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Morphological characteristics and phylogenetic analyses reveal *Scopuloides yunnanensis* (Polyporales, Basidiomycota), a new wood-decaying fungus from China

ZIRUI GU[1,3], HONGMIN ZHOU[1,2,4]* & CHANGLIN ZHAO[1,2,5]*

[1] *College of Forestry, Southwest Forestry University, Kunming 650224, P.R. China*
[2] *Yunnan Key Laboratory of Gastrodia and Fungal Symbiotic Biology, Zhaotong University, Zhaotong, 657000, China*
[3] *fungiziruig@163.com; https://orcid.org/0000-0003-1476-9040*
[4] *hongminzhou@foxmail.com; https://orcid.org/0000-0002-0724-5815*
[5] *fungichanglinz@163.com; https://orcid.org/0000-0002-8668-1075*
**Corresponding author: H.M. Zhou, e-mail: hongminzhou@foxmail.com; C.L. Zhao, e-mail: fungichanglinz@163.com*

Abstract

Wood-decaying fungi, as crucial decomposers in forest ecosystems, have been the subject of meticulous research. In this study, a new wood-decaying fungal species, *Scopuloides yunnanensis*, found in Yunnan, China, is proposed. *Scopuloides yunnanensis* was identified based on a comprehensive analysis of its membranaceous basidiomata, generative hyphae, lamprocystidia, projecting cystidia, and allantoid basidiospores. In addition to the morphology, phylogenetic analyses of ITS and nLSU rRNA markers were performed with Maximum Likelihood, Maximum Parsimony, and Bayesian inference methods, ensuring the robustness of our findings. The phylogenetic analysis showed that *S. yunnanensis* is sister to *S. hydnoides.* The new species' description, illustrations, and results of the phylogenetic analysis are provided. In addition, an identification key to all *Scopuloides* species worldwide is provided.

Key words: 1 new species, Molecular systematics, New taxon, Taxonomy, Yunnan Province

Introduction

Wood-decaying fungi are eukaryotic microorganisms that play fundamental ecological roles. They are not just decomposers but also mutualists of plants and animals in the fungal tree of life. They drive carbon cycling in forest soils, mediate plants' mineral nutrition, and alleviate other soil organisms' carbon limitations.

The wood-decaying fungal genus *Scopuloides* (Massee) Höhn. & Litsch. (1908: 57) was recovered as a monophyletic group with strong support within the family Meruliaceae Rea (1922: 620), including species from Asia, Europe, North America, and Neotropics, which is typified by *S. hydnoides* (Cooke & Massee) Hjortstam & Ryvarden (1979: 509) (Chen *et al.* 2021). *Scopuloides* species are typically ceraceous with white-buff basidiocarps, and odontioid or hydnoid grandinioid hymenophore, and the genus is characterized by having a subiculum of compact texture with agglutinated and fairly short-celled subicular hyphae, short and small basidia, small basidiospores, and the presence of lamprocystidia (Wu 1990, Gilbertson & Nakasone 2003, Bernicchia & Gorjón 2010). So far, 12 taxa have been addressed in the genus, but only seven species have been accepted worldwide (http://www.mycobank.org, accessed on 11 September 2024; http://www.indexfungorum.org, accessed on 11 September 2024), among them two species have been recorded from China (Lin & Chen 1990, Chen *et al*. 2021).

Species diversity, taxonomy and multi-gene phylogeny of phlebioid clade (Phanerochaetaceae Jülich (1982: 384), Irpicaceae Spirin & Zmitr. (2003: 48), Meruliaceae) of Polyporales Gäum. (1926: 503) showed that four species of *Scopuloides* grouped together based on the nuc rDNA ITS1-5.8S-ITS2, the 28S rDNA, the RNA polymerase II largest subunit, the RNA polymerase II second largest subunit, and the translation elongation factor 1-α, in which all species were clustered into the family Meruliaceae (Chen *et al.* 2021). Multi-gene phylogeny and taxonomy of the wood-rotting fungal genus *Phlebia* sensu lato (Polyporales, Basidiomycota) indicated that four *Scopuloides* taxa were nested into the family Meruliaceae belonging to the order Polyporales based on the ITS + nLSU + TEF1 + mt-SSU + GAPDH

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+ RPB1 + RPB2 sequences, and the genus *Scopuloides* was closely related to *Climacodon* P. Karst. (1881: 20) and *Luteochaete* C.C. Chen & Sheng H. Wu (2021: 425) (Zhao *et al.* 2023).

