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Morphological plasticity of silica scales of *Synura echinulata* (Synurophyceae) in crossed gradients of light and temperature – a geometric morphometric approach

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With 9 figures and 1 table

Abstract: Phenotypic plasticity is the phenomenon of a genotype producing different phenotypes in response to different environmental conditions, and is a ubiquitous aspect of organisms. In this study we used geometric morphometrics to describe qualitatively and quantitatively phenotypic plasticity of *Synura echinulata* silica scales cultured at four combinations of light intensity and temperature. Relative warps analysis revealed that the most important morphological trends were the disproportionality of distal and proximal parts of the scale, and the extent of the anterior labyrinthic pattern. All the groups of scales investigated differed significantly in shape. Silica scales of *S. echinulata* exhibited considerable environmentally induced plasticity, but taxonomically relevant characters remained unchanged. However, the scales from the group grown under dark/cold conditions were clearly the most distinctly discriminated by canonical variate analysis. This group also exhibited the highest extent of scale plasticity, and finally was the only one, where stomatocysts (resting stages) were encountered in culture. Lower temperature, combined with long-term exposure to low light conditions, decreased the scale size, but increased the morphological plasticity of the silica scales. These effects may be considered a response to environmental stress.

Key words: phenotypic plasticity, synuro phyceae, *Synura echinulata*, scale-shape, geometric morphometrics

Introduction

Synura echinulata was described by Korshikov (1929) who revealed, with the help of the light microscope, the different shapes of silica scales within the species of this genus. Biflagellated cells are enclosed in a case formed by imbricated silica scales. Scale morphology, studied under transmission or scanning electron microscopy, is of critical taxonomic importance within the synurophytes, and the scales are a valuable indicator for microfossils in paleolimnological studies. *Synura echinulata* represents a ubiquitous, widely distributed species, e. g. it was found on five out of six continents (Kristiansen 2000), including both tropical (Dürrschmidt & Croome 1985, Cronberg 1989) and arctic environments (Asmund 1968). *Synura echinulata* is one of the most

often reported species occurring in different biotopes with relatively wide ecological tolerances. Although the maximum frequency of occurrence has been reported from acidic localities low in specific conductance and nutrient levels (Siver 2005), the reports from eutrophic fishponds or mesotrophic, pH neutral, alluvial pools are not rare (Němcová et al. 2003). The scale case of *S. echinulata* is composed of several morphological types of scales related to their position on the cell. Body scales are nearly circular to oblong. Longer and narrower caudal scales, together with rearmost (slipper-like) scales, cover the elongated stalk of the cell attached to the colony center. The base plate of the body scales is perforated with pores, and an upturned rim encircles one-half to two-thirds of the scale. The distal part of the body scale is dominated by a hollow tapered spine with an opening to the inner side of the base plate. The distal third of the scale is strengthened by a characteristic vermiform or labyrinthic pattern (secondary layer), and a thin distal edge possesses short radiating ribs separated by a single pore (see Fig. 1). Caudal scales have an extended labyrinthic pattern and a more or less reduced spine. Extreme slipper-like scales lack both a labyrinthic pattern and a spine. Tubular scales were also observed (Wee 1982). A gradual transition between the caudal and rearmost scales is obvious, while body scales and caudal scales seem to remain more distinctive.

The extent of silica scale morphological plasticity within natural populations of synurophytes has been studied since the invention of the electron microscope (Asmund 1955, Fott 1955). As the classification of synurophytes relies almost exclusively on structural variation of scales, the definition of stabile, species-specific scale characters that are not subject to environmental change, is highly desirable. A few papers, based on field observations, dealt with the impact of environmental factors on variation of silica structures (Siver & Skogstad 1988, Siver 1991). Experiments conducted on clonal populations (with uniform genotype) subjected to different, controlled conditions may help to characterize scale shape plasticity within the species. Martin-Wagemann & Gutowski (1995) investigated the effect of temperature and culture age on scale morphology of three clones of *S. petersenii.* To describe the change in scale shape, linear measurements (e. g. scale length and width, central ridge length and width, pore diameter) were used. They found that higher cultivation temperature caused shortening of scales. Gavrilova et al. (2005) documented variation of scale shape in two *S. petersenii* strains in response to pH. Under suboptimal pH conditions scales were significantly shorter when compared to populations grown at optimum pH. Earlier, Gutowski (1996) had examined the relationship between the variability in scale and bristle length in clonal cultures of *M. tonsurata,* and observed that scales and bristles shortened with increasing temperatures. Cells grown in silica- and phosphorus-enriched cultures produced significantly larger scales, although the dimensions were still within the limits defined for *M. tonsurata* (Hahn et al. 1996).

