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Variability in scale shape between ancient and modern specimens of *Mallomonas insignis*

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With 5 figures and 3 tables

Abstract: *Mallomonas insignis*, a cosmopolitan freshwater alga belonging to the class Synurophyceae, has a cell covering consisting of three types of siliceous scales, apical spined scales, body scales, and caudal spined scales. Body scales comprise the majority of scales on an individual cell and form the basis of this study. Recently, fossil scales were uncovered from an Arctic Eocene locality known as Giraffe Pipe that appears identical to modern specimens of *M. insignis*. The objectives of this study were to use geometric morphometrics to compare body scale shape between modern populations of *M. insignis* from three continents and the fossil specimens from the Giraffe locality, and to assess variability in scale shape between all specimens. The primary differences in scale shape identified in the analysis included the outline of the scale, position of the V-rib, shape of the shield and window, and extent of the secondary reticulation on the distal end of the scale. Overall, there were no differences in scale shape when specimens were pooled by continent, and there was a large degree of overlap in the shape morphospaces between modern scales and the fossil specimens. This implies a high degree of evolutionary stasis with regards to scale shape and suggests that the morphospecies, *M. insignis*, has inhabited freshwaters since at least the Eocene. There were, however, some slight differences between a few of the modern sites, most notably ones in Germany and North America. The degree of variability in scale shape, estimated using Procrustes distances was found to be virtually identical for clonal cultures as it was for any modern or fossil sample, any grouping by continent, and even when all specimens were pooled. These findings support the hypothesis that the position of a scale on the cell covering yields the primary source of variation in scale shape found within the synurophyte algae.

Key words: *Mallomonas insignis*, geometric morphometrics, scale shape, Eocene, synurophytes

Introduction

The genus *Mallomonas* Perty 1852 includes unicellular organisms within the class Synurophyceae (Andersen 1987) that have one or two emergent flagella, a large bilobed chloroplast, and a highly organized cell covering consisting of overlapping siliceous scales (Siver 1991, Kristiansen 2005). In addition to scales, the vast majority of *Mallomonas* species have a second type of siliceous structure, the bristle. Bristles are long slender structures that consist of a main shaft and a flattened and slightly bent proximal end known as the foot (Siver 1991). The foot is

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tucked under the distal end of the scale such that the shaft is free to radiate out from the cell. The morphology of the scales and bristles are taxon-specific and form the basis by which species are described and delineated (Siver 1991, Kristiansen & Preisig 2007). Over 177 taxa have been described based on electron microscopy of the siliceous components (Kristiansen & Preisig 2007).

Scales are flat, more or less oval structures that are overlapped in a very precise and highly ordered manner over the cell (Siver & Glew 1990). Scales are arranged in spiral rows, with each row originating at the anterior end and terminating at the posterior end. An individual scale is overlapped by the scale positioned behind it in the same row, and by one or two scales in the spiral row above it (Siver 1991). The basic design of *Mallomonas* scales includes a base plate and a posterior upturned rim. The base plate is often perforated with pores. The location, spacing and density of the pores are constant for any taxon, but varies widely between species. Depending on the taxon, additional features, such as a dome, V-rib, wings, and secondary features are added to form a unique species-specific design. The dome is a raised portion of the distal end of the scale under which the bristle foot attaches. The V-rib is a prominent V-shaped ridge of silica positioned on the surface of the base plate and likely involved in spacing the scales on the cell covering (Siver & Glew 1990).

All scales on a cell have the same basic design typical of that species. However, the shapes of scales can vary depending on their position on the cell covering. The majority of scales are similar in shape and referred to as body scales. Scales that surround the emergent flagellum and those that cover the posterior end of the cell, i.e. those at the ends of the spiral rows, can have a much different shape. In addition, the distal end of the posterior scales is often drawn out to form a spine. In 2005, Siver and Wolfe uncovered numerous *Mallomonas* scales from a Middle Eocene Arctic locality known as Giraffe Pipe. Many of the scales appeared to be virtually identical to modern scales of *Mallomonas insignis* Penard, and the authors concluded that they could not visually distinguish the fossil scales from the modern specimens, implying long-term evolutionary stasis in regards to scale morphology and suggesting that this organism was an ancient species. In a subsequent study, Siver et al. (2013b) used geometric morphometrics to further illustrate the similarity in scale shape between fossil and modern specimens. The objectives of the current study are to further elaborate on the shape and design of *M. insignis* body scales among modern and fossil populations, and to examine variability in scale shape between modern populations from three continents, three clones and fossil specimens.

Materials and methods

Scales of *Mallomonas insignis* used in this study originate from eight modern localities, including three from North America, two from Asia, and three from Europe, and eight fossil assemblages (Table 1). Of these, the two Asian populations and one from North America, referred to as the Maine culture, are organisms isolated from lakes and grown in culture. Seven of the collections represent specimens from individual lakes. One collection, labeled “Various lakes”, represents isolated body scales from six ponds along the east coast of North America. Fossil specimens originate from the post-eruptive sedimentary sequence in the Giraffe kimberlite maar in Northern Canada (Siver & Wolfe 2009). These samples comprise middle Eocene (40–48 Ma) organic shales from depths between 97.1 m to 112.1 m in the core.

