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ARTICLE

Longistipes gen. nov. and four novel species of Hyphodiscaceae, along with six new collections of Leotiomycetes in Yunnan Province, China

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Abstract

During the investigation of discomycetes in Yunnan, China, ten saprotrophic species were discovered on fallen leaves and decaying wood. Among them, four species were identified as new species within Hyphodiscaceae based on phylogenetic analyses of the combined LSU and ITS datasets and morphological evidence. *Longistipes* is established as a new genus with two new species, *L. albus* and *L. niger*, typified by *L. niger*. This genus is distinguished by unique characteristics such as long stipes, white and long hairs, and broad lanceolate paraphyses that exceed the length of the asci. Additionally, two new species, *Hyphopeziza macrospora* and *Hyphodiscus pseudotanii*, are introduced. Three known species, *Chalara bambusicola*, *Nagrajchalara neonawawii* and *Nagrajchalara yongnianii*, are described for the first time in their sexual morphs, with *C. bambusicola* being reported for the first time on *Gleditsia* sp. Furthermore, *Dicephalospora chiangraiensis* is reported as a new geographical record from China, and new collections of *Erioscyphella hainanensis* and *Lachnellula subtilissima* are discovered in Yunnan Province. All described species are supported by detailed descriptions, illustrations, and multi-gene analyses.

Keywords – 5 new taxa – Helotiales – new geographical records – new sexual morphological records – one new genus

INTRODUCTION

Helotiales is the most abundant, widely distributed and well-studied order in Leotiomycetes, containing 27 families with 313 genera and over 4500 species (Quandt & Haelewaters 2021, Luo et al. 2024). Members of Helotiales have tiny apothecia (mostly less than 2 mm) or cleistothecia on the surface of the plant or buried under the epidermis (Quandt & Haelewaters 2021). They usually appear as saprobic fungi and include pathogenic, parasitic, and symbiotic species (Ekanayaka et al. 2019, Johnston et al. 2019, Quandt & Haelewaters 2021).

The taxonomic classification and phylogenetic relationships within the family Hyphodiscaceae Ekanayaka & K.D. Hyde (Helotiales) has been subjected to significant refinement

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and expansion in recent years, as elucidated by several pivotal studies (Han et al. 2014, Ekanayaka et al. 2019, Johnston et al. 2019, Quijada et al. 2022).

Initially proposed by Ekanayaka et al. (2019), Hyphodiscaceae was delineated to comprise six genera and placed in Helotiales (Leotiomycetes), namely *Fuscolachnum*, *Hyalopeziza*, which included only three species, including the synonymized species *Hyphopeziza pygmaea*, *Hyphodiscus*, *Soosiella*, *Scolecolachnum*, and *Venturiocistella*, based on a comprehensive phylogenetic analysis utilizing five genetic markers (LSU, SSU, ITS, *TEF-α* and *RPB2*). *Hyphodiscus* was designated as the type genus (Ekanayaka et al. 2019). Before the formal establishment of Hyphodiscaceae, Han et al. (2014) conducted a phylogenetic analysis utilizing ITS, LSU, and *RPB2* sequences, revealing the positioning of *Hyphodiscus*, *Hypopeziza*, and *Venturiocistella* within the Hyaloscyphaceae (Helotiales, Leotiomycetes), termed "Han Clade 4". Subsequently, the comprehensive phylogenetic analysis conducted by Johnston et al. (2019) further supported these findings of Han et al. (2014) and integrated *Gamarada* into this clade. Ekanayaka et al. (2019) reclassified 'Han Clade 4' as a distinct family comprising six genera while omitting *Gamarada*. However, only three members of *Hyalopeziza* were placed within Hyphodiscaceae, while *Hyalopeziza pygmaea*, *Hyalopeziza leuconica*, and *Hyalopeziza nectrioidea* being were misplaced in the phylogenetic analysis (Ekanayaka et al. 2019, Quijada et al. 2022). Subsequent research by Quijada et al. (2022) expanded the taxonomic concept using a 15-gene dataset for phylogenetic analyses based on the work of Johnston et al. (2019). Their investigation confirmed the close relationship between Hyphodiscaceae and Leptodontidiaceae and identified inaccuracies in the morphological summary of Hyphodiscaceae by Ekanayaka et al. (2019) (Quijada et al. 2022). There were notable discrepancies, including the limitation of substrate preference to saprobic dead plant materials, the absence of description regarding the hemi-amyloid reaction at the ascus tip, exclusive mention of an ellipsoid shape for ascospores, omission of one species harboring four spores per ascus, and oversight regarding hair surfaces, which exhibit not only granular but also coarse and spiny warts (Ekanayaka et al. 2019, Quijada et al. 2022).

The latest research reveals that Hyphodiscaceae includes elven genera: *Fuscolachnum* J.H. Haines, *Gamarada* D.J. Midgley & Tran-Dinh, *Glutinomyces* Nor. Nakam., *Helicoscypha* Baral, *Hyphodiscus* Kirschst., *Hyphopeziza* J.G. Han, Hosoya & H.D. Shin, *Microscypha* Syd. & P. Syd., *Scolecolachnum* Guatim., R.W. Barreto & Crous, *Soosiella* Hujslová & M. Kolařík, *Venturiocistella* Raitv., and *Venturioscypha* Baral, T. Kosonen & Polhorský (Barral et al. 2022, Quijada et al. 2022, Baral 2023). The sexual morph of Hyphodiscaceae is characterized by bright to dark coloured, sessile to stipitate apothecia with downy to hairy margins, thin-walled, angular to porrect cells in the medullary excipulum, cylindrical to lageniform, septate primary hairs covered with round tubercules or granules, occasionally appearing long and aseptate, conical to lageniform and thick-walled secondary hairs with granular lower parts, four to eight-spored asci with inamyloid or an apical ring, basally branched paraphyses with longer apical cells, and globose to filiform, aseptate to rarely septate ascospores varying in shape (Zhuang 1988, Haines 1989, Baral 1993, Hosoya & Harada 1999, Hosoya 2002, Chlebicki & Sukova 2005, Huhtinen et al. 2010, Hosoya et al. 2011, Pärtel & Põldmaa 2011, Han et al. 2014, Johnston et al. 2014, Guatimosim et al. 2016, Ekanayaka et al. 2019, Quijada et al. 2022). The asexual morph of Hyphodiscaceae is characterized by pale or rarely dark-colored colonies, septate and branched hyphae with occasional chlamydospore-like cells, solitary lateral phialides or conidiophores with one to three terminal phialides and one-celled, short-cuneiform to guttuliform conidia (Huhtinen et al. 2010, Guatimosim et al. 2016, Midgley et al. 2018, Nakamura et al. 2018, Quijada et al. 2022).

Members of Hyphodiscaceae have been predominantly distributed across Asia, Europe, and North America, with a limited number of taxa identified in Africa, South America, and Oceania (Quijada et al. 2022). The life modes of Hyphodiscaceae display a wide diversity; the majority of genera (*Fuscolachnum*, *Hyphodiscus*, *Hyphopeziza*, *Microscypha*, *Soosiella*, and *Venturiocistella*) were reported as saprotrophic taxa on decaying leaves and wood of angiosperms or gymnosperms or pteridophytes, bryophytes, fungi, and highly acidic soil (Zhuang 1988, Haines 1989, Baral 1993, Hosoya & Harada 1999, Hosoya 2002, Chlebicki & Sukova 2005, Huhtinen et al. 2010, Hosoya et

al. 2011, Pärtel & Põldmaa 2011, Han et al. 2014, Johnston et al. 2014, Ren & Zhuang 2016, Guatimosim et al. 2016, Midgley et al. 2018, Nakamura et al. 2018, Ekanayaka et al. 2019, Quijada et al. 2022). Additionally, *Gamarada* was described as a mycorrhizal fungus, and *Glutinomyces* as endophytes (Midgley et al. 2018, Nakamura et al. 2018, Quijada et al. 2022). Three genera, namely *Glutinomyces*, *Hyphodiscus*, and *Microscypha*, were recorded in China (Zhuang 1988, Ren & Zhuang 2016, Quijada et al. 2022).

Hyphodiscus was initially described by Kirschstein (1906), comprising a single species, *Hyphodiscus gregarius*. To date, 18 species have been listed in the Index Fungorum (2024), and 15 of them have been tentatively accepted. Among these species, three have been solely observed in their asexual morphs (transferred from *Catenulifera*), while five species have been documented to exist in both their sexual and asexual morphs (Han et al. 2014, Quijada et al. 2022). Distinct characteristics of *Hyphodiscus*, which are distinguished from its sister groups (*Hyphopeziza*, *Soosiella*, *Fuscolachnum sensu stricto*, and *Microscypha*) on the phylogenetic tree from Quijada et al. (2022), include strongly gelatinized, glass-like excipular cells, almost short clavate coarsely warted hairs with a longer apical cell, and pigmented exudates. *Hyphodiscus* includes saprotrophic taxa crucial in the decomposition of decaying wood, lichens and corticioid fungi (Kirschstein 1906, Zhuang 1988, Baral 1993, Hosoya 2002, Huhtinen et al. 2010, Hosoya et al. 2011, Han et al. 2014, Quijada et al. 2022). According to the Species 2000 China node database, only one species, *Hyphodiscus hymeniophilus*, has been recorded in China (http://www.sp2000.org.cn/).

Han et al. (2014) introduced *Hyphopeziza* with a single species, *Hyphopeziza pygmaea*, which was transferred from *Hyalopeziza*. Despite the legitimacy of *Hyphopeziza* being questioned by Ekanayaka et al. (2019), recent studies have presented more robust phylogenetic and morphological evidence supporting its independence. It is characterized by white-greyish, minutely pubescent apothecia, hyaline to pale brown and gelatinized thin-/thick-walled excipular cells, cylindrical-conical to lageniform and hyaline hairs without septate, cylindrical paraphyses with warty and glassy apices, and elliptic-clavate and aseptate ascospores (Han et al. 2014, Quijada et al. 2022). *Hyphopeziza* is primarily distributed throughout Europe and Asia, colonizing the leaves of angiosperm plants (Huhtinen 1987, Hosoya & Otani 1997, Han et al. 2014, Quijada et al. 2022). Currently, there is a lack of documented evidence for the presence of *Hyphopeziza* in China.

Helotiaceae, Lachnaceae and Pezizellaceae are rich, widely distributed and paraphyletic groups in Helotiales (Ekanayaka et al. 2019, Johnston et al. 2019). Helotiaceae is one of the abundant families in Helotiales, introduced by Rehm (1982) and currently includes 32 genera (Luo et al. 2024). *Dicephalospora* is a group of apothecial fungi established by Spooner, typified by *Dicephalospora calochroa*, and currently consists of 18 species (Luo et al. 2024). They are found as saprobes on leafy or woody substrates and are characterized by stipitate, yellow to red apothecia, *textura prismatica* ectal excipular cells, *textura intricata* medullary excipular hyphae, filiform paraphyses, clavate asci and sub-ellipsoid to fusoid ascospores with occasional mucilaginous caps at the ends (Zhuang et al. 2016, Luo et al. 2024).

Lachnaceae was established by Raitviir (2004) to accommodate lignicolous members and currently encompasses 17 genera (Li et al. 2022). The genus *Erioscyphella*, introduced by Kirschstein (1938), consists of hairy and saprobic apothecial fungi with lanceolate paraphyses and long ascospores (Li et al. 2022, Su et al. 2023). *Lachnellula*, established by Karsten (1884), includes species that are saprobic or parasitic on coniferous wood and leaves, characterized by hairy apothecia with white or brown granulate hairs and small spherical to large, elongated, elliptical ascospores (Saccardo 1888, Ellis & Everhart 1893, Engler 1902, Nannfeldt 1932, Hahn & Ayers 1934, Dennis 1949, Seaver 1951, Dennis 1962, Dharne 1965, Kar & Pal 1970, Raitviir 1970, Kohn 1980, Oguchi 1981, Baral 1985, Iturriaga & Korf 1998, Baral & Matheis 2000, Baral 2008, Hosoya et al. 2010, Šandová 2015).

Pezizellaceae, established by Velenovský (1934), currently includes 27 genera with apothecial and hyphomycetous chalara-like taxa (Baral et al. 2022, Wijayawardene et al. 2022, Baral 2023, Wu & Diao 2023). The life modes of Pezizellaceae include saprobes or pathogens on plants, and they are distributed worldwide (Wu & Diao 2023). *Chalara* was initially introduced by

Rabenhorst (1844). According to the latest study by Wu & Diao (2023), the genus concept is now restricted to rarely include phialidic hyphomycetous members (chalara-like taxa) with cylindrical collarettes and deeply seated sporulating loci, as well as hyaline, cylindrical conidia. *Nagrajchalara*, established by Wu & Diao (2023) based on phylogenetic analysis of ITS, LSU, and SSU datasets and morphological evidence of septate conidia, includes only saprobic chalara-like asexual taxa.

In our study exploring the diversity of saprobic Leotiomycetes in southwestern China, a total of 19 specimens were collected and analyzed. This investigation resulted in the identification of four species belonging to three genera within the rare Hyphodiscaceae family, three species in two genera of Pezizellaceae, two species in two genera of Lachnaceae, and one species in *Dicephalospora* (Helotiaceae) based on both morphological characteristics and phylogenetic analyses. We introduce a new genus, along with four new species within Hyphodiscaceae. Additionally, we have described three newly discovered sexual morph records, one new geographical record in China, and two new collections in Yunnan Province. Comprehensive morphological descriptions and illustrations, along with a phylogenetic tree, are presented herein.

MATERIALS AND METHODS

Sample collection

All specimens were collected from unidentified decayed wood and fallen leaves during field investigations in subtropical regions of Yunnan Province, China, from May to September 2022. Our collections were found in protected areas and primary forests. During the sampling period, temperatures at collection sites ranged from 11 ℃ to 25 ℃, with altitudes ranging from 1200 m to 2520 m (Kunming and Puer City, Yunnan Province), while sites were situated at an altitude of 3800 m (Lijiang City, Yunnan Province) exhibited temperatures ranging from 9 ℃ to 13 ℃. The samples and their attaching substrates were carefully enclosed in tissue paper, rotated at both ends and securely fastened at the periphery to prevent compression of the specimen's central region. All collections were naturally air-dried, then wrapped in a layer of tissue, placed in a paper envelope containing a small amount of silica gel, and finally rehydrated upon arrival at the laboratory for morphological examination. The dried specimens were deposited at the Herbarium of Cryptogams, Kunming Institute of Botany Academia Sinica (KUN-HKAS). Facesoffungi numbers were obtained as in Jayasiri et al. (2015), and Index Fungorum numbers were obtained as outlined in Index Fungorum (2024). As the specimens are from the Greater Mekong region, the details will be lodged in the GMS database (Chaiwan et al. 2021).

Morphological studies

The Canon EOS M100 camera (Canon Co. LTD, Japan) was used to capture fresh apothecia in the field. A Canon EOS 70D(W) digital camera attached to a C-PSN stereomicroscope (Nikon Corporation Tokyo, Japan) was utilized for photographing macro-morphological features of dried apothecia. Fresh and dried apothecia were manually sectioned with razor blades before and then observed through a charge-coupled device SC 2000C connected to a Nikon ECLIPSE Ni-U compound microscope (Nikon Corporation Tokyo, Japan). Examination of the excipulum and hymenium involved vertical sections. Squashed mature apothecia mounted in water were used for the observation of asci, ascospores, and paraphyses. The blue iodine reaction at the ascus apex was examined employing Melzer’s reagent. All measurements were conducted utilizing the Tarosoft (R) Image Framework software (IFW) and subsequently modified in Adobe Photoshop 2020 (Adobe system, USA). The Q value represents the ratio of length to width for ascospores, while n denotes the count of measured structures, and Q_m signifies the mean value of Q. The colors of apothecia, hymenium and excipulum were referenced from https://www.colorhexa.com/.

DNA Extraction, PCR Amplification, and Sequencing

Total genomic DNA was extracted from multiple mature fruiting bodies, which were cleaned

with sterilized water and a 75% alcoholic solution using the TriliefTM Plant Genomic DNA Kit (Tsingke Biological Technology Co., LTD, Beijing, China). The product amplification primers used for each of the genes were as follows: for the nuclear internal transcribed spacers (ITS), ITS1-F and ITS4 (White et al. 1990, Gardes & Bruns 1993); for the D1/D2 domain of the nuclear large subunit ribosomal RNA (LSU), LROR and LR5 (Vilgalys & Hester 1990). The total reaction volume (25 μl) contains 12.5 μl of 2 × Power Taq PCR MasterMix, 7.5 μl of sterile deionized water, 1 μl of each primer (100 μM stock), and 3 μl of DNA template. Amplifications were carried out in a TC-type gene amplifier (LifeECO) (Hangzhou Bori Technology Co., LTD, Hangzhou Province, China). Reactions include an initial denaturation condition at 98 °C for 5 min, followed by 35 cycles of denaturation at 98 °C for 20 s, annealing at 53 °C for 30 s for LSU and ITS, followed by extension at 72 °C for 45 s, and a final extension at 72 °C for 10 min. The PCR products were verified through electrophoresis on a 1% agarose gel, followed by staining using TS-GelRed Ver.2 at a concentration of 10,000 times in water (provided by Tsingke Biological Technology Co., LTD, Beijing, China). The products were subsequently sequenced by Tsingke Biological Technology Co., LTD, located in Beijing, China.

Sequence assembly and alignment

All forward and reverse sequences were assembled in ContigExpress (Invitrogen, USA), and checked and edited in BioEdit 7.2.5.0 (Hall 1999). The newly generated sequences in this study were subjected to a blastn search available at NCBI to search for the homologous sequences (https://blast.ncbi.nlm.nih.gov/Blast.cgi). All new and related sequences used in this study were obtained from GenBank and utilized for conducting phylogenetic analyses. Two species of Amicodiscaceae (Helotiales, Leotiomycetes) were selected as the outgroup taxa.

The individual datasets of LSU and ITS were aligned in the MAFFT v. 7 online server (https://mafft.cbrc.jp/alignment/server/) (Katoh et al. 2019) using the default settings and improved manually in BioEdit v. 7.2.5.0. Then, the datasets were trimmed in TrimAl v.1.3 using the gt 0.5 option for ITS data and the gappyout option for LSU data (Capella-Gutiérrez et al. 2009). Finalized datasets of each gene were concatenated into a combined dataset using SequenceMatrix 1.7.8. (Vaidya et al. 2011) in the order 'LSU-ITS'. The online tool ALTER (Alignment Transformation EnviRonment) was employed to transform the file format from ".fasta" to ".nexus". The sequences generated in this study have been submitted to GenBank (Table 1). The genus names are abbreviated as '*L*.' for *Longistipes*, '*H*.' for *Hyphopeiza*, '*Hy*.' for *Hyphodiscus*, '*C*.' for *Chalara*, '*N*.' for *Nagrajchalara*, '*D*.' for *Dicephalospora*, '*E*.' for *Erioscyphella*, and '*La*.' for *Lachnellula*.

Phylogenetic analyses

The maximum likelihood (ML) analysis was performed at the IQ-Tree web portal (http://iqtree.cibiv.univie.ac.at/). The substitution model options for each gene were automatically evaluated based on the partition file provided. The clade support for the ML analysis was evaluated by conducting a 1,000 replicate SH-aLRT test and utilizing the ultrafast bootstrap (UFB) method (Guindon et al. 2010, Hoang et al. 2018). The best-fitting models for each gene part were automatically evaluated and are shown in Table 2. Nodes with support values of both SH-aLRT ≥ 80 and UFB ≥ 95 were considered well-supported, nodes with either SH-aLRT < 80 or UFB < 95 were classified as moderately supported, and nodes with both SH-aLRT < 80 and UFB < 95 were deemed unsupported.

Bayesian inference (BI) analysis was performed using MrBayes v. 3.1.2. The posterior probabilities (PP) were assessed using the Markov Chain Monte Carlo sampling (MCMC). The best models for each gene part were selected using the Akaike information criterion (AIC) with MrModeltest v.2.3 (Nylander et al. 2004), as shown in Table 2. Four simultaneous Markov Chains were executed for 2,000,000 generations, with tree sampling occurring every 100th generation. The MCMC analysis commenced with a randomly selected tree, which was sampled at intervals of 1,000 generations. The average standard deviation < 0.01 for split frequencies was used to suggest convergence between parallel runs. The initial 25% of total trees were excluded as burn-in, while

Table 1 Detailed information and corresponding GenBank accession numbers for the taxa utilized in the phylogenetic analyses of this study are provided.

