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Morphological and molecular identification of a new species of *Cinereomyces* (Polyporales, Basidiomycota) in southern China

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Abstract

A new poroid wood-inhabiting fungal species, *Cinereomyces fimbriatus sp. nov.*, is proposed based on morphological and molecular characteristics. The species is characterized by resupinate, brittle basidiomata with cream pore surface, a dimitic hyphal system with clamped generative hyphae, broad allantoid, hyaline, thin-walled, smooth basidiospores (3.6–4.6 × 0.8–1.1 µm). The internal transcribed spacer (ITS) region of nuclear ribosomal RNA gene sequences of the studied samples were generated, and phylogenetic analyses were performed with maximum likelihood, maximum parsimony and bayesian inference methods. The phylogenetic analysis based on ITS sequences showed that *C. fimbriatus* formed a monophyletic lineage with strong supports (100% BS, 100% BT, 1.00 BPP) sister to *C. lindbladii*.

Keywords: Phylogenetic analyses, Polypores, Taxonomy, Wood-inhabiting fungi, Yunnan Province

Introduction

Cinereomyces Jülich (1982: 396) (Polyporales, Basidiomycota) was typed by *C. lindbladii* (Berk.) Jülich (1982: 396), which is characterized by resupinate, poroid basidiomata with white to cream to greyish pore surface, encrustation in trama or tube mouths, a dimitic hyphal system with clamp connections on generative hyphae, skeletal hyphae dissolving in KOH, cystidia absent and cylindrical to allantoid, hyaline, thin-walled, smooth, IKI–, CB–, basidiospores (Jülich 1982, Miettinen & Rajchenberg 2012, Ryvarden & Melo 2014). So far, 2 species have been accepted in this genus worldwide (Jülich 1982, Miettinen 2012).

Recently, the phylogenetic studies of *Cinereomyces* have been carried out (Miettinen & Larsson 2011, Miettinen & Rajchenberg 2012). Miettinen & Larsson (2011) introduced that *Obba* Miettinen & Rajchenb. (2012: 141) and *Sebipora* Miettinen (2012: 144) were related to *Cinereomyces* and *Gelatoporia* Niemelä (1985: 22), which showed that *C. lindbladii* nested into the *Cinereomyces* clade and grouped with *G. subvermispora*, *O. rivulosa* and *S. aquosa* Miettinen (2012: 144). Miettinen & Rajchenberg (2012) employed molecular study on Hymenochaetales with poroid and hydnoid species showed that *C. lindbladii* grouped with *G. subvermispora* and *Skeletocutis amorpha* (Fr.) Kotl. & Pouzar (1958: 103).

During investigations on wood-inhabiting fungi in southern China, a taxon was found which could not be assigned to any described species. In this study, the authors expand samplings from previous studies to examine taxonomy and phylogeny of this new species within the *Cinereomyces*, based on the internal transcribed spacer (ITS) region sequences.

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Materials and methods

Morphological studies.—The specimens studied are deposited at the herbarium of Southwest Forestry University (SWFC), Kunming, Yunnan Province, P.R. China. Macro-morphological descriptions are based on field notes followed Chen *et al*. (2016) and Sun *et al*. (2020). Special colour terms follow Petersen (1996). Micro-morphological data were obtained from the dried specimens, and observed under a light microscope following Dai (2010) and Cui *et al*. (2019). The following abbreviations were used: KOH = 5% Potassium hydroxide, CB = Cotton Blue, CB– = acyanophilous, IKI = Melzer's reagent, IKI– = both inamyloid and indextrinoid, L = mean spore length (arithmetic average of all spores), W = mean spore width (arithmetic average of all spores), Q = variation in the L/W ratios between the specimens studied, n (a/b) = number of spores (a) measured from given number (b) of specimens.