In the present study we use landmark-based methods of geometric morphometrics (GM) to investigate the effect of light and temperature on size and shape variation of *S. echinulata* silica scales. Geometric morphometrics is a recently developed approach that retains information on spatial covariation among biologically definable homologous points called landmarks (Rolf & Marcus 1993, Adams et al. 2004). Shape variables of objects may be described by differences in the coordinates of corresponding landmarks. Multivariate statistical procedures are complementary with GM methods, and may be used to test for statistically significant correlations between a shape and ecological factors (Rohlf & Marcus 1993, Dryden & Mardia 1998, Rohlf 2000, Zeldich et al. 2004). Geometric morphometrics allows one to directly visualize changes and transformations necessary to distinguish one shape from another. To date, only a few studies have used this methodology for analyzing phenotypic plasticity (Debat et al. 2003, Neustupa & Hodač 2005, Neustupa et al. 2008). In silica scaled chrysophyte research, landmark-based GM methods were used to characterize the patterns of variation in the shape of silica scales of *Mallomonas striata* (Neustupa & Němcová 2007). The shape differences between scales belonging to the two varieties – *M. striata* var. *striata* and *M. striata* var. *serrata* – were revealed. Until that study, these taxa had been distinguished only on the bases of their bristle structure.

Materials and methods

Synura echinulata was isolated from a drainage ditch in the southern part of the Břehyně-Pecopala National Nature Reserve, Czech Republic (pH 3.8). Stock clonal culture was grown in DY V medium (Keller & Andersen, https://ccmp.bigelow.org/node/73) without pH adjustment (pH 5.3) at 18 °C under continuous light. Diluted culture (200 cells.ml^{-1}) was pipetted to a 96 well culture plate. Cultivation in crossed gradients of temperature and light were performed in a Labio unit (Czech Republic); for details see Kvíderová & Lukavský (2001). Cells were cultivated for 14 days under continuous illumination (daylight fluorescent tubes Osram Dulux L). Four groups of cells growing at limit positions of a crossed gradients unit were designated as follows: 110 µmol.m^{-2}.s^{-1} and 22 °C – referred to as light/warm group in the text; 9 µmol.m^{-2}.s^{-1} and 22 °C – dark/warm group; 110 µmol.m^{-2}.s^{-1} and 12 °C – light/cold group; and 9 µmol.m^{-2}.s^{-1} and 12 °C – dark/cold group. The viability of colonies growing in wells was checked under a light microscope using low magnification (40×). Cells from one quarter of the culture plate (15 wells) were combined and treated as a single sample. Samples were fixed with Lugol's and prepared for transmission electron microscopy (TEM). Grids were examined with a JEOL 1010 electron microscope. Stomatocysts were oxidized in peroxide according to Kalina et al. (2000). Fifty randomly selected body scales from each group were photographed. Caudal and rear scales were omitted from the analyses. Twenty-one landmarks were defined on each investigated scale (Fig. 1) using the TpsDig ver. 2.05 (Rolf 2006) program. Thirteen of the semilandmarks were allowed to slide along the outline of the scale. The left and right sides of the scale may not be discerned on TEM pictures, so we symmetrized the landmarks in mirror positions as was recommended by Klingenberg et al. (2002) – see Neustupa & Němcová (2007), Neustupa et al., this volume. Original and mirrored landmark configurations were averaged and super-imposed by generalized Procrustes analysis (Bookstein 1991, Marcus et al. 1996) in tpsRelw ver.1.42 (Rohlf 2006). The centroid size (square root of the sum of squared distances from the landmarks to their centre) of each configuration prior to superimposition was used as a size variable. Differences among centroid size of individual groups were tested using permutation t-test (10 000 permutations) in PAST ver.1.74 (Hammer et al. 2001). The multivariate regression of shape data on centroid size was performed in tpsRegr ver. 1.31. (Rohlf 2006). The permutation test (1000 permutations) on Wilk's λ and Goodall's F-ratio was used to evaluate significance of the regression model.

The relative warp analysis (Rolf 1993) of the shape data was conducted on silica scales of individual groups and on all investigated silica scales. Variation in landmark positions spanned by the first two relative warps was visualized using the thin-plate spline approach. Canonical variate analysis (CVA) was conducted to distinguish individual groups using scores of the objects on the first ten relative warps. The CVA was performed in PAST ver.1.74. To test for scale shape differences between individual groups, the scores on the first ten relative warps were used for two-group multivariate permutation tests (10000 permutations) on Mahalanobis distance between all group pairs.

Shape configurations typical for individual groups were visualized as deformation grids, gener-ated from thin-plate spline analysis. Deformation grids demonstrate how the overall consensus configurations must be warped to fit average shapes determined for each group. A theoretical model based on original photographs of scales was created using tpsSuper, ver. 1.12 (Rohlf 2006).

To quantify the differences in the extent of scale plasticity among individual groups the values on partial morphological disparities were used (Zelditch et al. 2004). Morphological disparity was calculated using the formula:

$$M = \frac{\sum_{i=1}^{N}\left(D_i^2\right)}{(N-1)}$$

where Di is Procrustes distance of an individual object (scale) to a consensus shape and N is number of objects. The contribution of each scale to the overall morphological disparity of the set can be expressed as partial morphological disparity index:

$$PM = \frac{D_i^2}{N-1}$$

The significance of differences in morphological disparity among individual groups was evaluated by a permutation test with 10 000 permutations using partial morphological disparity *PM* as a computed value in R 2.3.1. routine (R Core Development Team 2007).