Taxon name		**Voucher**	**Gene accession No.**	
			ITS	**LSU**
Helotiaceae	*Amylocarpus encephaloides*	CBS 129.60	MH857920	MH869464
	Amylocarpus encephaloides	017cN	KM272369	KM272361
	Bryoscyphus dicrani	M141	EU940183	EU940107
	Connersia rilstonei	CBS 537.74	KJ755499	AF096189
	Crocicreas amenti	F-147481	FJ005093	FJ005124
	Crocicreas cacaliae	F-148706	FJ005107	FJ005126
	Crocicreas cyathoideum	MFLU 18-0698	MK584943	MK591970
	Crocicreas tomentosum	MFLU 17-0082	MK584988	MK592008
	Cudoniella clavus	AFTOL-ID 166	DQ491502	DQ470944
	Cyathicula microspora	M267	EU940165	EU940088
	Dicephalospora albolutea	HMAS 279693	MK425601	–
	Dicephalospora aurantiaca	HMAS 61850	DQ986486	–
	Dicephalospora chiangraiensis	MFLU 21-0019	MZ241818	MK591988
	Dicephalospora chiangraiensis†	MFLU 21-0018	MZ241817	–
	Dicephalospora chiangraiensis	**HKAS 131084**	**PP919441**	**PP917520**
	Dicephalospora chiangraiensis	**HKAS 131063**	**PP919443**	**PP917521**
	Dicephalospora chrysotricha	PDD:91762	KF727411	MZ241826
	Dicephalospora chrysotricha	PDD:58197	KF727409	–
	Dicephalospora chrysotricha	PDD:93932	MH578487	MZ241827
	Dicephalospora chrysotricha	PDD:81537	KF727410	–
	Dicephalospora dentata	3093	KP204263	–
	Dicephalospora huangshanica	MFLU 18-1828	MK584979	MK591979
	Dicephalospora huangshanica	KUS-F52405	JN033408	JN086711
	Dicephalospora inthanonensis†	MFLU 22-0050	ON606312	ON604634
	Dicephalospora inthanonensis	MFLU 22-0053	ON606313	ON604635
	Dicephalospora irregularis	CM 31	ON511117	ON514038
	Dicephalospora rufocornea	MFLU 16-1860	MK584989	MK592011
	Dicephalospora rufocornea	MFLU 19-2083	MZ241816	MZ241825
	Dicephalospora sagerae	BRIP 72428d	NR_182617	–
	Dicephalospora sessilis†	MFLU 18-1823	NR_163779	NG_068621
	Dicephalospora shennongjiana	HMAS 279698	MK425606	–

Table 1 Continued.

Taxon name		Voucher	Gene accession No.	
			ITS	LSU
	Dicephalospora xishuangbannaensis	HKAS 131157	OR948047	–
	Dicephalospora xishuangbannaensis†	HKAS 131164	OR948048	–
	Dicephalospora yunnanica	HMAS 279701	MK425609	–
	Dicephalospora yunnanica	HMAS 279700	MK425608	–
	Dicephalospora yunnanica	HMAS 279699	MK425607	–
	Endoscypha perforans	PDD:102231	KF727424	MK039717
	Glarea lozoyensis†	ATCC 20868	NR_137138	–
	Glarea sp.	C2B	KX610435	–
	Gloeotinia granigena	CBS 417.50	–	MH868212
	Hymenoscyphus fructigenus	CBS 186.47	MH856211	MH867741
	Hymenoscyphus occultus	KUS-F52847	KP068064	–
	Hymenoscyphus pseudoalbidus	Hokk 14	KJ511191	–
	Hymenotorrendiella eucalypti	PDD:70105	MH578483	–
	Hymenotorrendiella eucalypti†	CPC 11050	DQ195788	DQ195800
	Hymenotorrendiella madsenii	PRJ D672	AY755336	KJ606676
	Lanzia berggrenii	ICMP:19614	KC164645	KC164640
	Ombrophila violacea	WZ0024	AY789366	AY789365
	Phaeohelotium epiphyllum	TNS:F 40042	AB926061	AB926130
	Pirottaea palmicola	PDD:60282	KM677208	–
	Pirottaea palmicola	PDD:65971	KM677206	–
	Pleuroascus nicholsonii	CBS 345.73	KJ755519	AF096196
	Roesleria subterranea	CBS 339.96	EF060308	EF608074
	Roesleria subterranea	CBS 407.51	MH856922	–
Hyphodiscaceae	*Amicodisca castaneae*	KUS-F51377	JN033389	JN086692
	Amicodisca castaneae	KUS-F51917	JN033411	JN086714
	Dematioscypha dematiicola	NBRC108583/TNS-F-17834	JN033438	JN086739
	Fuscolachnum inopinatum	SBRH855	OL752697	OM203548
	Fuscolachnum misellum	SBRH799b	KX501124	KX501129
	Fuscolachnum misellum	SBRH943	OM203545	OM203549
	Fuscolachnum pteridis	SBRH831	OM203547	–
	Fuscolachnum pteridis	TUR215412	OM818501	OM818501
	Fuscolachnum aff. *misellum*	SBRH927	OM203544	OM203550

Table 1 Continued.

Taxon name	Voucher	Gene accession No.	
		ITS	**LSU**
Fuscolachnum aff. *misellum*	LE 305267	OM407389	–
Glutinomyces brunneus	ta2uD7	LC218306	LC315171
Glutinomyces inflatus†	TNS-F-80763	LC218289	LC315170
Glutinomyces takaragaikensis†	TNS-F-80765	LC218290	LC315176
Glutinomyces vulgaris†	TNS-F-80764	LC218288	LC315172
Hyphodiscus brachyconius	CBS:630.75	MH860958	MH872727
Hyphodiscus cajaniensis	M155	EU940189	EU940112
Hyphodiscus hyaloscyphoides†	TNS-F-13588	AB546944	AB546945
Hyphodiscus hymeniophilus	TAAM174997	ON241771	–
Hyphodiscus hymeniophilus	TUF117323	ON241772	–
Hyphodiscus hymeniophilus	TUF104970	ON241773	–
Hyphodiscus hymeniophilus	TNS-F-31801	AB546948	AB546946
Hyphodiscus cf. *hymeniophilus*	LE_305399	OM407386	–
Hyphodiscus luxurians	LE_304401	OM407387	–
Hyphodiscus luxurians	30BG69	OP585913	–
Hyphodiscus otanii†	TNS-F-7099	AB546949	AB546947
Hyphodiscus pseudotanii†	**HKAS 128322**	**PP499245**	**PP503195**
Hyphodiscus pseudotanii	**HKAS 128362**	**PP503191**	**PP503196**
Hyphodiscus theiodeus	TNS-F-31803	AB546953	AB546952
Hyphodiscus ucrainicus	PZ124	MH134512	MH134511
Hyphopeziza macrospora	**HKAS 128311**	**PP503193**	**PP503197**
Hyphopeziza macrospora†	**HKAS 128312**	**PP503192**	**PP503198**
Hyphopeziza macrospora	**HKAS 128313**	**PP503194**	**PP503199**
Hyphopeziza pygmaea	KUS-F51564	JN033410	JN086713
Hyphopeziza pygmaea	TNS-F-17940	JN033448	JN086748
Longistipes albus†	**HKAS 128338**	**PP915871**	**PP915873**
Longistipes albus	**HKAS 128361**	**PP915870**	**PP915872**
Longistipes niger†	**HKAS 128334**	**PP915874**	–
Longistipes niger	**HKAS 128358**	**PP919438**	–
Microscypha arenula	SBRH922	OM203546	–
Scolecolachnum nigricans†	MFLU 18-1817	MK584975	MK591973
Scolecolachnum pteridii	CPC:24666	KU597797	KU597764

Table 1 Continued.

Taxon name		Voucher	Gene accession No.	
			ITS	**LSU**
	Soosiella minima	MH_2012_1230	JX124327	JX124327
	Venturiocistella japonica	TNS-F-18030	JN033447	AB546954
	Venturiocistella sp.	KUS-F52028	JN033391	JN086694
Lachnaceae	*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	JN086695	JN033392
	Capitotricha bicolor	PRM:915564	–	LT904864
	Capitotricha rubi	TNS-F-65752	LC438573	LC438560
	Dasyscyphella montana	FC-2031	–	AB481241
	Dasyscyphella montana	FC-2070	–	AB481242
	Erioscyphella abnormis	TNS-F-80478	LC424837	LC424949
	Erioscyphella alba†	MFLU 16-0614	NR_163782	NG_066457
	Erioscyphella aseptata†	MFLU 16-0590	NR_163780	NG_066456
	Erioscyphella boninensis†	TNS-F-26520	UDB0779049	LC533151
	Erioscyphella brasiliensis	MFLU 16-0577a	MK584953	–
	Erioscyphella brasiliensis	MFLU 16-0577b	MK584967	MK591993
	Erioscyphella curvispora†	KL381	MH190414	MH190415
	Erioscyphella euterpes	PR147	U58640	–
	Erioscyphella fusiforme	MFLU 15-0230	NR_154122	NG_066455
	Erioscyphella fusiforme	MFLU 18-1824	MK584948	MK591975
	Erioscyphella hainanensis	TNS-F-35056	UDB0779065	LC533169
	Erioscyphella hainanensis	TNS-F-35049	UDB0779064	LC533168
	Erioscyphella hainanensis	**HKAS 135665**	**PP919440**	**–**
	Erioscyphella insutae	TNS-F-26500	UDB0779060	LC533149
	Erioscyphella insutae	TNS-F-39720	UDB0779063	LC533177
	Erioscyphella lataspora†	HKAS 124389	OP310823	OP113844
	Erioscyphella lunata	S.T. 13021602	KX501132	KX501133
	Erioscyphella lushanensis†	3631	AF505515	–
	Erioscyphella otanii	TNS-F-81472	UDB0779085	LC533179
	Erioscyphella papillaris	TNS-F-81272	UDB0779081	LC533161

Table 1 Continued.

Taxon name	Voucher	Gene accession No.	
		ITS	**LSU**
Erioscyphella paralushanensis	TNS-F-61920	UDB0779075	LC533141
Erioscyphella sasibrevispora	TNS-F-80399	UDB0779082	LC533173
Erioscyphella sasibrevispora	TNS-F-81401	UDB0779084	LC533174
Erioscyphella sclerotii	MFLU 18-0688	MK584969	MK591995
Erioscyphella sclerotii	MFLU 16-0569	MK584951	MK591980
Erioscyphella sinensis	TNS-F-16838	AB481280	LC533164
Erioscyphella sinensis	TNS-F-80354	UDB0779083	LC533143
Hymenoscyphus albidoides†	HMAS 264140	NR_154903	NG_059508
Hymenoscyphus fructigenus	TNS-F-44644	AB926057	AB926144
Incrucipulum capitatum	TNS-F81420	LC424838	LC424954
Incrucipulum ciliare	TNS-F-81516	LC438582	LC438565
Incrucipulum longispineum	FC-2323	AB481325	AB481256
Lachnellula calyciformis	CBS:189.66	MH870403	MH858771
Lachnellula flavovirens	CBS:191.66	KC492975	KC464637
Lachnellula pini	CBS:169.35	MH855623	MH867133
Lachnellula subtilissima	CBS:197.66	MH858774	MH870409
Lachnellula subtilissima	**HKAS 135664**	**PP919439**	**–**
Lachnum asiaticum	FC-2056	AB481297	AB481251
Lachnum pudibundum	FC-2058	AB481298	AB481259
Lachnum rachidicola	TNS-F-16647	–	AB745431
Lachnum virgineum	FC-2137	AB705275	AB705235
Neodasyscypha cerina	TNS-F-65625	LC424948	LC424836
Proliferodiscus alboviridis	TNS-F-17436	LC438558	LC424950
Proliferodiscus chiangraiensis†	MFLU 16-0588	NR_164304	NG_068622
Proliferodiscus dingleyae	ICMP:21730	MH682231	–
Proliferodiscus earoleucus	BHI-F624d	MF161304	–
Proliferodiscus ingens†	CBS:145519	NR_170767	NG_073756
Proliferodiscus longisporus	HKAS 124388	OP113842	OP264080
Proliferodiscus longisporus†	HKAS 124390	OP113840	OP310824
Proliferodiscus pulveraceus	G.M. 2017-03-21.3	MN066320	–
Proliferodiscus tricolor	CBS:122000	KC464643	KC492981
Proliferodiscus tricolor	CBS:128288	MH864846	MH876293

Table 1 Continued.

Taxon name		Voucher	Gene accession No.	
			ITS	LSU
Pezizellaceae	*Allophylaria subliciformis*	R.M.2374	MH221035	–
	Bispora pallescens	DMS-10078235T	MW203182	–
	Bloxamia discedens	MFLU 18-0691	MK584970	MK591996
	Bloxamia discedens	NN076346	ON993899	OP173629
	Bloxamia elongata†	NN047646	ON993905	OP173630
	Bloxamia truncata	NN050624	ON993907	OP173631
	Bloxamia truncata	FMR_11240	KY853426	KY853486
	Bloxamiella cyatheicola†	VIC 42563	NR_153617	NG_058691
	Calycellina fagina	SBRH925	OL752703	OM218631
	Calycellina leucella	MP150937	MT231682	–
	Calycina affinis	NN076476	ON993917	OP173634
	Calycina affinis	NN076333	ON993909	OP173632
	Calycina alstrupii†	NN010761	NR_154846	NG_068538
	Calycina brevipes	NN050639	ON993924	OP173639
	Calycina brevipes	NN050681	ON993925	OP173640
	Calycina citrina	HMAS 286993	OQ534198	OQ534484
	Calycina citrina	HMAS 285424	OQ534197	OQ534483
	Calycina claroflava	F132983	KC412006	–
	Calycina eucalypticola	NN043033	ON993929	–
	Calycina eucalypticola†	CPC 36078	NR_182461	NG_148969
	Calycina fungorum	NN050484	ON993930	–
	Calycina herbarum	KUS-F51458	JN033390	JN086693
	Calycina lactea	HB7224	KC412007	–
	Calycina lactea	iNAT:18000156	MZ209003	–
	Calycina languida	F116599	KC412002	–
	Calycina marina	TROM:F26093	KT185677	KT185670
	Calycina parilis	NN047902	ON993932	–
	Calycina parilis	NN047837	ON993931	–
	Calycina oxenbolliae	NN050633	ON993940	–
	Calycina parvispora	NN050487	ON993941	–
	Calycina populina	CBS:247.62	MH858147	MH869739
	Calycina riisgaardii†	NN047715	ON993963	OP173649

Table 1 Continued.

Taxon name	Voucher	Gene accession No.	
		ITS	**LSU**
Calycina shangrilana	HKAS 90655a	MK584972	MK591998
Calycina sulfurina	G.M.2015-10-23.6	MT435017	OP173636
Chalara africana	NN076574	ON993919	OP173635
Chalara africana	NN043684	ON993918	NG_228995
Chalara bambusicola†	CGMCC 3.23394	NR_187031	KX228321
Chalara bambusicola	**HKAS 128357**	**PP919444**	**PP917525**
Chalara bambusicola	**HKAS 128372**	**PP919445**	**PP919446**
Chalara clidemiae†	CPC 26423	NR_145313	AF222457
Chalara cylindrosperma	CBS:658.79	MH873005	OP173641
Chalara longiphora†	NN076442	ON993933	OP173642
Chalara longiphora	NN076442	ON993936	OP173643
Chalara pengii†	NN077176	ON993942	OP173645
Chalara pengii	NN077140	ON993951	OP173647
Chalara platanicola†	NN076305	ON993956	OP173648
Chalara qinlingensis†	NN076946	ON993959	MH869739
Chalara qinlingensis	NN076946	ON993958	–
Cylindrocephalum aureum	NN042820	ON993979	OP173654
Cylindrocephalum aureum	NN077404	ON993982	OP173655
Cylindrocephalum aureum	CBS:633.75	MH860959	MH872728
Cylindrocephalum clavatisetosum†	NN053375	ON993984	OP173656
Cylindrocephalum hughesii	NN076314	ON993990	OP173658
Cylindrocephalum kendrickii	NN043817	ON993995	OP173660
Cylindrocephalum zhejiangense†	CGMCC 3.23422	NR_187037	NG_229001
Hymenoscyphus albidus	CBS:126533	MH864151	MH875610
Micropeziza umbrinella	K(M):168087	MZ159422	–
Mollisina uncinata	KUS-F52307	JN033404	JN086707
Nagrajchalara acuaria	NN043205	ON994023	–
Nagrajchalara acuariella†	CGMCC 3.23454	NR_187039	–
Nagrajchalara agathidis	NN042964	ON994025	OP173672
Nagrajchalara agathidis	NN078321	ON994027	–
Nagrajchalara angustata†	CBS:231.96	NR_159786	NG_067438
Nagrajchalara aspera	NN042933	ON994031	OP173674

Table 1 Continued.

Taxon name	Voucher	Gene accession No.	
		ITS	LSU
Nagrajchalara aspera	NN076391	ON994037	OP173676
Nagrajchalara aunstrupii	NN078290	ON994047	OP173678
Nagrajchalara cannonii	NN076549	ON994049	OP173679
Nagrajchalara cannonii†	NN076454	ON994048	–
Nagrajchalara conifericola	NN050593	ON994052	OP173680
Nagrajchalara conifericola†	NN077636	ON994051	–
Nagrajchalara curviphora	NN076102	ON994056	OP173682
Nagrajchalara ejneri†	NN055373	ON994058	OP173683
Nagrajchalara ellipsoidea	NN047768	ON994060	OP173684
Nagrajchalara ellispoidea†	CGMCC 3.23402	NR_187042	NG_229004
Nagrajchalara guangcaii†	NN076112	ON994062	OP173686
Nagrajchalara haituoshanensis†	NN078171	NR_187044	OP173687
Nagrajchalara haituoshanensis	NN078174	ON994066	OP173688
Nagrajchalara hangzhouensis†	NN077457	ON994068	–
Nagrajchalara hangzhouensis	NN059055	ON994067	–
Nagrajchalara inflatipes	NN055517	ON994069	–
Nagrajchalara insignis	PRC 4658	LR963470	–
Nagrajchalara japonica	NN077460	ON994072	OP173692
Nagrajchalara japonica†	CGMCC 3.23402	NR_187045	OP173691
Nagrajchalara jonesii†	CGMCC 3.23460	NR_187046	OP173695
Nagrajchalara jonesii	NN076451	ON994073	OP173693
Nagrajchalara keginii†	NN076020	ON994076	OP173696
Nagrajchalara knudsonii†	CGMCC 3.23409	NR_187048	NG_229007
Nagrajchalara knudsonii	NN076023	ON994080	OP173697
Nagrajchalara neonawawii	NN078612	OQ145667	–
Nagrajchalara neonawawii	NN078611	OQ145666	–
Nagrajchalara neonawawii	**HKAS 128356**	**PP917529**	–
Nagrajchalara morganjonesii	NN043682	ON994082	OP173699
Nagrajchalara mubatilis	NN050725	ON994085	–
Nagrajchalara nawawii†	NN047788	ON994086	OP173700
Nagrajchalara panamensis†	PRC 3714	LT629155	–
Nagrajchalara paraunicolor†	NN050666	ON994090	–

Table 1 Continued.

Taxon name	Voucher	Gene accession No.	
		ITS	LSU
Nagrajchalara pseudoaurea†	NN050701	ON994091	OP173702
Nagrajchalara puerense†	NN076052	ON994092	OP173703
Nagrajchalara puerensis	NN076120	ON994093	OP173704
Nagrajchalara qingchengshanense†	NN077720	ON994094	OP173706
Nagrajchalara selaginellae	NN043153	ON994097	OP173707
Nagrajchalara sichuanensis	NN057632	ON994102	OP173710
Nagrajchalara sivanesanii†	NN076694	ON994103	OP173711
Nagrajchalara sp.	NN040628	ON994157	–
Nagrajchalara sp.	NN047517	ON994155	OP173730
Nagrajchalara sp.	NN076348	ON994153	OP173729
Nagrajchalara strobilina	NN09796	ON994160	–
Nagrajchalara tengii	NN044481	ON994104	OP173712
Nagrajchalara tropicalis	NN042844	ON994109	OP173713
Nagrajchalara tsuensis†	NN077469	ON994111	OP173714
Nagrajchalara tsukairakuensis†	NN077323	ON994112	OP173715
Nagrajchalara tubakii	NN076708	ON994119	OP173717
Nagrajchalara tubakii†	CGMCC 3.23431	NR_187060	–
Nagrajchalara unicolor	NN044014	ON994123	–
Nagrajchalara unicolor	NN045977	ON994122	OP173718
Nagrajchalara venicola	NN076015	ON994125	OP173720
Nagrajchalara venicola	NN076011	ON994124	OP173719
Nagrajchalara yinglaniae†	NN076400	ON994126	OP173721
Nagrajchalara yongnianii†	NN077394	ON994151	OP173728
Nagrajchalara yongnianii	NN046066	ON994134	OP173723
Nagrajchalara yongnianii	**HKAS 128367**	**PP917531**	–
Neochalara spiraeae†	CPC 39565	OK664715	OK663754
Orbiliopsis callistea	PDD:97932	HQ533049	HQ533050
Parachalara olekirkii†	NN043656	NR_187035	NG_228999
Pezizella epithallina	NN017411	KJ559552	KJ559572
Phialina lachnobrachyoides	KUS-F52183	JN033412	JN086715
Phialina lachnobrachyoides	KUS-F52576	JN033424	JN086727
Phialina ulmariae	K(M):200003	MZ159550	–

Table 1 Continued.

