KEW BULLETIN (2021) 76:549–555
DOI 10.1007/S12225-021-09959-Y

ISSN: 0075-5974 (print)
ISSN: 1874-933X (electronic)

Skvortzovia yunnanensis, a new species of corticioid fungus from southern China

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Summary. A new corticioid species, *Skvortzovia yunnanensis* sp. nov., is described based on morphological and molecular characters. *Skvortzovia yunnanensis* is characterised by annual, resupinate, very thin basidiomata with a smooth, white to pale cream hymenial surface and a monomitic hyphal system with clamped generative hyphae and allantoid, colourless, thin-walled, smooth basidiospores (5 – 6.5 × 1 – 1.5 µm) and larger halocystidia. Sequences of internal transcribed spacer (ITS) and large subunit nuclear ribosomal RNA gene (nLSU) regions of the studied samples were generated, and phylogenetic analyses were performed with maximum likelihood, maximum parsimony and Bayesian inference methods. The phylogenetic analyses based on molecular data of ITS and ITS+nLSU sequences showed that *S. yunnanensis* formed a single well-supported lineage (100% maximum likelihood bootstrap (BS), 100% parsimony bootstrap portion (BP) and 1.00 Bayesian posterior probability (BPP)), and then grouped with *S. furfurella* (Bres.) Bononi & Hjortstam and *S. meridionalis* (Burds. & Nakasone) G.Gruhn & Hallenb.

Key Words. molecular phylogeny, taxonomy, wood-inhabiting fungi, Yunnan Province.

Introduction

Skvortzovia Bononi & Hjortstam (Rickenellaceae, Hymenochaetales) is typified by *S. furfurella* (Bres.) Hjortstam & Bononi (Hjortstam & Bononi 1987). It is characterised by a combination of resupinate basidiomata with a light-coloured to pale yellowish brown hymenial surface and an odontioid to moderately fragile hymenophore, a monomitic hyphal system with clamp connections on generative hyphae, the presence of capitate cystida or other sterile elements, and allantoid or subreniform, thin-walled, smooth, *Melzer's reagent* negative (IKI–), cotton blue negative (CB–), acyanophilous basidiospores (Hjortstam & Bononi 1987). To date, about five species have been accepted in the genus worldwide (Hjortstam & Bononi 1987; Nakasone 2007; Gruhn *et al.* 2017; Gruhn & Hallenberg 2018; https://www.mycobank.org; http://www.indexfungorum.org/Names/Names.asp).

A molecular phylogeny inferred from 5.8S and nucLSU rDNA sequences with Bayesian analysis for the hymenochaetoid clade showed that five *Skvortzovia* species, *S. furfuracea* (Bres.) G.Gruhn & Hallenb., *S. furfurella*, *S. georgica* (Parmasto) G.Gruhn & Hallenb., *S. meridionalis* (Burds. & Nakasone) G.Gruhn & Hallenb. and *S. pinicola* (J.Erikss.) G.Gruhn & Hallenb., nested into the *Rickenella* clade and then grouped with *Resinicium* Parmasto (Larsson *et al.* 2006). Morphological and molecular studies on *Resinicium* revealed that three species of *Skvortzovia* closely formed a group and then clustered with a clade comprising *Cyphellostereum* D.A.Reid, *Resinicium* and *Rickenella* Raithelh. (Nakasone 2007).

During investigations on wood-inhabiting fungi in southern China, an additional species was found that could not be assigned to any described species. In this study, the authors use collections from other studies to examine the taxonomy and phylogeny of this new species within *Skvortzovia*. The new species is described and illustrated, compared to related species, and placed within the phylogeny on the basis of analysis of the internal transcribed spacer (ITS) regions and large subunit nuclear ribosomal RNA gene (nLSU) sequences.

Material and Methods

Morphological studies

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 dried specimens, and observed under a light microscope (Dai 2012). The following abbreviations are used: KOH = 5% potassium hydroxide, CB =

Accepted for publication 8 February 2021. Published online 27 August 2021

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Table 1. List of species, specimens and GenBank accession numbers of sequences used in this study.

