Journal of Species Research 15(3):212-223, 2026
https://doi.org/10.12651/JSR.2026.15.3.212

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Freshwater *Pseudanabaena* (Pseudanabaenales, Cyanobacteriophyta) species identified from Korea using a polyphasic approach

Do-Hyun Kim[1], Bora Jang[2], Jae-Shin Kang[3], Ga Yeon Cho[4] and Eun Hee Bae[5,*]

[1]*Research Institute of EcoScience Ewha Womans University, Seoul 03760, Republic of Korea*
[2]*Biodiversity Conservation Research Division, National Institute of Biological Resources, Incheon 22689, Republic of Korea*
[3]*National Biodiversity Center, National Institute of Biological Resources, Incheon 22689, Republic of Korea*
[4]*Biodiversity Education Division, National Institute of Biological Resources, Incheon 22689, Republic of Korea*
[5]*Biological Resources Utilization Division, National Institute of Biological Resources, Incheon 22689, Republic of Korea*

**Correspondent: ehbae@korea.kr*

Six filamentous cyanobacterial strains belonging to four species of the genus *Pseudanabaena*—*P. cinerea*, *P. gygaxiana*, *P. pruinosa*, and *P. yagii*—were isolated from freshwater habitats in Korea. The morphological characteristics of these strains, including trichome shape, cell dimensions, and pigmentation, were consistent with those of the respective type strains. Species identification was conducted using a polyphasic approach integrating morphology and 16S rRNA gene sequence data. All strains exhibited high sequence similarity ($\geq$98.7%) to the corresponding type strains, and phylogenetic analysis clearly supported their placement within their respective species. Among the identified taxa, *P. gygaxiana* and *P. pruinosa* are newly recorded species in Korea, while *P. cinerea* and *P. yagii* represent additional records that confirm their presence in the Korean freshwater cyanobacterial flora.

Keywords: 16S rRNA, Cyanobacteriophyta, freshwater, polyphasic approach

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Introduction

Cyanobacteriophyta are photosynthetic prokaryotes distributed worldwide, inhabiting diverse environments ranging from polar extreme habitats to tropical regions, including marine, freshwater, and terrestrial ecosystems (Komárek *et al*., 2014). Some genera exhibit broad environmental adaptability and can occur in almost all types of ecosystems, with the genus *Pseudanabaena* being a representative example (Komárek and Anagnostidis, 2005).

The genus *Pseudanabaena* was established as the family Pseudanabaenaceae by Anagnostidis and Komárek (1988) based on morphological features, characterized by very narrow cells usually less than 3 μm wide, distinct cell boundaries, and simple filamentous structures. Recent phylogenetic analyses further reclassified the order Pseudanabaenales into two families: Pseudanabaenaceae, occurring mainly in freshwater and soils, and Thalassoporaceae, distributed in marine and brackish environments (Strunecký *et al*., 2023).

However, many cyanobacterial genera, including *Pseudanabaena*, show considerable morphological variation depending on environmental conditions, making species-level identification difficult based solely on morphology (Aleksovski *et al*., 2024). To overcome this limitation, a polyphasic approach integrating morphological, ecological, and molecular data has been applied, and more reliable species delimitation can be achieved when 16S rRNA gene sequence analyses are combined with ITS secondary structures, ultrastructural features, and ecological informa-

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tion (Komárek, 2016). In particular, *P. cinerea* and *P. yagii* are recognized as ecologically important taxa because they produce odorous compounds such as 2-methylisoborneol (MIB), which negatively affect water quality (Tuji and Niiyama, 2018).

Globally, 48 species of the genus *Pseudanabaena* have been reported (Guiry and Guiry, 2025), but only nine species are recorded in Korean freshwater environments (NIBR, 2024), and these are confined to specific regions. This highlights the lack of sufficient studies on the diversity and distribution of *Pseudanabaena* in Korea.

In this study, morphological characteristics and phylogenetic analyses based on 16S rRNA gene sequences were conducted for *Pseudanabaena pruinosa* and *P. gygaxiana* strains isolated from freshwater habitats in Korea. The results revealed that both species are newly recorded taxa in Korea. The morphological, ecological, and phylogenetic evidence presented here contributes to a better understanding of cyanobacterial taxonomy in Korean freshwater ecosystems. Furthermore, by adding these two species to the previously recorded *P. cinerea* and *P. yagii*, this study demonstrates that the diversity of *Pseudanabaena* in Korea is greater than previously recognized.

Materials and Methods

Sample collection

Specimens were collected from freshwater habitats between August 2020 and November 2023 (Table 1) and were immediately transported to the laboratory in a cooled ice box. Preservation was performed in accordance with the standard procedures outlined in the institutional manual (NIBR, 2009). All specimens were registered in the NIBR Biological Resources Management System under unique serial numbers, and cultures were deposited in the National Biological Resources Biobank (NIBR Biobank).

Morphological analysis and characterization

Morphological characteristics of cyanobacterial strains were examined using an optical microscope (Axio Imager. A2, Carl Zeiss Microscopy GmbH, Germany) equipped with a digital camera (OPTIMIS-5M, Optinity, Korea), and images were obtained at 200−1000× magnification. For morphological measurements, trichome and cell dimensions were recorded from well-preserved filaments after two weeks of culture. Cell length and width were measured only from mature, non-dividing cells to minimize variation associated with cell division stages, and 50−100 cells per strain were measured to account for natural morphological variability. The taxonomic classification system of Cyanobacteriophyta followed Strunecký *et al*. (2023) throughout, and referred to Komárek *et al*. (2014) and AlgaeBase (Guiry and Guiry, 2025). Also, the identification of Cyanobacteriophyta was referred to Casamatta *et al*. (2005), Tuji and Niiyama (2018), and Aleksovski *et al*. (2024).

DNA extraction

For genomic DNA (gDNA) extraction, isolated single filaments of Cyanobacteriophyta were picked under a light microscope (Axio Imager.A2, Carl Zeiss Microscopy GmbH, Germany) equipped with a digital camera (OPTI MIS-5M, Optinity, Korea) and washed with distilled water. Each sample was transferred into a 1.5 mL microcentrifuge tube containing 100 μL of 10% Chelex 100 DW

Table 1. Strains with sampling sites and collection information in freshwater.

