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A new species of *Punctularia* (Punctulariaceae, Basidiomycota) from southwest China

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Abstract

A new wood-rotting fungal species, *Punctularia bambusicola*, is proposed based on a combination of morphological features and molecular data. The species is characterized by resupinate, lilac to purple basidiomata, pink to rose tuberculate hymenial surface, monomitic hyphal system with clamped generative hyphae, yellowish to brown dendrohyphidia and ellipsoid basidiospores measuring 6.5–8.5 × 3.5–5 µm. Phylogenetic analyses of combining ITS and LSU nrRNA gene regions demonstrated *that P. bambusicola* forms a single lineage sister to *P. atropurpurascens* with strong statistical supports (100% BS, 100% BT, 1.00 BPP).

Keywords: Phylogenetic analyses, Punctulariaceae, Taxonomy, Wood-rotting fungi

Introduction

Punctularia Patouillard (1895: 57) (Punctulariaceae, Corticiales), was typified with *P. tuberculosa* (Pat.) Pat. & Lagerh. (1895: 57) (current name *P. atropurpurascens* (Berk. & Broome) Petch (1916: 160), which is characterized by resupinate to effused-reflexed basidiomata gelatinous when fresh, rigid upon drying; hymenophore tuberculate or radial ridges; monomitic hyphal structure with clamped generative hyphae; yellowish to brown dendrohyphidia; and thin-walled, smooth, ellipsoid, acyanophilous basidiospores negative in Melzer's reagent (Patouillard 1895, Bernicchia & Gorjón 2010). So far, two species have been accepted in the genus worldwide (Patouillard 1895, Petch 1916, Talbot 1958, Hjortstam 1995).

Numerous studies on molecular phylogeny of *Punctularia* have been carried out (Larsson *et al*. 2004, Ghobad-Nejhad & Duhem 2013, Knijn & Ferretti 2018). Larsson *et al*. (2004) presented the high-level phylogenetic diversity among corticioid homobasidiomycetes and suggested that *P. strigosozonata* (Schwein.) P.H.B. Talbot (1958: 143) is nested within corticioid clade with *Dendrocorticium roseocarneum* (Schwein.) M.J. Larsen & Gilb. (1977: 115). *Vuilleminia nilsii* Ghobad-Nejhad & Duhem (2013: 5) and *Dendrominia* Ghobad-Nejhad & Duhem (2013: 7) in Corticiales were introduced by Ghobad-Nejhad & Duhem (2013) and it revealed that *P. strigosozonata* clusters with genera *Dendrocorticium* M.J. Larsen & Gilb. (1974: 225) and *Punctulariopsis* Ghobad-Nejhad (2010: 1529). Phylogenetic study of *Dendrocorticium*, *Punctularia*, *Punctulariopsis* and *Vuilleminia* Maire (1902: 81) using ITS sequence data reveled that *P. atropurpurascens* grouped with *P. strigosozonata* in the genus *Punctularia* (Knijn & Ferretti 2018).

Recently, we collected a basidiomycetous fungus from Yunnan Province of China, which could not be assigned to any described species in family Punctulariaceae. In this paper, we present morphological and molecular phylogenetic evidence to support the identification of the new species in *Punctularia*.

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Materials and methods

Morphological studies.—Macromorphological descriptions were based on field notes. Colour terms followed Petersen (1996). Micromorphological data were obtained from the dried specimens, and observed under a compound light microscope following Dai (2012) and Ma *et al*. (2020). The following abbreviations were used: KOH = 5% Potassium hydroxide, CB = Cotton Blue, CB– = acyanophilous, IKI = Melzer's reagent, IKI– = both inamyloid and indextrinoid, L = mean spore length (arithmetic average of all spores), W = mean spore width (arithmetic average of all spores), Q = variation in the L/W ratios between the specimens studied, n (a/b) = number of spores (a) measured from given number (b) of specimens. The studied specimens were deposited at the herbarium of Southwest Forestry University (SWFC), Kunming, Yunnan Province, China.

DNA extraction, amplification, sequencing and phylogenetic analyses.—The EZNA HP Fungal DNA Kit (Omega Biotechnologies Co., Ltd, Kunming) was used to obtain PCR products from dried specimens, according to the manufacturer's instructions (Xu *et al*. 2019). ITS region was amplified with primer pair ITS5 and ITS4 (White *et al*. 1990). Nuclear LSU region was amplified with primer pair LR0R and LR7 (http://www.biology.duke.edu/fungi/mycolab/primers.htm). 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 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 Company, Yunnan Province, China. All newly generated sequences were deposited at GenBank (Table 1).

