Effect of temperature on the variability of silicate structures in *Mallomonas kalinae* and *Synura curtispina* (Synurophyceae)

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With 14 figures and 4 tables

**Abstract:** Taxonomy of the class Synurophyceae is based on the morphology of the siliceous cell coat, especially of the scales that are found in samples of freshwater plankton or in sediments and examined by electron microscope (EM). Members of this class are known to be powerful indicators of environmental conditions and are often used for biomonitoring and paleoecological studies. We investigated the effect of temperature on scale shape using landmark-based geometric morphometric methods in two synrophycean species *Mallomonas kalinae* Řezáčová and *Synura curtispina* (Petersen & Hansen) Asmund. Clones of these species were grown at different temperatures (5, 10, 15, 20, 25, and 30 °C) in batch culture experiments. We found statistically significant differences in the shape and size variation of silicate structures corresponding to temperature changes, although a substantial part of shape variation was associated with the position of scales on the cell. The range of shape variation was characterised by wide rounded vs. tapered scales, and further by the extent of the dome area, and the secondary layer. Highly asymmetrical apical *Mallomonas* scales occurred only at low temperatures (10 and 15 °C). We also revealed a tendency for scale size to be reduced with increasing temperature; significant differences were found both in size of scales and in length of bristles or spines. Additionally, the present study provides evidence that geometric morphometrics is a powerful tool in analyses of temperature induced shape variation of silica scales.

**Key words:** Synurophyceae, culture experiments, shape variation, scale size, geometric morphometrics

**Introduction**

During the last decade, many studies on relationships among temperature, organismal growth, shape plasticity, and cell size in several taxa have been published. The temperature-dependent plasticity of body size has been observed in bacteria, protists, plants, and animals (e.g. Atkinson & Sibly 1997, Partridge & Coyne 1997, Kindleman et al. 2001, Angilleta et al. 2004, Yom-Tov & Geffen 2006). In this study, we have investigated the effect of water temperature on shape and plasticity of silicate structures in two synurophycean species. Members of the class Synurophyceae (Heterokontophyta) are characterized by silica scales that cover their cell body. Taxonomy of this group has been based on morphology of scales and bristles since the 1950’s, when EM became available. Most synurophycean species are classified into two genera. The genus *Mallomonas* Perty
includes unicellular species with about 30 to 150 scales per cell, depending on the species (Siver 1991). The bases of bristles are connected to the apical part of the scales (dome), but both bristles and scales develop independently in cells (Siver 1991, Wee 1997). The genus *Synura* Ehrenberg is colonial and, depending on the species, the number of scales per cell ranges from approximately 50 to 100 (Wee 1997). A number of synurophyte species are known to occur only in specific habitats that are generally characterized by temperature, pH, conductivity or trophic conditions. Therefore, synurophytes are frequently used in limnological and paleolimnological biomonitoring studies. In this respect, the study of morphological variation of siliceous structures could represent important information for biomonitoring studies (Smol 1995). Siver & Skogstad (1988) found a distinct relationship between temperature and the two morphological types (helmet and serrated) of siliceous bristles in populations of *Mallomonas crassisquama*. The serrated bristles were typical for cold water, whereas, helmet bristles occurred exclusively in a warm water environment. In laboratory conditions, the effect of temperature on the variability of scales was investigated in *Synura petersenii* (Martin-Wagenmann & Gutowski 1995) and in *Mallomonas tonsurata* (Gutowski 1996). All these studies were based on the analysis of conventionally measured distances (length, width, angles) of structurally homologous points on silica-scale structures. Here, we use methods of landmark-based geometric morphometrics for the analysis of temperature-dependent differences in *Mallomonas kalinae* and *Synura curtispina* scales. Geometric morphometrics is based on high-dimensional multivariate analyses of shape variables that retain all of the geometric information contained within the data and provides informative visualizations that are frequently not possible to obtain with alternative methods (Slice 2005). Geometric morphometric methods have recently become the most useful tool for the evaluation of shape in many branches of organismal biology (Adams et al. 2004).

