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Five newly recorded species of cyanobacteria in Korea

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Five newly recorded species of cyanobacteria in Korea are *Sphaerospermopsis reniformis* (Aphanizomenonaceae) from Chungju-si, Chungcheongbuk-do; *Pelatocladus maniniholoensis* (Hapalosiphonaceae) from Ulleung-gun, Gyeongsangbuk-do; *Tolypothrix carrinoi* (Tolypothrichaceae) and *Myxacorys chilensis* (Leptolyngbyaceae) from Suwon-si, Gyeonggi-do; and *Tildeniella torsiva* (Oculatellaceae) from Gunsan-si, Jeollabuk-do. These species are morphologically similar to each of its corresponding type species, and clustered in the same clade with respective type species in the phylogeny using 16S rRNA. The similarity of 16S rRNA sequences was more than 98.5% with each of its respective type species.

Keywords: 16S rRNA, cyanobacteria, freshwater, polyphasic, subaerophytic

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Introduction

Cyanobacteria can live in any environment where light is present. Their habitats range from land and water to extreme environments such as deserts and polar regions (Albertano, 2012). The classification of cyanobacteria is often difficult using only morphological and ecological features. Therefore, modern classification studies of cyanobacteria are conducted through a polyphasic approach by introducing molecular data along with morphological characteristics and ecological diversity (Castenholz, 1992; Komárek and Anagnostidis, 2005; Komárek *et al*., 2014; Komárek, 2016; Mareš, 2017). Komárek and colleagues proposed a new classification system for cyanobacteria which contain eight orders (Gloeobacteriales, Synechococcales, Spirulinales, Chroococcales, Pleurocapsales, Oscillatoriales, Chroococcidiopsidales, and Nostocales) based on electron microscope characteristics, molecular methods, and genetic methods, with classification supported by molecular sequence data (Komárek *et al*., 2014). Since new genera and species of cyanobacteria are continuously reported worldwide, more than 50 genera have been described since 2000, resulting in more than 300 genera to date (Mai *et al*., 2018). New species of cyanobacteria have been reported in Korea, including *Dolichospermum hangangense* (Choi *et al*., 2018), *Pinocchia daecheonga* (Kim *et al*., 2021), *Pseudoaliinostoc sejongens* (Lee *et al*., 2021), and *Wilmottia koreana* (Lee *et al*., 2020a). Unrecorded species have also been reported in Korea, such as *Aerosakkonema funiforme* (Kim *et al*., 2020), *Aphanizomenon gracile* (Lee *et al*., 2020b), *Leptolyngbya margaretheana* (Song and Lee, 2017), and *Toxifilum mysidocida* (Yim *et al*., 2018). So far, a total of 388 species of cyanobacteria have been reported (NIBR, 2022).

The genus *Sphaerospermopsis* (Aphanizomenonaceae, Nostocales) includes a total of seven species, including *S. reniformis* (Guiry and Guiry, 2022). It inhabits eutrophic lakes, rivers, and stagnant waters with high salinity (Lemmermann, 1898). *Pelatocladus maniniholoensis*, belonging to family Hapalosiphonaceae and order Nostocales, was isolated from the Maniniholo Cave wall in Hawaii (Miscoe *et al*., 2016). The genus *Tolypothrix* (Tolypothrichaceae, Nostocales) consists of 65 species, including *T. carrinoi* (Guiry and Guiry, 2022). It attaches to substrates of stones and plants in stagnant and flowing water bodies, and widely inhabits limestone areas, strongly alkaline waters, and cave walls (Bornet and Flahault, 1886; Miscoe *et al*., 2016). The genus *Myxacorys*, belonging to family Leptolyngbyaceae and order Synechococcales, has been isolated from desert soils (Alwathnani and Johansen, 2011; Patzelt *et al*., 2014). Species belonging to the genus *Myxacorys* were initially identified as a morphotype of *Pseudophormidium hollerbachianum*. However, they were later transferred to this genus through 16S rRNA sequences analysis and named *M. chilensis* and *M. californica* (Osorio-Santos *et al*., 2014; Pietrasiak *et al*., 2019). *M. almedinensis* isolated from a limestone surface of chapels of the Cathedral, Portugal was also added to this genus (Soares *et al*., 2019). The genus *Tildeniella*, belonging to Oculatellaceae, is a subaerophytic species that inhabits

soil around stone walls or plant roots. It has three reported species: *Tildeniella torsiva*, *T. nuda*, and *T. alaskaensis* (Mai *et al*., 2018; Strunecky *et al*., 2020).

In this study, subaerophytic cyanobacteria were isolated from a cement wall of a building and tree bark located in Suwon-si, Gyeonggi-do, Korea. Planktonic cyanobacteria were collected from a reservoir located in Gunsan-si, Jeollabuk-do and Chungju-si, Chungcheongbuk-do, Korea. Attached cyanobacteria were collected from a rock located in Dodong Port, Ulleungdo. They were isolated in a unialgal culture. They were identified through morphological characteristics through light microscopy and molecular analysis of the 16S rRNA gene sequences. As a result, five cyanobacteria (*Sphaerospermopsis reniformis*, *Pelatocladus maniniholoensis*, *Tolypothrix carrinoi*, *Myxacorys chilensis*, and *Tildeniella torsiva*) were added as unrecorded genera and species of cyanobacteria in Korea.

Materials and Methods

Sample collections and cultures

Samples were collected from July 2018 to August 2019 and cyanobacteria were isolated from a reservoir, stones around a river, cement wall, and tree bark (Tables 1, 2). Planktonic cyanobacteria were collected using a 25 μm mesh 30 cm diameter plankton net (Sournia, 1978) and attached cyanobacteria were isolated using a soft brush or spatula (Kiel and Gaylarde, 2006). The collected samples were sealed up in 50 mL conical tubes, stored in an icebox at 4°C, and transported to the laboratory. For unialgal culture, one strand of trichome was separated using a Pasteur pipette under a microscope and placed in a 24-well plate. After 1−2 weeks of culture in a 24-well plate, unialgal culture was checked, and transferred to a 50 mL cell culture flask (SPL, Korea) for mass culture (Lee *et al*., 2019). For the culture BG-11 medium was used (Stanier *et al*., 1971), and sustained at 20−25°C, under a 16 h : 8 h LD cycle with a photon flux density of 25 μmol $m^{-2}s^{-1}$ from white fluorescent lamps (Lee *et al*., 2019). The unialgal cultured strains were deposited to the Freshwater Bioresources Culture Collection (FBCC) of the Nakdonggang National Institute of Biological Resources of Korea.

