

ORIGINAL PAPER

Euglyphid Testate Amoebae (Rhizaria: Euglyphida) from an Arctic Eocene Waterbody: Evidence of Evolutionary Stasis in Plate Morphology For Over 40 Million Years

Andrew Barber, Peter A. Siver¹, and William Karis

Department of Botany, Program in Environmental Studies, Connecticut College, New London, CT 06320, USA

Submitted December 24, 2012; Accepted May 5, 2013
Monitoring Editor: Genoveva Esteban

Well-preserved siliceous plates representing multiple species of euglyphids are described from a Middle Eocene maar lake deposit located near the Arctic Circle in northern Canada. Siliceous plate morphotypes including scutiform, rectangular, hexagonal, oval and circular body forms, six apertural plate types containing from five to thirteen teeth and spine plates, are documented. Many plate types bear resemblance to those found on modern species as well as ones documented from Miocene sites. These findings extend the known geological record for euglyphids and support the concept of evolutionary stasis in regards to plate morphology over much of the Cenozoic. Future use of these euglyphid remains, in conjunction with other microfossil assemblages, for reconstructing historical conditions within the maar lake is discussed.

© 2013 Elsevier GmbH. All rights reserved.

Key words: Arctic; Eocene; *Euglypha*; evolutionary stasis; *Scutiglypha*; testate amoebae.

Introduction

Testate amoebae are heterotrophic protists encased in a protective structure known as the test (Wall et al. 2010; Wanner 1999). The test is produced of organic (protein) or inorganic (e.g. silica) components and consists of a single continuous covering, an agglutination of acquired organic and inorganic components, or a series of overlapping plates that are manufactured within, and precisely arranged around, the cell (Charman 2001; Hedley and Ogden 1974). As a group, testates are cosmopolitan, play important roles in microbial food and energy webs as consumers of

bacteria, algae, other protists and detritus (Mitchell et al. 2008; Wall et al. 2010), and inhabit a myriad of terrestrial and aquatic habitats, including organic-rich soils, moss beds, lakes, bogs, wetlands and peatlands (Medioli and Scott 1983; Mitchell et al. 2008; Ogden and Hedley 1980; Patterson et al. 2002). Many testates exhibit specific responses to environmental gradients such as pH, nutrient levels and moisture content, and as such inhabit discrete ecological niches (Booth and Zygmunt 2005). These responses, coupled with high reproductive rates, make testates sensitive bioindicators of environmental change (Heal 1964; Ogden 1981; Schönborn 1992; Charman 2001). In addition, the remains of the tests become archived in sediments making them useful for reconstructing historical conditions (Charman 2001; Warner 1990), such as

¹Corresponding author;
e-mail pasiv@conncoll.edu (P.A. Siver).

paleoclimates (Booth 2001, 2002, 2008; Charman 2001), the effects of land-use alterations (Patterson et al. 2002) and sea-level change (Gehrels et al. 2001).

Testate amoebae that bear lobopodia or filopodia are classified within the Amoebozoa or Rhizaria, respectively (Adl et al. 2012; Lahr et al. 2011). Within the Amoebozoa, the Arcellinida include organisms possessing an organic or mineralized test with a single opening, the pseudostome. Testate amoebae with filopodia that secrete siliceous plates, and bind them together with an organic cement to construct the test, are classified in the Euglyphida (Adl et al. 2012). The Euglyphida are, in turn, divided into five families, including the Euglyphidae, Trinematidae and Assulinidae that are largely freshwater or terrestrial, and the Cyphoderiidae and Paulinellidae that have freshwater and marine members (Adl et al. 2012; Heger et al. 2010; Meisterfeld 2002). Based on the SSU rDNA gene, the Euglyphida comprise a well-defined monophyletic clade (Heger et al. 2010; Wylezich et al. 2002).

The taxonomy of the Euglyphida is based primarily on characteristics of the test and siliceous plates (Foissner and Schiller 2001; Meisterfeld 2002). Species of *Euglypha* and *Scutiglypha*, two genera that are the primary focus of our study, are encased in siliceous tests composed of between 80 and 300 body plates and 5 to 15 apertural plates, and with some taxa bearing spine plates (De Smet and Gibson 2009; Foissner and Schiller 2001; Hedley and Ogden 1974; Meisterfeld 2002). Plates are highly ordered and precisely arranged on the test, with specific apertural plates surrounding the pseudostome, and body plates forming the majority of the structure. Body-plate morphotypes of Euglyphida include circular, elliptical, hexagonal, scutiform and crenulate forms, and the number of teeth on denticulate apertural plates can range from 3 to 13 (Ogden and Hedley 1980; Ogden 1981; De Smet and Gibson 2009). Body and apertural plates range in size from 4–16 μm \times 2.5–11 μm , with a limited number of species having spine-bearing body plates measuring up to 30 μm in length.

Documentation of testate amoebae in the fossil record, a topic reviewed by Schmidt et al. (2004) and Vincent (2012), is poor considering the antiquity of the group. The oldest known testate amoebae are of vase-shaped microfossils (VSMs) reported by Porter and Knoll (2000) and Porter et al. (2003) from Neoproterozoic rocks of marine origin (Chuar Group, \sim 750–700 Ma). The Chuar deposit contains a large number of taxa, representing at least five families of testate amoebae. In addition,

Bosak et al. (2011) described agglutinated tests, possibly representative of testate amoebae belonging to the Arcellinida, in Cryogenian deposits from Namibia and Mongolia (750–635 Ma), and Corsetti et al. (2003) uncovered probable testate amoebae specimens from the Kingston Peak Formation (750–700 Ma). Other than these Neoproterozoic fossils, that most likely represent lobose arcellinids (Berney and Pawlowski 2006), the geologic record for testate amoebae is sparse until the Mesozoic where numerous freshwater forms have been described largely from amber (e.g. Poinar et al. 1993; Schmidt et al. 2004, 2006; Schönborn et al. 1999; Vincent 2012; Waggoner 1996a).

According to Schmidt et al. (2004), van Hengstum et al. (2007) and Vincent (2012), the oldest definitive records of freshwater testate amoebae bearing siliceous plates are from the Middle Eocene (Loeblich and Tappan 1964; Schiller 1999) and near the Eocene-Oligocene boundary (Schiller 1998; Waggoner 1996b). Except for the microfossils uncovered by Waggoner (1996b) belonging to the family Cyphoderiidae, the other records from these deposits represent taxa from the family Euglyphidae. Exceptionally preserved siliceous plates representing the genus *Scutiglypha*, in the family Euglyphidae, are known from the Middle Miocene (Foissner and Schiller 2001; Williams 1985), and Beouf and Gilbert (1997) documented *Trinema linare* Penard from 3 Ma Pliocene deposits.

The primary purpose of this work is to document multiple, well-preserved, euglyphid species from a Middle Eocene maar lake deposit, referred to herein as the Giraffe Pipe, that was situated near the Arctic Circle in northern Canada during a very warm period in geologic history known as the Cenozoic hot house. Evolutionary stasis with regards to plate morphology within the family Euglyphidae, the relationships of the organisms to modern species, and the potential use of the microfossils for reconstructing historical conditions, are discussed.

Results

Site description. The Giraffe Pipe locality (64°44' N, 109°45' W) is a kimberlite diatreme that was emplaced into the Slave Craton in the Northwest Territories of Canada approximately 47.8 million years ago during the Middle Eocene (Siver and Wolfe 2005; Wolfe et al. 2006). The diatreme crater subsequently filled with water becoming a maar lake, which slowly infilled with a sequence of lacustrine, then paludal sediments, and was later capped

by a thick layer of Neogene glacial deposits (Siver and Wolfe 2005; Wolfe et al. 2006). The Giraffe Pipe is one of many kimberlites in the Lac de Gras field, most of which have Cretaceous or Paleogene emplacement ages (Heaman et al. 2004). A 163 m drilled core was uncovered from the Giraffe locality in 1999 by BHP Billiton Inc. (Siver and Wolfe 2009). A total of 113.1 m of the core contained well preserved stratified organic material, including 68.3 m of lacustrine lake mudstones, overlain with 44.8 m of peaty material. Within the 163 m core, the lacustrine remains range from near the bottom to a depth of approximately 95 m. An air-fall tephra bed located near the transition between lake and terrestrial sediments was dated using fission tracking to be 40 Ma (Doria et al. 2011), indicating that all of the lake sediments are Eocene in nature. We envisage that, after phreatomagmatic kimberlite emplacement, a waterbody formed within the deep crater that persisted for several million years before transitioning to a terrestrial environment.

