

## Proposal of *Purpurea* gen. nov. (Nostocales, Cyanobacteria), a novel cyanobacterial genus from wet soil samples in Tibet, China

Fangfang CAI<sup>1,3</sup>, Yilang WANG<sup>1,4</sup>, Gongliang YU<sup>1</sup>, Jie WANG<sup>2</sup>, Xin PENG<sup>1</sup>, Renhui LI<sup>1\*</sup>

<sup>1</sup> Key Laboratory of Algal Biology, State Key Laboratory of Freshwater Ecology and Biotechnology of China, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, People's Republic of China;

\*Correspondence author e-mail: reli@ihb.ac.cn, tel. (+86) 27 68780067, fax (+86) 27 68780123

<sup>2</sup> Department of Biology, Taiyuan Normal University, Jinzhong 030619, China

<sup>3</sup> Key Laboratory for Animal Nutrition and Feed Science of Hubei province, Wuhan Polytechnic University, Wuhan 430023, China

<sup>4</sup> University of Chinese Academy of Sciences, Beijing 100049, People's Republic of China

**Abstract:** A new cyanobacterial strain CHAB5880 morphologically identified to the *Nostoc*-like genus was isolated from wet soil samples in Tibet of China, and it was taxonomically and phylogenetically characterized based on the polyphasic approach combining morphological, genetic and ecological characteristics. Colonies of this strain were usually purple to black, irregularly clustering, forming diffluent mucilage. The phylogenetic tree based on 16S rRNA gene indicated that the novel strain formed a unique cluster, and separated from the '*Nostoc sensu stricto*' clade and from the clades of the morphologically similar genera *Aliinostoc*, *Desmonostoc* and *Holatia*. The secondary structure of ITS between 16S–23S rRNA gene in the strain CHAB5880 showed unique patterns of D1–D1', Box–B and V3 helix, which distinguished it from other heterocytous genera. *Purpurea tibecum* sp. nov. was designated as the type species of the genus.

**Key words:** cyanobacteria, *Purpurea*, new genus, polyphasic methods, 16S rRNA phylogeny, secondary structure of ITS, taxonomy

## INTRODUCTION

The taxonomic system of cyanobacteria has undergone several radical and significant revisions (RIPPKA et al. 1979; ANAGNOSTIDIS & KOMÁREK 1985; HOFFMANN et al. 2005a, 2005b). The polyphasic approach combining morphological, ecophysiological, biochemical and molecular characters have been proposed to classify cyanobacterial diversity since it is likely to select some satisfactory common criteria for the characterization of basic cyanobacterial taxonomic units. In 2014, an important update on the taxonomic system of cyanobacteria was proposed to better reflect the combination of phylogeny and morphology, and to effectively describe the cyanobacterial genus (KOMÁREK et al. 2014). Through much efforts on the application of polyphasic approach for cyanobacteria classification during the past decades, considerable progress has been made in systematics and taxonomy of cyanobacteria, resulting in descriptions of a large amount of novel cyanobacteria genera and species (FIORE et al. 2007; BOHUNICKÁ et al. 2015; GENUÁRIO et al. 2015; SCIUTO & MORO 2016).

The heterocytous taxa have been proven

difficult to characterize, and many morphologically well-defined genera are indeed polyphyletic based on molecular phylogenetic analyses. The taxonomy of these heterocytous taxa characterized by filaments with differentiated heterocytes and akinetes, has undergone considerable revision, even establishing new groups at higher level categories, such as new families including *Tolypothrichaceae*, *Godleyaceae* (HAUER et al. 2014), *Aphanizomenonaceae* (KOMÁREK et al. 2014) and *Dapisostemonaceae* (HENTSCHKE et al. 2016). At genus level, a variety of novel heterocytous cyanobacterial genera were proposed, such as the *Dolichospermum* (WACKLIN et al. 2009), *Sphaerospermopsis* (ZAPOMĚLOVÁ et al. 2009), *Chrysosporum* (ZAPOMĚLOVÁ et al. 2012), *Cyanocohniella* (KAŠTOVSKÝ et al. 2014), *Dactylothamnus* (FIORE et al. 2013), *Roholtiella* (BOHUNICKÁ et al. 2015), *Macrochaete* (BERRENDERO et al. 2016) and *Cyanomargarita* (SHALYGIN et al. 2017), etc.

*Nostocaceae*, the largest family in the Nostocales, has received significantly more studies in recent years. *Nostoc*, the type genus of this family, is a widespread, complicated and commonly encountered group of cyanobacteria, with 104 accepted species based on the updated

AlgaeBase (GUIRY & GUIRY 2019). Phylogenetic studies based on 16S rRNA gene sequence have shown that most strains identified as *Nostoc* do not form a monophyletic cluster (HROUZEK et al. 2005; ŘEHÁKOVÁ et al. 2007; KAŠTOVSKÝ & JOHANSEN 2008; LUKEŠOVÁ et al. 2009; JOHANSEN et al. 2014; CAI et al. 2019a, 2019b). In order to solve the polyphyletic problem of *Nostoc*, ŘEHÁKOVÁ et al. (2007) first described a new genus, *Mojavia*, which is morphologically similar to *Nostoc*, but phylogenetically separated from the '*Nostoc sensu stricto*' clade. Following this kind of revision, several novel genera including *Desmonostoc* (HROUZEK et al. 2013), *Halotia* (GENUÁRIO et al. 2015), *Komarekiella* (HENTSCHKE et al. 2017), *Aliinostoc* (BAGCHI et al. 2017), *Compactonostoc* (CAI et al. 2019a) and *Minunostoc* (CAI et al. 2019b), which morphologically appear to be *Nostoc*-like, have been proposed and separated from the '*Nostoc sensu stricto*' clade based on the phylogenetic analysis of the 16S rRNA gene. Despite these advances, further revisionary work of *Nostocaceae* is expected along more new strains isolated and characterized by using both molecular and morphological features.

In this study, a filamentous heterocytous cyanobacterial strain isolated from wet soils in Tibet, China, was characterized applying a polyphasic approach, by using morphological characters, phylogeny of 16S rRNA gene, 16S–23S internal transcribed spacer (ITS) secondary structure, and ecological data. The results allowed us to describe a novel genus *Purpurea* with the proposal of the type species as *Purpurea tibecum* sp. nov.