Modern sediment and Eocene mudstone samples were oxidized with a sulfuric acid-potassium dichromate solution according to Marsicano & Siver (1993), followed by a minimum of six washes with distilled water. Aliquots of each slurry were dried onto aluminum foil, trimmed, mounted on aluminum SEM stubs using Apiezon wax, coated with a mixture of gold and palladium with a Polaron model E5 100 sputter coater, and imaged with a Leo (Zeiss) 982 field-emission scanning electron microscope (FESEM). A total of 297 body scales was used for geometric morphometric analyses (Table 1). Each specimen imaged and used in the study was lying flat on the stub where all landmarks were easily identified.

Table 1. Origins of the *Mallomonas insignis* body scales used in the study.

Name	Sample description	Location	Number of scales	Source
Big Pond	Modern, surface sediment	North America, USA, Connecticut	10	P.A. Siver collection[1]
Andersen Culture	Modern, culture	North America, USA, Maine	18	R. Andersen[2]
Rökepipans Damm	Modern, plankton	Europe, Sweden	33	G. Cronberg[3]
Etang Cazaux	Modern, plankton & surface sediment	Europe, France	38	Y. Němcová[4]
Zeller Loch	Modern, plankton	Europe, Germany	17	Y. Němcová[4]
Beopsu Lake	Modern, culture	Asia, Korea	19	W. Shin[5]
Jeangsan Lake	Modern, culture	Asia, Korea	21	W. Shin[5]
Various Lakes	Modern, surface sediment	North America, east coast sites	11	P.A. Siver collection[1]
GP 19-2-10	Middle Eocene, 97.1 m in core	Giraffe fossil locality	12	P.A. Siver collection[1]
GP 20-3-105	Middle Eocene, 100.0 m in core	Giraffe fossil locality	21	P.A. Siver collection[1]
GP 20-1-40	Middle Eocene, 101.7 m in core	Giraffe fossil locality	20	P.A. Siver collection[1]
GP 20-1-60	Middle Eocene, 101.8 m in core	Giraffe fossil locality	22	P.A. Siver collection[1]
GP 20-1-70	Middle Eocene, 101.9 m in core	Giraffe fossil locality	20	P.A. Siver collection[1]
GP 23-1-15	Middle Eocene, 109.2 m in core	Giraffe fossil locality	5	P.A. Siver collection[1]
GP 23-2-90	Middle Eocene, 110.8 m in core	Giraffe fossil locality	10	P.A. Siver collection[1]
GP 23-2-116	Middle Eocene, 112.1 m in core	Giraffe fossil locality	20	P.A. Siver collection[1]

[1] Department of Botany, Connecticut College; [2] Strain CCMP2549 isolated Dec. 1, 2003 by R. Andersen from West Harbor Pond, Maine, USA; [3] Lund University, Lund, Sweden. Plankton samples were collected October 27, 2009 and January 5, 2010.; [4] Y. Němcová, Department of Botany, Charles University, Prague, Czech Republic. The plankton sample from Etang Cazaux was collected Feb. 2, 2010; [5] Department of Biology, Chungnam National University, Daejeon, Korea.

Geometric morphometric analyses were done using a suite of software including TpsUtility v.1.4, TpsDig v.2.05, TpsRelW v.1.42, TpsSmall v.1.2, and TpsSuper v.1.13 (Rohlf 2007), all available from http://life.bio.sunysb.edu/morph. Forty-four landmarks were digitized from each specimen, outlining the scale perimeter, V-rib, posterior rim, extent of the secondary reticulation on the scale anterior, and the "window" on the posterior flange (Fig. 1). Fourteen of the landmarks are fixed and the remaining 30 represent semilandmarks (Bookstein 1997). Semilandmarks are slid along the outline such as to minimize Procrustes distances. The alignment of landmarks (Procrustes superimposition) and subsequent relative warp analysis (a principal components analysis in linear tangent space) were performed using TpsRelW. Scale shapes plotted along the primary relative warp axes, including the consensus configuration, were visualized using thin-plate spline deformation grids. The final aligned matrix of coordinates for all landmarks and semilandmarks from each specimen was used to compute Procrustes distances (ρ) and distances in the tangent space (d). These distances were highly correlated ($r = 0.99$) justifying the use of the linear tangent space approximation for subsequent statistical analyses (Zelditch et al. 2004). Procrustes distances were used to investigate the degree of variation in scale shape for each of the 16 collections of specimens, as well as for pooled sets including by continent, only modern specimens, the three clones, and only fossil specimens. Procrustes distances are measured using the distances from each specimen to a reference or consensus shape. The larger the difference in shape of an individual specimen from the consensus shape, the larger the resulting Procrustes distance. The mean, standard deviation, minimum and maximum values of Procrustes distances obtained for each set of specimens are reported.

Analysis of variance (ANOVA) was used to test for differences in Procrustes distances between a) scales from modern lakes, clones and fossil samples; b) scales grouped by continent; c) all modern lakes and; d) all fossil samples using SPSS v.9.0.1. SPSS was also used to run a general linear model (GLM) multivariate analysis of variance to test for differences in scale shapes between fossil and modern specimens, and between all modern samples, using scores from principal components analysis (PCA) along the primary axes as dependent variables. Scheffé and Bonferroni *post hoc* tests were used to identify differences using a significance level of 0.05. Similar results were produced by both tests of significance. Only symmetrical body scales lying flat on the SEM stub and unobstructed from debris were used in this study. The vast majority of specimens from all samples observed with SEM were symmetrical in outline. The few asymmetric scales encountered are believed to represent transitional forms between body scales and either apical or caudal spined scales. Very small circular scales (presumed to be caudal scales) and scales with distal projections (caudal and apical scales) were also excluded.

