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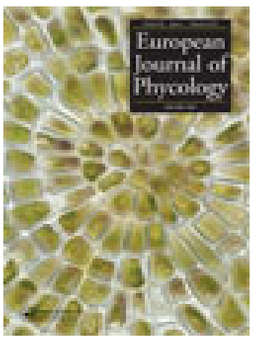

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Multigene phylogeny of *Synura* (Synurophyceae) and descriptions of four new species based on morphological and DNA evidence

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We used phylogenetic analyses based on multiple gene sequences (partial nr SSU and LSU rDNA, partial pt LSU rDNA, *psa*A and *rbc*L) from 148 strains (including three outgroups) and scale ultrastructure to examine phylogenetic relationships among species of the colonial genera *Synura* and *Tessellaria*. The phylogenetic tree based on the combined dataset was congruent with ultrastructural characteristics of the scales. *Synura* was divided into three major clades, two including species in section *Synura*, and one representing section *Peterseniae*. One clade, consisting of seven strains of *S. uvella* (section *Synura*), diverged at the base of the genus. The second clade consisted of the remaining species belonging to the section *Synura*. The third clade, containing organisms in the section *Peterseniae* and characterized by scales possessing a keel, was monophyletic with strong support values. Based on our findings, *S. uvella* needs to be in a separate section from other spine-bearing species, and we therefore propose new sectional ranks; *Synura, Peterseniae, Curtispinae* (presence of body scales with slender spines, tubular scales and caudal scales). We further propose four new species based on phylogenetic analyses and unique scale characters: *S. longitubularis* sp. nov., *S. sungminbooi* sp. nov., *S. soroconopea* sp. nov. and *S. lanceolata* sp. nov. Lastly, we propose a new genus name, *Neotessella*, to replace the invalid use of the name *Tessellaria*.

Key words: molecular phylogeny, morphology, *Neotessella*, scale, synurophytes, *Synura*, *Tessellaria*, ultrastructure

INTRODUCTION

The genus *Synura* Ehrenberg (1834) is an important component of the phytoplankton community in numerous freshwater habitats worldwide (Kristiansen & Preisig, 2007). There are approximately 46 species and subspecies of *Synura*, including two fossil taxa, described from Africa (Hansen, 1996), the Arctic region (Bradley, 1964; Asmund, 1968; Kristiansen, 1992), Asia (Takahashi, 1972, 1973; Kim, 1997; Boo *et al*., 2010), Europe (Cronberg & Kristiansen, 1980; Hartmann & Steinberg, 1989; Siver *et al*., 2005; Škaloud *et al*., 2013, 2014), and North and South America (Nicholls & Gerrath, 1985; Siver, 1987, 1988; Siver & Wujek, 1993, 1999; Siver & Vigna, 1997; Vigna & Munari, 2001; Siver & Wolfe, 2005; Siver, 2013). *Synura* species are colonial flagellates characterized by cells that are covered with siliceous scales, two visible and unequal length flagella and two plastids. Scale morphology and shape are dependent, in part, on their position on the cell surface, and often consists of apical tubular scales, body scales, transition scales and caudal scales. Because apical and caudal scales in some species are characterized by more cylindrical or elongated morphologies, respectively, distinguishing among species is based largely on differences in body scales (Kristiansen & Preisig, 2007).

The systematics of *Synura* were revolutionized once the ultrastructure of scales was resolved with the electron microscope and the new information led to modifications in the classification of *Synura*, from earlier sectional to detailed serial ranks (Petersen & Hansen, 1956, 1958; Fott & Ludvík, 1957; Asmund, 1968; Balonov & Kuzmin, 1974; Péterfi & Momeu, 1977; Takahashi, 1978; Cronberg, 1989; Škaloud *et al*., 2012, 2013, 2014). The classification scheme of Petersen & Hansen (1956) divided *Synura* into two major groups, section *Petersenianae* and section *Uvellae*. Species in *Petersenianae* were defined as

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having body scales with a central keel with either a blunt or spine-like projection. Within the section, the identification of species and infraspecific taxa is based primarily upon scale dimension, keel shape, size of the base plate holes and the character of struts. Species in *Uvellae* were defined as having body scales with a forward-projecting spine. Within the section, the identification of species and infraspecific taxa is based primarily upon scale dimension, spine shape and dimension, base plate pores, and the pattern of struts and meshwork on the base plate.

Subsequent to the work of Petersen & Hansen (1956), additional subgeneric distinctions were made (Balonov & Kuzmin, 1974; Péterfi & Momeu, 1977; Takahashi, 1978; Cronberg, 1989). Balonov & Kuzmin (1974) modified Petersen & Hansen's system and split the genus into three sections based on the morphological similarities in silica scales: *Lapponicae, Peterseniana*e and *Synura*. The section *Lapponicae* was established to account for *S. lapponica*, a taxon with oval to circular scales surrounding the whole colony instead of individual cells as is the case for *Synura*. Under the Balonov & Kuzmin (1974) system section *Synura* was equivalent to the section *Uvellae* proposed by Petersen & Hansen (1956). Péterfi & Momeu (1977) accepted the scheme of Balonov & Kuzmin (1974) and also used the name *Synura* for the type section. They further recognized series *Splendidae* within section *Synura*. Recently, Škaloud *et al*. (2013) transferred *S. lapponica* to the genus *Tessellaria*, and proposed a revised classification system that recognizes five sections: *Peterseniae, Spinosae, Echinulatae, Splendidae* and *Uvellae*.

Most molecular phylogenetic studies of *Synura* have focused on cryptic diversity within the *Synura petersenii* complex. The first molecular analyses were performed by Wee *et al*. (2001), who investigated genetic variability in 15 isolates of *S. petersenii* using the nuclear ITS region. Maximum parsimony (MP) analysis revealed the existence of two well-supported clades. Later, Kynčlová *et al*. (2010) extended the molecular dataset by adding a further 21 ITS sequences from newly isolated strains. The maximum likelihood (ML) analysis revealed six different clades within *S. petersenii*. Boo *et al*. (2010) published a seven-protein gene phylogeny of about 100 *S. petersenii* isolates, and confirmed a high degree of cryptic diversity within this species complex. Taxonomic assessment of observed cryptic diversity in the *S. petersenii* complex was first addressed by Škaloud *et al*. (2012), who recognized six lineages as separate species based on scale morphology and DNA sequence data, *S. americana, S. conopea, S. glabra, S. macropora, S. petersenii* and *S. truttae*. Most recently, Škaloud *et al*. (2014) described four additional species within the *S. petersenii* complex based on scale morphology and a combined ITS rDNA, *rbc*L and *cox*1 dataset.

The main objectives of this study are to (1) determine the phylogenetic relationships among species within *Synura* based on increased taxon sampling; (2) revise the family designations within the order Synurales; (3) revise the sections within the genus *Synura*; and (4) describe four new species.

MATERIALS AND METHODS

Strains and cultures

Collection information and culture accession numbers for 148 strains (including three outgroup species) used in this study are listed in Table S1. Strains were collected with a 20 µm mesh plankton net (Bokyeong Co., Pusan, Korea) from small ponds in Korea. Strains from outside South Korea were obtained from the following algal culture collections: The Provasoli-Guillard National Center for Culture of Marine Algae and Microbiota (NCMA), USA (http://ncma.bigelow.org/); Culture Collection of Algae at the University of Göttingen (SAG), Germany (http://www.epsag.uni-goettingen.de/); Culture Collection of Algae of Charles University in Prague (CAUP), Czech Republic (Department of Botany, Charles University in Prague). Culture methods followed those previously described (Jo *et al*., 2011, 2013). We omitted author citations of all *Synura* species in text, but listed full author citations in Table 3.

Morphological observations and statistical analyses

Cultured *Synura* strains were observed using an Axio Imager A2 microscope (Carl Zeiss Inc., Hallbergmoos, Germany) equipped with differential interference contrast (DIC) optics. Images were captured with an AxioCam HRc (Carl Zeiss Inc., Hallbergmoos, Germany) photomicrographic system. Cellular dimensions were determined by measuring 25–30 cells of each taxon from photographic images.

For field emission SEM, cells were filtered using nylon membrane filters (Whatman Ltd, Maidstone, UK), rinsed in distilled water, fixed in 1% OsO_4, dehydrated, prepared and viewed according to Jo *et al*. (2011). Voucher specimens were stored at the Chungnam National University (CNUK) herbarium in Daejeon, Korea. For TEM, cells were air dried onto Formvar coated copper grids. The grids were viewed in a JEM 1010 TEM (JEOL Ltd, Tokyo, Japan) at 80 kV. Images were recorded on Kodak EM Film 4489 (Eastman Kodak Co., Rochester, NY, USA) and scanned to digital format using an Epson Perfection V700 Photo scanner (Epson Korea Co. Ltd, Seoul, Korea). Terminology used to describe ultrastructural features of scales follows Škaloud *et al*. (2014). For each newly described species belonging to the section *Peterseniae*, the following characters were measured in 20 randomly selected scales (in *S. sungminbooi*, only 15 well-developed scales were analysed): (1) scale length, (2) scale width, (3) base hole (also referred to as a foramen pore) diameter, (4) average diameter of a keel pore, (5) average diameter of a base-plate pore, (6) keel width and (7) number of struts. The measurements were performed using the program ImageJ 1.45 s (Schneider *et al*., 2012). Average values of keel and base-plate pore diameters were calculated by measuring areas of approximately 30 randomly selected pores per scale. Measured data were compared with those obtained for 10 closely related *Synura* species by Škaloud *et al*. (2014). Canonical discriminant analysis of measured

data was performed using Statistica 8.0 (StatSoft, Inc., Tulsa, OK, USA). Graphs were generated using the R statistical software (http://www.r-project.org/). The position of *S. petersenii* f. *praefracta* in the canonical discriminant ordination space has resolved by measuring the above-mentioned characters in a scale illustrated by Asmund (1968), in her paper describing this taxon.

