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Morphological and molecular identification of a new species of *Fulvoderma* (Hymenochaetaceae, Basidiomycota) from the Yunnan-Guizhou Plateau, China

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Abstract

A new wood-inhabiting fungal species, *Fulvoderma yunnanense* , is proposed based on a combination of morphological features and molecular data . *Fulvoderma yunnanense* is characterized by annual, laterally stipitate basidiomata with greyish brown pore surface , a monomitic hyphal system with simple septate generative hyphae , tubular cystidia and broad ellipsoid to subglobose , thin-walled, smooth basidiospores measuring as 4.0– 5.4 × 3.3– 4.8 µm . Sequences of ITS and nLSU rRNA gene regions of the specimens were generated, and phylogenetic analyses were carried out with methods of maximum parsimony, maximum likelihood, and Bayesian inference. The phylogenetic analyses inferred from ITS+nLSU indicated that *F. yunnanense* nested in *Fulvoderma* within Hymenochaetaceae. Furthermore, the phylogenetic analysis based on the ITS dataset revealed that the new species formed a well-supported monophyletic lineage with *F. australe*. Full description, photo plates and a phylogenetic tree to show the placement of the new species are given.

Key words: 1 new species, Hymenochaetales, Taxonomy, Wood-rotting fungi, Yunnan Province

Introduction

Wood-rotting fungi are a cosmopolitan group and they have a rich diversity on the basis of growing on boreal, temperate, subtropical, and tropical vegetations (Gilbertson & Ryvarden 1986, Núñez & Ryvarden 2001, Bernicchia & Gorjón 2010, Dai 2012, Ryvarden & Melo 2014, Dai *et al.* 2015, Dai *et al.* 2021). *Fulvoderma* L.W. Zhou & Y.C. Dai, is one of the members of wood-rotting fungi and it was typified by *F. australe* L.W. Zhou & Y.C. Dai (Zhou *et al.* 2018). This genus is characterized by annual, sessile to laterally stipitate basidiocarps with yellowish brown pileal surface; a monomitic hyphal system with simple septate generative hyphae; hyphoid and hymenial setae absent; broadly ellipsoid basidiospores, hyaline, thin-walled, IKI–, CB– (Zhou *et al.* 201 8). So far about 2 species have been accepted in the genus worldwide (Zhou *et al.* 2018).

The molecular systematics involves *Fulvoderma* (2018: 876) based on the internal transcribed spacer (ITS) regions and the large subunit nuclear ribosomal RNA gene (nLSU) sequences were carried out recently (Zhou *et al.* 2018). To resolve the relationships among the species of *Pyrrhoderma* Imazeki (1955: 388) and other related taxa, specimens from China, Costa Rica, Singapore, and Thailand were studied for both morphological and phylogenetic perspectives, in which a new genus, *Fulvoderma*, was erected to accommodate *F. scaurum* (2018: 879) and *F. australe* (2018: 876) (Zhou *et al*. 2018).

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During investigations on wood-inhabiting fungi in southern China, an additional taxon of *Fulvoderma* was found, which could not be assigned to any described species. In this study, the authors introduce a new *Fulvoderma* species based on morphological characteristics and molecular phylogenetic evidence .

Materials and methods

Morphological studies

Fresh fruiting bodies of the fungi growing on the angiosperm trunk were collected from the Wenshan of Yunnan Province, China. The samples were photographed in situ. The fresh macroscopic details were recorded and the samples were taken to the field station where the fruiting bodies were dried by using an electronic food dryer at 45°C (Hu *et al.* 2022). Once dried, the specimens were stored in envelopes sealed in 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. The macro-morphological descriptions were based on field notes and photos captured in the field and lab. Special colour terms follow Petersen (1996). Micro-morphological data were obtained from the dried specimens, and observed under a light microscope following Dai (2010). 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.