Species name	Sample number	GenBank accession number		References
		ITS	nLSU	
Resinicium bicolor (Alb. & Schwein.) Parmasto	FP 133695	DQ826536	—	Nakasone 2007
R. bicolor	UC 2022858	KP814209	—	Unpublished
Resinicium confertum Nakasone	FP 102863	DQ826538	—	Nakasone 2007
Resinicium friabile Hjortstam & Melo	PR 1380	DQ826542	—	Nakasone 2007
R. friabile	ECCO 146	DQ826544	—	Nakasone 2007
Resinicium grandisporum G.Gruhn, S.Dumez & Schimann	GGGUY 13030	KY995326	—	Gruhn *et al.* 2017
R. grandisporum	GGMAR 12326	KY995329	—	Gruhn *et al.* 2017
Resinicium monticola Nakasone	FP 102832	DQ826550	—	Nakasone 2007
R. monticola	FP 150360	DQ826552	—	Nakasone 2007
Resinicium mutabile Nakasone	FP 102989	DQ826556	—	Nakasone 2007
R. mutabile	PR 1366	DQ826557	—	Nakasone 2007
Resinicium rimulosum Nakasone	FP 150328	DQ826546	—	Nakasone 2007
R. rimulosum	KUC 2013102212	KJ668464	—	Unpublished
Resinicium saccharicola (Burt) Nakasone	GGGUY 12118	KY995323	—	Gruhn *et al.* 2017
R. saccharicola	GGGUY 12158	KY995324	—	Gruhn *et al.* 2017
Resinicium tenue Nakasone	FP 150354	DQ826539	—	Nakasone 2007
R. tenue	FP 150251	DQ826540	—	Nakasone 2007
Rickenella fibula (Bull.) Raithelh.	AFTOL-ID 486	DQ241782	—	Gruhn *et al.* 2017
R. fibula	MES 950	MF319096	MF319096	Unpublished
Skvortzovia furfuracea (Bres.) G.Gruhn & Hallenb.	1604	DQ873648	DQ873648	Larsson *et al.* 2006
S. furfuracea	UC 2023081	KP814421	KP814421	Unpublished
S. furfurella (Bres.) Bononi & Hjortstam	8128	MK575274	MK575274	Unpublished
S. furfurella	MAR 12-286	MH248259	—	Unpublished
Skvortzovia georgica (Parmasto) G.Gruhn & Hallenb.	3169b	DQ873645	DQ873645	Larsson *et al.* 2006
Skvortzovia meridionalis (Burds. & Nakasone) G.Gruhn & Hallenb.	CFMR 4210	KX065952	—	Unpublished
Skvortzovia pinicola (J.Erikss.) G.Gruhn & Hallenb.	FC 2015102015	MF437009	MF437009	Park *et al.* 2017
S. pinicola	KHL 12224	EU118649	EU118649	Larsson 2007
Skvortzovia yunnanensis C.L.Zhao sp. nov.	*C. L. Zhao* 16084	MW472754	MW473473	Present study
S. yunnanensis	*C. L. Zhao* 16181	MW472755	MW473474	Present study

Cotton Blue, IKI = Melzer's reagent, 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 a given number (b) of specimens. CB– = acyanophilous, CB+ = cyanophilous, IKI– = both inamyloid and indextrinoid.

Molecular procedures and phylogenetic analyses

The EZNA HP Fungal DNA Kit (Omega Biotechnologies Co., Ltd, Kunming, Yunnan Province, P. R. China) was used to obtain PCR products from dried specimens, according to the manufacturer's instructions with some modifications. The ITS region was amplified with the ITS5 and ITS4 primer pair (White *et al.* 1990). The nuclear LSU region was amplified with the LR0R and LR7 primer pair (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 Company Limited, Kunming, Yunnan Province, P. R. 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 27247). Sequences of *Rickenella fibula* (Bull.) Raithelh. obtained from GenBank were used as an outgroup to root trees, following Nakasone (2007), in the ITS (Fig. 1) and ITS+nLSU (Fig. 2) analyses.

