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Research Letter

RESEARCH LETTER – Taxonomy & Systematics

Insights into the phylogeny of false-branching heterocytous cyanobacteria with the description of *Scytonema pachmarhiense* sp. nov. isolated from Pachmarhi Biosphere Reserve, India

Aniket Saraf[1,2], Himanshu G. Dawda[1], Archana Suradkar[2], Priyanka Batule[2], Isha Behere[2], Manasi Kotulkar[2], Ankita Kumat[2] and Prashant Singh[2,3,*]

[1]Department of Botany, Ramniranjan Jhunjhunwala College, Ghatkopar, Mumbai 400086, Maharashtra, India, [2]National Centre for Microbial Resource, National Centre for Cell Science, Pune-411021, Maharashtra, India and [3]Department of Botany, Banaras Hindu University, Varanasi 221005, Uttar Pradesh, India

*Corresponding author: Department of Botany, Banaras Hindu University, Varanasi 221005, Uttar Pradesh, India. Tel: +91-7755830009; E-mail: sps.bhu@gmail.com

One sentence summary: This paper reviews the phylogeny of the cyanobacterial genus *Scytonema* and includes the description of a new species.
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ABSTRACT

A false branching cyanobacterium (strain 10A1_PS) was isolated from a freshwater body of the Pachmarhi Biosphere Reserve, India and was characterised using the polyphasic approach. The detailed morphological examination indicated that the strain belonged to the complex genus *Scytonema* as it exhibited typical false branching character whose frequency increased with age of the culture. As the family Scytonemataceae and the genus *Scytonema* has been shown to be polyphyletic in many studies, we provide deep insights into the phylogenetic complexities within the family Scytonemataceae based on 16S rRNA gene phylogeny along with complete morphological, molecular and phylogenetic characterisation of the strain. The 16S rRNA gene phylogenetic tree inferred by Bayesian Inference, Neighbor-Joining and Maximum Parsimony methods showed that the strain clustered within the *Scytonema sensu stricto* clade. The phylogenetic distance and the positioning of the strain clearly indicated it to be different from other *Scytonema* species. Further analysis using *rbcL* phylogeny, folded secondary structures of the 16S-23S ITS, p-distance and percentage pairwise similarity matrix clearly distinguished the strain 10A1_PS from the other closely related species. In accordance with the International Code of Nomenclature for Algae, Fungi and Plants, we propose the name of the new species to be *Scytonema pachmarhiense*.

Keywords: cyanobacteria; *Scytonema*; taxonomy; phylogeny; 16S rRNA gene; ITS

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INTRODUCTION

The heterocytous group of cyanobacteria are morphologically diverse with respect to the branching of filaments in having true branched, false branched and unbranched filaments. The false branching heterocytous cyanobacteria are morphologically unique and are grouped under Scytonemataceae, Tolypothrichaceae, Godleyaceae and Rivulariaceae families of the order Nostocales according to the recent classification system (Komárek *et al.* 2014). Amongst the four families, Rivulariaceae exhibits facultative false branching, whereas the other three are strictly false branching types (Komárek *et al.* 2014). Along with the type of false branching, the polarity of the filament is also one of the major distinguishing morphological trait to differentiate Scytonemataceae, Tolypothrichaceae and Godleyaceae. The family Scytonemataceae shows isopolar filaments whereas Tolypothrichaceae displays heteropolar filaments, and in the case of Godleyaceae the younger filaments are isopolar and the older filaments are heteropolar (Hauer *et al.* 2014; Komárek *et al.* 2014). In order to achieve monophyly, the families Tolypothrichaceae and Godleyaceae were recently erected after dissolving the family Microchaetaceae (Hauer *et al.* 2014). Hence, the family Scytonemataceae can be considered to be the only traditional cyanobacterial family with the false branching phenotype and at present consists of *Scytonema*, *Scytonema* sect. *Myochrotes*, *Petalonema*, *Scytonematopsis*, *Brasilonema*, *Ophiothrix*, *Chakia*, *Iningainema*, *Ewamiania*; of which the last five new genera have been described in the last decade (Bornet and Flahault 1887; Correns 1889; Kiseleva 1930; Fiore *et al.* 2007; Sant'Anna *et al.* 2010; Komárková, Zapomělová and Komárek 2013; McGregor and Sendall 2017a,b).

Scytonema is considered to be one of the oldest genus of the phylum Cyanobacteria and was first described by Bornet and Flahault (1886). Subsequently, more than 300 species have been documented within this genus (Komárek 2013). *Scytonema* is also one of the most widely studied genus of the false branching group of heterocytous cyanobacteria and is predominantly observed in the tropical regions with only a few reports from the temperate and polar regions (Komárek *et al.* 2013). The typical feature of this genus is the appearance of filaments or thallus usually in the form of cluster, or prostrate with entangled filaments and frequently observed false branching. The trichomes are isopolar, uniseriate, usually constricted and mostly surrounded by a sheath that may be coloured or colourless. The cells primarily appear pale or olive green in colour and pinkish or yellowish in rare cases (Komárek *et al.* 2013). Although the morphological characters are well defined for the genus *Scytonema* but it has been reported to be polyphyletic based on phylogenetic studies (Komárek *et al.* 2013; McGregor and Sendall 2017a,b). Komárek *et al.* (2013) performed in depth morphological, ecological and phylogenetic studies on Brazilian isolates and established the *Scytonema sensu stricto* clade for the first time. Further they also proposed the existence of new genera from different clusters of *Scytonema* like isolates. However, due to relatively small number of sequenced taxa no new genus was erected in their study.