## MATERIALS AND METHODS

**Isolation and cultivation.** Samples used in this study were isolated from the wet soil samples in Tibet, China. Strains were smashed into small pieces and fully infiltrated with sterile water before isolation. Unialgal filaments from the visible growths of cyanobacterial thallus samples were isolated by lab-made pasteur pipette under a 100 times magnification microscope (Olympus C31, Japan) and then cultured in screw-capped tubes containing 5 ml of BG11 medium. Isolated strains were subsequently cultivated at 25 °C under a 12:12 h (light: dark) cycle with a photon flux density of 40  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  from white fluorescent lamps.

**Morphological characterization.** Fresh cultures of the studied strains were examined with a Nikon eclipse 80i microscope (Nikon, Tokyo, Japan). Trichome size was measured from  $\geq 50$  individuals using a Nikon eclipse 80i light microscope with DS-R1i digital camera (Nikon, Japan). The image was analyzed using the NIS-Elements D 3.2.

**DNA extraction and PCR amplification.** Total genomic DNA was extracted using the modified cetyltrimethylammonium bromide (CTAB) method adopted by NEILAN et al. (1995). Primers pA (EDWARDS et al. 1989) and B23S (GKELIS et al. 2005) were chosen for obtaining segment containing 16S ribosomal RNA gene and the associated 16S–23S internal transcribed spacer (ITS) region. The PCR reaction had a final

volume of 20  $\mu\text{l}$  with 1  $\mu\text{l}$  of template DNA (100  $\text{ng}\cdot\mu\text{l}^{-1}$ ), 0.5  $\mu\text{l}$  of each primer (10  $\mu\text{mol}\cdot\text{l}^{-1}$ ), 8  $\mu\text{l}$  of sterile water and 10  $\mu\text{l}$  of 2 $\times$  PCR mix with Taq polymerase (Cat TSE001; Beijing Tsingke Biotech Co., Ltd., Beijing, China). The amplification was performed using a BIO–RAD T100™ Thermal Cycler (Cat 186–1096; Bio–Rad Laboratories, Inc. California, USA) with a PCR profile of an initial denaturation at 95 °C for 3 min at beginning, then a total 35–cycle melting at 95 °C for 30 s, annealing at 58 °C for 30 s and extension at 72 °C for 60 s in the middle and final elongation at 72 °C for 5 min. The PCR products were analyzed on 1% agarose gels and DNA strips sized approximate 2200 bp were then purified using TSINGKE DNA Gel Extraction Kit (Cat GE0101–200; Beijing Tsingke Biotech Co., Ltd., Beijing, China) before cloning to vector pMD18–t (Cat D101A; TaKaRa Biotechnology (Dalian) Co., Ltd., Dalian, China). The cloning procedure employed by SAMBROOK & RUSSELL (2001) was performed. Sequencing was carried out using an ABI 3730 Automated Sequencer (PerkinElmer, Waltham, Massachusetts USA).

**Phylogenetic analysis.** In this study, the obtained 16S rRNA gene sequences, together with similar reference sequences from the GenBank database, were aligned using MAFFT v7.312 (KATO & STANDLEY 2013). Alignments finally formed a matrix of 123 sequences with 1128 nucleotide sites. The most appropriate models of DNA sequence evolution were selected for this dataset by a hierarchical likelihood ratio test using MrModel Test version 3.7 (POSADA & CRANDALL 1998). The final phylogenetic trees were constructed using maximum–parimony (MP), maximum likelihood (ML), and Bayesian inference (BI). The MP analysis using a heuristic search with 1000 replicates was run via MEGA version 7.0.26 (KUMAR et al. 2016), the ML algorithms were performed using PhyML 3.0 (GUINDON et al. 2010) and Bayesian inference were analyzed with MrBayes 3.2.6 (RONQUIST et al. 2012). The phylogenetic trees were viewed in FigTree v1.4.3 (<http://tree.bio.ed.ac.uk/software/figtree/>). *Chroococcidiopsis thermalis* PCC7203 was chosen as the outgroup taxon for 16S rRNA phylogeny. Accession numbers for GenBank sequences that were used for phylogenetic analyses were listed as a supplementary file. GenBank accession numbers for our sequences are MN381942, MN381943, MN381944 and MN381945.

**Analyses of 16S–23S Internal Transcribed Spacer (ITS).** 16S–23S rRNA ITS secondary structures of D1–D1', Box–B and V3 helices were determined using RNA structure, version 5.6 (MATHEWS LAB 2013).

## RESULTS

### *Purpurea* F. Cai et R. Li gen. nov.

**Description:** In nature, growing on the wet soil. Colonies usually purple to black, irregularly clustering, forming diffuent mucilage. In liquid media culture growing attached to the glass walls of the test tubes, filaments aggregated into macrocolonies (up to 1 mm in diameters). Filaments flexuous, freely entangled, sheath present or not, colorless. Vegetative cells purple, barrel-shaped to cylindrical. Heterocytes spherical or cylindrical, usually develop in intercalary position, 1 to 3, trichomes divided in the position of heterocyte, leading to 1–3



heterocytes in terminal. Akinetes oval, varying in color from brown, green to purple, easily distinguished from vegetative cells, up to 10 µm long, up to 7 µm wide (average size 7.95 µm long and 6.12 µm wide), usually in short chains (1 to 8). Akinetes develop intercalarly, usually distant from heterocytes, occasionally appear between two heterocytes.

**Type species:** *Purpurea tibecum* F. Cai et R. Li

**Etymology:** The name of genus '*Purpurea*' was chosen due to the purple black vegetative cells.

***Purpurea tibecum* F. Cai et R. Li sp. nov. (Fig. 1)**

**Description:** Colonies purple to black, forming diffuent mucilage. Filaments flexuous, freely entangled, sheath present or not, colorless. Vegetative cells barrel-shaped to cylindrical, 2.28–3.78–5.34 µm long, 2.66–3.36–4.2 µm wide. Heterocytes spherical or cylindrical, 2.79–3.71–4.92 µm long, 2.66–3.46–4.45 µm wide. Akinetes oval, brown,

green to purple, 7.95 µm long and 6.12 µm wide.

**Reference strains:** CHAB 5880.

**Type locality:** Isolated from the wet soil samples in Tibet, China (30°46'32.90"N, 90°52'14.78"E).

**Holotype here designated:** Dry material was stored at the Freshwater Algal Herbarium (HBI), Institute of Hydrobiology, Chinese Academy of Science, Wuhan, China, as specimen No. TBCN201701.

