

A geometric morphometric study of the variation in scales of *Mallomonas striata* (Synurophyceae, Heterokontophyta)

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J. NEUSTUPA AND Y. NĚMCOVÁ. 2007. A geometric morphometric study of the variation in scales of *Mallomonas striata* (Synurophyceae, Heterokontophyta). *Phycologia* 46: 123–130. DOI: 10.2216/05-61.1

Relative warps analysis was used to explore the patterns of variation in the shape of silica scales of the freshwater algal flagellate *Mallomonas striata* (Synurophyceae). Two data sets were analysed: individual worldwide reported scales and the scales of a single population from Trnová pond in the Czech Republic. The comparison of hyperplanes delimited by relative warps analysis of both data sets documented the striking similarity of the two morphospaces, indicating the importance of variation related to the position of individual scales on the cell body. However, there was a higher variability in the worldwide scales set and the analysis of group mean shapes differences revealed the variation in scales morphology in relation to infraspecific identification – *M. striata* var. *striata* or *M. striata* var. *serrata* – whose body scales have been considered identical so far.

KEY WORDS: Geometric morphometrics, *Mallomonas*, Relative warps analysis, Synurophyceae

INTRODUCTION

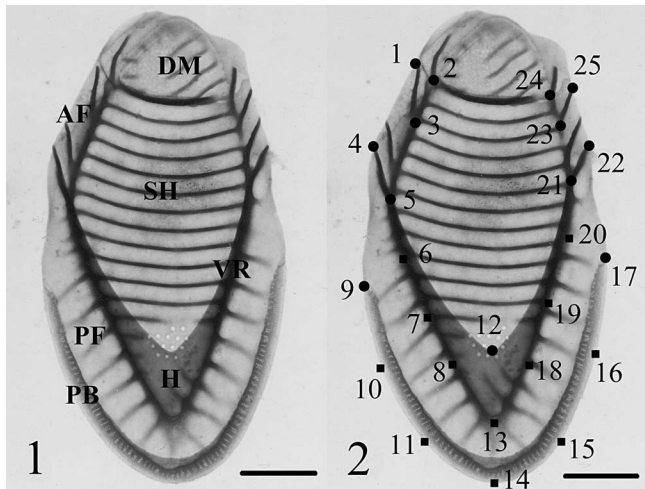
The members of the algal class Synurophyceae are characterized by the production of silica scales and bristles covering their flagellate cell body (Fig. 1). The dimensions of these scales reach about $3\text{--}6 \times 1.5\text{--}4 \mu\text{m}$ in most species. The number of scales covering a single cell ranges from 30 to 150 in different species of the genus *Mallomonas* Perty (Siver 1991). The bases of the bristles are connected to the apical part of the scales (the dome – Fig. 1), but both bristles and scales develop independently in the cells (Siver 1991; Wee 1997; Kristiansen 2005). Transmission electron microscopy (TEM) has been the essential method for the investigation of species-specific scale microstructure since the middle of the 20th century (Fott 1955; Asmund & Kristiansen 1986; Kristiansen 2002). Nowadays, the taxonomy of the group is based principally on the morphology of silica scales, which can be studied directly from the cells and also be found individually in mineralized samples of freshwater plankton or in sediments from freshwater pools and lakes and examined by EM. Currently, there are about 200 species and infraspecific taxa (about 150 of them belonging to *Mallomonas*) recognized within the class on the basis of differences in the silica scales structure (Kristiansen 2002; Siver 2003). The members of Synurophyceae inhabit freshwater environments worldwide and numerous species are known to have specific ecological requirements for water temperature, pH, trophic status and conductivity (Siver 1991, 2003). Consequently, the members of the class have become a favourite group for studies of microalgal palaeoecology (Smol 1995; Smol & Cumming 2000) and biogeography (Kristiansen 2001, 2002), as well as for biomonitoring (Roijackers & Kessels 1986; Siver 1991, 1993; Siver & Lott 2000). Smol (1995) suggested that morphological variability of silica structures within individual

species could represent an important piece of information for biomonitoring studies.