The phylogenetic status of the family Scytonemataceae is under constant debate in the recent past, and the polyphyletic nature of the family has been reported multiple times in different studies (Vaccarino and Johansen 2011; Komárek *et al.* 2013, 2014, McGregor and Sendall 2017a,b). One of the earliest reports indicating the polyphyletic nature of Scytonemataceae was provided by Vaccarino and Johansen (2011) in their study on the cyanobacterial population from the Hawaiian Islands. They observed distant phylogenetic positioning of *Scytonematopsis contorta* and *Petalonema* away from the other members of the family. Whereas in case of *Chakia* it was found to be phylogenetically close to the members of Scytonemataceae but showed unfamiliar morphological characters like isopolar filaments with frequent hormocytes (Komárková, Zapomělová and Komárek 2013). More importantly, *Scytonema*, which is the type genus of the family, has also been shown to be polyphyletic by Komárek *et al.* (2013) and this has further aggravated the phylogenetic complexity within the family. Recently, McGregor and Sendall 2017a,b) also reported the polyphyly within Scytonemataceae in their study and believed that the higher taxonomic status of their newly described genera *Iningainema* and *Ewamiania* may change.

Nevertheless, the taxonomic complexities within the family Scytonemataceae and the genus *Scytonema* can be resolved by employing polyphasic approach, which has proven to be effective in demarcating the taxonomic complexities within the families Nostocaceae (Řeháková *et al.* 2007; Hrouzek *et al.* 2013; Genuário *et al.* 2015; Bagchi, Dubey and Singh 2017) and Hapalosiphonaceae (Kabirnataj *et al.* 2018) in recent studies. In this study, a freshwater isolate 10A1_PS has been characterised on the basis of polyphasic approach and we intend to describe it as a new species of *Scytonema* in accordance with International code of Nomenclature for Algae, Fungi and Plants, with the name proposed being *Scytonema pachmarhiense*. Also, the taxonomic complexity within the family Scytonemataceae is discussed in detail, which may further contribute in resolving the existing disputes within the family.

MATERIAL AND METHODS

Sampling, isolation, purification and culturing

Strain 10A1_PS was isolated from a freshwater body situated in the hilly region of Pachmarhi, Madhya Pradesh, India. Pachmarhi is located at an altitude of 1100 metre and is a part of Pachmarhi Biosphere Reserve, which is spread across three districts of Madhya Pradesh in the Satpura Ranges. It is one of the largest and most conserved biosphere reserve in India and has been listed in the UNESCO Biosphere Reserve for its unique flora and fauna. The sample collection was done during the post-monsoon season in the month of November, and the physico–chemical parameters of the habitat and sampling area were measured on-site at the time of sampling. The isolation and purification step were performed using $BG11_0$ media (Rippka *et al.* 1979), pH of the media was adjusted to 7.2, and the strain was maintained in a culture room under a photoperiod of 14/10h light/dark cycle at 28 ± 2°C with light intensity of 50–55 $\mu Em^{-2}s^{-1}$. The purified strain was then grown in 15-ml tubes followed by transferring them to 250-ml Erlenmeyer flask.

Phenotypic analysis

Microscopy of the sample was performed immediately after sampling in order to get an idea about the overall microflora present. Further, extensive morphological assessment of the laboratory grown culture was performed using Nikon YS100 microscope (Nikon, Minato, Tokyo, Japan), and the microphotographs were taken on Olympus BX53 (Olympus Corporation, Shinjuku, Tokyo, Japan) fitted with ProgRes C5 camera (Jenoptik, Jena, Thuringia, Germany). Type of filament, occurrence and positioning of the specialised cells and type of branching were some of the important phenotypic characters that were

considered during microscopic observations. Measurements were taken at both ×40 and ×100 magnifications in order to get better resolutions. Initial identification of the strain was done using the keys of Desikachary (1959), and the keys of Komárek (2013) were also consulted for the final conclusion.

Genomic DNA extraction and PCR

The genomic DNA was extracted using 10–12 days old log phase culture using Himedia Ultrasensitive Spin Purification Kit (MB505). As the filaments were surrounded by sheath it hindered DNA isolation, hence modifications in the lysis step were done as described by Suradkar *et al.* 2017, in which the incubation time was increased for both the lysis solution. Amplification of the 16S rRNA gene was performed using the primers pA (5′-AGAGTTTGATCCTGGCTCAG-3′) and B23S (5′-CTTCGCCTCTGTGTGCCTAGGT-3′) (Edwards *et al.* 1989; Gkelis *et al.* 2005). The *rbc*L gene was amplified as described by Singh, Fatma and Mishra (2015).