**Etymology:** "*tibecum*" refers to the place Tibet where this species was located.

**Habitat:** Free-living on wet soil.

**Molecular and phylogenetic analysis**

The evolutionary distance based on the 16S rRNA gene showed that the *Purpurea* strain containing two clones shared 100% similarity with each other (Table 1), and similarities to *Aliinostoc*, *Trichormus*, *Desmonostoc*, *Halotia* and *Nostoc* were 94.4%–95.2%, 95.1%–95.2%,

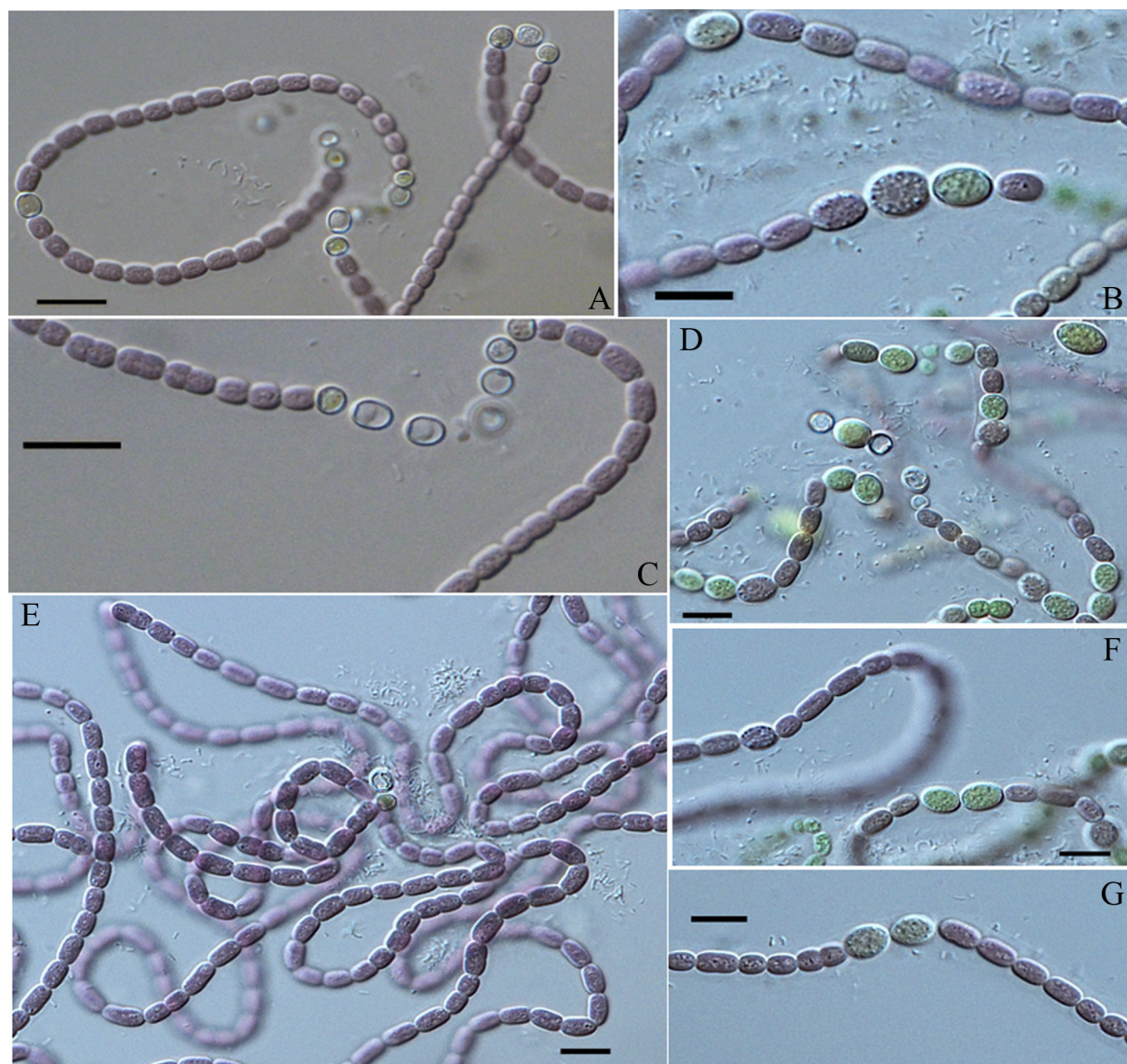


Fig. 1. Micrographs of *Purpurea tibecum* under the light microscopy (LM): (A) Filaments with internal and terminal heterocytes; (B) Akinetes with different color; (C) Filaments with terminal heterocytes; (B, D, E, F, G) Trichomes wrapped by thin sheath. Scale bars 10µm.

