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Morphological and Molecular Characterizations of Three Species of the Genus *Synura* (Synurales, Chrysophyceae) from China

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Abstract: Three species of the genus *Synura* from China are described here. Morphological observations and molecular phylogenetic analyses were conducted for three specimens collected at different locations in China. The described morphological features included cell size, scale size, spines, keels, and struts. Molecular analyses based on multiple genetic markers (SSU and LSU rDNA and internal transcribed spacer rDNA) were used to determine the phylogenetic positions of the three *Synura* species. Morphologically, specimen GZ201017 collected in Guizhou Province was characterized by a well-developed keel and lanceolate scales; specimen SX210304 collected in Shanxi Province was characterized by a less-developed keel and poor silicification; and specimen GD201126 collected in Guangdong Province was characterized mainly by spines with blunt ends or two small teeth on the tips. The morphotypes GZ201017, SX210304, and GD201126 corresponded to the original descriptions of *Synura petersenii*, *S. glabra*, and *S. longitubularis*, respectively. This discovery laid a foundation for the molecular phylogeny of the genus *Synura* and an enhanced understanding of *Synura* diversity and distribution in China.

Keywords: Chrysophyta; China; molecular phylogenetics; morphological characteristics; *Synura* species

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1. Introduction

The genus *Synura* was established by Ehrenberg in 1834 and contains colonial flagellates whose cells are covered with imbricate silica scales, the ultrastructure of which is the primary attribute used to distinguish species [1,2]. To date, the genus *Synura* has been recognized as a distinct genus which is exclusively distributed in freshwater. There are currently 57 species accepted in the AlgaeBase taxonomy, and molecular evidence is accessible for 34 species [3,4]. The cells of *Synura* often have two to four types of scales, consisting of caudal scales, spineless body scales, spine-bearing body scales, and tubular apical scales [5]. Species identification is conducted according to the following characteristics: scale size, keel size, number and distance of struts, spine size, spine tip, and hexagonal meshwork. Generally, body scales are mainly used to distinguish amongst species [6–8]. Early classifications of *Synura* species were made mainly by observing features such as cell size and shape using light microscopy [1]. Subsequently, the application of electron microscopy has revolutionized the classification of *Synura* species [9–17]. However, the accuracy of the systematics and biodiversity of *Synura* cannot be guaranteed by relying simply on traditional morphological observations. Indeed, with the rapid advances in molecular technology, the section divisions of the genus *Synura* have been improved. The first proposed classification scheme divided the *Synura* genus into two sections on the basis of scale ultrastructure: *Petersenianae* and *Uvellae* [9]. Molecular reconstruction data then showed that section *Synura* and section *Peterseniae* represented two distinct evolutionary subclades on the phylogenetic tree, which was consistent with their morphological groupings [18]. Additional subgenera have been identified in subsequent studies [10,13,15,17].

According to the classification in 1974, the genus contained three sections: *Synura*, *Petersenianae* Petersen et Hansen ex Balonov et Kuzmin (1974: 1682), and *Lapponicae* Balonov et Kuzmin (1974: 1685) wherein section *Lapponicae* replaced section *Uvellae* proposed in 1956 [7,9,10]. Péterfi and Momeu accepted the classification scheme proposed in 1974, and the series *Splendidae* was recognized [10,15]. In addition, in 2013, Škaloud et al. proposed a five-section classification comprising *Echinulatae*, *Peterseniae*, *Spinosae*, *Splendidae*, and *Synura*, but no formal descriptions were given [11]. In 2016, new sectional ranks were proposed wherein Jo et al. suggested that the genus *Synura* should be further divided into the following three sections: *Synura*, *Peterseniae*, and *Curtispinae* [7]. As currently recognized, these three sections are clearly distinguishable, and the molecular classification scheme is consistent with that based on morphology [7,8]. More recently, Škaloud et al. and Jo et al. studied the diversity within the genus *Synura*, but this genus needs to be further resolved [7,8,16,19,20]. Furthermore, genetic markers, including SSU, LSU, and ITS, have been widely used in the phylogenetic analysis of Chrysophyta [7,8,18–23]. Therefore, three genes were selected to investigate species within the *Synura* genus in this study.

The genus *Synura* is distributed widely around the world, but rarely has been reported in Asia [6,24]. Some species are widespread, while others are extremely rare and distributed in specific habitats, even inhabiting cold and acidic conditions [24–26]. The first report of the genus *Synura* in China was by Skvortsov (1961), but that report was not based on scale ultrastructure. Since then, several additional species of the genus *Synura* have been reported in China based on their ultrastructure [9,27–30]. In addition, Pang and Wang described the stomatocyst of *Synura petersenii* in 2012 and described a new species *S. morusimila* in 2013 [31,32]. Wei and Yuan reported *S. glabra* and *S. petersenii* in 2014 and *S. bjoerkii*, for the first time, in 2015 [33,34]. To date, the *Synura* studies in China have lacked the molecular evidence that is necessary to enhance and validate the research on *Synura* diversity and distribution in this region.

Three *Synura* specimens were collected in Guizhou, Guangdong, and Shanxi provinces, respectively. Molecular phylogenetic analysis was conducted on the basis of concatenated SSU rDNA, LSU rDNA, and ITS rDNA sequences, and the identification results were verified using morphological characteristics. The aims of this study were to (1) describe three species of the genus *Synura* using a combination of morphological and molecular techniques; (2) infer the phylogenetic relationships among *Synura* species in this study; (3) compare morphological characterizations among the three species and closely related species; and (4) comprehend the species diversity and geographical distribution of *Synura* in China. This study has significantly improved our knowledge of the diversity of *Synura* species and their regional distribution in China in addition to serving as a regional resource for the biodiversity of freshwater Chrysophyta.

