Environmental Variables Controlling Abundances of Testate Amoebae Bearing Siliceous Plates in Freshwater Lakes and Ponds on the East Coast of North America: Potential for Inferring Water Depth and pH

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ABSTRACT

Testate amoebae comprise a highly diverse and polyphyletic group of heterotrophic, free-living, amoeboid protists, where the cell is enclosed within a shell, or test. These organisms inhabit a broad range of habitats, including lakes, ponds, rivers, bogs, wetlands and peatlands, where they prey on bacteria, algae, other protists, and even small micrometazoans. One group of testate amoebae produce the test out of overlapping siliceous plates that are formed individually within the cell, and then secreted and glued together to make an organized shell. Upon death, the siliceous plates can accumulate in lake sediments, and in some cases eventually become part of the fossil record. The goals of the current study were to document the concentrations of siliceous plate morphotypes in waterbodies along the east coast of North America, examine distributional patterns, determine the environmental variables controlling the abundance of plates, and evaluate if plate concentrations would be useful for inferring and reconstructing historical conditions. Seven siliceous plate morphotypes representing remains of testate amoebae belonging largely to the Order Euglyphida were enumerated in surface sediments from 125 waterbodies situated from North Carolina to Newfoundland. Circular-shaped plates were the most widely distributed morphotype, found in 95% of the waterbodies, and coupled with oval-shaped plates accounted for 75% of all specimens enumerated. Other plate morphotypes, including quadrangular, rectangular, rhomboidal, and scutiform forms, were also common and all morphotypes exhibited distinctive distribution patterns. Five environmental variables significantly added to a forward linear regression model explaining the concentration of plates per dry weight of sediment in the following order: pH, water depth, concentration of potassium, concentration of sulfate, and latitude, and collectively accounted for 60% of the variation in plate abundance. Significantly higher concentrations of siliceous plates were found in shallow and acidic waterbodies,

indicating the possibility of reconstructing estimates of these two variables in ancient waterbodies.

Keywords: Euglyphida, North America, pH, siliceous plates, testate amoebae, water depth.

INTRODUCTION

Testate amoebae, often referred to as thecamoebians, form a highly diverse and polyphyletic group of, heterotrophic, free-living, amoeboid protists where the cell is enclosed within a structure called the test (Mitchell et al. 2008). Pseudopodia, which emerge from the test through an opening known as the pseudostome, are used for movement and feeding. Some testate amoebae construct the test from organic or inorganic (e.g. silica plates) components secreted by the cell, while others scavenge material and particles from the environment and glue them together to form the covering. Differences in the test, type of pseudopodia (e.g. filose or lobose), and other cellular features reflect the polyphyletic nature of the group (Mitchell et al. 2008).

Testate amoebae within the Class Imbricatae in the Phylum Cercozoa form tests out of siliceous components, or plates, that are produced within the cell, secreted to the outside of the cell membrane, and glued together to form an organized covering. The shapes of the siliceous plates on the body of the organism may be circular, square, rhomboid, or scutiform, depending on the species (Ogden and Hedley 1980). Some siliceous plates may bear a protruding spine, and the apertural plates that surround the pseudostome are usually differentiated from body plates where the anterior end is lined with teeth (Ogden 1981, Ogden and Hedley 1980). The most common and ecologically important thecamoebians forming siliceous plates are taxa within the Order Euglyphida Copeland 1956 emended Cavalier-Smith 1997, especially species encompassing the genera *Euglypha* Dujardin 1841, *Scutiglypha* Foissner and Schiller 2001, *Assulina* Ehrenberg 1872 and *Trinema* Dujardin 1841 (Wylezich et al. 2002). These organisms are commonly referred to as euglyphids. The majority of non-euglyphid testate amoebae belong to the Order Arcellinida within the Phylum Amoebozoa Lühe 1913 emended Cavalier-Smith

1998. Tests of arcellinids are formed from organic components and these organisms have lobose pseudopodia instead of the filose type possessed by euglyphids.

