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The pH-related morphological variations of two acidophilic species of Desmidiales (Viridiplantae) isolated from a lowland peat bog, Czech Republic

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Abstract Morphological variation related to pH was investigated in two acidophilic desmid species (*Euastrum binale* var. *gutwinskii* and *Staurastrum hirsutum*) utilizing geometric morphometric methods. Clones isolated from acidic habitats were cultured using a range of pH values from 3.5 to 6.5. The plasticity of ensuing populations was quantified and illustrated by the general Procrustes superimposition of landmarks placed along the outline of cells and subsequent statistical analyses of shape data. In both species, there was a significant effect of pH on the morphology of cells. In *Staurastrum hirsutum*, the pH-related morphological change was accompanied by a decrease in the size of cells cultured at a higher pH. However, in *Euastrum binale*, cell size did not differ in relation to pH, but cell shape was characterized by a deepening of the incisions between cell lobes at higher pH. In both species, cell complexity based on surface-to-volume ratio was positively correlated with increasing pH. We conclude that by manipulating their surface-to-volume ratios, these desmid species can respond to pH variations in their environment.

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Introduction

Desmids are typically unicellular or filamentous microorganisms belonging to the green algae. Generally, they occur in phytobenthos of standing freshwater habitats with the majority of the species inhabiting oligo-to mesotrophic, slightly acidic wetlands. Members of Desmidiales also often dominate in phytobenthos of pronouncedly acidic peat bogs with pH values varying from 3.5 to 5.0. The environmental conditions of these low-pH habitats are often correlated with low diversity of benthic microorganisms (Blouin 1989; Mataloni 1999; Ceosel and Meesters 2007). Highly acidic pH can be damaging to cell walls as it weakens hydrogen bonds in the cellulose strands comprising the walls and can result in uncontrolled cell expansion (Gross 2000). In addition, cells in these conditions may be placed under stress by the need to maintain the neutral pH of their cytoplasm as the H^+ ions continuously penetrate their plasmatic membrane (Gimmler and Weis 1992). Several adaptive physiological mechanisms of microalgae living in low-pH conditions were suggested (Gimmler 2001; Gerloff-Elias et al. 2005), e.g. maintaining a positive membrane potential and a positive charge outside the plasmatic membrane (Remis et al. 1994), decreasing permeability of protons through the plasmatic membrane or sustaining active proton pumping (Gross 2000; Gimmler 2001). Additionally, morphological adaptations of microalgae to conditions of extremely low pH were also proposed (Nixdorf et al. 2001). Generally, cells need to have low surface-to-volume ratios (S/V ratios) in order to minimize the stress posed by H^+ ions penetrating their walls and membranes. Clonal strains of protists with versatile cell shape are known to rapidly adjust their S/V ratios by expanding their cells (Weisse and Stadler 2006; Weisse et al. 2007). Similarly, the microalgae strains with cell wall may simplify their cell morphology in subsequent generations in order to adaptively manipulate the S/V ratio (Neustupa and Hodač 2005; Neustupa et al. 2008). The morphologically elaborate cells of many desmid species have high S/V ratios in compared with other microalgae (Padisák et al. 2003). This feature has been considered advantageous in oligotrophic conditions as it maximizes the surface area available for nutrients uptake (Coesel 1982). However, Coesel (1982) also mentioned that desmids inhabiting highly oligotrophic and acidic localities (e.g. elevated boreal peat bogs) tended to have lower S/V ratios than desmids from assemblages in localities with higher pH. In extremely acidic conditions, the pH may act as the critical environmental factor driving shape features of desmid cells. Species with a lower S/V ratio may have higher fitness under low-pH conditions, because of their lower surface area and reduced exposure to the acidic environment. However, pH of natural localities varies due to different biotic and abiotic processes (Rydin and Jeglum 2006). Photosynthesis and respiration of algae and other photosynthetic organisms cause diurnal changes in water pH and carbon/oxygen availability. During respiration, CO_2 is released into the water, causing the pH to decline. Conversely, during photosynthesis, CO_2 is utilized by photosynthetic organisms, and the pH increases (Odum 1956; Edwards and Owens 1965). Also, pH levels often vary within same locality. This variability is influenced by the moisture-aeration regime (Rydin and Jeglum 2006), or microhabitat types in the area (e.g. tychoplanktonic communities of the open water of peat-pits, benthic communities of the water-filled shallow hollows or emergent communities in mossy vegetation (Coesel 1982; Mataloni 1999). Interestingly, individual traditionally defined desmid species typically inhabiting acidic peat bog localities are known to occur at a range of different pHs e.g. *Closterium striolatum*—4.0 to 7.0, *Euastrum binale* var. *gutwinskii*—3.9 to 7.5, *Penium cylindrus*—4.5 to 6.8 and *Tetmemorus laevis*—3.8 to 7.0, (Růžička 1977, 1981; Negro et al. 2003; Ceosel and Meesters 2007; Neustupa et al. 2009). These very different H^+ concentrations produce quite different conditions for individual desmid populations, inducing them to manifest adaptive plastic responses.

