

Mycorrhizal associations in *Polylepis* woodlands of Central Argentina

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Abstract: *Polylepis* woodlands are one of the most threatened mountain ecosystems of South America, and their restoration is a high priority. To assess the mycorrhizal status in *Polylepis* woodlands of “Quebrada del Condorito” National Park (Córdoba Mountains, Central Argentina), we examined the roots of 22 plant species, belonging to 14 families and determined morphological types of arbuscular mycorrhiza (*Arum* and *Paris* type) and colonization level. The 22 species were colonized by arbuscular mycorrhizal fungi and dark septate endophytes. Different arbuscular mycorrhizal structures and colonization patterns were observed, although *Paris*-type colonization was predominant. Fourteen plant species are reported for the first time as hosts of arbuscular mycorrhizal fungi. We conclude that arbuscular mycorrhizal fungi and dark septate endophyte hosts are widespread in the *Polylepis* woodlands of Central Argentina and should receive special attention in future revegetation programs.

Key words: *Polylepis australis*, arbuscular mycorrhizal, dark septate fungi, ericoid mycorrhizae, *Arum* type, *Paris* type.

Résumé : Les forêts de *Polylepis* des montagnes sud-américaines sont parmi les écosystèmes forestiers les plus menacés du monde. Pour déterminer le statut mycorrhizien des bois de *Polylepis* du Parc National « Quebrada del Condorito » (situé dans les montagnes de Córdoba, Argentine), 22 espèces de plantes, appartenant à 14 familles, ont été examinées. Les types morphologiques des mycorrhizes arbusculaires (type paris et type arum) et le degré de colonisation ont été déterminés en examinant les échantillons des racines. Toutes les espèces de plantes ont présenté une colonisation des mycorrhizes arbusculaires et des endophytes foncés à septations. Des différentes structures et patrons de colonisation mycorrhiziens ont été observés, cependant la colonisation type-paris a été prédominante. Quatorze espèces de plantes sont reportées comme hôtes des champignons mycorrhiziens à arbuscules pour la première fois. Les résultats montrent que les champignons mycorrhiziens arbusculaires et les endophytes foncés à septations sont les hôtes prédominants des bois du *Polylepis* de l'Argentine Centrale.

Mots-clés : *Polylepis australis*, mycorrhizes arbusculaires, endophytes foncés à septations, mycorrhizes éricoïdes, type arum, type -paris.

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Introduction

Man has reduced and fragmented *Polylepis* (Rosaceae) mountain woodlands endemic to tropical and subtropical South America through burning, cutting, and browsing by livestock. These woodlands are one of the most threatened mountain ecosystems of South America, and their conserva-

tion and restoration is a high priority (Fjeldså and Kessler 1996). *Polylepis australis* Bitt. (“tabaquillo”) is the species with the southernmost distribution (Simpson 1979) and is currently being used to revegetate degraded areas, as it is especially useful because of its capacity to retain water and reduce soil erosion (Renison et al. 2005). Arbuscular mycorrhizae (hereinafter AM) have been found in *Polylepis besseri* Hieron. of the Andes of Bolivia (Hensen 1994) suggesting other *Polylepis* species including *P. australis* and their associated plant communities may also be associated with AM, but this has not been investigated.

The roots of most plant species are symbiotically associated with AM fungi. These associations are important in nutrient uptake, influencing the establishment and competitive capacity of plants (Moora and Zobel 1996; Smith and Read 1997; Callaway et al. 2001; Hart et al. 2003), and thus influencing community composition and ecosystem functioning (van der Heijden et al. 1998; van der Heijden 2002; Johnson et al. 2003). Roots are also frequently colonized by a diverse

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group of fungi called dark septate endophytes (DSE) (Jumpponen and Trappe 1998). The function of DSE is controversial, as they have been found to be pathogenic to mutualistic symbionts (Jumpponen and Trappe 1998; Jumpponen 2001).

Plant mycorrhizal status may vary on the basis of soil and climatic characteristics and mycorrhizal status of the dominant species; it is thus important to determine presence of mycorrhizae on the basis of these characteristics (Dhillon et al. 1995; Smith and Read 1997; Fontenla et al. 2001). We studied the mycorrhizal status of the most abundant plant species in a *P. australis* woodland of Central Argentina (recently protected by National Parks' legislation) to improve our knowledge on the ecology of *Polylepis* woodlands and the mycorrhizal biology and diversity of the native plants for future revegetation programs.

