Further observations on the scaled Chrysophycean and Synurophycean flora of the Ocala National Forest, Florida, U.S.A.

Peter A. Siver and Anne Marie Lott

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The distributions of silica-scaled chrysophytes in 30 freshwater ponds located in the Ocala National Forest, Florida, U.S.A., relative to chemical gradients are described. Phytoplankton, periphyton, and surface sediments from each of the 30 sites were collected in March of 2000 and later analyzed extensively with both scanning electron microscopy (SEM) and light microscopy (LM) for scaled chrysophytes. In addition, water samples were used to measure a suite of chemical characteristics, including specific conductance, pH, alkalinity, total phosphorus, total nitrogen, chlorophyll-a, chloride, sulfate and base cation concentrations. Overall, waterbodies included in this study are oligotrophic, dilute, poorly buffered and low in pH. Including six previously described species which are largely known only from the Ocala National Forest, we have identified forty-nine taxa of silica-scaled Chrysophyceae and Synurophyceae, 23 of which were present in 5 or more waterbodies, indicating that lakes in this region are quite diverse and abundant in scaled chrysophytes. The most important species included Synura petersenii, S. echinulata and one recently reported new species from this region, Mallomonas wujekii, which was found in 73% of the lakes in this survey. The number of taxa found per lake ranged from 2 to 23 and observations include new records of several rarely reported species. Although the flora includes species commonly found in more northern regions, it also includes a group of taxa that appear to be endemic to the region and others commonly found in more tropical regions. Lastly, a new form found in ten ponds, Mallomonas transsylvanica f. curvata, is described.

Peter A. Siver and Anne Marie Lott, Botany Department, Connecticut College, New London, CT 06320, U.S.A. E-mail: pasiv@conncoll.edu.

Introduction

Recent studies of silica scale-bearing Chrysophyceae and taxa in the Synurophyceae have clearly indicated that a rich flora of these organisms (hereafter referred to as the scaled chrysophytes), exists in Florida waterbodies (Wujek 1984; Wujek & Siver 1997; Siver 1991; Siver & Wujek 1999; Wujek & Moghedan 2001). Siver & Wujek (1999) reported

that 67 scaled chrysophytes had been previously recorded in a few limited EM-based studies of Florida waterbodies, including original descriptions of nine new organisms (Wujek 1983; Wujek & Gardnier 1985; Wee & Wujek 1986; Wujek & Bland 1988; Siver 1991, 1994). Since the Siver & Wujek (1999) study, five additional new *Mallomonas* taxa have been described (Siver 1999, 2002a, 2002b), advocating the idea that waterbodies in Florida

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Ocala National Forest

Fig. 1. Map of the Ocala National Forest region in north-central Florida showing the locations of the 30 study lakes. All numbers correspond with the lake names in Table 1.



support a rather unique scaled chrysophyte flora and one that differs from other regions of North America studied to date.

Based on preliminary examinations of a limited number of waterbodies in the Ocala National Forest, it was clear that this subtropical region harbored a rich and diverse assemblage of scaled chrysophytes (Siver & Wujek 1999). This preliminary work was based on single collections from a suite of ten lakes and ponds from within the Forest boundary. More recently, we were able to analyze multiple collections, including surface sediments, from 30 waterbodies within the Ocala National Forest and simultaneously measure a suite of physical and chemical characteristics for each lake in order to examine further the distribution and biodiversity of scaled chrysophytes. The purpose of this study is to provide a more in-depth analysis of the distribution and biodiversity of scaled chrysophytes from this subtropical region of North America, including the description of a new form, *Mallomonas transsyl*vanica f. curvata.

In conjunction with many partners, the World Wildlife Federation launched a large-scale effort to begin the process of identifying aquatic regions in need of conservation in North America (Abell et al.

Lake Lake Name No.		Latitude	Longitude	pH units	SC µS	Color Pt-Co	TP μg/L	TN mg/L	Na mg/L		
1	Baptist	N 29° 01' 19.30"	W 81° 40' 09.95"	4.9	44	13	15	0.553	5.38		
2	Blue Sink	N 29° 03' 39.92"	W 81° 40' 13.94"	5.3	26	0	7	0.080	3.48		
3	Bryant	N 29° 08' 28.09"	W 81° 50' 31.53"	7.0	101	20	18	1.286	9.02		
4	Cathead	N 29° 25' 02.26"	W 81° 40' 54.40"	6.0	41	275	25	0.344	4.35		
5	Catherine	N 29° 03' 56.66"	W 81° 50' 04.38"	4.5	68	3	11	0.336	5.57		
6	Charles	N 29° 13' 33.05"	W 81° 54' 32.97"	6.6	103	138	29	1.663	8.39		
7	Clay	N 29° 01' 44.66"	W 81° 27' 21.33"	4.6	54	5	9	0.294	5.79		
8	Clearwater	N 28° 58' 41.28"	W 81° 33' 16.14"	4.4	63	3	9	0.105	5.43		
9	Cowpen	N 29° 01' 20.23"	W 81° 27' 31.85"	4.7	57	21	20	0.593	5.57		
10	Crooked	N 29° 09' 12.00"	W 81° 36' 06.00"	5.5	46	10	10	0.699	4.95		
11	Delancy	N 29° 25' 16.03"	W 81° 45' 53.67"	5.3	41	13	18	0.685	4.97		
12	Doe	N 29° 02' 10.92"	W 81° 49' 30.29"	4.8	63	3	8	0.234	5.78		
13	Dorr	N 29° 00' 47.64"	W 81° 38' 05.30"	5.7	75	13	12	0.388	8.35		
14	Eaton	N 29° 15' 18.34"	W 81° 51' 54.84"	7.4	231	38	23	1.021	8.33		
15	Echo	N 29° 06' 16.15"	W 81° 39' 02.39"	5.3	47	11	17	0.502	4.96		
16	Farles Prairie	N 29° 06' 16.46"	W 81° 40' 23.06"	5.0	52	3	7	0.199	4.89		
17	Fore	N 29° 16' 14.06"	W 81° 54' 54.14"	5.4	49	10	18	0.571	5.11		
18	Gobbler	N 29° 09' 38.85"	W 81° 36' 39.90"	3.8	85	225	22	0.869	5.69		
19	Grasshopper	N 29° 08' 02.66"	W 81° 37' 09.80"	4.0	65	1	7	0.026	8.03		
20	Halfmoon	N 29° 09' 31.69"	W 81° 49' 18.65"	6.3	66	13	19	0.665	6.08		
21	Hopkins Prairie	N 29° 16' 27.54"	W 81° 41' 42.28"	4.4	65	38	70	1.903	4.29		
22	Lawbreaker	N 29° 09' 41.52"	W 81° 36' 53.53"	4.0	74	1	7	0.073	5.54		
23	Lou	N 29° 14' 04.09"	W 81° 50' 58.95"	5.4	60	100	14	1.065	4.94		
24	Mary	N 29° 04' 24.68"	W 81° 49' 56.80"	4.2	87	0	3	0.085	8.21		
25	Milldam	N 29° 10' 41.82"	W 81° 50' 01.01"	6.3	60	4	11	0.489	5.66		
26	Penner	N 29° 29' 27.15"	W 81° 49' 19.99"	5.1	32	38	14	1.387	4.29		
27	Sellers	N 29° 06' 36.24"	W 81° 37' 52.68"	4.7	42	1	4	0.055	5.97		
28	Tomahawk	N 29° 08' 05.61"	W 81° 54' 10.38"	4.3	51	3	7	0.090	5.08		
29	Trout	N 29° 03' 03.30"	W 81° 49' 38.10"	4.5	66	3	8	0.082	6.20		
30	Wildcat	N 29° 10' 13.30"	W 81° 37' 34.04"	4.8	52	3	10	0.168	6.22		

