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Phylogenetic, morphological, and ecological context of microevolution in pennate diatoms.

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Ph.D. Thesis

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Declaration: I hereby declare that I have written this thesis independently, using the listed references; or in cooperation with other paper co-authors (for this author's contributions to particular papers, see chapter 2). I have not submitted this thesis, or any of its parts, to acquire any other academic degree.

Prague, 8th June 2011 .....

Jana Veselá

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Abstract: Visual assessment of discontinuities in the morphological features of diatom cells has been widely used in the discovery and delimitation of diatom species. However, a multidisciplinary approach to species-level taxonomy has revealed hidden diversity within the traditional diatom morphospecies. Consequently, this work examined both the natural and clonal populations of diatoms by diverse traditional and modern approaches, in order to assess the diversity, ecology, and distribution of diatom species. Although a detailed investigation of natural diatom samples was confounded by uncertain morphological boundaries between the traditional diatom species, it recognized that the diversity was relatively high; even one new diatom species was described using the morphological species concept. The multivariate statistical analyses showed that the variation of natural communities of traditional diatom morphospecies reflected differences in the local environmental conditions, as well as microhabitat heterogenity within a region. Since each diatom morphospecies is most likely a complex of sibling species, the two model traditional morphospecies were investigated, in order to assess morphological variation, genetic diversity, and/or the reproductive compatibility of monoclonal cultures. Even though isolated strains were cultivated under controlled conditions, the morphological variability of the cells was relatively high within the strains, as well as within the phylogenetic lineages. The morphometric study indicated that shape changes associated with the size diminution of diatoms during their life cycle might obscure characteristic morphological features that are important for species identification. Furthemore, the morphological variation of genetically differentiated strains was relatively high, and in many cases the morphology between particular phylogenetic lineages overlapped. Nonetheless, there is good reason to believe that genetic differentiation within model diatom morphospecies represented meaningful information about diatom biology, as the phylogeny was congruent with cytological, reproductive, and/or ecological differentiation.

CONTENTS

1 INTRODUCTION

1.1 Biology of diatoms

Since various aspects of diatom biology have been described and reviewed in a number of publications (e.g., Round et al. 1990; van den Hoek et al. 1995; Edlund and Stoermer 1997; Mann 1999; Sims et al. 2006; Smol and Stoermer 2010), only those specific diatom characteristics which are relevant to the thesis topics will be mentioned in this chapter. Diatoms are unicellular or colonial photosynthetic protists, with unique cell walls (frustules), which are almost always silicified. In their diplontic life cycle, the cell sizes of populations generally decrease with each asexual mitotic division; and the maximum size is primarily restored by a sexual process. Diatoms occur in almost all aquatic (and many subaerial) habitats, and they are often remarkably abundant and taxonomically diverse in natural samples. Consequently, diatom assemblages have been widely used as environmental indicators.

1.2 Discovery and delimitation of diatom species

Morphological species concept

Diatom taxonomy and systematics has been based on frustule morphology since diatoms were first discovered; and discontinuities in size, shape, and ornamentation of the frustules were considered to be taxon specific. However, unstable taxonomy, phenotypic plasticity, and life cycle changes have complicated morphological delimitation of species (reviewed by Mann 1999, 2010). Diatomists have been detected species boundaries inconsistently, using either a wide or narrow morphological concept of species. The narrow morphological species concept (e.g. Ehrenberg 1838; Kützing 1844; Rabenhorst 1953) had largely been replaced by the wide species concept, which considered the differences between the described species to be continuous or minor (e.g. Hustedt 1927-1966; Krammer and Lange-Bertalot 1986-1991). Species-level taxonomy changed back again to the narrow species concept as scanning electron microscopy and multivariate morphometric analyses significantly improved the ability to study frustule morphology in detail (e.g., Round et al. 1990; Mann and Droop 1996). Conventional and geometric morphometry showed that visual assessment considerably underestimated the complexity of the morphological variation. Therefore, many traditional

species were suggested as possible species complexes (Theriot and Ladewski 1986; Droop et al. 2000; Rhode et al. 2001; Pappas and Stoermer 2003; Beszteri et al. 2005a). Conventional morphometry is based on measurements (e.g., cell length and width, density of ornamentation) as well as on verbally described features (cell shape, type of cell structures). On the other hand, geometric morphometrics quantifies the shape variation (cell outline, shape of cell structures); indicating the directions of maximal variation, and predicting the characteristic shapes (Rohlf and Marcus 1993; Zelditch 2004; Mitteroecker and Gunz 2009). Moreover, geometric morphometrics may decompose the overall shape variation into allometric and nonallometric component (Debat et al. 2003), or into symmetric variation and asymmetry (Savriama and Klingenberg 2007; Savriama et al. 2010). Although these morphometric techniques represent a powerful tool for morphological examination of natural populations, it was not clear whether the morphological differentiation reflected intraspecific (rather than interspecific) variation; as differences in the environmental conditions (Cerino et al.. 2005; Leterme et al. 2010) or the diminution of cell sizes in the populations (Edlund and Stoermer 1997; Meyer et al. 2001; Cox 2010) may significantly influence frustule morphology. Therefore, new approaches to diatom taxonomy and systematics were needed to evaluate whether morphospecies are meaningful units in the context of evolution.

A multidisciplinary approach

Molecular genetic techniques and breeding experiments supported an even narrower species concept (Behnke et al. 2004; Beszteri et al. 2005b, 2007; Mann and Chepurnov 2005). Diatom species distinguished on the basis of molecular and mating data appeared to be cryptic; however, subsequent detailed examinations often revealed that they exhibit subtle morphological differences (Mann et al. 2004; Amato et al. 2007; Vanormelingen et al. 2007, 2008), and therefore are somewhat pseudocryptic (Mann 1999; Mann and Evans 2007). Although molecular genetic methods play an important role in the discovery of diatom species (Mann and Evans 2007; Alverson 2008), and potential barcode genes were tested on diatoms (Evans et al. 2007; Evans and Mann 2009; Moniz and Katzmarska 2010), a multidisciplinary approach that combines several modern techniques was suggested to be appropriate for species delimitation (the Waltonian species concept, see Mann 1999). However, conflicting evidence regarding species boundaries (an example is given in Figure 1) were found by diatomists which had used morphological, biological, and phylogenetic species

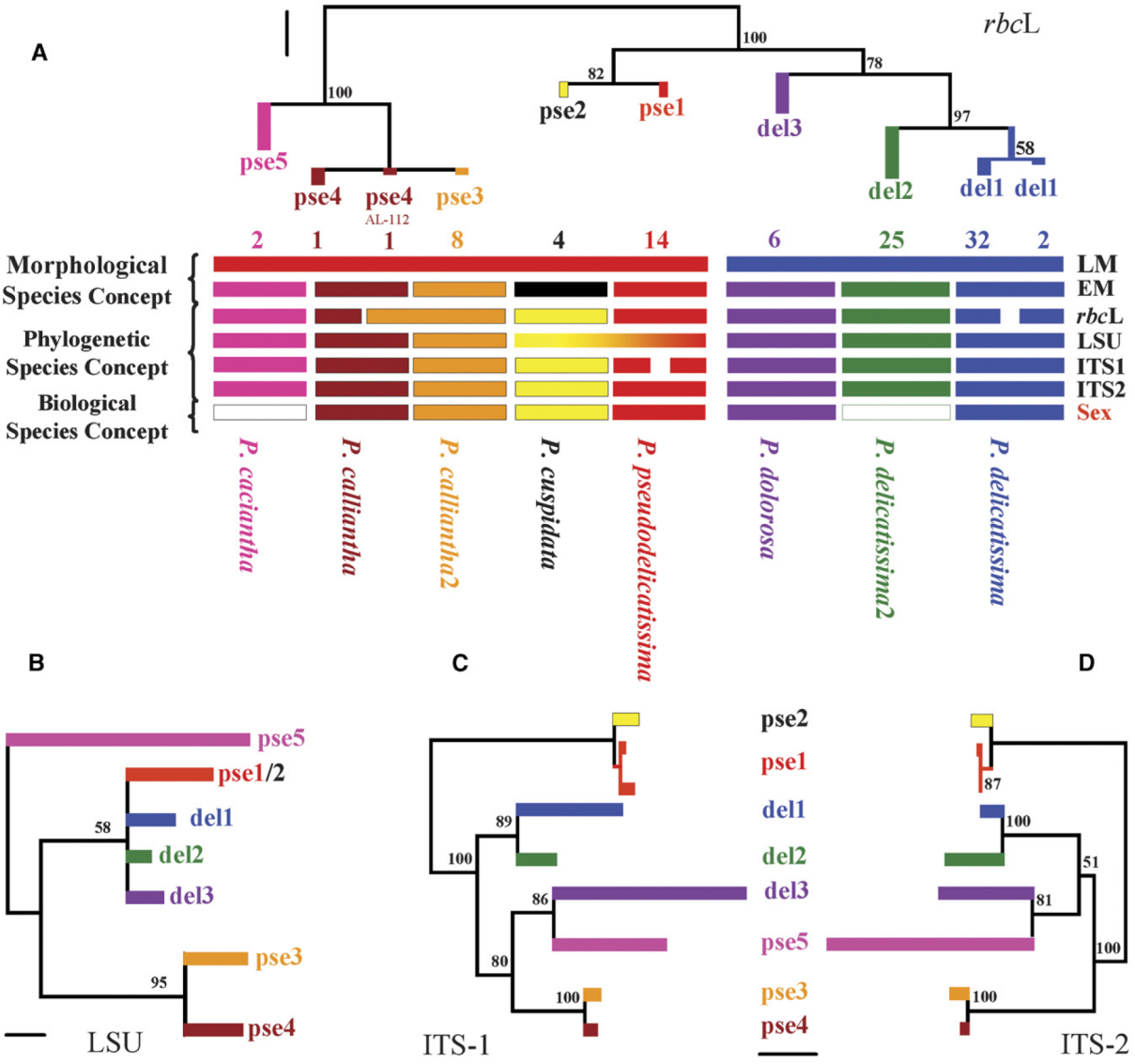

Fig. 1. ML tree inferred from rbcL (A), LSU (B), ITS-1 (C), and ITS-2 (D) sequences. Boldface numbers in each node indicate the bootstrap values. (A) Numbers in the first row represent the number of strains analysed. Horizontal bars indicate the clustering patterns recognized by gross (LM) and ultrastructural (TEM) morphology, molecular markers (listed on the right side), and sexual compatibility. The black solid rectangle indicates that ultrastructural analyses were not carried out, the green empty rectangle that mating experiments were performed but never produced sexual stages, and black empty rectangle that mating experiments were not performed. Scale bars: 0.01 (A), 0.005 (B), and 0.05 substitutions/site (C,D). After Amato et al. (2007).

definitions to discover and delimit diatom species (e.g. Amato et al. 2007; Evans et al. 2008; Trobajo 2009). Incongruence between datasets may be evidence for: i) the decoupled evolution of morphological and genetic data (Philippe et al. 1994; Alverson 2008); ii) the existence of hybrids with intermediate characteristics (Vanormelingen et al. 2008; Casteleyn et al. 2009; D'Alelio et al. 2009); and iii) reproductive isolation by spatial or temporal separation (i.e. phylogenetic lineages that can interbreed in culture possibly do not meet in nature) (Behnke et al. 2004; Casteleyn et al. 2008).

A unified species concept

One solution to the incompatible species definitions is to deny the differences between different approaches, i.e. to select just one species concept. A second solution is to accept all approaches as multiple lines of evidence for the speciation of metapopulation lineages, i.e. to adopt the unified species concept as postulated by de Quieroz (2005, 2007). The unified species concept considers species to be separately evolving metapopulation lineages, which during their divergence acquire different properties such as reproductive isolation, distinctive ecology, morphological differentiation, and the reciprocal monophyly of molecular markers (a simplified diagram is shown in Figure 2). Therefore, the diverse properties are equally

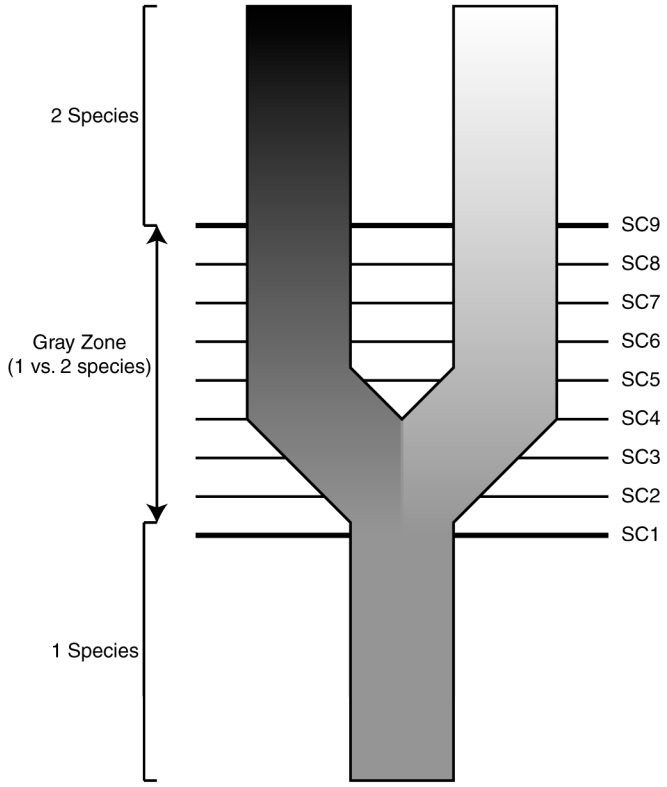

Fig. 2. This highly simplified diagram represents a single lineage (species) splitting to form two lineages (species). The gradations in shades of gray represent the daughter lineages diverging through time, and the horizontal lines labeled SC (species criterion) 1 to 9 represent the times at which they acquire different properties. The entire set of properties forms a gray zone within which alternative species concepts come into conflict. On either side of the gray zone, there will be unanimous agreement about the number of species. After de Quieroz (2007).

relevant to species delimitation, as those features used to distinguish species may or may not be achieved during the process of speciation. Nonetheless, the existence of separate species requires multiple lines of evidence. The de Quieroz approach to species definition is consistent with the attempts of David G. Mann "to encourage diatom taxonomy away from *exclusive* reliance on valve morphology and to take account also of other types of information, including insights derived ultimately from population genetics" (Mann 2010: 262).

1.3 Distribution and ecology of diatoms

Two controversial theories

Recent discussions on the diversity and distribution of diatoms have reflected upon the question of whether the biogeography of microbial species fundamentally differs from that of macroscopic organisms. The first theory, the ubiquity model, makes the following assumptions about microbial species: i) they are represented by a large number of individuals - perhaps 10^{16} protist cells beneath 1 ha of surface waters; ii) they are easily and randomly

Tab. 1. Comparison of distribution in macro-organisms and free-living protists. After Foissner (2008).

	Features	Macro-organisms	Protists (micro-organisms)	
			Ubiquity model	Moderate endemicity model
1	Absolute abundance of individuals within morphospecies	Low	High	Low in the majority (≥90%) of species, high only in some euryoecious species
2	Rates of migration	Low	High	Low for most of the rare species, high only for some euryoecious species
3	Proportion of global species pool found locally	Low	High	Moderate; usually highly over-estimated due to undersampling, see Foissner (1999) for an example
4	Rates of allopatric speciation	High	Low	Low, but see next entry
5	Rates of non-allopatric speciation	Low	?	High, e.g., parapatry, microallopatry, isolation-by-distance (Helbig 2005)
6	Cryptic persistence of species	Variable	High	High
7	Persistence of specific morphotypes over geological time scales	Low	High	Moderate
8	Large-scale distribution determined by historical contingencies, e.g., continental drift	High	Low	Moderate
9	Time for speciation	Low	?	High
10	Relative number of endemics	High	Low/none	Moderate (~30%)
11	Rates of species extinction	High	Low	Moderate
12	Global number of morphospecies	High	Low	High due to long time to speciate and non-allopatric speciation (see above)
13	Conservation	Needed	Not needed	Needed
14	Human introductions	Low	?	Likely high; see Foissner (2006) and several contributions in this issue

Based on Finlay et al. (2004), except for features (5, 9, 13) and the "moderate endemicity model"

dispersed across all spatial scales - cosmopolitan distribution prevails; iii) they maintain consistent patterns of local abundance or rarity on a global scale - abundant species have a higher rate of dispersal; iv) they grow wherever they have found a suitable habitat - their distribution is constrained only by ecological requirements; v) their global species richness is relatively low - they have a higher local/global species ratio (Finlay and Fenchel 1999; Finlay 2002; Finlay et al. 2004; Fenchel 2005). The second theory, the moderate endemicity model,

represents an alternative view proposing that: i) a much larger proportion of protists have biogeographies - contemporary or historically restricted distribution; ii) the real number of protist species is much larger than is known at present - new species are expected to be discovered in unexplored places on earth (Chao et al. 2006; Foissner 2006; 2008). The differences in these assumptions regarding the distribution of macro-organisms and free-living protists are summarized in Table 1.

Traditional diatom species

In the past, the distribution of species was not considered very important for diatom research, because it has been assumed that most diatoms are cosmopolitan (Kociolek and Spaulding 2000). Consequently, European taxonomic publications were used for the identification of diatom species in various parts of the world, and many potentially new species were force-fit into the already described species (e.g. Tyler 1996). Interest in the biogeography of diatoms has increased considerably since the second narrow species concept was adopted (details mentioned above in the section on the morphological species concept). A large number of new species, including endemic species, were described on the basis of detailed studies of regional diatom flora from diverse regions (for instance, see the Bibliotheca Diatomologica and Iconograhia Diatomologica series). The second wave of increased interest has been associated with the ubiquitous theory that was also applied to diatoms (Finlay et al. 2002). Empirical evidence about the distribution of diatom species at the global scale considerably supported the alternative hypothesis, i.e. that a number of species have a restricted distribution, influenced by historical processes. A high degree of endemism was revealed in ancient lake basins, isolated islands, and in diverse geographic regions (Mann and Droop 1996; Mann 1999; Kociolek and Spaulding 2000; Vanormelingen et al. 2008). The most striking evidence represents the unique diatom flora of the Australasian region (Tyler 1996; Kilroy 2007), human-mediated introductions of exotic diatom species (Vanormelingen et al. 2008), and the restricted distribution of species within the genera *Actinella, Eunophora*, and *Muelleria* (Vanormelingen et al. 2007, 2008).

The ubiquitous model has also been challenged at different spatial scales. The review and meta-analysis of Soininen (2007) indicated that the relative importance of environmental and spatial factors on diatom community structure is inversely related to the spatial distance. Environmental factors considerably influenced diatom assemblages at local spatial scales;

however, their effect decreased at the regional and continental scales. In contrast, the role of spatial factors increased with an increasing study area. Furthermore, interesting phenomenon emerged from those studies, which examined the relationship between local and regional diatom species richness within streams. There, it was found that local diatom richness is linearly dependent on regional species richness; hence, the diversity of diatom communities is enriched with the regional species pool (Passy 2009; Soininen et al. 2009). Other diatom studies have also failed to prove the assumptions of Finlay and co-workers regarding cell sizes and the frequencies of protist species. These studies suggested that although abundant diatom species were found more frequently at sampling sites, the distribution patterns at local, regional, and global scales primarily reflected the species' history of dispersal and establishment (Soininen and Heino 2005; Passy 2008); additionally, that the size of the diatom species was a less important predictor of distribution frequency (Heino and Soininen 2006; Passy 2008).

The metacommunity concept

The mechanisms underlying the ecological patterns and phenomena at both the local and regional spatial scales may be interpreted using metacommunity theory (a metacommunity is defined as a set of local communities that are linked by the dispersal of multiple potentially interacting species). Leibold and colleagues (2004) identified four simplified views of metacommunities, which can explain the observed patterns of distribution, abundance, and interactions of species: i) the patch-dynamic paradigm - competition or colonization trade-offs among species; ii) the species-sorting paradigm - trade-offs among species that allow them to specialize in different habitat conditions; iii) the mass-effects paradigm - source-sink relations between populations in different habitats; and iv) the neutral paradigm - the absence of differences between species in niche relationships, with local factors and/or their abilities to disperse or avoid local extinctions. A schematic representation of the four paradigms is illustrated in Figure 3. The assumptions of these four models differ in the character of differences among the habitats: differences in species pool (patch dynamic, neutral models) versus differences in environmental conditions (mass-effect, species-sorting perspectives); and in the ecological traits of species: the equal fitness of species (neutral model) versus the variability of ecological traits (the three other views). Nonetheless, it has been suggested that "it is unlikely that all the species that interact in a given set of real metacommunities will uniformly conform to any one of these perspectives. Instead, it is likely that each of these sets

of processes will play interactive roles in structuring real metacommunities." (Leibold et al. 2004: 608).

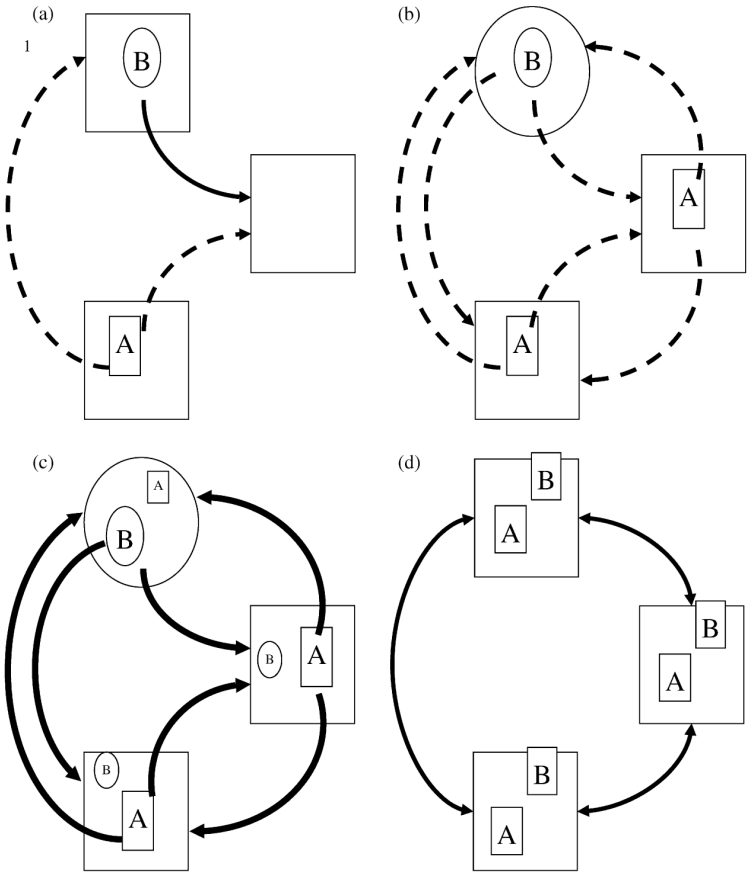

Fig. 3. Schematic representation of the four paradigms for metacommunity theory for two competing species with populations A and B. Arrows connect donor populations with potential colonization sites, shown as large boxes or ovals. Solid arrows indicate higher dispersal than dashed arrows and either unidirectional movement (single-headed arrows) or bidirectional movement (double-headed arrows). The degree to which a species is the competitive dominant in a site is shown by the matching of the smaller box or oval (denoting its habitat type niche) with the site symbol. The four paradigms illustrated are (a) patch-dynamics, (b) species-sorting, (c) mass-effects and (d) neutral. In (a) the patchdynamics paradigm is shown with conditions that permit coexistence: a competition-colonization trade-off is illustrated with species A being a superior competitor but species B being a superior colonist; the third patch is vacant and could become occupied by either species. In (b) species are separated into spatial niches and dispersal is not sufficient to alter their distribution. In (c) mass effects cause species to be present in both source and sink habitats; the smaller letters and symbols indicate smaller sized populations. In (d) all species are currently present in all patches; species would gradually be lost from the region and would be replaced by speciation. After Leibold et al. (2004)

Species complexes

In ecological studies, the diatom communities are predominantly represented by traditional morphospecies, because they are identified relatively easily. Recognition of cryptic or pseudocryptic species (sibling species) needs an incomparably larger effort; therefore,

investigations have been confined to the examination of several model diatom species from marine and freshwater environments (e.g., Amato et al. 2007; Evans et al. 2008; Kooistra et al. 2008). Nevertheless, molecular approaches will likely soon prevail in diatom ecology; as the overall diversity of environmental samples will be assessed by improved sequencing techniques, such as the DNA barcoding (Evans et al. 2007; Medlin 2007), the gene clone libraries (Šlapeta et al. 2005; Epstein and López-García 2008; Behnke et al. 2010), and parallel tag sequencing (Stoeck et al. 2009; Medinger et al. 2010; Nolte et al. 2010).

Although it has been assumed that the hidden diversity of traditional diatom species is accompanied by physiological, ecological, and geographical differentiation, few diatom studies have investigated these aspects. The case studies on sibling species have suggested that marine planktonic diatoms are widely distributed within a particular climatic zone, or their distribution is geographically restricted (Casteleyn et al. 2008; Kooistra et al. 2008). Furthermore, it has been shown that abundances of sympatric populations of model species have reflected seasonal changes (Orsini et al. 2004; Cerino et al. 2005; Kooistra et al. 2008), and that levels of gene flow among natural populations could markedly differ over similar spatial scales (Evans et al. 2005; Rynearson 2009). In freshwater habitats, sibling species of *Nitzschia palea* appeared to be widely distributed (Trobajo et al. 2009), although the different genetic structure of *Sellaphora capitata* populations was found on the medium and large scales (Evans et al. 2009). Partial information about the ecology and distribution of freshwater species complexes has also been acquired by the long-term study of *Sellaphora* species. Some species appeared to have specific environmental requirements, with respect to pH and trophy; several (to many) closely related and morphologically similar species frequently occurred together; several species (which can be recognized on the basis of photographs) mostly occurred in cool-temperate North Hemisphere localities. Additionally, almost no *Sellaphora* species that were recorded on the British Isles were found in South American non-temperate areas (Mann et al. 2008; Poulíčková et al. 2008).

In agreement with this, examples from other groups of microbial organisms have indicated that the distribution of sibling species may be cosmopolitan, as well as geographically restricted; further, that local environmental conditions considerably influenced the genetic diversification (see Dolan 2005 and Logares 2006). Since undersampling might significantly influence the observed distribution patterns (Finlay et al. 2004; Foissner 2008), a detailed biogeographic study of benthic heterotrophic flagellates (cercomonads) was conducted in order to assess the molecular diversity in environmental samples collected from soil,

freshwater, and marine habitats (Bass et al. 2007). Although increased sequencing efforts changed the distribution of some sequence-types from restricted to widely distributed, several sequences remained unique for the Australasian region. The ecological requirements of particular sibling species that were reconstructed from environmental conditions recorded at individual sampling sites are in contrast to the observations about morphospecies. Bass and colleagues (2007) recovered identical sequence-types from both marine and non-marine environments. Similarly, phylogenetic lineages identified in the study of Boo and colleagues (2010) did not reflect differences in pH values nor in the amounts of nutrients. In addition, experimental cultivation of heterotrophic protists demonstrated that the ecophysiological response (functional diversity) was related to the variations of local environmental conditions within habitats and/or to differences in climatic conditions; but not to genetic differentiation (e.g., Lowe et al. 2005a, 2005b, 2007; Weisse et al. 2008). In the context of these observations, advocates of the ubiquitous theory have concluded that genetic variation within microbial morphospecies reflected the accumulation of selectively neutral mutations (Fenchel 2005; Fenchel and Finlay 2006). Consequently, they have argued that species identification and delimitation should be based on phenotypic properties, on ecophysiology, and especially on morphology (Finlay 2004; Fenchel and Finlay 2006). One should compare this statement with the unified species concept.

Phylogenetic community ecology

Despite the fact that genetic differentiation within complexes of microbial species appeared to be neutral with regard to functional diversity (for details see the previous section), the phylogenetic structure of ecological communities is highly relevant to an understanding of the historical and ecological processes underlying species assemblages (reviewed by Webb et al. 2002). The two processes are traditionally considered important in shaping communities: competitive interactions among species with overlapping ecological niches, and the environmental filtering of species with a particular phenotype (for references, see Cavender-Bares et al. 2007). The relative importance of these processes may be inferred from the phenotypic and phylogenetic structures of communities, which can be described using the following terminology: i) phenotypic clustering (overdispersion) - species within a community are more (or less) phenotypically similar to each other than expected by chance; ii) phylogenetic clustering (overdispersion) - species within a community are phylogenetically more closely (or distantly) related to each other than expected by chance; iii) conserved

(convergent) trait evolution - closely (or distantly) related species are ecologically similar (Webb et al. 2002; Hardy and Senterre 2007; Pausas and Verdú 2010). The processes predicted from the relationships between phylogenetic distances, ecological trait similarity, and species co-occurrence are illustrated in Figure 4. Since the observed pattern of community structure is dependent on the spatial, temporal and taxonomic scales (Cavender-Bares et al. 2006; Swenson et al. 2006; Emerson and Gillespie 2008), other mechanisms such

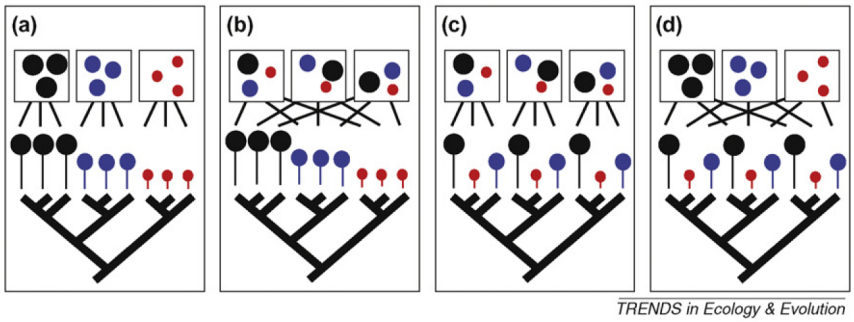

Fig. 4. Environmental filters, interspecific competition, trait lability and the structure of communities. Species composition within and among communities can be influenced by environmental filtering, interspecific interactions and the potential for evolutionary change of traits associated with niche occupancy. (a) Both phylogenetic and phenotypic clustering in three communities (squares) consistent with the existence of both the conserved evolution of traits associated with niche occupancy (represented by circle size and color) and strong environmental filtering. (b) Both phylogenetic and phenotypic overdispersion consistent with conserved evolution of traits associated with niche occupancy, and greater importance for species interactions over environmental filtering in determining species composition. (c) Phylogenetic clustering and phenotypic overdispersion consistent with evolutionary change in traits associated with niche occupancy and adaptive radiation. (d) Phylogenetic overdispersion and phenotypic clustering consistent with evolutionary change in traits associated with niche occupancy and strong environmental filtering. After Emerson and Gillespie (2008).

as dispersal, speciation, biogeographic history, and neutral processes may be involved in community assembly and structure (Cavender-Bares et al. 2009).

1.4 Research objectives of the thesis

In the present thesis, multiple approaches to diatom biology were used for the investigation of diatom species diversity, ecology, and distribution. The main objectives of the research were:

i) to estimate the diversity of diatoms adopting the morphological species concept (paper I), and a multidisciplinary approach to study morphospecies (papers III, IV);

ii) to analyze both the qualitative and quantitative morphological variability, using fine-grained investigations of frustules (papers II, III, IV);

iii) to identify the patterns of distribution and diversity at the regional, local, and microhabitat scales (papers I, III, IV);

iv) to assess whether environmental requirements considerably influence the diversity and distribution of diatoms (papers I, IV); and

v) to compare the morphological, phylogenetic, distributional, and ecological data sets within species complexes (papers III, IV).

1.5 Thesis outline

Morphology of traditional diatom species

In paper I, the morphological species concept was adopted to estimate diatom species diversity of natural samples taken from ephemeral streams in a sandstone region. Species diversity of the region was relatively high in comparison with similar studies. Moreover, several specimens differed considerably in morphological characteristics from any species that had already been described. Since intraspecific phenotypic variation of natural populations is influenced both by life cycle changes and genetic differences, the morphology of monoclonal cultures were examined, using conventional morphometry and geometric morphometrics; papers II-IV. Strains of *Navicula cryptocephala* sensu lato were analyzed, in order to quantify the differences in the morphology of diatom frustules at different stages of the life cycle (paper II). Although large frustules (postinitial cells) differed significantly from small frustules (sexually competent cells); the non-allometric component of valve shape variation was characteristic for each strain. The morphology of monoclonal cultures was also analyzed in the context of genetic differentiation within traditional species of *Navicula cryptocephala* sensu lato (paper III), and *Frustulia rhomboides* sensu lato (paper IV). It was

possible to determine the distinctive morphological features among strains, representing closely related phylogenetic lineages on the basis of the average characteristics of valve morphology.