Results

Description of *Mallomonas insignis*: Cells of *M. insignis* have three types of scales, apical, body and caudal scales that overlap to form a highly imbricated cell covering. The majority of scales comprising the cell covering are symmetrical body scales, each with a broad posterior

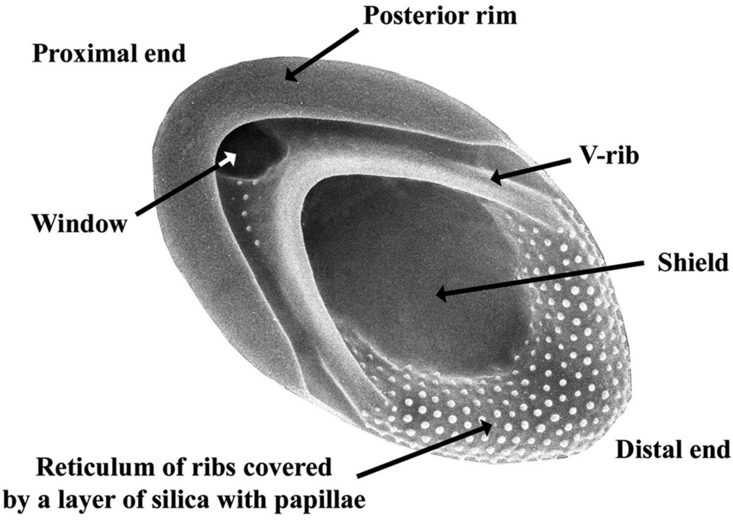

Fig. 1. Body scale of *Mallomonas insignis* depicting the posterior rim, V-rib, shield, window, and the anterior reticulum of ribs covered by a thin silica layer with small papillae.

rim, V-rib, and a well developed reticulum of ribs lining the distal end and inner portion of the V-rib (Fig. 1). A series of parallel ribs radiates from the V-rib onto the posterior flange. Both the reticulum of ribs on the distal end and the parallel ribs on the posterior flange are often covered by a thin layer of silica. Secondary structures, including ribs, are lacking on the posterior flange immediately behind the base of the V-rib forming an opening referred to as the window (Fig. 1). Small siliceous papillae are often found on the surface of the scale, especially atop the secondary reticulum. Apical scales surround the flagellar pore and each possesses a stout forward projecting and hollow spine. The caudal scales are smaller, more rounded and have a long slender spine protruding from the distal end. Unlike most species in the genus, *M. insignis* cells lack bristles.

Comparison of scale shapes between modern sites: The first two axes of a relative warp analysis based on all modern specimens included in this study accounted for 85 % of the shape variation (Fig. 2). Along axis 1, the V-rib moves closer towards the center of the scale, the shield becomes more circular, and the secondary reticulum shifts from intersecting with the V-rib near

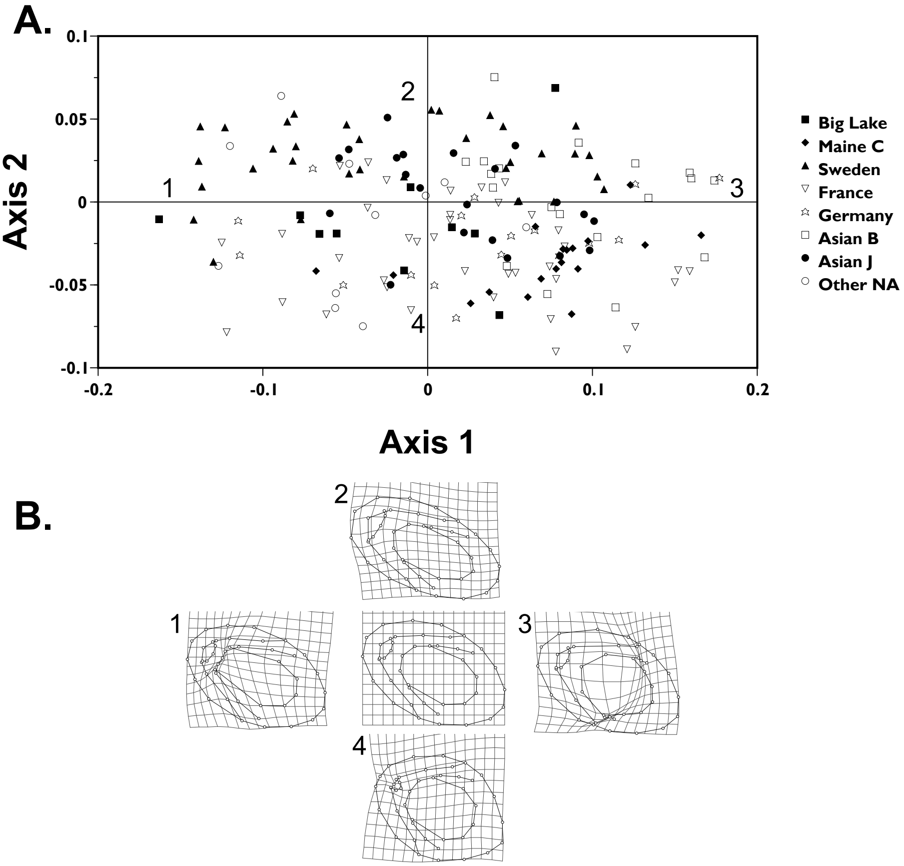

Fig. 2. Shape analysis of *Mallomonas insignis* scales. Relative warp analysis (A) and grid line deformation plots (B) for modern body scales of *Mallomonas insignis* from eight different localities. The first two axes accounted for 85 % of the total shape variation. The five deformation plots correspond to the positions marked with the numbers 1, 2, 3 and 4, and the center of the plot.

