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Investigations of two-celled colonies of *Synura* formerly described as *Chrysodidymus* with descriptions of two new species

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ABSTRACT
The two-celled colonial synurophyte genus *Chrysodidymus* was originally distinguished from its close relative *Synura* on the basis of the colonies always being of two cells, the shape of the cells and the odd swimming nature of the colony. Recently, based on gene sequence data, *Chrysodidymus* was found to be deeply nested within the *Synura* clade. As a result *Chrysodidymus* was placed in synonymy with *Synura* and a new combination, *S. synuroidea*, was made for the basionym *C. synuroideus*. Based on our observations and findings of two-celled colonies originally described under the genus *Chrysodidymus*, we propose descriptions of two new species *Synura papillosa* and *Synura prowsei* and an emended description for *Synura synuroidea*. Scales of *S. synuroidea* are significantly smaller than those of the two proposed species and possess an exceptionally wide posterior rim that is perforated with large holes of uneven diameters. Scales of the new species are significantly larger with narrow posterior rims that lack holes. Scales of *S. prowsei* possess anterior ribs that connect the ends of the posterior rim to the base of the spine and lack surface papillae, whereas scales of *S. papillosa* have surface papillae, but lack the anterior ribs. In addition, the base plate pore patterns are distinctive for each species. We further designate a neotype for *S. synuroidea* since the original holotype has been lost. All three species favour dilute, shallow, highly acidic water bodies often with highly coloured dissolved organic matter (CDOM).

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KEY WORDS Acid water bodies; *Chrysodidymus*; *Synura papillosa*; *Synura prowsei*; *Synura synuroidea*; two-celled colonies

Introduction

The two-celled colonial genus *Chrysodidymus* was originally described from an acidic swamp locality in Malaya, today part of Malaysia (Prowse, 1962). Prowse (1962) distinguished the genus from its close relative *Synura* on the basis of the colonies always being composed of two cells, the shape of the cells and the swimming nature of the colony. Prowse (1962) noted that the posterior ends of the cells that attach the two cells within the colony were wider than the anterior flagellated end, a pattern opposite to that observed in *Synura*. He further detailed the odd swimming style of the colony as 'forward and backwards in a straight line' more or less resembling a tug-of-war match, but distinctly different from the rolling and tumbling pattern observed in *Synura*. The back and forth oscillating movement, along the longitudinal axes of the colony, has also been observed by others including Nicholls & Gerrath (1985), Graham *et al.* (1993) and Pusztai *et al.* (2016). Details of the two flagella, parietal plastids and covering of scales were similar to those of *Synura*. Although not discussed by Prowse, he does illustrate what is likely a basal contractile vacuole similar to what is also observed in *Synura* (Prowse, 1962, Plate IV, figs m–n).

Prowse (1962) further described two species of *Chrysodidymus, C. synuroideus* and *C. gracilis*, differing in the size and shape of the cells. Cells of *C. synuroideus*, the type for the genus, were distinctly trapezoid-shaped with a wider posterior end, as compared with the longer and narrow cells described for *C. gracilis*. Unfortunately, Prowse did not comment on the nature of the siliceous scales and no drawings of these structures are given. His illustrations do note the forward projecting nature of the spines on the cell. It is also of interest that the type locality was apparently the same acid swamp for both species, and the holotypes were deposited by Prowse (1962) at the Tropical Fish Culture Research Institute in Malacca. This institution is no longer in existence, and materials are believed to have been moved to the University of Hawaii in 1972. Unfortunately, the types for the two *Chrysodidymus* species have not been located and are apparently no longer available (Alison Sherwood, personal communication).

Based on observations of living collections made from localities in Michigan and Minnesota, Wujek & Wee (1983) noted that cells within colonies were polymorphic, often changing shape from long and narrow to shorter and more spherical. The sizes and shapes of cells observed by Wujek & Wee (1983) were similar to

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those reported by Prowse (1962) for both *C. synuroideus* and *C. gracilis*, prompting these authors to synonymize the two species as *C. synuroideus* on the basis of priority. Wujek & Wee (1983) further synonymized *Synura microcrepis* Nygaard (Nygaard, 1978) with *C. synuroideus*. Graham *et al.* (1993) reported that in culture cells of recently formed colonies of *Chrysodidymus* were globose or spherical, but as colonies aged the cells become more elongated and vase-like, with the posterior portion being distinctly wider than the flagellated end. These observations supported the conclusions made by Wujek & Wee (1983), and all reports of *Chrysodidymus* made since have been as *C. synuroideus*.

On the basis of a multi-gene study including SSU rDNA, LSU rDNA and *rbc*L sequences, Pusztai *et al.* (2016) clearly demonstrated that a strain of *C. synuroideus* nested deep within the *Synura* clade, closest to *Synura sphagnicola*. As a result, they placed *Chrysodidymus* in synonymy with the genus *Synura* and proposed the new combination, *Synura synuroidea* (Prowse) Pusztai, Čertnerová, Škaloudová & Škaloud.

In our study, we examined 78 collections of two-celled colonies of *Chrysodidymus* (now *Synura*) over broad geographic regions of North America, Europe and Southeast Asia. We found consistent differences in the ultrastructure of the siliceous scales that imply at least three distinct species. The objectives of this paper are to describe two new species of *Synura*, and establish a neotype for *S. synuroidea*.

Materials and methods

Chrysodidymus populations examined as part of this study included 51 localities from North America, 25 from Vietnam and two from Europe (Table 1). The 51 North American localities were part of a larger survey of synurophytes that included 264 water bodies in nine regions spanning the east coast of the continent from Florida to Newfoundland (Siver & Lott, 2012). The North American observations were made prior to the discovery that *Chrysodidymus* belonged in *Synura*, and before specimens were separated according to scale type. We had photographic records and sufficient material to further study 31 of the 51 collections in order to identify and separate specimens into the three taxa. One of the European sites is in the Ukraine and the other one represents a culture isolated from a site in Scotland (Pusztai *et al.*, 2016). The 25 Vietnam localities were part of a hydrobiological survey of lakes and reservoirs under the project Ecolan 3.2 of the Russian-Vietnam Tropical Centre and the joint VAST-RFBR project on synurophyte diversity in the tropics. These studies were conducted in the central and southern parts of the country in 2008–2015.

