of the sources of pollution (urban or industrial). Mieczan, Adamczuk (2015) indicated that variables that significantly explained the variance in testate amoebae communities in mosses were moisture, temperature, pH, and dissolved oxygen.

There are few studies focusing on the distribution of testate amoebae with respect to individual bryophyte species. Smith (1986) reported ten species on *Drepanocladus* moss carpet near Rothera Station, Adelaide Island. Nguyen-Viet *et al*. (2004) studied the testacean communities in *Tortula ruralis* growing on wall, stones and other hard and dry substrata in the town of Besancon (France) and surrounding villages and recorded nine species. Mitchell *et al*. (2004) explored the patterns of testacean communities in *Hylocomium splendens* in the South-eastern Alps of Italy and found a significant correlation between testate amoebae community structure and macro- and micro-nutrients concentration in the bryophyte tissues. Mieczan, Adamczuk (2015) studied the structure and spatial distribution of moss-dwelling testate amoebae communities in four species of mosses – *Polytrichastrum alpinum*, *Sanionia georgico-uncinata*, *Sanionia uncinata* and *Brachythecium austrosalebrosum* on King George Island (Antarctic Peninsula) and found fifteen species.

The aims of the study are: 1) to determine whether a relationship exists between the species composition and richness of the testate amoebae and particular bryophyte species, which have not been investigated so far; 2) to specify the preferences of testate amoebae with respect to moisture in the bryophytes and type of substratum.

Material and Methods

Study area

The study covered testate amoebae associated with bryophytes in Zlatni Pyasatsi Natural Park (North-eastern Bulgaria). This park covers an area of 1324.7 hectares, parallel to the coastal line of the Black Sea, 17 km north-east from Varna. The area forms the west border of the resort Golden Sands and reaches the Kranevo Village to the north (43°17′00″ N, 28°02′00″ E). The terrain in the park is diverse with an altitude between 60 and 269.3 m a.s.l.. The climate in the area is transitional: from continental to continental-mediterranean. There is a forest type vegetation in the park, and the dominant species are *Carpinus orientalis*, *Quercus cerris*, *Quercus frainetto*, *Fraxinus oxycarpa*, *Abies pinsapo*, *Cedrus libani*, *Ulmus minor*, *Quercus robur*, *Populus alba*, *Fraxinus americana*, *Fraxinus angustifolia*, etc. (Kopralev *et al.* 2002).

Sampling and laboratory analysis

Samples were collected during July and August 2011 from 21 species of bryophytes (Table 1). Identification of the bryophyte species follows Grolle, Long (2000), Hill *et al*. (2006), Natcheva *et al*. (2006), Papp *et al*. (2011). Moss moisture values were estimated using the F-classification of Jung (1936). According to this scale the studied bryophytes belong to the following moisture classes: FIII – very wet: water drips from sample without pressure; FIV – wet: water drips after slight pressure; FV – semi-wet: water drips after moderate pressure; FVI – moist: little water is produced after high pressure; FVII – semi-dry: only a few drops of water can be squeezed out; FVIII – dry: no water (Meisterfeld 1977). The analysis of the preferences of testate amoebae to the degree of moisture was made using 21 bryophyte samples, grouped in three groups: wet, moist and dry bryophytes (Table 1). The analysis of the preferences of testate amoebae to the type of substratum on which the bryophyte grows, was made using 30 bryophyte samples, grouped as epiphytic, rocky and soil bryohytes (Table 1).

To study the relationships between testate amoebae communities and different bryophyte species, the type of substratum and other environment factors, should remain constant. Therefore, the analysis of the relation between testate amoebae fauna and particular bryophyte species, which they inhabit comprise only part of the studied bryophytes and samples, given in Table 2.