Testate amoebae inhabit a broad range of terrestrial and aquatic habitats, including organic rich soils, moss beds, lakes, ponds, rivers, bogs, wetlands, peatlands and even brackish environments (Mitchell et al. 2008, Ogden and Hedley 1980, Escobar et al. 2008, Amesbury et al. 2018, Barnett et al. 2017), where they prey on bacteria, algae, other protists, and even small micrometazoans (McKeown et al. 2019). Water table depth is an important factor regulating species abundances and vertical distributional patterns in wetlands and peatlands, while additional variables such as soil thickness and pore space are also important in terrestrial habitats (Ogden and Hedley 1980, Mitchell et al. 1999, Booth 2001, Charman 2001, Booth and Jackson 2003). Concentrations of organic matter in sediments, along with other factors such as pH, conductivity, and trophic status, are also important variables controlling the presence/absence and often concentrations of individual species (Patterson and Kumar 2002, Booth and Zygmunt 2005, Payne et al. 2006, Escobar et al. 2008, McKeown et al. 2019). Species responses to specific environmental conditions, coupled with high reproductive rates, make thecamoebians sensitive bioindicators of environmental change (Charman 2001, Ogden 1981, Schonborn 1992). As a result, the remains of tests in sediments and peats are useful in reconstructing historical conditions (Charman 2001, Warner 1990), such as paleoclimates (Booth 2001, 2002, 2008, Charman 2001), peatland hydrology (Payne et al. 2006, Amesbury et al. 2018), the impacts of land-use changes (Patterson et al. 2002), sea-level change and salt enrichment (Gehrels 2000, Gehrels et al. 2001, Whittle et al. 2018).

The oldest account of fossil specimens representing members of the Order Euglyphida is from the early Eocene Giraffe Pipe fossil locality near the Arctic Circle in Canada (Barber et al.

2013). Euglyphid fossil remains have also been uncovered from Middle Eocene (Loeblich and Tappan 1964), Middle Miocene (Williams 1985, Foissner and Schiller 2001), and Pliocene (Beouf and Gilbert 1997) deposits. Given the high abundances of well-preserved siliceous testate plates found in the Giraffe core, Barber et al. (2013) discussed the possibility of using these euglyphid remains to aid in reconstructing the history of the waterbody, which is represented by over 65 m of mudstone sediments. Since the Barber et al. (2013) study, we have uncovered euglyphid specimens in many additional strata from the Giraffe site, and believe they may serve as a valuable group of organisms for reconstructing lake water depth, pH and possibly other environmental variables.

Most, if not all, ecological studies of thecamoebians include members of both the Order Euglyphida and the Order Arcellinida (Whittle et al., 2018), and relate individual species to environmental variables. Since the fossil remains in the Giraffe Pipe locality represent isolated siliceous plates and not entire tests, especially after extraction from the mudstone rocks, we have abundance estimates for all plate morphologies, but not for specific species. In previous works we have used surface sediments from modern lakes to document and quantify scaled chrysophytes and diatoms in freshwater lakes and ponds along the east coast of North America (Siver and Hamilton 2011, Siver and Lott 2012). Since the remains of scaled chrysophytes and diatoms are siliceous, the preparation methods used in these studies also contain remains of siliceous testate amoebae plates. The primary goals of this study were to 1) identify siliceous plate morphotypes in 125 waterbodies from five regions along the east coast of North America; 2) examine relationships between abundances of siliceous testate plates and environmental variables and; 3) investigate the utility of using abundances of testate plates to aid in

reconstructing water depth, pH and possibly other environmental variables in ancient waterbodies such as the early Eocene Giraffe Pipe waterbody.

MATERIALS AND METHODS

In an earlier work, Siver and Lott (2012) reported on the biogeographic distributions of scaled chrysophytes in freshwater waterbodies spread along the east coast of North America. Waterbodies from five regions (n = 125) included in that work, coastal North Carolina, the Pinelands National Preserve in New Jersey (New Jersey), Connecticut, Nova Scotia and Newfoundland, were used in the current study. The sites from North Carolina and the Pinelands of New Jersey are situated in non-glaciated areas on the Atlantic Coastal Plain, while those from the three remaining regions are located in glaciated areas. Details of all regions are given in Siver and Lott (2012). Sediment cores were taken from each waterbody with a Glew gravity corer (Glew 1988) and sectioned into 1cm units using a mechanical extruder (Glew 1989). The 0-1cm section from the surface of each core was used to identify and quantify siliceous plates of testate amoebae from each site. Surface sediment samples are commonly used to study organism remains as they effectively integrate growth of the organisms over the course of a year or more (Smol 1995).

Twenty environmental variables were measured for each study site, including water color, maximum depth, Secchi disk depth, alkalinity, pH, specific conductivity, chlorophyll-*a* , total phosphorus, total nitrogen, chloride, sulfate, potassium, sodium, calcium, magnesium, latitude, mean maximum temperature in January and July, and the mean minimum temperature for January and July. Maximum depth was derived from existing bathymetric maps, government databases, or estimates made at the time of collection. Details for all other parameters, including all chemical analyses, were according to Canavan and Siver (1994), Ahrens and Siver (2000), Lott and Siver (2005) and Siver and Lott (2010), and are summarized in Siver and Lott (2012).

