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ORIGINAL PAPER

Molecular and morpho-anatomical description of mycorrhizas of *Lactarius rimosellus* on *Quercus* sp., with ethnomycological notes on *Lactarius* in Guatemala

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Abstract Guatemala is one of the richest biodiversity hotspots worldwide, bursting a wild array of ecosystems that range from pine and mixed forests in the highlands to tropical rain forests in the extensive El Petén area, bordering Belize and Mexico. Despite this biological wealth, however, current knowledge on the Guatemalan mycobiota is particularly scant, in part because of the prolonged civil war that has prevented exploration of many ecological niches. In the present paper, we report on the occurrence of *Lactarius rimosellus* Peck—a rarely discussed species—in oak-pine mixed forests in the Guatemalan highlands and describe the relevant ectomycorrhizae formed with *Quercus* sp. by means of molecular and morpho-anatomical tools. On the phylogenetic trees constructed on the basis of the partial LSU sequence, sporocarp- and ectomycorrhizae-derived sequences formed a common, statistically supported clade. The structural features of the ectomycorrhizae of *L. rimosellus* were generally found to match those described on various hosts for other *Lactarius* species belonging to the subgenus *Russularia*, where *L. rimosellus* has been traditionally assigned. These mycorrhizae are characterized by a pseudoparenchymatous outer mantle layer, with epidermoid or angular hyphal cells, and a plectenchymatous inner mantle layer; lactifers are embedded either in the middle and/or inner mantle layer. In the framework of a more general, ongoing study of the ethnomycology of the Maya populations in the Guatemalan highlands, we also report on the traditional knowledge about *Lactarius* mushrooms and their uses among native people.

Keywords *Lactarius* · DNA sequences · Ectomycorrhizal fungi · Neotropical fungi · Morphology · Ethnomycology

Introduction

Our knowledge on the mycobiota of the Neotropics, despite its potentially high biodiversity, is still very poor. Most studies have focused on selected areas, such as various states of Mexico and Costa Rica, thanks to the efforts of national and international research groups. Besides, other countries of the region have been almost totally neglected, for the lack of trained local mycologists and for other reasons. In Guatemala, for example, detailed mycological studies have been severely hampered by the prolonged civil war (1960–1996) and related political and social instability. As a consequence, only limited accounts of the mycota inhabiting the highly diversified

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Guatemalan ecological niches are available to date (Guzmán et al. 1985; Sommerkamp and Guzmán 1990; Flores and Simonini 2000; Flores et al. 2005).

The ecological importance played by the ectomycorrhizal (ECM) symbiosis is condensed in just a few data: It is estimated that 2–3% of all vascular plants (some 6000–10,000 species of angiosperm and gymnosperm species grouped in 26 families and 145 genera) are ECM (Smith and Read 2008; Brundrett 2009). Although these figures are much lower compared to the diversity of arbuscular mycorrhizal plants—estimated at over 200,000 species, or two thirds of all vascular plants—it should be recalled that most ECM plants are trees, which form the core structure of boreal forests in the northern hemisphere (e.g., Pinaceae, Fagaceace, Betulaceae), and are also often dominant elements in many temperate, sub-tropical, and tropical areas of the southern hemisphere (e.g., Nothofagaceae, Myrtaceae, Dipterocarpaceae, several cesalpinioid genera in Fabaceae) (Wang and Qiu 2006; Smith and Read 2008; Brundrett 2009). As new ECM plants are likely going to be identified in particular in the tropics, these figures are bound to be updated (i.e., increased) in the future. As for the fungal side, a detailed and critical survey of literature recently revealed that the global richness of ECM fungal species is estimable to be at around 7,750 species, and that, on the basis of estimates of knowns and unknowns in macromycete diversity, a final estimate of ECM species richness would likely be between 20,000 and 25,000 (Rinaldi et al. 2008).

One of the most prominent genera of ECM fungi is *Lactarius* (Hutchinson 1999). With more than 450 species described worldwide, *Lactarius* taxa play a significant role as late-stage colonizers of trees and shrubs in a vast range of ecosystems, from boreal coniferous forests to temperate Mediterranean-type maquis. In the Mesoamerican Neotropics, most of currently available information for this genus comes from Mexico—where Leticia Montoya and Victor Bandala have documented *Lactarius* diversity in coniferous, oak and mesophytic forests—and from Costa Rica, where Gregory Mueller and Roy Halling have been exploring the distribution and ecology of macrofungi in montane *Quercus* forests (Montoya and Bandala 2005, 2008; Mueller and Halling 1995, 2010). On the contrary, information on the occurrence of *Lactarius* in Guatemala is very scanty and mostly reported in gray literature.

