ISSN (print) 0093-4666 ISSN (online) 2154-8889

MYCOTAXON

DOI: 10.5248/113.355

Volume 113, pp. 355–364 *July–September 2010*

Occurrence of *Lentinula raphanica* in Amazonas State, Brazil

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Abstract — *Lentinula raphanica* (*Basidiomycota*, *Marasmiaceae*) has been found in the Amazonian region of Brazil for the first time. Sequencing of the LSU region of the nuclear ribosomal DNA confirms the identity of the species. Macro- and microscopic descriptions and illustrations are provided, and the American distribution of *Lentinula* species is summarized.

Key words — *Agaricales*, diversity

Introduction

Lentinula Earle had long been considered to be a synonym of the cosmopolitan genus *Lentinus* Fr., but it is now accepted as a distinct genus with significant morphological differences in hyphal structure (Pegler 1983a) and type of wood rot (Redhead & Ginns 1985). Molecular data also confirm the distinction between the genera (Molina et al. 1992, Hibbett & Vilgalys 1993).

The genus comprises only seven morphological species with Asian-Australasian and American distributions. According to Nicholson et al. (1997), *Lentinula edodes* (Berk.) Pegler comprises three phylogenetic species with biological compatibility (Shimomura et al. 1992): *L. edodes*, *L. novae-zelandiae* (G. Stev.) Pegler, and *L. lateritia* (Berk.) Pegler. However, Fukuda et al. (1994) and Hibbett et al. (1998) have identified five distinct molecular groups within the *L. edodes* complex, each specific to a particular geographic region.

The currently recognized American species are *L. boryana* (Berk. & Mont.) Pegler, described from material collected by Blanchet de Laurane in Bahia State,

Brazil; *L. guarapiensis* (Speg.) Pegler, known only from the type collection made by Balansa in Guarapi, Paraguay; and *L. raphanica*, described from Florida, U.S.A, and segregated from specimens previously identified as *L. boryana* or *L. aciculospora* J.L. Mata & R.H. Petersen from Costa Rica.

Lentinula boryana and *L. raphanica* are morphologically very similar. Thon & Royse (1999) first established the separation of these two phylogenetic independent lineages within *L. boryana* after which Hibbett (2001) showed that the lineages represented two phylogenetic species of *Lentinula*. One species ("group VI") had a Central American distribution, and the other ("group VII") had a Coastal-Caribbean-South American distribution. Mata & Petersen (2001) and Mata et al. (2001) formally described these groups as *L. boryana* and *L. raphanica*, respectively.

In Brazil, *L. boryana* has been reported previously for Bahia, the type locality [Berkeley & Cooke 1876, as *Agaricus boryanus*; Dennis 1951, as *Collybia boryana*; Pegler 1983a; Mata & Petersen 2001], Paraná State (Meijer 2001), Rio Grande do Sul State (Rick 1907, as *C. boryana*; Singer 1952a, 1952b, 1953, as *Lentinus puiggarii*), and São Paulo (Grandi et al. 1984, as *Lentinus cubensis*; Pegler 1983b, under *L. puiggarii*; Pegler 1988, 1997). In this paper, we report the first record of *Lentinula raphanica* for Amazonas State, supported by morphological and molecular (nLSU) data. This is the second record for Brazil; the voucher material mentioned by Thon & Royse (1999, ambiguously cited as "sp834, Instituto de Botânica Herbarium, São Paulo, Brazil") was not found there.

Material and methods

Sampling

The studied material was collected at the Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Amazonas State. The specimens were deposited at the Instituto de Botânica Herbarium, São Paulo, Brazil (SP) and at the Instituto Nacional de Pesquisas da Amazônia Herbarium, Manaus, Brazil (INPA).

Morphological study

Dried material was analyzed microscopically. Sections of basidiomata were first rehydrated with 70% ethanol and then with 5% KOH for 5–10 minutes and observed under a light microscope equipped with a drawing tube.

Molecular study

nLSU rDNA sequences were phylogenetically analyzed to compare the *Lentinula* species from Amazonas, Brazil, with sequences deposited in GenBank (Table 1).

DNA extraction —DNA extraction protocols were adapted from Ferreira & Grattapaglia (1995) using lyophilized mycelium previously ground to a fine powder in liquid nitrogen. The sample was re-suspended in 50 µL TE, incubated at 37°C for 30 min after the addition of RNase A (0.01 mg μL^{-1}), and stored at –20°C.

Table 1. Collection data and GenBank accession number of the taxa analyzed.