Distribution and diversity of diatoms at different spatial scales

In order to estimate diatom diversity, spatial distribution patterns were determined for traditional morphospecies at both the microhabitat and local scales (paper I), as well as for the phylogenetic lineages of morphospecies at the local and regional scales (paper III, IV). In paper I, significant differences in species diversity and similarity were found among the microhabitats, sampling sites, and localities within a single region. The phylogenetic identity of strains isolated from Europe and Australia indicated that lineages of *Navicula cryptocephala* are widely distributed (paper III). In paper IV, diversity and distribution of European populations of *Frustulia rhomboides* sensu lato were explored in detail, and the results showed that the distribution pattern varied in the frequencies of individual lineages among the localities and regions.

An ecological perspective on the distribution and diversity of diatoms

In paper I, the ecological requirements of diatom morphospecies, with respect to pH and conductivity, were estimated on the basis of multivariate statistical methods. Furthermore, the similarity of diatom assemblages among different localities was compared with the geographical and environmental distances. The spatial autocorrelation among diatom assemblages found at different localities was not significant; consequently, instead diatom species composition was influenced by environmental conditions at the local spatial scale. In paper IV, an obvious ecological signal emerged from the sequence frequencies of phylogenetic lineages of *Frustulia rhomboides* sensu lato; the distribution pattern of *F. rhomboides* lineages, most likely reflected their specific habitat requirements and their preferences for particular climatic conditions.

2 ORIGINAL PAPERS

I. Veselá J, Johansen JR (2009) The diatom flora of ephemeral headwater streams in the Elbsandsteingebirge region of the Czech Republic. *Diatom Research* 24: 443-477.

II. Veselá J, Neustupa J, Pichrtová M, Poulíčková A (2009) Morphometric study of *Navicula* morphospecies (Bacillariophyta) with respect to diatom life cycle. *Fottea* 9: 307-316.

III. Poulíčková A, Veselá J, Neustupa J, Škaloud P (2010) Pseudocryptic diversity versus cosmopolitanism in diatoms: a case study on *Navicula cryptocephala* Kütz. (Bacillariophyceae) and morphologically similar taxa. *Protist* 161: 353-369.

IV. Veselá J, Urbánková P, Černá K, Neustupa J (subm) Ecological variation within traditional diatom morphospecies; diversity of *Frustulia rhomboides* sensu lato (Bacillariophyceae) in European freshwater habitats. *Journal of Phycology*.

Author's contributions

Paper I. I had planned the study, done the sampling, identified most of the species, and statistically analyzed the data. Jeffrey R. Johansen helped me with the species identification and with the weighted averaging method. We jointly wrote the paper.

Paper II. Jiří Neustupa and I jointly planned the study. Aloisie Poulíčková isolated and cultivated the monoclonal cultures as well as provided the images of the cultures. Myself, J. Neustupa, and Martina Pichrtová were responsible for the morphometric analyses. The figures and tables were made by myself and by J. Neustupa. I wrote the paper, and J. Neustupa helped me with the final improvements on the manuscript.

Paper III. Aloisie Poulíčková planned the study, performed the sampling, isolated and cultivated the strains, got the images of the cultures, measured the cell characteristics, visualized the nuclear structure of cells, and examined the sexual reproduction of the strains. I performed the molecular reactions; and Pavel Škaloud helped me with the phylogenetic analyses as well as with the reconstruction of ITS2 secondary structure. I obtained the geometric morphometric data of the strains; and Jiří Neustupa analyzed the shape variability of strains. I wrote the molecular parts of the paper with the help of P. Škaloud; J. Neustupa wrote the morphometric portions, and A. Poulíčková wrote the remaining parts of the manuscript.

Paper IV. I planned the study. Pavla Urbánková and I isolated and cultivated the monoclonal cultures, performed the molecular analyses, and prepared the tables. I photographed the cells of the strains; and Kateřina Černá helped me with the morphometric analyses. I made the figures and wrote the paper. Jiří Neustupa together with P. Urbánková helped me to improve the text of the manuscript.

On behalf of all of the co-authors, we declare the keynote participation of Jana Veselá in the research of aspects of diatom biology and the writing the papers, as described above.

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Jiří Neustupa Pavel Škaloud

2.1 I. Manuscript

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THE DIATOM FLORA OF EPHEMERAL HEADWATER STREAMS IN THE ELBSANDSTEINGEBIRGE REGION OF THE CZECH REPUBLIC.

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The diatom flora of 15 headwater streams in the Elbsandsteingebirge region of the Czech Republic was studied. This region is dominated by sandstone, but a few areas have a mixed geology with some combination of sandstone, limestone, and granite. Diatoms were collected from a mixture of substrates whenever available, including stone, wood, bryophyte, and sediment. A total of 307 diatom taxa were observed, including one species new to science, *Chamaepinnularia rexii*. Taxa particularly characteristic for the region, or lacking previous indicator status, or rare or otherwise interesting are illustrated and discussed. Abundance weighted means for both pH and conductivity are reported for all taxa which occurred in more than four samples.

INTRODUCTION

The Elbsandsteingebirge (Elbe Sandstone Mountains) is a hilly region of north Bohemia and Saxony in the Elbe River drainage that is characterized by a massive Cretaceous sandstone formation about 300 m high. Deep gorges have been cut into the sandstone, and the streams draining the region are forested, shaded and cold. The region includes four protected areas: Bohemian Switzerland National park, Elbe Sandstone Mountains Protected Landscape Area, Saxon Switzerland National Park and Saxon Switzerland Protected Landscape Area.

Rabenhorst (1851) described Frustulia saxonica from wet-walls in the Elbsandsteingebirge. The cryptogamic communities (lichens, bryophytes, eukaryotic algae, cyanobacteria) of this region were first studied in some detail by Schorler (1915) and Schade (1923), who both examined wet walls. The diatoms of the wet walls were studied subsequently by Friedrich Hustedt, who described several diatom taxa from the region (Hustedt 1930). However, the flora of the region was not treated in a separate publication so we do not have a record of all the taxa Hustedt may have seen there.

More recently, peat bogs (Nováková 2003, Nováková *et al*. 2004) and some streams (Skácelová 1998, Heteša *et al*. 2000) were studied with respect to their algae, particularly siliceous heterokont algae. Veselá (2006) studied the seasonal succession and spatial variability of algae in one drainage, Suchá Bělá. She found 48 taxa in just two sites in the brook. These studies demonstrated that the region was of interest, particularly because it possess pristine habitat and clean water in much of its range. However, a systematic study of the diatoms of the Elbsandsteingebirge remains to be done.

In the present study such a systematic study is reported. We examined 161 samples in 30 sites in 15 streams in the region. Most of the streams are ephemeral headwater streams with either a cobbly, rocky channel or channel lying directly in sandstone bedrock. The purpose of this study is to 1) document diatom taxa present in the streams 2) illustrate rare and interesting taxa, 3) describe a taxon new to science, and 4) document optima and tolerance (pH, conductivity) of aquatic and aerophilic diatom taxa.

MATERIALS AND METHODS

Fifteen streams and brooks in the Czech part of Elbsandsteingebirge were sampled (Fig. 1). Most of the sites were within Bohemian Switzerland National Park. The streams were headwater streams (mostly first and second order), and most were ephemeral for much of their drainage. Many of the sites were in unnamed tributaries. Streams were sampled between October 2004 and June 2006, and both an upper reach near the origin and a lower reach above the confluence with a named stream were sampled. The distance between sites varied among streams. Conductivity, pH and temperature were measured using a Hanna Combo HI 98129 pH/EC/TDS.

Samples were taken from all available substrates, and included epilithon (from rocks), epixylon (from wood), epiphyton (among bryophytes and filamentous algae), and epipelon/epipsamnon (from sediment and sand). This yielded 5-6 samples per sampling site,

for a total of 161 samples in all (62 sediment, 49 rock, 23 wood, 4 filamentous algae and 23 bryophytes). Diatom samples were cleaned using the hydrogen peroxide-potassium permanganate method (Krammer & Lange-Bertalot 1986), rinsed, and mounted in Naphrax diatom mountant. Initial identification and estimation of species abundance was determined using an Olympus BX 31 brightfield microscope. Diatoms were scored on a semi-quantitative scale: rare (one or two cells per sample), uncommon (less than 5% of the cells), common (5-25%), frequent (25-50%) and dominant (more than 50%). Identification of difficult (small, finely striated, etc.) taxa was confirmed or established in an Olympus BX 60 photomicroscope with high resolution Nomarski DIC optics and Spot digital 5 camera. A scanning electron microscope, SEM JEOL 6380, was used to identify and illustrate the most difficult taxa, such as *Diadesmis* and *Chamaepinnularia*. Diatom taxa were identified according to Alles *et al*. (1991), Houk (2003), Houk & Klee (2007), Krammer (1992, 1997a,b, 2000, 2002, 2003), Krammer & Lange-Bertalot (1985, 1986, 1988, 1989, 1991a,b), Lange-Bertalot (1993, 2001), Lange-Bertalot & Metzeltin (1996), Lange-Bertalot & Moser (1994), Werum & Lange-Bertalot (2004).

Optima and tolerance values for both pH and conductivity were determined by using the weighted averaging program in WA CALIB (Line *et al*. 1994). Abundances of taxa were estimated using a semi-quantitative scale (see above). Saprobity and trophic state of streams were reconstructed from indices based on diatom composition (Lange-Bertalot 1979, Hofmann 1994, Van Dam *et al*. 1994), all of which use indicator values and abundance weighted means (AWM). Sites were ordinated using Detrended Correspondence Analysis in CANOCO (ter Braak & Šmilauer 1998). Bray-Curtis and Dice-Sørensen similarity indices and one-way analysis of variance (ANOVA) and non parametric Kruskal-Wallis test were calculated using PAST-software ver. 4.5 (Hammer *et al*. 2001). Box plot diagrams were constructed using STATISTICA-software ver. 8. A Mantel and partial Mantel test (zt-software, Bonnet & Van de Peer 2002) were used to uncover associations between pairs of measurements in consideration of spatial autocorrelation (geographic distance). The Mantel test is a nonparametric test for simultaneous comparisons between two dissimilarity matrices (Mantel, 1967; Legendre & Legendre, 1998). Partial Mantel analysis computed the partial correlation between two distance matrices while controlling for the effect of a third matrix (Smouse *et al*. 1986).

RESULTS

A total number of 307 intraspecific diatom taxa (Table 1) were recorded in 161 samples from 15 streams. Species richness was low in many samples, ranging from 3-72 taxa, with an average of 27 taxa (Table 2). The greatest number of species per sampling site (109 taxa) and stream (128 taxa) was found in Vlčí potok. The most diverse genera of diatoms in the streams were *Pinnularia* (34 taxa) and *Eunotia* (31 taxa).

Measured stream water parameters varied between 3.30 and 8.30 for pH and conductivity varied between 52 $\mu S \cdot cm^{-1}$ and 229 $\mu S \cdot cm^{-1}$ (Table 3).

Abundance weighted means (AWM) were calculated for both pH and conductivity for the taxa which occurred in more than four samples using the semi-quantitative scale described above (Table 1). Van Dam's ecological (indication) values of pH (Van Dam *et a*l. 1994) are listed in Table 1 for comparison with the calculated values of AWM pH optima. The species moisture preferences (desiccation resistant and aerophytic species) are also according to Van Dam's list. In total 45 diatom taxa were previously reported as subaerophytic, while 64 taxa were not listed in his paper. Aerophytic genera represented by the greatest number of intraspecific taxa were *Diadesmis* (8 taxa) and *Chamaepinnularia* (6 taxa).

The first four axes of detrended correspondence analysis (DCA) explained 25.4% of variance in species data. The first axis (13.3%) of the ordination (Fig. 207) appears to represent pH, trophic level, and saprobity (the latter two factors estimated from stream AWM indices), with higher value streams (localities 6, 10-13) to the left containing species characteristic of more alkaline and more nutrient rich conditions (*Amphora pediculus*, *Cocconeis placentula*, *Eolimna minima*, *Melosira varians*, *Navicula gregaria*, *N. lanceolata*, *Reimeria sinuata*). More acidic streams (3, 4, 7, 8) with both acidophilic and oligotrophic taxa are clustered to the right (characteristic species: *Chamaepinnularia soehrensis*, *Eunotia incisa*, *E. paludosa*, *E. rhomboidea*, *E. trinacria*, *E. ursamaioris*, *Frustulia saxonica*, *Microcostatus krasskei*). The second DCA axis explained 5.0% of variation in species data and no clear gradient is evident along this axis (Fig. 207).

The results of a Mantel and partial Mantel permutation test, used for assessment of the correlation between the species matrix and a matrix of geographical distance, were not significant ($p>0.05$). We interpret this to mean that localities in close proximity to one another are not more similar than localities that are spatially removed from one another. This finding is consistent with results from the DCA diagram that indicated the arrangement of localities along the first axis is rather dependent on the pH and concentration of nutrients than on

geographical distance. However, species similarity indices between sites were not significantly correlated with pH differences between sites.

Variability of similarity indices within and among groups was tested at the level of whole streams (Table 2) and sampling sites within streams (Table 3). Similarity within streams is significantly higher than between streams (one-way ANOVA, $p<0.001$). However, significant differences between upstream and downstream samples were still found in twelve of the fifteen streams (Kruskal-Wallis test).

Diatoms showed significant differences (Kruskal-Wallis test, $p<0.001$) in species diversity and species similarity on different substrate types (Fig. 208). Hard substrates (epilithon, epixylon) had lower species richness than soft substrates (epiphyton, epipelon; Table 2). Specificity of diatoms was higher for soft substrates than for hard substrates.

Taxonomic Part

In the interest of space, we have not illustrated all taxa observed in this study. We provide micrographs of taxa within the characteristic genera of the site (*Eunotia*, *Chamaepinnularia*, *Diadesmis*, *Neidium*, etc.), taxa lacking indicator values in Van Dam *et al*. (1994), aerophilic taxa, and rare or otherwise interesting taxa. We comment on these taxa below. Authorities for taxa given in Table 1 are not repeated here.

Eunotia Figs. 2-57.

This difficult genus was one of the most diverse and abundant taxa in the study, and for this reason most taxa are figured in this paper. The prevalence of *Eunotia* reflects the oligotrophic and acidic nature of the water in the studied streams. At least one *Eunotia* species was recovered from every stream (Table 1), with at least 6 species in 13 of the streams. Seven of the streams had 12 or more (up to 20) taxa. The most widespread species were *Eunotia bilunaris* (Figs. 6, 7), *E. botuliformis* (Figs. 42-44), *E. exigua* (Figs. 24-26), *E. tenella* (Fig. 27-30), *E. incisa* (Fig. 16), *E.* cf. *minor* (Fig. 2, 3), *E. paludosa* (Figs. 48, 49), and *E. rhomboidea* (Fig. 45). All taxa were acidophils, with only 3 of our taxa having an AWM > 6.0 for pH (Table 1). While *Eunotia bilunaris* is considered indifferent to pH in Van Dam *et al*. (1994), it was an acidophil in our study, with a pH optimum of 5.3 (Table 1). In agreement with our findings, Denys (2004) considered *Eunotia bilunaris* acidophilous instead of pH-indifferent after personal communication with H. van Dam and A. Mertens. According to Alles *et al*. (1991), *Eunotia bilunaris* has two optima, suggesting the existence of acidobiontic

and circumneutral populations or cryptic species. We suspect multiple species are contained in the current circumscription of *Eunotia bilunaris*, an idea which is in agreement with Vanormelingen *et al*. (2007, 2008), and that our populations belong to an acidobiontic species within the complex. Our pH optima were in most instances in good agreement with the optima found for the same *Eunotia* species in Alles *et al*. (1991). The most taxonomically problematic taxa were the small *Eunotia* species in the *E. exigua-E. tenella* complex (Figs. 24-30). Some of our specimens seemed transitional between the two taxa, although they clearly were in the complex. We illustrate specimens of which we are confident in identification. Given the cosmopolitan nature of this group, we suspect there may be cryptic diversity in the complex.

Eunotia ursamaioris was illustrated as *E. septentrionalis* Østrup in Krammer & Lange-Bertalot (1991a, Tafel 157:17-18) and Lange-Bertalot & Metzeltin (1996, Tafel 16:25-30). Lange-Bertalot & Genkal (1999) examined the type material of *E. septentrionalis*, and realized that Hustedt's concept of *E. septentrionalis* (Hustedt 1930, 1959) and their earlier concept was not consistent with the type. They described *Eunotia ursamaioris* to circumscribe the Siberian and European material. Our specimens (Figs. 11-13) are very similar to the material illustrated in Werum & Lange-Bertalot (2004, Plate 8:12-13). We suspect that others have reported *Eunotia septentrionalis* when in fact they had *E. ursamaioris*.

***Encyonopsis* Figs. 58-66.**

We observed two taxa of diminutive *Encyonopsis* which appear to be different from any described taxa. We do not name these taxa because they were very rare in the samples and we could not find any valves in the SEM. Given their fine structure that is scarcely visible in LM, we prefer to wait until such time that larger populations are found. It is possible that some of these specimens were teratological assymetrical forms of *Achnanthidium* (Figs. 58-60).

***Encyonema perpusillum* Figs. 67-68.**

This taxon has been observed as an aerophytic taxon in other studies. It was widespread in our samples, but always rare or uncommon (Table 1). It has been considered acidophilic (Van Dam *et al*. 1994), but had an optimum near neutrality in our study.

***Psammothidium altaicum* Figs. 69-70.**

Psammothidium altaicum was recently transferred from Achnanthes by Bukhtiyarova (Bukhtiyarova & Round 1996). It was found only in one stream, Písečná rokle.

***Eucocconeis laevis* (Figs 71-72)**

This taxon is typically found aerophilic on sandstone wet walls. It was common only in one site.

***Nupela lapidosa* (Figs 73-74)**

Nupela is commonly found in wet wall habitats. This species is the most commonly reported species in the genus. Siver & Hamilton (2005) suggested that heterovalvar *Nupela* species (e.g. N. lapidosa) needs to be established as a new genus, because the original description of the genus does not include the heterovalvar state and the type species for the genus is isovalvar.

***Sellaphora stauroneioides* (Lange-Bertalot) nov. comb. (Figs 75-76)**

Basionym: *Naviculadicta stauroneioides* Lange-Bertalot in Lange-Bertalot & Metzeltin 1996, p. 89, plate 109, fig. 22-27.

This taxon bears the hyaline areas at the apices and curved striae characteristic of *Sellaphora*. The striae are fine for this genus, but Lange-Bertalot published an internal view of the striae in *Naviculadicta stauroneioides* (Lange-Bertalot & Metzeltin 1996, plate 89, fig. 27), and the ultrastructure of the striae and central area is identical to that shown for *Sellaphora* (Round *et al*. 1990, p. 553, fig. j). Lange-Bertalot indicated his population contained specimens 5.5-6.5 μm wide by 20-27 μm long, with 35-40 striae in 10 μm. *Sellaphora stauroneioides* was rare in our material, and our specimens were slightly smaller (5.0-6.0 μm wide by 20-21 μm long, striae too fine to enumerate in LM).

***Luticola* (Figs 77-78)**

Most of the species within this genus are aerophilic. *Luticola acidoclinata* and *L. mutica* were both widely distributed in the streams of the Elbsandsteingebirge.

***Fallacia vitrea* (Figs 79, 197)**

This acidobiontic species typically occurs in very dry sites. The conopeum was evident in SEM (Fig. 197).

Diminutive naviculoid taxa (Figs 80-91, 140, 141)

A number of rare and interesting taxa in a diverse group of taxa from *Navicula* sensu lato occurred in the Elbsandsteingebirge. Most of the illustrated taxa were found in more than one stream, but only *Microcostatus krasskei* and *Stauroneis thermicola* were widespread.

***Chamaepinnularia soehrensis* (Figs 92-103, 195)**

Our specimens correspond well to the lectotype of *Navicula soehrensis* Krasske established by Lange-Bertalot et al. (1996), as well as to the type for *Navicula soehrensis* var. *septentrionalis* Hustedt (1924) illustrated by Simonsen (1987a, plate 123, figs 11-12). They were 10-13 µm long by 2.1-2.3 µm wide with 18-20 striae in 10 µm. During our studies we considered these varieties separate, but concluded that it is only the Krasskematerial from Chile attributed to *Navicula soehrensis* (Lange-Bertalot *et al*. 1996, plate 22, fig. 18) that likely represents a different species. The Chilean specimens have much broader, more distinctly capitate apices. Our specimens were all distinctly triundulate.

***Chamaepinnularia soehrensis* var. *capitata* (Figs 104-107)**

This taxon was less represented in the Bohemian Switzerland National Park than other *Chamaepinnularia* taxa. Our specimens correspond to the holotype (compare Figs. 104-105 with Lange-Bertalot *et al*. 1996, plate 22, figs 20-22) and to Krasske's material from *Sphagnum squarrosum* (compare Figs. 102-103 with Lange-Bertalot *et al*. 1996, plate 22, figs 23-26). They were 10.0-11.3 µm long by 2.3-2.7 µm wide with 18-20 striae in 10 µm. The latter material (Figs. 102-103) has flat parallel sides and is more distinct from var. soehrensis. In our material, *Chamaepinnularia soehrensis* var. *capitata* seems to be a set of forms intermediate between *C. soehrensis* and *C. tongatensis* (Hustedt) Lange-Bertalot. We question whether or not this taxon is truly genetically distinct from the nominate variety, but studies of cultured material of both are likely required before this question can be resolved.

***Chamaepinnularia tongatensis* (Figs 108-116)**

Our specimens correspond well to the type material of this species as illustrated in Simonsen (1987b). They are not triundulate, and are capitate in all but the smallest specimens, which are rostrate at the ends. Our specimens were 6.3-9.3 µm long by 2.3-3.0 µm wide, with 18-21 striae in 10 µm. There is a discrepancy between Hustedt's description of this taxon (Hustedt 1962, p. 227) and the holotype material shown in Simonsen (1987b, plate 728, figs. 21-32). Hustedt indicates the dimensions for this taxon as 6-8 µm long by 2.5 µm wide, with 26-28 striae in 10 µm. However, when we measured photographs of the specimens from the type material (Simonsen 1987b), they were 8.7-10.3 µm long by 2.3-3.3 µm wide, with 18-20 striae in 10 µm. The shape of our material and the type material are identical, with the exception of a few teratological forms asymmetric with regards to the apical axis. These forms could easily be confused with *Chamaepinnularia soehrensis* var. *capitata* if a large

population was not observed. The possibility certainly exists that minimal genetic distance exists between the two taxa, and that we have simply documented the morphology over the whole size range of one taxon.

***Chamaepinnularia mediocris* (Figs 117-119, 196)**

Our specimens were typical for this taxon. Dimensions were 10-12 μm long by 2.7 μm wide with 18-21 striae in 10 μm. Krasske reported finer striae 22-24 in 10 μm, but measurements of specimens from his type material (Lange-Bertalot *et al*. 1996) gave 18-20 in 10 μm.

***Chamaepinnularia rexii* nov. sp. (Figs 120-135, 192-194)**

Diagnosis:

Chamaepinnularia wiktoriae affinis, a qua differt apicibus acuminatioribus, area centrali grandiore et striis tenuioribus. *Chamaepinnularia evanida* aemulans, a qua differt amplitudine parviore et striis subparallelis. *Chamaepinnularia vyvermanii* aemulans, a qua differt amplitudine parviore, striis tenuioribus et depressionibus rotundatis conspicuis secus aream axialem destitutis.

Akin to *Chamaepinnularia wiktoriae*, from which it differs in its more acuminate ends, larger central area, and finer striation. Similar to *Chamaepinnularia evanida*, from which it differs by its smaller size and nearly parallel striae. Similar to *Chamaepinnularia vyvermanii*, from which it differs by smaller size, finer striae, and absence of conspicuous rounded areole-like depressions along the axial area.

Descriptio:

Valvae lanceolatae, apicibus acutirotundatis vel raro protractis, 5.7-9.7 μm longae, 2.5-3.0 μm latae. Area axialis angusta, linearis. Area centralis distincta, striis abbreviatis uno vel duobus in quoque lato formatibus. Raphe filiformis poris centralibus rotundatis leniter deflexis et fissuris ternimalibus uncatis. Striae indistincte radiatae, leviter distantiores in centro, membrana externo, depressionibus linearibus externis aream axialem contiguis, in partibus duobus area hyalina longitudinali prope limbum divisiae, 21-23-(24) in 10 μm.

Valves lanceolate with sharply rounded, rarely protracted ends, 5.7-9.7 μm, width 2.5-3.0 μm wide. Axial area narrow, linear. Central area distinct, formed by one or two shortened striae on each side. Raphe filiform with weakly deflected, rounded central pores and hooked terminal fissures. Striae slightly radiate, subtly more distantly placed in the

center, with an external hymen, with linear external depressions adjacent to the axial area, divided into two parts by a long hyaline area near the mantle, 21-23-(24) in 10 μm.

Type Locality: Písečná rokle (Sandy Gorge, Site 4, downstream locality, 50°52'05"N, 14°16'31"E), tributary of Kamenice River, Bohemian Switzerland National Park, Czech Republic. Holotype here designated: circled specimen, slide number B 40 0040624, pellet material number B 40 0040625, Berlin Collection (Botanic Garden and Botanical Museum Berlin-Dahlem).

Etymology: Named in honor Rex L. Lowe

In the light microscope, this species most closely resembles *Chamaepinnularia evanida* (Hustedt) Lange-Bertalot in shape, size, and striae density. However, *Chamaepinnularia evanida* is distinguished by the interruption of the valve striae along the mantle edge by a long hyaline area, as well as the distinctly radiate striae (Simonsen 1987, plate 396, figs. 12-18, Werum & Lange-Bertalot 2004, plate 83, fig. 6, Van de Vijver *et al*. 2002, plate 85, figs. 8-10). The interruption in *Chamaepinnularia rexii* is closer to the mantle edge, and generally visible only in TEM or in the SEM internal valve view (Fig. 194). A single specimen attributed to *Chamaepinnularia evanida* has been published with external hymen intact and linear depressions along the axial area (Werum & Lange-Bertalot 2004, plate 81, fig. 7). We think that this specimen likely belongs to *Chamaepinnularia rexii*, as the striae appear to be only slightly radiate. The valves illustrated in SEM by other authors all have missing (eroded?) hymens, and so direct comparison of depressions is difficult.

Chamaepinnularia rexii appears identical to *Chamaepinnularia* (?nov.) spec. Nr. 2 from the Finnish dystrophic lake Julma Ölkky (Lange-Bertalot & Metzeltin 1996, plate 28, fig. 37-39c). These specimens were recognized by the authors as distinctive, but not named in that work due to the paucity of specimens seen. *Chamaepinnularia rexii* was additionally compared to *Chamaepinnularia* species illustrated in Witkowski (1994), Metzeltin & Lange-Bertalot (1998), Moser *et al*. (1998), Rumrich *et al*. (2000), Witkowski *et al*. (2000), Lange-Bertalot *et al*. (2003), Reichardt (2004), and Siver *et al*. (2005).

A large population of *Chamaepinnularia rexii* was found in a headwater stream of low pH (3.30-3.95) and low conductivity (148 μS). The stream is subject to frequent drying, and so we suspect this diatom is a desiccation-tolerant aerophile, as is typical for many species in this genus. It co-occurred with *Chamaepinnularia septentrionalis*, *C. tongatensis*, *Microcostatus krasskei*, *Caloneis aerophila*, *Diadesmis paracontenta* and *Eunotia exigua*. It occurred very rarely in the other drainages examined in this study.

***Diadesmis* (Figs 136-139, 142-153, 199-206)**

This aerophilic genus is cosmopolitan in wet, acidic to neutral subaerial habitats. Species in the genus occur both epilithically and epiphytically on bryophytes. Surface ultrastructure of the valves is critical for correct species determination, and we provide SEM's of most of our taxa to verify their identification (Figs. 199-206).

***Neidium* (Figs 154-166)**

The *Neidium* species encountered in this study were problematic. While we had some taxa easily identifiable, there were a number of taxa we designated with cf. because they were not quite a match to the taxa to which they were identified as being similar. There is also some problem because even in the literature, *Neidium* are not always identified with certainty. For example, our *Neidium* cf. *ampliatum* was very similar species to specimens found in Julma Ölkky designated as *Neidium* spec. Nr. 4 cf. *ampliatum* (Lange-Bertalot & Metzeltin 1996, Plate 41, figs. 3-6). Our specimens had very similar size ranges and overlapping striae densities, although the densities in our specimens were lower (22-25 in 10 μm compared to 23-30 in 10 μm). *Neidium ampliatum* has been found to contain a number of reproductively isolated populations (Mann & Chepurnov 2005). Consequently, it represents several semicryptic species (a species complex in which minute variation has indeed taxonomic and phylogenetic significance). We also had a very diminutive form of *Neidium hercynicum* that we designated *N*. cf. *hercynicum* (Figs. 163, 165). We highly doubt this is the same species as *Neidium hercynicum* recovered from the Elbsandsteingebirge (Fig. 154).

***Cavinula* (Figs 167, 183)**

Four species in this genus were observed, all occurring only rarely. *Cavinula variostriata* and *C. lapidosa* are shown (Figs. 167, 183).

***Frustulia weinholdii* (Fig. 168)**

Four *Frustulia* species were observed. *Frustulia crassinervia* and *F. saxonica* are common species in acidic water and subaerial habitats. We illustrate only the rarest taxon, *Frustulia weinholdii* (Fig. 168).

***Caloneis* and *Pinnularia* (Figs 169-182, 188-191)**

Caloneis taxa typical of springs and subaerial sandstone wet substrates were observed, including *C. aerophila*, *C. vasileyevae* and *C. fontinalis*. With our populations of *Pinnularia*

subinterrupta we enlarge the range of length and width reported for this taxon by Krammer (2000), from 20-24 x 4.3-4.6 μm to 16.0-26.5 x 4.0-4.6 μm.

***Brachysira brebissonii* (Fig. 184)**

This taxon was common in one site, Písečná rokle (Table 1), but rare or absent in other sites.

***Muelleria* cf. *gibbula* (Fig. 185)**

This species was extremely rare, occurring in only one sample. We were not able to resolve the valve structure well in our specimens, but what we could see appeared to be consistent with *Muelleria gibbula*. Most *Muelleria* species are restricted to high latitudes of either the southern or northern hemisphere, except *M. terrestris* (Petersen) Spauding et Stoermer and the cosmopolitan *M. gibbula* (Cleve) Spauding et Stoermer (Spaulding *et al*. 1999).

***Diploneis fontanella* (Fig. 186)**

Diploneis fontanella was rare in our samples. This species was only recently described from springs in central Europe (Werum & Lange-Bertalot 2004).

***Placoneis hambergii* (Fig. 187)**

Bruder & Medlin (2007) recently transferred *Navicula hambergii* Hustedt to *Placoneis* based upon phylogenetic analysis of a number of diatom taxa. Three different loci (SSU rRNA, LSU rRNA, rbcL) all indicated that this taxon was at the base of the *Placoneis* clade. We were struck by the similarity of this taxon to *Geissleria*. In particular, the central area of this taxon bears a strong resemblance to that of *Geissleria decussis*. We were not able to resolve any apical irregularities in the striae such as those visible in other *Geissleria* species, but suspect that *Geissleria* and *Placoneis* may be sister taxa (for external SEM see Werum & Lange-Bertalot 2004: plate 87, figs 24-25). Bruder & Medlin (2007) did not have access to sequences of any *Geissleria* species, but this potential relationship is worthy of further analysis.

DISCUSSION

The diatom diversity of the 15 streams in the Elbsandsteingebirge area was relatively high (307 taxa). It is difficult to find comparable studies to make this evaluation, as species

richness is related to number of sites, number of samples, and level of taxonomic effort. However, Cantonati (1998) found only 254 diatom taxa in 30 streams in an area in the Southern Alps of very similar size and scale to the Elbsandsteingebirge, and with similar geological complexity (carbonates and siliceous). Cantonati's (1998) study is additionally similar to our study in that he sampled multiple substrates and the sites were headwater streams that sometimes dried down during the year. The scale of these two studies and the level of effort is likely similar, but the fact that we had half the number of streams and yet 20% more taxa indicates to us that the higher richness in Elbsandsteingebirge likely has some significance. In a study of much greater scope and effort (400 springs in south and central Hessen, Germany) only 416 taxa were found, despite the fact that this region is much more geologically complex and much larger in area (Werum & Lange-Bertalot 2004).

