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Biogeographic patterns in scaled chrysophytes from the east coast of North America

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SUMMARY

1. We assessed the distribution of scaled chrysophytes in fresh waters along 3200 km of the east coast of North America (29° to 48°N) to determine any biogeographic patterns in relation to chemical, physical, climatic and spatial variables.
2. Scaled chrysophytes were identified using scanning electron microscopy and counted from 264 waterbodies in nine regions (20 subregions). Eighteen chemical, physical and climate variables were determined for each waterbody. We used Sorensen's similarity index and analysis of similarity (ANOSIM) to evaluate whether the floras differed between regions, subregions, glaciated and non-glaciated areas, as well as within sets of waterbodies with similar chemical and physical characteristics but situated in different regions. Distance-based linear modelling (DISTLM) was used to evaluate the relative importance of the chemical, physical and climate factors in explaining the variability in the assemblages of scaled chrysophytes, and the resulting models were visualised using redundancy analysis (RDA).
3. Significant differences in the flora were found between all regions and most subregions, and between glaciated and non-glaciated areas. Significant differences were also recorded between waterbodies with similar chemical and physical characteristics but situated in different regions. Many species were abundant along specific sections of the latitudinal gradient, but lacking from others. A set of environmental variables explained significant and independent portions of the variation in scaled chrysophytes, with pH and mean minimum July temperature accounting for 20% of the total.
4. The distribution of scaled chrysophytes along the east coast of North America is not homogeneous and there are biogeographic patterns, despite apparent dispersal mechanisms (migratory birds and wind events) that might act to reduce differences between regions. Rather, differences exist even between neighbouring subregions containing sites with statistically similar chemical and physical attributes. Environmental variables clearly play a significant role in determining whether species will inhabit a given site. However, species were not always found in waterbodies likely to support growth, implicating inadequate dispersal, poor transportability or both.

Keywords: biogeography, dispersal mechanisms, environmental gradients, scaled chrysophytes, ubiquity hypothesis

Introduction

Scaled chrysophytes are freshwater heterokonts in the Chrysophyceae or Synurophyceae with cells covered by flat, overlapping, siliceous scales (Nicholls & Wujek, 2003; Siver, 2003a). They are unicellular or colonial planktonic flagellates commonly found in lakes, ponds, sluggish rivers and wetlands. The vast majority of species, including all of the Synurophyceae, are strict photoautotrophs, although some Chrysophyceae are mixotrophs and a few (e.g. *Paraphysomonas*) are strict heterotrophs (Siver, 2003a; Kristiansen, 2005). Scaled chrysophytes are ubiquitous

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(Siver, 2003a; Kristiansen, 2005) with the richest floras often recorded from habitats that are slightly acidic, dilute and weakly buffered, with low to moderate concentrations of nutrients and humic substances (Siver, 1995).

The ultrastructure of the scales is species-specific and used in their taxonomy (Siver, 1991; Kristiansen & Preisig, 2007). Upon death, the scales detach and can be preserved in sediments for tens (Smol, 1995) to millions (Siver & Wolfe, 2005) of years. These sedimented scales can be used to identify and estimate the abundances of species that grew in the waterbody recently (in surface sediments) or in the past (down core sediments), and to reconstruct historical conditions (Cumming *et al.*, 1992; Smol, 1995; Siver *et al.*, 1999).

According to the ubiquity hypothesis outlined by Baas-Becking (1934), and more recently developed by Finlay & Clarke (1999) and Finlay (2002), in eukaryotic microbial species, the size of scaled chrysophytes should be globally dispersed (everything is everywhere), with active populations maintained wherever there are habitats that will support growth and reproduction (the environment selects). Birds, other animals including humans and wind are viewed as important vectors in their dispersal (Proctor, 1959; Schlichting, 1960, 1961; Trainor & Gladych, 1995; Kristiansen, 2001) and, assuming such vectors are highly effective and that suitable habitats are available globally, the ubiquity hypothesis would predict that scaled chrysophytes should lack distinct biogeographic patterns. In contrast, based on a review of all known records, Kristiansen (2001) argued that scaled chrysophytes have distinct patterns and questioned the ubiquity hypothesis with regard to these organisms. However, factors that may result in discrete distributional patterns of scaled chrysophytes are not fully understood, and Kristiansen (2000, 2001) further stated that little is known about their dispersal.

We used surface sediments to quantify scaled chrysophytes, coupled with data on 18 physical, chemical and climatic variables, from 264 waterbodies distributed from Florida to Newfoundland (29° to 48°N) to investigate the biogeography of scaled chrysophytes along the east coast of North America. Our study area spans 3200 km and includes subtropical to northern coniferous forested zones. It is a major migratory bird route, a primary region over which storms and hurricanes routinely track, is home to millions of humans, and possesses thousands of freshwater habitats that support rich assemblages of scaled chrysophytes. Our hypothesis was that given ample habitats for growth, coupled with adequate dispersal vectors, assemblages of scaled chrysophytes would be similar along the east coast of North America.

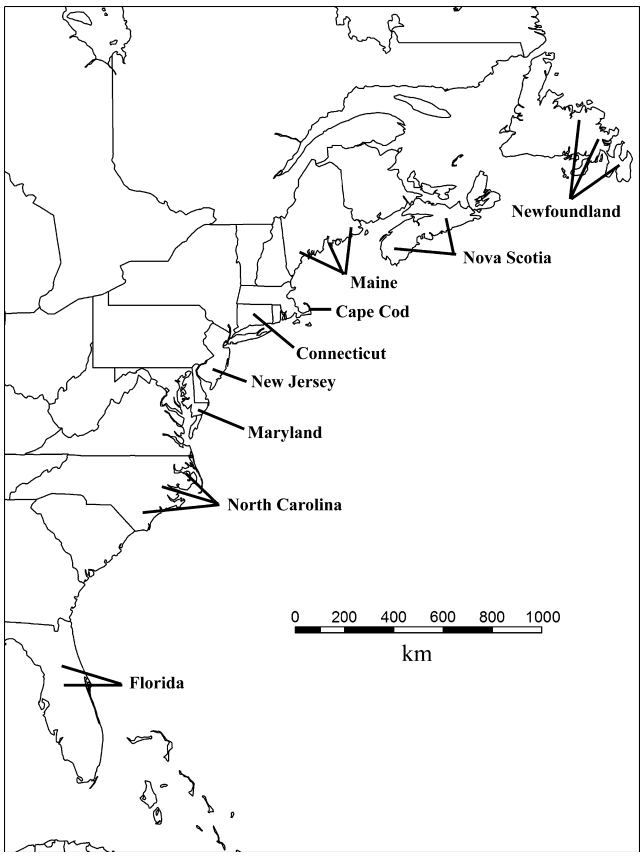

Fig. 1 Locations of the nine study regions along the east coast of North America.

Methods

The 264 study sites, sampled between 1991 and 2006, are distributed into nine regions, including the Ocala National Forest in Florida (Ocala), coastal North Carolina (North Carolina), sites on the Delmarva Peninsula in Maryland (Maryland), the Pinelands National Preserve in New Jersey (New Jersey), Connecticut, Cape Cod, coastal Maine, Nova Scotia and Newfoundland (Fig. 1). Waterbodies in some regions are further divided into subregions based on differences in geology, lakewater chemistry or geographic location. Canavan & Siver (1994) separated waterbodies in the Connecticut region into five subregions according to differences in geology, including the Coastal Slope, Eastern Uplands, Western Uplands, Central Valley and Marble Valley. In a similar fashion, lakes on the Cape Cod peninsula were divided into four subregions, the Bicep, Elbow, Forearm and Provincetown, identified by differences in water chemistry and location on the peninsula (Ahrens & Siver, 2000). Lakes on the Atlantic Coastal Plain within North Carolina (Lott & Siver, 2005) and those along the coastal Maine

region were each clustered into three different subregions. The three subregions in North Carolina represent waterbodies in the Croatan National Forest, the Pocosin National Wildlife Refuge and the Bladen Lakes State Forest, and those along coastal Maine in the China Lakes area, Acadia National Park and the Moosehorn National Wildlife Refuge. The remaining five regions, Ocala, Maryland, New Jersey, Nova Scotia and Newfoundland, were not further subdivided, yielding 20 total regions/subregions. We recognise that the number of ponds on the Delmarva Peninsula was small (n = 5), but we decided to maintain this region because of apparent differences in chemical characteristics. The study sites south of Connecticut are all in non-glaciated areas on the Atlantic Coastal Plain, while all sites north of the New Jersey localities are of glacial origin, with the exception of the small suite of post-glacial lakes on the outer tip of Cape Cod in Provincetown.

