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Article

A whole-cell reconstruction of *Mallomonas porifera* Siver & Wolfe from the Eocene: Implications for the evolution of Chrysophyte cell architecture

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With 5 figures

Abstract: *Mallomonas porifera* was originally described from Middle Eocene lake sediments based on isolated siliceous microfossils and named for the single large pore found centrally on the base plate. Both domeless body and domed scales were described, however it was not known how the scales were positioned on the cell covering nor if the organism possessed bristles. Since the original description, 15 additional samples taken from a long sequence of the sediment core and found to contain *M. porifera* have been examined, including fractured mudstone sections from each sample. The fractured surfaces were found to contain the remains of whole cells allowing for a detailed description of the siliceous cell covering. *Mallomonas porifera* contains three types of scales and one bristle morphology arranged in precise positions on the cell. Domed scales are restricted to the anterior of the cell and each is associated with a bristle. The domed scales transition abruptly to domeless body scales that surround the main portion of the cell and are not associated with bristles. The posterior part of the cell covering consists of large triangular-shaped and spine-bearing scales that lack the characteristic large base plate pore found on the other scale types. The relationship of *M. porifera* to modern taxa within the genus is discussed.

Key words: Arctic Canada, fossil, Eocene, Giraffe Pipe, *Mallomonas*, chrysophytes

Introduction

Until the recent work by Siver and Wolfe (2005a, b), fossil remains of siliceous scales and bristles representing species within the Chrysophyceae and Synurophyceae were not known older than the Holocene (< 11,800 years). During investigation of sediments from an ancient, freshwater Eocene (55.8–33.9 million years) lake that existed within a crater formed during emplacement of the Giraffe Pipe kimberlite, Siver and Wolfe (2005a) discovered exceptionally well preserved remains of scales representing members of the genera *Mallomonas*, *Synura*, *Chrysosphaerella* and *Spiniferomonas*. The Giraffe Pipe kimberlite exists near the Arctic Circle in the Northwest Territories of Canada at 64°44’ N, 109°45’ W (Hamblin et al. 2003). A core from the Giraffe Pipe kimberlite, originally obtained by BHP Billiton Diamonds Inc. in 1999 (BHP 99-01), was drilled at a 47 degree angle and measured 163 m in length, including 50 m of Neogene glacial till overlying 45 m of peaty terrestrial material and 68 m of lacustrine sediments. Conversion of

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these core depths to their vertical equivalent implies stratigraphic thicknesses of 32.7 m for the peat and 51.1 m for lake sediments. Using a ^{87}Rb/^{87}Sr model age from kimberlitic phlogopite, Creaser et al. (2003) estimated emplacement of the Giraffe kimberlite at 47.8±1.4 million years ago (Ma), providing a maximum age for the lacustrine sedimentary sequence. Two air-fall tephra zones located near the end of the lake phase were estimated at 40 Ma using fission tracking methodology (Westgate personal communication), constraining the age of the waterbody between 48 and 40 Ma. The findings reported by Siver and Wolfe (2005a, b) proved definitively that the most common genera of photosynthetic scaled chrysophytes found in freshwater habitats worldwide today were well established by the middle Eocene.

Mallomonas porifera was one of six species originally described from Giraffe Pipe sediments by Siver and Wolfe (2005b) and named for the single large pore found centrally on the base plate. Two different scale types, domeless body scales and domed anterior scales, were described both possessing the large base plate pore and uncovered together from sediments deposited when the waterbody was shallow just prior to its transition to a wetland environment. A large triangular-shaped scale that possibly represented a collar scale (see Fig. 2F in Siver & Wolfe 2005b) was also uncovered, but the identity of this microfossil was unknown and it was not thought to be related to *M. porifera* since it lacked a large base plate pore.

Since the original description of *M. porifera* we have had the opportunity to examine over 50 additional samples taken from throughout the lacustrine sediment zone of the Giraffe Pipe core. Of these samples, over 15 contained abundant concentrations of *M. porifera* scales allowing for further study of this taxon. Although isolated microfossil remains from oxidized samples provided significant structural detail, it was not possible to reconstruct whole intact cell coverings from these samples. In order to fully understand the siliceous structures that comprise the scale covering of *M. porifera* it was necessary to examine remains of whole specimens. As a result, we examined fractured samples of mudstone from sections of the core we knew contained large concentrations of *M. porifera* scales based on our observations of oxidized samples and were able to observe remains of whole specimens. The objective of this paper is to further describe scale and bristle types belonging to *M. porifera* along with the spatial relationships of the siliceous components on the cell surface. Potential relationships of *M. porifera* to other taxa within the genus are discussed.