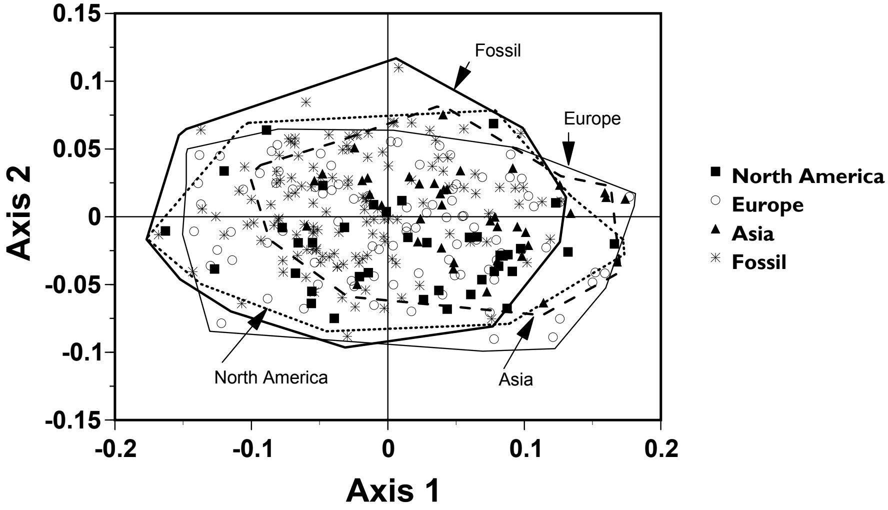

Fig. 3. Shape analysis of *Mallomonas insignis* scales. Relative warp analysis for modern and fossil body scales of *Mallomonas insignis*. The first two axes accounted for 80.4 % of the total shape variation. Overlying plots depict the positions of specimens from North America, Europe, Asia, and the Giraffe Pipe fossil locality.

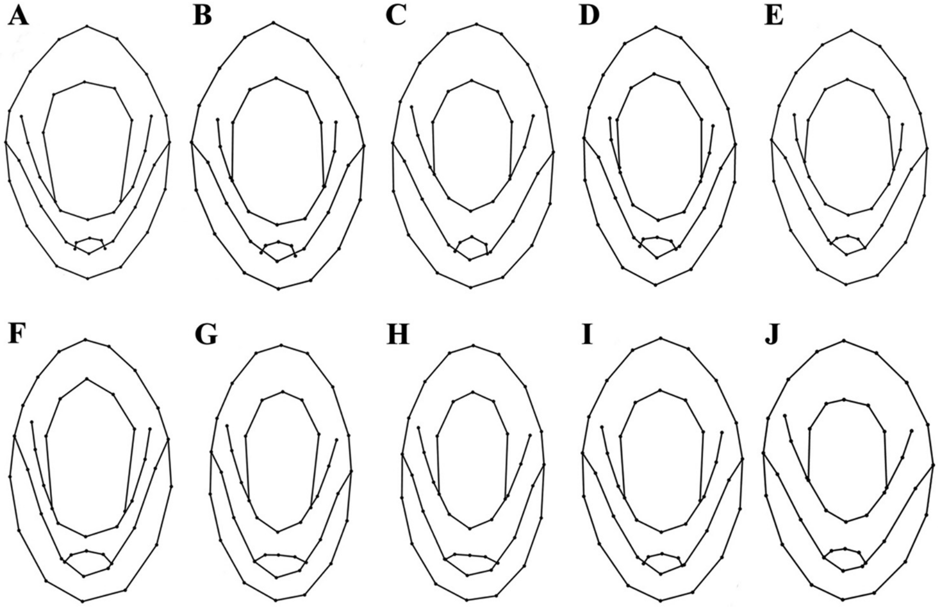

Fig. 4. Consensus or mean shapes of *Mallomonas insignis* body scales from each of eight modern collections, for all modern specimens pooled, and all fossil specimens imaged from the Giraffe Pipe locality. A) Germany. B) France. C) Sweden. D) Big Lake, CT, North America. E) Other North American waterbodies. F) Culture from Maine, North America. G) Culture from Beopsu Lake, Korea. H) Culture from Jeangsan Lake, Korea. I) The collection of all modern scales used in the study. J) The collection of all fossil specimens used in the study.

Table 2. Comparisons of body scale shapes between eight populations of *Mallomonas insignis*. Significant (+ or ++) and non-significant (–) differences are given based on a p = 0.01 (++) or p = 0.05 (+). The locations of each population are given in Table 1 and in the text.