Molecular procedures 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 that were slightly modified by grinding a small piece of dried fungal specimen (about 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 13,000 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 to the AC for 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 the adsorbed film to elute the genomic DNA. The internal transcribed spacer region (ITS) was amplified with primer pair ITS5 and ITS4 (White *et al.* 1990). The nuclear LSU region was amplified with primer pair LR0R and LR7 (http://lutzonilab.org/primer-sequences/). 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 at 94 °C for 30 s, 48 °C for 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 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 29599). *Hyphodontia pallidula* (Bres.) J. Erikss. (1958: 104) and *H . arguta* (Fr.) J. Erikss (1958: 104). obtained from GenBank were used as an outgroup to root trees (Zhou 2015) in the ITS+nLSU analysis (Fig. 1); *Coniferiporia sulphurascens* (Pilát) L.W. Zhou & Y.C. Dai (2016: 994) and *Phellinidium ferrugineofuscum* (P. Karst.) Fiasson & Niemelä (1984: 26) retrieved from GenBank were selected as an outgroup for phylogenetic analyses of the ITS dataset following the previous study of Zhou *et al.* (2018) (Fig. 2) .

Maximum parsimony analysis was applied to the ITS +nLSU dataset sequences. Phylogenetic analyses 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 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 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.

TABLE 1. Names, voucher numbers, references and corresponding GenBank accession numbers of sequences used in this study. The new species are in bold.

Species name	Voucher numbers	GenBank accession no.		References
		ITS	nLSU	
Coniferiporia qilianensis	Yuan 6424	NR_158318	KJ635808	Pan & Zhou (2016)
C. qilianensis	Yuan 6441	KR350562	KJ635809	Pan & Zhou (2016)
C. sulphurascens	Cui 10429	KR350565	—	Pan & Zhou (2016)
Cylindrosporus flavidus	Cui 10479	KP875563	KP875560	Zhou (2015)
C. flavidus	Dai 13213	KP875564	KP875561	Zhou (2015)
Fulvoderma australe	Cui 10342	MF860766	—	Zhou *et al.* (2018)
F. australe	Cui 10343	MF860767	—	Zhou *et al.* (2018)
F. australe	Dai 8125	MF860768	—	Zhou *et al.* (2018)
F. australe	Dai 11257	MF860769	MF860720	Zhou *et al.* (2018)
F. australe	Dai 11664	MF860770	—	Zhou *et al.* (2018)
F. australe	Dai 11671	MF860771	—	Zhou *et al.* (2018)
F. australe	LWZ 20160728-20	MF860772	MF860724	Zhou *et al.* (2018)
F. australe	Yuan 6120	MF860773	—	Zhou *et al.* (2018)
F. scaurum	Cui 9881	MF860774	MF860726	Zhou *et al.* (2018)
F. scaurum	Cui 10112	MF860775	—	Zhou *et al.* (2018)
F. scaurum	Cui 11160	MF860776	—	Zhou *et al.* (2018)
F. scaurum	Dai 7005	MF860777	—	Zhou *et al.* (2018)
F. scaurum	Dai 15020	MF860778	MF860729	Zhou *et al.* (2018)
F. scaurum	Dai 15492	MF860779	—	Zhou *et al.* (2018)
F. scaurum	LWZ 20130909-2	MF860780	—	Zhou *et al.* (2018)
F. scaurum	Wei 3067	MF860781	—	Zhou *et al.* (2018)
F. scaurum	Wei 5750	MF860782	—	Zhou *et al.* (2018)
F. yunnanense	**CLZhao 10624**	**OL619277**	**OL619272**	**Present study**
F. yunnanense	**CLZhao 10651**	**OL619278**	**OL619273**	**Present study**
F. yunnanense	**CLZhao 10836**	**OL619279**	**—**	**Present study**
F. yunnanense	**CLZhao 10841**	**OL619280**	**OL619275**	**Present study**
F. yunnanense	**CLZhao 10854**	**OL619281**	**OL619276**	**Present study**
Hyphodontia pallidula	SFC20180810-04	MK992852	—	Lupala *et al.* (2019)
H. arguta	KHL 11938	EU118632	EU118633	Larsson (2007)
Phellinidium ferrugineofuscum	Cui 10042	KR350573	KR350559	Pan & Zhou (2016)
P. ferrugineofuscum	LWZ 20130510	—	KR350560	Pan & Zhou (2016)
Phellinus adamantinus	Cui 6105	MF860784	MF860733	Zhou *et al.* (2018)
P. adamantinus	Dai 13084	MF860789	MF860735	Zhou *et al.* (2018)
Pyrrhoderma hainanense	LWZ 20150530-1	MF860794	MF860739	Zhou *et al.* (2018)
P. hainanense	IFP 019153	NR_158943	NG_064468	Zhou *et al.* (2018)
P. lamaense	Dai 16227	MF860802	MF860743	Zhou *et al.* (2018)
P. lamaense	Dai 17500	MF860804	MF860748	Zhou *et al.* (2018)
P. noxium	Dai 17754	MF860809	MF860752	Zhou *et al.* (2018)
P. noxium	LWZ 20150601-3	MF860810	MF860750	Zhou *et al.* (2018)
P. yunnanense	LWZ 20140719-12	MF860814	MF860755	Zhou *et al.* (2018)
P. yunnanense	LWZ 20140719-13	MF860815	MF860756	Zhou *et al.* (2018)

