



RESEARCH COMMUNICATION

# A new species of *Desertifilum* (Desertifilales, Cyanobacteria) from a monument of Western Odisha, India

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## Abstract

A new species of subaerophytic cyanobacteria under the genus *Desertifilum* is described from a stone pillar within a temple in western Odisha, India. The blackish crust known as biofilms was isolated from a sub-aerial habitat of Balangir district, western Odisha was characterised by light and electron microscopy. The new taxon was to be closest to *Desertifilum tharense* (Oscillatoriales). The taxon, *Desertifilum adhikarii* is morphologically distinguished from so far documented species under the genus. The novelty of the species is also supported by 16S rRNA sequencing analysis and habitat.

## Keywords

16S rRNA sequencing; cyanobacteria; subaerophytic; systematics

## Introduction

Cyanobacteria are photosynthetic prokaryotes, with high adaptability, thus distributed in the widest range of ecological niches. In the terrestrial sub-aerophytic habitats, they form a biological consortium along with lichens, micro-fungi, micro-algae etc. They thrive in these habitats under high temperature, irradiance and desiccation and their spatial distribution is influenced by these parameters and other climatic factors. The microbial biofilms or sub-aerial biofilms (SAB) on the archaeologically important monuments and building facades are adhered to their substratum by extracellular polymeric substances (EPS), secreted by the cyanobacteria as sheath or envelope (1). Cyanobacterial colonization on the monuments can cause weathering of the substrates, reducing their aesthetic qualities. This microorganism-mediated biodeterioration has been a growing concern across the globe for ages (2). In this quest, numerous cyanobacterial taxa were newly described and taxonomically revised in the last few decades. It has been believed that taxonomic enumerations of cyanobacteria must be polyphasic, considering the phenotypic, genotypic and ecological characterization (3). Specifically, the consideration of phylogenetic analyses solely for the identification of the taxa, like the authentication procedures in bacteriology, led to ambiguous conclusions in the case of cyanobacteria. It was observed in the recent past that many coccal, heterocystous or non-heterocystous filamentous cyanobacteria share high similarity in 16S rRNA gene sequence, though morphologically and ecologically distinct from each other. This can be exemplified by the recent descriptions of many non-heterocystous filamentous cyanobacteria (4-7).

The genus *Desertifilum* was erected describing a new thermophilic cyanobacterial species (*D. tharense*) growing as biological crusts in the Thar Desert of Rajasthan, India (8). Apart from the phylogenetic uniqueness, the species had distinct morphological and ecological characteristics to separate it from other

*Oscillatoria* members. The genus currently includes four species, including the type species. *Desertifilum fontinale* Dadheech, Mahmoud, Kotut & Krienitz was isolated and described from a warm spring near Lake Bogoria, Kenya (9). While describing this species, the characterization of the genus has been amended. *Desertifilum salkalinema* Cai & Li was described from an alkaline pool in Zhejiang Province, China (10). The most recent addition to the genus was *Desertifilum dzianense* Cellamare, Duval, Touibi, Djediat & Bernard, isolated from the stromatolites, distributed on the edge of crater lake Dziani Dzaha of Mayotte, Indian Ocean (11). Excluding these, several strains of *Desertifilum* were also recorded from a hot spring in Greece (12) and freshwater lake Shar-Nuur in Bayan-Ölgii Aymag of Mongolia (13). All these species and isolated strains are very closely related to each other both morphologically and phylogenetically. Phenotypic differentiation was limited up to filament width, cell length, apical cell shape and presence or absence of gas vacuoles. The 16S rRNA gene sequence similarity among them was  $\geq 98\%$  (11). However, significant uniqueness was observed in their habitat and ecological preferences.

