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Anatomical and molecular characterization of *Lactarius aff. omphaliformis*, *Russula alnijorullensis* and *Cortinarius tucumanensis* ectomycorrhizae on *Alnus acuminata*

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Abstract: Ectomycorrhizae (ECM) of *Lactarius aff. omphaliformis* Romagn., *Russula alnijorullensis* (Sing.) Sing. and *Cortinarius tucumanensis* Mos. on Andean alder (*Alnus acuminata* Kunth) were characterized and identified. The identification of the fungal symbionts was achieved by morpho-anatomical observations of mycorrhizae and by comparison of ITS-RFLP patterns obtained from ECM and fruitbodies. *L. aff. omphaliformis* ECM differed in some morphological details such as ramification and mantle type from ECM of the same species on *A. glutinosa*. *L. aff. omphaliformis* ECM show an orange to ochre mantle containing latex cells, which stain with sulpho-vanillin, emanating hyphae without clamps. *R. alnijorullensis* ECM represent a typical *Russula*-type-ECM, light yellow to pinkish, the outer mantle being composed of triangular latex-filled cells staining with sulpho-vanillin, emanating hyphae without clamps. *C. tucumanensis* ECM exhibit a white (silvery) to yellowish brown mantle covered with soil particles, emanating hyphae with clamps.

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INTRODUCTION

Species of *Alnus* colonize ecologically extreme or disturbed habitats. They grow rapidly and can improve soil fertility and stability, partially owing to their ability to form both ectomycorrhizal (ECM) and actinorhizal relationships (Brunner et al 1992). The distribution of *Alnus acuminata* Kunth, an ecologically valuable tree species in South America, ranges from the north of Venezuela to the Andes (Dawson 1990), reaching its southernmost distribution in the Catamarca province of Argentina.

Alnus ectomycorrhizae have been described from North America (Neal et al 1968; Froidevaux 1973; Molina 1979; Godbout and Fortin 1983; Massicotte et al 1986, 1989, 1994, 1999; Miller et al 1988, 1991, 1992; Murphy and Miller 1994; Molina et al 1994), South America (Becerra et al 2002, 2005a), Japan (Masui 1926), New Zealand (Mejstrik and Benecke 1969) and Europe (Treu 1990; Agerer et al 1993; Airaudi et al 1994; Pritsch et al 1997a, b; Wiedmer and Senn-Irlet 1998, 1999a, b, c, d, 2001a, b; Beenken 2001).

Trappe (1962) and Horak (1963) listed a number of fungi of the families Cortinariaceae and Russulaceae associated with different *Alnus* species. Several species of Cortinariaceae have been reported in mycorrhizal association with alders (Stangl 1970; Godbout and Fortin 1983, 1985; Brunner et al 1990; Miller et al 1991; Moser 2001). The ectomycorrhizae of *Cortinarius* cf. *helvelloides* (Fr.) Fr. and *C.* cf. *alneus* (Mos.) Mos. associated with *A. glutinosa* (L.) Gaertn, *C. bibulus* Quél. associated with *A. rubra* Bong., *C. pluvius* (Fr.) Fr., *C. alnobetulae* Kühn., *C. atropusillus* Frave, *C. badiovestitus* Mos., *C. kühneri* Mos., *C. bibulus* Quél. and *C. helvelloides* (Fr.) Fr. associated with *A. viridis* (Chaix) DC. and *C. helodes* Moser, Matheny & Daniele associated with *A. acuminata*, have been described respectively by Pritsch et al (1997b), Miller et al (1991), Wiedmer and Senn-Irlet (1998, 1999a, b, c, d, 2001a, b) and Becerra et al (2005a).

Within Russulaceae, Brunner et al (1992) found *Russula alnicrispa* Brunner, *R. subartica* Brunner and *Lactarius obscuratus* (Lasch) Fr. associated with *A. tenuifolia* Nutt.. Pritsch et al (1997b) described the

ectomycorrhizae of *L. obscuratus*, *L. omphaliformis* Romagn., *L. lilacinus* (Lasch) Fr. and *R. pumila* Rouzeau & Massart associated with *A. glutinosa*. The ECM of *L. obscuratus*, *L. alpinus* Peck and *R. alnetorum* were described in association with *A. viridis* (Airaudi et al 1994, Beenken 2001), and ectomycorrhizae of *L. obscuratus* Quél. in association with *A. rubra* (Miller et al 1991).

Two of the three species considered in this study (*Russula alnijorullensis* Sing., *Cortinarius tucumanensis* Mos.) have been reported to form fruitbodies under *A. acuminata* in Argentina (Moser and Horak 1975, Moser 2001).

In this study we complement reports related to *A. acuminata*-ECM associations (Becerra et al 2002, 2005a; Becerra 2002) by newly describing the ectomycorrhizae of *Lactarius aff. omphaliformis*, *Russula alnijorullensis* and *Cortinarius tucumanensis* on *A. acuminata* by morphological and anatomical parameters. The identification of the fungal symbionts was achieved by comparing ITS-PCR/RFLP patterns of mycorrhizae and fruitbodies.

