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# Morphological delineation and distribution patterns of four newly described species within the *Synura petersenii* species complex (Chrysophyceae, Stramenopiles)

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The *Synura petersenii* species complex represents a common, cosmopolitan and highly diverse taxon of autotrophic freshwater flagellates. In this paper, we describe and characterize four new species (*S. borealis*, *S. heteropora*, *S. hibernica* and *S. laticarina*) that have been identified during our extensive sampling of freshwater habitats in 15 European countries. Morphometric analyses of siliceous scales led to the significant phenotypic differentiation of all four newly described species, and their separation from other related species of the *S. petersenii* complex. Two of these newly described species (*S. hibernica* and *S. borealis*) can be clearly distinguished by characteristic large colonies consisting of elongated, lanceolate-shaped cells. Development of strongly elongated, narrow cells in *S. hibernica* could be explained by the adaptation of this species to oligotrophic conditions. Though morphologically distinct, *S. borealis* possesses an exceptionally high degree of genetic diversity, possibly indicating recent speciation and evolutionary diversification within this taxon. Three of the four newly described species exhibit restricted biogeographic distribution. The evolutionarily related *S. borealis* and *S. laticarina* occur only in Northern Europe, and seem to be adapted to colder areas. The most remarkable distribution pattern was observed for *S. hibernica*, which has a geographic distribution that is restricted to western Ireland.

**Key words:** biogeography, *cox*1, cryptic species, ITS rDNA, morphology, phylogeny, *rbc*L, speciation, *Synura*, taxonomy



## Introduction

The order Synurales (Chrysophyceae, Stramenopiles) contains several genera of scale-bearing flagellates that are important components of phytoplankton communities of various freshwater bodies (Kristiansen & Preisig, 2007; Škaloud *et al.*, 2013*a*). The most conspicuous genus of the order, *Synura*, is a colonial organism formed by a variable number of cells joined together at their posterior ends. Each cell is covered by imbricate silica scales (Wee, 1997), consisting of a perforated basal plate with various upturned or bent parts, and a secondary ornamentation, which is used to delimit the different species (Kristiansen, 1986). According to the recently published phylogeny of the Synurales, the genus *Synura* should be split into five sections: Echinulatae, Peterseniae, Spinosae, Splendidae and Synura (Škaloud *et al.*, 2013*a*).Taxa belonging to the section Peterseniae are well characterized by body scales with a central keel that may end in a spine-like projection. The most widely recognized species of this section, *Synura petersenii sensu lato*, is considered to be one of the most widely distributed freshwater chrysophytes (Kristiansen, 1975), even causing taste and odour problems in water supplies (Nicholls & Gerrath, 1985). This species has been subjected to various ecophysiological experiments (e.g. Saxby-Rouen *et al.*, 1997; Kim *et al.*, 2008; Pichrtová & Němcová, 2011).

Due to the species-specific morphology of silica scales, *Synura* could be supposed to have one of the best morphological species concepts within protists. *Synura* scales are often well preserved in benthic sediments and used in palaeoecological studies. Because many taxa are distributed within a narrow range of ecological variables (e.g. pH, temperature and nutrient concentration), investigation of fossilized *Synura* scales is used as a tool to assess eutrophication, acidification and shifts in climate (Smol, 1995; Smol & Cumming, 2000). In addition, the records of fossilized *Synura* scales discovered in Eocene sediments are used to date the diversification and evolution of the genus (Boo *et al.*, 2010; Siver, 2013).

Recent molecular phylogenetic investigations have revealed a conflict between the traditional morphological species concept based on ultrastructure of silica structures and the phylogenetic data. *Synura petersenii sensu lato* represents the most

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investigated species in this respect. First, Wee *et al.* (2001) demonstrated the existence of two well-supported *S. petersenii* clades with different distribution patterns. Later, Kynčlová *et al.* (2010) investigated a number of European isolates, and revealed the existence of six cryptic species. Almost concurrently, Boo *et al.* (2010) published a multigene phylogeny of approximately 100 *S. petersenii* isolates, confirming the high degree of hidden, species-level diversity within this species. A taxonomic assessment of observed cryptic diversity redefined the species concept within the *S. petersenii* morphotype, and recognized six cryptic lineages as separate species (Škaloud *et al.*, 2012).

Phylogenetic analyses performed by Boo *et al.* (2010) and Škaloud *et al.* (2012) clearly indicate that there are more than six hidden species within the *S. petersenii* complex. To further investigate the real level of species diversity within this complex, we performed extensive sampling in 15 European countries, including Greenland. According to the analyses of internal transcribed spacer (ITS) rDNA sequences, 58 of more than 800 investigated *Synura* isolates belonged to four novel, undescribed species. The principal aim of this study was to expand our knowledge and understanding of the diversity in the *S. petersenii* complex, and to describe and characterize all these new taxa. In addition, we performed a detailed morphological investigation of all cryptic species, and investigated whether they could be delineated by the morphology of silica scales.

## Materials and methods

### *Origin and cultivation of the investigated strains*

The strains used in this study were obtained during an investigation of silica-scaled chrysophytes in Europe and Greenland during the period 2008–2013 (Table 1). The material was usually sampled from plankton and metaphyton of various water bodies, using a plankton net with 20-µm mesh. To ensure the highest viability of *Synura* strains, individual colonies were isolated on the same day that they were sampled. The colonies were removed from the natural sample by micropipetting, and transferred into separate wells of a 96-well plate containing 400 µl of either MES- or HEPES-buffered DY IV liquid medium (Andersen *et al.*, 1997). The wells were kept at 15°C (fridge bag TK 51, Ardes SpA, Ponte Nossa, Italy), under constant illumination of 50–200 µmol $m^{-2}$ $s^{-1}$ provided by 6 W LED diodes (LB115A-6W-X, Yuyao Lianliang Electric Appliance Co Ltd, Ningbo, China). After returning them to the laboratory, the strains were cultivated at 15°C (cooling box C5G, Helkama Oy, Helsinki, Finland), under illumination of 40 µmol $m^{-2}$ $s^{-1}$ and a 16-h light : 8-h dark cycle (TLD 18W/33 fluorescent lamps, Philips, Amsterdam, the Netherlands). In addition, isolated colonies were separately transferred to 200 µl PCR tubes and subsequently frozen at −24°C.

### *DNA extraction, PCR and DNA sequencing*

For DNA isolation, 100 µl of the growing culture was centrifuged in PCR tubes (6000 rpm for 3 min), and 30 µl 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 12000 rpm for 2 min, and the supernatant was directly used as a PCR template. Three molecular markers were amplified by PCR: nuclear ITS rDNA, chloroplast *rbc*L and mitochondrial *cox*I. The amplification of ITS rDNA was performed as described in Kynčlová *et al.* (2010), using the primers Kn1.1 (5′-CAA GGT TTC CGT AGG TGA ACC-3′; Wee *et al.*, 2001) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′; White *et al.*, 1990). Amplification of the *rbc*L marker was performed according to Jo *et al.* (2011), using the primers rbcL_2F (5′-AAA AGT GAC CGT TAT GAA TC-3′; Daugbjerg & Andersen, 1997) and rbcL_R3 (5′-GTA ATA TCT TTC TTC CAT AAA TCT AA-3′; Jo *et al.*, 2011). The *cox*1 marker was amplified according to Boo *et al.* (2010), using the primers F692 (5′-TTG TDT GGT CAG TTT TAA TTA C-3′) and R1433 (5′-GGC ATA CCT GCW ARA CCT AA-3′; Boo *et al.*, 2010). The PCR products were purified and sequenced at Macrogen Inc. in Seoul, Korea.

### *Phylogenetic analyses*

A multiple alignment of the newly determined ITS rDNA, *rbc*L and *cox*I gene sequences and other sequences selected from the GenBank/EMBL/DDBJ databases was built using MAFFT, version 6, applying the Q-INS-i strategy (Katoh *et al.*, 2002). The sequences were selected to encompass all known lineages within the *Synura petersenii* species complex (Table S1). ITS rDNA sequences were then aligned on the basis of their rRNA secondary structure information (Kynčlová *et al.*, 2010) with MEGA 4 (Kumar *et al.*, 2008). The three loci were concatenated, yielding an alignment of 2308 bases. The final matrix contained 51 ITS rDNA, 36 *rbc*L and 33 *cox*I sequences. A suitable partitioning strategy and partition-specific substitution models were selected in a multistep process (Verbruggen *et al.*, 2010). Initially, a guide tree was obtained by carrying out a second-level maximum likelihood (ML) search on the unpartitioned dataset with a HKY + Γ8 model using TreeFinder (Jobb *et al.*, 2004). Then, the dataset was divided by 17 different partitioning strategies (combining different levels of locus segmentation). Subsequently, Bayesian information criterion (BIC) calculations were performed for all 17 potential partitioning strategies, assuming the guide tree and HKY + Γ8 model for each partition. The five best-scoring partitioning strategies (lowest BIC scores) were retained for further analysis. In the next step, models of sequence evolution were selected for individual partitions using BIC. For each partition present in the five retained partitioning strategies, 12 different nucleotide substitution models were evaluated (F81, HKY, GTR and their combinations with Γ, I and Γ + I). Finally, the partitioning strategies were re-evaluated using the selected models for the particular partitions. This BIC-based model selection procedure selected the following model with eight partitions: (1) internal transcribed spacers ITS1 and ITS2 – GTR + Γ; (2) 5.8 S ribosomal locus – F81; (3), first codon position of the *rbc*L gene – GTR + Γ; (4) second codon position of the *rbc*L

