TESTATE AMOEBAE (PROTISTS)
AS PALAEOENVIRONMENTAL INDICATORS IN PEATLANDS

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Abstract. Testate amoebae (or testaceans, \textit{Testacea}, \textit{Archellacea}) are unicellular eukaryotic organisms living in freshwater or most terrestrial habitats such as soils, mosses, lakes, rivers, as well as brackish habitats such as estuaries. They are very abundant in \textit{Sphagnum} mosses, where they live in the top part of mosses and the oxygenated part of the peat. The tests (shells) of \textit{Testacea} are well preserved in peat and to a lesser extent in lake sediments.

Efforts should be concentrated on constructing reliable regional transfer functions (mathematical representation of relation of species to environmental variables — presence of particular taxa in fossil material is the function of past environmental parameters), as they exist for some world areas, and there is a complete lack of them for central Europe. Polish data are exceptionally important because Poland is under several contrasted climatic influences — from oceanic to continental.

The comparison of our data on climatic tendencies with those from Western Europe and the rest of the world will show how similar or different the responses of Polish peatlands might be.

The paper has three aims: (a) to present the need for ecological studies on testate amoebae in Central Europe, (b) to show the potential of reconstruction of past environment on the basis of multiproxy studies that include testate amoebae as an integral part of the palaeoecology toolbox and (c) to put our research efforts on testate amoebae in Poland in a more global perspective.

Key words: \textit{Sphagnum}-dominated peatlands, testate amoebae, protozoa, transfer function, pollen analysis, Holocene, palaeoecology, hydrology, climate, Tuchola Pinewoods.

\textbf{Abstrakt:} Amelby skorupkowe (\textit{Testacea}) to jednokomórkowe organizmy, które żyją w środowiskach słodkowodnych, w większości siedlisk lądowych (glebie, mszakach, jeziorach, rzekach) oraz w siedliskach słonawych (np. w ujściowych partiach rzek). \textit{Testacea} występują licznie w mechach torfowisk (\textit{Sphagnum}), gdzie zasiedlają ich górna część oraz natreśniony stopowy fragment torfu. Skorupki ameb dobrze zachowują się w torfu oraz w mniejszym stopniu w osadach jeziornych.

Główny wysiłek powinien być skoncentrowany na budowaniu regionalnych funkcji transferu (transfer function; matematyczny obraz relacji organizmu w stosunku do środowiska - obecność poszczególnych taksonów w materiale kopalnym jest funkcją przeszłych parametrów środowiskowych). Dla kilku obszarów już one powstały ale brak tego rodzaju danych dla centralnej części Europy. Polskie dane są wyjątkowo istotne, ponieważ obszar ten znajduje się pod wpływem różnych wpływów klimatycznych — od klimatu oceanicznego do kontynentalnego. Porównanie naszych danych z informacjami z innych stanowisk w Europie i na świecie może wykazać w jak odmiennej sposób reagują polskie torfowiska na zmiany klimatyczne.

Artykuł ma trzy cele: (a) zaprezentowanie potrzeby badań ekologii ameb skorupkowych w Europie centralnej, (b) przedstawienie potencjału rekonstrukcji palaeośrodowisk na bazie wielu wskaźników, gdzie ameby skorupkowe stanowią integralną część metod badań palaeoekologii i (c) zaprezentowanie naszych badań nad \textit{Testacea} w Polsce w szerszej, globalnej perspektywie.

\textbf{Słowa kluczowe:} torfowiska mszarne (\textit{Sphagnum}), ameby skorupkowe, Protozoa, funkcja transferu, analiza pyłkowa, holocen, palaeoekologia, hydrologia, klimat, Bory Tucholskie.

