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Scytinostroma yunnanense sp. nov. (Russulales, Basidiomycota) evidenced by morphological characteristics and phylogenetic analyses in China

HUI WANG[1,3], XIAO HE[1,4] & CHANG-LIN ZHAO[1,2,5]*

[1] *College of Biodiversity Conservation, Southwest Forestry University, Kunming 650224, P.R. China.*
[2] *Key Laboratory of Forest Disaster Warning and Control of Yunnan Province, Southwest Forestry University, Kunming 650224, P.R. China.*
[3] *whfungi@163.com; https://orcid.org/0000-0002-5962-7049*
[4] *fungixiaohe@163.com; https://orcid.org/0000-0003-2189-937X*
[5] *fungichanglinz@163.com; https://orcid.org/0000-0002-8668-1075*
**Corresponding author: C.L. Zhao*
Corresponding authors: fungichanglinz@163.com

Abstract

A new wood-inhabiting fungal species, *Scytinostroma yunnanense*, is proposed based on morphological and molecular evidences. The species is characterized by an annual growth habit, resupinate basidiomata with cream hymenial surface, a monomitic hyphal system with generative hyphae bearing simple septa, fusiform to cylindrical cystidia and basidiospores (4.5–5.5 × 4.2–5.2 µm) are acyanophilous, subglobose to globose, hyaline, thin-walled, smooth, amyloid. The phylogenetic analyses based on molecular data of ITS sequences showed that the new species formed a monophyletic lineage with a strong support (100% BS, 100% BP, 1.00 BPP) and then was sister to *S. duriusculum*.

Keywords: Lachnocladiaceae, Phylogeny, Taxonomy, Wood-rotting fungi, Yunnan Province

Introduction

The genus *Scytinostroma* Donk (1956: 19) is characterized by resupinate, coriaceous basidiomata, smooth to tuberculate hymenophore and a dimitic hyphal structure with simple septa or clamps on generative hyphae, skeletal hyphae densely branched and sometimes forming dendrohyphae or dichohyphae, strongly dextrinoid and cyanophilous, and the presence of cystidia, basidia tubular to uniform, and subglobose to ellipsoid, smooth, thin-walled, variably amyloid basidiospores (Donk 1956, Bernicchia & Gorjón 2010). So far 35 species have been accepted in the genus worldwide (Donk 1956, Gilbertson 1962, Boidin 1967, Rattan 1974, Boidin & Lanquetin 1977, 1987, Lanquetin 1984, Boidin & Gilles 1988, Hjortstam 1990, Nakasone 2008). The type species of the genus is *S. portentosum* (Berk. & M.A. Curtis) Donk (1956: 20).

Recently, molecular studies involving *Scytinostroma* based on single-gene or multi-gene datasets have been carried out and the type species or related taxon of *Hyphoderella* was phylogenetically placed in Lachnocladiaceae (Larsson & Larsson 2003, Larsson *et al*. 2004, Binder *et al*. 2005, Larsson 2007). Another study of Larsson (2003) revealed the phylogenetic relationships of russuloid basidiomycetes with emphasis on aphyllophoralean taxa based on the internal transcribed spacer (ITS) regions and the large subunit nuclear ribosomal RNA gene (nLSU) sequences and showed that *Scytinostroma* grouped with *Gloiothele* Bres. (1920: 44). Larsson *et al.* (2004) presented the high phylogenetic diversity among corticioid homobasidiomycetes employing multi-gene datasets, in which *S. odoratum* (Fr.) Donk (1956: 20) was nested into the russuloid clade and then grouped with *Dichostereum* Pilát (1926: 223), *Peniophora* Cooke (1879: 20) and *Vararia* P. Karst. (1888: 2). The study on phylogenetic distribution of resupinate forms across the major clades of mushroom-forming fungi was analyzed using a four gene dataset, and showed that *Scytinostroma* clustered into the russuloid clade belonging to the order Peniophorales (Binder *et al*. 2005). Re-thinking the classification of corticioid fungi revealed that *Scytinostroma* was placed in Peniophoraceae within Russulales (Larsson 2007).

