

Gibellula aurea sp. nov. (Ascomycota, Cordycipitaceae): a new golden spiderdevouring fungus from a Brazilian Atlantic Rainforest

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https://doi.org/10.11646/phytotaxa.573.1.8

Ramaricium yunnanense sp. nov. (Gomphaceae, Gomphales) from China

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Abstract

A new corticioid fungus belonging to *Ramaricium* is described from China based on morphology and molecular phylogenetic analyses. It is characterized by annual, resupinate basidiomata with a white hymenial surface, a monomitic hyphal system with generative hyphae bearing clamp connections, and ellipsoid to subglobose basidiospores with thin, colorless, ornamented walls. Phylogenetic analyses of combined internal transcribed spacer (ITS) and large subunit nuclear ribosomal RNA gene (nLSU) regions showed that the corticioid fungus is a distinct species in *Ramaricium* and nested sister to *R. polyporoideum*. The new corticioid fungus is described and named as *Ramaricium yunnanense* in this study. A full description, illustration, and a phylogenetic tree to show the placement of *Ramaricium yunnanense sp. nov.* are provided.

Keywords: Basidiomycota, molecular phylogeny, taxonomy, wood-rotting fungi, Yunnan Province

Introduction

The corticioid genus *Ramaricium* J. Erikss. (1954: 189), was placed in the family Gomphaceae (Agaricomycetes, Basidiomycota), with *R. occultum* J. Erikss. (1954: 189) as the type. This genus is characterized by the resupinate basidiomata with smooth hymenophore-bearing rhizomorphic margin; a monomitic hyphal system with clamp connections on generative hyphae; cystidia absent; basidia terminal or pleural, clavate, more or less sinuous, and globose to ellipsoid or cylindrical, colorless, thin-walled, smooth or ornamented, IKI–, cyanophilous basidiospores. The genus is reported to cause white rot (Gray 1821, Jülich 1977, Hibbett & Binder 2002, Bernicchia & Gorjón 2010). Based on the MycoBank database (http://www.MycoBank.org, accessed on 4 October 2022) and the Index Fungorum (http://www.indexfungorum.org, accessed on 4 October 2022), five species are accepted in this genus *Ramaricium* (Eriksson 1954, Jülich 1977, Ginns 1979, Hibbett & Binder 2002).

Based on the evolution of complex fruiting body morphologies in Homobasidiomycetes, Hibbett & Binder (2002) revealed that phylogenetically, the species of *Ramaricium* clustered in the gomphoid-phalloid clade. The previous phylogenetic study inferred from the major clades of mushroom-forming fungi (Homobasidiomycetes), indicated that *Ramaricium* species nested within the gomphoid-phalloid clade, sister to *Lentaria micheneri* (Berk. & M.A. Curtis) Corner (1950: 441) (Binder *et al.* 2005).

The taxa of this genus are widespread in America, Belarus, Britain, Canada, China, Finland, Germany, Italy, Norway, Poland, Sweden, and Tanzania, and the taxa are primarily the wood decomposers, causing white-rot of both angiosperms and gymnosperms (Bernicchia & Gorjón 2010). Recently, many wood-decaying fungi were recorded in China (Wang *et al.* 2020, Guan *et al.* 2021, Ma & Zhao 2021, Zong *et al.* 2021a,b, Jiang *et al.* 2021, Wu *et al.* 2022a,b), but, no *Ramaricium* was found in China (Dai 2011, Dai *et al.* 2015).

During an investigation of corticioid fungi in southern China, an interesting fungus was encountered. To clarify the taxonomic placement of this species, both morphological characteristics and phylogenetic analyses of combined ITS and nLSU genes were done.

Materials and methods

Morphological studies

The studied specimens have been deposited at the herbarium of Southwest Forestry University (SWFC), Kunming, Yunnan Province, China. The macromorphological descriptions were based on field records and photos captured in the field and lab. Colour terms were determined following the methodology described by Petersen (1996). Micro-morphological data were observed based on dried specimens using a light microscope following Dai (2012). The following abbreviations were used in the description: KOH = 5% potassium hydroxide water solution, CB– = acyanophilous, IKI– = both inamyloid and indextrinoid, L = mean spore length (arithmetic average for all spores), W = mean spore width (arithmetic average for all spores), Q = variation in the L/W ratios between the specimens studied, and n = a/b (number of spores (a) measured from a given number (b) of specimens).

Molecular procedures and phylogenetic analyses

CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd, Beijing) was used to extract genomic DNA from dried specimens, according to the manufacturer's instructions. ITS region was amplified with primer pairs ITS5 and ITS4 (White *et al.* 1990). The nLSU region was amplified with primer pairs LR0R and LR7 (http://lutzonilab. org/nuclear-ribosomal-dna). 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 1LSU was as follows: initial denaturation at 94°C for 3 min, followed by 35 cycles at 94°C for 1.5 min, and a final extension of 72°C for 10 min. The PCR for 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, Kunming, Yunnan Province, P.R. China. All newly generated sequences were deposited at GenBank (Table 1).

