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Mallomonas enigmata sp. nov. (Synurales, Chrysophyceae), an Eocene fossil species with a second and unique scale morphotype attached to its cyst

Peter A. Siver

Botany Department, Connecticut College, New London, Connecticut, 06320, USA

ABSTRACT

A new and intriguing fossil *Mallomonas* species, *M. enigmata*, was uncovered from 10 strata in the Eocene Giraffe Pipe locality. The new species represents an ancient member of the section Punctiferae, and based on scale and bristle morphology is most closely related to the modern congener, *M. nieringii. M. nieringii* is a rare species known from a suite of acidic waterbodies on the Cape Cod peninsula, Massachusetts, USA. Only a few modifications of the body scales differ between *M. enigmata* and *M. nieringii*, and the new taxon was uncovered from a section of the Giraffe Pipe maar lake representing a shallow and acidic waterbody. Cysts of *M. enigmata* are oval, with a small pore surrounded by a narrow hyaline zone. The anterior ~ ¼ of the cyst surrounding the hyaline zone consists of a series of small, closely spaced, rimmed holes that penetrate the wall. Otherwise, the cyst wall is smooth and unornamented. Unlike any known modern species in the genus, a second type of body scale is produced, but found only attached to the cyst. It is hypothesized that these scales may represent unfinished body scales that became fused to the cyst during its development.

HIGHLIGHTS

- A new fossil Eocene species of *Mallomonas*.
- A unique second scale morphotype attached to the cyst.
- Thrived in a shallow, humic and acidic freshwater pond.

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KEYWORDS Cyst; Eocene; fossil; Giraffe Pipe; *Mallomonas*; Synurales

Introduction

Mallomonas Perty is the largest and most diverse genus within the order Synurales, a monophyletic clade within the class Chrysophyceae consisting of unicellular heterokont algae bearing highly organized cell coverings of siliceous scales (Kristiansen, 2005; Škaloud *et al*., 2013; Siver *et al*., 2015; Jadrná *et al*., 2021). Almost all *Mallomonas* species possess a second type of siliceous component referred to as the bristle. A bristle is an elongated linear structure with a flattened basal portion, or foot, that is tucked under the distal end of a scale such that the elongated shaft radiates out from the cell surface (Siver, 1991; Kristiansen, 2002). Although a few species have been reported from marine environments (Jeong *et al*., 2019), the vast majority are common elements of planktic communities in wetlands, ponds, lakes and slow-moving streams and rivers (Siver, 2015). Because many species are found to be restricted along different environmental gradients, including for example nutrient, pH and specific conductivity, they are often used as effective bioindicators (Siver, 1995). The specific environmental requirements, coupled with the fact that the species-specific siliceous components become archived in sediments and fossil rocks, make synurophytes a valuable resource for reconstructing past environments (e.g. Cumming *et al*., 1994; Siver & Lott, 2023).

According to AlgaeBase, which uses taxonomic information maintained in the Index Nominum Algarum at the Jepson Herbarium at the University of California, a total of 433 species and subspecific taxa of *Mallomonas* have been described, including both contemporary and fossil organisms (Guiry & Guiry, 2020). Historically, the delineation of taxa is based on differences in scale types and designs, and to a lesser degree on bristle structure. Of the 433 taxa, a fair number have been combined over time, and a few represent duplicates. Many early descriptions made with light microscopy (LM) had only a few, often rudimentary, drawings. Over time, electron microscopy (EM) became the primary tool used to describe the siliceous components used to identify species, and more recently gene sequencing has been implemented along with EM in species descriptions. Because it has been difficult, if not impossible, to link many of the earlier taxa described with LM to those described with EM and/or both EM and gene sequences, the status of some LM-based species remains unclear. Given these limitations, the number of *Mallomonas* species that are well studied and identifiable based on EM methods is closer to 250 (Siver,

CONTACT Peter A. Siver ✉ pasiv@conncoll.edu

2024). The genus is divided into approximately 19 sections with most sections further subdivided into different series (Kristiansen, 2002; Siver *et al*., 2015).

There is a wide range of scale morphology found among the numerous species, ranging from simple to highly elaborate designs (Siver, 2015 and numerous references therein). Although the basic design is common to all scales on a cell, some features, including shape, will differ depending on the position of the scale on the cell. The majority of scales covering the main portion of the cell are called body scales. Scales surrounding the flagellar pore or posterior end will differ in shape from body scales and often possess spines (Siver, 1991; Kristiansen, 2002). The flat portion of the scale, referred to as the base plate, and the posterior border of the scale, are turned upwards and bend slightly over the base plate forming the posterior rim. The base plate is usually perforated with pores, and the size, spacing and location of these pores can serve as diagnostic characters. Additional structures, referred to as secondary structures and including for example ribs, papillae, a V-rib, wings or spines, can be formed on top of the base plate. In addition, the anterior end can be moulded to form a dome, a raised portion of the base plate into which the bristle foot is secured and held in place.

