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Trechispora yunnanensis sp. nov. (Hydnodontaceae, Basidiomycota) from China

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Abstract

A new wood-inhabiting fungal species, *Trechispora yunnanensis* sp. nov., is proposed based on morphological characteristics and molecular phylogenetic analyses. The species is characterized by resupinate basidiomata, rigid and fragile up on drying, cream to pale greyish hymenial surface; a monomitic hyphal system with generative hyphae bearing clamp connections, IKI-, CB-; ellipsoid, hyaline, thick-walled, ornamented, IKI-, CB- basidiospores measuring as 7–8.5 × 5–5.5 µm. The internal transcribed spacer (ITS) and the large subunit (LSU) regions of nuclear ribosomal RNA gene sequences of the studied samples were generated, and phylogenetic analyses were performed with maximum likelihood (ML), maximum parsimony (MP) and bayesian inference methods (BPP). The phylogenetic analyses based on molecular data of ITS+nLSU sequences showed that *T. yunnanensis* formed a monophyletic lineage with a strong support (100% ML, 100% MP, 1.00 BPP) and was closely related to *T. byssinella* and *T. laevis*. Both morphological characteristics and results of molecular phylogenetic analyses confirmed the placement of the new species in *Trechispora*.

Keywords: Morphological characteristics, Phylogenetic analysis, Taxonomy, Wood-rotting fungi

Introduction

Trechispora P. Karst. (1890: 147) is a large cosmopolitan genus characterized by resupinate to effused basidiomata; smooth to hydnoid to poroid hymenophore; ampullaceous septa; short cylindric basidia and smooth to verrucose or aculeate basidiospores (Karsten 1890, Bernicchia & Gorjón 2010). Forty-eight species are currently known in the genus *Trechispora* worldwide (Liberta 1966, 1973, Larsson 1994, 1995, 1996, Ryvarden 2002, Trichiès & Schultheis 2002, Miettinen & Larsson 2006, Kirk *et al*. 2008, Bernicchia & Gorjón 2010, Ordynets *et al*. 2015).

Molecular study based on the large subunit nuclear ribosomal RNA gene (nLSU) datasets demonstrated the phylogeny of *T. thelephora* (Lév.) Ryvarden (2002: 32) and showed that molecular data support the transfer of *Hydnodon thelephorus* (Lév.) Banker (1913: 297) to the genus *Trechispora* (Albee-Scott & Kropp 2010). Molecular phylogenetic studies employing the nuclear ribosomal LSU and the internal transcribed spacer (ITS) regions have helped to investigate phylogenetic overview of the order Trechisporales and suggested that the genera *Porpomyces* Jülich (1982: 425), *Sistotremastrum* J. Erikss. (1958: 62), *Subulicystidium* Parmasto (1968: 120) and *Trechispora* belong to a highly supported clade and *Trechispora* belongs to Hydnodontaceae and is closely related to the genus *Brevicellicium* K.H. Larss. & Hjortstam (1978: 117) (Telleria *et al*. 2013). The phylogenetic study of *Trechispora* inferred from the combined data of the ITS and LSU datasets have demonstrated two new *Trechispora* species: *T. cyatheae* Ordynets, Langer & K.H. Larss. (2015: 3) and *T. echinocristallina* Ordynets, Langer & K.H. Larss. (2015: 5) that were found in La Réunion Island (Ordynets *et al*. 2015).

During our investigations on the diversity of wood-rotting fungi in southern China, an undescribed species belongs to *Trechispora* was found. To confirm the placement of the undescribed species of *Trechispora*, morphological examination and phylogenetic analyses based on the ITS and nLSU sequences were carried out.

