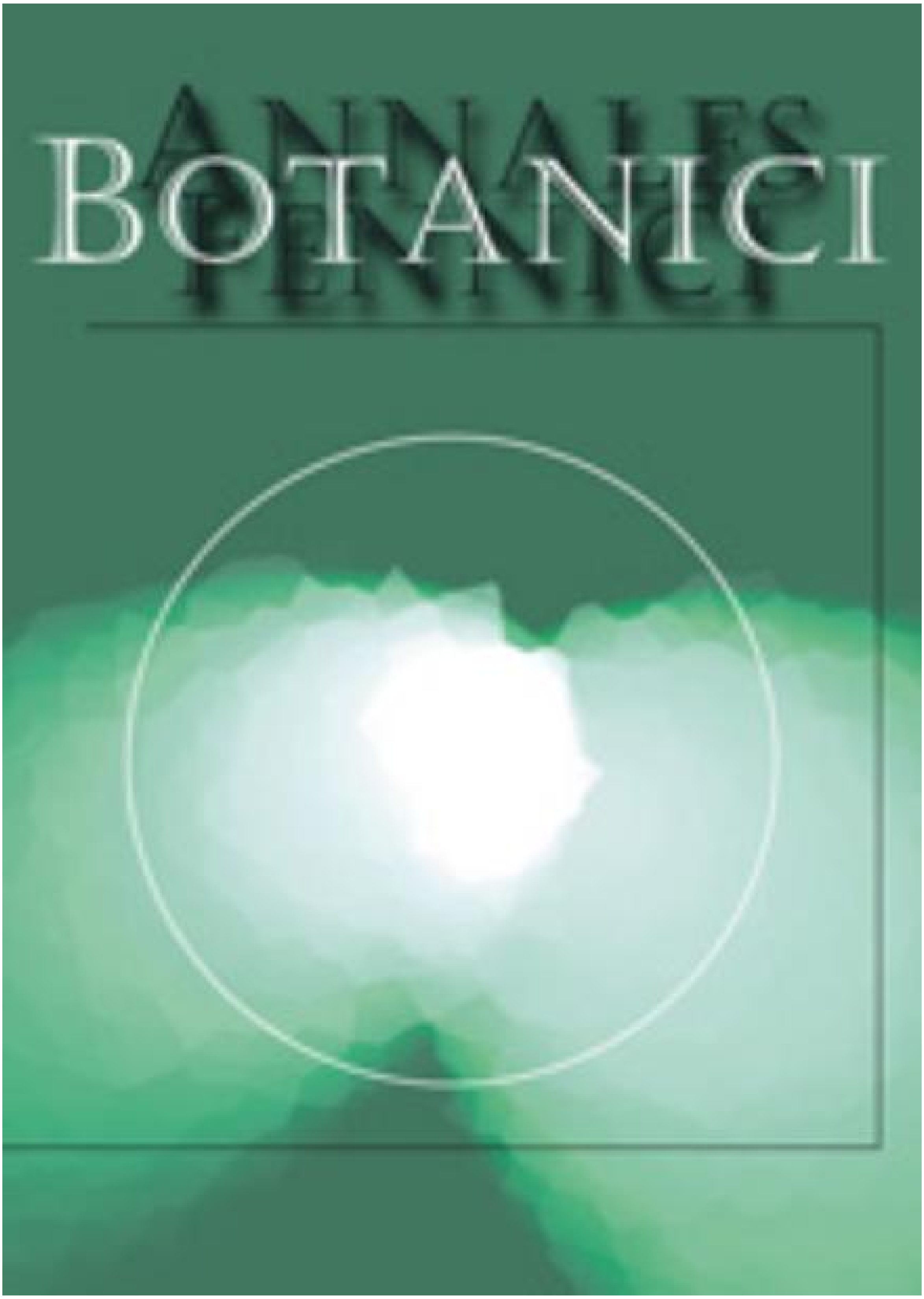
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Authors: Duan, Zi-Yan, and Zhao, Chang-Lin