Taxon name	Voucher	Gene accession No.	
		ITS	**LSU**
Porodiplodia livistonae†	CPC 32154	MH327809	MH327845
Porodiplodia vitis	CBS:144634	MK442616	MK442552
Porodiplodia vitis	CBS:144634	MK442616	MK442552
Rodwayella citrinula	KUS-F52443	JN033414	JN086717
Rodwayella sessilis	H.B. 9913	KT876974	–
Rubropezicula thailandica†	MFLU 16-0592	NR_163781	–
Scleropezicula alnicola	CBS:200.46	MH856161	MH867686
Triposporium cycadicola	CBS:137968	NR_156587	NG_067285
Triposporium deviatum	CBS:995.70	MH860021	MH871806
Zymochalara cyatheae†	CPC 24665	NR_154509	NG_059652
Zymochalara lygodii†	CPC 24699	NR_154510	NG_059653

[a]ITS: Part of rDNA 18S (3' end), the first internal transcribed spacer (ITS1), the 5.8S rRNA gene, the second ITS region (ITS2) and part of the 28S rRNA(5' end); LSU: Large subunit (28S).

[b]Abbreviations: AFTOL: Assembling the Fungal Tree of Life; ATCC: Herbarium of American Type Culture Collection, Virginia, U.S.A.; BRIP: Plant Pathology Herbarium, Queensland, Australia; CBS: Herbarium of Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands; CPC: Herbarium of Colegio de Postgraduados, Veracruz, Mexico; F: Herbarium of Field Museum of Natural History, Chicago, U.S.A.; FMR: Herbarium of Universitat Rovira i Virgili, Tarragona, Spain; HB: Herbarium Bradeanum, Rio de Janeiro, Brazil; HKAS: Herbarium of Cryptogams of Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China; HMAS: Herbarium of Chinese Academy of Sciences, Beijing, China; ICMP: International Collection of Microorganisms from Plants, Auckland, New Zealand; iNAT: Herbarium of National Institute of Agronomy, Tunisia, Tunis, Tunisia; K: Herbarium of Royal Botanic Gardens, Kew, England; KUS: the Mycological Herbarium of the Korea University; MFLU: Herbarium of Mae Fah Luang University, Chiang Rai, Thailand; MP: Herbarium of Východoceské muzeum Pardubice, Pardubice, Czech Republic; NBRC: Herbarium of National Institute of Technology and Evaluation, Chiba, Japan; PDD: Herbarium of Manaaki Whenua - Landcare Research, Auckland, New Zealand; PR: Herbarium of National Museum in Prague, Praha, Czech Republic; PRC: Herbarium of Charles University, Prague, Praha, Czech Republic; PRM: Herbarium of National Museum, Praha, Czech Republic; TAAM: Herbarium of Institute of Agricultural and Environmental Sciences of the Estonian University of Life Sciences, Tartu, Estonia; TNS: Herbarium of the National Museum of Nature and Science, Tsukuba, Japan; TROM: Herbarium of UiT The Arctic University of Norway, Troms, Norway; TUR: Herbarium of University of Turku, Turku, Finland; VIC: Herbarium of Universidade Federal de Viçosa, Minas Gerais, Brazil.

[c]The symbol '†' is used to denote type species. Newly generated sequences in bold font. The symbol '–' indicates that sequence data is unavailable.

Table 2 The model selections for each gene or dataset in ML analysis and BI analysis.

Figure	Gene type or dataset	Selected model for each gene or dataset	
		ML analysis	**BI analysis**
Fig. 1	LSU	TIM3e+I+G4	SYM+I+G
	ITS	TIM2e+I+G4	GTR+I+G
Fig. 2	LSU	TNe+I+G4	GTR+I+G
	ITS	GTR+F+I+G4	GTR+I+G
Fig. 3	LSU	TN+F+R3	GTR+I+G
	ITS	TIM2e+R3	GTR+I+G
Fig. 4	LSU	TIM2e+I+G4	GTR+I+G
	ITS	TN+F+I+G4	GTR+I+G

[a]Abbreviations: F: Empirical base frequencies; G4, G: discrete Gamma model with default 4 rate categories; GTR: General time reversible model with unequal rates and unequal base frequencies; I: allowing for a proportion of invariable sites; R2: FreeRate model with 3 rate categories; SYM: Symmetric model with unequal rates but equal base frequencies; TIM2e: AC = AT, CG = GT and equal base frequencies; TIM3e: AC = CG, AT = GT and equal base frequencies; TN: Unequal transition/transversion rates with unequal purine/pyrimidine rates and unequal base frequencies; Tne: Unequal transition/transversion rates with unequal purine/pyrimidine rates and equal base frequencies.

the remaining trees in each analysis were employed to estimate PP within the majority-rule consensus tree. PP ≥ 0.95 is deemed indicative of strong support. The phylogenetic tree obtained was visualized using Figtree v.1.4.0 (Rambaut 2009) and illustrated with the assistance of Adobe Illustrator 2020 and Adobe Photoshop 2020 (https://www.adobe.com/).

RESULTS

Phylogenetic analyses – Hyphodiscaceae (Fig. 1)

The combined LSU and ITS dataset comprises 31 taxa, representing 44 isolates and 1374 aligned nucleotide sites, including 803 bp for the LSU region and 571 bp for the ITS region with gaps. The combined alignment contained 283 parsimony-informative characters, 77 singleton sites, and 1014 constant characters. The ML and BI analyses yielded similar topologies. The maximum likelihood matrix had 432 distinct alignment patterns with 23.22% undetermined characters or gaps. The best maximum likelihood tree, with a final likelihood value of -6675.839, is shown in Fig. 1. *Fuscolachnum inopinatum* (strain: SBRH855) and *Scolecolachnum pteridii* (strain: CPC: 24666) formed a distinct clade within the Hyphodiscaceae family, positioned far from *Fuscolachnum sensu stricto*. *Longistipes* was closely related to *Fuscolachnum sensu stricto* and *Microscypha*, with 89% ML bootstrap support in the SH-aLRT test, 61% in the UFB method, and a Bayesian probability of 0.5. Additionally, *Longistipes* showed proximity to *Hyphopeziza*, with 69% ML bootstrap support in the SH-aLRT test, 74% in the UFB method, and a Bayesian probability of 0.90. *Longistipes albus* (strains: HKAS 128338 and HKAS 128361) and *Longistipes niger* (strains: HKAS 128334 and HKAS 128358) were confidently clustered together, with a strong ML bootstrap support of 96% in the SH-aLRT test, 99% in the UFB method, and a Bayesian probability of 1.0. *Hyphopeziza macrospora* was found to be closely related to *H. pygmaea* (strains: TNS-F 17940 and KUS-F51564) within *Hyphopeziza*, with ML bootstrap support of 93.4% in the SH-aLRT test, 94% in the UFB method, and a Bayesian probability of 0.99. *Hyphodiscus pseudotanii* clustered with *Hy. hymeniophilus* (strain: TAAM:174997 and TUF:117323), allied species of *Hy. hymeniophilus* (strain: TUF:104970 and LE 305399) and *Hy. brachyconius* (strain: CBS:630.75) with ML bootstrap support of 83% in the SH-aLRT test, 74% in the UFB method, and a Bayesian probability of 0.66.

Phylogenetic analyses – Pezizellaceae (Fig. 2)

The combined LSU and ITS dataset comprises 104 taxa, representing 139 isolates, and 1387

aligned nucleotide sites, including 921 bp for the LSU region and 466 bp for the ITS region with gaps. The combined alignment contained 463 parsimony-informative characters, 118 singleton sites, and 806 constant characters. The ML and BI analyses yielded similar topologies. The maximum likelihood matrix had 649 distinct alignment patterns with 21.85% undetermined characters or gaps. The best maximum likelihood tree, with a final likelihood value of -16436.044, is shown in Fig. 2. *Nagrajchalara yongnianii* HKAS 128367 was clustered with two strains of *N. yongnianii*, with ML bootstrap support of 98.6% in the SH-aLRT test, 100% in the UFB method, and a Bayesian probability of 1.0. *Nagrajchalara neonawawii* HKAS 128356 was nested with two strains of *N. neonawawii*, with ML bootstrap support of 79.2% in the SH-aLRT test, 99% in the UFB method, and a Bayesian probability of 0.96.

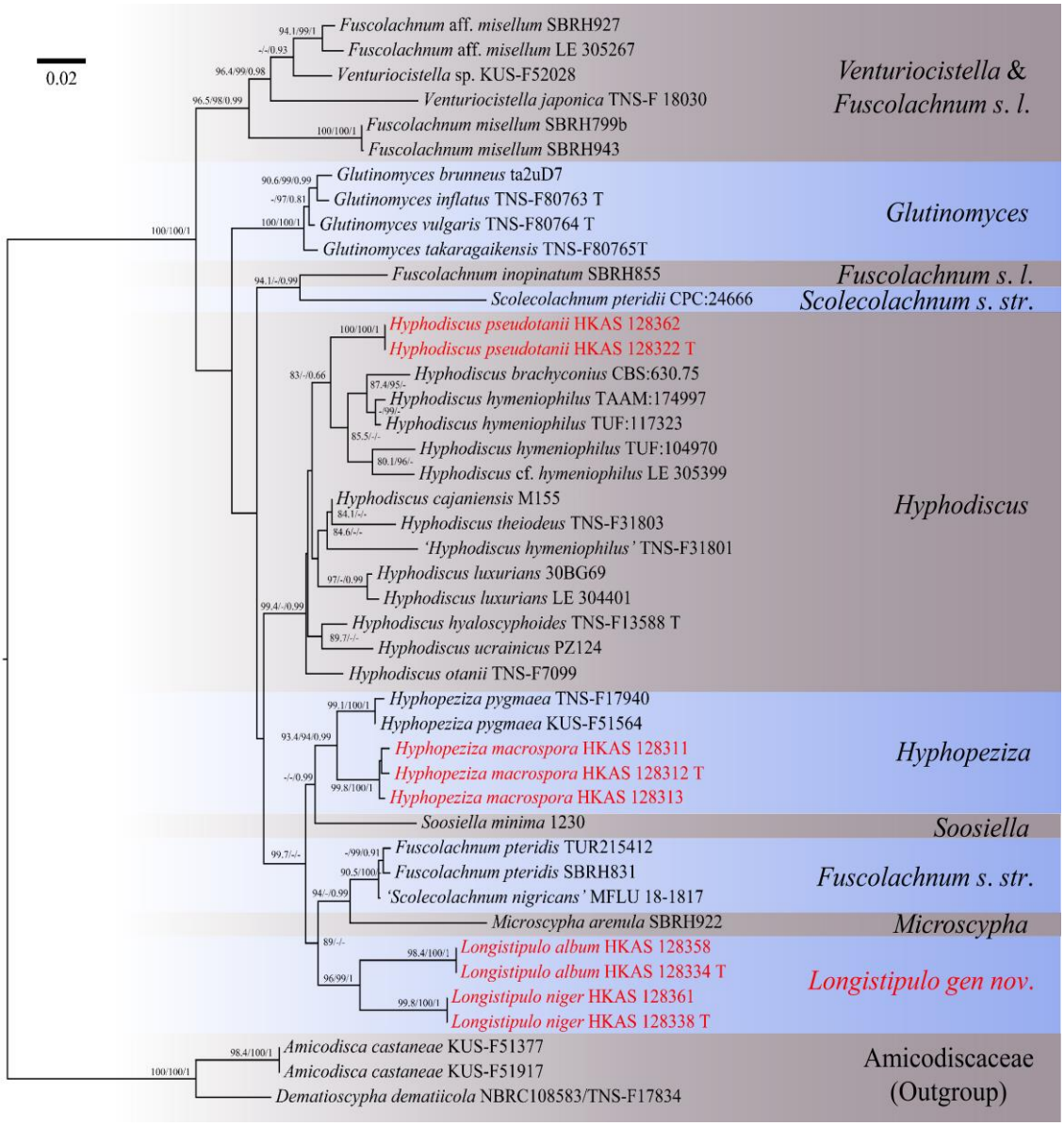

Figure 1 – Maximum likelihood tree based on a combined dataset of LSU and ITS sequences for Hyphodiscaceae. Bootstrap support values for ML ≥ 80 of SH-aLRT or ML > 95 of UFB and posterior probability for BI ≥ 0.95 are indicated above the nodes and separated by "-/-/-" (SH-aLRT/UFB/PP). The newly generated isolates of the current study are highlighted in red, whereas ex-types are denoted as 'T' following the strain number.

Phylogenetic analyses – *Dicephalospora* (Fig. 3)

The combined ITS and LSU dataset comprises 38 taxa, representing 54 isolates, and 1199

aligned nucleotide sites, including 791 bp for the LSU region and 408 bp for the ITS region with gaps. The combined alignment contained 299 parsimony-informative characters, 79 singleton sites, and 821 constant characters. The ML and BI analyses yielded similar topologies. The maximum likelihood matrix had 379 distinct alignment patterns with 30.56% undetermined characters or gaps. The best maximum likelihood tree, with a final likelihood value of -6847.338, is shown in Fig. 3. Our two collections of *D. chiangraiensis* (HKAS 131063 and HKAS 131084) were nested within two strains of *D. chiangraiensis* (MFLU 21-0018 and MFLU 21-0019) with ML bootstrap support of 99.7% in the SH-aLRT test, 100% in the UFB method, and a Bayesian probability of 1.0.

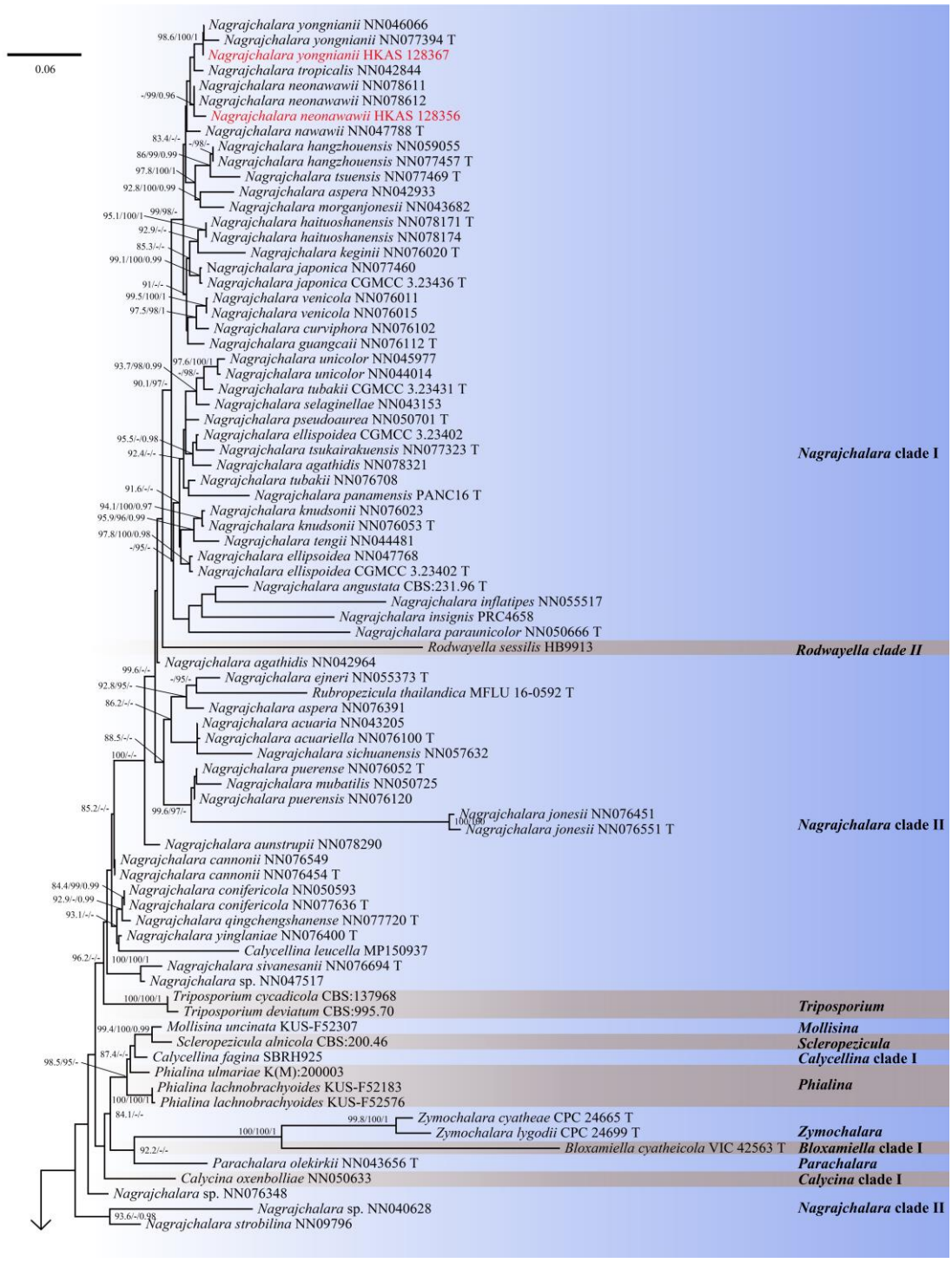

Figure 2 – Maximum likelihood tree based on a combined dataset of LSU and ITS sequences for Pezizellaceae. Bootstrap support values for ML ≥ 80 of SH-aLRT or ML > 95 of UFB and posterior

probability for BI ≥ 0.95 are indicated above the nodes and separated by "-/-/-" (SH-aLRT/UFB/PP). The newly generated isolates of the current study are highlighted in red, whereas ex-types are denoted as 'T' following the strain number.

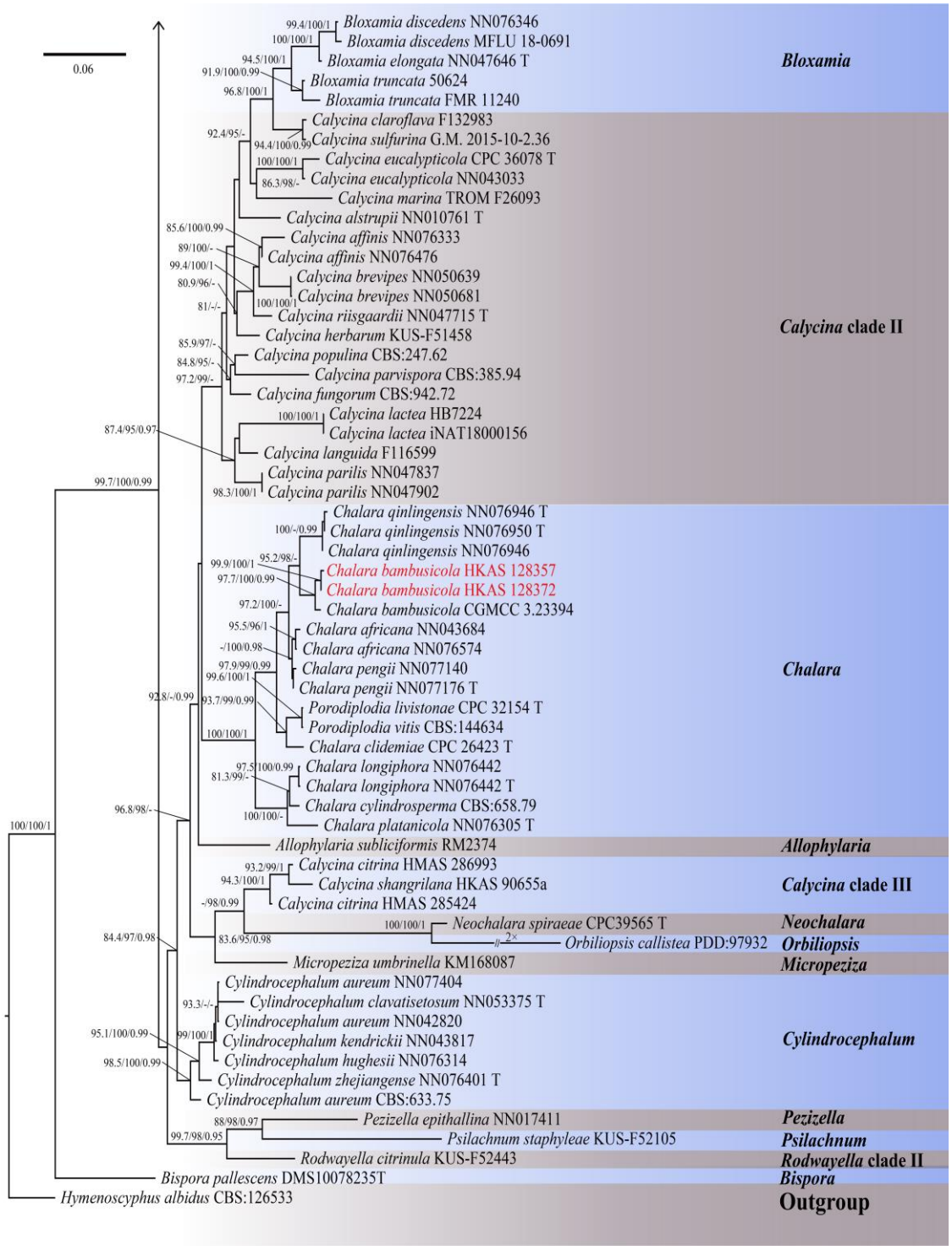

Figure 2 – Continued.