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During investigations on wood-inhabiting fungi in southern China, an additional taxon was found which could not be assigned to any described species. In this study, a new species under the genus *Scytinostroma*, is proposed on basis of macro-anatomical as well as phylogenetically (internal transcribed spacer (ITS) regions).

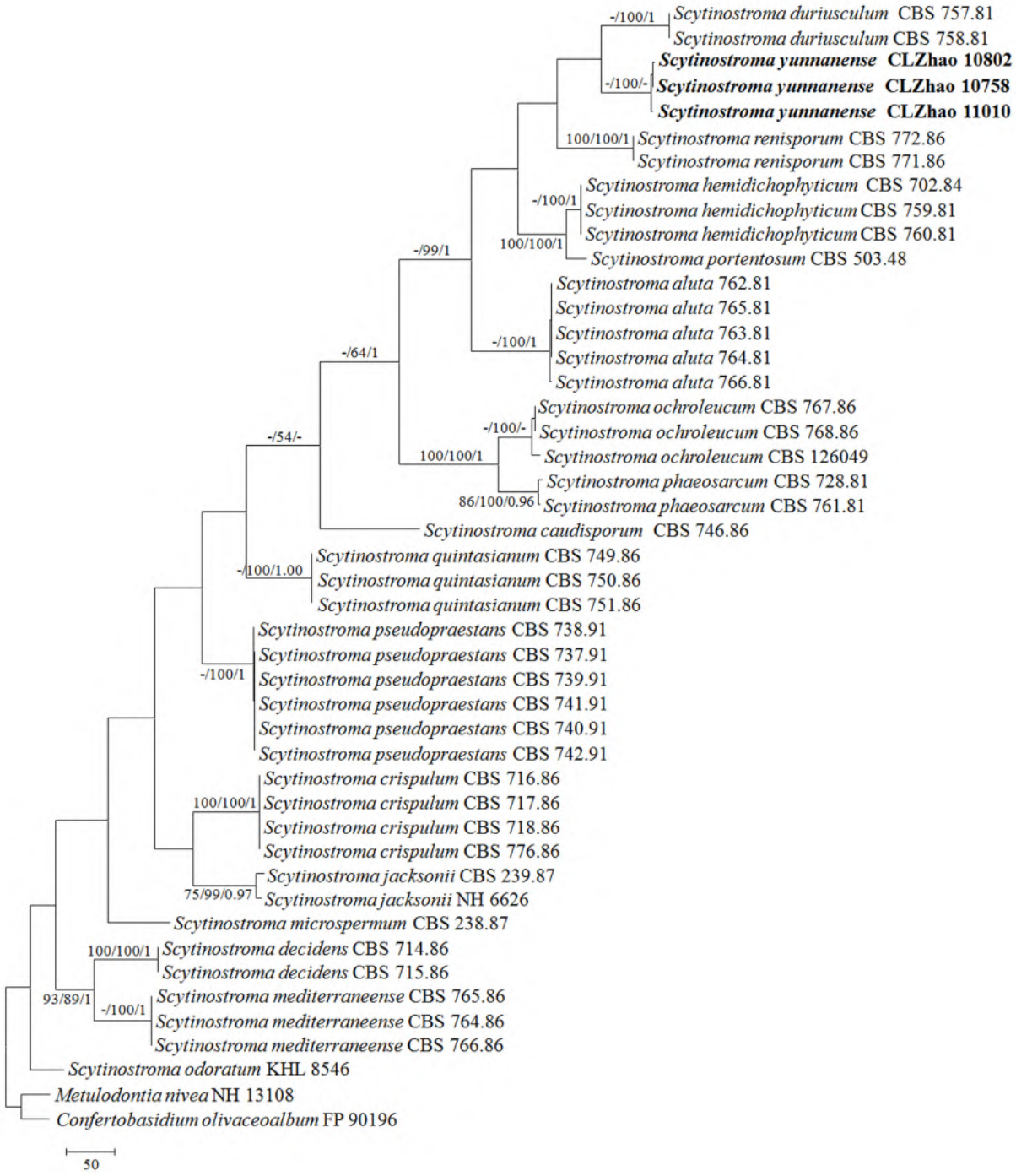

FIGURE 1. Maximum Parsimony strict consensus tree illustrating the phylogeny of *Scytinostroma yunnanense* and related species in *Scytinostroma* based on ITS sequences. Branches are labeled with maximum likelihood bootstrap higher than 70%, parsimony bootstrap proportions higher than 50% and Bayesian posterior probabilities more than 0.95 respectively.