Diatom diversity in the Elbsandsteingebirge area is possibly high due to heterogeneity of microhabitats (e.g. geology, trophic level, pH, amount of humic acids, desiccation, and anthropogenic impact). One of the key variables in this study was the sampling of four different substrate types at all sites where they were present. Preferences of diatom taxa to specific substrata have been observed in studies of Stevenson & Hashim (1989); Maier (1994); Pringle (1990); Sabater *et al*. (1998); Cantonati (2001), Potapova & Charles (2005). The specific substrate preferences of diatoms found in a single stream, Suchá Bělá (our locality 3) in the Elbsandsteingebirge are discussed elsewhere. Veselá (2009) found that algal substrate preferences fell into two main categories, hard substrates (wood, stone) and soft substrates (bryophytes, sediment). The hard substrates were poor in species richness and species fidelity, with only 20% of common taxa in Suchá Bělá specific for this substrate type. Soft substrates had higher richness, and a higher percentage (46%) of common algae was specific to those surfaces. In particular, Eunotia spp. and Pinnularia spp. were primarily found on soft substrates. In the streams studied, stone surfaces were typically covered by abundant green algae, while wood surfaces had high numbers of fungi. We suspect these other microscopic organisms prevented good epilithic and epidendric diatom communities from forming, a finding reported by others (Ledger & Hildrew 1998, Sabater *et al*. 1998, Soininen 2003, Potapova & Charles 2005, Greenwood & Lowe 2006). The patterns seen in Suchá Bělá were present in this study of fifteen streams as well (Fig. 208). One outcome of this study is consequently that bryophytes in particular should be studied in headwater streams if diversity is an issue, as they harbor many interesting and rare taxa.

The geographic distance among streams had a more minor effect on similarity of diatom assemblages than pH value, which was tied directly to the eutrophication levels of the

watershed. This result is not surprising given similar results in other studies where researchers found pH and enrichment to be determiners of diatom distribution (Mölder 1964; Cantonati 1998; Werum & Lange-Bertalot 2004; Soininen 2007). Casamatta et al. (2002) reported that the algal assemblages from sandstone wet walls also showed little geographical grouping of sites, and moisture levels appeared to have the greatest congruence with clustering of sites based on species composition. Significant effects on species composition attributable primarily to geographic distance have only been observed on larger spatial scales (Potapova & Charles 2002; Foerster *et al*. 2004; Charles *et al*. 2006).

Species concepts in diatoms based almost exclusively on the morphology of the frustules are frequently too broad (Mann 1999). In fact, many of the described species are complexes of different species within which have been found reproductive barriers, differences in life cycle, morphological variability, and genetic divergence (Droop *et al*. 2000; Pappas & Stoermer 2003; Mann *et al*. 2004; Poulíčková & Mann 2006; Vanomerlingen *et al*. 2007). If an evolutionary species concept (Simpson 1953, 1961; Wiley & Mayden 2000) or phylogenetic species concept (Mischler & Theriot 2000) is adopted, then it is possible to recognize even more species than is permitted by the biological species concept Mann (1999) and Mann *et al*. (2004) advocate. The ability for two lineages to reproduce sexually is a symplesiomorphy, and consequently not ideal to use as evidence of conspecificity. Reproductive barriers are apomorphic, and thus good for separation. By more modern species concepts, it is easy to imagine that two morphologically or ecologically similar but genetically divergent lineages could be recognized as separate species even if they retain the ability to reproduce sexually. Morphological dissimilarity within a species complex is sometimes detectable using morphometric techniques, and consequently the species are semicryptic (pseudocryptic) rather than cryptic. In this study we found many diatoms which obviously varied in minute ways from descriptions or illustrations of species in the literature, and these may indeed represent new taxa. Additionally, we had a number of "cf." taxa that, had they been part of larger populations, would likely have been considered worthy of taxonomic recognition.

The observed morphological divergence of our natural populations from published records is due to some combination of phenotypic plasticity and genetic differentiation, but it is difficult in the absence of experimental evidence to know the relative importance of these two sources of variation. Potapova & Hamilton (2007) studied natural populations within the *Achnanthidium minutissimum* species complex in North American rivers using morphometric and ecological data. Their morphometric analysis did not reveal discontinuities among all

morphological groups corresponding to the historical taxa. However, they did find six morphotypes which were correlated with water chemistry, and the implication is that at least some of the morphological variation observed is due to genetic differentiation. Evidence for plasticity was not given, although different stages of the life cycle may be responsible for description of too many species historically.

As we struggled with the identification of taxa in the Elbsandsteingebirge, we confirmed our opinion that diatom taxonomy and ecology is problematic. Valve outline, an important species criterion, changes during the life cycle as valves get progressively smaller. Cryptic species may have only minor (or no) morphological differences that are easily masked by life-cycle changes. Finally, there may be ecophenotypic variation, although beyond the formation of teratological valves this has not been extensively documented. There are instances of polymorphism in diatom cells which demonstrate species-level changes in morphology tied to environmental stimuli (Stoermer 1967). The combination of apparent tight genetic control of morphology within species combined with the morphological changes that occur during the life cycle and the possible impacts of environmental stressors on morphology make any morphology-based taxonomy difficult. Still, diatom morphotypes, whatever we call them, are regularly correlated with water chemistry and consequently are ecologically informative. Careful taxonomic work will be of high value at least for the foreseeable future.

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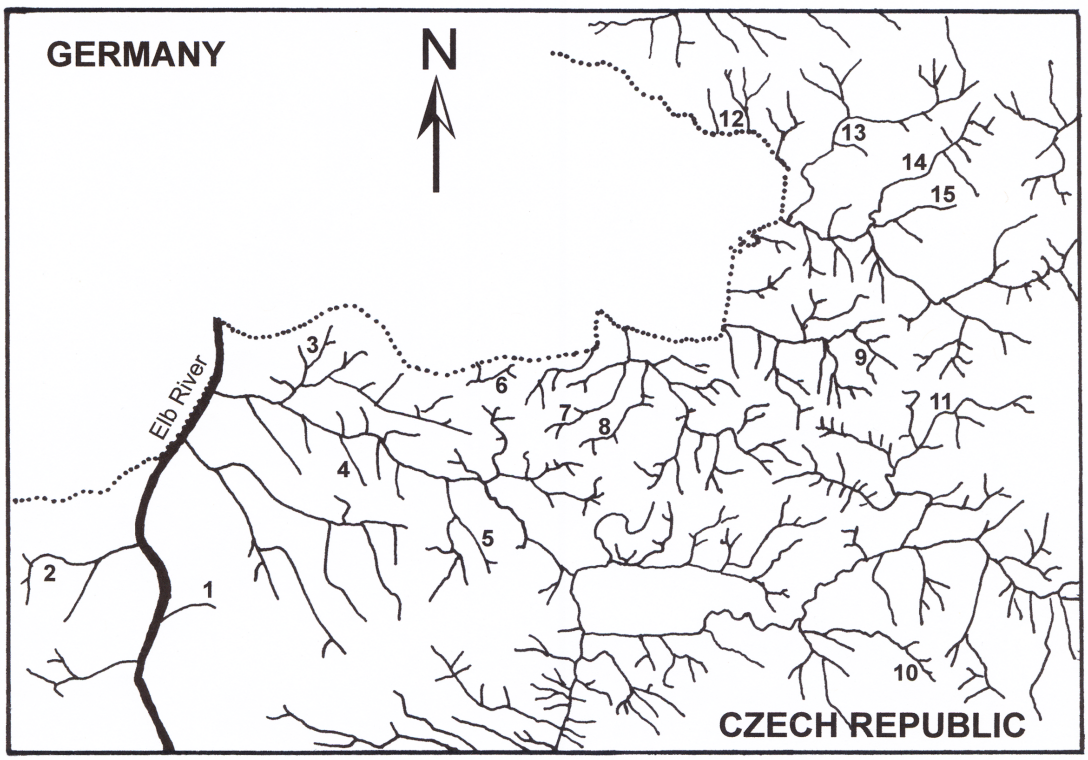
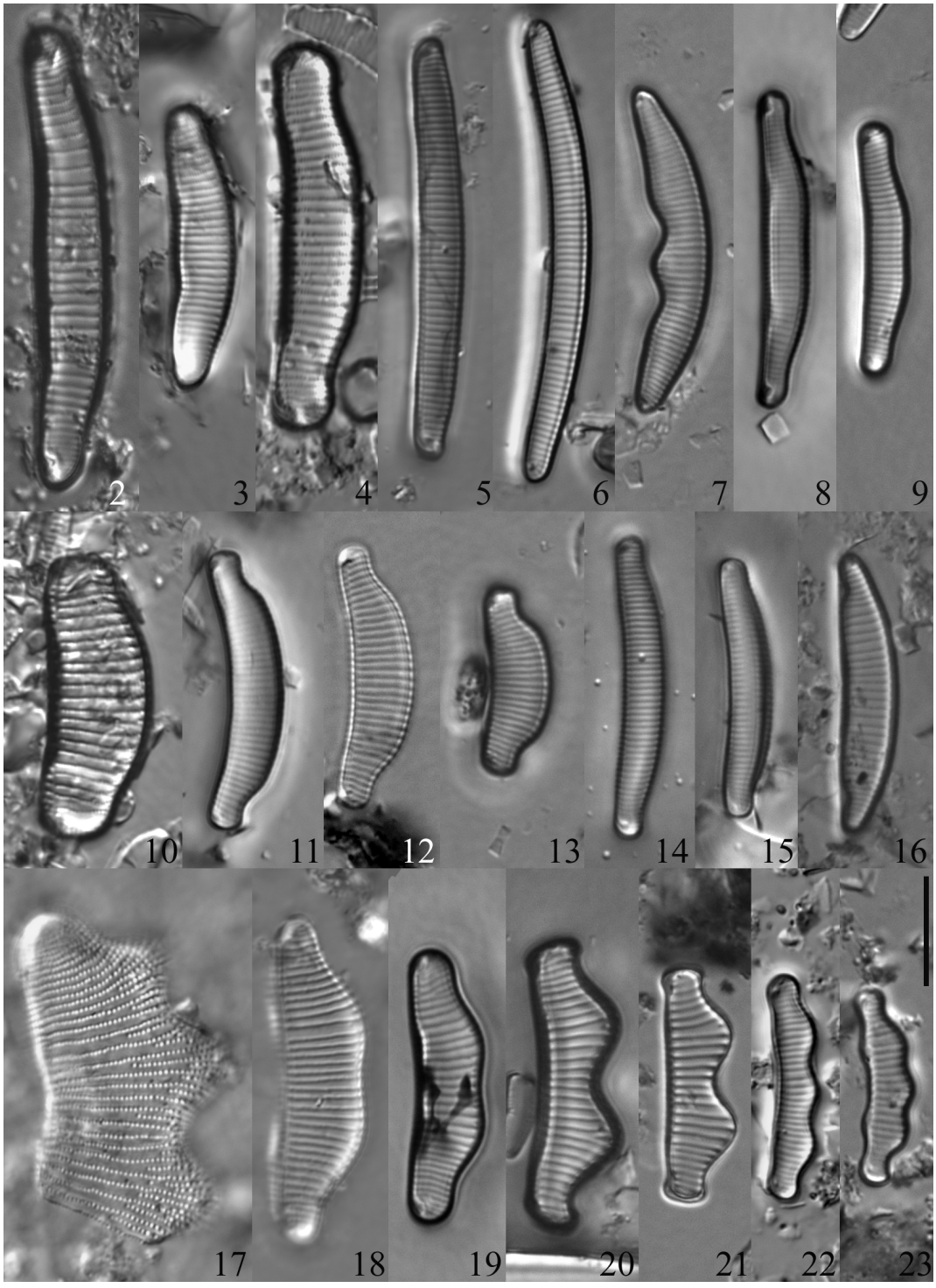


Fig. 1. Map of the study area. Geographic locations of the 15 headwater streams sampled in the Elbsandsteingebirge Region. **1**-Studený potok, **2**-Dolnožlebský p., **3**-Suchá Bělá, **4**-Písečná rokle, **5**-Kachní potok, **6**-Ponova louka, **7**- Hluboký důl, **8**-Mlýnská rokle, **9**-Červený potok, **10**-Studenec, **11**-Doubický potok, **12**-Bílý p., **13**-Brtnický p., **14**-Vlčí p., **15**-Panský p. Scale bar = 1.5 km.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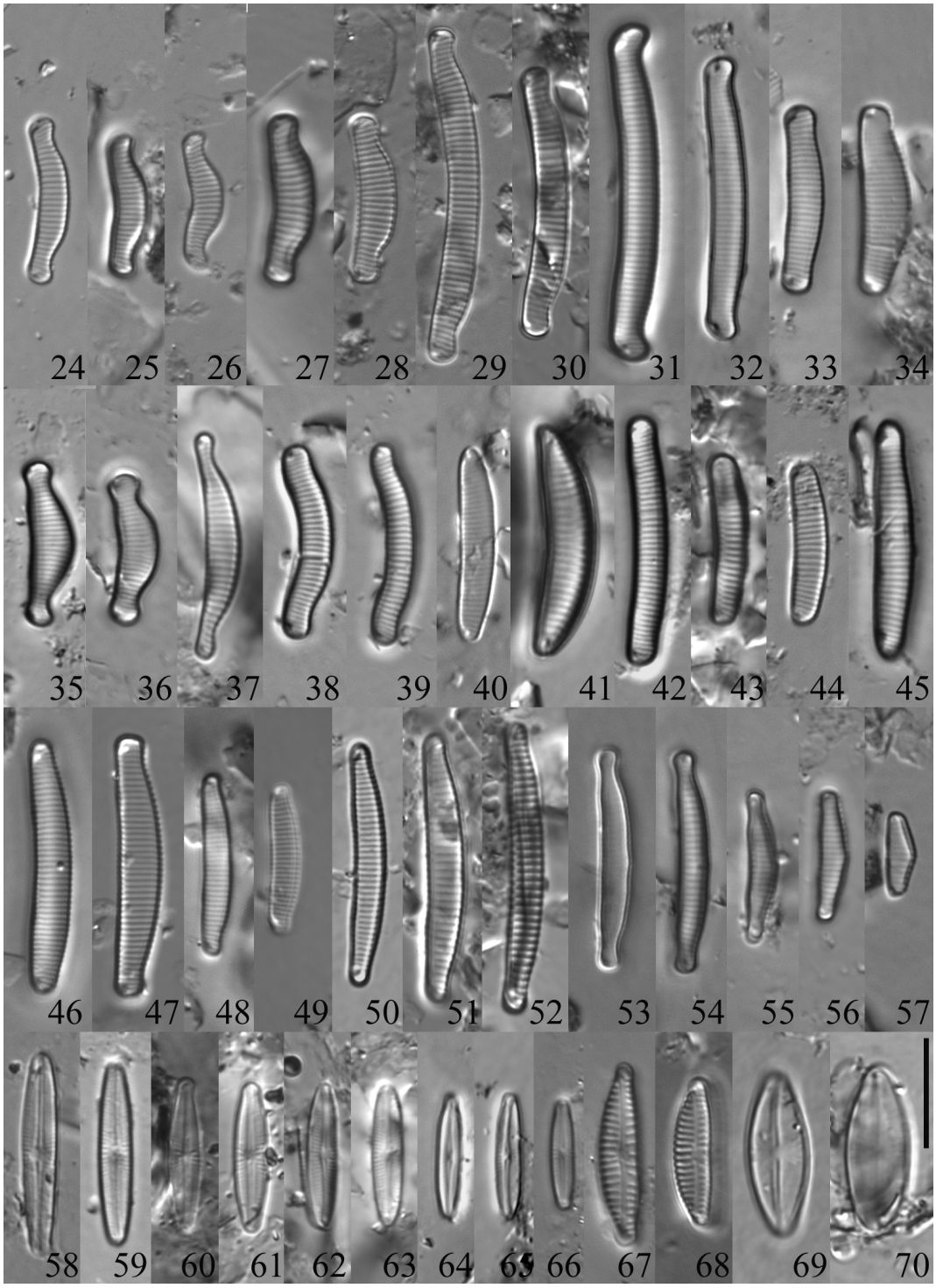
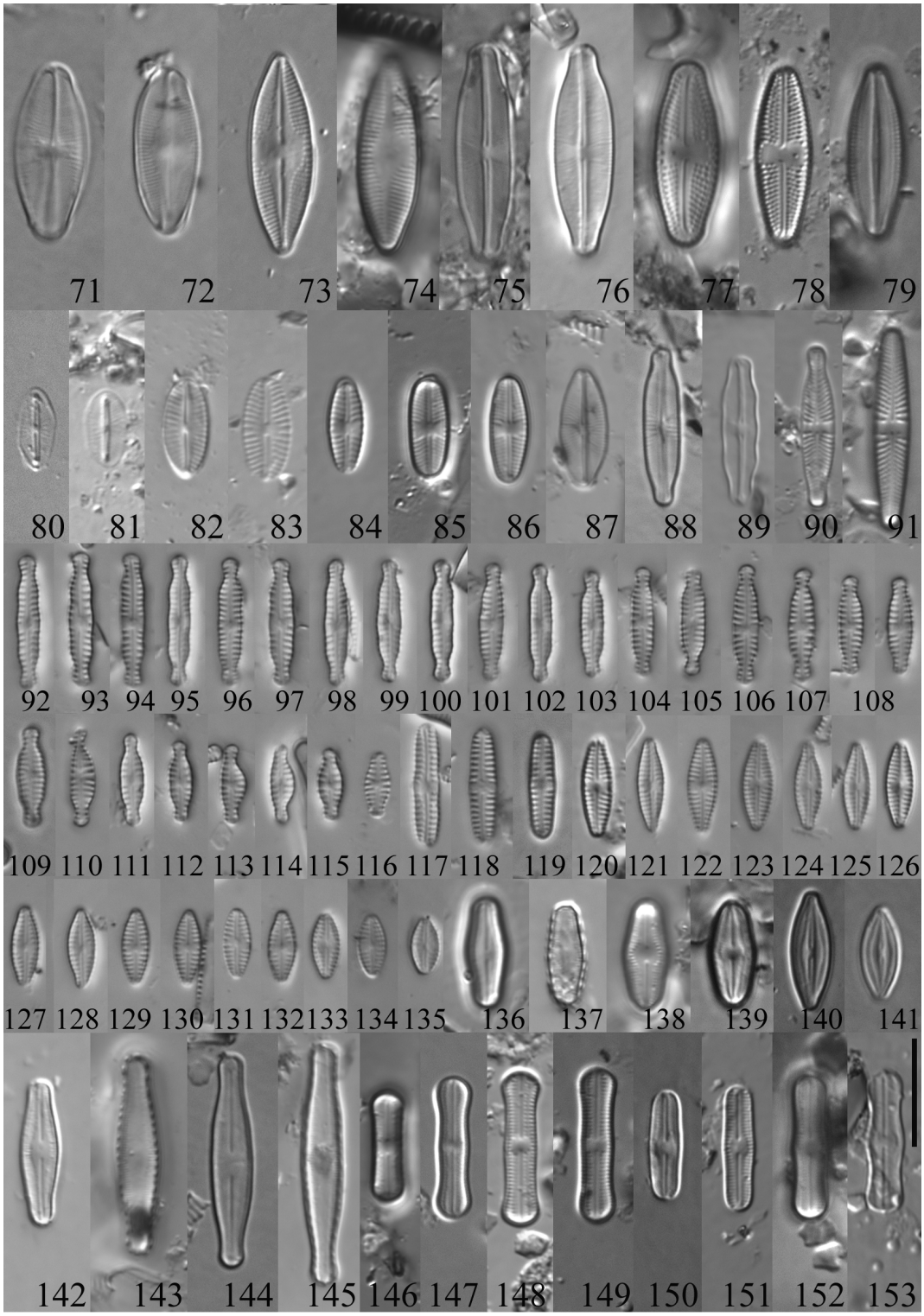
Figs 2-23. *Eunotia* species from the Elbsandsteingebirge Region. **Figs 2, 3**. *Eunotia* cf. *minor*. **Fig. 4**. *Eunotia* cf. *praerupta*. **Fig. 5**. *Eunotia valida*. **Figs 6, 7**. *Eunotia bilunaris*. **Figs 8, 9**. *Eunotia implicata*. **Fig. 10**. *Eunotia curtagrunowii*. **Figs 11-13**. *Eunotia ursamaioris*. **Figs 14, 15**. *Eunotia fennica*. **Fig. 16**. *Eunotia incisa*. **Fig. 17**. *Eunotia tetraodon*. **Fig. 18**. *Eunotia islandica*. **Fig. 19**. *Eunotia circumborealis*. **Figs 20, 21**. *Eunotia bigibba*. **Figs 22, 23**. *Eunotia muscicola* var. *tridentula*. Scale bar = 10 μm.


24 25 26 27 28 29 30 31 32 33 34
35 36 37 38 39 40 41 42 43 44 45
46 47 48 49 50 51 52 53 54 55 56 57
58 59 60 61 62 63 64 65 66 67 68 69 70

Figs 24-70. *Eunotia* species and other interesting species from the Elbsandsteingebirge Region. **Figs 24-26**. *Eunotia exigua*. **Figs 27-30**. *Eunotia tenella*. **Figs 31-33**. *Eunotia nymanniana*. **Fig. 34**. *Eunotia levistriata*. **Figs 35, 36**. *Eunotia meisterii*. **Fig. 37**. *Eunotia arculus*. **Figs 38, 39**. *Eunotia steineckei*. **Fig. 40**. *Eunotia subarcuatoides*. **Fig. 41**. *Eunotia* sp. **Figs 42-44**. *Eunotia botuliformis*. **Fig. 45**. *Eunotia rhomboidea*. **Fig. 46**. *Eunotia* sp. **Fig. 47**. *Eunotia* sp. **Figs 48, 49**. *Eunotia paludosa*. **Fig. 50**. *Eunotia groenlandica*. **Figs. 51, 52**. *Eunotia fallax*. **Figs. 53-54**. *Eunotia microcephala*. **Figs. 55-57**. *Eunotia trinacria*. **Figs 58-61**. *Encyonopsis* sp. 1. **Figs 62-66**. *Encyonopsis* sp. 2. **Figs 67, 68**. *Encyonema perpusillum*. **Figs 69, 70**. *Psammothidium altaicum*. Scale bar = 10 μm.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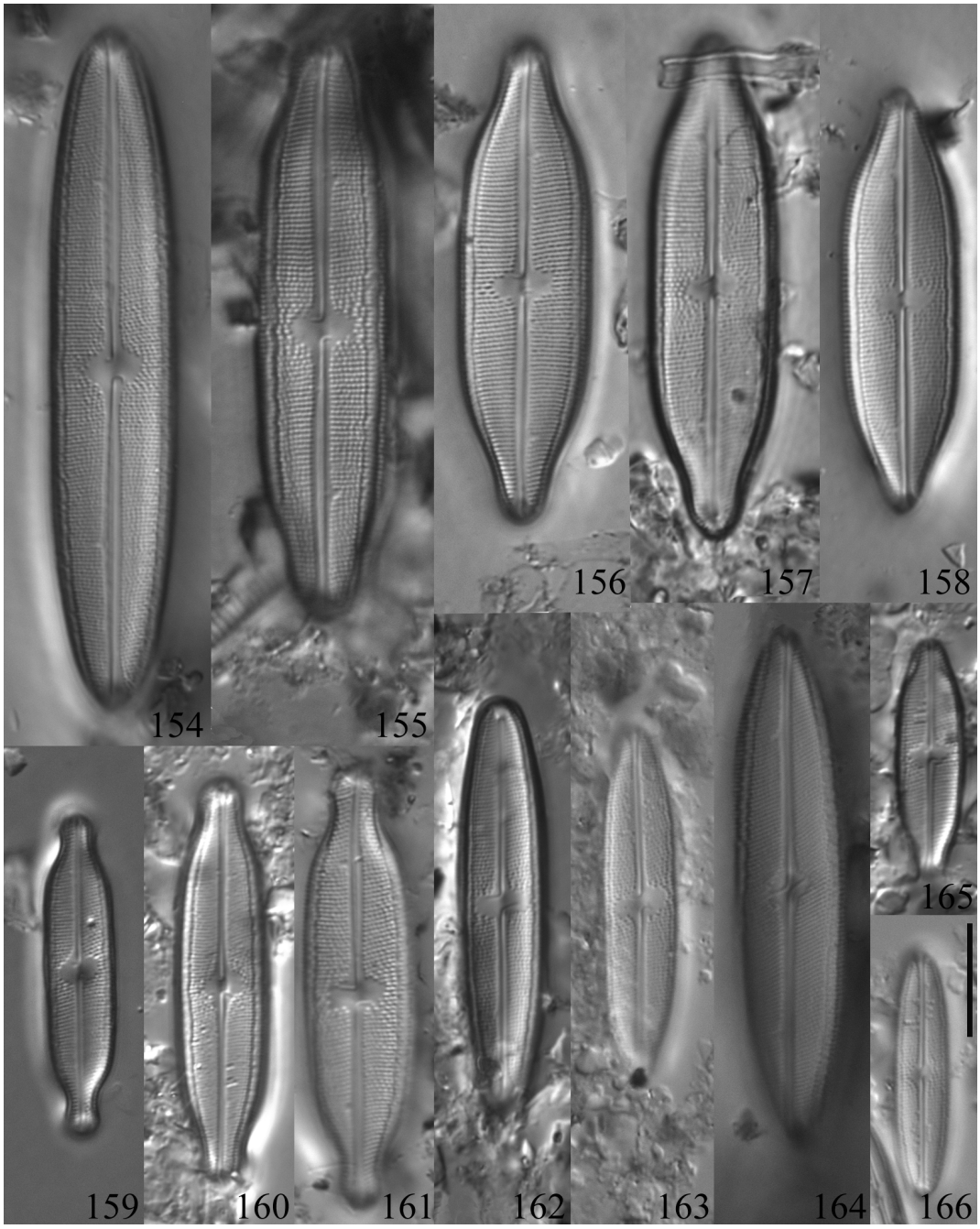
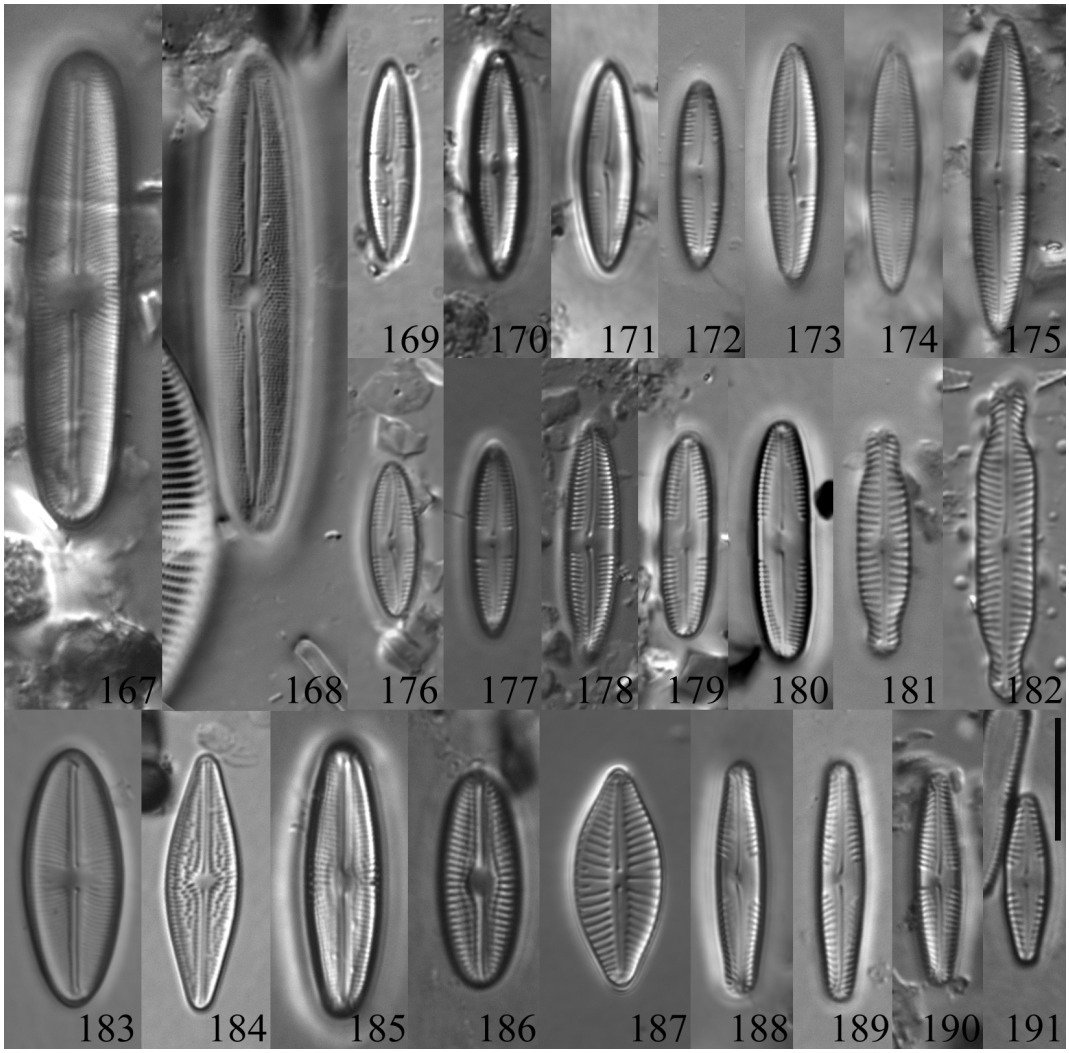
80 81 82 83 84 85 86 87 88 89 90 91
92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108
109 110 111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126
127 128 129 130 131 132 133 134 135 136 137 138 139 140 141
142 143 144 145 146 147 148 149 150 151 152 153

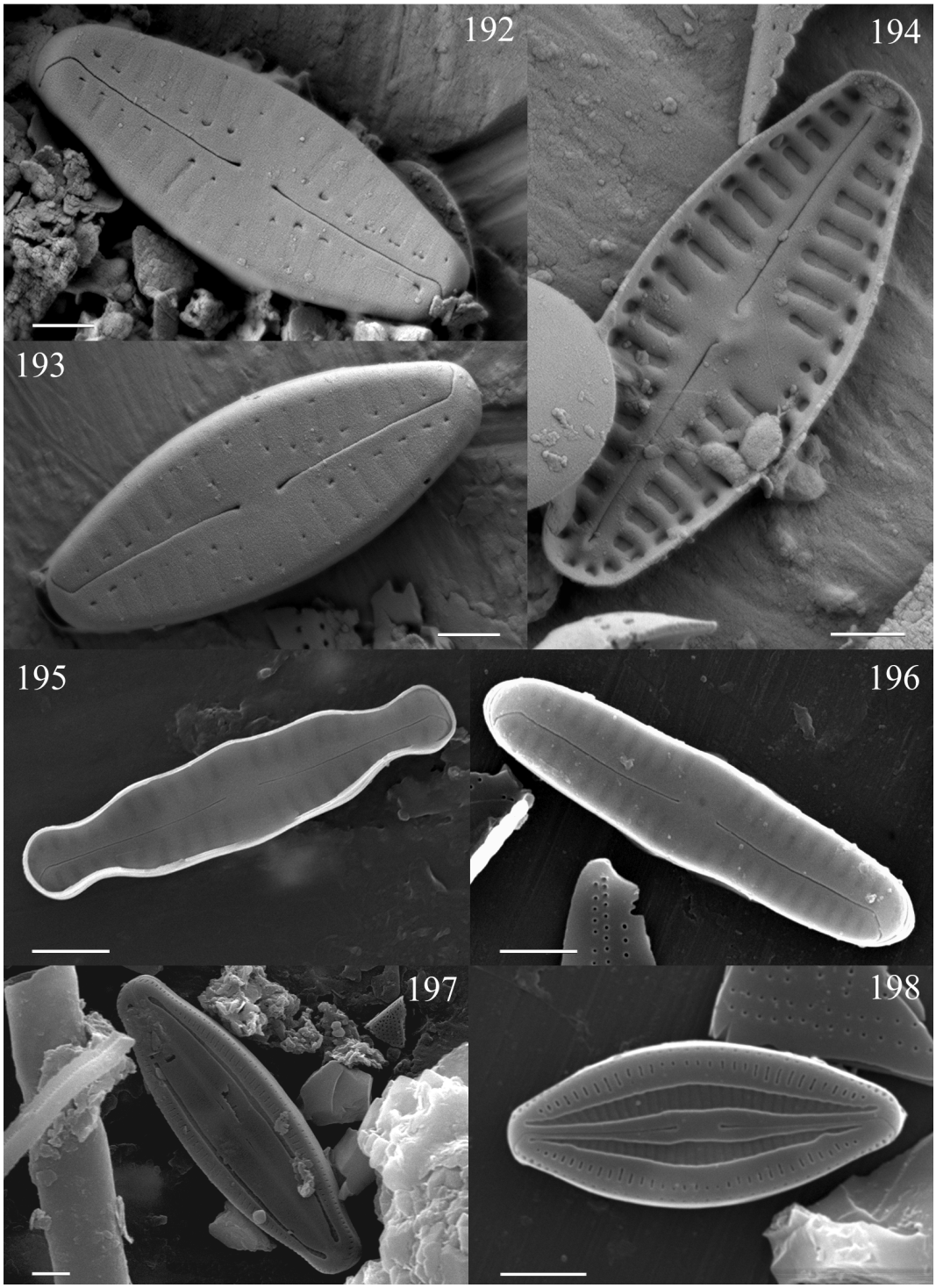
Figs 71-153. Finely striated diminutive taxa from the Elbsandsteingebirge Region. **Figs 71, 72**. *Eucocconeis laevis*. **Figs 73, 74**. *Nupela lapidosa*. **Figs 75, 76**. *Sellaphora stauroneioides*. **Fig. 77**. *Luticola acidoclinata*. **Fig. 78**. *Luticola mutica*. **Fig. 79**. *Fallacia vitrea*. **Figs 80, 81**. *Mayamaea atomus* var. *permitis*. **Figs 82, 83**. *Mayamaea recondita*. **Fig. 84**. *Sellaphora seminulum*. **Figs 85, 86**. *Eolimna minima*. **Fig. 87**. *Adlafia minuscula*. **Fig. 88**. *Adlafia bryophila*. **Fig. 89**. *Navicula tridentula*. **Fig. 90**. *Stauroneis thermicola*. **Fig. 91**. *Stauroneis thermicola* f. *lanceolata*. **Figs 92-103**. *Chamaepinnularia soehrensis*. Size diminution series. **Figs 104-107**. *Chamaepinnularia soehrensis* var. *capitata*. **Figs 108-116**. *Chamaepinnularia tongatensis*, size diminution series. **Figs 117-119**. *Chamaepinnularia mediocris*. **Figs 120-135**. *Chamaepinnularia rexii*, size diminution series. **Figs 136, 137**. *Diadesmis gallica*. **Figs 138, 139**. *Diadesmis perpusilla*. **Figs 140, 141**. *Microcostatus krasskei*. **Figs 142-145**.*Diadesmis laevissima*. **Fig. 146**. *Diadesmis contenta*. **Figs 147-149**. *Diadesmis paracontenta*. **Figs 150, 151**. *Diadesmis brekkaensis*. **Figs 152, 153**. *Diadesmis biceps*. Scale bar = 10 μm.