At each sampling, the living green top parts of the bryophytes were collected from an area of 0.5/0.5 m. The bryophyte samples were stored and transported in paper or nylon bags. In the laboratory the gathered bryophyte tufts were soaked in distilled water, after which they were 'squeezed' and washed in it. Then the water used for the soaking and washing was filtered carefully, removing vegetative bryophyte particles, for establishing the taxonomic

Table 1. Grouping of the investigated bryophyte species according to degree of moisture and type of substratum

Groups	Moisture class (F)	Number of samples	Bryophyte species
According to different degree of moisture			
Wet bryophytes	III, IV, V	7	*Platyhypnidium riparioides*, *Oxyrrhynchium speciosum*, *Kindbergia praelonga*
Moist bryophytes	VI	7	*Amblystegium serpens*, *Hypnum cupressiforme*, *Platyhypnidium riparioides*, *Bryum moravicum*
Dry bryophytes	VII, VIII	7	*Homalothecium lutescens*, *Brachytheciastrum velutinum*, *Hypnum cupressiforme*, *Radula complanata*, *Amblystegium serpens*
According to different type of substratum			
Epiphytic bryophytes	VII, VIII	10	*Amblystegium serpens*, *Homalothecium lutescens*, *Brachytheciastrum velutinum*, *Hypnum cupressiforme*, *Porella platyphylla*, *Radula complanata*, *Scorpiuruim circinatum*, *Homalothecium sericeum*
Rocky bryophytes	VII, VIII	10	*Scorpiuruim circinatum*, *Homalothecium sericeum*, *Anomodon viticulosus*, *Cirrhyphyllum crassinervium*, *Brachytheciastrum velutinum*,*Kindbergia praelonga*, *Oxyrrynchium hians*, *Grimmia pulvinata*, *Tortula muralis*, *Orthotrichum* sp.
Soil bryophytes	VII, VIII	10	*Platyhypnidium riparioides*, *Amblystegium serpens*, *Bryum moravicum*, *Brachytheciastrum velutinum*, *Fissidens taxifolius*, *Oxyrrynchium hians*, *Homalothecium lutescens*, *Brachythecium rutabulum*, *Hypnum cupressiforme*, *Sciuro-hypnum flotowianum*

Table 2. Characteristics of the bryophyte species used to analyse the relationship between species composition and richness of the testate amoebae and particular bryophyte species

Bryophyte species	Moisture class (F)	Group according to the substratum	Number of samples
Radula complanata	VII, VIII	Epiphytic bryophytes	5
Brachytheciastrum velutinum	VII, VIII	Epiphytic bryophytes	5
Amblystegium serpens	VII, VIII	Epiphytic bryophytes	5
Hypnum cupressiforme	VII, VIII	Epiphytic bryophytes	5
Homalothecium sericeum	VII, VIII	Epiphytic bryophytes	5
Scorpiurium circinatum	VII, VIII	Epiphytic bryophytes	5
Homalothecium lutescens	VII, VIII	Epiphytic bryophytes	5

composition and the number of testate amoebae. All samples were preserved with 4% formaldehyde and stored in polyethylene bottles. Testate amoebae were identified and counted at magnification of 200-400x. Identification of the testate amoebae is based on the works of Deflandre (1928, 1929), Gauthier-Lievre, Thomas (1958), Decloitre (1962), Ogden, Hedley (1980), Ogden (1983), Ogden, Živcovic (1983).

Data analysis

The relative abundance (D) of each taxon was calculated as a percentage of the total count. The relationships between testate amoebae communities and environmental variables were explored using the computer programs Statistica 10.0 (StatSoft inc., 2010) and PAST (Hammer *et al*., 2001). Detrended correspondence analysis (DCA) was carried out to explore the characteristics of the communities and to examine associations between species. The significance of differences between testate amoebae richness and particular bryophyte species and groups was tested using analysis of variance (ANOVA).

Results

A total of 70 testate amoebae taxa were found in bryophyte samples (Tables 3, 4).

Testate amoebae fauna in different bryophyte species. In the seven bryophyte species used to analyse the relation between the species composition and the richness of the testate amoebae and particular bryophyte species 36 testate amoebae taxa were established (Table 3). The highest number of species (22) occurred in the moss *H. lutescens*.