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Basidiodendron yunnanense (Auriculariales), a new species from southern China based on morphological and molecular evidence

Zi-Yan Duan[1,3] & Chang-Lin Zhao[1,2,3,4,*]

[1)] *Key Laboratory for Forest Resources Conservation and Utilization in the Southwest Mountains of China, Ministry of Education, Southwest Forestry University, CN-650224 Kunming, P.R. China (*corresponding author's e-mail: fungi@swfu.edu.cn)*
[2)] *Yunnan Key Laboratory of Plateau Wetland Conservation, Restoration and Ecological Services, Southwest Forestry University, CN-650224 Kunming, China*
[3)] *College of Biodiversity Conservation, Southwest Forestry University, CN-650224 Kunming, P.R. China*
[4)] *Yunnan Key Laboratory for Fungal Diversity and Green Development, Kunming Institute of Botany, Chinese Academy of Science, CN-650201 Kunming, P.R. China*

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A wood-decaying fungal species, *Basidiodendron yunnanense* Z.Y. Duan & C.L. Zhao *sp. nova* (Auriculariales) from the Yunnan-Guizhou Plateau, China, is described based on morphological and molecular evidence. *Basidiodendron yunnanense* has effused, monomitic basidiomata, clamped hyphae, abundant gloeocystidia and broadly ellipsoid or subglobose basidiospores (5–6.5 × 4.5–6 µm). According to a phylogenetic analysis based on ITS region sequences, as well as according to maximum likelihood analysis, maximum parsimony and Bayesian inference *B. yunnanense* is sister to *B. luteogriseum* in a monophyletic clade sister to a clade formed of *B. alni* and *B. eyrei*.

Introduction

Basidiodendron belongs in the order Auriculariales (Agaricomycetes, Basidiomycota). The *Basidiodendron* species are characterized by resupinate to effused basidiomata with a smooth or minutely warted hymenophore, monomitic hyphal structure, clamped generative hyphae, prominent gloeocystidia and longitudinally septate, predominantly four-celled basidia (Luck-Allen 1963). Kotiranta and Saarenoksa (2005) added a morphological trait shared by *Basidiodendron* spp., i.e. the hymenial surface which is bluish grey when fresh and upon drying turning pale cream or ochre with brownish spots. These wood-inhabiting fungi are found in Europe, Africa, the Hawaiian Islands, Oceania, and North and South America (Raitviir 1967, Wells & Raitviir 1975, Roberts 2001, Kotiranta & Saarenoksa 2005, Ordynets 2012, Spirin *et al*. 2020, 2021). The MycoBank database (http://www.MycoBank.org) contains 51 and *Index Fungorum* (http://www.indexfungorum.org) 49 specific and infraspecific names in *Basidiodendron*, but the actual number of accepted species is approximately 40 (Luck-Allen 1963, Spirin *et al*. 2020, 2021).

Spirin *et al*. (2020) studied *B. eyrei* and the similar-looking taxa using the nuclear DNA sequence data. *Basidiodendron* clustered with *Bourdotia*, *Protohydnum* and *Ductifera*. In total, 14 species from Eurasia and North and South America were recognized in the *B. eyrei* complex. Subsequently, Spirin *et al*. (2021) studied the *B. caesiocinereum* complex and showed that the basidiospore ornamentation is a key morphological character for species recognition in this group.

During investigation of wood-inhabiting fungi in Yunnan, southern China, we found a species of *Basidiodendron* that could not be assigned to any of the described species.

Material and methods

Sample collection

Fresh fruiting bodies were collected from the Daweishan National Nature Reserve in Yunnan Province, China. The specimens were dried in an electric food dehydrator at 40 °C, then sealed and stored in an envelope bag, and deposited in the herbarium of the Southwest Forestry University (SWFC), Kunming, Yunnan Province, China.