**Table 1.** Origin and sampling details of analysed strains.

| Taxon | Strain | Locality | Geographic coordinates | Sampling date | Temp. (°C) | pH | Cond. (µS cm$^{-1}$) |
|---|---|---|---|---|---|---|---|
| *S. americana* | S 81.C7 | Záplavy NR, Czech Republic | 50.14643° N, 14.01389° E | 19.3.2012 | 5 | 7.2 | 670 |
| *S. americana* | S 104.C4 | Schinkelbos, Netherlands | 52.29927° N, 4.80742° E | 7.12.2012 | 4 | 7.9 | 1257 |
| *S. borealis* | S 58.C7 | Lillesjön, Sweden | 56.95543° N, 14.71428° E | 22.4.2011 | 12 | 6.5 | 81 |
| *S. borealis* | S 62.D7 | unnamed lake, Disko island, Greenland | 69.28913° N, 53.49322° W | 28.7.2011 | 7 | 6.3 | 81 |
| *S. borealis* | S 90.F3 | Rutajärvi, Harjulahti, Finland | 61.90636° N, 26.02054° E | 5.5.2012 | 8 | 5.8 | – |
| *S. borealis* | S 90.G4 | Harjujärvi, Finland | 61.88547° N, 26.01479° E | 5.5.2012 | 7 | 4.9 | – |
| *S. borealis* | S 90.M34 | Jämsänjarvi, Finland | 62.25060° N, 25.18766° E | 5.5.2012 | 6 | 5.2 | – |
| *S. borealis* | S 110.E2/F3 | a small pool near Kalli jõgi, Estonia | 58.33539° N, 27.26385° E | 10.5.2013 | 21 | 6.7 | 157 |
| *S. borealis* | S 110.B9 | Apna jõgi, Estonia | 58.34305° N, 27.26793° E | 10.5.2013 | 19 | 6.7 | 110 |
| *S. borealis* | S 113.F4/F6 | Emajõgi, Estonia | 58.39636° N, 26.31094° E | 10.5.2013 | 16 | 7.5 | 322 |
| *S. borealis* | S 114.B8/B9/C8 | unnamed lake (A1), Sweden | 68.34805° N, 19.03784° E | 14.6.2013 | 13 | 6.3 | 134 |
| *S. borealis* | S 114.F3 | unnamed lake near Paittasjärvi, Sweden | 67.86223° N, 19.02746° E | 15.6.2013 | 16 | 6.5 | 23 |
| *S. borealis* | S 114.G6 | Syväjärvi, Sweden | 67.75664° N, 20.09297° E | 16.6.2013 | 12 | 6.6 | 49 |
| *S. borealis* | S 115.F4 | a pool near Arosnjarkajaute, Sweden | 68.42876° N, 18.35156° E | 19.6.2013 | 4 | 6.8 | 88 |
| *S. borealis* | S 115.G2/G3 | a pool near Vassijaure, Sweden | 68.43305° N, 18.23966° E | 19.6.2013 | 7 | 6.9 | 45 |
| *S. borealis* | S 115.G7 | Vassijaure, Sweden | 68.43072° N, 18.12755° E | 19.6.2013 | 11 | 6.9 | 24 |
| *S. borealis* | S 116.B6 | Kjerringdalsvatnet, Norway | 68.66019° N, 15.54052° E | 19.6.2013 | 16 | 6.2 | 37 |
| *S. borealis* | S 117.D3 | a pool near Paittasjärvi, Sweden | 67.85803° N, 19.02454° E | 15.6.2013 | 16 | 6.0 | 13 |
| *S. borealis* | S 118.C6 | a pool near Torneträsk, Sweden | 68.35576° N, 18.82491° E | 17.6.2013 | 14 | 7.4 | 173 |
| *S. borealis* | SC FIN 13A | Ojala, Finland | 61.84778° N, 26.29145° E | 5.5.2012 | 10 | 6.5 | – |
| *S. borealis* | SC FIN 22B | Heinäjärvi, Finland | 62.88112° N, 25.49724° E | 5.5.2012 | 7 | 5.9 | – |
| *S. borealis* | SC GRL 340 | Sanningassup Tasia, Greenland | 69.26939° N, 53.47820° W | 28.7.2011 | 15 | 6.8 | 72 |
| *S. borealis* | SC GRL 364 | a morraine lake, Disko island, Greenland | 69.29754° N, 53.51961° W | 28.7.2011 | 5 | – | – |
| *S. borealis* | SC SWE 9 | Helgassjön, Sweden | 56.95611° N, 14.71630° E | 22.4.2011 | 11 | 6.6 | 72 |
| *S. conopea* | S 103.B3 | a pool near Smědava, Czech Republic | 50.84606° N, 15.236750° E | 14.10.2012 | 6 | 5.0 | 40 |
| *S. glabra* | S 89.F5 | Saarijärvi, Finland | 62.70173° N, 25.26388° E | 5.5.2012 | 6 | 6.5 | – |
| *S. heteropora* | S 20.1 | Loch an Add, Scotland, UK | 56.03298° N, 5.534510° W | 4.6.2008 | – | – | – |
| *S. heteropora* | S 20.45 | Crinan Canal, Scotland, UK | 56.06051° N, 5.477146° W | 4.6.2008 | – | – | – |
| *S. heteropora* | S 40.F11 | Podhradská pool, Czech Republic | 50.46112° N, 14.91176° E | 3.4.2011 | 16 | 7.4 | 660 |
| *S. heteropora* | S 54.E11 | Klejnarka river, Czech Republic | 49.96956° N, 15.32123° E | 19.4.2011 | – | – | – |
| *S. heteropora* | S 86.F2 | Zbýšov pond, Czech Republic | 49.81160° N, 15.35409° E | 20.3.2012 | – | – | – |
| *S. heteropora* | S 87.C6 | an ephemeral puddle near Kufstein, Austria | 47.59791° N, 12.15115° E | 22.4.2012 | – | – | – |
| *S. heteropora* | S 101.F7 | a pond in Nygårdsparken, Bergen, Norway | 60.38614° N, 5.32447° E | 21.10.2012 | – | 6.2 | 117 |
| *S. heteropora* | S 112.E2 | Saarde paisjärv, Estonia | 58.14355° N, 24.97033° E | 10.5.2013 | 19 | 8.7 | 313 |
| *S. heteropora* | S 112.F5 | Rahumeri, Estonia | 58.18246° N, 25.05247° E | 10.5.2013 | 17 | 7.5 | 213 |
| *S. heteropora* | S 113.C8 | Karula Jarv, Estonia | 58.39858° N, 25.59201° E | 10.5.2013 | 15 | 6.9 | 458 |
| *S. heteropora* | S 117.G6 | Torneträsk, Sweden | 68.35573° N, 18.82172° E | 17.6.2013 | 12 | 7.5 | 53 |
| *S. heteropora* | SC IRL 8 | Garlan Lough, Ireland | 55.00343° N, 7.905883° W | 29.9.2011 | 16 | 6.1 | 121 |
| *S. hibernica* | S IE E4 | The Long Range, Ireland | 51.99725° N, 9.55089° W | 17.7.2009 | 16 | 8.0 | 23 |
| *S. hibernica* | S IE E8 | unnamed lake near Maam Cross, Ireland | 53.45049° N, 9.54407° W | 19.7.2009 | 16 | 6.2 | 56 |
| *S. hibernica* | S IE E11 | Glendollagh Lough, Ireland | 53.46658° N, 9.73863° W | 18.7.2009 | 17 | 7.9 | 53 |
| *S. hibernica* | S IE M38 | The Long Range, Ireland | 51.99725° N, 9.55089° W | 19.9.2008 | – | 7.6 | 32 |
| *S. hibernica* | S IE 103.C8/C9/C11 | Gowlaun Lough, Ireland | 51.78316° N, 9.76503° W | 6.3.2010 | 7 | 4.4 | 31 |

(*continued*)

**Table 1.** Continued.

| Taxon | Strain | Locality | Geographic coordinates | Sampling date | Temp. (°C) | pH | Cond. (µS cm$^{-1}$) |
|---|---|---|---|---|---|---|---|
| *S. hibernica* | S IE 104.D11 | Glanmore Lake, Ireland | 51.73901° N, 9.77194° W | 6.3.2010 | 9 | 5.2 | 40 |
| *S. hibernica* | S IE 105.F6 | Caha Lakes, Ireland | 51.72171° N, 9.65994° W | 6.3.2010 | 7 | 5.4 | 31 |
| *S. hibernica* | SC IRL 20a | Derrynaherriva Lough, Ireland | 54.07093° N, 9.51278° W | 30.9.2011 | 14 | 5.8 | 87 |
| *S. hibernica* | SC IRL 41 | Easky Lough, Ireland | 54.15717° N, 8.84404° W | 2.10.2011 | 14 | 6.1 | 51 |
| *S. hibernica* | SC IRL 60 | Maumwee Lough, Ireland | 53.47438° N, 9.54363° W | 19.7.2009 | – | 6.9 | 39 |
| *S. laticarina* | S 89.D5 | Ojala, Finland | 61.84778° N, 26.29145° E | 5.5.2012 | 10 | 6.5 | – |
| *S. laticarina* | S 90.C8 | Tehriselkä, Finland | 61.75652° N, 26.48581° E | 5.5.2012 | 6 | 5.5 | – |
| *S. laticarina* | S 110.C9 | Apna jõgi, Estonia | 58.34305° N, 27.26793° E | 10.5.2013 | 19 | 6.7 | 110 |
| *S. laticarina* | S 113.E5 | a pool near Emajõgi, Estonia | 58.39637° N, 26.31094° E | 10.5.2013 | 16 | 7.6 | 357 |
| *S. laticarina* | S 115.B2 | Barduelva, Norway | 69.01395° N, 18.48489° E | 17.6.2013 | 10 | 7.0 | 80 |
| *S. laticarina* | S 115.D2 | a pool near Arosnjarkajaute, Sweden | 68.42876° N, 18.35156° E | 19.6.2013 | 4 | 6.8 | 88 |
| *S. laticarina* | S 115.E5 | a pool near Vassijaure, Sweden | 68.43305° N, 18.23966° E | 19.6.2013 | 7 | 6.9 | 45 |
| *S. macropora* | S 71.B4 | Podhradská pool, Czech Republic | 50.46112° N, 14.91176° E | 4.3.2012 | 5.5 | 6.5 | 230 |
| *S. macropora* | S 104.D7 | a canal in Oosteinde, Netherlands | 52.28816° N, 4.80922° E | 7.12.2012 | 3.3 | 7.9 | 850 |
| *S. macropora* | S 104.F11 | a pool in Aalsmeer, Netherlands | 52.27148° N, 4.76143° E | 7.12.2012 | 4.1 | 7.9 | 975 |
| *S. petersenii* | S 89.D6 | Ojala, Finland | 61.84778° N, 26.29145° E | 5.5.2012 | 9.5 | 6.5 | – |
| *S. petersenii* | S 89.F9 | Heinäjärvi, Finland | 62.88112° N, 25.49724° E | 5.5.2012 | 7.0 | 5.9 | – |
| *S. truttae* | S 62.B5 | a peat bog near Přebuz, Czech Republic | 50.38308° N, 12.59668° E | 24.5.2011 | 12.6 | 4.7 | 107 |

gene – F81; (5) third codon position of the *rbc*L gene – GTR + Γ; (6) first codon position of the *cox*I gene – GTR + Γ; (7) second codon position of the *cox*I gene – F81 + Γ; and (8) third codon position of the *cox*I gene – GTR + Γ.

The phylogenetic tree was inferred with Bayesian inference (BI) using MrBayes version 3.1.2 (Ronquist & Huelsenbeck, 2003). Two parallel MCMC runs were carried out for 10 million generations. The dataset was divided into eight partitions, for which different substitution models were selected according to the BIC-based model selection. Trees and parameters were sampled every 100 generations. Convergence of the two runs was assessed during the run by calculating the average standard deviation of split frequencies (SDSF). The SDSF value between simultaneous runs was 0.003908. The burn-in was determined using the 'sump' command. Bootstrap analyses were performed with ML and weighted parsimony (wMP) criteria using GARLI version 0.951 (Zwickl, 2006) and PAUP* version 4.0b10 (Swofford, 2002), respectively. ML analyses consisted of rapid heuristic searches (100 pseudoreplicates) using automatic termination (genthreshfortopoterm command set to 100 000). The dataset was divided into eight partitions with different substitution models. The wMP bootstrapping (1000 replications) was performed using heuristic searches with 100 random sequence addition replicates, tree bisection reconnection swapping, random addition of sequences (the number limited to 10 000 for each replicate), and gap characters treated as a fifth character state. Character weights were assigned using the rescaled consistency index on a scale of 0 to 1000. New weights were based on the mean fit values for each character over all trees in the memory.

### *Morphological investigations and statistical analyses*

For morphological observations, the strains were cultivated in 50-ml Erlenmeyer flasks for 1–2 months. To avoid depletion of nutrients, the strains were grown under lower light and temperature regimes. Light microscopy (LM) observations were performed using an Olympus BX51 microscope. For scanning electron microscopy (SEM) observations, a drop of glutaraldehyde (GA)-fixed (2% GA overnight) cell suspension was sedimented for 60 min on polyL-lysine-coated glass coverslips to ensure appropriate cell adhesion. The coverslips were washed by repeated transfer into drops of deionized water dispensed onto the hydrophobic surface of a Parafilm strip, and subsequently dehydrated via acetone series. The cells were dried to a critical point with liquid carbon dioxide (Bal-Tec CPD 030), coated with platinum for 90 s with a Bal-Tec SCD 050 sputter coater, and observed with a JEOL JSM-740 1F FESEM scanning electron microscope. For transmission electron microscopy (TEM) investigations of silica scales, a drop from the living cultures was placed onto formvar-coated copper grids and dried. After washing in a series of water droplets, the grids were examined in a TEM JEOL 1011 electron microscope. At least three strains were randomly chosen from each of the clades identified by the molecular study, including the cultures deposited in the Culture Collection of Algae of Charles University in Prague (CAUP) and the National Center for Marine Algae and Microbiota in Maine (designated as CCMP). For each clade, the following characters were measured in 30 randomly selected scales: (1) scale length; (2) scale width; (3) area of a base hole; (4)

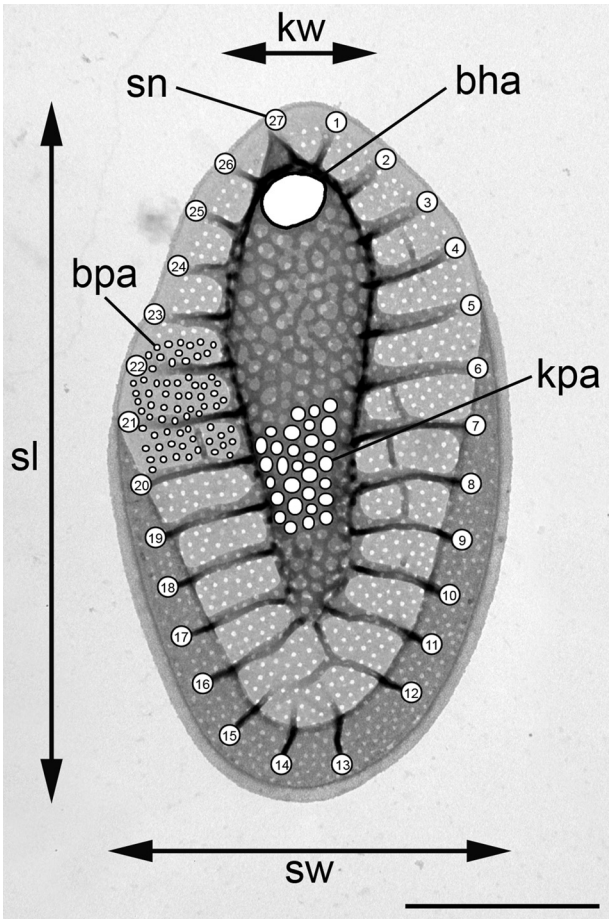



**Fig. 1.** Morphological features measured in *Synura* scales; scale length (sl), scale width (sw), base hole area (bha), keel pore area (kpa), base-plate-pore area (bpa), keel width (kw) and number of struts (sn). Scale bar represents 1 µm.

average area of a keel pore; (5) average area of a base-plate pore; (6) keel width; and (7) number of struts (Fig. 1). The measurements were performed using the program ImageJ 1.45 s (Schneider *et al.*, 2012). Average values of keel and base-plate pores were obtained by measuring areas of approximately 30 and 60 pores per scale, respectively. Statistical analyses of measured data (principal component and canonical discriminant analyses) were performed using Statistica 8.0 (StatSoft, Inc., Tulsa, Oklahoma, USA).

