Abundance and distribution of testate amoebae bearing siliceous plates in freshwater lakes and ponds along the east coast of North America: Importance of water depth and pH

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Abstract: Testate amoebae are a highly diverse and polyphyletic group of heterotrophic, free-living amoeboid protists that consist of a single cell 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 in some cases 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 amoebae death, the siliceous plates can accumulate in lake sediments and 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, and determine the relationships between environmental variables and abundances of plates. 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, USA, to Newfoundland, Canada. Circular-shaped plates were the most widely distributed morphotype, found in 95% of the waterbodies, and along with oval-shaped plates, they accounted for 75% of all specimens enumerated. Other plate morphotypes, including quadrangular, rectangular, rhomboidal, and scutiform, were also common, and all morphotypes exhibited distinctive distribution patterns. We used best subset regression to evaluate the relationships between environmental variables and the concentration of plates/dry mass of sediment and to assess differences among sites. Water depth and pH were consistently shown to explain the most variation in testate plate abundance. Concentrations of potassium and sulfate were of lesser importance. Higher concentrations of siliceous plates were found in shallow and acidic waterbodies, indicating the possibility of reconstructing estimates of these 2 variables in ancient waterbodies.

Key words: Euglyphida, North America, pH, siliceous plates, testate amoebae, water depth

Testate amoebae, often referred to as thecamoebians, form a highly diverse and polyphyletic group of heterotrophic, free-living amoeboid protists that consist of a single cell 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 components (e.g., silica plates) secreted by the cell, whereas others scavenge material and particles from the environment and glue them together to form a 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 (shield-shaped), depending on the species

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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 >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 Orders Euglyphida and Arcellinida (Whittle et al. 2018) and relate individual species to environmental variables. Because 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. The primary goals of this study were to: 1) identify and quantify siliceous plate morphotypes in 125 waterbodies from 5 regions along the east coast of North America, 2) document distribution patterns between regions, and 3) examine relationships between concentrations of siliceous testate plates and environmental variables.

METHODS

In an earlier work, Siver and Lott (2012) reported on the biogeographic distributions of scaled chrysophytes in freshwater bodies spread along the east coast of North America. Because the remains of scaled chrysophytes are siliceous, the preparation methods used by Siver and Lott (2012) also provided remains of siliceous testate amoebae plates. Waterbodies from 5 regions (n = 125) included in that work—coastal North Carolina, the Pinelands National Preserve in New Jersey, Connecticut, Nova Scotia, and Newfoundland—were used in the current study. We took sediment cores from each waterbody with a Glew gravity corer (Glew 1988) and sectioned the cores into 1-cm units using a mechanical extruder (Glew 1989). We used the 0 to 1-cm section from the surface of each core to identify and quantify siliceous plates of testate amoebae from each site. Surface sediment samples are commonly used to study organism remains because they effectively integrate growth of the organisms over the course of a year or more (Smol 1995).

We measured 20 environmental variables for each study site: water color, maximum depth, Secchi disk depth, alkalinity, pH, specific conductivity, chlorophyll a, total phosphorus, total nitrogen, chloride, sulfate, potassium, sodium, calcium, magnesium, latitude, and mean maximum and minimum temperatures in January and July. We derived maximum depth 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).

To prepare samples for examination with light and scanning electron microscopy, we processed surface sediment from the 125 cores as follows: 1) We thoroughly mixed wet sediment from the 0 to 1-cm section of each core and added a known amount to a beaker along with a mixture of sulfuric acid–potassium dichromate according to the procedure of Marsicano and Siver (1993). The amount of wet mass used/sample varied between samples, with a mean of 1.3 g. We gently heated the mixture to facilitate oxidation of organic matter. Once oxidized we transferred the material to a centrifuge tube and washed it with deionized water a minimum of 5×. We transferred the resulting slurry to a glass vial and brought the volume to 10 mL by adding deionized water. 2) We placed a 2nd wet-mass sample from the 0 to 1-cm section of the core onto an aluminum weighing boat and dried it in an oven at 105°C to a constant mass. We calculated the % dry mass and used that value to estimate the amount of dry mass of material used to derive the slurry. 3) We diluted a known volume of the slurry to 30 mL and slowly poured it into a Battarbee tray containing 5 wells, each well fitted with a 22-mm diameter circular glass cover slip (Battarbee 1986). We covered the Battarbee trays and placed them on a vibration-free table, and we allowed the solution to air dry such that the microfossils became affixed to the cover glasses within the tray. 4) We permanently mounted the cover glasses containing the dry sediment material onto glass slides using Hyrax or Naphrax mounting medium and labeled the slides with a diamond knife. 5) We scanned the permanent slides at 40× magnification with an Olympus BX51 microscope (Waltham, Massachusetts) using a phase contrast lens (numerical aperture = 0.65) and recorded the numbers of each testate plate morphotype within a known number of fields. The surface area of a field of view under 40× magnification was 0.29 mm². 6) Given the above parameters, we calculated the amount of dry mass of sediment/field at 40× magnification and reported concentrations of testate plates on a μg-dry-mass basis. The mean across all samples was 0.29 μg dry mass/field.