Morphological studies

The macromorphological descriptions are based on field notes and photos taken in the field and lab. The colour terms follow Petersen (1996). The micromorphological data were obtained from the dried specimens and observed under Nikon Eclipse E100 light microscope following Zhao and Wu (2017). The following abbreviations are used: KOH = 5% potassium hydroxide; CB = cotton blue; CB– = acyanophilous, CB+ = cyanophilous; IKI = Melzer's reagent; IKI– = non-amyloid and non-dextrinoid; *L* = mean spore length (arithmetic average of all spores); *W* = mean spore width (arithmetic average of all spores); *Q* = *L*/*W* ratio; *n* (*a*/*b*) = number of spores (*a*) measured from a given number (*b*) of specimens.

DNA extraction and sequencing

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. The ITS region was amplified with ITS5 and ITS4 primers (White *et al*. 1990). The PCR cycling procedure for ITS was as follows: initial denaturation at 95 °C for 3 min, followed by 35 cycles at 94 °C for 40 s, at 58 °C for 45 s and 72 °C for 1 min, and a final extension at 72 °C for 10 min. The PCR products were purified and directly sequenced at the Kunming Tsingke Biological Technology Limited Company, Yunnan Province, China. All the newly generated sequences were deposited in GenBank (*see* Table 1).

Phylogenetic analyses

Sequencher 4.6 (GeneCodes, Ann Arbor, MI, USA) was used to assemble and edit the generated sequence reads. Sequences were aligned in MAFFT 7 (https://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 29412). *Bourdotia galzinii* and *Protohydnum cartilagineum* were used selected as outgroups for the phylogenetic analysis (Spirin *et al*. 2021).

Maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) analyses were applied to the ITS data set sequences. Approaches to phylogenetic analyses followed Zhao and Wu (2017). MP analysis was performed in PAUP* ver. 4.0b10 (Swofford 2002). All the 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 most-parsimonious trees were saved. Clade robustness was assessed using bootstrap (BT) analysis with 1000 replicates (Felsenstein 1985). Descriptive tree statistics tree length (TL), the consistency index (CI), the retention index (RI), the rescaled consistency index (RC) and the homoplasy index (HI) were calculated for each most-parsimonious tree generated. ML was inferred using RAxML-HPC2 through the Cipres Science Gateway (www.phylo.org). Branch support (BS) for ML analysis was determined by 1000 bootstrap replicates and evaluated under the gamma model.

MrModeltest 2.3 (Nylander 2004) was used to determine the best-fit evolution model for the data set for Bayesian inference (BI). Bayesian inference was performed with MrBayes 3.1.2

Table 1. Species included in the phylogenetic analysis, ITS GenBank accession numbers, references, and country of origin of the samples.

