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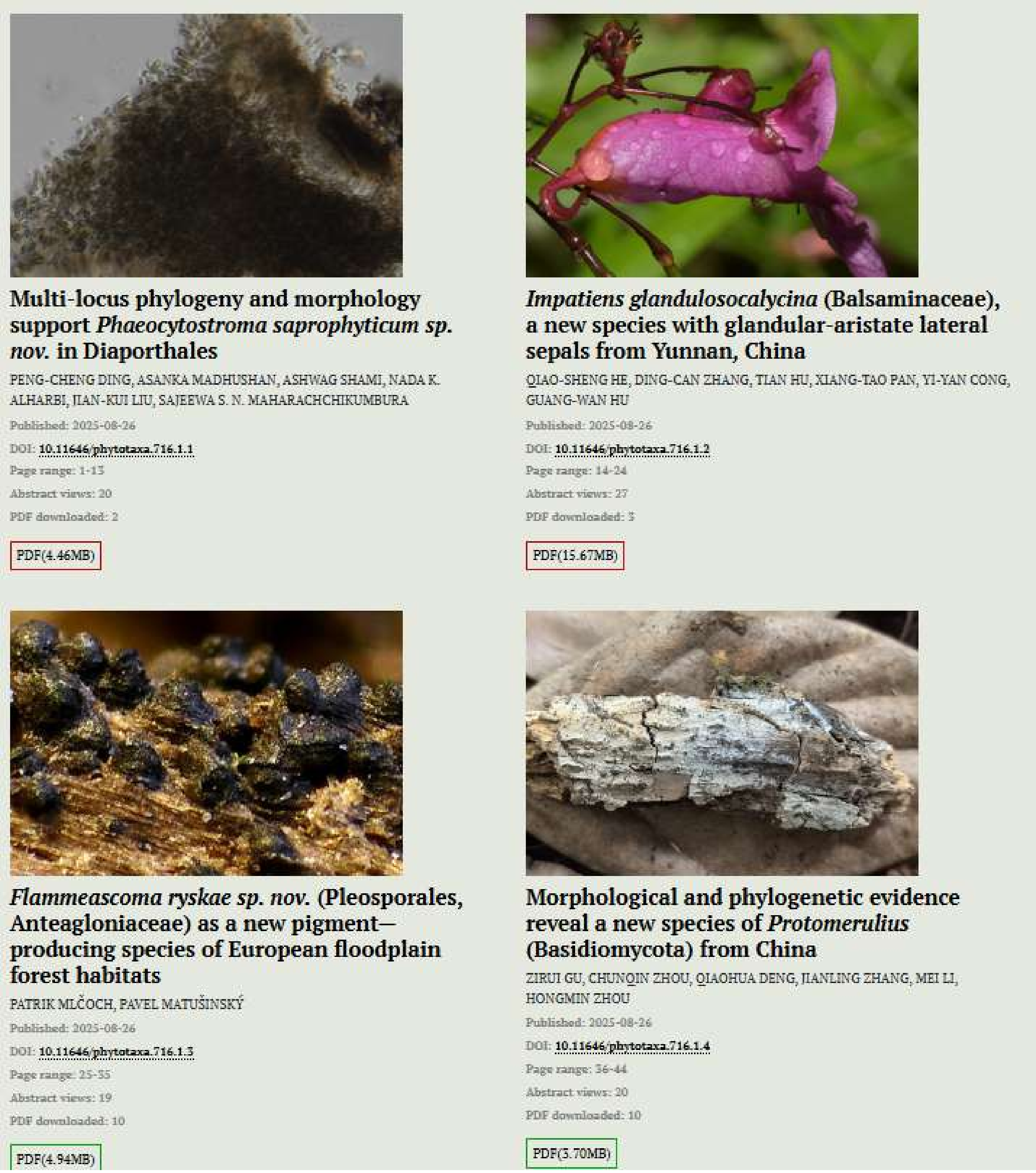
216



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PENG-CHENG DING, ASANKA MADHUSHAN, ASHWAG SHAMI, NADA K. ALHARBI, JIAN-KUI LIU, SAJEEWA S. N. MAHARACHCHIKUMBURA