In this study, we investigated two strains of frequently co-occurring acidophilic species of Desmidiales. The strains were isolated from acidic Central European peat bog habitats. They were cultivated at pH values ranging from 3.5 to 6.5. Our central question was whether these species exhibited a plastic response to varying the pH of their environment, and if the pH influenced their morphology and S/V ratios. We employed geometric morphometrics (e.g. Zelditch et al. 2004) to examine and quantify the morphological plasticity of experimental clonal populations cultured under various pH conditions, so that we could ascertain and separate their subsequent shape-related and size-related plastic responses.

Materials and methods

The strains of *Euastrum binale* var. *gutwinskii* (Schmidle) Homfeld (CAUP K 503) and *Staurastrum hirsutum* (CAUP K 302) used in this study were isolated as the single cell isolates from benthos of a Břehyně lowland peat bog near Doksy, Czech Republic (pH 3.8–5.5, conductivity 80–170 µS/cm) in September 2006. The isolated cells were cultured in 50-ml Erlenmeyer flasks with liquid CAUP oligotrophic medium (http://botany.natur.cuni.cz/algo/caup.html) in a series of seven different pH levels in gradations of 0.5 pH (3.5–6.5), at 17°C and day:night (14:10 hours) light regime for 30 days. The designated pH was maintained using 5-mM MES buffer. The initial inoculum was 0.5 ml of algal suspension (aprox. 100,000 cells), and there were ca. 20–45 divisions in individual pH levels. At each pH tested, 60 randomly chosen cells were photographed using Olympus BX51 light microscope and Olympus Z5060 digital photographic equipment. Thus, for each species, we photographed and analysed a total of 420 cells.

The two-dimensional morphology of cells was investigated using geometric morphometric methods, and for most of the analyses, the TPS-series software (Rohlf 2006) was used. In total, 60 landmarks depicting the frontal outline of the cells (Fig. 1), and length and width of the cells in the frontal view were digitized in TpsDig, ver. 2.05. The landmark configurations were superimposed by generalized Procrustes analysis (GPA) in TpsRelw, ver. 1.42. We used the generalized Procrustes superimposition with semilandmark registration, such that the resulting Procrustes coordinates described the outline of individual cells. The single fixed landmark was placed on the apex of an older semicell, and all the other 59 semilandmarks were placed regularly along the investigated outlines as the sliding algorithm (following e.g. Bookstein 1997 or Zelditch et al. 2004) optimizes the inter-landmark distances. The principal component analyses (PCA) were carried out using the Procrustes aligned data of all cells of each species grown at different pHs. The morphological variations related to the changing pH values were characterized by multivariate regression of shape data with pH taken as the independent variable; TpsRegr, ver. 1.31 was used for this analysis. Significance of a regression model was evaluated by permutation tests (with 1,000 permutations) on Wilks′ λ and Goodall′s F-ratio (Zelditch et al. 2004; Rohlf 2006). Scores of the objects on first 10 PC axes (spanning 98.52% of the total variation) for *E. binale* and 15 PC axes (spanning 95.84% of the total variation) for *S. hirsutum* were used for canonical variates analyses (CVA) in PAST, ver. 1.81 (Hammer et al. 2001) to test for and visualize the differences between pH values. The number of PC axes chosen was estimated, so that all the axes with eigenvalues higher than the Jolliffe cut-off value (Jolliffe 1986) were included. In addition, the scores on PC axes were also used in the two-group multivariate permutation tests (with 2,000 permutations) on Mahalanobis distance between all pairs of the populations from different pH values to measure the significance of their shape differences.

As a part of the Procrustes superimposition, landmark coordinates are scaled to a unit size, so that the resulting data describe "pure" shape properties of the investigated objects with size differences removed (Zelditch et al. 2004). Therefore, in geometric morphometrics, the variations in size and shape, i.e. allometric changes (Zelditch et al. 2004) can be investigated separately. It is often interesting to ascertain whether shape differences are allometric or are unrelated to size. To determine the effect of allometry, the Procrustes aligned data were regressed on centroid size of the cells. Then, we took the residuals from this multiple regression and tested for the effect of pH on the size-unrelated fraction of cell

