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A new species of *Lindernia* (Linderniaceae) from Tanzania

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Physisporinus yunnanensis sp. nov. (Polyporales, Basidiomycota) from Southern China

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Abstract

A wood-inhabiting fungal species, *Physisporinus yunnanensis* is proposed based on a combination of morphological features and molecular evidence. *Physisporinus yunnanensis* is characterized by annual, resupinate, bone-hard basidiomata with an olivaceous to black pore surface, a monomitic hyphal system, presence of hyphoid cystidia at dissepiment edge and subglobose, smooth basidiospores measuring as 4–5.5 × 3.5–5 µm, and causing a white rot. The phylogenetic analyses based on molecular data of ITS+nLSU sequences showed that *P. yunnanensis* is sister to *P. furcatus* with high support (100% BS, 100% BP, 1.00 BPP). Both morphological characteristics and molecular phylogenetic analyses confirmed the placement of the new species in *Physisporinus*.

Keywords: 1 new species, Basidiomycetes, Meruliaceae, Phylogeny, Taxonomy, Wood-inhabiting fungi

Introduction

Wood-rotting fungi are crucial components of kingdom fungi with their fundamental roles in carbon balance, soil formation and forest regeneration (Marcot 2017), in which the taxa of the order Polyporales (Basidiomycota) can efficiently degrade lignocellulose biomass on an attractive target for many biotechnological applications of the production of biofuel, bio-pulping, and bioremediation (Fisher & Fong 2014).

Physisporinus P. Karst. (1889: 324) was typified by *P. vitreus* (Pers.) P. Karst. (Karsten 1889). It was defined by annual to perennial, resupinate to pileate, soft to hard corky basidiocarps, changing color on bruising or drying, a monomitic hyphal structure with simple septa on generative hyphae, and absence or presence of cystidia, mostly broadly ellipsoid to globose basidiospores, and causing a white rot (Ryvarden & Melo 2014, Wu *et al.* 2017, Chen & Dai 2021). Based on the MycoBank database (http://www.mycobank.org, accessed on 4 January 2023) and the Index Fungorum (http://www.indexfungorum.org, accessed on 4 January 2023), the genus *Physisporinus* has registered 33 specific and infraspecific names, however, the actual number of species reaches 21 (Ipulet & Ryvarden 2005, Wu *et al.* 2017, Decock & Ryvarden 2021), of which 12 species have been recorded in China (Wu *et al.* 2017, Dai & Dai 2018, Chen & Dai 2021).

Recently, phylogenetic analyses of *Physisporinus* have been carried out to reveal the relationship among genera or species (Wu *et al.* 2017, Dai & Dai 2018, Chen & Dai 2021). Molecular phylogeny inferred from ITS and nLSU sequences data strongly supported *P. sulphureus* Y.C.Dai (2018: 147) as a distinct species belonging to the genus *Physisporinus* (Dai & Dai 2018). The phylogeny and diversity of the morphologically similar polypore genera *Rigidoporus* Murrill (1905: 478), *Physisporinus*, *Oxyporus* (Bourdot & Galzin) Donk. (1933: 202) and *Leucophellinus* Bondartsev & Singer (1941: 57), indicated that *Physisporinus* and related genera were nested into eight lineages, in

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which they belonged to orders Polyporales and Hymenochaetales (Wu *et al*. 2017). Based on the molecular phylogenetic analyses using rDNA ITS1-5.8S-ITS2 and 28S rDNA sequences, Chen and Dai (2021) revealed that *Physisporinus* is a heterogeneous assemblage, including two groups of the *P. furcatus* group and the *P. undatus* group.

The present study describes a new basidiomycetous fungus from Yunnan Province of China. In this paper, we present morphological and molecular phylogenetic evidence to support identifying the new species within *Physisporinus*.