Numerous rock samples taken from regular intervals throughout the 68.3 m long lacustrine phase have been fractured and the internal surfaces examined directly with SEM. The majority of these samples illustrate vast numbers of siliceous microfossils entombed within the rocks, including scales, cysts and bristles of chrysophytes (Siver et al. 2009), diatoms (Wolfe et al. 2006), sponges (Pisera et al. 2013), heliozoans, and with this communication, euglyphid testate amoebae. Three examples of rocks from the lacustrine phase, along with corresponding images of the fractured surfaces that illustrate euglyphid remains, are given in Figure 1.

Microfossils representing remains of chrysophyte algae dominate most sections of the Giraffe Pipe core, with 33.4% and 33.1% belonging to scales and cysts, respectively (Table 1). Other than chrysophyte remains, testate amoebae plates belonging to the Euglyphida were common within the core, found in 91 of the 100 sections enumerated for microfossil remains. On a relative basis, euglyphid plates accounted for 9.2% of all microfossils uncovered in the core (Table 1), and the highest concentrations were consistently found in the lower 1/3 of the lacustrine phase (162.5 m to 135.5 m) and near the end of the lacustrine phase just prior to formation of a terrestrial landscape (108.5 m to 95 m) (Fig. 2). Testates accounted for over 30% of the microfossils in nine of the 100 samples enumerated. In the lower sections of the lacustrine phase, testate scales were most commonly associated with chrysophyte cysts, sponge spicules and several species representing the chrysophyte genus *Mallomonas*. At the end of the lake phase, high

Table 1. Relative percentages of 11 categories of microfossils enumerated in 100 sections of the Giraffe Pipe core. Estimates of the numbers of different microfossil types included in each category are given. Raphe-bearing diatoms are divided into eunotioid diatoms and all others (noted below as raphid diatoms). The testates are represented by the 11 morphotypes listed in Table 2.

Microfossil category	Estimated # microfossil types	Relative %
Sponge megasclerids	15	1.9
Sponge gemmusclerids	15	0.05
Chrysophyte cysts	100	33.1
Chrysophyte scales	41	33.4
Centric diatoms	10	6.7
Araphid diatoms	5	0.8
Eunotioid diatoms	15	2.5
Raphid diatoms	3	0.8
Testate euglyphids	11	9.2
Heliozoans	3	6.2
Unknown protists	5	5.4

concentrations of euglyphids were again associated with chrysophyte cysts, and sponge remains, along with chrysophyte scales and heliozoan microfossils.

Euglyphid plates uncovered from the Giraffe core were grouped by morphotype, including two scutiform-shaped plate types with crenulate oral (part facing the pseudostome) and aboral margins, circular to oval plates, square to rectangular plates, hexagonal plates, plates with spines and five denticulate forms (Table 2; Figs 3–7). Scutiform, circular to oval, and denticulate plates were the most common morphotypes found in many of the samples examined throughout the core. Hexagonal, square and rectangular plate types were rare, and the latter two forms were found exclusively in the middle sections of the core.

Scutiform plates are shield-shaped plates where the wider or aboral end faces the posterior of the test, and the narrower oral end is oriented towards the pseudostome (Figs 3–5). Plates with a scutiform shape were divided into two morphotypes based on the degree of undulation along the oral and aboral margins. The first type, morphotype 1, has two indentations along each end of the plate that form undulating margins consisting of three projections or processes (Fig. 4). The undulations are well developed and the two outer processes are broadly rounded and extend from the margin the same distance or slightly less than the central

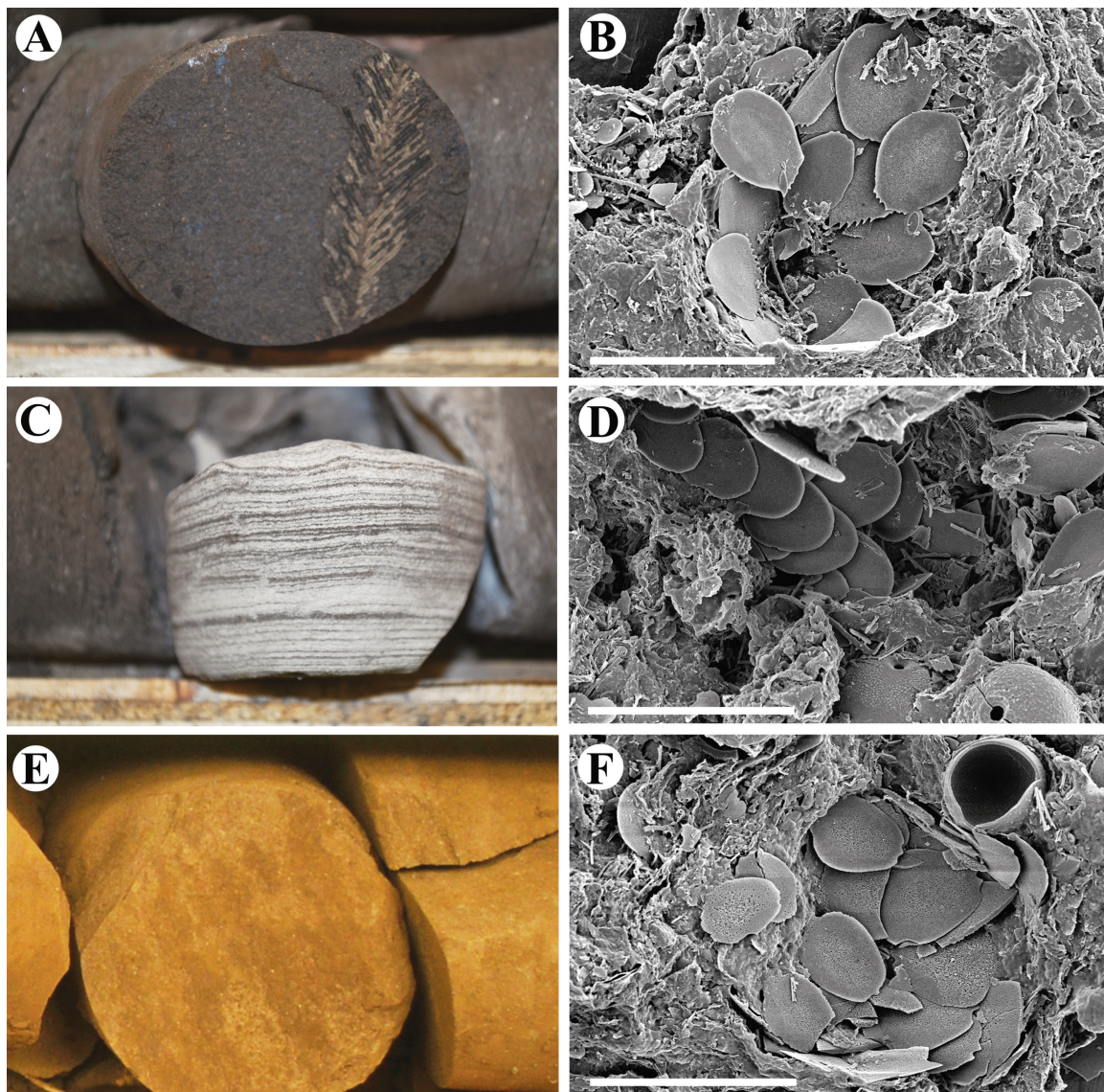


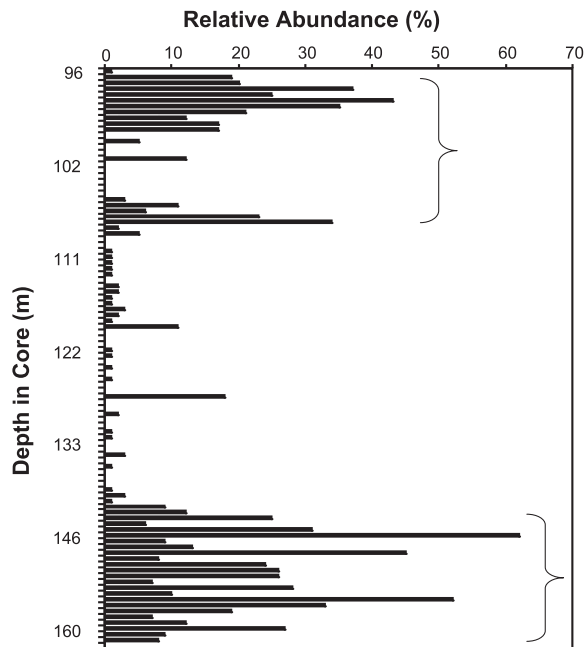
Figure 1. Three rock types from the Giraffe core representing different sections of the lacustrine phase (**A**, **C**, **E**), and corresponding images along fractured surfaces showing the remains of euglyphid testate amoebae (**B**, **D**, **F**). The remains in **B** may represent the pseudostome end of the test. Note the overlapping pattern of the plates in **D**. Chrysophyte cysts can be observed in **D** (two cysts with pores in the lower right) and in **F** (broken cyst in upper right).

projection, yielding an outline that resembles that of a butterfly (Fig. 4). The central projection is rounded on the aboral margin, but often pointed on the oral end. The sides of the plate that connect the undulated ends are straight, yielding a bilateral symmetry. The undulations on morphotype 2 are similar along the oral margin, but the outer projections are less pronounced or lacking altogether along the aboral margin (Fig. 5B, D, F). The central projection on the aboral margin is usually well formed, rounded, and extended from the margin

(Fig. 5D). Morphotype 2 plates also have a bilateral symmetry, but the sides are more rounded than those on morphotype 1, forming a shape that resembles a lemon. Some morphotype 2 plates have additional indentations along the oral margin forming more than three projections; this plate type appears to be a transitional form between denticulate and body plates. The ranges in length and width of plates for both scutiform morphotypes were similar, with mean values of $10.4 \times 8.1 \mu\text{m}$ and $10.6 \times 7.6 \mu\text{m}$ for morphotypes 1 and 2, respectively

Table 2. Mean, minimum and maximum sizes of 11 fossil euglyphid plate morphotypes uncovered from the Giraffe Pipe locality.

