



Micropaleontological observations on a *Sphagnum* bog in East Carpathian region – testate amoebae (Rhizopoda: Testacea) and their potential use for reconstruction of micro- and macroclimatic changes

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Abstract

We investigated the possibilities of reconstructing the hydrological history of an East Carpathian *Sphagnum* bog (Fenyves-tető, near Erzsébetbánya) based on the Testacea fauna and the humification. A Russian-type sampler was used to supply undisturbed cores of 400 cm in depth. The exotic marker method (*Lycopodium clavatum* as a marker) was used for quantitative estimation of the abundances. Humic acid concentration was measured by photometry. The age of the peat layers was determined by radiocarbon method. Altogether we identified 33 testate amoebae taxa; there were 10 taxa which were frequent in the samples and have remarkable faunistic and ecological significance. Based on the relative abundance of Testacea taxa, eight zones were identified. The following species were characteristic of dry conditions (*Assulina muscorum*, *Diffugia pulex*, and *Nebela militaris*), while wet conditions were indicated by *Amphitrema flavum* and *Hyalosphenia papilio*. We found strong qualitative correlation between the humification and the relative frequencies of the testate amoebae. Major changes of the moisture conditions can be appropriately reconstructed from the quantitative and qualitative analysis of testate amoebae, and offer a good characterization for the rapid changes of the moisture conditions and the stable periods. We have demonstrated that the analysis of Testacea and the study of humification are useful for reconstructing hydrological changes.

Introduction

Testate amoebae (Protozoa: Rhizopoda, Testacea) often occur in moist environments, and are particularly frequent in the soil of bogs. These organisms have been widely studied in European bogs (Schönborn, 1963; Tolonen, 1986; Foissner, 1987; Hendon et al. 2001), yet there is relatively little information available on their fauna in the Carpathian Basin (e.g., Ertl, 1960; Török, 1993). Situated near Erzsébetbánya Northern Transylvania, the bog (15 000 m²) of Fenyves-tető (1340 m) was subjected to a micropaleontological assessment by the help of the Testacea fauna. Relying on the quantitative and qualitative

conditions of testate amoebae, as well as radiocarbon dating and measurements on humification, the investigations were intent on the possibility of reconstructing macro- and microclimatic changes having occurred in the examined bog.

Materials and methods

Sampling was performed with a Russian-type sampler (Jowsey, 1966) that was able to supply undisturbed cores and reach to a depth of 400 cm in 40 cm portions. In the sediment filling up the basin, the peat layer was found to be 380 cm deep with an underlying 20 cm of clayey limnic deposit over the andesite,

constituting the base rock. The age of the peat layers was determined by radiocarbon method and the development of humification was examined alongside with the presence of testate amoebae (Rhizopoda: Testacea) in various layers. There were two subsamples of 1 cm³ volume each for every 4 cm portion to measure the humic acid concentration and the species composition of the testate amoebae.

For the study of humification, samples were dried to constant mass, and then weighed. 50 g of 0.1 M NaOH was added to each sample, and boiled in a sand bath at 100 °C for 1 h. Humic acid concentration was measured by photometry from the supernatants. Based on the absorbance values, humic acid concentration was provided for each layer with the help of preliminary calibration (Bahnsen, 1968).

For identification of the testate amoebae species, the samples were boiled in 100 ml of distilled water for 10 min, while they were occasionally stirred to dissipate the peat (Hendon & Charman, 1997). Before boiling, a *Lycopodium clavatum* L. tablet was added to the substance to obtain quantitative outcomes, according to Stockmarr's (1971) method. In order to remove the coarse plant debris, the samples were passed first through a 250 µm filter, then a 15 µm filter, and only the oversize fraction was retained. It was separated at 3000 rev for 5 min, and then, after decanting the supernatant, the remaining concentrate was coloured with two drops of safranin dye. Samples were stored in glycerine until microscopic identification. We used the identification guide of Charman et al. (2000) and Hendon et al. (2001), which is based on a recent synthesis of the paleoenvironmental literature. 150–200 tests per sample were counted. Zonation was prepared by the Psimpoll 4.10 program (Bennett, 1996). We used the optimal splitting by information content method to determine the zones.

