

Article

Description of *Cylindrospermum solincola* sp. nov. from Jammu and Kashmir, India and Further Insights into the Ecological Distribution and Morphological Attributes of *Cylindrospermum badium*

Naresh Kumar ¹, Aniket Saraf ² , Sagarika Pal ¹ and Prashant Singh ^{1,*} 

¹ Department of Botany, Institute of Science, Banaras Hindu University, Varanasi 221005, India

² Department of Biological Sciences, Ramniranjan Jhunjhunwala College, Mumbai 400086, India

* Correspondence: sps.bhu@gmail.com

Abstract: Two cyanobacterial strains KUT1-PS and 18C-PS were collected from the soil surface and vernal pool, respectively, from the Basantgarh village, Udhampur district of the union territory of Jammu and Kashmir, India and characterized by a polyphasic approach. The morphological characterization indicated that both the strains showed typical *Cylindrospermum*-like morphology and probably belonged to the genus *Cylindrospermum*. Further, phylogenetic interpretations at the genus level were made using the 16S rRNA gene while the 16S-23S ITS region phylogenetic analysis and secondary structure analysis were conducted to enhance the resolution at the species level. The results from the comparative morphological analysis, the 16S rRNA gene percent similarity and phylogenetic analyses, the 16S-23S ITS percent dissimilarity and the ITS secondary structure analyses provided enough evidence that the strain 18C-PS is a representative of *Cylindrospermum badium*, providing further insights into its ecological distribution and morphological attributes. Additionally, the strain KUT1-PS was a novel species of the genus *Cylindrospermum* and is referred to herein as *Cylindrospermum solincola* sp. nov., in accordance with the International Code of Nomenclature for algae, fungi and plants. This study also discusses the importance of comparing the newly sequenced strains with previously established species before making final taxonomic interpretations.

Keywords: cyanobacteria; *Cylindrospermum*; India; 16S rRNA gene; 16S-23S ITS; polyphasic approach



Citation: Kumar, N.; Saraf, A.; Pal, S.; Singh, P. Description of *Cylindrospermum solincola* sp. nov. from Jammu and Kashmir, India and Further Insights into the Ecological Distribution and Morphological Attributes of *Cylindrospermum badium*. *Diversity* **2023**, *15*, 592. <https://doi.org/10.3390/d15050592>

Academic Editors:

Christophe Destombe,
Gabrielle Zammit
and Elliot Shubert

Received: 14 February 2023

Revised: 20 March 2023

Accepted: 19 April 2023

Published: 25 April 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The genus *Cylindrospermum* was established by Kützing [1] with eight species (*C. majus* (= *C. maius*), *C. conglobatum*, *C. polyspermum*, *C. spirale*, *C. riparium*, *C. limicola*, *C. humicola* and *C. arenicola*), whereas Bornet and Flahault [2] included only five species, *C. stagnale* (including *C. conglobatum*, *C. riparium* and *C. limicola*), *C. majus*, *C. licheniforme* (= *C. spirale*), *C. muscicola* and *C. catenatum*, in their classification system. Furthermore, Bornet and Flahault [2] mentioned *C. arenicola* and *C. humicola* in the list of species to be investigated, and *C. polyspermum* in the list of species to be excluded. Interestingly, there has been a long-lasting debate around the type species of the genus *Cylindrospermum* and different studies have considered either *C. maius* or *C. stagnale* as the generitype [3–9]. Gardner [3] in his monograph chose *C. maius* to represent the type species of *Cylindrospermum* whereas; Geitler [4] chose *C. stagnale* as the type species. Since Gardner [3] has priority over Geitler [4], more recent treatments have considered *C. maius* CCALA 998 to be the generitype of *Cylindrospermum* [10,11].

The external morphology of the genus *Cylindrospermum* is a mostly amorphous, dull blue-green or brownish-coloured mucilaginous mat. The trichomes are short, unbranched, isopolar or uniformly broad, cylindrical, straight or slightly curved or coiled, and constricted at cross walls, only with very fine or thin, diffuent and colourless mucilage. The

vegetative cells are cylindrical, barrel-shaped, or conical (terminal), longer to isodiametric or shorter than wide. Heterocytes are mostly solitary at both terminal ends or seldom at one end only, spherical or oval to ovoid and slightly larger than vegetative cells. Akinetes are always larger than vegetative cells and heterocytes, usually solitary or rarely in a row, adjacent to the heterocyte at one end or both ends of the trichome, oval or cylindrical to ellipsoidal, thick-walled and either smooth or ornamental with spines and warts [2,5,6]. The morphological characteristics of vegetative cells, heterocytes and akinetes, such as their shape and dimensions, the position of heterocytes and akinetes and the texture of akinete walls are considered to be crucial in differentiating morphologically related inter or intra-genic taxa [5,6,11–13].

Taxonomic studies using phylogenetic methods on members of the genus *Cylindrospermum* are limited [11]. Johansen et al. [11] in their phylogenetic analysis using the 16S rRNA gene found that all the five foundational species described by Bornet et Flahault [2] along with other strains isolated from temperate regions formed a monophyletic clade. This clade was designated to represent *Cylindrospermum sensu stricto*. Further, the authors also reported two different clades (Y and Z) consisting of *Cylindrospermum* strains isolated from non-temperate regions [11]. These clades were phylogenetically distant from *Cylindrospermum sensu stricto*, indicating the polyphyletic nature of the genus. In a recent study, Pal et al. [10] created a novel genus, *Johanseniella*, to accommodate the *Cylindrospermum* strains of the clade Y (as designated in Johansen et al. [11]). Furthermore, Tawong et al. [14] in their study found that their strains, which showed morphological resemblance to the genus *Cylindrospermum*, clustered distantly from *Cylindrospermum sensu stricto*, *Johanseniella* and clade Z of Johansen et al. [11]. Based on the phylogenetic evidence, Tawong et al. created a novel genus, *Neocylindrospermum* [14]. The recent studies of Pal et al. [10] and Tawong et al. [14] have contributed to resolving the phylogenetic inconsistencies of *Cylindrospermum*-like taxa to a certain extent, indicating the importance of the polyphasic approach in cyanobacterial taxonomic studies. Johansen et al. [11] emphasized the need for further studies using a polyphasic approach within the genus *Cylindrospermum*, but unfortunately, there has been no such study conducted to date.

Continuing our long-term pursuit of studying and unravelling the taxonomic complexities of Indian cyanobacteria dwelling in different habitats, we started to sample different regions of the Jammu division (Jammu and Kashmir, Union Territory) in 2019. Amongst the many strains that have been isolated and characterized, two *Cylindrospermum*-like strains were found to be interesting and hence were evaluated extensively using a polyphasic approach. Out of these two strains, KUT1-PS, emerged as a novel species in the genus *Cylindrospermum* and is being described as a novel species of *Cylindrospermum*, following the norms of the International Code of Nomenclature for algae, fungi and plants (ICN). The other strain, 18C-PS, turned out to be *Cylindrospermum badium* after a thorough polyphasic evaluation. The case study of 18C-PS reflects an interesting example in modern cyanobacterial taxonomy, in which strains isolated from different geographical and ecological realms turned out to represent the same species. The strain 18C-PS also exhibited interesting life-cycle events that will serve to bring more clarity into the morphological intricacies of the genus *Cylindrospermum*. The current study continues to bring more information on the cyanobacterial diversity from different habitats across India, which we believe is just the tip of the iceberg.

2. Materials and Methods

2.1. Sampling, Isolation and Maintenance of the Isolates

Sampled specimens were collected in 50 mL falcon tubes containing 20 mL of sterile BG11₀ liquid medium [15]. Geographical coordinates, physicochemical parameters (pH, temperature, TDS and salinity) and the vegetation type of the habitat and surrounding areas were recorded at the time of sampling. The collected samples were observed under a microscope for the presence of cyanobacteria. Further, the samples were spread on solidified BG11₀ medium [15] with 1.2% agar. After an initial growth was observed, a

small portion of the mat was taken from the Petri plate and transferred to a clean and sterile culture tube containing 5 mL sterile BG11₀ liquid medium. This step of subculturing from solid to liquid medium and vice versa was done until uni-cyanobacterial strains were obtained for both samples. The uni-cyanobacterial strains, 18C-PS and KUT1-PS were maintained in slants of solidified BG11₀ medium and also in 100 mL cotton-stopped flasks containing 50 mL liquid BG11₀ medium, at 22 °C ± 2 °C under the cool white light of approximately 50–55 $\mu\text{Em}^{-2} \text{s}^{-1}$, with a photoperiod of a 14/10 h light/dark cycle. Both the strains, KUT1-PS and 18C-PS were deposited in the Global Collection of Cyanobacteria (<https://ccinfo.wdcm.org/details?regnum=1165> (accessed on 30 January 2023)), Varanasi, India and are available under the accession numbers GCC20227 and GCC202228, respectively.

2.2. Morphological Analysis

Morphological analysis of both the strains characterized in this study (KUT1-PS and 18C-PS) was carried out with the help of an Olympus BX53 light microscope. Morphological characters such as the size and shape of vegetative cells and the position, size and shape of heterocytes and akinetes, etc. were carefully observed. The morphologies of both strains were matched and compared with the other species of *Cylindrospermum*, as described in the keys of Desikachary [5] and Komárek [6] and the other related research articles.

2.3. Molecular Analysis

Approximately 100–120 mg exponentially growing, uni-cyanobacterial culture was taken for each isolate (KUT1-PS and 18C-PS), washed 6–8 times with de-ionized water and subjected to genomic DNA isolation. DNA was isolated with the help of a FastDNA Spin Kit for Soil (MP Biomedicals, Santa Ana, CA, USA) and visualized on 0.8% agarose gel. The 16S rRNA gene of both the strains was amplified with the primer pA (5'-AGAGTTTGATCCTGGCTCAG-3') [16] and a cyanobacteria-specific B23S primer (5'-CTTCGCCTCTGTGTGCCTAGGT-3') [17]. The PCR reaction was performed in a 25 μL volume consisting of dNTPs (250 μM) and Taq Buffer (1X), 1 μL of each primer (0.4 μM), 0.3 μL of Taq DNA polymerase (1U/ μL) and 2 μL of genomic DNA (15–40 ng/ μL). With the initial denaturation at 94 °C for 5 min and final extension at 72 °C for 3 min, the amplification of the 16S rRNA gene was performed for 34 cycles at 94 °C for 30 s, 55 °C for 30 s and 72 °C for 60 s with a Bio-Rad C1000TM Thermal Cycler. The 16S-23S ITS region was amplified using the primers and conditions mentioned by Iteman et al. [18]. The sequencing was performed by Sanger's method with a 3730xl DNA analyzer (Applied Biosystems, Foster City, CA, USA). Sequences were assembled into contigs, and their quality was monitored using ChromasPro (ver. 2.1.10, Technelysium Pty. Ltd. Unit 406, 8 Cordelia St., South Brisbane, QLD, Australia). Finally, the sequences were submitted to NCBI GenBank and are available under the accession numbers OQ055346 and OQ055347.

