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Morpho-molecular diversity of Linocarpaceae (Chaetosphaeriales): *Claviformispora* gen. nov. from decaying branches of *Phyllostachys heteroclada*

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Abstract

In this paper, *Claviformispora* **gen. nov.** in Linocarpaceae is introduced from *Phyllostachys heteroclada* in Sichuan Province, China. The new genus is characterised by its distinct morphological characters, such as ostiole with periphyses, asci with a thick doughnut-shaped, J- apical ring and clavate ascospore without septum-like band and appendage. Maximum Likelihood and Bayesian Inference phylogenetic analyses, based on DNA sequence data from ITS, LSU, SSU and TEF-1α regions, provide further evidence that the fungus is a distinct genus within this family. The new genus is compared with similar genera, such as *Linocarpon* and *Neolinocarpon*. Descriptions, illustrations and notes are provided for the new taxon.

Keywords

bambusicolous fungi, one new genus and species, phylogeny, taxonomy

Introduction

The order Chaetosphaeriales Huhndorf, A.N. Mill. & F.A. Fernández (Sordariomycetes) was introduced in Sordariomycetidae O.E. Erikss. & Winka, based on LSU sequence data (Huhndorf et al. 2004) and currently comprises four families viz. Chaetosphaeriaceae Réblová, M.E. Barr & Samuels, Helminthosphaeriaceae Samuels, Cand. & Magni, Leptosporellaceae S. Konta & K.D. Hyde and Linocarpaceae S. Konta & K.D. Hyde (Hongsanan et al. 2017; Konta et al. 2017; Wijayawardene et al. 2020). Recently, 43 genera were accepted within Chaetosphaeriaceae and seven genera within Helminthosphaeriaceae (Hyde et al. 2020; Wijayawardene et al. 2020). Based on morphology and combined analyses of ITS and LSU sequence data, Konta et al. (2017) accommodated *Linocarpon* Syd. & P. Syd. and *Neolinocarpon* K.D. Hyde in Linocarpaceae and *Leptosporella* Penz. & Sacc. in Leptosporellaceae. Leptosporellaceae and Linocarpaceae are morphologically similar in their dome-shaped ascomata and filiform ascospores (Hyde and Alias 1999; Cai et al. 2004; Konta et al. 2017). The former, however, can be delineated based on ascospores that are narrow, long filiform, with gradually tapering ends and indistinct mucilage (if present), whereas in Linocarpaceae, ascospores have a distinct appendage at the apex and are generally wider and differ in appearance at the ends (Konta et al. 2017).

The genus *Leptosporella* was introduced with *L. gregaria* Penz. & Sacc., 1897 as the type species by Penzig and Saccardo (1897). Lumbsch and Huhndorf (2010) referred it in Sordariomycetidae genera *incertae sedis*. Subsequently, the genus was referred to the Chaetosphaeriales, based on phylogenetic analysis of LSU sequence data (Huhndorf and Miller 2011; Dai et al. 2016; Wijayawardene et al. 2020). At present, 15 epithets of *Leptosporella* are recorded in Index Fungorum (http://www.speciesfungorum.org/Names/Names.asp). Sydow and Sydow (1917) introduced *Linocarpon* with *L. pandani* Syd. & P. Syd., 1917 as the type species. Hyde (1992a) introduced *Neolinocarpon* to accommodate a linocarpon-like species, *N. globosicarpum* K.D. Hyde, 1992. Currently in Index Fungorum (2020), 44 and 13 epithets are accommodated in *Linocarpon* and *Neolinocarpon*, respectively. Hyde (1997) and Jeewon et al. (2003) reported that *Linocarpon* and *Neolinocarpon* can be accommodated in Hyponectriaceae (Xylariales), while Bahl (2006) suggested a closer relationship with Chaetosphaeriales and Xylariales, based on their molecular data. However, with more taxon sampling and fresh collections, Konta et al. (2017) confirmed that *Linocarpon* and *Neolinocarpon* should be accommodated in a distinct family (Linocarpaceae) in Chaetosphaeriales.

The present research is a part of our investigations on the taxonomic and phylogenetic circumscriptions of pathogenic and saprobic micro-fungi associated with bamboo in Sichuan Province, China. In this paper, we introduce a new genus *Claviformispora* in Linocarpaceae, typified by *C. phyllostachydis* from *Phyllostachys heteroclada* Oliv., 1894 (Poaceae). The morphological differences and analyses of a combined ITS, LSU, SSU and TEF-1α sequence dataset support the validity of the new genus and its placement in Linocarpaceae. The new genus is compared with other genera in the family. The comprehensive descriptions and micrographs of new taxa are provided.

Materials and methods

Specimen collection and morphological study

Bamboo materials were collected from Ya'an City, China. Single ascospore isolations were carried out following the method described by Chomnunti et al. (2014) and the germinating spores were transferred to PDA, incubated at 25 °C in the dark and cultural characteristics were determined. Ascomata were observed and photographed using a dissecting microscope NVT-GG (Shanghai Advanced Photoelectric Technology Co. Ltd, China) matched to a VS-800C micro-digital camera (Shenzhen Weishen Times Technology Co. Ltd., China). The anatomical details were visualised using Nikon ECLIPSE *Ni* compound microscope fitted to a Canon 600D digital camera and an OPTEC BK-DM320 microscope matched to a VS-800C micro-digital camera (Shenzhen Weishen Times Technology Co. Ltd., China). Iodine reaction of the ascus wall was tested in Melzer's reagent (MLZ). Lactate cotton blue reagent was used to observe the number of septa. The gelatinous appendage was observed in Black Indian ink. Type specimens were deposited at the Herbarium of Sichuan Agricultural University, Chengdu, China (SICAU) and Mae Fah Luang University Herbarium (MFLU). The ex-type living cultures are deposited at the Culture Collection in Sichuan Agricultural University (SICAUCC) and the Culture Collection at Mae Fah Luang University (MFLUCC). Index Fungorum numbers (http://www.indexfungorum.org/Names/Names.asp) are registered and provided.

DNA extraction, PCR amplification and DNA sequencing

Total genomic DNA was extracted from mycelium that were grown on PDA at 25 °C for two weeks using a Plant Genomic DNA extraction kit (Tiangen, China) following the manufacturer's instructions. The primers pairs LR0R and LR5 (Vilgalys and Hester 1990), NS1 and NS4, ITS5 and ITS4 (White et al. 1990), EF1-983F and EF1-2218R (Rehner 2001) were used for the amplification of the partial large subunit nuclear rDNA (LSU), the partial small subunit nuclear rDNA (SSU), internal transcribed spacers (ITS) and translation elongation factor 1-alpha (TEF-1α), respectively.

Polymerase chain reaction (PCR) was performed in 25 µl final volumes containing 22 µl of Master Mix (Beijing TsingKe Biotech Co. Ltd.), 1 µl of DNA template, 1 µl of each forward and reverse primers (10 µM). The PCR thermal cycle programmes for LSU, SSU, ITS and TEF1-α gene were amplified as: initial denaturation 94 °C for 3 minutes, followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 55 °C for 50 seconds, elongation at 72 °C for 1 minute and final extension at 72 °C for 10 minutes. PCR products were sequenced with the above-mentioned primers at TsingKe Biological Technology Co. Ltd, Chengdu, China. The newly-generated sequences from the LSU, SSU, TEF-1α and ITS regions were deposited in GenBank (Table 1).

Table 1. Molecular data used in this study and GenBank accession numbers.

Species name	Strain	GenBank accession number			
		LSU	ITS	SSU	TEF
Chloridium aquaticum	MFLUCC 11-0212	MH476567	MH476570	MH476573	–
Chloridium aseptatum	MFLUCC 11-0216	MH476568	NR_158365	MH476574	–
Claviformispora phyllostachydis	**SICAUCC 16-0004**	**MT232720**	**MT232736**	**MT232735**	**MT240855**
Cryptophiale hamulata	MFLUCC 18-0098	MG386756	–	MG386757	–
Cryptophiale udagawae	MFLUCC 18-0422	MH758211	MH758198	MH758205	–
	MFLUCC 18-0428	MH758210	MH758197	MH758204	–
Dictyochaeta siamensis	MFLUCC 15-0614	KX609952	KX609955	–	–
Dictyochaeta assamica	CBS 242.66	MH870426	MH858788	–	–
Dictyochaeta pandanicola	MFLUCC 17-0563	MH376710	MH388338	MH388307	MH388373
Dictyochaeta terminalis	GZCC 18-0085	MN104624	MN104613	MN104633	–
Echinosphaeria canescens	SMH4666	KF765605	–	–	–
	SMH4791	AY436403	–	–	–
Endophragmiella dimorphospora	FMR_12150	KY853502	KY853442	HF937351	–
Gelasinospora tetrasperma	CBS 178.33	DQ470980	NR_077163	DQ471032	DQ471103
Helminthosphaeria clavariarum	SMH4609	AY346283	–	–	–
Hilberina caudata	SMH1542	KF765615	–	–	–
Infundibulomyces cupulata	BCC11929	EF113979	EF113976	EF113982	–
Infundibulomyces oblongisporus	BCC13400	EF113980	EF113977	EF113983	–
Kionochaeta castaneae	GZCC 18-0025	MN104621	MN104610	MN104630	–
Kionochaeta microspora	GZCC 18-0036	MN104618	MN104607	MN104627	–
Leptosporella arengae	MFLUCC 15-0330	MG272246	MG272255	MG366594	MG272259
Leptosporella bambusae	MFLUCC 12-0846	KU863122	KU940134	–	–
Leptosporella cocois	MFLUCC 15-0816	–	MG272256	–	–
Leptosporella gregaria	SMH4290	AY346290	–	–	–
	SMH4673	HM171287	–	–	–
Leptosporella elaeidis	MFLU 19-0669	MK659772	MK659767	MK659774	MN883560
Linocarpon arengae	MFLUCC 15-0331	MG272247	–	MG366596	–
Linocarpon cocois	MFLUCC 15-0812	MG272248	MG272257	MG272253	–
Menispora tortuosa	DAOM 231154	AY544682	KT225527	AY544723	–
	CBS 214.56	AF178558	AF178558	–	–
Menisporopsis anisospora	CBS 109475	MH874421	MH862827	–	–
Menisporopsis breviseta	GZCC 18-0071	MN104623	MN104612	MN104632	–
Menisporopsis dushanensis	GZCC 18-0084	MN104626	MN104615	MN104635	–
Menisporopsis pandanicola	KUMCC 17-0271	MH376726	MH388353	MH388320	MH388388
Menisporopsis theobromae	MFLUCC 15-0055	KX609954	KX609957	–	–
Neolinocarpon arengae	MFLUCC 15-0323	MG272249	MG272258	MG366597	–
Neolinocarpon rachidis	MFLUCC 15-0332	MG272250	–	MG366598	–
	MFLUCC 15-0814a	MK106353	MK106342	MK106367	–
	MFLUCC 15-0814b	MK106354	–	MK106368	
Neolinocarpon phayaoense	MFLUCC 17-0073a	MG581933	–	MG581936	MG739512
	MFLUCC 17-0073b	MG581934	–	MG581937	MG739513
	MFLUCC 17-0074	MG581935	–	MG581938	MG739514
Phialosporostilbe scutiformis	MFLUCC 17-0227	MH758207	MH758194	MH758201	–
	MFLUCC 18-1288	MH758212	MH758199	–	–
Ruzenia spermoides	SMH4606	AY436422	–	–	–
	SMH4655	KF765619	–	–	–
Synaptospora plumbea	ANM963	KF765620	–	–	–
	SMH3962	KF765621	–	–	–
Sordaria fimicola	CBS 508.50	MH868251	MH856730	–	–
Zanclospora iberica	FMR 11584	KY853544	KY853480	HF937360	–
	FMR 12186	KY853545	KY853481	HF937361	–

Notes. New species in this study is in bold. "–" means that the sequence is missing or unavailable.

Abbreviations. ANM: Collection of A.N. Miller; **BCC**: BIOTEC Culture Collection, National Center for Genetic Engineering and Biotechnology (BIOTEC), Bangkok, Thailand; **CBS**: Centraalbureau voor Schimmelcultures, Utrecht, Netherlands; **DAOM**: Canadian Collection of Fungal Cultures, Agriculture and Agri-Food Canada, Ottawa, Canada; **FMR**: Facultad de Medicina, Universitat Rovira i Virgili, Reus, Tarragona, Spain; **GZCC**: Guizhou Culture Collection, Guiyang, China; **KUMCC**: Kunming Institute of Botany Culture Collection, Chinese Academy of Sciences, Kunming, China; **MFLU**: Herbarium of Mae Fah Luang University, Chiang Rai, Thailand; **MFLUCC**: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; **SICAUCC**: Sichuan Agricultural University Culture Collection, Sichuan, China; **SMH**: Collection of S.M. Huhndorf.

Phylogenetic analyses

Taxa to be used for phylogenetic analyses were selected, based on results generated from nucleotide BLAST searches online in GenBank and recent publications (Lu et al. 2016; Konta et al. 2017; Senwanna et al. 2018; Wei et al. 2018; Lin et al. 2019). *Gelasinospora tetrasperma* (CBS 178.33) and *Sordaria fimicola* (CBS 508.50) were selected as the outgroup taxa. The sequences were downloaded from GenBank (http://www.ncbi.nlm.nih.gov/) and the accession numbers are listed in Table 1. A combined ITS, LSU, SSU and TEF-1α sequence dataset was used to construct the phylogenetic tree. DNA alignments were performed by using MAFFT v.7.429 online service (Katoh et al. 2019) and ambiguous regions were excluded with BioEdit version 7.0.5.3 (Hall 1999). Multigene sequences were concatenated by using Mesquite software (Maddison and Maddison 2019). Maximum Likelihood (ML) and Bayesian Inference (BI) analyses were performed. The best nucleotide substitution model was determined by MrModeltest v. 2.2 (Nylander 2004).

Maximum Likelihood analysis and Bayesian Inference analysis were generated by using the CIPRES Science Gateway web server (Miller 2010). RAxML-HPC2 on XSEDE (8.2.10) (Stamatakis 2014) with GTR+GAMMA substitution model with 1000 bootstrap iterations was chosen for Maximum Likelihood analysis. For BI analyses, the best-fit model GTR+I+G for ITS, LSU and SSU was selected in MrModeltest 2.2 and GTR+G for TEF. The analyses were computed with six simultaneous Markov Chain Monte Carlo (MCMC) Chains with 8,000,000 generations and a sampling frequency of 100 generations. The burn-in fraction was set to 0.25 and the run automatically ended when the average standard deviation of split frequencies reached below 0.01.

Phylogenetic trees were visualised with FigTree v.1.4.3 (Rambaut and Drummond 2016) and edited using Adobe Illustrator CS6 (Adobe Systems Inc., United States). Maximum Likelihood bootstrap values (MLBP) equal to or greater than 70% and Bayesian Posterior Probabilities (BYPP) equal to or greater than 0.95 were accepted. The finalised alignment and tree were deposited in TreeBASE (http://www.treebase.org), submission ID: 25996. The new taxa introduced follow the recommendations of Jeewon and Hyde (2016).

Results

Phylogenetic analyses

Phylogenetic analyses of a combined dataset (ITS, LSU, SSU, TEF-1α) comprises 51 taxa within the order Chaetosphaeriales (Table 1), including 24 taxa in family Chaetosphaeriaceae, nine taxa in Helminthosphaeriaceae, ten taxa in Linocarpaceae, six taxa in Leptosporellaceae and two outgroup taxa in Sordariales. The dataset consisted of 5,849 characters including gaps (LSU = 1,571, ITS = 736, SSU = 2,522, TEF = 1,020). The best scoring tree of RAxML analysis is shown in Fig. 1, with the support values of ML and BI analyses.

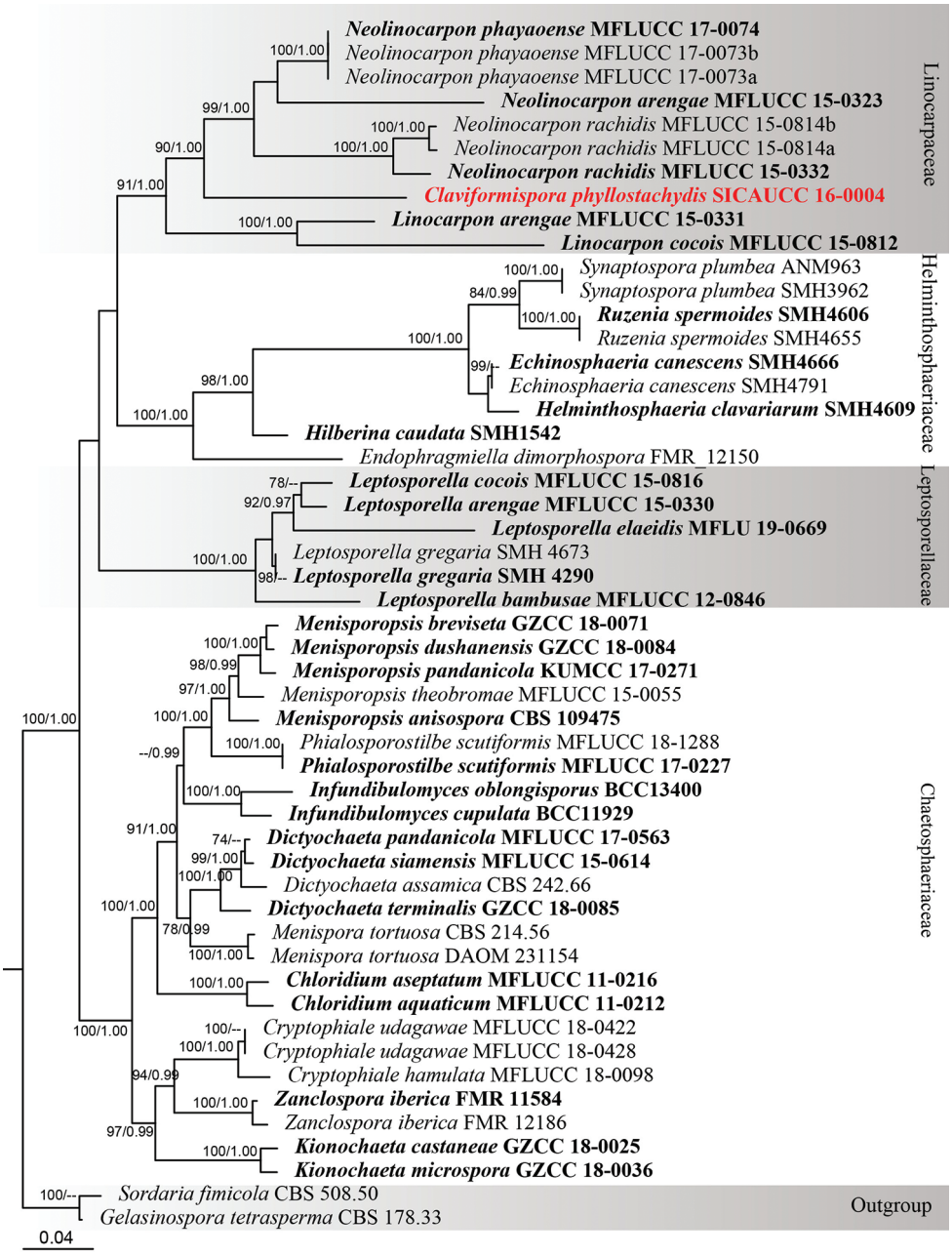

Figure 1. Phylogram of RAxML analysis based on a combined ITS, LSU, SSU and TEF-1α sequence dataset within order Chaetosphaeriales. Bootstrap support values for maximum likelihood (ML, left) greater than 70% and Bayesian posterior probabilities (PP, right) equal to or greater than 0.95 are indicated at the nodes. The tree is rooted to *Gelasinospora tetrasperma* (CBS 178.33) and *Sordaria fimicola* (CBS 508.50). All sequences from ex-type strains are in bold. The newly-generated sequence is in red.

The best scoring RAxML tree with the final optimisation had a likelihood value of -26,415.700648. The matrix had 1,751 distinct alignment patterns and 64.64% in this alignment is the gaps and completely undetermined characters. Estimated base frequencies were as follows: A = 0.236065, C = 0.261532, G = 0.295313, T = 0.207091, with substitution rates AC = 1.062535, AG = 1.855434, AT = 0.940219, CG = 1.052604, CT = 4.590285, GT = 1.000000. The gamma distribution shape parameter $\alpha = 0.311923$ and the Tree-Length = 2.281738. The Bayesian analysis resulted in 20,502 trees after 8,000,000 generations. The first 25% of trees (1,624 trees), which represent the burn-in phase of the analyses were discarded, while the remaining 4,878 trees were used for calculating posterior probabilities. Bayesian posterior probabilities were evaluated by MCMC with a final average standard deviation of split frequencies = 0.009877.

Phylogenetic trees generated from Maximum Likelihood (ML) and Bayesian Inference analyses were similar in overall topologies. Phylogeny from the combined sequence data analysis indicates that all families were monophyletic with strong bootstrap support values (Fig. 1). Phylogenetic results show that our novel species *Claviformispora phyllostachydis* (SICAUCC 16-0004) belongs to family Linocarpaceae with 91% ML and 1.00 BYPP support and close to genera *Neolinocarpon* and *Linocarpon* (Fig. 1). The new genus *Claviformispora* constituted a distinct lineage in the family Linocarpaceae (Fig. 1).

Taxonomy

Linocarpaceae S. Konta & K.D. Hyde, Mycosphere 8(10): 1962 (2017) emend.

Type genus. *Linocarpon* Syd. & P. Syd.

Description. *Saprobic* and *endophytic* fungi on monocotyledons and rarely dicotyledons. ***Sexual morph*:** *Ascomata* solitary or aggregated, superficial or immersed comprising black, dome-shaped or subglobose, slightly raised blistering areas with a central ostiole or immersed with a black shiny papilla. *Peridium* composed of dark brown to black cells of *textura angularis*. *Hamathecium* comprising septate paraphyses that are longer than asci, wider at the base, tapering towards the apex. *Asci* 8-spored, unitunicate, cylindrical, with a J-, apical ring, developing from the base and periphery of the ascomata. *Ascospores* parallel or spiral in asci, hyaline or pale yellowish in mass, filiform or claviform, straight or curved, unicellular with or without refringent bands, with or without polar appendages. ***Asexual morph*:** *Phialophora*-like spp. were found in *Linocarpon appendiculatum* and *L. elaeidis* cultures (Hyde 1992b), but no records are available for other species.

Notes. Linocarpaceae was introduced as a new family to accommodate *Linocarpon* and *Neolinocarpon* species, based on morphology and phylogeny (Konta et al. 2017). Appressoria were first recorded from *Neolinocarpon rachidis* (Hyde et al. 2019). The new genus *Claviformispora*, which is well-supported within Linocarpaceae suggests

that there is a need to amend the morphological circumscriptions of the family given that the ascomata (subglobose) and ascospore (claviform) characters are so different from the other two genera.

Claviformispora **X. L. Xu & C. L. Yang, gen. nov.**
Index Fungorum identifier: IF557395

Type species. *Claviformispora phyllostachydis* X. L. Xu & C. L. Yang

Etymology. Name reflects the claviform ascospores.

Description. *Saprobic* on dead branches. ***Sexual morph*****:** *Stromata* solitary or gregarious, black, erumpent. *Ascomata* solitary or aggregated, immersed, subglobose, slightly raised blistering areas with a central ostiole with periphyses. *Peridium* outer cells merging with the host tissues, composed of pale to dark brown cells of *textura angularis*. *Hamathecium* comprising hyaline, septate paraphyses, longer than asci, wider at the base, tapering towards the apex. *Asci* 8-spored, cylindrical to cylindric-clavate, unitunicate, short pedicellate, apically rounded, with a doughnut-shaped, refractive, J- apical ring. *Ascospores* overlapping uniseriate or 2-seriate, clavated with a thin pedicellate, 1-celled, hyaline, without appendage and refringent bands, smooth-walled. ***Asexual morph*****:** Undetermined.

Notes. *Claviformispora* resembles *Neolinocarpon* in having immersed ascomata and ostiole with periphyses, but differs in forming aggregated ascomata, cylindric-clavate, short pedicellate asci, clavate ascospores with thin pedicel and without septa-like bands and appendages, whereas the ascospores of *Neolinocarpon* and *Linocarpon* (Linocarpaceae) species are usually filiform with refringent bands and appendages (Hyde 1992b, 1997; Konta et al. 2017). The nature of the ascospore appendages appears to be phylogenetically significant for intergeneric delineation as has been seen in other studies (Poonyth et al. 2000; Jeewon et al; 2003, Thongkantha et al. 2003; Cai et al. 2004; Konta et al. 2017), but this warrants further investigations with more sampling and fresh collections of *Neolinocarpon* and *Linocarpon*. Differences in morphology between these genera in Linocarpaceae are summarised in Table 2.

Claviformispora phyllostachydis **X. L. Xu & C. L. Yang, sp. nov.**
Index Fungorum identifier: IF557396
Fig. 2

Type. China, Sichuan Province, Ya'an City, Yucheng Distinct, Kongping Township, alt. 1133 m, 29°50.14'N, 103°03'E, on dead branches of *Phyllostachys heteroclada* Oliv. (Poaceae), 11 December 2016, C. L. Yang and X. L. Xu, YCL201612002 (SICAU 16-0007, ***holotype***; MFLU 18-1217, ***isotype***), ex-type living culture, SICAUCC 16-0004 = MFLUCC 18-1230.

Etymology. The specific epithet refers to the host genus *Phyllostachys*.

Description. *Saprobic* on dead branches of *Phyllostachys heteroclada* Oliv. ***Sexual morph*****:** *Stromata* solitary comprising elliptical areas or aggregated in large black

Table 2. Morphological comparison of *Linocarpon*, *Neolinocarpon* and *Claviformispora*.

Morphology	***Linocarpon*** (Type: ***L. pandani***)	***Neolinocarpon*** (Type: ***N. globosicarpum***)	***Claviformispora*** (Type: ***C. phyllostachydis***)
Stromata	Absent	Absent	Solitary or aggregated, comprising elliptical areas and large black areas, with slit-like openings
Ascomata	Solitary, superficial, subglobose, comprising black, dome-shaped, raised blistering areas, central ostiole	Solitary, deeply immersed, oval to globose, with central raised, dark, shiny papilla, central ostiole with periphyses	Solitary or aggregated, deeply immersed, subglobose, slightly raised blistering areas, central ostiole with periphyses
Peridium	Textura angularis	Textura angularis	Textura angularis
Hamathecium	Hyaline, septate paraphyses, longer than asci	Hyaline, septate paraphyses, longer than asci	Hyaline, septate paraphyses, longer than asci
Asci	Cylindrical, unitunicate, a small non-amyloid apical ring	Long cylindrical, pedicellate, unitunicate, an oblong to wedge-shaped, refractive, apical ring and some with a refractive circular body below	Cylindrical to cylindric-clavate, unitunicate, pedicellate, doughnut-shaped, refractive, J- apical ring
Ascospores	Filiform, aseptate, hyaline or pale-yellowish in mass, parallel or spiral, with appendage and refringent septum-like bands or absent	Filiform, aseptate, hyaline or pale-yellowish in mass, parallel or spiral, with apical appendages and refringent bands or absent	Clavate, thin pedicellate, aseptate, hyaline, parallel, no appendage and refringent band
Asexual morph	Only found in *L. appendiculatum* and *L. elaeidis*, conidiophore arising from the aerial mycelium, conidiogenous cells phialidic, smooth, translucent brown, conidia clavate to fusiform, straight or slightly curved or slightly sinuous, unicellular, smooth, colourless	Undetermined	Undetermined
Others	Colonies on MEA and PDA growing slowly	Colonies on MEA growing slowly. Ascospores on MEA produced appressoria-like structures at each tip of germ tube, only found in *N. rachidis*	Colonies on PDA grow faster
References	Hyde (1992b), Konta et al. (2017), Thongkantha et al. (2003)	Hyde et al. (2019), Senwanna et al. (2018), Hyde et al. (1998)	This study

areas, slightly raised with slit-like openings presenting on host surface. *Ascomata* 120–240 µm high × 220–490 µm diameter ($\bar{x}$ = 189 × 345 µm, n = 20), perithecial, immersed, central, papillate ostiole with periphyses, oval-globose in section, the cells between the perithecia are composed with tissue of stromata and host. *Peridium* 20–40 µm wide ($\bar{x}$ = 33 µm, n = 10), outer cells merging with the host tissues, composed of pale to dark brown cells of *textura angularis*. *Hamathecium* comprising hyaline, hypha-like, septate paraphyses, occasionally branched, longer than asci, wider at the base, 2–4 µm diameter ($\bar{x}$ = 2.7 µm, n = 20) tapering towards the apex, 0.78–1.20 µm diameter ($\bar{x}$ = 0.98 µm, n = 20). *Asci* 90–160 × 9–15 µm ($\bar{x}$ = 118 × 13 µm, n = 20), 8-spored, cylindrical to cylindric-clavate, unitunicate, short pedicellate, apically rounded, with a massive, doughnut-shaped, refractive, J- reaction, apical ring. *Ascospores* 35–50 × 5.7–8.6 µm ($\bar{x}$ = 45.7 × 7.0 µm, n = 40), overlapping uniseriate or 2-seriate, claviform typically with a thin pedicel, aseptate, hyaline, straight or slight curved, without appendage and septum-like bands, guttulate when maturity. ***Asexual morph*:** Undetermined.

Culture characters. Ascospores germinated on PDA within 12 hours at both ends. Colonies on PDA reaching 5 cm diameter after 7 days at 25 °C, white to grey with strong radiations outwards on forward side. Colonies became dark brown and black on the reverse after a long time of cultivation. The hyphae are septate, branched, smooth.

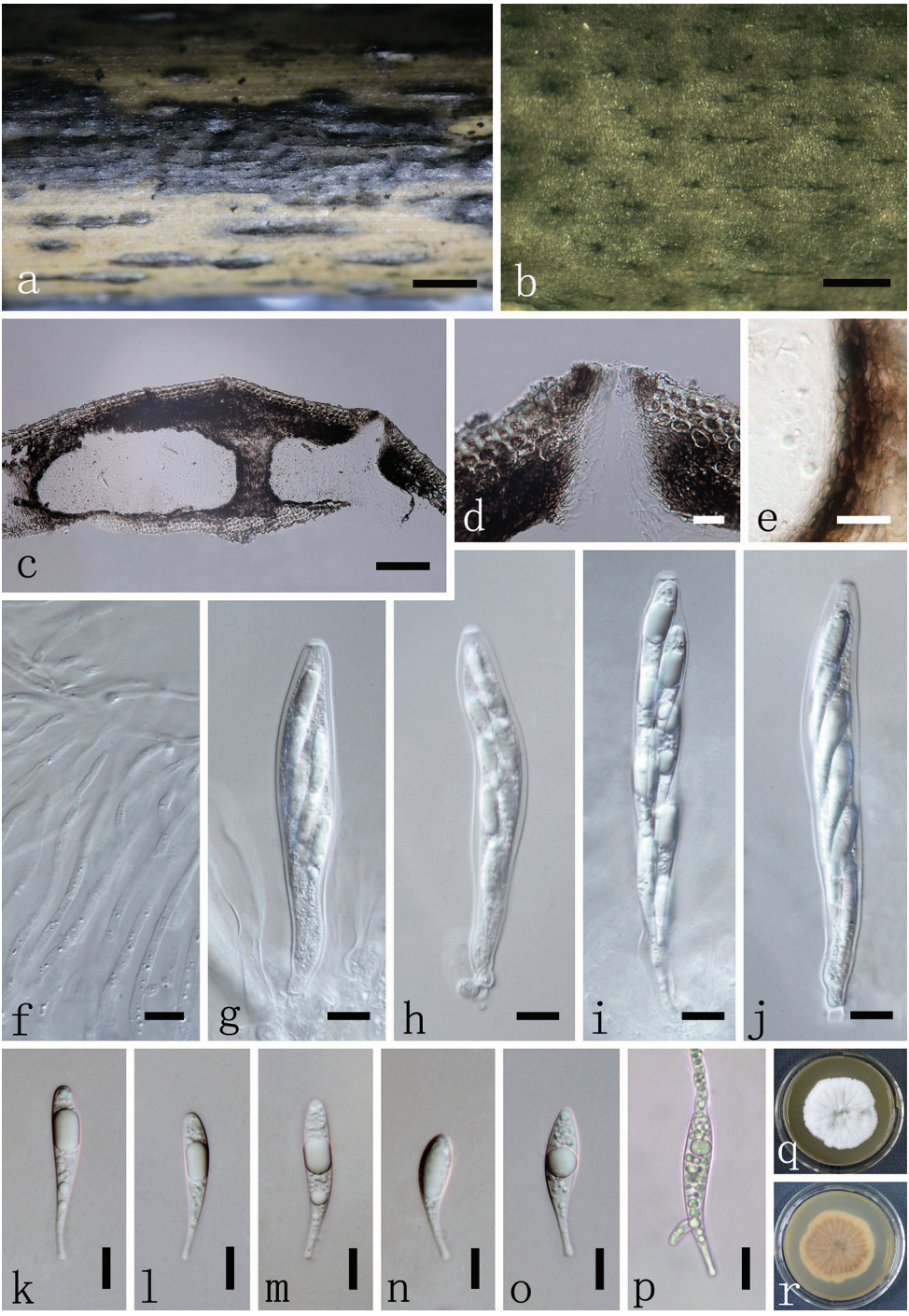

Figure 2. *Claviformispora phyllostachydis* (SICAU 16-0007, holotype) **a, b** *Stromata* on host substrate **c** section through ascoma with ascomata **d** ostiole with periphyses **e** peridium **f** paraphyses **g–j** asci **k–o** ascospores **p** germinated ascospore **q, r** colony on PDA after 7 days. Scale bars: 2 mm (**a**), 500 μm (**b**), 100 μm (**c**), 20 μm (**d, e**), 10 μm (**f–p**).

Discussion

This study establishes a new genus and also provides further insights into the phylogeny of members associated with Linocarpaceae. Morphologically-based examinations of *Claviformispora* (as discussed above) clearly show that the morphological circumscriptions (familial concept) of species should be broadened and possibly indicate that this family is much more diverse than expected. Our collection can be clearly distinguished from other groups of similar fungi in Linocarpaceae with its interesting ascospore morphology. In addition, we also noted some peculiarities in the DNA sequences we analysed. A comparison of ITS sequences, based on BLAST reveals 34%, 26% and 30% base pair differences with *L. cocois* (MFLUCC 15-0812), *N. arengae* (MFLUCC 15-0323) and *N. rachidis* (MFLUCC 15-0814a), respectively. There are more than 9% and 5% sequence differences with the three taxa when the LSU and SSU rDNA sequences were compared respectively. Following the guidelines recommended by Jeewon and Hyde (2016), there are therefore sufficient grounds to establish a new species at the genus rank.

Species of Linocarpaceae have been found on Arecaceae, Poaceae, Euphorbiaceae, Zingiberaceae, Pandanaceae, Fagaceae, Fabaceae and Smilacaceae, including *Arenga*, *Attalea*, *Calamus*, *Trachycarpus*, *Acrocomia*, *Archontophoenix*, *Cocos*, *Daemonorops*, *Licuala*, *Livistona*, *Plectocomia*, *Phoenix*, *Raphia*, *Sabal*, *Mauritia*, *Nypa*, *Elaeis*, *Pinanga*, *Eugeissona*, *Pennisetum*, *Gramineae*, *Stipa*, unidentified bamboo, *Hevea*, *Manihot*, *Alpinia*, *Pandanus*, *Quenrcus*, *Cajanus* and *Smilax* (Hyde 1988, 1992a, b; Dulymamode et al. 1998; Hyde et al. 1998; Hyde and Alias 1999; Thongkantha et al. 2003; Cai et al. 2004; Bhilabutra et al. 2006; Vitoria et al. 2013; Konta et al. 2017; Senwanna et al. 2018). More than 50% of the species were recorded from hosts of the Arecaceae. Species in Linocarpaceae are mostly saprobic, except *Linocarpon palmetto* which was discovered as a pathogen of *Sabal palmetto* in Florida (Barr 1978). Four species in Linocarpaceae from Poaceae have been reported so far, including *Neolinocarpon penniseti* on *Pennisetum purpureum* (Bhilabutra et al. 2006), *Linocarpon williamsii* on *Gramineae* sp. (Hansford 1954), *L. stipae* on *Stipa* sp. (Hansford 1954) and *L. bambusicola* on unidentified bamboo submerged in a river (Cai et al. 2004).

Phyllostachys heteroclada, mainly a food source and use as a material in the weaving industry, is distributed along the Yellow River Valley and the southern Provinces in China. It is common in the mountainous areas of Sichuan Province with distribution up to 1,500 m above sea level (Yi 1997; Yi et al. 2008). There is a large area of pure forest in Yibin, Leshan and Ya'an Cities and sporadic distribution in other areas. According to preliminary statistics, bambusicolous fungi from seven orders (excluding fungi referred to as Sordariomycetes *incertae sedis*) have been recorded on *P. heteroclada*, including Hypocreales, Ostropales, Pleosporales, Phyllachorales, Pucciniales, Ustilaginales and Xylariales, of which Pleosporales is the largest one. Most bambusicolous fungi in China were recorded with inadequate morphological descriptions or molecular data. The early known fungi on *P. heteroclada* are documented as *Aciculosporium take*, *Ellisembia pseudoseptata*, *Fusarium oxysporum*, *F. semitectum*, *Phyllachora gracilis*, *Ph. orbicular*, *Shiraia bambusicola*, *Stereostratum corticioides* and *Ustilago shiraiana* (Zhou et al. 2001; Xu et al. 2006). In recent years, some new records and taxa, viz. *Bambusicola subthailandica*,

B. sichuanensis, *Neostagonosporella sichuanensis*, *Parakarstenia phyllostachydis*, *Phyllachora heterocladae*, *Podonectria sichuanensis*, *Arthrinium yunnanum* and *A. phyllostachium* have been reported (Yang et al. 2019a, b, c, d, e, f). Here, we introduce a new genus in order Chaetosphaeriales, which is a contribution to fungal diversity on *P. heteroclada*.

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Supplementary material 1

Figure S1

Authors: Xiu-Lan Xu, Chun-Lin Yang, Rajesh Jeewon, Dhanushka N. Wanasinghe, Ying-Gao Liu, Qian-Gang Xiao

Data type: multimedia

Link: https://doi.org/10.3897/mycokeys.70.54231.suppl1

Supplementary material 2

Figure S2

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Taxonomy and phylogenetic appraisal of *Spegazzinia musae* sp. nov. and *S. deightonii* (Didymosphaeriaceae, Pleosporales) on Musaceae from Thailand

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Abstract

Tropical plants host a range of fungal niches including endophytes, pathogens, epiphytes and saprobes. A study undertaken to discover the saprobic fungal species associated with *Musa* sp. (banana) from northern Thailand found two hyphomycetous taxa of *Spegazzinia* (Didymosphaeriaceae, Pleosporales). These were collected during the dry season and their morpho-molecular taxonomic relationships were investigated. Based on phylogenetic analysis of combined SSU, LSU, ITS and TEF1-α sequence data (77% ML, 0.99 BYPP) and contrasting morphological features to the sister taxon, we introduce *Spegazzinia musae* as a

novel species from a decaying leaf of *Musa* sp. Details on the taxonomy, ecology and geographical distribution of *Spegazzinia* species are provided. In addition, we report *S. deightonii* as a new host record from *Musa* sp. Our data further validate the taxonomic placement of *Spegazzinia* in Didymosphaeriaceae.

