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# Newly recorded species of the genus Synura (Synurophyceae) from Korea

Bok Yeon Jo and Han Soon Kim\*

# **Abstract**

**Background:** Species in the heterokont genus *Synura* are colonial and have silica scales whose ultrastructural characteristics are used for classification. We examined the ultrastructure of silica scales and molecular data (nuclear SSU rDNA and LSU rDNA, and plastid *rbcL* sequences) to better understand the taxonomy and phylogeny within the section *Petersenianae* of genus *Synura*. In addition, we report the first finding of newly recorded *Synura* species from Korea.

**Results:** We identified all species by examination of scale ultrastructure using scanning and transmission electron microscopy (SEM and TEM). Three newly recorded species from Korea, *Synura americana*, *Synura conopea*, and *Synura truttae* were described based on morphological characters, such as cell size, scale shape, scale size, keel shape, number of struts, distance between struts, degree of interconnections between struts, size of base plate pores, keel pores, base plate hole, and posterior rim. The scales of the newly recorded species, which belong to the section *Petersenianae*, have a well-developed keel and a characteristic number of struts on the base plate. We performed molecular phylogenetic analyses based on sequence data from three genes in 32 strains (including three outgroup species). The results provided strong statistical support that the section *Petersenianae* was monophyletic, and that all taxa within this section had well-developed keels and a defined number of struts on the base plate.

**Conclusions:** The phylogenetic tree based on sequence data of three genes was congruent with the data on scale ultrastructure. The resulting phylogenetic tree strongly supported the existence of the section *Petersenianae*. In addition, we propose newly recorded *Synura* species from Korea based on phylogenetic analyses and morphological characters: *S. americana*, *S. conopea*, and *S. truttae*.

**Keywords:** Synura americana, S. conopea, S. truttae, Morphology, Ultrastructure, Scale, Molecular phylogeny, Taxonomy

# **Background**

Ehrenberg established the genus *Synura* in 1834 (Ehrenber 1834), with *S. uvella* as the type species. *Synura* is the most common and widespread genus in many phytoplankton floras (Kristiansen & Preisig 2007). The species in this genus are colonial flagellates with two visible flagella and two chloroplasts, and are covered by imbricate silica scales. Several scale morphologies (apical scales, body scales, transition scales, and caudal scales) occur at different locations on the surface of the same cell. These body scales are the most important character for species identification (Kristiansen & Preisig 2007).

Early classification of *Synura* species using light microscopy (LM) was based largely on features such as cell size and shape, general outline of scales, and the spine or keel (Ehrenber 1834). Previous taxonomical studies of *Synura* have traditionally stressed the distinguishing features of these scales.

The classification of *Synura* species using electron microscopy (EM) is based on scale ultrastructure (Korshikov 1929; Petersen & Hansen 1956; Petersen & Hansen 1958; Fott & Ludvík 1957; Asmund 1968; Balonov & Kuzmin 1974; Péterfi & Momeu 1977; Takahashi 1967; Takahashi 1972; Takahashi 1973; Takahashi 1978; Cronberg 1989; Škaloud et al. 2012; Škaloud et al. 2013; Škaloud et al. 2014). In fact, examination of the ultrastructural features of the silica scales has revolutionized *Synura* taxonomy. The first classification scheme to consider scale ultrastructure

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suggested that the genus *Synura* is divided into two sections: *Petersenianae* and *Uvellae* (Petersen & Hansen 1956). Subsequent classification schemes have made additional subgeneric distinctions (Balonov & Kuzmin 1974; Péterfi & Momeu 1977; Takahashi 1967; Takahashi 1972; Takahashi 1973; Takahashi 1978; Cronberg 1989).

The first molecular analyses investigated the genetic variability in 15 individuals of *Synura petersenii* by comparison of nuclear internal transcribed spacer (ITS) sequences (Wee et al. 2001). Subsequent molecular analyses examined ITS sequences from 21 other individuals (Kynčlová et al. 2010). Also, phylogenetic analyses investigated about 100 S. petersenii using seven-protein gene and confirmed the high degree of cryptic, species-level diversity within this nominal species (Boo et al. 2010). A recent taxonomic assessment of observed cryptic diversity redefined the species concept within the S. petersenii morphotype and recognized six cryptic lineages as separate species: Synura americana, Synura conopea, Synura glabra, Synura macropora, Synura petersenii, and Synura truttae (Škaloud et al. 2012). Most recently, the classification of Synura described an additional four new species within the S petersenii species complex based on scale morphology and sequence data (ITS, rbcL, and cox1) (Skaloud et al. 2014).