2. Materials and Methods

2.1. Collection and Culture

Three specimens (GD201126, GZ201017, and SX210304) were collected from Guangdong Province, Guizhou Province, and Shanxi Province in China (Figure 1, Table 1). A plankton net with a mesh size of 20 µm was used to collect samples, and the samples were transferred to the laboratory as soon as possible. Single strains were isolated by Pasteur capillary pipette under an inverted microscope and placed into a uni-algal culture in DY-IV medium with MES. The pH of the medium was 6.8. The cultures were maintained at 14–16 °C under a 12 h: 12 h of light: dark cycle with 1000 lux of illumination. The incubation period was 8 days, after which the culture was expanded. Voucher specimens were preserved in 4% formaldehyde. Voucher specimens were deposited in the Herbarium of Shanxi University (SXU), Shanxi University, Taiyuan, Shanxi Province, China.

Figure 1. Map of general collection locations of the samples investigated in this study. More detailed information on the collection of the *Synura* specimens is provided in Table 1.

Table 1. Collection information and voucher numbers for taxa analyzed in this study.

Isolate	Locality with Longitude and Latitude	Habitat Type	Collection Date	Collector	Voucher Number
GD201126	the Xianxia waterfall, Huizhou, Guangdong Province, China (23.6494° N, 113.8851° E)	a pond near a waterfall	26 November 2020	Nini Cui	SXU-GD201126
GZ201017	the Xinpu Wetland Park, Zunyi, Guizhou Province, China (27.7024° N, 107.0180° E)	a lake in the park	17 October 2020	Qi Liu	SXU-GZ201017
SX210304	the Long Korean Road, Changzhi, Shanxi Province, China (36.0619° N, 113.0049° E)	a lake near a factory	4 May 2021	Chen Su	SXU-SX210304

2.2. DNA Extraction, Amplification, and Sequencing

Algal sediments pellets were obtained after centrifugation of 1 mL culture in the exponential growth phase for 5 min at room temperature. A plant DNA extraction kit (Sangon Biotech, Shanghai, China) was used to extract the total DNA from the pellets. SSU, LSU, and ITS rDNA were amplified using polymerase chain reaction (PCR) in a total volume of 50 µL, containing 37.75 µL ddH_2O, 5.0 µL 10 × buffer, 4.0 µL 2.5 mM dNTPs, 0.25 µL Taq DNA polymerase (Sangon Biotech, Shanghai, China), 1.0 µL of each primer (10 mM), and 1.0 µL of genomic DNA. The SSU gene was PCR amplified using the 18S_1F, 18S_9R, 18S_4F, and 18S_12R primers, and the LSU gene used the 28S_25F, 28S_861R, 28S_736F, 28S_1440R, 28S_1228F, 28S_2160R, 28S_2038F, and 28S_2812R primers [35,36]. According to Wee et al. and White et al., the amplification of the ITS marker used the primers KN1.1 and ITS4 [22,37]. The SSU, LSU, and ITS genes all used the following cycle:

94 °C for 5 min, 35 cycles of 94 °C for 30 s, annealing temperature for 30 s and 72 °C for 2 min, and final 72 °C for 10 min. The reactions were undertaken in a MyCycler thermal cycler (Bio-Rad, Hercules, CA, USA). The annealing temperature changed depending on the primer. The temperature for 18S_1F, 18S_9R, and 28S_736F, and 28S_1440R was set to 49 °C and that for 28S_1228F, 28S_2160R, 28S_2038F, and 28S_2812R was set to 51 °C. The temperature for 18S_4F and 18S_12R was set to 54 °C and that for 28S_25F and 28S_861R was set to 47 °C. The annealing temperature of the primers KN1.1 and ITS4 was set to 48 °C. The PCR products, along with their amplification primers, were sent to BGI Tech Corporation (Beijing, China) where they were sequenced on an ABI 3730XL sequencer. The sequence data of SSU rDNA (OM267653, OM285147, and OM267663), LSU rDNA (OM267664, OM285146, and OM285148), and ITS rDNA (OP811172, OP811173, and OP811174) were submitted to GenBank.

2.3. Phylogenetic Analysis

Using MAFFT version 7, the sequence data obtained by sequencing in BGI Tech Corporation were aligned with those of other *Synura* species and outgroup taxa, downloaded from GenBank, by nucleotide blasting [38]. The SSU rDNA, LSU rDNA, and ITS rDNA molecular data of the genus *Synura* were collected (49 SSU rDNA sequences from 26 species, 44 LSU rDNA sequences from 24 species, and 56 ITS rDNA sequences from 30 species). The sequences of SSU, LSU, and ITS were concatenated on the basis of the methods of Zhang et al. (2020) [39]. The concatenated SSU, LSU, and ITS sequence set was 5418 base pairs, of which 1400 (25.84%) were variable and 1192 (22.00%) were parsimony informative. T, C, A, and G exhibited average compositions of 26.3%, 20.0%, 27.5%, and 26.2%, respectively. BioEdit v7.2.1 was used to cut the untrimmed ends to produce the same length alignments [40]. MEGA 5.0 was employed to calculate the pairwise genetic P-distances between individual samples [41]. The outgroup taxa *Neotessella volvocina* and *N. lapponica* were chosen on the basis of previous studies [7,8]. The appropriate model was built using the software PartitionFinder 2 with all algorithm and BIC criterion (for BI: Subset (1)(2)(3) = GTR + I + G; for ML: Subset (1)(2)(3) = GTR + I + G) [42]. Maximum likelihood (ML) phylogenies were inferred using the IQ-TREE under edge-linked partition model for 5000 ultrafast bootstraps, as well as the Shimodaira–Hasegawa-like approximate likelihood-ratio test [43–45]. Moreover, Bayesian inference (BI) phylogenies were inferred using MrBayes 3.2.6 under the partition model (2 parallel runs, 3,000,000 generations), in which the initial 25% of sampled data were discarded as burn-in [46]. The Figtree 1.4.2 software was applied to redact the resultant phylogenetic trees (http://tree.bio.ed.ac.uk/software/figtree/ (accessed on 5 December 2022)). Adobe Illustrator CS5 (Adobe System, San Jose, CA, USA) was used to optimize the graphics of all trees.

