Lactarius indigo and *L. deliciosus* form mycorrhizae with Eurasian or Neotropical *Pinus* species

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Nine new ectomycorrhizal associations between *Lactarius indigo* from Guatemala and the Eurasian pines *Pinus halepensis, P. nigra, P. pinaster* and *P. sylvestris* and between *L. deliciosus* from Spain and the Neotropical *P. ayacahuite, P. hartwegii, P. oocarpa, P. rudis* and *P. pseudostrobus* were obtained for the first time by *in vivo* synthesis under controlled conditions. Seedlings were inoculated with mycelial inoculum of *Lactarius* species obtained by culturing them in semi-liquid BAF medium. Morphological and anatomical characteristics of each combination are described. *L. indigo* and *L. deliciosus* mycorrhizae showed some similar features. They were yellowish or saffron to cinnamon when young, with a net of laticifers visible through mantle, secreting orange latex when injured and with plectenchymatous mantle. Cystidia-like emanating hyphae were observed on the mantle surface of young mycorrhizae.

Keywords: ectomycorrhiza, mycorrhiza description, shynthesis, edible fungi

Several species of the section *Dapetes* of the genus *Lactarius* are of great interest as edible mushrooms in many countries. *Lactarius deliciosus* (L.) Gray is an ectomycorrhizal species that occurs naturally in Europe, North- and Central America. It has also been introduced with pine plantations in Chile (Lazo 2001), India (Rao *et al.* 1997), Australia and New Zealand (Hall & Wang 2002). The fruiting bodies are highly appreciated by North American, Mexican and Guatemalan indigenous populations, as they are in Europe (Flores *et al.* 2002a, b). Its mycorrhizal association has been reported for several *Pinus* species such as *P. contorta* Dougl. ex Loud. (Parladé et al. 1996, Bradbury *et al.* 1998), *P. halepensis* Miller (Torres &

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Honrubia, 1994, Díaz *et al*. 2003), *P. pinaster* Aiton (Mousain *et al*. 1979, Pera & Alvarez 1995, Parladé *et al*. 2003), *P. pinea* L. (Rincón *et al*. 1999), *P. ponderosa* Dougl. ex D.Don (Riffle 1973, Massicotte *et al*. 1999) and *P. sylvestris* L. (Parladé *et al*. 2003, Guerin-Laguette *et al*. 2003). In contrast, despite the presence of *L. deliciosus* in Neotropical pine stands (Lodge *et al*. 2002, Reygadas *et al*. 1995, Flores *et al*. 2002b), there is little information about its mycorrhizal association.

Lactarius indigo (Schw.) Fr. is also a highly appreciated edible mushroom in northeast and Central America, especially in Mexico and Guatemala. This species is associated with several pines, oaks and other plant species (Montoya & Bandala 1996). Recently, Flores *et al*. (2005) described the mycorrhizal synthesis between *L. indigo* and the Neotropical pines *P. ayacahuite* Ehrenb.ex Schltdl., *P. hartwegii* Lindl., *P. oocarpa* Schiede ex Schltdl., *P. pseudostrobus* Lindl. and *P. rudis* Endl.. Other mycorrhizal associations have so far not been reported.

The Neotropical species *P. ayacahuite, P. rudis* and *P. hartwegii* have a scarce distribution in Mesoamerica from Mexico to Guatemala, growing in high environments up to 2200, 3200 or 3700 m altitude respectively. However, *P. pseudostrobus* and *P. oocarpa* are widely distributed in Mesoamerica, the latter representing about 50 % of the pine forests in Guatemala and 90 % in Nicaragua. *P. oocarpa* grows between 1800 and 2000 m altitude in low fertility soils, whereas the range for *P. pseudostrobus* is 1600–2800 m in acid and volcanic soils. On the other hand, the Eurasian *P. halepensis,* and *P. pinaster* are widely distributed in the Mediterranean region, growing between 0 and 1000 m altitude (up to 1500 in the case of *P. pinaster*) in calcareous and siliceous soils respectively. *P. nigra* grows at 800-1500 m altitude in South and Central Europe in several soil types and *P. sylvestris* present a wide distribution in Central and North Europe between 1300 and 2000 m altitude in sandy soils.

