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RESEARCH ARTICLE

Morphological characteristics and phylogenetic analysis reveal *Punctulariopsis fissurata* sp. nov. (Punctulariaceae, Basidiomycota) from Yunnan, China

Akmal Muhammad, Minglan Chen, Wen Li and ChangLin Zhao

College of Forestry, Southwest Forestry University, Kunming, People's Republic of China

ABSTRACT
A new wood-inhabiting fungal species, *Punctulariopsis fissurata*, is proposed based on morphological description and molecular verification. The species is characterised by the resupinate basidiomata with a pinkish buff salmon hymenial surface, a monomitic system with the generative hyphae bearing clamp connections and the presence of dendrohyphidia with broadly ellipsoid basidiospores measuring 13–15.5 × 8.5–11.5 µm. Phylogenetic analysis of the combined nrDNA-ITS and LSU gene sequences verified that *P. fissurata* formed an independent lineage sister to *P. efibulata* with strong statistical support. A full description, illustrations and phylogenetic analysis results of the new species are provided.

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New species; phylogenetic analysis; southwest China; taxonomy; wood-rotting fungi

Introduction

Wood-inhabited fungi have been considered important biological entities that play significant ecological roles in forest ecosystems. These wood-inhibiting fungi are macrofungi mostly grow on woody substances and belong to Basidomycota (Wang et al. 2021a). Many species of wood-inhabiting fungi are edible and medicinal, while others have toxic metabolites (Wu et al. 2019; Zhou et al. 2022; Cheng et al. 2023), which have properties to degrade lignocellulose in wood materials that play crucial ecological role in recycling and energy flow in forest ecosystems (Floudas et al. 2012; Zhou and Dai 2012; Zhao et al. 2023). Some wood-inhabiting fungi are also forest pathogens which cause economic losses to these biological resources. Species diversity is a key indicator for the conservation of both forests and wood-inhabiting fungi (Krah et al. 2018; Wang et al. 2022; Bai et al. 2023; Zhou and May 2023).

Corticioid fungi are a large distinctive group of Basidiomycota, which are mainly distributed in tropical and subtropical areas (Ghobad-Nejhad et al. 2010; Dai 2011; Cui et al. 2019; Wang et al. 2021a, 2021b; Ji et al. 2022; Luo et al. 2022; Luo and Zhao 2023; Liu et al. 2023; Yang et al. 2024; Zhao et al. 2023; Dong et al. 2024; Su et al. 2024; Wang et al. 2024; Zhang et al. 2024; Zhou et al. 2024). To date, only five species of

CONTACT ChangLin Zhao fungichanglinz@163.com
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Punctulariopsis have been identified (Ghobad-Nejhad et al. 2010; Ariyawansa et al. 2015a; Muhammad et al. 2024). A phylogenetically varied group of fungi with a relatively simple fruiting body structure is known as corticioid fungi (non-poroid resupinate). Corticioid lineages are present in all main clades of Agaricomycotina (Binder et al. 2005; Ghobad-Nejhad et al. 2021); additionally, the resupinate habit represents the primordial form of basidiomycete fruiting bodies (Hibbett and Binder 2002). The taxa of the order Corticiales exhibit notable ecological and nutritional heterogeneity since various substrata are inhabited by lineages that are lichenicolous, saprophytic, and plant pathogenic (Lawrey et al. 2008; Rungjindamai et al. 2008; Dong et al. 2024). Corticioid species with simple resupinate basidiomes growing on angiosperm wood are accounted for by the small family Punctulariaceae Donk in the Corticiales (Donk 1964). Every species has ellipsoid basidiospores that are inamyloid and dendrohyphidia. According to Ariyawansa et al. (2015a), this family includes three genera; *Punctularia* Pat., *Dendrocorticium* M.J. Larsen & Gilb., and *Punctulariopsis* Ghob. -Nejh.

