Mycorrhiza (2005) 15:7–15
DOI 10.1007/s00572-003-0278-x

ORIGINAL PAPER

Alejandra Becerra · Eduardo Nouhra · Graciela Daniele · Laura Domínguez · Donaraye McKay

Ectomycorrhizas of *Cortinarius helodes* and *Gyrodon monticola* with *Alnus acuminata* from Argentina

Received: 28 May 2003 / Accepted: 14 October 2003 / Published online: 26 November 2003

Abstract Field ectomycorrhizas of *Cortinarius helodes* Moser, Matheny & Daniele (sp. nov) and *Gyrodon monticola* Sing. on *Alnus acuminata* Kunth (Andean alder, aliso del cerro) are described based on morphological and anatomical features. Ectomycorrhizal roots were sampled beneath fruitbodies of *C. helodes* and *G. monticola* from two homogeneous *A. acuminata* forest sites located in Tucumán and Catamarca Provinces in Argentina. *C. helodes* ectomycorrhizas showed a thick white to beige mantle exuding a milky juice when injured, were bluish toward the apex, and had hyphal strands in the mantle. *G. monticola* ectomycorrhizas showed some conspicuous features like highly differentiated rhizomorphs, inflated brown cells on the mantle surface, and hyaline and brown emanating hyphae with dolipores. Restriction fragment length polymorphism analysis of the nuclear rDNA internal transcribed spacer provided a distinctive profile for each of the collections of fruitbodies and the mycorrhizal morphotypes.

Keywords Andean alder · *Cortinarius helodes* · *Gyrodon monticola* · Morphological characterization · Polymerase chain reaction/restriction fragment length polymorphism

A. Becerra (✉) · G. Daniele · L. Domínguez
Instituto Multidisciplinario de Biología Vegetal (CONICET),
C.C. 495, 5000 Córdoba, Argentina
e-mail: abecerra@imbiv.unc.edu.ar
Fax: +54-351-4331056

E. Nouhra
Department of Forest Science,
Oregon State University,
Corvallis, OR, 97331, USA

D. McKay
USDA Forest Service, Forestry Sciences Laboratory,
Pacific Northwest Research Station,
3200 SW Jefferson Way, Corvallis, OR, 97331, USA

Introduction

The distribution of *Alnus acuminata* Kunth ranges from the highlands of Mexico to the Andes (Dawson 1990), reaching its southernmost point in the Catamarca province of Argentina. Alders form ectomycorrhizas, and most alder species are used as forage and fuel sources, and quickly increase the forest biomass over previously deforested areas.

Species identification of mycorrhizal morphotypes is often required in biodiversity and ecological studies. The first approach is to characterize mycorrhizas based on morphological and anatomical features (Miller et al. 1991; Agerer 1991); in many cases, as stated by Agerer (1986, 1991, 1995) and Brundrett et al. (1996), hyphal connections can be traced from the fruitbody's stipe base to the mycorrhizas. Additionally, molecular techniques such as polymerase chain reaction coupled with restriction fragment length polymorphism analyses (PCR/RFLP) increase the resolution of identification to the species level (Pritsch and Buscot 1994; Egger 1995; Baldwin and Egger 1996; Eberhardt et al. 1999; Horton and Bruns 2001; Mah et al. 2001; Sakakibara et al. 2002).

Ectomycorrhizal diversity is low in alder forest (Molina 1979, 1981; Gardner and Barrueco 1999). Trappe (1962) and Horak (1963) list a number of fungi within the families Cortinariaceae, Russulaceae, and Boletaceae associated with different *Alnu* s species. Within Cortinariaceae several species have been reported as having a mycorrhizal association with alders (Stangl 1970; Godbout and Fortin 1983, 1985; Brunner et al. 1990; Miller et al. 1991; Moser 2001). Pritsch et al. (1997a, 1997b) described the ectomycorrhizas of *Cortinarius* cf. *helvelloides* (Fr.) Fr. and *Cortinarius* cf. *alneus* (Mos.) Mos. associated with *A. glutinosa* (L.) Gaertn. Within Boletaceae, four *Gyrodon* species have been reported from North America and Europe associated with *Alnus* spp. (Hayward and Thiers 1984). *Gyrodon monticola* Sing. was reported growing under *A. acuminata* in Mexico and Argentina (Singer and Morello 1960; Hayward and Thiers 1984; Singer and Gomez 1984) and ectomycorrhizas of

Gyrodon lividus (Bull.) Fr. with *Alnus incana* (L.) Moench were described by Agerer et al. (1993).

The aim of this work was to characterize the ectomycorrhizas formed by *Cortinarius helodes* Moser, Matheny & Daniele and *Gyrodon monticola* with *Alnus acuminata* through morphological and anatomical features, and to confirm their identity with molecular techniques (PCR/RFLP).

Materials and methods

Study site

Two forest sites located in the northwestern region of Argentina (NOA) were sampled. Quebrada del Portugués, Tafí del Valle (Tucumán Province), elevation 2,187 mm; 26°58′S, 65°45′W, average precipitation ranges between 800 and 1,200 mm. The Narvaez Range (Catamarca Province), elevation 1,820 m; 27°43′S, 65°54′W, average precipitation 1,620 mm. Mean annual temperatures range from 5.8 to 24°C for NOA. The vegetation is a nearly homogeneous *A. acuminata* forest (height 6–15 m, age 20–30 years) with a few herbaceous understory plants such as *Duchesnea* sp. (Rosaceae); *Conyza* sp. (Asteraceae), *Axonopus* sp. (Poaceae), *Selaginella* sp. (Selaginellaceae); *Prunella* sp. (Lamiaceae), etc. (Aceñolaza 1995). Soil types are Entisols with high organic matter content in both locations (Becerra et al. 2002). Two plots of 30×30 m were sampled at each study site.