Table 1. Physical and chemical data for the 30 Ocala National Forest lakes. Lake numbers correspond with those listed in Fig. 1.

2000). As an initial step, the east coast of the continent was partitioned into seven ecoregions based on the distributions of fishes, crayfishes, mussels, amphibians and aquatic insects. Each ecoregion was given a conservation priority status based on the biodiversity of the aquatic fauna, and current and future levels of habitat degradation (Abell et al. 2000). Based on conclusions for biological distinctiveness, much of the Atlantic Coastal Plain (including Florida) was scored as "globally outstanding" biological for distinctiveness. hut because of the high habitat threat assessment, this region received final ratings of either "endangered" or "vulnerable". Abell et al. (2000) recognized the need to eventually include data from other

organisms (besides animals) in order to more rigorously define and rate the ecoregions for conservation purposes. A secondary purpose of our study was to identify, for the National Forest Service, waterbodies that were particularly diverse and/or unique with respect to scaled chrysophytes and diatoms. The idea was for this type of data to be used, along with other studies, information and concerns, in making decisions regarding conservation of biological diversity within the boundary of the Forest. This study includes the scaled chrysophyte data to be considered in these conservation efforts. Table 2. The number of occurrences and maximum abundances for chrysophyte taxa found in 30 Ocala National Forest lakes. "X" indicates that no relative abundances were determined but that the taxon was observed with SEM. AWM pH is the abundant weighted mean pH for many of the species (Line et al. 1994). These scores were only calibrated for taxa found in 5 or more ponds.

Taxon name	Number of occurrences	Maximum abundance	AWM pH
Chrysodidymus synuroideus Prowse	10	1.0	4.7
Mallomonas acaroides Perty var. muskokana Nicholls	7	27.7	4.1
M. akrokomos Ruttner in Pascher	11	9.4	4.9
M. alpina Pascher & Ruttner	3	0.3	
M. binocularis Siver	4	8.0	
M. bronchartiana Compère	1	0.7	
M. caerula Siver	8	7.1	5.4
M. canina Kristiansen	11	56.3	4.8
M. caudata Ivanov	21	66.1	5.9
M. corymbosa Asmund & Hilliard var. corymbosa	1	1.7	
M. corymbosa Asmund & Hilliard var. poseidonii Siver	15	20.0	4.7
M. crassisquama (Asmund) Fott	5	24.3	6.1
M. cristata Dürrschmidt	3	1.6	
M. cyathellata Wujek & Asmund var. kenyana Wujek & Asmund	1	0.7	
M. delanciana Siver	3	13.1	
M. dickii Nicholls	15	25.8	4.6
M. duerrschmidtide Siver, Hamer & Kling	20	49.9	5.5
M. Javosa Nicholis	3	2.3	
M. guttata wujek	3	2.8	4.5
M. hamata Asmund	19	22.0	4.7
M. lychenensis Conrad	4	13.0	4 7
M. mangojera Harris & Bradley	20	10.3	4./
M. matvienkoae (Maiv.) Asmund & Kristiansen	8	4./	5.7
M. multisengera Durtschillia	2	41.1	4.0
M. multunca Asthuna M. coolensis Siver	2	8.9	
M. ocalensis Siver	2	9.0	
M. paranaidas (Harris) Momey & Déterfi von hangladashiga	4	1.7	
(Takahashi & Havakawa) Wujak & Timpana	1	6.6	
M paranaidas (Harris) Momey & Détarfi yar paranaidas	1	6.6	
M. pertonolides (mains) Monieu & Feleni vai, peronolides	1	0.0	
Gretz Sommerfeld & Wujek	3	2.0	
M pseudocoronata Prescott	5 4	40.6	
M. pseudocoronau Trescou M. pugio Bradley	2	34.0	
M. punctifera Korsh var brasiliensis Kristiansen & Menezes	3	86	
M. schwemmlei Glenk em Glenk & Fott	1	0.0	
M striata Asmund	3	0.3	
M. tonsurata Teiling em. Krieger	2	39.6	
M. torquata Asmund & Cronberg f. simplex Nicholls	5	54	47
M. transsvlvanica Péterfi & Momeu	10	29.0	49
M. wujekij Siver	22	41.0	4.9
Paraphysomonas vestita (Stokes) De Saedeleer	x	X	,
Spiniferomonas coronacircumspina (Wujek & Kristiansen) Nicholl	ls X	x	
Sp. serrata Nicholls	Х	x	
Sp. takahashii Nicholls	Х	X	
Ŝp. trioralis Takahashi	Х	X	
Synura echinulata Korsh.	24	67.6	4.7
S. petersenii Korsh.	29	64.0	4.9
S. petersenii f. truttae Siver	Х	Х	
S. sphagnicola Korsh.	12	6.6	5.0
S. spinosa Korsh.	15	12.6	5.2
S. uvella Stein em. Korsh.	3	2.7	

