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Two novel species of *Lachnaceae* (*Helotiales, Leotiomycetes*) from southwestern China

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Abstract
Two new species of *Lachnaceae*, *Erioscyphella latispora* and *Proliferodiscus longisporus* were discovered from a newly fallen trunk and the bark of *Liquidambar formosana*, respectively, in Yunnan Province, China. *Erioscyphella latispora* is distinguished from other species by its long stipe, scaled, tilted outward white hairs, poorly-developed ectal excipulum and wide, fusiform ascospores. *Proliferodiscus longisporus* is distinguished from other species by pale brownish orange hairs with resinous materials and larger asci and ascospores. Phylogenetic analyses of the nLSU-ITS-*RPB2* show that the *Erioscyphella latispora* and *Proliferodiscus longisporus* form monophyletic lineages with high ML bootstrap and BI posterior probability support. Detailed descriptions and illustrations are provided for *Erioscyphella latispora* and *Proliferodiscus longisporus*.

Keywords – 2 new taxa – *Erioscyphella* – phylogeny – *Proliferodiscus* – taxonomy

Introduction

Lachnaceae (Nannf.) Raitv. was raised by Raitviir (2004) from a tribe to a family based on suggested strong monophyly (Hosoya et al. 2010). Members of *Lachnaceae* are usually lignicolous, with hyaline or brown hairs, globose to filiform ascospores and lanceolate or cylindrical paraphyses (Raitviir 2004, Hosoya et al. 2010, Ekanayaka et al. 2019). The family presently accommodates 15 genera (Wijayawardene et al. 2022). Most genera of *Lachnaceae* are distributed in all climatic zones, especially in tropical and the north temperate zones (Tochihara & Hosoya 2022). A total of six genera, *Albotricha*, *Erioscyphella*, *Lachnellula*, *Lachnum*, *Perrotia* and *Proliferodiscus*, were reported in China (Zhuang 2000, Zhuang & Hyde 2001, Zhuang et al. 2002, Ye et al. 2006). Among them, *Erioscyphella* and *Proliferodiscus* comprised typical, lignicolous taxa that are usually found on the bark of wood and formed a sister clade with each other in the phylogenetic analysis based on 15 concatenated gene loci (Johnston et al. 2019).

Erioscyphella Kirschst. was initially established to accommodate two species, *Erioscyphella longispora* and *E. bambusina* (Kirschstein 1939), then some members of *Erioscypha* with

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lanceolate paraphyses and 'long-spored *Lachnum*' in which ascospores appeared pigmented were placed in *Erioscyphella* (Hosoya et al. 2010). Former studies (Hosoya et al. 2010, Jayasiri et al. 2015, Tochihara & Hosoya 2022) indicated the limitation of morphological characteristics and proposed a new concept based on the examination of Japanese materials. *Erioscyphella* is characterized by scattered and cupulate apothecia on leaves or bamboo sheaths, straight or irregularly curved, septate and granulated hairs, mostly covered by apical amorphous materials or resinous material, lanceolate or filiform paraphyses, 8-spored asci with an amyloid apical pore and fusiform to long needle-like ascospores (Kirschstein 1939, Perić & Baral 2014, Tochihara & Hosoya 2022). *Erioscyphella* is widely distributed in America, Asia, Australia and Europe, and a total of 19 legitimate species were reported (MycoBank 2022). In China, eight species have described, including *Erioscyphella abnormis E. brasiliensis, E. fusiforme* (Ekanayaka et al. 2019), *E. hainanensis* (Tochihara & Hosoya 2022), *E. lunata* (Zhuang 2000), *E. lushanensis* (Perić & Baral 2014), *E. sclerotii* (Ekanayaka et al. 2019) and *E. sinensis* (Yu & Zhuang 2002).

Proliferodiscus is distinguished from *Erioscyphella* by its lateral proliferous disc (Haines & Dumont 1983). It was erected to accommodate two species with proliferous hymenial discs, *Proliferodiscus inspersus* and *P. earoleuca*, and placed in *Lachnaceae* (Haines & Dumont 1983). The teleomorph of *Proliferodiscus* is usually characterized by colorful proliferous apothecia with a short stipe, hyaline, thin-walled curled hairs that are densely granulated or covered by hyaline, amorphous material, ectal excipulum consisting of *textura prismatica* cells, filiform paraphyses, 8-spored asci and hyaline ascospores (Haines & Dumont 1983, Spooner 1987, Popov 2013, Ekanayaka et al. 2019). Moreover, the only anamorph described is of *Proliferodiscus ingens* which was characterized by subglobose conidiomata on the medium, hyaline, septate, smooth-walled conidiophores, and cylindrical to ellipsoidal, hyaline, aseptate conidia (Bien & Damm 2020). Members of *Proliferodiscus* are distributed mainly in America, Asia, Australia and Europe, but only one species, *P. dingleyae*, was reported in China (Zhuang et al. 2002).

We collected four samples of *Erioscyphella* and *Proliferodiscus* during the investigation in southwest China. Based on the combination of morphological and molecular data, we propose them as two novel species and provide detailed morphological descriptions for our collections.

Materials & methods

Sample collection, morphological studies, and isolation

Fresh materials were collected from the newly fallen wood, decayed wood and a living tree of *Liquidambar formosana* in Kunming city, Yunnan Province, China in 2021. The dried collections are deposited at the Herbarium in Kunming Institute of Botany, Chinese Academy of Sciences (KUN). Macrophotographs were obtained from fresh and dried apothecia by a Canon EOS M100 camera in the field and laboratory. For microphotographs, dried apothecia were sectioned by hand using razor blades and photographed by a charge-coupled device SC 2000C attached to a Nikon ECLIPSE Ni-U compound microscope (Model Eclipse Ni-U Nikon Corporation Tokyo, Japan). Vertical sections were used to observe the excipulum and hymenium. Asci, ascospores and paraphyses were observed by mounting squashed mature apothecia in water. The blue iodine reaction of the ascus apex was checked using Melzer's reagent. The measurements of ascospores were shown as (a–)b–c(–d), where a and d indicate the maximum and minimum values observed, b–c indicates the 90% confidence interval, Q value indicates the length to width ratio of ascospores, Q_m indicates the average of Q values and n indicates the number of measured structures. Other measurements were shown as (a–d) and $\overline{x}$, which indicates the mean of all measured values. All the lengths and widths were obtained using a measurement software, Tarosoft (R) Image Framework program (IFW), and images were processed with Adobe Photoshop 2020 (Adobe system, USA). Colors of apothecia are determined following Kornerup & Wanscher (1967).

