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Research Letter

RESEARCH LETTER – Taxonomy & Systematics

Description of two new species of *Aliinostoc* and one new species of *Desmonostoc* from India based on the Polyphasic Approach and reclassification of *Nostoc punensis* to *Desmonostoc punense* comb. nov

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One sentence summary: This paper describes two new species of the cyanobacterial genus *Aliinostoc* and a new species of *Desmonostoc*.
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ABSTRACT

Three heterocytous cyanobacterial strains were isolated from different habitats of Central India, and initial morphological studies indicated them to be members of the genus *Nostoc* or other closely related genera. Subsequent studies using morphological, ecological, molecular and phylogenetic methods indicated the three strains to be new members of the genera *Aliinostoc* and *Desmonostoc*. Folding of the D1-D1' helix of the ITS region clearly differentiated the three strains from the other closely related strains, thus providing final indications of the strains being different and new additions to the genera *Aliinostoc* and *Desmonostoc*. In accordance with the International Code of Nomenclature for algae, fungi and plants, we establish three new species: *Aliinostoc tiwarii* sp. nov, *Aliinostoc soli* sp. nov. and *Desmonostoc magnisporum* sp. nov. along with reclassifying *Nostoc punensis* as *Desmonostoc punense* comb. nov.

Keywords: Cyanobacteria; taxonomy; phylogeny; *Aliinostoc*; *Desmonostoc*; 16S rRNA

INTRODUCTION

Nostoc is one of the most widely studied filamentous heterocytous genus with the first report dating back to the 19th century (Bornet and Flahault 1886). The diacritical features of *Nostoc* include the production of mucilaginous colonies with variable levels of slime production and a complex life cycle in some cases. The filaments of *Nostoc* are isopolar, uniseriate and unbranched with the occurrence of the nitrogen-fixing heterocyte at both terminal and intercalary positions along with chains of akinetes that are apoheterocytic. Vegetative propagation in *Nostoc* is achieved either by fragmentation of filaments into

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short few celled hormogonia or by sporogenic cycle (Lazaroff and Vishniac 1961; Lazaroff 1966; Komárek and Anagnostidis 1989; Mateo *et al.* 2011). However, *Nostoc* is considered to be a morphologically difficult genus as it lacks morphological synapomorphies (Řeháková *et al.* 2007). Moreover, with the emergence of the polyphasic approach, the heterogeneity within *Nostoc* was also reported in different studies (Hrouzek *et al.* 2005; Rajaniemi *et al.* 2005; Fernandez-Martinez *et al.* 2013). Rajaniemi *et al.* (2005), for the first time, reported the existence of more than a single cluster of *Nostoc*, while studying a limited number of temperate origin *Nostoc* species. Subsequently, numerous studies involving large numbers of isolates have also supported the findings of Rajaniemi *et al.* and more than three clusters of cyanobacteria sharing *Nostoc*-like morphology have been reported (Řeháková *et al.* 2007; Hrouzek *et al.* 2013; Genuário *et al.* 2015; Bagchi, Dubey and Singh 2017). In order to achieve monophyly, the *Nostoc* morphotypes forming clusters away from the *Nostoc sensu stricto* clade have been recommended to be described as new genera. This has led to the description of *Mojavia* (Řeháková *et al.* 2007), *Desmonostoc* (Hrouzek *et al.* 2013), *Halotia* (Genuário *et al.* 2015), *Aliinostoc* (Bagchi, Dubey and Singh 2017) and *Komarekiella* (Hentschke *et al.* 2017) that are morphologically similar to *Nostoc* but are phylogenetically distant and distinct from *Nostoc sensu stricto*.

The genus *Desmonostoc* was described by Hrouzek *et al.* in 2013 as a phylogenetically distinct taxon related to *Nostoc*. In their preliminary study involving a small number of taxa, the authors observed the clustering of a well-supported group of *Nostoc muscorum* strains away from *Nostoc sensu stricto*. Later in 2013, Hrouzek *et al.* came up with complete morphological and phylogenetic evidence and proposed the genus *Desmonostoc* with the type species being *Desmonostoc muscorum*. Even though *Desmonostoc* is morphologically quite similar to *Nostoc* but noteworthy morphological differences were observed between them. The filaments of *Desmonostoc* are long and densely coiled consisting of hundreds of cells throughout their life cycle. The akinetes were observed only in the initial stages of the life cycle, and the morphology of akinetes was found to be consistent among the *Desmonostoc* but different from the *Nostoc sensu stricto*. Unlike akinetes, it was observed that the hormogonia morphology varied amongst the strains of *Desmonostoc* (Hrouzek *et al.* 2013). Similarly, a phylogenetically distant clade of *Nostoc*-like taxa was described as a novel genus *Aliinostoc* with the type species being *Aliinostoc morphoplasticum* (Bagchi, Dubey and Singh 2017). This clade comprised of the strains isolated from water bodies in India, saline-alkaline lakes in Brazil and rice fields of Thailand (Papaefthimiou *et al.* 2008; Bagchi, Dubey and Singh 2017; Genuário *et al.* 2017). The morphological characters of *Aliinostoc* are almost indistinguishable from true *Nostoc*; however, most of the members of *Aliinostoc* formed motile hormogonia with gas vesicles. In case of *Aliinostoc* sp. PCC 8976 (AM711525), isolated from brackish marshland in France, hormogonia formation was not evident while the data for *A. elgonense* TH3S05 (AM711548) are unavailable. Therefore, the authors recommended that this morphological feature must be further evaluated when describing the new taxa within *Aliinostoc*. Both genera exhibit few morphologically unique characters, so distinguishing them from each other and from other *Nostoc*-like genera solely on the basis of morphological studies is challenging. Hence, the polyphasic studies with particular emphasis on the phylogenetic clustering are indeed essential to describe new species of *Desmonostoc* and *Aliinostoc*.

