Original Article

Microbial Biosystems 9(2) (2024) 2024.1117
10.21608/mb.2024.300002.1117

Contents lists available at Egyptian Knowledge Bank
Microbial Biosystems
Journal homepage: http://mb.journals.ekb.eg/

Phylogenetic insights into *Remototrachyna* (*Parmeliaceae*) and their *Trebouxia* symbionts found in the Western Ghats, India

Arsha S. Mohan[1], Parayelil A. Ansil[2,3], Kunhiraman C. Rajeshkumar[2,3]*, Ahmed M. Abdel-Azeem[4], Shahnoor Fatima[2], Bharati Sharma[2], Stephen Sequeira[1]

[1]*Department of Botany, Maharaja's College, Ernakulam 682011, Kerala, India.*
[2]*Biodiversity and Palaeobiology (Fungi and Lichens) Gr., MACS Agharkar Research Institute, G. G. Agarkar Road, Pune, 411 004, Maharashtra, India.*
[3]*Faculty of Science, Savitribai Phule Pune University, Pune, 411 005, Maharashtra, India.*
[4]*Botany and Microbiology Department, Faculty of Science, Suez Canal University, Ismailia 41522, Egypt.*

ARTICLE INFO

Article history
Received 28 June 2024
Received revised 19 July 2024
Accepted 27 July 2024
Available online 28 July 2024

Corresponding Editors
Sridhar KR.
Nayaka S.
Abo Nouh FA.

Keywords
Lichenization,
mycobiont,
Parmeliaceae,
photobiont,
phycobiont,
selectivity,
specificity.

ABSTRACT

This study investigates the phylogeny and symbiotic relationships of the foliose lichen genus *Remototrachyna*, *R. crenata* and *R. rhabdiformis* from the Western Ghats through morphological, chemical and molecular phylogenetic tools using concatenated ITS and LSU data analyses. The study further identified the photobiont *Trebouxia* species in *R. crenata* and *R. rhabdiformis* based on ITS sequence data and phylogeny. The *Trebouxia* species of *R. crenata* and *R. rhabdiformis* were delineated as a major clade closely related to *Trebouxia* sp. voucher SF31 sequenced from *Usnea ghattensis* belonging to *Trebouxia* Clade I. This study represents a pioneering effort to unravel the enigmatic lichen symbiosis that exists in the genus *Remototrachyna* from the Western Ghats of India.

Published by Arab Society for Fungal Conservation

Introduction

Lichens are composite organism and an excellent example for understanding the co-evolution and co-existence of organisms from two or more kingdoms. *Parmeliaceae*, the largest family of lichen-forming fungi, is represented by about 70 genera and 2734 species worldwide (Wijayawardene et al. 2020, 2022). In India, the family encompasses over 382 species (Singh & Sinha 2010; Sinha et al. 2018; Rai et al. 2019; Pandaya et al. 2020; Mishra et al. 2022; Sequeira et al. 2022; Joseph et al. 2023); among them, 207 species belonging to 26 genera are recorded from the Western Ghats, one of the lichen hotspots in India (Nayaka & Upreti 2005 Nayaka & Haridas 2024).

Molecular studies in the family *Parmeliaceae* revealed cryptic and semicryptic genera having similar morphological features (Crespo & Ortega 2009; Crespo et al. 2010). The genus *Remototrachyna* is one such genus in the section *Parmelina* segregated from the *Hypotrachyna* clade (Divakar et al. 2010). *Remototrachyna* is characterized by broad, sub-irregular lobes with rounded apices, short, mostly dichotomously branched rhizines, and scleroplectenchymatous exciple and large ellipsoid ascospores. *Remototrachyna* differs from *Hypotrachyna* in lobe morphology, rhizine length, hymenium height,

***Corresponding author Email address*: rajeshfungi@gmail.com (Kunhiraman C. Rajeshkumar)**

exciple structure, and ascospore size. The genus currently includes 20 species that are corticolous and/or saxicolous in higher elevations of the tropics (Divakar et al. 2010; Flakus et al. 2012; Masson et al. 2015). *Remototrachyna* is assumed to have originated in the Indian subcontinent, with the highest number of species (14 out of 20 known species) reported from India (Divakar & Upreti 2005; Divakar et al. 2010). It thrives high-altitude areas (700–3600 msl) throughout the para-tropical zones, including, Australia, Mexico, the West Indies, Venezuela, Brazil, Bolivia, Panama, Africa, Papua New Guinea, Guatemala, Taiwan, Sri Lanka, Colombia, Indonesia, Malaysia, the Philippines, Nepal and Thailand (Hale 1975; Sipman 1993; Elix 1994; Wolseley et al. 2002; Sipman et al. 2009; Louwhoff et al. 2012; Weerakoon & Aptroot 2014; Aptroot 2016; Paguirigan 2020). The genus is also reported from subtropical and temperate regions like Chile, Argentina, Uruguay, Japan, the Azores and the Canary Islands (Hale 1975; Hawksworth et al. 2008; Sipman et al. 2009). The species *R. crenata* shows the distribution across India, Thailand, Indonesia, Taiwan, Nepal and Japan (Kurokawa 1991; Kurokawa & Lai 2001; Wolseley et al. 2002; Divakar & Upreti 2005; Lauwhoff & Nimis 2012), whereas *R. rhabdiformis* is distributed in India, Nepal, Costa Rica, South America, Panama, Columbia, Venezuela, Ecuador and Peru (Hale 1975; Divakar & Upreti 2005; Sipman et al. 2009).

The phylogeny of *Trebouxia*, studied using multi-locus approach to reconstruct the evolutionary history of *Trebouxia*, revealing distinct lineages within the genus (Dal Grande et al. 2018). Muggia et al. (2014) investigated the diversity and evolution of *Trebouxia* in lichens, in the light of host specificity in shaping the phylogeny of these algae contribute to our understanding of the evolutionary relationships within *Trebouxia* and their lichen hosts.

The present study encompasses *Remototrachyna* species collected from the Western Ghats, identified using morphology, chemistry and molecular phylogeny. It unravels and authenticates the symbionts of *R. crenata* and *R. rhabdiformis* using molecular markers, the Internal Transcribed Spacer region (ITS), and ribosomal large subunit (LSU) sequence data for the mycobiont and ITS for the *Trebouxia* photobiont. This is a holistic approach to taxonomizing both the mycobiont and photobiont pioneered by Fatima et al. (2021) in fruticose, Sharma et al. (2023) in foliose and Ansil et al. (2023) in crustose lichens of India. This study aims to unravel the symbiosis in the genus *Remototrachyna* and is the first comprehensive study of symbiosis in the light of photobiont selectivity and specificity exhibited in *Remototrachyna* from India.