Phylogenetic analyses – Lachnaceae (Fig. 4)

The combined LSU and ITS dataset comprises 51 taxa, representing 65 isolates and 1422 aligned nucleotide sites, including 864 bp for the LSU region and 558 bp for the ITS region with gaps. The combined alignment contains 343 parsimony-informative characters, 108 singleton sites, and 970 constant characters. The ML and BI analyses yielded similar topologies. The maximum

likelihood matrix had 544 distinct alignment patterns with 26.15% undetermined characters or gaps. The best maximum likelihood tree, with a final likelihood value of -10133.243, is shown in Fig. 4. *Erioscyphella hainanensis* LCJY-471 was nested with two strains of *E. hainanensis* (TNS-F35049 and TNS-F35056) with ML bootstrap support of 99.4% in the SH-aLRT test, 100% in the UFB method, and a Bayesian probability of 1.0. *Lachnellula subtilissima* LCJY-976 was clustered with *La. subtilissima* CBS:197.66 with ML bootstrap support of 92.6% in the SH-aLRT test, 100% in the UFB method, and a Bayesian probability of 1.0.

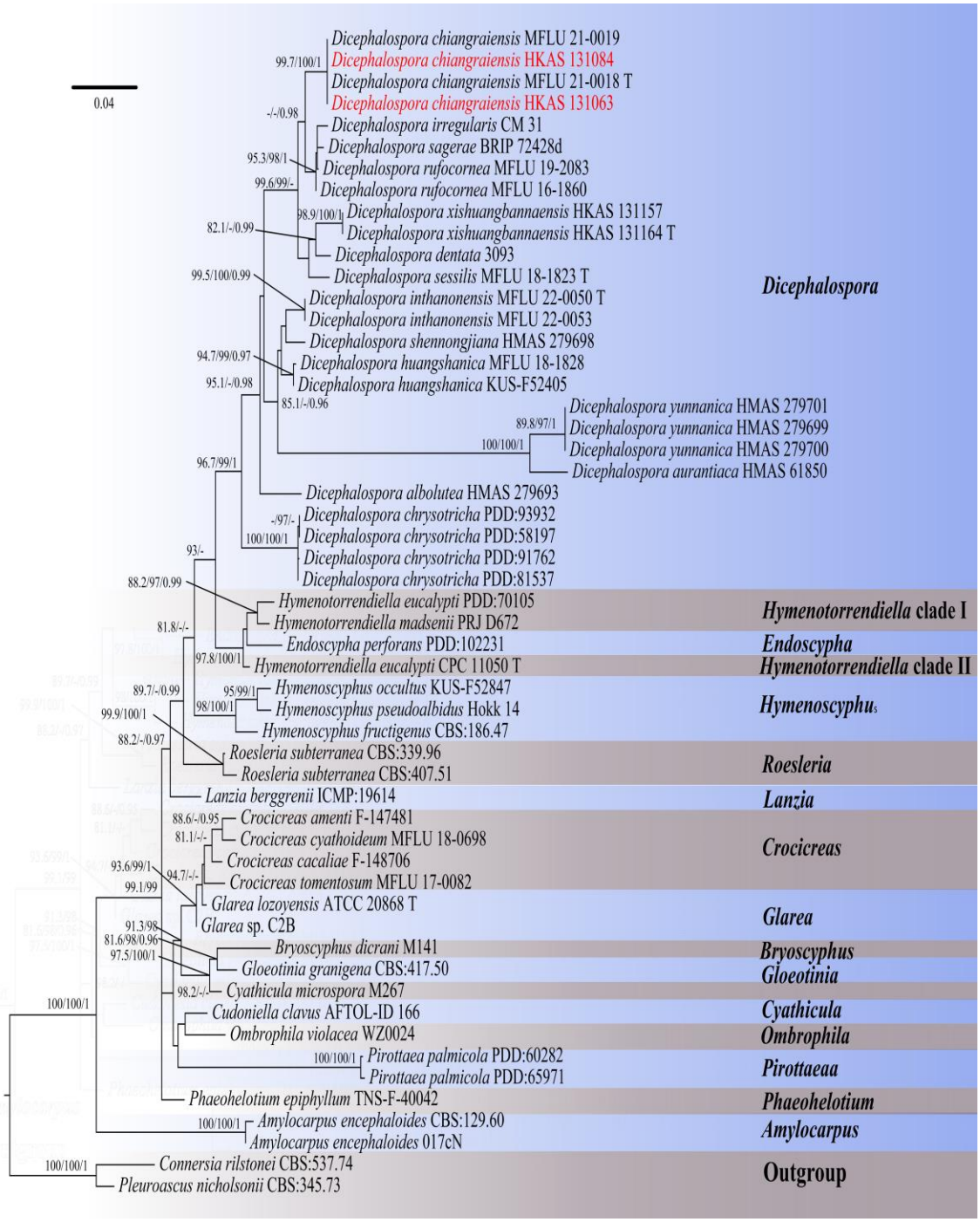

Figure 3 – Maximum likelihood tree based on a combined dataset of LSU and ITS sequences for *Dicephalospora*. Bootstrap support values for ML ≥ 80 of SH-aLRT or ML > 95 of UFB and posterior probability for BI ≥ 0.95 are indicated above the nodes and separated by "-/-/-" (SH-aLRT/UFB/PP). The newly generated isolates of the current study are highlighted in red, whereas ex-types are denoted as 'T' following the strain number.

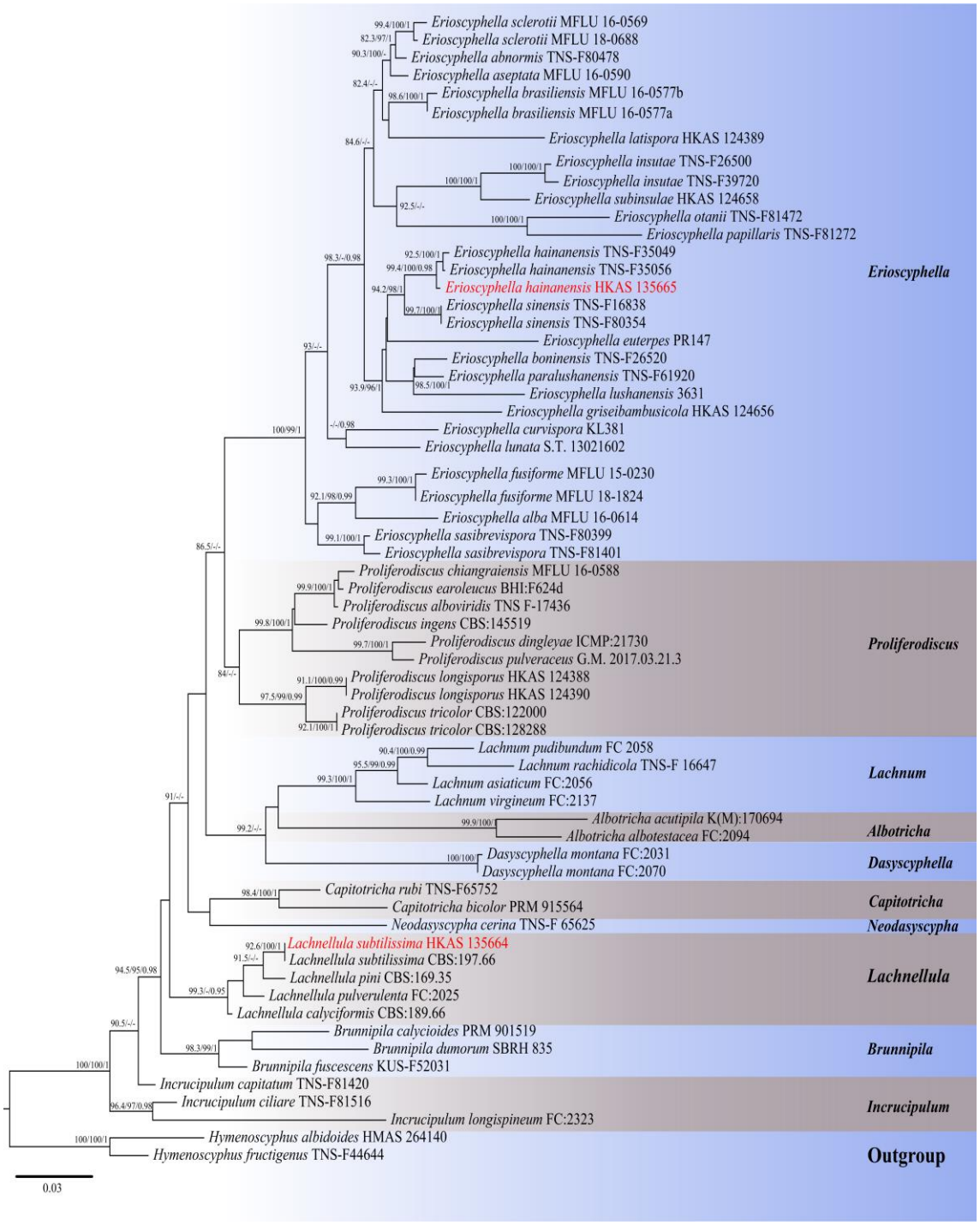

Figure 4 – Maximum likelihood tree based on a combined dataset of LSU and ITS sequences for Lachnaceae. Bootstrap support values for ML ≥ 80 of SH-aLRT or ML > 95 of UFB and posterior probability for BI ≥ 0.95 are indicated above the nodes and separated by "-/-/-" (SH-aLRT/UFB/PP). The newly generated isolates of the current study are highlighted in red, whereas ex-types are denoted as 'T' following the strain number.

Taxonomy

Hyphodiscus pseudotanii C.J.Y. Li, Q. Zhao & K.D. Hyde, sp. nov. Fig. 5

Index Fungorum number: IF902301; Faceoffungi number: FoF16027

Etymology – The specific epithet refers to the morphological similarity to *Hy. otanii*.

Saprobic on the decayed wood. Sexual morph: *Apothecia* 307–541 μm wide × 245–373 μm high ($\bar{x}$ = 445 × 303 μm, n = 15) when dry, scattered, superficial, cup-shaped with short-stipitate,

dark reddish brown (#422e1c), hairy. *Discs* flat, circular, dark grayish red (#574e4d) to dark grey (#353535), and the margins shrink when dry. *Receptacle* dark reddish brown (#422e1c), and the upper part covered with dark moderate green (#7b9e32) hairs. *Stipe* 96–213 μm wide × 177–321 μm long ($\overline{x}$ = 143 × 224 μm, n = 15), concolorous with the receptacles, cylindrical, glabrous. *Hairs* short, 18–25(–42) μm long × 2.9–4.3 μm wide ($\overline{x}$ = 25 × 3.9 μm, n = 15), arising from the outer layer of the excipulum, clavate with rounded apex, thin-walled, straight, usually (0–)1(–2)-septate, hyaline to pale-yellowish brown, covered with hyaline or yellowish, finely granules. *Hymenium* 23–36 μm ($\overline{x}$ = 29 μm, n = 40), hyaline. *Subhymenium* not obvious. *Excipulum* thick, hyaline, comprised of densely arranged *textura intricata* hyphae, the outermost layer mostly desaturated dark orange (#9d8b6c), septate and branched hyphae, 2.0–4.4 μm ($\overline{x}$ = 2.9 μm, n = 40) diam., strongly gelatinized. *Paraphyses* 22–32(–36) μm long × 1.1–2.4μm ($\overline{x}$ = 27 ×1.8 μm, n = 40), uninflated cylindric to slightly clavate, hyaline and aseptate, apically round, scarcely extending beyond the asci. *Asci* 27–40 × 4.4–5.6 μm ($\overline{x}$ = 35 × 5.1 μm, n = 20), 8-spored and almost filled the inner, unitunicate, clavate, rounded apex with J+ apical pore in Melzer's reagent, long-stipitate, croziers present. *Ascospores* 3.5–5.6 × 1.6–2.8 μm ($\overline{x}$ = 4.4 × 2.2 μm, n = 20), Q = 1.6–2.5 μm, Qm = 2.0 ± 0.3 μm, uniseriate or overlapping uniseriate, oval with two guttules, rounded at both ends, hyaline, thin-walled, smooth and aseptate. Asexual morph: Undetermined.

Material examined – China, Yunnan Province, Puer City, Jingdong County, altitude 1200 m, on the decayed wood, 18 ℃–25 ℃, 6 June 2022, Cuijinyi Li, LCJY-652 (HKAS 128322, holotype); *ibid*., Ailao Mountain, altitude 2520 m, on the decayed wood, 30 July 2021, Cuijinyi Li, 21-7-30-3 (HKAS 128362, paratype).

Note – Our collections were nested with *Hyphodiscus brachyconius* (specimens CBS 630.75), *Hy. hymeniophilus* (isolate TAAM 174997, TUF 17323 and TUF 104970) and *Hy*. cf. *hymeniophilus* (isolate LE 305399), with ML bootstrap support of 83% in the SH-aLRT test, 74% in the UFB method, and a Bayesian probability of 0.66 as shown in Fig. 1. *Hyphodiscus pseudotanii* exhibits similarities to *Hy. hymeniophilus* in terms of cylindrical hairs covered with granules, cylindrical paraphyses, and similar-sized, ellipsoidal ascospores; however, *Hy. hymeniophilus* can be distinguished by its pale orange apothecia and the presence of *textura prismatica* excipular cells (Hosoya 2002). *Hyphodiscus brachyconius* was only found in its asexual morph, hence, morphological characteristics could not be compared (Johnston et al. 2014). Meanwhile, *Hy. pseudotanii* shares most of its morphological characteristics with *Hy. otanii*; however, it exhibits broader apothecia (307–541 μm *vs*. 200–300 μm) and shorter asci (22–32 μm *vs*. 31–36 μm) compared to *Hy. otanii* (Hosoya 2002). Additionally, unlike *Hy. otanii*, no distinct white margins were observed in the apothecia of *Hy. pseudotanii* (Hosoya 2002). Furthermore, the differentiation between ectal and medullary excipulum is less pronounced in *Hy. Pseudotanii* than in *Hy. otanii* (Hosoya 2002). The phylogenetic analysis of *Hyphodiscus* revealed distinct findings in terms of topology compared to the previous study. Specifically, *Hy. otanii* was positioned between *Hy. hymeniophilus* clade I (consisting of isolates TAAM 174997 and TUF 17323) and *Hy. hymeniophilus* – *Hy.* cf. *hymeniophilus* clade II (isolate TUF 104970 and LE 305399). Our study demonstrated that *Hy. otanii* formed a basal clade within the genus, which can be clearly distinguished from the phylogeny based on LSU and ITS sequence comparisons. The ITS sequence of *Hy. pseudotanii* showed a 94.4% similarity with five gaps (525/556) to *Hy. hymeniophilus* (isolate: TUF:117323), and the LSU sequence displayed a 97.8% similarity with no gaps (541/553) to *Hy. hymeniophilus* (isolate: CBS 529.87). Therefore, using the guidelines of Chethana et al. (2021), we introduce a new species of *Hyphodiscus.*

Hyphopeziza macrospora C.J.Y. Li, Q. Zhao & K.D. Hyde, sp. nov. Fig. 6

Index Fungorum number: IF902302; Faceoffungi number: FoF16028

Etymology – The specific epithet refers to the large ascospores.

Saprobic on the underside of the fallen leaves of *Fagaceae*. Sexual morph: *Apothecia* 210–471 μm wide ($\overline{x}$ = 334 μm, n = 40) when fresh, 181–419 μm wide when dry, scattered, superficial, discoid to cup-shaped, light grey (mostly white) (#f5f5f5), transparent in high moisture, rough and

hairy, stipitate. *Discs* flat to bulge, circular, light grey (mostly white) (#f5f5f5) to hyaline, fur-like on the surface when fresh, margin concave inward and turning slightly hard waxy and yellowish brown (#564b2f) when dry. *Receptacles* rough and dark grayish-orange (#767163) when dry. *Stipe* 34–45 μm wide × 37–70 μm long ($\overline{x}$ = 39.5 × 50.4 μm, n = 10), rough and concolorous to the receptacle, short and broad. *Hairs* few and short, 16.5–32.6 μm long × 3.7–5.5 μm wide ($\overline{x}$ = 24.3 × 4.6 μm, n = 30), rising from the outermost cells of the marginal excipulum, cylindric with a blunt end, thin-walled, soft, mostly straight and sometimes slightly curved, aseptate, hyaline, covered with hyaline, finely granules. *Hymenium* 30–50 μm ($\overline{x}$ = 37 μm, n = 10), hyaline. *Subhymenium* not

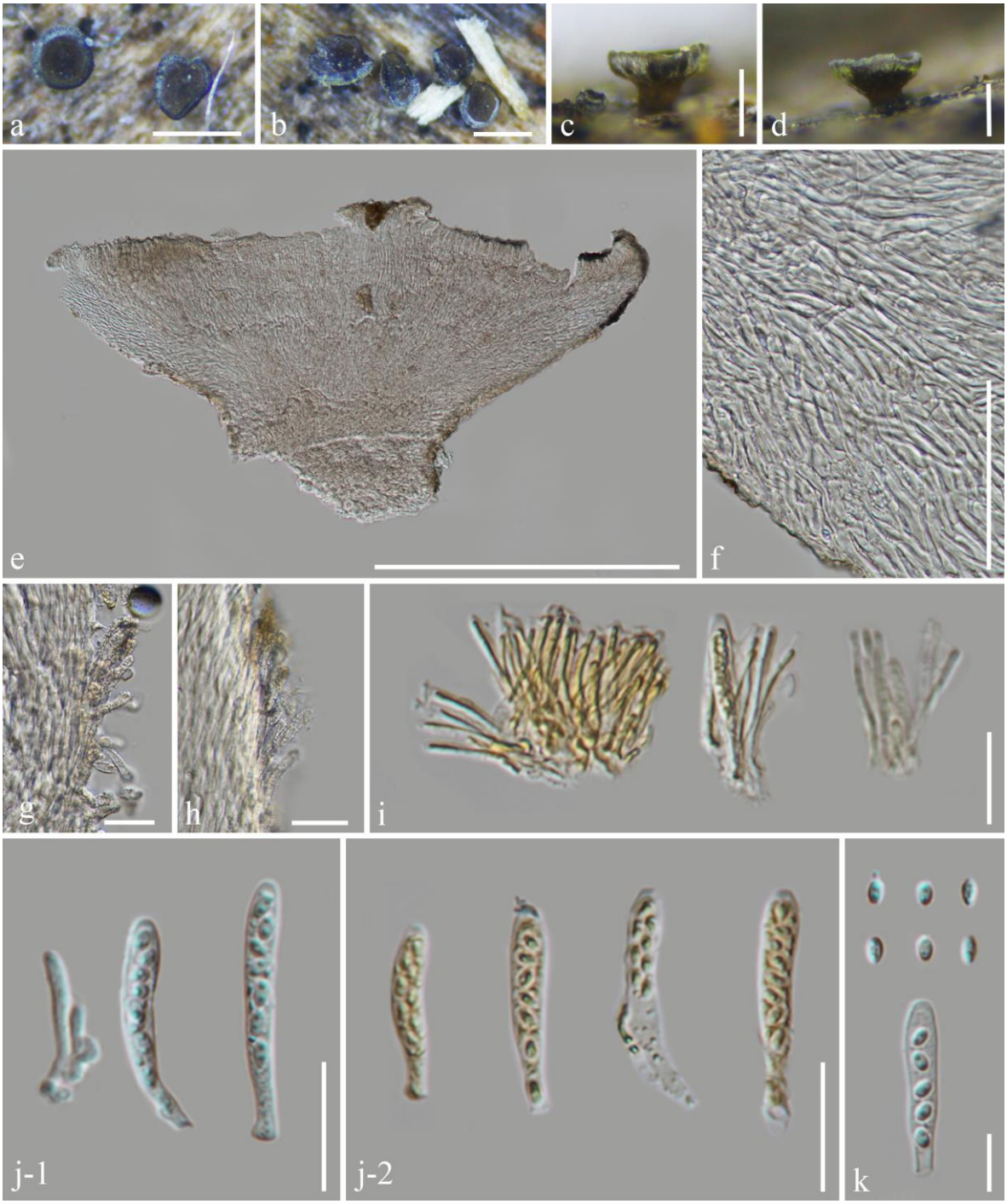

Figure 5 – *Hyphodiscus pseudotanii* (HKAS 128334, holotype). a–d Dried apothecia on the substrate. e Vertical section of an apothecium. f Excipulum. g, h Hairs. i Paraphyses. j–1 Asci in water. j–2 Asci in the Meltzer's reagent. k An ascus and ascospores. Scale bars: a–c, e = 400 μm, d = 300 μm, g, h = 20 μm, i = 15 μm, j–1, j–2 = 20 μm, k = 10 μm.

