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Clarifying the phylogenetic delimitation of *Ocellularia upretii*, the acid-deficient counterpart of *Ocellularia allosporoides* (*Graphidaceae*)

Parayelil A. Ansil[1], Kunhiraman C. Rajeshkumar[1,2*], Bharati Sharma[1] & Robert Lücking[3]

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Abstract. This study aims to clarify the phylogenetic delimitation of *Ocellularia upretii*, a recently described, acid-deficient counterpart of *Ocellularia allosporoides*, which contains the unusual substances norisonotatic and norsubnotatic acid. We obtained new data on the mitochondrial small subunit and the nuclear large subunit rDNA, as well as *rpb2*, from several specimens collected in the Western Ghats of India. In addition to the newly generated molecular data, we provide an updated assessment of the taxa currently distinguished in this complex, including morphology, ascospores, chemistry, and distribution. This paper is dedicated to our esteemed colleague, André Aptroot, on the occasion of his 65th birthday.

Key words: *Ascomycota*, *Graphidaceae*, Lichens, Phylogeny, Taxonomy

Introduction

The genus *Ocellularia* G. Mey. (Meyer 1825) belongs to *Graphidaceae* Dumort. (Dumortier 1822), a family of largely tropical, crustose lichens. For a long time, the genus was included in the family *Thelotremataceae* Stizenb. (Stizenberger 1862). However, Mangold et al. (2008), based on molecular phylogenetic analyses, reduced *Thelotremataceae* to synonymy under *Graphidaceae*, and this synonymy was subsequently supported by Rivas Plata et al. (2013) using an expanded data set. The genus has alternatively been classified in *Diploschistaceae* (Kraichak et al. 2018; Wijayawardene et al. 2020), but this is based on an inconsistent, unsupported topology; *Ocellularia* and its allies are not closely related to *Diploschistes* (Lücking 2019).

Ocellularia is characterized by a crustose, usually corticolous thallus, apothecia with a mostly carbonized excipulum, embedded in bark periderm and with a distinct pore often filled by a mostly carbonized columella, lack of periphysoids, and hyaline to rarely brownish, transversely septate to muriform ascospores (Hale 1980, 1981; Frisch et al. 2006; Mangold et al. 2009; Rivas Plata et al. 2012; Sipman et al. 2012; Kraichak et al. 2014). Species delimitation within the genus remains challenging due to often unresolved morphological and chemical variation, and many taxa turned out to represent species complexes (Lücking 2014; Lücking & Pérez-Ortega 2015; Marshall et al. 2020).

Ocellularia allosporoides (Nyl.) Patw. & Kulk. (Patwardhan & Kulkarni 1977) is characterized by a corticate thallus, conspicious, erumpent to prominent apothecia with a brownish, non-carbonized excipulum and a carbonized columella, large (80–150 × 10–20 µm), multiseptate ascospores, and the comparatively rare secondary chemistry of norisonotatic and norsubnotatic acids, visible as bright yellow spots on TLC plates (chonestoma unknowns according to Hale 1981). *Ocellularia allosporoides* is a widespread eastern paleotropical species, with two established synonyms, viz. *Thelotrema apayaoense* Vain. and *Ocellularia groenhartii* Hale (Mangold et al. 2009). The same authors indicated two additional potential synonyms, *O. verrucomarginata* Patw., Sethy & Nagarkar and *O. karnatakensis* Hale, both described from India and both producing norisonotatic acid. Phylogenetically, *O. allosporoides* belongs in the *O. baileyi* clade (Rivas Plata et al. 2012, 2013; Kraichak et al. 2014), including several eastern paleotropical species with norisonotatic acid chemistry.

In previous phylogenetic analyses, a closely related taxon lacking secondary compounds was recognized, known from Thailand and initially labelled *O.* aff. *allosporoides* (Rivas Plata et al. 2013; Kraichak et al. 2014). This taxon was subsequently described as *O. upretii* S. Joshi, Divakar, Lumbsch & Lücking (Joshi et al. 2018), but no molecular data were included in the protologue

[1] Biodiversity and Palaeobiology (Fungi and Lichens) Gr., MACS Agharkar Research Institute, G. G. Agarkar Road, Pune, Maharashtra, 411004, India
[2] Faculty of Science, Savitribai Phule Pune University, Ganeshkhind, Pune, Maharashtra, 411007, India
[3] Botanischer Garten und Botanisches Museum Berlin, Freie Universität Berlin, Berlin, Königin-Luise-Str. 6–8, 14195 Berlin, Germany
* Corresponding author e-mail: rajeshfungi@gmail.com

and no connection was made to the previously sequenced specimens.

A total of 44 species of *Ocellularia* have hitherto been reported from India, no less than 37 of which are known from the Western Ghats (Sinha et al. 2025). Although molecular phylogenetic studies have been carried out for *Graphidaceae* from the Western Ghats, such as *Allographa*, *Diorygma*, *Dyplolabia*, and *Graphis* (Ansil et al. 2023a, b, 2024, 2025; Manawasinghe et al. 2024), integrative taxonomic assessments remain scarce for Indian *Ocellularia*, warranting reassessment of these species using molecular approaches. Given the newly generated data and the unclear phylogenetic delimitation of *O. upretii* within the *O. allosporoides* clade, the present study provides an updated treatment of this complex using an integrative taxonomic approach combining morphological, chemical and phylogenetic data (mtSSU, nuLSU, and *rpb2*).

Materials and methods

Sample collection

Surveys were conducted and sampling was carried out during October to December 2023 in the areas of Charpa, Pithampetti and Karadichola in the Thrissur district of Kerala state, as well as in Pushpagiri Wildlife Sanctuary in the Kodagu district of Karnataka State, India. Minimalistic sampling methods were used to preserve the *in situ* diversity of the lichens. The samples were air-dried and stored in brown paper bags for further morpho-chemical studies. For molecular studies, fresh thalli were stored at 4°C upon return to the laboratory to prevent cross-contamination from fast-growing saprotrophic fungi.

Morphology and chemical analyses

Thallus morphology was studied using an Olympus SZX16 stereomicroscope with DP23 camera (Japan), using Olympus cellSens Entry 3.2 software to take and process the images. Sections of ascomata were prepared with a razor blade and subsequently placed in lactic acid (with mild heating over a flame), 10% KOH, water, and Lugol's iodine for microscopy. The micrometry was recorded in water mounts. Ascomata sections pretreated with 10% KOH were mounted in Lugol's iodine. Microscopic observations were done using an Olympus BX53 compound microscope with a DP74 camera (Japan) and Olympus cellSens Standard 3.2 software. Chemical profiles were studied by thin-layer chromatography (TLC) following standard protocols (Orange et al. 2001) with the solvent systems toluene-dioxane-acetic acid (TDA, 180:45:5 = solvent A) and toluene-ethyl acetate-formic acid (TEF, 139:83:8 = solvent G). The specimens were deposited in the Ajrekar Mycological Herbarium (AMH) at MACS Agharkar Research Institute, Pune, India.