Materials and methods

Morphological studies

Fresh fruiting bodies of a mushroom growing on the angiosperm stump, on the stump of angiosperm were collected from Lijiang (Yunnan Province, P.R. China). The samples were photographed in situ, and fresh macroscopic details were recorded. Photographs were recorded by a Canon 80D camera. All photos were focus-stacked and merged using Helicon Focus software. Macroscopic details were recorded and transported to a field station where the fruit body was dried on an electronic food dryer at 45 °C. Once dried, the specimens were sealed in envelopes and zip-lock plastic bags and labeled. The dried specimens were deposited in the herbarium of the Southwest Forestry University (SWFC), Kunming, Yunnan Province, P.R. China. Macromorphological descriptions are based on field notes and photos captured in the field and lab. Color terminology follows Petersen (Petersen 1996). Micromorphological data were obtained from the dried specimens following observation under a light microscope (Zhao *et al*. 2014). The following abbreviations were used: KOH = 5% potassium hydroxide water solution, CB = cotton clue, CB- = acyanophilous, CB+ = cyanophilous, IKI = Melzer's reagent, IKI- = both inamyloid and indextrinoid, L = means spore length (arithmetic average for all spores), W = means spore width (arithmetic average for all spores), Q = variation in the L/W ratios between the specimens studied, and *n* = a/b (number of spores (a) measured from given number (b) of specimens).

DNA extraction, PCR amplification, sequencing and phylogenetic analyses

A 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 that were slightly modified by grinding a smaller piece of the dried fungal specimen (30 mg) 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 thousand 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 13000 rpm for 0.5 min. Then, 0.5 mL inhibitor removal fluid was added to the AC for centrifugation at 12 thousand rpm for 30 s. After washing twice with 0.5 mL washing buffer, the AC was transferred to a sterile centrifuge tube, and 100 mL elution buffer was added to the middle of the adsorbed film to elute the genome DNA. The internal transcribed spacer region (ITS) was amplified with primer pair ITS5 and ITS4 (White *et al.* 1990). The nuclear large subunit region (nLSU) was amplified with primer pair LR0R and LR7 (https://sites.duke.edu/vilgalyslab/rdna_primers_for_fungi/). The PCR procedure for ITS was as follows: initial denaturation at 95 °C for 3 min, followed by 35 cycles of 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 procedure for LSU was as follows: initial denaturation at 94 °C for 1 min, followed by 35 cycles of 94 °C for 30 s, 48 °C 1 min, and 72 °C for 1.5 min; and a final extension of 72 °C for 10 min. The PCR products were purified and directly sequenced at the Kunming Tsingke Biological Technology Limited Company, Kunming, Yunnan Province, P.R. China. All newly generated sequences were deposited in GenBank (Table 1).

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 29913). Sequences of *Spongipellis spumeus* (Sowerby) Pat. (1887: 140) and *S. quercicola* Y.C. Dai and Chao G. Wang (2022: 13) retrieved from GenBank were used as an outgroup in ITS+nLSU (Fig. 1) analysis following previous study (Nakasone 2021, Chen & Dai 2021).

