

# Correspondence between morphology and ecology: morphological variation of the *Frustulia crassinervia-saxonica* species complex (Bacillariophyta) reflects the ombro-minerotrophic gradient

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**Abstract** – Two commonly reported *Frustulia* morphospecies from oligotrophic habitats, *F. crassinervia* and *F. saxonica*, showed overlapping morphological variation within natural populations and between clonal strains representing different genetic entities. Therefore, morphologies of natural populations of the *F. crassinervia-saxonica* complex were analyzed using geometric morphometric techniques regardless of species or genetic identity. It has been examined whether shape, centroid size, morphological diversity, and globularity of valves from three types of peatland habitats representing ombro-minerotrophic gradient reflected ecological differentiation within the *Frustulia crassinervia-saxonica* species complex. Morphometric analyses showed that valve differences within the species complex were significantly correlated with environmental conditions. In particular, allometric shape changes and size of valves showed strong relationships with different types of peatland habitats. It has been suggested that allometry probably acts as a constraint on morphological plasticity and is canalizing microevolutionary morphological differentiation within the *F. crassinervia-saxonica* complex.

**Allometry / diatoms / ecology / geometric morphometrics / morphotypes / shape / size**

## INTRODUCTION

The ongoing debate regarding the concept of species with respect to diatoms is illustrated by the problems in defining the morphological species boundaries and ecological preferences of putative species (Mann, 1999; Mann, 2010; Mann & Vanormelingen, 2013). Modern fine-grained morphological species identification uses ultrastructural features and/or morphometric methods to distinguish diatom species in natural populations (Van de Vijver *et al.*, 2010; English & Potapova, 2012). Ecology and distribution of these new species is often based on their occurrence in a single or very few localities. Another recent approach, a multidisciplinary approach, has been based on examination of clonal strains, and this uncovered a large amount of hidden species diversity within the traditional morphospecies (Amato *et al.*, 2007; Mann, 2010; Rimet *et al.*, 2014). Pseudo-cryptic or cryptic sibling species can occur in sympatry and, therefore, their niches are

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probably fine-tuned to the environment (Créach *et al.*, 2006; Vanellander *et al.*, 2009). The fine-grained taxonomy as well as the multidisciplinary approach is time-consuming for routine identification of diatoms. Consequently, analyses of the relationships between diatom communities and environmental parameters were performed at higher biological organization levels as an alternative approach. Passy & Legendre (2006) examined the relationships between taxonomic richness of diatoms at high categories (from genus to phylum). A number of studies investigated the response of diatom community size to various environmental factors such as nutrient content, pH values, and salinity concentrations (Snoeijs *et al.*, 2002; Sarthou *et al.*, 2005; Litchman *et al.*, 2009; Lavoie *et al.*, 2010; Neustupa *et al.*, 2013). Potapova & Hamilton (2007) examined morphology of natural populations within 12 historically recognized taxa to determine the indicator properties of newly defined morphological groups that were defined according to ecological preferences.

Here, we used an approach similar to that of Potapova & Hamilton (2007). Morphologies of natural populations of two commonly reported *Frustulia* morphospecies from oligotrophic habitats, *F. crassinervia* (Brebisson) Lange-Bertalot & Krammer and *F. saxonica* Rabenhorst, were analyzed using geometric morphometric techniques independently of species or genetic identity. Previous studies showed that the species boundaries of the natural populations (Siver & Baskette, 2004) and clonal strains representing different genetic entities (Veselá *et al.*, 2012) overlap between these two morphospecies. Furthermore, the latter study indicated that two common pseudo-cryptic lineages morphologically resembling the *F. crassinervia-saxonica* complex (lineages V and VI) had different ecological preferences. Although these lineages could live in sympatry, the difference in their abundances reflected the environmental conditions. Lineage V was common in habitats characteristic by higher pH and conductivity values (such as lakes and mires), whereas lineage VI was predominant in habitats with lower pH and conductivity values (such as peat bogs) and in ephemeral habitats (such as small desiccating pools and wet walls). In the present study, we examined whether shape, centroid size, morphological diversity, and globularity of valves from three types of peatland habitats representing ombro-minerotrophic gradient reflected ecological differentiation within the *F. crassinervia-saxonica* complex. We suppose that environmental conditions may have some particular relation to frustule morphology, and/or that there are the tight interactions between developmental and ecological factors in the evolution of morphological traits within the *F. crassinervia-saxonica* complex.