In this study, two freshwater isolates (LI_PS and AR6_PS) and one soil dwelling isolate (ZH1(3)_PS) have been characterized on the basis of polyphasic approach, and we describe them as new species of *Aliinostoc* and *Desmonostoc* with the names proposed being *A. tiwarii* (LI_PS), *A. soli* (ZH1(3)_PS) and *D. magnisporum* (AR6_PS) in accordance with the International Code of Nomenclature for algae, fungi and plants.

MATERIAL AND METHODS

Sampling, isolation and culturing of strains

The cyanobacterial strains were isolated from three different localities of central and western India having different climatic and geographical conditions. The strain LI_PS was isolated from a freshwater sample collected from the hilly area of Pachmarhi in the month of November. Pachmarhi is a hilly area located at a height of 1100 m in a valley of Satpura range in Hoshangabad district of Madhya Pradesh state of central India. The strain ZH1(3)_PS is a soil-dwelling cyanobacteria isolated from the hilly region located in Mumbra, a small town 30 Km away from Mumbai city in the month of February. The strain AR6_PS was isolated from the water sample collected in the month of October from a freshwater body located in the city of Shrirampur in the western part of Maharashtra. The physico-chemical parameters of the samples and the habitat were measured at the time of sample collection. Isolation and purification step was performed on BG-11_0 medium and the pH was adjusted to 7.2 (Rippka *et al.* 1979). The purified strains were maintained in a culture room illuminated with 50–55 $\mu Em^{-2}s^{-1}$ light at 28 ± 2°C with a photoperiod of 14/10 h light/dark cycle.

Morphological analysis

Morphological characterization of the strains LI_PS, ZH1(3)_PS and AR6_PS was performed using a Nikon YS100 microscope (Nikon, Minato, Tokyo, Japan), and the micrographs were taken using an Olympus BX53 (Olympus Corporation, Shinjuku, Tokyo, Japan) fitted with a ProgRes C5 camera (Jenoptik, Jena, Thuringia, Germany). The length of filaments, size and shape of vegetative cells and heterocytes, positioning of heterocytes, appearance of sheath, occurrence of akinetes and hormogonia were observed. Measurements were taken at both ×40 and ×100 magnifications. Approximately 100 measurements were taken for each of the morphological characters studied.

Genomic DNA extraction, PCR and sequence analysis

Total genomic DNA was extracted from 18- to 20-day-old log phase culture using Himedia Ultrasensitive Spin Purification Kit (MB505–250PR) with some modifications in the lysis step (Bagchi, Dubey and Singh 2017; Suradkar *et al.* 2017; Saraf *et al.* 2018). Amplification of 16S rRNA gene and 16S-23S ITS region was performed using primer pA (5′-AGAGTTTGATCCTGGC TCAG-3′) and cyanobacteria-specific primer B23S (5′-CTTC GCCTCTGTGTGCCTAGGT-3′) (Edwards *et al.* 1989; Gkelis *et al.* 2005). Direct sequencing of the amplified products was carried out by Sanger's method on a 3730xl DNA analyzer (Applied Biosystems, Foster City, CA, USA).

Phylogenetic analysis

The 16S rRNA gene phylogenetic tree was inferred by Bayesian inference (BI), maximum likelihood (ML) and maximum parsimony (MP) methods. jModeltest was used to determine the appropriate model for the construction of trees which led to the

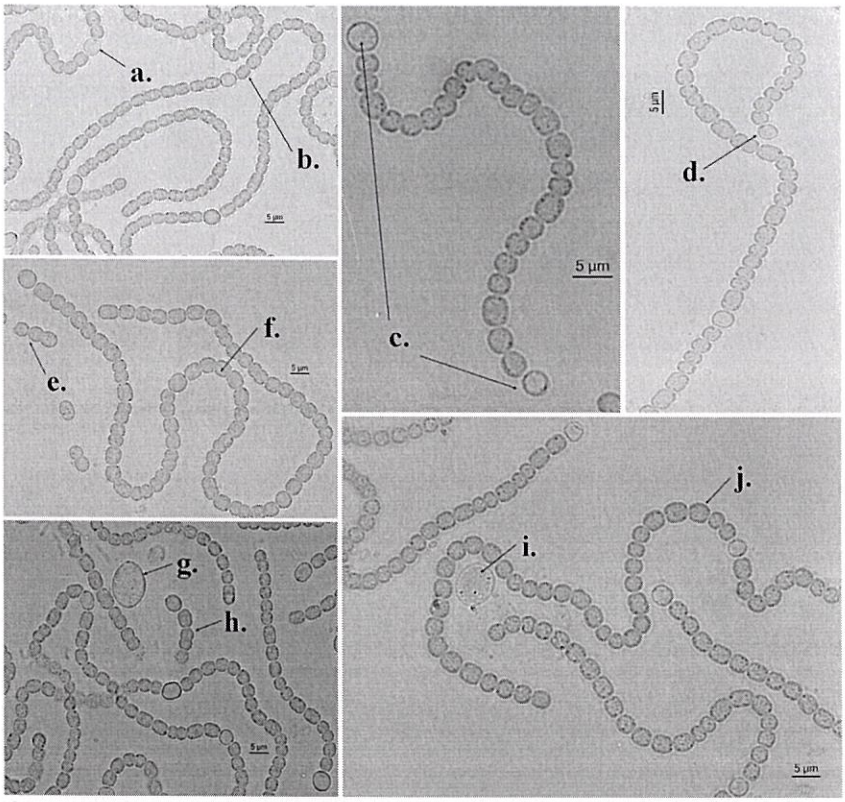

Figure 2. Morphological characteristics of *Aliinostoc soli*. (a) Intercalary heterocyte. (b) Barrel-shaped vegetative cell. (c) Typical filament with terminal heterocyte at both ends. (d) Filament with prominently curved terminal vegetative cells. (e) Hormogonia. (f) Typically curved filament with more than 60 cells. (g) Akinete. (h) Hormogonia. (i) Akinete. (j) Trichome with thin hyaline sheath.