DNA isolation, polymerase chain reaction and sequencing

DNA isolation and PCR were performed using the Sigma RED Extract-N-AmpTM Seed PCR Kit, following the manufacturer's instructions, in a thermocycler ProFlexTM PCR system (Applied Biosystems, Foster City, USA). Primers used for amplification were: i) mrSSU1 and mrSSU3R for the mtSSU marker (Zoller et al. 1999); ii) AL2R (Mangold et al. 2008) and LR6 (Vilgalys & Hester 1990) for the nuLSU marker; iii) GD1-*rpb2*-7cF and GD-*rpb2*-11aR (Kraichak et al. 2015) for the *rpb2* marker. Thermal cycling parameters used for amplification were: initial denaturation at 95°C for 5 min, followed by 35 cycles at 50°C for 1 min (mtSSU), 35 cycles at 58°C for 1 min (nuLSU), 35 cycles for 1 min from 57°C to 72°C, with an increase of 1°C per cycle (*rpb2*), and a final extension at 72°C for 10 min. The PCR products weree purified with Alphagen PCR Purification Kit (Alphagen Biotech Ltd., Ping-Tung, Taiwan) and sequenced with the same primers using the BigDye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems). The sequencing reactions were run on an ABI Prism® 3100 Genetic Analyzer (Applied Biosystems, USA).

Phylogenetic analyses

The NCBI GenBank nucleotide sequence database was searched using MegaBLAST (Morgulis et al. 2008) to identify the closest matching sequences, all in the genus *Ocellularia*. The phylogeny of *Ocellularia* was assembled following Kraichak et al. (2014). The sequences used for the phylogenetic analysis are given in the following table (Table 1).

The datasets were first individually aligned using MAFT (Kuraku et al. 2013; Katoh et al. 2019) trimmed and manually edited, after which mtSSU, nuLSU and *rpb2* were concatenated. The phylogeny tool AliView v. 1.28 (Larsson 2014) was used to manually edit the alignment and convert it to PHYLIP format. The alignment files are deposited in FigShare (https://figshare.com/articles/dataset/*Ocellularia*_concat/31441888?file=62282941). Marker partitioning was performed manually for the combined mtSSU+nuLSU+*rpb2* dataset (nuLSU: 1–991 bp; *rpb2*: 992–1879 bp; mtSSU: 1880–2631 bp). Phylogenetic analyses were performed using maximum likelihood (ML) in IQ-TREE v. 2.1.3 (Trifinopoulos et al. 2016) and RAxML v. 8.1.11 (Stamatakis 2006; Stamatakis et al. 2008). Nodal support was evaluated using 1,000 ultrafast bootstrap (UFboot) pseudo-replicates in IQ-TREE and 1,000 rapid bootstrap pseudo-replicates in RAxML. Model selection was done using the "auto" option available in IQ-TREE. TIM2+R4 (for nuLSU), HKY+I (for *rpb2*), and TVM+F+R3 (for mtSSU) were the best-fitting models for the dataset. The 'GTR G+I' model was determined a priori for RAxML analysis. The model test was performed using MrModeltest2 v. 2.4 (Nylander 2004) according to the Akaike Information Criterion (AIC) to select nucleotide substitution models for Bayesian posterior probability (PP) analysis, which was performed using MrBayes v. 3.2.7 (Ronquist et al. 2012). For Bayesian PP analysis 'GTR+G' was the best-fitting model for nuLSU and mtSSU; 'HKY+I' for *rpb2*. For phylogenetic trees generated using IQ-TREE, only clades with UFboot BS ≥ 96% were considered supported, and for RAxML,

Table 1. List of species with GenBank Accession numbers and voucher information for the sequences used in this study. Newly generated sequences are given in bold.

Name of taxa	Voucher/strain information	Country	mtSSU	nuLSU	*rpb2*
Ocellularia aff. *baileyi*	Papong 7982	New Caledonia	KJ435188	KJ435115	KJ435264
Ocellularia aff. *baileyi*	Lumbsch 19105a	Australia	JX421127	–	–
Ocellularia aff. *interposita*	Lumbsch 20203b	Thailand	JX421165	JX421566	–
Ocellularia aff. *Profunda*	Lumbsch 19123 k & Mangold (F)	Australia	EU075590	EU075636	–
Ocellularia allosporoides	Lumbsch 20200a	Thailand	–	JX421545	–
Ocellularia allosporoides	Lumbsch 20204a	Thailand	JX421119	JX421546	–
Ocellularia auberianoides	Lücking 26549	USA	JX421122	JX421548	–
Ocellularia auberianoides	Lücking 26548	USA	JX421123	JX421549	–
Ocellularia australiana	Lumbsch 19151 u & Mangold (F)	Australia	EU075595	EU075641	–
Ocellularia australiana	Mangold 25f	Australia	JX421126	JX421550	–
Ocellularia carassensis	Lücking 27015a	Panama	JX421132	–	–
Ocellularia cavata	Frisch & Tamnjong Idi 99/Ka392, 1999 [M]	Cameroon	DQ384878	–	–
Ocellularia cavata	Frisch & Tamnjong Idi 99/Ka403, 1999 [M]	Cameroon	DQ384879	DQ431935	–
Ocellularia cavata	A. Frisch No. 99/Ka1950 [M]	Tanzania	DQ384877	DQ431935	–
Ocellularia cicra	Rivas Plata 108Da	Peru	JX421139	JX421551	–
Ocellularia cicra	Rivas Plata 8extra	Peru	JX421138	–	JX420869
Ocellularia conformalis	Cáceres 6004a	Brazil	JX421142	–	–
Ocellularia conformalis	Cáceres 6004b	Brazil	JX421143	–	–
Ocellularia diacida	Lumbsch 19120 jb & Mangold (F)	Australia	EU075583	EU075630	–
Ocellularia dolichotata	Kalb 38892	Thailand	JX421146	JX421554	–
Ocellularia dolichotata	Lumbsch 20202d	Thailand	JX421149	JX421558	–
Ocellularia endomelaena	Lumbsch 19136l	Australia	JX421152	–	–
Ocellularia endomelaena	Mangold 29a	Australia	JF828966	–	–
Ocellularia fecunda	Lücking 27150	Panama	JX421154	–	–
Ocellularia fecunda	Lücking 32122	Venezuela	JX421155	–	–
Ocellularia fecunda	Lücking 32162	Venezuela	–	JX421562	–
Ocellularia fuscosporella	Papong 7757	New Caledonia	KJ435192	KJ435119	–
Ocellularia henatomma	Cáceres 6013a	Brazil	JX421160	–	–
Ocellularia henatomma	Cáceres 6013b	Brazil	JX421161	–	–
Ocellularia interposita	Lumbsch 20201c	Thailand	JX421164	JX421565	–
Ocellularia interposita	Lumbsch 20202b0	Thailand	JX421163	–	–
Ocellularia isidiza	Lücking 32059	Venezuela	JX421166	JX421567	JX420923
Ocellularia kalbii	Lumbsch 19085i	Australia	JX421196	–	–
Ocellularia kalbii	Lumbsch 19082 e & Mangold (F)	Australia	EU075570	EU075618	–
Ocellularia laeviusculoides	Lücking 32001	Venezuela	JX421167	JX421569	JX420896
Ocellularia massalongoi	Mangold 36m (F)	Australia	EU075584	EU075631	–
Ocellularia massalongoi	Frisch & Tamnjong Idi 99/Ka1799, 1999 [M]	Cameroon	DQ384882	–	–
Ocellularia microstoma	Lumbsch 19056m	Australia	–	JX421575	–
Ocellularia microstoma	Lumbsch 19125r	Australia	JX421108	JX421575	–
Ocellularia minutula	Lumbsch 19167t	Australia	JX421173	JX421578	–
Ocellularia neopertusariiformis	Mangold 35zf	Australia	JX421176	–	–
Ocellularia obturascens	Lücking 26553a	USA	JF828967	JF828979	–
Ocellularia obturascens	Lücking 26554	USA	JX421177	–	–
Ocellularia papillata	Mangold 43 o (F)	Australia	EU075585	EU075633	–
Ocellularia perforata	Lumbsch 19120 ja & Mangold (F)	Australia	EU075587	EU075634	–
Ocellularia perforata	Lumbsch 19849d	Fiji	JX421183	–	–
Ocellularia pertusariiformis	Rivas Plata 1074B	Philippines	JX421184	–	–
Ocellularia petrinensis	Lücking 32024	Venezuela	JX421185	JX421532	JX420910
Ocellularia philippina	Rivas Plata 1070	Philippines	JX421187	–	–
Ocellularia philippina	Rivas Plata 1135	Philippines	JX421188	–	–
Ocellularia praestans	Lücking 32200	Venezuela	JX421192	JX421581	JX420892
Ocellularia praestans	Lücking 32236	Venezuela	JX421195	JX421583	JX420911
Ocellularia profunda	Lumbsch 19100p & Mangold (F)	Australia	EU075589	–	–
Ocellularia profunda	Lumbsch 19100p	Australia	JX421198	JX421585	JX420825
Ocellularia pseudochapsa	Cáceres 11790	Brazil	KJ435210	KJ435134	–
Ocellularia pseudochapsa	Cáceres 11719	Brazil	KJ435229	–	–
Ocellularia pyrenuloides	Papong 8031	New Caledonia	KJ435203	KJ435127	KJ435268
Ocellularia pyrenuloides	K. Kalb 34019, 2002 [herb. Kalb]	Australia	DQ384896	–	–

