Comparing morphological and molecular estimates of species diversity in the freshwater genus *Synura* (Stramenopiles): a model for understanding diversity of eukaryotic microorganisms

Pavel Škaloud, Magda Škaloudová, Iva Jadrná, Helena Bestová, Martin Pusztai
Charles University, Faculty of Science, Department of Botany, Benátská 2, 128 00 Praha 2, Czech Republic

Dmitry Kapustin
Institute of Plant Physiology, Russian Academy of Sciences, Botanical Street 35, 127276, Moscow, Russia

and Peter A. Siver
Department of Botany, Connecticut College, New London 06320-4196, Connecticut, USA

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2 Author for correspondence: skaloud@natur.cuni.cz, +420 221 951 648

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ABSTRACT
We performed a comparison of molecular and morphological diversity in a freshwater colonial genus Synura (Chrysophyceae, Stramenopiles), using the island of Newfoundland (Canada) as a case study. We examined the morphological species diversity in collections from 79 localities, and compared these findings to diversity based on molecular characters for 150 strains isolated from the same sites. Of 27 species or species-level lineages identified, only one third was recorded by both molecular and morphological techniques, showing both approaches are complementary in estimating species diversity within this genus. Eight taxa, each representing young evolutionary lineages, were recovered only by sequencing of isolated colonies, whereas ten species were recovered only microscopically. Our complex investigation, involving both morphological and molecular examinations, indicates that our knowledge of Synura diversity is still poor, limited only to a few well-studied areas. We revealed considerable cryptic diversity within the core S. petersenii and S. leptorrhabda lineages. We further resolve the phylogenetic position of two previously described taxa, S. kristiansenii and S. petersenii f. praefracta, propose species-level status for S. petersenii f. praefracta, and describe three new species, S. vinlandica, S. fluviatilis and S. cornuta. Our findings add to the growing body of literature detailing distribution patterns observed in the genus, ranging from cosmopolitan species, to highly restricted taxa, to species such as S. hibernica found along coastal regions on multiple continents. Finally, our study illustrates the usefulness of combining detailed morphological information with gene sequence data to examine species diversity within chrysophyte algae.

Key index words: algae, biogeography, chrysophytes, diversity, molecular phylogeny, morphology, Synura, protists, taxonomy.

Abbreviations: BIC, Bayesian information criterion; eDNA, environmental DNA; LDA, linear discrimination analysis; MES, 2-(N-morpholino)ethanesulfonic acid; mt, mitochondrial; nu, nuclear; OTU, operational taxonomic unit; psaA, photosystem I P700 chlorophyll a apoprotein A1; pt, plastid.

INTRODUCTION
Protists represent a wide diversity of organisms that are distributed across the eukaryote tree of life and play critical roles in ecological and biogeochemical processes, including carbon fixation, decomposition, elemental transformations, energy transfer, and animal and plant diseases (Adl et al. 2019). Despite their significance to the functioning of aquatic and terrestrial ecosystems, species diversity, biogeography and ecological importance are poorly known for many groups of protists. In addition, contrasting views regarding protist diversity have emerged over the last several decades. On the one hand, global protist diversity is believed to be extraordinarily high and represented by a wide range of distribution patterns (Foissner 1999). In contrast, other studies suggest that protist diversity is much lower and fundamentally different than that of macroorganisms (Fenchel and Finlay 2003). The majority of recent surveys based on environmental DNA (eDNA) support the former opinion, often reporting an extremely high proportion of SSU rDNA sequences that could not be assigned to described species (e.g., Šlapeta et al. 2005, Howe et al. 2009, Behnke et al. 2011). Indeed, projections of the number of protist species globally have ranged from several tens of millions (Adl et al. 2012) to over 160 million, especially when parasitic and symbiotic taxa are considered (Larsen et al. 2017).

Tools used to estimate protist diversity, including advances in microscopical and molecular techniques, have evolved rapidly since the 1950’s (Pawlowski et al. 2012, Caron and Hu 2019). Morphology has always been and remains the central criterion for delineating protist species. However, several investigations indicate that for some groups morphospecies can fail to differentiate all species due to both the lack of discriminating characters, and convergent morphological evolution (Von Der Heyden et al. 2004, Krienitz et al. 2010, Škaloud and Rindi 2013, Pinseel et al. 2019). Recent development of molecular tools has advanced our ability to discriminate among cryptic taxa, improving overall diversity estimates. For some cryptic complexes a re-evaluation of the morphological characters supported the molecular findings (Škaloud et al. 2014).

Without question, eDNA metabarcoding surveys have yielded deep insights into the composition of protist communities in soil (Mahé et al. 2017), marine (De Vargas et al. 2015) and freshwater (Boenigk et al. 2018) habitats. However, despite its increasing application in estimating protist biodiversity, there are drawbacks to using metabarcoding to estimate species richness. First, the lack of morphological data associated with metabarcoding sequences prevents investigation of other facets of diversity, such as structural and functional aspects. Second, generation of short single loci...
sequences makes it difficult or even impossible to determine appropriate species boundaries. Therefore, the taxonomic interpretation of generated sequences greatly relies on the completeness and quality of existing reference databases. Third, environmental sequencing may often lead to creating molecular chimaeras and amplification of a number of alien organisms, transported into study sites from other systems. Studies that can improve our understanding of species boundaries based on molecular data, and effectively link the molecular data to morphospecies, would advance the use of metabarcoding in determining protist diversity, especially within closely related groups of organisms, and improve comparison with previous and historical studies based solely on morphological data.

*Synura* is a species-rich freshwater genus (Chrysophyceae, Stramenopiles) that has one of the best morphological species concepts among protists. A total of 90 *Synura* taxa have been described so far, from which 54 are recognized as currently accepted (see Škaloud et al. 2012 for the list of synonyms and taxa nomen nudum). Cells of *Synura* are covered with an organized layer of morphologically complex siliceous scales, each of which is produced under highly controlled conditions within a silica deposition vesicle (Leadbeater 1990, Kristiansen 2005). Scale shape, size and design are primary characters used to distinguish between species. Consistent differences in scale morphology have largely aligned nicely with differences between taxa identified using multiple gene sequences (Škaloud et al. 2014, Jo et al. 2016). The alignment of morphological and molecular traits makes *Synura* an excellent model organism for comparing the two methodologies as tools used to distinguish between species.