Sequence analysis

The amplified products were sequenced by Sanger's method on a 3730xl automated sequencer (Applied Biosystems). In case of 16S rRNA gene and 16S-23S internal-transcribed spacer (ITS) region, a clear 1904 bp sequence was obtained. Further, the similarity search was performed using both EzBioCloud (Kim *et al.* 2012) and NCBI BLAST database with only validly published cyanobacterial taxa in order to cross check the results from both the databases. For *rbc*L, the similarity search was performed using NCBI BLASTN tool, and finally all the sequences were submitted to NCBI. The protein coding sequence was annotated using ORF finder before submission.

Phylogenetic analysis

The phylogenetic analysis for both the 16S rRNA and *rbc*L genes was inferred by Bayesian Inference (BI), Neighbor-Joining (NJ) and Maximum Parsimony (MP) methods. In case of the 16S rRNA gene, the phylogenetic tree using BI method was constructed by MrBayes 3.2.6 (Ronquist *et al.* 2012), and the model for Bayesian analysis was inferred using jModeltest (Darriba *et al.* 2012), which led to the selection of GTR + I + G. In the analysis, two runs of eight Markov chains were applied for 15 million generations, and the sampling was done every 1000th generation. Twenty-five percent samples from the beginning of the chain were discarded, and the analysis was continued till the value of standard deviation of split frequency was below 0.01. In case of NJ and MP, the trees were constructed using Mega 5.2.2, and the appropriate model for NJ was found to be K2 + G (Tamura 1992; Tamura *et al.* 2011). The reliability of NJ and MP trees was tested using bootstrap resampling method with 1000 replications (Felsenstein 1985). The analysis involved 147 nucleotide sequences, and the robustness of the tree topology was assessed by mapping all the three trees into a single tree. Similarly for *rbc*L gene, the phylogenetic tree was constructed by all the three methods using MrBayes 3.2.6 for BI tree and Mega 5.2.2 for NJ and MP. The model selection was performed as mentioned above, and the appropriate model for BI and NJ were SYM + G and K2 + G, respectively.

16S-23S ITS secondary structure analysis

The sequences corresponding to the D1-D1' helix region of 16S-23S ITS and box-B helix region were transcribed, and the folded secondary structures were obtained using Mfold web server (Zuker 2003).

p-distance and percentage pairwise identity matrix

The p-distance value of the strain 10A1_PS with the closely related strains was calculated using MEGA 5.2.2, and the percentage pairwise identity matrix was obtained by Sequence Demarcation Tool (Muhire, Varsani and Martin 2014)

RESULTS

Morphological and ecological evaluation

The morphological assessment of the strain 10A1_PS was performed giving importance to the type of filaments and branching observed. 10A1_PS displayed isopolar filaments with prominent false branching with one or two lateral branches. The other characters that were evaluated involved size and shape of vegetative cells, positioning and appearance of heterocytes, occurrence of sheath, shape of terminal vegetative cells and hormogonia formation (Fig. 1). The morphological comparisons between strain 10A1_PS, *Scytonema hofmanni* and two newly described species of *Scytonema* from India, *Scytonema bilaspurense* and *Scytonema singhii*, were made along with the representative strains from other *Scytonema* clusters (Table 1). Further, the morphology of the strain 10A1_PS was also compared with the strains from other closely related genera within the family Scytonemataceae (Table S1, Supporting Information). The evaluation of the sampling site was also done in order to get a brief idea about the habitat. The sampling was done in the month of November, which is usually the beginning of winters in Pachmarhi with the day temperature in the range of 25°C–30°C and the temperature in the night falls to 15°C. The weather during the sample collection was bright and sunny with 27°C, and the humidity was as low as 10%. The pH of the water body was 7.3, the concentration of total dissolved solids was 30 mg/L and the electrical conductivity was found to be 100 μS.

Molecular evaluation

The pairwise percentage similarity of strain 10A1_PS in case of 16S rRNA gene was found to be 94.67% with *S. hofmanni* PCC 7110 (AM709637). The similarity search was conducted with only validly published sequences from EzBioclould database. In case of *rbc*L gene, the percentage similarity search was conducted from NCBI database and was found to be 92% with *S. hofmanni* PCC 7110 (AB075921).