Sampling and direct identification

Both locations were visited during summer and fall from 1999 through 2001. At every sampling time, soil cores of 15×15 cm to a depth of about 10 cm were concurrently collected below fruitbodies. The samples were placed in plastic bags leaving the sample as undisturbed as possible, and stored at 4°C during transport to the laboratory.

The samples were placed in water and examined for hyphal connections leading from fruitbodies to fungal mantles under a Zeiss stereo microscope at ×10–40 magnification according to the method of Agerer (1991). Alder roots (which are the only structures within these forests that present ectomycorrhizas) and mycorrhizas within soil cores were easy to identify due to their morphological appearance, although roots from herbaceous plants were also present. Within every morphotype, several tips were prepared for DNA extraction, while others were subjected to comparative anatomical studies. Photographs of mycorrhizas were taken with a Leica M420 stereo microscope. 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 mycorrhizas. Afterwards, mycorrhizal roots were fixed in 70% alcohol and stored at 4°C in the dark.

Microscopic analysis

Description of the ectomycorrhizas follows the terminology of Agerer (1991, 1999a) and Miller et al. (1991). Mantle views were examined and photographed with a Zeiss Axiophot light microscope at ×200–1,000 magnifications. Characterization of the Hartig net follows Godbout and Fortin's (1983) nomenclature. Fruitbodies were identified following Singer and Digilio (1957, 1960) and Moser (1978) protocols. Voucher specimens and mycorrhizas were deposited in the Museo Botánico de Córdoba Herbarium (CORD) (Holmgren et al. 1990).

DNA extraction and amplification

DNA was extracted from one to three root tips and from dried lamellae from two fruitbodies (Gardes and Bruns 1993) (both extractions were carried out twice). Species characterization of the fungi was based on PCR amplification of the internal transcribed spacer (ITS) region of the rDNA gene by using ITS-1F and ITS-4B primers (Gardes and Bruns 1993). The primer pairs preferentially amplify specific fragments of basidiomycete DNA from mixtures of plant and fungus DNA. We used reagents, protocols, and cycling parameters as described previously (Gardes and Bruns 1996).

RFLP analysis

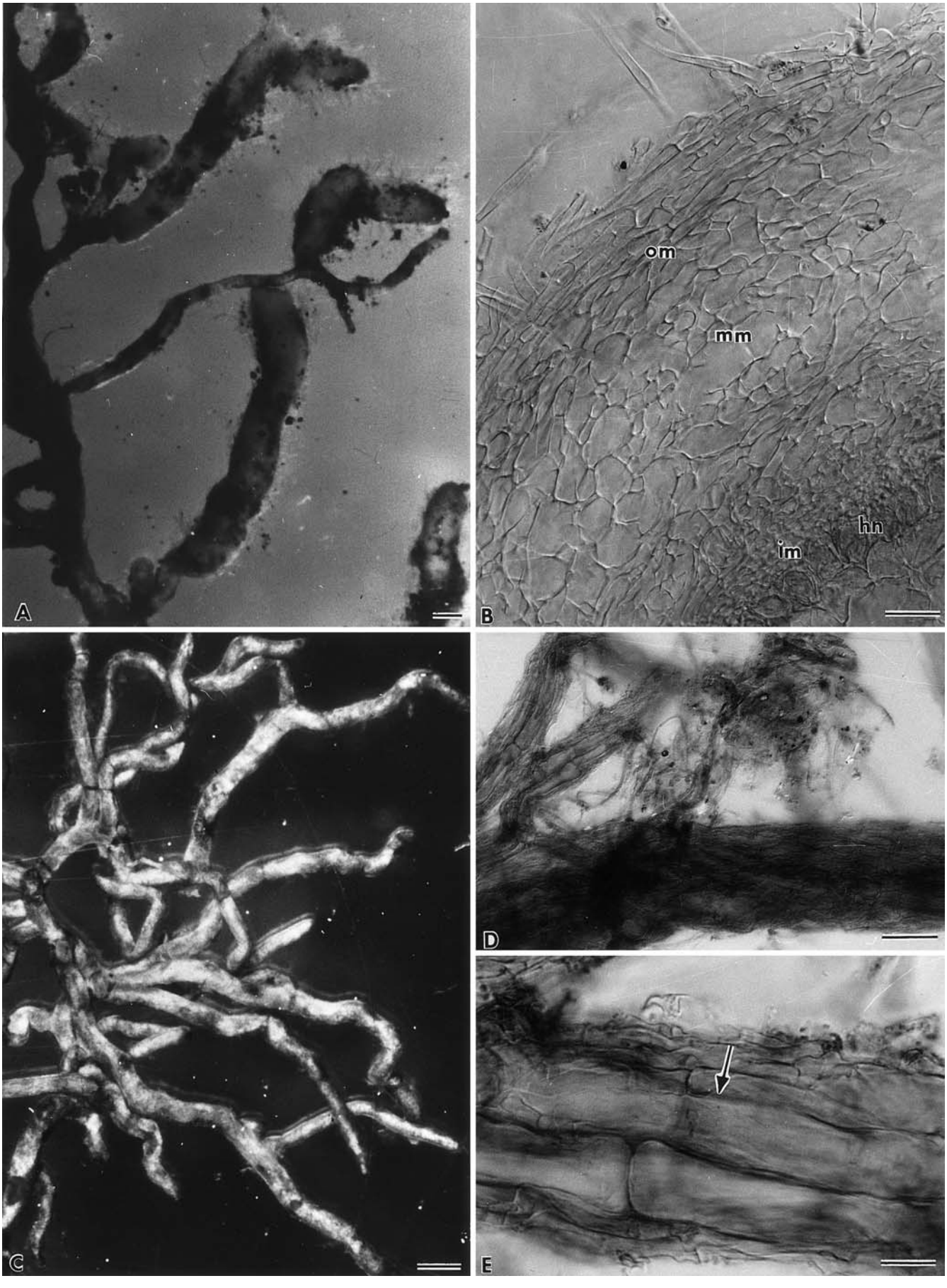
We characterized the ITS region by RFLP analysis, which was used to match mycorrhiza root samples and fruitbodies of voucher collections. Aliquots of DNA from mycorrhizas and fruitbodies of *C. helodes* were digested with *Alu* I, *Hinf* I and *Dpn* II enzymes, whereas *G. monticola* DNA was digested with *Hinf* I, *Dpn* II and *Hae* III enzymes loaded side by side for comparison onto a 1% agarose/2% Nusieve gel and separated by electrophoresis for 3 h at 100 V in a 1% TBE buffer. A 100-base pair DNA ladder (Promega, Madison, Wis.) was used to determine fragment size. The length of the complete ITS was estimated by comparing undigested PCR product run on a 1% agarose/2% Nusieve gel with the 100-base pair DNA ladder. Gels were stained in ethidium bromide and observed under ultraviolet light. Images were analyzed with Scanalytics, Gene Profiler 4.02 software using default parameters. The ITS base pair lengths are based on scores using a 100-base pair ladder. Scanalytics software calculates the logarithm of all molecular weight standards, then plots the log molecular weight values that intersect each standard data point. To calculate the molecular weight values of the unknown samples, the software substitutes each unknown band migration distance (pixel location) into the linear line equation ($y = mx + b$) for each piece (Hook 2002).

