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Nodosilinea hunanesis sp. nov. (Prochlorotrichaceae, Synechococcales) from a Freshwater Pond in China Based on a Polyphasic Approach

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Abstract: A cyanobacterial strain (ZJJ01), morphologically identified as a *Leptolyngbya*-like species was isolated from a freshwater pond in Zhangjiajie National Forest Park, China, and it was characterized through a polyphasic study based on morphological, ecological, and molecular data. Morphologically, the strain fits the description of *Leptolyngbya* well, but under further examination, it formed a distinctive structure, namely nodules, which confirmed that it belonged to the genus of *Nodosilinea*. The 16S rRNA gene threshold value and 16S rRNA phylogenetic analyses clearly confirmed that the studied strain belongs to the genus *Nodosilinea* but that it is phylogenetically distinct from the 10 other species of *Nodosilinea*. Furthermore, The D1–D1′ and Box–B helix of the 16S–23S ITS region of the strain ZJJ01 were also different from those of previously described *Nodosilinea* species. On the basis of this polyphasic approach, here, we provide a description of the new taxon: *Nodosilinea hunanesis* sp. nov.

Keywords: 16S rRNA gene; 16S–23S ITS; polyphasic approach; *Nodosilinea*

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1. Introduction

Cyanobacteria are considered the most ancient group of oxygenic photosynthetic organisms [1]. Traditional classification of cyanobacteria was based on morphological features, but the phylogenetic relationships of this group have recently been significantly modified [2–4]. Many morphological characteristics traditionally used as phylogenetic features have been shown to be plastic, thus requiring the use a of polyphasic approach, i.e., in addition to morphological features, it requires phylogenetic analyses based on 16S rRNA sequences and threshold values based on the 16S rRNA gene, the secondary structure of the 16S–23S ITS regions, and ecological data [5–10]. Researchers have also noted the presence of cryptic taxa that could not be identified on the basis of morphological features alone but also required molecular markers, such as 16S rRNA [4,11–14]. Furthermore, based on phylogenetic reconstructions, most morphologically coherent genera appear to be polyphyletic, and these genera are known as cryptogenera [3,4], which may be the result of frequent evolutionary convergence among many lineages of cyanobacteria [13].

The genus *Leptolyngbya* was described in 1988 by Anagnostidis and Komárek [15] and was originally placed in the Pseudanabaenaceae before being transferred to the Leptolyngbyaceae [4]. *Leptolyngbya* has 159 species names in the database at present, as well as three infraspecific ones, 138 of which have been flagged as accepted taxonomically on the basis of the literature listed under the species name [16]. The details of the thin

trichomes (0.5–3.5 μm), facultative to obligate sheaths, and parietal thylakoids are major traits common to all species in the *Leptolyngbya* genus. Morphological identification of *Leptolyngbya*-like strains is difficult due to the lack of diagnostic features and overlapping features with many taxa belonging to this genus or to related groups [2]. As *Leptolyngbya* is the largest genus in the Leptolyngbyaceae, many previous studies have already indicated the wide genetic diversity of this genus [2,5,17]. In addition, the genus has repeatedly been proven to be polyphyletic [2,5,7,18–21]. Therefore, many studies have suggested a revision of this genus, taking the phylogenetic data into account, and several *Leptolyngbya*-related morphotypes have been established as new genera. including *Halomicronema*, *Phormidesmis*, *Nodosilinea*, *Plectolyngbya*, *Oculatella*, *Haloleptolyngbya*, *Alkalinema*, *Pantanalinema*, *Scytolyngbya*, *Kovacikia*, *Thermoleptolyngbya*, *Timaviella*, etc. [7,20–29].

Nodosilinea Perkerson et Casamatta in Perkerson et al. (2011: 1404) [7] revealed a close morphological resemblance to the genus *Leptolyngbya* with almost indistinguishable differences, except for the nodules forming in the filaments under low light conditions. However, phylogenetic analysis clearly separated the *Nodosilinea* clade from *Leptolyngbya sensu stricto* with high bootstrap support. The species *Nodosilinea nodulosa*, as the type species, was isolated from a marine environment in the South China Sea. Other species were observed in the benthos of freshwater ponds (*Nodosilinea bijugata* Perkerson et Kovácik in Perkerson et al. [7] or on subaerial parts on rocks (*Nodosilinea epilithica* Perkerson et Kovácik in Perkerson et al. [7]. *Nodosilinea conica* Perkerson et Johansen in Perkerson et al. [7] and *Nodosilinea signiensis* Radzi et Merican in Radzi et al. [30] were found in soils. *Nodosilinea chupicuarensis* Gutierrez-Villagomez et Molina-Torres in Vázquez-Martínez et al. (2018) [31] grew on a stone monument in central Mexico. *Nodosilinea radiophila* Heidari et Hauer in Heidari et al. [32] and *Nodosilinea ramsarensis* Heidari et Hauer in Heidari et al. [32] occurred in thermal springs. *Nodosilinea svalbardensis* Davydov et Shalygin in Davydov et al. [33] was isolated from alluvium in the Mimer River valley. *Nodosilinea alaskaensis* in Strunecky et al. [34] was found on stones in Lake Toolik. Up to now, 10 species have been reported in this genus.

In the present study, we isolated one *Leptolyngbya*-like strain from a freshwater pond in Hunan province, China, and evaluated its taxonomic status via a polyphasic approach using its morphology, molecular characteristics, and ecological data. Morphologically, we concluded that it is a *Leptolyngbya*-related morphotype, and the molecular analyses confirmed its position in the *Nodosilinea* clade. The purpose of this study was to clarify the phylogenetic position of the strain ZJJ01, distinguish it from other *Nodosilinea* species, and prove that it belongs to a novel species. Nodosilinea hunanesis sp. nov. is taxonomically described here.

