

Geometric morphometric analyses of silica-scale variation in four *Mallomonas* species (Synurophyceae, Stramenopiles)

Martina Pichrtová and Yvonne Němcová

M. Pichrtová (pichrtov@natur.cuni.cz) and Y. Němcová, Dept of Botany, Faculty of Science, Charles Univ., Benátská 2, Praha 2, CZ-128 01, Czech Republic.

The tools of geometric morphometrics (GM) were used to examine morphological variation of silica-scales in four *Mallomonas* species (*M. calceolus*, *M. kalinae*, *M. flora* and *M. striata*). Relative warps analysis revealed the main trends in morphological variation to be associated with the width, length and shape of the V-rib, while the proximal border region was found to be more stable. Almost all of the scales investigated were successfully differentiated and assigned to an individual species by means of multivariate statistics, the classification experiments were done in a pairwise manner. Geometric morphometric based analyses did not corroborate the classification of these species into traditionally defined sections. *M. kalinae* and *M. striata* were found to be the most similar, although they are classified into different sections of the genus, while *M. kalinae* and *M. calceolus* that are both traditionally classified as belonging to the section Papillosae, proved to be the most dissimilar species pair.

The genus *Mallomonas* (Synurophyceae, Stramenopila) comprises unicellular freshwater flagellates. One of the main distinctive features of this genus is the presence of bilaterally symmetric and heteropolar scales forming a scale case around the cell. The ultrastructure of silica scales and bristles is crucial for species determination, and the taxonomy of *Mallomonas* is based almost exclusively on it. Since the middle of the 20th century, about 150 species and infraspecific taxa have been described. Scale morphology is species-specific and a single scale will usually suffice for making a species determination. Taxa with narrow ecological optimum may be considered valuable bioindicators of both recent and historical environments (Siver 1995, Smol 1995, Siver and Marsicano 1996).

The genus *Mallomonas* is traditionally divided into sections and series (Péterfi and Momeu 1996, Kristiansen 2002) based on morphological characteristics of scales. This classification is based on cluster analyses of a high number of phenetic morphological features (Péterfi and Momeu 1996). As we do not know which scale characters are stable and represent reliable taxonomical markers, this detailed classification scheme may be highly artificial. Only a few phylogenetic studies based on molecular data have been conducted to examine the infrageneric taxonomy and phylogeny (Lavau et al. 1997, Andersen et al. 1999), therefore, the phylogenetic reliability of individual morphological characteristics remains unclear.

Geometric morphometrics (GM) has recently become a popular tool in many fields of biology including systema-

tics, ecology or anthropology, as it affords new possibilities for the interpretation of shape and shape change in organisms. Methods of GM have been employed in phycology for various taxonomic studies (Beszteri et al. 2005, Verbruggen et al. 2005a, 2005b, Neustupa and Štastný 2006, Neustupa and Škaloud 2007). The shape analyses of *Mallomonas striata* scales helped to characterize two varieties that were described on the bases of different bristle morphology (Neustupa and Němcová 2007).

In the present study, we used the landmark-based GM methods to investigate the interspecific morphological variation of *Mallomonas* scales. We chose four species belonging to two different sections. *Mallomonas calceolus* Bradley and *M. kalinae* Řezáčová are classified in the section Papillosae (Kristiansen 2002, Řezáčová 2006), while *M. flora* Harris & Bradley and *M. striata* Asmund are included in the section Striatae. Bristles distributed all over the cell and tripartite scales are common features of all the investigated species. The tripartite scale is divided by a posterior sub-marginal rib, often called a V-rib, into a central shield and marginal flange area. The V-rib is often hooded, sometimes almost reaching the reflexed margin of the proximal border. A dome is the distal concavity, in which the bristle is lodged. The above mentioned structures are depicted in Fig. 1. The main difference between these two sections is the presence of shield papillae in the section Papillosae (Kristiansen 2002).