In this study, an unknown species of wood-decaying fungus was found in Yunnan Province, China. To clarify the taxonomic placement of this species, morphological, and phylogenetic analyses based on the ITS and LSU sequences were carried out. This species is identified as a new species of *Scopuloides*, and a detailed description, illustrations, and phylogenetic analysis results of the new species are provided.

Materials and methods

Sample Collection and Herbarium Specimen Preparation

The fresh fruiting bodies were collected from Dehong and Honghe of Yunnan Province, China. The samples were photographed *in situ*, and the fresh macroscopic details were recorded. Photographs were recorded by a Nikon D7100 camera. All the photos were focus-stacked using Helicon Focus software. The samples were transported to a field station and were dried by an electric dryer at 45 °C (Hu *et al.* 2022). Once dried, the specimens were sealed in envelopes and zip-lock plastic bags and labeled (Zhang *et al.* 2024). The dried specimens were deposited in the herbarium of the Southwest Forestry University (SWFC), Kunming, Yunnan Province, China.

Morphological studies

The macromorphological descriptions were based on field records and photos. The color terminology follows Petersen (1996). The micromorphological data were obtained from the dried specimens after observation under a light microscope with a magnification of 10 × 100 oil (Zhao *et al.* 2023). To show the variation in spore sizes, 5% of measurements were excluded from each end of the range and shown in parentheses. At least thirty basidiospores from each specimen were measured. The following abbreviations are used: KOH = 5% potassium hydroxide water solution, CB– = acyanophilous, IKI– = both inamyloid and indextrinoid, 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, and n = a/b (number of spores (a) measured from given number (b) of specimens).

Molecular procedures and phylogenetic analyses

The CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd., Beijing, China) was used to obtain genomic DNA from the 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 thousand 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 12,000 rpm for 0.5 min. Then, 0.5 mL inhibitor removal fluid was added to the AC for centrifugation at 12 thousand 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 genome DNA. The nuclear ribosomal of the internal transcribed spacer (ITS) region was amplified with ITS5 and ITS4 primers (White *et al.* 1990). The nuclear large subunit (nLSU) region was amplified with the LR0R and LR7 primer pair (http://lutzonilab.org/nuclear-ribosomal-dna/, accessed on 20 March 2024). 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; the PCR procedure for nLSU 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 (Yang *et al.* 2023). The PCR products were purified and sequenced at Kunming Tsingke Biological Technology Limited Company (Yunnan Province, P.R. China). All of the newly generated sequences were deposited in NCBI GenBank (Table 1).

The sequences were aligned in MAFFT version 7 (Katoh *et al.* 2019) using the G-INS-i strategy. The alignment was adjusted manually using AliView version 1.27 (Larsson 2014). The dataset was aligned first, and then the sequences of ITS and nLSU were combined with Mesquite version 3.51. The alignment datasets were deposited in TreeBASE (submission ID 31292). The combined ITS+nLSU sequences were used to infer the position of the new species in the genus *Scopuloides*. Sequences of *Climacodon septentrionalis* (Fr.) P. Karst. (1881: 20) was retrieved from GenBank and used as an outgroup in the phylogenetic analysis following Chen *et al.* (2021) and Zhao *et al.* (2023).

TABLE 1 Names, specimen numbers, and corresponding GenBank accession numbers of the taxa used in this study. The new species are in bold.