Prior to enumeration of testate plates, we first examined samples with a Leica DMR light microscope (Leica Microsystems, Buffalo Grove, Illinois) using a 100× plan apochromat lens (numerical aperture = 1.4) coupled with a Zeiss Axiocam 503 color camera (Carl Zeiss Microscopy, White Plains, New York) and with either a Zeiss Leo-982 or a Nova NanoSEM™ 450 (FET™, Hillsboro, Oregon) field emission scanning electron microscope. These analyses yielded initial qualitative estimates and images of the plate morphotypes found in each sample. For scanning electron microscopy, we air dried an aliquot of the oxidized slurry onto a piece of heavy-duty aluminum foil, trimmed it, and attached it to an aluminum scanning electron microscope stub with Apiezon® wax (M&I Materials, Suwanee, Georgia). We coated samples with a mixture of gold and palladium for 2 min with a Polaron Model E sputter coater (Quorum Technologies, East Sussex, UK).

We used PRIMER-e (version 7; Quest Research Limited, Auckland, New Zealand; Clarke and Warwick 2001, Anderson et al. 2008) to conduct non-metric multidimensional scaling (NMDS) to examine differences in testate plate morphotypes between sites and study regions. The organism matrix consisted of concentration data for each testate plate morphotype at each site. We square-root transformed plate abundances to downweight the influence of abundant morphotypes and calculated a Bray–Curtis similarity matrix, which was then used in the NMDS. The Bray–Curtis metric is commonly used for comparing sites with species count data.

We used simple linear regression, coupled with scatterplots, and best subset regression analysis to assess relationships between the abundances of testate plates/site and environmental variables. We used the Shapiro–Wilk test to evaluate normality, the Durbin–Watson statistic for independence of residuals, and Spearman rank correlation to test for constant variance. The best subset regression analysis evaluates all possible combinations of the independent variables and selects subsets of variables that best predict the dependent variable. We used the Mallows’ C_p as the criterion for inclusion of an independent variable into the final model. C_p estimates the bias added to the dependent-variable estimate when independent variables are removed from the model. C_p accounts for the difference between the true population mean for the dependent variable and the population mean based on the independent variables included in the model. For a given model, the optimum value of C_p is equal to the number of variables in the model plus the constant (C_p = k + 1, where k = the number of independent variables in the model). A value close to k + 1 indicates a low bias of the model regression coefficients used to infer the dependent variable. Following best subset regression, we then used multiple regression analyses to develop models for each of the top 10 subsets of variables.

We further used the predicted residual sum of squares (PRESS) statistic, which we computed using leave-1-out cross validation, to evaluate regression models for each of the top 10 subsets of variables identified in the best subset regression analysis. PRESS provides a measure of the potential predictive power of a regression model. Once a model has been developed, each observation of the dependent variable is removed, 1 at a time, and the model is refitted using the remaining observations. The model is then used to estimate the value of the observation that was removed, and the process is repeated across all observations. The PRESS statistic is calculated as the sum of squares of the residuals. Lower PRESS values indicate models with better prediction ability and that are less likely to be overfitted. We used SigmaPlot (version 12.5; Systat Software Inc., San Jose, California) for
all regression analyses and to calculate the PRESS statistic and standard error of estimation for each model as well as Pearson correlation coefficients between environmental variables.