The genus *Punctulariopsis* (Punctulariaceae, Corticiales) was recognised in 2010 and was typified by *P. subglobispora* (Hallenb. & Hjortstam) Ghob. -Nejh. (2010: 1529) (Ghobad-Nejhad et al. 2010). The genus is categorised by basidiocarp resupinate, strictly adnate, reddish-ochraceous, hymenium surface smooth when fresh, subgelatinous became corneous in a dried state, velutinose to pruinose under lens, abrupt margin, loosening and concolorous with the hymenium. The hyphal system is monomitic with the generative hyphae, and clamps are yellowish-brown and thin-walled in the hymenial layer while thickened and walled in the basal layer. The subiculum possessed clumped hyphae with a brown tint, which is horizontally arranged. Basidia elongate, large clavate, flexuous, with four sterigmata and a basal clamp, walls occasionally thickened, and cystidia are absent. Dendrohyphidia are smooth or irregularly encrusted and lightly branched. Basidiospores are broadly smooth, ellipsoid, contents granular with thick walls (Ghobad-Nejhad et al. 2010). Based on the combined ITS and LSU loci, three genera *Dendrocorticium*, *Punctulariopsis*, and *Punctularia* were described in Punctulariaceae (Ghobad-Nejhad et al. 2010). Based on the MycoBank (http://www.MycoBank.org, accessed on January 14, 2025) and the Index Fungorum (http://www.indexfungorum.org, accessed on January 14, 2025), the genus *Punctulariopsis* has five species, *viz. P. cremeoalbida* (M.J. Larsen & Nakasone) Ghob. -Nejh. (2015: 224), *P. efibulata* (M.J. Larsen & Nakasone) Ghob. -Nejh. (2015: 224), *P. obducens* (Hjortstam & Ryvarden) Ghob. -Nejh. (2010: 1529), *P. subglobispora* (Ghobad-Nejhad et al. 2010; Ariyawansa et al. 2015b) and *P. yunnanensis* (Muhammad et al. 2024) Morphologically, *Punctulariopsis* resembled *Punctularia* but had a thinner basidiocarp, less developed dendrohyphidia, and larger basidia and basidiospores (Ghobad-Nejhad et al. 2010). The LSU phylogeny presented that the taxa in Punctulariaceae included the three well-supported clades as *Punctulariopsis*, *Dendrocorticium* M.J. Larsen & Gilb., and *Punctularia* (Ghobad-Nejhad et al. 2010).

In this study, fruit bodies of the basidiomycetous fungus were collected from Yunnan Province of China, which could not be allocated to any designated species in Punctulariaceae. We present molecular phylogenetic analysis and morphological characteristics to introduce the fungus as a new species in *Punctulariopsis*.

Materials and methods

Morphological studies

Specimens were collected from Tuole Village, of Dapo Town located in Zhanyi District, Qujing Yunnan Province P.R. China having geographical coordinates of E 103°39′ 40″, N 25° 35′ 30″, alt. 1740m, on the fallen branch of angiosperm. Observations of collected fungi and substrate (host, type of rot, etc.) were recorded and photographed. For better understanding macroscopic details were captured and the samples were photographed in situ. The Nikon D7100 camera was used to take the pictures. Helicon Focus software was used to focus-stack all of the images. After recording macroscopic details, the fruit body was taken to a field station and dried at 45°C using an electronic food dryer (Hu et al. 2022; Dong et al. 2024c). The samples were labeled and sealed in zip-lock plastic bags and an envelope once they had dried (Senanayake et al. 2020d; Rathnayaka et al. 2024; Dong et al. 2024c).

Macromorphological studies were conducted on basis of field notes, while Petersen (1996) followed for the colour terminologies. Micromorphological descriptions were recorded from the dried specimens (Hu et al. 2022) and examined under a compound light microscope with a magnification of 10 × 100 oil (Zhao et al. 2023; Dong et al. 2024c). Sections mounted in 5% KOH and 2% phloxine B (C20H2Br4Cl4Na2O5), and we also applied other reagents, including Cotton Blue and Melzer's reagent to observe micromorphology following as per Wu et al. (2022b). The following abbreviations are used: KOH = 5% potassium hydroxide water solution, CB– = acyanophilous, IKI– = both inamyloid and non-dextrinoid, L = mean spore length (arithmetic average for all spores), W = mean spore width (arithmetic average for all spores), Q = variation in the L/W ratios between the specimens studied, Qm represented the average Q of basidiospores measured ± standard deviation, and n = a/b (number of spores (a) measured from given number (b) of specimens. The specimens studied in this experiment were deposited at the museum of the Southwest Forestry University (SWFC) Kunming, Yunnan P.R. China.