## MATERIALS AND METHODS

### Field sample collection and processing

Samples were obtained from the ombro-minerotrophic gradient in the Czech Republic in 2009 and 2012 (sampling details are provided in Table 1). Localities were classified according to a visual inspection of both hydrography and physiognomy of peatland habitat as described by Neustupa *et al.* (2013): ombrotrophic raised bogs, minerotrophic mires, and intermediate localities. Samples were obtained by squeezing water from the upper part of submerged *Sphagnum* thalli. Two separate samples were obtained from all localities except two (missing samples contain very

Table 1. Sampling sites and their environmental characteristics

Sample	Locality name	Sampling date [month/year]	Habitat type	Altitude [m]	Latitude [°]	Longitude [°]	pH	Conductivity [μS/cm]
1A	Velký močál	08/12	O	923	50.394775	12.638149	3.6	32
1B	Velký močál	08/12	O	925	50.394373	12.638697	3.5	26
2A	Břehyně	09/12	M	290	50.583217	14.716800	5.0	39
2B	Břehyně	09/12	M	295	50.583217	14.716800	5.0	39
3A	Swamp	07/12	M	268	50.575941	14.670911	4.0	66
3B	Swamp	07/12	M	265	50.578944	14.667742	3.8	119
4A	Černá jezírka	06/12	O	906	50.845517	15.303790	5.0	28
4B	Černá jezírka	06/12	O	915	50.845804	15.303156	5.5	7
5A	Rybí loučky	06/09	I	848	50.847776	15.337900	4.5	38
5B	Rybí loučky	06/09	I	848	50.847449	15.338668	4.3	53
6A	Klečové louky	10/09	O	971	50.831110	15.246694	3.9	37
6B	Klečové louky	11/09	O	972	50.831120	15.246695	4.0	45
7A	Cínovecké raš.	08/09	O	876	50.722881	13.742694	4.4	14
7B	Cínovecké raš.	08/09	O	874	50.722881	13.742695	4.3	14
8A	Přebuzské vřes.	09/12	I	893	50.380283	12.601617	4.4	25
8B	Přebuzské vřes.	09/12	I	891	50.380290	12.603504	4.9	11
9A	Černý rybník	07/12	M	334	50.688633	14.842366	5.1	81
9B	Černý rybník	07/12	M	318	50.688766	14.842733	4.5	55
10A	Hliniř	05/12	M	420	49.135880	14.679594	4.5	65
10B	Hliniř	05/12	M	421	49.135785	14.679584	4.4	42
11	Ostrov u Tisé	09/12	M	440	50.806664	12.045066	4.3	45
12	Horní Blatná	09/12	I	990	50.381066	12.738733	5.3	36
13A	Mariánský rybník	07/12	M	275	50.545661	14.678382	4.0	119
13B	Mariánský rybník	07/12	M	274	50.545784	14.680141	3.9	155

M, minerotrophic mires; I, intermediate localities; O, ombrotrophic bogs.

poor populations of *F. crassinervia-saxonica* complex). Conductivity and pH were measured in the field using a combined WTW 340i pH/conductometer (Weilheim, Germany). Geographic coordinates and altitude were recorded using Global Positioning System (GPS) apparatus (Garmin e-trex legend, Taiwan). Field samples were concentrated by centrifugation and frustules were mineralized by reaction of hydrogen peroxide with potassium permanganate (Krammer & Lange-Bertalot, 1986) or by incineration (Battarbee *et al.*, 2001). Cleaned material was mounted permanently on glass slides using Naphrax (Brunel Microscopes Ltd., Wiltshire, UK). The valves of *F. crassinervia* and *F. saxonica* morphospecies were viewed using an Olympus BX51 (Tokyo, Japan) light microscope with differential interference contrast (DIC). Images were obtained using an Olympus Z5060 (Tokyo, Japan) digital camera, which was connected via a camera adapter to the light microscope. Images were managed using Adobe Photoshop CS4 software.