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Materials and methods

Morphological studies.—The specimens studied are deposited at the herbarium of Southwest Forestry University (SWFC). Macro-morphological descriptions were based on field notes. Petersen (1996) was followed for the special colour terms. Micro-morphological data were obtained from the dried specimens, and observed under a light microscope (Nikon Eclipse E 80i) following Dai (2010). The following abbreviations were used for the micro characteristics description: 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, 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 of 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. ITS region was amplified with primer pairs ITS5 and ITS4 (White *et al*. 1990). Nuclear LSU region was amplified with primer pairs 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 Chen *et al*. (2016). The PCR products were purified and sequenced at Kunming Tsingke Biological Technology Limited Company. 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 6 (Katoh & Toh 2008, 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 22374). Sequences of *Fibrodontia alba* Yurchenko & Sheng H. Wu (2012: 339) and *F. gossypina* Parmasto (1968: 207) retrieved from GenBank were used as outgroups in the ITS+nLSU analyses by following Ordynets *et al*. (2015).

Maximum parsimony analysis was applied to the ITS+nLSU sequences dataset. Approaches to phylogenetic analyses followed Zhao & Cui (2014), and the phylogenetic tree construction 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 analysed using Maximum Likelihood (ML) with RAxML-HPC2 through the Cipres Science Gateway (www.phylo.org; Miller *et al*. 2009). Branch support 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). Bayesian inference was calculated with MrBayes3.1.2 with a general time reversible (GTR) 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 3 million generations (ITS+nLSU), 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 (BP) and Bayesian posterior probabilities (BPP) greater than or equal to 75 % (BP) and 0.95 (BPP) were considered as significantly supported, respectively.

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

Species name	Sample no.	GenBank accession no.		References
		ITS	nLSU	
Fibrodontia alba	TNMF 24944	KC928274	KC928275	Ordynets *et al*. 2015
F. gossypina	GEL 5042	DQ249274	AY646100	Ordynets *et al*. 2015
Trechispora alnicola	AFTOL-ID 665	AY635768	AY635768	Albee-Scott & Kropp 2010
T. araneosa	KHL 8570	AF347084	AF347084	Larsson *et al*. 2004
T. byssinella	UC 2023068	KP814481	-	Ordynets *et al*. 2015
T. cohaerens	TU 110332	UDB008249	-	Ordynets *et al*. 2015
T. cohaerens	TU 115568	UDB016421	-	Ordynets *et al*. 2015
T. cohaerens	UC 2023013	KP814144	-	Ordynets *et al*. 2015
T. confinis	KHL 11064	AF347081	AF347081	Larsson *et al*. 2004
T. cyatheae	FR-0219442	UDB024014	UDB024014	Ordynets *et al*. 2015
T. cyatheae	FR-0219443	UDB024016	UDB024014	Ordynets *et al*. 2015
T. echinocristallina	FR-0219445	UDB024018	UDB024018	Ordynets *et al*. 2015
T. echinocristallina	FR-0219448	UDB024022	-	Ordynets *et al*. 2015
T. echinocristallina	FR-0219449	UDB024023	UDB024023	Ordynets *et al*. 2015
T.farinacea	KHL 8454	AF347083	AF347083	Larsson *et al*. 2004
T. farinacea	TUB 011825	EU909231	EU909231	Ordynets *et al*. 2015
T. hymenocystis	TL 11112	UDB000778	UDB000778	Ordynets *et al*. 2015
T. hymenocystis	KHL 8795	AF347090	AF347090	Larsson *et al*. 2004
T. incisa	EH 24/98	AF347085	-	Larsson *et al*. 2004
T. kavinioides	KGN 981002	AF347086	AF347086	Larsson *et al*. 2004
T. laevis	TU 115551	UDB016468	-	Ordynets *et al*. 2015
T. mollusca	DLL 2010-077	JQ673209	-	Ordynets *et al*. 2015
T. mollusca	DLL2011-186	KJ140681	-	Ordynets *et al*. 2015
T. nivea	G. Kristiansen	AY586720	AY586720	Larsson *et al*. 2004
T. regularis	KHL 10881	AF347087	AF347087	Larsson *et al*. 2004
T. stellulata	UC 2022880	KP814437	-	Ordynets *et al*. 2015
T. stellulata	UC 2023099	KP814451	-	Ordynets *et al*. 2015
T. stevensonii	TU 115499	UDB016467	UDB016467	Ordynets *et al*. 2015
T. subsphaerospora	KHL 8511	AF347080	AF347080	Larsson *et al*. 2004
T. thelephora	UTC 252606	HM104485	-	Albee-Scott & Kropp 2010
T. yunnanensis	CLZhao 210	MN654918	MN654921	Present study
T. yunnanensis	CLZhao 214	MN654919	MN654922	Present study
T. yunnanensis	CLZhao 215	MN654920	MN654923	Present study