Table 3. List of testate amoebae taxa and their relative abundance (%) in particular bryophyte species*

Testate amoebae taxa	Bryophyte species						
	R. comp.	***B. velut.***	***A. serp.***	***H. cupr.***	***H. seric.***	***S. circin.***	***H. lutesc.***
***Arcella** arenaria compressa* Chardez, 1974	2.24	-	-	-	0.37	-	-
A.arenaria sphagnicola Deflandre, 1928	0.75	-	-	-	-	-	-
A.catinus Penard, 1890	-	-	-	-	-	-	2.08
***Assulina** muscorum* Greeff, 1888	2.24	2.73	-	1.46	-	-	2.08
***Centropyxis** aerophila* Deflandre, 1929	47.01	6.36	13.12	1.09	13.76	-	17.71
C. aerophila sphagnicola Deflandre, 1929	-	10.00	10.00	3.28	8.92	4.82	2.08
C.hirsuta Deflandre, 1929	-	0.91	-	-	-	-	
C. laevigata Penard, 1890	-	-	-	-	0.74	-	1.04
C.orbicularis Deflandre, 1929	-	-	-	-	-	-	1.04
C. platystoma (Penard, 1890) Deflandre, 1929	-	0.91	-	-	-	-	3.65
***Corythion** dubium* Taranek, 1881	3.73	5.45	-	18.43	-	0.61	3.65
C.orbicularis (Penard, 1910) Iudina, 1996	-	-	-	4.01	-	-	0.52
***Cyclopyxis** eurystoma* Deflandre, 1929	5.22	10.91	9.38	2.01	-	6.63	-
C. kahli Deflandre, 1929	-	-	1.25	0.18	-	-	1.04
***Cyphoderia** loevis* Penard, 1902	-	-	-	-	-	6.02	-
***Difflugia** lucida* Penard, 1890	0.75	4.55	3.12	4.94	1.12	1.20	3.13
D. pulex Penard, 1902	-	-	-	0.18	-	-	-
***Difflugiella** oviformis* Bonnet &Thomas, 1955	1.49	-	-	-	-	-	-
***Euglypha** ciliata* (Ehrenberg, 1848) Leidy, 1878	-	-	0.63	8.03	-	-	0.52
E. ciliata glabra Wailes, 1915	26.12	-	3.12	6.39	-	-	4.17
E. compressa Carter, 1864	-	-	-	2.93	-	-	-
E. filiffera Penard, 1890	-	-	-	-	0.74	-	-
E. laevis (Ehrenberg, 1845) Perty, 1849	-	2.73	-	3.29	-	-	0.52
E. rotunda Wailes & Penard, 1911	0.75	20.91	18.13	30.11	5.58	12.05	11.98
E. tuberculata Dujardin, 1841	-	0.91	-	-	-	-	-
***Microchlamys** patella* (Clap. & Lach., 1885) Cockerell, 1911	2.98	0.91	-	-	4.09	-	4.69
***Microcorycia** flava* (Greeff, 1866) Cockerell, 1911	-	-	-	-	-	-	2.08
***Paraquadrulla** irregularis* (Wallich, 1863)	-	-	-	0.18	-	-	-
***Phryganella** hemisphaerica* Penard, 1902	4.48	8.18	30.00	7.66	29.00	36.14	5.73
***Plagiopyxis** declivis* Thomas, 1955	-	2.73	-	-	22.30	23.49	15.10
Pl. minuta Bonnet, 1959	0.75	-	-	-	-	7.23	-
***Tracheleuglypha** acolla* Bonn. & Thomas, 1955	-	-	-	-	-	0.61	-
***Trinema** complanatum* Penard, 1890	-	-	-	0.36	3.72	-	-
T. enchelys (Ehrenb., 1838) Leidy, 1878	-	15.45	7.50	1.46	5.20	-	11.98
Tr. lineare Penard, 1890	-	6.36	0.63	4.01	-	1.20	0.52
Tr. penardi Thomas & Chardez, 1958	1.49	-	3.12	-	4.46	-	4.69
Number of species	**14**	**16**	**12**	**19**	**13**	**11**	**22**

**R. comp. – Radula complanata, B. velut. – Brachytheciastrum velutinum, A. serp. – Amblystegium serpens, H. cupr. – Hypnum cupressiforme, H. seric. – Homalothecium sericeum, S. circin. – Scorpiurium circinatum, H. lutesc. – Homalothecium lutescens*

Relatively high diversity was found also in *H. cupressiforme* and *B. velutinum* where 19 and 16 species were established, respectively. In the samples of *A. serpens*, *R. complanata*, *H. sericeum* and *S. circinatum* the number of testate amoebae species varied between 11 and 14. However, a comparison of species numbers of testate amoebae among investigated bryophytes showed that the differences found were not significant (ANOVA, P =0.159).