Surface sediment from the 125 cores was processed as follows to prepare samples for examination with light and scanning electron microscopy: 1) Wet sediment from the 0-1 cm

139 section of each core was thoroughly mixed, and a known amount added to a beaker along with a
140 mixture of sulfuric acid-potassium dichromate according to the procedure of Marsicano and
141 Siver (1993). The amount of wet weight used per sample varied between samples, with a mean
142 of 1.3 g. The mixture was gently heated in order to facilitate oxidation of organic matter. At the
143 completion of the oxidation step the material (slurry) was transferred to a centrifuge tube and
144 washed with DW a minimum of five times. The resulting slurry was transferred to a glass vial
145 and the volume brought to 10 ml. 2) A second wet weight sample from the 0-1 cm section of the
146 core was placed onto an aluminum weighing boat and dried in a drying oven at 105 ºC to a
147 constant weight. The percent dry weight was calculated and used to estimate the amount of dry
148 weight of material used to derive the slurry. 3) A known volume of the slurry was diluted to 30
149 ml and slowly poured into a Battarbee tray containing five wells, each well fitted with a 22 ml
150 diameter circular glass cover slip (Battarbee 1986). The Battarbee trays were placed on a
151 vibration-free table, covered, and the solution allowed to air dry such that the microfossils
152 became affixed to the cover glasses within the tray. 4) The cover glasses containing the dry
153 sediment material were permanently mounted onto glass slides using Hyrax or Naphrax
154 mounting medium and the slides labeled with a diamond knife. 5) The permanent slides were
155 scanned at 40x with an Olympus BX 51 microscope using a phase contrast lens (n.a. = 0.65).
156 The numbers of each testate plate morphotype within a known number of fields were recorded.
157 The surface area of a field of view under 40x magnification is 0.29 mm^2. 6) Given the above
158 parameters, the amount of dry weight of sediment per field at 40x magnification was calculated
159 for each sample. The mean across all samples was 2.9×10^{-1} µg dry weight per field.
160 Concentrations of testate plates are given on a µg dry weight basis.

Prior to enumeration of testate plates, samples were first examined with a Leica DMR light microscope using a 100x Plan Apo lens (numerical aperture = 1.4) and coupled with a Zeiss Axiocam 503 color camera, and with either a Leo (Zeiss) 982 FESEM or a FEI Nova NanoSEM 450 FESEM field emission scanning electron microscope (SEM). These analyses yielded initial qualitative estimates and images of the plate morphotypes found in each sample. For SEM, an aliquot of the oxidized slurry was air dried onto a piece of heavy duty aluminum foil, trimmed, and attached to an aluminum SEM stub with Apiezon® wax. Samples were coated with a mixture of gold and palladium for 2 min with a Polaron Model E sputter coater.

Non-metric multidimensional scaling (MDS) was performed using Primer-E (ver. 6.1.12, Clarke and Warwick 2001) in order to ordinate and display sites based on the rank order of Bray–Curtis measurements of testate plate morphotypes. The organism matrix consisted of concentration data for each plate morphotype at each site. Plate abundances were first transformed using a square root transformation, and a resemblance matrix subsequently formed using a Bray–Curtis measure. Plate abundances were also transformed using logarithmic algorithms, but because these yielded similar results, only results based on a square root transformation are presented. Shapiro-Wilk normality tests and all regression analyses, including forward stepwise regression, were performed using SigmaPlot ver 12.5. Based on the normality tests, the maximum water depth and water color variables were logarithmic transformed prior to analysis.

181 **RESULTS**

182 **Physical and Chemical Properties of the Study lakes**:

183 A detailed analysis of the differences in physicochemical properties of the waterbodies
184 from each region is given in Siver and Lott (2012), and summarized here. The study sites span
185 the east coast of North America from the non-glaciated Atlantic Coastal Plain (North Carolina
186 and the Pinelands of New Jersey) to glaciated areas that include Connecticut, Nova Scotia and
187 Newfoundland. On average, waterbodies situated on the Atlantic Coastal Plain (North Carolina
188 and the Pinelands of New Jersey) are more similar to each other, as are those in Nova Scotia and
189 Newfoundland, than they are to those in Connecticut (Table 1). Although statistically different
190 from the other four regions, waterbodies in Connecticut are more similar to those in the Canadian
191 Maritime that those along the Atlantic Coastal Plain. The mean maximum and mean minimum
192 January temperatures ranged from 12.8 ºC to -1.7 ºC, and 1.1 ºC to -9.4 ºC, respectively, across
193 all regions (Table 1). There is approximately a 10 to12 ºC difference in the mean maximum and
194 mean minimum temperatures during July in all regions.