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

Species name	Sample no.	GenBank accession no.		References
		ITS	LSU	
Physisporinus castanopsidis	Dai 20397	MT309487	MT309472	Chen & Dai (2021)
P. castanopsidis	Dai 20398	MT840113	MT840131	Chen & Dai (2021)
P. cinereus	Cui 3266	KY131844	KY131903	Wu *et al.* (2017)
P. crataegi	Dai 15497	KY131845	KY131904	Wu *et al.* (2017)
P. crataegi	Dai 15499	KY131846	KY131905	Wu *et al.* (2017)
P. crocatus	DLL 2009-061	JQ673152	-	Chen & Dai (2021)
P. eminens	Dai 11400	KY131852	KY131909	Chen & Dai (2021)
P. eminens	Dai 20832	MT279689	MT279910	Chen & Dai (2021)
P. eminens	Dai 20868	MT840117	MT840135	Chen & Dai (2021)
P. furcatus	Dai 20976	MT840118	MT840136	Chen & Dai (2021)
P. furcatus	Dai 20977	MT840119	MT840137	Chen & Dai (2021)
P. furcatus	TAA 15097	KY131853	KY131910	Nufiez *et al.* (2001)
P. lavendulus	Dai 9925	KY131858	KY131915	Wu *et al.* (2017)
P. lavendulus	Dai 13587A	KY131859	KY131916	Wu *et al.* (2017)
P. lineatus	Cui 9139	KY131860	KY131917	Wu *et al.* (2017)
P. lineatus	Dai 17986	MT840121	MT840139	Chen & Dai (2021)
P. longicystidius	PDD 70600	KY131863	-	Wu *et al.* (2017)
P. pouzarii	Dai 21043	MT840124	MT840142	Chen & Dai (2021)
P. pouzarii	JV 0511/23LR	JQ409465	KY131921	Chen & Dai (2021)
P. roseus	Dai 19877	MT840126	MT840144	Chen & Dai (2021)
P. sanguinolentus	KHL 11913	JX109843	JX109843	Dai & Dai (2018)
P. subcrocatus	Dai 12800	KY131869	KY131925	Wu *et al.* (2017)
P. subcrocatus	Dai 15917	KY131870	KY131926	Wu *et al.* (2017)
P. sulphureus	Dai 17839	MG132179	MG132181	Dai & Dai (2018)
P. sulphureus	Dai 17841	MG132180	MG132182	Dai & Dai (2018)
P. sulphureus	Dai 17869	MT840127	MT840145	Chen & Dai (2021)
P. tibeticus	Cui 9381	KY131871	KY131927	Wu *et al.* (2017)
P. tibeticus	Cui 9588	KY131873	KY131929	Wu *et al.* (2017)
P. vinctus	Cui 16903	MT840129	MT840147	Chen & Dai (2021)
P. vitreus	3163	JN710580	JN710580	Chen & Dai (2021)
P. vitreus	KHL 11959	JQ031129	-	Dai & Dai (2018)
P. yunnanensis	**CLZhao 21647**	**OP852340**	**OP852342**	**Present study**
P. yunnanensis	**CLZhao 21583**	**OP852341**	**OP852343**	**Present study**
Spongipellis spumeus	Miettinen X1068	KY415960	KY415960	Dai & Dai (2018)
S. quercicola	Cui_10009	OM971919	OM971899	Wang & Dai (2022)

Maximum parsimony analysis was applied to the ITS+nLSU dataset sequences. Approaches to Maximum followed Zhao and Wu (2017), and the tree construction 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 1000 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 generated. Sequences were also analyzed using Maximum Likelihood (ML) with RAxML-HPC2

through the Cipres Science Gateway (www.phylo.org, Miller *et al.* 2012). 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 Bayesian Inference (BI) data set. BI was calculated with MrBayes 3.1.2 with a general time reversible (GTR+I+G) model of DNA substitution and a gamma distribution rate variation across sites (Ronquist *et al.* 2012). Four Markov chains were run for 2 runs from random starting trees, for 600,000 generations (Fig. 1). The first one-fourth generations were discarded as burn-in. A majority rule consensus tree of all remaining trees was calculated. Branches were considered as significantly supported if they received maximum likelihood bootstrap (BS) ≥70%, maximum parsimony bootstrap (BT) ≥50%, or Bayesian posterior probabilities (BPP) ≥0.95.