DNA extraction, amplification, sequencing and phylogenetic analyses.— CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd, Beijing) was used to obtain genomic DNA from dried specimens, according to the manufacturer's instructions with some modifications that a small piece of dried fungal specimen (about 30 mg) was ground to powder with liquid nitrogen. The powder was transferred to a 1.5 mL centrifuge tube, suspended in 0.4 mL of lysis buffer, and incubated in a 65 °C water bath for 60 min. After that, 0.4 mL phenol-chloroform (24:1) was added to each tube and the suspension was shaken vigorously. After centrifugation at 13,000 rpm for 5 min, 0.3 mL supernatant was transferred to a new tube and mixed with 0.45 mL binding buffer. The mixture was then transferred to an adsorbing column (AC) for centrifugation at 13,000 rpm for 0.5 min. Then, 0.5 mL inhibitor removal fluid was added in AC for a centrifugation at 12,000 rpm for 0.5 min. After washing twice with 0.5 mL washing buffer, the AC was transferred to a clean centrifuge tube, and 100 mL elution buffer was added to the middle of adsorbed film to elute the genome DNA. ITS region was amplified with primer pairs ITS5 and ITS4 (White *et al*. 1990). The PCR procedure for ITS was as follows: initial denaturation at 95 °C for 3 min, followed by 35 cycles at 94 °C for 40 s, 58 °C for 45 s and 72 °C for 1 min, and a final extension of 72 °C for 10 min. The PCR products were purified and directly sequenced at Kunming Tsingke Biological Technology Limited Company. All newly generated sequences were deposited at GenBank (Table 1).

TABLE 1. List of species, specimens, and GenBank accession numbers of sequences used in this study.

Species name	Sample no.	GenBank no. ITS	References
Antrodiella pallescens	Miettinen 12611	FN907921	Miettinen & Larsson 2011
Antrodiella pallescens	Miettinen 12611	FN907921	Miettinen & Larsson 2011
Ceriporia viridans	—	AF347109	Larsson *et al*. 2004
Cinereomyces fimbriatus	CLZhao 10461	MT809039	Present study
Cinereomyces fimbriatus	CLZhao 10493	MT809040	Present study
Cinereomyces lindbladii	Kotiranta 19911	FN907909	Miettinen & Larsson 2011
Cinereomyces lindbladii	FBCC 117	HQ659223	Miettinen & Rajchenberg 2012
Cinereomyces lindbladii	KH Larsson 12078	FN907906	Miettinen & Larsson 2011
Coltricia perennis	AFTOL-ID 447	DQ234559	Direct submision
Coltriciella dependens	TU103611	AM412254	Tedersoo *et al.* 2007
Dentocorticium sulphurellum	FPL11801	AF026647	Hibbett 1997
Diplomitoporus crustulinus	Niemelä 6293	FN907907	Miettinen & Larsson 2011
Diplomitoporus flavescens	Sturm s.n.	FN907908	Miettinen & Larsson 2011
Donkioporia expansa	MUCL 35116	FJ411104	Robledo *et al.* 2009
Ganoderma adspersum	FGA1	AM269771	Guglielmo *et al.* 2007
Gelatoporia subvermispora	CBS 347.63	FJ349621	Miettinen & Rajchenberg 2012
Gelatoporia subvermispora	Dai 3120	HQ659226	Miettinen & Rajchenberg 2012
Gelatoporia subvermispora	Kotiranta 20823	FN907911	Miettinen & Larsson 2011
Hyphodermella corrugata	KHL 3663	EU118630	Larsson 2007
Hyphodontia curvispora	1591a	DQ873615	Larsson *et al.* 2006

.....continued on the next page

TABLE 1. (Continued)

Species name	Sample no.	GenBank no. ITS	References
Hyphodontia subalutacea	3136b	DQ873630	Larsson *et al.* 2006
Mensularia radiata	—	AF237732	Hamelin *et al.* 2002
Meruliopsis taxicola	Kuljok 00/75	EU118648	Larsson 2007
Obba rivulosa	JLL 10602	AY219363	Miettinen & Rajchenberg 2012
Obba rivulosa	FBCC 938	HQ659233	Miettinen & Rajchenberg 2012
Obba thailandica	Dai 16659	KY240089	Guangjuan Ren 2017
Obba valdiviana	Gates FF503	HQ659235	Miettinen & Rajchenberg 2012
Oxyporus corticola	5385b	DQ873641	Larsson *et al.* 2006
Oxyporus populinus	Miettinen 10802	FN907910	Miettinen & Larsson 2011
Phanerochaete sordida	KHL 12054	EU118653	Larsson 2007
Sebipora aquosa	Miettinen 12032	HQ659241	Miettinen & Rajchenberg 2012
Sebipora aquosa	Miettinen 9265	HQ659243	Miettinen & Rajchenberg 2012
Sidera lenis	Miettinen 11036.1	FN907914	Miettinen & Larsson 2011
Sidera lowei	Ryvarden 38817	FN907919	Miettinen & Larsson 2011
Sidera lowei	Ryvarden 40576	FN907917	Miettinen & Larsson 2011
Sidera vulgaris	Gates FF257	FN907922	Miettinen & Larsson 2011
Sidera vulgaris	Ryvarden 37198	FN907918	Miettinen & Larsson 2011
Sistotrema brinkmannii	NH11412	AF506473	Larsson & Larsson 2003
Skeletocutis amorpha	Miettinen 11038.1	FN907913	Miettinen & Larsson 2011
Skeletocutis chrysella	Miettinen 9472	FN907916	Miettinen & Larsson 2011
Sphaerobasidium minutum	1593a	DQ873652	Larsson *et al.* 2006
Steccherinum ochraceum	Ryberg s.n.	EU118669	Larsson 2007

