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tion of fungi 1: Practical guidelines



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Resinicium yunnanense sp. nov. (Resiniciaceae, Hymenochaetales), a new wood-inhabiting fungal species from southern China

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With 3 figures and 1 table

Abstract: A new wood-inhabiting fungal species, *Resinicium yunnanense* sp. nov., is proposed based on a combination of morphological features and molecular characters. It is characterized by an annual growth habit, resupinate basidiomata with white to pale cream, smooth hymenial surface, a monomitic hyphal system with nodose-septate hyphae, presence of astrocystidia and halocystidia, and ellipsoid, colorless, thin-walled, smooth basidiomata measuring as $3.7-5.2 \times 2.3-3.3 \mu m$. Sequences of the internal transcribed spacer (ITS) 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 sequences showed that the new species is nested into genus *Resinicium*, which demonstrated *R. yunnanense* was sister taxon to *R. friabile*.

Keywords: Basidiomycota; corticioid; molecular phylogeny; taxonomy; wood-rotting fungi

Introduction

Resinicium Parmasto, typified with *R. bicolor* (Alb. & Schwein.) Parmasto (Parmasto 1968), is a worldwide distributed genus of wood-inhabiting fungi. It is characterized by resupinate, thin basidiomes with smooth, grandinioid, odontioid to hydnoid hymenophore

with white to yellowish hymenial surface, a monomitic hyphal system with clamp connections or rarely with simple septa, presence of astrocystidia and halocystidia, and thinwalled, smooth, ellipsoid, cylindrical or allantoid basidiospores (Parmasto 1968, Bernicchia & Gorjón 2010). So far about 18 species have been accepted in the genus worldwide (Larsson et al. 2006, Nakasone 2007, Yu et al. 2021). A total of 24 species have been assigned to *Resinicium* (Index Fungorum: http://www.indexfungorum.org/Names/Names. asp).

Recently, molecular studies involving *Resinicium* have been carried out (Nakasone 2007, Gruhn et al. 2017, Park et al. 2017, Yu et al. 2021, Wang et al. 2023). Morphological and molecular studies on *Resinicium* s. str. showed that two groups of *Resinicium* species, *Resinicium* s. str. group (*R. bicolor*), and *Resinicium* s.l. group (*R. furfuraceum* (Bres.) Pann.) were supported as two distinct groups. The morphological research as well as phylogenetic analyses based on ITS rDNA sequences, indicated that three main *Resinicium* clades were included and nine species nested into *Resinicium* (Gruhn et al. 2017). A recent study revealed that ten species of *Resinicium* grouped together on the basis of ITS datasets (Park et al. 2017). The research from Asia-Pacific about the genus *Resinicium* sensu lato were carefully studied from the morphological and phylogenetic perspectives, in which 11 species of *Resinicium* were included and differentiated by ITS-based on phylogeny, and two new species were described (Yu et al. 2021).

During investigations on wood-inhabiting fungi in southern China, an additional taxon was found which could not be assigned to any described species. In this study, the authors examine the taxonomy and phylogeny of a new species within *Resinicium*, based on the internal transcribed spacer (ITS) regions sequences.

Materials and methods

The studied specimens are deposited at the herbarium of Southwest Forestry University (SWFC), Kunming, Yunnan Province, P.R. China. Macromorphological descriptions are based on field notes. Color terms follow Petersen (1996). The micro-morphological data were obtained from the dried specimens and observed under Nikon Eclipse E100 light microscope following (Chen & Zhao 2020). The following abbreviations were used: KOH = 5% potassium hydroxide, CB = Cotton Blue, CB– = acyanophilous, IKI = Melzer's reagent, IKI– = both non-amyloid and non-dextrinoid, 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

The CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd., Beijing, P.R. China) was used to obtain genomic DNA from the dried specimens (Chen & Zhao 2020). The nuclear ribosomal ITS region was amplified with primers 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. The PCR products were purified and directly sequenced at Kunming Tsingke Biological Technology Limited Company, Kunming, Yunnan Province, P.R. China. All newly generated sequences were deposited at GenBank (Table 1).

