



## Morphological characteristics and phylogenetic analyses revealed three new wood-inhabiting fungi (Agaricomycetes, Basidiomycota) in Southern China

QI YUAN<sup>1,3</sup>, KAI-YUE LUO<sup>1,4</sup>, YING ZHANG<sup>1,5\*</sup> & CHANG-LIN ZHAO<sup>1,2,6\*</sup>

<sup>1</sup> College of Biodiversity Conservation, Southwest Forestry University, Kunming 650224, P.R. China

<sup>2</sup> Yunnan Key Laboratory for Fungal Diversity and Green Development, Kunming Institute of Botany, Chinese Academy of Science, Kunming 650201, P.R. China

<sup>3</sup> [✉ fungiyuanqi@163.com](mailto:fungiyuanqi@163.com); [ORCID](https://orcid.org/0000-0002-6732-1656) <https://orcid.org/0000-0002-6732-1656>

<sup>4</sup> [✉ fungikaiyuel@163.com](mailto:fungikaiyuel@163.com); [ORCID](https://orcid.org/0000-0001-6145-4864) <https://orcid.org/0000-0001-6145-4864>

<sup>5</sup> [✉ yingb.zhang@swfu.edu.cn](mailto:yingb.zhang@swfu.edu.cn); [ORCID](https://orcid.org/0000-0002-1204-1278) <https://orcid.org/0000-0002-1204-1278>

<sup>6</sup> [✉ fungichanglinz@163.com](mailto:fungichanglinz@163.com); [ORCID](https://orcid.org/0000-0002-8668-1075) <https://orcid.org/0000-0002-8668-1075>

\*Corresponding author: C.L. Zhao, [✉ fungichanglinz@163.com](mailto:fungichanglinz@163.com)

### Abstract

Fungi are one of the most diverse groups of organisms on Earth, among which wood-inhabiting fungi play a crucial role in ecosystem processes and functions. Three wood-inhabiting fungi belonging to the genera *Ceraceomyces*, *Stereopsis*, and *Xenasmatella* were found in Southern China. Combined with morphological characteristics and molecular biological evidence, the present study describes them to be new fungal taxa *Ceraceomyces yunnanensis*, *Stereopsis yunnanensis*, and *Xenasmatella bambusicola*. *Ceraceomyces yunnanensis* is distinguished by the resupinate basidiomata with merulioid hymenial surface, monomitic hyphal system and cylindrical basidiospores; *S. yunnanensis* is characterized by the laterally stipitate basidiocarps with greyish brown to brown pilei, monomitic hyphal system and subglobose basidiospores; and *X. bambusicola* is characterized by the resupinate basidiomata, arachnoid hymenophore with white to greyish hymenial surface and warted basidiospores. Sequences of ITS and nLSU rRNA markers of the studied samples were generated, and phylogenetic analyses were performed with maximum likelihood, maximum parsimony, and Bayesian inference methods. The phylogenetic analyses of ITS+nLSU showed that three new species nested into families Amylocorticiaceae, Stereopsidaceae and Xenasmataceae (Agaricomycetes), in which *C. yunnanensis* grouped sister to *C. borealis*; *S. yunnanensis* grouped with *S. radicans*; and *X. bambusicola* grouped sister to *X. xinpingensis*.

**Keywords:** Biodiversity, Classification, Forest ecosystem, Molecular systematics, Taxonomy

### Introduction

Fungi are well-known as a diverse group of microorganisms, that play important roles in the forest ecosystems (Phookamsak *et al.* 2019). Wood-inhabiting fungi are essential to natural ecosystems for nutrient cycling, and maintaining plant diversity (Drinkwater *et al.* 2017, Horwath 2017, Hyde *et al.* 2018, Wu *et al.* 2022).

The wood-inhabiting fungal genus *Ceraceomyces* Jülich. (1972: 146) (Amylocorticiaceae, Amylocorticiales), typified by *C. tessulatus* (Cooke) Jülich. (1972: 154) (Jülich 1972) is characterized by annual, resupinate, pellicular basidiomata with a smooth or merulioid hymenial surface, thin hymenial layer and loose subiculum, a monomitic hyphal system with clamped generative hyphae, narrowly clavate basidia and subglobose to narrowly ovate to ellipsoid basidiospores (Chikowski *et al.* 2017). Based on the MycoBank database (<http://www.Mycobank.org>, accessed on 27 March 2023) and the Index Fungorum (<http://www.indexfungorum.org>, accessed on 27 March 2023), the genus *Ceraceomyces* contains 30 specific and registered names, but the legitimate number of species has reached 12 (Chikowski *et al.* 2017, Dhingra & Singh 2008, Dhingra *et al.* 2014, Larsson & Larsson 1998, Roberts 2000). *Stereopsis* D.A. Reid. (1965: 290), (Stereopsidaceae, Stereopsidales), typified by *S. radicans* (Berk.) D.A. Reid (Sjökvisst *et al.* 2014), is characterized by the spathulate-flabellate basidiomata with upperside smooth to radiately or irregularly rugose, a monomitic hyphal system with or without clamped generative hyphae, clavate basidia with 2–4 sterigmata, and ellipsoid to subglobose, smooth, thin-walled basidiospores (Eriksson 1984). The MycoBank database (<http://www.Mycobank.org>, accessed on 27 March 2023) and Index Fungorum (<http://www.indexfungorum.org>, accessed on 27

March 2023) have registered 23 specific and infraspecific names in *Stereopsis*, but the legitimate number of species has reached 21 (Ryvarden 2012; Sjökvist *et al.* 2014; Guo 1987). The genus *Xenasmatella* Oberw. (1966: 28), a typical wood-inhabiting fungal group is mainly distributed in the tropics (Oberwinkler 1966). This genus is characterized by a combination of resupinate to effused basidiomata with a ceraceous to subgelatinous consistency, smooth to porulose, reticulate, grandinoid hymenophore, a monomitic hyphal structure with clamped generative hyphae, pleural basidia, and colorless, thin to slightly thick-walled, warted, globose to cylindrical basidiospores (Oberwinkler 1966, Bernicchia & Gorjón 2010). Based on the MycoBank database (<http://www.Mycobank.org>, accessed on 27 March 2023) and the Index Fungorum (<http://www.indexfungorum.org>, accessed on 27 March 2023), the genus *Xenasmatella* contains 43 specific and registered names, but the legitimate number of species has reached 33. Recently, the genus *Xenasmatella* has been thoroughly studied in China, in which seven new species viz. *X. ailaoshanensis* C.L. Zhao ex C.L. Zhao & T.K. Zong (2020: 118), *X. gossypina* (C.L. Zhao) G. Gruhn & Trichies (2021: 40), *X. nigroidea* K.Y. Luo & C.L. Zhao (2022: 13), *X. rhizomorpha* C.L. Zhao (2020: 115), *X. tenuis* C.L. Zhao (2020: 116), *X. wuliangshanensis* (C.L. Zhao) G. Gruhn & Trichies (2021: 40), and *X. xinpingensis* C.L. Zhao were described (Huang *et al.* 2019, Luo & Zhao 2022, Zong *et al.* 2021).

These phylogenetic studies of re-thinking the classification of corticioid fungi including three genera viz. *Ceraceomyces*, *Stereopsis* and *Xenasmatella*, were just the prelude to the molecular systematics of the wood-inhabiting fungi group (Larsson 2007). The high phylogenetic diversity among corticioid homobasidiomycetes based on a data set with 178 taxa analysed using neighbour-joining and maximum parsimony methods revealed that *Ceraceomyces* nested into euagarics clade, in which it grouped with *Amylocorticium* Pouzar and *Hypochniciellum* Hjortstam & Ryvarden, but it did not cluster with *Byssomerulius*, and *Gloeoporus*, even though all of them have the typical morphological characteristics of a merulioid to poroid hymenophore (Larsson *et al.* 2004). Re-thinking the classification of corticioid fungi inferred from 5.8S and nuLSU rDNA sequences using Bayesian analysis showed that *Ceraceomyces* nested into the family Amylocorticiaceae (Larsson 2007). The phylogenetic classification for corticioid fungi at the family level carried out by Larsson (2007) proposed that nine genera viz. *Clavulicium* Boidin. (1957: 280), *Conohypha* Jülich. (1975: 303), *Crustoderma* Parmasto. (1968: 87), *Paullicorticium* J. Erikss. (1958: 66), *Phlyctibasidium* Jülich. (1974: 54), *Scotomyces* Jülich. (1978: 139), *Stereopsis*, *Thujacorticium* Ginns. (1988: 69), and *Trechinothus* E.C. Martini & Trichies. (2004: 262), were sequenced but they still could not be assigned to any of the orders accepted, in which based on the nuLSU phylogenetic analyses, and these taxa always occurred on long branches, and several of them were grouped at the base of the tree. The new order Stereopsidales and the new family Stereopsidaceae were described to accommodate *Stereopsis radicans* and *S. globosa* based on multi-gene phylogenetic analyses of SSU, nLSU, RPB 2 and tef 1- $\alpha$  (Sjökvist *et al.* 2014). The outline of all genera of Basidiomycota with combined SSU, ITS, nLSU, tef1- $\alpha$ , RPB1, and RPB2 datasets showed that *Xenasmatella* grouped with Xenasmataceae within the order Russulales (He *et al.* 2019). Recently, according to the internal transcribed spacer and nuclear large subunit analyses including 13 orders, *Xenasmatella* and related genera in the family Xenasmataceae formed a single lineage and grouped with orders Atheliales, Boletales, and Hymenochaetales, therefore, a new order Xenasmatales was introduced based on both morphology and multigene phylogeny to accommodate Xenasmataceae (Luo & Zhao 2022).