The analysis of the dominant structure of the testaceans in different bryophyte species showed that the highest number of dominants (eight) was established in *B. velutinum*, and the lowest (three) – in *R. complanata*. DCA revealed that in terms of the

Table 4. List of testate amoebae taxa and their relative abundance (%) in bryophytes growing on different type of substratum and in bryophytes with different degree of moisture

Testate amoebae taxon	Epiphytic bryophytes	Rocky bryophytes	Soil bryophytes	Wet bryophytes	Moist bryophytes	Dry bryophytes
Arcella *arenaria compressa* Chardez, 1974	0.24	0.61	0.26	-	-	0.40
A. arenaria sphagnicola Deflandre, 1928	0.08	-	-	-	-	0.13
A. catinus Penard, 1890	-	-	0.26	-	-	-
A. rotundata Playfair, 1917	-	-	-	0.18	0.18	-
Assulina *muscorum* Greeff, 1888	1.34	0.47	0.39	-	-	9.70
Centropyxis *aculeata* (Ehrenberg, 1830) Stein, 1857	-	-	-	1.43	-	-
C. aerophila Deflandre, 1929	10.99	6.12	7.92	2.67	6.18	15.09
C. aerophila sphagnicola Deflandre, 1929	6.56	10.79	6.62	-	6.80	7.41
C. cassis (Wallich, 1864) Deflandre, 1929	-	0.08	-	0.53	-	-
C. constricta (Ehrenberg, 1841) Deflandre, 1929	-	-	-	1.60	-	-
C. ecornis (Ehrenberg, 1841) Leidy, 1879	-	-	-	-	0.18	-
C. elongata (Penard, 1890) Thomas, 1959	-	0.31	-	-	-	-
C. hirsuta Deflandre, 1929	-	-	-	0.36	-	-
C. laevigata Penard, 1890	-	0.15	0.13	-	-	-
C. marsupiformis (Wallich, 1864) Deflandre, 1929	-	-		0.36	-	-
C. orbicularis Deflandre, 1929	-	-	0.13	-	-	-
C. platystoma (Penard, 1890) Deflandre, 1929	-	0.54	0.91	0.71	0.35	-
C. sylvatica (Deflandre, 1929) Bonnet & Thomas, 1955	-	-	-	0.36	0.09	-
Corythion *dubium* Taranek, 1881	9.72	0.23	1.82	-	0.71	8.63
C. orbicularis (Penard, 1910) Iudina, 1996	1.74	-	0.13	-	-	-
Cyclopyxis *eurystoma* Deflandre, 1929	6.96	4.67	0.39	1.25	8.75	6.60
C. kahli Deflandre, 1929	0.40	0.99	0.39	-	4.24	-
Cyphoderia *ampulla* (Ehrenberg, 1841) Leidy, 1870	-	-	-	6.06	-	-
C. loevis Penard, 1902	-	0.77	-	6.42	3.80	-
C. trochus Penard, 1899	-	-	-	0.71	-	-
Cucurbitella *mespiliformis* Penard, 1902	-	-	-	-	0.27	-
Difflugia *bryophila* (Penard, 1902) Jung, 1942	-	-	-	0.18	-	-
D. decloitrei Godeanu, 1972	-	-	-	-	0.35	-
D. glans Penard, 1902	-	-	-	0.18	-	-
D. lucida Penard, 1890	4.43	2.52	2.73	-	1.77	2.96
D. manicata Penard, 1902	-	-		0.18	0.09	-
D. minuta Rampi, 1950	-	-		0.18	-	-
D. penardi Hopkinson, 1909	-	-		-	0.09	-
D. pristis Penard, 1902	-	-	0.13	0.18	-	0.13
D. pulex Penard, 1902	0.08	-	1.17	0.18	0.80	-
D. tenuis (Penard, 1890) Ogden, 1983	-	-		0.18	-	-
Difflugiella *horrida* Schönborn, 1965	-	-	6.88	-	3.80	1.08
D. oviformis Bonnet &Thomas, 1955	0.16	-	1.82	2.50	-	1.35
D. pusilla Playfair, 1918	-	-	-	0.18	0.27	-
Euglypha *ciliata* (Ehrenberg, 1848) Leidy, 1878	3.64	0.23	0.13	-	-	1.89
E. ciliata glabra Wailes, 1915	6.32	0.84	1.43	-	1.24	4.18
E. compressa Carter, 1864	1.26	0.08	-	-	-	0.27
E. compressa glabra Cash et al., 1915	-	0.31	-	-	-	-
E. filiffera Penard, 1890	-	0.23	-	0.36	-	-
E. filiffera cylindracea Playfair, 1917	-	-	18.96	-	12.90	-
E. laevis (Ehrenberg, 1845) Perty, 1849	1.58	0.23	0.39	-	0.18	0.27