A sediment core and a phytoplankton sample taken at the time of collection were examined for *Chrysodidymus* remains in each of the North American water bodies. 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 0–1 cm surface section of each core was used in this study. A 10 µm mesh net was used to retrieve a phytoplankton sample from each North American site, and used in conjunction with the surface sediment sample to both establish the presence of *Chrysodidymus* and image all scale types. Samples from the Vietnam and Ukraine sites were collected from the surface water layer using a 20 µm mesh plankton net and fixed with Lugol's solution. For the North American sites, the pH was measured with a Fisher Acument 640-A pH meter, and water temperature and specific conductance with a Hydrolab DataSonde 4A. Water colour, largely reflecting the concentration of coloured dissolved humic material (CDOM), was estimated using the platinum-cobalt method (APHA, 1985). Additional physical and chemical data for each North American site is available at http://silicasecchidisk.conncoll.edu. For the Vietnam and Ukraine sites, specific conductance, pH and temperature measurements were performed using Hanna Combo (HI 98129 and HI 9828) devices.

For the North American samples, approximately 0.5–1.0 g of sediment from the 0–1 cm section of each core was oxidized 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. Aliquots of each phytoplankton sample were also dried directly onto aluminium foil strips immediately after collection. 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) 982 FESEM, a Leo 435V SEM or an FEI Nova FESEM. For the Vietnam and Ukraine samples, an aliquot of each plankton sample was initially washed by repeated centrifugation in deionized water to remove the Lugol's fixative. Drops of the washed sample were used directly, or digested 4–5 minutes in sulphuric acid with potassium dichromate and washed a second time. Samples were placed on aluminium stubs, coated with gold for 10 min, and observed with either a JEOL 6510 LV or a LEO-1420 SEM, or dried onto formvar-coated grids (EMS FF200-Cu-50, Electron Microscopy Sciences) and observed with a JEM-1011 TEM.

Results

Taxonomy

Based on our observations and findings of two-celled colonies originally identified under the genus *Chrysodidymus*, we propose an emended description for *Synura synuroidea* and descriptions of two new species, *Synura papillosa* and *Synura prowsei*. Our

Table 1. Locations and ecological characteristics of sites harbouring *Synura synuroidea* (n=18), *S. papillosa* (n=33) or *S. prowsei* (n=6) used in this study.