Materials and methods

Study area

The study was carried out in the higher Córdoba Mountain range (Central Argentina, north–south direction, rising up to 2884 m a.s.l., 31°34'S, 64°50'W). Mean temperature of the coldest and warmest months are 5.0 and 11.4 °C, respectively, with no frost-free period. Mean annual precipitation is 854 mm, with 83% of the rainfall concentrated in the warmer months, between October and April (Renison et al. 2002). We selected our study area in the headwaters of “La Ciénaga” stream (31°37'S; 64°49'W; 2190 m a.s.l.) in “Quebrada del Condorito” National Park (Córdoba, Argentina). Soil physicochemical characteristics are presented in Table 1. The area is a mixture of grazing lawns and tall tussock grasslands, interrupted by granite rock outcrops, areas with rock exposed by soil erosion, and *P. australis* individuals which are isolated or in groups of two to five individuals and with a total estimated cover of less than 5% of the area (Cingolani et al. 2004). The low vegetation cover is due to the area being traditionally used for livestock rearing until the creation of the National Park in 1997 when stocking rates were greatly reduced.

Field sampling

Root samples from *P. australis* individuals and most common associated plant species were collected during the autumn of 2003. In herbs, grasses, and ferns, the whole root system of five individuals was sampled. In trees and shrubs with deep root systems, root samples of five individuals were collected, the root systems were carefully excavated around each selected plant to confirm connection between roots and shoots. The samples were placed in plastic bags and stored at 4 °C.

Mycorrhizal analysis

Root samples were washed to remove soil and adhering organic particles. Roots that became detached from the main root system were assigned to their corresponding species by careful examination of morphological characteristics. The mycorrhizal analysis of each plant species was determined from fine-root samples of five individuals. The root system of each plant was preserved with FAA (formalin –

Table 1. Soil properties (mean ± SE values of 10 samples) of our study site (La Ciénaga, Quebrada del Condorito National Park).

Parameter *	Mean ± SE
Texture	Sandy loam
Soil depth (cm)	42.51±21.71
Soil water content (%)	39.18±17.94
Available P (ppm)	16.3±13.5
Organic matter (%)	2.53±0.97
Total C (%)	1.27±0.49
Total N (%)	0.11±0.04
C/N	11.39±0.83
Ca ²⁺ (cmolc/kg of soil)	10.91±3.03
Mg ²⁺ (cmolc/kg)	1.48±0.69
Na ⁺ (cmolc/kg)	0.83±0.72
K ⁺ (cmolc/kg)	1.13±0.76
Electric conductivity (dS/m)	0.2±0.09
pH 1: 2.5 (in H ₂ O)	4.93±0.31

Note: cmolc, centimols of charge.

*The analyses were as follows: Ca-lactate extract (pH 3.6) for available phosphorus; Dumas method for total C and N; electric conductivity and pH in 1:2.5 suspension of soil in water; cations after equilibrium of soil in 0.1 N NH₄Cl; percent water by weight for soil water content.

acetic acid – ethanol) and later cleared and stained for observation (Grace and Stribley 1991). Roots were cleared with 10% KOH (15 min at 90 °C). Dark roots were further bleached with 30% H₂O₂ (5 min, room temperature). The roots were then acidified with 1% HCl (1 min, room temperature) and stained in 0.05% aniline blue. Each individual root system (whole root system in herbs, grasses, and ferns and at least 30 cm of root in trees and shrubs) was first observed under a Leica M 420 microscope. To confirm mycorrhizal structures, we then mounted the roots on slides for examination under a Kyowa 4–100× microscopic lens. The presence of arbuscules, vesicles, hyphal coils, and intracellular and intercellular hyphae without septa was used to designate AM associations. The presence of intracellular dense hyphal complexes in the epidermal cells was used as a positive criterion for ericoid mycorrhizae (ER) (Smith and Read 1997). The roots colonized by DSE were characterized by melanized, septate hyphae, usually forming microsclerotia within root cells (Jumpponen 2001). The species that did not show any of the seven types of known mycorrhizal associations were considered non mycorrhizal (Smith and Read 1997).

We also determined the AM morphological type of the study species, *Arum* and *Paris* type (Smith and Smith 1997). *Arum* type: intercellular hyphae, intercellular or intracellular vesicles, and terminal arbuscules on intracellular hyphal branches. Low colonization by hyphal coils may be observed. *Paris* type: intracellular hyphae, intracellular vesicles, and hyphal coils. Quantification of AM root colonization was estimated visually as the proportion of the root that was colonized and characterized using five classes: class I, 1%–5%; class II, 6%–25%; class III, 26%–50%; class IV, 51%–75%; and class V, 76%–100% (Kormanik and McGraw 1982).

Plant species identification, functional type, and origin follow Zuloaga et al. (1994) and Zuloaga and Morrone (1996, 1999a, 1999b).

Table 2. Mycorrhizal status of the main plants associated with a *Polylepis* woodland in “La Ciénaga” (Quebrada del Condorito National Park).

