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COMPARING MORPHOLOGICAL AND MOLECULAR ESTIMATES OF SPECIES DIVERSITY IN THE FRESHWATER GENUS *SYNURA* (STRAMENOPILES): A MODEL FOR UNDERSTANDING DIVERSITY OF EUKARYOTIC MICROORGANISMS[1]

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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 resolved 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; protists; *Synura*; taxonomy**

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

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

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techniques, have evolved rapidly since the 1950s (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 km^2) 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. leptorrhabda, 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 this study was 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 dataset. 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 Peterseniana*e*). 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 were 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 (3,200*g* for 3 min), and 30 mL of InstaGene matrix (Bio-Rad Laboratories, Hercules, CA, USA) 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 12900*g* 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 Peterseniana e, 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, London, UK), 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, Düren, Germany) 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 Peterseniana e, 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 modeling. Homology modeling was performed by the custom modeling option, using

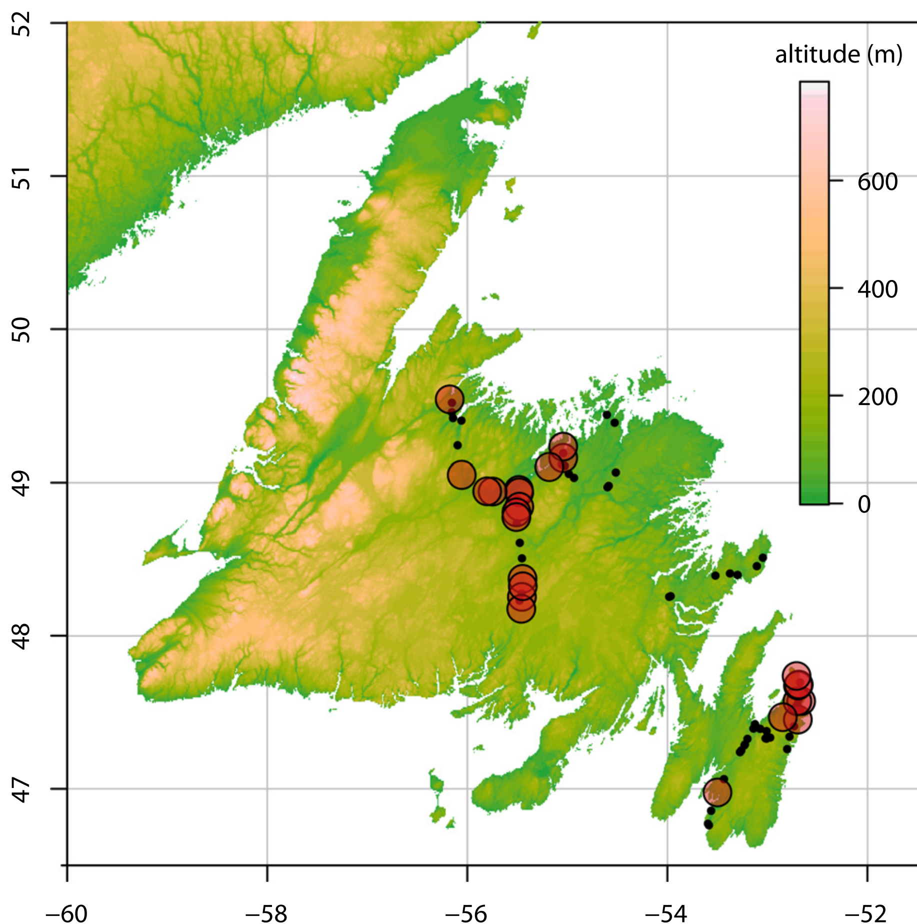

Fig. 1. Map of Newfoundland (Canada) showing the location of sampling sites. Those sites where *Synura* colonies were detected by light microscopy, and subsequently isolated into the cultures, are given by large circles. [Color figure can be viewed at wileyonlinelibrary.com]

the ITS 2 PAM 50 matrix and 20% threshold for the transfer of helices. Secondary structures were successfully obtained for all analyzed species, with the exception of *S. longitubularis*, *S. curtispina*, *S. sphagnicola*, *S. synuroidea*, and *S. spinosa*. These were manually folded with the help of modeled 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: https://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 earlier. 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 *psa*A gene, and the third codon positions of the pt *rbc*L and pt *psa*A genes, (ii) GTR + I for the first and second codon positions of the mt *cox*I and pt *psa*A 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 *rbc*L and mt *cox*I genes, respectively, (iv) JC for nu 5.8S rDNA and the second codon positions of the pt *rbc*L gene, and (v) F81 for the second codon positions of the mt *cox*I 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 *rbc*L 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 earlier, with the exception of fixing substitution rates of nu ITS2 and pt *rbc*L 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 earlier. For SEM investigations, aliquots of each *Synura* culture were air dried onto heavy duty aluminum foil. The aluminum foil samples were trimmed, attached to aluminum 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: (i) scale length; (ii) scale width; (iii) area of a base hole; (iv) average area of a keel pore; (v) average area of a base-plate pore; (vi) keel width; and (vii) number of struts. Measured data were compared with those we obtained in our previous investigations (Škaloud et al. 2014, Jo et al. 2016). Data visualization 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 the 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 20 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,