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QIAO-SHENG HE, DING-CAN ZHANG, TIAN HU, XIANG-TAO PAN, YI-YAN CONG, GUANG-WAN HU

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Morphological and phylogenetic evidence reveal a new species of *Protomerulius* (Basidiomycota) from China

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Morphological and phylogenetic evidence reveal a new species of *Protomerulius* (Basidiomycota) from China

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Abstract

The corticioid fungi, distinguished by their longitudinally septate basidia, represent a unique group within the Auriculares taxa. A new corticioid fungus, *Protomerulius wumengshanensis* was discovered in Yunnan Province, southwest China, and is described and illustrated based on morphological and molecular evidence. It is characterized by producing white and smooth basidiomata, a monomitic system with clamp connections, hyphoid cystidia, longitudinally septate basidia, and thin-walled and oblong-ellipsoid, ellipsoid to ovoid, basidiospores measuring 4.3–5.9 × 3.0–4.0 µm. The new species resembles *Protomerulius dubius* macroscopically, but the latter differs in having larger basidiospores (5.2–8.0 × 3.7–4.7 µm vs. 4.3–5.9 × 3.0–4.0 µm). In nuclear large subunit rDNA (nLSU) and internal transcribed spacer (ITS) based phylogenies, the new species formed a distinct lineage in the *Protomerulius* clade. A full description, illustrations and phylogeny analysis results of the new species are provided.

Keywords: 1 New species, Molecular systematics, Taxonomy, Yunnan Province

Introduction

Corticioid fungi form a cosmopolitan group characterized by their rich diversity, which is closely tied to the high diversity of plants found in boreal, temperate, subtropical, and tropical regions (Bernicchia & Gorjón 2010, Dai *et al.* 2015, Zhao *et al.* 2023, Dong *et al.* 2024a). The fungal order Auriculariales Bromhead is a group mainly composed of wood-inhabiting fungi in Agaricomycetes Doweld (Basidiomycota) (Hibbett *et al.* 2007, Deng *et al.* 2024a, Dong *et al.* 2024b). The type genus of Auriculariales is *Auricularia* Bull., which also includes several other gelatinous genera, such as *Exidia* Fr., *Guepinia* Fr., *Protomerulius* Möller, and *Pseudohydnum* P. Karst. (Dong *et al.* 2024b). Unlike the gelatinous genera, certain distinctive species in the order Auriculariales develop tough, resupinate, and effused to reflexed basidiomata, exhibiting morphological characteristic typical of corticioid and polyporoid fungi (Miettinen *et al.* 2012, Malysheva & Spirin 2017, Dong *et al.* 2024b). Whatever, the poroid or corticioid taxa were still unclear.

The old genus *Protomerulius* Möller represents a special taxon within the order Auriculariales. It is characterized by poroid or corticioid basidiomata, a monomitic or dimitic hyphal structure, as well as longitudinally septate basidia that resemble those jelly fungi.

Initial classification of *Protomerulius* species, based on the phylogenetic study of Auriculariales, suggested that the genus forms a strongly supported clade alongside various basidiomata types, *viz.* corticioid, hydnoid, and clavarioid

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taxa (Weiss & Oberwinkler 2001). Furthermore, the rather comprehensive research revealed that *Protomerulius* formed an independent clade, which is sister to the *Hyalodon* Malysheva & Spirin based on ITS+LSU phylogeny (Malysheva *et al*. 2018, Spirin *et al.* 2019). Recently, a species, *Protomerulius pedratalhadensis* R.L. Alvarenga, was introduced into the genus from South America (Mendes-Alvarenga *et al*. 2023). The authoritative sequences of *Protomerulius* are limited and require further exploration.

During investigations on wood-inhabiting fungi in Yunnan Province, one undescribed species was discovered and is introduced. Molecular data were utilized to infer relationships among groups of morphologically similar basidiomycetes. This study aims to confirm the taxonomic affinities of the new species and to elucidate the relationships among representative species of *Protomerulius*, and determine whether the genus is monophyletic or polyphyletic.