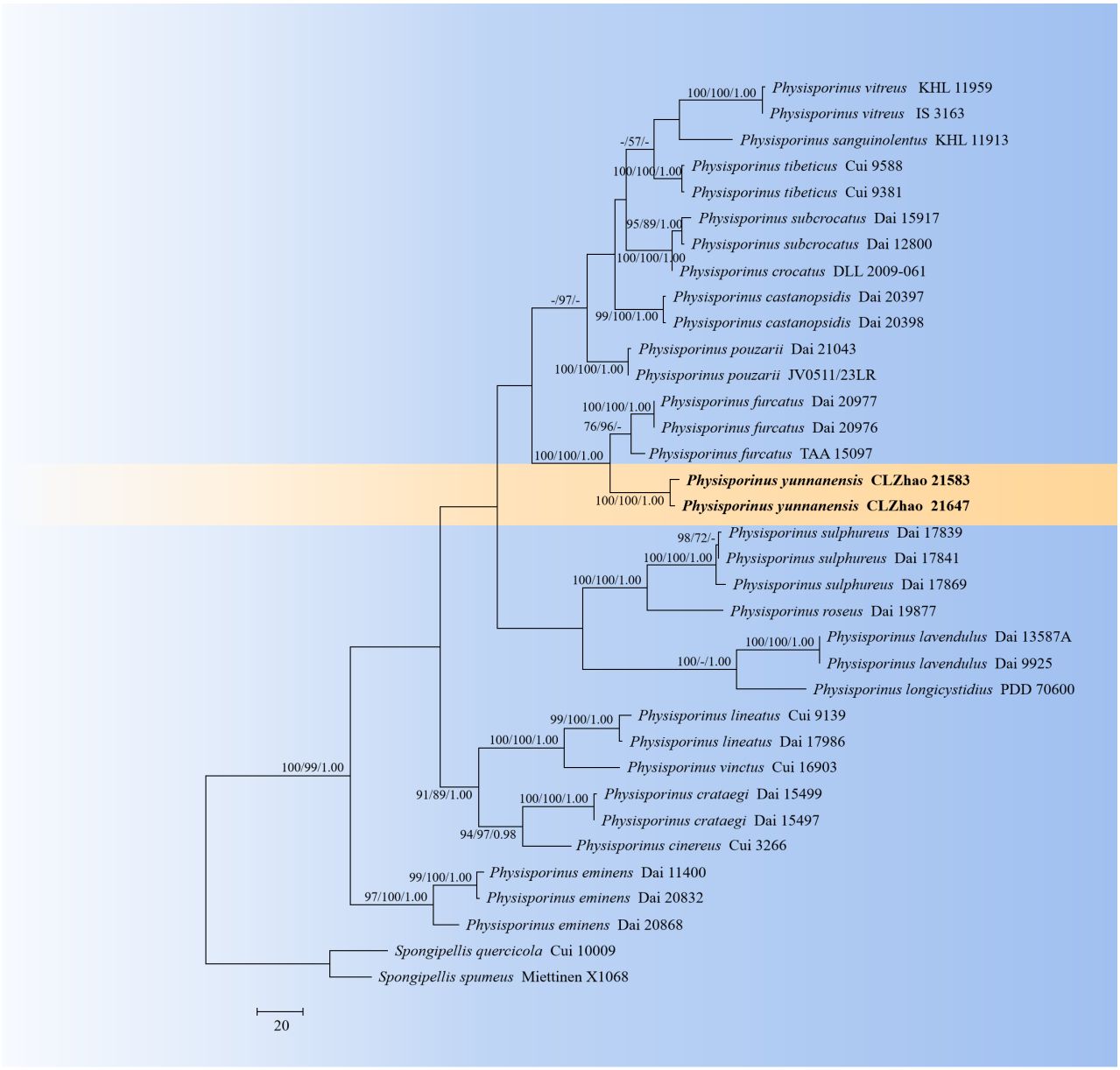

FIGURE 1. Maximum Parsimony strict consensus tree illustrating the phylogeny of one new species of *Physisporinus* based on ITS+nLSU sequences. Branches are labeled with maximum likelihood bootstrap values equal to or higher than 70%, parsimony bootstrap values equal to or higher than 50% and Bayesian posterior probabilities equal to or higher than 0.95.