Single spore isolation was performed following Senanayake et al. (2020). Cultures were grown on potato dextrose agar (PDA) medium at 25 ℃ for six weeks. The pure cultures were deposited at the Kunming Institute of Botany Culture Collection (KUNCC22-12441). Faces of

fungi number (Jayasiri et al. 2015) and Index Fungorum number (Index Fungorum 2022) were obtained, and the details are added to the Greater Mekong Subregion webpage (Chaiwan et al. 2021).

DNA Extraction, PCR Amplification, and Sequencing

Total genomic DNA was extracted using Trilief™ Plant Genomic DNA Kit (Tsingke biological technology Co., LTD, Beijing, China) from both dried apothecia and fresh mycelium. The D_1/D_2 domain of the nuclear large subunit ribosomal RNA (nLSU), the nuclear internal transcribed spacer (ITS) and RNA polymerase II second largest subunit regions (*RPB2*) were amplified using primer pairs LROR and LR5 (Vilgalys & Hester 1990), ITS1-F and ITS4 (Gardes & Bruns 1993), and f*RPB2*-5F and f*RPB2*-7cR (Liu et al. 1999), respectively. The total reaction volume was 25 µl containing 12.5 µl of 2 × Power Taq PCR MasterMix, 7.5 µl sterile deionized water, 1 µl of each primer (100 µM stock), and 3 µl of DNA template. Amplifications were performed in a TC-type gene amplifier (LifeECO) (Hangzhou Bori Technology Co., LTD, Hangzhou Province, China). The PCR amplification for all genes was performed by following Zeng et al. (2020). The PCR products were verified by 1% agarose gel electrophoresis followed by staining with TS-GelRed Ver.2 10,000 × in Water (Tsingke biological technology Co., LTD, Beijing, China). Products were sequenced at Tsingke biological technology Co., LTD, Beijing, China.

Sequence assembly and alignment

All reverse and forward sequences were assembled in ContigExpress (Invitrogen, USA) and edited in BioEdit 7.2.5.0 (Hall 1999). The homologous sequences were selected based on the results of the blastn search performed against the GenBank database available at NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The newly derived sequences and all related sequences of *Erioscyphella* and *Proliferodiscus* in *Lachnaceae* from previous studies (Kirschstein 1939, Haines & Dumont 1983, Spooner 1987, Popov 2013, Perić & Baral 2014, Ekanayaka et al. 2019, Tochihara & Hosoya 2022) were obtained from GenBank and used for phylogenetic analyses. Three species in *Hymenoscyphus* (*Helotiaceae*) were selected as the outgroup taxa (Hosoya et al. 2010, Tochihara & Hosoya 2022).

The datasets of nLSU and ITS were aligned in the MAFFT version 7 (http://mafft.cbrc.jp/align- mnet/server), with G-INS-i as the iterative refinement method with default parameters except for the gap penalty, which was changed to 1.00. The alignments were edited and adjusted manually in BioEdit 7.2.5.0, where necessary. Then datasets were trimmed in TrimAl v.1.3 using the gapthreshold option at 0.5 (Capella-Gutiérrez et al. 2009). Finalized datasets of each gene were concatenated to a combined dataset in Sequence Matrix 1.7.8. (Vaidya et al. 2011) with the order 'nLSU-ITS' for both genera.

Phylogenetic analyses

Maximum likelihood (ML) analysis was performed in RAxML-HPC2 on XSEDE (8.2.12) on the CIPRES Science Gateway platform (http://www.phylo.org/portal2) using the GTR model with 1,000 bootstrap replications. Bayesian inference analysis was performed using MrBayes v. 3.1.2 to evaluate the posterior probability by Markov Chain Monte Carlo sampling (MCMC). The symmetrical model with a discrete gamma distribution coupled with a proportion of an invariant (SYM+I+G) for RPB2 and the general time-reversible model with a discrete gamma distribution coupled with a proportion of an invariant (GTR+I+G) for nLSU and ITS were selected as the best models using MrModeltest v.2.3. (Nylander et al. 2004). Four simultaneous Markov Chains were run for 2,000,000 generations, with trees sampling at every 100th generation. The 25% of trees representing the burn-in phase were discarded, and the remaining trees were used to calculate the posterior probability. The finalized phylogenetic tree was visualized in Figtree v.1.4.0 (Rambaut 2009) and edited in Adobe Illustrator 2020 and Adobe Photoshop 2020 (https://www.adobe.com/). Sequences of new collections in this study were deposited in the GenBank (Table 1).

Table 1 Names, collection numbers and corresponding GenBank/UNITE accession numbers of the taxa used in this study.