Materials and methods

Morphological studies

Samples were collected from Yunnan Province of China and deposited in the herbarium of the Southwest Forestry University (SWFC), Kunming, Yunnan Province, China. The macro-morphology was based on fresh and dried specimens. The color terms in the description followed Anonymous and Petersen (Anonymous 1969, Petersen 1996). The micromorphological characteristics data were systematically analyzed from the dried specimens, and observed under a light microscope align with established methodologies (Dong *et al.* 2024c). Sections mounted in 5% KOH and 2% phloxine B dye (C20H2Br4Cl4Na2O5). Other reagents were also used, including Cotton Blue and Melzer's reagent. Micromorphology observation follows Dong *et al.* (2024c). The following abbreviations were used: CB = Cotton Blue, CB+ = cyanophilous; KOH = 5% potassium hydroxide; IKI = Melzer's reagent, IKI+ = both inamyloid and indextrinoid; W=mean spore width (arithmetic average for all spores); L=mean spore length (arithmetic average for 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 (Dong *et al.* 2024c).

DNA Extraction, Polymerase Chain Reaction (PCR), and Sequencing

Genomic DNA was extracted from dried basidiomata using a cetyltrimethylammonium bromide rapid plant genome extraction kit (Aidlab Biotechnologies Co., Ltd., Beijing) according to the manufacturer's instructions with some modifications (Zhao *et al.* 2012). ITS sequences were analyzed to determine the taxonomic status of *Protomerulius*. PCR was performed following Dong *et al.* (2024c). All newly generated sequences were deposited in the GenBank database. All the sequences were listed in Table 1.

TABLE 1. List of species, specimens, and GenBank accession numbers of sequences used in this study. The new species are in bold.

Species Name	**Sample No.**	**GenBank Accession No.**		**References**
		ITS	**nLSU**	
Protomerulius brachysporus	TAAM 102746	MK484037	-	Spirin *et al.* (2019)
Protomerulius brachysporus	VS 8351c	MK484034	MK480549	Spirin *et al.* (2019)
Protomerulius brachysporus	LR 23195	MK484038	MK480548	Spirin *et al.* (2019)
Protomerulius brachysporus	PV 2600	MK484033	MK480547	Spirin *et al.* (2019)
Protomerulius brachysporus	JN 7643	MK484035	-	Spirin *et al.* (2019)
Protomerulius brachysporus	O F293193	MK484036	MK480550	Spirin *et al.* (2019)
Protomerulius commotus	VS 11097	MK484039	MK480552	Spirin *et al.* (2019)
Protomerulius commotus	VS 11110	MK484040	MK480551	Spirin *et al.* (2019)
Protomerulius dubius	VS 3019	MK484041	MK480553	Spirin *et al.* (2019)
Protomerulius dubius	RS 14396	MK484042	-	Spirin *et al.* (2019)
Protomerulius madidus	VS 11053	MK484053	-	Spirin *et al.* (2019)
Protomerulius madidus	VS 11102	MK484043	MK480556	Spirin *et al.* (2019)

......continued on the next page

TABLE 1. (Continued)

