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TAXONOMIC DESCRIPTION

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Desikacharya gen. nov., a phylogenetically distinct genus of Cyanobacteria along with the description of two new species, *Desikacharya nostocoides* sp. nov. and *Desikacharya soli* sp. nov., and reclassification of *Nostoc thermotolerans* to *Desikacharya thermotolerans* comb. nov.

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Abstract

Two *Nostoc*-like strains have been isolated, purified, cultured and identified on the basis of the polyphasic approach using morphological, ecological, molecular and phylogenetic methods. Both strains were found to have morphology similar to the genus *Nostoc*, but clustered strongly in a group distant from the *Nostoc sensu stricto* clade. Further analysis, using the folded structures of the 16S–23S ITS region revealed strong differences from closely related members of the genus *Nostoc*. Distinct phylogenetic clustering and strong tree topologies using Bayesian inference, maximum-likelihood and maximum-parsimony methods indicated the need to revisit the taxonomy of the members of this particular clade with a clear need for giving a generic status distinct from the genus *Nostoc*. In accordance with the International Code of Nomenclature for Algae, Fungi and Plants, the name *Desikacharya* gen. nov. is proposed for the new genus along with the description of two new species, *Desikacharya nostocoides* sp. nov. and *Desikacharya soli* sp. nov., and reclassification of *Nostoc thermotolerans* to *Desikacharya thermotolerans* comb. nov.

INTRODUCTION

The genus *Nostoc* is one of the most complex and challenging groups to study from the perspective of cyanobacterial taxonomy and has undergone multiple taxonomic revisions in the recent past [1–5]. The first description of *Nostoc* dates back to the nineteenth century (*Nostoc commune*) [6] and since then approximately 300 species of *Nostoc* have been documented [7]. It must be noted that, out of these approximately 300 species, there are many that have been shifted or could be shifted in future to other genera closely related to *Nostoc*. A lack of synapomorphic characterization along with morphological crypticity within the members of the genus *Nostoc* makes it one of the most morphologically complex genus to study within the order Nostocales [1]. In addition, *Nostoc* was also reported to be polyphyletic by many authors [8–11]. One of the earlier reports indicating the heterogeneity within *Nostoc* was documented by Rajaniemi *et al.* [8] with a limited number of temperate origin *Nostoc* species. The above finding gained support from different studies in which the phylogenetic analysis was performed on large number of strains based on the 16S rRNA gene and other functional genes [1, 12–14]. The phylogenetic analysis clearly distinguished the *Nostoc sensu stricto* clade from different clades of cyanobacteria with morphologies similar to *Nostoc*. To overcome the polyphyly within *Nostoc*, many studies have recommended the revision of this complex genus [1–5]. Řeháková *et al.* [1], for the first time, described a novel genus, *Mojavia*, which was phylogentically distinct and distant from *Nostoc sensu stricto*. Further, Hrouzek *et al.* [2] also separated a phylogenetically distinct clade of *Nostoc muscorum* strains into a new genus, *Desmonostoc*. The authors also recommended describing all the morphologically similar clades of *Nostoc* which lie phylogenetically distinct from *Nostoc sensu stricto* into new genera. Later, three new genera, *Halotia* [3], *Aliinostoc* [4] and *Komarekiella* [5], were described as novel genera, although they exhibited morphologically similar characteristics to *Nostoc*. However, all these genera were phylogenetically

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Keywords: *Cyanobacteria*; taxonomy; 16S rRNA gene; ITS; *Nostoc*; *Desikacharya*.
Abbreviations: ITS, Internal Transcribed Spacer; BI, Bayesian Inference; ML, Maximum Likelihood; MP, Maximum Parsimony.
The accession numbers are as follows: 16S rRNA gene MH036167–MH036168; *rbcL* MH032584 and MH032587; *nifD* MH032583 and MH032586; *rpoC1* MH032585 and MH032588.
Supplementary material is available with the online version of this article.

distant from the *Nostoc sensu stricto* clade. The above studies, along with many other studies have demonstrated that as of now the correct way to solve the taxonomic complexities of *Nostoc* and other problematic genera is to rely on proper phylogenetic interpretations supplemented by morphological and ecological data [1–5, 15–20].

In this study, we propose the establishment of a novel genus, *Desikacharya* gen. nov., within the family Nostocaceae based on the polyphasic approach along with the description of two new species, *Desikacharya nostocoides* sp. nov. and *Desikacharya soli* sp. nov., and reclassification of *Nostoc thermotolerans* to *Desikacharya thermotolerans* comb. nov.