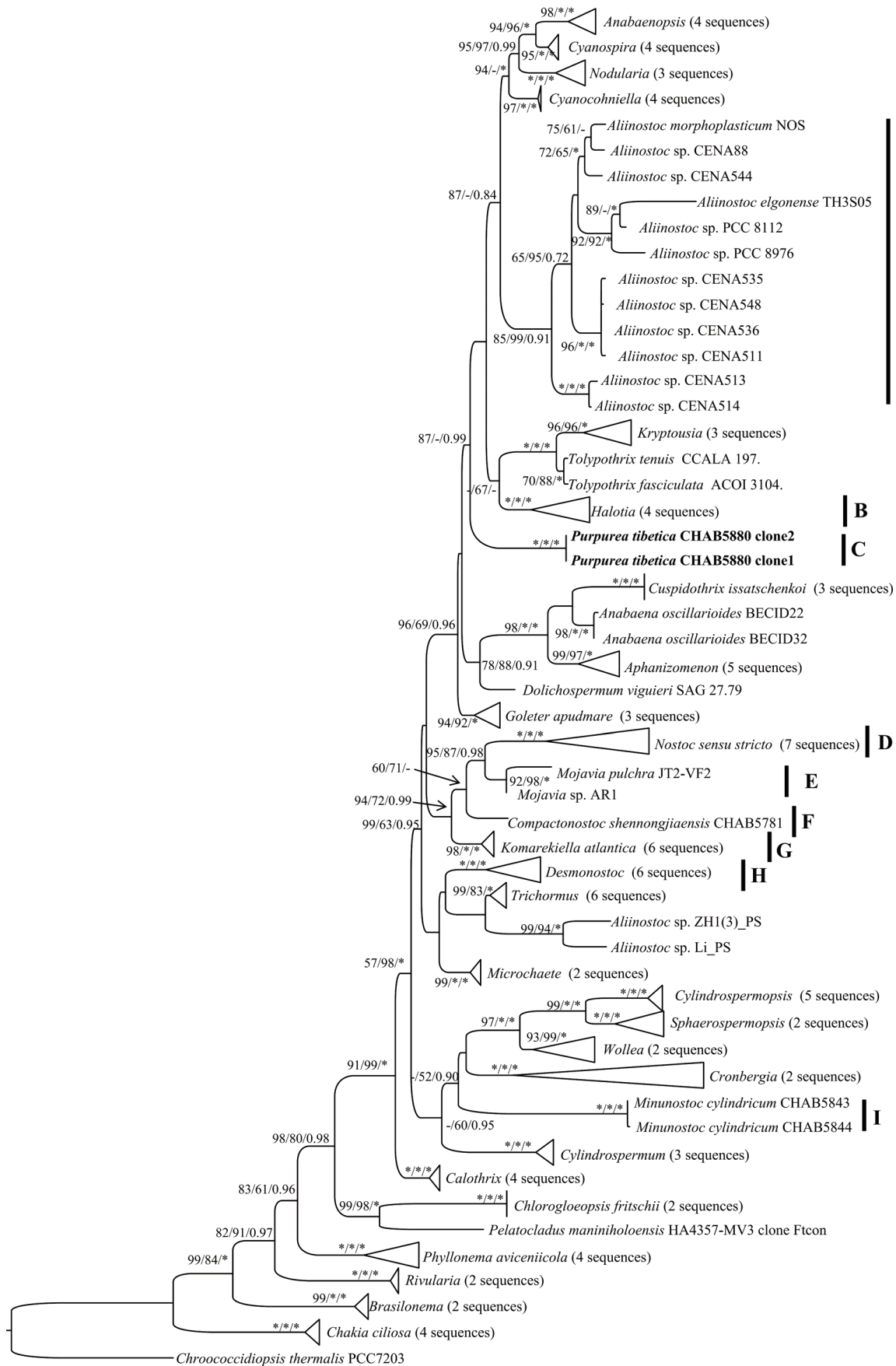


Fig. 2. Bayesian tree (BI) phylogenetic tree based on 16S rDNA sequences (1370bp) of *Purpurea tibecum* and other cyanobacterial strains. Bootstrap values greater than 50% with MP/ML/Mrbayes methods are indicated on the tree. The novel species is in bold font.

Table 1. Comparison of the 16S rRNA gene sequence similarity among *Purpurea* and its related taxa.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1. <i>Purpurea tibecum</i> CHAB5880 clone1	1																			
2. <i>Alinostoc morphoplasticum</i> NOS	0.944																			
3. <i>Alinostoc</i> sp. CENA88	0.952	0.989																		
4. <i>Alinostoc</i> sp. CENA544	0.949	0.985	0.986																	
5. <i>Alinostoc</i> sp. PCC 8112	0.947	0.976	0.977	0.977																
6. <i>Halotia branconii</i> CENA392	0.935	0.952	0.955	0.952	0.951															
7. <i>Halotia longispora</i> CENA184	0.949	0.952	0.958	0.956	0.955	0.976														
8. <i>Goleter apudmare</i> HA4356–MV2	0.952	0.956	0.962	0.957	0.955	0.957	0.964													
9. <i>Cylindropermum alatosporum</i> SAG 43.79	0.943	0.938	0.942	0.938	0.94	0.94	0.949	0.962												
10. <i>Desmonostoc muscorum</i> I	0.947	0.943	0.945	0.94	0.942	0.945	0.957	0.962	0.955											
11. <i>Desmonostoc geniculatum</i> HA4340–LM1	0.942	0.936	0.939	0.935	0.942	0.942	0.954	0.955	0.951	0.992										
12. <i>Trichormus variabilis</i> ATCC 29413	0.951	0.955	0.958	0.952	0.958	0.947	0.949	0.962	0.961	0.958	0.957									
13. <i>Trichormus variabilis</i> NIES23	0.952	0.949	0.953	0.948	0.953	0.944	0.955	0.963	0.963	0.964	0.959	0.99								
14. <i>Komarekiella atlantica</i> CCIBT 3307	0.946	0.949	0.949	0.945	0.953	0.954	0.967	0.977	0.957	0.966	0.965	0.961	0.963							
15. <i>Mojavia pulchra</i> JT2–VF2	0.935	0.935	0.939	0.934	0.937	0.946	0.956	0.96	0.946	0.951	0.946	0.959	0.958	0.963						
16. <i>Compactonostoc shennongjiaensis</i> CHAB5781	0.936	0.934	0.938	0.935	0.946	0.947	0.964	0.964	0.958	0.964	0.966	0.958	0.964	0.973	0.961					
17. <i>Nostoc punctiforme</i> strain PCC 73102	0.928	0.93	0.934	0.93	0.937	0.937	0.943	0.956	0.949	0.954	0.957	0.952	0.949	0.95	0.956	0.95				
18. <i>Nostoc commune</i> WY1KK1	0.919	0.918	0.92	0.916	0.923	0.928	0.935	0.948	0.941	0.945	0.949	0.938	0.938	0.943	0.95	0.94	0.979			
19. <i>Alinostoc</i> sp. ZH1(3)_PS	0.942	0.953	0.942	0.937	0.934	0.947	0.945	0.956	0.937	0.943	0.945	0.961	0.955	0.951	0.945	0.938	0.932	0.918		
20. <i>Minunostoc cylindricum</i> CHAB5843	0.92	0.923	0.923	0.924	0.93	0.919	0.927	0.933	0.937	0.933	0.936	0.939	0.94	0.937	0.923	0.939	0.927	0.921	0.926	
21. <i>Alinostoc</i> sp. Li_PS	0.92	0.934	0.934	0.926	0.936	0.938	0.939	0.948	0.937	0.943	0.944	0.955	0.957	0.946	0.94	0.943	0.931	0.921	0.967	0.94



94.2%–94.7%, 93.5%–94.9%, and 91.9%–92.8%, respectively. The phylogenetic trees, based on 16S rRNA gene from 123 cyanobacterial taxa including strain CHAB5880, were constructed using the MP, ML and Bayesian methods. A Bayesian tree, shown in Fig. 2, revealed that two clones of the strain CHAB5880 were grouped into a unique clade further supported by MP, ML and BI approaches with high bootstrap values as 100%, 100%, and 1.00, respectively. The phylogenetic tree supported phylogenetic monophyly for the *Nostoc* sensu stricto clade, as well as *Desmonostoc*, *Compactonostoc*, *Minunostoc*, *Komarekiella*, *Mojavia*, *Aliinostoc*, *Halotia*, etc.

