

Shape dynamics of silica scales (Chrysophyceae, Stramenopiles) associated with pH

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Abstract: We investigated the shape dynamics of silica scales in relation to environmental pH in two freshwater algal flagellates, *Mallomonas striata* and *Synura echinulata*, using landmark-based methods of geometric morphometrics. pH has been implicated as a primary factor controlling the occurrence and distribution of these protists. Moreover, scales are preserved in sediments and provide important proxy data of past and recent environmental changes. We concluded that the pH of the cultivation medium significantly affected scale biogenesis and influenced the final shape of produced silica scales. In addition, we asked how size of the scales was related to their shape (allometric effect). Scales produced under various levels of pH treatment were significantly different in shape in both investigated species, even though we eliminated the effect of size. We also investigated phenotypic plasticity (defined as the extent of scale shape variation) within the investigated pH treatments. Increased phenotypic plasticity is generally accepted to be induced by environmental stress. The impact of pH on *M. striata* and *S. echinulata* population growth was used to evaluate optimal/suboptimal conditions. We revealed increased scale shape plasticity in either suboptimal conditions (both species) or in high level pH treatments (pH 8.2 and 8.7, in *M. striata*). The inability of cells to compose a perfectly fitting, functional scale case, when the shape of individual scales varied considerably, may cause absence of Synurales in highly alkaline natural environments.

Key words: Shape dynamics, Geometric morphometrics, pH response, silica scales, Synurophyceae, Chrysophyceae, Synurales, phenotypic plasticity

Introduction

Synurales represent free living or colonial flagellates residing exclusively in fresh waters. In the multigene molecular phylogenetic analyses Synurales are included in Chrysophyceae/Synurophyceae clade (e.g. GRANT et al. 2009). Their cells are covered with an armor of imbricated silica scales attached to the plasma membrane. The scale cases are comprised of highly organized, flexible coats, the morphology of which is predetermined and controlled by the cell (LEADBEATER & BARKER 1995). In *Mallomonas* some or all scales may harbor a bristle. Silica structures (scales and bristles) are produced endogenously in a silicon deposition vesicle. Taxonomy within this group is still based almost exclusively on species-specific scales identified under the electron microscope. Scales are preserved in sediments for an extended period of time (tens to hundreds of years) depending on environmental conditions. However, the oldest preserved scales were reported from

Middle Eocene (ca. 47 million years old, SIVER & WOLFE 2005). Silica scales may provide important proxy data of past environmental change (e.g. eutrofication, acidification or shifts of climate; reviewed in ZEEB & SMOL 2001). Many well defined species have a definitive distribution along a pH gradient, and variations in the species composition are useful biological indicators of pH changes (SIVER 1991; SIVER 1995). Moreover, pH is implicated as a primary factor controlling occurrence and distribution of these protists. In natural waters, pH is regulated by carbonate equilibrium and it is influenced by a combination of geochemical and biological processes. Humic acids also act as a pH buffer, especially at lower pH in humic stained systems (GONDAR et al. 2005). Water in rivers and lakes show regional differences in pH depending on the geology and hydrology of the catchment area, input of acidifying substances and productivity of the system. Seasonal/diurnal pH fluctuation within a given water body is typically less or equal to 1 pH unit (BRÖNMARK &

HANSSON 2005).

Synurales are largely confined to neutral and/or slightly acidic waters (SIVER 1995). However, several species have been reported from alkaline habitats, e.g. *Mallomonas tonsurata* Teiling emend. W. Krieger, *M. crassisquama* Fott or *M. alpina* Pascher & Ruttner emend. Asmund & Kristiansen (KRISTIANSEN 1985; SIVER 1991). The growth restriction at high pH in *Synura petersenii* Korshikov was shown to be due to the inability to use bicarbonate as a source of inorganic carbon (SAXBY-ROUEN et al. 1997). Bhatti & Colman (2008) concluded that freshwater Synurales rely on the diffusive uptake of CO₂, maintaining a high internal pH relative to the pH of the external environment.

Growth responses of Synurales exposed to a gradient of experimental conditions (e.g. pH, temperature, light intensity) were found to be species-specific (LEE et al. 2007), or even strain-specific (WEE et al. 1991). As the silica scales represent important microfossils, the question arose: How is the scale biogenesis influenced by environmental conditions, and is this impact reflected in the scales' morphology? Pioneer works on scale phenotypic plasticity related to temperature were published by GUTOWSKI (1996) and MARTIN-WAGENMANN & GUTOWSKI (1995). They found that higher cultivation temperature caused a shortening of scales and bristles. The effect of pH on scale morphology was investigated by GAVRILOVA et al. (2005), who documented the strain-specific response to suboptimal pH conditions in *Synura petersenii* Korshikov. While in the strain CCAP 960/1c scale size was reduced at sub-optimally low pH and not affected at high pH, the response of strain CCAP 960/3c was the opposite.