Materials and methods

Morphological studies

Macro-morphological descriptions are based on field notes. Special colour terms follow Petersen (1996). Micro-

morphological data were obtained from the dried specimens, and observed under a light microscope followed by Dai (2012). 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. The specimens studied are deposited at the herbarium of Southwest Forestry University (SWFC), Kunming, Yunnan Province, P.R. China.

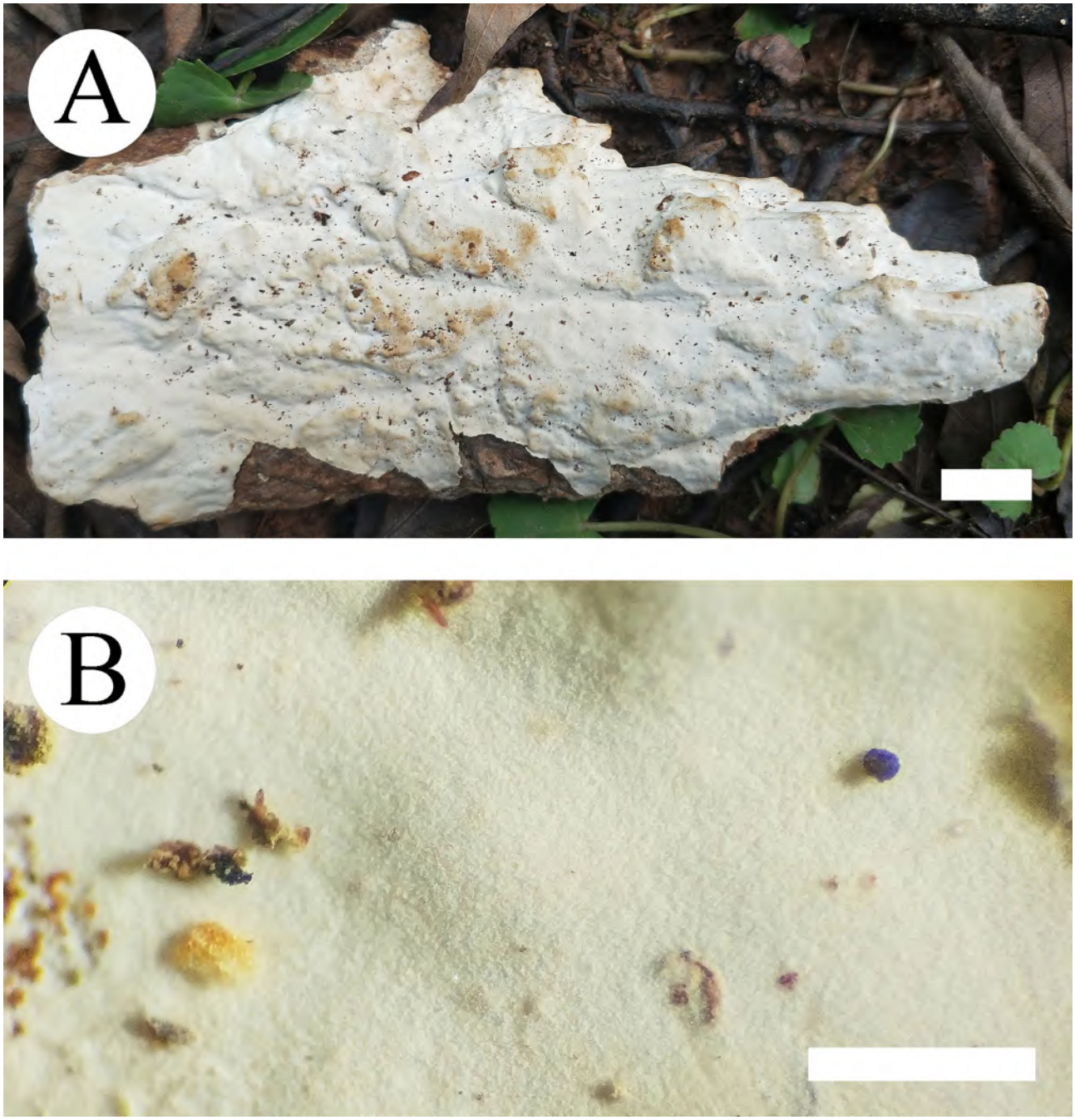

FIGURE 2. Basidiocarps of *Scytinostroma yunnanense.* Bars: A–1 cm; B–1 mm (holotype).

Molecular techniques 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 with some modifications that a small piece of dried fungal specimen (about 30 mg) was ground 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 in AC for a 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 adsorbed film to elute the genome DNA. ITS region was amplified with primer pairs ITS5 and ITS4 (White *et al.* 1990). The PCR procedure for ITS was as follows: initial denaturation at 95 °C for 3 min, followed by 35 cycles at 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 (Guan *et al*. 2020). The PCR products were purified and directly sequenced at Kunming Tsingke Biological Technology Limited Company. All newly generated sequences were deposited at NCBI GenBank database (Table 1).

TABLE 1. A list of species, specimens and GenBank accession number of sequences used in this study.

Species name	Sample no.	GenBank accession no. ITS	References
Confertobasidium olivaceoalbum	FP 90196	AF511648	Larsson & Larsson 2003