Sequencher 4.6 (GeneCodes, Ann Arbor, MI, USA) was used to edit the DNA sequence. 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 at TreeBase (submission ID 26756). Sequences of *Gloeophyllum abietinum* (Bull.) P. Karst. (1882: 80) and *Gloeophyllum sepiarium* (Bull.) P. Karst. (1882: 79) obtained from GenBank were used as an outgroup to root tree following Ghobad-Nejhad & Duhem (2012) in the ITS+nLSU analysis (Fig. 1). Maximum parsimony analysis was applied to the ITS+nLSU dataset sequences. Approaches to phylogenetic analyses followed Zhao & Wu (2017) and Wang *et al*. (2020), and the tree construction procedure was performed in PAUP* version 4.0b10 (Swofford 2002). All characters were equally weighted and gaps were treated as missing data. Trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Max-trees were set to 5000, branches of zero length were collapsed and all parsimonious trees were saved. Clade robustness was assessed using a bootstrap (BT) analysis with 1,000 replicates (Felsenstein 1985). Descriptive tree statistics tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated for each Maximum Parsimonious Tree (MPT) generated. Sequences were also analyzed using Maximum Likelihood (ML) with RAxML-HPC2 on Abe through the Cipres Science Gateway (www.phylo.org; Miller *et al*. 2009). Branch support (BS) for ML analysis was determined by 1000 bootstrap replicates.

MrModeltest 2.3 (Posada & Crandall 1998, Nylander 2004) was used to determine the best-fit evolution model for each data set for Bayesian inference (BI). BI was calculated with MrBayes3.1.2 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 run for 2 runs from random starting trees for 90 thousand generations (Fig. 1), and trees were sampled every 100 generations. The first one-fourth generations were discarded as burn-in. A majority rule consensus tree of all remaining trees was calculated. Branches that received bootstrap support for maximum likelihood (BS), maximum parsimony (BT) and Bayesian posterior probabilities (BPP) greater than or equal to 75 % (BS), 75 % (BT) and 0.95 (BPP) were considered as significantly supported, respectively.

Results

Molecular phylogeny

The ITS+nLSU dataset (Fig. 1) included sequences from 33 fungal samples representing 20 species. The dataset had an aligned length of 2225 characters, of which 1618 characters are constant, 142 are variable and parsimony-uninformative, and 465 are parsimony-informative. Maximum parsimony analysis yielded three equally parsimonious trees (TL = 1110, CI = 0.7009, HI = 0.2991, RI = 0.8304, RC = 0.5821). Best model for the ITS+nLSU dataset

estimated and applied in the Bayesian analysis: GTR+I+G. Bayesian analysis and ML analysis resulted in a similar topology as MP analysis, with an average standard deviation of split frequencies = 0.004254(BI).

A phylogeny (Fig. 1) inferred from the combined ITS+nLSU sequences, was obtained for 3 species within the genus *Punctularia* and showed that the new species formed a single lineage with a strong support (100% BS, 100% BT and 1.00 BPP) and was sister to the *P. atropurpurascens*.