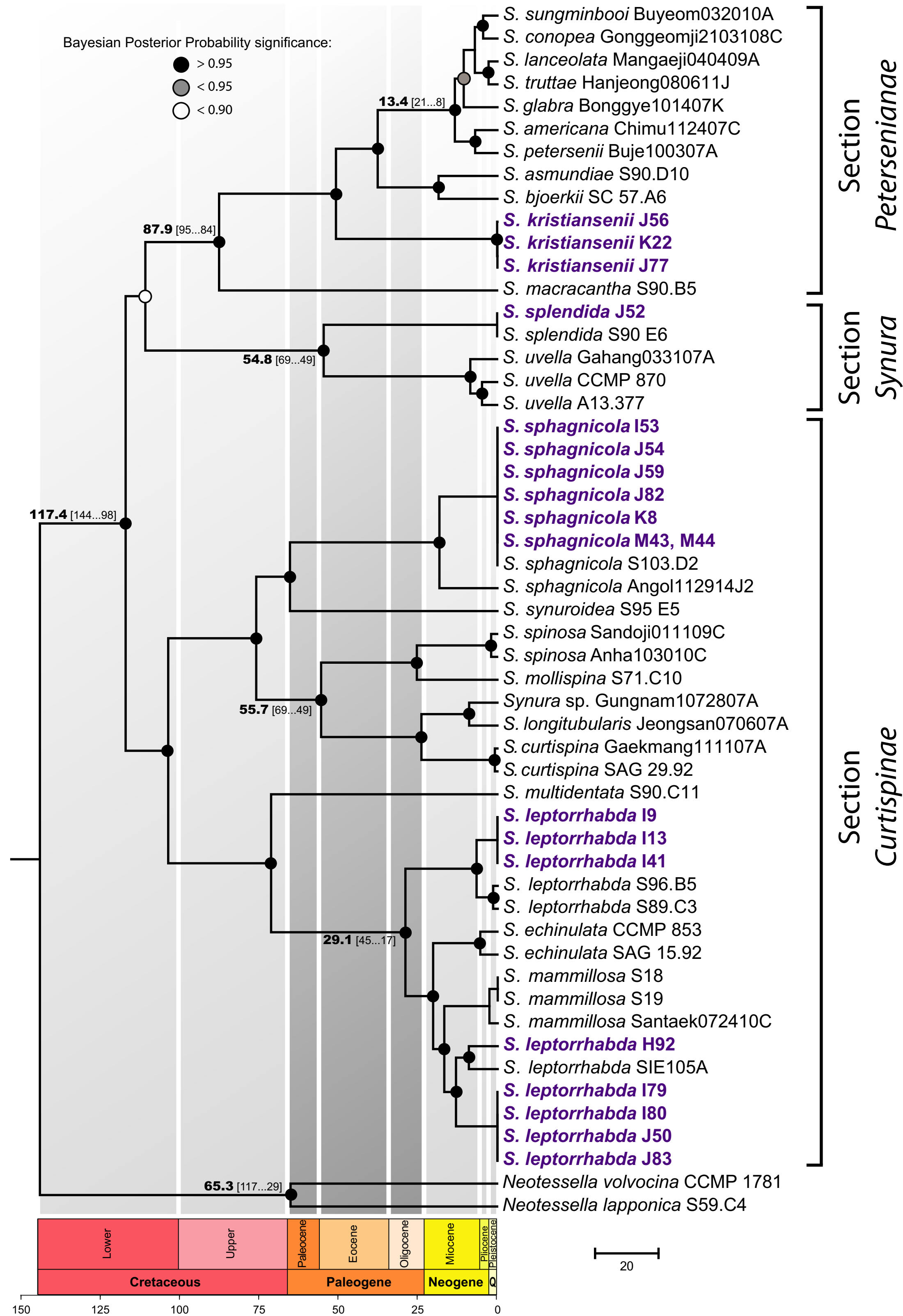

FIG. 2. Time-calibrated phylogeny of the genus *Synura* based on concatenated nu ITS rDNA, nu SSU rDNA, nu LSU rDNA, pt *rbc*L, pt *psa*A, and pt LSU rDNA sequences. Newly generated sequences are given in bold. Mean divergence times are given for selected nodes, along with 95% highest posterior density (HPD) values in square brackets. Time axis is Mya, along with chronological dating of geologic intervals. [Color figure can be viewed at wileyonlinelibrary.com