Plant-fungus specificity may vary considerably in ectomycorrhizal associations from narrow to broad host range fungi that associate with different species or families of host plants (Brundrett 2004). Members of *Russulaceae* are reported to associate with a wide range of hosts plants in natural ecosystems and therefore could be considered as "generalist fungi" (Bruns *et al*. 2002). Many *Lactarius* spp. form mycorrhizae with a variety of hosts plants (Hutchinson 1999). However, the edible species of the section *Dapetes* are reported to form ectomycorrhizae mainly with members of *Pinaceae* (Parladé *et al*. 2003). Taking into account that observation of the fruiting bodies of putative mycobionts near a potential host plant cannot be solely used to designate ECM associations (Brundrett 2004), it is necessary to experimentally confirm the relationship

between fungus and plant by *in vitro* or *in vivo* synthesis and/or to determine the mycorrhizal association by molecular tools.

Controlled inoculation with edible mycorrhizal fungi for fruiting body production is an economically valuable resource to increase forest productivity. In this context, knowledge about the plant-host range is important to select the most appropriate plant host for introduction of the fungus into different ecosystems.

Thus, the objective of this study was to establish the symbiotic capacity of two edible *Lactarius* species to form ectomycorrhiza with a range of Eurasian and Neotropical pine species. This study was specifically aimed at morphotyping and characterizing these *Lactarius* mycorrhizae.

Materials and Methods

Plant Material

Certified seeds of Neotropical *Pinus ayacahuite* var. *ayacahuite* Ehren, *P. oocarpa* var. *oocarpa* Schiede ex Schltdl. and *P. pseudostrobus* were obtained from the Instituto Nacional de Bosques de Guatemala (INAB). *P. hartwegii* and *P. rudis* seeds were collected in Tuicoj, Todos Santos Cuchumatán, northwest Guatemala, in 2000 and 2001.

Certified seeds of the Eurasian *P. halepensis, P. nigra, P. pinaster* and *P. sylvestris* were provided by the "Centro Nacional de Mejora Forestal El Serranillo", Guadalajara, Spain.

Seeds were disinfected for 30 min in 30 % H_2O_2, rinsed in sterile distilled water, placed in plastic trays with sterilized vermiculite (120 °C × 1h) for germination, and incubated in a growth chamber (19–23 °C, 65 % RH and 16/8 h light/dark cycle with 2500 Lux as maximum illumination). Once germinated, seedlings were transferred to 300 cm^3 containers which were previously filled with sterilized (120 °C, 1 h, 3 times on alternate days) peat-moss/vermiculite (1:1 vol/vol). They were maintained in the growth chamber for six months (under the same conditions but light intensity was increased to 12500 Lux) until short root development started. Plants were watered and fertilized (Peters Professional®, Scotts, Geldermalsen, The Netherlands 20-7-19 NPK) when required.

Fungal collections

Two strains of *L. indigo* from Guatemala and two strains of *L. deliciosus* from Guatemala and Spain respectively were used (Table 1). Voucher specimens have been deposited at the "Rubén Mayorga Peralta" Herbarium at the University of San Carlos, Guatemala. Voucher cultures are available from the Faculty of Chemical

Sciences and Pharmacy of the University of San Carlos, Guatemala and the Laboratory of Mycology-Mycorrizas of the University of Murcia. They were isolated in BAF medium (Moser 1960, Carrillo *et al.* 2004) by explants from basidiomata and later cultivated in flasks with semiliquid BAF medium (1.5 g agar/L) in the dark at 23 °C. The mycelia obtained were filtered through a 65 µm net and washed in sterile water to eliminate sugars and to reduce the growth of contaminants in the seedling root systems. The mycelium was fragmented and homogenized by manual agitation and then resuspended in distilled water (70–80 g mycelium/L) to obtain mycelial suspensions. On the other hand, the mycelia obtained were subjected to mechanical agitation with a shaker (GFL®, Gesellschaft für Labortechnik, Burgwedel, Germany) to obtain mycelial pellets.