MATERIALS AND METHODS

Sampling sites and characterization.—Samples were taken at Quebrada del Portugués, Tafí del Valle (Tucumán Province), and Narvaez Range (Catamarca Province) in northwestern Argentina (NOA). Details of the two study sites, including phytosociological and pedological aspects have been reported by Becerra (2002) and Becerra et al (2005b). Both locations were visited in Feb, Mar, May and Nov 2000–2002. At every sampling date, blocks of 15 × 15 cm were cut from the litter layer around fruitbodies with a sharp knife. The rooted soil samples were transferred to a plastic box together with the fruitbodies, leaving the sample as undisturbed as possible, and stored at 4 C during transport to the laboratory. Fruitbodies and mycorrhizae were examined within 2 d after sampling. Root samples were soaked and washed in water following Agerer's method (1991). Alder roots were distinguished easily from others roots by the presence of actinorhizal nodules and their morphological appearance. Hyphal connections from stipe bases to mycorrhizal tips were traced, when possible, under a Zeiss stereomicroscope at 10–40× magnification according to the method of Agerer (1991). Several tips of each morphotype were subjected to comparative anatomical studies. Photographs of ECM were taken with a Leica M420 stereomicroscope. Several chemical reagents (15% KOH, Melzer's reagent, cotton blue, 70% ethanol, sulpho-vanillin, NH_4OH and lactic acid) were used for studying specific color changes of mycorrhizae. In addition a portion of mycorrhizal roots was fixed in 70% alcohol and stored at 4 C in the dark. Description of the ectomycorrhizae follows the terminology of Agerer (1991, 1999) and Miller et al (1991). Fresh mycorrhizae were prepared for direct microscopy of the hyphal mantle (hand sections or mantle peeled off) according to Agerer (1991) or embedded in Leica Historesin kit after dehydration with series of 20, 30, 40, 50, 60, 70, 80, 90, 96, 100% ethanol. Semithin tangential and cross-sections of embedded ECM were cut with a microtome with steel knives. Total mantle views, peeled off mantles, and sections from resin-embedded mycorrhizae were examined and photographed with a Zeiss Axiophot light microscope at 200–1000× magnification. Characterization of the Hartig net follows the nomenclature of Godbout and Fortin (1983). Fruitbodies were identified according to Singer and Digilio (1951) and Moser (1978, 2001). Voucher specimen of fruitbodies and mycorrhizae were deposited in the Museo Botánico de Córdoba Herbarium (CORD) (Holmgren et al 1990).

Molecular identification.—Several tips of each morphotype were prepared for DNA extraction. Total DNA was extracted from small pieces of sporocarp tissue (1–4 mm^3) cut from the central part of the cap and from single mycorrhizal tips using DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany) according to manufacturer's instructions. Pelleted DNA was resuspended in 50 µL of Tris/HCl buffer (pH 8) containing 1 mM EDTA and stored at 4 C until use. DNA extraction from mycorrhizal tips was replicated at least twice. The primers ITS1 and ITS4 (White et al 1990) were used to amplify the ITS region. For PCR the protocol of Henrion et al (1994) was applied: 2 µL of DNA extract was mixed gently in 0.5 mL polypropylene tubes with 47.5 µL of the PCR-reactants at final concentrations of 1.5 mM $MgCl_2$, 200 µM each of dATP, dCTP, dGTP, dTTP, and 20 pmol of each primer in buffer for the Taq DNA polymerase. In every series of amplification reactions without DNA extracts served as controls for contaminants in the reagents. The tubes were placed immediately in a thermocycler (T3 Thermocycler, Biometra, Germany). 2.5 U Taq DNA polymerase was added after a denaturation step for 5 min at 94 C. PCR reaction was run in 30 cycles under these conditions: (i) denaturation of DNA for 1 min at 94 C; (ii) annealing of the primers for 1 min at 50 C; 3) elongation for 1 min at 72 C. A final step of 10 min at 72 C allowed elongation of uncompleted DNA strands. For RFLP analysis, 10 µL of the amplified ITS products were digested with the restriction endonucleases *Taq*I, *Hinf*I and *Eco*RI, in separate reactions, following manufacturer's recommendations. PCR and RFLP products were size-fractionated on 1.5% agarose gels (DNA Agarose, Biozym, Germany). The gels were stained with ethidium bromide and photographed under UV light. Gel images were analyzed visually by comparing RFLP patterns of mycorrhizal and fruitbody DNA on the same gel and by estimating the length of PCR products and RFLP fragments based on 100-bp ladder (MBI Fermentas) used as DNA marker.

RESULTS

Description of anatomotypes.—Lactarius aff. omphaliformis Romagn. FIGS. 1–6

Morphological characters: mycorrhizal systems up to 20 mm long, irregularly monopodial-pinnate to monopodial pyramidal; orders of ramification 0–2; systems occurring solitarily or in few numbers. Unramified ends bent to sinuous, preferentially tortuous, tip not inflated, up to 19 mm long and 0.2–0.45 mm diam. Entire surface of mycorrhiza orange to ochre, yellowish when young, brown when older (FIG. 1); surface of unramified ends smooth, covered with soil particles. Root tips blunt, covered by the mantle. Mantle not transparent; mycorrhiza not carbonizing. Rhizomorphs not observed.

Anatomical characters of mantle in plan view: mantle continuous over the root apex. Blue granules and a matrix lacking; needle-like contents after fixation present. Outer mantle layers pseudoparenchymatous with angular to epidermoid cells bearing a hyphal net (type P-Q, Agerer and Rambold 1998) (FIG. 2); mantle cells 13–23 μm long and 10–15 μm diam; walls of hyphal net faint, delicate, inconspicuous, rather thin, hyphal net not specialized. Cells of mantle membranaceously yellowish, thin-walled 0.3–0.5 μm, without clamps; septa as thick as walls; surface of cells smooth, more or less gelatinous (sticky); some cells filled with latex staining in sulpho-vanillin, shape rectangular, 9.5–28 μm long and 6.4–13 μm diam (FIG. 2). Middle mantle layer, plectenchymatous to pseudoparenchymatous without discernible pattern, 2.5–4 μm diam, hyphae without clamps (FIG. 3). Inner mantle layer, plectenchymatous without pattern; hyphae without clamps, membranaceously yellowish, 3–4 μm diam (FIG. 4); laticifers present, straight and even, 2–5 μm diam, ramification infrequent (FIG. 5).