Strain	Species	Location	GPS	Sampling date
NIBRCYC000514100	*Pseudanabaena cinerea*	601-4, Samhoe-ri, Cheongpyeong-myeon, Gapyeong-gun, Gyeonggi-do, Republic of Korea	37°39′43.7″N 127°22′45.1″E	23.07.03
NIBRCYC000514101	*P. cinerea*	354-16, Danhyeon-dong, Yeoju-si, Gyeonggi-do, Republic of Korea	37°16′28.6″N 127°40′58.9″E	23.11.20
NIBRCYC000514099	*P. cinerea*	San 29-2, Saeum-ri, Hoenam-myeon, Boeun-gun, Chungcheongbuk-do, Republic of Korea	36°25′60.0″N 127°33′02.9″E	21.07.27
NIBRCYC000514097	*P. gygaxiana*	602-1, Eosil-ro, Yangsan-si, Gyeongsangnam-do, Republic of Korea	35°24′58.6″N 128°59′56.9″E	20.08.25
NIBRCYC000514098	*P. pruinosa*	4, Acheon-dong, Guri-si, Gyeonggi-do, Republic of Korea	37°34′13.4″N 127°07′39.3″E	23.04.12
NIBRCYC000514102	*P. yagii*	354-16, Danhyeon-dong, Yeoju-si, Gyeonggi-do, Republic of Korea	37°16′28.6″N 127°40′58.9″E	23.03.21

solution (Chelex 100 Resin, Bio-Rad Laboratories, USA) (Walsh *et al*., 1991). The samples were vortexed for 10 sec and incubated at 99°C for 30 min. After incubation, the tubes were placed on ice for 5 min, followed by centrifugation at maximum speed for 5 min. The supernatant was carefully transferred to a new microcentrifuge tube using a micropipette, avoiding the Chelex 100 pellet, and used as the DNA template for PCR analysis.

Polymerase chain reaction (PCR) and sequencing

The 16S rDNA gene region was amplified using the primer pair CYA106F (Nübel *et al*., 1997) and FDSKS_CyaR1 (Shiels *et al*., 2019). PCR reactions contained 1 μM of each primer, 2.5 mM of each dNTP, 25 mM $MgCl_2$, 10 × buffer (Mg^{2+}-free), i-star^{TM} DNA polymerase (iNtRON Biotechnology, Korea), and template DNA in a final reaction volume prepared according to the manufacturer's protocol. Amplification was performed using two thermal cyclers: the ProFlex PCR System and the GeneAmp PCR System 9700 (both from Applied Biosystems, USA), under the following cycling conditions: initial denaturation at 95°C for 3 min; 35 cycles of denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 1 min 30 sec; followed by a final extension at 72°C for 10 min. PCR products were visualized on a 1% agarose gel containing dye and observed under UV illumination. Amplified products were submitted to Macrogen (Seoul, Korea) and Bioneer (Daejeon, Korea) for sequencing. Forward and reverse sequences were assembled and manually edited using Chromas (Technelysium Pty Ltd., Australia) and BioEdit ver. 7.2.5 (Hall, 1999).

Data analyses and phylogenetic analyses

For analysis of the complete 16S rRNA gene sequences, each sequence was analyzed using NCBI's Nucleotide BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to identify related species, and corresponding sequences of those species were retrieved from GenBank. To assess nucleotide sequence similarity and genetic distance, the retrieved sequences were aligned with MAFFT ver. 7 (Katoh and Standley, 2013), and based on this alignment, sequence similarity was measured in BioEdit ver. 7.2.5 (Hall, 1999), while genetic distances were calculated using the Kimura 2-parameter model in MEGA X (Kumar *et al*., 2018).

For Maximum Likelihood (ML) phylogenetic analysis, the retrieved sequences were analyzed in RAxML ver. 7.0.3 (Stamatakis, 2006) under the General Time Reversible (GTR) + MIX model, and the resulting phylogenetic tree was visualized with TreeView ver. 1.6.6 (Page, 1996). Bayesian inference (BI) phylogenetic analysis was conducted on the same dataset using MrBayes ver. 3.1.2 (Huelsenbeck and Ronquist, 2001), and posterior probabilities (PP) were estimated through a Markov Chain Monte Carlo (MCMC) process.

The final alignment contained 1,494 bp after trimming and was used for ML and BI phylogenetic analyses. Sequence identity values in Table 4 were calculated using an 810 bp fragment starting from the conserved CTCTTTT motif to maintain compatibility with previous comparative studies on *Pseudanabaena* (Aleksovski *et al*., 2024).

Nomenclatural and taxonomic standards

The nomenclatural framework of this study follows the International Code of Nomenclature for algae, fungi, and plants (ICN) (Turland *et al*., 2018). In accordance with recent ICN recommendations for cyanobacterial classification, the typified phylum name Cyanobacteriophyta Oren *et al*. (2022) is adopted for the taxa examined in this study (Aleksovski *et al*., 2025).

Results and Discussion

In this study, the morphological and ecological characteristics of four *Pseudanabaena* species collected from freshwater habitats in Korea were examined using unialgal cultures, and species identification was performed through 16S rRNA gene-based phylogenetic analyses. As part of the polyphasic approach, ecological information recorded during sampling—including habitat type (lake, stream, or wetland), GPS coordinates, and collection dates—was incorporated to provide environmental context for interpreting morphological variation among strains. These habitat-level observations served as the ecological component of the polyphasic framework. *Pseudanabaena gygaxiana* and *P. pruinosa* are newly recorded species in Korea, whereas *P. cinerea* and *P. yagii* were previously reported but additionally confirmed in this study.

1. Morphological and ecological characterization

Order Pseudanabaenales Hoffmann, Komárek and Kaštovský, 2005
Family Pseudanabaenaceae Anagnostidis and Komárek, 1988
Genus *Pseudanabaena* Lauterborn, 1915

***Pseudanabaena cinerea* Tuji and Niiyama, 2018 (Fig. 1)**

Trichomes brownish green or greenish gray to gray, straight, slightly curved, without sheath and calyptra, not attenuated toward the ends, distinctly constricted at cross-walls, usually consisting of 10−30 cells, rarely exceeding 30 cells. Cell cylindrical, mostly longer than wide (iso-