Species Name	Sample No.	GenBank Accession No.		References
		ITS	nLSU	
Protomerulius madidus	VS 11671	MK484046	MK480560	Spirin *et al.* (2019)
Protomerulius madidus	VS 11641	MK484044	MK480557	Spirin *et al.* (2019)
Protomerulius madidus	VS 11108	MK484048	MK480562	Spirin *et al.* (2019)
Protomerulius madidus	CWU 5998	MK484054	MK480561	Spirin *et al.* (2019)
Protomerulius madidus	VS 11588	MK484045	MK480558	Spirin *et al.* (2019)
Protomerulius madidus	VS 11045	MK484049	MK480563	Spirin *et al.* (2019)
Protomerulius madidus	VS 11094	MK484052	-	Spirin *et al.* (2019)
Protomerulius madidus	VS 12492	MK484050	-	Spirin *et al.* (2019)
Protomerulius madidus	VS 12425	MK484051	-	Spirin *et al.* (2019)
Protomerulius madidus	KHL 17664	MK484047	MK480559	Spirin *et al.* (2019)
Protomerulius microsporus	LR 23440	MK484055	MK480564	Spirin *et al.* (2019)
Protomerulius minor	OM 19586	MK484056	MK480565	Spirin *et al.* (2019)
Protomerulius minor	OM 15492	MK484057	MK480566	Spirin *et al.* (2019)
Protomerulius minor	OM 17461	MK484059	MK480568	Spirin *et al.* (2019)
Protomerulius minor	OM 15700	MK484058	MK480567	Spirin *et al.* (2019)
Protomerulius minor	KHL 15937	MK484060	MK480569	Spirin *et al.* (2019)
Protomerulius pertusus	VS 3081	MK484062	MK480574	Spirin *et al.* (2019)
Protomerulius pertusus	KHL 17572	MK484061	MK480573	Spirin *et al.* (2019)
Protomerulius pertusus	O F75913	MK484063	MK480570	Spirin *et al.* (2019)
Protomerulius pertusus	VS 11947	MK484064	MK580571	Spirin *et al.* (2019)
Protomerulius subreflexus	Otto Miettinen X1121	MK636542	-	Spirin *et al.* (2019)
Protomerulius subreflexus	Otto Miettinen X1597	MK636543	-	Spirin *et al.* (2019)
Protomerulius subreflexus	Otto Miettinen X1593	MG757508	-	Spirin *et al.* (2019)
Protomerulius substuppeus	JV 1407 104	MK434169	-	Spirin *et al.* (2019)
Protomerulius substuppeus	Otto Miettinen X917	MK636541	-	Spirin *et al.* (2019)
Protomerulius substuppeus	Otto Miettinen X899	MK636539	-	Spirin *et al.* (2019)
Protomerulius substuppeus	Otto Miettinen X913	MK636540	-	Spirin *et al.* (2019)
Protomerulius wumengshanensis	**CLZhao 31840**	**PQ362238**	**PQ363281**	**Present study**
Protomerulius wumengshanensis	**CLZhao 31860**	**PQ362239**	**PQ363282**	**Present study**
Protomerulius wumengshanensis	**CLZhao 32632 ***	**PQ362240**	**PQ363283**	**Present study**
Protomerulius wumengshanensis	**CLZhao 33259**	**PQ362241**	**PQ363284**	**Present study**
Psilochaete multifora	VS 11596	MK484066	MK480576	Spirin *et al.* (2019)

* is shown type material, holotype. - means that there are parts that we did not use.

Phylogenetic analyses

To determine the phylogeny, we compiled ITS and LSU sequences matrix, *Psilochaete multifora* Spirin & Malysheva was selected as the outgroup for *Protomerulius*. The sequences were initially aligned using MAFFT (https://mafft.cbrc.jp/alignment/server/) with the "G-INS-I" strategy, and then manually optimized in BioEdit (Hall 1999). ModelFinder was used to select the best-fit model based on the AIC criterion (Kalyaanamoorthy *et al.* 2017). Best-fit model according to AIC: SYM+G4 for *Protomerulius* dataset.

Maximum likelihood (ML) analysis was conducted through the Cipres Science Gateway (https://www.phylo.org/portal2/login!input.action). Bayesian inference (BI) was performed using MrBayes with two independent runs, each generating 590,000 generations for the *Protomerulius* dataset, with one tree sampled every 1000 generations (Ronquist *et al.* 2012). The first 25% of the sampled trees were discarded as burn-in, and the remaining trees were used to reconstruct a majority rule consensus and calculate the Bayesian posterior probabilities (BPP) of the clades.

Branches of the consensus tree that received bootstrap support for ML greater than or equal to 75%, and Bayesian posterior probabilities more than 0.95, respectively.

Results

Molecular phylogeny

In *Protomerulius* analyses, a total of 44 ITS and 20 LSU sequences were used in the phylogenetic analyses. The Bayesian analysis and Maximum likelihood analysis resulted in a similar topology. All samples of *Protomerulius* form a monophyletic clade. The species *P. wumengshanensis* has a close relationship with *P. dubius* (Bourdot & Galzin) Spirin & Malysheva with high support (99BS/0.99BPP) (Fig. 1).

FIGURE 1. Phylogeny of *Protomerulius* (in bold) and related species by ML analysis based on combined ITS rDNA sequences. Branches are labeled with maximum likelihood bootstrap equal to or higher than 75%, and Bayesian posterior probabilities equal to or more than 0.95, respectively.