Materials and Methods

Sample collection

Surveys were conducted in the Western Ghats region of Kollam (8˚58′24″N, 77˚05′47″E), Thrissur (10 ˚16′52″ N, 76˚50′47″ E), Kasargod (12°25′13″ N, 75°21′01″ E) districts in Kerala and Satara (17˚35′49″ N, 73˚50′50″ E) district in Maharashtra during 2021–2023. The study employed minimalistic sampling techniques to maintain the in-situ population of lichens in their natural environments. Fresh thalli were collected by gently detaching the lichen attached bark with a sharp knife and stored in brown paper bags. The samples were allowed to air dry and were stored in brown paper packs for further morpho-chemical studies. For molecular studies, fresh thalli were kept at 4 °C refrigerator to avoid cross-contamination from fast-growing saprotrophic fungi. A set of voucher specimens are deposited in the Ajrekar Mycological Herbarium, Agharkar Research Institute, Pune, India (AMH) and Maharajas College Herbarium, Kerala, India (MCH). In this work, morphological identification of mycobiont was performed by studying 04 accessions of *R. crenata* and 05 accessions of *R. rhabdiformis* samples, while molecular phylogenetic analyses of both mycobiont and photobiont were conducted using 03 *R. crenata* and 02 *R. rhabdiformis* samples.

Morphology and chemical analysis

Thallus morphology was studied using a stereomicroscope (Olympus SZX16 with Digi-CAM, Japan). Thallus sections were cut using a razor blade and mounted in lactic acid cotton blue, 10% KOH, and water for microscopy. Morphological characteristics were elaborated and compared with standard taxonomic references (Divakar & Upreti ,2005, Crespo et al. 2010). Chemical profiles were studied by spot tests and thin layer chromatography (TLC) following standard protocols (Orange et al. 2001) with the solvent systems toluene-dioxane-acetic acid (TDA, 180:45:5) and toluene-ethyl acetate-formic acid (TEA, 139:83:8).

DNA extraction, polymerase chain reaction and sequencing

Total genomic DNA from the lichen thalli was extracted by a modified CTAB method (Cubero et al. 1999; Porebski et al. 1997). ITS1T & ITS4T were the primers used for amplification of photobiont ITS (Kroken & Taylor 2000). Primers ITS5 & ITS4 (White et al. 1990) and LR0R & LR5 (Vilgalys & Hester 1990) were used for amplification of mycobiont ITS and LSU respectively. The PCR reactions (25 µl) contained 10X buffer (containing 100 mM Trizma/HCL, pH 8.3 at 25°C, 500 mM KCL, 15 mM MgCl2, 0.01% w/v gelatin), 0.2 mM each dNTP, 0.5 µM each primer, 1unit Taq DNA polymerase (Sigma-Aldrich) and 1–10 ng genomic DNA

extract. The amplifications were done in an automatic thermocycler ProFlexTM PCR system (Applied Biosystems, Foster City, USA).

Thermal cycling parameters used for amplification were initial denaturation at 95 °C for 5 min, 30 cycles at 94 °C for 1 min, 30 cycles at 50 °C for 1 min (ITS4 & ITS5), 30 cycles at 54 °C for 1 min (ITS1T & ITS4T), 30 cycles at 56 °C for 1 min (LR0R & LR5) and a final extension at 72 °C for 10 min. The PCR products were purified with the FavorPrep PCR Purification Kit (Favorgen Biotechcorp, Ping-Tung, Taiwan) and sequenced with the same primers using the BigDye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, USA). The sequencing reactions were run on an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, Foster City, USA).

Phylogenetic analysis

The newly generated sequences were subjected to Megablast searches in the NCBI GenBank nucleotide sequence database to identify the closest matching sequences. The phylogeny of *Remototrachyna* was assessed based on recent studies by Divakar et al. (2010), Masson et al. (2015), Michlig et al. (2024) and the photobiont *Trebouxia* was assessed following (Fatima et al. 2021) other available sequences of Remototrachyna ITS, LSU and Trebouxia ITS gene regions were retrieved from GenBank.

The individual datasets (ITS, LSU) were aligned and manually edited in MEGA v. 11.0.11 (Tamura et al. 2021) using MUSCLE. The phylogeny tool AliView v. 1.28 (Larsson 2014) was used to convert the FASTA alignment file into PHYLIP format for Maximum Likelihood (ML) analyses. The markers (ITS, LSU) were first analyzed separately, and a concatenated dataset was produced with ITS + LSU sequences. The phylogenetic analyses used the maximum likelihood (ML) and Bayesian analysis (PP) methods. Based on the J-Model test, the best-fit model of nucleotide substitution GTR G+I was performed. Phylogeny was inferred using RAxML v. 8.1.11 (Stamatakis 2006; Stamatakis et al. 2008) evaluating nodal support using 1000 bootstrap (BS) pseudo-replicates. The Bayesian posterior probability analysis of the individual and concatenated ITS + LSU dataset was performed using MrBayes v. 3.2.7a (Ronquist et al. 2012). For *Remototrachyna* and *Trebouxia* datasets, GTR G+I is applied as the best-fitting model and allows unlinked parameter estimation and independent rate variation. Posterior probabilities (PP) were estimated by sampling trees using a variant of the Markov Chain Monte Carlo (MCMC) method. For *Remototrachyna*, phylogenetic trees were sampled every 1000th generation (resulting in 1000 total trees) in 1000000 generations by running two simultaneous Markov chains. The first 363 trees containing the burn-in phase of the analyses were discarded. The remaining 637 trees were used to calculate the posterior probabilities (PP) in the majority rule consensus tree. For *Trebouxia*, phylogenetic trees were sampled every 1000th generation (resulting in 1110 total trees) in 1110000 generations by running two simultaneous Markov chains. The first 402 trees containing the burn-in phase of the analyses were discarded. The remaining 708 trees were used to calculate the posterior probabilities (PP) in the majority rule consensus tree. Only clades BS ≥ 50% under ML and PP ≥ 0.97 in the Bayesian framework were considered supported. Phylogenetic trees were visualized using the program FigTree 1.4.0 (Rambaut 2014). Trees were edited using Microsoft PowerPoint. DNA sequences newly generated in this study were deposited in GenBank.

Results

Phylogeny

Based on a Megablast search of NCBIs GenBank nucleotide database, the closest hits for *R. crenata* (AMH21.65, AMH21.67 and AMH21.68) had the highest ITS similarity (98%, 5 gaps) to two *R. awasthii* vouchers from India (MAF-Lich 15614, GQ919271; MAF-Lich 15615, GQ919272) and *R. rhabdiformis* MAF-Lich 15617 from India (96%, 4 gaps, GQ919284) and highest LSU similarity with *R. crenata* MAF-Lich 10377 from China (EU562683; 100%, no gap), *R. incognita* MAF 10384 from China (JN939642; 98%, no gap) and *R. infirma* MAF 10210 from China (AY785264; 98%, no gap).

Remototrachyna rhabdiformis (AMH21.66 and AMH23.664) had the highest ITS similarity to *R. rhabdiformis* MAF-Lich 15617 from India (98%, no gap, GQ919284), two *R. awasthii* vouchers from India (98%, no gap) (MAF-Lich 15614, GQ919271; MAF-Lich 15615, GQ919272) and the highest LSU similarity to *R. awasthii* MAF-Lich 15615 from India (GQ919248; 97%, 10 gaps), *Hypotrachyna* aff. *brevirhiza* MAF 10376 from China (EU562679; 97%, 8 gaps) and *R.* aff. *crenata* MAF-Lich 15616 from India (GQ919250; 97%,10 gaps).