Several researchers have studied the genus *Synura* from different regions in Korea by the use of EM (Kim 1997). These studies described nine species and provided very short descriptions based on scale ultrastructure (Kim 1997; Kristiansen 1990). Most recently, the first molecular multigene phylogeny of a large number of *S. petersenii* confirmed the high degree of cryptic, species-level diversity (Boo et al. 2010).

The purpose of the present study was to provide a better understanding of the taxonomy and molecular phylogeny within the section *Petersenianae* of genus *Synura* by analysis of the ultrastructure of the silica scales and molecular data (nuclear SSU rDNA and LSU rDNA, and plastid *rbc*L sequences) and to describe three species of *Synura* that are new to Korea.

#### **Methods**

#### Strains and cultures

The information and accession numbers for the 32 strains (including three outgroup species) examined in this study are in Table 1. Strains were either obtained from culture collections or collected with a 20- $\mu$ m mesh plankton net (Bokyeong Co., Pusan, Korea) from small ponds in Korea. The details of the culture methods were previously published (Jo et al. 2011; Jo et al. 2013).

#### Morphological investigations

For field emission scanning electron microscopy (SEM), cells were filtered using nylon membrane filters (Whatman Ltd., Maidstone, UK), rinsed in distilled water, fixed in 1%

OsO<sub>4</sub>, dehydrated, and then prepared and viewed as described previously (Jo et al. 2011). Voucher specimens were stored at the Kyungpook National University Herbarium. For field emission transmission electron microscopy (TEM), cells were prepared by air drying 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). The terminology used to describe scale ultrastructure follows a previous method (Škaloud et al. 2012).

# DNA extraction, amplification, sequence alignment, and phylogenetic analyses

DNA extraction, PCR amplification, PCR product purification, and sequence alignment were conducted as previously described (Jo et al. 2011; Jo et al. 2013). Phylogenetic analyses were performed using a combined dataset of 5011 characters (nr SSU rDNA = 1638, nr LSU rDNA = 2548, and pt rbcL = 825) by maximum likelihood (ML) and Bayesian inference (BI). Although nuclear ITS1 and ITS2 sequences were also determined, these sequences were used to examine groups of genetically identical strains and as a barcode to identify species. The sequences of three species of Chrysophyceae (Chromulina sp., Ochromonas danica, and Ochromonas sp.) were used as outgroups to root the tree. Primer regions and ambiguously aligned regions were removed prior to phylogenetic analyses. Prior to ML analysis, the best-fit model for individual and concatenated data sets was traced under Bayesian information criterion (BIC) using Modeltest 3.7 (Posada & Crandall 1998). GTR + I + G model for all the individual and concatenated data sets was selected. We used the GTR + I + G nucleotide model as implemented in RAxML v8 (Stamatakis 2014). Bayesian analyses were run using MrBayes 3.2 (Ronquist et al. 2012) with a random starting tree and ran for  $2 \times 10^6$ generations, keeping on tree every 1000 generations. The burn-in point was identified graphically by tracking the likelihoods in Tracer v.1.6 (Rambaut et al. 2013). Trees were visualized using the FigTree v.1.4.2 program (Rambaut A. FigTree v1.4.2 2014). Each analysis was conducted as previously described (Jo et al. 2011; Jo et al. 2013).

#### Results and discussion

# Morphological characteristics

We identified all species based on scale ultrastructure from SEM and TEM. This analysis led to identification of three species that are new to Korea: *S. americana*, *S. conopea*, and *S. truttae* (Figs. 1, 2 and 3 and Table 2). The scales of the newly recorded species, all in the section