Results

Altogether 33 testate amoeba taxa were found. The majority of them (*Amphitrema flavum* (Archer 1877) Penard 1902, *Amphitrema wrightianum* Archer 1869, *Assulina muscorum* Greeff 1888, *Diffflugia pulex* Penard 1902, *Hyalosphenia papilio* Leidy (1874) 1875, *Nebela militaris* Penard 1890, and *Nebela tincta* (Leidy 1879a) Awerintzew 1906) represented widely known cosmopolitan organisms. Beside these taxa, there were real rarities detected, including *Bullinularia indica* Penard 1907, *Diffflugia lanceolata* Penard

1890, *Diffflugia rubescens* Penard 1891, *Pontigulasia bigibbosa* Penard 1902, and *Pontigulasia elisa* Penard 1893. There were 10 out of 33 taxa which were frequent in the samples and have remarkable faunistic and ecological significance. Figure 1 shows the relative abundances of the 10 testate amoebae taxa with the humic acid concentrations, as well as the radiocarbon dating. There were 8 zones identified by the Psimpoll program (FT-1-FT-8). The quantity of humic acids is indicative of moisture conditions. Drier climate facilitates the decomposition of organic materials, which results in higher concentration values for humic acids. Table 1 summarizes the main features of the investigated core.

Discussion

In the FT-1 zone there were only a few testate amoebae species with a limited number of individuals. At the beginning of the zone there was a peak in density for the *Amphitrema wrightianum* and *Diffflugia lanceolata* species. *A. wrightianum* is a characteristic species of bog pools and wet hollows but also occurs in mesotrophic peats (Tolonen, 1986). *Diffflugia lanceolata* prefers bog pools (Cash & Hopkinson, 1909). In the zone FT-1, the concentration of humic acids starts to rise rapidly, indicating a process of drying; this observation is likely to indicate a transition towards a boggy state, which is supported by our observations on testate amoebae.

In the FT-2 zone the *Amphitrema flavum* was the most frequent species. This species is generally associated with wet conditions, sometimes with standing water (Tolonen, 1966; Meisterfeld, 1977; Warner, 1987), but extends to hummock tops in oceanic areas (Heal, 1964). Along the zone, the humic acid concentration strongly fluctuates.

At the beginning of the FT-3 zone a minor drop in the relative abundance of *A. flavum* is parallel to a smaller peak of *A. wrightianum*. *Hyalosphenia papilio* was also detected in FT-3 zone, which lives in wet *Sphagnum* (de Graaf, 1956) including wetter hummocks (Heal, 1961), but not in pools or hollows. In this zone the fluctuation of humic acid concentration becomes really pronounced. There are two pronounced peaks separated by a deep valley in the humic acid values. The second peak is succeeded by a very high peak, and then decreasing values prevail.

In the fourth zone, the *Amphitrema flavum* and *Amphitrema wrightianum* species were frequent. At