Sequencher 4.6 (GeneCodes, Ann Arbor, MI, USA) was used to edit the DNA sequence. Sequences were aligned in MAFFT 7 (http://mafft.cbrc.jp/alignment/server/) using the "G-INS-i" strategy and manually adjusted in BioEdit (Hall 1999). The sequence alignment was deposited in Treebase (submission ID 26679). Sequences of *Dentocorticium sulphurellum* obtained from GenBank was used as an outgroup to root tree following Miettinen & Rajchenberg (2012) in the ITS analysis and sequences of *Sistotrema brinkmannii* (Bres.) J. Erikss. obtained from GenBank was used as outgroup to root tree following Miettinen & Larsson (2011) in the ITS analysis.

Maximum parsimony (MP) analysis was applied to the ITS dataset sequences. Approaches to phylogenetic analysis followed Zhao & Wu (2017) and the tree construction procedure was performed in PAUP* version 4.0b10 (Swofford 2002). All characters were equally weighted and gaps were treated as missing data. Trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Max-trees were set to 5000, branches of zero length were collapsed and all parsimonious trees were saved. Clade robustness was assessed using a bootstrap (BT) analysis with 1,000 replicates (Felsenstein 1985). Descriptive tree statistics tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated for each Maximum Parsimonious Tree (MPT) generated. Sequences were also analyzed using Maximum Likelihood (ML) with RAxML-HPC2 through the Cipres Science Gateway (www.phylo.org; Miller *et al*. 2009). Branch support (BS) for ML analysis was determined by 1000 bootstrap replicates.

MrModeltest 2.3 (Nylander 2004) was used to determine the best-fit evolution model for each data set for bayesian inference (BI). Bayesian inference was calculated with MrBayes3.1.2 with a general time reversible (GTR) model of DNA substitution and a gamma distribution rate variation across sites (Ronquist & Huelsenbeck 2003). Four Markov chains were run for 2 runs from random starting trees for 120, 000 generations, and trees were sampled every 100 generations. The first one-fourth generations were discarded as burn-in. A majority rule consensus tree of all remaining trees was calculated. A majority rule consensus tree of all remaining trees was calculated. Branches were considered as significantly supported if they received maximum likelihood bootstrap values (ML) >75%, maximum parsimony bootstrap values (MP) >75%, or bayesian posterior probabilities (BPP) >0.95.

Results

Molecular phylogeny

The ITS dataset (Fig. 1) included sequences from 32 fungal specimens representing 28 taxa. The dataset had an aligned length of 988 characters, of which 355 characters were constant, 175 parsimony-uninformative, and 458 parsimony-informative. Maximum parsimony analysis yielded 2 equally parsimonious trees (TL = 3177, CI = 0.404, HI = 0.596, RI = 0.386, RC = 0.156). The best-fit model for ITS alignment estimated and applied in the Bayesian was GTR+I+G, lset nst = 6, rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1). Bayesian resulted in a similar topology with an average standard deviation of split frequencies = 0.009929 (BI).

The phylogeny (Fig. 1) in Hymenochaetales and Polyporales based on ITS sequences showed that *Cinereomyces fimbriatus* nested into Polyporales and grouped with *C. lindbladii*.