Materials and Methods

The current investigation is based on samples taken from throughout the lacustrine section of the core. Samples were processed for examination with scanning electron microscopy in two ways. First, small chips (~200 mg) of organic mudstone samples were oxidized in 30 % H_2O_2 under low heat, rinsed a minimum of four times with distilled water followed by repeated centrifugation and the resulting clean slurry stored in a glass vial. Aliquots of each clean slurry were air dried onto pieces of heavy duty aluminum foil. The aluminum foil samples were trimmed and attached to aluminum stubs with Apiezon® wax. Second, pieces of mudstone were fractured and the resulting surfaces examined with a dissecting microscope. The fractured mudstone pieces exposing the most microfossils were attached to aluminum stubs using double sided carbon tape. Silver paint was used to connect each mudstone sample to the aluminum stub in order to reduce charging effects. Both the oxidized aluminum and the fractured mudstone samples were coated with a mixture of gold and palladium for 1 min with a Polaron Model E sputter coater and observed with a Leo 982 field emission SEM.

A minimum of 25 specimens, mostly from the oxidized samples, were measured to establish morphometric means for each microfossil type.

Results

Examination of fractured mudstone samples:

We were able to locate and examine over 50 groups of undisturbed siliceous scales and bristles encased in mudstone from the Giraffe Pipe waterbody representing the remains of whole cells of *M. porifera* as they were originally deposited in the sediments (Fig. 1). It is clear from examination of these specimens that whole cells of *M. porifera* contain three primary types of scales and one bristle morphology arranged in precise positions on the cell. Domed scales are restricted to the anterior end of the cell where they are arranged in an ordered and overlapping pattern (Fig. 1A). Each dome scale is associated with a single bristle. The domed scales transition rather abruptly to domeless body scales that surround the main portion of the cell (Fig. 1A, D). The domeless body scales are not associated with bristles. Body scales transition, again rather abruptly, to large, triangular-shaped, spine-bearing scales surrounding the posterior of the cell (Figs 1B–C).