During investigations on wood-inhabiting fungi in the Yunnan Province of China, three potentially new species were collected. To clarify the taxonomic position of the three unidentified wood-inhabiting fungi, phylogenetic analyses based on the ITS and nLSU sequences and morphological studies were carried out.

## Materials and methods

### *Morphological studies*

The specimens studied are deposited at the herbarium of Southwest Forestry University (SWFC), Kunming, Yunnan Province, P.R. China (Herbarium numbers: *Ceraceomyces yunnanensis*: SWFCF00004112, SWFCF00018930, SWFCF00018992, SWFCF00019024, SWFCF00019054, SWFCF00019070; *Stereopsis yunnanensis*: SWFCF00003171, SWFCF00003277, SWFCF00003767; *Xenasmatella bambusicola*: SWFCF00010985). Macromorphological descriptions are based on field notes. Special color terms follow Petersen (1996). Micromorphological 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 nonamyloid and non-dextrinoid, 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. ITS region was amplified with primer pair 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. 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. The PCR products were purified and directly sequenced at Kunming Tsingke Biological Technology Limited (Yunnan Province, P.R. China). All of the newly generated sequences were deposited in NCBI GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>) (Table 1).

**TABLE 1.** List of species, specimens and the corresponding GenBank accession numbers of the sequences used in this study.

Species name	Sample no.	GenBank accession no.		References
		ITS	nLSU	
<i>Albatrellus subrubescens</i>	PV 154-95	AF506395	AF506395	Larsson & Larsson (2003)
<i>Aleurocystidiellum disciforme</i>	NH 13003	AF506402	AF506402	Larsson & Larsson (2003)
<i>A. subcruentatum</i>	NH 12874	AF506403	AF506403	Larsson & Larsson (2003)
<i>Aleurodiscus amorphus</i>	KHL 4240	AF506397	AF506397	Larsson & Larsson (2003)
<i>Amphinema byssoides</i>	EL 11-98	AY463375	AY586626	Larsson <i>et al.</i> (2004)
<i>Amylocorticium subincarnatum</i>	AS-95	AY463377	AY586628	Larsson <i>et al.</i> (2004)
<i>Amylostereum areolatum</i>	NH 8041	AF506405	AF506405	Larsson & Larsson (2003)
<i>Am. laevigatum</i>	NH 12863	AF506407	AF506407	Larsson & Larsson (2003)
<i>Amylocystis lapponica</i>	KHL 11755	EU118603	EU118603	Larsson (2007)
<i>Arrhenia retirugis</i>	EL 76/03	EU118604	EU118604	Larsson (2007)
<i>Athelia pyriformis</i>	Hjm 18581	EU118605	EU118605	Larsson (2007)
<i>At. epiphylla</i>	EL12-98	AY463382	AY586633	Larsson <i>et al.</i> (2004)
<i>Auriscalpium vulgare</i>	EL 33-95	AF506375	AF506375	Larsson & Larsson (2003)
<i>Bondarcevomyces taxi</i>	TAA 166337	AY463386	AY586637	Larsson <i>et al.</i> (2004)
<i>Ceraceomyces borealis</i>	KHL 8432	EU118610	EU118610	Larsson (2007)
<i>Ce. tessulatus</i>	KHL 8474	AY463391	AY586642	Larsson <i>et al.</i> (2004)
<i>Ce. violascens</i>	KHL 11169	EU118611	EU118612	Larsson (2007)
<b><i>Ce. yunnanensis</i></b>	<b>CLZhao 19070</b>	<b>OQ132516</b>	<b>OQ147006</b>	<b>This study</b>
<b><i>Ce. yunnanensis</i></b>	<b>CLZhao 4112</b>	<b>OQ132517</b>	—	<b>This study</b>
<b><i>Ce. yunnanensis</i></b>	<b>CLZhao 18930</b>	<b>OQ132518</b>	—	<b>This study</b>
<b><i>Ce. yunnanensis</i></b>	<b>CLZhao 18992</b>	<b>OQ132519</b>	<b>OQ147003</b>	<b>This study</b>
<b><i>Ce. yunnanensis</i></b>	<b>CLZhao 19024</b>	<b>OQ132520</b>	<b>OQ147004</b>	<b>This study</b>
<b><i>Ce. yunnanensis</i></b>	<b>CLZhao 19054</b>	<b>OQ132521</b>	<b>OQ147005</b>	<b>This study</b>
<i>Ceriporia reticulata</i>	KHL 11981	EU118613	EU118614	Larsson (2007)
<i>Chaetodermella luna</i>	NH 8482	EU118615	EU118615	Larsson (2007)
<i>Chondrostereum purpureum</i>	EL59-97	AY463393	AY586644	Larsson <i>et al.</i> (2004)
<i>Clavulina cinerea</i>	KHL 11694	EU118616	EU118616	Larsson (2007)
<i>Cl.cristata</i>	EL95-97	AY463398	AY586648	Larsson <i>et al.</i> (2004)
<i>Columnocystis abietina</i>	KHL 12474	EU118619	EU118619	Larsson (2007)
<i>Coronicium alboglaucum</i>	NH 4208	AY463400	AY586650	Larsson <i>et al.</i> (2004)
<i>Cristinia helvetica</i>	Kristiansen s.n.	EU118620	EU118620	Larsson (2007)
<i>Cystidiodontia lamifera</i>	KHL 13057	EU118622	EU118622	Larsson (2007)
<i>Cystostereum murrayi</i>	KHL 12496	EU118623	EU118623	Larsson (2007)
<i>Dacryobolus karstenii</i>	KHL 11162	EU118624	EU118624	Larsson (2007)

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TABLE 1. (Continued)