Plate Morphotype	Mean		Minimum		Maximum	
	Length (μm)	Width (μm)	Length (μm)	Width (μm)	Length (μm)	Width (μm)
Scutiform type 1	10.4	8.1	5.0	4.0	14.5	11.5
Scutiform type 2	10.6	7.6	5.5	4.0	16.0	11.5
Round/Oval	9.6	7.9	4.0	3.5	15.5	15.5
Rectangular	11	9.5	10.1	8	12.0	11.1
Hexagonal	9.9	6.1	6.5	4.0	18.0	12.0
Spine-bearing	18.7	7.8	12.5	10.0	28.5	10.0
All denticulate plates	11.7	8.0	7.5	6.0	16.0	10.0
5-toothed denticulate	12.5	7.5	10.0	7.0	15.0	8.0
7-toothed denticulate	10.7	7.6	7.5	6.0	15.0	10.0
9-toothed denticulate	11.7	8.0	9.0	6.5	16.0	10.0
11-toothed denticulate	12.5	8.6	11.0	7.0	13.0	10.0
13-toothed denticulate	12	8.4	10	7.0	12.9	9.9

**Figure 2.** The abundances of testate amoebae plates relative to all microfossils, including chrysophyte scales and cysts, diatom valves, sponge spicules and heliozoan scales, in the Giraffe Pipe core versus depth in the core. Testates were more abundant in the lower-most and upper-most sections of the core. Values are as relative percentages, and the brackets indicate regions of the core where testates accounted for more than 30% of all microfossils.

(Table 2). Scutiform plates were the most abundant euglyphid plate type found throughout the core with highest concentrations occurring in the upper sections of the lacustrine phase at 95 to 108 m, and near the bottom of the core between 135 to 158 m.

Plates with teeth aligning the oral or apertural edge, referred to as denticulate plates, were uncovered throughout the core and largely in association with scutiform-shaped body plates. Denticulate plates with 5, 7, 9, 11 and 13 teeth were found (Fig. 6), with plates containing 7, 9 or 11 teeth being the most common morphotypes and those with 5 or 13 teeth much rarer. All denticulate plates were bilaterally symmetric, with one larger apical tooth and even numbers of smaller teeth along each margin (Fig. 6). On the vast majority of denticulate plates, the teeth are extensions of the margin of the plate and are of similar thickness as the body of the plate. A much rarer type of denticulate plate consists of five thickened teeth that originate from below the plate margin (Fig. 7D). As a group, denticulate plates had a mean size of $11.7 \mu\text{m} \times 8.0 \mu\text{m}$ with a large degree of overlap between plates with different numbers of teeth (Table 2). Small numbers of plates with spines were consistently found and always in samples containing scutiform morphotype 1 plates (Fig. 5A, C, E). In all cases, the spines were broad, and protruded from the aboral margin. Spine plates ranged in size from $12.5\text{--}28.5 \mu\text{m} \times 6\text{--}10 \mu\text{m}$, with a mean size of $18.7 \mu\text{m} \times 7.8 \mu\text{m}$ (Table 2).

Many sections of the core contained oval to circular plates (Fig. 7A, B), the former being much more common with a mean size of $9.6 \mu\text{m} \times 7.9 \mu\text{m}$. Rectangular (Fig. 7C) and hexagonal (Fig. 7D) plates

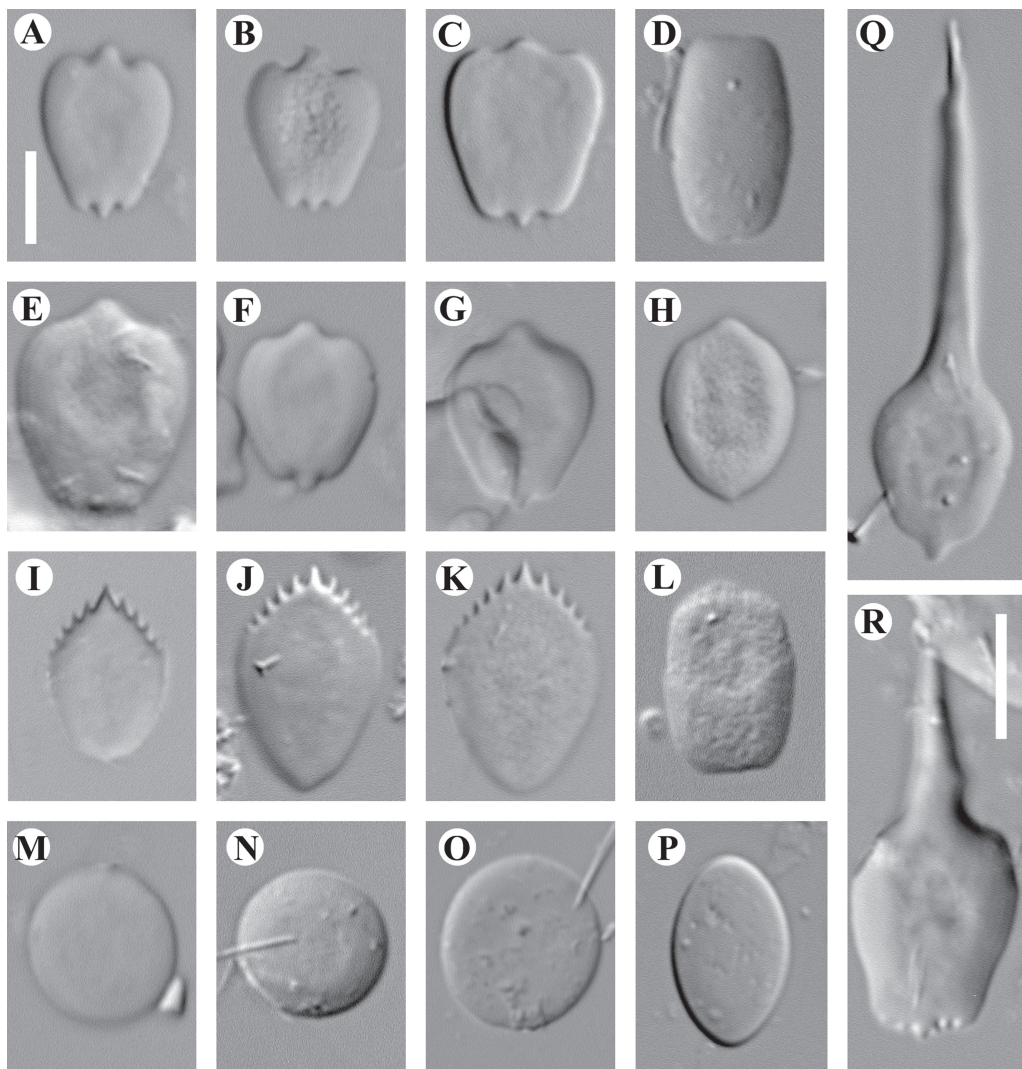


Figure 3. Light micrographs of the most abundant testate amoebae plate types uncovered in the Giraffe Pipe core. **A-C)** scutiform morphotype 1; **D)** hexagonal plate; **E-H)** scutiform morphotype 2; **I-K)** denticulate plates; **L)** rectangular plate; **M-N)** circular plates; **O-P)** oval plates and; **Q-R)** spined plates. Scale bars = 5 μm . The bar on A is for all images except R.

were also present, but much less abundant than other morphotypes. Hexagonal plates were associated with the denticulate plate type with thick teeth originating from below the margin (Fig. 7D). Rectangular plates had a mean size of 11 μm x 9.5 μm , and were mostly found in the middle of the lacustrine phase at 108–140 m depths. Hexagonal plates, with a mean size of 9.9 μm x 6.1 μm , were found in low concentrations at depths of 99.5 m to 108 m, 118 m to 122 m, and 149 m to 153.5 m.