METHODS

The soil and seepage samples were collected from the district of Mandsaur located in the state of Madhya Pradesh, India, and the physico-chemical parameters of the soil and seepage samples along with the habitat were recorded at the time of sampling (Table 1). The samples were then subjected to isolation and purification of cyanobacteria on 1.2 % solidified BG-11_0 medium [21]. Pure colonies of cyanobacterial strains were grown on sterilized Petri plates by the streak plate technique. After 14 days of growth, one or two colonies were picked up, washed thrice with deionized water and transferred to fresh liquid medium. For maintenance of bacteria-free cultures, the colonies, which appeared free of bacteria, were isolated and tested for bacterial contamination in dextrose/peptone broth and caseinate/glucose agar media. Only thereafter was axenicity established. The isolates were maintained in a culture room under ca. 50–55 $\mu Em^{-2}\ s^{-1}$ light with a photoperiod of 14 : 10 h light/dark cycle at 28 ±2 °C. Further, in depth morphological characterization of both strains was performed.

The genomic DNA was isolated from 12 to 14 day old log phase cultures using the HiMedia Ultrasensitive Spin Purification Kit (MB505-250PR). This was followed by the amplification of the 16S rRNA gene using primers pA (5′-AGAGTTTGATCCTGGCTCAG-3′) and B23S (5′-CTTCGCCTCTGTGTGCCTAGGT-3′) [22, 23]. The *rbc*L gene was amplified using the primers *rbc*L F (5′-GACTTCACCAAAGAYGACGAAAACAT-3′) and *rbc*L R (5′-GAACTCGAACTTRATYTCTTTCCA-3′) [14]. The *nif*D gene was amplified using the primers *nif*D F (5′-TCCGKGGKGTDTCTCAGTC-3′) and *nif*D R (5′-CGRCWGATRTAGTTCAT-3′) [13]. The *rpo*C1 gene was amplified using the primers *rpo*C1 F (5′-CCCGCNAARGAYTGGGAATG-3′) and *rpo*C1 R (5′-GCTTCYTGCARCATCCGYTTYTC-3′) [24]. The PCR products were directly sequenced by Sanger's method on the 3730xl DNA analyser. Further, a pairwise sequence similarity search was performed using the EzBioCloud server (www.ezbiocloud.net/) [25] for the 16S rRNA gene and the BLASTn tool for *rbc*L, *nif*D and *rpo*C1 genes.

The multiple sequence alignment was performed using CLUSTAL_X (version 1.81) [26] and the alignment was manually edited using DAMBE [27]. The phylogenetic tree was reconstructed using Bayesian inference (BI), maximum-likelihood (ML) and maximum-parsimony (MP) algorithms for all of the genes. The BI tree was reconstructed using MrBayes [28], whereas ML and MP trees were generated using MEGA 5.2.2

Table 1. Comparative morphological, ecological and physico-chemical assessment of strains 9C-PS^T, BHU1-PS^T and BHU2-PS^T

	Desikacharya thermotolerans	*Desikacharya nostocoides* (BHU1-PS^T)	*Desikacharya soli* (BHU2-PS^T)
Terminal vegetative cell	3.64–4.29 µm (length)	3.44–5.33 µm (length)	3.24–4.22 µm (length)
	3.30–3.49 µm (width)	3.95–4.04 µm (width)	3.57–3.74 µm (width)
	Perfectly curved ends	Usually barrel shaped	Usually barrel shaped
Intercalary vegetative cell	3.63–4.92 µm (length)	3.83–5.09 µm (length)	3.58–4.93 µm (length)
	3.46–3.81 µm (width)	3.44–5.33 µm (width)	3.79–4.48 µm (width)
	Usually barrel shaped	Usually barrel shaped	Usually barrel shaped
Terminal heterocyte	4.74–5.78 µm (length)	4.11–5.46 µm (length)	5.26–6.51 µm (length)
	3.39–4.49 µm (width)	4.41–5.21 µm (width)	4.21–5.21 µm (width)
	Spherical with slightly elongated ends	Spherical shaped	Oblong shaped
Intercalary heterocyte	4.74–6.00 µm (length)	4.72–6.32 µm (length)	4.91–6.89 µm (length)
	4.45–5.27 µm (width)	4.98–6.03 µm (width)	4.17–5.70 µm (width)
	Spherical shaped	Spherical shaped	Spherical shaped
Akinetes	Absent	Absent	Absent
Locality	Mandsaur, India	Mandsaur, India	Mandsaur, India
Co-ordinates	24.55° N, 75.75° E	24.56° N, 75.76° E	24.55° N, 75.79° E
Habitat	Soil	Seepage	Soil
pH	7.34	7.36	7.36
Temperature (°C)	43.20	43.20	43.11
EC ($\mu S\ cm^{-1}$)	1354	1358	1357
Humidity (%)	32	32	32