Metulodontia nivea	NH 13108	AF506423	Larsson & Larsson 2003
Scytinostroma aluta	CBS 762.81	MH861482	Vu *et al.* 2019
S. aluta	CBS 763.81	MH861483	Vu *et al.* 2019
S. aluta	CBS 764.81	MH861484	Vu *et al.* 2019
S. aluta	CBS 765.81	MH861485	Vu *et al.* 2019
S. aluta	CBS 766.81	MH861486	Vu *et al.* 2019
S. caudisporum	CBS 746.86	MH862030	Vu *et al.* 2019
S. crispulum	CBS 716.86	MH862013	Vu *et al.* 2019
S. crispulum	CBS 717.86	MH862014	Vu *et al.* 2019
S. crispulum	CBS 718.86	MH862015	Vu *et al*. 2019
S. crispulum	CBS 776.86	MH862053	Vu *et al.* 2019
S. decidens	CBS 714.86	MH862011	Vu *et al.* 2019
S. decidens	CBS 715.86	MH862012	Vu *et al.* 2019
S. duriusculum	CBS 757.81	MH861477	Vu *et al*. 2019
S. duriusculum	CBS 758.81	MH861478	Vu *et al.* 2019
S. hemidichophyticum	CBS 702.84	MH861818	Vu *et al.* 2019
S. hemidichophyticum	CBS 759.81	MH861479	Vu *et al*. 2019
S. hemidichophyticum	CBS 760.81	MH861480	Vu *et al.* 2019
S. jacksonii	NH 6626	AF506467	Larsson & Larsson 2003
S. jacksonii	CBS 239.87	MH862071	Vu *et al.* 2019
S. mediterraneense	CBS 764.86	MH862045	Vu *et al.* 2019
S. mediterraneense	CBS 765.86	MH862046	Vu *et al.* 2019
S. mediterraneense	CBS 766.86	MH862047	Vu *et al.* 2019
S. microspermum	CBS 238.87	MH862070	Vu *et al.* 2019
S. ochroleucum	CBS 767.86	MH862048	Vu *et al.* 2019
S. ochroleucum	CBS 768.86	MH862049	Vu *et al*. 2019
S. ochroleucum	CBS 126049	MH864062	Vu *et al.* 2019
S. odoratum	KHL 8546	AF506469	Larsson & Larsson 2003
S. phaeosarcum	CBS 728.81	MH861463	Vu *et al.* 2019
S. phaeosarcum	CBS 761.81	MH861481	Vu *et al.* 2019
S. portentosum	CBS 503.48	MH856447	Vu *et al.* 2019