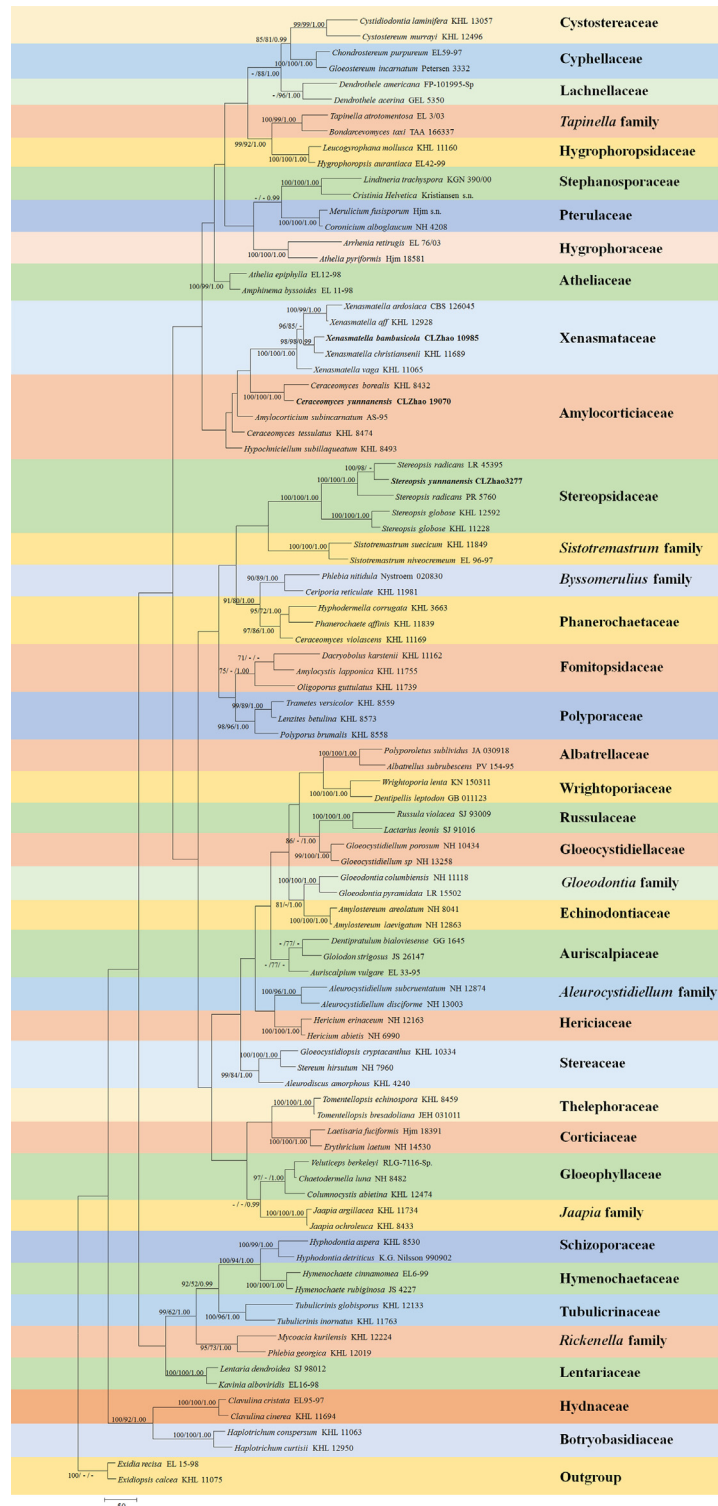
Species name	Sample no.	GenBank accession no.		References
		ITS	nLSU	
<i>Dendrothele acerina</i>	GEL 5350	—	AJ406581	Langer <i>et al.</i> (2001)
<i>D. americana</i>	FP-101995-Sp.	—	AY293178	Binder <i>et al.</i> (2005)
<i>Dentipellis leptodon</i>	GB 011123	EU118625	EU118625	Larsson (2007)
<i>Dentipratulum bialovisence</i>	GG 1645	AF506389	AF506389	Larsson & Larsson (2003)
<i>Erythricium laetum</i>	NH 14530	AY463407	AY586655	Larsson <i>et al.</i> (2004)
<i>Exidia recisa</i>	EL 15-98	AF347112	AF347112	Larsson <i>et al.</i> (2004)
<i>Exidiopsis calcea</i>	KHL 11075	AY463406	AY586654	Larsson <i>et al.</i> (2004)
<i>Gloeocystidiellum porosum</i>	NH 10434	AF310094	AF310094	Larsson & Hallenberg (2001)
<i>G. sp</i>	NH 13258	AF310089	AF310089	Larsson & Hallenberg (2001)
<i>Gloeocystidiopsis cryptacanthus</i>	KHL 10334	AF506442	AF506442	Larsson & Larsson (2003)
<i>Gloeodontia columbiensis</i>	NH 11118	AF506444	AF506444	Larsson & Larsson (2003)
<i>Gl. pyramidata</i>	LR 15502	AF506446	AF506446	Larsson & Larsson (2003)
<i>Gloeostereum incarnatum</i>	Petersen 3332	AF141637	AF141637	Parmasto & Hallenberg (2000)
<i>Gloiodon strigosus</i>	JS 26147	AF506449	AF506449	Larsson & Larsson (2003)
<i>Haplotrichum conspersum</i>	KHL 11063	AY463409	AY586657	Larsson <i>et al.</i> (2004)
<i>Haplotrichum curtisii</i>	KHL 12950	EU118629	EU118629	Larsson (2007)
<i>Hericium abietis</i>	NH 6990	AF506456	AF506456	Larsson & Larsson (2003)
<i>He. erinaceum</i>	NH 12163	AF506460	AF506460	Larsson & Larsson (2003)
<i>Hygrophoropsis aurantiaca</i>	EL42-99	AY463411	AY586659	Larsson <i>et al.</i> (2004)
<i>Hymenochaete cinnamomea</i>	EL6-99	AY463416	AY586664	Larsson <i>et al.</i> (2004)
<i>H. rubiginosa</i>	JS 4227	AY463417	AY586665	Larsson <i>et al.</i> (2004)
<i>Hyphodermella corrugata</i>	KHL 3663	EU118630	EU118630	Larsson (2007)
<i>Hyphodontia. aspera</i>	KHL 8530	AY463427	AY586675	Larsson <i>et al.</i> (2004)
<i>Hy. detritica</i>	K.G. Nilsson 990902	DQ677507	DQ677507	Larsson (2007)
<i>Hypochniciellum subillaqueatum</i>	KHL 8493	AY463431	AY586679	Larsson <i>et al.</i> (2004)
<i>Jaapia argillacea</i>	KHL 11734	EU118636	EU118636	Larsson (2007)
<i>J. ochroleuca</i>	KHL 8433	EU118637	EU118637	Larsson (2007)
<i>Kavinia alboviridis</i>	EL16-98	—	AY463434	Larsson <i>et al.</i> (2004)
<i>Lactarius leonis</i>	SJ 91016	AF506411	AF506411	Larsson & Larsson (2003)
<i>Laetisaria fuciformis</i>	Hjm 18391	EU118639	EU118639	Larsson (2007)
<i>Lentaria dendroidea</i>	SJ 98012	EU118640	EU118641	Larsson (2007)
<i>Lenzites betulina</i>	KHL 8573	AY463436	AY586683	Larsson <i>et al.</i> (2004)
<i>Leucogyrophana mollusca</i>	KHL 11160	EU118643	EU118643	Larsson (2007)
<i>Lindtneria trachyspora</i>	KGN 390/00	EU118646	EU118646	Larsson (2007)
<i>Merulicium fusisporum</i>	Hjm s.n.	EU118647	EU118647	Larsson (2007)
<i>Mycoacia kurilensis</i>	KHL 12224	EU118649	EU118649	Larsson (2007)
<i>Oligoporus guttulatus</i>	KHL 11739	EU118650	EU118650	Larsson (2007)
<i>Phanerochaete affinis</i>	KHL 11839	EU118652	EU118652	Larsson (2007)
<i>Phlebia georgica</i>	KHL 12019	DQ873645	DQ873645	Larsson <i>et al.</i> (2006)
<i>Ph. nitidula</i>	Nystroem 020830	EU118655	EU118655	Larsson (2007)
<i>Polyporoletus sublividus</i>	JA 030918	DQ389663	DQ389663	Unpublished
<i>Polyporus brumalis</i>	KHL 8558	AF347108	AF347108	Larsson <i>et al.</i> (2004)
<i>Russula violacea</i>	SJ 93009	AF506465	AF506465	Larsson & Larsson (2003)
<i>Sistotremastrum niveocremaum</i>	EL 96-97	AF347094	AF347094	Larsson <i>et al.</i> (2004)

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TABLE 1. (Continued)

Species name	Sample no.	GenBank accession no.		References
		ITS	nLSU	
<i>S. suecicum</i>	KHL 11849	EU118666	EU118667	Larsson (2007)
<i>Stereopsis radicans</i>	PR 5760	JN649370	JN649370	Sjökvisst <i>et al.</i> (2012)
<i>St. radicans</i>	LR 45395	JN649371	JN649371	Sjökvisst <i>et al.</i> (2012)
<i>St. globosa</i>	KHL 11228	JN649330	JN649330	Sjökvisst <i>et al.</i> (2012)
<i>St. globosa</i>	KHL 12592	JN649331	JN649331	Sjökvisst <i>et al.</i> (2012)
<i>St. yunnanensis</i>	<b>CLZhao 3277</b>	<b>MK268905</b>	<b>OQ147007</b>	<b>This study</b>
<i>St. yunnanensis</i>	<b>CLZhao 3171</b>	<b>OQ148432</b>	—	<b>This study</b>
<i>St. yunnanensis</i>	<b>CLZhao 3767</b>	<b>OQ148431</b>	<b>OQ147008</b>	<b>This study</b>
<i>Stereum hirsutum</i>	NH 7960	AF506479	AF506479	Larsson & Larsson (2003)
<i>Tapinella atrotomentosa</i>	EL 3/03	EU118673	EU118673	Larsson (2007)
<i>Tomentellopsis bresadoliana</i>	JEH 031011	EU118674	EU118674	Larsson (2007)
<i>To. echinospora</i>	KHL 8459	AY463472	AY586718	Larsson <i>et al.</i> (2004)
<i>Trametes suaveolens</i>	CBS 279.28	MH855012	MH866480	Vu <i>et al.</i> (2019)
<i>Tr. versicolor</i>	KHL 8559	AF347107	AF347107	Larsson <i>et al.</i> (2004)
<i>Tubulicrinis globisporus</i>	KHL 12133	DQ873655	DQ873655	Larsson <i>et al.</i> (2006)
<i>Tu. inornatus</i>	KHL 11763	DQ873659	DQ873659	Larsson <i>et al.</i> (2006)
<i>Veluticeps berkeleyi</i>	RLG-7116-Sp.	—	AY293219	Binder <i>et al.</i> (2005)
<i>Wrightoporia lenta</i>	KN 150311	AF506489	AF506489	Larsson & Larsson (2003)
<i>Xenasmattella ailaoshanensis</i>	CLZhao 4839	MN487106	—	Huang <i>et al.</i> (2019)
<i>X. ailaoshanensis</i>	CLZhao 3895	MN487105	—	Huang <i>et al.</i> (2019)
<i>X. aff.</i>	KHL 12928	EU118658	EU118658	Larsson (2007)
<i>X. ardosiacae</i>	CBS 126045	MH864060	MH875515	Vu <i>et al.</i> (2019)
<i>X. bambusicola</i>	<b>CLZhao 10985</b>	<b>OQ371469</b>	<b>OQ147009</b>	<b>This study</b>
<i>X. borealis</i>	UC 2022974	KP814210	—	Rosenthal <i>et al.</i> (2017)
<i>X. borealis</i>	UC 2023132	KP814274	—	Rosenthal <i>et al.</i> (2017)
<i>X. christiansenii</i>	KHL 11689	EU118659	EU118659	Larsson (2007)
<i>X. christiansenii</i>	TASM YGG 36	MT526342	—	Gafforov <i>et al.</i> (2020)
<i>X. christiansenii</i>	TASM YGG 26	MT526341	—	Gafforov <i>et al.</i> (2020)
<i>X. gossypina</i>	CLZhao 8233	MW545957	—	Zong & Zhao (2021)
<i>X. gossypina</i>	CLZhao 4149	MW545958	—	Zong & Zhao (2021)
<i>X. nigroidea</i>	CLZhao 18300	OK045679	OK045677	Luo & Zhao (2022)
<i>X. nigroidea</i>	CLZhao 18333	OK045680	OK045678	Luo & Zhao (2022)
<i>X. rhizomorpha</i>	CLZhao 9847	MT832953	—	Zong <i>et al.</i> (2021)
<i>X. rhizomorpha</i>	CLZhao 9156	MT832954	—	Zong <i>et al.</i> (2021)
<i>X. tenuis</i>	CLZhao 11258	MT832959	—	Zong <i>et al.</i> (2021)
<i>X. tenuis</i>	CLZhao 4528	MT832960	—	Zong <i>et al.</i> (2021)
<i>X. tulasnelloidea</i>	HHB-19281	MW740272	—	Unpublished
<i>X. tulasnelloidea</i>	FRDBI 20222550	OL828785	—	Unpublished
<i>X. vaga</i>	KHL 11065	EU118660	EU118661	Larsson (2007)
<i>X. vaga</i>	BHI-F 160a	MF161185	—	Larsson (2007)
<i>X. wuliangshanensis</i>	CLZhao 4080	MW545962	—	Zong & Zhao (2021)
<i>X. wuliangshanensis</i>	CLZhao 4380	MW545963	—	Zong & Zhao (2021)
<i>X. xinpingensis</i>	CLZhao 2216	MT832961	—	Zong <i>et al.</i> (2021)
<i>X. xinpingensis</i>	CLZhao 2467	MT832962	—	Zong <i>et al.</i> (2021)

