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VERTICAL MICRO-DISTRIBUTION OF TESTATE AMOEBAE AND CILIATES IN *SPHAGNUM* DOMINATED PEATLAND

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Abstract. Microzonation of testate amoebae and ciliates in relation to physical and chemical parameters in different species of mosses in peatland were studied. Regardless of the species of mosses, similar micro-vertical differentiation of these protozoa was ascertained. A similar number of species, like the abundance, significantly increased in the deepest sampling depth. The uppermost sampling of the mosses (0–5 cm) was dominated by mixotrophic taxons, whereas the deepest sampling level (5–10 cm) shown the increase of the proportion of bacterivore species. In peatlands the factors limiting the occurrence of ciliates and testate amoebae are physio-chemical suitablilty – mainly the total organic carbon content and water table depth, but with somewhat lower levels of pH and species of mosses.

Key words: testate amoebae, ciliates, micro-distribution, peatland

INTRODUCTION

Quantifying the diversity and distribution of protozoa (testate amoebae and ciliates) in aquatic habitats is important to gain a better understanding of microbial food webs in these systems. However, limnologists have paid little attention to peatlands, compared to other aquatic ecosystems [Gilbert *et al.* 2003]. Peatland ecosystems play an important role in the global C sequestration. They have been studied in the context of general climate change, and specifically ozone depletion, but research has focused largely on the peatland plants [Mitchell *et al.* 2000, Mieczan 2009]. In peatlands the animal communities, especially invertebrates, are sufficiently known [Borcard and Vaucher von Ballmoos 1997]. However micro-distribution of testate amoebae taxa has been observed along the *Sphagnum* stem only in South America. This spatial variation has been attributed primarily to gradients of light, temperature, oxygen concentration and food. Vertical micro-distribution is also a gradient from mostly live testate amoebae in the aerobic, upper portions and mostly empty test in the more anaerobic, lower portions [Booth 2002]. On the other hand, there are hardly any data concerning the vertical micro-distribution of ciliates. These comparisons can provide insights into the ecology of microorganisms, and may guide the collection of more representative calibration datasets. Investigations of testate amoebae have shown distinct differences in the vertical micro-distribution [Mitchell and Gilbert 2004, Mazei *et al.* 2007]. It seems that similar differences may also be expected to appear in the case of ciliates. Therefore, one of the aims of this study was the analysis of the distribution of the micro-vertical structure of Protozoa. To sum up, the research was undertaken to verify the following hypotheses: that the physio-chemical characteristics of waters in a significant way influence the species diversity of testate amoebae and ciliates in the peatlands; that the hydrological parameters and species variability of mosses have a significant influence on the abundance and structure of these microorganisms.

MATERIALS AND METHODS

The study was performed in Moszne peatland located in the western part of the Polesie Lubelskie (Eastern Poland, 51°N, 23°E) includes a unique territory, which is a miniature of tundra at its extreme southwest European location (Fig. 1). Its borders encompass the most precious parts of Poleski National Park, including lakes and floodplains, as well as swamps and peatlands, which survived until now in a relatively unaltered shape. 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. The vegetation is dominated by 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 & Molk., *Sphagnum magellanicum* Bird. and *Polytrichum* sp. Samples of testate amoebae and ciliates were collected from the different species of mosses (*Sphagnum angustifolium, Sphagnum cuspidatum, Sphagnum flexuosum, Sphagnum magellanicum*, *Polytrichum strictum* and *Polytrichum commune)*. From April to November 2015 from studied peatlands once a month, eight samples were collected. To assess the importance of the vertical distribution of testate amoebae and ciliates within the mosses, each sample was cut into two parts (sub-samples): living green part (0–5 cm) and dead brown part (5–10 cm) (Fig. 1). All samples were stored in a cooler and transported within 1 d to the laboratory. Microorganisms were identified in four subsamples, each equal to 5% of the original sample. The abundance of microorganisms was calculated on 1 g of the plant material. In order to determine the ciliates and testate amoebae four samples were preserved with Lugol solution. Ciliates were enumerated and identified with an inverted microscope at 400–1000 x magnification. Quantitative sampling and counting were performed with classical limnological methods using Utermöhl technique. Taxonomic identification was done using the method of Foissner and Berger [1996].

