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Ecology of testate amoebae (Protists) in a *Sphagnum*-dominated peat bog and the relationship between species assemblages and environmental parameters

by

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Abstract

Despite its ecological importance, little information is available regarding spatial and seasonal changes in the testate amoebae community in peat bogs. The objectives of this study were to examine the structure of communities and horizontal distribution of testate amoebae fauna, to improve the understanding of factors affecting the distribution of moss testate amoebae communities and to analyze the seasonal changes in testate amoebae communities in a *Sphagnum*-dominated peat bog (eastern Poland). A total of 45 testate amoebae species were identified in the samples. The highest species richness occurred in hollows dominated by *Sphagnum angustifolium*, much lower numbers of taxa were observed in hummocks dominated by *Sphagnum magellanicum* and *Polytrichum*. The Monte Carlo permutation test showed the significance of N_{tot}, temperature, pH, and the depth to the water table for the variability of testate amoebae in all microhabitats. Species found in spring samples were associated with the increased N_{tot} content. Species occurring in summer samples were associated with the increasing pH gradient and species developing in late spring and autumn preferred a greater depth to the water table.

Key words: peat bog, biodiversity, Protists, testate amoebae, seasonal dynamics

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Introduction

Peatlands are generally characterized by rich biodiversity and play an important role in the global carbon sequestration (Gilbert & Mitchell 2006). Due to the clear predominance of testate amoebae in peatlands, they are regarded as having the most important role in the matter and energy flow (Tolonen et al. 1992; Bobrov et al. 1999; Mitchell et al. 2000; Lamentowicz et al. 2010). They produce decay-resistant tests that protect cells from desiccation (Odgen & Hedley 1980). The morphology of tests is very often diagnostic, allowing species-level identification. Moreover, testate amoebae produce decay-resistant shells that are commonly used to reconstruct the past peatland hydrology (Payne 2011). These microorganisms are important consumers of bacteria, flagellates and algae; they also participate in the transformation of organic matter and the circulation of biogenic compounds. The short duration of their generations make them useful indicators of environmental changes (Lamentowicz & Mitchell 2005; Opravilova & Hajek 2006; Koenig et al. 2015). Animal communities, in particular invertebrates occurring in *Sphagnum*-dominated peat bogs are thoroughly researched (Borcard & Vaucher von Ballmoos 1997). On the other hand, species richness and abundance of Protists and their relationships with environmental parameters in these specific ecosystems have received little or no attention at all. The ecology of peat-bog testate amoebae is studied along a wide ecological gradient, i.e. from very wet to dry microsites where testate amoebae are often found to respond primarily to the depth of the water table (Buttler et al. 1996; Booth 2002; Kishaba & Mitchell 2005; Lamentowicz et al. 2010; Jassey et al. 2011; Payne 2011; Mieczan 2012). Relationships between testate amoebae and hydrological and chemical variables are well documented, much less is known about their seasonal dynamics in a peat bog. Numerous ecological studies dealing with recent responses of species to the water-level gradient in *Sphagnum* peat bogs provide a good basis for the use of testate amoebae as indicators of the past hydrological changes, and recently also as indicators of hydrological changes during wetland restoration (Tolonen et al. 1992). However, their response to seasonal changes in microhabitat conditions has not yet been studied thoroughly in peatlands. Mieczan (2007) found that moisture conditions, pH and the content of total organic carbon in the water were positively correlated with the abundance and biomass of testate amoebae at microsites with *Sphagnum palustre* L. In addition, Tolonen et al. (1992) found testate amoebae to be correlated with the concentration of nutrients. Even the temperature can co-determine the distribution pattern of the testacean fauna (Heal 1964). Testate amoebae require a minimum temperature at a specific time of the year to reproduce successfully (Tsyganov et al. 2011). In addition, factors such as light, oxygen and food availability may also affect the testate amoebae communities (Charman et al. 2000). Only a few publications focus on seasonal abundance of microorganisms living among *Sphagnum* (Heal 1964; Gilbert et al. 1998; Mieczan 2007; Lamentowicz et al. 2013; Marcisz et al. 2014). The seasonal patterns in the abundance of microorganisms are important to the understanding of nutrient and energy flows, as well as species-environment relationships. Since peat-bog ecosystems are deficient in inorganic nutrients and rich in organic matter, it is likely that microorganisms play a key role in their functioning (Gilbert et al. 1998). The objectives of this study were to test the hypotheses that testate amoebae communities vary in different seasons and the differences are related to moisture conditions and water chemistry as well as to examine the structure of communities and horizontal distribution of testate amoebae fauna in a *Sphagnum*-dominated peat bog, to improve the understanding of factors affecting the distribution of moss testate amoebae communities and to analyze the seasonal changes in testate amoebae communities.