2. Materials and Methods

2.1. Sampling, Isolation, and Culturing of Strains

The cyanobacterial sample presented in this study was collected from a freshwater pond in Zhangjiajie National Forest Park, Hunan Province, China (41°28′00.00″ N, 124°08′00.00″ E) on 3 October 2019. a Under microscope (Olympus IX73, Japan), a single filament from the cyanobacterial sample was separated by using a lab-made Pasteur pipette washing method and then cultured in screw-capped glass tubes containing 7 mL of CT medium [35]. All individual isolates were subsequently cultivated at 25 °C, in a 12 h:12 h light–dark cycle with a photon flux density of 35 μmol/(m^{-2}·s^{-1}) under white fluorescent light. The living cyanobacterial strain was maintained in the Chinese Harmful Algae Biology (CHAB) culture collection of the Institute of Hydrobiology (IHB), Chinese Academy of Sciences (CAS). The number of the studied strain is ZJJ01. Dry material of the strain ZJJ01 was freeze-dried at −50 °C and stored in the Freshwater Algal Herbarium (HBI), IHB, CAS.

2.2. Morphological Characterization

The morphological characteristics of the strain ZJJ01 were examined using a Nikon Eclipse 80i microscope (Nikon, Tokyo, Japan). The micrographs were taken using Nikon

NIS-Elements 3.2D software (Nikon, Tokyo, Japan). A Nikon Eclipse 80i microscope equipped with a Nikon DS-Ri1 digital camera was used to observe and describe the shape and sizes of vegetative cells, as well as the presence or absence of sheaths. The ultrastructure of the studied strain was examined by transmission electron microscopy (TEM). The sample was fixed and dehydrated according to Geng et al. [36]. Images of the processed sample were finally observed using a transmission electron microscope (Hitachi HT-7700, Tokyo, Japan) at an accelerating voltage of 80 kV.

2.3. DNA Extraction, PCR Amplification, and Sequencing

Total genomic DNA was extracted from cultured cyanobacterial cells using Clarke's method [37]. The 16S rRNA gene and the 16S–23S ITS region were PCR-amplified in a MJ Mini Personal Thermal Cycler (Bio–Rad, Hercules, CA, USA), using the primer sets pA and B23S [38,39]. The PCR reaction, with a total volume of 20 μL, contained: 8 μL of sterile water, 1 μL of genomic DNA (100 ng/μL), 0.5 μL of each primer (10 μmol/L), and 10 μL of 2× PCR mix with Taq polymerase (Cat TSE001, Beijing Tsingke Biotech Co. Ltd., Beijing, China). The resulting PCR products were purified using a Tsingke DNA Gel Extraction Kit (Cat GE0101-200, Beijing Tsingke Biotech Co. Ltd., Beijing, China) and subsequently cloned into the pMDTM18-T vector (TaKaRa, Japan) using the procedure of Sambrook and Russell [40]. All sequencing was performed by the ABI 3730 Automated Sequencer (PerkinElmer, Waltham, MA, USA).

2.4. Phylogenetic Analysis

The 16S rRNA gene sequences generated in this study and sequences retrieved from GenBank (a total of 153 sequences) were aligned using MAFFT v7.312 [41], trimmed (sequence data matrix with a 1094-bp length), and used to infer phylogenetic trees. The 16S rRNA gene's phylogenetic trees were inferred using maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI) methods. The MP analysis was conducted using the MEGA program package, version X [42], with 1000 repeated heuristic searches. ML analysis was performed on the IQ-TREE web server [43] with 10,000 bootstrap replicates by using ultrafast bootstrapping. The best fitting models, GTR+I+G, were selected for the MP, ML, and BI analyses via the Akaike Information Criterion (AIC) in ModelFinder [44]. The BI was conducted with MrBayes v3.2.6 [45] in the CIPRES Science Gateway V.3.3 [46]. In the BI analyses, two runs of eight Markov chains were run for 10,000,000 generations, sampling every 100 generations, with 25% of the sampled trees discarded as burn-in. The consensus phylogenetic trees thus obtained were visualized in FigTree, v1.4.4 [47], with *Gloeobacter violaceus* as the outgroup. Calculation of the p-distance in the 16S rRNA was carried out by MEGA software v.7.0.14 [48] and used to calculate the sequence identity ($100 \times (1 - p)$) for the 16S rRNA data. The 16S rRNA and 16S–23S ITS gene sequences of the cyanobacterial strain ZJJ01 were deposited in the NCBI (National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/, accessed on 1 May 2022) in the GenBank database under the accession numbers ON074585 and ON074584.

2.5. Secondary Structure Analysis of 16S–23S

The 16S–23S ITS region of the studied sequence was used for secondary structure folding. The secondary structures of the D1–D1′, Box–B, and V3 helices were determined using "RNAstructure", version 5.6 [49].

3. Results

3.1. Morphological Description

Nodosilinea hunanesis F. Cai et R. Li sp. nov. (Figures 1 and 2).