DNA extraction, amplification, sequencing, alignment and phylogenetic analyses

DNA extraction, PCR amplification, PCR product purification, sequencing and sequence alignments were conducted as described in Jo *et al.* (2011). Sequences for the nuclear-encoded SSU and LSU and plastid LSU rRNA genes were aligned by eye using the secondary structure of the rRNA gene sequences of *Mallomonas annulata* (Bradley) Harris (Wuyts *et al.*, 2001) as a guide. Conserved areas of these genes were readily aligned across taxa and were used for phylogenetic analyses, but unalignable nucleotides were excluded from subsequent phylogenetic analyses as well as from pairwise comparisons. The alignment of *rbc*L and *psa*A gene sequences was based on the alignment of inferred amino acid sequences. Only first and second codons of the *rbc*L gene were analysed since the third codon is known as a fast-evolving site (Škaloud *et al.*, 2013).

Phylogenetic analyses were performed using a combined dataset of 9178 characters (nr SSU = 1638, nr LSU = 2548, pt LSU = 2592, pt *psa*A = 1579 and pt *rbc*L = 825) by two different methods: maximum likelihood (ML) and Bayesian inference (BI). The combined dataset included sequences for all 148 strains (Table S1). Although nuclear ITS1 and ITS2 sequences were also determined, these sequences were used as a barcode to help identify species and were used only for phylogenetic analysis of most species within the section *Peterseniae* except for *S. asmundiae, S. bjoerkii* and *S. macracantha* (Škaloud *et al.*, 2012, 2014; see Supplementary Fig. S1). The sequences of three chrysophycean species were included as outgroup taxa and to root the tree. Primer regions and ambiguously aligned regions were removed from the alignment prior to phylogenetic analyses. Prior to ML analysis, the best-fit model for the concatenated dataset was estimated by Bayesian Information criterion (BIC) using MODELTEST 3.7 (Posada & Crandall, 1998), and the GTR + I + G model. We used the GTR + I + G nucleotide model as implemented in RAxML v.7.2.8 (Stamatakis, 2006), and used 200 independent tree inferences with the 'number of run' option, with default optimized SPR rearrangement and 25 distinct rate categories to identify the best tree. Statistical support for each branch was obtained from 1000 bootstrap replications using the same substitution model and RAxML program settings. Bayesian analyses were performed using MrBayes 3.2 (Ronquist *et al.*, 2012) with a random starting tree and ran for 2×10^6 generations, keeping one tree every 1000 generations. Each analysis was performed using the four Metropolis-coupled Markov Chain Monte Carlo (MC^3), with 2×10^6 generations for each chain. Burn-in point was identified graphically by tracking the likelihoods (Tracer v.1.6; http://tree.bio.ed.ac.uk/software/tracer/). The first 100 generations were discarded, and the remaining 1901 trees were used to calculate the posterior probabilities (PP) of each clade. Trees were visualized using FigTree v.1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

RESULTS

Phylogenetic analyses

The molecular data contained 436 new sequences, including 66 of nr SSU, 75 of nr LSU rDNA, 83 of nr ITS, 77 of pt LSU rDNA, 69 of pt *psa*A and 66 of pt *rbc*L data (Table S1), and 296 published sequences, including 76 of nr SSU, 36 of nr LSU rDNA, 27 of nr ITS and 26 of pt LSU rDNA, 55 of pt *psa*A and 76 of pt *rbc*L data (Table S1).

The consensus Bayesian tree was rooted with three species of Chromulinaceae serving as outgroups. The Bayesian and ML analyses recovered trees with almost identical topology (Fig. 1), except for positions of *Mallomonas insignis* Penard and *Synura uvella* isolates. The resulting phylogenetic tree showed that the colonial genus *Synura* was not closely related to *Tessellaria*, but to *Mallomonas*. The genus *Tessellaria* diverged at the base of the synurophyte clade with strong support values (pp = 1.00, ML = 100).

The genus *Synura* divided into three major lineages (clades A, B and C). The section *Synura* divided into two clades (B and C), each forming a monophyletic lineage. Clade A consisted of members of *S. uvella* and formed a strongly supported clade (pp = 1.00, ML = 100). Clade B consisted of the remaining members of the section *Synura* with moderate to strong support (pp = 1.00, ML = 73). Clade B was divided further into B1 and B2. Subclade B1 consisted of *S. splendida*, which diverged at the base of the clade. Subclade B2 consisted of *S. multidentata, S. echinulata, S. mammillosa, S. mollispina, S. spinosa, S. sphagnicola, S. curtispina*, a new species, *S. longitubularis* and several unidentified taxa. The single strain of *S. multidentata* showed a sister relationship to *S. echinulata* and *S. mammillosa* (pp = 1.00, ML = 100). *Synura mammillosa* was not monophyletic, and two strains, S96B5 and S89C3 showed sister relationships to two strains of *S. echinulata*. The single strain of *S. mollispina* was closely related to *S. spinosa* (pp = 1.00, ML = 100). The five strains of *S. spinosa* formed a monophyletic lineage with strongly supported values (pp = 1.00, ML = 100). The five strains of *S. sphagnicola* formed a monophyletic lineage with strong supported values (pp = 1.00, ML = 100). Five strains of *S. curtispina* were closely related to *S. longitubularis, Synura* sp. CCMP847, *Synura* sp. 450 and two Korean strains (pp = 1.00, ML = 100). The two strains of *S. longitubularis* formed a monophyletic lineage with well-supported values (pp = 1.00, ML = 100) and sister to two unidentified Korean strains.

The *Peterseniae* (C) clade formed a strongly supported monophyletic clade (pp = 1.00, ML = 100) and

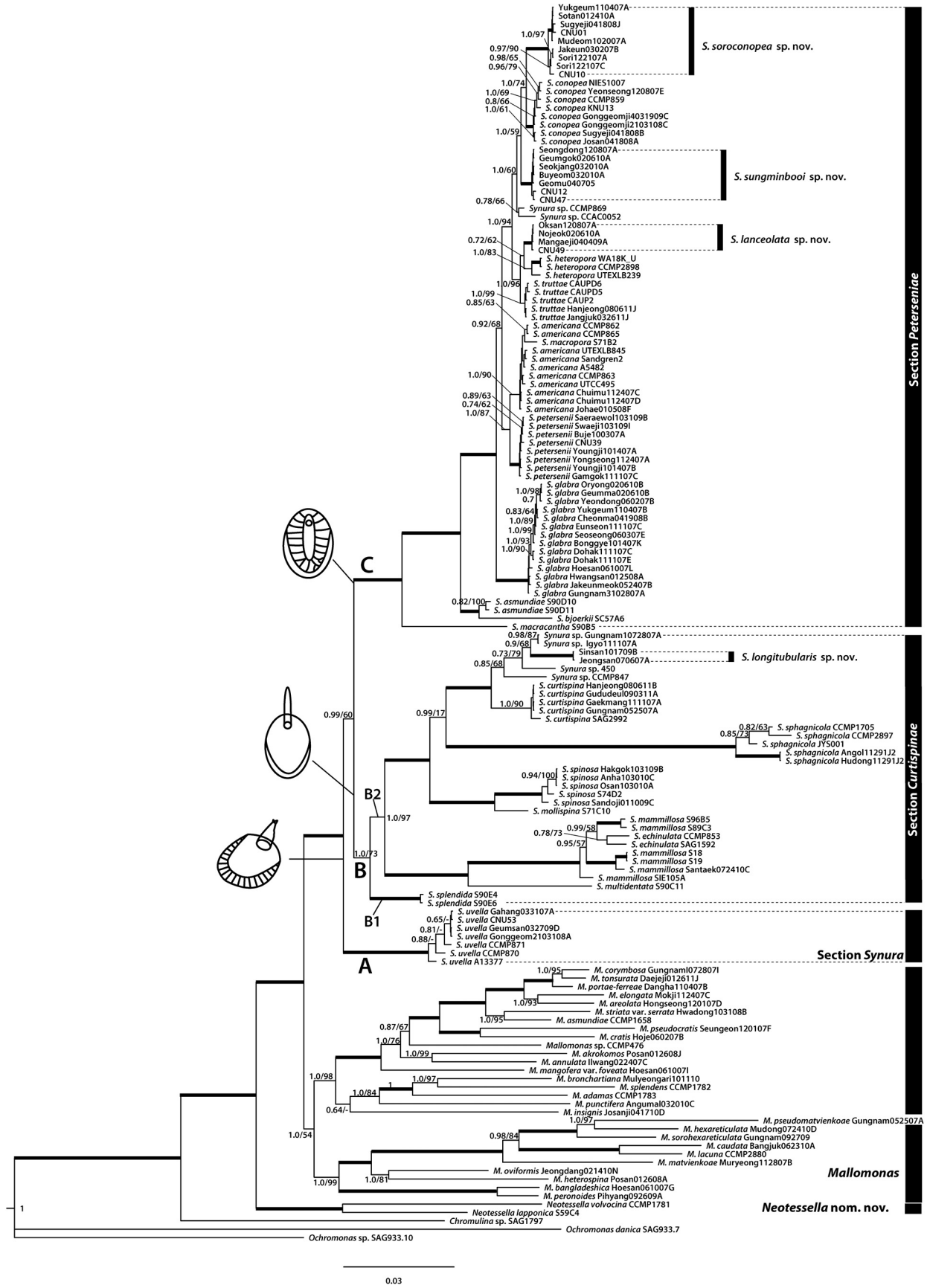


Fig. 1. Consensus Bayesian tree of *Synura* species based on a combined nuclear SSU and LSU rDNA, and plastid LSU rDNA, *psa*A, *rbc*L sequences data. Bayesian posterior probability (pp), maximum-likelihood (ML) bootstrap, maximum-parsimony (MP), and distance values (NJ) are shown above or below the branches. The bold branches indicate strongly supported values (pp = 1.00 and ML = 100%). Scale bar, 0.03 substitutions per site.