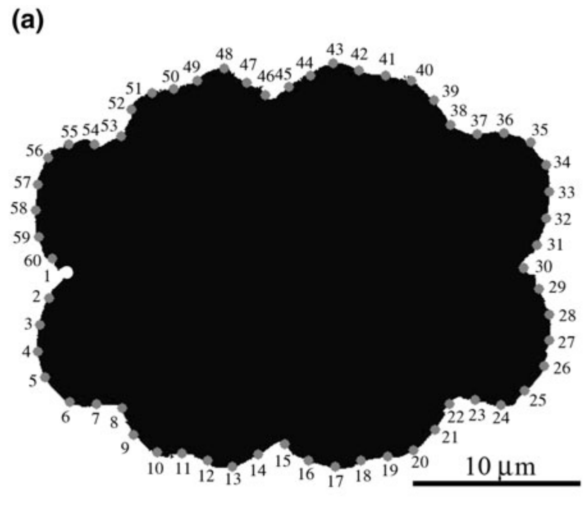

Fig. 1 Morphology of a vegetative cell of **a** *Euastrum binale* var. *gutwinskii* and **b** *Staurastrum hirsutum*, with landmark positions and numbers indicated. The single fixed landmark is shown by *white circle*. The semilandmarks are positioned counter clock-wise in regular intervals along the outlines

shape (Debat et al. 2003). The multivariate regression of size-unrelated shape data on pH (with cell size taken as a covariate) was conducted, and the canonical variates analysis (CVA) of size-unrelated shape data was made to test for the size-independent morphological separation of cells grown under specific pHs. Scores of the objects on first 10 PC axes (spanning 98.52% of the total variation) in *E.binale* and 13 PC axes (spanning 95.25% of the total variation) in *S. hirsutum* were used. Cell length, width and centroid size are illustrated as box plots.

Apart from multivariate datasets describing actual cell shape, the *complexity* of a cell's outline is also of interest. Generally, this measure indicates the deviation of a particular outline (spanned by landmark configuration) from a circular shape. We used the Procrustes aligned configurations (i.e. scaled to a unit size) and summed the Euclidean distances of all adjacent landmarks along an outline of all investigated cells. Then, we compared these values among individuals under specific pH conditions using permutation *t*-test with 10,000 permutations in PAST ver. 1.81 (Hammer et al. 2001).

Results

There was a significant effect of pH on the two-dimensional shape of both investigated strains. The multivariate regression of shape data in *Euastrum binale* populations, with pH as the independent variable, revealed a significant correlation (Wilks′ $\lambda = 0.356$, permutation p-value $= 0.001$; Goodall′s F-ratio $= 41.887$, permut. p-value $= 0.001$; proportion of unexplained variance: 91.1%). Thin-plate spline deformation grids depicting positive and negative deviations from the consensus (average) cell shape were calculated to visualize shape variation along the pH gradient. At lower pH, the cells were nearly cylindrical with shallow lobes; whereas, when grown at a higher pH, they had markedly deeper incisions (Fig. 2a). Specifically, at the highest pH (6.5), the apical cell incisions were ca. 78% deeper (average 1.33 µm at pH 6.5 vs. 0.75 µm at pH 3.5), and the sinuses were about 17% deeper (average 1.9 µm at pH 6.5 vs. 1.63 µm at pH 3.5) than those of cells grown at the lowest pH (3.5). The shape of the cells was also related to their size, albeit less than that with the pH.

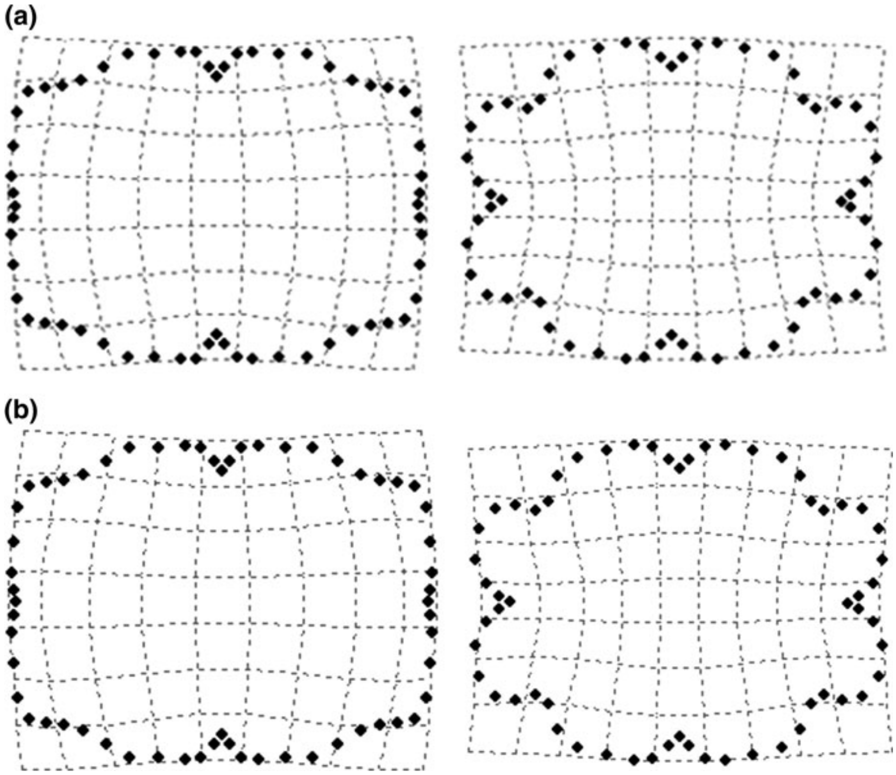

Fig. 2 The model for shape data **a** with the allometric component and **b** without the allometric component, illustrating the results of multivariate regression of cell shape on pH in *Euastrum binale*. Individual outlines illustrate the cell shapes in the lowest (*left*) and the highest (*right*) pH levels according to the regression model. Shapes are represented by thin-plate splines, and for better illustration, the deformations were extended three times