### ITS secondary structures

The ITS regions containing both tRNAs, from strain CHAB5880 in this study, together with the sequences derived from GenBank, were used to construct the ITS secondary structure. In total, three *Nostoc* species, two *Desmonostoc* species, one species for each genus of *Aliinostoc*, *Halotia*, *Mojavia*, *Minunostoc*, *Goleter*, *Trichormus*, *Compactonostoc*, *Minunostoc* and *Purpurea* strain CHAB5880 were used to infer their relationship within *Nostocaceae*.

Analyses on D1–D1' helix (Fig. 3) revealed similar arrangements in several genera. The basal stem of the strain CHAB5880 consisted of a 6–bp helix, followed by a 2:7 bp base bilateral bulge (2:6, 2:7 and 3:8 base bilateral bulge respectively in *Mojavia*, *Halotia* and *Komarekiella*), the bilateral bulge was connected by a side loop with a single base on the 3' side (other taxa do not have this structure), following this side loop was two bilateral bulges of 4:3, 4:5 bases; the terminal loop contained 4 bp bases (3 bases in *Halotia*, 5 bases in *Nostoc* and *Mojavia*, 6 bases in *Trichormus*).

Shown in Fig. 4, the Box–B helix of *Purpurea tibecum* CHAB5880 presented a unique structure when compared with other taxa. CHAB5880 consisted of 4 bp (AGCA) helix in the base of the stem, followed by a 1:2 base bilateral bulge, and then further followed by 2:4 and 2:2 base bilateral bulges, and the terminal loop contained 4 bp bases (GAAG) (Fig. 4N).

Furthermore, the V3 helix was also shown to be a variable helix, with the range of length from 35 bp to 103 bp (Fig. 5). The V3 region of *Purpurea tibecum* CHAB5880 was quite distinct from those in all other taxa, with the basal stem containing a 7–bp helix followed by six bilateral bulges, and the terminal loop consisted of six nucleoside bases (GUAAAA). The base of the stem of *Aliinostoc morphoplasticum* NOS consisted of an 8–bp helix, followed by a bilateral bulge of 2:3 bases, and the terminal loop consisted of six nucleoside bases (GAAAG). The basal stem of V3 in *Nostoc* species contained a 3–bp helix followed by one or two bilateral bulges (six in strains *Nostoc lichenoides* CNP–AK1). The V3 helices of recently established genera were also quite different from those of other taxa. The basal stem of V3 in *Halotia branconii* CENA392 contained a 9–bp

helix, followed by a side loop with 3 unpaired bases on the 5' side (Fig. 5G). Whereas the stem of *Trichormus anomalus* HA4352–LM2 consisted of a 2–bp helix, followed by four bilateral bulges (Fig. 5J), and the V3 helix of *Minunostoc* consisted of very long stems (10 bp), followed by a 1:2 base bilateral bulge, and further followed by a 5 bp terminal loop (Fig. 5M). The base stem of *Compactonostoc* consisted of a 5–bp helix (GCCAG), followed by a side loop with a single base on the 3' side, and then by one 4:3 bases bilateral bulge and the terminal loop consisting of a 4 nucleoside bases (GGUU) (Fig. 5L). These results showed that 16S–23S ITS region with both tRNA encoding genes varied not only in the secondary structures of D1–D1', Box B and V3 helix, but also in the length of regions of the ITS. The alignment of the ITS regions constructed among the taxa closely related to *Purpurea* genus showed that *Purpurea* genus differed from other genera in the length of the ITS region (Table 2).

## DISCUSSION

Molecular phylogenetic results have led the classification of cyanobacteria to be extensively revised and reconstructed in recent years, and all species within a genus in the revised taxonomic system of cyanobacteria would form a monophyletic cluster (ANAGNOSTIDIS & KOMÁREK 1985; KOMÁREK et al. 2014; MISCOE et al. 2016). However, many cyanobacterial groups are polyphyletic at different levels (KOMÁREK et al. 2014; GENUÁRIO et al. 2015; HENTSCHKE et al. 2017; BAGCHI et al. 2017; BUCH et al. 2017). The main mission of cyanobacterial taxonomic revision is to separate evolutionarily unrelated taxa within a genus, and to ensure that genus should be a monophyletic group. In this study, the strain CHAB5880 was characterized on the basis of the polyphasic approach. Morphological examination of this strain initially identified it to be the member of the morphological complex *Nostoc*–like taxa. Since the *Nostoc*–like taxa are very difficult to differentiate only on the basis of morphological features, a thorough phylogenetic study was carried out to determine the correct taxonomic characterization of this strain.

In 16S rRNA gene based phylogenetic tree, it was shown that the strain CHAB5880 was placed in a unique clade at a completely distinct and different node. The phylogenetic analysis showed that nostocacean genera resulted in the formation of 15 clusters (Fig. 2), and taxa with the *Nostoc* morphotype formed clades A, B, C, D, E, F, G, H and I. Clade A consisted of sequences from the recently proposed genus *Aliinostoc*, which morphologically appear to be *Nostoc*–like, but the phylogenetic placement is in a separate group with high bootstrap values (85%, 99% and 0.91 in MP, ML and Bayesian analyses, respectively). Clade B also contained sequences from the recently described genus *Halotia*, with its type species

Table 2. Nucleotide lengths of the regions of the 16S–23S ITS of several studied strains.

Strains	D1–D1'	Box–B	V3
<i>Compactonostoc shennongjiaensis</i> CHAB5781	67	29	42
<i>Nostoc commune</i> WY1–KK1	69	39	101
<i>Nostoc lichenoides</i> CNP–AK1	68	33	39
<i>Nostoc punctiforme</i> PCC73102	67	37	39
<i>Desmonostoc geniculatum</i> HA4340–LM1	65	50	90
<i>Desmonostoc danxiaense</i> CHAB5868	65	29	34
<i>Mojavia pulchra</i> JT2–VF2	64	28	103
<i>Halotia branconii</i> CENA392	65	39	44
<i>Komarekiella atlantica</i> CCIBT 3481	65	28	36
<i>Goleter apudmare</i> HA4340–LM2	65	30	35
<i>Minunostoc cylindricum</i> CHAB5843	107	50	36
<i>Trichormus anomalus</i> HA4352–LM2	66	28	82
<i>Aliinostoc morphoplasticum</i> NOS	93	25	35
<i>Purpurea tibecum</i> CHAB5880	70	56	98