The aim of this study was to quantitatively describe the shape difference in silica-scales among investigated species,

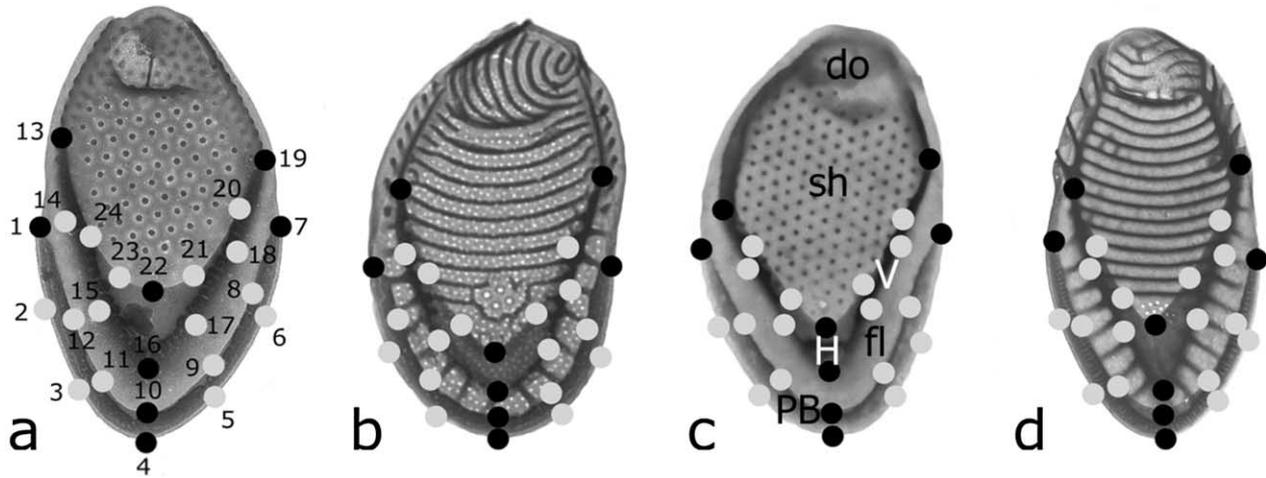


Figure 1. Positions of landmarks on the scales of all four selected species and the morphology of a tripartite *Mallomonas* scale. (a) *Mallomonas calceolus*, (b) *M. flora*, (c) *M. kalinae*, (d) *M. striata*. Fixed landmarks (numbers 1, 4, 7, 10, 13, 16, 19, 22) in black, semi-landmarks (no. 2, 3, 5, 6, 8, 9, 11, 12, 14, 15, 17, 18, 20, 21, 23, 24) in grey; do = dome, sh = shield, fl = flange, V = V-rib, H = hood, PB = proximal border.

and to compare the reliability of these data with those of traditional classification methods.

Material and methods

In total, 30 scales from each of the four investigated *Mallomonas* species (120 scales altogether) were digitalized

for GM analyses. We used the literature records, our own figures of the populations from the Czech Republic, and the scales from a cultured population of *M. kalinae* (CAUP B601) (Table 1). The *M. kalinae* strain was examined after 14 days of cultivation in DY V culture medium (<http://ccmp.bigelow.org/CI/DY-V_family.html>) at a temperature 15°C and illumination of 90 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The micrographs were taken by TEM JEOL 1011.

Table 1. The complete list of investigated scales accompanied by country of collection and references.