Keywords
Ascomycota, Dothideomycetes, fungi on banana, Hyphomycetes, Thai mycobiota

Introduction

Several taxonomic studies have been conducted to assess the saprobic fungal diversity in *Musa* species (Ellis 1971, 1976; Matsushima 1971; Photita et al. 2001b; Somrithipol 2007; Hernández-Restrepo et al. 2015; Crous et al. 2016; Hyde et al. 2017). Ellis (1971) described several species on *Musa* (i.e. *Arthrinium sacchari*, *Cladosporium musae*, *Cordana musae*, *Curvularia fallax*, *Deightoniella torulosa*, *Gliomastix elata*, *G. mutorum* var. *polychroma*, *G. musicola*, *Gyrothrix hughesii*, *Haplobasidion musae*, *Memnoniella subsimplex*, *Periconia digitata*, *P. lateralis*, *Periconiella musae*, *Pithomyces sacchari*, *Pyriculariopsis parasitica*, *Spegazzinia tessarthra*, *Stachylidium bicolor*, *Tetraploa aristata*, *Zygosporium gibbum*, *Z. masonii* and *Z. minus*). Ellis (1976) also described *Bidenticula cannae*, *Chlamydomyces palmarum*, *Cordana johnstonii*, *Parapyricularia musae* and *Veronaea musae* on *Musa* sp. Photita et al. (2001b) identified 46 saprobic fungal taxa from *Musa acuminata* in Hong Kong. Most of the saprobes reported by Photita et al. (2001b) belonged to the genera *Anthostomella*, *Deightoniella*, *Durispora*, *Hansfordia*, *Memnoniella*, *Nigrospora*, *Pyriculariopsis*, *Pseudopithomyces*, *Verticillium* and *Zygosporium*. In addition, *Dictyoarthrinium* (Somrithipol 2007) and *Ramichloridium* (Kirschner and Piepenbring 2014) were also recorded as saprobes on *Musa* sp. Considering the economic importance of *Musa* sp. there are not many studies on the saprobic fungal populations associated with this host. Few studies have molecular data for the identified strains. To address this research gap, we are investigating the saprobic fungal diversity of *Musa* sp. in the Asian region where the fungi are highly diverse (Hyde et al. 2018).

Spegazzinia was established by Saccardo (1880) based on *S. ornata*. Currently 17 taxa are listed in Species Fungorum (2020). Based on morphology, the genus was placed in Apiosporaceae (Sordariomycetes) by Hyde et al. (1998). Based on SSU, LSU, ITS and TEF1-α sequence data of *S. deightonii* and *S. tessarthra*, Tanaka et al. (2015) placed *Spegazzinia* in Didymosphaeriaceae (Dothideomycetes). This was supported by a phylogenetic analysis which placed *Spegazzinia* in a basal clade in Didymosphaeriaceae (Thambugala et al. 2017).

Hughes (1953) characterized *Spegazzinia* as a hypomycetous taxon with a unique basauxic conidiophore ontogeny (conidiophores that arise and elongate from a cup-shaped basal cell called a conidiophore mother cell). The conidia of *Spegazzinia* are brown to dark brown and dimorphic in most species, with a disc-shaped form and a stellate form (Ellis 1971; Manoharachary and Kunwar 2010). However, little molecular data for this genus is available in the GenBank (https://www.ncbi.nlm.nih.gov/). Therefore, for a better phylogenetic resolution of the genus in Didymosphaeriaceae,

the previously identified taxa should be recollected to obtain DNA sequence data and morphological descriptions.

In this present study, we introduce *Spegazzinia musae* sp. nov. and report the first occurrence of *Spegazzinia deightonii* from *Musa* sp. in Thailand. We provide detailed morphological descriptions, illustrations and molecular justification for the introduction of *Spegazzinia musae* sp. nov. Our molecular analyses further support the phylogenetic placement of *Spegazzinia* in Didymosphaeriaceae.

Materials and methods

Sample collection, morphological studies and isolation

Dead plant materials of *Musa* sp. (banana) were collected from Thailand during the dry season of 2018 to 2019. Specimens were transferred to the laboratory in cardboard boxes. Samples were examined with a Motic SMZ 168 Series microscope. Powdery masses of conidia were mounted in water for microscopic studies and photomicrography. The taxa were examined using a Nikon ECLIPSE 80i compound microscope and photographed with a Canon 550D digital camera fitted to the microscope. Measurements were made with the Tarosoft (R) Image Frame Work program and images used for figures processed with Adobe Photoshop CS3 Extended version 10.0 software (Adobe Systems, USA).

Single spore isolation was carried out following the method described in Chomnunti et al. (2014). Germinated spores were individually transferred to potato dextrose agar (PDA) plates and grown at 25 °C in daylight. Colony characteristics were observed and measured after 3 weeks. Specimens were deposited in the Mae Fah Luang University (**MFLU**) Herbarium, Chiang Rai, Thailand. Living cultures were deposited in the Culture Collection of Mae Fah Luang University (**MFLUCC**).

DNA extraction and PCR amplification

Fungal isolates were grown on PDA for 4 weeks at 25 °C and total genomic DNA was extracted from 50 to 100 mg of axenic mycelium of the growing cultures according to Wanasinghe et al. (2018). The mycelium was ground to a fine powder with liquid nitrogen and fungal DNA was extracted using the Biospin Fungus Genomic DNA Extraction Kit-BSC14S1 (BioFlux, P.R. China) according to the instructions of the manufacturer. Four gene regions, the internal transcribed spacer (ITS), partial 18S small subunit (SSU), partial 28S large subunit (LSU), and partial translation elongation factor 1-alpha gene (TEF1-α) were amplified using ITS5/ITS4 (White et al. 1990), NS1/NS4 (White et al. 1990), LR0R/LR5 (Vilgalys and Hester 1990) and EF1-983F/EF1-2218R (Rehner 2001) primers, respectively.

Polymerase chain reaction (PCR) was conducted according to the following protocol. The total volume of the PCR reaction was 25 μL containing 12.5 μL of 2 × Power

Taq PCR MasterMix (a premix and ready to use solution, including 0.1 Units/ μL Taq DNA Polymerase, 500 μm dNTP Mixture each (dATP, dCTP, dGTP, dTTP), 20 mM Tris-HCL pH 8.3, 100 mM KCl, 3 mM $MgCl_2$, stabilizer and enhancer), 1 μL of each primer (10 pM), 2 μL genomic DNA template and 8.5 μL double distilled water (ddH_2O). The reaction was conducted by running for 40 cycles. The annealing temperature was 56 °C for ITS and LSU, 57.2 °C for TEF1-α and 55 °C for SSU and initially 95 °C for 3 mins, denaturation at 95 °C for 30 seconds, annealing for 1 min, elongation at 72 °C for 30 seconds, and final extension at 72 °C for 10 mins for all gene regions. PCR amplification was confirmed on 1% agarose electrophoresis gels stained with ethidium bromide. The amplified PCR fragments were sent to a commercial sequencing provider (TsingKe Biological Technology (Beijing) Co., Ltd, China). The nucleotide sequence data acquired were deposited in GenBank.

Sequencing and sequence alignment

Obtained sequences were subjected to BLASTn search in GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi). BLASTn search results and initial morphological studies supported that our isolates belonged to Didymosphaeriaceae. Other sequences used in the analyses were obtained from GenBank based on recently published data (Tanaka et al. 2015; Jayasiri et al. 2019) (Table 1). The single gene alignments were automatically done by MAFFT v. 7.036 (http://mafft.cbrc.jp/alignment/server/index.html, Katoh et al. 2019) using the default settings and later refined where necessary, using BioEdit v. 7.0.5.2 (Hall 1999). The finalized alignment and tree were submitted to TreeBASE (submission ID: 25686, http://www.treebase.org/).

Phylogenetic analysis

Maximum likelihood (ML) trees were generated using the RAxML-HPC2 on XSEDE (8.2.8) (Stamatakis et al. 2008; Stamatakis 2014) in the CIPRES Science Gateway platform (Miller et al. 2010) using GTR+I+G model of evolution. Bootstrap support was obtained by running 1000 pseudo-replicates. Maximum likelihood bootstrap values (ML) equal or greater than 60% are given above each node in blue (Figure 1).

A Bayesian inference analysis was conducted with MrBayes v. 3.1.2 (Huelsenbeck and Ronquist 2001) to evaluate posterior probabilities (PP) (Rannala and Yang 1996; Zhaxybayeva and Gogarten 2002) by Markov chain Monte Carlo sampling (BMCMC). Two parallel runs were conducted, using the default settings, but with the following adjustments: four simultaneous Markov chains were run for 2,000,000 generations and trees were sampled every 100th generation and 20,000 trees were obtained. The first 4,000 trees, representing the burning phase of the analyses were discarded. The remaining 16,000 trees were used for calculating PP in the majority rule consensus tree. Branches with Bayesian posterior probabilities (BYPP) greater

Table 1. Taxa used in the phylogenetic analysis of *Spegazzinia* with the corresponding GenBank accession numbers. Type strains are superscripted with T and newly generated strains are indicated in bold.

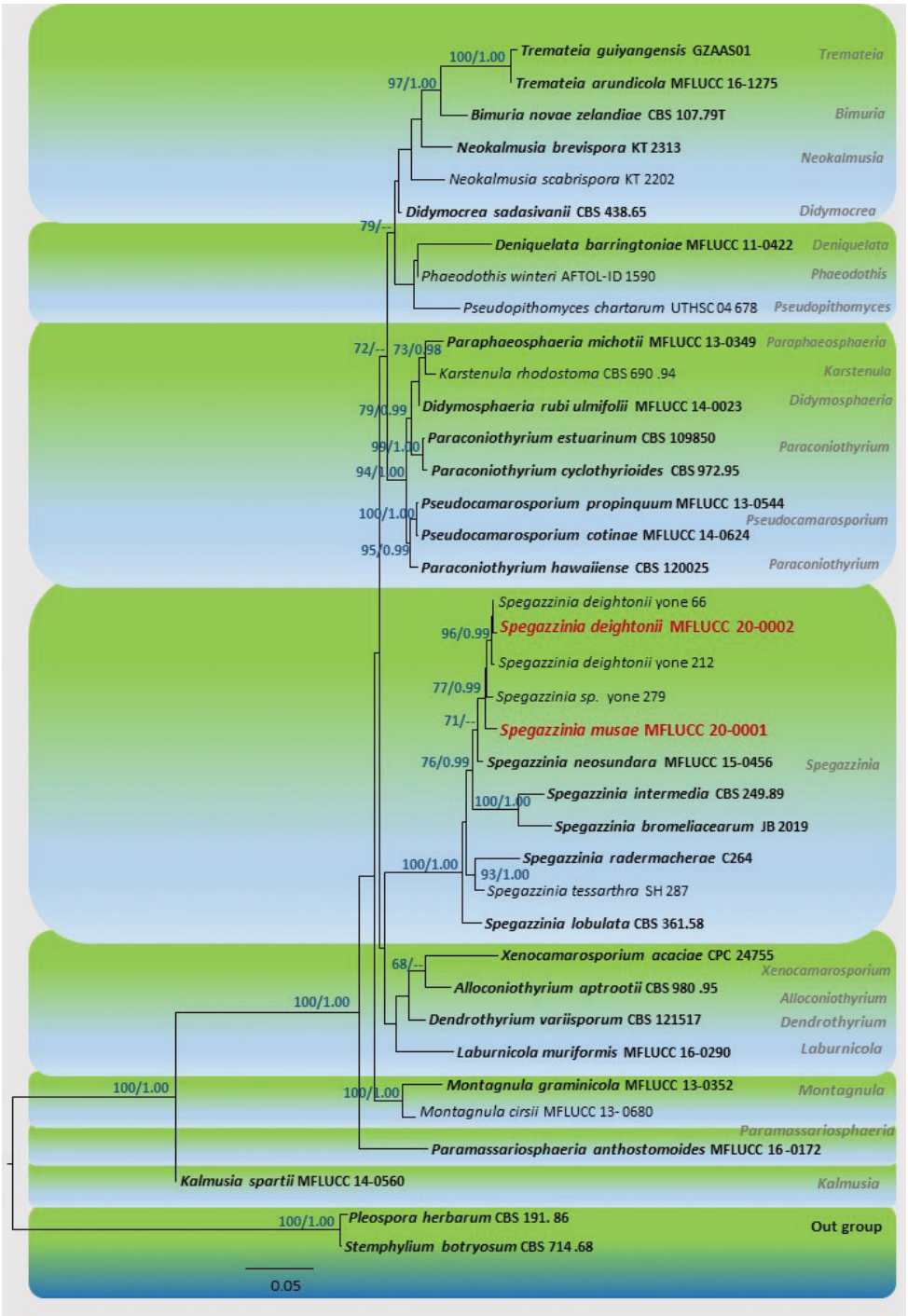
Species	Strains *	GenBank accession numbers				References
		LSU	SSU	ITS	TEF1-α	
Alloconiothyrium aptrootii	CBS 980.95^{T}	JX496234	–	JX496121	–	Verkley et al. (2014)
Bimuria novae zelandiae	CBS 107.79^{T}	AY016356	AY016338	–	–	Lumbsch and Lindemuth (2001)
Dendrothyrium variisporum	CBS 121517^{T}	JX496143	–	JX496030	–	Vu et al. (2019)
Deniquelata barringtoniae	MFLUCC 110422^{T}	JX254655	JX254656	JX254654	–	Ariyawansa et al. (2013)
Didymocrea sadasivanii	CBS 438.65^{T}	DQ384103	DQ384066	–	–	Vu et al. (2019)
Didymosphaeria rubi ulmifolii	MFLUCC 14-0023^{T}	KJ436586	KJ436588	–	–	Ariyawansa et al. (2014)
Kalmusia spartii	MFLUCC 14-0560^{T}	KP744487	KP753953	KP744441	–	Liu et al. (2015)
Karstenula rhodostoma	CBS 690.94	GU301821	GU296154	–	–	Schoch et al. (2009)
Laburnicola muriformis	MFLUCC 16-0290^{T}	KU743198	KU743199	KU743197	KU743213	Wanasinghe et al. (2016)
Montagnula cirsii	MFLUCC 13-0680^{T}	KX274249	KX274255	KX274242	KX284707	Hyde et al. (2016)
Montagnula graminicola	MFLUCC 13-0352^{T}	KM658315	KM658316	KM658314	–	Liu et al. (2015)
Neokalmusia brevispora	KT 2313^{T}	AB524601	AB524460	–	AB539113	Tanaka et al. (2009)
Neokalmusia scabrispora	KT 2202	AB524594	AB524453	–	AB539107	Tanaka et al. (2009)
Paracamarosporium hawaiiense	CBS 120025^{T}	JX496140	EU295655	JX496027	–	Verkley et al. (2014)
Paraconiothyrium cyclothyrioides	CBS 972.95^{T}	JX496232	AY642524	JX496119	–	Verkley et al. (2014)
Paraconiothyrium estuarinum	CBS 109850^{T}	JX496129	AY642522	JX496016	–	Verkley et al. (2014)
Paramassariosphaeria clematidicola	MFLU 16-0172^{T}	KU743207	KU743208	KU743206	–	Wanasinghe et al. (2016)
Paraphaeosphaeria michotii	MFLUCC 13-0349^{T}	KJ939282	KJ939285	KJ939279	–	Tennakoon et al. (2016)
Phaeodothis winteri	AFTOL-ID 1590	DQ678073	DQ678021	–	DQ677917	Schoch et al. (2006)
Pleospora herbarum	CBS 191.86^{T}	GU238160	GU238232	–	KC584731	Aveskamp et al. (2010)
Pseudocamarosporium cotinae	MFLUCC 14-0624^{T}	KP744505	KP753964	KP744460	–	Liu et al. (2015)
Pseudocamarosporium propinquum	MFLUCC 13-0544^{T}	KJ813280	KJ819949	KJ747049	–	Wijayawardene et al. (2014)
Pseudopithomyces chartarum	UTHSC 04-678	HG518065	–	HG518060	–	Da Cunha et al. (2014)
Spegazzinia bromeliacearum	URM 8084^{T}	MK809513	–	MK804501	–	Crous et al. (2019)
Spegazzinia deightonii	yone 212	AB807582	AB797292	–	AB808558	Tanaka et al. (2015)
Spegazzinia deightonii	**MFLUCC 20-0002**	**MN956772**	**MN956770**	**MN956768**	**MN927133**	This study
Spegazzinia deightonii	yone 66	AB807581	AB797291	–	AB808557	Tanaka et al. (2015)
Spegazzinia intermedia	CBS 249.89	MH873861	–	MH862171	–	Vu et al. (2019)
Spegazzinia lobulata	CBS 361.58	MH869344	–	MH857812	–	Vu et al. (2019)
Spegazzinia musae	**MFLUCC 20-0001^{T}**	**MN930514**	**MN930513**	**MN930512**	**MN927132**	This study
Spegazzinia neosundara	MFLUCC 15–0456^{T}	KX954397	KX986341	KX965728	–	Thambugala et al. (2017)
Spegazzinia radermacherae	MFLUCC 17-2285^{T}	NG_066308	MK347848	NR_163331	MK360088	Jayasiri et al. (2019)
Spegazzinia sp.	yone 279	AB807583	AB797293	–	AB808559	Tanaka et al. (2015)
Spegazzinia tessarthra	SH 287	AB807584	AB797294	–	AB808560	Tanaka et al. (2015)
Stemphylium botryosum	CBS 714.68^{T}	KC584345	KC584603	KC584238	KC584729	Woudenberg et al. (2013)
Tremateia arundicola	MFLU 16-1275^{T}	KX274248	KX274254	KX274241	KX284706	Tennakoon et al. (2016)
Tremateia guiyangensis	GZAAS01^{T}	KX274247	KX274253	KX274240	KX284705	Tennakoon et al. (2016)
Xenocamarosporium acaciae	CPC 24755^{T}	KR476759	–	KR476724	–	Tennakoon et al. (2016)

*Abbreviations of culture collections: **AFTOL-ID**: Assembling the Fungal Tree of Life, **CBS**: Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands, **CPC**: Working collection of Pedro Crous housed at CBS, **GZAAS**: Guizhou Academy of Agricultural Sciences herbarium, China, **KT**: K. Tanaka, **MFLU**: Mae Fah Luang University, Chiang Rai, Thailand, **MFLUCC**: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand, **SH**: Academia Sinica People's Republic of China. Shanghai, **URM**: Universidade Federal de Pernambuco, **UTHSC**: Fungus Testing Laboratory, University of Texas Health Science Center, San Antonio, Texas, USA, **Yone**: H. Yonezawa.

than 0.95 are indicated above each node in blue (Figure 1). Phylograms were visualized with FigTree v1.4.0 program (Rambaut 2011) and reorganized in Microsoft Power Point.

Data resources

The data underpinning the analysis reported in this paper are deposited in the Dryad Data Repository at https://doi.org/10.5061/dryad.2ngf1vhk6.


Trematosphaeria
Trematosphaeria guiyangensis GZAAS01
Trematosphaeria arundicola MFLUCC 16-1275
Bimuria
Bimuria novae zelandiae CBS 107.79T
Neokalmusia
Neokalmusia brevispora KT 2313
Neokalmusia scabrispora KT 2202
Didymocrea
Didymocrea sadasivanii CBS 438.65
Deniquelata
Deniquelata barringtoniae MFLUCC 11-0422
Phaeodothis
Phaeodothis winteri AFTOL-ID 1590
Pseudopithomyces
Pseudopithomyces chartarum UTHSC 04 678
Paraphaeosphaeria
Paraphaeosphaeria michotii MFLUCC 13-0349
Karstenula
Karstenula rhodostoma CBS 690 .94
Didymosphaeria
Didymosphaeria rubi ulmifolii MFLUCC 14-0023
Paraconiothyrium
Paraconiothyrium estuarinum CBS 109850
Paraconiothyrium cyclothyrioides CBS 972.95
Pseudocamarosporium
Pseudocamarosporium propinquum MFLUCC 13-0544
Pseudocamarosporium cotinae MFLUCC 14-0624
Paraconiothyrium
Paraconiothyrium hawaiiense CBS 120025
Spegazzinia deightonii yone 66
Spegazzinia deightonii MFLUCC 20-0002
Spegazzinia deightonii yone 212
Spegazzinia sp. yone 279
Spegazzinia musae MFLUCC 20-0001
Spegazzinia
Spegazzinia neosundara MFLUCC 15-0456
Spegazzinia intermedia CBS 249.89
Spegazzinia bromeliacearum JB 2019
Spegazzinia radermacherae C264
Spegazzinia tessarthra SH 287
Spegazzinia lobulata CBS 361.58
Xenocamarosporium
Xenocamarosporium acaciae CPC 24755
Alloconiothyrium
Alloconiothyrium aptrootii CBS 980 .95
Dendrothyrium
Dendrothyrium variisporum CBS 121517
Laburnicola
Laburnicola muriformis MFLUCC 16-0290
Montagnula
Montagnula graminicola MFLUCC 13-0352
Montagnula cirsii MFLUCC 13- 0680
Paramassariosphaeria
Paramassariosphaeria anthostomoides MFLUCC 16 -0172
Kalmusia
Kalmusia spartii MFLUCC 14-0560
Out group
Pleospora herbarum CBS 191. 86
Stemphylium botryosum CBS 714 .68
0.05

Results

Phylogenetic analysis

The combined SSU, LSU, ITS, TEF1-α matrix comprised 38 sequences including selected genera in Didymosphaeriaceae. A best scoring RAxML tree is shown in Figure 1. All trees (ML and BYPP) were similar in topology and did not differ (data not shown) at the generic relationships which are in agreement with multi-gene phylogeny of Tanaka et al. (2015). All *Spegazzinia* strains analyzed here were clustered as a highly supported monophyletic clade (100% ML, 1.00 BYPP) in Didymosphaeriaceae (Figure 1) sister to *Alloconiothyrium*, *Dendrothyrium*, *Laburnicola* and *Xenocamarosporium*. Our new species, *Spegazzinia musae* (MFLUCC 20-0001) clustered with *Spegazzinia* sp. (yone 279) and *S. deightonii* (yone 66, MFLUCC 20-0002, yone 212) with significant statistical support (77% ML, 0.99 BYPP). Strain MFLUCC 20-0002 grouped with *S. deightonii* (yone 66, yone 212) with high statistical support (96% ML, 0.99 BYPP).

Taxonomy

***Spegazzinia deightonii* (S. Hughes) Subram., J. Indian bot. Soc. 35: 78 (1956)**
Facesoffungi Number: FoF07238
Figure 2

Description. *Saprobic* on dead leaves of *Musa* sp. **Sexual morph** Undetermined. **Asexual morph** Hyphomycetous. *Sporodochia* powder like, dark, dense, dry, 1–3 mm diameter. *Conidiophore mother cells* 3.5–6.8 × 2.5–5.0 µm ($\bar{x}$ = 5.59 × 4.15 µm, n = 6), hyaline to light brown, subspherical or doliiform. *Conidiophores* long or short and give rise to two types of conidia referred here as α and β. *Conidiophores of a conidia* up to 48–120 × 1–2 µm ($\bar{x}$ = 95.3 × 1.6 µm, n = 20) long, erect or flexuous, narrow, verrucu-

Figure 1. Maximum likelihood tree revealed by RAxML from an analysis of SSU, LSU and ITS and TEF1-α sequence data of selected genera of family Didymosphaeriaceae, showing the phylogenetic position of *Spegazzinia musae* (MFLUCC 20-0001) and *S. deightonii* (MFLUCC 20-0002). ML bootstrap supports (≥60 %) and Bayesian posterior probabilities (≥ 0.95 BYPP) are given above in the branches, respectively. The tree was rooted with *Pleospora herbarum* and *Stemphylium botryosum* (Pleosporaceae). Strains generated in this study are indicated in red-bold. Ex-type species are indicated in bold. The scale bar represents the expected number of nucleotide substitutions per site. A best scoring RAxML tree is shown with a final ML optimization likelihood value of -13516.66. The matrix had 795 distinct alignment patterns, with 33.60% of undetermined characters or gaps. Estimated base frequencies were: A = 0.239862, C = 0.245185, G = 0.277025, T = 0.237927; substitution rates AC = 1.626982, AG = 2.468452, AT = 1.211822, CG = 1.092437, CT = 6.295657, GT = 1.000000; proportion of invariable sites I = 0.484119; gamma distribution shape parameter α = 0.445929.

Figure 2. *Spegazzinia deightonii* (MFLU 19-2908) **a–c** fungal colonies on host surface **d** conidiophore mother cell of α conidia **e–g** α conidia **i** a developmental stage of β conidia **h, k** conidia **l** colonies on PDA after 28 days showing sporulation **j, m–p** β conidia. Scale bars: 500μm (**a**), 200μm (**b**), 50 μm (**c**), 20μm (**e–h**), 10μm (**d, k, m–p**), 5 μm (**i, j**).

lose, unbranched, hyaline to golden-brown. *Conidiophores of β conidia* initially hyaline, light brown to brown at maturity, very short and slightly bent, 1.6–2 × 2.5–3 µm ($\bar{x}$ = 1.8 × 2.6 µm, n =10). *Conidiogenous cell development* basauxic, forming a single, terminal holoblastic conidium at the apex of conidiophore. *Conidial development* holoblastic. *Conidia* two types: α *conidia* stellate, 18–28 × 17–29 µm ($\bar{x}$ = 25.1 × 23.3 µm, n = 25), solitary, globose to variously shaped, with spines 4–6 µm long, 4–8-celled, frequently 4- to 6-celled, deeply constricted at the septa. β *conidia* disc-shaped, initially hyaline, light brown to dark brown at maturity, 8-celled, 16–21 × 11–14 µm ($\bar{x}$ = 19.2 × 14.6 µm, n = 25), flat from both sides with short and blunt spines, frequently with attached conidiogenous cells when splitting from the conidiophores.

Culture characteristics. Conidia germinating on PDA within 13–14 h. Colonies growing on PDA, reaching a diameter of 55 mm after 14 d at 25 °C, raised, moderately dense, undulate margin, middle grey, periphery brownish grey and olive green at immature stage; reverse white to greyish white.

Material examined. Thailand, Chiang Rai Province, Doi Thun, on a dead leaf of *Musa* sp. (Musaceae), 7 December 2018, M.C. Samarakoon, BNS 072 (MFLU 19-2908), living culture MFLUCC 20-0002.

Notes. *Spegazzinia deightonii* MFLUCC 20-0002 clustered with *S. deightonii* (yone 66, yone 212) with significant statistical support (Figure 1). All the strains of *S. deightonii* described in Ellis (1971) and Tanaka et al. (2015) have similar morphological features with our strain such as dark brown, 8-celled, disked-shaped, spiny conidia. With morphological and multigene phylogenetic support, we report a new host record of *S. deightonii* from *Musa* sp.

Spegazzinia musae **Samarakoon, Phookamsak, Wanas., Chomnunti & K.D. Hyde, sp. nov.**
MycoBank No: 835298
Facesoffungi Number: FoF07237
Figure 3

Etymology. The name reflects the host genus, *Musa* (Musaceae).

Holotype. MFLU 19-2907

Description. *Saprobic* on a dead leaf of *Musa* sp. **Sexual morph** Undetermined. **Asexual morph** Hyphomycetous. *Sporodochia* dark, dense, dry, powdery, velvety, 1–2 mm diameter. *Conidiophore mother cells* 3.4–5.8 × 3.7–4.7 µm ($\bar{x}$ = 4.6 × 4.1 µm, n = 10) subhyaline or light brown, doliiform or subspherical. *Conidiophores* usually short to long bearing two types of conidia referred to here as α and β. *Conidiophores* of α *conidia* up to 40–85 × 0.8–2.5 µm ($\bar{x}$ = 64 × 21.7 µm, n = 15), pale brown or dark golden brown, rough-walled, hyaline at bottom near the conidiophore mother cell, pale brown at middle, dark golden brown at top near conidial cells, erect or flexuous, narrow and long, generally unbranched, rarely branched. *Conidiophores* of β *conidia* 0.7–3.5 × 1.5–3 µm ($\bar{x}$ = 1.9 × 2.3 µm, n = 15) short, erect, unbranched, hyaline

Figure 3. *Spegazzinia musae* (MFLU 19-2907, holotype) **a–c** fungal colonies on host surface **d** mature conidia **e** conidiophore of α conidia with the mother cell **f, g** α conidia **h–q** β conidia **r** colony on PDA after 28 days. Scale bars: 200 µm (**a–c**), 20 µm (**d–g, j**), 10 µm (**h, i, k–q**).

when immature, subhyaline or hyaline at maturity. *Conidiogenous cell development* basauxic, forming a single, terminal holoblastic conidium at the apex of conidiophore. *Conidial development* holoblastic. *Conidia* solitary, dry, two types: α *conidia* stellate,

15–22.7 × 14.5–20.5 µm ($\bar{x}$ = 18.8 × 17.8 µm, n = 15), 4–6 celled, each cell globose to subglobose, deeply constricted at the septa, conspicuously spinulate, 4–6 spines, each 2–8 µm long arise from surface of each cell. β *conidia* disc-shaped, initially hyaline, 4-celled, each cell slightly turbinate in shape, rough-walled, crossed septate, becoming brown to dark brown at maturity, each cell turbinate, crossed-septate, smooth-walled, light brown at the center near the septa, dark brown at periphery in constricted areas, 9.3–14.2 × 8.4–12.5 µm ($\bar{x}$ = 12.7 × 10.8 µm, n = 40), somewhat obovoid, deeply constricted at the septa, flat from side view, frequently with attached conidiogenous cells when splitting from the conidiophores.

Culture characteristics. Conidia germinating on PDA within 12–15 h, germ tubes produced from one or several cells. Colonies growing on PDA, reaching a diameter of 46 mm after 14 d at 25 °C, greyish white, unevenly raised, surface rough, moderately dense, radially striated at center, margin crenulate; reverse white to greyish white.

Material examined. Thailand, Nan Province, on a dead leaf of *Musa* sp. (Musaceae), 12 September 2018, B.C. Samarakoon, BNS 069 (MFLU 19-2907, **holotype**), ex-type living culture MFLUCC 20-0001.

Notes. Based on BLASTn search results of SSU, LSU, ITS and TEF1-α sequence data, *Spegazzinia musae* showed a high similarity (SSU = 98.24%, LSU = 98.92%, ITS = 96.91%, TEF1-α = 98.11%) to *S. neosundara* (MFLUCC 15-0456). In the multigene phylogeny, *S. musae* groups as a sister taxon to *S. deightonii* with strong statistical support (77% ML, 0.99 BYPP) (Figure 1). Also, ITS sequence comparison revealed 3.75% base pair differences between *S. musae* and *S. deightonii*, which is in agreement with the species concept outlined by Jeewon and Hyde (2016). Besides, *S. musae* has contrasting morphological features to *S. deightonii* in both kinds of conidia. The disk-shaped conidia of *S. musae* are 4-celled and do not bear spines at the periphery of cells, while the disc-shaped conidia of *S. deightonii* are 8-celled and spiny. Based on contrasting morphological differences and significant statistical support from our molecular phylogeny, *Spegazzinia musae* is introduced as a new species.

Discussion

Spegazzinia is ubiquitous in the environment. Several taxa of *Spegazzinia* occur as saprobes on dead material of tropical, subtropical and temperate vascular plants (Ellis 1971; Subramanian 1988; Caretta et al. 1999; Delgado-Rodríguez et al. 2002; Bhat 2010; Leão-Ferreira and Gusmão 2010; Manoharachary and Kunwar 2010). In addition, *Spegazzinia* was also recorded from soil (Ellis 1971), dredged sediments of marine and brackish estuaries (Borut and Johnson 1962) and grassland vegetation (Caretta et al. 1999). *Spegazzinia tessarthra* was recorded as an endophyte from lichens (Manish et al. 2014) and recently *S. bromeliacearum* was introduced as an endophyte from the leaves of *Tilandsia catimbauensis* (Crous et al. 2019). Damon (1953) considered *S. tessarthra* to be an important decomposer of monocotyledonous plants

and other cellulose containing materials in tropical and subtropical areas. *Spegazzinia deightonii* was previously recorded on monocotyledons such as *Areca catechu* (China, Taiwan; Matsushima 1980), *Cocos nucifera* (China; Tianyu et al. 2009) and *Panicum maximum* (Hong Kong; Lu et al. 2000) (Farr and Rossman 2020). Our study presents the first report of *Spegazzinia deightonii* in Musaceae as a saprobe and introduces our new species, *S. musae*.

There does not appear to be any host-specificity as the genus is found on a wide range of hosts in various habitats and there are no records of a pathogenic lifestyle. Some *Spegazzinia* species (such as *S. tessarthra*) have been identified as saprobes and endophytes and therefore the genus may have the potential of switching nutritional modes during the degradation of plant material (Promputtha et al. 2007).

Spegazzinia is a unique taxon among other dematiaceous hyphomycetes due to its conidial morphology and basauxic conidiogenesis. Most *Spegazzinia* species have contrasting morphological features in the shapes of α and β conidia. Some taxa bear spines in both types of conidia while some taxa do not bear spines. Simultaneously, some species of *Spegazzinia* such as *S. radermacherae*, *S. tessarthra* show similar characters in morphology apart from dimensions of conidia. The length of conidiophores can be varied with the environmental stresses (Cole 1974). Therefore, the use of morphological data coupled with DNA sequence data (SSU, LSU, ITS and TEF-α) will be crucial for better taxonomic resolutions in this genus.

Dictyoarthrinium (Apiosporaceae) bears some similar morphological features to *Spegazzinia* such as basauxic conidiogenesis (Ellis 1971) and cross septate, 4-celled, dematiaceous conidia with warts (Rao and Rao 1964). However, generic placement of *Dictyoarthrinium* in Apiosporaceae was confirmed by Vu et al. (2019) based on the LSU sequence of *D. sacchari* strain CBS 529.73. Therefore, *Dictyoarthrinium* was treated as a distinct genus with *Spegazzinia* (Vu et al. 2019).

Microfungal studies in *Musa* sp. are mostly oriented towards pathogens and endophytes due to the economic value of the fruit crop. Most of the pathogenic species descriptively studied from *Musa* sp. are identified as *Colletotrichum*, *Fusarium*, *Mycosphaerella*, *Neocordana* and *Phyllosticta* (Giatgong 1980; Wulandari et al. 2010; Churchill 2011; Guarnaccia et al. 2017; Marin-Felix et al. 2019; Maryani et al. 2019). The endophytic fungal populations of *Musa* sp. were studied by Brown et al. (1998), Photita et al. (2001a, 2004) and Samarakoon et al. (2019). Few studies have documented the saprobic diversity of *Musa* sp. and as we believe that there are saprobic niches associated with *Musa* sp. that are still unrevealed, taxonomists should investigate this hidden diversity for conservation purposes.

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RESEARCH ARTICLE

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A new lichenized fungus, *Lecanora baekdudaeganensis*, from South Korea, with a taxonomic key for Korean *Lecanora* species

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Abstract

Lecanora baekdudaeganensis Lee & Hur is described as a new lichenized fungus from Baekdudaegan Mountains, South Korea. The new species is classified into the *Lecanora subfusca* group – *allophana* type and distinguishable from *Lecanora imshaugii* Brodo by a darker thallus, brownish disc, K–insoluble granules on the surface of the epihymenium, shorter hypothecium, and the presence of oil droplets in the apothecial section. Molecular analyses employing internal transcribed spacer (ITS) and mitochondrial small subunit (mtSSU) sequences strongly support *Lecanora baekdudaeganensis* as a distinct species in the genus *Lecanora*. A surrogate key is provided to assist in the identification of all 52 taxa in the genus *Lecanora* of Korea.

Keywords

biodiversity, Lecanoraceae, phorophyte, phylogeny, taxonomy

Introduction

The Baekdudaegan Mountains are the main mountain range stretching across the entire Korean Peninsula. The mountains stretch 1,400 km in length from North Korea to South Korea and encompass protected areas of approximately 2,750 km^2 (Korea Forest Service 2019). The Baekdudaegan Mountains, as the main mountain system for whole mountainous areas comprising 70 percent of Korea, are almost totally covered with forest and support a productive ecosystem for specialists as well as generalists,

represented by 27 percent endemic vascular plants (Korea Forest Service 2019) and 20 percent endemic lichens/lichenicolous fungi.

Although the genus *Lecanora* is one of the largest genera in lichens, just three new species in *Lecanora* were formerly discovered out of all 164 lichenized or lichenicolous fungi which were reported as new species from Korea. Specifically, all three species, *L. hafelliana* L. Lü, Y. Joshi & Hur, *L. loekoesii* L. Lü, Y. Joshi & Hur, and *L. pseudosambuci* S.Y. Kondr., Lőkös & Hur were detected from the bark of *Quercus* or other deciduous trees in the Baekdudaegan mountains or other mountainous areas in North Korea and South Korea (Lü et al. 2011; Kondratyuk et al. 2016) (Fig. 1).

This study describes a new lichenized fungus in the genus *Lecanora*. During three field trips to Mt. Munsu, Bonghwa in 2019 (Fig. 1), four specimens were collected but identified just to genus without matching any previously known species. We describe them below as a new corticolous lichen species, *Lecanora baekdudaeganensis*, and this discovery contributes to the taxonomy with overall 52 taxa in the genus *Lecanora* from North Korea and South Korea. All specimens are deposited in the herbarium of the Baekdudaegan National Arboretum (BDNA), South Korea.

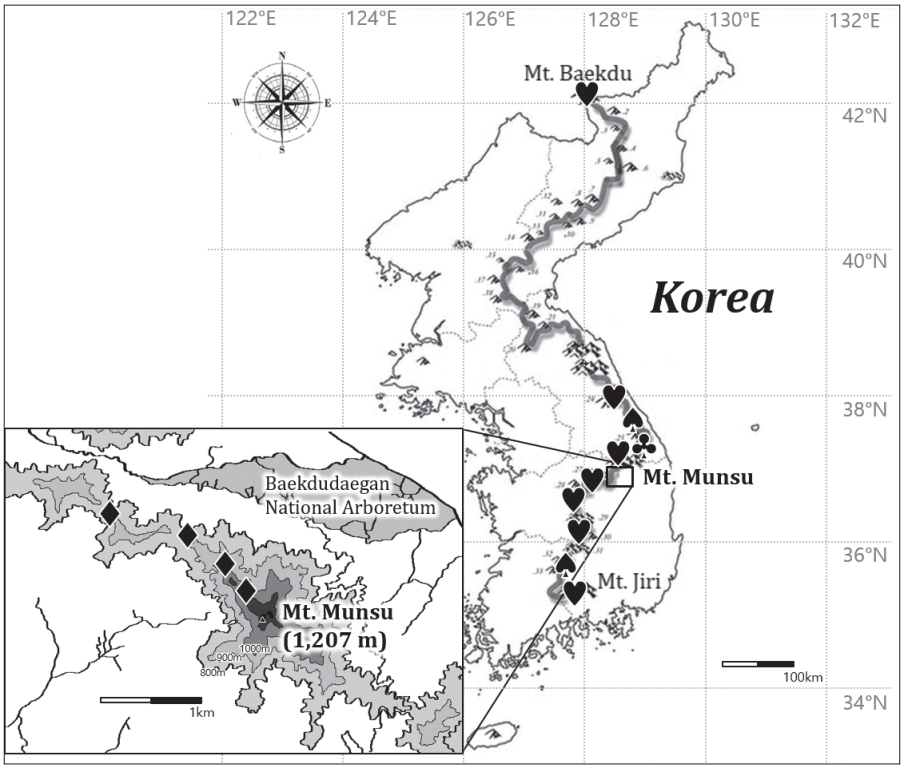

Figure 1. Specific collection sites (black symbols) on the Baekdudaegan Mountains (thick gray line on the entire Korea map), for the new species *Lecanora baekdudaeganensis* (♦), and previously discovered species *L. hafelliana* (♥), *L. loekoesii* (♣), and *L. pseudosambuci* (♠). All *Lecanora* species reported as new species were detected in the Baekdudaegan Mountains or other mountainous areas just close to the mountains in Korea.