All Chrysophyceae, including all species of *Mallomonas*, can produce a resting stage as part of their life cycle known as a stomatocyst, or simply a cyst. Except for a small opening, or pore, cysts are solid spherical to ovoid siliceous vessels produced within the parent cell in a silica deposition vesicle (SDV) and ultimately contain all vital cell organelles (Sandgren, 1981, 1988, 1989). Cysts are formed in stages, where the shape of the vessel wall, pore and surrounding collar are first produced resulting in a smooth outer wall. Additional or secondary structures, such as spines, ribs and papillae, are often added to the outer wall to form specific designs. Once the cyst is formed, the pore is sealed with a plug which will eventually be removed to allow the new vegetative cell to emerge from the pore. Cysts are typically induced to form in response to specific cues, including shifts in environmental conditions, predation pressure or population density, and can form asexually or via sexual reproduction (Sandgren, 1981, 1988; Holen, 2014). Detailed terminology describing the various structures and components often produced on the cyst wall and associated with the pore region are given in Duff *et al*. (1995) and Wilkinson *et al*. (2001). Like scales, the finished morphology of the mature cyst is specific to each species (Skogstad, 1984; Kristiansen, 2005).

The oldest known fossil synurophyte scales and bristles date to the late Cretaceous, with most fossil species having been described from Eocene mudstones (Siver, 2023). Some fossil species exhibit evolutionary stasis with respect to scale morphology, while others present very different designs and structures, and presumably represent extinct lineages. The objective of this communication is to describe a new fossil species, including information for scales, bristles and the cyst, from an Eocene deposit in northern Canada. The morphology of the body scales exhibits evolutionary stasis with a rarely reported modern species, however the new fossil species differs from all known *Mallomonas* species in forming a second type of body scale found only attached to the cyst surface.

Material and methods

Ten strata from the Giraffe Pipe core, each identified in previous investigations to contain the new species (Siver & Lott, 2023), were intensively examined in this study (Table 1). Pieces of rock from the 10 strata, each weighing approximately 100 mg, were oxidized using 30% H_2O_2 under low heat for a minimum of 2 h, rinsed five times with distilled water, and the resulting slurries stored in glass vials at 4°C. This procedure resulted in separation of siliceous microfossils from the mudstone/rock matrix. One ml aliquots of the clean slurries were air-dried onto flat pieces of heavy-duty aluminium foil, trimmed and attached to aluminium stubs with Apiezon wax. Samples were coated with a mixture of gold and palladium for 2 min with a Polaron Model E sputter coater, and examined with either a Leo 982 field emission SEM, or a FEI Nova NanoSEM 450 field emission SEM.

Samples from each of the 10 strata contained body scales, apical scales and bristles representing the new fossil taxon, and its corresponding cyst was uncovered in eight of the samples. Morphometric measurements of all siliceous components were made from digital images. For triangular-shaped apical scales, length measurements included the spine, and width measurements were made at the widest point.

Because cysts were ovoid, estimates of both length and width are given.

Location and identification of samples from the Giraffe core are given in Siver & Lott (2012). Each section of the core was immediately cut and placed into a core box. Each sample is identified with a three-part number (Table 1). The first number represents the core box. Deeper sections of the core correspond to larger box numbers. Each box contains three 1.5 m core lengths, stored in three channels marked 1, 2 and 3. The second number represents the channel. The third number is the length in cm measured from the top of a core length within the channel. Thus, sample 13-1-33 represents a sample taken from 33 cm down along the core length positioned in channel 1 from box 13.

Table 1. Strata in the Giraffe Pipe core containing remains of *Mallomonas enigmata*. The depth along the core is measured from the ground surface and a summary of the most important co-inhabiting organisms are given for each stratum containing *M. enigmata*. The percentage of the total number of microfossils uncovered in each stratum is listed in parentheses for each organism, and is according to Siver & Lott (2023). *Mallomonas* species listed with a 'GP' designation are undescribed species discussed in Siver *et al.* (2015).

Stratum	Depth in core (m)	Major cohabiting taxa
13-1-15	107.3	Chrysophyte cysts (22), *Mallomonas caverna* (25), *M. ampla* (6), *Synura cronbergiae* (29), eunotioid diatoms (8)
13-1-33	107.5	Chrysophyte cysts (38), *Mallomonas media* (3), *M. preisigii* (4), *M. caverna* (6), *Synura cronbergiae* (3), eunotioid diatoms (9), *paraphysomonads* (15)
13-1-50	107.7	Chrysophyte cysts (24), *Mallomonas pleuriforamen* (17), *M. preisigii* (29), *M.* GP11 (21), *Synura cronbergiae* (1), eunotioid diatoms (5)
13-1-130	108.5	Chrysophyte cysts (4), *Mallomonas elephantus* (11), *M. pleuriforamen* (2), *M. preisigii* (< 1), *Synura cronbergiae* (72), eunotioid diatoms (< 1)
13-1-144	108.6	Chrysophyte cysts (4), *Mallomonas ampla* (12), *M. porifera* (6), *M. preisigii* (4), *Synura cronbergiae* (68), eunotioid diatoms (< 1)
14-3-20	108.8	Chrysophyte cysts (18), *Mallomonas lychenensis* (36), *M. porifera* (22), M. *ampla* (1), *M. dispar* (6), *Mallomonas preisigii* (1), other *Mallomonas* spp. (4), *Synura cronbergiae* (10), eunotioid diatoms (< 1)
14-3-140	110.1	Chrysophyte cysts (6), *Mallomonas lychenensis* (48), *M. porifera* (26), *Mallomonas skogstadii* (4), *Synura cronbergiae* (5), paraphysomonads (2), eunotioid diatoms (2)
14-2-32	110.6	Chrysophyte cysts (25), *Mallomonas aperturae* (4), *M. media* (3), other *Mallomonas porifera* (15), *Mallomonas caverna* (2), *Mallomonas skogstadii* (8), *Synura cronbergiae* (4), araphid diatoms (4), euglyphids (3), heliozoans (3), eunotioid diatoms (25)
14-1-60	112.2	Chrysophyte cysts (9), *Mallomonas lychenensis* (16), *M. porifera* (21), *M. media* (3), *M. schumachii* (28), *Synura cronbergiae* (6), eunotioid diatoms (14)
15-3-75	113.9	Chrysophyte cysts (45), *Mallomonas porifera* (1), *Mallomonas* GP11 (12), *M. gigantica* (11), *Synura cronbergiae* (6), euglyphids (2), raphid diatoms (1), eunotioid diatoms (20)

The percentages of different taxa given in this communication represent the percentages of the total number of microfossils uncovered and enumerated in each sample as reported in the previous work by Siver & Lott (2023).

