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Peniophorella cremea sp. nov. from China

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Abstract

A new wood-inhabiting fungal species, *Peniophorella cremea sp. nov.* is proposed based on a combination of morphological features and molecular evidence. *Peniophorella cremea* is characterized by an annual growth habit, resupinate basidiomata with odontioid hymenial surface, a monomitic hyphal system with generative hyphae bearing clamp connections and ellipsoid, hyaline, thin-walled, smooth basidiospores measuring as 7–9.5 × 2.7–3.3 µm. The phylogenetic analyses based on ITS sequences showed that *P. cremea* forms a monophyletic lineage with high statistical supports (100% BS, 100% BP and 1.00 BPP).

Keywords: *Hyphoderma*, phylogeny, taxonomy, wood-inhabiting fungi, Yunnan Province

Introduction

Peniophorella P. Karst. (1889: 427) (Hyphodermataceae, Basidiomycota) was typified by *P. pubera* (Fr.) P. Karst. (1889: 427), which is characterized by resupinate to effuse-reflexed basidiomata with ceraceous to corneus, smooth to tuberculate to odontioid hymenophore with white to yellowish hymenial surface; hyphal system monomitic with generative hyphae bearing clamp connections; cystidia of several types as leptocystidia, gloeocystidia and echinulated cells (echinocysts or stephanocysts) present in some species; basidiospores cylindrical, ellipsoid or allantoid, smooth, thin-walled, IKI–, CB– (Karsten 1889, Bernicchia & Gorjón 2010). So far about 20 species have been accepted in the genus worldwide (Karsten 1889, Donk 1957, 1962, Jülich 1974, 1978, Malençon 1982, Gilbertson & Blackwell 1984, Wu 1990, Boidin & Gilles 1991, Larsson 2007, Hjortstam & Ryvarden 2009, Duhem & Buyck 2011, Nakasone 2012, Duhem 2013, Prasher 2015, Index Fungorum 2020; http://www.mycobank.org/Biolomics.aspx?Table=Mycobank).

Molecular phylogenetic studies of *Peniophorella* have been carried out by Larsson (2007), Telleria *et al.* (2012) and Justo *et al.* (2017). Molecular phylogeny of *Hyphoderma* Wallr. (1833: 576) and *Peniophorella* revealed nineteen new combinations in *Peniophorella* (Larsson 2007). Studies of *Hyphoderma* (Meruliaceae, Polyporales) and its closely related taxa showed eight *Peniophorella* species closely grouped and are distinct from *Hyphoderma* s.s (Telleria *et al.* 2012). A revised family-level classification of the Polyporales proposed twenty species of *Peniophorella* in Hyphodermataceae Jülich (Hymenochaetales) (Justo *et al.* 2017).

In this paper we describe a new species of *Peniophorella* collected from Yunnan Province, China, based on both morphological data and molecular phylogenetic analysis.

Materials and methods

Sample collection

All basidiomata were collected in Yeyahu Forestry Park, Kunming, Yunnan Province, China. The macro-morphological characteristics of basidiomata such as colour, size, texture and smell, were recorded in situ before they are collected. The host, type of decay and the habitat were also recorded (Dai & Xiong 2012).

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Morphological study

The specimens studied are deposited at the herbarium of Southwest Forestry University (SWFC), Kunming, Yunnan Province, P.R. China. Macromorphological descriptions are based on field notes. Colour terms follow Petersen (1996). Micromorphological data were obtained from the dried specimens, and observed under a light microscope following Dai (2011, 2012). 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.

DNA extraction, PCR amplification, sequencing 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 with some modifications that a small piece of dried fungal specimen (about 30 mg) was ground 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, 000 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 13, 000 rpm for 0.5 min. Then, 0.5 mL inhibitor removal fluid was added in AC for a centrifugation at 12, 000 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 adsorbed film to elute the genome DNA (Shen *et al.* 2018). The ITS region was amplified with primer pairs 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 (Zhao & Cui 2013). The PCR products were purified and directly sequenced at Kunming Tsingke Biological Technology Limited Company, Kunming, 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 (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 25350). Sequences of *Hyphoderma setigerum* (Fr.) Donk (1957: 15) and *H. subsetigerum* Sheng H. Wu (1997: 137) obtained from GenBank were used as outgroups to root trees following Larsson (2007) in the ITS analysis (Fig. 1).