However, there have still been relatively few studies investigating the population structure of individual synurophycean species, concentrating on the variation of scales and resolving taxonomic, biogeographical or ecological problems in this way. Siver & Skogstad (1988) studied a large number of *Mallomonas crassisquama* (Asmund) Fott collections in relation to temperature, pH and other environmental factors. They found a significant relationship between temperature and the respective proportions of the two morphological types of bristles in the population. Serrated bristles prevailed in cold waters, whereas helmet bristles were found mainly in warm environments. Martin-Wagenmann & Gutowski (1995) investigated the clonal variability of scales of *Synura petersenii* Korshikov. They found significant morphological differences between individual strains in experimental conditions, allowing their identification as different *formae* of the species [*S. petersenii* f. *petersenii* and *S. petersenii* f. *glabra* (Korshikov) Siver], thus indicating the appropriateness of their delimitation. Sandgren *et al.* (1996) studied morphological variability in a strain of *S. petersenii* under silica stress and documented variation of scale shape and ornamentation in response to this factor. Gutowski (1996) found a relation between the variability in scale form and in bristle length in an experimental study of a clonal culture of *Mallomonas tonsurata* Teiling. She demonstrated that scale length of this species decreased as water temperature increased. These studies were based on so-called “traditional morphometrics” (Adams *et al.* 2004), i.e. on the analysis of arbitrary collections of size or shape variables such as distances and angles between certain points on silica scales or bristles.

The tools of geometric morphometrics are currently considered the most powerful in biological shape analysis (Bookstein 1991; Dryden & Mardia 1998; Rohlf 2000; Zelditch *et al.* 2004) and are now widely used in almost all branches of organismal biology (Adams *et al.* 2004). So far in phycology,

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Figs 1–2. The morphological description of *Mallomonas striata* scales and the position of landmarks. Bars = 1 μm .

Fig. 1. Description of scale morphology. Abbreviations: DM, dome; AF, anterior flange; SH, shield; VR, V-rib; PF, posterior flange; H, hood; PB, proximal border.

Fig. 2. Position of landmarks (circles) and semilandmarks (squares).

the landmark-based geometric morphometric methods were used in taxonomic investigations of diatoms (Beszteri *et al.* 2005), macroscopic green algae (Verbruggen *et al.* 2005a, b) and in the experimental study of phenotypic plasticity to pH relation in *Pediastrum duplex* Meyen (Neustupa & Hodač 2005). In this study, we will investigate the scales of *Mallomonas striata* Asmund by means of geometric morphometrics, concentrating primarily on two issues: (1) the analysis of overall morphological variation in the set of investigated scales; (2) the analysis of scales in two infraspecific taxa – *M. striata* var. *striata* and *M. striata* var. *serrata* Harris & Bradley.

M. striata is distributed worldwide (Kristiansen 2002) and the structure of the scales provides a satisfactory set of landmarks (Fig. 2). The species occurs in a wide range of ecological conditions; it has been recognized as a species tolerating slightly acidic to alkaline waters with growth optimum in neutral to alkaline environments (Siver 1989, 1991).

We will use two sets of scales in our analyses. The first one is a worldwide set of *M. striata* scales documented in the literature (Table 1); the second represents a single natural population of the species (from Trnová pond in Central Bohemia, Czech Republic). The first issue treated here will be the analysis of overall morphological variation in the set of investigated scales. Relative warps analysis (RWA), a modification of principal component analysis for geometric morphometric shape variables, is the appropriate technique for the determination and visualisation of the principal axes (relative warps) of shape variation (Rohlf 1993). Apart from its relation to temperature (Siver & Skogstad 1988; Martin-Wagenmann & Gutowski 1995; Gutowski 1996), nutrients (Hahn *et al.* 1996) or other external factors, the shape of synurophycean scales varies considerably according to their position on the cells (Siver 1991; Kristiansen 2002) and, supposedly, also in consequence of developmental instabilities. However, this overall variation is not random and RWA will allow us to determine the main types of shape change in natural populations, without *a priori* attributing the observed variability to any particular

factor. In addition, the extent of morphological variation will be investigated and both data sets will be compared in this respect. To compare the morphospaces occupied by both data sets (the patterns of shape change in both groups), the investigation uses the recently introduced method of evaluating the angle between subspaces (Zelditch *et al.* 2004). In our study we use the subspaces defined by parallel RWA of the Trnová population and the worldwide scales data. Evaluating the angle between morphological subspaces spanned by the first few principal component (PC) axes allows comparison of the structure of morphological variability between two parallel data sets.