Table 4. Continued

Testate amoebae taxon	Epiphytic bryophytes	Rocky bryophytes	Soil bryophytes	Wet bryophytes	Moist bryophytes	Dry bryophytes
E. polylepis Bonnet, 1960	-	-	-	-	1.41	0.40
E. rotunda Wailes & Penard, 1911	20.16	12.55	7.79	19.96	17.49	7.55
E. strigosa glabra Wailes, 1898	-	-	0.13	-	0.09	-
E. tuberculata Dujardin, 1841	-	-	2.99	0.71	2.03	-
Heleopera *petricola* Leidy, 1879	-	-	0.13	0.36	-	-
H. sylvatica Penard, 1890	-	0.08	0.13	-	-	0.13
Microchlamys *patella* (Clap. & Lach., 1885) Cockerell, 1911	0.87	2.83	1.69	14.44	1.59	0.67
Microcorycia *flava* (Greeff, 1866) Cockerell, 1911	-	-	0.13	-	-	-
Paraquadrulla *irregularis* (Wallich, 1863)	0.08	0.46	-	1.96	-	-
Phryganella *hemisphaerica* Penard, 1902	8.77	27.08	6.88	0.36	14.13	7.14
Plagiopyxis *declivis* Thomas, 1955	1.66	12.01	12.73	0.18	1.41	4.31
P. minuta Bonnet, 1959	0.08	1.53	0.13	-	-	0.13
Pontigulasia *elisa* Penard, 1893	-	-		0.89	-	-
Psammonobiotus *linearis* Golemansky, 1970	-	-	2.21	0.18	-	2.29
Pseudodifflugia *fascicularis* Penard, 1902	-	-	0.13	-	0.09	-
Schoenbornia *viscicula* Schonborn, 1964	-	-	-	-	0.18	-
Trachelocorythion *pulchelum* Bonnet, 1979	0.16	-	0.13	1.96	-	0.27
Tracheleuglypha *acolla* Bonnet & Thomas, 1955	0.08	0.46	0.78	1.07	0.80	-
T. dentata Deflandre, 1938	-	-	-	2.14	3.09	-
Trinema *complanatum* Penard, 1890	0.24	0.23	-	-	-	-
T. enchelys (Ehrenberg, 1838) Leidy, 1878	6.01	6.04	7.14	11.41	4.15	9.43
T. lineare Penard, 1890	5.61	1.68	1.17	16.58	0.44	6.87
T. lineare truncatum Chardez, 1964	-	-		0.89	-	-
T. penardi Thomas & Chardez, 1958	0.79	4.90	2.73	-	0.09	0.67
Number of species	**28**	**31**	**38**	**39**	**35**	**27**

dominants, most specific and different was the testate amoebae fauna of *R. complanata*, while greatest similarity were found between the testacean faunas of *B. velutinum*, *A. serpens* and *H. sericeum* (Fig. 1A). Testate amoebae species *Plagiopyxis minuta*, *Cyphoderia loevis*, *Euglypha ciliata* and *Trinema lineare* dominated in only one of the studied mosses, respectively *Plagiopyxis minuta* and *Cyphoderia loevis* in *S. circinatum*, *Euglypha ciliata* in *H. cupressiforme* and *Trinema lineare* in *B. velutinum* (Fig. 1). *Euglypha ciliata glabra* and *Corythion dubium* were dominants in two bryophyte species – *R. complanata-H. cupressiforme* and *B. velutinum-H. cupressiforme*, respectively. Five of the established 13 dominant species dominanted in more than half of the bryophyte species. These were *Phryganella hemisphaerica* and *Euglypha rotunda* (dominants in six bryophyte species), *Centropyxis aerophila* (in five), *Cyclopyxis eurystoma* and *Trinema enchelys* in four (Fig. 1B).