In phycology, geometric morphometric methods were applied in taxonomic studies of closely related diatoms (Beszteri & Medlin 2005, Potapova & Hamilton 2007), and in several studies dealing with green algae (Neustupa & Hodač 2005, Verbruggen et al. 2005, Neustupa & Škaloud 2007). In synurophytes, Neustupa & Němcová (2007) investigated variation in scales of *Mallomonas striata* using geometric morphometric analyses of natural populations and data from the literature. They found significant differences in the shapes of scales occurring world-wide in two varieties – *M. striata* var. *striata* and *M. striata* var. *serrata* Harris & Bradley. Previously, both of these varieties had been delimited only on the basis of differences in bristles (Kristiansen 2002).

This report considers the effect of temperature on silicate structures (both scales and bristles) of clonal populations of *Mallomonas kalinae* and *Synura curtispina*. The aim of this study is to describe the main trends in scale shape variation, and to document these changes in relation to temperature.

**Materials and Methods**

*Mallomonas kalinae* was isolated from a peaty pool in the Czech Republic and is deposited in the Culture Collection of Algae at Charles University in Prague (CAUP B601). *Synura curtispina* (SAG 29.92) was obtained from the Culture Collection of Algae at the University of Göttingen. Both unialgal batch cultures were grown in DY IV medium (Andersen et al. 1997). Stock cultures were grown at 25 °C under continuous light. Initially, about 50 cells of each flagellate were transferred into Erlenmeyer flasks during their exponential phase of growth. Experiments were carried out simultaneously in different chambers at temperatures of 5, 10, 15, 20, 25, and 30 °C on a 16:8 light:dark cycle at 40 µmol photons m−2 s−1 for one month. Lugol’s fixed algal suspensions were then prepared for transmission electron microscopy (TEM) and examined with a JEOL 1010. For both species, about 60 randomly selected scales from each population growing at each of the temperatures, and which allowed for the delimitation of the landmarks, were photographed at the same magnification. This investigation included three to five electron microscopical grids.
Effect of temperature

per given temperature. Cultures at the lowest temperatures (5 °C for *M. kalinae*, 5 and 10 °C for *S. curtispina*) contained low numbers of scales due to poor cell growth, thus they were not included in the analyses. Altogether, there were 319 *Mallomonas* dome-bearing scales and 264 *Synura* scales bearing spines suitable for the landmark-based geometric morphometrics. Further, 60 randomly selected *Mallomonas* bristles from each of the temperatures were photographed at the same magnification.

The shape dynamics of silica scales were characterized with the landmark-based geometric morphometrics method. Twenty-three landmarks for *M. kalinae* and nineteen landmarks for *S. curtispina* were digitalized on each of the scales (Figs 1, 2) using the TpsDig ver. 1.40 program (Rohlf 2004a). Sixteen (for *M. kalinae*) and thirteen (for *S. curtispina*) of the landmarks were allowed to slide along the outline (semilandmarks). The scales of both flagellates are almost bilaterally symmetrical, the left and right sides of *M. kalinae* scales can be distinguished by asymmetric dome features. This is not true for *S. curtispina* scales, so the landmarks in mirror positions were symmetrised using the method recommended by Klingenberg et al. (2002). This involves reflecting each of the scales (by multiplication of x-coordinates of all landmarks by −1), re-labelling paired landmarks, and averaging the original and mirrored configurations in a Procrustes superimposition. The averages of original and mirrored/re-labelled scales are ideal symmetric shapes in which each half, together with landmarks lying on the median axis, bears all the information on the shape of that symmetric object. Thus, further analysis of these symmetrised configurations involves only the symmetric part of the shape variation and omits the asymmetric part. In symmetrised configurations, all the shape information is included in the coordinates of one half of the paired landmarks plus the landmarks lying on the median axis (Neustupa & Němcová 2007). The halved configurations were used for the canonical variates analysis; however, the thin-plate splines of extreme positions were made using entire configurations for better graphical illustration in TpsRegr ver. 1.28. (Rohlf 2003, Zelditch et al. 2004).