Materials and methods

Study site

The study was performed in the Moszne peat bog located in the western part of the Polesie Lubelskie region (Eastern Poland, 51°N, 23°E) (Fig. 1). It is one of the most valuable natural regions in Poland, which was not covered by the last glaciation. The mean monthly air temperatures of January and July were –4.1°C and 17.9°C, respectively. Mean annual rainfall is ca. 551 mm. Vegetation of this area is characterized by the presence of a number of rare species, such as *Scheuchzeria palustris* L., *Drosera anglica* Huds., *Drosera intermedia* Hayne, *Salix myrtilloides* L. and *Salix lapponum* L. and the dominance of graminoids such as *Eriophorum vaginatum* (L.), *Carex acutiformis* Ehrhart., *Carex gracilis* Curt. and *Sphagnum angustifolium* (C.C.O. Jensen ex Russow), *Sphagnum cuspidatum* Ehrh. ex Hoffm., *Sphagnum flexuosum* Dozy and Molk., *Sphagnum magellanicum* Bird. and *Polytrichum* sp.

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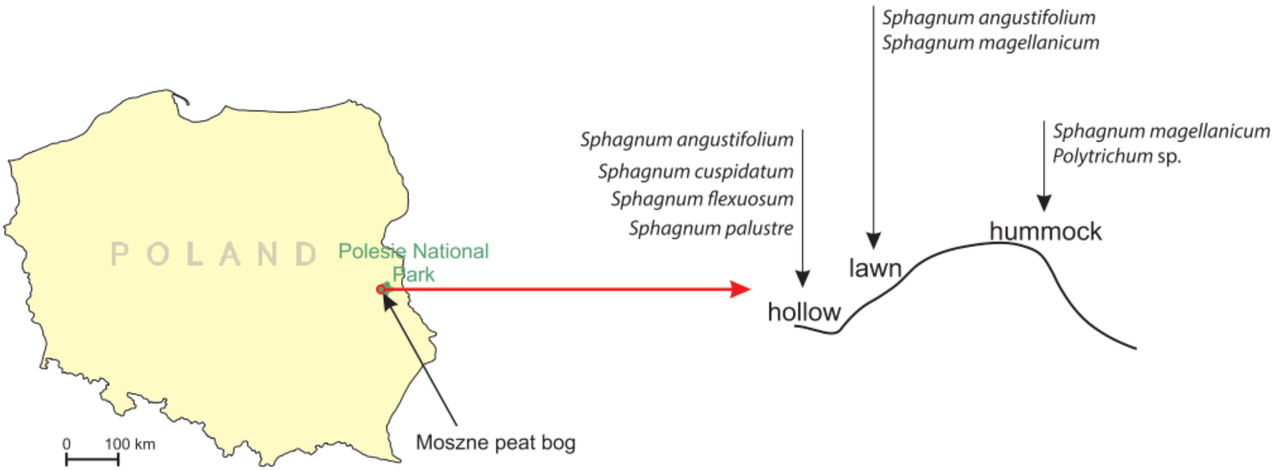