	Maine C	Asian B	Asian J	Big Lake	France	Other NA	Sweden	Germany
Maine C	_							
Asian B	_	_						
Asian J	_	_	_					
Big Lake	_	_	_	_				
France	_	_	_	_	_			
Other NA	++	+	+	_	+	_		
Sweden	+	_	_	_	+	+	_	
Germany	_	_	+	+	_	–	++	_

the base to near the ends of the V-rib arms (Fig. 2, compare thin splines 1, center and 3). Along axis 2, both the scale and shield become more circular, the posterior window reduces in size, and the secondary layer becomes broader. However, the point of intersection between the secondary layer and V-rib, and the position of the V-rib, remains relatively constant along axis 2.

When specimens were pooled by continent, there were no shape differences between those from North America, Europe and Asia (Fig. 3). There were also no differences among specimens from different continents along axis 3. However, there were some slight differences in scale shape between a few of the modern sites (Table 2; Fig. 4). The most notable differences were associated with the Zeller Loch site in Germany and the “other” sites in North America (Table 2). Specimens from Germany showed significant differences with those from North America and Sweden, while those from the other North American sites differed from all three clones, France and Sweden (Table 2). The shapes of scales from the Swedish lake were slightly different from the waterbody in France. Despite these few differences, there was overlap among populations (Fig. 2) and the consensus shapes were similar (Fig. 4) between all modern sites.

Comparison of fossil and modern specimens: The distributions of fossil scale shapes for *Mallomonas insignis* specimens collected from the eight levels of the Giraffe Pipe core and as depicted in a relative warp analysis, overlapped considerably (Fig. 5). The first two axes of the relative warp analysis accounted for 81 % of the total shape variation. In general, along axis 1 scales become more elongate (larger length to width ratio), have a broader secondary layer that extends farther down the V-rib, a smaller and more rectangular shield, and a smaller window (Fig. 5B). Along axis 2, scales become more circular with a wider and slightly more extensive shield, and with a larger window. The position and shape of the V-rib, and the intersection of the secondary reticulation with the V-rib, remained relatively constant along axis 2.

Differences between fossil and modern specimens are discussed and detailed in Siver et al. (2013b) and are only briefly summarized here. The shapes of the fossil scales were largely contained within the shape space represented by the modern specimens (Fig. 3). However, the collection of modern scales comprised a slightly larger shape space relative to the fossil specimens. Some modern specimens were more elongate with a broader V-rib base and a more extensive secondary layer as compared with the fossil scales. These features resulted in a slight, but significant, difference in the mean shapes of fossil and modern scales. Despite these overall differences, the shapes of fossil scales were not significantly different from modern specimens from waterbodies in France, Sweden, Big Pond in Connecticut, and the collection of lakes representing ponds along eastern North America.

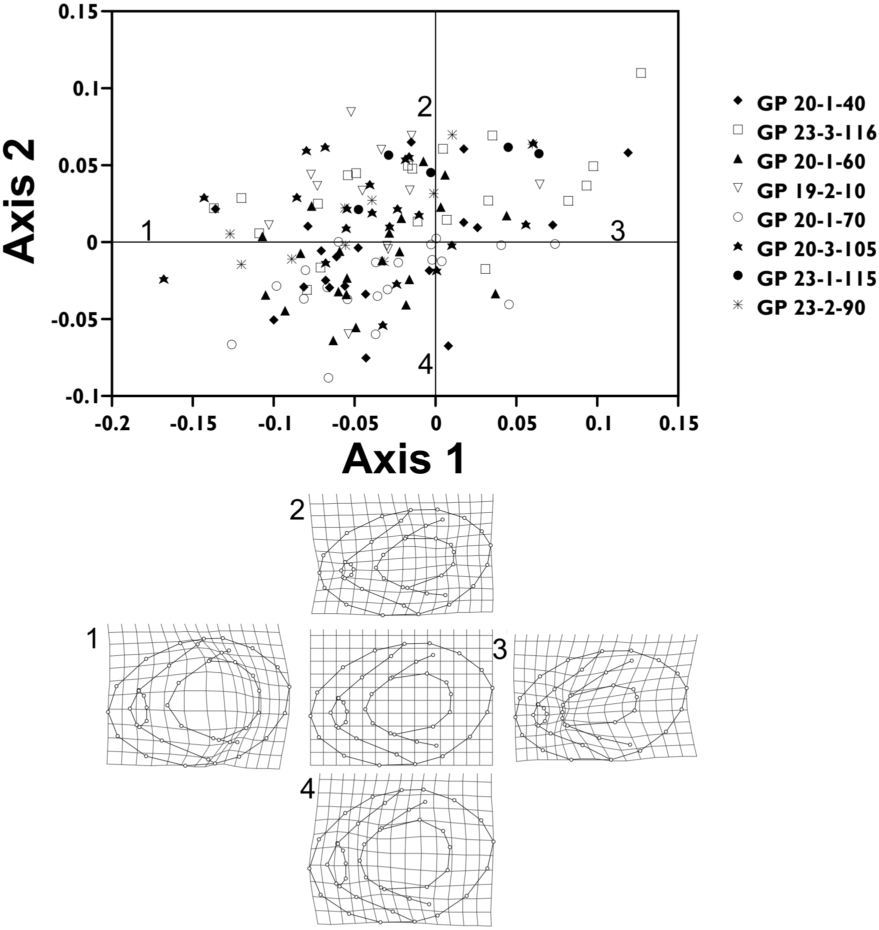

Fig. 5. Shape analysis of *Mallomonas insignis* scales. Relative warp analysis (A) and grid line deformation plots (B) for fossil body scales of *Mallomonas insignis* from eight levels of the Giraffe Pipe core. The first two axes accounted for 81 % of the total shape variation. The five deformation plots correspond to the positions marked with the numbers 1, 2, 3 and 4, and the center of the plot.