Inoculation

Seedlings were inoculated twice. The first inoculation was made by applying 30 mL of the mycelial suspension to the seedlings rootlets. A second inoculation was made one month later with mycelial pellets, placing 20–40 pellets on the rootlets. Neotropical pines were inoculated with *L. deliciosus* and the Eurasian ones were inoculated with *L. indigo*. *P. halepensis* was also inoculated with the Guatemalan strain of *L. deliciosus*.

Descriptions of mycorrhizae

Fresh mycorrhizae were described and photographed using an Olympus® SZH zoom stereo microscope. At least 10 to 20 root tips for each combination were selected. They were fixed in FAA (formol-alcohol-acetic acid), washed in distilled water and put into an ultrasonic cleaner (Fungilab S.A.) to detach substrate debris from the surfaces. Then, they were washed three times in cold phosphate buffer 0.2 M pH 7.2, included in 1 % OsO_4 for 2.30 h and maintained for 2 h in uracile acetate. Serial ethanol dehydration and Spurr resin embedding were carried out according to Roland & Vian (1991).

Semi-thin (1.0 µm) sections were sliced with a Reichert-Jung® Ultracut microtome, using crystal knives. Sections were stained with 1 % toluidine blue in 1 % acetic acid, and then observed and photographed using a light Olympus® BH-2 microscope.

Mycorrhizae for scanning electron microscopy (SEM) were cleaned, washed and prepared as described above. Gold coated samples were observed in a Jeol® T-300 scanning electron microscope, and photographs were saved by the Autobeam Link Issis Program. Voucher samples of the fixed mycorrhizae are deposited at the Laboratory of Mycology-Mycorrizas of the University of Murcia.

Mycorrhizae were described following the criteria of Agerer (1987–2002) and Ingleby *et al.* (1990). Measurements are given as follows: minimum – maximum, on a basis of 30 samples. The colours of mycorrhizae were described using the Colour Identification Chart of Royal Botanic Garden (Raynes 2005).

Tab. 1. – Mycorrhizal fungi with their voucher number, collection site, date of collection and co-occurring plants at the collection site. The inoculated *Pinus* species are given in the right column.

Fungal Strain	Collection Site	Date of Collection	Co-occurring plant	*Pinus* species inoculated
L. indigo USAC 8.00	San Carlos University Campus Guatemala 14°36'42" N 90°32'43" 1518 m altitude	06/2000	*P. oocarpa*	*P. halepensis*
L. indigo SM 58.00	San Mateo Ixtatán Huehuetenango Guatemala 15°49'53" N 91°28'46" 2590 m altitude	08/2000	*P. rudis* *P. hartwegii*	*P. halepensis* *P. nigra* *P. pinaster* *P. sylvestris*
L. deliciosus SM 63.00	San Mateo Ixtatán Huehuetenango Guatemala 15°49'53" N 91°28'46" 2590 m altitude	06/2000	*P. rudis* *P. hartwegii*	*P. halepensis* *P. ayacahuite* *P. hartwegii* *P. rudis* *P. pseudostrobus* *P. oocarpa*
L. deliciosus SO 10	Almazán, Soria, Spain 41°29'18" N 2°31'56" 960 m altitude	11/2000	*P. pinaster*	*P. ayacahuite* *P. hartwegii* *P. rudis* *P. pseudostrobus* *P. oocarpa*

Results and Discussion

A detailed description is given for one host plant-fungus combination. The distinguishing features of the other combinations are given below.