Figure 1

Location of the study area and sampling sites

Field sampling and laboratory analyses

Samples of testate amoebae were collected from the *Sphagnum*-dominated peat bog. The microsites sampled in this study include hummocks, lawns and hollows (Fig. 1). Similar habitats (six replicated plots, three at each microsites) were selected in the investigated peat bog. The habitat similarity was determined for the selected microsites based on the vegetation type. From April to October 2013-2014, upper segments of *Sphagnum* were sampled once a month. A long knife was used to cut the plants off the surrounding vegetation. Each sample was packed into a cylindrical plastic container, which was pressed into the moss carpet and cut with the knife. All samples were stored in a cooler and transported within 1 day to a laboratory. Testate amoebae were isolated from moss samples using sieves with a 250 μm mesh size, and the filtrate was used to analyze the testate amoebae. Shells were counted under a light microscope at 500 × magnification. The abundance of testate amoebae and their community composition were determined using Utermöhl's method (Utermöhl 1958). The abundance of microorganisms was calculated per 1 g of the plant material. Morphological identifications of testate amoebae were based mainly on the studies by Odgen & Hedley (1980), Charman et al. (2000) and Clarke (2003).

Various environmental variables (e.g. depth of the water table – DWT , dissolved oxygen, pH and conductivity) were measured at each sampling site on the day of sampling. DWT was measured to the nearest centimeter. The zero level was marked by the top part of the peat mosses. Temperature, dissolved oxygen, pH and conductivity were determined using a multiparameter instrument YSI 556 MPS. Total organic carbon (TOC) was determined using the PASTEL UV analyzer and the remaining factors ($N\text{-}NO_3$, $P\text{-}PO_4$, P_{tot}) were analyzed in the laboratory (Hermanowicz et al. 1976).

Data analysis

Diversity analysis [Shannon-Wiener diversity index ($\log_{10}$-based)] was performed using the Multivariate Statistical Package – MVSP (Kovach Computing Services 2002).

Differences in physical and chemical water parameters among the studied habitats were analyzed by one-way ANOVA. Tukey's multiple range test (at $P < 0.05$) was used to compare means when significant differences were found. The analysis was performed using the PAST software (Hammer et al. 2005). Ordination techniques were used to describe the abundance of testate amoebae in relation to habitats and environmental variables. The length of the gradient indicated by detrended correspondence analysis (DCA) of the communities was 3.482, which suggests that DCA and canonical correspondence analysis (CCA) were appropriate methods (ter Braak & Šmilauer 2002). Automatic forward selection of environmental variables with the Monte Carlo permutation test (999 permutations) was used to determine the most important variables (Lepš & Šmilauer 2003). Variables with the significance level above 0.05 were passively plotted on the diagrams. Arrows representing the physicochemical variables on the obtained plot indicate the direction of the maximum change of a given variable, and the length of each arrow is proportional to the rate of change.

The proportion of variance explained by environmental variables was quantified using variance partitioning (Borcard et al. 1992). The analysis was repeated for the whole sampling area and separately for each of the studied habitats. The ordination analyses were performed by CANOCO 4.5 for Windows (Ter Braak & Šmilauer 2002).

Results

Physical and chemical parameters

The highest water table was observed in spring and summer and the lowest one in autumn (ANOVA, $F = 12.36$, $P = 0.021$). The DWT gradient in the samples ranged from 6 to 23 cm. The pH of water ranged from 3.1 to 7.6. The conductivity significantly varied and ranged from 18 µS cm^{-1} to 68 µS cm^{-1} (ANOVA, $F = 22.74$, $P = 0.003$). The highest conductivity occurred in summer, much lower in spring or autumn. The highest water temperature was recorded in summer (18°C - 23.7°C) for all the studied peatlands, and decreased in autumn (3.6°C - 14.3°C). The concentration of total organic carbon fluctuated between 35 mg C l^{-1} in summer and 68 mg C l^{-1} in autumn. The highest values of nutrients were determined in spring and autumn, and considerably lower values in summer (ANOVA, $F = 28.11$, $P = 0.0021 - 0.033$) (Table 1).