Results

Molecular phylogeny

The ITS+nLSU dataset (Fig. 1) included sequences from 35 fungal specimens (Including 2 outgroup species) representing 20 species within the genus *Physisporinus*. The dataset had an aligned length of 2018 characters, of which

1577 characters were constant, 72 were variable and parsimony-uninformative, and 369 were parsimony-informative. Maximum parsimony analysis yielded 2 equally parsimonious trees (TL = 1084, CI = 0.5517, HI = 0.4841, RI = 0.7722, RC = 0.4260). The best model for the ITS+nLSU dataset estimated and applied in the Bayesian analysis: was GTR+I+G, lset nst = 6, rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1). Bayesian analysis and ML analysis resulted in a similar topology as MP analysis, with an average standard deviation of split frequencies = 0.006427 (BI) and the effective sample size (ESS) across the two runs is double the average ESS (avg. ESS) = 195.

The phylogenetic tree (Fig. 1) inferred from ITS+nLSU sequences revealed that the new taxon nested into the genus *Physisporinus* and was sister to *P. furcatus.*

Taxonomy

Physisporinus yunnanensis C.L. Zhao, ***sp. nov.*** Figs. 2, 3
MycoBank no.: MB 846536

Etymology:—*yunnanensis* (Lat.) refers to the location "Yunnan Province" where the type specimen was collected.

Holotype:—CHINA. Yunnan Province, Lijiang, Yulong County, Black Dragon Pool Park, E 100°13′57″, N 26°53′15″, elev. 2417 m, on the stump of angiosperm, 21 July 2021, CLZhao 21647 (SWFC).

***Fruiting body*:—**Basidiomata annual, resupinate, soft to juicy when fresh, without odor or taste, bone hard when dry, up to 3 cm long, 2.5 cm wide and 0.5 mm thick at centre. Pore surface olivaceous when fresh, olivaceous to black when dry, slightly staining when touched or bruised; sterile margin narrow, up to 1 mm wide; pores round, shallow, 2–3 per mm; dissepiments thin, entire. Subiculum very thin to almost lacking, olivaceous to brown, hard corky. Tubes concolourous with the pore surface, bone hard, up to 0.4 mm long.

***Hyphal structure*:—**Hyphal system monomitic; generative hyphae with simple septa, IKI-, CB+; tissues unchanged in KOH.

***Subiculum*:—**Subicular generative hyphae colorless, thin to slightly thick-walled, occasionally branched, more or less straight, regularly arranged, 4–6 µm in diam.

***Hymenium*:—**Tramal generative hyphae colorless, thin to slightly thick-walled, moderately branched, interwoven, 3.5–5.5 µm in diam; cystidia larger, strongly encrusted, colorless, thin-walled, 30–76 × 5–8.5 µm; basidia mostly short clavate to barrel-shaped, thin-walled, smooth, with four sterigmata and a simple basal septum, 8–11.5 × 5.5–7 µm ($\bar{x}$ = 9.8 × 6.3 µm); basidioles in shape similar to basidia, but smaller.

***Basidiospores*:—**Subglobose, colorless, thin-walled, smooth, bearing a larger guttule, IKI-, CB-, 4–5.5 × 3.5–5 µm, L = 4.5 µm, W = 4 µm, Q = 1.04–1.08 (n = 60/2).

***Additional specimens examined*:**—CHINA. Yunnan Province, Lijiang, Yulong County, Black Dragon Pool Park, E 100°13′57″, N 26°53′15″, elev. 2417 m, on the stump of angiosperm, 21 July 2021, CLZhao 21583 (SWFC).

***Habitat and ecology*:**—Climate of the sample collection site is monsoon humid, and the forest type is evergreen broad-leaved forest, and the samples were collected on an angiosperm stump.

Discussion

Recently several new wood-inhabiting fungal taxa have been reported from the subtropics and tropics, including the genus *Physisporinus* (Zhou *et al.* 2021). The present study reports a new *Physisporinus* species as *P. yunnanensis*, based on a combination of morphological features and molecular evidence. *Physisporinus* and its morphologically similar genera *Leucophellinus*, *Oxyporus* and *Rigidoporus*, which include some forest pathogens and medicinal species, are very essential groups of wood-decaying fungi, in which four genera include a monomitic hyphal structure, cyanophilous generative hyphae with simple septa, broadly ellipsoid to globose basidiospores, and causing a white rot and these characters have resulted in a complex of species in which generic limits are difficult to define (Wu *et al.* 2017).