2.4. Morphological Observations

For morphological observations, fresh specimens were observed under a BX-51 Olympus (Olympus, Tokyo, Japan) fitted with a digital camera (DP72) for imaging. For electron microscopy, the cultured *Synura* strains were directly transferred and dried onto aluminum stubs in an oven. The aluminum stubs were sputter-coated with gold for 40 s and examined with a scanning electron microscope (Phenom-prox, Eindhoven, The Netherlands).

3. Results

3.1. Molecular Analysis

Pairwise distances based on concatenated SSU, LSU, and ITS sequences between the examined species and the outgroup taxa are listed in Table S1. Two species: *Neotessella volvocina* and *N. lapponica* were used as outgroups to root the phylogenetic tree. Similar topologies were produced by both methods, i.e., BI and ML. Therefore, only the BI trees containing all of the supporting values obtained on the nodes are displayed in Figure 2.

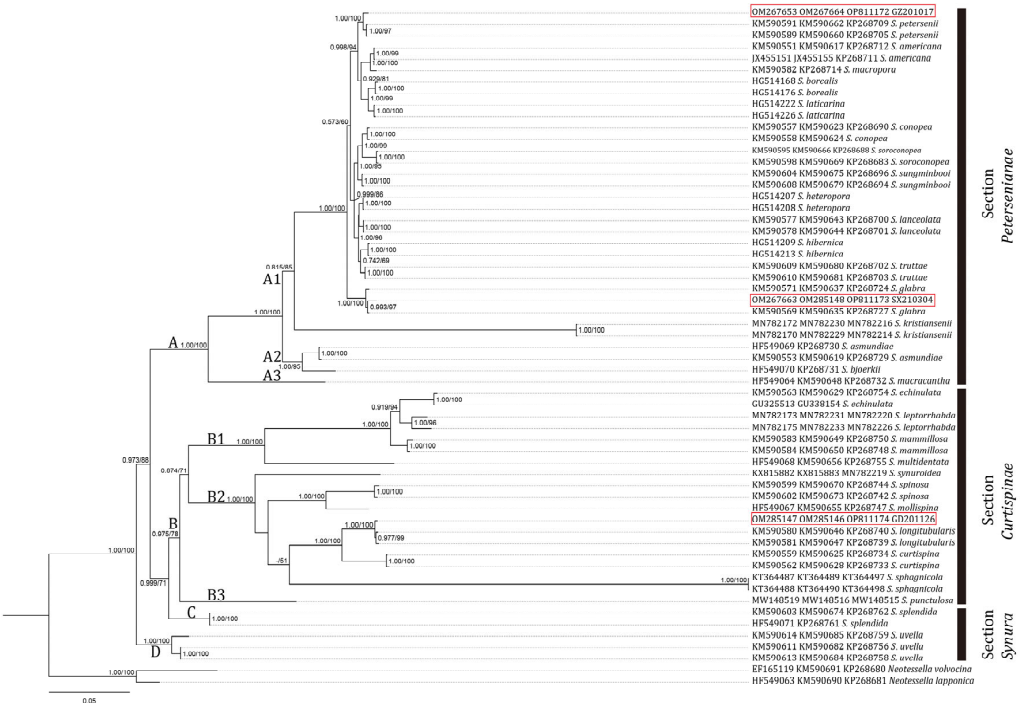

Figure 2. Bayesian inference (BI) tree based on concatenated SSU, LSU, and ITS sequences. Support values > 50% for all analyses are shown as follows: Bayesian posterior probabilities (BI)/maximum likelihood bootstrap values (ML). '-' denotes < 50% support for that analysis at that node. Red boxes indicate the *Synura* specimens used in this study.

In the phylogeny based on multiple genetic markers (SSU, LSU, and ITS) (Figure 2), *Synura* was segmented into four primary clades: A, B, C, and D. The section *Synura* was segmented into two clades: C and D. The two clades were composed of the members *Synura splendida* and *S. uvella*, both with strong supporting values of 1.00/100. Clade A was divided further into A1, A2, and A3. *S. macracantha* diverged at the bottom of Clade A. Subclade A1 consisted of *S. petersenii*, *S. americana*, *S. macropora*, *S. borealis*, *S. laticarina*, *S. conopea*, *S. soroconopea*, *S. sungminbooi*, *S. heteropora*, *S. lanceolata*, *S. hibernica*, *S. truttae*, *S. glabra*, and *S. kristiansenii*. The species collected from Guizhou was clustered together with *S. petersenii* with a high supporting value (1.00/100), and the distance between them was smaller than the intraspecific distance (0.0067 vs. 0.0087). *S. americana* was not monophyletic, and *S. macropora* was revealed as closely related to *S. americana* (1.00/100). *S. borealis* was closely related to *S. laticarina* (1.00/99). The *S. sungminbooi* strains shared a tight relationship with *S. soroconopea* and *S. conopea*. A high supporting value (1.00/96) indicated that the *S. heteropora* and *S. lanceolata* strains were strongly connected to *S. truttae* and *S. hibernica*. Specimen SX210304 collected from Shanxi Province was in a highly supported clade (1.00/100) with *S.glabra*, and they formed a monophyletic lineage. In addition, the distance between specimen SX210304 and *S. glabra* was smaller than the intraspecific distance within *Synura* (0.0064 vs. 0.0087). The monophyletic *S. kristiansenii* strains had strong supporting data (1.00/100). Subclade A2 comprised *S. asmundiae* and *S. bjoerkii*, which were related with strong supporting value (1.00/95). Clade B was further subdivided into B1, B2, and B3. *S. mammillosa* was closely related to *S. leptorrhabda* and *S. echinulata* with strong support (1.00/100). *S. multidentata* diverged at the base of Clade B1. *S. sphagnicola* represented a monophyletic lineage with strong supporting data (1.00/100). *S. mollispina* and *S. spinosa* were very closely related (1.00/100), and *S. curtispina* was closely related to *S. longitubularis* (1.00/100). The species collected from Guangdong Province was closely related to *S. longitubularis* with high support (1.00/100). Additionally, the distance between the species collected from Guangdong Province and *S. longitubularis* was smaller than the intraspecific distances within the *Synura* genus (0.0022 vs. 0.0087). *S. synuroidea* branched

