Current Research in Environmental & Applied Mycology (Journal of Fungal Biology)
13(1): 16–33 (2023) ISSN 2229-2225
www.creamjournal.org **Article**
Doi 10.5943/cream/13/1/2

Two new species of *Erioscyphella* (*Lachnaceae*) from southwestern China

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Su HL, Chethana KWT, Zeng M, Zhao Q 2023 – Two new species of *Erioscyphella* (*Lachnaceae*) from southwestern China. Current Research in Environmental & Applied Mycology (Journal of Fungal Biology) 13(1), 16–33, Doi 10.5943/cream/13/1/2

Abstract

Erioscyphella is a widely distributed genus characterized by white to orange discs, white to brown receptacles, granulated hairs with amorphous and/or resinous material without swelling apices and crystals, and long ascospores. We collected six specimens from southwestern China. Morphological and phylogenetic analyses based on the combined LSU, ITS, mtSSU and *RPB2* dataset show that our specimens represent two new species of *Erioscyphella* and are described and illustrated in this study. *Erioscyphella griseibambusicola* is characterized by minute, cupulate to globose, grey apothecia occurring on bamboo culms. *Erioscyphella subinsulae* is characterized by a yellow hymenium, white receptacles with short, white hairs, and long, filiform ascospores. In addition, a key to the species of *Erioscyphella* is provided.

Keywords – 2 novel species – *Helotiales* – *Leotiomycetes* – morphology – phylogeny – taxonomy

Introduction

Erioscyphella is a monophyletic genus within *Lachnaceae* (Dennis 1954, Spooner 1987, Cantrell & Hanlin 1997, Guatimosim et al. 2016, Tochihara & Hosoya 2022, Wijayawardene 2020, 2022). The genus is characterized by discoid, rarely urceolate and spherical, white to orange apothecial discs, white to brown, sometimes red receptacles, granulated hairs of uniform thickness, some with hyaline or brown apical amorphous material and/or resinous material, without crystals, filiform to lanceolate paraphyses, and fusiform to filiform ascospores (Korf 1978, Perić & Baral 2014, Han et al. 2021, Li et al. 2022, Tochihara & Hosoya 2022).

Erioscyphella was originally established without typification to accommodate *Erioscyphella longispora* (≡ *Lachnum longisporum*) and *E. bambusina* (≡ *Erinella bambusina*) (Kirschstein 1938), until Haines (1984) selected *E. longispora*, under which *Peziza abnormis* was later synonymized, as the lectotype of *Erioscyphella*. Kirschstein (1938) questioned the assignment of *Erinella rhabdocarpa* in this genus. Subsequently, *Erinella rhabdocarpa* was synonymized with *Trichobelonium rhabdocarpum* in *Hyaloscyphaceae* according to their similar morphologies (Dennis 1963). As *Trichobelonium* was synonymized with *Belonopsis*, *Trichobelonium rhabdocarpum* became *Belonopsis rhabdocarpum* (Nauta & Spooner 2000). Perić & Baral (2014) introduced *Erioscyphella curvispora*, and synonymized *Peziza abnormis*, *Cenangium brasiliense*,

Submitted 1 November 2022, Accepted 16 November 2022, Published 9 January 2023
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Lachnum lunatum and *Belonidium sclerotii* under *Erioscyphella abnormis, E. brasiliensis*, *E. lunata* and *E. sclerotii* based on morphological similarities and the neighbor-joining analysis of ITS, respectively. Since the first phylogenetic study of *Erioscyphella* (Perić & Baral 2014), four combinations and ten species were proposed successively based on morphology and multi-gene phylogeny (Guatimosim et al. 2016, Ekanayaka et al. 2019, Li et al. 2022, Tochihara & Hosoya 2022). Up to now, 20 species have been accepted in *Erioscyphella*. The members of *Erioscyphella* have different host preferences, which is a key characteristic for distinguishing its species (Tochihara & Hosoya 2022). *Erioscyphella abnormis, E. alba, E. aseptata, E. brasiliensis, E. fusiforme, E. insulae, E. latispora* and *E. sclerotii* are found on the wood of hardwood trees (Miyoshi et al. 2007, Hosoya et al. 2010, Zhao & Zhuang 2011, Zhao et al. 2012, Han et al. 2014, Ekanayaka et al. 2019, Li et al. 2022, Tochihara & Hosoya 2022); *E. boninensis, E. hainanensis* and *E. sinensis* are from the fallen leaves of broad-leaved trees (Yu & Zhuang 2002, Hosoya et al. 2013, Tochihara & Hosoya 2022); *E. curvispora* and *E. lunata* are on the damp, dead needles of pine trees (Perić & Baral 2014, Tello & Baral 2016); *E. euterpes* on the palm leaves (Cantrell & Hanlin 1997); *E. bambusina* and *E. paralushanensis* on the bamboo culm (Dennis 1954, Tochihara & Hosoya 2022); *E. sasibrevispora* on the fallen bamboo sheaths (Tochihara & Hosoya 2022); *E. otanii* and *E. papillaris* on the fallen bamboo leaves (Tochihara & Hosoya 2022); *E. lushanensis* on the dead leaf sheath at stem base of grass (Zhuang & Wang 1998, Guatimosim et al. 2016).

During the investigation on *Leotiomycetes* in China, six collections of *Erioscyphella* were made. We used morphological and phylogenetic analyses based on LSU, ITS, mtSSU and *RPB2* data to confirm that these six collections differ from all known species of *Erioscyphella*. We introduce two new species to accommodate these collections and present a key to the species of *Erioscyphella*.

Material & Methods

Sample collection

We collected six specimens from an evergreen broadleaf forest in the Ailao Mountain, Yunnan, China. The specimens were dried at 25–30 °C in a dehydrator. After studying the morphology of the specimens and getting their genomic DNA, they were deposited at the Cryptogamic Herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences (KUN-HKAS). Facesoffungi and Index Fungorum numbers were obtained as in Jayasiri et al. (2015) and Index Fungorum (2022), respectively. The newly produced data were added to the Greater Mekong Subregion webpage (Chaiwan et al. 2021).

Morphological studies

The specimens were examined using a Nikon C-PSN stereoscope (Nikon, Japan), and their micro-morphology was photographed using a Nikon ECLIPSE Ni-U microscope (Nikon, Japan) connected to a Canon EOS 70D (W) digital camera (Canon, Japan). Free-hand sections of the dried specimens were rehydrated in water. Cotton blue (CB) dissolved in lactic acid (LA) (CB/LA; 0.5 g CB and 99.5 mL LA) was used as a mounting fluid. Iodine reactions of the asci apical apparatus were checked using Melzer's reagent (MLZ) and Lugol's iodine (IKI) with or without 3% KOH pretreatment (Tochihara & Hosoya 2022). Microstructures were measured in water by Image Frame Work, and measurements were given as (*a*–)*b*–*c*(–*d*), where *a* denoted the minimum value, *d* the maximum value, and *b*–*c* the 90% confidence interval. The $\bar{x}$ indicated the average value of measurements. The measurements of ascospores were given as *[n/m/p]*, indicating that the *n* number of ascospores were measured from *m* ascomata of the *p* collections. The Q value denoted the length to width ratios of the ascospores, and ***Q*** denoted the average of the length to width ratios (Q) of all ascospores ± standard deviation (Calatayud et al. 2002). Finally, the illustrations were made in Adobe Photoshop 2020.