Anatomical characters of emanating elements: emanating hyphae smooth, wavy, shape not striking, 1.5–2.4 μm diam, cell walls thin (<0.5 μm), colorless to membranaceously yellowish, elbow-like protrusions lacking; distal ends of hyphae simple, septal distances 4–18 μm, septa without clamps, cross walls as thick as remaining walls.

Anatomical characters in longitudinal section: mantle 11–30 μm thick (FIG. 6), three distinct mantle layers discernible; outer mantle layer pseudoparenchymatous, hyphae tangentially 4–20 μm, radially 2–11 μm; middle mantle layer plectenchymatous to pseudoparenchymatous, hyphae tangentially 2–20 μm, radially 2–11 μm; inner mantle layer plectenchymatous, hyphae tangentially 2–22 μm, radially 1.5–4 μm; mantle of very tip 12–19 μm. Tannin cells lacking. Epidermal cells rectangular, parallel to root axis, tangentially 13–35 μm, radially 9–20 μm. Cortical cells rectangular to cylindrical, tangentially 22–44 μm, radially 10–18 μm. Hartig net not completely paraepidermal (FIG. 6), in plan view infrequently lobed; 5–6 μm wide lobes around epidermal cells.

Color reaction with different reagents: whole ectomycorrhizae bleaching with 15% KOH and lactic acid. Sulpho-vanillin: walls pink, contents of mantle cells with black droplets, laticifers black. Emanating hyphae and laticifers staining slightly blue with cotton blue; no reaction with Melzer's reagent, ethanol 70% and NH_4OH.

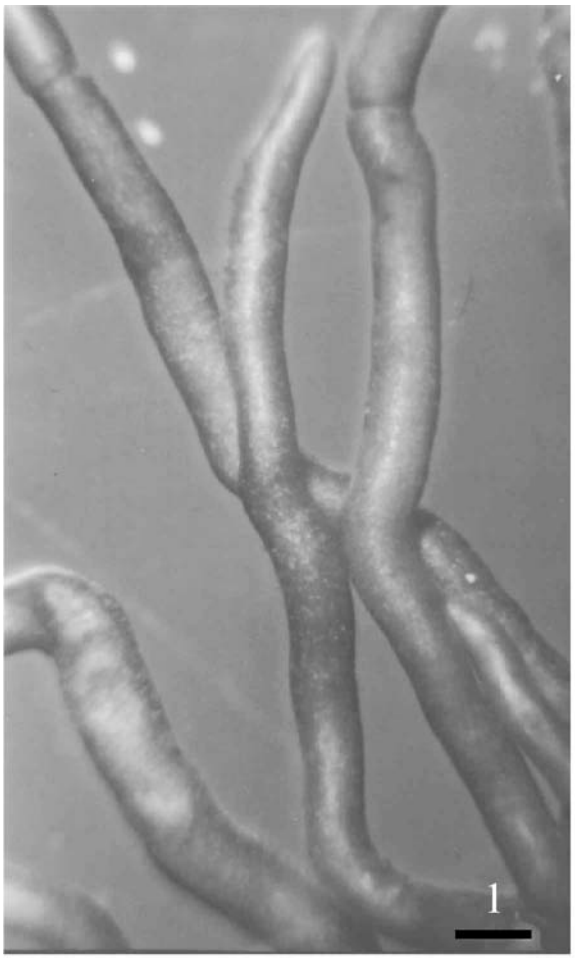
Specimens examined. ARGENTINA. Catamarca. Sierra de Narváez. 27°43′S, 65°54′W, elevation 1820 m. Ectomycorrhizae under *A. acuminata*, 7 Nov 2001, *A. Becerra* 04 (CORD); Tucumán. Quebrada del Portugués. Tafí del Valle. 26°58′S, 65°45′W, elevation 2187 m. Fruitbodies under *A. acuminata*, 11 Nov 2000, *G. Daniele* 191 (CORD), 16 Feb 2001, *G. Daniele* 203 (CORD). Catamarca. Sierra de Narváez. 27°43′S, 65°54′W, elevation 1820 m. Fruitbodies under *A. acuminata*, 7 May 2000, *G. Daniele* 186 (CORD), 15 Feb 2001, *G. Daniele* 204 (CORD), 7 Nov 2001, *G. Daniele* 210 (CORD), 27 May 2002, *G. Daniele* 215 (CORD).

Russula alnijorullensis (Sing.) Sing. FIGS. 7–12

Morphological characters: mycorrhizal systems up to 27 mm long, irregularly monopodial-pinnate (FIG. 7); orders of ramification 0–1; systems abundant, dense; unramified ends sinuous to tortuous, up to 10.4 mm long and 0.1–0.5 mm diam; entire mycorrhiza light yellow to pinkish when young, ochre, yellowish brown when older. Surface of unramified ends smooth, covered with few soil particles. Root tips blunt and covered by the mantle. Mantle not transparent; mycorrhizae not carbonizing. Rhizomorphs not observed.

Anatomical characters of mantle in plan views: mantle continuous over the root apex. Blue granules and a matrix lacking. Outer mantle layers pseudoparenchymatous with emanating hyphae on angular cells (type L, Agerer and Rambold 1998) (FIGS. 8–9); some cell mounds on the mantle, 10–21 μm long and 7–17 μm diam. Angular cells membranaceously yellowish, 5–32.2 μm long and 5–19 μm diam, thin-walled (<0.5 μm), 6–7 cells in a square of 20 × 20 μm. Cells filled with latex, staining in sulpho-vanillin; shape triangular, 6.5–26 μm long and 6.5–13 μm diam. Inner mantle layer plectenchymatous without discernible pattern; cells straight, membranaceously yellowish, without clamps, 2.5–5 μm diam (FIG. 10).