The second issue concerns variation in infraspecific taxa. There are two varieties of *M. striata* – the type variety *striata*, and var. *serrata* Harris & Bradley. The *M. striata* var. *serrata* differs from the type variety by having serrated bristles. Because the morphology of bristles has been found to vary in relation to external factors in other *Mallomonas* species (Siver & Skogstad 1988; Gutowski 1996), the taxonomic delimitation of the two varieties remains problematic. Differences in scale structure between var. *striata* and var. *serrata* could support the appropriateness of their distinct taxonomic status.

Both varieties are distributed worldwide and to date no ecological differences have been ascertained between these two taxa. In our set of *M. striata* scales found in different parts of the world, there are 26 scales with var. *striata* type bristles, 25 scales with bristles of var. *serrata* and 33 scales with no bristles documented. We will investigate whether there are any differences in the morphology of scales designated as *M. striata* var. *striata* and *M. striata* var. *serrata* on the basis of their bristles. Of course, a single scale or even a few scales representing some individual population reported in the literature do not represent the overall morphological variation of scales of *M. striata* cells from the reported locality. Much of the variation will supposedly be related to their position on the cell body – and as the scales are mostly found individually in TEM, their original position on the cell cannot be discerned. Therefore, the potential morphometric signal allowing the delimitation of shapes typical for different varieties has to be discernible across the morphological variation caused by the varying position of individual scales on cells.

MATERIAL AND METHODS

In total, 84 scales of *M. striata* extracted from the literature (Table 1) were analysed. Most consisted of TEM illustrations, but several scanning EM illustrations with the scales photographed on a perpendicular plane were also included. In addition, 50 scales from a single population of *M. striata* var. *striata* from a small pond in Trnová village, Central Bohemia (collected 9 March 1997, pond area 0.08 ha, temperature 10°C, pH 6.25, conductivity 360 $\mu\text{m cm}^{-1}$) were used for comparative analysis. The flora of silica-scaled chrysophytes and synurophytes of Trnová pond was investigated by Kalina *et al.* (2000). The standard method of removing the organic matter using hydrogen peroxide and potassium dichromate was used for the preparation of the silica scales for TEM (Řezáčová *et al.* 2004). Preparation grids were shadowcast with chromium in a Polaron high-vacuum evaporator and examined with a Philips 300 TEM.

Table 1. List of the set of worldwide *Mallomonas striata* scales with their respective references.