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TABLE 1. (Continued)

Species name	Sample no.	GenBank accession no. ITS	References
S. pseudopraestans	CBS 737.91	MH862322	Vu *et al*. 2019
S. pseudopraestans	CBS 738.91	MH862323	Vu *et al*. 2019
S. pseudopraestans	CBS 739.91	MH862324	Vu *et al*. 2019
S. pseudopraestans	CBS 740.91	MH862325	Vu *et al*. 2019
S. pseudopraestans	CBS 741.91	MH862326	Vu *et al*. 2019
S. pseudopraestans	CBS 742.91	MH862327	Vu *et al*. 2019
S. quintasianum	CBS 749.86	MH862031	Vu *et al*. 2019
S. quintasianum	CBS 750.86	MH862032	Vu *et al*. 2019
S. quintasianum	CBS 751.86	MH862033	Vu *et al*. 2019
S. renisporum	CBS 771.86	MH862051	Vu *et al*. 2019
S. renisporum	CBS 772.86	MH862052	Vu *et al*. 2019
S. yunnanense	CLZhao 10758	MT611445	Present study
S. yunnanense	CLZhao 10802	MT611446	Present study
S. yunnanense	CLZhao 11010	MT611447	Present study

Sequences were aligned with MAFFT 7 (http://mafft.cbrc.jp/alignment/server/) using the "E-INS-I" strategy for ITS sequence database (Luo *et al*. 2019) and manually adjusted in BioEdit (Hall 1999). Alignment datasets were deposited in TreeBase (submission ID 25287). *Confertobasidium olivaceoalbum* (Bourdot & Galzin) Jülich (1972: 167) and *Metulodontia nivea* (P. Karst.) Parmasto (1968: 118) were selected as outgroups for phylogenetic analyses of ITS phylogenetic tree (Larsson & Larsson 2003).

Maximum parsimony analysis was applied to the ITS dataset sequences. Approaches to phylogenetic analysis followed Zhao & Wu (2017), and the tree construction procedure 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 replicate.

MrModeltest 2.3 (Nylander 2004) was used to determine the best-fit evolution model for each data set for Bayesian inference (BI). Bayesian inference was calculated with MrBayes3.1.2 with a general time reversible (GTR) model of DNA substitution and a gamma distribution rate variation across sites (Ronquist & Huelsenbeck 2003). Four Markov chains were run for 2 runs from random starting trees for 30 thousand generations (Fig. 1) and trees were sampled every 100 generations. 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 (ML)>70%, maximum parsimony bootstrap (MP) >50%, or Bayesian posterior probabilities (PP) >0.95.

Results

Molecular phylogeny

The ITS dataset (Fig. 1) included sequences from 46 fungal specimens representing 19 species. The dataset had an aligned length of 828 characters, of which 294 characters are constant, 37 are variable and parsimony-uninformative, and 497 are parsimony-informative. Maximum parsimony analysis yielded 12 equally parsimonious trees (TL = 2088,

CI = 0.490, HI = 0.510, RI = 0.807, RC = 0.395). Best model for the ITS dataset estimated and applied in the Bayesian analysis: 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 = 0.009985 (BI).

The phylogeny (Fig. 1) inferred from ITS sequences was obtained for related taxa in *Scytinostroma* and showed that the new species was sister to *S. duriusculum* (Berk. & Broome) Donk (1956: 20) with a strong support (100% BS, 100% BP, 1.00 BPP) and then grouped with *S. renisporum* Boidin, Lanq. & Gilles. (1987: 97).