Maximum parsimony analyses were applied to the ITS and ITS+nLSU dataset sequences. Approaches used for phylogenetic analyses followed Zhao & Wu (2017), 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 tree bisection and reconnection (TBR) branch swapping and 1000 random sequence additions. Max-trees were set to 5000,

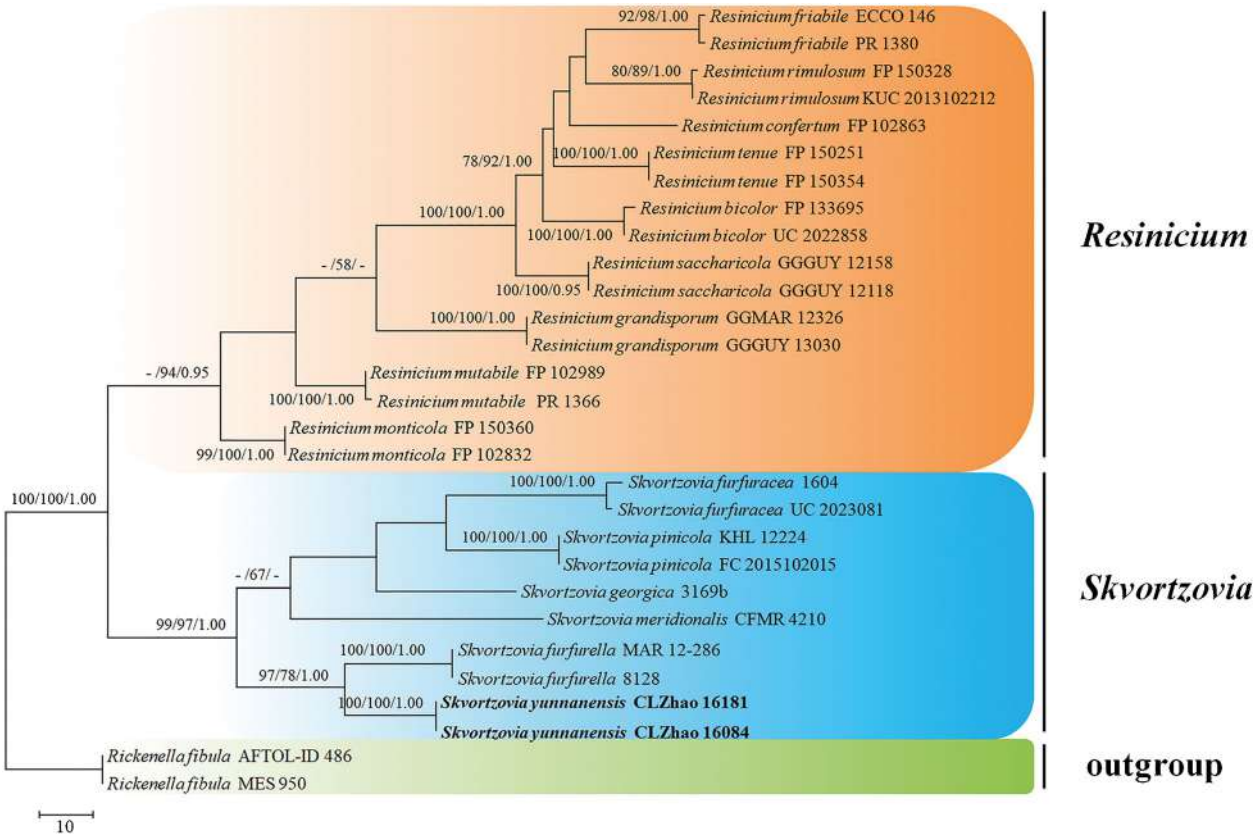

Fig. 1. Maximum parsimony strict consensus tree illustrating the phylogeny of *Skvortzovia yunnanensis* and related species in *Skvortzovia* and *Resinicium* based on ITS sequences. Branches are labelled with maximum likelihood bootstrap value >70%, parsimony bootstrap value >50% and Bayesian posterior probabilities >0.95, respectively.

branches of zero length were collapsed and all parsimonious trees were saved. Clade robustness was assessed using a bootstrap (BP) 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 (MPT) generated. Sequences were also analysed 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 (Nylander 2004) was used to determine the best-fit evolution model for each data set for Bayesian inference (BI). BI was calculated with MrBayes 3.1.2 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 two runs from random starting trees for 230,000 generations for ITS (Fig. 1), and for 160,000 generations for ITS+nLSU (Fig. 2), 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 were considered as significantly supported if they received maximum likelihood bootstrap value (BS) >75%, maximum parsimony bootstrap value (MP) >75%, or Bayesian posterior probability (BPP) >0.95.