## Morphometric data

The shapes of 30 randomly photographed valves from each sample were assessed by geometric morphometric analysis using TPS-series software (Rohlf, 2010). For the analysis, 44 points (landmarks and semi-landmarks) were placed regularly along the valve outline (TpsDig2 ver. 2.16). Forty of these were sliding landmarks - semi-landmarks (Bookstein, 1997) and the remainder were fixed. The fixed landmark positions were defined as the intersections of a cell outline with the apical and trans-apical axes. Configurations of both landmarks and semi-landmarks were superimposed by Generalized Procrustes Analysis (TpsRelw ver. 1.45). For all analyses, except calculation of disparity, the Procrustes aligned data were symmetrized along apical and trans-apical axes according to Savriama & Klingenberg (2011). Valve sizes were characterized by centroid size (TpsRelw ver. 1.45), which was defined as the square root of the sum of squared distances from the landmarks to their centroid (Zelditch *et al.*, 2004), and by the length and width of valves as calculated from the positions of fixed landmarks. All of the dimension variables were highly correlated ( $r = 0.99/0.96$ ,  $p < 0.001$ ); therefore, centroid sizes were selected to represent cell dimension in subsequent analyses. The globularity of the valves, which quantifies the deviation of a particular outline from a circular shape, was evaluated as the sum of Euclidean distances between adjacent landmarks in the Procrustes aligned data. Globularity was calculated according to the method of Černá & Neustupa (2010) using R software ver. 2.13.0 (R Development Core Team 2012). Partial morphological disparities from morphometric distances (Foote, 1993) were calculated using TpsSmall ver. 1.20 (Neustupa *et al.*, 2009). The non-allometric component of shape variability (dataset B) was calculated by multivariate regression of Procrustes coordinates on centroid size (R software); the residuals from this regression represented shape variation unrelated to size (Debat *et al.*, 2003). Dataset A contained original Procrustes aligned data (the shape changes related to both the allometric and non-allometric components), and dataset B represented non-allometric component only. The amount of shape variability explained by valve sizes was quantified and tested by 1000 permutations (TpsRegr ver. 1.38).

## Valve shape reconstructions

Configurations of landmarks for reconstruction of shapes along size gradients were obtained from multivariate regression of Procrustes aligned data (dataset A) on centroid size (TpsRegr ver. 1.38). The average configuration of valves from different types of biotopes and average configuration of the whole dataset (dataset A) were calculated using TpsRelw ver. 1.45. Thin-plate spline deformation grids were used for visualisation of shape changes between the two groups of landmark configurations (TpsSpline ver. 1.20).

## Statistical analysis of data

Principal component analyses (PCA) of partial warps (datasets A and B), which described shape variability, were performed using TpsRelw. Ordinations were visualised in PAST ver. 2.08 (Hammer *et al.*, 2001). Mean centroid size values (overall sizes), globularity (complexity of shape), and disparity (morphological diversity) of valves per locality were compared between habitats using Mann-Whitney tests with Bonferroni corrections and were visualised by box-plot diagrams

(PAST software). Correlations between univariate morphometric data were evaluated using Spearman's rank correlation coefficient (PAST software). Variance in both size and shape data attributed to pH, conductivity, altitude, habitat type, and spatial distance was partitioned by non-parametric (permutational) multivariate analysis of variance (MANOVA)(Anderson, 2001). Permutational MANOVA, which was based on Euclidean distance matrices, was performed with R software using the *adonis* function of the *vegan* package (Oksanen *et al.*, 2011). Valve sizes were characterized by centroid size. The scores on the first 15 axes, resulting from PCA of partial warps (dataset A and B), represented valve shapes. Habitats were coded as qualitative variables: O-ombrotrophic, M-minerotrophic, and I-intermediate. Values for pH, conductivity, and altitude (PAST software) were standardized to zero mean and unit variance (R software). Spatial distances were gleaned from GPS coordinates using PAST software. The similarity matrix based on geographical index was used for principle coordinates analysis (PCoA), and the scores on the first 12 axes from PCoA were used as a space variable. Significance of relationships was evaluated by permutation tests with pseudo F-ratios (999 permutations).