Anatomical characters of emanating elements: emanating hyphae tortuous to irregularly inflated, 2–4 μm diam, septal distances 12–32 μm, colorless,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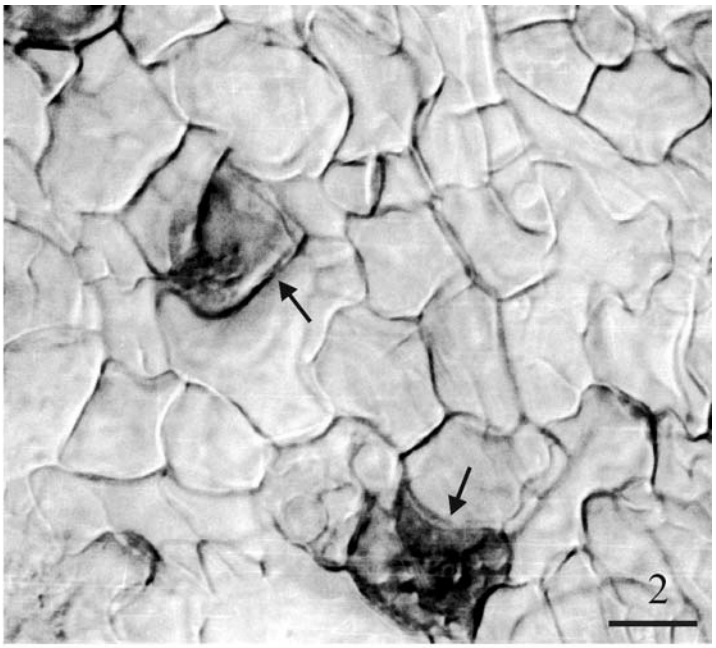
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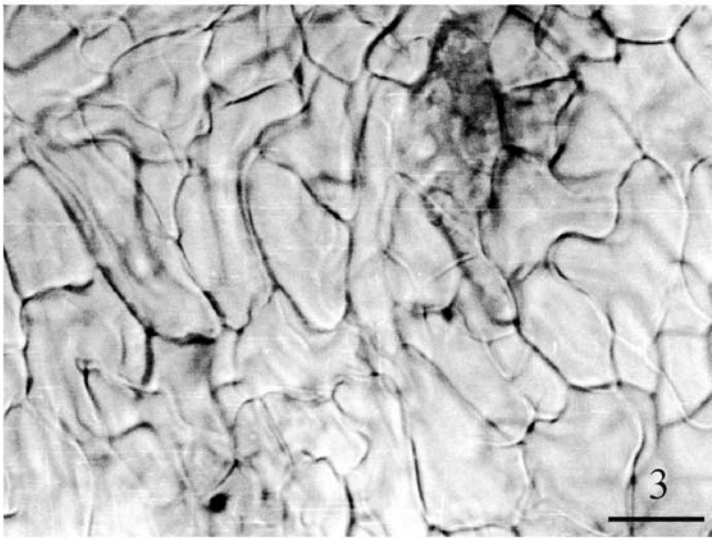
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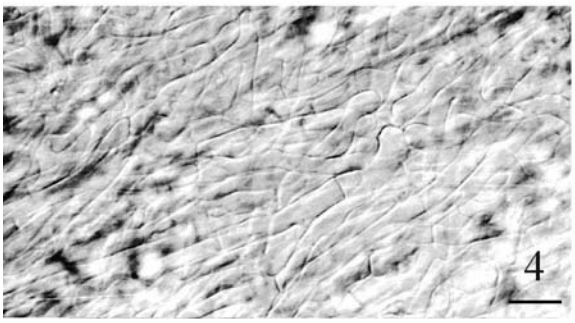
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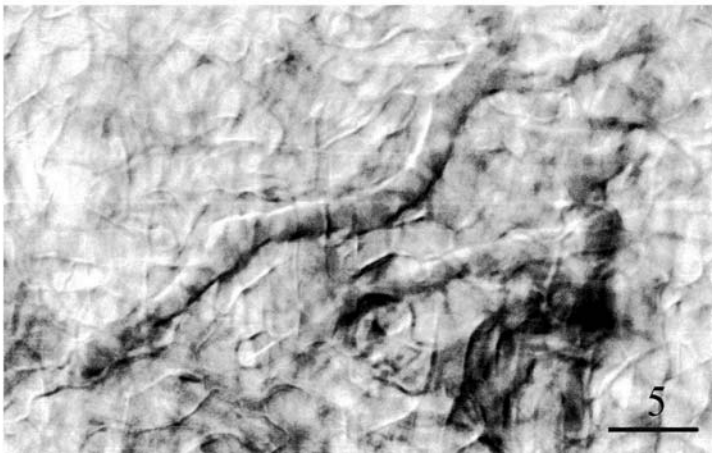
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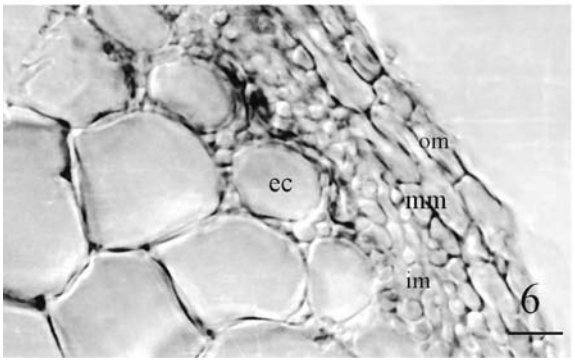
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hyphae even at septa, without clamps, elbow-like protrusions present, smooth, but agglutinated with soil particles (FIG. 11). Cystidia lacking.