Taxonomy

Protomerulius wumengshanensis Z.R. Gu, H.M. Zhou & C.L. Zhao, *sp. nov.* Figs. 2–4.
MycoBank no.: MB 856058

Etymology—*Wumengshanensis* (Lat.) refers to the type of location (Wumengshan National Nature Reserve).

Holotype—CHINA. Yunnan Province, Zhaotong, Wumengshan National Nature Reserve, GPS coordinates 27°77'N, 104°29'E, evel. 1800 m asl., on fallen angiosperm branch, 29 August 2023, leg. C.L. Zhao, CLZhao 32632 (SWFC).

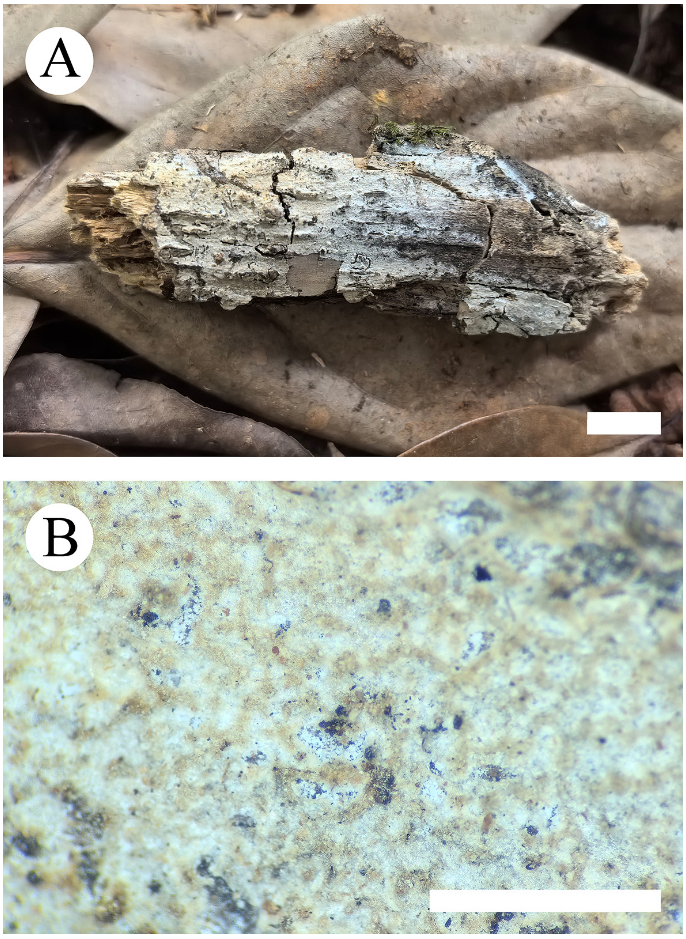

FIGURE 2. Basidiomata of *Protomerulius wumengshanensis* (holotype, CLZhao 32632). (A) The front of the basidiomata, (B) A section of the hymenophore. Scale bars: A = 1 cm; B = 1 mm.