In the present study, we describe the morpho-anatomical features of the mycorrhizae formed by *Lactarius rimosellus* Peck on *Quercus* as collected in the central Guatemalan highlands. The identity of the ECMs was also verified through molecular tools. Furthermore, we discuss the results of a survey on the traditional knowledge about *Lactarius* mushrooms and their uses among native Maya people.

Materials and methods

Study site and fungal collections

Sporocarps of *L. rimosellus* (Fig. 1) were harvested in Altavista (Guatemala City, 1,600 m above sea level) and in the Parque Ecológico Florencia (Antigua Guatemala, 1,530 m above sea level), in the central highlands of Guatemala; sporocarps were identified on the basis of published descriptions of macroscopic and microscopic characters (Hesler and Smith 1979; Montoya and Bandala 2005). Among the Mesoamerican *Lactarius* mycota, *L. rimosellus* is macroscopically distinctive for its small dimensions (pileus, 15–50 mm; stipe, 25–50 mm), the orange brown colors (brownish orange to vinaceous cinnamon), the rimulose areolate cuticle, and its scanty watery to whitish latex. In Guatemalan specimens, a dense growth of whitish-brown hyphae is visible at the base of stem (Fig. 1). Ectomycorrhizae were collected only in Altavista, under *Quercus* spp. in an oak-pine tree mixed forest. Several *Quercus* species (*Quercus brachystachis*, *Quercus oleoides*, *Quercus peduncularis*, *Quercus sapotifolia*, and *Quercus tristis*) were present on the collection spot, so that it was not possible to identify the host(s) of *L. rimosellus* at species level. Discrimination between potential host genera, i.e., *Quercus* vs. *Pinus*, was possible on the basis of obvious ECMs morphological characters, as ramification, which clearly indicated an angiosperm as host tree. Soil cores were excavated from beneath sporocarps and immersed overnight in water, and ectomycorrhizal roots were carefully separated under a dissecting microscope. Several tips were immediately transferred into 50% EtOH and stored at −20°C for subsequent DNA analysis. Reference material for sporocarps (OC-2008/1, OC-2008/2) and ectomycorrhizae (OC-2008/1-E) were deposited in the collection of the Department of Biomedical Sciences and Technologies, University of Cagliari, Cagliari, Italy.

Microscopy

Mantle preparations of fresh ectomycorrhizae were fixed on slides with polyvinyl lactophenol for light microscopy. Observations were made with a Zeiss Axioplan 2 bright field microscope and a Leica MZ 6 stereomicroscope. Images were acquired with a Leica DFC290 digital camera. For longitudinal sections (2.5 mm thick), ectomycorrhizae were embedded in LR white resin (Multilab), cut with a Leica Ultracut R ultramicrotome, and stained with toluidine blue in 1% sodium borate for 15 s at 60°C. The general methodology and terminology used to characterize ectomycorrhizae followed Agerer (1986, 1987–2006, 1991). Munsell Soil Color Charts (2000) were used as reference for the descriptions of the colors of ectomycorrhizae.