195 On average, the Connecticut waterbodies have higher pH and alkalinity, and are clearer
196 waterbodies with deeper Secchi disk depths. In contrast, sites on the Atlantic Coastal Plain are
197 more acidic, poorly buffered, with high concentrations of colored dissolved organic matter, and
198 low Secchi disk depths. A mixture of clear water and humic-stained waterbodies characterize
199 those in the Canadian Maritime regions. Although the pH ranged from 3.5 to 8.6, a total of 64 of
200 the waterbodies had a pH < 6. Lakes in Nova Scotia and Newfoundland have lower
201 concentrations of sulfate and potassium than those in the other regions. Based on chlorophyll-*a*,
202 total phosphorus, and total nitrogen concentrations, the most eutrophic lakes are situated in North

203 Carolina and New Jersey, while the more northern and glaciated sites become more oligotrophic
204 with lower nutrient and chlorophyll- *a* concentrations.
205
206 **Diversity and Abundances of Siliceous Shell Plates:**
207 Siliceous shell plates were separated into seven different morphotypes for quantitative
208 purposes (Table 2; Figs 1-3). Circular shaped plates (Figs 1H-K; 3E-F) were the most abundant
209 type, ranging from 32.6% (North Carolina) to 64% (Connecticut) of the total per region, and
210 accounting for 45% of plates from all sites. Except for North Carolina localities, circular plates
211 were the most abundant type in all other regions. Circular plates were widely distributed,
212 recorded in 95% of all study lakes. Oval plates (Figs 1D-G; 3G) were the second most abundant
213 plate type, with maximum abundances recorded from waterbodies along the Atlantic Coastal
214 Plain in New Jersey and North Carolina where they accounted for 39.7% and 37% of all plates,
215 respectively (Table 2). Collectively, circular and oval morphotypes comprised 75% of all plates.
216 Square or quadrangular shaped plates (Figs 1A-C; 3H-I) accounted for 8% of the total, were
217 relatively evenly distributed between regions, but were noticeably rare in Connecticut lakes. In
218 contrast, quadrangular plates were present in all of the New Jersey sites. Rhomboidal and
219 rectangular-shaped plates (Figs 1Q-S; 3A-D) were found in all regions, and in 46% of the study
220 sites, however accounted for only 2.7% of all plates.
221 Scutiform or shield-shaped plates with a bilateral symmetry were separated into two
222 groups based on the shape along the wider portion of the plate. The wider end of the plate,
223 referred to as the aboral end, faces the posterior of the test, while the narrower end faces the oral
224 end of the test. The first scutiform shaped plate, morphotype 1, has strongly undulating margins
225 along both the aboral and oral ends of the plate resulting in three projections on each end (Figs

226 1N-P; 2A-B). The two outer projections on the aboral end of the plate are highly accentuated,
227 and extend from the plate slightly further than the central process or projection, yielding a
228 "butterfly" shape. The margins of the plate connecting the aboral and oral ends are relatively
229 straight to slightly curved and tapering, completing the bilateral design. Except for the central
230 projection, the lateral margins along the aboral end of morphotype 2 are broadly rounded and not
231 accentuated and projected out from the plate as they are on morphotype 1(Figs 1U-Y; 2D-E, G-
232 H). Narrow scutiform morphotype 2 plates have a shape that resembles a "lemon" rather than a
233 butterfly (Figs 1U-V, Y; 3G). Scutiform morphotypes 1and 2 accounted for only 4% and 8% of
234 all plates enumerated, respectively. However, morphotype 1 was significantly more abundant
235 and accounted for between 5.6-11.8% of the total in the three northern regions, Connecticut,
236 Nova Scotia and Newfoundland. They were much less abundant in lakes situated on the Atlantic
237 Coastal Plain where they accounted for < 1% of all plates. Despite the low abundances,
238 morphotype 1 was present in 48% and 22% of the waterbodies in North Carolina and New
239 Jersey, respectively. Morphotype 2 was much more evenly distributed between regions (Table
240 2).