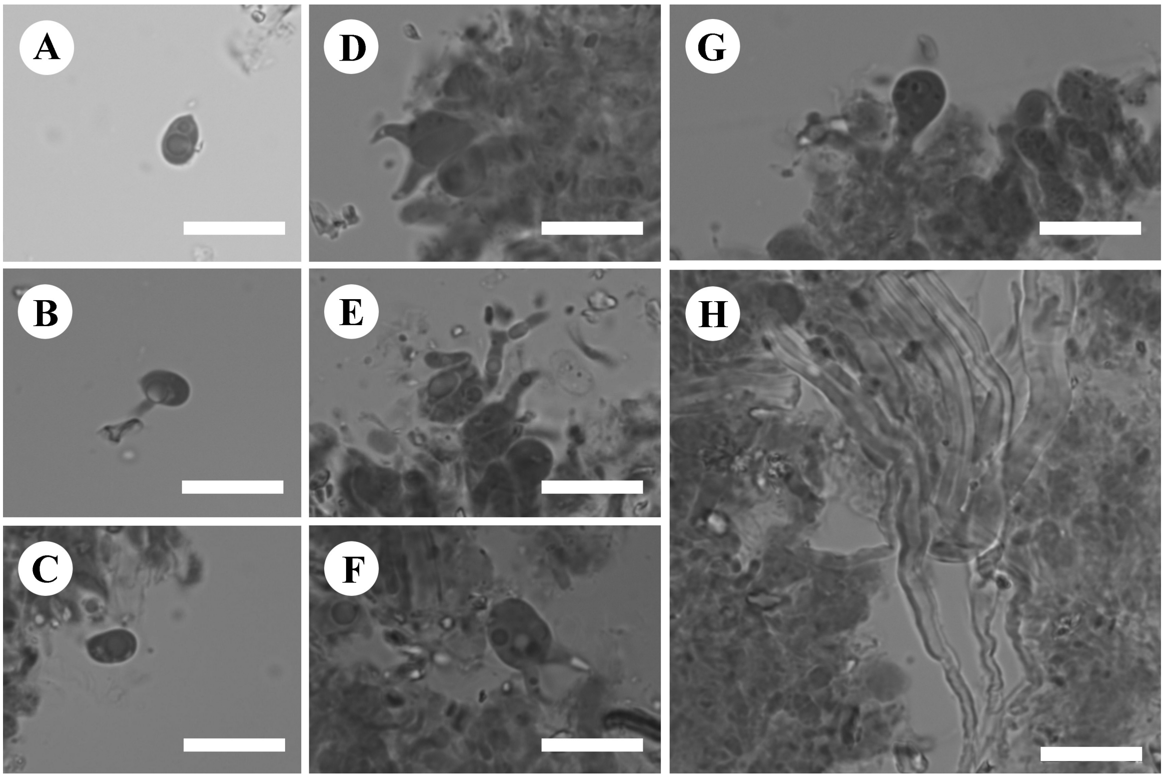

FIGURE 3. Sections of the hymenium of *Protomerulius wumengshanensis* (holotype, CLZhao 32632). (A–C) Basidiospores, (D–F) Basidia, (G) Basidioles, (H) Cystidia. Scale bars: (A–H) = 10 µm, 10 × 100 Oil.

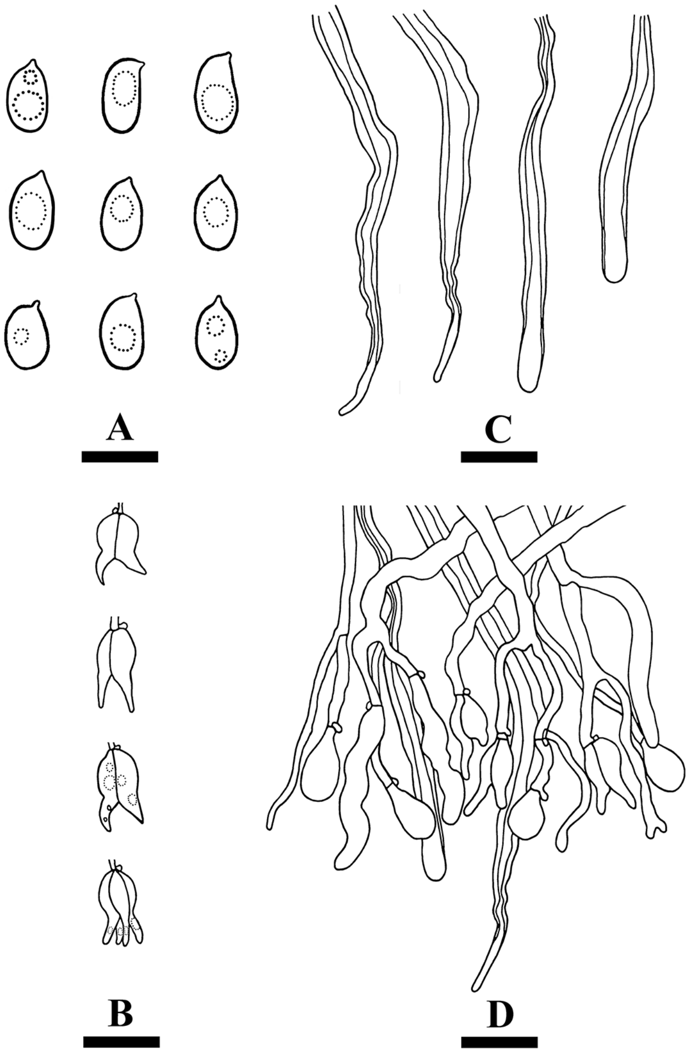

FIGURE 4. Microscopic structures of *Protomerulius wumengshanensis* (holotype, CLZhao 32632). (A) Basidiospores; (B) Basidia; (C) Hyphoid cystidia; (D) A cross section of basidiomata. Scale bars: A = 5 µm; B–D = 10 µm.