Morphological analysis

In this study, morphologies for our eight strains of five species (Table 3) were observed at 100−1,000× magnification using an optical microscope (Olympus BX53, Olympus, Japan) and photographed at 200−1,000× magnification (Axio Cam HRC camera, Carl Zeiss, Germany; Olympus UC-90, Olympus, Japan) (Lee *et al*., 2020). The taxonomic classification system of cyanobacteria in this study followed Komárek *et al*. (2014), and referenced AlgaeBase (Guiry and Guiry, 2022). Also, for the identification of cyanobacteria, we referred to Komárek and Anagnostidis (2005), Zapomělová *et al*. (2009), Komárek (2013), Miscoe *et al*. (2016), Mai *et al*. (2018), and Pietrasiak *et al*. (2019).

Molecular methods

After transferring 1 mL of the cultured sample to a 1.5 mL microcentrifuge tube, the cells were concentrated via centrifugation (13,000 rpm, 10 minutes). The supernatant was removed from the centrifuged sample and the cells were disrupted using a homogenizer. Then, genomic DNA (gDNA) was extracted using the i-genomic Plant DNA Extraction Mini Kit (iNtRON, Korea).

Nuclear 16S rDNA sequences were amplified by polymerase chain reaction (PCR), which was performed using primers 27F1 (Neilan *et al*., 1997) and 23S600R (Lee *et al*., 2019). Each PCR tube was prepared for gene amplification with a total of 20 μL including sterile tertiary distilled water (17 μL), extracted gDNA (1 μL), and each

Table 1. Sampling sites in Korea from July 2018 to August 2019.

Date	Locality	GPS	Strain
2018-07-05	Songam-ri, Sinni-myeon, Chungju-si, Chungcheongbuk-do, Republic of Korea	37°00′05.7″N/127°42′21.1″E	FBCC-A194
2019-02-13	Iui-dong, Yeongtong-gu, Suwon-si, Gyeonggi-do, Republic of Korea	37°18′18.2″N/127°02′04.0″E	FBCC-A216, FBCC-A220
2019-02-14	Iui-dong, Yeongtong-gu, Suwon-si, Gyeonggi-do, Republic of Korea	37°18′17.9″N/127°02′05.3″E	FBCC-A206, FBCC-A207, FBCC-A208
2019-04-24	Wonuri, Hoehyeon-myeon, Gunsan-si, Jeollabuk-do, Republic of Korea	35°55′42.8″N/126°46′22.1″E	FBCC-A1474
2019-08-01	Dodong-ri, Ulleung-eup, Ulleung-gun, Gyeongsangbuk-do, Republic of Korea	37°28′54.9″N/130°54′29.1″E	FBCC-A1476

primer (10 pmole, 1 μL), and Maxime™ PCR PreMix Kit (i-StarTaq™ GH) (iNtRON, Korea). PCR was carried out using a Mastercycler gradient (Eppendorf, Germany) instrument. The PCR reaction was initially denatured at 94°C for 5 minutes, and then repeated 33 cycles at 94°C for 1 minutes, 55°C for 2 minutes and 72°C for 3 minutes to amplify the target 16S rDNA gene region. After gene amplification was completed, the reaction was terminated after maintaining at 72°C for 10 minutes. The amplified PCR products were observed through electrophoresis in 1.0% agarose gel, stained with ethidium bromide, and visualized under ultraviolet light on a transilluminator. Then, amplicons were purified with a MEGAquick-spin™ Plus DNA Fragment Purification Kit (iNtRON, Korea), and DNA sequences were analyzed on an ABI 3730XL automated sequencer (Applied Biosystems, CA). Editing and contig assembly of the 16S rRNA sequences fragments were carried out in SeqMan Program (DNASTAR, WI), and all the sequences determined here were deposited in the GenBank database (ON411415−ON411422) (Table 3).

Phylogenetic analyses

The nucleotide sequences obtained through sequence analysis were entered into Nucleotide BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) of the National Center for Biotechnology Information (NCBI) to identify species with relatively close genetic distances, and nucleotide sequence information of related species was collected. The cyanobacterial nucleotide sequences obtained in this study and collected through the NCBI database were rearranged using BioEdit v. 7.2.5 (Hall, 1999), then trimmed to make the same length, and DNA similarity was analyzed. Genetic distances of the aligned sequences were calculated using the Kimura 2-parameter model in MEGA X (Kumar *et al*., 2018). Phylogenetic analysis was performed using the 16S rRNA gene sequences, comprised of our eight strains of five species and other 24 relative genera obtained from GenBank. These sequences were rearranged and trimmed to the same length using BioEdit, then they were analyzed. Maximum likelihood (ML) analysis was conducted on a 16S rRNA gene data matrix using the general time reversible (GTR) nucleotide substitution model with RAxML ver. 7.0.3 (Stamatakis, 2006). Additionally, Bayesian analysis was performed with the same dataset in MrBayes ver. 3.1.2 (Huelsenbeck and Ronquist, 2001). TreeView ver. 1.6.6 was used to visualize the results obtained with RAxML ver. 7.0.3 and MrBayes ver. 3.1.2 (Page, 1996).

Results and Discussion

In this study, *Sphaerospermopsis reniformis*, *Pelatocladus maniniholoensis*, *Tolypothrix carrinoi*, *Myxacorys chilensis*, and *Tildeniella torsiva* isolated from Korea were identified through morphological and molecular analyses. As a result, two species (*S. reniformis* and *T. carrinoi*) were added as unrecorded species and three species (*P. maniniholoensis*, *M. chilensis*, and *T. torsiva*) were added as unrecorded genera and species in Korea.

Morphology and description

Order Nostocales Borzì
Family Aphanizomenonaceae Elenkin
Genus *Sphaerospermopsis* Zapomelová, Jezberová,

Table 2. The morphological and ecological characters of five unrecorded species in Korea.

Species	Trichome	Sheath	Branching	Cell	Heterocyte /Akinete	Slime cap	Habitat
Sphaerospermopsis reniformis	Screw-like coiled or straight	Mucilaginous	−	Spherical or barrel shape	+/+	−	Freshwater
Pelatocladus maniniholoensis	Uniseriate	Facultative	+ (Tb)	Rounded, rounded at the base, becoming cylindrical	+/−	−	Rock
Tolypothrix carrinoi	Straight	Firm	+ (Fb)	Barrel-shaped to cylindrical	+/−	−	Tree bark
Myxacorys chilensis	Straight, curved	Firm, mucilaginous	+ (Fb)	Barrel-shaped	−/−	+	Cement wall
Tildeniella torsiva	Straight, curved, or sometimes coiled	Firm	+ (Fb)	Cylindrical	−/−	−	Freshwater