Results

Various fruitbodies and mycorrhizas were collected in the field, but we present here two fungi (*C. helodes* and *G. monticola*) associated with *A. acuminata* according to the RFLP matches obtained.

The direct attempt to identify ectomycorrhizas of these fungi by tracing hyphal connections between the stipe base and mycorrhizal root was difficult. Within the collected *C. helodes* soil samples only a few mycorrhizas were observed. On the other hand, mycorrhizas of *G. monticola* were abundant under the fruitbodies. Within the molecular analysis, the comparison of PCR/RFLP patterns showed a similar band size between ectomycorrhizas and fruitbodies when using three endonuclease

Fig. 1 Light micrographs of *Alnus acuminata* and *Cortinarius helodes* (**A**, **B**) collected from the Sierra de Narváez (Catamarca Province) and *Gyrodon monticola* (**C** – **E**) collected from the Quebrada del Portugués (Tucumán Province) and Sierra de Narváez (Catamarca Province) sites. **A** Simple (unramified) ectomycorrhizal root tip of *C. helodes*; *bar* 0.5 mm. **B** Cross-section showing the plectenchymatous to pseudoparenchymatous outer mantle layer (*om*) (see also Fig. 2 C–D), pseudoparenchymatous middle mantle layer (*mm*) and pseudoparenchymatous inner mantle layer (*im*); Hartig net (*hn*); *bar* 25 μm. **C** Monopodial to irregularly pinnate ectomycorrhizal root tip of *G. monticola*; *bar* 0.5 mm. **D** Rhizomorphs; *bar* 50 μm. **E** Central core of thick hyphae in the rhizomorph (➞); *bar* 10 μm