Site description

The Giraffe Pipe locality (64.73°N, 109.75°W) is a kimberlite diatreme that was emplaced into the Slave Craton in the Northwest Territories of Canada approximately 47.8 ± 1.4 Mya during the Eocene (Creaser *et al.*, 2004; Buryak *et al.*, 2024). Soon after formation, the diatreme crater filled with water becoming a maar waterbody and slowly infilled with a sequence of lacustrine sediments, then paludal sediments, that was later capped with Neogene glacial deposits (Wolfe *et al.*, 2006, 2017). The emplacement age estimate, based on a Rb-Sr model from kimberlitic phlogopite, provides a maximum age estimate for maar lake sedimentation (Creaser *et al.*, 2004). A 163 m long drilled core, collared at a 47° angle, was uncovered from the kimberlite maar in 1999 by BHP Billiton Inc. (Wolfe *et al.*, 2006). A total of 113.1 m of the core contained stratified material, including 68.3 m of lacustrine mudstones, overlain with 44.8 m of organic peaty material. Multiple air-fall tephra beds found within the core were used to further provide age estimates of the maar infill. Based on CA-ID-TIMS tephra zircon U-Pb dating of the tephra layers, a Bayesian model age of 47.995 ± 0.082|0.087 Ma was established for the upper portion of the lacustrine sediments, while a single zircon grain from tephra in the lowermost lacustrine sediments had an age of 48.72 ± 0.29|0.30 Ma (Buryak *et al.*, 2024).

Given the age estimates, the hypothesis is that after phreatomagmatic kimberlite emplacement, the waterbody that formed within the crater and varied in depth over time persisted for thousands of years before transitioning to a terrestrial environment (Siver & Lott, 2023).

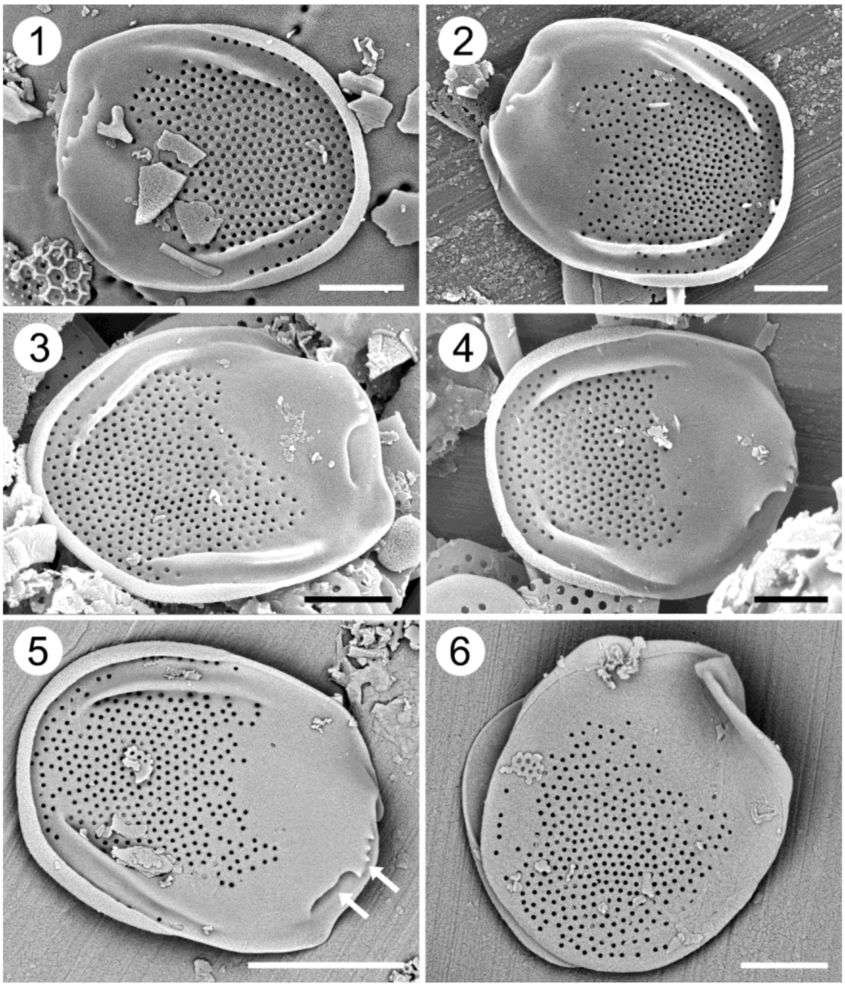
Results

During the last decade, over 200 strata of the long core taken from the Giraffe Pipe fossil locality have been thoroughly investigated using SEM for remains of microorganisms. Siliceous components of an undescribed species of *Mallomonas* were uncovered in 10 of 20 strata investigated over a 6.5 m length of the core estimated to span approximately 6000 years (Table 1). The organism was previously reported as *Mallomonas* GP4 (Siver *et al*., 2015; Siver & Lott, 2023), belonging to the series Punctiferae within the section Punctiferae (Siver, 2023). Each of the 10 strata was further examined as part of this study in order to fully characterize the undescribed species, the formal description of which is given below:

Mallomonas enigmata **Siver, sp. nov. (Figs 1–25)**

Description

Body scales are quadrate-shaped with a broad and rounded posterior end, a square-shaped anterior margin, and range in size from 3.6–4.8 µm × 2.8–4.1 µm (n = 25) with a mean of 4.0 × 3.4 µm (Figs 1–6). The posterior rim is narrow and encircles approximately one-half of the scale perimeter. There are two submarginal ribs, one on each side of the scale, that are parallel with the margin along the

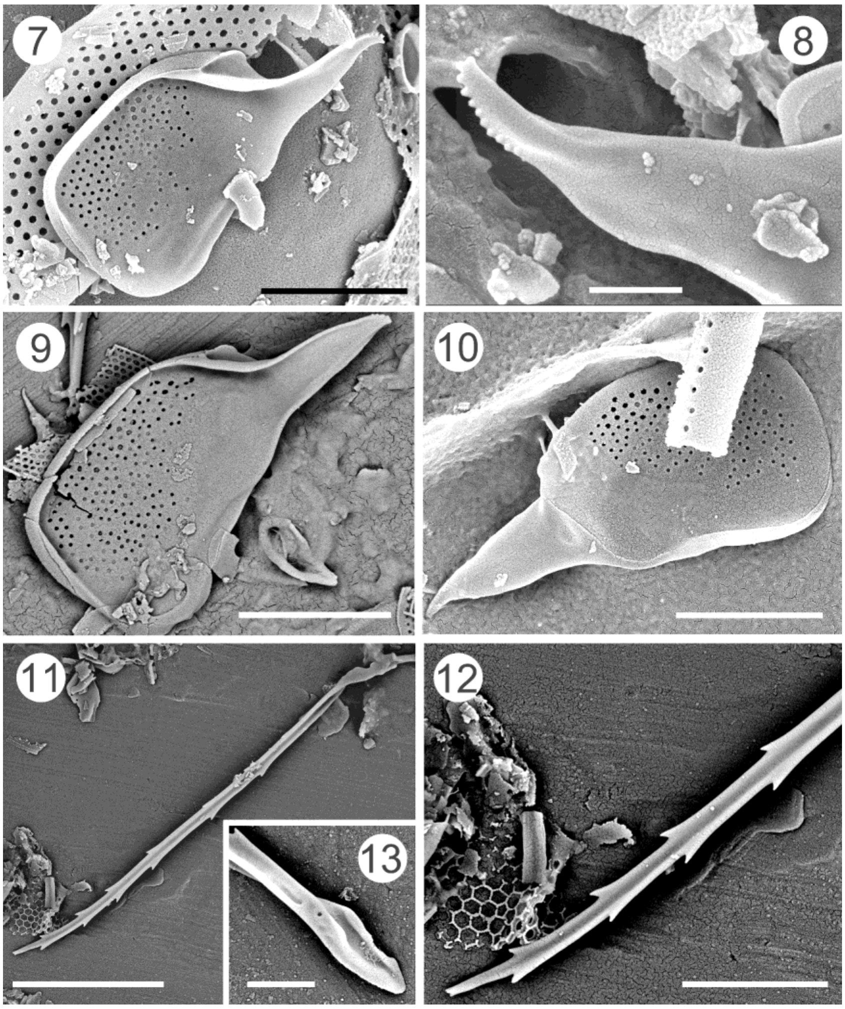


Figs 1–6. Body scales of *Mallomonas enigmata*. **Figs 1–5**. Surface views of scales showing the overall shape, distribution of base plate pores, pair of submarginal ribs, the narrow posterior rim, and both the linear depression (lower arrow on **Fig. 5**) and series of small teeth (upper arrow on **Fig. 5**) on top of the shallow dome. **Fig. 6**. Undersurface view depicting the U-shaped opening of the shallow dome, and distribution of base plate pores. Scale bars: Fig. 1–4, 6, 1 µm; Fig. 5, 2 µm.

longitudinal axis (Figs 1–5). Other than the pair of submarginal ribs, there are no additional secondary structures on the scale. Base plate pores cover approximately 2/3 of the area between the submarginal ribs, or shield, but are lacking in the anterior portion of the scale (Fig. 6). Additional pores can be found between each submarginal rib and the margin, often in a single row closer to the margin. All base plate pores are of a similar diameter. The dome is small, shallow, and with an inverted U-shaped opening to the right side of centre from which the bristle emerges (Fig. 6). There is a linear depression (not a hole) on the top of the dome above the U-shaped opening (lower arrow on the left, Fig. 5). A series of 2–5 (sometimes more) small teeth align with, and to the left of, the linear depression (upper arrow on the right, Fig. 5). A thin linear depression on the underside of scale is positioned slightly submarginal to the anterior margin.