Tb: True branching, Fb: False branching

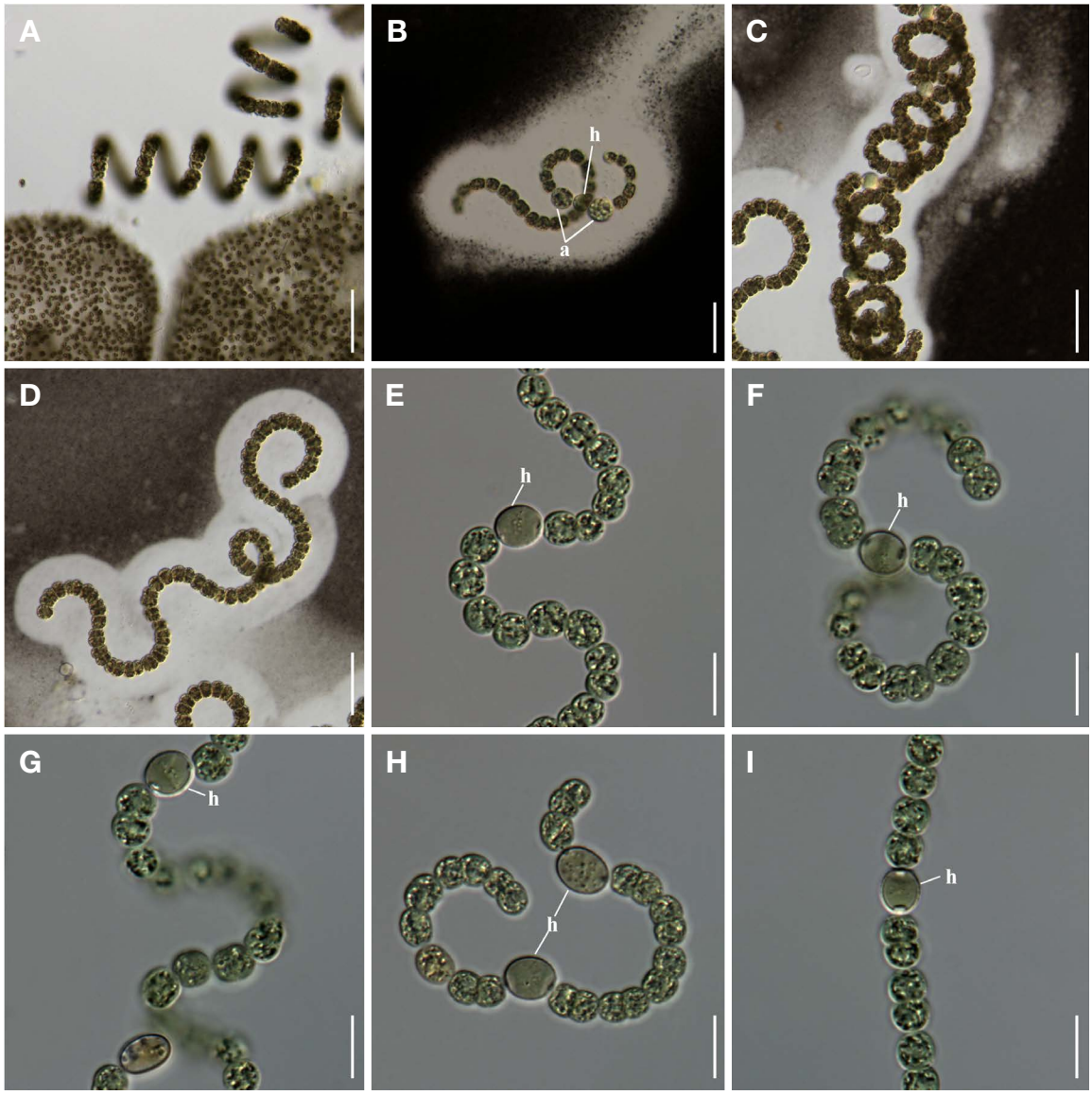

Fig. 1. Microphotographs of *Sphaerospermopsis reniformis* from the strain FBCC-A194. (A) Raw sample, (B) Akinetes (a) from both sides adjacent to heterocytes (h), (C, D) Mucilaginous sheaths, (E–I) Heterocytes and morphologies of trichomes. Scale bars = (A, C, D) 50 μm, (B) 20 μm, (E–I) 10 μm.

Hrouzek, Hisem, Reháková and Komárková, 2010

***Sphaerospermopsis reniformis* (Lemmermann) Zapomelová, Jezberová, Hrouzek, Hisem, Reháková and Komárková, 2010 (Fig. 1, Table 2)**

Anabaena reniformis Lemmermann, 1898.

Sphaerospermum reniforme (Lemmermann) Zapomelová, Jezberová, Hrouzek, Hisem, Reháková and Komárková, 2009.

Trichomes free-floating, rarely forming small clusters, irregularly screw-like coiled or circular fragments, clearly constricted at the cross-walls, not attenuated towards ends. Cells barrel-shaped to oval, sometimes slightly curved, with aerotopes, usually longer than wide, rarely isodiametric, 5.1–6.9 μm wide, 5.2–7.5 μm long. Apical cells similar to other vegetative cells, rarely a little longer, slightly curved and rounded. Heterocytes spherical or slightly elongated and barrel-shaped, solitary, intercalary, 5.9–8.7 μm wide, 6.2–9.9 μm long. Akinetes almost spherical, rarely slightly longer than wide, developing on both sides of heterocytes, solitary to 3 in row, 8.8–11.1 μm wide, 8.0–12.2 μm long.

Ecology. Planktonic in stagnant freshwater, rarely in running waters (Lemmermann, 1898), planktonic in freshwater in Korea.