MrModeltest 2.3 (Nylander 2004) was used to determine the best-fit evolution model for each data set for Bayesian Inference (BI). BI was calculated with MrBayes 3.2.7a with a general time reversible (GTR+I+G) model of DNA substitution and a gamma distribution rate variation across sites (Ronquist *et al.* 2003). Four Markov chains were run for 2 runs from random starting trees for 350 thousand generations for ITS+nLSU (Fig. 1), and for 350 thousand generations for ITS (Fig. 2). 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) ≥75%, maximum parsimony bootstrap (BT) ≥75%, or Bayesian posterior probabilities (BPP) ≥0.95.

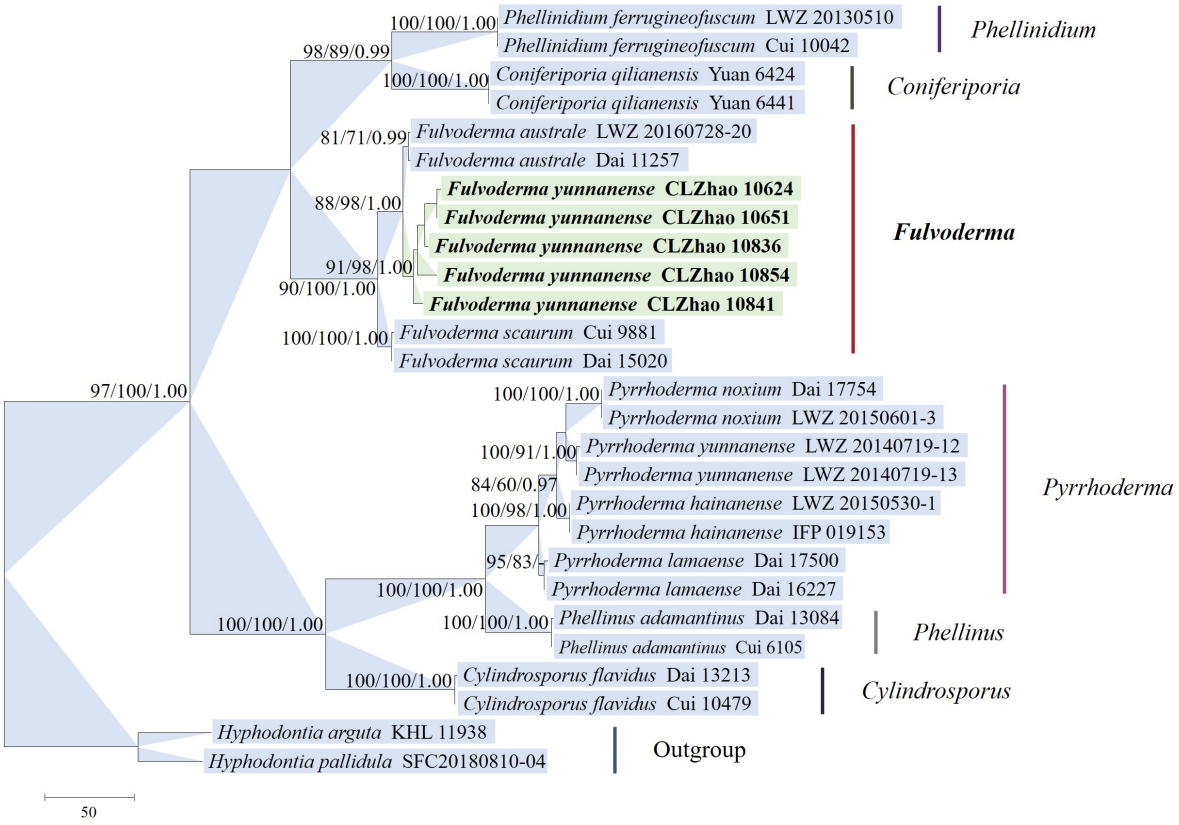

FIGURE 1. Maximum Parsimony strict consensus tree illustrating the phylogeny of *Fulvoderma yunnanense* and related genera in the family Hymenochaetaceae based on ITS+nLSU sequences. Branches are labelled with maximum likelihood bootstrap values ≥ 70%, parsimony bootstrap values ≥ 50% and Bayesian posterior probabilities ≥ 0.95, respectively, Scale bar = 20. The new species are in bold.

Results

Molecular phylogeny

The ITS +nLSU dataset (Fig. 1) included sequences from 27 fungal specimens representing 13 species between the *Fulvoderma* clade and related genera within the family Hymenochaetaceae. The dataset had an aligned length of 1 674 characters, of which 1106 characters were constant, 150 were variable and parsimony-uninformative, and 418 were parsimony-informative. Maximum parsimony analysis yielded 35 equally parsimonious trees (TL = 961, CI = 0. 