Testate amoebae fauna in bryophytes growing on different type of substratum. Species richness in soil bryophytes (38 testate amoebae species) was significantly higher in comparison with those from rocky and epiphytic bryophytes, where 31 and 28 species were established, respectively (ANOVA, P =0.001; Table 4).

In the epiphytic bryophytes, which were characterised by the lowest species richness a greater number of dominants (nine) were found, as four of them had a high relative abundance only in them (Fig. 2). These were *C. eurystoma*, *C. dubium*, *T. lineare* and *E. ciliata glabra*. In soil bryophytes eight dominant species were established, two of which were dominant only here: *Difflugiella horrida* and *Euglypha filifera cylindracea*. Species with high relative abundance in rocky bryophytes were six and one of them – *P. hemisphaerica* was dominant only in these bryophytes. The species *P. declivis* was dominant both in soil and in rocky bryophytes. The remaining four dominants – *C. aerophila*, *E. rotunda*, *C. aerophila sphagnicola* and *T. enchelys* showed little ecological preferences and were established with

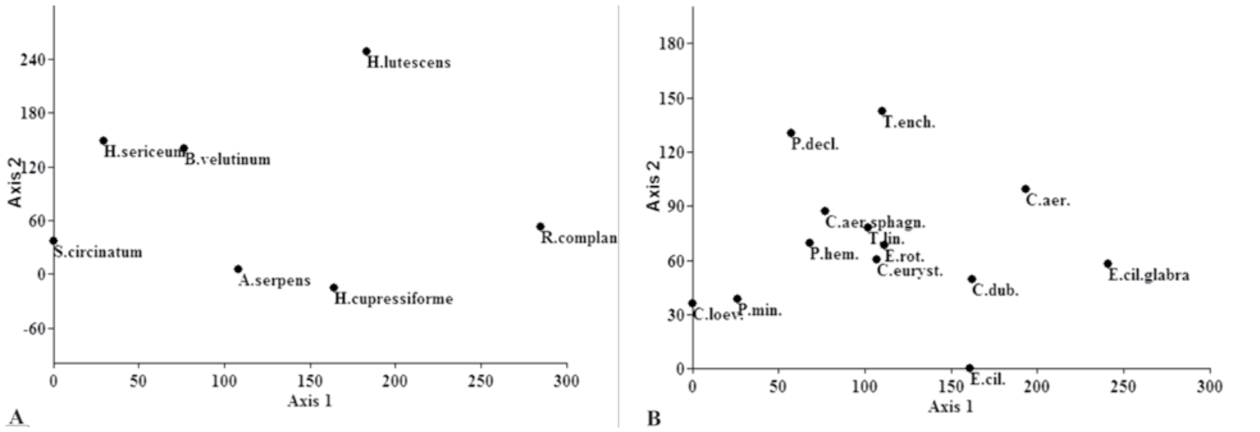

Fig. 1. Detrended correspondence analysis showing ordination of: A – particular species of bryophytes and B – dominant species of testate amoebae

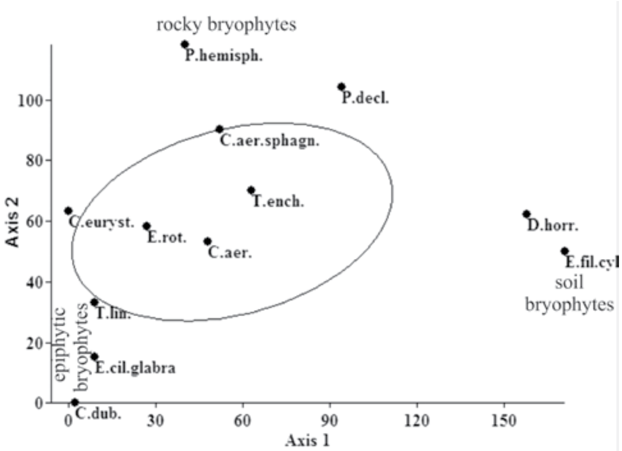

Fig. 2. Detrended correspondence analysis showing ordination of dominant species of testate amoebae and bryophytes growing on different type of substratum (soil, rocky and epiphytic bryophytes)