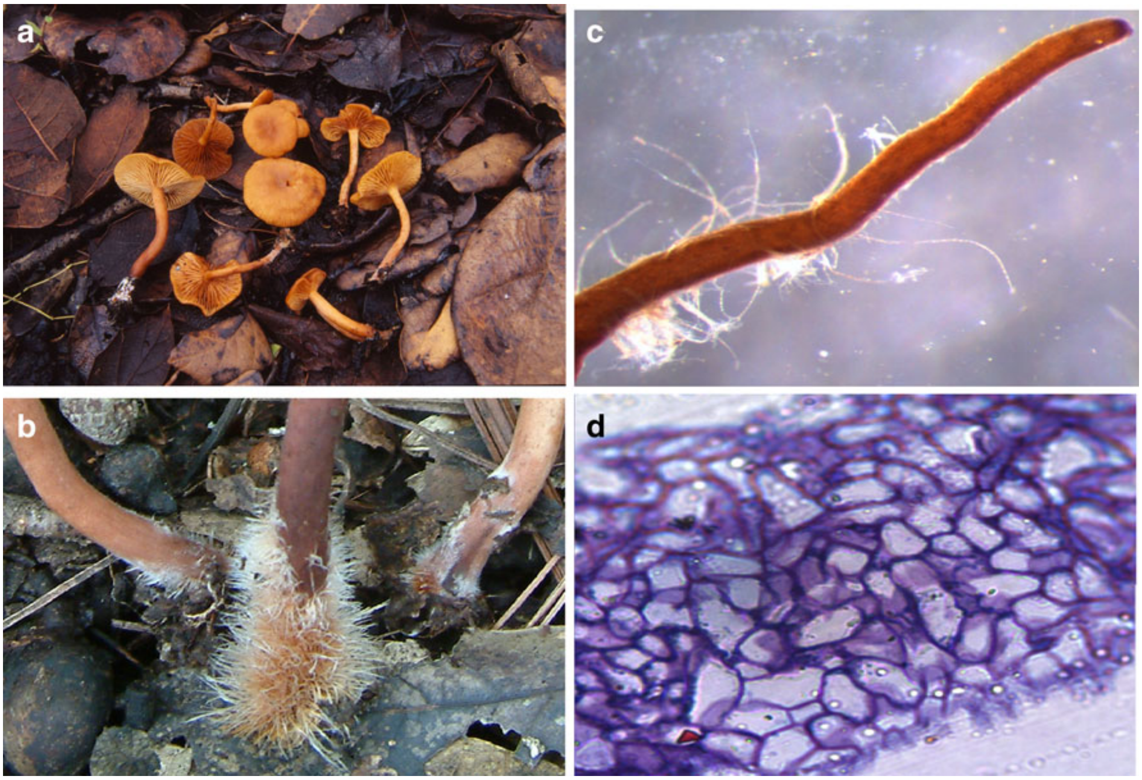

Fig. 1 *Lactarius rimosellus* from the Guatemalan highlands. **a** Habitus. **b** Particular of the stem base, showing a dense growth of whitish-brown hyphae. **c** Habitus of ectomycorrhizae, with abundant rhizomorphs. Magnification, ×32. **d** Pseudoparenchymatous outer mantle layer with polygonal cells. Magnification, ×400

DNA extraction, PCR, and sequencing

For the molecular phylogenetic analyses, DNA was acquired from both sporocarps and ECM root tips stored in ethanol, using the modified cetyl trimethylammonium bromide (CTAB) method as described previously (Erős-Honti et al. 2008). Prior to the DNA extraction, samples were incubated in CTAB solution overnight. Partial 28S rDNA region of the nrDNA was amplified by PCR using the primers LROR (Rehner and Samuels 1994) and LR5 (Vilgalys and Hester 1990) according to the following program: starting denaturation step at 94°C for 2 min, amplification cycles of 35 times with a denaturation for 1 min at 94°C, annealing at 56°C for 45 s, and elongation at 72°C for 2 min. The program closed with a final elongation step at 72°C for 14 min. Amplicons were sequenced by LGC Genomics (Berlin, Germany).

Phylogenetic inference

Sequence electrophoregrams were processed with the Pregap4 and Gap4 modules of the STADEN software package (Staden et al. 2000). Edited sequences were annotated in the GenBank molecular database. Closely related sequences were assembled from the GenBank and EMBL databases with BLAST (Altschul et al. 1990). Acquired sequences were aligned with those acquired from the sporocarps and ECM tips with ClustalW (Thompson et al. 1994), alignments were manually edited by ProSeq (Filatov 2002). Phylogenies were inferred with neighbor joining (NJ), Bayesian and maximum likelihood (ML) methods using MEGA (Tamura et al. 2007), Mr Bayes 3.1.1 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003), and PhyML softwares (Guindon and Gascuel 2003), respectively. For NJ analysis, maximum composite likelihood model was applied, and gaps were deleted only in pairwise comparisons; for rates of substitutions, gamma distribution was proposed (with a shape parameter of 1.00). For the Bayesian analysis, distribution of invariable sites was also estimated according to the gamma distribution. Priors were as follows: equal nucleotide frequencies, uniform prior shape parameter value and uniform proportion of invariable sites, non-constrained topology prior, and unconstrained branch length prior. The MCMC simulation ran for 12,000,000 generations and was sampled in every 100th step with a burn in at 2,500 sampled trees. For the ML method, equilibrium base frequencies were optimized, and four different substitution categories were used; both the proportion of invariable sites and the gamma distribution parameter were estimated. In case of NJ and ML analyses, the statistical support of the clades was tested by bootstrapping, applying 10,000 and 1,000 replicates respectively.

Ethnomycological survey

Fieldwork, developed by researchers from the University of San Carlos de Guatemala, involved repeated visits to 30 localities of the central, western, and northern Guatemalan highlands, in the departments of Alta Verapaz, Baja Verapaz, Chimaltenango, El Quiché, El Progreso, Guatemala, Huehuetenango, Quetzaltenango, Sacatepéquez, San Marcos, Sololá, and Totonicapán. Mushrooms were purchased at municipal markets and from vendors along main routes or obtained from hired local harvesters. In a few cases, mushrooms were also purchased from vendors along the interdepartmental Carretera Panamericana. Information on common names of mushrooms in the local idioms, traditional uses and edibility, methods of cooking, the period of the year mushrooms are found, the locations where they are found, and the prices at which they are sold in the community, were collected through interviews with vendors and harvesters. In each interview, fresh mushrooms and field guides were shown as stimuli and reference.