Materials and methods

Morphological and chemical analyses

Hand-cut sections were prepared with a razor blade under a stereomicroscope (Olympus optical SZ51; Olympus, Tokyo, Japan), examined under a compound microscope (Nikon Eclipse E400; Nikon, Tokyo, Japan) and imaged using a software program (AxioVision Release 4.8.2; Carl Zeiss, Jena, Germany) and an Axiocam ERc 5s camera (Carl Zeiss, Jena, Germany) mounted on a Zeiss Axioscope A1 microscope (Carl Zeiss, Jena, Germany). The ascospores were investigated at 1000× magnification in water. The length and width of the ascospores were measured and the range of spore sizes was shown with average, standard deviation, and number of measured spores. Thin-layer chromatography (TLC) was performed using solvent systems A and C according to standard methods (Orange et al. 2001).

Isolation, DNA extraction, amplification, and sequencing

Hand-cut sections of ascomata or thallus from all collected specimens were prepared for DNA isolation and DNA was extracted with a NucleoSpin Plant II Kit in line with the manufacturer's instructions (Macherey-Nagel, Düren, Germany). PCR amplification for the internal transcribed spacer region (ITS1-5.8S-ITS2 rDNA) and the mitochondrial small subunit genes was achieved using Bioneer's AccuPower PCR Premix (Bioneer, Daejeon, Korea) in 20-µL tubes and primers ITS5 and ITS4 (White et al. 1990), and mrSSU1 and mrSSU3R (Zoller et al. 1999), respectively. The PCR thermal cycling parameters used were 95 °C (15 sec), followed by 35 cycles of 95 °C (45 sec), 54 °C (45 sec), and 72 °C (1 min), and a final extension at 72 °C (7 min) based on Ekman (2001). DNA sequences were generated by the genomic research company GenoTech (Daejeon, Korea).

Phylogenetic analyses

All ITS and mtSSU sequences were aligned and edited manually using ClustalW in Bioedit V7.2.6.1 (Hall 1999). All missing and ambiguously aligned data and parsimony-uninformative positions were removed and only parsimony-informative regions were finally analyzed in MEGA X (Stecher et al. 2020). The final alignment comprised 564 (ITS) and 1032 (mtSSU) columns. In them, variable regions were 51 (ITS) and 100 (mtSSU). Finally, the phylogenetically informative regions were 359 (ITS) and 464 (mtSSU). Phylogenetic trees with bootstrap values were obtained in RAxML GUI 2.0 beta (Edler et al. 2019) using the maximum likelihood method with a rapid bootstrap with 1000 bootstrap replications and GTR GAMMA for the substitution matrix. The posterior probabilities were obtained in BEAUti 1.8.0

and BEAST 1.8.0 (Drummond et al. 2012) using the HKY (Hasegawa, Kishino and Yano) model, as the appropriate model of nucleotide substitution based on the Bayesian Information Criterion (BIC) (Schwarz 1978) as evaluated by bModelTest (Bouchaert and Drummond 2017), empirical base frequencies, gamma for the site heterogeneity model, four categories for gamma, and a 10,000,000 Markov chain Monte Carlo chain length with a 10,000-echo state screening and 1000 log parameters. Then, a consensus tree was constructed in TreeAnnotator 1.8.0 (Drummond and Rambaut 2007) with a burn-in of 5000, no posterior probability limit, a maximum clade credibility tree for the target tree type, and median node heights. All trees were displayed in FigTree 1.4.2 (Rambaut 2014) and edited in Microsoft Paint. The bootstrapping and Bayesian analyses were repeated three times for the result consistency and no significant differences were shown for the tree shapes and branch values. The phylogenetic trees and DNA sequence alignments are deposited in TreeBASE under the study ID 25859.

Results and discussion

Phylogenetic analyses

Two independent phylogenetic trees for the *Lecanora subfusca* group and related species were produced from 122 sequences (61 for ITS, and 61 for mtSSU) from GenBank and with two new sequences (each one for ITS and mtSSU) for the new species (Table 1). The new species was positioned in the *L. subfusca* group in both ITS and mtSSU trees. In the ITS tree, the new species was located in a clade with *L. achroa* Nyl., *L. allophana* (Ach.) Nyl., *L. cinereofusca* H. Magn., *L. horiza* (Ach.) Röhl., *L. layana* Lendemer, *L. saxigena* Lendemer & R.C. Harris and *L. tropica* Zahlbr. (Fig. 2). All species including the new species were in the *L. subfusca* group except for *L. layana* which was nevertheless the most closely located to the new species, represented by a bootstrap value of 89 and a posterior probability of 100 for the branch. Many other species, including *L. imshaugii* in the *L. subfusca* group, were positioned in different clades and our results did not reveal any close species in the *L. subfusca* group to the new species. In the mtSSU tree, the new species is located in a clade with *L. allophana*, *L. cenisia* Ach., *L. expersa* Nyl., *L. farinaria* Borrer, *L. horiza*, *L. imshaugii*, *L. layana*, *L. paramerae* I. Martínez, Aragón & Lumbsch, *L. pulicaris* (Pers.) Ach., *L. substerilis* Malíček & Vondrák, *L. tropica*, and *L. vainioi* Vänskä (Fig. 3). All species including the new species were in the *L. subfusca* group except for *L. layana*. Except for *L. layana*, a sorediate species (Lendemer 2015), *L. imshaugii* was the most closely positioned with the new species, represented by a bootstrap value of 90 and a posterior probability of 100 for the branch. Our analysis did not represent any species identical to the new species in the *L. subfusca* group.

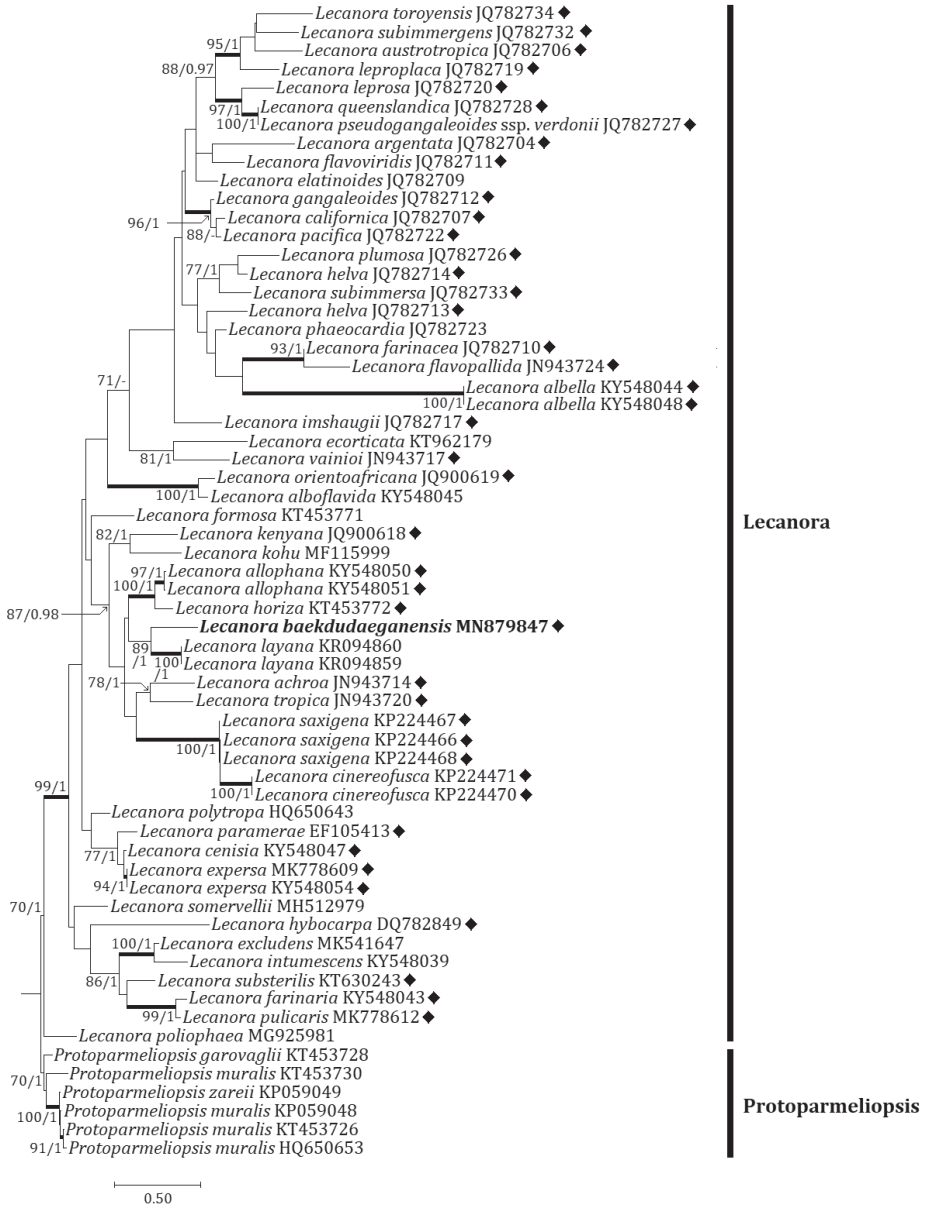

Figure 2. Phylogenetic relationships among comparable species related mainly with the *Lecanora subfusca* group based on a maximum likelihood analysis of the nuclear ribosomal ITS1-5.8S-ITS2 region. The tree was rooted with several sequences in the genus *Protoparmeliopsis*. Maximum likelihood bootstrap values ≥ 70% and posterior probabilities ≥ 95% are shown above internal branches. Branches with bootstrap values ≥ 90% are shown in bold. The new species *Lecanora baekdudaeganensis* is presented in bold, and all species names are followed by GenBank accession numbers. A dash indicates branches with posterior probabilities <95%. The *Lecanora subfusca* group is marked with a black diamond (♦). Reference Table 1 provides the GenBank accession numbers for the included species and voucher information.

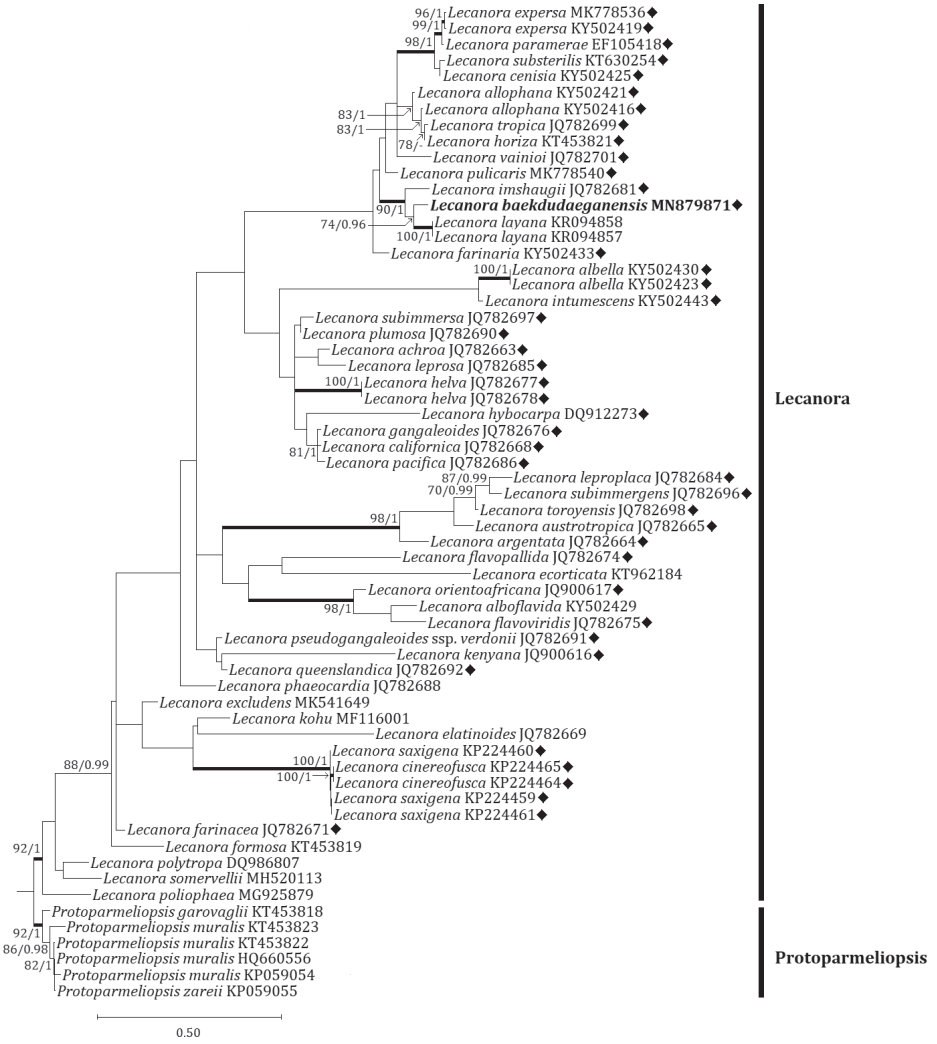

Figure 3. Phylogenetic relationships among comparable species related mainly with the *Lecanora subfusca* group based on a maximum likelihood analysis of the mitochondrial small subunit (mtSSU) sequences. The tree was rooted with several sequences in the genus *Protoparmeliopsis*. Maximum likelihood bootstrap values ≥ 70% and posterior probabilities ≥ 95% are shown above internal branches. Branches with bootstrap values ≥ 90% are shown in bold. The new species *Lecanora baekdudaeganensis* is presented in bold, and all species names are followed by GenBank accession numbers. A dash indicates branches with posterior probabilities <95%. The *Lecanora subfusca* group is marked with a black diamond (♦). Reference Table 1 provides the GenBank accession numbers for the included species and voucher information

Table 1. Species list and DNA sequence information employed for phylogenetic analysis.

No.	Species	ID (ITS)	ID (mtSSU)	Voucher
1	*Lecanora achroa*	JN943714	JQ782663	Papong 6458
2	*Lecanora albella*	KY548044	KY502430	Berger 29362
3	*Lecanora albella*	KY548048	KY502423	Malicek 7336
4	*Lecanora alboflavida*	KY548045	KY502429	Coppins s.n.
5	*Lecanora allophana*	KY548050	KY502421	Malicek 9626
6	*Lecanora allophana*	KY548051	KY502416	Malicek 9491
7	*Lecanora argentata*	JQ782704	JQ782664	Papong 6041(F)
8	*Lecanora austrotropica*	JQ782706	JQ782665	Papong 6407(F)
9	***Lecanora baekdudaeganensis***	**MN879847**	**MN879871**	**BDNA-L-0000065**
10	*Lecanora californica*	JQ782707	JQ782668	Lumbsch 19914a(F)
11	*Lecanora cenisia*	KY548047	KY502425	Malicek 5869
12	*Lecanora cinereofusca*	KP224470	KP224465	Lendemer 34944 (NY)
13	*Lecanora cinereofusca*	KP224471	KP224464	Lendemer 35007 (NY)
14	*Lecanora ecorticata*	KT962179	KT962184	NMW<GBR>:C.2015.005.77
15	*Lecanora elatinoides*	JQ782709	JQ782669	Lumbsch 19992d(F)
16	*Lecanora excludens*	MK541647	MK541649	Palice 21929
17	*Lecanora expersa*	KY548054	KY502419	Malicek 9625
18	*Lecanora expersa*	MK778609	MK778536	Vondrak 16033 (PRA)
19	*Lecanora farinacea*	JQ782710	JQ782671	Lumbsch 20022d(F)
20	*Lecanora farinaria*	KY548043	KY502433	Tonsberg 46170
21	*Lecanora flavopallida*	JN943724	JQ782674	Lumbsch 20031a
22	*Lecanora flavoviridis*	JQ782711	JQ782675	Papong 6539(F)
23	*Lecanora formosa*	KT453771	KT453819	ZX 20129045-2
24	*Lecanora gangaleoides*	JQ782712	JQ782676	Lumbsch 19923a(F)
25	*Lecanora helva*	JQ782713	JQ782677	Lumbsch 19809h(F)
26	*Lecanora helva*	JQ782714	JQ782678	Lumbsch 19843b(F)
27	*Lecanora horiza*	KT453772	KT453821	Zhao 2015
28	*Lecanora hybocarpa*	DQ782849	DQ912273	AFTOL-ID 639
29	*Lecanora imshaugii*	JQ782717	JQ782681	Lumbsch 19273b(F)
30	*Lecanora intumescens*	KY548039	KY502443	Malicek 8203
31	*Lecanora kenyana*	JQ900618	JQ900616	Kirika 1179 (F)
32	*Lecanora kohu*	MF115999	MF116001	UNITEC 7497
33	*Lecanora layana*	KR094859	KR094857	Lendemer 37519 (NY)
34	*Lecanora layana*	KR094860	KR094858	Lendemer 38131 (NY)
35	*Lecanora leproplaca*	JQ782719	JQ782684	Lumbsch 19815r(F)
36	*Lecanora leprosa*	JQ782720	JQ782685	Papong 6443(F)
37	*Lecanora orientoafricana*	JQ900619	JQ900617	Kirika 2205(F)
38	*Lecanora pacifica*	JQ782722	JQ782686	Lumbsch 19901c(F)
39	*Lecanora paramerae*	EF105413	EF105418	Lumbsch s.n. (F)
40	*Lecanora phaeocardia*	JQ782723	JQ782688	Papong 3473(F)
41	*Lecanora plumosa*	JQ782726	JQ782690	Papong 6965(F)
42	*Lecanora poliophaea*	MG925981	MG925879	O:L 200460
43	*Lecanora polytropa*	HQ650643	DQ986807	AFTOL-ID 1798
44	*Lecanora pseudogangaleoides* subsp. verdonii	JQ782727	JQ782691	Lumbsch 19103a(F)
45	*Lecanora pulicaris*	MK778612	MK778540	Malicek 10263
46	*Lecanora queenslandica*	JQ782728	JQ782692	Lumbsch 19113j(F)
47	*Lecanora saxigena*	KP224466	KP224459	Lendemer 32825 (NY)
48	*Lecanora saxigena*	KP224467	KP224460	Lendemer 25832 (NY)
49	*Lecanora saxigena*	KP224468	KP224461	Lendemer 33186 (NY)
50	*Lecanora somervellii*	MH512979	MH520113	YO 10109

No.	Species	ID (ITS)	ID (mtSSU)	Voucher
51	*Lecanora subimmergens*	JQ782732	JQ782696	Papong 6431(F)
52	*Lecanora subimmersa*	JQ782733	JQ782697	Lumbsch 19103b(F)
53	*Lecanora substerilis*	KT630243	KT630254	Malicek 202
54	*Lecanora toroyensis*	JQ782734	JQ782698	Papong 7197(F)
55	*Lecanora tropica*	JN943720	JQ782699	Papong 6440
56	*Lecanora vainioi*	JN943717	JQ782701	Papong 6957
57	*Protoparmeliopsis garovaglii*	KT453728	KT453818	Leavitt 089 (BRY-C)
58	*Protoparmeliopsis muralis*	HQ650653	HQ660556	Schmull s. n.
59	*Protoparmeliopsis muralis*	KP059048	KP059054	SK 765
60	*Protoparmeliopsis muralis*	KT453726	KT453822	Leavitt 143 (BRY-C)
61	*Protoparmeliopsis muralis*	KT453730	KT453823	Vondrak 9413
62	*Protoparmeliopsis zareii*	KP059049	KP059055	SK 480
	Overall	**62**	**62**	

DNA sequences for the new species *Lecanora baekdudaeganensis* (in bold) were generated in this study. All others were obtained from GenBank. The species names are followed by GenBank accession numbers and voucher information. ITS, internal transcribed spacer; mtSSU, mitochondrial small subunit; Voucher, voucher information.

Taxonomy

Lecanora baekdudaeganensis **B.G. Lee & J-.S. Hur, sp. nov.**
MycoBank No: 833845
Fig. 4

Diagnosis. *Lecanora baekdudaeganensis* differs from *L. imshaugii* by a darker thallus (bluish, olivish, or pale brownish gray vs. greenish or yellowish gray), brownish disc (vs. reddish brown disc), K-insoluble granules on the surface of epihymenium (vs. absence of granules), shorter hypothecium (15–25 μm vs. 50–75 μm), and the presence of oil droplets in the apothecial section.

Type. South Korea, North Gyeongsang Province, Bonghwa-gun, Chunyang-myeon, Mt. Munsu, 36°59.41'N, 128°48.24'E, 1,005 m alt., on bark of *Quercus mongolica* Fisch. ex Ledeb., 29 August 2019, B.G.Lee 2019-000065 (holotype: BDNA-L-0000065!; GenBank MN879847 for ITS and MN879871 for mtSSU); South Korea, North Gyeongsang Province, Bonghwa-gun, Chunyang-myeon, Mt. Munsu, 37°0.31'N, 128°47.39'E, ca 900 m alt., on bark of *Quercus dentata* Thunb., 26 September 2019, B.G.Lee 2019-000135 (paratype: BDNA-L-0000135); South Korea, North Gyeongsang Province, Bonghwa-gun, Chunyang-myeon, Mt. Munsu, 36°59.82'N, 128°46.81'E, 970 m alt., on bark of *Quercus mongolica*, 26 August 2019, B.G.Lee 2019-000147 (paratype: BDNA-L-0000147); South Korea, North Gyeongsang Province, Bonghwa-gun, Chunyang-myeon, Mt. Munsu, 36°59.35'N, 128°46.12'E, ca 1,075 m alt., on bark of *Quercus mongolica*, 26 August 2019, B.G.Lee 2019-000151 (paratype: BDNA-L-0000151).

Description. Thallus corticolous, crustose, without lobes, continuous or cracked, rimose to areolate or verruculose, usually rounded or irregular, bluish gray in the beginning (margin) and olivish– or pale brownish–gray when mature (center), not pruinose, 30–70 mm diam., 100–170 μm thick; cortex hyaline to pale yellow or pale brown,

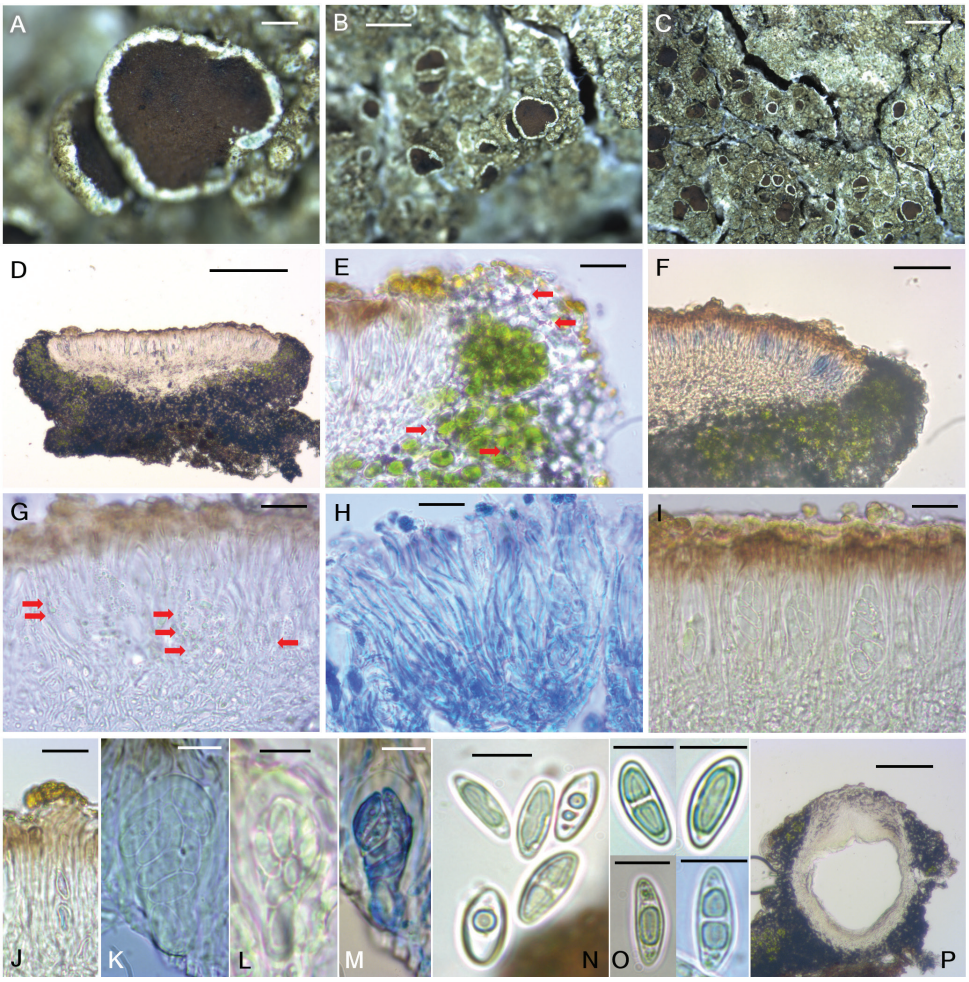

Figure 4. *Lecanora baekdudaeganensis* (BDNA-L-0000065, holotype) in morphology. **A–C** Habitus with dark thallus and epruinose apothecia **D** sessile apothecia with constricted base in section. Hypothecial base closed by medulla of amphithecium **E** well-developed amphithecium with small calcium oxalate crystals (red arrows) not dissolving in KOH **F** apothecial section in Iodine. I– reaction in the beginning then turning slowly to blue or purple–blue hymenium **G** oil droplets (red arrows) present in the apothecial section **H** anastomosing paraphyses shown in Lactophenol cotton blue **I** asci in hymenium **J** epihymenium with yellowish granules not dissolving in KOH **K–M** 8-spored, clavate asci (**M** in Lactophenol cotton blue) **N, O** ellipsoid ascospores in diverse development stages. Spores biguttulate in the beginning then having a long oil drop by assembly of guttules when mature **P** old pycnidia without pycnocodidia. Scale bars: 200 µm (**A, D**), 2 mm (**B, C**), 20 µm (**E, G–J**), 50 µm (**F**), 10 µm (**K–O**), 100 µm (**P**).

5–10 µm thick; medulla 20–75 µm thick; photobiont coccoid, forming a distinct algal layer, 45–80 µm thick, cells globose, 8.5–17 × 8–15 µm. Prothallus absent.

Apothecia abundant, rounded, smaller and scattered around the margin and larger and aggregated in the center, constricted at the base, 0.2–1.6 mm diam. Disc flat to

slightly concave, not pruinose, brown to dark brown from the beginning, 270–430 μm thick; margin persistent, prominent, generally entire or slightly flexuous, some a little crenulate when old, concolorous to thallus. Amphithecium well-developed, with numerous small crystals in both algal-containing and cortical parts (*allophana*-type) not dissolving in K, 60–100 μm thick laterally, 110–130 μm thick basally; amphithecial cortex distinct, 7–12 μm thick. Parathecium hyaline, indistinct in water, 15–25 μm thick in I. Epihymenium pale yellowish brown to pale brown, with small granules on the surface not dissolving in K, pigment slightly paler in K but not diluted, without oil droplets, 5–15 μm high. Hymenium hyaline, 50–75 μm high. Subhymenium hyaline, 20–40 μm high. Hypothecium hyaline, coarsely prosoplectenchymatous (periclinal) in the lower and marginal parts and prosoplectenchymatous (irregular) in the upper and central parts, 15–25 μm high. Oil droplets present in hypothecium, subhymenium and the base of hymenium. Hypothecial base not extending or a little extending to the substrate and always closed by medulla of amphithecium. Paraphyses septate, anastomosing, 1–2.5 μm wide, simple or sparsely branched at tips but not, or only slightly, swollen, 2.5–4 μm. Asci clavate, 8-spored, 41–51 × 13–20 μm (n = 10). Ascospores simple, often biguttulate in the beginning then having an oval-shaped oil drop by assembly of guttules when mature, narrowly or widely ellipsoid, or eye-shaped, 10–18.5 × 4.5–9.5 μm (mean = 15.2 × 6.5; SD = 1.58 (L), 1.10 (W); n = 128), wall ca 0.5 μm thick when exist. Pycnidia only once detected, pale brown at tip, ovoid, 315 × 330 μm, without conidia as old.

Chemistry. Thallus K+ yellow, KC+ yellow, C–, Pd–. Hymenium I– in the beginning but turning slowly blue or purple–blue, KI+ blue (reaction mainly starting from tholus then the whole ascus), C–, Pd–. UV–. Atranorin, zeorin and an unidentified minor constituent (Rf classes A3 and C3 in Culberson's standardized thin layer chromatography method (Culberson 1972)), UV– before heating, spot color slightly pale yellow-orange after heating, and UV+ pale pink-orange after heating) were detected by TLC.

Distribution and ecology. The species occurs on the bark of *Quercus mongolica* and *Q. dentata* which are the most dominant tree species on the mountain. This species is currently known from four different sites on the mountain.

Etymology. The species epithet indicates the lichen's geography, namely the main mountains called Baekdudaegan stretching from north to south in the entire Korean Peninsula.

Notes. The new species is classified to the *Lecanora subfusca* group – *allophana* type, representing the main characteristics of a crustose thallus without lobes containing atranorin as a major constituent, small calcium oxalate crystals in both algal-containing and cortical parts of the amphithecium, and trebouxioid photobionts in the thalline margin, dark brown discs, and colorless ellipsoid simple spores in the range of 10–20 × 6–9 μm (Brodo 1984; Miyawaki 1988; Lumbsch 1995; Lumbsch et al. 2003). The new species is compared with *Lecanora chionocarpa* Hue, *L. horiza*, *L. imshaugii*, *L. japonica* Müll. Arg., and *L. megalocheila* (Hue) H. Miyaw., as those species are in the *L. subfusca* group with only small crystals in the amphithecium (*allophana*

or campestris type) which is defined by the main characteristics such as K+ yellow thallus reaction (containing atranorin), small calcium oxalate crystals in algal-containing and/or cortical parts of amphithecium, and ascospores in the size of 10–20 × 6–9 µm (Hue 1915; Brodo 1984; Miyawaki 1988; Smith et al. 2009). The new species is most similar to *L. imshaugii* by a continuous, rimose, verruculose or areolate thallus, the absence of soredia, the absence of a prothallus, apothecia size, and ascospore size (Brodo 1984). However, *Lecanora baekdudaeganensis* differs from *L. imshaugii* by a darker thallus (bluish, olivish, or pale brownish gray vs. greenish or yellowish gray), brownish disc (vs. reddish brown disc), K–insoluble granules on the surface of epihymenium (vs. absence of granules), a shorter hypothecium (15–25 µm vs. 50–75 µm), and the presence of oil droplets in the apothecial section (Brodo 1984).

The new species is distinguishable from *L. chionocarpa* by a darker thallus (bluish, olivish or pale brownish gray vs. ash gray), the absence of a prothallus (vs. presence of white prothallus), crystals in the amphithecium not dissolving in K (vs. granular crystals dissolving in K), a shorter hymenium (50–75 µm vs. 75–100 µm), the presence of oil droplets in hypothecium, subhymenium and the base of hymenium (vs. oil droplets present in epihymenium), shorter asci (41–51 × 13–20 µm vs. 60–70 × 13–18 µm), smaller ascospores (10–18.5 × 4.5–9.5 µm vs. 15–20 × 8–11 µm), thinner ascospore walls (0.5 µm vs. 0.5–1 µm), and the Pd– reaction of the thallus and medulla (vs. Pd+ yellowish) (Hue 1915; Miyawaki 1988).

The new species differs from *L. horiza* by a darker thallus (bluish, olivish or pale brownish gray vs. yellowish white to whitish gray), and the crystals in amphithecium not dissolving in K (vs. crystals dissolving in K) (Smith et al. 2009).

The new species differs from *L. japonica* by thallus color (bluish, olivish or pale brownish gray vs. dirty greenish to ashy gray), the absence of a prothallus (vs. prothallus with white bundle of hyphae), larger apothecia (0.2–1.6 mm vs. up to 1 mm), a thicker amphithecium (60–100 µm laterally and 110–130 µm basally vs. 5–20 µm laterally and 20–50 µm basally), the presence of oil droplets in hypothecium, subhymenium and the base of hymenium (vs. oil droplets present in epihymenium), shorter hymenium (50–75 µm vs. 70–80 µm), a shorter subhymenium (20–40 µm vs. 180–220 µm), a granular epihymenium (vs. non-granular epihymenium), shorter (41–51 µm) and constantly 8-spored asci (vs. longer (50–80 µm) and 8- or 16-spored asci), the Pd– reaction of the thallus and medulla (vs. Pd+ pale brown thallus and medulla), and the absence of chloroatranorin (vs. presence of chloroatranorin) (Nylander 1891; Miyawaki 1988; Guderley and Lumbsch 1999).

The new species differs from *L. megalocheila* by a darker thallus (bluish, olivish or pale brownish gray vs. whitish gray or whitish with green tinge without brownish color), the absence of a prothallus (vs. blackish prothallus), crystals in the amphithecium not dissolving in K (vs. crystals dissolving in K), a shallower hypothecium (15–25 µm vs. 120–150 µm), wider asci (41–51 × 13–20 µm vs. 35–50 × 10–14 µm), larger ascospores (10–18.5 × 4.5–9.5 µm vs. 10–14 × 5–8 µm), , and the Pd– reaction of the thallus and medulla (vs. Pd+ pale yellow thallus and medulla) (Hue 1915; Miyawaki 1988).

Key to the species in *Lecanora* of Korea (52 taxa)

Overall, 56 species have been recorded in the genus *Lecanora* from Korea (i.e., South Korea (55 spp.) and North Korea (6 spp.) with sharing five species from both countries). However, four of these species are excluded in the key. *Lecanora fusanii* Hue is regarded as a *Caloplaca* species because *L. fusanii* (syn. *Caloplaca fusanii* (Hue) Zahlbr.) has yellow thalli, orange discs, and polarilocular ascospores (Hue 1915). *Lecanora subrugosa* Nyl. is identical to *L. argentata* (Ach.) Röhl. based on a molecular analysis (Malíček 2014). *Lecanora vulnerata* Hue (syn. *Caloplaca vulnerata* (Hue) Zahlbr.) is supposed to be classified into the family Teloschistaceae because *L. vulnerata* was compared with *L. heppiana* (Müll. Arg.) Hue as a quite similar species, and the former differs from the latter mainly by presence of soredia and KOH reaction (Hue 1915). The latter is classified in the family Teloschistaceae as a *Variospora* species at present (Arup et al. 2013). *Lecanora muralis* (Schreb.) Rabenh., a lobed species, is excluded from the key as it is classified into the genus *Protoparmeliopsis*. However, one species, *L. confusa* Almb., is included in the list as the species was discovered in North Korea. A further five species from North Korea, i.e., *L. chionocarpa*, *L. megalocheila*, *L. polytropa* (Ehrh.) Rabenh., *L. rubina* (Hoffm.) Ach., and *L. subrubra* Hue (syn. *L. japonica*), were previously discovered in South Korea as well.

