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Cystoderma lignicola, a new species from southwestern China

Liu JW[1,2,3], Luangharn T[2*] and Yu FQ[1*]

[1] The Germplasm Bank of Wild Species, Yunnan Key Laboratory for Fungal Diversity and Green Development, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, Yunnan, China
[2] Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand
[3] School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand

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Abstract

In this study, we describe a new species, *Cystoderma lignicola*, collected from rotten woods in the temperate region in Yunnan Province, southwestern China. Our new species was described based on macro- and micro-morphological characteristics and molecular data of combined internal transcribed spacers (ITS) and large subunit (nrLSU) of the nuclear ribosomal DNA (rDNA) sequences. *Cystoderma lignicola* is characterized by its light orange to brown cap, globose, subglobose, ellipsoid to broadly ellipsoid basidiospores, with 4–5 × 3–4 µm diam. Phylogenetically, *C. lignicola* showed a sister clade to *C. chocoanum* with statistical support of SH-aLRT/UFB = 90.9/90 in maximum likelihood analysis and statistical support of BPP = 0.99 in the Bayesian analysis.

Keywords – Amyloid basidiospore – phylogenetic analysis – saprotrophic – Squamanitaceae – taxonomy

Introduction

Cystoderma Fayod, a genus in the family Squamanitaceae (Vizzini et al. 2019, Kalichman et al. 2020, Liu et al. 2021), was established by Fayod in 1889 with *C. amianthinum* (Scop.) as the type species. Harmaja (2002) considered the results of the phylogenetic analysis of *Cystoderma*, which is not monophyletic in the study of Moncalvo et al. (2002), and divided *Cystoderma* into two genera, viz., *Cystoderma* and *Cystodermella* Harmaja, where *Cystoderma* contains species that possess amyloid basidiospores, and *Cystodermella* encompasses species with inamyloid basidiospores. *Cystoderma* members can be found in forest ecosystems in open areas, growing on soil, among mosses and litter, and some species grow on rotten wood (Saar 2012). Currently, 31 *Cystoderma* species have been reported worldwide (Li et al. 2021). In China, ten species have been recorded up to now, viz., *C. amianthinum*, *C. aureum* (Matt.) Kühner & Romagn., *C. carcharias* (Pers.) Fayod., *C. granosum*, *C. japonicum* Thoen & Hongo., *C. lilaceum* R.L. Zhao, M.Q. He & J.X. Li, *C. pseudoamianthinum* R.L. Zhao, M.Q. He & J.X. Li, *C. rugosolateritium* R.L. Zhao, M.Q. He & J.X. Li, *C. subglobisporum* R.L. Zhao, M.Q. He & J.X. Li, and *C. subvinaceum* A.H. Sm (Tai 1979, Li et al. 2015, 2021, Liu et al. 2021, Mao 2000).

In this study, we reported new knowledge of *Cystoderma* collected from the Ailao Mountains of Nanhua County in central Yunnan Province, China. Morphological comparison with all known species of the genus *Cystoderma* and phylogenetic analysis supported our collections as a new

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Corresponding Author: Fu-Qiang Yu – e-mail – fqyu@mail.kib.ac.cn,
Thatsanee Luangharn – email – thatsanee.lua@mfu.ac.th

taxon. Details of morphological characteristics and molecular analyses of combined ITS and LSU are described and illustrated here.

Materials & Methods

Sample collections and morphological studies

Fresh basidiomata were collected from Nanhua County in central Yunnan. The color codes were recorded following Kornerup & Wanscher (1981). All specimens were then dried at 45°C until they were completely dehydrated. Dried specimens were deposited in the Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences (HKAS), Yunnan Province, China.

To observe micro-morphological characteristics, dried specimens were sectioned by hand, mounted in 5% KOH, and observed using a compound microscope (Leica DM2500, Leica Microsystems, Wetzlar, Germany). Twenty basidiospores per collection were measured, and their sizes and shapes were recorded and photographed. The notations [n/m/p] indicated that the measurements were made with "n" basidiospores from "m" basidiomata of "p" collections with a minimum of 20 basidiospores. The basidiospore quotient was followed by [Q = L/W], where Q is the quotient of basidiospore length to width (L/W) of a basidiospore in side view, and Qm, the mean of Q-values ± SD, was calculated considering the mean value of the lengths and widths of basidiospores. The basidiospore size was measured by Tulloss (2005). Index Fungorum and Faces of Fungi numbers were registered as instructed in Index Fungorum (2023) and Jayasiri et al. (2015).