The Procrustes superimpositions and relative warps analyses (shape PCA) with parameter $\alpha$ set to 0 (Rohlf 1993) were performed with TpsRelw ver. 1.39 (Rohlf 2004b). The extreme positions of the individual relative warps axes were presented as deformation grids allowing the visualisation of

Figs 1–2. Positions of landmarks (circles) and semilandmarks (squares). Fig. 1. *Mallomonas kalinae*. Fig. 2. *Synura curtispina*. Scale bars = 1 µm.
principal trends of shape variation. The canonical variates analysis programme CVAGen6 (Sheets 2002) was used to find the set of axes that allows for the greatest possible ability to discriminate among groups. The tests of significance of the canonical variate axes in CVAGen6 are all based upon the Wilk’s lambda ($\lambda$) value and on the Bartlett’s test. In Mallomonas, a distinctly delimited clump of apical scales (for details see results) was removed from the input data for the CVA, in order to find other features that discriminated among groups.

TpsSuper ver. 1.13 (Rohlf 2004c) was used to reconstruct the shape of scales at different temperatures. Only one scale was chosen for shape reconstruction, and consensus for each of the temperatures was used as a fixed reference. For the shape reconstruction of apical scales, one apical scale was chosen and consensus of all apical scales was used as a fixed reference. Size of the scales is given as centroid size, which is defined as square root of the sum of squared distances from the landmarks to their centroid. Differences among centroid sizes of scales were tested using a one-way ANOVA and Tukey’s pairwise comparisons in PAST ver. 1.40 (Hammer et al. 2001). A multivariate regression testing the influence of two independent variables (centroid size and temperature) on shape variables was computed using TpsRegr ver. 1.28. Lengths of both Mallomonas bristles and Synura spines were measured conventionally. Again, differences among the values of a length were tested using a one-way ANOVA and Tukey’s pairwise comparisons in PAST ver. 1.40 (Hammer et al. 2001).

Results

*Mallomonas kalinae*

The first three relative warps explained 71.5% of the variance in shape (RW1 36.4%, RW2 19.8%, RW3 15.3%). The position of scales in the space of the first two warps is shown in a PCA plot (Fig. 3). A distinctly delimited clump of scales, corresponding to the apical scales, is enclosed in

![Fig. 3. Scatter plot from RWA (shape PCA) of 319 Mallomonas kalinae scales originating from five different temperatures. Distinctly asymmetrical apical scales associated with RW1 are enclosed in the ellipse, and depicted by their consensus reconstruction. Symbols: + 10 °C, × 15 °C, * 20 °C, ○ 25 °C, □ 30 °C.](image-url)
the ellipse and a consensus of these scales is reconstructed. The thin-plate splines of extreme positions corresponding to the first two relative warps from each of the temperatures are depicted in Fig. 4. The extreme values of the first axis that described the shape variation of scales are related to their position on the cell. Asymmetrical scales with a dome placed more to the right, towards the center of symmetry, correspond to apical scales, whereas a dome placed more to the left, is typical for rear scales (Fig. 5). As is obvious from the thin-plate splines of extreme positions, highly asymmetrical apical *M. kalinae* scales were found only at low temperatures (10 and 15 °C).

**Fig. 4.** *Mallomonas kalinae* – thin-plate splines of extreme positions of the first two relative warps (RW) for five temperatures. Highly asymmetrical apical scales appeared only at 10 and 15 °C. Slightly asymmetrical scales were revealed at higher temperatures (25 and 30 °C) on both the first and second axis. The most homogenous appearance of scales was found at 20 °C.
Slightly asymmetrical apical scales were revealed at higher temperatures (25 and 30°C), and the most homogenous appearance of scales was observed at 20°C. In the CVA, there were four significant canonical variates ($\lambda_1 = 0.0554, p < 0.0001$; $\lambda_2 = 445.7959, p < 0.0001$; $\lambda_3 = 0.4482, p = 0.0001$; $\lambda_4 = 0.6916, p = 0.0001$), that separated scales from each temperature treatment. The main distinguishing characteristics among groups were the extent of scale elongation (rounded vs. tapered), and the extent of dome area (Fig. 6). The scales originating at 10, 25, and 30°C were more rounded than scales originating at 15 and 20°C. At 10°C, the area of the dome was

**Fig. 5.** *Mallomonas kalinae* cell at 10°C. Apical scales (white arrows) and rear scales (black arrows) corresponding to the first relative warp. Scale bar = 5 µm.
Effect of temperature

clearly smaller, although the overall scale appearance was rather robust. The shape reconstruction of scales at particular temperatures corresponding to above mentioned shape changes are depicted in Fig. 7. Distinctly larger scales were found at 10°C, as is obvious from box plots of scales centroid sizes (Fig. 8), including a test of significance (Table 1). In multivariate regression, both temperature and centroid size explained a small amount (11.03%, p < 0.00001) of shape variation, and residual variation was larger for temperature. Significantly shorter bristles were revealed at 25 and 30°C (Fig. 9, Table 2).