2.4. Phylogenetic Analysis

Phylogenetic analysis of the strains KUT1-PS and 18C-PS was performed by using the 16S rRNA gene and 16S-23S ITS region. Phylogenetically related sequences of the 16S rRNA gene and the 16S-23S ITS region of both the strains under investigation were obtained by NCBI Blast search. The sequences for 16S rRNA gene phylogenetic analysis were aligned by MAFFT (version 7) [19] and manually curated to ensure that the secondary structure of the 16S rRNA gene was maintained. In addition, phylogenetic analysis was also inferred using the 16S-23S ITS region. The operons of the 16S-23S ITS region with both tRNAs were recovered for both KUT1-PS and 18C-PS and the phylogenetic analysis was inferred using the orthologous operons of the phylogenetically related strains. The sequences were aligned using MAFFT and manually curated to maintain the secondary structure of the conserved domains. Further, the phylogenetic trees for both analyses were reconstructed using Bayesian inference (BI), maximum likelihood (ML) and neighbour-joining (NJ) methods. The BI analysis was performed in Mr Bayes 3.2.2 [20]. For the

16S rRNA gene, the analysis was run for 4.7 million generations, whereas for the 16S-23S ITS region, the analysis was run for 0.5 million generations. A standard deviation of the split frequency of less than 0.01 was achieved for both analyses. The sampling frequency, print frequency and diagnostic frequency for both analyses were set to 1000. The estimated sample size (ESS) was above 100 and the potential scale reduction factor (PSRF) was near to 1 for all the parameters. The ML tree for each analysis was constructed in IQ-TREE [21], using the ultrafast bootstrap method with 1000 replications [22]. The BI and ML trees in both analyses were reconstructed using the GTR + G + I model, selected using jModelTest [23]. Furthermore, the NJ tree was inferred using MEGA X [24] for each analysis and the standard bootstrap method with 1000 replications was used to test the reliability of both the 16S rRNA gene and 16S-23S ITS region analyses [25]. Finally, the trees were visualised and edited with the help of iTOL (v6) [26] and Adobe Illustrator 2022 (version 23.0.2).

2.5. 16S-23S ITS Secondary Structure Analysis

The 16S-23S ITS regions of the strains under study along with their phylogenetically related strains were annotated by colour-coding the different ITS domains. The domains corresponding to the D1-D1', V2, BoxB and V3 regions were folded using Mfold on UNAFold web server [27]. The folded secondary structures were downloaded and redrawn in Adobe Illustrator 2022 (version 23.0.2). Furthermore, the lengthwise comparison of different ITS domains (leader, D1-D1', Spacer + D2, Spacer + D3 + Spacer, tRNA^{Ile}, Spacer + V2 + Spacer, tRNA^{Ala}, Spacer, BoxB + Spacer, BoxA, D4 + Spacer and V3 + end of ITS) was also done for our strains and their phylogenetically related taxa.

2.6. Calculations of 16S rRNA Gene Percent Similarity and 16S-23S ITS Percent Dissimilarity

p-distance values for calculating the 16S rRNA gene percent similarity and 16S-23S ITS percent dissimilarity was determined using MEGA X [24]. Further, the percent similarity of the 16S rRNA gene was calculated using the formula $(1-p\text{-distance}) \times 100$, whereas the percent dissimilarity of the 16S-23S ITS region was calculated using the formula $p\text{-distance} \times 100$.

2.7. Preparation of Holotype and Isotype

For holotype preparation, a portion of culture from each strain was preserved in a metabolically inactive form, provided with the accession number and stored in the Global Collection of Cyanobacteria (<https://ccinfo.wdcm.org/details?regnum=1165> (accessed on 30 January 2023)), Varanasi, India. Isotype and herbaria preparation in duplicate for each strain were performed by taking a liquid culture, sieving onto a filter paper followed by air drying, keeping the filter paper in a small Petri plate and storing it in an acid-free envelope after proper labelling.

3. Results

3.1. Site Studied

The cyanobacterial samples were collected from the Basantgarh village of Udhampur district, in the Union territory of Jammu and Kashmir, India. This village falls in the subtropical region, has hilly terrain and is located at an altitude of ~2000 m above sea level in the Shivalik range of the Himalayas. Climatic conditions generally remain pleasant in the summers, with the average minimum and maximum temperature ranging between 24–37 °C, whereas the winters are extremely cold, with the average minimum and maximum temperature ranging between 5–15 °C. The area receives snowfall in the months of December to March and rainfall occurs in the months of July to September, with the annual rainfall in the region being about 40 mm. The vegetation of the area includes Deodar Forests with *Berberis*, *Rubus*, *Rosa*, *Prinsepia*, *Sarcococca*, *Litsea*, *Wikstroemia*, etc. and many other shrubs and plants in the wild. Apples, pears, walnuts, plums, peaches, kiwis, etc. are grown in the orchards and maize is grown as an annual crop.

Cylindrospermum badium 18C-PS was isolated from the cyanobacterial sample collected in November 2019, from a vernal pool (32°48'23" N, 75°32'36" E). The depth of the vernal pool was around one ft. and the sample collection was carried out at nearly 0.5 ft. depth from the surface of the pool. The temperature at the time of sample collection was 12.7 °C and the pH, electrical conductivity, total dissolved solids (TDS) and salinity of the pool were recorded at 5.5, 54.0 µS, 38.5 ppm and 27.3 ppm, respectively. Further, *Cylindrospermum solincola* KUT1-PS was isolated from the cyanobacterial sample collected in June 2021, from the surface of the soil found near an agricultural field. The temperature at the time of sample collection was 26 °C.

3.2. Morphological Characterization

The morphology of the natural sample of *Cylindrospermum solincola* KUT1-PS showed two different heterocytous cyanobacterial forms. One of the morphological forms exhibited a typical *Cylindrospermum*-like morphology, with isopolar trichomes, cylindrical-shaped vegetative cells, terminal heterocytes and paraheterocytic akinetes, whereas the other morphological form exhibited a *Calothrix*-like morphology, with heteropolar filaments and heterocytes at the basal end. In culture conditions, the trichomes of *Cylindrospermum solincola* KUT1-PS were isopolar and flexuous, with cylindrical (many times isodiametric too) vegetative cells. Heterocytes were solitary and terminal, sometimes developing asymmetrically at both ends of the trichome. Akinetes were solitary, large and always formed adjacent to the heterocyte. The exospore of mature akinetes was covered with spines (Figure 1, Table 1).

Table 1. Comparison of morphological features of *Cylindrospermum solincola* KUT1-PS and *Cylindrospermum badium* 18C-PS with their morphologically, geographically and phylogenetically related taxa (based on 16S rRNA gene) of the genus *Cylindrospermum*. Note: Species studied in the present investigation are marked in bold.

Species	Habitat	Vegetative Cell (Size; LxW)	Heterocyte (Size; LxW)	Akinete (Size; LxW)
<i>Cylindrospermum solincola</i> KUT1-PS	Wet soil	Cylindrical, often longer than wide, some isodiametric and shorter than wide, 1.49–3.08 × 1.72–2.11 µm	Terminal, solitary, both terminal ends, oval, rarely spherical, 2.89–4.39 × 2.61–3.33 µm	Large, solitary, at one terminal end, adjoining to heterocyte, young cylindrical, mature oblong, wall serrated with spines, yellowish in colour, 10.21–13.98 × 5.04–6.41 µm
<i>Cylindrospermum gorakhpurence</i>	Moist soils in paddy fields	Cylindrical, 7–16.5 × 3.5–5 µm	Terminal, solitary, both terminal ends, ellipsoidal, 13.2–16.5 × 6–7.8 µm	Subterminal, ellipsoidal with rounded ends, wall thick, with delicate, needle-like projections, 26.4–33 × 13.2–20 µm
<i>Cylindrospermum dobrudjense</i>	Agricultural soils (<i>Medicago sativa</i>)	Cylindrical, longer than wide, 6.6–9.2 × 4 µm	Terminal, both terminal ends, elongated ellipsoidal, conical or subspherical, 9.4–13.4 × 6.7 µm	Solitary, elongated, ellipsoidal, with rounded ends, wall with very fine and short spines, 22.8–26.6 (28.8) × 9.2–10.5 µm
<i>Cylindrospermum ecballiisporum</i>	Edge of wetted, peaty, partly periodically submerged pools and ditches	Cylindrical, ± isodiametric or longer than wide, end cells (without heterocytes) cylindrical and rounded 2.5–7.5 (8) × 2–3.4 µm	Terminal, almost spherical, ellipsoidal, short cylindrical or ovoid, slightly wider than vegetative cells, 5–10 × 3.5–6 µm	Solitary, rarely in pairs, adjoining to heterocytes, cylindrical-oval, rarely ellipsoidal, wall thick, with spines, (14) 20–33 (40) × (9) 11–14.5 (16) µm

Table 1. Cont.