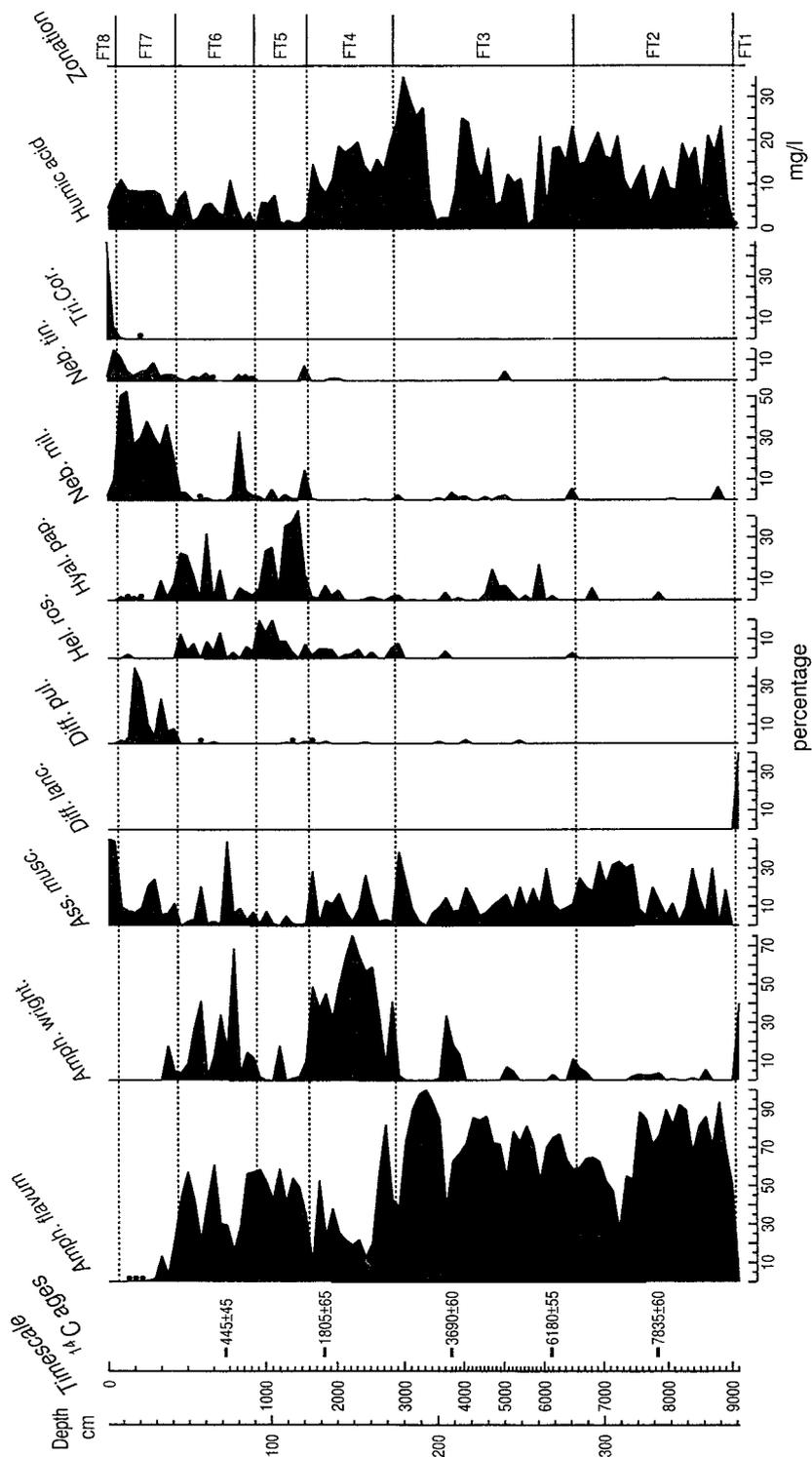


Figure 1. Relative abundance of testate amoebae taxa and humic acid concentrations plotted against depth/time. Radiocarbon dating is also represented on the diagram as ¹⁴C ages. Abbreviations: *Amph. flavum* – *Amphitrema flavum*; *Amph. wrighti* – *Amphitrema wrightianum*; *Ass. musc.* – *Assulina muscorum*; *Diff. lanc.* – *Diffugia lanceolata*; *Diff. pul.* – *Diffugia pulex*; *Hel. ros.* – *Helicoperla rosea*; *Hyal. pap.* – *Hyalosphenia papilio*; *Neb. mil.* – *Nebela militaris*; *Neb. ini.* – *Nebela tincta*; *Tri. Cor.* – *Trinema-Corythion* type.

Table 1. Main characteristics of the investigated core. Notations of the 'Characteristic species' column: 1 – *Amphitrema flavum*; 2 – *Amphitrema wrightianum*; 3 – *Assulina muscorum*; 4 – *Diffflugia lanceolata*; 5 – *Diffflugia pulex*; 6 – *Heleopera rosea*; 7 – *Hyalosphenia papilio*; 8 – *Nebela militaris*; 9 – *Nebela tincta*; 10 – *Trinema-Corythion* type

Zones	Depth (cm)	Age (years before present)	Mean concentrations of humic acids (mg l ⁻¹)	Characteristic species	Number of species	Mean individual concentrations (ind. cm ⁻³)
FT-1	381–379	9102–9050	8.6	2, 4	3	575.0
FT-2	379–283	9050–6568	16.8	1, 3	11	9613.5
FT-3	283–175	6568–2872	18.3	1, 2, 3	19	6964.6
FT-4	175–123	2872–1601	31.0	1, 2, 3	18	14032.2
FT-5	123–91	1601–876	31.7	1, 6, 7	13	13090.4
FT-6	91–43	876–263	32.1	1, 2, 3	22	13450.6
FT-7	43–7	263–38	41.2	3, 5, 8	29	19371.1
FT-8	7–0	38–0	16.2	3, 9, 10	11	3535.5