	Region	Site	Latitude	Longitude	pH	SC µS	Colour Pt-Co	Temp. °C
S. synuroidea	Cape Cod, MA, USA	Spectacle-F	N 41° 21′ 0″	W 70° 21′ 0″	6.6	74	5	25
	Maine, USA	Cranberry	N 45° 2′ 24″	W 67° 10′ 48″	6.4	23	87	17
	Maine, USA	Ledge	N 45° 1′ 12″	W 67° 10′ 12″	6.7	20	0	16
	Newfoundland, Canada	Deer Park	N 47° 9′ 36″	W 53° 9′ 36″	6.1	40	30	15
	Newfoundland, Canada	Grassy Pond	N 48° 17′ 24″	W 54° 0′ 36″	5.9	33	72	20
	Newfoundland, Canada	Minchin's Pond	N 48° 19′ 48″	W 53° 31′ 48″	6.0	24	50	19
	Newfoundland, Canada	Orchid Bog	N 47° 8′ 24″	W 53° 10′ 48″	3.9	29	212	16
	Newfoundland, Canada	Otter	N 49° 6′ 0″	W 55° 3′ 0″	6.4	32	75	20
	Newfoundland, Canada	Screech Pond	N 49° 4′ 12″	W 53° 26′ 24″	4.6	24	81	21
	New Hampshire, USA	Willard	N 43° 01′ 17″	W 72° 00′ 58″	6.1	22	NA	NA
	North Carolina, USA	Hannah's Pond	N 34° 25′ 48″	W 76° 34′ 12″	4.2	71	335	32
	Nova Scotia, Canada	Giants Pond	N 45° 13′ 48″	W 61° 31′ 48″	6.3	47	11	16
	Nova Scotia, Canada	Lower Beaver	N 45° 0′ 36″	W 62° 27′ 36″	4.5	28	90	18
	Ocala, FL, USA	Penner	N 29° 17′ 24″	W 81° 29′ 24″	5.1	32	38	23
	Pine Barrens, NJ, USA	Chatsworth	N 39° 28′ 48″	W 74° 19′ 12″	4.2	49	239	29
	Pine Barrens, NJ, USA	Egg Harbor	N 39° 19′ 48″	W 74° 21′ 36″	4.2	60	102	26
	Pine Barrens, NJ, USA	Harrisville Lake	N 39° 23′ 24″	W 4° 18′ 36″	4.4	40	63	26
	Rivne Region, Ukraine	Bushchanske Fen	N 50° 17′ 55″	E 26° 12′ 40″	NA	NA	NA	NA
S. papillosa	Cape Cod, MA, USA	Northeast	N 41° 56′ 35″	W 69° 59′ 55″	5.0	111	5	27
	Connecticut, USA	Emmons	N 41° 01′ 5″	W 75° 55′ 2″	4.6	53	15	23
	Maine, USA	James Pond	N 45° 01′ 55″	W 67° 16′ 46″	6.3	21	45	16
	Maine, USA	Upper Haddock	N 44° 18′ 59″	W 68° 17′ 29″	6.4	45	12	16
	Newfoundland, Canada	Butter Pot	N 44° 18′ 41″	W 65° 19′ 19″	5.8	36	28	17
	Newfoundland, Canada	Moon	N 47° 39′ 48″	W 53° 52′ 18″	6.2	176	25	15
	North Carolina, USA	Debbie's Pond	N 35° 43′ 14″	W 76° 57′ 56″	4.1	130	300	27
	Ocala, FL, USA	Clay	N 29° 01′ 45″	W 81° 27′ 21″	4.6	54	5	21
	Cam Ranh, Vietnam	Mangroves 5	N 12° 02′ 08″	E 109° 12′ 09″	6.9	1240	NA	33
	Cam Ranh, Vietnam	Mangroves 7	N 12° 02′ 10″	E 109° 12′ 09″	7.0	3000	NA	34
	Cat Tien NP, Dong Nai Province, Vietnam	Canh May Bay Lake	N 11° 28′ 11″	E 107° 20′ 30″	5.8	14	NA	27
	Cat Tien NP, Dong Nai Province, Vietnam	Dak Lua swamp	N 11° 30′ 43″	E 107° 20′ 30″	5.2	10	NA	24
	Cat Tien NP, Dong Nai Province, Vietnam	Dak Lua village	N 11° 30′ 26″	E 107° 24′ 24″	5.5	72	NA	28
	Cat Tien NP, Dong Nai Province, Vietnam	Old irrigation ponds	N 11° 24′ 27″	E 107° 24′ 24″	5.7	25	NA	32
	Cat Tien NP, Vietnam	Dau Ca Lake	N 11° 28′ 26″	E 107° 20′ 34″	5.9	22	NA	28
	Con Son Isl., Baria-Vung Tau Province, Vietnam	Pond by Quang Trung Res.	N 8° 41′ 33″	E 106° 36′ 20″	NA	NA	NA	NA
	Dak Lak Province, Vietnam	Eao Reservoir	N 12° 43′ 10″	E 108° 29′ 14″	7.3	89	NA	29
	Dak Lak Province, Vietnam	Ea Soup Reservoir	N 13° 03′ 56″	E 107° 53′ 43″	8.0	89	NA	34
	Dak Lak Province, Vietnam	Ea Dar Reservoir	N 12° 48′ 25″	E 108° 29′ 30″	7.3	73	NA	30
	Dong Nai Province, Vietnam	Ta Lai reservoir	N 11° 23′ 26″	E 107° 21′ 49″	6.4	37	NA	30
	Dong Nai Province, Vietnam	Temporary forest pool	N 11° 24′ 26″	E 107° 24′ 49″	6.1	29	NA	31
	Khanh Hoa Province, Vietnam	Pool in Cam Ranh Peninsula	N 12° 4′ 39″	E 109° 11′ 08″	4.6	79	NA	31
	Khanh Hoa Province, Vietnam	Pool in Cam Ranh Peninsula	N 12° 4′ 42″	E 109° 11′ 06″	6.2	64	NA	33
	Khanh Hoa Province, Vietnam	Pool in Cam Ranh Peninsula	N 12° 4′ 41″	E 109° 11′ 05″	5.0	82	NA	29
	Khanh Hoa Province, Vietnam	Sandpit-Cam Ranh Peninsula	N 12° 04′ 43″	E 109° 11′ 26″	6.0	75	NA	34
	Khanh Hoa Province, Vietnam	Sandpit-Cam Ranh Peninsula	N 12° 05′ 02″	E 109° 10′ 56″	5.5	54	NA	33
	Khanh Hoa Province, Vietnam	Dzua River	N 12° 15′ 01″	E 109° 09′ 05″	6.6	246	NA	30
	Khanh Hoa Province, Vietnam	Cau Doi reservoir	N 12° 15′ 45″	E 109° 04′ 01″	6.8	110	NA	29
	Khanh Hoa Province, Vietnam	Pond near Da Ban Reservoir	N 12° 37′ 45″	E 109° 6′ 41″	6.3	54	NA	31
	Khanh Hoa Province, Vietnam	Dzua River	N 12° 15′ 01″	E 109° 09′ 05″	6.6	246	NA	30
	Khanh Hoa Province, Vietnam	Sandpit-Cam Ranh Peninsula	N 12° 04′ 43″	E 109° 11′ 23″	6.0	75	NA	34
	Phu Quoc Island, Vietnam	Swamp	N 10° 13′ 54″	E 103° 59′ 07″	4.7	25	NA	29
	Phu Quoc Island, Vietnam	Pond	N 10° 19′ 51″	E 103° 51′ 32″	6.0	117	NA	33
S. prowsei	North Carolina, USA	Salters	N 34° 41′ 55.6″	W 78° 37′ 35.97″	4.1	62	125	28
	North Carolina, USA	White	N 34° 39′ 18″	W 78° 30′ 19.27″	4.8	62	2	29
	Ocala, FL, USA	Blue Sink	N 29° 03′ 40″	W 81° 40′ 13.94″	5.3	26	0	26
	Ocala, FL, USA	Tomahawk	N 29° 08′ 06″	W 81° 54′ 10.38″	4.3	51	2.5	23
	Pine Barrens, NJ, USA	Absegami	N 39° 37′ 36″	W 74° 25′ 33.7″	4.3	66	47	30
	Pine Barrens, NJ, USA	Batsto	N 39° 38′ 49″	W 74° 39′ 11.69″	5.8	47	188	28

observations of colony and cell shape of *S. synuroidea* agree with those recently published by Pusztai *et al.* (2016). Here we provide additional observations on the ultrastructure of the siliceous scales, and propose a neotype for *S. synuroidea.*

***Synura synuroidea* (Prowse) Pusztai, Čertnerová, Škaloudová & Škaloud (Figs 1–10)**

BASIONYM: *Chrysodidymus synuroideus* Prowse 1962. In: *Further Malayan freshwater flagellata*, Garden Bull., Singapore, 19(1): 128–129 (Plate IV, Figure n). Type locality: Malaya, Malacca, acid swamps.

SYNONYM: *Synura microcrepis* Nygaard 1978.