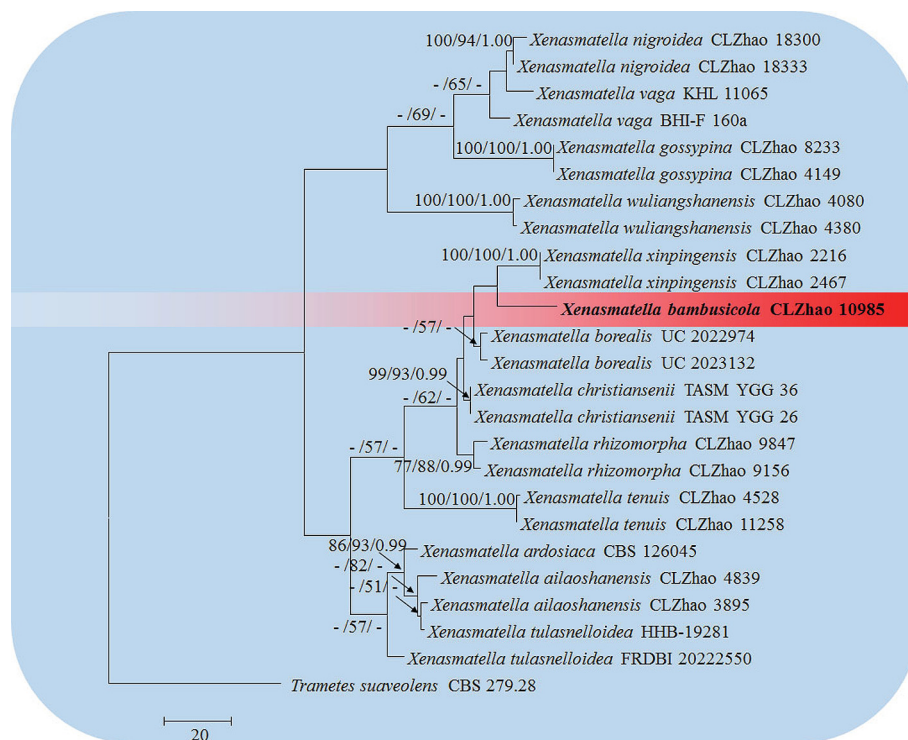
Sequencher 4.6 (GeneCodes, Ann Arbor, MI, USA) was used to edit the DNA sequences. Sequences were aligned in MAFFT 7 (<http://mafft.cbrc.jp/alignment/server/>) using the “G-INS-i” strategy and manually adjusted in BioEdit (Hall 1999). The sequence alignment was deposited in TreeBase (submission ID 30000). Sequences of *Exidia recisa* (ITS: AF347112 , LSU: AF347112) (Ditmar) Fr. and *Exidiopsis calcea* (ITS: AY463406, LSU: AY586654) (Pers.) K. Wells obtained from GenBank were used as an outgroup to root the tree following Larsson (2007) (Fig. 1). Sequence of *Trametes suaveolens* (L.) Fr. obtained from GenBank was used as the outgroup to root the tree following a previous study (Luo & Zhao 2022) (Fig. 2).



**FIGURE 1.** Maximum Parsimony strict consensus tree illustrating the phylogeny of three new species and related species based on ITS+nLSU sequences. Branches are labelled with maximum likelihood bootstrap equal to or greater than 70%, parsimony bootstrap proportions equal to or greater than 50% and Bayesian posterior probabilities equal to or greater than 0.95, respectively, Scale bar = 50.

Maximum parsimony analysis was applied to the ITS and nLSU datasets. Approaches to phylogenetic analyses followed Zhao and 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 1,000 random sequence additions. Max-trees were set to 5,000, 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. Datamatrix was also analyzed using Maximum Likelihood (ML) approach with RAxML-HPC2 through the Cipres Science Gateway (www.phylo.org, Miller *et al.* 2009). Branch support (BS) for ML analysis was determined by 1,000 bootstrap replicates.

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 3.1.2 (Ronquist & Huelsenbeck 2003). Four Markov chains run for 2 runs from random starting trees for 3.5 million generations for ITS+nLSU (Fig. 1), 400 thousand generations for ITS (Fig. 2) and trees were sampled every 100 generations. The first one-fourth generations were discarded as burn-in. The majority rule consensus tree of all remaining trees was calculated. Branches were considered as significantly supported if they received maximum likelihood bootstrap (BS) equal to or greater than symbol 70%, maximum parsimony bootstrap (BT) equal to or greater than symbol 50%, or Bayesian posterior probabilities (BPP) equal to or greater than symbol 0.95.



**FIGURE 2.** Maximum Parsimony strict consensus tree illustrating the phylogeny of one new species and related species in *Xenasmatella* based on ITS sequences. Branches are labelled with maximum likelihood bootstrap equal to or greater than 70%, parsimony bootstrap proportions equal to or greater than 50% and Bayesian posterior probabilities equal to or greater than 0.95, respectively, Scale bar = 20.

## Results

### Molecular phylogeny

The combined ITS+nLSU dataset (Fig. 1) comprises sequences from 93 specimens and 91 species. The dataset had an aligned length of 1731 characters, of which 739 characters were constant, 274 were variable and parsimony-uninformative and 718 were parsimony-informative. Maximum parsimony analysis yielded one equally parsimonious tree (TL = 7158, CI = 0.250, HI = 0.750, RI = 0.407, RC = 0.102). Best model suggested by MrModeltest and applied in BI was GTR+I+G. Bayesian analysis and ML analysis resulted in a similar topology as MP analysis; BI had the average standard deviation of split frequencies = 0.006466, and the effective sample size (ESS) across the two runs is

double that of the average ESS (avg ESS) = 290. The phylogeny reconstruction based on the ITS+nLSU dataset (Fig. 1) demonstrated that three new species nested into families Amylocorticiaceae, Stereopsidaceae and Xenasmataceae, in which *C. yunnanensis* clustered into the genus *Ceraceomyces* sister to *C. borealis*; and *S. yunnanensis* clustered into genus *Stereopsis* grouped with *S. radicans*.

The ITS dataset (Fig. 2) comprises sequences from 25 specimens and 14 species. The dataset had an aligned length of 651 characters, of which 410 characters were constant, 76 were variable and parsimony-uninformative and 165 were parsimony-informative. Maximum parsimony analysis yielded 8 equally parsimonious trees (TL = 456, CI = 0.675, HI = 0.325, RI = 0.792, RC = 0.535). The best model for the ITS+28S dataset estimated and applied in the Bayesian analysis was GTR+I+G. Bayesian analysis and ML analysis resulted in a similar topology as MP analysis; BI had the average standard deviation of split frequencies = 0.009316, and the effective sample size (ESS) across the two runs is double that of the average ESS (avg ESS) = 203.5. The ITS dataset of genus *Xenasmatella* demonstrated that *X. bambusicola* formed a distinct clade sister to *X. xinpingsensis* that is similar to a previous study.