Newfoundland (Canada) is a large island (area 108 860 km²) situated off the east coast of the North American mainland. The morphological diversity of *Synura* in Newfoundland was documented by four previous studies, investigating 37 different water bodies by means of transmission or scanning electron microscopy (Wawrzyniak and Andersen 1985, Siver and Lott 2016, 2017, Siver et al. 2018). These studies recorded 14 *Synura* taxa (*S. bjoerkii, S. curtispina, S. echinulata, S. kristiansenii, S. leptorrhhabda, S. mammillosa, S. mollispina, S. papillosa, S. petersenii sensu lato, S. sphagnicola, S. spinosa, S. spinosa f. nygaardii, S. synuroidea, S. uvella*). A high morphological diversity of scales within several *Synura* species complexes was reported by Siver and Lott (2017), who mentioned the perforce of future molecular analyses of these cryptic species complexes to enhance our understanding of scaled chrysophyte diversity.
The aim of the current study is to estimate species diversity of *Synura* in Newfoundland using a combination of morphological and molecular techniques. We characterized morphological and molecular data for 150 *Synura* strains isolated from 79 localities on the island in order to estimate species diversity, and examine how the morphological diversity reported in the previous works compares with an investigation based on a combined data set. A total number of 16 *Synura* species has been molecularly detected (*S. americana, S. borealis, S. conopea, S. hibernica, S. kristiansenii, S. lanceolata, S. leptorrhabda/ S. mammillosa sensu lato, S. petersenii, S. sphagnicola, S. splendida, S. truttae* and four new taxa within *Synura petersenii* species complex (section Petersenianae). In addition, the *Synura* species diversity was further investigated using electron microscopy, and the list of *Synura* species in Newfoundland waterbodies has increased to 31.

**MATERIALS AND METHODS**

*Collection, isolation and cultivation of Synura strains.*

On May 24-29, 2017 samples of phytoplankton were collected from 79 lakes and ponds in Newfoundland (Fig. 1 and Table S1 in the Supporting Information) using a plankton net with 20 μm mesh. Standard measurements of water temperature, pH and specific conductivity was carried out using a combined pH/conductometer (WTW 340i; WTW GmbH, Weilheim, Germany). Samples were examined with an Olympus CX 31 light microscope and the individual *Synura* colonies were isolated by micropipetting. Each colony was placed into a separate well of a 96-well polypropylene plate filled with approximately 300 μl of MES buffered DY IV liquid medium (pH ≈ 6; Andersen et al. 2005). In the laboratory, the well growing cultures were transferred from wells into 50 mL Erlenmeyer flasks filled with the same medium. They were cultivated in a cooling box (C5G, Helkama Oy, Helsinki, Finland) at 15°C, under constant illumination of 40 μmol photons · m⁻² · s⁻¹ (TLD 18W/33 fluorescent lamps, Philips, Amsterdam, the Netherlands).

*Sequencing and phylogenetic analysis.*

For DNA isolation, 100-200 mL of living cultures were centrifuged in PCR tubes (6,000 rpm for 3 min), and 30 mL of InstaGene matrix (Bio-Rad Laboratories) was added to the pellet. The solution
was vortexed for 10 s, incubated at 56°C for 30 min, and heated at 99°C for 8 min. After vortexing a second time, the tubes were centrifuged at 12,000 rpm for 2 min, and the supernatant was directly used as a PCR template. A total of seven molecular loci were sequenced. First, all strains were genetically characterized by sequencing their nu ITS rDNA. This molecular locus has been shown to represent an ideal DNA barcode to distinguish among Chrysophycean species, including those belonging to the genus *Synura* (Jost et al. 2010, Škaloud et al. 2012, Bock et al. 2017). For the selection of strains having a unique nu ITS rDNA barcode, additional loci were amplified to obtain robust, well-resolved phylogenies. For the strains belonging to Petersenianae, we additionally sequenced pt *rbc*L and mt *cox*I loci. For other strains, we further sequenced nu SSU rDNA, nu LSU rDNA, pt LSU rDNA, pt *psa*A and pt *rbc*L loci. The amplifications were performed in 10 μL reaction volumes (6.1 μL sterile Milli-Q Water, 2 μl MyTaq™ Buffer; Bioline LAB MARK), 0.4 μL each of the forward and reverse primer (25 nM), 0.1 μL of MyTaq™ DNA polymerase (5 U · μL⁻¹; Bioline LAB MARK), and 1 μL DNA template (not quantified), using the primers and amplification conditions listed in Table S2 in the Supporting Information. The PCR products were purified by NucleoMag® NGS Clean-up and Size Select kit (Macherey-Nagel) and sequenced with an ABI3730XL DNA Analyzer at Macrogen Inc. in Seoul, Korea.

Multiple alignments of nuclear nu ITS, nu SSU, nu LSU rDNA and organellar mt *cox*I, pt *rbc*L, pt *psa*A and pt LSU rDNA loci sequences were either manually built in MEGA6 (Tamura et al. 2013) or constructed using MAFFT v6, applying the Q-INS-i strategy (Katoh et al. 2002). The newly determined sequences were aligned to other sequences from the GenBank database, selected to encompass all known lineages (Tables S3 and S4 in the Supporting Information). The positions with deletions prevailing in a majority of sequences were removed from the alignment. Two alignments were constructed for the phylogenetic analyses: (i) a concatenated nu ITS rDNA + pt *rbc*L + mt *cox*I alignment of 60/187 unique/total sequences of Petersenianae, and (ii) a concatenated nu ITS rDNA + nu SSU rDNA + nu LSU rDNA + pt LSU rDNA + pt *rbc*L + pt *psa*A alignment of 39/54 unique/total sequences of the genus *Synura*. In Synurales, the nu ITS rDNA alignment consisted of the 5.8S rDNA and ITS2 rDNA regions only, due to high genetic divergence among the strains. ITS2 rDNA sequences were aligned with the help of their secondary structure information, using the ITS2 database V (Ankenbrand et al. 2015). The ITS2 secondary structures of *Synura americana*
(HG514166.1), S. petersenii (AF308832.1), S. conopea (FM178506.1), S. truttae (FM178508.1), S. borealis (HG514174.1), S. glabra (FM178511), S. macropora (FM178494.1), Pedospumella encystans (EF577176), P. sinomuralis (EF577170) and Ellipsoidion sp. (HE586522) were used as a template for homology modelling. Homology modelling was performed by the custom modelling option, using the ITS 2 PAM 50 matrix and 20% threshold for the transfer of helices. Secondary structures were successfully obtained for all analysed species, with the exception of S. longitubularis, S. curtispina, S. sphagnicola, S. synuroidea, and S. spinosa. These were manually folded with the help of modelled secondary structures of closely related taxa. The alignments were generated using the ITS2 database V, by both sequences and structures. DNA alignments are freely available on Mendeley Data: http://dx.doi.org/10.17632/jjfmp6nv4b.1.