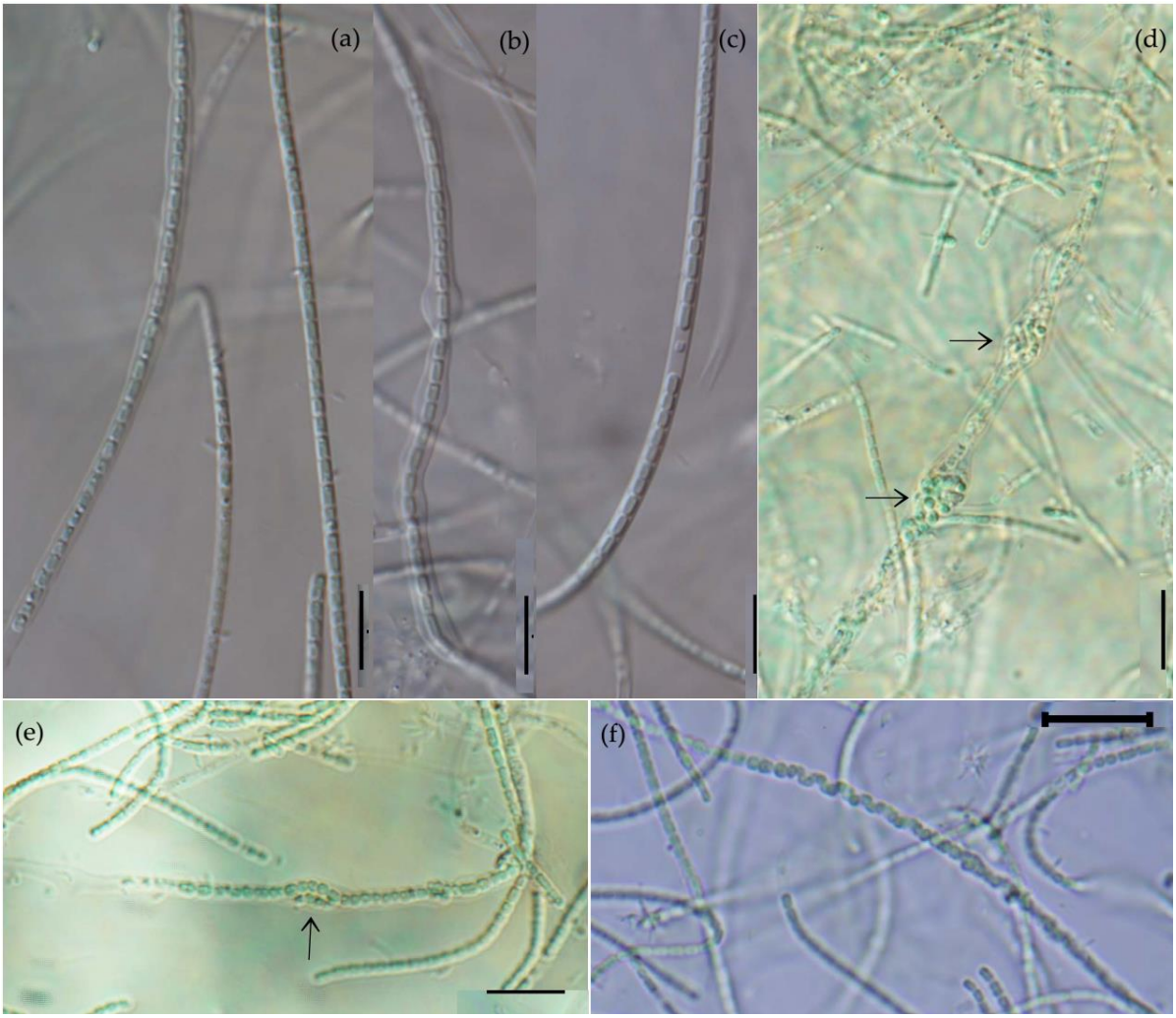

Figure 1. Light microscopy of *Nodosilinea hunanesis* strains. (**a**–**c**) A single trichome with a sheath. (**d**,**e**) Characteristic nodule (arrow). (**f**) Filaments forming loose spirals. Scale bars: 10 μm.

Description: The thallus is blue-green, macroscopic, in aggregates. Filaments are straight or bent, unbranched, solitary, and free-floating. The sheath is soft, layered, colorless, adherent to the edges of the cells, often becoming wide. Trichomes are isodiametric, constricted at the cross walls, and forming long, loose spirals under normal light condition; nodules are present under low light intensity (<4 μmol/(m^{-2}·s^{-1})). Cells are green to blue-green, lacking a gas vesicle, slightly cylindrical, more or less longer than they are wide, 1.02–1.75–2.74 μm long, and 1.10–1.10–1.34 μm wide, with a length: width ratio of 1.0–2.42; the cell content is divided into peripheral chromatoplasma and central nucleoplasma. The thylakoids are parietally arranged (four or five per cell) (Figure 3). Reproduction is by hormogonia or trichome breakage.

Reference strains: ZJJ01 (Freshwater Algal Herbarium (HBI), Institute of Hydrobiology, Chinese Academy of Science).

Type locality: Isolated from a water sample in Hunan Province, China (41°28′00.00″ N, 124°08′00.00″ E).

Holotype designated here: Dry material of the strain ZJJ01 was stored at the Freshwater Algal Herbarium (HBI), Institute of Hydrobiology, Chinese Academy of Science, Wuhan, China, as specimen No. HN201910.

Etymology: *hunanesis* refers to the Hunan province where the strain was isolated, transliterated into Latin.

Habitat: Free-living in water.