241 Plates with projecting teeth that surround the pseudostome opening varied in the number
242 and position of the teeth (Figs 1L-M, T; 2C, F). Denticulate plates with five to 13 teeth were
243 observed. Although combined in abundance estimates, denticulate plates can be separated into
244 two types. One type typically had 5-7 teeth, each of which originated from the undersurface of
245 the plate, and possessed a more accentuated aboral end (Figs 1L-M). The second type of
246 denticulate plate usually had more (e.g. 9-11) and smaller teeth that projected from the margin of
247 the plate in the same plane as the body of the plate. This plate type has a less projecting aboral
248 margin (Figs 1T; 2C, F).

249 The totality of differences in siliceous plate types between regions was further illustrated
250 with a MDS analysis (Figs 4-5A-F). The distribution of sites clearly shows the high degree of
251 similarity between New Jersey and North Carolina, the two most southern localities included in
252 the study. The majority of Connecticut sites also separate from those in other regions, are more
253 similar to other northern regions (Nova Scotia and Newfoundland), but very different from the
254 southern regions. Differences in the distributions of plate types between regions noted above are
255 supported in the MDS analysis. First, quadrangular plates have the most limited distribution, are
256 clearly more abundant in New Jersey and North Carolina, and largely lacking in Connecticut
257 sites (Fig 5D). Second, the distributions and abundances of scutiform morphotype 2 and
258 rectangular plate types largely overlap (Figs 5E-F). Third, butterfly-shaped scutiform
259 morphotype 1 plates have greater abundances in northern regions, and are noticeably less
260 abundant in North Carolina and New Jersey. Fourth, circular plates exhibit the widest
261 distribution, and can be found in high abundances in lakes from multiple regions (Fig. 5A). Fifth,
262 oval plates are also widely distributed with highest abundances in more southern regions, and
263 lower concentrations in many Connecticut localities (Fig. 5B). Sixth, the distribution of
264 rectangular plates (Fig. 5C) is most similar to that for oval specimens.

265 **Siliceous Plate Abundance versus Environmental Variables**:

266 Individual linear regression analyses were initially performed to investigate the
267 relationships between plate abundance and each of the 20 environmental variables. Based on
268 these analyses, plate abundance was significantly related ($p < 0.05$) to twelve variables (Table 3).
269 The strongest relationships were with pH, water depth, and water color. The relationship with
270 pH is highly significant ($p < 0.001$; $r^2 = 0.45$), where the abundance of plates increased with a
271 decrease in pH (Fig. 6B). Most waterbodies with pH above 7 had less than one plate per µg dry

weight sediment. In contrast, most waterbodies with a pH below 5 had three to over ten plates per µg dry weight sediment. With respect to water depth ($p < 0.001$; $r^2 = 0.43$), deeper lakes had significantly lower abundances of euglyphid plates (Fig. 6A). The highest abundances of plates were found in waterbodies below 3m in depth, with the greatest numbers in sediments from shallow ponds less than 1m deep.

A stepwise forward multiple regression analysis was then performed to determine the suite of variables most important in determining abundance of siliceous testate plates, where each variable independently accounts for a significant portion of the total variation. Five variables were added to the regression model in the following order: pH, water depth, concentration of potassium, concentration of sulfate, and latitude (Table 4). The resulting model explained 60% of the variation in plate abundance (Fig. 6C). The pH variable accounted for 45% of the variance in plate abundance, while adding water depth explained an additional 7% (52% total for both variables). The concentrations of potassium and sulfate, and latitude accounted for an additional 8% of the variance. Due to the covariance between color and both pH and water depth, the former variable did not add to the final model. If the pH variable was removed from the analysis, water depth became the most important variable, accounting for 43% of the variance in plate abundance. If both pH and water depth were removed, color becomes the strongest factor.