Table 1 List of strains used in the molecular study and GenBank accession number

T ( ) :	GenBank accession					
Taxa/strain	Nuclear ITS	Nuclear SSU	Nuclear LSU	Plastid <i>rbc</i> L		
S. americana Kynčlová and Škaloud						
Chimu112407C	KP268712	KM590551	KM590617	KM590838		
Johae010508F	KP268711	JX455151	JX455155	JX455147		
CCMP862	GU338124	GU325583	_	GU325485		
CCMP863	GU338125	GU325584	_	GU325486		
KNUJO-CM20151226	KX610938	KX610941	KX610944	KX6109447		
<i>S. asmundiae</i> (Cronberg and Kristianser Kristiansen and Škaloudová	n) Škaloud,					
S90D10	KP268729	KM590553	KM590619	KM590840		
S90D11	KP268730	HF549069	_	HF549079		
S. bjoerkii (Cronberg and Kristiansen) Šk Kristiansen and Škaloudová	kaloud,					
SC57A6	KP268731	HF549070	_	HF549080		
S. conopea Kynčlová and Škaloud						
Sugyeji041808B	KP268690	KM590557	KM590623	KM590844		
Yeonseong120807E	KP268689	KM590558	KM590624	KM590845		
CCMP859	GU338121	GU325580	_	GU325482		
NIES1007	GU338119	GU325578	_	GU325479		
KNUJO-YG20160117	KX610939	KX610942	KX610945	KX6109448		
S. glabra Korshikov emend. Kynčlová a	nd Škaloud					
Bonggye101407K	KP268722	KM590564	KM590630	KM590851		
Cheonma041908B	KP268716	KM590565	KM590631	KM590852		
Dohak111107C	KP268721	JX455149	JX455153	JX455145		
Geumma020610B	KP268718	KM590568	KM590634	KM590855		
Hwangsan012508A	KP268724	KM590571	KM590637	KM590858		
S. macracantha (Petersen and Hansen)	Asmund					
S90B5	KP268732	HF549064	KM590648	HF549075		
S. petersenii Korshikov emend. Škaloud	and Kynčlová					
Buje100307A	KP268710	KM590586	KM590657	KM590873		
Gamgok111107C	KP268707	KM590587	KM590658	KM590874		
Swaeji103109l	KP268705	KM590589	KM590660	KM590876		
Yongseong112407A	KP268706	KM590590	KM590661	KM590877		

**Table 1** List of strains used in the molecular study and GenBank accession number (Continued)

Youngji101407A	KP268708	JX455150	JX455154	JX455146
S. truttae (Siver) Škaloud and Kynčlová				
Hanjeong080611J	KP268702	KM590609	KM590680	KM590896
Jangjuk032611J	KP268703	KM590610	KM590681	KM590897
CAUP2	GU338138	GU325598	_	GU325500
CAUPD5	GU338140	GU325600	_	GU325502
KNUJO-HJ20151222	KX610940	KX610943	KX610946	KX610949
Chromulina sp.				
SAG 17.97	<u>—</u>	EF165103	GU935638	EF165151
Ochromonas danica Pringsheim				
SAG 933.7	<del>_</del>	JQ281514	GU935636	GU935657
Ochromonas sp.				
SAG 933.10	_	EF165109	GU935637	GU935658

New sequences are indicated in italic type

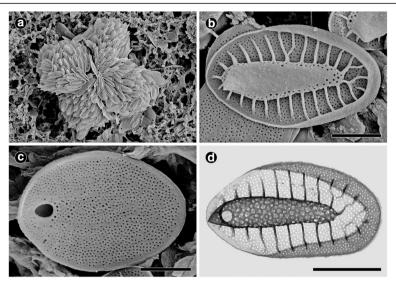


Fig. 1 Morphology of the colony and scales of Synura americana (a–c: SEM, d: TEM). All scale bars, 1 μm. a SEM image of colony forming cells. b Top surface of a body scale. c Bottom surface of a body scale. d TEM image of body scale

Petersenianae, have well-developed keels and a number of struts on the base plate. The terminology used to describe the ultrastructure of these scales follows a previous method (Škaloud et al. 2012). Other studies have described newly recorded species of Synura from Korea based on morphological characters, such as cell size, scale shape, scale size, keel shape, number of struts, distance between struts, degree of interconnections between struts, size of the base plate pores, keel pores, base plate hole, and posterior rim (Škaloud et al. 2012). Two of our species (S. americana and S. conopea) are morphologically similar to S. petersenii, suggesting a close relationship. S. conopea was most similar to

*S. petersenii* in terms of cell shape and transverse folds, although these species differ in keel reticulation. *S. conopea* is distinguished by its smaller scales and its large and closely arranged keel pores. *S. americana* is characterized by rounded scales, a near absence of transverse folds, an occasionally triangular keel, and long rear scales. *S. truttae* is characterized by small scale size, keel tips, large base plate hole, and short distance between struts.

# Taxonomic description

*S. americana* Kynčlová and Škaloud 2012 (Fig. 1) Reference: Škaloud et al. 2012, p. 320, Figs. 62–69.

