ABSTRACT: The research was made on the largest (ca 500 ha) peatland complex in the western Poland ("Chlebowo" mire) (N 52°44'14.3" E 16°45'20.7"), that exists under considerable anthropogenic pressure. Species composition of testate amoebae in selected microhabitats was described. The fundamental environmental factors influencing the structure of assemblages were determined. Twenty four samples were taken from 10 sites (Sphagnum lawn, hollow and hummock) dominated by Sphagnum and brown mosses. Each of them was analysed in relation to its taxonomical composition. Nine environmental parameters (acidity (pH), conductivity, colour, NH$_4^+$, NO$_3^-$, PO$_4^{3-}$, SO$_4^{2-}$, Ca$^{2+}$ and Mg$^{2+}$) were measured in the field and laboratory. Thirty two testate amoebae species of 13 genera were identified within the 24 sites. In most of the sites species composition was dominated by Hyalosphenia papilio Leidy, Cyclopyxis arcelloides Leidy and Hyalosphenia elegans Leidy, however the most numerous were Amphitrema flavum Archer, Hyalosphenia papilio Leidy and Cyclopyxis arcelloides Leidy. The particular parameters, i.e. pH, depth to the water table (DWT) and SO$_4^{2-}$ content significantly explained the species variability. Multivariate analyses showed that species tend to group especially along the pH gradient; to a smaller extent along the DWT and SO$_4^{2-}$, together with pH.

KEY WORDS: Protists, Testate Amoebae, Sphagnum peatland, conductivity, pH, nutrients

1. INTRODUCTION

Testate amoebae (Protists) are unicellular organisms numerously occurring in water and peatland environments (Bobrov et al. 1999); they can also be found in soil, on tree bark and roofs of buildings (Ogden and Hedley 1980). Characteristic feature of their anatomy is the presence of a proteinaceous, calcium or silica shell. They also agglutinate organic material, diatoms or minerals (Ogden and Hedley 1980). Another distinctive morphological feature of the shell is the pseudostome (aperture), through which pseudopodia emerge. Shell structure is an important criterion taken into consideration in taxonomical research on this group of organisms (Meisterfeld 2001a, 2001b, Bobrov and Mazei 2004).

The most significant parameters determining species distribution of testate amoebae in peatlands is the habitat moisture (Tolonen 1986, Warner 1987, 1990) and pH (Charman and Warner 1992, Booth 2001). Nevertheless, acidity is correlated with other environmental parameters and it may often be a combination of numerous factors (Charman et al. 2000). Yet, literature data is scarce, as far as the relationship of amoebae
and chemical parameters of their habitats is concerned.

Testate amoebae respond dynamically to environmental changes (Mitchell and Gilbert 2004) and reveal well-defined ecological preferences (Tolonen *et al.* 1992, 1994, Bobrov *et al.* 1999, Lamentowicz and Mitchell 2005). Therefore, these organisms are regarded as important bioindicators of environmental changes. The correlation between the occurrence of testate amoebae and the moisture is often used in paleoenvironmental reconstructions (Mitchell *et al.* 2001, Charman 2001).

Studies of testate amoebae were not common in Poland. First information about this group of protists was described from lake environments (Moraczewski 1961, Schönborn 1966, 1981, 1984). Moreover, some papers were published about morphology and species composition of testate amoebae in peatland habitats (Offierska 1978, 1984, Offierska-Wawrzyniak 1993). Studies including relation of environmental parameters and testate amoebae were made by Lamentowicz and Mitchell (2005) and Mieczan (2007).

This study is one of the few taking into consideration several environmental variables governing the distribution of testate amoebae in various microhabitats on the peatland surface.

The paper has two aims: a) to describe the taxonomic composition of testate amoebae in various mire habitats, and b) to determine the most important environmental parameters that influence the amoebae communities structure in peatlands.

2. STUDY AREA

Studied peatland “Chlebowo” (local name) is located in Toruń-Eberswald ice marginal valley in western Poland (N 52°44'14.3” E 16°45'20.7”). The peatland complex occupies the area of ca. 500 ha and is located in a depression with a slight western slope, from the north, west and south surrounded by dunes. In its external parts, meadow complexes and fens are present; central part is occupied by a mostly degraded fen (Celka and Szkudlarz 2000). The whole area is a subject of anthropogenic transformation, especially peat exploitation carried out for over a hundred years (Ilnicki 1996, 2002). In the 19th century the peatland was drained by the ditch (Ludomicki canal) and several other ditches, changing the structure of the area. Numerous exploitation ponds (over 50) left after peat exploitation can be found here.