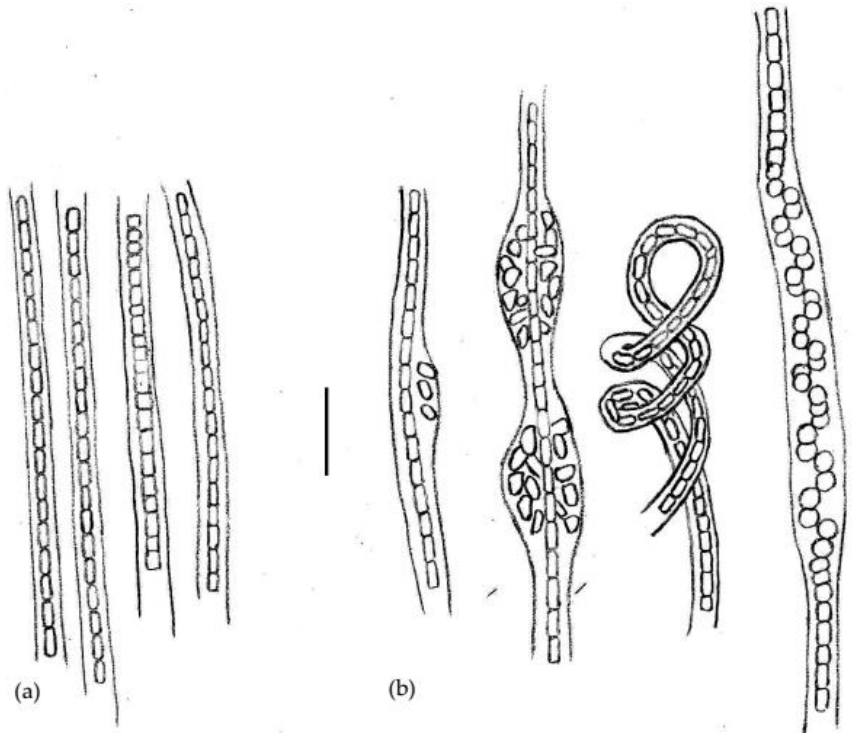

Figure 2. Line drawings of *Nodosilinea hunanesis.* (**a**) Single trichomes with sheaths. (**b**) Trichomes with nodules, and filaments forming loose spirals. Scale bars: 10 μm.

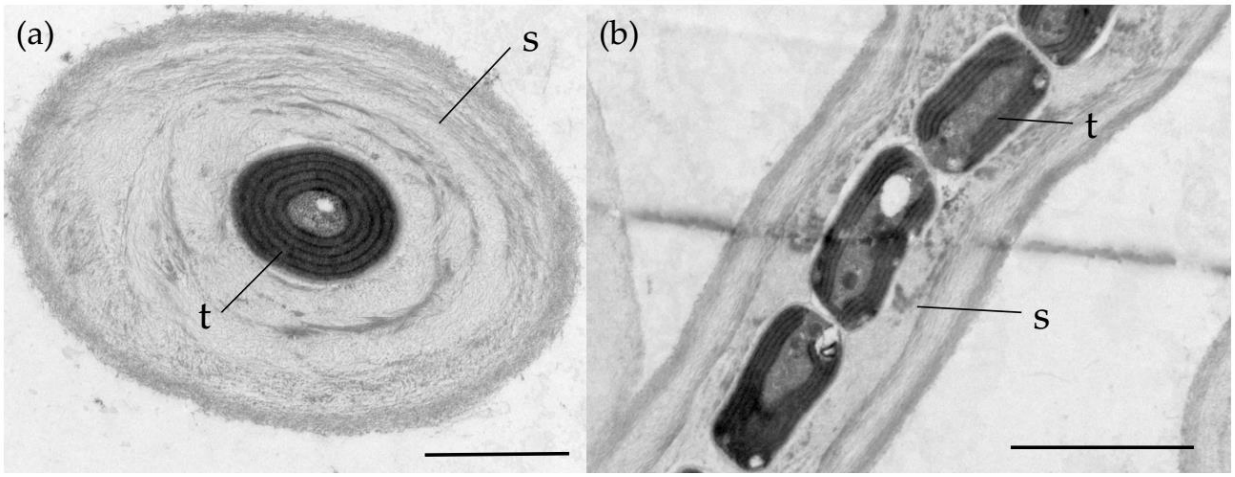

Figure 3. TEM micrographs of *Nodosilinea hunanesis*. (**a**) Cross-section of a cell within its surrounding sheath. (**b**) Longitudinal section of a filament. Scale bar: (**a**) = 1 μm, (**b**) = 2 μm. s, sheath; t, thylakoids. Thylakoids are arranged more or less parallel in a parietal position.

3.2. Molecular and Phylogenetic Analysis

The distance matrix based on the 16S rRNA gene showed that the *Nodosilinea hunanesis* strain shared 95.6–97.1% similarity with other *Nodosilinea* species (Table 1). In total, 153 representative taxa sequences were included in the phylogenetic analysis to assess the placement of the *Nodosilinea* clade within the cyanobacteria (Figure 4). MP, ML, and Bayesian inference analyses produced similar tree topologies in our phylogenies. The 16S rRNA phylogeny clearly indicated that our strain was nested within the genus *Nodosilinea* (100% and 100% MP and ML bootstrapping percentage (BP) and 1.00 posterior probability (PP)). *Nodosilinea hunanesis* is sister to *Nodosilinea svalbardensis* 3220 and *Nodosilinea nodulosa* PCC7104.

Table 1. Sequence similarity comparison of the 16S rRNA gene between the *Nodosilinea hunanesis* strain and its close species. Similarity = [1 − (p-distance)] × 100.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
1. *N. hunanesis* ZJJ01															
2. *N. alaskaensis* T21	0.956														
3. *N. alaskaensis* T29	0.956	1													
4. *N. bijugata* KOVACIK1986/5a	0.955	0.956	0.956												
5. *N. chupicuarensis* PC471	0.967	0.979	0.979	0.969											
6. *N. conica* strain SEV4-5-c1	0.957	0.96	0.96	0.964	0.975										
7. *N. epilithica* str. Kovacik 1990/52	0.962	0.963	0.963	0.961	0.971	0.964									
8. *N.* sp. FI2-2HA2	0.957	0.958	0.958	0.977	0.973	0.983	0.966								
9. *N. nodulosa* PCC 7104	0.968	0.976	0.976	0.966	0.984	0.974	0.969	0.972							
10. *N. nodulosa* UTEX 2910	0.966	0.978	0.978	0.968	0.996	0.974	0.974	0.972	0.983						
11. *N. radiophila* TM S2B clone cl3	0.958	0.98	0.98	0.961	0.978	0.966	0.972	0.963	0.975	0.977					
12. *N. ramsarensis* KH-S S2.6 clone cl2	0.964	0.98	0.98	0.966	0.986	0.968	0.974	0.968	0.983	0.989	0.979				
13. *N. ramsarensis* KH-S S2.6 clone cl1	0.965	0.981	0.981	0.967	0.987	0.969	0.975	0.969	0.984	0.99	0.98	0.994			
14. *N. signiensis* USMFM	0.971	0.978	0.978	0.971	0.984	0.973	0.98	0.973	0.984	0.983	0.981	0.987	0.988		
15. *N.* sp. FI2-2HA2	0.957	0.958	0.958	0.977	0.973	0.983	0.966	1	0.972	0.972	0.963	0.968	0.969	0.973	
16. *N. svalbardensis* 3220	0.96	0.961	0.961	0.953	0.966	0.959	0.96	0.959	0.971	0.964	0.962	0.969	0.97	0.978	0.959