8106, HI = 0. 1894, RI = 0. 9297, RC = 0. 7537). 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 = 0.009 962 (BI) , and the effective sample size (ESS) across the two runs is the double the average ESS (avg ESS) = 108. The phylogram based on ITS+nLSU sequences (Fig. 1), demonstrated that the new taxon clustered into genus *Fulvoderma* .

The ITS dataset included 24 fungal specimens representing 5 species within the genus *Fulvoderma*. The dataset had an aligned length of 664 characters, of which 513 characters are constant, 80 are parsimony-informative, and 71 are variable and parsimony-uninformative. Maximum parsimony analysis yielded 42 equally parsimonious trees (TL = 181 , HI = 0. 0718 , RI = 0. 9340 , CI = 0. 9282 , RC = 0. 8669). The best model for the ITS dataset estimated and applied in the Bayesian analysis was GTR+I+G (lset nst = 6, rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1)). ML analysis and Bayesian analysis resulted in a similar topology to MP analysis and Bayesian analysis has an average standard deviation of split frequencies = 0.009 871 (BI), and the effective sample size (ESS) across the two runs is double the average ESS (avg ESS) = 184 . The phylogenetic tree (Fig. 2) inferred from ITS sequences revealed that *Fulvoderma yunnanense sp. nov.* formed a monophyletic lineage with high supports of 100% BS, 100% BP, and 1.00 BPP and then grouped with *F. australe* L.W. Zhou & Y.C. Dai .