Table 1. *Remototrachyna* species with GenBank accession numbers and voucher information for the sequences used in this study. Newly generated sequences are given in bold.

Species	Specimen voucher	Country	ITS	LSU
Remototrachyna adducta	MAF 10206	China	AY785270	AY785263
Remototrachyna aff. *crenata*	MAF-Lich 15616	India	GQ919275	GQ919250
Remototrachyna aff. *infirma*	MAF-Lich 15611	India	GQ919278	GQ919254
Remototrachyna awasthii	MAF-Lich 15614	India	GQ919271	GQ919247
Remototrachyna awasthii	MAF-Lich 15615	India	GQ919272	GQ919248
Remototrachyna costaricensis	2090	Argentina	OQ971993	-
Remototrachyna costaricensis	MAF 10211	Costa Rica	AY785269	AY785262
Remototrachyna crenata	**AMH21.67**	**India**	**PP911596**	**PP915990**
Remototrachyna crenata	**AMH21.68**	**India**	**PP911595**	**PP915989**
Remototrachyna crenata	**AMH21.65**	**India**	**PP911594**	**-**
Remototrachyna crenata	MAF-10377	China	DQ279495	EU562683
Remototrachyna dodapetta	MAF-Lich 15613	India	GQ919277	GQ919252
Remototrachyna dodapetta	MAF-Lich 15612	India	GQ919276	GQ919251
Remototrachyna flexilis	MAF 13974	India	DQ279499	EU562685
Remototrachyna flexilis	MAF 13975	India	DQ279500	-
Remototrachyna incognita	MAF 10385	China	DQ279506	EU562687
Remototrachyna infirma	MAF 10386	China	DQ279508	-
Remototrachyna kingii	MAF-Lich 15610	India	GQ919280	GQ919255
Remototrachyna kingii	MAF-Lich 15609	India	GQ919281	GQ919256
Remototrachyna koyaensis	MAF 10388	China	DQ279509	EU562688
Remototrachyna pandani	LG S3286	Reunion	KP098543	KP098551
Remototrachyna pandani	LG S3350	Reunion	KP098545	KP098553
Remototrachyna rhabdiformis	**AMH21.66**	**India**	**PP911597**	**PP915991**
Remototrachyna rhabdiformis	**AMH23.664**	**India**	**PP911598**	**PP915992**
Remototrachyna rhabdiformis	MAF-Lich 15617	India	GQ919284	-
Remototrachyna scytophylla	MAF 10410	China	DQ279525	EU562694
Bulbothrix isidiza	MAF-Lich 15511	Republic of the Congo	GQ919262	GQ919237

The combined sequence data of *Remototrachyna* were analyzed together with other available sequences of the genus in NCBI database to determine the placement of the species (Fig. 1). The tree was rooted with *Bulbothrix isidiza* MAF-Lich 15511. The analyzed dataset comprised ITS (491 bp) and LSU (798 bp) for a total of 1289 characters, including gaps for 27 taxa. The best-scoring RAxML tree with a final likelihood value of -4131.271 was presented. The matrix had 279 distinct alignment patterns, with 16.06% of undetermined characters or gaps. Substitution rates: AC = 2.23449, AG = 3.49355, AT = 2.23449, CG = 1.00000, CT = 11.12483, GT = 1.00000; gamma distribution shape parameter $\alpha = 0.548$. Maximum likelihood and Bayesian analyses resulted in similar topologies. *Remototrachyna crenata* formed a well-supported monophyletic clade sister to *R. rhabdiformis*, allied to *R. awasthii* (Fig. 1). The concatenation of the genes ITS and LSU resulted in a similar topology to the individual data.

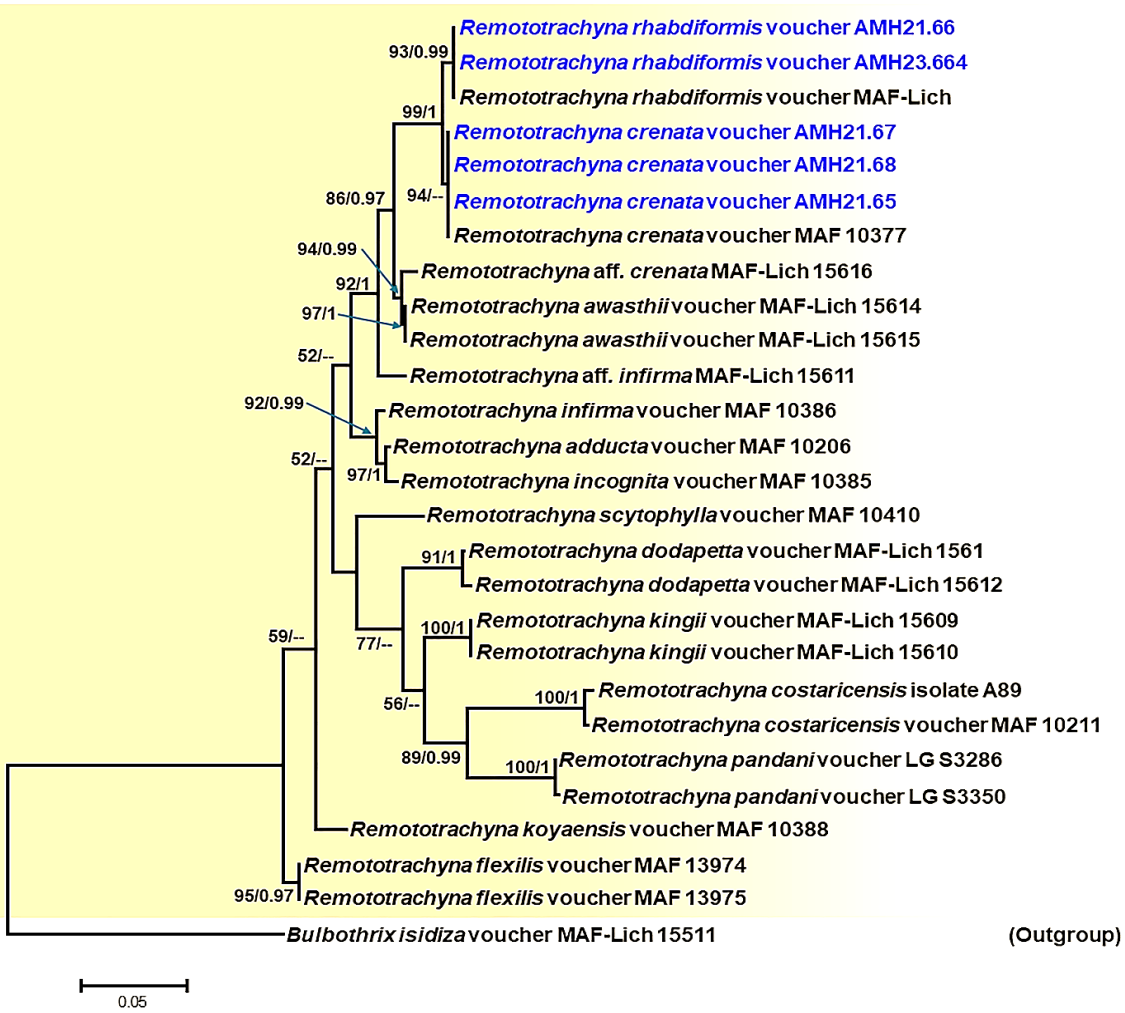

Fig. 1. Phylogram generated from Maximum Likelihood (ML) analyses based on concatenate ITS+LSU sequence data for the genus *Remototrachyna* (*Parmeliaceae*). Branch support values from 1000 non-parametric bootstraps for RAxML (ML-BS) and posterior probability (PP) from the Bayesian analysis are shown at the nodes (ML-BS≥50% / PP≥0.97). The tree is rooted with *Bulbothrix isidiza* MAF-Lich 15511 (GQ919262, GQ919237). The sequences generated in this study are highlighted in blue.