Species	Habitat	Vegetative Cell (Size; LxW)	Heterocyte (Size; LxW)	Akinete (Size; LxW)
<i>Cylindrospermum maius</i> CCALA 998	Recultivated soil after coal mining	Cylindrical, longer than wide, isodiametric to shorter than wide, apical cell rounded to cylindrical, $3.7\text{--}6.5 \times 3.9\text{--}5 \mu\text{m}$	Terminal, spherical to elongated, $4\text{--}10 \times 4.5\text{--}6 \mu\text{m}$	Solitary, adjoining to heterocyte, cylindrical, oval to ellipsoidal, wall smooth, initially colourless, later brown and layered $1\text{--}1.5 \mu\text{m}$ wide, (18) $21\text{--}36 \times 10\text{--}15$ (16) μm
<i>Cylindrospermum badium</i> 18C-PS	Vernal pool	Cylindrical, isodiametric to longer than wide, $1.44\text{--}2.92 \times 1.47\text{--}1.88 \mu\text{m}$	Terminal, solitary, one or both terminal ends, oval to ovoid with broad base, $2.59\text{--}3.74 \times 2.04\text{--}2.51 \mu\text{m}$	Large, solitary, at one terminal end, adjoining to heterocyte, young oval to oblong, covered with a thin, transparent and diffuent layer of mucilage, mature oblong, wall thick, smooth and chestnut brown, $6.57\text{--}10.63 \times 4.31\text{--}7.19 \mu\text{m}$
<i>Cylindrospermum badium</i> CCALA 1000	Recultivated soil after coal mining with sweet gum	Cylindrical, isodiametric to longer than wide, $3.5\text{--}7.5 \times 3.0\text{--}4.8 \mu\text{m}$	Terminal, solitary, almost spherical, elongated or slightly conical, $5\text{--}10$ (13) $\times$, $3\text{--}5 \mu\text{m}$	Solitary, paraheterocytic, oval broadly, flattened at ends, wall should be granulated or coarse instead of smooth, wide and chestnut-coloured, $17\text{--}30 \times 10.0\text{--}14.4 \mu\text{m}$
<i>Cylindrospermum musicola</i> Var. <i>kashmiriensis</i>	Epiphytic on <i>Myriophyllum</i> in shallow pond	Barrel-shaped, longer than broad, $2.6\text{--}8.4 \times 2.6\text{--}3.9 \mu\text{m}$	Terminal and intercalary, terminal, solitary, one or both terminal ends, oval or ellipsoidal, intercalary, solitary or in pairs, shorter or longer than vegetative cells, $5.2\text{--}10.5 \times 3.9\text{--}5.9 \mu\text{m}$	Adjoining to terminal heterocyte, barrel-ellipsoidal, wall thick and smooth, $9.5\text{--}13.6 \times 5.2\text{--}7.8 \mu\text{m}$
<i>Cylindrospermum stagnale</i>	Marshes, pools, ponds, lakes	Cylindrical, isodiametric or double longer than wide, (3) $3.8\text{--}4.5$ (6?) μm wide, apical cylindrical-rounded	Usually oval, ovoid, or cylindrical, with rounded ends, rarely almost spherical, $7\text{--}16 \times$ (4.5) $6\text{--}7 \mu\text{m}$	Solitary, cylindrical or cylindrical-oval, rounded at the ends, smooth-walled, $32\text{--}40 \times 10\text{--}16 \mu\text{m}$
<i>Cylindrospermum skujae</i>	Wet sandy soils or sand, sometimes among mosses, in drying pools with a sandy bottom	Cylindrical, rarely isodiametric mainly $1.5\text{--}2$ (4) $\times$ longer as wide, $1.6\text{--}5$ (7) $\times 2.2\text{--}3 \mu\text{m}$, terminal conical or cylindrical and rounded	Terminal, at one end only, oval, ovoid to cylindrical with rounded ends, (4.8) $6\text{--}8 \times 2.8\text{--}4.5 \mu\text{m}$	Solitary or pairs or up to 3 in a row oval-cylindrical, wall smooth, (12) $14\text{--}36 \times 7\text{--}11.5 \mu\text{m}$
<i>Cylindrospermum muscicola</i>	Wet soils, among mosses, shallow ditches and pools with water vegetation	More or less cylindrical, $\pm$ isodiametric or slightly longer or shorter than wide, terminal conical rounded or cylindrical and rounded, $3.6\text{--}5.4$ (6) $\times 3\text{--}5.3 \mu\text{m}$	Elongated, $5\text{--}7$ (10.5) $\times$ (3.6) $4\text{--}5$ (7) μm	Solitary, oval or ovoid, rounded at ends, wall smooth, $10\text{--}20$ (21) $\times$ (8.2) $9\text{--}12 \mu\text{m}$
<i>Cylindrospermum alatosporum</i>	Soil or guts of earthworms	Cells isodiametric or longer than wide, rounded at ends, $3\text{--}7$ (8) $\times 3.5\text{--}5.0 \mu\text{m}$	Rounded cylindrical, elongated or almost spherical, $4\text{--}9$ (11) $\times 3.5\text{--}7.0 \mu\text{m}$	Solitary or in pair, oval-rhomboid, wall smooth, $20\text{--}32 \times$ (6.5) $10.0\text{--}13.0$ (17.5) μm

Table 1. Cont.

Species	Habitat	Vegetative Cell (Size; LxW)	Heterocyte (Size; LxW)	Akinete (Size; LxW)
<i>Cylindrospermum licheniforme</i>	Prairie remnant soil	Cylindrical, shorter than wide to isodiametric, $3.1\text{--}5.1 \times 3.6\text{--}4.8 \mu\text{m}$	Terminal, solitary, oval to elongated to bluntly conical, $5.4\text{--}9 \times 4.0\text{--}5.2 \mu\text{m}$	Solitary, paraheterocytic, oval broadly to elongated, wall smooth, $13\text{--}23 \times 7.0\text{--}12.4 \mu\text{m}$
<i>Cylindrospermum moravicum</i>	Cave sediment	Cylindrical, rarely concave or irregular, isodiametric, longer than wide, $2.7\text{--}5 \times 3.5\text{--}7 \mu\text{m}$	Terminal, solitary, spherical to cylindrical elongated, $5.0\text{--}9.0 (11) \times 2.7\text{--}6.0 \mu\text{m}$	Solitary, paraheterocytic, granulated, cylindrical, with widened end toward the heterocyte, wall wide with colourless to golden-brown, smooth, internally structured or lamellate exospores, (18) $22\text{--}32 \times 9\text{--}13 \mu\text{m}$

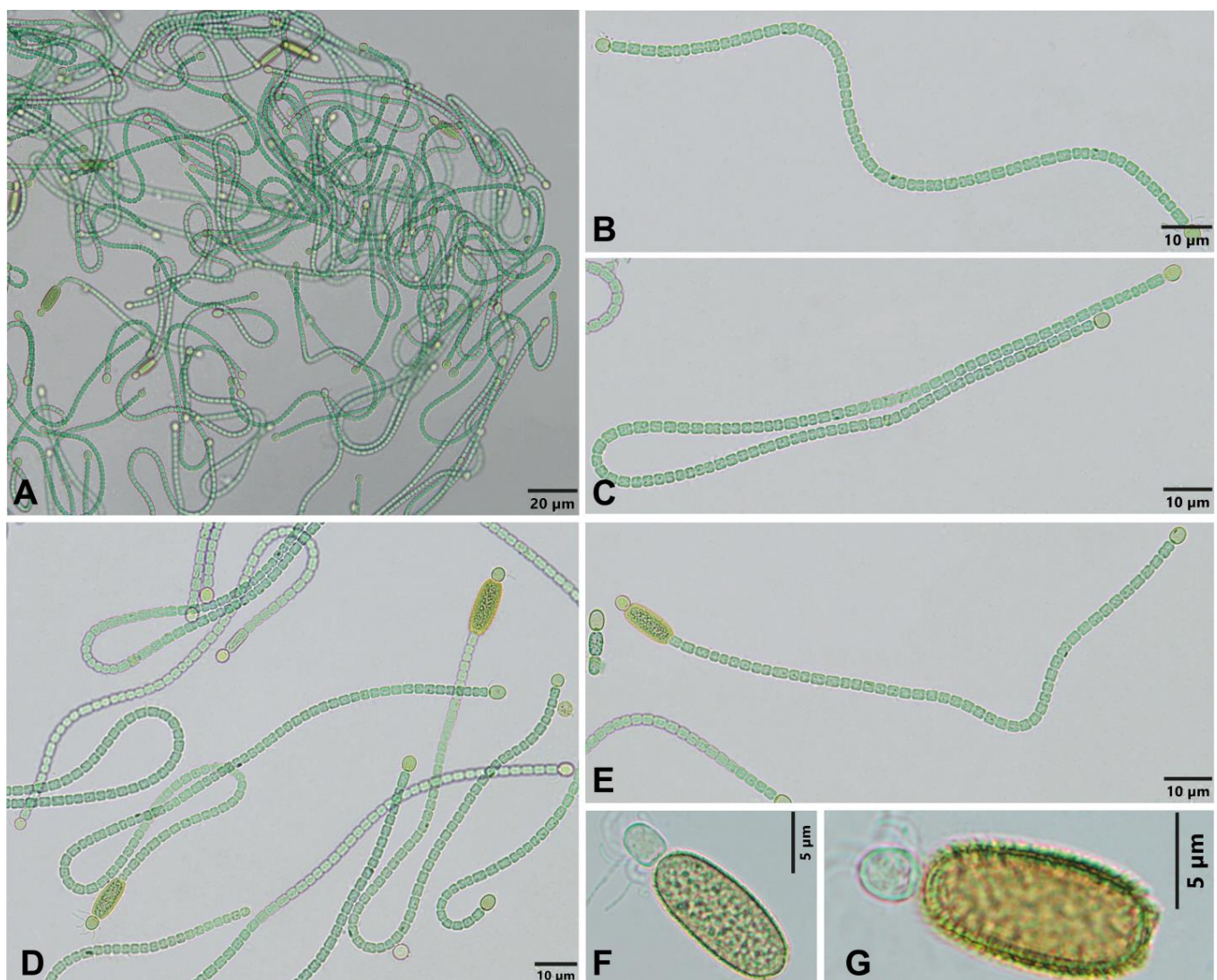


Figure 1. Microscopic plate showing the morphological features of *Cylindrospermum solincola* KUT1-PS. (A) trichomes short, isopolar, slightly bent or irregularly coiled; (B,C) vegetative cells cylindrical, longer or shorter than wide or isodiametric, and heterocytes terminal, solitary at both ends of the trichome; (D,E) heterocytes oval or spherical and a large, young, solitary akinete adjoining to the heterocyte at one end of the trichome; (F,G) mature, oblong, granulated, exospore thick akinete, serrated with short spines. Scale bars: (A) 20 µm; (B–E) 10 µm and (F,G) 5 µm.

The morphology of the natural sample of *Cylindrospermum badium* 18C-PS also showed an assemblage of greenish and bluish-green isopolar and heteropolar filaments with heterocytes. The isopolar filaments were either long with terminal and intercalary heterocytes or short with only terminal heterocytes at both ends. The trichomes of *Cylindrospermum badium* 18C-PS were short, straight or slightly bent with cylindrical vegetative cells. Heterocytes were solitary, present either at one terminal end or both terminal ends of the trichome. Akinetes were large, solitary and always formed adjacent to heterocyte. Young akinetes appeared greenish in colour and were covered with a thin, transparent and diffuent layer of mucilage. The exospore of the mature akinete was thick, chestnut brown and did not have spines (Figure 2, Table 1).