Results

In all individual groups, a sufficient number of new colonies/cells was revealed. Colonies (mostly ca. 15–25 cells) were in a good physiological state. Under dark/cold treatment more colonies were disintegrated to smaller ones (2–3 cells) or to individual cells, compare to other treatments. Furthermore, the population grown under dark/cold conditions was the only one in which the process of encystation was initiated and stomatocysts (resting stages) were observed (Figs 3, 4). In all investigated groups some weakly silicified scales occurred. Typically, these scales were composed of an extremely thin basal plate with no secondary structure (Fig. 2). These scales were probably released from silicon deposition vesicles before silification was completed when the cell was fixed. Weakly silicified scales were excluded from the shape analyses.

No significant differences were found in centroid size values of groups growing in high temperature (light/warm × dark/warm groups; permutation p = 0.684), while the lower temperature groups differed significantly between each other and also among the other groups (with the permutations p << 0.006 in all t-tests). The scales from the dark/cold group were the smallest (Fig. 5). Shape appeared to be weakly, but still significantly, related to centroid size. Shape-to-
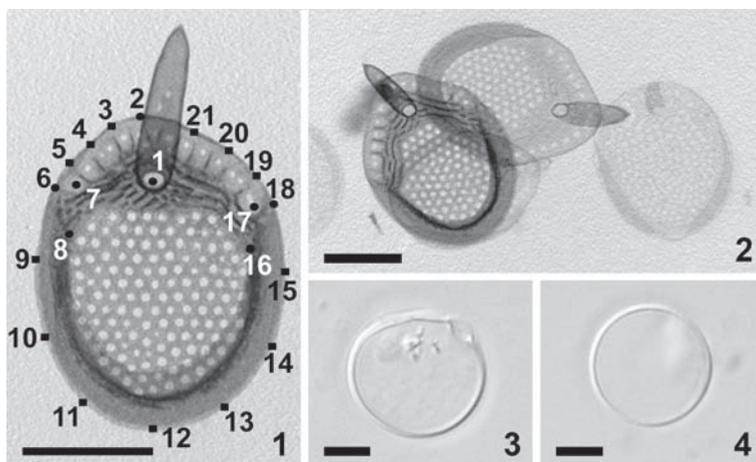
Figs 1–4. 1: Position of landmarks (circles) and semilandmarks (squares) on a body scale of *Synura echinulata* (bar = 1 μm). **2**: Various degrees of silification in *S. echinulata* scales, only well silicified scales (left) were used for shape analyses (bar = 1 μm). **3–4**: stomatocysts revealed in a culture growing in dark/cold conditions (bar = 5 μm).
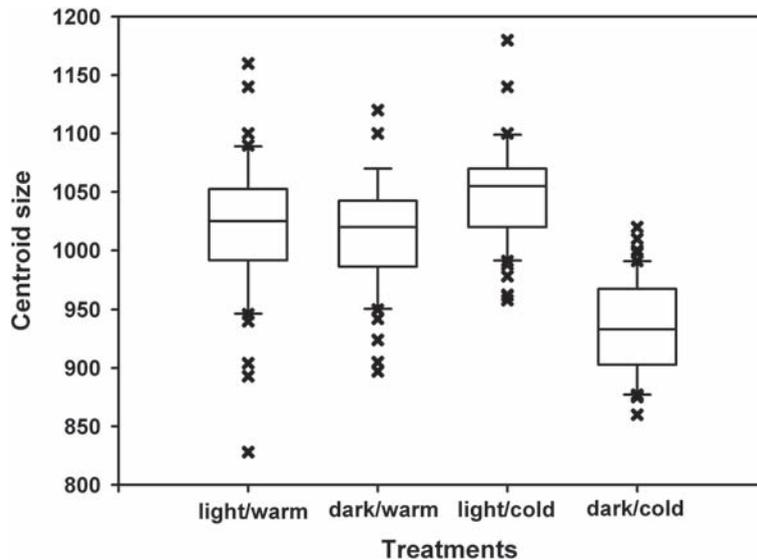
Fig. 5. Centroid size values of scales from four ecologically determined groups. × represents outlying values.

size multivariate regression model of the entire data set was significant with p < 0.001 for Wilk's λ and p < 0.01 for Goodall's F-ratio, but it explained only 2.0 % of the variation. The shape changes associated with centroid size may have corresponded to the position of the scale on the cell surface. The shape variation related to the position of scales on cells was not tested in this study. (However, for details on the shape variation related to the position of scales on cells in the genus *Mallomonas* see Neustupa et al. this volume).

The relative warp analysis (RWA) revealed the most important trends in the overall shape variation for the entire data set (all groups treated together). Relative warps 1–4 accounted for 93.2 % of the total variation in shape (RW1 46.5, RW2 24.0, RW3 15.9 and RW4 6.8). Shape changes of scales associated with extreme positions along the first two RW axes are illustrated in Fig. 6e. The first relative warp described the change from wide rounded scales with a pronounced distal part to guttiform oval scales with a reduced distal part. The second RW emphasized extension of the characteristic labyrinthic pattern (or secondary layer). Shape changes of scales in individual groups along the first two relative warps are depicted in Fig. 6a–d. Similarity in a pattern of shape dynamics was obvious in the first axes of light/warm and dark/warm groups (Fig 6a, b), while slightly different morphological trends were revealed in the first axes of cold groups (Fig 6c, d). Compare, e. g. the pronounced distal part of the rounded scales in the light/cold group to the reduced distal part of the rounded scales in the dark/cold group.