DNA extraction, PCR amplifications and sequencing

Genomic DNA was extracted from the dried apothecia using a TSP101 DNA extraction kit (TSINGKE, China). Following the latest study of Tochihara & Hosoya (2022), LSU, ITS, mtSSU and *RPB2* were used for PCR amplification, using the primers LROR/LR5 (Vilgalys & Hester 1990), ITS1F/ITS4 (White et al. 1990, Gardes & Bruns 1993), mrSSU1/mrSSU3R (Zoller et al. 1999) and fRPB2-5F/fRPB2-7cR (Liu et al. 1999), respectively. For LSU, ITS and *RPB2*, the total volume of PCR amplifications was 25 μL, including 12.5 μL 2×PCR G013 Taq MasterMix with Dye (abm, Canada), 1 μL of each primer (10 μM), 2 μL genomic DNA, and 8.5 μL of sterilized, distilled water. Amplifications of LSU, ITS and *RPB2* were conducted under the following conditions: pre-denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 20 sec, annealing at 56 °C (LSU)/53 °C (ITS & *RPB2*) for 10 sec, elongation at 72 °C for 20 sec, and final elongation at 72°C for 7 min. For mtSSU, the total volume of PCR amplifications was 25 μL, including 21 μL 1×PCR TSE101 Mix (TSINGKE, China), 1 μL of each primer (10 μM), and 2 μL genomic DNA. Amplifications of mtSSU were conducted under the following conditions: initial denaturation at 98 °C for 3 min, followed by 40 cycles of denaturation at 98 °C for 1 min, annealing at 52 °C for 1 min, elongation at 72°C for 1 min, and final elongation at 72 °C for 10 min. Gel electrophoresis with 1% TAE and TSJ003 GoldView nucleic acid dye (TSINGKE, China) was used to test the obtained PCR products. Finally, the PCR products were sequenced at the Tsingke Biotech (Beijing, China).

Phylogenetic analyses

All newly generated sequences were proofread in BioEdit v. 7.0.9 (Hall 1999). The corresponding forward and reverse sequences were concatenated in DNASTAR Lasergene SeqMan Pro v. 7.1.0 (Swindell & Plasterer 1997). The concatenated sequences were used to search for the close relatives in the NCBI (Johnson et al. 2008). According to the close relatives and recent studies, the newly generated sequences and some published sequences were used for the phylogenetic analyses (Table 1). *Capitotricha bicolor* (TNS-F-65670), *C. rubi* (TNS-F-65752), *Lachnellula calyciformis* (TNS-F-81248), *L. suecica* (TNS-F-16529) and *Neodasyscypha cerina* (TNS-F-65625) were used as the outgroup taxa in this study. The dataset of each gene region was aligned using MAFFT v. 7.49 (Katoh & Standley 2013). Then the individual datasets of LSU were trimmed with 'gapthreshold' set to 0.8, ITS with 'gapthreshold' set to 0.6, mtSSU and *RPB2* with 'gapthreshold' set to 0.5 in TrimAl v. 1.3 (Capella-Gutiérrez et al. 2009). The four datasets were assembled into a matrix using SequenceMatrix v. 1.8 (Vaidya et al. 2011). File formats were converted using AliView v. 1.19, the phylip format for Maximum likelihood (ML) analyses, and the nexus format for Bayesian inference (BI) analyses (Larsson 2014). The ML and BI analyses were conducted in the CIPRES Science Gateway v. 3.3 (Miller et al. 2010). The ML analyses were constructed using the RAxML-HPC2 on XSEDE v. 8.2.12 tool with 1000 replicates and other default parameters (Stamatakis 2014). For the BI analyses, the best-fit evolutionary model for each dataset was determined by MrModeltest v. 2.3. The best-fit model of LSU and *RPB2* was GTR+I+G, SYM+G for ITS, and HKY+I+G for mtSSU (Nylander et al. 2004). The BI analysis was performed using MrBayes on XSEDE v. 3.2.7a tool, the trees were sampled at every 100^{th} generation, and six Markov chains were run for 1.5 million generations. When the average standard deviation of split frequency is less than 0.01 and the effective sample size is greater than 200, the results indicated convergence (Huelsenbeck & Ronquist 2001, Ronquist et al. 2012). Finally, phylogenetic trees were viewed in FigTree v. 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/). Decisions as to whether species are new followed the polyphasic approach recommended by Chethana et al. (2021).

Results

Phylogenetic analysis

The phylogenetic analyses were based on 33 *Erioscyphella* taxa, including *Capitotricha rubi*

(TNS-F-65752), *C. bicolor* (TNS-F-65670), *Lachnellula calyciformis* (TNS-F-81248), *L. suecica* (TNS-F-16529) and *Neodasyscypha cerina* (TNS-F-65625) as the outgroup taxa. The alignment comprised 3147 base pairs (bp) (LSU: 1−839 bp; ITS: 840−1372 bp; mtSSU: 1373−2326 bp; *RPB2*: 2327−3147 bp). The data matrix is composed of 1120 distinct alignment patterns, with 17.28% gaps and completely undetermined characters. The ML tree has the same topology as the BI tree. The best ML tree is displayed in Fig. 1. For the ML analyses, the final optimization likelihood is -18037.205375. In BI analyses, the final average standard deviation of split frequencies was 0.007666, which revealed convergence.

In the multi-gene phylogenetic tree based on the combined LSU, ITS, mtSSU and *RPB2* dataset, all taxa of *Erioscyphella* clustered together. *Erioscyphella subinsulae* was sister to *E. insulae* with 100% maximum likelihood bootstrap (MLBP) and 1.00 Bayesian posterior probabilities (BPP) support. Besides, *E. griseibambusicola* was sister to the clade comprising *E. hainanensis*, *E. sinensis*, *E. euterpes*, *E. lushanensis*, *E. paralushanensis*, *E. boninensis* with 100% MLBP and 1.00 BPP.

Table 1 Taxa included in molecular phylogenetic analyses and the GenBank accession numbers of LSU, ITS, mtSSU and *RPB2* sequences.

Fungal taxon	**Specimen voucher**	**Collection site**	**Host plants and parts**	**UNITE/GenBank accession number**				**Reference**
				LSU	**ITS**	**mtSSU**	***RPB2***	
Capitotricha bicolor	TNS-F-65670	Switzerland	Twigs of *Prunus spinosa*	LC424942	LC424834	LC533244	LC425011	Tochihara & Hosoya (2022)
Capitotricha rubi	TNS-F-65752	Switzerland	Twigs of *Rubus idaeus*	LC438573	LC438560	LC533243	LC440395	Tochihara & Hosoya (2022)
Erioscyphella abnormis	TNS-F-16609	Japan	Wood of *Cephalotaxus harringtonia*	LC533175	AB705234	LC533256	LC533184	Tochihara & Hosoya (2022)
Erioscyphella abnormis	TNS-F-38452	Japan	Wood of unidentified tree	LC533171	UDB0779069	LC533262	LC533210	Tochihara & Hosoya (2022)
Erioscyphella alba (T)	MFLU 16-0614	Thailand	Dead stems of unidentified tree	MK591990	MK584965	–	–	Ekanayaka et al. (2019)
Erioscyphella aseptata (T)	MFLU 16-0590	Thailand	Dead stems of unidentified tree	MK591986	MK584957	–	MK388223	Ekanayaka et al. (2019)
Erioscyphella boninensis (T)	TNS-F-26520	Japan	Fallen leaves of *Pittosporum boninense*	LC533151	UDB0779049	LC533254	LC533196	Tochihara & Hosoya (2022)
Erioscyphella brasiliensis	MFLU 16-0577b	–	Wood of unidentified tree	MK591993	MK584967	–	–	Ekanayaka et al. (2019)
Erioscyphella brasiliensis	TNS-F-46419	China	Wood of unidentified tree	LC533133	UDB0779068	LC533278	LC549672	Tochihara & Hosoya (2022)
Erioscyphella curvispora (T)	KL 381	Montenegro	Damp dead needles of *Pinus heldreichii,*	–	MH190414	–	–	Perić & Baral (2014)
Erioscyphella euterpes	PR147	Puerto Rico	–	–	U58640	–	–	Cantrell et al. (1997)

Table 1 Continued.