Description

Colonies consisting of two cells attached by their bases at 180°. The shape of cells is highly variable, displays a high degree of phenotypic plasticity, and commonly ranges from elongate to ovoid, trapezoid or pyriform. The basal width of the cells at the point of attachment is equal to or wider than that of the distal end. Scales are elongate-oval in shape, range in size from 1.6–2.8 × 0.7–1.6 µm with a mean of 2.1 × 1.0 µm, and possess a posterior rim and anterior spine. The posterior rim encircles two-thirds to three-quarters of the scale and forms a broad hood that extends over approximately one-third of the scale (Figs 1, 2, 4–6). The basal portion of the hood is perforated with one to ten or more holes of variable diameters. Spines are cylindrical in shape, range in length from 0.45 to 1.3 µm, and bend slightly forward extending a short distance beyond the base plate (Figs 1–6). The diameter of the spine is remarkably similar over most of its length, terminating in a blunt, sometimes pointed, apex. The majority of scales possess short spines about one-quarter to one-third the length of the scale, however spines on the anterior end of the cell are often longer (Fig. 8, black arrows). Base plate pores cover the scale, but are often lacking along the very anterior end distal to the spine perforation (Fig. 4). There is a large difference in diameter of the base plate pores. Pores on the anterior one-third to one-half of the scale are small, increase in diameter in the posterior half of the scale, and with the largest pores situated under the rim often forming a single row along the margin (Fig. 4, white arrows; Fig. 10, black arrows). The diameters of the pores situated under the rim are usually 5–10 times as wide as those around the base of the spine. A uniform secondary layer encircles each base plate pore (Figs 1–2).

NEOTYPE: Portion of a single gathering of cells from strain CAUP B712 on SEM stub #NEOTYPE-B712 deposited at the Culture Collection of Algae of the Charles University in Prague (CAUP). Strain S95.E4 was isolated from a small unnamed lake near Loch Garten in the Grampian Mountains, Scotland (57°13′32.55″N, 3°43′20.71″W), and is deposited in the Culture Collection of Algae of Charles University, Prague, Czech Republic, CAUP B712. Original reference: Pusztai *et al.* (2016). *Cryptogamie, Algologie* 37: 297–307.

NEOTYPE LOCALITY: Strain S95.E4 was isolated from a small unnamed lake near Loch Garten in the Grampian Mountains, Scotland (57°13′32.55″N, 3° 43′20.71″W).

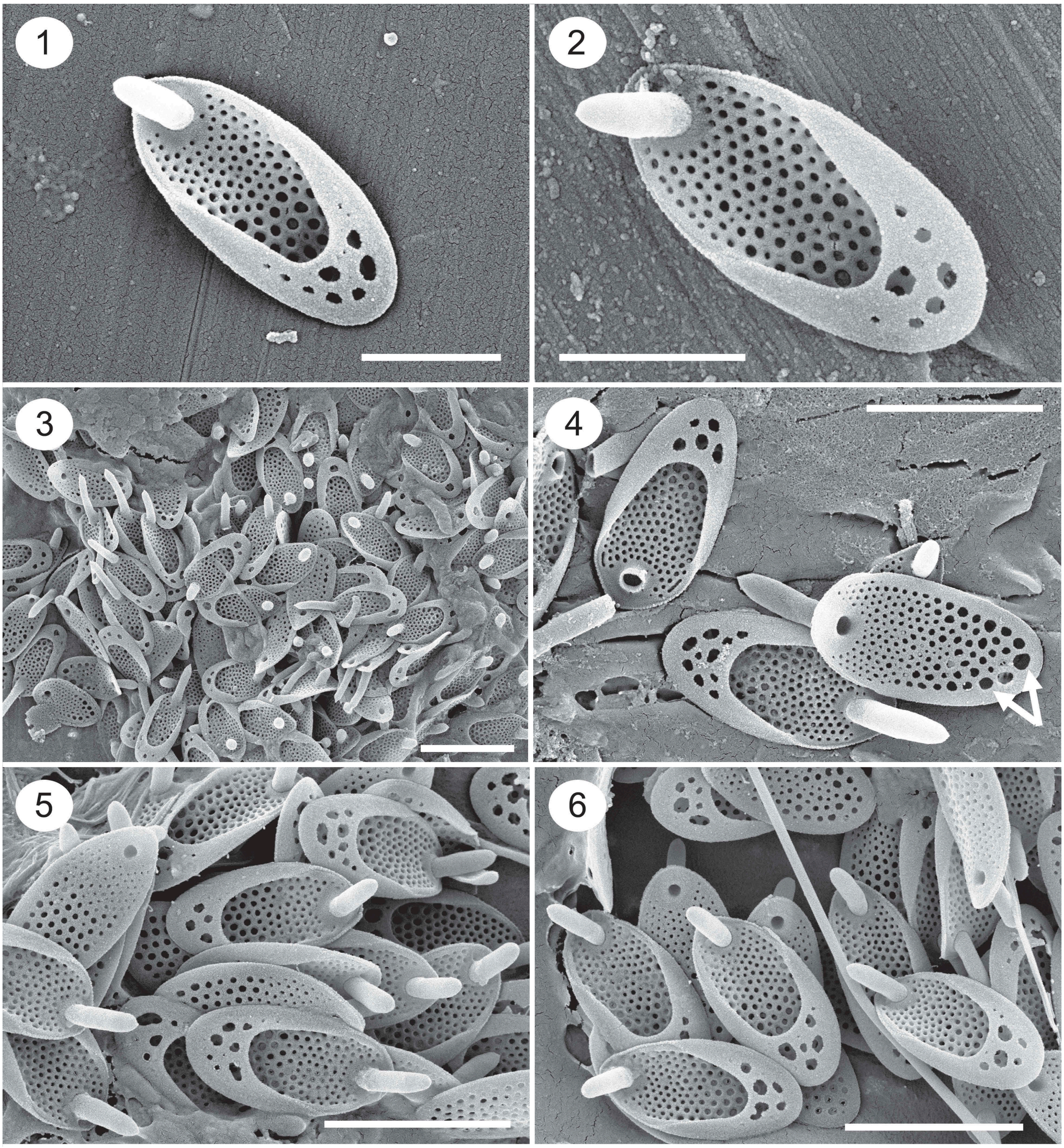
Additional information

We observed cells from living populations (not cultures) with variable shapes ranging from spherical, elongated, oval, trapezoid to pyriform, similar to findings by Graham *et al.* (1993) and Pusztai *et al.* (2016). In the vast majority of populations studied, the posterior ends of the cell are wider than the anterior end. However, we did observe one population in a small bog pond (Orchid Bog) in Newfoundland, Canada, where the cells were club-shaped and attached by the tapered ends as is common among species of *Synura* (Figs 7–10). To our knowledge, this morphology has not been reported for *Synura synuroidea*, and it differs from all previous findings where the basal portion of the cell is wider than the distal flagellated end. Scales from these club-shaped cells were similar in size, shape and morphology to those from all other populations of *S. synuroidea*, including the row of large base plate pores aligning the posterior margin (Fig. 10, black arrows). However, most scales had a single large hole in the posterior rim (Figs 8, 9, 10, white arrows), instead of a collection of holes.