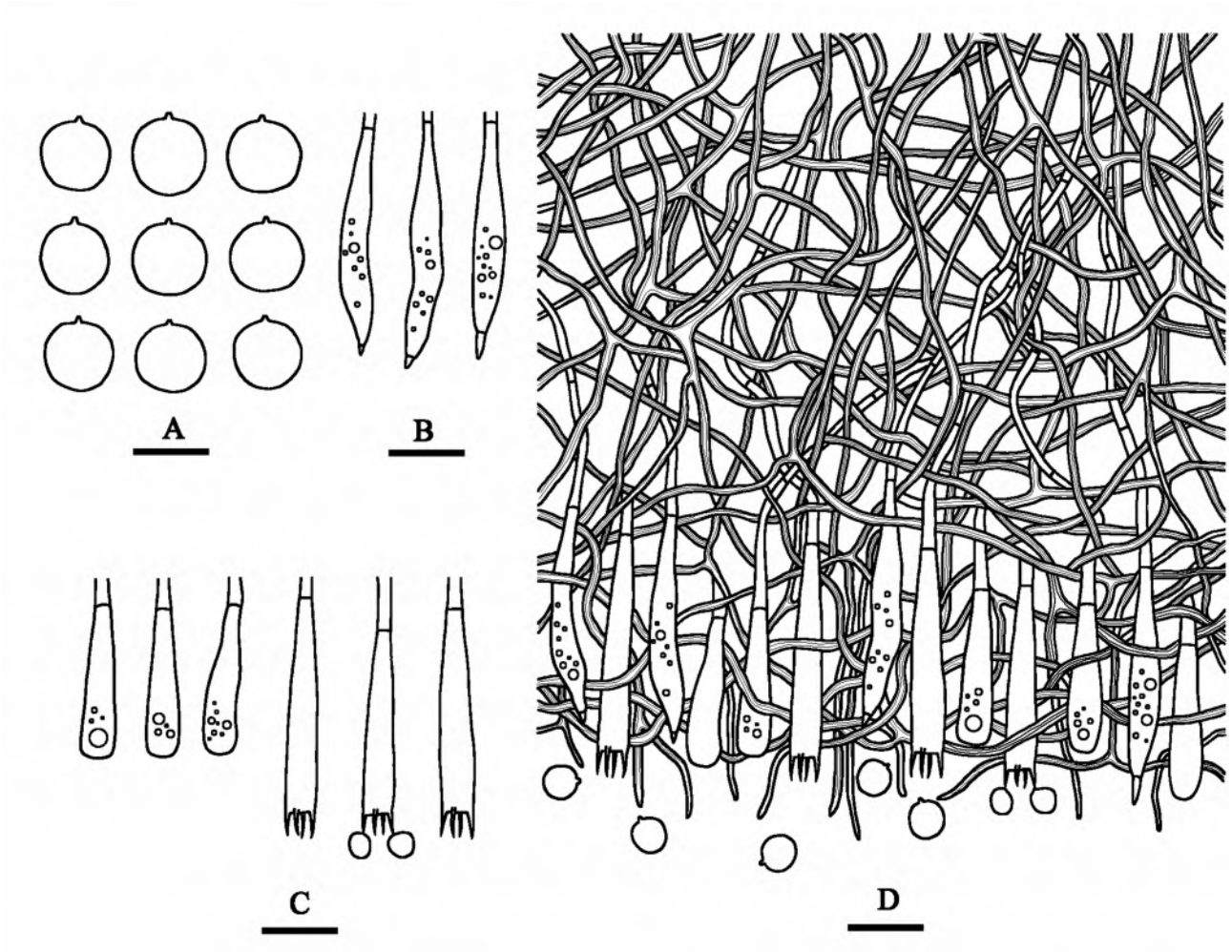

FIGURE 3. Microscopic structures of *Scytinostroma yunnanense* (drawn from the holotype). A. Basidiospores. B. Cystidia. C. Basidia and basidioles. D. A section of hymenium. Bars: A–5 µm; B, C, D–10 µm.

Taxonomy

Scytinostroma yunnanense C.L. Zhao, *sp. nov.* (Figs. 2, 3)
MycoBank no.: MB 835865

Holotype:—China. Yunnan Province: Wenshan, Xichou County Xiaoqiaogou, Xiaoqiaogou National Nature Reserve, E 103°53′-104°10′, N 23°16′-23°25′, on the angiosperm trunk, 15 January 2019, *CLZhao 11010* (SWFC!).

Etymology:—*Yunnanense* (Lat.): referring to the locality (Yunnan Province) of the type specimens.

