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Laccaria rubroalba sp. nov. (Hydnangiaceae, Agaricales) from Southwestern China

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Abstract

The novel species *Laccaria rubroalba* is described from Southwestern China by using both morphological characteristics and molecular data. It is characterized by small basidiomata; reddish white pileus when moist or young, white to pale when dry; 4-spored basidia and globose to broadly ellipsoid, hyaline, moderately echinulate basidiospores. Phylogenetic relationships inferred from ITS sequence data confirmed the separation of this new species from other closely related species in the genus *Laccaria*. The new species is compared with similar taxa. A description, line drawings and colour photographs of the new species, and phylogenetic tree to show the placement of the new species are provided.

Keywords: Mushrooms, Mycorrhizal fungi, Phylogeny, Taxonomy, Yunnan Province

Introduction

Laccaria Berk. & Broome (Hydnangiaceae, Agaricales) is an ectomycorrhizal genus with a cosmopolitan distribution. Species in this genus range from host-generalists to host-specialists (Giachini *et al.* 2004, Sheedy *et al.* 2013, Wilson *et al.* 2013, Sheedy *et al.* 2015). Some *Laccaria* species, such as *L. amethystine* Cooke (Wilson *et al.* 2013) and *L. laccata* (Scop.: Fr.) Cooke (Osmundson *et al.* 2005), are pioneers in harsh conditions (Wadud *et al.* 2014). *Laccaria* species also have potential for high altitude land recovery (Osmundson *et al.* 2005). Species of *Laccaria* have been used in experimental systems for studies of ectomycorrhizal basidiomycetes. Lately, several new species of *Laccaria* have been described worldwide.

Laccaria is one of the most readily identifiable mushroom genera and is characterized by thick, widely spaced lamellae, purple or orange to brown or flesh-coloured fruiting bodies with adnate to slightly decurrent gills and hyaline and ornamented basidiospores in most species. Combined morphological and molecular characteristics can provide useful data to identity *Laccaria* species (Gardes *et al.* 1990, Mueller 1991, Osmundson *et al.* 2005).

Yunnan Province, located in Southwestern China, is known to be one of the most important biodiversity hotspots of the world (Xu *et al.* 2009, Popa *et al.* 2014). Six species have been recorded in Yunnan province, China, among the 39 known *Laccaria* species in the world. During the last decade, numerous new *Laccaria* species have been described from this region, such as, *L. alba* Zhu L. Yang & L. Wang and *L. angustilamella* Zhu L. Yang & L. Wang (Wang *et al.* 2004), *L. fulvogrisea* F. Popa, Rexer & G. Kost (Popa *et al.* 2014). *L. aurantia* F. Popa, Rexer & G. Kost and *L. yunnanensis* F. Popa, Rexer & G. Kost (Popa *et al.* 2014). Our survey on macrofungi in Yunnan Province, China has been carried out from 2012 (Guo *et al.* 2014, Li *et al.* 2014, Ye *et al.* 2014). Under the framework of this survey, we studied four collections of *Laccaria* from Mengsong, Yunnan Province. The collections were identified as a new species based on both morphological characteristics and molecular data. In this paper, we introduce *L. rubroalba* with a full description, line drawings, colour photographs and a phylogenetic tree to show the placement and uniqueness of the new species.

Materials and methods

Collection site and collections

Four collections of the new *Laccaria* species were found in mixed forest dominated by *Fagaceae* and other broad-leaf trees between August and September of 2012 and 2013 in Mengsong, Yunnan Province, Southwestern China.



FIGURE 1. Basidiomata of Laccaria rubroalba A-C: KUN-HKA 90766, E-G: KUN-HKA 90753.

Morphological studies

Each collection was photographed in *situ*, gathered, wrapped in aluminum foil, and placed in a collection box with compartments in order to avoid mixing or crushing. The macro-morphological description of fresh samples was carried out soon after returning from the field following the methodology described in Lodge *et al.* (2004) (fig. 1). Colour coding was according to Kornerup and Wanscher (1978). Specimens were dried using an electric food dryer at 35 °C. Dry specimens are deposited at herbarium of Kunming Institute of Botany, Chinese Academy of Sciences. Index

Fungorum (2016), Faces of fungi database (Jayasiri *et al.* 2015, Index Fungorum 2016) and MycoBank (Robert *et al.* 2014) numbers are registered. Microscopic structures were examined from dry specimens following the protocols of Largent and Thiers (1977) (fig. 2). Detailed measurements of at least 20 basidiospores, basidia and cystidia, were taken from at least three collections. Basidiospore dimensions were given excluding ornamentation and the apiculus. In addition, the length/wide ratio (Q) was calculated.