We expanded upon previous research of pH-related scale shape plasticity by implementation of an intriguingly efficient tool – landmark based methods of geometric morphometrics (GM). Shape in GM is formalized using digitalized coordinates of biologically definable homologous points – landmarks (BOOKSTEIN 1991). Relative position of landmarks among various objects may be used for multivariate analysis of shape. Extent of plasticity (the variety of shapes) may be quantified using a morphological disparity measure (ZELDITCH et al. 2004). In silica scaled chrysophyte research, landmark based GM methods have been successfully implemented to investigate temperature induced morphological variation of

scales in clonal populations of *Mallomonas kalinae* Řezáčová, *Synura curtispina* (Petersen J.B. & Petersen J.B.) Asmund (ŘEZÁČOVÁ-ŠKALOUDOVÁ et al. 2010), *M. tonsurata* Teiling emend. Willi Krieger and *S. petersenii* (PICHRTOVÁ & NĚMCOVÁ 2011), to characterize the patterns of scale shape variation in *S. echinulata* Korshikov in combined gradients of light and temperature (NĚMCOVÁ et al. 2010), to describe scale shape variation in relation to their position within the scale-case (NEUSTUPA et al. 2010), and to emphasize the shape differences between scales belonging to the two distinct varieties – *Mallomonas striata* var. *striata* Asmund and *M. striata* var. *serrata* Harris K. & Bradley D.E. (NEUSTUPA & NĚMCOVÁ 2007).

In the present study we describe the effect of pH on shape of silica scales in two flagellates *Mallomonas striata* and *Synura echinulata*. We have primarily concentrated on scale shape plasticity across the entire range of viable pH. In addition, we asked how size of scales may influence their shape (allometric effect).

Materials and methods

The 2059 strain of *Mallomonas striata* var. *serrata* (referred to as *M. striata*) used in this study was obtained from the Provasoli–Guillard National Center for Culture of Marine Phytoplankton (CCMP); the strain of *Synura echinulata* (CAUP B702) was originally isolated from a peat bog pool in the National Nature Monument Swamp (Czech Republic) in May 2008.

The experimental cultures were selected from exponentially growing clones and inoculated into 50 ml Erlenmeyer flasks with buffered fresh medium at an initial cell density 300 cells ml⁻¹. Derived sub clones were grown at different pH levels. Experiments were carried out in triplicate. The tested pH range was from 3.5 to 8.7. Fresh DY V medium (<https://ccmp.bigelow.org/node/73>) was buffered with 1 mM MES (2-[N-morpholino] ethanesulphonic acid) to pH 4.5, 5.5, or 6.5, and with 1 mM HEPES (N-[2-hydroxyethyl] piperazine-N'-2-ethanesulfonic acid) hemisodium salt to pH 7.5, 8.3 or 8.7. Strains were cultivated at an optimum growth temperature of 22 °C (*M. striata*) and 17 °C (*S. echinulata*) under continuous white fluorescent illumination of 20 μmol m⁻² s⁻¹ for 14 to 40 days until stationary phase was observed. The duration of the experiments differed to allow a sufficient number of new scales to be formed under the test conditions. Cell growth was measured at intervals of 2–3 days (later 5–7 days) by absorbance at 750 nm (Spekol 11, Analytik Jena), and recalculated to cell density. The pH was checked and adjusted with NaOH or HCl every three days (InoLab pH/conductometer 720, WTW).

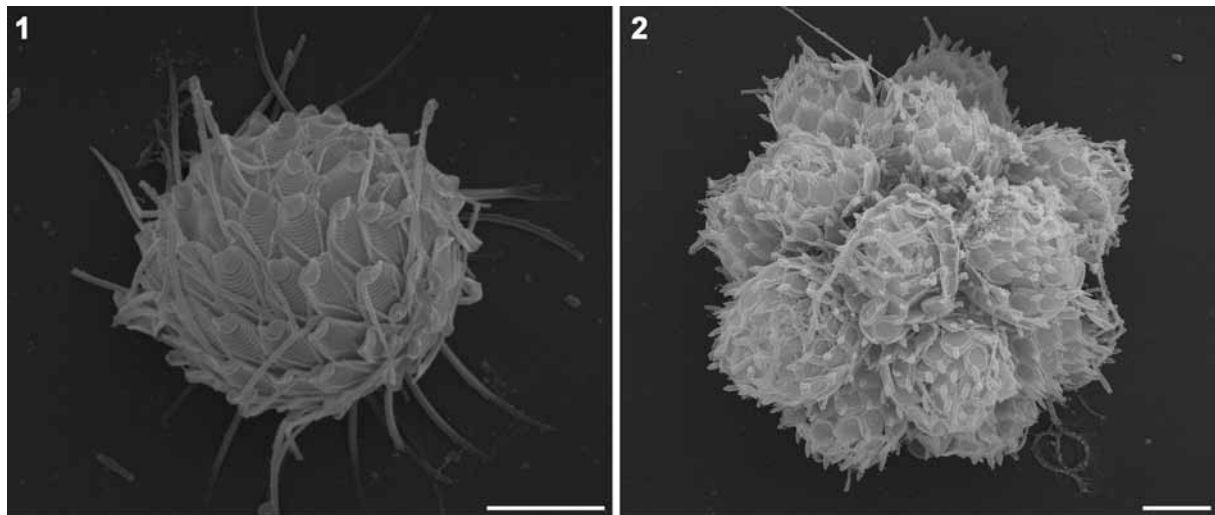
During the exponential growth phase, 10 ml of algal suspension from every target pH was pooled and fixed with Lugol's solution.