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

Species name	Sample no.	GenBank accession no.		References
		ITS	nLSU	
Australovuilleminia coccinea	MG75	—	HM046931	Ghobad-Nejhad *et al.* 2010
A. coccinea	MG74	HM046875	HM046930	Ghobad-Nejhad *et al.* 2010
Cytidia salicina	MG49	GU590881	HM046921	Ghobad-Nejhad & Hallenberg 2011
C. salicina	CBS 727.85	—	DQ915478	Lawrey *et al.* 2007
Dendrocorticium roseocarneum	FPL1800	—	AF393053	Binder & Hibbett 2002
D. polygonioides	MG48	HM046877	—	Ghobad-Nejhad *et al.* 2010
Dendrothele maculata	HHB1062	—	AY586652	Larsson *et al.* 2004
Gloeophyllum sepiarium	FP 125002-T	—	AY333806	Ghobad-Nejhad & Ginns 2012
G. abietinum	P254	—	AJ583431	Moreth & Schmidt 2005
Punctularia atropurpurascens	UC 2022981	KP814559	—	Knijn & Ferretti 2018
P. atropurpurascens	T 6	MG755209	—	Knijn & Ferretti 2018
P. bambusicola	CLZhao 9098	MW559983	MW559985	Present study
P. bambusicola	CLZhao 4133	MW559982	MW559984	Present study
P. strigosozonata	CBS 34534	MH855559	MH867064	Vu *et al*. 2019
P. strigosozonata	BHIF 586b	MH558554	MH558554	Knijn & Ferretti 2018
P. strigosozonata	AFTOL 1248	DQ398958	DQ398958	Knijn & Ferretti 2018
Punctulariopsis obducens	MG70	HM046918	HM046933	Ghobad-Nejhad *et al.* 2010
P. subglobispora	GB 12761	NR119827	—	Schoch *et al.* 2014
P. subglobispora	FCUG 2535	HM046917	HM046932	Ghobad-Nejhad *et al.* 2010
Veluticeps abietina	KHL 12474	—	EU118619	Larsson 2007
Vuilleminia comedens	MG79	HM046880	—	Ghobad-Nejhad *et al.* 2010
V. comedens	FCUG 2595	HM046891	—	Ghobad-Nejhad *et al.* 2010
V. coryli	FCUG 1038A	HM046903	—	Ghobad-Nejhad *et al.* 2010
V. coryli	FCUG 2280	HM046901	—	Ghobad-Nejhad *et al*. 2010
V. cystidiata	FCUG 2145	HM046912	HM046925	Ghobad-Nejhad *et al.* 2010
V. cystidiata	FCUG 2596	HM046909	HM046923	Ghobad-Nejhad *et al.* 2010
V. erastii	MG139	JN387999	JN388008	Ghobad-Nejhad & Ginns 2012
V. erastii	MG96	JN388000	JN388009	Ghobad-Nejhad & Ginns 2012
V. macrospora	MG60	HM046885	HM046927	Ghobad-Nejhad *et al.* 2010
V. megalospora	FCUG 3210	HM046913	—	Ghobad-Nejhad *et al.* 2010
V. megalospora	FCUG 3211	HM046914	—	Ghobad-Nejhad *et al.* 2010
V. pseudocystidiata	MG69	HM046888	HM046928	Ghobad-Nejhad *et al.* 2010
V. pseudocystidiata	FCUG 2600B	HM046916	—	Ghobad-Nejhad *et al.* 2010