The ITS dataset (Fig. 2) included sequences from 14 fungal specimens representing 8 taxa. The dataset had an aligned length of 815 characters, of which 475 characters were constant, 211 parsimony-uninformative, and 129 parsimony-informative. Maximum parsimony analysis yielded 3 equally parsimonious trees (TL = 455, CI = 0.875, HI = 0.125, RI = 0.841, RC = 0.736). The best-fit model for ITS alignment estimated and applied in the Bayesian was GTR+I+G, lset nst = 6, rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1). Bayesian resulted in a similar topology with an average standard deviation of split frequencies = 0.009958 (BI).

The further phylogeny (Fig. 2) inferred from ITS sequences demonstrated that *Cinereomyces fimbriatus* recognized in *Cinereomyces* and it was sister to *C. lindbladii*.

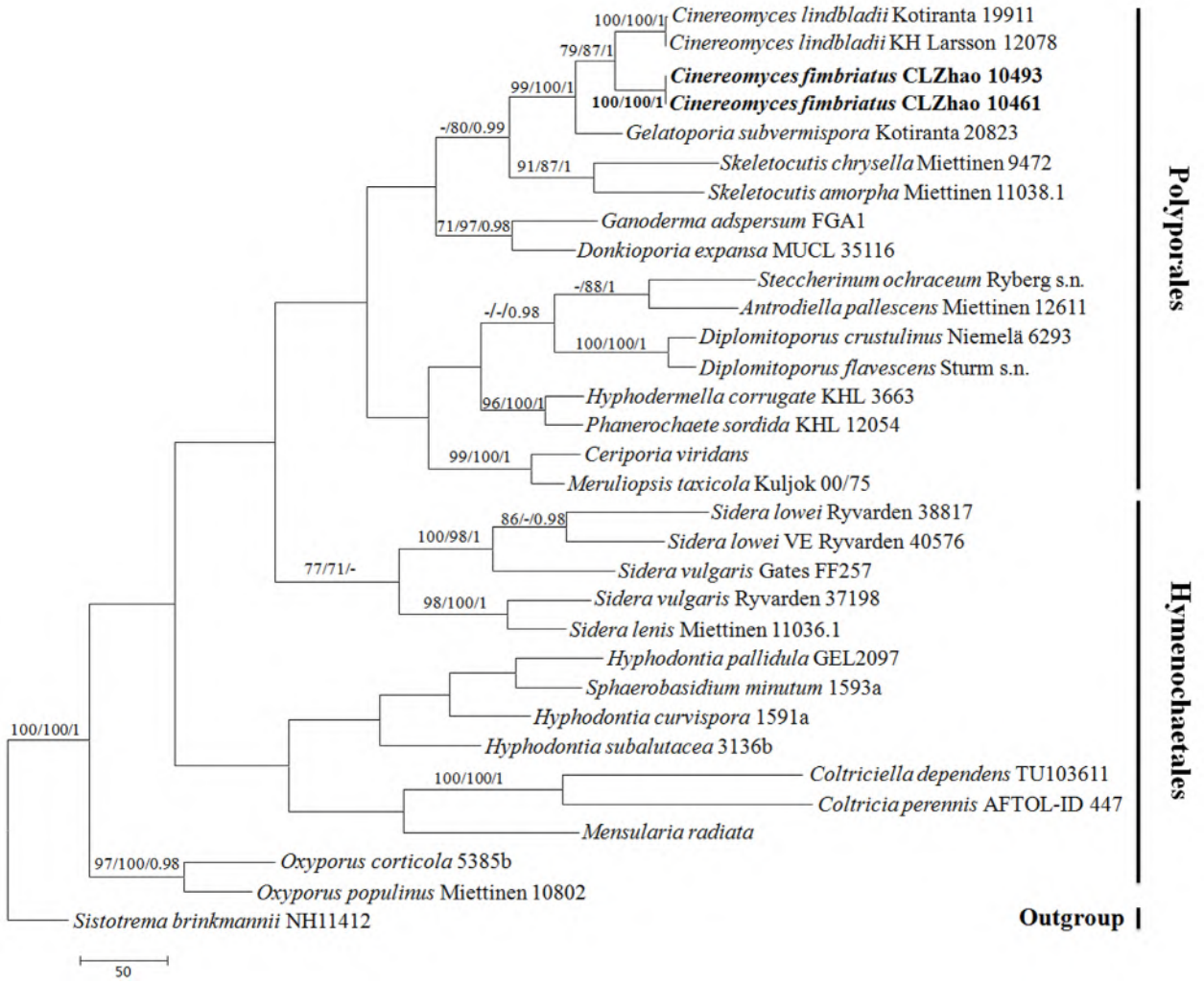

FIGURE 1. Maximum parsimony strict consensus tree illustrating the phylogeny of *Cinereomyces fimbriatus* and related taxa in Polyporales and Hymenochaetales based on ITS sequences. Branches are labelled with maximum likelihood bootstrap values > 75%, parsimony bootstrap values > 75% and bayesian posterior probabilities > 0.95, respectively. Clade names follow Miettinen & Larsson (2001).

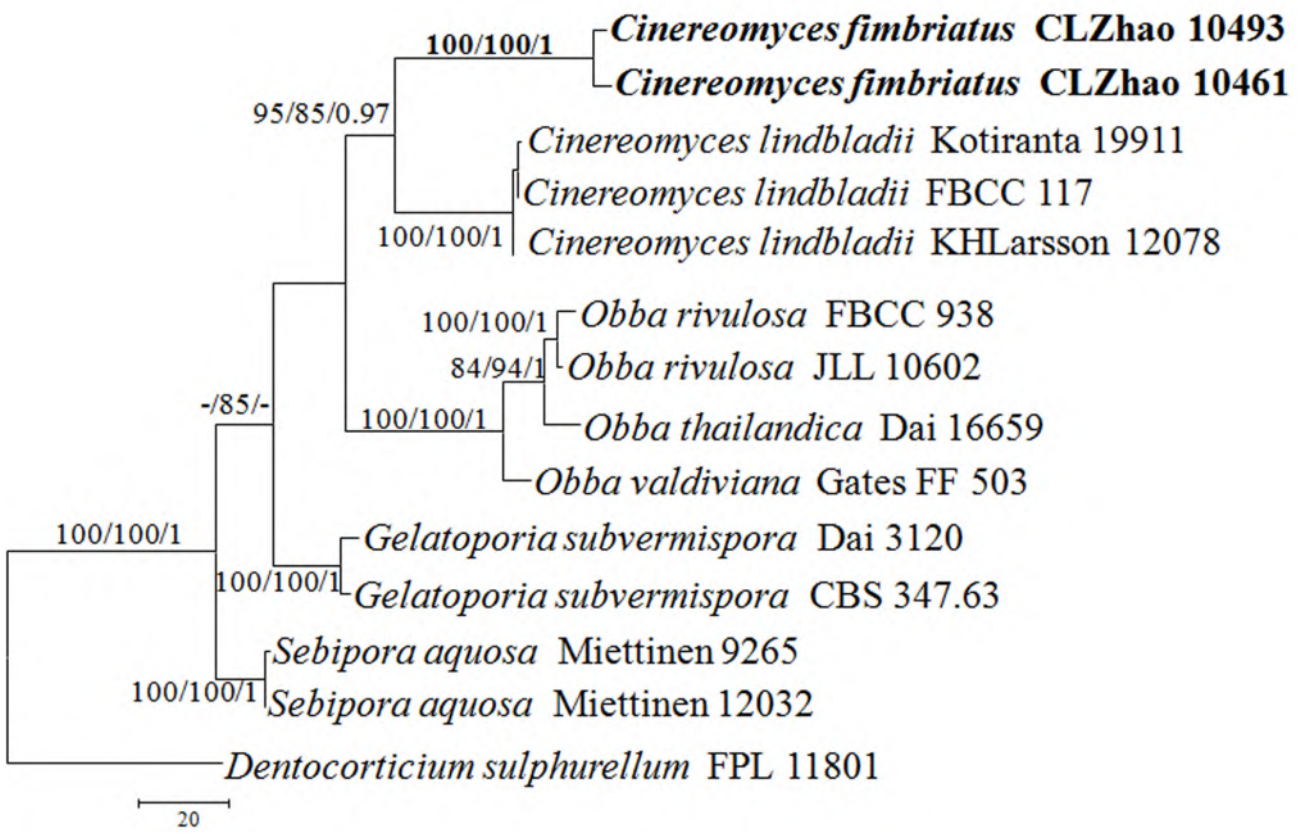

FIGURE 2. Maximum parsimony strict consensus tree illustrating the phylogeny of *Cinereomyces fimbriatus* and related taxa based on ITS sequences. Branches are labelled with maximum likelihood bootstrap values > 75%, parsimony bootstrap values > 75% and bayesian posterior probabilities > 0.95, respectively.