## Materials and Methods

### Study site and sample collection

The present species was sampled while surveying sub-aerophytic cyanobacteria colonizing on different archaeologically important monuments and building facades of western Odisha districts, in February 2021. It was found growing as a thin blackish crust on a stone sculpture (Fig. 1A) in the premises of Surda Shiv temple, located in Surda village of Dasapur, Balangir (83.51299° N; 20.70019° E). The cyanobacterial crust was carefully sampled following non-destructive methods using adhesive tape stripes (14) and brought to the laboratory.

### Culture and light microscopy

The cyanobacterium was isolated to culture in BG-11 medium with nitrogen (15) and maintained at  $25 \pm 1^\circ\text{C}$  under continuous light with fluorescent tubes at an intensity of  $7.5\text{ Wm}^{-2}$ . The phenotypic features were observed microscopically at different growth phases of the organism, using a Nikon microscope Ni-11 fitted with Nikon Digital Camera DS-Ri1-U3 and operated by Nikon Imaging Software NIS-D + EDF. The type specimen of the taxon was deposited at the Central National Herbarium (CAL), Howrah.

### Scanning electron microscopy

The dried cyanobacterial sample was analyzed for surface morphology and size, which was further accessed using Fe-SEM (JSM-IT800 Jeole, Japan). The samples were first dried properly then after the dried sample was spread on coverslip without containing any moisture. After completion of drying the coverslip was fixed by using a carbon tap (double adhesive tape) on a Brass Scab followed by coating with gold (70%) and palladium (30 %) sputter at 20kb (Hitachi EMITECH-SC 7620) to make them conductive. In the final step, the sample in the scab was then placed on a sample holder with high vacuum pressure for acquisition of images.

### Molecular characterization and phylogenetic analysis

The genomic DNA was isolated following a standardized DNA isolation protocol for bacteria. The fragment of the 16s rRNA

gene was amplified by PCR. The PCR amplicon was purified by column purification to remove contaminants. DNA sequencing reaction of PCR amplicon was carried out with 357F and 1391R primers using BDT V3.1 Cycle Sequencing Kit on ABI 3500x1 Genetic Analyzer. The genome sequence was deposited at NCBI GenBank with accession no. ON358232.1 (initially mislabelled as *Desertifilum dzianense*). The sequence was aligned with ten 16S rDNA sequences with the highest homology score (identity  $\geq 96\%$ , coverage  $\geq 92\%$ ) which were identified using BLASTn. Alignment was made with MUSCLE in MEGA 11(16). The phylogenetic tree was also constructed in MEGA 11 using the maximum likelihood method based on the Tamura-Nei model with 1000 bootstrap repetitions.

## Results and Discussion

### Taxonomic treatment

***Desertifilum adhiarii* Sudipta K. Das, S. Mahanandia & L. Singh sp. nov.** (Fig. 1 B-E)

### Type

India: Dasapur, Odisha, 83.51299° N, 20.70019° E, 03.02.2021, 067, *SmrutiMahanandia* (Holotype CALI, ALG/058), culture is maintained at the cyanobacterial culture collection at the Department of Botany, College of Basic Science and Humanities, Odisha University of Agriculture and Technology, Bhubaneswar, with reference Voucher /Strain no.BOT/CBSH/67.

### Diagnosis

The newly described species is morphologically compared with other *Desertifilum* species (Table 1). Phenotypically, *D. adhiarii* resembles more of the type species of the genus, *D. tharense*. However, our species has wider filaments with shorter cells and the elongated apical cell in *D. adhiarii*, separates it from all other species.

Analysis of 16S rRNA sequence data revealed that *D. adhiarii* shares 97.65 %, 97.49 %, 97.19 % and 96.17 % sequence similarities with *D. salkalinema*, *D. dzianense*, *D. tharense* and *D. fontinale*, respectively. The newly described species *D. adhiarii* is placed in a common cluster with most of the *Desertifilum* species and strains yet positioned as a separate lineage.