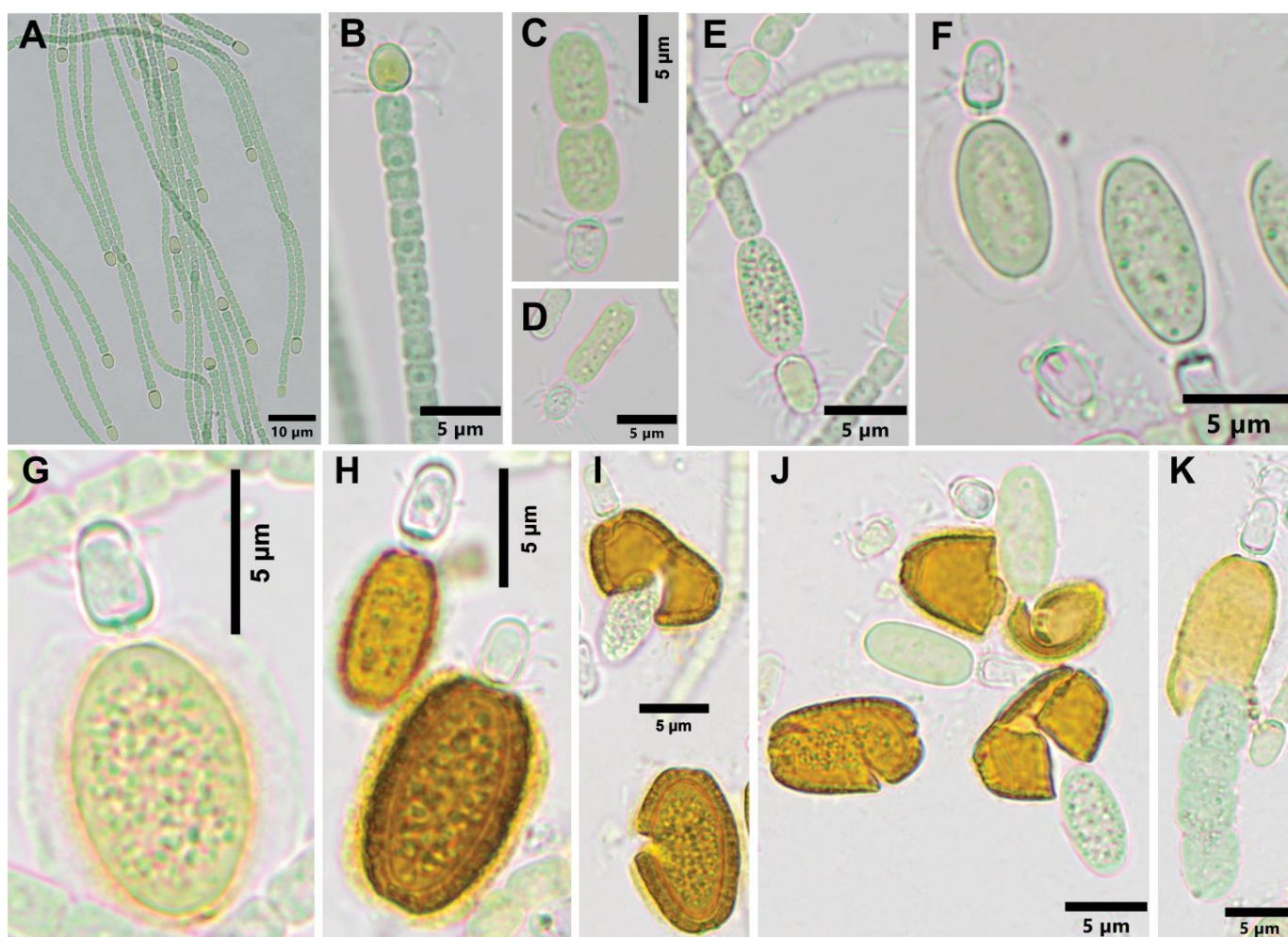


Figure 2. Microscopic plate showing the morphological features of *Cylindrospermum badium* 18C-PS. (A) trichomes short, isopolar, straight or slightly bent, cells cylindrical, isodiametric to longer than wide, heterocytes solitary, terminal, one or both ends, oval to ovoid with a broad base; (B) heterocyte with thread-like projections; (C) development of proakinetes; (D,E) proakinetes, cylindrical, free or attached, adjoining to heterocyte at one end of trichome, constricted or unconstricted at the centre; (F,G) young, oval to oblong akinetes, solitary with a thin, transparent, diffuent layer of mucilage; (H) mature, oblong akinetes, exospore chestnut brown, thick and smooth; (I,J) rupturing of akinetes wall, release of akinetes; (K) germination of akinete. Scale bars: (A) 10 µm and (B–K) 5 µm.

3.3. Phylogenetic Analysis Based on 16S rRNA Gene and 16S-23S ITS Region

The 16S rRNA gene phylogenetic tree was reconstructed using 216 nucleotide sequences belonging to the heterocytous cyanobacteria and *Chroococcidiopsis* was used as an outgroup (Figure 3). Among the 216 nucleotide sequences corresponding to the order Nos-

tales, 43 sequences belonged to *Cylindrospermum sensu stricto* (Figure 3). The clustering of strains—KUT1-PS and 18C-PS within the *Cylindrospermum sensu stricto* indicated that both the strains under investigation were representatives of the genus *Cylindrospermum* (Figure 4).

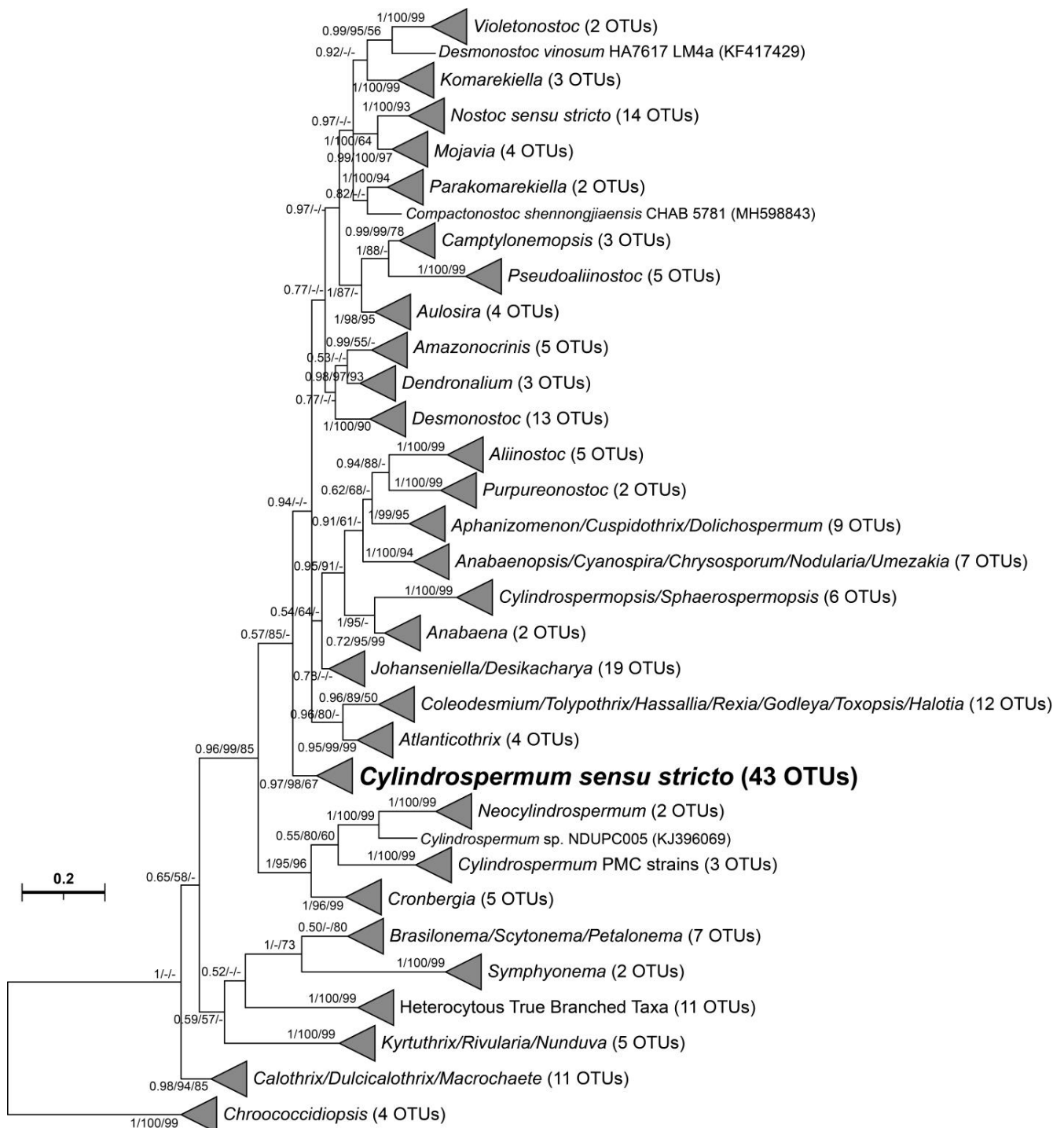


Figure 3. Phylogenetic position of *Cylindrospermum sensu stricto* with morphologically related and unrelated taxa of heterocytous cyanobacteria based on 16S rRNA gene sequences of 216 OTUs of heterocytous cyanobacteria, rooted with 4 OTUs of *Chroococcidiopsis* and posterior probability values/bootstrap values (0.50/≥50%), representing BI/ML/NJ mapped on to the nodes in the BI analysis. Scale bar represents 0.2 changes per nucleotide position.