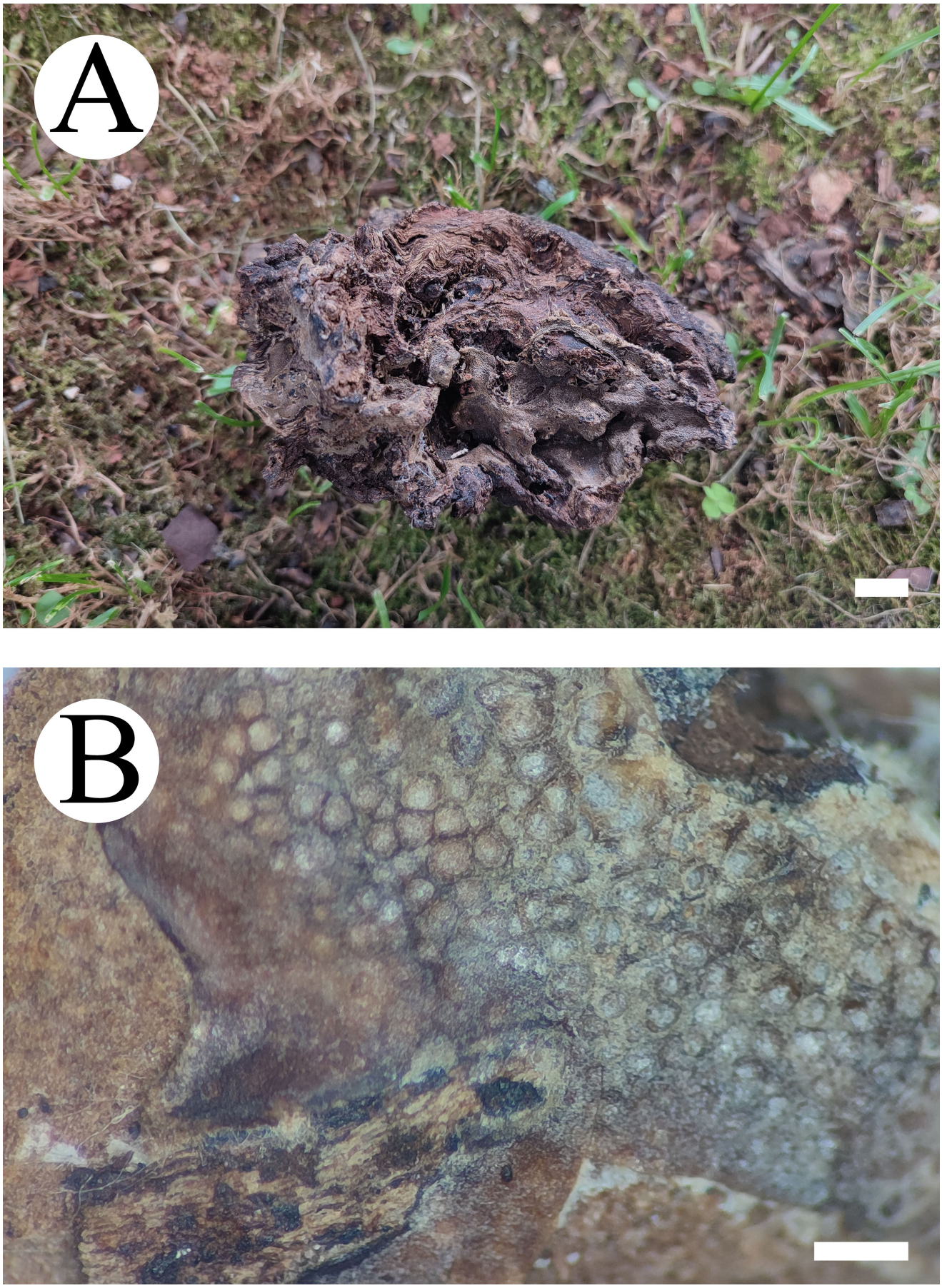

FIGURE 2. Basidiomata of *Physisporinus yunnanensis*. Bars: A = 1 cm, B = 1 mm (Holotype: CLZhao 21647). Photoplate by: Jia Cai.