Species	ITS GenBank accession number	Reference	Country
Basidiodendron alni	MT040878	Spirin *et al.* 2020	Russia
B. alni	MT040869	Spirin *et al.* 2020	Russia
B. caesiocinereum	MW136103	Spirin *et al.* 2021	Norway
B. caesiocinereum	MW136101	Spirin *et al.* 2021	Norway
B. caesiocinereum	MW136105	Spirin *et al.* 2021	Italy
B. caucasicum	MT040877	Spirin *et al.* 2020	Russia
B. cinerellum	MW136104	Spirin *et al.* 2021	Norway
B. cinerellum	MW136088	Spirin *et al.* 2021	Belgium
B. cinerellum	MW136086	Spirin *et al.* 2021	Italy
B. deminutum	MT040885	Spirin *et al.* 2020	Slovenia
B. eyrei	MT040868	Spirin *et al.* 2020	Canada
B. eyrei	MT040874	Spirin *et al.* 2020	Russia
B. eyrei	MT040866	Spirin *et al.* 2020	Russia
B. glaucum	MW136079	Spirin *et al.* 2021	Norway
B. glaucum	MW136078	Spirin *et al.* 2021	Norway
B. globisporum	MT040872	Spirin *et al.* 2020	Russia
B. globisporum	MT040884	Spirin *et al.* 2020	Russia
B. globisporum	MT040888	Spirin *et al.* 2020	Slovenia
B. grandinioides	MT040873	Spirin *et al.* 2020	Norway
B. grandinioides	MT040887	Spirin *et al.* 2020	Norway
B. groningae	MW139278	Spirin *et al.* 2021	Netherlands
B. groningae	MW139276	Spirin *et al.* 2021	Netherlands
B. inconspicuum	MW136098	Spirin *et al.* 2021	USA
B. iniquum	MT040876	Spirin *et al.* 2020	Brazil
B. luteogriseum	MT040881	Spirin *et al.* 2020	Brazil
B. mexicanum	MW136068	Spirin *et al.* 2021	Mexico
B. olivaceum	MT040870	Spirin *et al.* 2020	Canada
B. olivaceum	MT040883	Spirin *et al.* 2020	Russia
B. olivaceum	MT040867	Spirin *et al.* 2020	Russia
B. parile	MT040890	Spirin *et al.* 2020	Norway
B. parile	MT040889	Spirin *et al.* 2020	Norway
B. pelinum	MT040875	Spirin *et al.* 2020	Brazil
B. robenae	MW270998	Spirin *et al.* 2021	USA
B. robenae	MW270997	Spirin *et al.* 2021	USA
B. salebrosum	MT040871	Spirin *et al.* 2020	Russia
B. salebrosum	MT040891	Spirin *et al.* 2020	Russia
B. spiculosum	MW136076	Spirin *et al.* 2021	Mexico
B. trachysporum	MW136080	Spirin *et al.* 2021	Russia
B. trachysporum	MW136077	Spirin *et al.* 2021	Russia
B. trachysporum	MW136081	Spirin *et al.* 2021	Norway
B. walleynii	MW136066	Spirin *et al.* 2021	Russia
B. widdringtoniae	MW136073	Spirin *et al.* 2021	Malawi
B. yunnanense	OM677311	this study	China
B. yunnanense	OM677312	this study	China
Bourdotia galzinii	MG757511	Spirin *et al.* 2021	Spain
Protohydnum cartilagineum	MG735419	Spirin *et al.* 2021	Brazil