Phylogenetic analysis

The 16S rRNA gene phylogenetic tree was constructed using Mega 5.2.2 with 147 nucleotide sequences and it was found that the strain 10A1_PS clustered within the *Scytonema sensu stricto* clade at a completely different node with a strong probability/bootstrap support. Moreover, the phylogenetic distance between the strain 10A1_PS and other members of the clade was substantial. The sequences from Nostocaceae, Tolypothrichaceae, Godleyaceae and Hapalosiphonaceae families clustered separately with strong probability/bootstrap support indicating the robustness of the complete 16S rRNA gene tree. The genus *Scytonema* was found to be polyphyletic as many sequences of *Scytonema* were clustered into multiple groups clearly separating them from *Scytonema sensu stricto*. A clade containing

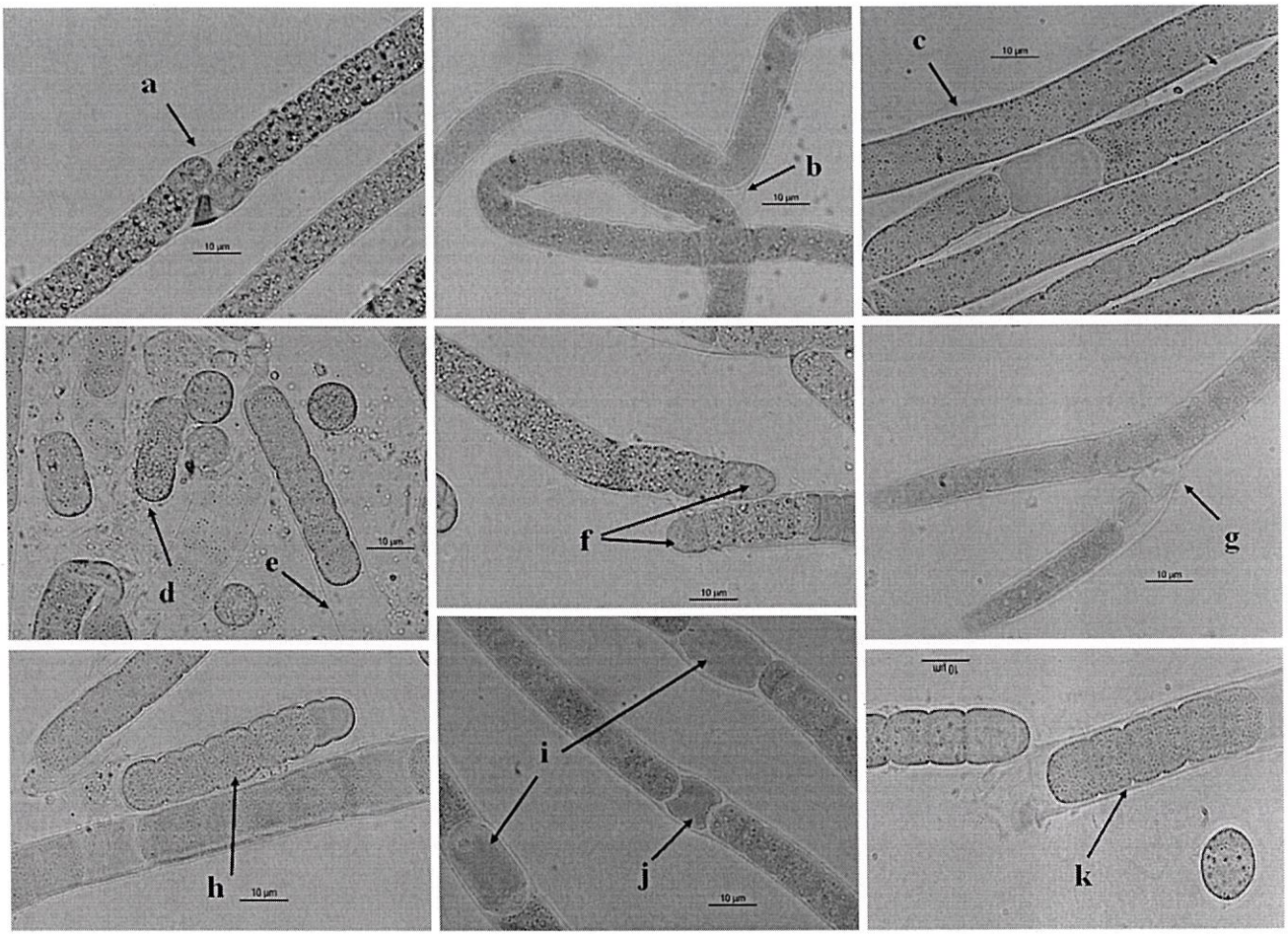

Figure 1. (a) Filament showing development of false branching. (b) Double false branching. (c) Old trichome having no constriction. (d) Hormogonia. (e) Newly developed hormogonia still inside the parent sheath. (f) Tapering ends of the terminal cells. (g) False branching. (h) Hormogonia with prominent constrictions and tapering terminal cells. (i) Heterocytes with convex and concave ends. (j) Separation disc. (k) Hormogonia inside the sheath having prominent constrictions.

the sequences of *Scytonema* cf *crispum* was clustered away from the *Scytonema sensu stricto*. Also, the phylogenetic positioning of *Scytonema hyalinum* cluster was distant from *Scytonema sensu stricto*. Further, the taxonomic positioning of *Scytonematopsis contorta*, *Iningainema* and *Chakia* indicated the polyphyletic nature of Scytonemataceae. Similarly, the family Rivulariaceae was observed to be polyphyletic as *Calothrix* and *Macrochaete* were clustered distantly from *Rivularia*, the type species of the family (Fig. 2). In case of the *rbc*L gene, strain 10A1_PS was clustered in the clade of *S. hofmanni*. The phylogenetic positioning and the distance of the strain 10A1_PS with the other members of the clade in *rbc*L gene tree supported the 16S rRNA gene phylogeny (Fig. S1, Supporting Information).