subdivided into 14 lineages. The single strain of *S. macracantha* diverged at the base of the *Peterseniae* clade, followed by *S. bjoerkii* and *S. asmundiae*. The single strain of *S. bjoerkii* formed sister relationships with *S. asmundiae*, which included two strains (pp = 1.00, ML = 100). *Synura glabra* diverged next to *S. bjoerkii* and *S. asmundiae*, forming a monophyletic group with strong support (pp =1.00, ML = 100). Members of *S. americana* and *S. petersenii* diverged after *S. glabra*, but the single strain of *S. macropora* was intermixed together with *S. americana* (pp = 1.00, ML = 90), and their relationship was not resolved. The eight strains of *S. petersenii* formed a monophyletic lineage with strong supported values (pp = 1.00, ML=100) and showed sister relationships with *S. americana* and *S. macropora*. The five strains of *S. truttae* formed a monophyletic lineage with strong support values (pp = 1.00, ML = 99), and showed sister relationships with three strains of *S. heteropora* and four strains of *S. lanceolata* (pp = 1.00, ML = 96). A third newly described species, *S. sungminbooi*, was related to *S. conopea* and a fourth new taxon, *S. soroconopea* (pp = 1.00, ML = 57). The seven strains of *S. sungminbooi* formed a monophyletic lineage with strongly supported values (pp = 1.00, ML = 100). The new species, *S. soroconopea* was closely related to *S. conopea* (pp = 1.00, ML = 75), and formed a monophyletic lineage with strong support values (pp = 1.00, ML = 100).

Morphological characteristics

The two sections of the genus *Synura* were distinguishable by ultrastructural characteristics of the scales. Section *Synura* was characterized by a hollow forward-projecting spine at the anterior end of the scale; section *Peterseniae* by a keel (hollow ridge) in the middle of the scale. All species included in this study were identified using scale ultrastructural features based on SEM and TEM (Tables 1 and 2). In addition, we described four new species; *S. longitubularis, S. sungminbooi, S. soroconopea* and *S. lanceolata* based on morphology of body scales (Figs 2–19).

Taxonomic descriptions

Synura lanceolata B.Y. Jo, W. Shin, J.I. Kim & P. Siver sp. nov. (Figs 2–5).

Description: Colonies are globular (40–51 µm in diameter). Cells are pyriform or obovate (16–20 × 10–13 µm), entirely covered with lanceolate to ellipsoidal-shaped scales. Body scales are 2.7–3.94 × 1.2–1.7 µm in dimension, with a posterior rim encircling half to two-thirds of the scale perimeter and with small, evenly spaced basal plate pores 20–36 nm in diameter. A round to oblong-shaped base plate hole is 0.24–0.47 µm in diameter, on the anterior end of the scale. Keel is 1.6–2.7 × 0.4–0.7 µm, marked through the vertical scale axis and elliptical in shape, with a blunt tip often bearing two to three very short teeth. Meshworks along the base of the keel vary from 54 to 82 nm in diameter. Transverse struts are 24–28 in number, extend from the keel to near the edge of the scale perimeter, and the spacing between struts ranges from 0.15–0.34 µm.

Holotype Specimen: Portion of a single gathering of cells on SEM stub CNU056357, deposited at the Herbarium of Chungnam National University, Daejeon, Korea (CNUK). Fig. 5 is a representative scale from the specimen.

Representative DNA sequences: GenBank accession no. KP268701.

Isotype Specimen: Portion of a single gathering of cells on SEM stub CNU056362, deposited at the Herbarium of Chungnam National University, Daejeon, Korea (CNUK).

Strain Information: The live culture of the holotype, deposited as Nojeok020610A at the Culture Collection of Algae of Chungnam National University, Daejeon, Korea.

Type locality: Nojeok, Chunpo-myeon, Iksan-si, Jeollabuk-do, Korea (35°56’48”N, 127°01’43”E).

Etymology: The specific epithet ‘*lanceolata*’ refers to the lanceolate shape of the scale.

Synura longitubularis B.Y. Jo, W. Shin, J.I. Kim & P. Siver sp. nov. (Figs 6–11).

Description: Colonies are globular (40–50 µm in diameter). Cells are pyriform or obovate (22–28 × 8–12 µm), entirely covered with ovate or ellipsoidal scales. Body scales are 2.2–3.9 × 1.6–2.4 µm in dimension with a posterior thickened rim encircling half to two-thirds of the scale perimeter. There are two different-sized base plate pores on the scale except along the peripheral margin. Larger and rimmed pores with a diameter of 100–135 nm are situated at the posterior part of scale. Smaller pores with a diameter of 60–75 nm are found in the anterior part of scale. Hexagonal secondary meshwork exists in the distal one-third to half of the scale. Each mesh encloses a small pore and struts extend from the meshwork near to anterior edge of the scale. Spine is 0.9–1.6 × 0.3–0.5 µm in dimension, with distal end tapered to an acute point or bearing two to three small teeth on the tip. Caudal scales are spineless, small, slipper-like, 2.0–2.4 × 0.3–0.6 µm. Tubular scales are linear or slightly bent, 5.1–6.3 × 0.2–0.4 µm.

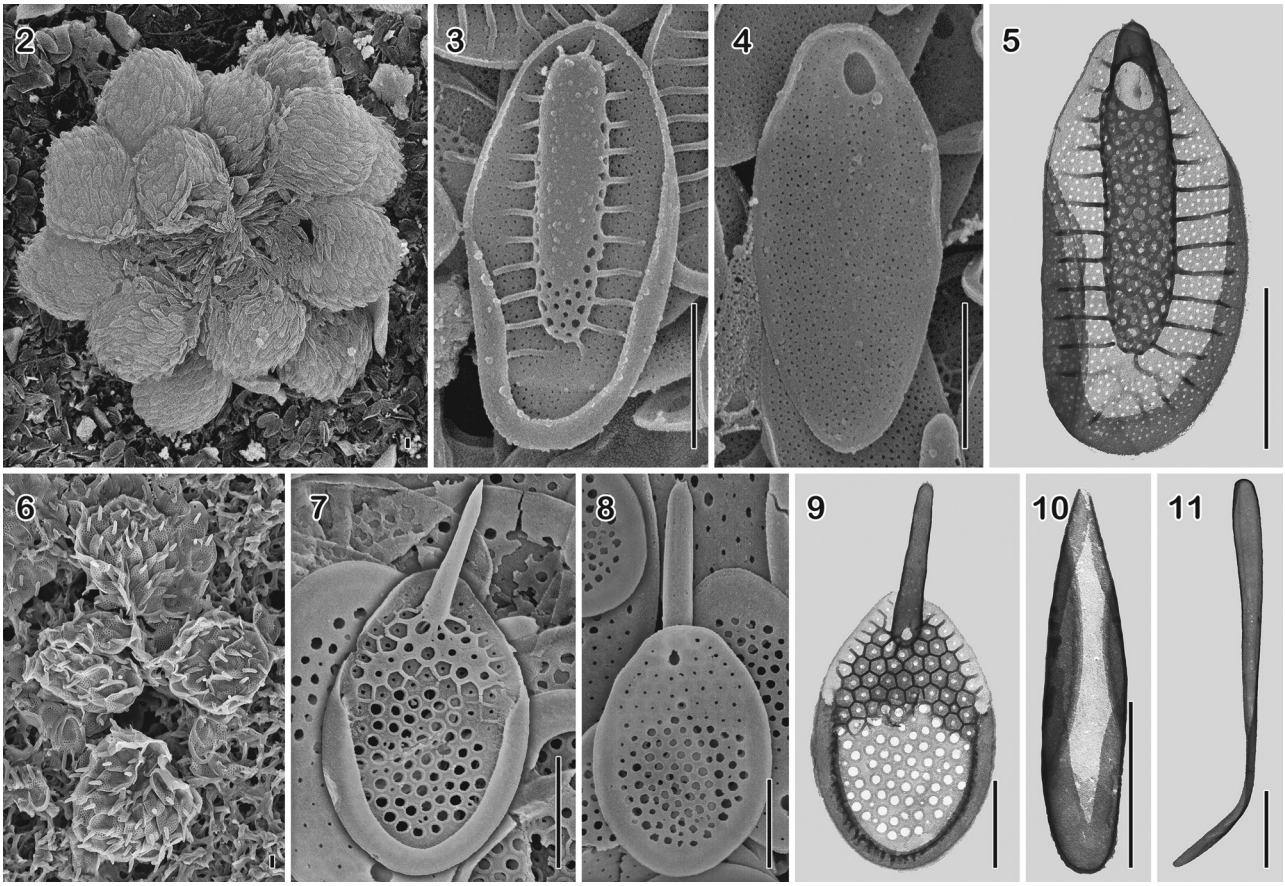
Holotype Specimen: Portion of a single gathering of cells on SEM stub CNU056359, deposited at the Herbarium of Chungnam National University,

Table 1. Summary of the major characteristic features that distinguish taxa of the section *Peterseniae*.