Multivariate regression of shape data based on centroid size revealed a significant effect (although with relatively little variance explained by the regression model) (Wilks′ $\lambda = 0.751$, permut. p-value $= 0.001$; Goodall′s F-ratio $= 6.925$, permut. p-value $= 0.001$; proportion of unexplained variance: 98.42%). In order to determine the significance of the size change on the shape of the cells, we asked whether there was any pH-related shape change completely independent from the size-related (allometric) change in the cells' shape. We took the residuals from multivariate regression of shape data on size and analysed their relation to pH. The relationship remained clearly significant (Wilks′ $\lambda = 0.368$, permut. p-value $= 0.01$; Goodall′s F-ratio $= 15.488$, permut. p-value $= 0.01$; proportion of unexplained variance: 96.53%), and there were no visible differences between the morphological changes associated with this regression model (Fig. 2b) and the regression of size-uncontrolled data on the pH (Fig. 2a). Thus, the allometry based on size and shape was probably not related to pH and shape relationship. We detected no correlation between pH and centroid size but a weak positive one between pH and cell length and pH and width (Fig. 3).

The significance of cell shape difference between individual populations from varying pH environments was evaluated by multivariate permutation tests on Mahalanobis distance. The cell shape of all group pairs (populations from individual pH levels) significantly differed in size effect-uncontrolled, as well as in shape data with the allometric effect removed (Table 1).

The multivariate regression of *Staurastrum hirsutum's* shape data on pH changes revealed a significant effect of pH on cell shape (Wilks′ $\lambda = 0.363$, permut. p-value $= 0.01$; Goodall′s F-ratio $= 96.11$, permut. p-value $= 0.01$; proportion of unexplained variance: 82.0%). The semicells of *S. hirsutum* were more globular under low-pH conditions; but markedly elliptical at a higher pH (Fig. 4a). The multivariate regression revealed a significant relationship between the shape and size of cells of *S. hirsutum* (Wilks′ $\lambda = 0.265$, permut. p-value $= 0.01$; Goodall′s F-ratio $= 118.868$, permut. p-value $= 0.01$; proportion of unexplained variance: 78.6%). Thus, the shape data were regressed on the pH with the allometric effect controlled, and the multivariate regression revealed a significant relation (Wilks′ $\lambda = 0.907$, permut. p-value $= 0.049$; Goodall′s F-ratio $= 5.858$, permut. p-value $= 0.003$; proportion of unexplained variance: 98.71%). The shape variation related to this regression model (Fig. 4b) was similar to those of allometric effect-uncontrolled regression, but the corresponding morphological changes were barely visible (Fig. 4b). The cell dimensions were significantly correlated with pH (Fig. 5). Irrespective of the size measures used, the cell size decreased with increasing pH. The multivariate permutation tests on Mahalanobis distance revealed significant shape differentiation among most data pairs, both with and without an allometric component. However, there were a few exceptions. No differences were detected between shapes in populations at pH of 3.5 and 4.0 or between pH 5.0 and 5.5 in size-controlled comparisons; likewise, no differences were detected between shapes in populations at pH 4.0 and 5.5 in size-uncontrolled data (Table 1).

In addition to multivariate regressions, we completed CVA analyses of shape data in both species in order to discriminate positions of populations at different pHs. We asked whether the position of groups in the ordination space would differ in size effect-uncontrolled data and in data with the allometric effect removed. The CVA ordination plot of the size

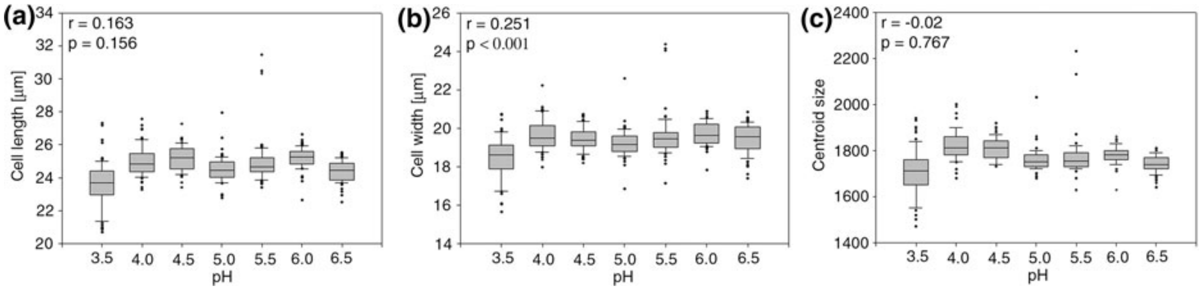

Fig. 3 Experimental pH levels correlated with **a** cell length, **b** cell width and **c** centroid size in *Euastrum binale*