## Taxonomy

*Ceraceomyces yunnanensis* Q. Yuan & C.L. Zhao, *sp. nov.* Figs. 3, 4  
Mycobank no.: MB 846982

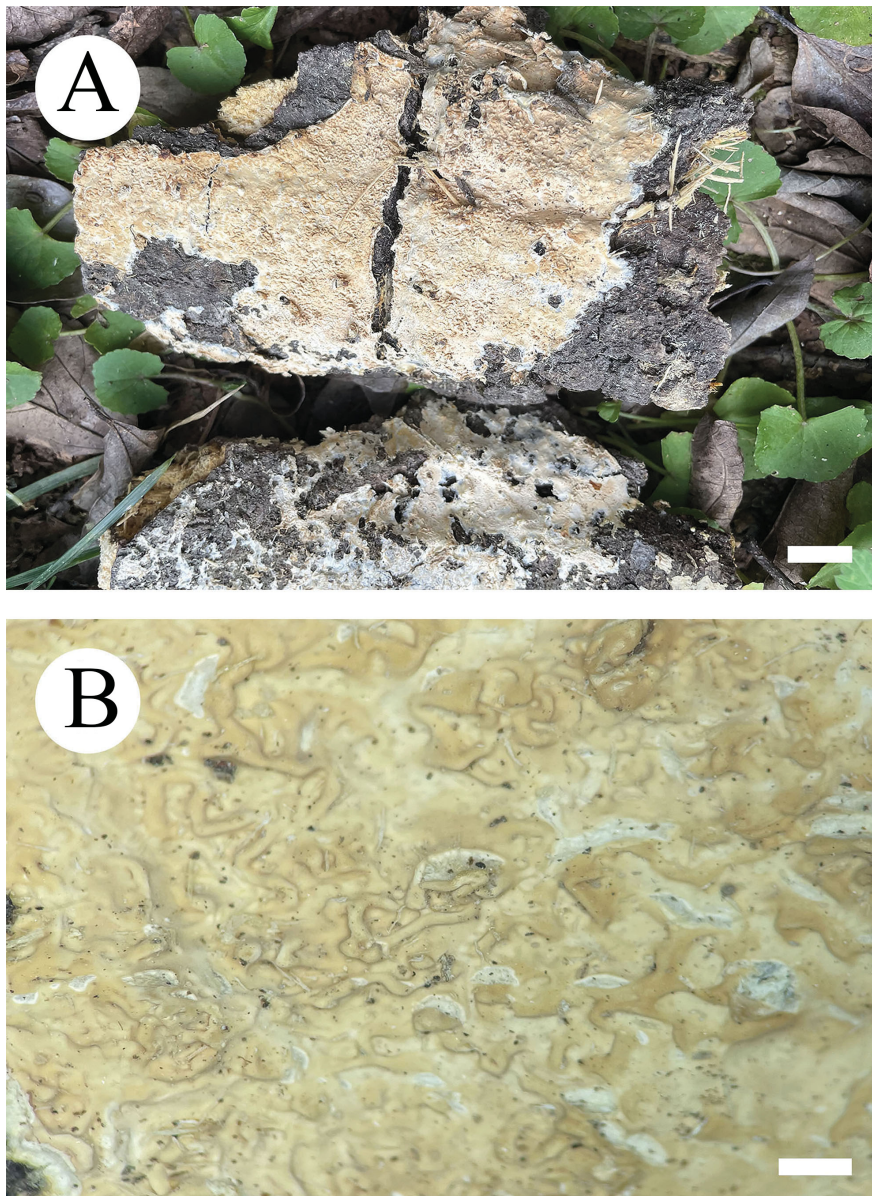
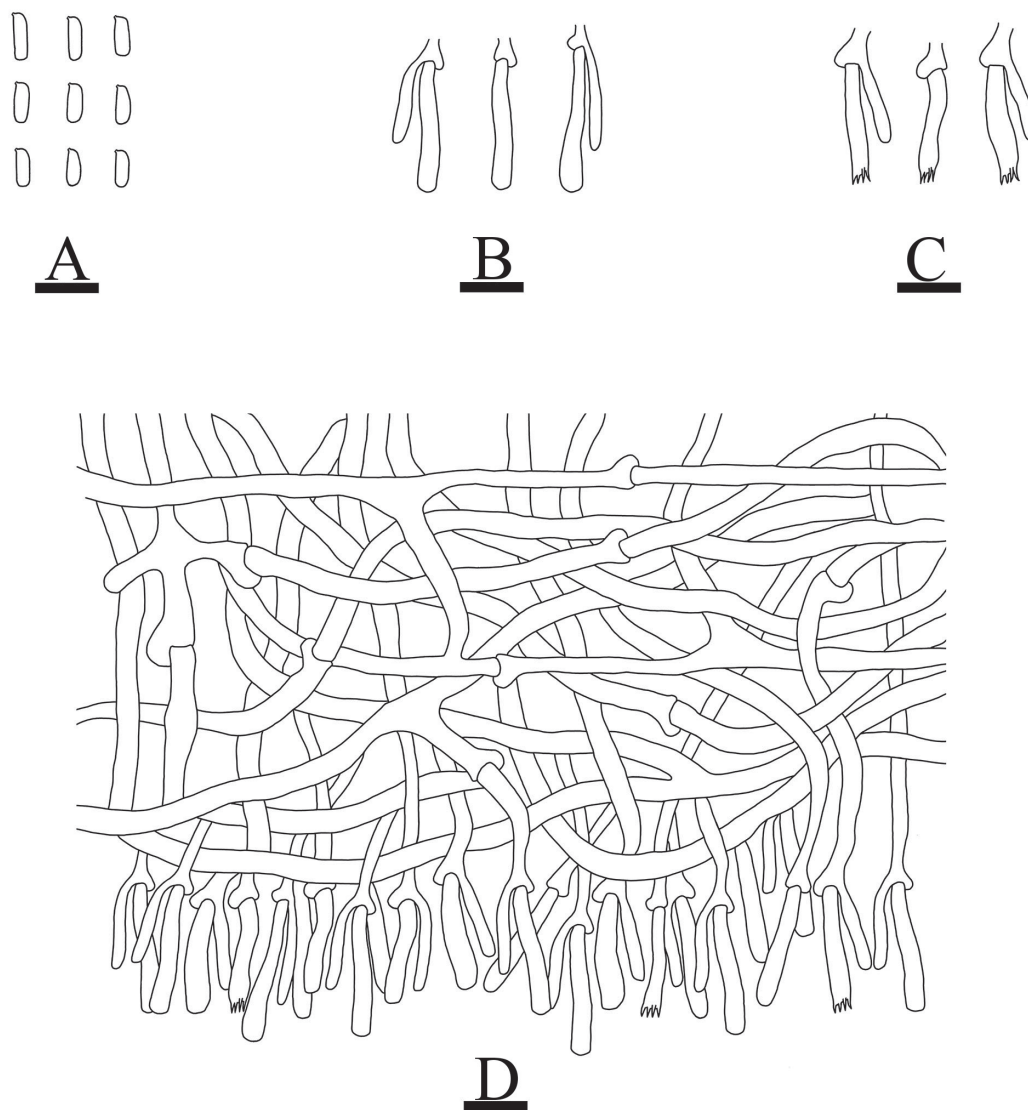


FIGURE 3. Basidiomata of *Ceraceomyces yunnanensis* (holotype). Bars: A = 1 cm, B = 1 mm.





**FIGURE 4.** Microscopic structures of *Ceraceomyces yunnanensis* (holotype). A Basidiospores. B basidioles. C Basidia. D A section of hymenium. Bars: A = 5  $\mu\text{m}$ , B–D = 10  $\mu\text{m}$ .

**Holotype:**—CHINA, Yunnan Province, Honghe, Pingbian County, Daiweishan National Nature Reserve, E 103°42', N 22°57', elev. 2100 m, on the fallen branch of angiosperm, 9 June 2020, CLZhao 19070 (SWFC).

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

**Fruiting body:**—Basidiomata resupinate, adnate, pellicular when fresh, membranous upon drying, without odor or taste when fresh, up to 10 cm long, 4 cm wide, 100–200  $\mu\text{m}$  thick. Hymenial surface meruliod, cream when fresh, turn to cream to slightly buff upon drying. Margin sterile, white, fimbriate.

**Hyphal structure:**—Hyphal system monomitic, generative hyphae with clamp connections, colorless, thin-walled, branched, interwoven, 2.2–6.4  $\mu\text{m}$  in diameter, IKI–, CB–; tissues unchanged in KOH.

**Hymenium:**—Cystidia and cystidioles absent; basidia narrowly clavate, in a dense palisade, with 4 sterigmata and a basal clamp connection, 14–19  $\times$  2.5–4  $\mu\text{m}$ .

**Spores:**—Basidiospores cylindrical, colorless, smooth, thin-walled, IKI–, CB–, 3–4  $\times$  1–1.5  $\mu\text{m}$ , L = 3.54  $\mu\text{m}$ , W = 1.33  $\mu\text{m}$ , Q = 2.56–2.73 (n = 180/6).

**Additional specimens examined (paratypes):**—China, Yunnan Province, Honghe, Pingbian County, Daiweishan National Nature Reserve, E 103°42', N 22°57', elev. 2100 m, on angiosperm trunk, 9 Jun 2020, CLZhao 19024 (SWFC), CLZhao 19054 (SWFC), 8 Jun 2020, CLZhao 18930 (SWFC), on fallen angiosperm branch, 9 Jun 2020, CLZhao 18992, CLZhao 6132 (SWFC); Puer, Jingdong County, Wuliangshan National Nature Reserve, E 100°08', N 24°04', elev. round to 3000 m, on the stump of angiosperm, 5 Oct 2017, CLZhao 4112 (SWFC).