Diagnosis—Differs from *Protomerulius dubius* by the larger basidiospores.

Basidiomata—Annual, resupinate, smooth, without odor or taste when fresh, up to 8 cm long, 4 cm wide, and 0.1 mm thick. Hymenial surface farinaceous, white when fresh, become white to pale cream when dry.

Hyphal system—Monomitic; generative hyphae distinct, with clamp connections, hyaline, thin-walled, frequently branched, interwoven, IKI+, CB+, 1.5–2.5 µm in diam; tissues unchanged in KOH.

Hymenium—Hyphoid cystidia, thick-walled, 23.5–84 × 3.5–4.5 µm; Cystidioles absent. Hyphidia present, simple or branched, 1–2 µm in diam. Crystals present in young basidiomata, variable, up to 10 µm in widest dimension. Basidia ellipsoid to ovoid, longitudinally septate, with two or four sterigmata, hyaline, thin-walled, with several guttules, 6.6–8.3 × 5.4–6.8 µm; basidioles dominant, similar to basidia in shape, but slightly smaller, without longitudinal septa.

Basidiospores—Basidiospores oblong-ellipsoid, ellipsoid to ovoid, hyaline, thin-walled, smooth, with one or two guttules, more or less CB+, (4.0–)4.3–5.9(–7.0) × (2.7–)3.0–4.0(–5.9) µm, L = 5.06 µm, W = 3.47 µm, Q = 1.42–1.50 (n = 120/4).

Type of rot—White rot.

Additional specimens examined (paratypes)—CHINA. Yunnan Province, Zhaotong, Wumengshan National Nature Reserve, GPS coordinates 27°77′N, 104°29′E, evel. 1800 m asl., on an angiosperm stump, leg. C.L. Zhao, 26 August 2023, CLZhao 31840 (SWFC); leg. C.L. Zhao, 27 August 2023, CLZhao 31860 (SWFC); on fallen angiosperm branch, leg. C.L. Zhao, 19 September 2023, CLZhao 33259 (SWFC).

Discussion

In this study, the new species was discovered in Yunnan Province, China, and is described based on morphological characteristics and phylogenetic analyses.

Protomerulius wumengshanensis is characterized by the white and smooth basidiomata, a monomitic system, and oblong-ellipsoid basidiospores. In phylogeny, *P. wumengshanensis* clustered with *P. dubius* (Bourdot & Galzin) Spirin & Malysheva, *P. minor* (Möller) Spirin & O. Miettinen, and *P. pertusus* Malysheva & Spirin, with high support (95%BS/1BPP). However, *Protomerulius dubius* has larger basidiospores (5.2–8.0 × 3.7–4.7 µm vs. 4.3–5.9 × 3.0–4.0 µm, (Spirin *et al.* 2019); *P. pertusus* has longer basidiospores (5.1–8.1 µm vs. 4.3–5.9 µm, (Spirin *et al.* 2019); while *P. minor* has the dimitic hyphal system (Spirin *et al.* 2019).

Corticium fungi represent a frequently overlooked resupinate group of wood-inhabiting basidiomycetes. However, many species are widely distributed across diverse habitats due to their remarkable adaptability (Yang *et al.* 2023, Dong *et al.* 2024c, Deng *et al.* 2024b, Yang *et al.* 2024). Furthermore, corticium fungi possess a strong capacity to degrade fallen branches in broad-leaved forests (Zhao *et al.* 2023, Gu *et al.* 2024, Zhou *et al.* 2024). Those advances distinguish them from other common macrofungi, such as those with gills and poroids (Wang *et al.* 2024, Zhao *et al.* 2024). Our findings will enhance the understanding of fungal diversity in these ecosystems.

Acknowledgements

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