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Cyphellostereum indicum (Hygrophoraceae), a new species of basidiolichen from India

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Abstract

Cyphellostereum indicum, an interesting basidiolichen is described as a new species. The species was collected from Arunachal Pradesh, an Indian state located within the Eastern Himalayas. The species is characterized by a terricolous habitat, the cyanobacteria *Rhizonema* as the photobiont that is loosely wrapped by fungus hyphae, white to cream-coloured, flabellate, solitary hymenophores, and thin-walled, smooth, simple, irregular to pip-shaped basidiospores 3.18–4.9 × 2.06–2.9 µm. The phylogenetic analysis clearly shows its position in a separate branch within the genus *Cyphellostereum* making it a species distinct from any previously reported species. *Cyphellostereum indicum* is the third basidiolichen reported from India.

Keywords: Asia, biodiversity hotspot, lichenized fungi, Lichenomphalinoideae, mycobiota

Introduction

Lichens are prominent symbiotic organisms in terrestrial ecosystems and dominate approximately 8 % of the Earth's land surface (Nash 2008). At present approximately 20,000 species of lichens are known to occur and are represented mostly by the Ascomycota group whereas the lichenized Basidiomycota amount to only 0.9 % of the total (Lücking *et al.* 2017). Recent molecular phylogenetic analysis has revealed the existence of high diversity within the basidiolichens that is otherwise masked by traditional methods of identification. For example, Dal-Forno *et al.* (2016) discovered that the widespread neotropical *Acantholichen pannarioides* P.M. Jørg. (1998: 444) included at least six distinct species. Lücking *et al.* (2017a) recognized 189 taxa within the genus *Cora* (1825: 300) and described 70 new species. The genus *Cora* was previously considered to contain only a single species. The *Dictyonema* C. Agardh ex Kunth (1822: 1) clade has been shown to contain many more species (Parmasto 1978). Currently *Cora* is the largest genus (Dal-Forno *et al*. 2022, Lücking *et al.* 2017) while subtribe Dictyonematinae is the largest group (Dal-Forno *et al*. 2022).

In India, *Dictyonema irrigatum* (Berk & M.A. Curtis) R. Lücking (2013: 30) and *Multiclavula vernalis* (Schwein.) R.H. Petersen (1967: 216) are the only two basidiolichens reported so far (Jagadeesh Ram & Singh 2014, Mishra *et al.* 2019). In our recent lichen exploration at East Kameng district of Arunachal Pradesh we encountered large patches of a basidiolichen and after detailed study it turns out be a new species of *Cyphellostereum* D.A. Reid (1965: 336).

The genus *Cyphellostereum* was initially established based on the stipitate, cyphelloid or flabellate fruiting body (Reid 1965). However, its association with a photobiont was observed later. Oberwinkler (1970) while studying *Cyphellostereum pusiolum* (Berk. & M.A. Curtis) D.A. Reid (1965: 342) acknowledged that the mycelium was surrounded by a bunch of trichomes of filamentous cyanobacteria. The genus *Cyphellostereum* has the *Rhizonema* Lücking and Barrie (2014: 265) as the cyanobacterial photobiont, where the cells are mostly square-shaped, 10–12 µm broad (Dal-Forno *et al.* 2013, 2017, Lawrey *et al.* 2009, Lücking *et al.* 2009, 2014, Oberwinkler 1970, 1984, Parmasto 1978). Phylogenetically, *Cyphellostereum* is nested within a well-supported clade previously referred to as the '*Dictyonema s.l.* clade' but now recognized as the subtribe Dictyonemateae (Dal Forno *et al.* 2020), which is entirely lichenized with *Rhizonema* and made up of five genera, *Acantholichen* P.M. Jørg. (1998: 444), *Cora, Corella* Vainio (1890: 242), *Cyphellostereum* and *Dictyonema s. str.* (Lawrey *et al.* 2009, Dal-Forno *et al.* 2013, Lücking & Timdal 2016, Dal Forno

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et al. 2020). The genus *Cyphellostereum* is the most basal of these genera and the least complex morphologically (Dal-Forno 2013). According to Indexfungorum.org 13 out of 15 species epithets recorded for *Cyphellostereum* are valid (Table 1). Most of the species of *Cyphellostereum* are distributed in North and South America, while *C. muscicola* (Pat.) D.A. Reid (1965: 345)*, C. phyllogenum* (Müll. Arg.) Lücking, Dal-Forno & Lawrey (2013: 24) and *C. ushimanum* H. Masumoto & Y. Degawa (2022: 177) are known to occur in Asia.