Variability in scale shape: Procrustes distance provides a measure of how far the suite of landmarks for a given specimen is from the centroid. The greater or smaller the distance, the greater or smaller is the variability in shape for that specimen. Procrustes distances were estimated for a) the three major groupings consisting of all modern scales, scales from clones, and fossil specimens; b) modern specimens grouped by continent (North America, Europe and Asia) and; c) for each modern site and stratum from the Giraffe Pipe locality (Table 3). In general, the Procrustes distances were very similar for all groups of scales tested, with means ranging only between 0.094 and 0.137. The mean values for all modern specimens, all specimens from clones and all fossil specimens were 0.116, 0.121 and 0.106, respectively, yielding an overall mean of 0.111 (Table 3). The variation in scale shape for specimens from culture isolates was virtually identical to that estimated from any individual modern or fossil sample, any grouping by continent, and even when all modern specimens were pooled together. As a result, there were no significant dif-

Table 3. Mean, standard deviation, minimum and maximum Generalized Procrustes Distance measurements for all scales, modern scales, scales only from clones and fossil scales.

	Generalized Procrustes Distance			
	Mean	**Minimum**	**Maximum**	**SD**
Major Groups				
All specimens	0.11	0.05	0.21	0.03
All modern	0.12	0.05	0.2	0.03
Modern, clones	0.12	0.07	0.18	0.02
Fossils	0.11	0.06	0.21	0.03
Continent				
North America	0.127	0.063	0.189	0.029
Europe	0.111	0.052	0.197	0.033
Asia	0.115	0.071	0.170	0.024
Lake or Culture				
Culture, Maine	0.135	0.105	0.178	0.019
Culture, Asia B	0.114	0.071	0.162	0.024
Culture, Asia J	0.114	0.084	0.170	0.024
Big Pond	0.114	0.063	0.188	0.036
Sweden	0.101	0.061	0.164	0.031
France	0.110	0.057	0.171	0.029
Germany	0.137	0.074	0.197	0.039
Various Lakes	0.126	0.087	0.189	0.035
Fossil Samples				
GP 19-2-10	0.106	0.068	0.134	0.020
GP 20-3-105	0.102	0.062	0.184	0.032
GP 20-1-40	0.110	0.083	0.153	0.021
GP 20-1-60	0.108	0.070	0.134	0.019
GP 20-1-70	0.093	0.056	0.155	0.025
GP 23-1-115	0.094	0.072	0.108	0.017
GP 23-2-90	0.105	0.073	0.146	0.026
GP 23-3-116	0.110	0.064	0.180	0.031

ferences in the mean Procrustes distances between the three major groupings – all modern scales, all scales from clones and all fossil specimens, scales grouped by continent, and any of the fossil samples. Procrustes distances were slightly higher for the Maine culture and the Zeller Lock site in Germany, but these were not significant.

Discussion

The largest amount of shape variability in *Mallomonas insignis* scales was associated with the outline of the scale, the extent of the secondary layer and where it intersects with the V-rib, and in the position of the V-rib on the scale. In addition, as the secondary layer increases or decreases in size, the relative size of the shield decreases or increases, respectively. Other characters, including the shapes of the posterior rim and V-rib, and the position of the posterior window, were more stable and showed less difference between all groups of scales examined.

Despite the range in scale shape observed on *Mallomonas insignis* scales, much of the variability can be found on specimens collected from the same site, and even from cells harvested from clonal cultures. Thus, the overall differences in the shapes of scales collected from sites representing North America, Europe and Asia, as well as from the fossil record, were remarkably small, and it was not possible to distinguish the origin of individual scales. This implies both a high degree of evolutionary stasis in regards to the overall ultrastructure and shape of scales, and that this morphospecies has probably inhabited freshwater lakes for much of the Cenozoic, if not longer. The similarity in scale structure between fossil remains from the Giraffe Pipe locality and modern specimens was first recognized by Siver and Wolfe (2005), and the antiquity of this species initially estimated by Siver et al. (2013b). The current study further documents that the shapes of fossil scales from the Giraffe locality are similar to modern specimens, especially ones from European and North American sites. Shapes of *M. insignis* body scales were not significantly different between the most of modern sites compared. The group of scales representing multiple lakes in North America had the greatest difference, being significantly different from the Maine and Asian clones, as well as those from the localities in France and Germany. This difference is likely related, in part, to the fact that the scales are from six waterbodies and probably represent different genetic strains. Scales from the German locality were also different from those in North America, but interestingly they were most different from specimens collected in other European localities, most notably Sweden. Scales from the Swedish site were very similar to specimens from the North American localities. In summary, we could not detect consistent differences in scale shape relative to the geographic locations of the sites.