FIGURE 2. Line drawings of microstructures of *Laccaria rubroalba*. a. Pileipellis b. Hymenium with basidia c. Pleurocystidia d. Basidiospores

Phylogenetic analysis

DNA extraction, PCR amplification and sequencing

DNA was extracted from dry basidiomata by using the Biospin Fungus Genomic DNA Extraction Kit (BSC14S1) according to the manufacturer's protocol. The internal transcribed spacer (ITS) region of the nuclear ribosomal DNA (rDNA) were amplified using the primers ITS1-F (5'CTTGGTCATTTAGAGGAAGTAA3') and ITS4 (5'TCCTCCGCTTATTGATATGC3') following the studies of Gardes *et al.* (1990) and Popa *et al.* (2014). DNA sequencing was performed at BGI Sequencer, Beijing, China.

Sequence alignment and phylogenetic analyses

Contig assembly for generating one consensus sequences from forward and reverse sequences was conducted using SeqMan Pro (DNASTAR, Madison, WI). Sequences generated for this study plus those retrieved from GenBank were initially aligned using the program MAFFT (Katoh & Standley 2013) with default settings, and then manually adjusted in BioEdit v. 7.0.4. The ITS alignment was treated with Gblocks version 0.91 b (Castresana 2000), eliminating poorly aligned positions and ambiguous regions. Maximum likelihood (ML) analysis was performed with RAxML-HPC BlackBox (8.0.0) (Stamatakis 2006, Stamatakis *et al.* 2008) under GTRGAMMA model with one thousand rapid bootstrap (BS) replicates. Bayesian analysis was performed with MrBayes 3.2 (Ronquist & Huelsenbeck 2003, Ronquist *et al.* 2012) using GTR+I+G model selected by AIC in MrModeltest 2.2 (Nylander 2004). Bayesian analysis included two independent runs of 5,000,000 generations with four nchains per run, a sampling frequency of 100 and print frequency to 1000 generations. A burn-in of 10% with nst = 6, rates = invgamma, statefreqpr = dirichlet (1,1,1,1), and, for other parameters, default settings were used. As a result, all compatible groups consensus tree from both analyses was selected.

Results

Phylogenetic analyses

The expanded ITS dataset comprised 77 sequences and 646 positions. Four sequences were newly obtained for this study and 73 sequences were retrieved from GeneBank (Benson *et al.* 2013). *Mythicomyces corneipes* was used as the out group following Popa *et al.* (2016). Sequences for all known *Laccaria* species were retrieved from GenBank for phylogenic analyses as in the studies of Popa *et al.* (2016) and Montoya *et al.* (2015). Table 1 shows an overview of all sequences used in the phylogenetic analyses. Maximum likelihood (ML) and Bayesian analyses (BI) generated very similar topologies, and thus only that from the ML analysis is presented along with the bootstrap statistics (BS) and posterior probabilities (PP) values more than 60% and 0.8, respectively, at the nodes (fig. 3). Phylogenetic analyses revealed that four newly sampled specimens formed a distinct lineage with strong support (97BS/1PP).

Taxon	Location	Voucher specimen	Genbank accession no.
L. acanthospora TYPE	China: Xizang	-	JX504102
L. acanthospora	China: Tibet	-	JX504119
L. acanthospora	China: Tibet	-	JX504161
L. affinis	France	GMM7619	KM067853
L. alba	China	-	JX504131
L. alba	China: Yunnan	-	JX504094
L. alba	China	-	JX504126
L. amethysteo-occidentalis	Canada	f16573	FJ627031
L. amethysteo-occidentalis	USA	-	JX504112
L. amethystina	Denmark: Zealand	MC01-520	AM113954
L. amethystina	France	-	JX504150
L. anglica	France	-	GQ406459
L. angustilamella	China: Yunnan	-	JX504168
L. angustilamella	China:Yunnan	-	JX504118
L. angustilamella	China:Yunnan	-	JX504132
L. aurantia	China: Yunnan	MB-FB-001109	JQ681209
L. aurantia	China: Yunnan	MB-FB-001106	JQ670895

TABLE 1. Taxa information and GenBank accession numbers of Laccaria specimens used in the molecular phylogenetic analyses

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TABLE 1. (Continued)