For scanning electron microscopy (SEM) acetone-washed glass coverslips were coated three times with a poly-L-lysine solution (1:10 in deionized water) to ensure appropriate adhesion of the cells. Then, a drop of the glutaraldehyde-fixed cell suspension was placed on the glass, transferred into 30% acetone, and dehydrated by an acetone series. Subsequently, cells were dried to a critical-point with liquid CO₂. The glass coverslips were mounted onto an SEM stub with double-sided adhesive carbon tape, coated with gold for 5 min (3 nm layer) with a Bal-Tec SCD 050 sputter coater and observed with a JEOL 6380 LV scanning electron microscope. Samples for transmission electron microscopy (TEM) were oxidized in peroxide and potassium dichromate to disintegrate the scale case, washed carefully, transferred to grids, and examined with a JEOL 1010 electron microscope. For each pH level, 70 body scales were chosen and photographed. The experimental organism *M. striata* possesses relatively uniform spirally arranged scales (Fig. 1). All scales are provided with a dome, where a slightly curved serrated bristle is attached (Fig. 3). Slightly curved scales from the most anterior ring of scales (surrounding the flagella pore) were excluded from the analyses. Colonies of *Synura echinulata* typically consist of 10–25 cells. The scale case of a single pear shaped cell is composed of several morphological types of scales (Fig. 2). Body scales, caudal and rearmost (slipper-like) scales are arranged from the anterior to posterior of the cell. Scales positioned on the anterior of the cell have the longest spines, and length of the spine decreases as scales are traced from the anterior to the posterior of the cell (SIVER 1999). We used the length of the spine to distinguish the scales of transitional morphology between caudal and body scales. All scales with a spine shorter than 0.9 µm were excluded from analyses. Moreover, relatively large scale sets were investigated to minimize the effect of shape variation related to scale position on the cell surface. Length and width of scales was measured in the tpsDig program (ROHLF 2006). To identify significant pair wise differences in length measurements among individual pH treatments a T-test was used (PAST, ver. 1.81; HAMMER et al. 2001). Results were considered significant if $p < 0.05$. Twenty-nine landmarks (LM) were defined on each scale of *M. striata* (Fig. 5) using the TpsDig program. Twelve landmarks were allowed to slide along the outline of the scale (so called semilandmarks). Thirty-two landmarks, including twenty-five semilandmarks were depicted on *Synura echinulata* scales (Fig. 6). The silica scales represent flat, bilateral, symmetrical or almost symmetrical objects. The side of the scale adjacent

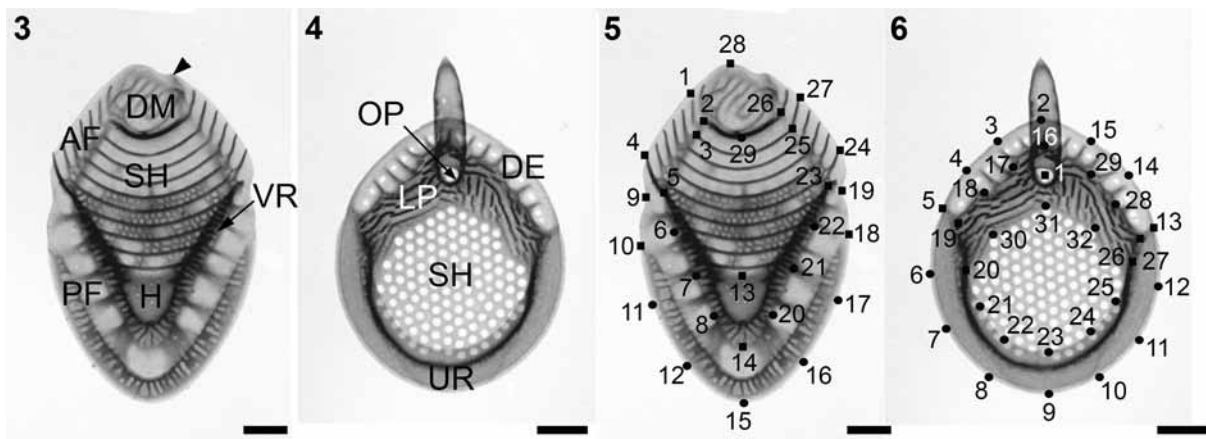
to the plasma membrane is smooth, while the other side is structured. Nevertheless, we were not able to discern the left and right sides of an *S. echinulata* scale on TEM images, so we symmetrized the landmarks in mirror positions, as recommended by KLINGENBERG et al. 2002. Original and mirrored landmark configurations were averaged and superimposed by Generalized Procrustes analysis–GPA (BOOKSTEIN 1991) in tpsRelw (ROHLF 2006). There is, however, a protrusion on an apical portion of the scale (dome) which enables one to distinguish the sides (Fig. 3, arrowhead) in *M. striata* scales. Original LM coordinates were subjected to GPA.

The principal component analyses (PCA) of the shape data was conducted on all silica scales across a pH gradient (overall shape variation). Scores for the objects on the first 15 PC axes (spanning 95.4% of the total variation for *Mallomonas striata* and 98.2% of the total variation for *Synura echinulata*) were used for canonical variates analyses (CVA) in PAST. To test for scale shape differences between individual groups, the scores on the first 15 PC axes were used for two-group multivariate permutation tests (2000 permutations) on the Mahalanobis distance between all group pairs. Shape configurations typical for individual pH treatments were visualized as thin-plate splines of average group shapes from overall consensus configurations. The multivariate regression of shape data (the Procrustes coordinates) on pH was performed in tpsRegr (ROHLF 2006). In addition, the multivariate regression of shape data on centroid size was designed to test for the effect of size on shape (allometric effect). The centroid size (square root of the sum of squared distances from the landmarks to their centroid) of each configuration prior to superimposition was used as a size variable. To examine the relation of scale shape on pH, with size-effect controlled for, residuals from this regression (R-2.6.2, R Development Core Team 2008) were regressed on pH (DEBAT et al. 2003). The permutation test (1000 permutations) on Wilk's λ and Goodall's F-ratio was used to evaluate significance of all regression models.