Testate amoebae communities – general results

A total of 45 testate amoebae species were identified in the samples. The highest species richness (28-41 taxa) occurred in hollows dominated by *Sphagnum angustifolium, Sphagnum cuspidatum Sphagnum flexuosum* and *Sphagnum palustre*. Much lower numbers of taxa (4-8) were observed in hummocks dominated by *Sphagnum magellanicum* and *Polytrichum*. The most frequent species were *Assulina muscorum*, *Arcella discoides* type, *Hyalosphenia papilio, Hyalosphenia elegans* and *Archerella flavum*. The species distribution pattern also showed a higher variation in acid habitats, from wet assemblages with *Hyalosphenia papilio* to *Assulina muscorum* assemblages in drier microhabitats. The species richness and abundance was different for *Sphagnum* (*Sphagnum angustifolium*, *Sphagnum cuspidatum, Sphagnum flexuosum* and *Sphagnum palustre*); however, only a slight difference was determined for the abundance of testate amoebae among *Polytrichum.* The diversity analysis resulted in a mean value of the Shannon-Wiener diversity index (H) of 2.45. The highest diversity was measured in *Sphagnum cuspidatum* (H = 2.8), and the lowest one in *Polytrichum* sp. (H = 0.65).

Unconstrained ordination (DCA)

DCA axes 1 ($\lambda = 0.465$) and 2 ($\lambda = 0.198$) explained 44% of the total variance in testate composition and showed clear spatial separation of species. Testate showed similar species composition in hollows and lawns and different composition on hummocks (Fig. 2A). *Heleoptera sphagni*, *Cyclopyxis arcelloides* type, *Hyalosphenia papilio, Hyalosphenia elegans, Hyalosphenia ovalis, Heleoptera rosea, Nebela flabellulum, Nebela collaris, Centropyxis platystoma*

Table 1

Seasonal changes in physical and chemical parameters of the investigated peat bog

Parameters	units	2013								2014							
		months															
		IV	V	VI	VII	VIII	IX	X	XI	IV	V	VI	VII	VIII	IX	X	XI
Temp.	°C	18.0	21.6	23.4	21.7	20.2	14.4	14.3	14.3	21.2	21.2	14.8	23.7	18.8	17.4	3.6	3.6
pH		3.1	3.1	3.9	3.3	6.2	6.5	3.9	3.9	3.5	3.5	4.6	5.2	7.7	3.9	3.9	3.9
Conductivity	µS cm^{-1}	44.0	44.0	24.0	35.0	60.0	23.0	59.0	59.0	31.0	31.0	18.0	50.0	53.0	67.0	68.0	68.0
Oxygen	mg O_2 l^{-1}	5.9	5.9	5.6	7.0	13.1	3.8	9.4	9.4	5.6	5.6	8.7	5.2	7.5	5.9	11.1	11.1
N-NH_4^+	mg N-NH_4 l^{-1}	0.135	0.155	0.125	2.696	0.256	0.012	0.070	0.070	0.020	0.020	0.020	0.016	0.021	0.011	0.023	0.023
N-NO_3	mg N-NO_3 l^{-1}	1.156	1.156	0.740	1.014	0.805	1.602	1.030	0.030	0.658	0.658	0.096	0.757	0.533	0.582	0.651	0.651
Ntot	mg N l^{-1}	4.318	4.318	1.134	0.159	1.158	1.523	2.57	2.567	4.318	4.318	1.134	0.16	1.16	1.52	2.57	2.57
Ptot	mg P l^{-1}	0.434	0.434	1.262	0.590	0.402	0.278	0.569	0.569	0.739	0.739	0.449	0.500	0.612	0.551	0.774	0.774
Chlorophyll *a*	µg l^{-1}	185.6	160.6	277.4	265.2	95.1	170.3	118.0	118.0	33.5	33.5	24.2	103.7	77.1	26.9	47.1	47.1
TOC	mg C l^{-1}	75.3	75.3	37.8	74.0	126.5	37.8	60.5	60.5	38.5	38.5	23.8	42.0	27.7	38.0	38.8	38.8
DWT	cm	7.0	6.0	9.0	11.0	11.0	17.0	23.0	17.0	9.0	9.0	7.0	11.0	9.0	18.0	19.0	22.0