Taxon name	**Collection number**	**Gene accession numbers**		
		nLSU	**ITS**	***RPB2***
Albotricha acutipila	K(M):170694	MZ159436	-	-
Albotricha albotestacea	FC-2094	AB481235	-	-
Brunnipila calycioides	PRM:901519	LT904855	-	-
Brunnipila dumorum	SBRH 835	KX501125	-	-
Brunnipila fuscescens	KUS-F52031	JN033392	JN086695	JN086846
Capitotricha bicolor	PRM:915564	LT904864	-	-
Capitotricha rubi	TNS-F-65752	LC438560	LC438573	-
Dasyscyphella montana	FC-2031	AB481241	-	-
Dasyscyphella montana	FC-2070	AB481242	-	AB481336
Erioscyphella abnormis	TNS-F-80478	LC424949	LC424837	LC425009
Erioscyphella alba^{T}	MFLU 16-0614	NG_066457	NR_163782	-
Erioscyphella aseptata^{T}	MFLU 16-0590	NG_066456	NR_163780	MK388223
Erioscyphella boninensis^{T}	TNS-F-26520	LC533151	UDB0779049	LC533196
Erioscyphella brasiliensis	MFLU 16-0577a	-	MK584953	MK388221
Erioscyphella brasiliensis	MFLU 16-0577b	MK591993	MK584967	-
Erioscyphella curvispora^{T}	KL381	MH190415	MH190414	-
Erioscyphella euterpes	PR147	-	U58640	-
Erioscyphella fusiforme	MFLU 15-0230	NG_066455	NR_154122	MK388220
Erioscyphella fusiforme	MFLU 18-1824	MK591975	MK584948	-
Erioscyphella hainanensis	TNS-F-35056	LC533169	UDB0779065	LC533206
Erioscyphella hainanensis	TNS-F-35049	LC533168	UDB0779064	LC533205
Erioscyphella insutae	TNS-F-26500	LC533149	UDB0779060	LC533194
Erioscyphella insutae^{T}	TNS-F-39720	LC533177	UDB0779063	LC533207
Erioscyphella latispora	**HKAS 124391**	**OP113850**	**OP113849**	**OP715727**
***Erioscyphella latispora*^{T}**	**HKAS 124389**	**OP113844**	**OP310823**	**OP715728**
Erioscyphella lunata	S.T. 13021602	KX501133	KX501132	-
Erioscyphella lushanensis^{T}	3631	-	AF505515	-
Erioscyphella otanii^{T}	TNS-F-81472	LC533179	UDB0779085	LC533226
Erioscyphella papillaris	TNS-F-81272	LC533161	UDB0779081	LC533204
Erioscyphella paralushanensis^{T}	TNS-F-61920	LC533141	UDB0779075	LC533220
Erioscyphella sasibrevispora	TNS-F-80399	LC533173	UDB0779082	LC533216
Erioscyphella sasibrevispora^{T}	TNS-F-81401	LC533174	UDB0779084	LC533217
Erioscyphella sclerotii	MFLU 18-0688	MK591995	MK584969	-
Erioscyphella sclerotii	MFLU 16-0569	MK591980	MK584951	MK388219
Erioscyphella sinensis	TNS-F-16838	LC533164	AB481280	AB481364
Erioscyphella sinensis	TNS-F-80354	LC533143	UDB0779083	LC533187
Hymenoscyphus albidoides^{T}	HMAS 264140	NG_059508	NR_154903	-
Hymenoscyphus fructigenus	TNS-F-44644	AB926144	AB926057	AB926189
Incrucipulum ciliare	TNS-F-81516	LC438565	LC438582	LC438595
Incrucipulum longispineum	FC-2323	AB481256	AB481325	AB481362
Lachnellula calyciformis	CBS:189.66	MH858771	MH870403	-
Lachnellula flavovirens	CBS:191.66	KC464637	KC492975	-
Lachnum asiaticum	FC-2056	AB481251	AB481297	-
Lachnum pudibundum	FC-2058	AB481259	AB481298	AB481335
Lachnum rachidicola	TNS-F-16647	AB745431	-	-
Lachnum virgineum	FC-2137	AB705235	AB705275	-
Neodasyscypha cerina	TNS-F-65625	LC424836	LC424948	-
Proliferodiscus alboviridis	TNS-F-17436	LC424950	LC438558	LC425014
Proliferodiscus chiangraiensis^{T}	MFLU 16-0588	NG_068622	NR_164304	-
Proliferodiscus dingleyae	ICMP:21730	-	MH682231	-

Table 1 Continued.

Taxon name	Collection number	Gene accession numbers		
		nLSU	**ITS**	***RPB2***
Proliferodiscus earoleucus	BHI-F624d	-	MF161304	-
Proliferodiscus ingens[T]	CBS:145519	NG_073756	NR_170767	-
Proliferodiscus longisporus	**HKAS 124388**	**OP264080**	**OP113842**	**OP715729**
Proliferodiscus longisporus[T]	**HKAS 124390**	**OP310824**	**OP113840**	**OP715730**
Proliferodiscus pulveraceus	G.M. 2017-03-21.3	-	MN066320	-
Proliferodiscus tricolor	CBS:122000	KC492981	KC464643	-
Proliferodiscus tricolor	CBS:128288	MH876293	MH864846	-

* The newly generated sequences are shown in bold black and T indicates the type specimens.

Results

Phylogenetic analyses

In Fig. 1, the combined nLSU+ITS+*RPB2* dataset consists of 46 taxa, represented by 57 isolates, and 2159 aligned nucleotide sites, including 866 bp for the nLSU region, 537 bp for the ITS region and 756 bp foe the *RPB2* region with gaps. The combined alignment contained 595 parsimony-informative characters, 135 variable and parsimony uninformative characters, and 1429 constant characters. The maximum likelihood matrix had 847 distinct alignment patterns with 31.19% undetermined characters and gaps. Estimated base frequencies were as follows: A = 0.248057, C = 0.220105, G = 0.274656, T = 0.257181; substitution rates AC = 1.685678, AG = 4.732248, AT = 1.685697, CG = 1.156650, CT = 10.270895, GT = 1.000000; gamma distribution shape parameter α = 0.198851. Final likelihood value of the best tree given in Fig. 1 is -15062.835812. In Fig. 2, the combined nLSU+ITS dataset consists of 30 taxa, represented by 42 isolates, and 1385 aligned nucleotide sites, including 849 bp in the nLSU region and 533 bp in the ITS region. The combined alignment contained 270 parsimony-informative characters, 84 variable and parsimony uninformative characters, and 1031 constant characters. The maximum likelihood matrix had 433 distinct alignment patterns with 13.76% undetermined characters and gaps. Estimated base frequencies were as follows: A = 0.242848, C = 0.219653, G = 0.281623, T = 0.255876; substitution rates AC = 0.918309, AG = 2.107016, AT = 1.191742, CG = 0.589855, CT = 6.130315, GT = 1.000000; gamma distribution shape parameter α = 0.186399. Final likelihood value of the best maximum likelihood tree (Fig. 2) is -6700.256351. In our study, *Erioscyphella latispora* formed an independent clade sister to *Erioscyphella otanii* and *E. papillaris* with 82% ML bootstrap and 0.99 BI posterior probability support. *Proliferodiscus longisporus* clustered separately, sister to *P. tricolor* with 100% ML bootstrap and 1.00 BI posterior probability support. Therefore, these provide strong evidence for our collections as new species. *Erioscyphella* spp. and two members of *Proliferodiscus* clustered in a clade with low statistical support, and *Proliferodiscus* taxa clustered as a paraphyletic group in the phylogenetic analysis (Fig. 1). However, further analysis with genes, nLSU and ITS (Fig. 2) showed that members of *Proliferodiscus* clustered with high statistical support (97% ML-BP and 1.00 BI-PP).