To quantify the differences in the extent of scale plasticity among individual pH treatments the values on partial morphological disparities (measured by a variance) were used (BALTANÁS et al. 2002, FOOTE 1993, ZELDITCH et al. 2004). The significance of differences among individual pH treatments was evaluated by a permutation test with 10 000 permutations using partial morphological disparity as a computed value in R-2.6.2. routine.



Figs 1–2. Scanning electron micrographs of the investigated strains: (1) *Mallomonas striata*, scale bar 5 μm ; (2) *Synura echinulata*, scale bar 10 μm .



Figs 3–6. Morphological description of scales and the position of landmarks: (3) *Mallomonas striata*, scale morphology [(AF) anterior flange, (DM) dome, (H) hood, (PF) posterior flange, (SH) shield, (VR) V-rib, (arrowhead) protrusion on an apical part of the scale enables to distinguish the left and right sides]; (4) *Synura echinulata*, scale morphology [(DE) distal edge with short ribs separated by a single pore, (LP) labyrinthic pattern, (OP) spine opening, (SH) shield, (UR) upturned rim]; (5–6) position of landmarks (squares) and semilandmarks (circles), (5) *M. striata*, (6) *S. echinulata*. Scale bar 0.5 μm .

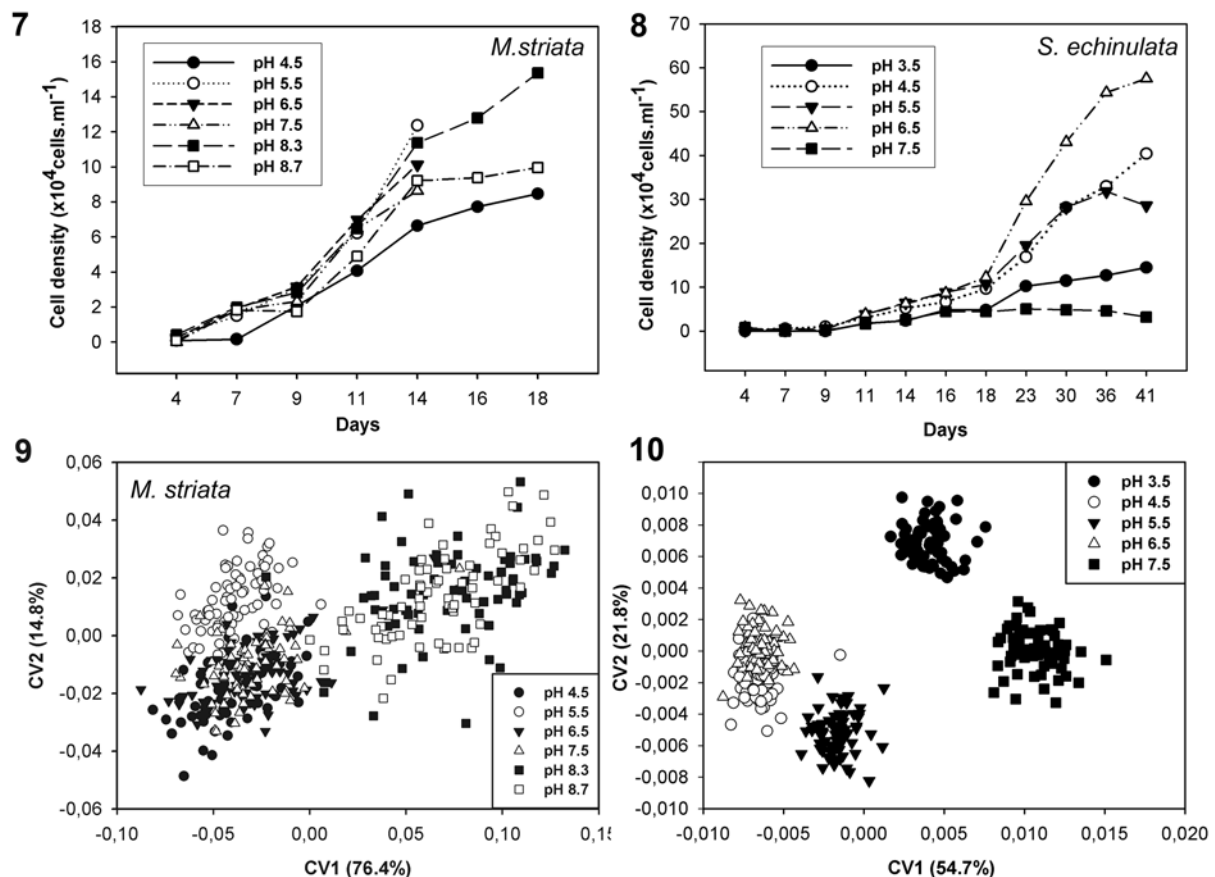
Results

Mallomonas striata

Mallomonas striata exhibited a growth response at a wide pH range (4.5 – 8.7 pH). *M. striata* showed rapid population growth at all investigated pH levels except pH 4.5 (Fig. 7). No apparent pH effect on *M. striata* scale size could be concluded. Scale size (expressed as centroid size) was only weakly, however significantly ($r = 0.16$; permutation $p = 0.007$) positively correlated to pH (not shown). The scale size measurements were significantly correlated (CS vs. length of the scale $r = 0.93$; CS vs. width of the scale $r = 0.81$ and length vs. width of the scale $r = 0.62$; all permutation $p < 0.0001$).