DISCUSSION

We are confident that except for the quadrangular-shaped plates, the remaining specimens in our work represent species belonging to the Order Euglyphida. Because the plates are disarticulated from the original test when uncovered from the sediment samples, assigning them to species, and even to genus level, is difficult. It is especially difficult to determine from which taxa the circular or oval plates were derived. Plates with these shapes are common morphotypes formed by some species belonging to *Euglypha* Dujardin 1841, *Assulina* Ehrenberg 1982, *Trinema* Dujardin 1841, *Sphenoderia* Schlumberger 1845, *Corythion* Taranek 1918, *Tracheleuglypha* Deflandre 1928, and possibly *Puytoracia* Bonnet 1970. Most of the rectangular or rhomboidal-shaped scales are believed to belong to members of the genus *Euglypha* (e.g. *E. strigosa* Ehrenberg 1848), and possibly the genus *Assulina* (e.g. *A. scandinavica* Penard 1890). The more rectangular-shaped plates with rounded margins likely belong to *Euglypha compressa* Carter 1864, especially since denticulate plates matching this species were also found in the samples. The quadrangular-shaped plates belong to species in the genus *Quadrullela* Cockerell 1909, which also form tests with a highly organized arrangement of siliceous plates. However, unlike taxa in the Order Euglyphida that produce filose pseudopodia, *Quadrullela* belongs to the Order Arcellinida Kent 1880 that includes species with lobose pseudopodia (Ogden and Hedley 1980).

The bilateral and scutiform-shaped plate morphotypes belong to the genus *Scutiglypha* Foisner and Schiller 2001. *Scutiglypha* was erected to include species of *Euglypha* that bear bilateral, shield-shaped plates (Foisner and Schiller 2001), and currently the genus includes at least six species (DeSmet and Gibson 2009). The tests of *Scutiglypha* species also include plates that are intermediate in shape between those bearing teeth that surround the pseudostome, the

typical shield-shaped body plates, and smaller and more circular plates that cover the posterior end of the test (Foissner and Schiller, 2001; DeSmet and Gibson 2009; Schiller and Wuttke 2015). Based on our findings, the two shield-shaped morphotypes probably represent two different *Scutiglypha* species. Although both morphotypes were found together in a few samples, they were not in many collections leading us to conclude they represent closely related, but different species.

Although some authors question the validity of the genus *Scutiglypha*, the arguments for separating it from *Euglypha* were reviewed by DeSmet and Gibson (2009). In addition to the obvious differences in plate morphology, our findings indicate that there is also a significant difference in the structure of the denticulate plates that surround the pseudostome. The denticulate plates of *Euglypha* usually have 5 to 7 teeth, a large and prominent median tooth, and the teeth clearly originate from the undersurface of the plate. In our study, this type of denticulate plate is always found in samples with circular and/or oval plates, and indicates that many of the latter plate morphotypes may indeed belong to *Euglypha* since other genera in the Euglyphidae besides *Scutiglypha* lack these distinctive denticulate plates. On the other hand, denticulate plates found in samples with *Scutiglypha* body scales usually have 7 to 11(13) smaller teeth, a less prominent median tooth, and the teeth originate at, and project from, the margin of the plate. As additional species are described, it will be interesting to see if the differences in the structures of denticulate plates form an additional character that can be valuable for distinguishing between the two genera.

Although rare, two additional plate types are worth mention and likely indicate the present of additional taxa in the collections. First, a few of the numerous oval plate specimens had distinctively thickened rims. Plates that match this morphotype have been illustrated for

337 *Assulina muscorum* Greef 1888 (Ogden and Hedley 1980). Another rare type of plate uncovered
338 in a few samples was a small, oval, plate bearing a single tiny tooth. This plate morphotype is
339 typical of the genus *Trinema*, where it aligns the margin of the pseudostome.
340 Numerous studies have reported that the abundance, diversity, and distribution of testate
341 amoebae in freshwaters are related to a combination of environmental variables including, but
342 not limited to, water depth, pH, trophic status, conductivity, organic content, temperature,
343 moisture content and substrate type (e.g. Ogden and Hedley 1980, Collins et al. 1990, Roe and
344 Patterson 2014, Ju et al. 2014, Amesbury et al. 2018, Tsyganov et al. 2019). Of these factors,
345 water depth is often reported as the most important variable controlling diversity, abundance, and
346 species composition in freshwater lakes and peatlands (Mitchell et al. 1999, Booth 2002,
347 Patterson et al. 2012, McKeown et al. 2019, Tsyganov et al. 2019). Given the importance of
348 water depth, Sonnenburg et al. (2013) attempted to use species assemblages to infer this variable
349 over time, but concluded that additional data relating specific species to specific water depths
350 were needed. In a more recent study, Tsyganov et al. (2019) described distinctive assemblages
351 of testate amoebae species relative to lake depth, further demonstrating that these organisms have
352 great promise for inferring historical lake water levels.
353 Using testate amoebae to reconstruct paleohydrological conditions in peatlands is more
354 advanced than their use in inferring lake depth (Charman 1997, Mitchell et al. 1999, Booth
355 2002). By combining regional-scale datasets, Amesbury et al. (2018) developed transfer
356 functions applicable for inferring peatland palaeohydrology not only throughout North America,
357 but for the Holarctic. In an interesting work based on New Zealand peatlands, McKeown et al.
358 (2019) showed that testate amoebae-based inference models could be improved by dividing
359 species into subsets based on size. In their study, smaller species were related to different