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Table 3. Scaled chrysophyte observations from 30 Ocala National forest lakes. Lakes are arranged in order of increasing pH and lake number corresponds to those in Fig. 1. VR = very rare; R = rare; C = common; A = abundant; D = dominant; X = noted in SEM observations. See text for details.



Study area

The Ocala National Forest is located in north-central Florida within the subtropical zone of North America (Lincoln et al. 1982) and includes a mixture of central highlands, lowlands and wetlands. The Forest is situated on a thick lens of coarse and porous sand, resulting in well drained soils. The vegetation is largely dominated by the genus Pinus, including the sand pine, slash pine and longleaf pine, but also includes a mixture of hardwoods. The Forest encompasses 430 000 acres and includes over 600 lakes and ponds. Historically, most of the lakes and ponds are solution basins that subsequently became lined with clay, and lack drainage streams (Greis 1985). The clay linings are often thick, cohesive, and impermeable (Sinclair & Stewart 1985), resulting in little or no interaction with the high pH groundwater in contact with the calcareous bedrock (Fellows & Brezonik 1980; Greis 1985). Because the majority of the waterbodies are surrounded by acidic, sandy soils with low acid neutralizing capacities and are not influenced by the alkaline groundwater, many of the lakes in the Ocala National Forest are acidic with low alkalinity. In general, most of the waterbodies are low in nutrients, dilute, and have NaCl as the most abundant dissolved salt (Greis 1985). Below, we further characterize lakes investigated in this study.

Materials and methods

Plankton, periphyton and surface sediment samples were collected from 30 waterbodies within the Ocala National Forest in March 2001 (Fig. 1). Plankton samples were made with a 10 μ m mesh net from the center of each pond or lake. Fifteen or more periphyton samples were taken from habitats around the perimeter of each lake. For the purposes of this study, aliquots of each periphyton sample were combined and prepared for SEM observation. Surface sediments were taken from the deep basin of each lake using a Glew gravity corer (Glew 1988) and sectioned with a mechanical extruder (Glew 1989). Water samples for chemical analysis were taken at a depth of 1 m from the center of each waterbody. Samples were collected in acid-washed olyethylene bottles and those for pH and alkalinity analysis were kept in bottles with air-excluding lids. The Secchi disk depth and geographic coordinates were taken from the center of each lake with a 20 cm black and white disk and Trimble Geoexplorer GPS unit, respectively.

Chemical analyses followed the procedures of Ahrens & Siver (2000). Briefly, pH was measured on the same day of collection with a Fisher Acument 640-A pH meter. Conductivity was measured with a YSI 33 SCT meter and standardized to 25 °C. Alkalinity was measured by the Gran titration method (Wetzel & Likens 1991) using Fisher reagent-grade 0.02N acid titrant. Chlorophyll-a was extracted in acetone and measured using the trichlorometric method (APHA 1985). Total phosphorus determined using the stannous chloridewas ammonium molybdate colorimetric assay after a persulfate digestion (APHA 1985). For total nitrogen, samples were first digested using the alkaline persulfate oxidation method (D'Elia, 1977) and then analyzed using the N-(1-napthyl)-ethylenediamine dihydrochloride method (U.S. EPA method 353.2, 1983). In this procedure, nitrate, which is the sole N product of the persulfate digestion, is reduced to nitrite with cadmium and coupled with sulfanilamide and N-(1-napthyl)-ethylenediamine dihydrochloride under acidic conditions to produce a colored dye that is quantified spectrophotometrically. Sulfate and chloride were estimated with anion chromatography (U.S. EPA 1983). Base cations were measured using flame atomic absorption spectroscopy with a Perkin Elmer 2380 spectrophotometer. Water color was determined by the platinum-cobalt method (APHA 1985) using water samples taken in January, 2001. The results for only pH, specific conductance, color, total phosphorus, total nitrogen and sodium concentrations are given in Table 1; data for all chemical parameters can be found at our web site, http://silicasecchidisk.conncoll.edu.

Approximately 1-2 ml of each plankton sample was air dried onto heavy duty aluminum foil the same day of collection. The surface sediment (0-1 cm) samples and combined periphyton samples were oxidized with a sulfuric acid-potassium dichromate solution according to Marsicano & Siver (1993), and aliquots of each resulting slurry were air dried onto both glass coverslips and aluminum foil. The

Fig. 2. (A) Chrysodidymus synuroideus. Scale bar = 1 μ m. (B) Close up of two body scales of Mallomonas acaroides var. muskokana. Scale bar = 2 μ m. (C) Mallomonas akrokomos body scales. Scale bar = 1 μ m. (D) Domed body scale with portion of the bristle of Mallomonas alpina. Scale bar = 2 μ m. (E) A scale and associated smooth bristle of Mallomonas binocularis. Scale bar = 1 μ m. (F) Mallomonas binocularis. Scale bar = 1 μ m. (G) Mallomonas bronchartiana. Scale bar = 2 μ m. (H) Scales of Mallomonas caerula. Scale bar = 5 μ m.



aluminum foil samples were used for observation with scanning electron microscopy (SEM) according to the procedures of Siver (1987). Essentially, samples were attached onto an aluminum stub with Apiezon[®] wax, coated with a gold and palladium mixture for one minute with a Polaron model E5100 sputter coater and observed with a Leo 982 SEM or a Leo 435V SEM. Glass coverslips were mounted onto glass slides with Naphrax mounting medium.