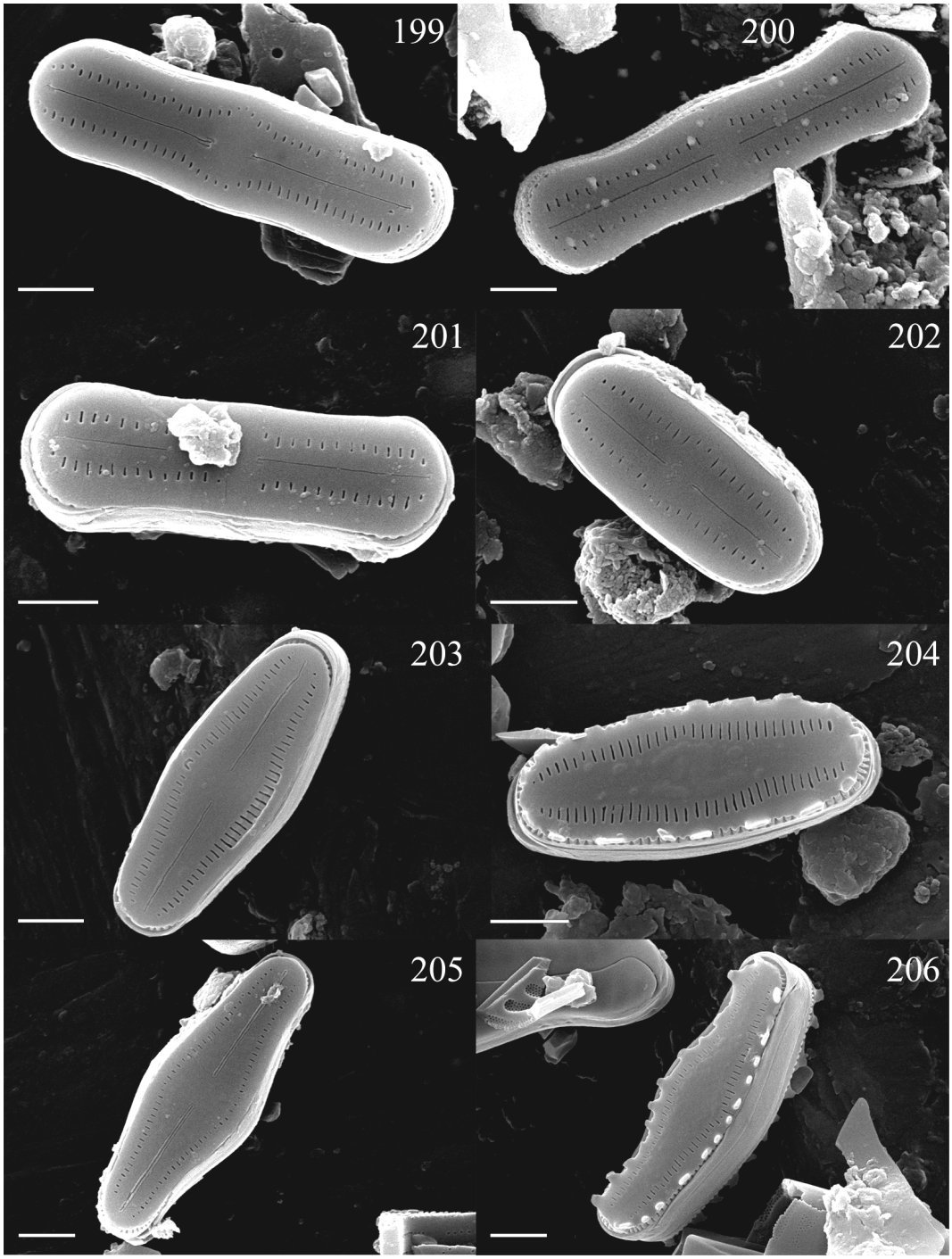
Figs 154-166. *Neidium* species from the Elbsandsteingebirge Region. **Fig. 154**. *Neidium hercynicum*. **Figs 155-158**. *Neidium* cf. *ampliatum*. **Fig. 159**. *Neidium longiceps*. **Fig. 160**. *Neidium* sp. **Fig. 161**. *Neidium affine* var. *amphirhynchus*. **Fig. 162**. *Neidium bisulcatum*. Fig. 163. *Neidium* cf. *hercynicum*. **Fig. 164**. *Neidium carterii*. **Fig. 165**. *Neidium* cf. *hercynicum*. **Fig. 166**. *Neidium alpinum*. Scale bar = 10 μm.



Figs 167-191. Naviculoid taxa from the Elbsandsteingebirge Region. **Fig. 167**. *Cavinula variostriata*. **Fig. 168**. *Frustulia weinholdii*. **Figs 169-171**. *Caloneis aerophila*. **Figs 172-178**. *Caloneis vasileyevae*. **Figs 179, 180**. *Caloneis fontinalis*. **Figs 181, 182**. *Pinnularia subinterrupta*. **Fig. 183**. *Cavinula lapidosa*. **Fig. 184**. *Brachysira brebissonii*. **Fig. 185**. *Muelleria* cf. *gibbula*. Fig. 186. *Diploneis fontanella*. **Fig. 187**. *Placoneis hambergii*. **Figs 188-191**. *Pinnularia perrirorata*. Scale bar = 10 μm.



Figs 192-198. SEM views of finely striated taxa. **Figs 192-194**. *Chamaepinnularia rexii*. **Fig. 195**. *Chamaepinnularia soehrensis*. **Fig. 196**. *Chamaepinnularia mediocris*. **Fig. 197**. *Fallacia vitrea*. **Fig. 198**. *Microcostatus krasskei*. Figs 192-194: scale bars = 1 μm. Figs 195-198: scale bars = 2 μm.



Figs 199-206. SEM views of *Diadesmis* species. **Fig. 199**. *Diadesmis biceps*. **Figs 200, 201**. *Diadesmis paracontenta*. **Fig. 202**. *Diadesmis contenta* var. *paralella*. **Fig. 203.** *Diadesmis* cf. *perpusilla*. **Fig. 204**. *Diadesmis gallica*. **Figs 205, 206**. *Diadesmis laevissima*. Scale bars = 2 μm.

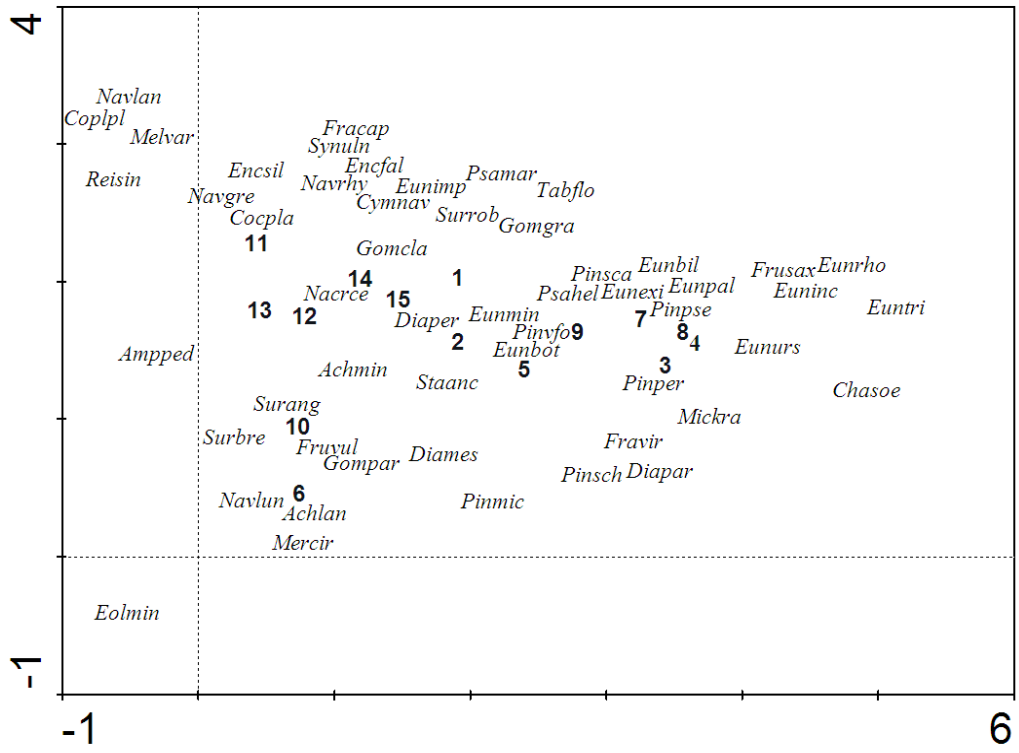


Fig. 207. DCA ordination biplot of 53 most important diatom taxa and streams as supplementary variables in the ordination space of the first (13.3%) and the second axes (5.0%). Ampped = *Amphora pediculus*. Achlan = *Planothidium lanceolatum* sensu lato. Achmin = *Achnanthidium minutissimum* sensu lato. Chasoe = *Chamaepinnularia soehrensis*. Cocpla = *Cocconeis placentula*. Cocplpl = *C. placentula* var. *placentula*. Cymnav = *Cymbopleura naviculiformis*. Diames = *Diatoma mesodon*. Diapar = *Diadesmis paracontenta*. Diaper = D. *perpusilla*. Encfal = *Encyonopsis falaisensis*. Encsil = *Encyonema silesiacum* + E. *minutum*. Eolmin = *Eolimna minima*. Eunbil = *Eunotia bilunaris*. Eunbot = *E. botuliformis*. Eunexi = *E. exigua* + *E. tenella*. Euninc = *E. incisa*. Eunimp = *E. implicata*. Eunmin = *E. minor*. Eunpal = E. *paludosa*. Euntri = *E. trinacria*. Eunrho = *E. rhomboidea*. Eunurs = *E. ursamaioris*. Fracap = *Fragilaria capucina*. Fravir = *Fragilariforma virescens*. Frusax = *Frustulia saxonica*. Fruvul = *F. vulgaris*. Gomcla = *Gomphonema clavatum*. Gomgra = *G. gracile*. Gompar = *G. parvulum*. Melvar = *Melosira varians*. Mercir = *Meridion circulare*. Mickra = *Microcostatus krasskei*. Navcrce = *Navicula cryptocephala*. Navgre = *N. gregaria*. Navlan = *N. lanceolata*. Navlun = *N. lundii*. Navrhy = N. *rhynchocephala*. Pinmic = *Pinnularia microstauron*. Pinper = *P. perrirorata* + P. *silvatica*. Pinpse = *P. pseudogibba*. Pinsca = *P. subcapitata* + *P. sinistra*. Pinsch = *P. schoenfelderii*. Pinvfo = *P. viridiformis*. Psahel = *Psammothidium helveticum*. Psamar = *P. marginestriatum*. Reisin = *Reimeria sinuata*. Staanc = *Stauroneis anceps*. Surang = *Surirella angusta*. Surbre = *S. brebissonii*. Surrob = *S. roba*. Synuln = *Synedra ulna*. Tabflo = *Tabellaria flocculosa*.

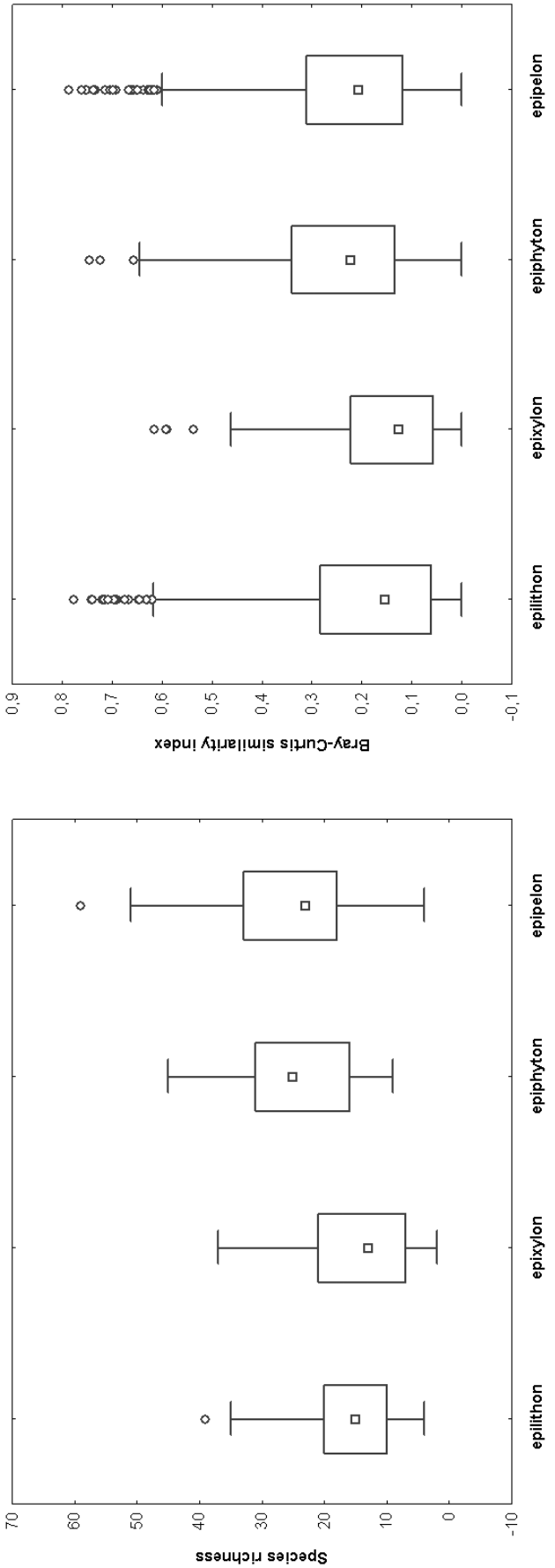


Fig. 208. Box plot diagrams. Variation in species richness and Bray-Curtis similarity index in four microhabitats from 15 headwater streams. Both between-habitat pair-wise comparisons (Kruskal-Wallis test) were statistically significant at $p < 0.001$.

Table 1. List of diatom taxa and their semi-quantitative scale of abundance in streams: r = rare. u = uncommon. c = common. f = frequent. d = dominant. Van Dam's indication values: pH. 1 = acidobiontic. 2 = acidophilic. 3 = neutrophilic. 4 = alkaliphilous. 5 = alkalibiontic. 6 = indifferent. Moisture. dry = moist or temporarily dry places. ae = aerophytic. un = not listed. Calculated AWM optimum ± tolerance values.

	Studený potok	Dolnožlebský p.	Suchá Bělá	Písečná rokle	Kachní potok	Ponova louka	Hluboký důl	Mlýnská rokle	Červený potok	Studenec	Doubický potok	Bílý potok	Brtnický potok	Vlčí potok	Panský potok	pH indication values	moisture indication v.	abundance weighted mean pH ± tolerance values	abundance weighted mean conductivity ± tolerance values
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15				
Adlafia bryophila (Petersen) Moser, Lange-Bertalot et Metzeltin	-	**r**	-	-	**u**	-	-	-	-	-	-	**r**	**r**	**c**	**u**	3	ae	6.9 ± 0.5	146 ± 22
Adlafia minuscula (Grunow) Lange-Bertalot	-	-	-	-	-	**u**	-	-	-	**u**	-	**r**	**u**	-	-	4	dry	7.1 ± 0.6	166 ± 34
Achnanthes lutherii (Hustedt)	-	-	-	-	-	-	-	-	**r**	-	-	-	-	-	-	2	dry	-	-
Achnanthes spp.	**u**	**u**	-	**u**	-	**u**	-	-	-	**u**		**u**	**u**	-	**u**	-	-	-	-
Achnanthidium exiguum (Grunow) Czarnecki	-	-	-	-	-	-	-	-	-	**r**	-	-	-	-	-	4	-	-	-
Achnanthidium minutissimum (Kützing) Czarn. *sensu lato*	**f**	**f**	-	**r**	**u**	**c**	**r**	-	**u**	**f**	**u**	**c**	**f**	**f**	**f**	3	-	6.8 ± 0.7	159 ± 32
Amphipleura pellucida (Kützing) Kützing	-	-	-	-	-	-	-	-	-	-	**r**	-	-	-	-	4	-	-	-
Amphora libyca Ehrenberg	-	-	-	-	-	**r**	-	-	-	**r**	**r**	**r**	**u**	**u**	-	4	-	7.2 ± 0.3	172 ± 20
Amphora normanii Rabenhorst	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**r**	3	-	-	-
Amphora pediculus (Kützing) Grunow	**r**	**r**	-	-	**r**	-	-	-	-	**u**	**u**	**c**	**f**	**u**	**u**	4	-	7.3 ± 0.5	172 ± 33
Asterionella formosa Hassall	-	-	-	-	-	-	-	-	-	-	**u**	-	-	-	-	4	-	7.2 ± 0.1	198 ± 11
Aulacoseira ambigua (Grunow) Simonsen	-	-	-	-	-	-	-	-	-	-	**f**	-	-	-	-	4	-	7.1 ± 0.2	189 ± 14
Aulacoseira italica (Ehrenberg) Simonsen	-	-	-	-	-	-	-	-	-	-	-	**u**	-	-	-	3	-	-	-
Aulacoseira cf. *laevissima* (Grunow) Krammer	-	-	-	-	-	-	-	-	-	-	-	-	-	**u**	-	-	un	7.3 ± 0.2	160 ± 5
Aulacoseira cf. *perglabra* (Østrup) Haworth	-	-	-	-	-	-	-	-	-	**r**	-	-	-	-	-	-	un	-	-
Brachysira brebissonii Ross	**r**	-	**u**	**c**	-	-	**r**	-	-	-	-	-	**r**	-	-	2	-	4.3 ± 1.1	123 ± 42
Brachysira vitrea (Grunow) Ross	-	-	-	-	-	-	-	-	-	-	**u**	**r**	-	**u**	**u**	2	-	7.0 ± 0.1	150 ± 23
Brachysira spp.	-	-	**r**	-	-	**r**	-	-	-	-	-	-	-	-	-	-	-	-	-

Caloneis aerophila Bock	-	-	-	**c**	-	-	-	-	-	-	-	-	-	**r**	**r**	-	ae	4.3 ± 1.5	147 ± 7
Caloneis fontinalis (Grunow) Lange-Bertalot et Reichardt	-	-	-	-	-	**r**	-	-	-	-	-	**r**	**r**	**r**	-	-	un	7.3 ± 0.2	153 ± 22
Caloneis silicula (Ehrenberg) Cleve	-	-	-	-	**r**	**r**	-	-	-	-	**r**	-	-	-	-	4	-	-	-
Caloneis tenuis (Gregory) Krammer	**r**	-	-	-	-	-	-	-	-	-	-	-	-	-	**r**	3	dry	-	-
Caloneis vasileyevae Lange-Bertalot, Genkal et Vekhov	-	-	**u**	-	**r**	-	-	**u**	**r**	-	-	-	-	-	**r**	-	un	5.0 ± 1.3	94 ± 49
Caloneis spp.	-	-	-	-	-	**r**	-	-	-	-	-	-	-	-	**r**	-	-	-	-
Cavinula cocconeiformis (Gregory) Mann et Stickle	-	-	-	-	**u**	-	-	**r**	-	-	-	-	-	**r**	-	3	-	5.8 ± 0.8	113 ± 34
Cavinula lapidosa (Krasske) Lange-Bertalot	-	-	-	-	-	-	-	-	**r**	-	-	**r**	-	**r**	**r**	2	dry	6.9 ± 0.2	143 ± 13
Cavinula variostriata (Krasske) Mann	-	-	-	-	-	-	-	-	-	-	-	**r**	-	-	-	2	dry	-	-
Cavinula sp.	-	-	-	-	**r**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chamaepinnularia mediocris (Krasske) Lange-Bertalot	-	**r**	**r**	**u**	-	-	-	-	**u**	-	-	-	-	-	-	2	un	4.2 ± 1.0	134 ± 34
Chamaepinnularia rexii nov. sp.	-	**r**	**r**	**c**	-	-	-	-	-	-	-	-	-	-	-		un	4.0 ± 0.1	143 ± 22
Cham. soehrensis (Krasske) Lange-Bert. et Krammer	**r**	**r**	**c**	**c**	-	-	**u**	**c**	**u**	-	-	-	-	-	-	2	dry	4.6 ± 1.0	101 ± 47
Chamaepinnularia soehrensis var. *capitata* (Krasske) Lange-Bertalot et Krammer	-	-	**r**	**r**	-	-	-	-	-	-	-	-	-	-	-	-	un	-	-
Chamaepinnularia soehrensis var. *muscicola* (Petersen) Lange-Bertalot et Krammer	-	-	-	-	-	-	-	-	**r**	-	-	-	-	-	-	2	dry	-	-
Chamaepinnularia tongatensis (Hustedt) Lange-Bertalot	-	-	**u**	**u**	-	-	**r**	**r**	**r**	-	-	-	-	**r**	-	-	un	4.5 ± 0.9	121 ± 45
Cocconeis placentula Ehrenberg var. *placentula*	-	-	-	-	-	-	-	-	-	-	**u**	**c**	**f**	-	-	4	-	7.4 ± 0.2	173 ± 18
Cocconeis placentula var. *klinoraphis* Geitler	-	-	-	-	-	-	-	-	-	-	-	-	-	**r**	-	4	-	-	-
Cocconeis placentula var. *lineata* (Ehrenberg) Cleve	**u**	**r**	-	-	-	**r**	**r**	-	-	**u**	-	**c**	**c**	**u**	**u**	4	-	-	-
Cocconeis pseudolineata (Geitler) Lange-Bertalot and *C. euglypta* (Ehrenberg) Cleve	**r**	-	-	-	-	**r**	-	**r**	-	**u**	**u**	**f**	**u**	**u**	**u**	4	-	7.2 ± 0.5	165 ± 34
Craticula molestiformis (Hustedt) Lange-Bertalot	-	-	-	-	-	**u**	-	-	-	-	**r**	-	-	-	-	4	-	-	-
Craticula riparia (Hustedt) Lange-Bertalot var. *riparia*	-	-	-	**r**	-	-	-	-	**u**	-	-	-	-	-	-	2	-	4.8 ± 0.9	72 ± 42
Cymatopleura solea (Brébisson) W. Smith	-	-	-	-	-	-	-	-	-	-	**r**	-	-	-	-	4	-	-	-
Cymbella subaequalis Grunow	-	-	-	-	-	-	-	-	-	-	-	-	-	**u**	**u**	3	-	7.1 ± 0.2	146 ± 15
Cymbopleura naviculiformis (Auerswald) Krammer	**r**	**u**	-	-	**r**	**r**	**r**	-	**u**	**u**	**u**	**u**	**r**	**c**	**c**	3	-	6.9 ± 0.6	146 ± 32
Denticula tenuis Kützing	**u**	-	-	-	-	-	-	-	-	-	**r**	-	-	**r**	-	4	-	6.7 ± 0.5	155 ± 27
Diadesmis biceps Arnott	-	-	**r**	**r**	-	-	-	-	-	-	-	-	-	-	-	-	un	-	-
Diadesmis brekkaensis (Petersen) Mann	-	-	**u**	-	-	**r**	-	**r**	-	-	-	**r**	-	-	-	3	un	5.5 ± 1.5	97 ± 66
Diadesmis contenta (Grunow) Mann	-	-	**r**	-	-	**u**	-	-	-	-	-	-	-	-	-	4	dry	-	-
Diadesmis contenta var. *parallela* (Petersen) Spaulding	-	-	**r**	-	-	-	-	-	-	-	-	-	-	-	-	-	un	-	-
Diadesmis gallica W. Smith	-	-	**u**	-	-	-	-	-	-	-	-	-	-	-	-	-	ae	-	-
Diadesmis laevissima (Cleve) Mann	-	-	**c**	-	-	-	-	-	-	-	-	-	-	-	**r**	-	ae	4.8 ± 0.7	69 ± 16
Diadesmis paracontenta Lange-Bertalot et Werum	**u**	-	**c**	**u**	-	**u**	-	**r**	**r**	**r**	-	**u**	**r**	**r**	**u**	-	un	5.4 ± 1.3	107 ± 55

Diadesmis perpusilla (Grunow) Mann	-	u	u	-	u	u	u	-	r	r	u	r	u	u	u	3	ae	6.6 ± 1.1	145 ± 49
Diadesmis cf. *perpusilla* (Grunow) Mann	-	-	r	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Diatoma mesodon Kützing	c	d	u	-	u	-	-	-	-	c	-	u	u	u	r	3	-	6.4 ± 0.5	152 ± 29
Diploneis fontanella Lange-Bertalot	r	u	-	-	-	u	-	-	r	u	-	r	r	u	u	-	un	6.9 ± 0.6	158 ± 36
Diploneis cf. *fontium* Reichardt et Lange-Bertalot	-	r	-	-	-	r	-	-	-	u	-	u	r	u	r	-	un	7.3 ± 0.3	157 ± 5
Diploneis oculata (Brebisson) Cleve	u	u	-	-	r	-	-	-	-	r	-	r	r	u	r	3	-	6.8 ± 0.4	154 ± 21
Diploneis ovalis (Hilse) Cleve	-	-	-	-	-	-	-	-	-	r	-	-	-	-	-	4	dry	-	-
Discostella pseudostelligera (Hustedt) Houk et Klee	-	-	-	-	-	-	-	-	-	-	u	r	-	r	-	3	-	7.3 ± 0.2	187 ± 21
Discostella steligera (Cleve et Grunow) Houk et Klee	-	-	-	-	-	-	-	-	-	-	u	-	-	-	-	-	-	-	-
Encyonema minutum (Hilse) Mann and *E. silesiacum* (Bleisch) Mann	-	u	-	-	r	-	r	r	-	c	f	u	c	f	c	-	-	7.0 ± 0.4	166 ± 28
Encyonema perpusillum (A. Cleve) Mann	-	r	-	r	r	u	-	r	r	u	u	r	r	r	r	2	dry	6.9 ± 1.3	162 ± 59
Encyonopsis falaisensis (Grunow) Krammer	c	u	-	-	-	-	-	-	-	r	r	-	-	c	c	-	-	6.8 ± 0.4	144 ± 21
Encyonopsis sp. 1	-	-	-	-	-	-	-	-	-	-	-	-	-	r	r	-	-	-	-
Encyonopsis sp. 2	-	-	-	-	-	u	-	-	-	-	u	u	u	r	r	-	-	7.6 ± 0.4	190 ± 31
Encyonopsis sp. 3	r	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Entomoneis ornata (Bailey) Reimer	-	-	-	-	-	-	-	-	-	-	r	-	-	r	-	3	-	-	-
Eolimna minima (Grunow) Lange-Bertalot	-	-	-	-	-	c	r	r	-	c	u	u	c	-	u	4	-	7.1 ± 0.7	167 ± 42
Epithemia adnata (Kützing) Brébisson	-	-	-	-	-	-	-	-	-	-	-	r	-	-	-	5	-	-	-
Eucocconeis laevis (Østrup) Lange-Bertalot	-	-	-	u	-	-	-	-	-	-	-	-	-	c	u	4	dry	6.4 ± 1.7	163 ± 27
Eunotia angusta (Grunow) Berg	-	-	-	-	r	-	-	-	-	-	-	-	-	-	-	-	un	-	-
Eunotia arculus Lange-Bertalot et Nörpel	-	-	-	-	r	-	-	-	-	-	-	-	-	-	-	2	un	-	-
Eunotia bigibba Kützing	-	-	u	r	r	-	-	-	-	-	-	-	-	-	r	2	dry	5.0 ± 1.2	99 ± 37
Eunotia bilunaris (Ehrenberg) Mills	c	u	u	u	r	-	d	f	u	r	-	-	r	u	u	6	-	5.3 ± 1.2	110 ± 46
Eunotia botuliformis Wild, Nörpel et Lange-Bertalot	r	r	u	-	u	r	-	-	u	r	-	r	r	u	r	-	un	6.2 ± 0.9	124 ± 45
Eunotia circumborealis Lange-Bertalot et Nörpel	-	-	-	r	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Eunotia curtagrunowii Nörpel-Schempp et Lange-Bert.	-	-	-	-	r	-	-	-	-	-	-	-	-	-	-	-	un	-	-
Eunotia exigua (Bréb.) Rabenh. and *E. tenella* (Grun.) Hust.	c	u	f	f	c	-	f	c	c	u	u	u	u	u	u	-	-	5.5 ± 1.2	115 ± 45
Eunotia faba Ehrenberg	-	r	-	-	-	-	-	r	-	-	-	-	-	-	-	2	-	-	-
Eunotia fallax A. Cleve	r	-	-	-	-	-	r	u	-	r	-	-	-	-	r	2	dry	4.7 ± 1.1	89 ± 37
Eunotia fennica (Hustedt) Lange-Bertalot	-	-	-	-	-	-	-	u	-	-	-	-	-	-	-	-	un	-	-
Eunotia glacialis Meister	-	r	-	-	-	-	-	r	-	-	-	-	-	-	-	2	-	4.9 ± 1.2	123 ± 37
Eunotia groenlandica (Grun.) Nörpel-Sch. et Lange-Bert.	-	-	-	r	r	-	-	-	-	-	-	-	-	-	-	2	dry	-	-
Eunotia implicata Nörpel, Alles et Lange-Bertalot	c	u	-	-	-	-	-	-	-	-	-	r	r	u	u	2	-	6.6 ± 0.5	144 ± 19