Erioscyphella latispora C.J.Y. Li & Q. Zhao, sp. nov. Fig. 3

Index Fungorum number: IF 559355; Facesoffungi Number: FoF 12759

Etymology – The specific epithet refers to the wide ascospores.

Holotype – HKAS 124389.

Saprobic on the newly fallen trunk. Sexual morph: *Apothecia* 1–2.5 mm wide × 1.5–3.8 mm high ($\bar{x}$ = 1.6 × 2.5 mm, n = 50) when dry, superficial, arising scattered or in clusters, cup-shaped, long-stipitate, covered with long, white hairs. *Disc* circular, concave and smooth, deep yellow to orange-yellow (4A8-4B8). *Receptacle* cupulate, concolorous, covered with white hairs. *Stipe* 0.3–0.7 mm wide × 1–2.8 mm long ($\bar{x}$ = 0.5 × 1.7 mm, n = 30), concolorous with receptacle, long and narrow with whitish hairs, blackish and hairless base. *Hairs* 69–89 μm long × 3.4–4.4 μm wide ($\bar{x}$ =

80 × 3.8 mm, n = 10), scaled and tilted outward, arising from the outermost layer cells of ectal excipulum, slightly curved, cylindric with blunt ends, thin-walled, densely covered with colorless granules, septate, hyaline. *Hymenium* 154–173 µm, hyaline to pale yellow. *Ectal excipulum* 23–29 µm thick, comprised of 1–3 layers, thin-walled, poor-developed hyaline cells of *textura angularis* at the receptacle, comprised of thin-walled, septate, hyaline hyphae of *textura intricata* with yellowish contents at stipe, non-gelatinous. *Medullary excipulum* 152–267 µm thick, 1.9–3.9 µm wide, comprised of thin-walled, septate, hyaline, loosely packed hyphae of *textura intricata* with yellowish contents, non-gelatinous. *Subhymenium* 41–50 µm, hyaline to pale yellow (4A3), comprising tightly packed hyphae of *textura intricata* with yellowish contents. *Paraphyses* 2.2–3.8 µm ($\overline{x}$ = 2.9 µm, n = 30) in the widest, hyaline, thick-walled, narrow lanceolate, septate, unbranched, observed conspicuous contents, extending beyond the asci. *Asci* 121–142 × 9–12 µm ($\overline{x}$ = 132 × 10 µm, n = 40), 8-spored, cylindric or subclavate, rounded to subconical apex with J+ apical ring in Melzer's reagent, tapering to subtruncate base. *Ascospores* (21–)22–27(–31) × (4.1–)4.4–5.4(–5.8) µm ($\overline{x}$ = 25 × 4.9 µm, n = 100), Q = (4.2–)4.4–5.7(–6.1) µm, Q_m = 5.0 ± 0.2 µm, overlapping uniseriate, fusiform with sharp ends, hyaline, thin-walled, smooth, 3–4-septate, four or more large guttules.