## Results

### *Phylogenetic analyses*

The Bayesian analysis based on the concatenated dataset (ITS rDNA, *rbc*L and *cox*1 sequences) revealed the existence of at least 16 lineages within the *Synura petersenii* species complex (Fig. 2). In addition to the six described species (*S. americana*, *S. conopea*, *S. glabra*, *S. macropora*, *S. petersenii* and *S. truttae*) and lineages resolved in previous studies, the phylogenetic analysis detected the existence of four distinct, novel lineages. The first two lineages (here referred to as *S. borealis sp. nov.* and *S. laticarina sp. nov.*) formed a well-supported clade together with *S. americana*, *S. macropora* and *S. petersenii*. The latter two newly recognized lineages (here referred to as *S. heteropora sp. nov.* and *S. hibernica sp. nov.*) were inferred to be members of another well-supported clade including *S. truttae* and the strains UTEX LB 239 and KNU01. *Synura borealis* represented the most diverse novel lineage, containing a number of isolates clustering into several distinct lineages. The most common genotype was represented by eight isolates originating from Estonia, Finland, Norway and Sweden. By contrast, *S. laticarina* was represented by seven sequences clustered into two related sub-clades. The third novel lineage, *S. heteropora*, consisted of 13 isolates originating from Austria, Czech Republic, Estonia, Great Britain, Ireland, Sweden and Norway. The strain CCMP 2898, which was isolated from an Austrian lake, was inferred as a member of this lineage. The last newly recognized lineage, *S. hibernica*, consisted of 12 isolates that all originated from western Ireland.

### *Morphological observations and taxa descriptions*

Molecular phylogenetic analyses show the existence of four novel lineages in the genus *Synura*, section Peterseniae. The detailed TEM investigation of siliceous scales demonstrated their clear distinctness from all species without molecular characterization (*S. obesa*, *S. australiensis*, '*S. petersenii*' f. *columnata*, '*S. petersenii*' f. *praefracta*, and '*S. petersenii*' f. *taymyrensis*). Therefore, these novel lineages represent four new species, which we describe and illustrate below.

### *Synura borealis* Škaloud & Škaloudová, *sp. nov.*

(Figs 3–10)

Description: Colonies are spherical, up to 86 µm in diameter, consisting of approximately 16–38 cells associated by their posterior ends. Cells are significantly elongated, lanceolate-shaped, posteriorly elongated into the tail, 31–42 µm long and 7–12 µm wide (Fig. 3). Each cell is surrounded by a layer of imbricate siliceous scales (Fig. 4). Body scales are 4.0–5.8 µm long and 1.6–2.6 µm wide, consisting of a basal plate with a centrally raised keel protruding into an acute tip (Figs 5 and 6). The keel is often anteriorly widened, ornamented by medium-sized pores (diameter, 54–88 nm). Anteriorly, keel pores are produced on both sides, so that the keel pore pattern is notably over-layered (Figs 6, 7). The ratio between scale and keel width varies from 1.7 to 2.9. The basal plate is ornamented by numerous small pores (diameter, 17–26 nm), and anteriorly perforated by a large, rounded or elongated base hole (diameter, 0.27–0.55 µm). Numerous struts (28–38), often interconnected by transverse folds, extend regularly from the keel to the scale perimeter. Apical scales are 3.2–4.2 µm long and 1.7–2.5 µm wide (Fig. 8). The keel of the apical scales ends in a prominent, acute tip (Fig. 9). Rear scales are long and narrow, 4.3–5.7 µm long and 1.1–1.6 µm wide (Fig. 10).

Etymology: The specific epithet 'borealis' refers to the northern (boreal) occurrence of the species, which is found in northern Europe and Greenland.

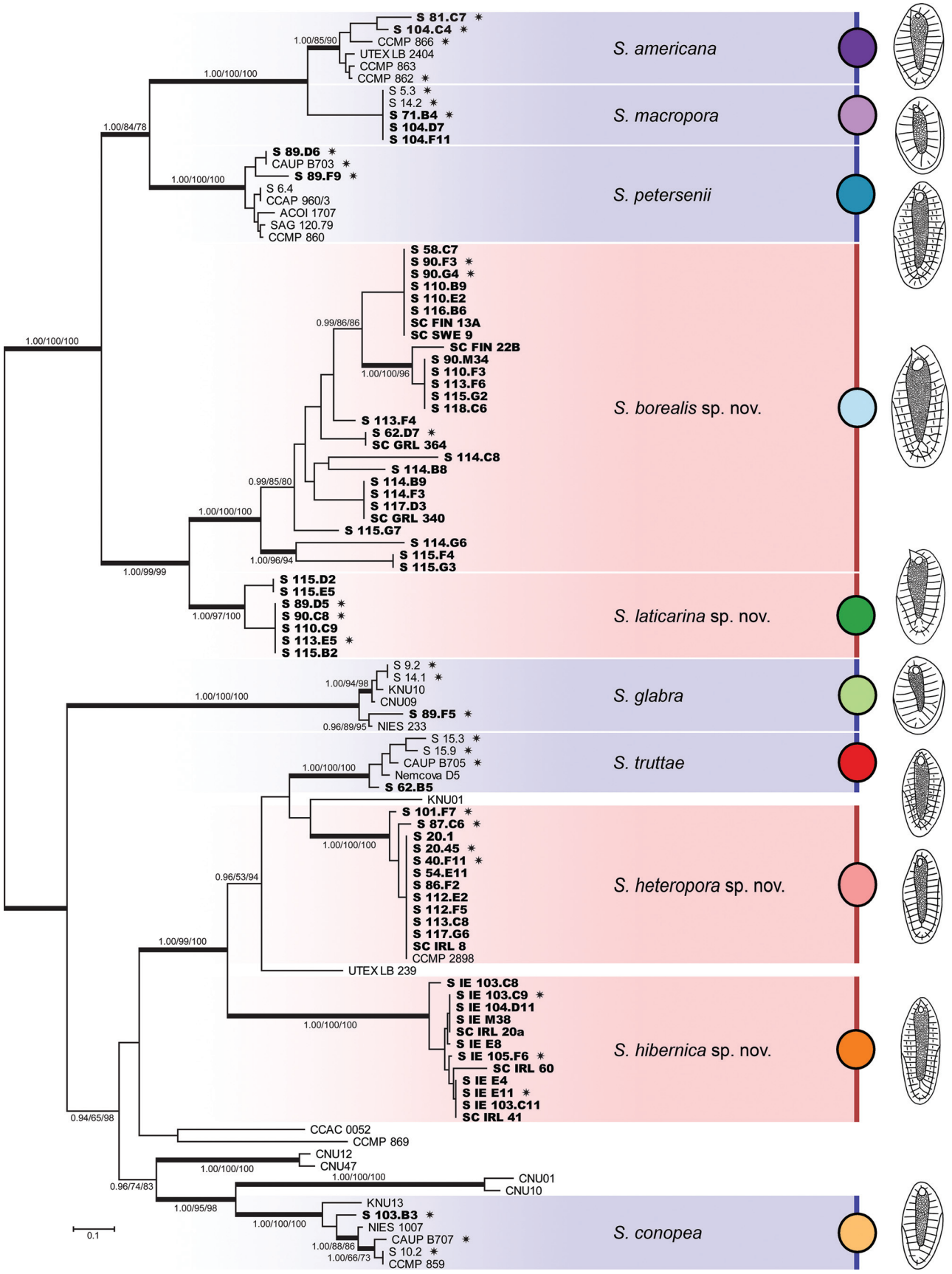
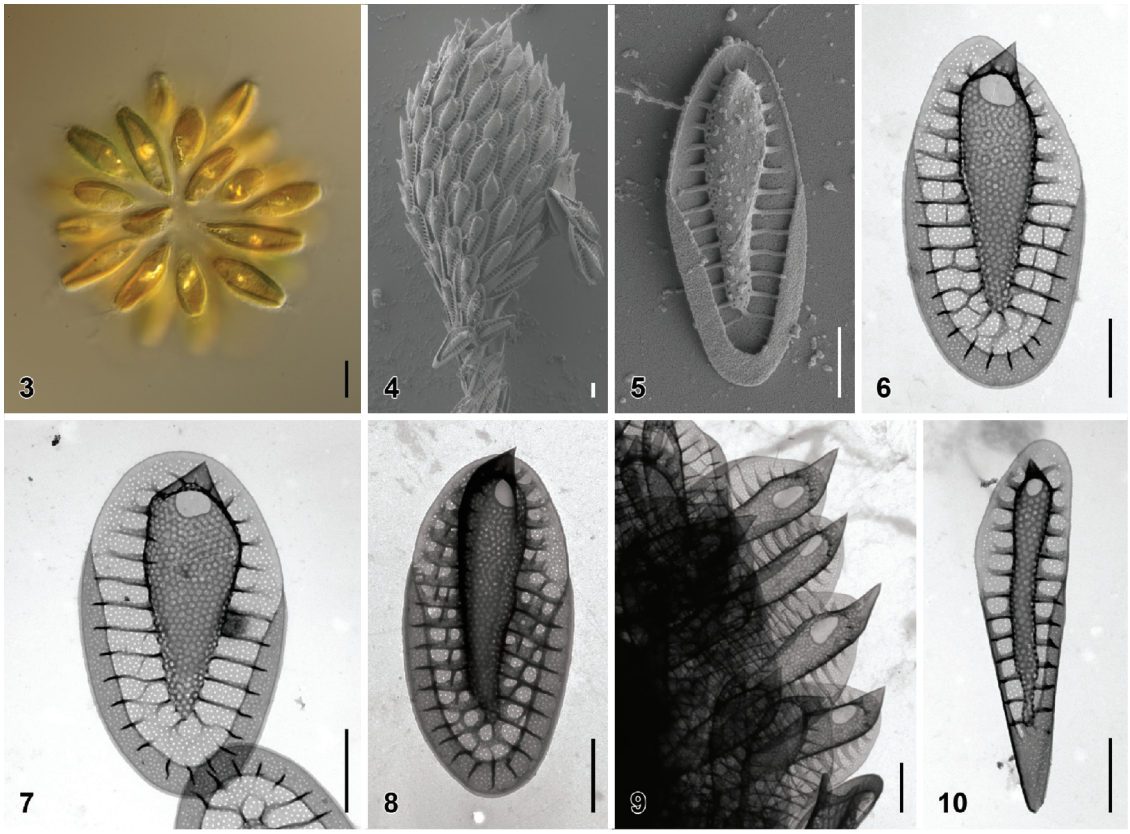



**Fig. 2.** Phylogeny of the genus *Synura*, section Peterseniae, obtained by Bayesian inference of the concatenated ITS rDNA, *rbc*L and *cox*1 dataset. The analysis was performed under a partitioned model, using different substitution models for each partition. Values at the nodes indicate statistical support estimated by three methods; MrBayes posterior node probability (left), maximum likelihood bootstrap (middle), and maximum parsimony bootstrap (right). Only statistical supports higher than 0.90/50/50 are shown. Thick branches highlight nodes receiving the highest posterior probability (PP) support (1.00). Newly sequenced strains are marked in bold. Strains used for the statistical analyses of morphological features are marked by asterisks. Scale bar represents the expected number of substitutions per site.




**Figs. 3–10.** Scale morphology of *Synura borealis sp. nov.* (Fig. 3: LM; Figs 4, 5: SEM; Figs 6–10: TEM). Scale bars represent 10 µm (Fig. 3) and 1 µm (Figs 4–10). **Fig. 3.** Colony consisting of elongated, lanceolate-shaped cells (strain S 90.G4). **Fig. 4.** Single cell surrounded by a layer of siliceous scales (S 90.G4). **Fig. 5.** Body scale (S 90.G4). **Fig. 6.** Body scale with transverse folds interconnecting the struts. Note the over-layered pore pattern at the scale keel (S 62.D7). **Fig. 7.** Body scale with obviously anteriorly widened keel (S 62.D7). **Fig. 8.** Apical scale (S 62.D7). **Fig. 9.** Apical scales with prominently protruding keel tips (S 58.C7). **Fig. 10.** Rear scale (S 62.D7).



Type locality: Unnamed lake, Disko Island, Greenland (69.28913°N, –53.49322°W).

Holotype: *Synura borealis* strain S 62.D7, frozen material deposited in the Culture Collection of Algae of Charles University in Prague (CAUP). Figure 6 presents an illustration of the holotype.

Holotype DNA barcode: GenBank Accession no. HG514176.

Distribution (Table 1): Estonia, Finland, Greenland, Norway and Sweden.