Table 1. Continued.

Name of taxa	Voucher/strain information	Country	mtSSU	nuLSU	*rpb2*
Ocellularia rhabdospora	Mercado F35	Puerto Rico	KJ435171	–	–
Ocellularia rhabdospora	Mercado F75	Puerto Rico	KJ435172	KJ435108	KJ435254
Ocellularia rhabdospora	Mercado F76	Puerto Rico	KJ435176	–	KJ435255
Ocellularia rondoniana	Cáceres 11335	Brazil	KJ435216	–	–
Ocellularia rondoniana	Cáceres 11982	Brazil	KJ435222	–	–
Ocellularia salmonea	Papong 7954	New Caledonia	KJ435191	KJ435118	–
Ocellularia thelotremoides	Lumbsch 19108 I & Mangold (F)	Australia	EU075592	EU075638	–
Ocellularia upretii	Kalb 38813	Thailand	JX421118	JX421544	JX420925
Ocellularia upretii	Gueidan 3076G	Vietnam	KJ435157	–	–
Ocellularia upretii	Lumbsch 19756c	Thailand	–	JX421535	–
Ocellularia upretii	Lumbsch 19756e	Thailand	–	JX421536	–
Ocellularia upretii	AMH23.379	India	**PZ288646**	**–**	**–**
Ocellularia upretii	AMH23.432	India	**PZ288647**	**PZ288651**	**–**
Ocellularia upretii	AMH23.438	India	**PZ288648**	**–**	**–**
Ocellularia upretii	AMH23.440	India	**PZ288649**	**PZ288652**	**PZ289844**
Ocellularia upretii	AMH23.596	India	**PZ288650**	**–**	**PZ289845**
Ocellularia upretii	Lücking 24110	Thailand	JX421116	–	–
Ocellularia upretii	Lücking 24130	Thailand	JX421117	–	–
Ocellularia upretii	YN210737	China	–	OQ676637	–
Ocellularia urceolaris	Lücking 21053	Costa Rica	JX421224	–	–
Ocellularia violacea	Cáceres sn DNA3496a	Brazil	JX421225	–	–
Ocellularia violacea	Cáceres sn DNA3496b	Brazil	JX421226	–	–
Ocellularia vulcanisorediata	Mercado 76	Puerto Rico	KJ435174	–	–
Ocellularia vulcanisorediata	Mercado 4451	Puerto Rico	KJ435165	KJ435106	–
Ocellularia xanthostromiza	Cáceres 11982	Brazil	KJ435219	–	–
Ocellularia xanthostromiza	Papong 8559	Thailand	KJ435243	–	–
Ocellularia xanthostromiza	Rivas Plata 809canopy	Peru	JX421171	–	–
Rhabdodiscus isidiifer	Lücking 32212	Venezuela	JX421300	JX421621	JX420903
Rhabdodiscus neocaledonicus	Papong 7630	New Caledonia	KJ435204	KJ435128	–
Rhabdodiscus saxicola	Papong 8042	New Caledonia	KJ435197	KJ435123	–

BS ≥ 70% were considered supported. Posterior probabilities (PP) were estimated, allowing unlinked parameter estimation and independent rate variation. Trees were sampled using a variant of the Markov Chain Monte Carlo (MCMC) method. For inferring the PP, phylogenetic trees were sampled every 1,000th generation (resulting in 1,056 total trees) in 1,056,000 generations from running four simultaneous Markov chains. The first 25% of trees containing the burn-in phase of the analyses were discarded. The remaining 792 trees were used to calculate the posterior probabilities (PP) in the majority rule consensus tree. Only clades with PP ≥ 0.97 in a Bayesian framework were considered supported. Phylogenetic trees were visualized using the program FigTree 1.4.0. (Rambaut 2014). DNA sequences newly generated in this study were deposited in GenBank (Table 1).

Results and discussion

Phylogeny

A total of 12 mtSSU and 9 nuLSU sequences were available for the target taxa, representing 15 terminals, five with both markers and seven with either only mtSSU or nuLSU. However, the mtSSU sequence JX421102 turned out to be erroneous, as it blasted with the unrelated *O. viridipallens* and OQ676627 due to misalignment, whereas the nuLSU sequence of the same sample belonged in the *O. allosporoides* complex. Accession JX421102 and OQ676627 were therefore excluded, leaving ten mtSSU and eight nuLSU sequences and four terminals with both markers.

The mtSSU marker displayed two main patterns, one corresponding to accession JX421119 (Lumbsch 20204a, Thailand), unfortunately a short sequence, the other to the remaining ten terminals. These could further be divided into three subgroups based on specific indel patterns in the terminal portion of the marker: JX421118 (Kalb 38813, China), the five accessions from India, and the remaining accessions from Thailand, Vietnam, and China. The nuLSU marker showed two rather distinct phylogroups (Fig. 2): one formed by the accessions JX421545 (Lumbsch 20200a, Thailand) and JX421545 (Lumbsch 20204a, Thailand), the other by all other accessions. In the second phylogroup, the two Indian accessions are set apart by a single substitution. Available TLC results showed that the two accessions Lumbsch 20200a and 20204a from Thailand produced norisonotatic and norsubnotatic acid, whereas all the tested accessions of the second group (seven accessions tested) lacked secondary compounds.

The combined sequence data of *Ocellularia* was analyzed with other available sequences in the genus in NCBI to determine the placement of the species (Table 1, Fig. 1). The tree was rooted with *Rhabdodiscus* spp. The results of the IQ-TREE and RAxML-ML analyses carried out in this study are represented in the following Table 2.