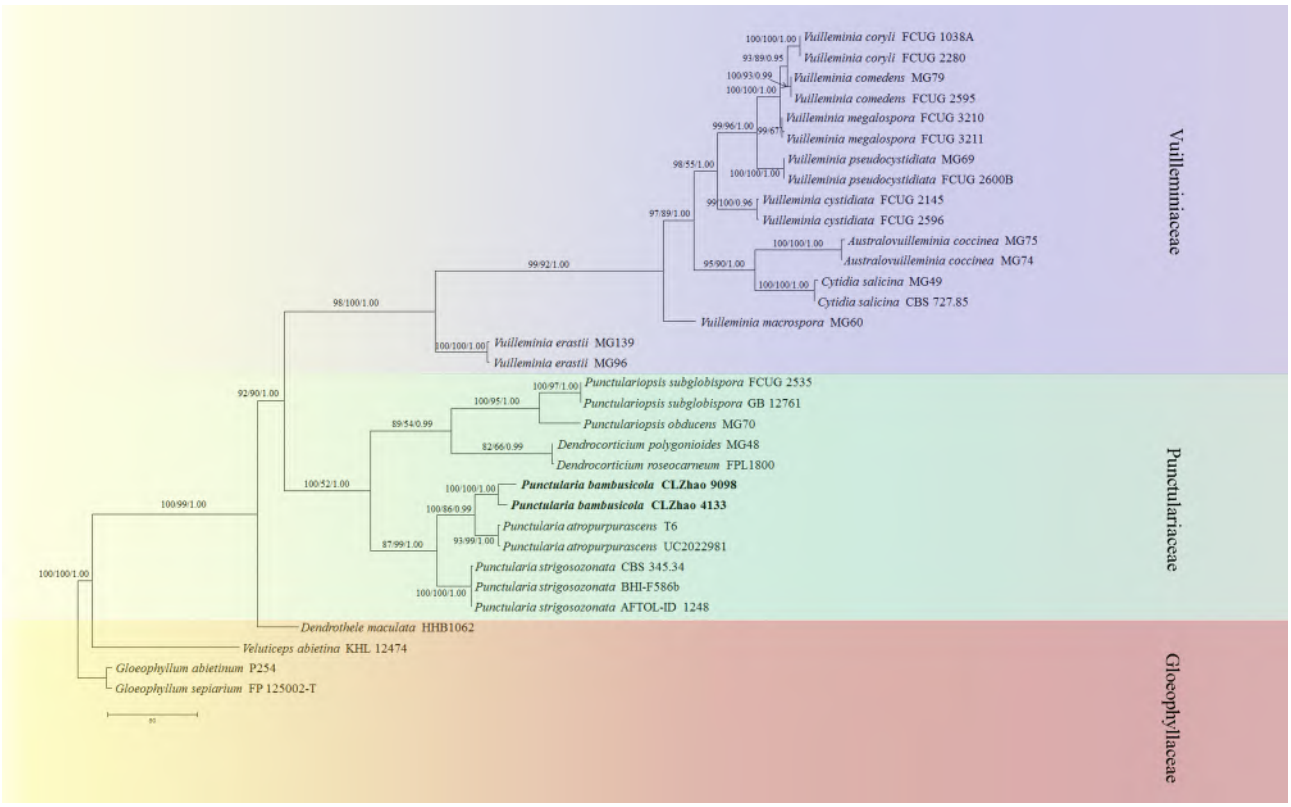

FIGURE 1. Maximum parsimony strict consensus tree illustrating the phylogeny of *Punctularia bambusicola* and related species in *Punctularia* based on ITS+nLSU sequences. Branches are labeled with maximum likelihood bootstrap equal to or higher than 70%, parsimony bootstrap proportions equal to or higher than 50% and Bayesian posterior probabilities equal to or higher than 0.95 respectively.

Taxonomy

Punctularia bambusicola C.L. Zhao, *sp. nov.* (Figs. 2, 3)
MycoBank no.: MB 838694

Holotype.—CHINA. Yunnan Province, Puer, Zhenyuan County, Heping Town, Ailaoshan National Nature Reserve, E 100°54′52″, N23°46′32″, alt. 2136 m, on dead bamboo, 1 January 2019, *CLZhao 9098* (SWFC).
Etymology.—*Bambusicola* (Lat.): referring to the host of bamboo.

Basidiomata.—Annual, resupinate, adnate but easily separable, gelatinosus when fresh, becoming rigid upon drying, up to 27 cm long, up to 5 cm wide, 100–300 µm thick. Hymenial surface tuberculate with rose tints, pink to rose when fresh, turn to lilac to purple upon drying. Sterile narrow, white.

Hyphal structure.—Hyphal system monomitic; generative hyphae with clamp connections, IKI–, CB–; tissues unchanged in KOH. Subiculum absent or indistinct, suhymenial generative hyphae hyaline, more or less interwoven, thin-walled, frequently branched, 2–3 µm in diam., abhymenial generative hyphae brown, thick-walled, branched, 3–5 µm in diam.

Hymenium.—Cystidia and cystidioles absent; dendrohyphidia numerous, yellowish to brown, thin-walled, with short branches, usually forming a catahymenium, 19–42 × 1.5–3 µm; basidia clavate, flexuose, with four sterigmata and a basal clamp connection, 17.5–21.5 × 4–5.5 µm; basidioles dominant, in shape similar to basidia, but slightly smaller.

Spores.—Basidiospores ellipsoid, yellowish, thin-walled, smooth, IKI–, CB–, 6.5–8.5(–9) × 3.5–5 µm, L = 7.62 µm, W = 4.48 µm, Q =1.7 7 (n = 60/2)..

Additional specimen examined.—CHINA. Yunnan Province, Puer, Jingdong County, Wuliangshan National Nature Reserve, , E 100°54′46″, N23°46′45″, alt. 2169 m, on dead bamboo, 5 October 2017, *CLZhao 4133* (SWFC).