Molecular studies

The DNA extraction and PCR of fungal specimens followed by using the EZNA HP Fungal DNA Kit (Omega Biotechnologies Co., Ltd., Kunming) (Xu et al. 2019; Zhang et al. 2023). Newly created sequences were deposited to GenBank (Table 1).

Sequences were manually modified in BioEdit after being aligned using the "G-INS-I" method in MAFFT 7 (https://mafft.cbrc.jp/alignment/server/) (Hall 1999; Katoh et al. 2019). *Gloeophyllum abietinum* (Bull.) P. Karst. (1882: 80) and *G. sepiarium* (Bull.) P. Karst. (1882: 79) sequences were applied as the outgroup (Ghobad-Nejhad and Duhem 2014). Maximum parsimony (MP), Maximum Likelihood (ML), and Bayesian Inference (BI) analyses were applied to the combined three datasets following a previous study (Felsenstein 1985; Posada and Crandall 1998; Swofford 2002; Nylander 2004; Larsen and Nakasone 1984; Zhao and Wu 2017; Wang et al. 2020), while the MrBayes3.1.2 model of DNA substitution was computed using BI (Ronquist and Huelsenbeck 2003).

Table 1. Names, sample numbers and corresponding GenBank accession numbers of the taxa used in this study.

Species Name	Sample no.	GenBank accession no		References
		ITS	nLSU	
Dentocorticium taiwanianum	He 4615	MF626362	MF626386	Liu et al. (2018)
D. taiwanianum	He 3777	—	MF626388	Liu et al. (2018)
D. portoricense	personal: Alden Dirks: ACD0358	ON364085	ON369537	Unpublished
D. portoricense	personal: Alden Dirks: ACD0365	ON364088	ON369540	Unpublished
D. portoricense	He 2202	MF626357	MF626381	Liu et al. (2018)
D. portoricense	He 2161	MF626356	MF626380	Liu et al. (2018)
D. bicolor	He 2772	MF626354	MF626378	Liu et al. (2018)
D. bicolor	He 2757	MF626355	MF626379	Liu et al. (2018)
D. sulphurellum	DLL2011-016	KJ140542	—	Brazee et al. (2014)
D. sulphurellum	FP11801	JN165018	—	Justo and Hibbett (2011)
D. ussuricum	CLZhao 16063	MW582752	—	Unpublished
D. ussuricum	He 3278	MF626358	MF626382	Liu et al. (2018)
D. ussuricum	KUC20121102-32	KJ668553	KJ668406	Unpublished
D. ussuricum	He 3322	MF626360	MF626384	Liu et al. (2018)
Gloeophyllum sepiarium	FP 125002-T	—	AY333806	Ghobad-Nejhad and Ginns (2012)
G. abietinum	P254	—	AJ583431	Moreth and Schmidt (2005)
Punctularia strigosozonata	AFTOL-ID 1248	DQ398958	—	Unpublished
P strigosozonata	CBS 345.34	MH855559	MH867064	Vu et al. (2019)
P strigosozonata	BHI-F586b	MH558554	—	Haelewaters et al. (2018)
P bambusicola	CLZhao 4133	MW559982	MW559984	Guan et al. (2023)
subhepatica	He 5695	MW507075	MW507015	Unpublished
P. subhepatica	He 4436	MW507074	MW507014	Unpublished
Punctulariopsis obducens	MG70	HM046918	—	Ghobad-Nejhad et al. (2010)
P?obducens	He 5343	—	MW507017	Unpublished
P. cremeoalbida	Burdsall 9616	KR494275	—	Ariyawansa et al. (2015a)
P. efibulata	Burdsall 8824	KR494276	—	Ariyawansa et al. (2015b)
P. efibulata	He 2027	MW507077	—	Ariyawansa et al. (2015b)
P. efibulata	CLZhao 2860	PP998459	—	Unpublished
P. efibulata	CLZhao 3478	PP998460	PP998481	Unpublished
P. efibulata	CLZhao 9842	PP998461	PP998482	Unpublished
P. efibulata	CLZhao 9869	PP998462	PP998483	Unpublished
P. fissurata	**CLZhao 27430**	**PQ062036**	**—**	**Present Study**
P. fissurata	**CLZhao 27439**	**PQ062037**	**PQ109089**	**Present Study**
P. fissurata	**CLZhao 27463**	**PQ062038**	**PQ109090**	**Present Study**
P. fissurata	**CLZhao 27503**	**PQ062039**	**PQ109091**	**Present Study**
P. fissurata	**CLZhao 27548**	**PQ062040**	**PQ109092**	**Present Study**
P. subglobispora	FCUG 2535	HM046917	HM046932	Ghobad-Nejhad et al. (2010)
P. yunnanensis	CLZhao 21333	PP425903	PP572972	Muhammad et al. (2024)
P. yunnanensis	CLZhao 21393	PP425904	PP572969	Muhammad et al. (2024)
P. yunnanensis	CLZhao 21443	PP425905	PP572972	Muhammad et al. (2024)
P. yunnanensis	CLZhao 21683	PP425907	PP572971	Muhammad et al. (2024)
P. yunnanensis	CLZhao 21718	PP425908	PP572973	Muhammad et al. (2024)
P. yunnanensis	CLZhao 29618	PP190238	PP190235	Muhammad et al. (2024)
P. yunnanensis	CLZhao 29620	PP190239	PP190236	Muhammad et al. (2024)
P. yunnanensis	CLZhao 29632	PP190240	PP190237	Muhammad et al. (2024)