Species Name	Sample No.	GenBank Accession No.		References
		ITS	nLSU	
Climacodon septentrionalis	CBS 131.40	MH856064	MH867555	Vu *et al.* (2018)
Scopuloides allantoidea	GC 1602-11	MZ637080	MZ637278	Chen *et al.* (2021)
Scopuloides allantoidea	WEI 16-060	MZ637081	MZ637279	Chen *et al.* (2021)
Scopuloides dimorpha	WEI 17-227	MZ637083	MZ637281	Chen *et al.* (2021)
Scopuloides dimorpha	WEI 19-073	MZ637084	MZ637282	Chen *et al.* (2021)
Scopuloides hydnoides	FP-150473	KP135355	KP135284	Floudas and Hibbett (2015)
Scopuloides hydnoides	Wei 17-569	MZ637085	MZ637283	Chen *et al.* (2021)
Scopuloides leprosa	FRDBI 17584773	MW487975	—	Unpublished
Scopuloides rimosa	KHL 11916	EU118665	EU118665	Larsson (2007)
Scopuloides rimosa	Wu 1507-117	MZ637087	MZ637284	Chen *et al.* (2021)
Scopuloides yunnanensis	**CLZhao 18588**	PP511312	PP511315	**Present study**
Scopuloides yunnanensis	**CLZhao 30079**	PP511313	PP511316	**Present study**
Scopuloides yunnanensis	**CLZhao 30213 ***	PP511314	PP511317	**Present study**

***** is shown type material, holotype.

Maximum Parsimony (MP), Maximum Likelihood (ML), and Bayesian Inference (BI) analyses were applied to the combined ITS+nLSU dataset following Zhao & Wu (2017), and the tree construction procedure was performed in PAUP* version 4.0b10 (Swofford 2002). All of the characters were equally weighted, and gaps were treated as missing data. Using the heuristic search option with TBR branch swapping and 1000 random sequence additions, trees were inferred. Max trees were set to 5000, branches of zero length were collapsed, and all parsimonious trees were saved. Clade robustness was assessed using bootstrap (BT) analysis with 1000 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. The multiple sequence alignment was also analyzed using maximum likelihood (ML) in RAxML-HPC2 (Miller *et al.* 2012). Branch support (BS) for ML analysis was determined by 1000 bootstrap replicates.

MrModeltest 2.3 (Nylander 2004) was used to determine the best-fit evolution model for the dataset for Bayesian inference (BI), which was performed using MrBayes 3.2.7a with a GTR+I+G model of DNA substitution and a gamma distribution rate variation across sites (Ronquist *et al.* 2012). The first one-fourth of all generations were discarded as burn-ins. The majority-rule consensus tree of all the remaining trees was calculated. Branches were considered significantly supported if they received a Maximum Likelihood bootstrap value (BS) of ≥70%, a Maximum Parsimony bootstrap value (BT) of ≥50%, or Bayesian posterior probabilities (BPP) of ≥0.95.

Results

Molecular phylogeny

The aligned dataset comprised 13 specimens representing seven species. Four Markov chains were run for two runs from random starting trees, each for one million generations for the combined ITS+nLSU dataset with trees and parameters sampled every 1,000 generations. The dataset had an aligned length of 1,548 characters, of which 1,336 characters are constant, 104 are variable and parsimony uninformative, and 108 are parsimony informative. Maximum parsimony analysis yielded two equally parsimonious trees (TL = 216, CI = 0.7639, HI = 0.2361, RI = 0.7682, and RC = 0.5868). The best model for the combined 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). Both Bayesian analysis and ML analysis resulted in a similar topology to MP analysis with an average standard deviation of split frequencies = 0.002596 (BI), and the effective sample size (ESS) for Bayesian analysis across the two runs is double the average ESS (avg ESS) = 1165.

The phylogram based on the combined ITS+nLSU dataset (Fig. 1) indicated that the species *Scopuloides yunnanensis* is nested in the genus *Scopuloides*, sister to *S. hydnoides*.