Table 1 Differences between populations from pH levels expressed as Mahalanobis distance for both strains

	3.5	4.0	4.5	5.0	5.5	6.0	6.5
3.5		*0.075/0.217****	*0.144*****/0.239****	*0.203*****/0.142****	*0.193*****/0.156****	*0.221*****/0.094***	*0.212*****/0.09**
4.0	0.145***/0.106*		*0.154****/0.13****	*0.188*****/0.13****	*0.179*****/0.085*	*0.216*****/0.094**	*0.443*****/0.12****
4.5	0.236***/0.216***	0.25***/0.251***		*0.39*****/0.229****	*0.341*****/0.184****	*0.511*****/0.252****	*0.443*****/0.215****
5.0	0.29***/0.304***	0.345***/0.354***	0.195***/0.209***		*0.085/0.097***	*0.168*****/0.128****	*0.14*****/0.014****
5.5	0.3***/0.315***	0.371***/0.373***	0.258***/0.27***	0.131***/0.144***		*0.137*****/0.11****	*0.137*****/0.118****
6.0	0.348***/0.386***	.463***/0.457***	0.346***/0.355***	0.272***/0.281***	0.152***/0.159***		*0.129*****/0.121****
6.5	0.259***/0.289***	0.371***/0.341***	0.332***/0.315***	0.323***/0.309***	0.252***/0.245***	0.182***/0.183***	

Significance was evaluated using two-group permutation tests

Values of Mahalanobis distances for shape data with allometric component and shape data without allometric component are separated by a slash

Values for Euastrum binale are written in normal, values for Staurastrum hirsutum are written in italics

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Underlined values are statistically not significant

effect-uncontrolled group's centroids in *Euastrum binale* (Fig. 6a) illustrated a clear division among those at lower pHs (3.5–4.5) from those at higher pHs (5.0–6.5) along the first CV axis. The second CV axis separated those at "extreme" pH conditions (3.5, 6.0 and 6.5) from the other groups. The CVA of size effect-controlled shape data illustrated identical positioning of this group of centroids and similar trends along the first two CV axes (Fig. 6b). Pattern for *Staurastrum hirsutum* differed: the CVA plot of centroids of populations from particular pH values confirmed their clustering into three groups (pH 3.5 and 4.0; pH 5.0, 5.5, 6.0, 6.5; and the isolated pH 4.5 group; Fig. 7a). The first CV axis primarily contrasted the low-pH groups (3.5–4.5) from the others, whereas the second, CV2 axis, was largely defined by the difference among pH 4.5 and other groups. However, after removing the allometric component from the shape data, the CVA revealed a considerably different positioning of group centroids (Fig. 7b). The first CV axis clearly separated pH 4.5, and the second CV axis essentially separated the low from the high pH groups (Fig. 7b).

The complexity of cell outlines was evaluated, as the sum of Euclidean distances between adjacent landmarks in size-standardized data. Indirectly, this measure establishes the deepness of a cell's incisions in relation to its area. In both investigated strains, there was a significant positive correlation of cell outline measure with pH (Fig. 8), thus, indicating a greater complexity of cell shapes with more pronounced incisions under conditions of higher pH.

Discussion

Morphological changes that were related to differences in environmental pH were revealed for both of the investigated desmid species. All of the two-dimensional shape and cell size changes observed in the low-pH environment clearly diminished the S/V ratio of cells. We see this pattern as a possible adaptive response of these populations that restricts the osmotic pressure exerted on their cells. These findings generally confirm the field observations of Coesel (1982), who reported a gradual change in overall average cell shape of desmids along a pH gradient in peat bogs. Under conditions of low pH, the cylindrical cells with a lower S/V ratio were