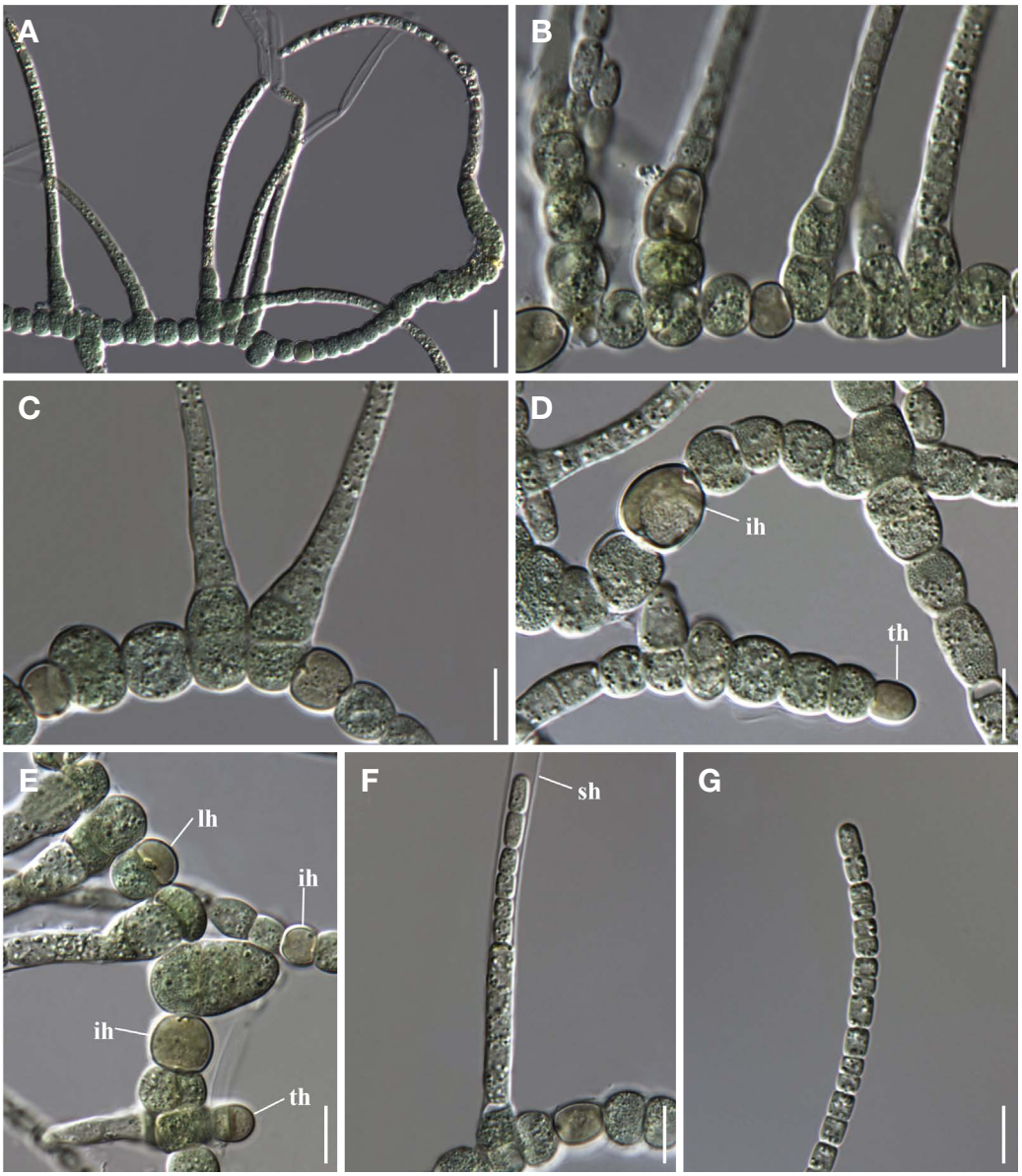

Fig. 2. Microphotographs of *Pelatocladus maniniholoensis* from the strain FBCC-A1476. (A–C) Various branching patterns of trichomes, (D, E) Intercalary (ih), lateral (lh), and terminal (th) heterocytes, (F) Hormogonia sheath (sh) branched from the main axis, (G) Hormogonia. Scale bars = (A) 20 μm, (B–G) 10 μm.

Distribution. Czech Republic (Zapomělová *et al*., 2009).
Site collection. Songam-ri, Sinni-myeon, Chungju-si, Chungcheongbuk-do (37°00′05.7″N/127°42′21.1″E).
Date of collection. July 05, 2018.

Family Hapalosiphonaceae Elenkin, 1916
Genus *Pelatocladus* Johansen and Vaccarino, 2016

Filaments of main axis uniseriate, densely laterally branched, clearly constricted at the cross-walls, lacking sheaths. Cells located in the main axis rounded. At the base cells of branched filaments rounded, becoming cylindrical and elongated. Heterocytes spherical or elongate-cylindrical, intercalary, lateral, or terminal.

Pelatocladus maniniholoensis
Johansen and Vaccarino, 2016 (Fig. 2, Table 2)

Colony bright blue-green in color. Filaments of main axis straight, uniseriate, mostly one-sided laterally branched, distinctly constricted cross-walls, lacking sheaths.

Fig. 3. Microphotographs of *Tolypothrix carrinoi* from the strains FBCC-A206, FBCC-A207, and FBCC-A208. (A−D) False branching, (E, F) Heterocytes (h), (G, H) Morphologies of trichomes, (I) Morphology of sheath. Scale bars = 10 μm.

Cells of main axis filaments 5−8 μm wide, 6−11 μm long. Filaments of branches never branching when attached to main axis, rounded at base, becoming cylindrical and elongated. Cells of branch filaments 3.5−5.1 μm wide, 4−13 μm long. Heterocytes intercalary, lateral and terminal, 5.1−7.7 μm wide, 9−12 μm long in the main axis, typically more elongated in the branched regions. Sometimes hormogonia with distinct sheaths.

Ecology. On the walls of a cave (Miscoe *et al*., 2016), attached to rocks in Korea.

Distribution. Hawaii (Miscoe *et al*., 2016).

Site of collection. Dodong-ri, Ulleung-eup, Ulleung-gun, Gyeongsangbuk-do (37°28′54.9″N/130°54′29.1″E).

Date of collection. August 01, 2019.