The canonical variate analysis clearly segregated the dark/cold group scales from all other groups (Wilk's λ = 0.0351, p << 0.0001). A certain degree of overlap was observed among the light/warm, dark/warm and light/cold groups. The first two canonical axes explained 96.6 % and 2.2 % of the variation, respectively (Fig. 7). All the investigated groups of scales were significantly different in shape (p << 0.001). We compared the scale shape similarity between pairs of groups using the values on Mahalanobis distance. The smaller the value, the more similar in scale shape were the groups. Light/warm and dark/warm groups were the most similar, while light/cold and dark/cold groups showed the most dissimilar scale shapes (Tab. 1).
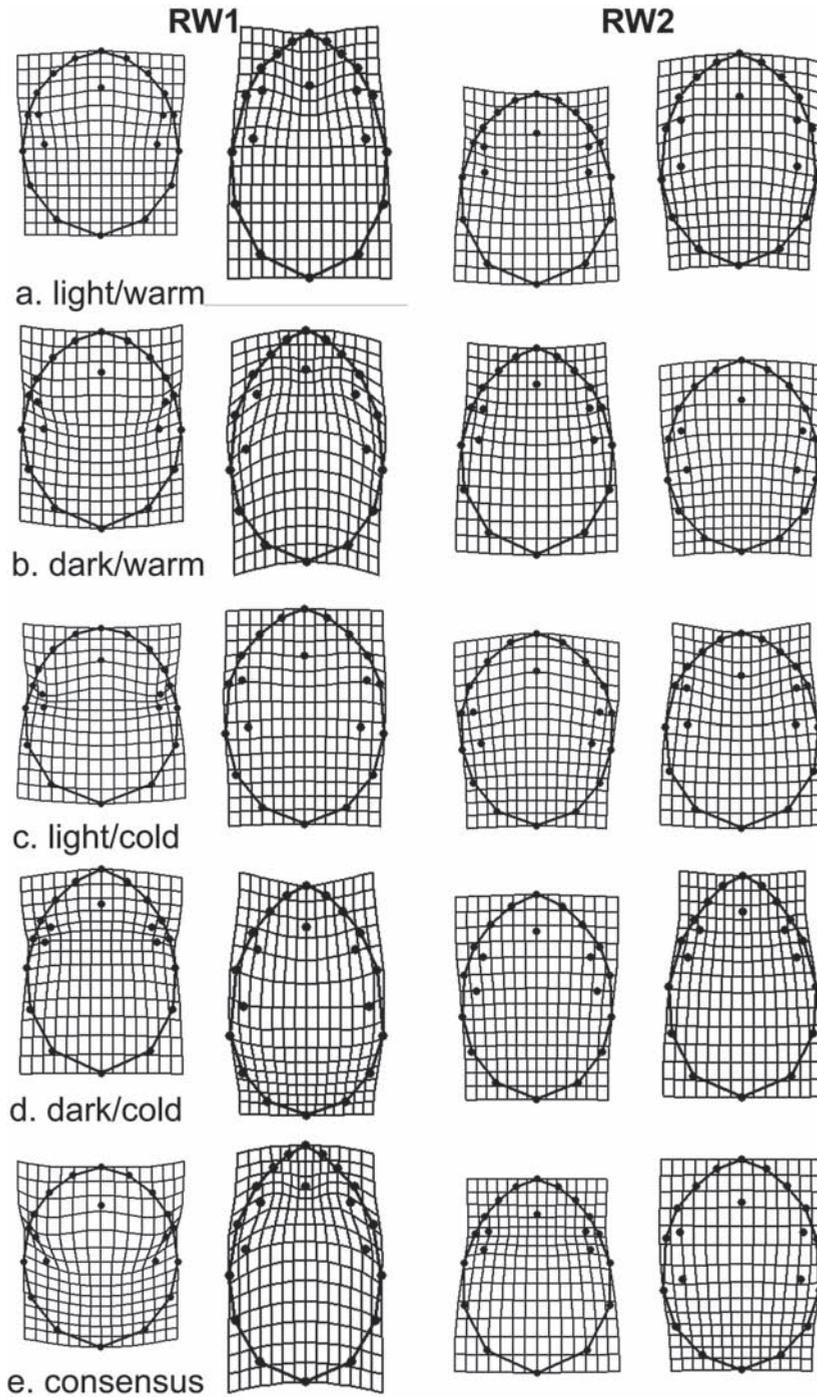
Fig. 6. Shape changes of scales associated with extreme positions along first two relative warps. **a**: a light/warm group. **b**: a dark/warm group. **c**: a light/cold group. **d**: a dark/cold group. **e**: all groups treated together (e. g. overall morphological variation within all investigated scales).
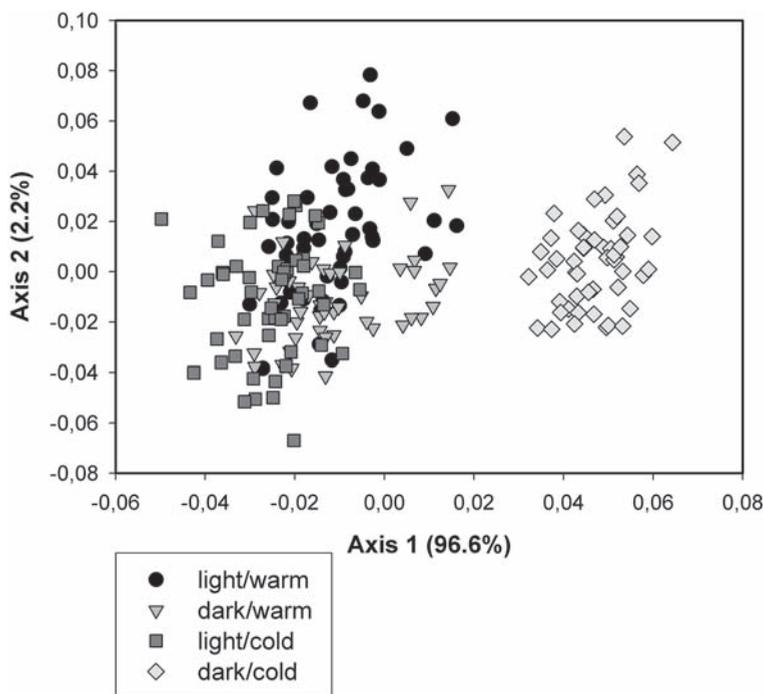
Fig. 7. Discrimination of shapes from four ecologically determined groups. CVA ordination plot shows individual scale scores along canonical axes. Values in brackets indicate the proportion of total variation described along each axis.