Materials and methods

Morphological observation:—The lichen samples were collected from the East Kameng district of Arunachal Pradesh in Eastern Himalayas which is considered as a biodiversity hotspot. The colour photographs of the specimen were taken in the field using a Nikon DSLR camera. The morphological and anatomical characters of the dry, rehydrated lichenized thallus and hymenophore were examined using stereo zoom Leica S8APO and a compound Leica DM2500 microscope equipped with digital camera and image processing software. The squash preparations of rehydrated thallus containing the photobiont were mounted in distilled water to study the mycobiont association with the photobiont cells. The free-hand sections of hymenophore were mounted in 3 % KOH solution and stained with Congo Red to observe the basidia, basidioles and spores. The measurements of 25 spores stained with Congo Red were recorded and range of length and width are presented. To identify the presence of any secondary lichen substances, thin layer chromatography (TLC) in solvent system C was performed following Orange *et al.* (2001). The type specimen has been deposited in the lichen herbarium of CSIR-National Botanical Research Institute (LWG, Lucknow, India).

DNA extraction, Polymerase Chain Reaction and sequencing:—Genomic DNA was isolated directly from a portion of the thallus and basidiome by the CTAB method as described by Doyle & Doyle (1987). The PCR amplification of Internal Transcribed Spacer (ITS) region 1 and 2, and the 5.8S rDNA, was achieved using pair ITS1 forward (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 reverse primers (5′-TCCTCCGCTTATTGATATGC-3′) (White *et al*. 1990). The DNA fragments were amplified on a thermal cycler. The initial denaturation was done for 2 minutes at 95 °C and final denaturation at 95 °C for 30 seconds; annealing was at 50 °C for 30 seconds and elongation was at 72 °C for 1 minute, for 30 cycles. The final elongation was done at 72 °C for 10 minutes then held at 4 °C. The amplified PCR yields were visualized with the help of agarose gel using ethidium bromide through a gel documentation system. The PCR products were purified and subjected to Sanger Sequencing using primers identical with the amplification for ITS regions. The newly generated sequence obtained were saved as AB1 format which was viewed by using BioEdit v. 7.2.5 (Hall 1999) software to check the quality of the produced sequence data by editing the chromatogram. Finally, the sequence was deposited in GenBank nucleotide database with the accession number OQ544589 (www.ncbi.nlm.nih.gov).

Phylogenetic analysis:—The Basic Local Alignment Search Tool (BLAST) was performed with the newly generated ITS sequence to find taxa with similar sequences in the database. The maximum percent identity and query coverage of sequence with related taxa were examined and downloaded from GenBank nucleotide database. Further, the ITS sequences of other *Cyphellostereum* species were also retrieved from GenBank for phylogenetic analysis. In total a dataset of 30 nrITS sequences were used in the analyses, of which 29 were downloaded from GenBank and one sequence generated in the present study. Multiple sequence alignment was performed with the help of online software MAFFT on XSEDE (7.505) on the CIPRES Science Gateway portal (Miller *et al.* 2015) and then trimmed manually in AliView (Larsson 2014). The only values ≥ 50 % are considered. *Eonema pyriforme* (M.P. Christ.) Redhead, Lücking & Lawrey (2009: 1169) was selected as outgroup taxa based on Lawrey *et al.* (2009), Dal-Forno *et al.* (2019) and Masumoto & Degawa (2022). TIM2ef+I+G was determined as the best-fit model for the data set using jMODELTEST 2.1.10 v20160303 having the lowest Bayesian information criterion (BIC) value. The Maximum Likelihood (ML) analysis was performed with RAxML-HPC2 on XSEDE v8.2.12 (Stamatakis 2006) software using 1000 bootstrap replicates on the CIPRES Science Gateway. FigTree v.1.4.4 was used to visualize the generated tree.

Results

Taxonomy

Cyphellostereum indicum S. Nayaka and A. Debnath *sp. nov.* Figs. 1, 2.