All samples were used to identify, record and image scaled chrysophytes with SEM. The majority of images reported here were taken from the plankton samples. Abundances of scales for each species were made using the surface sediment glass slide preparations with light microscopy (LM). Approximately 300 scales were enumerated for each sample after the sample was thoroughly examined with SEM. In this manner all identifications were confirmed with SEM. For samples that contained specimens with morphologically similar scales that would be difficult or impossible to separate with LM, ratios were established with SEM and used to separate the LM counts. Percentages of scales for each sample can be found at our web site. For the purposes of this paper, we used the scale abundances to score each taxon as dominant, abundant, common, rare or very rare in each sample using the following criteria: Dominant = scale abundance >39.9%; Abundant = 20-39.9%; Common= 5-19.9%; Rare = 1-4.9% and; Very Rare = <1%. Taxa in the genera Paraphysomonas and Spiniferomonas were not included in the counts, but their presence was noted (Table 3). For taxa found in five or more ponds abundance weighted mean (AWM) pH values were calculated using WACALIB 3.3 (Line et al. 1994).

Results

Water chemistry

The study lakes were low in pH and dissolved salts, and poorly buffered. The lakes ranged in pH from 3.8 to 7.4, with 25 localities having a pH less than 6.0, and 16 with a pH less than 5.0 (Table 1). Twenty and 70% of the waterbodies had alkalinity values below 0 μ eq/L and 50 μ eq/L, respectively. Lake Eaton, the one waterbody in the study known to be seasonally influenced by the highly alkaline groundwater, had a high alkalinity value of 1134 μ eq/L. Except for Lake Eaton (231 μ S), all of the waterbodies were dilute lakes with specific conductance values between 26-103 μ S (Table 1). With few exceptions, sodium was the dominant cation in the study lakes with a mean concentration of 0.26 meq/L. Mean concentrations of chloride, sulfate and alkalinity were 0.28 meq/L, 0.18 meq/L, and 106 μ eq/L, respectively, for all lakes.

Most lakes were clearwater and oligotrophic, but our sites did range from clear to highly colored (0-275 Pt-Co units). The mean Secchi disk depth for the study lakes was 2.4 m, with a range from 0.5 to 7.4 m. With the exceptions of Hopkins Prairie (36.6 μ g/ L), Charles (21.8 μ g/L), Bryant (8.3 μ g/L) and Gobbler (6.7 μ g/L), chlorophyll-*a* concentrations were relatively low and below 4 μ g/L. Excluding Hopkins Prairie (70 μ g/L), total phosphorus concentrations were below 30 μ g/L, and 19 waterbodies were below 15 μ g/L. Total nitrogen ranged from 0.026 to 1.903 mg/l. Hopkins Prairie had a large *Peridinium* bloom at the time of collection.

It is of interest to note that the pH, specific conductance and total phosphorus concentrations of the ten waterbodies also surveyed in 1993 (Siver & Wujek 1999) were very similar to values observed in this study, indicating little change over the eight year period. The only exception was Lake Eaton which had a specific conductance value about $2.5 \times$ higher in 2000, most likely due to differences in the degree of contact with the highly alkaline groundwater prior to sampling. Although the alkalinity was not measured in 1993, the idea that Lake Eaton had more contact with the alkaline groundwater in 2000 is supported by the fact that the calcium concentration was almost five times higher than in 1993.

Scaled chrysophytes

Taxonomic authorities and AWM pH values for those taxa found in five or more ponds are given in Table 2. The relative abundances of the scaled chrysophytes found in the study lakes are listed in Table 3. A total of 49 taxa, representing five genera, were identified in the 30 study lakes. Two additional organisms of unknown identity were also observed.

Fig. 3. (A) Close up of domed body scales of *Mallomonas caerula*. Scale bar = 2 μ m. (B) Remains of the siliceous cell covering of one cell depicting body scales and hooked bristles of *Mallomonas canina*. Scale bar = 2 μ m. (C) Scales of *Mallomonas canina*. Scale bar = 2 μ m. (C) Scales of *Mallomonas canina*. Scale bar = 2 μ m. (C) Scales of *Mallomonas canina*. Scale bar = 2 μ m. (C) Scales of *Mallomonas canina*. Scale bar = 2 μ m. (C) Scales of *Mallomonas canina*. Scale bar = 2 μ m. (C) Scales of *Mallomonas canina*. Scale bar = 2 μ m. (C) Scales of *Mallomonas canina*. Scale bar = 2 μ m. (C) Scales of *Mallomonas canina*. Scale bar = 2 μ m. (C) Scales of *Mallomonas canina*. Scale bar = 2 μ m. (E) Close up of domed body scale of *Mallomonas corymbosa* var. *poseidonii*. Scale bar = 2 μ m. (F) *Mallomonas corymbosa* var. *poseidonii* bristle. Scale bar = 2 μ m. (G) *Mallomonas crassisquama*. Scale bar = 2 μ m. (H) Two scales with bristles of *Mallomonas cristata*. Scale bar = 2 μ m.



The number of species per lake ranged from 2 to 23, with a mean of 12. The majority of the species belonged to the genera *Mallomonas* or *Synura*, accounting for 38 and 5 taxa, respectively.