It was not uncommon for euglyphid plates to contain pitted areas most often situated in the center of the structure (e.g. Fig. 4B, C). This pattern was observed on all scale types, but not observed on all specimens.

Discussion

Vincent (2012) noted that the tests of numerous specimens discovered in Mesozoic amber deposits were morphologically similar, if not identical, to modern species, and represented extensive evolutionary stasis. Schmidt et al. (2006) uncovered specimens in Triassic amber from Italy that they assigned to the modern taxon *Centropyxis hirsuta* Deflandre. Since most of the Cretaceous specimens of testate amoebae found in Schliersee amber are also thought to represent extant taxa, they too demonstrate long-term stasis (Vincent 2012). Evolutionary stasis of morphological forms for testate amoebae was further documented by

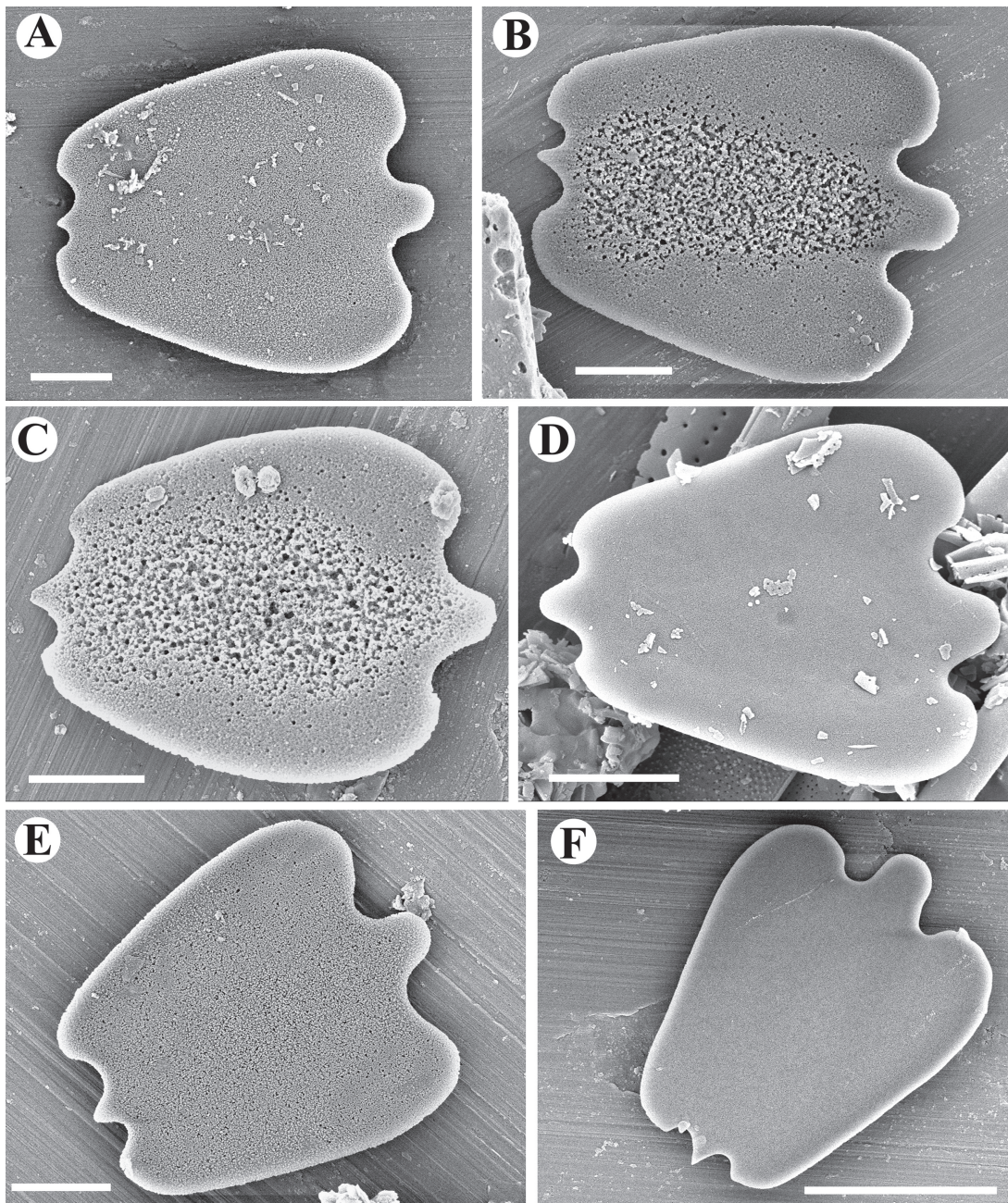


Figure 4. Scanning electron micrographs of plates representing scutiform morphotype 1 in the genus *Scutiglypha* (A-F). Note the larger undulations along the aboral margin (on the right), the smaller undulations along the oral margin (on the left), and the overall butterfly-shape of the plates. Plates in B and C show pitting in the central region. Scale bars = 2 μm .

van Hengstum et al. (2007), who reported seven modern arcellinids in Cretaceous clay-silt deposits from Nebraska, U.S.A.

Remains of euglyphids from Cenozoic deposits, especially of the siliceous plates, also suggest long-term evolutionary stasis. Foissner and Schiller (2001) documented well preserved whole tests

containing scutiform-shaped plates from a middle Miocene volcanic crater lake in Germany approximately 15 Ma old. The specimens represented two distinct species and these authors made a convincing argument that they match the modern species, *S. crenulata* and *S. scutigera*. This means that the shapes of the siliceous plates for these

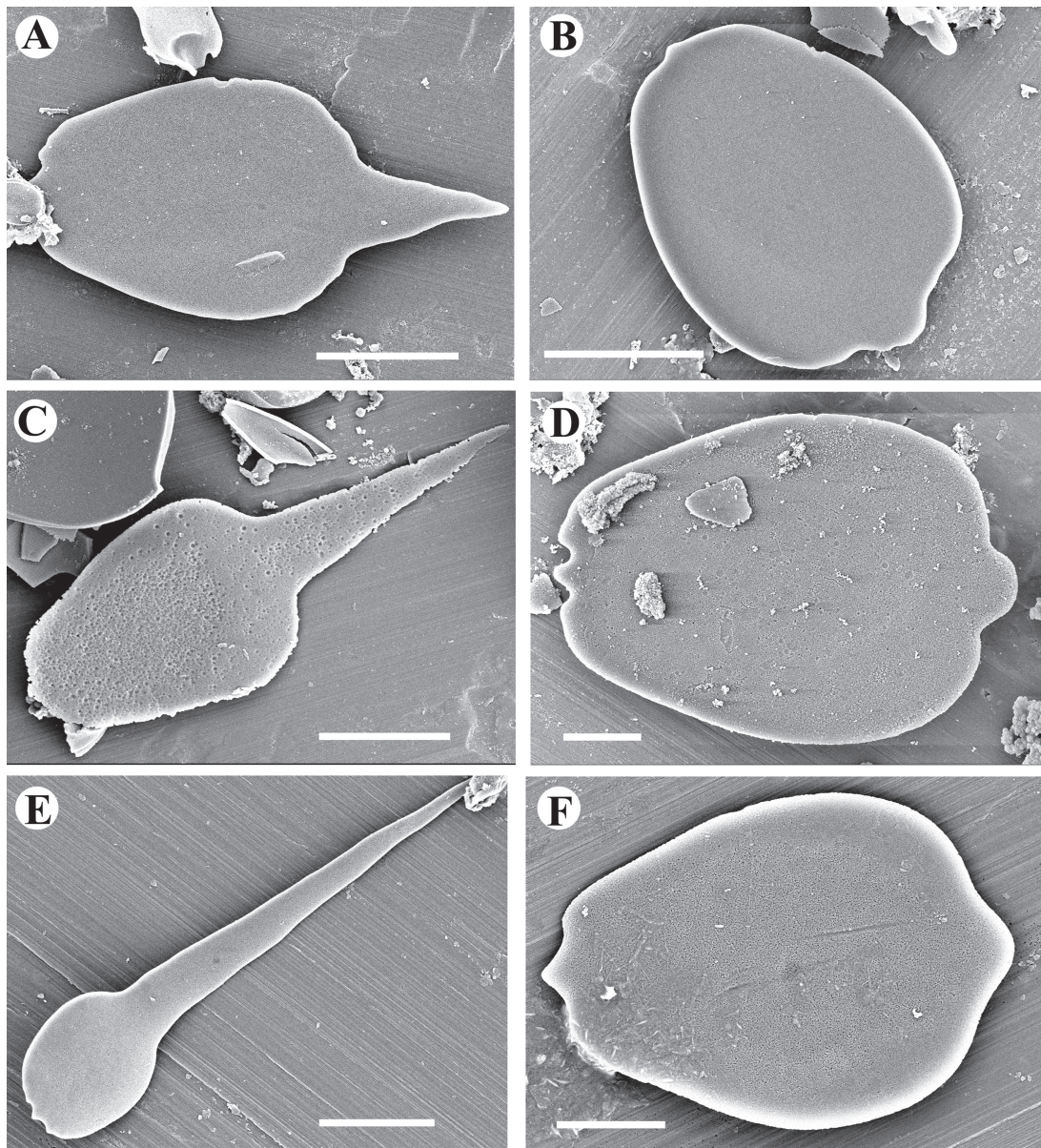


Figure 5. Scanning electron micrographs of spine-bearing plates (**A, C, E**) and scutiform morphotype 2 plates (**B, D, F**) representing the genus *Scutiglypha*. Note that the longer the spine length, typically the smaller is the base plate. The undulations along both the aboral and oral margins on the scutiform scales are much less pronounced than on morphotype 1. Scale bars = 2 μm (D, F) and 5 μm (A-C, E).

