



## Epibryic diatoms from ombrotrophic mires: diversity, gradients and indicating options

Aloisie Pouličková<sup>1\*</sup>, Klára Bergová<sup>1</sup>, Radek Hnilica<sup>1</sup> and Jiří Neustupa<sup>2</sup>

<sup>1</sup> Department of Botany, Faculty of Science, Palacký University in Olomouc, Šlechtitelů 11, CZ-78371 Olomouc, Czech Republic

<sup>2</sup> Department of Botany, Charles University of Prague, Benátská 2, CZ-12801 Prague, Czech Republic

With 2 figures and 5 tables

**Abstract:** The present study focuses on comparison of recent diatom diversity within the unique Central European ombrotrophic wetlands in two regions with contrasting levels of past atmospheric depositions (Jizerské hory Mts. and Jeseníky Mts.). Further, two methods of mire diatom sampling (squeezing and mineralization of the entire moss sample) are compared at the same localities to determine whether bryophyte herbarium specimens could be used for studies on the historical development of peat bog areas.

There were a total of 47 diatom species at 59 sites within 13 ombrotrophic mires along the Czech-Polish-German border areas. The regions did not differ in diatom species richness. The two-group pattern along the Axis 1 in NMDS ordination analysis did not correspond with the regional distribution. The inter-site variation in diatom assemblages was lower than the intra-site variation. The combination of three environmental variables (conductivity, pH, and height above water table – HWT) appears to be able to explain the diatom distribution patterns. No significant differences were found between the composition of diatom species of moss and squeezed samples. However, moss samples can be characterized by significantly greater species richness, most likely because the mineralization of the whole bryophyte minimizes the losses of diatom frustules caused by the squeezing method. These results allow us to recommend herbarized bryophytes as a source of missing information on the sub-recent diatom diversity of mires.

**Key words:** epibryon, diatoms, peat bogs, sampling.

### Introduction

The peat bogs in the Jizerské hory and in the Jeseníky Mts. (both in the northern parts of the Czech Republic), represent unique ecosystems within Central Europe, characterized by ombrotrophic conditions, a very low pH (Table 1), and an unaffected water regime (Hájková et al. 2011b).

The regions, 150 km distant mountain ridges in the Sudeten Mts., differ in their air contamination and deposition loads, especially from the past. The Jizerské hory Mts. are located westwards (crossborder area between Czech Republic, Poland, and Germany; Fig. 1), and characterised by high atmospheric depositions of nitrogen and sulphates, the eastern areas (Jeseníky Mts.) seem to be less affected, especially by sulphates (Bragazza et al. 2004, Jiroušek et al. 2011). The depositional load was high, particularly at the end of the 1980s and only recently have the first signs of environment recovery been observed (Štěpánková et al. 2008, 2012).

Permanent sampling plots were established in both areas in 1991–1993 (Rybníček 2000), in order to monitor environmental changes. Although peat bog vegetation and environmental variables (pH, conductivity) have been monitored annually in the Jizerské hory Mts. as well as the Jeseníky Mts. since 1991 (Hájková et al. 2011a), algae (mostly desmids and euglenophytes) have only occasionally been studied (reviewed by Štěpánková et al. 2008, 2012). Diatoms, dominating the assemblages of wet bryophytes, have not yet been studied in these areas. The majority of studies on the ecology of epibryic diatoms have been done in circumpolar areas (e.g. Van de Vijver et al. 2004). Some studies have been done in temperate zone (Nováková 2002, Buczkó & Wojtal 2005).

Diatoms are one of the most important indicators of recent and past water quality, eutrophication, and acidification (Smol & Stoermer 2010). Apart from other applications of diatom bioindications, epiphytic diatoms from a dried specimen of aquatic macrophytes from herbaria have been used to assess the reference situation in acidified or eutrophized waters (Van Dam & Beljaars 1984, Van Dam & Mertens 1993, Denys 2009, Yallop et al. 2009). For this reason, dried specimens of bryophytes (*Sphagnum*) herbarized and deposited in museums could likewise be used for comparison of recent and sub-recent diatomic flora, as well as historical development of the peat bog areas. However, there is no methodology for such a comparative study on subrecent to recent diatom diversity. The usual method of peat bog diatom sampling is by squeezing of bryophytes into plastic bottles. The water samples are usually concentrated by sedimentation or centrifugation and mineralized in hydrogen peroxide or a mixture of acids. In contrast, entire dried herbarium bryophyte specimens need to be mineralized together with epiphytic assemblage. The important question is to what extent the data obtained by the two methods will be comparable.

The present paper focuses on a comparison of both epibryon sampling methods with the aim of testing whether bryophytes sampled and preserved as "herbarium specimens" contain representative diatom samples indicating the diversity of a site. Moreover, as the materials used represent unique samples from two ombrotrophic wetland areas contrasting in their levels of past atmospheric deposition (Jizerské hory Mts. and Jeseníky Mts.), their recent diatom diversity as well as environmental gradients are compared and discussed.