*Synura heteropora* Škaloud, Škaloudová & Procházková, *sp. nov.*

(Figs 11–19)

Description: Colonies are spherical, up to 50 µm in diameter, consisting of approximately 16–36 cells associated by their posterior ends (Fig. 11). Cells are pyriform, posteriorly elongated into the tail, 20–25 µm long and 7–11 µm wide. Each cell is surrounded by a layer of imbricate siliceous scales (Fig. 12). Body scales are 2.5–3.8 µm long and 1.1–1.9 µm wide, consisting of a basal plate with a centrally raised keel protruding into an acute tip (Fig. 13). The keel is cylindrical, occasionally slightly widened anteriorly, and ornamented by larger pores (diameter, 49–100 nm) (Fig. 14). The ratio between scale and keel width varies from 1.7 to 3.9. The basal plate is ornamented by numerous small pores (diameter, 20–31 nm), and anteriorly perforated by a rounded base hole (diameter, 0.19–0.42 µm). Numerous struts (22–28), often interconnected by transverse folds, extend regularly from the keel to the scale perimeter (Fig. 15). Apical scales are 2.6–3.0 µm long and 1.3–1.6 µm wide (Fig. 16). The keel of the apical scales ends in a rounded tip (Fig. 17). Rear scales are 1.8–3.2 µm long and 0.6–1.0 µm wide (Figs 18, 19).

Etymology: Named for the different sizes of the keel- and base-plate pores.

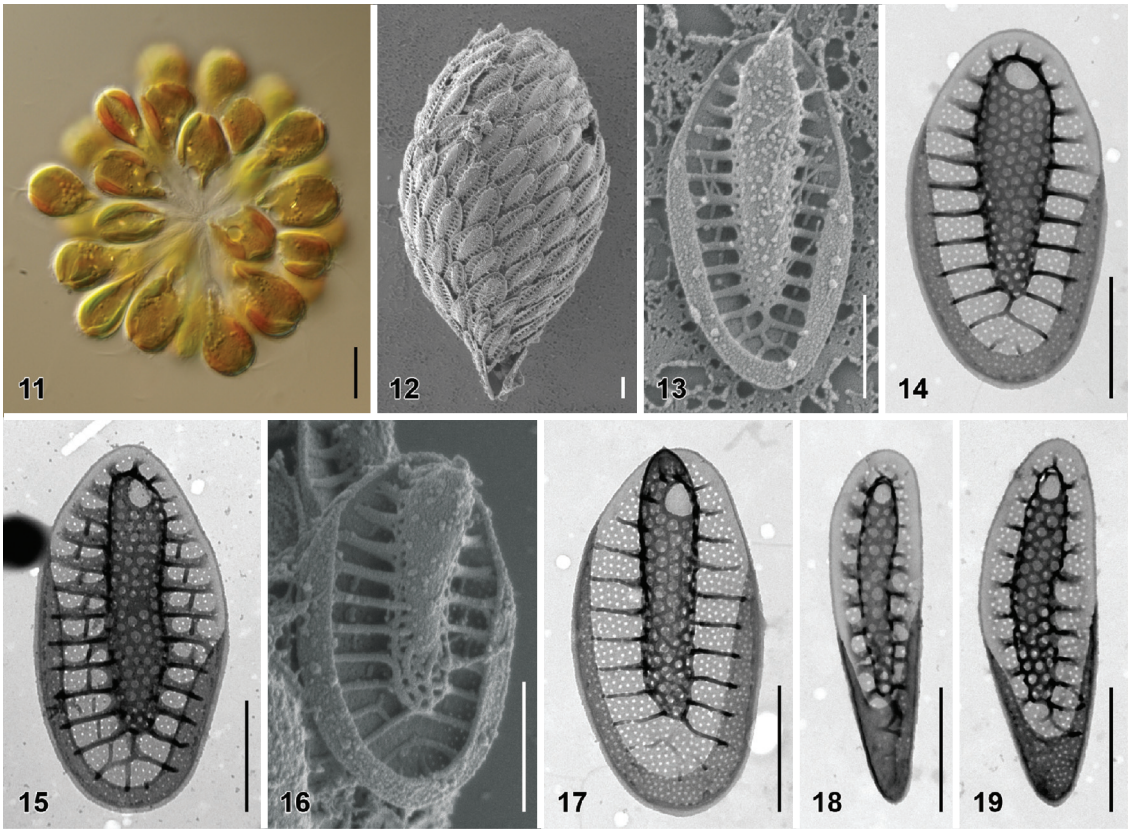
Type locality: Crinan Canal, Scotland, UK (56.060461° N, 5.481813° W).

Holotype: *Synura heteropora* strain S 20.45, frozen material deposited in the Culture Collection of Algae of Charles University in Prague (CAUP). Figure 14 presents an illustration of the holotype.

Holotype DNA barcode: GenBank Accession no. HG514198.

Distribution (Table 1): Austria, Czech Republic, Estonia, Ireland, Norway, Sweden and the UK.

*Synura hibernica* Škaloud & Škaloudová, *sp. nov.*




**Figs. 11–19.** Scale morphology of *Synura heteropora* sp. nov. (Fig. 11: LM; Figs 12, 13, 16: SEM; Figs 14, 15, 17–19: TEM). Scale bars represent 10 µm (Fig. 11) and 1 µm (Figs 12–19). **Fig. 11.** Colony consisting of densely grouped, pyriform cells (strain S 20.45). **Fig. 12.** Single cell surrounded by a layer of siliceous scales (S 54.E11). **Fig. 13.** Body scale (S 54.E11). **Fig. 14.** Body scale (S 20.45). **Fig. 15.** Body scale with transverse folds interconnecting the struts (S 87.C6). **Fig. 16.** Apical scale (S 54.E11). **Fig. 17.** Apical scale with a pronounced, rounded keel tip (S 101.F7). **Figs 18, 19.** Rear scales (S 101.F7).

(Figs 20–28)

Description: Colonies are spherical, up to 94 µm in diameter, consisting of approximately 12–52 cells associated by their posterior ends (Fig. 20). Cells are significantly elongated, anteriorly cylindrical, posteriorly tapering into the tail, 26–47 µm long and 6–12 µm wide. Each cell is surrounded by a layer of imbricate siliceous scales (Fig. 21). Body scales are 3.4–5.6 µm long and 1.2–2.0 µm wide, consisting of a basal plate with a centrally raised keel protruding into an acute tip (Fig. 22). The keel is cylindrical, ornamented by medium-sized pores (diameter, 49–89 nm) (Figs 23, 24). The ratio between scale and keel width varies from 1.9 to 3.9. The basal plate is ornamented by numerous small pores (diameter, 18–27 nm), and anteriorly perforated by a rounded base hole (diameter, 0.14–0.38 µm). A large number of struts (30–47), often interconnected by transverse folds, extend regularly from the keel to the scale perimeter (Figs 22, 23). Apical scales are 2.9–3.3 µm long and 1.6–1.9 µm wide (Fig. 25). The keel of the apical scales ends in a prominent, acute tip (Fig. 26), sometimes terminated by two short teeth. Rear scales are long and narrow, 3.0–4.9 µm long and 0.9–1.0 µm wide (Figs 27, 28).

Etymology: The specific epithet 'hibernica' refers to the common occurrence of the species in Ireland.

Type locality: Glanmore Lake, Ireland (51.732724° N, 9.767121° W).

Holotype: *Synura hibernica* strain S IE 104.D11, frozen material deposited in the Culture Collection of Algae of Charles University in Prague (CAUP). Figure 22 presents an illustration of the holotype.

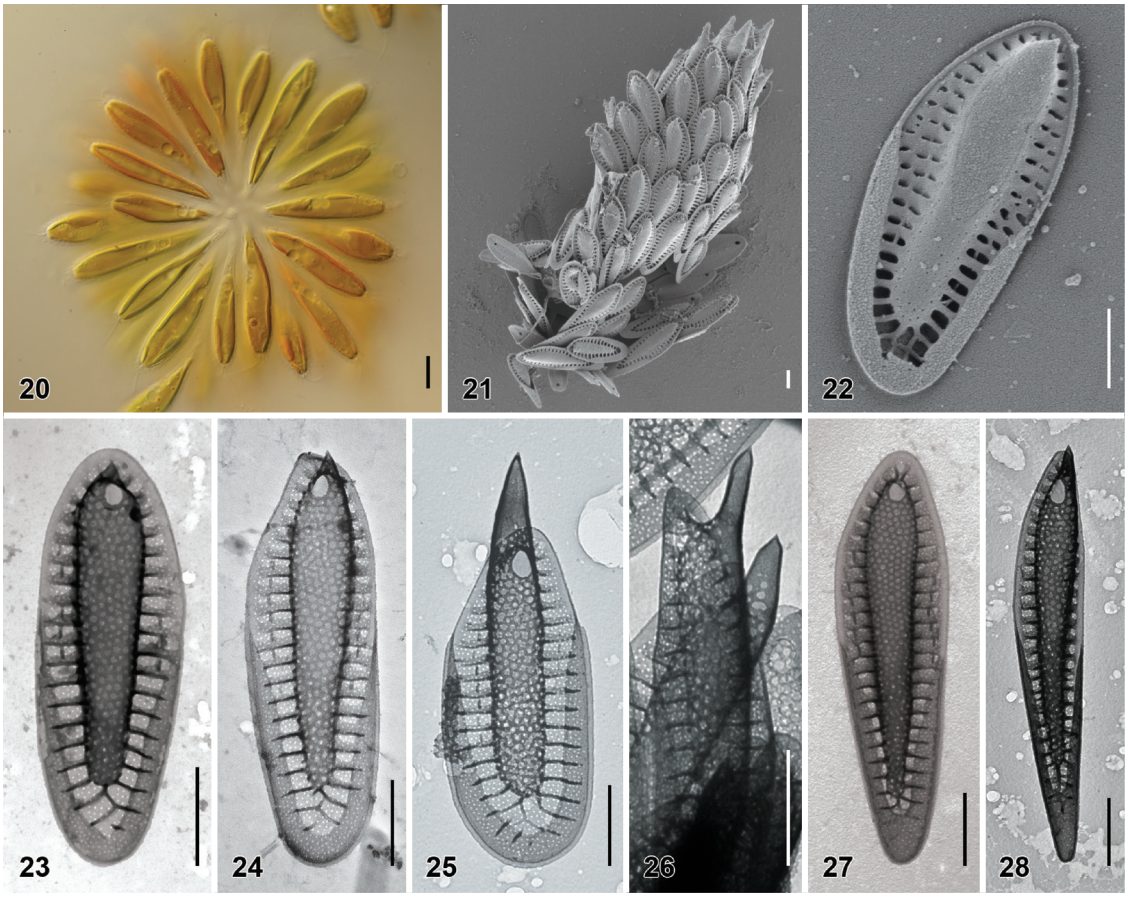
Holotype DNA barcode: GenBank Accession no. HG514216.

Distribution (Table 1): Currently known only in Ireland.

*Synura laticarina* Škaloud & Škaloudová, *sp. nov.*

(Figs 29–36)

Description: Colonies are spherical, up to 64 µm in diameter, consisting of approximately 12–28 cells associated by their posterior ends (Fig. 29). Cells are pyriform, posteriorly elongated into the tail, 21–32 µm long and 7–13 µm wide. Each cell is surrounded by a layer of imbricate siliceous scales (Fig. 30). Body scales are 3.1–4.3 µm long and 1.6–2.1 µm wide, consisting of a basal plate with a centrally raised keel protruding into an acute tip (Fig. 31). The keel is often anteriorly widened, ornamented by medium-sized pores (diameter, 52–76 nm) (Figs 32, 33). Anteriorly, keel pores are produced on both sides, so that the keel pore pattern is notably over-layered (Fig. 33). The ratio between scale and keel width varies from 1.9 to 2.9. The basal plate is ornamented by numerous small pores (diameter, 19–25 nm), and anteriorly perforated by a rounded base hole (diameter, 0.20–0.33 µm). Numerous struts (24–32), often interconnected by transverse folds, extend regularly from the




**Figs. 20–28.** Scale morphology of *Synura hibernica* sp. nov. (Fig. 20: LM; Figs 21, 22: SEM; Figs 23–28: TEM). Scale bars represent 10 µm (Fig. 20) and 1 µm (Figs 21–28). **Fig. 20.** Colony consisting of significantly elongated cells (strain S IE 104.D11). **Fig. 21.** Single cell surrounded by a layer of siliceous scales (S IE 104.D11). **Fig. 22.** Body scale (S IE 104.D11). **Fig. 23.** Body scale (S IE 103.C9). **Fig. 24.** Body scale (S IE E11). **Fig. 25.** Apical scale with prominently protruding keel tip (S IE E11). **Fig. 26.** Lateral view on apical scales with protruding keel tips (environmental sample, Lough Anillaun, Co. Galway, Ireland). **Fig. 27.** Rear scale (S IE M38). **Fig. 28.** Rear scale (S IE E11).

keel to the scale perimeter (Fig. 34). Apical scales are 2.5–3.0 µm long and 1.7–2.0 µm wide (Fig. 35). The keel of the apical scales ends in a prominent, sometimes rounded tip. Rear scales are 2.7–4.3 µm long and 0.7–1.3 µm wide (Fig. 36).