## RESULTS

### Ordination and reconstruction of valve shapes

PCA ordination was performed with both non-allometric and allometric components of shape variability (dataset A). In this analysis, mean valve shapes from different sampling sites clustered according to ombro-minerotrophic gradient (Fig. 1). Nevertheless, valve shapes varied between sampling sites from the same localities, e.g., samples 1A-1B, 3A-3B, 7A-7B, and 10A-10B (Fig. 1, Table 1). Along the first PC axis (91.5% of variability), valve outline changed from narrowly to broadly lanceolate (Figs 2, 3). The second PC axis (4.4% of variability) described changes at the apices (more or less protracted) and the central parts of the valves (parallel or lanceolate outline; Figs 4, 5). The mean shape of valves (dataset A) from minerotrophic mires was narrowly lanceolate (Fig. 6), whereas the average valve representing the ombrotrophic raised bogs had a broadly lanceolate shape with protracted valve ends (Fig. 7). The mean valve shape from intermediate habitats was similar to the average valve shape from minerotrophic mires (see positions of

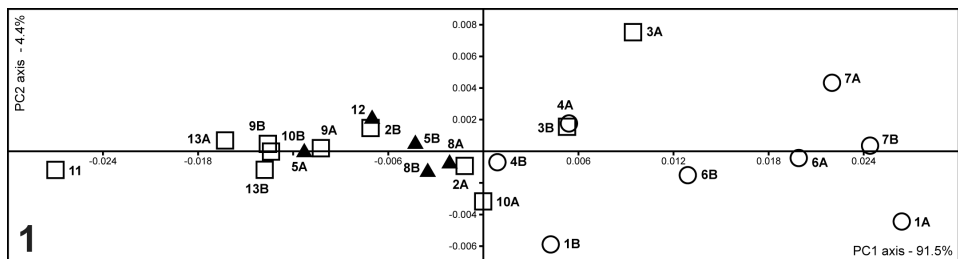
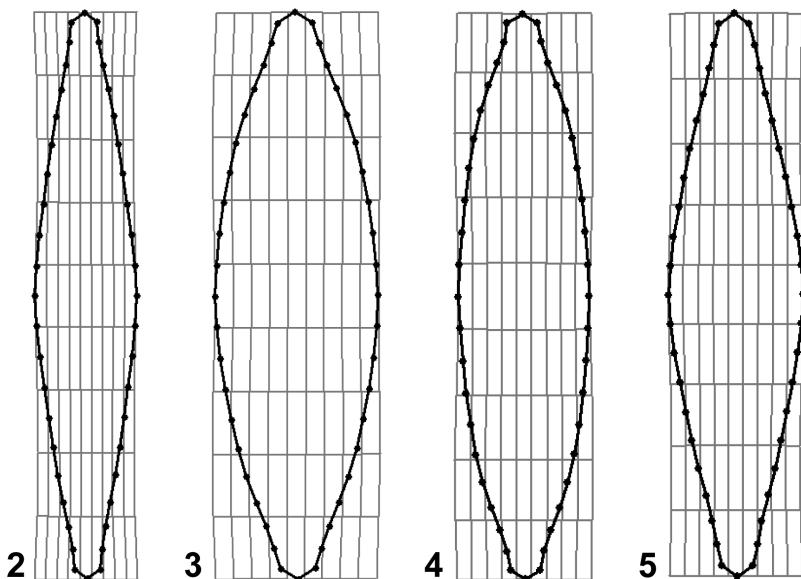
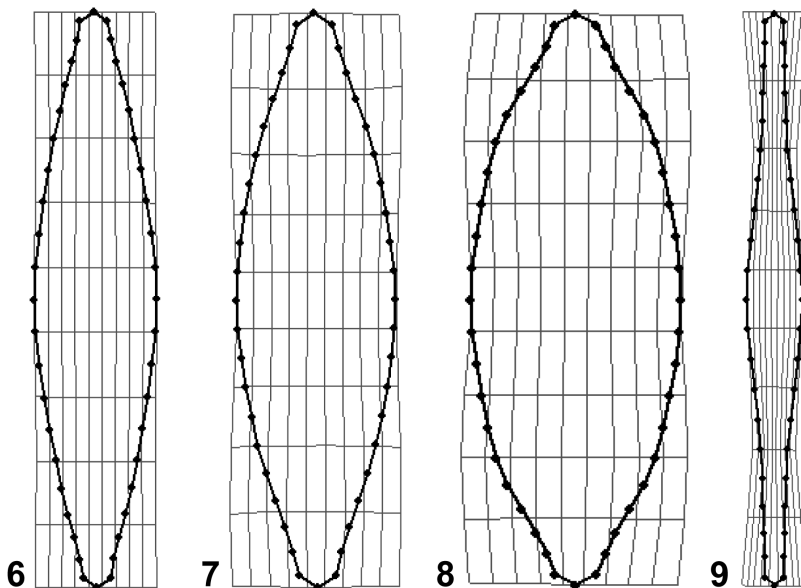


Fig. 1. Principal component analysis of non-allometric and allometric components of shape variability in the *F. crassinervia-saxonica* complex (dataset A). Symbols indicate the mean shape of populations at sampling sites. Circles correspond to ombrotrophic bogs (O), squares to minerotrophic mires (M), and filled triangles to intermediate localities (I). Sampling site details are in Table 1.