The closest hits for *Trebouxia* phycobiont (AMH2.66, AMH21.67, AMH21.68 and AMH23.664) using the ITS were *Trebouxia* sp. CUPVOUCHER_HP38_P_2020_01 from India (98%, 2 gaps, MT187588), *Trebouxia* sp. clone A012539 from Korea (97%, 2 gaps, MH258954), and *Trebouxia* sp. strain XTM2017112 from China (97%, 2 gaps, OL331682). The ITS sequence data of *Trebouxia* sp. were analyzed together with other available sequences in the genus *Trebouxia* in NCBI to determine the placement of the species (Fig. 2). The tree was rooted with *Asterochloris glomerata* voucher Alonso506. The analyzed dataset comprised ITS sequence data for 614 bp characters, including gaps for 34 taxa. The best RAxML tree with a final likelihood value of -3953.632 is presented (Fig. 2). The matrix had 302 distinct alignment patterns, with 12% undetermined characters or gaps. Substitution rates: AC = 1.82087, AG = 6.94617, AT = 3.66549, CG = 1.00648, CT = 6.94617, GT = 1.000000; gamma distribution shape parameter $\alpha = 0.325$. Maximum likelihood and Bayesian analyses resulted in similar topologies. The *Trebouxia* spp. associated with *R. crenata* and *R. rhabdiformis* were delineated as a major clade belonging to *Trebouxia* Clade I allied to *Trebouxia* sp. (voucher SF31) sequenced from *Usnea ghattensis* (Fatima et al. 2021) from the Western Ghats, India.

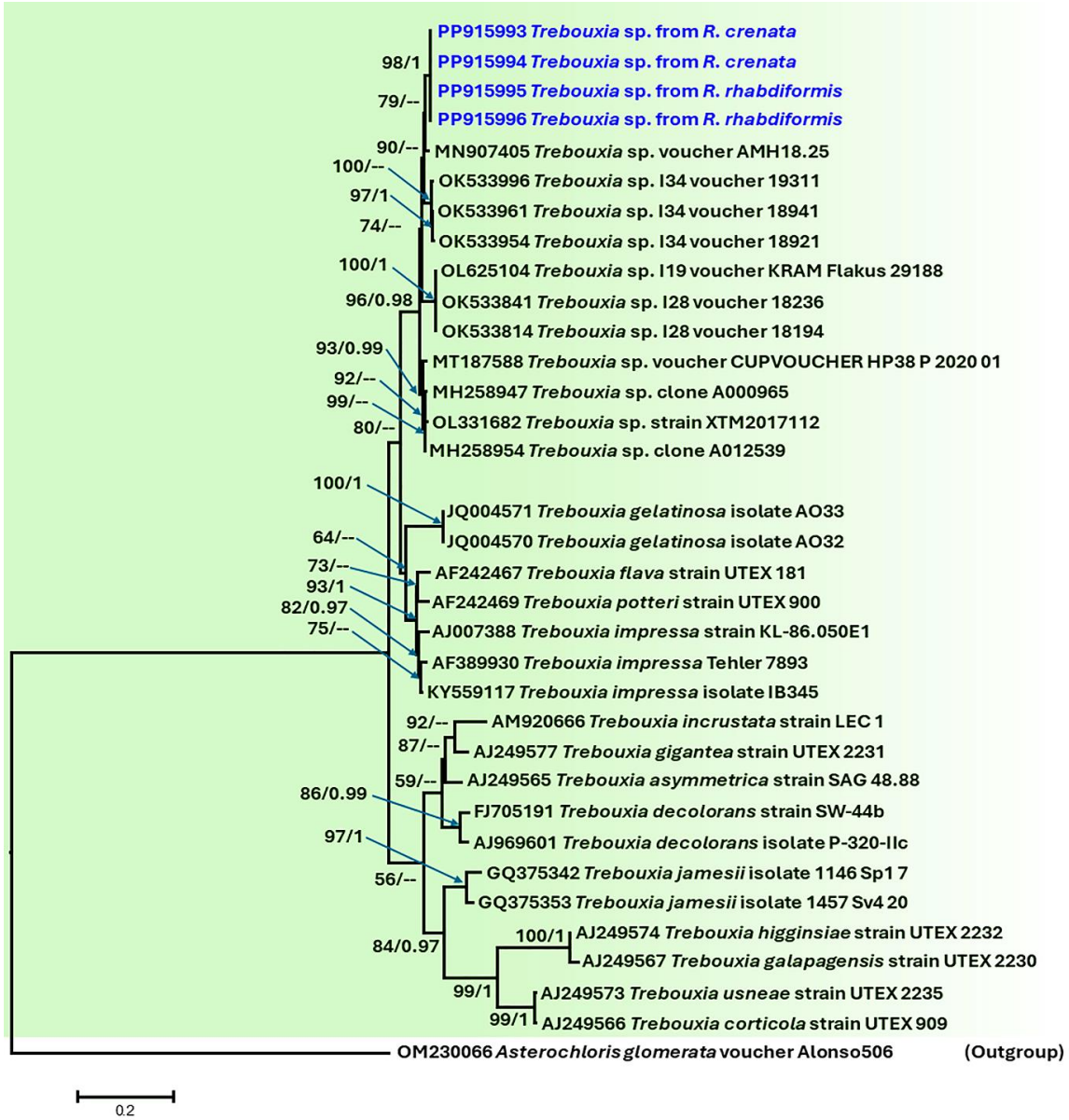

Fig. 2. Phylogram generated from Maximum Likelihood (ML) analyses based on concatenate ITS+LSU sequence data for the genus *Trebouxia* (*Trebouxiaceae*). Branch support values from 1000 non-parametric bootstraps for IQ-TREE (UFboot-BS) and posterior probability (PP) from the Bayesian analysis are shown at the nodes (Ufboot-BS≥50% / PP≥0.97). The tree is rooted with *Asterochloris glomerata* Alonso506 (OM230066). The sequences generated in this study are highlighted in blue.

Taxonomy

Remototrachyna crenata (Kurok.) Divakar & A. Crespo, *Am. J. Bot.*, 97-4: 586. (2010) (Fig. 3)

Basionym: *Parmelia crenata* Kurok., Contr. U. S., *Nat. Herb.*, 36: 168. 1964.