A
B
om
mm
hn
im
C
D
E

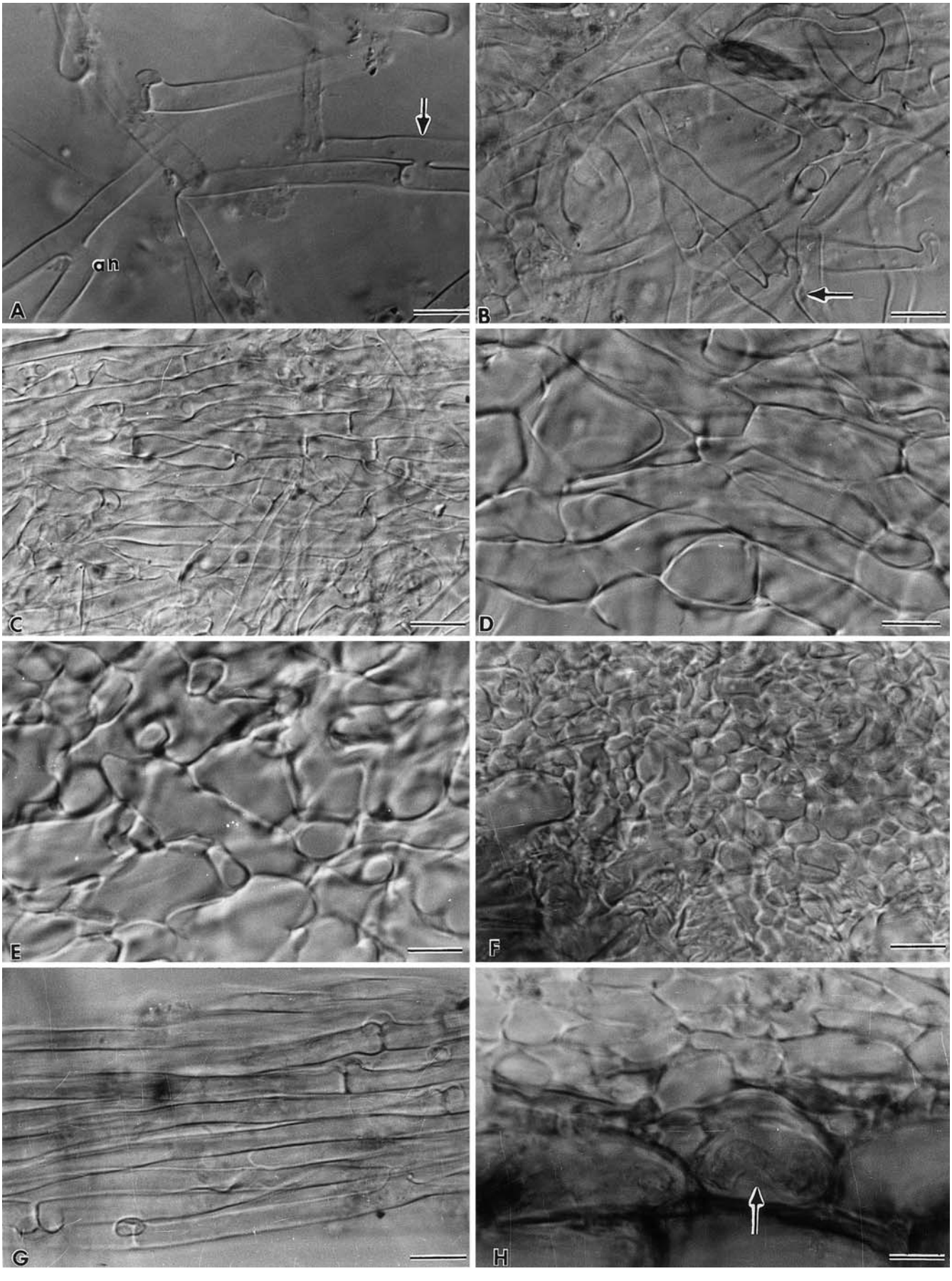

ah
A
B
C
D
E
F
G
H

Table 1 Internal transcribed spacer (*ITS*) length and restriction fragment band size for *Cortinarius helodes* collected from Sierra de Narváez (Catamarca Province) and *Gyrodon monticola* collected from Quebrada del Portugués (Tucumán Province) and Sierra de Narváez (Catamarca Province) obtained by using, respectively, *Alu*I, *Hinf*I, *Dpn*II and *Hae*III, *Hinf*I, and *Dpn*II restriction enzymes. *bp* Base pairs

Structure	Collection no.	Samples (*n*)	ITS length (bp)	*Alu*I	*Hinf*I	*Dpn*II
C. helodes	GD19	2	730	455/135/85	330/125/[a]	325/215/195
Mycorrhiza	AB01	1–3	775	455/135/85	330/125/[a]	325/210/190
Structure	Collection no.	Samples (*n*)	ITS length (bp)	*Hae*III	*Hinf*I	*Dpn*II
G. monticola	GD173	2	895	367/164/138	375/193/75/58	312/149/124/70
Mycorrhiza	AB05	1–3	905	362/162/136	378/196/75/60	310/151/124/70

[b] Small fragments probably not detected

restriction fragment enzymes, thus confirming the identification of *C. helodes* and *G. monticola* as symbionts of *A. acuminata*. The ITS was about 750 base pairs for *C. helodes* and about 900 base pairs for *G. monticola* (Table 1).

Description of the morphotypes

Cortinarius helodes ectomycorrhizas

Simple (unramified), straight to bent, 1.2–9.2 mm in length and 0.4–0.8 mm in diameter, some tips not entirely colonized (Fig. 1A). Mantle, white when young with bluish to lilac tints toward the apex, pale yellow to beige when older. Root tips are blunt, some acute, covered by the mantle. Mantle surface with soil particles (Fig. 1A); smooth to woolly with few to many emanating hyphae and hyphal strands. Young mycorrhizas exudate whitish substance when damaged.

Emanating hyphae (Fig. 2A, B): hyaline, few or abundant, straight to bent, branched, thin walled, 2.5–6.5 μm in diameter, regularly septate with some clamps. Hyphae with simple anastomoses without septa (Agerer 1991, Type A).

Hyphal strands (Fig. 2G): hyaline to pale yellow, straight, agglutinated, up to 47 μm in diameter at the base with a few thin-walled and clamped emanating hyphae, 3.2–5 μm in diameter.

Mycorrhiza mantle (plane view): mantle continuous over the root apex. Plectenchymatous outer layer of loosely interwoven, hyaline, thin-walled, tangentially oriented hyphae with clamp connection, 2.4–5 μm in diameter (Fig. 2C), turning inward into pseudoparenchymatous tissue with elongated hyphae (Fig. 2D); middle layer with a pseudoparenchymatous arrangement of angular to spherical cells 6–24 μm in diameter (Fig. 2E); inner layer with a pseudoparenchymatous arrangement of small, spherical and thin-walled cells, 3.2–11 μm in diameter (Fig. 2F).

Mycorrhiza (cross section): mantle 130–220 μm thick (Fig. 1B), differentiated into a plectenchymatous outer layer of tangentially arranged hyphae 3.2–6.5 μm in diameter, a middle pseudoparenchymatous layer of angular to globose cells 6.5–27.5 μm in diameter, and a pseudoparenchymatous inner layer of small, isodiametric cells 4–16 μm in diameter; paraepidermical Hartig net, hyphae lined up in one row between epidermal cells 1.6–5 μm in diameter; epidermal cells spherical to elongated in outline, 8–30.5 μm×6.5–19.5 μm. Intracellular hyphae are present at the epidermal cells (Fig. 2H).