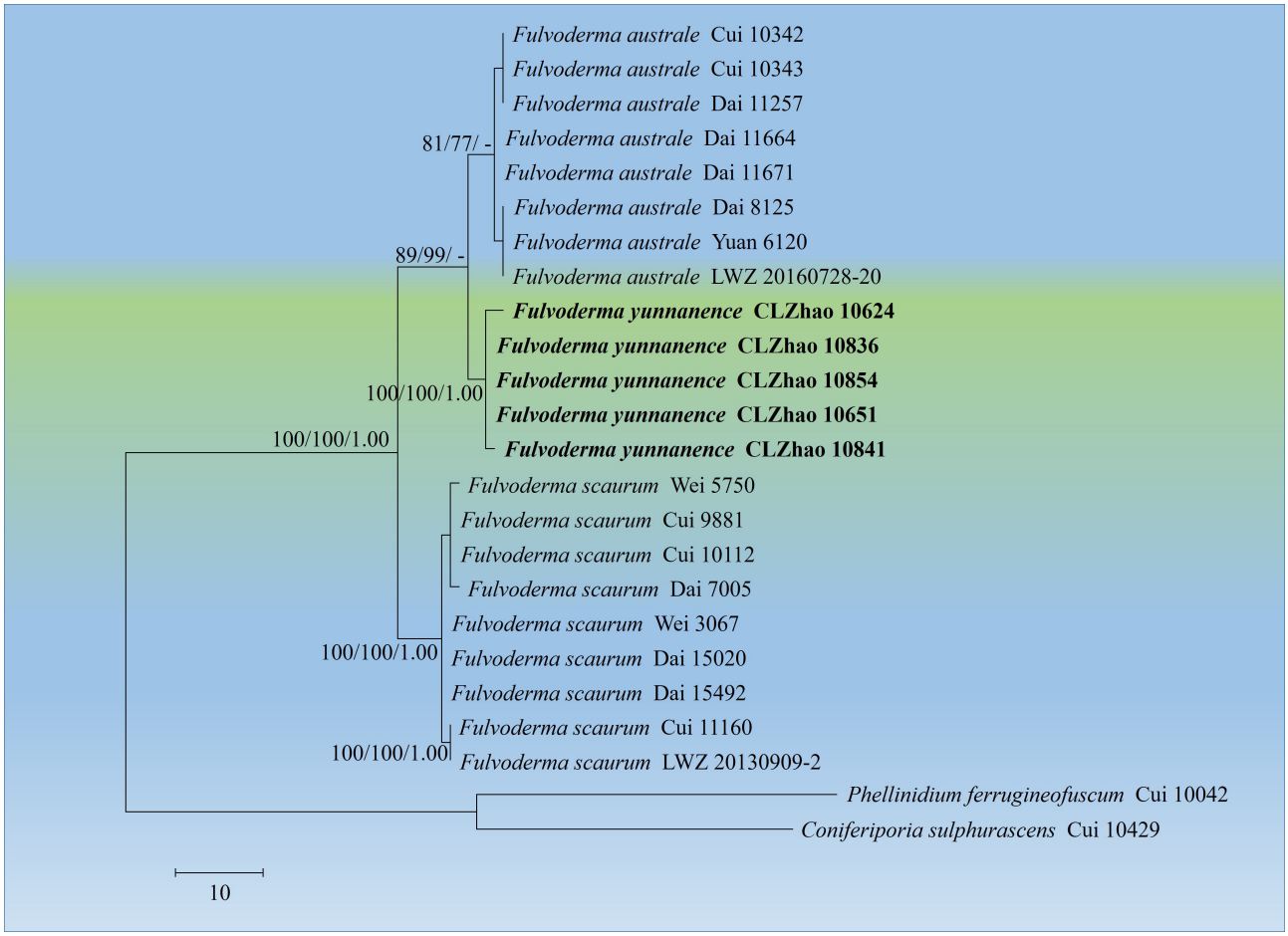

FIGURE 2. Maximum Parsimony strict consensus tree illustrating the phylogeny of *Fulvoderma yunnanense* and related species in the genus *Fulvoderma* based on ITS sequences. Branches are labelled with maximum likelihood bootstrap values ≥ 70%, parsimony bootstrap values ≥ 50% and Bayesian posterior probabilities ≥ 0.95, respectively , S cale bar = 20. The new species are in bold.