## Material and methods

**STUDY AREA:** Two mountain regions were chosen for detailed study: the Jeseníky and the Jizerské hory Mts. (Fig. 1) where 59 permanent plots were established in the period 1991–1993 for monitoring

changes in bog vegetation and water chemistry during chronosequencing of decreasing industrial emissions (Rybníček 2000). The permanent plots are situated at 13 ombrotrophic peat bogs (A–M, Fig. 1, Table 1). The altitudes and selected environmental data of the sites are summarized in Table 1. The diversity of desmids, bryophytes, and higher plants at the same localities have been published elsewhere (Štěpánková et al. 2008, 2012, Hájková et al. 2011a). Most of the mires have the characteristics of oligotrophic montane raised bogs (Józsa & Vonička 2004), with *Sphagnum* growths and several shallow pools or puddles. A large pool (bog lake) can often be found in the open, central part. The regions differ in vascular plant and bryophyte vegetation. Groups of plants with a subatlantic and boreoatlantic distribution (e.g. *Erica tetralix* L., *Trichophorum caespitosum* (L.) Hartman, *Sphagnum papillosum* Lindberg, *S. majus* (Russow) C.E.O.Jensen, *S. rubellum* Wilson) is much more common in the western region (the Jizerské hory Mts.). Almost all of the peat bogs are protected as small-scale conservation areas within the Protected Landscape Area of the Jizerské hory Mts. (Peat bog Jizerky, Na Čihadle; Józsa & Vonička 2004) and Jeseníky Mts. (Šerák-Keprník, Praděd).

Samples were taken in August 2008 at 59 sites and selected environmental variables (pH, conductivity) were measured *in situ* using field instruments (WTW, Germany). Conductivity caused by H<sup>+</sup> ions was subtracted according to the formula of Sjörs (1952). We used only corrected conductivity in this study. Water level (HWT) with respect to the bog surface was measured directly in the field. Water samples for chemical analyses were filtered to remove mechanical particles and were transported in plastic bottles to the laboratory. Iron (Fe) was measured using atomic absorption spectrometry directly in the samples. Humic acids were detected by spectrophotometry, and ammonium ions (NH<sub>4</sub><sup>+</sup>) were measured by spectrometry after reaction of the ammonium ions with salicylate. Nitrites (NO<sub>2</sub><sup>-</sup>) were detected by a photometric method with sulphanyl acid and N-1 naphthyl ethylenediamine dihydrochloride. Phosphates (PO<sub>4</sub><sup>3-</sup>) were measured spectrophotometrically as phosphomolybdenum blue. Nitrates (NO<sub>3</sub><sup>-</sup>) were measured using Capillary Zone Electrophoresis (CZE). Sulphates (SO<sub>4</sub><sup>2-</sup>) were detected using an ITP analyser, and the chlorides (Cl<sup>-</sup>) using potentiometric titration with silver nitrate solution.

**DIATOM SAMPLING AND PREPARATION:** Diatom samples (called "squeezed samples"), collected by the squeezing of *Sphagnum* tufts, were concentrated by sedimentation, cleaned with a mixture of concentrated sulphuric and nitric acids, and mounted in Naphrax as previously described by Poulíčková & Mann (2006). Additionally, 13 *Sphagnum* samples (10 × 10 cm) were collected at the same mires (A–M, Table 1) into plastic bags. The bryophytes were dried by the same means as were the "herbarium specimens". The dry biomass (cca. 9 cm<sup>3</sup>) was mineralized in Erlenmayer flasks using the same acid mixture as described above. The diatom samples obtained by this method (called "moss samples") were again mounted in Naphrax. The diatoms were identified according to Krammer & Lange-Bertalot (1986, 1988, 1991a, b), Lange-Bertalot et al. (2011) and the nomenclature was updated according to Algaebase. The relative abundance of individual diatom species were estimated by counting 400 valves from each Naphrax slide.

**DATA ANALYSIS:** The statistical analyses involved non-metric multidimensional scaling (NMDS) of the species composition data in the samples, using the Bray–Curtis similarity measure in Primer, ver. 6.1.5 (Clarke & Gorley 2006). The significance of differences in the diatom species' composition between groups of samples taken from different regions or between moss and squeezed samples were evaluated by the non-parametric ANOSIM (Analysis of Similarities), using the Bray–Curtis similarity measure in PAST, ver. 2.01 (Hammer et al. 2001, Clarke & Gorley 2006). In both tests, we used 10,000 permutations for the computation of the *p*-values. Species that characteristically discriminated between individual groups were identified using the SIMPER (Similarity Percentage) method based on the Bray–Curtis measure in PAST, ver. 2.01. The SIMPER method illustrates which particular taxa are primarily responsible for a difference between groups of samples, e.g. illustrated by ANOSIM tests. The method computes the overall percentage contribution made to the dissimilarity of each species between groups (Clarke & Gorley 2006). The differences in the species richness of groups of samples were evaluated by permutation tests (10,000 permutations). The BEST analysis (BIOENV procedure) in Primer, ver. 6.1.5 was used to relate the patterns of the environmental variables to the patterns of the diatom assemblages. This analysis illustrates which environmental variables best predict the observed patterns of species composition in a complex (Clarke & Gorley 2006, Kovtun et al. 2009). A global BEST match permutation test (1000 permutations) was run to evaluate the statistical significance of the observed weighted Spearman rank correlation between the environmental variables and biotic patterns. In addition, the relationships between the abiotic and