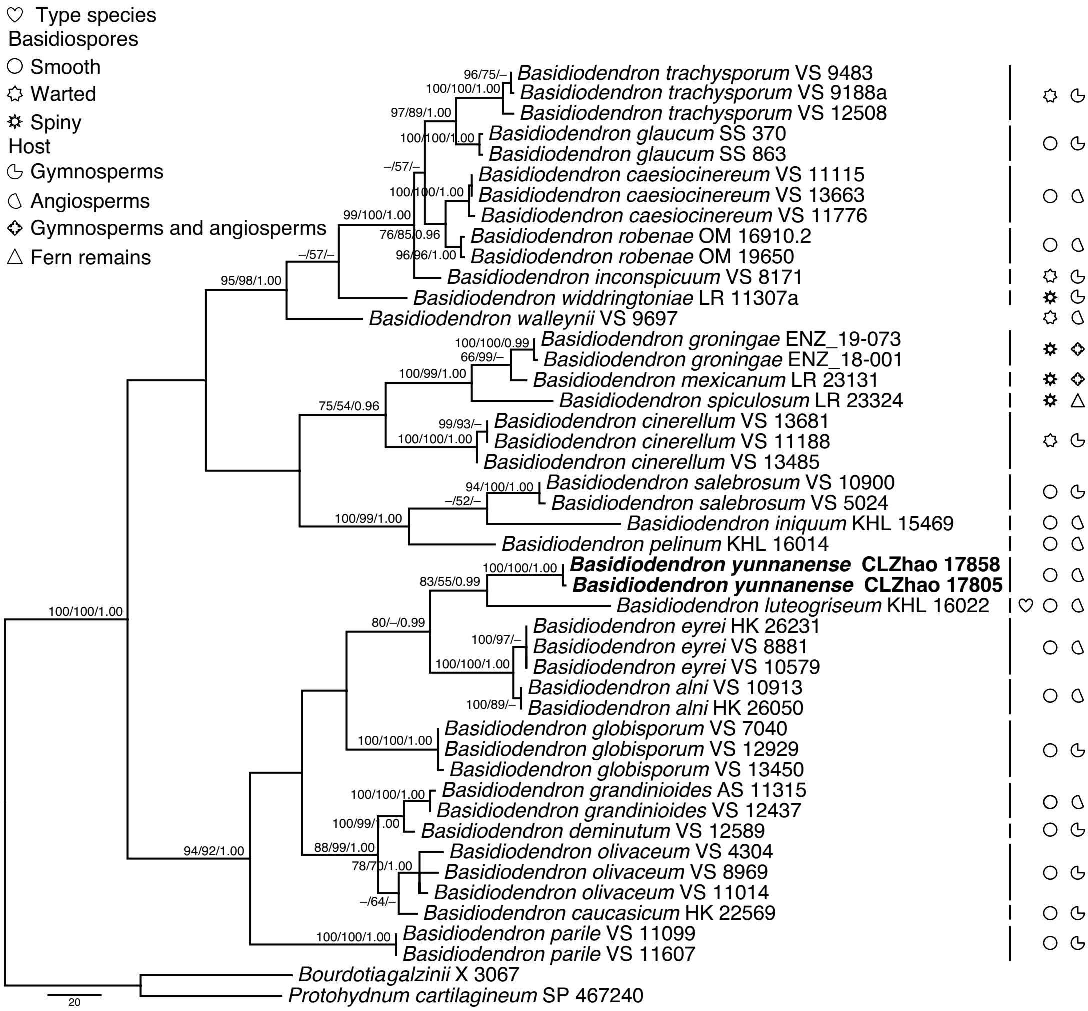

Fig. 1. Maximum parsimony strict consensus tree illustrating the phylogeny of *Basidiodendron yunnanense* and congenerics based on the ITS sequences. Branches are labelled with maximum likelihood bootstrap values > 70%, parsimony bootstrap values > 50%, and Bayesian posterior probabilities > 0.95, respectively. Sample numbers are from Spirin *et al.* (2020, 2021), except those for *B. yunnanense*.

with a general time reversible (GTR + I + G) model of DNA substitution and a gamma distribution rate variation across sites (Ronquist & Huelsenbeck 2003). Four Markov chains were used in each of two runs from random starting trees for 600 000 generations (Fig. 1), with trees and parameters sampled every 100 generations. The first quarter of all the generations were discarded as burn-ins. A majority rule consensus tree of all remaining trees and posterior probabilities were calculated. Branches were considered significantly supported if they received a maximum likelihood bootstrap value (BS) of > 70%, a maximum parsimony bootstrap value (BT) of > 70%, or Bayesian posterior probabilities (BPP) of > 0.95.

Results

Molecular phylogeny

The ITS data set included sequences from 46 fungal specimens representing 26 species. The dataset had an aligned length of 572 characters, of which 226 characters were constant and 284 parsimony-informative. The MP analysis yielded nine equally parsimonious trees (TL = 1340, CI = 0.4724, HI = 0.5276, RI = 0.7714, RC = 0.3644). 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). The Bayesian and ML analyses resulted in a similar topology to that of the MP analysis, with an average standard deviation of split frequencies = 0.009013 (BI), and the effective sample size (ESS) across the two runs was double the average ESS (avg ESS) = 190.5. The phylogenetic tree (Fig. 1) inferred from ITS sequences revealed that two specimens of *B. yunnanense* formed a monophyletic lineage sister to *B. luteogriseum* in a clade sister to a clade consisting of *B. alni* and *B. eyrei*.