All the former exploitation ponds are progressively overgrown with peat-accumulating communities advancing from their edges. The process of succession is initiated both by swamp communities with *Phragmites australis* (Cav.) Trin. ex Steud and *Typha latifolia* L., transforming into poor fens dominated by *Sphagnum fallax* K. Kilinggr. Particular water basins are separated from each other with dikes – remains of former mire surface composed of desiccated peat, in 50–60% overgrown with pine-birch stands. In 1959, a small (4.42 ha) nature reserve was created in the central part of the mire, protecting a fragment of coniferous forest *Vaccinium uliginosì –Pinetum*, nowadays strongly desiccated. An information about the vegetation and fauna of the analysed area can be found in the study of Celka and Szkudlarz (2000).

Basic physical and chemical parameters of ground water in “Chlebowo” peatland complex indicate high nutrients concentration and considerable water colour. The water was poor in Ca²⁺ and Mg²⁺ (Table 1).

3. METHODS

In order to investigate the species assemblages of the living testate amoebae, surface samples of *Sphagnum* were taken in July and August of 2006. Study sites were located in *Sphagnum* habitats with most characteristic plant communities. The material was randomly taken, from habitats with a significant participation of *Sphagnum fallax*, and also from dry habitats of hummocks with *Sphagnum magellanicum* Brid. One sample was taken from the brown moss habitat dominated by *Caliergonella* sp. and *Drepanocladus* sp., occurring together with vascular plants (e.g. *Eleocharis palustris* (L.) Roem. & Schult, typical species of eutrophic habitats). In total, 24 samples were taken from various microhabitats.
Testate amoebae in *Sphagnum* peatland

Samples were cut with a serrated knife from *Sphagnum* carpet, hollows, floating mat edge and hummocks. The mosses were placed in plastic containers 6 cm in diameter and 8 cm tall. Only the living parts of the mosses were taken to obtain species associations in aim to assess the quantity of each species in a sample. Abundance of each species was based on the percentage value represented by each taxon. In each of the study sites pH, depth of water table (DWT) and conductivity were measured in the field, and the water samples were taken for laboratory chemical analyses. Depth of water table (DWT) was measured with a centimetre measure. Zero level was marked by the top parts of the *Sphagnum* peatmosses. Ground water samples from the microhabitats was taken up to the depth of 0.35 m. In the laboratory, living parts of the peatmosses were stirred with 200 ml of hot water in a beaker, and then sieved with plastic sieves of the 300 μm mesh diameter in order to separate big parts of plants. The obtained fraction was later washed with the sieve of the 20 μm mesh diameter. The material that left on the sieve was taken for analysis. Species were identified with the biological microscope with ×100 and ×400 magnification. According to methods applied to other studies (Hendon and Charman 1997; Payne and Mitchell 2007) testate amoebae were counted to reach the total 150 shells. Physical and chemical analyses were performed according to standard methods for hydrochemical analyses (Hermanowicz *et al.* 1999). Nine parameters of water were analysed: acidity (pH), conductivity (portable apparatus Elmetron CX742), colour (platinum-cobalt standard method), $\text{NH}_4^+$ (by Nessler's colorimetric method), $\text{NO}_3^-$ (by cadmium reduction method), $\text{PO}_4^{3-}$ (by the colorimetric ascorbic acid method), $\text{SO}_4^{2-}$ (by nephelometric method), $\text{Ca}^{2+}$, $\text{Mg}^{2+}$ (calmagite colorimetric method). Paper filters were used for analyses of anions and nutrients.

We applied an unconstrained indirect multivariate method: detrended correspondence analysis DCA (ter Braak and Šmilauer 1998) for the description of main gradients. Furthermore, this method allowed us to explore the size of ecological gradient. It occurred to be over 4 s.d. Considering wide gradient we decided to use the canonical correspondence analysis (CCA, the method assuming unimodal relation of species and environment) (Legendre and Legendre 1998, ter Braak and Šmilauer 1998) for the investigation of relation of particular species and environment. Logarithmic transformation [ln (x+1)] was performed on species data to normalize the distribution. Significance of particular environmental parameters was tested in the forward selection procedure of environmental variables by means of Monte Carlo permutation test with 499