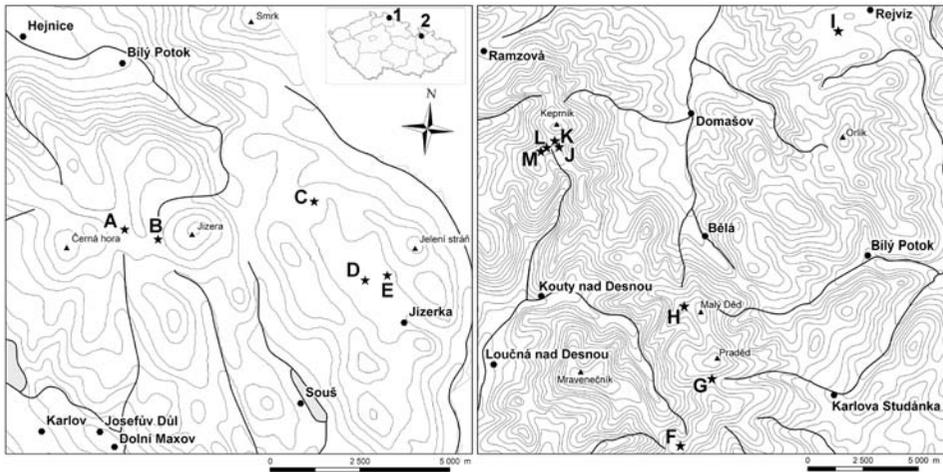


Fig. 1. Map of sampling sites in the Jizerské hory Mts. (1; A–E), and Jeseníky Mts. (2; F–M); for localities, see Table 1.

species data, as well as species richness of the samples were evaluated by linear correlation analyses, with the significance assessed by permutation  $p$ -values. The homogeneity of the species composition within samples taken from a single locality were evaluated by comparisons of the Bray-Curtis similarities of within-group samples and with the B-C similarities in species composition of group samples from samples taken from differing localities. Similarly, the position of moss samples, with respect to the squeezed samples of localities, were evaluated by comparison of the B-C similarity values of a moss sample to squeezed samples of a locality with B-C values of a moss sample to other samples of the investigated set. The significance of these models was evaluated by permutation tests on the B-C similarity values, with 10,000 permutations.

## Results

A total of 47 diatom species occurred at 59 sites from 13 ombrotrophic mires within the Jizerské and Jeseníky Mts. The greatest species richness was found at the Trojmezí A locality in the Jeseníky Mts. which was characterized by the highest pH (around 5) and microsite moisture (low HWT – height of water table; Table 1). The most frequent and abundant species in both areas were *Frustulia saxonica* and *Eunotia paludosa* (Table 2), representing 40–90% of all diatom frustules present. The Na Čihadle, Klečová Louka, Tetřeví louka, and Trojmezí B localities were dominated by *Frustulia saxonica*, while the Máj, Barborka, Rejvíz, Pod Vozkou, and Malá Jizerská Louka localities were dominated by *Eunotia paludosa*. *Eunotia nymmanniana* occurred as a codominant species at the Trojmezí A locality.

NMDS ordination analysis of species composition data, using the Bray-Curtis similarity values among samples (Kruskall stress = 0.1313), generally illustrated a two-group pattern along Axis 1. However, it clearly did not correspond with either the regional distribution, nor with the moss-squeezed sample differences (Fig. 2a). Indeed, the

Table 1. Selected environmental variables of 13 ombrotrophic mires in the Jizerské hory Mts. (A-E), and Jeseníky Mts. (F-M). Locality numbers indicate mires (A-M) and the sampling sites within mires (1-30). HWT = height of the water table.