Further shape-to-size analyses were conducted using CS as a size measure in both investigated species.

The shape of the scales was significantly related to pH (Wilk's $\lambda = 0.294$, permutation $p = 0.001$; Goodall' F-ratio = 160.5, permutation $p = 0.001$; 27.6% of variance explained by the regression model). The CVA revealed statistically significant scale shape differences among individual pH treatments (Wilk's $\lambda = 0.057$, $p \ll 0.0001$). All pair wise comparisons on Mahalanobis distance were significantly different (all permutation $p < 0.007$). In an ordination space of the first two CV axes high pH groups (pH 8.3 and 8.7) clustered together and were

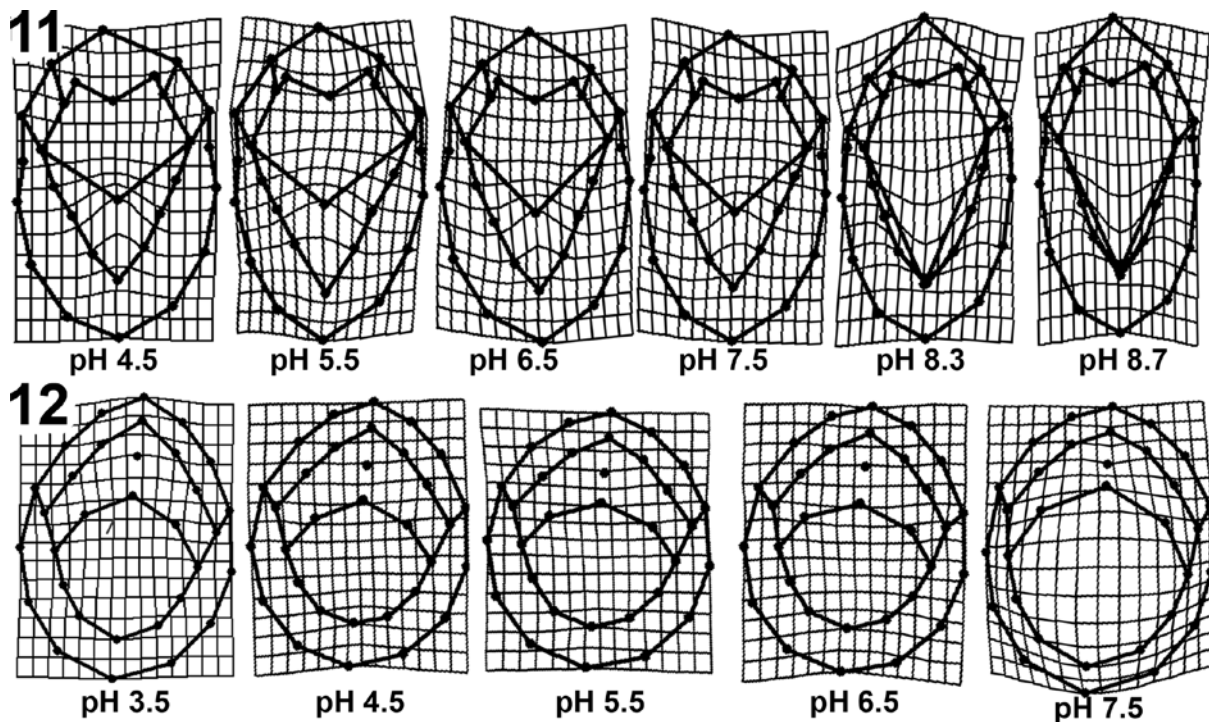


Figs 7–10. (7–8) the growth response curves expressed as cell density vs. cultivation period, (7) *Mallomonas striata*, (8) *Synura echinulata*; (9–10) discrimination of shapes from individual pH treatments. Canonical variate analysis ordination diagram shows individual scale scores along first two canonical axes. Values in brackets indicate the proportion of total variation described along each axis, (9) *Mallomonas striata*, (10) *Synura echinulata*.

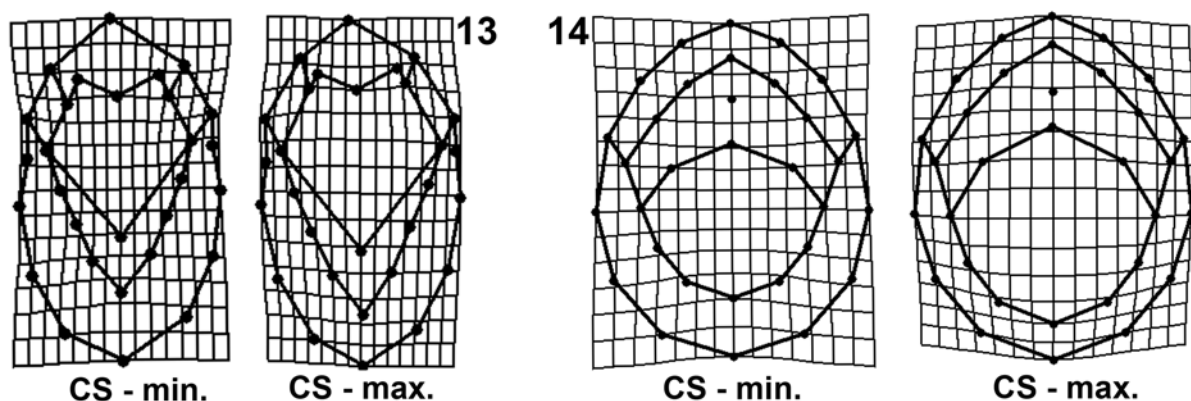
distinctly separated from the remaining groups (Fig. 9). Mean scale–shapes for each pH treatment were demonstrated by thin–plate splines (Fig. 11). Silica scales produced under low, neutral and slightly alkalic pH treatments tended to be wider, with a pronounced V–rib. Scales from treatments at higher pH (pH 8.3 and 8.7) were thinner and their V–rib was reduced considerably. The multivariate regression of Procrustes coordinates on centroid size confirmed the occurrence of a weak, albeit significant, allometric effect on scale shape accounting for only 2.3% of the total shape variance (Wilk's $\lambda = 0.216$, permutation $p = 0.001$; Goodall' F–ratio = 9.8, permutation $p = 0.001$). The scale shape variations along the size gradient (Fig. 13) were minimal. The smallest scales typically had a slightly reduced shield, short pronounced anterior flanges, and expanded posterior flanges, while the largest scales were characterized by an extensive shield and slightly reduced posterior flanges. The relation of scale shape to pH, with size effect