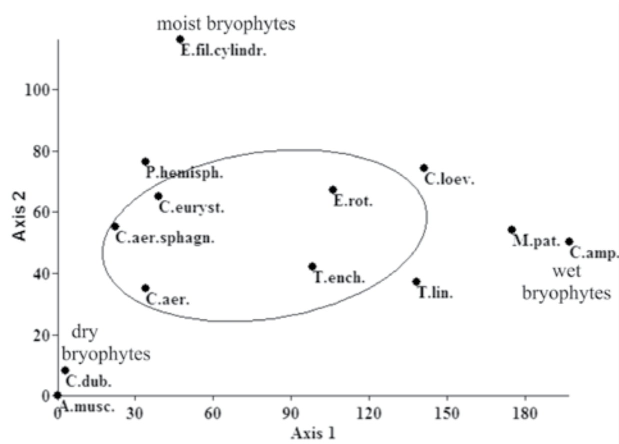

Fig. 3. Detrended correspondence analysis showing ordination of dominant species of testate amoebae and bryophytes with different degree of moisture (wet, moist and dry bryophytes)

high relative abundance in bryophytes growing on the three types of substratum (Fig. 2).

Testate amoebae fauna in bryophytes with different degree of moisture. The greatest species diversity was found in wet bryophytes (FIII, FIV and FV), where 39 testate amoebae were established (Table 4). Slightly smaller was the number of species found in moist bryophytes (FVI) – 35 species, while the dry bryophytes were characterised by the lowest taxonomic diversity (FVII and FVIII), where 27 species were identified. Comparing species richness of testate amoebae in wet, moist and dry bryophytes showed that the differences were not significant (ANOVA, P =0.687).

The analysis of the community structure of testate amoebae in bryophytes with different moisture showed that the species *Cyphoderia ampulla*, *C. loevis* and *Microchlamys patella* dominated in very wet bryophytes (Fig. 3). *E. filifera cylindracea* and *P. hemisphaerica* were abundant and dominated in moist bryophytes. In dry bryophytes the community was predominantly composed of *C. dubium* and *Assulina muscorum*. The DCA plot indicated that five of the established 13 dominant species – *C. aerophila*, *E. rotunda*, *C. aerophila sphagnicola*, *C. eurystoma* and *T. enchelys* did not show preferences to particular moisture content in the bryophytes and were found with greater relative abundance at different levels of moisture (Fig. 3).

Disscusion

The species richness of testate amoebae in particular bryophyte species varied but these differences were not significant. The higher species diversity in mosses *H. lutescens* and *H. cupressiforme* was due to the presence of species *Corythion orbicularis*, *Difflugia pulex*, *Euglypha compressa*, *Microcorycia flava* and *Paraquadrula irregularis*, which were found only in these mosses, but in single specimens, which was not enough to define them as typical to a particular bryophyte species. These results do not confirm the observations of Mieczan, Adamczuk (2015). Analysing testate amoebae fauna inhabiting four different species of bryophytes, the authors found statistically significant differences between testate amoebae richness, abundances and biomass in the studied bryophytes and indicated that these characteristics were related significantly to the bryophyte species. However, the authors explained that these differences were a consequence of the different level of moisture in bryophyte samples. The lack of any statistically significant difference in testacean richness in the present investigation may be related to the fact that bryophyte species were in similar conditions, the degree of moisture of the bryophytes was constant. Our results agree well with other research by Mieczan (2012). Studying protozoa living in Sphagnum mosses, the author found that species richness levels and abundances of testate amoebae were similar between different Sphagnum species and indicated that the lack of any statistically significant difference was because of the fact that all Sphagnum species were situated in similar physical and chemical conditions.

Analysing the structure of the testacean communities in different bryophytes, we found that they were dominated mainly by the same species. Nevertheless, some differences were observed. In *S. circinatum* along with the common to other investigated bryophyte dominants, with high relative abundance were *P. minuta* and *C. loevis*. In the other bryophytes these species were with very low relative abundance or were never established. In *H. cupressiforme* one of the five dominant species was *E. ciliata*, which was found only in two other bryophyte species, but with single specimens (Fig. 1). A similar variability in terms of the dominant species reported Mieczan, Adamczuk (2015) and indicated that in *P. alpinum*, the community was predominantly composed of *C. dubium*, whereas in *B. austrosalebrosum*, *S. georgico-uncinata* and *S. uncinata* dominated *T. lineare*, *C. aerophila* and *Nebela lageniformis*. Török (1993) established that *Phryganella acropodia* had the highest occurrence in *Brachythecium velutinum*, and *Trinema penardi* was a characteristic species to *Cirriphyllum tommasinii*. Vincke *et al.* (2004) indicated that many testate amoebae showed a preference for a specific bryophyte species.