Taxon	Cell size (μm)	Scale size (μm)	Keel size (μm)	Base hole diameter (μm)	Base-plate pore diameter (nm)	Keel pore diameter (nm)	Number of struts	Distance of struts (μm)	Interconnection of struts by transverse ribs
[a]*S. americana* Kynčlová & Škaloud	22–28 × 8–12	3.0–4.2 × 1.7–2.3	1.8–2.3 × 0.5–0.7	0.17–0.34	27–63	54–94	21–24	0.27–0.30	very rare
[a]*S. borealis* Škaloud & Škaloudová	31–42 × 7–12	4.0–5.8 × 1.6–2.6	3.1–3.6 × 0.9–1.2	0.27–0.55	17–26	54–88	28–38	0.27–0.33	Frequent
[a]*S. conopea* Kynčlová & Škaloud	20–28 × 8–12	3.3–4.1 × 1.4–1.9	1.6–2.8 × 0.5–0.6	0.19–0.44	25–51	70–125	24–30	0.23–0.26	rare
[a]*S. glabra* Korshikov emend. Kynčlová & Škaloud	19–28 × 10–14	2.4–3.4 × 1.5–2.4	1.3–1.7 × 0.4–0.5	0.14–0.33	29–41	44–100	17–22	0.25–0.29	none
[a]*S. heteropora* Škaloud, Škaloudová & Procházková	20–25 × 7–11	2.5–3.8 × 1.1–1.9	2.0–2.8 × 0.5–0.7	0.19–0.42	20–31	49–100	22–28	0.18–0.25	frequent
[a]*S. hibernica* Škaloud & Škaloudová	26–47 × 6–12	3.4–5.6 × 1.2–2.0	3.4–4.1 × 0.7–0.9	0.14–0.38	18–27	49–89	30–47	0.20–0.25	frequent
***S. lanceolata* Jo, Kim, Siver & Shin sp. nov.**	**16–20 × 10–13**	**2.7–3.9 × 1.2–1.7**	**1.6–2.7 × 0.4–0.7**	**0.24–0.47**	**20–36**	**54–82**	**26–35**	**0.15–0.34**	**none**
[a]*S. laticarina* Škaloud & Škaloudová	21–32 × 7–13	3.1–4.3 × 1.6–2.1	2.5–2.9 × 0.7–1.0	0.20–0.33	19–25	52–76	24–32	0.23–0.26	frequent
[a]*S. macropora* Škaloud & Kynčlová	17–25 × 8–12	2.2–3.4 × 1.5–2.2	1.6–1.8 × 0.4–0.6	0.16–0.39	50–78	69–137	15–22	0.27–0.36	none
[a]*S. petersenii* Korshikov emend. Škaloud & Kynčlová	20–31 × 8–12	3.6–4.6 × 1.8–2.3	1.7–2.5 × 0.4–0.6	0.24–0.46	18–30	45–82	26–34	0.24–0.28	frequent
***S. soroconopea* Jo, Kim, Siver & Shin sp. nov.**	**18–28 × 10–15**	**2.0–2.9 × 1.0–1.5**	**1.3–2.3 × 0.5–0.9**	**0.20–0.53**	**26–34**	**65–104**	**21–26**	**0.22–0.34**	**rare**
***S. sungminbooi* Jo, Kim, Siver & Shin sp. nov.**	**20–31 × 10–15**	**2.5–3.4 × 1.5–1.8**	**1.3–2.1 × 0.3–0.8**	**0.20–0.38**	**15–24**	**41–120**	**28–32**	**0.19–0.41**	**rare**
[a]*S. truttae* (Siver) Škaloud & Kynčlová	22–31 × 11–13	3.3–3.8 × 1.5–1.8	1.3–2.0 × 0.4–0.5	0.27–0.56	18–25	47–70	27–33	0.19–0.24	frequent

Table 2. Summary of the major characteristic features observable with EM used in this study to distinguish between taxa of the section *Curtispinae*.

Taxon	Cell size (μm)	Scale size (μm)	Spine size (μm)	Spine tip	Secondary structure, diameter of hexagonal meshwork (nm)	Base plate pore diameter (nm)	Posterior rim	Length of tubular scale (μm)	Other
S. curtispina (Petersen & Hansen) Asmund	20–25 × 8–12	3.0–4.3 × 1.3–3.1	1.0–1.4 × 0.3–0.4	blunt or with 2–3 apical teeth	distal part of scale covered with hexagonal meshwork, 163–200	85–114	upturned rim	4.1–6.1 × 0.2–0.3 (n = 18)	each hexagonal meshwork including a small pore
S. echinulata Korshikov	11–20 × 8–10	2.5–3.0 × 1.6–2.3	up to 1.2	pointed spine	short and radiating struts	58–76	upturned rim	3.8–7.2 × 0.2–0.3[a]	distal part of scale with a sinuous pattern
S. korshikovii D. Kapustin & E. S. Gusev	–	4.0–4.9 × 2.4–3.1	1.5–2.0 × 0.6–0.8	flat with 2–3 rows of rounded teeth	whole scale covered with hexagonal meshwork, 197–242	60–106	upturned rim encircling 2/3 of scale perimeter	–	each hexagonal meshwork including a small pore
***S. longitubularis* Jo, Kim, Siver & Shin sp. nov.**	**22–28 × 8–12**	**2.2–3.9 × 1.6–2.4**	**0.9–1.6 × 0.3–0.5**	**blunt or with 2–3 apical teeth**	**distal part of scale covered with hexagonal meshwork, 150–240**	**80–135**	**upturned rim**	**5.1–6.9 × 0.2–0.3 (n = 13)**	**each hexagonal meshwork including a small pore**
S. mammillosa Takahashi	10–14 × 8–10	2.1–3.6 × 1.8–2.5	1.0–1.6 × 0.2–0.4	pointed spine	short and radiating struts	50–70	upturned rim	2.2–2.7 x0.2–0.3 (n = 7)	distal part of scale with a sinuous pattern
S. morusimila W. Pang & Q. Wang	23–25 × 17–18	2.8–3.6 × 2.1–2.6	1.1–1.8	round with 10–14 teeth	short and radiating struts	43–60	upturned rim	–	stout, cylindrical spine with ribs at its base
S. sphagnicola (Korshikov) Korshikov	11–15 × 8–10	2.3–2.8 × 1.5–2.2	up to 1.6	pointed spine	no further ornamentation	60–75	uninterrupted upturned rim	5.6 × 0.2 (n=1)	–
S. spinosa Korshikov	19–27 × 8–12	2.4–5.2 × 2.0–3.8	0.8–2.1 × 0.3–0.4	with two small teeth	distal part of scale covered with hexagonal meshwork, 115–173	regularly arranged large and small pores, between 28–92	upturned rim	5.2–9.7 × 0.2–0.3 (n = 20)	individual hexagonal meshwork does not contain a pore



Figs 2–11. Colony and scale morphology of *Synura lanceolata* Nojeok020610A (Figs 2–5) and *Synura longitubularis* Sinsan101709B (Figs 6–11). **Fig. 2**. SEM image of colony forming cells. **Fig. 3**. SEM image showing top surface of body scale with a median keel, transverse strut and posterior rim. **Fig. 4**. SEM image showing bottom surface of body scale with a base plate hole and numerous base plate pores. **Fig. 5**. TEM image of body scale. **Fig. 6**. SEM image showing the colony of *Synura longitubularis* Sinsan101709B. **Fig. 7**. SEM image showing top surface of body scale with secondary layer near the spine with acute end. **Fig. 8**. SEM image showing bottom surface of body scale with smaller pores and larger pores. **Fig. 9**. TEM image of body scale showing hexagonal secondary meshwork. **Fig. 10**. TEM image of caudal scale. **Fig. 11**. TEM image of apical tubular scale. All scale bars = 1 μm.

Daejeon, Korea (CNUK). Fig. 9 is a representative scale from the specimen.

Representative DNA sequences: GenBank accession no. KP268739.

Isotype Specimen: Portion of a single gathering of cells on SEM stub CNU056364, deposited at the Herbarium of Chungnam National University, Daejeon, Korea (CNUK).

Strain Information: The live culture of the holotype, deposited as Sinsan101709B at the Culture Collection of Algae of Chungnam National University, Daejeon, Korea.

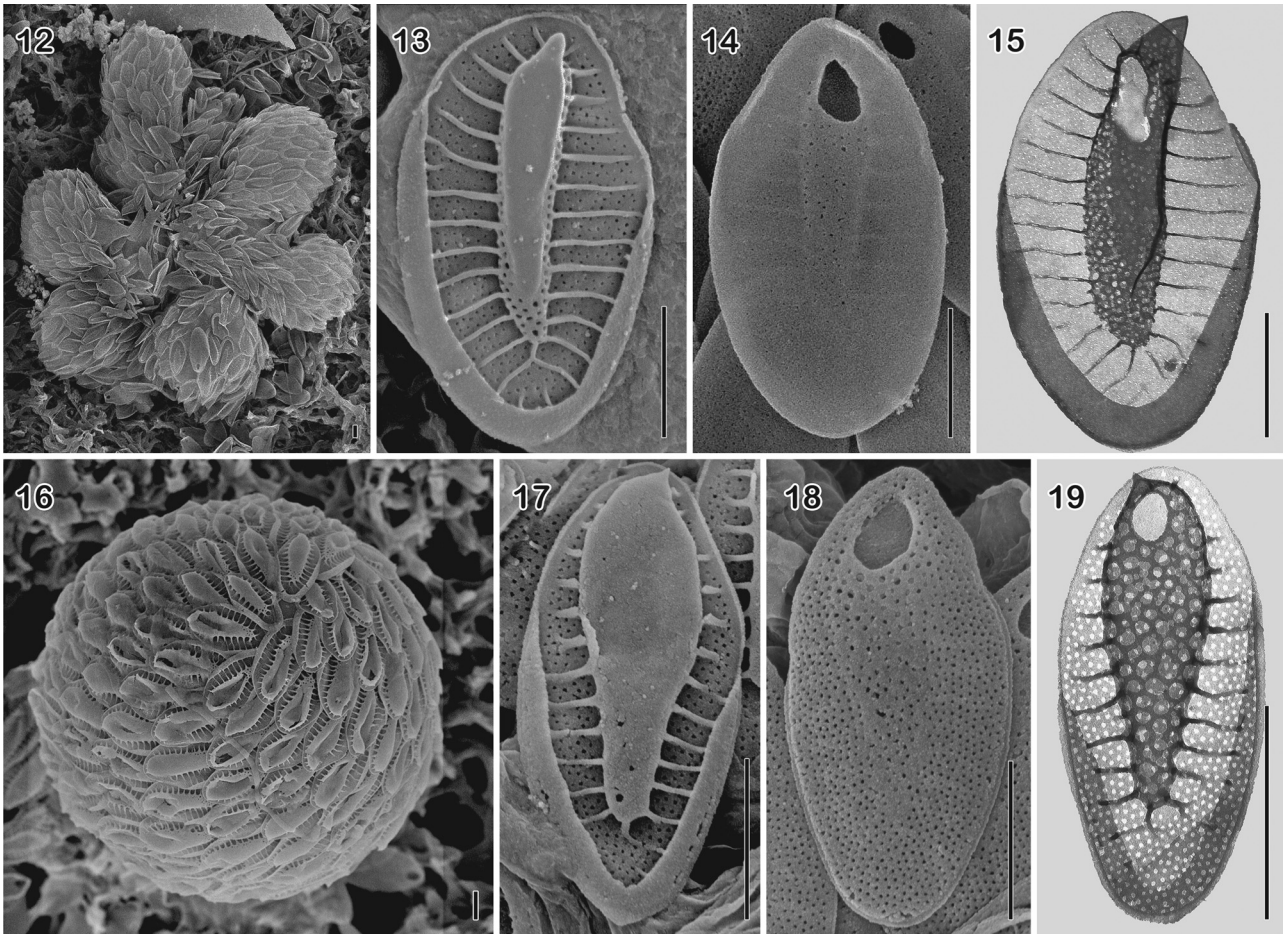
Type locality: Sinsan, Dain-myeon, Uiseong-gun, Gyeongsangbuk-do, Korea (36°25'24"N, 128° 22'52"E).