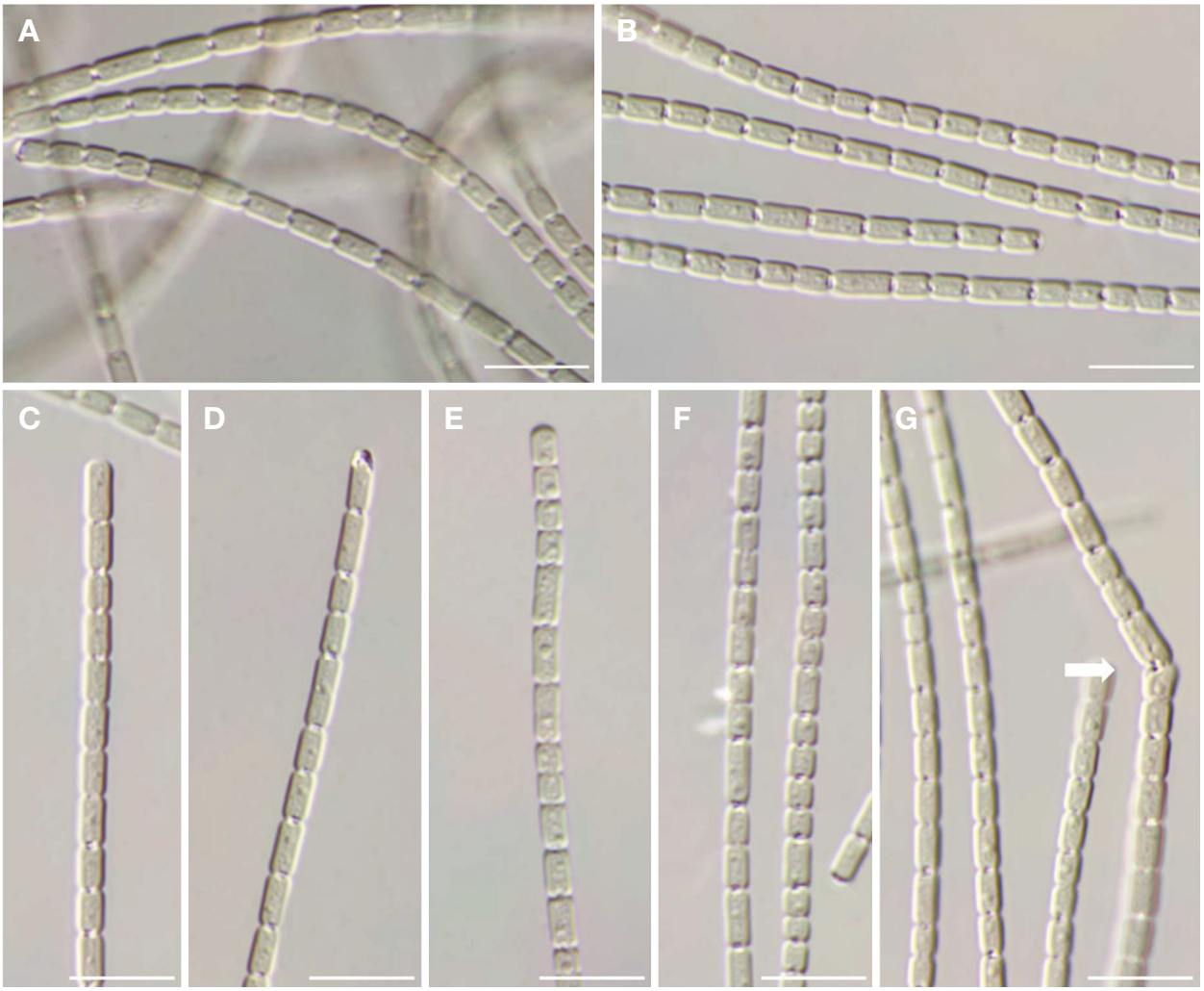


Fig. 1. Microscopic photographs of *Pseudanabaena cinerea* from the strain NIBRCYC000514099. (A, B) Trichomes straight or slightly curved, without sheath, (C–E) Rounded to conical apical cells, (F) Detail of trichomes, (G) Trichomes showing branching trichomes (arrow). Scale bars = 10 μm.

diametric at the early stage of cell division), 2.0–2.5 μm wide and 2.0–6.5 μm long, ratio of length to width 1.0 (3.0), with aerotopes at both ends of cells, sometimes with one to several small granules. Apical cells mostly rounded, rarely conical, 2.0–3.0 μm wide and 3.0–6.0 μm long. Reproduction by separation of trichomes at intercellular junctions or by fragmentation without necridic cells.

Ecology. This species was previously reported as plankton in lakes and reservoirs in Japan (Tuji and Niiyama, 2018), and in this study, it was also isolated from freshwater lakes and streams in Korea. Additionally, this species is known to produce the odorous compound 2-methylisoborneol (2-MIB), as reported from Japanese strains (Tuji and Niiyama, 2018).

Distribution. Japan (Lake Ogawara, Aomori; Serikawa reservoir) (Tuji and Niiyama, 2018), Korea (Bukhan River basin; Uiam Lake) (Kim *et al*., 2020).

Site of collection. Saeum-ri, Hoenam-myeon, Boeun-gun, Chungcheongbuk-do (36°25′60.0″N, 127°33′02.9″E); Samhoe-ri, Cheongpyeong-myeon, Gapyeong-gun, Gyeonggi-do (37°39′43.7″N, 127°22′45.1″E); Danhyeon-dong, Yeoju-si, Gyeonggi-do (37°16′28.6″N, 127°40′58.9″E).

Date of collection. July 27, 2021; July 3, 2023; November 20, 2023.

Specimen deposit No. NIBRCYC000514099, NIBRCYC000514100, NIBRCYC000514101.

Pseudanabaena gygaxiana (Casamatta and Johansen) Aleksovski, Krstic, and Strunecký, 2024 (Fig. 2)

Trichomes bright blue-green, straight, slightly curved, without sheath and calyptra, not attenuated toward the ends, distinctly constricted at cross-walls, usually consisting of 20–30 cells, rarely exceeding 50 cells. Cell cylindrical to