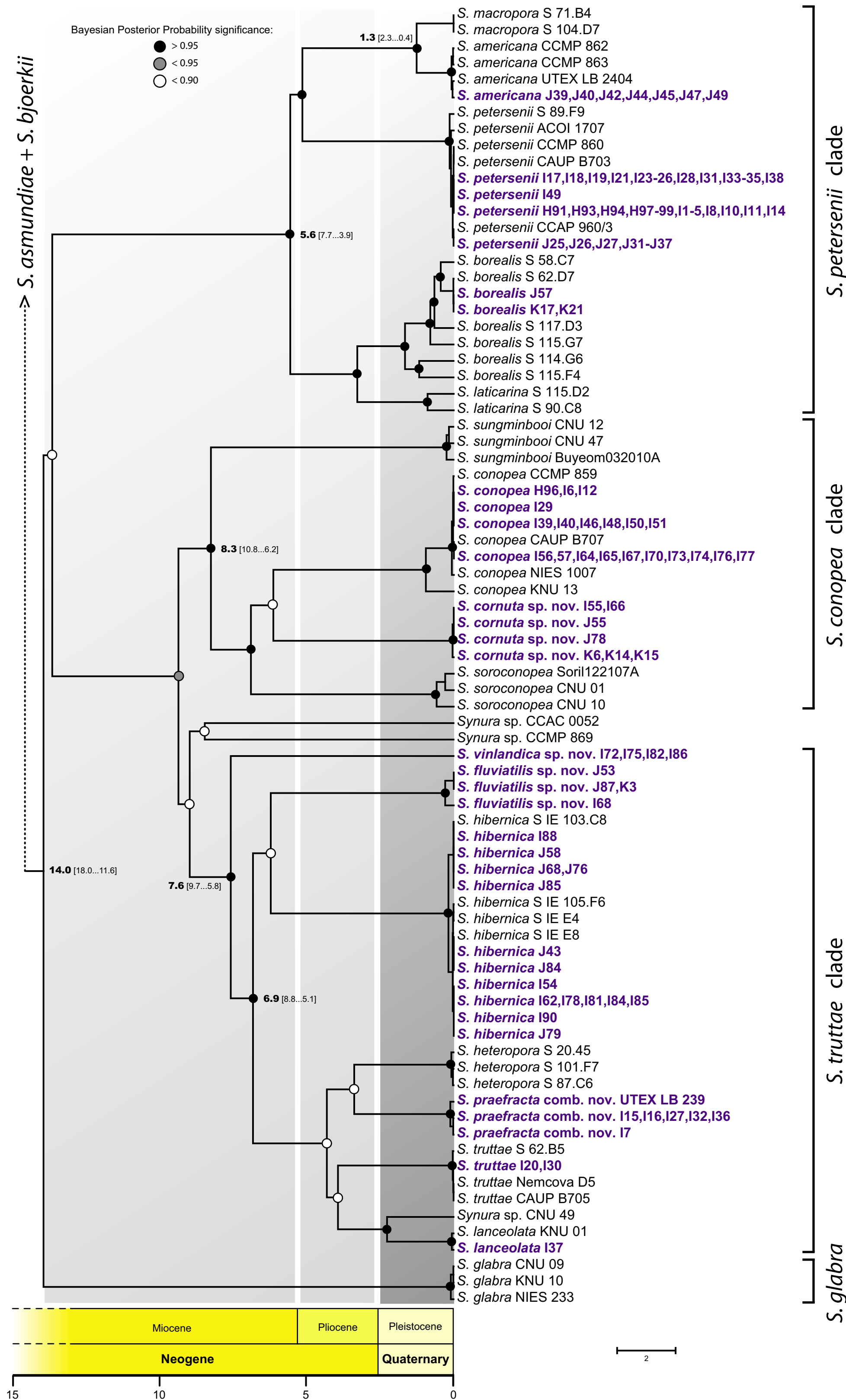

FIG. 3. Time-calibrated phylogeny of the genus *Synura*, section Peterseniananae, based on concatenated nu ITS rDNA, pt *rbc*L and mt *cox*I sequences. Newly generated sequences are given in bold. Mean divergence times are given for selected nodes, along with 95% highest posterior density (HPD) values in square brackets. Time axis is Mya, along with chronological dating of geologic intervals. [Color figure can be viewed at wileyonlinelibrary.com]