Etymology: The specific epithet is derived from the Latin 'latus' (broad) and 'carina' (keel), referring to the remarkable anterior widening of the keel.

Type locality: Ojala Lake, Finland (61.847778° N, 26.291447° E).

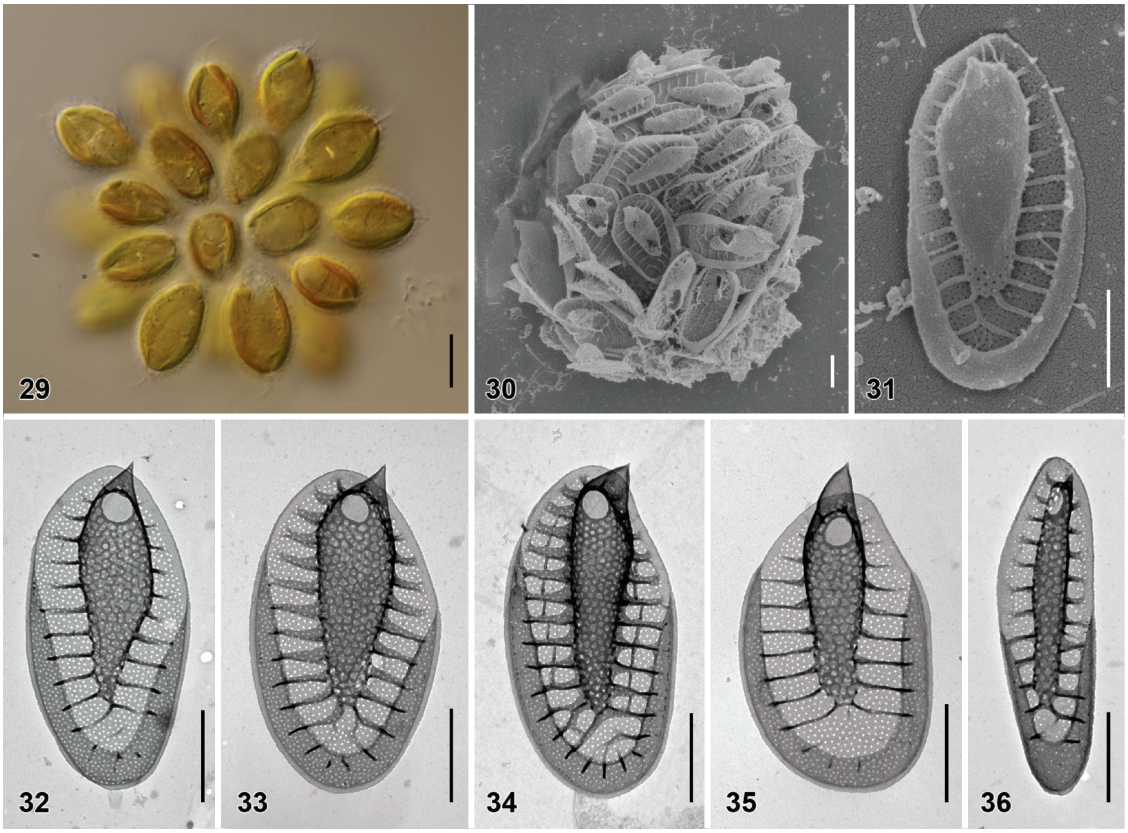
Holotype: *Synura laticarina* strain S 89.D5, frozen material deposited in the Culture Collection of Algae of Charles University in Prague (CAUP). Figure 33 presents an illustration of the holotype.

Holotype DNA barcode: GenBank Accession no. HG514221.

Distribution (Table 1): Estonia, Finland, Norway and Sweden.

### *Morphological analyses*

To investigate morphological differences among the 10 recognized, closely related *Synura* species in detail, we morphologically characterized each species by measuring 30 randomly chosen body scales. The species were obviously heterogeneous in the size of body scales (Fig. 37). *Synura borealis*, *S. hibernica* and *S. petersenii* had significantly longer scales than those of the remaining species. In addition, two groups of species could be recognized by the scale width: *S. conopea*, *S. heteropora*, *S. hibernica* and *S. truttae* had much narrower scales than those of the other species. Obvious morphological differences also were observed in the number of struts (Fig. 38), which represented the least variable morphological character. Therefore, some species could be clearly recognized only by counting the number of struts in a few scales. Heterogeneity also was observed in the size of all three pores measured. The base-plate pore area was the most discriminating character among the species (Fig. 38), with exceptionally large pores presented in *S. macropora*. All four newly recognized species were characterized by rather small base-plate pores. Both the keel pore and the base hole area showed relatively high size variability, which restricted their use as good discriminant features



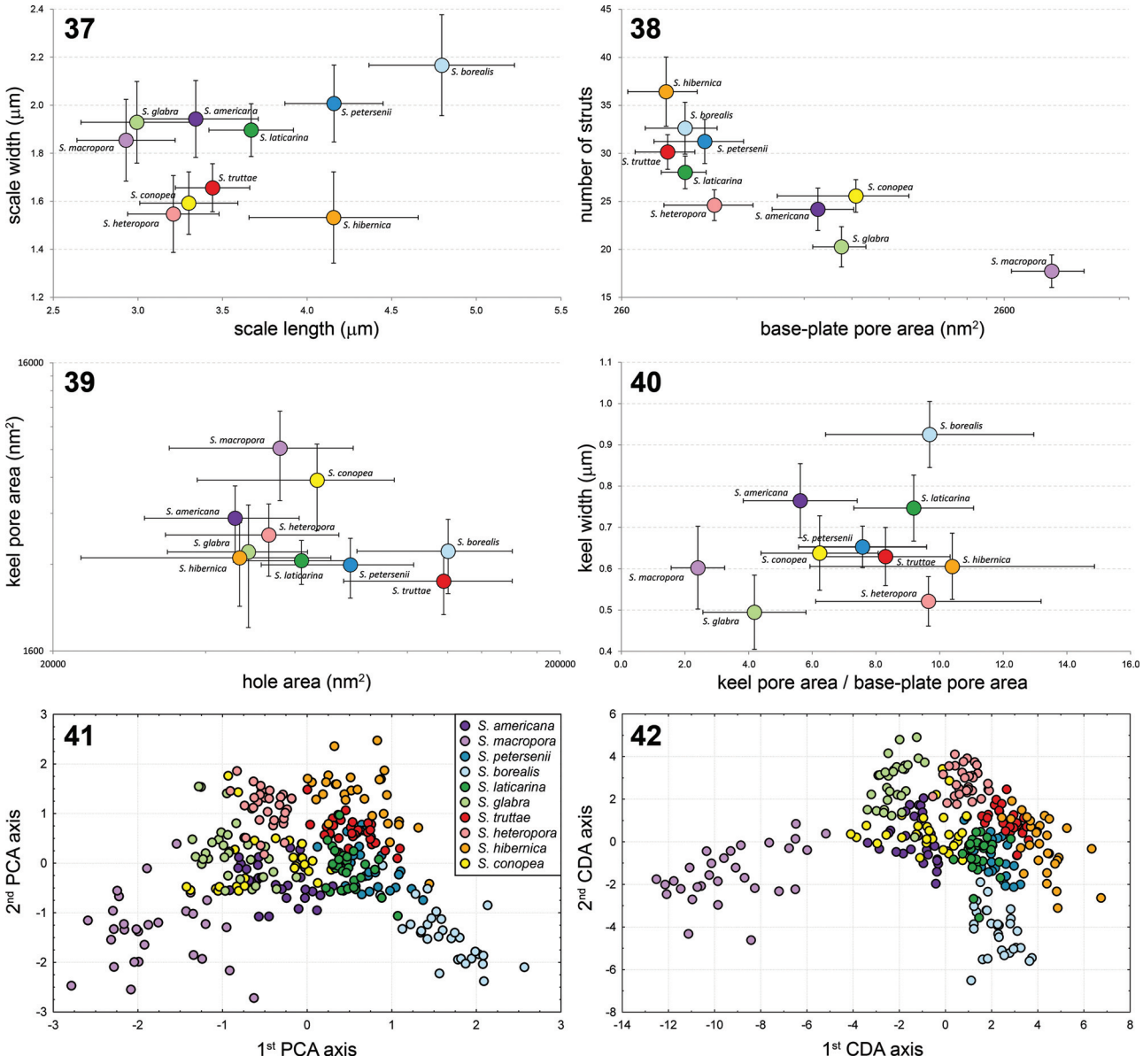

**Figs. 29–36.** Scale morphology of *Synura laticarina* sp. nov. (Fig. 29: LM; Figs 30 and 31: SEM; Figs 32–36: TEM). Scale bars represent 10 µm (Fig. 29) and 1 µm (Figs 30–36). **Fig. 29.** Colony consisting of pyriform cells (strain S 90.C8). **Fig. 30.** Single cell surrounded by a layer of siliceous scales (S 90.C8). **Fig. 31.** Body scale (S 90.C8). **Fig. 32.** Body scale with anteriorly widened keel (S 89.D5). **Fig. 33.** Body scale. Note over-layered pore pattern at the scale keel (S 89.D5). **Fig. 34.** Body scale with transverse folds interconnecting the struts (S 90.C8). **Fig. 35.** Apical scale (S 89.D5). **Fig. 36.** Rear scale (S 89.D5).

(Fig. 39). *Synura borealis* and *S. truttae* were well characterized by a notably large base hole. Strong heterogeneity was observed in the base-plate area to keel area ratio (Fig. 40). The keel pores of *S. macropora* and *S. glabra* were approximately comparable in size to the base-plate pores; however, those of *S. borealis*, *S. heteropora*, *S. hibernica* and *S. laticarina* were usually distinctively larger. *Synura americana*, *S. hibernica* and *S. laticarina* were well characterized by an anteriorly widened keel (Fig. 40). The central morphological characteristics of these 10 lineages, and other members of the genus *Synura*, section Peterseniae, are presented in Table 2. A key to these species is given in Table S2.

The principal component analysis (PCA) of the entire dataset resulted in a relatively well-defined grouping of scales belonging to the particular species (Fig. 41). Scales of *S. macropora* and *S. borealis* formed two distinct clusters with negative values on the first PCR axis. By contrast, scales of *S. conopea* were mostly intermixed with other species. The canonical discriminant analysis (CDA) yielded much better grouping of scales based on their morphological data (Fig. 42). With the exception of *S. conopea* and *S. americana*, all species formed distinct, separate clusters. The discriminant analysis (DA) indicated strongly significant differentiation among the 10 species (Wilk's $\lambda = 0.0008$; $P < 0.00001$). The forward stepwise analysis indicated that all tested morphological characters were significant for species recognition ($P < 0.00001$), and selected the base-plate pore area (Partial Wilk's $\lambda = 0.20$), the keel width (Partial Wilk's $\lambda = 0.41$) and the number of struts (Partial Wilk's $\lambda = 0.46$) as the three best discriminating characters. The discrimination of species was highly significant even when only these three characters were analysed (Wilk's $\lambda = 0.0058$; $P < 0.00001$). Congruently, whereas the first CDA axis was principally correlated with the base-plate pore area and the number of struts (correlation coefficients −0.82 and 0.63, respectively), the second CDA axis correlated with keel and scale widths (correlation coefficients 0.68 and 0.64, respectively). The average correct discrimination of individual scales on the basis of their morphology reached 92% (Table 3). The lowest correct discrimination levels were recovered in *S. conopea* (70.0%) and *S. laticarina* (80.0%).

### *Biogeography*

A 6-year sampling of *Synura* isolates performed in 15 different countries enabled us to determine their




**Figs. 37–42.** Morphological comparisons and statistical analyses of siliceous scales. **Figs 37–40.** Scatterplots of morphological features measured in the 10 investigated species; average values and standard deviations are given. **Fig. 37.** Scatterplot of scale length versus scale width. **Fig. 38.** Scatterplot of base-plate pore area versus number of struts. **Fig. 39.** Scatterplot of base hole area versus keel pore area. **Fig. 40.** Scatterplot of keel pore to base-plate pore area ratio versus keel width. **Fig. 41.** Principal component analysis (PCA) of the entire measured morphological features dataset. **Fig. 42.** Canonical discriminant analysis (CDA) of the same dataset.

biogeographic distribution across the European continent. Different distribution patterns can be recognized in four newly described *Synura* species (Figs 43–46). *Synura heteropora* is distributed across much of Europe, whereas the remaining three species are more restricted in their occurrence. Evolutionarily related *S. borealis* and *S. laticarina* exhibit similar biogeographic patterns, occurring in the northern regions (Figs 43, 46). *Synura laticarina* is regionally more restricted, currently reported only from Estonia, Finland, Norway and Sweden (Fig. 46). The most restricted distribution pattern was observed in *S. hibernica*. This species was found only in the blanket peat bogs located along the western coast of Ireland (Fig. 46).