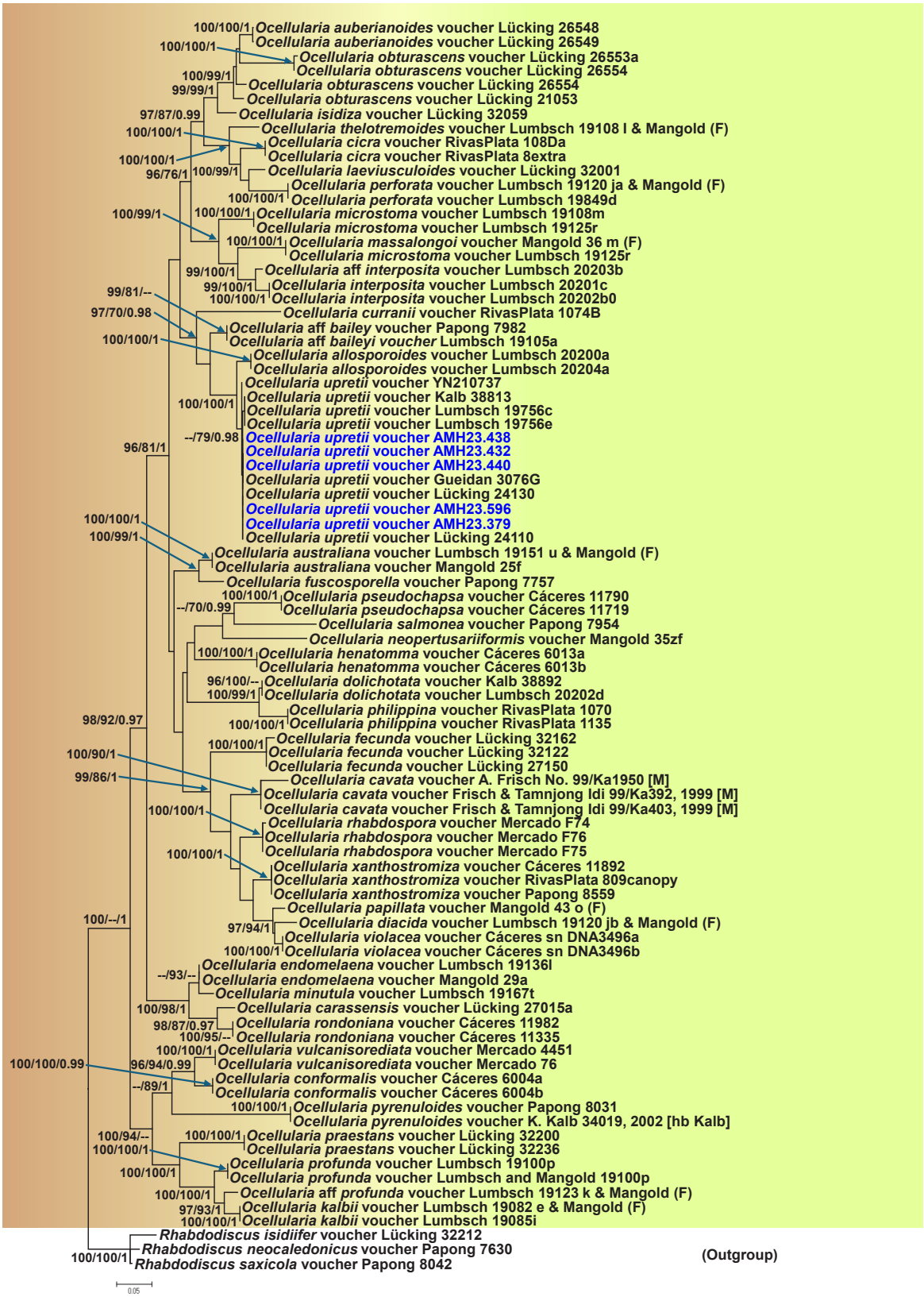

Figure 1. Phylogram generated from Maximum Likelihood (ML) analyses based on concatenate mtSSU, nuLSU and *rpb2* sequence data for the genus *Ocellularia* (*Graphidaceae*). Branch support values from 1,000 non-parametric bootstraps for IQ-TREE (UFboot-BS), RAxML (R-BS) and posterior probability (PP) from the Bayesian analysis are shown at the nodes (Ufboot-BS ≥ 96% / R-BS ≥ 70% / PP ≥ 0.95). Low support values are not represented. The tree is rooted with *Rhabdodiscus* spp. The sequences generated for *Ocellularia upretii* in this study are highlighted in blue.

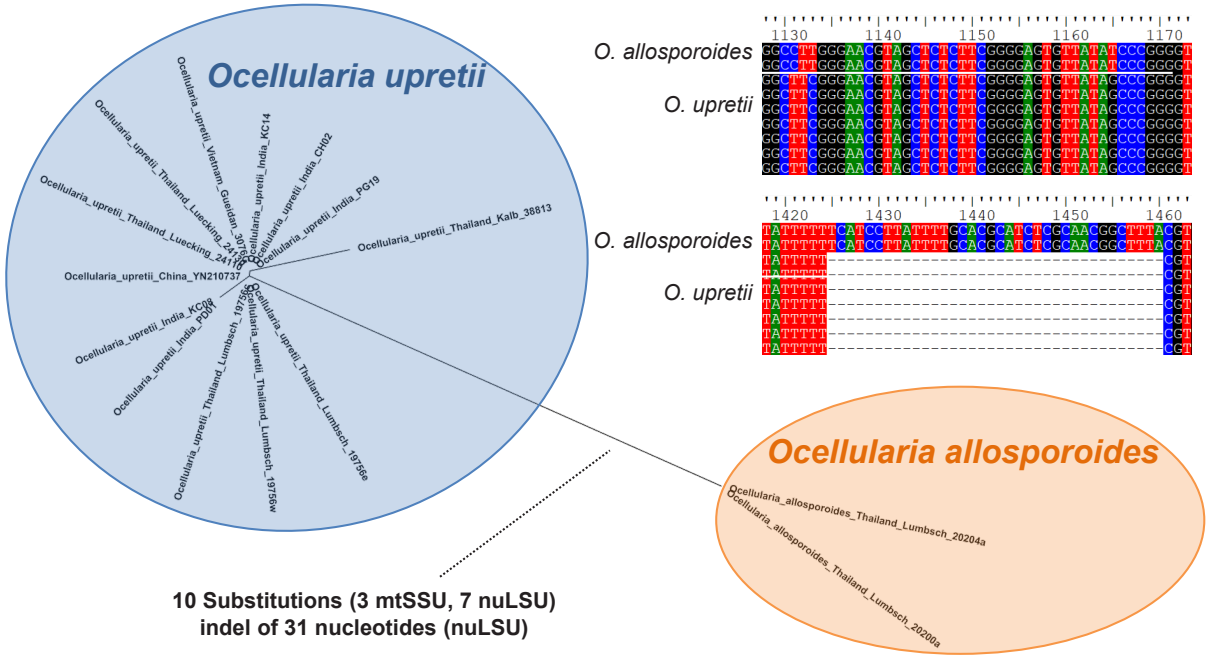

Figure 2. Phylogram generated based on mtSSU and nuLSU markers showing relationships among studied *Ocellularia allosporoides* and *Ocellularia upretii* accessions. The dataset revealed two distinct phylogroups. Nucleotide substitutions of 10 bases (3 mtSSU, 7nuLSU) and an indel of 31 nucleotides (nuLSU) are also depicted.