Sediment cores were taken from the deep basin of each site with a Glew gravity corer (Glew, 1988) and sectioned into 1-cm units using a mechanical extruder (Glew, 1989). The 0- to 1-cm surface section of each core was used to identify and quantify scaled chrysophytes from each site. Surface sediment samples are commonly used to study chrysophytes (and diatoms) as they effectively integrate growth of all organisms over the course of a year from the entire water column (Smol, 1995). Temperature, dissolved oxygen, chlorophyll-*a* (relative fluorescence) and specific conductivity profiles were made with a Hydrolab Datasonde 4a (Hach Hydromet, Loveland, CO, U.S.A.), and the Secchi depth was measured with a 20-cm black-and-white disc. All water samples used for chemical analyses were taken with a van Dorn water sampler at a depth of 1 m. Geographic coordinates were made with a Trimble Geoexplorer GPS unit.

Chemical analysis followed the procedures of Canavan & Siver (1994), Ahrens & Siver (2000) and Lott & Siver (2005). Briefly, samples for pH were taken in polyseal containers and measurements made the day of collection using a Fisher Accumet 640-A pH Meter (Fisher Scientific, Hampton, NH, U.S.A.). Samples for alkalinity were also collected in polyseal containers and measured by the Gran titration method using a 0.2-N reagent grade acid titrant (Wetzel & Likens, 1991). For absolute chlorophyll-*a* analysis, approximately 0.5–1 L was filtered and the pigment extracted in acetone and estimated using the trichlorometric method (APHA, 1985). Total phosphorus was determined using the stannous chloride-ammonium molybdate colorimetric assay after a persulphate digestion (APHA, 1985). Total nitrogen samples were digested using alkaline persulphate oxidation (D'Elia, Steudler & Corwin, 1977) and analysed using the *N*-(1-naphthyl)-ethylenediamine dihydrochloride method (US EPA method 353.2, 1983). Sulphate and chloride concentrations were measured using anion chromatography (US EPA, 1983), and base cations (sodium, potassium, calcium and magnesium) were estimated using flame atomic absorption spectroscopy with a Perkin Elmer 2380 spectrophotometer (PerkinElmer, Waltham, MA, U.S.A.). Water colour, largely determined by the concentration of dissolved humic material, was estimated using the platinum-cobalt method (APHA, 1985). All chemical and physical data are available at http://silicasecchidisk.conncoll.edu.

Mean minimum and maximum temperatures for the months of January and July were estimated for each study lake using either the GHCN Global Climate database or the NCDC Summary of the Day (Eastern United States) database published by EarthInfo Inc (Huntington Beach, CA, U.S.A.). For each lake, we determined the closest weather station within the EarthInfo database. Practically, all of the weather stations we used had 100% coverage for at least the last 20-year period, and in all cases, we used no more than the last 30 years of temperature records to estimate means. In a few areas with limited coverage (coastal North Carolina and on the Cape Cod peninsula), the closest weather station was the same for multiple sites.

For the analyses of scaled chrysophytes, after thoroughly mixing the 0- to 1-cm section of each core, approximately 10% (0.5–1.0 g) of the sediment was removed, oxidised with a sulphuric acid-potassium dichromate solution according to Marsicano & Siver (1993) and washed with distilled water. Aliquots of each sediment slurry were dried onto aluminium foil and evaporated onto circular glass coverslips using Battarbee trays (Battarbee, 1986). The aluminium foil samples were trimmed, attached onto aluminium stubs using Apiezon wax, coated with a mixture of gold and palladium with a Polaron model E5 100 sputter coater and observed with a Leo (Zeiss, Thornwood, NY, U.S.A.) 982 FESEM or a Leo 435V scanning electron microscope (SEM). Glass coverslips were mounted in Naphrax mounting medium and examined at 100× with an Olympus BX-51 or a Leica DMR light microscope (Leica Microsystems, Wetzlar, Germany).

All samples were first thoroughly examined with SEM, and identifications, images and relative abundances of all scaled chrysophytes were made. Specific care was taken to identify scales from species that cannot be either identified or separated from similar taxa using light microscopy. These species were noted and relative abundances calculated. After SEM analysis, a minimum of 300 scales were counted using the glass slide mounts. Scales of

species that could not be separated with light microscopy were initially lumped and later divided based on the SEM estimates. On average, over 95% of the taxa observed with SEM were found and included during counting with light microscopy. Occasionally, scales of rare species verified with SEM were not found using light microscopy. Since these were confirmed species records and their presence of importance in the biogeography analysis, we added the taxa to the counts and each was scored a value of 1.

We used Sorensen's similarity index as a measure of beta diversity, to evaluate the differences in species diversity between regions along the latitudinal study area. Two data matrices, a species matrix and an environment matrix, were constructed and imported into Primer-E (ver. 6.1.12, Clarke & Warwick, 2001) for further analyses. The species matrix consisted of relative abundances of each scaled chrysophyte taxon at each site (75 × 264). The environment matrix consisted of values for all chemical, physical, location and climate variables for each site (18 × 264). Species abundances were first transformed using a square root transformation, and a resemblance matrix subsequently formed using a Bray–Curtis measure. For the environment matrix, all variables except pH and alkalinity were log (X + 1) transformed and normalised, and a Euclidean distance metric was used to construct a corresponding resemblance matrix. The transformed matrices and resulting resemblance matrices were used for all further analyses within Primer-E. Species abundances were also transformed using fourth root and logarithmic algorithms, but because these yielded similar conclusions, only results based on a square root transformation are presented.

Cluster analysis was used to identify groups of waterbodies with similar chemical and physical characteristics. Analyses were performed for the entire suite of lakes (n = 264), as well as based on averages for regions or subregions. In each case, the cluster routine used a resemblance matrix constructed from only chemical and physical characters of the waterbodies (location and climate variables were removed), group-average linkage, and a SIMPROF (Primer-E) test was applied to detect significant nodes (groups). We then used analysis of similarity (ANOSIM, Primer-E), and additional cluster analyses coupled with SIMPROF tests, to see whether the scaled chrysophyte floras differed within groups of lakes found to have similar environmental characteristics. The ANOSIM routine uses *a priori* structured data (e.g. groups of lakes determined to have similar chemical and physical attributes or ones located in the same subregion) and a test statistic, R, derived from the resemblance matrix. Essentially, R is a measure of the difference in the mean rank between groups and the mean rank within a group (Clarke & Warwick, 2001; Anderson, Gorley & Clarke, 2008). In Primer-E, the calculated value for R is compared to a null distribution generated by a permutation routine (n = 1000) that randomly shuffles the data (e.g. species abundances) associated with each site. The probability that R is part of the null distribution is then estimated.

Principal component analysis (PCA) and non-metric multidimensional scaling (MDS) were used to ordinate and display sites based on Euclidean distance for environmental characteristics or rank order of Bray–Curtis measurements for species data, respectively. SIMPER (Primer-E), similarity percentage analysis, was used to identify sets of species that best characterise the scaled chrysophyte flora within and between regions or subregions, as well as between glaciated and non-glaciated zones. The DIVERSE routine in Primer-E was used to calculate three alpha diversity indices, Shannon diversity, Simpson's diversity and Pielou's Evenness index.

The relationships between the species-site data cloud (matrix) and predictor variables (environmental matrix) were analysed using distance-based linear modelling (DISTLM), and the resulting models were visualised using redundancy analysis (db-RDA) (Primer-E, Permanova + ver 1.02, Anderson *et al.*, 2008). DISTLM partitions the variation in the species-site matrix, as described by a resemblance matrix, according to a regression model based on quantitative predictor variables (environmental variables). This routine can also be used to analyse partial (conditional) tests, where the amount of variation explained by a given predictor variable is determined after other variables have been fitted into the model. Primer-E uses a permutation routine to derive a null distribution for test statistic values from which a P-value is calculated. The db-RDA runs an eigen analysis and produces an ordination that is constrained to be a linear combination of the predictor variables responsible for explaining significant portions of the variation within the data cloud.