A total of two million generations were conducted using four Markov chains, with random beginning trees for each run (Figure 1). Trees were sampled every 1,000 generations, with the first 1/4 of the generations being deleted as burn-in. For every tree that remained, a majority-rule consensus tree was computed. Branches were deemed significantly supported if they obtained bootstrap support for maximum likelihood (BS),

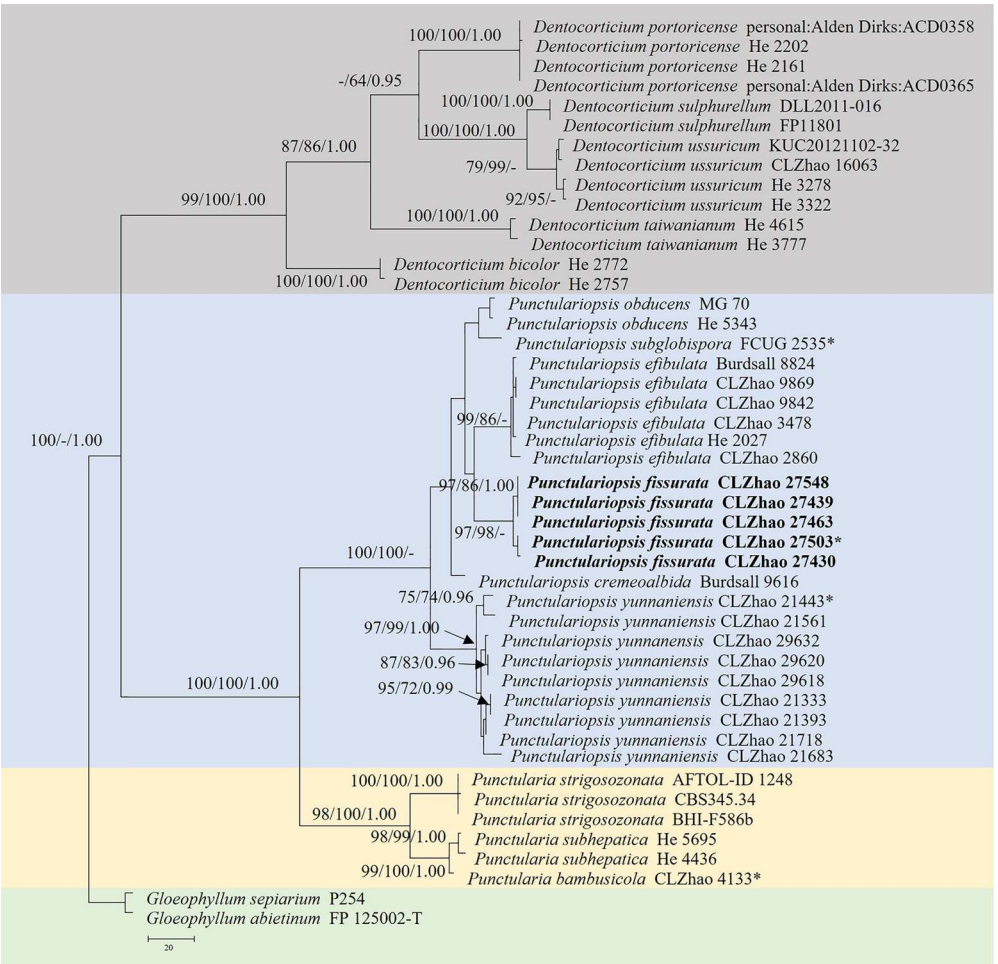

Figure 1. Maximum parsimony strict consensus tree illustrating the phylogeny of *Punctulariopsis fissurata* and related species in *Punctulariopsis* based on ITS + nLSU sequences. Branches are labeled with maximum likelihood bootstrap equal to or higher than 70%, parsimony bootstrap proportions equal to or higher than 50%, and Bayesian posterior probabilities equal to or more than 0.95 respectively. * Indicates type specimens.

maximum parsimony (BT), and Bayesian posterior probabilities (BPP) of at least 75% for BS, 75% for BT, and 0.95 for BPP, respectively.

Results

Molecular phylogeny

Punctulariopsis fissurata sp. nov. is introduced based on molecular phylogeny analysis and morphological data. The new species was grouped into the genus *Punctulariopsis*, in which *P. fissurata* was retrieved as a sister to *P. efibulata*, and formed a clade with five known species (Figure 1).

For the combined ITS+nLSU (Figure 1) data set, trees, and parameters were sampled every 1,000 generations, while four Markov chains were run for two runs from random starting trees, each for 2 million generations. The dataset has a representative aligned length of 2,081 characters with a constant of 1,643 characters on the other side; 40 are parsimony-uninformative and variable, and 398 are parsimony-informative. The maximum parsimony analysis produced three equally parsimonious trees (TL = 879, CI = 0.6951, HI = 0.3049, RI = 0.9135, RC = 0.9135).