Pinus halepensis × *Lactarius indigo* SM 58.00

Morphological characters. – Mycorrhizal systems dichotomous, up to 3.8 mm long, stiped, often forming dense clusters. – Main axes 0.4–0.5 mm in diameter and up to 1.2 mm in length. – Unramified ends rather straight, up to 1.2 mm in length and 0.4–0.5 mm in diameter, yellowish to saffron, with or without green spots, net of laticifers visible through outer mantle, apices lighter or

orange, older parts of ectomycorrhizae rusty tawny to cinnamon, whole mycorrhiza turning dark green to brown with age; mantle and rhizomorphs secreting orange latex when injured. – Surface of unramified ends short spiny (acute cystidia-like emanating hyphae, up to 35.0 µm in length) in young mycorrhizae, especially at the tips but becoming pruinose-grainy (whitish crystalline granules) in older ones; bases becoming fibrillose with age. – Rhizomorphs frequent, emanating from the bases and middle portions of the ectomycorrhizae; up to 94.0 µm in diameter, roundish to flattened in cross-section, surface rugulose and sometimes sparsely short spiny, especially near the base of main rhizomorphs, frequently with monopodial ramification; young rhizomorphs thin and yellowish, older ones thicker and coloured like mantle, even with green colour.

Anatomical characters. – Mantle in cross section 20.0–50.0 µm thick, densely plectenchymatous, with abundant interhyphal gelatinous matrix material, two different layers distinguishable, laticifers frequent in the inner layer; all hyphae without clamps. Outermost layer hyphae tightly packed to dispersed in the gelatinous matrix; hyphal cells rather straight, 3.0–4.0 µm in diameter, frequently branched; laticifers rare, 4.0–6.0 µm in diameter. Innermost layer hyphae 3.0–4.0 µm in diameter, tightly agglutinated in the gelatinous material; laticifers frequent, 5.0–8.8 µm in diameter, septate and ramified.

Hartig net surrounding two of the three-four rows of cortical cells with one-two chains of irregular hyphae, 3.0–4.0 µm in diameter and 4.0–5.6 µm in length; tannin cells surrounded by irregular hyphae, 3.0 µm in diameter and 8.0–14.0 µm in length.

In *Pinus halepensis* × *Lactarius indigo* USAC 8.00 mycorrhizal structure, size and colour were similar, but there were some slight differences in cross-section: the mantle thickness was 30–46 µm, with tightly packed laticifers in the inner layer, a poorly developed Hartig net and narrower rhizomorphs (44–47 µm in diameter).

Pinus nigra × *Lactarius indigo* SM 58.00

Morphological characters. – Mycorrhizae sometimes coralloid, up to 5.0 mm long. – Unramified ends up to 0.7 mm in length and 0.3–0.4 (0.6) mm in diameter, saffron to rusty tawny; acute cystidia-like hyphae up to 47 µm in length. – Rhizomorphs quite rare, up to 55 µm in diameter.

Anatomical characters. – Mantle in cross-section 20–60 µm thick. – Hartig net with one or two chains of irregular hyphae (3.0–4.0 µm in diameter and 5.0–12.0 µm length); tannin cells

surrounded by irregular hyphae 4.0–6.0 µm in diameter × 6.0–8.0 µm in length; some hyphae show epidermoid features.

***Pinus pinaster* × *Lactarius indigo* SM 58.00**

Morphological characters. – Mycorrhizal systems sometimes coralloid, yellowish to saffron-peach; acute cystidia-like emanating hyphae up to 23–31 µm long in. – Rhizomorphs quite rare, up to 78 µm in diameter.

Anatomical characters. – Mantle in cross-section 20–64 µm thick, two to three different layers distinguishable, laticifers frequent in the middle and inner layers. Middle layer,with hyphae 3.0–4.0 µm in diameter and frequent laticifers, 4.0–7.0 µm in diameter, ovaled and flattened, ramified and septated. – Hartig net oligoseriate, hyphae 3.0–8.0 µm length; some Palmetti-like cells around tannin cells and the first cortical layer.