| Locality No. | Lokalita                  | Altitude m a.s.l. | HWT cm | pH      | Conductivity $\mu\text{S}\cdot\text{cm}^{-1}$ | $\text{NO}_3^-$ $\text{mg}\cdot\text{l}^{-1}$ | $\text{PO}_4^{3-}$ $\text{mg}\cdot\text{l}^{-1}$ | Humic acids $\text{mg}\cdot\text{l}^{-1}$ |
|--------------|---------------------------|-------------------|--------|---------|---|---|--|---|
| A1-4,        | 28 Na Čihadle             | 975-980           | 3-15   | 3.8-4.3 | 0.01-10.9                                     | <0.15-0.6                                     | 0.1-0.2  | 4.3-27                                    |
| B5-9,        | 29 Klečová louka          | 984-986           | 4-14   | 3.6-4.1 | 0.01-27.6                                     | <0.15   | 0.1-0.2  | 25-74                                     |
| C10-13       | Tetřeví louka             | 907-912           | 9-18   | 3.9-5.1 | 7.7-33.4                                      | <0.15   | 0.1  | 6-25                                      |
| D14-20       | Malá Jizerská South       | 866-871           | 1-22   | 4.1-4.5 | 8.2-19.2                                      | <0.15-1.1                                     | 0.1-0.3  | <1-35                                     |
| E21-27       | Malá Jizerská North       | 866-872           | 5-20   | 4.2-4.4 | 21.9-49.2                                     | <0.15-0.8                                     | 0.1  | 2.5-19                                    |
| F1-3         | Máj, Jeseníky Mts.        | 1366-1380         | 14-16  | 4.1-4.3 | 23.5-40.0                                     | <0.15-2.1                                     | 0.1-0.2  | 42-72                                     |
| G4-5         | Barborka, Jeseníky Mts.   | 1314-1316         | 0-10   | 3.8     | 45.6-45.7                                     | 0.8-3.5                                       | 0.2  | 44-64                                     |
| H6-9         | Slatě, Jeseníky Mts.      | 1307-1312         | 5-10   | 3.8-3.9 | 38.5-63.3                                     | 0.9-3.5                                       | 0.1-0.3  | 49-76                                     |
| I10-13       | Rejvív, Jeseníky Mts.     | 475-771           | 0-20   | 3.6-3.8 | 56.6-86.4                                     | <0.15-0.5                                     | 0.1-0.5  | 75-185                                    |
| J14-18       | Trojmezí A, Jeseníky Mts. | 1297-1300         | 0-15   | 4.6-5.3 | 19.6-27.1                                     | <0.15-1.2                                     | 0.1-0.4  | 62-120                                    |
| K19-23       | Trojmezí B, Jeseníky Mts. | 1319-1323         | 0-10   | 4.0-4.2 | 25.2-39.1                                     | <0.15-0.4                                     | 0.1-0.3  | 39-130                                    |
| L24-25       | Pod Vozkou, Jeseníky Mts. | 1304              | 0-13   | 4.0     | 33.9-42.2                                     | 0.6-0.7                                       | 0.1-0.5  | 54-140                                    |
| M26-30       | Vozka, Jeseníky Mts.      | 1320-1326         | 0-14   | 4.0-4.7 | 25.3-44.5                                     | <0.15-0.4                                     | 0.1-0.2  | 68-120                                    |

ANOSIM test on species composition of the moss and squeezed samples revealed no significant differences ( $R = -0.04$ ,  $p > 0.05$ ). On the other hand, there was a significantly greater species richness in the moss samples than in squeezed samples (moss samples = 8.69, squeezed samples = 6.22,  $p = 0.0231$ ). There were significant differences between species composition of the samples taken in the Jizerské hory and Jeseníky Mts. (ANOSIM,  $R = 0.186$ ,  $p = 0.0001$ ), even if there was some overlap in the position of the samples from these regions in the ordination space (Fig. 2a). The SIMPER method revealed that *Eunotia paludosa* (24.3% contribution to the total dissimilarity of two sets), *E. nymanniana* (2.3%) and *Pinnularia subcapitata* (2.2%) distinguished the Jizerské hory Mts. region, and *Frustulia saxonica* (22.9%), *E. fennica* (2.3%) and *Pinnularia rupestris* (1.6%) occurred more frequently in the Jeseníky Mts. region.

Out of these species, *Eunotia paludosa* was significantly correlated with conductivity ( $r = 0.55$ ,  $p < 0.001$ ) and with pH ( $r = -0.38$ ,  $p < 0.003$ ). *Eunotia nymanniana* significantly correlated with pH ( $r = 0.39$ ,  $p < 0.002$ ), and *Frustulia saxonica* with conductivity ( $r = -0.46$ ,  $p < 0.001$ ). There were no significant differences in values of species richness of peat bog samples from either the Jizerské hory or Jeseníky Mts.

There was no single abiotic variable with a dominant influence on the diatom assemblages. On the other hand, the combined effect of environmental variables proved to be related significantly to the species composition of the samples (BEST analysis,

Table 2. List of the most frequent diatom species (+ presence) at mires in Jizerské hory Mts. (A-E) and Jeseníky Mts. (F-M). For locality names see Table 1.