controlled for, remained significant (Wilk's $\lambda = 0.335$, permutation $p = 0.001$; Goodall' F–ratio = 99.8, permutation $p = 0.001$; 19.1 % of variance explained by the regression model). All pair wise comparisons on Mahalanobis distance remained significantly different (all permutation $p < 0.002$). Mean scale–shapes, with the effect of size eliminated (not shown), were very similar to those in Fig. 11.

The values on partial morphological disparity for each of the pH treatments were used to quantify the extent of scale plasticity. The scales produced under higher pH (8.3 and 8.7) in *Mallomonas striata* were significantly more morphologically diverse (2.06×10^{-3} and 1.79×10^{-3}) compared to all the other groups, although slightly increased plasticity at pH 4 was also observed (1.08×10^{-3}). Moreover, the differences between adjacent pairs in the 7.5 pH and lower groups were not statistically significant (permutation $p = 0.1029$ to 0.1672 ; Fig. 15).



Figs 11–12. Mean shape configurations of individual pH treatments illustrated by thin-plate splines. Comparisons are expressed as the deformation of the landmarks of overall consensus configuration into those of mean shapes determined for each pH. Scale factor: two times exaggerated to emphasize differences, (11) *Mallomonas striata*, (12) *Synura echinulata*.



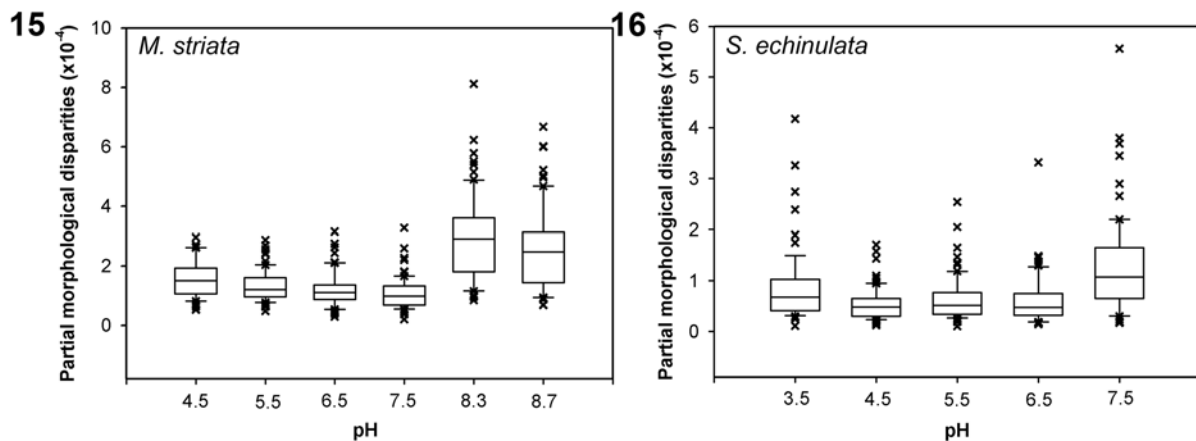
Figs 13–14. Shape configurations reconstructed by the regression model of shape variables on size. Deformations associated with minimum (CS – min.) and maximum (CS – max.) size of the scale are shown, (13) *Mallomonas striata*, (14) *Synura echinulata*.

Synura echinulata

Synura echinulata exhibited a growth response at a pH range of 3.5 – 7.5 (Fig. 8). *S. echinulata* growth was reduced in pH treatments (3.5 and 7.5 pH). The effect of pH on *S. echinulata* centroid size was very similar to that described for *Mallomonas striata*. The positive correlation was rather weak, however significant ($r = 0.13$; permutation $p = 0.0142$). Scale size measurements were highly correlated (CS vs. length of the scale $r = 0.84$; CS vs. width of the scale $r = 0.73$ and length vs. width

of the scale $r = 0.48$; all permutation $p < 0.0001$).