Figs 2-5. Changes from the mean shape within the entire dataset along the first and the second principal component (PC) axes of the diagram shown in Fig. 1. 2-3. A negative and a positive position in morphospace along the PC1 axis, respectively. 4-5. A positive and a negative position along the PC2 axis, respectively.

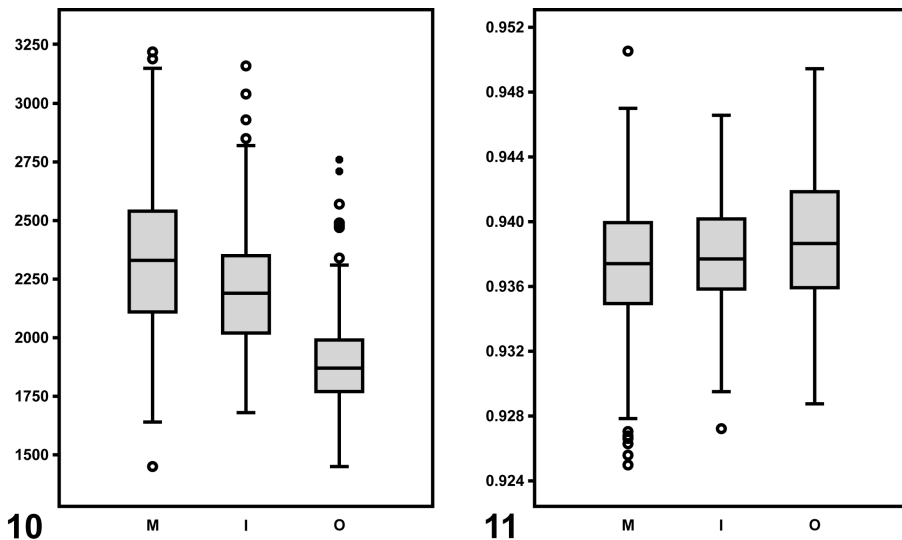


Figs 6-9. Deformation grids depicting differences in *F. crassinervia-saxonica* valve shape between the overall mean configuration of landmarks of the entire dataset and mean shapes. Deformation grid representing: 6. minerotrophic mires (M), 7. ombrotrophic peat bogs (O), and 8-9. allometric trends associated with increase in centroid size. Shape reconstructions are based on dataset A.

triangles in Fig. 1). A multivariate regression of morphometric data (dataset A) on centroid sizes explained 61.3% of the variability ( $p < 0.001$ ). The allometric variation associated with increase in centroid size involved a shape change from oval valves with protracted apices (Fig. 8) to narrowly lanceolate valves with elongate apices (Fig. 9). Ordination of sampling sites based on non-allometric morphometric data (dataset B) did not show a clear ecological signal (plot not shown).

### The relationship between biotic and abiotic variables

Comparisons of valve centroid sizes demonstrated that significant variability in valve size occurred between different peatland habitats (Mann-Whitney test,  $p < 0.001$ ). Large frustules were found in minerotrophic mires, small frustules in ombrotrophic raised bogs, and middle-sized frustules in intermediate localities (Fig. 10). The relative change in valve size between populations from raised bogs and mires was 81.8% (mean) and 80.4% (median). Globularity differed significantly between minerotrophic and ombrotrophic sites (Mann-Whitney test,  $p < 0.001$ ; Fig. 11). Frustules from ombrotrophic bogs had valves with a more pronounced globular shape (outline almost circular) than those from minerotrophic mires. Morphological diversity (disparity) among all pairs of habitats was not significantly different ( $p > 0.05$ ). Spearman correlation analysis showed that centroid size was not significantly correlated with globularity or disparity ( $p > 0.05$ ). A significant relationship was found between disparity and globularity ( $r = 0.56$ ,  $p < 0.01$ ). Valve sizes were affected most by habitat type, spatial distance and pH (Table 2). The variability associated with the pure effect of these variables on valve sizes was



Figs 10-11. **10.** Box-plot diagrams showing the centroid sizes of valves within three types of peatland habitats (M, minerotrophic; I, intermediate; and O, ombrotrophic). Centroid size represents the square root of the sum of squared distances from the landmarks to their centroid. **11.** Box-plot diagrams showing globularity of valves within three types of peatland habitats. Globularity represents the sum of Euclidean distances between adjacent landmarks within size-standardized data.