The Bayesian evolutionary analyses were performed to infer a phylogeny and simultaneously estimate branch divergence times for the investigated strains, using the program BEAST v1.10.4 (Suchard et al. 2018). The analyses were performed on the two concatenated and partitioned alignments as specified above. For each of the 15 specified alignment partitions, the most appropriate substitution model was estimated using the Bayesian information criterion (BIC) as implemented in jModelTest 2.1.4 (Darriba et al. 2012). This BIC-based model selection procedure selected the following models: (i) GTR + Γ for the nu ITS1 rDNA, the first codon positions of the pt psaA gene, and the third codon positions of the pt rbcL and pt psaA genes, (ii) GTR + I for the first and second codon positions of the mt cox1 and pt psaA genes, respectively, (iii) GTR + I + Γ for the nu ITS2 rDNA, nu SSU rDNA, nu LSU rDNA, pt LSU rDNA, and the first and third codon positions of the pt rbcL and mt cox1 genes, respectively, (iv) JC for nu 5.8S rDNA and the second codon positions of the pt rbcL gene, and (v) F81 for the second codon positions of the mt cox1 gene. Lognormal relaxed clock models were selected for the partitions, and a birth-death diversification process was used as a prior on the distribution of node heights. Three temporal constraints were used to calibrate the Synurales phylogeny, based on the fossil scales found in lacustrine mudstones from the Giraffe (Siver et al. 2015) and Wombat (Siver et al. 2013) cores, respectively. These constrains include (i) the lineage comprising Synura uvella and S. splendida (Giraffe core), (ii) the stem of S. curtispina + S. longitubularis lineage (Giraffe core), and (iii) the lineage of all Petersenianae taxa including S. macracantha (Wombat core). The splits were based on an offset of either 48 (Giraffe core) or 83 Ma.
(Wombat core), a mean of 8.0 and a standard deviation of 6.0. Six Markov Chain Monte Carlo (MCMC) analyses were run for 50 million generations, sampling every 10,000 generation. After the diagnosis for convergence using Tracer 1.6, the log files were merged using the burn-in set to 10 million generations. Accordingly, the substitution rates were obtained for the nu ITS2 rDNA (8.857E-4) and three codon partitions of the pt rbcL gene (1.875E-4, 4.318 E-5, 0.001663), respectively. The estimated rates were then applied to infer the Petersenianae phylogeny, since the fossil calibrations are unrealistic due to high morphological similarity of cryptic species. The Bayesian evolutionary analyses were performed as described above, with the exception of fixing substitution rates of nu ITS2 and pt rbcL partitions instead of defining temporal constrains. The analyses were run on the CIPRES ScienceGateway v.3.3 web portal (Miller et al. 2010).

Morphological investigations and statistical analyses.

To assess the morphological diversity of Synura, selected samples (those with numerous living Synura colonies detected by light microscopic examinations) were investigated with transmission electron microscopy (TEM). After a gentle mix, a drop of the sample was placed onto formvar-coated copper grids and dried. After washing in a series of distilled water droplets, the grids were examined in a TEM JEOL 1011 electron microscope. In four new taxa, morphology of colonies and ultrastructure of silica scales were observed by light microscopy (LM), as well as by TEM and scanning electron microscopy (SEM). For TEM investigations, a drop from the living cultures was placed onto formvar-coated copper grids, dried, and investigated as described above. For SEM investigations, aliquots of each Synura culture were air dried onto heavy duty aluminium foil. The aluminium foil samples were trimmed, attached to aluminium stubs with Apiezon® wax, coated with a mixture of gold and palladium for 2 min with a Polaron Model 5100E sputter coater, and examined with a FEI Nova NanoSEM 450 field emission SEM. For each strain, seven morphological characters of 30 randomly selected silica scales were measured using the program ImageJ 1.45s (Schneider et al. 2012). The seven morphological characters, as described in Škaloud et al. (2014), include: (1) scale length; (2) scale width; (3) area of a base hole; (4) average area of a keel pore; (5) average area of a base-plate pore; (6) keel width; and (7) number of struts. Measured data were compared with those we obtained in our previous investigations (Škaloud et al. 2014, Jo et al. 2016). Data visualisation and
statistical analyses (principal component analysis, linear discrimination analysis and phylomorphospace plots) were performed in R 3.5.2 (R Development Core Team), using the packages phytools (Revell 2012), and MASS (Venables and Ripley 2002). Principal component analysis (PCA) and linear discrimination analysis (LDA) were performed using the functions prcomp and lda, respectively.

RESULTS

Analyses of molecular data.

Our six-loci phylogeny of Synurales resolved three major clades identified here as sections Synura, Curtispinae, and Petersenianae (Fig. 2). All three clades were strongly supported (Bayesian posterior probabilities 1.00), but their relationship remain unresolved probably due to their concurrent origin. On the basis of our time calibration, the genus Synura originated near the onset of the Cretaceous (approximately 145 Mya), and split into the three major clades during the Early Cretaceous at about 117 Mya. During the late Neogene, the major radiation occurred within the core Petersenianae, leading to the origin of about twenty species-level lineages (Fig. 3).

We have successfully sequenced a total of 150 Synura strains, forming 17 well-resolved lineages. Ten of these lineages were well attributed to previously described and genetically characterized species S. americana, S. borealis, S. conopea, S. hibernica, S. lanceolata, S. leptorrhabda, S. petersenii, S. sphagnicola, S. splendida and S. truttae. We resolved the phylogenetic position of S. kristiansenii, a putative Newfoundland endemic species recently described by Siver and Lott (2016). It represents a distinct lineage within the section Petersenianae that originated ca 51 Mya (Fig. 3).

Significant cryptic diversity has been detected within the S. leptorrhabda clade of section Curtispinae and within the core Petersenianae. We identified three and four genetically novel lineages from these two sections, respectively. The ones from Petersenianae are proposed here as S. praefracta comb. nov., S. vinlandica sp. nov., S. fluviatilis sp. nov. and S. cornuta sp. nov.