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INTRODUCTION

Testate amoebae (or testaceans, Testacea, Arcellacea) are unicellular eukaryotic organisms living in freshwater or most terrestrial habitats such as soils, mosses, lakes, rivers, as well as brackish habitats such as estuaries. They are very abundant in Sphagnum mosses, where they live in the top part of mosses and the underlying oxygenated part of the peat. The tests (shells) of Testacea are well preserved in peat and to a lesser extent in lake sediments. The abundance and distribution of species in mires is correlated to factors such as moisture, water table depth and pH for which many species have a limited ecological tolerance (stenotopy). This stenotopy makes it possible to use testate amoebae for palaeoenvironmental reconstruction (Charman, 2001) and indeed, they are now becoming increasingly popular in multi-proxy palaeoenvironmental studies of peatlands, especially for inferring moisture changes over the Holocene. Such studies can be used for land management and nature conservation purposes. Indeed, knowledge on the developmental history of a site and its responses to past natural and anthropogenic changes allows to assess how the ecosystem may respond to present and future changes (Scott et al., 2001; Davis, Wilkinson, 2004). However, so far, ecological and palaeoecological investigation on testate amoebae are not very common in central Europe and are very rare in Poland.

In Poland, modern studies of testate amoebae from Sphagnum peatlands commenced in 2002 in the Department of Biogeography and Palaeoecology in Adam Mickiewicz University in Poznan in cooperation with Department of Biological Sciences of the University of Alaska Anchorage (former affiliation of E. Mitchell). The research began with the intention to combine palaeoecological (testate amoebae, pollen and plant macrofossil) methods used in mire studies.

This publication presents some potential uses of testate amoebae analysis in palaeoecological reconstructions. We present results of a palaeoecological study of two peatlands from NE Poland (Tuchola Pinewoods) (Lamentowicz, 2005) as well as results of an ecological investigation used to build two transfer functions (Lamentowicz, Mitchell, 2004). Currently, our efforts are concentrated on two particular factors — depth to the water table and pH, but further developments are possible. The paper has three aims: (a) to present the need for ecological studies on testate amoebae in Central Europe, (b) to show the potential of reconstruction of past environment on the basis of multiproxy studies that include testate amoebae as an integral part of the palaeoecology toolbox and (c) to put our research efforts on testate amoebae in Poland in a more global perspective.

ECOLOGICAL STUDIES

Testate amoebae are generally regarded as soil organisms (Foissner, 1987). They are highly diverse in the acid, oligotrophic habitat represented by Sphagnum mosses growing on peatlands where they represent one of the main components of the soil microbial loop (Gilbert et al., 1998).

In Sphagnum mires, living testate amoebae are restricted to top (living) part of the mosses (Fig. 1) and the underlying dead part. In the top part (1–5 cm long) the testate amoebae community is composed of both mixotrophic taxa (e.g. Hyalosphenia papilo, Amphirema flavum or A. wrightianum) that contain endosymbiotic photosynthetic algae and heterotrophic taxa (Meisterfeld, 1977; Mitchell, Gilbert, 2004). Below that level, only heterotrophic species occurs and the assemblage is dominated by species such as Nebela sp. sp. and Hyalosphenia elegans (Schönborn, 1963; Grospietsch, 1990). The highest diversity is found in the transition zone between the upper and lower part of Sphagnum shoots (Meisterfeld, 1977). Under the living Testacea zone empty tests of dead individuals remain. Those necrocenoses are the main area of interest of palaeoecology (Fig. 1 A).

Knowledge on the distribution of species in microhabitats and the relationship between species assemblages and environmental factors is a prerequisite for future studies of soil ecology and palaeoecology. Each taxonomic group responds to more or less specific environmental gradients. Once the present ecological preferences of the species are known, the link between the present and the past is achieved using transfer functions (Birks, 1995).

For testate amoebae, the best-studied factors, and those for which the highest correlation are usually found, are moisture and water table depth. Testacea are very sensitive to moisture changes and this factor is also one of the most important in peatland ecology. Ombrotrophic bogs depend solely on precipitation for their water supply and the relative importance of precipitation versus evaporation has a profound influence on the structure and functioning of the ecosystem. Climate therefore influences the micro-topography, and also the composition of plant, animal and microbial communities. This link between climate and the structure and functioning of the system make ombrotrophic bogs especially sensitive to climate change (Charman, 2001, 2002 b; Charman et al., 2001; Wilmshurst et al., 2003; Charman et al., 2004).