DNA extraction, PCR amplification, and sequencing

Dried internal specimen tissue was used for extracting DNA by the CTAB method, following the instructions by Doyle (1987). Sequences of the internal transcribed spacers 1 and 2 with the 5.8S rDNA (ITS) and the large nuclear ribosomal RNA subunit (nrLSU) were amplified by using universal primers for ITS1F/ITS4 (White et al. 1990, Gardes & Bruns 1993) and LROR/LR5 (Vilgalys & Hester 1990), respectively. Total volume (25 μL) of PCR mixture containing 12.5 μL of 2X Taq MasterMix (Kangweishiji Company, Beijing), 1 μL of each primer (10 μM), 1 μL genomic DNA extract, and 9.5 μL double distilled water. The PCR thermal cycle for the amplification of ITS and nrLSU was 94 ℃ for 3 min initially, followed by 35 cycles of denaturation at 94 ℃ for 40 s, annealing at 52 ℃ for 40 s, elongation at 72 ℃ for 50 s, and a final extension at 72 ℃ for 7 min. PCR products were sent for sequencing at Qingke Company, Kunming City, Yunnan Province, China.

Sequence alignment

The ITS and nrLSU sequences obtained in this study were compiled with *Cystoderma* sequences listed in Li et al. (2021) and are shown in Table 1. Two species of *Crucibulum* were used as outgroups. Single-gene alignments were obtained using MAFFT v.6.8 (Katoh et al. 2005), and BioEdit v. 7.0.9 was used to refine where necessary (Hall 1999). The ITS and nrLSU datasets were exported with missing ends of alignment as Ns. and concatenated by using Geneious Prime 2020.0.3 (BioMatters, Ltd., Auckland, New Zealand).

Table 1 Names, voucher numbers, locations and corresponding GenBank numbers of the taxa used in the phylogenetic analyses of the present study, newly generated sequences are shown in bold.

Species	**Voucher**	**Location**	**GenBank accession no.**	
			ITS	**nrLSU**
Cystoderma amianthinum (epitype)	TU101287	Estonia	AM946480	AM946424

Table 1 Continued.

Species	Voucher	Location	GenBank accession no.	
			ITS	nrLSU
C. amianthinum	HMAS291341	Heilongjiang, China	MW242929	–
C. andinum (isotype)	C57998	Southern America	AM946481	AM946425
C. andinum	C58476	Southern America	AM946482	AM946426
C. aureum	TAAM146976	Estonia	AM946522	–
C. aureum	C27851	Denmark	AM946523	AM946459
C. aureum	HMAS255933	Yunnan, China	MZ424458	MZ413916
C. carcharias var. *carcharias* (epitype)	TAAM172011	Sweden	AM946483	AM946428
C. carcharias var. *carcharias*	TU106011	Estonia	UDB015074	–
C. carpaticum (holotype)	IB19750290	Poland	LT592276	–
C. chocoanum (isotype)	NY00775586	Colombia	–	U85302
C. carpaticum	CNF1/7034	Croatia	LT592274	LT592277
C. chocoanum	SP393641	São Paulo, Brazil	–	EU727143
C. granosum	HMAS291346	Gansu, China	MW242933	MW242945
C. granosum	HMAS291347	Gansu, China	MW242931	MW242943
C. granosum	HMAS291348	Gansu, China	MW242932	MW242944
C. granosum	HMAS255934	Gansu, China	MZ424459	MZ413917
C. japonicum (holotype)	BR5020079022647	Japan	AM946491	AM946435
C. japonicum	TU101697	Estonia	UDB011137	LT592278
C. jasonis	TU118180	Estonia	UDB015579	–
C. lignicola (holotype)	**HKAS 125915**	**Yunnan, China**	**OP881487**	**OP881489**
C. lignicola	**HKAS 125914**	**Yunnan, China**	**OP881486**	**OP881488**
C. lilaceum (holotype)	HMAS291349	Gansu, China	MW242922	MW242948
C. lilaceum	HMAS291350	Gansu, China	MW242923	MW242946
C. pseudoamianthinum (holotype)	HMAS255932	Yunnan, China	MZ424460	MZ413918
C. pseudoamianthinum	HMAS291343	Heilongjiang, China	MW242928	MW242940
C. pseudoamianthinum	HMAS291344	Heilongjiang, China	MW242927	MW242939
C. pseudoamianthinum	HMAS291345	Heilongjiang, China	MW242926	MW242938
C. pseudoamianthinum	HMAS255935	Heilongjiang, China	MZ424461	MZ413919
C. rugosolateritium (holotype)	HMAS291351	Gansu, China	MW242925	MW242937
C. simulatum	PDD83705	New Zealand	AM946490	AM946434

Table 1 Continued.