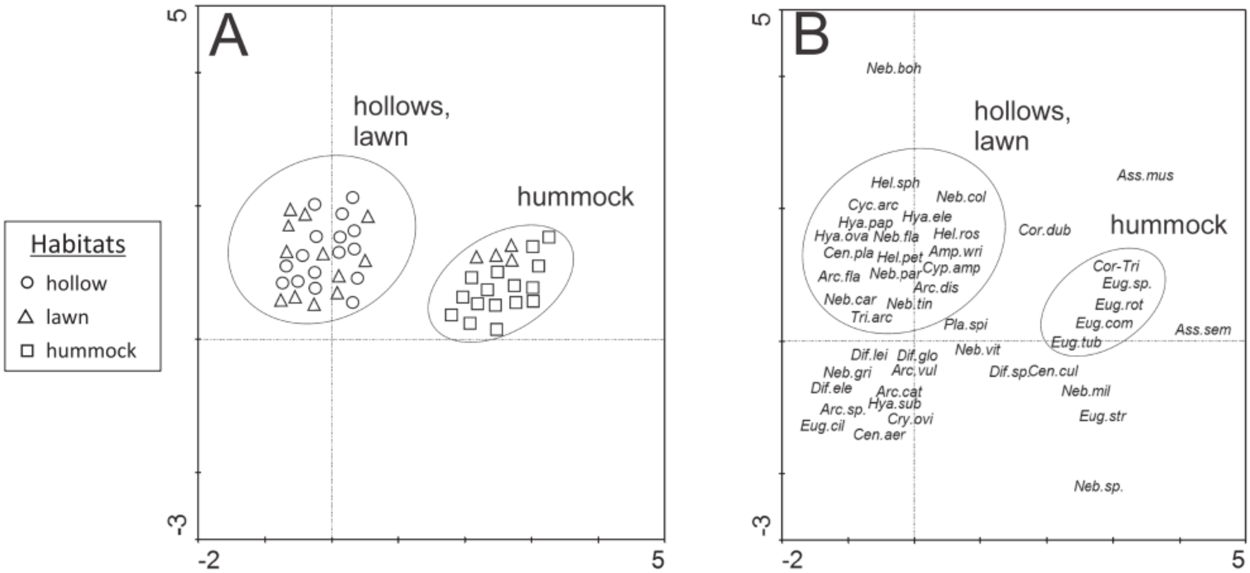

Figure 2

Biplots of DCA axes 1 and 2 showing species populations of testate amoebae (A) and habitats (B)

type, *Heleoptera petricola, Amphitrema wrightianum, Cyphoderia ampulla, Arcella disoides* type, *Archerella flavum, Nebela carinata, Nebela tincta, Nebela parvula* and *Trigonopyxis arcula* showed affinity with hollows and lawns. The group of species related to hummocks were represented by *Corythion-Trinema* type and *Euglypha ciliata* (Figs 2A, B).

Constrained ordination (CCA)

CCA for spatial distribution of testate amoebae showed that all environmental variables together explained 82.5% of the total variance. The Monte Carlo permutation test at $P < 0.05$ showed the significance of N_{tot} ($\lambda = 0.14$, $F = 1.61$, $P = 0.001$), temperature ($\lambda = 0.29$, $F = 1.38$, $P = 0.002$), pH ($\lambda = 0.07$, $F = 0.76$, $P = 0.025$), and DWT ($\lambda = 0.09$, $F = 0.56$, $P = 0.036$) in explaining the variability of testate amoebae in all habitats. Samples on the CCA biplot are divided seasonally into three groups. Summer samples (July-September) were separated by axis 1 from the early spring (April-May) samples and late spring and autumn (June, October-November) samples (Fig. 3A). Species occurring in summer samples (*Assulina seminulum*, *Arcella disoides* type, *Hyalosphenia elegans, Hyalosphenia subflava, Nebela*) were associated with the increasing pH gradient. Species found in spring samples (*Cyphoderia ampulla, Assulina muscorum, Heleoptera petricola, Difflugia elegans, Placocista spinosa* type, *Centropyxis platystoma* type, *Difflugia globulosa*, *Euglypha*) were associated with the increased N_{tot} content. Species developing in late spring and autumn (*Centropyxis aerophila, Heleoptera sphagnii, Nebela bohemica, Hyalosphenia ovalis, Hyalosphenia papilio, Amphitrema wrightianum, Difflugia leidyi, Heleoptera rosea*, *Nebela flabellulum, Archerella flavum*) preferred higher DWT (Fig. 3B).