Results

Differences in the physicochemical characteristics of lakes and climate between regions

Fourteen physicochemical parameters, including Secchi depth, alkalinity, pH, specific conductivity, water colour, and concentrations of chlorophyll-*a*, total nitrogen, total phosphorus, chloride, sulphate, sodium, potassium, calcium and magnesium, were estimated for all waterbodies included in the study (Table 1). Although most of the data have been published previously (Canavan & Siver, 1994, 1995; Ahrens & Siver, 2000; Lott & Siver, 2005; Siver &

Table 1 Mean values for chemical, physical and climate-related variables for waterbodies from all nine regions included in this study

Parameter	Ocala	NC	MD	NJ	CT	CC	ME	NS	NFL
SD (m)	2.53	0.7	2	1.19	3.32	4.38	3.31	2.68	1.46
ALK (μeq L^{-1})	81	−6	130	58	431	39	70	−13	89
pH	5.13	4.31	5.44	5.16	7.06	6.15	6.46	4.99	5.71
Spec Cond (μS)	66	100	82	75	98	106	39	35	53
CHL-*a* (μg L^{-1})	4.2	23.5	13.3	9	6.5	3.1	3.1	1.7	1.5
Colour (Pt-Co)	34	537	151	118	30	18	39	92	74
TP (μg L^{-1})	15.1	47.3	25.2	17.8	33.1	14.2	1.5	5.1	12
TN (mg L^{-1})	0.55	1.25	0.91	1.02	0.44	0.25	0.26	0.18	0.37
Cl^- (meq L^{-1})	0.29	0.31	0.39	0.34	0.27	0.6	0.18	0.19	0.33
SO_4^{-2} (meq L^{-1})	0.16	0.2	0.1	0.19	0.16	0.11	0.07	0.04	0.04
K^+ (meq L^{-1})	0	0.02	0.02	0.03	0.03	0.02	0.09	0	0
Na^+ (meq L^{-1})	0.26	0.38	0.21	0.28	0.29	0.52	0.2	0.2	0.29
Ca^{+2} (meq L^{-1})	0.12	0.16	0.15	0.1	0.36	0.08	0.1	0.02	0.1
Mg^{+2} (meq L^{-1})	0.12	0.11	0.13	0.13	0.2	0.15	0.06	0.04	0.05
January T_{max} (°C)	20.9	12.8	6.5	5	1.7	3.1	−1.2	0.4	−1.7
January T_{min} (°C)	6.5	1.1	−3.3	−5.6	−7.8	−4.3	−11.4	−7.3	−9.4
July T_{max} (°C)	32.4	31.7	29.1	29.4	28.6	24.6	26.2	21.6	20.8
July T_{min} (°C)	21.3	21.7	19.4	18.3	16.9	16.7	14.4	12	10.6
Latitude	29.1	34.9	39.3	39.4	41.4	41.5	44.3	44.5	48.1
Longitude	81.4	77	75.1	74.4	72.3	70.1	68.4	64.1	53.6

Average latitude and longitude values are also given.
NC, North Carolina; MD, Delmarva Peninsula; NJ, New Jersey Pine Barrens; CT, Connecticut; CC, Cape Cod; ME, Maine; NS, Nova Scotia; NFL, Newfoundland; SD, Secchi disc depth; ALK, alkalinity; Spec Cond, specific conductivity; CHL-*a*, chlorophyll-*a*; Colour, platinum-cobalt water colour; TP, total phosphorus; TN, total nitrogen.

Lott, 2006, 2010), a few generalisations comparing regions are in order. The clearest lakes with the highest Secchi depths and lowest water colour were in Southern New England and coastal Maine. Lakes on the Atlantic Coastal Plain had darker waters with significantly lower Secchi depths. A mixture of clear water and humic-stained waterbodies characterise the Ocala National Forest and Canadian Maritime regions. Most of the waterbodies were acidic, and the mean pH values of sites in regions south of Connecticut and north of Maine were below 6. In general, lakewater alkalinity followed pH, with lowest mean values in North Carolina and Nova Scotia. Lakes in the Marble Valley and Central Valley subregions of Connecticut were highly buffered with raised alkalinity and pH values.

Based on chlorophyll-*a*, total phosphorus and total nitrogen concentrations, the most eutrophic lakes are situated along the Atlantic Coastal Plain from North Carolina to New Jersey, while sites north of New Jersey become more oligotrophic with lower nutrient and chlorophyll-*a* concentrations. With the exception of lakes in the Marble Valley and Central Valley subregions of Connecticut, sodium is the most abundant base cation, with concentrations often twice those of either calcium or magnesium. Concentrations of chloride generally follow those of sodium, and lakes with the lowest concentrations of sulphate were along the more northern latitudes.

A PCA showed considerable overlap in the physicochemical characteristics between lakes, although many within a given region clustered relatively closely together, indicating differences along the latitudinal gradient (Fig. 2). Based on this initial PCA analysis, physicochemical parameters were averaged by both region and subregion and used in separate cluster analyses, coupled with SIMPROF tests, to identify further major differences between geographic areas (Figs 3 & 4). Based on the 14 variables used in the analysis, the average waterbodies from all nine regions were significantly different from each other (Fig. 3). Dividing the waterbodies by subregion resulted in five significantly different clusters of lakes largely, but not exclusively, separated according to location along the study region (Fig. 4). Lakes from Newfoundland, Nova Scotia and all subregions of Maine formed one distinct cluster. The second group consisted of lakes from two areas on Cape Cod, the Elbow and Forearm. Lakes from the Ocala National Forest clustered with ones from the low alkaline subregions of Connecticut and the Bicep area of Cape Cod. The fourth cluster contained all of the subregions along the Atlantic Coastal Plain and the non-glaciated lakes on outer Cape Cod (Provincetown). Cluster five contained all of the waterbodies within the Marble Valley and Central Valley of Connecticut.

The mean minimum air temperature for January ranged from 6.5 °C (Ocala) to −14.2 °C (Moosehorn) (Table 1).

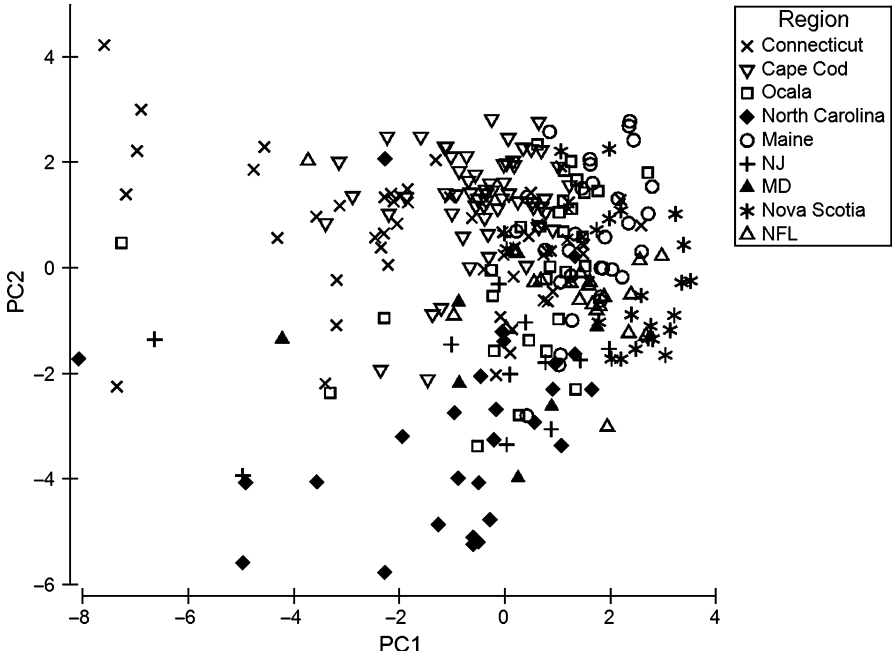

Fig. 2 Results of a principal component analysis (PCA) analysis based on physicochemical characteristics of waterbodies from nine regions along the east coast of North America.