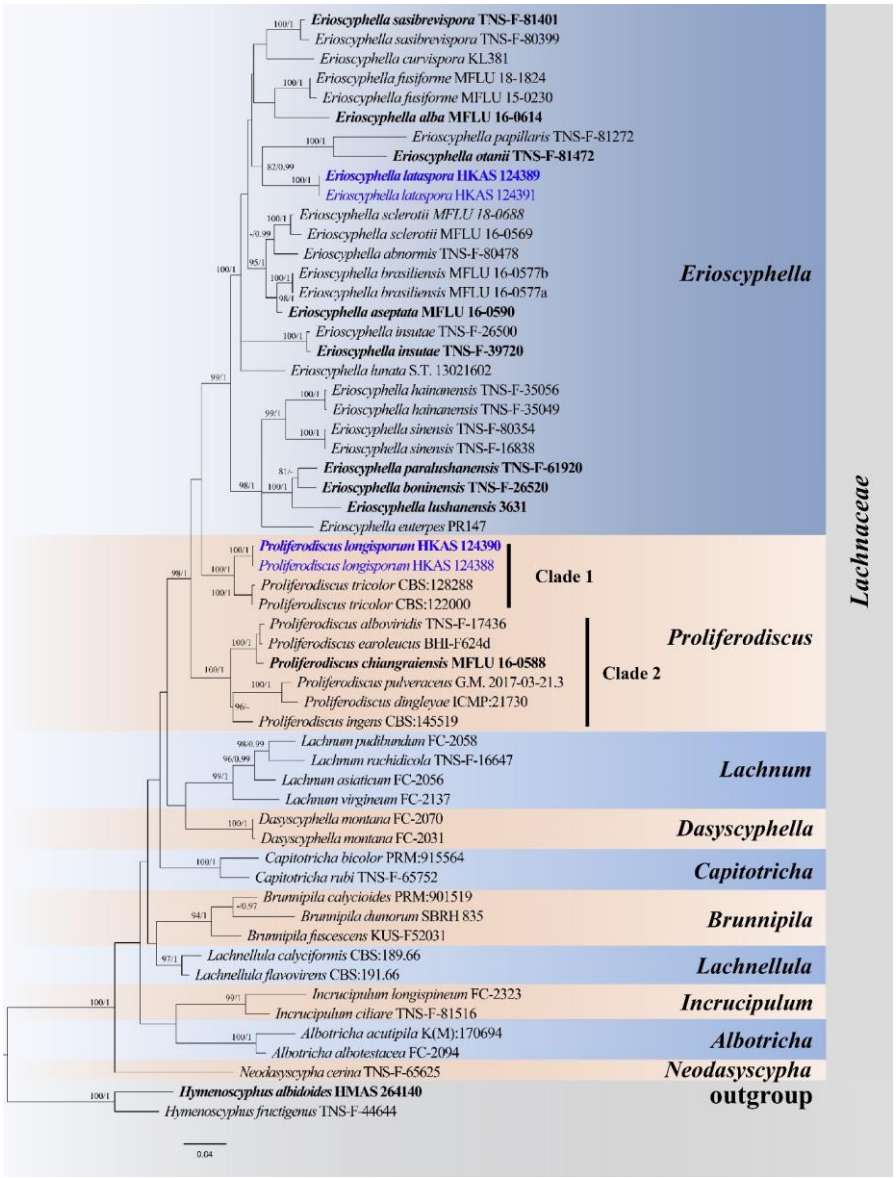

Fig. 1 – Maximum likelihood tree generated from RAxML analyses based on the combined dataset of nLSU, ITS and *RPB2* sequences, showing the phylogenetic position of *Erioscyphella latispora*

and *Proliferodiscus longisporus*. The ML bootstrap proportions (ML-BP) higher than 80% and Bayesian posterior proportions (BI-PP) higher than 0.95 are shown above the branches on the phylogenetic tree with the order 'MP/BI/ML'. Newly generated sequences are shown in blue. Ex-types are shown in bold black.

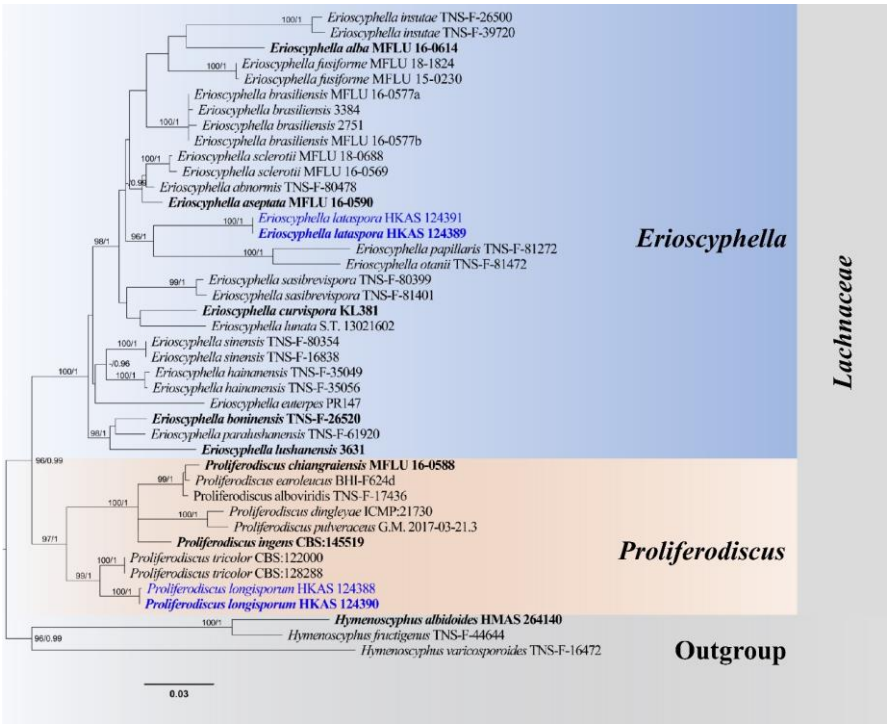

Fig. 2 – Maximum likelihood tree generated from RAxML analyses based on the combined dataset of nLSU and ITS sequences, showing the phylogenetic position of *Erioscyphella latispora* and *Proliferodiscus macrospora*. The ML bootstrap proportions (ML-BP) higher than 90% and Bayesian posterior proportions (BI-PP) higher than 0.95 are shown above the branches on the phylogenetic tree with the order 'ML/ BI'. Newly generated sequences are shown in blue. Ex-types are shown in bold black.

Asexual morph – Undetermined.

Material examined – China, Yunnan Province, Puer City, Jingdong County, altitude 2500m, on the newly fallen trunk, 30 July 2021, Cuijinyi Li, LCJY-269 (HKAS 124389, holotype); *ibid*, Kunming City, Sanjian mountain, altitude 1950m, on decayed wood, 18 December 2021, Cuijinyi Li, LCJY-359 (HKAS 124391, paratype).

Notes – The distinctive characteristics of *Erioscyphella latispora* are large, cup-shaped apothecia, long stipe, scaly and long white hairs with dense, hyaline granules, lanceolate and septate paraphyses and wide, fusiform, 3-septate ascospores. Morphologically, *Erioscyphella latispora* resembles *E. sasibrevispora* with regards to the receptacle shape, yellow to pale orange discs and lanceolate paraphyses. In contrast to *E. sasibrevispora*, *E. latispora*, has longer stipes (1.7 mm *vs.* 0.8 mm) and larger asci (121–142 × 9–12 µm vs. 82.5–90 × 6.6–8.1 µm) (Tochihara & Hosoya 2022). *Erioscyphella paralushanensis* exhibits characteristics similar to *E. latispora*, such as the apothecial shape and size but differs in red hairs and shorter asci (61.4–70.2 × 4.7–5.6 µm *vs.* 121–142 × 9–12 µm) (Tochihara & Hosoya 2022). *Erioscyphella alba* shares some similar characteristics with regards to the apothecia of *E. latispora* but is distinguished by having straight hairs, shorter asci (42.1–48.9 × 4.9–6.3 µm *vs.* 121–142 × 9–12 µm) and smaller ascospores (11.1–13.4 × 1.8–2.4 µm *vs.* 22–27 ×4.4–5.4) (Ekanayaka et al. 2019).