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

Species name	Sample no.	GenBank accession no.	References
		ITS	-
Peniophorella praetermissa	KHL 13164	DQ873597	Larsson et al. (2006)
Resinicium austroasianum	LWZ (2017)1014-3	MW414503	Zhou et al. (2021)
R. austroasianum	LWZ 20180518-2	MW414507	Zhou et al. (2021)
R. austroasianum	LWZ 20180417-5	MW414504	Zhou et al. (2021)
R. austroasianum	LWZ 20180417-28	MW414505	Zhou et al. (2021)
R. austroasianum	LWZ 20180517-42	MW414506	Zhou et al. (2021)
R. bicolor	O.Miettinen 14049	MF319079	Zhou et al. (2021)
R. bicolor	TENN57741	AF518763	Krüger (2002)
R. bicolor	FP-133575	DQ826533	Nakasone (2007)
R. bicolor	HHB10731	DQ826534	Nakasone (2007)
R. bicolor	JLL13731	DQ826535	Nakasone (2007)
R. bicolor	UC2022858	KP814209	Zhou et al. (2021)
R. bicolor	FP-133695	DQ826536	Nakasone (2007)
R. bicolor	GEL2071	DQ340321	Zhou et al. (2021)
R. confertum	FP-102863	DQ826538	Nakasone (2007)
R. friabile	FP-102983	DQ826545	Nakasone (2007)
R. friabile	FP-102803	DQ826541	Nakasone (2007)
R. friabile	PR-1380	DQ826542	Nakasone (2007)
R. friabile	FP-150153	DQ826543	Nakasone (2007)
R. friabile	ECCO-146	DQ826544	Nakasone (2007)
R. grandisporum	GGGUY13-008	KY995325	Gruhn et al. (2017)

Table 1. cont.

Species name	Sample no.	GenBank accession no.	References
		ITS	
R. grandisporum	GGGUY13-031	KY995327	Gruhn et al. (2017)
R. grandisporum	GGMAR12-326	KY995329	Gruhn et al. (2017)
R. grandisporum	GGGUY13-030	KY995326	Gruhn et al. (2017)
R. lateastrocystidium	LWZ 20180414-13	MW414508	Zhou et al. (2021)
R. lateastrocystidium	LWZ 20180416-10	MW414510	Zhou et al. (2021)
R. lateastrocystidium	LWZ 20180414-15	MW414509	Zhou et al. (2021)
R. monticola	FP-150360	DQ826552	Nakasone (2007)
R. monticola	FP-150407	DQ826554	Nakasone (2007)
R. monticola	FP-150332	DQ826555	Nakasone (2007)
R. monticola	FP-102832	DQ826550	Nakasone (2007)
R. monticola	FP-150355	DQ826553	Nakasone (2007)
R. mutabile	FP-102989	DQ826556	Nakasone (2007)
R. mutabile	PR-1366	DQ826557	Nakasone (2007)
R. mutabile	GGMAR15-175	KY995331	Gruhn et al. (2017)
R. mutabile	GGMAR15-174	KY995330	Gruhn et al. (2017)
R. mutabile	GGGUY12-087	KY995322	Gruhn et al. (2017)
R. rimulosum	FP-150328	DQ826546	Nakasone (2007)
R. rimulosum	KUC20131022-12	KJ668464	Zhou et al. (2021)
R. saccharicola	GGMAR12-230	KY995328	Gruhn et al. (2017)
R. saccharicola	FP-102754	DQ826547	Nakasone (2007)
R. saccharicola	FP-102841	DQ826548	Nakasone (2007)
R. saccharicola	FP-102843	DQ826549	Nakasone (2007)
R. saccharicola	GGGUY12-118	KY995323	Gruhn et al. (2017)
R. saccharicola	GGGUY12-158	KY995324	Gruhn et al. (2017)
R. tenue	FP-150354	DQ826539	Nakasone (2007)
R. tenue	FP-150251	DQ826540	Nakasone (2007)
R. yunnanense	CLZhao 18543	MZ810958	Present study
R. yunnanense	CLZhao 17889	MZ810959	Present study
Skvortzoviella lenis	LWZ 20180921-17	MW414522	Zhou et al. (2021)



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

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 in TreeBase (submission ID 28764). *Peniophorella praetermissa* (P. Karst.) K.H. Larss and *Skvortzoviella lenis* Jia Yu, Xue W. Wang, S.L. Liu & L.W. Zhou were used as an outgroup to root trees following Yu et al. (2021) in the ITS analyses (Fig. 1).