| Country | No. | Variety | Reference |
|------------------------------|------------|----------------|--------------------------------|
| Argentina | 1, 2 | <i>striata</i> | Vigna 1988 |
| Australia, New South Wales | 3 | — | Furlotte <i>et al.</i> 2000 |
| Brazil | 4, 5 | <i>serrata</i> | Couté & Franceschini 1998 |
| Canada, Alberta | 6 | — | Kristiansen 1975 |
| Canada, Ontario | 7 | — | Nicholls 1982 |
| Canada, Ontario | 8 | <i>striata</i> | Nicholls 1982 |
| Canada, Ontario | 9 | — | Kling & Kristiansen 1983 |
| Chile | 10 | — | Dürschmidt 1980 |
| Chile | 11, 12 | — | Dürschmidt 1982 |
| China | 13, 14 | <i>striata</i> | Kristiansen 1989 |
| China | 15 | <i>striata</i> | Kristiansen & Tong 1989 |
| China | 16, 17 | — | Kristiansen 1990 |
| China | 18, 19 | — | Wei & Kristiansen 1994 |
| Colombia | 20 | — | Vigna <i>et al.</i> 2005 |
| Costa Rica | 21 | — | Wujek 1984a |
| Czech Republic | 22, 23 | <i>striata</i> | Kalina <i>et al.</i> 2000 |
| Czech Republic | 24 | — | Němcová <i>et al.</i> 2002 |
| Czech Republic | 25, 26 | — | Němcová <i>et al.</i> 2003 |
| Czech Republic | 27 | — | Nováková <i>et al.</i> 2004 |
| Denmark | 28, 29 | <i>striata</i> | Asmund 1959 |
| Denmark | 30, 31, 32 | <i>striata</i> | Asmund & Kristiansen 1986 |
| Denmark | 33 | — | Kristiansen 1988 |
| Denmark, Greenland | 34 | — | Nygaard 1978 |
| Denmark, Greenland | 35, 36 | <i>serrata</i> | Jacobsen 1985 |
| Denmark, Greenland | 37 | — | Kristiansen 1992 |
| Denmark, Greenland | 38 | <i>serrata</i> | Wilken <i>et al.</i> 1995 |
| Denmark, Greenland | 39 | — | Ikävalko <i>et al.</i> 1996 |
| Ecuador | 40 | <i>serrata</i> | Wujek & Dziedzic 2005 |
| Finland | 41 | <i>serrata</i> | Eloranta 1895 |
| Finland | 42, 43 | — | Ikävalko 1994 |
| Finland | 44 | — | Hansen & Kristiansen 1997 |
| Finland | 45 | <i>serrata</i> | Hansen & Kristiansen 1997 |
| Germany | 46 | <i>serrata</i> | Dürschmidt 1984 |
| Germany | 47 | <i>striata</i> | Gutowski 1989 |
| Germany | 48 | <i>striata</i> | Hartmann & Steinberg 1989 |
| Germany | 49 | <i>serrata</i> | Hickel & Maass 1989 |
| Germany | 50, 51 | <i>striata</i> | Gutowski 1997 |
| Germany | 52 | <i>striata</i> | Günzl 2001 |
| Hungary | 53, 54 | — | Barreto <i>et al.</i> 2000 |
| Hungary | 55 | — | Barreto 2005 |
| Iceland | 56 | — | Bradley 1964 |
| Iceland | 57, 58 | <i>striata</i> | Kristiansen 1995 |
| Jamaica | 59, 60 | <i>striata</i> | Cronberg 1989 |
| Jamaica | 61 | <i>serrata</i> | Cronberg 1989 |
| Korea | 62 | — | Kristiansen <i>et al.</i> 1990 |
| Madagascar | 63 | <i>serrata</i> | Hansen 1996 |
| Papua–New Guinea | 64 | <i>serrata</i> | Vyverman & Cronberg 1993 |
| Portugal | 65, 66 | <i>serrata</i> | Santos <i>et al.</i> 1996 |
| Romania | 67, 68 | <i>serrata</i> | Péterfi & Momeu 1976 |
| Russia, Taymyr peninsula | 69 | <i>striata</i> | Kristiansen <i>et al.</i> 1997 |
| Russia, Bolshezemels. tundra | 70 | — | Siver <i>et al.</i> 2005 |
| Sweden | 71 | — | Cronberg & Kristiansen 1980 |
| Sweden | 72 | <i>striata</i> | Asmund & Kristiansen 1986 |
| United Kingdom | 73, 74 | <i>serrata</i> | Harris & Bradley 1960 |
| United Kingdom | 75, 76 | <i>serrata</i> | Harris 1967 |
| USA, Arkansas | 77, 78 | <i>striata</i> | Andersen & Meyer 1977 |
| USA, Carolina | 79, 80 | <i>serrata</i> | Wujek 2000 |
| USA, Florida | 81 | <i>serrata</i> | Wujek 1984b |
| USA, Louisiana | 82 | — | Wee <i>et al.</i> 1993 |
| USA, Michigan | 83 | <i>serrata</i> | Wujek <i>et al.</i> 1975 |
| USA, Michigan | 84 | — | Wujek & Hamilton 1973 |

Twenty-five landmarks were digitalized on each of the investigated scales (Fig. 2) using the TpsDig ver. 1.40 program (Rohlf 2004a). The landmarks were positioned in two dimensions throughout the scale body with the exception of the dome, which is inadequately developed in numerous scales and thus does not allow sufficient landmark delimitation. The

three-dimensional information was not used in our analyses as the digitalisation in three dimensions had not been possible on TEM images showing scales as flat structures.