MycoBank MB 849435
GenBank accession number: OQ544589

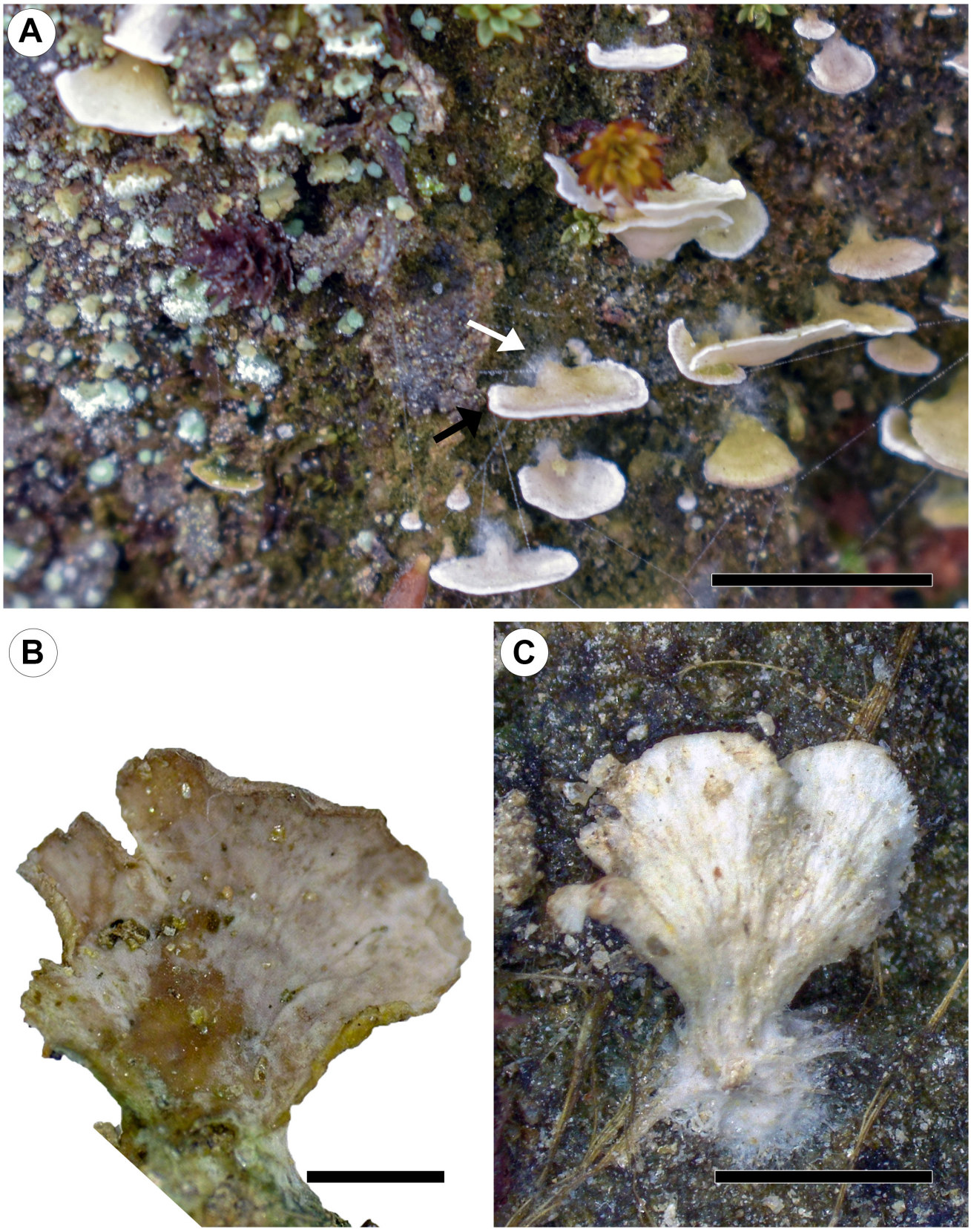

FIGURE 1. Morphological features of the hymenophore of *Cyphellostereum indicum* (Holotype). **A**. Flabellate basidiome (black arrow) radiating out of the lichenized thallus (white arrow), **B**. Ventral view of the mature basidiome, **C**. Dorsal view of the mature basidiome. Scale bars **A** = 1mm; **B** = 0.5mm; **C** = 1mm.