Species	Voucher	Location	GenBank accession no.	
			ITS	nrLSU
C. simulatum	PDD75555	New Zealand	AM946489	AM946432
C. subglobisporum (holotype)	HMAS281432	Tibet, China	MW242934	MW242947
C. subvinaceum	WU19742	Austria	AM946501	AM946441
C. subvinaceum	WU10567	Austria	AM946502	-
C. subvinaceum	HMAS291342	Inner Mongolia, China	MW242924	MW242941
C. superbum	BR22288-75	Belgium	AM946504	AM946442
C. superbum	REG (Oct 1976)	Germany	AM946503	AM946443
C. tricholomoides (holotype)	BR5020125408845	Germany	UDB011633	–
C. tricholomoides	BR De Meyer 597	The Netherlands	UDB011634	–
C. tuomikoskii (holotype)	H6026179	Finland	AM946505	AM946444
C. tuomikoskii	O153775	Norway	AM946507	–
Crucibulum leave	CBS168.37	Sweden	MH855872	MH867377
Cr. leave	SWFC21261	Ningxia, China	DQ463357	–

Phylogenetic analyses

Substitution model evaluation and phylogenetic tree construction for maximum likelihood (ML) analysis were performed using IQ-TREE 2.0.3 (Minh et al. 2020) on the Linux system, and the substitution model for the concatenated ITS-nrLSU dataset was GTR+F+I+G4, which was determined according to the Akaike information criterion (AIC) by IQ-TREE. Bayesian Inference (BI) analysis was performed using MrBayes v.3.1.6 (Ronquist et al. 2012), and the substitution model was determined using the Akaike Information Criterion (AIC) implemented in MrModeltest v.2.4 (Nylander 2004). The selected model was GTR+I+G for the concatenated ITS-nrLSU dataset. Clade support for the ML analyses was assessed using the Shimodaira–Hasegawa-like approximate likelihood ratio test (SH-aLRT) with 1,000 replicates (Guindon et al. 2010) and 1,000 replicates of the ultrafast bootstrap (UFB) (Hoang et al. 2018). BI analyses were conducted with generations set to 2,000,000 and trees sampled every 100 generations. The average standard deviation of split frequencies (0.01) and effective sample size (ESS) values (> 200) were used to assess convergence. The trees were summarized after omitting the first 25% of trees as burn-in using the "sump" and "sumt" commands. Nodes with support values of both SH-aLRT ≤ 80 and UFB ≤ 95 were considered to be supported, nodes with one of SH-aLRT ≤ 80 or UFB ≤ 95 were weakly supported, and nodes with both SH-aLRT < 80 and UFB < 95 were unsupported in the maximum likelihood (ML) analysis. Nodes with support values of Bayesian posterior probabilities (BPP) ≤ 0.95 were considered strongly supported in BI analyses.

The concatenated ITS-nrLSU dataset alignment included 42 ITS and 32 nrLSU sequences, of which four sequences were newly generated and comprised 1,569 characters (667 characters for ITS and 902 characters for nrLSU) with gaps, of which 1,131 characters were constant, and 420 characters were variable. The phylogenetic tree topologies generated from ML and BI analyses were almost identical with minimal variation in statistical support, and thus only the tree inferred from ML analysis was displayed.