Results

Molecular phylogeny

The ITS+nLSU dataset comprised sequences from 33 fungal specimens representing 22 species. The dataset had an aligned length of 2205 characters, of which 1499 characters are constant, 129 are variable and parsimony-uninformative, and 577 are parsimony-informative. Maximum parsimony analysis yielded two equally parsimonious trees (TL = 1984, CI = 0.551, HI = 0.449, RI = 0.638, RC = 0.351). 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 = 0.003782 (BI).

The phylogeny (Fig. 1) inferred from combined ITS+nLSU sequences demonstrated that *Trechispora yunnanensis* formed a monophyletic lineage with a strong support (100% ML, 100% MP, 1.00 BPP) and is closely related to *T. byssinella* (Bourdot) Liberta and *T. laevis* K.H. Larss.

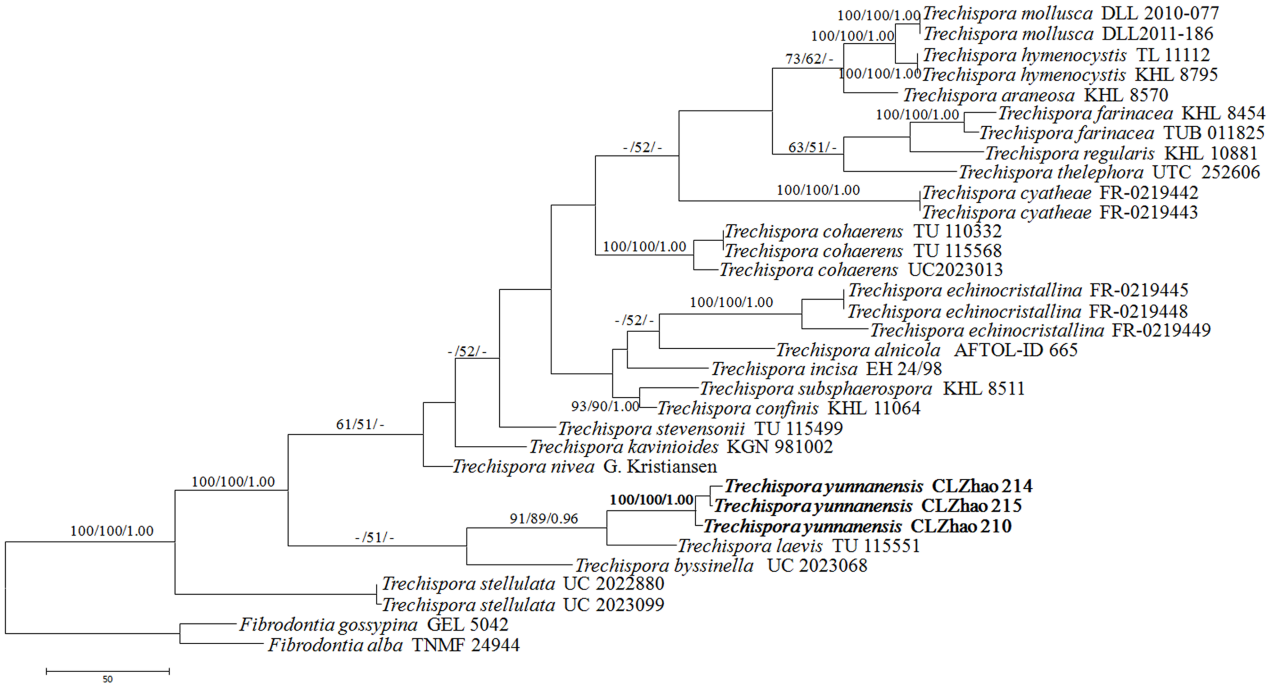

FIGURE 1. Maximum parsimony strict consensus tree illustrating the phylogeny of *Trechispora yunnanensis* and related species based on combined ITS+nLSU sequences. Branches are labelled with maximum likelihood bootstrap equal to or higher than 70%, parsimony bootstrap proportions equal to or higher than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