Color reaction: whole ectomycorrhizas are whitish with 15% KOH and 70% ethanol; emanating hyphae and hyphal strands stain slightly blue with Cotton blue; and show no reactions with sulpho-vanillin, lactic acid, and Melzer's reagent.

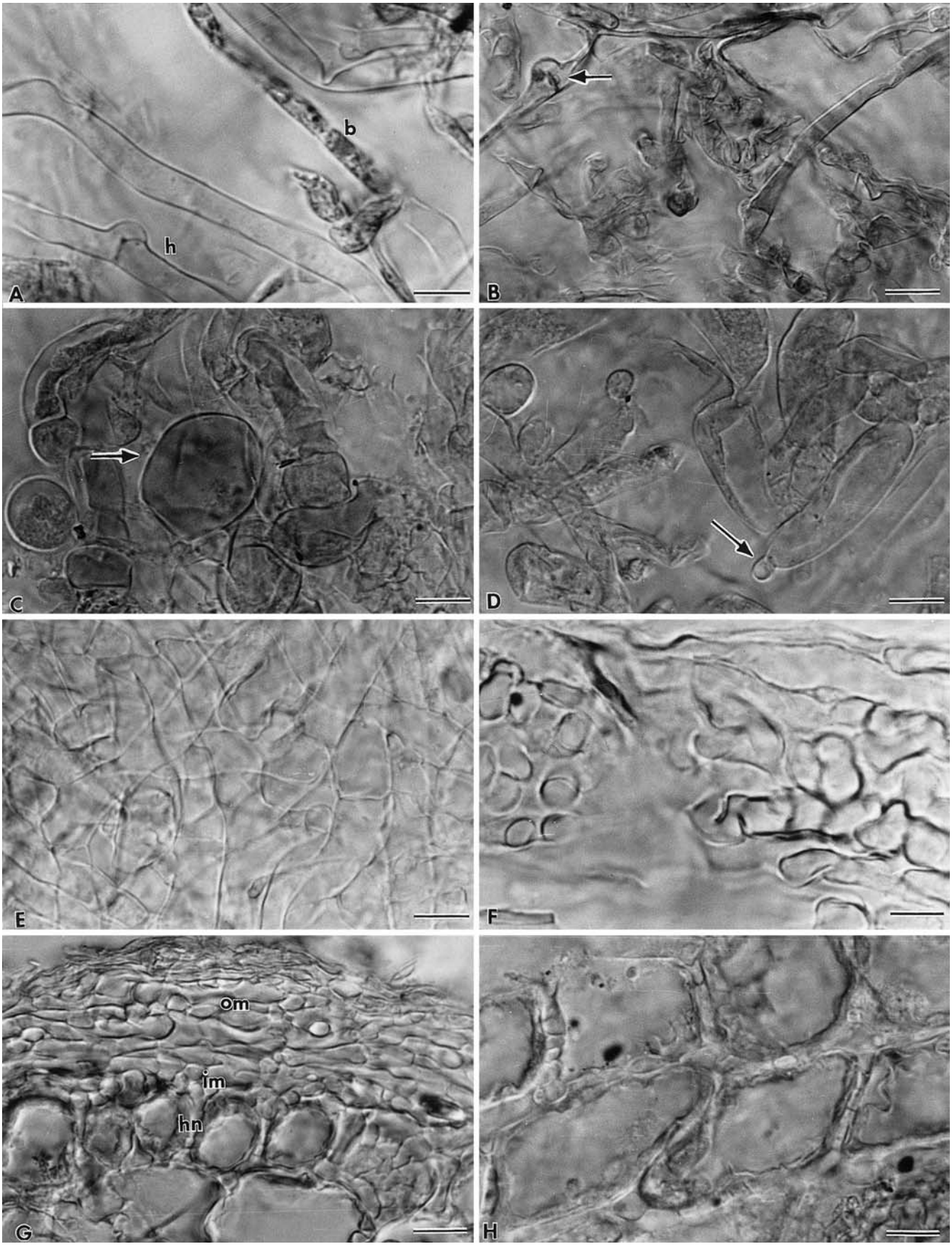
Voucher specimen: ectomycorrhizas under *A. acuminata*, in herbarium A. Becerra AB 01 (CORD); fruitbodies examined: Argentina: Sierra de Narváez (Catamarca Province), elevation 1,820 m; 27°43′S, 65°54′W; 27 May 1999, G Daniele GD 187 (CORD), 17 February 2000, GD 19 (CORD), 20 March 2001, GD 211 (CORD).

Gyrodon monticola ectomycorrhizas

Monopodial to irregularly pinnate (Fig. 1C), straight, tortuous, some bent, 0.6–6.3 mm long and 0.2-0.3 mm in diameter, golden yellow when young, brown when old, rhizomorphs concolorous with a shiny surface. Root tips are blunt, mantle surface has few globose to ellipsoid brown cystidia and many soil particles.

Emanating hyphae of two types (Fig. 3A, B): hyaline and brown pigmented, loosely interwoven. Hyaline hyphae are branched, thin walled, 2.4–4 μm in diameter, regularly septate, clamped. Brown hyphae are branched, thin walled, 3.2–5 μm in diameter, some completely collapsed; clamp connections are present with conspicuous dolipores (Fig. 3B). Simple anastomoses are present without septa (Agerer 1991, Type A).