The ML trees of the combined mtSSU+nuLSU+*rpb2* dataset obtained with IQ-TREE, RAxML, and the trees obtained from the PP analysis were topologically congruent and hence, the phylogenetic tree obtained using IQ-TREE is presented (Fig. 1), with superimposed support values from the RAxML and Bayesian posterior probability analysis. *Ocellularia upretii*, formed a sister clade with *Ocellularia allosporoides*.

Table 2. Summary of results of the ML analyses performed.

Summary of results of the ML analyses	mtSSU+nuLSU+*rpb2*
Number of strains (incl. Outgroup)	88
Length incl. gaps	2631
Distinct alignment patterns	989
Undetermined characters (Gaps)	55.96%
Estimated base frequencies	A: 0.276192 C: 0.197261 G: 0.243368 T: 0.283178
Substitution rates	AC: 0.998562 AG: 3.853318 AT: 2.367180 CG: 0.639215 CT: 6.326263 GT: 1.000000
IQ-TREE analysis	
Model (BIC)	TIM2+R4 (nuLSU) HKY+I (*rpb2*) TVM+F+R3 (mtSSU)
Final likelihood value	−15044.557
RAxML analysis	
Model (BIC)	GTR G+I
Final likelihood value	−15287.799026
Gamma distribution shape parameter (α)	0.638811

Taxonomy

Key to the species of the *Ocellularia allosporoides* complex

1 Thallus with stout isidia; norisonotatic and norsubnotatic acid present; excipulum uncarbonized; columella carbonized; ascospores 80–150 × 10–15 µm; India . . . . . ***O. karnatakensis***

Thallus without isidia 2

2(1) Thallus and apothecial margins coarsely verrucose to bumpy; ascospores 70–100 × 10–15 µm; norisonotatic and norsubnotatic acid present; eastern Paleotropics (India, Malaysia) . . . . . ***O. verrucimarginata***

Thallus and apothecial margins even to slightly uneven; ascospores mosty >100 µm long; chemistry variable 3

3(2) Norisonotatic and norsubnotatic acid present; excipulum uncarbonized; columella carbonized; ascospores 70–150 × 10–20 µm; eastern Paleotropics (Solomon Islands, India, Thailand, Malaysia, Vietnam, Indonesia, Philippines, Australia) . . . . . ***O. allosporoides***

No substances detected; excipulum uncarbonized; columella carbonized; ascospores 80–180 × 15–25 µm; eastern Paleotropics (India, Thailand, Vietnam, China) . . . . . ***O. upretii***

Ocellularia upretii S. Joshi, Divakar, Lumbsch & Lücking (Fig. 3)

Description. Thallus crustose, corticolous, moderately thick (<400 µm), surface smooth to uneven or verruculose, corticate, greyish green, olivaceous green to pale green, thallus margin not observed, thallus in section 95–145 µm thick, cortex 14–50 µm high, algal layer trentepohlioid 50–70 µm high, medulla white, crystalline, 120–150 µm high; Apothecia round to slightly angular,

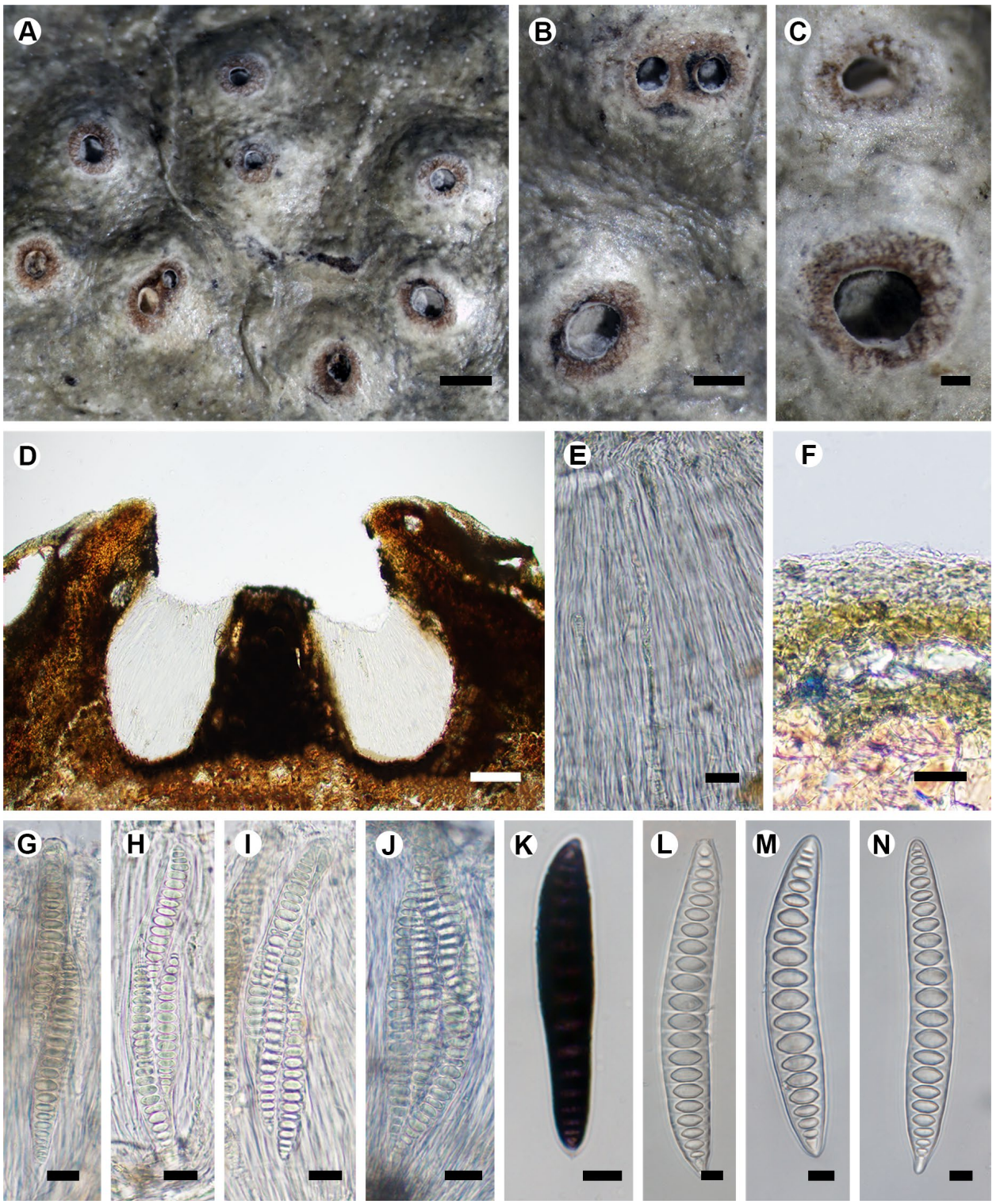

Figure 3. Morphological characteristics of *Ocellularia upretii* (AMH23.596). A–C – thallus; D – section of ascocarp; E – hymenium; F – section of the thallus; G–J – ascus showing ascospores; K – I+ ascospore; L–N – ascospore. Scales: A = 1 mm, B = 500 µm, C = 200 µm, D = 100 µm, F–J = 20 µm, K–N = 10 µm. Photos: P. A. Ansil.

dispersed, separate, 0.1–2.5 mm diam., immersed to prominant, pore rounded to oval, 0.1–1 mm, with mottled or pale-colored complete thalline margin; Disc more or less filled with blackish columella, often immersed; proper margin entire, visible as brownish rim around the pore; Thalline margin smooth, concolorous to thallus; Excipulum entire, fused with margin, brownish to reddish brown, non-carbonized, (50–)100–185 µm thick; Columella present, simple, entire, carbonized, conical, apically sometimes pruinose, sometimes protruding out of the pore, (50–)120–225 µm thick; Epithecium indistinct to greyish, granular, crystalline, 10–15 µm high; Hymenium hyaline, clear, I–, 200–312 µm high; Subhymenium hyaline, clear, I–, 15–30 µm high; Asci 2–8 spored, fusiform, hyaline, 188–264 × 28–50 µm: Ascospores hyaline, fusiform to oblong, transversly 10–23-septate, with lens-shaped lumina (80–)100–180(–240) × 15–25(–35) µm, septa I+ blue; Conidiomata not observed.