***Synura papillosa* Kapustin, Gusev & Siver, sp. nov.** (Figs 11–17)

Description

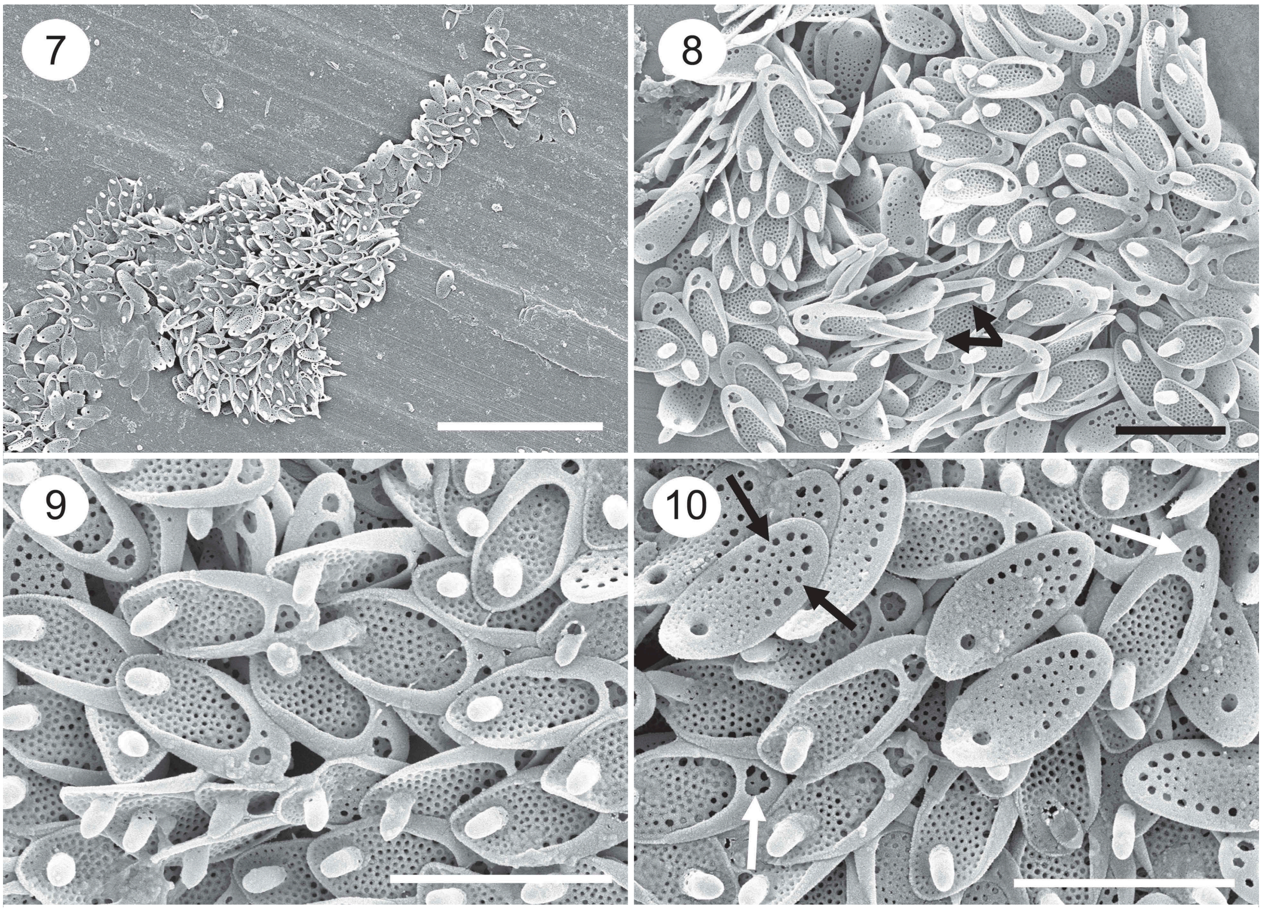
Colonies consisting of two cells attached by their bases at 180°. The shape of cells is highly variable, ranging from elongate to ovoid, trapezoid or pyriform. The basal width of the cells at the point of attachment is equal to or wider than that of the distal end. Scales are elongate-oval in shape, range in size from 2.2–3.4 × 1.2–1.8 with a mean of 2.6 × 1.4 µm, and possess a posterior rim and anterior spine (Figs 11–16). The posterior rim encircles two-thirds to three-quarters of the scale, extends a short distance over the base plate, and lacks perforations. Spines are cylindrical in shape, range in length from 0.41 to 1.2 µm, bend slightly forward extending a short distance beyond the base plate (Figs 11–16), and are larger on the apical-most



Figs 1–6. SEM images of *Synura synuroidea*. **Figs 1–2**. Individual scales illustrating the large posterior rim bearing holes, details of the base plate pores and forward projecting spine. **Figs 3–6**. Groups of scales from individual cells depicting different numbers and sizes of holes penetrating the posterior rim, and lengths of spines. The undersurface of the scale in Fig. 4 illustrates the differences in the sizes of the base plate pores, and the posterior-most row of large pores situated immediately below the posterior rim (white arrows). Scale: 1 µm (Figs 1–2) and 2 µm (Figs 3–6).

portion of the cell. The diameter of the spine is similar over most of its length, terminating in a blunt, sometimes pointed, apex. Base plate pores over most of the scale are large, evenly spaced and of similar diameter (Figs 11–16). The diameter of the base plate pores decreases slightly on the anterior end of the scale (Figs 12, 16). The base plate is thickened around each pore, often forming a pentagonal or hexagonal pattern (Figs 12, 17). Surface papillae are present, positioned at the corners of the polygon patterns (Fig. 17).