Eunotia incisa Gregory	**r**	**r**	**c**	**c**	**r**	**-**	**c**	**r**	**r**	**-**	**-**	**r**	**-**	**-**	**-**	2	-	5.1 ± 1.1	108 ± 53
Eunotia islandica Østrup	**-**	**-**	**-**	**-**	**u**	**-**	**-**	**r**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	2	dry	5.3 ± 0.9	95 ± 40
Eunotia levistriata Hustedt	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**r**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	-	un	-	-
Eunotia meisteri Hustedt	**-**	**-**	**-**	**-**	**-**	**-**	**r**	**r**	**u**	**-**	**-**	**-**	**-**	**-**	**-**	2	dry	5.2 ± 0.5	65 ± 42
Eunotia microcephala Krasske	**-**	**-**	**-**	**-**	**-**	**-**	**r**	**r**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	2	dry	-	-
Eunotia minor (Kützing) Grunow	**-**	**c**	**u**	**r**	**r**	**-**	**u**	**r**	**u**	**u**	**-**	**u**	**u**	**u**	**r**	2	dry	4.2 ± 0.8	129 ± 47
Eunotia cf. *minor* (Kützing) Grunow	**-**	**r**	**-**	**-**	**-**	**-**	**-**	**r**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	2	dry	-	-
Eunotia muscicola var. *tridentula* Nörpel et Lange-Bert.	**r**	**c**	**u**	**r**	**-**	**-**	**-**	**-**	**-**	**r**	**-**	**-**	**-**	**-**	**-**	-	un	5.8 ± 0.8	140 ± 58
Eunotia nymanniana Grunow	**-**	**r**	**-**	**u**	**-**	**-**	**-**	**-**	**u**	**-**	**-**	**-**	**-**	**-**	**-**	2	un	4.8 ± 0.9	96 ± 55
Eunotia paludosa Grunow	**u**	**u**	**u**	**c**	**-**	**-**	**u**	**c**	**u**	**-**	**-**	**r**	**r**	**r**	**u**	1	-	4.9 ± 1.3	112 ± 48
Eunotia praerupta Ehrenberg	**-**	**-**	**r**	**r**	**u**	**-**	**r**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	2	-	5.4 ± 1.0	119 ± 45
Eunotia cf. *praerupta* Ehrenberg	**-**	**-**	**r**	**-**	**r**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	2	-	-	-
Eunotia rhomboidea Hustedt	**-**	**r**	**u**	**c**	**-**	**-**	**f**	**-**	**c**	**-**	**-**	**r**	**r**	**-**	**-**	2	-	4.9 ± 1.2	130 ± 50
Eunotia steineckei Petersen	**-**	**r**	**-**	**r**	**-**	**-**	**-**	**r**	**-**	**-**	**-**	**-**	**-**	**r**	**-**	-	un	5.2 ± 1.5	140 ± 30
Eunotia subarcuatoides Alles, Nörpel et Lange-Bertalot	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**r**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	1	-	-	-
Eunotia sudetica Muller	**-**	**r**	**-**	**-**	**r**	**-**	**-**	**-**	**-**	**-**	**-**	**u**	**u**	**-**	**-**	2	-	6.8 ± 1.0	155 ± 43
Eunotia tenella (Grunow) Hustedt - long cells	**-**	**r**	**u**	**-**	**r**	**-**	**u**	**u**	**u**	**-**	**-**	**-**	**-**	**r**	**-**	-	-	-	-
Eunotia tetraodon Ehrenberg	**-**	**-**	**-**	**-**	**r**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	2	-	-	-
Eunotia trinacria Krasske	**-**	**-**	**u**	**-**	**-**	**-**	**c**	**c**	**u**	**-**	**-**	**-**	**-**	**-**	**-**	1	dry	4.7 ± 0.8	82 ± 40
Eunotia ursamaioris Lange-Bertalot et Nörpel-Schempp	**u**	**-**	**f**	**-**	**u**	**-**	**f**	**u**	**r**	**-**	**-**	**-**	**-**	**-**	**-**	-	un	5.1 ± 0.8	93 ± 39
Eunotia valida Hustedt	**-**	**r**	**-**	**r**	**r**	**-**	**r**	**r**	**-**	**-**	**-**	**-**	**-**	**-**	**r**	2	-	5.0 ± 1.2	96 ± 45
Eunotia spp.	**-**	**-**	**r**	**r**	**-**	**r**	**-**	**u**	**-**	**-**	**-**	**-**	**-**	**r**	**-**	-	-	-	-
Fallacia insociabilis (Krasske) Mann	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**r**	**-**	**-**	**-**	**-**	**-**	**-**	3	dry	-	-
Fallacia vitrea (Østrup) Mann	**-**	**r**	**r**	**u**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	1	dry	4.2 ± 1.1	137 ± 34
Fragilaria capucina Desmazieres *sensu lato*	**c**	**c**	**u**	**r**	**r**	**-**	**-**	**-**	**r**	**r**	**c**	**u**	**u**	**c**	**c**	3	-	6.8 ± 0.7	157 ± 30
Fragilaria vaucheriae (Grunow) Jørgensen	**-**	**-**	**-**	**-**	**r**	**-**	**-**	**-**	**-**	**-**	**r**	**-**	**c**	**-**	**-**	4	-	-	-
Fragilaria sp.	**-**	**-**	**-**	**r**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	-	-	-	-
Fragilariforma virescens (Ralfs) Williams et Round	**-**	**-**	**f**	**u**	**c**	**-**	**u**	**u**	**-**	**u**	**-**	**u**	**r**	**-**	**r**	3	-	5.5 ± 1.0	102 ± 44
Frustulia crassinervia (Bréb.) Lange-Bert. et Krammer	**-**	**-**	**u**	**u**	**-**	**-**	**-**	**r**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	1	-	4.0 ± 0.7	116 ± 45
Frustulia saxonica Rabenhorst	**r**	**-**	**u**	**c**	**-**	**-**	**u**	**u**	**u**	**-**	**-**	**-**	**-**	**-**	**u**	1	-	4.9 ± 1.1	110 ± 48
Frustulia vulgaris (Thwaites) De Toni	**r**	**c**	**-**	**-**	**u**	**u**	**-**	**-**	**-**	**u**	**r**	**u**	**u**	**c**	**-**	4	-	6.9 ± 0.5	164 ± 26
Frustulia weinholdii Hustedt	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**r**	**-**	**u**	**-**	3	-	7.1 ± 0.2	154 ± 4
Geissleria decussis (Oestrup) Lange-Bertalot et Metzeltin	**-**	**-**	**-**	**-**	**-**	**-**	**r**	**-**	**-**	**-**	**-**	**r**	**-**	**-**	**-**	4	-	-	-
Gessleria paludosa (Hustedt) Lange-Bertalot et Metzeltin	**-**	**-**	**-**	**-**	**-**	**u**	**-**	**-**	**-**	**-**	**-**	**r**	**-**	**-**	**-**	3	dry	-	-
Gomphonema acuminatum Ehrenberg	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**r**	**r**	**r**	**u**	**-**	4	-	7.4 ± 0.6	184 ± 34

Gomphonema angustum (Kützing) Rabenhorst	-	-	-	-	-	u	r	-	-	-	-	-	u	-	r	4	un	6.6 ± 0.4	167 ± 29
Gomphonema clavatum Ehrenberg	u	u	r	r	r	-	-	-	r	u	-	u	u	c	u	3	-	6.7 ± 0.7	146 ± 24
Gomphonema gracile Ehrenberg	u	u	r	r	r	-	u	-	u	r	r	-	-	u	u	3	-	6.4 ± 0.7	141 ± 33
Gomphonema minutum (Agardh) Agardh	u	-	-	-	-	-	-	-	r	-	u	r	u	u	-	3	un	6.8 ± 0.7	146 ± 42
Gomphonema olivaceoides Hustedt	-	-	-	-	-	-	-	-	-	u	-	r	u	r	-	3	-	6.8 ± 0.4	168 ± 26
Gomphonema parvulum (Kützing) Kützing *sensu lato*	c	u	u	r	u	f	-	r	u	c	u	c	c	f	u	3	-	6.8 ± 0.8	153 ± 37
Gomphonema truncatum Ehrenberg	-	-	-	-	-	-	-	-	-	-	-	-	u	u	-	4	-	7.3 ± 0.2	178 ± 17
Hannaea arcus (Ehrenberg) Patrick	r	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-
Hantzschia amphioxys (Ehrenberg) Grunow	-	-	-	-	-	-	-	r	-	-	-	-	-	r	-	3	dry	-	-
Hantzschia graciosa Lange-Bertalot	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	un	-	-
Hippodonta capitata (Ehrenberg) Lange-Bertalot, Metzeltin et Witkowski	-	-	-	-	-	-	-	-	-	r	u	-	u	u	-	4	-	7.2 ± 0.3	164 ± 20
Karayevia clevei (Grunow) Round et Bukhtiyarova	-	-	-	-	-	-	-	-	-	r	r	r	u	-	-	4	-	7.4 ± 0.3	163 ± 21
Karayevia laterostrata (Hustedt) Round et Bukhtiyarova	-	-	-	-	-	-	-	-	-	-	r	r	u	-	-	3	-	7.4 ± 0.2	174 ± 12
Kobayasiella cf. *jaagii* Meister	-	-	-	-	-	-	-	-	-	r	-	r	-	-	-	-	un	-	-
Kobayasiella subtilissima (Cleve) Lange-Bertalot	-	-	-	u	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Luticola acidoclinata Lange-Bertalot	-	-	-	-	-	r	r	-	r	-	-	r	r	-	-	-	un	-	-
Luticola mutica (Kützing) Mann	-	-	u	-	-	u	r	u	r	-	-	r	r	r	-	3	dry	5.9 ± 1.8	125 ± 74
Mayamaea recondita (Hustedt) Lange-Bertalot	-	-	r	-	-	u	-	-	-	-	r	-	-	-	r	-	ae	7.2 ± 1.0	154 ± 52
Mayamaea atomus var. *permitis* (Hustedt) Lange-Bertalot	-	-	-	-	-	u	-	-	-	u	r	-	u	-	-	4	-	6.9 ± 0.6	174 ± 36
Melosira varians Agardh	-	-	-	-	-	-	-	-	-	-	u	u	f	f	-	4	-	7.3 ± 0.2	169 ± 16
Meridion circulare (Greville) Agardh	r	r	r	-	u	f	r	r	u	c	u	c	c	c	u	4	-	7.0 ± 0.6	156 ± 33
Microcostatus krasskei (Hustedt) Johansen et Sray	r	r	c	c	u	r	-	r	u	-	-	-	r	r	r	2	dry	5.1 ± 1.2	115 ± 47
Microcostatus sp.	-	u	-	-	-	-	-	-	-	-	-	-	-	r	-	-	-	-	-
Muelleria sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	r	-	-	-	-	-
Navicula exilis Kützing	-	u	-	-	-	-	-	-	-	-	-	-	-	-	-	-	un	-	-
Navicula angusta Grunow	-	u	-	-	-	-	-	-	-	-	-	-	-	u	c	2	-	6.8 ± 0.3	144 ± 25
Navicula antonii Lange-Bertalot	-	r	-	-	-	c	-	-	-	-	-	u	-	-	-	4	-	7.8 ± 0.6	191 ± 43
Navicula cryptocephala Kützing	-	-	-	-	u	u	-	-	u	c	u	u	u	c	c	3	-	6.9 ± 0.5	156 ± 31
Navicula cryptotenella Lange-Bertalot	-	r	-	-	r	-	-	-	-	-	-	r	-	u	r	4	-	6.9 ± 0.6	146 ± 18
Navicula difficillima Hustedt	-	r	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-
Navicula gregaria Donkin	-	-	-	-	u	c	-	-	u	c	c	c	f	c	u	4	-	7.2 ± 0.5	168 ± 34
Navicula lanceolata (Agardh) Ehrenberg	-	-	-	-	-	r	r	-	-	u	d	f	f	c	-	4	-	7.2 ± 0.4	171 ± 28
Navicula cf. *lundii* Reichardt	-	r	r	-	-	u	-	-	r	u	u	u	u	u	u	4	-	7.2 ± 0.6	161 ± 36
Navicula medioconvexa (Hustedt)	-	-	-	r	-	-	-	-	-	-	-	-	-	r	-	3	un	-	-

Navicula protracta (Grunow) Cleve	-	-	-	-	-	-	-	-	-	u	-	-	u	-	-	3	-	6.9 ± 0.4	126 ± 31
Navicula radiosa Kützing	-	-	-	-	-	-	-	-	-	-	u	r	r	c	-	3	-	7.2 ± 0.2	168 ± 19
Navicula reinhardtii Grunow	-	-	-	-	-	-	-	-	-	-	u	-	-	-	-	5	-	-	-
Navicula rhynchocephala Kützing	-	r	-	-	u	-	-	-	r	u	u	c	u	c	u	4	-	7.0 ± 0.5	146 ± 27
Navicula slesvicensis Grunow	-	-	-	-	-	-	-	-	-	-	r	-	-	-	-	4	-	-	-
Navicula tridentula Krasske	-	r	-	-	-	r	-	r	-	r	-	-	-	r	-	2	dry	6.8 ± 1.2	145 ± 56
Navicula tripunctata (Müller) Bory	-	-	-	-	-	u	-	-	-	-	-	r	r	-	-	4	-	7.9 ± 0.5	201 ± 33
Navicula ventralis Krasske	-	-	-	-	-	-	-	-	-	-	-	r	-	-	-	2	-	-	-
Navicula spp.	-	u	-	-	-	-	-	-	-	r	-	u	-	r	-	-	-	-	-
Neidium affine (Ehrenberg) Pfitzer	-	u	-	r	c	-	-	-	r	r	-	-	-	u	-	3	-	6.1 ± 0.7	140 ± 25
Neidium affine var. *amphirhynchus* (Ehrenberg) Cleve	-	-	-	-	-	-	-	-	-	-	-	-	-	r	-	-	un	-	-
Neidium alpinum Hustedt	-	-	r	r	c	-	r	r	r	-	-	-	-	r	u	2	-	5.9 ± 0.8	117 ± 34
Neidium ampliatum (Ehrenberg) Krammer	-	-	-	-	-	-	-	-	-	r	u	-	r	-	-	3	-	-	-
Neidium cf. *ampliatum* (Ehrenberg) Krammer	-	r	-	-	c	-	r	r	u	r	-	-	-	r	r	3	-	6.2 ± 0.7	129 ± 39
Neidium binodeforme Krammer	-	-	-	-	-	r	-	-	-	-	-	-	-	-	-	-	un	-	-
Neidium bisulcatum (Lagerstedt) Cleve	-	-	-	-	-	-	-	-	r	-	r	-	-	r	-	3	-	6.3 ± 0.6	135 ± 33
Neidium carterii Krammer	-	-	-	r	-	-	-	-	-	-	-	-	-	-	-	2	un	-	-
Neidium hercynicum A. Mayer	-	r	-	r	u	-	r	u	u	-	r	-	-	-	u	2	un	5.5 ± 1.1	103 ± 48
Neidium cf. *hercynicum* A. Mayer	-	-	-	-	r	-	-	-	-	-	-	-	-	-	-	2	un	-	-
Neidium longiceps (Gregory) Cleve-Euler	-	-	-	-	u	-	-	-	-	-	-	-	-	-	-	2	-	-	-
Neidium productum (W.M. Smith) Cleve	-	-	-	-	r	-	-	-	-	-	-	-	-	-	-	-	un	-	-
Neidium cf. *productum* (W. M. Smith) Cleve	-	-	-	-	-	r	r	-	-	-	-	-	-	-	-	-	un	-	-
Neidium sp.	-	-	r	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nitzschia acicularis (Kützing) W. M. Smith	-	-	-	-	-	-	-	-	-	-	u	-	r	r	-	4	-	7.3 ± 0.3	177 ± 13
Nitzschia debilis (Arnott) Grunow	-	-	-	-	-	r	-	-	-	-	-	r	r	u	-	4	dry	7.3 ± 0.4	166 ± 22
Nitzschia dissipata (Kützing) Grunow	-	r	-	-	-	r	-	-	-	r	u	u	u	c	r	4	-	7.3 ± 0.4	172 ± 26
Nitzschia linearis (Agardh) W. Smith	-	-	-	-	-	u	-	-	-	-	-	-	-	-	r	4	-	-	-
Nitzschia sinuata var. *sinuata* (Thwaites) Grunow	-	-	-	-	-	-	-	-	-	-	-	-	-	r	-	4	-	-	-
Nitzschia sinuata var. *tabellaria* (Grunow) Grunow	-	-	-	-	-	-	-	-	-	-	r	-	-	-	-	3	-	-	-
Nitzschia cf. *subconstricta* Grunow	-	-	-	-	u	-	-	-	r	-	-	-	-	-	-	-	un	6.1 ± 0.3	129 ± 11
Nitzschia spp.	-	u	-	r	u	c	u	-	u	f	c	u	c	c	u	-	-	-	-
Nupela lapidosa (Krasske) Lange-Bertalot	u	-	-	-	-	-	-	-	-	r	-	u	-	-	-	2	dry	6.6 ± 0.8	136 ± 24
Pinnularia borealis Ehrenberg	-	-	u	-	-	u	r	u	r	r	r	-	r	r	-	4	dry	-	-
Pinnularia borealis var. *scalaris* (Ehrenberg) Rabenhorst	-	-	-	-	r	-	-	r	-	-	-	-	-	r	r	-	un	6.1 ± 1.3	126 ± 52
Pinnularia brebisonii (Kützing) Rabenhorst	-	-	-	-	-	-	-	-	u	-	u	-	-	u	r	3	dry	6.9 ± 0.6	154 ± 38

Pinnularia divergens (W. Smith) var. *media* Krammer	-	-	-	-	-	-	-	-	-	r	-	r	-	-	-	-	-	-	-	-	-
Pinnularia dornii Metzeltin	-	-	-	-	-	-	-	r	-	-	-	-	-	-	-	-	un	-	-	-	-
Pinnularia frequentis Krammer	r	u	-	r	-	r	-	r	-	r	-	u	r	r	r	-	-	6.3 ± 1.2	136 ± 42	-	-
Pinnularia gibba Ehrenberg	-	-	-	-	u	-	-	-	r	-	r	-	-	-	-	3	-	6.1 ± 0.7	130 ± 49	-	-
Pinnularia grunowii Krammer	-	r	-	-	r	r	-	-	-	r	-	-	-	u	r	-	un	6.8 ± 0.5	149 ± 21	-	-
Pinnularia lata (Brébisson) Rabenhorst	-	-	-	-	r	-	r	r	-	-	-	-	-	-	-	2	ae	-	-	-	-
Pinnularia lundii var. *linearis* Krammer	-	-	-	-	-	-	-	-	-	-	-	-	-	r	-	-	un	-	-	-	-
Pinnularia macilenta Ehrenberg	-	-	-	-	-	-	u	-	-	-	-	-	-	-	-	-	un	-	-	-	-
Pinnularia microstauron (Ehrenberg) Cleve	-	r	-	-	u	u	c	-	r	u	u	r	r	u	r	3	-	6.2 ± 1.1	127 ± 40	-	-
Pinnularia neomajor Krammer	-	r	-	-	-	-	-	-	-	-	-	-	-	-	r	-	un	-	-	-	-
Pinnularia nodosa (Ehrenberg) W. Smith	-	-	-	-	-	r	r	-	-	-	-	-	-	r	r	2	-	-	-	-	-
Pinnularia notabilis Krammer	-	-	-	-	-	-	-	-	-	-	-	-	-	u	r	-	un	7.2 ± 0.3	162 ± 31	-	-
Pinnularia obscura Krasske	r	-	-	c	u	r	-	r	u	u	-	r	-	u	-	3	dry	5.3 ± 1.5	137 ± 29	-	-
Pinnularia perrirorata Krammer and *P. silvatica* Petersen	u	u	c	f	u	r	-	u	u	u	-	u	r	r	u	-	un	5.2 ± 1.3	115 ± 46	-	-
Pinnularia pseudogibba Krammer	-	u	-	u	c	-	-	u	u	-	r	-	-	r	-	-	un	5.2 ± 1.2	120 ± 41	-	-
Pinnularia rabenhorstii var. *franconica* (Grun.) Krammer	-	-	-	-	-	-	-	-	-	-	-	-	-	r	-	-	un	-	-	-	-
Pinnularia renata Krammer	-	-	-	-	-	-	r	-	-	-	-	r	-	-	-	-	un	-	-	-	-
Pinnularia rupestris Hantzsch	-	r	r	-	-	-	u	c	r	r	-	-	-	-	-	3	-	4.2 ± 1.0	92 ± 36	-	-
Pinnularia schoenfelderi Krammer	-	r	u	u	u	u	r	u	u	u	r	u	-	u	u	-	un	5.5 ± 1.4	116 ± 46	-	-
Pinnularia schroederii (Hustedt) Krammer	r	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	un	-	-	-	-
Pinnularia sinistra Krammer	-	-	-	-	r	u	u	-	-	-	r	r	r	-	-	-	un	4.0 ± 0.6	81 ± 26	-	-
Pinnularia stomatophora (Grunow) Cleve	-	r	-	-	-	-	-	-	-	-	-	r	-	-	-	2	dry	-	-	-	-
Pinnularia subcapitata Gregory and *P. sinistra* Krammer	u	u	c	c	c	-	f	f	c	u	u	u	u	u	c	-	-	5.6 ± 1.3	112 ± 46	-	-
Pinnularia subcommutata Krammer	-	-	-	-	-	u	-	-	-	r	-	-	-	r	-	-	un	7.4 ± 0.7	165 ± 49	-	-
Pinnularia subgibba Krammer	-	-	-	-	-	-	-	-	-	-	u	-	r	u	u	-	un	7.1 ± 0.2	158 ± 26	-	-
Pinnularia subinterrupta Krammer et Schroeter	-	-	r	r	-	-	r	u	-	-	-	-	-	-	-	1	dry	-	-	-	-
Pinnularia submicrostauron Schroeter	-	-	-	-	-	-	u	r	u	-	-	-	-	-	-	-	un	4.8 ± 0.4	62 ± 13	-	-
Pinnularia cf. *subrupestris* Krammer	-	-	-	-	-	-	-	-	-	r	-	-	-	-	-	-	un	-	-	-	-
Pinnularia viridiformis Krammer	-	r	-	u	r	-	c	-	u	u	r	r	r	u	u	-	un	5.8 ± 1.3	125 ± 41	-	-
Pinnularia viridis (Nitzsch) Ehrenberg	-	-	-	-	u	r	r	-	-	r	r	-	-	r	r	3	-	6.3 ± 1.0	124 ± 37	-	-
Pinnularia spp.	-	r	-	-	r	r	-	-	r	-	-	u	-	-	u	-	-	-	-	-	-
Placoneis hambergii (Hustedt) Bruder	-	-	-	-	-	r	-	-	-	-	-	-	-	u	u	2	dry	7.2 ± 0.4	149 ± 31	-	-
Placoneis ignorata (Schimanski) Lange-Bertalot	-	-	-	-	-	-	-	-	-	-	-	r	-	r	-	4	-	-	-	-	-
Placoneis paraelginensis Lange-Bertalot	-	-	-	r	-	c	-	-	r	u	r	r	r	r	r	-	-	7.2 ± 1.0	169 ± 41	-	-

Planothidium lanceolatum (Brébisson) Round et Bukhtiyarova *sensu lato*	**u**	**u**	**-**	**r**	**u**	**f**	**u**	**u**	**u**	**f**	**c**	**f**	**f**	**c**	**c**	4	-	7.0 ± 0.7	161 ± 37
Psammothidium altaicum Bukhtiyarova	**-**	**-**	**-**	**u**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	2	-	-	-
Psammothidium bioretii (Germain) Bukht. et Round	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**c**	**-**	**-**	**-**	**-**	3	dry	7.1 ± 0.2	189 ± 14
Psammothidium helveticum (Hustedt) Bukht. et Round	**-**	**u**	**u**	**c**	**f**	**u**	**r**	**-**	**c**	**u**	**c**	**-**	**-**	**c**	**-**	4	-	5.9 ± 1.0	120 ± 39
Psammothidum marginulatum (Grunow) Bukht. et Round	**f**	**u**	**-**	**u**	**-**	**-**	**-**	**-**	**-**	**r**	**r**	**-**	**-**	**r**	**u**	2	dry	5.9 ±1.3	138 ± 14
Psammothidium oblongellum (Oestrup) Van de Vijver	**-**	**c**	**r**	**r**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**r**	**-**	**r**	**-**	3	-	6.1 ± 0.9	157 ± 37
Psammothidium subatomoides (Hust.) Bukht. et Round	**-**	**r**	**-**	**-**	**r**	**-**	**-**	**-**	**-**	**r**	**-**	**r**	**c**	**u**	**c**	2	-	7.1 ± 0.4	151 ± 26
Puncticulata comta (Ehrenberg) Håkansson	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**r**	**-**	**-**	**-**	**-**	4	-	-	-
Reimeria sinuata (Gregory) Kociolek et Stoermer	**-**	**-**	**-**	**-**	**-**	**r**	**-**	**-**	**-**	**u**	**c**	**u**	**c**	**u**	**u**	-	un	7.3 ± 0.4	176 ± 29
Reimeria uniseriata Sala, Guerrero et Ferrario	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**r**	-	un	-	-
Rhoicosphenia abbreviata (C. Agardh) Lange-Bertalot	**r**	**-**	**-**	**-**	**-**	**r**	**-**	**-**	**-**	**u**	**-**	**u**	**c**	**-**	**-**	4	-	7.1 ± 0.5	180 ± 19
Rhopalodia gibba (Ehrenberg) O. Muller	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**r**	**-**	**-**	5	-	-	-
Sellaphora psedopupula (Krasske) Lange-Bertalot	**-**	**-**	**-**	**-**	**r**	**u**	**-**	**-**	**-**	**-**	**r**	**-**	**-**	**-**	**-**	-	un	-	-
Sellaphora pupula (Kützing) Mereschkovsky *sensu lato*	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**r**	**u**	**r**	**-**	**r**	**u**	**-**	3	-	6.9 ± 0.5	155 ± 29
Sellaphora seminulum (Grunow) Mann	**-**	**-**	**-**	**-**	**-**	**c**	**-**	**-**	**-**	**u**	**-**	**r**	**u**	**-**	**r**	3	-	7.3 ± 0.6	170 ± 38
Sellaphora stauroneioides (Lange-Bertalot) nov comb.	**-**	**-**	**-**	**-**	**u**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	-	un	-	-
Sellaphora sp.	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**r**	**-**	-	-	-	-
Stauroneis anceps Ehrenberg *sensu lato*	**-**	**r**	**-**	**u**	**u**	**u**	**u**	**-**	**u**	**u**	**r**	**u**	**u**	**u**	**u**	3	-	6.5 ± 1.0	141 ± 33
Stauroneis gracillima Hustedt	**-**	**-**	**-**	**-**	**-**	**r**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	-	un	-	-
Stauroneis kriegeri Patrick	**-**	**-**	**-**	**-**	**-**	**r**	**-**	**-**	**-**	**r**	**u**	**r**	**u**	**r**	**u**	3	-	7.4 ± 0.5	184 ± 37
Stauroneis legumen (Ehrenberg) Kützing	**-**	**-**	**-**	**u**	**u**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	3	-	4.9 ± 1.2	124 ± 34
Stauroneis obtusa Lagerstedt	**-**	**-**	**-**	**-**	**-**	**u**	**-**	**r**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	3	dry	-	-
Stauroneis phoenicenteron (Nitzsch) Ehrenberg	**-**	**-**	**-**	**-**	**u**	**-**	**r**	**-**	**-**	**u**	**-**	**u**	**r**	**u**	**u**	3	-	6.8 ± 0.5	143 ± 24
Stauroneis smithii Grunow	**-**	**-**	**-**	**-**	**r**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**r**	**-**	4	-	-	-
Stauroneis thermicola (Petersen) Lund	**-**	**u**	**r**	**u**	**u**	**u**	**-**	**r**	**r**	**r**	**-**	**r**	**r**	**r**	**u**	3	dry	6.4 ± 1.4	148 ± 43
Stauroneis thermicola fo. *lanceolata* (Hust.; Hust.) Hust.	**-**	**r**	**-**	**-**	**r**	**-**	**-**	**-**	**r**	**-**	**-**	**-**	**r**	**r**	**-**	-	un	6.6 ± 0.5	161 ± 24
Staurosirella lapponica (Grunow) Williams et Round	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**r**	**-**	**-**	**-**	4	-	-	-
Staurosirella martyi (Héribaud) Morales et Manoylov	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**r**	**-**	**-**	**-**	4	-	-	-
Stephanocyclus menenghiniana (Kützing) Skabitschevsky	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**r**	**u**	**r**	**-**	**r**	**-**	4	-	7.1 ± 0.3	181 ± 18
Stephanodiscus hantzschii Grunow	**-**	**-**	**-**	**-**	**-**	**-**	**r**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	5	-	-	-
Surirella angusta Kützing	**-**	**u**	**-**	**-**	**r**	**c**	**-**	**-**	**r**	**u**	**u**	**u**	**c**	**c**	**u**	4	-	7.1 ± 0.4	159 ± 28
Surirella brebisonii Krammer et Lange-Bertalot	**-**	**-**	**-**	**-**	**r**	**u**	**-**	**-**	**-**	**u**	**r**	**u**	**c**	**c**	**u**	4	un	7.3 ± 0.4	153 ± 24
Surirella helvetica Brun	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**u**	**u**	**-**	**-**	-	un	-	-
Surirella linearis W. M. Smith	**-**	**-**	**-**	**u**	**-**	**r**	**-**	**-**	**-**	**-**	**r**	**-**	**-**	**-**	**-**	3	-	-	-

Surirella minuta Brébisson	-	-	-	-	**u**	**u**	-	-	**r**	**u**	**u**	-	-	-	-	4	-	6.8 ± 0.7	144 ± 33
Surirella ovalis Brébisson	-	-	-	-	-	-	-	-	-	-	**c**	-	**r**	-	-	4	-	7.2 ± 0.2	190 ± 15
Surirella roba Leclercq	**f**	**c**	-	-	**u**	-	**r**	-	**u**	**r**	-	-	-	**c**	**c**	2	un	6.5 ± 0.5	138 ± 20
Surirella splendida (Ehrenberg) Kützing	-	-	-	**r**	-	-	**u**	-	-	-	-	-	**r**	-	-	4	-	5.9 ± 1.4	174 ± 14
Surirella tenera Gregory	-	**u**	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-
Synedra ulna (Nitzsch) Ehrenberg	**c**	**r**	-	-	-	-	**r**	-	-	-	**r**	**c**	**c**	**f**	**c**	4	-	7.0 ± 0.5	150 ± 20
Tabellaria flocculosa (Roth) Kützing	-	**u**	**u**	**u**	**u**	-	**f**	**c**	**u**	**u**	**c**	-	**r**	**c**	-	2	-	5.8 ± 1.1	123 ± 54

Table 2. Basic statistics of diatom taxa per sample, sampling site and locality and Bray-Curtis similarity index between and within localities and within different substrate types.

	min	max	mean	SD	n
number of diatom taxa					
sample	3	72	27	14	161
sampling site	28	109	59	19	30
locality	53	128	82	21	15
Bray-Curtis index					
between localities	0.00	0.711	0.192	0.127	12080
within localities	0.04	0.839	0.465	0.155	760

Table 3. Chemical and physical parameters of the sampling sites (upstream headwaters site = US, downstream site near confluence with larger stream =DS) in 15 streams in the Elbsandsteingebirge of Bohemian Switzerland National Park. Results of the Kruskal-Wallis test between Bray-Curtis similarity index of different reaches of the stream are indicated as: ns = $p>0.05$. * = $p<0.05$. ** = $p<0.01$. *** = $p<0.001$.

	locality	pH (upstream/ downstream)		conductivity (US/DS, $\mu S \cdot cm^{-1}$)		temperature (US/DS, °C)		species similarity index (US vs. DS)
1	Studený potok	6.27	6.20	136	135	9.2	9.5	***
2	Dolnožlebský p.	6.31	6.31	184	155	12.2	14.1	***
3	Suchá Bělá	5.31	4.43	53	70	6.1	5.8	***
4	Písečná rokle	3.30	3.95	147	148	9.1	9.3	*
5	Kachní potok	6.13	5.91	135	118	7.9	8.7	*
6	Ponova louka	7.30	8.30	130	229	11.5	9.4	***
7	Hluboký důl	4.74	6.20	78	181	9.0	9.1	***
8	Mlýnská rokle	3.62	4.39	82	52	5.1	5.5	***
9	Červený potok	6.71	5.22	146	53	9.5	7.8	**
10	Studenec	6.54	6.66	184	109	15.4	16.0	***
11	Doubický potok	7.28	6.95	202	175	13.9	11.3	ns
12	Bílý potok	7.21	7.61	154	153	14.6	14.5	ns
13	Brtnický potok	7.36	7.50	190	173	15.3	15.7	ns
14	Vlčí potok	7.35	6.94	162	152	5.8	6.9	**
15	Panský potok	7.84	7.02	229	125	7.3	7.4	***

2.2 II. Manuscript

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Morphometric study of *Navicula* morphospecies (Bacillariophyta) with respect to diatom life cycle

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Abstract: Quantitative and qualitative variation in valve morphology in strains of *Navicula cryptocephala* and *N. trivialis* were examined using conventional valve measurements (length, width, striae density) and landmark-based geometric morphometric method. The ability of morphometric techniques to distinguish individual strains on the basis of postinitial and/or sexually competent cells was assessed by multivariate analyses and permutation tests. The data showed that each strain had unique valve characters, which were shared by both large postinitial and small sexually competent cells. Landmarks representing central area and apical ends of the valves were primarily the most useful for discrimination between strains. The geometric morphometric techniques appeared to be enough sensitive to distinguish subtle morphological differences between *Navicula* morphospecies clonal cultures withouth any interference of diatom life cycle stage.

Key words: canonical variate analysis, geometric morphometry, life cycle, *Navicula cryptocephala*, *Navicula trivialis*, sliding landmarks

Introduction

Taxonomy of diatoms has primarily been based on the morphology of diatom frustules observed in light microscope. Electron microscopy revealed other diverse valve structures, which have been used for description of new diatom species and genera (e.g., ROUND *et al*. 1990; VAN DE VIJVER 2002). However, molecular data demonstrated higher diatom species diversity than recognizable using traditional morphology (MANN *et al*. 2003; BEHNKE *et al*.