Taxonomy

Cinereomyces fimbriatus C.L. Zhao, *sp. nov.* (Figs. 3, 4)
MycoBank no.: MB 836322

Type.—**CHINA**. Yunnan Province, Dali, Nanjian County, Lingbaoshan National Forest Park, on angiosperm trunk, 10 January 2019, *CLZhao 10461* (holotype, SWFC!)

Etymology.—*Fimbriatus* (Lat.): referring to on the fimbriate sterile margin of the basidiomata.

Basidiomata.—Annual, resupinate, soft, without odor or taste when fresh, becoming brittle upon drying, up to 8 cm long, 5 cm wide, 2 mm thick at centre. Pore surface white to cream when fresh, cream upon drying; pores round, 4–6 per mm; dissepiments thin, entire. Sterile margin narrow, white, fimbriate, up to 2 mm wide. Subiculum white to cream, thin, up to 0.5 mm thick. Tubes cream, corky, up to 1.5 mm long.

Hyphal structure.—Hyphal system dimitic; generative hyphae with clamp connections, skeletal hyphae IKI–, CB–; tissues swelling in KOH.

Subiculum.—Generative hyphae infrequent, hyaline, thin-walled, unbranched, encrusted, 2.5–4.5 µm in diam.; skeletal hyphae dominant, hyaline, thick-walled with a wide lumen, unbranched, interwoven, encrusted, 3.5–6.5 µm in diam.

Tubes.—Generative hyphae infrequent, hyaline, thin-walled, unbranched, 1.5–3 µm in diam.; skeletal hyphae dominant, hyaline, thick-walled with a wide lumen, unbranched, interwoven, encrusted, 3–5.5 µm. Cystidia absent, fusoid cystidioles present, hyaline, thin-walled, 14–24 × 2.5–4 µm; basidia barrel-shaped, with four sterigmata and a basal clamp connection, 11.5–18.5 × 2.5–5 µm; basidioles dominant, mostly barrel-shaped, but slightly smaller than basidia.