Fungal taxon	Specimen voucher	Collection site	Host plants and parts	UNITE/GenBank accession number				Reference
				LSU	ITS	mtSSU	*RPB2*	
Erioscyphella fusiforme (T)	MFLU 15-0230	China	Dead stems of unidentified tree	MK591975	MK584948	–	MK614728	Ekanayaka et al. (2019)
***Erioscyphella griseibambusicola* (T)**	**HKAS 124656**	**China**	**Culms of unidentified bamboo**	**OP451790**	**OP451796**	**OP451843**	**OP432251**	**This study**
Erioscyphella griseibambusicola	**HKAS 124657**	**China**	**Culms of unidentified bamboo**	**OP451791**	**OP451797**	**OP451844**	**OP432252**	**This study**
Erioscyphella hainanensis	TNS-F-35056	Japan	Leaves of *Quercus serrata*	LC533169	UDB0779065	LC533275	LC533206	Tochihara & Hosoya (2022)
Erioscyphella hainanensis	TNS-F-35049	Japan	Leaves of *Quercus glauca*	LC533168	UDB0779064	LC533274	LC533205	Tochihara & Hosoya (2022)
Erioscyphella insulae (T)	TNS-F-39720	Japan	Bark of unidentified tree	LC533177	UDB0779063	LC533261	LC533207	Tochihara & Hosoya (2022)
Erioscyphella insulae	TNS-F-26500	Japan	Bark of unidentified tree	LC533149	UDB0779060	LC533252	LC533194	Tochihara & Hosoya (2022)
Erioscyphella latispora (T)	HKAS 124389	China	Newly fallen trunks of unidentified tree	OP113844	OP310823	–	OP715728	Li et al. (2022)
Erioscyphella latispora	HKAS 124391	China	Newly fallen trunks of unidentified tree	OP113850	OP113849	–	OP715727	Li et al. (2022)
Erioscyphella lunata	JA-CUSSTA 8292	Spain	Fallen needles of *Pinus nigra*	KX501133	KX501132	–	–	Tello & Baral (2016)
Erioscyphella lushanensis	HMAS 81575	China	–	–	JF937582	–	–	Zhao et al. (2011)
Erioscyphella otanii (T)	TNS-F-81472	Japan	Leaves of *Sasa senanensis*	LC533179	UDB0779085	LC533226	LC533286	Tochihara & Hosoya (2022)
Erioscyphella papillaris (T)	TNS-F-81272	Japan	Leaves of unidentified bamboo	LC533161	UDB0779081	LC533285	LC533204	Tochihara & Hosoya (2022)
Erioscyphella paralushanensis (T)	TNS-F-61920	Japan	Culms of *Pleioblastus argenteostriatus*	LC533141	UDB0779075	LC533267	LC533220	Tochihara & Hosoya (2022)
Erioscyphella sasibrevispora (T)	TNS-F-81401	Japan	Culms of *Sasa nipponica*	LC533174	UDB0779084 /LC669472	LC533269	LC533217	Tochihara & Hosoya (2022)

Table 1 Continued.

Fungal taxon	Specimen voucher	Collection site	Host plants and parts	UNITE/GenBank accession number				Reference
				LSU	ITS	mtSSU	*RPB2*	
Erioscyphella sasibrevispora	TNS-F-80399	Japan	Sheaths of *Sasa veitchii*	LC533173	UDB0779082 /LC669470	LC533268	LC533216	Tochihara & Hosoya (2022)
Erioscyphella sclerotii	TNS-F-26492	Japan	Wood of unidentified tree	LC533152	UDB0779050 /LC669438	LC533255	LC533197	Tochihara & Hosoya (2022)
Erioscyphella sclerotii	TNS-F-38480	China	Twigs of unidentified tree	LC533134	UDB0779070	LC533263	LC549673	Tochihara & Hosoya (2022)
Erioscyphella sinensis	TNS-F-32161	Japan	Leaves of *Quercus myrsinifolia*	LC533167	UDB0779061 /LC669449	LC533273	LC533219	Tochihara & Hosoya (2022)
Erioscyphella sinensis	TNS-F-16838	Japan	Leaves of unidentified broad-leaved tree	LC533164	AB481280	LC533235	AB481364	Tochihara & Hosoya (2022)
***Erioscyphella subinsulae* (T)**	**HKAS 124658**	**China**	**Bark of unidentified tree**	**OP451792**	**OP451798**	**OP4518435**	**OP432253**	**This study**
Erioscyphella subinsulae	**HKAS 124659**	**China**	**Bark of unidentified tree**	**OP451793**	**OP451799**	**OP4518436**	**OP432254**	**This study**
Erioscyphella subinsulae	**HKAS 124660**	**China**	**Bark of unidentified tree**	**OP451794**	**OP451800**	**OP4518437**	**OP432255**	**This study**
Erioscyphella subinsulae	**HKAS 124661**	**China**	**Bark of unidentified tree**	**OP451795**	**OP451801**	**OP4518438**	**OP432256**	**This study**
Lachnellula calyciformis	TNS-F-81248	Japan	Twigs of *Abies sachalinensis*	LC438574	LC438561	LC533247	LC438590	Tochihara & Hosoya (2022)
Lachnellula suecica	TNS-F-16529	Japan	Twigs of *Larix kaempferi*	LC424944	AB481248	LC533231	AB481341	Tochihara & Hosoya (2022)
Neodasyscypha cerina	TNS-F-65625	Switzerland	Twigs of *Crataegus* sp.	LC424948	LC424836	LC533242	LC425013	Tochihara & Hosoya (2022)

Names in bold indicate the specimens from the current study. Names with (T) indicate type specimens.
Abbreviations: HKAS: Herbarium of Cryptogams of Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China; JA: Herbarium of Consejería de Medio Ambiente (Junta de Andalucía), Sevilla, Spain; MFLU: Mae Fah Luang University Herbarium, Chiang Rai, Thailand; PR: Herbarium of National Museum in Prague, Praha, Czech Republic; TNS: Herbarium of the National Museum of Nature and Science, Tsukuba, Japan.

Taxonomy

Erioscyphella griseibambusicola H.L. Su & Q. Zhao sp. nov. Fig. 2

Diagnosis – It is characterized by minute, cupulate to globose apothecia, abundant, long, brown hairs with resinous material on the apex.