***Pinus sylvestris* × *Lactarius indigo* SM 58.00**

Morphological characters. – Mycorrhizal systems sometimes coralloid, up to 9.0 mm long. – Main axes up to 2.1 mm long. – Unramified ends sometimes beaded, up to 3.4 mm in length and 0.3–0.4 mm in diameter; acute cystidia-like emanating hyphae up to 39.0 µm length. – Rhizomorphs quite rare, up to 78 µm in diameter.

Anatomical characters. – Mantle in cross section 20–30 µm thick. – Innermost mantle layer with laticifers frequent, 4.0–8.0 µm in diameter towards the outside and and 4.0–6.0 µm in diameter towards the inside. – Hartig net surround three of the four-five rows of cortical cells with irregular and polygonal hyphae 3.0–4.0 µm 4.0–10.0 (14.0) µm in length.

***Pinus oocarpa* × *Lactarius deliciosus* SO 10**

Morphological characters. – Mycorrhizal systems dichotomous, up to 3.4 mm long, stiped, often forming clusters in the substrate. – Main axes 0.45–0.5 mm in diameter. – Unramified ends rather straight, up to 0.7 mm in length and 0.35–0.5 mm in diameter, yellowish to orange, net of orange laticifers visible through outer mantle, apices lighter, whitish to yellowish orange, older parts of ectomycorrhizae greenish to cinnamon, whole mycorrhiza turning brown with or without dark green spots with age; mantle and rhizomorphs secreting orange latex when injured. – Surface of unramified ends short spiny, especially in young mycorrhizae

(acute cystidia-like emanating hyphae, 31.0–47.0 µm long) but becoming reticulated-grainy (crystal-like granules up to 23.0 µm in diameter) in old mycorrhizae. – Rhizomorphs emanating from the basal and middle portions of the ectomycorrhiza, up to 78.0 µm in diameter, roundish to flattened in cross-section, rugulose surface, ramified, young rhizomorphs thin and transparent, older ones thicker, bright yellow-orange and coloured like mantle.

Anatomical characters. – Mantle in cross section 16.0–60.0 (80.0) µm thick, densely plectenchymatous, with abundant interhyphal matrix material, two-three different layers distinguishable, laticifers frequent in middle and innermost layers; all hyphae without clamps. Outermost layer compact and thick, hyphae densely to loosely arranged in the gelatinous matrix material, hyphae rather straight, 2.5–3.0 µm in diameter, and branched; laticifers 4.0–5.0 µm in diameter, ramified and septate. Middle layer, when distinguishable, with hyphae 3.0–4.0 µm in diameter; laticifers frequent, 4.0–8.0 µm in diameter, dichotomously branched. Innermost layer hyphae 3.0–4.0 µm in diameter, tightly agglutinated and compacted by gelatinous matrix material; laticifers frequent, 4.0–8.0 µm in diameter.

Hartig net uniseriate, surrounding two-three of the four-five rows of cortical cells, 3.0–4.0 µm in diameter and 4.0–10.0 µm in length; Hartig net around tannin cells uniseriate 4.0–5.0 µm in diameter, and 6.0–12.0 µm in length.

Pinus ayacahuite × *Lactarius deliciosus* SO 10

Morphological characters. – Mycorrhizal system up to 6.3 mm long, stiped. – Unramified ends up to 1.5 mm in length, saffron to peach in maturity; laticifers yellowish to saffron; acute cystidia-like emanating hyphae up to 31 µm long. – Rhizomorphs emanating from the middle portion of the ectomycorrhizae, up to 50 µm in diameter.

Anatomical characters. – Mantle in cross section 14–74 µm thick, laticifers rare. – Hartig net oligoseriate around the first cortical row but uniseriate around the lower layers, hyphae up to 12.0 µm in length; tannin cells are surrounded by bigger and irregular hyphae, 4.0–8.0 µm in diameter, and 4.0–10.0 µm length; some Palmetti-like hyphae were also present, especially in mycorrhizae with thicker mantles.