2004; AMATO *et al*. 2007; VANORMELINGEN *et al*. 2007). Many phenotype-based species were proved to be complexes of cryptic species, with almost identical morphology (POULÍČKOVÁ & MANN 2006; MANN *et al*. 2008). However, quantitative morphometric analyses of valve outlines revealed the small-scale shape differences between individual micro-species (reviewed by MANN *et al*. 2004). Traditionally, the conventional morphometric measurements of length, width and other valve characters as e.g., striae or areolae density, qualitative description of raphe ends or central area, were used (e.g., DROOP *et al*. 2000; SIVER & BASKETTE 2004; KNAPP *et al*. 2006). Morphometrics underwent "scientific revolution" in 1990's (ROHLF & MARCUS 1993; ADAMS *et al*. 2004) that resulted in what is now called geometric morphometrics (ADAMS *et al*. 2004; ZELDITCH *et al*. 2004). Geometric morphometrics is based on analyses of configurations of homologous points (landmarks) or landmark-registered outlines (BOOKSTERIN 1997). In diatom research, geometric morphometric methods were recently used in taxonomic (BESZTERI *et al*. 2005; FRÁNKOVÁ *et al*. 2009), phylogenetic (EDGAR & THERIOT 2004) and ecological applications (POTAPOVA & HAMILTON 2007). One particular difference of the landmark-based geometric morphometrics from traditional measurements relies in inherent separation of size differences from the landmarks data *prior* to the statistical analysis (data reflecting shape variation with size of the cells are standardized to a unit dimension). Thus, differences in size and shape of different populations or species can be analysed separately. However, the allometric shape dynamics caused by the size change related to vegetative diatom life cycle may still influence the shape of the investigated populations (MANN & CHEPURNOV 2005). Therefore, in the present study, we analysed valve morphology of cells in particular, well-identified stages of their life cycle. We used the large postinitial and small sexually competent cells in eight strains of *Navicula cryptocephala* Kützing and *N. trivialis* Lange-Bertalot using conventional and landmark-based morphometric approaches. Both cell types represented the size extremes of the usual variation of each strain within life cycle. We aimed to address the question whether the individual strains could be correctly identified at various stages of their life cycle using the morphometric data.

Material and Methods

Altogether eight monoclonal cultures of *Navicula cryptocephala* and *N. trivialis* isolated from Czech, Scottish and Australian lake epipelon were examined (Tab. 1). Isolation, cultivation and preparation of strains followed methods described previously by POULÍČKOVÁ & MANN (2006). *Navicula* species

under study are homothalic (*N. cryptocephala*) and automictic (*N. trivialis*), thus both exhibit sexual reproduction within monoclonal cultures. Sexually competent / postinitial cells were obtained from monoclonal cultures under sexual reproduction, containing both cell types. Valves were cleaned in acids and mounted in Naphrax as descibed many times elsewhere (POULÍČKOVÁ & MANN 2006).

Micrographs of approximately 30 postinitial and 30 sexually competent cells of each strain (a total of 464 valves) were obtained using a light microscope Zeiss Axioimager with a Zeiss Axiocam HRc digital camera (Carl Zeiss, Jena) capable of 1388 × 1040 pixel resolution. Images were captured and managed using Zeiss Axiovision Version 4.5 imaging software. Differential interference contrast (DIC) optics was used at ×100 planapochromat lens, nominal numerical aperture 1.4.

In all frustules, length, width and striae density along the axial area immediately above the central area were recorded. Landmarks were placed along valve outline (36) and along the central area, at the raphe and striae endings (12). Of these 48 landmarks, 10 landmarks were in fixed positions (no. 1-4: intersections of a cell outline with apical and transapical axis; no. 37-42: the raphe central endings and ends of the longest striae in the central area; Fig. 1) and remaining landmarks were semilandmarks that were allowed to slide along the abscissa connecting adjacent landmarks (BOOKSTEIN 1997). The TPS-series software (ROHLF 2007) was used for most geometric morphometric analyses. The landmarks were digitized using TpsDig ver. 2.05. Valves were symmetrised along both the apical and transapical axes because valve symmetry (MANN 1983) could not always be determined from the micrographs focused primarily on the valve outline. Therefore, the asymmetric component of shape variation in valves was eliminated from data and subsequent analyses were conducted on symmetric configurations (KLINGENBERG *et al*. 2002). Following the general Procrustes superimposition, the thin-plate spline analysis based on tangent space projections was conducted (BOOKSTEIN 1991; ZELDITCH *et al*. 2004). The mean configurations of individual strains were illustrated using the deformation grids based on the thin-plate spline interpolation (ZELDITCH *et al*. 2004).

The principal component analyses (PCA) of partial warps and the uniform component were separately conducted on three sets of objects: the postinitial cells, the sexually competent cells and the entire set of 464 cells. For these analyses, we used the TpsRelw ver 1.45. In all three sets, we used the scores of the objects on all the 15 non-zero PC axes for further analyses. Conventional measurements of the postinitial cells, sexual cells and the combined set were standardized before their use in statistical analyses. Geometric morphometric characters, conventional measurements and combined geometric and conventional morphometric data of the three datasets were used in the canonical variate analyses (CVA), two-group multivariate permutation tests on Mahalanobis distance (1000 permutations), and series of linear discrimination analyses assessing the percentage of correct classification of individual valves into their appropriate groups (strain) on the basis of the linear discriminant function. The geometric morphometric and conventional data were combined by normalization by the column-wise subtracting the mean and dividing by standard deviation (geometric morphometric data represented

first 15 PC axes of the entire set of 464 cells, conventional data represented measurements of individual cells). The discriminative power of individual valve areas was evaluated using the F-value of NPMANOVA (non-parametric MANOVA, ANDERSON 2001). The Procrustes-superimposed coordinates of individual landmarks were separately used in series of NPMANOVA tests in all three investigated sets (the postinitial cells, the sexually competent cells and the combined set). The F-value resulting from NPMANOVA, indicating the degree of separation between groups was used for evaluation of discriminative power of individual landmarks. Statistical analyses were performed using PAST ver. 1.85 (HAMMER *et al*. 2001).

Results

Differences in shape between postinitial and sexually competent cells were evident from the PCA ordination plot of geometric morphometric data, relative warp analysis (Fig. 2). The ordination plot representing the first and second RW axes accounted for 95.7% of the total shape variability. Along the first axis (spanning 64.7% of the variability), the postinitial cells were clearly separated from the sexually competent cells. Shape dynamics along this axis reflected the change from thin, elongated postinitial cells to more rounded sexual cells (Fig. 2). The second RW axis (31.0% of the variation) did not discriminate between postinitial and sexual cells, but it reflected the shape change of the central part of the valves. Not surprisingly, ranges of width and length in postinitial and sexually competent cells within the strains did not overlap. In addition, *Navicula trivialis* strains (D, E, G) had larger cells than *N. cryptocephala* strains (Tab. 1, Fig. 3). The mean valve shapes of individual groups (postinitial cells, sexual cells and combined sets in all strains) within strains are visualised by thin-plate splines as deformation from the overall mean configuration of landmarks (Fig. 4).

The separate canonical variates analyses (CVA) of geometric morphometric data (Fig. 5a), conventional measurements (Fig. 5b) and of the combined set of standardized geometric morphometric data and measurements (Fig. 5c) demonstrate shape and form separation of strains. Strain centroids clustered differently in the ordination plots produced from different morphometric data sets. However, three *N. trivialis* strains (D, E, G) were in each CVA diagram separated from *N. cryptocephala* strains (Fig. 5). The degree of separation (evaluated by Mahalanobis distance) and the percentage of correct discrimination between strains differed among different data sets (postinitial and/or sexual cells, geometric and/or conventional morphometrics; Tabs. 2a, 2b). The shape differentiation of strains was very high in sets of exclusively postinitial or sexually competent cells. When using the geometric

morphometric data, there was an average 98.3% correct strain assignment for postinitial cells and 98.4% for sexual cells. As for the traditional measurements, the average correct strain assignment was 98.6% for postinitial cells and 95.4% for sexual cells. In the combined set of both postinitial and sexually competent cells, discrimination values between strains were slightly lower. There was average correct discrimination of 95.0% cells in geometric morphometric data and 93.9% in measurements. The combined standardized geometric morphometric and measurements data in average correctly discriminated 97.9% of cells in two-group tests.

The apical parts of cells and the central areas were clearly the most strain-specific in postinitial cells (Fig. 6a) Characteristic shape features of strains were more regularly distributed along the cell outline in sexually competent cells (Fig. 6b). In the combined set, shape of central area was the most important valve feature for discrimination between strains (Fig. 6c).

Discussion

Species concept in diatoms has often been too wide and that there are many more species than are currently recognized, even in areas of the world that are comparatively well known, such as Europe (MANN 1999; MANN *et al*. 2004). The modern morphometric techniques appeared to be sensitive tool for distinguishing subtle morphological differences between morphotypes of diatom strains traditionally assigned to a single species (RHODE *et al*. 2001; PAPPAS & STOERMER 2003; MANN *et al*. 2004). In this study, we were able to characterize and distinguish quantitatively and qualitatively all of the investigated clones of *Navicula cryptocephala* and *N. trivialis* by means of conventional and geometric morphometric methods. The data obtained from morphometric analyses of postinitial and sexually competent cells showed that each strain of *N. cryptocephala* and *N. trivialis* had unique valve characters shared by both stages of the life cycle. Shape of the central area and apical ends of valves were particularly the most useful parts of the valve. In accordance, the importance of central area for identification of naviculoid diatoms was suggested in other studies (e.g., BARBER & HAWORTH 1981; ROUND 2001). The traditional measurements reflected mostly differences in size of the valves and were much less successful in discrimination of strains in data set including postinitial and sexually competent cells, where most of the size variation was within, rather than between the strains. On the other hand, the geometric morphometric data (discriminating the postinitial and/or the sexual cells between pairs of strains solely on

the basis of shape differences and the quantitative shape properties spanned by the geometric morphometric descriptors) were quite powerful even in these sets with cells taken from the opposite stages of the diatom life cycle combined into single group in each strain. Shape differences were distinctly less dependent on the life cycle stage and the geometric morphometric data included strong strain-specific signal in most pair comparisons. However, the average correct assignment of individual cells still increased in discrimination analyses of strain pairs using the normalized geometric morphometric and traditional data. Phenotypic variation may be influenced by several factors, including growth at different environmental conditions, changes during life cycle stage, and genetic differentiation. Although morphological and physiological variation has been observed in different protists under influence of temperature (MONTAGNES & DANIEL 2001; GÄCHTER & WEISSE 2006; NEUSTUPA *et al*. 2008), pH (WEISSE *et al*. 2007) and salinity (CLAVERO *et al*. 2000; FINLAY *et al*. 2006), a few such studies were done in diatoms (SCHULTZ 1971; SCHMID 1976; COX 1994). Changes in valve shapes during ontogeny are discussed, e.g., by PAPPAS & STOERMER (2003) and MANN & CHEPURNOV (2005). The separation of both species *N. cryptocephala* and *N. trivialis* was expected, however, geometric morphometrics clearly separated also 3 clones of *N. cryptocephala* (A, C, F) from two others (B, H). *N. cryptocephala* populations have been previously found to be polymorphic with respect to cytological characteristics and reproduction (GEITLER 1952, 1958; POULÍČKOVÁ & MANN 2006). The latter authors hypothesized the existence of pseudocryptic diversity within *N. cryptocephala*, which can be supported by our morphometric analysis. Nevertheless, to what extent the observed morphometric patterns reflected the microevolutionary differentiation of strains remains to be confirmed by the molecular methods.

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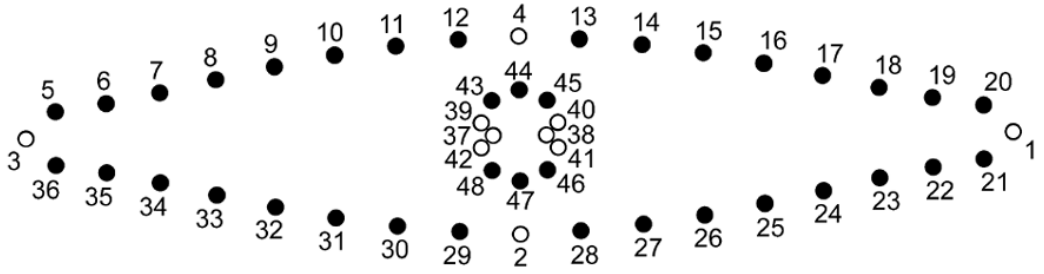

Fig.1. Positions of landmarks. Fixed landmarks are represented by the empty circles and semilandmarks by the filled circles.

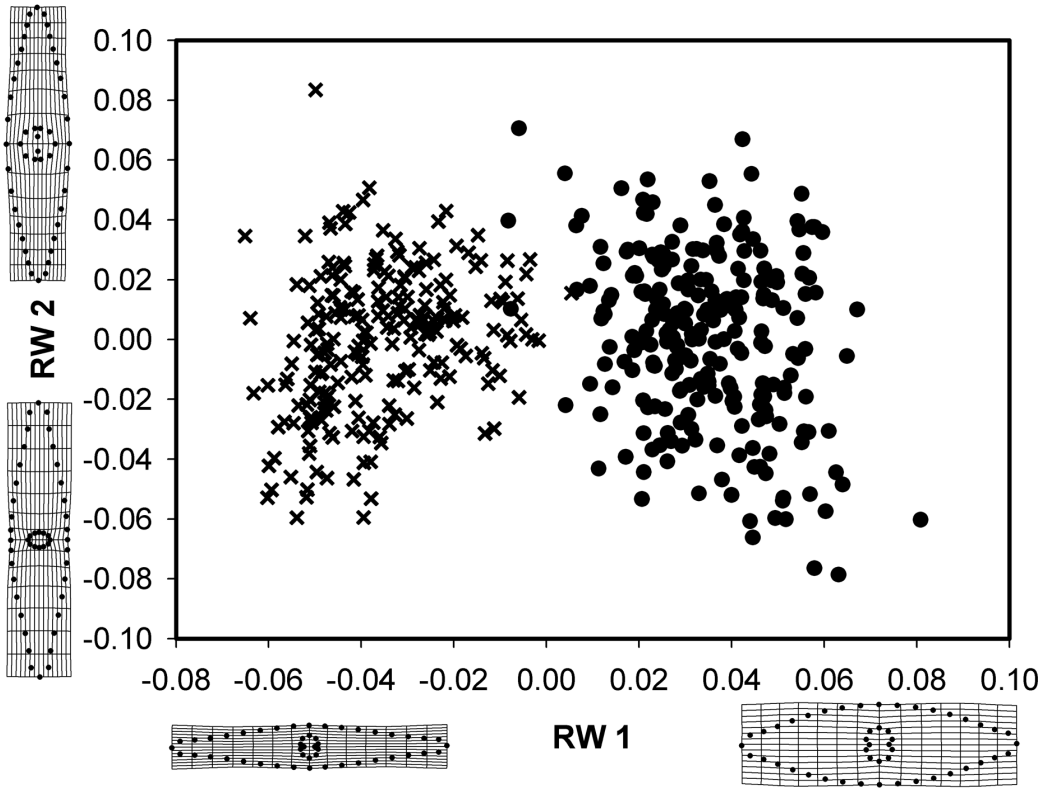

Fig. 2: Relative warp analysis (RWA). Postinitial cells are represented by crosses and sexually competent cells by circles. Shape changes along the first (64.7%) and second (31.0%) RW axes are demonstrated.

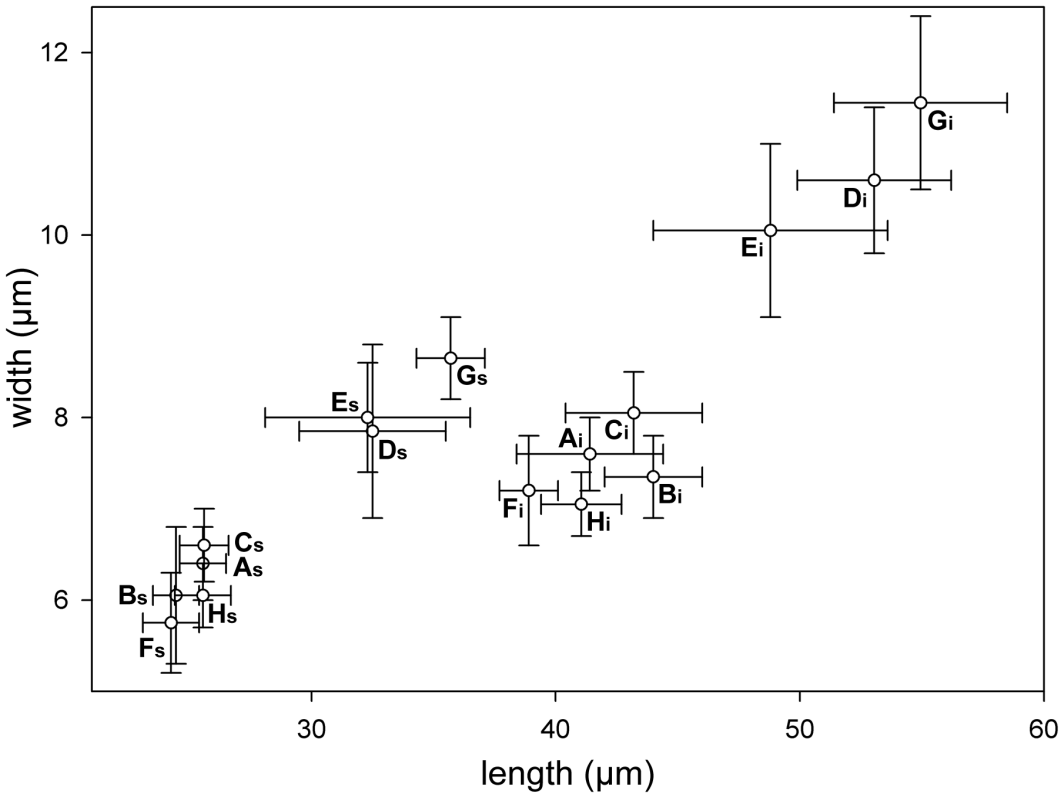

Fig. 3: Scatter plot of length versus width of *Navicula* strains. For clones (A-H) see Table 1, small letters indicate sexually competent cells (s) or postinitial cells (i).

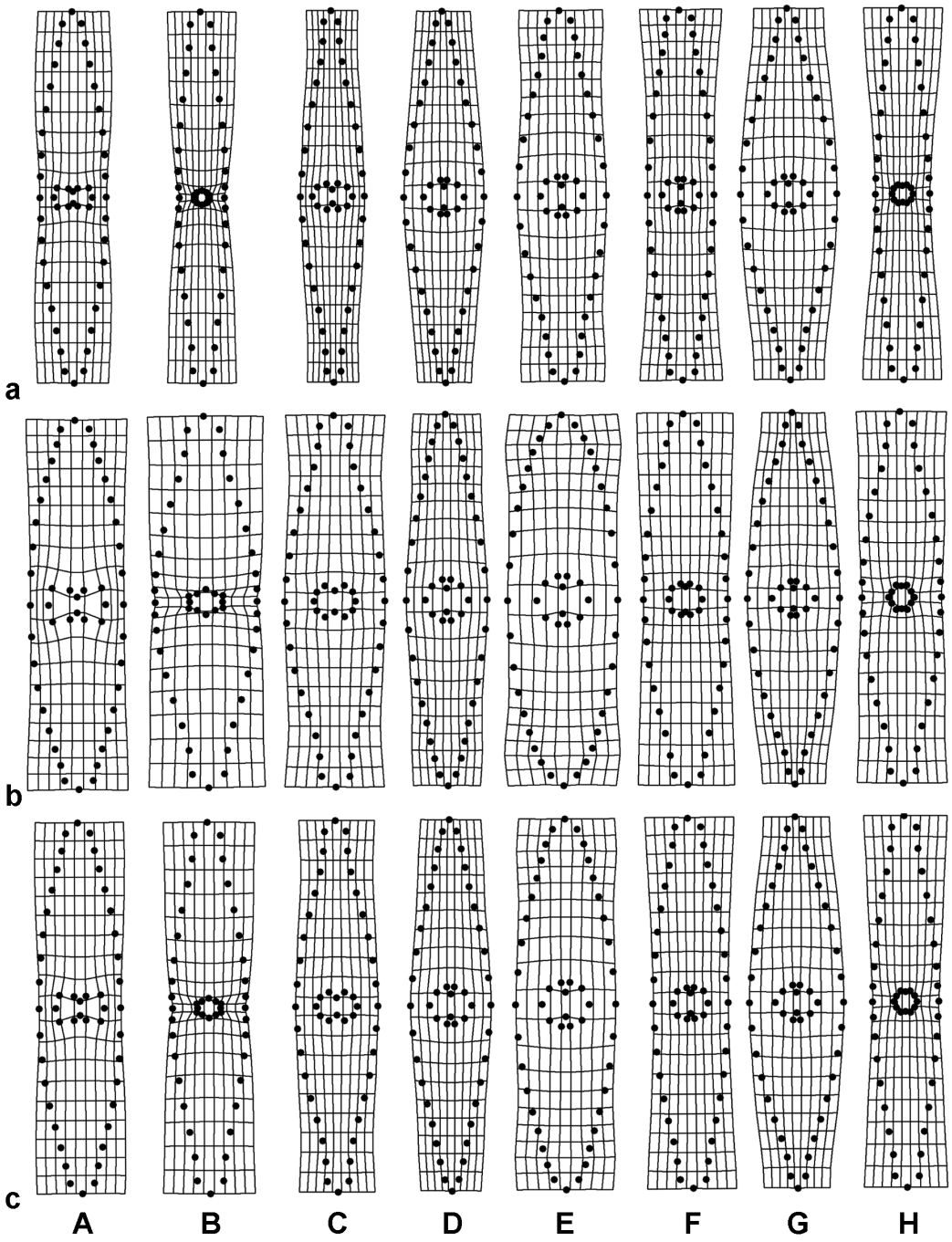

Fig. 4: Reconstruction of characteristic shape of strains by the deformation of thin-plate spline from mean configuration: a) postinitial cells, b) sexually competent cells, c) postinitial + sexual cells. For clones (A-H) see Table 1.

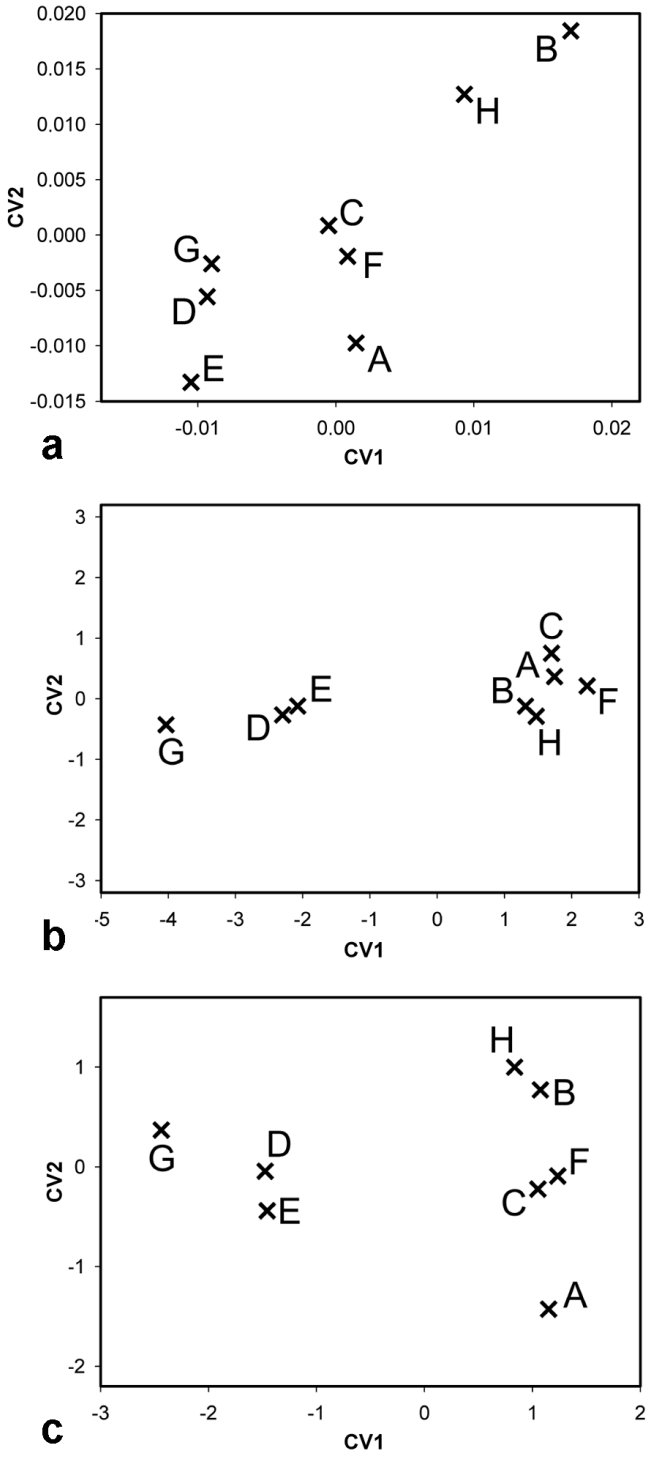

Fig. 5: Centroids of strains calculated from canonical variates analysis (CVA) using: a) geometric morphometric data, b) conventional morphometric data, c) geometric + conventional morphometric data. For clones (A-H) see Table 1.

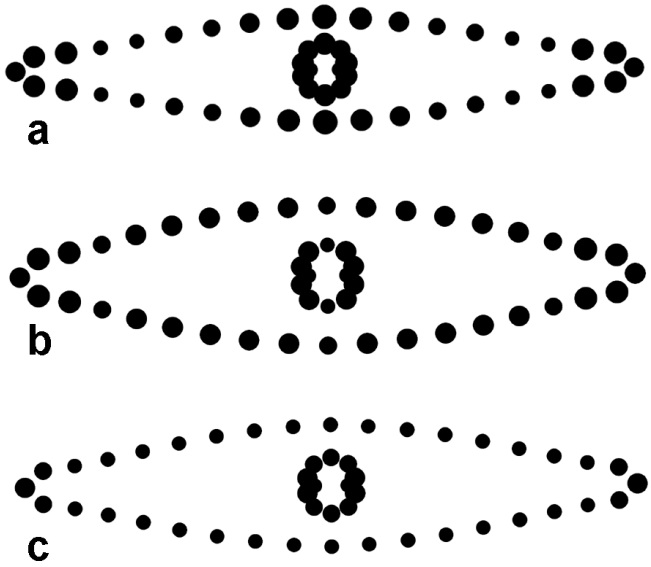

Fig. 6: The importance of landmarks for discrimination between strains. Sizes of landmarks correspond to amount of dissimilarity between strains within postinitial cells (a), sexually competent cells (b), and both together (c).

2.3 III. Manuscript

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Pseudocryptic Diversity versus Cosmopolitanism in Diatoms: a Case Study on *Navicula cryptocephala* Kütz. (Bacillariophyceae) and Morphologically Similar Taxa

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Despite the significance of diatoms in biomonitoring, many aspects of their biodiversity and geographical distribution are poorly understood. Recent evidence from molecular data has shown that traditional cosmopolitan and euryvalent morphospecies are often heterogeneous, containing cryptic or pseudo-cryptic species. It is important to establish whether these more finely differentiated species are also cosmopolitan or show restricted distributions.

According to the standard freshwater diatom floras, *Navicula cryptocephala* and morphologically similar species (*N. veneta*, *N. trivialis*, *N. gregaria* and *N. cryptotenella*) are common, cosmopolitan freshwater pennate diatoms. Although allopatric and even sympatric populations of *N. cryptocephala* are extremely similar morphologically, they have previously been found to be highly polymorphic with respect to reproductive and nuclear characteristics; however, molecular data supporting the existence of cryptic diversity were lacking. Phylogenetic analyses (LSU rDNA, ITS of the rRNA operon) of 52 strains of *N. cryptocephala*-like diatoms

confirmed the existence of genetically distinct lineages within *N. cryptocephala*, and revealed a close relationship between *N. trivialis* and *N. cryptocephala*. Cytological, reproductive and morphological variation, investigated by means of landmark-based geometric morphometrics, were in congruence with molecular data. Two pseudocryptic species within *N. cryptocephala* coexist sympatrically and are widely distributed, occurring in both European and Australian lakes.

Key words: cytology; geometric morphometrics; *Navicula*; molecular phylogeny; reproduction; cryptic diversity; biogeography.

Introduction

Because speciation is not always accompanied by morphological change, the true number of biological species is likely to be greater than the current tally of nominal species, most of which are delineated on purely morphological grounds (Bickford et al. 2007). Recent taxonomic research on diatoms suggests that traditional species boundaries, based largely on variation in the morphology of the siliceous exoskeleton (the frustule), have been drawn too widely and that real species diversity has probably been greatly underestimated (Evans et al. 2009; Mann 1999).

Several recent studies have revealed that phenotype-based species each consisted of two or more genetically distinct demes (cryptic/semicryptic/ pseudocryptic species; for terminology see Mann and Evans 2007) with identical or subtly different morphologies (e.g. Kooistra et al. 2008). The genetic differences were confirmed as biologically relevant when reproductive barriers were found among the distinct demes (Amato et al. 2007; Behnke et al. 2004; Mann 1999; Vanormelingen et al. 2008). In several cases, ecological differences were also detected among cryptic or semicryptic species (de Vargas et al. 1999, 2002; Kooistra et al. 2008; Mann et al. 2008; Poulíčková et al. 2008; Rodriguez et al. 2005; Špačková et al. 2009). There is a need for a narrower species concept if contentious issues such as distribution, dispersal and biogeography are to be resolved (Evans et al. 2007).

Navicula cryptocephala Kütz. is a common, cosmopolitan benthic diatom of moderate size (20–40 mm long), well described and illustrated in the standard freshwater diatom floras by Hustedt (1930) and Krammer and Lange-Bertalot (1997). At least under light microscopy, frustule morphology is very similar in all *N. cryptocephala* populations, including those recorded by Geitler (1958) in Austria. However, this species has been found to be

polymorphic with respect to interphase nuclear structure (heterochromatin distribution) and reproductive characteristics (Geitler 1951, 1952a, b, 1958; summarized by Poulíčková and Mann 2006). Unfortunately, taxonomic changes over the past five decades did not allow us to distinguish exactly which of the recently accepted *N. cryptocephala*-like species were included by Geitler (1951, 1952a, b, 1958) under his tentative names: *N. cryptocephala typica* I and II; *N. cryptocephala* var. *veneta* I and II; *N. cryptocephala* var. *intermedia*. This is why morphologically similar species (*N. trivialis*, *N. veneta*, *N. gregaria*, *N. cryptotenella*; Fig. 1; for morphological characteristics see Table 1) were included in this study, to cover the entire cytological variability described by Geitler (1951, 1952a, b, 1958) in *N. cryptocephala*-like diatoms, and to verify specific cytological characteristics and their congruence with molecular data.

For quantitative morphometric evaluation of the *N. cryptocephala*-like strains, we used the methodology of geometric morphometrics of cell shapes (Zelditch et al. 2004). This method is based on the simultaneous analysis of landmarks and semi-landmarks, i.e. series of points delimiting outlines of objects (Zelditch et al. 2004). The Procrustes analysis, at the basis of the geometric morphometrics, standardizes position, rotation and scale of the objects, thus minimizing the distances between corresponding landmarks and semi-landmarks. These residual distances are then used in subsequent statistical analyses aimed at delimitation of shape differences among groups. Geometric morphometric methods are increasingly being used in diatom studies, both in taxonomic (Beszteri et al. 2005; Fránková et al. 2009; Veselá et al. 2009) and ecological contexts (Potapova and Hamilton 2007).

The present study aims to test the hypothesis that *N. cryptocephala* is a complex of pseudocryptic species, which have restricted (non-cosmopolitan) distributions. To identify genetically distinct clades, we determined the D1-D2 domains of the 28S rDNA (LSU) and ITS2 rDNA (ITS) sequences for a variety of *N. cryptocephala*-like strains.

Results

Genetic and Morphological Diversity of Navicula cryptocephala-like Species

Navicula cryptocephala and morphologically similar small naviculoid diatoms were collected systematically in Great Britain and the Czech Republic. In addition, a few samples were obtained from Austria, including ‘Lunz Untersee’, the main locality where Geitler (1958) studied polymorphism in *N. cryptocephala* sensu lato, and Australia. These diatoms were

common and often abundant (Poulíčková et al. 2008, 2009). In total, 52 strains from 11 localities (Tables 2 and 3) were selected and identified as *N. cryptocephala* (22 strains), *N. veneta* (4), *N. gregaria* (3), *N. cryptotenella* (2) and *N. trivialis* (21). Basic morphometric data of all morphospecies are given in Table 1. The relative abundance of these species in the epipelon of the source localities is given in Table 2.

The phylogenetic relationships amongst 49 strains of *Navicula* sensu stricto (29 sequences obtained in our laboratory and 20 GenBank sequences) were reconstructed from LSU rDNA sequences. The alignment length based on secondary rRNA structure was 528 base pairs (bp). The resulting tree shows several strongly supported clades (Fig. 2): *N. cryptocephala* I, *N. cryptocephala* II, *N. trivialis*, *N. cryptotenella*, *N. veneta* and *N. gregaria*. However, relationships among groups of clades remained largely unresolved. All clades corresponded to different morphospecies except for *N. cryptocephala*. *Navicula cryptocephala* strains were paraphyletic and, together with *N. trivialis*, they comprised a well-supported clade.