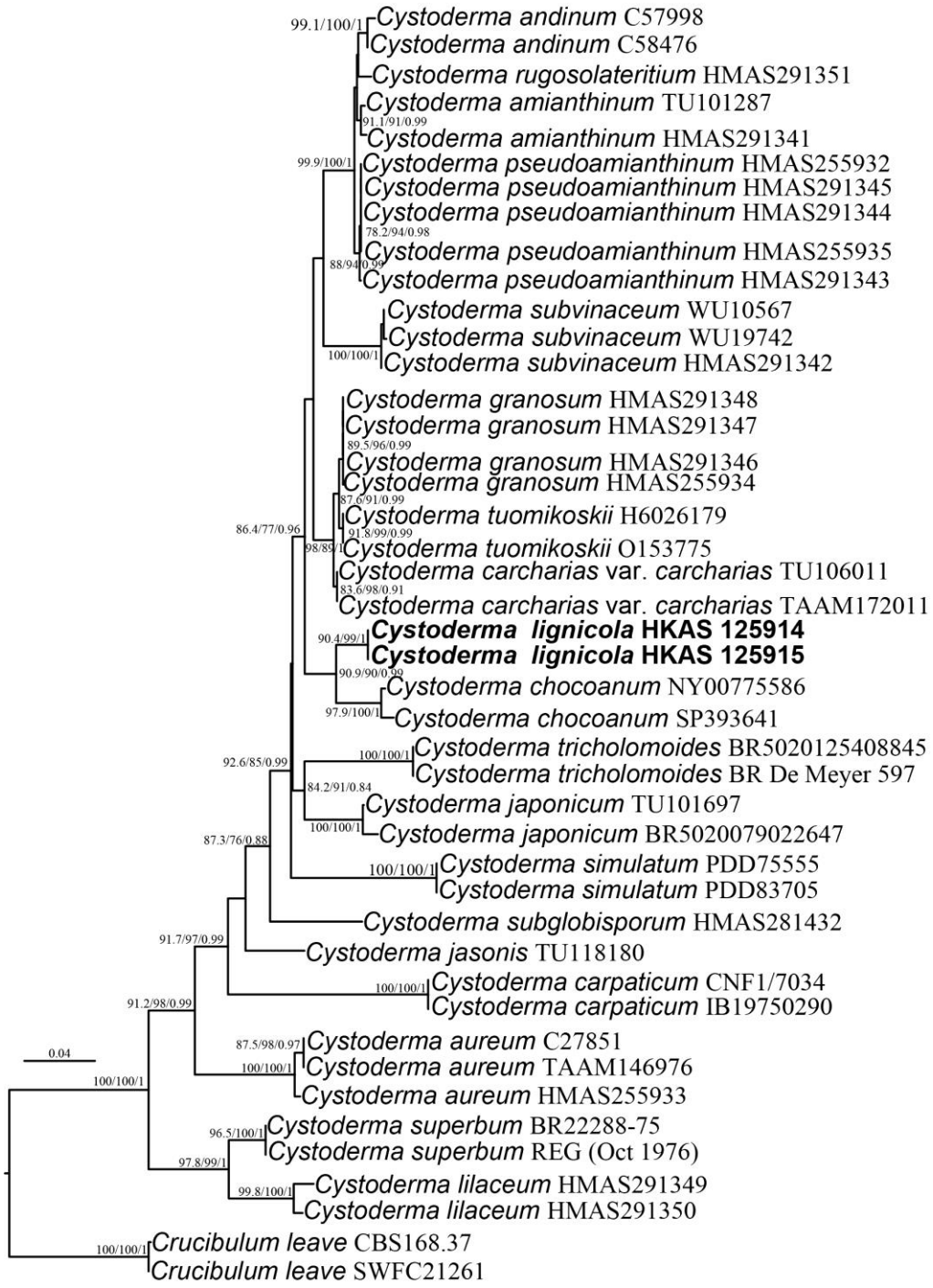

Fig. 1 – Maximum-Likelihood (ML) phylogenetic tree of *Cystoderma* inferred from the ITS-nrLSU dataset, with SH-aLRT (left), ultrafast bootstrap (UFB) (middle), and PPs values (right) near the corresponding node. The values of SH-aLRT ≥ 80 or UFB ≥ 95 for ML and BPP ≥ 0.90 for BI are indicated along the branches (SH-aLRT/UFB/BPP). New species, *Cystoderma lignicola* is in black bold.

Results

Phylogenetic results

The phylogenetic tree generated from the ITS-nrLSU dataset indicated that two *Cystoderma lignicola* collections represented a distinct clade (Fig. 1) and were relatively close to *C. chocoanum*, with strong statistical support in the Bayesian analysis (BPP = 0.99).

Cystoderma lignicola J. W. Liu & F. Q. Yu, *sp. nov.* Figs 1–3

Index Fungorum: IF559660; Facesoffungi number: FoF14112

Diagnosis – Similar to *C. chocoanum* but differs in having a light orange to a brown cap, larger basidia, and abundant smaller globose and subglobose basidiospores.

Type – China, Yunnan Province, Chuxiong Prefecture, Nanhua County, Tujie Town, Ailaoshan Natural Reserve, 24°53′40.13″N, 100°46′51.64″E, alt. 2,644 m, single or scattered on rotten logs of a Fagaceous tree, 21 August 2022, LJW3216 (HKAS 125915, holotype, GenBank accession numbers: ITS = OP881487, nrLSU = OP881489).