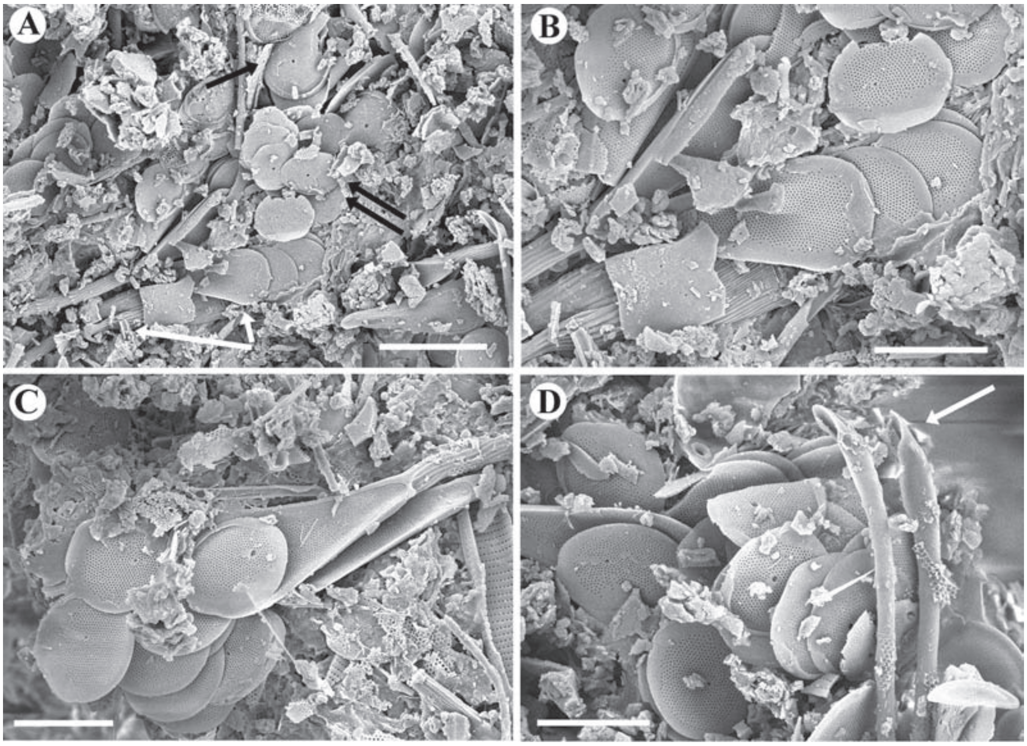

Fig. 1. Remains of *Mallomonas porifera* cells in fractured mudstone from the Giraffe Pipe core. A) Siliceous components of a whole cell depicting anterior domed scales (black arrow), body scales (two black arrows) and posterior spined scales (white arrow). B) Close up of the cell remains in "A" showing the protruding spines. C) Posterior portion of a cell. D) Anterior section of cell remains. Note two bristles (white arrow). Scale bars = 5 µm (B–D) and 10 µm (A).