Discussion

The existing comparative data on seasonal changes in the distribution of testate amoebae in the micro-horizontal transect (hummock – lawn – hollow) are very scarce. This study suggests significant relationships between the species richness of testate amoebae and the type of habitat. The highest species richness occurred in hollows dominated by *Sphagnum angustifolium, Sphagnum cuspidatum, Sphagnum flexuosum* and *Sphagnum palustre*. The lowest species richness occurred in hummocks dominated by *Sphagnum magellanicum* and *Polytrichum*. At all three studied sites, a decline in the abundance of testate amoeba was observed in summer, particularly pronounced in microhabitats with the lowest moisture content. These results are consistent with the results of other seasonal studies (Marcisz et al. 2014). Testate amoeba assemblages are similar to those found at other sites in Europe (Jauhiainen 2002; Lamentowicz & Mitchell 2005). The similarity of testate amoeba assemblages between our study sites and other sites is not surprising given the cosmopolitan distribution of many taxa. Testate amoebae occurring in the studied peat bog were mainly represented by:

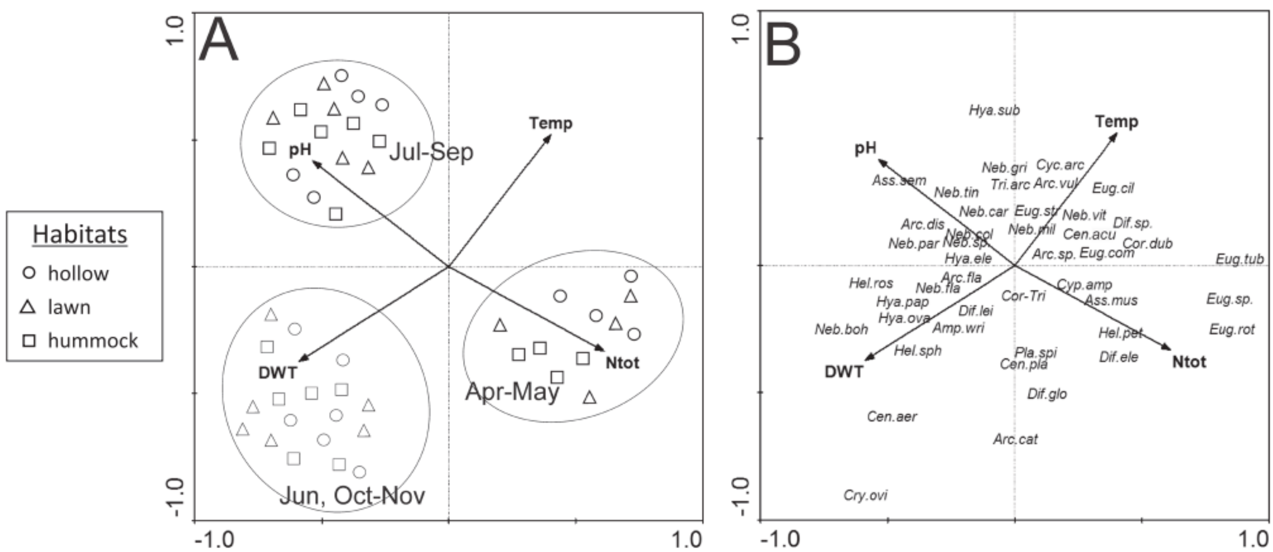

Figure 3

CCA biplots showing relationships between environmental variables and habitats (A) and testate amoebae populations (B). Arrows indicate significant parameters in the Monte Carlo permutation test at $P < 0.05$. Species codes: *Amp.wri, Amphitrema wrightianum; Arc.cat, Arcella catinus type; Arc.dis, Arcella disoides type; Arc.sp., Arcella* sp.*; Arc.vul, Arcella vulgaris; Ass.mus, Assulina muscorum; Arc.fla, Archerella Flavum; Ass.mus, Assulina muscorum; Ass.sem, Assulina seminulum; Cen.acu, Centropyxis aculeata type; Cen.aer, Centropyxis aerophila; Cen.pla, Centropyxis platystoma type; Cor.dub, Corythion dubium; Cor.tri, Corythion-Trinema type; Cry.ovi, Cryptodifflugia oviformis; Cyc.arc, Cyclopyxis arcelloides type; Cyc.amp, Cyphoderia ampulla; Dif.ele, Difflugia elegans; Dif.glo, Difflugia globulosa; Dif.lei, Difflugia leidyi; Dif.sp., Difflugia* sp.*; Eug.cil, Euglypha ciliata; Eug.com, Euglypha compressa; Eug.rot, Euglypha rotunda type; Eug.sp., Euglypha* sp.