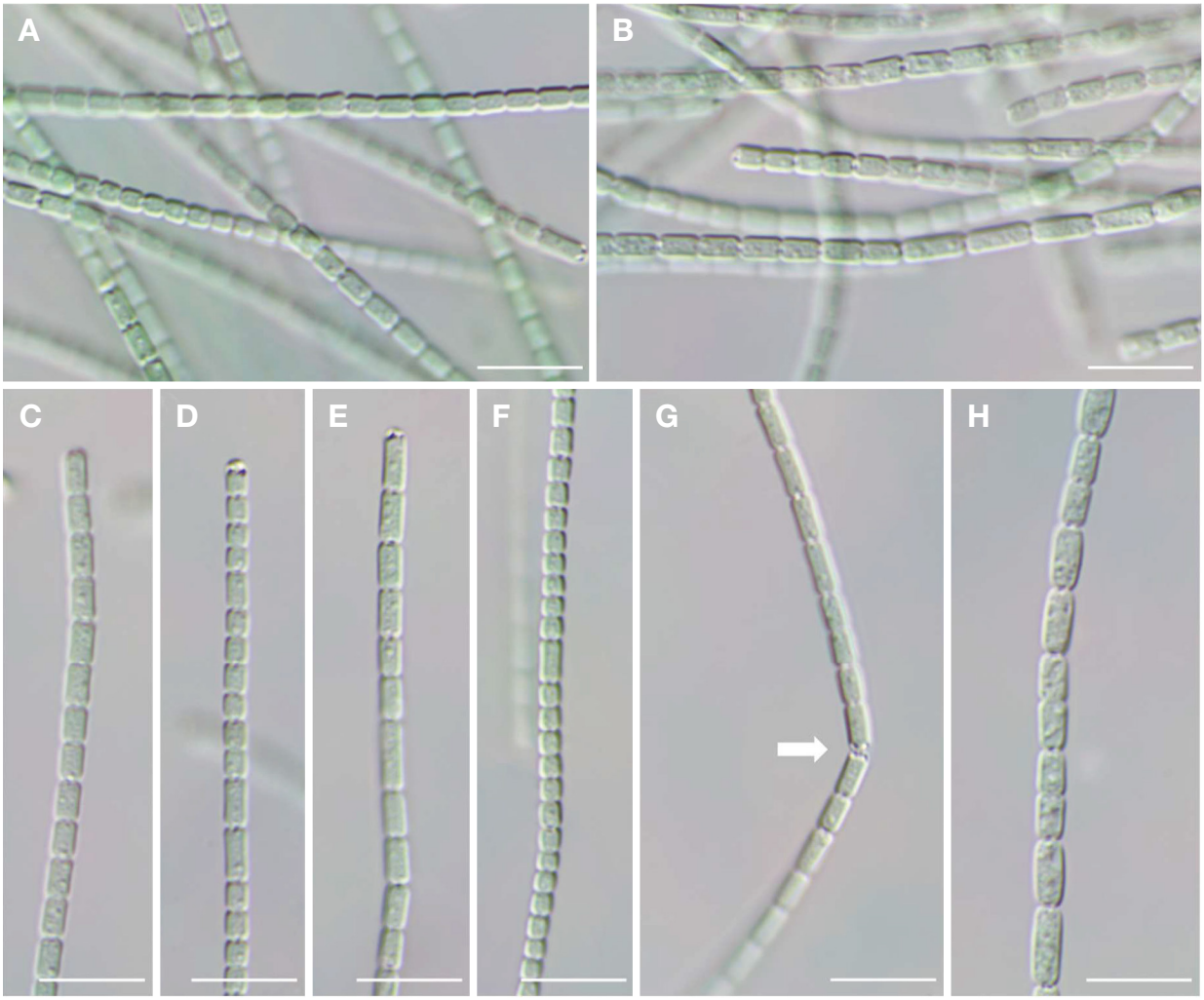


Fig. 2. Microscopic photographs of *Pseudanabaena gygaxiana* from the strain NIBRCYC000514097. (A, B) Trichomes straight or slightly curved, (C–E) Apical cells, (F) Detail of trichome, (G) Trichomes showing branching trichomes (arrow), (H) Cells observed in old cultures. Scale bars = 10 μm.

barrel-shaped, rarely spherical, mostly longer than wide (isodiametric at the early stage of cell division), 1.5–2.0 μm wide and 2.0–6.5 μm long, ratio of length to width 1.5 (3.0), with aerotopes at both ends of cells. Apical cells mostly rounded, rarely conical, 1.5–2.5 μm wide and 2.0–6.5 μm long. Reproduction by separation of trichomes at intercellular junctions or by fragmentation without necridic cells.

Ecology. This species was recorded as an epiphyte on floating leaves of *Nuphar variegata* Durand in freshwater habitats of Canada (Casamatta *et al*., 2005). In this study, it was collected from wetlands in Korea. Odorous compound production (geosmin or 2-MIB) has not been reported for this species, and this characteristic was not evaluated in the present study.

Distribution. Canada (Tasso Lake, Lake of Bays Township, Ontario) (Casamatta *et al*., 2005).

Site of collection. Eosil-ro, Yangsan-si, Gyeongsangnam-do (35°24′58.6″N, 128°59′56.9″E).

Date of collection. August 25, 2020.

Specimen deposit No. NIBRCYC000514097.

Pseudanabaena pruinosa Aleksovski, Krstic, Komárek, and Strunecký, 2024 (Fig. 3)

Filaments 1.0–2.0 μm wide, with facultative, fine, colourless sheath. Trichomes blue-green, uniseriate, isopolar, solitary, not attenuated toward the ends, distinctly constricted at cross-walls, either short (3–5 cells) or long (20–40 cells). Cell cylindrical to barrel-shaped, rarely spherical. Mostly isodiametric, occasionally longer than wide, with aerotopes at both ends of cells. Cell 1.0–1.5 μm wide and 1.5–3.0 μm long, ratio of length to width 1.5 (3.0). Apical cell spherical to slightly oval, sometimes slightly conical