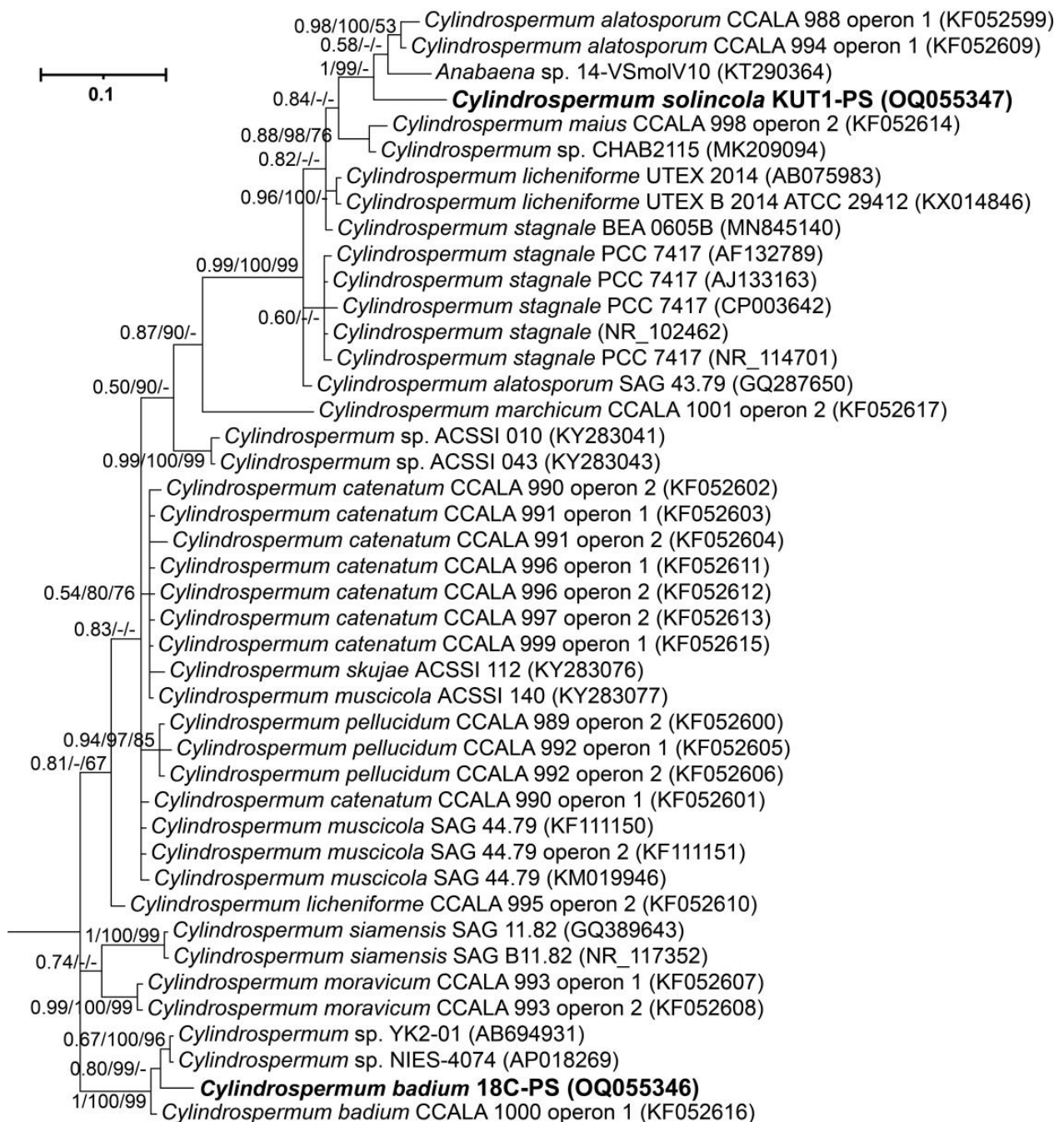


Figure 4. Phylogenetic position of *Cylandrospermum solincola* KUT1-PS and *Cylandrospermum badium* 18C-PS within the *Cylandrospermum sensu stricto* clade, based on 16S rRNA gene and posterior probability values/bootstrap values (0.50/ $\geq 50\%$), representing BI/ML/NJ mapped onto the nodes in the BI analysis. Scale bar represents 0.1 changes per nucleotide position.

C. solincola KUT1-PS clustered with *C. alatosporum* (CCALA 988/CCALA 994), *C. maius* CCALA 998 (reference strain), *Cylandrospermum* sp. CHAB2115 and *Anabaena* sp. 14-VSmolV10 (Figure 4). The strains of *C. alatosporum* were isolated from wildfire-impacted soil of the Riding Mountains National Park, Canada and from the gut of earthworms collected from soils above Amaterská Cave, Czech Republic. *C. maius* CCALA 998 was isolated from recultivated (top) soil after coal mining and with loblolly pine in the Pyramid State Recreation Area, Illinois, USA. The strain *Cylandrospermum* sp. CHAB2115 was isolated from benthic areas of freshwater Dali, China, while *Anabaena* 14-VSmolV10 was isolated from the eastern part of the Smolnice mine waste dump in the Czech Republic.

Cylindrospermum badium 18C-PS, along with *Cylindrospermum badium* CCALA 1000, *Cylindrospermum* sp. NIES-4074 and *Cylindrospermum* sp. YK2-01, clustered together at a single node (Figure 4). *C. badium* CCALA 1000 was isolated from the recultivated topsoil after coal mining with sweet gum in Pyramid State Recreational Area, Illinois, USA, whereas *Cylindrospermum* sp. YK2-01 was isolated from the *Nostoc commune* crust, Kanagawa, Japan and *Cylindrospermum* sp. NIES-4074 was isolated from soil in Yokohama, Kanagawa, Japan.

Furthermore, the 16S-23S ITS phylogenetic tree was reconstructed using 12 nucleotide sequences belonging to *Cylindrospermum sensu stricto*. *C. badium* 18C-PS, along with *C. badium* CCALA 1000, clustered together with strong probability /bootstrap support, whereas *C. solincola* KUT1-PS clustered separately at the bottom of the tree (Figure 5).

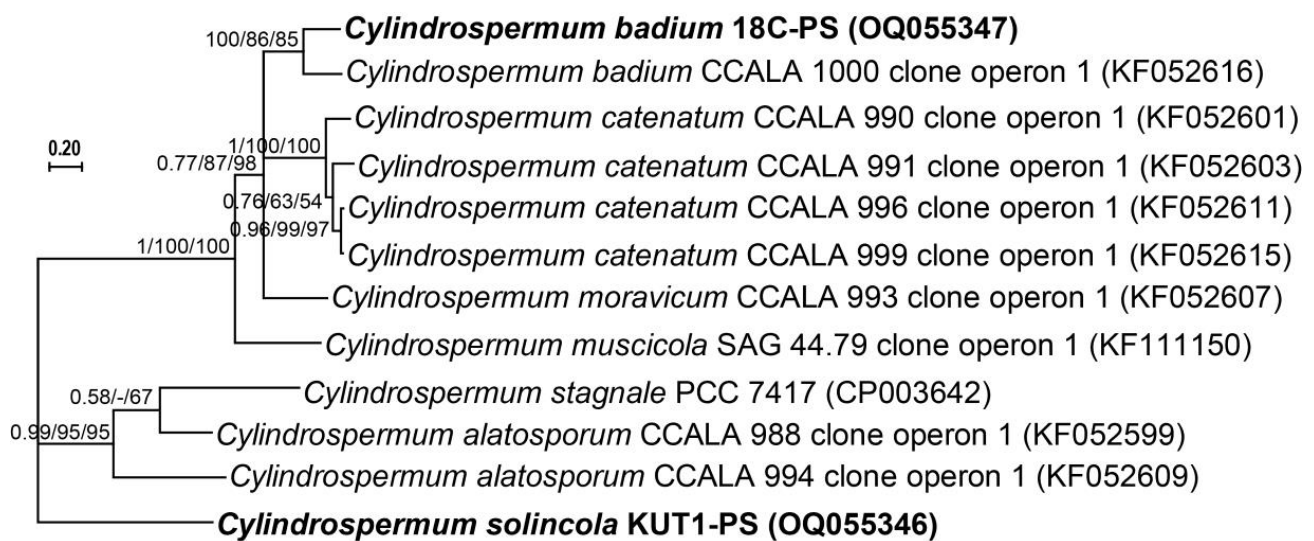


Figure 5. Phylogenetic analysis based on 16S-23S ITS region with both tRNA operons, showing the phylogenetic positioning of *Cylindrospermum solincola* KUT1-PS and *Cylindrospermum badium* 18C-PS and posterior probability values/bootstrap values (0.50/ $\geq 50\%$), representing BI/ML/NJ mapped onto the nodes in the BI analysis. Scale bar representing 0.20 changes per nucleotide position.

3.4. Percent Similarity Analysis of 16S rRNA Gene

The 16S rRNA gene percent similarity of *C. solincola* KUT1-PS and *C. badium* 18C-PS with the other members of *Cylindrospermum sensu stricto* was in the range of 96.3–98.9% and 96.5–99.7% (Table S1), respectively, whereas the percent similarity between *C. solincola* KUT1-PS and *C. badium* 18C-PS was 96.3% (Table S1).

3.5. Secondary Structure Analysis of 16S-23S ITS Region

The D1-D1' helix region of all the *Cylindrospermum* strains consisted of 64 nucleotides (Table S2). Furthermore, the folded secondary structures of all the strains were similar and consisted of a 5 bp basal stem followed by a 3' unilateral bulge formed by 7 nucleotides. The unilateral bulge was followed by a bilateral bulge of 9 nucleotides and a terminal loop of 5 nucleotides (Figure 6). The lengths of the V2 helices and their folded secondary structures were variable among the *Cylindrospermum* species (Figure 7, Table S2). The folded secondary structure of the V2 helix of *C. solincola* KUT1-PS consisted of an 11 bp basal stem followed by two bilateral bulges and a terminal loop (Figure 7). On the other hand, the folded secondary structure of *C. badium* 18C-PS consisted of an 11 bp basal stem followed by a single bilateral bulge and a terminal loop (Figure 7).