Table 1. Pair wise comparisons were performed using Mahalanobis distances. Significant scale shape differences were revealed between all tested groups (p << 0.001)

n scales	group	Mahalanobis distance			
		light/warm	dark/warm	light/cold	dark/cold
50	light/warm	–	0.1903	0.2598	0.9641
50	dark/warm		–	0.2692	0.9404
50	light/cold			–	0.9887
50	dark/cold				–

The light/warm group was characterized by oval scales with a rim encircling two-thirds of the scale and a well developed labyrinthic pattern (Fig. 8a). The scales from the dark/warm group were oval to rounded with a rim encircling slightly more than half of the scale, also a labyrinthic pattern was well developed (Fig. 8b). The light/cold group was characterized by almost circular body scales with a rim encircling slightly more than half of the scale, and with an extremely extended labyrinthic pattern (Fig. 8c). The scales of the dark/cold group were
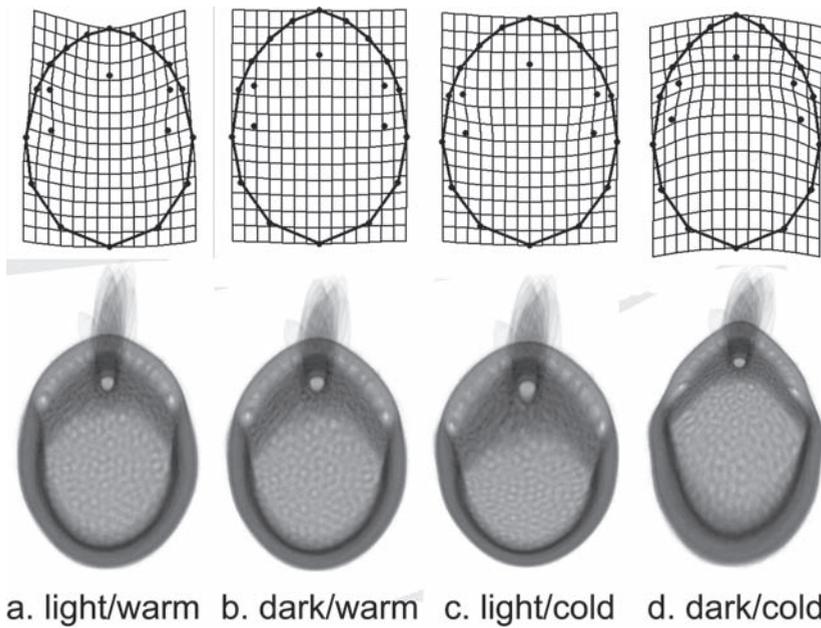
a. light/warm   b. dark/warm   c. light/cold   d. dark/cold

Fig. 8. Mean shape configurations of individual groups accompanied by theoretical shape models. Deformation grids demonstrate how consensus configuration must be warped to fit mean shapes determined for each group. Scale factor: three times exaggerated to emphasize differences.

oval to guttiform with a rim encircling two-thirds of the scale, and their labyrinthic pattern was considerably reduced (Fig. 8d).

The values of partial morphological disparity for each of the groups were used to quantify the extent of scale plasticity (Zelditch et al. 2004). The dark/cold group was significantly more morphologically diverse than all the other groups (permutation p < 0.0066 in all paired tests). The differences among the other groups were not statistically significant (Fig. 9).