Etymology: The specific epithet '*longitubularis*' refers to longer tubular scale than that of a closely related species, *Synura curtispina*.

Synura sungminbooi B.Y. Jo, W. Shin, J.I. Kim & P. Siver sp. nov. (Figs 12–15).

Description: Colonies are globular (30–55 μm in diameter). Cells are pyriform or obovate (20–31 × 10–15 μm), entirely covered with ellipsoidal or obovate scales. Body scales are 2.5–3.4 × 1.5–1.8 μm in dimension, with a posterior rim encircling half to two-thirds of the scale perimeter and with small, evenly spaced and finely perforating base plate pores (11–20 nm in diameter). An oval or irregular-shaped base plate hole is 0.32–0.49 μm at the longest axis, on the anterior end of the scale. Keel is 1.3–2.1 × 0.3–0.8 μm in size, marked through the vertical scale axis and sub-ulate or oblong in shape, with an acute tip. Meshworks along the base of the keel vary from 41 to 120 nm in diameter. Transverse struts are 28–32 in number, extending from the keel near to the edge of the scale perimeter. They are usually not interconnected by transverse ribs and distance between struts ranges from 0.19–0.41 μm.

Holotype Specimen: Portion of a single gathering of cells on SEM stub CNU056356, deposited at the Herbarium of Chungnam National University,



Figs 12–19. Colony and scale morphology of *Synura sungminbooi* Geumgok020610A (Figs 12–15) and *Synura soroconopea* Jakeun030207B (Figs 16–19). **Fig. 12**. SEM image showing the colony of *Synura sungminbooi* Geumgok020610A. **Fig. 13**. SEM image showing top surface of body scale with keel, transverse strut and posterior rim. **Fig. 14**. SEM image showing bottom surface of body scale with a big base plate hole and small pores beneath the keel. **Fig. 15**. TEM image of body scale. **Fig. 16**. SEM image showing whole cell of *Synura soroconopea* Jakeun030207B. **Fig. 17**. SEM image showing top surface of body scale with a wide median keel with acute tip and simple strut. **Fig. 18**. SEM image showing bottom surface of body scale with one big hole and a lot of small pores on the plate. **Fig. 19**. TEM image of body scale. All scale bars = 1 μm.

Daejeon, Korea (CNUK). Fig. 15 is a representative scale from the specimen.

Representative DNA sequences: GenBank accession no. KP268698.

Isotype Specimen: Portion of a single gathering of cells on SEM stub CNU056361, deposited at the Herbarium of Chungnam National University, Daejeon, Korea (CNUK).

Strain Information: The live culture of the holotype, deposited as Geumgok020610A at the Culture Collection of Algae of Chungnam National University, Daejeon, Korea.

Type locality: Geumgok, Duyeo-ri, Yeosan-myeon, Iksan-si, Jeollabuk-do, Korea (36°04'10"N, 127° 05'02"E).

Etymology: The species is named after Prof. Sung Min Boo, who has greatly contributed to phycological taxonomy in Korea.

Synura soroconopea B.Y. Jo, W. Shin, J.I. Kim & P. Siver sp. nov. (Figs 16–19).

Description: Colonies are globular (22–48 μm). Cells are pyriform or obovate 18–28 × 10–15 μm, entirely covered with ellipsoidal or obovate scales. Body scales are 2.0–2.9 × 1.0–1.5 μm in dimension, with a posterior rim encircled half to two-thirds of the scale perimeter and with small sized, evenly spaced basal plate pores (26–34 nm in diameter). A round to oblong-shaped base hole is 0.20–0.53 μm in diameter, marked to the anterior end of the scale. Keel is 1.3–2.3 × 0.5–0.9 μm, marked through vertical axis of the scale and obolanceolate or obovate in shape, with an acute tip. Large, evenly spaced keel meshworks are 65–104 nm in diameter at keel base atop the base plate. Transverse struts are 21–26 in number, extending from the keel near to the edge of the scale and lacking interconnection between transverse struts, and spacing between struts, ranges from 0.22–0.34 μm.

Holotype Specimen: Portion of a single gathering of cells on SEM stub CNU056355, deposited at the Herbarium of Chungnam National University, Daejeon, Korea (CNUK). Fig. 19 is a representative scale from the specimen.

Representative DNA sequences: GenBank accession no. KP268686.

Isotype Specimen: Portion of a single gathering of cells on SEM stub CNU056360, deposited at the Herbarium of Chungnam National University, Daejeon, Korea (CNUK).

Strain Information: The live culture of the holotype, deposited as CNU01 (Okgeum121810C) at the Culture Collection of Algae of Chungnam National University, Daejeon, Korea.

Type locality: Okgeum pond, Jaenam-ri, Yeosan-myeon, Iksan-si, Jeollabuk-do, Korea (36°24'73"N, 128°25'13"E).

Etymology: The specific epithet '*soroconopea*' is derived from the species *S. conopea* and Latin soro- (=a sister of).

Morphological analyses

Three newly described species belonging to the section *Peterseniae* (*S. lanceolata, S. soroconopea, S. sungminbooi*) are evolutionarily young (Fig. 1) and morphologically highly similar. To differentiate them from each other and closely related taxa, we morphologically characterized them by measuring several traits associated with the silica scale structure (Figs 20–23). Obvious morphological differences were observed in several traits. All three strains produced relatively small silica scales (Fig. 20). The scales of *S. soroconopea* and *S. lanceolata* were the narrowest, about 1.24 ± 0.12 µm and 1.42 ± 0.14 µm in width, respectively. In addition, *S. soroconopea* could be well recognized by having the shortest scales among all the species analysed, measuring 2.51 ± 0.20 µm in length. *Synura sungminbooi* was distinct by having exceptionally small base plate pores (15.8 ± 1.6 nm in diameter) and the largest ratio of keel pore to base plate pore diameter (Figs 21, 22). Finally, the newly described species significantly differed by the scale width/keel width ratio (Fig. 23). Whereas the scales of *S. sungminbooi* had a very narrow keel compared with the basal plate (width ratio 3.1 ± 0.5), *S. soroconopea* is well characterized by an anteriorly widened keel (width ratio 1.9 ± 0.2).