### Morphological description

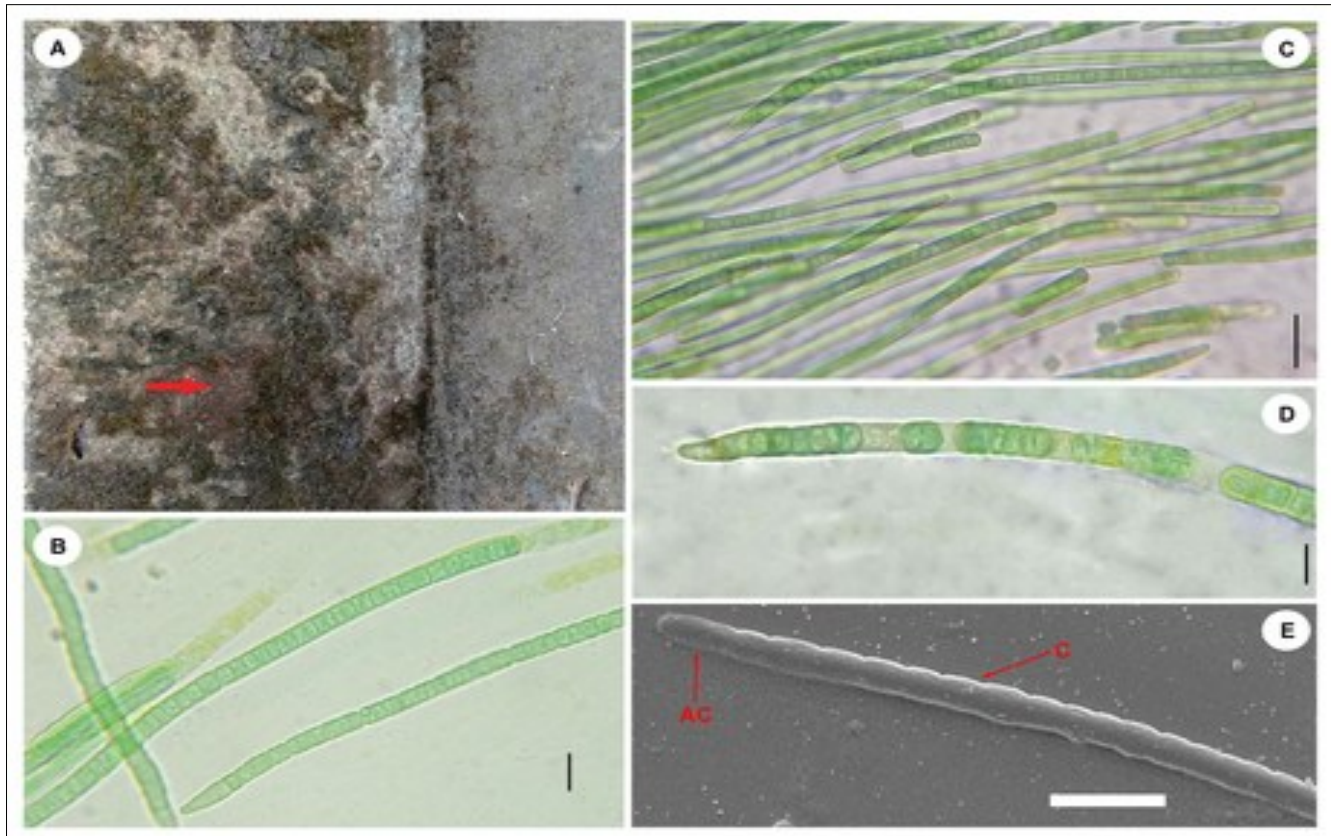
Thallus bright blue-green, with solitary or loosely entangled filaments. Filaments straight,  $3.32 \pm 0.82\text{ }\mu\text{m}$  wide. Constrictions at the cross walls are prominent in most parts of the filaments and almost unconstricted towards the apices. Filaments are motile with both gliding and oscillation movements. The sheath is thin, colourless and agglutinated. The apical portion of the filament is attenuated. The apical cell is elongated, with rounded apices, sometimes slightly bent. No extrusions from the apical cells are observed. Cells are cylindrical or barrel-shaped,  $2.94 \pm 1.88\text{ }\mu\text{m}$  long. Gas vacuoles are present and Necridial cells are also observed. Reproduction is by hormogonia.