360 environmental variables than larger taxa, demonstrating an even greater utility to use these
361 organisms in reconstructing efforts.
362 The pH is another important variable controlling both abundance and species
363 composition of testate amoebae (Escobar et al. 2008, Patterson et al. 2013). On a broad scale,
364 many testate amoebae species are limited by pH, with some taxa being found to be more
365 abundant in acidic habitats, and others in more alkaline sites (Ogden and Hedley 1980). A
366 similar result was reported by Patterson and Kumar (2002), who found some species of
367 euglyphids thriving at low pH, and other species mostly of Centropyxids more abundant at
368 higher pH sites. In a study of subtropical lakes in Florida, Escobar et al. (2008) reported the
369 highest diversity of testates in lakes with a high pH near 8. Although our findings largely agree
370 with those of Patterson and Kumar (2002), they are seemingly in contrast to those of Escobar et
371 al (2008). Whereas we report a significant increase in silica plate abundance with decreasing
372 pH, Escobar et al. (2008) reported greater species diversity at high pH. Many of our study lakes
373 with a pH below ca. 5.5 had abundances of silica plates five to ten times higher than in lakes
374 with a pH above 7. The difference between the Escobar et al (2008) study and our findings is
375 probably due to a difference in the species composition. Interestingly, lakes in the Escobar et al.
376 (2008) study contained primarily species that do not construct their tests out of idiosomes,
377 including internally-produced siliceous plates. In contrast, our study focused solely on species
378 that build highly organized tests using silica plates (e.g. euglyphids) formed internally within
379 cytoplasmic vesicles, and then deposited externally to form the test. Another difference is that
380 our study was based on the abundances of plates and not species diversity.
381 Lake trophic status can be another important variable determining the assemblage of
382 testate amoebae species found in a given waterbody (Schönborn 1992, Patterson et al. 2012,

Tsyganov et al. 2019). As a result of such relationships, Reinhardt et al. (2005) and Drljepan et al. (2014) used these organisms to track shifts in trophic status. Highest species diversities of testate amoebae have been found in mesotrophic to eutrophic lakes (Escobar et al. 2008, Ju et al. 2014), and greater abundances of specimens are usually associated with organic-rich sediments (Patterson and Kumar 2002, Roe and Patterson 2006). Although we did not observe a relationship between plate abundance and the trophic-related variables total phosphorus and chlorophyll-*a* concentrations, there were significantly greater concentrations in sites from North Carolina and New Jersey that have higher total nitrogen levels and elevated water color relative to most localities. These are also sites that most likely have higher concentrations of organic matter.

A major difference between our approach and previous works using testate amoebae to infer environmental conditions is that our results are based on a) only species that produce siliceous plates and; b) abundances of siliceous plates and not numbers of individual species. None of the previous studies relating testate amoebae to specific environmental variables, or focused on inferring historical conditions, relied on abundances of siliceous plates. Nor are there studies that include only the subset of species that produce siliceous plates, although some studies do rely solely on Arcellacea taxa (Patterson et al. 2012, Roe and Patterson 2014). Our interest in focusing on abundance of siliceous plates is because this is the metric we can best estimate in modern lake sediments as well as in fossil mudstones, especially if acidic oxidation procedures are needed to prepare and/or extract the microfossils. Despite not using a metric based specifically on species, our model still accounted for 60% of the variation in plate concentrations, which is comparable to previous works. Further, our results clearly indicate that significantly higher abundances of siliceous testate plates, regardless of species diversity, are

406 found in shallow, acidic ponds and lakes. Undoubtedly, if other variables, such as biotope, food
407 supply, and predator concentration, were included, a greater percentage of variation could
408 probably be explained. With additional study, it may also be possible to improve the models by
409 linking specific plate morphotypes to specific conditions in a similar fashion as McKeown et al.
410 (2019) did based on test size.