Pinus hartwegii × *Lactarius deliciosus* SO 10

Morphological characters. – Unramified ends up to 1.1 mm in length, saffron-peach to cinnamon; acute cystidia-like emanating hyphae up to 31.0 µm long and with hirsute hairs up to

47.0 μm long, especially at the base. – Rhizomorphs up to 62.0 μm in diameter.

Anatomical characters. – Laticifers rare, 3.0–5.0 μm in diameter on the outermost mantle layer and frequent, 5.0–6.0 μm in diameter, on the middle mantle layer. – Hartig net surrounding three of the three-four rows of cortical cells, 3.0–6.0 μm in diameter and 4.0–12.0 μm in length; tannin cells surrounded by irregular hyphae, 5.0–6.0 μm in diameter and 4.0–8.0 μm in length; palmetti-like hyphae up to 8.0 μm in diameter are present in the first cortical row and around tannin cells in mycorrhizae with well-developed mantles.

***Pinus pseudostrobus* × *Lactarius deliciosus* SO 10**

Morphological characters. – Mycorrhizal systems up to 2.7 mm long. – Unramified ends up to 1.1 mm in length, saffron-peach to rusty tawny-cinnamon; acute cystidia-like emanating hyphae 23.0–55.0 μm long. – Rhizomorphs up to 55 μm in diameter.

Anatomical characters. – Mantle (in plan view): Tannin cells surrounded by roundish hyphae, 4.0 μm in diameter, and irregular hyphae up to 4.0 × 10.0 μm.

***Pinus rudis* × *Lactarius deliciosus* SO 10**

Morphological characters. – Mycorrhizal systems sometimes coralloid. – Unramified ends up to 1.3 mm in length, saffron-peach to sienna color, older parts of ectomycorrhizae dark cinnamon with gray-greenish spots; acute cystidia-like emanating hyphae, 31.0–39.0 μm long; short hirsute hairs are also present in the bases of mature mycorrhizae. – Rhizomorphs emanating from the middle and apices of ectomycorrhizae, up to 63.0 μm in diameter.

Anatomical characters. – Mantle in cross-section 20–40 μm thick, two different layers distinguishable; laticifers on the outermost mantle layer 3.0–6.0 μm in diameter but 4.0–5.0 μm on the innermost mantle layer.

Mycorrhizae were obtained between 20 and 25 days after the inoculation of seedlings. Except the Guatemalan *L. deliciosus* SM 63.00, which only formed ectomycorrhizae on *P. hartwegii*, the mycorrhizal synthesis was successful in all the plant-host combinations assayed. Probably, the lack of mycorrhizal capacity of *L. deliciosus* SM 63.00 was due to either a low efficiency of this particular strain to colonize roots, or to the low viability of the mycorrhizal

inoculum, being even unable to colonize seedlings of *P. rudis*, the putative original host of the collected fruiting bodies. This strain showed a slow growth rate in culture conditions compared with *L. deliciosus* SO 10 (data not shown). Quantitative determination of the mycorrhizal colonization was not done in order to avoid root system destruction. However, the observation of the root systems allowed the conclusion that mycorrhizae formation was successful in all the seedlings inoculated with *L. indigo* and *L. deliciosus* SO 10 (100 % of the seedlings mycorrhized). The degree of mycorrhizal colonization was varied among fungal-plant combinations. *L. deliciosus* was more efficient to form mycorrhizae than *L. indigo*. *L. indigo* colonization was extensive on *P. halepensis* and *P. pinaster* whereas it was weak on *P. sylvestris* and *P. nigra*. *L. deliciosus* developed abundant mycorrhizae on *P. oocarpa*, *P. hartwegii, P. pseudostrobus* and *P. rudis*. However, less mycorrhizal root tips were observed on *P. ayacahuite*, probably due to the scarce short root formation that is characteristic of this pine specie (Donahue *et al*. 1991). The capacity to form mycorrhizae was unrelated to the origin (Eurasian or Neotropical) of fungal and pine strains. This result demonstrates the host compatibility of these pine species with the mycobionts *L. indigo* or *L. deliciosus* despite their geographically different origins. Moreover, this finding extends the known host range of these two important edible fungi. This information could be important for the practical use of these fungal species in controlled inoculations of pines for the production of fruiting bodies as a secondary crop.