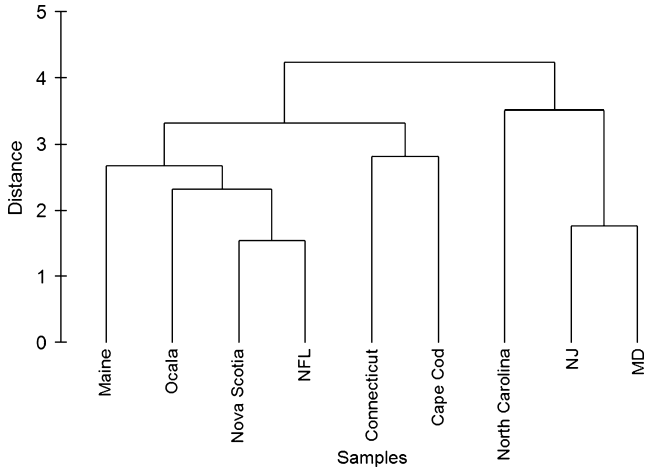

Fig. 3 Results of a cluster analysis based on physicochemical characteristics of waterbodies from nine regions along the east coast of North America. Based on a SIMPROF test, waterbodies from all nine regions were significantly different.

The mean maximum January temperature ranged from 20.9 °C (Ocala) to −2.2 °C (China Lakes subregion). The minimum and maximum July temperatures were highest in coastal North Carolina (21.7 °C) and Ocala (32.4 °C), respectively, and lowest in Newfoundland. The largest range in mean temperature over the course of a year was 40.8 °C for the Moosehorn subregion, while the lowest range of 25.9 °C was recorded in Ocala. There was a much wider difference in winter temperature (over 20 °C) than summer temperature (near 11 °C) over the latitudinal span of the study sites.

General characteristics of the scaled chrysophyte flora

Three of the 264 waterbodies studied were removed owing to limited numbers of scaled chrysophytes. A total of 75 taxa from the genera *Mallomonas*, *Synura*, *Chrysosphaerella* and *Chrysodidymus* were identified and quantified from the 261 remaining study sites (Table S1). Fifteen additional species from the genera *Spiniferomonas* and *Paraphysomonas* were also identified with SEM, but since disarticulated scales from these taxa cannot always be assigned to the correct organism, they were not included in further analyses. Of the 75 species, only 11 were found in all nine regions (Table S1). Fourteen species each accounted for over 2% of the total number of scales, with *Synura echinulata* (18.4%), *Mallomonas duerrschmidtiae* (12.7%), *Synura petersenii* (8.2%), *M. caudata* (6.8%) and *S. sphagnicola* (6.1%) representing the most abundant species along the entire study area.

Four diversity estimates, including the number of species (*S*), Pielou's Evenness measure (*J'*), Shannon diversity (*H'*) and Simpson's estimate (1−*λ'*), were calculated for each of the nine regions (Table 2). The mean number of species varied from 28 in Maryland to 49 in New Jersey, although there were only slight differences in the other diversity estimates between regions (Table 2).

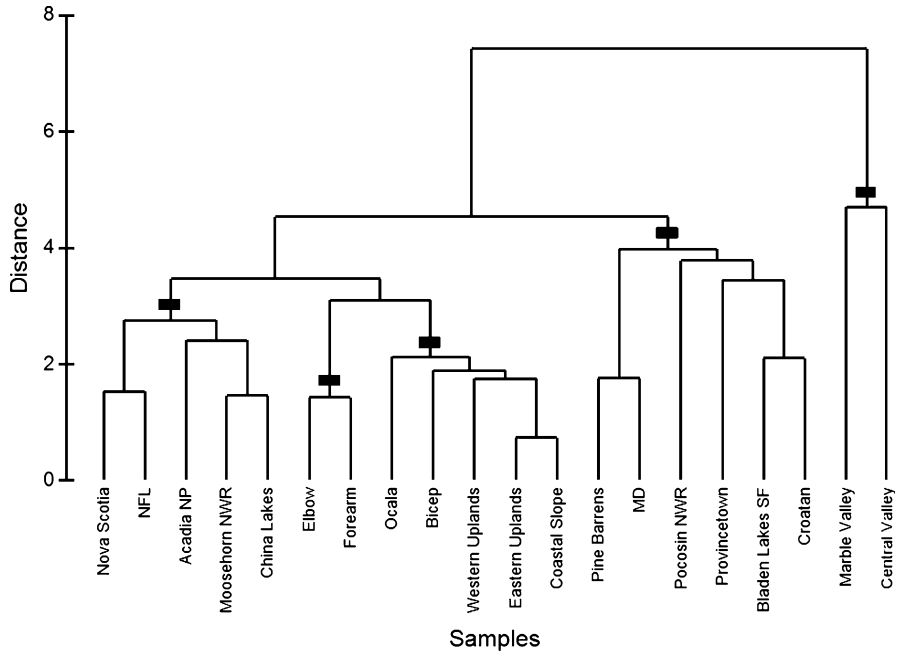

Fig. 4 Results of a cluster analysis based on physicochemical characteristics of waterbodies from 20 subregions along the east coast of North America. Five significant groups (denoted by the black bars) were detected using a SIMPROF test.

Table 2 Mean diversity measures of scaled chrysophytes in waterbodies from nine regions along the east coast of North America

Region	No. of species (*S*)	Pielou's Evenness (*J'*)	Shannon (log e) (*H'*)	Simpson's (1−*λ'*)
Newfoundland	37	0.69	2.49	0.87
Nova Scotia	38	0.64	2.32	0.84
Maine	47	0.66	2.56	0.9
Cape Cod	37	0.77	2.77	0.92
Connecticut	46	0.74	2.83	0.92
New Jersey	49	0.62	2.42	0.8
Maryland	28	0.66	2.21	0.84
North Carolina	30	0.7	2.38	0.87
Ocala	43	0.75	2.81	0.92

Measurements include the number of species (*S*), Pielou's Evenness measure (*J'*), Shannon diversity based on log e (*H'*) and Simpson's diversity estimate (1−*λ'*).

New Jersey and Nova Scotia had more species but slightly lower evenness scores, indicating that these regions contained more sites dominated by fewer species. Connecticut, Cape Cod and Ocala were slightly more diverse based on *J'*, *H'* and (1−*λ'*) estimates.

Comparison of scaled chrysophytes between regions and subregions

Initially, we used Sorensen's similarity index as a measure of beta diversity (*β*-diversity) to examine differences in the species compositions between the nine regions (Fig. 5).

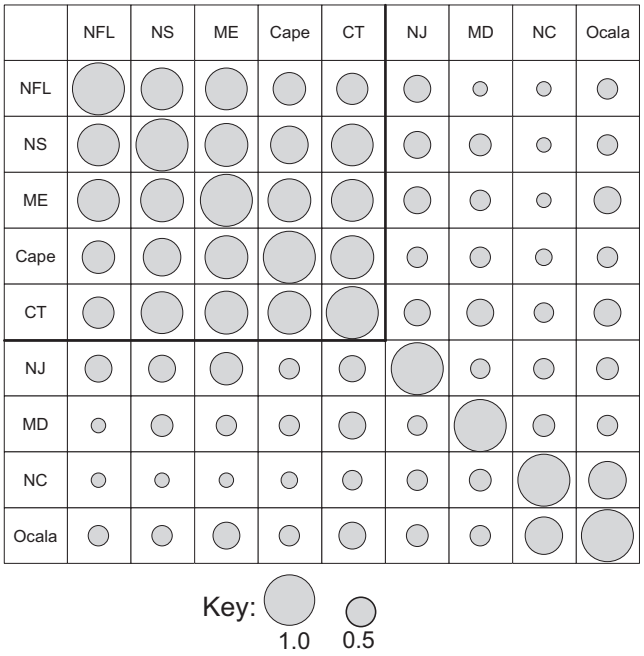

Fig. 5 Sorensen's similarity values denoting beta diversity in the scaled chrysophyte floras between all nine regions along the east coast of North America. The larger the circle, the greater the similarity between any two regions. In general, the highest similarity values are between regions within the glaciated zone (demarcated by thicker lines).