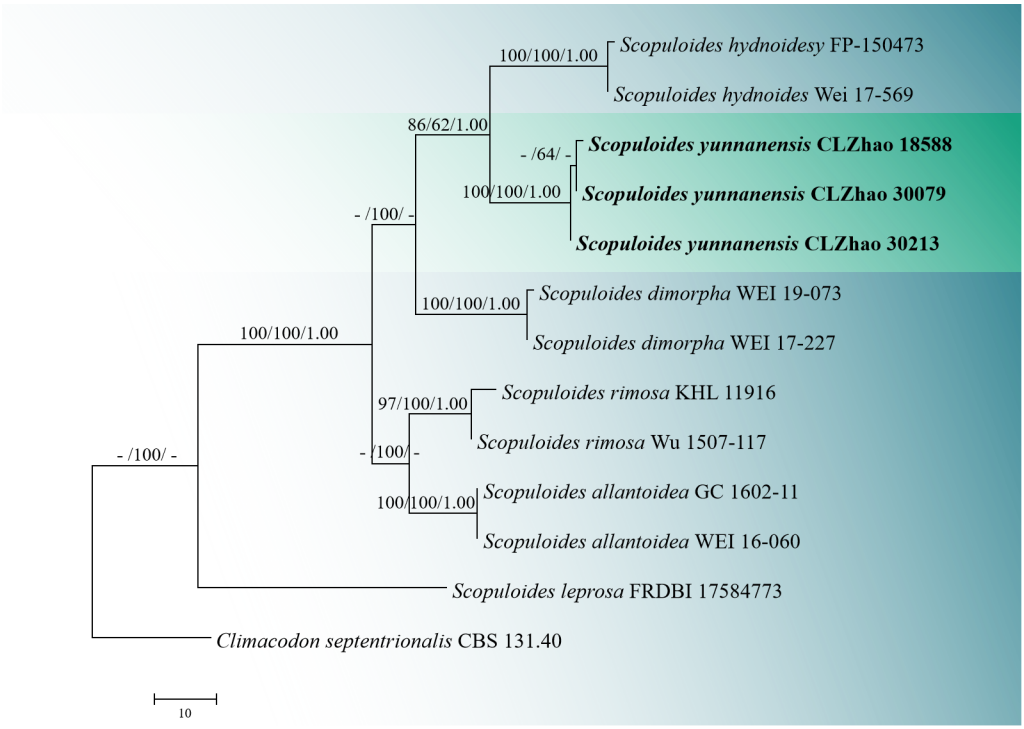

FIGURE 1. Maximum Parsimony strict consensus tree illustrating the phylogenetic relationships of *Scopuloides* species based on the combined ITS+nLSU dataset. Branches are labeled with Maximum Likelihood bootstrap values equal to or above 70%, Maximum Parsimony bootstrap values equal to or above 50%, and Bayesian posterior probabilities equal to or above 0.95. The new species is in bold.

Taxonomy

Scopuloides yunnanensis Z.R. Gu & C.L. Zhao, *sp. nov.* Figs. 2, 3, 4
MycoBank no.: MB 852964

Etymology*:**—yunnanensis*** (Lat.) refers to the locality (Yunnan Province) of the holotype.

***Holotype*:**—CHINA. Yunnan Province, Dehong, Yingjiang County, Tongbiguan Nature Reserve, 24°42′N, 97°56′E, elev. 850 m, on fallen angiosperm branch, 19 July 2023, CLZhao 30213 (SWFC!).

***Basidiomata*:**—Annual, resupinate, adnate, membranaceous, without odor or taste when fresh, up to 19 cm long, 2.5 cm wide, 0.4 mm thick. Hymenial surface grandinoid, white when fresh, turning to white to pale cream upon drying. Sterile margin white to pale cream, up to 1 mm wide.

***Hyphal structure*:**—Hyphal system monomitic, generative hyphae simple septate, regularly arranged, colorless, thin- to thick-walled, rarely branched, 2.5–5.5 µm in diameter; IKI–, CB–, tissues unchanged in KOH.

***Hymenium*:**—Lamprocystidia numerous, conical or subulate, colorless, simple-septate at base, 15–34 × 5–12 µm, heavily encrusted, originating from trama or subiculum, immersed or projecting; septate cystidia usually projecting from hymenium, some encrusted, cylindrical, apically capitate, rounded or narrow, 18.5–40.5 × 4.5–7.5 µm, with several secondary septa. Basidia cylindrical, 7–14.5 × 3–5 µm, thin-walled, 4-sterigmate; basidioles, similar to basidia in shape, but slightly smaller.

Basidiospores:—Cylindrical to allantoid, colorless, smooth, thin-walled, occasionally with 1–2 oil drops, IKI–, CB–, (2.5–)2.8–3.7(–3.9) × (1.3–)1.4–2(–2.3) µm, L = 3.28 µm, W = 1.74 µm, Q = 1.81–1.97 (n = 90/3).