Lactarius indigo ectomycorrhizae on Eurasian pines were similar in all cases, the main morphological and anatomical features being the same in each combination (Fig. 2). Only some slight differences in colour or size were observed among species. Mycorrhizae were yellowish or saffron to cinnamon when young and dark green to brown at maturity, with a net of laticifers visible through mantle, secreting orange latex when injured. This orange colour contrasts with the characteristic blue latex of fruiting bodies of *L. indigo*. Plectenchymatous mantle was observed in cross-section. These main features were close to the features described by Flores *et al*. (2005) on Neotropical pines species. However, we have observed a dark green colour in mature mycorrhizae of the Eurasian species, whereas they tended to turn brown in Neotropical species. The outermost mantle layer described here for Eurasian pines mycorrhizae were thicker than those reported for Neotropical species. Nevertheless, mantle thickness may change in response to environmental conditions and can not be considered a diagnostic character. Another remarkable feature is the thinner mantle observed on *P. sylvestris,* a feature also reported for mycorrhizae of the Neotropical *P. rudis* and *P. hartwegii* (Flores *et al*. 2005). These pine species have a similar habitat in high

mountains in Europe and Mesoamerica respectively. On the other hand, *P. rudis* and *P. hartwegii* are currently considered as the same species by several authors (Matos 1995). This taxonomical affinity could explain the anatomical similarity of their mycorrhizae, as previously reported by Flores *et al.* (2005).

L. deliciosus mycorrhizae on Neotropical pines were similar to those described for pines in Europe (Torres & Honrubia 1994, Rincón *et al.* 1999, Díaz *et al.* 2003). The main morphological and anatomical characteristics were the saffron to cinnamon colour of the mycorrhizae similar to that of fruiting bodies but changing to green with age, the secretion of orange latex, the presence of laticifers and the plectenchymatous mantle (Fig. 1). A plectenchymatous mantle has been reported as a common feature of ectomycorrhizae of *Lactarius* species belonging to the section *Dapetes* (Agerer 1987–2002) (Figs. 4, 6). The presence of plectenchymatous mantle on all our *L. indigo* and *L. deliciosus* mycorrhizae confirms such previous observation.

One interesting feature of *L. indigo* and *L. deliciosus* mycorrhizae is the presence of cystidia-like emanating hyphae with acute or beaded ends (Figs. 1, 3). Up to date, the presence of cystidia on *Lactarius* mycorrhizae was reported for *L. indigo* (Flores *et al.* 2005), *L. acris* (Brand 1992), *L. lignyotus* (Kraigher *et al.* 1995), *L. mitissimus* (Weiss 1991), *L. rubrocintus* (Brand 1991) and *L. scrobiculatus* (Amiet & Egli 1991), but this kind of hyphae or cystidia was still unreported for *L. deliciosus*. The differences between emanating hyphae and cystidia are sometimes not clear, as transitional forms may exists: whereas emanating hyphae connect surface mantle and soil, cystidia are generally considered as hyphae of finite length on the surface mantle (Agerer, 1991). The short hyphae described in this study resemble cystidia by their limited and constant length, but they can also be considered as emanating hyphae as they disappear with age, the short-spiny surface in young mycorrhiza becoming grainy in mature ones (Fig. 5). That observation agrees with the description of *L. mitissimus* mycorrhiza on *Picea abies* (Weiss 1991). More studies are required to determine the nature and function of these mycelial structures.