scutiform taxa have remained virtually unchanged for at least 15 Ma. Remains of euglyphid plates from the Miocene Clarkia fossil beds in northern Idaho, U.S.A. (Williams 1985), support the conclusions of Foissner and Schiller (2001). Our findings from the Giraffe locality significantly extend those of Foissner and Schiller (2001) and Williams (1985) to the Middle Eocene. The two scutiform taxa represented in the Giraffe core also match those of the modern species *S. crenulata* and *S. scutigera*, and

as concluded by Foissner and Schiller (2001), we have no reason to believe they are not the same taxa. Thus, evolutionary stasis of these two morphospecies with scutiform-shaped plates can be extended to at least 40 Ma, far beyond the Middle Miocene, and given the precise match in shape it is likely that these morphotypes are considerably older. In fact, with the discovery of testate amoebae in Neoproterozoic rocks (Porter and Knoll 2000), some of which may represent the Euglyphidae, it is

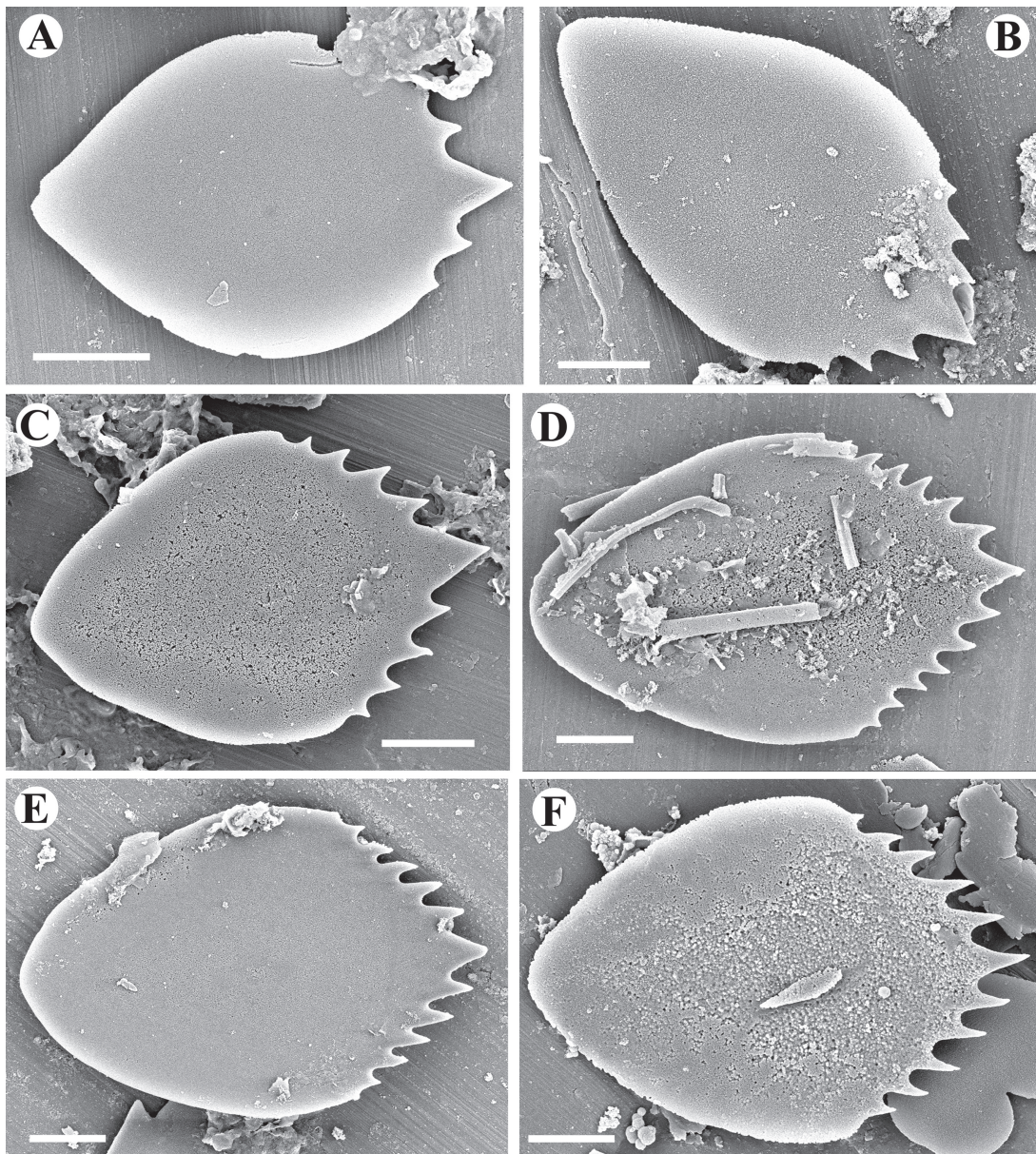


Figure 6. Scanning electron micrographs of denticulate plates with five (A), 7 (B), 9 (C), 11 (E-F) and 13 (D) teeth. Plates are believed to represent the genus *Scutiglypha*. Scale bars = 2 μm .

likely that evolutionary stasis of siliceous plates is much older.

Although the mechanisms resulting in evolutionary stasis of morphological structures in testate amoebae remain unclear, Schmidt et al. (2010) proposed convergent evolution and/or a predominance of asexual reproduction as potential agents driving this process. Evolutionary stasis would be perpetuated in protists with rapid rates of asexual reproduction coupled with large population sizes, traits often assigned to testate amoebae. However, Lahr et al. (2011) presented evidence

that at least some testate lineages are ancestrally sexual, suggesting that the predominance of asexual reproduction may not be a mechanism promoting stasis. It is also possible that a common morphology (e.g. plate structure) may mask or hide genetically distinct (cryptic) species. For example, extensive genetic diversity was found in several synurophycean algal species bearing similar siliceous scale structures (Siver et al. 2013; Skaloud et al. 2012), and it is possible that cryptic species with similar morphological structures will be uncovered within testate amoebae.