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

The secondary structures corresponding to D1-D1' and box-B helix region for the strain 10A1_PS and *Scytonema javanicum* (HF911525) were evaluated and are presented in Fig. 3. It was observed that the secondary structures of 10A1_PS were completely different from *S. javanicum* in both the cases (Fig. 3). Moreover, the p-distance value and percentage pairwise identity matrix showed the close relatedness of strain 10A1_PS with the members of the *S. hofmanni* clade (Figs S2 and S3, Supporting Information). These results were also in congruence with our phylogenetic and 16S-23S ITS analysis.

DISCUSSION

The initial aim of this study was to identify the freshwater strain 10A1_PS isolated from Pachmarhi, India by using the polyphasic approach. The in-depth morphological characterisation of the strain indicated it to be a member of the genus *Scytonema*. The morphological characters of the strain 10A1_PS were compared with the members of the *Scytonema sensu stricto* and the representative members of the other *Scytonema* clusters (Table 1). The strain 10A1_PS exhibited the typical morphological characters of *Scytonema*; however, the shape of heterocytes and constrictions between the cells seemed interesting. The heterocytes in most of the filaments exhibited distinct convex or concave shaped ends, which is relatively unusual in *Scytonema*. Moreover, prominent constrictions were observed in the younger filaments, which became less or even absent in the older filaments (Fig. 1). Both the above mentioned features can be considered as unique for the strain 10A1_PS, which distinguish it from other members of *Scytonema sensu stricto*. Moreover, due to the polyphyletic nature of *Scytonema* as reported in many studies (Vaccarino and Johansen 2011, 2012; Komárek *et al.* 2013; Komárková, Zapomělová and Komárek 2013; Hoad *et al.* 2017; McGregor and

Table 1. Comparison between *Scytonema pachmarhiense* with the other closely related species of *Scytonema*.

Strain	Filament	Vegetative cell	Heterocyte	Sheath	Habitat
Scytonema pachmarhiense	Isopolar filaments with solitary and frequent binary false branching	±Cylindrical; wider than length; terminal cell have curved ends L: 4.9–7.6 μm; W: 8.6–9.1 μm	Solitary; long; cylindrical; with concave and convex ends L:7.8–15.2 μm; W: 8.7–9.7 μm	Thin and narrow sheath; present throughout the trichome; colourless	Freshwater
Scytonema bilaspurense	Isopolar filaments with solitary false branching	±Cylindrical; wider than length; terminal cell have curved ends L: 5.5–11.6 μm; W: 5.6–9.7μm	Solitary; square to cylindrical; never have rounded ends L: 3.1–4.7 μm; W: 6.6–8.8 μm	Thin and narrow sheath; present throughout the trichome; colourless	Freshwater
Scytonema singhii	Isopolar filaments with solitary false branching	±Cylindrical; wider than length; terminal cell have curved ends L: 3.6–6.9 μm; W: 5.6–6.9 μm	Solitary; square to cylindrical; never have rounded ends L: 4.8–13.1 μm; W: 5.2–6.9 μm	Thin and narrow sheath: present throughout the trichome; colourless	Freshwater
Scytonema hofmanni	Isopolar filaments with geminate or solitary false branching	±Cylindrical; slightly widened at ends; Older cells ± isodiametric; W: 4.6–9.5 μm	Solitary or in pairs; cylindrical with rounded ends or ellipsoid W: 4.6–9.5 μm	Thin and narrow, ±slightly lamellated; colourless or yellow up to yellow-brown on wet wood.	Wet walls; soils; stones
Scytonema crispum	Short to long isopolar filaments; solitary or binary false branching	Barrel shaped to disc-like; slightly narrowed at the ends L: 2–10 μm; W: 10–17 μm	Solitary; flattened; different shapes; yellow-green coloured W: 10–17 μm	Thick; colourless-yellow; orange-brownish; slightly lamellated; closed at ends	Aquatic
Scytonema stuposum	Short; densely arranged; single or double false branching	Cylindrical to disc-like; wider; terminal cell rounded or conical L: 2–6 μm; W: 8–18 μm	Single or in pairs; cylindrical beige to yellow coloured L: 4–10 μm; W: 10–15 μm	Thin up to 4 μm wide; unstructured; colourless; closed at trichome ends	Wet soils; among mosses; on rocks
Scytonema hyalinum	Densely entangled; single or double false branched	Barrel to cylindrical shaped; terminal cell have conical ends L: 4.6–8 μm; W: ± 9 μm	Cylindrical with rounded ends; shorter than wide L: 5–18 μm; W: 6–12 μm	Firm and thin; slightly lamellated; colourless; yellow-brown when old less on wet soil	Stony substrates; L: Length; W: Width