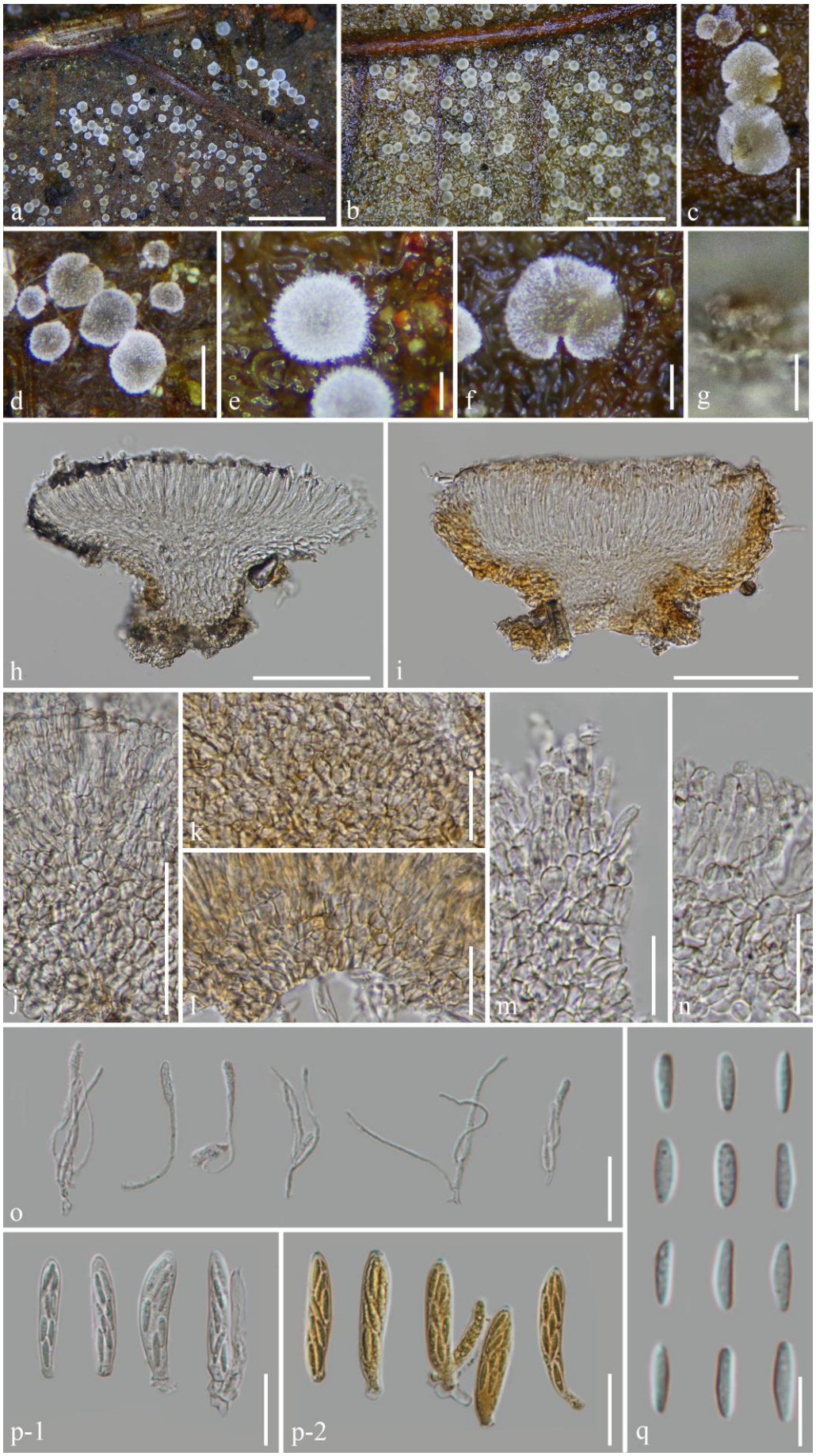

Figure 6 – *Hyphopeziza macrospora* (HKAS 128312, holotype; HKAS 128310, paratype; HKAS 128311, paratype; HKAS 128313, paratype). a–f Fresh apothecia on the wood. g Dried apothecia

on the wood. h, i Vertical sections of an apothecia. j–l Excipulum. m, n Hairs. o Paraphyses. p-1 Asci in water. p-2 Asci in the Meltzer's reagent. q Ascospores. Scale bars: a, b = 2 mm, c, d = 300 μm, e = 100 μm, f = 150 μm; g = 90 μm, h, i = 60 μm, j = 50 μm, k–p-2 = 20 μm, q = 12 μm.

obvious. *Medullary excipulum* comprised of hyaline *textura intricata* hyphae, hyphae 1.7–2.8 μm diam., well-developed, thin-walled and non-gelatinous. *Ectal excipulum* 15–41.5 μm ($\overline{x}$ = 26.4 μm, n = 20), comprised of *textura angularis* cells, 3.6–8.0 μm diam. ($\overline{x}$ = 5.6 μm, n = 70), walls hyaline, smooth on the surface, 0.37–0.69 μm ($\overline{x}$ = 0.53 μm, n = 20) thick, usually moderate orange (#b79651) and slightly elongated at the outermost layer and enlarged to 6.6–11.8 μm ($\overline{x}$ = 9.0 μm, n = 20) diam. at the stipe. *Paraphyses* 0.7–1.1 μm ($\overline{x}$ = 0.9 μm, n = 30) at the middle, filiform, hyaline, upper parts of some swollen to 1.6–3.3 μm, usually 7.7–17.8 μm long, and covered with hyaline, finely granules same as hairs, extending beyond the asci to visible on macroscopic. *Asci* 31.7–43.5 × 5.0–7.9 μm ($\overline{x}$ = 37.4 × 6.2 μm, n = 40), 8-spored filled the inner, unitunicate, stubby clavate with short truncated base, subconical apex with J+ apical pore in Melzer's reagent, croziers present. *Ascospores* (6.9–)8.3–11.5 × 1.8–3.4 μm ($\overline{x}$ = 9.4 × 2.5 μm, n = 70), Q = 3.0–4.9(–5.3) μm, Qm = 3.8 ± 0.48 μm, biseriate, obovate to oblong, slightly asymmetrical at the side view but not obvious, rounded at the apex, slightly pointed at the base, hyaline, thin-walled, smooth and aseptate. Asexual morph: Undetermined.

Material examined – China Yunnan Province, Kunming City, Panlong District, Heilongtan Mountain, altitude 1920 m, on the underside of the fallen leaves of Fagaceae, 11 ℃–22 ℃, 25 May 2022, Cuijinyi Li, LCJY-473 (HKAS 128312, holotype); *ibid*., LCJY-460 (HKAS 128310); *ibid*., LCJY-472 (HKAS 128311); *ibid*., LCJY-476 (HKAS 128313, paratype); *ibid*., LCJY-488 (HKAS 128314); *ibid*., 29 May 2022, Cuijinyi Li, LCJY-490 (HKAS 128315); *ibid*., LCJY-493 (HKAS 128316).

Note – Our collections were nested with *Hyphopeziza pygmaea* (TNS-F-17940 and KUS-F-51564), with ML bootstrap supports of 93.4% in the SH-aLRT test, 94% in the UFB method, and a Bayesian probability of 0.99 as shown in Fig. 1. The life mode of *H. macrospora* is similar to that of *H. pygmaea*, as it grows on the leaves of angiosperms and exhibits similar characteristics such as small apothecia, apically swollen and granular paraphyses, stubby clavate asci and obovate to oblong ascospores (Huhtinen 1987, Hosoya & Otani 1997, Johnston et al. 2014, Quijada et al. 2022). However, there are some distinguishing features between the two species: *H. pygmaea* has grey discs when fresh but turns white when dry, and possesses cylindrical-conical hairs, larger cells in the ectal excipulum (15 μm *vs*. 5.6 μm), shorter asci (24 μm *vs*. 37.4 μm), and smaller ascospores (3.5–7 μm × 1.2–1.8 μm *vs*. 8.3–11.5 × 1.8–3.4 μm) (Huhtinen 1987, Hosoya & Otani 1997, Quijada et al. 2022). We, therefore, describe *Hyphopeziza macrospora* as a new species according to the guidelines of Chethana et al. (2021). *Hyphopeziza* is clustered with *Soosiella* with ML bootstrap supports of 68.6% in the SH-aLRT test, 74% in the UFB method, and a Bayesian probability of 0.99, similar to previous studies. However, it is impossible to make any morphological comparisons as *Soosiella* was found only in its asexual morph (Johnston et al. 2014, Quijada et al. 2022).

Longistipes C.J.Y. Li, Q. Zhao & K.D. Hyde, gen. nov. Figs 7–8

Index Fungorum number: IF902303; Faceoffungi number: FoF16032

Etymology – The name refers to the distinguishing long stipes in this genus.

Saprobic on the underside of the fallen leaves of *Rhododendron* sp. Sexual morph: *Apothecia* scattered, superficial, cup-shaped with long-stipitate, white to translucence or black receptacles, covered with white hairs. *Discs* flat, surrounded by white hairs at the margin. *Receptacle* white to pale whitish-yellow to black when dry, covered with white hairs. *Stipe* long and thin, black or white at the upper and black at the base, almost glabrous to cover with white hairs. Hairs long, cylindric with blunt-end, thin-walled, soft, flexible or straight, septate, hyaline, and covered with hyaline, finely granules. *Subhymenium* not obvious. *Medullary excipulum* comprised of hyaline, *textura intricata* hyphae. *Ectal excipulum* comprised of hyaline to brown, *textura porrecta* cells, rough and

hyaline, finely granules, and a fasciculate arrangement at the stipe. *Paraphyses* broad lanceolate, 1–2-septate, unbranched, extending beyond the asci. *Asci* clavate, with short truncated base, J+, croziers present. *Ascospores* drop-shaped to obovate to oblong, overlapping uniseriate to biseriate and aseptate. Asexual morph: Undetermined.

Type species – *Longistipes niger* C.J.Y. Li, Q. Zhao & K.D. Hyde

Note – Based on the combined phylogenetic analysis of LSU-ITS genes, *Longistipes* is located within *Hyphodiscaceae* and clusters with *Scolecolachnum sensu stricto* and *Microscypha*, supported by ML bootstrap support of 89% in the SH-aLRT test, 61% in the UFB method, and a Bayesian probability of 0.5 as shown in Fig. 1. Based on the presence of tiny apothecia covered with dense hairs, clavate asci and small cylindrical to clavate-fusoid ascospores, we accept *Longistipes* as a member of *Hyphodiscaceae* (Quijada et al. 2022). All members of *Hyphodiscaceae* have short stipes or are sessile to subsessile, but *Longistipes* is an exception, with long and thin stipes and long hairs at the margin (Quijada et al. 2022). *Longistipes* possesses *textura porrecta* hyphae in the ectal excipulum and lanceolate paraphyses. *Scolecolachnum sensu stricto* is distinguished from *Longistipes* by its short and brown hairs, *textura angularis* to *prismatica* cells of the ectal excipulum and filiform paraphyses (Quijada et al. 2022). It can be differentiated from *Microscypha* by its translucent apothecia, smooth hairs with 0–3 septa, *textura angularis* to *primatica* cells of non-gelatinized ectal excipulum, filiform paraphyses, surrounded by hyaline gels that do not exceed the asci (Quijada et al. 2022).

Longistipes albus C.J.Y. Li, Q. Zhao & K.D. Hyde, sp. nov. Fig. 7

Index Fungorum number: IF902304; Faceoffungi number: FoF16030

Etymology – The name refers to the white apothecia.

Saprobic on the underside of the fallen leaves of *Rhododendron* sp. Sexual morph: *Apothecia* 260–370 μm wide × 625–804 μm high ($\overline{x}$ = 298 × 684 μm, n = 15) when dry, scattered, superficial, cup-shaped with long-stipitate, pale grayish-blue (#9eb3ca) to translucence, covered with thick white hairs. *Discs* flat, subcircular, light greyish blue (#d1d8e6), covered by white marginal hairs when dry. *Receptacles* covered with long, white hairs, 83–131 μm long, white to pale whitish-yellow when dry. *Stipe* 59–91 μm wide × 431–599 μm long ($\overline{x}$ = 78 × 506 μm, n = 15), long and thin, covered with slight white hairs, pale greyish-blue (#9eb3ca) to translucence at the upper and gradually darkened to dark greyish-blue (#34373b) toward the base. *Hairs* long, 3.1–5.9 μm wide ($\overline{x}$ = 4.5 μm, n = 40), extending from the hyphae at the excipulum, cylindrical with a blunt end, thin-walled, soft, usually straight, short and curved at stipe part, multi-septate and the distance between two septa gradually increasing, hyaline, covered with hyaline, finely granules. *Hymenium* 58–76 μm ($\overline{x}$ = 68 μm, n = 20), hyaline. *Subhymenium* not obvious. *Medullary excipulum* comprised of hyaline, *textura intricata* hyphae, well-developed, septate and branched, thin-walled and non-gelatinous. Ectal excipulum comprised of hyaline *texture porrecta* hyphae, 20–35(–38) μm × 4.9–6.3(–7.1) μm, hyaline, septate and branched, the surface of the outer hyphae rough and covered with hyaline, finely granules, the surface of the inner hyphae smooth, part of the hyphal wall fused and connected, fasciculate arrangement and at the color gradually deepening to very dark (mostly black) yellow (#070704) at the stipe. *Paraphyses* 63–79 × 3.3–5.3(–6.7) μm ($\overline{x}$ = 71 × 4.3 μm, n = 30), broad lanceolate with sharp apex, hyaline, thin-walled, 1-septate at the base, unbranched, with some tiny oil drops inside, tips occasionally sticking together, extending (3.9–)7.0–15.9 μm beyond the asci. *Asci* 47–62 × 6.2–8.4 μm ($\overline{x}$ = 53.5 × 7.2 μm, n = 35), 8-spored, filled the inner, unitunicate, clavate with short truncated base, subconical apex with J+ apical pore in Melzer's reagent, croziers present. *Ascospores* 8.7–11.5 × 2.1–4.0 μm ($\overline{x}$ = 10.2 × 2.9 μm, n = 50), Q = 2.8–4.4 μm, Qm = 3.6 ± 0.4 μm, biseriate, obovate to oblong, slightly asymmetrical at the side view but not obvious, both ends almost equally blunt, hyaline, thin-walled, smooth and aseptate. Asexual morph: Undetermined.

Material examined – China, Yunnan Province, Lijiang City, Naxi Autonomous County, Laojun Mountain, 99 Longtan scenic spot, altitude 3800 m, on the underside of the fallen leaves of

Rhododendron sp., 9 ℃–13 ℃, 23 July 2022, Cuijinyi Li, LCJY-979 (HKAS 128338, holotype); *ibid*., 25 July 2022, Cuijinyi Li, 22-7-25-A (HKAS 128361, paratype).

Note – Our collections clustered with *Longistipes niger*, another newly introduced taxa in this study, with a high ML bootstrap support of 96% in the SH-aLRT test, 99% in the UFB method, and a Bayesian probability of 1.0 as shown in Fig. 1. *Longistipes niger* and *L. albus* share similarities in terms of white hairs covering the surface of apothecia, long and thin stipes, *textura intricata* hyphae of the medullary excipulum, *textura porrecta* hyphae of the ectal excipulum, broad lanceolate paraphyses, and small ascospores; however, *L. albus* can be easily distinguished from *L. niger* by its white apothecia covered with thick white hairs, brown *textura porrecta* of the ectal excipulum, the acute apex of paraphyses, and obovate to oblong ascospores.

Longistipes niger C.J.Y. Li, Q. Zhao & K.D. Hyde, sp. nov. Fig. 8

Index Fungorum number: IF902305; Faceoffungi number: FoF16031

Etymology – The name refers to the black apothecia.

Saprobic on the underside of the fallen leaves of *Rhododendron* sp. Sexual morph: *Apothecia* 0.4–0.8 mm wide × 0.5–0.6 mm high ($\overline{x}$ = 0.55 × 0.55 mm, n = 15) when dry, scattered, superficial, cup-shaped with long-stipitate, dark desaturated blue (nearly black) (#22303d), covered with sparse white hairs. *Discs* flat, circular, white hairs margin around, dark grayish yellow (#828278) when fresh, dark greyish green (#a8ac9d), marginal hairs covered inwards when dry. *Receptacles* covered with thin layers of white hairs, black (#22303d), most hairs concentrating on the upper part near the margin, about 50–64 μm long. *Stipe* 30–53 μm wide × 229–376 μm long ($\overline{x}$ = 45 × 288 μm, n = 10), concolorous to the receptacle, slightly contracted at the connection with the receptacle, covered with slightly short white hairs or almost glabrous. *Hairs* 50–64 μm ($\overline{x}$ = 56 μm, n = 15) long, 2.8–4.1 μm wide ($\overline{x}$ = 3.4 μm, n = 30), extending from the outer layers of hyphae at the excipulum, cylindrical with blunt-end, thin-walled, soft and flexible, multi-septate, hyaline, slightly desaturated orange (#c3b48b) at the base, covered with hyaline, finely granules. *Hymenium* 88–98 μm ($\overline{x}$ = 92 μm, n = 20), hyaline. *Subhymenium* not obvious. *Medullary excipulum* comprised of hyaline to mostly desaturated brown (#94835d), *textura intricata* hyphae, well-developed, 30–40 μm thick, septate and branched, thin-walled, hyphae 2.2–4.1 μm ($\overline{x}$ = 3.0 μm, n = 20) diam., non-gelatinous. *Ectal excipulum* comprised of dark brown (#563d2b), *textura porrecta* hyphae, septate and branched, cells of (9.1–)11.3–21.5(–24.1) μm × (2.1–)2.6–5.7(–6.2) μm (x = 16.0 × 3.8 μm, n = 70) diam., becoming lighter at the margin, almost smooth on the surface, fasciculate arrangement at the stipe, non-gelatinous. *Paraphyses* 3.6–5.9 μm ($\overline{x}$ = 4.7 μm, n = 40) at widest, broadly lanceolate or cylindrical with a blunt apex, hyaline, thin-walled, 2-septate at the lower part, unbranched, no obvious oil drops inside, tips usually sticking together, extending 26–45 μm beyond the asci. *Asci* 38–54 × 5.0–7.5 μm ($\overline{x}$ = 45.5 × 6.2 μm, n = 50), 8-spored, filled the inner, unitunicate, clavate with short truncated base, subconical apex with J+ apical pore in Melzer's reagent, croziers present. *Ascospores* 6.9–9.6 × 1.5–2.8 μm ($\overline{x}$ = 8.2 × 2.1 μm, n = 50), Q = (2.8–)3.1–4.6(–5.6) μm, Qm = 4.0 ± 0.7 μm, overlapping uniseriate, drop-shaped, obtusely rounded at the apex, sharp at the base, hyaline, thin-walled, smooth and aseptate. Asexual morph: Undetermined.

Material examined – China, Yunnan Province, Lijiang City, Naxi Autonomous County, Laojun Mountain, 99 Longtan scenic spot, altitude 3800 m, on the underside of the fallen leaves of *Rhododendron* sp., 9 ℃–13 ℃, 23 July 2022, Cuijinyi Li, LCJY-942 (HKAS 128334, holotype); *ibid*., 24 July 2022, Cuijinyi Li, FM-1 (HKAS 128358, paratype).

Note – Compared to another newly introduced species (*L. albus*) proposed in this study, *L. niger* has dark-colored apothecia that are almost glabrous with shorter and thinner stipes (30–53 μm wide × 229–376 μm long *vs*. 59–91 μm wide × 431–599 μm long), shorter hairs at the margin (50–64 μm *vs*. 83–131 μm), apical blunt paraphyses, and drop-shaped ascospores.