The most common and/or abundant taxa observed in the Ocala National Forest lakes were the common cosmopolitan species, Synura petersenii (Fig. 9B-D) and S. echinulata (Fig. 9A), found in 97% and 80% of the lakes, respectively. Synura petersenii represented a wide range of relative abundances between 0.3 and 64%; in 9 of the lakes it represented at least 30% or more of the scale assemblage. Synura echinulata, the second most observed taxon, was found in slightly fewer lakes with a similar range (1 to 68%). In addition, eight other species, Mallomonas wujekii (Fig. 7F-H), M. caudata (Fig. 3D), M. duerrschmidtiae (Fig. 4G), M. mangofera (Fig. 5F), M. hamata (Fig. 5D), M. corymbosa var. poseidonii (Fig. 3E-F), S. spinosa (Fig. 9F-G), and M. dickii (Fig. 4E), were reported from 50% or more of the sites. Five taxa, M. multiunca, M. ocalensis (Fig. 6A-B), M. pugio (Fig. 7B), M. tonsurata (Fig. 7E), and Spiniferomonas takahashii (Fig. 8G), were observed in only two of the waterbodies. In addition, nine taxa including Mallomonas alpina (Fig. 2D), M. bronchartiana (Fig. 2G), M. corymbosa, M. cyathellata var. kenvana (Fig. 4A-B), M. peronoides, M. peronoides var. bangladeshica (Fig. 6D-E), M. schwemmlei, Spiniferomonas coronacircumspina (Fig. 8C), and S. serrata (Fig. 8F), were very rare and each recorded at only one site. Two species, M. heterospina and M. papillosa, found in the Siver & Wujek (1999) study, were not observed in this survey.

Of the 49 recorded species in this study, six represent recently described species and one variety which are largely known only from the Ocala National Forest. In particular, it is of special interest to note that *M. wujekii* (Siver 1994) was found in 73% of the study lakes in this survey; in 7 ponds *M. wujekii* accounted for over 30% of the scales in surface sediments. *Mallomonas wujekii* was the third most abundant species in terms of both relative abundance and the number of lakes in which it was found. In addition, this taxon was not found in Charles, Bryant, or Eaton, the three lakes with the highest pH values in our study (6.6, 7.0, and 7.4, respectively). *Mallomonas corymbosa* var. *poseidonii*, another recently described taxon from this

region (Siver 1999), was observed in 15 of the waterbodies and accounted for up to 15% of the scale count. Other recently described species include M. binocularis (Fig. 2E-F), M. caerula (Figs 2H, 3A), M. delanciana (Fig. 4C-D), and M. ocalensis, which were found in 4, 8, 3, and 2 of the sites, respectively. Included in the Ocala flora was a small group of taxa, including M. bronchartiana, M. cyathellata var. kenvana, M. guttata (Fig. 5A-B), M. peronoides and M. peronoides var. bangladeshica, that are more commonly known from tropical regions. Isolated scales of two additional Mallomonas taxa, noted as M. cf. peronoides/stellata (Fig. 6F) and M. cf. serrata (Fig. 8A) were found, but could not be positively identified; these organisms were not included in the totals.

In terms of taxa from the other genera represented in the Ocala region, *Chrysodidymus synuroideus* (Fig. 2A), was recorded in 10 study sites, and *Paraphysomonas vestita* (Fig. 8B) from 7 sites. In addition, four species of the genus *Spiniferomonas* were also observed. *Spiniferomonas trioralis* (Fig. 8H), the most common species of this genus in the world (Siver 1988), was observed in 7 of the Ocala lakes (23%), but the remaining taxa, *S. coronacircumspina*, *S. serrata*, and S. *takahashii*, were found in only one or two of the lakes.

We could not find a clear relationship between the number of species found in a waterbody and any of the chemical variables measured. Lakes with 16 or more taxa had a range in pH from 4.4 to 5.3, but lakes with only slightly fewer species had pH values ranging from 4 (Grasshopper) to 7.4 (Eaton). On the other hand, the two sites with the fewest scaled chrysophytes, Lawbreaker and Charles, had pH values of 4 and 6.6, respectively. The most alkaline lakes in this study, especially Eaton and Bryant, had distinctly different floras from the majority of sites (Table 3). Eaton and Bryant were the only lakes containing M. tonsurata in the survey and both lakes also contained populations of M. guttata. In addition, M. pseudocoronata (Fig. 7A) was found in 4 ponds ranging in pH from 5.5 to 7.4, but was clearly most abundant in Eaton and Bryant. Mallomonas crassisquama (Fig. 3G) was also important in the more alkaline sites, found only in 5 lakes between pH 5.5 and 7. In terms of the most acidic lakes in the study, Gobbler (pH 3.7) contained an

Fig. 4. (A) Scale of Mallomonas cyanthellata var. kenyana. Scale bar = 2 μ m. (B) Group of scales from Mallomonas cyanthellata var. kenyana. Scale bar = 2 μ m. (C) Mallomonas delanciana. Scale bar = 2 μ m. (D) Close up of scales of Mallomonas delanciana. Scale bar = 2 μ m. (E) Portion of a cell of Mallomonas dickii. Scale bar = 1 μ m. (F) Mallomonas dickii with slightly wavy ribs. Scale bar = 2 μ m. (G) Portion of a cell of Mallomonas duerrschmidtiae. Scale bar = 2 μ m. (H) Mallomonas favosa. Scale bar = 2 μ m.



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"abundant" population of *M. acaroides* var. *musko-kana* (Fig. 2B). Although this taxa was found in 6 other lakes, it was only noted as rare or very rare in each. *Mallomonas delanciana*, one of the recently described taxa from the Ocala National Forest, was observed in only three lakes, two of which, Grasshopper and Hopkins Prairie, were very acidic. In each instance, *M. delanciana* was observed as "common". Finally, the only observation of *M. schwemmlei* was in Mary Lake (pH 4.2), as very rare.

Mallomonas transsylvanica forma curvata Siver & Lott

Latin Diagnosis: A f. transsylvanica costis transversalibus scuti curvatis et irregulariter dispositis differt.

Iconotype: Figure 10A.

Dimensions: Cells: $31-39 \times 12-16 \mu m$; Scales: 5.5-6.7 × 4.8-5.3 μm ; Density of ribs on the shield: $6/\mu m$.

Type material: Cleaned sediment (Canadian Museum of Nature Accession # CANA 43823), collector: P. A. Siver, 11 March 2000.

Type locality: Catherine Lake, Ocala National Forest, Florida, U.S.A., (N 29° 03' 57", W 81° 50' 04").

Etymology: The epithet is based on the Latin word "curvo", referring to the curving nature of the transverse ribs on the shield.