Sendall 2017a,b), it was important to perform a thorough phylogenetic evaluation before providing final taxonomic identity to the strain 10A1_PS. The phylogenetic tree constructed using the 16S rRNA gene clearly indicated the strain to be a new member of the genus *Scytonema*. Also, it is proposed to change the name of *Scytonema bilaspurensis* (Singh *et al.* 2016) to *Scytonema bilaspurense* as it contradicts Article 23(5) of the International Code of Nomenclature for Algae, Fungi and Plants. Further, the 16S-23S ITS secondary structure analysis was performed as it has been shown to be effective in distinguishing the closely related taxa in many studies (Bohunicka *et al.* 2015; Genuário *et al.* 2015; Berrendero *et al.* 2016; Alvarenga *et al.* 2017). Surprisingly, the secondary structure of only one member of *Scytonema sensu stricto* was available for comparison. Hence, the secondary structure analysis was not conclusive enough in distinguishing the closely related taxa in case of *Scytonema*. The presence of limited number of secondary structures may be attributed to the fact that the use 16S-23S ITS as a tool for demarcating the closely related taxa has gained popularity only in the recent years. Although the ITS analysis in this study was not conclusive, but we believe that continuous efforts in this direction may eventually pave the way for better comparative studies using the ITS-folded structures for differentiating closely related species of the same genus.

The phylogenetic tree constructed using the 16S rRNA gene in this study not only proved to be helpful in providing taxonomic identity to our strain but many noteworthy observations were made, which must be discussed here. In congruence with Komárek *et al.* (2013), we also observed different clusters of *Scytonema* phylogenetically distant from *Scytonema sensu stricto*, but the phylogenetic positioning of these clusters in our study were different. A cluster containing the sequences represented as *Scytonema* cf *crispum* was found to be close to the families Tolypothrichaceae and Godleyaceae rather than the members of Scytonemataceae. Similarly, *Scytonema hyalinum* cluster was found to be relatively distant from *Scytonema sensu stricto*. Difference