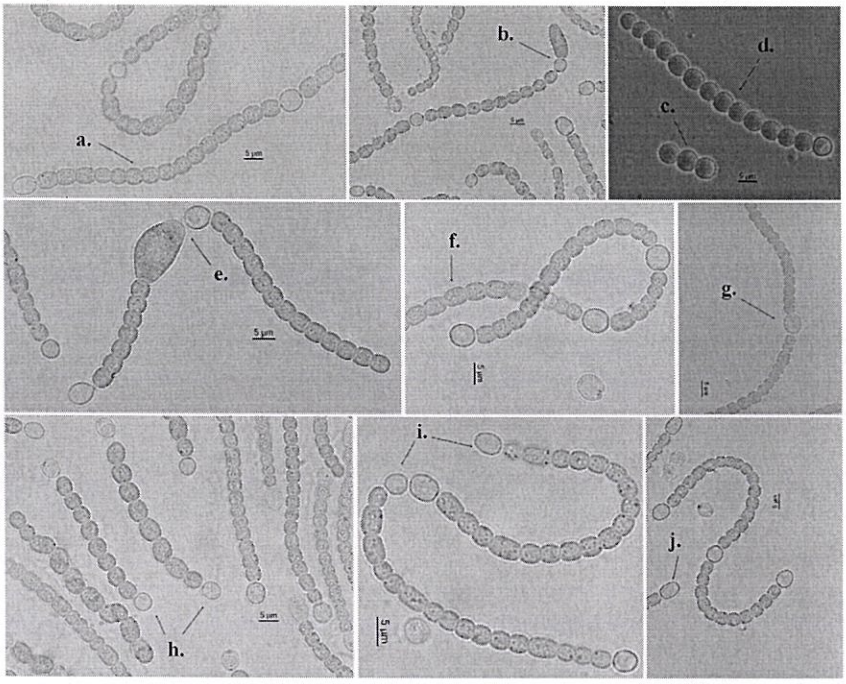

Figure 3. Morphological characteristics of *Desmonostoc magnisporum*. (a) Well constricted filament. (b) Filament with heterocyte adjacent to akinete. (c) Hormogonia. (d) Trichome with thin hyaline sheath. (e) Large irregularly shaped akinete just adjacent to intercalary heterocyte. (f) Typical curved filament with barrel shaped vegetative cells. (g) Intercalary heterocyte. (h) Terminal heterocyte. (i) Typical filament with two adjacent intercalary heterocyte and terminal heterocyte at both the ends. (j) Terminal heterocyte with elongated ends.

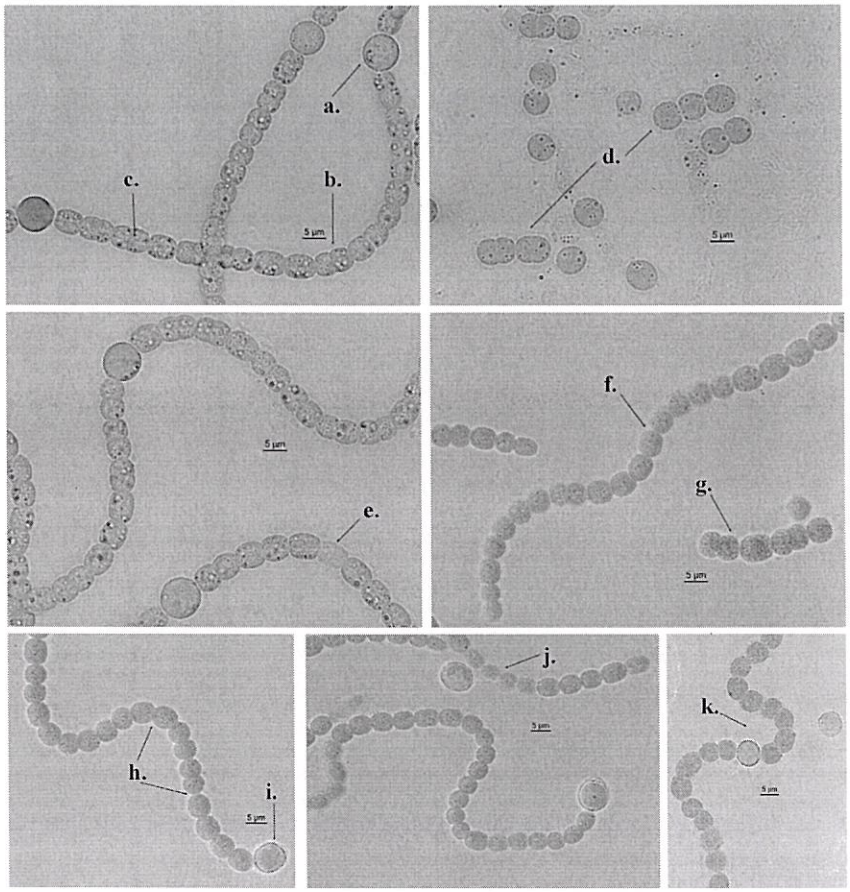

Figure 1. Morphological characteristics of *Aliinostoc tiwarii*. (a) Intercalary heterocytes. (b) Dividing vegetative cells. (c) Vegetative cells with granular cytoplasm. (d) Hormogonia. (e) Just developing akinete. (f) Well constricted vegetative cells. (g) Hormogonia. (h) Trichome with thin hyaline sheath. (i) Terminal heterocyte. (j) Dying vegetative cells in chain. (k) Typical curved filament having prominent constricted vegetative cells with intercalary heterocyte.