No. of specimen	Species	Country of origin	Source (reference)
1	<i>M. calceolus</i>	Iceland	Bradley (1964)
2	<i>M. calceolus</i>	Denmark	Kristiansen (2002)
3	<i>M. calceolus</i>	USA	Wee et al. (1993)
4	<i>M. calceolus</i>	Denmark	Kristiansen (2002)
5	<i>M. calceolus</i>	The Netherlands	Wujek and van der Veer (1976)
6	<i>M. calceolus</i>	Ireland	own sampling
7–30	<i>M. calceolus</i>	Czech Republic	own sampling
31	<i>M. flora</i>	Korea	Kristiansen et al. (1990)
32–33	<i>M. flora</i>	China	Yin-Xin and Xiu-Ping (2001)
34	<i>M. flora</i>	Korea	Kristiansen et al. (1990)
35	<i>M. flora</i>	Sweden	Kristiansen (2002)
36	<i>M. flora</i>	Denmark	Kristiansen (2002)
37	<i>M. flora</i>	Argentina	Vigna and Kristiansen (1996)
38	<i>M. flora</i>	Russia	Siver et al. (2005)
39–60	<i>M. flora</i>	Czech Republic	own sampling
61–90	<i>M. kalinae</i>	Czech Republic	culture collection CAUP
91	<i>M. striata</i>	Denmark	Kristiansen (1988)
92–95	<i>M. striata</i>	USA	Wee 1982
96	<i>M. striata</i>	UK	Harris and Bradley (1960)
97	<i>M. striata</i>	Germany	Gutowski (1997)
98	<i>M. striata</i>	Hungary	Barreto et al. (2000)
99	<i>M. striata</i>	China	Kristiansen (1990)
100	<i>M. striata</i>	Denmark	Kristiansen (2002)
101–102	<i>M. striata</i>	Iceland	Kristiansen (1995)
103	<i>M. striata</i>	Canada	Nicholls (1982)
104	<i>M. striata</i>	USA	Wee et al. (1993)
105	<i>M. striata</i>	Denmark	Asmund (1959)
106	<i>M. striata</i>	Hungary	Baretto (2005)
107	<i>M. striata</i>	Greenland	Wilken et al. (1995)
108	<i>M. striata</i>	USA	Wujek and Hamilton (1973)
109	<i>M. striata</i>	Colombia	Vigna et al. (2005)
110	<i>M. striata</i>	Denmark	Asmund (1959)
111–120	<i>M. striata</i>	Czech Republic	own sampling

Twenty four landmarks were defined on each *Mallomonas* scale and digitized using TpsDig, ver. 2.10 (Rohlf 2006). The landmarks were located on a proximal border and a V-rib (Fig. 1). These structures can be identified on the scales of all four species and they provide structurally corresponding points. Sixteen of the landmarks (called semi-landmarks) defined outlines by being allowed to slide between two other landmarks (Bookstein 1997). The area of the dome was omitted from the analysis as a hypothesis of an independent construction mechanism was addressed by Hahn et al. (1996). As the scales are symmetric objects, where left and right sides may not be discerned, we symmetrized the landmarks (Klingenberg et al. 2002, Neustupa and Němcová 2007). A Procrustes superimposition that minimizes the squared distances between corresponding landmarks was used, and the shape variation was illustrated by principal component analysis (PCA) of the shape data using TpsRelw, ver. 1.45 (Rohlf 2007). The thin-plate splines were used for graphical representation of shape variation spanned by the individual PC axes.

The scores on the first ten PC axes were used as the source data for the canonical variates analysis (CVA). This analysis depicted specimens in an ordination space defined by canonical axes leading in the direction of highest

between-groups variability. In addition, linear discrimination analysis was performed in each pair of species in PAST, ver. 1.56b (Hammer et al. 2001).

The similarity in the shape of the scales in the investigated species was evaluated in three different ways. Firstly, we counted the average coordinates of each species from the scores on the first ten RWA axes and compared the Euclidean distances between these average positions for all species pairs. Secondly, we performed the analogous procedure using the scores on the first three canonical axes. Thirdly, we used the Procrustes distances of the consensus configurations of individual species. All of these three data sets provided information in relation to distances among these species based on the shape of their scales. The shape characteristics of individual groups were illustrated as deformations of the consensus configuration.