Maximum parsimony (MP), Maximum Likelihood (ML) and Bayesian Inference (BI) analyses were applied to the combined three datasets. Approaches to phylogenetic analyses followed Zhao & Wu (2017). MP analysis was performed in PAUP* version 4.0b10 (Swofford 2002). All of 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 1,000 random sequence additions. Max-trees were set to 5,000, 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 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 (BS) for ML analysis was determined by 1,000 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 run for 2 runs from random starting trees for 1.1 million generations 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 (ML) > 70%, maximum parsimony bootstrap value (MP) > 50%, or Bayesian posterior probabilities (BPP) > 0.95.

Results

Molecular phylogeny

The ITS dataset (Fig. 1) included sequences from 50 fungal specimens representing 14 taxa. The dataset had an aligned length of 707 characters, of which 338 characters were constant, 81 parsimony-uninformative and 246 parsimony-informative. MP analysis yielded 100 equally parsimonious trees (TL = 747, CI = 0.6841, HI = 0.3159, RI = 0.9128, RC = 0.6244). The best-fit model for ITS alignment estimated and applied in the BI was GTR+I+G, lset nst = 6, rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1). BI resulted in a similar topology with an average standard deviation of split frequencies = 0.008921.

A phylogenetic tree (Fig. 1) inferred from the ITS sequence of *Resinicium*, showed that the sampled specimens of *R. yunnanense* sp. nov., were sistered to *R. friabile* Hjortstam & Melo with supports (94% BS, 91% BP, 1.00 BPP), and it formed a well-supported lineage distinct from the other species.

Taxonomy

Resinicium yunnanense J. Cai & C.L. Zhao, sp. nov. Figs. 2, 3

MycoBank no.: 841273

Holotype: CHINA. Yunnan Province, Honghe, Pingbian County, Daweishan National Nature Reserve, E 116°24', N 39°57', alt. 2265 m, on angiosperm trunk, 3 August 2019, CLZhao 18543 (SWFC), GenBank MW810958 (ITS).



Fig. 2. Basidiomata of *Resinicium yunnanense* (holotype). Bars: A = 1 cm; B = 1 mm.



Fig. 3. Microscopic structures of *Resinicium yunnanense* (drawn from the holotype). A. Basidiospores; B. Basidia and basidioles; C. Astrocystidia; D. Halocystidia; E. A vertical section of hymenium. Bars: $A = 5 \mu m$; B, C, D, E= 10 μm .

Etymology: Yunnanense (Lat.): referring to the locality (Yunnan) of the type specimens.

Description: Basidiomata annual, resupinate; hymenial surface smooth to irregular poroid; hyphal system monomitic, generative hyphae with clamp connections; basidiospores ellipsoid, colorless, thin-walled, smooth, IKI–, CB–, $3.7-5\times2.3-3.3$ µm.

Fruiting body: Basidiomata annual, resupinate, farinaceous without odor or taste when fresh, becoming fragile when drying, up to 9 cm long, 3.5 cm wide, $50-100 \mu m$ thick. Hymenial surface smooth to irregular poroid, white to pale cream when fresh, turn to buff to cinnamon-buff upon drying. Margin sterile, white to cream, 1-2 mm wide.

Hyphal structure: Hyphal system monomitic, generative hyphae with clamp connections, colorless, thin-walled, frequently branched, $1.5-4.5 \mu m$ in the diameter of the cross section; CB–, IKI–.

Hymenium: Cystidia of two kinds: (1) Astrocystidia numerous, colorless, thin-walled, 7–12.8 × 1–1.5 μ m, with an acute or bulbous apex, the star-shaped crystals are easily crushed by tapping; becoming black in Melzer's reagent (IKI+). (2) Halocystidia rare, colorless, thin-walled, capitate, 19.1–23.2 × 15.1–20.9 μ m; tapering to 2–3 μ m diameter at base, outer globules 10–20 μ m diameter, covered by a large hyaline guttulate mass. Larger crystals usually free in the hymenium. Basidia clavate, with four sterigmata and a basal clamp connection, 7–10 × 3–4.5 μ m. Basidioles dominant, in shape similar to basidia, but slightly smaller.

Spores: Basidiospores ellipsoid, colorless, thin-walled, smooth, IKI–, CB–, $(3.5–)3.7-5(-5.3) \times (2-)2.3-3.3(-3.5) \ \mu\text{m}$, L = 4.50 μm , W = 2.82 μm , Q = 1.54–1.65 (n = 60/2).

Rot type: A white rot.

Additional specimen (paratype) examined: CHINA. Yunnan Province: Honghe, Pingbian County, Daweishan National Nature Reserve, E 116°24', N 39°57', alt. 2265 m, on fallen angiosperm branch, 1 August 2019, CLZhao 17889 (SWFC).