Basidiomata:—Annual, resupinate, coriaceous, up to 13 cm long, 3.5 cm wide, 300–500 µm thick. Hymenial surfaces smooth to tuberculate, white to cream when fresh, cream upon drying.

Hyphal structure:—Hyphal system dimitic; generative hyphae bearing simple septa, thin-walled, 1.5–2.5 µm in diameter, IKI–, CB–; tissues unchanged in KOH; skeletal hyphae branched, interwoven, hyaline to yellowish, narrow and thick-walled (0.6–1 µm thick), 2–3 µm in diameter, dextrinoid, cyanophilous.

Hymenium:—Cystidia fusiform to cylindrical, 28–33 × 4–5 µm, thin-walled; basidia clavate, 21–28 × 4–5.5 µm, with four-spored, smooth, thin-walled, guttulate, basidioles dominant, in shape similar to basidia, but slightly smaller.

Spores:—Basidiospores 4.5–5.5 × 4.2–5.2 µm, L = 4.93 µm, W = 4.74 µm, Q =1.04–1.08 (n = 90/3), subglobose to globose, hyaline, thin-walled, smooth, amyloid, acyanophilous.

Ecology and distribution:—Lignicolous, causing a white rot. Found in China.

Additional specimens (paratypes) examined:—China. Yunnan Province, Wenshan, Xichou County, Xiaoqiaogou National Nature Reserve, E 103°53′-104°10′, N 23°16′-23°25′, on the angiosperm trunk, 14 January 2019, *CLZhao 10758*, *CLZhao 10802* (SWFC!).

Discussion

In the present study, a new species *Scytinostroma yunnanense* is described based on phylogenetic analyses and morphological characters.

Phylogenetically, *Scytinostroma yunnanense* is closely related to *S. duriusculum* based on the ITS sequence data (Fig. 1). However, morphologically *S. duriusculum* differ from *S. yunnanense* by its membranous basidiomata and larger cystidia (50–100 × 6–9 µm; Donk 1956).

Morphologically, *Scytinostroma alutum* Lanq. (1984: 187), *S. arachnoideum* (Peck) Gilb. ((1963) [1962]: 660), *S. cystidiatum* Boidin (1960: 285), *S. hemidichophyticum* Pouzar (1966: 217) and *S. portentosum* (Berk. & M.A. Curtis) Donk are similar to *S. yunnanense* based on the characters by amyloid basidiospores. However, *S. alutum* differs from *S. yunnanense* by resupinate to effuse-reflexed basidiomata with cracked hymenophore and larger basidiospores (5–7 × 5–7.5 µm; Bernicchia & Gorjón 2010). *Scytinostroma arachnoideum* is separated from *S. yunnanense* by cottony basidiomata with white rhizomorphs and smaller basidiospores (3.5–4.5 × 2.5–3.5 µm; Gilbertson 1962). *Scytinostroma cystidiatum* differs in its thick-walled, incrusted, larger cystida (40–100 × 7–14 µm; Boidin 1960). *S. hemidichophyticum* differs from *S. yunnanense* by cream to pale yellow or pale ochraceous hymenial surfaces and larger cystida (25–100 × 2.5–5 µm; Bernicchia & Gorjón 2010). *Scytinostroma portentosum* is separated from *S. yunnanense* by its resupinate to effuse-reflexed basidiomata with cream to plae yellow to light ochraceous hymenial surfaces (Donk 1956).

Wood-rotting fungi is an extensively studied group of Basidiomycota (Gilbertson & Ryvarden 1987, Núñez & Ryvarden 2001, Bernicchia & Gorjón 2010, Dai 2010, 2011, 2012, Ryvarden & Melo 2014), and some economically important species are found in this group (Dai *et al*. 2003, 2007, Cui *et al*. 2011, Wu *et al*. 2019). Yunnan Province is very rich for wood-decaying fungi, although many new taxa have been found from the Province (Yuan & Dai 2008, Chen *et al*. 2016, Zheng *et al*. 2019, Zhao *et al*. 2019), the wood-rotting fungi diversity is still not well known. The new species in the present study, *Scytinostroma yunnanense*, is from Yunnan Province, too. It is possible that new taxa will be found after further investigations and molecular analyses.

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