Anatomical characters in longitudinal section: mantle 40–60 μm thick (FIG. 12), two distinct mantle layers discernible; outer mantle layer pseudoparenchymatous, hyphae tangentially 7.3–27 μm, radially 5–10 μm; inner mantle layer plectenchymatous, hyphae tangentially 6–11 μm, radially 2–7 μm; mantle of very tip 30–35 μm. Tannin cells lacking. Epidermal cells roundish to rectangular, parallel to root axis. Cortical cells roundish to cylindrical, tangentially 25–47 μm, radially 11–22 μm. Hartig net paraepidermal, in plan view infrequently lobed with 3.5–6 μm wide lobes.

Color reaction with different reagents: whole ectomycorrhizae bleaching with 15% KOH, lactic acid, NH_4OH and ethanol 70%. Sulpho-vanillin: walls red, contents of mantle cells with droplets becoming black. Emanating hyphae stain slightly blue with cotton blue; no reaction with Melzer's reagent.

Specimens examined. ARGENTINA. Catamarca. Sierra de Narváez. 27°43′S, 65°4′W, elevation 1820 m. Ectomycorrhizae under *A. acuminata*, 20 Mar 2002, *A. Becerra* 09 (CORD); Catamarca. Sierra de Narváez. 27°43′S, 65°54′W, elevation 1820 m. Fruitbodies under *A. acuminata*, 7 May 2000, *G. Daniele* 188 (CORD), 27 May 2001, *G. Daniele* 216 (CORD), 20 Mar 2002, *G. Daniele* 222 (CORD).

Cortinarius tucumanensis Mos. FIGS. 13–19

Morphological characters: mycorrhizal systems up to 8 mm long, simple (unramified), rarely monopodial-pinnate; orders of ramification 0–1; systems occurring solitarily or in few numbers; unramified ends sinuous to tortuous, tapering or with cylindric tips, up to 9 mm long and 0.2–0.5 mm diam; entire surface of mycorrhiza white (silvery) when young, ochre, yellowish brown when older (FIG. 13). Surface of unramified ends cottony to densely cottony, covered with soil particles. Root tips blunt, covered by the mantle. Mantle not transparent; mycorrhizae not carbonizing.

Rhizomorphs infrequent, up to 150 μm diam, concolorous to mantle, without specific origin, connection to the mantle fan-like, repeatedly ramified into smaller filaments with fan-like margins.

Anatomical characters of mantle in plan views: mantle continuous over the root apex. Blue granules and a matrix lacking. Outer mantle layers plectenchymatous with irregularly arranged hyphae, without any special pattern (type B, Agerer and Rambold 1998) (FIG. 14); hyphae undifferentiated, cylindric and slightly constricted at septa, colorless, 3–5 μm diam, thin-walled (<0.5 μm), always with clamps, anastomoses open (type A, Agerer 1991), septa as thick as walls. Inner mantle layer, plectenchymatous to pseudoparenchymatous without discernible pattern; inflated cells colorless, 6–15 μm diam, without clamps (FIG. 15).

Anatomical characters of emanating elements: rhizomorphs up to 150 μm diam (FIG. 16), undifferentiated; hyphae rather loosely woven and of uniform diameter (type A; Agerer and Rambold 1998), central and peripheral hyphae not specialized to irregularly sinuous, 3–5 μm diam, colorless cell walls up to 0.5 μm, septa with clamps as thick as hypha, surface of peripheral hyphae smooth and with soil particles. Emanating hyphae smooth, straight to wavy, 3.2–8 μm diam, slightly constricted at septa; cell walls thin (<0.5 μm), colorless, in few cases slightly thick-walled, with soil particles (FIG. 17), elbow-like protrusions lacking; distal ends of hyphae simple; septal distances 3–12 μm; septa with backward-oriented clamps, clamps in lateral view thinner than the hypha; secondary septa lacking; anastomoses open with a short bridge or bridge almost lacking, anastomosal bridge as thick as hyphae, with cell walls as thick as remaining walls.

Anatomical characters in longitudinal section: mantle 62–160 μm thick (FIG. 18), two distinct mantle layers discernible; outer mantle layer loosely plectenchymatous; hyphae tangentially 23–78 μm, radially 3–7 μm; inner mantle layer pseudoparenchymatous, hyphae tangentially 7–27 μm, radially 4–20 μm; mantle of very tip 30–35 μm. Tannin cells lacking. Epidermal cells rectangular, parallel to root axis. Cortical cells roundish to cylindrical, tangentially 30–45 μm, radially 13–45 μm. Hartig net periepidermal, rarely paraepidermal, in plan view of palmetti type