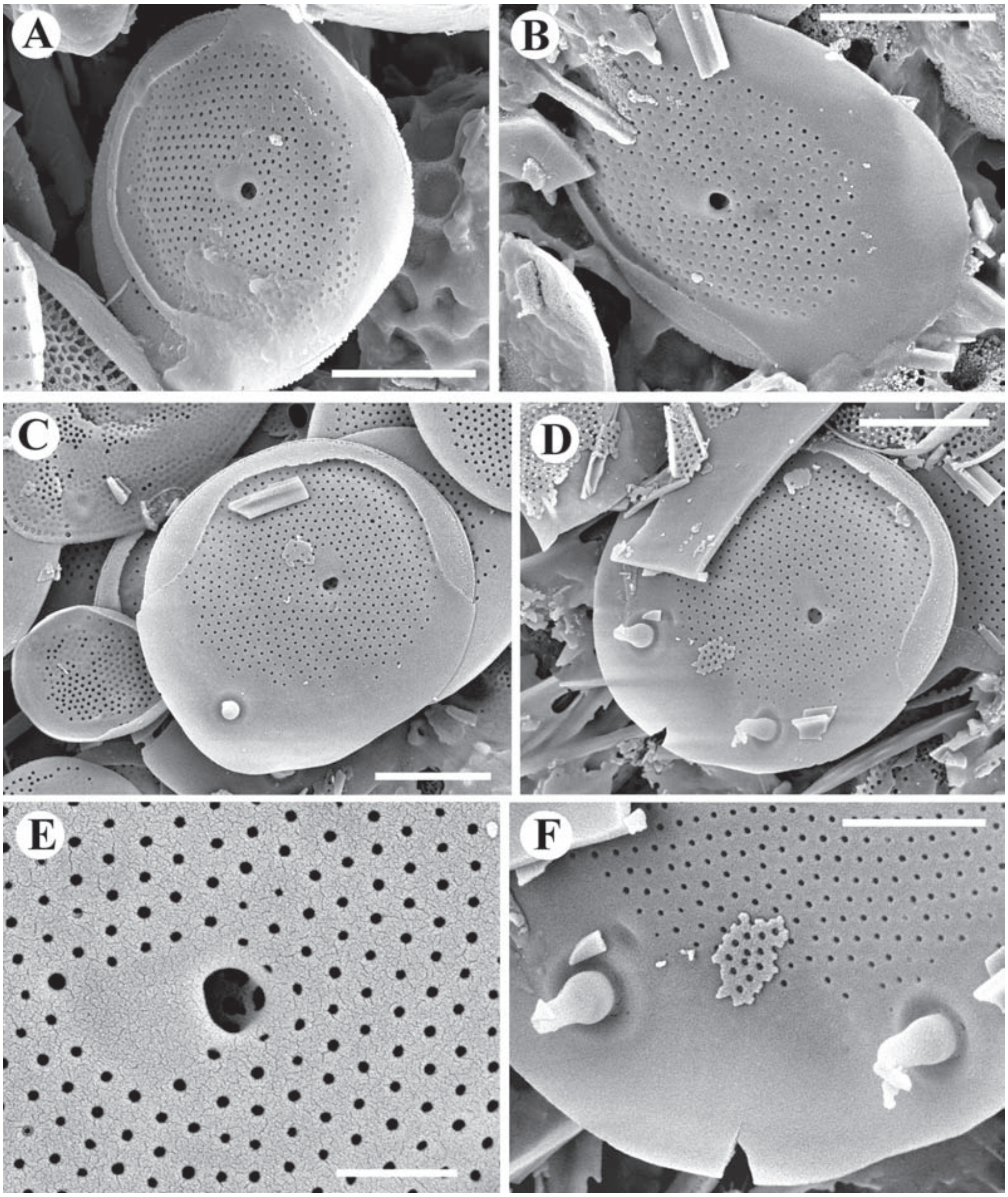

Fig. 2. Body scales of *Mallomonas porifera*. A–B) Typical body scales depicting small base plate pores, the large central pore and a posterior rim, but lacking anterior papillae. C–D) Body scales with one (C) and two (D) anterior papillae. E) Close up of a large pore and associated hyaline zone. F) Close up of the scale depicted in "D". Scale bars = 500 nm (E), 1 µm (F) and 2 µm (A–D).

Revised description of Mallomonas porifera Siver & Wolfe:

Body scales are oval to subcircular, sometimes slightly asymmetrical with a posterior rim that encircles approximately half of the scale perimeter and a broad hyaline region that encircles the anterior end of the scale (Figs 2A–D). The base plate is covered with small, evenly-spaced, circular pores that form linear rows that can be traced in several directions (Fig. 2). A single, large, circular pore is present close to the geometric center of the scale. Each large pore has a small hyaline zone positioned on the side facing the posterior rim (Figs 2A–E). A few body scales are often found that possess one or two large depressions within the anterior hyaline zone, each of which contains a single spine-like papilla (Figs 2C–D,F). The papilla-bearing scales are most often found in the posterior half of the cell.

Anterior domed scales are triangular-shaped, asymmetrical, and with a broad, hyaline dome positioned along the left side of the scale (Fig. 3). The large base plate pore is found near the proximal end of the dome and opens along the internal surface within a funnel-shaped structure. Interestingly, the hyaline zone associated with the large pore usually faces the dome and not the posterior rim as noted on body scales (Figs 3A–B). The dome cavity is elongated along the left