Diversity of Hyphodiscaceae in China

As mentioned above, three genera, *Glutinomyces*, *Hyphodiscus*, and *Microscypha*, were

reported in China (Zhuang 1988, Ren & Zhuang 2016, Quijada et al. 2022). Among them, the strain found in *Glutinomyces* remains uncertain (Kuang et al. 2022). While only one species of

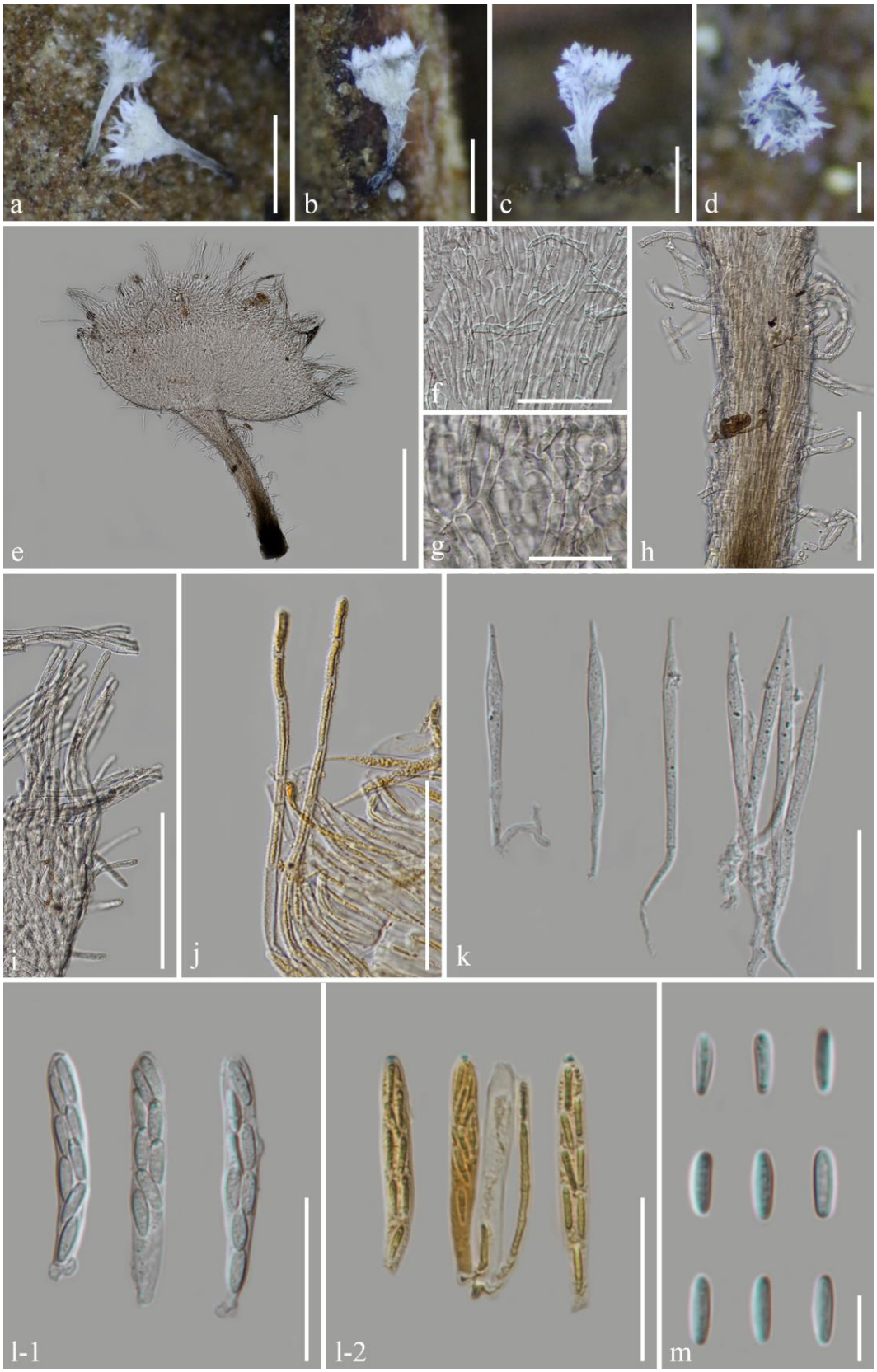

Figure 7 – *Longistipes albus* (HKAS 128338, holotype) a–d Dried apothecia on the substrate. e A squeeze mount of an apothecium. f, g Excipulum. h Stipe. i, j Hairs. k Paraphyses. l-1 Asci in water. l-2 Asci in the Meltzer's reagent. m Ascospores. Scale bars: a = 500 μm, b, c = 400 μm, d =

150 μm, e = 300 μm, f, j = 60 μm, g = 30 μm, h, i = 100 μm, k = 40 μm, l-1, l-2 = 30 μm, m = 10 μm.

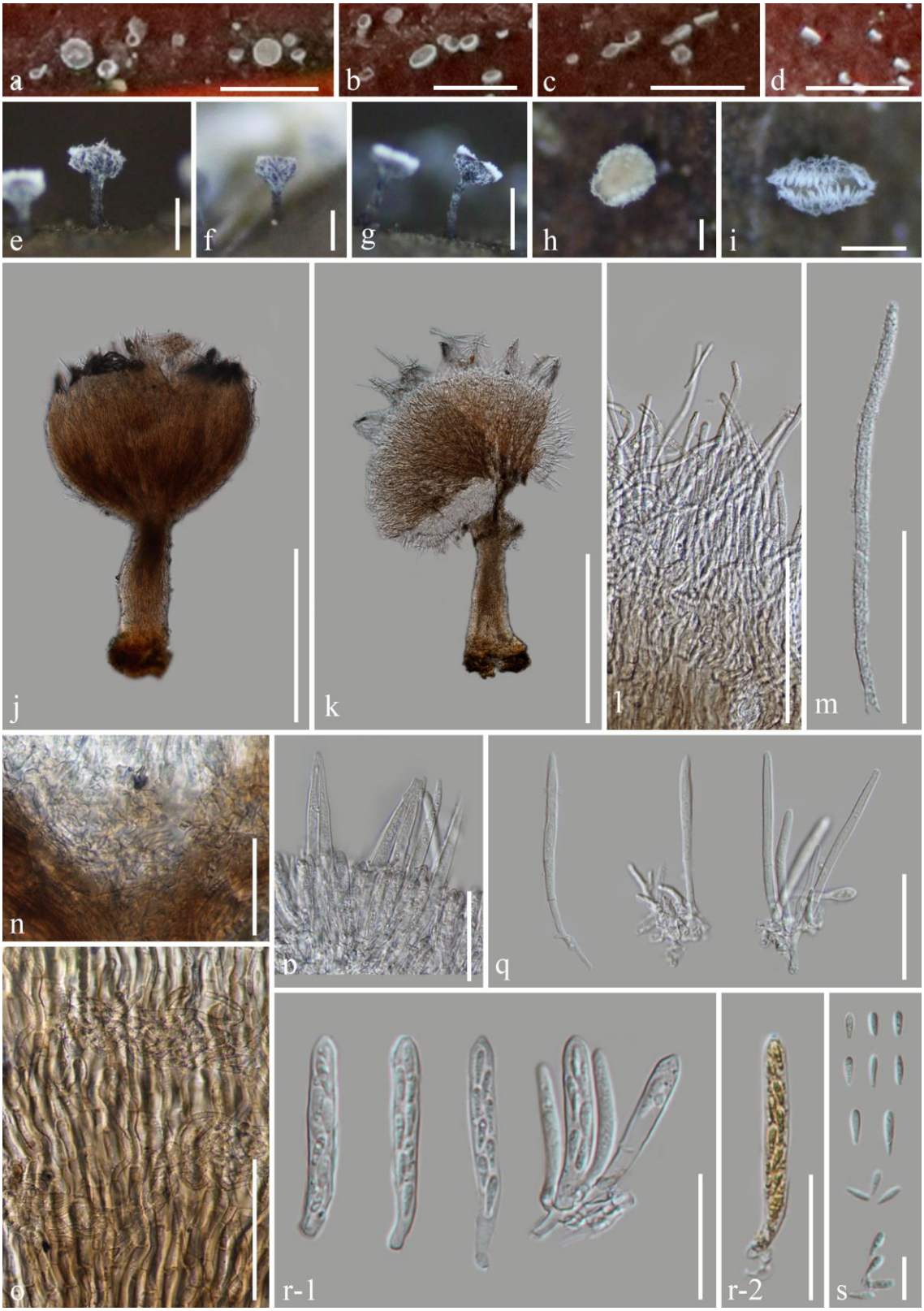

Figure 8 – *Longistipes niger* (HKAS 128334, holotype). a–d Fresh apothecia on the substrate. e–i Dried apothecia on the wood. j, k The squeeze mount of Apothecia. l, m Hairs. n, o Excipulum. p, q Paraphyses. r-1 Asci in water. r-2 Asci in the Meltzer's reagent. s Ascospores. Scale bars: a–d = 2 mm, e, f = 200 μm, g = 400 μm, h = 150 μm, i = 300 μm, j, k = 400 μm, l = 80 μm, m = 60 μm, n, o = 40 μm, p, q = 40 μm, r-1, r-2 = 30 μm, s = 12 μm.

Hyphodiscus has been previously documented in China, our study has identified a new species, *Hy. pseudotanii* (Quijada et al. 2022). Prior to this study, *Hyphopeziza* had not been reported in China. The discovery of *H. macrospora* fills a gap in the knowledge of this genus within China. A comprehensive checklist detailing Hyphodiscaceae species in China is provided in Table 3.

Table 3 Species checklist of Hyphodiscaceae in China.

Genus name	Species name	Location (Province in China)	Substrate	Reference
Glutinomyces	*Glutinomyces* sp.	Guangdong	Fermenting soysauce	Kuang et al. (2022)
Hyphodiscus	*Hyphodiscus hymeniophilus*	Gansu; Xizang; Qinghai; Sichuan; Yunnan	Unknown	Yao (2023)
	Hyphodiscus pseudotanii	Yunnan	The decayed wood	This study
Hyphopeziza	*Hyphopeziza macrospora*	Yunnan	the fallen leaves of *Fagaceae*	This study
Longistipes	*Longistipes albus*	Yunnan	the fallen leaves of *Rhododendron* sp.	This study
	Longistipes niger	Yunnan	the fallen leaves of *Rhododendron* sp.	This study
Microscypha	*Microscypha septospora*	Qinghai	the rotten wood	Ren & Zhuang (2016)

New host, geographical and sexual morphological records of Leotiomycetes

Chalara bambusicola W.P. Wu & Y.Z. Diao, Fungal Diversity 119: 291 (2023) Fig. 9

Classification – Pezizellaceae, Helotiales, Leotiomycetes, Ascomycota

Index Fungorum number: IF845201; Faceoffungi number: FoF16025

Saprobic on the fallen and decaying legume of *Gleditsia* sp. Sexual morph: *Apothecia* 0.3–1.0 μm wide ($\overline{x}$ = 0.56 μm, n = 20) when fresh, 297–553 μm wide ($\overline{x}$ = 414 μm, n = 20) when dry, scattered, superficial, discoid, sessile to subsessile. *Discs* flat, circular, slightly desaturated yellow (#bdad71) when fresh with a paler and slightly curled inward margin, light pink to dark grayish violet (#8f91a9) to dark grayish cyan (#62706e) when dry, the color deepening in the center. *Receptacles* rough and glabrous, dark grayish orange (#948d7d) with paler flanks when dry. *Hymenium* 39–63 μm ($\overline{x}$ = 49 μm, n = 20), hyaline or moderate orange (#c37946) to dark orange [Brown tone] (#a0420d). Subhymenium comprised of thin, *textura intricata* hyphae. *Excipulum* 21–61 μm ($\overline{x}$ = 41 μm, n = 20), cells tightly and regularly arranged, comprised of *textura prismatica* cells of 2.0–4.2 μm wide × 4.9–10.8 μm long ($\overline{x}$ = 3.1 × 8.3 μm, n = 40), the innermost three layers of cells elongate to 1.8–2.8 μm wide × 10.5–16.8 μm long ($\overline{x}$ = 13.8 × 2.3 μm, n = 20), the middle matrix comprised of *textura angularis* cells, the outermost cells slightly elongated with rounded end, cell wall slightly desaturated orange (#b79f6b). *Paraphyses* 2.1–3.0 μm ($\overline{x}$ = 2.5 μm, n = 15), cylindric, almost hyaline, or rarely brownish yellow pigment, soft, 4–5-septate from middle to the base. *Asci* 42–58 μm × 5.3–7.7 μm ($\overline{x}$ = 49 × 6.4 μm, n = 30), 8-spored, unitunicate, clavate to cylindric with a short-truncated stipe, subconical apex with J+ apical pore in Melzer's reagent, croziers present. *Ascospores* 9.0–14.2 × 1.5–2.3 μm ($\overline{x}$ = 11.2 × 1.9 μm, n = 70), Q = 4.9–7.3(–8.2) μm, Qm = 6.0 ± 0.8 μm, overlapping uniseriate or biseriate, obovate to oblong, slightly pointed-ends, thin-walled, hyaline, smooth and 1-septate. Asexual morph: see Wu & Diao (2023).

Material examined – China, Yunnan Province, Xishuangbanna City, Menghai County, altitude 1661m, on the fallen and decayed legume of *Gleditsia* sp., 17 °C–27 °C, 8 September 2022, Cuijinyi Li, LCJY-1405 (HKAS 128357); *ibid*, 22-9-5-B (HKAS 128372).

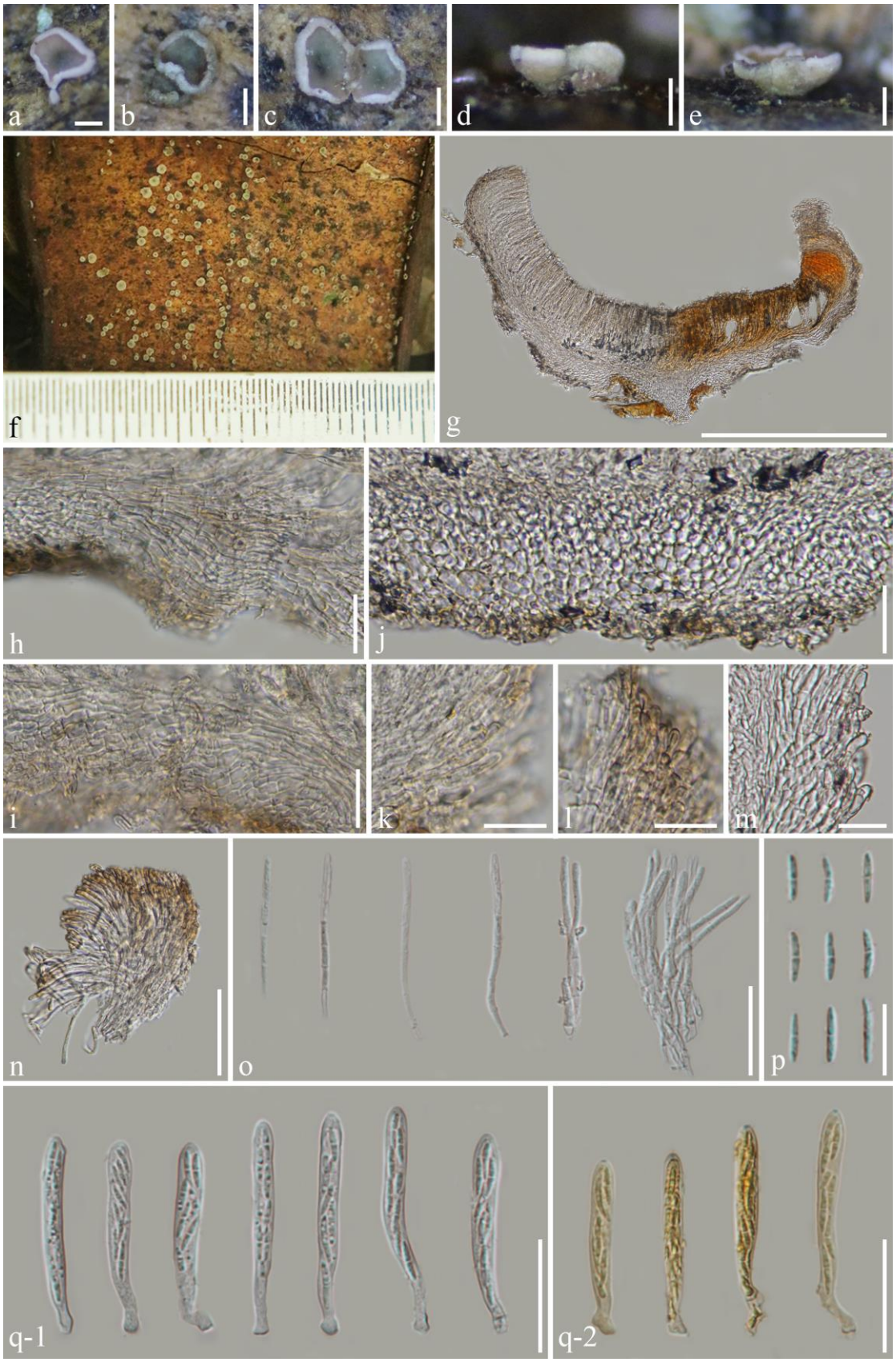

Figure 9 – *Chalara bambusicola* (HKAS 128357, new sexual morphology and host records). a–d Dried apothecia on the substrate. f Fresh apothecia on the substrate. g A vertical section of an apothecium. h, i Excipulum. k–m Outermost excipular cells. n Marginal hyphae. o Paraphyses. p Ascospores. q-1 Asci in water. q-2 Asci in the Meltzer's reagent. Scale bars: a–g = 200 μm, h, i, m = 20 μm, k, l, p = 15 μm, n = 40 μm, o, q-1, q-2 = 30 μm.

Known distribution – ASIA: China (Guangdong) (Wu & Diao 2023).

Notes – Our collections were nested with *Chalara bambusicola* (CGMCC 3.23394), supported by ML bootstrap support of 97.7% in the SH-aLRT test, 100% in the UFB method, and a Bayesian probability of 0.99. Previous studies have not described the sexual morph of *Chalara* (Wu & Diao 2023). The apothecia excipulum of *Calycina* consists of parallel or interwoven, thick-walled hyphae, but *Chalara* has regularly arranged *textura prismatica* cells (Wu & Diao 2023). *Bloxamiella* has black apothecia, *textura intricata* at the basal stroma and *textura epidermoidea* hyphae at the medullary excipulum, filiform paraphyses with swollen tips, and broader ascospores (4–7 μm *vs*. 1.5–2.3 μm) (Guatimosim et al. 2016). *Chalara bambusicola* is very similar to *Calycellina* because of the broad concept of *Calycellina* (Raitviir 2004). However, *C. bambusicola* lacks hair of varying lengths and brown basal rings, and its paraphyses are mostly hyaline and 4–5-septate (Raitviir 2004). *Phaeoscypha cladii* is similar to *Chalara bambusicola* on sessile to subsessile apothecia, *prismatica* excipular cells with brown walls, but is different in having pale brown hairs and ellipsoid ascospores (Raitviir 2004). Based on the findings of our phylogenetic analysis, we have adopted the taxonomic result that *C. bambusicola* is a member of *Chalara sensu lato*, shown in Fig. 2. This study represents the first report of the sexual morph of *C. bambusicola*, its first occurrence on *Gleditsia* sp., and marks its first record in Yunnan Province, China.

Dicephalospora chiangraiensis Phutthacharoen & K.D. Hyde, in Phutthacharoen, Chethana, Lestari, Stadler & Hyde, Diversity 14(no. 645): 10 (2022) Fig. 10

Classification – Helotiaceae, Helotiales, Leotiomycetes, Ascomycota

Index Fungorum number: IF558614; Facesoffungi number: FoF09715

Saprobic on dead twigs. Sexual morph: *Apothecia* 80–110 μm high × 700–840 μm diam. ($\overline{x}$ = 96 × 768 μm, n = 10), when dry arising solitary or gregarious, scattered on wood, central short stipitate, superficial, orange when fresh, flat cupulate. *Stipe* 320–420 μm ($\overline{x}$ = 363 μm, n = 10). *Receptacles* flat. *Discs* flat, orange, slightly curled when dry with smooth, orange to light brown margins. *Ectal excipulum* 40–72 μm ($\overline{x}$ = 42 μm, n = 10), multi-layered, thin-walled, with clear to pale yellow *textura porrecta* compact, tiny cells. *Medullary excipulum* 24–57 μm ($\overline{x}$ = 36 μm, n = 10), multi-layered, with hyaline to light orange cells of *textura intricata*, condensed, small cells. *Hymenium* yellow or orange, dense with enclosed asci and paraphyses. *Paraphyses* 1.2–3.1 μm wide ($\overline{x}$ = 1.75 μm, n = 30), thin-walled, numerous, filiform, aseptate, and swollen at the apex. *Asci* 80–105 × 5.7–10 μm ($\overline{x}$ = 95 × 8 μm, n = 20), 8-spored, unitunicate, inoperculate, long cylindrical, thin-walled, with a tapered long stipitate base, blunt apices, and an apical apparatus bluing in the Melzer's reagent. *Ascospores* 16–30 × 1.2–3.3 μm ($\overline{x}$ = 20.5 × 2.4 μm, n = 20), biseriate, hyaline, fusiform, thin-walled, with a non-mucilaginous cap. Asexual morph: unknown.

Material examined – China, Yunnan Province, Xishuangbanna City, on a dead twig, 9 September 2022, Le Luo, ly973 (HKAS 131063); *ibid*., ly969 (HKAS 131084).

Known distribution – ASIA: Thailand (Phutthacharoen et al. 2022).