RESULTS

Physical and chemical properties of the study lakes

The sites from North Carolina and the Pinelands of New Jersey are situated in non-glaciated areas on the Atlantic Coastal Plain, whereas those from the 3 remaining regions were previously glaciated. Waterbodies on the Atlantic Coastal Plain are more similar to each other, as are those in Nova Scotia and Newfoundland, than they are to those in Connecticut (Table 1). Although different from the other 4 regions, waterbodies in Connecticut are more similar to those in the Canadian Maritime than those along the Atlantic Coastal Plain. The mean maximum and mean minimum January temperatures ranged from 12.8 to \(-1.7^\circ C\) and 1.1 to \(-5.6^\circ C\), respectively, across all regions (Table 1). There is \(\sim 10\) to \(12^\circ C\) difference in the mean maximum and mean minimum temperatures during July in all regions.

On average, the Connecticut waterbodies have higher pH and alkalinity and are clearer waterbodies with deeper Secchi disk depths than waterbodies in the other 4 regions. In contrast, sites on the Atlantic Coastal Plain are more acidic and poorly buffered, have high concentrations of colored dissolved organic matter, and have low Secchi disk depths. A mixture of clear water and humic-stained waterbodies characterize those in the Canadian Maritime regions. Although the pH ranged from 3.5 to 8.6, a total of 64 Canadian Maritime waterbodies had a pH <6. Lakes in Nova Scotia and Newfoundland have lower concentrations of sulfate and potassium than those in the other regions. Based on chlorophyll \(a\), total phosphorus, and total nitrogen concentrations, the most eutrophic lakes are situated in North Carolina and New Jersey, whereas the more northern and glaciated sites become more oligotrophic with lower nutrient and chlorophyll \(a\) concentrations.

Diversity and abundances of siliceous shell plates

Siliceous shell plates were separated into 7 different morphotypes for quantitative purposes (Table 2, Figs 1A–Y, 2A–H, 3A–I). Circular shaped plates (Figs 1H–K, 3E, F) were the most abundant type, ranging from 32.6 (North Carolina) to 64% (Connecticut) of the total/region and accounting for 45% of plates from all sites. Except for North

<table>
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<th>Parameter</th>
<th>NC (m)</th>
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<th>CT</th>
<th>NS</th>
<th>NFL</th>
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Table 2. Relative abundances of 7 euglyphid plate morphotypes in surface sediments of waterbodies from 5 regions along the east coast of North America. The percentage of waterbodies containing each plate type/region is also given, and data is summarized across all regions. Types include plates that are circular (cir), oval (ova), square (squ), rhomboid or rectangular (rho), scutiform (scu1, scu2) and toothed (too). The regions include Connecticut (CT), North Carolina (NC), New Jersey (NJ), Nova Scotia (NS), and Newfoundland (NFL).

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<th>ova</th>
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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.
Carolina localities, circular plates were the most abundant type in all other regions. Circular plates were widely distributed, recorded in 95% of all study lakes. Oval plates (Figs 1D–G, 3G) were the 2nd most abundant plate type, with maximum abundances recorded from waterbodies along the Atlantic Coastal Plain in New Jersey and North Carolina, where they accounted for 39.7 and 37% of all plates, respectively (Table 2). Collectively, circular and oval morphotypes comprised 75% of all plates. Square- or quadrangular-shaped plates (Figs 1A–C, 3H, I) accounted for 8% of the total and were relatively evenly distributed between regions, but they were noticeably rare in Connecticut lakes. In contrast, quadrangular plates were present in all of the New Jersey sites. Rhomboidal and rectangular-shaped plates (Figs 1Q–S, 3A–D) were found in all regions and in 46% of the study sites; however, they accounted for only 2.7% of all plates.

Scutiform plates with a bilateral symmetry were separated into 2 groups based on the shape along the wider portion of the plate. The wider end of the plate, referred to as the aboral end, faces the posterior of the test, whereas the narrower end faces the oral end of the test. The 1st scutiform-shaped plate, morphotype 1, has strongly undulating margins along both the aboral and oral ends of the plate, resulting in 3 projections on each end (Figs 1N–P, 2A, B). The 2 outer projections on the aboral end of the plate are highly accentuated and extend slightly further than the central process or projection, yielding a butterfly shape. The margins of the plate connecting the aboral and oral ends are relatively straight to slightly curved and tapering, completing the bilateral design. Except for the central projection, the lateral margins along the aboral end of morphotype 2 are broadly rounded, and they are not as accentuated and projected out from the plate as they are on morphotype 1 (Figs 1U–Y, 2D, E, G, H). Narrow scutiform morphotype 2 plates have a shape that resembles a lemon rather than a butterfly (Figs 1U, V, Y, 3G). Scutiform
morphotypes 1 and 2 accounted for only 4 and 8% of all plates enumerated, respectively. However, morphotype 1 was significantly more abundant and accounted for between 5.6 and 11.8% of the total in the 3 northern regions, Connecticut, Nova Scotia, and Newfoundland. They were much less abundant in lakes situated on the Atlantic Coastal Plain where they accounted for <1% of all plates. Despite low abundances, morphotype 1 was present in 48 and 22% of the waterbodies in North Carolina and New Jersey, respectively. Morphotype 2 was much more evenly distributed among regions (Table 2).