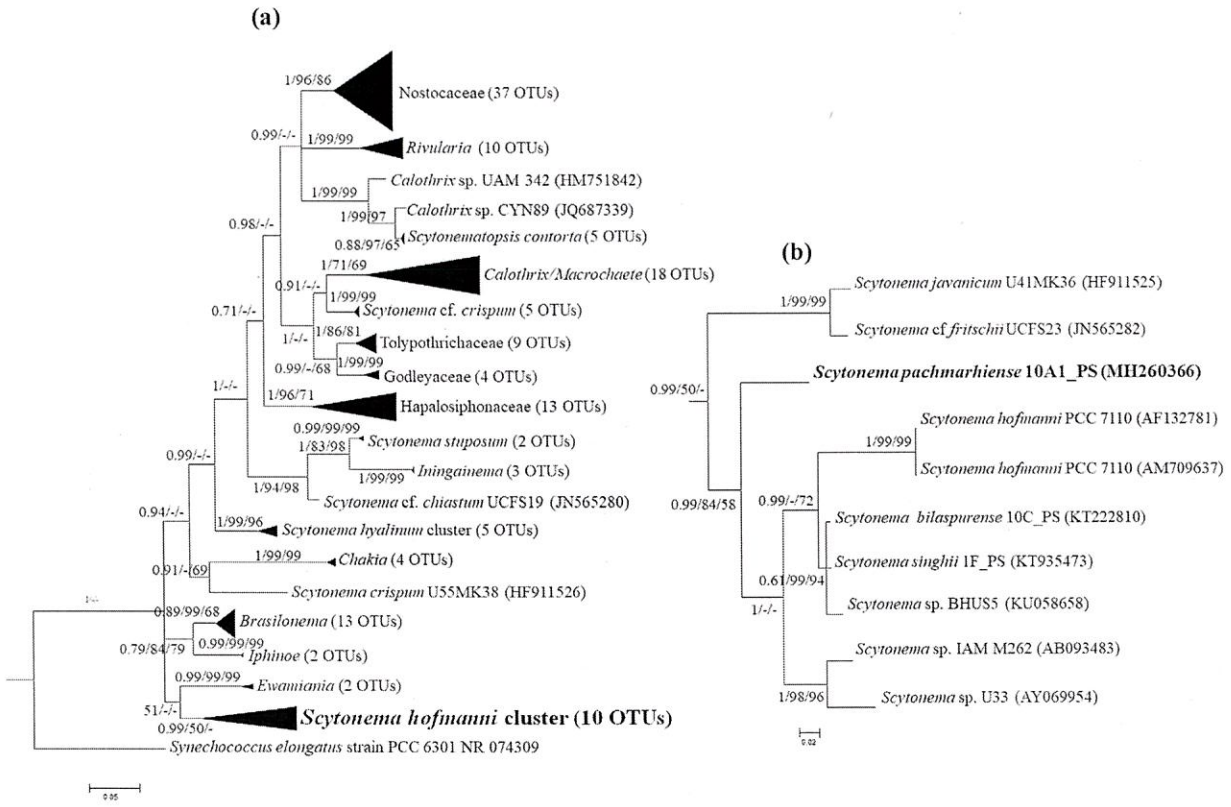

Figure 2. (a) Phylogenetic positioning of *Scytonema pachmarhiense* based on 16S rRNA gene inferred by BI tree with the probability scores/bootstrap values representing BI, NJ and MP, respectively. Bar, 0.05 changes per nucleotide position. For NJ and MP, all bootstrap values below 50 were deleted. (b) Complete *Scytonema* clade based on 16S rRNA gene with the probability scores/bootstrap values representing BI, NJ and MP, respectively. Bar, 0.02 changes per nucleotide position.

in the phylogenetic positioning of both these clusters may be because of the absence of the sequences corresponding to the families Tolypothrichaceae and Godleyaceae in Komárek *et al.* (2013). Further, the phylogenetic positioning of *Iningainema* is not in agreement with McGregor and Sendall (2017a) but may be attributed to the absence of *Scytonema stuposum* sequences in their study. Interestingly, the authors in their study pointed out the possibility of change in the higher taxonomic status of *Iningainema*. This study provides first instance of phylogenetic clustering of *Iningainema* away from the members of Scytonemataceae, and we feel that the higher taxonomic status must be revisited. The repeated observation of phylogenetic relatedness between *Brasilonema* a well-established false branching genus and *Iphinoe* a true branching genus (Bohunická *et al.* 2013; Rodarte *et al.* 2014; Bagchi, Dubey and Singh 2017; Singh *et al.* 2017) raises few questions over the higher taxonomic status of both these genera. *Iphinoe* was described by Lamprinou *et al.* (2011) as a member of Stigonematales; however, after dissolution of the order Stigoneamtales by Komárek *et al.* (2014), the taxonomic status of *Iphinoe* has been debated. Moreover, Komárek *et al.* included *Brasilonema* as a member of Scytonemataceae but did not provide a confirmed taxonomic status to *Iphinoe*.

As a concluding note, we believe that the classification of false branching heterocytous cyanobacteria needs to be revisited as the family Scytonemataceae seems to be highly polyphyletic. From our 16S rRNA gene phylogenetic evaluation, which to the best of our knowledge has included the maximum number of false-branching heterocytous genera, we observed that only the families Tolypothrichaceae and Godleyaceae form monophyletic clades, whereas Scytonemataceae and Rivulariaceae are highly polyphyletic. The polyphyletic nature of the family Rivulariaceae has also been reported in different studies where they have shown the distant phylogenetic clustering of *Calothrix* and *Macrochaete* from *Rivularia* (Berrendero *et al.* 2016; Shalygin *et al.* 2017). Similar observations were also made in our study. In the classical taxonomic scheme, the unique morphological traits were considered to be the basis of differentiating one species from other, and the classification was developed on the same principle. However, Shalygin *et al.* (2017) in their study showed that the tapering of the filaments, which was once considered to be the distinguishing character for the family Rivulariaceae, had actually evolved many times during the course of evolution. This same theory of convergent evolution may hold true for Scytonemataceae with the possibility of existence of more than one family, which could arise from Scytonemataceae. However, at the present moment, we believe that before major revisions at the family level are made, it is prudent to think and plan of constructing a phylogenetic scheme consisting of most of the genera of the entire heterocytous clade. This task with the combined efforts of majority of the currently active groups could eventually pave the way for a well sampled consensus tree, which in turn could serve as a guiding principle in the next few decades. At the present moment, as it was beyond the scope of this study, we are not sure about the proposal to erect new family/families from Scytonemataceae. An interesting observation is the study of Johansen *et al.* (2017) where using clone libraries, they reported multiple operons in *S. hyalinum*. While the study carefully indicates the divergence of the 16S rRNA gene in this particular taxa, this occurrence is yet to be established properly in the *Scytonema sensu stricto* clade to which our strain belongs to.