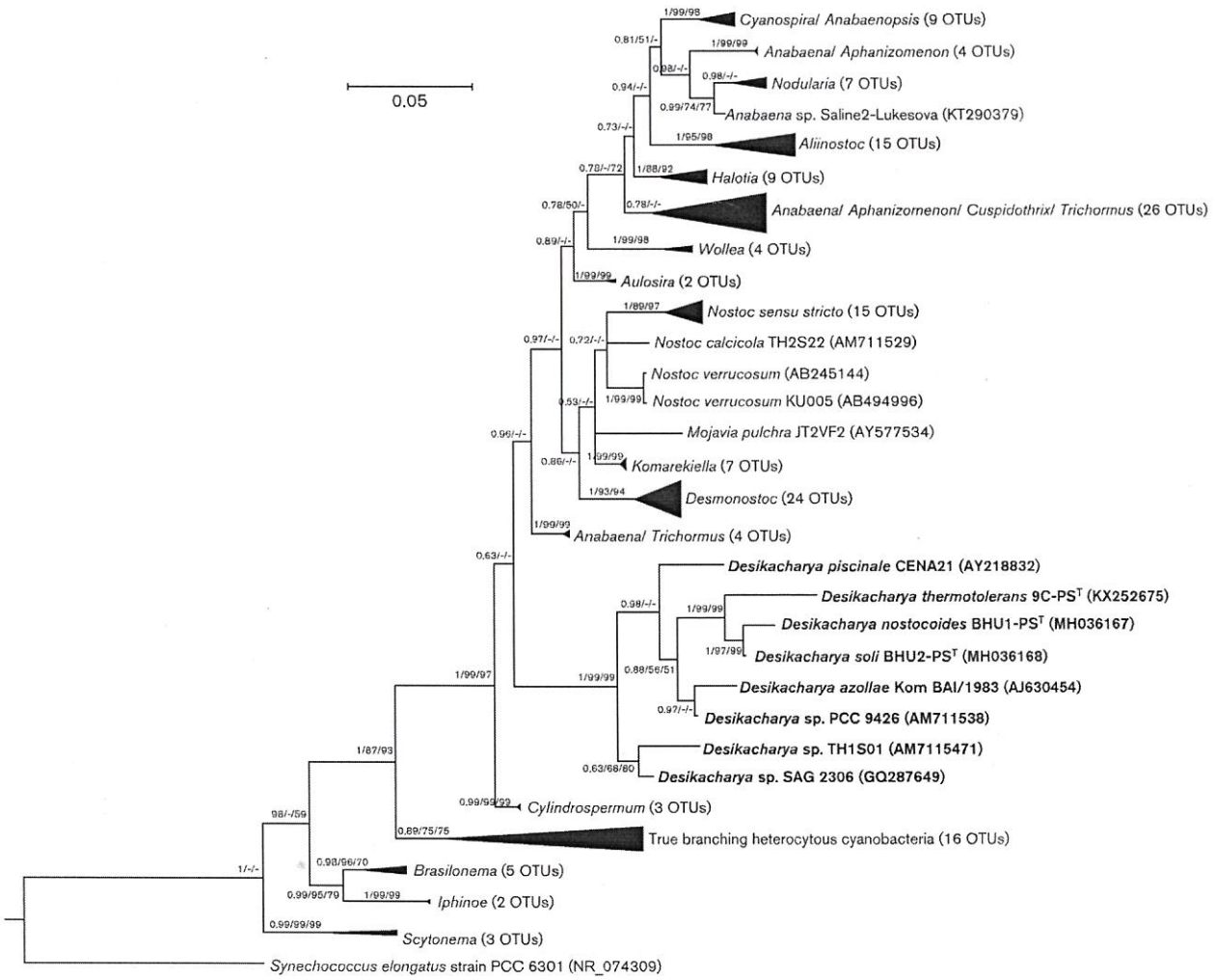

Fig. 1. Phylogenetic positioning of the genus *Desikacharya* based on 16S rRNA gene inferred by the Bayesian inference tree with the probability scores/bootstrap values representing BI, ML and MP, respectively. Bar, 0.05 changes per nucleotide position. For ML and MP, all bootstrap values below 50 were deleted.

[29]. Models with the lowest BIC scores were selected as they are considered to describe the substitution pattern the best. For the reconstruction of the BI tree, two runs of eight Markov chains were applied until the standard deviation of the split frequency was lower than 0.01. The diagnostics were calculated after every 1000 generations. The sampling was done every 1000th generation. The initial 25 % of the sampled trees were discarded as burn-in and the rest was used to calculate the posterior probabilities. For all the functional genes, nucleotide sequences were used for phylogenetic analyses. For the ML and MP trees [30–32], the bootstrap method was used as the test of phylogeny with 1000 replications.

Further, the secondary structures of the D1–D1′ helix region, boxB and V3 helix region of the 16S–23S internal transcribed spacer (ITS) were determined using Mfold [33] for strains BHU1-PS^T and BHU2-PS^T. In addition, the secondary structures were obtained for the closely related taxa based on the phylogenetic analysis and compared with the structures of strains BHU1-PS^T and BHU2-PS^T. The *p*-distance values for the strains and the closely related taxa were determined using MEGA 5.2.2 [29].