TABLE 1. Comparison of different species of *Cyphellostereum* with *C. indicum* and sequence data. The new species is in bold.

	Species	Hymenophore	Basidia	Basidiospores	Cyanobacterial filaments	Hyphal sheath	Intracellular haustoria	Clamp connections	Type locality	Sequence data	References
1	*C. bicolor* Lücking & Timdal	Resupinate, irregular	—	—	7–9 × 5–6 µm	Irregular, leaving interspaces	Absent	Absent	Mauritius	—	Lücking & Timdal (2016)
2	*C. brasiliense* Ryvarden	Pileate, round to slightly spatulate	Clavate; 18–25 × 5–6 µm	Oblong ellipsoid: 7–8 × 3.5–4.5 µm	—	—	—	Absent	Brazil	—	Ryvarden (2010)
3	*C. galapagoense* (Yánez, Dal-Forno & Bungartz) Dal-Forno, Bungartz & Lücking	—	—	—	7–10 × 5–10 µm	Jigsaw puzzle-shaped completely closed	Present	Absent	Ecuador	ITS	Dal-Forno *et al.* (2017)
4	*C. georgianum* Dal-Forno, McMullin & Lücking	Resupinate to corticioid	—	—	7–12.5 × 5–10 µm	Irregular, leaving interspaces	Absent	Present	USA	ITS	Dal-Forno *et al.* (2019)
5	*C. imperfectum* Lücking, Barillas & Dal-Forno	—	—	—	7–10 × 3–6 µm	Irregular, leaving interspaces	—	Absent	Guatemala	ITS, nuLSU, *RPB2*	Yánez *et al.* (2012)
6	***C. indicum* S. Nayaka and A. Debnath**	**Stipulate, flabelliform**	**Clavate, 16.7–20.11 × 2.5–5.5 µm**	**Irregular to pip shaped, smooth, simple, 3.18–4.9 × 2.06–2.9 µm**	**6–8 × 5.1–6.9 µm**	**Loosely wrapping photobiont filament**	**Superficial**	**Absent**	**India**	**ITS**	**This article**
7	*C. jamesianum* Dal-Forno & Kaminsky	Resupinate	—	—	11–13 × 4–6 µm	Irregular, leaving interspaces	—	Present	USA	ITS	Dal-Forno *et al.* (2019)
8	*C. mucuyense* V. Marcano	Resupinate to corticioid	Basidia scattered 25–40 × 5–7 µm	Subglobose, non-septate, hyaline, 5-7 µm	8–10 × 4–9 µm	Regular in lateral outline	Not penetrating	Present	Venezuela	—	Marcano (2022)
9	*C. muscicola* (Pat.) D.A. Reid	Spathulate	20 × 4 µm	—	—	—	—	Absent	Java	—	Reid (1965)
10	*C. nitidum* (Lücking) Lücking	—	—	—	—	strongly appressed photobiont, loose hyphal sheath	—	—	Galapagos	*RPB2*	Yánez *et al.* (2012)
11	*C. phyllogenum* (Müll. Arg.) Lücking, Dal-Forno & Lawrey	—	—	—	6–12 × 7µm	With a regular hyphal sheath	—	Absent	Borneo	ITS, nuLSU	Parmasto (1978)
12	*C. rivulorum* (Berk. & M.A. Curtis) D.A. Reid	Shortly stipitate	Clavate; 30 × 5 µm	2.5–3,5 × 2–2.5 µm	—	—	—	Absent	Cuba	—	Reid (1965)
13	*C. unoquinoum* Dal-Forno, Bungartz & Lücking	—	—	—	7.5–11 × 5–7 µm	Irregular, leaving interspace	Absent	Absent	Ecuador	ITS	Dal-Forno *et al.* (2017)
14	*C. ushimanum* H. Masumoto & Y. Degawa	Resupinate	Clavate to short-cylindrical; 9.4–13.0 × 5.4– 6.5 µm	Ellipsoid, 5.3–6.3 × 3.4–4.0 µm	5.5–8.6 x 3.3–7.3 µm	Irregular, leaving interspaces	Present	Absent	Japan	ITS, nuLSU	Masumoto & Degawa (2022)

Diagnosis:—Differing from other species of *Cyphellostereum* with distinct stipulate, flattened, flabelliform hymenophores, irregular to pip-shaped basidiospores and loose mycobiont hyphae encircling the cyanobacterial filaments.