The best model for the ITS+nLSU dataset is assessed and useful in the Bayesian analysis: GTR+I+G. ML and Bayesian analysis resulted in a similar topology as MP analysis with an average standard deviation of split frequencies = 0.007534 (BI); the effective sample size (ESS) for Bayesian analysis across the two runs is double the average ESS (avg ESS) = 335.5.

Phylogenetic analysis (Figure 1) was conducted from the combined ITS+nLSU sequences. This resulted in one species within the genus *Punctulariopsis* and showed that the new species formed a single lineage with strong support sister to *P. efibulata*. The taxon *P. efibulata* reported from southwest China is also included in the dataset (Figure 1).

Taxonomy

Punctulariopsis fissurata A. Muhammad & C.L. Zhao, *sp. nov.* (Figures 2 and 3)

MycoBank no: MB 855566

Etymology: —*fissurata* (Lat.) refers to the cracking hymenial surface of the type species.

Holotype: —CHINA. Yunnan Province, Qujing, Zhanyi District, Dapo Town, Tuole Village, E 103°39′ 40″, N 25° 35′ 30″, alt. 1740m, on the fallen branch of angiosperm, 23 March 2023, CLZhao 27503 (SWFC!).

Basidiomata: —Annual, resupinate, adnate but easily separable, coriaceous when fresh, becoming rigid upon drying, up to 17 cm long, up to 3 cm wide, 300–500 µm thick. Hymenial surface smooth, slightly brown when fresh, turned slightly brown to yellowish brown upon drying. Sterile narrow edged, slightly brown, up to 1 mm.

Hyphal structure: —Hyphal system monomitic; thick-walled, colorless, generative hyphae having clamp connections, subhymenial generative hyphae are 2.5–3.5 µm in diameter, crystals present among generative hyphae.

Hymenium: —Dendrohyphidia are colorless, thin-walled; basidia clavate, flexuous, with size of 85–109 × 10–11.5 µm; basidioles dominant in shape similar to basidia, but slightly smaller.

Spores: —Basidiospores are broad with thin walls, CB–, 13–15.5 × 8.5–11.5 µm, L = 14 µm, W = 9.8 µm, Q = 1.28–1. 6 (n = 150/5).