Etymology – The species epithet "lignicolous" refers to the habit of this fungus.

Description – *Pileus* 25–55 mm in diam., hemispherical at first, then becoming convex to plano-convex at maturity, densely covered with obtuse and pyramidal warts, an incurved margin at first, then applanate to plane with age. Margin and stipe are connected by a veil at the beginning, then the veil is broken and forms blocky remnants of the veil attached to the edge of the pileus, which usually present a dried surface. *Lamellae* 1–2 lamellulae, adnexed, some narrowly adnate or emarginate, crowded middle, cream (1A1), with smooth edge. *Stipe* 20–30 × 2.5–5 mm, up to 6.5 mm, sub-cylindrical or broadened at the base, solid, with evanescent floccose-scaly ring zone, whitish (1A1) to reddish-golden (6C7) above the ring zone, and concolorous with pileus below the ring zone, numerous small squamules attached. Context is whitish when fresh. *Odour* and taste are undocumented.

Fig. 2 – Basidiomata of *Cystoderma lignicola*. a–b HKAS 125914. c–d HKAS 125915, holotype. Scale bars: a–d = 1.5 cm.

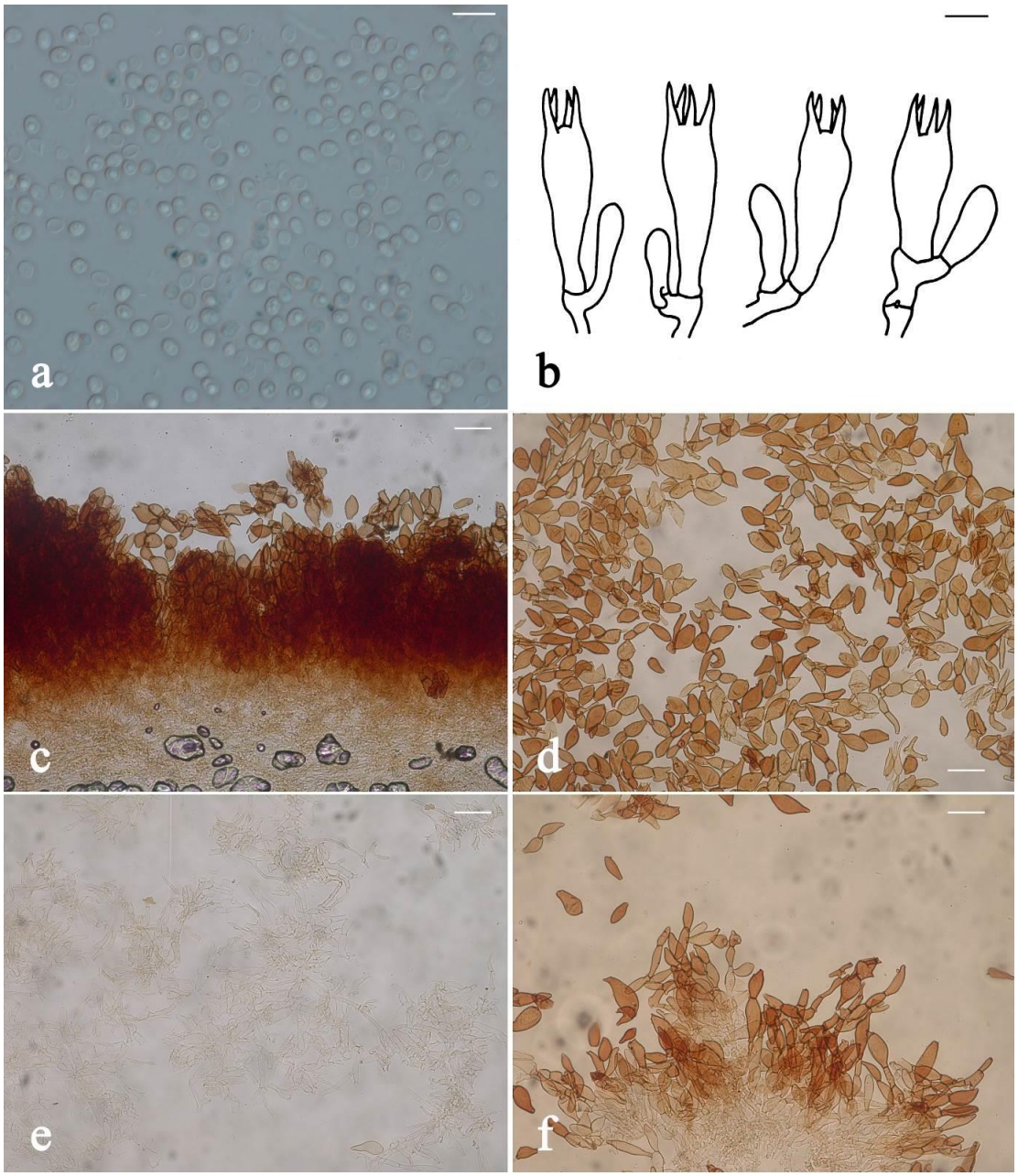

Fig. 3 – Micro-morphological characteristics of the *Cystoderma lignicola* (holotype, HKAS 125915). a Basidiospores. b Basidia. c Pileipellis section. d Upper layer of pileipellis. e Lower layer of pileipellis. f Longitudinal section of the evanescent floccose-scaly ring zone. Scale bars: a = 10 μm, b = 5 μm, c–f = 40 μm.

Basidiospores [40/2/2] (3.5–)4–5 × 3–4 μm, Q = 1–1.33 (1.56), Qm =1.12 ± 0.15, subglobose to globose, broadly ellipsoid to ellipsoid, smooth, thin-walled, hyaline, with amyloid. *Basidia* 17–30 × 6–9 μm, clavate, hyaline, with 4-spored. *Cystidia* absent. *Pileipellis* composed of chains of numerous sphaerocytes, fusiform, baseball bat-like to subglobose, 18–50 × 7–14 μm, sometimes lotus-shaped, smooth, and brownish yellow on the upper layers, while the lower layers are 4–6 μm in width, smooth and hyaline, scattered, fasciculate to branched hyphae, tightly interwoven, occasionally mixed with similar elements in the upper pileipellis, and discolored in KOH. *Arthrospores* absent. The composition of the evanescent floccose-scaly ring zone and pileipellis is identical. All tissues have an abundance of clamp connections.

Ecology – Solitary as a single or scattered on rotten woods of Fagaceous or *Rhododendron delavayi* tree.