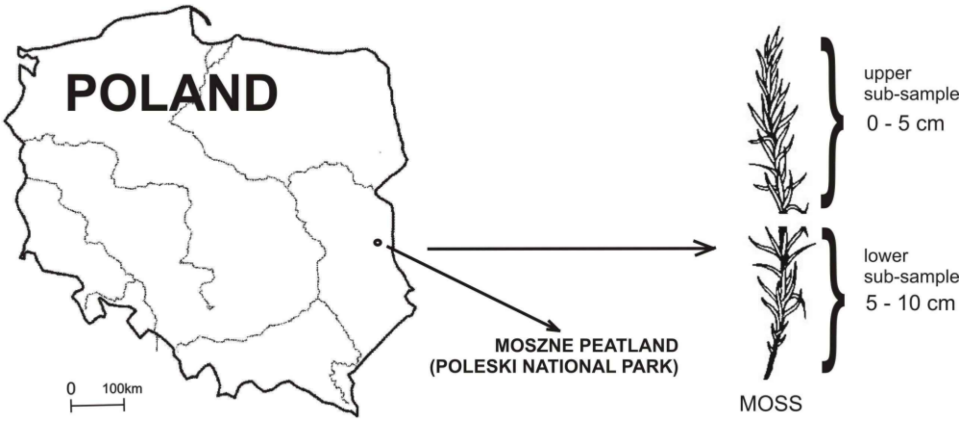

Fig. 1. Study area and scheme of the sampling procedure

During each sampling occasion physical and chemical factors (e.g. depth of the water table – DWT, pH, temperature, conductivity, $N\text{-}NO_3$, $P\text{-}PO_4$, P_{tot}, total organic carbon – TOC) were examined. Depth of water table (DWT) was measured within a centimetre measure. Temperature, pH and conductivity were determined *in situ* using the electrode JENWAY 3405, TOC was determined using the PASTEL UV and the remaining factors were analysed in the laboratory with standard methods [Hermanowicz *et al.* 1976]. Diversity analysis [Shannon Wiener diversity index (log_{10}-based)] was performed using the Multivariate Statistical Package – MVSP All statistical analyses were made using SAS Programme [2001]. All abiotic and biotic data were log-transformed to approximate a normal distribution and to linearize bivariate relationships. Full-factorial ANOVA was used to test for significant effect of the three independent factors (type of microsites, vertical micro-distribution and time) on testate amoebae and ciliate species richness and abundance. The correlation between abundance of ciliates and environmental factors (seasonal aspect) was assessed using Spearman correlation coefficient.

RESULTS AND DISCUSSION

Depth to the water table fluctuated significantly during the sampling periods. The water table was highest in spring, with the sampling sites being the wettest, whereas the water table was lowest and the sites were driest during summer. In the samples, the DWT gradient ranged from 6 (i. e. submerged) to 43 cm, with a pH

Table 1. Description of the sampling sites (physical and chemical characteristics of water – average values for spring, summer and autumn 2015)

Site name	Season*	DWT cm	pH	Temp. °C	Conduct. µS cm^{-1}	N-NO_3 mgNO_3 dm^{-3}	PO_4^{3-} mgPO_4 dm^{-3}	P_{tot} mgP dm^{-3}	TOC mgC dm^{-3}	Moss species sampled
	spring	6	3.8	7.9	69	0.762	0.439	0.753	61.2	*Sphagnum flexuosum* Dozy & Molk., *Sphagnum cuspidatum* Ehrh. ex Hoffm.
Moszne 1	summer	7	2.6	16.0	73.6	0.449	0.060	0.180	58.8	
	autumn	9	3.9	2.6	54.5	0.058	0.040	0.358	28.1	
	spring	29	3.2	7.3	34.6	0.622	0.110	0.336	52.3	*Sphagnum magellanicum* Bird., *Poly-trichum strictum* Men-zies *ex* Brid., *Polytrichum commune* Hedw.
Moszne 2	summer	43	2.7	18.3	71.8	0.299	0.288	0.320	66.9	
	autumn	29	3.3	2.1	45.3	0.662	0.122	0.366	79.2	
	spring	7	5.2	8.7	48.6	0.367	0.191	0.423	79.7	*Sphagnum flexuosum* Dozy & Molk., *Sphagnum palustre* L.
Moszne 3	summer	9	3.4	8.6	81.3	1.195	0.081	0.092	46.5	
	autumn	9	3.9	3.3	54.4	0.058	0.040	0.353	76.8	