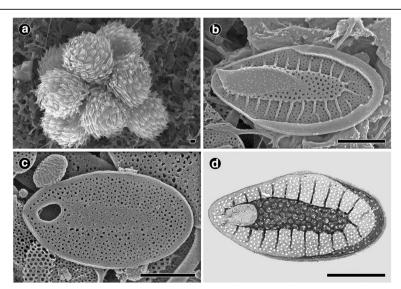


Fig. 2 Morphology of the colony and scales of *Synura conopea* (**a–c**: SEM, **d**: TEM). All *scale bars*, 1 μm. **a** SEM image of colony forming cells. **b** Top surface of a body scale. **c** Bottom surface of a body scale. **d** TEM image of a body scale

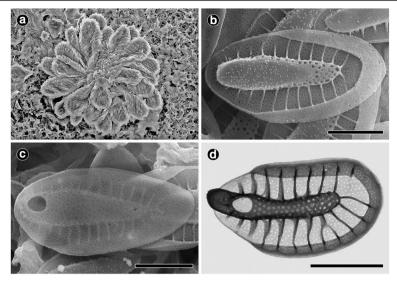


Fig. 3 Morphology of the colony and scales of Synura truttae (a–c: SEM, d: TEM). All scale bars, 1 μm. a SEM image of colony forming cells. b Top surface of a body scale. c Bottom surface of a body scale. d TEM image of a body scale

Specimens examined: KNUJO-CM20151226.

Description: Colonies globular and  $22-51~\mu m$  in diameter (Fig. 1a). Cells pyriform ( $22-28\times8-12~\mu m$ ) and entirely covered by rounded scales (Fig. 1a). Body scales  $3.0-4.2\times1.7-2.3~\mu m$  (Fig. 1b–d). The keel often terminates at an acute tip (Fig. 1b) and is ornamented by medium-sized pores (Fig. 1d). In some cases, the keel is wider in the anterior region, giving it a triangular shape (Fig. 1b). The basal plate, ornamented by numerous small pores, is anteriorly perforated by a rounded base plate hole that is  $0.08-0.27~\mu m$  in diameter (Fig. 1b–d). Numerous struts (21-24) extend regularly from the keel to the edge of the scale but almost never interconnect the transverse folds (Fig. 1b and d). The spacing between struts is  $0.27-0.30~\mu m$  (Fig. 1b and d).

Site of collection: Chimu, Daesan-myeon, Haman-gun, Gyeongsangnam-do, Korea (35°20′21"N, 128°25′47"E).

Date of collection: 26 Dec 2015.

Distribution: Widely distributed. Canada (Wee et al. 2001), Colombia (Cronberg 1989), Czech Republic (Škaloud et al. 2012; Kynčlová et al. 2010), Denmark (Kristiansen 1988), Germany (Kies & Berndt 1984), Korea (Boo et al. 2010, this study), North America (Kling & Kristiansen 1983; Kristiansen 1975; Wee 1981), and USA (Wee et al. 2001; Boo et al. 2010).

#### S. conopea Kynčlová and Škaloud 2012 (Fig. 2)

Reference: Škaloud et al. 2012, p. 324, Figs. 78-85.

Specimens examined: KNUJO-YG20160117, NIBRFL 0000131748, and NIBRFL0000131749.

Description: Colonies globular and 25–47  $\mu m$  in diameter (Fig. 2a). Cells pyriform (20–28  $\times$  8–12  $\mu m$ ) and entirely covered by lanceolate scales (Fig. 2a). Body scales 3.3–4.1  $\times$ 

 $1.4{-}1.9~\mu m$  (Fig. 2b–d). The keel terminates at an acute tip (Fig. 2b) and is usually broadened apically and ornamented by medium to large-sized pores (Fig. 2d). The basal plate, ornamented by numerous medium-sized pores, is anteriorly perforated by a round to oblong base plate hole that is  $0.19{-}0.32~\mu m$  in diameter (Fig. 2b–d). Numerous struts (24–30) extend regularly from the keel to the edge of the scale but are usually not interconnected by transverse folds (Fig. 2b and d). The spacing between struts is  $0.23{-}0.26~\mu m$  (Fig. 2b and d).

Site of collection: Yongji, Yongchon-ri, Toseong-myeon, Goseong-gun, Gangwon-do, Korea (38°13′43″N, 128° 33′49″E).

Date of collection: 17 Jan 2016.