Type:—INDIA. Arunachal Pradesh: East Kameng district, Bameng, near Kasturba Gandhi Balika Vidyalaya School, (N 27°32’38.95”, E 92°57’19.00”), on soil, elev. 1301 m, 09-11-2022, *S. Nayaka and A. Debnath 22-047553* (LWG-holotype).

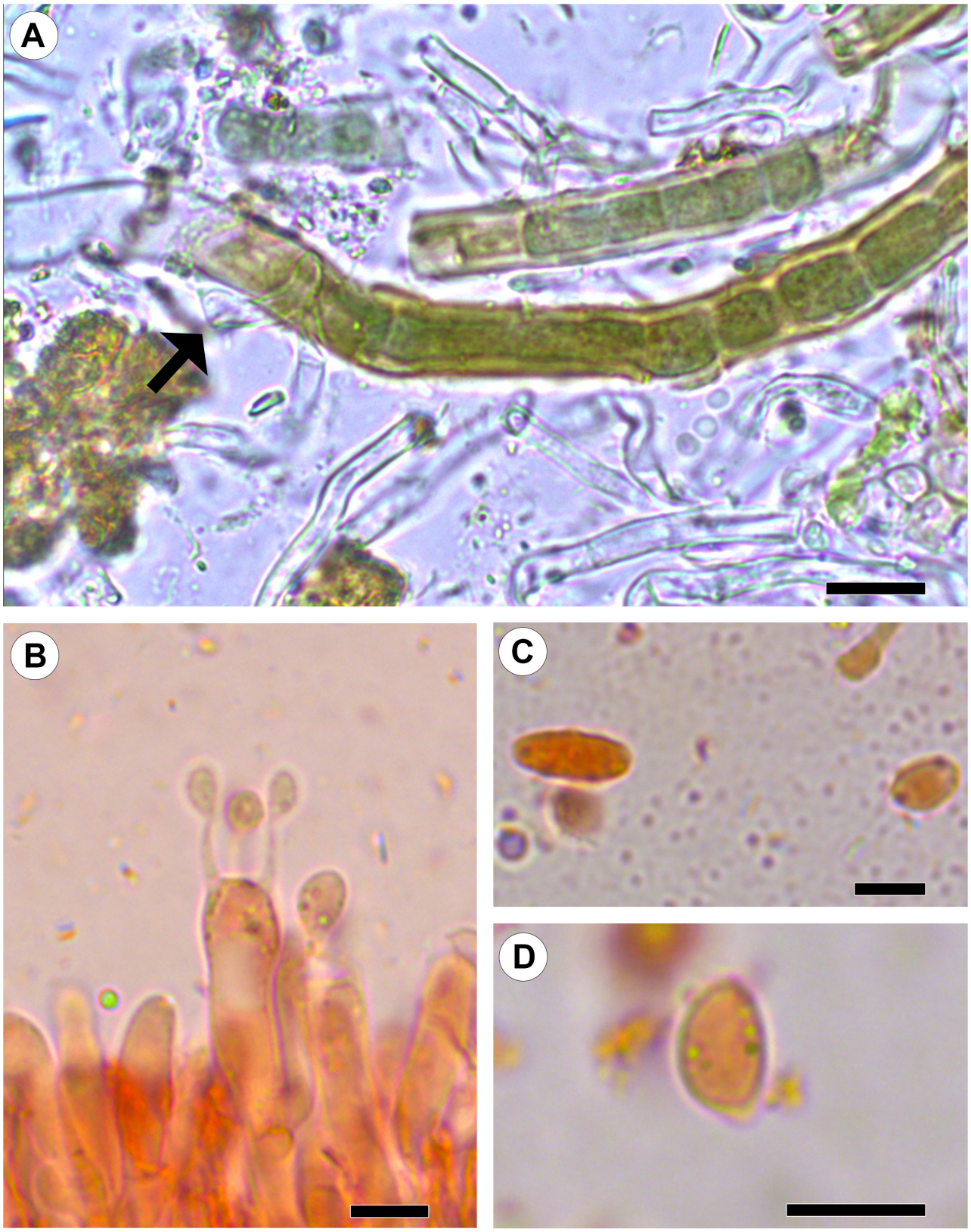

FIGURE 2. Microscopical features of the of *Cyphellostereum indicum* (Holotype). **A**. Mycobiont hyphae surrounding the photobiont *Rhizonema* (black arrow), **B**. Basidium with sterigmata and basidiospore. **C–D**. Basidiospores. Scale bars: **A–B**= 5µm; **C–D** = 3µm.

Description:—*Thallus* terricolous, on wet soil, crustose, olive green to brownish, filamentous, interwoven with algal mat, undifferentiated, homomerous; hyphae dense at the base of hymenophores, cottony, loose, irregular, simple, hyaline, thin, 2.2–2.9 µm; *photobiont* cyanobacteria, *Rhizonema*, filamentous, unbranched, cells thin, in chains, square to cylindrical, 6–8 µm long and 5.1–6.9 µm wide, heterocysts intercalary, pale yellow, 7–8 µm long, 5–6 µm wide; fungal hyphae of mycobiont loosely wrapped around photobiont filaments, encircling the filaments at several points, lacking haustoria, *clamp connections* lacking or not seen.

Hymenophores (basidiomata) stipulate, arising from the primary thallus, solitary, stipe narrow, expanding upwards, thin, dorsiventrally flattened, flabelliform, smooth, white to cream-coloured, 1.87–3.77 × 1–3.66 mm; *hyphae* thick, compactly