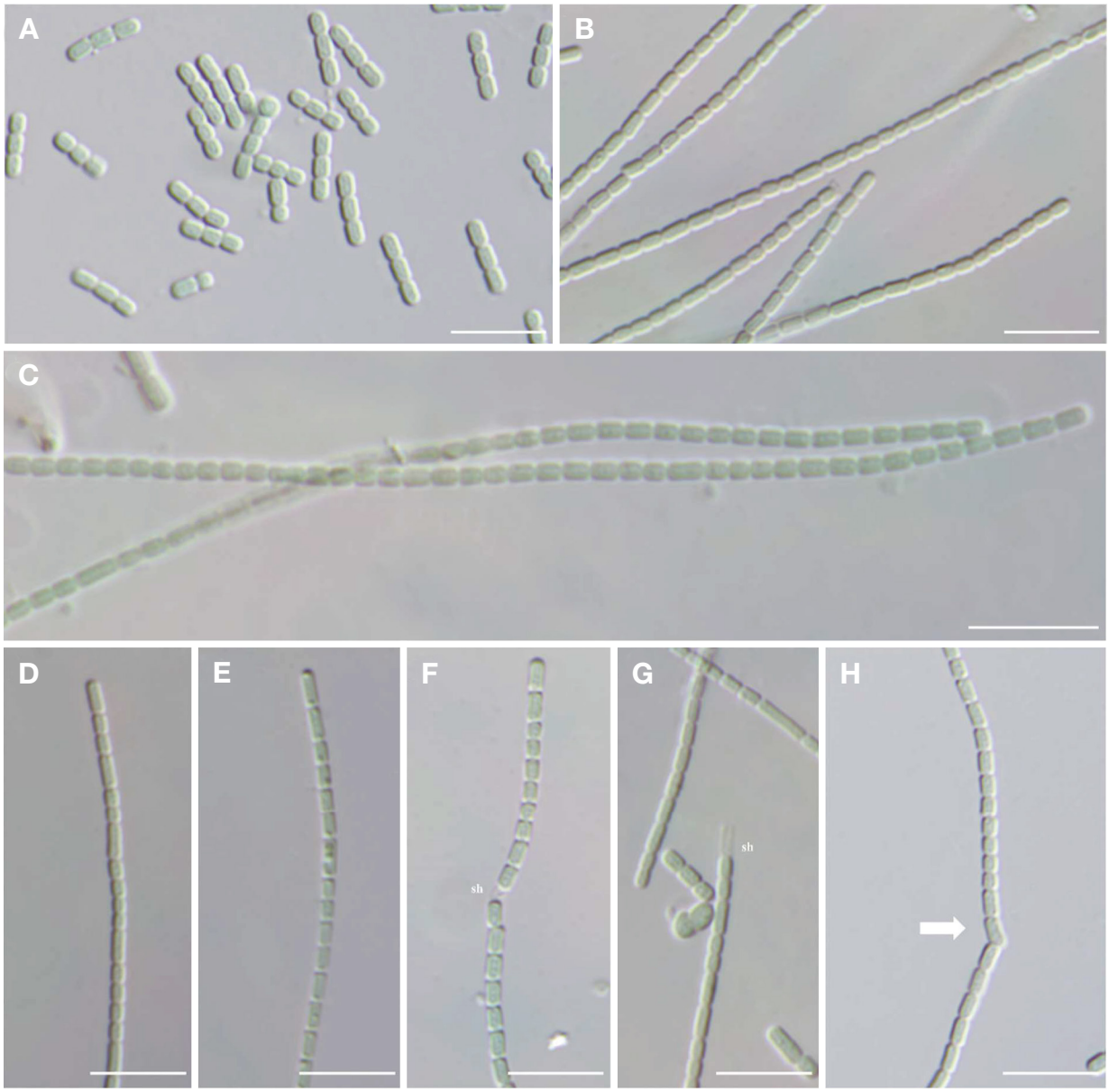


Fig. 3. Microscopic photographs of *Pseudanabaena pruinosa* from the strain NIBRCYC000514098. (A, C) Trichomes consisting of few cells (3–5) or long filaments (20–40 cells), (B) Trichomes straight or slightly curved, (D, E) Apical cells, (F, G) Sheaths (sh), (H) Trichomes showing branching trichomes (arrow). Scale bars = 10 μm.

or elongated. 1.0–1.5 μm wide and 2.0–4.3 μm long. Reproduction by formation of few-celled hormogonia. Necridic cells absent.

Ecology. This species was found on icy soil crusts in the foreland of a valley-type glacier (Hørbyebreen) in polar environments (Aleksovski *et al*., 2024). In this study, it was collected from freshwater habitats in Korea. Odorous compound production (geosmin or 2-MIB) has not been documented for this species, and this characteristic was not assessed in this study.

Distribution. Arctic region, Svalbard Archipelago, Spitsbergen Island (Billefjorden, northwest of Petuniabukta Bay) (Aleksovski *et al*., 2024).

Site of collection. Acheon-dong, Guri-si, Gyeonggi-do (37°34′13.4″N, 127°07′39.3″E).

Date of collection. April 12, 2023.

Specimen deposit No. NIBRCYC000514098.

Pseudanabaena yagii Tuji and Niiyama, 2018 (Fig. 4)

Trichomes yellow-green to gray, straight or curved, constrictions at cross-walls, occasionally with sheath, not atten-



Fig. 4. Microscopic photographs of *Pseudanabaena yagii* from the strain NIBRCYC000514102. (A, B) Arrangement of trichomes, (C–F) Cells of various shapes and terminal cells, (G) Trichomes showing branching trichomes (arrow). Scale bars = 10 μm.

uated nor differentiated at the ends, without calyptra, usually consisting of 5–10 cells, rarely exceeding 20 cells. Cells cylindrical, rounded, mostly longer than wide, sometimes isodiametric, 1.0–2.0 μm wide and 2.0–5.0 (7.0) μm long, ratio of length to width 1.0 (5.0), with aerotopes. Apical cells mostly rounded, conical, 1.0–2.0 μm wide and 2.0–6.0 μm long. Reproduction by formation of hormogonia. Necridic cells absent.

Ecology. This species occurs as plankton in lakes. The type strain (NIVA-CYA 111) was originally isolated from Lake Biwa, Japan, in 1982, and additional strains were later collected from Lake Kasumigaura, Japan (Tuji and Niiyama, 2018). In this study, it was collected from a freshwater stream in Korea. This species has also been documented as a 2-MIB-producing taxon based on physiological observations of Japanese strains (Tuji and Niiyama, 2018).

Distribution. Japan (Lake Biwa, Lake Kasumigaura) (Tuji and Niiyama, 2018), Korea (Bukhan River basin; Uiam Lake) (Kim *et al*., 2020).

Site of collection. Danhyeon-dong, Yeoju-si, Gyeonggi-do, Korea (37°16′28.6″N, 127°40′58.9″E).

Date of collection. March 21, 2023.

Specimen deposit No. NIBRCYC000514102.

The Korean strains *Pseudanabaena gygaxiana* (NIBR CYC000514097) and *P. pruinosa* (NIBRCYC000514098) both exhibited cell dimensions that were smaller than those of their respective type strains, but with slightly higher length-to-width ratios, indicating a tendency toward more slender and elongated cells (Table 2). In *P. gygaxiana* (NIBRCYC000514097), cells measured 1.5–2.0 μm in width and 2.0–6.5 μm in length, compared with 2.0–2.6 μm wide and (2.3) 3.0–13 (22) μm long in the type strain. Similarly, *P. pruinosa* (NIBRCYC000514098) showed cells 1.0–1.5 μm wide and 1.5–3.0 μm long, whereas the

Table 2. Morphological characteristics of *Pseudanabaena pruinosa* and *P. gygaxiana* compared with their type strains.