Another important factor is pH, which was we also modelled. But because pH is very variable not only during the year but also daily (Tahvanainen, Tuomala, 2003), the interpretation is more difficult than for water table depth. Thus pH is less extensively discussed in the literature on Testacea and further studies are required to better understand the relationship between testate amoebae and this factor. The scarcity of extensive studies, covering the entire range of pH encountered in peatland is partly due to the natural rarity of transitional situations between highly acidic and circum-neutral conditions resulting in a bimodal distribution of pH in peatlands (Sjörs, Gunnarson, 2002; Tahvanainen, Tuomala, 2003).

Testate amoebae are very sensitive to habitat changes and the heterogeneity of the environment, but are nevertheless
adapted to living in unstable conditions. The answer to the apparent paradox lies in two traits that characterize these organisms: their ability to encyst when conditions become unfavorable and their small size allowing easy long-distance passive dispersal and contributing to their cosmopolitan distribution (despite some exceptions and many open questions on this topic). Although testate amoebae could potentially occur everywhere thanks to their dispersal ability, the narrow ecological tolerance of the species limits the actual colonization and allows their use as reliable bioindicators (of soil humus type, lake water quality, soil chemistry, atmospheric pollution) (Foissner, 1996; Meisterfeld, 1997; Foissner, 1999; Nguyen et al., 2004).

The bioindication value of testate amoebae should be based on thorough ecological studies. The ecology of peatland testate amoebae has been well studied in Great Britain (Woodland et al., 1998; Charman, 2001), Switzerland (Mitchell et al., 1999, 2000 a), France (Mitchell et al., 2000 b), United States (Booth, 2001, 2002), Canada (Warner, 1987; Beyens, Chardez, 1994; Charman, Warner, 1997) and New Zealand (Wilmshurst et al., 2003), but until recently not in Poland.

Modern Polish studies were conducted on oligotrophic, but not exclusively ombrotrophic, peatlands dominated in larger part by Sphagnum species. Those habitats were supposed to provide good environmental data on testate amoebae ecology because a wide range of microforms (hummocks, lawns, and hollows) was sampled. The pilot study presents the results for three small Sphagnum dominated peatlands, and describes three ecological factors and species assemblages from surface samples from Tuchola Pinewoods (Pomerania, N Poland) (Lamentowicz, Mitchell, 2004). The samples were taken mainly from oligotrophic habitats in Sphagnum dominated ecosystems. Some mesotrophic habitats were also sampled. The results are in general agreement with previous research on testate amoebae ecology and strengthen the case for using them in palaeoenvironmental studies. Statistical models WA and WA(tol) (Birks, 1998, 2001) (with the use of CANOCO (ter Braak, Šmilauer, 1998) and WACALIB (Line, Birks, 1990; Line et al., 1994; Birks, 2001) applications) were used to describe species — environment relationship. The correlation was presented with the use of Redundancy Analysis (RDA) in which linear relationships between species data and ecologi-
cal factors are assumed (Birks, 1995). The variable having the largest influence was water table depth (DWT) (sampled sites spanned 3 to 40 cm of DWT), which explained the majority of variation (21%). Taxa preferring wet habitat are e.g.: Nebela carinata and Amphithrema wrighti, whereas on the opposite side of the RDA biplot are projected “dry” taxa e.g.: Nebela militaris and Trigonopyxis arenula. It should be also mentioned that Assulina muscorum is described in this study as a species of dry hummock. Our results are in agreement with previous studies (Warner, 1987; Tolonen et al., 1992; Charman, 1997; Charman, Warner, 1997; Mitchell et al., 2000b; Booth, 2001, 2002), in which hydrology (usually as moisture or water table depth) was also the most important factor governing the distribution of testate amoebae. Currently, our model allows to reconstructing water table depth with an accuracy 9.89 cm (RMSEP) represented by weighted averaging model with tolerance downweighting of rare species. In the future, comparisons with other statistical models e.g. WA-PLS or PLS (Birks, 1995, 1998) are planned.

The range of acidity in the sites we sampled ranged between 3.26 and 6.67. As mentioned above, this factor is difficult to interpret and changes daily and annually. In the Tuchola Pine-woods area, some Testacea species occur in circum-neutral habitats e.g. Quadrirrella symetrica (idiomorphic taxa) or Centropyxis ecoris (idiomorphic pseudochitinous taxa) in some exclusively in acid habitats: Nebela militaris and N. griseola (both xenomorphic taxa). The results for this factor also relate well to the other world studies, despite an imbalance in the pH range covered. Further investigations are required to fill the gap in transitional habitats. The last factor modeled was conductivity, but it was not statistically significant and was highly correlated with pH.