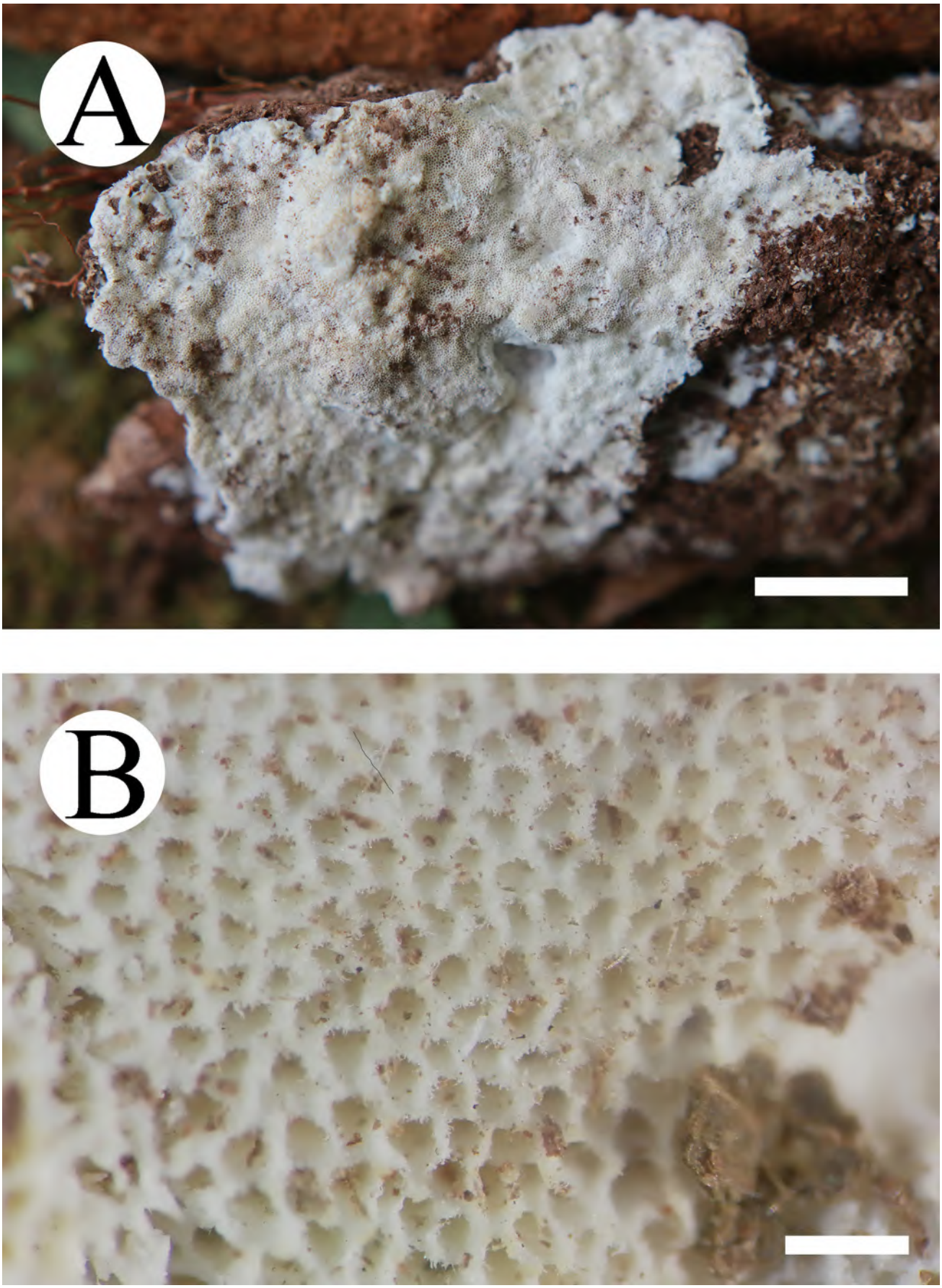

FIGURE 3. Basidiomata of *Cinereomyces fimbriatus* (holotype). Scale bars: A–1 cm; B–0.5 mm.