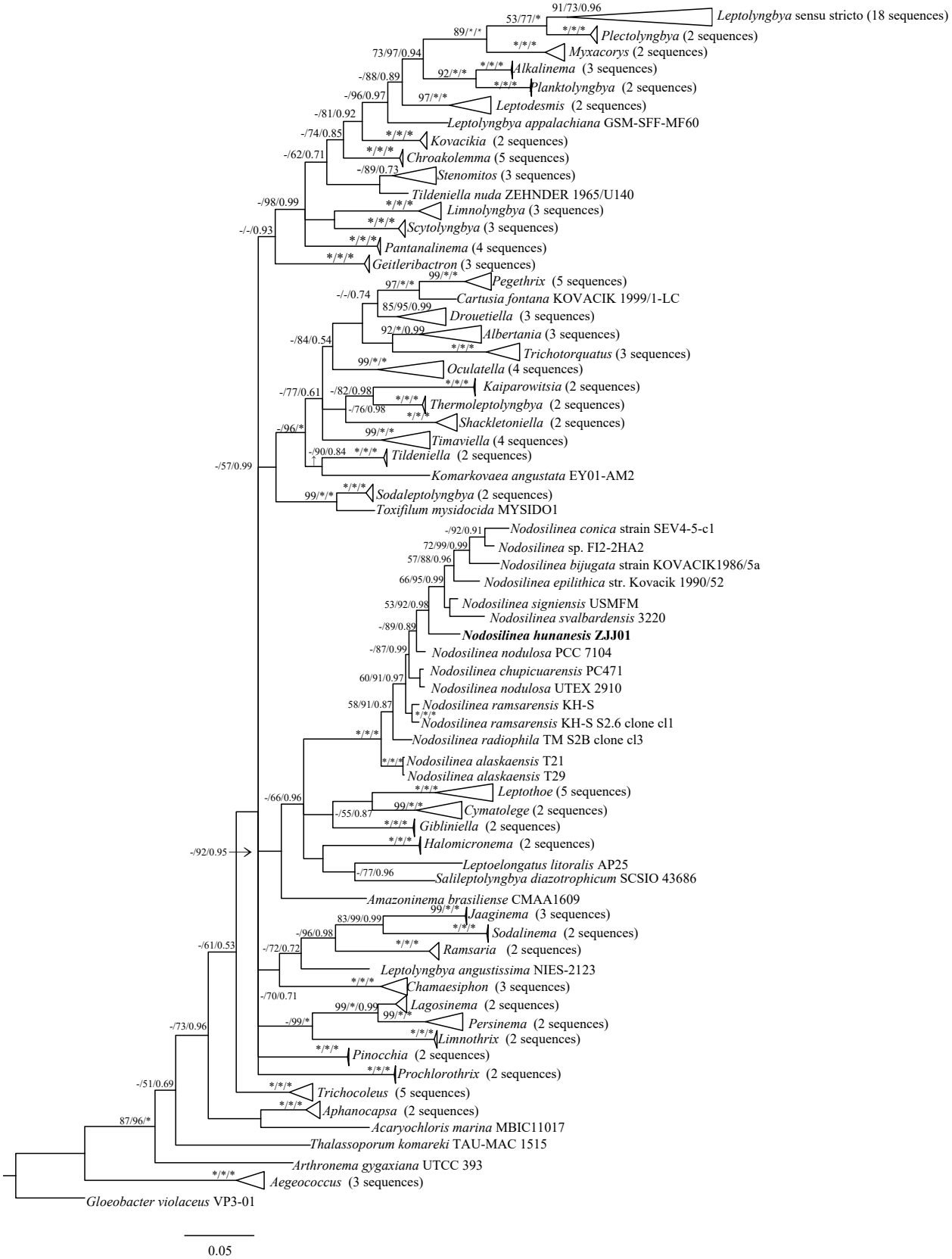

Figure 4. Bayesian inference (BI) phylogenetic tree of *Nodosilinea hunanesis* ZJJ01 based on 16S rRNA gene sequences. Bootstrapping values higher than 50% are shown on the BI tree for MP/ML methods and Bayesian posterior probabilities. * indicates bootstrapping values of 100 in MP, ML, and BI posterior probabilities of 1.00. The novel filamentous strain of this study is indicated in bold.

3.3. The 16S–23S ITS Region

In total, 10 existing *Nodosilinea* species and *Nodosilinea hunanesis* (ZJJ01) were used to compare the secondary structures within *Nodosilinea*. The secondary structures of the conserved ITS domains in *Nodosilinea* were highly similar (Figures 5 and 6), particularly in the V3 helix, which was structurally identical and had the same patterns. Except for *N. conica*, *N. bijugata*, and *N. ramsarensis*, the other eight existing *Nodosilinea* species and *N. hunanesis* had identical D1–D1′ helices, while *N. hunanesis* showed different nucleotides in the D1–D1′ helix in comparison with other *Nodosilinea* species.