Results

Molecular phylogeny

The ITS dataset (Fig. 1) included sequences from 29 fungal specimens, representing 16 taxa. The dataset had an aligned length of 639 characters, of which 294 characters are constant, 38 are variable and parsimony-uninformative, and 307 are parsimony-informative. Maximum parsimony analysis yielded four equally parsimonious trees (TL = 965, CI = 0.6280, HI = 0.3720, RI = 0.7946, RC = 0.4990). The best model for ITS estimated and applied in the Bayesian analysis had the following parameters: GTR+I+G, lset nst = 6, rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1). Bayesian analysis and ML analysis resulted in a

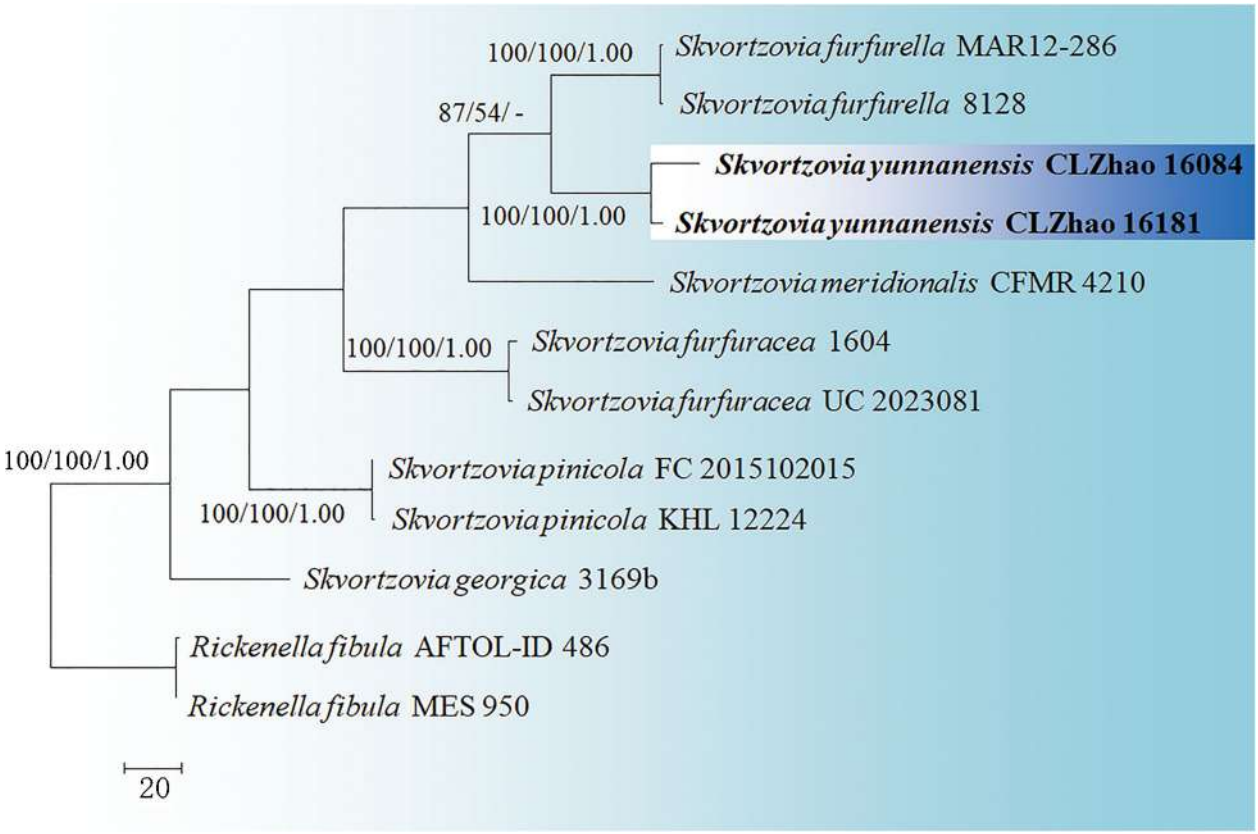

Fig. 2. Maximum parsimony strict consensus tree illustrating the phylogeny of *Skvortzovia yunnanensis* and related species in *Skvortzovia* based on ITS+nLSU sequences. Branches are labelled with maximum likelihood bootstrap value >70%, parsimony bootstrap value >50% and Bayesian posterior probabilities >0.95, respectively.

topology similar to that provided by MP analysis, with an average standard deviation of split frequencies = 0.008733 (BI).