from Clade B2 at its base. *S. punctulosa* made up Subclade B2 and the strain diverged at the bottom of Clade B.

3.2. Morphological Characterization

We observed the scale ultrastructures of the specimens (GZ201017, SX210304, and GD201126) by light microscope and scanning electron microscope. The results were consistent with the molecular results, which assisted in confirming the taxonomic status of these three new species records as *Synura petersenii*, *S. glabra*, and *S. longitubularis*. Of these, two species (*S. petersenii* and *S. glabra*) belonged to section *Peterseniae* and the other belonged to section *Curtispinae*. The scale ultrastructural characteristics of section *Peterseniae* and section *Curtispinae* of the genus *Synura* considered in this study are listed in Tables S2 and S3.

Specimen GD201126 of *S. longitubularis* was collected from the Xianxia waterfall (23.6494° N, 113.8851° E) in Guangdong Province. Its morphological features are shown in Figure 3 (1–3) and Figure 4. Its colonies were oval and 44–94 × 28–53 μm, and its cells were globular and 11–19 × 10–15 μm in diameter, with ovate or ellipsoidal scales covering the entire cell surface. Its body scales, 2.7–3.5 × 2.0–2.9 μm, were arranged in the shape of petals with a thickened posterior rim surrounding roughly one-half of the scale perimeter. A hexagonal meshwork was present on the distal areas of its scales. Its spines were 0.6–1.5 × 0.2–0.4 μm, with distal ends tapering to acute angles or terminating in two small teeth on the tip.

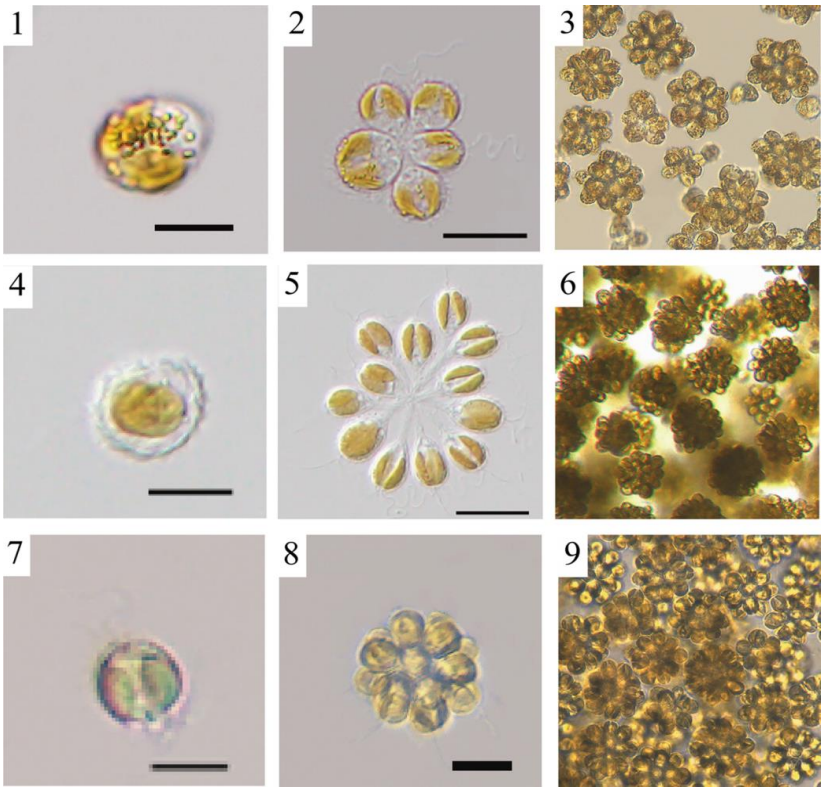

Figure 3. Light micrographs of *Synura* species. (**1**) A single cell of *Synura longitubularis*; (**2**) Five-celled colony of *S. longitubularis*; (**3**) Group of colony (*S. longitubularis* culture); (**4**) A single cell of *S. petersenii* collected from Guizhou, China; (**5**) Twelve-celled colony of *S. petersenii* collected from Guizhou, China. Long colorless cytoplasmic stalks connecting individual cells are visible; (**6**) Group of colony (Culture of *S. petersenii* collected from Guizhou, China); (**7**) A single cell of *S. glabra* collected from Shanxi, China; (**8**) Colony of *S. glabra* collected from Shanxi, China. Compact colony formed by densely grouped cells; and (**9**) Group of colony (Culture of *S. glabra* collected from Shanxi, China). Scale bars: (**2, 5, 8**) = 20 μm, (**1, 4, 7**) = 10 μm.