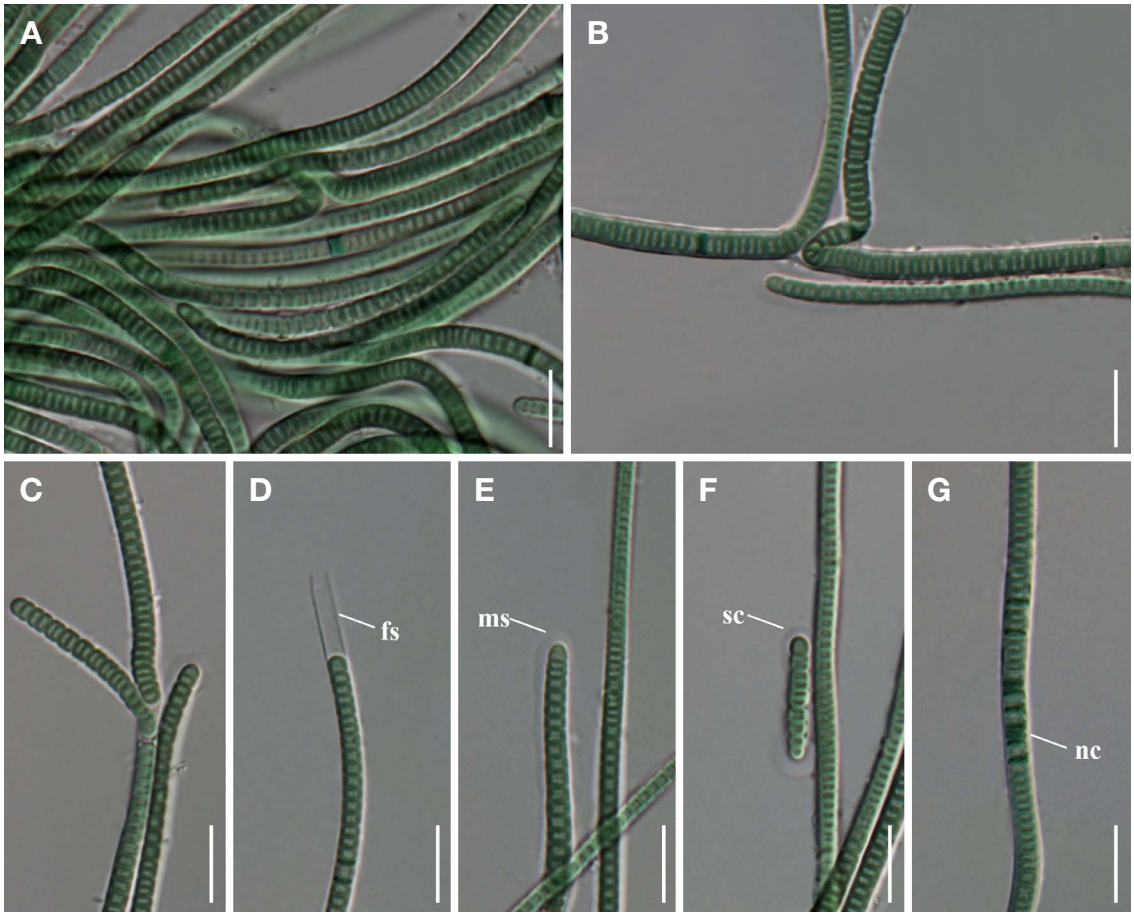

Fig. 4. Microphotographs of *Myxacorys chilensis* from the strains FBCC-A216 and FBCC-A220. (A) Arrangement of filament in the colony, (B, C) False branching, (D, E) Firm (fs) or mucilaginous (ms) sheaths, (F) Hormogonia and slime caps (sc), (G) Necridic cells (nc). Scale bars = 10 μm.

Family Tolypothrichaceae Hauer, Bohunická, J.R. Johansen, Mareš and Berrendero-Gomez, 2014
Genus *Tolypothrix* Kützing ex Bornet and Flahault, 1886

***Tolypothrix carrinoi* Miscoe, Pietrasiak and J.R. Johansen, 2016 (Fig. 3, Table 2)**

Filaments straight, heteropolar, with rare single and double false branching. Trichomes slightly to distinctly constricted at the cross-walls. Sheaths thin, colorless, clearly visible when cells are absent. Cells blue-green in color, compressed disc-like to shorter than wide when rapidly dividing, becoming barrel-shaped to cylindrical and longer than wide in old cultures, with finely granular, 11.1−13.3 μm wide, 4.1−12.8 μm long. Apical cells hemispherically rounded. Heterocytes spherical, located in filaments basal, one to three in a series, 11.2−14.0 μm wide, 9.6−12.5 μm long.
Ecology. Dry wall exposed to sunlight in the sinkhole of a cave (Miscoe *et al*., 2016), on tree bark in Korea.
Distribution. Hawaii (Miscoe *et al*., 2016).
Site collection. Iui-dong, Yeongtong-gu, Suwon-si, Gyeonggi-do (37°18′17.9″N/127°02′05.3″E).
Date of collection. February 14, 2019.

Order Synechococcales Hoffmann, Komárek and Kastovsky, 2005
Family Leptolyngbyaceae Komárek, J. Kastovsky, Mareš and J.R. Johansen, 2014
Genus *Myxacorys* Pietrasiak and J.R. Johansen, 2019

Filaments wavy, olive green in color, often form false branching. Trichomes straight, sometimes twisted, slightly constricted at the cross-walls. Sheaths colorless, thin. In young cultures, filaments with single trichome without sheaths, and later with one to two trichomes in a sheath. Cells shorter than wide to isodiametric, occasionally longer than wide when stressed. Apical cells rounded to conical, sometimes forming irregularly shaped involution