The phylogeny (Fig. 1) inferred from ITS sequences demonstrated that *Skvortzovia yunnanensis* nested into the *Skvortzovia* group and then clustered with *Resinicium*.

The ITS+nLSU dataset (Fig. 2) included sequences from 12 fungal specimens representing seven taxa. The dataset had an aligned length of 751 characters, of which 467 characters are constant, 62 are variable and parsimony-uninformative, and 222 are parsimony-informative. Maximum parsimony analysis converged on a single parsimonious tree (TL = 564, CI = 0.7482, HI = 0.2518, RI = 0.7280, RC = 0.5447). In the best model for the ITS+nLSU dataset, esticqpr = dirichlet (1,1,1,1). Bayesian analysis and ML analysis resulted in a topology similar to that provided by the MP analysis, with an average standard deviation of split frequencies of 0.008922 (BI)

Further phylogeny (Fig. 2) inferred from the combined ITS+nLSU sequences demonstrated that *Skvortzovia yunnanensis* formed a single well-supported lineage (100% BS, 100% BP and 1.00 BPP) and then grouped with *S. furfurella* and *S. meridionalis*.

Taxonomic Treatment

Skvortzovia yunnanensis *C.L.Zhao* **sp. nov.** Type: China, Yunnan Province, Wenshan, Pingba Town, Wenshan National Reserve Nature, on a fallen branch of an angiosperm, 25 July 2019, *C. L. Zhao* 16084 (holotype SWFC). Figs 3 and 4.

MycoBank no.: MB 838504.
GenBank no.: *C. L. Zhao* 16084 (ITS MW472754; nLSUMW473473); *C. L. Zhao* 16181 (ITS MW472755; nLSU MW473474).

Basidiomata annual, resupinate, very thin, adnate, membranaceous to ceraceous, without odour or taste when fresh, becoming membranaceous upon drying, up to 12 cm long, 4.5 cm wide, 50 – 100 µm thick. Hymenial surface smooth, white to pale cream when fresh, becoming pale cream upon drying. Margin sterile, concolorous with the hymenial surface, up to 1 mm wide. *Hyphal structure.* Hyphal system monomitic; generative hyphae with clamp connections, colourless, thin-walled, frequently branched, 2 – 4 µm in diameter; IKI–, CB–, tissues unchanged in KOH. *Hymenium.* Halocystidia larger, numerous, cylindri-

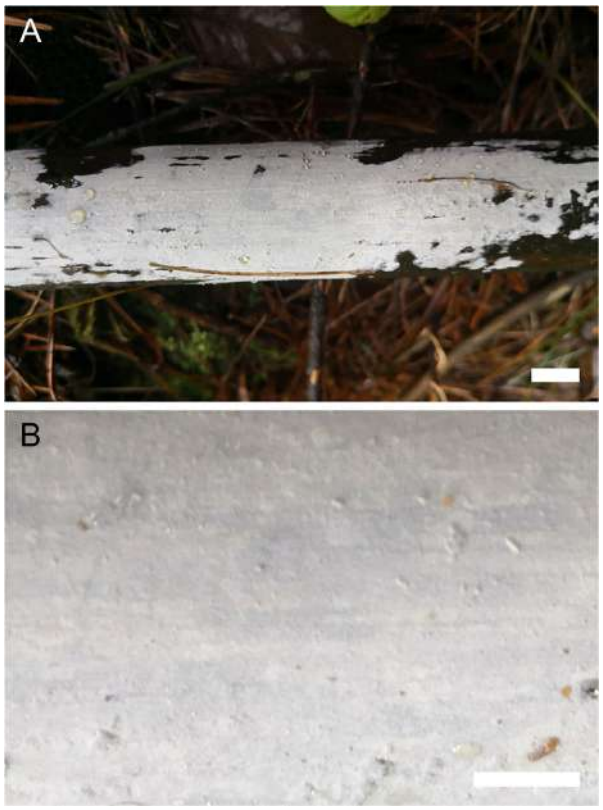

Fig. 3. Basidiomata of *Skvortzovia yunnanensis* (holotype). Scale bars: **A** 1 cm; **B** 0.5 cm. PHOTOS: JUN-HONG DONG.

cal with a capitate apex, colourless, thin-walled, 21 – 32 × 2 – 4 µm and cystidioles absent; basidia clavate, thin-walled, with four sterigmata and a basal clamp connection, 9 – 12 × 2 – 4.5 µm; basidioles dominant, similar in shape to the basidia, but slightly smaller. *Spores*. Basidiospores allantoid, colourless, thin-walled, smooth, IKI–, CB–, (4.5 –) 5 – 6.5 (– 7) × 1 – 1.5 µm, L = 5.90 µm, W = 1.30 µm, Q = 4.21 – 4.77 (n = 60/2).