<table>
<thead>
<tr>
<th>Mean</th>
<th>SD</th>
</tr>
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<tbody>
<tr>
<td>pH</td>
<td>4.7</td>
</tr>
<tr>
<td>Conductivity (μS cm⁻¹)</td>
<td>98.6</td>
</tr>
<tr>
<td>DWT* (cm)</td>
<td>11.8</td>
</tr>
<tr>
<td>Colour (mg Pt l⁻¹)</td>
<td>185.0</td>
</tr>
<tr>
<td>$\text{Ca}^{2+}$ (mg l⁻¹)</td>
<td>0.9</td>
</tr>
<tr>
<td>$\text{Mg}^{2+}$ (mg l⁻¹)</td>
<td>0.8</td>
</tr>
<tr>
<td>$\text{N-NH}_4^+$ (mg l⁻¹)</td>
<td>2.9</td>
</tr>
<tr>
<td>$\text{N-NO}_3^-$ (mg l⁻¹)</td>
<td>0.29</td>
</tr>
<tr>
<td>$\text{P-PO}_4^{3-}$ (mg l⁻¹)</td>
<td>0.23</td>
</tr>
<tr>
<td>$\text{SO}_4^{2-}$ (mg l⁻¹)</td>
<td>2.6</td>
</tr>
</tbody>
</table>

*DWT – depth to the water table
permutations. Variables were removed until the level of significance $P < 0.05$ was reached.

Testate amoebae identification was based on the available literature (Grospietsch 1958; Ogden and Hedley 1980, Charman et al. 2000, Clarke 2003, Hoogenraad and de Groot 1940).

4. RESULTS

Thirty-two testate amoebae species of 13 genera were identified within the 24 sites (Appendix). Two representatives of amoebae were marked only to the genus. Species of Archerella, Cyclopyxis and Hyalosphenia were the most numerous represented. In most of the sites, the species composition was dominated by Hyalosphenia papilio, Cyclopyxis arcelloides and Hyalosphenia elegans.

The detrended correspondence analysis (DCA) (Fig. 1) was used to describe gradients in species composition of the analysed communities. The diagram shows differences in species distribution along the gradients, represented by ordination axes. Considering the appearance of species in ordination space, environmental gradients can be inter-
Testate amoebae in *Sphagnum* peatland

![Graph showing canonical correspondence analysis biplots for species (A) and samples (B). Arrows represent significant environmental parameters explaining species composition.](image-url)

Fig. 2. Canonical correspondence analysis biplots for species (A) and samples (B). Arrows represent significant environmental parameters explaining species composition.
Fig. 3. Pictures of six testate amoeba taxa from various habitats in investigated mire: A – *Trinema lineare* (low pH, dry sites) SEM micrograph, B – *Amphitrema (Archerella) flavum* (low pH, wet sites) SEM micrograph, C – *Difflugia rubescens* (high pH, sites with open water, rich in SO₄), D – *Lesquereusia spiralis* (high pH, wet sites with brown mosses), E – *Arcella catinus* (species recorded in dry and acid habitat), F – *Nebela (Physochila) griseola* (found in acid *Sphagnum* lawn).
Testate amoebes in Sphagnum peatland

interpreted indirectly, primarily by the first, and then by the second ordination axis.

The DCA diagram shows that amoebae associations, are connected with two parameters: pH and moisture. The first axis reflects pH gradient, while the second axis – moisture of the habitat. On the left side of the diagram Archerella flavum, Diffugia leidyi and Hyalosphaenia papilio are located that are associated with acid habitat, while on the right side of the biplot alkaline species, such as Lesquerusia spiralis, Diffugia rubescens and Centropyxis aculeata are situated. The number of alkaline species increases to the right along the gradient.

Between these two groups, in the DCA diagram other species are distributed, such as Nebela militaris, Nebela tincta and Assulina muscorum, that tend to occupy the acidic habitats. Most probably, the second axis shows the gradient of moisture. Species occurring in dry habitats, e.g. Centropyxis aerophila, Euglypha rotunda and Corythion-Trinema type have low species score. The remaining species, located in the upper part of the diagram, appear usually in wet habitats. Most of the samples were taken in sites with low DWT value (high water table); three samples only were taken from hummocks with the water level between 25–35 cm.

The CCA ordination diagram shows the location of species in the ordination space of the axes I and II and the direction and intensity of changes in the value of environmental variables (Fig. 2). The vector length of a given variable shows its relative importance for the shaping of species variety; its angle is related to the particular ordination axis (the smaller the angle, the greater the relation), and the direction reflects the direction of the increase in its value. For the CCA analysis, after forward selection procedure only three significant parameters were used for calculations: SO\text{4}^{2−}, pH and DWT. Those three parameters explained 28 % of the variation in the species composition. Mean values of all measured parameters are presented in Table 1.