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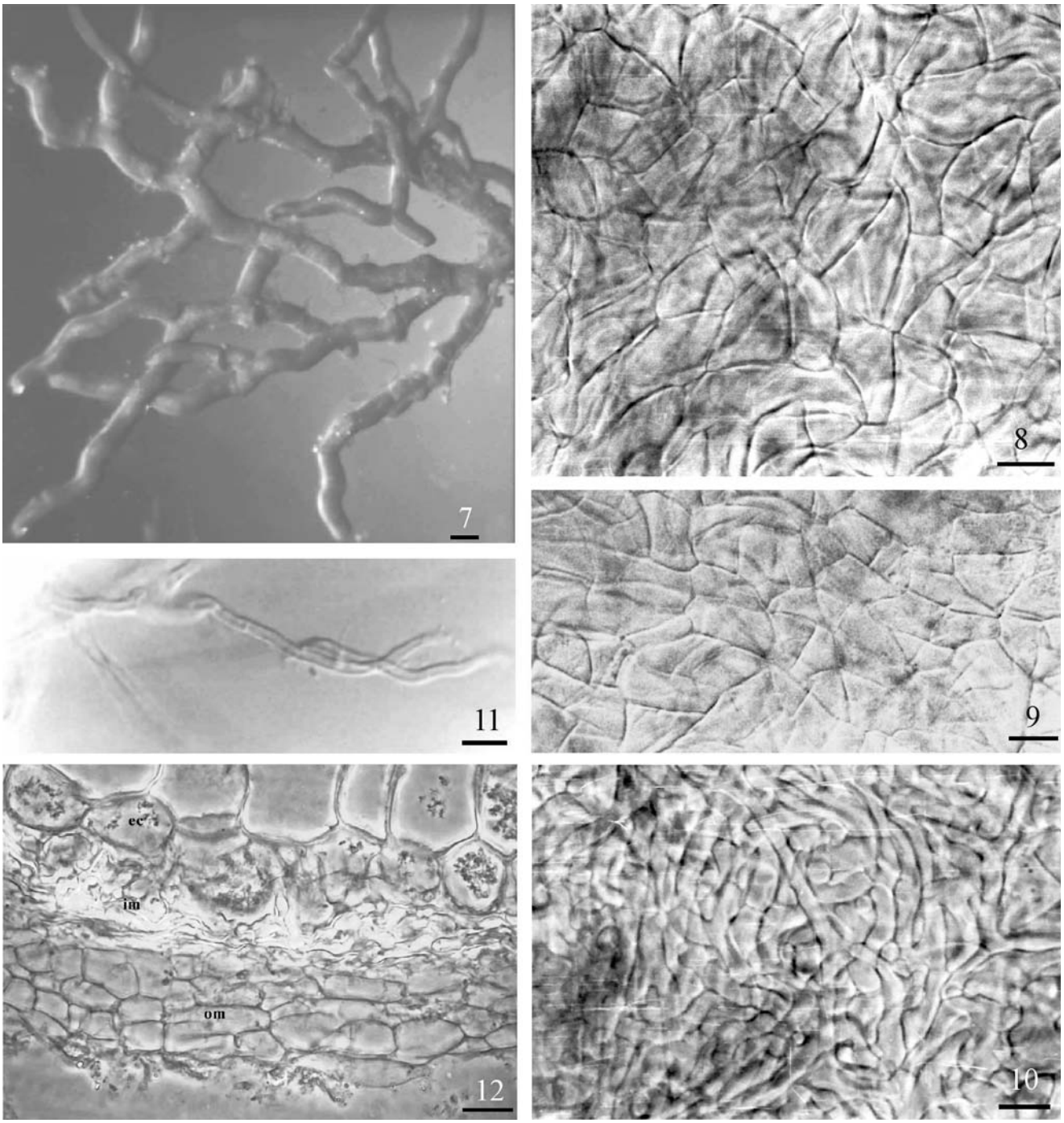
FIGS. 1–6. Light micrographs of ECM of *A. acuminata* and *L. aff. omphaliformis*. 1. Ectomycorrhizal system. 2. Outer mantle layer, pseudoparenchymatous with angular to epidermoid cells, some of them stained with sulpho-vanillin (→). 3. Middle mantle layer, plectenchymatous to pseudoparenchymatous, without discernible pattern. 4. Inner mantle layer, plectenchymatous without pattern. 5. Middle layer, straight laticifers. 6. Longitudinal section of the mantle with three distinct layers; outer mantle layer pseudoparenchymatous (om); middle mantle layer plectenchymatous to pseudoparenchymatous(mm); inner mantle layer plectenchymatous (im); epidermal cells rectangular (ec). Bars: 1 = 0.5 mm, 2–6 = 10 μm.