Spacies nome	Sample no.	GenBank accession no.		Deferences
Species name		ITS	LSU	- Kelerences
Beenakia fricta	K 2083	_	AY574693	Giachini et al. 2010
Clavariadelphus occidentalis	OSC 37018		AY574648	Giachini et al. 2010
C. Truncatus	OSC 67280		AY574649	Giachini et al. 2010
Gautieria crispa	OSC 61308	—	DQ218484	Hosaka et al. 2006
G. otthii	REG 636		AF393058	Binder & Hibbett 2002
Gloeocantharellus novae-zelandiae	ZT 68-657		AF261547	Moncalvo et al. 2002
Hydnocristella latihypha	He 20120911-3	KM489521	KM489523	Chen et al. 2015
H. latihypha	He 20120911-4	KM489522	KM489524	Chen et al. 2015
Kavinia alboviridis	EL 16-98		AY463434	Larsson et al. 2004
K. alboviridis	O 102140		AY574692	Giachini et al. 2010
Lentaria dendroidea	SJ 98012	EU118640		Larsson 2007
Phaeoclavulina argentea	AGK 042	JQ408234	JQ408234	Unpublished
P. argentea	AGK 036	JQ408231	JQ408231	Unpublished
Ramaria araiospora	OSC 81497	EU669297	EU669297	Unpublished
R. araiospora	OSC 108292	EU669293	EU669293	Unpublished
R. aurantiisiccescens	OSC 65703	EU669298	EU669298	Unpublished
R. foetida	AGK 058	JQ408239	JQ408239	Unpublished
R. vinosimaculans	Trappe 23383	EU669348	EU669348	Unpublished
Ramaricium polyporoideum	ECV 4163	MF992160		Unpublished
R. polyporoideum	X 4595	KY352646		Unpublished

TABLE 1. Names, voucher numbers, and corresponding GenBank accession numbers of the taxa used for the phylogenetic analyses in this study. The new species are in black bold.

.....continued on the next page

TABLE 1. (Continued)

Species name	Sample no.	GenBank accession no.		Defenences	
		ITS	LSU	Kelerences	
R. yunnanense	CLZhao 4158	MZ746097	MZ881948	Present study	
R. yunnanense	CLZhao 4179	ON054914	MZ881949	Present study	
Russula violacea	SJ 93009	AF506465	AF506465	Larsson & Larsson 2003	

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 28754). *Russula violacea* Quél. (1883: 397) was used as an outgroup following Chen *et al.* (2015) in the ITS+nLSU analyses (Fig. 1).



FIGURE 1. Maximum parsimony strict consensus tree illustrating the phylogeny of *Ramaricium yunnanense* and related species in the order Gomphales, based on ITS+nLSU sequences. Branches are labeled with maximum likelihood bootstrap \ge 70%, parsimony bootstrap proportions \ge 50% and Bayesian posterior probabilities \ge 0.95.

Maximum parsimony analysis was applied to the ITS+nLSU dataset sequences. Approaches to phylogenetic analysis were followed Zhao and 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 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 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 240 thousand 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 a maximum likelihood bootstrap value (BS) \ge 70%, maximum parsimony bootstrap value (BT) \ge 50%, or Bayesian posterior probabilities (BPP) \ge 0.95.

Results

Molecular phylogeny

The ITS+nLSU dataset (Fig. 1) included sequences from 23 fungal specimens representing 17 taxa. The dataset had an aligned length of 1028 characters, of which 1410 characters were constant, 237 parsimony-uninformative and 342 parsimony-informative. MP analysis yielded one equally parsimonious tree (TL = 1028, CI = 0.7121, HI = 0.2879, RI = 0.6542, RC = 0.4658). The best-fit model for ITS+nLSU 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 = 0.008967.

The phylogenetic tree (Fig. 1) inferred from ITS+nLSU sequences includes 17 species from the order Gomphales. The new taxon *Ramaricium yunnanense* was sister to *R. polyporoideum* (Berk. & M.A. Curtis) Ginns (1979: 98).

Taxonomy

Ramaricium yunnanense C.L. Zhao, *sp. nov.* Figs. 2, 3 MycoBank no.: MB 841259

Holotype:—CHINA. Yunnan Province, Puer, Jingdong County, Wuliangshan National Nature Reserve, E 100°32', N 24°36', alt. 1600 m, on the angiosperm trunk, 5 October 2017, *CLZhao 4179* (SWFC!), GenBank No. (ITS ON054914; nLSU MZ881949).

Etymology:—*yunnanense* (Lat.): the epithet refers to the Province in China "Yunnan" where the type species was collected.