* spring – average values from period April–June, summer – average values from period July–August, autumn – average values from period September–November

gradient from 2.6–5.2, a conductivity gradient from 46–123 µS cm^{-1} and a total organic carbon gradient from 28.1 mg C dm^{-3} to 79.7 mg C dm^{-3}. Conductivity and nutrients reached the highest values at high pH micro-sites. TOC content reached the highest values in low pH micro-sites (Table 1). Species richness among both testate amoebae and ciliates showed micro-vertical diversity. The number of taxa of the protozoans was the highest in the lower level (34 and 12 taxa, respectively) and became much lower in upper level where merely 15 testate amoebae taxa and 5 ciliate taxa were identified. Ciliates and testate amoeba assemblages are similar to those found at other sites in Europe [Jauhiainen 2002, Lamentowicz and Mitchell 2005]. The similarity in Protozoa assemblage between the sites from this study and other sites is not surprising given the cosmopolitan distribution of many taxa. The species richness of testate amoebae increased with depth and was significantly higher for the deepest level. *Assulina muscorum, Hyalosphenia papilio* and *Hyalosphenia elegans* were relatively more abundant in the uppermost, photosynthetic part of the mosses, and decreased in relative abundance with depth. *Arcella discoides* and *Archerella flavum* were relatively more abundant at the deepest sampling depth. In the microenvironment dominated by *Polytrichum,* in both the layer above the surface and the layer below the surface, *Assulina muscorum* occurred in the highest numbers. The species diversity, minimal in the uppermost 5-cm layer of sphagnum moss (mean Shannon index, 1.0), remained at approximately the same level in the deeper layers (2.3–2.8). Moreover, regardless of the moss species, the highest numbers of testate amoebae occurred in the deepest sampling site (Fig. 2 a-f). Several factors probably contribute to this. According to Booth [2002], some taxa occur predominantly on the upper portions of the mosses, and these taxa eventually become incorporated into the lower assemblage by vertical transport and growth, and senescence of the moss. Likewise, a distinct increase in moisture in the lower parts of the mosses may result in increased abundance of testate amoebae. Taxa with symbiotic zoochlorellae were more common at the top of the stem, and agglutinate taxa more common on lower portions of the stem [Meisterfeld 1977]. A vertical micro-distribution in community of testate amoebae was clear among the *Sphagnum* moss (*Sphagnum angustifolium*, *Sphagnum cuspidatum, Sphagnum flexuosum* and *Sphagnum palustre*)*;* on the other hand, it was blurred in dry environments dominated by *Polytrichum strictum* and *Sphagnum magellanicum*. The vertical distribution patterns of testate amoebae in this study are similar to studies from Europe [Heal 1962, Buttler *et al.* 1996]. The species richness of ciliates increased with depth and was higher for the deepest level, compared with the living green part. The Shannon-Wiener diversity index ranged from 1.33 for the deepest part to 0.33 for the green, living part. As follows from the full-factorial ANOVA, the vertical micro-distribution, site and season of the year in which material was collected from research, all had a statistically significant influence on the number of ciliates. In all species of mosses examined, there were appreciable vertical differences in the abundance of protozoa (Fig. 2 a-f). In the uppermost sampling of mosses the abundance of ciliates was the lowest and in general only the mixotrophic *Paramecium* dominated, whereas in the deepest sampling the abundance was appreciably higher with domination of *Colpidium colpoda, Chilodonella uncinata* and *Cinetochilum margaritaceum.* The uppermost sampling of the mosses was dominated by mixotrophic taxons, whereas the deepest sampling level shown the increase of the proportion of bacterivore species. Similar results were observed in sphagnophilus ciliates in Germany [Strüdel-Kypke 1999]. Such a significant difference in the vertical distribution was probably a result of the degree of dampness and fertility of the micro-sites. A clear paucity of mixotrophic taxa in the lower part of the mosses could also be a consequence of unutilized light conditions. A similar regularity was observed in *Sphagnum* mats in dystrophic peat bog lakes in Germany [Strüdel-Kypke 1999]. The studies showed that testate amoebae and ciliate abundance were more dependent on WTD and pH in summer (Spearman coefficient of correlation $r = 0.62$, $p \leq 0.01$). In spring and autumn contents of total organic carbon and nutrients are probably the major regulators of abundance of ciliates ($r = 0.32–0.64$ and $r = 0.41–0.51$, $p \leq 0.05$, respectively) (Table 2).