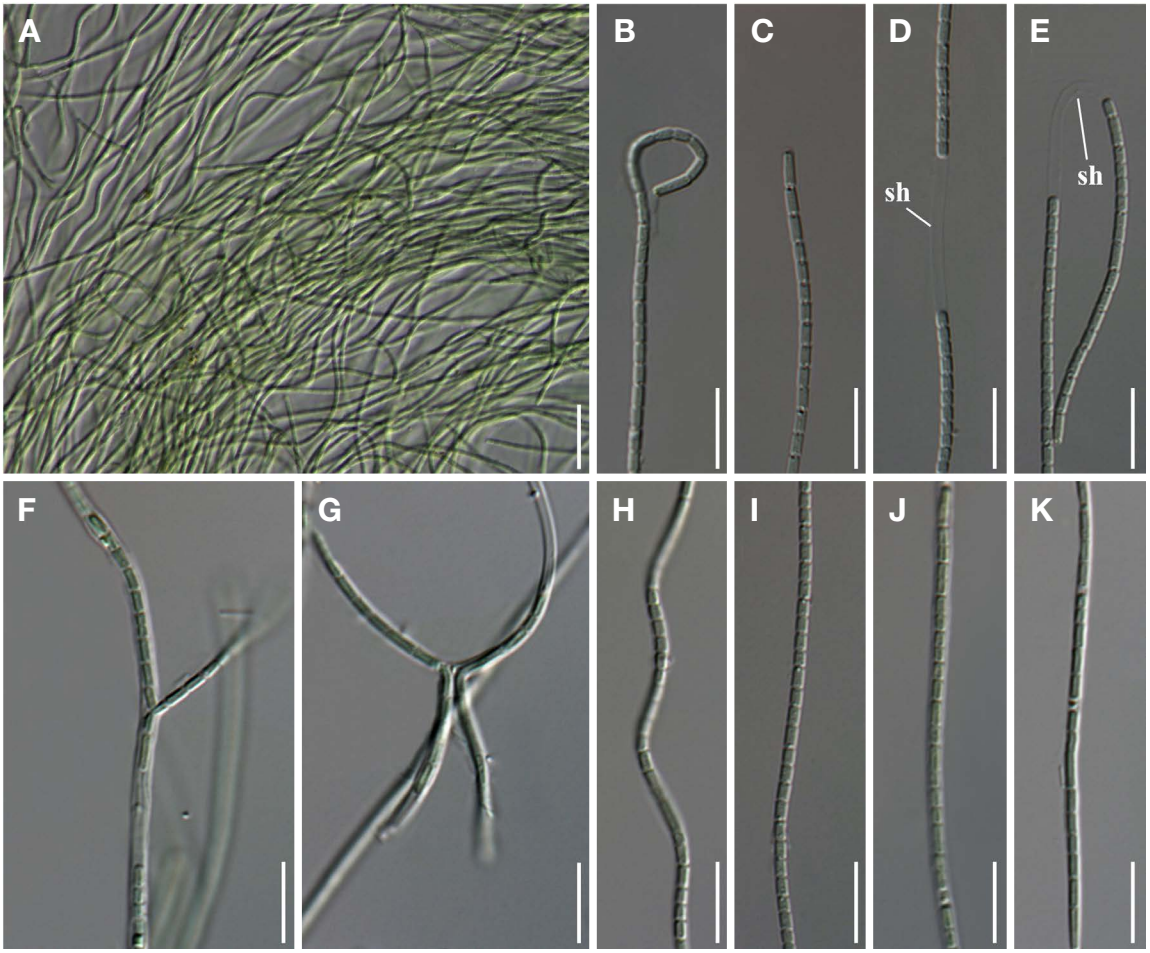

Fig. 5. Microphotographs of *Tildeniella torsiva* from the strain FBCC-A1474. (A) Arrangement of filament in the colony, (B) Curved terminal cells, (C) Apical cell, (D, E) Sheaths (sh), (F, G) False branching, (H–J) Morphologies of trichomes, (K) Diversity of cells morphology. Scale bars = (A) 20 μm, (B–K) 10 μm.

cells. Hormogonia widened at one end, tapering toward the opposite end. Slime caps lack in young cultures, and present in old cultures. Calyptra absent.

Myxacorys chilensis Pietrasiak and J.R. Johansen, 2019 (Fig. 4, Table 2)

Filaments with single trichome without sheaths in young cultures, and with one to two trichomes in a sheath in mature cultures, often form false branching. Trichomes straight, slightly curved, and constricted at the cross-walls. Sheaths firm or mucilaginous, colorless. Cells mostly shorter than wide or isodiametric, 1.8–3.3 μm wide, 1.0–2.7 μm long. Apical cells rounded, up to 3.9 μm long. Involution cells slightly widened, rare, indistinctive. Slime caps extending outward from the apical cells, relatively clear.

Ecology. Biological soil crust on fine sand (Pietrasiak *et al*., 2019), on cement wall in Korea.

Distribution. Chile (Pietrasiak *et al*., 2019).

Site collection. Iui-dong, Yeongtong-gu, Suwon-si, Gyeonggi-do (37°18′18.2″N/127°02′04.0″E).

Date of collection. February 13, 2019.

Family Oculatellaceae Mai and J.R. Johansen, 2018

Genus *Tildeniella* Mai, J.R. Johansen and Pietrasiak, 2018

Filaments straight, flexuous or spirally coiled. Trichomes not attenuated towards ends, not constricted to slightly constricted at the cross-walls, with thin translucent cross-walls. Sheaths thin, firm, colorless. Cells longer than wide, apical cells rounded. Hormogonia and necridic cells absent.

Tildeniella torsiva Mai, J.R. Johansen and Pietrasiak, 2018 (Fig. 5, Table 2)

Colony forming irregular fascicules, bright blue green in color, olive green with maturity. Filaments often entan-

gled, form false branching in older cultures. Trichomes straight, curved, waved, or sometimes coiled, not attenuated towards ends, slightly constricted at cross-walls. Sheaths thin, firm, colorless. Cells mostly longer than wide, rarely isodiametric, ungranulated, 1.1−1.9 μm wide, 1.6−4.9 μm long. Apical cells rounded. Hormogonia and necridic cells absent.

Ecology. On a limestone wall (Mai *et al*., 2018), planktonic in freshwater in Korea.

Distribution. Slovakia (Mai *et al*., 2018).

Site collection. Wonuri, Hoehyeon-myeon, Gunsan-si, Jeollabuk-do (35°55′42.8″N/126°46′22.1″E).

Date of collection. April 24, 2019.

16S rRNA and phylogenetic affiliation

In this study, 16S rRNA gene sequences of cyanobacteria were obtained. Molecular phylogenetic analysis of ML and Bayesian was performed on 16S rRNA gene sequences of the most similar and closely related species registered in NCBI (National Center for Biotechnology Information). As a result, two phylogenetic trees were completed, one tree containing species belonging to order Nostocales, and the other containing species belonging to order Synechococcales (Figs. 6, 7). Additionally, nucleotide sequence similarity and genetic distance were analyzed. When the similarity of the 16S rRNA gene sequence is 98.5% or more, it can be determined as the same species (Kim *et al*., 2014).

In the case of *Sphaerospermopsis reniformis* (FBCC-A194), molecular phylogenetic analysis was performed based on 16S rRNA gene sequences of *S. reniformis* and related taxa registered in NCBI. As a result, similar branch patterns were shown in all trees (Fig. 6). *S. reniformis* (FBCC-A194) collected in Korea was included in the same cluster as previously reported *S. reniformis* (06-01, 07-01). Results of sequence similarity and genetic distance analysis revealed that it showed a sequence similarity of 99.9−100% and a genetic distance of 0.00% to *S. reniformis* (06-01, 07-01) (Table 3). In this study, *S. reniformis* (FBCC-A194) also showed high sequence similarity and close genetic distance to *S. kisseleviana* and *S. aphanizomenoides* (similarity: 99.9% and 99.4%, respectively; genetic distance: 0.00% and 0.01%, respectively). However, for the morphology of trichomes, *S. kisseleviana* and *S. aphanizomenoides* are straight or slightly curved, whereas *S. reniformis* has a screw-like coil. Thus, they can be clearly distinguished morphologically (Zapomělová *et al*., 2009). *S. reniformis* (FBCC-A194) can prove that it is *S. reniformis* because it has screw-like coiled trichomes. The high 16S rRNA gene sequence similarity of *S. reniformis*, *S. kisseleviana*, and *S. aphanizomenoides* supports the previously reported content that the presence or absence of coiling of trichomes is not divided in the phylogenetic tree (Rajaniemi *et al*., 2005a; 2005b).

In the case of *Pelatocladus maniniholoensis* (FBCC-A1476), a phylogenetic analysis was performed through 16S rRNA gene sequences of *P. maniniholoensis* and related taxa registered in NCBI. All trees showed similar branching patterns (Fig. 6). Since *P. maniniholoensis* (FBCC-A1476) collected from Ulleungdo was included in the same cluster as the previously reported *P. maniniholoensis* (HA4357-MV3), the species could be clearly distinguished from the phylogenetic tree based on 16S rRNA gene sequences. As a result of additional sequence similarity and genetic distance analysis, it showed a sequence similarity of 99.5% and a genetic distance of 0.00% to *P. maniniholoensis* (HA4357-MV3) (Table 3). Also, *Hapalosiphon hibernicus* (BZ-3-1), which has the highest sequence similarity in the NCBI database, is 100% identical to 16S rRNA gene sequences of *P. maniniholoensis* (HA 4357-MV3). *H. hibernicus* (BZ-3-1) has been considered as a misidentification of *P. maniniholoensis* (Miscoe *et al*., 2016; Casamatta *et al*., 2020).