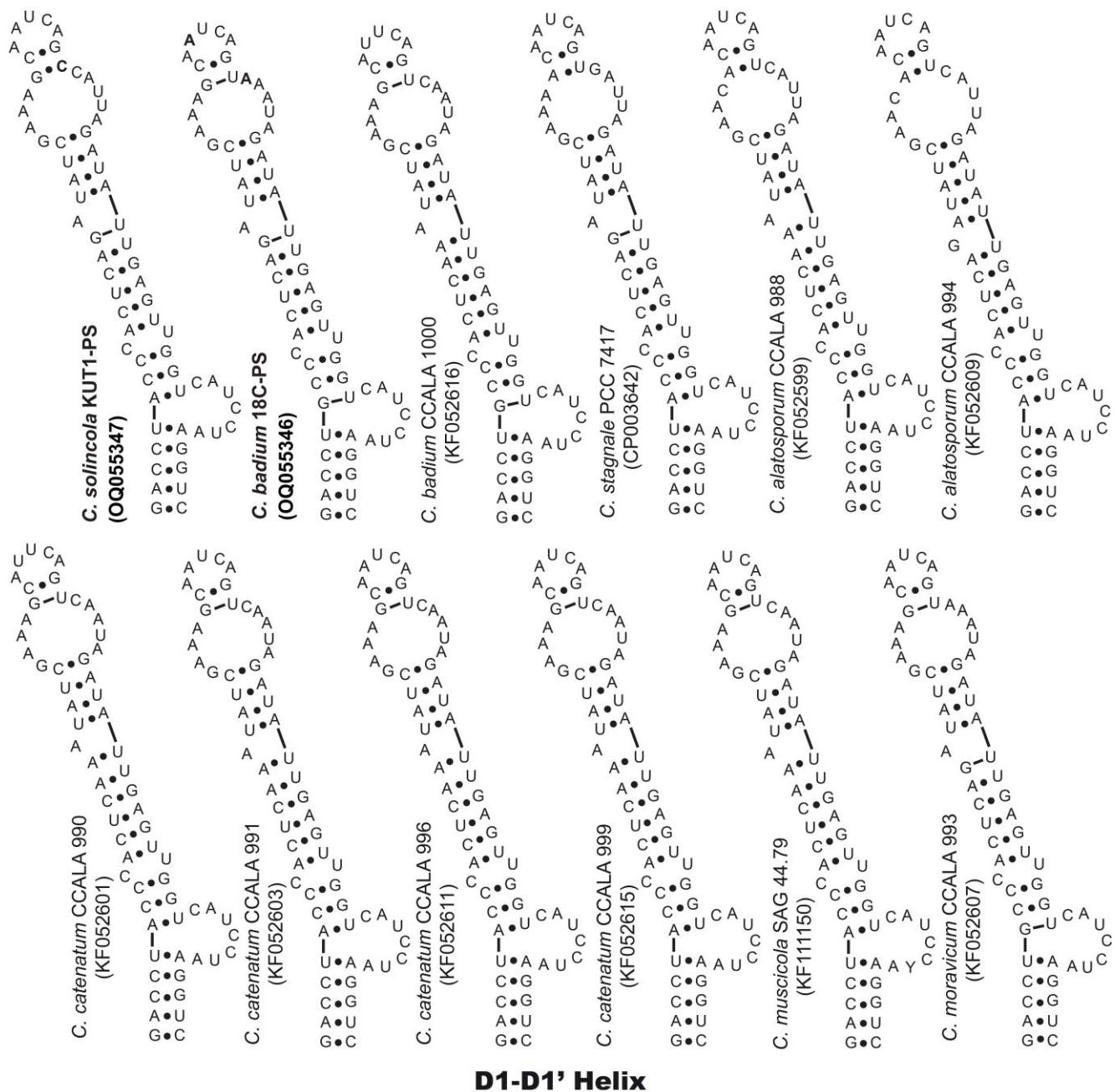


Figure 6. Comparative folded secondary structure analysis of D1-D1' helix of *Cyindrospermum solincola* KUT1-PS and *Cyindrospermum badium* 18C-PS, with their phylogenetically related strains based on the 16S rRNA gene.

Furthermore, the BoxB region consisted of 27 nucleotides in all the strains included in the analysis, except in *C. stagnale* PCC 7417, which consisted of 28 nucleotides (Figure 7). The folded secondary structure of the BoxB region of *C. solincola* KUT1-PS was found to be similar to that of *C. alatosporum* (CCALA 988, CCALA 994), consisting of a 4 bp basal stem followed by a bilateral bulge and a terminal loop (Figure 7). The folded secondary structure of *C. badium* 18C-PS was found to be similar to *C. badium* CCALA 1000, *C. catenatum* (CCALA 990, CCALA 991 and CCALA 996), *C. muscicola* SAG 44.79 and *C. moravicum* CCALA 993, comprising a 4 bp basal stem followed by a unilateral bulge opposed by adenine and a terminal loop (Figure 7). The length of the V3 helix within the *Cyindrospermum* varied from 33–41 nucleotides (Table S2). The folded secondary structures of the strains included in the analysis were similar, having a 3 bp basal stem followed by a bilateral bulge and a terminal

loop, except for the folded secondary structure of *C. moravicum*, which consisted of two bilateral bulges (Figure 7).

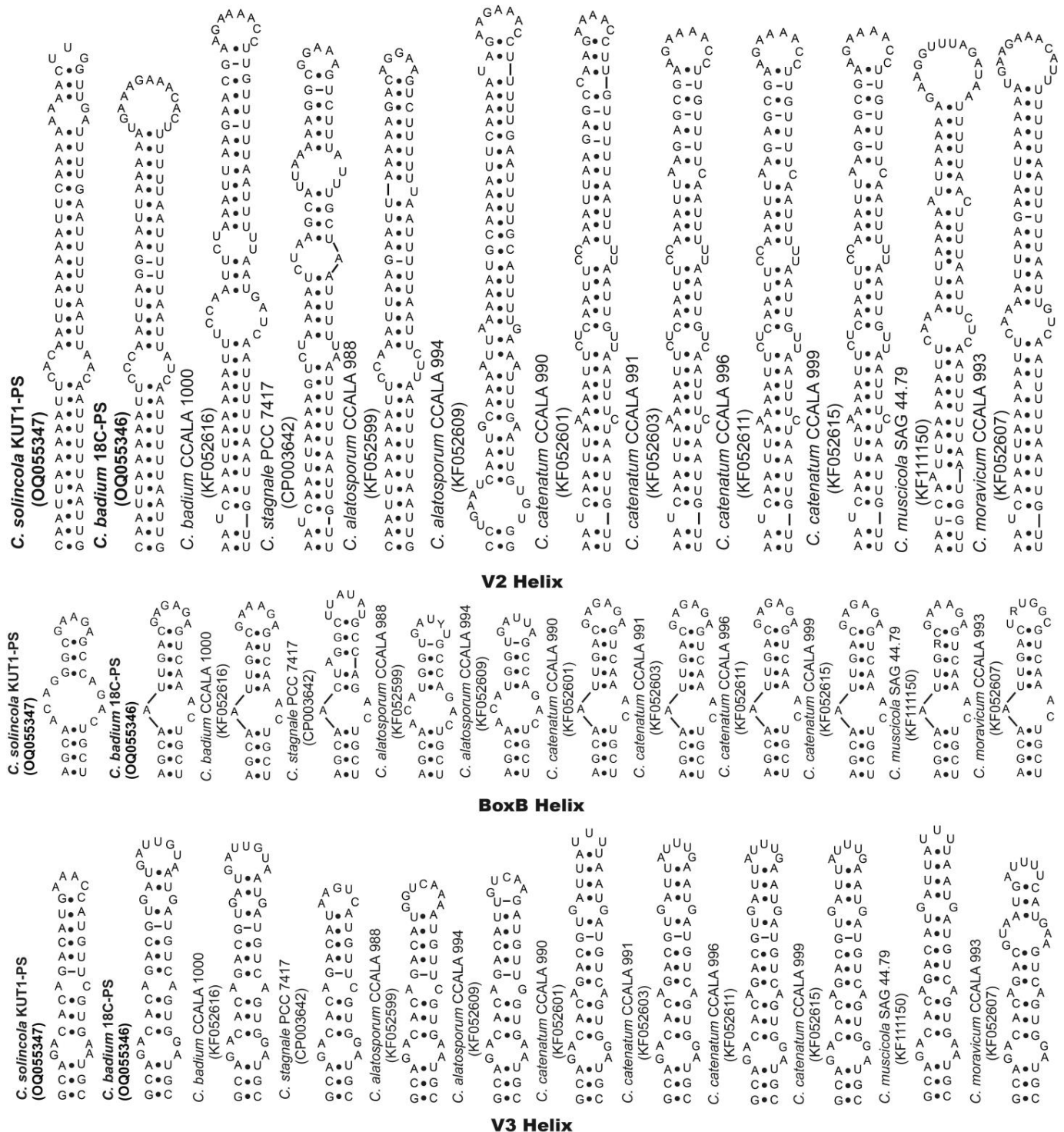


Figure 7. Comparative folded secondary structure analysis of V2, BoxB and V3 helices of *Cylindrospermum solincola* KUT1-PS and *Cylindrospermum badium* 18C-PS, with their phylogenetically related strains based on the 16S rRNA gene.

3.6. Percent Dissimilarity Analysis of 16S-23S ITS Region

Furthermore, the percent dissimilarity of the 16S-23S ITS region of *C. solincola* KUT1-PS and *C. badium* was in the range of 12.2–15.5% and 4.1–16.6%, respectively, with their phylogenetically related strains (Table S3).

3.7. Taxonomic Description

Cylindrospermum solincola. N Kumar and P Singh sp. nov. (Figure 1A–G).

Description: Thallus dark blue-green mucilaginous mass in the natural environment and under the microscope, typical *Cylindrospermum*-like trichomes with a large paraheterocytic akinete were observed. In culture conditions, a greenish, thick, gelatinous mat observed on the surface of a Petri plate, olive at the centre and greenish spreading out. In liquid medium, a greenish, gelatinous mat formed, which turns brownish in older cultures. Trichomes cylindrical, uniseriate, isopolar, unbranched, short, flexuous, not straight, loosely tangled, greenish in colour and without any sheath. Vegetative cells slightly constricted at the ends, cylindrical, granulated and usually longer than wide, but some cells shorter than wide to isodiametric; 1.49–3.08 µm long and 1.72–2.11 µm wide. Heterocytes terminal, pale coloured, solitary, at both ends, usually oval, and very rarely spherical, 2.89–4.39 µm long and 2.61–3.33 µm wide. Akinetes large, yellowish, solitary, paraheterocytic, granulated, thick-walled, serrated with spines and with only one per filament; cylindrical when young and oblong when mature, 10.21–13.98 µm long and 5.04–6.41 µm wide.

Etymology: *solincola* (sol.in'co.la. L. neut. n. *solum*, soil; L. masc./fem. n. *incola*, an inhabitant; N.L. n. *solincola*, an inhabitant of soil).

Type locality: the sample was collected by Naresh Kumar in June 2021 from the village Basantgarh, Udhampur, Union Territory of Jammu and Kashmir, India (32°48'27'' N 75°32'37'' E).

Ecology of type locality: wet soil, found near a small agricultural field in which maize is cultivated annually. The sampling site was surrounded by a coniferous forest of Deodar, spiny bushes and grasses. Dark bluish-green mucilage found at the surface of the soil was collected as a sample.

Holotype (here designated): A portion of a culture of *Cylindrospermum solincola* has been preserved in a metabolically inactive form in the Global Collection of Cyanobacteria (<https://ccinfo.wdcm.org/details?regnum=1165> (accessed on 30 January 2023)), Varanasi, India, and is available under the accession number GCC20227.

Isotype (here designated): Herbaria was deposited in the Global Collection of Cyanobacteria with the number GCC-botanybhu-20227.