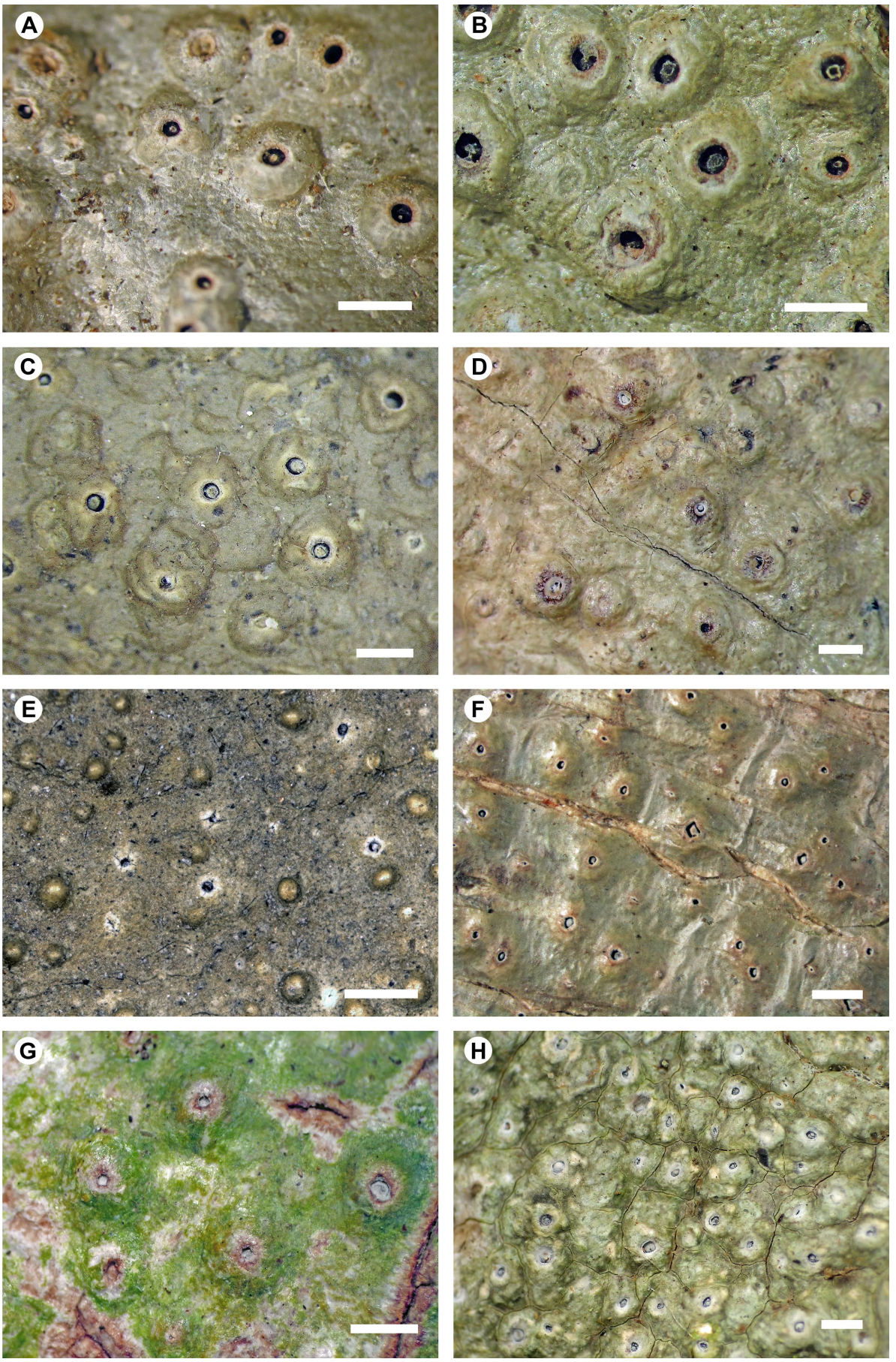

Figure 4. Thallus morphology of A – *Ocellularia allosporoides* Type of *Thelotrema apayoense*; B – *Ocellularia allosporoides* Holotype of *Thelotrema apayoense* (TUR 7093); C – *Ocellularia allosporoides* Holotype of *O. groenhartii* from Java (US 0639); D – *Ocellularia allosporoides* Lectotype H 1982; E – *Ocellularia karnatakensis* Type 25 F1; F – *Ocellularia upretii* India Lumbsch 19745-l3; G – *Ocellularia upretii* Thailand Lücking 24110; H – *Ocellularia upretii* Thailand Lücking 24130. Photos: B–D, F–H – by Robert Lücking; A, E – by Armin Mangold. Scales = 1 mm.

Chemistry. No substances detected by TLC, UV–, K–.

Distribution and ecology. Within India, the species is currently known from the forest fringes of the Western Ghats of Kerala and Karnataka, India (collections ranging from 214 msl to 1,700 msl), as well as from the Andaman and Nicobar Islands (see also Joshi et al. 2018). In addition, it has been collected (confirmed by sequencing data) in Thailand, Vietnam, and China. In all instances, the lichens grew on bark substrate in tropical forest.

Remarks. *Ocellularia upretii* was described as the acid-deficient counterpart of *O. allosporoides* (Joshi et al. 2018), with reference to a previously published study that had phylogenetically separated this taxon from the latter (Kraichak et al. 2014). A possible split in specimens identified as *O. allosporoides* was already noted in Rivas Plata et al. (2013), where three collections from Thailand (Lumbsch 19756c, 19756e, 19756w) formed a clade clearly separate from the remaining specimens. However, as outlined above, this was caused by an erroneous mtSSU accession for Lumbsch 19756w. In the subsequent analysis by Kraichak et al. (2014), the problematic sequence was removed, and the resulting, rather compact clade showed a small, emerging subclade including two specimens from Thailand (Lumbsch 20200a, 20204a). Integration of all available data supports this separation and indicates that the *O. allosporoides* complex can be divided into two subclades (see above): one containing the two collections Lumbsch 20200a, 20204a, with norisonotatic and norsubnotatic acid, representing *O. allosporoides* s.str., and the other comprising all remaining sequenced accessions, including the newly studied material from India.

Although in the description of *Ocellularia upretii*, the authors made reference to the phylogenetic study by Kraichak et al. (2014), the actually sequenced specimens representing that taxon, from Thailand and Vietnam, were not mentioned or included in the list of examined material. Given that the type and other material mentioned in the protologue has not been sequenced, a direct connection cannot be made, but it agrees well with the now sequenced material from the same region, especially in the rather broad ascospores (15–25 µm). Our expanded analysis thus demonstrates that the clade interpreted as *O. upretii* has a rather wide, eastern paleotropical distribution, found in India (most collections), Thailand, Vietnam, and China.