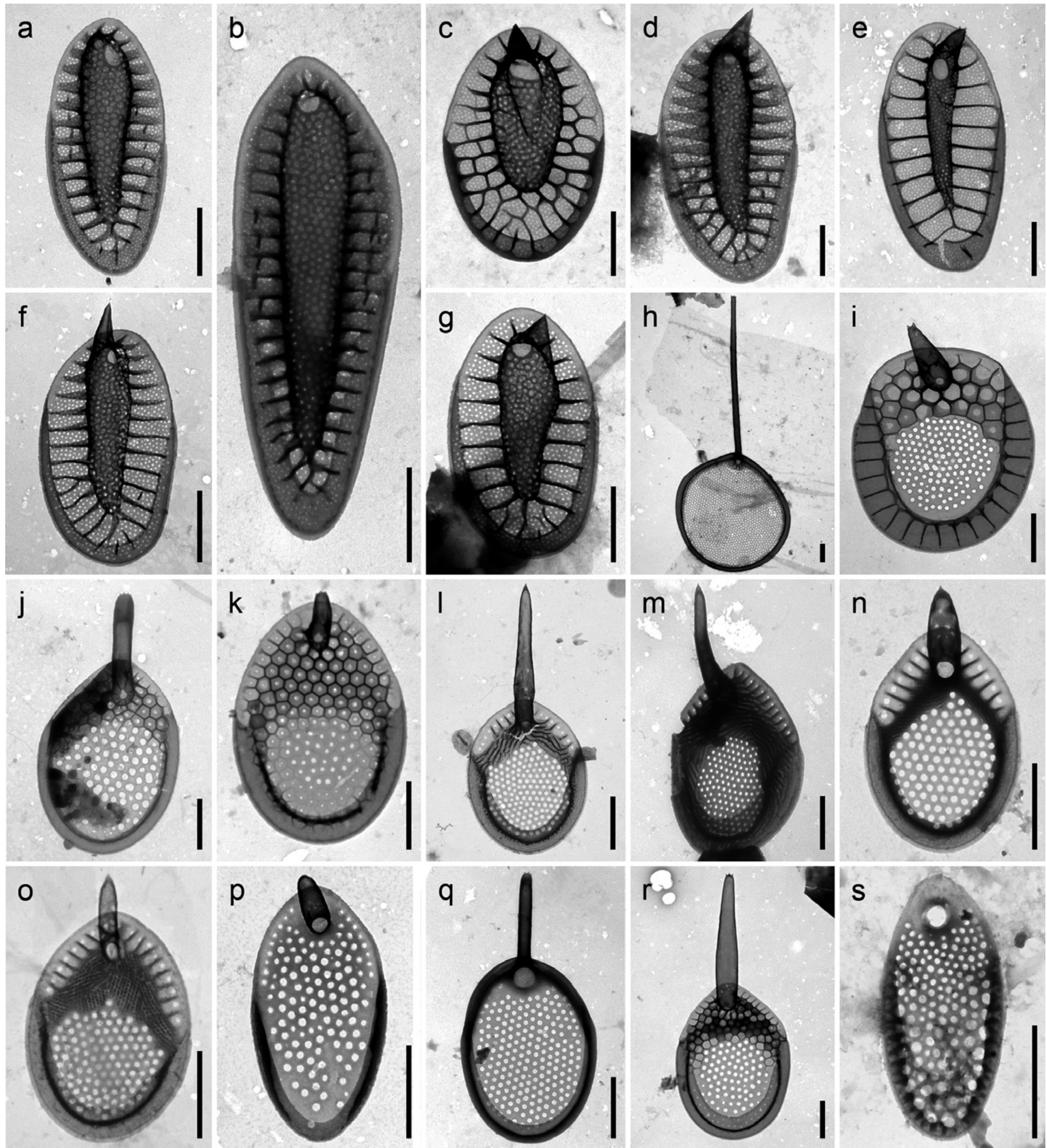

Fig. 4. *Synura* morphotypes identified in natural populations. a. *S. conopea*. b. *S. hibernica*. c. *S. kristiansenii*. d. *S. petersenii*. e. *S. petersenii* f. *"praefracta"* sensu Wujek and Igoe 1989, f. *Synura* sp. 1. g. *Synura* sp. 2. h. *S. splendida*. i. *S. uvella*. j. *S. curtispina*. k. *S. curtispina* f. *reticulata*. l. *S. echinulata*. m. *S. "echinulata"* sensu Nicholls and Gerrath 1985. n. *S. leptorrhabda*. o. *S. mammillosa*. p. *S. papillosa*. q. *S. sphagnicola*, r. *S. spinosa* f. *longispina*. s. *S. synuroidea*. Scale bars represent 1 μm.