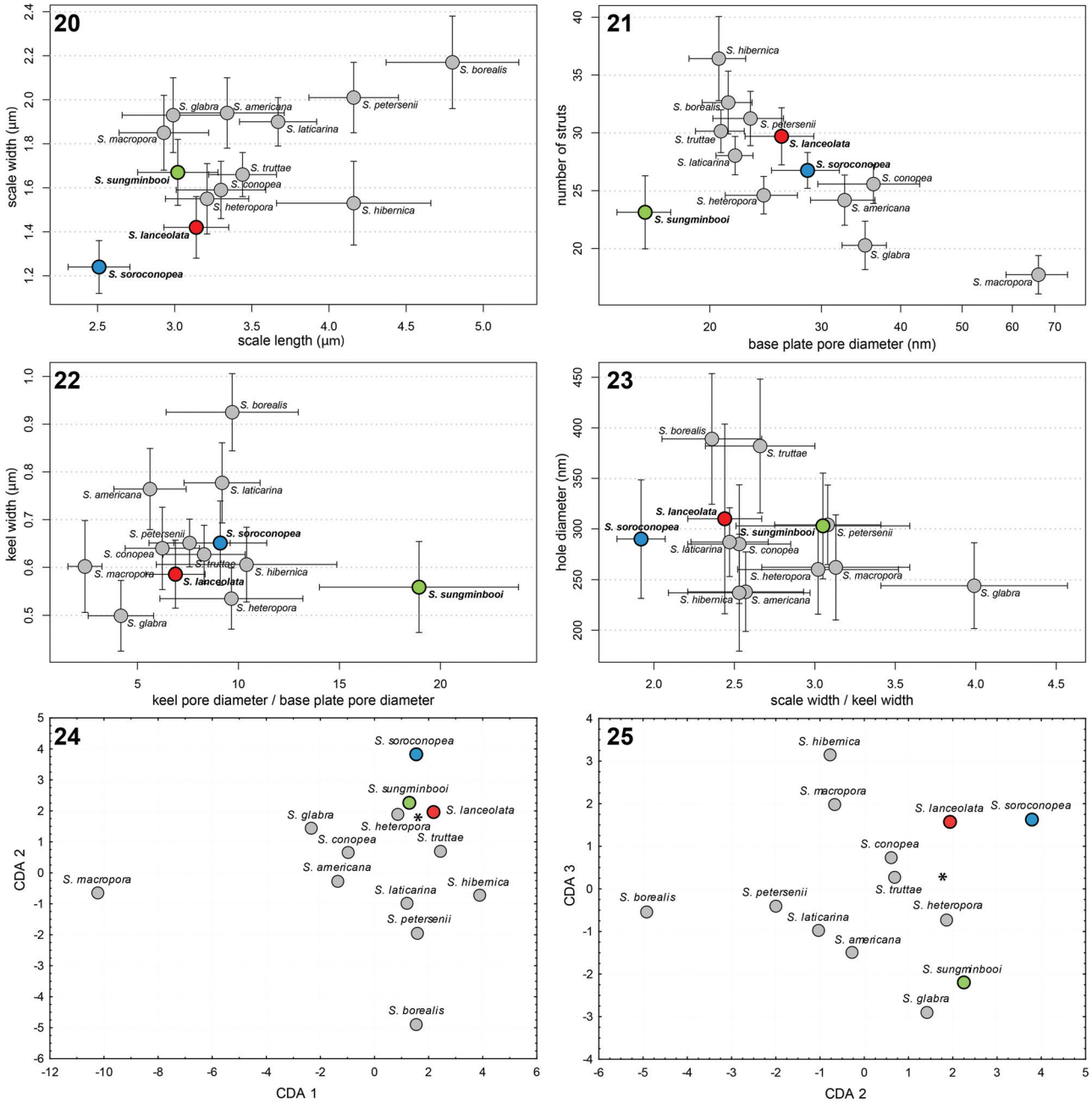
The canonical discriminant analysis (CDA) yielded well-defined groupings based on their morphological data. The analysis indicated strongly significant differentiation among the 13 species (Wilk's $\lambda = 0.0008$; $P < 0.00001$). The forward stepwise analysis indicated that all tested morphological characters were significant for species recognition ($P < 0.00001$), and selected the base-plate pore diameter as the best discriminating morphological trait (Partial Wilk's $\lambda = 0.18$). The average correct discrimination of individual scales on the basis of their morphology reached 90%. The correct discrimination level recovered for *S. lanceolata, S. soroconopea* and *S. sungminbooi* was 85%, 95% and 73%, respectively. Specifically, three *S. lanceolata* scales were incorrectly classified as *S. truttae* (2) or *S. heteropora* (1), a single *S. soroconopea* scale was classified as *S. lanceolata*, and four *S. sungminbooi* scales were incorrectly classified as *S. truttae* (2) or *S. heteropora* (2). Overall morphological similarity of *S. lanceolata, S. sungminbooi, S. truttae* and *S. heteropora* based on the suite of morphometric characters is also evident by their close affinity in CDA plots showing the species correlation with canonical axes (Figs 24, 25). Indeed, the smallest squared Mahalanobis distances were observed between *S. sungminbooi – S. heteropora*, and *S. lanceolata – S. truttae* species pairs (3.98 and 5.37, respectively). The first CDA axis was principally correlated with the base-plate pore diameter (correlation coefficient –0.85), whereas the second and third axes were correlated with the scale length and number of struts, respectively (correlation coefficients –0.81 and 0.58).

DISCUSSION

Phylogenetic relationships between families

Our phylogenetic analyses, combining sequence data for five genes from 115 *Synura* strains and two *Tessellaria* strains, confirmed that *Synura lapponica* is a member of *Tessellaria*, and that the genus *Synura* is divided into three major clades. Section *Peterseniae* forms a monophyletic clade, however taxa belonging to section *Synura* as currently described are split into two groups, one containing *S. uvella* and one containing the remaining species of the section. As reported by Škaloud *et al*. (2013), *Tessellaria* (*Synura*) *lapponica* groups with *T. volvocina*, supporting its removal from the family Synuraceae. Other characteristics support this finding. *Tessellaria* species have radially symmetrical scales with a rim that surrounds the entire scale (Goldstein *et al*., 2005). In addition, *Tessellaria* differs from *Synura* in terms of disposition of the scales around the colony and not around individual cells, the manner in which the colony undergoes asexual fission without mother cell elongation and the formation of compact colonies within a multilayered thick wall (Goldstein *et al*., 2005). Therefore, both genera are clearly separated based on molecular and morphological characters.

Playfair (1921) first recognized that a new family was required for the genus *Tessellaria* and created the



Figs 20–25. Morphometric analysis of siliceous scales. Newly described species *S. lanceolata, S. soroconopea* and *S. sungminbooi* are highlighted. **Figs 20–23**. Comparison of eight morphological traits measured in 13 species belonging to the section *Peterseniae*; average values and standard deviations are given. **Fig. 20**. Scatterplot of scale length versus scale width. **Fig. 21**. Scatterplot of base plate pore diameter versus number of struts. **Fig. 22**. Scatterplot of keel pore to base-plate pore diameter ration versus keel width. **Fig. 23**. Scatterplot of scale width to keel width ratio versus base hole diameter. **Figs 24–25**. Canonical discriminant analysis (CDA) of the entire dataset of measured morphological traits, showing the centroids of species displayed in the first and second (**Fig. 24**) and second and third (**Fig. 25**) CDA coordinate system. Asterisk in Figs 24–25 indicate the position of an iconotype of *S. petersenii* f. *praefracta* (Asmund, 1968).

family Tessellariaceae, lacking a description. However, algal taxonomists overlooked this family (Starmach, 1985; Kristiansen, 1986, 2005; Preisig, 1995) and classified *Tessellaria* as a genus in the family Synuraceae (Kristiansen & Preisig, 2007). In addition, the name *Tessellaria* has been found to be invalid. Given our findings, we propose the following three families within the order Synurales Andersen 1987.

Family I. Mallomonadaceae Diesing 1866.

Type genus: *Mallomonas* Perty 1852.

Family II. Synuraceae Lemmermann 1899 emended B.Y. Jo, J.I. Kim, W. Shin, P. Škaloud & P. Siver.

Emended diagnosis: Spherical or elongate colonies. Cells associated by their posterior ends to free-swimming colonies. Each cell covered by two to four discernible types of the silica scales; apical tubular scales, body scales, transition scales and caudal scales;

two flagella being equal or unequal in length; chloroplasts positioned at the periphery of the cell.

Type genus: *Synura* Ehrenberg 1834.

Family III. Neotessellaceae B.Y. Jo, J.I. Kim, W. Shin, P. Škaloud & P. Siver fam. nov.

Diagnosis: Spherical colony covered by a silica scale case; scales with rims that encircle the perimeter; scales with or without protuberance. Cells united by their tails (or stalks) to free-swimming colonies. Each cell naked; two flagella being equal or unequal in length; chloroplasts positioned at the periphery of the cell.

Type genus: *Neotessella* B.Y. Jo, J.I. Kim, W. Shin, P. Škaloud & P. Siver nom. nov.

Basionym: *Tessella* Playfair 1915 (*Proc. Linn. Soc. N. S.W.*, 40:315–316, Pl. xlv, Figs 6–7).

Type species: *Neotessella volvocina* (Playfair 1915) B. Y. Jo, J.I. Kim, W. Shin, P. Škaloud & P. Siver comb. nov.

Basionym: *Tessella volvocina* Playfair 1915 (*Proc. Linn. Soc. N.S.W.*, 40:315–316, Pl. xlv, Figs 6–7).

Synomym: *Tessellaria volvocina* Playfair 1918 (*Proc. Linn. Soc. N.S.W.*, 43:508–509, Pl. lvi, Fig. 4).

Type: Playfair 1915, Pl. xlv, Figs 6–7 (iconotype).

Type Locality: Lagoons at Pott's Hill near Auburn, Australia.

Included species: *Neotessella lapponica* (Skuja 1956) B.Y. Jo, J.I. Kim, W. Shin, P. Škaloud & P. Siver comb. nov.

Basionym: *Synura lapponica* Skuja 1956 (*Nov. Act. Reg. Soc. Sci. Upsal*. IV, 16: 275–276).

Synonym: *Tessellaria lapponica* (Skuja) Škaloud, Kristiansen & Škaloudová 2013 (*Nord. J. Bot.*, 31: 400).

Type: Skuja 1956, pp. 275–276, Pl. 47, Figs 10–14, Pl. 48, Figs 1–2p (iconotype).

Type Locality: Swampy ponds and lakes around Abisko, Lappland, Sweden.

Remarks: Playfair (1915) erected the genus *Tessella*, based on *Tessella volvocina*. The genus name was illegitimate due to homonymy with a diatom genus *Tessella* (Ehrenberg 1838). Playfair (1918) renamed the genus, *Tessellaria*. However, the name *Tessellaria* is an illegitimate homonym of a fossil cycadophyte genus *Tessellaria* (*Tessellaria* (Schimper & Mougeot) C.E.I. von Eichwald; see Eichwald 1860). Therefore, since the original genus *Tessella* was validly published, but with an illegitimate name, we propose the genus *Neotessella* nomen novum as the new replacement name.

Included species: *Neotessella volvocina* (Playfair 1915) B.Y. Jo, J.I. Kim, W. Shin, P. Škaloud & P. Siver comb. nov.; *Neotessella lapponica* (Skuja 1956) B.Y. Jo, J.I. Kim, W. Shin, P. Škaloud & P. Siver comb. nov.