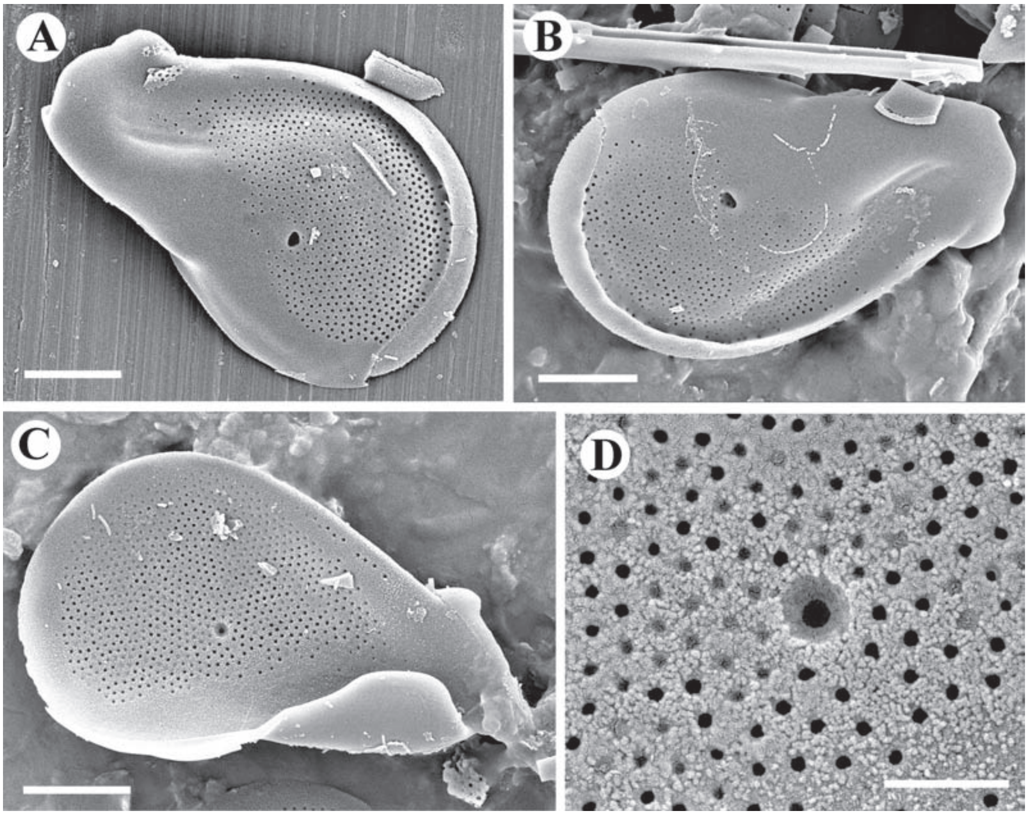

Fig. 3. Anterior domed scales of *Mallomonas porifera*. A–B) Exterior surface depicting the asymmetrical nature of the scale, elongated dome and position of the large pore. C) Undersurface of the scale showing the curved nature of the left anterior margin that wraps around the bristle and the opening of the large pore. D) Close up of the scale in “C”. Scale bars = 500 nm (D) and 2 µm (A–C).