Species	GenBank nr.	Voucher/ Strain	Reference
Gymnopus biformis	AF261336	RV98/32	Moncalvo et al. 2002
G. menehune	AY639423	AWW02-SFSU	Wilson & Desjardin 2005
Lentinula boryana	AF356151	R.G. Thorn 960624/09	Hibbett 2001
	AF356152	R38	Hibbett 2001
L. lateritia	AF356160	RHP3577	Hibbett 2001
	AF356162	TMI1172	Hibbett 2001
L. raphanica	AF356147	DUKE HN2002	Hibbett 2001
	GQ865600	SP394008	This study

PCR amplification — The 5' end of the nLSU rDNA was targeted for amplification. The nLSU region was amplified using the primer set LR16 and LR5 (Moncalvo et al. 2000). PCR reactions containing 2.0 U of Platinum® Taq DNA Polymerase - Brazil (Invitrogen), 0.2 mM of each dNTP, 1.5 mM of $MgCl_2$ and 0.2 µM of each primer in 100 µL were performed in an Eppendorf thermocycler. The program was initiated by a 5-min denaturation step at 94°C, followed by 40 cycles of 40 sec at 94°C, 30 sec at 55°C and 60 sec at 72°C. Polymerization was completed by a 5-min incubation at 72°C. Amplification products were electrophoresed in a 1.5% agarose gel containing 0.1 µg ml^{-1} ethidium bromide. PCR products were then purified using the PureLink PCR Purification Kit (Invitrogen).

DNA sequencing —PCR product was sequenced in both directions using the same amplification primers and the DYEnamic ET Dye Terminator Kit in a MegaBACE 1000 DNA sequencer (GE Healthcare) according to the manufacturer's instructions. The sequence was deposited in GenBank as GQ865600.

Data analysis — Initially, a blast search was conducted in GenBank to compare the sequence obtained from the Amazonas material with existing sequence data. Subsequently, phylogenetic analysis was performed using the nLSU sequence determined in this study and five sequences available on GenBank (Table 1).

The sequences were analyzed using BioEdit version 7.0.5.3 (Hall 1999) and automatically aligned in Clustal W (Thompson et al. 1994). Parsimony analysis was performed using PAUP* version 4.0b10 (Swofford 2001). The most parsimonious tree was obtained by a heuristic search with 1000 replicates of simple sequence addition, employing the tree-bisection-reconnection (TBR) branch-swapping algorithm. Characters from the extreme 5' and 3' ends of the sequences were deleted from all taxa to obtain individual datasets that had identical start and end positions. Gaps were treated as missing data, all characters were unordered and equally weighted, and multistate taxa were interpreted as uncertainty.

Branch support values were determined using 1000 bootstrap (BS) replicates. Estimated levels of homoplasy and phylogenetic signal (retention and consistency indexes) were determined. Trees were rooted using *Gymnopus biformis* (Peck) Halling and *G. menehune* Desjardin et al. as outgroups.

Results and discussion

Molecular analysis

Eight sequences were aligned — two each from three *Lentinula* taxa, and one per outgroup. The alignment consisted of 1426 characters, including gaps. Prior to analysis, 719 characters were excluded from the 5' and 3' ends of the sequences. Of 707 characters included in the analysis, 654 characters were constant, 10 variable characters were parsimony uninformative, and 43 were parsimony informative.

The heuristic search with 1000 BS replicates resulted in a single most parsimonious tree with the following scores: tree length = 60 steps, consistency index = 0.933, retention index = 0.934.

The most parsimonious tree generated from the nLSU sequence data from *Lentinula* species revealed three clades (Fig. 1) according with species identification. *L. boryana* is the sister species of *L. raphanica* with 75% BS support. Neighbor joining analysis (data not shown) showed the same topology. This result and a two-base pair difference between the Amazonas sequence (GQ865600) and the *L. raphanica* sequence (AF356147, obtained from the same material (DUKE HN2002) used for ITS analysis by Mata et al. 2001) support the Amazonas material as *L. raphanica*.