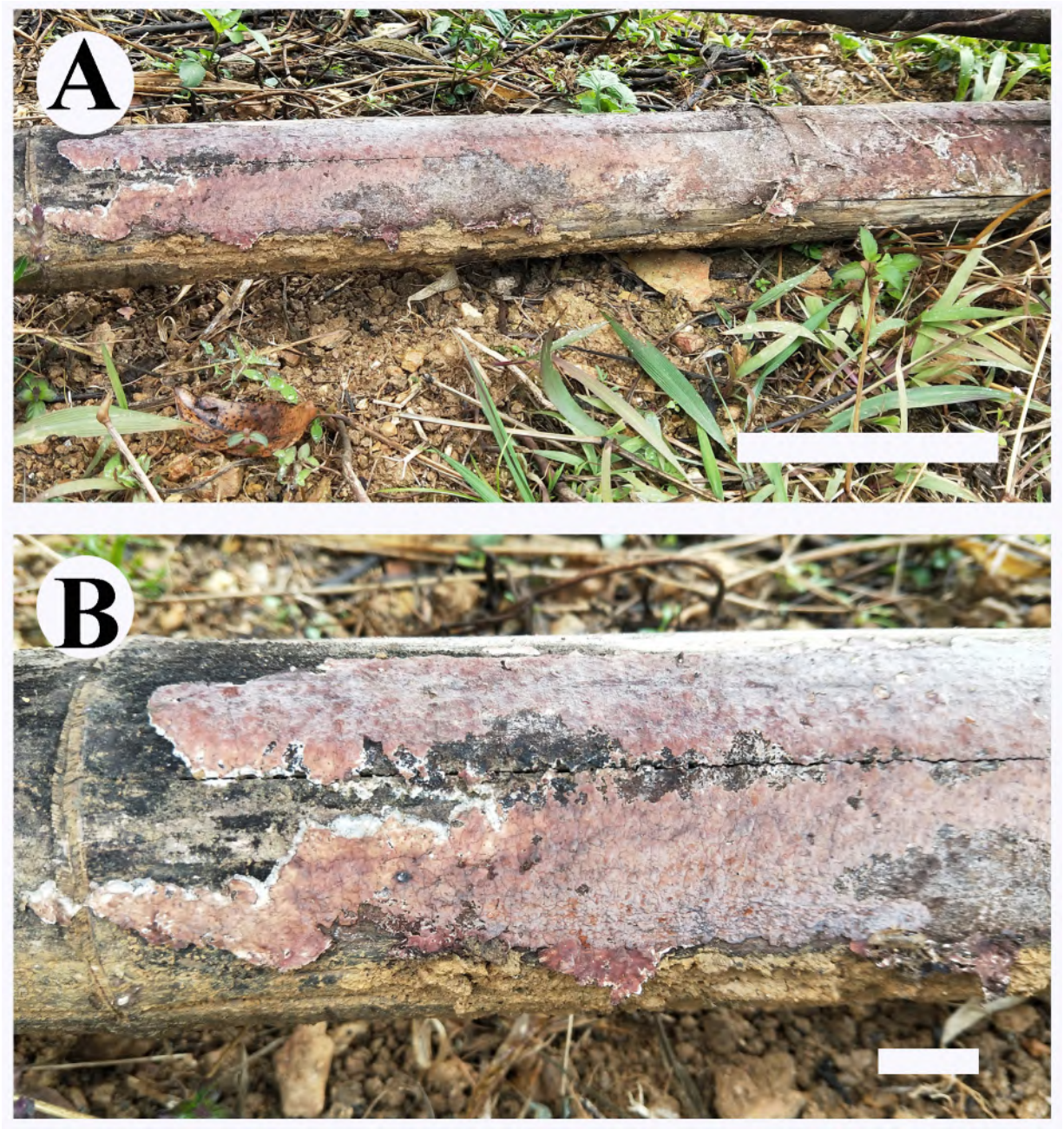

FIGURE 2. Basidiomata of *Punctularia bambusicola* (holotype Bars: A = 7.5 cm, B = 3 cm. Drawings by: Qian-Xin Guan.

Discussion

In the present study, a new species, *Punctularia bambusicola*, is described based on phylogenetic analyses and morphological characteristics.

Phylogenetically, *Punctularia bambusicola* is closely related to *P. atropurpurascens* and *P. strigosozonata* based on ITS+LSU-nrRNA gene analyses (Fig. 1), which is similar to the previous study of Knijn & Ferretti (2018). However, morphologically *P. atropurpurascens* differs from *P. bambusicola* by the effuse-reflexed, thicker basidiomata (up to 1 mm) with reddish brown to dark purplish brown or bluish hymenial surface and presence of fibrillose margin and larger basidia measuring 40–65 × 5–6 µm (Petch 1916). *Punctularia strigosozonata* differs in its resupinate to effuse-reflexed basidiomata with brown, velutinous margin and brown to dark violaceous hymenial surface (Bernicchia & Gorjón 2010).