Additional examined specimens (paratypes): —CHINA. Yunnan Province, Qujing, Zhanyi District, Dapo Town, Tuole Village, E 103°55″, N 25°68″, alt. 1740m, 23 March 2023, CLZhao 27430, CLZhao 27439, and CLZhao 27463, (SWFC!); Lijia Tomp Village, E 103°39′40″, N 25°67″, alt. 1960m, 23 March 2023, CLZhao 27548 (SWFC!).

Punctulariopsis efibulata (M.J. Larsen & Nakasone)

CHINA. Yunnan Province, Kunming, Xishan District, Haikou Forestry Park, E 102° 37″, N 24°57″, alt. 2133 m, 17 September 2023, CLZhao 2860 (SWFC!); Puer, Jingdong

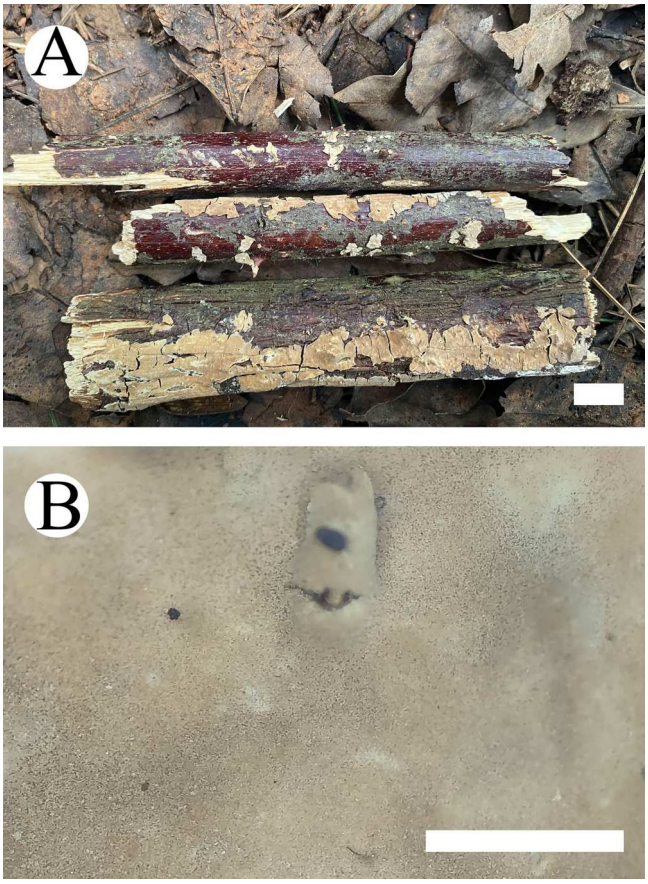

Figure 2. Basidiomata of *Punctulariopsis fissurata* (holotype). Bars: A = 1 cm, B = 1 mm.

County, Wuliangshan National Nature Reserve, E 100°22″, N 23°57″, alt. 3370 m, 17 October 2023, CLZhao 3478, 19 January CLZhao 9842, and CLZhao 9869 (SWFC!).

Discussion

In this work, *Punctulariopsis fissurata* sp. nov. is introduced on the basis of molecular phylogeny analysis and morphological data. The ITS+nLSU phylogeny (Figure 1) showed that the new species is grouped as a sister to *P. efibulata*, and formed a clade with five known species (Figure 1). *Punctulariopsis cremeoalbida* and *P. efibulata* were originally described by Larsen and Nakasone (1984) for two corticioid species with flow out basidiomes growing on *Vitis* (the former), and on *Vaccinium* (the latter) in North America. The light-colored, thin, adnate basidiomes of both species, their little-branched dendrohyphidia, large and largely ellipsoid spores with blunt apiculus, and their growth on hardwood match well with the concept of *Punctulariopsis* (Ghobad-Nejhad et al. 2010). Both species are apparently known only from their type localities in the USA. With the addition of two species in *Punctulariopsis* the number of species in this genus has grown to four that time, while *P. efibulata* was categorised as the only species in the genus with the absence of clamp connections on its hyphae