Index Fungorum number: IF 557880; Facesoffungi number: FoF 12851

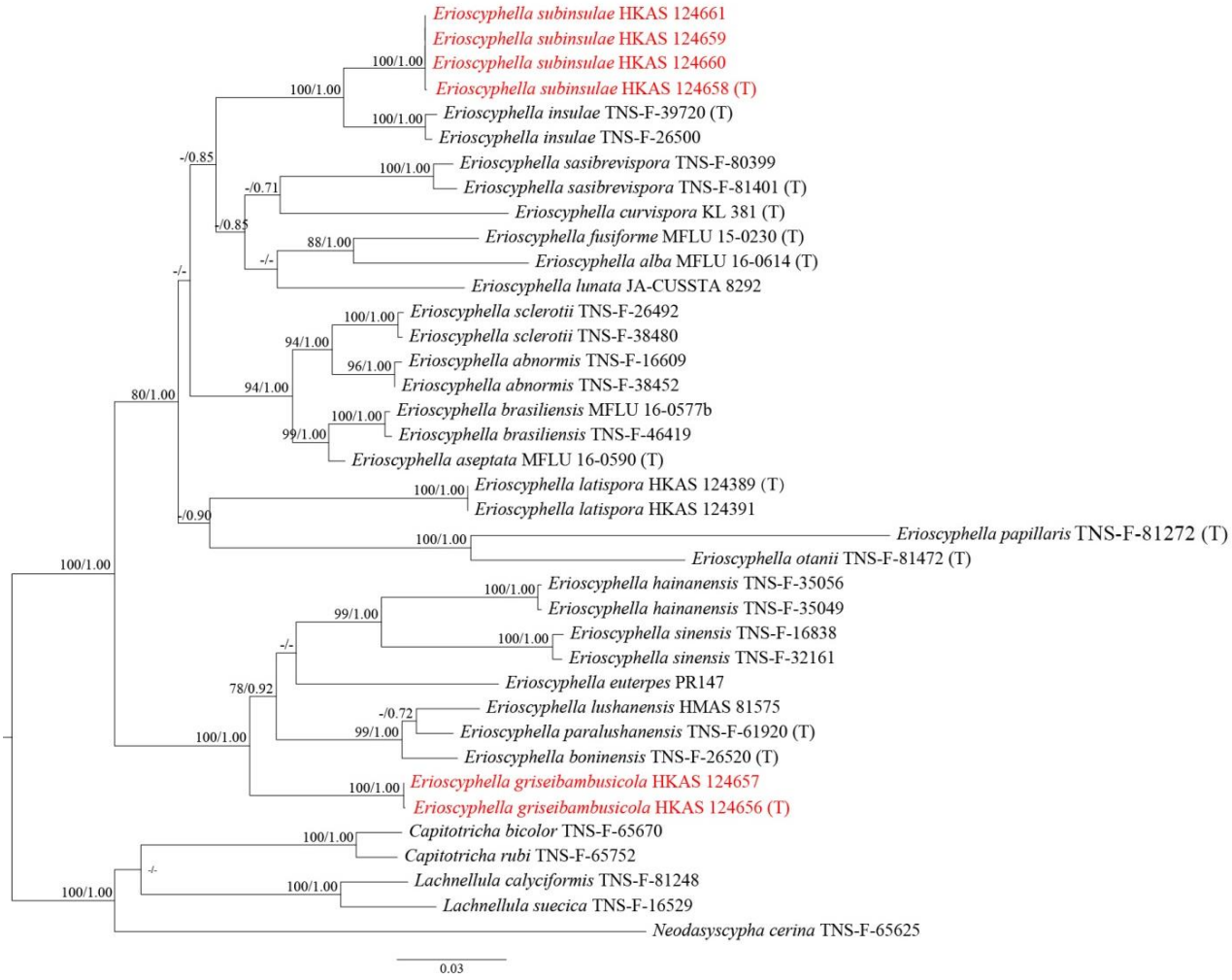

Fig. 1 – The ML tree based on the combined LSU, ITS, mtSSU and *RPB2* dataset. MLBP ≥ 70% and BPP ≥ 0.70 are shown at the nodes as MLBP/BPP. MLBS < 70% and BPP < 0.70 are expressed as a hyphen ("-"). Names with (T) indicate type specimens. Names in red indicate new species.

Etymology – "grisei" means grey, "bambusicola" means bamboo. The species has grey apothecia occurring on bamboo culm.

Holotype – HKAS 124656

Saprobic on dead bamboo culms. Sexual morph: *Apothecia* scattered to partly gregarious, superficial, 0.2−0.4 mm in diameter, 0.3−0.5 mm high when dry, cupulate when fresh, globose when dry, shortly stipitate, leathery, externally covered with abundant, long and greyish yellow hairs. *Disc* concave, surface slightly rough, white to grey. *Margin* involute, white to grey, clothed with abundant, white to brown hairs. *Receptacle* cupulate to globose, brown, clothed with abundant, long, greyish yellow hairs entirely. *Stipe* 0.1−0.2 mm in diameter (including hairs), 0.1−0.2 mm long when dry, cylindric, most solitary, some gregarious with the common base, dark brown, clothed with abundant, long, white to brown hairs. *Hairs* 60−160 × 2.5−5.0 µm ($\bar{x}$ = 96 × 3.9 µm, n = 47), cylindric, straight to slightly curved, multiseptate, light brown to dark brown, thick-walled, covered with hyaline granules, obtuse apex crowned with light brown amorphous material, not dissolved with CB/LA. *Hymenium* 115–140 µm ($\bar{x}$ = 130 µm, n = 10), concave, surface slightly rough, white to grey. *Medullary excipulum* 55–100 µm ($\bar{x}$ = 80 µm, n = 15) thick, hyaline, thin-walled, smooth cells of *textura intricata*, 2.0–4.5 µm ($\bar{x}$ = 2.8 µm, n = 50) in diameter. *Ectal excipulum* 15–35 µm ($\bar{x}$ = 23 µm, n = 22) thick, thick-walled, smooth, brownish cells of *textura porrecta*, 5.5–18.5 × 3.0–6.5 µm ($\bar{x}$ = 10.5 × 4.3 µm, n = 51). *Paraphyses* 100–150 × 2.0–3.0 µm ($\bar{x}$ = 123 × 2.5 µm, n = 50), longer than asci, filiform, straight, septate on the base, hyaline,

thin-walled, slightly rough, with slightly acute apex. *Asci* (95–)100–120 × 10–15 µm ($\bar{x}$ = 107 × 12 µm, n = 47), 8-spored, clavate, straight to slightly curved, inoperculate, hyaline, unitunicate, wall apically thickened, laterally relatively thin, slightly rough, with an apical, amyloid pore and slightly tapered ends, croziers absent at the basal septa, blue in MLZ or IKI with and without 3% KOH pretreatment. *Ascospores* (90/14/2) (57.0–)59.0–76.0(–81.5) × (2.0–)2.5–4.0 µm, ($\bar{x}$ = 67.0 × 2.9 µm, Q = 16.5–31.7, ***Q*** = 23.43±3.45), fascicled, filiform, 7–8-septate, thin-walled, hyaline, and slightly smooth when mature, rough with some oil guttules in immature, with taper, obtuse ends. Asexual morph: Unknown.

Material examined – China, Yunnan, Ailao Mountain, alt. 2460 m, on the fallen bamboo culm, 29 August 2021, H.L. Su, SHL70 (HKAS 124656, holotype); *ibid.*, alt. 2464 m, on the fallen bamboo culm, 28 August 2021, H.L. Su, SHL44 (HKAS 124657, paratype).