Our training set from Poland is expanding along with the predictive power of the transfer function. We gathered more than 60 samples from different habitats that will be used in future studies from Pomeranian Sphagnum dominated peatlands (Lamentowicz, Obremksa, Mitchell in prep; Lamentowicz, Tobolski in prep). Further sampling in central Poland and mountain regions are also planned.

PALAEOECOLOGICAL STUDIES

Testate amoebae have been used in ecological and palaeoecological studies since the beginning of the 20th century. Frey (1964) describes the development of early research on Testacea, in which Lagerheim (1901) was the first who recorded the tests in freshwater sediment. Steinbeck (1914) was the first to recognize and describe the presence of rhizopods in Sphagnum habitat. Hesmer (1929) interpreted the lack of some testate amoebae at some depth in peat profiles as the result of test decomposition, and not of past changes in ecological conditions. Granlund (1932) related the degree of peat decomposition to testate amoebae taxa assemblages. Harmisch (1927, 1951) started working on peat cores, and consequently described testate amoebae from intercalary peat. Following semiquantitative methods of palaeoenvironmental reconstruction developed by Tolonen (1966), quantitative methods were applied (Woodland et al., 1998; Wilmshurst et al., 2002; Hendon, Charman, 2004) along with the development of multivariate statistics (Birks, 1995, 1998).

Testate amoebae (taxa such as Assulina sp. sp. and Amphithrema flavum) were sometimes counted along with pollen and their abundance related to the total pollen percentage (Aaby, Tauber, 1975; Aaby, 1976; Latalowa, 1999). Charman et al. (2000) pointed out that although this may be convenient it is not logical because “there is no ecological process that connects pollen influx with the living conditions of testate amoebae”. The good representation of Assulina muscorum in pollen preparations is due to the high durability of its test (Charman, 2001). However, this taxon, which is often regarded as a dry indicator by palynologists, is in fact represented by three distinct species - Assulina muscorum, A. seminulum, and A. scandinavica, each with its own ecological preferences (in this sequence, a decreasing tolerance to dryness with increasing size). These differences were revealed in a number of studies outside Poland (Warner, 1987; Tolonen et al., 1992, 1994; Mitchell et al., 2000b; Booth, 2001) as well as in our Polish data (Lamentowicz, Mitchell, 2004). The low number of testate amoebae species in pollen counts is explained by the pollen preparation method (acetolysis) of peat samples. This treatment destroys most tests, especially the agglutinated (e.g. Diffuglas) or siliceous (e.g. Euglypha) taxa (Hendon, Charman, 1997).

Multiproxy palaeoclimatic reconstruction studies in peatland are rare in Poland. Past changes in water level have mostly been obtained by using the peat humification index (Zurek, Pazdur, 2000; Zurek, Michczynska et al., 2002). In Poland there is presently a lack of comprehensive studies, comprising many bioindicators (Charman et al., 1999; McCarroll et al., 2003). The multiproxy approach allows reconstructing a much wider range of environmental changes in the Holocene (Barber et al., 1998; Mauquoy, Barber, 2002; Mauquoy et al., 2004). Testate amoebae were used similarly in some studies (Charman et al., 1999, 2001; Mitchell et al., 2001; Langdon et al., 2003). Changes in peat accumulation rates and the communities of organisms living at the surface of peatlands are the key indicators in palaeoclimatic research.