Distribution: Widely distributed. Argentina (Vigna & Munari 2001), Brazil (Couté & Franceschini 1988), Czech Republic (Škaloud et al. 2012; Kynčlová et al. 2010), Greenland (Jacobsen 1985), Ireland (Řezáčová & Škaloud 2005), Japan (Boo et al. 2010), and Korea (Boo et al. 2010, this study).

*S. truttae* (Siver 1987) Škaloud and Kynčlová 2012 (Fig. 3) Basionym: *S. petersenii* f. *truttae* (Siver 1987), p. 111, Figs. 12–14.

Reference: Škaloud et al. 2012, p. 318, Figs. 52–61. Specimens examined: KNUJO-HJ20151222.

Description: Colonies globular and  $35-48~\mu m$  in diameter (Fig. 3a). Cells pyriform ( $22-31\times11-13~\mu m$ ) and entirely covered by lanceolate scales (Fig. 3a). Body scales elongated and  $3.3-3.8\times1.5-1.8~\mu m$  (Fig. 3a–d). The keel of the body scales has no apparent tip or a much reduced tip and is ornamented by small pores (Fig. 3b). The keel tip frequently has several (two to

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

Taxon	Cell size (µm)	Scale size (µm)	Base plate hole size (µm)	Number of struts	Distance of struts (µm)	Interconnection of struts by transverse folds	Other
S. americana Kynčlová & Škaloud	22-28 × 8-12	*3.0-4.2 × 1.7-2.3	0.08-0.27	21–24	0.27-0.30	Almost never interconnected	Occasional triangular shape of the keel
S. conopea Kynčlová & Škaloud	$20-28 \times 8-12$	*3.3-4.1 × 1.4-1.9	0.19-0.32	24-30	0.23-0.26	Usually not interconnected	Large and closely arranged keel pores
S. glabra Korshikov emend. Kynčlová & Škaloud	19-28 × 10-14	*2.4-3.4 × 1.5-2.4	0.14-0.32	17–22	0.25-0.29	Never interconnected	With a small, narrow and sometimes bent keel
S. petersenii Korshikov emend. Škaloud & Kynčlová	20-31 × 8-12	*3.6-4.6 × 1.8-2.3	0.24-0.36	26–34	0.24-0.28	Mainly interconnected	Large scale dimensions, common presence of transverse folds
S. truttae (Siver) Škaloud & Kynčlová	22-31 × 11-13	*3.3-3.8 × 1.5-1.8	0.32-0.56	27–33	0.19-0.24	Mainly interconnected	Keel tip has several (two to four) very short teeth on its top end and large base plate hole

<sup>\*</sup>The dimensions of body scales

four) very short teeth on its top (Fig. 3d) and is covered by a number of small bumps. The basal plate, ornamented by numerous small pores, is anteriorly perforated by a large, round to oblong base plate hole that is 0.32–0.56  $\mu m$  in diameter (Fig. 3b–d). Numerous struts (27–33), which are often interconnected, regularly extend from the keel to the edge of the scale (Fig. 3b and d). Scales with nearly absent transverse folds (Fig. 3b–d). The spacing between struts is 0.19–0.24  $\mu m$  (Fig. 3b and d).

Site of collection: Hanjeong, Girin-ri, Soseong-myeon, Jeongeup-si, Jeollabuk-do, Korea (35°33′55″N, 126°46′ 30″E).

Date of collection: 22 Dec 2015.

Distribution: Widely distributed. Czech Republic (Škaloud et al. 2012; Kynčlová et al. 2010), Korea (This study), and USA (Siver 1987; Siver & Wujek 1993; Siver & Lott 2004).

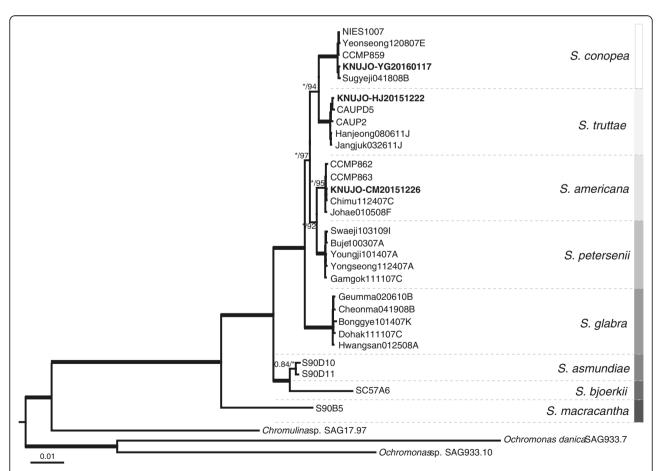
#### Molecular data

The 5011 nucleotides of the combined data set (nuclear SSU and LSU rDNA, and plastid *rbc*L) were determined