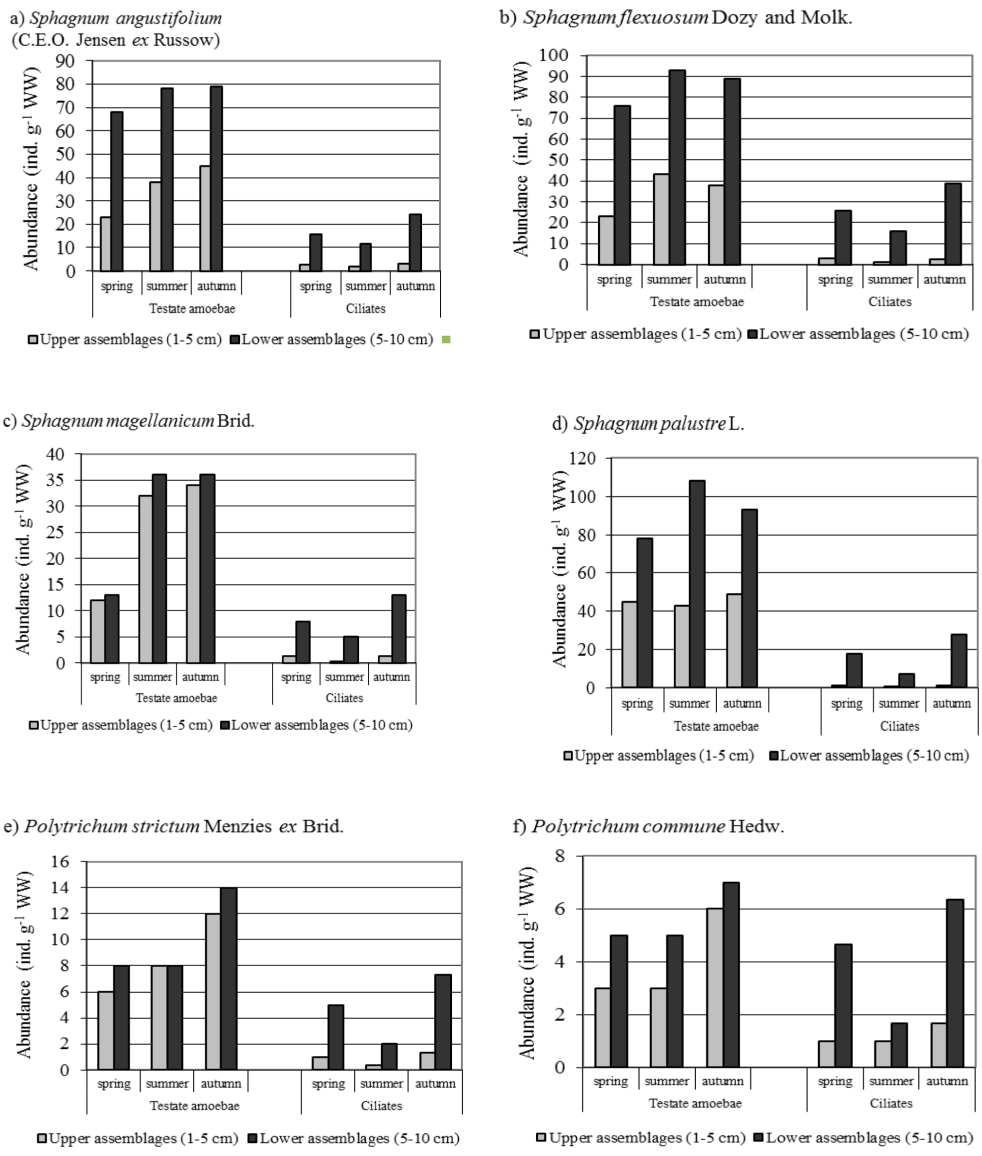

Fig. 2. Average (April-November 2015) density of testate amoebae and ciliates associated with investigated species of mosses along the vertical gradient (WW – wet weight, 0–5 cm; 5–10 cm)

Table 2. Spearman correlation coefficients between protozoan abundance and physical and chemical factors of the investigated peatland,

Site name	Season	DWT	pH	Temp.	Conduct.	$N\text{-}NO_3$	PO_4^{3-}	P_{tot}	TOC
Moszne 1 (M1)	spring	0.40*	0.38*	-	-	0.42*	0.32*	-	0.48*
	summer	0.62**	0.62**	-	-	-	-	-	-
	autumn	0.40*	0.40*	-	-	-	-	-	0.55*
Moszne 2 (M2)	spring	0.35*	0.38*	-	-	0.39*	0.32*	-	0.47*
	summer	0.62**	0.62**	-	-	-	-	-	0.32*
	autumn	0.33*	0.36*	-	-	0.41*	0.42*	-	0.51**
Moszne 3 (M3)	spring	0.51**	0.51**	-	-	-	-	-	0.51**
	summer	0.62**	0.62**	-	-	-	-	-	0.48*
	autumn	0.50**	0.48*	-	-	0.48*	0.51**	-	0.51**

** $P \leq 0.01$, * $P \leq 0.05$, – not significant

The number of species of testate amoebae and ciliates increased together with a decrease in pH, and increase of concentrations of total organic carbon, and moisture conditions. The opposite tendency, however, was observed in a lakes ecosystem where low pH clearly limited the number of ciliate taxa [Crisman and Brezonik 1980, Mieczan 2007].

CONCLUSIONS

It therefore seems that in peatland relationship between testate amoebae, ciliates and species of mosses does not necessarily imply a direct ecological link between two types of organisms, but is explained by the fact that the moisture conditions of micro-sites primarily define