***Type of rot*:**—White rot.

***Additional specimens examined (paratypes)*:**—CHINA. Yunnan Province, Honghe, Pingbian County, Daweishan National Forest Park, 22°49′N, 103°24′E, elev. 1500 m, on angiosperm trunk, 6 June 2020, CLZhao 18588 (SWFC!); Dehong, Yingjiang County, Tongbiguan Nature Reserve, 24°42′N, 97°56′E, elev. 850 m, on fallen angiosperm branch, 18 July 2023, CLZhao 30079 (SWFC!).

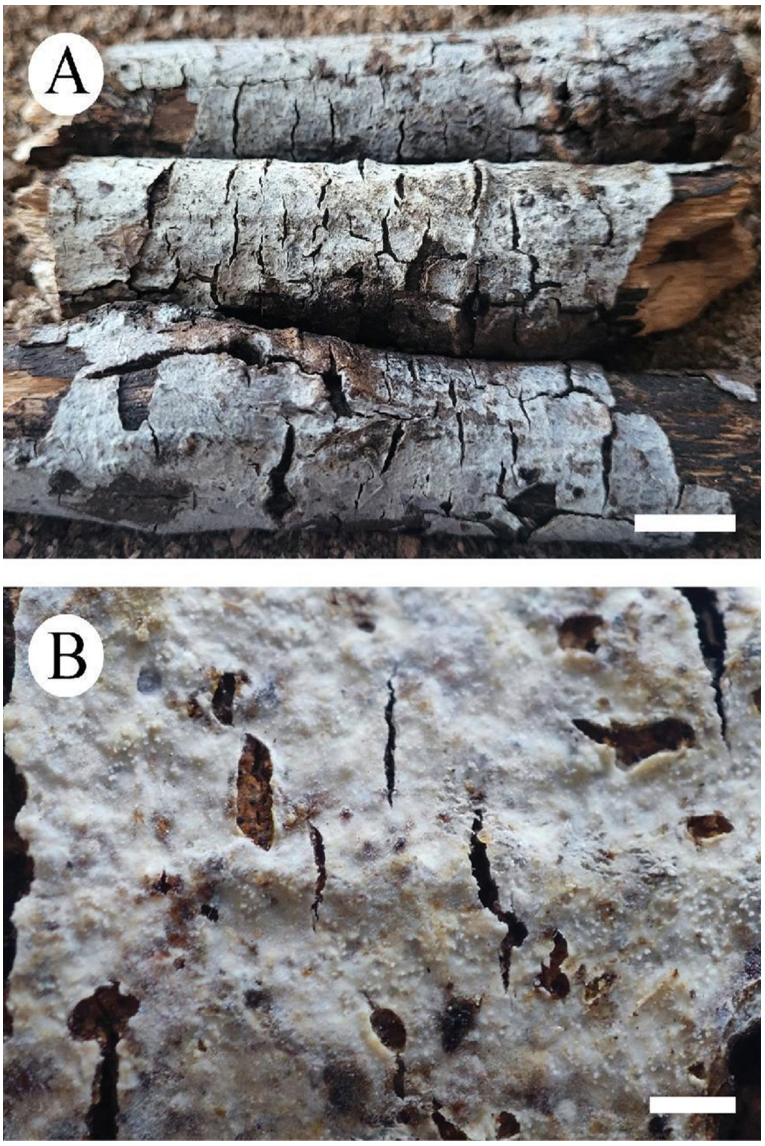

FIGURE 2. Basidiomata of *Scopuloides yunnanensis* (holotype, CLZhao 30213). Bars: A= 1 cm; B= 1 mm.

Discussion

In the present study, one new species, *Scopuloides yunnanensis,* is described based on phylogenetic analyses and morphological characteristics.

Scopuloides allantoidea C.C. Chen & Sheng H. Wu (2021: 432), *S. dimorpha* (Sang H. Lin & Z.C. Chen) C.C. Chen & Sheng H. Wu (2021: 432), *S. hydnoides,* and *S. rimosa* (Cooke) Jülich (1982: 422) group together phylogenetically based on the ITS + nLSU + TEF1 + mt-SSU + GAPDH + RPB1 + RPB2 sequences (Chen *et al*. 2021, Zhao *et al*. 2023). Our phylogenetic results based on the combined ITS+nLSU dataset (Fig. 1) demonstrated that *S. yunnanensis* is sister to *S. hydnoides*, and both species are closely related to *S. dimorpha*. However, *Scopuloides hydnoides* differs from *S. yunnanensis* by having the hydnoid basidiomata with ceraceous and cracked hymenial surface, and larger lamprocystidia (16–55 × 8–16 µm vs. 15–34 × 5–12 µm), and narrower basidiospores (3–3.5 × 1–1.25 µm vs. 2.8–3.7 ×1.4–2 µm, Cunningham 1959). *Scopuloides dimorpha* differs from *S. yunnanensis* by having distinctly thicker basidiocarps with long and cylindrical spines and cartridge buff, ivory buff or pale pinkish buff hymenial surface and ellipsoid basidiospores (Lin & Chen 1990). Table 2 presents a morphological comparison of all *Scopuloides* species, including the new species.