Results

Descriptions of ectomycorrhizae

Morphological characters. Mycorrhizal system (1.5) 4–10 (20) mm long, only sometimes simple, mainly monopodial-pinnate or irregularly pinnate, with 0–1 orders of ramification (Fig. 1). *Main axes* 0.6–0.8 mm diameter. *Unramified ends* straight or slightly tortuous 1.5–3 mm long and 0.5 mm in diameter; mycorrhizae, including very tip, reddish yellow to yellowish red (5YR 7/6-5/8), older mycorrhizae dark reddish brown. *Surface of unramified ends* smooth, not secreting latex when injured; emanating hyphae not observed; mantle not trasparent. *Rhizomorphs* in some systems are scanty, in others very abundant somehow resembling hyphae at stem base, up to 80 µm in diameter, connected to mantle in restricted points, colour is lighter with respect to mycorrhizal surface, no ramifications, smooth margin (Fig. 2). *Cystidia* lacking. *Sclerotia* lacking.

Anatomical characters of mantle in plan views (Fig. 2) *Outer mantle layer* pseudoparenchymatous with polygonal cells, hyphae irregularly shaped, with main axes 16–20 (30) µm, minor axes 7–8 µm; hyphal cells colourless, with very thin cell walls (<1 µM); no lactifers observed (see also Fig. 1). *Middle mantle layer* pseudoparenchymatous with the same pattern observed for the outer part. Here, hyphal cells start to slightly reduce their dimensions. Lactifers probably lie in this part of the mantle, but they are scanty and quite thin, and observable only from the inner mantle layer (see below). *Inner mantle layer* transitional type, in some parts a clear hyphal arrangement is observed, hyphae are 3–4 µm thick and do not form a specific hyphal pattern; in some other parts, a more pseudoparenchymatous pattern is observable. Here, hyphal cells are shaped as the ones in the outer part, but are smaller (main axes, 14–18 µm and minor axes, 5–7 µm). Lactifers are scant, quite thin (3–4 µm), and very long. Unequivocally determining the position of the lactifers has not been possible because of their thinness. The thickness of the outer mantle layer confused observation, and lactifers were observable only from the thinner inner mantle layer; possibly, lactifers lie in the middle mantle layer. *Very tip* hyphal arrangement as in the other parts of the mantle.

Anatomical characters of emanating elements. Rhizomorphs up to 80 µm in diameter, pale yellow, margin smooth with very scanty emanating hyphae, in the thinner parts rhizomorphs are undifferentiated (type A or B), with hyalinae hyphae compactly arranged or sometimes loosely woven, uniform in diameter (2–3 µm); thicker hyphae are slightly differentiated. Central hyphae somewhat enlarged (4–5 µm thick). Very few, thin (4–4.5 µm diameter) lactifers can be observed. Peripheral hyphae are parallel and very compact in some parts, curled to twisted in others; all the hyphae are clampless and thin walled.

Anatomical characters, longitudinal section. Mantle (15) 25–30 (35) µm thick, compact, two layers can be distinguished due to the shape and dimension of the relative elements. The outer layer, 15–20 µm thick, is formed by larger and more elongated hyphal cells, up to 20 µm tangentially and 5–7 µm radially; more roundish elements, 7–8 µm in diameter, are also observable. The inner part of the mantle is definitely more compact, and hyphae are 2–3.5 µm radially; lactifer cells are difficult to distinguish from the other hyphal cells probably because of their particular thinness; mantle of very tip with the same thickness and hyphal organization, but structures are more compact, single elements difficult to measure. *Tannin cells* lacking. *Caliptra cells* in one discontinuous row, tangentially elongated, collapsed, parallel orientated to root axes, 2.5–4 µm µm thick. *Epidermal cells* rectangular or tangentially oval, (13) 17–22 (28) µm tangentially, 15–20 (22) µm radially. *Hartig net* paraepidermal, one row of roundish, 3–4 µm thick hyphal cells, palmetti-type, lobes 1–1.5 µm wide.

Phylogenetic inference

We managed to amplify the partial LSU region of both the sporocarp and the ectomycorrhiza of *L. rimosellus*.