RESULTS AND DISCUSSION

This study is an extension of our previous report of *Nostoc thermotolerans* sp. nov. (9C-PS^T) [20]. In the present study, two more strains isolated from almost similar ecology were studied using the polyphasic approach. Both the strains showed a very close phenotypic resemblance to *N. thermotolerans*. Phenotypic characteristics such as the appearance of the sheath, the shape of heterocytes and the vegetative cells were found to be cryptic. Therefore, it was difficult to differentiate among the three species only on the basis of morphological characteristics. However, minor differences in the appearance of colonies, size of

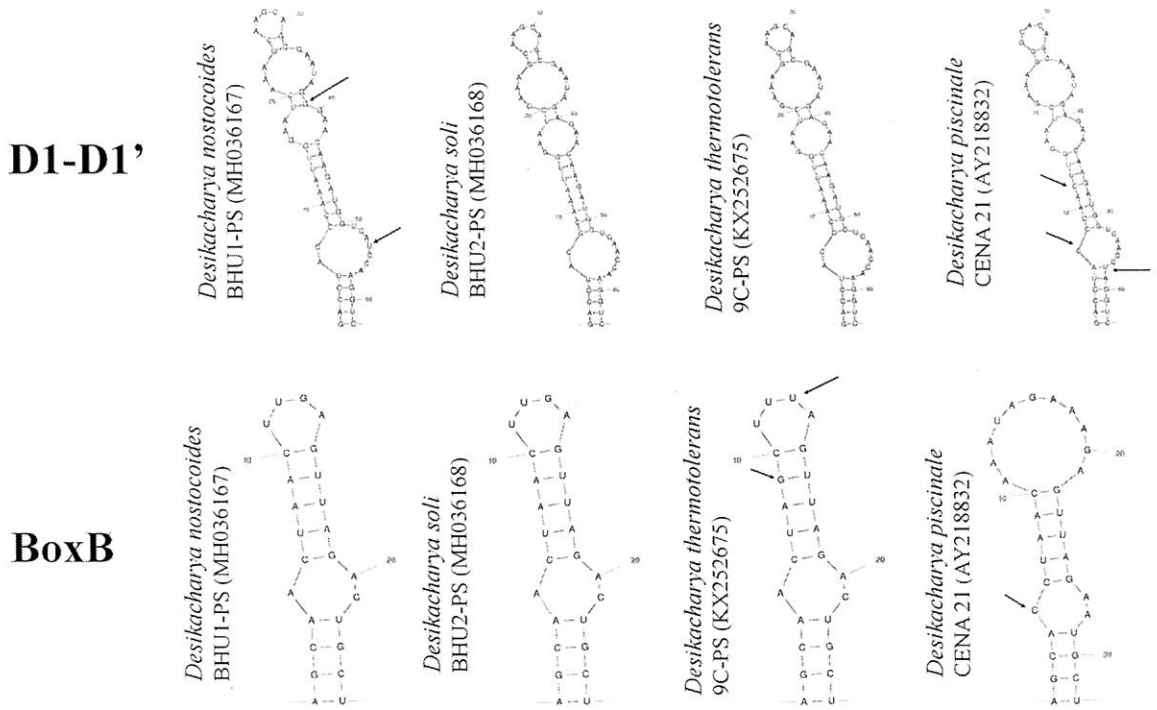

Fig. 2. Comparison between the folded secondary structures of the D1–D1′ and BoxB helix regions of the members of the *Desikacharya* clade. The arrow indicates the differences observed between the compared strains.

heterocytes and vegetative cells were observed between the closely related species (Table 1 and Fig. S1, available with the online version of this article). Further, the 16S rRNA gene sequence similarities were determined. The pairwise sequence similarity within the clade was found to be in the range of 95 –98 % and with the other closely related genera was found to be less than 94 %. Although percent similarity values are important from the taxonomic perspective, recent studies have indicated that these values should not be considered as a primary criterion to establish taxon levels in cyanobacteria [34–36]. We also believe that the phylogenetic positioning of the strains should be given more importance rather than the percentage similarity values in case of cyanobacteria.