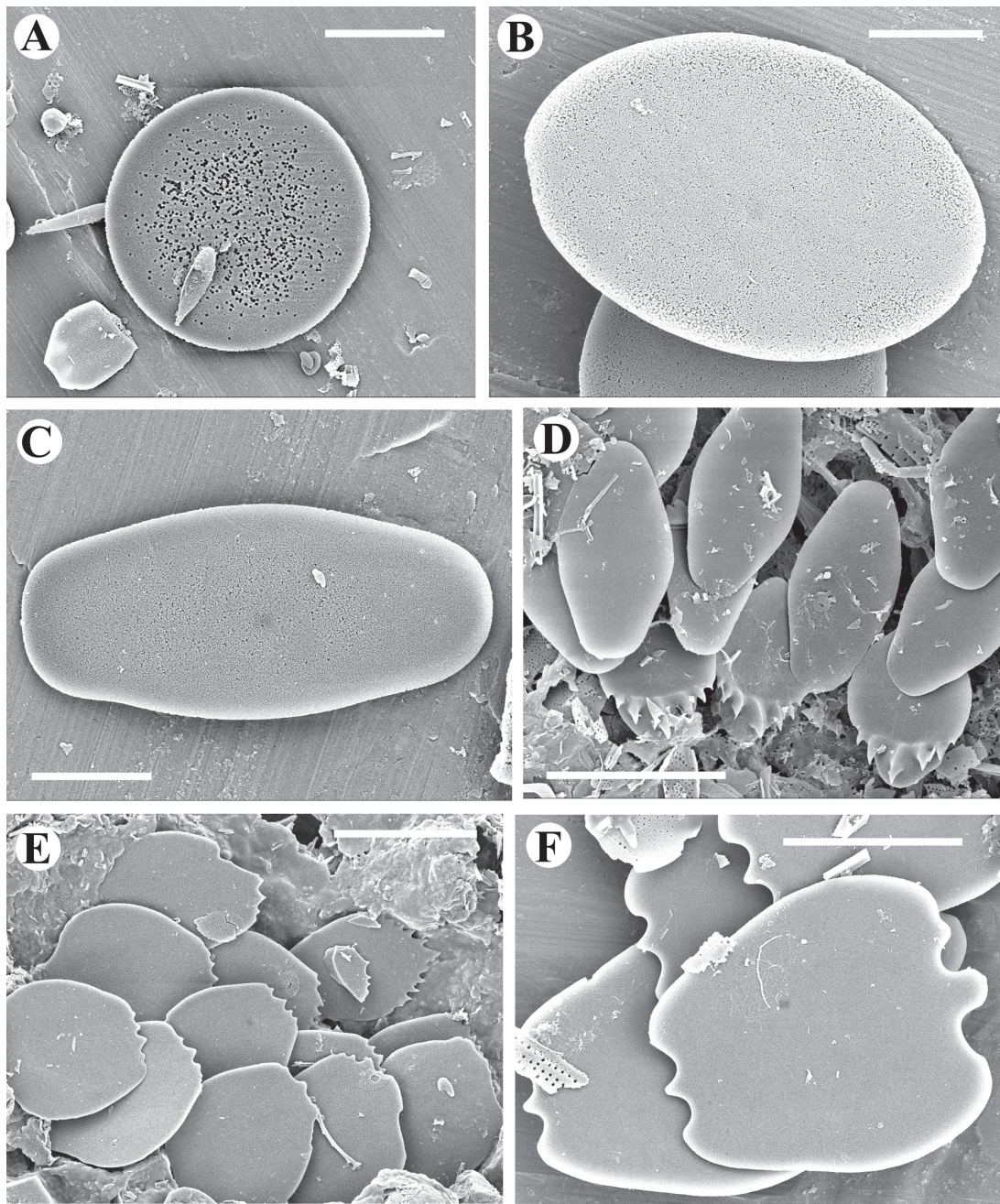


Figure 7. Scanning electron micrographs of circular (A), oval (B), rectangular (C), hexagonal (D), scutiform morphotype 2 (E), and scutiform morphotype 1 (F) plates. D-E represent direct observations from fractured mudstone surfaces where the overlapping nature of scales can still be resolved. Note the denticulate plates with thick teeth originating from below the margin associated with the hexagonal plates (D). Scale bars = 2 μm (B-C), 5 μm (A, D, F) and 10 μm (E).

Until recently, taxa in the order Euglyphida with scutiform-shaped plates were placed in the genus *Euglypha* (Ogden 1981; Ogden and Hedley 1980). However, Foissner and Schiller (2001) made the argument, supported by De Smet and Gibson (2009), that the shape and morphology

of test plates was a very stable character and proposed a new genus, *Scutiglypha*, for species with scutiform-shaped plates. Use of silica-plate characters to distinguish between euglyphid taxa was further supported by Wylezich et al. (2002) and Meisterfeld (2002). Based on this concept,

Foissner and Schiller (2001) transferred a number of species into *Scutiglypha*, including *S. crenulata* (formerly *Euglypha crenulata* Wailes and sometimes identified as *E. acanthophora* Ehrenberg) and *S. scutigera* (formally *Euglypha scutigera* Penard). Tests with scutiform-shaped plates matching these *Scutiglypha* species have also been identified in the literature as *E. acanthophora* (e.g. Ogden 1981; Wylezich et al. 2002), which Foissner and Schiller (2001) claim represent misidentifications.

We have uncovered quite a few partial tests of the scutiform taxa, especially morphotype 2, in the Giraffe core to be able to conclude that the geometric pattern formed by the overlapping of plates in the Eocene specimens matches that observed on modern specimens, and that the rows of plates surrounding the pseudostome end of the cell have teeth. It is important to note that since many of our specimens represent individual plates we cannot always be certain that they represent two different species. However, even though both types of scutiform plates were often found in the same samples, there were enough samples that contained only one of the morphotypes, to suggest that they indeed represent different species. In addition to scutiform body plates, we also uncovered denticulate aperture plates containing the same number of teeth typically found on modern specimens of *S. crenulata* and *S. scutigera*, and body plates with extended spines similar to those found on *S. crenulata* (Foissner and Schiller 2001; Ogden and Hedley 1980). Lastly, the size of scutiform-shaped plates uncovered from the Giraffe core are similar to those reported on modern species (Foissner and Schiller 2001; Ogden 1981; Ogden and Hedley 1980).

Denticulate plates are commonly associated with species in the genera *Euglypha* and *Scutiglypha*, where they are found in two or more rows surrounding the pseudostome (De Smet and Gibson 2009; Foissner and Schiller 2001; Ogden 1981; Ogden and Hedley 1980; Wylezich et al. 2002). The toothed margins of the overlapping denticulate plates align the pseudostome opening forming a zagged margin from which the pseudopodia emerge. In the Giraffe core, denticulate plates were routinely found with scutiform plates and we surmise that these plate types represent the same taxa. The shape, size, number of teeth and origin of the teeth as extensions of the apical margin all match nicely modern denticulate scales from *Scutiglypha crenulata* and *S. scutigera*. *Scutiglypha crenulata* and *S. scutigera* typically have denticulate plates bearing 7 to 9, and 9 to 11 teeth, respectively (Foissner and Schiller 2001; Ogden and Hedley 1980), matching the majority of those

uncovered in the Eocene core. In addition to denticulate plates, spine-bearing plates were also found with scutiform plates and although we cannot be absolutely certain, it is likely they belong to the *S. crenulata* morphotype. According to Foissner and Schiller (2001), *S. crenulata* bear 2 to 6 spine plates per test that range in length from 20 to 50 μm , while other species of *Scutiglypha* lack such plates. The majority of spine scales found in the Giraffe core were on the lower end of this size range, but always uncovered with scutiform-shaped plates.

Most modern *Euglypha* have denticulate scales that differ slightly from those observed on *Scutiglypha* species in that the apical-most tooth is much larger and thicker than the lateral teeth, the teeth originate from below the plate margin, and the number of teeth is usually closer to five (Ogden 1981; Ogden and Hedley 1980). This type of denticulate plate, also uncovered in Giraffe, is found on many modern specimens of *Euglypha* including *E. rotunda* Wailes, *E. strigosa* Ehrenberg, *E. compressa* Carter and *E. cristata* Leidy. Although not noted in the original description of *Scutiglypha* given by Foissner and Schiller (2001), this difference in morphology of denticulate plates may serve as another potential character for distinguishing *Scutiglypha* from *Euglypha*. Denticulate plates with thickened teeth originating below the margin were rare in the Giraffe core and only found in association with hexagonal plates, giving us further reason to believe that the more abundant type of denticulate plate indeed represents *Scutiglypha* species.

Identifying species from isolated circular, oval, hexagonal or rectangular plates is problematic since the taxa containing these types of plates were originally described using characters of the test and not the plates. *Euglypha filifera* Penard (sensu Ogden and Hedley 1980), *E. mucronata* Leidy, and *E. tuberculata* Dujardin all have oval plate scales that are similar in size to Giraffe specimens. Of these species, *E. tuberculata* has denticulate plates that resemble the more common type uncovered from Giraffe sediments, while the denticulate plates bearing thickened teeth are characteristic of *E. filiform* and *E. mucronata*. Oval to circular plates are also produced by species in the genera *Assulina*, *Sphenoderia*, *Tracheleuglypha* and *Trinema* (Meisterfeld 2002; Ogden and Hedley 1980), and it is possible that some of the plates represent species from these genera. Lastly, we cannot rule out the possibility that some of the oval plates represent those found on the posterior-most region of *Scutiglypha* tests (De Smet and Gibson 2009; Foissner and Schiller 2001). For example, De Smet and Gibson (2009) documented that the

scutiform-shaped body plates of *Scutiglypha cabro-lae* transitioned to smaller ellipsoidal-shaped plates along the posterior end of the test. Rectangular-shaped siliceous plates similar to those uncovered in Giraffe are produced by *E. strigosa* Ehrenberg (Ogden and Hedley 1980).

Using similar reasoning as they did regarding species with scutiform plates, Foissner and Schiller (2001) suggested that euglyphids with hexagonal-shaped plates also be placed into a separate genus. Hexagonal plates uncovered in Giraffe are similar to those found on *Euglypha compressa*, *E. filifera* (sensu Wylezich et al. 2002), and possibly those on *E. rotunda*. All three species have denticulate plates with thickened teeth originating from the under-surface of the plate as we observed on Giraffe specimens. *Euglypha compressa* and *E. filifera* also have slender siliceous spines. Although we uncovered slender siliceous spines in Giraffe of unknown origin, we are not certain that these spines represent euglyphids.