Phylogenetically, our collections formed a clade and sister to *Erioscyphella otanii* and *E. papillaris* with 96% ML bootstrap support and 1.00 Bayesian probability in the nLSU-ITS multi-gene phylogeny (Fig. 2). Although *Erioscyphella latispora* has a closer phylogenetic relationship

and similar traits of fusiform ascospores with *E. otanii* and *E. papillaris*, the latter two are distinguished from *E. latispora* by having narrow ascospores, small apothecia and ascospores and obvious ectal excipulum cells (Tochihara & Hosoya 2022).

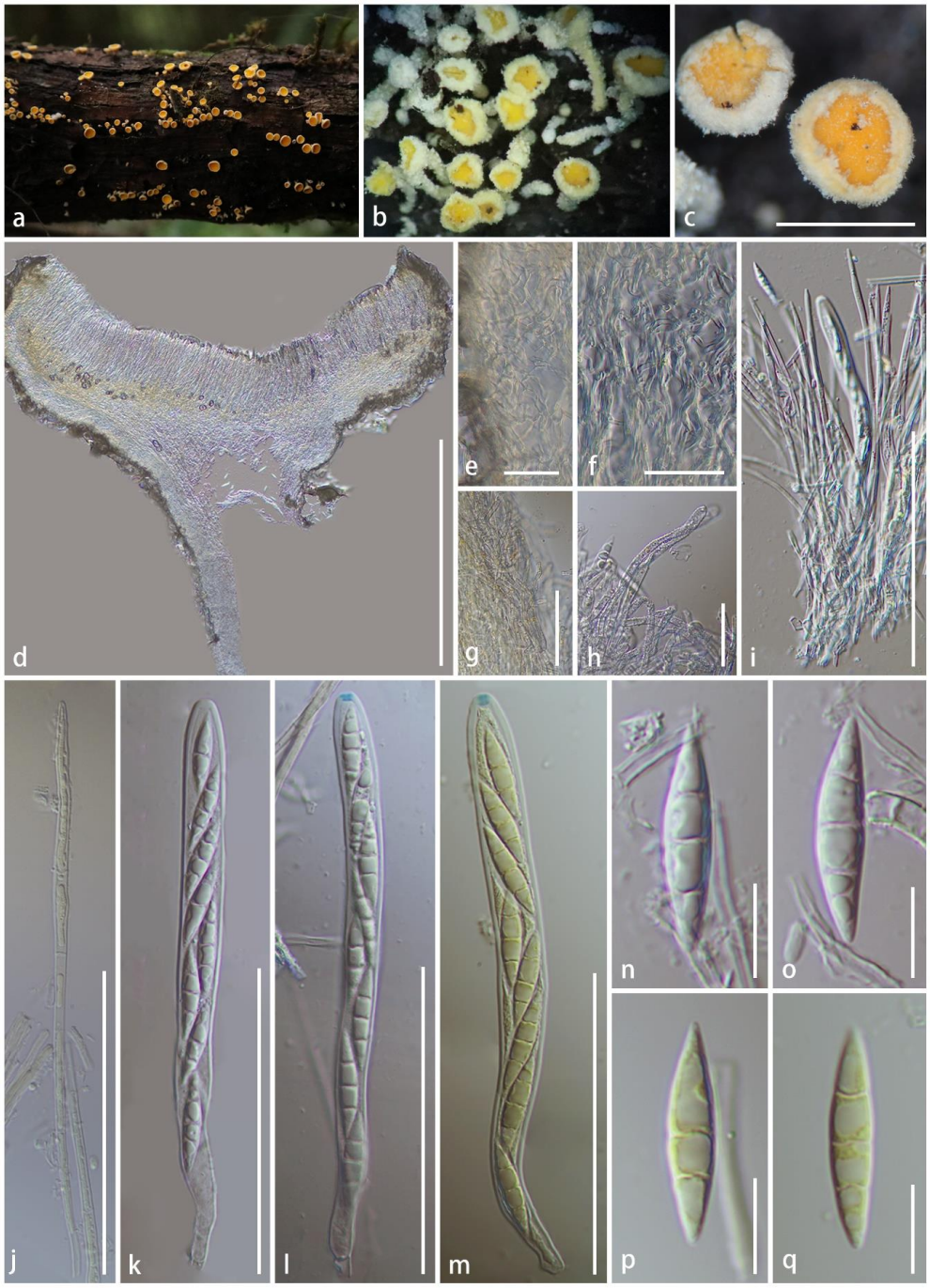

Fig. 3 – *Erioscyphella latispora* (HKAS 124389, holotype). a–b Fresh ascomata on the wood. c Dried ascomata on the wood. d Vertical section of an ascoma. e Ectal excipulum. f Medullary excipulum. g–h Hairs. i–j Paraphyses. k–m Asci (m Asci in Meltzer's reagent). n–q Ascospores (p-q Ascospores in Meltzer's reagent). – Scale bars: c = 1500 μm, d = 500 μm, e = 25 μm, f = 35 μm, g = 50 μm, h = 30 μm, i = 100 μm, j = 50 μm, k–m = 70 μm, n–q = 10 μm.

Proliferodiscus longisporus C.J.Y. Li, K. D. Hyde & Q. Zhao, sp. nov. Fig. 4

Index Fungorum number: IF 559356; Facesoffungi Number: FoF 12760

Etymology – The specific epithet refers to the long ascospores.

Holotype – HKAS 124390.