Morphological analyses of natural populations.
Based on TEM investigations of natural samples, we identified a total of 19 Synura morphotypes (Fig. 4, Table S5 in the Supporting Information). Seven morphotypes were identified within the section Petersenianae, of which only four might be assigned to any of described species (S. conopea, S. hibernica, S. kristiansenii, S. petersenii). One morphotype was distinct by a very narrow keel (Fig. 4e) and corresponds well to the scales found by Wujek and Igoe (1989) in Michigan, USA (Little Tom Lake, fig. 12) determined as Synura petersenii f. praefracta. However, this morphotype does not correspond to the iconotype of S. petersenii f. praefracta (Asmund 1968) in both keel morphology and strut number and therefore very probably represents a novel yet undescribed species. In addition, two Petersenianae morphotypes did not fit into any of previously described, morphologically similar taxa. Synura sp. 1 (Fig. 4f) differs by having medium-sized scales possessing a large number of struts (29-32). Synura sp. 2 (Fig. 4g) is distinct by rather broad scales with a wide keel and a low number of struts (26-28).

A remarkable diversity has been recognized within the section Curtispinae, as well. Along with well-characterized Synura curtispina, S. echinulata, S. leptorrhabda, S. mammillosa, S. papillosa, S. sphagnicola, S. spinosa f. longispina and S. synuroidea we observed some yet undescribed species or taxa with uncertain taxonomic status. S. curtispina f. reticulata (Fig. 4k) was found in several investigated localities. Although this taxon has been synonymized with S. curtispina (Kristiansen and Lind 1995), we are convinced it represents a distinct taxon since the honeycomb reticulation extends to the proximal end of the scale. Probably the most conspicuous morphotype was found in Great Rattling Brook (locality K70), distinct by a curved spine and a large area of distinctive labyrinthic pattern, spreading almost to the proximal part of the scale (Fig. 4m). The scale corresponds well to those presented by Nicholls and Gerrath (1985) from Ontario, Canada, determined there as S. echinulata. However, the labyrinthic pattern differs a lot from the sculpture present in the iconotype of S. echinulata (Korshikov 1929). We therefore presume the morphotype shown in Figure 4m, which has been observed in other North American localities (P.A. Siver, unpub. data), represents a distinct species, not identical to S. echinulata.

Morphological analyses of cultured strains.

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From a morphological perspective, the majority of strains fit the circumscription of described taxa, forming single, genetically distinct lineages. However, our investigations revealed a striking morphological similarity of strains belonging to the novel lineages within the *Synura leptorrhabda* clade of section Curtispinae and within the core Petersenianae. Three lineages morphologically corresponding to *S. leptorrhabda* were highly similar in their sculpture and dimensions of silica scales (Fig. 5). In addition, we revised the morphology of closely related strains we originally determined as *S. mammillosa* in Škaloud et al. (2013a), concluding they either morphologically better fit with *S. leptorrhabda* (strains S89.C3 and S96.B5) or represent a transient morphotype between these two taxa (the strain SIE.105A). Accordingly, the *S. leptorrhabda* clade probably consists of numerous cryptic lineages. Since we do not currently possess enough material to investigate in detail the morphological properties of *S. leptorrhabda* lineages, we do not treat them taxonomically in this paper, and will focus on this cryptic complex in a separate study.

On the other hand, we analysed in detail the morphological properties of four novel lineages inferred within the core Petersenianae, along with all previously described, closely related taxa (Fig. 6). Morphological comparisons of silica scales revealed the general similarity of all novel clades to the previously described taxa (Fig. 6, a and b). Indeed, only 73, 60, 70 and 63 percent of *Synura praefracta*, *S. vinlandica*, *S. fluviatilis* and *S. cornuta* scales were correctly recognized by the discrimination function, respectively (Table S6 in the Supporting Information). However, all four novel lineages could be clearly differentiated by the combination of scale dimensions, basal/keel pore sizes and specific morphological features. The scales of *S. vinlandica* are characterized by the shortened, eccentrically positioned keel, observed in the majority of apical and even some of the body scales. The three remaining lineages can be well recognized by the unique shapes of their keel tips. Whereas the scales of *S. praefracta* possess a rounded tip terminated by several short teeth, the keels of *S. fluviatilis* and *S. cornuta* protrude into acute tips, which are either very long and tapering (*S. fluviatilis*) or shorter and much narrow (*S. cornuta*), respectively (see the taxonomic revision below for more details). The toothed keel tips observed in the scales of *S. praefracta* are characteristic of *Synura petersenii* f. *praefracta*, described by Asmund (1968) from Alaska. Indeed, the scale morphology is in correspondence with the taxon iconotype, showing the morphology of three apical silica scales (Fig. 6c). Therefore, we can unquestionably assign this species to *Synura petersenii* f.
praefracta. Since this lineage represents a distinct species within the core Petersenianae, we are proposing a new combination, *S. praefracta*, comb. nov. (see below). The remaining three novel lineages could not be assigned to any *Synura* taxon with known morphology of silica scales but lacking molecular characterization (*S. obesa*, *S. australiensis*, ‘*S. petersenii*’ f. *columnata*, and ‘*S. petersenii*’ f. *taymyrensis*). To avoid introduction of superfluous names, we also carefully considered all previously described species with unknown ultrastructure of silica scales. According to Škaloud et al. (2012), six of these taxa can be affiliated to the section Petersenianae according to either the presence of keel or the absence of distal spines on the scales: *S. adamsii*, *S. adamsii* f. *malabrica*, *S. caroliniana*, *S. elipidosa*, *S. intermedia* and *S. virescens*. The former three taxa are well differentiated by their very long cells, *S. elipidosa* is distinct by very small cell dimensions (up to 12 µm in length), and *S. virescens* has very large colonies (up to 137 µm in diameter). *S. intermedia* can be distinguished by a strongly prolonged keel resembling the spine. Even though a similarly prominent keel tip has been observed in *S. cornuta*, the spiny keel in *S. intermedia* is much longer, exceeding in its length the entire length of the scale. Consequently, given the fact three novel lineages are not identical to any previously described species, we are proposing that they represent three new species (*S. vinlandica*, *S. fluviatilis* and *S. cornuta*) described below.

Consequently, the core Petersenianae now includes 17 ultrastructurally and molecularly well-defined species distributed in four clades (Fig. 3). The phylomorphospace plots (projections of the species trees into the morphospaces based on silica scale morphology) show the morphological similarity of species belonging to particular clades, though in *Synura macropora* and *S. borealis* a significant morphological shift occurred during the evolution of the genus (Fig. 6d). In general, whereas clade 1 is composed by the species possessing rather broad scales, clade 2 comprises those species having the smallest scale dimensions (Fig. 6, e and f).

Taxonomic revisions and diagnoses.