	P. pruinosa NMCCC001*	*P. pruinosa* NIBRCYC000514098	*P. gygaxiana* UTCC 393†	*P. gygaxiana* NIBRCYC000514097
Cell length	(0.7) 1.2–2.4 (3.0)	1.5–3.0	(2.3) 3.0–13 (22)	2.0–6.5
Cell width	1.2–3.5 (8.2)	1–1.5	2.0–2.6	1.5–2.0
L : W ratio	Usually isodiametric or 1.5× longer than wide	Isodiametric or 1.5 (3.0)× longer than wide	Mostly longer than wide	1.5 (3.0)× mostly longer than wide
Cell shape	Spherical to distinct barrel-shaped	Cylindrical to barrel-shaped, rarely spherical	Cylindrical	Cylindrical to barrel-shaped
Cell color	Blue-green to vivid green	Blue-green	Bright greenish blue-green	Bright blue-green
Sheaths	Facultative, widened and branched	Colorless	Lacking	Lacking
Aerotopes	Polar and central	Polar and central	Polar	Polar

*: Aleksovski *et al*., 2024; †: Casamatta *et al*., 2005

Table 3. Morphological characteristics of *Pseudanabaena cinerea* and *P. yagii* from type strains, previously reported Korean strains, and this study.

	P. cinerea Ak1550*	*P. cinerea* NHUA202007-7†	*P. cinerea* NIBRCYC000514099	*P. yagii* NIVA-CYA 111*	*P. yagii* NHUA202007-8†	*P. yagii* NIBRCYC000514102
Cell length	3.2–9.9	3.9 (±0.6)	2.0–6.5	1.9–5.0	2.7 (±0.8)	2.0–5.0 (7.0)
Cell width	1.4–1.9	2.3 (±0.1)	2.0–2.5	1.5–2.3	1.5 (±0.1)	1.0–2.0
L:W ratio	2.0–6.3	1.2–2.4	1.0 (3.0)	0.9–3.3	1.4–3.4	1.0 (5.0)
Cell shape	Cylindrical	Cylindrical, straight, or slight flexuous	Cylindrical	Cylindrical with rounded ends	Cylindrical	Cylindrical, rounded
Cell color	Greenish gray to gray	–	Brownish green, or greenish gray to gray	Bright blue-green	–	Yellow-green to gray
Cells per trichome	–	10–25	10–30	–	10–15	5–10 (20)

*: Tuji and Niiyama, 2018; †: Kim *et al*., 2020

type strain was reported to have wider but shorter cells, 1.2–3.5 μm in width and (0.7) 1.2–2.4 (3.0) μm in length. Despite these differences in cell size, other morphological features of both species were largely consistent with previous descriptions, including cylindrical to barrel-shaped cells, rounded to occasionally conical terminal cells, and trichomes that were straight or slightly curved with distinct cross-wall constrictions. In *P. pruinosa* (NIBRCYC000514098), the occurrence of both short (3–5 cells) and elongated (20–40 cells) filaments was observed, while *P. gygaxiana* (NIBRCYC000514097) consistently lacked sheaths and typically formed trichomes of 20–30 cells. These findings suggest that the Korean strains fall within the morphological variability of the species but tend to develop relatively slender cell forms under local conditions.

In this study, the Korean strains *P. cinerea* (NIBRCYC000514099) and *P. yagii* (NIBRCYC000514102) also showed morphological features consistent with their type strains and previously reported Korean isolates (Table 3). *P. cinerea* (NIBRCYC000514099) formed trichomes of 10–30 cylindrical cells, 2.0–2.5 × 2.0–6.5 μm in size, similar to the type strain Ak1550 and NHUA202007-7, though the Korean isolates tended to have slightly smaller cells. *P. yagii* (NIBRCYC000514102) produced short trichomes of 5–10 cells (rarely up to 20), with cell dimensions of 1.0–2.0 × 2.0–5.0 (7.0) μm, largely corresponding to the