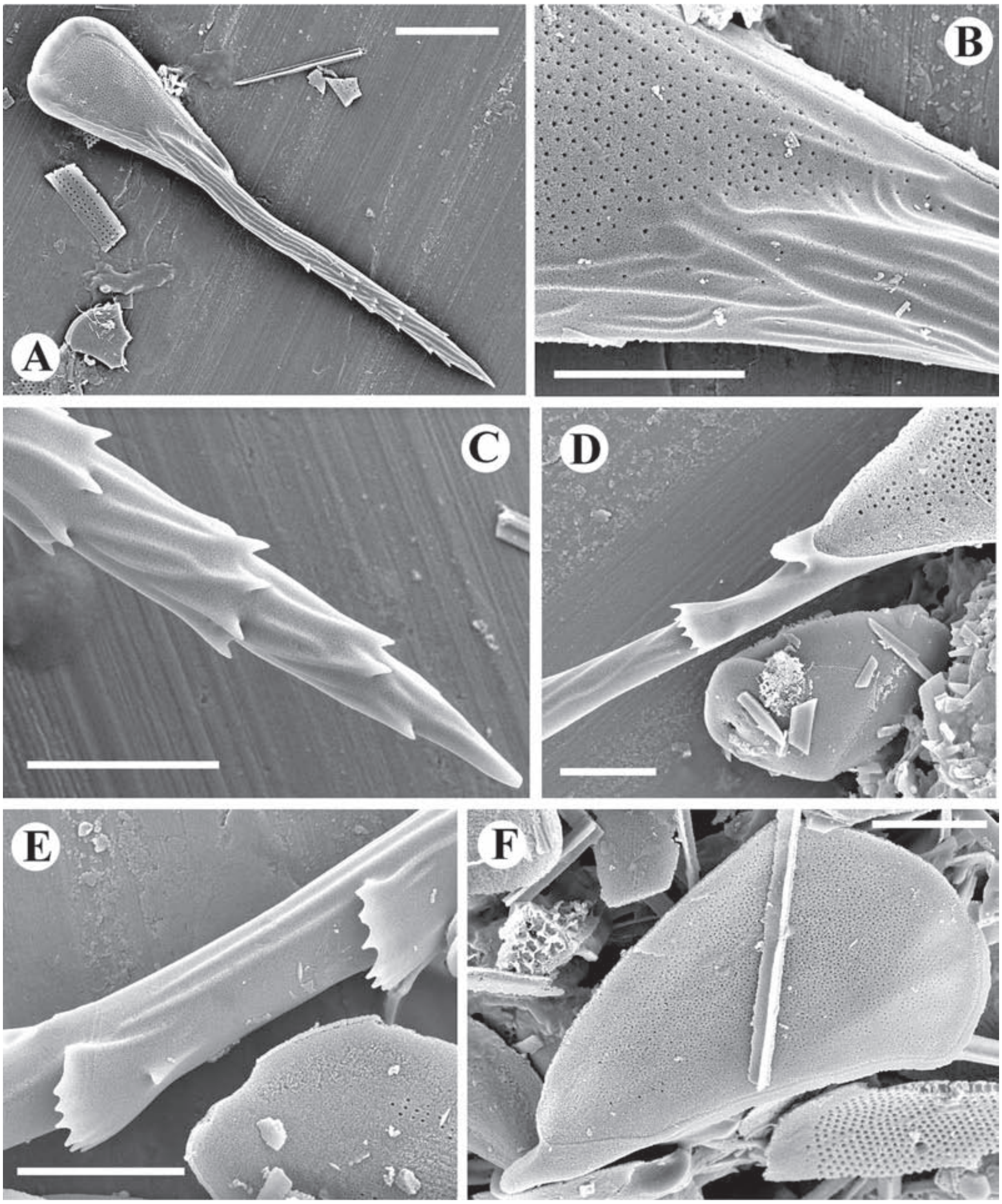

Fig. 4. Spine scales of *Mallomonas porifera*. A) Whole scale depicting the triangular shape of the scale and the robust nature of the spine. B–C) Close ups of the scale in "A" showing the base (B) and tip (C) of the spine. D) Undersurface of a spine scale. E) Spatula-shaped extensions along the botton of a spine. F) Scale with a short spine. Scale bars = 2 µm (B–F) and 5 µm (A).

side of the scale. The left anterior margin of the scale is bent down and wraps partially around the bristle, presumably to help hold it in place (Fig. 3C).

Posterior spine scales are large, triangular-shaped, possess an asymmetrically placed rim that encircles the posterior and entire left margins of the scale (Fig. 4A) and are positioned such that the spines protrude from the posterior of the cell (Figs 1B–C). Scales can have short spines (e. g. Fig. 4F), but the vast majority possess thick, solid and long spines (Figs 4A–E). The spines are pointed and covered with a series of wavy ridges that each ends as a tooth along the distal half of the shaft (Figs 4A–E). The teeth along the bottom of the spine often form small spatula-shaped projections (Figs 4D–E).

Bristles are thick, long, hollow and slightly curved with a highly modified proximal foot (Fig. 5). The shaft of the bristle consists of a rolled sheet of silica with the edges meeting to form a groove along the concave surface of the shaft (Figs 5B–D). The shaft is smooth with series of small teeth that sometimes form in rings (Fig. 5F). The foot consists of a flattened disc connected at an angle of approximately 20–45° with the shaft, a thickened rib connected to the convex surface of the shaft and a small ear-like flap of silica that protrudes at 90° from the bristle (Figs 5B–E).

Siliceous components of *M. porifera* cells ranged in size as follows – Body scales: 4.7–7 x 4.5–5.6 µm; Domed scales: 7–10.1 x 5.9–6.5 µm; Spine scales (without the spine): 8.8–11.8 x 4.7–5.3 µm; Spines: up to 20 µm; Bristles: 31–42 µm.

Discussion

Strong oxidation of any sediment sample, including Giraffe Pipe mudstones, yields isolated microfossil specimens that are excellent for study, but are no longer associated with other microfossil components from the original organism. When we examined oxidized samples containing *M. porifera* scales we always also found the rolled bristles with the small ear-like flap and the triangular-shaped spined scales. The consistent findings of these microfossil morphotypes together with *M. porifera* scales indicated a possible association, but until remains of whole cells were studied such assignment of these microfossil types was not possible. Initially, we did not anticipate that the triangular-spined scales were associated with *M. porifera* cells since they lacked the large base plate pore. Likewise, we did not know whether the bristles with ear-like flaps were associated with domed *M. porifera* scales and could not prove it based on isolated microfossils.