**Synura praefracta** (Asmund) Škaloud & Škaloudová comb. nov. (Fig. 7, a-i)


*Observations:* Colonies are spherical, up to 57 µm in diameter, consisting of approximately 6–28 cells associated by their posterior ends (Fig. 7a). Cells are elongated, anteriorly cylindrical,
posteriorly tapering into the long tail, 21–29 µm long and 6.5–8.5 µm wide (Fig. 7b). Each cell is surrounded by a layer of imbricate siliceous scales (Fig. 7, c and d). Body scales are 2.8–3.9 µm long and 1.5–2.0 µm wide, consisting of a basal plate with a centrally raised keel protruding into a very short acute tip (Fig. 7e). The keel is cylindrical and ornamented by larger pores (diameter, 51–93 nm). The basal plate is ornamented by numerous small pores (diameter 19–37 nm), and anteriorly perforated by an elongated or rounded base hole (diameter 0.19–0.74 µm). Numerous struts (27–36, rarely 40), interconnected by transverse ribs, extend regularly from the keel to the scale perimeter (Fig. 7f). Apical scales are 2.6-3.3 µm long and 1.6-1.8 µm wide (Fig. 7g). The keel of the apical scales with rounded spine terminated by several short teeth (Fig. 7, g and h). Rear scales are 2.5-2.6 µm long and x 1 x 0.9-1.1 µm wide (Fig. 7i).

**Holotype:** material deposited in Statens Naturhistoriske Museum, Copenhagen, Denmark (currently lost).

**Epitype** (here designated): Strain I7 permanently cryopreserved in a metabolic inactive state in the Culture Collection of Algae of Charles University in Prague (CAUP) as the item TYPE-B714.

**Reference strain:** The live culture of the epitype (strain I7) has been deposited as CAUP B714 in the Culture Collection of Algae of Charles University in Prague, Czech Republic.

**Distribution:** The scales with rounded spine terminated by minute teeth at the apex designated as *Synura petersenii f. praefracta* have previously been reported from different areas, e.g. Canada (Nicholls and Gerrath 1985), USA (Siver 1987), Chile (Dürrschmidt 1982), Hungary (Barreto 2005), Ireland (Řezáčová and Škaloud 2005), Netherlands (Wujek and Van Der Veer 1976, Roijackers and Kessels 1981), Russia (Balonov 1976).

**Synura vinlandica Škaloud, Škaloudová & Siver sp. nov.** (Fig. 7, j-r)

**Description:** Colonies are spherical, up to 65 µm in diameter, consisting of approximately 20–42 cells associated by their posterior ends (Fig. 7j). Cells are drop-shaped, anteriorly cylindrical, posteriorly tapering into the tail, 21–32 µm long and 7–12 µm wide (Fig. 7k). Each cell is surrounded by a layer of imbricate siliceous scales (Fig. 7, l and m). Body scales are 2.9-4.1 µm long and 1.4-2.2 µm wide, consisting of a basal plate with a centrally raised keel, which is rounded or mostly protruding into an acute tip (Fig. 7n). The keel is cylindrical, usually narrow, rarely slightly widened
anteriorly, and ornamented by larger pores (diameter 56–115 nm). The basal plate is ornamented by numerous medium-sized pores (diameter 25–37 nm), and anteriorly perforated by an elongated base hole (diameter 0.17–0.46 μm). Struts (23–34), sometimes interconnected by transverse ribs, extend regularly from the keel to the scale perimeter (Fig. 7, n-p). Apical scales are 2.9-3.6 μm long and 1.6-2.2 μm wide (Fig. 7q). The keel of the apical scales usually ends in a prominent, acute tip. The keel of apical scales is shortened and positioned eccentrically to one side of the scale (Fig. 7q). Such eccentric keel positioning was observed in some of the body scales, as well (Fig. 7p). Rear scales are 1.9-4.6 μm long and 0.8-1.5 μm wide (Fig. 7r). Differ from other Synura species by the ultrastructure of silica scales and by nu ITS rDNA (GenBank Accession MN782206), pt rbcL (MN783119) and mt coxI (MN783144) sequences.

*Holotype* (here designated): Strain I82 permanently cryopreserved in a metabolic inactive state (cryopreservation in liquid nitrogen) at the Culture Collection of Algae of Charles University in Prague (CAUP) as the item TYPE-B715. Figure 7n presents an illustration of the holotype.

*Reference strain*: The live culture of the epitype (strain I82) has been deposited as CAUP B715 in the Culture Collection of Algae of Charles University in Prague, Czech Republic.

*Etymology*: The specific epithet ”vinlandica” refers to the Viking name for the Canadian Island of Newfoundland (Vinland), where the species has been discovered.

*Type locality*: Shoe Cove Pond, Newfoundland, Canada (47.74186, -52.74175).

*Distribution*: Currently only known from Newfoundland, Canada.

**Synura fluviatilis Škaloud, Škaloudová & Siver sp. nov.** (Fig. 8, a-i)

*Description*: Colonies are spherical, up to 56 μm in diameter, consisting of approximately 16–24 cells associated by their posterior ends (Fig. 8a). Cells are lanceolate, widest in their middle part, posteriorly tapering into the tail, 20–28 μm long and 7–10 μm wide (Fig. 8b). Each cell is surrounded by a layer of imbricate siliceous scales (Fig. 8c). Body scales are 2.7-4.0 μm long and 1.3-1.8 μm wide, consisting of a basal plate with a centrally raised rounded keel. Body scales in the anterior part of the cell have a keel which protruding into an acute tip (Fig. 8d). The keel is cylindrical, occasionally slightly widened anteriorly, and ornamented by larger pores (diameter, 64–105 nm; Fig. 8, e and f). The basal plate is ornamented by numerous medium-sized pores (diameter 22–39 nm), and
anteriorly perforated by a rounded or elongated base hole (diameter 0.15–0.38 µm). Numerous struts (26–37), sometimes interconnected by transverse folds, extend regularly from the keel to the scale perimeter (Fig. 8e). Apical scales are 2.0-2.9 µm long and 1.4-1.8 µm wide. The keel of the apical scales ends in a long, prominent, usually acute tip (Fig. 8, g and h). Rear scales are 1.9-2.5 µm long and 0.9-1.1 µm wide (Fig. 8i). Differs from other *Synura* species by the ultrastructure of silica scales and by nu ITS rDNA (GenBank Accession MN782209), pt *rbc*L (MN783121) and mt *cox* I (MN783146) sequences.

*Holotype* (here designated): Strain J87 permanently cryopreserved in a metabolic inactive state (cryopreservation in liquid nitrogen) at the Culture Collection of Algae of Charles University in Prague (CAUP) as the item TYPE-B716. Figure 8f presents an illustration of the holotype.