Discussion

The present study describes a new species, *Resinicium yunnanense* sp. nov., based on phylogenetic and morphological evidence.

Previously, based on the ITS sequences, the phylogeny and taxonomy of *Resinicium* s. str. indicated that ten species nested into the core group, including *R. friabile*, *R. tenue* Nakasone, *R. confertum* Nakasone, *R. rimulosum* Nakasone, *R. bicolor*, *R. saccharicola* (Burt.) Nakasone, *R. monticola* Nakasone, *R. mutabile* Nakasone, *Rickenella fibula* Bull, *R. mellea* Singer & Clémençon and the generic species of *R. bicolor* (Alb. & Schwein.) Parmasto, in which all of them formed a single lineage (Nakasone 2007). In the present study, the new species, *R. yunnanense* clusters into *Resinicium* s. str., in which it sisters to *R. friabile* with high support. However, morphologically *R. friabile* differs from *R. yunnanense* by its subceraceous to ceraceous, denticulate to spinose basidiomata and bigger basidia (10–15 × 4–5.5 µm, Nakasone 2007).

Morphologically, *Resinicium yunnanense* resembles *R. grandisporum* G. Gruhn, S. Dumez & E. Schimann, *R. mutabile* and *R. tenue* by having farinaceous basidiomata. However, *R. grandisporum* differs in its odontioid hymenophore, and larger basidiospores (8–9.6 × 4.3–5.5 μ m, Gruhn 2017); *R. mutabile* differs in its odontoid to spinose basidiomata, and larger basidia (11–16 × 3.5–6 μ m, Nakasone 2007). *R. tenue* differs in its odontoid to grandinioid hymenophore with short conical or cylindrical aculei (4–6 per mm) and larger basidiospores (5–6 × 3.5–4 μ m, Nakasone 2007).

Resinicium yunnanense is similar to *R. aculeatum* Tellería, Melo & Dueñas, *R. monticola* and *R. rimulosum* based on the character of the ellipsoid basidiospores. However, *R. aculeatum* differs in its grandinioid to odontioid basidiomata with conical to almost semiglobose teeth and halocystidia with a finely aculeolate wall $(20 \times 6.5-12 \ \mu\text{m}, \text{Telleria et al.} 2008)$; *R. monticola* differs in its short conical, broad tubercules hymenophore, and aculeate to lageniform astrocystidia $(10-30 \times 1.5-4 \ \mu\text{m}, \text{Nakasone} 2007)$; *R. rimulosum* differs in its odontoid hymenophore with the short, conical aculei (4–6 per mm), and larger basidia $(11-22 \times 4-5 \ \mu\text{m}, \text{Nakasone} 2007)$.

In geographical distribution, *Resinicium* is a widespread group and six *Resinicium* species were recorded in China (Dai 2011), in which two species were found and described in this country, *R. austroasianum* J. Yu, X. W. Wang, S.L. Liu & L.W. Zhou and *R. lateastrocystidium* J. Yu, X. W. Wang, S.L. Liu & L.W. Zhou (Yu et al. 2021). However, morphologically, *R. austroasianum* differs in its grandinioid to odontoid basidiomata with the small cylindrical aculei, usually with round apex (5–6 per mm), and cylindrical leptocystidia (10–30 × 2–3 μ m, Yu et al. 2021); *R. lateastrocystidium* differs in having the grandinioid to odontoid basidiomata with small conical apex (3–4 per mm), and larger basidia (13–20 × 5–6 μ m, Yu et al. 2021).

Wood-inhabiting fungal species are an extensively studied group of Basidiomycota (Bernicchia & Gorjón 2010, Dai 2012, Dai et al. 2015, Zhao et al. 2023). However, the Chinese wood-inhabiting fungus diversity is still not well known, especially in subtropics and tropics, many recently described taxa of this group were from these areas (Cui & Dai 2006, Dai 2011, Dai 2012, Yuan 2013, Zhao et al. 2015, Zhao et al. 2016, Zhao et al. 2019, Zhao et al. 2023, Yuan et al. 2016, Shen et al. 2018, Cui et al. 2019, Luo & Zhao 2022, Cai et al. 2023, Zhao & Zhao 2023). A new species in the present study is from subtropics, too, supporting the possibility of finding more new taxa belonging to the wood-inhabiting fungi.

Acknowledegments

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