*Halotia branconii* isolated from biofilms in Antarctica. Clade D is regarded as ‘*Nostoc sensu stricto*’, including seven species, and this core *Nostoc* clade was supported by high bootstrap values. Clades E, F, G, H and I contained sequences from the newly established genera *Mojavia*, *Compactonostoc*, *Komarekiella*, *Desmonostoc* and *Minunostoc*, respectively. *Purpurea* strain CHAB5880 was placed in clade C, clearly outside the existing *Nostoc*–like clades, suggesting that this strain do not belong to any *Nostoc*–like genera. The *Purpurea* clade showed <95% similarities to most of *Nostoc*–like genera such as *Nostoc*, *Desmonostoc*, *Halotia*, *Komarekiella*, *Mojavia*, *Compactonostoc* and *Minunostoc*, and similarities to *Aliinostoc* and *Trichormus* were 94.4%–95.2% and 95.1%–95.2%. In bacteriology, the 95% similarity has been recommended as a cut-off for identification at the genus level (WAYNE et al. 1987; STACKEBRANDT & GOEBEL 1994; STACKEBRANDT & EBERS 2006). However, many researchers suggested that the 95% classification basis proposed by STACKEBRANDT & GOEBEL (1994) cannot be used as an absolute criterion for separation of genera for most cyanobacteria (FLECHTNER et al. 2002; CASAMATTA et al. 2006; JOHANSEN et al. 2014; ŘEHÁKOVÁ et al. 2014a; HENTSCHKE et al. 2017). The similarity of 16S rRNA between the new proposed genus *Komarekiella*

and other related genera in *Nostocaceae* (*Anabaena*, *Cylindrospermum*, *Desmonostoc*, *Dolichospermum*, *Goleter*, *Halotia*, *Mojavia* and *Nostoc*) were greater than 96%. Some studies recognized that new genera can be proposed and established even with higher levels of 16S rRNA gene similarities to the existing genera, as long as phylogenetic or morphological evidences prove this recognition. The phylogenetic analysis revealed that *Purpurea tibecum* CHAB5880 stood alone and supported its separation from the existing *Nostoc*–like genera clades to become a new genus. However, in our study, we found that *Aliinostoc soli* ZH1(3)\_PS and *Aliinostoc tiwarii* LI\_PS were phylogenetically placed outside of the *Aliinostoc* cluster composed of the type species *Aliinostoc morphoplasticum* and other *Aliinostoc* strains. Furthermore, the 16S rRNA gene sequence similarities between *Aliinostoc soli* ZH1(3)\_PS, *Aliinostoc tiwarii* LI\_PS and other *Aliinostoc* strains were shown as nearly less than 95%, below the bacterial genus cut-off. Thus, we suggest that the taxonomic status of *Aliinostoc soli* and *Aliinostoc tiwarii* should be separated from the *Aliinostoc* genus.

The establishment of *Purpurea* is also supported by the 16S–23S ITS secondary structure. The secondary structure of ITS between 16S–23S rRNA gene has been considered to be a powerful tool for delimitation of cyanobacterial species, among which D1–D1', Box–B and V3 helix are specifically important for separation of strains in different species (ITEMAN et al. 2000; VACCARINO & JOHANSEN 2012; OSORIO–SANTOS et al. 2014; PIETRASIAK et al. 2014; MAREŠ 2018). The secondary folding patterns of the 16S–23S ITS were very different from those of closely related taxa within *Nostocaceae*. D1–D1', Box–B and V3 helix of *Purpurea* were quite unique in comparison with those of *Aliinostoc*, *Desmonostoc*, *Nostoc*, *Halotia*, *Minunostoc*, *Mojavia* and *Compactonostoc*, enabling us to support the establishment of this new genus.

Considering the morphological features, CHAB5880 resembles the morphotypes of the related genera, *Nostoc sensu stricto*, *Aliinostoc*, *Desmonostoc* and *Halotia*. That being said, the identification of diacritical morphological features among these genera and species is apparently highly complex (GENUÁRIO et al. 2015). However, *Purpurea* differs from *Nostoc*, because the filaments do not form the spherical colonies which are characteristic of *Nostoc*, and *Purpurea* can be distinguished from *Halotia* since it does not form the aggregated filaments of microcolonies. When compared to *Desmonostoc* and *Aliinostoc*, it is shown that both *Purpurea* and *Desmonostoc* possess long, flexuous filaments and chains of akinetes, and *Purpurea* and *Aliinostoc* even possess the same type of cell division in trichomes. However, it is important that the purple black colour within vegetative cells and the formation of terminal heterocytes can be assumed to be important morphological traits distinguishing it from members of the genus *Aliinostoc* and *Desmonostoc*.

Many studies have emphasized the importance of ecological surveys in the classification of cyanobacteria

(KOMÁREK et al. 2014; KOMÁREK 2016; BAGCHI et al. 2017; CAI et al. 2017; SABER et al. 2017), and it is worthy of mention that CHAB5880 is the first strain of the genus *Purpurea* isolated from wet soils in Tibet, China. The new cyanobacterial taxa described in this study emphasize the importance of exploration of underexplored environments to reveal the biodiversity of cyanobacteria.

When considered together, morphological, ecological, molecular and phylogenetic evidences obtained here make it reasonable to propose the novel cyanobacterial genus *Purpurea*, which represents a new monophyletic group in the cyanobacterial systematics.