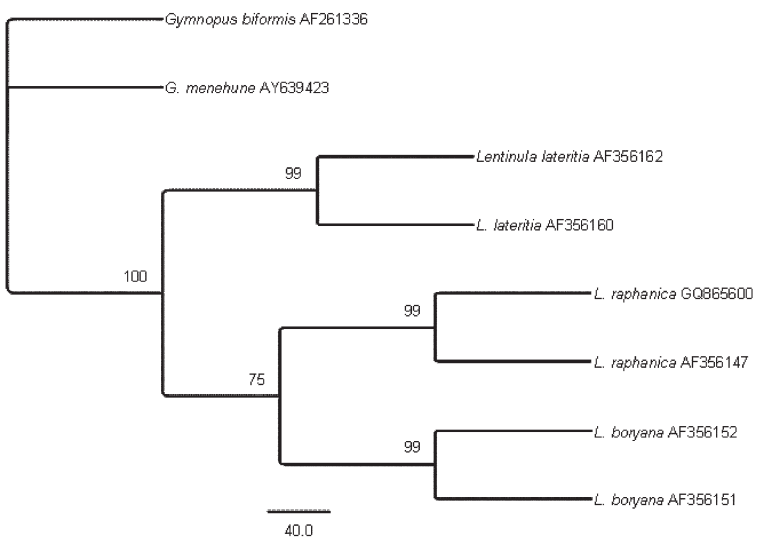

Figure 1. MP tree generated by parsimony analysis of partial LSU rDNA sequences. BS values are shown above branches. GenBank accession numbers are shown after each taxon name.

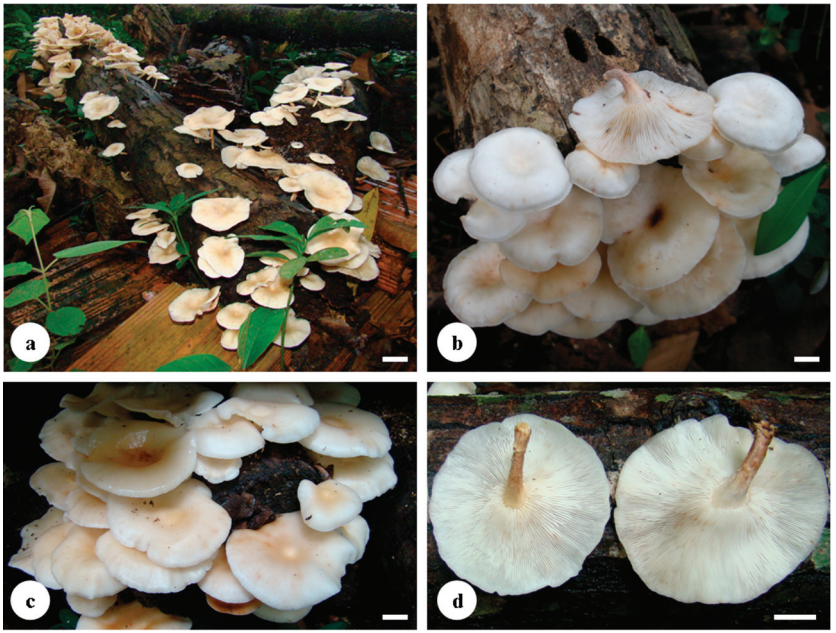

Figure 2. *Lentinula raphanica* (INPA230870, SP394008). Bar = 1 cm.

Taxonomy

Lentinula raphanica (Murrill) J.L. Mata & R.H. Petersen, Mycotaxon 79: 228. 2001. Figs. 2, 3

≡ *Armillaria raphanica* Murrill, Mycologia 35: 423. 1943.

≡ *Lentinula raphanica* (Murrill) J.L. Mata & R.H. Petersen, Mycologia 93: 1107. 2001 (superfluous combination).

= *Gymnopus alliaceus* Murrill, Mycologia 35: 425. 1943.

Pileus 3–6 cm diam., convex at first with an involute margin, then applanate with a depressed center to infundibuliform when fully expanded, glabrous, some slightly viscous, smooth, hygrophanous, white to dirty white, center sometimes cinnamon brown to brownish, sometimes with cinnamon brown patches, fleshy. Lamellae free, crowded, white, thin, smooth-edged, with lamellulae. Stipe 40 × 3 mm, central to slightly eccentric, curved, equal to tapering at the base, surface with some floccose fibrillose small scales, dirty white to pinkish, with brownish base, firm. Annulus absent. Basidiospores not seen. Basidia not seen. Basidioles numerous, mostly ventricose, 13–18 × 4–5 µm. Pleurocystidia absent. Cheilocystidia 21.4–28.5 × 5–7 µm, versiform, contorted, clavate with diverticulate outgrowths, hyaline, thin-walled, clamped at the base. Lamellar trama regular, becoming interwoven towards the edge,

hyphae 3–14 µm, hyaline, thin to slightly thick-walled, with clamp connections. Caulocystidia 14.2–50 × 2–7 µm, abundant, cylindrical, clavate or flexuous, apex obtuse or with outgrowths, hyaline. Lignicolous, growing on *Bertholletia excelsa* Humb. & Bonpl. (*Lecythidaceae*, castanha da amazônia).