1 Thallus saxicolous or lignicolous **2**
– Thallus corticolous **31**
2 Thallus lobate or sublobate **3**
– Thallus not lobed **5**
3 Disc dark, ruby-colored ..... ***L. rubina***
– Disc light-colored, pale pink, greenish brown to yellow–brown **4**
4 Thallus usually areolate or sometimes sublobate, paraphyses tips swollen up to 3 μm wide, conidia 20–25 × 1 μm, thallus Pd+ orange ..... ***L. albescens* (*Myriolecis albescens*)**
– Thallus lobate, resetting, paraphyses tips hardly swollen, conidia absent, thallus Pd– ..... ***L. valesiaca***
5 Thallus inconspicuous, immersed or with dispersed areoles, ascospores 10–14 × 5–6.5 μm, thallus UV– ..... ***L. polytropa***
– Thallus clearly visible **6**
6 On calcareous rocks or wood/logs **7**
– On non-calcareous rocks **9**
7 Common on wood/logs, thalline margin excluded finally ..... ***L. anopta***
– Only on calcareous rocks, thalline margin persistent **8**
8 Thallus starkly white or pale gray, apothecia 0.1–0.7 mm diam., thallus Pd+ orange ..... ***L. albescens* (*Myriolecis albescens*)**
– Thallus gray to blackened, apothecia 0.5–1.4 mm diam., thallus Pd– ..... ***L. semipallida* (*Myriolecis semipallida*)**
9 Disc pruinose **10**
– Disc not pruinose **13**

10 Prothallus green–black, thalline margin ±excluded, disc densely gray pruinose, epihymenium green– or blue–gray, containing zeorin, ±gangaleoidin, and usnic acid ..... ***L. sulphurea***

– Prothallus whitish, thalline margin persistent, containing small or large crystals, disc slightly or faintly pruinose, epihymenium brownish, containing ±chloratranorin **11**

11 Thalline margin with small, irregular crystals (<10 µm diam.) not dissolving in K, thallus white to yellow–white, K+ indistinct yellow ..... ***L. horiza***

– Thalline margin with large crystals (>10 µm diam.), thallus grayish, yellowish or brownish, K+ yellow or yellow turning to red **12**

12 Thallus not glossy, apothecia 1–2 mm diam., disc yellow–brown, red–brown to black, ascospores 9–15 × 6–8.5 µm, containing ±roccellic acid and norstictic acid ..... ***L. cenisia***

– Thallus somewhat glossy, apothecia 0.4–0.7 mm diam., disc waxy or pale to greenish orange, ascospores 8–11.5 × 4–6.5 µm ..... ***L. plumosa***

13 Disc blackish, epihymenium bluish or greenish, not reddish, orangish or brownish **14**

– Disc yellowish to brownish, epihymenium yellowish to brownish **16**

14 Thallus grayish white to white, prothallus blackish when present, ascospores smaller, 7–13 × 4–6.5 µm, thallus Pd+ yellow, medulla Pd+ pale yellow .... ***L. oreinoides***

– Thallus green–yellow to yellowish, prothallus absent, ascospores larger, 11–15 × 6–9 µm, thallus Pd– **15**

15 Thallus not shining, apothecia 0.7–4 mm diam., disc not shining, hymenium 80–110 µm high, ascospores 11–15 × 6–9 µm ..... ***L. decorata***

– Thallus somewhat shining and waxy, apothecia ca 0.5 mm diam., disc shining, hymenium 55–65 µm high, ascospores 11–13 × 6–7 µm .. ***L. marginata***

16 Disc pale to green–brown or black–green **17**

– Disc pale brown, red–brown to dark brown **19**

17 Thallus somewhat glossy, prothallus whitish to whitish gray when present, apothecia 0.4–0.7 mm diam., disc waxy, pale to greenish orange, ascospores 8–11.5 × 4–6.5 µm, thallus Pd+ pale orange, containing atranorin and chloratranorin ..... ***L. plumosa***

– Thallus not glossy, prothallus black when present, apothecia 0.3–1 mm diam., disc not waxy, pale yellow to greenish brown or greenish black, ascospores 10–14 × 5–7 µm, thallus Pd–, containing usnic acid and zeorin **18**

18 Thallus continuous and well-developed, disc green–brown to green–black, epihymenium green–brown to brown, hymenium 60–70 µm high, pycnoconidia 23–25 × 0.5–1 µm, thallus UV+ dull orange ..... ***L. intricata***

– Thallus inconspicuous with dispersed areoles, disc pale yellow to pale brown, epihymenium hyaline to yellow– or red–brown, hymenium 45–60 µm high, pycnoconidia 18–22 ×1 µm, thallus UV–, containing rangiformic acid and ±eulecanoral ..... ***L. polytropa***

19 Thallus richly sorediate, disc dark brown and shiny..... ***L. ussuriensis***
– Thallus not sorediate, disc not shiny **20**
20 Thallus pale to medium yellow or yellow–green, not white or gray .....
.....***L. frustulosa***
– Thallus pale to white, gray or dark gray.....**21**
21 Apothecia smaller, up to ca 0.5 mm diam. **22**
– Apothecia larger, 0.5–2.5 mm diam. **23**
22 Thallus medium to dark gray, epihymenium yellow to brown, ascospores 9–15 × 4–6 µm, often guttulate and appearing 1-septate, reaction all negative else epihymenium K+ yellow .....***L. helicopis***
– Thallus grayish white, epihymenium brownish red, ascospores 12–13 × 5–6 µm, thallus K+ pale yellow, epihymenium K–..... ***L. subimmersa***
23 Prothallus whitish **24**
– Prothallus absent.....**28**
24 Thallus pale, gray to dark gray, thalline margin with irregular or large crystals.....**25**
– Thallus white, grayish white or yellowish white, thalline margin with small crystals.....**27**
25 Ascospores narrower, 10–15 × 5–7 µm, thallus Pd–.....***L. subimmergens***
– Ascospores wider, 9–15 × 6–8.5 µm, thallus Pd+ weakly yellow or yellow turning to red **26**
26 Epihymenium pale orange to red–brown without granules, paraphyses tips red–brown, asci 50–60 × 12–21 µm, containing zeorin .....***L. campestris***
– Epihymenium brown to olivaceous brown with coarse granules dissolving in K, paraphyses tips olivaceous, asci 45–50 × 7–9 µm, containing ±roccellic acid and ±norstictic acid.....***L. cenisia***
27 Disc orange, red–orange to red–brown, thalline margin with small irregular crystals not dissolving in K, hypothecium without oil droplets, thallus Pd–, containing ±chloratranorin..... ***L. horiza***
– Disc brown to dark brown, thalline margin with small and large crystals dissolving in K, hypothecium inspersed with oil droplets, thallus Pd+ light orange, containing zeorin .....***L. melacarpella***
28 Ascospores smaller, 8–12 × 4.5–5 µm ..... ***L. orientalis***
– Ascospores larger, 9–15 × 5–8.5 µm.....**29**
29 Thalline margin with only large crystals not dissolving in K, thallus yellowish gray to whitish gray, ascospores narrower, 9–15 × 5–7 µm, prothallus absent...
.....***L. pseudistera***
– Thalline margin with small crystals, thallus whitish to grayish white, ascospores wider, 10.5–15 × 6.5–8.5 µm, prothallus white when present.....**30**

30 Disc orange, red–orange or red–brown, thalline margin with small irregular crystals not dissolving in K, ascospores 12–15 × 6.5–8.5 µm, thallus K+ indistinct yellow, Pd–, containing ±chloratranorin ..... ***L. horiza***

– Disc brown to dark brown, thalline margin with small and large crystals dissolving in K, ascospores 10.5–13.5 × 7.5–8.5 µm, thallus K+ yellow, Pd+ light orange, containing zeorin..... ***L. melacarpella***

31 Thallus sorediate **32**

– Thallus not sorediate **39**

32 Apothecia absent or rarely seen **33**

– Apothecia present **35**

33 Thallus UV–, apothecia not seen, containing stictic acid..... ***L. layana***

– Thallus UV+ pale orange or ice blue, apothecia rarely seen..... **34**

34 Thallus pale gray, prothallus pale gray, apothecia not pruinose, ascospores 7–10 × 3–4 µm, thallus Pd± yellow, UV+ pale orange, containing chloratranorin..... ***L. barkmaniana***

– Thallus yellow to greenish, occasionally with blue or gray tints, prothallus white and fibrous, often with one or two blue–gray zones, apothecia faintly or heavily white pruinose, ascospores 11–14 × 6–9 µm, thallus Pd–, UV+ ice blue (or violet), containing usnic acid and porphyrilic acid ..... ***L. thysanophora***

35 Apothecia pruinose **36**

– Apothecia not pruinose..... **38**

36 Ascospores smaller, 11–14 × 6–9 µm ..... ***L. thysanophora***

– Ascospores larger, 15–24 × 7–12 µm..... **37**

37 Thallus white or yellowish white, asci 8- or 6-spored, ascospores 15–22 × 7–12 µm..... ***L. pachycheila***

– Thallus yellowish–blue green, asci 8-spored, ascospores 15–20 × 9–12 µm ... . ***L. sibirica***

38 Thallus pale gray, apothecia 0.4–0.7 mm diam., ascospores 7–10 × 3–4 µm . ..... ***L. barkmaniana***

– Thallus yellowish gray to greenish gray, apothecia 0.2–0.6 mm diam., ascospores 12–15.5 × 6–8.5 µm, containing chodatin, demethlchodatin, and thiophanic acid..... ***L. leproplaca***

39 Prothallus distinct, whitish, grayish or blackish..... **40**

– Prothallus indistinct or absent..... **47**

40 Prothallus whitish or grayish, but not blackish..... **41**

– Prothallus blackish..... **44**

41 Thalline margin with large crystals (> 10 µm diam.) not dissolving in K... **42**

– Thalline margin with small crystals (< 10 µm diam.)..... **43**

42 Apothecia pale orange to yellowish brown, sometimes slightly pruinose on disc, epihymenium hyaline to yellow–brown with numerous small crystals

(*chlarotera*-type), ascospores 9–13 × 5–7 μm, containing gangaleoidin, chloratranorin, chlorolecideoidin, leoidin, and norgangaleoidin ..... ***L. leprosa***

– Apothecia dark reddish brown to brownish black, not pruinose, epihymenium reddish brown with fine brown granules (*pulicaris*-type), ascospores 11–14 × 6–8 μm, containing fumarprotocetraric acid, ±roccellic acid ..... ***L. pulicaris***

43 Subhymenium 60–80 μm high, asci consistently 8-spored, ascospores 15–20 × 8–11 μm, pycnidia brown–black with pycnoconidia 20–25 × 0.5 μm, containing zeorin ..... ***L. chionocarpa***

– Subhymenium 180–220 μm high, asci 8-spored or 16-spored, ascospores 12–16 × 6–8 μm, pycnidia absent ..... ***L. japonica***

44 Thalline margin with granular crystals dissolving in K ..... ***L. megalocheila***

– Thalline margin with large crystals not dissolving in K..... **45**

45 Disc paler, orange–brown or pale red–brown, flat to slightly convex, epihymenium inspersed with coarse granules, thallus Pd–, containing pannarin, ±placodialic acid, and ±roccellic acid ..... ***L. cinereofusca***

– Disc darker, dark reddish brown to brownish black, flat to slightly concave, epihymenium without granules, thallus Pd+ faintly yellow **46**

46 Paraphyses tips reddish brown (or faintly yellow), asci wider, 45–55 × 18–22 μm, ascospores larger, 11.5–14.5 × 6–8.5 μm, containing gangaleoidin and usually traces of californin ..... ***L. argentata***

– Paraphyses tips dark brown, asci narrower, 50–60 × 8–12 μm, ascospores smaller, 9–14 × 5–8 μm, containing zeorin ..... ***L. iseana***

47 Thalline margin finally excluded **48**

– Thalline margin permanent..... **51**

48 Disc pruinose, thallus not corticate, containing decarboxysquamatic acid ..... ***L. strobilina***

– Disc not pruinose, thallus corticate, decarboxysquamatic acid absent..... **49**

49 Asci 16- or 32-spored, thallus C–, K–, KC–, containing no substance ..... ***L. sambuci***

– Asci 8-spored, thallus C± orange, KC± yellow to orange, containing usnic acid, zeorin and xanthones..... **50**

50 Thallus yellow–green to gray–green, disc pale yellow to greenish, when young the exciple crenulate and containing algae..... ***L. confusa***

– Thallus yellowish–white to greenish black, disc pinkish brown to greenish black, when young the exciple smooth and lacking algae ..... ***L. symmicta***

51 Disc and epihymenium darker, brown, red–brown to dark brown, not pruinose **52**

– Disc or epihymenium paler, pinkish, pale orangish, green–brown, yellow–brown, orange–brown to pale red–brown, pruinose or not..... **57**

52 Thalline margin with small crystals (*allophana*-type) not dissolving in K... **53**

– Thalline margin with large crystals (*pulicaris*-type) **54**

53 Thallus darker, bluish, olivish or pale brownish gray, disc brownish, amphithecial cortex present, epihymenium with granules on the surface not dissolving in K, hypothecium shorter, 15–25 µm high, oil droplets present in apothecial section..... ***L. baekdudaeganensis***

– Thallus paler, greenish or yellowish gray, disc reddish brown, amphithecial cortex indistinct or absent, epihymenium without granules, hypothecium taller, 50–75 µm high, oil droplets absent ..... ***L. imshaugii***

54 Epihymenium without granules, ascospores 10.5–16.5 × 6–9.5 µm..... **55**

– Epihymenium with coarse, hyaline to brown granules (*chlarotera*-type), ascospores 8–12 × 4.5–7 µm..... **56**

55 Apothecia smaller, 0.4–0.8 mm diam., ascospores 11.5–14.5 × 6–8.5 µm, thallus Pd+ weakly yellow, containing gangaleoidin ..... ***L. argentata***

– Apothecia larger, up to 1.6 mm diam., ascospores 10.5–16.5 × 6–9.5 µm, thallus Pd–, containing zeorin..... ***L. perplexa***

56 Apothecia not constricted at base, disc slightly convex when mature, hymenium including subhymenium 80–100 µm high, hypothecium 120–150 µm high, asci 40–50 × 7–12 µm, ascospores 8–10 × 4.5–6 µm, pycnidia absent, thallus Pd+ orange ..... ***L. nipponica***

– Apothecia slightly constricted at base, disc extremely convex when mature, hymenium including subhymenium 150–200 µm high, hypothecium 70–110 µm high, asci 50–60 × 10–15 µm, ascospores 9–12 × 6–7 µm, pycnidia present, thallus Pd– ..... ***L. sulcata***

57 Asci 12- or 16-spored..... ***L. loekoesii***

– Asci 8-spored **58**

58 Ascospores smaller, 7–10 × 4–5 µm, thallus C–, K–, KC– (or KC+ yellow), Pd–..... ***L. saligna***

– Ascospores larger, 9–15 × 5–9.5 µm, thallus K+ yellow, Pd+ yellow–orange to orange..... **59**

59 Ascospores 9–11.5 × 5–7 µm, thallus K+ weakly yellow, Pd+ sulphur yellow, thallus areoles somewhat shiny, containing psoromic acid and usnic acid..... ..... ***L. varia***

– Ascospores 9–15 × 5–9.5 µm, thallus K+ distinct yellow or yellow turning to red, Pd+ pale yellow to yellow orange, thallus not shiny..... **60**

60 Thalline margin with numerous small crystals (*allophana*-type), containing ±stictic acid **61**

– Thalline margin with large crystals not dissolving in K (*pulicaris*-type)..... **62**

61 Disc carneous to pinkish, flat to convex, heavily pruinose, epihymenium gray–brown, thallus K+ yellow or yellow turning to red, Pd+ orange, containing chloratranorin, ±norstictic acid, ±protocetraric acid, ±virensic acid, ±connorstictic acid, ±conprotocetraric acid, and ±salazinic acid .... ***L. caesiorubella***

– Disc yellowish brown to dark reddish brown, flat to slightly concave, slightly pruinose, epihymenium dark brown to blackish brown, thallus K+ yellow,

Pd+ pale yellow, containing hafellic acid, zeorin, and usnic acid..... ..... ***L. hafelliana***

62 Thallus pale to medium gray, apothecia larger, 0.7–1.5 mm diam., disc not pruinose, epihymenium red–brown, paraphyses anastomosed, ascospores larger, 10–14.5 × 7–8.5 µm, containing pannarin and ±placodialic acid..... ..... ***L. cinereofusca***

– Thallus yellow–white to yellow gray, apothecia smaller, 0.2–1 mm diam., disc slightly pruinose, epihymenium pale to red–brown, paraphyses sparsely branched, ascospores smaller, 9–13.5 × 5–7 µm..... **63**

63 Apothecia 0.5–1 mm diam., prothallus absent, thallus Pd–, containing ±roccellic acid and ±fatty acid ..... ***L. hybocarpa***

– Apothecia 0.2–0.7 mm diam., prothallus gray when present, thallus Pd+ pale orange, containing gangaleoidin, chloratranorin, chlorolecideoidin, leoidin, and norgangaleoidin ..... ***L. leprosa***

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RESEARCH ARTICLE

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Additions to Phaeosphaeriaceae (Pleosporales): *Elongaticollum* gen. nov., *Ophiosphaerella taiwanensis* sp. nov., *Phaeosphaeriopsis beaucarneae* sp. nov. and a new host record of *Neosetophoma poaceicola* from Musaceae

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Abstract

A novel ascomycetous genus, *Elongaticollum*, occurring on leaf litter of *Hedychium coronarium* (Zingiberaceae) in Taiwan, is described and illustrated. *Elongaticollum* is characterized by dark brown to black, superficial, obpyriform, pycnidial conidiomata with a distinct elongate neck, and oval to oblong, hyaline, aseptate conidia. Phylogenetic analyses (maximum likelihood, maximum parsimony and Bayesian) of combined ITS, LSU, SSU and *tef1-α* sequence data revealed *Elongaticollum* as a distinct genus within the family Phaeosphaeriaceae with high statistical support. In addition, *Ophiosphaerella taiwanensis* and *Phaeosphaeriopsis beaucarneae* are described as new species from dead leaves of *Agave tequilana* and *Beaucarnea recurvata* (Asparagaceae), respectively. *Neosetophoma poaceicola* is reported as a new host record from dead leaves of *Musa acuminata* (Musaceae). Newly described taxa are compared with other similar species and comprehensive descriptions and micrographs are provided.

Keywords
Asparagaceae, Dothideomycetes, leaf litter, new taxa, Zingiberaceae

Introduction

Plant litter is considered as one of the main contributors to net above-ground primary productivity of terrestrial ecosystems (Swift et al. 1979; Berg and McClaugherty 2008; Krishna and Mohan 2017). Since plant litter is returned back to the soil, it represents a major source of organic carbon in forest soils (Berg 2003). Plant litter can be defined as a collection of fallen leaves, twigs, seeds and other woody debris that accumulate on the ground as a natural part of the forest ecosystem (Johnson and Catley 2002; Berg and McClaugherty 2008). In particular, leaf litter is the main source of organic matter and nutrients of the soil, compared to other litter types (Robertson and Paul 1999; Berg and McClaugherty 2008; Krishna and Mohan 2017). Leaf litter decomposition is a key process contributing to biogeochemical cycles in any forest ecosystem. Microorganisms are the primary agents in this process (Purahong et al. 2016; Mlambo et al. 2019). Fungi are considered as the "key players" in leaf litter decomposition, because of their ability to produce a wide range of extracellular enzymes (Pointing et al. 2005; Berg and McClaugherty 2008; Bani et al. 2018). Many researchers have been carrying out studies of fungal species inhabiting leaf litter and have described numerous new species in Dothideomycetes (Hyde et al. 2019; Phookamsak et al. 2019; Tennakoon et al. 2019).

The family Phaeosphaeriaceae is considered to be one of the most species-rich families in Dothideomycetes and includes species that inhabit a wide range of ecosystems (i. e., marine, terrestrial, and mangroves) (Phookamsak et al. 2014, 2017; Bakhshi et al. 2019; Jones et al. 2019; Luo et al. 2019; Tennakoon et al. 2019). Phaeosphaeriaceae was established by Barr (1979), who designated *Phaeosphaeria* I. Miyake as the generic type of the family. Phaeosphaeriaceae species have immersed to superficial, globose to subglobose ascomata, short papilla, bitunicate asci and hyaline to pigmented, fusiform to ellipsoidal, filiform, or muriform ascospores (Bakhshi et al. 2019; Chaiwan et al. 2019; Maharachchikumbura et al. 2019; Yang et al. 2019). Members of Phaeosphaeriaceae are cosmopolitan, since they exhibit diverse lifestyles as saprobes, endophytes and pathogens of economically important plants (Barr 1992; Phookamsak et al. 2014, 2017; Yang et al. 2016; Hyde et al. 2020; Mapook et al. 2020). Apart from being cosmopolitan in nature, it appears that this family is phylogenetically highly diverse. Thus, recent studies have revealed a large number of new genera in this family. For instance, in the space of two years, eleven genera have been introduced, *viz*. *Bhagirathimyces* S.M. Singh & S.K. Singh (Hyde et al. 2020), *Hydeomyces* Maharachchikumbura et al. (Maharachchikumbura et al. 2019), *Hydeopsis* J.F. Zhang et al. (Zhang et al. 2019), *Neostagonosporella* C.L. Yang, et al. (Yang et al. 2019), *Parastagonosporella* M. Bakhshi, Arzanlou & Crous (Bakhshi et al. 2019), *Pseudoophiosphaerella* J.F. Zhang

et al. (Zhang et al. 2019), *Murichromolaenicola* Mapook & K.D. Hyde (Mapook et al. 2020), *Neoophiobolus* Mapook & K.D. Hyde (Mapook et al. 2020), *Paraleptospora* Mapook & K.D. Hyde (Mapook et al. 2020), *Pseudostaurosphaeria* Mapook & K.D. Hyde (Mapook et al. 2020) and *Vittaliana* Devadatha et al. (Devadatha et al. 2019). Currently, more than 70 genera are accommodated in this family (Wanasinghe et al. 2018; Bakhshi et al. 2019; Maharachchikumbura et al. 2019; Phookamsak et al. 2019; Hongsanan et al. 2020; Hyde et al. 2020).

We are investigating the diversity of microfungi on leaf litter in the tropics with the aim of clarifying their taxonomy based on morphology coupled with multi-gene phylogeny. As a part of this study, we have collected and isolated four taxa from Taiwan, which belong to the family Phaeosphaeriaceae. We present herein comprehensive morphological descriptions and an in-depth phylogenetic investigation of the newly introduced species.

Materials and methods

Sample collection, morphological studies and isolation

Decaying leaf litter samples of *Agave tequilana* F.A.C. Weber (Asparagaceae), *Beaucarnea recurvata* Lem. (Asparagaceae), *Hedychium coronarium* J.Koenig (Zingiberaceae), and *Musa acuminata* Colla (Musaceae) were collected from Dahu Forest Area in Chiayi, Taiwan and taken to the laboratory in Zip lock plastic bags. Specimens were examined with a LEICA EZ4 stereomicroscope. Micro-morphological characters were determined using AXIOSKOP 2 PLUS compound microscope and images were captured with a Zeiss AXIOCAM 506 COLOR digital camera. Observations and photomicrographs were made from materials mounted in water. Permanent slides were preserved in lactoglycerol, sealed by applying nail-polish around the margins of cover slip. All measurements were made with ZEN2 (blue edition) and images used for figures were processed with Adobe Photoshop CS3 Extended version 10.0 software (Adobe Systems, USA).

Single ascospore and conidial isolation was carried out following the method described in Phookamsak et al. (2014). The single germinated spore was picked up and transferred to potato dextrose agar (PDA) and incubated at 25 °C in natural light. Subsequent sub-culturing was done carefully to obtain pure culture and ensure absence of contaminants. Culture characteristics were observed after three weeks. Colonies were photographed and colonial characters were noted and described. Type specimens of new taxa were deposited at the herbarium of Mae Fah Luang University (MFLU) and National Chiayi University Herbarium (NCYU). Living cultures were deposited in Mae Fah Luang University Culture Collection (MFLUCC) and National Chiayi University Culture Collection (NCYUCC). Faces of Fungi and Index Fungorum numbers were provided as in Jayasiri et al. (2015) and Index Fungorum (2020).

DNA extraction and PCR amplification

Total genomic DNA was extracted from scraped fresh fungal mycelium using the DNA extraction kit E.Z.N.A Fungal DNA Mini Kit (D3390-02, Omega Bio-Tek) following the manufacturer's protocol. The DNA product was kept at 4 °C for DNA amplification and maintained at -20 °C for long term storage. DNA was amplified by polymerase chain reaction (PCR) for four genes, the large subunit (28S, LSU), small subunit (18S, SSU), internal transcribed spacers including the 5.8s rDNA (ITS1-5.8S-ITS2) and translation elongation factor 1 alpha (*tef1*-α). The partial LSU gene was amplified by using the primer combination LR0R and LR5 (Vilgalys and Hester 1990; Rehner and Samuels 1994); partial SSU was amplified with NS1 and NS4 (White et al. 1990), nuclear ITS was amplified with primers ITS5 and ITS4 (White et al. 1990), and *tef1*-α gene was amplified using the primers EF1-983F and EF1-2218R (Rehner et al. 2001). Amplification reactions were performed in 25 µl of total reaction that contained 9.5 µl of sterilized water, 12.5 µl of 2×Power Taq PCR MasterMix (Tri-I Biotech, Taipei, Taiwan), 1 µl of each forward and reverse primers and 1 µl of DNA template. The PCR thermal cycle program of ITS, LSU, SSU and *tef1*-α gene was processed initially at 94 °C for 3 minutes, followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 55 °C for 50 seconds, elongation at 72 °C for 1 minute and a final extension at 72 °C for 10 minutes and a holding temperature of 4 °C. The PCR products were analyzed by 1.5% agarose gels containing the Safeview DNA stain (GeneMark, Taipei, Taiwan) to confirm their expected molecular weight. PCR products were purified and sequenced with primers mentioned above by Tri-I Biotech, Taipei, Taiwan. Nucleotide sequences were deposited in GenBank (Table 1).

Phylogenetic analysis

Phylogenetic analyses were performed using a combined LSU, SSU, ITS and *tef1*-α sequence dataset. Newly generated sequence data were initially subjected to blast search in NCBI to obtain the closest matches in GenBank. Sequences generated from this study were analyzed with related taxa in the family Phaeosphaeriaceae, which were obtained from GenBank and from recently published data (Bakhshi et al. 2019; Hyde et al. 2019; Maharachchikumbura et al. 2019; Yang et al. 2019; Mapook et al. 2020) (Table 1). The combined dataset consisted of 168 sequences including our newly generated sequences. Multiple alignments were automatically made with MAFFT v. 7 at the web server (http://mafft.cbrc.jp/alignment/server), using default settings (Katoh and Standley 2013). The alignment was refined manually with BioEdit v. 7.0.5.2 (Hall 1999), where necessary.

Evolutionary models for phylogenetic analyses were selected independently for each locus using MrModeltest v. 3.7 (Posada and Crandall 1998) under the Akaike Information Criterion (AIC). Phylogenetic trees were obtained from Randomized Accelerated Maximum Likelihood (RAxML), maximum parsimony analysis (MP) and

Table 1. GenBank and culture collection accession numbers of species included in the present phylogenetic study. Newly generated sequences are shown in bold.

Species	Strain/Voucher no.	GenBank accession no.			
		LSU	SSU	ITS	*tef1–α*
Acericola italica	MFLUCC 13-0609	MF167429	MF167430	MF167428	–
Allophaeosphaeria muriformia	MFLUCC 13-0277	KX910089	KX950400	KX926415	–
Alloneottiosporina thailandica	MFLUCC 15-0576	–	–	–	–
Amarenographium ammophilicola	MFLU 17-2571	MN017847	MN017913	MN047087	MN077065
Amarenomyces dactylidis	KUMCC 18-0154	MK356345	MK356359	MK356371	–
Arezzomyces cytisi	MFLUCC 15-0649	KT306950	KT306954	KT306947	–
Banksiophoma australiensis	CBS 142163	KY979794	–	KY979739	KY979889
Bhagirathimyc es himalayensis	AMH 10127	MK836020	MN121697	MK836021	–
Bhatiellae rosae	MFLUCC 17-0664	MG828989	MG829101	MG828873	–
Brunneomurispora lonicerae	KUMCC 18-0157	MK356346	MK356360	MK356373	MK359065
Camarosporioides phragmitis	MFLUCC 13-0365	KX572345	KX572350	KX572340	KX572354
Chaetosphaeronema achilleae	MFLUCC 16-0476	KX765266	–	KX765265	–
C. hispidulum	CBS 216.75	KF251652	EU754045	KF251148	KF253108
Dactylidina shoemaker	MFLUCC 14-0963	MG829003	MG829114	MG828887	MG829200
Dematiopleospora cirsii	MFLUCC 13-0615	KX274250	–	KX274243	KX284708
D. mariae	MFLUCC 15-0612	KJ749653	KJ749652	KX274244	KJ749655
Didymocyrtis xanthomendozae	CBS 129666	–	–	KP170651	KP170677
Diederichomyces ficuzzae	CBS 128019	JQ238616	–	KP170647	KP170673
Dlhawksworthia clematidicola	MFLUCC 17-0693	MG829038	MG829144	MG828929	–
D. lonicera	MFLUCC 14-0955	MG829012	MG829121	MG828902	MG829203
Edenia gomezpompae	JLCC 34533	–	–	KC193601	–
	LVPEI 3225	–	–	KU578033	–
Elongaticollum hedychii	**MFLUCC 18-1638**	**MT321810**	**MT321803**	**MT321796**	**MT328753**
E. hedychii	**MFLUCC 17-2653**	**MT321811**	**MT321804**	**MT321797**	**MT328754**
	NCYUCC 19-0286	**MT321812**	**MT321805**	**MT321798**	**MT328755**
Embarria clematidis	MFLUCC 14-0652	KT306953	KT306956	KT306949	–
	MFLUCC 14-0976	MG828987	MG829099	MG828871	MG829194
Equiseticola fusispora	MFLUCC 14-0522	KU987669	KU987670	KU987668	MG520895
Galiicola baoshanensis	HKAS 102234	MK356348	MK356362	MK356374	MK359066
G. pseudophaeosphaeria	MFLU 14-0524	–	–	–	MG520896
Hydeomyces desertipleosporoides	SQUCC 15259	MK290839	MK290843	MK290841	MK290848
	SQUCC 15260	MK290840	MK290844	MK290842	MK290849
Hydeopsis verrucispora	SD 2016-5	MK522498	MK522504	MK522508	MK523388
Italica achilleae	MFLUCC 14-0955	MG829012	MG829121	MG828902	MG829203
I. luzulae	MFLUCC 14-0932	KT306951	–	–	–
Jeremyomyces labinae	CBS 144617	MK442529	–	MK442589	MK442695
Juncaceicola italica	MFLUCC 13-0750	–	–	KX500110	MG520897
J. luzulae	MFLUCC 13-0780	KX449530	KX449531	KX449529	–
Kwanghwaensis miscanthi	FU31017	MK503823	MK503829	MK503817	MT009126
Leptosphaeria doliolum	CBS 505.75	GU301827	GU296159	JF740205	GU349069
Leptospora rubella	CPC 11006	DQ195792	DQ195803	DQ195780	–
L. thailandica	MFLUCC 16-0385	KX655549	KX655554	KX655559	KX655564
Longispora clematidis	MFLU 15–1277				
Loratospora aestuarii	CBS 117592	–	–	MH863024	–
Mauginiella scaettae	CBS 239.58	MH869303	–	MH857770	–
Melnikia anthoxanthii	MFLUCC 14-1011	KU848204	KU848205	–	–
Murichromolaenicola chiangraiensis	MFLUCC 17-1488	MN994559	MN994605	MN994582	MN998163
M. chromolaenae	MFLUCC 17-1489	MN994560	MN994606	MN994583	MN998164
Muriphaeosphaeria galatellae	MFLUCC 14-0614	KT438329	KT438331	KT438333	MG520900
	MFLUCC 15-0769	KT438330	KT438332	–	–
Neoophiobolus chromolaenae	MFLUCC 17-1467	MN994562	MN994606	MN994583	MN998164

Species	Strain/Voucher no.	GenBank accession no.			
		LSU	SSU	ITS	*tef1–α*
N. chromolaenae	MFLUCC 17-1449	MN994561	MN994607	MN994584	MN998165
Neosetophoma sp.	MFLUCC 17-0844	MG829035	MG829141	MG828926	MG829219
N. aseptata	CBS 145363	MK540024	–	MK539953	–
N. camporesii	MFLUCC 15-0682	KU302778	–	KU302779	–
N. clematidis	MFLUCC 13-0734	KP684153	KP684154	KP744450	–
N. garethjonesii	MFLUCC 14-0528	–	KY501126	–	KY514402
N. guiyangensis	GZ13	MH018132	MH018136	MH018134	MH051889
N. italica	MFLU 14-0809	KP711361	KP711366	KP711356	–
N. lonicerae	KUMCC 18-0155	MK356349	MK356363	MK356375	MK359067
N. lunariae	CPC 26671	KX306789	–	KX306763	–
N. miscanthi	MFLU 18-2675	MK503826	MK503832	MK503820	–
N. phragmitis	CBS 145364	MK540025	–	MK539954	MK540148
N. poaceicola	MFLUCC 16-0886	KY550382	KY550383	KY568986	–
	MFLUCC 18-1632	**MT321809**	**MT321802**	**MT321795**	**–**
N. rosae	MFLUCC 17-0844	MG829035	MG829141	MG828926	MG829219
N. rosaena	MFLUCC 17-0768	MG829037	MG829143	MG828928	–
N. rosarum	MFLU 17-0308	MG829036	MG829142	MG828927	–
N. salicis	MFLU 17-0118	MK608026	–	MK608025	–
N. samarorum	CBS 138.96	KF251664	GQ387517	MH862569	KF253119
N. sambuci	CBS 145365	MK540026	–	MK539955	MK540149
N. shoemakeri	MFLU 16-1606	MG602199	MG602201	MG602203	MG844352
	MFLUCC 17-0780	MG844348	MG844350	MG844346	MG844352
N. tienshanensis	MFLUCC 17-0844	MG829035	MG829141	MG828926	MG829219
N. xingrensis	GZAAS18 0100	MH018133	–	MH018135	–
Neosphaerellopsis thailandica	CPC 21659	KP170721	–	KP170652	KP170678
Neostagonospora caricis	CBS 135092	KF251667	–	KF251163	–
N. phragmitis	MFLUCC 16-0493	KX910090	KX950401	KX926416	MG520902
Neostagonosporella sichuanensis	MFLUCC 18-1228	–	–	–	MK313854
	MFLUCC 18-1231	–	–	–	MK313851
Neosulcatispora agaves	CPC 26407	KT950867	–	KT950853	–
Nodulosphaeria multiseptata	MFLUCC 15-0078	KY496728	–	KY496748	–
N. scabiosae	MFLUCC 14-1111	KU708846	KU708842	KU708850	KU708854
Ophiobolopsis italica	MFLUCC 17-1791	MG520959	MG520977	MG520939	MG520903
Ophiobolus disseminans	MFLUCC 17-1787	MG520961	MG520980	MG520941	MG520906
O. rossicus	MFLU 17-1639	MG520964	MG520983	MG520944	MG520909
Ophiosimulans tanaceti	MFLUCC 14-0525	KU738891	KU738892	KU738890	MG520910
Ophiosphaerella agrostidis	MFLUCC 11-0152	KM434281	KM434290	KM434271	KM434299
	MFLUCC 12-0007	KM434282	KM434291	KM434272	KM434300
	MFLUCC 16-0895	MF197563	MF351604	MF351996	–
	IGM35	MF197563	MF351604	–	–
	MFLUCC 11-0152	KM434281	KM434290	KM434271	KM434299
O. aquatica	MFLUCC 14-0033	KX767089	KX767090	KX767088	MG520911
	MFLUCC 14-0033	KX767089	KX767090	KX767088	MG520911
O. herpotricha	k28	–	–	KP690992	KP691016
	KS29	–	–	KP690986	KP691015
O. korrae	ATCC 56289	–	–	KC848509	KC848515
O. narmari	ATCC 64688	–	–	KC848510	KC848516
	ATCC 201719	–	–	KC848508	KC848514
O. taiwanensis	**MFLU 18-2534**	**MT321815**	**MT321808**	**MT321801**	**MT328758**
O. taiwanica	NTUCC 17-024	MN082419	–	MN082417	–
	NTUCC 17-025	MN082420	–	MN082418	–
Paraleptosphaeria dryadis	CBS 643.86	GU301828	KC584632	JF740213	GU349009
Paraleptospora chromolaenae	MFLUCC 17-1481	MN994563	MN994609	MN994587	MN998167

Species	Strain/Voucher no.	GenBank accession no.			
		LSU	SSU	ITS	*tef1–α*
P. chromolaenicola	MFLUCC 17-1450	MN994564	MN994610	MN994588	MN998168
Paraophiobolus arundinis	MFLUCC 17-1789	MG520965	MG520984	MG520945	MG520912
P. plantaginis	MFLUCC 17-0245	KY815010	KY815012	KY797641	MG520913
Paraloratospora camporesii	MFLU 18-0915	MN756637	MN756635	MN756639	–
Paraphoma chrysanthemicola	CBS 522.66	KF251670	GQ387521	KF251166	KF253124
P. radicina	CBS 111.79	KF251676	EU754092	KF251172	KF253130
Parastagonospora dactylidis	MFLUCC 13-0375	KU058722	–	KU058712	–
Parastagonosporella fallopiae	CBS 135981	MH460545	–	MH460543	MH460549
P. fallopiae	CCTU 1151-1	MH460546	–	MH460544	MH460550
Phaeopoacea muriformis	MFLUCC 17-0372	MF611638	MF611639	MF611637	–
P. festucae	MFLUCC 17-0056	KY824767	KY824769	KY824766	–
Phaeoseptoriella zeae	CBS 144614	MK442547	–	MK442611	MK442702
Phaeosphaeria musae	MFLUCC 11-0133	KM434277	KM434287	KM434267	KM434296
P. oryzae	CBS 110110	KF251689	GQ387530	KF251186	–
P. papayae	CBS 135416	–	–	MH866082	–
Phaeosphaeriopsis agapanthi	CPC 26303	KX228311	–	KX228260	–
P. agavacearum	CPC 29122	KY173520	–	KY173430	–
P. agavensis	CBS 102206	KY090669	KY090693	KY090635	–
P. aloes	CBS 145367	MK540030	–	MK539959	MK540153
P. aloicola	CBS 145368	MK540031	–	MK539960	MK540154
P. amblyospora	CBS 110131	–	–	MH862851	–
P. beaucarneae	**MFLU 18-2586**	**MT321813**	**MT321806**	**MT321799**	**MT328756**
	MFLU 18-2587	**MT321814**	**MT321807**	**MT321800**	**MT328757**
P. dracaenicola	MFLUCC 11-0157	KM434283	KM434292	KM434273	KM434301
P. glaucopunctata	MFLUCC 13-0265	KJ522477	KJ522481	KJ522473	MG520918
P. grevilleae	CBS 145369	MK540032	–	MK539961	MK540155
P. nolinae	CBS 102205	KY090667	KY090694	KY090637	–
P. obtusispora	CBS 246.64	JX681119	–	KY090644	–
P. omaniana	SQUCC:14333	MT075849	–	MT075840	–
P. phacidiomorpha	CBS 198.35	AF275496	AF275515	FJ462742	–
P. pseudoagavacearum	CBS 145370	MK540033	–	MK539962	–
	MFLU 17-1800A	MN750592	MN750607	MN750613	MN756837
P. triseptata	MFLUCC 13-0271	KJ522479	KJ522484	KJ522475	MG520919
P. yuccae	MFLUCC 16-0558	KY554481	KY554480	KY554482	MG520920
Piniphoma wesendahlina	CBS 145032	MK442551	–	MK442615	MK442706
Populocrescentia ammophilae	MFLUCC 17-0665	MG829059	MG829164	MG828949	MG829231
P. rosacea	MFLU 17-0128	MG829060	MG829165	–	MG829232
Pseudoophiobolus achilleae	MFLU 17-0925	MG520966	–	MG520946	–
P. galii	MFLUCC 17-2257	MG520967	MG520989	MG520947	MG520926
Pseudoophiosphaerella huishuiensis	HS13	MK522499	MK522505	MK522509	MK523389
Pseudophaeosphaeria rubi	MFLUCC 14-0259	KX765299	KX765300	KX765298	MG520934
Pseudostaurosphaeria chromolaena	MFLUCC 17-1490	MN994570	MN994616	MN994593	MN998174
P. chromolaenicola	MFLUCC 17-1491	MN994571	MN994617	MN994594	MN998175
Poaceicola arundinis	MFLU 16-0158	MG829057	MG829162	MG828947	MG829229
P. bromi	MFLUCC 13-0739	KU058727	–	KU058717	–
Sclerostagonospora rosicola	MFLUCC 15-0129	MG829068	MG829172	MG828957	MG829237
Scolicosporium minkeviciusii	MFLUCC 12-0089	KF366382	KF366383	–	–
Septoriella phragmitis	CPC 24118	KR873279	–	KR873251	–
S. pseudophragmitis	CBS 145417	–	–	MK560161	MK559452
Setomelanomma holmii	CBS 110217	GU301871	GU296196	KT389542	GU349028
Setophoma antiqua	LC6594	MK511947	–	MK511909	MK525070
S. chromolaenae	CBS 135105	KF251747	–	KF251244	KF253195
S. endophytica	LC3163	MK511956	–	MK511931	MK525092

Species	Strain/Voucher no.	GenBank accession no.			
		LSU	SSU	ITS	*tef1–α*
S. longinqua	LC6593	MK511946	–	MK511908	MK525069
S. pseudosacchari	CBS 145373	MK540039	–	MK539969	
S. sacchari	MFLUCC 11-0154	KJ476146	KJ476148	KJ476144	KJ461319
	MFLUCC 12-0241	KJ476147	KJ476149	KJ476145	KJ461318
S. terrestris	CBS 335.29	KF251749	GQ387526	KF251246	KF253196
S. vernoniae	CBS 137988	KJ869198	–	KJ869141	MK540162
S. yingyisheniae	LC12696	MK511950	–	MK511914	MK525075
S. yunnanensis	LC6532	MK511945	–	MK511907	MK525068
Stagonospora foliicola	CBS 110111	KF251759	EU754118	KF251256	KF253206
Sulcispora sp.	MFLUCC 14-0995	KP271444	KP271445	KP271443	MH665366
Sulcispora pleurospora	CBS 460.84	–	–	AF439498	–
Tintelnotia destructans	CBS 127737	KY090664	KY090698	KY090652	–
T. opuntiae	CBS 376.91	GU238123	GU238226	KY090651	–
Vagicola vagans	CBS 604.86	KU058727	–	KF251193	KF253149
Vittaliana mangrovei	NFCCI 4251	MG767312	MG767313	MG767311	MG767314
Vrystaatia aloeicola	CBS 135107	KF251781	–	KF251278	–
Wingfieldomyces cyperi	CBS 141450	KX228337	–	KX228286	MK540163
Wojnowiciella eucalypti	CPC 25024	KR476774	–	KR476741	LT990617
W. kunmingensis	KUMCC 18-0159	MK356354	MK356368	MK356380	MK359071
Xenophoma puncteliae	CBS 128022	JQ238619	–	–	KP170686
Xenoseptoria neosaccardoi	CBS 120.43	KF251783	–	KF251280	KF253227
	CBS 128665	KF251784	–	KF251281	KF253228
Yunnanensis chromolaenae	MFLUCC 17-1486	MN994573	MN994619	MN994596	MN998177
	MFLUCC 17-1487	MN994574	MN994620	MN994597	MN998178
Yunnanensis phragmitis	MFLUCC 17-0315	MF684863	MF684867	MF684862	MF683624
	MFLUCC 17-1361	MF684865	MF684864	MF684869	–

Bayesian inference analyses (BI). ML trees were generated using the RAxML-HPC2 on XSEDE (8.2.8) (Stamatakis et al. 2008; Stamatakis 2014) in the CIPRES Science Gateway platform (Miller et al. 2010) using GTR+I+G model of evolution. The MP analysis was performed using PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b10 (Swofford 2002), with parameters as described in Tennakoon et al. (2019). Descriptive tree statistics for parsimony, such as Tree Length (TL), Consistency Index (CI), Retention Index (RI), Relative Consistency Index (RC) and Homoplasy Index (HI) were calculated.