Phylogenetic relationships within *Synura*

Classification within the genus *Synura* is based mainly on ultrastructure of body scales and historically taxa have been divided into two or three infrageneric ranks (Petersen & Hansen, 1956; Balonov & Kuzmin, 1974; Péterfi & Momeu, 1977). A key diagnostic character is the presence of a keel or spine on the base plate of the scale. Péterfi & Momeu (1977), following the infrageneric classification system of Balonov & Kuzmin (1974), recognized three sections: *Lapponica, Peterseniae* and *Synura*, the latter section further divided into two series: *Splendidae* and *Synura*. They included *S. splendida* and *S. sphagnicola* in the series *Splendidae*, and the remaining taxa in the series *Synura*. Further, Péterfi & Momeu (1977) noted that species in series *Splendidae* have scales with a long spine and lack additional secondary structures on the base plate. Our results, which indicate that *S. sphagnicola* is more closely related to strains of *S. longitubularis* and *S. curtispina* than to *S. splendida*, do not support this infrageneric concept. More recently, Škaloud *et al*. (2013) proposed five sections corresponding to monophyletic units on their phylogenetic tree based on nuclear SSU rRNA and plastid *rbc*L gene sequences (*Peterseniae, Spinosae, Echinulatae, Splendidae* and *Uvellae*). Although the monophyletic units are the same as in our study, this system needs to be reevaluated to determine which taxonomic level is more reasonable and acceptable for infrageneric rankings. In addition, the section *Uvellae* cannot be accepted according to Article 22 of International Code of Nomenclature for algae, fungi and plants (McNeill *et al*., 2012; see http://www.iapt-taxon.org/nomen/main.php) because its epithet has not repeated the generic name even though the section includes the type of the adopted and legitimate name of the genus. Therefore, the section *Synura* should be established as the corresponding autonym and include the type species, *S. uvella.*

Taxonomic revision of subgeneric ranks for *Synura*

With the addition of four new species there are now 49 accepted species and subspecific taxa for *Synura* (Table 3). Based on our findings, we propose that the 49 taxa be divided into the following three sections: *Peterseniae, Synura* and *Curtispinae.*

Section I. *Peterseniae* Petersen & Hansen ex Balonov & Kuzmin 1974, *Bot. Jurn. Leningrad*, 59, 11, p. 1682.

Table 3. Check-list of all previously described species of the genus *Synura*. New species are given in bold.

Section	Taxon	Taxonomic status
Lapponica	*Synura lapponica* Skuja 1956	Basionym (= *Tessellaria lapponica* (Skuja) Škaloud, Kristiansen & Škaloudova; Škaloud *et al*. 2013)
Peterseniae	*S. adamsii* (Smith 1924) Nygaard 1949	Valid
	S. adamsii f. *malabarica* Philipose 1953	Valid*
	S. americana Kynčlová & Škaloud 2012	Valid (Škaloud *et al*. 2012)
	S. australiensis Playfair 1915 emend. Croome & Tyler 1985	Valid
	S. borealis Škaloud & Škaloudová 2014	Valid (Škaloud *et al*. 2014)
	S. caroliniana Whitford 1943	Valid*
	S. conopea Kynčlová & Škaloud 2012	Valid (Škaloud *et al*. 2012)
	S. elipidosa Skvortsov 1961	Valid*
	S. glabra Korshikov 1929 emend. Kynčlová & Škaloud 2012	Valid (Škaloud *et al*. 2012)
	S. heteropora Škaloud, Škaloudová & Procházková 2014	Valid (Škaloud *et al*. 2014)
	S. hibernica Škaloud & Škaloudová 2014	Valid (Škaloud *et al*. 2014)
	S. intermedia Bioret ex Kufferath in Conrad 1946	Valid*
	***S. lanceolata* B.Y. Jo, W. Shin, J.I. Kim & P. Siver**	**Newly described in this study**
	S. laticarina Škaloud & Škaloudová 2014	Valid (Škaloud *et al*. 2014)
	S. longisquama Wujek & Elsner 2000	Valid
	S. macracantha (Petersen & Hansen 1958) Asmund 1968	Valid
	S. macropora Škaloud & Kynčlová 2012	Valid (Škaloud *et al*. 2012)
	S. petersenii Korshikov 1929 emend. Škaloud & Kynčlová 2012	Valid (Škaloud *et al*. 2012)
	S. petersenii f. *asmundiae* Cronberg & Kristiansen 1980	Basionym (=*S. asmundiae* (Cronberg & Kristiansen) Škaloud, Kristiansen & Škaloudova; Škaloud *et al*. 2013)
	S. petersenii f. *bjoerkii* Cronberg & Kristiansen 1980	Basionym (= *S. bjoerkii* (Cronberg & Kristiansen) Škaloud, Kristiansen & Škaloudova; Škaloud *et al*. 2013)
	S. petersenii f. *bonaerensis* Vigna 1979	Synonym (=*S. petersenii*; Škaloud *et al*. 2012)
	S. petersenii f. *columnata* Siver 1988	Valid
	S. petersenii f. *glabra* (Korshikov 1929) Kristiansen & Preisig 2007	Synonym (=*S. glabra* Korshikov 1929 emend. Kynčlová & Škaloud; Škaloud *et al*. 2012)
	S. petersenii f. *kufferathii* Petersen & Hansen 1958	Synonym (=*S. petersenii* Korshikov 1929 emend. Škaloud & Kynčlová; Škaloud *et al*. 2012)
	S. petersenii f. *macracantha* Petersen & Hansen 1958	Basionym (=*S. macracantha* (Petersen & Hansen) Asmund; Asmund 1968)
	S. petersenii f. *praefracta* Asmund 1968	Valid
	S. petersenii f. *taymyrensis* Kristiansen 1995	Valid
	S. petersenii f. *truttae* Siver 1987	Synonym (=*S. truttae* (Siver 1987) Škaloud & Kynčlová; Škaloud *et al*. 2012)
	S. petersenii var. *glabra* (Korshikov 1929) Huber-Pestalozzi 1941	Synonym (=*S. glabra* Korshikov 1929 emend. Kynčlová & Škaloud; Škaloud *et al*. 2012)
	***S. soroconopea* B.Y. Jo, W. Shin, J.I. Kim & P. Siver**	**Newly described in this study**
	***S. sungminbooi* B.Y. Jo, W. Shin, J.I. Kim & P. Siver**	**Newly described in this study**
	S. truttae (Siver 1987) Škaloud & Kynčlová 2012	Valid (Škaloud *et al*. 2012)
	S. virescens (Bory de Saint Vincent 1824) Playfair 1921	Valid*
Curtispinae	*S. bioretii* Huber-Pestalozzi 1941	Valid (probably = *S. sphagnicola* or *S. curtispina*: Nicholls & Gerrath 1985)
	S. biseriata Balonov 1976	Valid*
	S. cronbergiae Siver 2013	Valid (fossil species)
	S. conradii Kufferath in Conrad 1946	Synonym (=*S. echinulata* Korshikov 1929; Petersen & Hansen 1958)
	S. curtispina (Petersen & Hansen 1956) Asmund 1968	Valid
	S. curtispina f. *reticulata* Asmund 1968	Synonym (=*S. curtispina*; Kristiansen & Lind 1995)
	S. danubiensis (Schiller 1929) Starmach 1968	Invalid (=*Synuropsis danubiensis*; Wujek & Thompson 2001)
	S. echinulata Korshikov 1929	Valid*
	S. echinulata f. *leptorrhabda* Asmund 1968	Synonym (=*S. leptorrhabda*; Nicholls in Nicholls & Gerrath 1985)
	S. falcata Skvortsov 1961	Valid*
	S. favus Bradley 1964	Synonym (=*S. curtispina*; Kristiansen & Lind 1995)
	S. globosa (Schiller 1929) Starmach 1968	Valid
	S. granulosa Playfair 1915	Valid*
	S. granulosa var. *pusilla* Playfair 1915	Valid*
	S. korshikovii Kapustin & Gusev 2015	Valid

(*continued*)

Table 3. Continued.