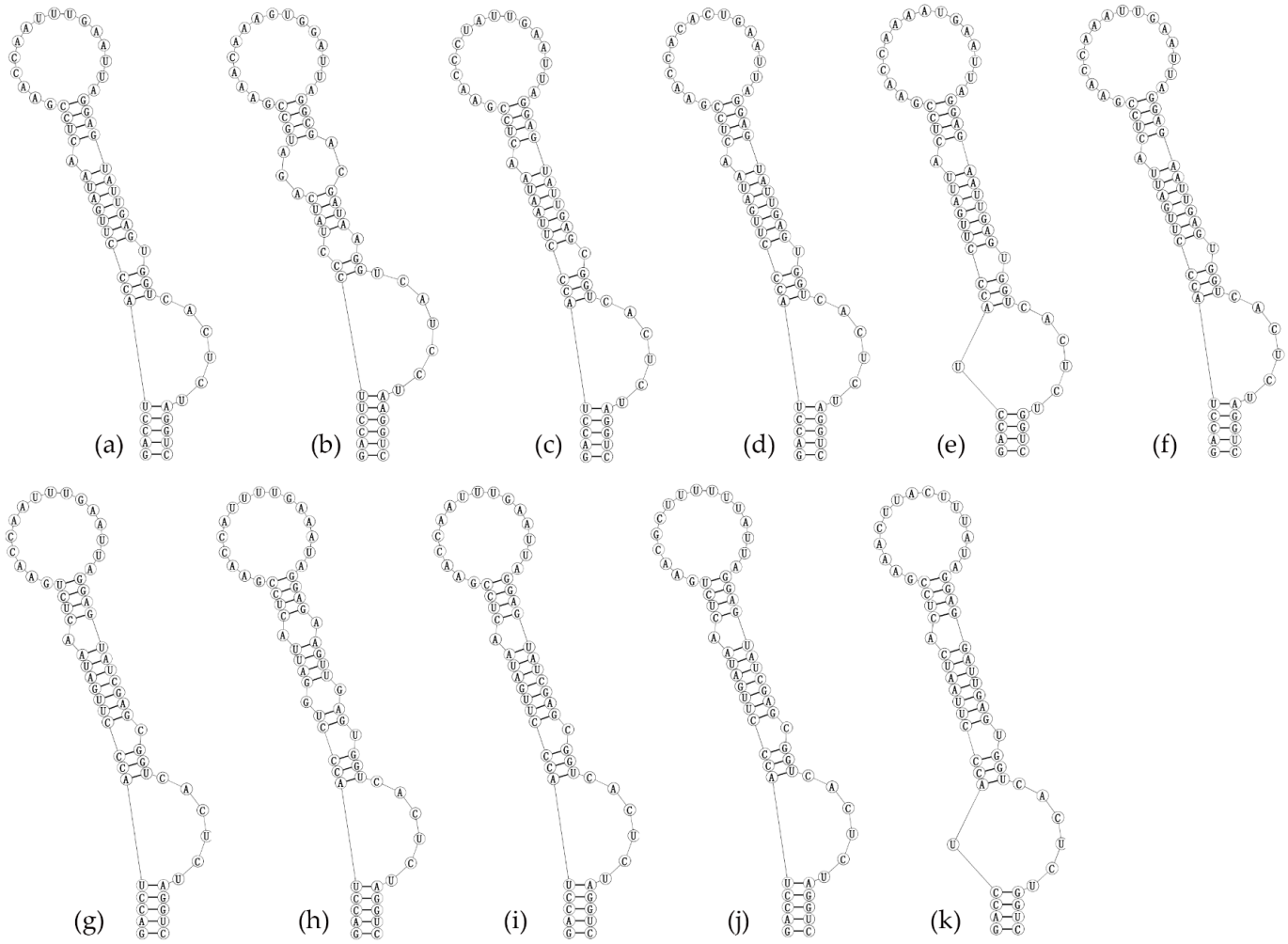

Figure 5. D1–D1′ helices of 11 representative strains of *Nodosilinea*. (**a**) *N. hunanesis* ZJJ01. (**b**) *N. conica* strain SEV4-5-c1. (**c**) *N. epilithica* str. Kovacik 1990/52. (**d**) *N. nodulosa* PCC 7104. (**e**) *N. signiensis* USMFM. (**f**) *N. svalbardensis* 3220. (**g**) *N. alaskaensis* T21. (**h**) *N. bijugata* KOVACIK1986/5a. (**i**) *N. chupicuarensis* PC471. (**j**) *N. radiophila* TM S2B clone cl3. (**k**) *N. ramsarensis* KH-S S2.6.

The Box–B helix is more variable in sequence, length, and structure (Figure 6), which could be considered as clear evidence for the differentiation of different species. *Nodosilinea hunanesis* had its own unique Box–B helix: the base stem of *N. hunanesis* (Figure 6a) consisted of a 4 bp helix, followed by a 1:1 base bilateral bulge, further followed by a 1:2 base bilateral bulge; the terminal loop contained 4 bp bases (GGGA). However, the other nine related species had nine Box–B helix types.