Synonym: *Hypotrachyna crenata* (Kurok.) Hale, *Phytologia*, 28-4: 341. 1974.

Thallus foliose, 2–7 cm wide, closely adnate to the substratum. Upper surface pale, smooth, dull, emaculate, greenish grey, isidiate. Isidia moderately dense, short, cylindrical, simple to branched, 0.3–1mm long. Lobes sub-irregular, divaricate, sinuate, imbricate, 3–5 mm wide, apices rotund. Margins ascending, crenate. Lower surface shiny, black towards center, dark brown towards margin, margin 1–3 mm wide papillate, margin erhizinate. Rhizines moderately present towards center and sparse towards periphery, dichotomously branched towards center, either branched or simple towards periphery, short, black, 0.2–0.5 mm long. Apothecia and pycnidia absent. Upper cortex 14.3–15.2 µm thick. Photobiont *Trebouxia* sp. The algal layer is uneven, continuous and 13.3–34.4

µm thick. Medulla white, 50.4–71.3 µm thick and lower cortex 23.3– 42.8 µm thick.

Chemistry. Cortex K+ yellow; Medulla K+ yellow, C–, KC–, P+ pale orange. Containing atranorin, constictic, stictic and norstictic acids.

Specimens examined: — India. Kerala: Thrissur District, Malakkappara, Perumpara, elev. 930 m, 10° 16′ 52″ N, 76° 50′ 47″ E, 17 March 2021, *S.M. Arsha & S. Stephen* (MCH 3596, AMH21.65). Maharashtra: Satara District, Thosaeghar, elev. 1049 m, 17˚35′49″ N, 73˚50′50″ E, 30 September 2022, *P.A. Ansil & K.C. Rajeshkumar* (AMH21.67, AMH21.68).

Notes: This species is mainly corticolous in nature, found growing on the barks of different trees.

Remark: The species is characterized by the presence of an isidiate thallus with crenate lobes. Thallus shares resemblances with *R. awasthii*, but the species is distinct from the later in the absence of cilia in lobe margins, black-tipped isidia and in chemistry. Unlike *R. crenata*, *R. awasthii* differs in the presence of salazinic acid in the medulla while constictic acid and stictic acid are absent.

Remototrachyna rhabdiformis (Kurok.) Divakar & A. Crespo, Am. J. Bot., 97-4: 586. (2010) (Fig. 4)
Basionym: *Parmelia rhabdiformis* Kurok., Contr. U. S., Nat. Herb. 36: 183. 1964.
Synonym: *Hypotrachyna rhabdiformis* (Kurok.) Hale, Smithson, Contr. Bot. 25: 62. 1975.

Thallus foliose, 6.5–10 cm wide, closely adnate to the substartum. Upper surface pale, smooth, shiny at peripheral regions, older parts rugose, emaculate to minutely maculate, greenish grey, isidiate. Lobes sub-linear, imbricate to crowded, 2–6 mm wide. Margins ascending, black-rimmed, eciliate. Lobe apices rotund and outwardly curved. Isidia laminal, moderately dense to dense, short, globose to cylindrical, simple to branched (coralloid), 0.8–1.1 mm long. Isidia germinate to form tiny eciliate lobules with rotund apices. Lower surface black, rugulose towards center, shiny smooth and dark brown towards margin, margin erhizinate. Rhizines black, dichotomously branched, short, up to 0.8 mm long. Pycnidia not observed. Upper cortex 15.1–17.1 µm thick. The algal layer is uneven, discontinuous 16.6–29 µm thick. Medulla white, 58.4–75.1 µm thick, lower cortex 21.4–26.6 µm thick. Apothecia laminal, sessile, developed towards the center and the periphery of the thallus. 1–3 mm diameter. Margins inflexed. Disc brown, concave, imperforate. Amphithecium dull, smooth to rugose. Epithecium 19.3–23.7 µm thick, hymenium 63.4–68.6 µm high. Asci clavate, 48.5–55.7 × 23–28.4 µm. Ascospores hyaline, oval, 14.5–20 × 6–8 µm.

Chemistry. Cortex K+ yellow; Medulla K+ yellow turning red, C–, KC–, P+ orange; containing atranorin, stictic and norstictic acids.

Specimen examined: — India. Kerala: Kollam District, Kazhuthurutty, Harrisons Malayalam Isfield Estate, elev. 312 m, 8˚58′24″N 77˚05′47″E, 24 February 2023, *S.M. Arsha & S. Stephen* (MCH 3596, AMH 22.18). Kasargod District, Panathadi, Ranipuram Hills, elev. 1020 m, 12°25′13″N 75°21′01″E, 9 March 2021, *S.M. Arsha & S. Stephen* (MCH 5268) India. Maharashtra: Satara District, Thosaeghar, elev. 1049 m, 17˚35'55"N 73˚50'53"E, 30 September 2022, *P.A. Ansil & K.C. Rajeshkumar* (AMH21.66, AMH 23.664).

Note: The species is mainly corticolous. Saxicolous specimens are also observed (MCH 5268).

Remark: *Remototrachyna rhabdiformis* shows similarities with *R. infirma* in external morphology, but the species is distinct from *R. infirma* in having simple to branched, cylindrical to club-shaped isidia (strictly cylindrical, simple to coralloid branched in the case of *R. infirma*), in negative colour reactions to spot tests, and the presence of protolichesterinic acid, caperatic acid and atranorin.

Discussion

In the present study, *Remototrachyna* (*Parmeliaceae*) was identified based on morphology, chemotaxonomy and molecular analyses. This is a comprehensive approach to taxonomizing Indian *Parmeliaceae*, including mycobiont and phycobiont phylogeny, to understand their diversity and symbiosis with different microhabitats. Lichens colonize by associating with locally adapted photobionts (Muggia et al. 2014). The ITS sequence-based phylogeny of the phycobiont revealed the existence of an identical *Trebouxia* species in *R. crenata* and *R. rhabdiformis* allied to *Trebouxia* sp. voucher SF31 sequenced from *Usnea ghattensis* by Fatima et al. (2021). The presence of the same photobiont in *R. crenata* and *R. rhabdiformis* agrees with the concept of locally adapted photobionts by Muggia et al. (2014) in the Western Ghats. Further, exploration, multigene sequencing and phylogeny of the *Trebouxia* species are crucial for species-level authentication and understanding the degree of photobiont specificity in Indian habitats.