Section	Taxon	Taxonomic status
	S. leptorrhabda (Asmund 1968) Nicholls in Nicholls & Gerrath 1985	Valid
	S. lohammarii Skuja 1956	Synonym (=*S. splendida*; Péterfi 1967)
	***S. longitubularis* B.Y. Jo, W. Shin, J.I. Kim & P. Siver**	**Newly described in this study**
	S. mammillosa Takahashi 1972	Valid
	S. mollispina (Petersen & Hansen 1956) Péterfi & Momeu 1977	Valid
	S. multidentata (Balonov & Kuzmin 1974) Péterfi & Momeu 1977	Valid
	S. nygaardii (Petersen & Hansen 1956) Kristiansen *et al*. 1997	Valid
	S. punctulosa Balonov 1976	Valid*
	S. reticulata Lemmermann 1904	Synonym (=*S. uvella*; Fott & Ludvík 1957)
	S. reticulata var. *minor* Chodat, Raineri & Drew 1926	Valid*
	S. reticulata var. *verrucosa* Pascher 1908	Synonym (=*S. uvella*; Fott & Ludvík 1957)
	S. sphagnicola (Korshikov 1927) Korshikov 1929	Valid*
	S. spinosa Korshikov 1929	Valid*
	S. spinosa f. *curtispina* Petersen & Hansen 1956	Synonym (=*S. curtispina*; Asmund 1968)
	S. spinosa f. *longispina* Petersen & Hansen 1956	Valid
	S. *spinosa* f. *mollispina* Petersen & Hansen 1956	Basionym (=*S. mollispina*; Péterfi & Momeu 1977)
	S. spinosa f. *nygaardii* Petersen & Hansen 1956	Synonym (=*S. nygaardii*; Kristiansen *et al*. 1997)
	S. spinosa var. *striata* Cronberg 1989	Valid
	S. splendida Korshikov 1942	Valid*
Synura	*S. morusimila* Pang & Wang 2013	Valid
	S. recurvata Siver & Wolfe 2005	Valid (fossil species)
	S. uvella Ehrenberg 1834 emend. Korshikov 1929	Valid
	S. uvella f. *turfaceae* Steinecke 1916	Combined (=*S. sphagnicola*; Fott & Ludvík 1957)
	S. uvella f. *typica* Pascher 1910	Synonym (=*S. uvella*; Korshikov 1929)
	S. uvella var. *longipes* Virieux 1916	Valid
	S. uvella var. *punctata* Averintsev 1901	Synonym (=*S. sphagnicola*; Fott & Ludvík 1957)
	S. uvella var. *reticulata* (Lemmermann 1904) Pascher 1910	Synonym (=*S. uvella*; Fott & Ludvík 1957)
	S. uvella var. *tiszaensis* Kiss 1978	Invalid
	S. verrucosa Pascher 1913	Synonym (=*S. uvella*; Fott & Ludvík 1957)
Uncertain taxa	*S. hyalina* Skvortsov 1958	Invalid (no species description in the paper and colonial cell without scales)
	S. hyalina var. *aculeata* Skvortsov 1958	Invalid (no species description in the paper and cell without scales)
	S. hyalina var. *rotundata* Skvortsov 1958	Invalid (no species description in the paper and cell without scales)
	S. klebsiana (O. Zacharias 1897) Lemmermann 1899	Invalid (=*Chrysosphaerella* sp.; Kristiansen & Preisig 2007)
	S. microcrepis Nygaard 1978	Synonym (=*Chrysodidymus synuroideus*; Kristiansen & Preisig 2007)
	S. rotundata Skvortsov 1958	Invalid (no species description in the paper, but colonial cells with scales)
	S. urogleniformis (Kisselev 1931) Starmach 1968	Invalid (=*Synuropsis danubiensis*; Wujek & Thompson 2001)
	S. uvella f. *turfosa* Steinecke 1922	Invalid (no description)

Type species: *Synura petersenii* Korshikov 1929, *Arch. Protistenkd*. 67, pp. 283, 284, 285, Pl 11, Figs 31–37.

Section II. *Synura.*

Emended Diagnosis: Body scales with or without a short, stout, conical-shaped spine bearing teeth on the distal end; larger pores present in the base plate, but lacking along the scale perimeter and under the portion of the scale with secondary ornamentation; a thick posterior rim encircling approximately two-thirds of the scale perimeter and supported by distinct struts; tubular and caudal (slipper) scales absent.

Type species: *Synura uvella* Ehrenb. emend. Korshikov 1929, *Arch. Protistenkd*. 67, pp. 279, 280, 281, Pl 11, Figs 31–37.

Remarks: Key morphological features include the heavy rim subtended by well-developed secondary struts; a stout, conical spine with 1–4 apical teeth and lack of tubular and caudal (slipper) scales. At present this section consists of at least three taxa: *Synura uvella, S. morusimila* and *S. recurvata*.

Section III. *Curtispinae* Jo, Kim, Shin, Škaloud & Siver sect. nov.

DIAGNOSIS: Body scales with a more or less slender spine bearing an acute tip or teeth at its end; smaller and larger pores present on the base plate and secondary meshwork; the posterior rim encircling approximately two-thirds to nearly the entire scale perimeter and lacking supporting struts; tubular and caudal scales may be present.

TYPE SPECIES: *Synura curtispina* (Petersen & Hansen, 1956) Asmund 1968, *Hydrobiologia* 31, pp. 506, 507, 508.

REMARKS: The section is a monophyletic lineage, consisting of all members of previous section *Synura* except for three taxa of the section *Synura* including one fossil species.

Comparison of the new species with closely related species

The three new species included in section *Peterseniae, Synura lanceolata, S. soroconopea* and *S. sungminbooi*, have been considered as genetic variants of *S. petersenii* with very low sequence divergences within a 9-gene DNA alignment (Boo *et al*., 2010). In our phylogeny *S. lanceolata* was closely related to *S. heteropora*, but was morphologically differentiated from this species by having smaller cell and scale size, more struts on the base plate, and lacking transverse ribs between the struts. *Synura petersenii* f. *praefracta* seems to be similar to *S. lanceolata* in terms of a bluntly pointed keel end with a few minute teeth. However, *S. petersenii* f. *praefracta* is characterized by having longer spine of the keel, presence of transverse ribs between struts, and broader scale. Moreover, the ordination analysis of a scale illustrated by Asmund (1968) along with the description of *S. petersenii* f. *praefracta* revealed its slight morphological differentiation from *S. lanceolata*, as well as other analysed *Synura* species (Figs 24, 25). Strains of *S. soroconopea* formed sister relationships with those of *S. conopea*, but the former species is characterized by a smaller scale size, broader keel, smaller keel meshwork diameter and fewer number of struts. Strains of *S. sungminbooi* have scales typical of the section *Peterseniae*, but they have the smallest base plate pores of all known species. In addition, the pores were irregular in shape under TEM. As a member of the section *Curtispinae, S. longitubularis* was closely related to *S. curtispina* in the phylogenetic tree, and morphological characters of both species do overlap. However, the new species was differentiated by slightly bigger hexagonal meshworks and longer tubular scales than those of *S. curtispina*. In addition, *S. longitubularis* differed from *S. spinosa* by means of larger diameter of hexagonal meshworks and wider base plate pores.

Cryptic diversity and taxonomic uncertainty within *Synura*

Although our results further clarify the phylogenetic relationships of species within *Synura*, there still remain areas of uncertainty. The topologies of trees obtained by both ML and Bayesian analyses were identical except for relationships among isolates of each species, but differed from those based on datasets containing nuclear SSU rDNA and plastid *rbc*L (Škaloud *et al*., 2013), or a concatenated ITS rDNA, *rbc*L and *cox*1 combination (Škaloud *et al*., 2014). For example, in clade B of our tree *Synura mollispina* was closely related to *S. spinosa*, but in a tree presented by Škaloud *et al*. (2013) the two species were not related. *Synura macropora* in the section *Peterseniae* did not separate as a monophyletic lineage from *S. americana* based on a dataset consisting of ITS, *psa*A, *rbc*L and *cox*1 (see Figure 1, Škaloud *et al*., 2012). In our ITS tree (see supplement tree) *S. macropora* clearly separated from strains of *S. americana* as a new lineage. Although *S. macropora* is differentiated from *S. americana* based on the sizes of the keel and base plate pores, our phylogenetic analyses support the inclusion of *S. macropora* in the *S. americana* clade. This discrepancy may be due to omitting fast-evolving genes (e.g. ITS, *cox*1) in our concatenated phylogenetic analyses (Fig. 1). Because these sequences are too variable to align for all *Synura* species, they cannot be used to understand phylogenetic relationships among all taxa of the genus *Synura*, but are more useful for understanding relationships between taxa of the section *Peterseniae*.

Recent molecular analyses of the *Synura petersenii* complex based on either nuclear ITS or multigene sequence data show that *S. petersenii* divides into many well-supported clades (Wee *et al*., 2001; Kynčlová *et al*., 2010; Boo *et al*., 2010), and confirms a high degree of cryptic diversity within this species complex. More recently, Škaloud *et al*. (2012, 2014) performed substantial taxonomic studies on cryptic diversity within *S. petersenii*, and recognized 10 cryptic lineages as separate species. Our results are in good agreement with the species concepts of Škaloud *et al*. (2012, 2014), and we additionally include three new species of this complex, based on combined molecular and morphological evidence. In addition, our multigene phylogeny suggests that the sections *Peterseniae* and *Curtispinae* may include more taxa that need to be designated taxonomically, suggesting even further cryptic diversity.

In summary, the phylogenetic tree based on multigene dataset showed that the colonial genera *Synura* and *Neotesella* do not share a sister relationship, but are paraphyletic, and support a separate family distinction for *Neotessella*. The genus *Synura* divided into three

major clades herein designated as *Peterseniae, Synura* and *Curtispinae. Synura uvella*, one of two modern species within section *Synura*, diverged at the base of the genus, followed by the evolution of the *Peterseniae* and *Curtispinae* clades. Except for *S. macropora*, our multigene data supported all other species as monophyletic lineages, and indicate further cryptic diversity exists within the genus. Lastly, we propose *Neotessella* to replace the invalid use of the name *Tessellaria*.

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DISCLOSURE STATEMENT

No potential conflict of interest was reported by the author(s).

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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/10.1080/09670262.2016.1201700

Supplementary table S1. Strains of the genus *Synura* used in this study and the GenBank accession numbers for their nr SSU, nr LSU rDNA, ITS, pt LSU rDNA, *psa*A and *rbc*L gene sequences.

Supplementary fig. S1. Unrooted Bayesian tree based on 225 nuclear ITS sequences of the *Peterseniae* species using the GTR + I + G evolutionary model [BaseFreq (πA = 0.2842, πC = 0.2301, πG = 0.1956, πT = 0.2902); RateMatrix (2.5444, 5.4851, 7.0150, 0.8953, 5.4851, 1.0000); Gamma shape = 0.6967; Invariant = 0.3744]. Sequences generated in the present study are in red-coloured bold italic.

AUTHOR CONTRIBUTIONS

B.Y. Jo and J.I. Kim: algal collection, isolation and culture, planning of experiments, molecular analyses and drafting manuscript; P. Škaloud: image and statistical analyses and editing manuscript; P.A. Siver: original concept, drafting and editing manuscript; W. Shin: original concept, algal collection, planning of experiments, drafting and editing manuacript.

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