Values ranged from a minimum of 0.5, indicating that only half of the species are common between regions, to a maximum of 0.82, where over 80% of the species are

shared. In general, glaciated regions have the most species in common, and β-diversity increases with distance with the largest differences observed at the demarcation with non-glaciated regions (Fig. 5). The Ocala, North Carolina, Maryland and New Jersey regions have the most distinct floras and share far fewer species with any other region. The range in mean β-diversity values, comparing any non-glaciated region with all glaciated regions, is 0.54–0.67, similar to the range of 0.6–0.67 when compared to other non-glaciated areas. In contrast, the floras from regions within the glaciated area have a range in mean β-diversity of 0.76–0.82, indicating more similar assemblages.

We next used ANOSIM to identify differences in the assemblages between regions and subregions, and between glacial and non-glacial areas. Whereas the Sorensen's similarity index is based solely on the presence or absence of species, the ANOSIM analyses is based on a Bray–Curtis measure that incorporates abundance information for all taxa. Numerous highly significant differences were observed, further indicating that the scaled chrysophyte assemblages are not uniform along the east coast of North America. Of the 36 pairwise tests made between the nine regions, only three were statistically similar: Ocala and Maryland, North Carolina and New Jersey, and North Carolina and Maryland. All of the remaining 33 comparisons were significantly different, with the largest differences noted between regions previously glaciated versus regions further south in the non-glaciated zone. The scaled chrysophyte floras were also significantly different between all pairwise comparisons of regions situated within the glaciated zone.

Based on the ANOSIM analysis, numerous differences in the scaled chrysophyte floras were also observed between subregions. Of the 190 pairwise tests, 143 yielded significant differences (each with only one or no estimated values for R smaller than the observed value out of 1000 permutations), while 47 comparisons indicated similar assemblages. Based on the 47 similar comparisons, five generalisations can be made. First, there were no differences between any of the three North Carolina subregions and Maryland. Second, the flora from the Eastern Uplands of Connecticut was similar to the other geologically similar subregions in Connecticut (Western Uplands and Coastal Slope), the Bicep subregion of Cape Cod and all three subregions along the Maine Coast. Third, the two more northern subregions of Maine, Acadia National Park and the Moosehorn Wildlife Refuge, shared similar scaled chrysophyte floras with Nova Scotia and Newfoundland although, interestingly, the floras from the latter two regions were significantly different. Fourth, the flora within the suite of post-glacial waterbodies in Provincetown on outer Cape Cod shared similarities with those from all other non-glaciated subregions. Fifth, the flora in the Ocala National Forest was similar to all other subregions along the Atlantic Coastal Plain, the Coastal Slope of Connecticut and the Moosehorn Wildlife Refuge in northern Maine.

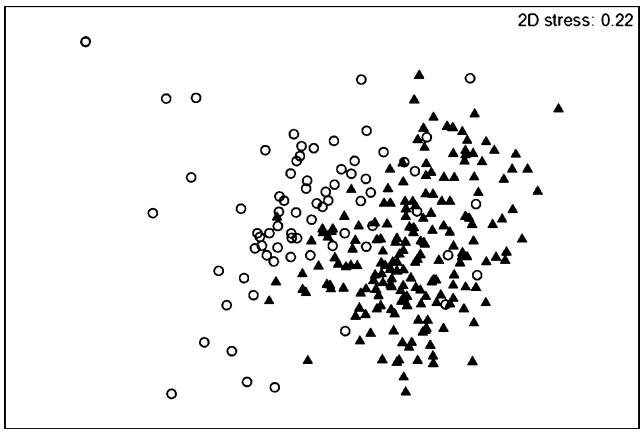

Fig. 6 A non-metric multidimensional scaled (MDS) ordination of all sites based on scaled chrysophytes. Note the strong separation of waterbodies between glaciated (solid triangles) and non-glaciated areas (open circles).

We also used an ANOSIM analysis to test for a difference between the flora in glaciated and non-glaciated areas. The Provincetown lakes on Cape Cod were included in the non-glaciated group. The floras were significantly different between these two categories, and the majority of sites were clearly separated when plotted in an MDS ordination (Fig. 6). This relationship was further investigated using a SIMPER analysis to identify differences in the species assemblages between the two floras. A total of 32 species accounted for over 90% of the difference, and 10 taxa accounted for 52% of the variation (Table 3). *Mallomonas wujekii*, *Chrysosphaerella longispina*, *M. galeiformis*, *M. multiunca* var. *pocosinensis* and *M. corymbosa* var. *poseidonii* were abundant in either the glaciated or non-glaciated area, but absent from the other (Table 3). *Mallomonas wujekii* accounted for over 12% of the scales counted in the Ocala region, was the most abundant species in the North Carolina sites (26%), was recorded in the New Jersey Pinelands, but was not found any further north. *Chrysosphaerella longispina* had maxima in Connecticut (11%) and coastal Maine (11%) but was not present south of Connecticut. Similarly, *M. galeiformis* was only observed in waterbodies north of New Jersey, with maxima in the Canadian Maritime Provinces (14%). The distributions of *Mallomonas multiunca* var. *pocosinensis*

Table 3 Results of a SIMPER analysis using scaled chrysophytes to determine the differences between waterbodies situated within versus outside of glacial regions along the east coast of North America

	Mean % abundance					
Species	Glacial	Non-glacial	Average dissimilarity	Diss/SD	Contribution (%)	Cumulative (%)
Mallomonas duerrschmidtiae	3.11	1.09	5.88	1.05	7.69	7.69
Synura echinulata	2.46	3.47	5.51	1.24	7.2	14.89
Mallomonas wujekii	0	2.41	4.83	0.71	6.32	21.21
Synura petersenii	2.41	2.46	4.45	1.12	5.82	27.02
Mallomonas caudata	2.24	1.27	4.4	1.14	5.75	32.77
Synura sphagnicola	1.05	1.75	3.48	0.98	4.54	37.31
Mallomonas muskokana	1.24	0.85	2.87	0.89	3.75	41.06
Synura spinosa	1.47	0.86	2.78	1	3.64	44.7
Mallomonas hamata	1.42	0.59	2.66	1.09	3.48	48.18
Chrysosphaerella longispina	1.42	0	2.63	0.68	3.44	51.61
Mallomonas crassisquama	1.16	0.3	2.42	0.73	3.16	54.77
Mallomonas tonsurata	0.87	0.49	2.22	0.68	2.9	57.67
Synura uvella	0.85	0.62	2.08	0.83	2.72	60.4
Mallomonas galeiformis	1.11	0	2.08	0.7	2.71	63.11
Mallomonas pseudocoronata	0.9	0.15	1.86	0.64	2.43	65.54
Mallomonas akrokomos	0.83	0.37	1.73	0.81	2.26	67.8
Mallomonas canina	0.21	0.8	1.69	0.59	2.21	70.01
Mallomonas mangofera	0.01	0.92	1.69	0.71	2.21	72.22
Mallomonas matvienkoae	0.08	0.81	1.55	0.6	2.03	74.24
Mallomonas punctifera	0.69	0.29	1.47	0.79	1.92	76.17
Mallomonas torquata	0.51	0.41	1.35	0.72	1.76	77.93
Mallomonas lychenensis	0.57	0.18	1.24	0.59	1.62	79.55
Mallomonas multiunca var. *pocosinensis*	0	0.55	1.24	0.27	1.62	81.17
Mallomonas elongata	0.58	0.01	1.08	0.61	1.41	82.58
Mallomonas dickii	0.23	0.44	1.06	0.59	1.38	83.96
Chrysodidymus synuroideus	0.19	0.38	0.9	0.52	1.18	85.14
Mallomonas corymbosa	0.37	0.06	0.8	0.37	1.04	86.19
Synura lapponica	0.34	0.09	0.74	0.4	0.97	87.16
Mallomonas transsylvanica	0.09	0.3	0.63	0.4	0.82	87.98
Mallomonas paludosa	0.11	0.27	0.62	0.52	0.81	88.78
Mallomonas corymbosa var. *poseidonii*	0	0.33	0.57	0.4	0.74	89.53
Mallomonas binocularis	0.01	0.31	0.57	0.39	0.74	90.27

The 32 most important scaled chrysophyte taxa accounting for 90% of the variance are listed in order of importance.

and *M. corymbosa* var. *poseidonii* were much more restrictive, with maxima in North Carolina (11.6%) and Ocala (1.9%), respectively.