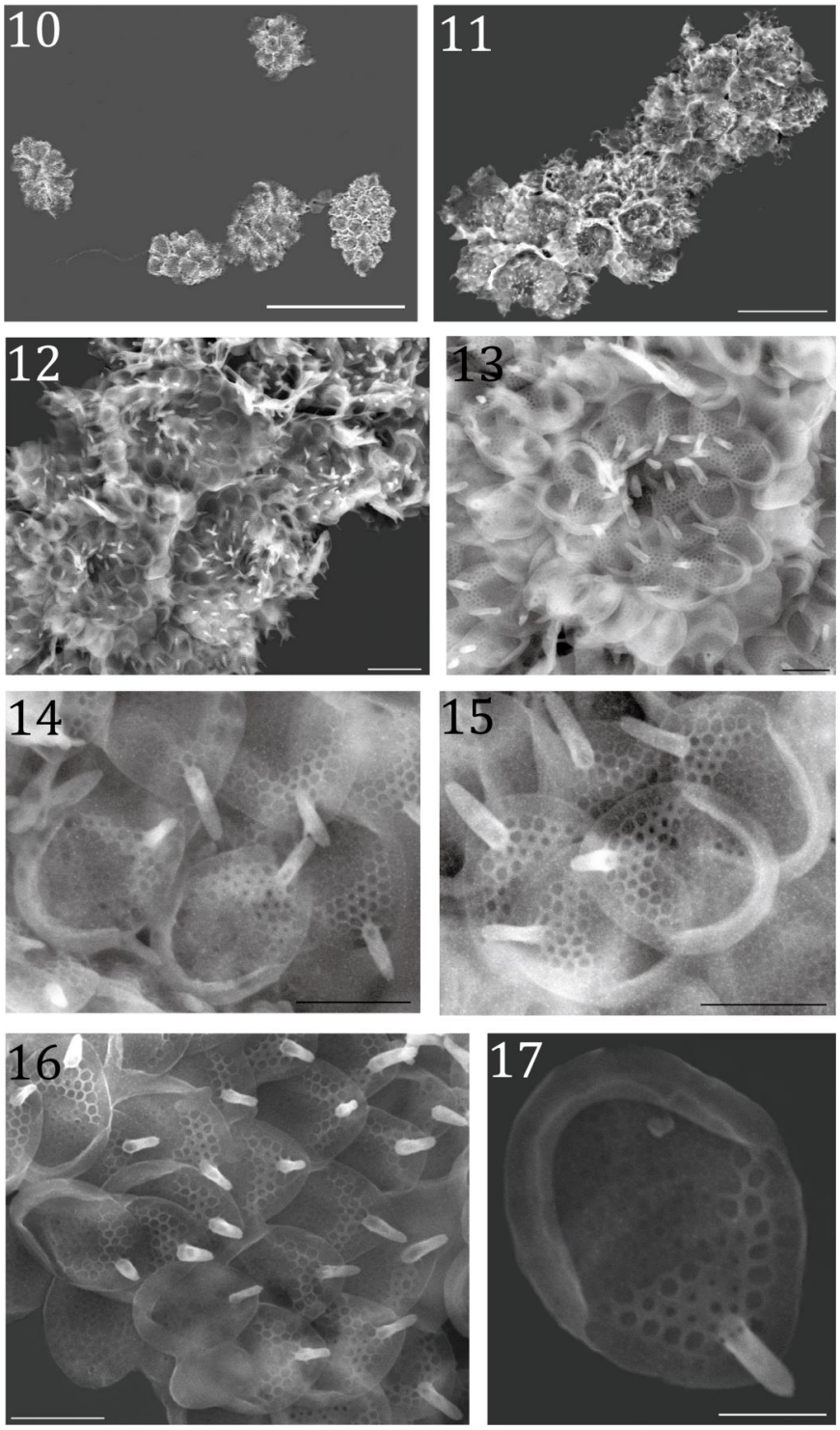

Figure 4. Morphological structures of *Synura longitubularis*. (**10**) Group of colony (SEM); (**11–12**) Colony (SEM); (**13**) A single cell (SEM); (**14–15, 17**) Body scales (SEM); and (**16**) A single body scale and spine (SEM). Scale bars: (**10**) = 100 μm, (**11**) = 20 μm, (**12**) = 5 μm, (**13–16**) = 2 μm, and (**17**) = 1 μm.

S. petersenii samples collected from the Xinpu Wetland Park (27.7024° N, 107.0180° E) in Guizhou were saved in the Herbarium of Shanxi University (SXU), Shanxi University, Taiyuan, Shanxi Province, China. The morphological characteristics of the sample are illustrated in Figure 3 (4–6) and Figure 5. Its colonies were petaloid and 29–38 μm in

diameter, and cells were pyriform, 12–17 × 11–14 μm, and entirely covered by lanceolate scales. Its body scales, 2.7–4.2 × 1.2–2.2 μm, had well-developed keels and a great many struts on the base plate. A posterior rim covered one-half to two-thirds of the scale perimeters. The keels, 2.0–3.2 × 0.4–0.8 μm, frequently ended in acute tips and were adorned by medium-sized pores. The keels were wider in the anterior region. Numerous small pores decorated its basal plate. Numerous struts (19–26) extended regularly from the keel to the edge of the scale and interconnected the transverse folds irregularly. Struts were spaced 0.13–0.34 μm apart.