411 An ultimate long-term goal is to use the abundances of siliceous testate plates to
412 reconstruct water depth and pH not only in modern waterbodies using recently deposited
413 sediment, but also in fossil waterbodies including the extensive mudstone core from the Giraffe
414 Pipe fossil locality (Siver and Wolfe 2009, Barber et al. 2013, Wolfe et al. 2017). This fossil site
415 is of particular interest because it represents an important deep-time freshwater analog of an
416 Arctic lake that existed under a warm greenhouse climate (Siver and Wolfe 2009, Wolfe et al.
417 2017). Reconstructed mean annual temperature and mean annual precipitation values for the
418 Giraffe locality are 17 ºC higher and 4 times greater, respectively, than present, and the region
419 supported a warm mixed forest (Wolfe et al. 2017). Tracing the history of the Giraffe waterbody
420 can potentially help us understand how freshwater Arctic habitats will respond to future warming
421 scenarios. The Giraffe core contains numerous siliceous testate plates, including most of the
422 morphotypes uncovered in our modern lake study. In addition, the concentrations of plates
423 ranges widely over the length of the core, including periods of extensive numbers alternating
424 with periods with few to no testate remains (Barber et al. 2013). Preliminary results indicate that
425 concentrations of testate plates in the Giraffe core are positively correlated with remains of acidic
426 and periphytic diatoms, sponge sclerids, heliozoans, and specific types of chrysophyte cysts,
427 while low concentrations are found concurrent in strata where planktonic diatoms dominate
428 (Barber et al. 2013, Siver 2019). Based on these results, our current hypothesis is that abundant

concentrations of testate plates correspond to time periods represented by a shallow waterbody, and vice versa. Inference models for water depth and pH based on concentrations of testate plates would yield much needed independent verification of reconstructions based on other fossil proxies.

In summary, remains of siliceous plates from testate amoebae are a common type of microfossil found in many lakes and ponds, including those distributed along a wide expanse of eastern North America. Most of the plate morphotypes represent taxa in the Order Euglyphida. Greater concentrations of plates are significantly associated with shallower and more acidic waterbodies, indicating that this metric could be used to infer historical conditions. The plate morphotypes found in modern waterbodies have all been uncovered in fossil localities. This finding indicates that the lineages of organisms producing these plate morphotypes had already evolved by at least the Eocene (Barber et al. 2013), further supporting their use in reconstructing conditions found in these ancient waterbodies.

AUTHOR CONTRIBUTIONS

PAS developed the concept for the project, collected and analyzed data, imaged specimens, wrote and edited the manuscript; AML collected and analyzed data, imaged specimens, edited the manuscript; PT collected and analyzed data.

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FIGURE LEGENDS

Figure 1. Light micrographs of siliceous plate morphotypes from freshwater testate amoebae. Morphotypes include quadrangular (A-C), oval (D-G), circular (H-K), denticulate (L-M, T), scutiform morphotype 1 (N-P), rectangular to rhomboid (Q-S), and scutiform morphotype 2 (U-Y). Scale bar = 10 μm.

Figure 2. Scanning electron micrographs of siliceous plate morphotypes from freshwater testate amoebae. Morphotypes include scutiform morphotype 1 (A-B), denticulate (C-F), and scutiform morphotype 2 (D-E, C-H). Denticulate plates illustrated have 11 (C) and nine (F) teeth. The lemon-shaped plate with seven teeth (H) is situated on the test just behind the row of denticulate plates. Scale bars = 3 μm (F-G), 4 μm (E) and 5 μm (A-D, H).

Figure 3. Scanning electron micrographs of siliceous plate morphotypes from freshwater testate amoebae. Morphotypes include rectangular to rhomboid (A-D), circular (E-F), oval (G), and quadrangular (H-I). Scale bars = 2 μm (A, C, F, H) and 3 μm (B, D-E, G, I).

Figure 4. Result of a non-metric multidimensional scaling analysis indicating the ordination of 125 freshwater sites from five regions along the east coast of North America based on the concentrations of different testate amoebae plate morphotypes.

Figure 5. Non-metric multidimensional scaling results depicting the occurrences and concentrations of six testate amoebae plate morphotypes in freshwater sites representing five regions along the east coast of North America. Morphotypes include circular (A), oval (B), rectangular to rhomboid (C), quadrangular (D), scutiform morphotype 1 (E), and scutiform morphotype 2 (F). See Figure 4 for locations of sites in each of the five regions.

676 Figure 6. Abundance of testate amoebae plates versus lake depth (A) and pH (B) in 125
677 freshwater sites along the east coast of North America. C) Predicted versus actual abundances of
678 testate amoebae plates using a multiple regression model based on five variables, pH, water
679 depth, concentration of potassium, concentration of sulfate, and latitude.