Do waterbodies with similar physicochemical conditions support similar assemblages regardless of location?

To address this question, we initially ran a cluster analysis using the first 14 variables listed on Table 1 (excludes temperature variables), coupled with a SIMPROF test, to identify groups of lakes with statistically similar physicochemical characteristics, regardless of location. This resulted in 15 clusters, each with eight or more lakes, that had similar conditions. Significant differences in the scaled chrysophyte assemblages were found within all 15 groups. Next, we tested for differences in the scaled chrysophyte floras within each of the five groups of subregions identified above to have statistically similar physicochemical attributes (Fig. 4). Again, significant differences in the scaled chrysophyte assemblages were also found within each of these five sets of sites. The largest differences in scaled chrysophyte assemblages were found within the group that included the Upland and Coastal Slope subregions of Connecticut, the portion of the Cape Cod peninsula closest to the mainland (Bicep) and the Ocala National Forest (Fig. 7). Eleven significant clusters were identified, many of which contained waterbodies from more than one subregion.

Which variables have the most influence on the distribution of scaled chrysophytes?

We used DISTLM to identify physicochemical and climatic variables that described significant and indepen-

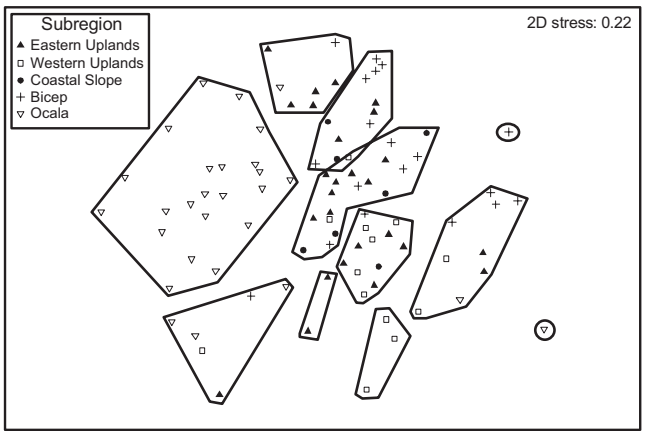

Fig. 7 A non-metric multidimensional scaled (MDS) ordination of sites from five subregions determined to have similar physicochemical conditions. The ordination is based on scaled chrysophyte assemblages. Sites found to have similar assemblages are enclosed within polygons.

dent proportions of the variance in the scaled chrysophyte flora along the east coast of North America. A DISTLM analysis based on all sites regardless of geographic location along the latitudinal gradient identified 12 significant factors that collectively accounted for 43% of the variance in the flora (Table 4). The two most important variables were pH (12%) and the mean minimum July temperature (8%) (Fig. 8). Other chemical variables included specific conductivity, alkalinity, and concentrations of potassium, chloride and sulphate. Interestingly, even after the mean minimum July temperature was included in the model, three other climate-related factors, mean maximum July and January temperatures and latitude, were still found to explain small, but significant, portions of the remaining variation (Table 4). Lastly, two variables related to water clarity, Secchi depth (5%) and water colour (2%), were also significant. When results from this analysis are plotted in RDA ordination, sites in glaciated areas clearly separate from those in non-glaciated zones (Fig. 8).

Table 4 Results of a distance-based linear modelling analysis determining the suite of environmental variables that describe significant and independent proportions of the variation in scaled chrysophyte floras between all sites

Variable	R^2	SS (trace)	Pseudo-F	P-value	Proportions
pH	0.12	79 628	35.44	0.001	0.12
July T_{min}	0.2	52 713	25.69	0.001	0.08
Secchi depth	0.25	31 945	16.5	0.001	0.05
Latitude	0.27	16 727	8.9	0.001	0.03
July T_{max}	0.29	14 802	8.1	0.001	0.02
Colour	0.31	12 670	7.1	0.001	0.02
Specific conductivity	0.33	11 656	6.67	0.001	0.02
Potassium	0.35	10 361	6.05	0.001	0.02
January T_{max}	0.36	9438.7	5.61	0.001	0.01
Chloride	0.37	5565.2	3.34	0.001	0.01
Alkalinity	0.38	6898.6	4.19	0.001	0.01
Sulphate	0.39	5760.3	3.54	0.001	0.01

Variables are listed in order of importance, and they are added to the model. The R^2 values are cumulative. This suite of 12 variables described a total of 39% of the variation. Total SS (trace) = 6.6×10^5. SS, sum of squares.

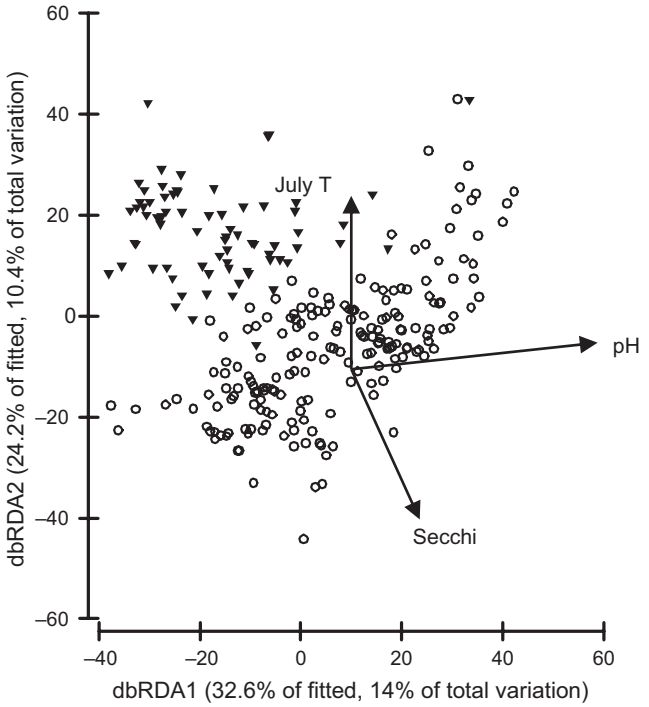

Fig. 8 The result of a db-RDA ordination of all study sites labelled as either within a glaciated region (open circles) or within a non-glaciated zone (solid triangles). The ordination is constrained to be a linear combination of environmental variables. The three most significant factors, pH (12%), mean July minimum temperature (8%) and Secchi depth (5%), are displayed.

A second DISTLM model was developed based on the 20 subregions (Table 5). The same two variables, pH (31%) and mean minimum July temperature (16%), were identified as the most influential factors. Coupled with total nitrogen, specific conductivity, water colour, alkalinity and latitude, this suite of seven variables accounted for 77% of the variation in the scaled chrysophyte floras along our study area.

Discussion

Biogeographic patterns are the result of the interplay of a complex matrix of variables and processes that include environmental and habitat constraints (often referred to as

Table 5 Results of a distance-based linear modelling analysis determining the suite of environmental variables that describe significant and independent proportions of the variation in scaled chrysophyte floras between subregions

Variable	R^2	SS (trace)	Pseudo-*F*	*P*-value	Proportions
pH	0.31	8411.6	8.031	0.001	0.31
July T_{min}	0.47	4354.4	5.106	0.001	0.16
Total nitrogen	0.55	2187.0	2.842	0.005	0.08
Specific conductivity	0.63	2185.7	3.238	0.001	0.08
Colour	0.68	1467.7	2.373	0.007	0.05
Alkalinity	0.73	1206.3	2.105	0.017	0.04
Latitude	0.77	1110.8	2.102	0.040	0.04
Chloride	0.80	923.8	2.036	0.037	0.03
Sulphate	0.83	880.2	1.773	0.050	0.03

Variables are listed in order of importance, and they are added to the model. The R^2 values are cumulative. This suite of nine variables described a total of 83% of the variation. Total SS (trace) = 27 264. SS, sum of squares.

local variables), the density of suitable habitat, dispersal mechanisms, factors preventing or facilitating dispersal, transportability of the organisms, historical factors and geological considerations (Kristiansen, 1996, 2001; Potapova & Charles, 2002; Vyverman *et al*., 2007; Verleyen *et al*., 2009; Boo *et al*., 2010). In addition, factors involved with the collection of data, such as sampling design, taxonomic resolution and taxonomic bias, can potentially influence findings and resulting conclusions (Kristiansen, 2001; Verleyen *et al*., 2009). The Baas-Becking ubiquity hypothesis (Baas-Becking, 1934), championed more recently by Finlay & Clarke (1999) and Finlay (2002), states that in organisms, the size of microscopic algae is dispersed 'everywhere', but they will be found only in habitats that subsequently support growth, i.e. the 'environment selects'. If this is true, microbes should have cosmopolitan distributions, provided suitable habitats exist, and little difference with latitude. This means that the distribution of a microbe should match the distribution of suitable habitat (Fenchel & Finlay, 2004), and other variables, such as dispersal mechanisms, are unimportant. One of the goals of the current work was to understand better the interplay between chemical, physical and climatic variables in shaping scaled chrysophyte floras and to understand whether the ubiquity hypothesis could account for observed patterns expressed by these organisms.