Taxonomy

Trechispora yunnanensis C.L. Zhao, *sp. nov.* (Figs. 2, 3)

MycoBank no.: MB 833229

Type.—CHINA. Yunnan Province, Kunming, Shuanglong county, Yeyahu Forestry Park, alt. 1980 m, on fallen angiosperm branch, 2 August 2016, *CLZhao 210* (holotype, SWFC!).

Etymology.—*Yunnanensis* (Lat.): referring to the locality (Yunnan) of the type specimen.

Basidiomata.—Annual, resupinate, thin, growing adnate but easily separable, soft without odor or taste when fresh, becoming rigid and fragile up on drying, up to 10 cm long, 100–500 µm thick. Hymenial surface smooth to farinaceous with white to cream tints, white to cream (60, 21) when fresh, turn to cream to pale greyish (21, 55) upon drying. Sterile determined, concolorous with hymenial surface.

Hyphal structure.—Hyphal system monomitic; generative hyphae with clamp connections, IKI-, CB-; tissues unchanged in KOH. Subiculum absent or indistinct, hymenium thickening, hyphae colorless, more or less interwoven, thin-walled, branched, 2.5–4.5 µm in diam.

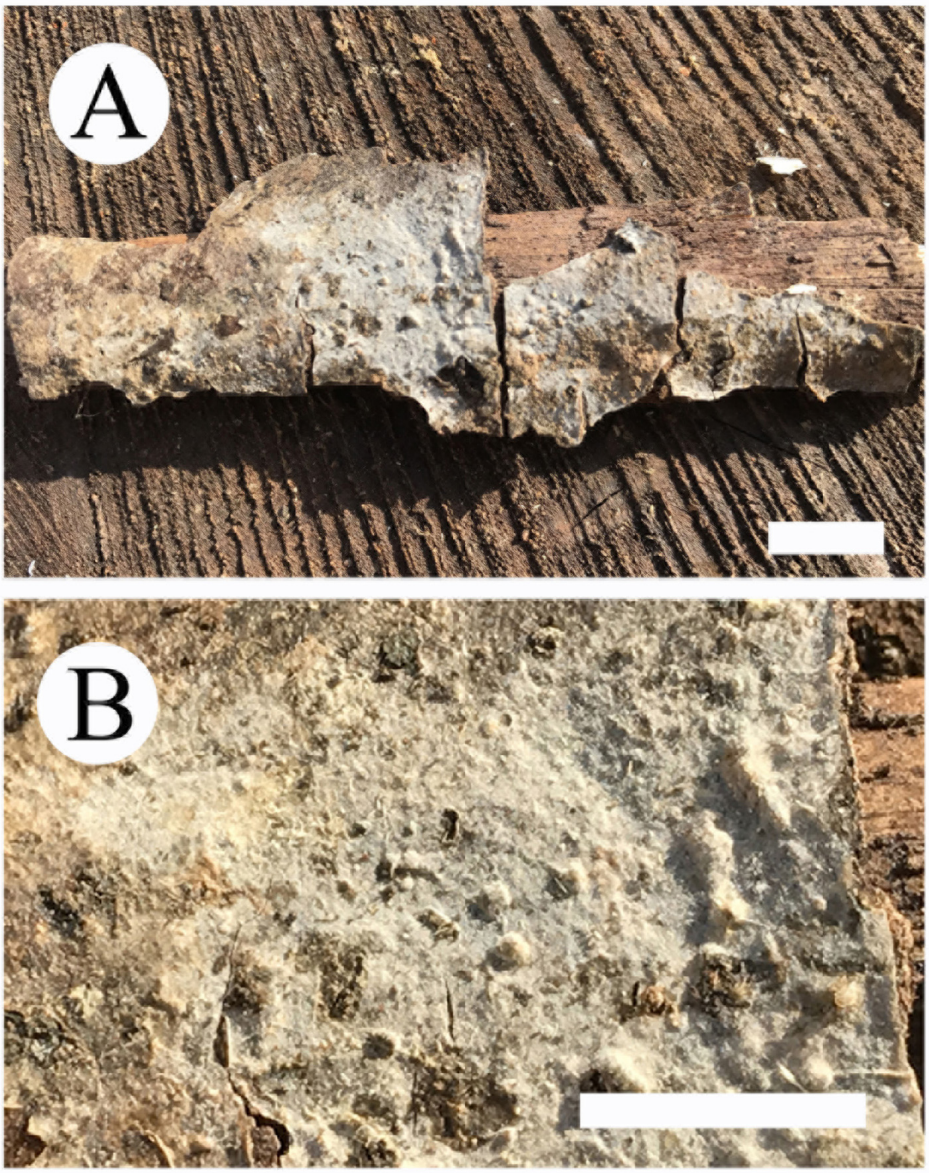

FIGURE 2. Basidiomata of *Trechispora yunnanensis* (holotype). Scale bars: a—2 cm, b—5 mm.