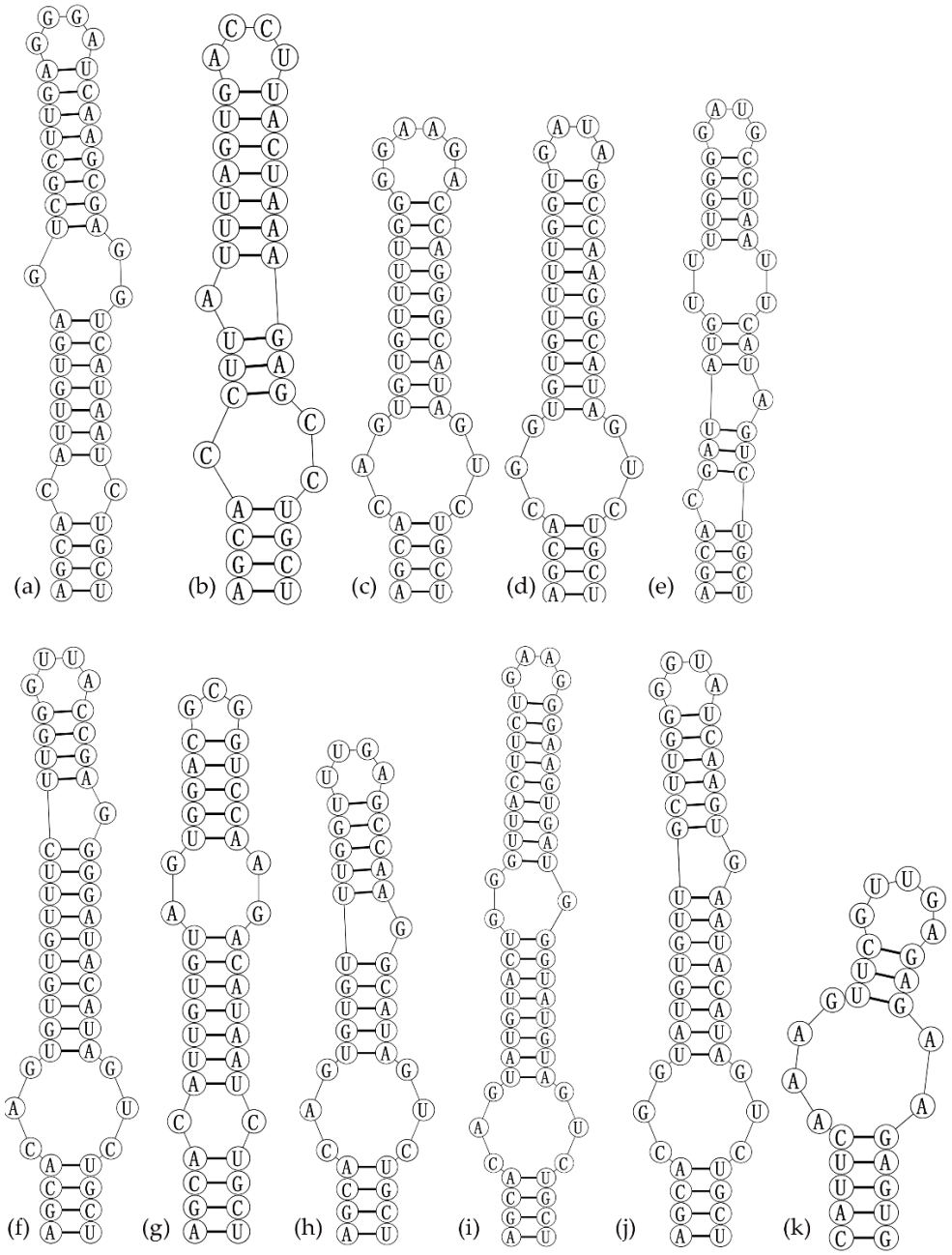

Figure 6. Secondary structure of the conserved domains of the 16S–23S ITS regions for eight representative strains of *Nodosilinea*. (**a**–**j**) Box–B helices: (**a**) *N. hunanesis* ZJJ01. (**b**) *N. conica* strain SEV4-5-c1. (**c**) *N. epilithica* str. Kovacik 1990/52. (**d**) *N. nodulosa* PCC 7104. (**e**) *N. signiensis* USMFM. (**f**) *N. svalbardensis* 3220. (**g**) *N. bijugata* KOVACIK1986/5a. (**h**) *N. chupicuarensis* PC471. **(i)** *N. radiophila* TM S2B clone cl3. (**j**) *N. ramsarensis* KH-S S2.6. (**k**) V3 helix for *Nodosilinea hunanesis* ZJJ01.

4. Discussion

Oscillatorian cyanobacteria with filaments less than 3.5 µm in width and covered by a sheath, mainly belong to the genus *Leptolyngbya* Anagnostidis et Komárek [2,15]. Based on molecular taxonomic studies, Komárek et al. divided the Oscillatoriales sensu auct. into two orders: Oscillatoriales and Synechococcales, with the latter including the genus *Leptolyngbya* [4]. It is difficult to put forward a morphological feature to distinguish the phylogenetically distinct taxa from the groups characterized by these thin filamentous cyanobacteria, such as *Leptolyngbya*. Many genetically distinct species have been classified as *Leptolyngbya* based only on morphology, prompting many taxonomists to attempt to

split this polyphyletic group into new genera to better understand the classification of *Leptolyngbya* [7,20,21,24–29,50–52]. Building the genus *Nodosilinea* [7] by splitting it from *Leptolyngbya* was another significant example, representing the effort to narrowly define the genus. Since the separation of the *Nodosilinea* genus from *Leptolyngbya* in 2011, 10 species have been described.

In this study, we isolated a cyanobacterial strain morphologically resembling *Leptolyngbya* from a freshwater pond in Zhangjiajie, Hunan province, and consequently identified it as a new species of *Nodosilinea*. The morphological features of the strain ZJJ01 were in accordance with those of family Leptolyngbyaceae. From ecology to cytology, to the introduction of recent molecular biological data (mainly the 16S rRNA gene and 16S–23S ITS), it has been shown that morphological characteristics alone do not reflect the evolutionary history of cyanobacteria [4]. Nevertheless, the combination of molecular evidence and morphological, biogeographical, and ecological data can better distinguish species with similar morphologies. This is the essence of the polyphasic approach, which has been followed for species-level identification [50,53,54]. Phylogenetic analysis in this study, combined with cut-off values of species separation, clearly indicated that the strain under investigation belonged to the genus *Nodosilinea*. In the 16S rRNA gene's phylogenetic tree, *N. hunanesis* clustered within the *Nodosilinea* branch and formed a distinct clade with moderate to high bootstrapping support. The species *N. hunanesis* was well defined on the basis of molecular criteria. Within *Nodosilinea*, *N. hunanesis* sp. nov. genetically differed from other species by at least 97.1% in terms of 16S rRNA similarity. Stackebrandt and Goebel [55] initially suggested that strains with a 16S rRNA sequence similarity of less than 97.5% should be considered as independent species. Stackebrandt and Ebers (2006) [56] changed the cutoff point to a higher value, which was supported by the cut-off proposed by Yarza et al. (98.7% for species) [57]. Therefore, ZJJ01 is below any of the proposed threshold values for 16S rRNA comparisons. On this basis, *N. hunanesis* sp. nov. represents a distinct species from the 10 previously described *Nodosilinea* species.