Results of our study conclusively demonstrate that significant differences in the scaled chrysophyte floras, as well as with the geographic distributions of individual species, exist in freshwater habitats along the east coast of North America. In fact, scaled chrysophyte assemblages were significantly different between all regions and between the majority of subregions. For many scaled chrysophytes, sites in regions that had statistically similar physicochemical conditions to those that supported growth in other areas often lacked these species. In other words, there were significant differences in the scaled chrysophytes found in waterbodies with the same physicochemical attributes (not including temperature), regardless of latitude, supporting a biogeography concept for these microbes.

Our initial hypothesis that scaled chrysophyte floras would be homogenous and lack a biogeography along the east coast of North America given ample habitat and adequate dispersal vectors was not supported. However, for a number of reasons, it is not possible to reject the ubiquity hypothesis. First, an argument can always be made that a species is not absent from a locality, but rather very rare and simply missed during the sampling process (Finlay, Esteban & Fenchel, 2004). Second, it could be argued that lakes with similar physicochemical conditions, but geographically separated along the latitudinal gradient, supported different scaled chrysophytes because of a difference in climate. Indeed, we found that climate-related variables significantly influenced scaled chrysophyte distributions. Third, it could also be argued that lakes from the same geographic area with apparently similar physicochemical conditions supported different scaled chrysophytes because of an unmeasured variable. Thus, a case could always be made for the 'environment selects' argument, making it virtually impossible to test the ubiquity hypothesis. Such arguments prompted Williams & Reid (2006) to refer to the ubiquity hypothesis as an 'opinion' and not a 'scientific statement.'

Other microbes have been found to express distinct biogeographic patterns as the result of environmental and climatic variables, and dispersal limitations (Whitfield, 2005; Martiny *et al*., 2006). Vyverman *et al*. (2007) and Vanormelingen *et al*. (2008) documented dispersal limitations in lacustrine diatoms, and Verleyen *et al*. (2009) concluded that both dispersal and local environmental variables were instrumental in shaping global distribution patterns for these microbes. Using molecular markers, Boo *et al*. (2010) reported both cosmopolitan and regionally endemic cryptic species of the scaled chrysophyte *S. petersenii*, suggesting that neither the ubiquity hypothesis nor endemism fully explains the distribution of protists. Similarly, Potapova & Charles (2002) demonstrated that even though environmental factors were most important in structuring diatom communities in rivers across the continental United States, geographic variables were also significant and many diatom species expressed limited spatial distributions. Based on a thorough review of studies of freshwater diatoms that included high-resolution

morphological characters, crossing experiments, molecular markers and impacts of humans, Vanormelingen *et al.* (2008) concluded that for these organisms, 'dispersal limitation is significant and the endemism observed in isolated areas is real'. Our findings also suggest dispersal limitation for scaled chrysophytes.

Understanding taxonomic limitations, including inconsistencies between counters, identification biases, lumping versus splitting of species and the degree of resolution used in a study (e.g. genus versus species level), can affect conclusions regarding biogeographic patterns of microbes (Heino & Soininen, 2007; Verleyen *et al*., 2009). Taxonomic inconsistencies between counters or research groups make it challenging to compare findings and perhaps impossible to combine data sets for testing hypotheses over large geographic regions. Indeed, identifying the same taxon as a different species, either because of differences in taxonomic ability or philosophy (e.g. splitting), could result in rejection of the ubiquity hypothesis when, in fact, it is potentially correct. On the other hand, lumping different species together, a practice often performed in studies of scaled chrysophytes when only light microscopy is used, could mask differences in biogeographic patterns (Verleyen *et al*., 2009). Similarly, if higher taxonomic units are employed in biogeographical works (e.g. genus versus species level), a larger study area may be needed to detect trends (Heino & Soininen, 2007).

We took a number of steps in the development of our database to reduce taxonomic inconsistencies and biases. First, all of the identifications and counts were made jointly only by the authors. Second, all identifications were made or verified using SEM, a technique that allows close inspection of fine ultrastructural detail and separation of some taxa not possible using light microscopy. Third, a substantial reference library of images was developed, continuously added to during the course of the study and used routinely. Lastly, many scaled chrysophyte species have seasonalities and grow during specific times of the year (Siver, 2003a; Kristiansen, 2005). To avoid missing species that were not actively growing at the time of collection, we analysed scaled chrysophyte remains in surface sediments, a method routinely used to investigate diatoms, chrysophytes and invertebrates in palaeolimnological works (Siver, 1993; Smol, 1995; Bennion, Juggins & Anderson, 1996; Lotter *et al*., 1997). The idea is that remains of all species that grew in the waterbody over the last few years would be part of the surface sediment record and uncovered during analysis.

Several works specifically examining the spatial patterns of freshwater diatoms over large geographic areas are noteworthy in terms of taxonomic issues and their findings regarding the ubiquity hypothesis. Verleyen *et al.* (2009) assembled a large database consisting of 1039 sites spanning 19 000 km to examine biogeographic patterns in freshwater diatoms. The large data set combined 15 smaller ones developed by 12 different research groups. To assure better taxonomic consistency and avoid identification biases, Verleyen *et al.* (2009) transformed the final data set to the genus level. Even though this reduced taxonomic resolution substantially, significant distributional patterns were still detected, demonstrating the importance of dispersal variables in shaping global diatom communities. Potapova & Charles (2002) used species-level data from 897 sites to examine distributional patterns of diatoms in continental U.S. rivers. This data set, assembled as part of the USGS NAWQA program, contained results from eight counters mostly from the same research group made over a number of years. These authors needed first to correct for inconsistencies by re-examining the taxonomic concepts of all counters and ultimately needed to lump some species into broader groups. Even after lumping, a process that would enhance acceptance of the ubiquity hypothesis, distinct geographic patterns were documented.

Many species of scaled chrysophytes have limited distributions along environmental gradients, making them excellent bioindicators (Kristiansen, 1986; Smol, 1995; Siver, 2003a). Lake pH has been found repeatedly as a key variable controlling assemblages of scaled chrysophytes in freshwater habitats (Siver & Hamer, 1989; Siver, 2003a and references therein), and numerous species have well-defined distributions along this gradient (Siver, 1991, 2003a; Kristiansen, 2005). Temperature, dissolved salt content and trophic conditions also play important roles in structuring the scaled chrysophyte flora within individual waterbodies (Siver & Marsicano, 1996; Nicholls & Wujek, 2003; Siver, 2003a; Kristiansen, 2005).

Given the findings from these previous investigations, it is not surprising that the factor describing the maximum variation in the distribution of scaled chrysophytes along the east coast of North America was pH, and that specific conductance and total nitrogen content also accounted for significant and independent portions of the total variation in the data. In addition, Secchi depth and water colour played significant roles in determining the structure of the scaled chrysophyte assemblages. Thus, lakewater chemistry and physical attributes clearly play dominant roles in shaping biogeographic assemblages of scaled chrysophytes. However, geographic-related variables, especially the mean minimum July temperature, consistently accounted for the second largest portion of variation after pH. In addition, the mean maximum July and

January temperatures, along with latitude, also explained significant and independent proportions of the variation. Collectively, this confirms that a strong latitudinal element exists which, along with chemical and physical features of the lake water, serves to shape scaled chrysophyte assemblages into the patterns observed in nature.