The scale shape of *Synura echinulata* was significantly influenced by pH (Wilk's $\lambda = 0.013$, permutation $p = 0.001$; Goodall' F-ratio = 30.0, permutation $p = 0.001$; 8.0% of variance explained by the regression model). On a CVA scatter plot the scales from an individual pH treatment formed distinctive clusters. All pair wise comparisons on Mahalanobis distance were significantly different (all permutation $p < 0.0005$). The first CV axis separated the groups of scales produced at pH



Figs 15–16. Quantitative extent of scale shape plasticity expressed as partial morphological disparity in individual pH treatments. ×: outlying values, (15) *M. striata*, (16) *S. echinulata*.

3.5 and 7.5 from the rest (Fig. 10). The shapes of mean configurations are illustrated by thin-plate splines (Fig. 12). The low pH group (3.5) was characterized by oval scales with reduced shield and expanded secondary labyrinthic pattern area. The scales from 4.5, 5.5 and 6.5 pH appeared to be relatively uniform in shape, and their outline became rounder with increasing pH. The high pH group (7.5) was distinguished by wider scales with an expanded shield, reduced labyrinthic pattern area and considerably reduced rim. The effect of scale size on shape variables (allometric effect) in *Synura echinulata* was more apparent than in *Mallomonas striata* (Wilk's $\lambda = 0.575$, permutation $p = 0.001$; Goodall' F-ratio = 22.7, permutation $p = 0.001$; 6.2% of variance explained by the regression model). The shape models reconstructed by multivariate regression revealed similarity between the high pH group (7.5) scales and the largest scales (Fig. 14). Multivariate regression of the shape component on pH, with size effect controlled for, confirmed a significant relation (Wilk's $\lambda = 0.03$, permutation $p = 0.001$; Goodall' F-ratio = 26.6, permutation $p = 0.001$; 7.1 % of variance explained by the regression model). All pair wise comparisons on Mahalanobis distance were significantly different (all permutation $p < 0.0005$). Mean scale-shapes, with the effect of size eliminated (not shown), were very similar to those in Fig. 12.

Synura echinulata scales exhibited the highest plasticity in marginal pH 3.5 and 7.5 (5.79×10^{-4} and 8.95×10^{-4}), where the growth of cells was restricted (Fig. 16). Differences among the other pH groups (4.5; 5.5; 6.5) were not statistically significant (permutation $p =$

0.1099 to 0.6076).

Discussion

We investigated the pH reaction norm in two freshwater flagellates: *Mallomonas striata* and *Synura echinulata*. The reaction norm is defined as a set of phenotypes produced by a genotype over a range of environments. We examined the traits concerning silica scale shape over a range of pH. The impact of pH on population growth was used to evaluate optimal/suboptimal conditions. In *M. striata* a pH of 4.5, and in *S. echinulata* pHs of 3.5 and 7.5 were considered suboptimal. The pH tolerance observed under laboratory conditions differed slightly from the results of field studies. *Mallomonas striata* represents an alkaliphilic to pH indifferent species, absent only in extremely acidic conditions (SIVER 1995). It was previously found in localities ranging from 5.2 to 9.0 pH (weighted mean pH 7.8). In our experiments, *M. striata* tolerated the pH of 4.5, 5.5, 6.5, 7.5, 8.3 and 8.7 well. Surprisingly, a growing population was obtained at pH of 4.5 (Fig. 7). This indicates that even though *M. striata* is able to survive under low pH conditions, in combination with other factors (e.g. resource competition) in natural localities it may result in population densities so low that they are undetectable. *Synura echinulata* was described as an acidophilic taxon that easily tolerates habitats with low pH and alkalinity (weighted mean pH 5.9; SIVER 1995). Of the taxa found at low pH, *S. echinulata* has been reported over the largest pH range. It has been observed, although rarely, above pH 8 (KRISTIANSEN 1985;

NĚMCOVÁ et al. 2003). *Synura echinulata* grew well at 3.5, 4.5, 5.5, 6.5 and 7.5 pH, with the optimum at pH 6.5 (Fig. 8), which is consistent with the data from large populations found in nature.

The relationship between pH and size of scales was not clearly ascertained by this study (data not shown). The scale size in *Mallomonas* and *Synura* was found to be significantly affected by the cultivation temperature in previous studies. The size of scales decreased constantly with increasing temperature within a natural range of conditions (ŘEZÁČOVÁ–ŠKALOUDOVÁ et al. 2010; PICHRTOVÁ & NĚMCOVÁ 2011) in accordance with the “temperature rule” (ATKINSON et al. 2003). While the “temperature rule” indicating reduction in cell size with increasing temperature, was verified in a wide range of aquatic protists, data on the pH–cell size relationship is still limited. A wide range of responses have been observed under controlled experimental conditions, as well as in natural populations (BOOTH 2001; NEUSTUPA & HODAČ 2005; WEISSE & STADLER 2006; ČERNÁ & NEUSTUPA 2010).