Results

The ordination plot illustrating the distribution of all investigated scales along the first two PC axes is shown in Fig. 2. The first axis explained 36.5% of the total variation in shape. The second PC axis, describing the variation

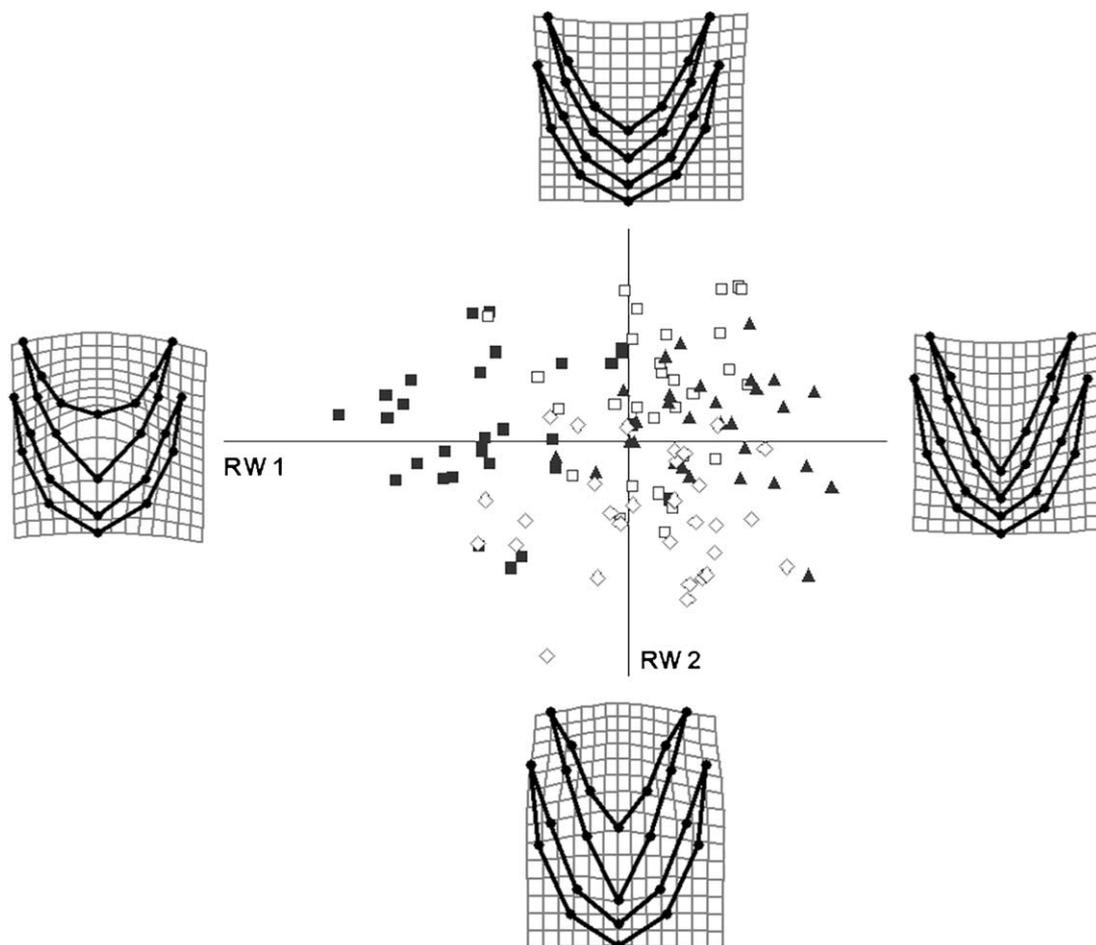


Figure 2. Ordination plot showing all scales scattered along first two relative warps (principal component axes). ■ *Mallomonas calceolus*, □ *M. flora*, ▲ *M. kalinae*, ◇ *M. striata*. The deformation grids of the extreme positions show main trends of morphological variability described by particular relative warp. Left and right deformation grids represent the extreme values of the first relative warp, top and bottom ones represent the extreme values of the second RW. The first axis explains 36.5% of the total variance, the second one 27.8%.