for 32 strains (Table 1). Although the nuclear ITS1, 5.8S, and ITS2 sequences were also determined, these sequences were only used for to confirm identification, not to assess phylogenetic relationships. The combined sequences had 5011 nucleotides, 4039 variable sites, and 725 parsimoniously informative sites. The molecular data contained 12 new sequences (3 new nr SSU rDNA sequences, 3 new nr LSU rDNA sequences, 3 new nr ITS sequences, and 3 new pt *rbc*L sequences) and 102 published sequences (29 nr SSU rDNA sequences, 20 nr LSU rDNA sequences, 25 nr ITS sequences, and 28 pt *rbc*L sequences).

#### Phylogenetic analyses

We analyzed nr SSU and LSU rDNA, and pt *rbc*L sequences from 32 strains (including three outgroup species). The phylogenetic tree based on the Bayesian analysis was rooted with three species of Chromulinaceae serving as outgroups. The Bayesian and ML analyses recovered a tree with identical topologies (Fig. 4). The phylogenetic tree consisted of species of the section *Petersenianae*, each of which has a well-developed keel and a number of struts on



**Fig. 4** Consensus Bayesian tree of the genus *Synura* based on a combined nuclear SSU and LSU rDNA, and plastid *rbcL* sequences data. Bayesian posterior probability (pp) and maximum-likelihood (ML) bootstrap values are shown above or below the branches. The bold branches indicate strongly supported values (pp = 1.00 and ML = 100). *Scale bar*, 0.01 substitutions/site

the base plate. The section *Petersenianae* formed a strongly supported monophyletic lineage (pp = 1.00 and ML = 100). The single strain of Synura macracantha diverged at the base of the tree, followed by Synura bjoerkii and Synura asmundiae. The single strain of S. bjoerkii was closely related to S. asmundiae, which included two strains (pp = 1.00 and ML = 100). Synura glabra formed a sister group with S. americana, S. conopea, S. petersenii, and S. truttae (pp = 1.00 and ML = 100), and S. americana and S. petersenii diverged at the next S. glabra. The five strains of S. petersenii formed a strongly supported monophyletic lineage (pp = 1.00 and ML = 100) and was a sister group to the five strains of S. americana, which included KNUJO-CM20151226 (pp = 1.00 and ML = 92). The five strains of S. americana were monophyletic group (pp = 1.00 and ML = 95), and the intraspecific similarity based on nuclear ITS rDNA sequence data ranged from 99.9% to 100.0%. The five strains of S. truttae (including KNUJO-HJ20151222) were a sister group to the five strains of S. conopea, which included KNUJO-YG20160117 (pp = 1.00 and ML = 94). The five strains of S. conopea formed a monophyletic lineage with strong support values (pp = 1.00 and ML = 100), and the intraspecific similarity based on nuclear ITS rDNA sequence data ranged from 98.5% to 100.0%. The five strains of S. truttae formed a monophyletic lineage with strong support values (pp = 1.00 and ML = 100), and the intraspecific similarity based on nuclear ITS rDNA sequence data was 100.0%.

### **Conclusions**

In summary, we used molecular analysis of three genes and data on the scale ultrastructure to investigate the phylogenetic relationships within *Synura*, with a focus on the section *Petersenianae*. The phylogenetic tree based on a combined dataset was well congruent with the ultrastructural characteristics of scales. The phylogenetic tree was comprised of members of the section *Petersenianae*. The section *Petersenianae* was monophyletic with strong support values and characterized by a well-developed keel and a number of struts on the base plate. In addition, our morphological observations and molecular analyses confirmed unambiguously that this is the first report of *S. americana*, *S. conopea*, and *S. truttae* in Korea.

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#### Availability of data and materials

The sequence data from this study were deposited in GenBank with the accession codes KX610938-KX610949.

#### Authors' contributions

Both authors read and approved the final manuscript.

#### Competing interests

The authors declare that they have no competing interests.

#### Consent for publication

Not applicable.

#### Ethics approval and consent to participate

Not applicable.

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