The BI analysis was conducted with MrBayes v. 3.1.2 (Huelsenbeck and Ronquist 2001) to evaluate posterior probabilities (PP) (Rannala and Yang 1996; Zhaxybayeva and Gogarten 2002) by Markov Chain Monte Carlo sampling (MCMC). Six MCMC chains were run simultaneously, starting from random trees for 3,000,000 generations. Trees were sampled every 100th generation for a total of 30,000 trees. The first 6,000 trees were discarded as the burn-in phase of each analysis. Posterior probabilities (Rannala and Yang 1996) were determined from a majority-rule consensus tree generated with the remaining 24,000 trees. Phylograms were visualized with FigTree v1.4.0 (Rambaut 2012) and annotated in Microsoft Power Point (2010). Sequences of the new strains generated in this study are deposited in GenBank. The final alignment and trees were deposited in TreeBASE, submission ID: 26088.

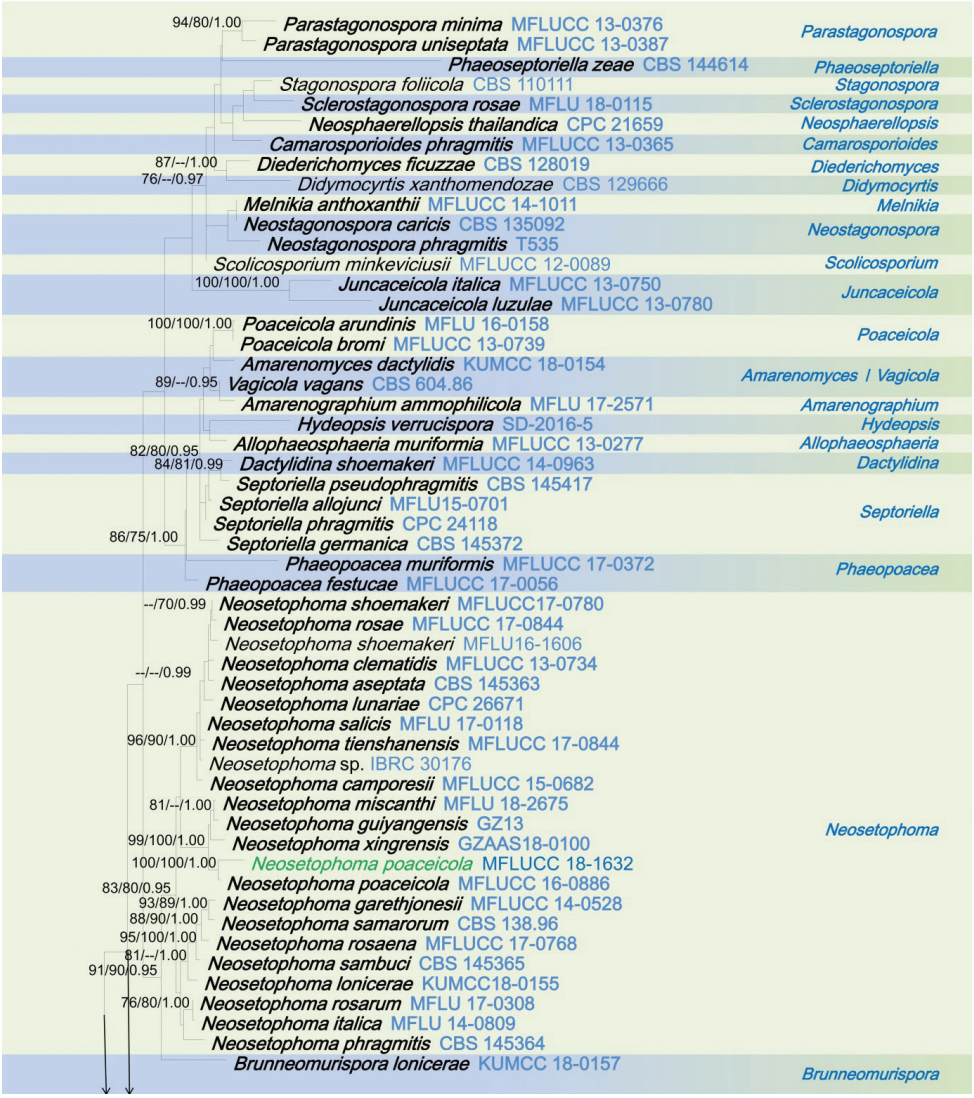

Figure 1. RAxML tree inferred from combined dataset of ITS, LSU, SSU and *tef1-α* partial sequences of 168 strains of Phaeosphaeriaceae. Bootstrap support values for maximum likelihood (ML), maximum parsimony (MP) values ≥70%, and Bayesian posterior probabilities (BYPP) ≥0.95 are given above each branch respectively. The new species are highlighted in red, and the new record in green. Ex-type strains are in bold. The tree is rooted by *Leptosphaeria doliolum* (CBS 505.75) and *Paraleptosphaeria dryadis* (CBS 643.86).

Results

Phylogenetic analysis

The combined dataset of ITS, LSU, SSU and *tef1-α* sequences comprised 3423 characters, of which 2418 characters are constant, 697 characters are parsimony-in-

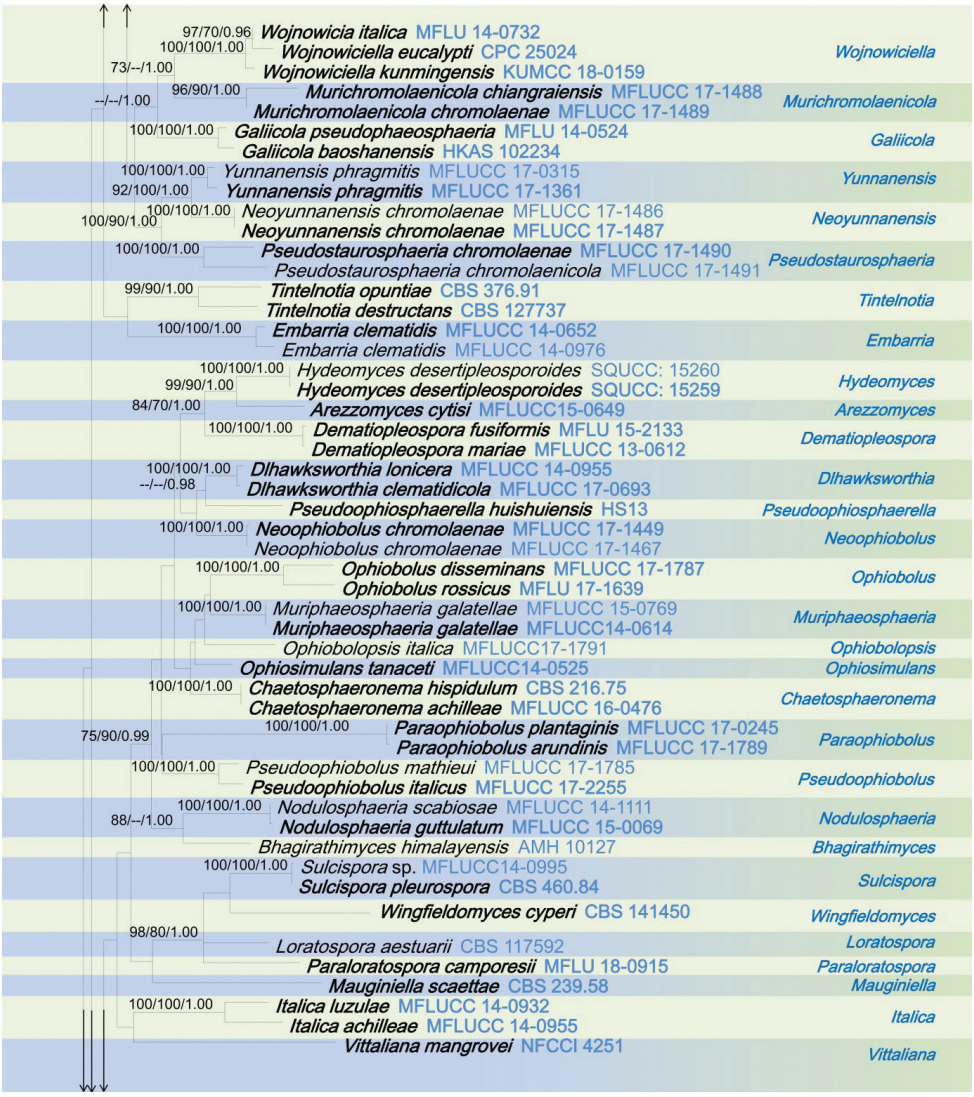

Figure 11. Continued.

formative, while 308 variable characters are parsimony-uninformative in the maximum parsimony (MP) analysis (TL = 6364, CI = 0.250, RI = 0.657, RC = 0.164, HI = 0.750). The RAxML analysis of the combined dataset yielded a best scoring tree (Figure 1) with a final ML optimization likelihood value of – 34492.801018. The matrix had 1331 distinct alignment patterns, with 37.25% of undetermined characters or gaps. Estimated base frequencies are; A = 0.247120, C = 0.228182, G = 0.268238, T = 0.256459; substitution rates AC = 1.250439, AG = 3.526348, AT = 2.517351, CG = 0.798250, CT = 6.907432, GT = 1.000; proportion of in-

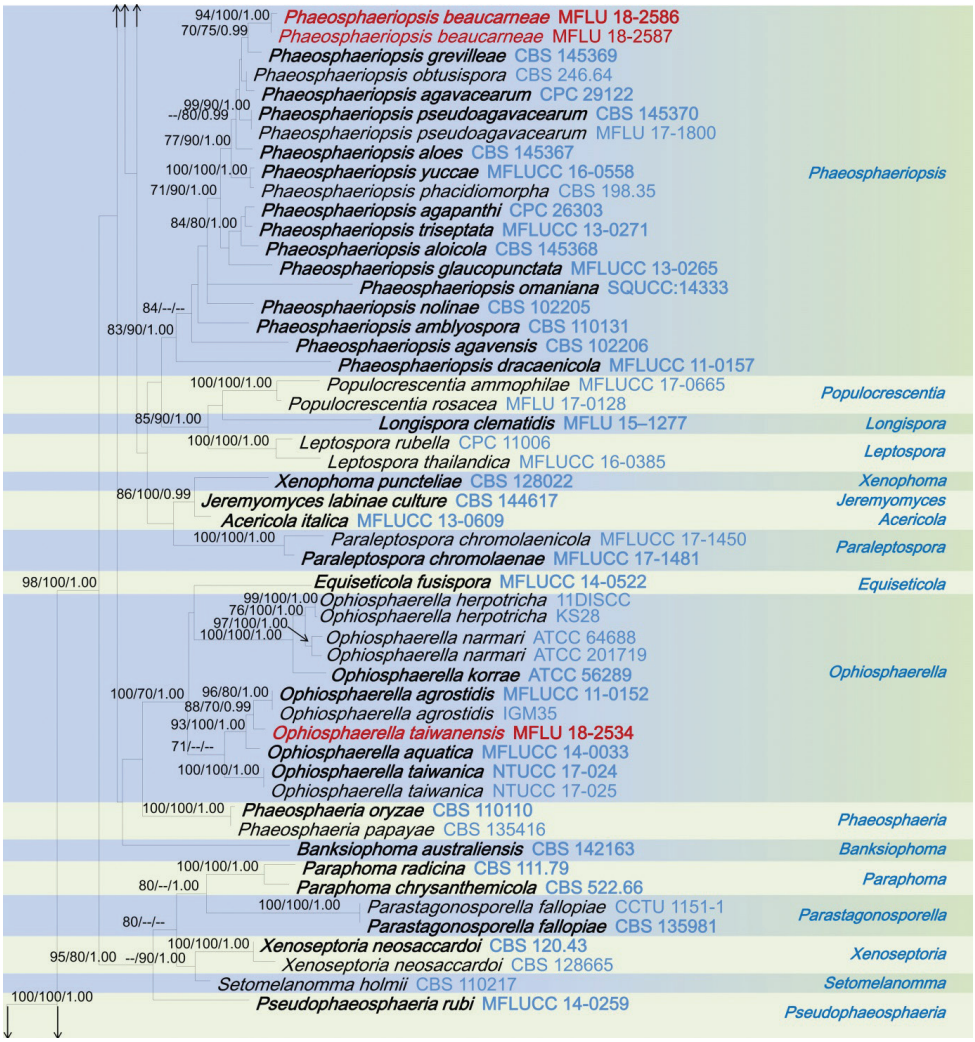

Figure 12. Continued.

variable sites I = 0.596400; gamma distribution shape parameter α = 0.492378. All analyses (ML, MP and BI) gave similar results and are in agreement with previous studies based on multi-gene analyses (Hyde et al. 2019, 2020; Phookamsak et al. 2019). Phylogenetic analyses of the combined data matrix resulted in well-resolved clades, many of which had considerably high statistical support (Figure 1). Bootstrap support values for maximum likelihood, maximum parsimony ≥70%, and Bayesian posterior probabilities (BYPP) ≥0.95 are given above each branch in that order (Figure 1). Phylogenetic position and statistical support are noted in the taxonomy section.

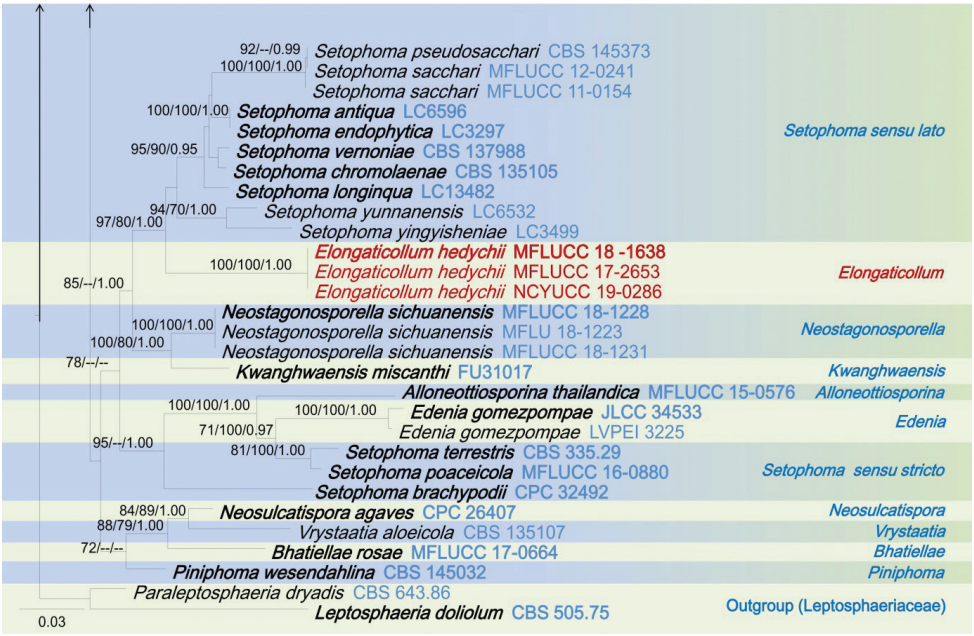

Figure 13. Continued.

Taxonomy

Elongaticollum **Tennakoon, C.H. Kuo & K.D. Hyde, gen. nov.**
Index Fungorum number: IF 557486
Facesoffungi number: FoF07849

Etymology. Refers to the fact that the pycnidia have elongated necks.

Diagnosis. *Saprobic* on dead leaves of *Hedychium coronarium* J. Koenig. **Sexual morph:** Undetermined. **Asexual morph:** Coelomycetous. *Conidiomata* pycnidial, solitary, superficial, dark brown to black, obpyriform, papillate. *Neck* elongate, dark brown, usually straight, but sometimes slightly curved. *Conidiomatal wall* composed of 4–5 layers of light brown cells, arranged in *textura angularis*. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* hyaline, aseptate, smooth, ampulliform, arising from the inner cell wall of the apex. *Conidia* oval to oblong, smooth and thin-walled, hyaline, aseptate, with 1–2-minute guttules.

Type species. *Elongaticollum hedychii* Tennakoon, C.H. Kuo & K.D. Hyde.

Elongaticollum hedychii **Tennakoon, C.H. Kuo & K.D. Hyde, sp. nov.**
Index Fungorum number: IF 557487
Facesoffungi number: FoF07850
Figure 2

Etymology. Name reflects the host *Hedychium coronarium* J. Koenig, from which the holotype was collected.

Holotype. MFLU 18-2542.

Diagnosis. *Saprobic* on dead leaves of *Hedychium coronarium* J. Koenig. **Sexual morph:** Undetermined. **Asexual morph:** Coelomycetous. *Conidiomata* 120–140 µm high, 60–70 µm diam., pycnidial, solitary, scattered, superficial, visible as small black spots on host surface, dark brown to black, obpyriform, papillate. *Neck* up to 80–100 µm long, 20–30 µm diam., elongated, dark brown, usually straight, but sometimes slightly curved. *Conidiomatal wall* 10–20 µm wide, composed of 4–5 layers of light brown, thick-walled cells, arranged in *textura angularis*. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 3–4 × 3–3.5 µm ($\bar{x}$ = 3.6 × 3.2 µm, n = 10), arising from the inner cell wall of the apex, hyaline, aseptate, smooth, ampulliform. *Conidia* 4–5 × 1.8–2.2 µm ($\bar{x}$ = 4.6 × 2.1 µm, n = 30), oval to oblong, smooth, thin-walled, hyaline, aseptate, with 1–2-minute guttules.

Culture characteristics. Colonies on PDA reaching 30 mm diameter after 3 weeks at 20–25 °C, colonies medium sparse, circular, raised, surface slightly rough with entire edge, margin entire, colony from above: light brown to grey at the margin, dark brown at middle, dark brown to black at the center; reverse, light brown to yellowish at the margin, brown at middle, dark brown to black at the center; mycelium light brown to grey with tufts; not producing pigments in PDA.

Material examined. Taiwan, Chiayi, Fanlu Township area, Dahu Forest, dead leaves of *Hedychium coronarium* J. Koenig (Zingiberaceae), 15 August 2018 (23°27.514'N, 120°36.302'E), D.S. Tennakoon, TLF031-A (MFLU 18-2542, ***holotype***), ex-type living culture (MFLUCC 18-1638 = NCYUCC 19-0163); *ibid*. 20 August 2018 (23°27.530'N, 120°36.314'E), TLF031-B (NCYU19-0139, ***paratype***), living culture (NCYUCC19-0286); *ibid*. 25 August 2018 (23°27.512'N, 120°36.301'E), TLF031-C (NCYU19-0140, ***paratype***), living culture (NCYUCC 19-0287).

Notes. The genus *Elongaticollum* differs from other asexual morphs in Phaeosphaeriaceae in dark brown to black, superficial, obpyriform, pycnidial conidiomata with distinct elongate necks (80–100 µm) and a globose base and oval to oblong, hyaline, aseptate conidia (Figure 2). Multi-gene phylogenetic analyses (LSU, SSU, ITS, *tef1*-α), show *Elongaticollum* strains constitute a highly supported independent lineage nested between *Setophom*a *sensu lato* and *Neostagonosporella* (97% ML, 80% MP, 1.00 BYPP, Figure 1). However, the asexual morph of *Setophoma* can be distinguished from *Elongaticollum* in having setose conidiomata without elongate necks and oblong to ellipsoidal conidia, whereas, *Elongaticollum* have conidiomata with distinct elongate necks and lacking setae and oval to oblong conidia (De Gruyter et al. 2010; Phookamsak et al. 2014). Despite some *Setophoma* species not having setae (i.e. *S. antiqua*, *S. endophytica*, and *S. yunnanensis*) (Liu et al. 2019), *Elongaticollum* species can be distinguished by its superficial conidiomata with elongate necks.

The asexual morph of *Neostagonosporella* differs from *Elongaticollum* in having multiloculate conidiomata without distinct elongate necks and two types of conidia (macroconidia: subcylindrical to cylindrical, transversely multi-septate, hyaline and microconidia oval, ellipsoidal or long ellipsoidal, aseptate, hyaline), whereas *Elongaticollum* has uni-loculate conidiomata with distinct elongate necks and oval to oblong conidia (Figure 2, Yang et al. 2019).

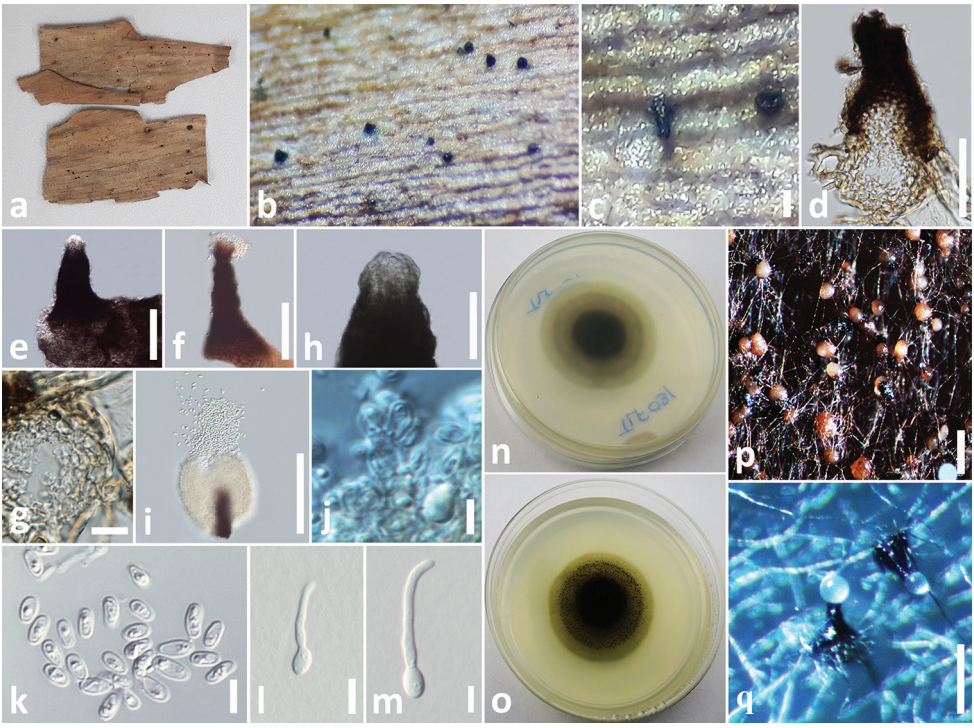

Figure 2. *Elongaticollum hedychii* (MFLU 18-2542, holotype) **a** specimen **b** appearance of conidiomata on host **c** close up of conidiomata on host **d** vertical section through conidioma **e, f** squash mount of conidioma **g** conidioma wall **h, i** elongated conidiomatal necks **j** conidiogenous cells **k** conidia **l, m** germinated conidia **n** colony from below **o** colony from above **p, q** pycnidia formed on PDA. Scale bars: 100 µm (**c**), 50 µm (**d–h**), 10 µm (**g**), 30 µm (**i**), 3 µm (**j–m**), 100 µm (**p, q**).

Phylogenetic investigations herein provide insights into the taxonomy of *Setophoma* as well (Figure 1). Two major clades of *Setophoma* are recovered (*Setophoma sensu stricto* and *Setophoma sensu lato*. The *Setophoma sensu stricto* clade includes *S. brachypodii*, *S. poaceicola* and *S. terrestris* (type species). *Setophoma sensu lato* comprises *S. antiqua*, *S. chromolaenae*, *S. endophytica*, *S. pseudosacchari*, *S. sacchari*, *S. vernoniae*, *S. yingyisheniae* and *S. yunnanensis* (Figure 1). *Elongaticollum*, differs from *Setophoma sensu lato* in having distinct superficial, obpyriform, pycnidial conidiomata with a globose base and distinct elongated necks (Figure 2, Liu et al. 2019). Further work is needed to resolve relationships between *Setophoma sensu stricto* and *Setophoma sensu lato*.

Ophiosphaerella Speg., Anal. Mus. nac. B. Aires, Ser. 3 12: 401 (1909)

Notes. *Ophiosphaerella* was introduced by Spegazzini (1909) to accommodate *O. graminicola* Speg. as the type species. The species of this genus are characterized by papillate ascomata bearing fissitunicate, cylindrical asci frequently narrower near the

base, with a short furcate pedicel and filamentous, pale brown, multi-septate ascospores without swollen cells or separating into part spores. Barr (1987) placed *Ophiosphaerella* in Phaeosphaeriaceae and this was confirmed by Zhang et al. (2009, 2012) and Hyde et al. (2013) based on molecular phylogeny. Most *Ophiosphaerella* species are often found as pathogens or saprobes worldwide on Poaceae and Cyperaceae (Camâra et al. 2000). Currently, twelve *Ophiosphaerella* species are listed in Index Fungorum (2020). In this study, we introduce *Ophiosphaerella taiwanensis* from *Agave tequilana* F.A.C. Weber (Asparagaceae) as a new species.

Ophiosphaerella taiwanensis **Tennakoon, C.H. Kuo & K.D. Hyde, sp. nov.**
Index Fungorum number: IF 557488
Facesoffungi number: FoF07851
Figure 3

Etymology. Named after Taiwan, where this fungus was collected.

Holotype. MFLU 18-2534.

Diagnosis. *Saprobic* on dead leaf of *Agave tequilana* F.A.C. Weber (Asparagaceae). **Sexual morph:** *Ascomata* 270–310 µm high, 220–260 µm diam., solitary, scattered, immersed to slightly erumpent through host tissue with papilla, visible as raised, small black dots in host surface, globose to subglobose, uniloculate, glabrous, dark brown to black, ostiole central, periphysate. *Peridium* 20–25 µm wide, thick-walled, of equal thickness, composed of 6–7 layers of small, flattened, brown to dark brown pseudoparenchymatous cells, hyaline towards the inside, arranged in a *textura angularis*, fusing and indistinguishable from the host tissues. *Hamathecium* of 1.5–2.5 µm wide, cellular, septate, rarely branching, pseudoparaphyses, anastomosing mostly above the asci and embedded in a mucilaginous matrix. *Asci* 115–140 × 8.5–10 µm ($\bar{x}$ = 121.6 × 9.2 µm, n = 20), 8-spored, bitunicate, fissitunicate, cylindrical to cylindric-clavate, short pedicellate, apically rounded, with a well-developed ocular chamber. *Ascospores* 110–132 × 2.2–2.7 µm ($\bar{x}$ = 117.2 × 2.4 µm, n = 20), fasciculate, parallel, scolecosporous, filiform, 12–13-septate, narrowing towards ends, pale brown to brown, smooth-walled. **Asexual morph:** Undetermined.

Culture characteristics. Colonies on PDA reaching 25 mm diameter after 3 weeks at 20–25 °C, colonies medium sparse, circular, raised, surface slightly rough with entire edge, margin well-defined, colony from above: gray to light brown at the margin, gray to cream at the center; reverse, gray to light brown at the margin, dark brown to black at the center; mycelium whitish gray with tufting; not producing pigments in PDA.

Material examined. Taiwan, Chiayi, Fanlu Township area, Dahu Forest, dead leaf of *Agave tequilana* F.A.C. Weber (Asparagaceae), 15 August 2018 (23°27.520'N, 120°36.310'E), D.S. Tennakoon, TLF016 (MFLU 18-2534, ***holotype***); *ibid*. (NCYU19-0131, ***isotype***), ex-type living culture, NCYUCC 19-0152.

Notes. The scolecosporous specimen was collected from dead leaves of *Agave tequilana* (Asparagaceae) in Taiwan. The multi-gene phylogenetic analysis (Figure 1)

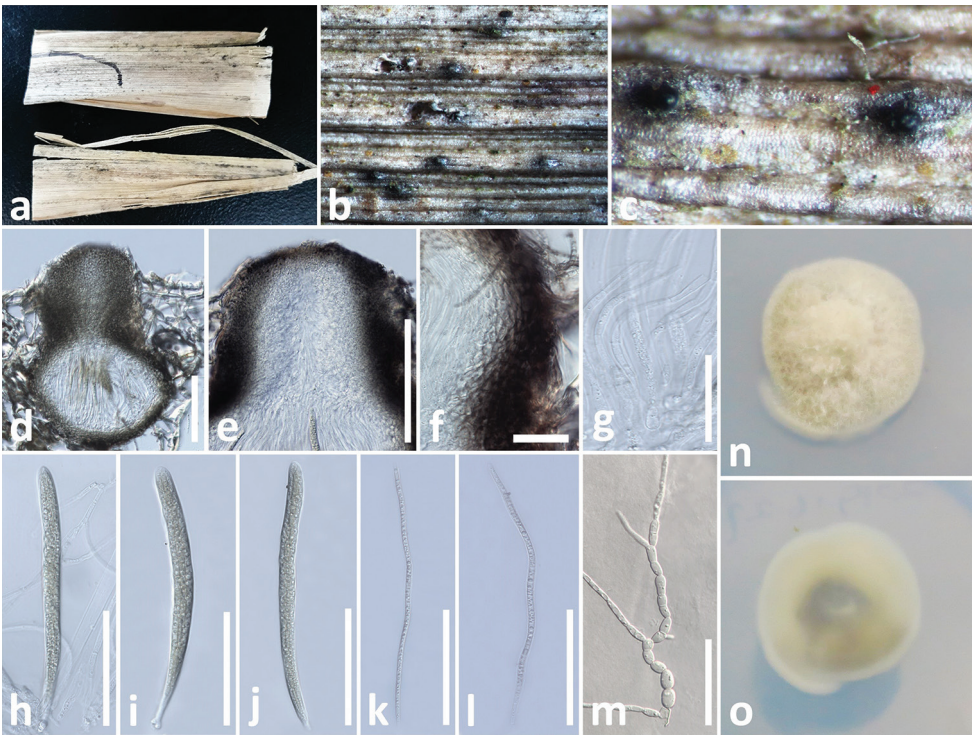

Figure 3. *Ophiosphaerella taiwanensis* (MFLU 18-2534, holotype) **a, b** appearance of ascomata on host **c** close-up of ascomata **d** vertical section through ascoma **e** apex of ascoma **f** peridium **g** pseudoparaphyses **h–j** asci **k, l** ascospores **m** germinated ascospore in PDA **n** colony from above **o** colony from below. Scale bars: 100 µm (**d, e**), 15 µm (**f**), 50 µm (**g–m**).

shows our strain (*Ophiosphaerella taiwanensis*, NCYUCC 19-0152), cluster with other *Ophiosphaerella* species, in particular with close affinity to *Ophiosphaerella agrostidis* with high bootstrap support (88% ML, 70% MP, 0.99 BYPP, Figure 1). Morphological characters of our collection (NCYUCC 19-0152) differ from *Ophiosphaerella agrostidis* in having periphyses in the ostiole, 12–13 septate ascospores and host occurrence (Asparagaceae). *Ophiosphaerella agrostidis* was introduced by Camâra et al. (2000) on *Agrostis palustris* (Poaceae), and is lacking periphyses, comprises 15-septate ascospores (Phookamsak et al. 2014). A comparison of the 619 nucleotides across the *tef1-α* gene region of *Ophiosphaerella taiwanensis* and *O. agrostidis* (MFLUCC 11-0152) reveals 17 base pair differences (2.74%).

Phaeosphaeriopsis M.P.S. Câmara, M.E. Palm & A.W. Ramaley, Mycol. Res. 107(5): 519 (2003)

Notes. The genus *Phaeosphaeriopsis* was introduced by Câmara et al. (2003) to accommodate *Paraphaeosphaeria*-like taxa, viz. *P. agavensis* A.W. Ramaley, M.E. Palm & M.E.

Barr, *P. glaucopunctata* (Grev.) Shoemaker & C.E. Babc., *P. nolinae* A.W. Ramaley, *P. obtusispora* (Speg.) O.E. Erikss, *Phaeosphaeriopsis amblyspora* A. W. Ramaley and *Phaeosphaeriopsis amblyspora* A. W. Ramaley. The genus is typified by *P. glaucopunctata* and characterized by having immersed, sub-epidermal, globose to subglobose to pyriform ascomata, cylindric asci and septate, punctate or verrucose ascospores (Câmara et al. 2003; Phookamsak et al. 2014; Thambugala et al. 2014; Tibpromma et al. 2017). Currently, 17 *Phaeosphaeriopsis* species are accepted in Index Fungorum (2020). In this paper, *Phaeosphaeriopsis beaucarneae* is introduced from *Beaucarnea recurvata* (Asparagaceae) as a new species and the sexual/asexual morph connection between strains isolated from the natural habitat was established based on molecular sequence data.

Phaeosphaeriopsis beaucarneae **Tennakoon, C.H. Kuo & K.D. Hyde, sp. nov.**
Index Fungorum number: IF 557489
Facesoffungi number: FoF07852
Figures 4, 5

Etymology. Name reflects the host *Beaucarnea recurvata* Lem., from which the holotype was collected.

Holotype. MFLU 18-2586.

Diagnosis. *Saprobic* on dead leaf of *Beaucarnea recurvata* Lem. (Asparagaceae). **Sexual morph:** *Ascomata* 160–200 µm high, 220–250 µm diam., scattered, solitary, gregarious, coriaceous, immersed to semi-immersed, slightly raised, erumpent, visible as black spots on host surface, uniloculate, dark brown to black, globose to subglobose, ostiolate. *Ostiole* central, papillate. *Peridium* 20–30 µm wide, thick-walled, of equal thickness, composed of 4–5 layers of dark brown to brown, thick-walled, pseudoparenchymatous cells of *textura angularis*. *Hamathecium* of 1.5–2.5 µm wide, cellular, septate, rarely branching, pseudoparaphyses, anastomosing mostly above the asci and embedded in a mucilaginous matrix. *Asci* 80–90 × 9–10 µm ($\bar{x}$ = 86.5 × 9.6 µm, n = 25), 8-spored, bitunicate, fissitunicate, cylindrical to cylindric-clavate, short pedicellate, apically rounded, with a well-developed ocular chamber. *Ascospores* 20–25 × 5.5–7 µm ($\bar{x}$ = 22.6 × 6.2 µm, n = 20), overlapping 1–2-seriate, oblong to cylindrical, yellowish to light brown, slightly narrowing towards the end cells, mostly 5-septate, constricted at the septa, enlarged at the 4th cell from above, verruculose, straight to curved, lacking a mucilaginous sheath. **Asexual morph:** *Conidiomata* 180–200 µm high, 140–160 µm diam., pycnidial, solitary, immersed to erumpent, small black spots on host surface, globose to subglobose with centrally placed ostiole. *Conidiomatal wall* 28–34 µm wide, composed of 6–7 layers of dark brown cells, arranged in *textura angularis*. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 3–4 × 2.6–3.1 µm, holoblastic, phialidic, single, discrete, sometimes integrated, ampulliform or cylindric-clavate, hyaline, arising from basal stratum. *Conidia* 6.8–7.4 × 3–4 µm ($\bar{x}$= 7.1 × 3.4 µm, n = 30), 1-celled, globose to subglobose, initially hyaline, becoming brown to dark brown, aseptate, rough-walled.

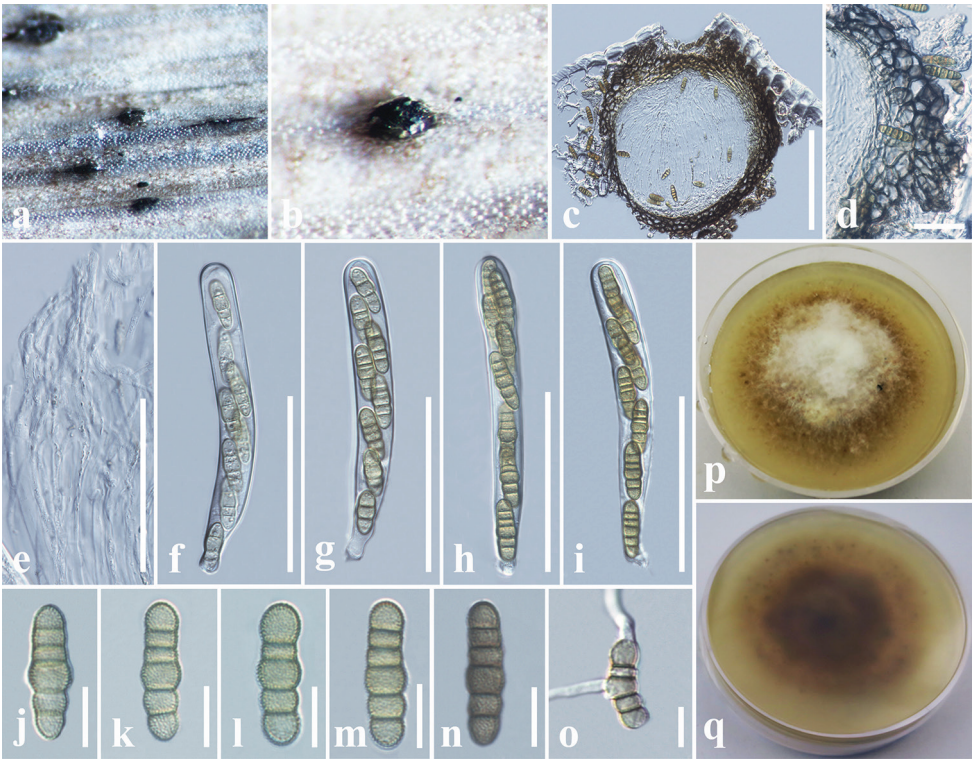

Figure 4. *Phaeosphaeriopsis beaucarneae* (MFLU 18-2586, holotype) **a** appearance of ascomata on host **b** close up of ascoma **c** vertical section through ascoma **d** peridium **e** pseudoparaphyses **f–i** asci **j–n** ascospores **o** germinated ascospore in PDA **p** colony from above **q** colony from below. Scale bars: 100 µm (**c**), 15 µm (**d**), 50 µm (**e–i**), 10 µm (**j–o**).