Analysis of the 16S–23S ITS secondary structure has been used as an effective tool to distinguish taxa at the species level [6,19,58–62]. The analyses of the secondary structures of the ITS further supported the distinctiveness of *N. hunanesis.* The secondary structures of the ITS, including the D1–D1′ and Box–B helices, distinguished *N. hunanesis* from other ten *Nodosilinea* species. It is worth mentioning that the Box–B helix of *N. hunanesis* (Figure 5a) was significantly different from that of the other *Nodosilinea* species in its stem–loop structures.

Morphologically, most *Nodosilinea* species are very similar (Table 2), and due to the simple morphological characteristics of this genus, some features overlap between species. Nevertheless, some morphological features may be helpful for diagnosing *N. hunanesis*, such as the formation of long, loose spirally coiled filaments under normal light conditions, and the layered, widened sheath. Furthermore, the presence of this species in a freshwater habitat in China further supports its separation from most of the previously described species, based on its significantly different biome. Most of other species were found in soils, stones, rocks, thermal springs, and oceans, and *Nodosilinea bijugata* was observed in the benthos of a eutrophic lake in the littoral zone in Poland.

Table 2. Comparison of the characteristics of the 10 previously described species of *Nodosilinea* and *N. hunanesis* ("?" indicates that the feature is unknown).

Characters	***N. hunanesis***	***N. signiensis***	***N. epilithica***	***N. bijugata***	***N. conica***	***N. chupicuarensis***	***N. nodulosa***	***N. radiophila***	***N. ramsarensis***	***N. alaskaensis***	***N. svalbardensis***
Cell length (μm)	1.02–2.74	1.0–2.0 (2.3)	1.0–8.0	1.5–6.2	0.9–2.4	1.1–1.3	1.1–1.5	1.0–2.0	(0.8) 1.0–1.5	2–4.1	1.2–2.1
Cell width (μm)	1.10–1.34	1.0 (1.5)	1.5–2.5	1.5–1.7	2.5–2.7	1.2	1.2–2.4	2.0–5.0	1.0–2.0	1.4–1.8	1.2–1.7
Cell shape	Cylindrical, longer than wide	Isodiametric, longer than wide/barrel shape	Barrel shaped, shorter to longer than wide	Isodiametric, longer than wide	Isodiametric, shorter than wide	Isodiametric	Isodiametric, longer than wide	Isodiametric, longer than wide	Isodiametric, longer than wide	More or lesslonger than wide	Shorter to longer than wide
Cross-wall	Strongly constricted	Slightly constricted to strongly constricted	Distinctly constricted	Slightly constricted	Slightly constricted	Constricted	Slightly to strongly constricted	Distinctly constricted	Distinctly constricted	Not constricted	Strongly constricted
Filaments	Forming nodules	Solitary, immotile, forming spirals	Forming nodules in low light	Rarely forming nodules	Rarely forming nodules	Multiseriate, motile, forming nodules	Forming nodules	No formation of nodules	Rrarely forming nodules	Forming nodules	Forming nodules
Apical cells	Dome-shapedor elongated	Rounded	Rounded	Rounded	Rounded	Dome-shaped	Rounded	Rounded or elongated	?	Rounded	Rounded
Sheaths	Soft, layered, colorless, often becoming wide	Very thin, colorless	Thin, colorless, occasionally becoming wide and diffluent	Often absent, thin, colourless	Soft, thin, colorless	Thin, clear	Thin, colourless, occasionally becoming wide and diffluent	Thin, colorless	Thin, colorless	Usually present; thin, soft, colorless	Soft, thin, colorless, sometimes widened, hyaline
Habitat	Freshwater pond, China	Soil, Signy Island, Antarctica	House wall, Peninsula Gargano, town of Vieste (Foggia), Italy	Littoral zone, eutrophic Lake Piaseczno, Poland	Sevilleta long- term ecological research, New Mexico; Soil, Chihuahuan Desert, USA	Stone monument surface, Central Mexico	Marine, South China Sea	Benthic mat in a thermal spring (<27 °C), Talesh Mahalleh, Ramsar Iran	Soil around a thermal spring (<32 °C), Khaksefid, Ramsar, Iran	Periphyton in a lake	Biocrust on sand

In conclusion, one new species of *Nodosilinea* was separated on the basis of a combination of the 16S rRNA gene threshold, the 16S rRNA gene's phylogeny, the secondary structures of the ITS, and the morphological features. On the basis of all of this evidence, we concluded that our strain belongs to a novel species in the genus *Nodosilinea*, and named it *Nodosilinea hunanesis*. In the past few years, polyphasic culture-dependent approaches aiming to investigate cyanobacterial diversity in previously underexplored habitats have resulted in the description of several new genera/species in China [27,36,62–71]. The taxonomic investigation of *Leptolyngbya*-like cyanobacteria is seriously insufficient in China. In the past 2 years, we have carried out a survey on the diversity of *Leptolyngbya*-like cyanobacteria in different habitats in China, hoping that more novel groups will be discovered in future studies.

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