the beginning of this zone the appearance of *Heleopera rosea* Penard 1890 is remarkable. This taxon indicates bog hummocks and drier *Sphagnum* (Jung, 1936, quoted in Tolonen, 1986). *Assulina muscorum* is widespread but generally in greatest abundance in relatively dry conditions (Tolonen, 1986), although it is often regarded as cosmopolitan (Warner, 1990). The relative abundance of *A. muscorum* shows two higher peaks straddling two smaller, vicinal peaks. The humic acid content gradually decreases in the whole zone, but is still quite high. The FT3/4 transition is very close to the major climatic transition at BP 2650 (van Geel et al., 1996).

The relative abundances of *Heleopera rosea* and *Hyalosphenia papilio* become higher in the fifth and sixth zones. Zones 5 and 6 are characterized by low humic acid concentrations but FT-6 has moderately higher humic acid concentrations as compared to the previous zone.

In the FT-7 zone the dominant species include *Assulina muscorum*, *Diffflugia pulex* and *Nebela militaris*. *Diffflugia pulex* is a rare taxon; there is no published hydrological data available for this species regarding the recent occurrence and/or distribution. On the basis of associations with other taxa in fossil samples, Hendon (1998) suggests that it is a relatively dry indicator. Most authors agree that *N. militaris* is a relatively dry indicator of drier mosses and bog hummocks (de Graaf, 1956; Heal, 1961; Corbet, 1973). In the FT-7 zone the humic acid concentrations are relatively high and stable.

The FT-8 zone contains an extremely high peak of the *Trinema-Corythion* type. This taxon is combined from *Corythion dubium* Taranek 1881 and *Trinema enchelys* Leidy 1878, due to the small size of the test and transparent plates, following Corbet (1973). It occurs in variable conditions, but is most typical of dry or moderately dry conditions (de Graaf, 1956; Schönborn, 1962; Meisterfeld, 1977). Data from the humification measurements also reflect mainly dry conditions.

Conclusion

We have provided data about the Testacea fauna of a *Sphagnum* bog in the Carpathian Basin, and there is only limited data about the fauna of this area and surrounding mountains (Speranza et al., 2000a, 2000b). There is a strong qualitative correlation between the humic acid concentration and the relative frequencies of testate amoebae. Our results demonstrated that the major changes of the moisture conditions of the climate can be appropriately reconstructed from the quantitative and qualitative analysis of testate amoebae. These organisms provided a good characterization, both for the rapid changes of the moisture conditions and the stable periods of the climate (Barber, 1981; Hughes et al., 2000; Hendon et al., 2001; Korhola, 1995). We have demonstrated that the qualitative and quantitative analysis of testate amoebae and the study of humification are useful for reconstructing the hydrological changes of the Fenyves-tető bog

(Northern Transylvania). Our results stress the importance of a long-term research project on the bogs of the Carpathian Basin.