selection of GTR + I + G, and the analysis was accomplished using Mr Bayes 3.2.6 (Darriba *et al.* 2012; Ronquist *et al.* 2012). Two runs of eight Markov chains were executed for 10 million generations until the value of standard deviation of split frequency was below 0.01. The sampling was done every 1000th generation, and the burn-in value was set to 25%. ML and MP trees were constructed using Mega 5.2.2, and the best fit model for ML was selected using Mega 5.2.2 which led to the selection of K2 + G + I (Tamura 1992; Tamura *et al.* 2011). The model with the lowest Bayesian inference criterion value was selected to be the most suitable model. Bootstrap resampling method with 1000 replications was used to test the reliability of the ML and MP trees (Felsenstein 1985). The analysis involved 155 sequences and all the three trees were mapped into one single tree to assess the robustness of the analysis. The p-distance values were calculated using Mega 5.2.2, and pairwise percentage similarity matrix was determined using SDT software (Muhire, Varsani and Martin 2014)

16S-23S ITS secondary structure determination and p-distance

Folded secondary structures of 16S–23S ITS region were determined for all the three strains and compared with the closely related taxa. All the secondary structures were transcribed and folded using Mfold web server (Zuker 2003). The p-distance for all the three strains along with the closely related taxa was calculated using Mega 5.2.2.

RESULTS

Habitat and morphological evaluation

The morphological characterization of all three strains was performed giving emphasis on the type of filaments, shape and size of vegetative cells, occurrence of akinetes, shape and size of heterocytes, occurrence of sheath, etc. (Figs 1–3). Furthermore, morphological comparisons were made for the strains LI_PS and ZH1(3)_PS with the type species of *Aliinostoc*, i.e. *A. morphoplasticum* (Table 1). Similarly, the strain AR6_PS was compared with the type species of *Desmonostoc*, i.e. *D. muscorum* (Table 1). The physico-chemical parameters of the soil and water samples along with the habitat were also recorded at the time of sample collection (Table S1, Supporting Information).

Phylogenetic analysis and pairwise percentage similarity matrix

The 16S rRNA gene phylogenetic tree was constructed with 155 nucleotide sequences, and it was observed that the strains LI_PS and ZH1(3)_PS were clustered strongly within the *Aliinostoc* clade. In contrast, the strain AR6_PS was clustered within the D1

Table 1. Morphological comparison of *A. dulciaquae*, *A. soli* and *D. magnisporum* with the type species of *Aliinostoc* and *Desmonostoc*, respectively.

Strain	Vegetative cell	Heterocyte	Sheath	Akinete	Hormogonia
Aliinostoc dulciaquae (LI_PS)	Barrel shaped L: 5.3–5.6 μm W: 5.2–5.6 μm	Spherical L: 3.8–4.3 μm W: 3.7–4.9 μm	Thin hyaline	Present	Present; with gas vacuoles
Aliinostoc soli (ZH1 (3)_PS)	Barrel shaped L: 3.6–4.0 μm W: 3.2–3.7 μm	Spherical L: 3.8–4.3 μm W: 3.7–4.9 μm	Thin hyaline	Present	Present
Aliinostoc morphoplasticum	Barrel shaped to spherical to oblong L: 2.99–5.11 μm W: 2.84–3.71 μm	Spherical to elliptical to ovate to oblong L: 3.72–5.71 μm W: 3.26–4.01 μm	Thin colourless	Present	Present; with gas vacuoles
Desmonostoc magnisporum (AR6_PS)	Barrel shaped L: 4.0–4.5 μm W: 4.1–4.7 μm	Spherical to oblong L: 5.3–6.0 μm W: 4.8–5.6 μm	Thin hyaline	Present	Present
Desmonostoc muscorum	Barrel shaped to cylindrical L: 3.2–7.9 μm W: 3.8–6.3 μm	Barrel shaped to spherical L: 4.5–8.7 μm W: 5.5–11.3 μm	Colourless to yellow–brown	Present	

subcluster of *Desmonostoc* clade (Fig. 4). This observation was consistent in all the 16S rRNA gene trees constructed using different methods. The sequences corresponding to *Nostoc sensu stricto*, *Halotia*, *Brasilonema*, *Scytonema*, etc. also clustered tightly with strong probability/bootstrap support indicating the robustness of the complete 16S rRNA gene tree (Fig. S1, Supporting Information). The pairwise percentage similarity matrix was constructed using SDT tool with the sequences from the *Aliinostoc* and *Desmonostoc* clade. The percentage similarity matrix also indicated the close relatedness of the strains LI_PS and ZH1(3)_PS with *Aliinostoc* and the strain AR6_PS with *Desmonostoc* which further supported the 16S rRNA gene phylogeny findings (Fig. S2, Supporting Information).