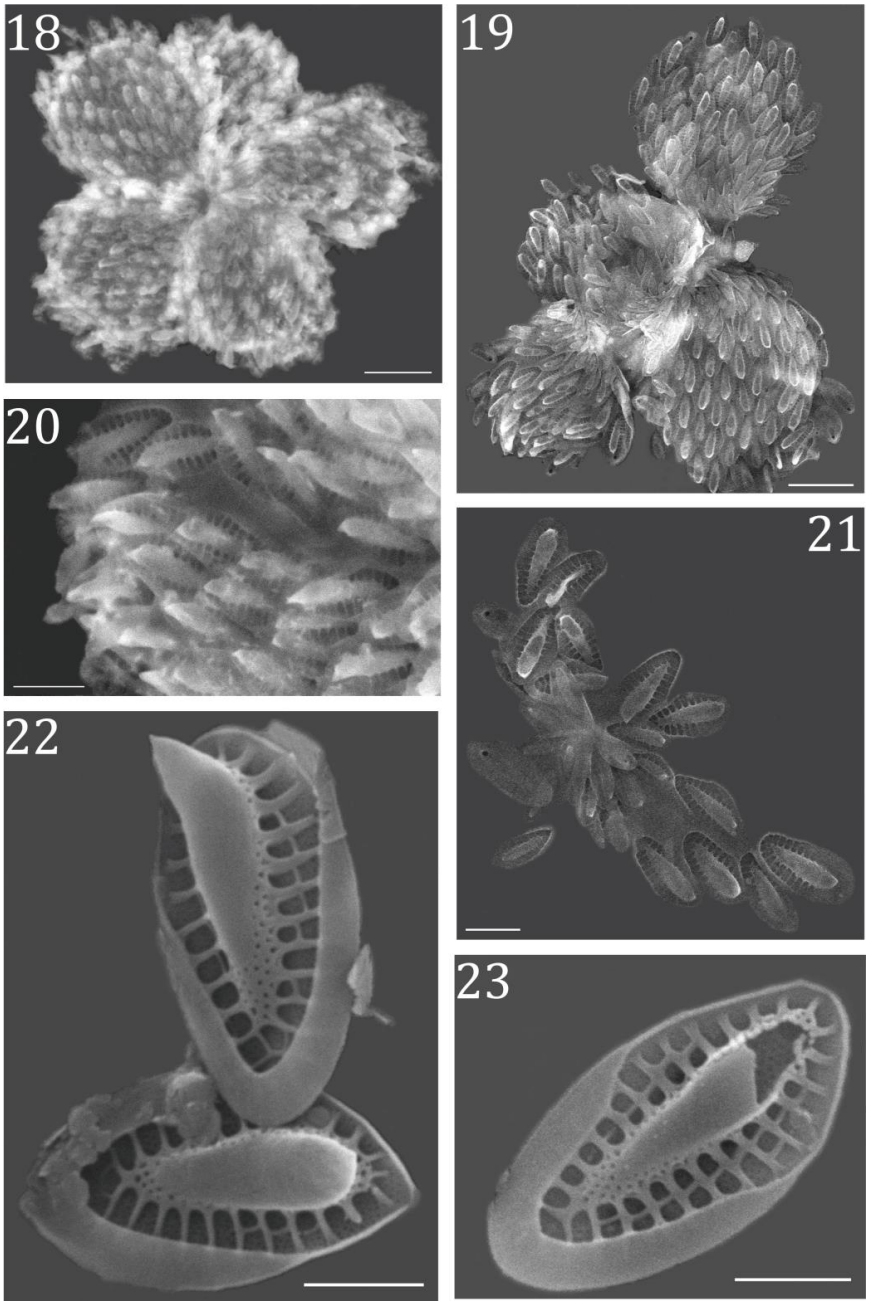

Figure 5. Morphological structures of *Synura petersenii* collected from Guizhou, China. (**18–19**) Colony (SEM); (**20–21**) Body scales (SEM); (**22**) Two body scales with well-developed keels, a great many struts, and posterior rim (SEM); and (**23**) A single body scale (SEM). Scale bars: (**18–19**) = 5 μm, (**20–21**) = 2 μm, and (**22–23**) = 1 μm.

The morphological characteristics of the *S. glabra* samples collected from the Long Korean Road (36.0619° N, 113.0049° E) in Changzhi, Shanxi Province, are illustrated in Figure 3 (7–9) and Figure 6. Its colonies were globular, 47–58 µm in diameter, and its cells were oval, 7–11 × 6–9 µm. Its body scales were ovate, 2.2–3.1 × 1.3–2.2 µm, with narrower edges. The keels, 1.1–2.1 × 0.3–0.6 µm, were less-developed and narrow. Its keels were decorated with medium-sized pores and were concave. The transverse folds were not connected with any of the struts (16–20), which extended regularly from the keels to the edges of the scales. Struts were spaced 0.14–0.28 µm apart.