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

Siliceous plate types differed among regions, as illustrated by NMDS. The ordination of sites based on plate morphotype composition (Fig. 4) clearly shows the high degree of similarity between New Jersey and North Carolina, the 2 southernmost localities included in the study. Most Connecticut sites also separate from the more southern regions and are more similar to other northern regions (Nova Scotia and Newfoundland). Differences in the distributions of plate types among regions are supported by the NMDS visualization. First, quadrangular plates have the most limited distribution, are clearly more abundant in New Jersey and North Carolina, and are largely lacking in Connecticut sites (Fig. 5D). Second, the distributions and abundances of scutiform morphotype 2 and rectangular plate types largely overlap (Fig. 5C, F). Third, butterfly-shaped scutiform morphotype 1 plates have greater abundances in northern regions and are noticeably less abundant in North Carolina and New Jersey (Fig. 5E). Fourth, circular plates exhibit the widest distribution and can be found in high abundances in lakes from multiple regions, but less so in Connecticut (Fig. 5A). Fifth, oval plates are also widely distributed with highest abundances in more southern regions and lower concentrations in many Connecticut localities (Fig. 5B).

Siliceous plate abundance and environmental variables

Water depth had a substantial influence on testate plate concentration (linear regression, $p < 0.001$; $R^2 = 0.43$), where deeper lakes had substantially lower abundances of plates (Fig. 6A). The highest abundances of plates were found in waterbodies <3 m in depth, with the greatest numbers in sediments from shallow ponds <1 m deep. The relationship between pH and testate plate concentration was also important (linear regression, $p < 0.001$; $R^2 = 0.45$), where abundance of plates increased with a decrease in pH (Fig. 6B).

Most waterbodies with pH >7 had <1 plate/µg dry-mass sediment. In contrast, waterbodies with a pH <5 often had 3 to over 10 plates/µg dry-mass sediment.

The best subset regression analyses confirmed the importance of pH and water depth in determining testate plate abundance and highlighted other potentially important environmental variables. All of the top 10 models identified with best subset regression included the variables pH, log water depth, and potassium, and the model with only these 3 variables yielded an $R^2$ of 0.56 and a $C_p$ value of 3.95, almost equal to the number of variables in the model + 1. Each of the 3 variables was highly important ($p < 0.001$) and the model yielded a low standard error of estimation (0.31). In addition, of the 10 best subset regression models, this model yielded the lowest PRESS statistic (18.9) and best relationship for predicting plate concentration (Fig. 6C). Another model identified in the best subset analysis included a 4th variable, sulfate. However, this model yielded a slightly larger standard error of estimation (0.34) and a larger PRESS statistic (19.1). Several of the remaining models added additional variables, including specific conductivity and total nitrogen, but these models did not improve $R^2$ values and yielded higher $C_p$ values (indicating increased bias), higher standard error of estimation values, and higher PRESS values. Although water color was strongly correlated with other variables included in the models (Table 3), it was not included in any of the top models identified in the best subset regression analysis. Based on the best subset regression analysis, the
A simpler model including pH, log depth, and potassium was deemed best supported.

**DISCUSSION**

We documented the distributions and concentrations of siliceous plate morphotypes in freshwater sites along the east coast of North America. We further investigated the relationships between environmental variables and concentrations of siliceous plates, with a particular focus on pH and water depth. Because similar plate morphotypes have been uncovered in lake mudstones dating to the warm Eocene, we discuss the future possibility of using these remains to reconstruct ancient environmental conditions.