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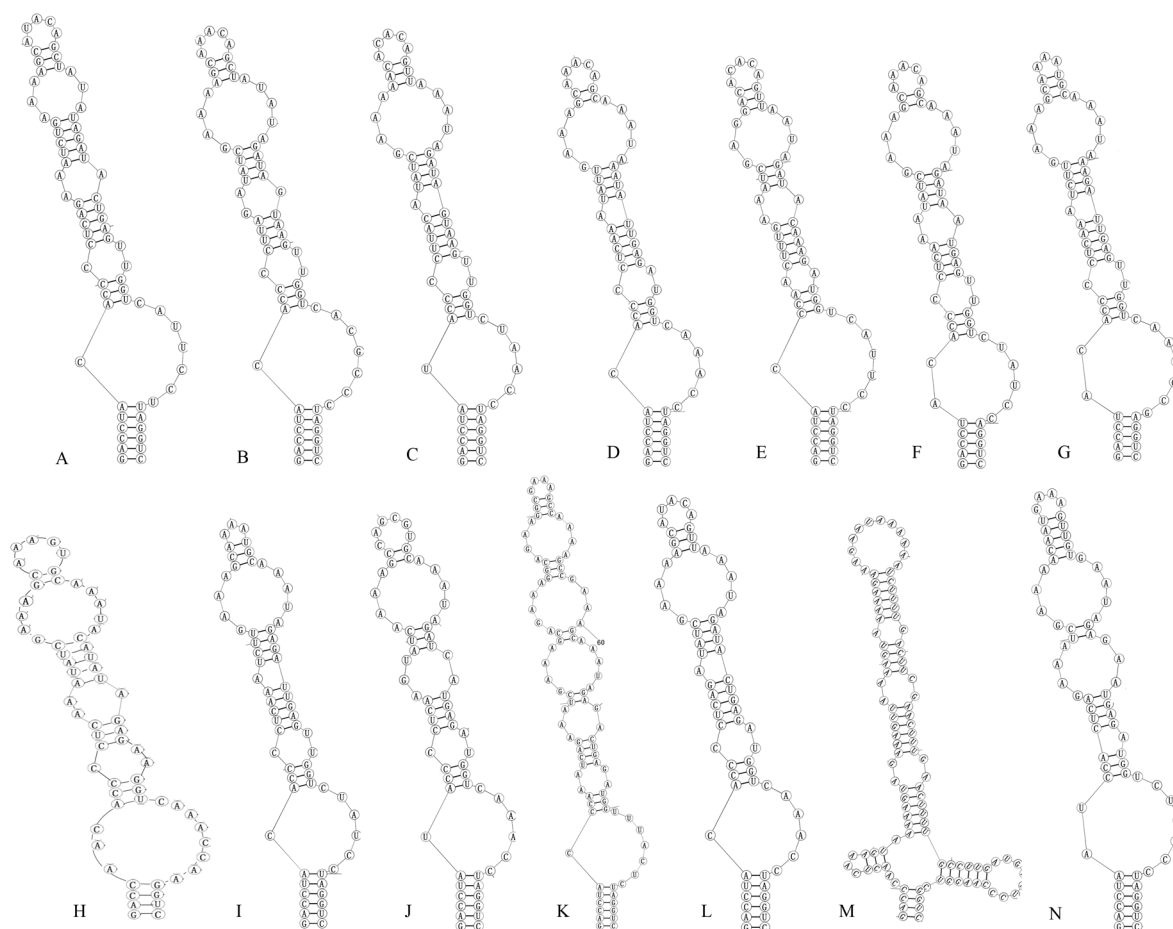


Fig. 3. D1–D1' helix in *Purpurea tibecum* and other heterocytous cyanobacteria: (A) *Nostoc commune* WY1–KK1; (B) *Nostoc lichenoides* CNP–AK1; (C) *Nostoc punctiforme* PCC73102; (D) *Desmonostoc geniculatum* HA4340–LM1; (E) *Desmonostoc danxiaense* CHAB5868; (F) *Mojavia pulchra* JT2–VF2; (G) *Halotia branconii* CENA392; (H) *Komarekiella atlantica* CCIBT 3481; (I) *Goleter apudmare* HA4340–LM2; (J) *Trichormus anomalus* HA4352–LM2; (K) *Aliinostoc morphoplasticum* NOS; (L) *Compactonostoc shennongjiaensis* CHAB5781; (M) *Minunostoc cylindricum* CHAB5843; (N) *Purpurea tibecum* CHAB5880.



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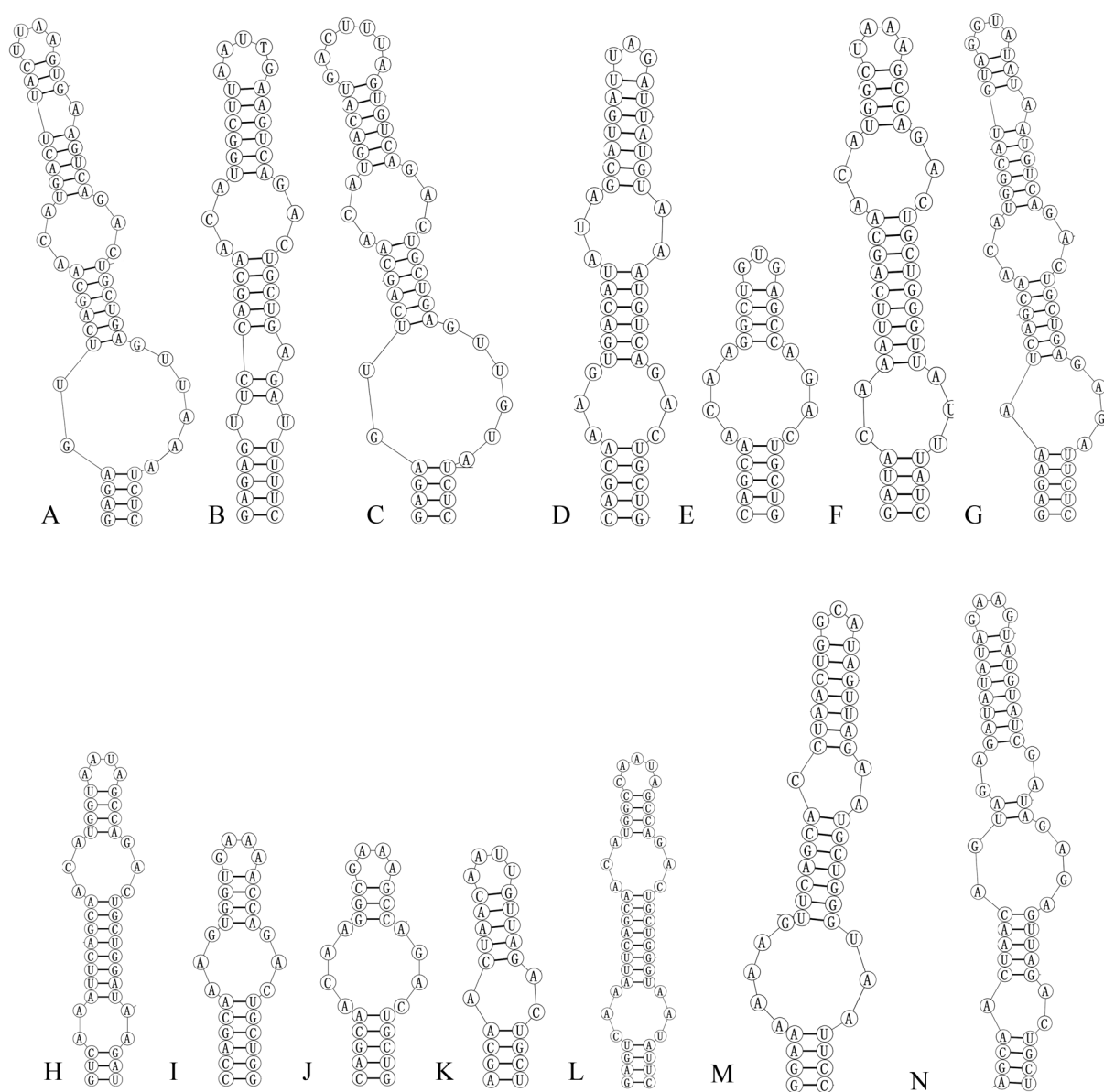