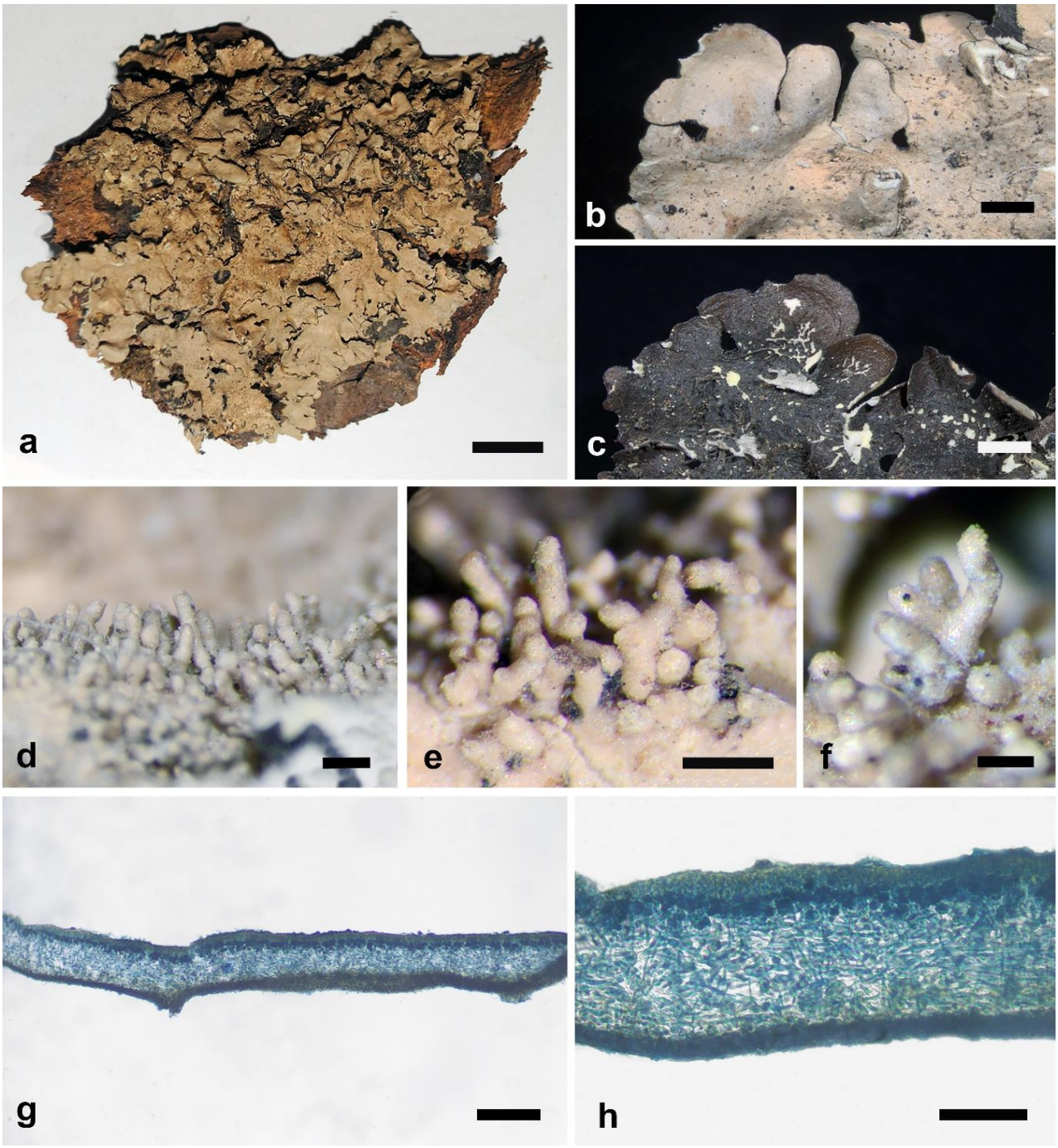

Fig. 3. ***Remototrachyna crenata*** (AMH21.65): **A, B –** Thallus, **C –** Thallus reverse, **D–F –** Thallus showing isidia, **G, H –** Section of thallus, Scale bars: a = 1 cm, b, c = 1 mm, d, e = 200 µm, f, g = 100 µm, h = 20 µm.

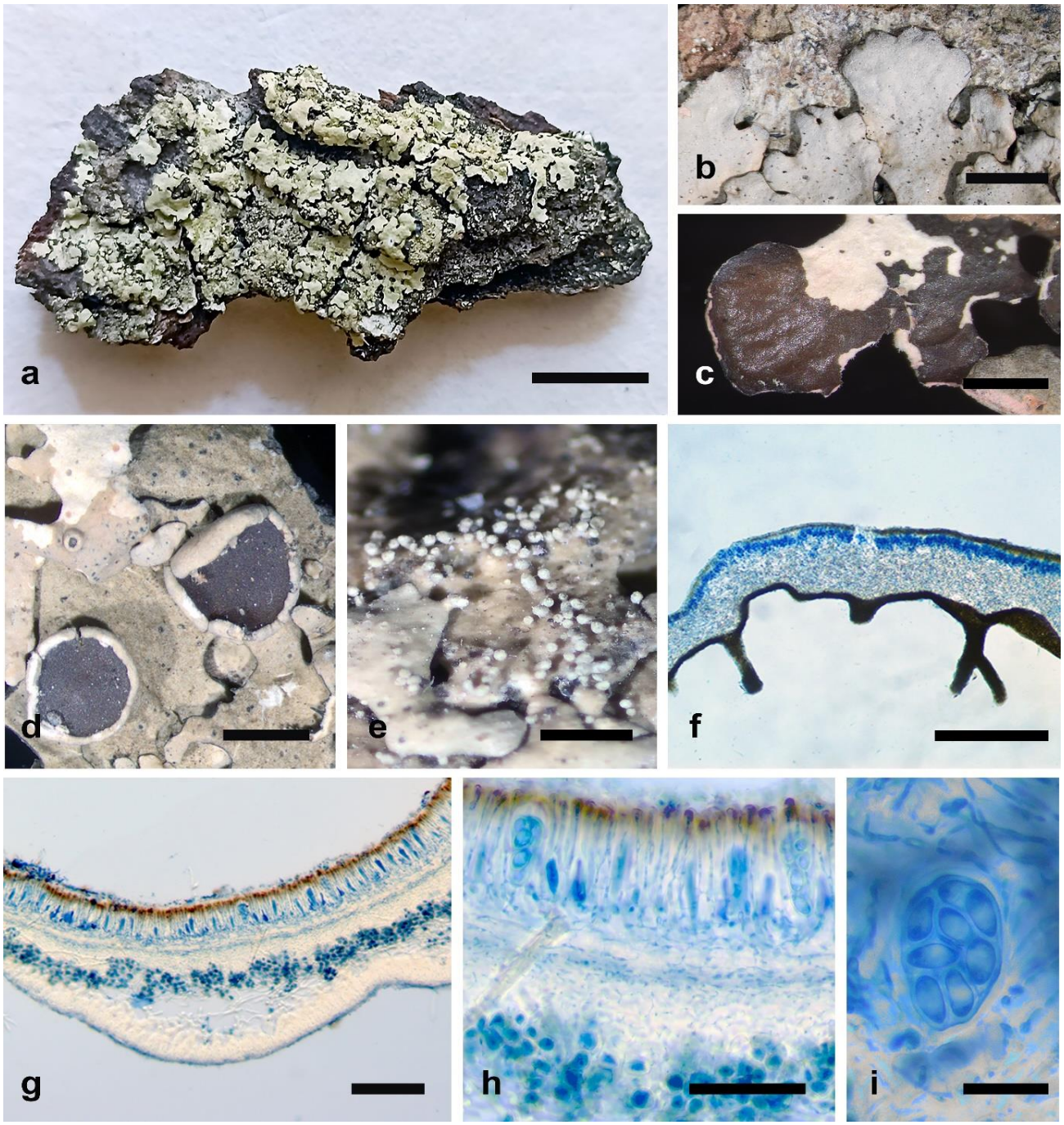

Fig. 4. ***Remototrachyna rhabdiformis*** (AMH23.664): **A, B –** Thallus, **C –** Thallus reverse, **D –** Thallus showing apothecia, **E –** Thallus showing isidia, **F –** Section of thallus, **G, H –** Section of apothecium, **I –** Ascus showing ascospores. Scale bars: a = 1 cm, b, c = 1 mm, d, g = 500 µm, e = 2 mm, f = 200 µm. g = 100 µm, h = 50 µm, i = 25 µm.