Apical scales are distinctly asymmetric and triangular with a prominent forward-projecting spine (Figs 7–10). Including the spine, the scales range in length from 5.2–5.9 µm × 2.4–2.6 µm (n = 10) with a mean of 5.6 µm × 2.5 µm. The posterior rim is extended along the left side of the scale essentially reaching the anterior margin, whereas the right side terminates at the base of the scale, highlighting the scale asymmetry (Figs 7, 9). The submarginal ribs are lacking, and the base plate pores are restricted to about half of the scale area. A rib on the left side of the scale, presumably analogous to the submarginal rib on body scales, and the right margin of the scale, extend forward to form the anterior spine (Figs 7, 9). A series of small teeth align the tip of the spine (Fig. 8).

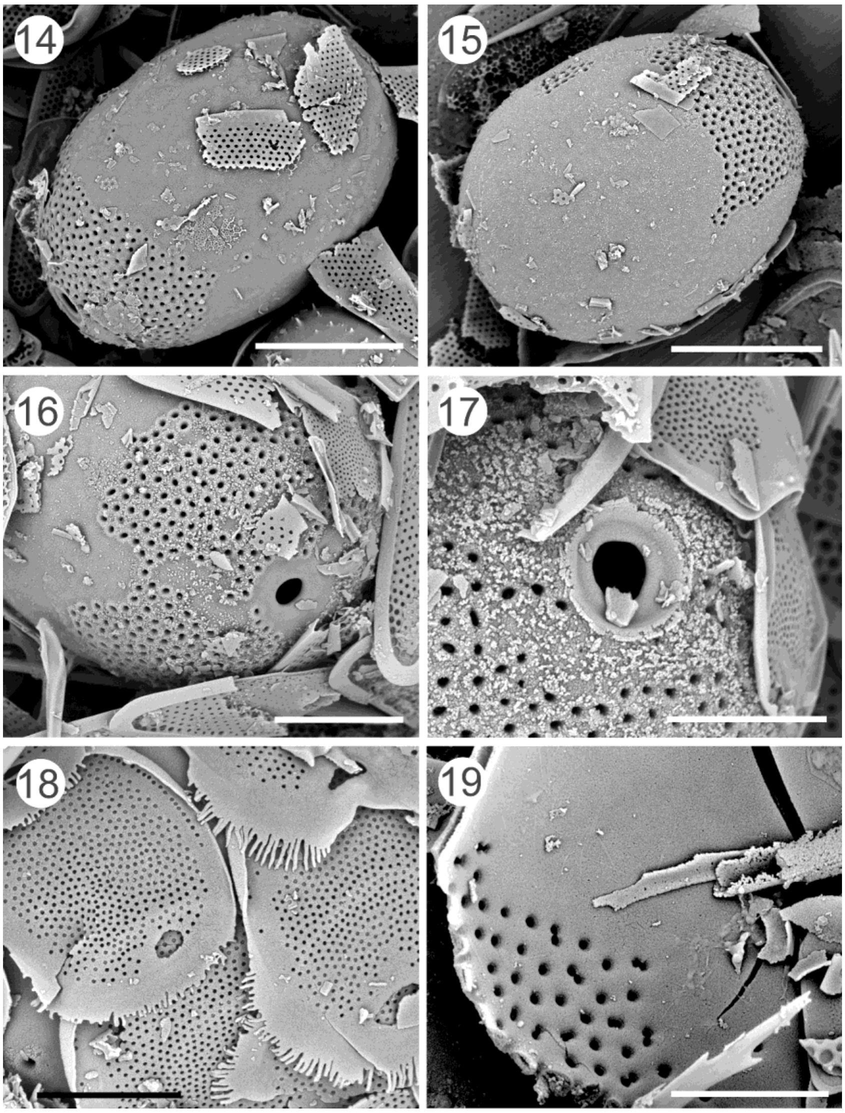
Bristles range in length from 14–18 µm (n = 10), are straight to slightly curved, ribbed, and with a pointed distal tip (Fig. 11). The shaft consists of three ridges, each with a series of sharp teeth that are more or less equally spaced along the distal 2/3 portion (Fig. 12). The proximal foot is flat and at a slight angle with the shaft (Fig. 13).



Figs 7–13. Apical scales and bristles of *Mallomonas enigmata*. **Figs 7, 9**. Surface views illustrating the triangular-shape and asymmetric nature of the scales, and forward-projecting spine. Note the asymmetric position of the posterior rim and small teeth aligning the apical end of the spine. **Fig. 8**. Close up of the apical end of a spine showing the row of small teeth. **Fig. 10**. Undersurface of a scale showing the distribution of base plate pores. Figure 11. An intact bristle comprised of a flat foot, a ribbed and toothed shaft, and a pointed distal tip. **Fig. 12**. Close up of the bristle in **Fig. 11** depicting the teeth in the middle and distal end of the shaft. Fig. 13. Close up of a bristle foot. Scale bars: Figs 7, 9–10, 12, 2 µm.

Cysts are oval-shaped, range in size from 11.7–14.8 µm × 9.3–10.7 µm (n = 10), with a surface that lacks any prominent projecting secondary structures (Figs 14–15, 20–23). The cyst surface is usually smooth, but many specimens also possessed patches of randomly scattered siliceous deposits (arrow on the right, Fig. 21). The pore is circular with a mean diameter of 0.8 µm (n = 10), more or less flush with the outer surface, and surrounded by a shallow hyaline zone that is approximately 0.7 µm wide (Figs 16–17, 24). Although not present on all cysts, a very shallow collar can surround the pore and hyaline region (e.g. Fig. 17). The anterior approximately ¼ portion of the cyst up to and surrounding the collar is covered with a series of closely spaced holes, each with a thickened rim and a mean diameter of 0.14 µm (Figs 14–16, 20). The spaces between the holes can be smooth (e.g. Fig. 14), but often contain small siliceous deposits (e.g. Fig. 16). The linear distance that the holes extend along the cyst surface varies with respect to cyst circumference, with some areas of holes extending further than others. The series of holes penetrate the cyst wall and open to the inside (Fig. 19). Smaller groups of isolated holes, as well as single holes, can be found on more proximal regions of the cyst surface.