## Discussion

*Synura petersenii sensu lato* is one of the most widely distributed and common groups of freshwater microorganisms. It is relatively easily cultivated and investigated with molecular methods (Wee *et al.*, 2001; Boo *et al.*, 2010; Kynčlová *et al.*, 2010; Škaloud *et al.*, 2012, 2013*a*), so it represents an ideal model taxon for investigating evolutionary patterns in protists. Particular species are relatively young in evolutionary terms, diverging on the order of several million years ago (Jo *et al.*, 2013). Therefore, it allows us to investigate speciation processes, rates of morphological and ecological differentiation of species, and overall species diversity within this recently diverging group

**Table 2.** Scale characteristics of selected *Synura* species (section Petersenieae) with similar scale morphologies. Newly described species are given in bold. For terminology of measured characteristics, see Fig. 1.

| Taxon | Cell dimensions (µm) | Scale dimensions (µm) | Number of struts | Rear scales longer than body scales? | Keel width | Base hole diameter (nm) | Base-plate pore diameter (nm) | Keel pore diameter (nm) | Body scale length to width ratio | Connection of struts |
|---|---|---|---|---|---|---|---|---|---|---|
| *S. americana* | 22–28 × 8–12 | 2.8–4.1 × 1.6–2.2 | 20–28 | yes | 0.6–0.9 | 167–336 | 27–43 | 54–94 | 1.4–2.3 | very rare |
| *S. conopea* | 20–28 × 8–12 | 2.6–3.7 × 1.3–1.9 | 22–29 | no | 0.5–0.8 | 189–438 | 25–51 | 70–125 | 1.5–2.5 | rare |
| *S. glabra* | 19–28 × 10–14 | 2.2–3.6 × 1.6–2.2 | 17–25 | no | 0.4–0.7 | 144–327 | 29–41 | 44–100 | 1.3–1.9 | none |
| *S. macropora* | 17–25 × 8–12 | 2.2–3.4 × 1.5–2.2 | 15–22 | yes | 0.4–0.8 | 156–391 | 50–78 | 69–137 | 1.3–2.1 | none |
| *S. petersenii* | 20–31 × 8–12 | 3.4–4.7 × 1.7–2.3 | 26–37 | no | 0.6–0.8 | 244–461 | 18–30 | 45–82 | 1.7–2.5 | frequent |
| *S. truttae* | 22–31 × 11–13 | 3.0–3.9 × 1.5–1.9 | 27–34 | no | 0.5–0.8 | 270–557 | 18–25 | 47–70 | 1.8–2.4 | frequent |
| ***S. borealis*** | **31–42 × 7–12** | **4.0–5.8 × 1.6–2.6** | **28–38** | **no** | **0.8–1.3** | **266–554** | **17–26** | **54–88** | **1.7–2.9** | **frequent** |
| ***S. heteropora*** | **20–25 × 7–11** | **2.5–3.8 × 1.1–1.9** | **22–28** | **no** | **0.4–0.7** | **187–415** | **20–31** | **49–100** | **1.8–2.6** | **frequent** |
| ***S. hibernica*** | **26–47 × 6–12** | **3.4–5.6 × 1.2–2.0** | **30–47** | **no** | **0.5–0.8** | **138–378** | **18–27** | **49–89** | **2.2–3.5** | **frequent** |
| ***S. laticarina*** | **21–32 × 7–13** | **3.1–4.3 × 1.6–2.1** | **24–32** | **no** | **0.6–1.0** | **200–334** | **19–25** | **52–76** | **1.7–2.4** | **frequent** |
| '*S. petersenii*' *f. columnata* | 15–23 × 8–11 | 2.7–3.5 × 1.5–2.2 | 22–27 | – | 2.6–3.1 | – | – | – | 1.6–1.8 | none |
| '*S. petersenii*' *f. praefracta* | – | 3.2–3.4 × 1.8–2.0 | 24–27 | – | 3.0–3.5 | – | – | – | 1.7–1.8 | frequent |
| '*S. petersenii*' *f. taymyrensis* | – | 2.7–2.8 × 1.2 | 23–25 | no | 1.7 | – | – | – | 2.3 | none |

of microorganisms. This study was the next step necessary for uncovering the real species diversity and to delimit and characterize particular species within this complex.

### *Overall species diversity*

Biological variation within *Synura petersenii* is very complex. Before information was available about genetic differentiation, *S. petersenii* was generally considered as a single species, with recognition of a few forms or varieties (Kristiansen & Preisig, 2007). In fact, this taxon contains a number of morphologically similar, yet genetically distinct species. In addition to the four newly proposed species, *S. petersenii* s.l. now includes 10 well-defined, genetically characterized species. Phylogenetic analysis clearly recognizes six lineages comprised of several strains deposited in various culture collections, which very probably represent new, yet undescribed species (Fig. 2). Several taxa belonging to the section Peterseniae are currently uncharacterized with molecular markers, and their phylogenetic position is still unknown. All these taxa, including *S. australiensis* (Playfair, 1915), '*S. petersenii*' f. *columnata* (Siver, 1988), '*S. petersenii*' f. *praefracta* (Asmund, 1968), '*S. petersenii*' f. *taymyrensis* (Kristiansen *et al.*, 1997), and *S. obesa* (Němcová *et al.*, 2008), could form additional lineages within *S. petersenii* s.l. Therefore, it is very difficult to estimate the total number of species in this taxon. In an extensive molecular investigation of more than 100 *S. petersenii* s.l. strains, Boo *et al.* (2010) identified a large number of genotypes clustered into seven groups. The strains originated from different areas located in four continents. Of the four new species proposed in the present study, only *S. heteropora* was identified with a previously recognized genotype, the strain CCMP 2898 belonging to clade G I Boo *et al.* (2010). Obviously, there is still a large degree of hidden diversity that cannot be fully resolved without additional molecular and morphological data. However, even if we can predict that several tens of currently undiscovered taxa exist, it is probable that the most frequently occurring species are already known and characterized.

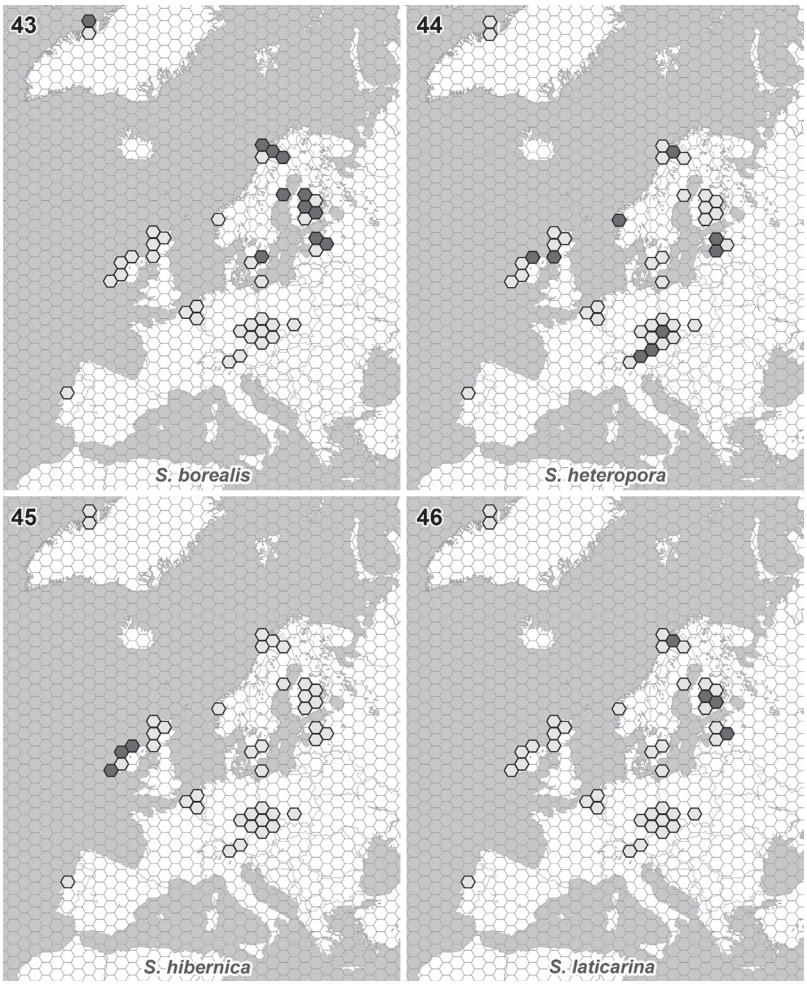
### *Species delineation and morphological evolution*

The six species delineated in previous studies (Kynčlová *et al.*, 2010; Škaloud *et al.*, 2012) were shown to be distinguishable by the siliceous scale morphology. However, the present study includes descriptions of four additional, morphologically highly similar taxa. Morphometric analyses of siliceous scales enabled the significant phenotypic differentiation of all species of the *S. petersenii* complex, including the newly described species. The CDA analysis significantly recognized all 10 investigated

**Table 3.** Classification matrix of the canonical discriminant analyses of all seven morphological characters/three best discriminating characters (base-plate pore area, keel width, number of struts). Rows: observed classification. Columns: predicted classification.

| | Predicted classification | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Species | (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) | (9) | (10) | % correctly classified |
| (1) *S. americana* | **27/25** | 0/0 | 2/2 | 1/1 | 0/1 | 0/0 | 0/1 | 0/0 | 0/0 | 0/0 | 90/83 |
| (2) *S. borealis* | 0/0 | **29/27** | 0/0 | 0/0 | 0/0 | 0/0 | 1/3 | 0/0 | 0/0 | 0/0 | 97/90 |
| (3) *S. conopea* | 2/3 | 0/0 | **21/17** | 0/0 | 4/3 | 0/0 | 3/6 | 0/0 | 0/1 | 0/0 | 70/57 |
| (4) *S. glabra* | 0/0 | 0/0 | 0/1 | **29/29** | 1/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 97/97 |
| (5) *S. heteropora* | 0/1 | 0/0 | 0/0 | 0/1 | **29/28** | 0/0 | 0/0 | 0/0 | 0/0 | 1/0 | 97/93 |
| (6) *S. hibernica* | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | **30/24** | 0/0 | 0/0 | 0/2 | 0/4 | 100/80 |
| (7) *S. laticarina* | 1/1 | 1/0 | 0/0 | 0/0 | 0/0 | 1/0 | **24/25** | 0/0 | 3/4 | 0/0 | 80/83 |
| (8) *S. macropora* | 1/1 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | **29/29** | 0/0 | 0/0 | 97/97 |
| (9) *S. petersenii* | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 1/2 | 1/1 | 0/0 | **27/17** | 1/10 | 90/57 |
| (10) *S. truttae* | 0/0 | 0/1 | 0/0 | 0/0 | 0/1 | 0/1 | 0/1 | 0/0 | 0/7 | **30/19** | 100/63 |
| Total | 31/31 | 30/28 | 23/20 | 30/31 | 34/33 | 32/27 | 29/37 | 29/29 | 30/31 | 32/33 | 92/80 |






**Figs. 43–46.** Distribution of newly described *Synura* species. Light grey hexagons show all studied regions where the occurrence of any *Synura* species has been recorded. Filled hexagons show the known distribution pattern of the new species. **Fig. 43.** *S. borealis*. **Fig. 44.** *S. heteropora*. **Fig. 45.** *S. hibernica*. **Fig. 46.** *S. laticarina*. The hexagon edge length corresponds to 80 km (hexagon area ≈ 16 600 km$^2$).

species (Fig. 42), and identified *S. conopea* as the species with the lowest correct discrimination level (70%). When analysing only the three best discriminating characters (base-plate pore area, keel width and number of struts), the correct discrimination level decreased to 57% in *S. conopea* and *S. petersenii*, but remained quite high in the majority of species (Table 3). Because the presented percentages represent post-hoc classifications, we can expect a lower accuracy when new scales will be classified. However, the CDA analyses clearly indicated that simply measuring three morphological features in four body

scales should be sufficient to correctly classify each of the species.

We detected a new lineage sister to *S. americana*, *S. glabra* and *S. petersenii* that we interpreted as two novel species, the genetically diverse *S. borealis* and the more molecularly homogeneous *S. laticarina* (Fig. 2). *Synura borealis* possessed an exceptionally high degree of genetic diversity, particularly in the *cox*1 gene, which could be interpreted as resulting from several independent species. However, the recognition of just these two species was strongly supported by our morphological data. The silica scales of all *S. borealis* strains shared characteristic morphological features, including the anteriorly widened keel with notably over-layered pore pattern, and large scale dimensions. Further, *S. borealis* and *S. laticarina* species could be undoubtedly recognized under the light microscope. All investigated *S. borealis* strains were characterized by large colonies consisting of significantly elongated, lanceolate-shaped cells, whereas *S. laticarina* produced notably smaller colonies containing rounded, pyriform cells. Therefore, we interpret the high genetic diversity in *S. borealis* as an indication of recent speciation and ongoing evolutionary differentiation. Further investigation of *S. borealis* genotypes, including their ecophysiological differentiation, ecological preferences and distributional patterns could provide important insights into the drivers of species diversification and mechanisms of population-level structuring in *Synura* species.