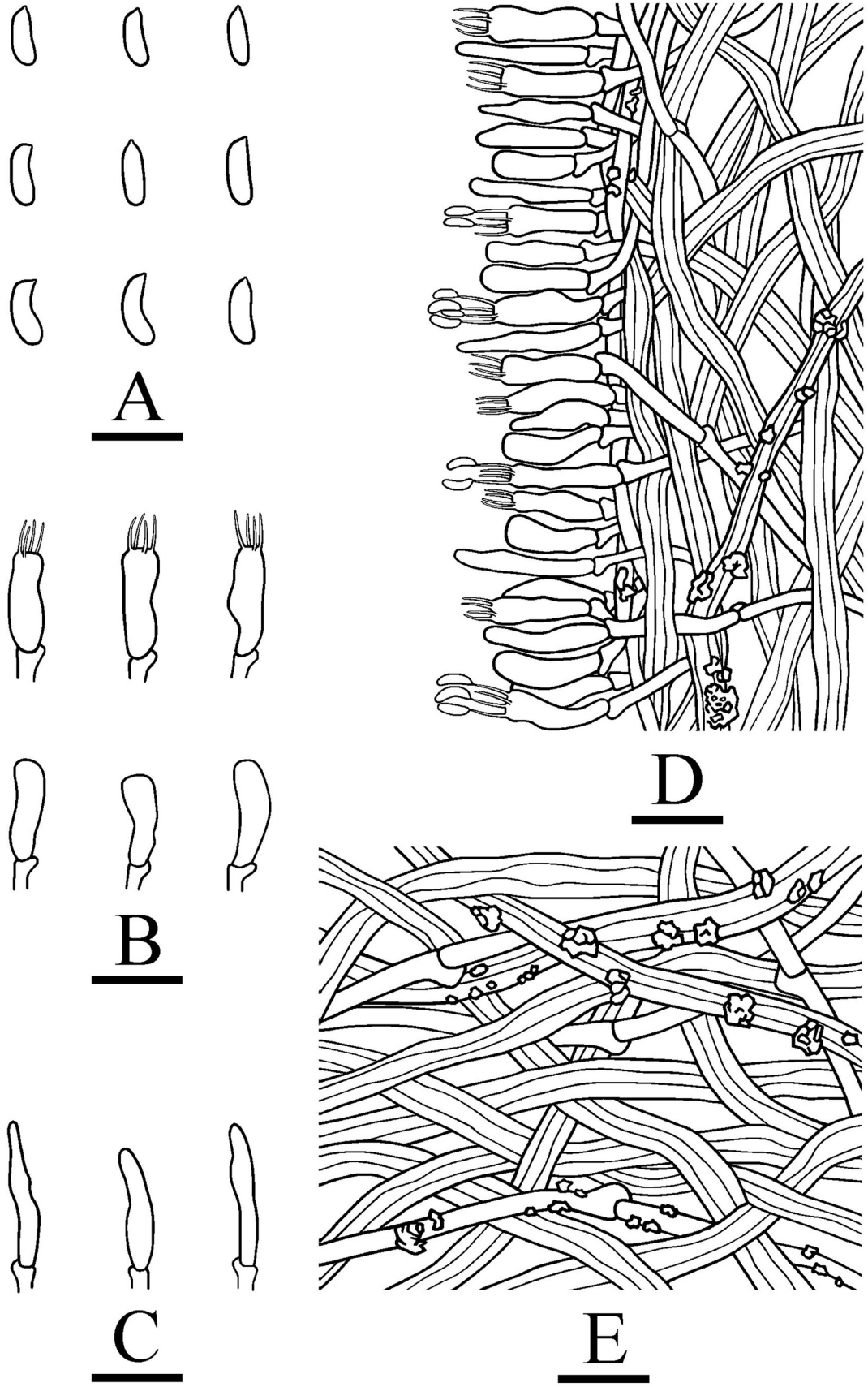

FIGURE 4. Microscopic structures of *Cinereomyces fimbriatus* (drawn from the holotype). A. Basidiospores. B. Basidia and basidioles. C. Cystidioles. D. Hyphae from trama. E. Hyphae from subiculum. Bars: A–5 µm; B–E–10 µm.

Spores.—Basidiospores allantoid, hyaline, thin-walled, smooth, IKI–, CB–, (3.4–)3.6–4.6(–4.8) × 0.8–1.1 µm, L = 4.20 µm, W = 0.99 µm, Q = 4.13–4.63 (n = 60/2).

Additional specimen examined.—**CHINA.** Yunnan Province, Dali, Nanjian County, Lingbaoshan National Forestry Park, on angiosperm trunk, 10 January 2019, *CLZhao 10493* (paratype, SWFC!).

Discussion

In the present study, a new species, *Cinereomyces fimbriatus*, is described based on phylogenetic analysis and morphological characters.

Miettinen & Larsson (2011) studied *Sidera* in Hymenochaetales with poroid and hydnoid species based on ITS sequences and revealed that *C. lindbladii* grouped with *Gelatoporia subvermispora* (Pilát) Niemelä, which belonged to the order Polyporales. In the present study based on the ITS analysis (Fig. 1), *C. fimbriatus* groups with *C. lindbladii* and clusters into Polyporales, in addition, *Sidera* Miettinen & K.H. Larss. and *Skeletocutis* Kotl. & Pouzar are distinct from the new species, *C. fimbriatus*. Further phylogenetic analysis (Fig. 2) between *C. fimbriatus* and related taxa showed it forms a monophyletic lineage with strong supports (100% BS, 100% BT, 1.00 BPP) and groups with *C. lindbladii*. However, morphologically, *C. lindbladii* differs from *C. fimbriatus* by its greyish pore surface and larger basidiospores (5–7 × 1.5–2 µm, Ryvarden & Melo 2014).

Morphologically, *Cinereomyces dilutabilis* (Log.-Leite & J.E. Wright) Miettinen (2013: 345) differs from *C. fimbriatus* by the light brownish pore surface, smaller pores (6–8/mm) and larger basidiospores (4.8–5.5 × 2.4–2.8 µm, Miettinen 2012).

Ecologically, the species is a white-rot causing saprotrophs growing in coarse woody debris and distributes in temperate-boreal on northern hemisphere (Miettinen & Rajchenberg 2012).

Acknowledgements

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