Taxon	Location	Voucher specimen	Genbank accession no.
L. bicolor	China: Yunnan	-	JX504159
L. bicolor	USA	-	JX504114
L. bicolor	USA	-	JX504105
L. bicolor	North America	-	JX504115
L. bullipellis TYPE	China : Xizang	-	JX504100
L. canaliculata	Australia: Victoria	-	JX504136
L. canaliculata	Australia	-	JX504137
L. cf. canaliculata	Australia	MEL:2360196	JX270720
L. cf. lateritia	Australia	MEL:2359691	JX270695
L. cf. lateritia	Australia	MEL:2359692	JX270736
L. cf. masoniae	Australia	MEL:2359694	JX270741
L. fulvogrisea	China: Yunnan	MB-FB-001110	JQ681210
L. fulvogrisea	China: Yunnan	MB-FB-001101	JQ670896
L. glabripes	New Zealand	-	JX504140
L. himalayensis TYPE	China: Xizang	-	JX504101
L. himalayensis	China: Xizang	-	JX504104
L. laccata	France	-	JX504147
L. laccata	Austria	IB2004243	EF644110
L. laccata	USA	JMP0036	EU819477
L. lateritia	Malaysia	RBG Kew K(M) 166658	JN235950
L. lateritia	India	RBG Kew K(M) 166659	JN235949
L. lateritia	Australia	-	FJ168596
L. masoniae	Australia: Tasmania	-	JX504139
L. montana	North America	TENN 42885	DQ149866
L. montana	North America	TWO 319	DQ149862
L. murina	Japan	-	AB211271
L. negrimarginata TYPE	China: Tibet	-	JX504120
L. nobilis	North America	TENN 42527	DQ149861
L. nobilis	North America	CLC 1445	DQ149863
L. oblongospora	France	-	GQ406466
L. ochropurpurea	USA: Illinois	-	JX504169
L. ochropurpurea	Canada	-	KM406966
L. ohiensis	Europe	-	JX504106
L. proxima	Europe	C19 EC174	AY750156
L. proxima	France	-	JX504152
L. proxima	Germany	FP-98555	DQ499641
L. proxima	North America	TENN 42922	DQ149852
L. pseudomontana	North America	Cripps 1771	DQ149870

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TABLE 1.	(Continued)
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Taxon	Location	Voucher specimen	Genbank accession no.
L. pseudomontana	North America	Cripps 1625	DQ149871
L. pumila	North America	CLC 1252	DQ149864
L. pumila	North America	TWO 501	DQ149873
L. roseoalbescens	Mexico	LM5099	KJ874328
L. roseoalbescens	Mexico	LM5042	KJ874327
L. rubroalba sp.nov.	China: Yunnan	KUN-HKA 90758	KX449357
L. rubroalba sp.nov.	China: Yunnan	KUN-HKA 90753	KX449358
L. rubroalba sp.nov.	China: Yunnan	KUN-HKA 90766	KX449359
L. rubroalba sp.nov.	China: Yunnan	KUN-HKA 90751	KX449360
L. salmonicolor TYPE	China: Tibet	-	JX504143
L. stellata	Panama	SYC 207	KP877339
L. stellata	Panama	SYC 109	KP877340
L. tortilis	Europe	DBGH 20904	DQ149872
L. tortilis	France	GMM7635	KM067859
L. trichodermophora	USA: Texas	-	JX504157
L. trullisata	-	-	JX504170
L. vinaceoavellanea	Japan	TNS:A2984	JN942785
L. vinaceoavellanea	Japan	TNS:A0559	JN942803
L. yunnanensis	China: Yunnan	MB-FB-001108	JQ681208
L. yunnanensis	China: Yunnan	MB-FB-001107	JQ670897
Mythicomyces corneipes	Germany	ES11.10.2.A	KC964108
Mythicomyces corneipes	Germany	AFTOL-ID 972	DQ404393

Taxonomy

Laccaria rubroalba X. Luo, L. Ye, Mortimer & K.D. Hyde, *sp. nov. Index Fungorum number*: IF552302; *Facesoffungi number*: FoF 02078

Etymology:-The species epithet "rubroalba" refers to the pileus colour of the new species.

Diagnosis:—The new species differs from known species of *Laccaria* by medium sized basidiomata; pileus 22–40 mm in diameter, translucent-striate, reddish white when moist or young, becoming white to paler when dry; lamellae distant, adnate; globose to subglobose to broadly ellipsoid, hyaline, moderately echinulate basidiospores (5) $6-9(10) \times 5-7(8) \mu m$, Q = 0.97-1.31; basidia, 4-spored, clavate. Pileipellis a cutis, 4–9(13) μm diameter thickness.

Description:—Basidiomata medium size. Pileus 22–40 mm in diam., less than 1 mm thick at disc., hemispherical to convex, flattened with age, centrally depressed to umbilicate, smooth with translucent-striate ridges, reddish white (10A2) when moist or young, becoming white to paler when dry. Context soft. Lamellae up to 4 mm broad, distant, adnate, ventricose to broadly ventricose, flesh-coloured. Stipe $20-31 \times 2-4$ mm, cylindrical with an enlarged base, centrally inserted, fistulose, fleshy brown, becoming pale brownish with age, and with rhizomorphs.