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 Peterseniane 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. 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 Peterseniane. 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 analyzed in detail the morphological properties of four novel lineages inferred within the core Peterseniane, 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

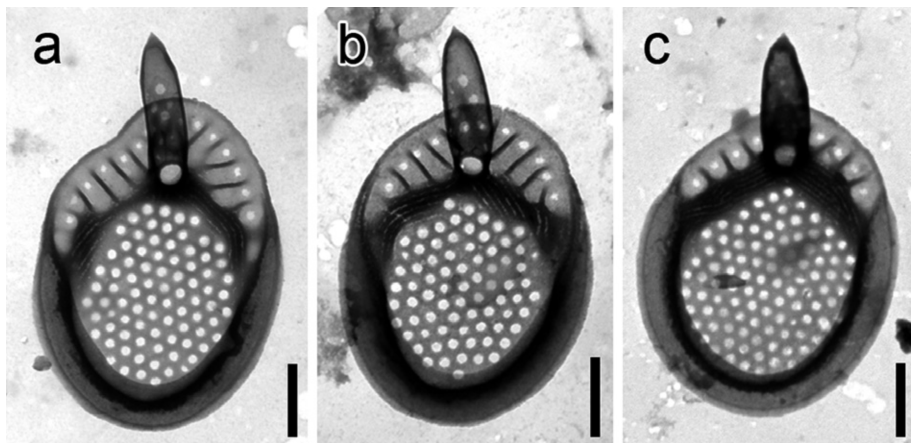

Fig. 5. Silica scales of three *Synura leptorrhabda* lineages. a. strain I13. b. strain H92. c. strain J83. Scale bars represent 0.5 μm.

much narrow (*S. cornuta*), respectively (see the taxonomic revision in the following section 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 the next section). 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 in the following section.

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)

Basionym: *Synura petersenii* Korshikov f. *praefracta* Asmund (1968), Hydrobiologia, 31: 501.

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 × 1 × 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