### Habitat

Occurred as a blackish crust on a stone sculpture in the premises of Surda Shiv temple, located in Surda village of Dasapur, Balangir.

**Table 1.** Phenotypic comparison of *Desertifilum adhikarii* with other *Desertifilum* species

Characters	<i>D. tharense</i>	<i>D. fontinale</i>	<i>Desrtifilum</i> sp. Strain no. IPPAS B-1220	<i>D. dzianense</i>	<i>D. salkalinema</i>	<i>D. adhikarii</i>
<b>Locality and habitat</b>	Desert biological crust, Thar desert, Rajasthan, India	Warm spring, Lake Bogoria National Reserve, Kenya	Strain isolated from Lake Shar-Nuur, Bayan-Ölgii Aymag, Mongolia	Stromatolites, saline-alkaline crater lake DzianiDzaha, Mayotte	Alkaline cultivated pool, Zhejiang Province, China	Stone sculpture, Balangir, Odisha, India
<b>Filament colour</b>	Pale to bright blue green	Blue green	Green or bright blue-green	Pale blue green	Pale to bright blue-green	Bright blue green
<b>Filament width (µm)</b>	2.88 ± 0.78	5.5 ± 1.5	2.8 ± 0.3	2.4–3.4	2.08 ± 0.8	3.32 ± 0.82
<b>Cell length (µm)</b>	4.29 ± 1.53	Varying	3.8 ± 1	Varying	5.14 ± 0.51	2.94 ± 1.88
<b>Constriction at cross walls</b>	Unconstricted or slightly constricted	Unconstricted or slightly constricted	Unconstricted or slightly constricted	Unconstricted or slightly constricted	Unconstricted or slightly constricted	Slightly constricted
<b>Motility</b>	Gliding and oscillation	Gliding and oscillation	Motile	Motile	Motile	Gliding and oscillation
<b>Sheath</b>	Thin, colourless, attached to trichome	Thin, colourless	Thin, colourless, observed only at the end of some trichomes	Thin, colourless	Thin, colourless, attached to trichome	Thin, colourless, attached to trichome
<b>Apical attenuation</b>	Present	Present	Present	Present	Present or absent	Present
<b>Cell shape</b>	Cylindrical, isodiametric or elongated	Isodiametric	Barrel, cylindrical, isodiametric or elongated	Longer than wide (rarely isodiametric)	Cylindrical	Cylindrical, barrel shaped
<b>Apical cell shape</b>	Conical with rounded apices	Conical shaped with rounded apices with extrusions	Circular or conical, sometimes with extrusions	Conical-rounded, slightly hooked or bent, sometimes with extrusions	Slightly elongated	Elongated with rounded apices, no extrusion
<b>Gas vacuoles</b>	Present	Absent	Present	Absent	Absent	Present

**Fig. 1.** Illustration of Habitat and microscopy of *Desertifilum adhikarii*. A: Stone surface of slider wall in SurdaShiv Temple in Odisha showing the sampling point (pointed with red arrow). B: Filaments of *Desertifilum adhikarii*. C: Filament showing elongated apical cell. D: Matured filament showing formation of hormogonia. E: Scanning electron microscopy image of the filament showing the distinct cellular constriction (C) and apical cell (AC). (Scale bar: B-E = 10 µm).