intertwined; *holobasidia* scattered 16.7–20.11 × 2.5–5.5 µm, lacking a basal clamp; *basidia* clavate, bearing four prominent sterigmata; sterigmata thin, slender, 3.5–4.2 × 0.8–1.1 µm; *basidiospore*s with hilar appendage, simple, asymmetric, thin-walled, smooth, irregular to pip-shaped (tear drop), 3.18–4.9 × 2.06–2.9 µm, inamyloid; basidioles numerous, palisade, clavate 10–15 × 1.5–3.8 µm, lacking hymenial cystidial elements.

Chemistry:—Thallus and hymenophore K-, C-, KC-, P-, UV-; no substances detected by TLC.

Distribution and ecology:—*Cyphellostereum indicum* is currently recorded only in East Kameng district of Arunachal Pradesh. It was found growing on a wet, vertical surface of a mound of soil on a roadside at an elevation of 1300 m.

Etymology:—The species epithet refers to its type locality country, 'India'.

Phylogenetic analysis:—The phylogenetic analyses confirmed that *Cyphellostereum indicum* belongs to the *Cyphellostereum* clade and is unambiguously distinct from other taxa. The sequence of *Cyphellostereum indicum* formed a well-supported (BS >75 %) separate clade outside a group comprising *C. ushimanum* H. Masumoto & Y. Degawa (2022: 177), *C. unoquinoum* Dal-Forno, Bungartz & Lücking (2017: 59), and *C. phyllogenum* (Müll. Arg.) Lücking, Dal-Forno & Lawrey (2013: 24) signifying its position as an autonomous, independent and well-supported monophyletic lineage (Fig. 3).