| Species  | A | B | C | D | E | F | G | H | I | J | K | L | M |
|--|---|---|---|---|---|---|---|---|---|---|---|---|---|
| <i>Eunotia exigua</i> (Brébisson ex Kützing) Rabenhorst                      |   | + | + | + |   |   |   |   |   |   |   |   |   |
| <i>E. fennica</i> (Hustedt) Lange-Bertalot                                   | + | + | + | + | + | + |   |   | + | + | + | + | + |
| <i>E. groenlandica</i> (Grunow) Nörpel-Schempp et Lange-Bertalot             |   | + |   | + |   |   |   |   |   |   |   |   |   |
| <i>E. juettnerae</i> Lange-Bertalot  |   | + |   | + | + | + |   |   |   | + | + | + | + |
| <i>E. nymanniana</i> Grunow  | + |   |   |   | + |   |   |   |   | + | + |   |   |
| <i>E. paludosa</i> Grunow  | + | + | + | + | + | + | + | + | + | + | + | + | + |
| <i>Frustulia saxonica</i> Rabenhorst   | + | + | + | + | + | + |   |   |   |   | + | + | + |
| <i>Gomphonema parvulum</i> (Kützing) Kützing                                 |   |   |   |   |   |   |   |   |   |   | + | + | + |
| <i>Hantzschia amphioxys</i> (Ehrenberg) Grunow                               | + |   |   | + |   | + | + | + |   | + | + |   | + |
| <i>Kobayasiella parasubtilissima</i> (H.Kobayasi et T.Nagumo) Lange-Bertalot | + |   | + | + |   |   |   |   |   |   | + |   |   |
| <i>Pinnularia microstauron</i> (Ehrenberg) Cleve                             | + |   |   | + |   |   |   |   |   | + |   | + | + |
| <i>P. rupestris</i> Hantzsch   | + | + |   |   |   | + | + |   |   | + |   |   | + |
| <i>P. subcapitata</i> W.Gregory  |   |   |   |   |   |   | + |   | + | + | + | + | + |
| <i>Tabellaria flocculosa</i> (Roth) Kützing                                  |   |   |   | + | + |   |   |   |   |   |   |   |   |
| <i>T. quadrisepitata</i> Knudson   | + | + |   | + |   |   |   |   |   |   |   |   |   |

$\rho_w = 0.244$ ,  $p < 0.001$ ). The set of three environmental variables (conductivity, pH, height of the water table = HWT) appeared to be the best explanatory combination (Fig. 2). Any addition of further environmental variables with the species composition data decreased the total correlation (Table 3). There were also significant relationships of pH, conductivity, and HWT values with the NMDS axes representing the species compositions of the samples (Table 4). Additionally, the concentrations of  $\text{NO}_3^-$  and  $\text{Cl}^-$  were also marginally significantly related to the NMDS axes. Conductivity was strongly correlated with Axis 1; therefore, related to the two-group distribution of the investigated peat bog diatom samples (Fig. 2). In addition, conductivity was also significantly positively correlated with the values of  $\text{Cl}^-$  ( $r = 0.42$ ,  $p = 0.0006$ ), as well as with  $\text{NO}_3^-$  values ( $r = 0.38$ ,  $p = 0.0033$ ). Conductivity was also negatively correlated with the pH ( $r = -0.36$ ,  $p = 0.0047$ ). No other correlations among the abiotic data, as well as between the abiotic data and NMDS axes were significant. Species richness of the samples was most closely related to pH values ( $r = 0.50$ ,  $p = 0.0001$ ). However, there were also significant negative relationships with HWT ( $r = -0.38$ ,  $p = 0.0022$ ) and conductivity ( $r = -0.36$ ,  $p = 0.0049$ ). Of the 13 investigated mires/localities, there were only three that had significantly greater similar within-locality samples, than among-locality samples (Table 5). Additionally, there were only two of these in which the moss sample was significantly related more to the squeezed sample of the same locality, than to the squeezed samples of the other peat bog localities.

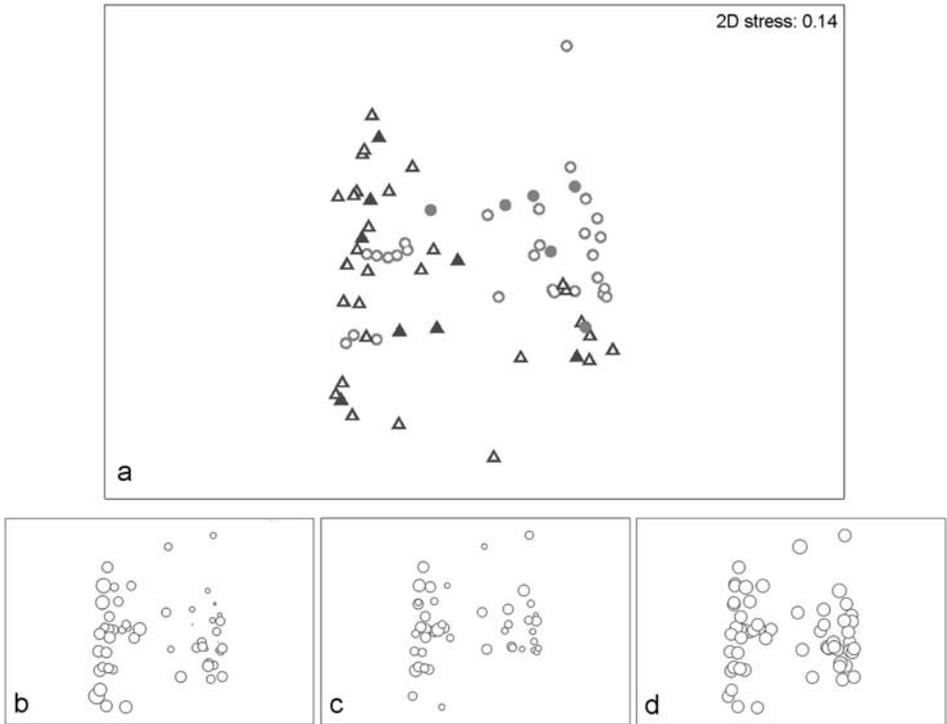


Fig. 2. The non-metric multidimensional scaling ordination plots of samples on the basis of their species composition; a. all characteristics, b. conductivity, c. height of the water table (HWT), d. pH. Fig 2 a: circles – samples A–E = Jizerské hory Mts, triangles – samples F–M = Jeseníky Mts; empty symbols – squeezed samples, filled symbols – moss samples. Fig 2b–d: circles are displaying factor value.