The current study addressed only shape differences in body scales and not for apical or caudal scale types. Even though body scales cover the majority of the cell in all *Mallomonas* species, including *M. insignis*, the largest changes in scale shape occur between body scales and scale types that cover the ends of the cell (Siver & Glew 1990, Siver 1991). Although scale shape was not quantified for apical or caudal scales, the overall morphology of these scale types appeared very similar between modern and fossil populations. Except for the fact that some fossil caudal scales had splayed tips, Siver et al. (2013b) observed no differences between fossil and modern specimens.

Evolutionary stasis in regards to scale shape is not restricted to *Mallomonas insignis*. Striking similarities between other *Mallomonas* and *Synura* taxa uncovered from Paleogene cores has also been demonstrated (e.g. Siver & Wolfe 2005, 2009, Siver et al. 2009, Siver et al. 2013a), and some of the modern morphospecies have been estimated to be tens of millions of years old (Siver et al. 2013b, Jo et al. 2013). For example, scales virtually identical to modern specimens of *M. bangladeshica* (Siver & Wolfe 2009), *Synura nygaardii* and *S. macracantha* (Siver et al. 2013a) have also been uncovered from the Giraffe Pipe core, and it is believed that evolutionary stasis of scale structure is a common phenomenon for other synurophycean algae. Despite evolutionary stasis in scale structure for some species, it should be kept in mind that the Paleogene cores also contain synurophycean taxa that are clearly extinct. Thus, not all of the lineages represented in the early Cenozoic survive today.

Several details of synurophyte biology are critical to fully understanding shape variation in scale structure. First, after scales are formed internally within silica deposition vesicles (SDVs) and positioned externally within the scale coat, they do not change in size, shape or design (Siver 1991). Second, scales of *Mallomonas* fit into a precise and highly organized pattern on the outside of the cell membrane (Siver & Glew 1990). Third, some degree of scale shape change is necessary in order to accommodate the curvature of the cell, especially at each apex. As noted by Leadbeater (1986, 1990), in order to produce a precise scale coat, the shapes and sizes of individual scales must be constrained to fit the overall pattern. That is, changes in scale shape and size from one position on the cell to another must be coordinated between all spiral rows. For example, if the shape and sizes of scales within one row begin to change in order to conform to cell shape, then a similar change must occur within all rows (Siver & Glew 1990). Conversely, an abnormally large (or small) scale, or an oddly-shaped scale, will disrupt the overall organization of the scale coat.

Given the constraints of fitting scales into an organized pattern, much of the variation in scale shape within a given species of *Mallomonas* should be related, in part, to the position of the scale on the cell. In a study comparing scales of *Mallomonas striata* cells from a single locality with ones taken from localities around the world, Neustupa & Němcová (2007) found virtually identical patterns of shape variation and suggested that the range of shape variation was primarily due to location of the scale on the cell. In a more recent study based on a culture of *Mallomonas kalinae* and using similar geometric morphometric analyses, Řezáčová et al. (2010) found that the position of the scale on the cell accounted for a significant percentage of the total variation in scale shape, supporting the earlier observations made for *M. striata* by Neustupa & Němcová (2007).

Results from the current study clearly support the findings by Neustupa & Němcová (2007) and Řezáčová et al. (2010) that scale position on the cell covering is a primary source of shape variation within the synurophytes. The assumption is that variation in scale shape measured from cultures derived from single cell isolates would represent the maximum variation capable of that genotype. Estimates made from culture isolates could then be compared to ones measured for specimens from different sites or combinations of sites (e.g. all continents) in order to better appreciate the extent of shape variability for that morphospecies. The range in scale shape found in all three *M. insignis* cultures tested in the current study was similar and not significantly different from the range in shape found in the majority of modern sites. In addition, the amount of variability in scale shape, as measured by Procrustes distance, for *Mallomonas insignis* cells grown from isolates was virtually identical to that estimated from any modern or fossil site, as well as for estimates made for all specimens grouped together regardless of origin. This implies that for *Mallomonas insignis* the variation in scale shape on a single cell can be as high as that found within an entire population or even multiple populations from different geographic regions. Since the degree of shape variability observed for fossil specimens throughout the Giraffe Pipe core was not significantly different from that measured for the clonal isolates, position of the scales on the cell covering was probably also the primary source of shape variation for this taxon during the Eocene.

The shape and size of synurophyte scales has also been found to vary with respect to environmental variables, including temperature, light, nutrient concentrations and pH. Hahn et al. (1996) reported that *M. tonsurata* produced larger scales when grown under high phosphorus or silica concentrations, and Gavrilova et al. (2005) found that *Synura petersenii* produced smaller and differently-shaped scales at pH extremes. Němcová et al. (2010) demonstrated that *Synura echinulata* produced differently shaped scales when grown under varying temperature-light regimes. Although Němcová et al. (2010) concluded that *S. echinulata* exhibited considerable phenotypic plasticity in regards to scale shape; all taxonomically relevant characters remained stable. Changes in temperature have been observed to impact scale size, although the effect is apparently not consistent between taxa. Martin-Wagenmann & Gutowski (1995) and Gutowski (1996) found that an increase in temperature resulted in significantly smaller scales for cultures of *Synura petersenii* and *Mallomonas tonsurata*, respectively. In contrast, Němcová et al. (2010) reported a decrease in scale size under lower temperatures when coupled with a long light exposure. Siver et al. (2013b) found that even though scale shape was similar, *M. insignis* scales from the Eocene were significantly smaller than modern specimens. Perhaps the smaller *Mallomonas insignis* scales from the Giraffe core reflect the significantly warmer Arctic climate that existed during the Eocene (Zachos et al. 2008, Greenwood et al. 2010), coupled with long summer days

(Jahren 2007). If a relationship between scale size and temperature can be further developed for *M. insignis*, it could serve as an important proxy for reconstructing temperature during the warmer climates of the Paleogene.