Preservation of microfossils within the Giraffe Pipe core is indeed exquisite and the fact that we have been able to uncover remains of whole organisms that still contained associations of their exterior siliceous components indicates that mixing was minimal to virtually nonexistent as organisms were deposited into the sediment. Although tedious, as demonstrated here for *M. porifera*, examination of fractured pieces of mudstone have yielded new insights regarding associations of different siliceous microfossil types. In addition to *Mallomonas* taxa, this technique has uncovered groups or piles of scales representing colonies of *Synura*, whole tests of the testate amoebae *Euglypha*, remains of sponge spicules presumably originating from the same specimen and whole diatom frustules including filaments of *Eunotia* and *Aulacoseira*. We conclude that examination of isolated microfossils along with fractured mudstone surfaces will significantly aid in our description and understanding of organisms that once lived in this Eocene waterbody.

Some morphological features of *M. porifera* resemble those of other extant species within the genus, but no modern taxon shares the full complement of siliceous structures found on the Eocene organism. Two species within the Sectio Planae, *Mallomonas caudata* Iwanoff emend. Krieger and *M. matvienkoae* (Matvienko) Asmund & Kristiansen, have scales that are similar to the body scales of *M. porifera* in lacking a V-rib and dome and in possessing a large base plate pore (Siver 1991, Kristiansen 2002, Kristiansen & Preisig 2007). However, the overall scale morphology and the complement of siliceous structures forming the cell covering is very different in *M. porifera*. All of the scales on cells of *M. caudata* and *M. matvienkoae* show little variation along the cell