Notes – The new species bears slight similarities to *Erioscyphella papillaris* and *E. otanii* in having minute apothecia and white to light yellow hymenium, while the latter two reference species are phylogenetically distant from the former. However, the three species exhibit morphological differences as well. *Erioscyphella griseibambusicola* has grey to greyish yellow apothecia, and light brown to dark brown hairs with the apex crowned with light brown amorphous material. However, *E. papillaris* has white to lemon yellow apothecia, hyaline hairs with hyaline amorphous material and lacks any crystals or resinous matters. Furthermore, *E. griseibambusicola* has longer hairs, asci, ascospores, and paraphyses than those of *E. papillaris* (Table 3). *Erioscyphella otanii* has pure white to pale yellow apothecia, hyaline, shorter hairs, asci, ascospores, and paraphyses compared to *E. griseibambusicola* (Table 3). Furthermore, *E. griseibambusicola* is found on the dead bamboo culm, while the other two species are on fallen leaves of bamboo.

The phylogenetic analyses based on the combined LSU, ITS, mtSSU and *RPB2* dataset showed that *E. griseibambusicola* was sister to a clade containing six species, *E. hainanensis*, *E. sinensis*, *E. euterpes*, *E. lushanensis*, *E. paralushanensis* and *E. boninensis* with 100% maximum likelihood bootstrap and 1.00 Baysean posterior probability support (Fig. 1). When comparing *E. griseibambusicola* with the six species, our new species differs morphologically from the six species, which are shown in Table 3. Based on the nucleotide sequence comparisons, *E. griseibambusicola* (HKAS 124656) significantly differs from the six species as shown in the Table 2. Due to these differences in the morphology, phylogeny and molecular sequences, we introduce our specimens as a new species.

Table 2 Comparison of nucleotide sequence differences among *Erioscyphella griseibambusicola* (HKAS 124656), *E. hainanensis* (TNS-F-35056), *E. sinensis* (TNS-F-32161), *E. euterpes* (PR147), *E. lushanensis* (HMAS 81575), *E. paralushanensis* (TNS-F-61920) and *E. boninensis* (TNS-F-26520).

Taxa for comparison	**LSU**	**ITS**	**mtSSU**	***RPB2***
Erioscyphella griseibambusicola* vs. *E. hainanensis	2.80% (3 gaps)	9.44% (2 gaps)	10.33% (30 gaps)	13.73% (25 gaps)
E. griseibambusicola* vs. *E. sinensis	2.80% (3 gaps)	10.0% (4 gaps)	11.16% (29 gaps)	14.03% (25 gaps)
E. griseibambusicola* vs. *E. euterpes	–	9.20% (1 gaps)	–	–
E. griseibambusicola* vs. *E. lushanensis	–	9.51% (2 gaps)	–	–
E. griseibambusicola* vs. *E. paralushanensis	2.91% (4 gaps)	9.60% (3 gaps)	8.27% (28 gaps)	13.38% (21 gaps)
E. griseibambusicola* vs. *E. boninensis	2.84% (4 gaps)	16.43% (26 gaps)	7.96% (28 gaps)	12.58% (21 gaps)

Table 3 A morphological comparison of eight *Erioscyphella* species.

Species	Apothecia	Hairs	Asci	Ascospores	Paraphyses	References
Erioscyphella griseibambusicola	• Cupulate to globose • 0.2−0.4 mm in diameter, 0.3−0.5 mm high • Short-stipitate • Grey to greyish yellow	• Cylindric • 60−160 × 2.5−5.0 µm • Light brown to dark brown • Multiseptate • Granulated • Apex crowned with light brown amorphous material, obtuse apex crowned with light brown amorphous material	• Clavate • 95–110 × 8.5–12 µm • Croziers absent	• Fusiform • 57.0–81.5 × 2.0–4.0 µm • 7–8-septate	• Filiform • 100–150 × 2.0–3.0 µm • Septate	This study
E. boninensis	• Discoid • 0.5–1.0 mm in diameter, 1.5 mm high • Long-stipitate • Cream to pale brown	• Cylindrical • 38–62 × 2.5–4.0 µm • Hyaline • 2–3-septate • Completely covered by brown granules • Lacks crystals or apical amorphous material	• Cylindrical-clavate • 37.7–44 × 3.6–4.2 µm • Croziers absent	• Fusiform • 10–12.3 × 1.2–1.7 µm • Aseptate	• Narrowly lanceolate • Exceeding the asci up to 5 µm, up to 2.5 µm wide • Septate	Tochihara & Hosoya (2022)
E. euterpes	• Cupulate • Up to 0.5 mm in diameter • Stipitate • Orange-buff to salmon-buff	• Cylindrical or slightly tapered towards the tips to a hemispherical apex • Up to 110 µm long, 3.0–4.5 µm wide • Hyaline • Sparingly septate • Granulate • Lacks apical resin masses or crystals	• Cylindric-clavate • 50–65 × 6.0–7.5µm • Crozier arise	• Elongate-ellipsoid with hemispheric tips • 15–18 × 2.5–3.5 µm • Hyaline • 40% of spores one-septate	• Cylindric-clavate • 5–20 µm beyond the asci, 2–3 µm wide at the broadest point, extending • Septate in the lower portion • Hyaline	Cantrell & Haines (1997)
E. hainanensis	• Discoid • 0.2–0.45 mm in diameter, 0.4–0.85 mm high • Stipitate • Disc dark orange when dried, pale orange when	• Cylindrical, obtuse apex • 44–99 × 2.0–2.5 µm • White • Multi-septate • Granulated	• Cylindrical-clavate • 30–42 × 3.5–5.5 µm • Crozier arise	• Fusiform to elongate ellipsoid • 11.5–17.0 × 1.5–2.5 µm • Hyaline • Aseptate	• Narrowly lanceolate • 34–42.5 × 1.5–3.0µm • Aseptate	Hosoya et al. (2013)

Table 3 Continued.

Species	Apothecia	Hairs	Asci	Ascospores	Paraphyses	References
	rehydrated; receptacle light orange					
E. lushanensis	• Discoid • 0.4–1.2 mm in diameter • Long-stipitate • White	• Cylindrical • 45–100 × 3 µm • Hyaline • 2–5-septate • Granulated • Covered with or upper portion capped by red, amorphous, resinous matter	• Clavate • 45–50 × 3.5–4.5 µm	• Narrowly fusoid • 12–18 × 1.0–1.5 um • Hyaline • Aseptate	• Lanceolate • Exceeding asci 5–8 um, 1.5–2.0 um wide	Zhuang & Wang (1988)
E. otanii	• Spherical at first and urceolate later • 0.1–0.3 mm in diameter, up to 0.3 mm high • Long-stipitate • Pure white, disc cream to pale yellow when dry	• Cylindrical or tapering toward the apices • Up to 60 µm long, up to 5 µm wide near the bases and 2.5–3.0 µm wide near the apices • Hyaline • 3-septate (usually 1- or 2-septate) • Granules dense near the apices and coarse toward the bases • Apex sometimes with a hyaline and inconspicuous apical amorphous material, lacks any crystals or resinous material	• Cylindrical-clavate • 34–38.8 × 4–5 µm • Croziers absent	• Fusiform • 12.3–14.6 × 1.36–1.7 µm • Aseptate	• Narrowly lanceolate to lanceolate • Exceeding the asci up to 10 µm, up to 2.5 µm • Septate	Tochihara & Hosoya (2022)
E. papillaris	• Discoid • 0.1–0.3 mm in diameter, up to 0.25 mm high • Short-stipitate • White to lemon yellow when fresh and dry	• Cylindrical with papillary apex, apex sometimes swelling • 45–75 × 3–5 µm • Hyaline • 2–3-septate • Totally granulated • Apex with hyaline and	• Cylindrical-clavate • 59.8–66 × 7.6–8.3 µm • Croziers absent	• Fusiform • 17.5–21.7 × 2.3–2.8 µm • Aseptate, or one-septate (rarely two-septate)	• Cylindrical • Equal or scarcely exceeding the asci, up to 3 µm wide • Septate	Tochihara & Hosoya (2022)

Table 3 Continued.