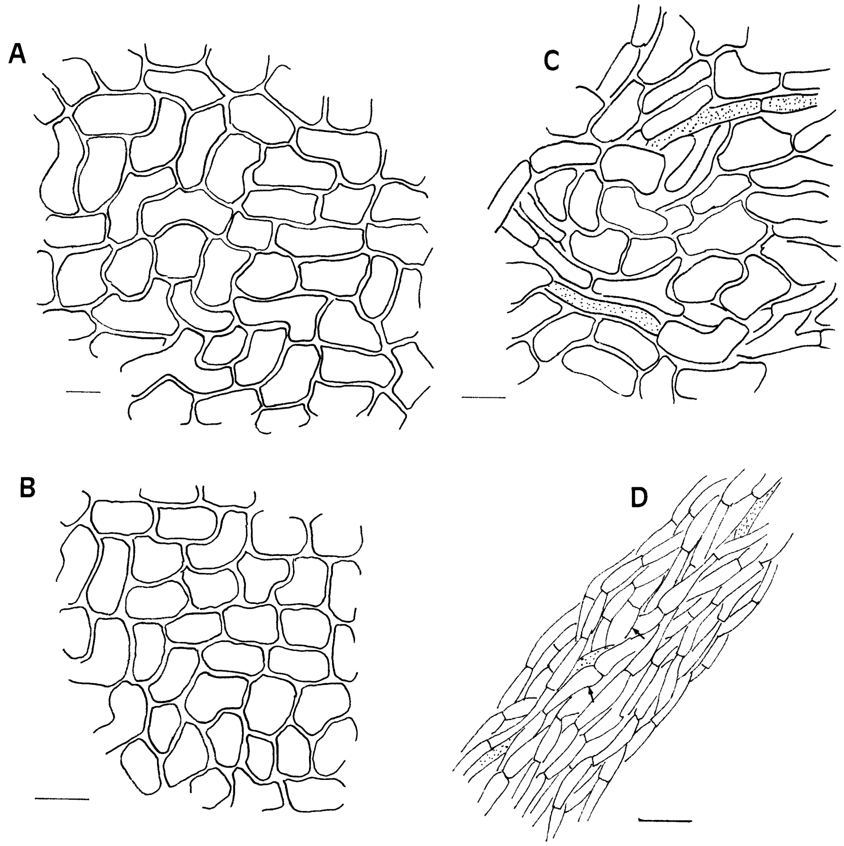

Fig. 2 Anatomical characters of *L. rimosellus* + *Quercus sp.* ectomycorrhizae. **a** Outer mantle layer with a pseudoparenchymatous structure with polygonal cells. **b** Middle mantle layer with a pseudoparenchymatous structure and polygonal cells of smaller dimensions with respect to those of the outer mantle layer. **c** Inner mantle layer, transitional type with hyphae, and hyphal cells. Scant and thin lactifers can be observed. **d** Surface view of thinner rhizomorphs, showing an undifferentiated structure, and central thicker hyphae that appear somewhat enlarged (*arrows*). Scarce and thin lactifers can be observed. All scale bars=10 µm

Sequences have been annotated in GenBank under the accession numbers JF906755-JF906757. In all the phylogenetic analyses, these formed a common, statistically well supported clade on the trees (Fig. 3). However, the sequences of this species did not form a common group of acceptable statistical support with those of any other *Lactarius* species.

Traditional knowledge and use of *Lactarius*

We found that three species of *Lactarius* are commonly gathered and sold for human consumption in the Guatemalan highlands. These are *Lactarius deliciosus* (L.:Fr.) Gray, *Lactarius indigo* (Schweiniz) Fries, and *Lactarius salmonicolor* R. Heim & Leclair (Table 1). In particular, according to the recorded interviews, *L. deliciosus* and *L. indigo* are among the most popular edible mushrooms among Maya populations in Guatemala alongside several species of the *Amanita caesarea* complex. These two species are often offered together, mixed, and sold in form of “medida,” i.e., fixed amount, which equals the content of a small basket. Common names for *L. deliciosus* and *L. indigo* have been recorded in several Maya idioms, not only in the widely spoken K’iche’, Kaqchikel, and Mam but also in the rarer Chuj and Uspanteko (the latter language, closely related to K’iche’, is spoken by as few as 3,000 people in Guatemala). *L. salmonicolor* is restricted to a few mountain sites in the Sierra de los Cuchumatanes (western Guatemala, in the departments of Huehuetenango and El Quiché) where its *Abies guatemalensis* (pinabete) host grows, and is locally appreciated. Besides the widely popular milk-caps, we have been able to record the occasional consumption of other *Lactarius* species in specific communities, such as *L. rubrilacteus* and *L. volemus* in the Chichicastenango (El Quiché) area. Also *L. rimosellus*, the main object of the current study, was found on sale only in the market of Jacaltenango, Huehuetenango. More generally, mushrooms are gathered and sold by women, often in familiar groups spanning three generations. These observations are part of a more general, ongoing study of the ethnomycological knowledge of the Maya populations in the Guatemalan highlands (Bran et al. 2002, 2003, 2005).