## Discussion

### Diatom assemblage variations on environmental gradients

The relationships of species to environmental gradients within mires are well known for: vegetation (Glaser et al. 1990), *Sphagnum* (Hájková & Hájek 2007), brown mosses (Hájková et al. 2007), and invertebrates (Borcard & von Ballmoos 1997). Two of the main environmental gradients which explain the distribution of microalgae, bryophytes, and vascular plants are the acidity-alkalinity and the fertility gradients (Bridgham et al. 1996, Fránková et al. 2009).

Most *Sphagnum* species are adapted to strongly acidic conditions (Clymo & Hayward 1982) as well as to the low availability of nutrients (Proctor 1995). On a regional scale, altitude and continentality can also be considered gradients of regional importance, since they affect the pool of plant species within a particular region (Glaser 1992). The chemical composition of precipitation also differs along the oceanic-continental gradient and can effect the ombrotrophic vegetation (Bragazza et al. 2005).

Table 3. Results of the global BEST match permutation test, illustrating the statistical significance of the relationships between environmental variables and species composition. Ten best models are shown. HWT = height of the water table.

| Model no. | Weighted Spearman $\rho_w$ | Variables included  |
|-----------|----------------------------|---|
| 1         | 0.244                      | conductivity, HWT, pH                                       |
| 2         | 0.242                      | conductivity, HWT, pH, $\text{SO}_4^{2-}$                   |
| 3         | 0.232                      | conductivity, HWT, pH, $\text{Cl}^-$                        |
| 4         | 0.228                      | conductivity, HWT, pH, $\text{NO}_3^-$                      |
| 5         | 0.227                      | conductivity, HWT, pH, $\text{NO}_3^-$ , $\text{SO}_4^{2-}$ |
| 6         | 0.226                      | conductivity, HWT   |
| 7         | 0.225                      | conductivity, HWT, pH, humic acids                          |
| 8         | 0.223                      | conductivity, HWT, pH, $\text{NO}_2^-$                      |
| 9         | 0.221                      | conductivity, HWT, pH, humic acids, $\text{SO}_4^{2-}$      |
| 10        | 0.216                      | conductivity, HWT, pH, $\text{NO}_3^-$ , $\text{Cl}^-$      |

The conductivity, HWT, and pH were determined as the primary abiotic correlates of the diatom epibryon of the peatbogs of the Sudeten Mts. (Jeseníky and Jizerské hory Mts.; this study). There was also a strong positive correlation of pH values with the species richness of the assemblages. Two diatoms which dominated most of the samples (*Frustulia saxonica* and *Eunotia paludosa*) were found to be vicariant species on pH/conductivity gradients, as previously observed at spring fens (Pouličková et al. 2004). Both are known to be associated with *Sphagnum* spp. (Cantonati et al. 2011). Several times, a similar pattern has also been illustrated in the peatbog assemblages of desmids (Štěpánková et al. 2008, Neustupa et al. 2009). On the other hand, there is no significant relationship of species richness to nutrient data, which is often known to be significantly negative in desmid assemblages (Neustupa et al. 2009).

In contrast to the conspicuous differences in vascular plants and bryophyte vegetation of the ombrotrophic mires between the regions (cf. Rybníček 2000), diatom species richness is comparable; although diatom species composition differed significantly, the two-group pattern along Axis 1 did not correspond with the regional distribution. There are two possible explanations why the differences in diatom assemblages between the regions are lower than for higher plants. First, there is no clear biogeographical group of diatom species that would correspond to the group of higher plants with a subatlantic and boreoatlantic distribution within Europe (see Introduction). In part, this is because of the better dispersal abilities of diatom species than to vascular plants (Finlay et al. 2002, Hájek et al. 2011). Second, diatoms may differ in the sensitivity and/or speed of their responses to past environmental fluctuations compared to higher plants because of their shorter life cycle, larger population sizes, with good dispersal ability allowing for ease of re-colonisation of formerly disturbed sites as well as other traits (Štěpánková et al. 2012).