In the case of *Tolypothrix carrinoi* (FBCC-A206, FBCC-

Table 3. 16S rRNA gene sequence similarity and *p*-distance of five unrecorded species in Korea.

Order	Family	Most closely related species	Similarity	*p*-distance	Strain	Accession number
Nostocales	Aphanizomenonaceae	*Sphaerospermopsis reniformis*	99.9−100%	0.00%	FBCC-A194	ON411418
	Hapalosiphonaceae	*Pelatocladus maniniholoensis*	99.5%	0.00%	FBCC-A1476	ON411417
	Tolypothrichaceae	*Tolypothrix carrinoi*	98.9−99.2%	0.01%	FBCC-A206	ON411420
					FBCC-A207	ON411421
					FBCC-A208	ON411422
Synechococcales	Leptolyngbyaceae	*Myxacorys chilensis*	99.0−99.5%	0.00−0.01%	FBCC-A216	ON411415
					FBCC-A220	ON411416
	Oculatellaceae	*Tildeniella torsiva*	99.8%	0.00%	FBCC-A1474	ON411419

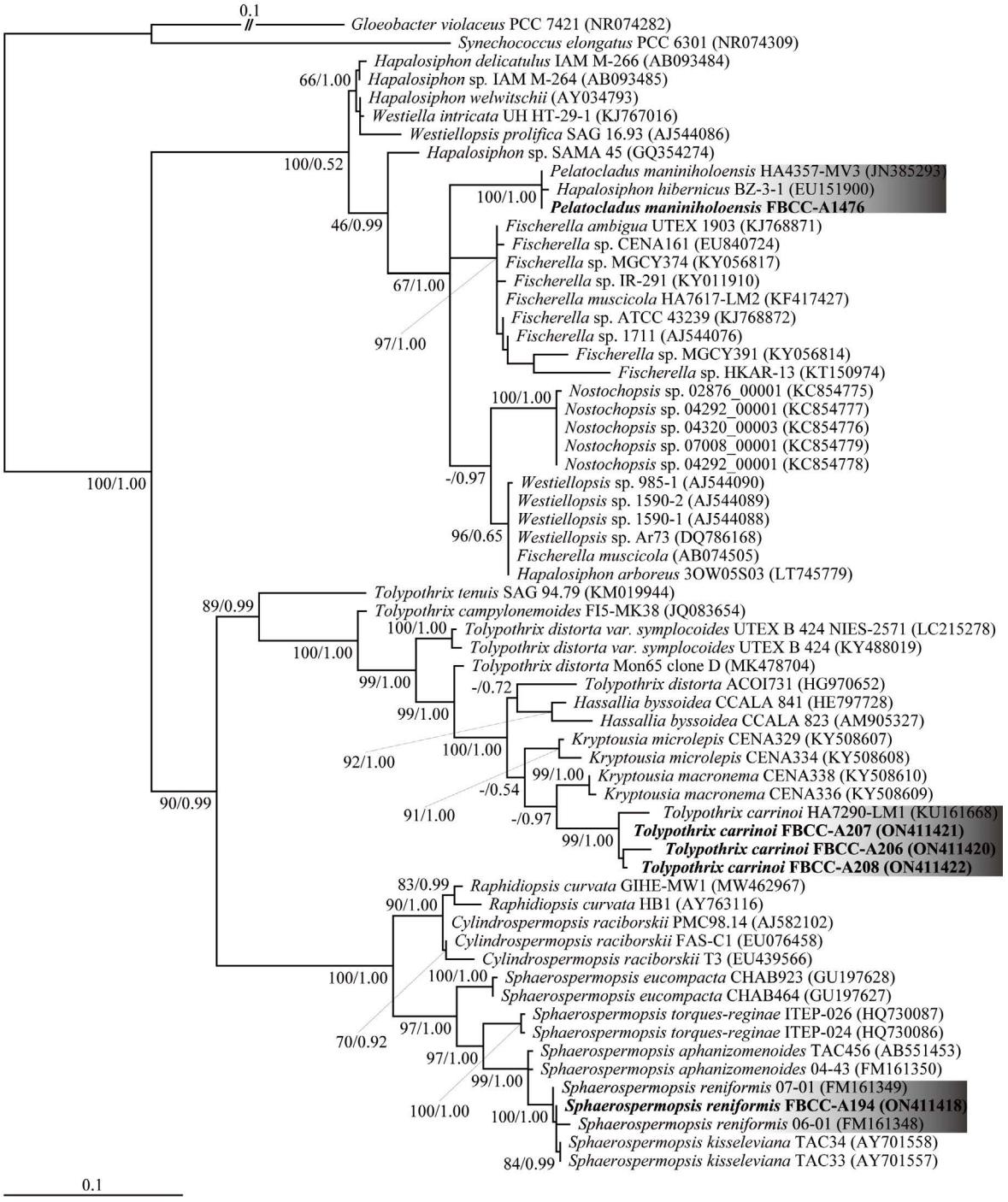

Fig. 6. Maximum-Likelihood (ML) phylogenetic tree based on 16S rRNA gene sequences of *Sphaerospermopsis reniformis*, *Pelatocladus maniniholoensis*, *Tolypothrix carrinoi* and other cyanobacterial strains. 16S rRNA gene sequences of genera (*Gloeobacter violaceus* PCC 7421, *Synechococcus elongatus* PCC 6301) were included as the outgroups. Additionally, the probability of Bayesian analysis was incorporated into the ML tree to support the strength of each branch. The first and second numbers at the nodes display the bootstrap proportions (>50%) in ML and posterior probabilities (>0.50) in Bayesian analysis, respectively. The branch lengths are proportional to the scale given.