Maximum parsimony analysis was applied to the ITS dataset sequences. Approaches to phylogenetic analyses followed Wu *et al.* (2019), 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 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. Sequences were also analyzed using Maximum Likelihood (ML) with RAxML-HPC2 through the Cipres Science Gateway (www.phylo.org, Miller *et al.* 2009). Branch support (BS) for ML analysis was determined by 1000 bootstrap replicate.

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 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 run for 2 runs from random starting trees for 340 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 trees of all remaining trees was calculated. Branches were considered as significantly supported if they received maximum likelihood bootstrap values (ML) >75%, maximum parsimony bootstrap values (MP) >75%, or Bayesian posterior probabilities (PP) >0.95.

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

Species name	Sample no.	GenBank accession no. ITS	References
Hyphoderma setigerum	NH 8211	AJ534273	Nilsson *et al.* 2003
H. subsetigerum	FCUG 2935	AJ534276	Nilsson *et al.* 2003
Peniophorella cremea	CLZhao 1606	MT955162	This study
P. cremea	CLZhao 1719	MT955163	This study
P. cremea	CLZhao 1493	MT955164	This study
P. cremea	CLZhao 1768	MT955165	This study
P. cremea	CLZhao 1667	MT955166	This study
P. cremea	CLZhao 1261	MT955167	This study
P. echinocystis	KHL 6284	DQ677494	Larsson 2007
P. guttulifera	NH 12012	DQ647501	Hallenberg *et al.* 2007
P. guttulifera	NH 7813	DQ647502	Hallenberg *et al.* 2007
P. odontiiformis	TMIC 34389	DQ647496	Hallenberg *et al.* 2007
P. odontiiformis	TMIC 50047	DQ647500	Hallenberg *et al.* 2007
P. pertenuis	NH 15115	DQ647487	Hallenberg *et al.* 2007
P. pertenuis	WU 960613	DQ647477	Hallenberg *et al.* 2007
P. pertenuis	TMI 16118	DQ647484	Hallenberg *et al.* 2007
P. pertenuis	NH 11063	DQ647485	Hallenberg *et al.* 2007
P. pertenuis	NH 12429	DQ647486	Hallenberg *et al.* 2007
P. pertenuis	NH 3868	DQ647479	Hallenberg *et al.* 2007
P. praetermissa	NH 3494	DQ647453	Hallenberg *et al.* 2007
P. praetermissa	NH 9815	DQ647454	Hallenberg *et al.* 2007
P. praetermissa	AN 3031	DQ647463	Hallenberg *et al.* 2007
P. praetermissa	NH 7827	DQ647460	Hallenberg *et al.* 2007
P. praetermissa	NH 11451	DQ647455	Hallenberg *et al.* 2007
P. praetermissa	NH 11803	DQ647456	Hallenberg *et al.* 2007
P. pubera	NH 10380	DQ647504	Hallenberg *et al.* 2007
P. pubera	NH 10512	DQ647505	Hallenberg *et al.* 2007
P. pubera	EL 4439	DQ647503	Hallenberg *et al.* 2007
P. pubera	NH 3950	DQ647506	Hallenberg *et al.* 2007
P. pubera	NH 12069	DQ647507	Hallenberg *et al.* 2007
P. pubera	FCUG 3126	GQ409535	Hallenberg *et al.* 2007
P. rude	Wu 9307-39	DQ647499	Hallenberg *et al.* 2007
P. rude	Wu 0104-3	DQ647495	Hallenberg *et al.* 2007
P. subpraetermissa	Wu 950627	DQ647493	Hallenberg *et al.* 2007