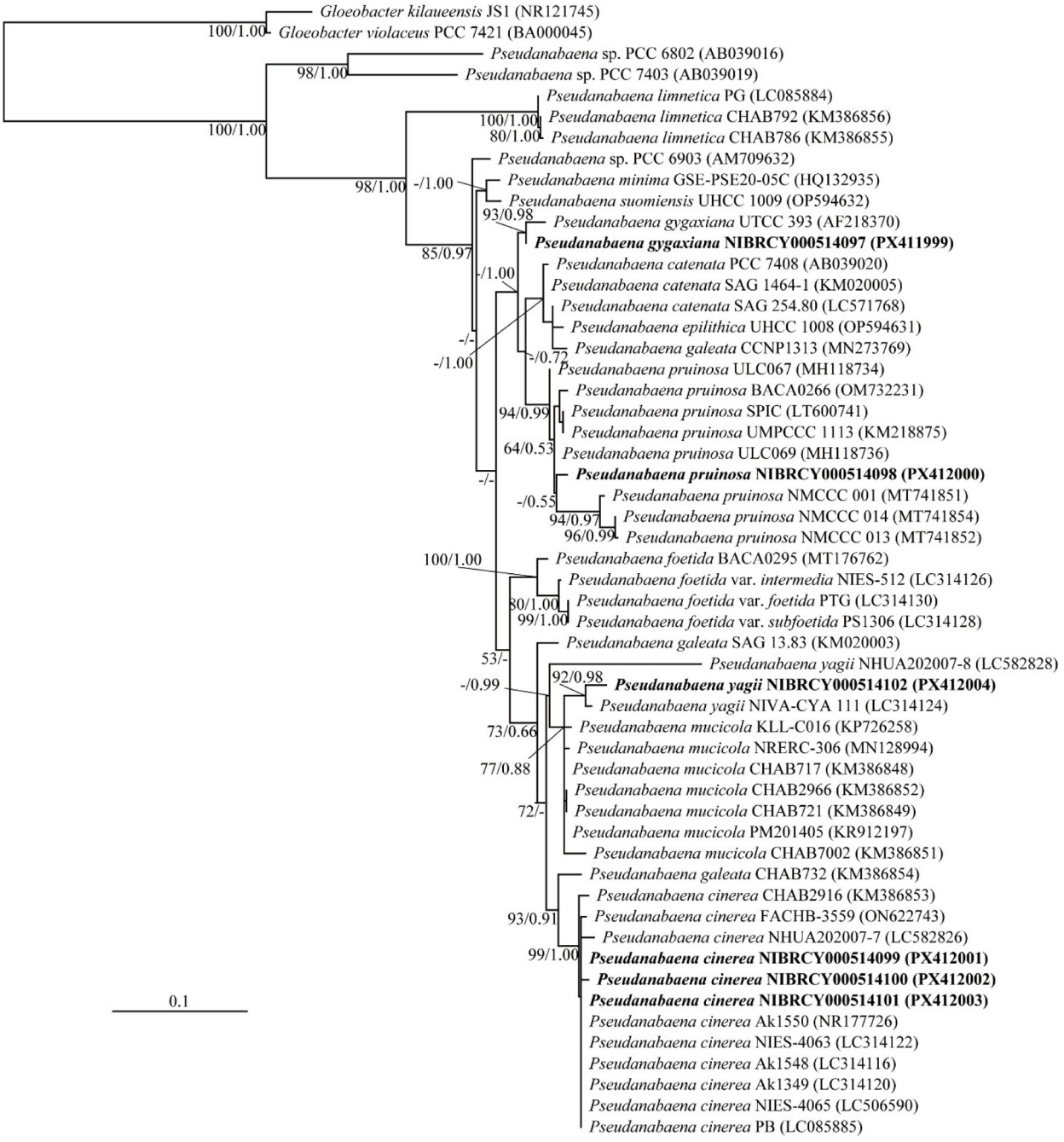


Fig. 5. Maximum-Likelihood (ML) phylogenetic tree based on 16S rRNA gene sequences (1,494 bp) of the genus *Pseudanabaena*. *Gloeobacter violaceus* PCC7421 and *Gloeobacter kilaueensis* JS1 were used as outgroups. Node support values are indicated as ML/Bayesian, with values shown only for >50% ML bootstrap and >0.5 Bayesian posterior probability. Branch lengths are proportional to the scale bar. Bold indicates sequences obtained in this study.

type strain NIVA-CYA 111 and NHUA202007-8, but again with somewhat smaller cell sizes. Overall, both species fall within the morphological variability of their taxa, but the Korean isolates tend to represent the lower end of the type range.

2. 16S rRNA and phylogenetic affiliation

Phylogenetic analyses based on 16S rRNA gene sequences of six cultured strains, together with related sequences retrieved from GenBank, revealed clear species-level affiliations (Fig. 5; Table 4). The ML and BI trees exhibited consistent topologies, and all Korean strains were placed with-

Table 4. 16S rRNA gene sequence identities (%) between type strains, previously reported Korean strains, and strains examined in this study. Sequence identity analyses were conducted on an 810 bp fragment of the 16S rRNA gene starting from the CTCTTTT sequence.

No.	Species and strains	1	2	3	4	5	6	7	8	9	10	11	12
1	*Pseudanabaena gygaxiana* NIBRCYC000514097 (PX411999)		**99.1**	97.4	97.1	97.4	97.4	97.3	97.4	96.9	97.9	97.6	94.2
2	*P. gygaxiana* UTCC 393 (AF218370)			98.2	98	97.7	97.7	97.6	97.7	97.3	98	97.7	94.3
3	*P. pruinosa* NIBRCYC000514098 (PX412000)				**99.6**	96.4	96.4	96.3	96.4	95.9	96.6	96.4	93
4	*P. pruinosa* NMCCC 001 (MT741851)					96.3	96.3	96.2	96.3	95.8	96.9	96.6	92.7
5	*P. cinerea* NIBRCYC000514100 (PX412002)						**100**	**99.8**	**100**	**99.5**	97.1	96.9	93.4
6	*P. cinerea* NIBRCYC000514101 (PX412003)							**99.8**	**100**	**99.5**	97.1	96.9	93.4
7	*P. cinerea* NIBRCYC000514099 (PX412001)								**99.8**	**99.3**	97	96.8	93.2
8	*P. cinerea* Ak1550 (NR177726)									99.5	97.1	96.9	93.4
9	*P. cinerea* NHUA202007-7 (LC582826)										96.7	96.4	93.5
10	*P. yagii* NIBRCYC000514102 (PX412004)											**99.3**	**94**
11	*P. yagii* NIVA-CYA 111 (LC314124)												**93.7**
12	*P. yagii* NHUA202007-8 (LC582828)												

Strains obtained in this study are shown in bold.

in their respective species clades with strong statistical support.

The Korean unrecorded species *Pseudanabaena gygaxiana* (NIBRCYC000514097) clustered with the type strain UTCC 393 with strong support (ML/BI = 93%/0.98) and showed 99.1% sequence similarity. Similarly, *P. pruinosa* (NIBRCYC000514098) grouped with the type strain NMC CC001 and other conspecific strains in a well-supported clade (ML/BI = 94%/0.99), exhibiting 99.6% similarity. These results provide molecular evidence confirming both species as newly recorded in Korea.

Among the previously reported taxa, Korean strains of *P. cinerea* (NIBRCYC000514099, NIBRCYC000514100, NIBRCYC000514101) formed a single clade with the type strain Ak1550 and the previously reported Korean strain NHUA202007-7, with maximal support (ML/BI = 99%/1.00). The sequence similarities among these strains ranged from 99.3% to 100%, supporting their identification as the same species. Likewise, *P. yagii* (NIBRCYC000514102) clustered with the type strain NIVA-CYA 111 with strong support (ML/BI = 99%/1.00), sharing 99.3% similarity. In contrast, the previously reported Korean strain NHUA 202007-8 exhibited lower similarity (93.7%) to the type strain. This indicates that the strain analyzed in this study is more closely related to the type strain.

Overall, all six Korean strains analyzed in this study exhibited $\geq$98.7% similarity to their respective type strains, thereby meeting or approaching the 98.65% species-level threshold proposed by Kim *et al*. (2014). These findings provide robust molecular evidence confirming *P. gygaxiana* and *P. pruinosa* as newly recorded species in Korea, while supporting *P. cinerea* and *P. yagii* as additional records.