Reference strain: KUT1-PS.

NCBI GenBank Accession number: OQ055347.

4. Discussion

The aim of this study was to characterize two *Cylindrospermum*-like strains isolated from the Jammu division of the Jammu and Kashmir Union territory of India. This is the first study after the work of Yajnavalkya Bharadwaja, in which a novel species of *Cylindrospermum* from Jammu and Kashmir is described. Bharadwaja in 1931 described *C. muscicola* var. *kashmiriensis* growing on *Myriophyllum* in a shallow pond situated in Srinagar, Kashmir [28]. Both the strains investigated in this study differ from *C. kashmiriensis* in more than one feature, such as the shape and size of the vegetative cells, heterocytes and akinetes, the position of the heterocytes and the ecology of the habitat (Table 1).

C. solincola KUT1-PS was related to *C. gorakhpurensis*, *C. dobrudjense* and *C. ecballiisporum* in terms of the ecology and morphology of the akinetes. The aforementioned species, including *C. solincola* KUT1-PS, were isolated from wet/moist soils (except *C. ecballiisporum*, which was isolated from the edge of wet peat) and had spines on their akinetes. However, the smaller size of vegetative cells, heterocytes and akinetes along with minor differences in their shapes distinguished *C. solincola* KUT1-PS from *C. gorakhpurensis*, *C. dobrudjense* and *C. ecballiisporum* (Table 1).

Further, *C. badium* 18C-PS was morphologically similar to *C. badium* CCALA 1000 in terms of trichome features, the shape of vegetative cells, the basal position and shape of heterocytes, the paraheterocytic position and the very typical chestnut brown smooth exospore of large akinetes in later stages of growth. Both the strains differed from each other only in the dimensions of vegetative cells, heterocytes and akinetes, this is understandable given the slight differences in ecology. Thus, this particular instance of the two strains representing *C. badium* casts intriguing reflections on the biogeographical importance of these findings. The evolution of cyanobacterial species and their subsequent spread to different geographical regions is something that has still not been clearly understood and hence must be studied in the near future. Another interesting observation related to *C. badium* 18C-PS was the extremely clear exhibition of different life cycle events (Figure 2). Various events were observed, including the rupturing of the exospore, the release of the spore and the germination of the spore into hormogonia (Figure 2I–K).

In the 16S rRNA gene phylogenetic analysis, *C. solincola* KUT1-PS and *C. badium* 18C-PS clustered within the *Cylindrospermum sensu stricto* clade with strong and well-supported posterior probabilities/bootstrap support. Unlike all the previous phylogenetic studies based on the 16S rRNA gene on the genus *Cylindrospermum* or its related taxa [7,10,11,14], *Cylindrospermum sensu stricto* clustered with good posterior probabilities/bootstrap support in the current phylogenetic analysis (Figure 3). However, similar to the above studies, *C. alatosporum*, *C. licheniforme*, *C. stagnale*, *C. catenatum* and *C. muscicola* were found to be polyphyletic (Figure 4). The clustering of these strains again clearly emphasizes the need for taxonomic revisions inside the *Cylindrospermum sensu stricto*.

All the phylogenetically related strains of *C. solincola* KUT1-PS and *C. badium* 18C-PS were isolated from different habitats located in different localities. Amongst the phylogenetically related strains of *C. solincola* KUT1-PS, two strains, *Anabaena* sp. 14-VSmolV10 and *Cylindrospermum* sp. CHAB2115, were not identified up to the rank of species level. Further, the absence of oval or rhomboidal-shaped large-sized akinetes with smooth walls and smaller sizes of vegetative cells and heterocytes differentiated *C. solincola* KUT1-PS from *C. alatosporum* (Table 1). Similarly, the smaller size of vegetative cells, heterocytes and akinetes and the presence of spines on the wall of akinetes differentiated *C. solincola* KUT1-PS from *C. maius* (Table 1).

The 16S rRNA gene percent similarity of *C. solincola* KUT1-PS was found to be the highest, at 98.8%, with *C. alatosporum* CCALA 994. This value is higher than the threshold value recommended by Yarza et al. [29] for the delimitation of prokaryotic species (Table S1). However, Johansen et al. [11] also reported exceptionally high 16S rRNA gene percent similarity within the *Cylindrospermum sensu stricto*. The authors concluded that the morphological diversity within the genus *Cylindrospermum* exceeds variations observed in the 16S rRNA gene, suggesting recent rapid divergence within the genus [11]. The present study also supports the observations made in the study of Johansen et al. [11].

Furthermore, the phylogenetic positioning of 18C-PS in the 16S rRNA gene tree and the percent similarity of 18C-PS with *C. badium* CCALA 1000 was 99.7%. The results from the 16S rRNA gene phylogenetic analysis and 16S rRNA gene percent similarity indicate that the strain 18C-PS is a representative of *C. badium*, supporting the results obtained from morphological characterization. Moreover, the results from the 16S-23S ITS phylogenetic analysis also indicated that 18C-PS is a representative of *C. badium* and *C. solincola*. KUT1-PS is a novel species of the genus *Cylindrospermum* (Figure 5), providing further support to the results from the 16S rRNA gene phylogenetic analysis.

Furthermore, the 16S-23S ITS region's folded secondary structure analysis was conducted to enhance the resolution at the species level. The D1-D1' helix of *C. solincola* KUT1-PS was highly similar to other *Cylindrospermum* species and differed in only one base at the top of the bilateral bulge (Figure 6). However, the folded secondary structures of the V2, BoxB and V3 helices of *C. solincola* KUT1-PS were completely different from the other phylogenetically related strains included in the analysis (Figure 7).

The folded secondary structure of the D1-D1' helix of *C. badium* 18C-PS was identical to *C. moravicum* CCALA 993 and was similar to *C. badium* CCALA 1000, with minor differences in the middle region, the bilateral bulge and the terminal loop (Figure 6). Similarly, the folded secondary structure of the BoxB region of *C. badium* 18C-PS was identical to *C. catenatum* (CCALA 990, CCALA 991, CCALA 996, CCALA 999) and showed one nucleotide difference in the terminal loop of *C. badium* CCALA 1000 (Figure 7). Further, the folded secondary structure of the V3 region of *C. badium* 18C-PS was completely identical to *C. badium* CCALA 1000 (Figure 7). Amongst all the compared motifs of the 16S-23S ITS, only the V2 helix was found to be unique in *C. badium* 18C-PS (Figures 6 and 7).

Additionally, the percent dissimilarity of the 16S-23S ITS of *C. solincola* KUT1-PS was >7% from the other phylogenetically related members of the *Cylindrospermum sensu stricto* (Table S3). Notably, percent dissimilarity values $\geq 7\%$ strongly indicate that the strains belong to separate species [30–32]. However, the percent dissimilarity of *C. badium* 18C-PS was <7% with *C. badium* CCALA 1000 (4.1%) and *C. moravicum* CCALA 993 (5.5%) and >7% with the other members of the *Cylindrospermum sensu stricto* (Table S3).

Thus, based on the results obtained from the comparative morphological analysis, phylogenetic analysis based on the 16S rRNA gene and 16S-23S ITS region, the 16S-23S ITS secondary structure analysis and the 16S-23S ITS percent dissimilarity, we gathered enough evidence indicating that *C. solincola* KUT1-PS represents a novel species of the genus *Cylindrospermum*. On the other hand, based on the morphological similarities, the phylogenetic positioning in the 16S rRNA gene and 16S-23S ITS region, the percent similarity of the 16S rRNA gene and the percent dissimilarity of the 16S-23S ITS region, it was established that strain 18C-PS was a representative of *C. badium*.

This study serves as an important reminder, especially to modern cyanobacterial taxonomists, to always compare newly sequenced strains with previously established species before going ahead with the creation of a novel species. Molecular studies should always be supplemented with morphological assessments in order to avoid ambiguous situations that could arise in the future. This fact has been mentioned very clearly in Komárek [33] and we fully agree that the polyphasic approach must always have molecular criterion as the primary and morphological/ecological evidence as a secondary but essential criterion in order to properly establish any taxonomic entity. In a similar vein, we also feel that phylogenomic interpretations could be employed to bring an enhanced resolution and separate the various strains that are present inside the *Cylindrospermum* cluster. However, unless and until all the taxa inside this cluster are sequenced, taxa-limited phylogenomic studies will bring in additional confusing interpretations. Issues related to limited taxon sampling and ambiguities emerging even after phylogenomic interpretations have already started to emerge in heterocytous cyanobacteria (the *Amazonocrinis* story, Alvarenga et al. [34], and Kumar et al. [35]), and we believe that with time, as more data emerges from the tropical and subtropical regions, taxonomic efforts will fuel interesting stories and debates. Hence, caution must be adopted while deriving taxonomic conclusions from whole genome efforts. Over-reliance on any method, modern or classical, must always be avoided and in fact, striking a careful balance between these methods constitutes the basic spirit of the polyphasic approach.

Further, recent studies on *Cylindrospermum* and *Cylindrospermum*-like taxa [7,10,11,14] clearly indicate that the diversity of these taxa is underrepresented and more taxonomic studies, based on a polyphasic approach, on the cyanobacterial strains that are morphologically similar to the genus *Cylindrospermum* need to be conducted in the future.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/d15050592/s1>, Table S1. Comparative percent similarity matrix of the 16S rRNA gene of *C. solincola* KUT1-PS and *C. badium* 18C-PS with their phylogenetically related taxa based on 16S rRNA gene; Table S2. Comparison of nucleotide length of the 16S-23S ITS regions of *C. solincola* KUT1-PS and *C. badium* 18C-PS with their phylogenetically related taxa based on 16S rRNA gene; Table S3. Comparative percent dissimilarity matrix of the 16S-23S ITS regions

of *C. solincola* KUT1-PS and *C. badium* 18C-PS with their phylogenetically related taxa based on 16S rRNA gene.

Author Contributions: Conceptualization, N.K., A.S., S.P. and P.S.; methodology, N.K. and P.S.; software, N.K. and S.P.; validation, A.S. and P.S.; formal analysis, A.S., S.P. and P.S.; investigation, A.S., S.P. and P.S.; resources, A.S. and P.S.; data curation, A.S., S.P. and P.S.; writing—original draft preparation, N.K.; writing—review and editing, A.S. and P.S.; visualization, N.K., A.S., S.P. and P.S.; supervision, P.S.; project administration, P.S.; funding acquisition, P.S.; All authors have read and agreed to the published version of the manuscript.