the niches of the moss species. There was a distinct vertical micro-differentiation of testate amoebae and ciliates occurring among *Sphagnum* moss, but only a small difference ascertained among *Polytrichum*. The number of testate amoebae and ciliate species significantly increased at the deepest sampling depth. Regardless of the micro-vertical mingling of Protozoa, their abundance to a high degree was limited by WTD, pH and TOC. The uppermost sampling of the mosses was dominated by mixotrophic taxons, whereas put the deepest sampling level the increase of the proportion of bacterivore species was shown.

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WERTYKALNE MIKROROZMIESZCZENIE AMEB SKORUPKOWYCH I ORZĘSKÓW NA TORFOWISKU SPHAGNOWYM

Streszczenie. Mikrorozmieszczenie ameb skorupkowych i orzęsków w powiązaniu z właściwościami fizycznymi i chemicznymi wód analizowano na torfowisku mszarnym wśród różnych gatunków mchów. Niezależnie od gatunku mchu stwierdzono wyraźne mikrowertykalne rozmieszczenie pierwotniaków, przy czym zarówno liczba gatunków, jak i liczebność wzrastały wraz z głębokością. Górna, fotosyntetyzująca część mchów (0–5 cm) zdominowana była przez taksony miksotroficzne, natomiast wraz z głębokością (5–10 cm) wzrastał udział taksonów bakteriożernych. Czynnikami determinującymi występowanie orzęsków i ameb skorupkowych były głównie poziom wód i całkowity węgiel organiczny, w mniejszym zaś stopniu pH i gatunek mchu.

Słowa kluczowe: ameby skorupkowe, orzęski, mikrorozmieszczenie, torfowiska