Table 2. Permutational MANOVA analysis of the relationships between environmental factors and centroid size within the *Frustulia crassinervia-saxonica* complex (variable R2). The first row (raw effect) quantifies the variation in valve size that is shared with other variables (the effect of correlations is included). Subsequent rows (Habitat-Altitude) show covariations between variables (variable in the first column was taken as the covariate; e.g., the effect of “Habitat type” on centroid size independent from “Spatial distance” is 0.144). The last row (pure effect) shows the amount of total variation accounted for by the factor alone (the effect of all variables included in the model was removed prior to quantification)

	<i>Habitat</i>	<i>Spatial</i>	<i>pH</i>	<i>Conductivity</i>	<i>Altitude</i>
Raw effect	0.327***	0.240***	0.144***	0.124***	0.078***
Habitat	–	0.057***	0.022***	0.000 <sup>n.s.</sup>	0.001 <sup>n.s.</sup>
Spatial	0.144***	–	0.079***	0.096***	0.097***
pH	0.205***	0.175***	–	0.016***	0.012***
Conductivity	0.204***	0.212***	0.036***	–	0.038***
Altitude	0.250***	0.259***	0.077***	0.083***	–
Pure effect	0.075***	0.065***	0.013**	0.007**	0.006**

\*\*\* P < 0.001; \*\* P < 0.01; \* P < 0.05; <sup>n.s.</sup> P > 0.05

higher than the effect on shape variability (see Table 3): habitat type explained 7.5% ( $p < 0.001$ ), spatial distance 6.5% ( $p < 0.001$ ), and pH 1.3% ( $p < 0.01$ ) of the variability in centroid size. In general, the permutational MANOVA, which was performed on allometric and non-allometric data (dataset A), and non-allometric data alone (dataset B), demonstrated that habitat type and environmental conditions had a more substantial effect on shape data within dataset A than within dataset B (Table 3). The percentage of variability explained by the pure effect of altitude decreased from 0.9% ( $p < 0.01$ ) to 0.5% ( $p < 0.05$ ), conductivity decreased from 0.9% ( $p < 0.01$ ) to 0.3% ( $p > 0.05$ ), and pH increased from 0.3% ( $p > 0.05$ ) to 0.4% ( $p < 0.05$ ). Minerotrophic, ombrotrophic, and intermediate habitat type explained 4.1% ( $p < 0.001$ ) of the variability within dataset A, but only 0.6% ( $p > 0.05$ ) of the variability within dataset B. The amount of shape variability that was accounted for by geographical distance was lower in dataset A ( $p < 0.001$ ) than in dataset B ( $p < 0.001$ ), with calculated values of 5.7% and 6.1%, respectively.

## DISCUSSION

Statistical analyses showed that morphological valve variability within the *Frustulia crassinervia-saxonica* complex was significantly correlated with environmental factors. In particular, allometric valve shape changes and size showed strong relationships to different types of peatland habitat. Samples from minerotrophic mires were characterized by large narrowly lanceolate valves, whereas valves from ombrotrophic raised bogs were about 20% smaller and broadly lanceolate with protracted apices. Although morphology of diatoms is affected by changes in life cycle (Round *et al.*, 1990), where frustule size gradually diminishes during the vegetative phase of the life cycle, it is unlikely that spatially and temporally diverse populations exhibit synchronized life cycles. For example, it is unlikely that a



Table 3. Permutational MANOVA analysis of the relationships between environmental factors and valve shape within the *Frustulia crassinervia-saxonica* complex (variable R2). Tests were performed using both allometric and non-allometric data (dataset A), and only non-allometric data (dataset B). The first row (raw effect) quantifies the variation in the shape data that is shared with other variables (the effect of correlations is included). Subsequent rows (Habitat-Altitude) show covariations between variables (variable in the first column was taken as the covariate; e.g., the effect of “Habitat type” on dataset A independent from “Spatial distance” is 0.103). The last row (pure effect) shows the amount of total variation accounted for by the factor alone (the effect of all variables included in the model was removed prior to quantification)