Fig. 4. Box-B helix in *Purpurea tibecum* and other heterocytous cyanobacteria: (A) *Nostoc commune* WY1-KK1; (B) *Nostoc lichenoides* CNP-AK1; (C) *Nostoc punctiforme* PCC73102; (D) *Desmonostoc geniculatum* HA4340-LM1; (E) *Desmonostoc danxiaense* CHAB5868; (F) *Mojavia pulchra* JT2-VF2; (G) *Halotia branconii* CENA392; (H) *Komarekiella atlantica* CCIBT 3481; (I) *Goleter apudmare* HA4340-LM2; (J) *Trichormus anomalus* HA4352-LM2; (K) *Aliinostoc morphoplasticum* NOS; (L) *Compactonostoc shennongjiaensis* CHAB5781; (M) *Minunostoc cylindricum* CHAB5843; (N) *Purpurea tibecum* CHAB5880.

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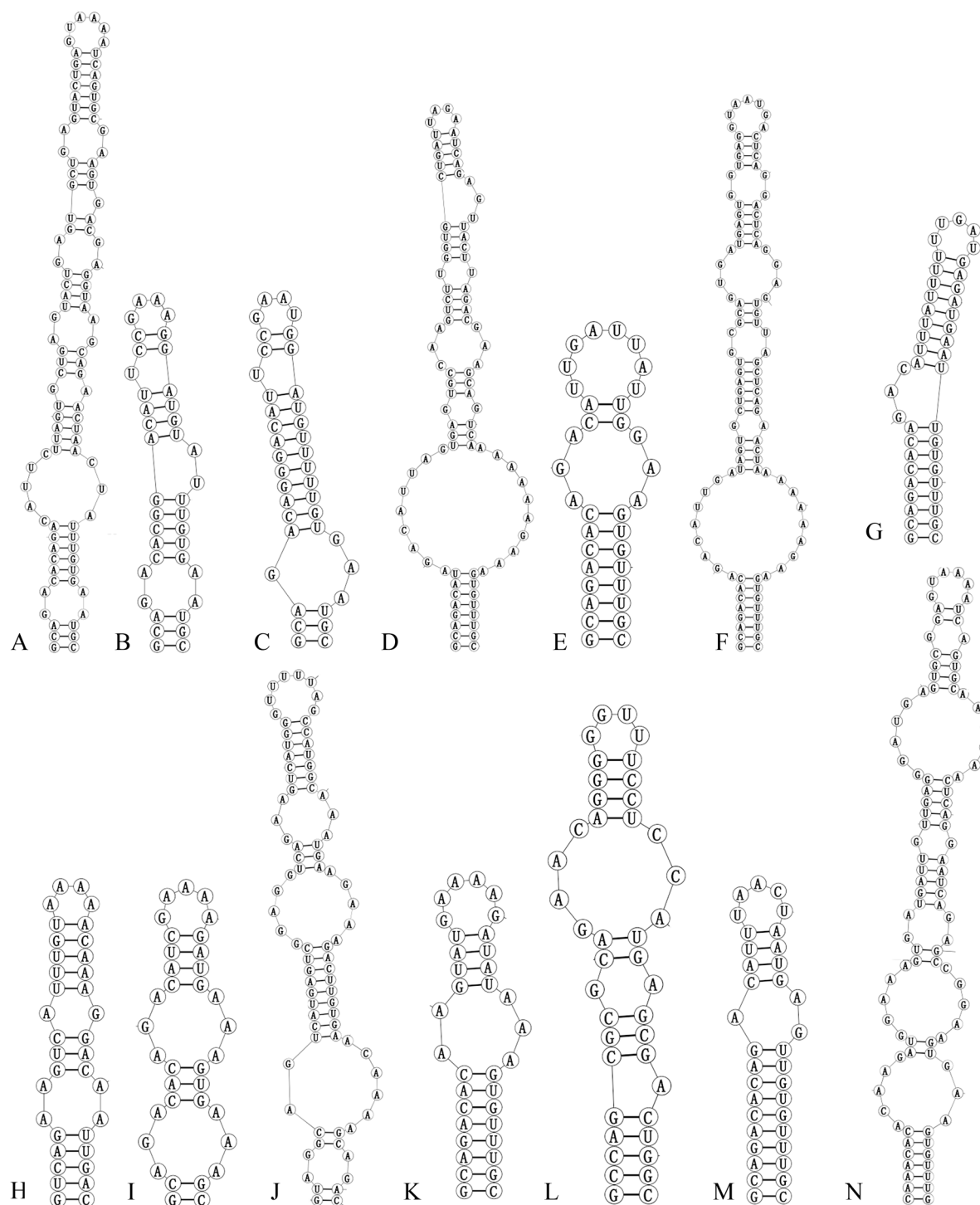


Fig. 5. V3 helix in *Purpurea tibecum* and other heterocytous cyanobacteria: (A) *Nostoc lichenoides* CNP-AK1; (B) *Nostoc commune* WY1-KK1; (C) *Nostoc punctiforme* PCC73102; (D) *Desmonostoc geniculatum* HA4340-LM1; (E) *Desmonostoc danxiaense* CHAB5868; (F) *Mojavia pulchra* JT2-VF2; (G) *Halotia branconii* CENA392; (H) *Komarekiella atlantica* CCIBT 3481; (I) *Goleter apudmare* HA4340-LM2; (J) *Trichormus anomalus* HA4352-LM2; (K) *Aliinostoc morphoplaticum* NOS; (L) *Compactonostoc shennongjiaensis* CHAB5781; (M) *Minunostoc cylindricum* CHAB5843; (N) *Purpurea tibecum* CHAB5880.

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