Fig. 2A–H Light micrographs of *A. acuminata* and *C. helodes* ectomycorrhiza collected from the Sierra de Narváez (Catamarca Province) site. **A** Emanating hyphae with soil particles and simple anastomoses (*an*) (*arrow*); *bar* 10 μm. **B** Emanating hyphae branched (→); *bar* 10 μm. **C** Plectenchymatous outer mantle of loosely interwoven hyphae; *bar* 10 μm. **D** Pseudoparenchymatous transitional mantle layer of elongated hyphae; *bar* 10 μm. **E** *mm* of angular to spherical cells; *bar* 6.25 μm. **F** *im* of spherical cells; *bar* 10 μm. **G** Hyphal strands of clamped hyphae in a parallel arrangement; *bar* 10 μm. **H** Detail of intracellular hyphae at epidermal cells (→); *bar* 6.25 μm. For abbreviations, see Fig. 1


b
h
A
B
C
D
E
F
om
im
hn
G
H

Cystidia (Fig. 3C, D): spherical to clavate with a small globule at the distal end, brownish, 4.8–11.2 μm in diameter.

Rhizomorphs (Fig. 1D, E): usually more or less perpendicularly oriented, with a restricted point of connection to the mantle, orange to brown, frequently thick (10.1–30.5 μm in diameter), with a central core of very thick hyphae (6.4–13 μm in diameter) (Agerer 1991, type E), septate, without clamp connections. The remaining hyphae are thin (2.4–4 μm in diameter), clamped, and thin walled. Few hyphae emerge from the rhizomorphs, and are thin walled, with or without clamps, hyaline to brownish, 3.2–5 μm in diameter.

Mycorrhiza mantle (plane view): usually continuous over the root apex. Outer layer is plectenchymatous with patches of roundish to ellipsoidal brown cells (mantle type F, Agerer 1991), hyphae interwoven, hyaline, thin walled, with some clamped septa, 3.2–6 μm in diameter (Fig. 3E). Toward the Hartig net, hyphae are smaller in diameter and length, spherical to elongated, with a pseudoparenchymatous and tangential arrangement, 2.4–8 μm in diameter, thick walled (Fig. 3F).

Mycorrhiza (cross section): mantle 24–57 μm thick (Fig. 3G), differentiated into a plectenchymatous outer layer of tangentially orientated hyphae 3.2–6.5 μm in diameter, and a pseudoparenchymatous inner layer of roundish cells 2.5–9.1 μm in diameter. Periepidermal Hartig net has hyphae between epidermal cells in one row, 1.6–2.5 μm in diameter (Fig. 3H). Epidermal cells are spherical to elliptical in outline, thick walled, 11–17 μm×6–16 μm.

Color reaction: whole ectomycorrhizas and emanating hyphae stain slightly blue with Cotton blue; the root and the Hartig net stain reddish with sulpho-vanillin; bleach with NH_4OH and lactic acid; and show no reaction with 15% KOH, Melzer's reagent and 70% ethanol.

Voucher specimen: ectomycorrhizas under *A. acuminata*, in herbarium A. Becerra AB 05 (CORD); fruitbodies examined: Argentina: Quebrada del Portugués, Tafí del Valle (Tucumán Province), elevation 2,187 m; 26°58′S, 65°45′W and Sierra de Narváez (Catamarca Province), elevation 1,820 m; 27°43′S, 65°54′W; 19 March 1999, G Daniele GD 173 (CORD), 20 March 2000, GD 207 (CORD).

Fig. 3A–H Light micrographs of *A. acuminata* and *G. monticola* ectomycorrhiza collected from the Quebrada del Portugués (Tucumán Province) and Sierra de Narváez (Catamarca Province) sites. **A** Emanating hyaline (*h*) and brown hyphae (*b*) from mantle surface; *bar* 6.25 μm. **B** Emanating *b* with conspicuous dolipore (→); *bar* 10 μm. **C** Spheric brownish cystidia (→); *bar* 8 μm. **D** Clavate light brown cystidia with a small distal globule (→); *bar* 8 μm. **E** Plectenchymatous outer mantle of interwoven hyphae; *bar* 8 μm. **F** *im*; *bar* 6.25 μm. **G** Cross-section showing plectenchymatous outer mantle (*om*), *im* and *hn*; *bar* 10 μm. **H** *hn* surrounding epidermal cells (tangential section); *bar* 8 μm. For other abbreviations, see Fig. 1

Discussion

In this study, the combined approach of morphotyping and molecular biology allowed us to identify the ectomycorrhizas of *C. helodes*, *G. monticola* with *A. acuminata* in the field.

The PCR/RFLP analyses were used successfully for the identification of the freshly harvested fruitbodies and the morphotypes. The two mycorrhizas were separated by their molecular profiles, supporting the morphological observations. Band size estimates made by using agarose gels are approximate, with an error rate of about 3%. We obtained an ITS length of approximately 750 base pairs for *C. helodes*, in contrast to a 610-base pair ITS for *Cortinarius* ectomycorrhizas obtained by Pritsch et al. (1997a). The larger ITS found in our study is directly a function of using the primers ITS-1F and ITS-4B, which amplify a larger fragment through the priming sites (ITS-1 and ITS-4) employed by Pritsch et al. (1997a). Differences in band size patterns between fruitbodies and mycorrhizas in the *G. monticola* RFLP analysis (around 1%) probably are due to band size estimation error rate. No other Boletales were observed during the sampling, nor are there reports of other species from the area.

Cortinarius and *Gyrodon* species, like *Cortinarius bibulus* Quel.; *C.* cf. *saturninus* (Fr.) Fr.; *C.* cf. *alneus* (Mos.) Mos. and *Gyrodon lividus* have been found associated with North American and European *Alnus* spp. (Horak 1963, 1985; Schmid-Heckel 1985; Miller et al. 1987; Brunner et al. 1990; Pritsch et al. 1997b).