CCA analysis showed that the most important parameters were SO\text{4}^{2−} and pH. The DWT vector is shorter and plays a less significant role than the remaining two. Both SO\text{4}^{2−} and pH indicate the increase in trophic state of the habitat, and this relation is confirmed by the presence of amoebae located in the vicinity of these vectors. Such species as Diffugia rubescens, Arcella vulgaris and Diffugia gramen are directly connected with increased sulphate concentration. Diffugia urceolata is situated next to the pH vector, this species is an indicator of minerotrophic habitat. Towards the centre of the diagram the concentration of acidophilous species increases.

Considering DWT parameter, two groups of amoebae are apparent. Indicators of dry habitats such as Arcella catinus, Corythion-Trinema type and Centropyxis aerophila are located below DWT vector, while above this vector species either preferring moist habitats, or those with a wider ecological tolerance are located. The only exception here are Assulina muscorum and Nebela militaris, which, although clearly preferring dry conditions seem to be more related to pH gradient. The sulphate and pH vectors alone can indicate the increase in habitat alkalinity, and they sufficiently demonstrate that the distribution of particular amoebae species is selected to these variables. Fig. 3 presents testate amoebae taxa encountered during the study that corresponds to various habitat conditions.

5. DISCUSSION

The aim of the study was to present the relationships between species composition of testate amoebae and environmental conditions in area mostly disturbed by man. The study shows that testate amoebae respond to two main habitat parameters, i.e. ground water level and pH. Particular amoebae species occurrence may be more strongly correlated with pH (first axis) than with the DWT, that would suggest that pH has important influence on the formation of amoebae communities. In the analysed case, the gradient represented by the other ordination axis is hard to interpret clearly. Low pH value is correlated with low water level, while high pH value – with high DWT.

Our results confirm the earlier published results showing that depth of water table (DWT) and pH play a the main role in the formation of testate amoebae communi-
ties in *Sphagnum* peatlands (Tolonen et al. 1992, Mitchell et al. 1999, Booth 2001, Bobrov et al. 2002, Booth 2002, Mieczan 2007). The location of species in the DCA ordination space points out that the amoebae react most strongly to trophic gradient, while the DWT gradient is less marked. The hummocks dominated by *Sphagnum magellanicum* and *Aulacomnium palustre* (Hedw.) Schwägr. hosted species connected with dry habitats, i.e. *Arcella catinus*, *Assulina muscorum*, *Centropyxis aerophila* and *Corythion-Trinema* typ, while on the *Sphagnum* mat dominated by *S. fallax* species preferring higher ground water level (i.e. *Arcella vulgaris*, *Arcella discoides* and *Centropyxis aculeata*) appeared.

The CCA analysis suggests that sulphates can play an important role in the shaping of species richness of amoebae communities. High sulphate level was observed in three sites, with values ranging from 20 to 66 mg SO$_4^{2-}$ l–1, while in the other sites its values were below detection. In contrast to the remaining parameters, this one essentially explains species diversity, and therefore was included in the diagram. The content of SO$_4^{2-}$ in water and peatland ecosystems is primarily a result of natural processes of ground water supply and of the redox processes controlled by bacteria. High values of sulphate ions are often regarded as a sign of anthropogenic activity (Dojlido 1995, Jedrysek 2003). The clear preference of some species of testate amoebae for habitats rich in SO$_4^{2-}$, observed in the analysed peatland complex, requires further investigation. It has to be pointed out, however, that such species as *Diffugia rubescens* and *Arcella vulgaris* appeared mainly in microhabitats rich in sulphates. We assume that the most important factors shaping the species composition in studied sites are the depth of water table, acidity and SO$_4^{2-}$.

Towards the centre of the DCA diagram, acidophilous species such as *Amphitrema (Archerella) flavum* and *Hyalosphenia papilio* can be found, while on the right side appear alkaline species, e.g. *Lesquereusia spiralis*, *Diffugia rubescens* and *Centropyxis aculeata*. Low ground water level is linked with low pH. This link can be explained by higher concentration of humic acids in habitats with low ground water level than in sites immersed in water. Humic acids are created during peat decomposition in sites with low ground water level. Low pH also results from habitat acidification caused by *Sphagnum*. Therefore, it plays an important habitat-forming role and substantially influence the structure of the peatland vegetation (Clymo 1983, van Breemen 1995).

As the studied mire peatland has been strongly transformed by man, we may assume that testate amoebae may prove to be useful in the estimation of the results of this transformation as well as secondary succession in exploitation ponds.