Discussion

L. rimosellus is currently classified within the subgenus *Russularia*, section *Olentes*. Leticia Montoya and Victor Bandala have recently described and illustrated material collected in the Gulf of Mexico area (State of Veracruz), after comparison with North American specimens (Montoya and Bandala 2005). This species is reported to occur “[o]n bare

Fig. 3 Bayesian tree demonstrating the relationships of the large subunit rDNA (LSU) sequences derived from the sporocarp and ectomycorrhiza of *L. rimosellus* and those acquired from public databases (accession numbers are indicated in the brackets). *Numbers above the branches* (or the *horizontal lines*) represent the bootstrap values (>50%) of the NJ analysis; *numbers below the branches* (*horizontal lines*) stands for the posterior probability values (>0.5) of the Bayesian analysis (*left*) and the bootstrap values (>50%) of the MP analysis (*right*) (outgroups: *Russula campinensis* and *R. discopus*). Scale stands for one change per 100 characters

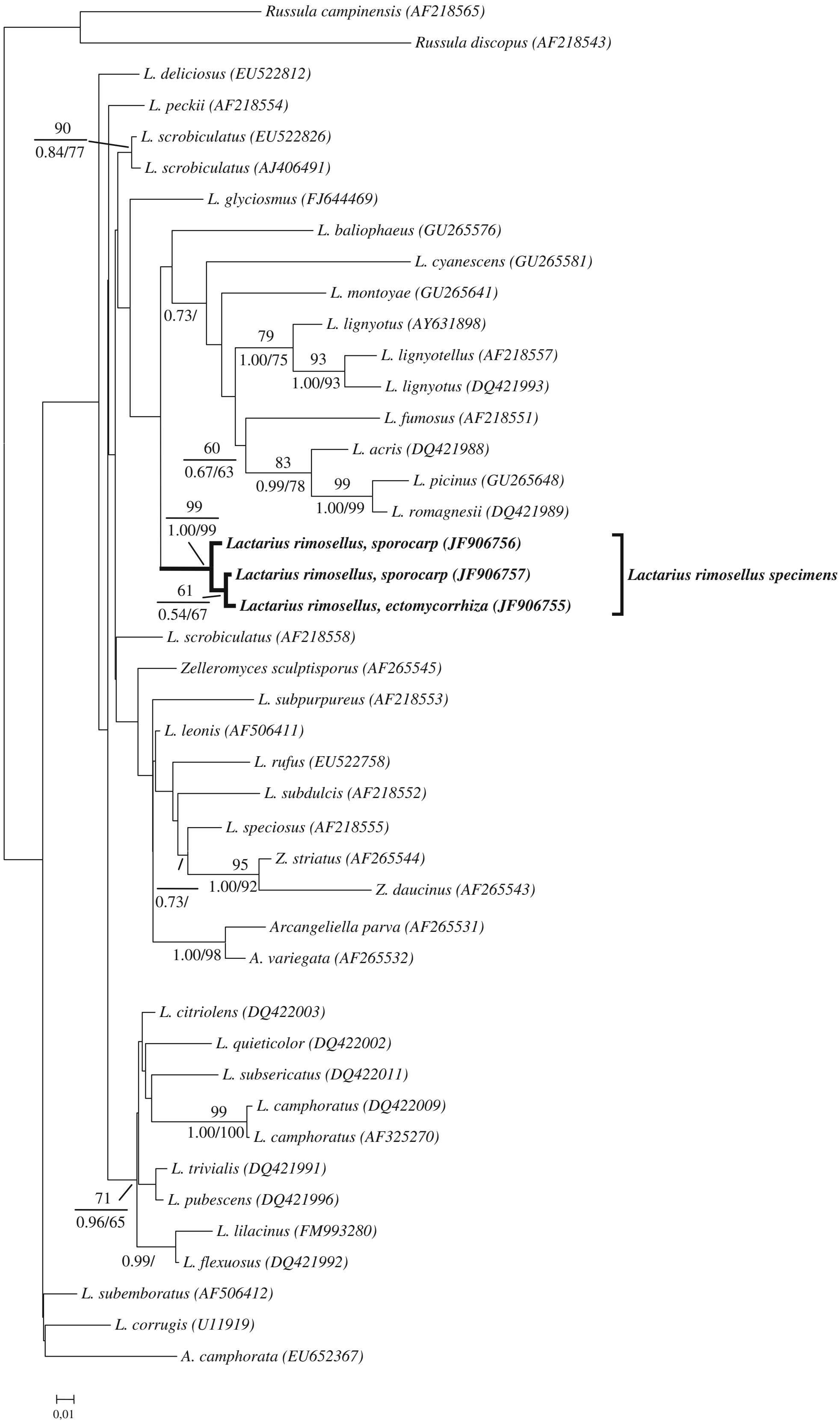

Table 1 *Lactarius* species used as food in the Guatemalan highlands

Species	Locality	Common name in Mayan idiom(s)[a]
L. deliciosus[b]	Chichicastenango, Cobán, Comalapa, Nentón, Patzún, San Juan Ostuncalco, San Juan Sacatepéquez, San Martín Jilotepeque, San Mateo Ixtatán, San Rafael La Independencia Tactic, Tecpán, Totonicapán, Uspantán	"Kanxul" (Nentón; *Chuj*), "Mancel" (San Mateo Ixtatán; *Chuj*), "Kaqix" (Patzún, Tecpán; *Kaqchikel*), "Q'än Xar" (San Martín Jilotepeque; *Kaqchikel*), "Tolor" (Comalapa; *Kaqchikel*), "Q'o'tz (Totonicapán; *K'iche'*), "Kaqiix" (Chichicastenango; *K'iche'*), "Liklich" (San Juan Ostuncalco; *Mam*)
L. indigo[c]	Chichicastenango, Cobán, Comalapa, Mixco, Patzún, Purulhá, San Juan Ostuncalco, San Juan Sacatepéquez, San Martín Jilotepeque, Tactic; Uspantán	"Xar" (San Martín Jilotepeque; *Kaqchikel*), "Ruwi' Xar" (Comalapa; *Kaqchikel*), "Räx Kaqiix" (Chichicastenango; *K'iche'*), "Xew" (San Juan Ostuncalco; *Mam*), "Naah Sekek" (Tactic, *Poqomchi'*), "Sekek" (Uspantán; *Uspanteko*)
L. rimosellus	Jacaltenango	No particular name recorded[d]
L. rubrilacteus	Chichicastenango	No particular name recorded
L. salmonicolor	Todos Santos Cuchumatán, Totonicapán	"Keqix" (Totonicapán; *K'iche'*), "Chuk-chuk" (Todos Santos Cuchumatán; *Mam*)
L. volemus	Chichicastenango	No particular name recorded

[a] For each vernacular name (idiom is in italics), the locality where it was recorded is mentioned

[b] Known as "Shara" (or "Xara"), "amarilla," or "Cabeza de Xara" in local Spanish. Sharas, also known as "urracas" are birds, variously colored, living in different parts of the country

[c] Known as "Shara" (or "Xara"), "azul," or "Cabeza de Xara," in local Spanish. According to Bran et al. (2002), who reports previous studies, this species is also known as "Räx Kaqix," "Räx Okox," and "Raxwach Kaqix" in the Kaqchikel language as spoken in Tecpán and as "Upawi' Xar" in Kaqchikel as spoken in Chipotón

[d] In several instances, no particular name is attributed locally to a given mushroom; this frequently occurs in the case of those species that are collected and consumed only occasionally