Dataset	Habitat type		Spatial distance		pH		Conductivity		Altitude	
	A	B	A	B	A	B	A	B	A	B
Raw effect	0.203***	0.012**	0.166***	0.066***	0.067***	0.018***	0.050***	0.012**	0.030***	0.008**
Habitat	—	—	0.066***	0.072***	0.022***	0.014***	0.002 <sup>n.s.</sup>	0.005*	0 <sup>n.s.</sup>	0.003 <sup>n.s.</sup>
Spatial	0.103***	0.018***	—	—	0.059***	0.014***	0.072***	0.016***	0.075***	0.014**
pH	0.158***	0.008*	0.159***	0.062***	—	—	0.005*	0.002 <sup>n.s.</sup>	0.003 <sup>n.s.</sup>	0.002 <sup>n.s.</sup>
Conductivity	0.154***	0.005 <sup>n.s.</sup>	0.188***	0.070***	0.021***	0.009***	—	—	0.020***	0.004*
Altitude	0.173***	0.008*	0.212***	0.072***	0.040***	0.012***	0.041***	0.008**	—	—
Pure effect	0.041***	0.006 <sup>n.s.</sup>	0.057***	0.061***	0.003 <sup>n.s.</sup>	0.004*	0.009**	0.003 <sup>n.s.</sup>	0.009**	0.005*

\*\*\* P < 0.001; \*\* P < 0.01; \* P < 0.05; <sup>n.s.</sup> P > 0.05.

significant proportion of the minerotrophic *F. crassinervia-saxonica* populations was near the start of the vegetative phase and that ombrotrophic populations were at the end of the vegetative phase. Furthermore, we included living diatoms as well as empty frustules in our analyses, and the examined valves, therefore, represented population histories. We propose that a specific size class of *F. crassinervia-saxonica* cells is favoured over other size classes according to type of peatland habitat. Long-term studies of diatom populations reported several mechanisms, e.g., abrupt size reduction, different growth rates within size classes, and prevention of size reduction through modified girdle band arrangement, that could lead to the distribution of cell sizes differing from that expected by gradual size diminution (Mann, 1988; Edlund & Stoermer, 1997; Rose & Cox, 2013). These changes in size reduction pattern might be associated with adaptation to ecophysiological parameters such as the amount of available nutrients, light intensity, and evasion of parasites or predators (Tall *et al.*, 2006; Kagami *et al.*, 2007; Finkel *et al.*, 2009; Litchman *et al.*, 2015). In our study, large cells were found predominantly in minerotrophic lowland mires, which had generally higher values of both pH and conductivity than other habitats, and small cells were characteristic for ombrotrophic peat bogs, which had lower pH and conductivity values. The combined effect of habitat type and measured environmental factors on morphology of valves was evident from the variance partitioning. When habitat type was included in the model, the effect of pH, conductivity, and altitude were significantly reduced (Tables 2, 3). Small cell size might be beneficial in dystrophic conditions for at least two reasons, e.g., high surface-to-volume ratio enhances effective nutrient uptake, and cells with smaller biovolumes have lower biosynthetic demands than larger cells (Sarhou *et al.*, 2005; Naselli-Flores *et al.*, 2007; Finkel *et al.*, 2010; Vilmi *et al.*, 2015). In contrast with our results, a previous geometric morphometric study examined the mean biovolume of the whole diatom assemblage in peatland samples (Neustupa *et al.*, 2013) and found a positive correlation of diatom biovolume with a pH of 3.3-5.3 (pH range similar to that the present study) and an inverse correlation of volume with conductivity. Furthermore, the ombro-minerotrophic gradient was not strongly correlated with the mean biovolume of diatoms (Neustupa *et al.*, 2013). The response of benthic diatom assemblage biovolumes to nutrient enrichment was examined in other studies, but the results were ambiguous (Snoeijs *et al.*, 2002; Lavoie *et al.*, 2010). It is likely that the complex response of benthic diatom assemblages is affected by differences in life history and/or ecological strategies among phylogenetically distant taxa (Pappas & Stoermer, 1995; Edlund & Stoermer, 1997; Morin *et al.*, 2008). More studies are needed to ascertain whether size variation within species complexes is a more sensitive parameter than the biovolume of the whole diatom assemblage for assessing ecological conditions.