Funding: The work was funded by the Department of Science and Technology (DST-SERB), India, through the Core Research Grant Project CRG/2018/004111. The Banaras Hindu University is also acknowledged for providing Seed Grant under the Institution of Eminence (IoE) Scheme.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: We thank the Head of the Department of Botany at Banaras Hindu University (BHU) for providing the necessary facilities and encouragement. NK thanks the University Grants Commission (UGC) for the Senior Research Fellowship (SRF). SP thanks the Department of Science and Technology for the DST-INSPIRE Senior Research Fellowship (SRF). We thank Aharon Oren for his help with the Latin etymology of the scientific name.

Conflicts of Interest: The authors declare that they have no conflict of interest.

References

1. Kützing, F.T. *Phycologia Generalis Oder Anatomie, Physiologie und Systemkunde der Tange: Mit 80 Farbigen Gedruckten Tafeln*; Brockhaus: Munich, Germany, 1843.
2. Bornet, E.; Flahault, C. *Revision des Nostocacées Hétérocystées Contenues Dans les Principaux Herbiers de France (Quatrième et Dernier Fragment)*; Annales des Sciences Naturelles Botanique Septième Série 1886–1888; G. Masson: Paris, France, 1890; pp. 177–262.
3. Gardner, N.L. *The Myxophyceae of Porto Rico and the Virgin Islands*; New York Academy of Sciences: New York, NY, USA, 1932.
4. Geitler, L.S. Klasse schizophyceae. In *Natürliche Pflanzenfamilien 1b*; Engler, A., Prantl, K., Eds.; Duncker and Humblot: Berlin, Germany, 1942; pp. 1–232.
5. Desikachary, T.V. Cyanophyta. In *ICAR Monograph on Algae*; Indian Council of Agricultural Research: New Delhi, India, 1959.
6. Komárek, J. *Süßwasserflora von Mitteleuropa*; Bd. 19/3: Cyanoprokaryota. 3. Teil/3rd part: Heterocytous genera. Süßwasserflora von Mitteleuropa; Spektrum Akademischer Verlag: Heidelberg, Germany, 2013.
7. Genuário, D.B.; Sant’Anna, C.L.; Melo, I.S. Elucidating the *Cronbergia* (Cyanobacteria) dilemma with the description of *Cronbergia amazonensis* sp. nov. isolated from Solimões river (Amazonia, Brazil). *Algal Res.* **2018**, *29*, 233–241. [CrossRef]
8. Guiry, M.D.; Guiry, G.M. *AlgaeBase*; World-Wide Electronic Publication, National University of Ireland: Galway, Ireland, 2023; Available online: <https://www.algaebase.org> (accessed on 10 January 2023).
9. Hauer, T.; Komárek, J. *CyanoDB 2.0—On-Line Database of Cyanobacterial Genera*; World-Wide Electronic Publication, University of South Bohemia and Institute of Botany AS CR: Ceske Budejovice, Czech Republic, 2023; Available online: <http://www.cyanodb.cz> (accessed on 10 January 2023).
10. Pal, S.; Saraf, A.; Kumar, N.; Singh, A.; Talukdar, U.; Kohar, N.; Singh, P. Digging deeper into the taxonomy of *Cylindrospermum* and description of *Johanseniella tripurensis* gen. et sp. nov. from India. *FEMS Microbiol. Lett.* **2022**, *369*, fnac074. [CrossRef] [PubMed]
11. Johansen, J.R.; Bohunická, M.; Lukešová, A.; Hřčková, K.; Vaccarino, M.A.; Chesarino, N.M. Morphological and molecular characterization within 26 strains of the genus *Cylindrospermum* (Nostocaceae, Cyanobacteria), with descriptions of three new species. *J. Phycol.* **2014**, *50*, 187–202. [CrossRef] [PubMed]
12. Komárek, J.; Zapomělová, E.; Hindák, F. *Cronbergia* gen. nov., a new cyanobacterial genus (Cyanophyta) with a special strategy of heterocyte formation. *Cryptogam. Algol.* **2010**, *31*, 321–341.
13. Komárek, J.; Kaštovský, J.; Mareš, J.; Johansen, J.R. Taxonomic classification of cyanoprokaryotes (cyanobacterial genera) 2014, using a polyphasic approach. *Preslia* **2014**, *86*, 295–335.
14. Tawong, W.; Pongcharoen, P.; Saijuntha, W. *Neocylindrospermum variakinetikum* gen. & sp. nov. (Nostocales, Cyanobacteria), a novel genus separated from *Cylindrospermum* using a polyphasic method. *Phycologia* **2022**, *61*, 653–668.
15. Rippka, R.; Deruelles, J.; Waterbury, J.B.; Herdman, M.; Stanier, R.Y. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *Microbiology* **1979**, *111*, 1–61. [CrossRef]
16. Edwards, U.; Rogall, T.; Blöcker, H.; Emde, M.; Böttger, E.C. Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. *Nucleic Acids Res.* **1989**, *17*, 7843–7853. [CrossRef] [PubMed]

17. Gkelis, S.; Rajaniemi, P.; Vardaka, E.; Moustaka-Gouni, M.; Lanaras, T.; Sivonen, K. *Limnothrix redekei* (Van Goor) Meffert (Cyanobacteria) strains from Lake Kastoria, Greece form a separate phylogenetic group. *Microb. Ecol.* **2005**, *49*, 176–182. [\[CrossRef\]](#) [\[PubMed\]](#)
18. Iteman, I.; Rippka, R.; de Marsac, N.T.; Herdman, M. Comparison of conserved structural and regulatory domains within divergent 16S rRNA–23S rRNA spacer sequences of cyanobacteria. The GenBank accession numbers for the sequences reported in this paper are AF180968 and AF180969 for ITS-L and ITS-S, respectively. *Microbiology* **2000**, *146*, 1275–1286. [\[CrossRef\]](#) [\[PubMed\]](#)
19. Katoh, K.; Rozewicki, J.; Yamada, K.D. MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. *Brief. Bioinform.* **2019**, *20*, 1160–1166. [\[CrossRef\]](#) [\[PubMed\]](#)
20. Ronquist, F.; Teslenko, M.; Van Der Mark, P.; Ayres, D.L.; Darling, A.; Höhna, S.; Larget, B.; Liu, L.; Suchard, M.A.; Huelsenbeck, J.P. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* **2012**, *61*, 539–542. [\[CrossRef\]](#) [\[PubMed\]](#)
21. Trifinopoulos, J.; Nguyen, L.T.; Von Haeseler, A.; Minh, B.Q. W-IQ-TREE: A fast online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Res.* **2016**, *44*, 232–235. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Minh, B.Q.; Nguyen, M.A.T.; Haeseler, A.V. Ultrafast approximation for phylogenetic bootstrap. *Mol. Biol. Evol.* **2013**, *30*, 1188–1195. [\[CrossRef\]](#)
23. Darriba, D.; Taboada, G.L.; Doallo, R.; Posada, D. jModelTest 2: More models, new heuristics and parallel computing. *Nat. Methods* **2012**, *9*, 772. [\[CrossRef\]](#)
24. Kumar, S.; Stecher, G.; Li, M.; Knyaz, C.; Tamura, K. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* **2018**, *35*, 1547. [\[CrossRef\]](#)
25. Felsenstein, J. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **1985**, *39*, 783–791. [\[CrossRef\]](#)
26. Letunic, I.; Bork, P. Interactive Tree of Life (iTOL): An online tool for phylogenetic tree display and annotation. *Bioinformatics* **2007**, *23*, 127–128. [\[CrossRef\]](#)
27. Zucker, M. Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acid Res.* **2003**, *31*, 3406–3415. [\[CrossRef\]](#)
28. Bharadwaja, Y. Contributions to Our Knowledge of the Myxophyceae of India. *Ann. Bot.* **1933**, *47*, 117–143. [\[CrossRef\]](#)
29. Yarza, P.; Yilmaz, P.; Pruesse, E.; Glöckner, F.O.; Ludwig, W.; Schleifer, K.H.; Whitman, W.B.; Euzéby, J.; Amann, R.; Rosselló-Móra, R. Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. *Nat. Rev. Microbiol.* **2014**, *12*, 635–645. [\[CrossRef\]](#) [\[PubMed\]](#)
30. Bohunická, M.; Pietrasiak, N.; Johansen, J.R.; Gómez, E.B.; Hauer, T.; Gaysina, L.A.; Lukešová, A. *Roholtiella*, gen. nov. (Nostocales, Cyanobacteria)—A tapering and branching cyanobacteria of the family Nostocaceae. *Phytotaxa* **2015**, *197*, 84–103. [\[CrossRef\]](#)
31. González-Resendiz, L.; Johansen, J.R.; Alba-Lois, L.; Segal-Kischinevsky, C.; Escobar-Sanchez, V.; Jimenez-Garcia, L.F.; Hauer, T.; León-Tejara, H. *Nunduva*, a new marine genus of Rivulariaceae (Nostocales, Cyanobacteria) from marine rocky shores. *Fottea* **2018**, *18*, 86–105. [\[CrossRef\]](#)
32. Pal, S.; Saraf, A.; Kumar, N.; Singh, P. Phycological exploration of the global biodiversity hotspots of Northeast India: Discovery of a new species of soil-dwelling cyanobacteria, *Desikacharya kailashaharensis* sp. nov. *FEMS Microbiol. Lett.* **2022**, *369*, fnac099. [\[CrossRef\]](#)
33. Komárek, J. A polyphasic approach for the taxonomy of cyanobacteria: Principles and applications. *Eur. J. Phycol.* **2016**, *51*, 346–353. [\[CrossRef\]](#)
34. Alvarenga, D.O.; Andreote, A.P.D.; Branco, L.H.Z.; Delbaje, E.; Cruz, R.B.; Varani, A.D.M.; Fiore, M.F. *Amazonocrinis nigriterrae* gen. nov., sp. nov., *Atlanticothrix silvestris* gen. nov., sp. nov. and *Dendronalium phyllosphericum* gen. nov., sp. nov., nostocacean cyanobacteria from Brazilian environments. *Int. J. Syst. Evol. Microbiol.* **2021**, *71*, 004811. [\[CrossRef\]](#)
35. Kumar, N.; Saraf, A.; Pal, S.; Mishra, D.; Singh, P. Insights into the phylogenetic inconsistencies of the genus *Amazonocrinis* and description of epilithic *Amazonocrinis malviyai* sp. nov. (Cyanobacteria, Nostocales) from Jammu and Kashmir, India. *Int. J. Syst. Evol. Microbiol.* **2022**, *72*, 005658. [\[CrossRef\]](#)

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.