16S-23S ITS analysis and p-distance

The folded secondary structures of D1-D1' helix region were obtained for all the three strains using Mfold web server and were compared with available structures of their respective genera. The secondary structures of the strains LI_PS and ZH1(3)_PS were compared amongst themselves and also with *A. morphoplasticum* (KY403996). Also, the secondary structure of the strain AR6_PS was compared with *Desmonostoc* sp. CCIBT 3489 (KX638490), *Desmonostoc* sp. 81 NMI ANAB (KF761562), *Desmonostoc* sp. 111 CR4 (KF761564) and *D. geniculatum* (KU161662). Comparable differences were observed indicating the strains LI_PS and ZH(3)_PS to be new members of the genus *Aliinostoc* and AR6_PS to be new member of *Desmonostoc* (Fig. 5). Furthermore, the p-distance values also supported the results obtained from 16S rRNA gene phylogenetic tree and 16S-23S ITS analysis (data not shown).

DISCUSSION

In this study, the strains LI_PS, ZH1(3)_PS and AR6_PS were characterized on the basis of the polyphasic approach. Initial morphological characterization of the strains indicated them to be the members of the morphologically complex *Nostoc*-like taxa (Figs 1–3). Since the *Nostoc*-like taxa are very difficult to differentiate on the basis of morphological characterization, in-depth phylogenetic studies were performed to determine the correct taxonomic identity of all the strains. In all the 16S rRNA gene phylogenetic trees, it was observed that the strains LI_PS and ZH1(3)_PS clustered strongly within the *Aliinostoc* clade at a completely different and distinct node. Furthermore, the strong bootstrap support for the entire *Aliinostoc* clade as well as the node consisting of the strains LI_PS and ZH1(3)_PS indicated them to be new members of the genus *Aliinostoc* (Fig. 4). In case of the strain AR6_PS, it was found to be clustered in close proximity of *N. punensis* and *Desmonostoc* sp. PCC 7422 within the D1 subcluster of the *Desmonostoc* clade. The phylogenetic positioning of the strain AR6_PS along with strong bootstrap support for the subcluster D1 and the entire *Desmonostoc* clade indicated the strain AR6_PS to be a new member of the genus *Desmonostoc* (Fig. 4). The strong clustering of *Nostoc*, *Halotia*, *Scytonema*, *Brasilonema*, *Iphinoe* and true branching heterocytous cyanobacteria indicated the robustness of the entire 16S rRNA gene tree presented in this study (Fig. S1, Supporting Information). Furthermore, it was observed that *D. vinosum* (Miscoe *et al.* 2016) reported from the caves on Kauai, Hawaii Island, clustered well outside the *Desmonostoc* clade in all the three trees (Fig. S1, Supporting Information). Based on the apparent phylogenetic positioning, we suggest that the taxonomic status of *D. vinosum* should be revisited. Oren and Ventura (2017) in their review recommended the need for reclassification of *N. punensis* as it appeared to be closely related to the genus *Desmonostoc*. In our phylogenetic analysis, we also observed the clustering of *N. punensis* within the D1 subcluster of *Desmonostoc* clade indicating it to be actually a member of the genus *Desmonostoc*. Therefore, we propose to reclassify this strain to the genus *Desmonostoc* along with the change of name from *N. punensis* (Singh *et al.* 2016) to *D. punense* comb. nov. as suggested by Oren and Ventura (2017).

Furthermore, the 16S-23S ITS secondary structure analysis was performed as it has been demonstrated to be effective in differentiating the closely related species (Boyer, Johansen and Flechtner 2002; Bohunická *et al.* 2015; Berrendero *et al.* 2016; Shalygin *et al.* 2017; Kabirnataj *et al.* 2018; Mareš *et al.* 2018). In case of the strains LI_PS and ZH1(3)_PS, the secondary structures obtained from D1-D1' helix region were compared amongst themselves and with *A. morphoplasticum*. The size, shape and number of loops clearly differentiated the three strains. *Aliinostoc morphoplasticum* exhibited six loops, whereas the strains LI_PS and ZH1(3)_PS had only five loops. Also, the sizes of all the loops