Description:—*Basidiomata* annual, resupinate, cottony, without odor or taste when fresh, becoming flocculent on drying, up to 7 cm \times 4 cm (length \times breadth), up to 300 μ m thick. *Hymenial surface* smooth, white when fresh and drying. *Margin* sterile, fimbriate, white, up to 1.5 mm wide.

Hyphal system monomitic, generative hyphae colorless, thin-walled, bearing clamp connections, IKI-, CB-; tissues unchanged in KOH.

Subiculum generative hyphae colorless, more or less interwoven, thin-walled, rarely branched, presence of encrusted hyphae, $2.5-5 \mu m$ in diameter.

Hymenium cystidia and cystidioles absent; *basidia* pleural, clavate, with four sterigmata, and a basal clamp, slightly constricted in the middle to somewhat sinuous, $14-23 \times 6-8\mu$ m; basidioles dominant, in shape similar to basidia, but slightly smaller.

Basidiospores ellipsoid to subglobose, colorless, thin-walled, ornamented, IKI–, cyanophilous, $(3.8)4–5(-5.4) \times 3-4.5(4.7) \mu m$, L = 4.53 μm , W = 3.70 μm , Q = 1.22 (n = 30/1).

Additional specimen (paratype) examined:—CHINA. Yunnan Province, Puer, Jingdong County, Wuliangshan National Nature Reserve, E 100°32', N 24°36', alt. 1600 m, on the angiosperm trunk, 5 October 2017, *CLZhao 4158* (SWFC!), GenBank No. (ITS MZ746097; nLSU MZ881948).

Discussion

The previous morphological and molecular study of Binder *et al.* (2005) strongly supported the placement of *Ramaricium* in the gomphoid-phalloid clade. In the present study, based on the ITS and nLSU sequence data, the newly described species, *R. yunnanense* clustered within *Ramaricium* (Fig. 1) (100% BS, 89% BT, 1.00 BPP), and grouped with a clade comprising *Beenakia* D.A. Reid (1955: 635), *Clavariadelphus* Donk (1933: 72), *Hydnocristella* R.H. Petersen. (1971: 130), *Kavinia* Pilát (1938: 3), and *Lentaria* Corner (1950: 696). Phylogenetically, the new species is sister to *R. polyporoideum* and both taxa form a monophyletic lineage. However, morphologically *R. polyporoideum* differs from *R. yunnanense* by having a pale greyish hymenial surface with a light yellowish tint, larger basidia (25–50 × 7–10 µm) and basidiospores (7–9 × 5–6 µm, Ginns 1979).

Ramaricium yunnanense resembles R. alboflavescens (Ellis & Everh.) Ginns (1979: 94) and R. albo-ochraceum

(Bres.) Jülich (1977: 417) by sharing the ornamented basidiospores. However, *R. alboflavescens* differs in having the pale olive drab to pale olive-brown hymenophore both larger basidia ($33-45 \times 8-10 \mu m$) and thick-walled, ellipsoid to ovoid basidiospores ($5.9-7.8 \times 5.5-7.3 \mu m$, Ginns 1979); while *R. albo-ochraceum* differs in its pale ochraceous hymenial surface, larger basidia ($30-40 \times 5-7 \mu m$) and narrowly ovoid to subfusiform basidiospores ($5.5-8 \times 3-3.5 \mu m$, Jülich 1977).



FIGURE 2. Basidiomata of *Ramaricium yunnanense*. A. Habit; B. A close up view. Bars: A = 1 cm, B = 1 mm (holotype). Photos by: Jin-Ying Gu



FIGURE 3. Microscopic structures of *Ramaricium yunnanense* (drawn from the holotype). A. Basidiospores; B. Basidia and basidioles; C. Encrusted generative hyphae; D. A section of hymenium. Bars: $A = 5 \mu m$; $B-D = 10 \mu m$. Drawings by: Jin-Ying Gu

Key to 5 accepted species of Ramaricium worldwide

1.	Basidiospores smooth	R. flavomarginatum
-	Basidiospores ornamented	2
2.	Basidiospores <5.5 µm in length, thin	R. yunnanense
-	Basidiospores >5.5 µm in length, thin to thick-walled	3
3.	Hymenophore smooth, white to pale ochraceous	R. albo-ochraceum
-	Hymenophore cracking, pale greyish to pale olive brown	4
4.	Hymenophore pale buff, basidiospores yellow, broadly ellipsoid	R. polyporoideum
-	Hymenophore pale olive brown, basidiospores hyaline or pale yellow, globose	R. alboflavescens

Acknowledgements

The research was supported by the National Natural Science Foundation of China (Project No. 32170004), Yunnan Fundamental Research Project (Grant No. 202001AS070043), and the High-level Talents Program of Yunnan Province (YNQR-QNRC-2018-111).

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