Many of the cysts uncovered in specific strata had scales still attached to, and aligned on, the surface (Figs 18, 20–25). Although some of the scales within the alignment were of the typical body scale morphology (Figs 21–25, arrows), the majority had a very different structure, representing a second type of body scale referred to herein as the fringe-type scale (e.g. Fig. 18). Like the normal scale type, these scales were quadrate-shaped with a similar posterior rim and base plate pore pattern. However, the fringe scales differed from the normal type in four significant ways. First, the fringe scales lacked the two submarginal ribs. Second, they lacked domes. Third, the scales have a large hole of varying diameter positioned just inside of the anterior edge close to where the dome would theoretically be



Figs 14–19. SEM images of *Mallomonas enigmata* cysts. **Figs 14–15**. Whole cysts lacking attached scales. Note the elongate- shape, dense series of small holes surrounding the cyst pore on the apical end, and the otherwise smooth nature of the cyst wall. **Figs 16–17**. Close ups of the apical pore and surrounding cluster of holes. Note the hyaline area surrounding the apical pore, the shallow collar (**Fig. 17**), and the thickenings surrounding each small hole. **Fig. 18**. Fringed scale type still attached to the surface of a cyst. **Fig. 19**. Internal view of a broken cyst illustrating that the cluster of small holes penetrate the cyst wall. Scale bars: Figs 14–15, 5 µm; Figs 16–19, 2 µm.

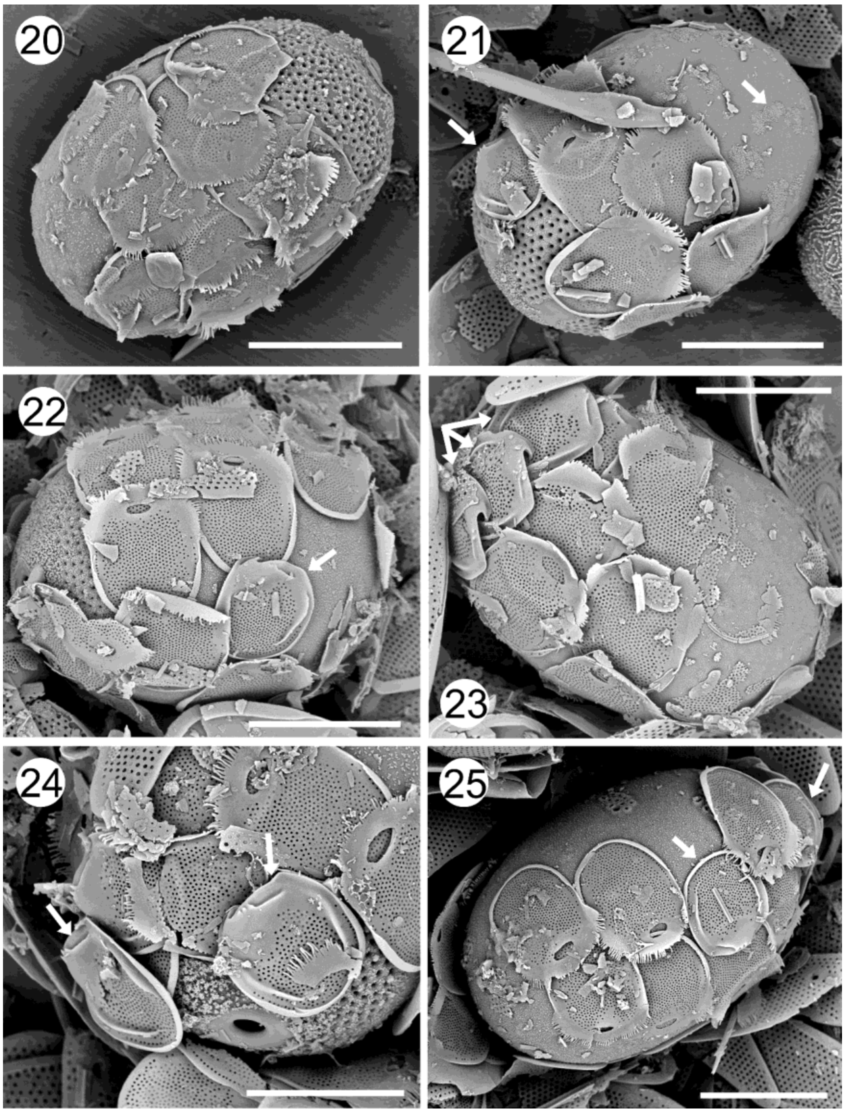
located (e.g. Fig. 18). Fourth, the anterior margin is lined with a series of closely spaced finger-like extensions of various lengths, resulting in a frayed fringe (e.g. Fig. 18).

HOLOTYPE SPECIMEN: Portion of a single gathering of cells on SEM stub deposited at the Canadian Museum of Nature, CANA 131536. Material from section 13-1-33 of the Giraffe Pipe core collected by P.A.Siver. Fig. 2 is a representative scale from the specimen. ETYMOLOGY: The name refers to the puzzling fact that the species produces a second type of body scale only found fused to the cyst wall.