In total, 12 of the landmarks were allowed to slide along the outline they depicted to arrive at the optimal superimposition [so called semilandmarks according to Bookstein

(1997)]. The Procrustes superimposition and subsequent shape principal component analysis (PCA) [relative warps analysis with parameter α set to 0 (Rohlf 1993)] were carried out using TpsRelw ver. 1.39 software (Rohlf 2004b). The extreme positions of the individual relative warps axes were visualised as deformation grids allowing the visualisation of principal trends of shape variation.

The scales of *M. striata* are close to bilateral symmetry. The left and right sides of the scales can be discerned only on some scales preferably by asymmetric dome features. However, as the dome was not developed properly on all the analysed scales, we were not able to discern the respective left and right halves of the scales. Therefore, we symmetrized the landmarks in mirror positions using the method recommended by Klingenberg *et al.* (2002). This involves reflecting each of the scales (e.g. by multiplication of x -coordinates of all landmarks by -1), relabelling paired landmarks and averaging the original and mirrored configurations in the Procrustes superimposition. The averages of original and mirrored/relabelled scales are ideal symmetric shapes where 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 symmetrized configurations involves only the symmetric part of the shape variation and omits the asymmetric part. Because the size measures in various TEM illustrations may be inaccurate and, in addition, a measurement reference was not included in a number of illustrations, no analyses concerning size and shape relationships were conducted.

To analyse the differences in the extent of morphological variation between investigated sets, the Procrustes distances (D) of individual objects from the joint reference forms were computed from landmark configurations using TpsSmall 1.19. (Rohlf 1998). Procrustes distance increases with increasing shape difference between an individual scale and the consensus reference shape.

As a measure of variation we used Foote's index of morphological disparity (Foote 1993; Zelditch *et al.* 2004):

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

where D_i is Procrustes distance of an individual object to reference form and N is number of objects. The contribution of each object to the overall morphological disparity of the set can be expressed as partial morphological disparity index:

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

The sum of PM values of all objects equals the value of morphological disparity index of the whole set. The differences in morphological disparity between individual groups were evaluated by a permutation test with 10,000 permutations using partial morphological disparity PM as a computed value in R 2.1.1. routine (R Development Core Team 2005).

The pattern of the shape change in subspaces described by the PCA of individual data sets was compared using the method of evaluating the angle between hyperplanes (Zelditch *et al.* 2004). The method allows the evaluation of the significance of differences in morphological subspaces described by a set of PC axes in several groups. The significance was evaluated by comparison of the observed angle between groups

with the distribution of angles produced by a random bootstrap procedure, which consists in the random subdivision of either group into two subgroups. PCA based on the partial warp and uniform component scores is carried out independently for the two groups. The angle between the hyperplanes defined by the first x principal components is determined. Pairs of the bootstrap sets are formed by resampling the data from both groups separately. PCA is carried out and the angle between hyperplanes is determined for each pair of bootstrap sets in the same way as it was computed in the original data set. This is done many times for different bootstrap sets from both groups. Hence, we get a bootstrap distribution of random within-group angles for both groups. Then we can compare our observed angle between the original hyperplanes and the 95% range of within-group angles for both groups from a bootstrap distribution. If the observed angle between the hyperplanes exceeds the 95% bootstrap range of either group, we can assume that the observed angle has not arisen from a random subdivision of a single group into two parts. In such a case, the observed angle between hyperplanes is significant and, therefore, the investigated groups do not occupy a common morphospace (Zelditch *et al.* 2004). The IMP Space-Angle program (Sheets 2002) was used for this analysis.