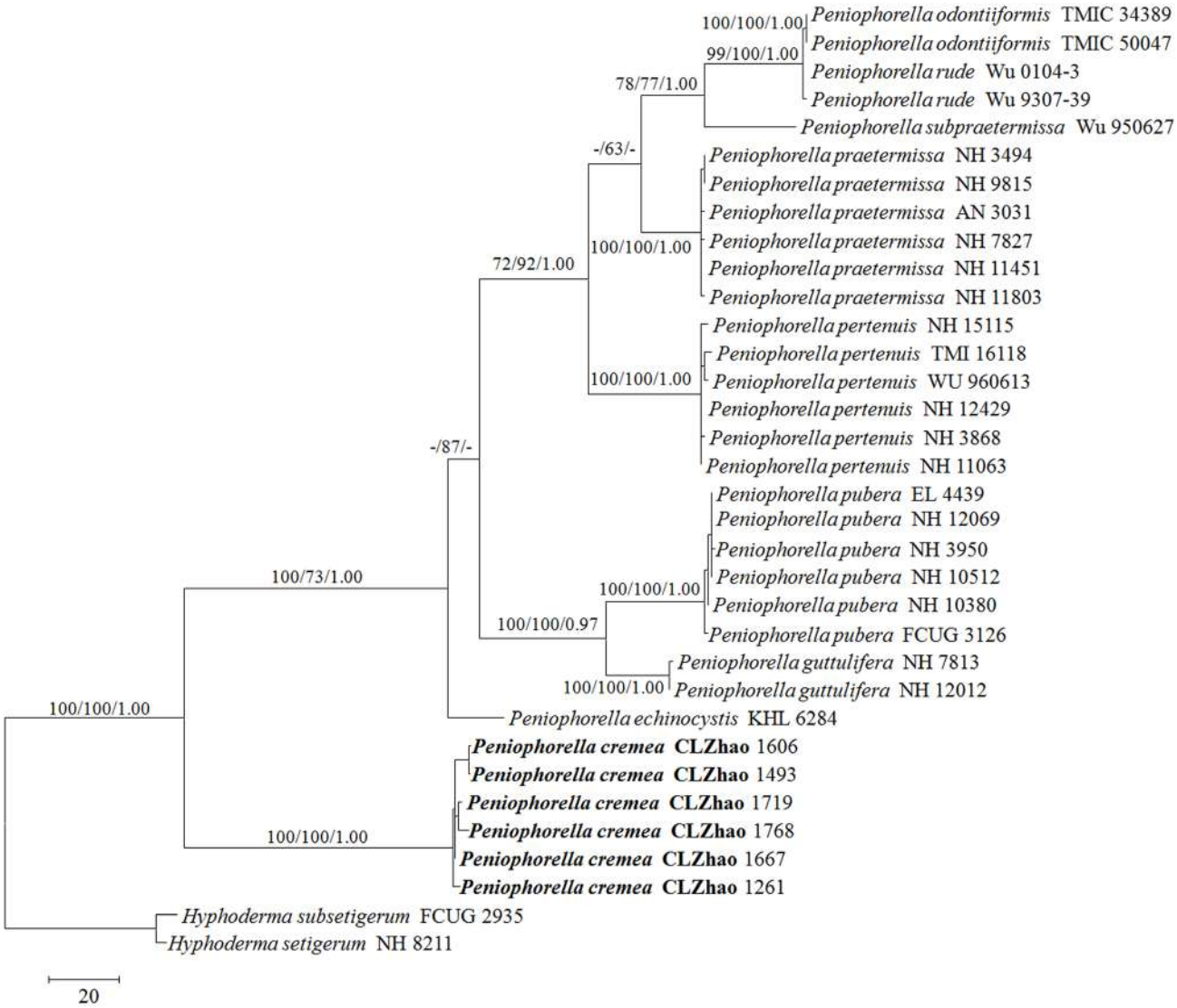

FIGURE 1. Maximum Parsimony strict consensus tree illustrating the phylogeny of *Peniophorella cremea* and related species in *Peniophorella* based on ITS sequences. Branches are labelled with maximum likelihood bootstrap higher than 70%, parsimony bootstrap proportions higher than 50% and Bayesian posterior probabilities more than 0.95, respectively.