In the 16S rRNA gene phylogenetic tree, the strains BHU1-PS^T and BHU2-PS^T, along with *N. thermotolerans* 9C-PS^T (KX252675), *Nostoc piscinale* CENA21 (AY218832), *Trichormus azollae* Kom BAI/1983 (AJ630454), *Nostoc* sp. TH1S01 (AM711547), *Nostoc* sp. SAG 2306 (GQ287649) and *Nostoc* sp. PCC 9426 (AM711538) formed a distant and distinct clade away from *Nostoc sensu stricto* and other related genera (Fig. 1). The taxonomic status of the members of this clade has been debated for a long time. *Nostoc piscinale* was initially reported to be a member of the soil community [37], however, Elenkin [38] included this as a form of *Nostoc linckia* which is usually found in freshwater. Sant'anna in 1991 [39] and Fiore *et al.* in 2005 [40] in their study on cyanobacteria from Brazil, again reported *Nostoc piscinale* to be a member of the soil community. Further, Fiore *et al.* [40] also indicated the possibility that *Nostoc piscinale* CENA 21 would undergo reclassification on the basis of its phylogenetic positioning. However, Komárek [7] persisted with the classification proposed by Elenkin even after the work of Sant'anna [39] and Fiore *et al.* [40]. This has led to complications and uncertainty about the taxonomic status of *Nostoc piscinale*. Our phylogenetic tree is in congruence with Fiore *et al.* [40] and we also believe that *Nostoc piscinale* CENA 21 is definitely not a member of *Nostoc*. Similarly, the strain *Trichormus azollae* Kom BAI/1983 (AJ630454) is another interesting case study which has been analysed for quite some time now. This particular strain was assessed specifically by Rajaniemi *et al.* [8], in which they concluded that the genus *Trichormus* is not monophyletic and seems to be split genetically into three groups. Considering the confusion in taxonomy at that time, they had very correctly proposed the proximity of this strain with *Nostoc* rather than *Trichormus* or *Anabaena*. Later, Komárek [7] clearly mentioned that the taxonomy of *Trichormus azollae* is debatable. In 2014, Pereira and Vasconcelos [41] studied the *Azolla* symbionts and indicated that these endobionts could be representatives of a new genus of the family Nostocaceae. Our study clearly indicates that *Trichormus azollae* Kom BAI/1983 does not belong to the genera *Anabaena* or *Trichormus* and also reflects that it is definitely not a member of the *Nostoc sensu stricto* clade. In our previous study, strain 9C-PS^T, which was phylogenetically close to both the above discussed species, was described as a new member of the genus because of its

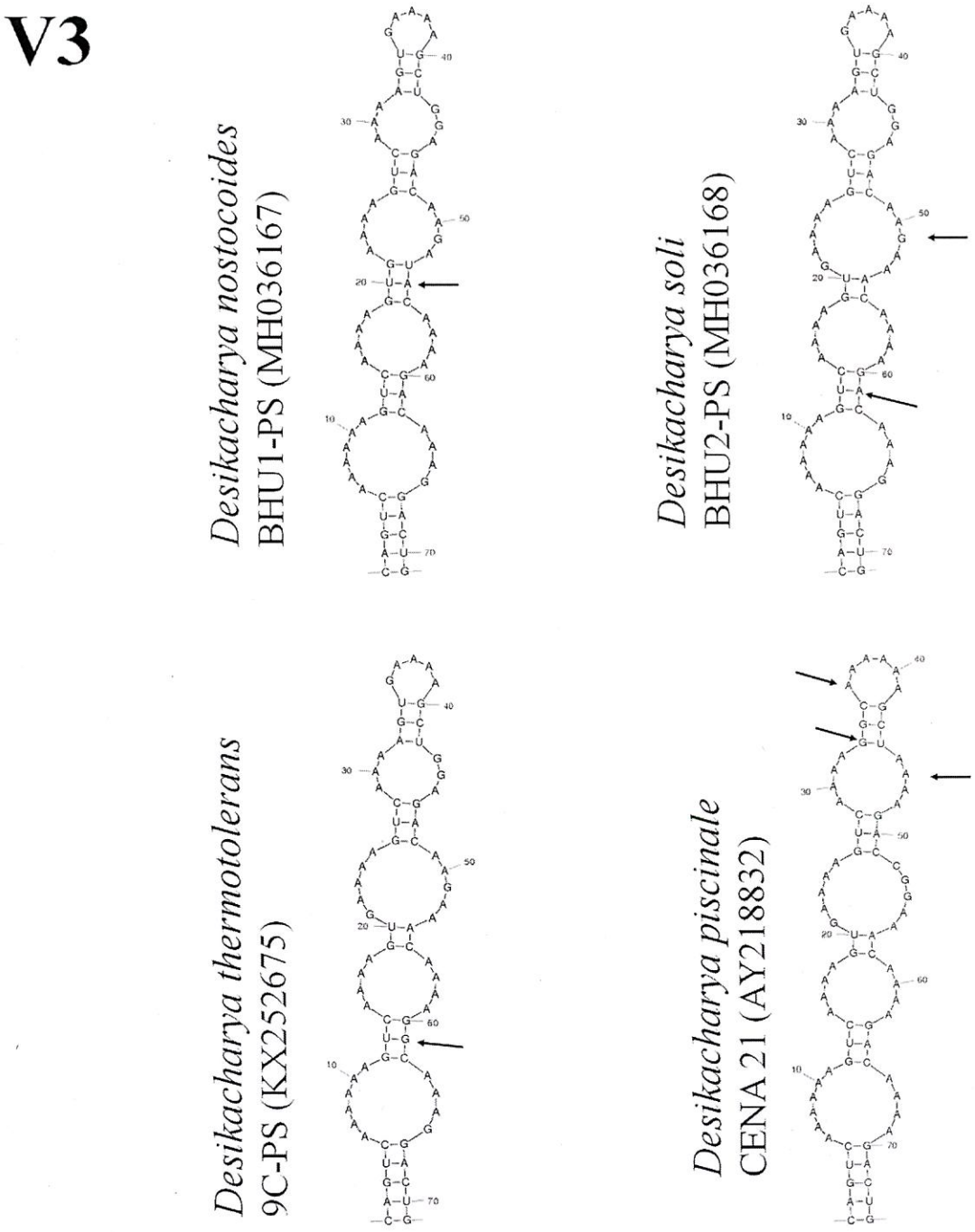

Fig. 3. Comparison between the folded secondary structures of the V3 helix region of the members of the *Desikacharya* clade. The arrow indicates the differences observed between the compared strains.

morphological relatedness to *Nostoc*. Although the idea of erecting a new genus was not proposed in the earlier study, this may be attributed to the lack of taxon sampling in the previous 16S rRNA gene phylogenetic tree.