The new form differs from the nominate form in the structure and size of the scales (Fig. 10A-F). Body scales have secondary shield ribs that curve in different directions becoming somewhat disorganized, and usually do not transverse the dome (Fig. 10C; E-F). In addition, scales are slightly larger than those of the nominate form and have base plate pores of two different diameters (Fig. 10D). The ribs are more or less parallel with respect to the transverse axis only on the sides of the shield near the V-rib, but become discontinuous near the center of the scale where they curve, change orientation and are more randomly arranged. The sizes of cells and the structure of bristles agrees with that of the nominate form. The unique character of the scales was consistent among

and between cells of all ten populations we observed in Ocala. Further, because cells lacked scales with regularly arranged ribs more typical of the nominate form, we believe the Ocala organism represents a new taxon and is best described at the form level. Hälfors & Hälfors (1988) do show a few scales of M. *transsylvanica* with slightly disorganized transverse ribs, but not to the same degree and consistency as found in the Ocala populations.

Mallomonas transsylvanica f. curvata was found in ten ponds (Table 3). The ponds were primarily oligotrophic, acidic and poorly buffered. The AWM pH value for this taxon was 4.9.

Discussion

There are thousands of freshwater ponds and lakes in the State of Florida representing a broad spectrum of aquatic habitats within a subtropical setting of North America (Pollman & Canfield 1991; Terrell et al. 2000). In many respects, the majority of waterbodies in the Ocala National Forest are chemically different from most of subtropical Florida. The majority of waterbodies within the Ocala National Forest are dilute and acidic, with low nutrient concentrations (Greis 1985; this paper). Although the specific conductance is generally below 150 µS, Na⁺ and Cl⁻ are the dominant cation and anion species in these waterbodies. In contrast, aquatic ecosystems in surrounding regions are normally well buffered, CaCO, - based systems, with high pH and elevated total phosphorus concentrations; these chemical characteristics are more the norm in many regions of Florida (Terrell et al. 2000). Thus, given the differences in water chemistry, it is not surprising that the scaled chrysophyte flora of the Ocala region is very different from that of other areas of Florida (e.g. Wujek 1984; Wujek & Bland 1991; Wujek & Siver 1997; Siver & Wujek 1993, 1999). Since habitats that support the most diverse floras of scaled chrysophytes are generally ones that are dilute, poorly buffered, slightly acidic and relatively low in nutrients (Siver 1995, 2003), it is not surprising that the Ocala region was found to be rich in scaled chrysophytes with ten or more species in most of the lakes.

In a previous work (Siver & Wujek 1999), and confirmed in the current study, a component of the

Fig. 5. (A) Mallomonas guttata. Scale bar = 1 μ m. (B) Mallomonas guttata. Scale bar = 2 μ m. (C) Whole cell of Mallomonas hamata. Scale bar = 5 μ m. (D) Group of scales and bristles of Mallomonas hamata. Scale bar = 2 μ m. (E) Whole cell of Mallomonas lychenensis. Scale bar = 10 μ m. (F) Mallomonas mangofera scales. Scale bar = 1 μ m. (G) Several scales of Mallomonas matvienkoae. Scale bar = 5 μ m. (H) Mallomonas multisetigera. Scale bar = 2 μ m.



flora from the Ocala region was identified that was similar to more northern areas of North America (e.g. Nicholls 1981; Nicholls & Geratth 1985; Siver 1987, 1988, 2001, Siver 2003 and references therein) and Europe (Kristiansen 2002 and references therein). However, Siver & Wujek (1999) did not observe any similarities between the Ocala flora and other subtropical and tropical regions (e.g. Cronberg 1989, 1996; Sara & Wujek 1990; Siver & Vigna 1997). Such a finding was intriguing since other studies in Florida (e.g. Wujek & Bland 1991; Siver & Wujek 1993) and slightly to the north in Louisiana (Wee et al. 1993) revealed a group of taxa commonly associated with more tropical regions. In the Siver & Wujek (1999) study, it was suggested that a possible connection to a more tropical flora was missed because the samples were not taken during the warmer parts of the year. To overcome the seasonality issue, we took and thoroughly analyzed surface sediments from all study ponds for scaled chrysophyte remains. The idea was that the surface sediments would incorporate remains of scaled chrysophytes that grew over the course of the entire year. Using this strategy, we found the remains of a few taxa commonly associated with tropical regions, including M. bronchartiana, M. guttata, M. peronoides var. bangladeshica and M. portae-ferreae var. reticulata (Figs 6G-H), but they were rare and restricted to the more eutrophic localities that were high in pH and lacked sodium as the primary cation. Mallomonas bronchartiana, found previously in Florida (Wujek & Bland 1991) and along the Atlantic Coastal Plain (Wujek 2000), along with M. peronoides var. bangladeshica, are known tropical species (Kristiansen 2002). Although Mallomonas guttata is more widely distributed, it is also most often reported from warmer tropical localities (Cronberg 1989; Kristiansen 2002). Mallomonas portae-ferreae var. reticulata, originally described from Arizona, U.S.A., is known largely from tropical and subtropical areas of South America (e.g. Siver & Vigna 1997; Vigna & Escobar 1999; Vigna & Munari 2001). Our concept of tropical scaled chrysophytes is based almost solely on a relatively few collections from localities that are more eutrophic and not as acidic as those in Ocala (Cronberg 1989; Saha & Wujek 1990; Vyverman & Cronberg 1993; Siver & Vigna 1997). Perhaps as

more acidic localities from subtropical and tropical regions are surveyed for scaled chrysophytes, we will be able to begin to determine more precisely the relative contributions water chemistry, in particular pH, and geographic location (e.g. tropics vs. temperate zone) play in controlling the distributions of this algal group.