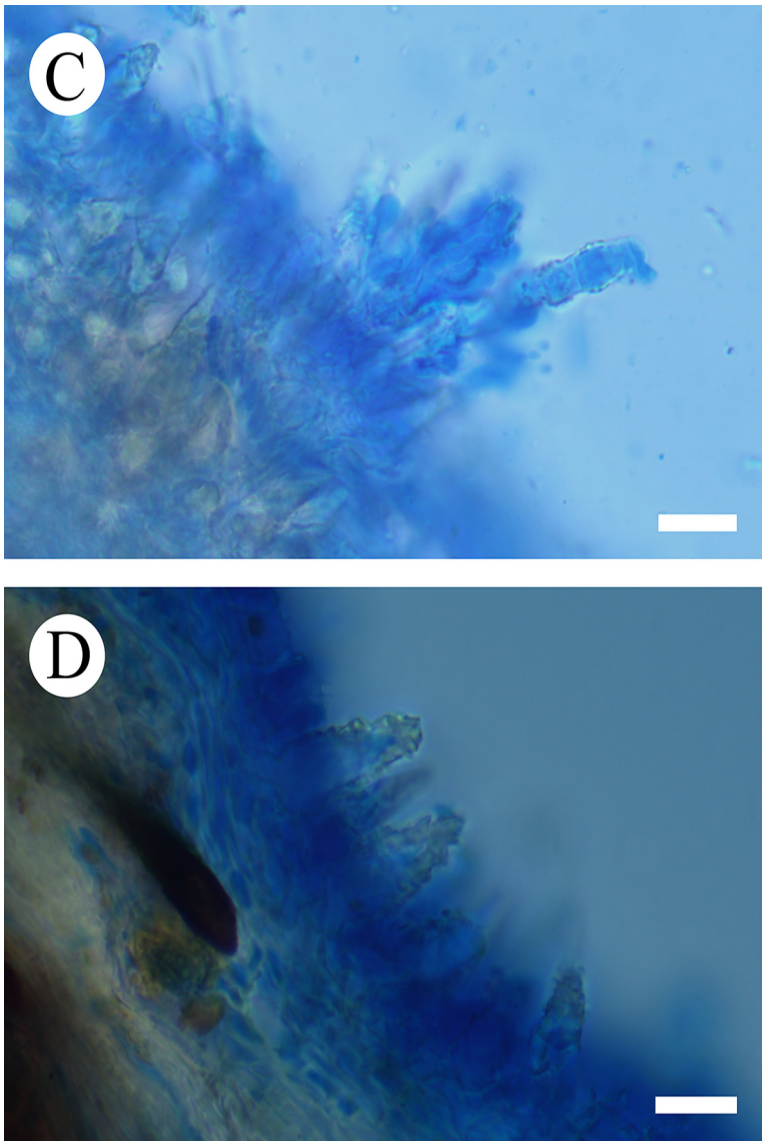

FIGURE 3. Sections of hymenium of *Scopuloides yunnanensis* (holotype, CLZhao 30213). Scale bars: C, D= 10 µm, 10 × 100 Oil.

The wood-decaying fungi are very rich in Yunnan Province of China, and many new taxa have been recently described from the province ((Dai *et al.* 2021, Wu *et al.* 2022, Wang *et al*. 2021, 2023, Dong *et al.* 2023, Duan *et al.* 2023, Mao *et al*. 2023, Yang *et al.* 2023, 2024, Yuan *et al.* 2023, Zhang *et al*. 2023, 2024, Zhou *et al*. 2024). However, there are still unknown taxa of wood-decaying fungi in the province. Previously, two *Scopuloides* species (*S. allantoidea* and *S. dimorpha*) have been recorded in China (Chen *et al.* 2021). The present paper reports the third species of *Scopuloides* from Yunnan, and more new species could be found after further investigations in different areas in Yunnan.