HOLOTYPE: Portion of a single gathering of cells on SEM stub #DL2/3 deposited at the Herbarium of the I.D. Papanin Institute for Biology of Inland Waters RAS, Borok (IBIW). Material is from a phytoplankton sample collected by E.S. Gusev on 10 March 2014. Figs 13–15 are representative scales from the specimen.



Figs 7–10. SEM images of a population of *Synura synuroidea* from Orchid Bog, Newfoundland, Canada, where cells of the two-celled colonies were club-shaped and attached by the tapered ends. **Fig. 7**. Remains of a whole cell showing the club-shape morphology. **Figs 8–10**. Groups of scales depicting the large posterior rim, base plate pores and forward projecting spine. Scales with longer spines representing the anterior-most portion of the cell are depicted in Fig. 8 (black arrows). The majority of scales on cells in this population have a single large hole in the posterior rim (Fig. 10, white arrows). The row of large base plate pores aligning the posterior marginal are illustrated in Fig. 10 (black arrows). Scale: 2 µm (Figs 8–10) and 10 µm (Fig. 7).

TYPE LOCALITY: Dak Lua swamp, situated at 11°30′43″ N, 107°22′56″E in Cat Tien National Park, Dong Nai Province, Vietnam.

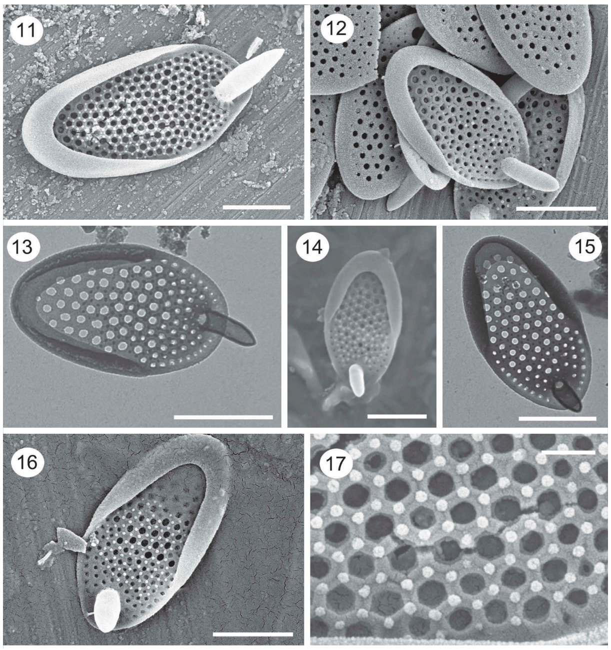
ETYMOLOGY: The name *papillosa* reflects the presence of surface papillae on the scale surface.

***Synura prowsei* Siver, Kapustin & Gusev, sp. nov.** (Figs 18–23)

Description

Colonies consisting of two cells attached by their bases at 180°. The shape of cells is highly variable, ranging from elongate to trapezoid to pyriform, and where the width of the cells at the point of attachment is equal to or wider than that of the distal end. Scales are elongate-oval in shape, range in size from 1.9–3.3 × 1.1–1.7 with a mean of 2.8 × 1.5 µm, and possess a posterior rim and anterior spine (Figs 18, 19, 21). The posterior rim is narrow, encircles approximately three-quarters of the scale margin, and lacks perforations. Spines are cylindrical in shape, range in length from 0.74 to 1.70 µm, with a mean of 1.09 µm, and bend slightly forward extending a short distance beyond the base plate (Figs 18, 19, 21, 22). The diameter of the spine is similar over most of its length, terminating in a blunt, sometimes pointed, apex. Apical-most scales are smaller and with longer spines that can be as long as the base plate (Fig. 20). Base plate pores over most of the scale are large, evenly spaced, and of similar diameter (Figs 20–23). The diameter of the base plate pores decreases slightly on the anterior end of the scale. The base plate is thickened around each pore, often forming a pentagonal or hexagonal pattern (Figs 18, 19). Thick anterior submarginal ribs originate at the ends of the posterior rim and connect to the base of the spine (Figs 18–21). Additional thickenings often cross the base plate and connect the anterior submarginal ribs (Figs 19, 22). A single row of base plate pores is found between the anterior submarginal ribs and the scale margin (Figs 18, 19, 21). Surface papillae are lacking.

HOLOTYPE: Portion of a single gathering of cells on SEM stub deposited at the Canadian Museum of Nature, CANA (127506). Material is from a



Figs 11–17. SEM and TEM images of *Synura papillosa*. Images of isolated scales observed with SEM (**Figs 11, 14, 16**) and TEM (**Figs 13, 15**) depict the shallow posterior rim, large base plate pores, the stout anterior spines and the arrangement of surface papillae. Figs 14, 16, 17. Note the hexagonal-shaped thickenings, and corresponding surface papillae, surrounding the base plate pores. Figs 13–15 are from the holotype specimen. Scale: 200 nm (Fig. 17), 1 µm (Figs 11–16).

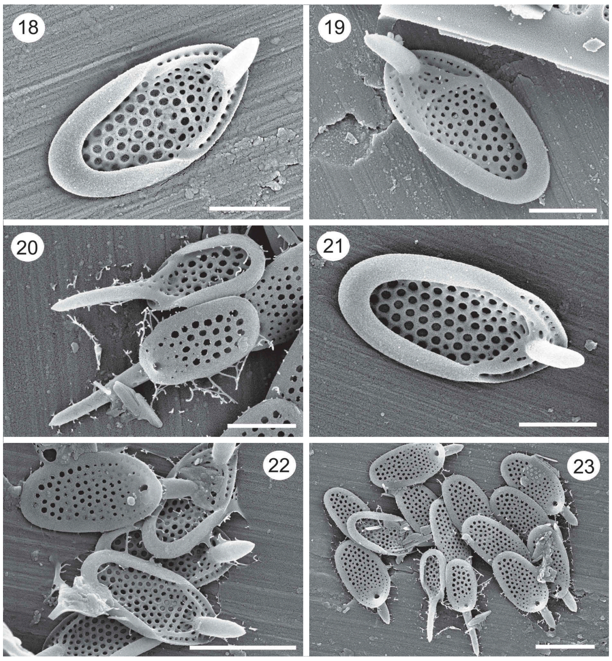
phytoplankton sample collected by P.A. Siver on 7 June 2001. Figures 20, 22 and 23 are representative scales from the specimen.