Table 4. Results of the correlation analyses between the NMDS axes and individual abiotic data. HWT = height of the water table, humic = humic acids, a = mg.l<sup>-1</sup>

|                | pH               | conductivity<br>( $\mu\text{S}\cdot\text{cm}^{-1}$ ) | HWT<br>(cm)        | Fe<br>a       | NH <sub>4</sub> <sup>+</sup><br>a | NO <sub>2</sub> <sup>-</sup><br>a | NO <sub>3</sub> <sup>-</sup><br>a | SO <sub>4</sub> <sup>2+</sup><br>a | PO <sub>4</sub> <sup>3-</sup><br>a | Cl <sup>-</sup><br>a | Humic<br>a    |
|----------------|------------------|--|--------------------|---------------|-----------------------------------|-----------------------------------|-----------------------------------|------------------------------------|------------------------------------|----------------------|---------------|
| NMDS<br>Axis 1 | <b>0.32</b><br>* | <b>-0.51</b><br>***                                  | <b>-0.38</b><br>** | 0.05<br>n.s.  | 0.03<br>n.s.                      | -0.07<br>n.s.                     | <b>0.30</b><br>*                  | 0.03<br>n.s.                       | -0.22<br>n.s.                      | -0.19<br>n.s.        | 0.17<br>n.s.  |
| NMDS<br>Axis 2 | 0.23<br>n.s.     | <b>-0.28</b><br>*                                    | 0.18<br>n.s.       | -0.06<br>n.s. | 0.07<br>n.s.                      | 0.11<br>n.s.                      | 0.02<br>n.s.                      | -0.07<br>n.s.                      | -0.09<br>n.s.                      | <b>-0.26</b><br>*    | -0.11<br>n.s. |

\*\*\* permutation  $p$ -value < 0.001, \*\* 0.001 <  $p$  < 0.01, \* 0.01 <  $p$  < 0.05, n.s.  $p$  > 0.05

Kokfelt et al. (2009) found that diatoms continue to thrive or even flourish when ambient mire vegetation is stressed and/or dies. More specialized diatom species seem to benefit from either low pH or partially aerated conditions (possibly from improved light conditions and increased nutrient availability), which arise when the ambient surface vegetation dies and starts to decay. Kokfelt et al. (2009) showed that diatoms can capitalize on a changing environment in boreal wetlands, thus creating isolated hotspots for nutrients, even in peats characteristic of poor fen and bog vegetations. As the deposition load in the Jizerské Mts. has decreased in several of the recent years (Jiroušek et al. 2011), the recovery of microalgal flora could correspond with the slowing down of acidification and the slow recovery of the mires (Štěpánková et al. 2012). Using herbarized mosses from Central European peat bog mires, verification of a second hypothesis should be possible due to the lack of historical studies for subrecent-recent diatom flora comparisons.

The development of *Sphagnum*-dominated mires is controlled by a balance between precipitation and evapotranspiration, as well as the chemistry of the water feeding into it (Glaser et al. 1997). However, even if all ombrotrophic mires rely on atmospheric depositions for their nutrient and mineral supplies, ombrotrophy only indicates the source of the mire water (Proctor et al. 2009). The major consequence is that comparisons on a regional or continental scale must take into consideration that mires in geographically distinct regions are not necessarily equivalent from an ecological point of view (Sjörs & Gunnarsson 2002).

One of the most important features of *Sphagnum*-dominated mires is a surface microtopography (Sjörs 1948) reflecting the position of the mire surface, relative to the water table position along a major gradient from wet hollows to dry hummocks (Rydin et al. 1999). Micro-topographical heterogeneity favours plant, protist (Lamentowicz et al. 2010, Mitchell et al. 2008), desmid (Štěpánková et al. 2012), and diatom diversity (Pouličková et al. 2004, Hájková et al. 2011a, Buczkó & Wojtal 2005). Indeed, inter-locality variations in diatom assemblages in our study were mostly lower than the intra-locality variations. This is caused by variations in the microsites on the microtopographical gradient (HWT).

However, the regions (Jeseníky and Jizerské hory Mts.) differed significantly in their diatom species composition, suggesting that the large-scale effects also influenced the investigated assemblages.

Table 5. Results of the permutation tests, evaluating the differences between Bray-Curtis similarities of the samples.

|   | within-group B-C similarities<br>vs.<br>B-C similarities of a group samples to<br>samples from different localities | B-C similarities of a moss sample to other<br>samples of a locality<br>vs.<br>B-C similarities of a moss sample to<br>samples from different localities |
|---|---|---|
| A | n.s.  | n.s.  |
| B | n.s.  | n.s.  |
| C | n.s.  | n.s.  |
| D | mean B-C value 1: 0.62; mean B-C value 2:<br>0.39<br><b>permutation p-value = 0.0009</b>                            | n.s.  |
| E | n.s.  | n.s.  |
| F | mean B-C value 1: 0.83; mean B-C value 2:<br>0.40<br><b>permutation p-value = 0.0327</b>                            | mean B-C value 1: 0.83; mean B-C value 2:<br>0.41<br><b>permutation p-value = 0.0169</b>  |
| G | n.s.  | n.s.  |
| H | n.s.  | n.s.  |
| I | mean B-C value 1: 0.80; mean B-C value 1:<br>0.41<br><b>permutation p-value = 0.0063</b>                            | mean B-C value 1: 0.88; mean B-C value 2:<br>0.40<br><b>permutation p-value = 0.0122</b>  |
| J | n.s.  | n.s.  |
| K | n.s.  | n.s.  |
| L | n.s.  | n.s.  |
| M | n.s.  | n.s.  |