### ***Etymology***

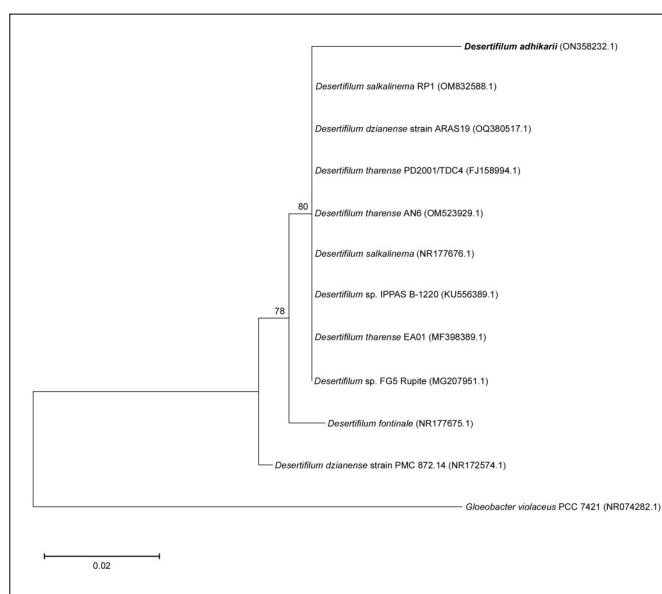
The specific epithet is named in honour of Prof. Siba Prasad Adhikary, for his contribution towards the exploration of sub-aerial cyanobacteria from Odisha and other parts of India.

The Kenyan freshwater species *D. fontinale* has the widest filaments among all the *Desertifilum* species. Along with

this, the isodiametric cells in it also differentiate it from *D. adhikarii*. Similarly, the new species is also distinguishable in its appearance from *D. dzianense*, *D. salkalinema* and *Desertifilum* strain IPPAS B-1220. Ecologically, whereas most of the species of the genus are aquatic in occurrence, *D. adhikarii* is sub-aerophytic like *D. tharense* which was isolated from desert crusts. Phylogenetically, the existing species of *Desertifilum* share



high 16S rRNA sequence similarity among themselves. *D. salkalinema*, *D. fontinale* and *D. dzianense* showed 100 %, 99 % and  $\geq 98$  % of sequence similarity, respectively with the type species *D. tharense*. *Desertifilum* strain IPPAS B-1220 displayed 99.9% pairwise sequence similarity with several strains (11). Our BLAST search analysis also revealed that *D. adihikarii* shares 97.65 %, 97.49 %, 97.19 % and 96.17 % sequence similarities with *D. salkalinema*, *D. dzianense*, *D. tharense* and *D. fontinale*, respectively. The Maximum Likelihood phylogenetic tree, constructed for *D. adihikarii* using nine other *Desertifilum* species and strains with *Gloeobacter violaceus* PCC 7421 as an out group (Fig. 2), exhibited that *D. adihikarii* belongs to a subclade including most of the species and strains supported by 80 % bootstrap value. Both *D. fontinale* and *D. dzianense* strain PMC 872.14 form their distinct clades. *D. adihikarii*, though placed in a common cluster with most of the *Desertifilum* species and strains, is positioned as a separate lineage.



**Fig. 2.** Maximum likelihood phylogenetic tree of 16S rRNA sequences of *Desertifilum adihikarii* (in bold font) with other species and strains of *Desertifilum* with high homology and *Gloeobacter violaceus* PCC 7421 as an out group. Bootstrap values ( $> 50\%$ ) are presented above the branches. 101x90mm (300 x 300 DPI)

## Conclusion

In conclusion, the morphological characterization and 16S rRNA-based phylogeny evidenced the consideration of our species under the genus *Desertifilum* and exhibited adequate uniqueness for its segregation from all other existing species. Consequently, we describe the new cyanobacterial species *Desertifilum adihikarii* sp. nov.

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## Authors' contributions

SM contributed to sample collection, workouts; SKD Prepared the Manuscript, corrected and edited the original manuscript, LK Supervised and edited the original manuscript. All authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interest to declare.

**Ethical issues:** None

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