Taxonomy

Fulvoderma yunnanense C.L. Zhao, *sp. nov.* Figs . 3, 4

MycoBank no.: MB 843524

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

Holotype:—CHINA. Yunnan Province, Wenshan, Xichou County, Xiaoqiaogou, Wenshan National Nature Reserve, E 104°46′, N 23°22′, elev. 1541.5 m , on the angiosperm trunk, 14 January 2019, CLZhao 10854 (SWFC), Genbank No. (ITS OL619281; nLSU ON075542).

Description:—*Basidiomata* annual, solitary, laterally stipitate , without odor or taste when fresh, corky to woody hard. Pilei applanate, projecting up to 8 cm long, 6 cm wide, and 2.6 cm thick at the base. *Pileal surface* reddish brown to fuscus, distinctly concentrically zonate and sulcate. Margin acute, honey-yellow. *Pore surface* greyish brown.

Sterile margin cinnamon-buff, up to 3 mm wide; pores mostly circular, 4–6 per mm; dissepiments thick, entire. *Context* yellowish brown, hard, up to 1.5 cm thick, indistinctly concentrically zonate. *Tubes* greyish brown, hard, up to 0.2 cm long, tissue unchanged in KOH. *Stipe* up to 3 cm long and 1.7 cm diameter.

Hyphal structure:—Hyphal system monomitic; generative hyphae with simple septate, IKI–, CB–; tissues unchanged in KOH.

Subiculum:—Subicular generative hyphae colorless, hyaline, thin to thick-walled, branched, 2.5 – 6 µm in diameter.

Hymenium:—Cystidia tubular , 35–60 × 3. 0– 4.0 µm ; basidia clavate, with 4 sterigmatas and a septum, 1 8.0–39.4 × 5.6–9.1 µm; basidioles dominant, in shape similar to basidia, but slightly smaller.

Spores:—Basidiospores broad ellipsoid to subglobose, colorless , thin-walled, IKI–, CB–, (3.7–)4.0–5.4(– 5.5) × (3.0–)3.3– 4.8(– 5.0) µm , L = 4.70 µm, W = 4.15 µm, Q = 1.13– 1.18 (n = 150/ 5).

Rot type:—A white rot.

Additional specimens (paratypes) examined:—CHINA. Yunnan Province, Wenshan, Xichou County, Xiaoqiaogou, Wenshan National Nature Reserve, E 104°46′, N 23°22′, elev. 1541.5 m, on the angiosperm trunk, 14 January 2019, CLZhao 10624, CLZhao 10651, CLZhao 10836, CLZhao 10841(SWFC).