TABLE 2. Morphological comparisons of *Scopuloides* species.

Species name	Hymenial surface	Hyphae	Cystidia	Basidia	Basidiospores	References
S.allantoidea	Ceraceous to pruinose, odontioid, not cracked; grayish, white to cream	Thick-walled, branched	Lamprocystidia, conical or subulate, 30–70 × 7–17 µm; septate cystidia, cylindrical, apically capitate, rounded or narrow, 5–13 µm diam, Thick-walled	Cylindrical to clavate, 9–12 × 3–4 µm	Allantoid, 3.3–3.7 × 1.2–1.4 µm	Chen *et al.* 2021

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TABLE 2. (Continued)

Species name	Hymenial surface	Hyphae	Cystidia	Basidia	Basidiospores	References
S. dimorpha	Membranaceous, odontioid, not completely cracked; white, catridge buff, ivory buff or pale pinkish buff	Thin-walled, unbranched	Lamprocystidia, 35 × 5–17 µm; septate cystidia, cylindrical, fimbriatus apice, Thin-walled	Subclavate to cylindrical, 15 × 3.5–3.8 µm	Ellipsoid, 3–3.2 × 1.2–1.8 µm	Lin & Chen 1990
S. hydnoides	Ceraceous, odontoid, distinctly cracked; whitish or greyish	Thick-walled, branched	Lamprocystidia, 40–60 × 8–12 µm; septate cystidia, cylindrical, Thick-walled	Subclavate, 12–15 × 3.5–4.5 µm	Short-allantoid, 3.5–4(–5) × 1.8–2(2.2) µm	Hjortstam & Ryvarden 1979
S. leprosa	Ochraceous, smooth to irregular, rarely slightly grandinioid, deeply cracked; whitish to yellowish	Thin- to thick- walled, branched	Lamprocystidia, fusiform, 40–80(100) × (5)8–14 µm; cylindrical leptocystidia with obtuse or slightly swollen apex, infrequent, 150(200) × 8–10 µm	Narrowly clavate, 20–30(–40) × 4–6 µm	Ellipsoid, (3.5)4–5.5(6) × (2)2.5–3 µm	Boidin *et al*. 1993
S. magnicystidiata	Gray to white, cracking moderately; pale smoke gray to pale olive gray, older areas cartridge buff	Thick-walled, infrequently branched	Cylindrical with simple septa, often constricted at septa, thin-walled, 150 × 20 µm	Clavate, 11–14 × 4–5.5 µm	Oblong to short-cylindric, 4–5 × 2–2.5 µm	Gilbertson & Nakasone 2003
S. rimosa	Ceraceous, odontoid; more or less greysh	Thin- to thick-walled	Lamprocystidia, coical, 40–50 × 8–10 µm	Subclavate, 10–12 × 3–4 µm	Suballantoid, 3.5–4.5(5) × 1.5–2(2.5) µm	Jülich 1982
S. subgelatinosa	Ceraceous, widely hydnaceous to odontoid, cracks locally abundant; greyish brown, light brown, greyish brown, light brown, or brown	Thick-walled, moderately branched	Pseudocystidia narrowly clavate with an obtuse apex or fusiform with an acute apex; 50–70 × 7–8 µm; metuloid hymenial cystidia, clavate to broadly fusiform with acute or rounded apices, 25–40 × 5.5–8 µm	—	Ellipsoid, 2.7–3 × 1.3–1.8(–2) µm	Nakasone 2003
S.yunnanensis	Membranaceous, grandinoid; white when fresh, turning to white to slightly cream upon drying	Thin- to thick-walled, rarely branched	Lamprocystidia conical or subulate, 15–34 × 5–12 µm; septate cystidia, cylindrical, apically capitate, rounded or narrow, 18.5–40.5 × 4.5–7.5 µm	Cylindrical, 7–14.5 × 3–5 µm	Cylindrical to allantoid, (2.5–)2.8–3.7(–3.9) × (1.3–)1.4–2(–2.3) µm	**Present study**