RECOGNITION. *Skvortzovia yunnanensis* differs from all its congeners in having the following combination of traits: annual, resupinate, very thin, smooth basidiomata (up to 12 cm long, 4.5 cm wide, 50 – 100 µm thick) with a white to pale cream hymenophore; a monomitic hyphal system with clamped generative hyphae (2 – 4 µm in diam.); halocystidia larger (21 – 32 × 2 – 4 µm) and cylindrical with a capitate apex; clavate basidia (9 – 12 × 2 – 4.5 µm); allantoid basidiospores, 5 – 6.5 × 1 – 1.5 µm. In addition, *S. yunnanensis* is placed in the genus *Skvortzovia* because of the white to pale cream hymenial surface, and the presence of capitate cystida and allantoid basidiospores.

DISTRIBUTION. Known only from the type locality.

SPECIMEN EXAMINED. CHINA. Yunnan Province, Wenshan, Pingba Town, Wenshan National Reserve Nature, on the fallen branch of angiosperm, 25 July 2019, *C. L. Zhao* 16181 (SWFC).

HABITAT. Lignicolous.

CONSERVATION STATUS. Not evaluated.

ETYMOLOGY. Refers to the location of the specimens (Yunnan Province).

NOTES. Larsson *et al.* (2006) provided a comprehensive study on the hymenochaetoid clade that involved both morphological analyses and phylogenetic analyses based on ITS rDNA and 28S sequences, in which five species were clustered into *Skvortzovia* in the phylogenetic tree. In the present study, *S. yunnanensis* nested into *Skvortzovia*, which formed a single well-supported lineage (100% BS, 100% BP and 1.00 BPP), and then grouped with *S. furfurella* and *S. meridionalis* (Figs 1 and 2). Morphologically, however, *S. furfurella* differs from *S. yunnanensis* by its odontioid hymenophore and smaller basidiospores (4 – 5 × 1 µm; Hjortstam & Bononi 1987). *Skvortzovia meridionalis* differs in its light yellow to greyish-orange to brownish-orange hymenial surface and ellipsoid, wider basidiospores (4.5 – 5.5 × 2.5 – 3 µm; Burdsall & Nakasone 1981).

Morphologically, other three species of *Skvortzovia* are similar to *S. yunnanensis*. However, *S. georgica* is separated from *S. yunnanensis* by its smaller basidiospores (4.5 – 5 × 2 – 2.5 µm; Bernicchia & Gorjón 2010). *Skvortzovia furfuracea* differs from *S. yunnanensis* by its purple hymenial surface and wider basidiospores (4 – 6 × 2.5 – 3 µm; Bresadola 1925). *Skvortzovia pinicola* differs in its hydnoid hymenophore with brownish hymenial surface and wider basidiospores (4 – 5 × 2 – 2.5 µm), and because it also grows on coniferous trees (Eriksson *et al.* 1981).

Key to the known species of *Skvortzovia* worldwide

1. Hymenophore smooth 2
1. Hymenophore odontoid 4
2. Basidiospores < 2 µm in width . . . . . *S. yunnanensis*
2. Basidiospores > 2 µm in width 3
3. Basidia > 12 µm in length, basidiospores allantoid . . . . . *S. georgica*
3. Basidia < 12 µm in length, basidiospores ellipsoid . . . . . *S. furfuracea*
4. Aculei > 1 mm in length, basidia > 15 µm in length . . . . . *S. pinicola*

4. Aculei < 1 mm in length, basidia < 15 µm in length 5
5. Hymenial surface brownish orange, basidiospores > 2 µm in width . . . . . *S. meridionalis*
5. Hymenial surface white to cream, basidiospores < 2 µm in width . . . . . *S. furfurella*

Acknowledgements

The research is supported by the Yunnan Fundamental Research Projects (Grant No. 202001AS070043), the Key Laboratory of Forest Resources Conservation and Utilization in the Southwest Mountains of China Ministry of Education, Southwest Forestry University (KLESWFU-202003), and the National College Students Innovation and Entrepreneurship Training Program (No. 202010677015).