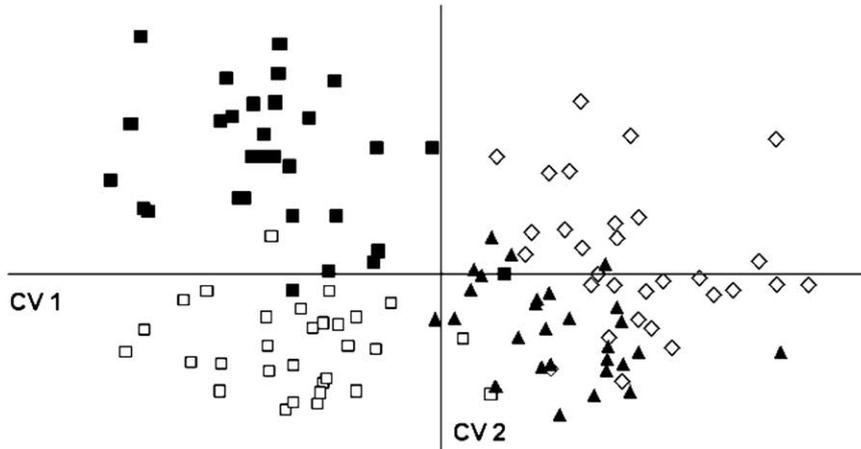


Figure 3. CVA ordination plot showing positions of scales along CV axes. ■ *Mallomonas calceolus*, □ *M. flora*, ▲ *M. kalinae*, ◇ *M. striata*.

which is not represented by the first PC axis, accounted for 27.8% of the total. Thus, greater than 99% of total variance was accounted for by the first ten axes. The greatest variation among specimens, described by the first PC axis, is associated with the middle region of the V-rib varying from the widely opened U-shaped V-rib to the acutely angled thin V-rib. The second PC axis described variations in length and width of the investigated scales.

The canonical variates analysis illustrated significant differentiation between groups of scales belonging to different species (Wilk's lambda = 0.02081, p-value = 1.146×10^{-72}). The positions of all the scales in the canonical space are illustrated in Fig. 3. The first CV axis (61.5% of the total variation), differentiated pairs of species *Mallomonas kalinae* and *M. striata* from the *M. flora* and *M. calceolus* species pair, although a slight degree of overlap between scales of *M. striata* and *M. calceolus* was observed. The second CVA axis (23.2% of the total variation) separated the *M. calceolus* from *M. kalinae* and *M. flora*. All the species pairs were significantly differentiated by the linear discrimination analysis of the shape data (Table 2). In three pair-analyses, all the scales were correctly classified; in the remaining three, a single scale was misidentified. The scale shapes of *M. flora* and *M. kalinae* were found the most similar by all the distance measures, and, conversely,

M. calceolus and *M. kalinae* were consistently found to be the most remote species (Table 2).

The average shape of *Mallomonas calceolus* scales possessed, in comparison to the overall consensus, a wide, rather U-shaped V-rib with a distinct hood (Fig. 4). The V-rib of *M. flora* was also U-shaped, but it was placed closer to the proximal border. The outline of the *M. kalinae* scales was rather acute. The V-rib of this species was wide open and formed an acute angle. The *M. striata* scales were characterized primarily by a V-shaped V-rib. In contrast to that of *M. kalinae*, it was more distant from the proximal border and its central area was thickened.

Discussion

The landmark-based GM analysis of interspecific shape variation proved itself to be a valuable tool in scale shape evaluation. Almost all of the scales examined were correctly distinguished and assigned to an individual species, even though landmarks were placed on the proximal portion of the scales only (excluding the dome). Similarly to that achieved with some desmids (Neustupa and Štátný 2006), geometric morphometric analysis provided reliable discrimination between species originally determined by

Table 2. Results of the discriminant analysis and the shape similarity measures for each species pair. c = *Mallomonas calceolus*, f = *M. flora*, k = *M. kalinae*, s = *M. striata*. The discriminant analysis shows, how successfully the scales were matched with particular species. Group distances: Euclidean distances among average species scales counted as average positions of specimens in an ordination system defined by first 3 CVA (10 PCA axes respectively). The last column shows Procrustes distances among particular consensus configurations. Bold values highlight the highest and lowest numbers, i.e. species pairs with shortest and longest distances.