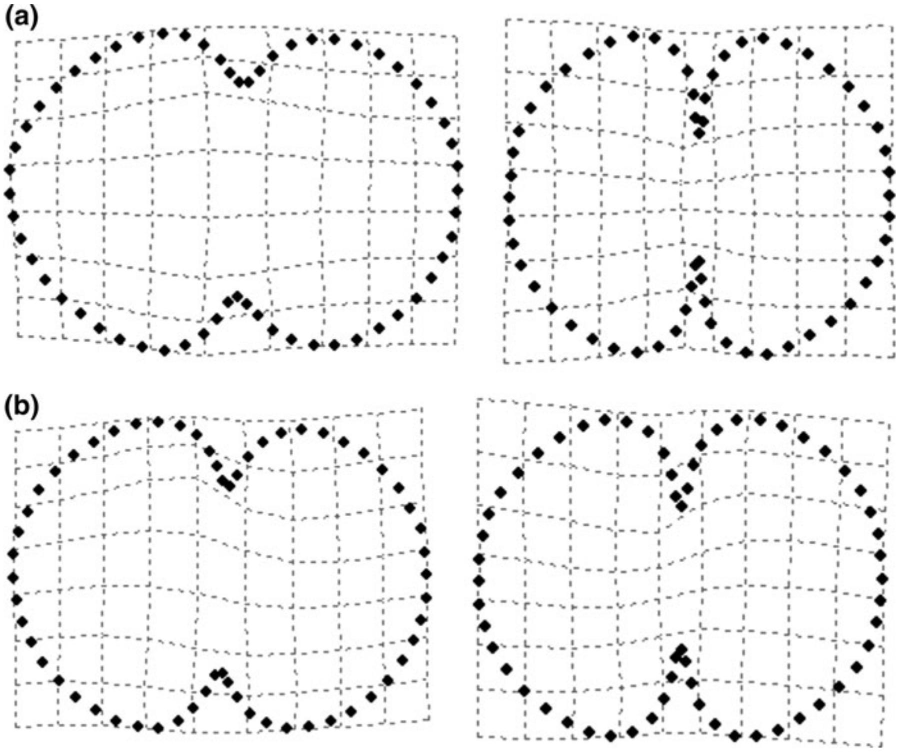

Fig. 4 The model for shape data **a** with the allometric component and **b** without the allometric component, illustrating the results of multivariate regression of cell shape on pH in *Staurastrum hirsutum*. Individual outlines illustrate the cell shapes in the lowest (*left*) and the highest (*right*) pH levels according to the regression model. *Shapes* are represented by thin-plate splines, and the deformations were extended three times for better illustration (Fig. 4b)

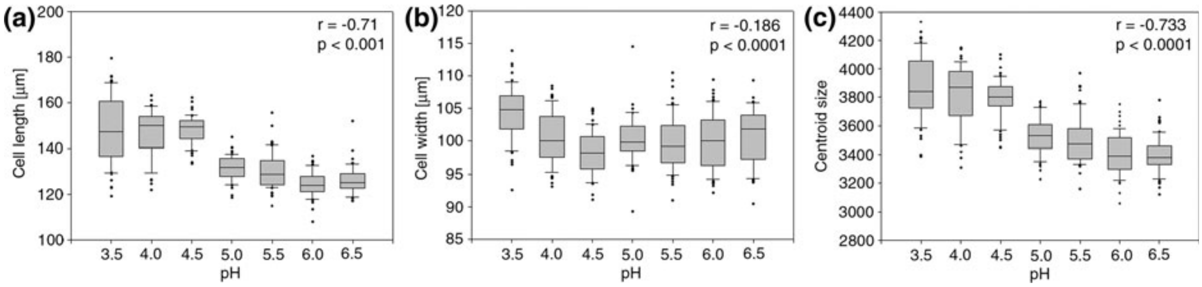

Fig. 5 Experimental pH levels correlated with **a** cell length, **b** cell width and **c** centroid size in *Staurastrum hirsutum*

abundant but were gradually replaced by cells with a higher S/V ratio when the pH of the environment increased.

In *E. binale* var. *gutwinskii*, the shallowing of the lateral incisions was generally similar to differences between this variety and several further infraspecific taxa (*E. binale* var. *binale*, *E. binale* var. *groenbladii* (Messikommer) Willi Krieger) characteristic by less-ornamented cell outline. Similarly, in *S. hirsutum*, the observed size and shape trends related to pH level resembled transition between the type variety of this species and *S. hirsutum* var. *muricatum* (Brébisson ex Ralfs) Kurt Förster, which is characteristic by larger cells similar to those observed in low-pH conditions. However, the taxonomic relevance of these observations can only be assessed by molecular comparisons of multiple strains corresponding to these traditionally defined varieties.