Type locality: White Lake, a shallow Carolina Bay water body situated at 35°39′18″N, 76°30′20″W in the Bladen Lakes State Forest, North Carolina, USA. The pond is located along Route 701 in the Town of White Lake.

Etymology: *Synura prowsei* is in honour of G.A. Prowse who originally described the genus *Chrysodidymus*.

Ecological and distribution findings

Siver & Lott (2012) examined scaled chrysophyte remains in 262 water bodies along the east coast of North America, ranging from Florida to Newfoundland. A total of 51 of those sites were found to have populations of *Chrysodidymus* that at the time were all included under the epithet, *C. synuroideus*. *Chrysodidymus* populations were found in each of the major regions examined by Siver & Lott (2012), were most common in water bodies from the Pine Barrens of southern New Jersey, coastal Maine and Newfoundland, and rarest in localities in Connecticut and on Cape Cod. In fact, populations were found in all of the water bodies examined from the New Jersey Pine Barrens.



Figs 18–23. SEM images of *Synura prowsei*. **Figs 18–19, 21.** Note the shallow posterior rim lacking holes, large and reinforced base plate pores, the stout nature of the forward projecting spines, and the ribs connecting the ends of the posterior rim with the base of the spine. **Fig. 20.** Two scales with long spines from the anterior portion of the scale (also shown in Fig. 23). Note the large size of the base plate pores on the undersurface of the lower scale. **Figs 22–23.** Groups of scales. Note the smaller size of the two anterior scales with longer spines (Fig. 23). Figs 20, 22 and 23 are from the holotype specimen. Scale: 1 µm (Figs 18–21) and 2 µm (Figs 22–23).

As noted above, we had sufficient material from 31 of the 51 North American sites to further study and determine which species were present (Table 1). *Synura synuroidea* was the most common species, found in 17 of the localities, while *S. prowsei* and *S. papillosa* were present in six and eight sites, respectively. Interestingly, none of these collections contained more than one of the three species. Populations of *S. synuroidea* and *S. papillosa* were widespread, found from Florida to Newfoundland. In contrast, populations of *S. prowsei* represented a more southern distribution being found only in the three warmest regions, Ocala (Florida), coastal North Carolina and the New Jersey Pine Barrens (Table 1).

All of the 31 North American localities, representing all three species, were low in dissolved salt content with specific conductance values ranging from 20 to 176 µS cm^{-1} (Table 1). All but two sites had specific conductance values less than 74 µS cm^{-1}, and 21 of the localities had values below 50 µS cm^{-1}. The two sites with the highest specific conductance, Debbie's Pond and Moon Lake, are situated close to the ocean and are directly influenced by sea spray that results in elevated NaCl concentrations. All of the localities harbouring the three species were acidic with pH values ranging from 3.9 to 6.7, and 50% of the sites below pH 5. *Synura prowsei* had the smallest and more acidic, range in pH, from 4.1 to 5.8, relative to the other two taxa. Even though seven of the sites containing these species were clearwater localities low in CDOM, 66% had water colour values >20 Pt-Co units and half of the localities were darkly stained with values above 50 Pt-Co units (Table 1). All three species were found in water bodies with low as well as high dissolved humic matter.

In addition to the ecological records from North America, we also had similar data from 23 sites in Vietnam for *Synura papillosa* (Table 1). In general, at the Vietnam sites, this species was found over a broader pH gradient ranging from 4.6–8.0. Still, 12 of the 23 sites had pH ≤6. Except for three sites, the distribution of *S. papillosa* along a specific conductance gradient in the Vietnam sites was similar to that observed in North America where 20 sites had a range from 10–117 $\mu S\ cm^{-1}$ (Table 1). Isolated scales of *S. papillosa* were also found in two mangrove swamps with high specific conductance values of 1240 and 3000 $\mu S\ cm^{-1}$. At the time of collection, these two sites had a freshwater layer on top of a more saline layer. It is also possible that *S. papillosa* grew during the rainy season or were possibly washed in from a neighbouring locality.

Based on a review of the literature, we found 29 records of *Chrysodidymus synuroideus* that represent either *S. synuroidea* (n=12) or *S. papillosa* (n=17) (Table 2). The literature records for *S. synuroidea* represent sites primarily in North America, Europe and Greenland. Records of *S. papillosa* are more widespread, including sites from North and South America, Europe, Asia and New Zealand.

Table 2. Previous literature records based on EM of *Chrysodidymus synuroideus* that can be assigned to either *Synura synuroidea* or *S. papillosa*. See text for details.