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References

Andersen, R. A. (1987): Synurophyceae classis nov., a new class of algae. – Amer. J. Bot. **74**: 337–353.

Bookstein, F. L. (1997): Landmark methods for forms without landmarks: morphometrics of group differences in outline shape. – Med. Im. Anal. **1**: 225–243.

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

Greenwood, D. R., Basinger, J. F. & Smith, R. Y. (2010): How wet was the Arctic Eocene rain forest? Estimates of precipitation from Paleogene Arctic macrofloras. – Geology **38**: 15–18.

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

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

Jahren, A. H. (2007): The Arctic forest of the Middle Eocene. – Ann. Rev. Earth Plan. Sci. **35**: 509–540.

Jo, B. Y., Shin, W., Kim, H. S., Siver, P. A. & Andersen, R. A. (2013): Phylogeny of the genus *Mallomonas* (Synurophyceae) and descriptions of five new species on the basis of morphological evidence. – Phycologia **52**: 266–278.

Kristiansen, J. (2005): Golden Algae: A Biology of Chrysophytes. Gantner Verlag K.G.

Kristiansen, J. & Preisig, H. R. (2007): Chrysophyte and Haptophyte Algae. Part 2; Synurophyceae. – In: Büdel, E. et al. (eds): Süsswasserflora von Mitteleuropa Vol. 1/2. Spektrum Verlag, Berlin, Heidelberg.

Leadbeater, B. S. C. (1986): Scale-case construction in *Synura petersenii* Korsh. (Chrysophyceae). – In: Kristiansen, J. & Andersen, R. A. (eds): Chrysophytes: Aspects and problems, pp. 121–131. Cambridge Univ. Press, Cambridge, UK.

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

Marsicano, L. J. & Siver, P. A. (1993): A paleolimnological assessment of lake acidification in five Connecticut lakes. – J. Paleolimnol. **9**: 209–221.

Martin-Wagenmann, B. & Gutowski, A. (1995): Scale morphology and growth characteristics of clones of *Synura petersenii* (Synurophyceae) at different temperatures. – In: Sandgren, C. D., Smol, J. P. & Kristiansen, J. (eds): Chrysophyte algae: Ecology, phylogeny and development, pp. 345–360. Cambridge Univ. Press, Cambridge, UK.

Němcová, Y., Neustupa, J., Kvíderová, J. & Řezáčová-Škaloudová, M. (2010): Morphological plasticity of silica scales of *Synura echinulata* (Synurophyceae) in crossed gradients of light and temperature – a geometric morphometric approach. – Nova Hedwigia Beih. **136**: 21–32.

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

Řezáčová-Škaloudová, M., Neustupa, J. & Němcová, Y. (2010): Effect of temperature on the variability of silicate structures in *Mallomonas kalinae* and *Synura curtispina* (Synurophyceae). – Nova Hedwigia Beih. **136**: 55–69.

Rohlf, F. J. (2007): TPS Series. Department Ecology & Evolution, SUNY-Stony Brook, N.Y.

Siver, P. A. (1991): The Biology of *Mallomonas*. Morphology, Taxonomy and Ecology. – Kluwer Acad. Publ. Dordrecht, Netherlands.

Siver, P.A. & J. R. Glew (1990): The arrangement of scales and bristles on *Mallomonas*: A proposed mechanism for the formation of the cell covering. – Can. J. Bot. **68**: 374–380.

Siver, P. A. & Wolfe, A. P. (2005): Eocene scaled chrysophytes with pronounced modern affinities. – Int. J. Plant Sci. **166**: 533–536.

Siver, P. A. & Wolfe, A. P. (2009): Tropical ochrophyte algae from the Eocene of northern Canada: a biogeographic response to past global warming. – Palaios **24**: 192–198.

Siver, P. A., Lott, A. M. & Wolfe, A. P. (2009): Taxonomic significance of asymmetrical helmet and lance bristles in the genus *Mallomonas* (Synurophyceae) and their discovery in Eocene lake sediments. – Eur. J. Phycol. **44**: 447–460.

Siver, P. A., Lott, A. M. & Wolfe, A. P. (2013a): A summary of *Synura* taxa in early Cenozoic deposits from northern Canada. – Nova Hedwigia Beih. **142**: 181–190, this volume.

Siver, P. A., Wolfe, A. P., Rohlf, F. J., Shin, W. & Jo, B. Y. (2013b): Combining geometric morphometrics, molecular phylogeny, and micropaleontology to assess evolutionary patterns in *Mallomonas* (Synurophyceae: Heterokontophyta). – Geobiology **11**: 127–138.

Zachos, J. C., Dickens, G. R. & Zeebe, R. E. (2008): An early Cenozoic perspective on greenhouse warming and carbon-cycle dynamics. – Nature **451**: 279–283.

Zelditch, M. I., Swiderski, D. L., Sheets, D. H. & Fink, W. L. (2004): Geometric Morphometrics for Biologists: a Primer. Elsevier Acad. Press, London.