Results

Molecular phylogeny

The ITS dataset (Fig. 1) included sequences from 34 fungal specimens representing 11 species. The dataset had an aligned length of 694 characters, of which 364 characters are constant, 28 are variable and parsimony-uninformative, and 302 are parsimony-informative. Maximum parsimony analysis yielded 5000 equally parsimonious trees (TL = 577, CI = 0.805, HI = 0.194, RI = 0.947, RC = 0.763). 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). Bayesian analysis and ML analysis resulted in a similar topology as MP analysis, with an average standard deviation of split frequencies = 0.009962 (BI).

The phylogenetic tree (Fig. 1) inferred from ITS sequences revealed that *Peniophorella cremea* formed a monophyletic lineage with a high supports (100% BS, 100% BP and 1.00 BPP).

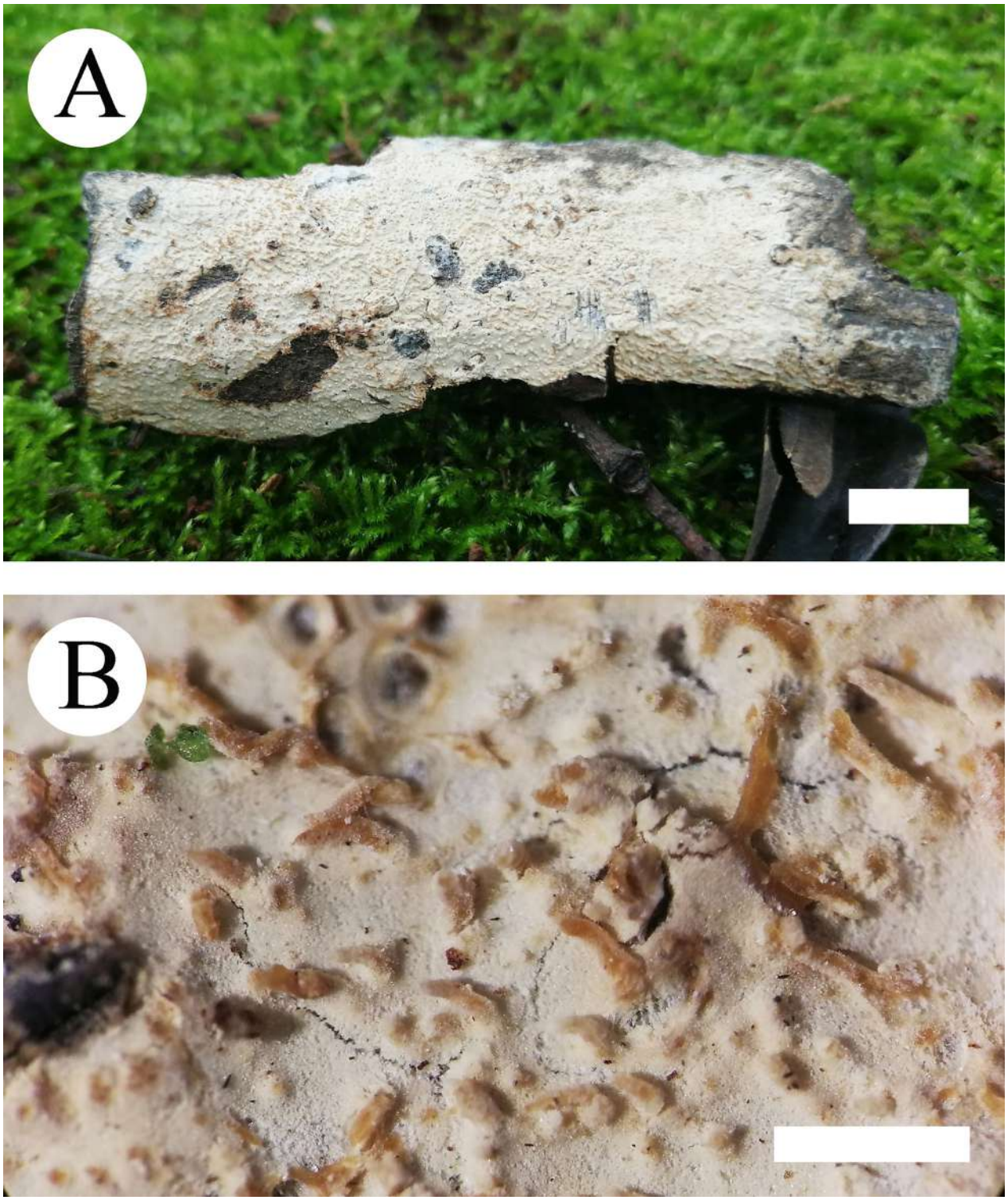

FIGURE 2. Basidiomata of *Peniophorella cremea* (holotype). Scale bars: A=1 cm, B=1 mm.

Taxonomy

Peniophorella cremea C.L. Zhao, ***sp. nov.*** (Figs. 2, 3)
MycoBank no.: MB 836887

Type.—**CHINA**. Yunnan Province, Kunming, Yeyahu Forestry Park, on angiosperm trunk, 23 June 2017, *CLZhao 1606* (holotype, SWFC!)

Etymology.—*Cremea* (Lat.): refers to cream hymenial surface.