Taxonomy

Basidiodendron yunnanense Z.Y. Duan & C.L. Zhao, *sp. nova* (Figs. 2 and 3)

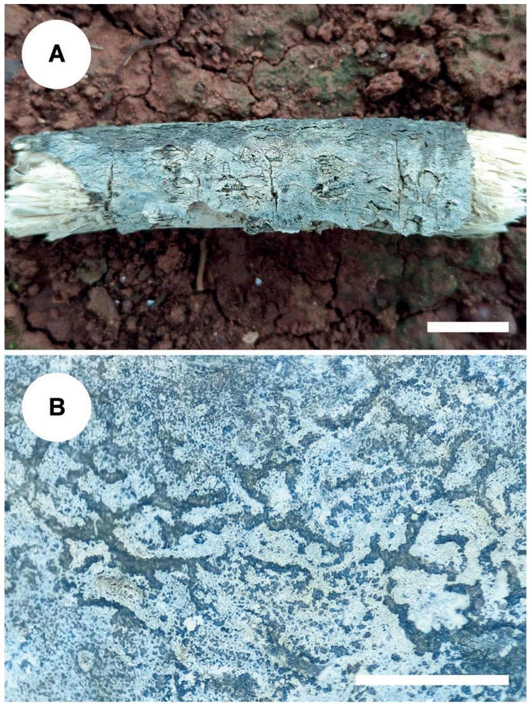

Fig. 2. Basidiomata of *Basidiodendron yunnanense* (holotype). Scale bars: **A** = 1 cm, **B** = 1 mm.

MycoBank MB 843087. — Holotype: China. Yunnan Province, Honghe, Pingbian County, Daweishan National Nature Reserve, 22.98°N, 103.67°E, on fallen branches of angiosperm, 1 August 2019 *C.L. Zhao 17805* (SWFC 017805). — Paratype: Same locality, substrate and date, *C.L. Zhao 17858* (SWFC 017858).

Etymology: *yunnanense* (Lat.), referring to the provenance (Yunnan Province) of the type specimens.

Basidiomata annual, resupinate, pruinose, up to 20 cm long and 3 cm wide, 100–200 µm thick. Hymenial surface reticulate to porulose, greyish blue when fresh, turning cream-coloured upon drying, cracking with age. Margin narrow, slightly grey. Hyphal system monomitic, hyphae with clamp connections, moderately CB+, IKI–, tissues unchanged in KOH; subicular hyphae hyaline, thin- to slightly thick-walled, unbranched, subparallel, 1.5–3 µm in diameter; subhymenial generative hyphae hyaline, thin-walled, interwoven, rarely branched, 0.5–1.5 µm in diameter, fishbone-like basidia-bearing hyphae present. Gloeocystidia variably shaped, of three types: (1) tapering cystidia, hyaline, thin-walled, smooth, subfusiform to bottle-shaped, with a hyphoid apical outgrowth, 13.5–44 × 1.5–5.5 µm; (2) normally developed gloeocystidia, hyaline, thin-walled, smooth, subcylindrical to tubular, apically slightly tapering or obtuse, mostly widest at basal part, encrusted or not on the apex, 9.5–32 × 3–6.5 µm; (3) capitate cystidia, rarely present, hyaline, thin-walled, smooth, subcapitate, 5.5–8 × 4–7.5 µm. Hyphidia rare, hyaline, thin-walled, smooth, branched, 18.5–27 × 2.5–3.5 µm. Basidia two or four-celled, urniform, thin-walled, 6.5–12.5 × 5–9 µm; involucres well-developed, often covering basidia cells up to the middle part. Basidiospores broadly ellipsoid to subglobose, hyaline, thin-walled, smooth, moderately CB+, IKI–, with a short, often eccentric and asymmetric apiculus, 5–6.5 × 4.5–6 µm, L = 5.97 µm, W = 5.58 µm, Q = 1.1 (n = 60/2).