The current proposition of conceptualizing strongly supported monophyletic clades into different genera finds robust support from previous works [1–5, 15–20, 42]. The distinct phylogenetic positioning along with strong posterior probability/bootstrap support indicates conclusively that the entire clade of *Nostoc piscinale* CENA21 (AY218832), *Trichormus azollae* Kom BAI/1983 (AJ630454), *Nostoc thermotolerans* 9C-PS^T (KX252675), BHU1-PS^T, BHU2-PS^T, *Nostoc* sp. TH1S01

Table 2. *p*-distance values of the 16S rRNA gene of the genus *Desikacharya* with the species of closely related genera based on phylogenetic analysis

Accession	Strain	1	2	3	4	5	6	7	8	9	10	11	12
MH036167	*Desikacharya nostocoides*												
MH036168	*Desikacharya soli*	0.008											
KX252675	*Nostoc thermotolerans*	0.038	0.029										
AY218832	*Nostoc piscinale* CENA21	0.042	0.032	0.057									
AJ630454	*Trichormus azollae* Kom BAI/1983	0.037	0.028	0.057	0.034								
EU586722	*Nostoc commune* NC3-K1	0.067	0.058	0.089	0.057	0.065							
KY403996	*Aliinostoc morphoplasticum*	0.066	0.058	0.088	0.064	0.056	0.070						
AM711524	*Desmonostoc muscorum* NIVA-CYA 818	0.060	0.052	0.083	0.052	0.050	0.049	0.061					
KJ843310	*Halotia branconii* CENA390	0.083	0.074	0.106	0.078	0.069	.059	0.040	0.050				
DQ234832	*Trichormus variabilis* KCTC AG10180	0.075	0.067	0.096	0.060	0.062	0.057	0.046	0.030	0.040			
AY577534	*Mojavia pulchra* JT2-VF2	0.082	0.073	0.099	0.070	0.069	0.043	0.069	0.049	0.059	0.051		
NR_074309	*Synechococcus elongatus* PCC 6301	0.177	0.164	0.201	0.164	0.158	0.155	0.158	0.152	0.155	0.147	0.142	

(AM711547), *Nostoc* sp. SAG 2306 (GQ287649) and *Nostoc* sp. PCC 9426 (AM711538) need to be recognized as a new genus separate from existing *Nostoc*-like genera. Further, the phylogenetic distance between strains BHU1- PS^T, BHU2-PS^T and *Nostoc thermotolerans* 9C-PS^T indicates that all three strains are indeed different species. The phylogenetic tree reconstructed using *rbc*L, *nif*D and *rpo*C1 genes proved to be inconclusive in erecting a new genus (Figs S2–S4). This is primarily due to the lack of robust databases for the functional genes. However, the phylogenetic distance between strains BHU1-PS^T, BHU2-PS^T and *Nostoc thermotolerans* 9C-PS^T in all the trees indicated that they are different species.

In addition, the secondary structures of the D1–D1′, BoxB and V3 helix regions of the 16S–23S ITS were determined using Mfold [33] for strains BHU1-PS^T and BHU2-PS^T along with the closely related taxa based on phylogenetic analysis (Figs 2 and 3). In the case of the D1–D1′ helix region, all the strains exhibited similar topology; however, there were minute differences, which are discussed further in detail (Fig. 2). The basal stem of the D1–D1′ region in *Nostoc piscinale* consists of six nucleotide pairs, whereas the other strains had only five nucleotide pairs. The topology of the first loop was similar in strains BHU1-PS^T, BHU2-PS^T and *Nostoc thermotolerans*. However, BHU1-PS^T had a uracil whereas BHU2- PS^T and *Nostoc thermotolerans* had adenine at the same position. The topology of the first loop was completely different in *Nostoc piscinale*. The stem connecting the first and the second loop was identical in BHU1-PS^T, BHU2-PS^T and *Nostoc thermotolerans*; whereas, *Nostoc piscinale* had a base replacement of cytosine instead of adenine. Similarly, there is a base difference in BHU1-PS^T in the stem connecting the second and the third loop. Furthermore, the number of nucleotides in the third loop of BHU1-PS^T is lower as compared to the other strains (12 instead of 13). Although, the secondary structures of the D1–D1′ helix region had similar topology, the above differences indicate clearly that these structures are in fact different from each other.