*Stereopsis yunnanensis* Q. Yuan & C.L. Zhao, *sp. nov.* Figs. 5, 6  
MycoBank no.: MB 846983

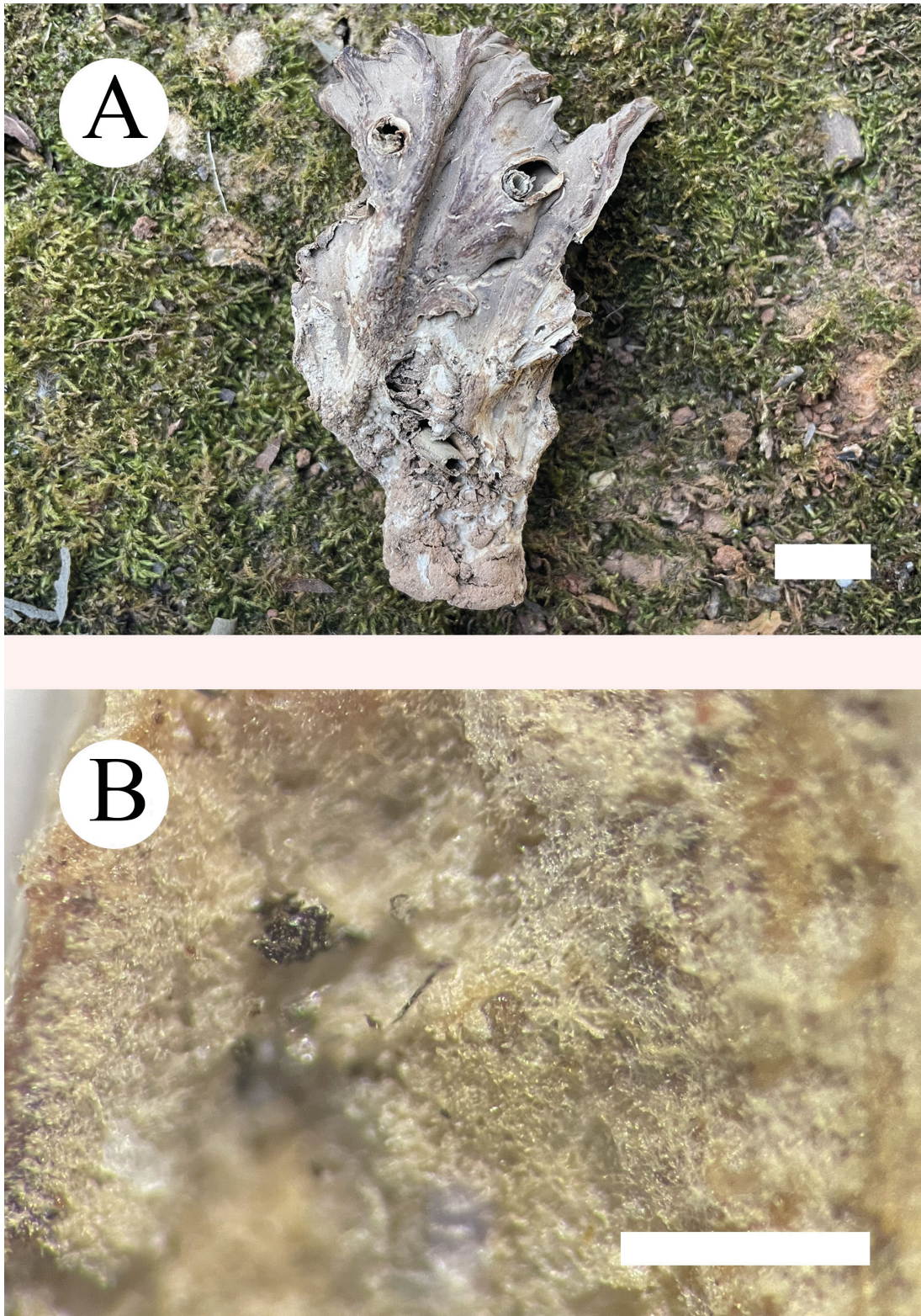


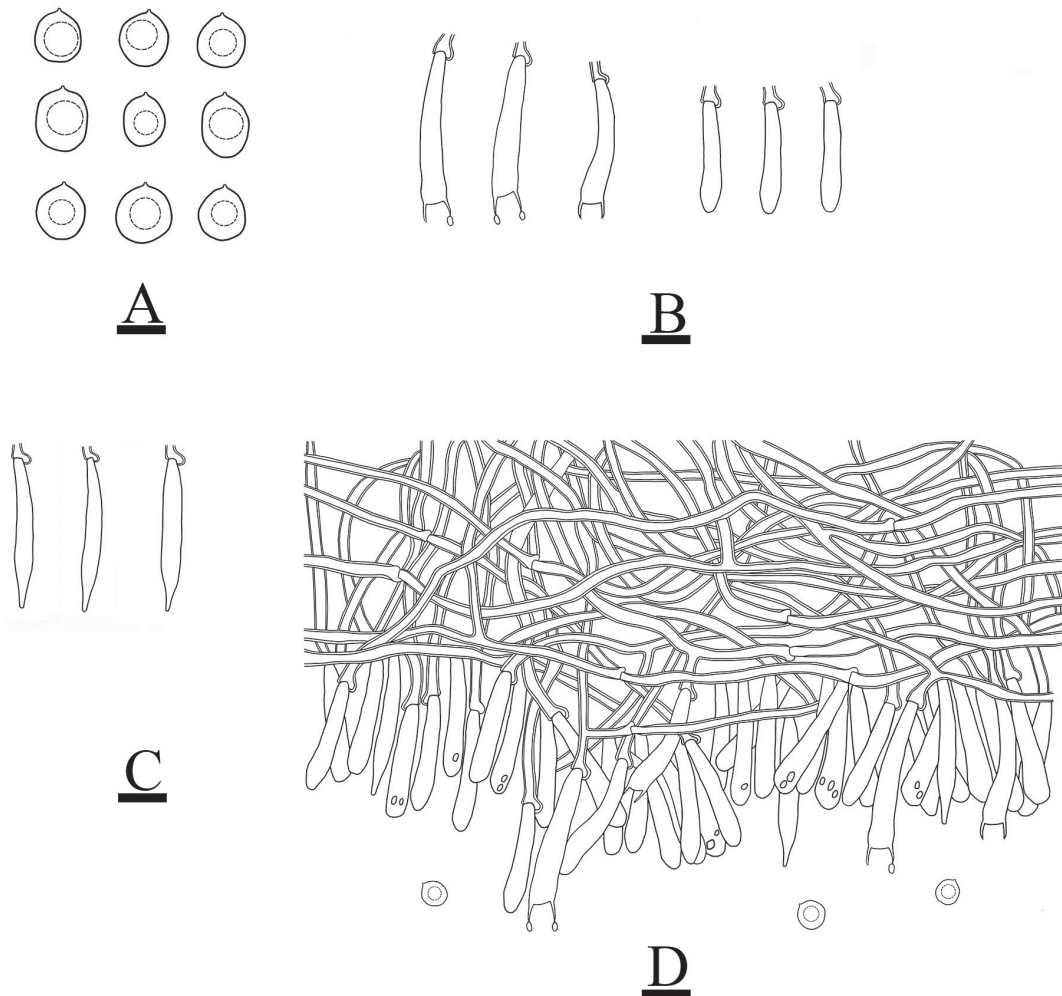
FIGURE 5. Basidiomata of *Stereopsis yunnanensis* (holotype). Bars: A = 1 cm, B = 1 mm.

**Holotype:**—CHINA, Yunnan Province, Puer, Jingdong County, Kongqeshan Forestry Park, E 100°08', N 24°04', elev. 3002 m, on dead bamboo, 3 October 2017, CLZhao 3767 (SWFC).

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

**Fruiting body:**—Annual, laterally stipitate, gregarious. Pilei medium-sized, infundibuliform, brittle when fresh, hard on drying, up to 3 cm long, 4 cm wide, 1.5 mm thick; fawn to isabelline when fresh, greyish brown to brown on

drying; proliferous from a central common base, usually with several laterally confluent spatulate to flabelliform or valves; the surface radially striate; margin thin, wavy. Hymenial surface stereoid, buff to clay-buff when fresh, clay-buff to slightly brown on drying. Stipe cylindrical, up to 2 cm long, up to 1 cm in diameter. Context leathery in fresh condition, corky to leathery in dried condition, up to 1 mm thick at the thickest portion of pileus, thinner at margin and thicker toward the base, buff. Odor mild when fresh, somewhat smelly when dried, or with the beef jerky flavor.



**FIGURE 6.** Microscopic structures of *Stereopsis yunnanensis* (holotype). A Basidiospores. B Basidia and basidioles. C Cystidia. D A section of hymenium. Bars: A = 5  $\mu$ m, B–D = 10  $\mu$ m.

**Hyphal structure:**—Hyphal system monomitic, hyphae thick-walled, with clamp connections, colorless, 1.5–3.5  $\mu$ m in diameter, IKI–, CB–; tissues unchanged in KOH.