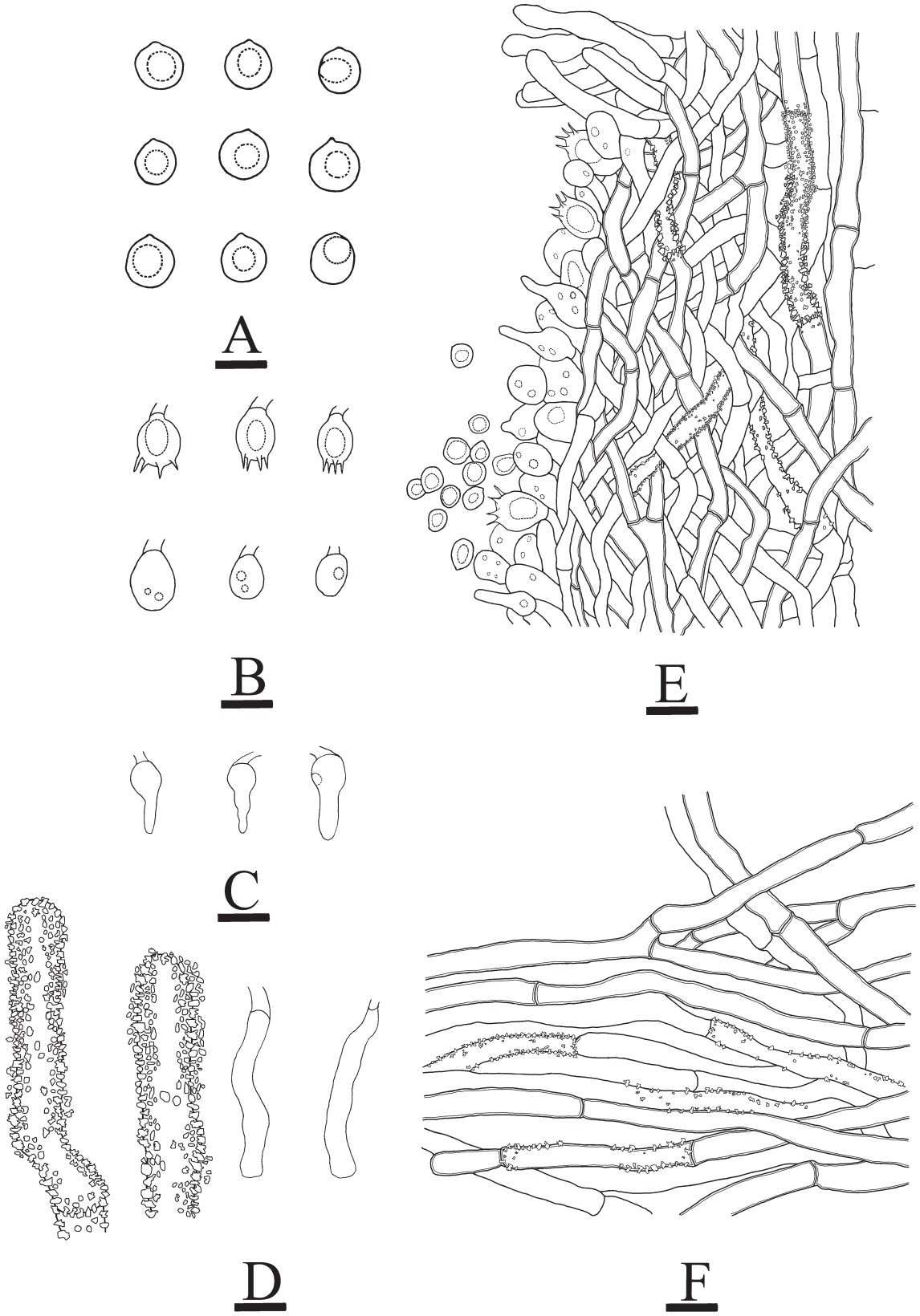

FIGURE 3. Microscopic structures of *Physisporinus yunnanensis* (drawn from the holotype, CLZhao 21647). A: Basidiospores. B: Basidia and basidioles. C: Cystidioles. D: Cystidia and hypha-like cystidia. E: Hyphae from subiculum. F: Hyphae from trama. Bars: A = 5 µm; B–F = 10 µm. Drawings by: Jia Cai.

Phylogenetically, *Physisporinus yunnanensis* was sister to *P. furcatus* and then grouped with a clade comprising *P. cinereus* (Núñez & Ryvarden) F. Wu, Jia J. Chen & Y.C. Dai (2017: 759), *P. crocatus* (Pat.) F. Wu, Jia J. Chen & Y.C. Dai (2017: 760), *P. lineatus* F. Wu, Jia J. Chen & Y.C. Dai (2017: 760), and *P. vitreus*. Morphologically, *P. furcatus* differs from *P. yunnanensis* by its ochraceous pore surface with angular pores, and the presence of forked cystidia (Núñez *et al.* 2001); *P. cinereus* is distinguished from *P. yunnanensis* by its pileate basidiocarps, and smaller pores (5–6 per mm, Núñez & Ryvarden 1999); *P. lineatus* differs in its pileate basidiomata, bright orange-red pore surface, and smaller pores (6–9 per mm, Ryvarden 2016); *P. vitreus* is delimited from *P. yunnanensis* by its white to bluish white pore surface and smaller pores (4–6 per mm, Karsten 1889).

Morphologically, *Physisporinus yunnanensis* is similar to *P. eminens* (Y.C. Dai) F. Wu, Jia J. Chen & Y.C. Dai (2017: 760), *P. roseus* Jia J. Chen & Y.C. Dai (2021: 7) and *P. sulphureus*, by sharing the resupinate basidiomata and subglobose basidiospores, bearing a