Discussion

Phenotypic plasticity is the phenomenon of a genotype producing different phenotypes in response to different environmental conditions, and it is a ubiquitous aspect of organisms (Travis 1994, Trainor 1998, West-Eberhard 2003). In this study we used geometric morphometrics to describe, both qualitatively and quantitatively, phenotypic plasticity of *Synura echinulata* silica scales in four combinations of light intensity and temperature. We designed the experiment to simulate natural conditions, so that neither extremely high nor low light intensities nor temperatures were applied. Cultures were illuminated continuously not to restrict the growth (Healey 1983, Saxby-Rouen et al. 1997, Gutowski 1996), although the light-dark cycle would probably represent more natural illumination. All the investigated groups of scales differed significantly in shape. However, the scales from the group growing in dark/cold conditions were most different in shape from all the other groups; furthermore, they were clearly the ones most distinctly discriminated by CVA (Fig. 7). This group was also more variable in shape than all the other groups (Tab. 1). Finally, the dark/cold population
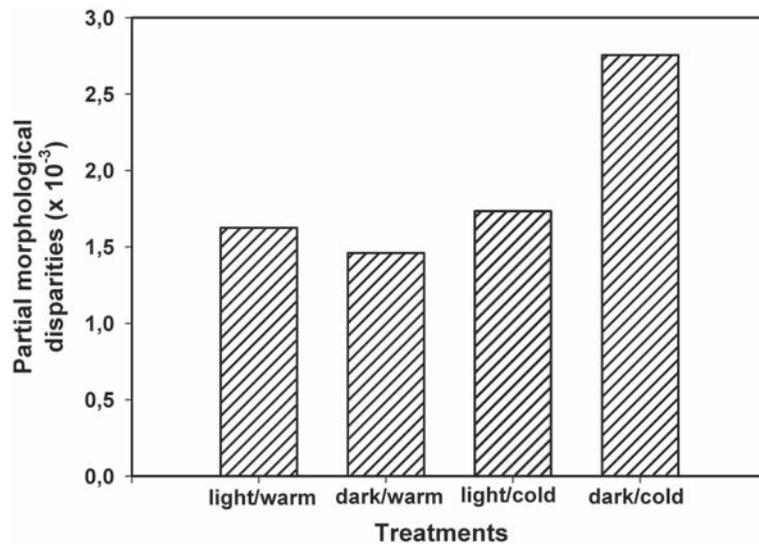
Fig. 9. Partial morphological disparities indicating quantitative extent of scale shape plasticity.

was the only one where stomatocysts were observed in cultures (Figs 3, 4). As the *Synura echinulata* clone was derived from a single colony, only one mating type was present, so the production of sexual cysts (Sandgren & Flanagin 1986) could be eliminated. Vegetative cysts represent resting stages, and their formation has also been reported as a strategy of the cells to survive unfavorable conditions (Sandgren 1983), although the process of encystment and excystment is still poorly understood (Agbeti & Smol 1995). We consider that stomatocyst formation may be interpreted as an indicator of stressful conditions in this case. The 12 °C temperature of the dark/cold population certainly represents the temperature normally encountered in natural localities. However, when combined with long-term exposure to low light intensities (9 μmol.m^{-2}.s^{-1}), stress may occur. Saxby-Rouen et al. (1997) also reported the reduction of the growth rate of *Synura petersenii* at a light intensity of 14 μmol.m^{-2}.s^{-1}.

Different types of phenotypic plasticity play a role in the adaptation of populations to new environments. Adaptive phenotypic plasticity reflects the ability of a population to respond to the normal range of conditions (e.g. conditions normally encountered in natural localities), while non-adaptive plasticity acts in "environmental stress" situations (Bradshaw & Hardwick 1989, Pigliucci 2001, Ghalambor et al. 2007). Stress-induced plasticity is thought to reveal cryptic genetic variation that results in an increase in the genotypic and phenotypic variance that is "hidden" or unexpressed under normal environmental conditions (e.g. Rutherford 2000, Ghalambor et al. 2007). This is probably the case in the dark/cold group where the greatest extent of scale plasticity was observed. Increased phenotypic plasticity has been correlated to environmental stress (Kristensen et al. 2003, Neustupa et al. 2008).

However, certain limitations to this interpretation must be considered, primarily that the scale shape represents a single trait and we do not know how it is related to fitness of an organism. The shape changes of scales are hardly biologically interpretable. The structure of scales, and of the scale coat as a whole provides an obvious space limitation on shape variation consistent with the universal pattern of scale arrangement on cells. Thus, certain construction principles probably are required to be followed:

1) The scale must retain its firmness. Reduction of a labyrinthic pattern was compensated for by extension of an upturned rim (see Fig. 8d).
2) The change in scale shape is constrained by a precise arrangement of the individual scale within the scale-case (Leadbeater 1990).

Paasche (1973a and 1973b) revealed the relationship between growth rate and cellular silica content which had direct impact to frustule morphology in a marine plankton diatom *Thalassiosira pseudonana*. The author speculated that the growth rate in silicate limited populations is governed by the uptake rate of silicate. In Synurophytes, by contrast to diatoms, the growth rate is not restricted by silica stress (Sandgren et al. 1996). It is not known, whether the decreased growth rate caused by other limitations than Si (temperature, pH) may influence silicate-uptake rate and thus impact the scale morphology in silica-scaled chrysophytes.