Group shape differences of two different subgroups (scales of *M. striata* var. *striata* and *M. striata* var. *serrata*) were evaluated by multivariate analysis of variance and Goodall's F -test using TpsRegr ver. 1.28. (Rohlf 2003). Statistical significance was evaluated by permutation tests on Wilk's λ and F -ratio (Sheets *et al.* 2004). In symmetrized configurations, all the shape information is included in the coordinates of one-half of the paired landmarks plus the landmarks lying on median axis. Therefore, halved configurations only were used for the statistical tests (Klingenberg, personal communication). However, the reconstructions were made using entire configurations for better graphical illustration of subsequent shape changes (Zelditch *et al.* 2004). TpsSuper ver. 1.13. (Rohlf 2004c) was used for reconstruction of shapes. In addition, we did the discriminant analysis of scales of two varieties using the first 10 axes of PCA to evaluate the percentage of correct group membership classification according to the scale shape data. We used PAST ver. 1.40 (Hammer *et al.* 2001) for this analysis.

RESULTS

The results of RWA for the first three relative warps in both investigated sets are summarised in Table 2 and the shape changes associated with these axes can be seen in Fig. 3. There is a noticeable similarity in the pattern of shape dynamics revealed by RWA in both sets (because the sign orientation of axes is arbitrary, the comparison relies rather on the pattern of extreme configurations than on their position in the negative or positive rank). In the first axis we can observe the change from relatively short and wide scales with a wide anterior flange to long narrow scales with a narrow anterior flange in both Trnová and worldwide sets. In the worldwide set the additional pattern thickening of the V-rib in wider scales can be observed. The second relative warp emphasises a pronounced shortening of the anterior flange. In the worldwide set there is an additional widening of the V-rib in scales

Table 2. Singular values and percentages of explained variability for first, second and third relative warps in both data sets.

| | RW1 | | RW2 | | RW3 | |
|--------------------|----------------|-------------|----------------|-------------|----------------|-------------|
| | Singular value | % explained | Singular value | % explained | Singular value | % explained |
| Trnová pond scales | 0.37 | 47.42 | 0.24 | 19.47 | 0.19 | 12.36 |
| Worldwide scales | 0.51 | 37.25 | 0.43 | 25.86 | 0.32 | 14.98 |

with longer anterior flanges. The third relative warp emphasises shortening of the V-rib connected with widening of the posterior flange, a trend seen in both data sets. The third relative warp also illustrates a simultaneous widening of the anterior and posterior flanges in scales from the Trnová data set.

To determine how different the morphological subspaces described by the first few axes of RWA in both groups are, the angle between them was computed. This angle was compared with 95% confidence values resulting from the bootstrap procedures determining the distribution of angles obtained from random splitting of each of the groups into two subgroups and their subsequent comparison (Zelditch et al. 2004). The results are summarised in Table 3. The analysis was performed using first two, five and nine relative warps in each group. In all of these analyses the observed angle between subspaces spanned by RWA was lower than the 95% confidence value for within-group angle in both groups. This indicates that our two samples (Trnová pond scales and worldwide scales) do not occupy different morphospaces.

To evaluate the differences in the extent of morphological

variation in our sets, we used the permutation tests for the differences in partial morphological disparities in three pairs of data sets. There was statistically supported higher morphological variation in the worldwide set of all *M. striata* scales in comparison to the Trnová pond set (composed only of *M. striata* var. *striata* population) ($P = 0.0232$). In addition, there also was higher variation in *M. striata* var. *striata* worldwide set, when compared to the Trnová set ($P = 0.0239$). Finally, there was no significant difference in the extent of morphological variation between the worldwide sets of *M. striata* var. *striata* and *M. striata* var. *serrata* ($P = 0.1605$). However, testing the actual shape differences between scales belonging to the two varieties, significant differences were revealed (Wilk's $\lambda = 0.22$, P after 1000 permutations = 0.002; Goodall's F -ratio = 8.14, P after 1000 permutations = 0.001). The configurations characterising the shape differences between the varieties and the subsequent reconstruction of these shapes are shown in Fig. 4. The scales of *M. striata* var. *striata* are characterised by a narrower and longer anterior flange connected with a wider shield area and a thinner V-rib. On the other hand, *M. striata* var. *serrata* is characterised by a wide and short anterior flange, a narrower shield and a thicker V-rib, particularly in the hood area.