Ecology and distribution: Lignicolous, causing white rot on fallen angiosperm branches. To date found only in China.

Basidiodendron yunnanense is described based on phylogenetic analyses and morphological characters. It is sister to *B. luteogriseum*, the generic type of *Basidiodendron*. The latter species differs from *B. yunnanense* by having

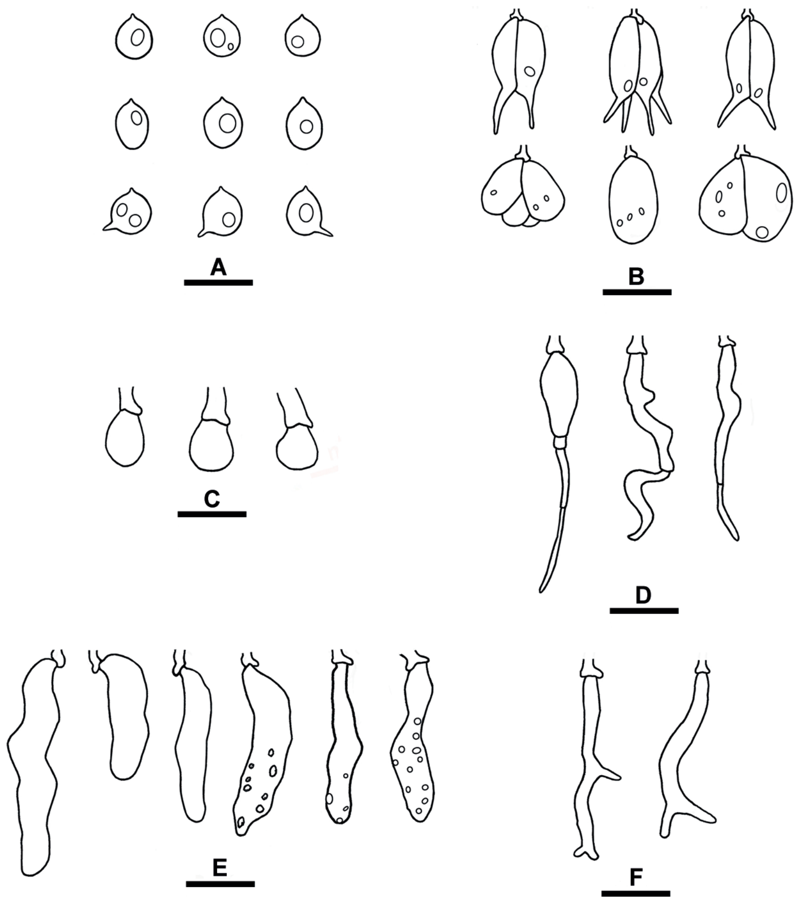

Fig. 3. Microscopic structures of *Basidiodendron yunnanense* (from the holotype). — **A**. Basidiospores. — **B**. Basidia and basidioles. — **C**. Capitate cystidia. — **D**. Tapering cystidia. — **E**. Normally developed gloeocystidia. — **F**. Hyphidia. Scale bars 10 μm.

thinner basidiomata (50–100 µm) with smooth hymenophore and slightly thick-walled basidiospores (Spirin *et al*. 2020). Two further species closely related to *B. yunnanense* are *B. alni* and *B. eyrei*. *Basidiodendron alni* has thinner basidiomata (30–50 µm) and smaller basidiospores than *B. yunnanense* (4.1–5 × 4.2–5 µm; Spirin *et al*. 2020). *Basidiodendron eyrei* differs from *B. yunnanense* by having pale ochraceous or fawn, thinner basidiomata (40–100 µm), and globose, smaller basidiospores (4.3–5 × 4.6–5 µm; Kotiranta & Saarenoksa 2005, Spirin *et al*. 2020).

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