Recent publications of Obremksa and Lamentowicz (2002) and Lamentowicz (2005) represent pilot studies in Polish peatland research in which, in addition to standard palaeoecological methods (palynological and macrofossils analysis), an analysis of testate amoebae was applied. Polish material was analyzed qualitatively (Lamentowicz, 2005) and was compared with other studies. Two cores from two Sphagnum mires were studied. Figure 1 (B and C) presents the results of analysis of peat monolith from Jelenia Wyspa Sphagnum sampled in high resolution. Stratigraphic changes in sediment are noticeable and they are also reflected by testate amoebae taxa composition. The lower part of the monolith is characterized by the occurrence of Amphithrema flavum — species recorded in wet peat moss habitats. The testate amoebae data shows a gradual drying of the peatland surface connected with the onset of Sphagnum domination (Lamentowicz, 2003 & in prep.). The drying trend is also indicated by
the appearance of *Heleopera sylvatica*, *Nebela militaris* and *Nebela griseola* and the disappearance of *Amphitrema flavum*. This short core thus gives much information about past local hydrological conditions. Recently, Jelena Wysha bog has been dominated by *Sphagnum* species and its shift to ombrotrophy or oligotrophy should be regarded as local, therefore we probably cannot interpret amoebae changes in the context of regional palaeohydrology. Although non-ombrotrophic peatlands may also be useful for palaeoclimatic reconstructions (Booth et al., 2004), strictly ombrotrophic bogs nevertheless give a better palaeoclimatological signal because they are dependent exclusively on rainfall (Charman, 2001). The analysed core from Tuchola kettle-hole bog (Tuchola Pine-woods, N Poland) reveals a connection between changes in testate amoebae and vegetation inferred from pollen and macrofossils analyses (Lamentowicz, 2004). In this study three palaeoecological methods were used: testate amoebae, pollen and plant macrofossils analyses. The resulting data will be presented in a wider regional context (Lamentowicz, Obreniska, Mitchell in prep). By combining several methods a more accurate picture of past environmental changes can be obtained and regional climate changes can be separated from the global signal. A critical aspect of this research is the use of dendrochronological methods (Krapiec, 1998; Cedro, 2003; Martinelli, 2004) that allow high precision temporal calibration of the signals obtained through other methods and can be correlated with peat stratigraphy (Gunnarson et al., 2003; McCarroll et al., 2003).

**SUMMARY AND CONCLUSIONS**

Peatlands represent the most significant terrestrial carbon sink (Borren et al., 2004; Grace, 2004), are very sensitive to climate changes, and have recorded past climatic fluctuations since the beginning of the Holocene (Blackford, 1993; Chambers, Charman, 2004).

Currently, there are only few multi-proxy studies of bogs in Poland. Most of the research is based on one or two methods, usually represented by the analysis of diatoms, cladocerans, plant macrofossils or pollen and spores. No attempt has yet been made to correlate several methods (e.g. testate amoebae, palynology, plant macrofossils and dendrochronology). Consequently, the testate amoebae method has been little used in Poland (Oferska, 1978, 1984; Ofierska, Okuniewska, 1986; Ofierska-Wawrzynak, 1993).

Although testate amoebae are mostly believed to be cosmopolitan, the total number of regions in which the ecology of peatland testate amoebae has been studied is relatively low. It therefore remains possible that in some regions the composition of the testate amoebae fauna, or the ecological preference of some species, may be different from those of better-studied regions. A locally produced transfer function is therefore needed, and to fill the existing gap in central Europe an effort has begun to build the required data set of Polish, modern testate amoebae (Lamentowicz, Mitchell, 2004). This data set now needs to be extended to improve the accuracy of the reconstruction and to extend the habitat types covered by the model. In view of the ongoing and predicted future change in climate, we need more high-resolution palaeoclimatological studies in order to gain a better understanding of the response of natural ecosystems to past climatic changes. This is particularly true in areas where few similar studies have been done until today. Palaeoecological data inferred from testate amoebae can be correlated with other signals (Charman, 2001, 2002a). We have the opportunity to reconstruct temperature and moisture changes during the Holocene because of the high sensitivity of tree species and soil microbes to climatic conditions and atmospheric chemistry (especially CO₂ concentrations). This period is exceptionally significant in palaeoclimatic studies (Follard, Karl, 2001).

Charman (2001) stated that the knowledge on peatland testate amoeba is in its infancy in Quaternary science. Efforts should be concentrated on constructing reliable regional transfer functions, as they exist for some world areas, and there is a complete lack of them for central Europe. Polish data are exceptionally important because Poland is under several contrasted climatic influences — from oceanic to continental. The comparison of our data on climatic tendencies with those from Western Europe and the rest of the world will show how similar or different the responses of Polish peatlands might be (Charman, Hendon, 2000; Follard, Karl, 2001).

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