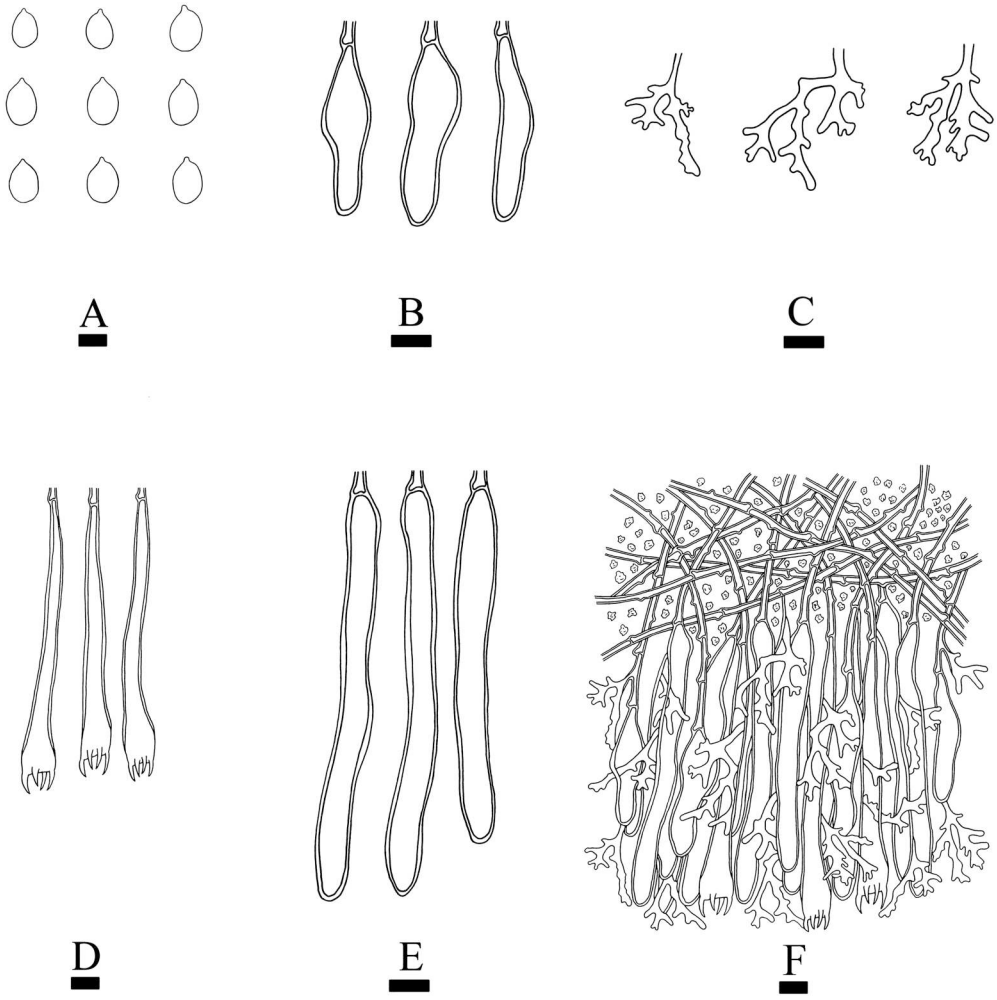

Figure 3. Microscopic structures of *Punctulariopsis fissurata* (drawn from the holotype). A Basidiospores. B Cystidioles. C Dendrohyphidia. D basidia. E Basidioles. F A cross-section of basidiomata. Scale bars: A–F 10 µm.

(Ariyawansa et al. 2015b). Based on the ITS + LSU phylogenetic analysis, *Dendrocorticium*, *Punctulariopsis*, and *Punctularia* genera were included in Punctulariaceae (Ghobad-Nejhad et al. 2010).

The diversity of *Punctulariopsis* in China is still not well studied. This paper reports a new species, *P. fissurata*, from China, which enhances our understanding of wood-inhabiting fungi in China. It is expected that more new wood-inhabiting fungal taxa will be discovered in Yunnan Province, with extensive field surveys.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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ORCID

Akmal Muhammad http://orcid.org/0000-0002-4556-3558
Minglan Chen http://orcid.org/0009-0006-7890-2604
Wen Li http://orcid.org/0009-0005-1394-4945
ChangLin Zhao http://orcid.org/0000-0002-8668-1075

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