Parasitic on the bark of *Liquidambar formosana*. Sexual morph: *Apothecia* 1–2.5 mm wide × 0.3–0.5 mm high ($\bar{x}$ = 1.7 × 0.4 mm, n = 20) when fresh, gregarious or scattered, disc-shaped, stipitate, growing on the superficial bark or proliferating from the center of the old apothecia. *Disc* flat and circular with light orange (5A3-5A4) or persion orange (6A6-6B6) when fresh, and brownish-orange (6C8) when dry. *Receptacle* pulvinate, reddish white to reddish-grey (7A2-7B2) with bushy hairs. *Stipe* 0.5–0.7 mm wide × 0.1–0.4 mm long ($\bar{x}$ = 0.6 × 0.3 mm, n = 20), short and broad with white hairs. *Hairs* 167–185 μm long × 2.4–3.1 μm wide ($\bar{x}$ = 180 × 2.8 mm, n = 20), rising from the outermost cells of ectal excipulum, cylindric, irregularly curled, hyaline, pale brownish-orange (5A5) on receptacle, white on stipe, septate, thin-walled, smooth, apex covered by irregular hyaline resinous materials. *Hymenium* 114–128 μm, pale orange (5A3). *Ectal excipulum* 77–93 μm thick, comprised of thin-walled, hyaline to pale brownish-orange (5A5) cells of *textura prismatica*, non-gelatinous. *Medullary excipulum* 110–160 μm thick, comprised of thin-walled, hyaline to pale brownish-orange (5A5) cells of *textura intricata*, non-gelatinous. *Subhymenium* not obvious. *Paraphyses* 2–3 μm ($\bar{x}$ = 2.7 μm, n = 20) wide, hyaline, filiform, cylindrical, rounded apex, branched and septate at base, extending beyond the asci. *Asci* 92–114 × 7–10 μm ($\bar{x}$ = 101 × 8 μm, n = 40), 8-spored, cylindric or subclavate, rounded to subconical apex with J+ apical ring in Melzer's reagent, tapering to subtruncate base, observed croziers at the stipitate base. *Ascospores* (21–)22–30(–40) × (2.9–)3.1–4.3(–4.7) μm ($\bar{x}$ = 25.5 × 3.7 μm, n = 100), Q = (4.8–)5.5–9.1(–10) μm, Q_m = 7.0 ± 1.1 μm, overlapping uniseriate or biseriate, fusoid-clavate with blunt ends, hyaline, slightly asymmetrical and sinuous, thin-walled, smooth, 0–3-septate, with multiple granules in the living state and four or more large guttules when dry.

Asexual morph – Undetermined.

Culture characteristics – Colonies growing on PDA medium, reaching 3 cm diam. in 6–7 weeks at 25℃, circular, umbonate elevation, entire margin, rough surface, white mycelia in the front view, reverse view brownish-yellow in suitable humidity and some red mycelium appear in medium with low humidity, white at the margin, center dark brown; no sporulation.

Material examined – China, Yunnan Province, Kunming City, Panlong District, altitude 1918m, on the bark of *Liquidambar formosana*, 14 September 2021, Cuijinyi Li, LCJY-286 (HKAS 124390, holotype) – ex-type living culture KUNCC22-12441; *ibid*, Yeya lake, altitude 1900m, on the bark of *Liquidambar sp.*, 3 July 2021, Cuijinyi Li, LCJY-123 (HKAS 124388, paratype).

Notes – *Proliferodiscus longisporus* is characterized by its disc-shaped, light orange apothecia, receptacles covered with pale brownish orange hairs, short stipe covered with white hairs, filiform, septate and branched paraphyses, large cylindric asci with amyloid pore and large fusoid-clavate ascospores. Morphologically, *Proliferodiscus longisporus* resembles *P. tricolor* in having similar-sized apothecia, shorter stipes, bushy hairs and cylindric asci, but differs by having light orange discs when fresh and pale brownish orange discs when dry, pale brownish orange hairs with resinous material on receptacles, branched paraphyses and larger ascospores (*P. longisporus* 22–30 × 3.1–4.3 μm *vs. P. tricolor* 10–14 × 3–3.5 um) (Popov 2013). Phylogenetically, our collections formed a clade sister to *Proliferodiscus tricolor* with 99% ML bootstrap support and 1.00 Bayesian probability in nLSU-ITS multi-gene phylogeny (Fig. 2).

Discussion

Based on the previous research on the phylogeny, morphology and ecology of *Lachnaceae* spp. (Hosoya et al. 2010), delimiting generic boundaries in *Lachnaceae* can no longer be defined by morphological characteristics alone, especially for *Erioscyphella*, pigmented ascospores and lanceolate paraphyses have been eliminated as the characteristic features for the genus (Chethana et al. 2021).

The latest concept for *Erioscyphella* was proposed as the typical taxa are recognized mainly by the hair structures (the swollen apices, apical anamorph material/resinous material) and ascospore length (Tochihara & Hosoya 2022). *Lachnum sensu stricto* was distinguished by having anamorph material, and long-spored *Lachnum* members by having rarely swollen apices of hairs

(Tochihara & Hosoya 2022). For the paraphyletic members of long-spored *Lachnum* within *Erioscyphella*, UNITE Species Hypotheses (SH) system analysis based on ITS gene fragment, morphological and ecological data are not enough to clarify the species boundaries (Tochihara & Hosoya 2022). Our collections of *Erioscyphella latispora* can be classified under *Erioscyphella*, distinguished by their 1) wider ascospores than *P. tricolor*'s and lower length to width ratios than other members, 2) poorly differentiated ectal excipulum than other members and 3) scaled and tilted outward white hairs.