Species	Apothecia	Hairs	Asci	Ascospores	Paraphyses	References
		globose apical amorphous material, lacking any crystals or resinous matters				
E. paralushanensis	• Discoid • 0.7–1.5 mm in diameter, up to 2.0 mm high • Long-stipitate • Disc cream to pale yellow	• Cylindrical • Up to 160 µm long, 2.0–3.0 µm wide • Pale brown but hyaline near the bases • Septate • Granulated • Apex with amber-colored resinous material and amber-colored apical amorphous material, lacking any crystals	• Cylindrical-clavate • 61.4–70.2 × 4.7–5.6 µm	• Fusiform • 15.8–20.7 × 1.7–2.0 µm • Septate • Containing conspicuous guttules; guttules hyaline but sometimes red	• Initially cylindrical to clavate, later becoming narrowly lanceolate • Exceeding the asci 5–10 µm, up to 2 µm wide • Septate	Tochihara & Hosoya (2022)
E. sinensis	• Discoid to shallow • 0.2–0.8 mm in diameter • Stipitate • Disc cream-coloured to pale yellow; receptacle whitish	• Cylindrical • 35–90 × (1.8–)2.5–4.5 µm. • Hyaline to pale brownish, • Septate • Granulated	• Subcylindrical • 53–65 × 4.8–5.5 µm • Croziers absent	• Filiform to aciculate • 40–52 × 0.6–1 µm • Aseptate • Multi-guttulate	• Lanceolate • Exceeding the asci 5–13 µm, 1.8–2.8 µm wide • Septate	Yu & Zhuang (2002)

Erioscyphella subinsulae H.L. Su & Q. Zhao sp. nov. Fig. 3

Diagnosis – Its characteristics are yellow hymenium and white receptacle, short, white hairs without apical amorphous or resinous material, and long, filiform ascospores. The macro- and micromorphology is similar to *E. insulae* but has different colored discs, larger asci and ascospores.

Index Fungorum number: IF 557885; Facesoffungi number: FoF 12852

Etymology – Referring to its similarity with *E. insulae*.

Holotype – HKAS 124658

Growing on the bark of a living tree. Sexual morph: *Apothecia* scattered, superficial, 0.5−1.7 mm in diameter, about 0.4−0.8 mm high when dry, discoid to cupulate, shortly stipitate, leathery, externally covered with short, white hairs. *Disc* concave, surface slightly rough, yellow. *Margin* flat to slightly involute, white to pale yellow, clothed with white to pale yellow hairs. *Receptacle* discoid to cupulate, white to pale yellow, clothed with short, white to yellowy hairs entirely. *Stipe* 0.2−0.3 mm in diameter, 0.1−0.4 mm long when dry, cylindric, solitary, white to pale yellow, clothed with white

to yellowy hairs. *Hairs* 30−80 × 2.0−4.0 µm ($\bar{x}$ = 52 × 2.9 µm, n = 30), clavate to cylindric, straight to slightly curved, multiseptate, hyaline, thin-walled, covered with hyaline granules, obtuse apex, lacks apical amorphous or resinous material. *Hymenium* 100–150 µm ($\bar{x}$ = 120 µm, n = 12), concave, surface slightly rough, light yellowish brown in fresh, yellow in dry. *Medullary excipulum* 70–180 µm ($\bar{x}$ = 135 µm, n = 18), thick, hyaline to light brown, thin-walled, smooth cells of *textura intricata*, 1.5–3.5µm ($\bar{x}$ = 2.4 µm, n = 50) in diameter. *Ectal excipulum* 40–80 µm ($\bar{x}$ = 57 µm, n = 18) thick, thick-walled, smooth, light brown cells of *textura prismatica*, 4.5–19.0 × 3.0–12.5 µm ($\bar{x}$ = 9.8 × 6.0 µm, n = 60). *Paraphyses* 85–130 × 2.0–3.0 µm ($\bar{x}$ = 100 × 2.4 µm, n = 25), longer than asci, filiform, straight, multiseptate, hyaline, thin-walled, slightly rough, with slightly acute apex. *Asci* 95–110 × (8.5–) 8.0–10 (–12) µm ($\bar{x}$ = 100 × 9.3 µm, n = 34), 8-spored, clavate, straight to slightly curved, inoperculate, hyaline, unitunicate, wall apically thickened, laterally relatively thin, slightly rough, with a apical, amyloid pore and tapered ends, croziers absent at the basal septa, blue in MLZ or IKI with and without 3% KOH pretreatment. *Ascospores* (165/12/3) (43.0–)48.5–74.5(–80.0) × 2.0–3.0 (–3.5) µm, ($\bar{x}$ = 62.1 × 2.4 µm, Q = 16.6–42.9, ***Q*** = 26.34±4.94), fascicled, filiform, 1–3-septate, thin-walled, hyaline, rough with taper, obtuse ends, some hyaline oil guttules, ends with globules, (1.5–)2.0–3.0(–3.5) µm (x = 2.5 µm, n = 115), subspherical, hyaline, slightly rough. Asexual morph: Unknown.

Material examined – China, Yunnan, Ailao Mountain, alt. 2460 m, on the bark of tree, 30 August 2021, H.L. Su, SHL131 (HKAS 124658, holotype); *ibid*, alt. 2434 m, on the bark of tree, 1 September 2021, H.L. Su, SHL180 (HKAS 124659, paratype); *ibid*, alt. 2478 m, on the bark of tree, 29 August 2021, H.L. Su, SHL55 (HKAS 124660, paratype); *ibid*, alt. 2428 m, on the bark of tree, 2 September 2021, H.L. Su, SHL244 (HKAS 124661, paratype).

Notes – *Erioscyphella subinsulae* is closely related to *E. insulae* in having white receptacles with white hairs, and they form sister groups with 100% MLBP and 1.00 BPP in the phylogenetic tree (Fig. 1). Morphologically, *E. insulae* has cream to pale yellow discs in the dry state, while *E. subinsulae* has yellow discs. Besides, *E. subinsulae* has larger asci and ascospores than *E. insulae*. Furthermore, *E. insulae* has apical amorphous material, while *E. subinsulae* lacks it (Table 3). Phylogenetically, *E. subinsulae* (HKAS 124658) and *E. insulae* (TNS-F-39720) differ by 132 base pairs (including 40 gaps) in the LSU region (969/1101, 88.01%), 32 base pairs (including 2 gaps) in the ITS region (468/500, 93.60%), 21 base pairs in the mtSSU region (913/934, 97.75%) and 77 base pairs in the *RPB2* region (691/768, 89.97%).