*; Eug.str, Euglypha strigosa; Eug.tub, Euglypha tuberculata type; Hel.pet, Heleopera petricola; Hel.ros, Heleopera rosea; Hel.sph, Heleopera sphagni; Hya.ele, Hyalosphenia elegans; Hya.ova, Hyalosphenia ovalis; Hya.pap, Hyalosphenia papilio; Hya.sub, Hyalosphenia subflava; Neb.boh, Nebela bohemica; Neb.car, Nebela carinata; Neb.col, Nebela collaris; Neb.par, Nebela parvula; Neb.fla, Nebela flabellulum; Neb.gri, Nebela griseola type; Neb.mil, Nebela militaris; Neb.sp., Nebela* sp.*; Neb.tin, Nebela tincta; Neb.vit, Nebela vitraea type; Pla.sci, Placocista spinosa type; Tri.arc, Trigonopyxis arcula*

Arcellidae and Hyalosphenidae. The genus Arcella was represented mainly by *Arcella discoides* (the largest number of individuals), and the genus Hyalosphenia by 3 species: *Hyalosphenia elegans*, *Hyalosphenia ovalis*, and *Hyalosphenia papilio*. The taxa are referred to as α-hydrophiles and dominate in habitats with significant humidity. A similar dominance structure was determined, among others, in peatlands of northern Russia (Bobrov et al. 1999). In the present study, the occurrence of 5 to 45 species of testate amoebae was determined in a single species of moss. Mitchell et al. (2004) determined the occurrence of 25 species of testate amoebae among *Hylocomium splenders*. By comparison, Bonnet (1973) recorded a total of 49 species of testate amoebae living in mosses. However, those were 13 different moss species growing on a range of substrates. Nguyen et al. (2004) studied the testate amoebae communities colonizing the moss *Tortula ruralis* and recorded 8 species in 30 samples. The remarkably low diversity of testate amoebae among *Polytrichum* is probably the consequence of low moisture content in this microenvironment. The number of species increased with decreasing pH and increasing humidity of the habitats. Testate amoebae also have to find the required material to build their test, and this requirement may be another constraint that determines their distribution (Meisterfeld 1977). Payne & Mitchell (2007) emphasized the significant influence of the water table level and pH on the occurrence of testate amoebae in Greek peat bogs. Such a relation also exists in the studied peat bog. In each of the study periods, the highest water level was determined in the hollows, which coincided with the highest abundance of testate amoebae.