Basidiomata.—Annual, resupinate, ceraceous when fresh, becoming corneus upon drying, up to 15 cm long, 3 cm wide, 100–300 µm thick. Hymenial surface odontioid, pale cream when fresh, turning to cream when drying.

Hyphal structure.—Hyphal system monomitic, generative hyphae with clamp connections, hyaline, thin-walled, frequently branched, 2.5–4 µm in diameter, IKI–, CB–; tissues unchanged in KOH.

Hymenium—Cystidia of two types: (i) subcylindrical, sinuous, 38–65 × 5–8.5 µm, (ii) cylindrical, thin-walled, 20–48 × 3–4.5 µm; basidia clavate to subclavate, constricted, with 4-sterigmata and a basal clamp connection, 17–25 × 4–6.5 µm, basidioles dominant, in shape similar to basidia, but slightly smaller.

Basidiospores—Ellipsoid, hyaline, thin-walled, smooth, IKI–, CB–, (6.8–)7–9.5(–10) × (2.4–)2.7–3.3(–3.6) µm, L = 8.46 µm, W = 3.08 µm, Q = 2.55–2.97 (n = 180/6).

Additional specimens examined.—CHINA. Yunnan Province, Kunming, Yeyahu Forestry Park, on fallen branch of an angiosperm, 23 June 2017, *CLZhao 1667*, *CLZhao 1719*, *CLZhao 1768* (SWFC!); Kunming, Xishan District, Haikou Forestry Park, on fallen branch of angiosperm, 22 April 2017, *CLZhao 1261* (SWFC!), 23 April 2017, *CLZhao 1493* (SWFC!).