*Reference strain:* The live culture of the epitype (strain J87) has been deposited as CAUP B716 in the Culture Collection of Algae of Charles University in Prague, Czech Republic.

*Etymology:* The specific epithet "fluviatilis" refers to the common habitat of the species, i.e., various running water bodies such as rivers and brooks.

*Type locality:* Oxbow lake of Exploits River, Newfoundland, Canada (48.94234, -55.76928).

*Distribution:* Currently only known from Newfoundland, Canada.

**Synura cornuta** Škaloud, Škaloudová & Siver sp. nov. (Fig. 8, j-r)

*Description:* Colonies are spherical, up to 55 µm in diameter, consisting of approximately 8–16 cells associated by their posterior ends (Fig. 8j). Cells are spherical, anteriorly rounded, posteriorly tapering into the tail, 13–27 µm long and 9–15 µm wide (Fig. 8k). Each cell is surrounded by a layer of imbricate siliceous scales (Fig. 8l). Body scales are 3.2-4.9 µm long and 1.4-1.9 µm wide, consisting of a basal plate with a centrally raised rounded keel, which protruding into either a short, tapering, acute tip (Fig. 8, m and n), or rarely a very specific, narrow and prominent tip resembling a horn (Fig. 8o). The keel is cylindrical, occasionally slightly widened anteriorly, and ornamented by larger pores (diameter, 47–92 nm). The basal plate is ornamented by numerous small pores (diameter 17–30 nm), and anteriorly perforated by a rounded or elongated base hole (diameter 0.16–0.39 µm). Numerous struts (22–35), not interconnected by transverse rims, extend regularly from the keel to the scale perimeter. Apical scales are 2.2-3.2 µm long and 1.3-1.6 µm wide (Fig. 8, p and q). Similar to
some of the body scales, the keel of the apical scales ends in a very prominent, narrow tip resembling a horn (Fig. 8, p and q). Rear scales are 2.3-3.1 µm long and 0.8-1.2 µm wide (Fig. 8r). Differs from other *Synura* species by the ultrastructure of silica scales and by nu ITS rDNA (GenBank Accession MN782210), pt *rbc*L (MN783122) and mt *cox*1 (MN783147) sequences.

**Holotype** (here designated): Strain K6 permanently cryopreserved in a metabolic inactive state (cryopreservation in liquid nitrogen) at the Culture Collection of Algae of Charles University in Prague (CAUP) as the item TYPE-B717. Figure 8o presents an illustration of the holotype.

**Reference strain**: The live culture of the epitype (strain K6) has been deposited as CAUP B717 in the Culture Collection of Algae of Charles University in Prague, Czech Republic.

**Etymology**: The specific epithet ”*cornuta*” refers to the specific shape of the keel tip on the silica scales.

**Type locality**: Unnamed lake, Newfoundland, Canada (48.94364, -55.82329).

**Distribution**: Currently only known from Newfoundland, Canada.

DISCUSSION

*Comparing morphological and molecular diversity estimates.*

Despite increasing popularity of cultivation-independent molecular methods to determine the overall diversity and distribution of protists, accurate comparative studies between morphological and molecular approaches remain very rare. DNA metabarcoding, primarily focusing on overall diversity of aquatic protist communities, usually reveals five to ten times higher diversity than the microscopic examinations (Abad et al. 2016, Groendahl et al. 2017, Rippin et al. 2018), though some studies have reported comparable taxon richness (Bazin et al. 2014). However, only a limited number of taxa are being identified by both molecular and microscopical approaches. For example, of 180 protist taxa morphologically determined by Groendahl et al. (2017) in the estuary of Bilbao River, only 44 of them were detected in the DNA-based datasets consisting of hundreds to thousands of OTUs.

Obviously, metabarcoding of protist communities is severely limited by i) overestimation of species diversity by accounting erroneous sequences and PCR chimaeras as new, distinct species (Behnke et al. 2011, Lücking et al. 2014), ii) incompleteness of reference DNA databases linked to morphology.
of described species (Leray and Knowlton 2015), and iii) a still high portion of cryptic taxa in protist morphospecies (Howe et al. 2009, Škaloud and Rindi 2013).

More accurate molecular estimations of protist diversity are obtained when distinct, morphologically well recognized protist lineages are analysed. For example, Bachy et al. (2013) studied the diversity of the marine ciliate order Tintinnida, characterized by production of a species-specific secreted shell, the lorica. The morphological observations were supplemented by classical DNA cloning and metabarcoding, using the modern, more complex algorithms of OTU generation. The molecular approaches congruently detected the vast majority of morphologically observed taxa, and additionally revealed numerous novel lineages hidden to the traditional approaches.

Our study utilized a similar approach to Bachy et al. (2013) in combining molecular information with detailed morphological data for a taxon that forms highly distinctive siliceous scales. Although we did not employ the DNA metabarcoding approach, we investigated the molecular diversity by nu ITS rDNA sequencing of 150 isolated Synura colonies grown over a short time period. Contrary to Bachy et al. (2013), we focused on a much more narrowly defined protist lineage - the single genus Synura, comparing the molecular diversity with the traditional ultrastructural investigations of silica scales retrieved from water samples. Of the 27 identified species or species-level lineages, only one third were recorded by both molecular and morphological investigations (Fig. 9a). Eight taxa were recovered just by sequencing of isolated colonies. All of them represented rather young evolutionary lineages within the S. petersenii and S. leptorrhabda clades (Fig. 9b). Their presence in the samples might be masked by their morphological similarity to closely related species discovered by morphological investigations. A total of ten species were recovered only microscopically, four of them lack previous molecular characterization. These four taxa may represent rare, locally distributed, ecologically specialized, seasonally restricted, or hard-to-culture species. However, the remaining six taxa generally represented common species, widely distributed in temperate or boreal regions of North Hemisphere. It is possible that we were unsuccessful isolating colonies representing these ten taxa because they were rare, difficult to establish in culture, or absent altogether in the collections at the time of sampling. It is worth noting that silica scales, typically used as a sole sign of species occurrences in many algal diversity studies, can remain in the water column after the demise of their carrier cells. Consequently, isolated silica scales can be effectively used to provide detailed insight
into the species composition at a given locality, however their applicability to study the short-term temporal dynamics of species composition can be limited.