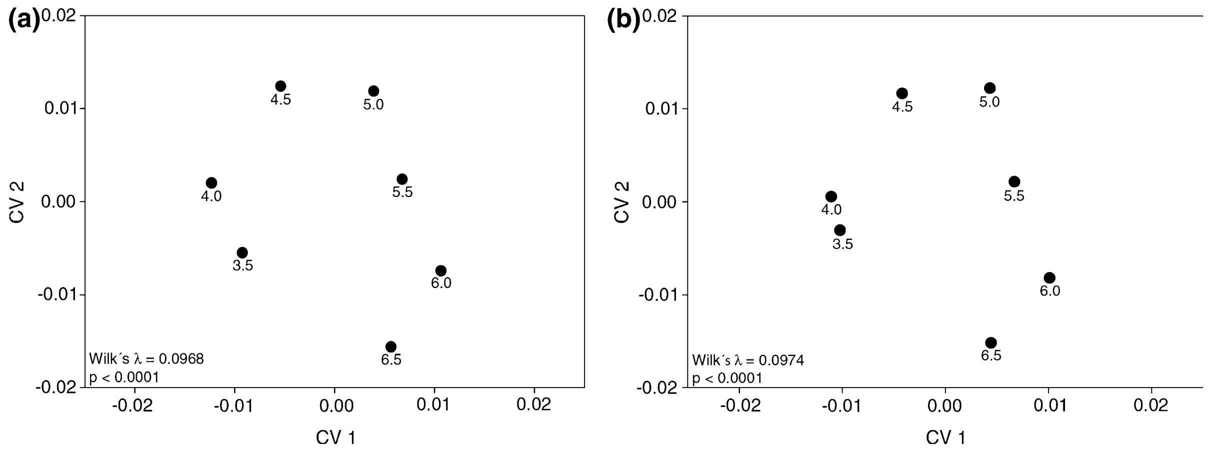

Fig. 6 Discrimination of cell shapes from different pHs in *Euastrum binale*. The CVA ordination plot of shape data **a** with allometric component (CV1 spanned 59.4% and CV2 31.8% of the variation) and **b** without the allometric component (CV1 spanned 63.2% and CV2 28.7% of the variation). Each group is represented by its centroid

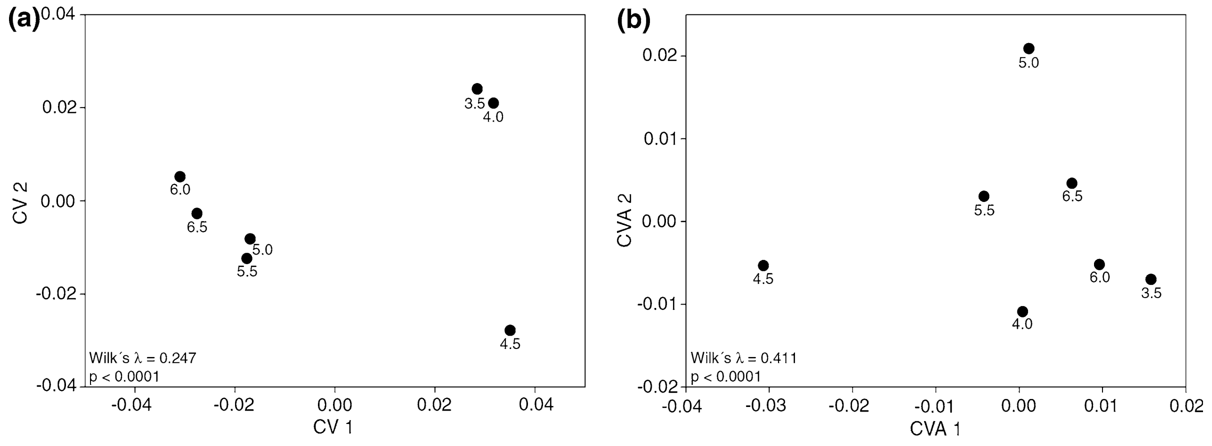

Fig. 7 Discrimination of cell shapes from different pHs in *Staurastrum hirsutum*. The CVA ordination plot of shape data **a** with allometric component (CV1 spanned 52.5% and CV2 17.8% of the variation) and **b** without the allometric component (CV1 spanned 73.1% and CV2 13.6% of the variation). Each group is represented by its centroid

A comparison of the investigated species

The two species we investigated differed notably in their pattern of pH-related response. In *Staurastrum hirsutum*, the cells' S/V ratio (indirectly estimated from complexity data and size values) at low-pH environments was clearly diminished, presumably by changes in cell size. The cells are generally larger in low pH than when in a nearer neutral environment. Although we did not specifically measure growth rates, an increase in cell size in unicellular protists is commonly known to indicate a decrease in their growth rates (Reynolds 1984; Niklas 1994; Nielsen et al. 1996). In addition to changes in size, the cells of *Staurastrum hirsutum* also alter their shape in relation to pH. The semicells were noticeably more globular like (as confirmed by complexity measure, Fig. 8b) under conditions of low pH that further decreased their S/V ratio. Our observations for the various cell size measurements in *Euastrum binale* are quite different. We find that cell length and width slightly increase with increasing pH, and this is associated with the cell lobes becoming more shallow, or conversely, deepening. Notably, the overall cell area, expressed as centroid size, does not seem to differ in relation to the pH. It is likely that the adaptive