The discriminant analysis of the data set comprising the scales of two investigated varieties was done on the scores of the first 10 PC axes that described 98.08% of the total morphological variability. The Hotelling T^2 test for the differences between the shape characteristics of two varieties was highly significant ($P = 2.06 \cdot 10^{-5}$) and there were 92.86% of the scales correctly classified into their appropriate varieties using the discriminant function.

DISCUSSION

The relative warps analyses have demonstrated the striking similarity of patterns of morphological variation in both data sets. The first axis described the most part of the variation (Table 2) and the morphological pattern correlated with this

Table 3. Angles between subspaces defined by RWA axes of Trnová and worldwide groups. The 95% confidence values result from bootstrap procedures with 900 repetitions.

| | Observed angle | 95% confidence value for TPS ¹ | 95% confidence value for WS ¹ | Different subspaces – yes/no |
|---------------------|----------------|---|--|------------------------------|
| Two relative warps | 51.61 | 76.31 | 65.32 | no |
| Five relative warps | 61.05 | 84.29 | 88.55 | no |
| Nine relative warps | 82.40 | 95.51 | 93.20 | no |

¹ TPS = Trnová pond scales; WS = worldwide scales.

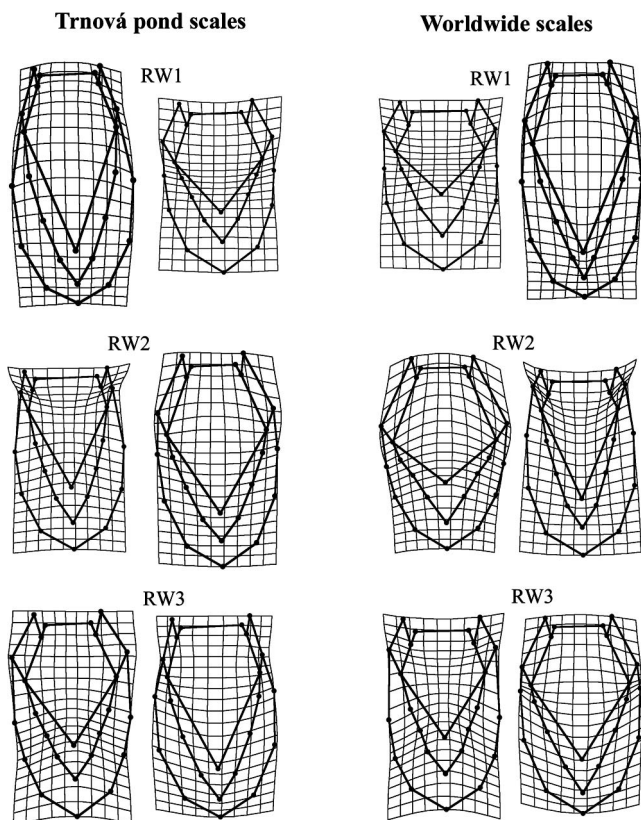


Fig. 3. The thin-plate splines of extreme positions of the first three relative warps in both data sets.

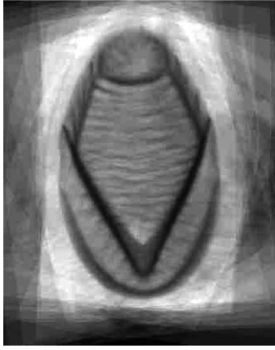
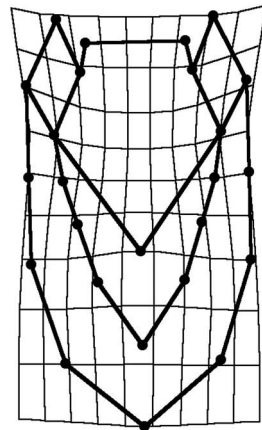
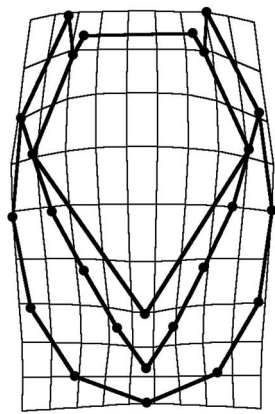
M. striata var. *striata**M. striata* var. *serrata*