Culture characteristics. Colonies on PDA reaching 27 mm diameter after 3 weeks at 20–25 °C, colonies medium sparse, circular, raised, surface slightly rough with entire edge, margin irregular, colony from above: light brown at the margin, white to cream at the center; reverse, yellow to light brown at the margin, light brown to brown at the center; mycelium white to cream with tufting; not producing pigments in PDA.

Material examined. Taiwan, Chiayi, Fanlu Township area, Dahu Forest, dead leaf of *Beaucarnea recurvata* Lem. (Asparagaceae), 21 July 2018 (23°27.514'N, 120°36.302'E), D.S. Tennakoon, SV027 (MFLU 18-2586, ***holotype***); *ibi*. (NCYU19-0184, ***isotype***), ex-type living culture, NCYUCC 19-0106; *ibid*., Dahu forest, dead leaf of *Beaucarnea recurvata* Lem. (Asparagaceae), 25 July 2018 (23°26.534'N, 120°36.220'E), D.S. Tennakoon, SV028 (MFLU 18-2587, ***paratype***); living culture, NCYUCC 19-0107.

Notes. *Phaeosphaeriopsis beaucarneae* is similar to other *Phaeosphaeriopsis* species in having scattered, semi-immersed to erumpent, globose to subglobose, ostiolate ascomata and cylindrical to clavate asci and light brown, verrucose ascospores (Phookamsak et al. 2014; Thambugala et al. 2014; Hyde et al. 2020). According to

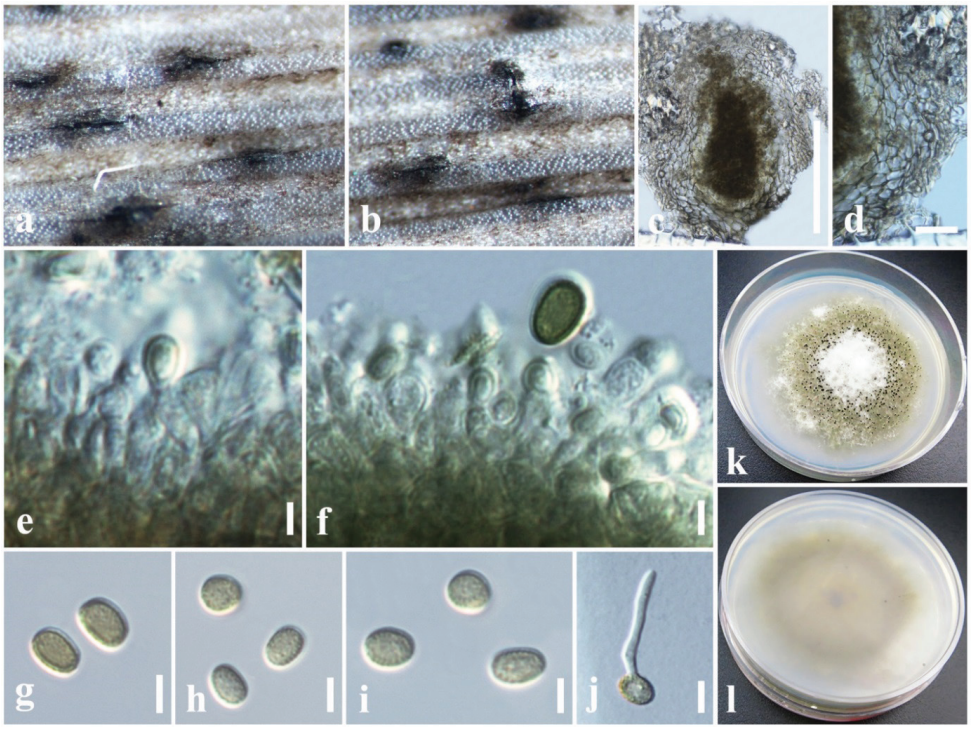

Figure 5. *Phaeosphaeriopsis beaucarneae* (MFLU 18-2586, paratype) **a** appearance of conidiomata on host **b** close up of conidiomata **c** vertical section through conidioma **d** conidiomatal wall **e, f** conidiogenous cells and developing conidia **g–i** conidia **j** germinated conidium in PDA **k** colony from above **l** colony from below. Scale bars: 100 μm (**c**), 20 μm (**d**), 3 μm (**e, f**), 5 μm (**g–j**).

the present multi-gene phylogenetic analyses (Figure 1), *Phaeosphaeriopsis beaucarneae* is grouped with other *Phaeosphaeriopsis* species, in particularly closely to *P. grevilleae* (CBS 145369) with high statistical support (70% ML, 75% MP, 0.99 BYPP, Figure 1). The asexual morph of *P. grevilleae* was isolated from leaves of *Grevillea* sp. (Proteaceae) and introduced by Marin-Felix et al. (2019). *Phaeosphaeriopsis beaucarneae* differs from *P. grevilleae* in having larger conidia (6.8–7.4 × 3–4 μm), whereas *P. grevilleae* has comparatively smaller conidia (5 × 3.5 μm). A comparison of the 516 nucleotides across the ITS (+5.8S rDNA) gene region of *Phaeosphaeriopsis beaucarneae* and *P. grevilleae* (CBS 145369) revealed 16 base pair differences (3.10%). In addition, we compared our new taxon with *P. grevilleae* based on base pair differences in the *tef1-α* gene region. We found a total of 19 base pair differences (3.06%) across 619 nucleotides.

Recent studies have revealed that *Phaeosphaeriopsis* is a species rich genus and numerous *Phaeosphaeriopsis* species have been described during the last few years (Thambugala et al. 2014; Tibpromma et al. 2017; Marin-Felix et al. 2019; Al-Jaradi et al. 2020; Hyde et al. 2020). With this study, the number of *Phaeosphaeriopsis* species increases to 18.

Neosetophoma Gruyter, Aveskamp & Verkley, Mycologia 102(5): 1075 (2010)

Notes. *Neosetophoma* was introduced by de Gruyter et al. (2010), typified by *N. samararum* (Desm.) Gruyter, Aveskamp. & Verkley. Species of *Neosetophoma* are characterized by globose to irregular conidiomata, with papillate ostioles, and yellowish conidia that are attenuate at one end (De Gruyter et al. 2010; Liu et al. 2015). Tibpromma et al. (2017) introduced *Neosetophoma garethjonesii* Tibpromma, E.B.G. Jones & K.D. Hyde as the first report of the sexual morph of *Neosetophoma*. *Neosetophoma* species have a diverse distribution as saprobes, endophytes, plant pathogens and soil fungi (Phookamsak et al. 2014; Hernandez-Restrepo et al. 2016; Karunarathna et al. 2017; Tibpromma et al. 2017; Wanasinghe et al. 2018). Currently, 19 *Neosetophoma* species are accepted in Index Fungorum (2020). In this study, we found *Neosetophoma poaceicola* Goonas., Thambug. & K.D. Hyde from dead leaves of *Musa acuminata* Colla in Taiwan. This is the first *Neosetophoma* species recorded from the plant family Musaceae.

Neosetophoma poaceicola Goonas., Thambug. & K.D. Hyde. Mycosphere 8: 742 (2017)

Index Fungorum number: IF552974
Facesoffungi number: FoF00262
Figure 6

Diagnosis. *Saprobic* on dead leaf petioles of *Musa acuminata* Colla (Musaceae). **Sexual morph:** *Ascomata* 70–100 µm high, 90–130 µm diam., solitary, gregarious, coriaceous, immersed to semi-immersed, slightly raised, visible as black spots on host surface, uni-loculate, dark brown to black, globose to ovoid. *Peridium* 15–20 µm wide, thick-walled, of equal thickness, composed of several layers of dark brown to brown, pseudoparenchymatous cells of *textura angularis*. *Hamathecium* of 1–2 µm wide, cellular, rarely branching, pseudoparaphyses, anastomosing mostly above the asci and embedded in a mucilaginous matrix. *Asci* 60–80 × 7–8 µm ($\bar{x}$ = 70.6 × 7.6 µm, n = 30), 8-spored, bitunicate, fissitunicate, cylindric-clavate with a short, rounded pedicel, apically rounded. *Ascospores* 20–30 × 3–4 µm ($\bar{x}$ = 25.5 × 3.7 µm, n = 40), overlapping 1–2-seriate, hyaline, fusiform, with acute ends, 1-septate, 3–4 eu-septate, cell near the septum slightly larger, slightly constricted at the septum, straight to curved, smooth-walled, guttulate. **Asexual morph:** Undetermined.

Culture characteristics. Colonies on PDA reaching 30 mm diameter after 3 weeks at 20–25 °C, colonies medium sparse, circular, flat, surface slightly rough with entire edge, margin well-defined, colony from above: yellow to light brown at the margin, brown at the center; reverse, yellow to light brown at the margin, dark brown at the center; mycelium light brown to whitish grey with tufting; not producing pigments in PDA.

Material examined. Taiwan, Chiayi, Fanlu Township area, Dahu Forest, dead leaf petiole of *Musa acuminata* Colla (Musaceae), 21 July 2018 (23°27.530'N, 120°36.340'E), D.S. Tennakoon, SV049 (MFLU 18-2597, **new host record**), living culture, MFLUCC 18-1632, NCYUCC 19-0119.

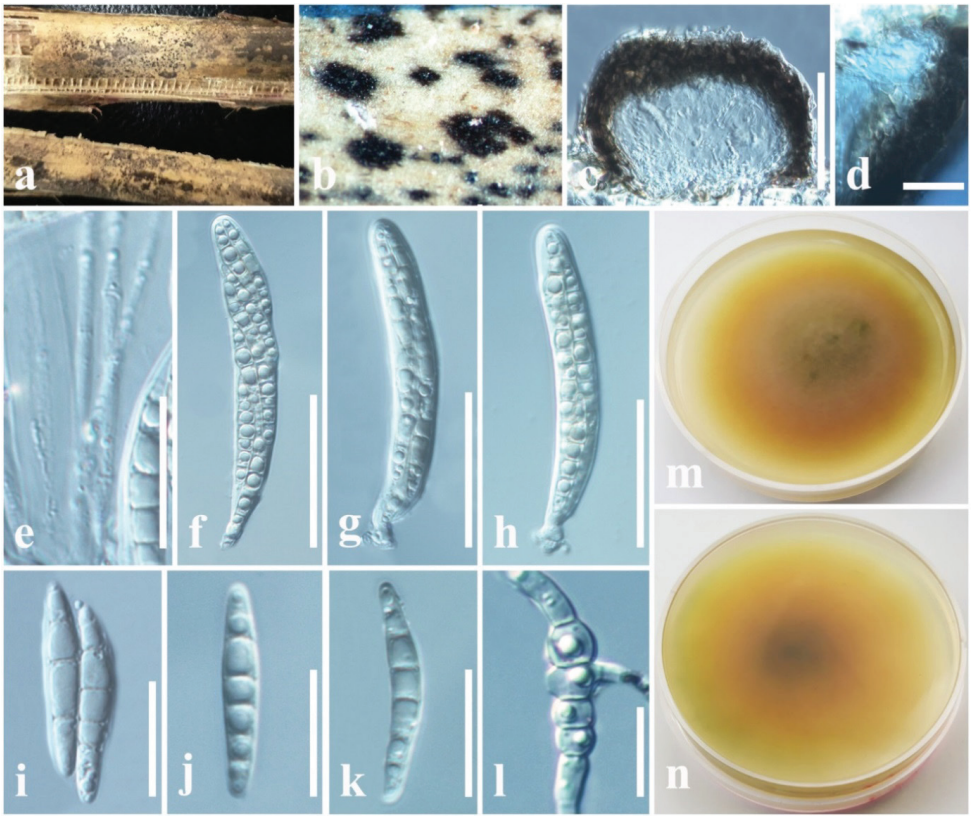

Figure 6. *Neosetophoma poaceicola* (MFLU 18–2597, new host record) **a** appearance of ascomata on host **b** close up of ascomata **c** vertical section through ascoma **d** peridium **e** pseudoparaphyses **f–h** asci **i–k** ascospores **l** germinated ascospore in PDA **m** colony from above **n** colony from below. Scale bars: 50 µm (**c**), 20 µm (**d**), 30 µm (**e–h**), 15 µm (**i–l**).

Notes. As morphological characters (immersed to semi-immersed ascomata, cylindric-clavate, apically rounded asci with short rounded pedicel and hyaline, fusiform, 1-septate ascospores) largely overlap with those of *Neosetophoma poaceicola* (MFLUCC 16–0886), we report our collection (MFLUCC 18-1632) as a new host record of *N. poaceicola* from dead leaves of *Musa acuminata* (Musaceae) in Taiwan. Combined multi-gene (LSU, SSU, ITS and *tef1-α*) based phylogenies also showed that our collection clustered with *Neosetophoma poaceicola* (MFLUCC 16-0886), with high bootstrap support (100% ML, 100% MP, 1.00 BYPP, Figure 1). *Neosetophoma poaceicola* was introduced by Thambugala et al. (2017) from dead leaves of grass species in Thailand. However, our collection slightly differs from *Neosetophoma poaceicola* (MFLUCC 16-0886) in having comparatively slightly larger ascospores (20–30 × 3–4 µm, versus 18.5–22.5 × 3.5–5 µm).

Neosetophoma species have been recorded from various host families, viz. Brassicaceae, Caprifoliaceae, Iridaceae, Malvaceae, Ranunculaceae, Salicaceae, but most are reported from Poaceae (Phookamsak et al. 2014; Karunarathna et al. 2017; Tibpromma et al. 2017, Wanasinghe et al. 2018; Marin-Felix et al. 2019). Interestingly, this is the first *Neosetophoma* species record (MFLU 18-2597) from the plant family Musaceae.

Discussion

The taxonomy of Phaeosphaeriaceae has been subjected to several changes in recent years. Traditionally, morphology-based identification was the main means for identifying Phaeosphaeriaceae species (Barr 1979, 1992; Tomilin 1993). However, species identification has been revolutionized by the application of molecular based approaches incorporating DNA sequence data in Phaeosphaeriaceae (Phookamsak et al. 2014, 2017; Tennakoon et al. 2016; Wanasinghe et al. 2018; Bakhshi et al. 2019; Chethana et al. 2020; Hyde et al. 2020). Phaeosphaeriaceae species are adapted to a wide range of ecological environments and are present in soils, fresh and marine habitats and cause infections in humans (Yuan 1994; Phookamsak et al. 2014, 2017; Ahmed et al. 2017; Maharachchikumbura et al. 2019; Valenzuela-Lopez et al. 2019). Members of the Phaeosphaeriaceae have also been recorded from both temperate and tropical countries (i.e. Austria, Belgium, Bulgaria, Canada, China, Germany, Italy, Japan, Norway, Poland, Thailand, Sweden, Switzerland) and from different host families (i. e. Acoraceae, Arecaceae, Cyperaceae, Asparagaceae, Brassicaceae, Fabaceae, Poaceae, Marantaceae) (Shoemaker and Babcock 1989; Phookamsak et al. 2014, 2019; Wanasinghe et al. 2018; Maharachchikumbura et al. 2019; Farr and Rossman 2020). Due to their cosmopolitan distribution, in the last few years, many researchers have paid significant attention to the Phaeosphaeriaceae (Phookamsak et al. 2014, 2019; Tennakoon et al. 2016; Wanasinghe et al. 2018; Bakhshi et al. 2019; Hyde et al. 2020).

The fungi that decay leaf litter are highly diverse and may be host-specific (Parangao et al. 2002). Several studies have examined the succession of leaf degrading communities and found unique sets of species on different types of litter (Promputtha et al. 2002, 2017; Duong et al. 2008). Additional ecological studies are therefore needed to establish whether these fungi are generalists or specialists. This study provides evidence to indicate the fungal diversity in leaf litter, even within a single family, Phaeosphaeriaceae. Additional work is necessary to identify if the newly described species are host specific.

Acknowledgments

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RESEARCH ARTICLE

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The genus *Clavariadelphus* (Clavariadelphaceae, Gomphales) in China

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Abstract

Clavariadelphus species (Clavariadelphaceae, Gomphales) in China were examined using morphology, molecular phylogenetic analyses of ITS data and chemical reactions. Eleven taxa were identified in China, including four species known previously to occur in China (*C. griseoclavus*, *C. ligula*, *C. sachalinensis* and *C. yunnanensis*), two new record species from China (*C. elongatus* and *C. himalayensis*), four novel species (*C. alpinus*, *C. amplus*, *C. gansuensis* and *C. khinganensis*) and one species that could not be described due to the paucity of material. Finally, we also provided a taxonomic key for the identification of *Clavariadelphus* species in China.

Keywords

Clavarioid fungi, taxonomy, molecular systematics, new taxa, species diversity

Introduction

Clavariadelphus Donk (Clavariadelphaceae, Gomphales, Basidiomycota), typified by *C. pistillaris* (L.) Donk, is a group of fungi characterised by erect, simple, club-shaped basidiomes with rhizomorphs at the stipe base, hymenium with (2–) 4-spored basidia,

* Have equally contributed to this work.

clavate leptocystidia, ellipsoid to amygdaliform, thin-walled, inamyloid basidiospores and clamp connections at the septa of the hyphae (Methven 1990). The genus is widely distributed in temperate regions of the Northern Hemisphere and 24 species were described before this study.

Clavariadelphus has been studied in Europe and North America and important taxonomic works are available (Corner 1950, 1970; Welden 1966; Smith and Corner 1967; Petersen 1967, 1972; Smith 1971; Petersen et al. 1974; Methven 1989; Methven and Guzmán 1989). The genus has not received as much attention in Asia, except for a couple of novel species described from Pakistan (Hanif et al. 2014; Sher et al. 2018). In China, two novel taxa have been described (Methven 1989; Lu and Li 2020). To date, only seven *Clavariadelphus* species have been reported in China, namely *C. griseoclavus* L. Fan & L. Xia, *C. ligula* (Schaeff.) Donk, *C. pallido-incarnatus* Methven, *C. pistillaris*, *C. sachalinensis* (S. Imai) Corner, *C. truncatus* Donk and *C. yunnanensis* Methven (Methven 1989, 1990; Mao et al. 1993; Yuan and Sun 1995; Zang 1996; Bau et al. 2003; Mao 2009; Tang and Yang 2014; Tang 2015; Lu and Li 2020). The studies, in which these species were identified, are comparatively brief and solely based on morphological criteria except *C. griseoclavus*.

Although *Clavariadelphus* can be readily distinguished from other members of the Gomphales, the delimitation of infrageneric taxa is difficult in many cases due to subtle variations in morphological characteristics and growth habits (Methven 1990). Recently, molecular techniques have been widely applied and have provided useful information for species delimitation in systematic fungal studies (Hibbett 2007; Yang 2011). Chemical reactions are also helpful in delimiting species of many macrofungal groups besides *Clavariadelphus*, including *Agaricus*, *Boletopsis*, *Chroogomphus*, *Cortinarius*, *Hygrophorus*, *Leucoagaricus* and *Leratiomyces* (Corner 1950; Hanif et al. 2014; Siegel and Schwarz 2016). Scanning electron microscopy (SEM) has been applied to the identification of other macrofungal groups (Zeng et al. 2013; Tang et al. 2014; Huang et al. 2018). However, SEM of structures of *Clavariadelphus* has not yet been reported. We mainly examined Chinese *Clavariadelphus* collections through analysis of morphological characteristics using light microscopy and SEM, as well as molecular phylogenetic data, ecological data and chemical reactions, to better understand species diversity of *Clavariadelphus* in China.

Materials and methods

Morphological studies

Aside from one collection from the Czech Republic, most specimens of *Clavariadelphus* in this study were collected from coniferous forests or mixed coniferous and broad-leaved forests in North (N) China, Northwest (NW) China and Southwest (SW) China during the rainy seasons (July–September). Collections and field records are deposited in the Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences (HKAS), Mycological Herbarium, Institute of Mycology, Chinese Academy of Sciences (HMAS), Mycological Herbarium of Hunan Normal University (MHHNU)

and Mycological Herbarium of Pharmacy College, Kunming Medical University (MHKMU) (Appendix 1). Specimens and their habitats were photographed *in situ*. Relevant metadata, such as altitude, latitude, longitude and nearby tree associates were recorded in the field. Detailed notes on macro-morphological descriptions were taken from fresh material and colour codes were from Kornerup and Wanscher (1981).

Light microscopy

Micro-morphological characteristics were observed under a light microscope (Leica DM 2500). Preparations were made from dried specimens. Tissue fragments of dried materials were sectioned, mounted in 10% KOH and observed. The abbreviation [n/m/p] means n basidiospores measured from m basidiomes of p collections. Dimensions for basidiospores are given as (a) b–c (d). The range of b–c contains a minimum of 90% of the measured values. Extreme values, i.e. a and d are given in parentheses. Q is used to denote the length/width ratio of basidiospores in the side view, whereas Q_m refers to the average Q value of all basidiospores ± standard deviation.

Scanning electron microscopy

The material was sampled and directly used from herbarium collections. The hymenium and basal mycelium from dried specimens were mounted on to aluminium stubs coated with gold palladium. Basidiospores and hyphae of the basal mycelium were observed and micrographs were taken with a ZEISS Sigma 300 scanning electron microscope at 7.0 kV accelerating voltage.

Chemical reactions

Seven chemical reagents were used: 10% (w/v) KOH, 10% (w/v) $FeCl_3$, 10% (w/v) $FeSO_4$, 10% NH_4OH, 10% (w/v) phenol, Melzer's reagent and 95% (v/v) ethanol. Small slices of tissue were taken from the hymenium of the basidiomes. The reagents were systematically added to the depression in plates so that each piece of tissue was submerged in several drops of a single reagent. Positive colour reactions were recorded immediately following the application of reagents.

DNA extraction, PCR and DNA sequencing

Total genomic DNA was isolated from dried materials using a modified CTAB method (Doyle 1987) with a prolongation of the extraction period as necessary. For PCR reactions, the nuclear ribosomal DNA internal transcribed spacer (ITS) region was amplified using primers ITS5 and ITS4 (White et al. 1990). The PCR amplification mix consisted of a

total volume of 25 μl containing 2.5 μl of 10 × amplification buffer (with $MgCl_2$), 0.5 μl dNTP (200 μM), 0.2 μl Taq DNA polymerase (5 U/μl), 1 μl of each primer (10 μM), 1 μl DNA template and 18.8 μl sterile water. PCR reactions were performed with an initial denaturation at 94 °C for 4 min; 38 cycles of denaturation at 94 °C for 40 s, annealing at 54 °C for 40 s, extension at 72 °C for 60 s; and a final extension at 72 °C for 8 min. PCR products were checked on 1% agarose gel. Successful reactions were sequenced using an ABI 3730 DNA Analyzer (Sangon, Shanghai, China) with both PCR primers. The DNA sequences were used as queries in NCBI BLAST searches to rule out contamination. The forward and reverse sequences were assembled with SeqMan (DNASTAR Lasergene 9) and their quality controlled according to the guidelines of Nilsson et al. (2012). Novel and already available sequences were aligned by using MAFFT version 7 (Katoh and Standley 2013). The alignment was manually adjusted in BioEdit version 7.0.9 (Hall 1999) and trimmed in trimAl version 1.2 (Capella-Gutiérrez et al. 2009).

Phylogenetic analyses

Two phylogenetic tree inference methods, Randomised Accelerated Maximum Likelihood (RAxML) and Bayesian Analysis (BA), were used to analyse the ITS sequence data. The programme RAxML version 7.0.3 (Stamatakis et al. 2008) was used to infer a maximum likelihood tree with bootstrap support values and the GTRGAMMA was selected as a default model. The programme MrBayes version 3.2.6 (Ronquist et al. 2012) was run using a Markov Chain Monte Carlo (MCMC) tree sampling procedure. The ITS1, 5.8S and ITS2 loci were extracted from the aligned ITS dataset, allowing the selection of substitution models for each partition. Aligned sequences were partitioned into ITS1 (1–270), 5.8S (271–429) and ITS2 (430–703). Nucleotide substitution models based on the Akaike Information Criteria (AIC) data were obtained in PartitionFinder 2 (Lanfear et al. 2016). The selected models were GTR+G for ITS1, K80 for 5.8S and HKY+G for ITS2. After four simultaneous Markov chains running with 7,000,000 generations and sampling every 100 generations, the average deviation of split frequencies was 0.004022 at the end of the run. Burn-in values were determined in Tracer v1.7 (Rambaut et al. 2018). Effective sample sizes were well over 200 for all sampled parameters for each run and the initial 20% of the samples was discarded. Bayesian Posterior Probabilities (PP) were calculated for a majority consensus tree of the retained Bayesian trees.

Results

Taxonomic identification based on morphological data

Fifty specimens of *Clavariadelphus* were examined in this study. Six species were previously reported from China, except the late described one, *C. griseoclavus*. However,

the re-examination of available vouchers confirmed the occurrence of only three of these species, specifically *C. ligula*, *C. sachalinensis* and *C. yunnanensis*. Our morphological observations revealed that nine taxa, including three species previously identified in China (*C. ligula*, *C. sachalinensis* and *C. yunnanensis*), two species that have not been previously reported from China (*C. elongatus* and *C. himalayensis*) and four novel species (*C. alpinus*, *C. amplus*, *C. gansuensis* and *C. khinganensis*), were identified on the basis of morphological characters. So far, there are ten described taxa in China, including *C. griseoclavus* which is recently published.

Taxonomic identification based on molecular data

The ITS dataset comprised 27 ingroup taxa including the type species *C. pistillaris* and three outgroup taxa, with 64 sequences in total. The length of the alignment was 703 aligned bases (TreeBASE accession 24163). Three species of *Lentaria* Corner and *Kavinia* Pilát were chosen as outgroups in the dataset, based on a previous study (Giachini et al. 2010).

In the phylogeny, based on ITS sequences, few differences in the topology of major clades were detected between the ML and Bayesian analyses. Twenty-seven phylogenetic species were recovered, amongst which, eleven species were from China, including one with a GenBank accession JQ991679 from Zhejiang Province, China, which might represent a separate species in the tree (Fig. 1). *Clavariadelphus sachalinensis* formed a distinct lineage with high support and was sister to the rest of the genus. Seven Chinese lineages, namely *C. amplus*, *C. elongatus*, *C. griseoclavus*, *C. himalayensis*, *C. ligula*, *C. khinganensis* and *C. yunnanensis*, were strongly supported as monophyletic groups. The other two species from China, namely *C. alpinus* and *C. gansuensis*, were each represented by only one specimen in the phylogenetic tree. The sister of each Chinese taxon is discussed below.

Taxonomic identification based on chemical reactions

Steglich et al. (1984) proposed that a positive ferric salts reaction of the basidiomes was indicative of the presence of pistillarin in the basidiomes of *Clavariadelphus*. To a large extent, Methven's study (1990) supported this hypothesis, excluding one exception (*C. cokeri* V.L. Wells & Kempton). Methven (1990) mentioned the negative ferric salts reaction of some species might be the result of pistillarin being present in too low concentrations or the result of samples affected by pesticides during storage. In our study, most species have positive reactions with four reagents ($FeCl_3$, KOH, NH_4OH and phenol), but all species from China showed a negative reaction to $FeSO_4$, Melzer's reagent and ethanol. The results of the chemical testing in this study are summarised in Table 1. As those specimens are preserved, pesticides are used regularly. Thus, we agree with Methven's argument (1990).

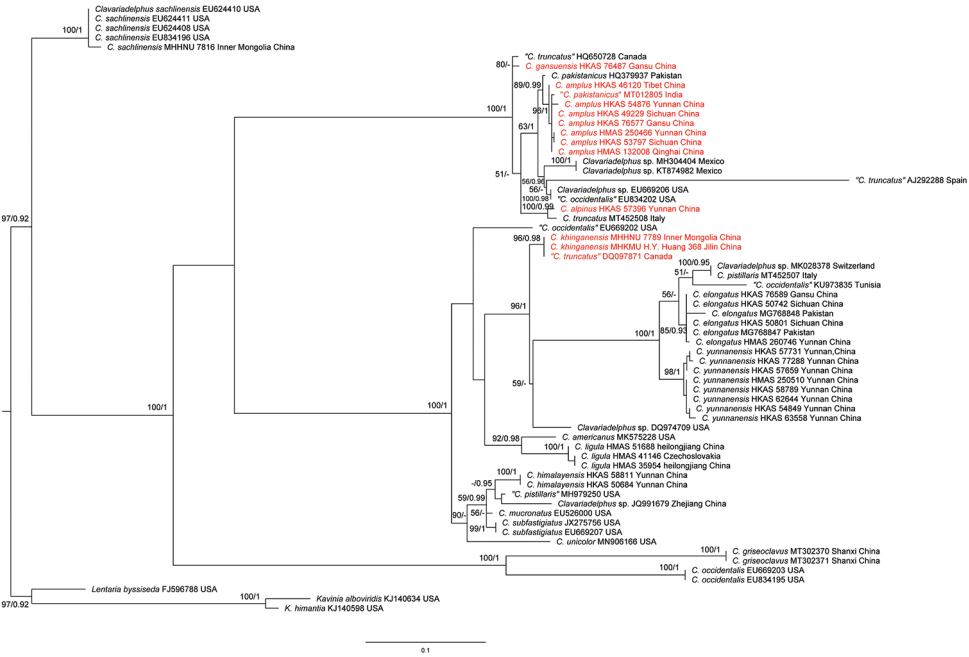

Figure 1. Phylogenetic tree of *Clavariadelphus* based on ITS sequence data. RAxML BP values (≥ 50%) are shown above branches, Bayesian posterior probabilities (≥ 0.90) are shown above branches; new taxa are marked in red.

Taxonomy

1. *Clavariadelphus alpinus* J. Zhao & L.P. Tang, sp. nov.
MycoBank No: 830258
Figs 2a, 3a, 4a, 5a, 6a, b

Diagnosis. This species is distinguished from other taxa in *Clavariadelphus* by the light yellow, clavate basidiomes with enlarged apex, broadly ellipsoid basidiospores, hyphae of the basal mycelium with nipple-shaped protuberances and basidiomes turning lemon-chiffon in KOH.

Etymology. Latin "*alpinus*" refers to this species occurring in high-altitude areas.

Description. *Basidiomes* up to 12 cm high, 0.9 cm diam. at the base, enlarged upwards to 2 cm diam., simple, initially cylindrical to subcylindrical, then narrowly clavate to clavate, laterally compressed in age; *hymenium* initially smooth, then longitudinally rugose, light yellow (4A4–5) to yellow or yellowish-orange, apricot-yellow, light orange-yellow (4A6–7) or (5A5–6); *apex* subacute to obtuse, smooth to rugose, concolorous with the hymenium; surface not staining when cut or bruised; *base* terete, smooth, white to cream; *mycelial hyphae* white; *flesh* initially solid, then soft and spongy upwards as the apex enlarges, white not staining on exposure. *Odour* and *taste* not recorded. *Spore deposit* not recorded.

Table 1. Chemical reactions of representative species of *Clavariadelphus* from China.

Taxa	KOH	$FeCl_3$	NH_4OH	Phenol	Ethanol	Melzer's reagent	$FeSO_4$
C. alpinus	3B8	–	6A8	–	–	–	–
C. amplus	12A4	1A8	2A8	2A5	–	–	–
C. elongatus	2A5	1A8	6A8	–	–	–	–
C. gansuensis	9B7	1A8	2A8	2A8	–	–	–
C. himalayensis	5B7	30A8	6A8	–	–	–	–
C. khinganensis	2A5	–	–	–	–	–	–
C. ligula	3B8	–	6A8	–	–	–	–
C. sachalinensis	2A5	30A8	6A8	–	–	–	–
C. yunnanensis	5B7	30A8	2A8	2A5	–	–	–

Note: "–" indicates negative reactions.

Hymenium extending over the apex of basidiomata, composed of basidia and leptocystidia. *Basidia* 65–85 × 8–10 µm, clavate, hyaline, thin-walled, (2–, 3–) 4-spored, sterigmata 8–12 µm in length. *Basidiospores* [20/1/1] (7.4–) 7.8–9.6 (–10.1) × 5.5 (–5.1)–7.4 µm, Q = 1.25–1.55 (–1.58), Q_m = 1.38 ± 0.10, broadly ellipsoid, ovate or amygdaliform, with a small apiculus, inamyloid, thin-walled, hyaline in KOH, smooth. *Leptocystidia* 45–55 × 2.8–4.2 µm, scattered amongst and scarcely projecting beyond the basidia, cylindrical to narrowly clavate, thin-walled, smooth, hyaline, non-pigmented, clamped, inflated apically at maturity and at times, with apical or subapical branches. *Mycelial hyphae* 2–4 µm diam., interwoven or aggregated into rhizomorphic strands, branched, clamped; hyphal walls echinulate with light microscopy, covered with massive nipple-shaped protuberances without crystals with SEM.

Chemical reactions (dried basidiomes). KOH = positive, lemon-chiffon; NH_4OH = positive, orange; ethanol, $FeCl_3$, $FeSO_4$, Melzer's reagent and phenol = negative.

Known distribution and ecology. SW China, Yunnan Province. Solitary on the ground in forests dominated by conifers (e.g. *Abies georgei*) at elevations of approximately 3700 m.

Materials examined. China. Yunnan Province: Shangri-la Prefecture, Bita Lake, 24 August 2009, approximately 3700 m elev., *B. Feng 667* (HKAS 57396, ***Holotype***).

Comments. *Clavariadelphus alpinus* is well characterised by its yellow basidiomes, broadly ellipsoid basidiospores, hyphae of the basal mycelium with nipple-shaped protuberances, the apex of the basidiomes having a positive reaction to NH_4OH and KOH and distribution at high elevations in SW China in association with conifers.

Morphologically, this taxon is similar to *C. khinganensis*. However, *C. khinganensis* has light brown-tan basidiomes, more elongated basidiospores (Q = 1.6–2.2), negative reaction to NH_4OH and distribution at lower elevations in NE China.

In the ITS phylogeny, this species is a sister species of *C. truncatus* with strong support (Fig. 1). However, *C. truncatus* differs from *C. alpinus* by having dark coloured basidiomes from yellow to cinnamon-brown or brown, broader apices (up to 3.5 cm) and larger basidiospores (10.3–12.6 × 5.5–7.1 µm from neotype; Methven 1990).

Figure 2. *Clavariadelphus* species in China. **a** *C. alpinus* (HKAS 57396, holotype) **b, c** *C. amplus* (HKAS 54876, holotype) **d, e** *C. elongatus* (**d** from HKAS 50742 **e** from HKAS 76589) **f** *C. gansuensis* (HKAS 76487, holotype) **g** *C. himalayensis* (HKAS 58811) **h, i** *C. khinganensis* (**h** from MHHNU 7789, holotype **i** from MHKMU H.Y. Huang 368) **j** *C. sachalinensis* (MHHNU 7816) **k, l** *C. yunnanensis* (**k** from HKAS 49398 **l** from HKAS 58789).

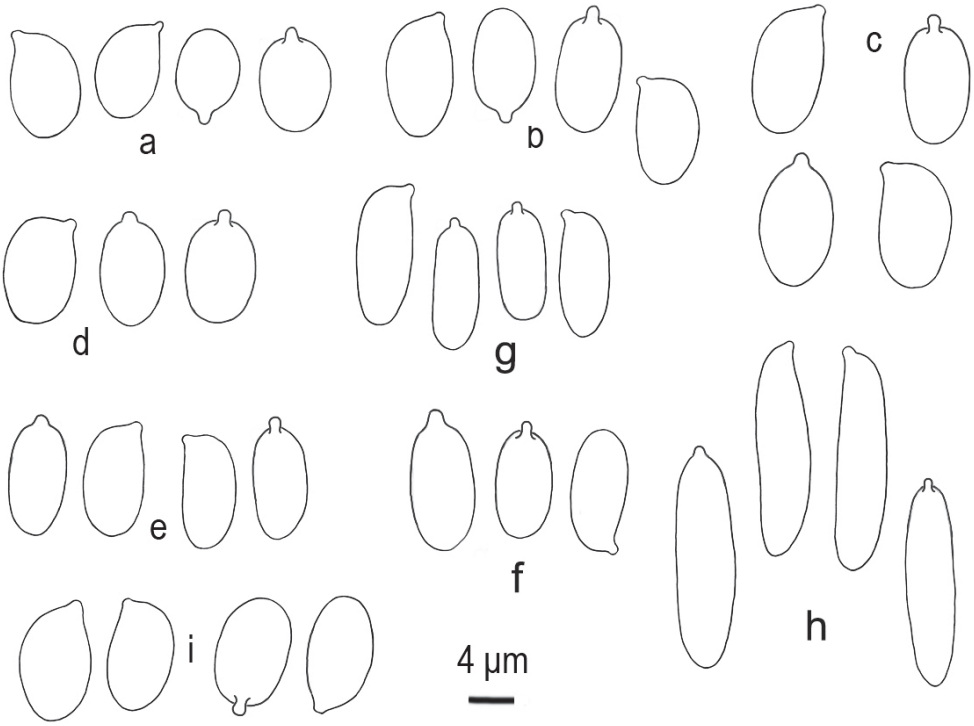

Figure 3. Basidiospores of *Clavariadelphus* under light microscope. **a** *C. alpinus* (HKAS 57396, holotype) **b** *C. amplus* (HKAS 54876, holotype) **c** *C. elongatus* (HKAS 76589) **d** *C. gansuensis* (HKAS 76487, holotype) **e** *C. himalayensis* (HKAS 58811) **f** *C. khinganensis* (MHHNU 7789, holotype) **g** *C. ligula* (HKAS 35954) **h** *C. sachalinensis* (MHHNU 7816) **i** *C. yunnanensis* (HKAS 57659).

2. *Clavariadelphus amplus* J. Zhao, L.P. Tang & Z.W. Ge, sp. nov.
MycoBank No: 830271
Figs 2b, c, 3b, 4b, 5b, 7a, b.

Diagnosis. This species is unique in its pink-orange basidiomes with enlarged, truncate and sterile apices, ellipsoid basidiospores, hyphae of the basal mycelium with nipple-shaped protuberances and prism-like crystals and basidiomes turning cherry-red in KOH. It differs from *C. truncatus* by the latter's darker coloured basidiomes, narrower apices and larger basidiospores.

Etymology. Latin "*amplus*" refers to the enlargement of the apex of the basidiomes.