Species	Region, country or island	Reference
Synura synuroidea sensu stricto	Québec, Canada	de Puytorac *et al.* 1972
	South Greenland	Nygaard 1978
	Michigan & Minnesota, USA	Wujek & Wee 1983
	Ontario, Canada	Nicholls & Gerrath 1985
	The Netherlands	Roijackers & Kessels 1986
	New Hampshire, USA	Siver & Lott 2000
	Elbe Sandstone Region, Czech Republic	Pichrtová & Veselá 2009
	North America	Siver & Lott 2012
	Ukraine	Kapustin & Gusev 2016
	Scotland	Pusztai *et al.* 2016
Synura papillosa	Yamagata Prefecture, Japan	Takahashi 1967
	Chile	Dürrschmidt 1982
	Michigan & Minnesota, USA	Wujek & Wee 1983
	Malaysia	Dürrschmidt & Croome 1985
	Australia	Croome & Tyler 1988
	Madagascar	Hansen 1996
	Argentina	Vigna & Kristiansen 1996
	Brazil	Couté & Franceschini 1998
	Brazil	Kristiansen & Menezes 1998
	Buenos Aires, Argentina	Vigna & Munari 2001
	Tierra del Fuego, Argentina	Kristiansen & Vigna 2002
	Argentina	Vigna & Siver 2003
	Belize	Carty & Wujek 2003
	Ecuador	Wujek & Dziedzic 2005
	North America	Siver & Lott 2012
	Aquitaine, France	Němcová *et al.* 2012
	New Zealand	Wujek 2013
	Vietnam	Gusev 2013
	China	Wei et al. 2014
	Vietnam	Gusev *et al.* 2017

Discussion

Our findings confirm that there are at least three species of *Synura* with two-celled colonies, suggesting that previous records of *Chrysodidymus synuroideus* probably represent more than one taxon (Table 2). The differences in scale ultrastructure between the three taxa are distinct and were observed consistently across all populations examined in this study. Details of the posterior rim, the pore pattern on the base plate, the presence of surface papillae, and presence of anterior ribs serve to easily separate the three species. The posterior rim on *S. synuroidea* is significantly wider and bears one or more large holes compared with the posterior rims on *S. prowsei* and *S. papillosa* scales. The degree to which the posterior rim overlaps the base plate on *S. synuroidea* scales is often twice that of *S. prowsei* and *S. papillosa*, and the posterior rims on the latter two species lack holes. The difference in the diameters of the base plate pores is greatest on *S. synuroidea* scales and more similar on *S. prowsei* and *S. papillosa*. On *S. synuroidea*, the base plate pores are large in the posterior region of the scale, especially under the rim, and become very small on the distal end near the spine. In contrast, the base plate pores on both *S. prowsei* and *S. papillosa* scales are large and of similar diameter over most of the scale, reducing in size only around the base of the spine. Scales of *S. papillosa* possess surface papillae that often match the thickened pentagonal or hexagonal pattern around each base plate pore, and lack anterior ribs connecting the posterior rim to the base of the spine. In contrast, surface papillae are lacking on *S. prowsei* scales, but these scales have thick secondary ribs connecting the posterior rim to the base of the spine. Although scales of *S. prowsei* and *S. papillosa* are similar in size, those of *S. synuroidea* are significantly smaller. The differences observed in the scales between the three species are of similar magnitude and character used to describe and distinguish other *Synura* species (Nicholls & Gerrath, 1985; Siver, 1987; Kristiansen & Preisig, 2007).

As noted by previous workers (e.g. Graham *et al*., 1993; Pusztai *et al*., 2016) and further documented in our study, cell shape is highly polymorphic within these two-celled *Synura* taxa making this character not useful for distinguishing between species which was the primary reason for Wujek & Wee (1983) proposing synonymy of *C. synuroideus* and *C. gracilis*. Interestingly, these authors noted scales that had posterior rims with and without holes (perforations). Indeed, the TEM images of scales illustrated in Wujek & Wee (1983) represent both *S. synuroidea* and *S. papillosa*. In our opinion, the scales in Wujek & Wee (1983, figs 3, 4) clearly have posterior rims and base plate pore patterns that represent *S. synuroidea* and *S. papillosa*, respectively. Based on our findings, these scale types are never found on the same cell and clearly represent two distinct species.

These findings beg the question as to whether Prowse (1962) did indeed observe two distinct species when he originally described *Chrysodidymus*. Unfortunately, although Prowse (1962) mentioned the covering of siliceous scales, he did not discuss or illustrate them, so we do not know if his observations represent one or more species. Further, we do not know which scale type really represents the type, *C. synuroideus*. It could be any of the three species discussed in this paper, or possibly a different one altogether. The culture used by Pusztai *et al.* (2016) to establish that *Chrysodidymus* belongs in *Synura* clearly represents *S. synuroidea* as discussed in our paper, and further represents the only strain to date with detailed molecular data. Given that we will never know what species Prowse (1962) originally observed, coupled with the fact that his types have been lost, we designated the neotype for *S. synuroidea* based on the culture used by Pusztai *et al.* (2016) and originally isolated from a lake in Scotland as noted above.

One population of *Synura synuroidea* reported from Orchid Bog, Newfoundland, Canada, possessed two traits that are not common for this species. First, cells within the two-celled colony were attached by their tapered ends, meaning that the cells were wider at the anterior ends. Second, cells of this population had scales with a single large hole in the posterior rim, instead of multiple holes. Although this combination of characters has only been observed on cells from the Orchid Bog site, it is interesting that this site is also the type locality for three new species of *Mallomonas* and *Synura* (Siver & Lott, 2016, 2017). Perhaps this form of *S. synuroidea* will also prove to be a separate and new species.

All three of the species clearly favour habitats that are dilute and acidic, in agreement with previous findings for *Chrysodidymus* (Siver, 1987; Charles & Smol, 1988). That said, *S. papillosa* was found over a wider pH gradient than the other two species, while *S. prowsei* was reported only from very acidic localities. Although each species was recorded from a relatively clear-water site, the vast majority of records were from localities with elevated CDOM concentrations. Based on our findings, *S. synuroidea* and *S. papillosa* were widespread and found in warm climates as well as in temperate and more northern localities. However, *S. papillosa* is the most widely distributed of the three taxa, and the only one found in both hemispheres. To date, *S. synuroidea* is also widely distributed in, but restricted to, the northern hemisphere. In contrast, *S. prowsei* was restricted to the warmer localities along the Atlantic Coastal Plain in North America. As more records are added for the three species, differences in habitat preferences may emerge that would improve their use as bioindicators (Siver, 2015).

In summary, previous records for the genus *Chrysodidymus* represent at least three different species, all of which represent two-celled colonies where the cells attach and align at 180°, but possess very different scale types. The differences in scale morphology were consistently observed in collections examined from three continents, and are consistent with characteristics used to distinguish between species of *Synura*.

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Author contributions

All authors contributed equally to the findings presented in this paper.

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