Several other comparisons with more northern regions of North America are noteworthy. First, Mallomonas duerrschmidtiae, M. acaroides var. muskokana and M. galeiformis are all North American endemics known almost exclusively from north temperate climates, or in the case of M. duerrschmidtiae as far north as Greenland (Jacobsen 1985). These three taxa are also found in acidic, poorly buffered and dilute waterbodies, characteristics common to many of the localities we surveyed in Ocala. Two of the taxa, M. acaroides var. muskokana and especially M. duerrschmidtiae, were well represented in Ocala ponds and their geographic ranges now extend to subtropical Florida. Mallomonas duerrschmidtiae was one of the more common taxa, found in 67% of the sites and accounting for as much as 49% of the scales found in some ponds. Mallomonas acaroides var. muskokana was also common and accounted for over 27% of the scale abundance in one pond. Interestingly, M. galeiformis was not found in Ocala, despite the fact that it is often found along with these other two species. It is also noteworthy that Chrysosphaerella longispina, another very common organism throughout much of the Northern Hemisphere, including northeastern North America (e.g. Siver 1993), was also absent in our study lakes. Lastly, the genus Spiniferomonas, which has been shown to be a common and often abundant genus in northern climates of North America (Nicholls 1981; Siver 1988), was poorly represented in Ocala. Further work along the Atlantic Coastal Plain of North America, especially between Florida and New England, is needed in order to more adequately determine the geographic ranges of some of these organisms.

A few taxa were difficult to identify based on isolated scales. Although both *M. peronoides* var. *bangladeshica* and the nominate variety were identified, these determinations were based on isolated scales or groups of scales and not whole cells (Siver & Vigna 1996). If a sample had scales

Fig. 6. (A) Whole cell of Mallomonas ocalensis. Scale bar = 5 μ m. (B) Mallomonas ocalensis scales. Scale bar = 1 μ m. (C) Single scale of Mallomonas paludosa. Scale bar = 2 μ m. (D) Mallomonas peronoides var. bangladeshica. Scale bar = 2 μ m. (E) Mallomonas peronoides var. bangladeshica. Scale bar = 2 μ m. (G) Mallomonas portae-ferrae var. reticulata. Scale bar = 1 μ m. (H) Group of scales of Mallomonas portae-ferrae var. reticulata. Scale bar = 2 μ m.



each with a transverse rib, but lacked an ornament, we referred to it as the nominate variety. If scales with ornaments were found they were referred to as var. bangladeshica (Siver & Vigna 1996). In addition, we found a scale type (Fig. 6F) that lacked both a thick transverse rib and an ornament, had a large anterior depression, and may possibly represent one of the scale types found on cells of Mallomonas stellata (Cronberg 1988). However, because we did not observe scales with an ornament and lacking a transverse rib, we hesitate to identify these scales as M. stellata. In addition, Siver & Vigna (1996) noted that scales of *M. peronoides* can also possess large anterior depressions and cells of this taxon can have scales lacking transverse ribs. As a result, we referred to these scales as M. cf. peronoides/stellata. We also found a collar scale (Fig. 8A) possibly from M. serrata or M. eoa. The scale had five or more small pits at the base of the V-rib, a feature more consistent with M. serrata than M. eoa (Kristiansen 2002).

The ponds in the Ocala National Forest had their origins as sink holes, or solution basins, but over time (millions of years) it is believed that the waterbodies became isolated from the highly alkaline groundwater via formation of clay lens within the basins (Greis 1985). During the isolation process there was a transition from high pH, well buffered systems to low pH, poorly buffered systems. It can not be certain if the change in chemistry occurred rapidly, slowly, or oscillated with respect to changes in climatic conditions. Nevertheless, the increase in acidity was most likely 3 to 4 pH units, orders of magnitude greater than the decline in pH attributed to acidic deposition in sensitive lake regions throughout North America (Charles 1991 and references therein) and Europe (Battarbee et al. 1999, and references therein). It is well known that changes in lakewater acidity can significantly alter scaled chrysophyte (Siver 2003) and diatom (Battarbee et al. 1999) floras, and it is this fact that has resulted in the successful use of both groups of organisms as bioindicators of lakewater pH. Over time, as the pH historically declined, the Ocala waterbodies offered habitats for aquatic organisms that differed from those in surrounding areas, and ones that would sequentially favor species that were more pHindifferent, then more acidophilous and lastly acidobiontic in nature. Although we can not be certain without paleolimnological evidence, it seems reasonable that the changing pH has historically played a significant role in shaping the present-day scaled chrysophyte flora of the Ocala region. Even more intriguing is the idea that some of the species originally present when the ponds were highly alkaline adapted to the more acidic conditions, and perhaps eventually evolved into new species.

The results of our study indicate that the scaled chrysophyte flora of the Ocala region is governed in large part by pH. The acidic nature of the majority of ponds is clearly mirrored in this flora (Table 3). Many of the more abundant taxa, including Mallomonas acaroides var. muskokana, M. canina (Fig. 3B-C), M. duerrschmidtiae, M. hamata, M. paludosa (Fig. 6C), M. pugio, Chysodidymus synuroideus, Synura sphagnicola (Fig. 9E) and S. echinulata, are all well-known acidobiontic species; all of these taxa had AWM pH values below 5.5. Mallomonas canina, found in 11 sites all with pH < 5.3 and with an AWM pH of only 4.8, has consistently been reported from very acidic waterbodies (Kristiansen 1982; Eloranta 1989; Hartmann & Steinberg 1989; Siver 2001) and represents one of the most acidobiontic scaled chrysophytes known. Synura sphagnicola has previously been reported to have AWM pH values of 5.3 (Eloranta 1989), 5.4 (Siver 1989) and 5.9 (Charles & Smol 1988), consistent with the value of 5 found in Ocala waterbodies. Mallomonas acaroides var. muskokana, M. pugio, M. paludosa and C. synuroideus, were only found at sites with pH < 5.4, supporting the idea that these taxa are also best described as acidobiontic species (Siver 1989, 1991; Charles & Smol 1988). Although M. hamata, M. duerrschmidtiae and S. echinulata can be found over a wider pH gradient, these species are also more often reported from acidic localites (Charles & Smol 1988; Siver 1989; Eloranta 1989; Siver 2003), which is supported by our findings. The impact that pH has on the distribution of scaled chrysophytes is further supported by examination of the higher pH sites. Several species, including Mallomonas pseudocoronata, M. tonsurata, M. corymbosa and M. crassisguama, known to be more widely distributed at higher pH (Siver 1989, 1991), were indeed most abundant in the ponds with higher pH.