From this study, *C. helodes* was identified as a new species, described by Moser (2001). *C. helodes* ectomycorrhiza showed similar features to *C.* cf. *helvelloides* ectomycorrhiza on *A. glutinosa* (Pritsch et al. 1997a, 1997b). The most conspicuous differences were the absence of refractive vacuoles on mantle cells and collapsed emanating hyphae in *C. helodes*. Similarly to the type A mycorrhiza of *A. japonica* Stend. and *Cortinarius* sp. (Masui 1926), *C. helodes* hyphae invade the epidermal cells (Fig. 2H). *Cortinarius* cf. *alneus* (Pritsch et al. 1997b) and *C. bibulus* (Miller et al. 1991) showed completely different anatomical features in mantle coloration (whitish, silvery to brown when old); mantle surface (woolly, cottony) and mantle thickness (20–50 μm).

G. monticola showed mantle coloration, emanating hyphae with brown pigments and unpigmented hyphae, cystidia arranged in a nest-like manner on mantle, and highly differentiated rhizomorphs similar to *G. lividus* and *A. incana* ectomycorrhizas (Agerer et al. 1993). Nevertheless the latter type is distinguished by sclerotia lying on ramifications, not present in *G. monticola* mycorrhiza. Brunner et al. (1990) found that *G. lividus* did not form a Hartig net on *A. tenuifolia* Nutt. in synthesis in vitro. *G. monticola* ectomycorrhizas on *A. acuminata* form an obvious Hartig net, although the mantle morphological features such as color are quite similar to *G. lividus* ectomycorrhizas.

Using direct identification by tracing mycelia from the fruitbodies to mycorrhizas (Agerer 1991) [successfully done by Agerer (1999b, 1999c), Agerer and Beenken (2001) with *Eucalyptus*, *Quercus*, *Pinus* and other species, among other authors] in a natural mature stand of *A. acuminata* was unsuccessful, because more than one morphotype was found below fruitbodies, as also found by Sakakibara et al. (2002). Nevertheless, *C. helodes* and *G. monticola* were identified molecularly (ITS PCR/RFLP analysis) and morphologically as symbionts of *A. acuminata* in native Argentinean forests.

Acknowledgements This work was partially supported by Agencia Córdoba Ciencia (ex-CONICOR) (nos. 1416/97; 1655/99) and PROYUNGAS (1999, 2001). We thank Tom Horton and Joyce Eberhart for their assistance in the molecular analysis. We also particularly thank Dr M. Moser for the identification of *Cortinarius helodes* sp. nov. Special thanks to Dr Efren Cázares, Oregon State University and Marcelo Zak for critically reading the manuscript. A. B. is grateful to FOMEC and CONICET for the fellowship provided, E. N. holds a postdoctoral position from CONICET.

References

Aceñolaza PG (1995) Estructura y Dinámica de bosques de aliso (*Alnus acuminata* HBK subsp. *acuminata*) de la Provincia de Tucumán. PhD thesis. Facultad de Ciencias Naturales e Instituto Miguel Lillo, Universidad Nacional de Tucumán, Tucumán

Agerer R (1986) Studies on ectomycrrhizae. III. Mycorrhizae formed by four fungi in the genera *Lactarius* and *Russula* on spruce. Mycotaxon 27:1–59

Agerer R (1991) Characterization of ectomycorrhiza. In: Norris JR, Read DJ, Varma AK (eds) Techniques for the study of mycorrhiza. (Methods in microbiology, vol 23) Academic Press, London, pp 25–73

Agerer R (1995) Anatomical characteristics of identified ectomycorrhizas: an attempt towards a natural classification. In: Varma AK, Hock B (eds) Mycorrhiza. Structure, function, molecular biology and biotechnology. Springer, Berlin Heidelberg New York, pp 605–734

Agerer R (1999a) Anatomical characteristics of identified ectomycorrhizas: an attempt towards a natural classification. In: Varma AK, Hock B (eds) Mycorrhiza. Structure, function, molecular biology and biotechnology, 2nd edn. Springer, Berlin Heidelberg New York, pp 633–682

Agerer R (1999b) *Elaphomyces aculeatus* Tul.. + *Quercus robur* L. Descr Ectomycorrhiza 4:37–41

Agerer R (1999c) *Rozites caperatus* (Pers.:Fr) Karst. + *Pinus sylvestris* L. Descr Ectomyc 4:109–113

Agerer R, Beenken L (2001) *Descomyces albus* (Klotzsch) Bougher & Castellano + *Eucalyptus* spec. Descr Ectomyc 5:41–47

Agerer R, Waller K, Treu R (1993) Die Ektomykorrhizen und sklerotien von *Gyrodon lividus*. Z Mykol 59:131–140

Baldwin QF, Egger KN (1996) Protocols for analysis of DNA from mycorrhizal roots. In: Goodman DM, Durall DM, Trofymow JA, Berch SM (eds) Concise descriptions of North American ectomycorrhizae, forest resource development agreement. Mycologue Publications, Canadian Forest Service, Victoria, BC, pp 3C.1–3C.2

Becerra A, Daniele G, Domínguez L, Nouhra E, Horton T (2002) Ectomycorrhizae between *Alnus acuminata* H. B. K. and *Naucoria escharoides* (Fr.:Fr.) Kummer from Argentina. Mycorrhiza 12:61–66

Brundrett M, Bougher N, Dell B, Grove T, Malajczuk N (1996) Working with mycorrhizas in forestry and agriculture. ACIAR, Canberra, Australia, 374 pp

Brunner IL, Brunner F, Miller OK Jr (1990) Ectomycorrhizal synthesis with Alaskan *Alnus tenuifolia*. Can J Bot 68:761–767