soil, on mossy, decaying logs and stumps, and in deep pine needle humus" in the USA by Hesler and Smith (1979), while in Mexico, it has been recorded only in a singular location, associated with *Pinus*. Having extensively studied the *Lactarius* mycota in Costa Rica montane *Quercus* forests, Roy Halling discussed this species as of north temperate origin, having migrated with oaks to Costa Rica (Halling 2001), as a component of the guild of obligate ectomycorrhizal fungal species that are believed to have migrated from north to south from the North Temperate zone with their associated phanerogams, in particular *Quercus* and *Alnus* (Mueller and Halling 1995; Halling 2001). By the results of the molecular phylogenetic analyses of sporocarp- and mycorrhiza-derived sequences, we clearly demonstrated the ECM-forming ability of *L. rimosellus* on the roots of oak trees. These results did not reveal any statistically supported grouping of this species with other *Lactarius* species, so that drawing firm taxonomic inferences is not possible at this stage. Detecting the ECM of *L. rimosellus* on the roots of oaks, together with our observations in the Guatemalan highlands, coincide with the findings in Costa Rica, indicating that in the Neotropics *L. rimosellus* is preferentially associated with oaks. More in general, the *Lactarius* mycota of montane forests of Costa Rica and Colombia—and probably of Guatemalan highlands and other Mesoamerican montane areas—closely resembles that of North America, while it differs significantly from those of Lesser Antilles and tropical South America (Mueller and Halling 1995).

In recent years, we have initiated a long-term study of *Lactarius* ectomycorrhizal diversity, using an approach that involves the full characterization—from both a morpho-anatomical and molecular point of view—of the relevant ECMs and the comparison of the recorded anatomical characters with those known from the ECMs formed by related taxa. While previous studies from our group have dealt with *Lactarius* mycobionts occurring in selected ecosystems in Europe (Eberhardt et al. 2000; Nuytinck et al. 2004; Comandini and Rinaldi 2008), this is the first time we apply this method to a Neotropical *Lactarius*. Several descriptions of ECMs formed by *Lactarius* species belonging to the *Russularia* subgenus (sensu lato) are currently available, the vast majority of which based on European samples (see DEEMY, http://www.deemy.de). Despite the differences in accuracy among the various descriptions and the variations of the terminology used for the description of specific characters, it stands out that all the ECMs of this group are rather homogeneous from an anatomical point of view. Mycorrhizae are characterized by a pseudoparenchymatous outer mantle layer, with epidermoid or angular hyphal cells, and a plectenchymatous inner mantle layer; lactifers are embedded either in the middle and/or inner mantle layer. The ECMs that most closely resemble those we observed for *L. rimosellus* are those described for *L.*

decipiens and *L. theiogalus* (=*tabidus*), both on *Picea* (Gronbach 1988), which share the presence of rhizomorphs and the absence of a hyphal net. A peculiar morphological aspect that characterizes *L. rimosellus* mycorrhizae is the presence of several unramified rhizomorphs that can be quite abundant on the same ECM tip, and which may be anatomically related to the similarly frequent mycelia strands originating from the base of the sporocarp of the mycobiont (Fig. 1). Another ECM aspect, also present in the correspondent fungal symbiont, is the scanty watery latex both in the mantle and in the rhizomorphs.