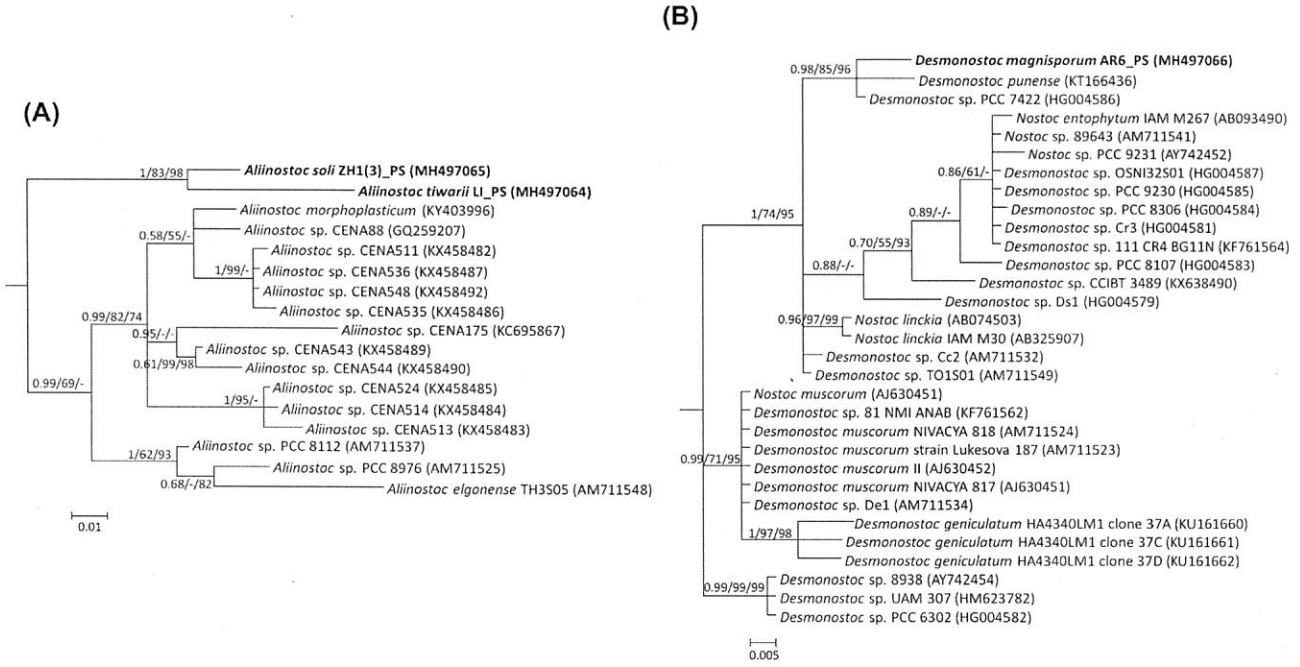

Figure 4. (A) Complete *Aliinostoc* clade showing the phylogenetic positioning of *A. tiwarii* (LI_PS) and *A. soli* (ZH1(3)_PS) based on 16S rRNA gene inferred by Bayesian inference tree with the probability scores/bootstrap values representing BI, ML and MP, respectively. Bar, 0.01 changes per nucleotide position. (B) Complete *Desmonostoc* clade showing the phylogenetic positioning of *D. magnisporum* (AR6_PS) and *D. punense* based on 16S rRNA gene inferred by Bayesian inference tree with the probability scores/bootstrap values representing BI, ML and MP, respectively. Bar, 0.005 changes per nucleotide position.

in *A. morphoplasticum* were smaller as compared to the loops of both the strains. Although both the strains LI_PS and ZH1(3)_PS exhibited five loops, the shape and the number of nucleotides of the basal loop clearly differentiated them. The basal loop of LI_PS consists of 12 nucleotides, whereas ZH1(3)_PS has 14 nucleotides (Fig. 5). In case of the strain AR6_PS, the secondary structure of D1-D1' helix region was compared with the closely related species of *Desmonostoc* from D1 and D2 subclusters. The overall secondary structures of all the strains were somewhat similar in having three prominent loops. The major difference between these strains was observed in the stem between the basal loop and the second loop. A unilateral bulge in the stem was observed in the strains AR6_PS, *Desmonostoc* sp. 111 and *Desmonostoc* sp. 81, whereas *Desmonostoc* sp. CCIBT 3489 and *D. geniculatum* exhibited a fourth loop instead of bulge. However, the shape and the number of nucleotides forming the bulge differentiated the strains (Fig. 5). The box B and V3 regions were not obtained in this study; however, D1-D1' helix region clearly distinguished all the three strains from their closely related taxa. The phylogenetic analysis along with the comparison of 16S-23S ITS secondary structures provided enough proof for us to conclude that the strains LI_PS, ZH1(3)_PS and AR6_PS should be described as new species of the genera *Aliinostoc* and *Desmonostoc*, respectively. Our study further emphasizes the importance of 16S-23S ITS secondary structure analysis in differentiating closely related taxa.

Furthermore, the morphological characters of all the three strains were compared with the respective strains of *Aliinostoc* and *Desmonostoc* (Table 1). Minor differences were observed which also indicated our strains to be new members of the genera *Aliinostoc* and *Desmonostoc*. An interesting observation was made in case of *D. magnisporum* AR6_PS which needs special attention. The akinetes in some of the filaments were very large in size with unusual irregular shapes (Fig. 3). Recently, many studies have emphasized the importance of ecological investigation in the cyanobacterial taxonomy (Komárek *et al.* 2014; Komárek 2016; Bagchi, Dubey and Singh 2017; Saber *et al.* 2017). The members of the genus *Aliinostoc* were reported from different habitats, namely eutrophic pond, alkaline lakes and rice fields which are usually having alkaline pH and higher concentration of dissolved ions, salts, etc. (Papaefthimiou *et al.* 2008; Bagchi, Dubey and Singh 2017; Genuário *et al.* 2017). The strains LI_PS and ZH1(3)_PS were also isolated from the habitat which had slightly alkaline pH (Table S1, Supporting Information). Although the strains of *Aliinostoc* are found in diverse habitats, Bagchi, Dubey and Singh (2017) believed that most of the strains may be attributed to a specific nutrient requirement. The authors also indicated a possible pattern of biogeographical distribution centering on the tropical regions. This study provides further evidence for the proposed biogeographical distribution of the members of the genus *Aliinostoc*. However, the above idea needs to be further confirmed by exploring similar habitats from the temperate and polar regions.

DESCRIPTION OF *ALIINOSTOC TIWARII* SARAF AND SINGH *ET AL.* SP. NOV. UNDER THE PROVISIONS OF THE INTERNATIONAL CODE OF NOMENCLATURE FOR ALGAE, FUNGI AND PLANTS

Description: Growing like soft macroscopic mats in the natural habitat with easily visible greenish blue color; mats may range from 3 to 6 mm in diameter, usually soft texture when collected from the nature; on culturing in the laboratory, the filaments appear less coiled with more of a scattered view; filaments not entangled and not coalescent together; coiling is definitely not as dense as present in *Nostoc*; sheath present around the entire filament and appears more [illegible] in older cultures;