Fig. 4. The thin-plate splines and corresponding shape reconstructions in *M. striata* var. *striata* and *M. striata* var. *serrata*. The shape changes were three times magnified to emphasize the observed shape patterns.

axis is in good accordance with the scales shape change connected with their position on the cells. The shorter scales with broader central area representing the extreme on the first axis are typical for apical position on cell body (Kristiansen 2002). This pattern was shared by both Trnová pond and worldwide collected scales data sets.

The comparison of the angle between subspaces formed by the RWA of two data sets of *M. striata* scales (a single sample from the Trnová pond vs worldwide collected scales) revealed that these do not belong to different morphospaces. In other words, the differences of the shape pattern revealed by the RWA were not significant. Thus, the qualitative dynamics of morphological variation in these two sets of quite different nature should be considered to be approximately the same. Probably, the position of individual scales on the cell body, influencing their actual morphology, acts as the main factor determining the principal shape trends in a data set of scales. This pattern could be shared worldwide by the populations of *M. striata*. However, the extent of morphological variation is higher in the worldwide data set than in the scales from a single pond, which indicates that there is some additional morphological variation in the worldwide scales that is related to other factors. In general, the higher morphological variability of the worldwide data could be ascribed to the presumed much higher variability of ecological factors in the worldwide localities than in the Trnová pond or to infraspecific diversity of the species on the global level. The possible infraspecific

taxonomic nonuniformity of *M. striata* has been supported by the multivariate analysis of shape differences between the worldwide scales of the two investigated varieties.

The taxonomic delimitation of the varieties of *M. striata* has been based on the differences in the structure of their bristles. However, as has been shown in other *Mallomonas* species (Siver & Skogstad 1988; Gutowski 1996), the morphology of bristles can vary considerably, depending on environmental conditions. Certainly we cannot reject the hypothesis that ecologically induced morphological variation takes place in developmentally independent structures such as bristles and scales of the species – and this could only be tested by a study investigating the clonal variability in cultures. The shape differences in scales between both groups, currently considered to be different varieties (Kristiansen 2002), can nonetheless be useful for synurophycean taxonomy. We know now that the scales of *M. striata* in natural samples cluster into two groups with characteristic shape patterns described in this study and that these groups coincide with varieties delimited on the basis of differences in bristles, which certainly supports their taxonomic status.

There is, no doubt, a wide field for geometric morphometric analyses in the investigation of both synurophycean taxonomy and ecology, especially in species with rich and complex morphology of scales bearing structures allowing the delimitation of landmarks (most *Synura* species, members of the sections *Papillosae*, *Heterospinae*, *Striatae*, *Pseudocoronatae* of the genus *Mallomonas* and others).

Particularly, the environmental causation of the infraspecific variability of *Mallomonas* scales can be investigated in detail in future geometric morphometric studies with useful conclusions for palaeoecological applications. The synurophycean scales are, in fact, far more suitable for palaeoecological reconstructions than their bristles, because, for the most part, the latter cannot be used for species determination. The usefulness of synurophycean scale variability data at the population level for inferring environmental factors was demonstrated by several authors (Siver & Skogstad 1988; Gutowski 1996; Sandgren et al. 1996). The application of geometric morphometric methods in the investigation of morphological variability in the scales of synurophycean protists may be critical for evaluating the Smoll (1995) proposal concerning wider application of their variation at the population level to palaeoecological research.

ACKNOWLEDGEMENTS

The study has been supported by the research project of the Czech Ministry of Education no. 0021620828. We would like to thank to two anonymous reviewers whose comments were very valuable to improve the manuscript quality.

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Received 9 November 2005; accepted 20 September 2006
Associate editor: Niels Daugbjerg