Notes – The characters of our sexual morph are similar to the holotype of *D. chiangraiensis* (MFLU 21-2018). According to the phylogenetic analyses, our collections (HKAS 131163 and HKAS 131084) clustered with other *D. chiangraiensis* strains (MFLU 21-2018 and MFLU 21-2019) with ML bootstrap support of 99.7% in the SH-aLRT test, 100% in the UFB method, and a Bayesian probability of 1.0 shown in Fig. 3. The current study presents a new geographical record of *D. chiangraiensis* in China.

Nagrajchalara neonawawii W.P. Wu & Y.Z. Diao, Fungal Diversity 119: 365 (2023) Fig. 11

Classification – Pezizellaceae, Helotiales, Leotiomycetes, Ascomycota

Index Fungorum number: IF845234; Faceoffungi number: FoF16033

Saprobic on the decayed unknown wood. Sexual morph: *Apothecia* 0.5–0.85 μm wide ($\overline{x}$ = 0.63 μm, n = 10) when fresh, 330–500 μm wide ($\overline{x}$ = 406 μm, n = 10) when dry, scattered, superficial, discoid, and sessile. *Discs* flat, circular, pale flesh color or light grayish orange (#ede7db) when

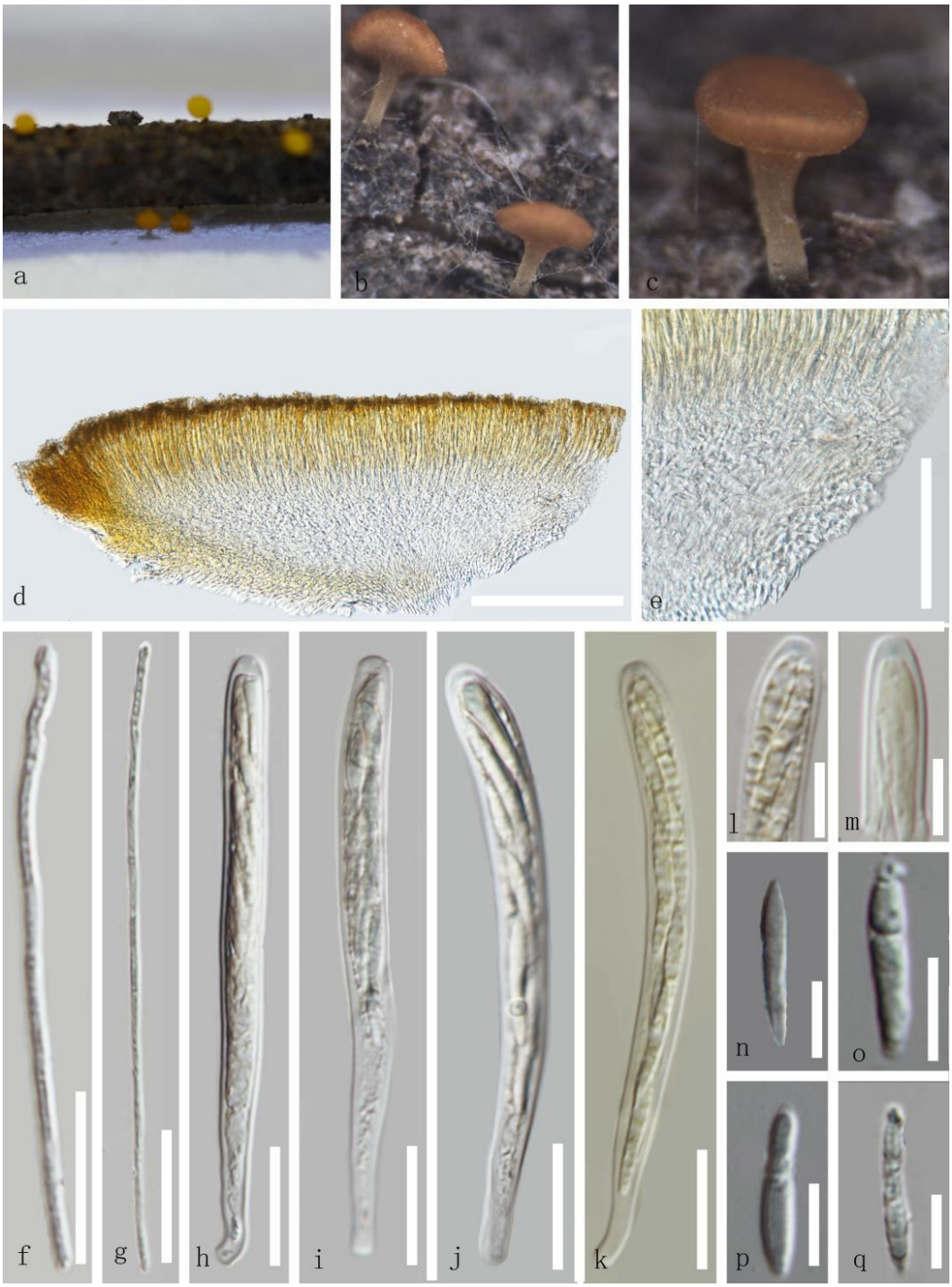

Figure 10 – *Dicephalospora chiangraiensis* (HKAS 131063). a–c Dried apothecia on the substrate. d A vertical section of an apothecium. e Excipulum. f, g Paraphyses. h–j Asci in water. k Asci in the Meltzer's reagent. l, m Apex of asci in the Melzer's reagent. n–q Ascospores. Scale bars: d = 200 µm, e = 100 µm, f–k = 20 µm, l–q = 10 µm.

fresh, light dull yellow or dark grayish yellow (#aea886) when dry with a thick and curled inward margin. *Receptacles* concolorous with the disc, slightly hairy. *Hairs* 3.2–5.4 µm wide, short and cylindric, septate, hyaline. *Hymenium* 63–119 µm ($\bar{x}$ = 85 µm, n = 10), hyaline, the top layer attached with golden brown pigment. *Subhymenium* not obvious. *Medullary excipulum* 20–80 µm ($\bar{x}$ = 47 µm, n = 30), comprised of hyaline *textura intricata* hyphae, 2.1–3.4 µm diam. ($\bar{x}$ = 2.7 µm, n = 30). *Ectal excipulum* 31–66 µm ($\bar{x}$ = 50 µm, n = 20), comprised of hyaline and large *textura angularis* cells, loosely arranged, 7.1–15.0 µm diam. ($\bar{x}$ = 10.5 µm, n = 40), the

outermost layer attached with golden brown pigment, the connection with the substrate consisting of brown *textura intricata* hyphae, 1.9–4.8 μm diam. ($\overline{x}$ = 3.0 μm, n = 30). *Paraphyses* 1.5–2.5 μm ($\overline{x}$ = 1.9 μm, n = 30), cylindric, hyaline, soft, 3-septate from middle to the base, branched near the middle. *Asci* 65–80 μm × 6.5–10 μm ($\overline{x}$ = 73 × 8.2 μm, n = 25), 8-spored, unitunicate, clavate to cylindric with a short-truncated stipe, semicircular apex with J+ apical pore in Melzer's reagent, croziers absent. *Ascospores* 12.2–18.2(–19.8) × 2.7–3.6 μm ($\overline{x}$ = 12.2 × 3.1 μm, n = 40), Q = 4.1–6.6(–7.0) μm, Qm = 5.2 ± 0.8 μm, overlapping uniseriate or biseriate, fusoid, slightly curved with pointed ends, thin-walled, hyaline, smooth and 1-septate. Asexual morph: see Wu & Diao (2023).

Material examined – China, Yunnan Province, Xishuangbanna City, Menghai County, altitude 1838 m, on the decayed unknown wood, 17 ℃–27 ℃, 5 September 2022, Cuijinyi Li, LCJY-1361 (HKAS 128356).

Known distribution – ASIA: China (Yunnan) (Wu & Diao 2023).

Notes – Our collections were nested with *Nagrajchalara neonawawii* (strains: NN078612 and NN078611), supported by ML bootstrap support of 79.2% in the SH-aLRT test, 99% in the UFB method, and a Bayesian probability of 0.96. *Nagrajchalara* differed from *Calycellina* by having pale flesh or light greyish-orange large apothecia, loosely arranged angularis excipular cells, branched and septate paraphyses, and croziers of asci are absent (Raitviir 2004). *Mollisina* can be easily distinguished from *Nagrajchalara* by having finger-shaped hairs, apical J- without KOH reagent, paraphyses branched at the base with an obtuse to swollen apex, and ellipsoid ascospores (Raitviir 2004). *Mollisinopsis* differs from *Nagrajchalara* by having *textura intricata* hyphae at the ectal excipulum (Raitviir 2004). *Nagrajchalara neonawawii* differs from *N. yongnianii* in its hairy apothecia, thick and curled inward margin, and apically semicircular asci. However, almost all measurements of these two species are within indistinguishable bounds. At the same time, although *N. neonawawii* and *N. yongnianii* appear to belong to two separate groups, these two species are still closely related to the phylogenetic tree shown in Fig. 2. Due to the lack of additional sexual morphological data for *Nagrajchalara*, we continue to acknowledge and uphold the findings of the current classification. This study represents the first report of the sexual morph of *N. neonawawii*.

Nagrajchalara yongnianii W.P. Wu & Y.Z. Diao, Fungal Diversity 119: 408 (2023) Fig. 12

Classification – Pezizellaceae, Helotiales, Leotiomycetes, Ascomycota

Index Fungorum number: IF845254; Faceoffungi number: FoF16034

Saprobic on the decayed unknown wood. Sexual morph: *Apothecia* 390–690 μm wide ($\overline{x}$ = 470 μm, n = 10) when dry, scattered, superficial, discoid, and sessile. *Discs* flat, circular, wavy and slightly curled inward edge, whitish or grayish-yellow (#a4a59f) when dry, dull yellow or mostly desaturated dark yellow (#978f6a) near the center. *Receptacle* whitish or grayish-yellow (#a4a59f), glabrous. *Hymenium* 65–95 μm ($\overline{x}$ = 74 μm, n = 10), hyaline, the top layer attached with golden brown pigment. *Subhymenium* not obvious. *Medullary excipulum* 57–68 μm ($\overline{x}$ = 57 μm, n = 10), comprised of hyaline and loosely *textura intricata* hyphae, 2.3–3.3(–4.0) μm diam. ($\overline{x}$ = 2.9 μm, n = 15). *Ectal excipulum* 27–44 μm ($\overline{x}$ = 34 μm, n = 15), comprised of hyaline and large *textura angularis* cells, loosely arranged, 5–12 μm diam. ($\overline{x}$ = 8.4 μm, n = 1.9), walls hyaline, appearing with brown pigment and brown *textura intricata* hyphae at the apothecia base and substrate attachment, 2.0–4.3 μm diam. ($\overline{x}$ = 3.3 μm, n = 30). *Paraphyses* 1.5–2.6 μm ($\overline{x}$ = 2.1 μm, n = 30), cylindric to filiform, hyaline, soft, 3-septate from middle to the base, branched near the middle. *Asci* 63–86 μm × 7.1–10.5 μm ($\overline{x}$ = 73 × 9.3 μm, n = 30), 8-spored, unitunicate, clavate to cylindric with a short-truncated stipe, obconical apex with J+ apical pore in Melzer's reagent, croziers present. *Ascospores* 12.3–18.9 × 2.2–3.6 μm ($\overline{x}$ = 16.0 × 3.1 μm, n = 35), Q = 4.4–6.0(–7.7) μm, Qm = 5.2 ± 0.7 μm, overlapping uniseriate to biseriate, fusoid, slightly curved with pointed ends, thin-walled, hyaline, smooth and 1-septate. Asexual morph: see Wu & Diao (2023).

Material examined – China, Yunnan Province, Puer City, Jingdong County, altitude 2455 m, on the fallen unknown leaves, 25 August 2022, Cuijinyi Li, 22-8-25-3 (HKAS 128367).

Known distribution – ASIA: China (Guangdong, Hainan, Hunan, Jiangsu, Yunnan, Zhejiang), Japan (Mie Prefecture) (Wu & Diao 2023).

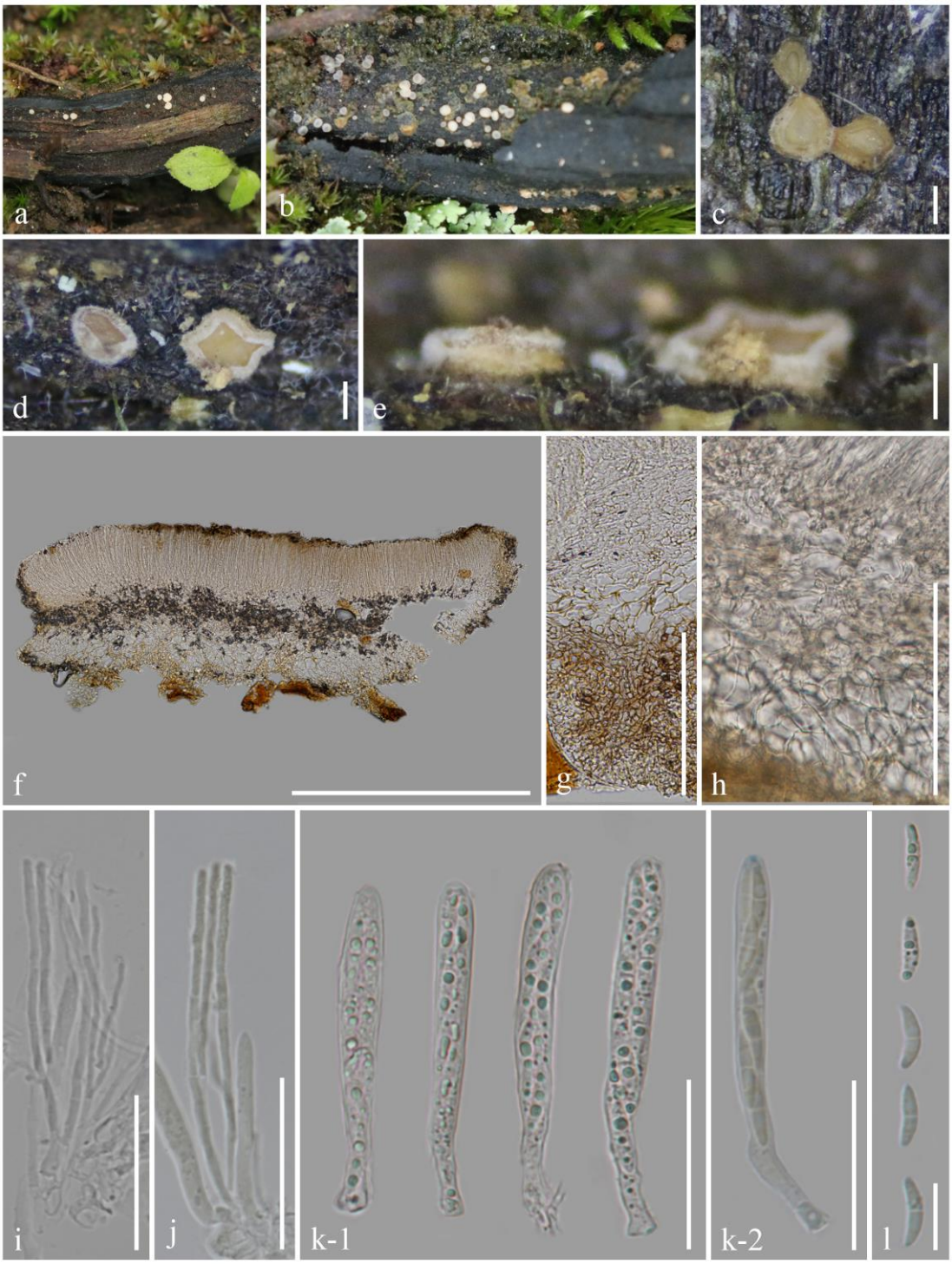

Figure 11 – *Nagrajchalara neonawawii* (HKAS 128356, a new sexual morph record). a, b Fresh apothecia on the substrate. c–e Dried apothecia on the substrate. f A vertical section of an apothecium. g, h Excipulum. i, j Paraphyses. k-1 Asci in water. k-2 Asci in the Meltzer's reagent. l Ascospores. Scale bars: c, d = 200 μm, e = 100 μm, f = 300 μm, g, h = 80 μm, i–k-2 = 35 μm, l = 15 μm.

Notes – Our collections were nested with *Nagrajchalara yongnianii* (NN046066 and NN077394), supported by ML bootstrap support of 98.6% in the SH-aLRT test, 100% in the UFB method, and a Bayesian probability of 1.0, as shown in Fig. 2. *Nagrajchalara yongnianii* shares similar characteristics with *N. neonawawii*, including yellowish apothecia, loosely arranged angular excipular cells attached with brown pigmented, loosely arranged *textura intricata* hyphae at the medullary excipulum, cylindric to filiform and branched paraphyses with 3-septate, clavate to

cylindric asci, and slightly curved and fusoid ascospores with a septum. But it can be distinguished from *N. neonawawii* by its glabrous apothecia, thin edges that roll inward when dry, and apically obconical asci. A detailed discussion between *N. neonawawii* and *N. yongnianii* was provided in the notes of *N. neonawawii*. This study represents the first report of the sexual morph of *N. yongnianii*.

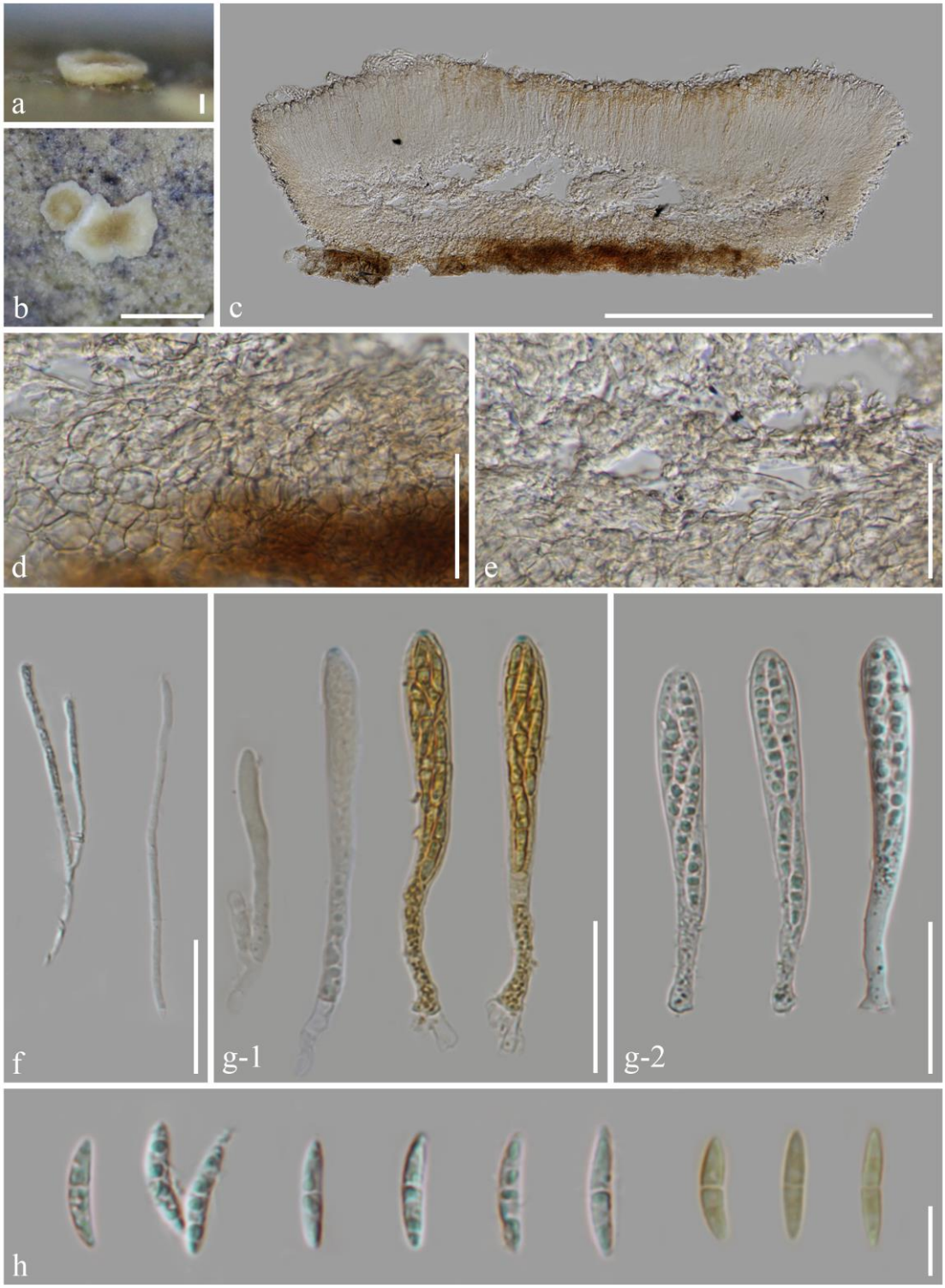

Figure 12 – *Nagrajchalara yongnianii* (HKAS 128367, a new sexual morph record). a, b Dried apothecia on the substrate. c A vertical section of an apothecium. d, e Excipulum. f Paraphyses. g-1 Asci in the Meltzer's reagent. g-2 Asci in water. h Ascospores. Scale bars: a = 100 μm, b = 600 μm, c = 300 μm, d–g-2 = 30 μm, h = 10 μm.