In addition to the more common, well known and widespread taxa, a group of organisms recently described from Ocala and most likely with a much

Fig. 7. (A) Mallomonas pseudocoronata scale with broken wing. Scale bar = 2 μ m. (B) Mallomonas pugio. Scale bar = 2 μ m. (C) Close up of a portion of Mallomonas punctifera var. brasiliensis. Scale bar = 1 μ m. (D) Mallomonas striata scales. Scale bar = 2 μ m. (E) Mallomonas tonsurata. Scale bar = 2 μ m. (F) Domed and domeless scales of Mallomonas wujekii. Scale bar = 2 μ m. (G) Mallomonas wujekii scales. Scale bar = 2 μ m. (H) Single domed scale of Mallomonas wujekii. Scale bar = 1 μ m.



more restricted geographic range were also found to thrive in the acidic sites. Mallomonas wujekii (Siver 1994) was the third most abundant species in the Ocala lakes, found in 73% of the study sites and with an AWM pH of only 4.9. Mallomonas binocularis and M. caerula (Siver 2002a), found in 4 and 8 of the waterbodies respectively, also had clear preferences for the more acidic sites. Unlike the nominate variety, which is more common in alkaline habitats, Mallomonas corymbosa var. poseidonii (Siver 1998) was also found in more acidic sites and had an AWM pH of only 4.7. Of these four taxa, we have now found M. wujekii and M. binocularis in other very acidic waterbodies along the Atlantic Coastal Plain in the southeast United States (unpub. data). Mallomonas caerula was recently reported from two slightly acidic lakes in Ontario (Nicholls 2001; his species #3), but Mallomonas corymbosa var. poseidonii remains known only from Ocala. With further work it will be interesting to see how widespread each of these species is, if they are endemic to North America, and if they are always indicative of acidic localities.

Including Mallomonas transsylvanica f. curvata, seven new synurophycean taxa have now been described from the Ocala National Forest, making it one of the more unique aquatic regions examined in North America and qualifying it as a globally outstanding region for biological diversity according to the World Wildlife Federation (Abell et al. 2000). This is further supported by the fact that new and unique diatom species have also been described from the region (Shayler & Siver 2004; Stachura-Suchoples et al. 2004; Siver & Baskette 2004), and we know that others await description. One of the more interesting questions has to do with how widely distributed are these newly described taxa? The idea that many of the new scaled chrysophyte species may be quite restricted in their geographic ranges is supported by the fact that, except for M. caerula (Nicholls 2001), none of the taxa were found in previous studies focusing on the distributions of scaled chrysophytes from Florida (Wujek 1984; Wujek & Bland 1991; Siver & Wujek 1993; Wujek & Siver 1997), other regions of the Atlantic Coastal Plain (Wee et al. 1993; Wujek 2001) or in more northern regions (Kristiansen 2002).

Since the ponds surveyed in this project are all within the boundary of the area managed by the U.S.

Forest Service, it is possible for the Forest Service to add restrictions that would aid in conserving individual ponds and thus help protect the more unique aquatic habitats in the region. Of the ponds examined, Echo, Cowpen, Hopkins Prairie, Cathead and Baptist contained the most species of scaled chrysophytes and are the most different from all other waterbodies examined in Florida for scaled chrysophytes. In addition to these ponds, we believe that Blue Sink, Grasshopper, Echo and Delancy would also be especially good candidates for conservation efforts because in addition to having relatively high diversity, each harbours 3 to 4 of the new taxa. In addition, Blue Sink is the Type locality for Mallomonas ocalensis and M. caerula, Delancy the Type locality for M. delanciana, and Grasshopper the Type locality for several diatom species. Making decisions regarding the protection of habitats in order to help conserve biodiversity is a complex and difficult issue. The Forest Service is currently considering our findings and recommendations in their conservation planning efforts. To our knowledge, this would be the first time a federal agency used microscopic algal data, in part, to help make decisions regarding the protection of biological diversity.

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Fig. 8. (A) Scale of *M*. cf. serrata. Scale bar = 1 μ m. (B) Paraphysomonas vestita. Scale bar = 2 μ m. (C) Spiniferomonas coronacircumspina. Scale bar = 2 μ m. (C) Spiniferomonas serrata. Scale bar = 2 μ m. (F) Spiniferomonas serrata. Scale bar = 2 μ m. (G) Spiniferomonas takahashi. Scale bar = 1 μ m. (H) Remains of a whole cell depicting both spined and non-spined scales of Spiniferomonas trioralis. Scale bar = 2 μ m.





Fig. 10 (A-F). Mallomonas transsylvanica f. curvata forma nov. (A) Remains of a whole cell depicting scales and bristles. Scale bar = 20 μ m. (B) Close-up of the body scales of the specimen in Fig. A. Secondary ribs never transverse the dome. Scale bar = 5 μ m. (C) Close-up of several scales. Note the disorganized nature of secondary ribs as they near the center of the scale, particularly in the apical scale located at the bottom-center of the micrograph. Scale bar = 5 μ m. (D) Close-up documenting the pores of two distinct diameters. Scale bar = 500 nm. (E) Single body scale. Scale bar = 2 μ m. (F) Single body scale. Scale bar = 2 μ m.

Fig. 9. (A) Synura echinulata scales. Scale bar = 2 μ m. (B) Group of Synura petersenii scales. Scale bar = 2 μ m. (C) Apical spined scales of Synura petersenii f. truttae. Scale bar = 2 μ m. (D) Synura petersenii f. truttae. Scale bar = 2 μ m. (E) Synura spinosa. Scale bar = 2 μ m. (F) Synura spinosa. Scale bar = 2 μ m. (G) Synura spinosa. Scale bar = 2 μ m. (H) Synura uvella. Scale bar = 2 μ m.

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