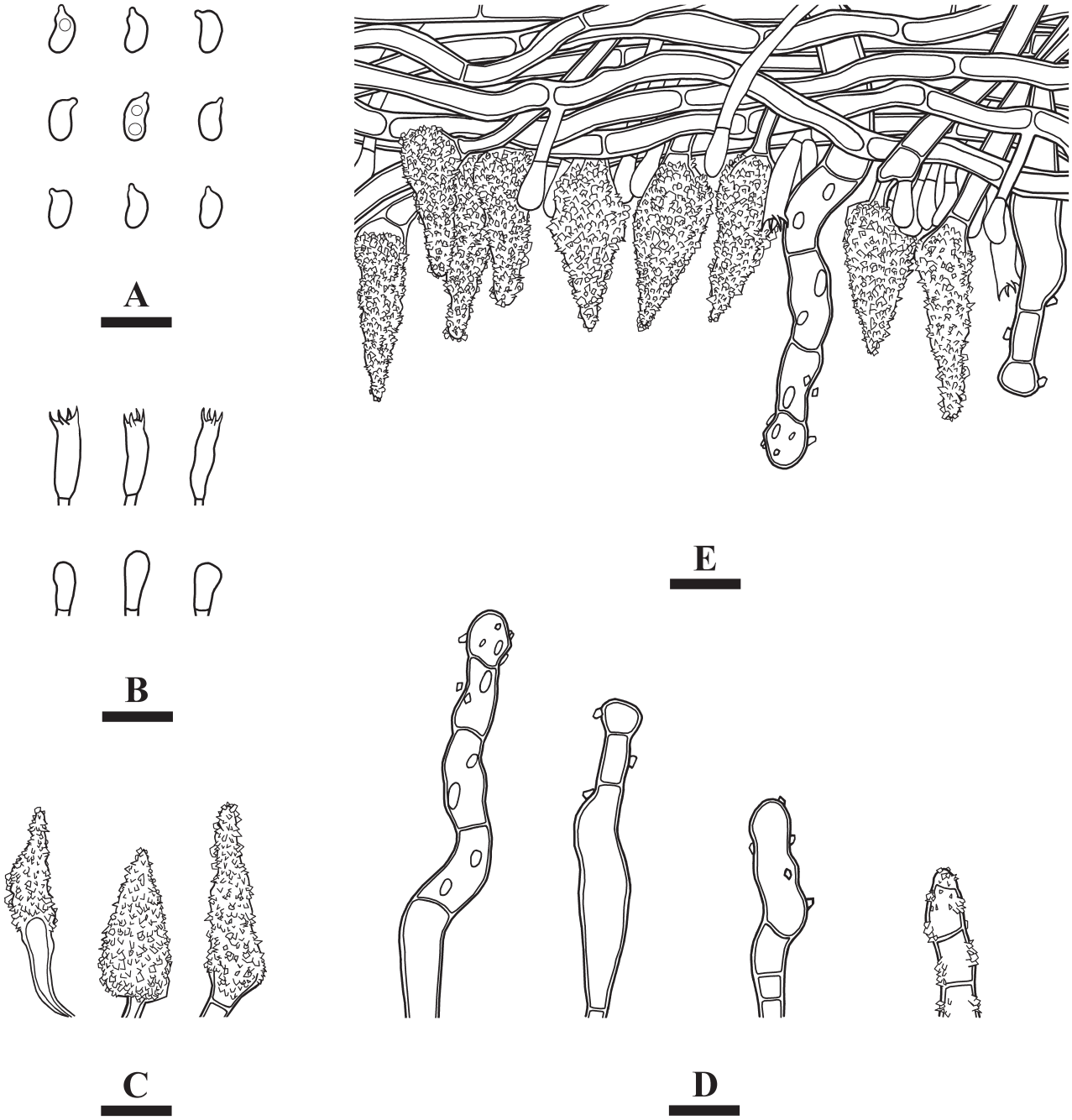

FIGURE 4. Microscopic structures of *Scopuloides yunnanensis* (holotype, CLZhao 30213). (A) Basidiospores; (B) Basidia and basidioles; (C) Lamprocystidia; (D) Septate cystidia; (E) Part of the vertical section of hymenium. Bars: A = 5 μm; B–E = 10 μm.

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