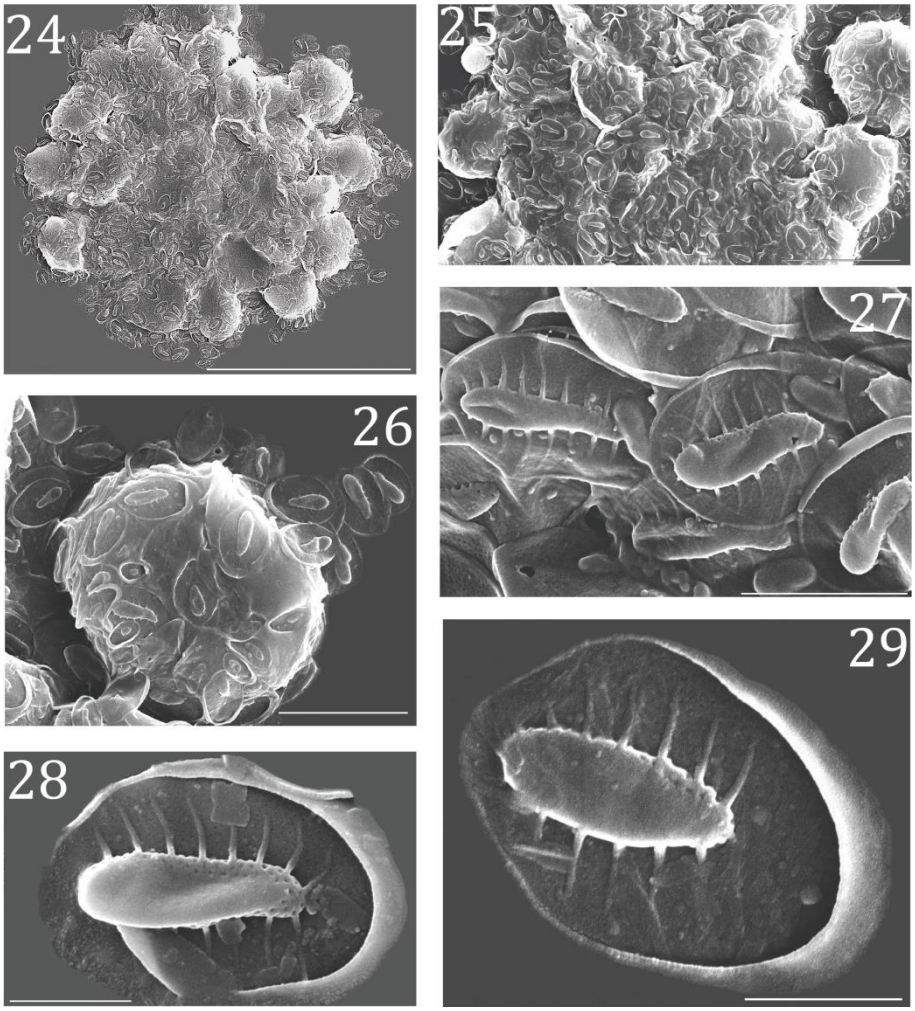

Figure 6. Morphological structures of *Synura glabra* collected from Shanxi, China. (**24–25**) Colony (SEM); (**26**) A single cell (SEM); (**27**) Body scales (SEM); (**28–29**) A single body scale with less-developed keels and several struts (SEM). Scale bars: (**24**) = 30 µm, (**25**) = 10 µm, (**26**) = 5 µm, (**27**) = 2 µm, (**28–29**) = 1 µm.

4. Discussion

The classification of *Synura* species has been based mainly on the ultrastructure of body scales, and the classification scheme changed four times from 1956 to 2016 [7,9–11]. Similarly, the classification of *Tessellaria* also changed continually in the last century. Playfair first established the genus *Tessella* in 1915 but changed the illegitimate genus name to *Tessellaria* in 1918 [47,48]. Subsequently, Playfair created a new family Tessellariaceae for the genus in 1921 [49]. Updating the classification scheme in 1974, Petersen and Hansen created section *Lapponicae* for *Synura lapponica* [10]. Kristiansen reclassified the genus *Tessellaria* as part of the family Synuraceae in 2007 [6]. However, this family classification was generally ignored [50–52]. In 2013, *S. lapponica* was transferred to *Tessellaria* [11]. Škaloud et al.

revised the previous classification scheme and removed the genus *Tessellaria* from the family Synuraceae on the basis of ultrastructural differences in scales and molecular evidence [11]. In 2016, Jo et al. proposed replacing the illegitimate name *Tessellaria* with *Neotessella* and proposed new sectional ranks [7]. In this study, we selected two species of the genus *Neotessella* as outgroups in our molecular phylogenetic analysis.

Previous taxonomic studies of the genus *Synura* have been based primarily on traditional morphology focusing on the ultrastructure of body scales [2]. In recent years, molecular tools have made great contributions to assessing phylogenetic placement [7,21]. The first molecular analysis was performed by Wee et al. to investigate genetic variability in *S. petersenii* [22]. In 2010, Boo et al. confirmed the high degree of cryptic, species-level diversity in the *S. petersenii* complex, creating the first multigene phylogeny [53]. The first taxonomic assessment was performed by Škaloud et al.; six genetic lineages of the *S. petersenii* species complex were recognized as separate species on the basis of morphological data and multiple genetic markers [20]. Škaloud et al. proposed three new species: *S. vinlandica*, *S. fluviatilis*, and *S. cornuta*, and discovered significant cryptic diversity within the core lineages of *Synura* [8]. Four new species, including *S. borealis*, *S. heteropora*, *S. hibernica*, and *S. laticarina*, were described and characterized in 2014 [16]. Jo et al. performed phylogenetic analyses on the basis of multiple gene sequences and proposed new sectional divisions: *Synura*, *Peterseniae*, and *Curtispinae* [7]. These publications clearly show that phylogenetic analyses using gene sequences have become increasingly popular and important in the identification and classification of *Synura* species.