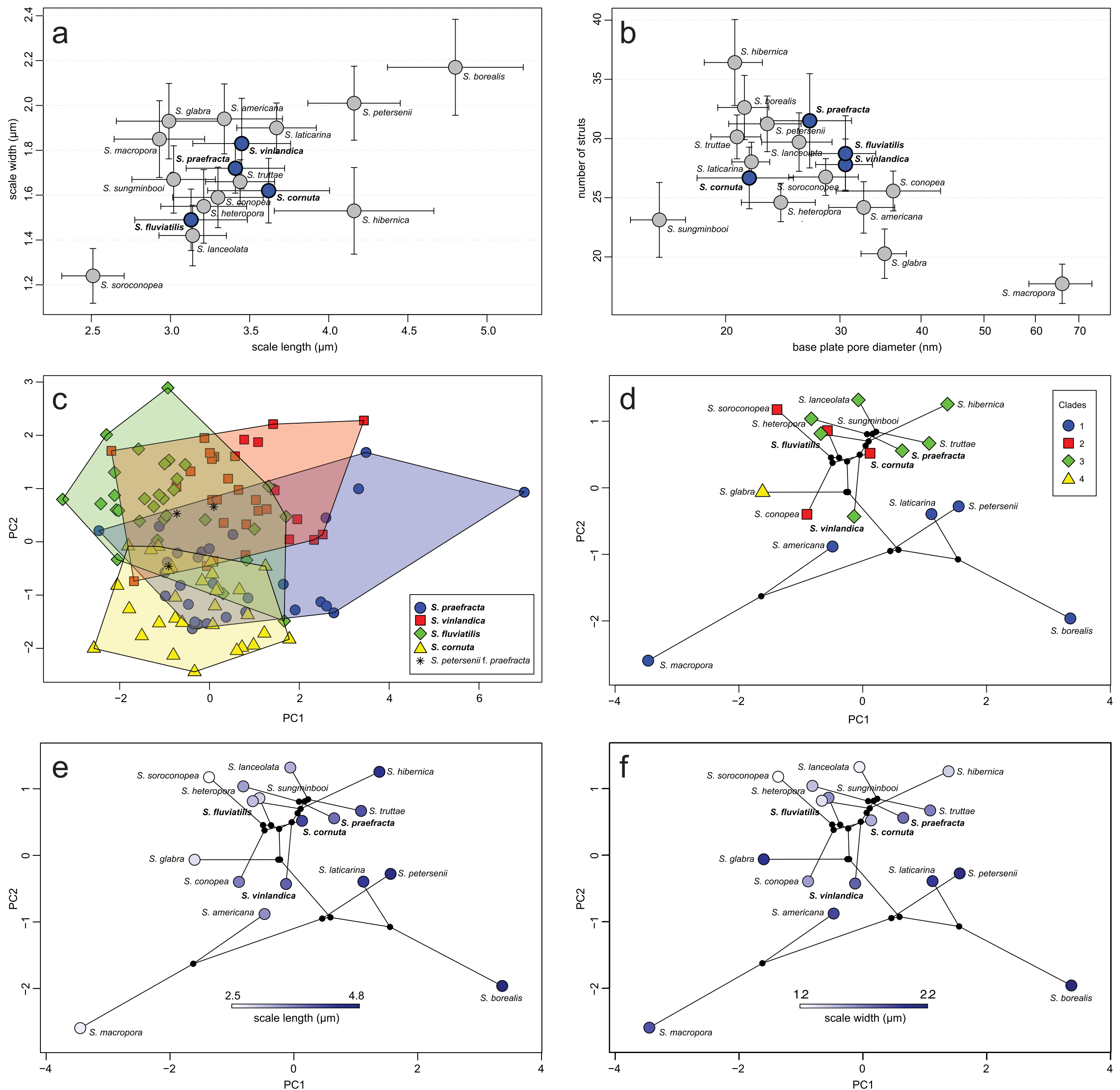

FIG. 6. Morphological analyses of 17 core Peterseniane species. (a–b). Comparison of four morphological traits (scale length, scale width, base plate pore diameter, number of struts); average values and standard deviations are given, with the four newly characterized species highlighted. (c) PCA ordination 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 colored/shaded by their clade affiliation (d), scale length (e), and scale width (f). Lines connect related species through hypothetical ancestors (small black dots). [Color figure can be viewed at wileyonlinelibrary.com]

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). Differs from other *Synura* species by the ultrastructure of silica scales and by nu ITS rDNA (GenBank Accession MN782206), pt *rbc*L (MN783119) and mt *cox*I (MN783144) sequences.