larger guttule. However, *P. eminens* is distinguished from *P. yunnanensis* by its white to cream pore surface, smaller pore (7–8 per mm) and weakly cyanophilous basidiospores (Dai 1998); *P. roseus* is readily separated from *P. yunnanensis* by its perennial basidiomata, rose pore surface, and smaller pores (5–6 per mm, Chen & Dai 2021); *P. sulphureus* differs from *P. yunnanensis* by its sulphurous pore surface, smaller pores (8–9 per mm) and strongly gelatinized generative hyphae (Dai & Dai 2018).

Morphologically, *Physisporinus yunnanensis* resembles *P. lavendulus* F. Wu, Jia J. Chen & Y.C. Dai (2017: 753), and *P. vinctus* (Berk.) Murrill (1942: 595) by sharing round pores and the presence of cystidia. However, *P. lavendulus* differs from *P. yunnanensis* by its pleate, purple basidiomata, smaller pores (9–10 per mm), and cyanophilous basidiospores (Wu *et al.* 2017); *P. vinctus* differs in its pileate basidiomata, and smaller pores (6–12 per mm, Ryvarden 2016).

To date, 13 species (including the new species in this study) of *Physisporinus* have been recorded in China (Wu *et al.* 2017, Dai & Dai 2018, Chen & Dai 2021), but the species diversity of *Physisporinus* is still not well known in China, especially in subtropical and tropical areas (Wenshan, Puer, Honghe, Baoshan regions). This study enriches our knowledge of fungal diversity in this area, and more new taxa will likely be found with further fieldwork and molecular analyses.

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