Acknowledgments

KC Rajeshkumar thanks the Science and Engineering Research Board, Department of Science and Technology, Government of India, for financial support under the SERB-CRG project No. CRG/2020/000668 and the Director, ARI, for providing the facility. Ansil P. A. acknowledges CSIR-HRDG, India, for the financial support under the SRF fellowship (09/670(0093)/2021-EMR-I). Stephen Sequeira thanks the principal of Maharaja's College for providing the facility. Arsha S. Mohan acknowledges Mahatma Gandhi University, Kottayam, for financial support under the University JRF fellowship (10512/AC A 6/2023/MGU). The authors are grateful to the Kerala and Maharashtra Forest

Departments for granting permission (KFDHQ-1262/2019/CWW/WL10; MSBB/Desk-5/Research/828/2022-23) to collect the specimens. We extend our deepest gratitude to the Arab Society for Fungal Conservation for their generous support in covering the publication fee for this research.

Conflict of interest statement

The authors declare that they have no conflicts of interest to disclose.

References

Ansil PA, Rajeshkumar KC, Sruthi OP, Gaikwad S, Sharma B. (2023). Decoding the evolutionary association among lichen symbionts in *Dyplolabia afzelii* from the Western Ghats, India. Microbial Biosystems, 8(2): 17-24. https://doi.org/10.21608/mb.2023.241013.1084

Aptroot A. (2016). Nine new lichen species and 64 new records from Sri Lanka. Phytotaxa, 280(2): 152-162. https://doi.org/10.11646/phytotaxa.280.2.5

Crespo A, Pérez-Ortega S. (2009). Cryptic species and species pairs in lichens: a discussion on the relationship between molecular phylogenies and morphological characters. In: Anales del Jardín Botánico de Madrid, 66(1): 71-81. https://doi.org/10.3989/ajbm.2225

Crespo A, Kauff F, Divakar PK, del Prado R, Pérez-Ortega S, de Paz GA, Ferencova Z, Blanco O, Roca-Valiente B, Núñez-Zapata J, Cubas P, Argüello A, Elix JA, Esslinger TL, Hawksworth DL, Millanes A, Molina MC, Wedin M, Ahti T, Aptroot A, Barreno E, Bungartz F, Calvelo S, Candan M, Cole M, Ertz D, Goffinet B, Lindblom L, Lücking R, Lutzoni F, Mattsson J, Messuti MI, Miadlikowska J, Piercey-Normore M, Rico VJ, Sipman HJM, Schmitt I, Spribille T, Thell A, Thor G, Upreti DK, Lumbsch HT. (2010). Phylogenetic generic classification of parmelioid lichens (*Parmeliaceae*, *Ascomycota*) based on molecular, morphological and chemical evidence. Taxon, 59(6): 1735-1753. https://doi.org/10.1002/tax.596008

Cubero OF, Crespo AN, Fatehi J, Bridge PD. (1999). DNA extraction and PCR amplification method suitable for fresh, herbarium-stored, lichenized, and other fungi. Plant Systematics and Evolution, 216(3-4): 243-249. https://doi.org/10.1007/BF01084401

Dal Grande F, Rolshausen G, Divakar PK, Crespo A, Otte J, Schleuning M, Schmitt I. (2018). Environment and host identity structure communities of green algal symbionts in lichens. New Phytologist, 217(1):277-89. https://doi.org/10.1111/nph.14770

Divakar PK, Upreti DK. (2005). *Parmelioid lichens in India: a revisionary study*. Dehra Dun: Bishen Singh Mahendra Pal Singh.

Divakar PK, Lumbsch HT, Ferencova Z, Del Prado R, Crespo A. (2010). *Remototrachyna*, a newly recognized tropical lineage of lichens in the *Hypotrachyna* clade (*Parmeliaceae*, *Ascomycota*), originated in the Indian subcontinent. American Journal of Botany, 97(4): 579-590. https://doi.org/10.3732/ajb.0900140

Elix JA. (1994). *Hypotrachyna*. Flora of Australia. 55: 49-59.

Fatima S, Ansil PA, Rajeshkumar KC, Sharma B, Gaikwad S, Mohan A, Sequeira S. (2021). Deciphering the symbiosis of endemic *Usnea ghattensis* and their photobiont *Trebouxia* sp. through molecular tools from the northern Western Ghats, India. Microbial Biosystems, 6(2): 30-42. https://doi.org/10.21608/mb.2022.121262.1050

Flakus A, Rodriguez Saavedra P, Kukwa M. (2012). A new species and new combinations and records of *Hypotrachyna* and *Remototrachyna* from Bolivia. Mycotaxon, 119(1): 157-166. https://doi.org/10.5248/119.157

Hale ME. (1975). A revision of the lichen genus *Hypotrachyna* (*Parmeliaceae*) in tropical America. Smithsonian Contributions to Botany, 25: 1-73. https://doi.org/10.5479/si.0081024X.25

Hawksworth DL, Blanco O, Divakar PK, Teuvo AHTI, Crespo A. (2008). A first checklist of parmelioid and similar lichens in Europe and some adjacent territories, adopting revised generic circumscriptions and with indications of species distributions. The Lichenologist, 40(1): 1-21. https://doi.org/10.1017/S0024282908007329

Joseph B, Sinisha EK, Jaseela VT, Pulparambil H, Pradeep NS. (2023). The study of biogeographic patterns of the genus *Parmotrema* in Wayanad District, Kerala with a new record in India. Journal of Threatened Taxa, 15(10): 24092-24103. https://doi.org/10.11609/jott.8570.15.10.24092-24103

Kroken S, Taylor JW. (2000). Phylogenetic species, reproductive mode and specificity of the green algae *Trebouxia* forming lichens with the fungal genus *Letharia*. Bryologist, 103(4): 645-660. https://doi.org/10.1639/0007-2745(2000)103[0645:PSRMAS]2.0.CO;2

Kurokawa S. (1991). Japanese species and genera of *Parmeliaceae*. J. Jap. Bot. 66: 152-159.

Kurokawa S, & Lai MJ. (2001). Parmelioid lichen genera and species in Taiwan. Mycotaxon, 77: 225-284.