**Testate amoebae assemblage characterization from plate morphotypes**

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, 1872; *Trinema* Dujardin, 1841; *Sphenoderia* Schlumberger, 1845; *Corythion* Taranek, 1918; *Tracheleuglypha* Deflandre, 1928; and possibly *Puytoracia* Bonnet, 1970. We believe that most of the
rectangular-shaped or rhomboidal scales 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 because 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 6 species (De Smet 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 (Foisner and Schiller 2001, De Smet and Gibson 2009, Schiller and Wuttke 2015). Based on our findings, and especially because both morphotypes were not always uncovered in the same collections, the 2 shield-shaped morphotypes probably represent 2 different *Scutiglypha* species.

Although some authors question the validity of the genus *Scutiglypha*, the arguments for separating it from *Euglypha* were reviewed by De Smet and Gibson (2009). In addition to the obvious differences in plate morphology, our findings indicate that there is also a major difference in the structure of the denticulate plates that surround the pseudostome. The denticulate plates of *Euglypha* usually have 5 to 7 teeth and 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 was always found in samples with circular or oval plates, which indicates that many of the latter plate morphotypes may indeed belong to *Euglypha* because 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 smaller teeth and a less prominent median tooth, and the teeth

Figure 6. Abundance of testate amoebae plates vs lake depth (A) and pH (B) in 125 freshwater sites along the east coast of North America. Predicted vs actual abundances of testate amoebae plates using a multiple-regression model based on 3 variables: pH, water depth, and concentration of potassium (C). Coefficient of determination $r^2$, $p$-value = $p$, standard error of estimation $\text{SEE}$.

Table 3. Pearson correlation coefficients for the environmental variables pH, log water depth (depth), log color (color), and concentrations of potassium (K) and sulfate (SO$_4$). $^*$ $p < 0.001$

<table>
<thead>
<tr>
<th>Variable</th>
<th>pH</th>
<th>Depth</th>
<th>Color</th>
<th>K</th>
<th>SO$_4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depth</td>
<td>0.52*</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Color</td>
<td>$-0.34^*$</td>
<td>$-0.62^*$</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>0.09</td>
<td>$-0.25^*$</td>
<td>0.16</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>SO$_4$</td>
<td>0.1</td>
<td>0.10</td>
<td>$-0.07$</td>
<td>0.18</td>
<td>1</td>
</tr>
</tbody>
</table>
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 2 genera.

Although rare, 2 additional plate types are worth mentioning and likely indicate the presence 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 *Assulina muscorum* Grev. 1888 (Ogden and Hedley 1980). Another rare type of plate uncovered in a few samples was a small oval plate bearing a single small tooth. This plate morphotype is typical of plates that align the pseudostome of species in the genus *Trinema*.

**Siliceous plate abundance and environmental variables**

Numerous studies have reported that the abundance, diversity, and distribution of testate amoebae in freshwater are related to a combination of environmental variables including but not limited to water depth, pH, trophic status, conductivity, organic content, temperature, moisture content, and substrate type (e.g., Ogden and Hedley 1980, Collins et al. 1990, Ju et al. 2014, Roe and Patterson 2014, Amesbury et al. 2018, Tsyganov et al. 2019). Of these factors, water depth is often reported as the most important variable controlling diversity, abundance, and species composition in freshwater lakes and peatlands (Mitchell et al. 1999, Booth 2002, Patterson et al. 2012, McKeown et al. 2019, Tsyganov et al. 2019). Our findings confirm that water depth is strongly associated with testate plate abundance. Given the importance of water depth, Sonnenburg et al. (2013) attempted to use species assemblages to infer this variable over time but concluded that additional data relating specific species to specific water depths were needed. In a more recent study, Tsyganov et al. (2019) described distinctive assemblages of testate amoebae species relative to lake depth, further demonstrating that these organisms have great promise for inferring historical lake water levels.

Using testate amoebae to reconstruct paleohydrological conditions in peatlands is more advanced than using these organisms to infer lake depth (Charman 1997, Mitchell et al. 1999, Booth 2002). By combining regional-scale datasets, Amesbury et al. (2018) developed transfer functions applicable for inferring peatland paleohydrology for the Holartic. In a work based on New Zealand peatlands, McKeown et al. (2019) showed that testate amoebae-based inference models could be improved by dividing species into subsets based on size. In their study, smaller species were related to different environmental variables than larger taxa, demonstrating an even greater utility to use these organisms in historical reconstruction efforts.