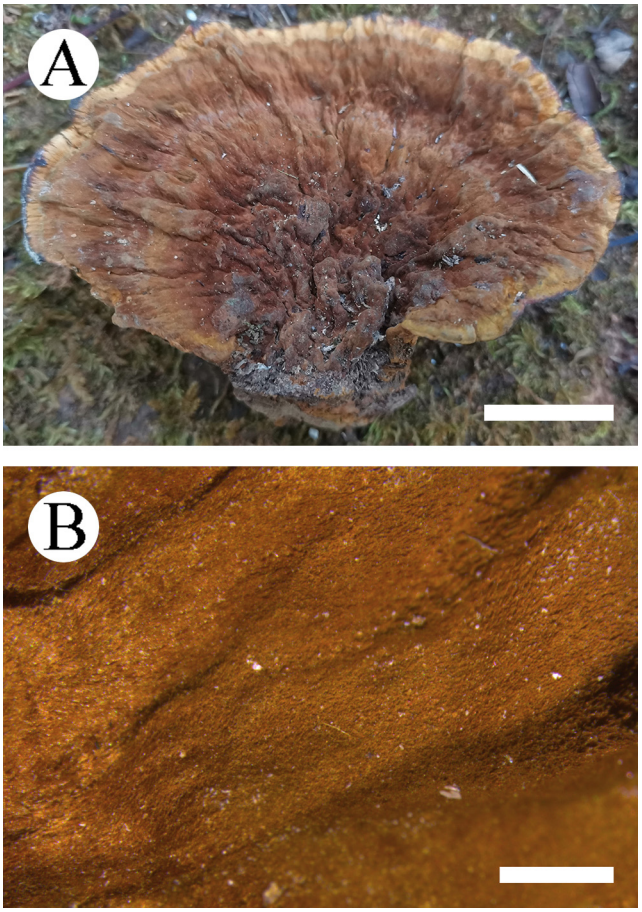

FIGURE 3. Basidiomata of *Fulvoderma yunnanense*. A. The front of the basidiomata . B. The back of the basidiomata . Bars: A = 2 cm; B = 2 mm (Holotype: CLZhao 10854). Photoplate by: Jia-Jin Li.

Discussion

In the present study, *Fulvoderma yunnanense sp. nov*., is described based on phylogenetic analyses and morphological characteristics.

Phylogenetically, Zhou *et al.* (2018) focused on the phylogenetic position of *Fulvoderma* and *Pyrrhoderma* within Hymenochaetaceae inferred from the 28S dataset and revealed that the *Fulvoderma* species grouped together and clustered with *Coniferiporia* and *Phellinidium*. In the present study, the species of *Fulvoderma* group together and then group closely with *Coniferiporia* and *Phellinidium* as well as previous study. The topology constructed from the