Larsson A. (2014). AliView: a fast and lightweight alignment viewer and editor for large datasets. Bioinformatics, 30(22): 3276-3278. https://doi.org/10.1093/bioinformatics/btu531

Louwhoff, SHJJ, Nimis PL, Wolseley PA, Saipunkaew W. (2012). *A key to the parmelioid lichens of northern Thailand*. Chian Mai University. 39 pp.

Masson D, Divakar PK, Sérusiaux E. (2015). *Hypotrachyna penduliloba* and *Remototrachyna pandani*, two new species in the hyperdiverse lichen family *Parmeliaceae* from Réunion in the Mascarene Archipelago. Mycological Progress, 14: 1-15. https://doi.org/10.1007/s11557-015-1039-x

Michlig A, Moreno S, Rodríguez MP, Niveiro N, Neffa VS. (2024). The genera *Hypotrachyna* and *Remototrachyna* (*Parmeliaceae*, *Ascomycota*) in northeastern Argentina. Folia Cryptogamica Estonica, 61: 13-24. https://doi.org/10.12697/fce.2024.61.02

Mishra GK, Maurya P, Upreti DK. (2022). *Cetrelia isidiata* (Asahina) WL Culb. & CF Culb.(*Parmeliaceae*)-an addition to the Indian lichen biota. Journal of Threatened Taxa, 14(7): 21467-21469. https://doi.org/10.11609/jott.7891.14.7.21467-21469

Mishra GK, Nayaka S, Upreti DK, Kondratyuk SY, Thell A, Kärnefelt I. (2022). Cetrarioid lichens from India revised, including *Nephromopsis awasthii* sp. nov. and new records. Mycotaxon, 137(2): 283-334. https://doi.org/10.5248/137.283

Muggia L, Pérez-Ortega S, Kopun T, Zellnig G, Grube M. (2014). Photobiont selectivity leads to ecological tolerance and evolutionary divergence in a polymorphic complex of lichenized fungi. Annals of Botany, 114(3): 463-475. https://doi.org/10.1093/aob/mcu146

Nayaka S, Upreti DK. (2005). Status of lichen diversity in Western Ghats, India. Sahyadri E-News, Western Ghats Biodiversity Information System, 16: 1-28.

Nayaka S, Haridas B. (2024). Lichen-Forming and Lichenicolous Fungi of the Western Ghats, India. In Biodiversity Hotspot of the Western Ghats and Sri Lanka. Apple Academic Press. 89-107. https://doi.org/10.1201/9781003408758-6

Orange A, James PW, White FJ. (2001). Microchemical methods for the identification of lichens. London: British Lichen Society.

Paguirigan JAG. (2020). A checklist of lichens known from the Philippines. Current Research in Environmental & Applied Mycology, 10(1): 319-376. https://doi.org/10.5943/cream/10/1/29

Pandava G, Mohabe S, Devi A, Reddy AM. (2020). Assessment of the lichen diversity from Koundinya Wildlife Sanctuary, Andhra Pradesh, India. Tropical Plant Research, 7(2): 427-429. https://doi.org/10.22271/tpr.2020.v7.i2.051

Porebski S, Bailey LG, Baum BR. (1997). Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. Plant Molecular Biology Reporter, 15(1): 8-15. https://doi.org/10.1007/BF02772108

Rai H, Nayaka S, Upreti DK, Gupta RK. (2019). A new record of *Canomaculina* (*Parmeliaceae*, *Ascomycota*) from Western Himalaya, India. National Academy Science Letters, 42: 429-431. https://doi.org/10.1007/s40009-018-0760-9

Rambaut A. (2014). FigTree. Available at: http://tree.bio.ed.ac.uk/software/figtree [Accessed 6 June 2024].

Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology, 61(3): 539-542. https://doi.org/10.1093/sysbio/sys029

Sequeira S, Christy A, Anilkumar A, Arsha SM. (2022). *Parmotrema sahyadrica* (*Parmeliaceae*): a new species of parmelioid lichen from Wayanad, Southern Western Ghats, India. Phytotaxa, 539(3): 287-292. https://doi.org/10.11646/phytotaxa.539.3.8

Sharma B, Fatima S, Rajeshkumar KC, Škaloud P, Divakar PK, Gaikwad S, Ansil PA, Mohan AS, Sequeira S. (2023). Molecular studies of *Flavopunctelia* and *Punctelia* species and their *Trebouxia* photobiont from the Himalayas, India. Mycotaxon, 137(4): 853-869. https://doi.org/10.5248/137.853

Singh KP, Sinha GP. (2010). *Indian Lichens: An Annotated Checklist*. Botanical Survey of India.

Sinha GP, Nayaka S, Joseph S. (2018). Additions to the checklist of Indian lichens after 2010. Cryptogam Biodiversity and Assessment Special, 197: 206. https://doi.org/10.21756/cab.esp16

Sipman HJM. (1993). Lichens from Mount Kinabalu. Tropical Bryology, 8: 281-314. https://doi.org/10.11646/bde.8.1.29

Sipman HJM, Elix JA, Nash TH. (2009). *Hypotrachyna* (*Parmeliaceae*, Lichenized Fungi). Flora Neotropica, 104: 1-176.

Stamatakis A. (2006). RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics, 22(21): 2688-2690. https://doi.org/10.1093/bioinformatics/btl446

Stamatakis A, Hoover P, Rougemont J. (2008). A rapid bootstrap algorithm for the RAxML web servers.

Systematic Biology, 57(5): 758-771. https://doi.org/10.1080/10635150802429642

Tamura K, Stecher G, Kumar S. (2021). MEGA11: molecular evolutionary genetics analysis version 11. Molecular Biology and Evolution, 38(7): 3022-3027. https://doi.org/10.1093/molbev/msab120

Vilgalys R, Hester M. (1990). Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology, 172(8): 4238-4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990

Wijayawardene NN, Hyde KD, Al-Ani LK, Tedersoo L, Haelewaters D, Rajeshkumar KC, Zhao RL, Aptroot A, Leontyev DV, Saxena RK, Tokarev YS. (2020). Outline of Fungi and fungus-like taxa. Mycosphere, 11(1):1060-456. https://doi.org/10.1128/jb.172.8.4238-4246.1990

Wijayawardene NN, Hyde KD, Dai DQ, Sánchez-García M, Goto BT, Magurno F. (2022). Outline of Fungi and fungus-like taxa–2021. Mycosphere, 13(1): 53-453. https://doi.org/10.5943/mycosphere/11/1/8

Weerakoon G, Aptroot A. (2014). Over 200 new lichen records from Sri Lanka, with three new species to science. Cryptogamie, Mycologie, 35(1): 51-62. https://doi.org/10.7872/crym.v35.iss1.2014.51

White TJ, Bruns T, Lee S, Taylor J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: M. A. Innis, et al. eds. PCR protocols: a guide to methods and applications. San Diego, CA: Academic Press, 315-322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Wolseley PA, Aguirre-Hudson B, McCarthy PM. (2002). Catalogue of the lichens of Thailand. Bulletin of the Natural History Museum, London. 32(1): 13-59. https://doi.org/10.1017/S0968044602000038