In the case of the BoxB region, it was observed that the structure of *Nostoc piscinale* was remarkably different from the other closely related strains (Fig. 2). Further, the structures of strains BHU1-PS^T, BHU2-PS^T and *Nostoc thermotolerans* were identical. However, a change of base in the stem and the loop region of *Nostoc thermotolerans* indicated that it is different from the other two strains. In the case of the V3 region, it was observed that the number of nucleotides in the third loop of BHU1-PS^T had 12 nucleotides whereas, the other strains had 14 nucleotides (Fig. 3). Also, the fourth and the fifth loop of *Nostoc piscinale* was found to be large as compared to the other strains. The stem connecting the second and the third loop had an extra nucleotide pair in the case of strain BHU1-PS^T. The structures of BHU2-PS^T and *Nostoc thermotolerans* were also similar in this case; however, a change of base in the stem connecting the second and third loop differentiated the two strains. The comparative 16S–23S ITS secondary structure analysis provides further evidence that BHU1-PS^T, BHU2-PS^T and *Nostoc thermotolerans* are indeed different species. Also, the *p*-distance values supported the results obtained from the phylogenetic analysis and 16S–23S ITS secondary structure analysis (Table 2).

The origin of *Trichormus azollae* Kom BAI/1983 is unknown; whereas, the rest of the cyanobacterial strains belonging to the proposed *Desikacharya* clade have been recorded to be members of the soil communities. These cyanobacterial strains are reported from tropical regions such as India, Brazil, Egypt and Thailand. Thus, there is a possibility of a typical biogeographic distribution of the members of the proposed genus. The morphological characterization did not provide enough conclusive proof to differentiate the closely related strains as they were found to be cryptic and could not be easily distinguished from the other *Nostoc*-like genera. However, it is notable that one of the characteristic features could be the presence of well-defined constrictions between the cells, thus enhancing the barrel shape of the vegetative cells. This character could can be subject to

environmental conditions and that is why the use of morphology alone cannot be considered as a reliable feature. However, based on the polyphasic approach employed in this study, we propose the creation of the new genus *Desikacharya* gen. nov. and two species, *Desikacharya nostocoides* sp. nov. and *Desikacharya soli* sp. nov. Also we recommend reclassifying the existing members of the clade into the proposed new genus including *Desikacharya thermotolerans* 9C-PS^T comb. nov., *Desikacharya piscinale* CENA 21 comb. nov. and *Desikacharya azollae* Kom BAI/1983 comb. nov.

Comparisons of cyanobacterial taxonomy with contemporary bacterial taxonomy are quite vociferous and through this work we would like to emphasize that as of now the taxonomy of cyanobacteria, especially the usage of methods like DNA–DNA hybridization or whole-genome sequencing, may create a very complex situation where there is a greater chance of confusion being created rather than settling the debate. Hence, it is strongly recommended to use the polyphasic approach prudently as per the taxa involved, rather than trying to create a single scheme of work which may vary at different levels in different taxa.

DESCRIPTION OF *DESIKACHARYA* SARAF AND SINGH *ET AL.* GEN. NOV.

Desikacharya (De.si.ka.cha′ry.a. N.L. fem. n. *Desikacharya* a genus named in honour of T. V. Desikachary, a great and well known cyanobacterial taxonomist from India).

Genus showing typical *Nostoc*-like morphology with rounded terminal cells and presence of both terminal and intercalary heterocytes. Coiling is easily visible though may vary to different degrees. As of now, one of the characteristic features is the presence of well-defined constrictions between the cells, thus giving the barrel-shaped vegetative cells an enhanced look. The genus may occur in soil, freshwater and seepages. The filaments can be very long in some cases, while others may have a moderate number of cells. The most important criterion for the establishment as a new genus is the distinct phylogenetic clustering based on the 16S rRNA gene.

DESCRIPTION OF *DESIKACHARYA NOSTOCOIDES* SARAF AND SINGH *ET AL.* SP. NOV.

Desikacharya nostocoides (nos.to.co'i.des. N.L. neut. n. *Nostoc* a cyanobacterial genus; L. suff. *-oides* (from Gr. suff. *eides* from Gr. n. *eidos* that which is seen, form, shape, figure), resembling, similar: N.L. fem. adj. *nostocoides Nostoc*-like).

Macroscopic greenish mat dwelling in the natural habitat; grows more or less in seepages where the confluence of little amount of water and soil is seen. Appears with a small amount of mucilage with a soft texture. Collection from nature is easy as the mucilage is not very dense. The laboratory-grown culture also has a mat-like appearance, although colonies at the ends may appear more discrete. Under the microscope, usually appears light green in colour. Extremely long filaments have a large amout of visible cells. Vegetative cells are usually barrel shaped, though sometimes may also appear a bit longer than the width. Constrictions are very prominent in between the cells. Cellular contents seem to appear slightly granular giving the entire filament a slightly textured appearance. The terminal vegetative cells range in size from 3.44 to 5.33 µm long to 3.95–4.04 µm wide. The intercalary vegetative cells range in size from 3.83 to 5.09 µm long to 3.44–5.33 µm wide. Sheaths are distinct, appearing throughout the trichome, with a slightly mucilaginous appearance. In most of the cases observed, the heterocytes appear at both the ends of the filament. Heterocytes are yellowish in colour and usually appear almost spherical. The terminal heterocytes range in size from 4.11 to 5.46 µm long to 4.41–5.21 µm wide. The intercalary heterocytes range in size from 4.72 to 6.32 µm long to 4.98–6.03 µm wide. Akinetes were not observed in any stage of the life cycle.