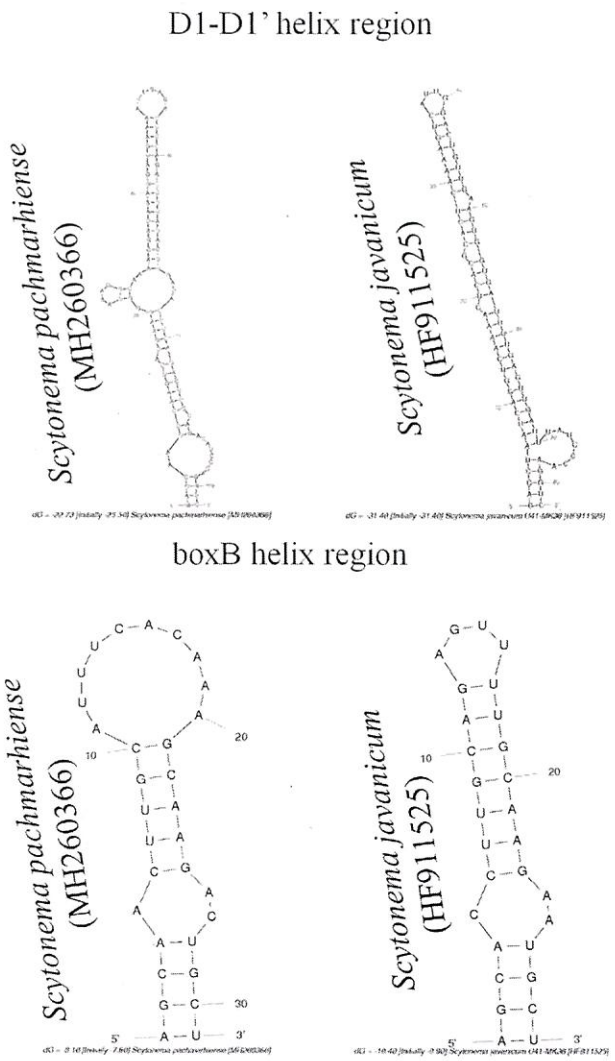

Figure 3. Presentation of the 16S-23S ITS folded secondary structures of the D1-D1' and box-B helix region of *Scytonema pachmarhiense* and *Scytonema javanicum* U41MK36.

Our phylogenetic assessment indicates clearly that *Scytonema* is indeed a polyphyletic genus whose taxonomy needs to be revised using the same approach as is nowadays being done in case of another highly diverse genus *Nostoc*. The creation of new generic entities of clusters outside the *Scytonema sensu stricto* clade is strongly recommended as this will eventually result into lesser complexities. But, extreme caution should be adopted while creating these entities as erroneous and superficial phylogenetic schemes could cause greater distortions in the final taxonomic conclusions.

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

Description: occur as mats in the nature with dark bluish green colour and mucilaginous texture; dark blue green filaments in the laboratory grown culture, which on long time incubation may start to appear yellowish in the microscope; prominently false branched with the frequency of false branching increasing with the age of culture; false branching appears both solitary and binary; branches are less in width as compared to the main filament; tapering towards the terminal cells seen prominently in all branches; sheath is prominent, thick and colourless but perfectly distinct in both texture and colour; sheath present throughout the length of trichome with constrictions at the cross walls; constrictions between cells prominent in younger filaments, while the constrictions become less evident in older filaments; hormogonia present and may serve as reproductive propagules; younger vegetative cells appear dark bluish green in colour, while the older ones become a bit yellowish; textured cytoplasm prominent; vegetative cells vary a lot in shape ranging from cylindrical to barrel shaped; width is usually more than the length; terminal cell have curved ends, which may or may not be tapering; curved ends of the terminal cells of a trichome both in the main filaments and the false branches; length varies from 4.9 to 7.6 μm, while the width ranges from 8.6 to 9.1 μm; heterocytes appear solitary and never in pairs; long and cylindrical with concave and convex ends; colour of the heterocytes is usually yellowish; position of the heterocytes is usually intercalary; length varies from 7.8 to 15.2 μm, while the width ranges from 8.7 to 9.7 μm. Akinetes not observed.

Diagnosis: The morphological differences with other closely related species of the genus *Scytonema* has been documented in Table 1. Phylogenetically, it clusters inside the *Scytonema* clade occupying a distinct position with well-supported bootstrap and consistent topology with other members in the cluster being *Scytonema javanicum* U41MK36 (HF911525), *Scytonema* cf *fritschii* UCFS23 (JN565282), *Scytonema hofmanni* PCC 7110 (AF132781), *Scytonema hofmanni* PCC 7110 (AM709637), *Scytonema bilaspurense* 10C_PS (KT222810), *Scytonema singhii* 1F_PS (KT935473), *Scytonema* sp. BHUS5 (KU058658), *Scytonema* sp. IAM M262 (AB093483) and *Scytonema* sp. U33 (AY069954).

Etymology: *Scytonema pachmarhiense* (pach.mar.hi.en'se. N.L. neut. adj. *pachmarhiense* pertaining to Pachmarhi Biosphere Reserve in Central India from where this species has been isolated).

Habitat: The sampling was done in the month of November, which is usually the beginning of winters in Pachmarhi with the day temperature being in the range of 25°C–30°C, and the temperature in the night temperature falling to 15°C. The weather during the sample collection was bright and sunny with 27°C, and the humidity was as low as 10%. The pH of the water body was 7.3, the concentration of total dissolved solids was 30 mg/L, and the electrical conductivity was found to be 100 μS.

Site location: Pachmarhi, Madhya Pradesh, 22° 28′ 41′ N, 78° 26′ 34′ E, Nov 2016. Holotype: Actively growing culture has been preserved in both live and cryopreserved form in National Centre for Microbial Resource (NCMR), formerly Microbial Culture Collection (MCC), National Centre for Cell Science (NCCS), Pune, India under the accession number MCC 3343 as *Scytonema* sp.

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSLE* online.

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