Examined material — BRAZIL. AMAZONAS: Manaus, Instituto Nacional de Pesquisas da Amazônia — 04.XII.2007, N.K. Ishikawa s.n. (INPA230870, SP394008; GenBank GQ865600); 31.VIII.2007, T.A. Silva s.n. (INPA230868, SP394011); 21.XI.2007, T.A. Silva s.n. (INPA230869, SP394010).

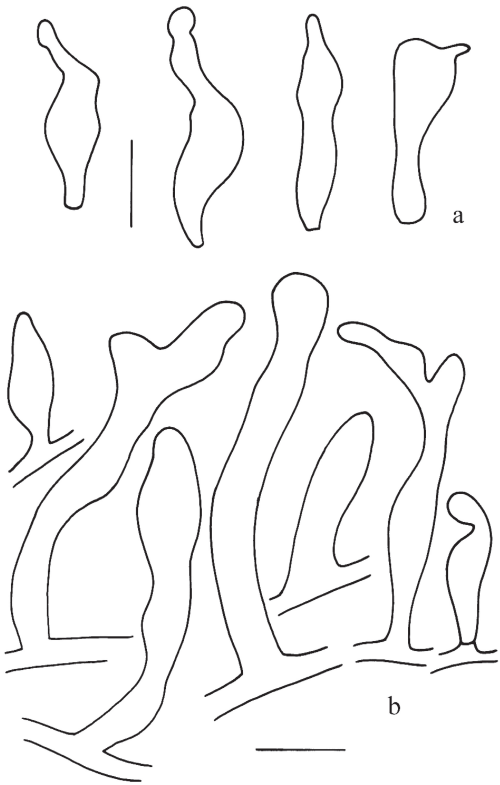

Figure 3. *Lentinula raphanica*. a. Cheilocystidia. b. Caulocystidia. (INPA230869, SP394010). Bar = 10 µm.

Except for the lighter pileus colour, the Amazonas specimens fit very well macroscopically with the description by Mata et al. (2001). *Lentinula raphanica* greatly resembles *L. boryana*, differing mainly in the cheilocystidia shape and basidiospore dimensions (Mata et al. 2001). According to Mata et al. (2001), in *L. raphanica* basidiospores "have a narrower shape, more subcylindrical than oblong", measuring 4.8–7.2 × 2.0–3.6 µm, Q = 1.50–3.00, Q_x = 2.16,

while in *L. boryana* they measure 4.8–8.0 × 2.4–4.0 µm, Q = 1.30–2.67, Q_x = 1.91. Unfortunately, the three Amazonas collections examined were sterile, lacking basidia on the lamellae, and the cheilocystidia were very difficult to see. Nevertheless, it was possible to confirm the species identity by matching the caulocystidia shape to those depicted by Mata et al. (2001). In *L. boryana*, the caulocystidia are cylindrical to clavate, while in *L. raphanica*, they are cylindrical, clavate, or flexuous, with an obtuse apex, and knobbed or with outgrowths. This difference in shape seems to be a constant and reliable diagnostic character. A more complete description of this species can be found in Mata et al. (2001) and Mata & Petersen (2001).

Figure 4. Geographic distribution of the American species of *Lentinula*.

The geographic distribution of the American species of *Lentinula* is shown in Fig. 4. After Mata & Petersen (2000) and Mata et al. (2001), *L. raphanica* was known from Brazil (probably from São Paulo State), Costa Rica, Puerto Rico, Trinidad, United States of America, and Venezuela; *L. boryana* was known from Brazil, Costa Rica, Cuba, Guadeloupe, Guyana, Mexico and Panama; *L. aciculospora* was known from Costa Rica; and *L. guarapiensis* was known from Paraguay. Subsequently, Vasco-Palacios et al. (2005) have reported *L. raphanica* from Colombia and Piepenbring (2008) has recorded *L. aciculospora* in Panama. Except for *L. guarapiensis*, which should be re-collected at or near its type locality to establish its biological and phylogenetic identity, the remaining species are well defined and probably occur throughout Central and South America. Further explorations will add *Lentinula* collections and may improve understanding of its distribution and diversity in the Americas.

Acknowledgments

The authors would like to thank Dr. Marília Gaspar, Instituto de Botânica, for allowing us to use her laboratory, Ruby Vargas-Isla for the help in field and laboratory, and Klei Sousa for inking the illustrations. We also thank Dr. Laura Guzmán-Dávalos, Universidad de Guadalajara, and Dr. Juan Luis Mata, University of South Alabama, for the critical review of the manuscript. We also thank Dr. Lorelei L. Norvell for editorial review. This study was supported by Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM).

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