Similar relationships among microorganisms (testate amoebae), macroorganisms (vegetation), and water chemistry were observed in *Sphagnum*-dominated peatlands across Europe (Mitchell et al. 2000, Lamentowicz et al. 2010). Contrasting higher plant vegetation with small within-site variations, the overlap among sites was found to be much higher in testate amoebae. Among-site differences in the assemblages of testate amoebae, therefore, are less pronounced (Mitchel et al. 2000). However, they have also been found to reflect large-scale gradients; e.g. from the least polluted sites (Finland), through the intermediate site (Sweden) to the more polluted sites in Britain and the Netherlands (Mitchel et al. 2000). Indeed, the distribution and biology of testate amoebae (except for heterotrophic nutrition) in many respects corresponds with that of the diatoms. Both are microorganisms (10–300 µm), with similar patterns of distribution in the upper few centimetres of the moss carpet. Thus they are strongly related to the moss community data, hydrochemical variables, and water levels (Pouličková et al. 2004, Lamentowicz et al. 2010, Hájková et al. 2011a). Moreover, testate amoebae and diatoms are both abundant and diverse in *Sphagnum* mires (Pouličková et al. 2004), and their ecological preferences are well-defined (Tolonen 1986, van Dam et al. 1994).

Both, testate amoebae (Mitchell et al. 2000) and diatoms (this study) have been shown to be sensitive to both the micro-topography gradient and to macro-scale variations between geographical regions.

Our conclusions are also in congruence with diatom distributions in spring fen ecosystems (Fránková et al. 2009, Hájek et al. 2011). In a more complex study (Hájek et al. 2011), the assumptions that pH and conductivity are important for many groups of organisms (vascular plants, bryophytes, molluscs, and diatoms) were confirmed, although spatial effects cannot be overlooked in both macro- and microorganisms. However, the spatial effects are stronger for large-propagule organisms (vascular plants, molluscs) than for small-propagule organisms (bryophytes, diatoms) (Hájek et al. 2011).

### **Implications for bioindication sensu "paleolimnology without a core"**

Substrate-associated diatoms are routinely used as bioindicators to assess the water quality of rivers and streams or lakes (Kelly et al. 2008, Smol & Stoermer 2010). While stratigraphic diatom analyses allow for the reconstruction of former lake conditions (Smol & Stoermer 2010), stratified sediments are rare in lotic ecosystems. Silica-organic-acid complexes may be the cause of the poor preservation of diatoms in decomposed peat (Bennett & Siegel 1987). Thus, any paleolimnological reconstruction based upon diatoms from peat bog cores are also problematic.

Submerged macrophytes carry epiphytic diatom communities, and reflect the environmental conditions at the site (Rothfritz 1997, Vogel 2004). Historic herbarium macrophyte specimens, preserved in museums the world over, often retain the epiphytic diatom communities which have the potential to be used to interpret the former water quality (van Dam & Mertens 1993, Cocquyt & De Wever 2002). Denys (2009) reconstructed 153 years of lake history using herbarium specimens of macrophytes, and called this approach "paleolimnology without a core". The fear of negative influences of the process of herbarium specimen conservation (drying and pressing) has been rejected experimentally (Vogel et al. 2005). No differences in species richness, diversity, or evenness have been found between "fresh" epiphyton and the corresponding samples from "herbarized" macrophytes. However, when using historical herbarium macrophytes for water quality reconstructions by means of epiphytic diatoms, one has to bear in mind that some of the former botanists or collectors may have cleaned their macrophytes (Vogel 2005). Moreover, bryophyte samples (at least those soaked with water) are likely to have been squeezed by the collectors before drying, leading to a loss of epibryic diatoms.

We compared the moss samples (*Sphagnum*) and the squeezed samples taken in parallel from the same bogs. Although the number of moss samples representing the 13 mires was lower than for the squeezed samples (representing 59 plots within these mires), no significant differences were found in the species composition. However, species richness in the moss samples was significantly greater than in the squeezed samples. The reason could be the method of mineralization of the whole bryophyte tuft, eliminating or minimizing losses in comparison to bryophyte squeezing. In our previous study on spring fens, we used the more careful squeezing method, and tested its effectiveness. The fresh bryophyte samples were washed in 100 ml of distilled water and thoroughly

squeezed. The effectiveness of the washing procedure was experimentally tested by mineralizing the whole bryophyte sample, and reached 80% (Poulíčková et al. 2004). This suggests that losses >20% should be expected in the case of the squeezing method. However, these losses are not selective as we found no significant differences in the species compositions.

Thus, the use of herbarized bryophytes to assess subrecent diatom diversity in mires across the Europe can be recommended. Both the epibryon sampling and the preparation methods provide comparable results. However, there remains a problem with correct sample localization. Historic herbarium bryophyte descriptions (scheda) never include the GPS coordinates, and rarely include any additional information concerning the microsite (position on micro-topographical gradient). The only information usually available is the mire name, sampling date, collector and bryophyte species. As the intra-site variation of peatbog diatoms was found to be significant, any conclusions about the ecological state of a locality should be based on multiple samples from different bryophyte species which may represent different microsite conditions due to their individual autecological demands (Dierssen 2001, Poulíčková et al. 2004).

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