As well as *Frustulia crassinervia-saxonica* valve size, globularity and shape of valve outlines could be differentiated according to the two types of peatland habitat. Shape simplification towards oval forms is a well-known phenomenon of the diatom life cycle and occurs as frustule size decreases during the vegetative phase (Geitler, 1932; Round *et al.*, 1990). Nevertheless, correlation between globularity and valve sizes was not significant, and morphological disparity was positively correlated with globularity. Thus, it is likely that globularity of *F. crassinervia-saxonica* complex was associated not only with overall sizes of valves but also with environmental conditions and specific population morphologies. Decomposition of valve shape variability confirmed the association of different valve sizes with distinct habitats. The percentage of explained variability accounted for by habitat type considerably decreased within the non-allometric dataset

(dataset B). By contrast, the spatial component of variability was almost equally important for shape variability in both datasets. In other words, valve shapes in populations from different samples were not primarily influenced by allometric shape changes. Spatial variability, which correlated with valve shape, might be associated with the geographical distances between populations as well as with biotic or spatially autocorrelated abiotic factors that were not assessed in our study.

In the present study, the natural populations represented at least two genetic entities with pseudo-cryptic diversity resembling *Frustulia crassinervia* and *F. saxonica* morphospecies (Veselá *et al.*, 2012). Molecular genetic data (almost 300 sequences of partial LSU rDNA; cca 50 samples from the Czech Republic) indicated that the most commonly occurring lineage at most localities was lineage VI, while lineage V showed a common or rare occurrence at minerotrophic localities (Veselá *et al.*, 2012; Urbánková & Veselá, 2013). Therefore, morphological differences between populations from different peatland habitats might be affected by both phenotypic plasticity and genotypic variability. It is likely that more than one genotype was present in several of the minerotrophic samples; however, correlations between morphological disparity and habitat type were not significant. We noted congruence in visual comparisons of valve outlines of natural populations and of European cultures (Veselá *et al.*, 2012). Lineage V valves typically had a narrowly lanceolate shape with more or less protracted apices (similar to the mean shape of minerotrophic and intermediate populations). Lineage VI exhibited a broadly lanceolate valve shape (similar to the mean shape of ombrotrophic populations). Despite similarities between the natural populations and the different lineages, we do not presume that genotype had a dominant effect on morphology; the phenotypes of clonal cultures might be significantly influenced by environmental conditions at sampling sites, i.e., cultures retained their morphology from field conditions. The results of the experimental study on co-occurring *Asterionella formosa* Hassall genotypes (Gsell *et al.*, 2012) support our conclusion. Although *A. formosa* genotypes reacted not uniformly to the temperature change, phenotypic plasticity of strains (changes in cell width, cell volume, and SA/V ratio per generation) showed a consistent pattern across all temperatures and no direct genotype effect on cell size has been proved (except change in mean cell length). Comparisons of traditional *Frustulia* species with shape reconstructions that were based on natural populations were not straightforward. Protracted apices, which are characteristic for *F. crassinervia* (Lange-Bertalot, 2001), were apparent along the first and second axes of the ordination plot (Figs 2-5). Similarly, clonal strains with protracted apices were observed in more than one phylogenetic lineage (Veselá *et al.*, 2012). Valve size and undulations on the valve margin constitute additional characteristic features of *F. crassinervia*, but these could not be taken into account in our dataset. It is likely that landmarks and semi-landmarks used in this study were not sensitive to detection of subtle shape variation (Pappas *et al.*, 2014). Furthermore, we assume that differences in habitats influenced valve sizes at the intra- as well as the interspecific level.

## CONCLUSIONS

This study demonstrated that the most of the variation in shape, related to environmental factors, could be attributed to allometry. Rate and timing of development are important determinants of life history, which reflects the relationship

between an organism's ontogeny and its environmental conditions (Klingenberg, 1998). Several studies indicates that ontogenetic allometries can evolve according to functional and ecological aspects (Klingenberg, 1998, 2010). It means that the association between size and shape probably acts as a constraint on morphological plasticity and is canalizing microevolutionary morphological differentiation within the *F. crassinervia-saxonica* complex.

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