Fig. 6. Scatter plot from CVA of 296 *Mallomonas kalinae* scales originating from five different temperatures. Symbols: + 10°C, × 15°C, ⊙ 20°C, ○ 25°C, □ 30°C.

Fig. 7. Shape reconstruction in *Mallomonas kalinae*. The scales arising at 20°C appear the narrowest with a slightly tapering dome. Very similar scales are seen at 15°C. At 25, 30 and especially at 10°C the scales are distinctly broader and more rounded. The posterior rim seems to be rather shorter at lower temperature (10 and 15°C), indicating an inverse trend than that of *Synura curtispina* (see Fig. 12).
Table 1. Tukey’s pairwise comparison of centroid sizes for *Mallomonas kalinae* scales (n = number of scales, significant differences on a 0.1% level with three asterisks and on a 1% level with two asterisks).

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Table 2. Tukey’s pairwise comparison of lengths for *Mallomonas kalinae* bristles (n = number of bristles, significant differences on a 0.1% level with three asterisks and on a 1% level with two asterisks).

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Table 3. Tukey’s pairwise comparison of centroid sizes for *Synura curtispina* scales (n = number of scales, significant differences on a 0.1% level with three asterisks and on a 5% level with one asterisk).

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Table 4. Tukey’s pairwise comparison of lengths for *Synura curtispina* spines (n = number of spines, significant differences on a 0.1% level with three asterisks).

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**Synura curtispina**

The first three relative warps accounted 93.6% of shape variation (RW1 61.9%, RW2 21.0%, RW3 10.7%). The thin-plate splines of extreme positions from each of the temperatures corresponding to the first two relative warps are depicted in Fig. 10. The narrow scales from the caudal part of the cell were associated with the first axis (RW1) at 20, 25 and 30 °C, whereas caudal scales grown at 15 °C were related to the second axis (RW2). In the CVA, there were three distinct canonical variates ($\lambda_1 = 0.3480$, $p < 0.0001$; $\lambda_2 = 0.6184$, $p = 0.0001$; $\lambda_3 = 0.8186$, $p = 0.0001$). The distinguishing characteristics among groups were the change from wide and rounded scales to narrow oval scales, i.e. the breadth of scales, as well as the extent of a secondary layer covering the scales. It also depended on how tapered both the proximal and distal parts of the scales became (Fig. 11). The shape reconstruction of scales, showing a narrowing trend with increasing temperature, is depicted in Fig. 12. The range of centroid sizes, including a test of significance (Fig. 13, Table 3), shows that the smallest scales appeared at 25 °C. In multivariate regression, both temperature and centroid size explained 23.1% of shape variation ($p < 0.0001$), and residual variation was larger for centroid size. The pattern of spine lengths correspond to the centroid sizes of scales (Fig. 14, Table 4). The shortest spines were found at 25 °C and the longest at 15 °C.

![Box plots of centroid sizes for *Mallomonas kalinae* scales.](image)

**Fig. 8.** Box plots of centroid sizes for *Mallomonas kalinae* scales.

![Box plots of lengths for *Mallomonas kalinae* bristles.](image)

**Fig. 9.** Box plots of lengths for *Mallomonas kalinae* bristles.
Discussion

In both species studied, the substantial shape variation of scales could be explained by their position on the cell. The pattern of an inverse relationship between size of siliceous structures and temperature was highly visible in the thermal range of 10–25°C. Generally, the scales became narrower and bristles and spines were shorter with increasing temperature. Both in *M. kalinae* and *S. curtispina* a small increase in scale size was observable at 30°C. However, since we also detected several deformed scales at this highest temperature, this increase in scale size was possibly caused by thermal stress.

To date, there are only a few studies elucidating the range of morphological variability of siliceous structures in different conditions within individual species or populations of synurophtyes. Several experiments have been hitherto performed with *Mallomonas tonsurata*, *M. striata*, *Synura petersenii* and *S. echinulata* (Martin-Wagenmann & Gutowski 1995, Gutowski 1996, Hahn et al.)