The identification of *N. veneta*, *N. gregaria*, *N. cryptotenella* and *N. trivialis* on the basis of frustule morphology was possible using LM (Fig. 1A-C, R-U); however we could not distinguish the strains of *N. cryptocephala* which belonged to two distinct clades (I and II; Fig. 1D-G versus N-Q). Thus we tried to separate them on the basis of interphase nuclei structure. There was an obvious congruence between nuclear shape and heterochromatin distribution of the strains and their position in the LSU rDNA phylogeny (Figs 1H-M, 2). Each LSU rDNA clade had a different nuclear structure (Fig. 1H-M) and the same structure was shared by all strains within each clade (not illustrated). *N. cryptocephala* I had two densely staining peripheral plaques of heterochromatin, lying opposite each other, one adjacent to each chloroplast (Fig. 1K). Nucleoli were not obvious. By contrast, *N. cryptocephala* II had a central mass of heterochromatin that occupied well over half the diameter of the nucleus (Fig. 1L) and one or two peripheral nucleoli. *N. trivialis* had a rhombic nucleus with one nucleolus in the centre surrounded by heterochromatin (Fig. 1M). A small, rounded and pale nucleus (Fig. 1H) without a nucleolus was characteristic for *N. cryptotenella*, whereas *N. gregaria* had a large, rounded nucleus with one nucleolus in the centre (Fig. 1J). *Navicula veneta* had an oval-shaped nucleus with irregular heterochromatin knots (Fig. 1I).

In contrast to *N. cryptocephala* and *N. trivialis*, auxosporulation was not observed in *N. veneta*, *N. gregaria* and *N. cryptotenella*. However, the number of strains under study in *N.*

veneta, *N. gregaria* and *N. cryptotenella* was insufficient for any conclusions to be made regarding their modes of reproduction and mating systems.

Navicula cryptocephala/N. trivialis Species Complex

The secondary structures of ITS2 rRNA were compared among the three lineages in the *N. cryptocephala*/*N. trivialis* species complex (Fig. 4) to determine compensatory base changes (CBCs, including hemi-CBCs) according to Coleman (2000, 2003). Between the two N. cryptocephala clades (I, II) and the N. trivialis clade (III), two CBCs and 12 or 14 hemi-CBCs were found, respectively. One CBC and 9 hemi-CBCs were identified between the two N. cryptocephala clades.

Strains of *N. cryptocephala* and *N. trivialis* exhibited homothallic and automictic auxosporulation, suggesting that they are reproductively isolated.

Strains of both clades of *N. cryptocephala* (I, II) were homothallic and their reproductive modality was exactly the same as previously described in 6 populations from the UK by Poulíčková and Mann (2006). Only three strains belonging to clade I (HV10, 228LIS and 340LU) did not exhibit any reproduction, because their cells were above the sexual size range (the upper threshold for sexual reproduction in *N. cryptocephala* is approximately 20 μm; Poulíčková and Mann 2006). Briefly, gametangia paired via the girdle, one gamete was formed per gametangium and hence one zygote (auxospore) was produced per pair of gametangia. All possible pairwise combinations of 4 strains within the sexual size range (clade I – 461R and 463R; clade II – 28L and 29L) were crossed. Although strains differed in cell size, thus potential interclonal pairing/auxosporulation should be possible to recognize, no changes in sexual behaviour were observed during crossing experiments.

Navicula trivialis strains reproduced by vigorous intra-clonal auxosporulation, with auxospores being produced by single, unpaired cells (Fig. 5). Uniparental (automictic) sexual reproduction in *N. trivialis* is more accurately classified as paedogamy, because cytokinesis was observed after the first meiotic division in a few cases under inverted microscope (not illustrated). This is the first time that this type of reproduction has been observed in *Navicula* sensu stricto. One parental cell of *N. trivialis* (Fig. 5A, B) produced 2 gametes (with 2 nuclei in each); these gametes fused again (without gamete rearrangement – not illustrated) within slightly pushed apart parental thecae. The single zygote (Fig. 5C, D) expanded to an auxospore (Fig. 5E-G). The superfluous nuclei from meiosis survived sufficiently long so that zygotes had 2 functional and 2 slowly degenerating pycnotic nuclei. The two unfused nuclei

were also visible in the expanding auxospore (Fig. 5E-G). On the 13th May 2008 all possible pairwise combinations of the 4 strains of *N. trivialis* with cells in the sexual size range (strains B145, O/70, HV5 and HV25) were crossed. However, no changes in the pattern or intensity of sexual behaviour or auxospore formation were observed.

Due to the similar valve morphologies of both *N. cryptocephala* clades and due to unresolved phylogenetic relationships among the *N. cryptocephala*/*N. trivialis* clades, we also investigated the morphology of selected strains in detail, using cytological characters (interphase nuclei structure see above) and a geometric morphometric approach. Cells at comparable life cycle stages were not available for all investigated strains, consequently the allometric shape change, i.e. the change in the shape of the frustules that is related to their size dynamics, was evaluated by multivariate regression of GPA-aligned shape data on centroid size of frustules. It spanned 17.1% of the total variation and was strongly significant on the basis of permutation tests (Wilk's λ=0.279, permutation p-value=0.001). Therefore, the shape variation related to size was removed using the multivariate regression; the allometry-free mean landmarks configurations of strains are illustrated in Figure 6. The strains subjected to these analyses (altogether 12 strains, approximately 30 cells of each strain) are marked by asterisks in Table 3. There were some obvious shape differences between individual configurations. The four strains of *N. trivialis* (clade III) were typified by relatively wide frustules with indistinctly capitate apical valve ends and wide radial central areas. In contrast, the frustules belonging to both *N. cryptocephala* clades had more pronounced capitate apical ends and a more variably shaped central area, but there were no obvious clade specific shape features visible on the mean configurations of individual strains assigned as *N. cryptocephala*. The principal component analysis (PCA) of the entire dataset resulted in 17 non-zero PC axes, which were used in all subsequent analyses (Fig. 7a). Strains belonging to the *N. trivialis* clade formed a distinct cluster; in contrast, the eight strains belonging to the two *N. cryptocephala* clades were not clearly separated from each other. The canonical variate analysis yielded similar results (Fig. 7b). However, the underlying MANOVA indicated strongly significant differentiation between the three clades (Wilk`s λ=0.283, p-value<10^{-20}). Furthermore, all the individual strains differed significantly in pair-wise comparisons based on the shape data. The Hotelling's T2 test was highly significant ($p<0.0001$) in all pairs of strains and the average correct discrimination of individual cells on the basis of shape data reached 99.2% (Table 4). The lowest correct discrimination level was between strains O/71 and O/26 (91.4%) and between strains 460R and 647K (91.5%), which belonged to different

N. cryptocephala clades. The clade-level differences in shape were also highly significant (Hotelling's T^2, $p<10^{-10}$ in all three pairs), but the correct discrimination values differed strongly. There were 98.3% and 94.9% correctly discriminated frustules between the two *N. cryptocephala* clades and *N. trivialis*. On the other hand, just 78.1% of frustules were correctly discriminated between the two *N. cryptocephala* clades.

Discussion

It is much debated whether microorganisms are easily dispersed globally or whether they have historical biogeographies. The ubiquitous dispersal hypothesis states that microorganisms <1 mm in length are so abundant and so easily dispersed that all should be globally distributed and found wherever growing conditions suit them (Fenchel and Finlay 2004; Finlay 2002). The alternative view is that at least some microbial species show restricted distributions, as demonstrated in freshwater habitats (Telford et al. 2006, 2007; Theriot et al. 2006; Vyverman et al. 2007).

Finlay et al. (2002) discussed four common diatom species as an example of diatom cosmopolitanism within a dataset based on published works, surveyed from the Web of Science and the Fritsch Collection of Freshwater Algal Illustrations (http://www.fritschalgae.info). These included *Cyclotella meneghiniana* Kütz., *Gomphonema parvulum* (Kütz.) Kütz., *Nitzschia palea* (Kütz.) W. Sm and *Navicula cryptocephala* Kütz. All four traditional species have been considered widely distributed and ecologically euryvalent (Krammer and Lange-Bertalot 1997). The former three species have been recognized as taxonomically complex, as reflected in the number of varieties and forms that have been described within them according to the standard freshwater diatom flora (Krammer and Lange-Bertalot 1997). Furthermore, Beszteri et al. (2005, 2007) have shown that *C. meneghiniana* is highly diverse genetically, probably containing several or many biological species. Contrary to the three previously mentioned species, frustule morphology is very similar in *N. cryptocephala* populations (Geitler 1958, Poulíčková and Mann 2006) and consequently, on the basis of LM frustule morphology, cryptic diversity would not be expected. However, this species has been found to be polymorphic with respect to nuclear structure (Geitler 1951, 1952a, b, 1958; Poulíčková and Mann 2006). Geitler (1951, 1952a, b, 1958) was able to distinguish several *N. cryptocephala*-like populations (*N. cryptocephala typica* I, II, *N. cryptocephala* var. *veneta* I, II, N. *cryptocephala* var. *intermedia*) at just a few

Austrian localities (Lunz Untersee, Altewasser Donau) on the basis of nuclear cytology and/or reproductive characters (reviewed by Poulíčková and Mann 2006, Table 2). Our results correspond to Geitler's observations with respect to species-specific cytological characteristics. However, we demonstrated that his concept of *N. cryptocephala* was too wide and therefore all his nuclear structure types do not belong to *N. cryptocephala* sensu stricto. All diatom species in our study (*N. cryptocephala*, *N. trivialis*, *N. cryptotenella*, *N. veneta*, *N. gregaria*) can be characterized by specific interphase nuclear structure types (i.e. shape and heterochromatin distribution, and presence/absence and position of nucleoli, see Fig. 1). Moreover, on the basis of nuclear cytology we were able to distinguish pseudocryptic species within *N. cryptocephala* (clades I and II) which were not recognizable by classic frustule morphology.

The molecular data presented here confirmed the clear separation of *Navicula* sensu stricto morphospecies and revealed a close relationship between *N. trivialis* and *N. cryptocephala*, even closer than that of *N. veneta* and *N. cryptocephala*. *Navicula veneta*, previously *N. cryptocephala* var. *veneta* (Kützing) Rabenhorst is morphologically similar to *N. cryptocephala* in contrast to *N. trivialis*, previously *N. lanceolata* sensu Kützing, which is a significantly more robust diatom (Table 1). Moreover molecular data confirmed pseudocryptic diversity within *N. cryptocephala*. Two lineages of *N. cryptocephala*, clades I and II, which were revealed by LSU rDNA and ITS2 phylogenies, seem to represent two different species. The presence of compensatory base changes (CBCs) and hemi-CBCs among sequences of *N. cryptocephala* also suggests that the *N. cryptocephala* lineages could be reproductively isolated (Amato et al. 2007; Casteleyn et al. 2008; Coleman 2005), which agrees with our limited data on compatibility during sexual reproduction. Clones of both clades were clearly within the sexual size range, because reproduction occurred in monoclonal cultures (both clades are homothallic), but no inter-clonal pairing was observed in crosses of clones (461R, 463R, 28L, 29L).

The variation in the number of auxospores per pairing cells found by Geitler between different populations of *N. cryptocephala*-like diatoms (Poulíčková and Mann 2006, Table 2) can be explained on the basis of the mode of reproduction, described here for *N. trivialis*. The most recent studies on automictic and homothallic pennate diatoms (Poulíčková 2008; Poulíčková and Mann 2008) indicate that these methods of reproduction evolved from allogamy. Reproduction in *N. trivialis* differs from that in *N. cryptocephala* clades I and II essentially by the presence of cytokinesis during gametogenesis; therefore ancestral species

with allogamous reproduction would have 2 gametes per gametangium and thus 2 zygotes/auxospores per pairing gametangia. Two auxospores per pair have been recorded by Geitler (1958) in N. *cryptocephala* ‘‘typica II’’ with long gametangia (up to 34.5 mm), corresponding with gametangial length in *N. trivialis* (a larger diatom than N. cryptocephala, cf. Table 1). However, the nuclear structure type does not correspond with *N. trivialis*, as well as the reproduction method. Unfortunately, because of the wide *N. cryptocephala* concept used by Geitler, we cannot be exactly sure which species of *Navicula* Geitler observed.

Although all investigated clades can be characterized by nuclear cytology (heterochromatin distribution), such a characteristic is not very useful for clade/species identification, because it requires special techniques and equipment not commonly used by diatomists (DAPI staining, FM). Thus we aimed to find a useful method for their identification. In accordance with the results of Veselá et al. (2009), the geometric morphometric data illustrated the size-independent strain-specific shape differences in naviculoid diatoms. The strains from the two clades of traditionally recognised *N. cryptocephala* were more similar to each other than to strains of *N. trivialis*. Veselá et al. (2009) demonstrated that the strain-specific differences in shape were identifiable even between cells in different stages of the life cycle. However, discrimination was more successful in cells at identical life cycle stages (large post-initial or small sexually competent cells). Here, we removed variation that was possibly caused solely by differences in life cycle stages (cell size) among investigated strains. We believe that allometry-free geometric morphometrics could become one of the most useful methods in quantitative studies of morphological variation of diatoms. This method represents the only suitable technique for comparison of both *N. cryptocephala* clades with type material of *N. cryptocephala* deposited in the Natural History Museum London, as the type material is already mounted in permanent slides and molecular and cytological methods cannot be used. Although morphological differences between *N. cryptocephala* clades were less pronounced (78.1% correctly discriminated frustules) than differences between frustules of individual strains (the average correct discrimination 99.2%), comparisons with the type material will show which of our *N. cryptocephala* clades is more similar to *N. cryptocephala* and which of them needs to be described as a new species. Geometric morphometrics has been successfully used for comparison with type slides in other pennate diatoms (Fránková et al. 2009).

Detection of cryptic/pseudocryptic diversity in microalgae introduced doubts concerning their cosmopolitanism. Genetic, reproductive and morphological variation has been studied in diatoms to assess potential intraspecific variation and biogeographic distribution patterns

(Casteleyn et al. 2008; Kooistra et al. 2008). Despite some limitation (e.g. only 52 strains), our molecular data demonstrated that the cosmopolitanism of diatom species should not be rejected solely on the basis of their pseudocryptic diversity. Both pseudocryptic species of *N. cryptocephala* (clades I and II) were found in British, central European and Australian localities. A similar distribution pattern has been documented for *Sellaphora capitata* (Evans et al. 2009). What we do not yet know is whether these wide distributions were achieved naturally, or whether humans have mediated dispersal. For example, *N. cryptocephala* could have been introduced to Australia when lakes were stocked with European fish in the 1880s (Crowl et al. 1992; Evans et al. 2009) since there is evidence that other freshwater diatoms have been introduced over the same time-scale (Harper 1994; Kilroy et al. 2008). Paleolimnological methods again in combination with geometric morphometrics can be used to confirm/reject this hypothesis. Moreover, both *N. cryptocephala* clades coexist sympatrically at Obectov (Czech Republic) and Kew Billabong (Australia). Sympatric coexistence of different demes was observed in *N. cryptocephala* sensu lato by Geitler (1951, 1952a, b, 1958), and in other epipelic or epiphytic freshwater diatoms (e.g. the *Sellaphora pupula* complex, Evans et al. 2008; the *Eunotia bilunaris* complex, Vanormelingen et al. 2008). The frequent co-occurrence of several to many closely related and morphologically similar species in lake/pond epipelon is paradoxical, given conventional niche theory (Hutchinson 1961; Mann et al. 2008). The possibility that sympatric semicryptic species may co-occur because they have different seasonal occurrences has been tested by Špačková et al. (2009). Morphotypes within the *S. pupula* species complex observed in a mesotrophic pond in a temperate climate, differed in their seasonal occurrence and relations to temperature. Most of the *Sellaphora* morphotypes were negatively correlated with water temperature; positive correlation was exhibited only by the ‘‘elliptical’’ morphotype. Morphospecies without any temperature/seasonal preferences seemed to be morphologically heterogeneous (Špačková et al. 2009).

Material and methods

Isolates, their origin and culture: We used 52 strains belonging to five morphologically similar species: *Navicula cryptocephala* Kützing; *N. veneta* Kützing; *N. gregaria* Donkin; *N. cryptotenella* Lange-Bertalot; and *N. trivialis* Lange-Bertalot. All strains were isolated by A. Poulíčková except those from Australia which were isolated by Prof. D.G. Mann (Royal Botanic Garden Edinburgh). Culture Poulíčková). Samples of lake/pond littoral epipelon were collected between 2004 and 2008 in

Great Britain (Poulíčková et al. 2008), the Czech Republic (Poulíčková et al. 2009), Austria and Australia (Table 2). Information on environmental variables and species composition of algal assemblages at the different sampling sites have been published elsewhere (Hašler et al. 2008; Hašler and Poulíčková 2010; Poulíčková et al. 2008, 2009).

Sediment samples were collected using a glass tube, as described by Round (1953), and transported to the laboratory in polyethylene bottles. The mud-water mixtures were then poured into plastic boxes and allowed to stand in the dark for at least 5 h. The supernatant was removed by suction and the mud covered with lens tissue on which a coverslip was placed. Under continuous low-level illumination (c. 5 µ mol photons m^{-2} s^{-1}), epipelic algae moved up through the lens tissue (trapping detritus and inorganic particles) and became attached to the cover slips placed on top. Captured diatoms were isolated by streaking onto WC medium with silicate (Guillard and Lorenzen 1972; adjusted to pH 7 with drops of 1 M HCl) solidified with 2% agar. After 3 weeks, single strains of small naviculoid diatoms were subcultured from discrete colonies and transferred to liquid WC medium. Cultures were kept in 50 mm petri dishes, at 15–20 °C, with an irradiance of 5–20 µmol photons m^{-2} s^{-1} provided by cool-white tubes; the photoperiod was usually 12:12 h light:dark.

Identification and microscopy: Voucher material for each strain was cleaned with a mixture of concentrated sulphuric and nitric acids and mounted in Naphrax as described by Poulíčková and Mann (2006); vouchers are deposited at the Department of Botany, Palacký University Olomouc. Diatom morphospecies were identified according to Krammer and Lange-Bertalot (1997). Relative abundances of individual diatom species were estimated by counting 400 individuals from each sample. Living cells and sexual stages were observed using light microscopy (LM) following the methods described by Poulíčková and Mann (2006) and Poulíčková et al. (2007).

Samples for nuclear cytology studied using epifluorescence microscopy (FM) were fixed with PGA solution (2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.0; Karnovsky 1965). Interphase cells were obtained from cultures synchronized by photoperiod and identified using an inverted microscope on the basis of nucleus and chloroplasts arrangement (Poulíčková and Mann 2006). Nuclei were stained with DAPI (4,6-diamino-2-phenylindole.2HCl; Sigma, St. Louis, MO, USA) as described by Poulíčková et al. (2007). Photomicrography (LM and FM) was carried out using a Zeiss Axioimager with a Zeiss Axiocam HRc digital camera (Carl Zeiss, Jena) at 1388_1040 pixel resolution. Images were captured and edited using Zeiss Axiovision Version 4.5 imaging software. Bright field (BF) or differential interference contrast (DIC) optics were used at ×100, ×40 or ×63 (planapochromat lenses, nominal numerical aperture 1.32, 1.4 and 0.95). For FM a Zeiss DAPI filter set 001 and 49 with the same objectives was used.

DNA extraction, PCR and DNA sequencing: For molecular analyses, cells were harvested from the bottom of petri dishes by removing the overlying medium, scraping off the cells and transferring them into sterile plastic Falcon tubes. Harvested cells were mechanically broken using glass beads and

undiluted and 100× diluted samples were preserved at -20 °C. In order to minimize the loss of DNA, a ‘‘single-cell’’ polymerase chain reaction (PCR) approach was followed (e.g. Duff et al. 2008) using samples with broken cells. Two nuclear rDNA regions (D1-D2 28S, LSU and ITS1–5.8S–ITS2, ITS) were amplified using primers D1R-D2C (Yeung et al. 1996) and newly designed primers (ITS1-D forward: 5′-CCTGCGGAAGGATCATTA-3′ and LSU-DR1 reverse: 5′-CTTCAGTCGCCCTTACT-3′). PCR conditions for the LSU rDNA region followed Yeung et al. (1996). PCR conditions for ITS were 94 °C for 3 min; 35 cycles of 94 °C for 1 min, 51 °C for 1 min, 72 °C for 1 min; and final extension at 72 °C for 10 min. Direct sequencing of the ITS region was in many cases problematic, not only because of the presence of substitutions, but also because of insertions/deletions (indels) longer than 1 bp in sequences within the strain. For example, three or 13 bp long indels were found in ITS2 variants within Navicula veneta strains (sequences not published). ITS intra-clonal sequence variation was investigated by cloning the PCR products (amplification primers 1617F: Prof. T. Friedl, unpubl. and LSU-DR1) into pJET1.2 cloning vector following the Sticky-End Cloning Protocol (JET PCR Cloning Kit, Fermentas). Amplification primers 1617F are available on request at University of Göttingen. Plasmid DNA was re-amplified using the ITS1-D and LSU-DR1 primers. PCR products were purified by Invisorb Fragment Clean Up Kit or JET quick PCR product purification Spin Kit and sequenced by Macrogen Inc (South Korea). The resulting sequences were edited using the SeqAssem software (Hepperle 2004).

Sequence alignment, rRNA secondary structure construction and DNA analyses: The alignments of the LSU rDNA (http://botany.natur.cuni.cz/algo/align/01_cryptocephala-like.fas) and ITS2 (.../02_cryptocephala-like.fas) regions were done manually on the basis of their rRNA secondary structures using MEGA 4 (Kumar et al. 2008). The secondary structure of the LSU rDNA region (HV10 strain; Supplementary Fig. 1) was constructed in accordance with the published secondary structure of *Psammoneis japonica* (Sato et al. 2008). The secondary structures of ITS2 (Fig. 4) were constructed for closely related taxa of *Navicula cryptocephala* and *N. trivialis* using the mfold software version 2.3 (Zuker 2003), with folding temperature set to 25 1C. The common secondary structures of the ITS2 rRNA were created using RnaViz version 2 (de Rijk et al. (2003)) and used to identify compensatory base changes (CBCs) and hemi-CBCs (Coleman 2000, 2003). The ITS2 region was chosen because ITS2 appears to be an appropriate marker for the discrimination of biological species (Amato et al. 2007).

The phylogenetic trees were inferred with Bayesian inference (BI) using MrBayes version 3.1 (Ronquist and Huelsenbeck 2003). Two parallel MCMC runs were carried out for 2 million generations, each with one cold and three heated chains. Trees and parameters were sampled every 100 generations. Convergence of the two cold chains was checked and burn-in (100 trees) was determined using the ‘sump’ command. In both LSU rDNA and ITS2 datasets, different substitution models were selected for stem and loop partitions, as extracted from the RNA secondary structure

information. For the loop regions, a 4-state, single-nucleotide substitution model was selected, while for the paired stem regions, the doublet model (a 16-state RNA stem substitution model; Schöniger and von Haeseler 1994) was selected (Leliaert et al. 2007; Verbruggen and Theriot 2008). In the ITS2 dataset, the highly variable region between the 2nd and 3rd stem was deleted, resulting in an alignment of 22 bp. In the LSU rDNA dataset, the invariable regions B22 and B23 were deleted to discard stem partitions paired with non-sequenced LSU regions, resulting in an alignment of 52 bp. The most appropriate substitution model was estimated for each partition using the Akaike Information Criterion (AIC) with PAUP/MrModeltest 1.0b (Nylander 2004). In the LSU rDNA dataset, HKY+G and GTR+G substitution models were estimated for stems and loops, respectively. In the ITS2 dataset, the GTR model was estimated for stems, whereas the HKY model was chosen for loops. Bayesian posterior probability values were obtained for phylogenies that included identical sequences, but the Bayesian consensus tree was reconstructed without identical sequences.

Bootstrap analyses were performed adopting maximum likelihood (ML) and weighted parsimony (wMP, character weighting) criteria using PAUPn, version 4.0b10 (Swofford 2002). ML analyses consisted of heuristic searches with 1000 random sequence addition replicates and Tree Bisection Reconnection (TBR) swapping. Reliability of the resulting topology was tested using bootstrap analysis (100 replications) consisting of heuristic searches with 10 random sequence addition replicates, TBR swapping, and a rearrangement limit of 5000 for each replicate. The wMP bootstrapping was performed using heuristic searches with 100 random sequence addition replicates, TBR swapping, random addition of sequences (the number limited to 10,000 for each replicate), and gap characters treated as missing data.

The LSU rDNA sequences were rooted using two alternative outgroups, Hippodonta capitata (Ehrenberg) LangeBertalot, Metzeltin et Witkowski (GenBank accession AM710521) and Nitzschia palea (Kützing) W. Smith (AM183244) selected on the basis of recently published phylogenies of pennate diatoms (Bruder and Medlin 2008). Since both rooting strategies resulted in uniform BI (Bayesian inference) topologies, we selected Hippodonta capitata, the closer relative of Navicula sensu stricto species, as the root. In contrast, unrooted BI analysis was chosen to infer the ITS2 rDNA phylogeny of the Navicula cryptocephala/N. trivialis species complex, since no related Navicula species could be aligned to our sequences.

Geometric morphometrics: The morphometric differentiation of diatoms belonging to three related clades (*N. cryptocephala* I ‘‘RBG’’, *N. cryptocephala* II ‘‘Lubnaig’’ and *N. trivialis* III) was evaluated using landmark-based geometric morphometrics (Zelditch et al. 2004). In total, 48 landmarks were digitalized on approximately 30 cells of each strain. Ten landmarks were depicted in fixed positions: intersections of a cell outline with apical (2) and transapical (2) axis; the raphe central endings (2) and ends of the longest striae in the central area (4). The remaining points placed along the outline (32) and central area (6) were sliding landmarks (semi-landmarks sensu Bookstein 1997). For

most analyses the TPS-series software (Rohlf 2007) was used. As the valve symmetry could not always be determined from the micrographs focused primarily on the valve outline, we symmetrized the landmark configurations both along the apical and transapical axes. The symmetrized data aligned by general Procrustes analysis (GPA) were used for the thin-plate spline analysis that resulted in partial warp scores and the uniform component spanning the shape variation of the data set (Zelditch et al. 2004). Size differences among cells resulted in strong allometric effects that spanned 17.1% of the total variation in multivariate regression of the entire data set. Shape allometry in pennate diatoms has repeatedly been ascribed to the diminution series resulting from the vegetative life cycle (Round et al. 1990; Veselá et al. 2009). As these allometric shape changes may obscure actual differences between individual clades, the allometric effect was removed by multivariate regression of Procrustes aligned data. The residuals this regression were added to the overall consensus configuration so that the resulting data set did not involve allometric shape variation (Debat et al. 2003). The principal component analysis (PCA) of this data set was conducted and the non-zero PC axes were used in all the subsequent analyses. The pattern of discrimination between individual clades was illustrated by the canonical variates analysis (CVA) in PAST, ver. 1.89 (Hammer et al. 2001). Significance of discrimination was evaluated by the multivariate analysis of variance (MANOVA) with permutation p-values based on Wilk's λ (1000 permutations). The discrimination of frustules from individual strains and percentages of correctly discriminated cells were evaluated by series of linear discrimination analyses accompanied by the Hotelling's T^2 tests in all pairs of strains.

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Appendix. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.protis.2009.12.003.

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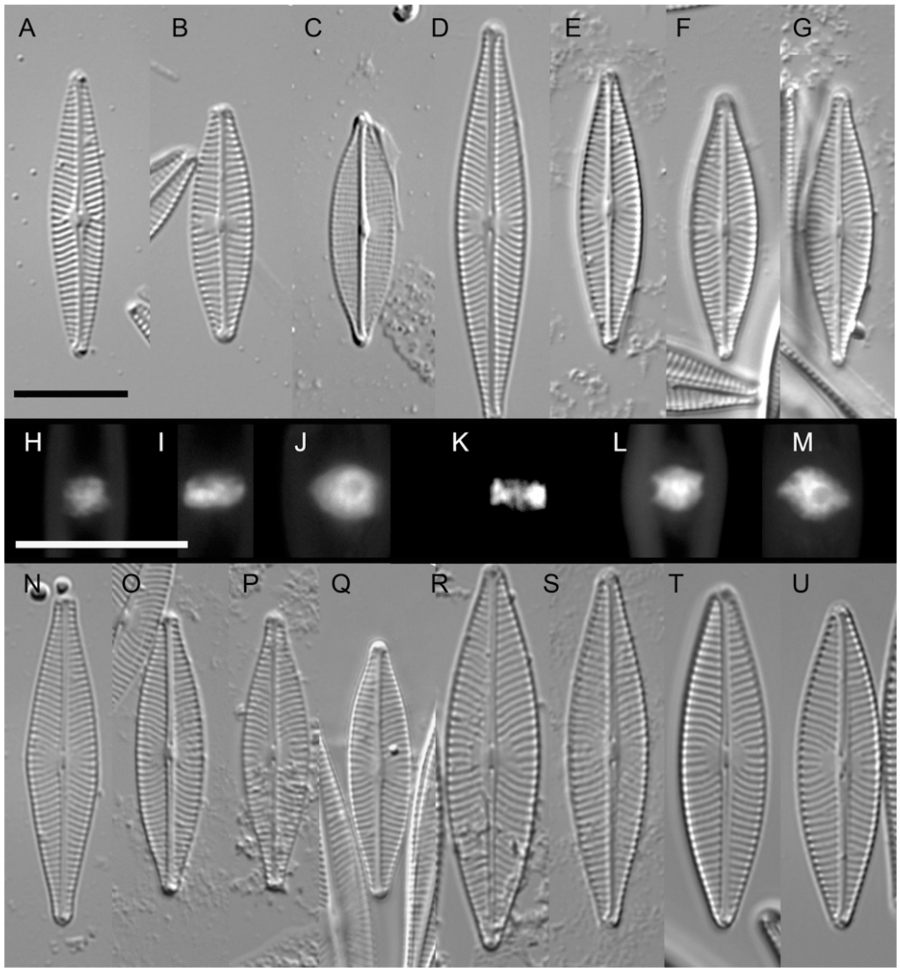


Figure 1. Light micrographs of cleaned valves (**A-G**, **N-U**) and interphase nuclear structure in epifluorescence (**H-M**) of the studied *Navicula* species: **A**,**H** – *Navicula cryptotenella*, **B**,**I** - *N. veneta*, **C**,**J** – *N. gregaria*, **D-G**,**K** - *N. cryptocephala* clade I, **N-Q**,**L** – *N. cryptocephala* clade II, **R-U**,**M** – *N. trivialis* clade III. Scale bar = 10 μm

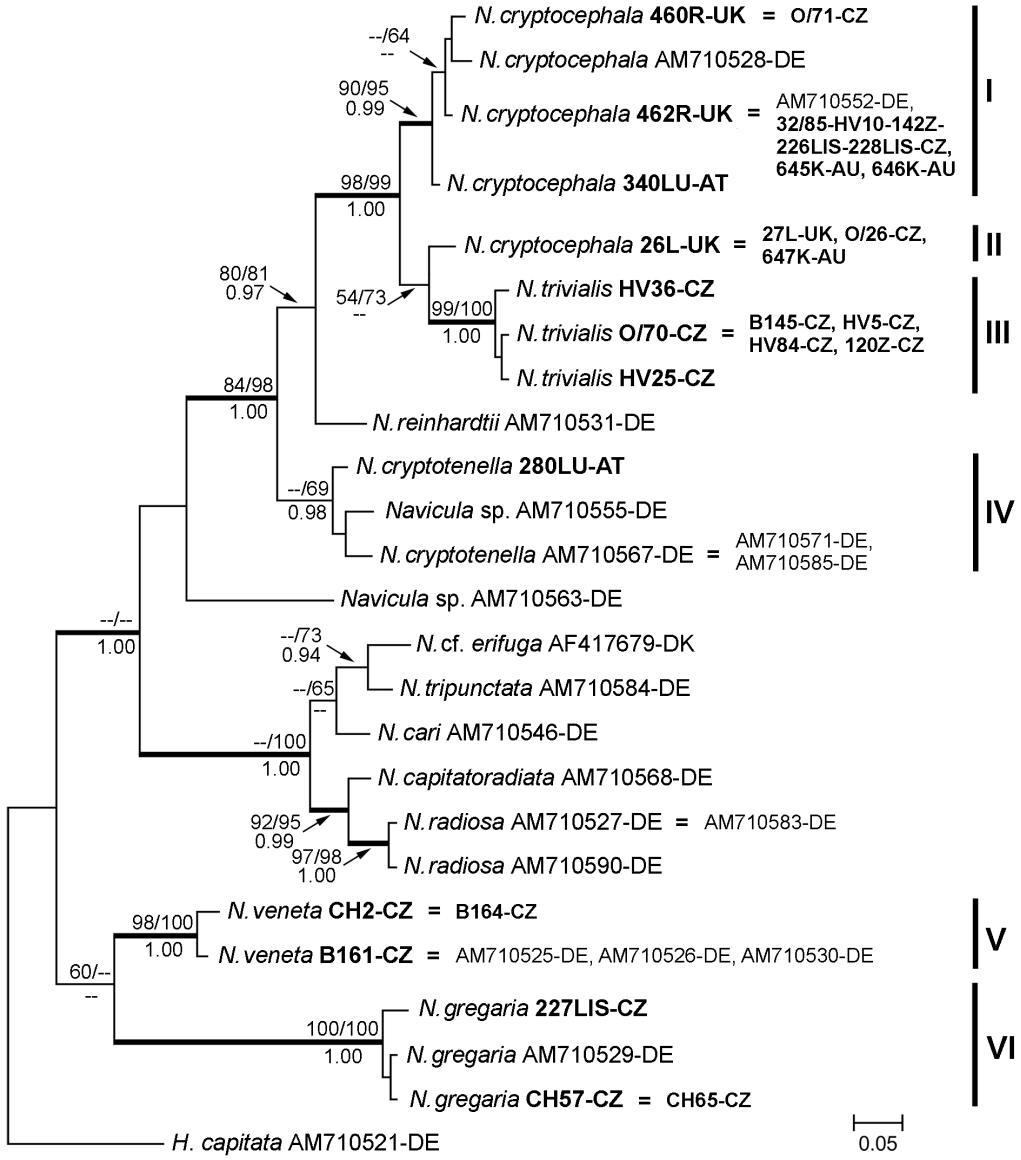


Figure 2. Bayesian tree of D1-D2 28S rDNA sequences from *Navicula* sensu stricto strains with *Hippodonta capitata* as outgroup. Published sequences are identified by their GenBank accession numbers and sequences from this study are indicated by the strain code in bold. Equals signs indicate identical sequences. Bootstrap values below 50% (ML/MP) or Bayesian posterior probability values (PP) below 0.9 are not given. The thick lines indicate the branches with PP > 0.98. Clade I - *N. cryptocephala* I, clade II - *N. cryptocephala* II, clade III - *N. trivialis*, clade IV - *N. cryptotenella*, clade V - *N. veneta*, clade VI - *N. gregaria*.