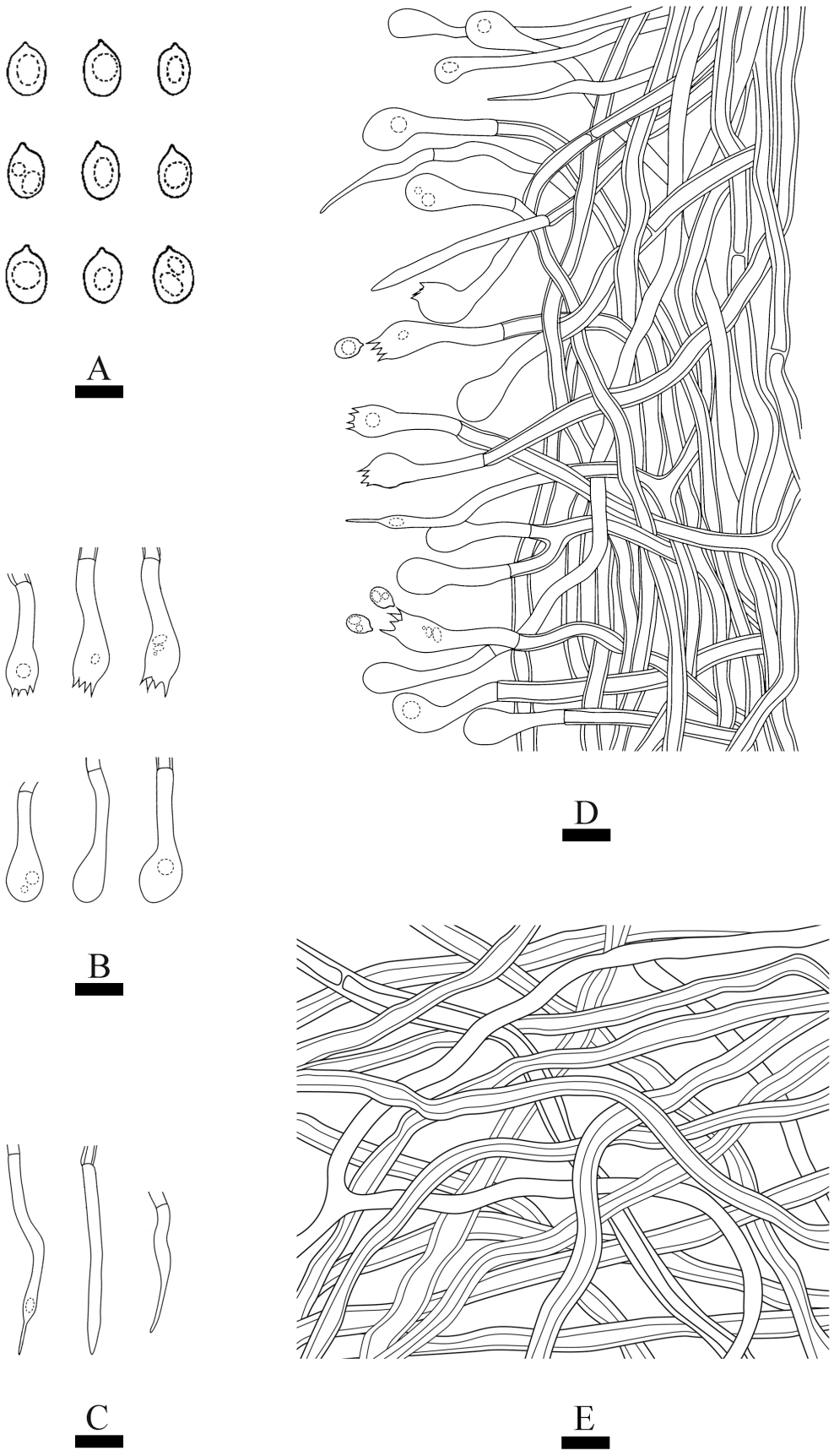

FIGURE 4 . Microscopic structures of *Fulvoderma yunnanense* (drawn from the holotype, CLZhao 10854). A. Basidiospores; B. Basidia and basidioles; C. Cystidia; D. Hyphae from context; E. Hymenium and hyphae from trama. Bars: A = 5 μm ; B–E = 10 μm . Drawings by : Jia-Jin Li.

maximum likelihood method and Bayesian inference method showed that *F. australe* was sister to *F. scaurum* (Lloyd) L.W. Zhou & Y.C. Dai, which nested into *Fulvoderma*. The present study reveals that *F. yunnanense* groups with *F. australe* and then clusters with *F. scaurum*. However, morphologically *F. australe* differs from *F. yunnanense* by the distinct cuticle on the pileal surface, angular pores with thin dissepiments, and unbranched contextual hyphae (Zhou *et al.* 2018). The species *F. scaurum* differs in its yellow to blackish brown upper surface and yellowish brown to brown pore surface with smaller pores round (6–8/mm) (Lee *et al.* 2020).

In geographical distribution, two species of *Fulvoderma* were reported from China (Dai 2012; Zhou *et al.* 2018). The diversity of *Fulvoderma* in China is still not well known, especially in the subtropical and tropical regions and previously numerous new species of wood-rotting fungi have been found in China (Dai *et al.* 2021; Huang *et al.* 2020; Ma *et al.* 2020; Petersen J. H. 1996; Wu *et al.* 2020), and present paper confirmed the fungal diversity is very rich in the Chinese ecosystem. I believe more species of *Fulvoderma* occur in subtropical and tropical Asia, because wood-inhabiting fungi are a well-studied group of Basidiomycota, and they are much rich in tropical China (Cai *et al.* 2023; Dai Y. C. 2010; Dai *et al.* 2021; Duan *et al.* 2023; Martin *et al.* 2015; Pan & Zhou 2016; Wang *et al.* 2020), and it is very possible the same phenomenon for *Fulvoderma*.

Acknowledegments

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