A207, FBCC-A208), a molecular phylogenetic analysis was performed based on 16S rRNA gene sequences of *T. carrinoi* and related taxa registered in NCBI. As a result, all trees showed similar branching patterns (Fig. 6). *Tolypothrix carrinoi* (FBCC-A206, FBCC-A207, FBCC-A208) collected in Korea was included in the same clus-

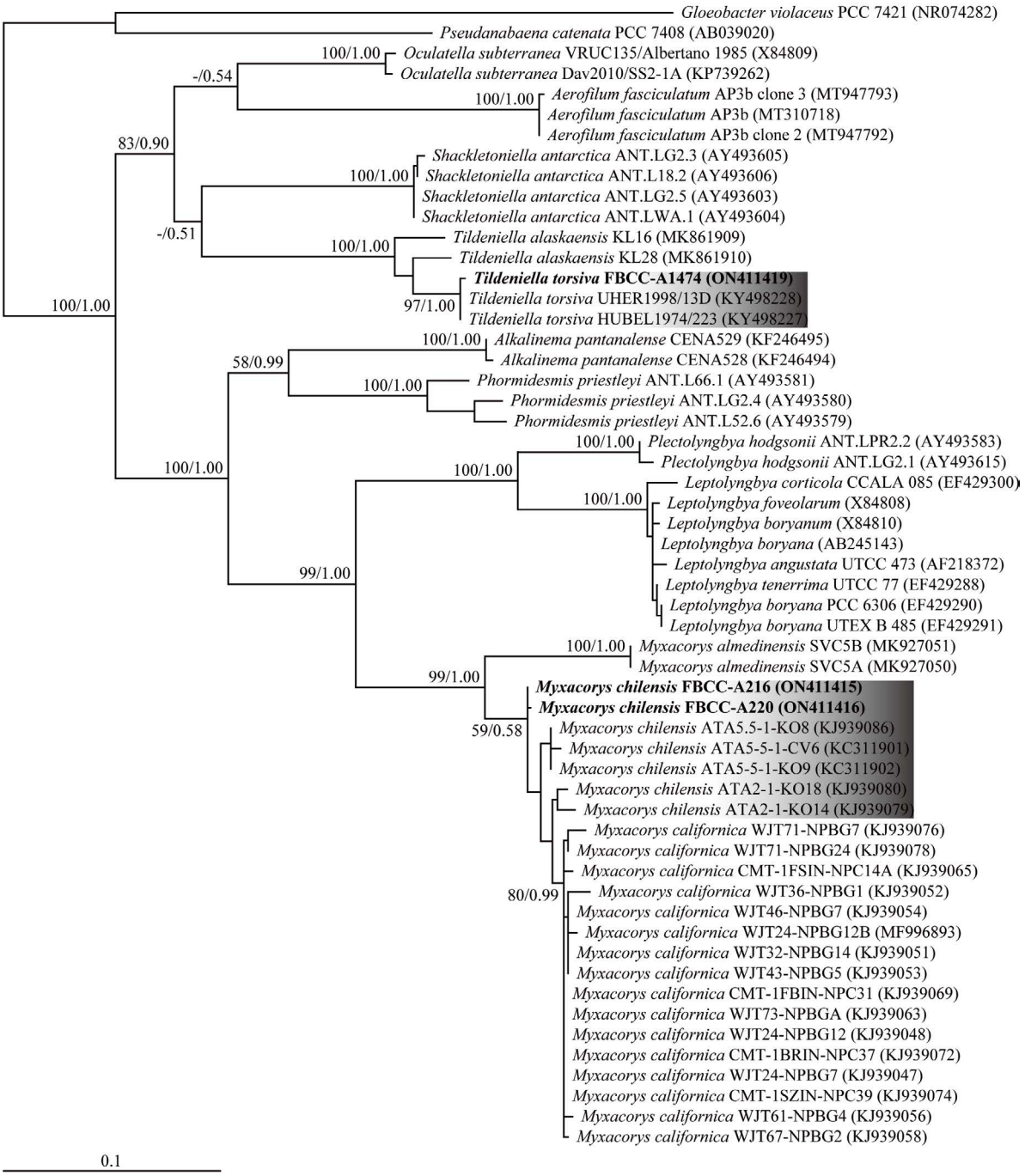

Fig. 7. Maximum-Likelihood (ML) phylogenetic tree based on 16S rRNA gene sequences of *Myxacorys chilensis*, *Tildeniella torsiva* and other cyanobacterial strains. 16S rRNA gene sequences of genera (*Gloeobacter violaceus* PCC 7421, *Pseudanabaena catenata* PCC 7408) were included as the outgroups. Additionally, the probability of Bayesian analysis was incorporated into the ML tree to support the strength of each branch. The first and second numbers at the nodes display the bootstrap proportions (>50%) in ML and posterior probabilities (>0.50) in Bayesian analysis, respectively. The branch lengths are proportional to the scale given.

ter as previously reported *T. carrinoi* (HA7290-LM1). As a result of additional sequence similarity and genetic distance analysis, it showed 98.9−99.2% similarity and 0.01% genetic distance with *Tolypothrix carrinoi* (HA7290-LM1) (Table 3).

In the case of *Myxacorys chilensis* (FBCC-A216, FBCC-A220), molecular phylogenetic analysis based on 16S rRNA gene sequences of *M. chilensis* and related

taxa registered in NCBI was performed. All trees showed similar branching patterns (Fig. 7). *M. chilensis* (FBCC-A216, FBCC-A220) collected in this study was included in the same cluster as previously reported *Myxacorys chilensis* (ATA2-1-KO14). It is clearly separated from *M. californica* and *M. almedinensis*, other species included in the genus *Myxacorys*. Therefore, it could be clearly distinguished through the phylogenetic tree based on the 16S rRNA gene sequences. Additional nucleotide sequence similarity and genetic distance analysis results showed a similarity of 99.0–99.5% and a genetic distance of 0.00–0.01% with *M. chilensis* (Table 3).

In the case of *Tildeniella torsiva* (FBCC-A1474), molecular phylogenetic analysis was performed based on 16S rRNA gene sequences of *T. torsiva* and related taxa registered in NCBI. All trees showed similar branching patterns (Fig. 7). The culture strain of this study, *T. torsiva* (FBCC-A1474), was included in the same cluster as previously reported *T. torsiva* (UHER1998/13D). It is clearly distinguished from *T. alaskaensis* included in the genus *Tildeniella*. Another species included in the genus *Tildeniella*, *T. nuda*, was excluded because it was included in a distant cluster from the genus *Tildeniella*. Additionally, as a result of analysis of nucleotide sequence similarity and genetic distance, it showed a similarity of 99.8% and a genetic distance of 0.00% with *T. torsiva* (Table 3). Stackebrandt and Goebel (1994) have stated that a taxon is classified as a different genus when the 16S rRNA gene sequence similarity is less than 95%. In this study, *T. nuda* (Zehnder 1965/U140) showed 91.8% nucleotide sequence similarity and 0.07% genetic distance to *T. torsiva* (UHER1998/13D). Strunecky *et al*. (2020) have mentioned that *T. nuda* shows 91.3% similarity to *T. torsiva*. It should be classified as a different genus because it has less than 95% sequence similarity to *T. torsiva*, a type species of genus *Tildeniella*. Mai *et al*. (2018) first reported *T. nuda* and stated that *T. nuda* 16S rRNA gene shared 99.1% sequence similarity with that of *T. torsiva*. This result is considered to be in error.

As described above, the five species collected in Korea were identified through morphological characteristics and phylogenetic analysis based on 16S rRNA gene sequencing. They were added to unrecorded genus and species in Korea.

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