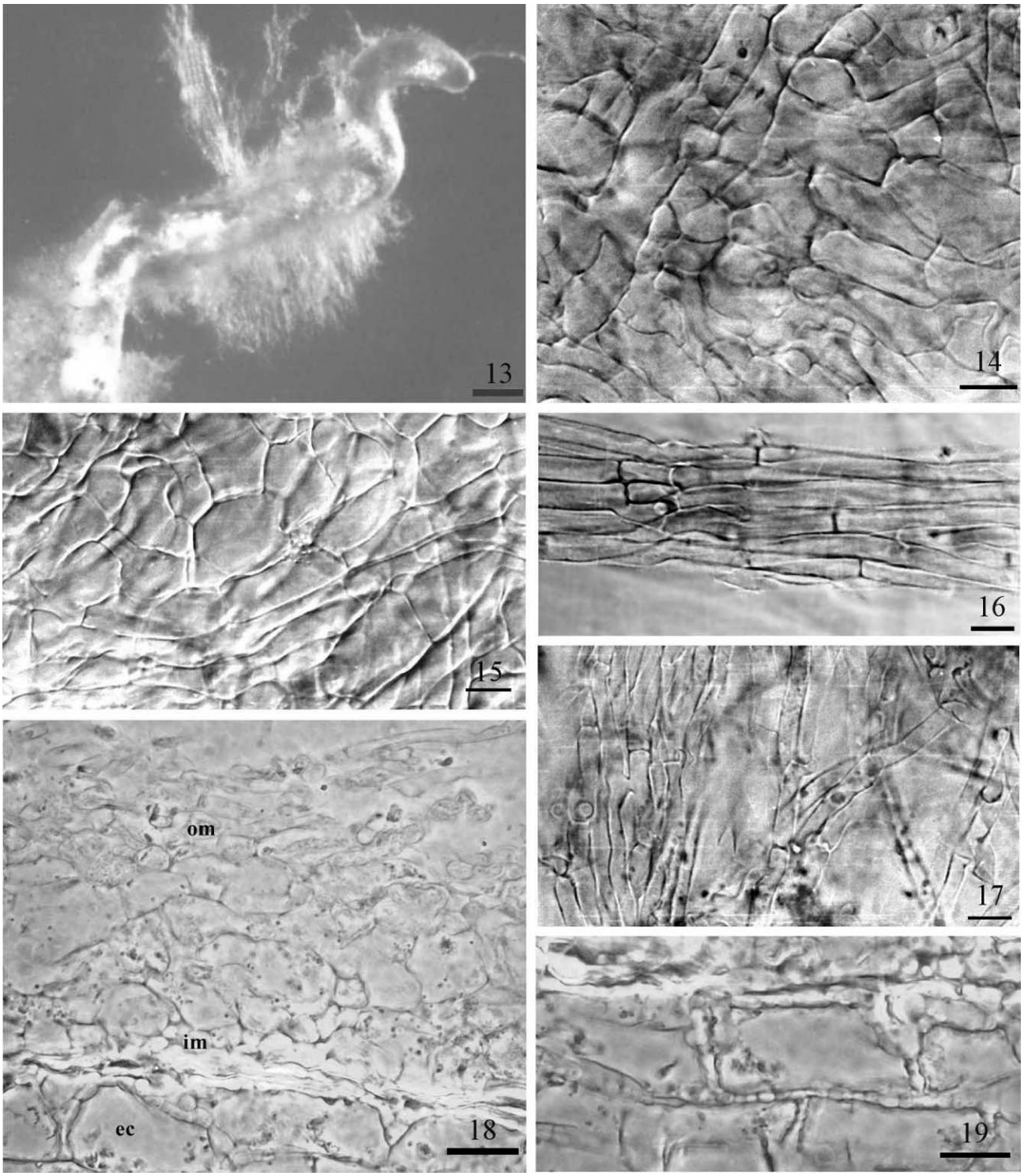


FIGS. 7–12. Light micrographs of ECM of *A. acuminata* and *R. alnijorullensis*. 7. Ectomycorrhizal systems. 8–9. Outer mantle layers pseudoparenchymatous with discernible angular cells. 10. Inner mantle layer plectenchymatous, without discernible pattern. 11. Emanating hyphae tortuous to irregularly inflated. 12. Longitudinal section of mantle with two distinct layers discernible; outer mantle layer pseudoparenchymatous (om), inner mantle layer plectenchymatous (im), epidermal cells (ec). Bars: 7 = 0.5 mm, 8–12 = 10 µm.

with 1.5–4 µm wide lobes (FIG. 19); 1–2 rows around epidermal cells, roundish in cross-section.

Color reaction with different reagents: whole ectomycorrhizae dark with 15% KOH. Lactic acid: orange. Emanating hyphae stain slightly blue with cotton blue; tips of unramified ends stain black with NH_4OH; no reaction with Melzer's reagent, sulphovanillin and ethanol 70%.

Specimens examined. ARGENTINA. Tucumán. Quebrada del Portugués. Tafí del Valle, 26°58′S,



FIGS. 13–19. Light micrographs of ECM of *A. acuminata* and *C. tucumanensis*. 13. Ectomycorrhizal systems. 14. Outer mantle layer densely plectenchymatous with rather irregularly arranged hyphae. 15. Inner mantle layer, plectenchymatous to pseudoparenchymatous. 16. Rhizomorphs with loosely woven hyphae. 17. Emanating hyphae smooth, straight to wavy. 18. Longitudinal section of mantle with two distinct layers; outer mantle layer densely plectenchymatous (om); inner mantle layer pseudoparenchymatous (im); epidermal cells (ec). 19. Hartig net surrounding epidermal cells (tangential section). Bars: 13 = 0.5 mm, 14–19 = 10 μm.

TABLE I. ITS length and restriction fragment band size (in base pairs) for *Lactarius aff. omphaliformis* collected from Sierra de Narváez (Catamarca Province), *Russula alnijorullensis* collected from Sierra de Narváez (Catamarca Province) and *Cortinarius tucumanensis* collected from Quebrada del Portugués (Tucumán Province) obtained by using *Taq*I, *Hinf* I and *Eco*RI enzymes

	Collection number	ITS length	*Taq*I	*Hinf* I	*Eco*RI
Lactarius aff. omphaliformis					
Fruitbody	GD 210	700	270/210	400/270	450/350
Mycorrhiza	AB04	700	270/210	400/270	450/350
Russula alnijorullensis					
Fruitbody	GD 222	700	390/170	380/320	400/310
Mycorrhiza	AB09	700	390/170	380/320	400/310
Cortinarius tucumanensis					
Fruitbody	GD 221	600	320/240	290/190	330/270
Mycorrhiza	AB02	600	320/240	290/190	330/270

65°45′W, elevation 2187 m. Ectomycorrhizae under *A. acuminata*, 19 Mar 2002, *A. Becerra* 02 (CORD); Tucumán. Quebrada del Portugués. Tafí del Valle, 26°58′S, 65°45′W, elevation 2187 m. Fruitbodies under *A. acuminata*. 19 Mar 2002, *G. Daniele* 221 (CORD). Catamarca. Sierra de Narváez. 27°43′S, 65°54′W, elevation 1820 m, 17 Feb 2000, *G. Daniele* 206 (CORD).

DNA analysis and restriction fragment patterns.—Single ITS-PCR products were obtained for all three species, with an approximate ITS length of 700 bp for *Lactarius aff. omphaliformis* and *Russula alnijorullensis*, and of 600 bp for *C. tucumanensis* (TABLE I). PCR/RFLP profiles revealed similar restriction fragment patterns for the three described anatomotypes with fruitbodies of *L. aff. omphaliformis, R. alnijorullensis* and *C. tucumanensis*, allowing the identification of these ECM (TABLE I).