In this study, we focused on the lineage of the genus *Synura*. According to the phylogenetic trees based on concatenated SSU, LSU, and ITS sequences, specimen GD201126 and *S. longitubularis*, a new species described by Jo et al. in 2016, were closely related [7]. Moreover, phylogenetic studies showed that *S. longitubularis* and *S. curtispina* shared a close relationship, which was consistent with phylogenetic trees presented by Jo et al. and Škaloud et al. [7,8]. Additionally, the results of maximum likelihood (ML) and Bayesian inference (BI) phylogenies based on concatenated SSU, LSU, and ITS sequences positioned GZ201017 and *S. petersenii* and SX210304 and *S. glabra*, respectively, within the same clades. Furthermore, both *S. petersenii* and *S. glabra* were assigned to section *Peterseniae*. The taxa collected from Guizhou was identified as *S. petersenii*, specimen SX210304 was confirmed to be *S. glabra*, and specimen GD201126 was identified as the first report of *S. longitubularis* in China, mainly on the basis of the molecular evidence. Note, however, these species identifications require more specimens and molecular data to improve their reliability, a task to be completed in future studies.

Traditional taxonomy relies on morphological observations. A key classification feature of the genus *Synura* is the presence or absence of keels or spines on the base plates of the scales. Therefore, the taxonomic statuses of the three *Synura* species in this study were further verified by morphological observations. The typical characteristics of *S. petersenii* are a well-developed keel and many struts, whereas the body scales in *S. glabra* have a less-developed keel and low silicification in *S. glabra*. *S. longitubularis* is characterized mainly by a blunt spine tip or a spine tip with 2–3 teeth. In 1929, Korshikov measured the size of *S. petersenii* scales to be 4 μm × 2 μm, which was consistent with the observations of Petersen and Hansen (3.6–4.7 × 2.2–2.5 μm) and Škaloud et al. (3.8–4.6 × 1.8–2.3 μm) [9,20,54]. The dimensions of *S. glabra* were 2.5–3.3 × 1.7–2.1 μm and 2.7–3.0 × 2.0–2.2 μm in the description of Petersen and Hansen and Škaloud et al., respectively [9,20]. In the description by Jo et al. in 2016, the body scales of *S. longitubularis* were 2.2–3.9 × 1.6–2.4 μm, and the spines were 0.9–1.6 × 0.3–0.5 μm [7]. The morphologies of GZ201017, SX210304, and GD201126 collected in this study were consistent with the characteristics of *S. petersenii*, *S. glabra*, and *S. longitubularis*, respectively. The scales of specimen GZ201017 and *S. petersenii* were similar in size, but the specimen in this study had smaller scales, longer keels (2.0–3.2 × 0.4–0.8 μm), and fewer struts (19–26). Specimen SX210304 was similar to *S. glabra*, exhibiting a less-developed keel and several struts (16–20), and the struts were spaced 0.14–0.28 μm apart and were not interconnected by transverse

ribs. In addition, the specimen representing the first report of *S. longitubularis* in China had body scales arranged in the shape of petals. Its spines were 0.6–1.5 × 0.2–0.4 μm in size, and the distal ends were blunt or had two small teeth on the tips. Morphological comparisons of silica scales revealed that all novel clades were broadly similar to the previously described taxa, with minor differences. Although morphology has historically been the central basis of *Synura* classification, similar morphological features and cryptic taxa may lead to incorrectly estimating the species diversity. Siver and Lott reported a high morphological diversity in scales of the genus *Synura* and recognized the necessity for molecular analyses [55]. Molecular approaches are highly sensitive to species, and morphological and molecular approaches can be used to complement each other. In some cases, molecular evidence must be referenced in the identification of the species' taxonomic statuses. Indeed, it is necessary to conduct molecular analyses of the *Synura* species to fully comprehend the species diversity of the genus in China.

The SSU, LSU, and ITS genes are frequently used as DNA barcodes in molecular phylogenetic analyses of freshwater Chrysophyta, which have also been widely used in the molecular studies of the genus *Synura* [7,8,16,20,21]. However, our knowledge of *Synura* diversity remains meager and limited. *S. elipidosa* and *S. falcata* collected in China were validly described by Skvortzov in 1961, but without electron microscopy analysis [27]. Until 2012, *S. elipidosa* was assigned to section *Peterseniae* and *S. falcata* to section *Synura*, but these assignments were not based on molecular data [20]. Similarly, the studies of statocysts by Pang and Wang did not involve molecular evidence [31,32]. The history of phylogenetic revision based on morphological observations emphasizes the critical need for molecular phylogenetic analyses of *Synura* species worldwide, especially in China. This study, by supplying the molecular sequences of three *Synura* species from China, has contributed data and a theoretical foundation for future taxonomic studies and further revealed the *Synura* species and geographic diversity in China.

5. Conclusions

Morphological features and molecular phylogenetic studies both agree in their assignments of taxonomic statuses of three *Synura* species in China. GZ201017, a specimen collected in Guizhou Province, was morphologically distinguished by a well-developed keel and lanceolate scales, whereas poor silicification and less-developed keels were exhibited by specimen SX210304. In addition, specimen GD201126 was distinguished mainly by spines whose distal end was blunt or had two small teeth on the tips. Given that the morphologies of the three specimens in this study were slightly different from their respective type specimens, the identification of these taxa collected from China was based primarily on molecular evidence and further verified by morphological characteristics. The molecular information of three *Synura* species from China was supplied in this study, providing molecular evidence and a theoretical basis for molecular phylogenetic analyses of the freshwater Chrysophyta genus. This will help enrich our knowledge of the species diversity and geographical distribution of the genus *Synura* in China.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d14121092/s1, Table S1: Pairwise distance (lower left matrix) and the number of nucleotide variance (upper right matrix) of concatenated SSU, LSU, and ITS sequences among the taxa in this study. Table S2: Summary of the ultrastructural features that distinguish apart the *Synura* taxa in the section *Peterseniae*. Table S3: Summary of the ultrastructural features that distinguish apart the *Synura* taxa in the section *Curtispinae*.

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