Silica scales of *Synura echinulata* exhibited considerable plasticity with respect to different combinations of light intensity and temperature. Taxonomically relevant characters remained unchanged, e. g. all scales were clearly identifiable as *S. echinulata*. The anterior part of the scales seem to be more variable, especially the extension of a labyrinthic pattern. Our results support the findings of Gutowski (1996), who showed proximal border and posterior flange of *M. tonsurata* scales insensitive to temperature, while an anterior part varied considerably. The posterior part of the scale is overlapped by adjacent scales and thus probably more stabile.

Geometric morphometrics provides us with an incomparably powerful tool with which to describe and quantify subtle shape variation. Experimental data should be compared to those obtained in the field. We must consider the fact that environmental impact on scale plasticity is very complex, and we are able to measure only a limited number of parameters.

Further study of phenotypic plasticity in experimental populations of synurophytes will help to reveal some general morphological trends related to certain environmental changes. Data on scale shape plasticity of widely distributed species, particularly those with wide ecological tolerances, will increase their potential in paleoecological research.

Acknowledgements

References

Adams, D.C., F.J. Rohlf & D.E. Slice (2004): Geometric morphometrics: ten years of progress following the 'revolution'. – Italian J. of Zool. **71**: 5–16.

Agbeti, M.D. & J.P. Smol (1995): Chrysophyte population and encystment patterns in two Canadian lakes. – J. Phycol. **31**: 70–78.

Asmund, B. (1955): Electron microscope observations on *Mallomonas caudata* and some remarks on its occurrence in four Danish ponds. – Bot. Tidsskrift **52**: 163–168.

Asmund, B. (1968): Studies on Chrysophyceae from some ponds and lakes in Alaska. VI. Occurrence of *Synura* species. – Hydrobiol. **31**: 497–515.

Bookstein, F.L. (1991): Morphometric tools for landmark data: geometry and biology. – Cambridge University Press, Cambridge.

Bradshaw, A.D. & K. Hardwick (1989): Evolution and stress: genotypic and phenotypic components. – Biol. J. of the Linnean Soc. **37**: 137–155.

Cronberg, G. (1989): Scaled chrysophytes from the Tropics. – Beih. Nov. Hedw. **95**: 191–232.

Debat, V., M. Begin, H. Legout & J.R. David (2003): Allometric and nonallometric components of *Drosophila* wing shape respond differently to developmental temperature. – Evolution **57**: 2773–2784.

Dryden, I.L. & K.V. Mardia (1998): Statistical shape analysis. John Wiley & Sons, New York.

Dürrschmidt, M. & R. Croome (1985): Mallomonadaceae (Chrysophyceae) from Malaysia and Australia. – Nord. J. Bot. **5**: 285–298.

Fott, B. (1955): Scales of *Mallomonas* observed in the electron microscope. – Preslia **27**: 280–282.

Gavrilova, O.V., N.V. Nogina & L.N. Voloshko (2005): Scale structures and growth characteristics of *Synura petersenii* (Synurophyceae) under different pH conditions. – Beih. Nov. Hedw. **128**: 249–256.

Ghalambor, C.K., J.K. McKay, S.P. Carroll & D.N. Reznick (2007): Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. – Functional Ecol. **21**: 394–407.

Gutowski, A. (1996): Temperature dependent variability of scales and bristles of *Mallomonas tonsurata* Teiling emend. Krieger (Synurophyceae). – Beih. Nov. Hedw. **114**: 125–146.

Hahn, A., A. Gutowski & U. Geissler (1996): Scale and bristle morphology of *Mallomonas tonsurata* (Synurophyceae) in cultures with varied nutrient supply. – Botanica Acta **109**: 239–247.

Hammer, Ø., D.A.T. Harper & P.D. Ryan (2001): PAST: paleontological statistics software package for education and data analysis. Palaeont Electr **4**:1–9.

Healey, F.P. (1983): Effects of temperature and light intensity on the growth rate of *Synura sphagnicola*. – J. of Plankton Res. **5**: 767–774.

Kalina, T., Y. Němcová & J. Neustupa (2000): Silica-scaled chrysophytes of the Czech Republic 1. District Česká Lípa (Northern Bohemia) and part of the Central Bohemia. – Arch. Hydrobiol. 131/Algol. Stud. **96**: 29–47.

Klingenberg, C.P., M. Barluenga & A. Meyer (2002): Shape analysis of symmetric structures: quantifying variation among individuals and asymmetry. Evolution **56**: 1909–1920.

Korshikov, A.A. (1929): Studies on the Chrysomonads. I. – Arch. Protistenk. **67**: 253–290.

Kristensen, T.N., C. Pertoldi, D.H. Andersen, V. Loeschcke (2003): The use of fluctuating asymmetry and phenotypic variability as indicators of developmental instability: a test of a new method employing clonal organisms and high temperature stress. – Evol. Ecol. Res. **5**: 53–68.

Kristiansen, J. (2000): Cosmopolitan chrysophytes. – Syst. Geogr. Pl. **70**: 291–300.

Kvíderová, J. & J. Lukavský (2001): A new unit for crossed gradients of temperature and light. – Beih. Nov. Hedw. **123**: 541–550.