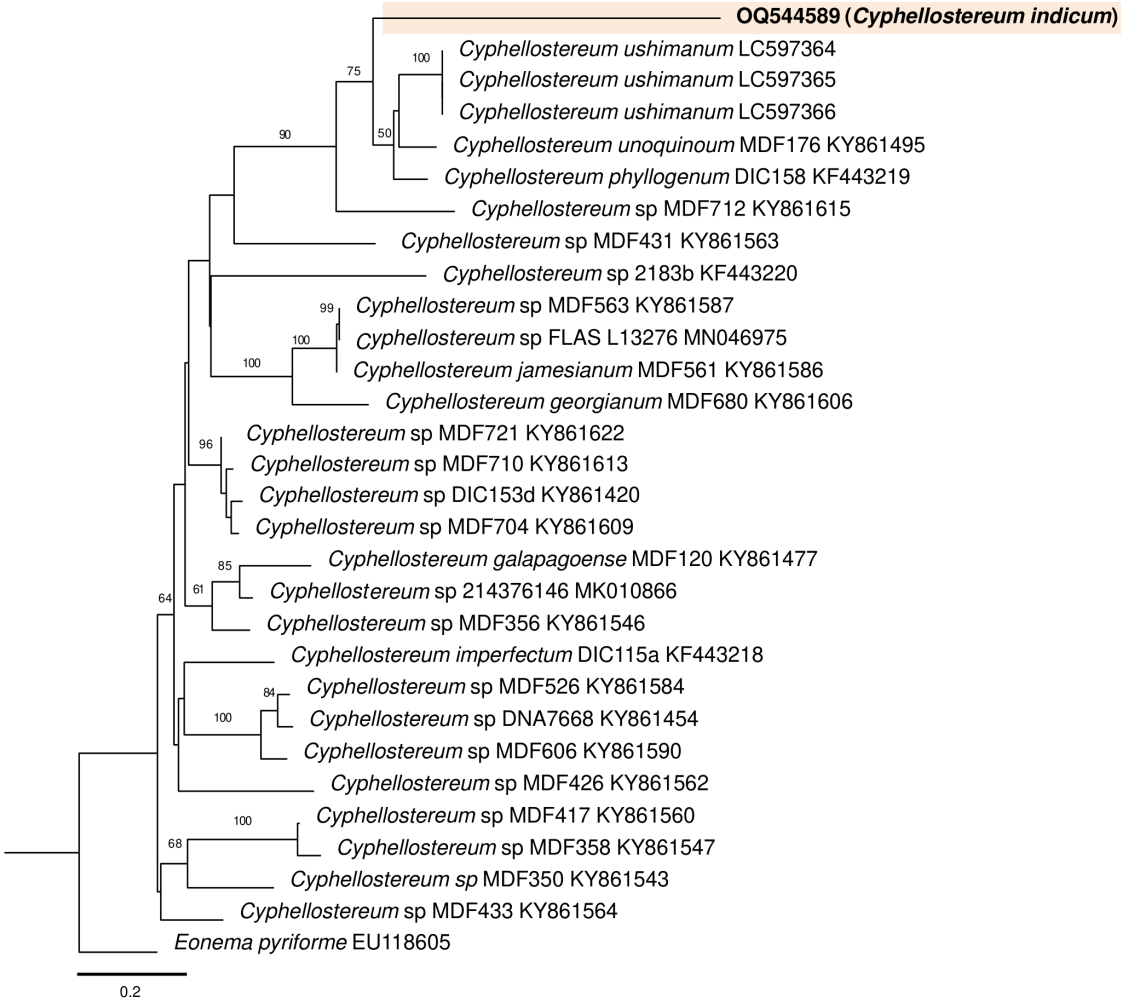

FIGURE 3. Molecular phylogenetic analysis of *Cyphellostereum* by the Maximum Likelihood method, only values ≥ 50% are shown in the tree. The scale bar signifies the nucleotide substitutions per sites.

Discussion

Cyphellostereum indicum is characterized by its thallus growing over the algal mat on wet soil, where the mycobiont hyphae loose, wraps around the cyanobacteria *Rhizonema* without clamp connections. The lichen is visible through its prominent, white to cream coloured flabelliform hymenophores that bear single, irregular to pip-shaped, aseptate, basidiospores on basidia. The hymenophore is glabrous and smooth with continuous margins when fresh, but gradually becomes wrinkled and curls as it dries. *Cyphellostereum indicum* is compared with other species of *Cyphellostereum* (Table 1). Phylogenetically *C. indicum* is close to *C. ushimanum*, however, macromorphologically, *C. ushimanum* differs in having resupinate hymenophores. The species *C. pusiolum* was synonymized as *Cotylidia pusiola* (Berk. & M.A. Curtis) A.L. Welden (2010: 27), by Welden (2010) and it is further accepted as valid name by He *et al.* (2019). Therefore, it is not included in the present study.

Thus, the number of basidiolichen species recorded from India has risen to a total of three species. The present study is significantly essential in better understanding the distribution, diversity and ecology of basidiolichens in India.

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