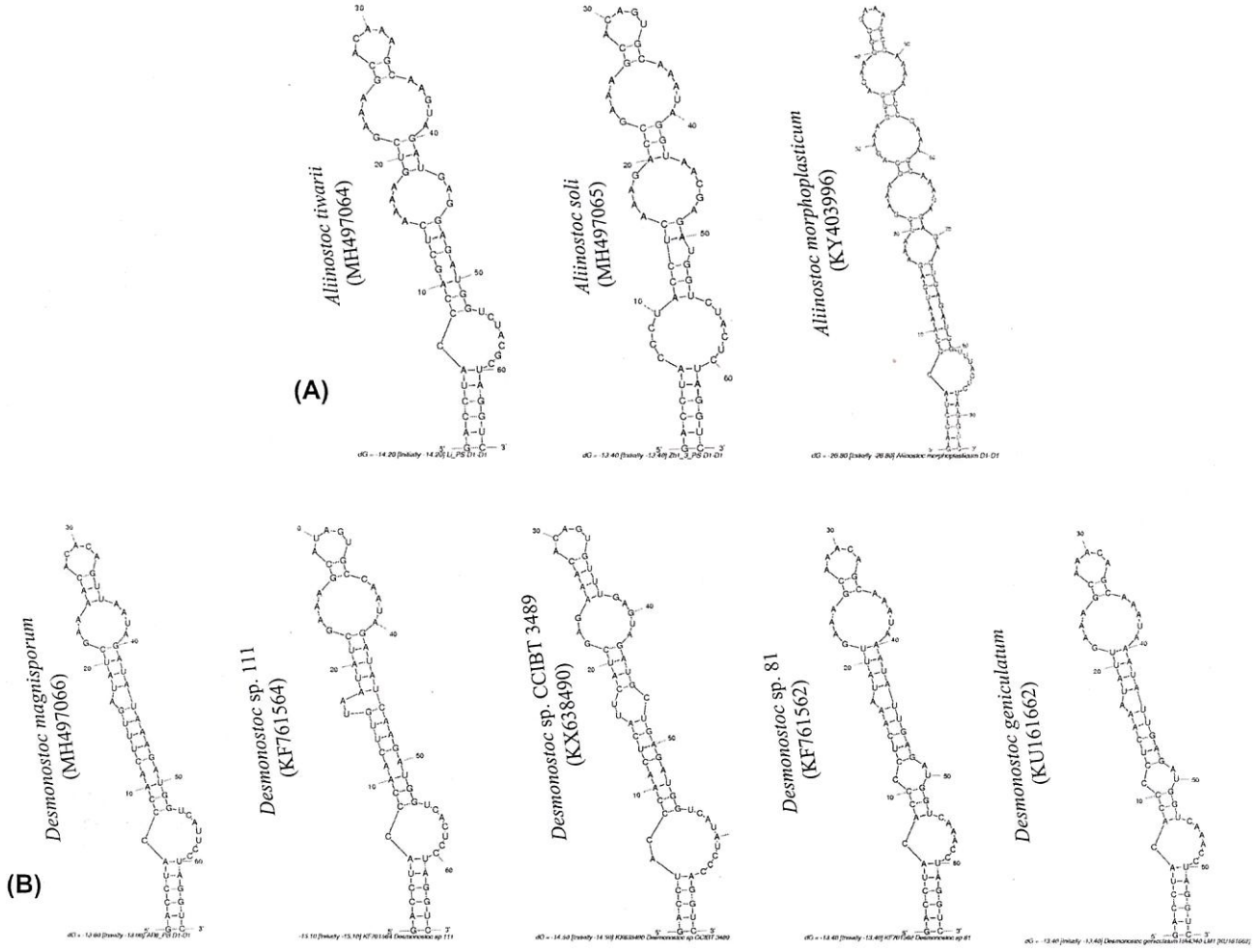

Figure 5. Comparison of folded secondary structures of the D1-D1' helix region of *A. tiwarii*, *A. soli* and *D. magnisporum* with the representative strains from the genera *Aliinostoc* and *Desmonostoc*, respectively.

presence of sheath is around the entire filament even at the ends and around the terminal cells; gas vesicles seen; hormogonia formation clearly evident; loosely arranged filaments with variable tendencies for coiling; protoplasm appears clearly granular; cells almost barrel shaped to sometimes even appearing isodiametric; constriction prominent at the cross walls in between all the cells; cells prominently constricted at the cross walls; vegetative cells range in length from 5.3 to 5.6 μm while the width is around 5.2–5.6 μm; heterocytes at both terminal and intercalary positions; heterocytes almost spherical in shape measuring about 3.8–4.3 μm in length to 3.7–4.9 μm in width; old decaying cells in chains in vegetative filaments also visible.

Diagnosis: Phylogenetically and morphologically most similar to *A. soli*, from which it differs by having distinctly wider cells. Differing from *A. morphoplasticum* by having both wider vegetative cells and less oval heterocyte. Differentiated from both species in the secondary structure of the D1-D1' helix. Strong phylogenetic support is evident for its distinct positioning and establishment as a new species of the genus *Aliinostoc*. Detailed morphological differences have been documented in Table 1.

Etymology: *Aliinostoc tiwarii* (ti.wa'ri.i. N.L. gen. n. *tiwarii* named in honor of Prof. D.N. Tiwari of the Department of Botany, Banaras Hindu University, India; a well-known researcher of cyanobacterial physiology and genetic studies along with being a hugely respected teacher).

Habitat: Freshwater dwelling strain with the pH of the water body being measured at 7.3. The temperature at the time of sampling was 17.5°C and the electrical conductivity was 100 μS.

Site location: Pachmarhi, Madhya Pradesh, India, 22°27'09.1"N 78°26'40.6"E.

Holotype here designated: An actively growing culture of strain *A. tiwarii* (LI_PS) was preserved in cryopreserved form in the National Centre for Microbial Resource (NCMR), formerly Microbial Culture Collection (MCC), National Centre for Cell Science (NCCS), Pune, India, and is available under the accession number MCC 3346.