Another important variable controlling both abundance and species composition of testate amoebae is pH (Escobar et al. 2008, Patterson et al. 2013). On a broad scale, many testate amoebae species are limited by pH, with some taxa being found to be more abundant in acidic habitats and others in more alkaline sites (Ogden and Hedley 1980). A similar result was reported by Patterson and Kumar (2002), who found some species of euglyphids thriving at low pH and other species, mostly of Centropyxids, more abundant at higher pH sites. In a study of subtropical lakes in Florida, USA, Escobar et al. (2008) reported the highest diversity of testates in lakes with a high pH near 8. Although our findings of increasing silica plate abundance with decreasing pH largely agree with those of Patterson and Kumar (2002), they contrast with those of Escobar et al. (2008). Many of our study lakes with a pH <5.5 had abundances of silica plates 5 to 10 times higher than in lakes with a pH >7, but a difference in species composition between the Escobar et al. (2008) study and ours (silica plate-forming euglyphids vs species that do not construct tests out of idiosomes) is likely the reason for the difference between the 2 studies. Another difference is that our study was based on the abundances of plates and not species diversity.

Lake trophic status can be another important variable determining the assemblage of 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 Driljepan 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 concentration, there were greater concentrations of testate plates in sites from North Carolina and New Jersey with 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.

Our results indicate that higher abundances of siliceous testate plates, regardless of species diversity, are found in shallow acidic ponds and lakes. This finding supported our original hypothesis that the number of testate amoebae would increase as the surface area for attachment/unit volume increased. Because the surface-area-to-volume ratio increases with decreasing water depth, it makes sense that the highest abundances of siliceous plates would be found in sediments from shallow sites. Water depth across our study region was, in turn, correlated with pH and water color. Because the vast majority of sites were in woodland settings, the shallower sites were typically more darkly stained with dissolved humic matter and had lower pH. This raises
questions such as: 1) are the concentrations of testate plates related more to water depth, pH, color, or a combination of these correlated variables? and 2) do the numbers of testates with siliceous plates increase in shallow sites simply because there is more surface area, because of lower pH, or both? Water color did not add to any of the models if either pH or water depth were included; however, all models included both pH and water depth. These findings support the idea that both water depth and pH play an important role in the growth and occurrence of these organisms.

A major difference between our approach and previous works using testate amoebae to infer environmental conditions is that our results are based on: 1) only species that produce siliceous plates, and 2) abundances of siliceous plates rather than numbers of individual species. None of the previous studies relating testate amoebae to specific environmental variables, or studies 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 abundance 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 extract the microfossils. Despite not using a metric based specifically on species, our model still accounted for a majority of the variation in plate concentrations, which is comparable to previous works based on species abundances. Undoubtedly, if other variables, such as biotope, food supply, and predator concentration, were included, a greater percentage of variation could probably be explained. With additional study, it may also be possible to improve the models by linking specific plate morphotypes to specific conditions in a similar fashion used by McKeown et al. (2019) to relate testate size classes to specific conditions.

An ultimate long-term goal is not only to use the abundances of siliceous testate plates to reconstruct water depth and pH in modern waterbodies using recently deposited sediment, but also in fossil waterbodies, including the extensive fossil lake from the Giraffe Pipe fossil locality (Siver and Wolfe 2009, Barber et al. 2013, Wolfe et al. 2017). This fossil site is of particular interest because it represents an important deep-time freshwater analog of an Arctic lake that existed under a warm greenhouse climate (Siver and Wolfe 2009, Wolfe et al. 2017). Reconstructed mean annual temperature and mean annual precipitation values for the Giraffe locality are 17°C higher and 4× greater, respectively, than present, and the region supported a warm mixed forest (Wolfe et al. 2017). Tracing the history of the Giraffe waterbody can potentially help us understand how freshwater Arctic habitats will respond to future warming scenarios. The Giraffe core contains numerous siliceous testate plates, including most of the morphotypes uncovered in our modern lake study. In addition, the concentrations of plates range widely over the length of the core, including periods of extensive numbers alternating with periods with few to no testate remains (Barber et al. 2013). Preliminary results indicated that concentrations of testate plates in the Giraffe core were positively correlated with remains of acidic and periphytic diatoms, sponge sclereids, heliozoans, and specific types of chrysophyte cysts, whereas low concentrations were found concurrent in strata where planktonic diatoms dominate (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 substantially 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 period (Barber et al. 2013), further supporting their use in reconstructing conditions found in these ancient waterbodies.

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

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