Leadbeater, B.S.C. (1990): Ultrastructure and assembly of the scale case in *Synura* (Synurophyceae Andersen). – Br. Phycol. J. **25**: 117–132.

Marcus, L.F., M. Corti, A. Loy, G.J.P. Naylor & D.E. Slice (1997): Advances in Morphometrics. Plenum Press, New York.

Martin-Wagenmann, B. & A. Gutowski (1995): Scale morphology and growth characteristics of clones of *Synura petersenii* (Synurophyceae) at different temperatures. – In: Sandgren, C.D., J.P. Smol & J. Kristiansen (eds.): Chrysophyte algae: 232–268. Cambridge University Press.

Němcová, Y., J. Neustupa, S. Nováková & T. Kalina (2003): Silica-scaled chrysophytes of the Czech Republic. – Acta Univ. Carolinae Biologica **47**: 285–346.

Neustupa, J. & L. Hodač (2005): Changes in shape of the coenobial cells of an experimental strain of *Pediastrum duplex* var. *duplex* (*Chlorophyta*) reared at different pHs. – Preslia **77**: 439–452.

Neustupa, J. & Y. Němcová (2007): A geometric morphometric study of the variation in scales of *Mallomonas striata* (Synurophyceae, Heterokontophyta). – Phycologia **46**: 123–130.

Neustupa, J., J. Šťastný & L. Hodač (2008): Temperature-related phenotypic plasticity in the green microalga *Micrasterias rotata*. – Aquatic microbial ecology **51**: 77–86.

Paasche, E. (1973a): Silicon and the ecology of marine plankton diatoms. I. *Thalassiosira pseudonana* Hasle and Heimdal (Cyclotella nana Hust.) grown in a chemostat with silicate as the limiting nutrient.– Mar. Biol. **19**: 117–126.

Paasche, E. (1973b): Silicon and the ecology of marine planktonic diatoms. II. Silicate-uptake kinetics in five diatom species.– Mar. Biol. **19**: 262–269.

Pigliucci, M. (2001): Phenotypic Plasticity: Beyond Nature and Nurture. Johns Hopkins University Press, Baltimore.

R Core Development Team (2007): R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.

Rohlf, F.J. (2000): Statistical power comparisons among alternative morphometric methods. – Am. J. Phys. Anthropol. **111**: 463–478.

Rohlf, F.J. (2006): Tps series. Department of Ecology and Evolution, State University of New York at Stony Brook, New York.

Rohlf, F.J. & L.F. Marcus (1993): A revolution in morphometrics. – Trends in Ecology and Evolution **8**: 129–132.

Rutherford, S.L. (2000): From genotype to phenotype: buffering mechanisms and the storage of genetic information. – BioEssays **22**: 1095–1105.

Sandgren, C.D. (1983): Survival strategies of chrysophyte flagellates: reproduction and formation of resistant resting cysts. – In: Fryxell, G. (ed.): Survival strategies of algae: 23–48. Cambridge University Press.

Sandgren, C.D. & J. Flanagin (1986): Heterothallic sexuality and density dependent encystment in the chrysophyte alga *Synura petersenii* Korshikov. – J. Phycol. **22**: 206–216.

Sandgren, C.D., S.A. Hall & S.B. Barlow (1996): Siliceous scale production in chrysophyte and synurophyte algae. I. Effects of silica-limited growth on cell silica content, scale morphology, and the construction of the scale layer of *Synura petersenii*.– J. Phycol. **32**: 675–692.

Saxby-Rouen, K.J., B.S.C. Leadbeater & C.S. Reynolds (1997): The growth response of *Synura petersenii* (Synurophyceae) to photon flux density, temperature, and pH. – Phycologia **36**: 233–243.

Siver, P.A. (1991): The biology of *Mallomonas*. Morphology, taxonomy, ecology. – Developm. Hydrobiol. **63**: 1–228. Kluwer Academic Publishers, Dordrecht.

Siver, P.A. (1995): The distribution of chrysophytes along environmental gradients: their use as biological indicators. – In: Sandgren, C.D., J.P. Smol & J. Kristiansen (eds.): Chrysophyte algae: 232–268. Cambridge University Press.

Siver, P.A. & A. Skogstad (1988): Morphological variation and ecology of *Mallomonas crassisquama* (Chrysophyceae). – Nord. J. Bot. **8**: 99–107.

Trainor, F.R. (1998): Biological aspects of *Scenedesmus* (Chlorophyceae) – phenotypic plasticity. – Beih. Nov. Hedw. **117**: 1–367.

Travis, J. (1994): Evaluating the adaptive role of morphological plasticity. – In: Wainwright, P.C. & S.M. Reilly (eds.): Ecological Morphology: 99–122. University of Chicago Press, Chicago.

Wee, J.L. (1982): Studies on the Synuraceae (Chrysophyceae) of Iowa. – Bibl. Phycol. **62**: 1–183.

West-Eberhard, M.J. (2003): Developmental Plasticity and Evolution. – Oxford University Press, New York.

Zelditch, M.L., D.L. Swiderski, D.H. Sheets & W.L. Fink (2004): Geometric morphometrics for biologists: a primer. – Elsevier Academic Press, London.