Etymology: *nostocoides* (nos.to.co'i.des. N.L. neut. n. *Nostoc* a cyanobacterial genus; L. suff. *-oides* (from Gr. suff. *eides* from Gr. n. *eidos* that which is seen, form, shape, figure), resembling, similar: N.L. fem. adj. *nostocoides Nostoc*-like).

Type locality: Bhanpura, Mandsaur, Madhya Pradesh, India (24.56° N 75.76° E).

Ecology of type locality: the samples were collected during the early summer season in which extreme hot and dry conditions prevail. The month of May is in fact one of the hottest months with extreme dryness and humidity dropping to around 32 %. Atmospheric temperature at the time of collection was 43.20 °C and the light intensity was around 8800 lux. The pH of the soil was 7.36 and EC was 1358 µS cm^{-1}.

Habitat: seepage-dwelling cyanobacteria found to be growing as a mat having influence of both soil, a nearby small rocky shade and dripping water.

Holotype here designated: the culture of *Desikacharya nostocoides* has been stored and deposited in the Global Collection of Cyanobacteria (GCC; http://www.wfcc.info/ccinfo/detail; Registered Number 1165), Varanasi, India as *Nostoc* species after identification and authentication on the basis of the full-length sequencing of the 16S rRNA gene along with folding of the secondary structures of the 16S–23S ITS region. After proper identification and authentication, the culture is being maintained in the GCC under the accession number GCC 20181.

DESCRIPTION OF *DESIKACHARYA SOLI* SARAF AND SINGH *ET AL.* SP. NOV.

Desikacharya soli (so'li. L. gen. n. *soli* of soil).

Macroscopic mat with a dark greenish colour in the natural habitat. Appears very clearly in almost dry soil as mats having feathery exterior appearance. The mats have little mucilage around them and picking the mat from nature is easy. Laboratory-grown cultures appear slightly leathery in appearance and grow as discrete colonies on the plates. Appears dark greenish blue in the microscope with cellular

contents being densely granular. Extremely long filaments with a large number of cells are visible and even the comparatively shorter filaments also have more than 15 cells and terminal heterocytes. The vegetative cells appear barrel shaped with some cells being larger than the routine vegetative cells. The presence of a small amount of mucilaginous sheath is evident all around the trichome. Constrictions are prominent between the adjacent cells. The heterocytes appear yellowish in colour. Heterocytes are present both at the intercalary and terminal positions. The shape of the heterocyte varies, with the intercalary heterocytes being spherical while the terminal heterocytes appear slightly oblong in shape. Terminal heterocytes appear at both the ends of the trichome in many cases. The terminal vegetative cells range in size from 3.24 to 4.22 µm long to 3.57–3.74 µm wide. The intercalary vegetative cells range from 3.58–4.93 µm long to 3.79–4.48 µm wide. Terminal heterocytes range in size from 5.26 to 6.51 µm long to 4.21–5.21 µm wide. Intercalary heterocytes range in size from 4.91 to 6.89 µm long to 4.17–5.70 µm wide. Neither the natural occurring sample nor the laboratory-grown culture showed any evidence of akinete formation.

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

Type locality: Bhanpura, Mandsaur, Madhya Pradesh, India (24.55° N 75.79° E).

Ecology of type locality: the sample occurred as macroscopic mat on the soil with a small amount of mucilage around the entire mat. Sample collection was done in May with the humidity being close to 32 %. Temperature at the time of collection was 43.11 °C and the light intensity was around 8800 lux. The pH of the soil was 7.36 and EC was 1357 µS cm^{-1}.

Habitat: typical soil-dwelling cyanobacteria which were found to be growing as a macroscopic mat.

Holotype here designated: the culture of *Desikacharya soli* has been stored and deposited in the GCC as *Nostoc* species after identification and authentication on the basis of the full-length sequencing of the 16S rRNA gene along with folding of the secondary structures of the 16S–23S ITS region. After proper identification and authentication, the culture is being maintained in the GCC under the accession number GCC 20182.

DESCRIPTION OF *DESIKACHARYA THERMOTOLERANS* SARAF AND SINGH *ET AL.* COMB. NOV.

Basionym: *Nostoc thermotolerans* Suradkar *et al.* 2014.

The description is as given by Suradkar *et al.* [20].

The type strain is 9C-PS^T (=MCC 3156).

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Conflicts of interest
The authors declare that there are no conflicts of interest

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