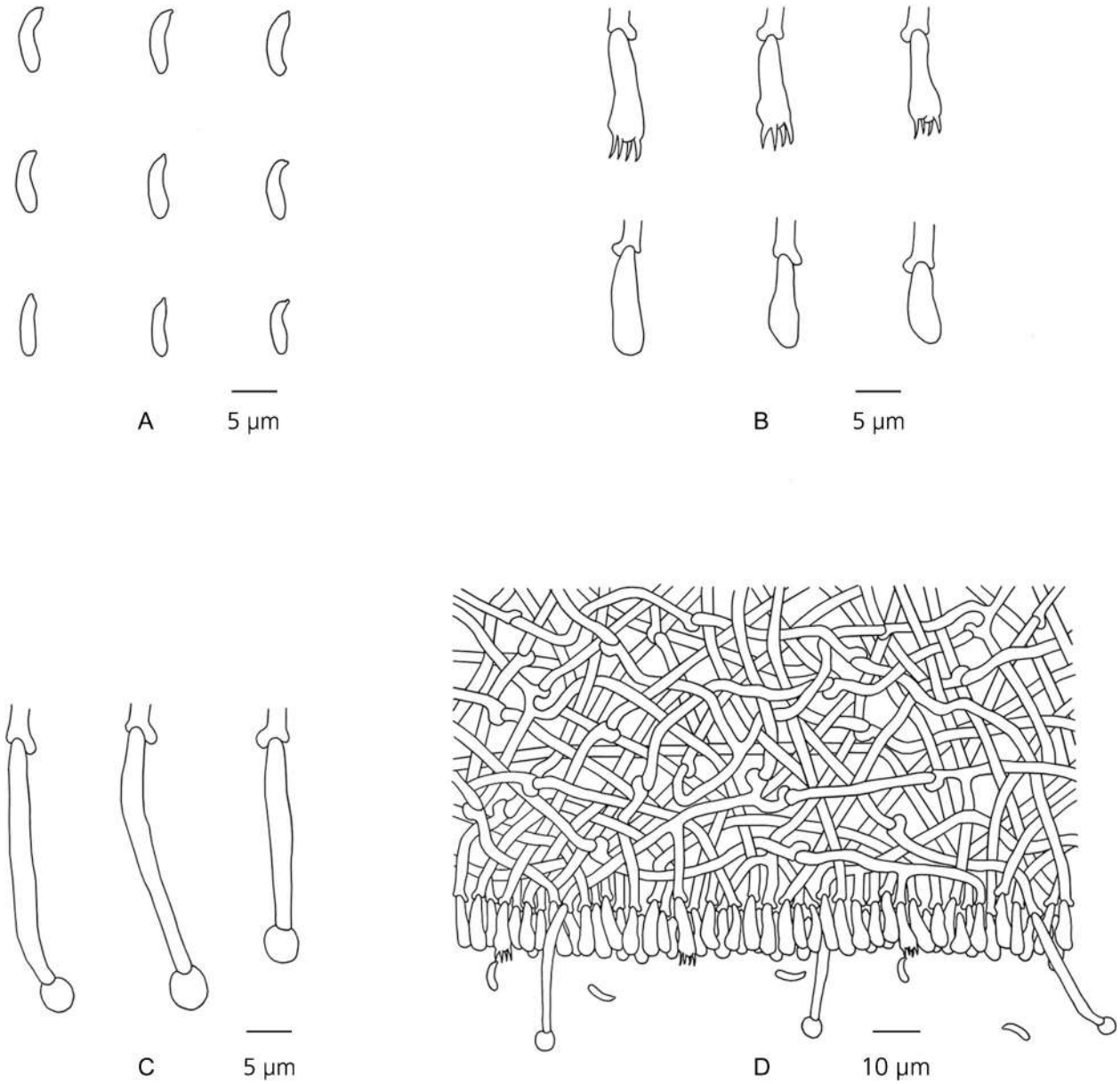

Fig. 4. Microscopic structures of *Skvortzovia yunnanensis* (drawn from the holotype). **A** basidiospores; **B** basidia and basidioles; **C** halocystidia; **D** a section of hymenium. DRAWN BY JUN-HONG DONG.

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