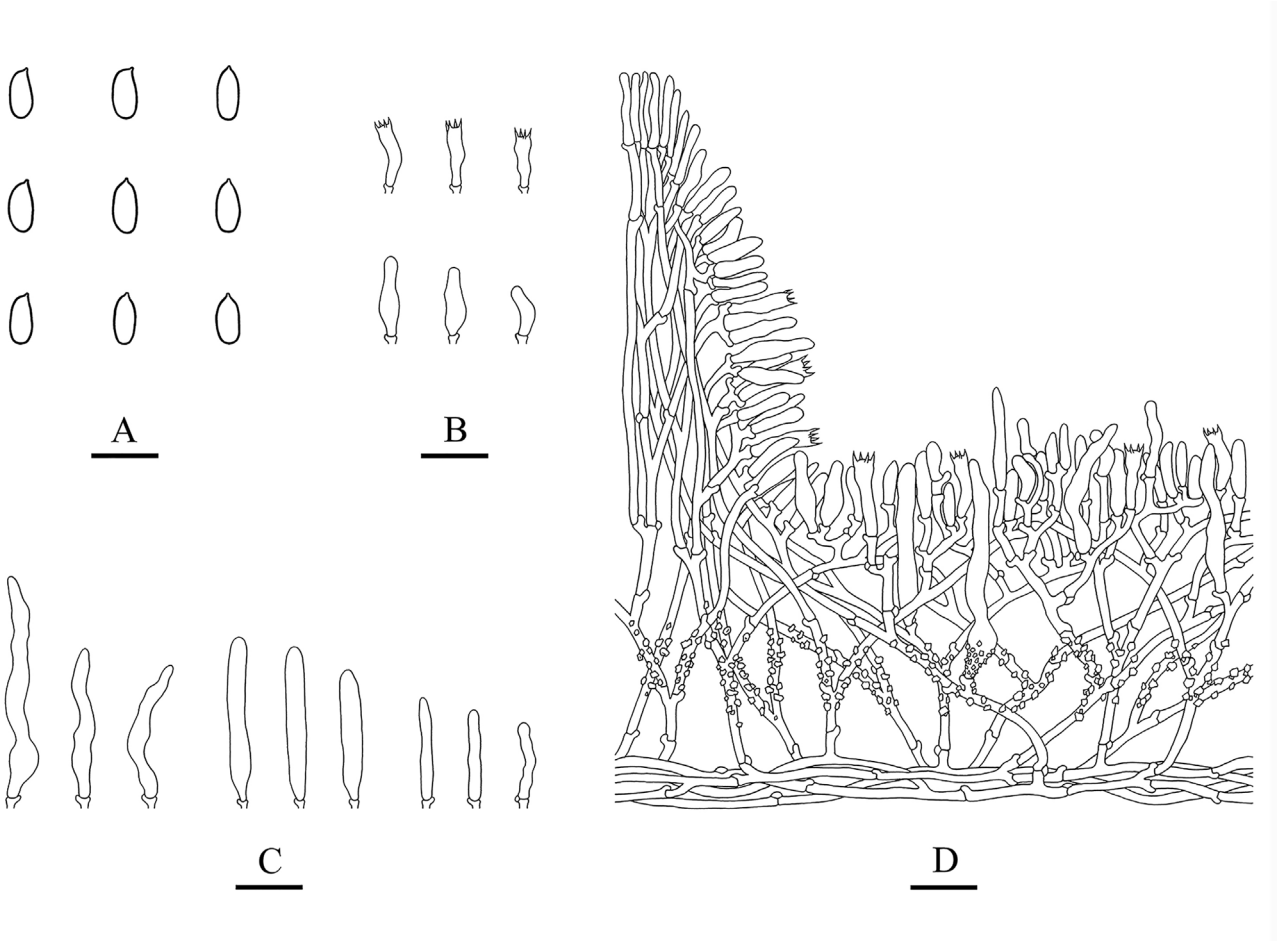

FIGURE 3. Microscopic structures of *Peniophorella cremea*. (drawn from the holotype). A. Basidiospores. B. Basidia and basidioles. C. Cystidia. D. A section of hymenium. Bars: A=5 µm; B, C, D = 10 µm.

Discussion

In the present study, a new species, *Peniophorella cremea sp. nov.*, is described based on phylogenetic analyses and morphological characteristics.

Phylogenetically, *Peniophorella cremea* formed a monophyletic lineage in the ITS phylogeny (Fig. 1), and grouped with *P. echinocystis* (J. Erikss. & Å. Strid) K.H. Larss. (2007: 191). But morphologically *P. echinocystis* differs from *P. cremea* by having the globose echinocysts and allantoids and larger basidiospores (9–12 × 2.5–2 µm, Larsson 2007, Bernicchia & Gorjón 2010).

Morphologically, *Peniophorella comptopsis* (Burds. & Nakasone) K.H. Larss. (2007: 191) is similar to *P. cremea* based on odontioid hymenophore, but *P. comptopsis* can be separated from *P. cremea* by yellowish white to greyish yellow hymenial surface and smaller basidiospores (4.5–5.5 × 3–4 µm, Burdsall & Nakasone 1981).

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