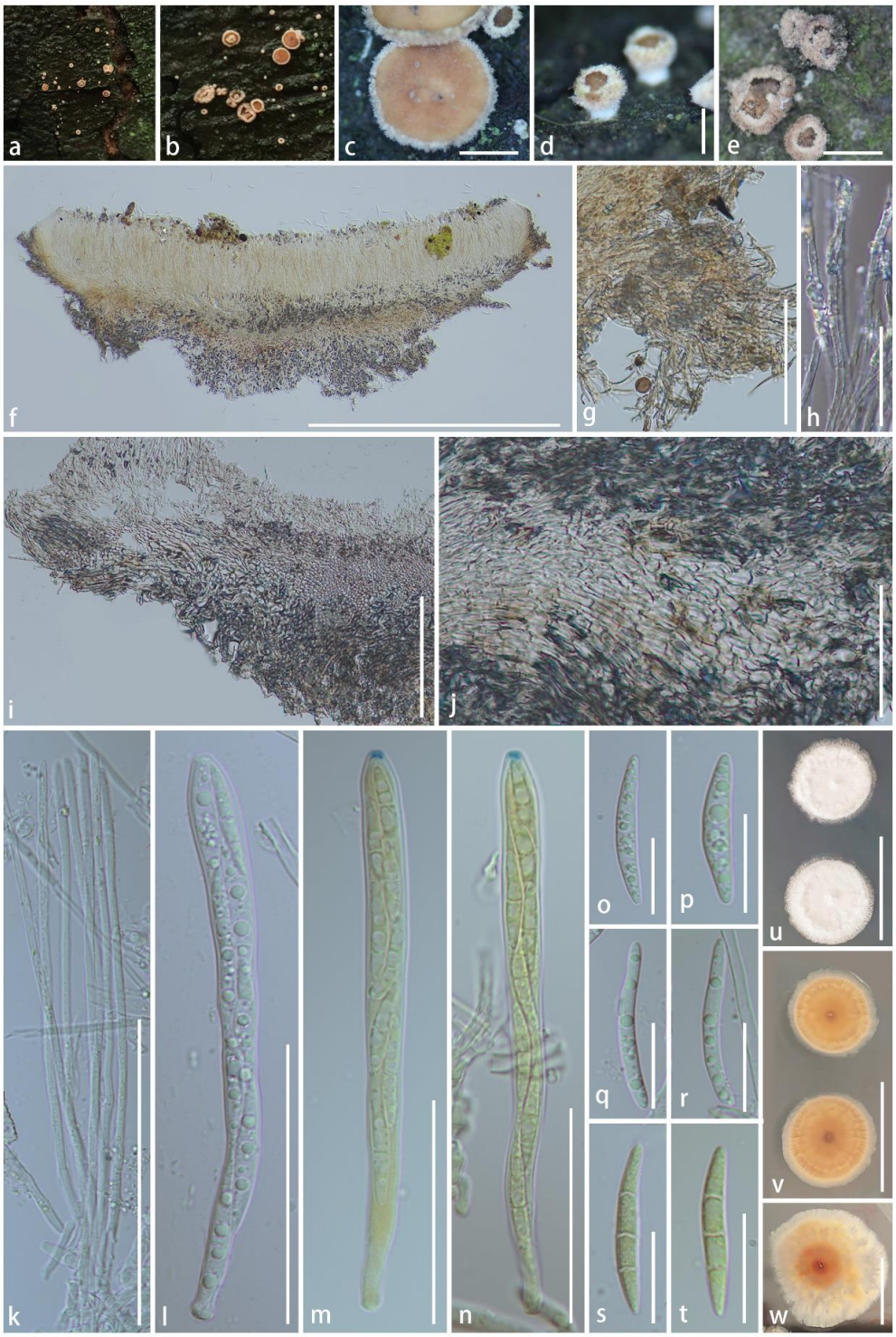

Fig. 4 – *Proliferodiscus longisporus* (HKAS 124390, holotype). a–d Fresh ascomata on the bark. e Dried ascomata on the bark. f Vertical section of ascoma. g–h Hairs. i–j Excipulum. k Paraphyses. l–n Asci (m–n Asci in Meltzer's reagent). o–t Ascospores (s–t Ascospores in

Meltzer's reagent) u–w Colonies on PDA. Scale bar: c = 1 mm, d = 500 μm, e 1500 μm, f = 600 μm, g = 100 μm, h = 30 μm, i = 100 μm, j = 40 μm, k = 80 μm, l = 50 μm, m–n = 40 μm, o–t = 15 μm, u–v = 3 cm, w = 1.5 cm.

As an important generic member of *Lachnaceae*, the most distinctive character of *Proliferodiscus* is the proliferative apothecia (Haines & Dumont 1983). *Proliferodiscus longisporus* has the proliferative apothecia, hence classified as a member of *Proliferodiscus* based on the molecular data. The concept of *P. longisporus* is distinguished from other species by having 1) larger asci, longer ascospores and 2) pale brownish orange hairs with resinous material. The proliferation of *Proliferodiscus* appears from the margin at the lateral aera of the disc (Haines & Dumont 1983, Spooner 1987), especially in *P. inspersus*, *P. earoleucus* and *P. dingleyae*, but the proliferative apothecia of *P. longisporus* only occurred in the center of the disc. Aiming the placement of members in *Proliferodiscus*, we recognized *Proliferodiscus* as a paraphyletic group rather than introducing *P. tricolor* and *P. longisporus* as an independent genus here. The reason that we infer thus is that there are 1) a large number of ambiguous sites in nLSU and *RPB2* genes fragments, 2) morphological features, as well as proliferative apothecia of Clade 1, are closer to *Proliferodiscus*, and 3) more abundant species of Clade 1 provide more evidence for further classification.

Most records of *Erioscyphella* and *Proliferodiscus* are from the tropics (Haines & Dumont 1983, Spooner 1987, Tochihara & Hosoya 2022). Although sample collections have extended to subtropic, temperate and cold-temperate regions (Bien & Damm 2020, Tochihara & Hosoya 2022), collected samples are still scarce. Further, it was found that the lack of available sequences of these species led to *Lachnaceae* and *Helotiaceae* exhibiting paraphyletic nature in the ITS-LSU phylogeny (Johnston et al. 2019, Quandt & Haelewaters 2021). Although Johnston et al. (2019) solved this issue using 15 loci phylogenetic analyses, obtaining genes sequences for multiple loci *Lachnaceae* is still one of the critical problems.

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