Thus, our study supports the conclusion that some testate amoebae species show a preference for a particular species of bryophytes. However, the reasons for this are not clear enough and it may be related to the presence of specific conditions required for the development of population of these species. Therefore, additional studies on the diversity and ecology of testate amoebae in bryophytes from different parts of the world are needed.

Our results show that the soil bryophytes testacean fauna is characterised by statistically significant higher species diversity in comparison with the epiphytic and rocky species. Moreover, in soil bryophytes a presence of typical species was found, e.g. found only in these bryophytes and with a high relative abundance were *D. horrida* and *E. filifera cylindracea* (Fig. 2). In contrast the fauna of the epiphytic and rocky bryophytes consisted mainly of eurybionts and widely spread species. The species *C. eurystoma*, *C. dubium*, *T. lineare*, *E. ciliata glabra* and *P. hemisphaerica*, which dominated these bryophytes, were found in soil bryophytes, as well. In their studies of the testate amoebae fauna of the epiphytic and soil bryophytes in different parts of Bulgaria Golemansky (1967), Golemansky, Todorov (1990), Todorov (1998) have found that the soil bryophytes are characterised by a much wider species diversity compared to the epiphytic ones. According to Golemansky (1967) this is due to a considerably constant greater wetness of soil bryophytes, which enables them to contain a larger number of rhizopodes. Bonnet (1973) distinguished the bryophytes growing on trees, which were drier and usually colonised by a ubiquitous and cosmopolitan fauna, and the bryophytes growing on soils, which, in addition were also colonised by soil-specific taxa. The same observation has been made by Nguyen-Viet *et al.* (2004), who indicated that bryophytes growing on vertical surfaces, such as trees and walls, where water drains fast, represent an extreme environment for testate amoebae.

Species richness of testate amoebae was highest in bryophytes samples with FIII moisture values and decreased towards FVIII bryophytes. Thus, our results agreed with other studies on bryophytes. Warner (1987) indicated that an increase in species diversity was connected to an increase in the moisture content of the habitat. Vincke *et al.* (2004) and Mieczan *et al.* (2012) observed a growth in the variety of species testate amoebae in bryophytes located in the wettest habitats. Mieczan, Adamczuk (2015) found that the number of species of testate amoebae increased along with increasing moisture conditions.

The community structure of testate amoebae varied greatly between bryophytes with different moisture. The results clearly indicate that *A. muscorum* and *C. dubium* are associated with the driest bryophytes and agree well with other studies. *Corythion dubium* has been reported as an indicator taxon of drier bryophyte habitats (Beyens *et al*. 1986, 1990, 1992, Tolonen *et al*. 1994, Van Kerckvoorde *et al*. 2000, Vincke *et al*. 2004, 2006, Booth, Zygmunt 2005, Mieczan 2012). *Assulina muscorum* is generally regarded as a species with xerophilous tendencies (Beyens *et al*. 1986, 1990, Vincke *et al*. 2004). In the present study interest represents the high relative abundance of the species *C. ampulla*, *C. loevis* and *M. patella* in wet bryophytes, which is reported for the first time. This is not surprising given the ecological preference of these species: they are often found in different aquatic habitats (Vincke *et al*. 2006, Todorov *et al*. 2009, Payne, Mitchell 2007). We should note also that in the present study the highest relative abundance of the species *C. aerophila* and *C. aerophila sphagnicola* are observed in the range of moisture from FVI to FVIII. The establishment of these two species as dominants and in dry environments indicated that they are not strictly hydrophilous as has been previously mentioned by some authors (Smith 1982, Vincke *et al*. 2006). According Vincke *et al*. (2006) the highest abundance of *C. aerophila* is observed in the range from FIII to FV. Species in the centre of the diagram, such as *E. rotunda*, *C. eurystoma* and *T. enchelys* which are abundant in bryophytes with varying degree of moisture, have wide ecological preferences and can be found in both dry and moist biotopes.

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