Conclusion

In conclusion, six *Pseudanabaena* strains isolated from freshwater environments in Korea were identified through a polyphasic approach combining morphological, ecological, and molecular analyses. Two taxa, *P. gygaxiana* and *P. pruinosa*, are reported here as newly recorded species in Korea, while *P. cinerea* and *P. yagii* are confirmed as additional records. All strains exhibited $\geq$98.7% sequence similarity with their respective type strains, supporting their species-level affiliations. These findings expand the inventory of *Pseudanabaena* diversity in Korea and provide a molecular phylogenetic framework for future taxonomic and ecological studies of this genus in freshwater ecosystems.

Conflicts of Interest

The authors of this paper have no affiliation with any interests and are solely responsible for the paper.

Acknowledgements

This work was supported by a grant from the National Institute of Biological Resources (NIBR) (NIBR202111103, NIBR202102109, NIBR202314101, NIBR202512101, NIBRE202501) funded by the Ministry of Climate, Energy, and Environment (MCEE) of the Republic of Korea.

References

Aleksovski, B., S. Krstić and M.D. Guiry. 2025. The names of phyla and classes of blue-green algae (cyanobacteria, cyanophytes) and a typified name: Cyanobacteriophyta Oren, Mareš et Rippka. Fottea 25(2):212-217.

Aleksovski, B., S. Krstić, J. Komárek, K. Nguyen, K. Pakovski, S. Kiprijanovska, A. Dimovski, A. Vuchurević and O. Strunecký. 2024. *Pseudanabaena pruinosa* sp. nov. (Pseudanabaenales, Cyanobacteria): an Arctic *Pseudanabaena* species with branched sheaths and central aerotopes. European Journal of Phycology 59(3):311-331.

Anagnostidis, K. and J. Komárek. 1988. Modern approach to the classification system of the cyanophytes 3: Oscillatoriales. Algological Studies 50(53):327-472.

Casamatta, D.A., J.R. Johansen, M.L. Vis and S.T. Broadwater. 2005. Molecular and morphological characterization of ten polar and near-polar strains within the Oscillatoriales (Cyanobacteria). Journal of Phycology 41(2):421-438.

Guiry, M.D. and G.M. Guiry. 2025. AlgaeBase. World-wide electronic publication. National University of Ireland, Galway [Available from: http://www.algaebase.org, accessed 01 December 2025].

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41:95-98.

Hoffmann, L., J. Komárek and J. Kaštovský. 2005. System of cyanoprokaryotes (cyanobacteria) state in 2004. Algological Studies/Archiv für Hydrobiologie Supplement Volumes 117:95-115.

Huelsenbeck, J.P. and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17(8):754-755.

Katoh, K. and D.M. Standley. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30(4):772-780.

Kim, K., S. Lee, K. Seo and S.J. Hwang. 2020. Molecular identification of *Pseudanabaena* strains and analysis of 2-MIB production potential in the North Han River system. Korean Journal of Environmental Biology 53(4):344-354.

Kim, M.C., H.S. Oh, S.C. Park and J.S. Chun. 2014. Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. International Journal of Systematic and Evolutionary Microbiology 64(2):346-351.

Komárek, J. 2016. A polyphasic approach for the taxonomy of cyanobacteria: principles and applications. European Journal of Phycology 51(3):346-353.

Komárek, J. and K. Anagnostidis. 2005. Cyanoprokaryota. 2. Teil/2nd Oscillatoriales. In: B. Büdel, L. Krienitz, G. Gärtner and M. Schagerl (eds.), Süβwasserflora von Mitteleuropa vol. 19/2. Elsevier/Spektrum, Heidelberg. pp. 1-759.

Komárek, J., J. Kaštovský, J. Mareš and J.R. Johansen. 2014. Taxonomic classification of cyanoprokaryotes (cyanobacterial genera) 2014, using a polyphasic approach. Preslia 86(4):295-335.

Kumar, S., G. Stecher, M. Li, C. Knyaz and K. Tamura. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 35(6):1547-1549.

Lauterborn, R. 1915. Die sapropelische Lebewelt. Ein Beitrag zur Biologie des Faulschlammes natürlicher Gewässer. Verhandlungen des Naturhistorisch-Medizinischen Vereins zu Heidelberg, Neue Folge 13:395.

National Institute of Biological Resources (NIBR). 2009. Optimization and standardization of storage and management process of biological resource collections at the National Institute of Biological Resources (II). 225 pp.

National Institute of Biological Resources (NIBR). 2024. National List of Species of Korea (2024) [Available from: http://kbr.go.kr, accessed 01 July 2025].

Nübel, U., F. Garcia-Pichel and G. Muyzer. 1997. PCR primers to amplify 16S rRNA genes from cyanobacteria. Applied Environmental Microbiology 63(8):3327-3332.

Oren, A., J. Mareš and R. Rippka. 2022. Validation of the names Cyanobacterium and Cyanobacterium stanieri, and proposal of Cyanobacteriota phyl. nov. International Journal of Systematic and Evolutionary Microbiology 72(10):005528.

Page, R.D.M. 1996. Tree View: an application to display phylogenetic trees on personal computers. Bioinformatics 12(4):357-358.

Shiels, K., N. Browne, F. Donovan, P. Murray and S.K. Saha. 2019. Molecular characterization of twenty-five marine Cyanobacteria isolated from coastal regions of Ireland. Biology 8:59.

Stamatakis, A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22(21):2688-2690.

Strunecký, O., A.P. Ivanova and J. Mareš. 2023. An updated classification of cyanobacterial orders and families based on phylogenomic and polyphasic analysis. Journal of Phycology 59(1):12-51.

Tuji, A. and Y. Niiyama. 2018. Two new *Pseudanabaena* (Cyanobacteria, Synechococcales) species from Japan, *Pseudanabaena cinerea* and *Pseudanabaena yagii*, which produce 2-methylisoborneol. Phycological Research 66(4): 291-299.

Turland, N.J., J.H. Wiersema, F.R. Barrie, W. Greuter, D.L. Hawksworth, P.S. Herendeen, S. Knapp, W.H. Kusber, D.Z. Li, K. Marhold, T.W. May, J. McNeill, A.M. Monro, J. Prado, M.J. Price and G.F. Smith (eds.). 2018. International code of nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. Regnum Vegetabile 159. Koeltz Botanical Books, Glashütten, pp. [i]-xxxviii, 1-253.

Walsh, P.S., D.A. Metzger and R. Higuchi. 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 10(4):506-513.

Submitted: October 15, 2025
Revised: December 17, 2025
Accepted: March 25, 2026