DESCRIPTION OF *ALIINOSTOC SOLI* SARAF AND SINGH ET AL. SP. NOV. UNDER THE PROVISIONS OF THE INTERNATIONAL CODE OF NOMENCLATURE FOR ALGAE, FUNGI AND PLANTS

Description: Found growing on soil as macroscopic mats with the presence of ample amount of water around the mats too; soft leathery texture in the natural habitat with sufficient light

at the time of collection; color of mats is distinctly bluish green; laboratory-grown cultures appear proficiently as mats on plates retaining the bluish green of the natural sample in the initial stages of growth; prolonged growth in nitrogen-deficient media gradually makes the cultures turn yellowish and later brownish on the plates; gas vesicles evident: hormogonia formation also seen; thin hyaline sheath is present all around the filament with the sheath being evident even at the ends; prominently constricted cells; coiling of the filaments is average and overall the filaments are usually not entangled with each other; protoplasm appears clearly granular; vegetative cells almost barrel shaped to sometimes even appearing isodiametric; vegetative cells range in length from 3.6 to 4.0 μm while the width is around 3.2–3.7 μm; heterocytes are present at both the terminal and intercalary positions; heterocytes almost spherical in shape measuring about 3.8–4.3 μm in length to 3.7–4.9 μm in width; large sized spherical or oval akinetes prominently present; usually exhibit a prominent yellowish coloration.

Diagnosis: Phylogenetically and morphologically most similar to *A. tiwarii* from which it differs by having less wide vegetative cells. Differing from *A. morphoplasticum* by having both wider vegetative cell and less oval heterocyte. Secondary structure of D1-D1' helix region easily distinguishes *A. soli* from *A. tiwarii* and *A. morphoplasticum*. Strong phylogenetic support is evident for its distinct positioning and establishment as a new species of the genus *Aliinostoc*. Detailed morphological differences have been documented in Table 1.

Etymology: *Aliinostoc soli* (so'li. L. gen. n. *soli* of soil).

Habitat: Soil dwelling strain with the pH being measured at 7.3. The temperature at the time of sampling was 29.5°C while the electrical conductivity was 105 μS.

Site location: Mumbra, Maharashtra, India, 19°10′14.2″N 73°00′53.1″E.

Holotype here designated: An actively growing culture of strain *A. soli* (ZH1(3)_PS) was preserved in cryopreserved form in the National Centre for Microbial Resource (NCMR), formerly Microbial Culture Collection (MCC), National Centre for Cell Science (NCCS), Pune, India, and is available under the accession number MCC 3342.

DESCRIPTION OF *DESMONOSTOC MAGNISPORUM* SARAF AND SINGH *ET AL.* SP. NOV. UNDER THE PROVISIONS OF THE INTERNATIONAL CODE OF NOMENCLATURE FOR ALGAE, FUNGI AND PLANTS

Macroscopic amorphous colonies but not exactly mat like in the natural conditions; exhibit greenish color while the older colonies may start to appear dull green also; on being grown in the laboratory, the cultures appear light bluish in color; filaments are not very long and usually covered with thin transparent hyaline sheath all across the filament with the visibility being even at the ends; granular cytoplasm visible; cells appear prominently constricted; hormogonia formation is evident; vegetative cells appear almost barrel shaped or sometimes may even appear isodiametric; size of the vegetative cells may range from 4.0–4.5 μm in length to 4.1–4.7 μm in width; heterocytes prominently present at both terminal and intercalary positions; shape of the heterocytes may vary from being spherical to oblong; size of the heterocytes may range from 5.3–6.0 μm in length to 4.8–5.6 μm in width; akinetes distinctly visible with large size; irregular shape and dense protoplasmic contents at both terminal and intercalary positions; akinetes are also present just adjacent to the heterocyte.

Diagnosis: Morphologically differs from *D. muscorum* in having comparatively wider and longer vegetative cells. The heterocyte of *D. magnisporum* differs from *D. muscorum* in having longer and less wide heterocyte. The characteristic feature of this species is the presence of large sized and irregularly shaped akinetes at both the intercalary and terminal positions. Strong phylogenetic support is evident for its distinct positioning and establishment as a new species of the genus *Desmonostoc*. Detailed morphological differences have been documented in Table 1.

Etymology: *Desmonostoc magnisporum* (mag.ni.spo'rum. L. adj. *magnus* large; Gr. n. *spora*, a seed and, in biology, a spore; N.L. neut. adj. *magnisporum* with large spores (akinetes)).

Habitat: Freshwater dwelling strain with the pH being measured at 7.3. The temperature at the time of sampling was 32.4°C while the electrical conductivity was 90 μS.

Site location: Shrirampur, Maharashtra, India, 19°37′23.0″N 74°39′26.7″E

Holotype here designated: An actively growing culture of strain *D. magnisporum* (AR6_PS) was preserved in cryopreserved form in the National Centre for Microbial Resource (NCMR), formerly Microbial Culture Collection (MCC), National Centre for Cell Science (NCCS), Pune, India, and is available under the accession number MCC 3345.

DESCRIPTION OF *DESMONOSTOC PUNENSE* SARAF AND SINGH *ET AL.* COMB. NOV. UNDER THE PROVISIONS OF THE INTERNATIONAL CODE OF NOMENCLATURE FOR ALGAE, FUNGI AND PLANTS

Basionym: *Nostoc punense* Singh *et al.* (2016), p. 1392 (Figs 1–3).

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSLE* online.

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Conflict of interest. None declared.

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