DISCUSSION

In this study the combined approach of anatomotyping and molecular biology let us identify the field-collected ectomycorrhizae of *L. aff. omphaliformis*, *R. alnijorullensis* and *C. tucumanensis* on *A. acuminata*.

Ectomycorrhizal diversity is low in alder forests (Molina 1979, 1981; Gardner and Barrueco 1999). As other *Alnus* species, *A. acuminata* is associated with a number of ECM fungi belonging to the genera *Alpova*, *Cortinarius*, *Gyrodon*, *Inocybe*, *Laccaria*, *Lactarius*, *Naucoria* and *Russula* (Singer and Morello 1960; Raithelhuber 1988; Moser 2001; Becerra et al 2002, 2005a, b; Nouhra et al in press). ECM described within the present study belong to some of the genera known from fruitbodies commonly associated with *A. acuminata*.

L. aff. omphaliformis ectomycorrhizae on *A. acuminata* showed high similarity to *L. obscuratus* on *A. rubra* (Miller et al 1991) and to *L. obscuratus* and *L. omphaliformis* ectomycorrhizae on *A. glutinosa* (Pritsch et al 1997b) but also differed in some anatomical features. The pseudoparenchymatous mantle structure of *L. aff. omphaliformis* ECM differed from the plectenchymatous mantle of *L. obscuratus* on *A. rubra* (Miller et al 1991) and from the intermediate mantle type between compact plectenchymatous to irregularly pseudoparenchymatous of *L. obscuratus* and *L. omphaliformis* ectomycorrhizae on *A. glutinosa* (Pritsch et al 1997b).

Within the genus *Russula*, *R. alnijorullensis* belongs to a group of species with an angular pseudoparenchymatous mantle structure such as *R. fellea* (=*Fagirhiza granulosa*) on *Fagus sylvatica* (Brand and Agerer 1988), *R. mairei* on *F. sylvatica* (Brand 1991), *R. ochroleuca* on *Picea abies* (Agerer 1986, Gronbach 1988), *R. pumila* on *A. glutinosa* (Pritsch et al 1997b) and *R. alnetorum* on *A. viridis* (Beenken 2001). *R. alnijorullensis* on *A. acuminata* differed from other *Russula* ECM on *Alnus*: It did not exhibit hyaline, grouped dead cells of variable irregular shape on the outer mantle layer as *R. pumila* on *A. glutinosa* (Pritsch et al 1997b) or highly organized rhizomorphs and three mantle layers as *R. alnetorum* on *A. viridis* (Beenken 2001).

Cortinarius tucumanensis ECM showed similar features to *C. bibulus* ECM on *A. rubra* (Miller et al 1991) and *Alnirhiza violacea* on *A. glutinosa* (Pritsch et al 1997b), such as color (whitish) and two mantle layers (loosely plectenchymatous outer layer and a compactly arranged inner layer). *C. tucumanensis* ECM differed from *C.* cf. *alneus* (mantle 20–50 µm) and *C.* cf. *hevelloides* (mantle < 150 µm), both on *A. glutinosa* (Pritsch et al 1997b), in that these show a mycorrhizal system with simple ramification, and

plectenchymatous inner and outer mantle layers. Other *Cortinarius* ECM can be discriminated from *C. tucumanensis* ECM by a completely plectenchymatous mantle, differing mantle thickness or ramification of mycorrhizal systems: *C.* cf. *saturninus* on *A. tenuifolia*, mantle up to 300 µm and monopodial ramification (Brunner et al 1990); *C. atropusillus* on *A. viridis*, mantle up to 120 µm and simple ramification (Wiedmer and Senn-Irlet 1999b); *C. badiovestitus* on *A. viridis*, mantle up to 160 µm and simple to irregularly pinnate ramification (Wiedmer and Senn-Irlet 1999c); *C. kühneri* and *C. alnobetulae*, both on *A. viridis*, mantle up to 80 and 95 µm respectively, and simple to irregularly monopodial-pinnate ramification (Wiedmer and Senn-Irlet 1999d, a respectively); *C. helvelloides* mantle up to 100 µm and *C. pluvius*, mantle up to 180 µm on *A. viridis*, both lacking ramification (Wiedmer and Senn-Irlet 2001b, 1998 respectively); *C. bibulus* on *A. viridis* with a mantle up to 90 µm and simple ramification (Wiedmer and Senn-Irlet 2001a). *C. tucumanensis* ECM also differ from *C. helodes* on *A. acuminata* (Becerra et al 2005a) with the latter forming a three layered mantle (inner pseudoparenchymatous, middle and outer plectenchymatous) of up to 200 µm thickness and a simply ramified mycorrhizal system.

ITS-PCR/RFLP analyses with the three endonucleases *Taq*I, *Hinf*I and *Eco*RI let us identify and distinguish three anatomically distinct ectomycorrhizae, thus supporting the anatomical observations. The approximate band size estimates based on agarose gels resulted in similar values as observed in other studies. With an ITS length of ca 600 bp of *C. tucumanensis*, similar ITS lengths of 610 bp were obtained by Pritsch et al (1997a) for *Cortinarius* ectomycorrhizae. ITS lengths of approximately 700 bp of *R. alnijorullensis* and *L. aff. omphaliformis* similar to 710 bp of ITS for Russulaceae ectomycorrhizae that have been published by Pritsch et al (1997a).

The anatomical and molecular characterization of *L. aff. omphaliformis*, *R. alnijorullensis* and *C. tucumanensis* ECM reported here contribute to a more detailed knowledge on the symbionts of *A. acuminata* in native Argentinean forests.

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