Known distribution – Currently known from southwestern China.

Additional material examined – China, Yunnan Province, Chuxiong Prefecture, Nanhua County, Tujie Town, Ailaoshan Natural Reserve, 24°53′43.44″N, 100°46′56.29″E, alt. 2639 m, scattered on rotten woods of *Rhododendron delavayi*, 21 August 2022, LJW3214 (HKAS 125914, GenBank accession no.: ITS = OP881486, nrLSU = OP881488).

Discussion

In this study, we introduce a new species of *Cystoderma lignicola* growing on the Fagaceous or *Rhododendron delavayi* trees from Yunnan Province, China. The phylogenetic tree revealed two collections of *C. lignicola* close to *C. chocoanum* collections from Brazil and Columbia, with statistical support of SH-aLRT/UFB/BPP = 90.9/90/0.99 in the maximum likelihood analysis and Bayesian analysis (Fig. 1). However, morphologically, *C. lignicola* is distinctively light orange and melon to brown on the cap, while *C. chocoanum* is usually brown. Meanwhile, the basidiospore size of *C. lignicola* was 4–5 × 3–4 μm diam., with subglobose to globose or ellipsoid to broadly ellipsoid shapes, while *C. chocoanum* basidiospores were 5.4–6.3 × 2.7–3.6 μm in diam. and ellipsoid to oblong in shape (Franco-Molano 1993). Besides, *C. lignicola* has larger basidia (17–30 × 6–9 μm) than *C. chocoanum* (14.5–18 × 4.5–6.5 μm). Moreover, *C. lignicola* is morphologically similar to *C. gruberianum*; however, *C. gruberianum* has smaller ellipsoid basidiospores (3.5–5 × 2.5–3 μm).

Twelve *Cystoderma* species have been reported in China, with eleven growing on moss, litter, or soil, except for *C. lignicola*, which grows on decayed wood. In other parts, most species of *Cystoderma* were also reported growing on moss, litter, or soil, and only a few lignicolous *Cystoderma* species have been reported, such as *C. caucasicum* and *C. subornatum*. It was obvious that this lignicolous *Cystoderma* belongs to saprophytic fungi, for most species growing on moss, litter, or soil, it is still unknown if they have a saprotrophic and/or biotrophic lifestyle.

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Accessibility of data

Dried specimens were deposited in the Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences (HKAS), Yunnan Province, China. The alignment of ITS-nrLSU sequences was submitted to TreeBase (Study No. 29961).

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