Description. *Basidiomes* up to 15 cm high, 0.5–1 cm diam. at the base, enlarged upwards to 3–7.5 cm diam. near apex; *hymenium* initially smooth, longitudinally rugulose in age, pruinose, pinkish-orange (7A5–7), paler downwards, greyish-orange (5B4–5); *apex* initially obtuse or broadly rounded, finally truncate, depressed, surface rugose to rugulose, more or less darker than the hymenium, apricot-yellow (5B6–7) to pink-orange, reddish-orange (7A7–8) or red-orange (7B7–8) at maturity; surface slowly staining light brown or light leather-brown (7D6–7) to brown (7E6–7) when cut or bruised, staining more conspicuously downwards; *base* simple, terete, nearly

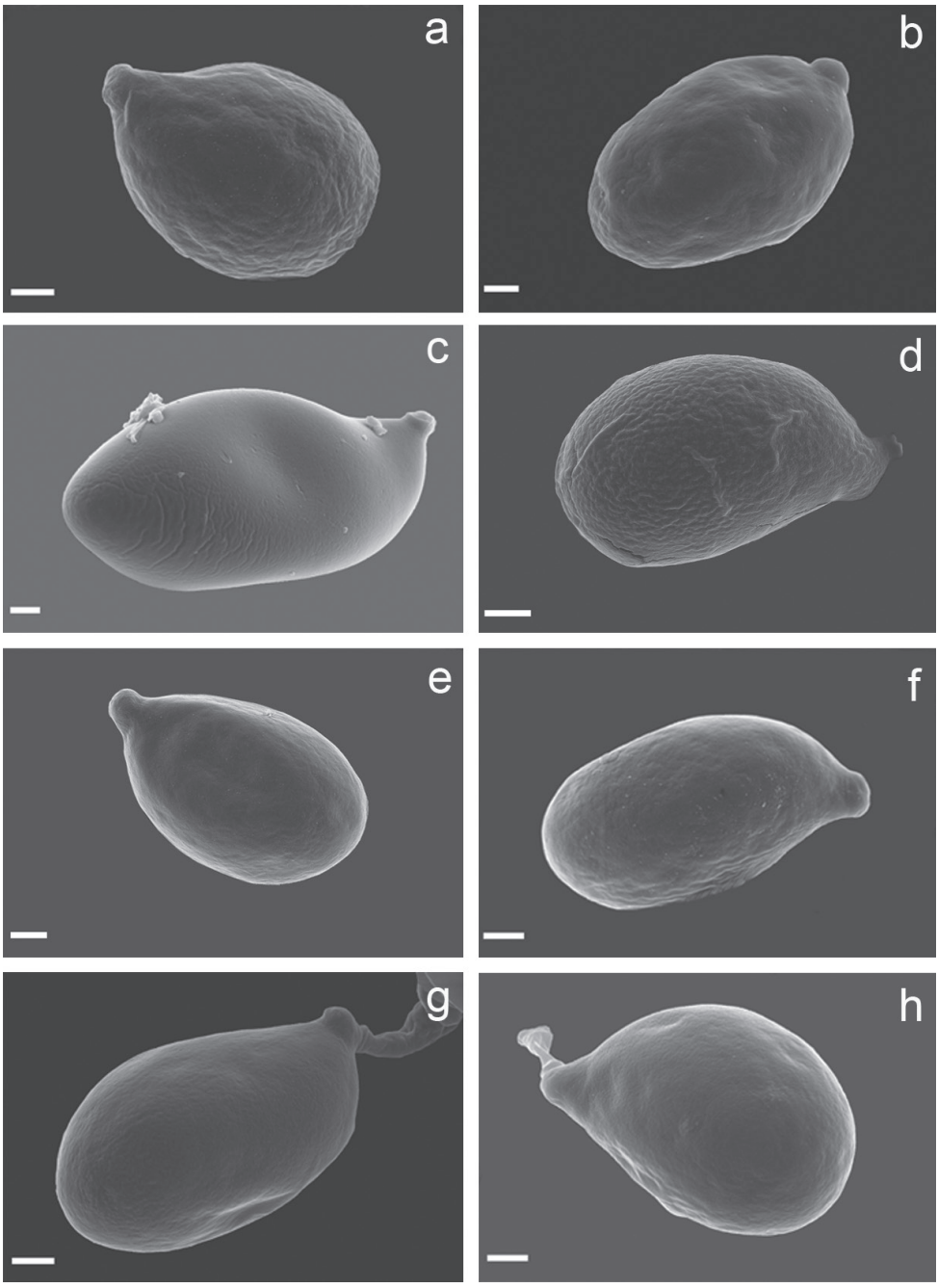

Figure 4. Basidiospores of *Clavariadelphus* under SEM. **a** *C. alpinus* (HKAS 57396, holotype) **b** *C. amplus* (HKAS 54876, holotype) **c** *C. elongatus* (HKAS 76589) **d** *C. gansuensis* (HKAS 76487, holotype); **e, f** *C. himalayensis* (HKAS 58811) **g** *C. khinganensis* (MHHNU 7789, holotype) **h** *C. yunnanensis* (HKAS 57659).

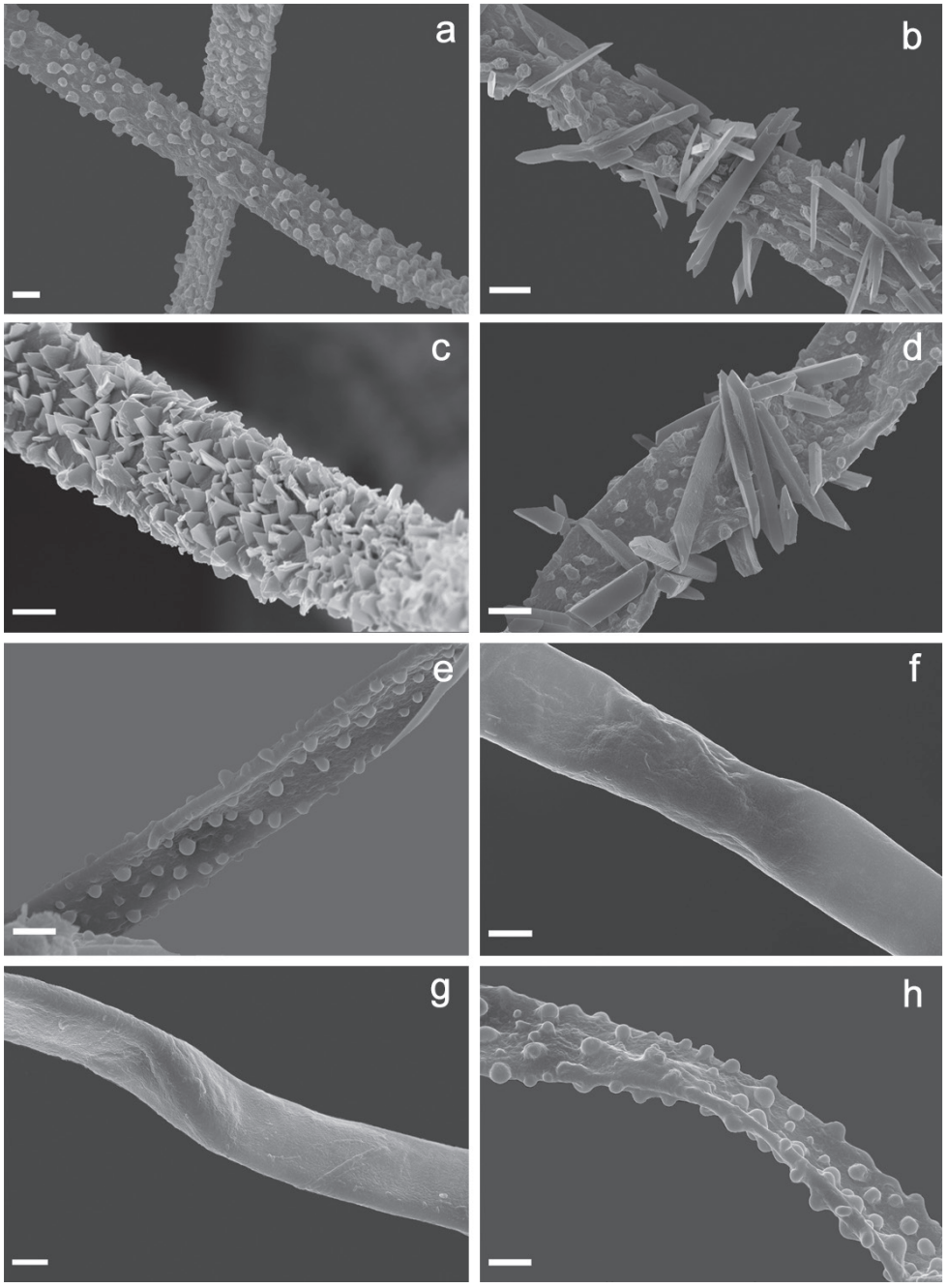

Figure 5. Hyphae of basal mycelium from *Clavariadelphus* under SEM. **a** *C. alpinus* (HKAS 57396, holotype) **b** *C. amplus* (HKAS 54876, holotype) **c** *C. elongatus* (HKAS 76589) **d** *C. gansuensis* (HKAS 76487, holotype) **e** *C. himalayensis* (HKAS 58811) **f, g** *C. sachalinensis* (**f** from HKAS 33844; **g** from MHHNU 7816) **h** *C. yunnanensis* (HKAS 57659).

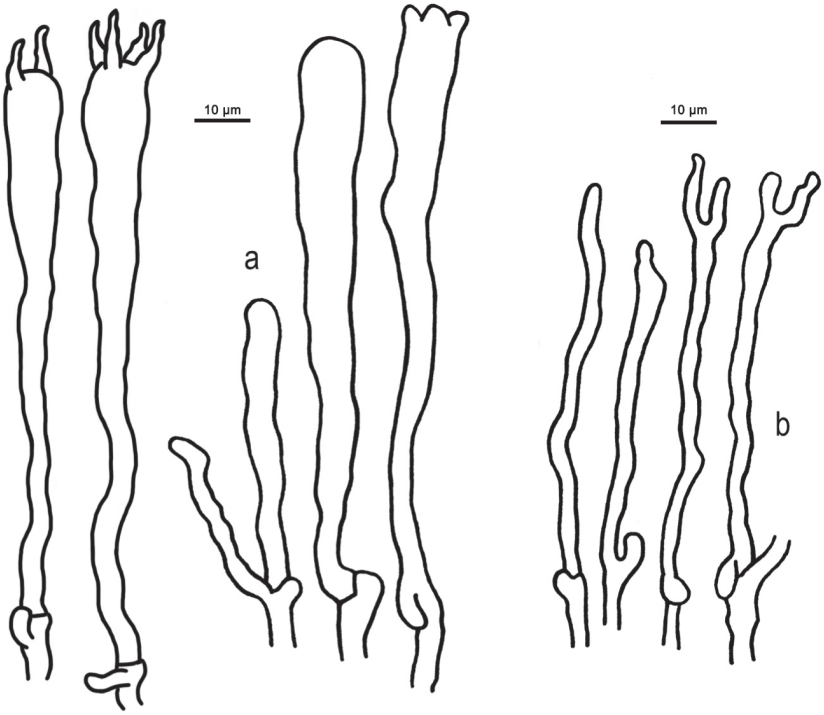

Figure 6. Microscopic features of *Clavariadelphus alpinus* (HKAS 57396, holotype). **a** Basidia **b** Leptocystidia.

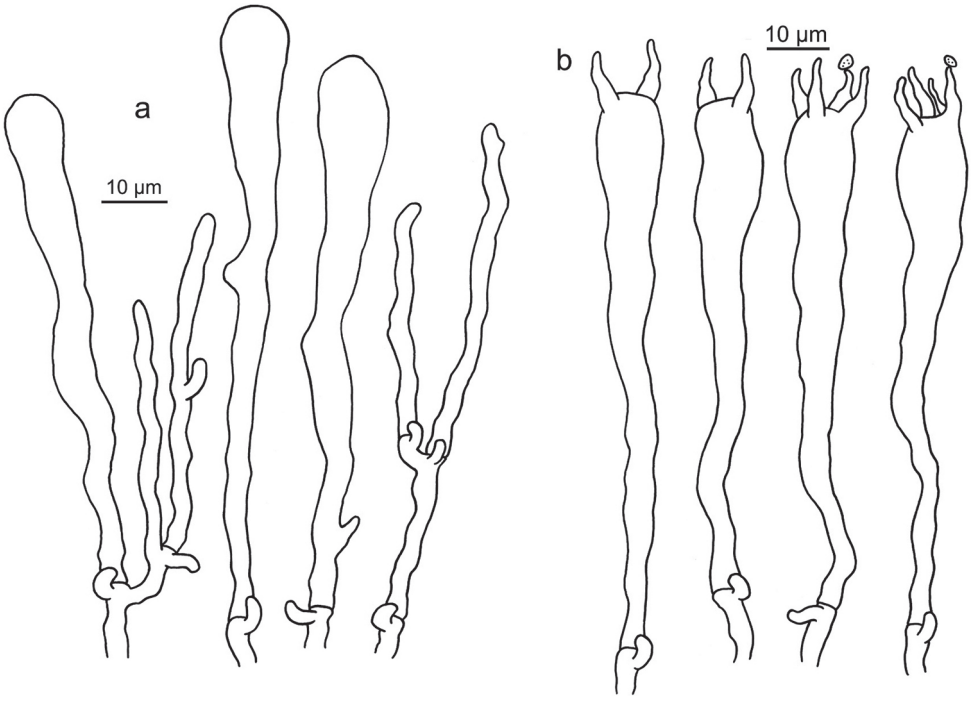

Figure 7. Microscopic features of *Clavariadelphus amplus* (HKAS 54876, holotype). **a** Leptocystidia and immature basidia **b** Basidia.

smooth, cylindrical to subcylindrical, pruinose; *mycelial hyphae* interwoven, white; *flesh* solid initially, then soft and spongy upwards as the apex enlarges, white, slowly staining light leather-brown (7D6–7) to brown (7E6–7) on exposure. *Odour* pleasant. *Taste* not distinctive. *Spore deposit* not recorded.

Hymenium limited to the sides of basidiomes, composed of basidia and leptocystidia; the apex of basidiomata is composed of sterile elements 18–28 × 5–8 µm, clavate, thin-walled, smooth, clamped. *Basidia* 85–95 × 8–12 µm, clavate, hyaline, thin-walled, (2–) 4-spored, sterigmata 9–11 µm in length. *Basidiospores* [40/2/2] 8.2–11.0 × 5.1–6.4 µm, *Q* = (1.36–) 1.38–2.00 (–2.18), Q_m = 1.75 ± 0.17, ellipsoid to broadly ellipsoid, ovate or amygdaliform, with a small apiculus, inamyloid, thin-walled, hyaline in KOH, smooth. *Leptocystidia* 45–70 × 2.8–3.8 µm, scattered amongst and scarcely projecting beyond the basidia, cylindrical to narrowly clavate, thin-walled, smooth, hyaline, non-pigmented, clamped, inflated apically at maturity and at times, with apical or subapical branches. *Mycelial hyphae* 2–4 µm diam., parallel, interwoven or aggregated into rhizomorphic strands, branched, clamped; walls thin or irregularly slightly thickened, the hyphal walls echinulate with light microscopy, covered with nipple-shaped protuberances, as well as encrusted with prism-like crystals (up to 6 µm long) that are insoluble in KOH.

Chemical reactions. (dried basidiomes): $FeCl_3$ = positive, green-yellow; KOH = positive, cherry-red or pink; NH_4OH = positive, golden-rod or vivid yellow; phenol = positive, light yellow; ethanol, $FeSO_4$, and Melzer's reagent = negative.

Known distribution and ecology. NW China and SW China, and India. Gregarious habit on the ground in conifer or mixed conifer forests (e.g. *Abies* spp. and *Picea* spp.) at elevations ranging from 3000–3950 m.

Materials examined. China. Gansu Province: Zhouqu Prefecture, under *Abies* spp., 6 August 2005, *X.T. Zhu 728* (HKAS 76577). Qinghai Province: Qilian mountains, 38°6.00'N, 100°7.03'E, alt. 3000 m, 21 August 2004, *H.A Wen 4305* (HMAS 132008); same location and date, *Q.B. Wang 438* (HMAS 97090). Sichuan Province: Seda Prefecture, *Picea-Juniperus* forests, 31°43.20'N, 100°43.17'E, alt. 3775–3925 m, 6 August 2005, *Z.W. Ge 783* (HKAS 49278); Litang Prefecture, 5 August 2007, *Z.W. Ge 1712* (HKAS 53797). Tibet: Linzhi City, 29°20.07'N, 094°18.00'E, alt. 3850 m, 19 July 2004, *Y.H. Wang 125* (HMAS 97248); Jilong Prefecture, on the ground in coniferous woods, 12 September 1990, *J.Y. Zhuang 3814* (HMAS 59867); Chengdu City, under forests dominated by *Picea* spp., 31°30.43'N, 097°20.07'E, alt. 3480–3550 m, 17 August 2004, *Z.W. Ge 381* (HKAS 46160); Riwoqe Prefecture, under *Picea* spp., 31°14.27'N, 096°31.92'E, alt. 3890 m, 12 August 2004, *Z.W. Ge 340* (HKAS 46120). Yunnan Province: Shangri-La Prefecture, Haba Snow Mountains, alt. 2800 m, 15 August 2008, *L.P. Tang 645* (HKAS 54876, ***Holotype***); Shangri-La Prefecture, 27°28.13'N, 099°25.03'E, alt. 3600 m, 15 August 2008, *T.Z. Wei* 172 (HMAS 250466).

Comments. *Clavariadelphus amplus* is distinctive by its pink-orange to red-orange, bright basidiomes, obviously enlarged, truncate, depressed, sterile apices (up to 7.5 m diam.) at maturity, large basidiospores (8.2–11.0 × 5.1–6.4 µm), gregarious habit at high elevations, base mycelial hyphae with nipple-shaped protuberances and prism-

like crystals and a cherry-red staining reaction to KOH. It is sold as an edible mushroom in markets in SW China. This taxon has a wide distribution in NW and SW China, including Gansu, Qinghai, Sichuan, Tibet and Yunnan Provinces. The data from GenBank (accession MT012805) also indicated its distribution of India.

This species was previously referred to as either *C. pallido-incarnatus* (Yuan and Sun 1995) or *C. truncatus* (Mao et al., 1993; Zang 1996; Mao 2009; Tang and Yang 2014; Tang 2015). *Clavariadelphus pallido-incarnatus*, a species described from the Pacific Northwest in North America, has pale pinkish-cinnamon basidiomes with fertile, non-truncated apices, no reactivity to KOH and habitat preference for coastal forests of *Sequoia sempervirens* and *Picea sitchensis* (Methven 1990). *Clavariadelphus truncatus* from Europe is readily confused with *C. amplus* as they have similar size and truncate sterile apex. However, *C. truncatus* has dark coloured basidiomes from yellow to cinnamon-brown or brown, narrower apices (up to 3.5 cm) and larger basidiospores (10.3–12.6 × 5.5–7.1 μm from neotype; Methven 1990). *Clavariadelphus unicolor* (Berk. & Ravenel) Corner, is also from North America and has enlarged sterile apices, but it is distinct in its reddish-brown to violet-brown basidiomes, narrow basidiospores with Q_m 2.1, a golden-yellow reaction to KOH and association with deciduous trees (Methven 1990).

So far, there are two species with sterile apices found in China, *C. amplus* and *C. gansuensis*. However, *C. gansuensis* has a narrower apex (up 1.6 cm), slightly broader basidiospores with a lower Q value (8.3–10.1 × 5.3–6.3 μm, Q = 1.47 –1.78, Q_m = 1.60) and a solitary growth habit. Except for the mentioned species, *C. amplus* is also similar to *C. pakistanicus*. *Clavariadelphus pakistanicus*, another species also from Asia, is distinct in smaller basidiomes (up to 12 cm high), with narrower fertile apices (up to 1.4 cm), smaller basidiospores (7.5–9.2 × 4.0–5.6 μm), solitary growth habit at lower elevations and violet-brown staining reactions to KOH (Hanif et al. 2014).

In the ITS tree, *C. amplus* exhibits a sister relationship with *C. pakistanicus* with strong support (Fig.1).

3. *Clavariadelphus elongatus* J. Khan, Sher & Khalid, Phytotaxa 365: 184, 2018

Figs 2d, 2e, 3c, 4c, 5c, 8a, 8b

Note. The following description is taken from Sher et al. (2018), field notes of the Chinese material including macro-morphology, growth habit, distribution, host plants and our examination of the specimens.

Description. *Basidiomes* up to 28 cm high, 0.5–1.0 cm diam. basally, enlarged upwards to 1.5 cm diam., subcylindrical to fusiform, simple or occasionally branched, laterally compressed in age; *hymenium* longitudinally rugose, plum colour (13C2–4) or light purple to greyish-purple (14C2–3) or dull-lilac (15D2–3); *apex* tapered, subacute to obtuse, initially smooth, rugulose in age, caramel-brown to sandy-brown or sienna (6C5–6); *base* terete, smooth, white; *mycelial hyphae* scant, white; *flesh* initially solid, then soft and spongy in age. *Odour* and *taste* not recorded.

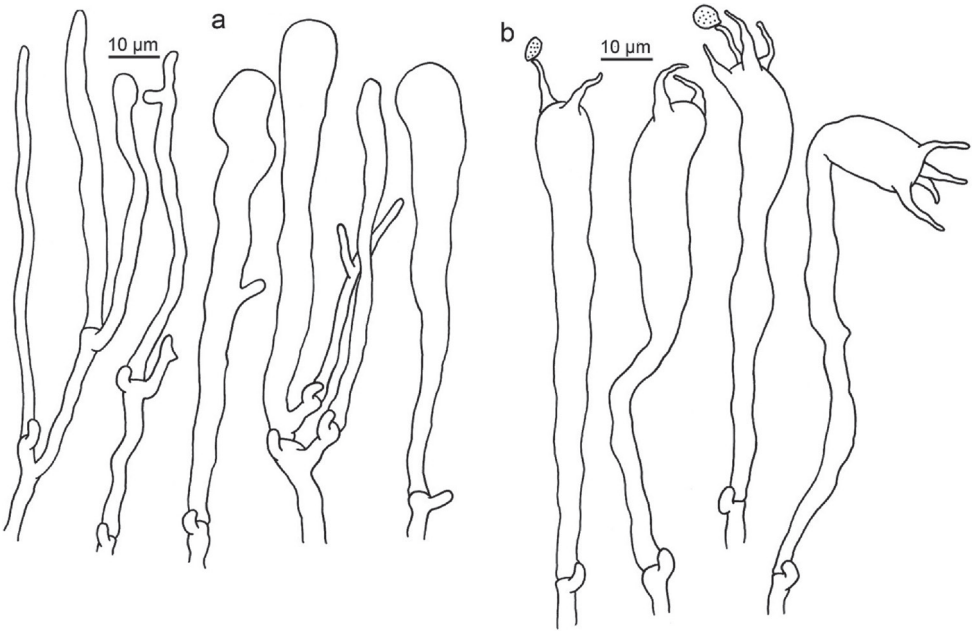

Figure 8. Microscopic features of *Clavariadelphus elongatus* (HKAS 76589). **a** Leptocystidia and immature basidia **b** Basidia.

Hymenium extending over the apex of the basidiomata, composed of basidia and leptocystidia. *Basidia* 75–95 × 6–10 µm, clavate, hyaline, thin-walled, 4-spored, sterigmata 7–10 µm in length. *Basidiospores* [40/2/2] (8.3–) 9.0–11.0 (–12.0) × (5.5–) 5.7–7.4 µm, Q = (1.43–) 1.44–2.04 (–2.31), Q_m = 1.71 ± 0.16, narrowly ellipsoid to ellipsoid, ovate or amygdaliform, with a small apiculus, inamyloid, thin-walled, hyaline in KOH, smooth. *Leptocystidia* 70–75 × 3.5–4.5 µm, scattered amongst and scarcely projecting beyond the basidia, cylindrical to narrowly clavate, thin-walled, smooth, hyaline, non-pigmented, clamped, inflated apically at maturity, at times with apical or subapical branches. *Mycelial hyphae* 2–3 or 6–8 µm diam., interwoven or aggregated into rhizomorphic strands, branched, clamped; the hyphal walls echinulate with light microscopy, encrusted with massive triangular or irregular, flaky crystals up 1 µm high, which are insoluble in KOH.

Chemical reactions. (dried basidiomes): KOH = positive, light yellow; $FeCl_3$ = positive, green-yellow; NH_4OH = positive, orange; ethanol, $FeSO_4$, phenol and Melzer's reagent = negative.

Known distribution and ecology. NW and SW China (in this study), Pakistan (Sher et al. 2018). Solitary to scattered on the ground in coniferous woods (*Abies* spp. and *Picea* spp.) or mixed with broad-leaved trees (*Quercus* spp., *Rhododendron* spp. and *Salix* spp.) at elevations ranging from 3000–4350 m.

Materials examined. China. Gansu Province: Zhouqu Prefecture, Shatan National Forest Park, *Abies* spp. woods, 16 August 2012, *X.T Zhu 740* (HKAS 76589). Sichuan

Province: Litang Prefecture, Gaowa, Kobresia-Bistorta meadows with extensive areas of dwarf *Rhododendron* and *Salix* scrub with *Picea* spp., 30°10.10'N, 100°35.12'E, alt. 4300–4350 m, 8 August 2006, *Z.W. Ge 1221* (HKAS 50801); Yajiang Prefecture, meadows with shrub thickets and *Picea* spp. forests, 30°2.67'N, 101°18.48'E, alt. 3850–3870 m, 4 August 2006, *Z.W. Ge 1162* (HKAS 50742). Yunnan Province: Yulong Prefecture, Lizui Village, mixed coniferous and broad-leaved forests of *Picea* spp. and *Quercus* spp., alt. 3000 m, 23 August 2007, *Y. Zhang 36* (HKAS 52425); Shangri-La Prefecture, 27°29.00'N, 99°25.00'E, alt. 3600 m, 13 August 2008, *T.Z. Wei 150* (HMAS 260746).

Comments. *Clavariadelphus elongatus* was originally described from Pakistan (Sher et al. 2018). In this study, it was found in NW and SW China. This species is unique in its greyish-purple basidiomes with acute to subacute, non-enlarged apex, hyphae of the basal mycelium encrusted with massive, flaky crystals and basidiomes having a light yellow reaction to KOH. *Clavariadelphus himalayensis*, another Asian taxon, might be confused with *C. elongatus* since both have a tinge of grey-purple when young. However, *C. himalayensis* is distinct in having smaller basidiomes, pastel-red colouration at maturation, shorter basidiospores (8.2–9.4 × 5.0–5.5 μm), hyphae of the basal mycelium covered nipple-shaped protuberances without crystals and basidiomes having a brown-yellow reaction to KOH.

Phylogenetically, *C. elongatus* is related to *C. pistillaris* and the sequence of "*C. occidentalis*" from GenBank with weak support (Fig. 1).

4. *Clavariadelphus gansuensis* J. Zhao & L.P. Tang, sp. nov.

MycoBank No: 830272
Figs 2f, 3d, 4d, 5d, 9a, b

Diagnosis. This species is characterised by its orange, clavate basidiomes with slightly enlarged, truncate, sterile apex, broadly ellipsoid to ellipsoid basidiospores, hyphae of the basal mycelium with nipple-shaped protuberances and prism-like crystals and basidiomes that turn pink or light cherry-red in KOH. It differs from *C. truncatus* by the latter's robust, darker basidiomes with enlarged apices, and larger basidiospores.

Etymology. Latin "*gansuensis*" refers to the holotype location in Gansu Province.

Description. *Basidiomes* up to 9 cm high, enlarged upwards to 1.6 cm diam., simple, clavate; *hymenium* longitudinally rugose, pruinose, light yellow to greyish-orange at maturity; *apex* initially obtuse or broadly rounded, flattening laterally, then truncate, slightly rugose, light orange or melon-orange (5A5–7) to orange (6A6–7) in age; *base* terete, smooth, pruinose, dirty white or pallid where covered, otherwise pruinose, pale orange or light orange (5A3–4); *mycelial hyphae* white; *flesh* initially solid, then soft and spongy upwards as the apex enlarges, white to pallid. *Odour* and *taste* not recorded.

Hymenium limited to the side of basidiomata, composed of basidia and leptocystidia; the apex of basidiomata composed of sterile elements 15–25 × 5–7 μm, clavate, thin-walled, smooth, clamped. *Basidia* 75–90 × 8–10 μm, clavate, hyaline, thin-walled to thick-walled, 4-spored, sterigmata 7–10 μm in length. *Basidiospores* [20/1/1] 8.3–10.1 (–10.3) × 5.3–6.3 (–6.4) μm, Q = (1.34–) 1.47 –1.78 (–1.83), Q_m = 1.60 ± 0.09,

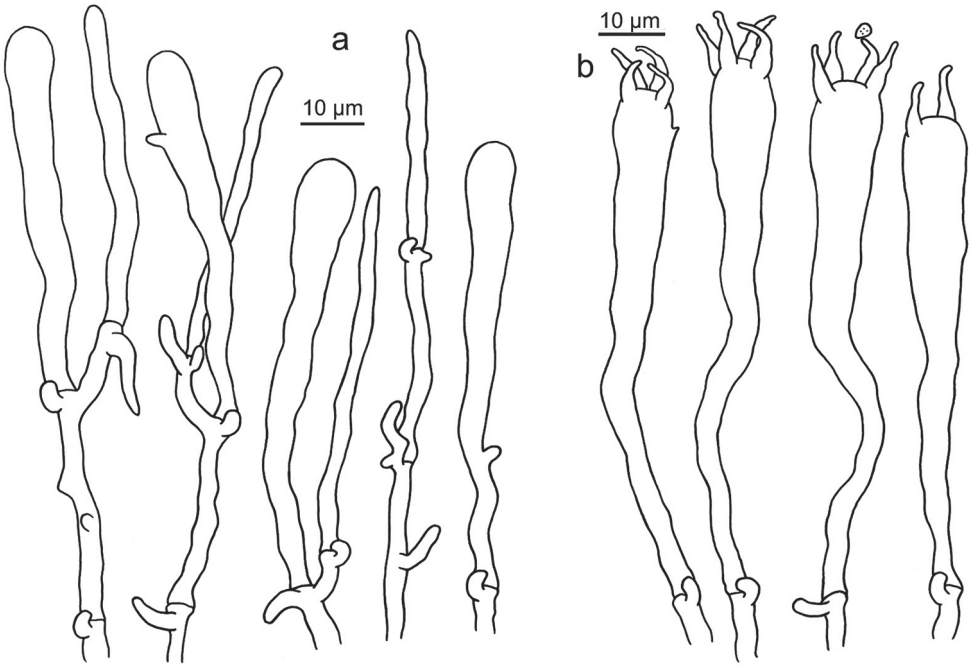

Figure 9. Microscopic features of *Clavariadelphus gansuensis* (HKAS 76487, holotype). **a** Leptocystidia and immature basidia **b** Basidia.

ellipsoid to broadly ellipsoid, ovate or amygdaliform, with a small apiculus, inamyloid, thin-walled, hyaline in KOH. *Leptocystidia* 50–65 × 3–5 μm, scattered amongst and scarcely projecting beyond the basidia, cylindrical to narrowly clavate, thin-walled, smooth, hyaline, non-pigmented, clamped, inflated apically at maturity, at times with apical or sub-apical branches. *Mycelial hyphae* 2–3 μm diam., interwoven or aggregated into rhizomorphic strands, branched, clamped; the hyphal walls echinulate with light microscopy, covered with massive nipple-shaped protuberances, as well as encrusted with prism-like crystals up 5 μm long that are insoluble in KOH.

Chemical reactions. (dried basidiomes): KOH = positive, pink, light coral or light cherry-red; $FeCl_3$ = positive, green-yellow; NH_4OH = positive, golden-rod or vivid yellow; phenol = positive, yellow; ethanol, $FeSO_4$ and Melzer's reagent = negative.

Known distribution and ecology. NW China, Gansu Province. Solitary on the ground in coniferous woods (*Abies* spp.) or mixed with broad-leaved trees (*Betula* spp. and Rosaceae) at elevations of approximately 3000 m.

Materials examined. China. Gansu Province: Lintan Prefecture, Yeliguan National Forest Park, coniferous woods (*Abies* spp.) or mixed with *Betula* spp. and Rosaceae plants, alt. 3000 m, 10 August 2012, *X.T. Zhu 638* (HKAS 76487, ***Holotype***); Wudu Prefecture, September 1992, *M.L. Tian M6465* (HMAS 63052).

Comments. *Clavariadelphus gansuensis*, currently known only from NW China, is distinct by its slender, clavate, orange basidiomes with truncate apex, ellipsoid basidiospores (8.3–10.1 × 5.3–6.3 μm), pink staining reaction to KOH, hyphae of the

basal mycelium with nipple-shaped protuberances and prism-like crystals and solitary growth habit in coniferous or mixed forests.

This species is most likely to be confused with several taxa, including *C. amplus*, *C. pallido-incarnatus*, *C. pakistanicus*, *C. truncatus* and *C. unicolor*. The comparison between *C. gansuensis* and *C. amplus* can be found in our treatment of *C. amplus*.

According to our phylogenetic analyses, *C. gansuensis* is allied with the sequence of "*C. truncatus*" from GenBank with strong support (Fig. 1).

5. *Clavariadelphus himalayensis* Methven, Mem. New York Bot. Garden 49: 152, 1989

Figs 2g, 3e, 4e, f, 5e, 10a, b

Note. The following description is mainly from Methven (1989), combined with our field notes, including macro-morphology, growth habit, distribution, host plants and examination.

Description. *Basidiomes* up to 15 cm high, 1–1.5 cm diam. basally, slightly enlarged towards to 2 cm diam., simple, narrow clavate, ligulate to spathulate, laterally compressed in mature specimens; *hymenium* initially smooth, longitudinally rugose in age, greyish-red to pastel-red; *apex* obtuse, smooth, concolorous with the hymenium; surface not staining where cut or bruised; *base* terete, smooth, pruinose, pallid-white; *mycelial hyphae* interwoven, white to pallid; *flesh* soft and spongy, hollow apically in age, white to cream colour, not staining on exposure. *Odour* and *taste* not recorded.

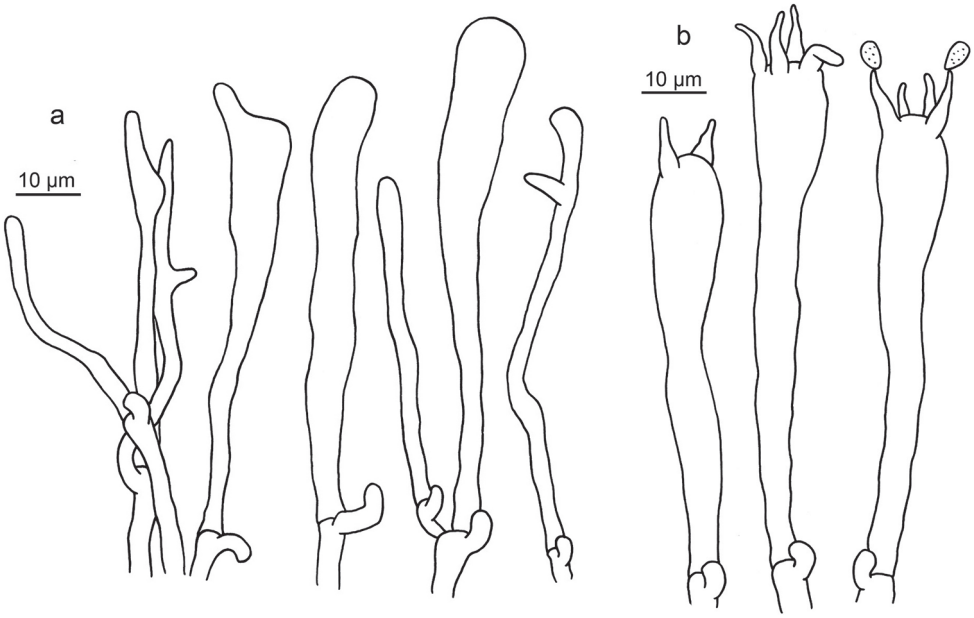

Figure 10. Microscopic features of *Clavariadelphus himalayensis* (HKAS 58811). **a** Leptocystidia and immature basidia **b** Basidia.

Hymenium extending over the apex of basidiomata, composed of basidia and leptocystidia. *Basidia* 75–95 × 8–11 μm, clavate, hyaline, thin-walled, (2–) 4-spored, sterigmata 8–10 μm in length. *Basidiospores* [20/1/1] (7.8–) 8.2– 9.4 (–9.6) × (4.6–) 5.0–5.5 (–6.0) μm, Q = 1.50–1.82 (–1.90), Q_m = 1.56 ± 0.08, ellipsoid to broadly ellipsoid or ovate, with a small apiculus, inamyloid, thin-walled, hyaline in KOH, smooth. *Leptocystidia* 50–70 × 2.5–3.5 μm, scattered amongst and scarcely projecting beyond the basidia, cylindrical to narrowly clavate, thin-walled, smooth, hyaline, non-pigmented, clamped, inflated apically at maturity, at times with apical or subapical branches. *Mycelial hyphae* 1–2 or 3–5 μm diam., interwoven or aggregated into rhizomorphic strands, branched, clamped; walls thin or irregularly slightly thickened, the hyphal walls echinulate under light microscopy, covered nipple-shaped protuberances with SEM.

Chemical reactions. (dried basidiomes): KOH = positive, golden-yellow; $FeCl_3$ = positive, green-yellow; NH_4OH = positive, orange; ethanol, $FeSO_4$, Melzer's reagent and phenol = negative.

Known distribution and ecology. SW China (in this study) and India (Methven 1989). Solitary to gregarious habit on the ground in mixed woods at elevations above 3200 m.

Materials examined. China. Yunnan Province: Shangri-La Prefecture, mixed coniferous (*Pinus* spp.) and broad-leaved forests (*Caragana* spp., dwarf *Quercus monimotricha* and *Sanguisorba* spp.), 27°28.55'N, 99°53.05'E, alt. 3280 m, 27 June 2006, *Z.W. Ge 1113* (HKAS 50684). Lijiang Prefecture, mixed conifers, alt. 3300 m, 27 August 2009, *Q. Cai 146* (HKAS 58811).

Comments. *Clavariadelphus himalayensis* was originally described from India (Methven 1989). It is the first report from China. Chinese collections match the original descriptions except for slightly smaller basidiospores (8.2–9.4 × 5.0–5.5 μm). The difference in basidiospore size might be from measurement error or the collections being from different geographical regions. *Clavariadelphus himalayensis* is distinct by its pastel-red to greyish-red, ligulate to spathulate basidiomes flesh that does not stain where bruised or cut, broadly ellipsoid basidiospores (9–11 × 5–6 μm from the holotype; Methven 1989), hyphae of the basal mycelium with nipple-shaped protuberances and a negative reaction with phenol. Other taxa from Asia, which might be confused with *C. himalayensis* include *C. mirus* (Pat.) Corner and *C. yunnanensis*. Although similar in size to those of *C. himalayensis*, the basidiomes of *C. mirus* are light brown to brown and produce broadly ovate, larger basidiospores (10–13 × 6–8 μm; Methven 1990). *Clavariadelphus yunnanensis*, known from northern India and SW China, is distinct by its larger basidiomes that are light brown, larger basidiospores (10–13.5 × 6.5–8 μm), hyphae of the basal mycelium covered by massive nipple-shaped protuberances and a light yellow staining reaction with phenol. Additionally, the flesh of *C. himalayensis* does not stain where bruised or cut, whereas the flesh of *C. mirus* and *C. yunnanensis* slowly stains brunnescent to russet on exposure.

The phylogenetic analyses show that *C. himalayensis* is allied with the sequence of "*C. pistillaris*" and *Clavariadelphus* (JQ991679 from Zhejiang Province, China) from GenBank with weak support (Fig. 1). More data are needed for understanding the phylogenetic relationship of the three species.

6. *Clavariadelphus khinganensis* J. Zhao, L.P. Tang & P. Zhang, sp. nov.
MycoBank No: 830273
Figs 2h–i, 3f, 4g, 11a, b

Diagnosis. This species is distinct from other taxa in *Clavariadelphus* by the yellowish-brown, clavate basidiomes with slightly enlarged apex, narrowly ellipsoid basidiospores and basidiomes that turn very light yellow in KOH.