Some euglyphid plates from the Giraffe core were observed to contain pits, mostly situated in the central portion of the structure. This same type of pit patterning can be observed on living specimens, plates found in surface (recent) lake sediments (Douglas and Smol 1987), and on Miocene specimens illustrated by Williams (1985) and Foissner and Schiller (2001). It is not clear if these pits represent a deterioration of the silica, or are simply an artifact of plate production. In our case, the pits are not a result of acid cleaning since they are also observed on untreated specimens found along fractured rock surfaces. Since the pits can be observed on plates from living cells, we do not believe those found on the Giraffe specimens are a result of post-depositional processes. Further, pitted euglyphid plates are often observed alongside exceptionally preserved plates, siliceous chrysophyte scales, cysts and diatom valves that all lack any indication of dissolution. Regardless of their origin, the pits did not interfere with identification or size estimates of the plates.

Habitats containing large numbers of euglyphids typically include shallow ponds or swamps with submerged vegetation, including *Sphagnum* and other bryophytes (Booth 2001; Charman 2001; Decloitre 1962; Leidy 1879; Ogden and Hedley 1980; Williams 1985). These habitats also tend to be acidic, are usually high in dissolved and particulate organic carbon, and often with elevated nutrient concentrations. Based on the high concentrations of euglyphid remains in the lower sections of the core, coupled with significant chrysophyte cysts and sponge spicules, we envisage that the

initial waterbody was a shallow pond situated at the base of a relatively deep crater and that this condition remained for thousands of years, slowly infilling part of the crater. Then, over a short period of time, the crater filled to form a deep lake as evidenced by sudden appearances of large concentrations of planktonic diatoms and planktonic colonial chrysophytes, coupled with a significant decline in abundances of euglyphids and sponges (Siver et al. 2009; Wolfe et al. 2006). Despite the overall decline in euglyphid plates as the lake deepened, this represents the portion of the core where rectangular-shaped scales were found. Over time, the deep lake became progressively infilled once again becoming a shallow pond or wetland with significant numbers of testate amoebae.

The euglyphid remains in the Giraffe core represent one of the oldest, richest, and most diverse deposits known from the geological record. Based on the large number of scales uncovered, euglyphids were an integral component of the aquatic community in the Middle Eocene maar waterbody where they thrived over extensive time intervals. There are only limited reports of euglyphid plates from Eocene deposits, including one from the Eckfeld maar lake in Germany with a similar age as Giraffe (Schiller 1999), and another report by Loeblich and Tappan (1964). Other than the Giraffe locality, the best known deposits containing well preserved euglyphids are Miocene in age (Foissner and Schiller 2001; Williams 1985). Williams (1985) reported a rich fauna of euglyphid plates from the Clarkia locality, Idaho, U.S.A. (22 to 15 Ma) that bears a striking resemblance to the fauna uncovered in Giraffe, including both types of scutiform-shaped plates, spine and denticulate plates, circular to oval plates and rectangular-shaped plates. As noted above, equally impressive euglyphid remains, including whole tests of *Scutiglypha crenulata* and *S. scutigera*, were reported from 15 Ma Miocene deposits from the kieselgur pit near the village of Beuern, Germany (Foissner and Schiller 2001). In summary, the fauna of euglyphid plates uncovered from Giraffe is significant in that it demonstrates that this group of protozoans was well established by the Eocene, and that the morphologies of many types of plates has remained largely unchanged for much of the Cenozoic.

Methods

Mudstone chips (0.1-0.5 g) from 100 sections of the Giraffe core were oxidized using 30% H₂O₂ under low heat for a minimum of an hour, rinsed with distilled water, and the resulting

slurries stored in glass vials. This procedure results in separation of many siliceous microfossils from the mudstone matrix. In addition, small fragments of mudstone, each containing many embedded microfossils, remain within each preparation. Aliquots of each slurry were air dried onto pieces of heavy duty aluminum foil and directly onto circular glass coverslips. The aluminum foil samples were trimmed, attached to aluminum stubs with Apiezon® wax, 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 scanning electron microscope (SEM) or a Leo 435VP SEM. The coverslips were mounted in Naphrax onto glass slides and examined with a Leica DMR or Olympus BX51 light microscope (LM). Morphometric measurements of specimens were made during examination with SEM. A minimum of 300 microfossils were enumerated from each of the 100 samples using LM and all identifications were verified with SEM.

Each sample from the Giraffe core is identified with a three-part number. The first number represents the core box. Each box contains three 1.5 m core lengths, identified as channels 1, 2 and 3. Larger box numbers correspond to deeper sections of the core. The second part of the number represents the sample's channel within the box. The third number represents the length, in cm, measured down from the top of a core length. Thus, sample 18-3-31 represents a sample taken from 31 cm down along the core length positioned in channel 3 from box 18. The lacustrine phase of the maar lake is contained within boxes 26 (deepest) to 11 (youngest). The current investigation is based on examination of 100 mudstone samples taken from the core's lacustrine phase, with an average of 7 samples examined per box.

Acknowledgements

We thank Anne-Marie Lott for assistance in preparing samples and Alex Wolfe for useful discussions on geology and evolution. This work was funded with support to PAS from the National Science Foundation (DEB-0716606, DEB-1049583 and DEB-1144098).

References

- Adl SM, Simpson AGB, Lane CE, Lukes J, Bass D, Bowser SS, Brown MW, Burki F, Dunthorn M, Hampl V, Heiss A, Hoppenrath M, Lara E, Gall ILE, Lynn DH, McManus H, Mitchell EAD, Mozley-Stanridge SE, Parfrey LW, Pawlowski J, Rueckert S, Shadwick L, Schoch CL, Smirnov S, Spiegel FW (2012) The revised classification of eukaryotes. *J Eukaryot Microbiol* **59**:429–493
- Berney C, Pawlowski K (2006) A molecular time-scale for eukaryote evolution recalibrated with the continuous microfossil record. *Proc Roy Soc B* **273**:1867–1972
- Boeuf O, Gilbert D (1997) Présence de thécamoebiens du genre *Trinema*, au pliocène supérieur, découverte à Chilhac (Haute-Loire, France). *C r hebdomadaire Séances Acad Sci, Paris* **325**:623–627
- Booth RK (2001) Ecology of testate amoebae (Protozoa) in two Lake Superior coastal wetlands: implications for paleoecology and environmental monitoring. *Wetlands* **21**:564–576
- Booth RK (2002) Testate amoebae as paleoindicators of surface-moisture changes on Michigan peatlands: modern ecology and hydrological calibration. *J Paleolimnol* **28**:329–348
- Booth RK (2008) Testate amoebae as proxies for mean annual water-table depth in *Sphagnum*-dominated peatlands of North America. *J Quaternary Sci* **23**:43–57
- Booth R, Zygmunt J (2005) Biogeography and comparative ecology of testate amoebae inhabiting *Sphagnum*-dominated peatlands in the Great Lakes and Rocky Mountain regions of North America. *Diversity Distrib* **11**:577–590
- Bosak T, Lahr DJG, Pruss SB, Macdonald FA, Dalton L, Matys E (2011) Agglutinated tests in post-Sturtian cap carbonates of Namibia and Mongolia. *Earth Planetary Sci Lett* **308**:29–40
- Charman DJ (2001) Biostratigraphic and paleoenvironmental applications of testate amoebae. *Quaternary Sci Rev* **20**:1753–1764
- Corsetti F, Awramik SM, Pierce D (2003) A complex microbiota from snowball Earth times: microfossils from the Neoproterozoic Kingston Peak formation, Death Valley, USA. *Proc Natl Acad Sci USA* **100**:4399–4404
- Decloitre L (1962) Le genre *Euglypha* Dujardin. *Arch Protistenkd* **106**:51–100
- De Smet WH, Gibson JAE (2009) On a new species of euglyphid testate amoeba, *Scutiglypha cabrolae*, from the Licancabur Caldera Lake, central Andes. *Acta Protozool* **48**:119–126
- Doria G, Royer DL, Wolfe AP, Fox A, Westgate JA, Beerling DJ (2011) Declining atmospheric CO₂ during the late Middle Eocene climate transition. *Am J Sci* **311**:63–75
- Douglas MSV, Smol JP (1987) Siliceous protozoan plates in lake sediments. *Hydrobiologia* **154**:13–23
- Foissner W, Schiller W (2001) Stable for 15 million years: scanning electron microscope investigation of Miocene euglyphid thecamoebians from Germany, with description of the new genus *Scutiglypha*. *Eur J Protistol* **37**:167–180
- Gehrels WR, Roe HM, Charman DJ (2001) Foraminifera, testate amoebae and diatoms as sea-level indicators in UK salt-marshes: a quantitative multiproxy approach. *J Quaternary Sci* **16**:201–220
- Heal OW (1964) Observations on the seasonal and spatial distribution of testaceans (Protozoa: Rhizopoda) in *Sphagnum*. *J Animal Ecol* **33**:395–412
- Heaman LM, Kjarsgaard BA, Creaser RA (2004) The temporal evolution of North American kimberlites. *Lithos* **76**:377–397
- Hedley RH, Ogden CG (1974) Adhesion plaques associated with the production of a daughter cell in *Euglypha* (Testacea; Protozoa). *Cell Tiss Res* **153**:261–268
- Heger TJ, Mitchell EAD, Todorov M, Golemansky V, Lara E, Leander BS, Pawlowski J (2010) Molecular phylogeny of euglyphid testate amoebae (Cerczoza: Euglyphida) suggests transitions between marine supralittoral and freshwater/terrestrial environments are infrequent. *Mol Phylogenet Evol* **55**:113–122