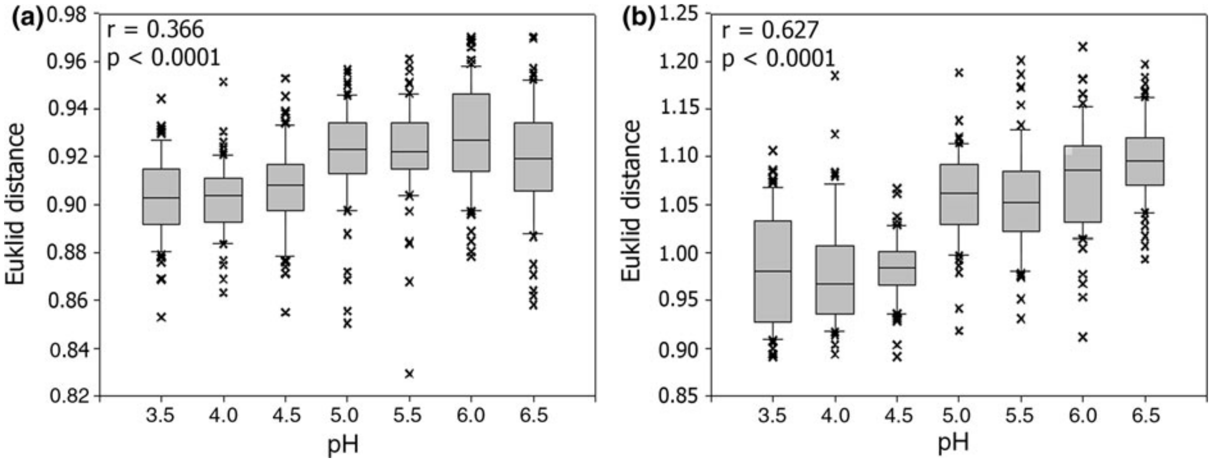

Fig. 8 The cell outlines at different pHs in **a** *Euastrum binale* and **b** *Staurastrum hirsutum*. The cell outline complexity was evaluated as a sum of Euclidean distances between landmarks in Procrustes aligned cells

decrease in S/V ratio in this species is achieved solely by changes in cell morphology. There is a clear pattern of incisions between a cell's individual lobes becoming shallower for cells grown at low pH; whereas, the cells from a higher pH environment typically indicate more pronounced incisions (Fig. 2). Consequently, the complexity of *Euastrum binale* cells increases with increasing pH. Thus, due to the intricate morphology of its cells, *E. binale* is able to manipulate its S/V ratio without increasing its cell sizes (and subsequently lowering its growth rates). A similar phenomenon was observed in *Micrasterias rotata* by Neustupa et al. (2008), who described a desmid species with extremely complex cells as its adaptive temperature-related plastic response. In this species, incisions seem to play a key role in affecting the S/V ratio of cells in response to temperature that influenced the population's growth rates.

Adaptive significance of morphological responses

The possible adaptive value of the intricate desmid morphologies was not recognized for a long time. However, the field observations desmid on communities made by Coesel (1982) and the experiments related to temperature effects (Neustupa et al. 2008) and pH-related plasticity of individual strains (this study) demonstrate the intriguing possibility that the morphological features of desmid cells may be involved in plastic response of a single species to environmental factors. Both the composition of desmid assemblages in natural wetlands and the plasticity observed by us in individual species illustrate that plastic desmid species may shape their cells in relation to environmental conditions. The desmids may thus optimize their S/V ratio, as this parameter seems to have a critical effect on the interface between the outer and inner cell environments. In oligotrophic conditions, desmid species have high S/V ratio as it maximizes the surface area available for nutrients uptake. However, in oligotrophic and acidic environment, species with a lower S/V ratio may have higher fitness, because of their lower surface area and reduced exposure to the acidic environment (Coesel 1982). Notably, in *Micrasterias rotata*, as well as in the species investigated here, plasticity is expressed at the level of individual clones. We presume that the variability between different populations of a particular species could be even more considerable. Thus, the strikingly morphologies of desmids, which have long intrigued biologists like Ralfs as early as in mid-nineteenth-century, could also be interpreted and evaluated in the context of their life strategies and reaction norms.

The future studies evaluating presented plastic responses of clonal strains should involve not only the morphometric analyses of the natural populations from which the strains were derived, but also the evaluation of their taxonomic homogeneity using molecular methods to provide further insight into the ecology of desmid species and clarify the relevance of our findings with the "real world". The investigated Břehyně lowland peat bog provides varying pH conditions, resembling those used by us in experiments (with pH > 4.5 < 6.0). Thus, the evolutionary and ecological relevance of the phenotypic adaptation in the investigated species could be tested in respect to the range of morphologies exhibited in the natural environment.

In conclusion, we revealed the significant effect of pH level on cell shape in two investigated desmid species. In *Euastrum binale*, the allometric effect on shape was not correlated with shape change related to pH level. On the other hand, in *Staurastrum hirsutum*, the cell size-related shape change correlated with the pH-related shape change. However, both species demonstrated similar morphological response to changing pH level of the environment. In low-pH conditions, the cells tended minimize the surface-to-volume ratio. In *Euastrum binale*, this effect was primarily connected with shallowing the incisions. In *Staurastrum hirsutum*, a species without any incisions on semicells, this response was mediated through more globular shape and higher size of the semicells. We conclude that with manipulating their surface-to-volume ratio (either by shallowing the incisions—in *E. binale* or by changes in cell size and globularity—in *S. hirsutum*) these desmid species can respond to pH conditions of the environment.

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