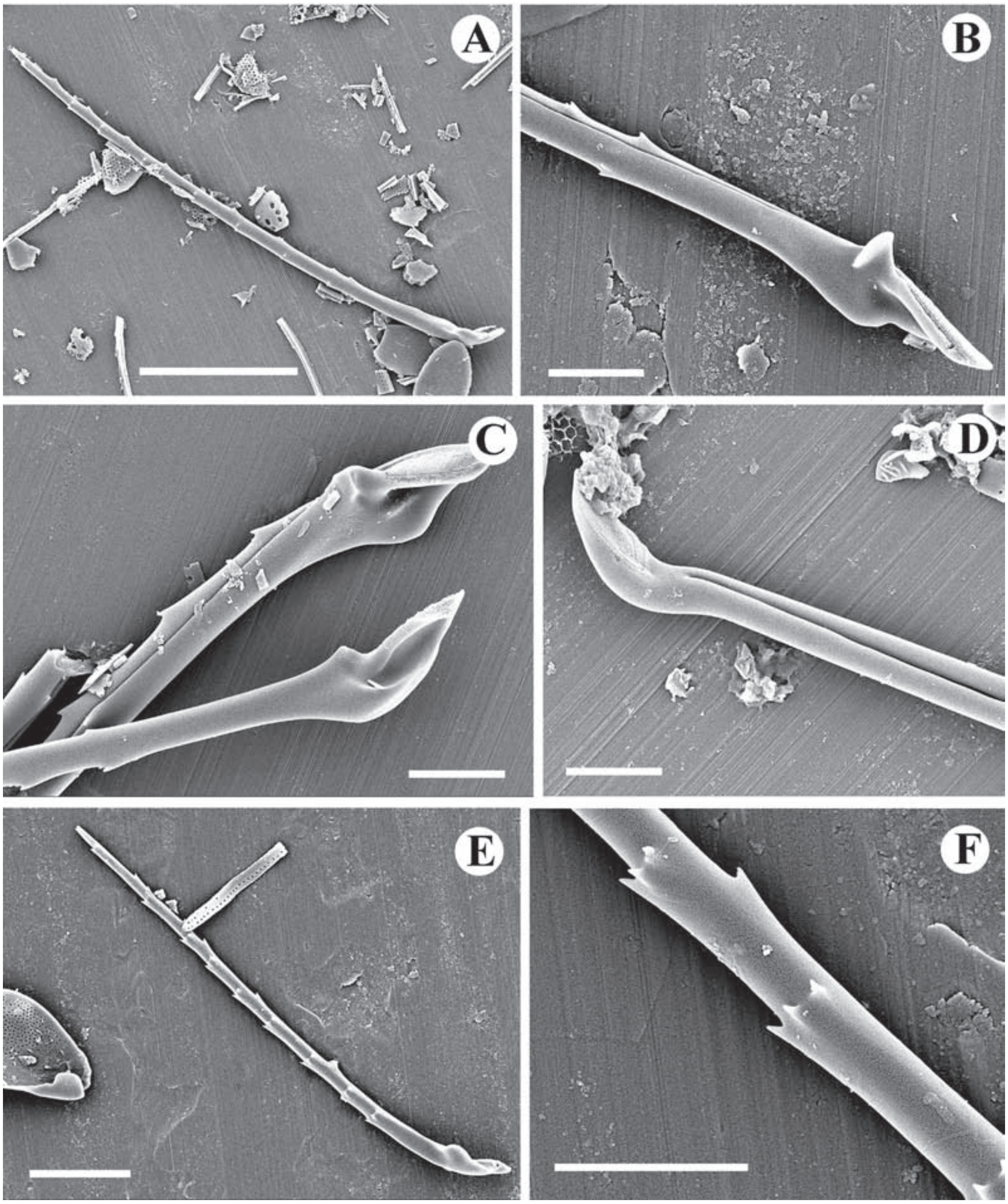

Fig. 5. Bristles of *Mallomonas porifera*. A, E) Whole bristles showing the distribution of teeth and proximal foot. B–D) Close ups depicting different views of the bristle foot. Note the flat portion (C–D), the ridge connecting the flat surface to the shaft (B–C), the ear-like flap (B–C) and the ventral groove. F) Close up illustrating the smooth surface of the shaft and associated teeth. Scale bars = 2 μm (B–D, F), 5 μm (E) and 10 μm (A).

and all scales are coupled with bristles. Further, both of the modern species lack dome-bearing scales surrounding the flagellum as well as spine-bearing posterior scales. Thus, although the simpler scale structure with a large base plate pore found on *M. porifera* body scales resembles those of *M. caudata* and *M. matvienkoae*, the former taxon differs significantly from the latter two organisms and does not belong in the Sectio Planae.

We hypothesize, based on comparisons with modern taxa, that the domed bristle-bearing scales of *M. porifera* formed a ring around an emergent flagellum and that the spine-bearing scales covered the posterior end of the cell. Two Sections of the genus *Mallomonas*, Sectio Torquatae and Sectio Akrokomae, contain species where the siliceous cell covering is composed of anterior domed scales associated with bristles, domeless body scales lacking bristles and posterior scales modified into spines or siliceous extensions (Asmund & Kristiansen 1986, Kristiansen 2002, Kristiansen & Preisig 2007). The distribution of scale types and bristles found on *M. porifera* cells is similar to those in both of these Sections, but the fine detail of the siliceous components clearly differ. It is tempting to consider *M. porifera* within the Sectio Torquatae whose taxa possess a ring of dome-bearing collar scales sharply delineated from domeless body scales which, in turn, are distinct from the spine-bearing posterior scales, a condition found in *M. porifera*. Like *M. porifera*, some species within the Sectio Torquatae also have posterior scales with very long spines. However, since Sectio Torquatae scales are tripartite (ie. have a V-rib and dome), from an evolutionary point of view they are presumably quite different from *M. porifera* and as a result the latter species would not belong in this Section. On the other hand, scales of *M. akrokomos* Ruttner in Pascher, the sole species within the Sectio Akrokomae, lack V-ribs and are fundamentally more similar to those of *M. porifera* than ones representing Sectio Torquatae. However, there are differences in the fine structure of the scales between *M. porifera* and *M. akrokomos* and the posterior scales on the latter species elongate to form a long caudal tail and are not considered by Kristiansen (2002) as spines. Nonetheless, the general morphology of scales and the overall design of the cell covering in *M. porifera* appears most closely aligned with the Sectio Akrokomae (ie. *M. akrokomos*) and not with any of the other 19 Sections recognized by Kristiansen & Preisig (2007).

Based on our examination of the Giraffe Pipe core, *Mallomonas porifera* was clearly one of the dominant species within the lacustrine ecosystem, especially in samples from core boxes 14–16 corresponding to lake depths of 11 to 19 m deep. In some of these samples, numerous remains of *M. porifera* cells can be found and its not uncommon to find cells piled on top of each other within the mudstone fractures. This organism was less abundant as the waterbody became shallower with time. Our assumption is that *M. porifera* is now extinct, but it is also possible that it is rare today and has simply not yet been discovered from modern aquatic ecosystems.

Acknowledgements

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