Punctularia atropurpurascens is normally found in the subtropical and tropical areas and it has been observed

on branch of *Acacia*, *Mangifera*, *Pinus* and *Quercus* (Petch 1916, Knijn & Ferretti 2018). *Punctularia strigosozonata* is mainly distributed in the North Europe and it was found on branch of *Pistacia*, *Populus* and *Quercus* (Talbot 1958, Bernicchia & Gorjón 2010). In the present study, the new species is found on a dead bamboo.

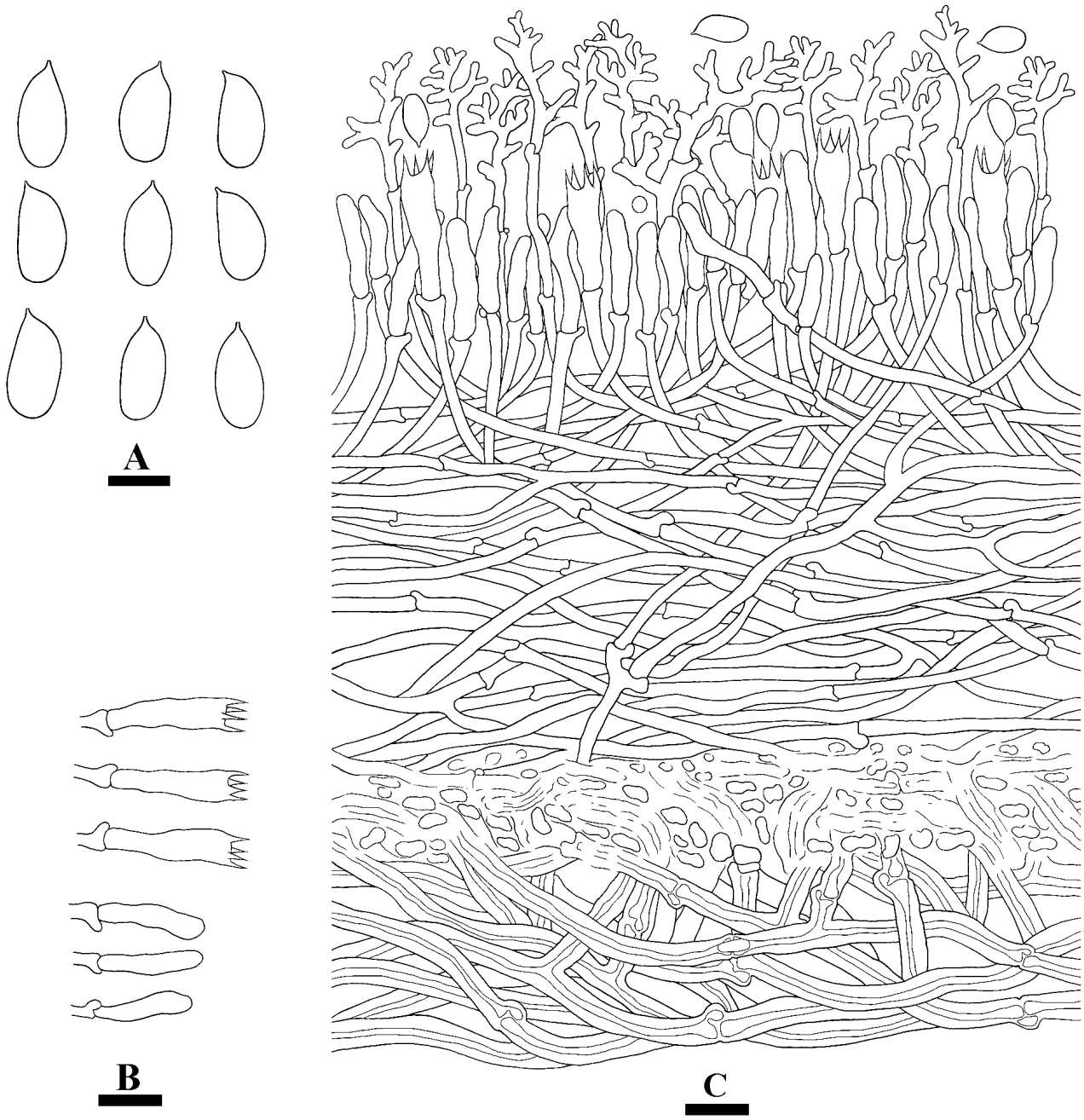

FIGURE 3. Microscopic structures of *Punctularia bambusicola* (drawn from the holotype). A: basidiospores. B: basidia and basidioles. C: a section of hymenium. Bars: A = 5 µm, B–C = 10 µm. Drawings by: Qian-Xin Guan.

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