Species pair	Discriminant analysis		Group distances		
	p-value	% of correctly classified scales	Euclidean distance (CVA)	Euclidean distance (PCA)	Procrustes distance
c-f	2.20E-17	98.33	0.04826	0.02972	0.09370
c-k	1.64E-15	98.33	0.05680	0.03500	0.11007
c-s	7.45E-18	100	0.04625	0.03043	0.09665
f-k	3.69E-20	100	0.03423	0.02016	0.06431
f-s	7.77E-14	98.33	0.04369	0.02797	0.08931
k-s	1.10E-19	100	0.03459	0.02199	0.06938

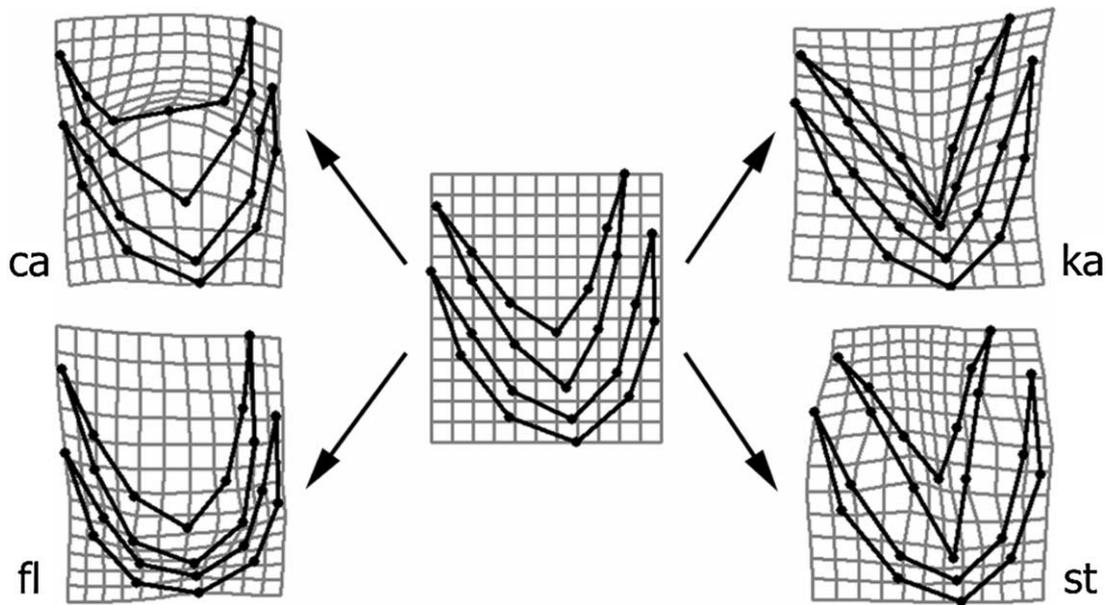


Figure 4. Thin-plate splines showing morphological trends connected with the deformation of the consensus configuration to particular species consensus configurations. ca = *Mallomonas calceolus*, fl = *M. flora*, ka = *M. kalinae*, st = *M. striata*. Scale factor: three times exaggerated to emphasize differences.

qualitative morphological assessments. The principal component analysis revealed that the main trends in morphological variation were associated with the width, length and shape of the V-rib, while the proximal border region was more stable. These results are in congruence with those of Neustupa and Němcová (2007) studying *M. striata* scales, where the first PC axis also illustrated a thickening of the V-rib. Therefore, it appears this could indicate a more general trend in morphological variation among scales of the genus *Mallomonas*.

Our GM based analysis did not support the classification of the four species investigated into sections as defined by Péterfi and Momeu (1996). *Mallomonas kalinae* and *M. striata* were the most similar, although they are classified into different sections of the genus. In addition, *M. kalinae* and *M. calceolus* that are both traditionally classified into the section Papillosae were shown to be the most remote species in our pair comparisons. This incongruence between the traditional experience-based infrageneric taxonomy and recent geometric morphometric results provides intriguing possibilities for future studies of this genus. While the traditional taxonomy is based on the presence and absence of subjectively chosen morphological features, the geometric morphometrics provides us the overall shape information including angles or relative distances. However, it may be difficult to find a sufficient number of homologous points (landmarks) on all of the investigated scales.

To evaluate which of the discussed classifications reflects the natural history, methods of molecular phylogeny have to be employed.

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