DISTRIBUTION: *Mallomonas enigmata* was found in 10 strata over a 6.5 m laminated sequence in the Giraffe Pipe core (Table 1). This section of the core contained a high diversity of synurophyte species and eunotioid diatoms, and many strata harboured large populations of *Mallomonas lychenensis* Conrad, *M. porifera* Siver & Wolfe, and/or *Synura cronbergiae* Siver. *Mallomonas media* Siver & Lott, *M. ampla* Siver & Lott, and *M. preisigii* Siver were also common in more than one of these strata, while other synurophyte species were abundant in at least one of the samples (Table 1). Eunotioid diatoms, mostly species of *Eunotia* Ehrenberg and *Actinella* Lewis, were also common in this section of the core.

Discussion

Mallomonas enigmata is clearly most closely related to *M. nieringii*, a modern congener only known from a suite of small acidic ponds on the outer tip of the Cape Cod peninsula, Massachusetts, USA (Siver, 2001). In fact, the morphological features of the scales



Figs 20–25. SEM images of *Mallomonas enigmata* cysts with attached scales. **Fig. 20**. Cyst with attached fringed scales that are still neatly arranged on the wall. Note the fringed scales lack submarginal ribs. **Fig. 21**. Cyst with six fringed scales lacking submarginal ribs, and a typical body scale with a dome and submarginal ribs (arrow on the left). The arrow on the right depicts an area on the cyst wall where silica has been deposited. **Fig. 22**. Cyst with fringed scales lacking submarginal ribs, and each with a large apical pore, and one fully formed and typical body scale (arrow). **Fig. 23**. Cyst with both fringed and typical body scales (arrows) still precisely arranged on the cyst wall. The inverted U-shaped and shallow dome, and linear feature are clearly visible on the body scales. **Fig. 24**. Apical end of a cyst showing the apical pore and a portion of the cluster of surrounding small holes, and both fringed and typical body scales (arrows). **Fig. 25**. Cyst with both fringe and typical body scales (arrows) still attached in a neat overlapping pattern. Each fringed scale has a large apical hole. Scale bars: Figs 20–22, 25, 5 µm; Figs 23–24, 4 µm.

comparing the Eocene and modern taxa are strikingly similar. In both cases, the body scales share a similar overall shape, are of similar size, have a similar base plate pore pattern, and except for the pair of submarginal ribs lack additional secondary structure (Siver, 2001). In addition, scales of both species have shallow domes with an inverted U-shaped opening from which the bristles emerge. Two distinct differences were observed between the body scales. First, the submarginal ribs of *M. nieringii* are thicker, extend further along the length of the scale, and sometimes connect in the proximal region. Second, the linear feature on top of the dome is only slightly developed on *M. nieringii* scales as compared with those of *M. enigmata*. In addition to the body scales, the asymmetric shape and forward-projecting spine with terminal teeth of the apical scales are also similar in the two taxa. Lastly, both species have bristles that are three-ribbed and serrated. In conclusion, there is no doubt that *M. enigmata* belongs in the series Punctiferae within the section Punctiferae, and that the morphological features of the siliceous components exhibit evolutionary stasis with its modern congener over the last 48 Ma. A new species is proposed based on the differences found in the body scales and the fact it can produce a second scale type, coupled with the 48 Ma age difference.

Possessing a second scale morphotype only found attached to the cyst, in this case the scale type with the frayed anterior end and open hole of varying diameter, is indeed unique and not known for any modern or fossil *Mallomonas* species. Despite an extensive search of thousands of synurophyte scales of *M. enigmata*, including many specimens of the mature scale type, no isolated scales with frayed margins were found in any of the 10 strata containing the fossil taxon examined. In contrast, over 40 cysts with one to many frayed scales were uncovered indicating that this scale type is oddly only found still attached to the cyst. Because these samples were extensively acid cleaned under heat to remove organic material, and washed five or more times with distilled water, there is no doubt that the frayed scales are indeed fused to each other and to the cyst wall. Although cysts of other synurophyte taxa have also been found in the Giraffe Pipe locality with scales still attached (Siver, 2018, 2020), in each case the scales consisted of the same morphological type typical of those forming the outer cell covering. What makes the current case so different and intriguing is the fact that the majority of the fringed scales attached to the cysts have a uniquely different morphology from the typical body scales found on vegetative cells.

Cysts are produced inside an SDV within the parent cell that has a covering over the outer plasmalemma consisting of scales typical of the species (Siver, 1991; Kristiansen, 2005). Once the cyst is formed, the parent scale covering typically disarticulates and the isolated scales and bristles sink separately to the sediments, leaving the cyst without identifiable scales (Duff *et al*., 1995). Once the cyst is formed it would not be possible for it to produce scales and move them to its outer surface. Because it is extremely rare to find cysts with attached scales in modern collections, the abundant specimens found in this 48 Ma fossil locality are intriguing. Even more interesting is the question of how did the fossil cysts end up with scales of a different morphology?