We observed differences in testate amoeba communities in different seasons. In spring, the most abundant species were hydrophiles *Arcella vulgaris*, *Hyalosphenia ovalis*, *Hyalosphenia papilio*, *Amphitrema wrightianum* and *Nebela* sp. *Hyalosphenia* and *Amphitrema* were primarily observed in the top part of mosses and/or in the surface water, where their endosymbionts can photosynthesize (Mieczan 2010). *Nebela* consumes mainly algae, particularly diatoms (45%), spores and mycelia of fungi (36%), and also large ciliates, rotifers, and small amoebae (Gilbert et al. 2003). An increase in the contribution of species from the genus *Arcella* may result from their relatively wide ecological tolerance. As evidenced by the study of Nicolau et al. (2005), species of this genus also occur in sewage with a high content of biogenic compounds. The spring peak may be associated with the increase in nutrient concentration and the water level. These patterns are also partly consistent with the previous observations. For example, Lamentowicz et al. (2013) observed a peak in the number of active testate amoebae in spring and autumn and a lower activity in mid-winter. In summer, *Hyalosphenia papilio*, *Arcella vulgaris* and *Amphitrema wrightianum* were the most abundant species. The water level and pH were the most important environmental variables among the measured ones. The importance of the water level and pH was also reported by other studies (Bobrov et al. 1999; Booth 2002; Mitchell et al. 2000; Lamentowicz et al. 2010). Differences in testate amoebae communities in spring and summer were also observed by Heal (1964) and Gilbert et al. (1998). Warner et al. (2007) observed different testate amoeba communities in summer (higher proportion of wet indicators associated with high water-table conditions following the snowmelt) compared to those occurring in autumn. In autumn, the relative abundance of several species (e.g. *Centropyxis aerophila*, *Heleoptera sphagnii*, *Nebela bohemica*, *Hyalosphenia ovalis*, *Amphitrema wrightianum*, *Difflugia leidyi*, *Heleoptera rosea*, *Nebela flabellulum*, *Archerella flavum*) was correlated with a greater water table depth (WTD). Moisture may affect testate amoebae and their potential food resources – bacteria, microalgae, other protozoa, micrometazoa and fungi (Mitchell et al. 2000). Field observations, supported by experiments with *Difflugia tuberculata*, indicate that chemical properties of water, in particular pH, are likely more important factors separating fen and bog pool species than the availability of food (Heal 1964). This study shows that testate amoebae respond to the same major environmental gradients in Poland as in other parts of the world. The strongest relationship was found between testate amoebae communities and both the water table depth and pH. Also a significant effect of total nitrogen on the occurrence of testate amoebae was determined. Similar results, i.e. a correlation between testate amoebae and some nutrients, were determined in drained and restored *Sphagnum* peatlands in Finland (Jauhiainen 2002). In *Sphagnum* peatlands, testate amoebae communities were associated with NO_3^- (Mitchell et al. 2000) and a combination of physical variables, e.g. moisture and chemical variables such as pH, N and DOC (Tolonen et al. 1994, Mieczan et al. 2015). Heal (1964) did not attribute the seasonal changes observed in testate amoebae communities to the seasonal changes in soil moisture factors. Instead, he believes that changes in light conditions may affect symbiotic zoochlorellae in the organisms. There might be other biological factors, such as differences in the organism body size, correlated with soil moisture conditions and hence seasonal changes. Taxa with a smaller body size tend to be more common in drier habitats where perhaps the water film on moss stems and leaves is thinner compared to larger taxa that occur with wetter mosses (Jassey et al. 2011). In the growing season, when mean daily temperature increased and light intensity was higher, an increase in the abundance of mixotrophic taxa was observed. According to Schönborn (1965), the symbiosis between testate amoebae and zoochlorellae in the peat bog is an adaptation to oligotrophic conditions.

Conclusions

It therefore appears that the relationship between testate amoebae and species of mosses in the peat bog does not necessarily imply a direct ecological connection between the two types of organisms, but is explained by the fact that the habitat moisture conditions primarily define the niches of the moss species. Regardless of the microhabitat type, the number of testate amoebae was determined mainly by WTD, pH, temperature and total nitrogen. Species found in spring samples were associated with the increased N_{tot} content. Species occurring in summer samples were associated with the increasing pH gradient, whereas species developing in late spring and autumn preferred a greater depth to the water table.

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