Morphologically and chemically, *Ocellularia upretii* differs from *O. allosporoides* chiefly in the lack of secondary compounds and in the relatively broader ascospores [15–25(–35) µm vs. 10–15(–20) µm]. The type of *Thelotrema allosporoides* Nyl. contains norisonotatic acid and ascospores about 50–130 × 10–15(–20) µm in size (Mangold et al. 2009). The type material of *Thelotrema apayoense* Vain. from the Philippines (Vainio 1921), considered a synonym of *O. allosporoides* (Mangold et al. 2009), features a more or less smooth thallus and apothecial margins, contains norisonotatic and norsubnotatic acid and its ascospores are 50–120 × 10–12 µm large, fitting the concept of *O. allosporoides* well (Fig. 4A, B, D). The same applies to *O. groenhartii* Hale [Fig. 4C; ascospores 80–150 × 10–15(–20) µm]. Hale (1975) originally did not compare his taxon with *O. allosporoides*, and Nagarkar et al. (1987) distinguished it from the latter assuming that *O. allosporoides* did not contain secondary compounds. However, this was based on comparison with acid-deficient material from the Andaman Islands (Nagarkar et al. 1986) that should now be referred to *O. upretii* (Fig. 4F–H).

The two other species considered putative synonyms of *Ocellularia allosporoides* by Mangold et al. (2009), namely *O. karnatakensis* Hale (Fig. 4E) and *O. verrucimarginata* (as "*verrucomarginata*") Patw., Sethy & Nagarkar, are here kept separately. Both contain norisonotatic and norsubnotatic acid. *Ocellularia karnatakensis* is distinguished by the numerous, conspicuous, robust and stout isidia, starting out as prominent warts. *Ocellularia verrucimarginata* has unusually bumpy apothecial margins and relatively small ascospores, but more material is needed to see whether this distinction will hold.

Specimens examined. INDIA. Kerala State, Thrissur District, Charpa. 10°18′10.8″N, 76°34′46.9″E, 214 m, on tree bark, 23 Oct. 2023., Ansil P.A. & Rajeshkumar K.C. (AMH23.379); Karadichola, 10°17′37.5″N, 76°48′05″E, 954 m, on tree bark, 24 Oct. 2023., Ansil P.A. & Rajeshkumar K.C. (AMH23.432, AMH23.438); Karnataka State, Kodagu District, Pushpagiri Wildlife Sanctuary, 12°39′37.4″N, 75°42′01.9″E, 1,125 m, on tree bark, 05 Dec. 2023, Ansil P.A. & Rajeshkumar K.C. (AMH23.596).

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Author Contributions

Conceptualization, KCR, RL; fieldwork, PAA and KCR; formal analysis, PAA, RL and KCR; writing: original draft preparation: PAA, KCR and RL; figures, sequence submission, PAA, RL and KCR; writing, review and editing, PAA, KCR, and RL; lab work, PAA; provided sequences, PAA and KCR; funding acquisition, KCR and BS. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors have declared that no competing interests exist.

ORCID

Parayelil A. Ansil https://orcid.org/0000-0002-6772-7736
Robert Lücking https://orcid.org/0000-0002-3431-4636
Kunhiraman C. Rajeshkumar https://orcid.org/0000-0003-0401-8294
Bharati Sharma https://orcid.org/0000-0001-6511-5421

References

Ansil, P. A., Rajeshkumar, K. C., Sharma, B., Lücking, R. & Hawksworth, D. L. 2023a. Phylogenetic placement and reappraisal of *Diorygma karnatakense* including the new synonym, *Diorygma dandeliense*, from Maharashtra, India. *The Lichenologist* 55: 59–67. https://doi.org/10.1017/S0024282923000087

Ansil, P. A., Rajeshkumar, K. C., Sruthi, O. P., Gaikwad, S. & Sharma, B. 2023b. Decoding the evolutionary association among lichen symbionts in *Dyplolabia afzelii* from the Western Ghats, India. *Microbial Biosystems* 8: 17–24. https://doi.org/10.21608/mb.2023.241013.1084

Ansil, P. A., Rajeshkumar, K. C., Lücking, R., Paraparath, S. O. & Sharma, B. 2024. Molecular studies of *Allographa effusosoredica* sp. nov. (*Graphidaceae*) along with its *Trentepohlia* photobiont and a comprehensive checklist for Indian *Allographa*. *Phytotaxa* 664: 31–45. https://doi.org/10.11646/phytotaxa.664.1.3

Ansil, P. A., Rajeshkumar, K. C., Lücking, R., Gaikwad, S. & Sharma, B. 2025. Resolving the phylogeny of *Diorygma aeolum*, along with its photobiont *Trentepohlia* species from the Western Ghats, India. *Studies in Fungi* 10: 1–11. https://doi.org/10.48130/sif-0025-0022

Dumortier, B. C. J. 1822. *Commentationes botanicae: observationes botanique.* Tournay: C. Casterman-Dieu.

Frisch, A., Kalb, K. & Grube, M. 2006. *Contributions towards a new systematics of the lichen family-Thelotremataceae*. Schweizerbart Science Publishers, Stuttgart, Germany. *Bibliotheca Lichenologica* 92: 3–370.

Hale Jr, M. E. 1975. Studies on the lichen family *Thelotremataceae*. 3. *Mycotaxon* 3(1): 173–181. https://doi.org/10.5962/p.413973

Hale Jr, M. E. 1980. Generic delimitation in the lichen family *Thelotremataceae* (*Thelotrema*, *Myriotrema*, *Ocellularia*). *Mycotaxon* 11: 130–138.

Hale Jr, M. E. 1981. A revision of the lichen family *Thelotremataceae* in Sri Lanka. *Bulletin of the British Museum (Natural History), Botany* 8: 227–332.

Joshi, S., Upreti, D. K., Divakar, P. K., Lumbsch, H. T. & Lücking, R. 2018. A re-evaluation of thelotremoid *Graphidaceae* (lichenized *Ascomycota*: *Ostropales*) in India. *The Lichenologist* 50: 627–678. https://doi.org/10.1017/S0024282918000439

Katoh, K., Rozewicki, J. & Yamada, K. D. 2019. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* 20: 1160–1166. https://doi.org/10.1093/bib/bbx108

Kraichak, E., Parnmen, S., Lücking, R., Rivas Plata, E., Aptroot, A., Cáceres, M. E. S., Ertz, D., Mangold, A., Mercado-Díaz, J. A., Papong, K., Broeck, D. V. D., Weerakoon, G. & Lumbsch, H. T. 2014. Revisiting the phylogeny of *Ocellularieae*, the second largest tribe within *Graphidaceae* (lichenized *Ascomycota*: *Ostropales*). *Phytotaxa* 189: 52–81. https://doi.org/10.11646/phytotaxa.189.1.6

Kraichak, E., Lücking, R., Aptroot, A., Beck, A., Dornes, P., John, V., Lendemer, J. C., Nelsen, M. P., Neuwirth, G., Nutakki, A. & Parnmen, S. 2015. Hidden diversity in the morphologically variable script lichen (*Graphis scripta*) complex (*Ascomycota*, *Ostropales*, *Graphidaceae*). *Organisms Diversity and Evolution* 15: 447–458. https://doi.org/10.1007/s13127-015-0219-5

Kraichak, E., Huang, J. P., Nelsen, M., Leavitt, S. D. & Lumbsch, H. T. 2018. A revised classification of orders and families in the two major subclasses of *Lecanoromycetes* (*Ascomycota*) based on a temporal approach. *Botanical Journal of the Linnean Society* 188(3): 233–249. https://doi.org/10.1093/botlinnean/boy060

Kuraku, S., Zmasek, C. M., Nishimura, O. & Katoh, K. 2013. aLeaves facilitates on-demand exploration of metazoan gene family trees on MAFFT sequence alignment server with enhanced interactivity. *Nucleic Acids Research* 41: W22–W28. https://doi.org/10.1093/nar/gkt389

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

Lücking, R. 2014. A key to species of the *Ocellularia papillata*, *perforata* and *terebrata* morphodemes (*Ascomycota*: *Graphidaceae*). *Glalia* 6(3): 1–35.