New collections in Yunnan Province

Erioscyphella hainanensis (W.Y. Zhuang and Zheng Wang) Hosoya and Tochihara, in Tochihara & Hosoya, MycoKeys 87: 28 (2022) Fig. 13

Classification – Lachnaceae, Helotiales, Leotiomycetes, Ascomycota

Index Fungorum number: IF835707; Faceoffungi number: FoF16026

Saprobic on the fallen leaves of *Quercus* sp. Sexual morph: *Apothecia* 690–860 μm wide ($\overline{x}$ = 780 μm, n = 10) when fresh, 280–360 μm wide × 350–630 μm high ($\overline{x}$ = 310 × 490 μm, n = 10) and margin incurved when dry, scattered, superficial, discoid and stipitate. *Discs* whitish-yellow (#868c7f) to translucent when fresh, dark moderate yellow (#878b37) when dry, edges shrink inward. *Receptacles* dark grayish-green (#869b7a) or light grayish-cyan-lime green (#c2e2d2) when dry, and hairy. *Stipes* 310–420 μm wide × 50–60 μm long ($\overline{x}$ = 380 × 57 μm, n = 10), concolorous to the receptacle, hairy. *Hairs* 2.4–3.4 μm diam., cylindrical, very long, subhyaline to pale yellow, growing from the outermost cells of the excipulum, thin-walled, 3–4-septate. *Hymenium* 28–36 μm thick ($\overline{x}$ = 32 μm, n = 20), hyaline. *Subhymenium* not obvious. *Ectal excipulum* 14–25 μm thick ($\overline{x}$ = 18 μm, n = 40), comprised of *textura prismatica* or *oblita* cells, 10–28(–40) μm × 3.2–5.5(–9.3) μm diam. ($\overline{x}$ = 18 × 4.9 μm, n = 70), thin-walled, smooth, slightly glassy, cells elongated significantly towards the stipe. *Medullary excipulum* 13–52 μm thick ($\overline{x}$ = 24 μm, n = 20), comprised of loosely arranged and hyaline *textura intricata* hyphae, 2.0–4.2 μm diam. ($\overline{x}$ = 2.8 μm, n = 20). *Paraphyses* 49–55 μm long × 2.3–3.2 μm ($\overline{x}$ = 52 × 2.7 μm, n = 20), lanceolate, pointed at ends, usually 1-septate, branched at the base, exceeding asci by 3.4–8.6 μm. *Asci* 31–40 μm × 4.0–5.2 μm ($\overline{x}$ = 35.5 × 4.5 μm, n = 30), 8-spored, unitunicate, cylindrical, semicircular apex with J+ apical pore in Melzer's reagent, croziers present. *Ascospores* 11.8–16.3 × 1.2–2.1 μm ($\overline{x}$ = 13.5 × 1.6 μm, n = 70), Q = 6.5–9.8(–11.7) μm, Qm = 8.4 ± 0.8 μm, overlapping uniseriate or biseriate, narrowly fusoid with both sharply pointed ends, hyaline, smooth, thin-walled and aseptate. Asexual morph: Unknown.

Material examined – China, Yunnan Province, Kunming City, Panlong District, altitude 1918 m, on the fallen leaves of *Quercus* sp., 13 ℃–22 ℃, 25 May 2022, Cuijinyi Li, LCJY-471 (HKAS 135664).

Known distribution – ASIA: China (Hainan, Yunnan), Japan (Gunma, Kanagawa and Niigata) (Tochihara & Hosoya 2022).

Notes – The ITS sequence of our collection showed a 99.62% similarity with *Erioscyphella hainanensis* (TNS:F-65722 and TNS:F-61941), and the LSU sequence of our collection displayed a 99.91% similarity with *E. hainanensis* (TNS-F25579 and TNS-F65752). Our collections were nested with *E. hainanensi* (strains: TNS-F35049 and TNS-F35056), supported by ML bootstrap support of 99.4% in the SH-aLRT test, 100% in the UFB method, and a Bayesian probability of 1.0 shown in Fig. 4. All the morphological characteristics are consistent with the description of *E. hainanensis* in previous studies, and the molecular data also support them as one species (Tochihara & Hosoya 2022). This is the first reported collection in Yunnan Province, China, and we have also provided a new colorful illustration and a detailed description of *E. hainanensis*.

Lachnellula subtilissima (Cooke) Dennis, Persoonia 2(2): 184 (1962) Fig. 14

Classification – Lachnaceae, Helotiales, Leotiomycetes, Ascomycota

Index Fungorum number: IF332862; Faceoffungi number: FoF16029

Saprobic on the decayed wood. Sexual morph: *Apothecia* 3.2–5.3 μm wide × 2.8–4 μm high ($\overline{x}$ = 3.9 × 3.5 μm, n = 10) when fresh, 1.1–2.2 μm wide × 1.7–2.2 μm high ($\overline{x}$ = 1.8 × 2.0 μm, n = 10) when dry, bright color with white flank, cup-shaped, stipitate, incurved when dry. *Discs* circular, concave, saucer-shaped, bright orange (#fbb93e) or soft orange (#feca5a) when fresh, strong yellow (#b79c27) to strong orange (#cd9605) when dry. *Receptacles* very soft orange (#f1d5b0) when fresh, white with grayish-yellow color (#beb8a2) when dry, marginal dentition, rough and hairy. *Stipe*s 290–420 μm wide × 420–680 μm long ($\overline{x}$ = 380 × 590 μm, n = 8), short and black. *Hairs* 2.3–3.6 μm diam. ($\overline{x}$ = 2.8 μm, n = 30), cylindrical, densely covered, with hyaline

granules, growing from the outermost cells of the excipulum, hyaline, 5–7-septate and thin-walled. *Subhymenium* 20–45 μm thick ($\bar{x}$ = 31 μm, n = 10), comprised of pale yellow, *textura intricata* hyphae. *Ectal excipulum* 37–70 μm thick, comprised of hyaline, *textura angularis* or *oblita* cells, 4.7–11.2 μm diam. ($\bar{x}$ = 7.5 μm, n = 80), thin-walled, smooth. *Medullary excipulum* 65–160 μm thick, comprised of loosely arranged, hyaline, *textura intricata* hyphae. *Paraphyses* 73–87 μm long × 1.7–2.8 μm ($\bar{x}$ = 80 × 2.2 μm, n = 60), cylindrical, hyaline, smooth and thin-walled, branched near the base, slightly swollen at the apex, containing yellow oil globules, slightly longer than asci. *Asci* 60–73 μm × 4.7–7.3 μm ($\bar{x}$ = 67 × 5.8 μm, n = 40), 8-spored, unitunicate, cylindrical to clavate, oval apex with J+ apical pore in Melzer's reagent, croziers present.

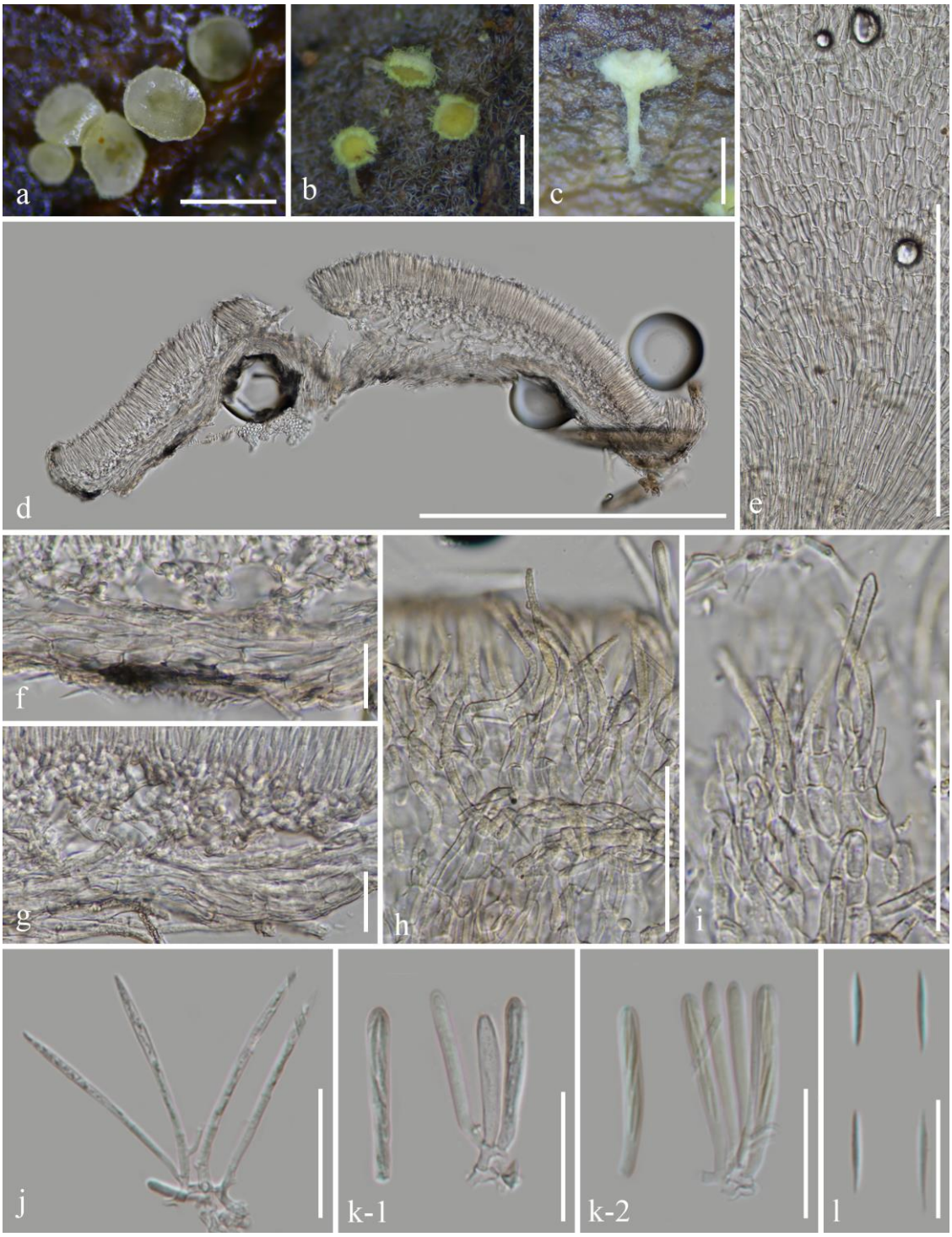

Figure 13 – *Erioscyphella hainanensis* (HKAS X). a Fresh apothecia on the substrate. b, c Dried apothecia on the substrate. d A vertical section of an apothecium. e–g Excipulum. h, i Hairs.

j Paraphyses. k-1 Asci in water. k-2 Asci in the Meltzer's reagent. l Ascospores. Scale bars: a = 1 mm, b = 500 µm, c = 400 µm, d = 300 µm, e = 200 µm, f–g, l = 20 µm, h–i = 50 µm, j–k-2 = 30.

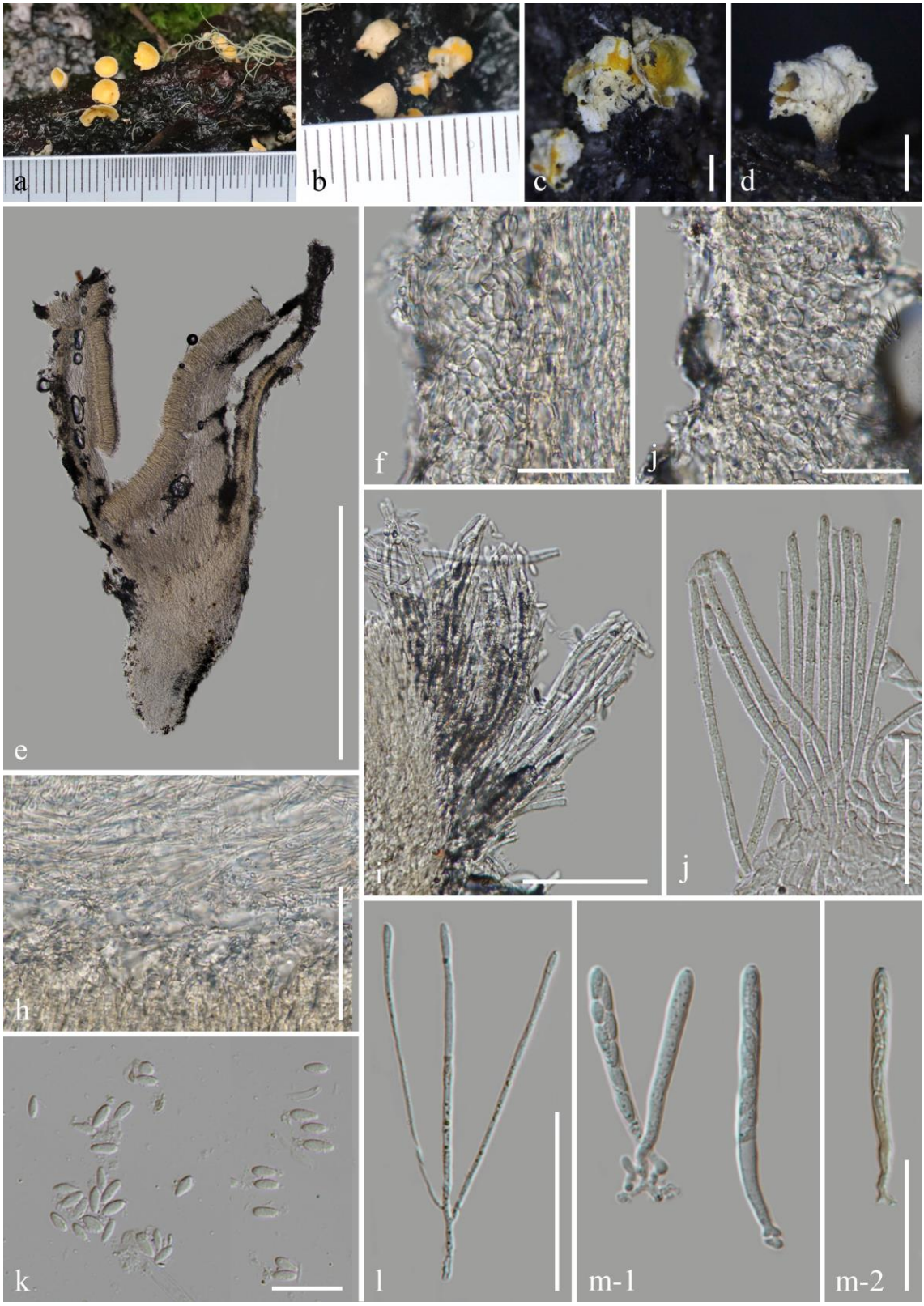

Figure 14 – *Lachnellula subtilissima* (HKAS X). a, b Fresh apothecia on the substrate. c, d Dried apothecia on the substrate. e A vertical section of an apothecium. f–h Excipulum. i, j Hairs. k Ascospores. l Paraphyses. m–1 Asci in water. m-2 Asci in the Meltzer's reagent. Scale bars: c–e = 1 mm, f, j, m-1, m-2 = 30 µm, h–j, l = 50 µm, k = 20 µm.

Ascospores 6.4–9.5(–11.1) × 2.4–3.4(–4.1) μm ($\bar{x}$ = 7.8 × 2.9 μm, n = 40), Q = 2.2–3.7 (–4.7) μm, Qm = 2.7 ± 0.3 μm, oval with one slightly pointed end and one rounded end, hyaline, smooth, thin-walled and aseptate. Asexual morph: see Minter (http://ascofrance.fr/uploads/forum_file/20063068720-0001.pdf).

Material examined – China, Yunnan Province, Lijiang City, Naxi Autonomous County, Laojun Mountain, 99 Longtan scenic spot, altitude 3800 m, on the decayed unknown wood, 9 ℃–13 ℃, 25 May 2022, Cuijinyi Li, LCJY-976 (HKAS 135664).

Known distribution – AUSTRALASIA: New Zealand; ASIA: China (Heilongjiang, Jilin, Sichuan, and Yunnan), Georgia, India, Japan, Pakistan, Russia, and South Korea; EUROPE: Austria, Czech Republic, Denmark, Estonia, Finland, France, Germany, Great Britain, Greece, Hungary, Ireland, Italy, Netherlands, Norway, Poland, Romania, Russia, Slovakia, Spain, Sweden, Switzerland; CENTRAL AMERICA: Dominican Republic. SOUTH AMERICA: Chile. NORTH AMERICA: Canada, USA (Minter 2005).

Notes – The ITS sequence of our collection exhibited a 99.88% similarity with *Lachnellula subtilissima* (CBS:197.66), while the LSU sequence displayed a 100.00% similarity with *La. subtilissima* (CBS:197.66). Our collepctions were nested with *La. subtilissima* (CBS:197.66), supported by ML bootstrap support of 92.6% in the SH-aLRT test, 100% in the UFB method, and a Bayesian probability of 1.0, shown in Fig. 4. *Lachnellula subtilissima* has been extensively documented in diverse regions worldwide, and our collection conforms morphologically to the established characteristics of *La. subtilissima*. This represents the first reported collection in Yunnan Province, China, and we have also provided a new colorful illustration and a detailed description of *La. subtilissima*.

DISCUSSION

In this study, we have introduced a novel genus, *Longistipes*, and expanded the concept of Hyphodiscaceae to include long-stalked apothecia, exceptionally lengthy and pale hairs, as well as broad lanceolate paraphyses. *Longistipes* is characterized by cup-shaped, white to translucent or black apothecia with long-stipitate, pale-colored discs, long, white hairs covered with hyaline, finely granules at the margins and receptacles, hyaline, *textura intricata* medullary hyphae, hyaline to brown, *textura porrecta* excipular cells, broad lanceolate paraphyses, exceeding the asci, and drop-shaped to obovate to oblong ascospores without septa.

Previous phylogenetic analyses have significantly contributed to the establishment of large-scale phylogenetic analyses through the combined analysis of four genes (ITS, LSU, mtSSU, and *RPB2*). However, in this study, we followed the results of Quijada et al. (2022) by utilizing the LSU and ITS gene combination as the marker for phylogenetic analysis due to the scarcity of protein-coding genes within the family. Simultaneously, we analyzed the phylogenetic trees using the mtSSU and *RPB2* genes. However, the limited number of sequences hindered the effectiveness of the combined analysis. Consequently, this combined analysis failed to significantly influence the topology of the phylogenetic tree or the support rate of individual nodes. Hence, obtaining additional gene fragments for known species is crucial. Expanding both the diversity and quantity of gene fragments would yield a more comprehensive genetic panorama, thereby enhancing the precision and depth of phylogenetic analyses.

In our analysis, the placement of *Hyphodiscus* on the phylogenetic tree aligns with previous studies. In an earlier study, *Hy. otanii* and *Hy. hymeniophilus* were grouped, leading Quijada et al. (2022) to propose that they may represent distinct genotypes of the same species, despite the noted morphological differences. However, our investigation reveals that *Hy. otanii* forms a distinct clade separated from *Hy. hymeniophilus* at the base of the genus, prompting further deliberation on whether they should be considered the same species. Meanwhile, concerns persist about potential errors in sequence interpretation within the NCBI database, which could impact phylogenetic studies. Previously, Wang et al. (2023) have advocated for implementing third-party error correction mechanisms within the NCBI database to uphold data accuracy. The addition of new

species has expanded the concept of *Hyphopeziza* to include short, cylindrical, and thin-walled hairs with a blunt tip that are covered with hyaline, finely granules.

The distribution of Hyphodiscaceae sampling sites primarily spans the Eurasian and American plates (Quijada et al. 2022). Presently, fungal sample collection has extended to virtually all continents, including harsh environments such as polar regions. However, the diminutive size and moisture sensitivity of fungal structures pose challenges to sample collection.

In a previous taxonomic study of *Chalara* and *Nagrajchalara* in Pezizellaceae, only asexual species were included. In our investigation of the diversity of discomycetes in Yunnan Province, China, we described the sexual morphs of three species (*Chalara bambusicola*, *Nagrajchalara neonawawii*, and *N. yongnianii*) in these two genera, providing detailed morphological illustrations and measurements to supplement the deficiencies in existing morphological records. Our findings not only enhance the morphological understanding of these two genera but also highlight the importance of considering both sexual and asexual forms when studying fungal diversity. Furthermore, our study reported a new geographical record of *D. chiangraiensis* in China. Additionally, we expanded the knowledge of two species in the Lachnaceae (*Erioscyphella hainanensis* and *Lachnellula subtilissima*) from Yunnan. While these species had been recorded in China, our research provided more detailed morphological descriptions and clear, colorful illustrations.

Therefore, broadening the sampling locations is imperative, aiming to engage more collectors. Collaboration with fungal enthusiasts is also sought to further augment the sampling range.

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