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References

- Bahnson, H., 1968. Kolorimetriske bestemmelser af humificeringstal i højmosetørv fra Fuglsø mose på Djursland. Meddelelser fra Dansk Geologisk Forening 18: 55–63.
- Barber K. E., 1981. Peat Stratigraphy and Climatic Change: a Palaeoecological Test of the Theory of Cyclic Peat Bog Regeneration. Balkema, Rotterdam.
- Bennett, K. D., 1996. Determination of the number of zones in a biostratigraphical sequence. *New Phytologist* 132: 155–170.
- Cash, J. & J. Hopkinson, 1909. The British Freshwater Rhizopoda and Heliozoa. Vol. II Rhizopoda part 2. The Ray Society, London, 166 pp.
- Charman, D. J., D. Hendon & W. A., Woodland, 2000. The identification of testate amoebae (Protozoa: Rhizopoda) in peats. QRA Technical Guide No. 9, Quaternary Research Association, London, 147 pp.
- Corbet, S. A., 1973. An illustrated introduction to the testate rhizopods in *Sphagnum*, with special reference to the area around Malham Tarn, Yorkshire. *Field Studies* 3: 801–838.
- de Graaf, F., 1956. Studies on Rotatoria and Rhizopoda from the Netherlands. *Biologisch Jaarboek Dodonea* 23: 145–217.
- Ertl, M., 1960. Beiträge zur Kenntnis der moosbewohnenden Thekamöben Ungarns. *Opuscula Zoologica Budapest* 4: 31–37.
- Foissner, W., 1987. Soil protozoa: fundamental problems, ecological significance, adaptations in ciliates and testaceans, bioindicators and a guide to the literature. *Progress in Protistology* 2: 69–212.
- Heal, O. W., 1961. The distribution of testate amoebae (Rhizopoda: Testacea) in some fens and bogs in northern England. *Journal of Linnean Society, Zoology* 44: 369–382.
- Heal, O. W., 1964. Observations on the seasonal and spatial distribution of testacea (Protozoa: Rhizopoda) in *Sphagnum*. *Journal of Animal Ecology* 33: 395–412.
- Hendon, D., 1998. Robustness and precision of Holocene palaeoclimatic records from peatlands using testate amoebae analysis. PhD thesis, University of Plymouth, UK.
- Hendon, D. & D. J. Charman, 1997. The preparation of testate amoebae (Protozoa: Rhizopoda) samples from peat. *The Holocene* 7: 199–205.
- Hendon, D., D. J. Charman & M. Kent, 2001. Palaeohydrological records derived from testate amoebae analysis from peatlands in northern England: within-site variability, between-site comparability and palaeoclimatic implications. *The Holocene* 11: 127–148.
- Hughes, P. D. M., D. Mauquoy, K. E. Barber & P. G. Langdon, 2000. Mire development pathways and palaeoclimatic records from a full Holocene peat archive at Walton Moss, Cumbria, England. *The Holocene* 10: 465–479.
- Korhola, A., 1995. Holocene climatic variations in southern Finland reconstructed from peat-initiation data. *The Holocene* 5: 43–58.
- Jowsey, P. C., 1966. An improved peat sampler. *New Phytologist* 65: 245–248.
- Jung, W., 1936. Thekamöben ursprünglicher, lebender deutscher Hochmoore. *Abhandlungen Ländesmuseum der Provinz Westfalen Museum für Naturkunde* 7: 1–87.
- Meisterfeld, R., 1977. Die horizontale und vertikale Verteilung der Testaceen (Rhizopoda, Testacea) in *Sphagnum*. *Archiv für Hydrobiologie* 79: 319–356.
- Schönborn, W., 1962. Zur Ökologie der sphagnikolen, bryokolen und terrikolen Testaceen. *Limnologica* 1: 231–254.
- Schönborn, W., 1963. Die Stratigraphie lebender Testaceen im *Sphagnetum* der Hochmoore. *Limnologica* 1: 315–321.
- Speranza, A., J. Hanke, B. van Geel & J. Fanta, 2000a. Late-holocene human impact and peat development in the Cerna Hora bog, Krkonose Mountains. *The Holocene* 10: 575–585.
- Speranza, A., J. van der Plicht & B. van Geel, 2000b. Improving the time control of the Subboreal/Subatlantic transition in a Czech peat sequence by ¹⁴C wiggle-matching. *Quaternary Science Reviews*, 19 (16): 1589–1604.
- Stockmarr, J., 1971. Tablets with spores used in absolute pollen analysis. *Pollen et Spores* 13: 615–621.
- Tolonen, K., 1966. Stratigraphic and rhizopod analyses on an old raised bog, Varrassuo, in Hollola, south Finland. *Annales Botanici Fennici* 3: 147–166.
- Tolonen, K., 1986. Rhizopod analysis. In Berglund, B. E. & J. Wiley (ed.), *Handbook of Holocene Palaeoecology and Palaeohydrology*. Chichester: 645–666.
- Török, J. K., 1993. Study on moss-dwelling testate amoebae. *Opuscula Zoologica Budapest* 26: 95–104.
- van Geel B., J. Buurman & H. T. Waterbolk, 1996. Archaeological and palaeoecological indications of an abrupt climate change in The Netherlands, and evidence for climatological teleconnections around 2650 BP. *Journal of Quaternary Science* 11: 451–460.
- Warner, B. G., 1987. Abundance and diversity of testate amoebae (Rhizopoda, Testacea) in *Sphagnum* peatlands in southwestern Ontario, Canada. *Archiv für Protistenkunde* 133: 173–189.
- Warner, B. G., 1990. Testate amoebae (Protozoa). *Methods in Quaternary Ecology* no. 5. *Geoscience Canada* 5: 65–74.