In conclusion, morphological and molecular approaches are clearly complementary in estimating the species diversity of protists, even for a narrowly defined, morphologically distinct lineage. When used separately, both approaches have their limitations. Molecular approaches using metabarcoding can be highly sensitive to species abundances at the time of sampling. Indeed, protist species richness, composition and abundance may fluctuate greatly due to changes in environmental factors, grazing pressure, and parasitism. Morphological approaches, on the other hand, can fail to recognize cryptic taxa and lead to underestimation of overall species diversity. In the case of Synura, morphological differences, especially of siliceous scales, have been successfully assigned to the majority of the cryptic species detected by molecular techniques. For cryptic Synura species, the morphological differences were always present, but simply needed to be recognized as important characters.

Building datasets that combine morphological and molecular species data, as we strive to do for Synura, will provide a more complete reference baseline that will ultimately aid future metabarcoding investigations.

Towards a more complete understanding of the global diversity within the genus Synura.

With respect to global diversity estimates, there remains a significant biogeographical bias in chrysophyte studies, where many more studies have taken place in Europe and North America than in most other regions of the world (Kristiansen 2005). Even in regions such as Newfoundland previously thought to be relatively well-studied, we documented much higher diversity using a combination of morphological and molecular techniques. The combination of techniques gave a better understanding of the boundaries used to delineate between either morphospecies or molecular species, thus improving the estimate of species diversity. Based on previous works using morphology alone (Wawrzyniak and Andersen 1985, Siver and Lott 2016, 2017, Siver et al. 2018), species diversity for Synura was considered high for Newfoundland with 14 species recorded. By simultaneously examining molecular data with a finer analysis of differences in morphological structure, the number of Synura species has more than doubled to 31 (Table S7 in the Supporting Information), illustrating the advantage of combining both techniques. Given the considerable cryptic diversity within the S.
lepotrrhabda lineage of the section Curtispinae that remains to be described, and finding additional distinct morphotypes that remain molecularly uncharacterized, Newfoundland waterbodies undoubtedly harbour even greater Synura species diversity.

In addition to differences used to distinguish between species (i.e., morphology versus molecular data), we recognize potential limitations in comparing species diversity estimates made between studies that employ different sampling methods. We further recognize that since only a small fraction of the aquatic habitats has been investigated to date, that it is highly likely additional Synura taxa reside in Newfoundland waterbodies. In the current study, and in the earlier investigation by Wawrzyniak and Andersen (1985), sampling was based on organisms actively growing at the time of collection. Since many chrysophytes present seasonal growth strategies and do not actively grow over the entire year, the time of collection can bias species diversity estimates (Siver 2015). This issue was less of a problem in the studies of Siver and colleagues (Siver and Lott 2016, 2017, Siver et al. 2018) since they incorporated remains of organisms found in both plankton and surface sediment samples. However, even though the top centimetre of surface sediments usually contains remains of organisms that grew over the last few years, only isolated scales are uncovered making it potentially difficult to capture the full complement of species. Given differences in methodologies between studies, differences in the seasonal occurrences between species, and since many waterbodies on the island have not been sampled, the full complement of species residing in Newfoundland is most likely still not realized.

Care also needs to be taken when comparing between morphological studies from different time periods, because the degree of variation in scale morphology used to differentiate between some taxa may have changed. We now recognize that the range of morphological scale variation associated with some species concepts was overly broad and representative of multiple taxa, and that other characters, such as size and distribution of base plate pores, can further aid in distinguishing between species. This was especially true for taxa in section Petersenianae where small, but consistent, differences in scale morphology are now recognized at the species level (e.g., Škaloud et al. 2012, 2014, Jo et al. 2016). Since the majority of published surveys illustrate only a few scales (and often only one) for each taxon, even for species found in numerous sites, it is highly unlikely that newly recognized differences could be fully evaluated using only the published illustrations in these previous works.
Despite thousands of localities investigated and thousands of sequences generated so far, our knowledge of global *Synura* diversity remains incomplete (Siver et al. 2010, Škaloud et al. 2013b). Geographic coverage needs to be broadened, and whenever possible investigations should strive to provide both morphological and molecular data. Most floristic and diversity studies for scaled chrysophytes are based solely on morphological investigations of silica scales, with no sequence data provided (Kristiansen and Preisig 2007). Accordingly, the molecular data are available only for about 16% of all currently accepted species and infraspecific taxa of silica-scaled chrysophytes. Considering the low genetic characterization of described taxa, and the here documented level of unknown genetic diversity, we propose that generating sequence data should become a gold standard in diversity studies of silica-scaled chrysophytes.

*Biogeographic implications.*

The biogeography of protists has become a highly controversial topic over the last two decades (Martiny et al. 2006, Caron 2009, Ryšánek et al. 2015). Finally, supported by a number of studies based on detailed molecular investigations, the ubiquitous dispersal hypothesis has been rejected in favour of a moderate endemity model (van der Gast 2015), proposing that although some protists may have cosmopolitan distributions, others are restricted in their distribution to particular regions and/or specific habitats (Foissner 2006). Indeed, very different distributional patterns have been highlighted previously for *Synura* (Boo et al. 2010). For chrysophytes, including the genus *Synura*, distribution patterns depend on dispersal capacity of the species, resistance level of resting cysts, available vectors, and suitable available habitats (Boo et al. 2010, Siver and Lott 2012, Kristiansen and Škaloud 2017).

Distribution patterns of particular *Synura* species are especially diverse, ranging from cosmopolitan distribution (e.g., *S. petersenii*, *S. glabra*) to much restricted patterns. In this respect, we point out the restricted distributional patterns of two taxa found in Newfoundland, *S. kristiansenii* and *S. hibernica*. The former species, which has a highly distinctive keel and base plate pore, was described quite recently by Siver and Lott (2016) from a small oligotrophic and highly acidic (pH 3.9) bog in Newfoundland. During our investigations, we found this species at three additional oligotrophic localities, with pH ranging 7.3-7.6. This species thus seems to occur quite frequently in
Newfoundland, spanning various habitats. Interestingly, it was never found outside of Newfoundland in numerous studies performed on the North American continent in the past, despite its very distinct scale morphology. Though *S. kristiansenii* represents a deep and evolutionary old lineage that originated ca 51 Mya, it seems to be highly restricted in its distribution to a small area in North America, possibly due to limited dispersal capacities.