A possible hypothesis for how the cysts ended up with a seemingly different scale type is as follows. Perhaps the frayed scales were in the process of being produced by the parent cell, each in an individual SDV and aligned along the inside of the cell membrane, when conditions, such as a change in temperature or nutrient content, arose that triggered cyst formation. The unfinished scales in various stages of development, including some which were close to or fully formed, were situated between the forming cyst SDV and the cell plasmalemma. Once the cyst was formed within the parent cell, the unfinished scales became fused to the outer cyst surface, while the mature scales and bristles of the parent cell covering disarticulated from the cell as is normally the case. Perhaps the chemical nature of the material within the scale SDVs and the cyst SDV, now in contact with each other, facilitated the ultimate fusion of the unfinished scales to the outer surface of the cyst. It is further interesting to note that most, and perhaps all, of the cysts with attached scales have deposits of siliceous material on the outer surface that presumably represent amorphous silica that was initially being used to build the unfinished scales (e.g. arrow on the right side, Fig. 21). These deposits were only observed on *M. enigmata* cysts, and not found on the isolated synurophyte scales and cyst types from other species that were abundant in the same samples. This indicates that the siliceous deposits found on the *M. enigmata* cysts were unique to these specimens and not deposited post cyst formation through other processes within the sediments or as a result of the cleaning procedure. In summary, it is clear that *M. enigmata* is capable of producing both scale types.

The first few specimens of the cyst that I uncovered only had the frayed scales attached, and my initial thinking was they represented a new and unidentified Eocene species. As I searched for additional frayed scales, I eventually found cysts with both frayed scales and typical body *M. enigmata* scales. The samples also contained numerous isolated and typical *M. enigmata* scales (e.g. Figs 1–6), but I never observed isolated frayed scales. Although both scale types present seemingly different morphologies, upon closer examination it is possible to align characteristics between both scale types. First, the basic shape of the base plate, the pattern of base plate pores, and details of the posterior rim are similar between the two scale types. Second, there are linear hyaline (missing pores) areas on the frayed scales that align with the position where the two elongated submarginal ribs of mature scales would be found. The hypothesis is that the secondary submarginal ribs had not yet been formed on the unfinished frayed scales. A few specimens did contain scales with weakly or partially formed submarginal ribs, and represent an in-between form. Third, the anterior hole on the frayed scales is closely aligned to the position where the dome cavity and exit point of the attached bristle would be found on a finished and typical body scale. Perhaps the frayed fingers, most likely points of deposition of amorphous silica, were in the process of forming the finished dome? On a number of frayed scales there is a faint crease that the scale margin appears to follow that is positioned to the inside of the fringe fingers, curves, and aligns with the large hole (e.g. Fig. 22). Could this faint crease represent the eventual margin on the fully formed scale, and the hole the space where the dome cavity will form? Perhaps the portion of the forming scale outside of the crease will eventually be folded under to strengthen this portion of the scale, leaving the linear indented feature and small marginal spines found on mature scales (arrows, Fig. 5).

Although the hypothesis proposed herein is that the frayed scales represent unfinished scales that were in the process of being fully formed when cyst formation was triggered, it may not be correct. However, this is the only known *Mallomonas* species with multiple and very different scale morphologies, and where one morphotype is only found attached to the cyst. It will be interesting to see if such a condition is uncovered for other species, modern or fossil, and if the function of the frayed finger-like projections can be definitively determined.

A review of the literature did not uncover any cyst morphotypes that even remotely match the cyst produced by *M. enigmata*. Several features of the *M. enigmata* cyst are especially noteworthy. First, the series of closely spaced holes that surround the collar/ pore and can cover ¼ or more of the cyst surface is unique among any modern or fossil cysts previously reported. Second, the asymmetry of the cluster of holes with respect to their position around the collar/pore. More typically, structures surrounding the pore/collar complex on chrysophyte cysts have a symmetric design (Duff *et al*., 1995; Wilkinson *et al*., 2001). Third, the fact that the clusters of holes penetrate the cyst wall also appears to be unique to this taxon. Except for the primary cyst pore, no other structures have been reported that penetrate the cyst wall. There are over 200 cyst morphotypes found throughout the extensive Eocene Giraffe Pipe core, and all but one other morphotype lack structures that penetrate the wall.

It is also interesting that the inferred conditions of the waterbody at the time period represented by the strata where *M. enigmata* was uncovered were closely aligned with those of waterbodies where *M. nieringii* is found today. Remains of *M. enigmata* were found over an approximately 6.5 m section of the Giraffe Pipe core representing what is referred to as the Eunotioid Zone (Siver & Lott, 2023). This zone, comprising organic-rich mudstones, harboured a wide diversity of synurophyte algae, eunotioid diatoms representing the genera *Eunotia* and *Actinella*, and species of *Oxyneis* (Ehrenberg) Round and was estimated to have covered thousands of years (Siver & Lott, 2023). A reconstruction based on microfossil remains inferred the waterbody as shallow, acidic, high in dissolved humic material, and low in nutrients. Interestingly, the shallow ponds harbouring *M. nieringii* are humic-stained, acidic, and low in dissolved salts and nutrients (Siver, 2001).

In summary, *M. enigmata* is an Eocene representative of the section Punctiferae that closely resembles the modern, but rare, taxon *M. nieringii*. The similarity of body scales between the two species displays a large degree of evolutionary stasis since the Eocene. It is also of interest that both species have been found under similar environmental conditions. What makes *M. enigmata* unique and especially intriguing is the fact that a second type of body scale was found only attached to the cyst wall, and presumably not on swimming vegetative cells. Such a condition has never been reported for species of *Mallomonas* and the function of the second scale morphotype is unknown.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Author contributions

P.A. Siver: performed all of the analyses associated with this manuscript, including microscopy, identifications and writing the manuscript.

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ORCID

Peter A. Siver http://orcid.org/0000-0001-5956-8889

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