Lücking, R. 2019. Stop the abuse of time! Strict temporal banding is not the future of rank-based classifications in fungi (including lichens) and other organisms. *Critical Reviews in Plant Sciences* 38(3): 199–253. https://doi.org/10.1080/07352689.2019.1650517

Lücking, R. & Pérez-Ortega, S. 2015. Four new species of *Ocellularia* (lichenized *Ascomycota*: *Graphidaceae*) from Cuba, with a revised taxonomy of the O. bahiana complex and a key to thelotremoid taxa with small, brown, (sub-) muriform ascospores. *The Lichenologist* 47(5): 305–322. https://doi.org/10.1017/S0024282915000286

Manawasinghe, I. S., Hyde, K. D., Wanasinghe, D. N., Karunarathna, S. C., Maharachchikumbura, S. S., Samarakoon, M. C., Voglmayr, H., Pang, K. L., Chiang, M. W., Jones, E. G., Saxena, R. K. et al. 2024. Fungal diversity notes 1818–1918: taxonomic and phylogenetic contributions on genera and species of fungi. *Fungal Diversity* 130: 1–261. https://doi.org/10.1007/s13225-024-00541-y

Mangold, A., Martín, M. P., Lücking, R. & Lumbsch, H. T. 2008. Molecular phylogeny suggests synonymy of *Thelotremataceae* within *Graphidaceae* (*Ascomycota*: *Ostropales*). *Taxon* 57: 476–486. https://doi.org/10.2307/25066016

Mangold, A., Elix, J. A. & Lumbsch, H. T. 2009. *Thelotremataceae*. In: McCarthy, P. M. (ed.), *Flora of Australia* Volume 57. Lichens 5. ABRS and CSIRO Publishing, Canberra and Melbourne. xx + 687 pages, 195–420.

Marshall, A. J., Blanchon, D. J., Lücking, R., de Lange, T. J. & de Lange, P. J. 2020. A new *Ocellularia* (lichenized *Ascomycota*: *Graphidaceae*) from New Zealand indicates small-scale differentiation of an Australasian species complex. *New Zealand Journal of Botany* 58: 223–235. https://doi.org/10.1080/0028825X.2019.1701504

Meyer, G. 1825. *Nebenstudien meiner Beschäftigungen im Gebiete der Pflanzenkunde*. Goettingen, Vandenhoeck und Ruprecht.

Morgulis, A., Coulouris, G., Raytselis, Y., Madden, T. L., Agarwala, R. & Schäffer, A. A. 2008. Database indexing for production MegaBLAST searches. *Bioinformatics* 24: 1757–1764. https://doi.org/10.1093/bioinformatics/btn322

Nagarkar, M. B., Sethy, P. K. & Patwardhan, P. G. 1986. Materials for a lichen flora of the Andaman Islands. I. *Mycotaxon* 27: 71–82. https://doi.org/10.5962/p.418885

Nagarkar, M. B., Sethy, P. K. & Patwardhan, P. G. 1987. Materials for a lichen flora of the Andaman Islands. V. *Mycotaxon* 29: 335–343. https://doi.org/10.5962/p.418949

Nylander, J. A. A. 2004. MrModeltest 2.3. Program distributed by the author. Evolutionary Biology Centre, Uppsala University. Available from: http://www.abc.se/~nylander/ [accessed 27 Feb. 2026]

Orange, A., James, P. W. & White, F. J. 2001. *Microchemical methods for the identification of lichens*. British Lichen Society, London.

Patwardhan, P. G. & Kulkarni, C. R. 1977. A contribution to our knowledge of the lichen flora of India. I. Family *Thelotremataceae. Kavaka* 5: 1–17.

Rambaut, A. 2014. FigTree. Available from: http://tree.bio.ed.ac.uk/software/figtree/ [accessed 27 Feb. 2026]

Rivas Plata, E., Lücking, R. & Lumbsch, H. T. 2012. Molecular phylogeny and systematics of the *Ocellularia* clade (*Ascomycota*: *Ostropales*: *Graphidaceae*). *Taxon* 61(6): 1161–1179. https://doi.org/10.1002/tax.616001

Rivas Plata, E., Parnmen, S., Staiger, B., Mangold, A., Frisch, A., Weerakoon, G., Hernandez, J., Caceres, M., Kalb, K., Sipman, H. & Common, R. S. 2013. A molecular phylogeny of *Graphidaceae* (*Ascomycota*, *Lecanoromycetes*, *Ostropales*) including 428 species. *MycoKeys* 6: 55–94. https://doi.org/10.3897/mycokeys.6.3482

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A. & Huelsenbeck, J. P. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542. https://doi.org/10.1093/sysbio/sys029

Sinha, G. P., Nayaka, S. & Mishra, G. K. 2025. A comprehensive checklist of lichens from India – 2024. *Cryptogam Biodiversity and Assessment* 8: 1–34.

Sipman, H. J. M., Lücking, R., Aptroot, A., Chaves, J. L., Kalb, K. & Umaña-Tenorio, L. 2012. A first assessment of the Ticolichen biodiversity inventory in Costa Rica and adjacent areas: the thelotremoid *Graphidaceae* (*Ascomycota: Ostropales*). *Phytotaxa* 55: 1–214. https://doi.org/10.11646/phytotaxa.55.1.1

Stamatakis, A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 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: 758–771. https://doi.org/10.1080/10635150802429642

Stizenberger, E. 1862. Beitrag zur Flechtensystematik. *Bericht über die Thätigkeit der St. Gallischen Naturwissenschaftlichen Gesellschaft* 1861–1862: 124–182.

Trifinopoulos, J., Nguyen, L. T., von Haeseler, A. & Minh, B. Q. 2016. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Research* 44: W232–W235. https://doi.org/10.1093/nar/gkw256

Vainio, E. A. 1921. Lichenes Insularum Philippinarum III. *Annales Academiae Scientiarum Fennicae*, Ser. A, 15(6): 1–368.

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

Wijayawardene, N. N., Hyde, K. D., Al-Ani, L. K. T., Tedersoo, L., Haelewaters, D., Rajeshkumar, K. C., Zhao, R. L., Aptroot, A., Leontyev, D. V., Saxena, R. K. & Tokarev, Y. S. 2020. Outline of Fungi and fungus-like taxa. *Mycosphere Online: Journal of Fungal Biology* 11(1): 1060–1456. https://doi.org/10.5943/mycosphere/11/1/8

Zoller, S., Scheidegger, C. & Sperisen, C. 1999. PCR primers for the amplification of mitochondrial small subunit ribosomal DNA of lichen-forming ascomycetes. *The Lichenologist* 31: 511–516. https://doi.org/10.1006/lich.1999.0220