Even more striking was our frequent observation of *Synura hibernica* in Newfoundland localities. Indeed, this species represented one of the most observed and sequenced species, being detected in 11 localities. Since its description in 2014, *S. hibernica* was considered to have a very restricted distribution pattern, occurring only in western Ireland (Škaloud et al. 2014). Despite our extensive sampling in Europe, including a total of 71 ecologically highly similar and geographically close localities in north-western Scotland, we did not discover a single colony of *S. hibernica* outside of Ireland. However, our present results show that *S. hibernica* has a much broader distribution, extending to North America. Moreover, it is possible that *S. hibernica* was observed in North America much earlier, almost a hundred years ago. The distinctive shape of colonies resembles that of *S. adamsii*, a species originally described by Smith (1924) from two ponds in the Palisades Interstate Park, New York, USA. *Synura adamsii* was subsequently reported in several ponds along coastal North Carolina (Whitford and Schumacher 1973). Since the morphology of silica scales was neither illustrated nor described in the protologue given by Smith (1924), the identity of *S. adamsii* is unclear relative to *S. hibernica* and it is possible that these taxa are conspecific. Notwithstanding the taxonomic status of *S. hibernica*, the distribution pattern of this species now spans coastal sites along both sides of the North Atlantic. Perhaps the distribution may be related to ionic chemistry as influenced by proximity to the ocean.

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REFERENCES


diversity assessments: Morphology compared with cloning and direct pyrosequencing of 18S rRNA genes and ITS regions using the conspicuous tintinnid ciliates as a case study. *ISME J.* 7:244–55.


USA, 324 pp.


Fig. 1. Map of Newfoundland (Canada) showing the location of sampling sites. Those sites where *Synura* colonies were detected by light microscopy, and subsequently isolated into the cultures, are given by large circles.

Fig. 2. Time-calibrated phylogeny of the genus *Synura* based on concatenated nu ITS rDNA, nu SSU rDNA, nu LSU rDNA, pt *rbcL*, pt *psaA* and pt LSU rDNA sequences. Newly generated sequences are given in bold. Mean divergence times are given for selected nodes, along with 95% highest posterior density (HPD) values in square brackets. Time axis is Mya, along with chronological dating of geologic intervals.

Fig. 3. Time-calibrated phylogeny of the genus *Synura*, section Petersenianae, based on concatenated nu ITS rDNA, pt *rbcL* and mt *cox1* sequences. Newly generated sequences are given in bold. Mean divergence times are given for selected nodes, along with 95% highest posterior density (HPD) values in square brackets. Time axis is Mya, along with chronological dating of geologic intervals.


Fig. 5. Silica scales of three *Synura leptorrhabda* lineages. a. strain I13. b. strain H92. c. strain J83. Scale bars represent 0.5 µm.

Fig. 6. Morphological analyses of 17 core Petersenianae species. a-b. Comparison of four morphological traits (scale length, scale width, base plate pore diameter, number of struts); average values and standard deviations are given, with the four newly characterized species highlighted. c. PCA ordination diagram showing the morphological diversity of 120 silica scales belonging to four
novel lineages, along with the three iconotype scales of *Synura petersenii f. praefracta*. d-f. Phylomorphospace plots of PCA axes obtained by the analysis of seven measured morphological traits. The circles represent individual species coloured/shaded by their clade affiliation (d), scale length (e) and scale width (f). Lines connect related species through hypothetical ancestors (small black dots).

**Fig. 7.** Scale morphology of *Synura* species. a-i. *S. praefracta*. a. Colony consisting of elongated, drop-shaped cells. b. Colony with one encysting cell. c. Single cell surrounded by a layer of siliceous scales. d-f. Body scales. g-h. Apical scales with rounded spine terminated by several short teeth. i. Rear scale. j-r. *S. vinlandica*. j-k. Colonies consisting of spherical, drop-shaped cells. l. Layer of silica scales covering one cell. m-o. Body scales. p. Body scale with eccentric keel. q. Apical scale with shortened and eccentric keel. r. Rear scale. Scale bars represent 10 \( \mu m \) (a-b, j-k) and 1\( \mu m \) (c-i, l-r). a-b, j-k: LM; c-d, l-m: SEM; e-i, n-r: TEM.

**Fig. 8.** Scale morphology of *Synura* species. a-i. *S. fluviatilis*. a-b. Colonies consisting of lanceolate cells. c. Single cell surrounded by a layer of siliceous scales. d-f. Body scales. g-h. Apical scales with prominent spines. i. Rear scale. j-r. *S. cornuta*. j-k. Colonies consisting of spherical cells. l. Single cell surrounded by a layer of siliceous scales. m-n. Body scales. o. Body scale with a prominent, narrow tip resembling a horn. p-q. Apical scales with the keels ending by prominent horn-like tips. r. Rear scale. Scale bars represent 10 \( \mu m \) (a-b, j-k) and 1\( \mu m \) (c-i, l-r). a-b, j-k: LM; d, m, p: SEM; c, e-i, l, n, o, q-r: TEM.

**Fig. 9.** Summary of taxa found by morphology and ITS rDNA sequencing (a) and their phylogenetic position along the *Synura* species tree (b). Estimated positions of taxa lacking molecular data (according to the morphology of silica scales) are visualized by dashed arrows. Taxa are coded by symbols in accordance to the Venn’s diagram: Morphologically detected taxa are indicated by circles, those recovered by DNA barcoding are depicted by rectangles, and those detected by both approaches are indicated by hexagons. Unrecovered taxa accompanied by no symbols.

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Table S1. List of sampling sites along with geographic coordinates, physico-chemical water parameters as well as the indication of those sites where Synura colonies were detected.

Table S2. Primers and PCR conditions for amplifying and sequencing of the nuclear nu SSU rDNA, nu LSU rDNA and nu ITS rDNA, the mitochondrion-encoded mt coxI gene, and the plastid-encoded pt rbcL, pt psaA and pt LSU rDNA genes.

Table S3. Strains of the genus Synura used in this study and the GenBank accession numbers for their nu ITS rDNA, nu SSU rDNA, nu LSU rDNA, pt LSU rDNA, pt psaA and pt rbcL gene sequences. Those strains sharing identical DNA sequences are marked with lower case letters.

Table S4. Strains of the genus Synura, section Petersenianae, used in this study and the GenBank accession numbers for their nu ITS rDNA, pt rbcL and mt coxI gene sequences. Those strains sharing identical DNA sequences are marked with lower case letters.

Table S5. List of species recovered by TEM investigations of selected localities.

Table S6. The linear discriminant analysis confusion matrix summarizing the reclassification of the silica scales based on their morphological features. The proportions of correctly classified scales are given in the diagonal.

Table S7. A summary of Synura taxa recorded in Newfoundland island, Canada.

DNA alignments are freely available on Mendeley Data: http://dx.doi.org/10.17632/jjfmp6nv4b.1.