Particular species of the *S. petersenii* group obviously underwent some degree of morphological differentiation, although they are relatively young in evolutionary terms. Some species were more significantly differentiated with respect to siliceous scales than others, and can be recognized at first glance. For example, *S. macropora* can be unambiguously identified by its extremely large base-plate pores (Fig. 38). We can only speculate about the causes of this rapid morphological evolution. Large pores could significantly decrease the density of the cell, and hence reduce the sinking rate. However, cells with larger pores could be more susceptible to viral infection (Losic *et al.*, 2006). *Synura macropora* seems to occur only in eutrophic habitats characterized by high conductivity values (Table 1; Škaloud *et al.*, 2013*b*). It was shown that eutrophic conditions decreased the availability of silica, which often caused a shift from heavily silicified to less silicified diatoms in freshwater biotopes (Rabalais *et al.*, 1996). Similarly, adaptation of *S. macropora* to eutrophic conditions could lead to the formation of less silicified scales characterized by large base-plate pores.

*Synura hibernica* represents another morphologically well-defined species characterized by very narrow, long siliceous scales bearing a large number of struts (Figs 37, 38). This species can even be distinguished under the light microscope by the characteristically large colonies consisting of strongly elongated, narrow cells. The significantly different morphology of this Irish taxon has been discussed previously by Řezáčová & Škaloud (2005), who emphasized its striking similarity with the tropical species *S. australiensis*. Obviously, the characteristically elongated shape of the siliceous scales is related to the elongated cell shape in both species. *Synura australiensis* and *S. hibernica* represent another example of rapid morphological evolution within the *S. petersenii* group, leading to the speciation of organisms producing big colonies consisting of long and narrow cells. Unfortunately, in the absence of molecular data for *S. australiensis*, it remains unclear whether these two taxa are evolutionarily related to each other. Significant morphological transformation may have been caused by an ecological niche-shift during the speciation of both taxa, similarly to that of *S. macropora*. In protists, a negative correlation between cell size and temperature has been proposed by Atkinson *et al.* (2003). However, *S. hibernica* is currently known only from Ireland, whereas the distribution of *S. australiensis* is restricted to tropical and subtropical regions (Kristiansen & Preisig, 2007). Therefore, we can rule out temperature-related morphological speciation of these two taxa.

The development of elongated cells in *S. australiensis* and *S. hibernica* could be explained by adaptation to oligotrophic conditions. It is known that most oligotrophs achieve a high surface-to-volume ratio, which increases the organisms' capacity to scavenge available nutrients (Reynolds, 2006). Elongation of *Synura* cells would be the most effective way to significantly increase this ratio. All 12 isolated strains of *S. hibernica* originated from nutrient-poor localities, with conductivity values ranging between 23–87 µS $cm^{-1}$ (Table 1). To our knowledge, all records of *S. australiensis* have been associated with clear, oligotrophic water bodies (Croome & Tyler, 1985; Cronberg, 1989, 1996; Saha & Wujek, 1990; Wee *et al.*, 1993; Hansen, 1996). The measured conductivity of these water samples was 10–123 µS $cm^{-1}$.

### *Biogeographic patterns*

The biogeography of protists has become a highly controversial topic over the last 10 years (Martiny *et al.*, 2006; Caron, 2009). It has been postulated that the small size, extremely large populations and high dispersal potential of protists result in the cosmopolitan distribution of the vast majority of species (Finlay, 2002; Finlay & Fenchel, 2004). Conversely, limited geographical distributions have been implied by Foissner (1999), primarily based on the observed restricted distribution of flagship species with easily recognizable morphologies and easily demonstrable presence/absence (Foissner, 2006, 2008). *Synura petersenii* s.l. represents a common group of

freshwater microorganisms, known to occur in all continents except Antarctica (Kristiansen, 2008). Because its molecular diversity has been investigated in three continents (Wee *et al.*, 2001; Boo *et al.*, 2010; Škaloud *et al.*, 2012), it represents an ideal taxon to study the distribution patterns in protists.

The first evaluation of biogeographic patterns in the *S. petersenii* group was published by Boo *et al.* (2010), who described the presence of restricted biogeographic distributions for many species. However, Škaloud *et al.* (2012) compared the genetic data with published morphological observations, and reported much broader distributions for some of the investigated taxa. For example, putative North American endemic *S. americana*, although dominantly occurring in the USA and Canada, was also reported in South America and Europe (Cronberg, 1989; Škaloud *et al.*, 2012). However, the restricted distribution of several lineages is evident (e.g. clades D and F *sensu* Boo *et al.* 2010). Our results support the restricted distribution of *Synura* species, including the strict regional endemism observed in *S. hibernica*. To the best of our knowledge, all four newly proposed species have been reported only in Europe and Greenland (Figs 43–46). A comparison of the morphological characteristics with previously published reports reveals that *S. borealis* and *S. hibernica* have been observed previously in Greenland and Ireland, respectively (Kristiansen, 1992; Řezáčová & Škaloud, 2005); however, we found no other reports outside Europe.

Three of four newly described species exhibit restricted biogeographic distribution. Evolutionarily related *S. borealis* and *S. laticarina* seem to be adapted to colder areas, and their distributions appear to be limited by high summer temperatures (Figs 43, 46). The occurrence of *S. borealis* in Greenland indicates no obvious dispersal limitations, so we expect its circumboreal distribution. The most remarkable distribution pattern, however, is that of *S. hibernica*. This species is restricted in its geographic distribution to western Ireland (Fig. 45). Although we found *S. hibernica* in nine localities located along the western coast of Ireland (Table 1), it was never detected in any of the other sampled localities. To check this highly unusual distribution pattern, we undertook two expeditions in 2008 and 2012 to investigate the diversity of *Synura* species in neighbouring Scotland. Ecological and limnological conditions are very similar in western Ireland and Scotland: both areas are characterized by the occurrence of extensive peat deposits restricted to a cool oceanic climate, known as blanket bogs (Moore, 1982). Moreover, the British Isles lie at the intersection of major bird migration corridors, and several bird species frequently pass through one part of the Isles to reach another (Newton, 2010). Therefore, the algal flora of the western British lakelands, including western Ireland and northwestern Scotland, are highly similar. The significant similarity of western British desmid flora was reported previously (West & West, 1909). Although we sampled a total of 71 localities in northwestern Scotland, we did not discover a single colony of *S. hibernica*. This distribution pattern, which is restricted to an extremely small biogeographic area, is remarkable and entirely outstanding. Future work should study the mechanisms underlying the obvious dispersal limitation of *S. hibernica*, including ecological and habitat requirements, desiccation tolerance and mechanisms of cyst formation.

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## Supplementary information

The following supplementary material is accessible via the Supplementary Content tab on the article's online page at http://dx.doi.org/10.1080/09670262.2014.905710

**Table S1.** Strain names, geographic origins, and the corresponding ITS rDNA, *rbc*L, and *cox*1 GenBank accession numbers for the taxa used in the phylogenetic analyses. Newly obtained sequences are given in bold.

**Table S2.** Key to species of the genus *Synura*, section Peterseniae.

**Alignment S1.** The final, partitioned alignment of concatenated ITS rDNA, *rbc*L, and *cox*1 sequences used for phylogenetic reconstructions.

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**Key to species of the genus *Synura*, section Peterseniae**

1. Base plate covered by struts extending from the keel to the scale perimeter ..... 2

   Struts missing, base plate with fine regular reticulation .....*S. longisquama*

2. Transverse struts connected in a dense net..... 3

   Transverse struts unconnected, or interconnected by single transverse struts ..... 4

3. Keel narrow, with a large base plate hole .....*S. macracantha*

   Keel extremely broad, with a very small base plate hole.....*S. obesa*

4. Scales long and narrow (scale length to width ratio higher than 3.6).....*S. australiensis*

   Scale length to width ratio less than 3.5..... 5

5. Scales with a very broad rim..... 6

   Rim of scales narrower..... 7

6. Struts of different length, irregularly spacered .....*S. asmundiae*

   Struts of similar length, regularly spacered.....*S. bjoerkii*

7. Inner portion of the rim ornamented by row of posts (in SEM) or dots (in TEM) .....

   ..... "*S. petersenii"* f. *columnata*

   Rim not ornamented..... 8

8. Keel ornamented by a hexagonal pattern ..... "*S. petersenii"* f. *taymyrensis*

   Keel not ornamented ..... 9

9. Keel of apical scales terminates into a rounded tip, provided with a number of small teeth10

   Keel of apical scales terminates into an acute tip ..... 11

10. Body scales narrower, with 27-34 thickened and conspicuous struts.....*S. truttae*

    Body scales broader, with 24-27 inconspicuous struts ..... "*S. petersenii"* f. *praefracta*

11. Body scales rounded to oval, struts never or very rarely connected, large base-plate pores (diameter 25-78 nm)..... 12

Body scales lanceolate, struts frequently connected by transverse ribs, small base-plate pores (diameter 17-31 nm) ..... 15

12. Rear scales longer than body scales ..... 13

    Rear scales short ..... 14

13. Base-plate pores very large (diameter 50-78 nm) ..... *S. macropora*

    Base-plate pores smaller (diameter 27-43 nm) ..... *S. americana*

14. Keel and ribs less developed, scales often almost rounded ..... *S. glabra*

    Keel and ribs well developed, scales oval ..... *S. conopea*

15. Keel anteriorly greatly widened, with the over-layered pore pattern (in TEM) ..... 16

    Keel more or less cylindrical, ornamented by a single layer of pores ..... 17

16. Body scales short (length 3.1-4.3 μm), cells shorter than 32 μm ..... *S. laticarina*

    Body scales long (length 4.0-5.8 μm), cells longer than 32 μm ..... *S. borealis*

17. Keel anteriorly slightly widened, scale width up to 1.8(-2) μm ..... 18

    Keel cylindrical, scale width greater than (1.7-)1.9 μm ..... *S. petersenii*

18. Body scales short (length 2.5-3.8 μm), with 22-28 struts ..... *S. heteropora*

    Body scales long (length 3.4-5.6 μm), with 30-47 struts ..... *S. hibernica*

| Taxon | Strain number | Origin | GenBank accession | | |
|---|---|---|---|---|---|
| | | | ITS | *rbc* L | *cox* 1 |
| ***Synura americana*** | | | | | |
| *Synura americana* Kynčlová & Škaloud | CCMP 862 | Winter's Creek, MI, USA | GU338124 | GU325485 | GU295529 |
| *Synura americana* Kynčlová & Škaloud | CCMP 863 | road ditch near Winter's Creek, MI, USA | GU338125 | GU325486 | GU295530 |
| *Synura americana* Kynčlová & Škaloud | CCMP 866 | Newfoundland, Canada | AF308840 | - | - |
| *Synura americana* Kynčlová & Škaloud | UTEX LB 2404 | Sportsman's Lake, WA, USA | GU338132 | GU325495 | GU295538 |
| *Synura americana* Kynčlová & Škaloud | S 81.C7 | Záplavy NR, Czech Republic | **HG514166** | - | - |
| *Synura americana* Kynčlová & Škaloud | S 104.C4 | Schinkelbos, Netherlands | **HG514167** | - | - |
| ***Synura borealis*** | | | | | |
| *Synura borealis* Škaloud & Škaloudová | S 58.C7 | Lillesjön, Sweden | **HG514168** | **HG514234** | **HG514255** |
| *Synura borealis* Škaloud & Škaloudová | S 90.F3 | Rutajärvi, Harjulahti, Finland | **HG514169** | - | - |
| *Synura borealis* Škaloud & Škaloudová | S 90.G4 | Harjujärvi, Finland | **HG514170** | - | - |
| *Synura borealis* Škaloud & Škaloudová | S 110.B9 | Apna jõgi, Estonia | **HG514171** | - | - |
| *Synura borealis* Škaloud & Škaloudová | S 110.E2 | a small pool near Kalli jõgi, Estonia | **HG514172** | - | - |
| *Synura borealis* Škaloud & Škaloudová | S 116.B6 | Kjerringdalsvatnet, Norway | **HG514173** | - | - |
| *Synura borealis* Škaloud & Škaloudová | SC FIN 13A | Ojala, Finland | **HG514174** | - | - |
| *Synura borealis* Škaloud & Škaloudová | SC SWE 9 | Helgassjön, Sweden | **HG514175** | - | - |
| *Synura borealis* Škaloud & Škaloudová | S 62.D7 | a small lake, Disko island, Greenland | **HG514176** | **HG514235** | **HG514256** |
| *Synura borealis* Škaloud & Škaloudová | SC GRL 364 | a morraine lake, Disko island, Greenland | **HG514177** | - | - |
| *Synura borealis* Škaloud & Škaloudová | S 90.M34 | Jämsänjarvi, Finland | **HG514178** | **HG514236** | **HG514257** |
| *Synura borealis* Škaloud & Škaloudová | S 110.F3 | a small pool near Kalli jõgi, Estonia | **HG514179** | - | - |
| *Synura borealis* Škaloud & Škaloudová | S 113.F6 | Emajõgi river, Estonia | **HG514180** | - | - |
| *Synura borealis* Škaloud & Škaloudová | S 115.G2 | a pool near Vassijaure, Sweden | **HG514181** | - | - |
| *Synura borealis* Škaloud & Škaloudová | S 118.C6 | a pool near Torneträsk, Sweden | **HG514182** | - | - |
| *Synura borealis* Škaloud & Škaloudová | S 113.F4 | Emajõgi river, Estonia | **HG514183** | - | - |
| *Synura borealis* Škaloud & Škaloudová | S 114.B8 | unnamed lake, Sweden | **HG514184** | **HG514237** | **HG514258** |
| *Synura borealis* Škaloud & Škaloudová | S 114.C8 | unnamed lake, Sweden | **HG514185** | **HG514238** | **HG514259** |
| *Synura borealis* Škaloud & Škaloudová | S 114.B9 | unnamed lake, Sweden | **HG514186** | - | - |
| *Synura borealis* Škaloud & Škaloudová | S 114.F3 | unnamed lake near Paittasjärvi, Sweden | **HG514187** | - | - |
| *Synura borealis* Škaloud & Škaloudová | S 117.D3 | a pool near Paittasjärvi, Sweden | **HG514188** | **HG514239** | **HG514260** |
| *Synura borealis* Škaloud & Škaloudová | SC GRL 340 | Sanningassup Tasia, Greenland | **HG514189** | - | - |
| *Synura borealis* Škaloud & Škaloudová | S 114.G6 | Syväjärvi, Sweden | **HG514190** | **HG514240** | **HG514261** |
| *Synura borealis* Škaloud & Škaloudová | S 115.F4 | a pool near Arosnjarkajaute, Sweden | **HG514191** | **HG514241** | **HG514262** |
| *Synura borealis* Škaloud & Škaloudová | S 115.G3 | a pool near Vassijaure, Sweden | **HG514192** | - | - |
| *Synura borealis* Škaloud & Škaloudová | S 115.G7 | Vassijaure, Sweden | **HG514193** | **HG514242** | **HG514263** |