Discussion

Phylogenetic relationships in *Erioscyphella* gradually became clear with species expansion and amendment at the genus level. Almost all species of *Erioscyphella* have stable phylogenetic positions with strong statistical support, with the exception of *E. bambusina*, which lacks sequences in public databases. We compared the morphology of *E. bambusina* and our two new species in Table 3. *Erioscyphella griseibambusicola* has grey and smaller apothecia, while *E. bambusina* has light brown and larger apothecia. *Erioscyphella griseibambusicola* also has larger asci and ascospores compared to *E. bambusina* (Dennis 1954). *Erioscyphella subinsulae* has white receptacles, hairs and apothecia on the tree bark. However, *E. bambusina* has light brown receptacles, brown hairs, and apothecia on bamboo culm (Dennis 1954). The key to the *Erioscyphella* is given below.

Erioscyphella is similar to *Lachnum* in their white to yellow or reddish hymenium, granulated, hyaline or brown hairs, lanceolate to nearly cylindrical paraphyses (Hosoya et al. 2010, Tochihara & Hosoya 2022). While *Erioscyphella* and *Lachnum* are distinguishable in their hairs, the former has hairs with apical amorphous material and lacks swelling apices, and the latter has hairs without the typical characteristics (Perić & Baral 2014, Tochihara & Hosoya 2022). However, the phylogenetic relationships of *Erioscyphella* and *Lachnum* were ambiguous. There were reports that *Erioscyphella* was a monophyletic genus (Dennis 1954, Spooner 1987, Cantrell & Hanlin 1997, Guatimosim et al. 2016, Tochihara & Hosoya 2022). However, based on the broader taxon sampling, some *Lachnum* species were interspersed in the *Erioscyphella* clade, such as *Lachnum*

palmae and *L. pteridophyllum* (Ekanayaka et al. 2019, Tochihara & Hosoya 2022). The inadequate phylogenetic studies on *Lachnum* resulted from the lack of sequences in the public databases. Up to now, *Lachnum* has above 250 species listed in Species Fungorum (2022) but only about a tenth of species have credible sequences in NCBI (2022). In order to clarify the relationship between *Lachnum* and *Erioscyphella* and identify their members, it is necessary to re-study the species of *Lachnum* in detail and systematically, especially the type specimens.

Key to the species of *Erioscyphella*

1. Ectal excipular cells have granulate walls……*E. boninensis*
1. Ectal excipular cells have smooth walls……2
2. Hairs have papillate apices……*E. papillaris*
2. Hairs lack papillate apices……3
3. Apothecia have red hairs……4
3. Apothecial hairs are not red……5
4. Ascospores have some red guttules……*E. paralushanensis*
4. Ascospores have no red guttules……*E. lushanensis*
5. Host is palm……*E. euterpes*
5. Host is not palm……6
6. Host is bamboo……7
6. Host is not bamboo……8
7. Apothecia are on the leaves of bamboo…… *E. otanii*
7. Apothecia are not on the leaves of bamboo……9
8. Apothecia are on the damp dead needles of pine tree……11
8. Host is not pine……12
9. Apothecia are on the fallen sheaths of bamboo……*E. sasibrevispora*
9. Apothecia are on the culm of bamboo……10
10. Apothecia are light brown……*E. bambusina*
10. Apothecia are grey……*E. griseibambusicola*
11. Ascospores are shorter than 12 µm……*E. curvispora*
11. Ascospores are longer than 12 µm……*E. lunata*
12. Apothecia are on the leaves of tree……13
12. Apothecia are on the wood of tree……14
13. Ascospores are shorter than 20 µm……*E. hainanensis*
13. Ascospores are longer than 20 µm……*E. sinensis*
14. Ascospores are shorter than 15 µm……*E. alba*
14. Ascospores are longer than 15 µm……15
15. Apothecia have brown hairs……16
15. Apothecia have white to yellowish hairs……17
16. Ascospores are shorter than 40 µm……*E. sclerotii*
16. Ascospores are longer than 40 µm……*E. abnormis*
17. Asci are shorter than 50 µm……*E. fusiforme*
17. Asci are longer than 50 µm……18
18. Most ascospores are aseptate……19
18. Most ascospores are septate……20
19. Asci lack croziers……*E. brasiliensis*
19. Asci have croziers……*E. aseptata*
20. Ascospores are shorter than 40 µm……21
20. Ascospores are longer than 40 µm……*E. subinsulae*
21. Ascospores are narrower than 4 µm……*E. insulae*
21. Ascospores are wider than 4 µm……*E. latispora*