**Fig. 10.** *Synura curtispina* – thin-plate splines of extreme positions of the first two relative warps (RW) for four temperatures. The shape of the scales is connected to their positions on the cell. The narrow scales are from the caudal part of the cell. This phenomenon holds for the first axis (RW1) at 20, 25 and 30°C, but only at 15°C for the second axis (RW2). The first axis (RW1) demonstrates dominant occurrence of the broadly oval scales at 15°C. In contrast, the narrowly oval scales are typical for higher temperatures.
Fig. 11. Scatter plot from CVA of 264 *Synura curtispina* scales originating from four different temperatures. Symbols: ×15 °C, ★20 °C, ○25 °C, □30 °C.

Fig. 12. Shape reconstruction in *Synura curtispina*. The scales gradually become narrower with increasing temperature. At 15 and 20 °C, the rim of the basal plate slightly exceeds half of the scale perimeter and the extent of the secondary layer is less than at 25 and 30 °C.
Gutowski (1996) cultivated a *Mallomonas tonsurata* clone at different temperatures (5, 10, 15, 20, and 25 °C) under continuous light and measured length and breadth of scales, length and breadth of domes (for dome-bearing scales), and length and breadth of anterior area (for domeless scales). A multiple range test showed that the scales were shorter at 15, 20, and 25 °C, with a tendency to have a larger dome. However, the domeless scales had the tendency to develop a smaller anterior area (Gutowski 1996). These significant changes in *M. tonsurata* correspond to the main distinguishing characteristics among groups revealed by CVA for *M. kalinae* (Fig. 6), i.e., rounded vs. tapered scales, and the extent of the dome area. *Mallomonas kalinae* scales, similar to those of *M. tonsurata*, were distinctly larger at 10 °C than at other temperatures tested. Similar trends in

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**Fig. 13.** Box plots of centroid sizes for *Synura curtispina* scales.

**Fig. 14.** Box plots of lengths for *Synura curtispina* spines.
response to temperature were found in bristles of both species. Gutowski (1996) found two types of bristles at 5 °C – shorter apical bristles and longer lateral bristles. Above 15 °C lateral bristles had a tendency to shorten and both bristle types were indistinguishable at 25 °C. This phenomenon is also in accordance with our data on *M. kalinae* where bristles were significantly shorter at 25 and 30 °C and their length was correlated with scale size.

Martin-Wagenmann & Gutowski (1995) investigated three clones of *Synura petersenii*, in relation to temperature, and reported changes in scale morphology only at 5 and 20 °C. Since, *S. petersenii* scales are considerably distinct from those of *S. curtispina*, it is only possible to compare overall shape and/or size of the scales. Interestingly, Martin-Wagenmann & Gutowski (1995) found that only the breadth (not the length) of the scales of the *S. petersenii f. petersenii* clone was significantly influenced by temperature. The *f. petersenii* clone developed narrower scales at higher temperature. The same pattern was revealed by our *S. curtispina* scales.

In summary, a tendency to reduce the size of siliceous structures with increasing temperature seems to be a general attribute of *Synura* and *Mallomonas* species. Even though we did not investigate the cell size and growth characteristics of the strains, we suppose that the reduction of scale size could be related to a higher cell growth rate. Higher temperatures result in faster cell growth, and subsequently in a smaller size of dividing cells. This phenomenon, known as a temperature-size rule, is applicable for a majority of ectotherms (Angiletta & Dunham 2003). Additional investigations focused on the relationship between cell size and temperature will be necessary to confirm the relevance of the temperature-size rule in chrysophytes. In addition, nutrient limitation can also result in larger cells, which in turn, may result in a significant effect on the development of siliceous structures. Hahn et al. (1996) reported shape change of *M. tonsurata* scales and bristles in relation to phosphorus, nitrogen and silica content.

The suggested relationships among shape, size and temperature may be useful from a paleoecological point of view. Chrysophyte microfossils have considerable potential in paleoclimatic reconstructions, especially with heightened interest in possible future climate modifications (Smol 1995). Temperature dependent morphological variability (mainly length of bristles and spines and size of scales) could potentially be used in the reconstruction of climatic histories.

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