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6. REFERENCES


Celka Z., Szkudlarz P. 2000 – Anthropogenic transformations of the Bagno Chlebowo
Testate amoebae in Sphagnum peatland


Grospietsch T. 1958 – Wechseltierchen (Rhizopoden) – Stuttgart, Kosmos (in German).


Offierska J. 1984 – Zmiennosc skorup u niektórych gatunków pełzaków skorupkowych (Testacea) w Wielkopolskim Parku Narodowym [Variability of testate amoebae


Received after revising July 2007
Testate amoebe in *Sphagnum* peatland

APPENDIX: List of testate amoebae taxa noted in “Chlebowo” peatland according to number of individuals recorded in all samples (n=24). Number of specimens recorded: ××× – Abundant (> 100), ×× – Less abundant (10–100), Rare (<10).

<table>
<thead>
<tr>
<th>Species</th>
<th>Abundance</th>
</tr>
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<tbody>
<tr>
<td><em>Amphitrema (Archerella) flavum</em> Archer, 1877</td>
<td>×××</td>
</tr>
<tr>
<td><em>Hyalosphenia papilio</em> Leidy, 1875</td>
<td>×××</td>
</tr>
<tr>
<td><em>Arcella catinus</em> Penard, 1890</td>
<td>××</td>
</tr>
<tr>
<td><em>Corythion – Trinema</em> type</td>
<td>××</td>
</tr>
<tr>
<td><em>Hyalosphenia elegans</em> Leidy, 1874</td>
<td>××</td>
</tr>
<tr>
<td><em>Arcella vulgaris</em> Ehrenberg, 1830</td>
<td>××</td>
</tr>
<tr>
<td><em>Assulina muscorum</em> Graff, 1888</td>
<td>××</td>
</tr>
<tr>
<td><em>Centropyxis aculeata</em> Ehrenberg, 1830</td>
<td>××</td>
</tr>
<tr>
<td><em>Nebela (Physochila) grisola</em> Penard, 1911</td>
<td>××</td>
</tr>
<tr>
<td><em>Nebela tinta</em> (Leidy 1879a) Awerintzew, 1906</td>
<td>××</td>
</tr>
<tr>
<td><em>Arcella discoides</em> Ehrenberg, 1843</td>
<td>××</td>
</tr>
<tr>
<td><em>Euglypha rotunda</em> Wailes, 1911</td>
<td>××</td>
</tr>
<tr>
<td><em>Lesquerusia spiralis</em> Ehrenberg, 1840</td>
<td>××</td>
</tr>
<tr>
<td><em>Nebela bohemica</em> Taranek, 1881</td>
<td>××</td>
</tr>
<tr>
<td><em>Centropyxis aerophila</em> Deflandre, 1929</td>
<td>××</td>
</tr>
<tr>
<td><em>Nebela militaris</em> Penard, 1890</td>
<td>××</td>
</tr>
<tr>
<td><em>Euglypha compressa</em> Carter, 1864</td>
<td>××</td>
</tr>
<tr>
<td><em>Diffugia rubescens</em> Penard, 1891</td>
<td>××</td>
</tr>
<tr>
<td><em>Assulina seminulum</em> Ehrenberg, 1848</td>
<td>××</td>
</tr>
<tr>
<td><em>Cryptodiffugia oviformis</em> Penard, 1890</td>
<td>×</td>
</tr>
<tr>
<td><em>Nebela parvula</em> Cash &amp; Hopkinson, 1909</td>
<td>×</td>
</tr>
<tr>
<td><em>Heleopera rosea</em> Penard, 1890</td>
<td>×</td>
</tr>
<tr>
<td><em>Diffugia leidy</em> Wailes, 1912</td>
<td>×</td>
</tr>
<tr>
<td><em>Diffugia oblonga</em> Ehrenberg, 1838</td>
<td>×</td>
</tr>
<tr>
<td><em>Tracheleuglypha dentata</em> Moniez, 1888</td>
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<tr>
<td><em>Euglypha strigosa</em> Ehrenberg, 1872</td>
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<tr>
<td><em>Arcella hemisphaerica</em> Perty, 1852</td>
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</tr>
<tr>
<td><em>Cyclopyxis arcelloides</em> Leidy, 1879</td>
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<tr>
<td><em>Diffugia globulosa</em> Dujardin, 1837</td>
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<td><em>Euglypha tuberculata</em> Dujardin, 1841</td>
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<td><em>Diffugia urceolata</em> Carter, 1864</td>
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