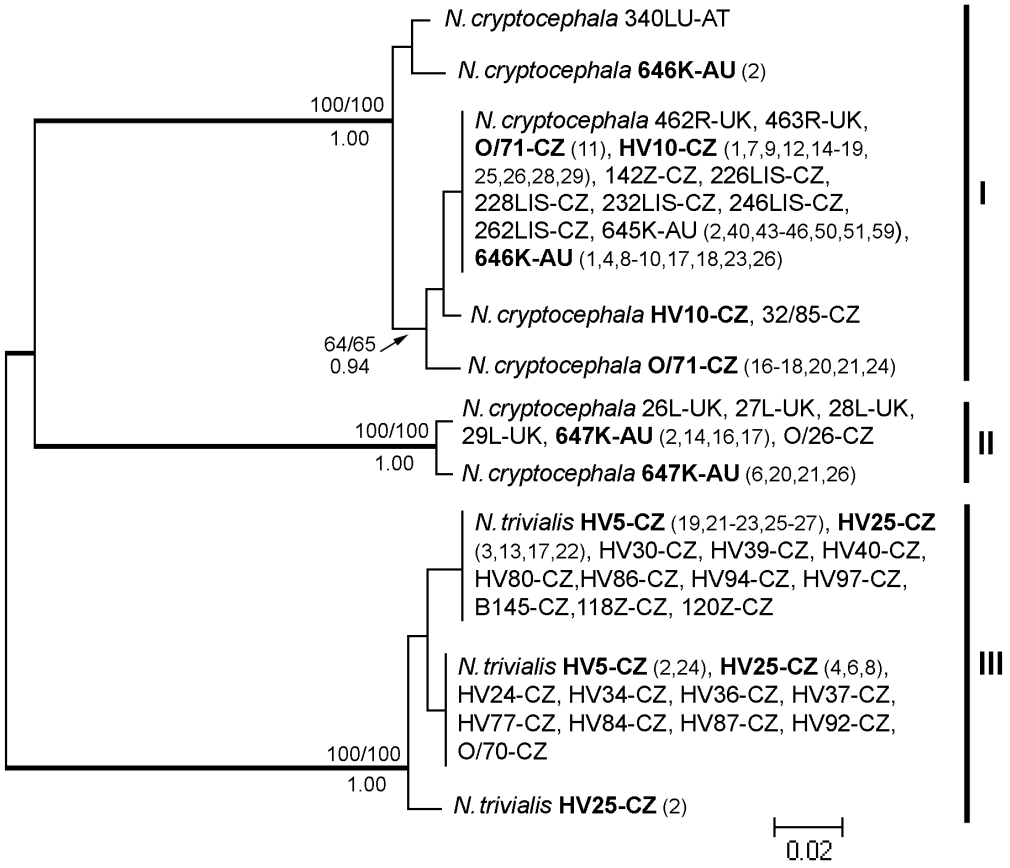


Figure 3. Unrooted Bayesian tree of ITS2 rDNA sequences from inter- and intra-genomic sequence variants of *Navicula cryptocephala* and *N. trivialis*. Smaller numbers in brackets indicate the different bacterial clones sequenced; intra-isolate ITS2 variants are in bold. Bootstrap values below 50% (ML/MP) or Bayesian posterior probability values (PP) below 0.9 are not given. The thick lines indicate the branches with PP > 0.98. Clade I - *N. cryptocephala* I, clade II - *N. cryptocephala* II, clade III - *N. trivialis*.

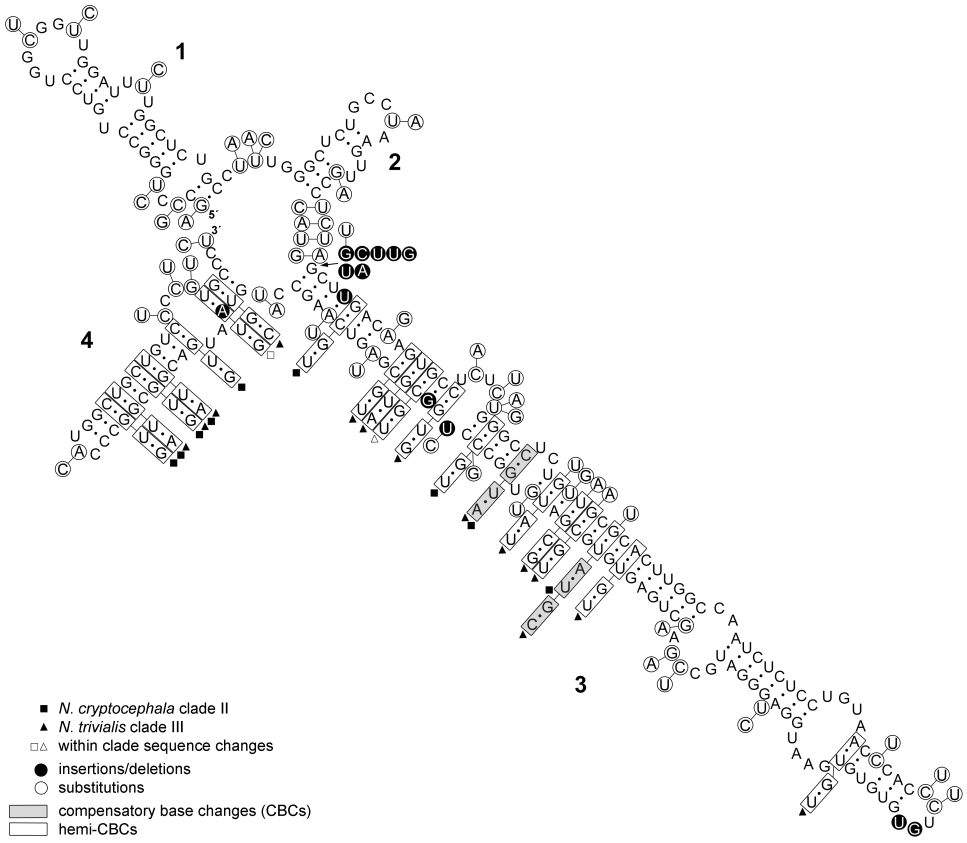


Figure 4. Diagram of ITS2 secondary structure of *Navicula cryptocephala* (HV10, clade I) derived by comparisons among inter- and intra-clonal variants of *N. cryptocephala*/*N. trivialis*. Base changes between different genotypes are indicated and explained in the figure.

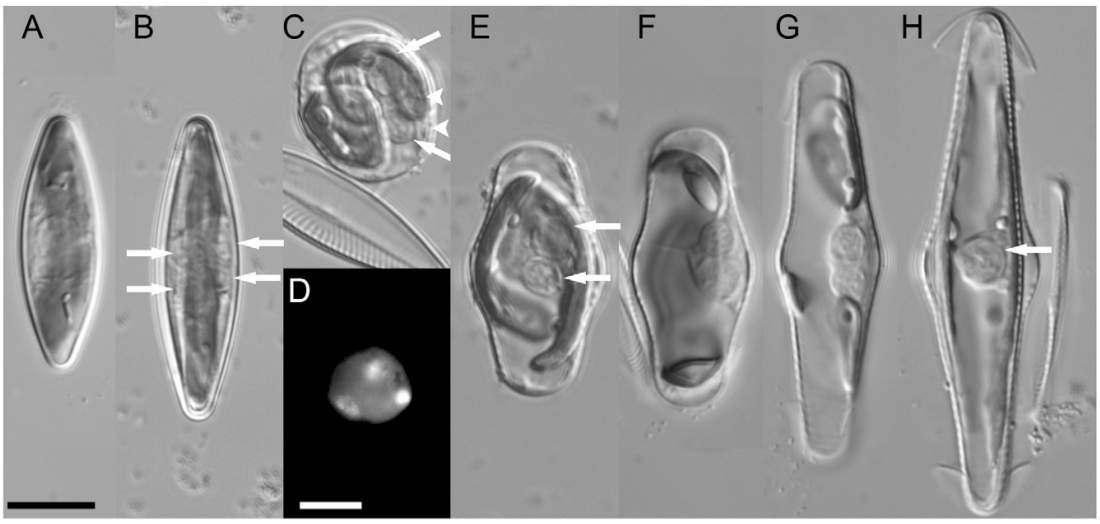


Figure 5. Light micrographs (DIC, except for **D**) illustrating uniparental (automictic) auxosporulation in *N. trivialis*. **A**, **B**: gametangium with 4 nuclei (arrows) after meiosis II, **C**: zygote with 2 functional (arrows) and 2 pycnotic (arrowheads) nuclei, **D**: epifluorescence micrograph of a zygote with 3 nuclei (fourth out of focus), **E-G**: expanding auxospore with 2 unfused nuclei (arrows), **H**: mature auxospore with initial cell inside and 1 nucleus after fusion (arrow).

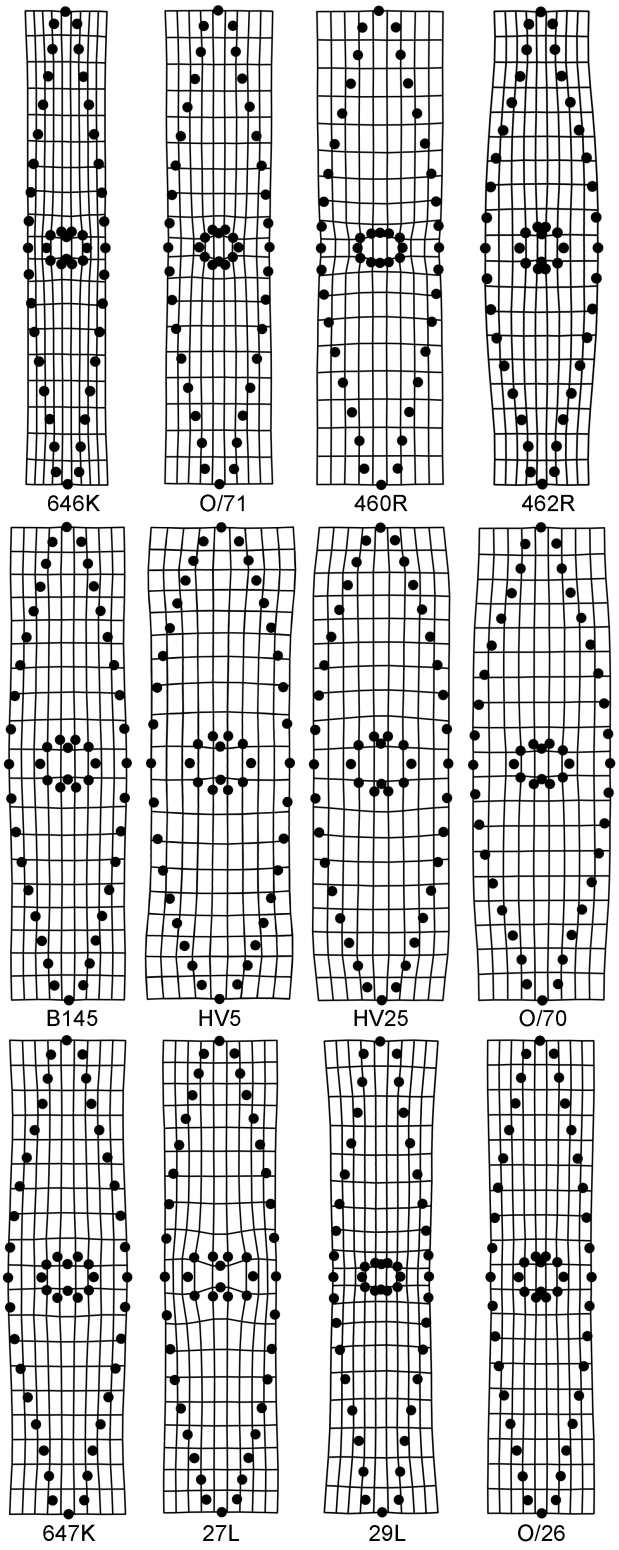


Figure 6. Characteristic non-allometric shape of strains is reconstructed by the deformation of thin-plate spline from mean configuration. First four strains *Navicula cryptocephala* clade I, second four strains *N. trivialis* clade III, the last four strains *N. cryptocephala* clade II.

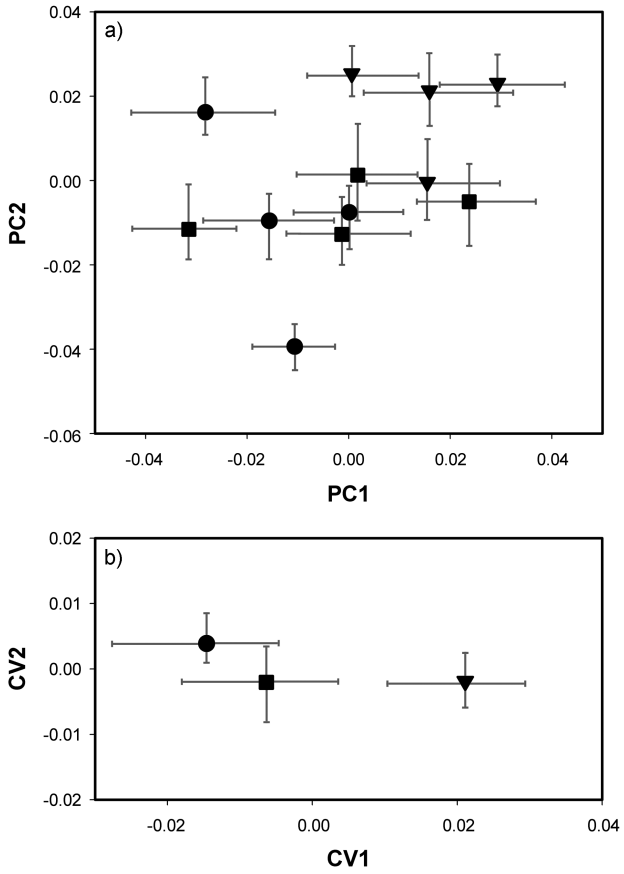
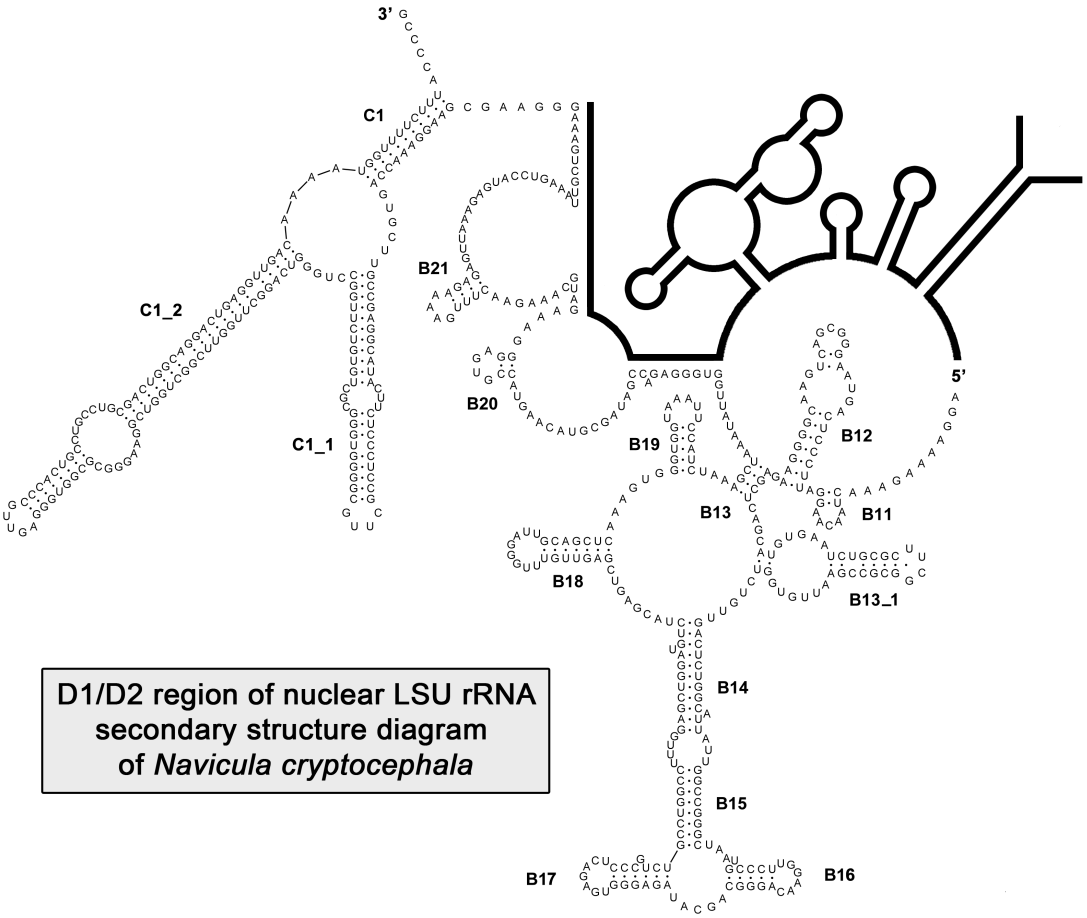


Figure 7. Centroids of strains with the 25th and 75th percentile calculated from a) principal component analysis (PCA) and b) canonical variate analysis (CVA) using geometric morphometric data. Circles represent *Navicula cryptocephala* clade I, squares *N. cryptocephala* clade II and triangles *N. trivialis* clade III.



Supplement. D1/D2 region of nuclear 28S rRNA secondary structure diagram of *Navicula cryptocephala* (HV10 strain).

Table 1. Valve morphological characteristics of *Navicula* species under study in comparison with published data (terminology follows Cox 1995). Abbreviations: L - valve length (µm), B – valve breadth (µm), S – stria density (10 µm^{-1}).

Species	Valve outline shape	Valve apices	Striae pattern	Krammer and Lange-Bertalot (1997)	Poulíčková and Mann (2006)	Strains under study
N. cryptocephala	narrowly lanceolate	subcapitate	radiate central striae	L 20-40 B 5-7 S 14-17	"RBG" L 23.5-48 B 5-8 S 14-18 "Lubnaig" L 25-44 B 5.5-8.5 S 14-18	Clade I: L 24-46 B 6-8 S 14-18 Clade II: L 24-45 B 5.5-8 S 14-18
N. trivialis	broadly lanceolate	slightly drawn out, gently tapered	radiate central striae	L 25-65 B 8-12.5 S11-13	nd	L 28.5-67 B 8.5-13.5 S 9-12
N. cryptotenella	lanceolate	rounded, very slightly drawn out	readiate central striae	L 14-40 B 5-7 S (12)14-16(18)	nd	L 18-25 B 4.5-5 S 16-18
N. veneta	linear-lanceolate	broadly sub-rostrate	central striae weakly radiate	L13-30 B 5-6 S 13.5-15	nd	L 21.5-35 B 5-6 S 12-16
N. gregaria	broadly lanceolate	rostrate	central striae transverse and parallel	L 13-42 B 5-10 S 13-22	nd	L 20-26 B 6-6.5 S 20-22

Table 2. Location (GPS coordinates) and main characteristics (altitude, depth, area, pH, conductivity) of the localities at which strains were isolated and percentage composition of the ivestigated species within epipelic assemblages (counted from voucher slides Poulíčková et al. 2008, 2009). Abbreviations: cond. – conductivity, UK – United Kingdom, CZ – Czech Republic, AU – Australia, AT – Austria, Navcry – *N. cryptocephala*, Navven – *N. veneta*, Navtri – *N. trivialis*, Navgre – *N. gregaria*, Navcrt – *N. cryptotenella*, * strains were isolated, nd – no data.

Locality number	Sampling locality	Sampling date	GPS coordinates	Altitude (m a.s.l.)	Depth (m)	Area (ha)	pH	Cond. $\mu S.cm^{-2}$	Navcry %	Navven %	Navtri %	Navgre %	Navcrt %
1	Royal Botanic Garden Pond UK	02.12.04	N 55°58' W 3°12'	15	2	0.09	7.5	374	2.5*	0	0	0.5	0
2	Loch Lubnaig UK	29.09.05	N 56°16' W 4°17'	123	44.5	249	6.76	48	40.4*	7.7	0	0.9	0
3	Bezedník CZ	01.05.07	N 49°18' E 17°43'	323	2	0.4	9.1	461	8.3	5.2*	3.5*	0.2	0
4	Horní Ves CZ	01.05.07	N 49°17' E 17°42'	316	2	1	8.1	429	14.3*	2.1	8.3*	0	0
5	Záhlinice CZ	10.05.07	N 49°17' E 17°28'	198	1.5	13	7.78	770	12.5*	3.6	22.7*	0.5	0
6	Chropyně CZ	10.05.07	N 49°21' E 17;22'	207	1.5	19	7.68	422	18.7	5.7*	2.4	6.5*	0
7	Hradčanský CZ	15.05.07	N 50°37' E 14°42'	287	2	8	7.57	245	14.8*	0	0	0	0
8	Líšnice CZ	31.05.07	N 49°45' E 16°51'	320	2.5	1.5	7.56	457	9.1*	4*	0.6	4	0
9	Obectov CZ	31.05.07	N 49°43' E 16°55'	329	0,8	0.05	7.26	296	0.2*	0	0*	0	0
10	Lunz Untersee AT	18.05.08	N 47°51' E 15°02'	608	33.7	10	7.6	nd	nd*	nd	nd	nd	nd*
11	Kew Billabong AU	02.12.07	S 37°47' E145°02'	10	1	1	nd	nd	nd*	nd	nd	nd	0

Table 3. Identities and sources of isolates, type of auxosporulation observed, and GenBank accession numbers for D1-D2 28S rDNA (LSU) and ITS1-5.8S-ITS2 rDNA (ITS) sequences. Abbreviations: for locality numbers see Table 1; * strains analyzed by geometric morphometrics; ** cells above sexual size range; auxosporulation: H – homothallic, A – automictic, nd – no auxosporulation observed; ITS accesion numbers: clones numbers in brackets=different bacterial clones sequenced (see Methods).

Strain	species	Locality	Auxo	LSU acc. #	ITS acc. #
460R-UK*	*N. cryptocephala*	1	H	FN397595	–
461R-UK	*N. cryptocephala*	1	H	–	–
462R-UK*	*N. cryptocephala*	1	H	FN397588	FN397596
463R-UK	*N. cryptocephala*	1	H	–	ITS2 as 462R
26L-UK	*N. cryptocephala*	2	H	FN397572	ITS2 as 27L
27L-UK*	*N. cryptocephala*	2	H	As 26L	FN397607
28L-UK	*N. cryptocephala*	2	H	–	ITS2 as 27L
29L-UK*	*N. cryptocephala*	2	H	–	ITS2 as 27L
B147-CZ	*N. veneta*	3	nd	–	–
B161-CZ	*N. veneta*	3	nd	FN397578	–
B164-CZ	*N. veneta*	3	nd	FN397576	–
B145-CZ*	*N. trivialis*	3	A	FN397584	FN397610
HV5-CZ*	*N. trivialis*	4	A	FN397582	FN397611 (clone 21)
HV25-CZ*	*N. trivialis*	4	A	FN397580	FN397614 (clone 6) FN397615 (clone 2)
HV24-CZ	*N. trivialis*	4	A	–	ITS2 as HV25 (clone 6)
HV30-CZ	*N. trivialis*	4	A	–	ITS2 as HV5 (clone 21)
HV34CZ	*N. trivialis*	4	A	–	ITS2 as HV25 (clone 6)
HV36-CZ	*N. trivialis*	4	A	FN397581	ITS2 as HV25 (clone 6)
HV37-CZ	*N. trivialis*	4	A	–	ITS2 as HV25 (clone 6)
HV39-CZ	*N. trivialis*	4	A	–	ITS2 as HV5 (clone 21)
HV40-CZ	*N. trivialis*	4	A	–	ITS2 as HV5 (clone 21)
HV77-CZ	*N. trivialis*	4	A	–	ITS2 as HV25 (clone 6)
HV80-CZ	*N. trivialis*	4	A	–	ITS2 as HV5 (clone 21)
HV84-CZ	*N. trivialis*	4	A	As HV5	ITS2 as HV25 (clone 6)
HV10-CZ	*N. cryptocephala*	4	nd**	FN397590	FN397598 (clone 7) FN397606
HV86-CZ	*N. trivialis*	4	A	–	ITS2 as HV5 (clone 21)
HV87-CZ	*N. trivialis*	4	A	–	ITS2 as HV25 (clone 6)
HV92-CZ	*N. trivialis*	4	A	–	ITS2 as HV25 (clone 6)
HV94-CZ	*N. trivialis*	4	A	–	ITS2 as HV5 (clone 21)
HV97-CZ	*N. trivialis*	4	A	–	ITS2 as HV5 (clone 21)
142Z-CZ	*N. cryptocephala*	5	H	FN397591	FN397599
118Z-CZ	*N. trivialis*	5	A	–	ITS2 as 120Z
120Z-CZ	*N. trivialis*	5	A	FN397585	FN397612
CH2-CZ	*N. veneta*	6	nd	FN397577	–
CH57-CZ	*N. gregaria*	6	nd	FN397579	–
CH65-CZ	*N. gregaria*	6	nd	As CH57	–

32/85-CZ	*cryptocephala*	7	H	FN397589	FN397605
226LIS-CZ	*cryptocephala*	8	H	FN397592	ITS2 as 228LIS
228LIS-CZ	*cryptocephala*	8	nd**	As 226LIS	FN397600
232LIS-CZ	*N. cryptocephala*	8	H	–	ITS2 as 228LIS
246LIS-CZ	*N. cryptocephala*	8	H	–	ITS2 as 228LIS
262LIS-CZ	*N. cryptocephala*	8	H	–	ITS2 as 228LIS
227LIS-CZ	*N. gregaria*	8	nd	FN397586	–
O/26-CZ*	*N. cryptocephala*	9	H	FN397573	FN397616
O/70-CZ*	*N. trivialis*	9	A	FN397583	FN397613
O/71-CZ*	*N. cryptocephala*	9	H	FN397594	FN397597 (clone 11) FN397602 (clone 16)
340LU-AT	*N. cryptocephala*	10	nd**	FN397587	FN397604
280LU-AT	*N. cryptotenella*	10	nd**	FN397575	–
311LU-AT	*N. cryptotenella*	10	nd	–	–
645K-AU	*N. cryptocephala*	11	H	FN397593	FN397601 (clone 40)
646K-AU*	*N. cryptocephala*	11	H	As 645K	FN397603 (clone 2)
647K-AU*	*N. cryptocephala*	11	H	FN397574	FN397608 (clone16) FN397609 (clone 26)

Table 4. Results of linear discriminant analyses (DA, $p < 10^{-5}$) based on quantitative shape characters of strains obtained from geometric morphometrics. Lower part of matrix: number of displaced cells, upper part of matrix: percentage of correctly identified cells into appropriate strain. First four strains *Navicula cryptocephala* clade I, second four strains *N. trivialis* clade III, the last four strains *N. cryptocephala* clade II.

	646K	O/71	460R	462R	B145	HV5	HV25	O/70	647K	27L	29L	O/26
646K	-	100	100	100	100	100	100	100	100	100	100	100
O/71	0	-	98.3	100	98.3	100	100	100	96.6	100	100	91.4
460R	10	1	-	100	93.3	98.3	100	98.3	91.5	100	100	98.3
462R	0	0	0	-	100	100	100	100	100	100	100	100
B145	0	1	4	0	-	98.3	100	94.8	94.9	100	100	100
HV5	0	0	1	0	1	-	100	98.3	100	100	100	100
HV25	0	0	0	0	0	0	-	100	100	100	100	100
O/70	0	0	1	0	3	1	0	-	100	100	100	100
647K	0	2	5	0	3	0	0	0	-	98.3	100	100
27L	0	0	0	0	0	0	0	0	1	-	100	96.6
29L	0	0	0	0	0	0	0	0	0	0	-	100
O/26	0	5	1	0	0	0	0	0	0	2	0	-

2.4 IV. Manuscript

3 CONCLUSIONS

The main results of this thesis can be summarized as follows:

Careful morphological studies of natural populations of diatoms are valuable in the contexts of both taxonomy and ecology, as the broad morphological species concept was not supported by the multidisciplinary approach to species delimitation. Detailed microscopic investigations of natural samples made it possible to assess the morphological variability of rare morphospecies, and even to describe one new species to science.

Morphological changes associated with the size diminution of diatom cells during their life cycle considerably influenced the variability of cell shape; differences between large and small cells were more important than differences between individual strains. The allometric component of shape variability should therefore be considered in species delimitation.

Morphologically similar strains of two model diatom morphospecies were separated by molecular genetic analyses, into two and six phylogenetic lineages, respectively.

Although the traditional morphological species concept underestimates diatom diversity, fine-grained examination of the diatom cell morphology of monoclonal cultures revealed significant variability that was correlated with the genetic diversity.

Genetic differentiation of closely related model diatom species was congruent with reproductive and cytological variations.

Species complexes, within traditional morphospecies, were widely distributed; and in many cases lived sympatrically. Nonetheless, individual lineages showed different distribution patterns.

A thorough molecular sampling of sympatric and allopatric populations of model diatom morphospecies suggested that individual phylogenetic lineages differ in their ecological requirements.

The species diversity of diatoms was associated with both environmental and spatial heterogeneity. The particular roles of the environmental and spatial factors in the structuring of diatom communities are scale dependent; differences in microhabitats, biotopes, local environmental conditions, and/or regional climatic conditions considerably influenced both the diversity and distribution of diatoms.

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5 CURRICULUM VITAE

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Study and practice:

From 2008: Position of research worker at the Department of Botany, Faculty of Science, Charles University in Prague.

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2002 - 2007: Undergraduate study in Biology, specialization: Systematics and ecology of non-vascular plants, Department of Botany, Faculty of Science, Charles University in Prague. Title of the diploma thesis: Ecology and distribution of cyanobacteria and algae in small streams of Bohemian Switzerland National Park.

Study abroad:

16th January - 12th February 2007, and 9th September - 1st October 2007: Dr. Jeffrey R. Johansen research group, Department of Biology, John Carroll University, Cleveland, Ohio, USA.

Publications in SCI journals:

Svoboda D, Peksa O, Veselá J (2011) Analysis of the species composition of epiphytic lichens in Central European oak forests. *Preslia* 83: 129–144.

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Abstracts, posters and presentations:

Veselá J, Urbánková P (2010) Rozšíření fylotypů *Frustulia saxonica* ve sladkovodních evropských biotopech [The distribution of *Frustulia saxonica* phylotypes in the freshwater European habitats] (presentation). *51st Working Conference of Czech Phycological Society*, Olomouc, Czech Republic.

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