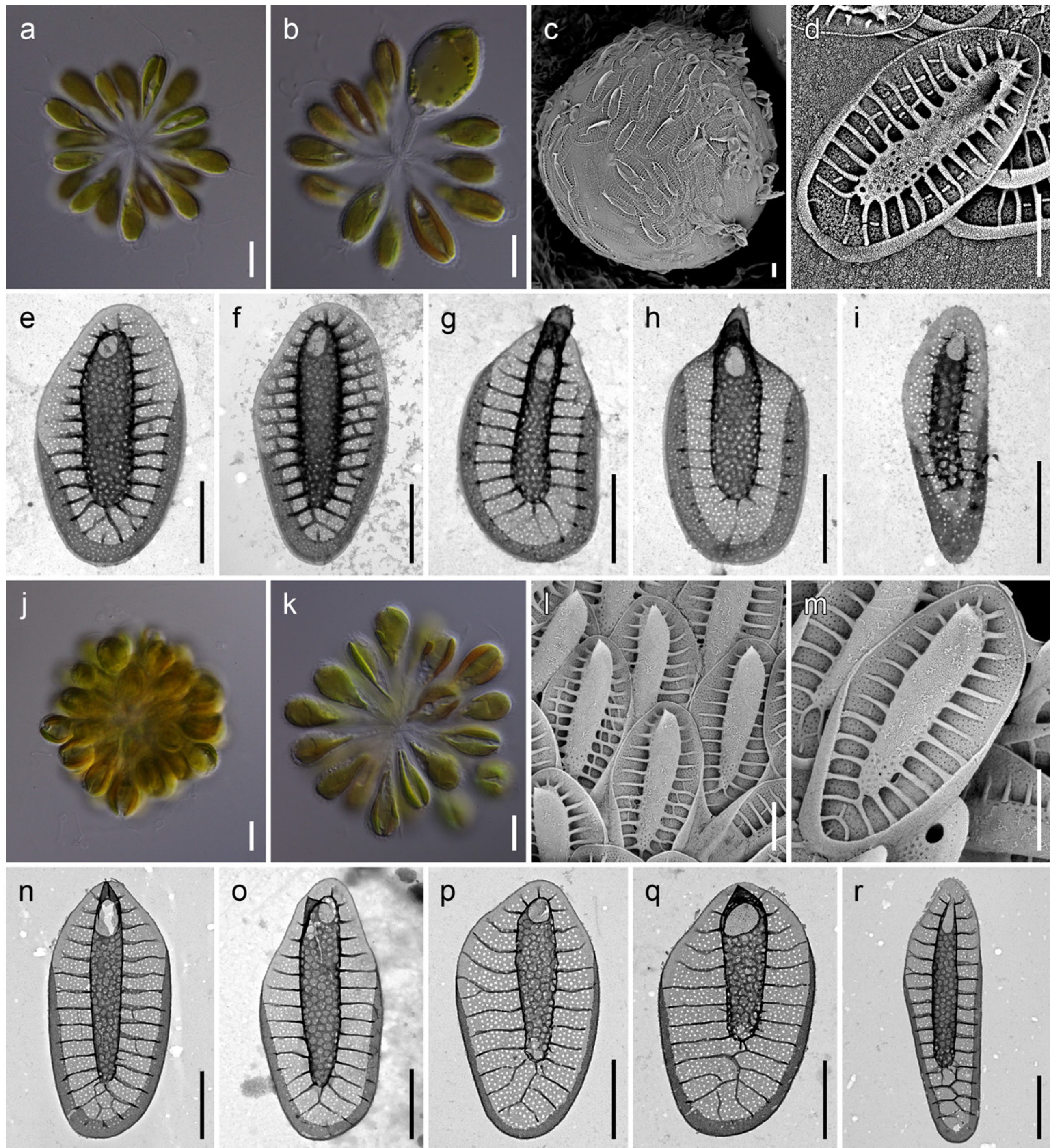

Fig. 7. Scale morphology of *Synura* species. a–i. *S. praefracta*. a. Colony consisting of elongated, drop-shaped cells. b. Colony with one encysting cell. c. Single cell surrounded by a layer of siliceous scales. d–f. Body scales. g–h. Apical scales with rounded spine terminated by several short teeth. 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 eccentrical keel. q. Apical scale with shortened and eccentrical keel. r. Rear scale. Scale bars represent 10 μm (a–b, j–k) and 1μm (c–i, l–r). a–b, j–k: LM; c–d, l–m: SEM; e–i, n–r: TEM. [Color figure can be viewed at wileyonlinelibrary.com]

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*I (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