Basidiospores (5) 6–9 (10) × 5–7 (8) μ m, Q = 0.97–1.31, globose, to subglobose to broadly ellipsoid, hyaline, moderately echinulate, 1.2–2.7 μ m long, 0.73–0.96 μ m broad at the base. Basidia 28–56 × 9–10 (11) μ m, 4-spored, some 2-spored, hyaline. Pleurocystidia (20) 25–40 (55) × 4–6 (7) μ m, flexuous to narrowly-cylindrical. Cheilocystidia rare, (11) 12–26 (26) × 5–9 μ m, cylindrical to capitate; margin cell 18–33 × 3–6 μ m, cylindric to clavate. Pileipellis a cutis to interwoven, 4–9 (13) μ m diam thickness, consisting of radiating interwoven hyphae, with rounded top and thick-walls (up to 1.20 μ m). Hyphae 3–6 μ m diam, Clamp connections present.



FIGURE 3. Maximum-likelihood (ML) phylogram based on ITS rDNA sequence data showing the phylogenetic position of *Laccaria rubroalba* with selected *Laccaria* species. The right number on the nodes denotes the bootstrap values $\geq 60\%$. The left number on the nodes indicates the Bayesian analysis probability values ≥ 0.80 in percent.

Habitat and Distribution:—Scattered on a trunk of *Castanopsis mekongensis* in a tropical forest dominated by mixed *Fagaceae* and broad-leaf trees in Xishuangbanna, Yunnan, China.

Material examined:—CHINA. Yunnan Province: Xishuangbanna, Mengsong village, elevation 1734 m, N21° 30' 52", E100° 28' 44.76", 18 August 2013, Xia Luo (KUN-HKA 90753, **Holotype**). CHINA. Yunnan Province: Xishuangbanna, Mengsong village, elevation 1694 m, N21° 30' 53", E100° 28' 49.1", 5 September 2012, Xia Luo (KUN-HKA 90758, Paratype). CHINA. Yunnan Province: Xishuangbanna, Mengsong village, elevation 1635 m, N21° 30' 14.4", E100° 29' 22.5", 12 October 2012, Xia Luo (KUN-HKA 90766, Paratype). CHINA. Yunnan Province: Xishuangbanna, Mengsong village, elevation 1635 m, N21° 30' 14.4", E100° 29' 22.5", 20 September 2012, Xia Luo (KUN-HKA F90751, Paratype).

Discussion

Laccaria rubroalba is morphologically characterized by its medium sized basidiomata; reddish white to pale white pileus (22–40 mm); globose to subglobose to broadly ellipsoid, hyaline, with moderately echinulate basidiospores; and flesh-coloured distant lamellae.

Laccaria yunnanensis (Popa *et al.* 2014) resembles *L. rubroalba*, however, *L. yunnanensis* differs from the latter in having bigger basidiomata, brownish to flesh-coloured basidiomata, convex to hemispherical, large, striate pileus (60–100 mm), and large pleurocystidia, (50) 55–65 (70) × (10) 15–25(30) μ m, and pileipellis consisting of radiating interwoven thin-walled hyphae. *Laccaria fulvogrisea* (Popa *et al.* 2014) differs from *L. rubroalba* by having grey to brownish basidiomata, large echinulate basidiospores and long cheilocystidia. *Laccaria salmonicolor* A.W. Wilson & G.M. Muell. (Wilson *et al.* 2013) is morphologically similar to *L. rubroalba*, but differs from the latter by having reddishbrown basidiomata with salmon-pink lamellae, smaller, globose, moderately echinulate basidiospores, and pileipellis a cuits. *Laccaria rubroalba* is morphologically similar to *L. laccata* (Osmundson *et al.* 2005), but the basidiomata of *L. laccata* convex or nearly omphaloid, pale orange, lamellae thick and broadly attached. *Laccaria himalayensis* A.W. Wilson & G.M. Muell. (Wilson *et al.* 2013) differs from *L. rubroalba* by having the characteristics such as brown at the disk to orange-pink at margin of the pileus, adnate to decurrent lamellae, orange-pink, moderately echinulate, globose basidiospores, pileipellis cutis of cylindrical and irregularly inflated hyphae. Furthermore, *L. acanthospora* A.W. Wilson & G.M. Muell. (Wilson *et al.* 2013) differs from *L. rubroalba* in having orange basidiomata, broad and distant lamellae, pink-lavender hues on the stipe, longer and broad echinulate basidiospores.

According to our phylogenetic results (fig. 3), the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA of *L. rubroalba* differs from the corresponding sequences known form other *Laccaria* species. *Laccaria rubroalba* is phylogenetically closely related to *L. aurantia*, but the latter differs from *L. rubroalba* at seven positions in ITS sequence with strong support (82 BS/1 PP). Morphologically, *L. aurantia* (Popa *et al.* 2014) has broad and orange lamellae, globose and balloon-like, large and short densely echinulate basidiospores, pileipellis semper hypha intertextis, and short pleurocystidia.

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