However, we conclude there was a significant effect of pH on the scale shape in both investigated species, although the proportion of the shape variance explained by regression models differed (27.6% in *Mallomonas striata* and 8.0% in *Synura echinulata*). The relationship between shape and pH remained significant, and even when we controlled for the size effect on scale shape a considerable portion of the shape variance was still explained by regression analysis (19.1% in *M. striata* and 7.1% in *S. echinulata*). The shape determined for *Mallomonas striata* var. *serrata* (CCMP 2059) scales across the tested pH gradient was congruent with the model based on the GM landmark method proposed by NEUSTUPA & NĚMCOVÁ (2007) for this variety. In that study *Mallomonas striata* var. *serrata* scale shape reconstruction was based on 25 images of scales collected worldwide. *Mallomonas striata* var. *serrata* differed from the type variety in having wide and short anterior flanges, narrower shield and thicker V–rib (NEUSTUPA & NĚMCOVÁ 2007). As we investigated the scale shape plasticity of *M. striata* var. *serrata* over a wide pH range we concluded that the appearance of anterior flanges was the most stable character, and it is not subject to change. The anterior flanges were always short and wide, while the area of the shield and the thickness of the V–rib differed with respect to pH (Fig. 11). Scale shape variation of *Synura*

echinulata in crossed gradients of light and temperature was studied by NĚMCOVÁ et al. (2010). Interestingly, scales produced in low illumination combined with low temperature exhibited very similar shape patterns to those from the high pH treatment in this study. In both studies, these treatments represented suboptimal conditions, where scales were characterized by an extended shield area and a considerably reduced labyrinthic pattern. Nevertheless, we think it is still premature to consider this shape pattern to emerge as a consequence of suboptimal conditions.

The ordination pattern of the canonical analysis of *Mallomonas striata* scale shape showed a strong differentiation between low and neutral vs. high pH treatments (Fig. 9); however, in *Synura echinulata* extreme pH treatments were distinct from the others (Fig. 10). While clouds of scores spanned by the first two canonical axes in *S. echinulata* were clearly segregated (with the exception of pH 4.5 and 6.5 groups), a certain degree of overlap was observed among groups of low and neutral treatments, and among groups of high pH treatment in *M. striata*. Nevertheless, all the investigated groups of scales were significantly different in shape in both *M. striata* and *S. echinulata*.

As a consequence of a symmetrisation procedure, where an asymmetric part of variation was eliminated, the mean value on disparity (the extent of scale shape plasticity) was almost three times lower in *Synura echinulata* compared to *Mallomonas striata*. Under the suboptimal conditions, characterized by low growth, the extent of scale plasticity in *S. echinulata* tended to increase. Conversely, this could not be said to apply to *M. striata*, where slightly increased plasticity at pH 4.5 was observed, congruent with slower growth. Nevertheless, the greatest scale shape plasticity was revealed at higher pH treatments, where growth of populations was optimal. Increased phenotypic plasticity has been correlated to environmental stress (KRISTENSEN et al. 2003; NEUSTUPA et al. 2008), generally characterized by reduced growth. We propose that increased scale shape plasticity of synurales in environments approaching pH 9 is not associated with stress, but to a decreased availability of dissolved reactive silicon. Silicon in the form of the weak monosilicic acid (H_4SiO_4) is exploitable by chrysophytes in environments below pH ~ 9 (REYNOLDS 2006). Moreover, *M. striata* cells from higher pH

treatments unmistakably economized on the silicon, thus producing significantly thinner scales with considerably reduced V-rib hoods (Fig. 11; pH 8.3 and 8.7). Investigation of the correlation between scale–shape patterns that develop under high pH with those of scales produced in Si-impoverished medium with an optimal pH, would help to clarify the cause(s) of scale shape variability. Increased scale shape variation associated with either pH stress or reduced H_4SiO_4 availability may decrease the ability of cells to compose a perfectly fitting, functional scale case. Imperfectly armoured cells may become more susceptible to infections, parasites and predators. The protective role of “armor” in the development and turnover of algal populations was emphasised by HAMM & SMETACEK (2007). Besides the inability of synurophytes to use bicarbonate as a source of inorganic carbon (BHATTI & COLMAN 2008; SAXBY–ROUEN et al. 1997), increased scale shape plasticity may represent the other reason why these organisms avoid highly alkaline natural environments.

Finally, we asked, how may the size of scales influence their shape (the effect of allometry). Multivariate regression of shape on centroid size explained 2.3% and 6.2% of variance in *Mallomonas striata* and *Synura echinulata*, respectively. Among photosynthetic protists, shape changes associated with cell size were described in *Micrasterias* semicells (NEUSTUPA et al. 2008; 37.5% of shape variation was explained by size), cenobial cells of *Pediastrum duplex* (NEUSTUPA & HODAČ 2005; size accounted for 13.9% of total shape variation) and in frustules of a pennate diatom *Achnanthes* (POTAPOVA & HAMILTON 2007). In these organisms allometric effect was obviously associated with cellular growth. After the silica scales mature, silicify and are exocytosed to the cell surface their shape is fixed and invariable (LEADBEATER & BARKER 1995), consequently the allometric effect is weaker (PICHRTOVÁ & NĚMCOVÁ 2011; this study). Size accounted for 5.3% and 12.2% of total scale shape variation in *S. petersenii* and *M. tonsurata*, respectively (PICHRTOVÁ & NĚMCOVÁ 2011). Allometry in synurophyte scales is not related to growth of scales but may reflect their position within the scale case (NEUSTUPA et al. 2010).

Silica scales of both investigated species expressed distinctive phenotypes over a range of tested pH, yet all of the observed shapes fitted within original descriptions of the taxa. Organisms

are evolutionarily designed to survive under the multifactorial conditions of nature, and can respond to a large number of factors that surely differ from the conditions in a test tube (GIMMLER 2001). Additional data collected on silica scales of natural populations will further advance our understanding of the relationship between scale shape and key environmental factors, and may help us to evaluate the potential of scale shape in paleoecological reconstructions. Application of partial least-squares analysis within geometric morphometrics (ROHLF 2006) will enable us to visualize correlations of shape changes with a set of variables and to compare those shape patterns to the pH related shape plasticity revealed in this study.

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