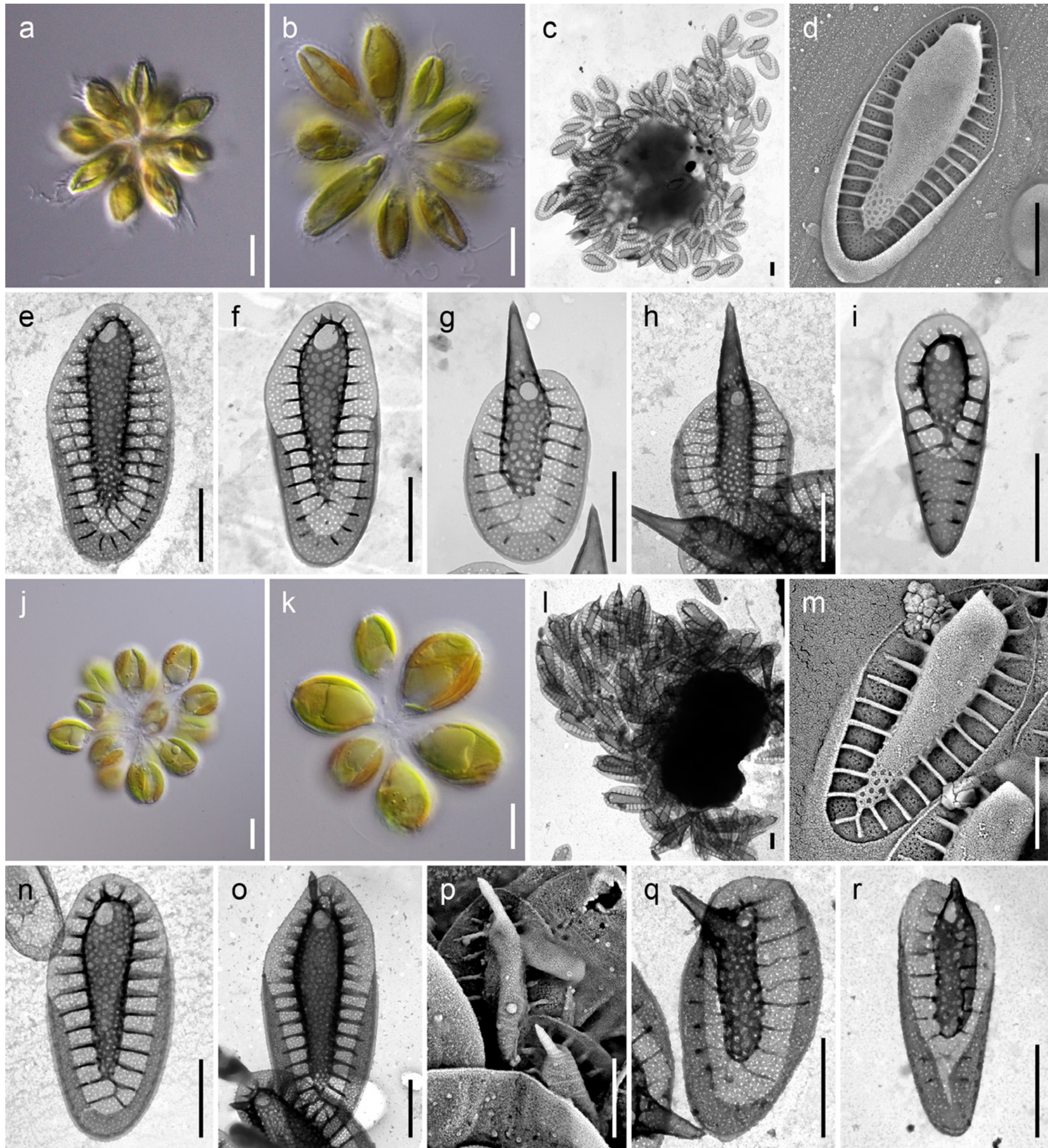

Fig. 8. Scale morphology of *Synura* species. a–i. *S. fluviatilis*. a–b. Colonies consisting of lanceolate cells. c. Single cell surrounded by a layer of siliceous scales. d–f. Body scales. g–h. Apical scales with prominent spines. i. Rear scale. j–r. *S. cornuta*. j–k. Colonies consisting of spherical 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 μm (a–b, j–k) and 1 μm (c–i, l–r). a–b, j–k: LM; d, m, p: SEM; c, e–i, l, n, o, q–r: TEM. [Color figure can be viewed at wileyonlinelibrary.com]

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

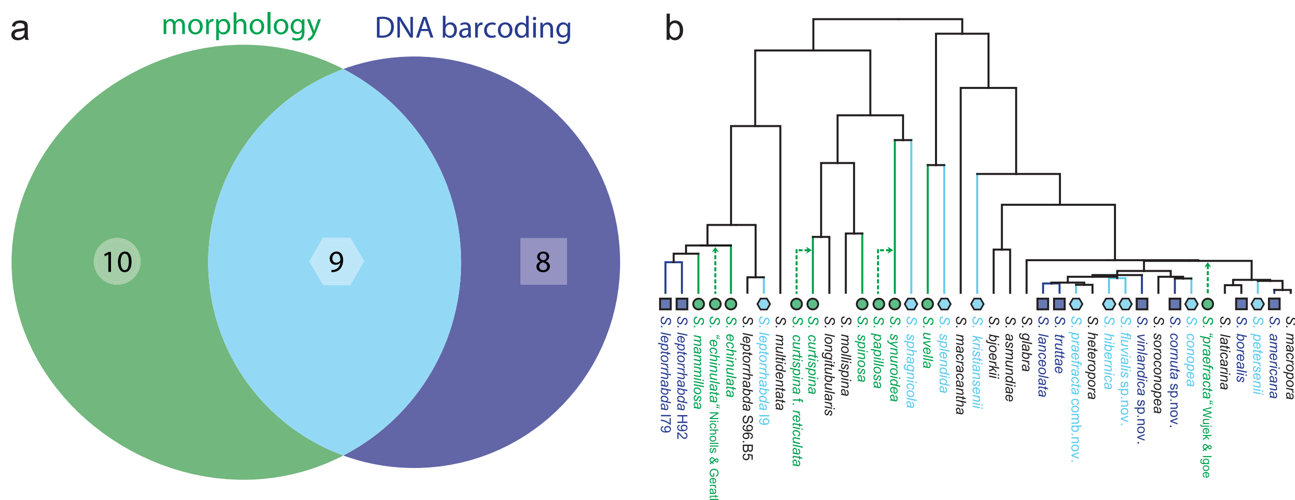

FIG. 9. Summary of taxa found by morphology and ITS rDNA sequencing (a) and their phylogenetic position along the *Synura* species tree (b). Estimated positions of taxa lacking molecular data (according to the morphology of silica scales) are visualized by dashed arrows. Taxa are coded by symbols in 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 are accompanied by no symbols. [Color figure can be viewed at wileyonlinelibrary.com]

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 in (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 lacking 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 in 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.

Toward 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. leptorrhabda* lineage of the section Curtispinae that remains to be described, and finding additional distinct morphotypes that remain molecularly uncharacterized, Newfoundland waterbodies undoubtedly harbor 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 this 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 centimetrer 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 favor of a moderate endemicity 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* remains 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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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

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 *cox*I gene, and the plastid-encoded pt *rbc*L, pt *psa*A, 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 *psa*A, and pt *rbc*L 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 *rbc*L, and mt *cox*I 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: https://doi.org/10.17632/jjfmp6nv4b.1.