Dawson JO (1990) Interactions among actinorhizal and associated plant species. In: Schwintzer CR, Tjepkema JD (eds) The biology of *Frankia* and actinorhizal plants. Academic Press, San Diego, Calif., pp 299–316

Eberhardt U, Walter L, Kottke I (1999) Molecular and morphological discrimination between *Tylospora fibrillose* and *Tylospora asterophora* mycorrhizae. Can J Bot 77:11–21

Egger KN (1995) Molecular analysis of ectomycorrhizal fungal communities. Can J Bot 73:S1415-S1422

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes: application to the identification of mycorrhizae and rusts. Mol Ecol 2:113–118

Gardes M, Bruns TD (1996) ITS-RFLP matching for identification of fungi. Methods Mol Biol 50:177–186

Gardner IC, Barrueco CR (1999) Mycorrhizal and actinorhizal biotechnology-problems and prospects. In: Varma AK, Hock B (eds) Mycorrhiza: structure, function, molecular biology and biotechnology, 2nd edn. Springer, Berlin Heidelberg New York, pp 469–495

Godbout C, Fortin JA (1983) Morphological features of synthesized ectomycorrhizae of *Alnus* crispa and Alnus rugosa. New Phytol 94:249–262

Godbout C, Fortin JA (1985) Synthesized ectomycorrhizae of aspen: fungal genus level of structural characterization. Can J Bot 63:601–603

Hayward D, Thiers HD (1984) *Gyrodon lividus* in California. Mycologia 76:573–575

Holmgren PK, Holmgren NH, Barnett LC (1990) Index Herbariorum. I. The herbaria of the world. Regnum Veg 120:1–693

Hook L (2002) Theory of operation for gene profiler and ONE-Dscan molecular weight analysis. Scanalytics, Fairfax, VA

Horak VE (1963) Pilzökologische Untersuchungen in den subalpinen Stufen (*Picetum subalpinen* and *Rhodoreto vaccinietum*) der rätischen Alpen. Mitte Schweiz Anst Forstl Versuchswes 39:1–112

Horak E (1985) Die Pilsflora (Macromyceten) und ihre Ökologie in fünf Pflanzengesellshaften der montan-subalpinen Stufe des Unterengadins (Schweiz). Ergeb Wiss Unters Schweiz Nationalpark 12:337–476

Horton TR, Bruns TD (2001) The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. Mol Ecol 10:1855–1877

Mah K, Tackaberry L, Egger KN, Massicotte HB (2001) The impacts of broadcast burning after clear-cutting on the diversity of ectomycorrhizal fungi associated with hybrid spruce seedlings in central British Columbia. Can J For Res 31:224–235

Masui K (1926) A study of the ectotrophic mycorrhiza of *Alnus*. Mem Coll Sci Kyoto Imp Univ Ser B 2:189–209

Miller SL, Koo CD, Molina R (1987) Characterization of red alder ectomycorrhizae. In: Sylvia DM, Hung LL, Graham JH (eds) Mycorrhizae in the next decade. University of Florida, Gainsville, Fla.

Miller SL, Koo CD, Molina R (1991) Characterization of red alder ectomycorrhizae: a preface to monitoring belowground ecological responses. Can J Bot 69:516–531

Molina R (1979) Pure culture synthesis and host specificity of red alder mycorrhizae. Can J Bot 57:1223–1228

Molina R (1981) Ectomycorrhizal specificity in the genus *Alnus*. Can J Bot 59:325–334

Moser M (1978) Keys to agarics and boleti.

Moser M (2001) Some aspects of *Cortinarius* associated with *Alnus*. J J E C 3:47–101

Pritsch K, Buscot F (1994) Biodiversity of ectomycorrhizas from morphotypes to species. In: Azcon Aguilar C, Barea JM (eds) Proceedings of the fourth European Symposium On Mycorrhizas. Mycorrhizas in integrated systems from genes to plant development. pp 9–14

Pritsch K, Boyle H, Munch JC, Buscot F (1997a) Characterization and identification of black alder ectomycorrhizas by PCR/

RFLP analyses of the rDNA internal transcribed spacer (ITS). New Phytol 137:357–369

Pritsch K, Munch JC, Buscot F (1997b) Morphological and anatomical characterisation of black alder *Alnus glutinosa* (L.) Gaertn. ectomycorrhizas. Mycorrhiza 7:201–216

Sakakibara SM, Jones MD, Gillespie M, Hagerman SM, Forrest ME, Simard SW, Durall DM (2002) A comparison of ectomycorrhiza identification based on morphotyping and PCR-RFLP analysis. Mycol Res 106:868–878

Schmid-Heckel H (1985) Zur Kenntnis der Pilze in den Nördlichen Kalkapen. Nationalpark Berchtesgaden, Forschungsberichtung 8:1–201

Singer R, Digilio APL (1957) Las Boletaceas Austrosudamericanas. Lilloa 28:247–268

Singer R, Digilio APL (1960) Las Boletaceas de Sudamérica tropical. Lilloa 30:141–165

Singer R, Gómez LD (1984) The basidiomycetes of Costa Rica. III. The genus *Phylloporus* (*Boletaceae*). Brenesia 22:163–181

Singer R, Morello JH (1960) Ectotrophic forest tree mycorrhizae and forest communities. Ecology 41:549–551

Stangl J (1970) Das Pilzwachstum in alluvialen Schotterebenen und seine Abhängigkeit von Vegetationgesellschaften. Z Pilzkd 31:209–255

Trappe JM (1962) Fungus associates of ectotrophic mycorrhizae. Bot Rev 28:538–606