Our study site spans 2140 km between the latitudes of 29°N and 48°N on one continent, representing what Vyverman *et al.* (2007) would refer to as 'regional'. However, our findings firmly support the limited observations that have been postulated on a more global scale for scaled chrysophytes. Using numerous electron microscope-based surveys from all continents, Kristiansen (2001) concluded that scaled chrysophytes have distinct distribution patterns, and he classified many taxa as 'cosmopolitan', 'widely distributed', having a 'north–temperate–subarctic–arctic distribution', 'bipolar', 'tropical' or 'disjunct'. Those species deemed either cosmopolitan or widely scattered accounted for only 19% of the taxa examined and fit the definition of being ubiquitous (Finlay, 2002). On the other hand, 58% had north temperate, tropical or bipolar distributions, and 36% of the taxa were classified as endemics, meaning that the majority of species indeed have biogeographies. In our study, many species were only present along specific portions of the latitudinal gradient, with the most intriguing taxa being those found either within, or totally excluded from, previously glaciated areas. Four of these species, *C. longispina*, *M. galeiformis*, *M. wujekii* and *M. multiunca* var. *pocosinensis*, were among the most abundant scaled chrysophytes in one or more regions. The historical significance of the role that past glaciers may have played in shaping freshwater communities remains unclear, but it is possible that they served as large-scale dispersal agents for microbes or as a means of homogenising surface (till) deposits over large areas which, in turn, reduce differences in contemporary lakewater chemistry.

Several factors are noteworthy regarding the transportability of scaled chrysophytes. First, there are two dominant parts of the life cycle, the vegetative phase and the cyst phase (Asmund & Kristiansen, 1986; Siver, 2003a). Vegetative cells exist as single cells (e.g. *Mallomonas*) or small collections of cells referred to as colonies (e.g. *Synura*). Individual cells are covered with scales that normally disarticulate once the vegetative cell forms a cyst or when the cell dies. Cysts are siliceous vase-shaped structures formed within the vegetative cell as a result of either sexual or asexual reproduction and represent an encysting or resting phase for the organism (Sandgren, 1988). Cysts accumulate in, and subsequently germinate from, surface sediments. As noted by Kristiansen (2001), it is unlikely that vegetative cells are the primary means by which cells are dispersed to new sites since this phase presumably cannot survive desiccation. Indeed, vegetative cells often disarticulate and burst within hours of collection even while staying hydrated. Thus, the cyst stage is probably the phase most readily transported.

Cysts are presumed to be able to withstand desiccation and are often referred to as being highly resistant. However, the fact is we know virtually nothing about the ability of the cyst stage to withstand desiccation (Kristiansen, 2005). Resting stages of other freshwater microalgae, for example chlorophytes, are known to be able to withstand desiccation for decades (Trainor & Gladych, 1995), but it could very well be that the cyst stage of scaled chrysophytes is far less resistant to drying out and a key factor limiting dispersal of these organisms. If so, this may limit the more long-distance modes of dispersal for scaled chrysophytes relative to chlorophytes and yield different outcomes in regard to the ubiquity hypothesis.

Historical factors, including past distributional patterns and the antiquity of species, should be considered when evaluating modern biogeographic patterns (Vyverman *et al.*, 2007). In this regard, recent discoveries of scaled chrysophytes from a 40-Ma Eocene maar lake, referred to as Giraffe Pipe, situated near the Arctic Circle in Northern Canada are noteworthy (Siver & Wolfe, 2005, 2009; Siver, Lott & Wolfe, 2009). The Giraffe Pipe core contains several species, including *Mallomonas bangledeschica* (Siver & Wolfe, 2009), *Mallomonas multiunca* var. *pocosinensis* (P. A. Siver, unpubl. data) and *Mallomonas insignis* (Siver & Wolfe, 2005), with siliceous scales that are essentially identical to those formed by modern populations. Today, *M. bangledeschica* is considered a hallmark tropical chrysophyte with a distribution range encompassing only warm regions of the world (Cronberg, 1989; Kristiansen, 2001, 2002). Yet, unambiguous scales from this tropical organism found in the Giraffe Pipe core document that it was once distributed far north of its current range. This finding does not question the organism's requirement for high temperature, as it existed near the Arctic Circle during the Cenozoic hot house (Zachos *et al.*, 2001; Zachos, Dickens & Zeebe, 2008) when warm, subtropical-like conditions reached far above the Arctic Circle (Greenwood & Basinger, 1994). Perhaps, *M. bangledeschica* was dispersed northward as climates warmed, survived in the Arctic and eventually perished at high latitudes as ice house conditions returned. Alternatively, it is the possibility that this organism evolved in the Arctic during the Cenozoic hot house and then retracted to more equatorial regions resulting in the distributional pattern we observe today. Remains of *M. multiunca* var. *pocosinensis* in Giraffe Pipe also offer a new perspective on the

modern distribution of this organism. *Mallomonas multiunca* var. *pocosinensis*, known today only from a suite of sites along the Atlantic Coastal Plain (Siver, 2003b), was granted 'endemism' status (Kristiansen, 2001). However, given its much wider distribution during the Eocene, extending at least to the lower Arctic, *M. multiunca* var. *pocosinensis* represents a true palaeoendemic. This also implies that dispersal mechanisms may be less of a limitation, or perhaps very different, over time scales of millions of years.

Kristiansen (2001) classified *Mallomonas insignis* as having a 'disjunct' or 'scattered' distribution, meaning that it is a scarce taxon found over an extensive geographic area, but without any apparent pattern relative to climate or other factors. As was the case in the current study, when found, *M. insignis* never formed extensive populations. On the contrary, remains of *Mallomonas insignis* dominate many sections of the Giraffe core, often accounting for practically all of the microfossils and implying that it grew extensively, forming monoculture-like populations. Is this ancient species less competitive today, resulting in smaller populations that are more difficult to document, yielding its current disjunct status?

The historical data from Giraffe Pipe support the concept that scaled chrysophytes can be transported long distances, but how they are moved and at what incremental distances remains a mystery (Kristiansen & Lind, 2005). What are the potential dispersal mechanisms for moving scaled chrysophytes from site to site along the east coast of North America? Dispersal by birds is often cited as a likely mechanism for distributing microalgae to new localities (Proctor, 1959; Schlichting, 1960; Kristiansen, 2001), including for chrysophytes (Wee, Booth & Bossier, 1993). All of the study sites included in the current investigation are situated along one of the most important fly zones, the Atlantic Fly Zone, in North America, which also connects directly to migratory routes in South America (Berthold, 2001; Newton, 2008). If waterfowl are indeed important dispersal vectors for scaled chrysophytes, then it stands to reason that cysts are routinely moved between all regions investigated in our study.

In addition to birds, wind and insects are other common factors often attributed as important dispersal vectors for microalgae (Schlichting, 1961; Kristiansen, 2001). Larger animals, including deer and moose, could easily transport hydrated sediment and material from one site to another and may prove to be important vectors over shorter distances, and clearly humans can serve in the same capacity, but over even longer distances. Lastly, large events such as hurricanes and glaciers have the ability to move massive material over long distances, but over very different time scales. In fact, many of the hurricanes that originate in the tropical Atlantic track directly along our study regions (NOAA, 2005). Thus, one could speculate that scaled chrysophytes are easily and often transported from sites south to north along the entire latitudinal gradient included in our study. Yet 'everything' is not found 'everywhere', not even in lakes situated close together with similar characteristics, supporting the likelihood that viable cysts (or cells) are simply not being dispersed with regularity between sites, and calling into question the universally held idea that birds and wind are effective dispersal mechanisms for all microbes. Whether the transportability of cysts is poor, the dispersal mechanisms lacking, or both are true, remains unclear. As pointed out by Kristiansen (2001), it is a fact 'that almost nothing is known about the dispersal of chrysophytes'.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. The percentages of the total number of scales enumerated by region, the mean percentage for all regions, and the number of regions (#) where each species was present.

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