The ectomycorrhizal features may reflect relationships within the different sections of the genus *Lactarius* (Treu 1991). In our study, the mycorrhizae formed by *L. indigo* and *L. deliciosus* were morphologically and anatomically very similar irrespective of the origin of fungi or pine species. This similarity is probably due to the close relationship of both fungi, belonging to the section Dapetes.

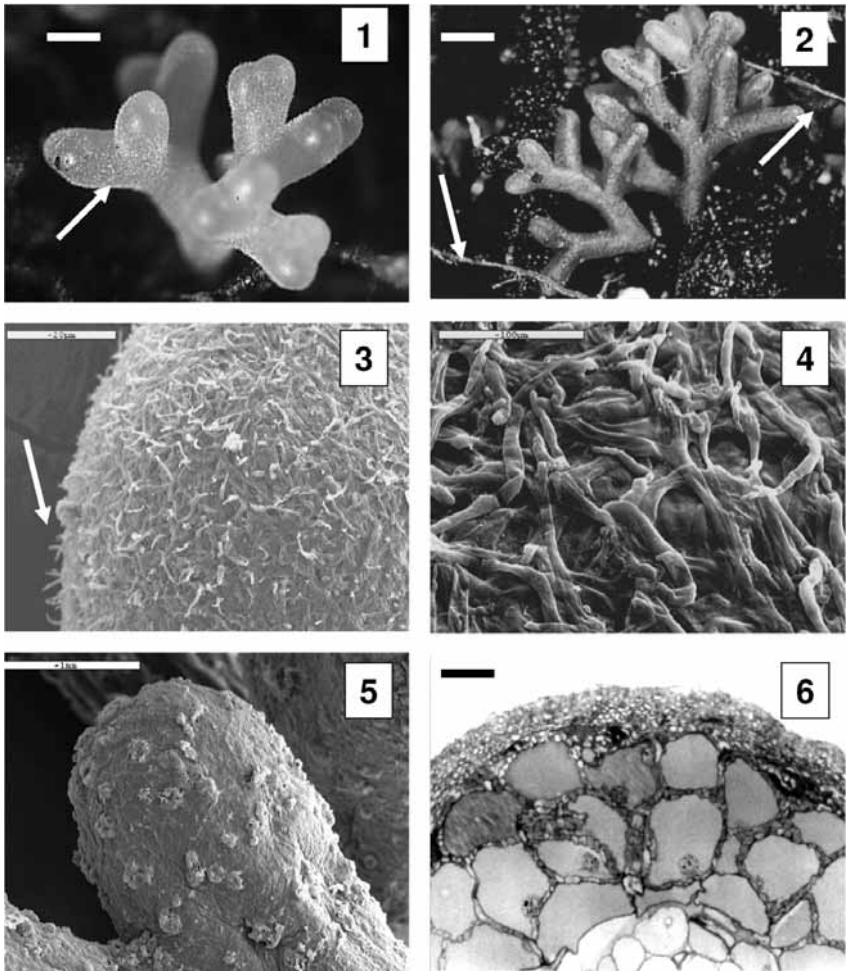

Fig. 1–6. *Lactarius indigo* and *L. deliciosus* mycorrhizae on Eurasian or Neotropical pines: **1.** *P. pseudostrobus* × *L. deliciosus* young mycorrhiza with cystidia-like hyphae. **2.** *P. halepensis* × *L. indigo* mature mycorrhiza with rhizomorphs. **3.** *P. pseudostrobus* × *L. deliciosus*, acute cystidia-like emanating hyphae in young mycorrhiza. **4.** *P. hartwegii* × *L. deliciosus*, plectenchymatous mantle. **5.** *P. oocarpa* × *L. deliciosus*, reticulate to grainy surface in mature mycorrhiza with crystal-like grains. **6.** *P. pinaster* × *L. indigo*, cross section showing mantle and Hartig net. Bars: 1 = 0.5 mm, 2, 5 = 1 mm, 3, 6 = 20 μm, 4 = 100 μm.

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