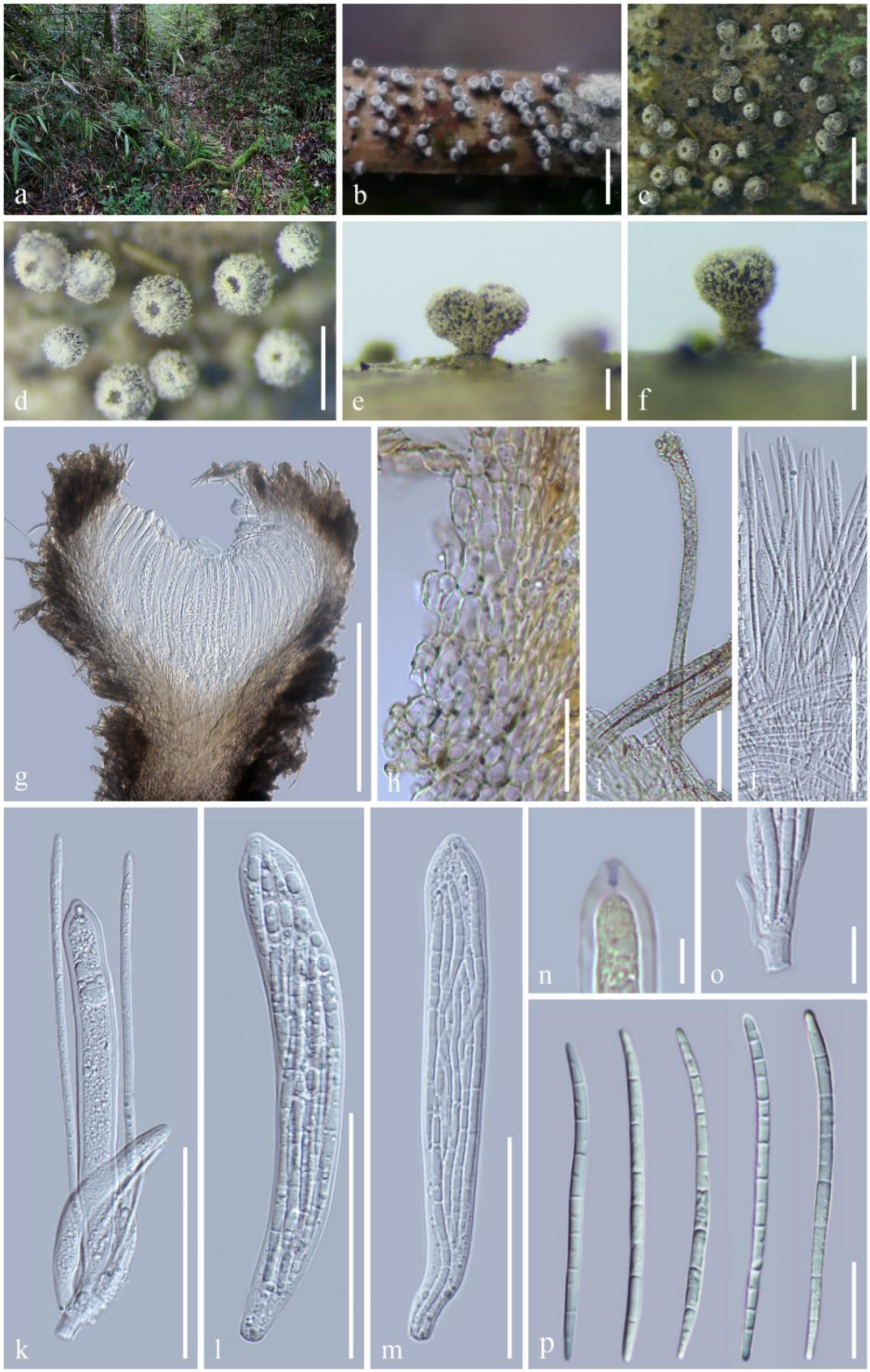

Fig. 2 – *Erioscyphella griseibambusicola* (HKAS 124656, holotype). a Habitat. b Fresh apothecia on host surface. c–f Dried apothecia. g Vertical section of an apothecium. h Section of the excipulum. i Hairs. j Paraphyses. k An immature ascus and paraphyses. l, m Asci. n Apex of an ascus in MLZ. o Base of an ascus. p Ascospores. Scale bars: b = 2 mm, c = 1 mm, d = 500 µm, e–g = 200 µm, h–i = 20 µm, j–m = 50 µm, n = 5 µm, o = 10 µm, p = 20 µm.

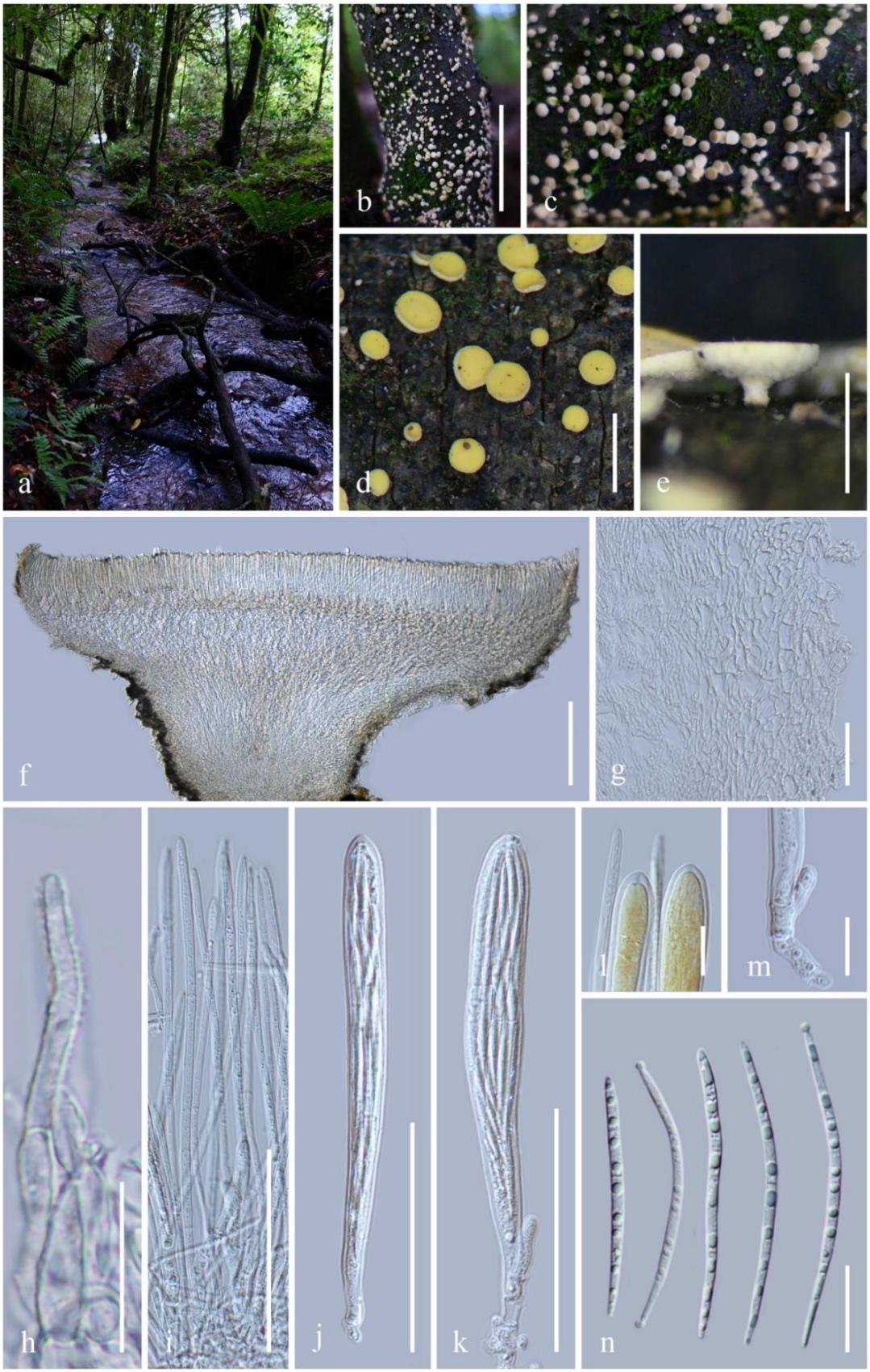

Fig. 3 – *Erioscyphella subinsulae* (HKAS 124658, holotype). a Habitat. b, c Fresh apothecia on host surface. d, e Dried apothecia. f Vertical section of an apothecium. g Section of the excipulum. h Hairs. i Paraphyses. j, k Asci. l Apexes of asci in MLZ. m Base of an ascus. n Ascospores. Scale bars: b = 5 cm, c = 1 cm, d = 2 mm, e = 1 mm, f = 200 µm, g = 50 µm, h = 20 µm, i–k = 50 µm, l–m = 10 µm, n = 20 µm.

Acknowledgments

We thank Dr. Shaun Pennycook (Manaaki Whenua-Landcare Research, New Zealand) for his valuable assistance with the Latin binomial. We also thank Kevin D. Hyde (School of Science, Mae Fah Luang University, Thailand) for the help in improving the manuscript. This study is supported by the Second Tibetan Plateau Scientific Expedition and Research (STEP) Program (Grant No. 2019QZKK0503), the open research project of "Cross-Cooperative Team" of the Germplasm Bank of Wild Species, Kunming Institute of Botany, Chinese Academy of Sciences (Grant No. 292019312511043), Major science and technology projects and key R&D plans/programs, Yunnan Province (202202AE090001), Natural Science Foundation of Guizhou Province (Grant No. Qian Ke Zhong Yin Di [2021]4031, Qian Ke He Zhi Cheng [2021] Generally 200), Science and Technology Service Network Initiative of the Chinese Academy of Sciences (KFJ-STS-QYZD-171), and the Biodiversity Survey and Assessment Project of the Ministry of Ecology and Environment, PR China (2019HJ2096001006).

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