Hymenium—Cystidia and cystidioles absent; basidia clavate, with two sterigmata and a basal clamp connection, 9.5–12.5 × 4.5–6 µm; basidioles dominant, in shape similar to basidia, but slightly smaller. Basidiospores ellipsoid, hyaline, thick-walled, ornamented, IKI-, CB-, (6.5–)7–8.5(–9) × (4.5–)5–5.5(–6) µm, L = 7.77 µm, W = 5.17 µm, Q = 1.49–1.53 (n = 90/3).

Additional specimens examined.—CHINA. Yunnan Province, Kunming, Shuanglong county, Yeyahu Forestry Park, alt. 1980 m, on fallen angiosperm branch, 2 August 2016, *CLZhao 214, 215* (paratypes, SWFC!).

Discussion

In the present study, a new species, *Trechispora yunnanensis*, is described based on phylogenetic analysis and morphological characteristics. In the ITS+nLSU analyses (Fig. 1), our new species formed a monophyletic lineage together with *T. byssinella* and *T. laevis* (100% ML, 100% MP, 1.00 BPP). However, morphologically *T. byssinella* differs from *T. yunnanensis* by having arachnoid to pellicular hymenophore and generative hyphae encrusted with numerous bipyramidal aggregated crystals and smooth basidiospores (Bernicchia & Gorjon 2010). *Trechispora laevis* differs from *T. yunnanensis* by having the arachnoid to smooth, bluish white hymenophore and smaller basidiospores (3.5–4 × 2.7–3.2 µm, Bernicchia & Gorjon 2010).