**Hymenium:**—Cystidia fusiform, 34.5–39  $\times$  3.5–4.5  $\mu$ m; basidia narrowly clavate, with 2 sterigmata and a basal clamp connection, 28–35  $\times$  3.5–5.5  $\mu$ m.

**Spores:**—Basidiospores subglobose, with large droplet of oil, colorless, thin-walled, smooth, IKI–, CB–, 4.5–6.5  $\times$  4–5.5  $\mu$ m, L = 5.56  $\mu$ m, W = 4.90  $\mu$ m, Q = 1.09–1.17 (n = 60/2).

**Additional specimens examined (paratypes):**—China, Yunnan Province, Puer, Laiyanghe National Forestry Park, E 101°6', N 22°35', elev. 1207 m, on the dead bamboo, 30 Sep 2017, CLZhao 3277 (SWFC), CLZhao 3171 (SWFC).

*Xenasmatella bambusicola* Q. Yuan & C.L. Zhao, *sp. nov.* Figs. 7, 8  
MycoBank no.: MB 846984

**Holotype:**—CHINA, Yunnan Province, Wenshan, Xiaoqiaogou National Nature Reserve, E 104°41', N 23°21', elev. 1900 m, on dead bamboo, 15 January 2019, CLZhao 10985 (SWFC).

**Etymology:**—*Bambusicola* (Lat.)—refers to the host from which the holotype occurrence on dead bamboo.

**Fruiting body:**—Basidiomata resupinate, adnate, thin, very hard to separate from substrate, membranous, without

odor or taste when fresh, up to 11 cm long, 1 cm wide, 30–80  $\mu\text{m}$  thick. Hymenial surface arachnoid, white when fresh, white to greyish when dry. Sterile margin thin, white.

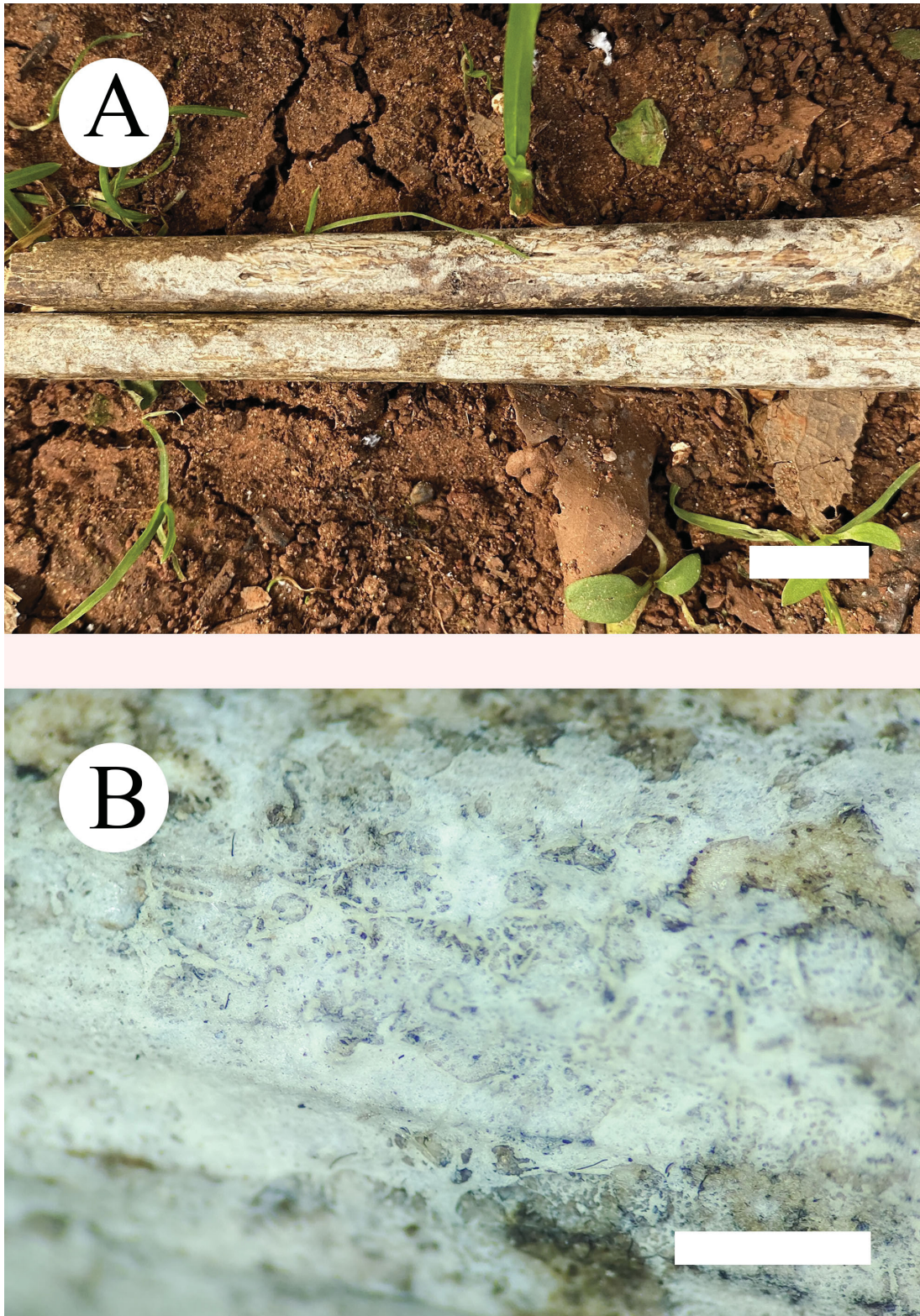
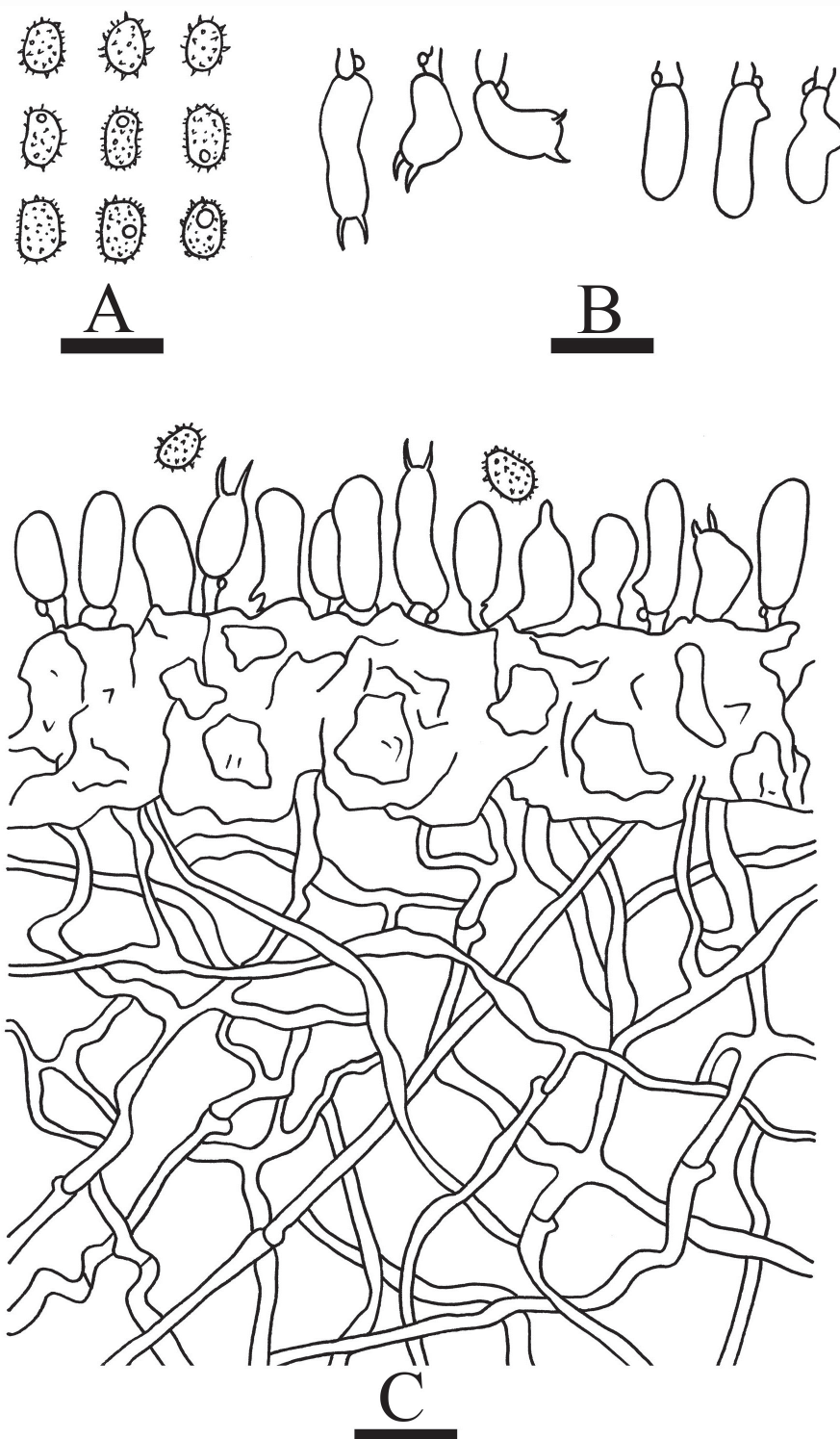


FIGURE 7. Basidiomata of *Xenasmatella bambusicola* (holotype). Bars: A = 1 cm, B = 1 mm.