- Lahr DJG, Parfrey LW, Mitchell EAD, Katz LA, Lara E** (2011) The chastity of amoebae: re-evaluating evidence for sex in amoeboid organisms. *Proc Roy Soc B* **278**:2081–2090
- Leidy J** (1879) Fresh-water Rhizopods of North America. Report of the United States Geological Survey of the Territories, Vol. XIII, p. 324. Government Printing Office, Washington, DC
- Loeblich A, Tappan H** (1964) Sarcodina, chiefly "Thecamoebians" and Foraminiferida. In Moore RC (ed) *Treatise on Invertebrate Paleobiology C(2) vol. 1*. Geological Society of America and University of Kansas Press, Lawrence, Kansas, pp 16–54
- Medioli FS, Scott DB** (1983) Holocene Arcellacea (Thecamoebians) from Eastern Canada. Cushman Foundation for Foraminiferal Research, Special Publication 21, Washington, D.C., pp 5–63.
- Meisterfeld R** (2002) Testate Amoebae with Filopodia. In Lee JJ, Leedale GF, Bradbury P (eds) *The Illustrated Guide to the Protozoa Vol. 2*, 2nd edn Society of Protozoologists, Lawrence, Kansas, USA, pp 1055–1084
- Mitchell EAD, Charman DJ, Warner BG** (2008) Testate amoebae analysis in ecological and paleoecological studies of wetlands: past, present and future. *Biodivers Conserv* **17**:2115–2137
- Ogden CG** (1981) Observations of clonal cultures of Euglyphidae (Rhizopoda, Protozoa). *Bull Br Mus Nat Hist (Zool.)* **41**:137–151
- Ogden CG, Hedley RH** (1980) An Atlas of Freshwater Testate Amoebae. *British Museum (Nat. Hist.) London and Oxford University Press, London*, 222 p
- Patterson RT, Dalby A, Kumar A, Henderson LA, Boudreau REA** (2002) Arcellaceans (thecamoebians) as indicators of land-use change: settlement history of the Swan Lake area, Ontario as a case study. *J Paleolimnol* **28**:297–316
- Pisera A, Siver PA, Wolfe AP** (2013) A first account of freshwater potamolepid sponges (Demospongiae, Spongillina, Potamolepidae) from the Middle Eocene: Biogeographic and paleoclimatic implications. *J Paleontol* **87**:373–378
- Poinar GOJ, Waggoner BM, Bauer U** (1993) Description and paleoecology of a Triassic amoeba. *Naturwissenschaften* **80**:566–568
- Porter SM, Knoll AH** (2000) Testate amoebae in the Neoproterozoic era: evidence from vase-shaped microfossils in the Chuar Group, Grand Canyon. *Paleobiology* **26**:360–385
- Porter S, Meisterfeld R, Knoll A** (2003) Vase-shaped microfossils from the Neoproterozoic Chuar Group, Grand Canyon: a classification guided by modern testate amoebae. *J Paleontol* **77**:409–429
- Schiller W** (1998) Kieselige Mikrofossilien aus dem Unter-Oligozän von Sieblos/Rhön. *Geol Abh Hessen* **104**:173–199
- Schiller W** (1999) Kieselige Thekamöben aus dem Mittel-Eozän des Eckfelder Maars in der Eifel. *Mainzer naturwiss Arch* **37**:55–62
- Schmidt AR, Schönborn W, Schäfer U** (2004) Diverse fossil amoebae in German Mesozoic amber. *Palaeontology* **47**:185–197
- Schmidt AR, Girard V, Perrichot V, Schönborn W** (2010) Testate amoebae from a Cretaceous forest floor of France. *J Eukaryot Microbiol* **57**:245–249
- Schmidt AR, Ragazzi E, Coppellotti O, Roghi G** (2006) A microworld in Triassic amber. *Nature* **444**:835
- Schönborn W** (1992) Comparative studies on the production biology of protozoan communities in freshwater and soil ecosystems. *Arch Protistenkd* **141**:187–214
- Schönborn W, Dörfelt H, Foissner W, Krienitz L, Schäfer U** (1999) A fossilized microcosmos in Triassic amber. *J Eukaryot Microbiol* **46**:571–584
- Siver PA, Wolfe AP** (2005) Scaled chrysophytes in Middle Eocene lake sediments from Northwestern Canada, including descriptions of six new species. *Nova Hedwigia, Beih* **128**:295–308
- Siver PA, Wolfe AP** (2009) Tropical ochrophyte algae from the Eocene of northern Canada: a biogeographic response to past global warming. *Palaios* **24**:192–198
- Siver PA, Lott AM, Wolfe AP** (2009) Taxonomic significance of asymmetrical helmet and lance bristles in the genus *Malomonas* (Synurophyceae) and their discovery in Eocene lake sediments. *Eur J Phycol* **44**:447–460
- Siver PA, Wolfe AP, Rohlf FJ, Shin W, Jo BY** (2013) Combining geometric morphometrics, molecular phylogeny, and micropaleontology to assess evolutionary patterns in *Malomonas* (Synurophyceae: Heterokontophyta). *Geobiology* **11**:127–138
- Škaloud P, Kynčlova A, Benada O, Kofroňova O, Škaloudova M** (2012) Toward a revision of the genus *Synura*, section Petersenianae (Synurophyceae, Heterokontophyta): morphological characterization of six pseudo-cryptic species. *Phycologia* **51**:303–329
- van Hengstum PJ, Reinhardt EG, Medioli FS, Gröcke DR** (2007) Exceptionally preserved late Albian (Cretaceous) arcellaceans (Thecamoebians) from the Dakota Formation near Lincoln, Nebraska, USA. *J Foram Res* **37**:300–308
- Vincent G** (2012) Fossil amoebae (Hemiarcherellidae fam. nov.) from Albian (Cretaceous) Amber of France. *Palaeontology* **55**:653–659
- Waggoner BM** (1996a) Bacteria and protists from Middle Cretaceous amber of Ellsworth County, Kansas. *Paleobios* **17**:20–26
- Waggoner BM** (1996b) The first fossil cyphoderiid testate amoeba, in Dominican Republic amber (Eocene-Oligocene). *Paleobios* **17**:17–19
- Wall AA, Gilbert JD, Magny M, Mitchell EAD** (2010) Testate amoeba analysis of lake sediments: impact of filter size and total count on estimates of density, species richness and assemblage structure. *J Paleolimnol* **43**:689–704
- Wanner M** (1999) A review on the variability of testate amoebae: Methodological approaches, environmental influences and taxonomical implications. *Acta Protozool* **38**:15–29
- Warner BG** (1990) Testate Amoebae (Protozoa). In Warner BG (ed), *Methods in Quaternary Ecology*. Geoscience Canada Reprint Series 5. Geological Association of Canada, St. John's, Canada, pp 65–74

Williams JL (1985) Thecamoebian Scales from a Miocene Lacustrine Deposit in Northern Idaho. In Smiley CJ (ed) Late Cenozoic history of the Pacific northwest. Am Assoc Adv Sci and Cal Acad Sci, San Francisco, CA, pp 67–71

Wolfe AP, Edlund MB, Sweet AR, Creighton S (2006) A first account of organelle preservation in Eocene nonmarine

diatoms: observations and paleobiological implications. *Palaios* **21**:298–304

Wylezich CR, Meisterfeld S, Schlegel M (2002) Phylogenetic analyses of small subunit ribosomal RNA coding regions reveal a monophyletic lineage of euglyphid testate amoebae (Order Euglyphida). *J Eukaryot Microbiol* **49**:108–118

Available online at www.sciencedirect.com

SciVerse ScienceDirect