The deep mycophily of Mesoamerican indigenous people has been known for many years now. This is not only due to the spread use of macrofungi as highly appreciated food (and more recently, as a source of economic income) by many ethnic groups living in the area but also to the ancient involvement of mushrooms within the complex network of religious beliefs that inextricably linked ancient Mesoamericans with their natural surroundings. This sort of "mycolatry" reaches its peak with the hallucinogenic mushroom cult (Wasson 1980)—with its reflections in Mesoamerican art, culture, and myth—but certainly extends to macrofungi as a whole, considered sacred and therefore worth of the highest respect. More prosaically and limiting discussion to the edible fungi and to the eventual use of these as medicines, a systematic ethnomycological study of the area is still lacking, and most observations pertain to Mexico (e.g., Montoya et al. 2003; Ruán-Soto et al. 2006).

In Guatemala, most ethnomycological efforts have been devoted so far to the study of Maya mycolatry in the preclassic and classic periods (approximately 1500 BC–900 AD), either through the investigation of the meaning and origin of the so called "mushroom stones"—sculptures of pre-hispanic origin probably associated in Maya culture with religious ceremonies wherein hallucinogenic fungi played a major role (Lowy 1971, 1977; Ohi and Torres 1994)—and of the sacred use of mushrooms as depicted in Maya codices (Lowy 1972, 1974; Guzmán 2001). The artifacts now known as mushroom stones first came to the attention of archaeologists at the beginning of the last century. Relatively common in Guatemala (only a few have been reported from El Salvador and Mexico), mushroom stones have been thought to have been used as land markers, but the more likely interpretation is "that the mushroom stones had ritualistic significance possibly associated with a mushroom cult that disappeared in Guatemala centuries ago," to go with Bernard Lowy (1971), suggesting that there may be "a kinship between such a cult and the surviving divinitory rite of the inebriating mushrooms in Mexico" (Wasson and Wasson 1957). Furthermore, Lowy has clearly shown the central role played by *Amanita muscaria* in the mycolatry of the Maya people inhabiting current Guatemala. Among the Maya, this poisonous species was linked to the "thunderbolt legend" (in many cultures, some fungi were believed to spring from the ground in places struck by a thunderbolt) (Lowy 1974) and is depicted in several Maya codices in what appear to be ceremonial offerings, either as a death symbol and/or possibly because of its hallucinogenic properties (Lowy 1972). Although the existence of an autochthonous hallucinogenic mushroom culture among present-day Mayas is not proved, Lowy has reported the occurrence of *Psilocybe mexicana* in Guatemala, either growing in the meadows and offered for sale in at least one location (Lowy 1977).

Only more recently, attention has been devoted to ascertaining the mycological knowledge of contemporaneous Guatemalan indigenous people (Sommerkamp 1990). In the frame of a widespread, ongoing study of the ethnomycology of the Maya populations in the Guatemalan highlands, we have been able to document the common use of scores of different species of wild edible fungi (Bran et al. 2002, 2003, 2005; Flores et al., unpublished data; Morales et al. 2002). This, on one hand, pinpoints not only the wealth of traditional mycological knowledge of these populations, certainly not lower than that recorded in several areas of bordering Mexico (Montoya et al. 2003; Boa 2004; Ruán-Soto et al. 2006), but also the need for more detailed and accurate studies to record this aspect of indigenous culture before social and habit changes may condemn it to oblivion. In the current report, we describe the regular collection and use of three main *Lactarius* species (*L. deliciosus*, *L. indigo*, and *L. salmonicolor*) in many areas of the Guatemalan highlands, with three additional species (including *L. rimosellus*) consumed only locally and occasionally. These observations match those available for different indigenous Mexican ethnic groups and confirm the appreciation of Mesoamerican people for this group of ECM fungi. Boa (2004) lists over 50 species of *Lactarius* that are regularly collected and eaten in at least 17 countries worldwide, with seven species confirmed from Mexico and four more from this country that are reported as edible but without confirmed consumption. It is well thus possible that further ethnomycological surveys will expand the range of *Lactarius* species that have a traditional use among Guatemalan indigenous people.

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