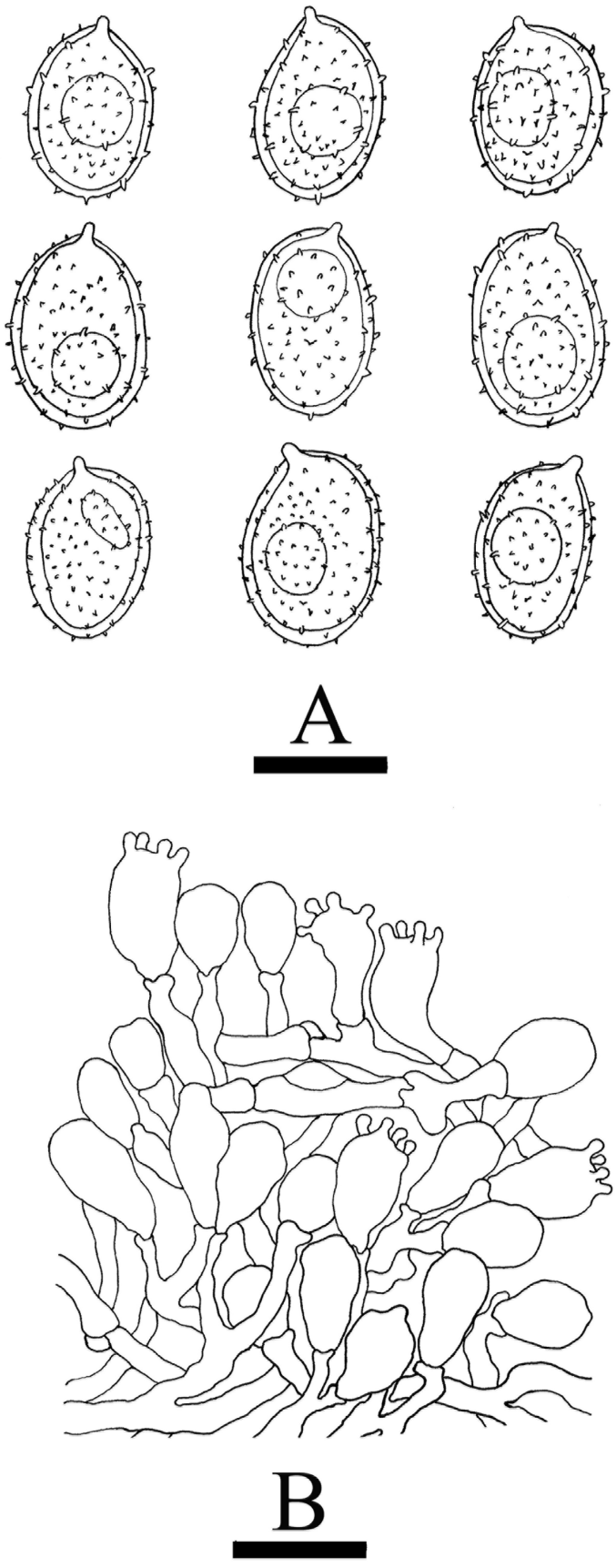

FIGURE 3. Microscopic structures of *Trechispora yunnanensis* (drawn from the holotype). a. Basidiospores. b. A cross section of basidiomata. Bars: a—5 μm; b—10 μm.

Presence of ornamented, larger basidiospores (> 6 µm in length) and a monomitic hyphal system bearing clamp connections of our new species remind three morphologically similar species of *Trechispora*: *T. clancularis* (Park.-Rhodes) K.H. Larss., *T. fastidiosa* (Pers.) Liberta, *T. praefocata* (Bourdot & Galzin) Liberta. *Trechispora clancularis* differs from *T. yunnanensis* by having reticulate to poroid to irpicoid hymenophore, presence of pleurobasidia and subglobose to ovoid, slightly cyanophilous basidiospores (Bernicchia & Gorjon 2010). *Trechispora fastidiosa* differs from *T. yunnanensis* by having smooth to membranaceous hymenophore; generative hyphae encrusted and larger basidia (20–30 × 5–6 µm, Bernicchia & Gorjon 2010). *Trechispora praefocata* differs from *T. yunnanensis* by pale grey to greenish hymenophore with rhizomorphic margin and generative hyphae covered with crystals (Bernicchia & Gorjon 2010).

Wood-rotting fungi are an extensively studied group of Basidiomycota (Gilbertson & Ryvarden 1987, Núñez & Ryvarden 2001, Bernicchia & Gorjon 2010, Ryvarden & Melo 2014), but the wood-rotting fungal diversity in China is still not well known, especially in subtropical and tropical China, many recently described taxa of wood-rotting fungi were found from subtropical and tropical China (Zhou & Dai 2012, 2013; Zhao & Cui 2013, 2014, Chen *et al*. 2017, Ren & Wu 2017, Wu *et al*. 2017, Yuan *et al*. 2017a, b). Most *Trechispora* species have been described from boreal and northern temperate zones, thus, it is possible that more new *Trechispora* taxa will be found after further investigations and molecular analyses.

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