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| *Synura borealis* Škaloud & Škaloudová | SC FIN 22B | Heinäjärvi, Finland | **HG514194** | - | - |
| ***Synura conopea*** | | | | | |
| *Synura conopea* Kynčlová & Škaloud | CCMP 859 | southeast bridge pond, AR, USA | GU338121 | GU325482 | GU295526 |
| *Synura conopea* Kynčlová & Škaloud | S 10.2 | Huťský pond, Czech Republic | FM178507 | - | - |
| *Synura conopea* Kynčlová & Škaloud | NIES 1007 | Tomakomai, Japan | GU338119 | GU325479 | GU295524 |
| *Synura conopea* Kynčlová & Škaloud | KNU13 | Dalseonggyo, Korea | GU338069 | GU325430 | GU295472 |
| *Synura conopea* Kynčlová & Škaloud | CAUP B707 (S 7.10) | Babín pool, Czech Republic | FM178506 | - | - |
| *Synura conopea* Kynčlová & Škaloud | S 103.B3 | a pool near Smědava, Czech Republic | **HG514195** | - | - |
| ***Synura glabra*** | | | | | |
| *Synura glabra* Korshikov | NIES 233 | Higashiyata River, Japan | GU338118 | GU325480 | GU295523 |
| *Synura glabra* Korshikov | CNU09 | Jiokji pond, Korea | GU338077 | GU325438 | GU295480 |
| *Synura glabra* Korshikov | KNU10 | Bomun reservoir, Korea | GU338066 | GU325427 | GU295469 |
| *Synura glabra* Korshikov | S 9.2 | confluence of the Morava and Dyje rivers, Czech R | FM178513 | - | - |
| *Synura glabra* Korshikov | S 14.1 | Swamp NR, Czech Republic | FM178514 | - | - |
| *Synura glabra* Korshikov | S 89.F5 | Saarijärvi, Finland | **HG514196** | - | - |
| ***Synura heteropora*** | | | | | |
| *Synura heteropora* Škaloud, Škaloudová & Kynčlová | CCMP 2898 | Wallersee, Austria | GU338136 | GU325498 | - |
| *Synura heteropora* Škaloud, Škaloudová & Kynčlová | S 20.1 | Loch an Add, Scotland, UK | **HG514197** | - | - |
| *Synura heteropora* Škaloud, Škaloudová & Kynčlová | S 20.45 | Crinan Canal, Scotland, UK | **HG514198** | **HG514243** | - |
| *Synura heteropora* Škaloud, Škaloudová & Kynčlová | S 40.F11 | Podhradská pool, Czech Republic | **HG514199** | - | - |
| *Synura heteropora* Škaloud, Škaloudová & Kynčlová | S 54.E11 | Klejnarka river, Czech Republic | **HG514200** | - | - |
| *Synura heteropora* Škaloud, Škaloudová & Kynčlová | S 86.F2 | Zbýšov pond, Czech Republic | **HG514201** | - | - |
| *Synura heteropora* Škaloud, Škaloudová & Kynčlová | S 112.E2 | Saarde paisjärv, Estonia | **HG514202** | - | - |
| *Synura heteropora* Škaloud, Škaloudová & Kynčlová | S 112.F5 | Rahumeri, Estonia | **HG514203** | - | - |
| *Synura heteropora* Škaloud, Škaloudová & Kynčlová | S 113.C8 | Karula Jarv, Estonia | **HG514204** | - | - |
| *Synura heteropora* Škaloud, Škaloudová & Kynčlová | S 117.G6 | Torneträsk, Sweden | **HG514205** | - | - |
| *Synura heteropora* Škaloud, Škaloudová & Kynčlová | SC IRL 8 | Garlan Lough, Ireland | **HG514206** | - | - |
| *Synura heteropora* Škaloud, Škaloudová & Kynčlová | S 87.C6 | an ephemeral puddle near Kufstein, Austria | **HG514207** | **HG514244** | **HG514264** |
| *Synura heteropora* Škaloud, Škaloudová & Kynčlová | S 101.F7 | a pond in Nygårdsparken, Bergen, Norway | **HG514208** | **HG514245** | **HG514265** |
| ***Synura hibernica*** | | | | | |
| *Synura hibernica* Škaloud & Škaloudová | S IE E4 | The Long Range, Ireland | **HG514209** | **HG514246** | **HG514266** |
| *Synura hibernica* Škaloud & Škaloudová | S IE E11 | Glendollagh Lough, Ireland | **HG514210** | - | - |
| *Synura hibernica* Škaloud & Škaloudová | S IE 103.C11 | Gowlaun Lough, Ireland | **HG514211** | - | - |
| *Synura hibernica* Škaloud & Škaloudová | SC IRL 41 | Easky Lough, Ireland | **HG514212** | - | - |
| *Synura hibernica* Škaloud & Škaloudová | S IE E8 | unnamed lake near Maam Cross, Ireland | **HG514213** | **HG514247** | **HG514267** |
| *Synura hibernica* Škaloud & Škaloudová | S IE 103.C8 | Gowlaun Lough, Ireland | **HG514214** | - | - |
| *Synura hibernica* Škaloud & Škaloudová | S IE 103.C9 | Gowlaun Lough, Ireland | **HG514215** | - | - |
| *Synura hibernica* Škaloud & Škaloudová | S IE 104.D11 | Glanmore Lake, Ireland | **HG514216** | **HG514248** | **HG514268** |

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| *Synura hibernica* Škaloud & Škaloudová | S IE M38 | The Long Range, Ireland | **HG514217** | - | - |
| *Synura hibernica* Škaloud & Škaloudová | SC IRL 20a | Derrynaherriva Lough, Ireland | **HG514218** | - | - |
| *Synura hibernica* Škaloud & Škaloudová | S IE 105.F6 | Caha Lakes, Ireland | **HG514219** | **HG514249** | **HG514269** |
| *Synura hibernica* Škaloud & Škaloudová | SC IRL 60 | Maumwee Lough, Ireland | **HG514220** | - | - |
| ***Synura laticarina*** | | | | | |
| *Synura laticarina* Škaloud & Škaloudová | S 89.D5 | Ojala, Finland | **HG514221** | - | - |
| *Synura laticarina* Škaloud & Škaloudová | S 90.C8 | Tehriselkä, Finland | **HG514222** | **HG514250** | **HG514270** |
| *Synura laticarina* Škaloud & Škaloudová | S 110.C9 | Apna jõgi, Estonia | **HG514223** | - | - |
| *Synura laticarina* Škaloud & Škaloudová | S 113.E5 | a pool near Emajõgi, Estonia | **HG514224** | - | - |
| *Synura laticarina* Škaloud & Škaloudová | S 115.B2 | Barduelva river, Norway | **HG514225** | - | - |
| *Synura laticarina* Škaloud & Škaloudová | S 115.D2 | a pool near Arosnjarkajaute, Sweden | **HG514226** | **HG514251** | **HG514271** |
| *Synura laticarina* Škaloud & Škaloudová | S 115.E5 | a pool near Vassijaure, Sweden | **HG514227** | - | - |
| ***Synura macropora*** | | | | | |
| *Synura macropora* Škaloud & Kynčlová | S 5.3 | alluvial pool, Prague, Czech Republic | FM178496 | - | - |
| *Synura macropora* Škaloud & Kynčlová | S 14.2 | Swamp NR, Czech Republic | FM178497 | - | - |
| *Synura macropora* Škaloud & Kynčlová | S 71.B4 | Podhradská pool, Czech Republic | **HG514228** | **HG514252** | **HG514272** |
| *Synura macropora* Škaloud & Kynčlová | S 104.D7 | a canal in Oosteinde, Netherlands | **HG514229** | - | - |
| *Synura macropora* Škaloud & Kynčlová | S 104.F11 | a pool in Aalsmeer, Netherlands | **HG514230** | - | - |
| ***Synura petersenii*** | | | | | |
| *Synura petersenii* Korshikov | ACOI 1707 | Abrantes, Portugal | GU338150 | GU325512 | GU295552 |
| *Synura petersenii* Korshikov | CCAP 960/3 | Priest Pot, England, UK | GU338143 | GU325505 | GU295545 |
| *Synura petersenii* Korshikov | S 6.4 | Horni Luznice NR, Czech Republic | FM178501 | - | - |
| *Synura petersenii* Korshikov | CCMP 860 | Cedar Lake, IL, USA | GU338122 | GU325483 | GU295527 |
| *Synura petersenii* Korshikov | SAG 120.79 | Lüneburger Heide, Meadow pool, Germany | GU338144 | GU325506 | GU295546 |
| *Synura petersenii* Korshikov | CAUP B703 (S 7.7) | Babín pond, Czech Republic | FM178504 | - | - |
| *Synura petersenii* Korshikov | S 89.D6 | Ojala, Finland | **HG514231** | - | - |
| *Synura petersenii* Korshikov | S 89.F9 | Heinäjärvi, Finland | **HG514232** | - | - |
| ***Synura truttae*** | | | | | |
| *Synura truttae* (Siver) Škaloud & Kynčlová | Nemcova D5 | Kachní potok, Czech Republic | GU338140 | GU325502 | GU295542 |
| *Synura truttae* (Siver) Škaloud & Kynčlová | S 15.3 | Úpské rašeliniště, Czech Republic | FM178508 | - | - |
| *Synura truttae* (Siver) Škaloud & Kynčlová | S 15.9 | Úpské rašeliniště, Czech Republic | FM178510 | - | - |
| *Synura truttae* (Siver) Škaloud & Kynčlová | CAUP B705 (S 34.1) | forest pool near Mezní louka, Czech Republic | FR819749 | **HG514253** | - |
| *Synura truttae* (Siver) Škaloud & Kynčlová | S 62.B5 | a peat bog near Přebuz, Czech Republic | **HG514233** | **HG514254** | - |
| **clade D** | | | | | |
| *Synura* sp. | CNU01 | Samhwa pond, Korea | GU338070 | GU325431 | GU295473 |
| *Synura* sp. | CNU10 | Bihaksan, Korea | GU338078 | GU325439 | GU295481 |
| **clade F** | | | | | |
| *Synura* sp. | CNU12 | Geomuyeokri, Korea | GU338080 | GU325441 | GU295483 |

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| *Synura* sp. | CNU47 | Seongdong pond, Korea | GU338114 | GU325475 | GU295518 |
| **others** | | | | | |
| *Synura* sp. | KNU01 | Uksu reservoir, Korea | GU338057 | GU325418 | GU295460 |
| *Synura* sp. | UTEX LB 239 | unknown | GU338135 | GU325493 | GU295536 |
| *Synura* sp. | CCAC 0052 | Cologne, Germany | GU338146 | GU325508 | GU295548 |
| *Synura* sp. | CCMP 869 | Tobacco River, MI, USA | GU338128 | GU325489 | GU295533 |