**Hyphal structure:**—Hyphal system monomitic, generative hyphae with clamp connections, colorless, thin-walled, 1.5–2  $\mu\text{m}$  in diameter, IKI–, CB–; tissues unchanged in KOH.

**Hymenium:**—Cystidia and cystidioles absent; basidia pleural, clavate, with 2 sterigmata and a basal clamp connection, 9–12.5  $\times$  3.5–5  $\mu\text{m}$ .

**Spores:**—Basidiospores ellipsoid, colorless, thin-walled, warted, with one oil drop, IKI–, CB–,  $3\text{--}7 \times 2\text{--}4.5\mu\text{m}$ ,  $L = 5.45 \mu\text{m}$ ,  $W = 3.65 \mu\text{m}$ ,  $Q = 1.5$  ( $n = 30/1$ ).



**FIGURE 8.** Microscopic structures of *Xenasmatella bambusicola* (holotype). A Basidiospores. B Basidia and basidioles. C A section of hymenium. Bars: A–C = 10  $\mu\text{m}$ .

## Discussion

In the present study, *Ceraceomyces yunnanensis*, *Stereopsis yunnanensis* and *Xenasmatella bambusicola* are described as new species based on phylogenetic analyses and morphological characteristics.

Based on the multi-gene phylogenetic analyses of nLSU, SSU, 5.8s, RPB1, RPB2, and *tef1- $\alpha$* , an outline of all genera of Basidiomycota including 241 families, 68 orders, 18 classes and four subphyla, revealed that *Ceraceomyces* clustered into family Amylocorticiaceae Jülich (Amylocorticiales); *Stereopsis* grouped into family Stereopsidaceae (Stereopsidales); and *Xenasmatella* grouped into family Xenasmataceae (He *et al.* 2019). In the present study (Figs. 1, 2), three new species nested into families Amylocorticiaceae, Stereopsidaceae and Xenasmataceae (Agaricomycetes), in which *C. yunnanensis* clustered into the genus *Ceraceomyces* sister to *C. borealis*; *S. yunnanensis* clustered into genus *Stereopsis* with *S. radicans*; and *X. bambusicola* nested into the genus *Xenasmatella* sister to *X. xinpingensis*. However, morphologically *C. borealis* differs from *C. yunnanensis* by its whitish basidiomata and larger basidiospores ( $6\text{--}8 \times 1.8\text{--}2 \mu\text{m}$ , Hjortstam *et al.* 1977); *S. radicans* is separated from *S. yunnanensis* by both larger basidia ( $40\text{--}70 \times 5\text{--}8 \mu\text{m}$ ) and basidiospores ( $7.5\text{--}10 \times 7\text{--}7.5 \mu\text{m}$ , Reid 1965); and *X. xinpingensis* differs from *X. bambusicola* by the ceraceous basidiomata and 4-sterigmatis basidia (Zong *et al.* 2021).

Morphologically, *Ceraceomyces atlanticus* K.H. Larss. & Chikowski, *C. tessulatus* (Cooke) Jülich and *C. corymbatus* (G. Cunn.) Stalpers, are similar to *C. yunnanensis* by having the resupinate basidiomata with the membranous to subceraceous hymenophore. However, *Ceraceomyces atlanticus* differs in its cracked hymenophore and wider basidiospores ( $4\text{--}5 \times 2\text{--}2.5 \mu\text{m}$ , Chikowski *et al.* 2017); *C. tessulatus* differs in its thick hymenium and obliquely ellipsoid to pip-shaped basidiospores (Hjortstam *et al.* 1977); and *C. corymbatus* differs in its arachnoid hymenophore and wider basidiospores ( $8\text{--}9.5 \times 3.5\text{--}4.5 \mu\text{m}$ , Stalpers 1985). *Ceraceomyces yunnanensis* resembles *C. americanus* Nakasone, C.R. Bergman & Burds, *C. bizonatus* Dhingra & Avn.P. Singh and *C. simulans* (Berk. & Broome) Hjortstam in having a monomitic hyphal system. However, *Ceraceomyces americanus* is distinguished from *C. yunnanensis* by having a rimous hymenial surface and larger basidia ( $22\text{--}36 \times 4\text{--}5 \mu\text{m}$ , Nakasone *et al.* 1994). *Ceraceomyces bizonatus* differs in having a yellowish brown hymenial surface and wider basidiospores ( $2.5\text{--}3.3 \times 1.8\text{--}2.2 \mu\text{m}$ , Dhingra & Singh 2008). *Ceraceomyces simulans* differs in having ochraceous to pale brown basidiomata and wider basidia ( $17\text{--}20 \times 4\text{--}5 \mu\text{m}$ , Hjortstam 1989).

*Stereopsis burtianum* (Peck) D.A. Reid, *S. cartilaginea* (Masse) D.A. Reid, *S. reidii* (Losi & A. Gennari), and *S. vitellina* (S. Lundell) D.A. Reid, are similar to *S. yunnanensis* in having the stipitate infundibuliform to spatulate basidiomata. However, *Stereopsis burtianum* differs in its submembranous basidiomata and narrower basidiospores ( $3.5\text{--}4.5 \times 2.5\text{--}3.5 \mu\text{m}$ , Reid 1965); *S. cartilaginea* in its waxy hymenial surface and larger basidia ( $48\text{--}65 \times 7\text{--}8 \mu\text{m}$ , Reid 1965); and *S. vitellina* in its glabrous hymenium and smaller basidiospores ( $3\text{--}4 \times 2.2\text{--}2.5 \mu\text{m}$ , Eriksson 1984). *Stereopsis yunnanensis* resembles *S. sparassoides* (D.A. Reid, K.S. Thind & Adlakha) D.A. Reid and *S. hiscens* (Berk. & Rav.) Reid in having a monomitic hyphal system. However, *Stereopsis sparassoides* is distinguished from *S. yunnanensis* by having the violet-grey hymenial surface and narrower basidiospores ( $4.5\text{--}5.5 \times 2\text{--}4 \mu\text{m}$ , Reid 1965); and *S. hiscens* differs in having the dark bluish-grey hymenial surface and larger basidia ( $90\text{--}150 \times 7\text{--}10 \mu\text{m}$ , Reid 1965).

*Xenasmatella bambusicola* is similar to *X. californica* (Liberta) Hjortstam, *X. christiansenii* (Parmasto) Stalpers and *X. wuliangshanensis* in having its membranous basidiomata. However, *Xenasmatella californica* can be distinguished by the bluish-gray hymenial surface and wider basidia ( $7\text{--}13 \times 5\text{--}6 \mu\text{m}$ , Liberta 1965); *X. christiansenii* differs from *X. bambusicola* by having the effused basidiomata with radially arranged rhizomorphs and wider basidia ( $10\text{--}18 \times 5\text{--}7 \mu\text{m}$ , Bernicchia & Gorjón 2010); and *X. wuliangshanensis* differs from *X. bambusicola* by having a clay-pink sterile margin and 4-sterigmatis basidia (Zong & Zhao 2021). *Xenasmatella bambusicola* is similar to *X. nigroidea*, *X. odontioidea* Ryvarden & Liberta and *X. vaga* (Fr.) Stalpers in having the ellipsoid basidiospores. However, *Xenasmatella nigroidea* differs from *X. bambusicola* by its black sterile margin and larger basidia ( $12\text{--}18 \times 4.5\text{--}6 \mu\text{m}$ , Luo & Zhao 2022); *X. odontioidea* can be distinguished from *X. bambusicola* by its colliculosa hymenial surface and larger basidia ( $17.5\text{--}20 \times 4.5\text{--}5 \mu\text{m}$ , Luo & Zhao 2022) and smaller basidiospores ( $2.5\text{--}3.5 \times 2\text{--}3 \mu\text{m}$ , Ryvarden & Liberta 1978); and *X. vaga* differs from *X. bambusicola* by its hymenial surface reddish in KOH and larger basidia ( $15\text{--}20 \times 5\text{--}6 \mu\text{m}$ , Stalpers 1996).

The Basidiomycota constitutes a major phylum of the kingdom Fungi (He *et al.* 2019, Wijayawardene *et al.* 2020). Wood-rotting fungi are an extensively studied group of Basidiomycota (Bernicchia & Gorjón 2010, Dai 2012, Gilbertson & Ryvarden 1987, Núñez & Ryvarden 2001, Ryvarden & Melo 2014, Wu *et al.* 2022), but the wood-rotting fungal diversity is still not well known in China, especially in subtropical and tropical areas, and many recently described taxa of this ecological group were from China (Bian *et al.* 2016, Guan *et al.* 2020, Huang & Zhao 2020, Ma & Zhao 2019, Zhao *et al.* 2014, Zhao *et al.* 2015, Zhao *et al.* 2016). Three new species in the present study are described based on morphological and molecular phylogenetic analyses, which are also from the subtropics. This study enriches the wood-rotting fungal diversity in China and the world.

## Acknowledgments

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