Etymology. Latin "*khinganensis*" refers to the holotype location, Greater Khingan Mountains or Da Xing'an Ling, in NE China.

Description. *Basidiomes* up to 12.5 cm high, around 0.8 cm diam. basally, 2.5 cm diam. apically, simple, initially subcylindrical to subfusiform, enlarged upwards in age, then clavate to broadly clavate, finally irregularly laterally compressed; *hymenium* initially smooth, longitudinally rugose to rugulose in age, pale yellow-brown (4A3) or pale orange (5A 4–6) to greyish-orange (5B4–5, 6B4–5); *apex* obtuse or broadly rounded, rugose, concolorous with the hymenium at maturity; *base* terete, smooth, white to pallid when covered, otherwise pale yellow (4A4–5) to light orange (5A4–6); *mycelial hyphae* interwoven, white; *flesh* initially solid, becoming soft and spongy upwards as the apex enlarges in age, dirty white. *Odour* and *taste* not recorded. *Spore deposit* not recorded.

Hymenium extending over the apex of basidiomata, composed of basidia and leptocystidia. *Basidia* 85–105 × 8–11 μm, clavate, hyaline, thin-walled, 4-spored, sterigmata 9–10 μm in length. *Basidiospores* [20/1/1] 9.2–12.0 × 4.6–6 μm, Q = 1.6–2.2, Q_m = 1.97 ± 0.17, narrowly ellipsoid or amygdaliform, with a small apiculus, inamyloid, thin-walled, hyaline in KOH, smooth. *Leptocystidia* 60–70 × 3–4 μm, scattered amongst and scarcely projecting beyond the basidia, cylindrical to narrowly clavate, thin-walled, smooth, hyaline, non-pigmented, clamped, inflated apically at maturity, at times with apical or subapical branches. *Mycelial hyphae* lacking material.

Chemical reactions. (dried basidiomes): KOH = positive, very light yellow; ethanol, $FeCl_3$, $FeSO_4$, phenol, Melzer's reagent and NH_4OH = negative.

Known distribution and ecology. N China. Solitary on the ground in broad-leaved forests at around 800 m altitude.

Materials examined. CHINA. Jilin Province: Antu Prefecture, Er-dao-bai-he Town, Changbai Mountains, mainly broad-leaved forests (*Betula platyphylla*, *Corylus mandshurica*, and *Quercus monimotricha*), mixed with the coniferous tree (*Pinus koraiensis*), 42°24.05'N, 128°6.00'E, alt. 753 m, 18 August 2019, *H.Y. Huang 368* (MHKMU H.Y. Huang 368). Inner Mongolia: De-er-bu-er Town, Greater Khingan Mountains, alt. 800 m, 6 August 2013, *P. Zhang 1289* (MHHNU 7789 ***Holotype***); Ku-ti-he Town, Zha-lan-tun City, 24 July 1985, *W. Huang s. n.* (HMAS 49920).

Comments. *Clavariadelphus khinganensis*, known from broad-leaved forests in N China, is distinct by its solitary habit at low elevations (around 800 m), small size, pale brown-orange basidiomes, ellipsoid basidiospores and very pale yellow reaction in KOH.

Morphologically, *C. khinganensis* is quite similar to two Asian taxa, *C. mirus* and *C. yunnanensis*. However, *C. mirus* was originally described from northern Vietnam and has larger basidiomes, broader basidiospores and a tropical distribution (Butan, India,

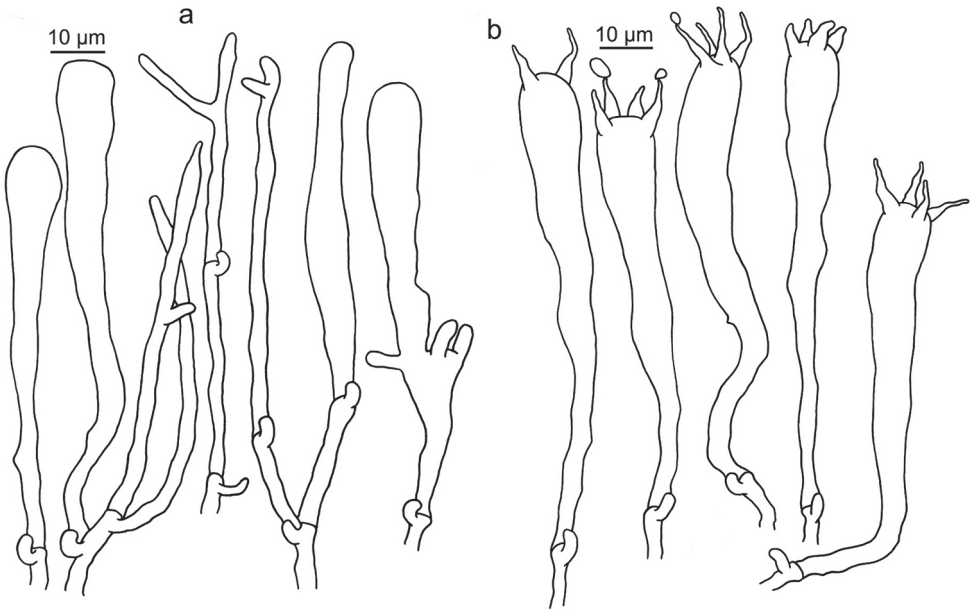

Figure 11. Microscopic features of *Clavariadelphus khinganensis* (MHHNU 7789, holotype). **a** Leptocystidia and immature basidia **b** Basidia.

Nepal; Methven 1990). *Clavariadelphus yunnanensis* is unique in its habit, growing with conifers at high elevations (above 3000 m), has darker colouration and larger basidiomes (up to 20 cm high), broader basidiospores and basidiomes with yellow reactivity in KOH.

Interestingly, *C. khinganensis* is clustered with a collection labeled as "*C. truncatus*" from Canada, the GenBank accession DQ097871 (Durall et al. 2006) and there are no genetic differences on ITS (Fig. 1). It indicates *C. khinganensis* may be distributed in Canada. More data from North America are needed to confirm the distribution pattern of this species. The sister relationship of *C. khinganensis* cannot be resolved according to the present data.

7. *Clavariadelphus ligula* (Schaeff.) Donk, Rev. Niederl. Homob. Aphyll. 2: 73, 1933

Figs 3g, 12a, b

Note. The following taxonomic description is drawn from Methven (1990) and our observations.

Description. *Basidiomes* up to 10 cm high, 0.2–0.8 cm diam. basally, slightly enlarged upwards, simple, narrowly clavate to clavate; *hymenium* longitudinally rugose in age, light yellow, brownish-orange to light brown at maturity; *apex* subacute to obtuse or broadly rounded, surface slightly rugulose, concolorous with the hymenium; surface

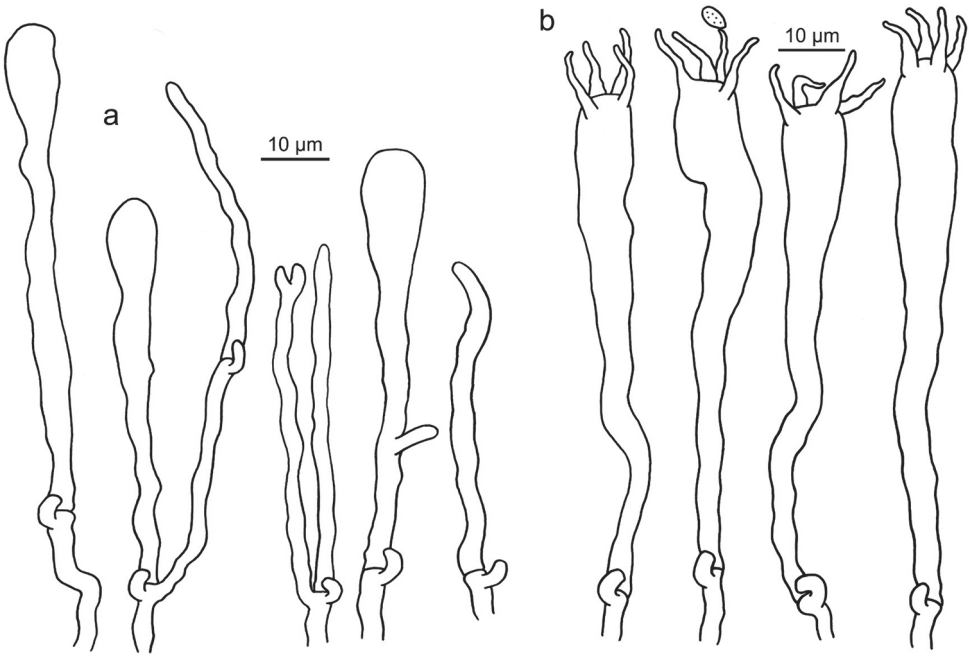

Figure 12. Microscopic features of *Clavariadelphus ligula* (HMAS 35954). **a** Leptocystidia and immature basidia **b** Basidia.

slowly staining brownish-orange to brownish-grey where cut or bruised; *base* terete, initially pale yellow to light yellow, then brownish-orange to light brown to brown; *mycelial hyphae* white to pallid; *flesh* initially solid, becoming soft and spongy upwards as the apex enlarges in age, white to pallid. *Odour* not distinctive. *Taste* not distinctive or slightly sweet. *Spore deposit* yellowish-white to light buff in mass.

Hymenium extending over the apex of basidiomata, composed of basidia and leptocystidia. *Basidia* 45–85 × 8–11 μm, clavate, hyaline, thin-walled, 4-spored, sterigmata 9–10 μm in length. *Basidiospores* 11.0–14.0 × 4.0–5.5 μm, Q = 2.4–3.1, Q_m = 2.7, narrowly ellipsoid, with a small apiculus, inamyloid, thin-walled, hyaline in KOH, smooth. *Leptocystidia* 40–80 × 2.5–5 μm, scattered amongst and scarcely projecting beyond the basidia, cylindrical to narrowly clavate, thin-walled, smooth, hyaline, non-pigmented, clamped, inflated apically at maturity, at times with apical or subapical branches. *Mycelial hyphae* 2–4 μm diam., interwoven or aggregated into rhizomorphic strands, branched, clamped. Insufficient material to perform SEM.

Chemical reactions. (dried basidiomes): KOH = positive, lemon-chiffon; NH_4OH = positive, orange; ethanol, $FeCl_3$, $FeSO_4$, Melzer's reagent and phenol = negative.

Known distribution and ecology. Widespread in the Northern Hemisphere, including in North America, Bulgaria, NE China, England, Estonia, Finland, Germany, India, Italy, Sweden and Switzerland (Methven 1990). Scattered to gregarious habit on the ground in mixed woods (*Abies*, *Picea*, *Pinus*, *Thuja* and *Tsuga*).

Materials examined. China. Heilongjiang Province: Linkou Prefecture, 19 August 1972, *X.L. Mao*, *s. n.* (HKAS 35954); same location, *Q.X. Wu*, *s. n.* (HMAS 51688). Czech: 2 September 1960, *M. Geesteranus 13290* (HMAS 41146).

Comments. *Clavariadelphus ligula* was originally described from Germany, but was also reported in China (Mao 2009). Our study confirms that this species is mainly found in N China, whereas our data do not support the previous report of the distribution in SW China (Mao et al. 1993; Mao 2009). The basidiospores of Chinese collections (11.0–14.0 × 4.0–5.5 µm, Q = 2.4–3.1, Q_m = 2.7) are smaller and broader than the neotype of *C. ligula* from Germany (12.0–16.5 × 3.5–4.5 µm, Q = 2.9–4.6, Q_m = 3.7; Methven 1990).

Morphologically, *C. ligula* and *C. sachalinensis* are similar in the field. However, *C. sachalinensis* has more elongated, narrower basidiospores (21–24 × 4–6 µm, Q = 3.5–5.0, Q_m = 4.2). Additionally, *C. ligula* lacks any reaction with $FeCl_3$, whereas *C. sachalinensis* turns green-yellow in $FeCl_3$. *Clavariadelphus yunnanensis* is likely to be confused with *C. ligula* when young. However, *C. yunnanensis* differs in that it has larger basidiomes (up to 20 cm high), smaller and broader basidiospores (9.0–11.0 × 4.6–6.4 µm, Q = 1.32–1.72, Q_m = 1.56) and a positive reaction with phenol.

The phylogenetic analyses show that *C. ligula* is allied with the sequence of *C. americanus* from GenBank with strong support (Fig. 1).

8. *Clavariadelphus sachalinensis* (S. Imai) Corner, Ann. Bot. Mem. I: 282, 1950
Figs 2j, 3h, 5f, g, 13a, b

Note. The following taxonomic description is drawn from Methven (1989), combined with our field notes, including macro-morphology, growth habit, distribution, host plants and observations.

Description. *Basidiomes* up to 8 cm high, 0.3–0.6 cm diam. basally, slightly enlarged upwards 0.8–1.2 cm diam., simple, initially cylindrical to subcylindrical, then narrowly clavate to clavate; *hymenium* longitudinally rugose in age, tawny or light walnut-brown to light brown at maturity; *apex* subacute, obtuse to broadly rounded, surface smooth to slightly rugulose, concolorous with the hymenium; surface slowly staining, brown or dark brown where cut or bruised, staining more conspicuously; *base* terete, pubescent to tomentose, initially pale yellow to light yellow, then brownish-orange to light brown; *mycelial hyphae* greyish to pallid; *flesh* initially solid, becoming soft and spongy upwards, white to pallid, staining on exposure. *Odour* and *taste* not distinctive. *Spore deposit* yellowish-white to light buff.

Hymenium extending over the apex of basidiomata, composed of basidia and leptocystidia. *Basidia* 65–105 × 8–12.5 µm, clavate, hyaline, thin-walled, (2–) 4-spored, sterigmata 8–10 µm in length. *Basidiospores* 21–24 × 4–6 µm, Q = 3.5–5.0, Q_m = 4.2, narrowly ellipsoid, boletoid or sway-backed in profile, with a small apiculus, inamyloid, thin-walled, hyaline in KOH, smooth. *Leptocystidia* 50–70 × 2.5–5 µm, scattered amongst and scarcely projecting beyond the basidia, cylindrical to narrowly clavate, thin-walled, smooth, hya-

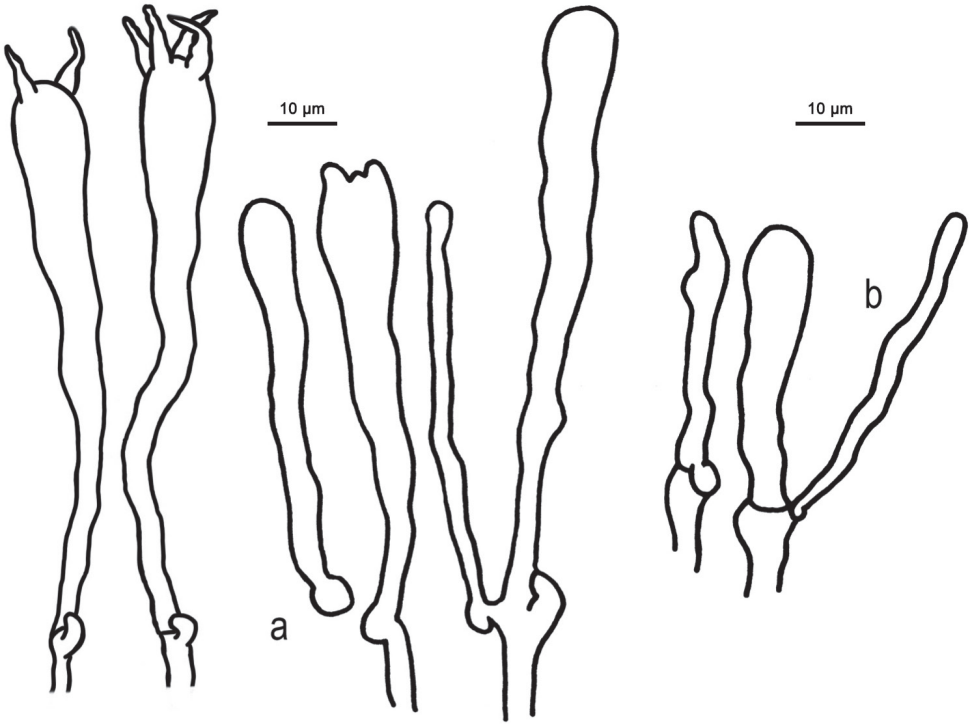

Figure 13. Microscopic features of *Clavariadelphus sachalinensis* (MHHNU 7816). **a** Basidia **b** Leptocystidia and immature basidia.

line, non-pigmented, clamped, inflated apically at maturity, at times with apical or subapical branches. *Mycelial hyphae* 2–8 µm diam., interwoven or aggregated into rhizomorphic strands, branched, clamped; hyphal walls smooth with light microscopy and SEM.

Chemical reactions. (dried basidiomes): KOH = positive, light yellow; $FeCl_3$ = positive, green-yellow; NH_4OH = positive, orange; ethanol, phenol, $FeSO_4$ and Melzer's reagent = negative.

Known distribution and ecology. N China (in this study) and SW China (Methven 1990). Gregarious habit on fallen needles and other debris under conifers, especially pine at elevations ranging from 2000–3600 m.

Materials examined. China. Inner Mongolia: Mo er dao ga National Forests, Great Khingan Mountains, 8 August 2013, *P. Zhang 1316* (MHHNU 7816). Sichuan Province: Hongyuan Prefecture, Kangle Town, alt. 3600 m, 14 August 1998, *M.S. Yuan 3361* (HKAS 33844).

Comments. *Clavariadelphus sachalinensis* was proposed by Imai, based on Japanese collections as a species of *Clavaria* and then transferred to genus *Clavariadelphus* (Imai 1930, Corner 1950). In China, *C. sachalinensis* was previously reported with distribution in SW China (Methven 1990; Zang 1996) and is also found in northern regions of China. *Clavariadelphus sachalinensis* is similar to *C. ligula* and *C. yunnanensis*. Their differences are described in our discussion of *C. ligula*.

9. *Clavariadelphus yunnanensis* Methven, Mem. New York Bot. Garden 49: 156 1989
Figs 2j–l, 3i, 4h, 5h, 14a, b

Note. The following taxonomic description is mainly drawn from Methven (1989). Field notes including macro-morphology, growth habit, distribution and host plants, SEM characteristics and chemical tests are from this study.

Description. *Basidiomes* up to 20 cm high, 0.5 cm diam. basally, enlarged upwards 2 cm diam., simple, initially cylindrical to subcylindrical, then narrowly clavate, subolanceolate; *hymenium* initially smooth, longitudinally rugose to rugulose in age, light brown to cinnamon at maturity; *apex* obtuse, smooth to rugose, concolorous with the hymenium; surface slowly staining, russet to umber; *base* terete, smooth, pale cinnamon or pale ochraceous-buff; *mycelial hyphae* white; *flesh* initially solid, becoming soft and spongy upwards as the apex enlarges, white to pinkish-buff. *Odour* not distinctive. *Taste* slightly bitter. *Spore deposit* white.

Hymenium extending over the apex of the basidiomata, composed of basidia and leptocystidia. *Basidia* 70–80 × 8–9 µm, clavate, hyaline, thin-walled, (2–) 4-spored, sterigmata 7–10 µm in length. *Basidiospores* [40/2/2] (8.8–) 9.0–11.0 × 4.6–6.4 (–7.4) µm, Q = (1.29–) 1.32–1.72 (–1.76), Q_m = 1.56 ± 0.11, ellipsoid to broadly ellipsoid, ovate or amygdaliform, smooth. *Leptocystidia* 40–60 × 2.5–3.5 µm, scattered amongst and scarcely projecting beyond the basidia, cylindrical to narrowly clavate, thin-walled, smooth, hyaline, non-pigmented, clamped, inflated apically at maturity, at times with apical or subapical branches. *Mycelial hyphae* 2–4 µm diam., parallel,

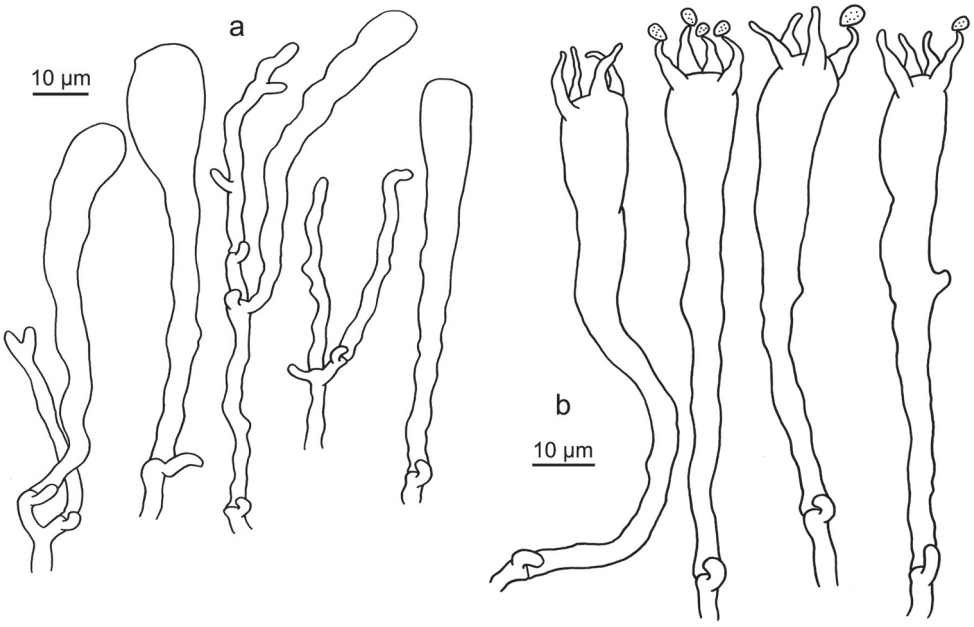

Figure 14. Microscopic features of *Clavariadelphus yunnanensis* (HKAS 57731). **a** Leptocystidia and immature basidia **b** Basidia.

interwoven or aggregated into rhizomorphic strands, branched, clamped; walls thin or irregularly slightly thickened, the hyphal walls echinulate with light microscopy, covered with massive nipple-shaped protuberances and lacking crystals with SEM.

Chemical reactions. (dried basidiomes): KOH = positive, golden-yellow; $FeCl_3$ = positive, green-yellow; NH_4OH = positive, golden-rod or vivid yellow; phenol = positive, light yellow; ethanol, $FeSO_4$ and Melzer's reagent = negative.

Known distribution and ecology. SW China and northern India (Methven 1989). Either solitary, scattered or gregarious habit on the ground in mixed deciduous-coniferous forests in association with several genera (e.g. *Abies*, *Berberis*, *Picea*, *Pinus*, *Quercus*, *Rosa* and *Salix*) at elevations ranging from 2200–3600 m.

Materials examined. China. Sichuan Province: Hongyuan Prefecture, Shuajing Temple, *Picea*, alt. 3400 m, 3 August 1996, *M.S. Yuan 2375* (HKAS 30752); Kangding Prefecture, Liuba, alt. 3500 m, 9 September 1996, *M.S. Yuan 2686* (HKAS 31136); Kangding Prefecture, Zheduo Mountains, shrubs dominated by *Berberis*, *Quercus*, *Rosa*, *Salix*, alt. 3585 m, 14 August 2008, *Z.W. Ge 903* (HKAS 49398). Yunnan Province: Shangri-La Prefecture, 19 August 2008, 28°18.00'N, 98°33.00'E, alt. 3100 m, *T.Z. Wei 270* (HMAS 250510); Deqing Prefecture, Xiaruo, 18 September 2010, *HBB2010-D15* (HKAS 62644); Jianchuan Prefecture, Shibao Mountains, 14 August 2003, *Z.W. Ge 4* (HKAS 43816); same location, 30 August 2009, *G. Wu 199* (HKAS 57731); Kunming City, Yeya Lake, alt. 2200 m, 22 September 2012, *Z.L. Yang 5629* (HKAS 77288); Shangri-La Prefecture, Haba Mountains, 13 August 2008, *L.P. Tang 618* (HKAS 54849); Shangri-La Prefecture, 16 August 2008, *T.Z. Wei 271* (HMAS 250466); Shangri-La Prefecture, Bita Lake, 24 August 2009, *Q. Cai 122* (HKAS 58789); same location and date, *G. Wu 127* (HKAS 57659); Weixi Prefecture, Qizong, 19 September 2010, *HBB2010-W21* (HKAS 61417); Yulong Prefecture, Yulong Snow Mountains, Sandaowan, under *Abies* spp., alt. 3200 m, 1 August 1995, *M. Zang 12514* (HKAS 30038); Yulong Prefecture, Lizui Village, 20 August 2008, *Q. Zhao 8262* (HKAS 55244); Yulong Prefecture, Jiuhe, 20 August 2010, *G. Wu 327* (HKAS 63558); Yulong Prefecture, Yulong Snow Mountains, Ganhaizi, under *Picea* spp., alt. 3100 m, 3 September 1986, *M. Zang 10739* (HKAS 17788); Yulong Prefecture, Yulong Snow Mountains, Yu Lake, *Abies* forests, alt. 3000 m, 1 August 1985, *M. Zang 10220* (HKAS 15063); Yulong Prefecture, Yulong Snow Mountains, 6 September 1986, *R.H. Petersen s. n.* (HKAS 20067); same location and date, *R.H. Petersen s. n.* (HKAS 20068); Yulong Prefecture, Wenhai, 17 September 2012, *G. Wu 1054* (HKAS 77226).

Comments. *Clavariadelphus yunnanensis* is quite common in SW China where it was previously reported as *C. ligula* or *C. pistillaris* (Mao et al. 1993; Yuan and Sun 1995; Zang 1996; Mao 2009). It is well characterised by its cinnamon buff, large basidiomes, broadly ellipsoid basidiospores, hyphae of the basal mycelium covered with nipple-shaped protuberances and occurrence at high elevation forests. This taxon is also similar to *C. ligula* and *C. sachalinensis*, but differs microscopically in the size and shape of the basidiospores (see the comments under *C. ligula*). Immature fruit bodies of *C. yunnanensis* are similar to *C. griseoclavus*. However, the latter can be dis-

tinguished from smaller basidiomata (less than 13 cm high), narrower apex (less than 1.5 cm diam.) and narrower basidiospores (Q_m 1.89) (Lu and Li 2020). Although *C. yunnanensis* might be confused with the Asian taxon *C. mirus*, the latter is distinct by its slender cylindrical, light brown basidiomes and broader basidiospores (Methven 1990). The presence of *C. mirus* in China needs to be ascertained. In the phylogenetic analyses, *C. yunnanensis* has a joint relationship with *C. elongatus*, *C. pistillaris* and the sequence of "*C. occidentalis*" from Tunisia, but the sister relationship cannot be resolved (Fig. 1).

Taxonomic key to species of *Clavariadelphus* in China

1 Basidiospores narrowly ellipsoid, Q_m > 2 ..... **2**
– Basidiospores broadly ellipsoid to ellipsoid, Q_m < 2 ..... **3**
2 Basidiospores 11.0–14.0 × 4.0–5.5 µm, Q_m 2.7 ..... ***C. ligula***
– Basidiospores 21–24 × 4–6 µm, Q_m 4.2 ..... ***C. sachalinensis***
3 Basidiomes orange; apex sterile, truncate **4**
– Basidiomes without orange tinge; apex fertile, not truncate **5**
4 Basidiomata apex 3–7.5 cm diam. ..... ***C. amplus***
– Basidiomata apex < 2 cm diam. ..... ***C. gansuensis***
5 Basidiomes usually 20–30 cm high **6**
– Basidiomes usually < 20 cm high **7**
6 Basidiomes grey-purple; basidiospores narrowly ellipsoid, 9.0–11.0 × 5.7–7.4 µm, Q_m 1.71 ..... ***C. elongatus***
– Basidiomes cinnamon; basidiospores broadly ellipsoid, 9.0–11.0 × 4.6–6.4 µm, Q_m 1.56 ..... ***C. yunnanensis***
7 Basidiomes greyish-red to pastel-red ..... ***C. himalayensis***
– Basidiomes grey or yellow, without red colouration **8**
8 Basidiomes grey; basidiospores ellipsoid 10–11 × 5–6.5 µm, Q_m 1.89 ..... ***C. griseoclavus***
– Basidiomes yellow colouration **9**
9 Basidiomes yellow; basidiospores broadly ellipsoid 7.8–9.6 × 5.5–7.4 µm, Q_m 1.38 ..... ***C. alpinus***
– Basidiomes pale yellow-brown; basidiospores narrowly ellipsoid 9.2–12.0 × 4.6–6 µm, Q_m 1.97 ..... ***C. khinganensis***

Discussion

The taxonomic importance of comprehensive data in *Clavariadelphus*

Many studies have verified that molecular methods are effective in resolving relationships in complicated groups of fungi (Zeng et al. 2013; Tang et al. 2017; Huang et al.

2018; Yang et al. 2018). In a pre-study analysis, we evaluated four DNA gene markers: ITS, large subunit of nuclear ribosomal RNA (nrLSU), translation elongation factor 1α gene (*tef1-a*) and DNA-directed RNA polymerase II second subunit (*rpb2*). Compared to the others, ITS offered the highest probability of successful identification in *Clavariadelphus*. By contrast, other markers displayed a lower success rate of PCR amplification or inferior species resolution in some close or sibling taxa. ITS sequences acquired from this study are listed in Appendix 1.

Macro-morphological, micro-morphological and SEM characteristics are very important in the taxonomy of *Clavariadelphus*. Although *Clavariadelphus* can be readily distinguished from other clavarioid genera, the delimitation of infrageneric taxa is difficult in many cases, especially without critical observation and examination (Methven 1990). Basidiomata colour is a diagnostic characteristic, although it must be used in conjunction with other morphological features. In China, basidiomata colour ranges from yellow to orange, grey-purple, pastel-red or brown. Despite the inherent variability in shape, size and other characteristics of the basidiomes, these features are important diagnostic characteristics in *Clavariadelphus*, as well as the size and shape of basidiospores. The basidiospores of Chinese taxa of this genus are summarised in Fig. 3 and 4. Additionally, some SEM characteristics, especially hyphae of the basal mycelium, are of taxonomic value. The variations of basal hyphae of Chinese taxa range from smooth, having nipple-shaped protuberances to crystals or both at the same time (Fig. 5).

Chemical reactions also are helpful in distinguishing *Clavariadelphus* species. Doty (1948) distinguished this genus using $FeSO_4$ reactions. Corner reported that chemical reactions, especially KOH, are useful for delimiting *Clavariadelphus* taxa (Corner 1950). In this study, chemical colour reactions were conducted using seven chemical reagents (Table 1). Amongst these, four reagents were found to be discriminatory with some species, specifically KOH, $FeCl_3$, NH_4OH and phenol. Three additional reagents, namely ethanol, Melzer's reagent and $FeSO_4$, were found to lack discriminatory value.

Metadata supply taxonomic information, such as habit, distribution and host plants. The growth habit of Chinese taxa includes solitary, scattered and gregarious. Growth habit is of taxonomic value only when used in conjunction with other features (Methven 1990). The Chinese specimens were collected in mixed or coniferous forests in association with *Abies*, *Berberis*, *Quercus*, *Pinus*, *Picea*, *Rhododendron*, *Rosa*, *Salix*, *Thuja* and *Tsuga*. The distribution of a species usually correlates with that of its host plant. Although the Chinese taxa exhibit no apparent preference of host plants, the so-called cosmopolitan species within *Clavariadelphus* seem to be rare in this study.

Clavariadelphus species diversity in China

Many new fungal taxa have been discovered in the last ten years in China (Zhang et al. 2005; Zeng et al. 2013; Tang et al. 2014; Huang et al. 2018; Yang et al. 2018). However, there are still a large number of undescribed fungal taxa in this country. This study indicates that there are at least ten known taxa of *Clavariadelphus* in China,

including four previously described (*C. griseoclavus*, *C. ligula*, *C. sachalinensis* and *C. yunnanensis*), two not previously reported in China (*C. elongatus* and *C. himalayensis*) and four novel species (*C. alpinus* sp. nov., *C. amplus* sp. nov., *C. gansuensis* sp. nov. and *C. khinganensis* sp. nov.). Several taxa, previously reported from China, need to be confirmed, including *C. mirus*, *C. pistillaris* and *C. truncatus*. In China, there are still some species that need to be discovered, such as GenBank accession JQ991679. To date, with the four novel taxa described in this study, there are twenty-eight species of *Clavariadelphus* worldwide. Although the taxonomy of *Clavariadelphus* has received much attention in the past, this group needs to be further examined with molecular methods. More reliable sequence data, especially those species from North America and Europe, are needed to understand phylogenetic relationships better.

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Appendix 1

Sequences used or produced in our phylogenetic analyses of Clavariadelphus in China.

Taxon	Voucher	Locality	GenBank Accession No. (ITS)
Clavariadelphus alpinus	**HKAS 57396**	**China, Yunnan**	**MK705888***
C. americanus	MycoMap # 1288	USA, Indiana	MK575228
C. amplus	HKAS 76577	China, Gansu	MK705851*
C. amplus	**HKAS 54876**	**China, Yunnan**	**MK705857***
C. amplus	HMAS 132008	China, Qinghai	MK705852*
C. amplus	HMAS 97090	China, Qinghai	MK705853*
C. amplus	HKAS 49229	China, Sichuan	MK705854*
C. amplus	HKAS 49278	China, Sichuan	MK705855*
C. amplus	HKAS 53797	China, Sichuan	MK705856*
C. amplus	HMAS 250466	China, Yunnan	MK705858*
C. amplus	HMAS 97248	China, Tibet	MK705859*
C. amplus	HMAS 59867	China, Tibet	MK705860*
C. amplus	HKAS 46160	China, Tibet	MK705861*
C. amplus	HKAS 46120	China, Tibet	MK705862*
C. elongatus	HKAS 76589	China, Gansu	MK705842*
C. elongatus	HKAS 50742	China, Sichuan	MK705843*
C. elongatus	HKAS 50801	China, Sichuan	MK705844*
C. elongatus	HMAS 260746	China, Yunnan	MK705845*
C. elongatus	HKAS 52425	China, Yunnan	MK705846*
C. elongatus	LAH 31397	Pakistan, Khyber Pakhtunkhwa	MG768847*
C. elongatus	SWAT 000559	Pakistan, Khyber Pakhtunkhwa	MG768848*
C. gansuensis	**HKAS 76487**	**China, Gansu**	**MK705847***
C. griseoclavus	BJTC FM964	China, Shanxi	MT302370
C. griseoclavus	BJTC FM965	China, Shanxi	MT302371

Taxon	Voucher	Locality	GenBank Accession No. (ITS)
C. himalayensis	HKAS 50684	China, Yunnan	MK705863*
C. himalayensis	HKAS 58811	China, Yunnan	MK705864*
C. khinganensis	**MHHNU 7789**	**China, Inner Mongolia**	**MK705865***
C. khinganensis	MHKMU H.Y. Huang 368	China, Jilin	MT447468*
C. ligula	HMAS 51688	China, Heilongjiang	MK705848*
C. ligula	HMAS 35954	China, Heilongjiang	MK705849*
C. ligula	HMAS 41146	Czech, –	MK705850*
C. mucronatus	OSC 1064138	USA, Oregon	EU526000
C. occidentalis	OSC 104664	USA, the Pacific Northwest	EU669308
C. occidentalis	OSC 112861	USA, the Pacific Northwest	EU669202
C. occidentalis	OSC 114250	USA, the Pacific Northwest	EU834202
C. occidentalis	OSC 114281	USA, the Pacific Northwest	EU846242
C. occidentalis	H21536	Tunisia, Aïn Draham	KU973835
C. pistillaris	NAMA 2017-123	USA, Wisconsin	MH979250
C. pistillaris	AMB 18611	Italy, Aquila	MT452507
C. pakistanicus	MH 129901	Pakistan, Khyber Pakhtunkhwa	HQ379937
C. pakistanicus	SR1742	India, –	MT012805
C. sachalinensis	MHHNU 7816	China, Inner Mongolia	MK705866*
C. sachalinensis	p061i	USA, the Pacific Northwest	EU624408
C. sachalinensis	p059i	USA, the Pacific Northwest	EU624410
C. sachalinensis	p058i	USA, the Pacific Northwest	EU624411
C. sachalinensis	OSC 96213	USA, the Pacific Northwest	EU834196
Clavariadelphus sp.	src121	USA, California	DQ974709
Clavariadelphus sp.	OSC 105674	USA, the Pacific Northwest	EU669206
Clavariadelphus sp.	HC-PNNT-268	Mexico, Mexico State	KT874982
Clavariadelphus sp.	ECM54	China, Zhejiang	JQ991679
Clavariadelphus sp.	MushroomObserver.org/254047	Mexico, Queteraro	MH304404
Clavariadelphus sp.	Montri-108	Switzerland, Montricher	MK028378
C. subfastigiatus	OSC 119587	USA, the Pacific Northwest	EU669207
C. subfastigiatus	MICH 73554	USA, Clackamas County	JX275756
C. truncatus	MA-Fungi 48062	Spain, –	AJ292288
C. truncatus	OUC99108	Canada, British Columbia	DQ097871
C. truncatus	SIM278	Canada, British Columbia	HQ650728
C. truncatus	AMB 18612	Italy, Belluno	MT452508
C. unicolor	Mushroom Observer #112193	USA, Indiana	MN906166
C. yunnanensis	HKAS 49398	China, Sichuan	MK705867*
C. yunnanensis	HKAS 31136	China, Sichuan	MK705868*
C. yunnanensis	HKAS 54849	China, Yunnan	MK705869*
C. yunnanensis	HKAS 63558	China, Yunnan	MK705870*
C. yunnanensis	HKAS 57731	China, Yunnan	MK705871*
C. yunnanensis	HKAS 58789	China, Yunnan	MK705872*
C. yunnanensis	HKAS 55244	China, Yunnan	MK705873*
C. yunnanensis	HMAS 250510	China, Yunnan	MK705874*
C. yunnanensis	HMAS 250471	China, Yunnan	MK705875*
C. yunnanensis	HKAS 62644	China, Yunnan	MK705876*
C. yunnanensis	HKAS 61417	China, Yunnan	MK705877*
C. yunnanensis	HKAS 43816	China, Yunnan	MK705878*
C. yunnanensis	HKAS 30752	China, Yunnan	MK705879*
C. yunnanensis	HKAS 30083	China, Yunnan	MK705880*
C. yunnanensis	HKAS 20068	China, Yunnan	MK705881*
C. yunnanensis	HKAS 20067	China, Yunnan	MK705882*
C. yunnanensis	HKAS 17788	China, Yunnan	MK705883*
C. yunnanensis	HKAS 15063	China, Yunnan	MK705884*
C. yunnanensis	HKAS 57659	China, Yunnan	MK705885*
C. yunnanensis	HKAS 77226	China, Yunnan	MK705886*
C. yunnanensis	HKAS 77288	China, Yunnan	MK705887*
Lentaria byssiseda	TENN 61159	USA, Tennessee	FJ596788
Kavinia himantia	CFMR:DLL2011-079	USA, Wisconsin	KJ140598
K. alboviridis	CFMR:DLL2011-131	USA, Wisconsin	KJ140634

* indicates sequences generated in this study, fontbold indicates type material for the new species.