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Morphological and molecular identification of a new species of *Fulvoderma* (Hymenochaetaeae, 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 Hymenochaetaeae. 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 & Górrón 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; tubular 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 Hymenochaetaeae. 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.
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) 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) 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.

<table>
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<tr>
<th>Species name</th>
<th>Voucher numbers</th>
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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 (iset 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 = 0.009871 (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.

**Taxonomy**

*Fulvoderma yunnanense* C.L. Zhao, *sp. nov.* Figs. 3, 4
MycoBank no.: MB 843524

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

**Holotype:** — CHINA. Yunnan Province, Wenshan, Xichou County, Xiaqiaogou, 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.

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**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. Scale bar = 20. The new species are in bold.
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 sterigmata 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).


**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. australis 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. australis and then clusters with F. scaurum. However, morphologically F. australis 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.

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