Family	Species	FT	PC	CT	AM %
Apiaceae	<i>Eryngium agavifolium</i> Griseb.	PH	iah, arc, iav, c, ep	<i>Paris</i>	II
	<i>Oreomyrrhis andicola</i> (Kunth) Hook. f.	PH	ieh, iav, iev c, ep	<i>Arum</i>	III
Asteraceae	<i>Achyrocline satureioides</i> (Lam.) DC.	S	iah, iav, c, ep	<i>Paris</i>	II
	<i>Gamochaeta americana</i> (Mill.) Wedd	PH	iah, iav, c, ep	<i>Paris</i>	IV
	<i>Hypochaeris radicata</i> L.	PH	iah, iav, ep	<i>Paris</i>	III
Berberidaceae	<i>Berberis hieronymi</i> C.K. Schneid.	S	iah, iav, c, ep	<i>Paris</i>	II
Blechnaceae	<i>Blechnum penna-marina</i> (Poir.) Kuhn	F	iah, arc, iav, c, ep	<i>Paris</i>	III
Cyperaceae	<i>Carex fuscua</i> d’Urv.	PH	iah, ep	*	II
Dryopteridaceae	<i>Polystichum montevidense</i> (Spreng.) Rosenst.	F	iah, iav, ep	<i>Paris</i>	III
Ericaceae	<i>Gaultheria poeppigii</i> D.C.	S	iah, iav, ep	<i>Paris</i>	V
Gentianaceae	<i>Gentianella achalensis</i> (Hieron.)T.N. Ho & S.W. Liu	AH	iah, ieh, arc, c, ep	<i>Paris</i>	III
Lamiaceae	<i>Lepechinia meyenii</i> (Walp.) Epling	PH	iah, ieh, c, ep	<i>Paris–Arum</i>	II
	<i>Satureja odora</i> (Griseb.) Epling	S	iah, ieh, iav, iev, ep	<i>Arum–Paris</i>	II
Lycopodiaceae	<i>Huperzia saururus</i> (Lam.) Trevis	F	ieh, ar, iev, c, ep	<i>Arum</i>	III
Poaceae	<i>Deyeuxia hieronymi</i> (Hack.) Türpe	G	iah, iav, iev, c, ep	<i>Paris–Arum</i>	IV
	<i>Festuca tucumanica</i> E.B. Alexeev	G	iah, iav, c, ep	<i>Paris</i>	IV
	<i>Poa stuckertii</i> (Hack.) Parodi	G	iah, ieh, iav, c, ep	<i>Paris</i>	V
Polypodiaceae	<i>Polypodium bryopodum</i> Maxon	F	iah, iav, c, ep	<i>Paris</i>	III
Rosaceae	<i>Alchemilla pinnata</i> Ruiz & Pav.	PH	ieh, iev, c, ep	<i>Arum</i>	III
	<i>Duchesnea indica</i> (Andrews) Focke	PH	ieh, iev, c, ep	<i>Arum</i>	IV
	<i>Polylepis australis</i> Bitt.	S/A	ieh, ar, iev, c, ep	<i>Arum</i>	V
Scrophulariaceae	<i>Bartsia crenoloba</i> Wedd.	PH	iah, ieh, ar, iav, iev, ep	<i>Arum–Paris</i>	III

Note: Indicated are the family, functional type (FT): A, arboreal; AH, annual herbaceous; PH, perennial herbaceous; G, grass; F, ferns; S, shrub. Patterns of AM colonization (PC): iah, intracellular aseptate hyphae; ieh, intercellular aseptate hyphae; ar, arbuscules; arc, arbuscules coils; iav, intracellular vesicles; iev, intercellular vesicle; c, coils; ep, entry point. AM colonization type (CT): *Arum* type, *Paris* type. Arbuscular mycorrhizal (AM) colonization (AM %), class: I, 1%–5%; II, 6%–25%; III, 26%–50%; IV, 51%–75%; V, 76%–100%.

*The pattern could not be defined.

Results

We determined the mycorrhizal status of 22 plant species from 14 families and six functional types (Table 2). Nine species were perennial herbs, 1 was an annual herb, 4 were shrubs, 1 was a tree, 3 were grasses, and 4 were ferns. Except for *Hypochaeris radicata* L. and *Duchesnea indica* (Andrews) Focke, all other plants were native to the area. All species had AM colonization and DSE, except *Polystichum montevidense* (Spreng.) Rosenst., which had only AM. *Gaultheria poeppigii* D.C. was also colonized by ericoid mycorrhiza.

Arbuscular mycorrhiza colonization was dominated by hyphae and vesicles, 22 and 19 species, respectively (Table 2). Vesicle shapes ranged from spherical and intracellular (Fig. 1A), to oval and intercellular (Fig. 1B); irregular shaped vesicles were also observed. Coils were found in 16 species with variable abundance, including *Polypodium bryopodum* (Fig. 1C) and *Berberis hieronymi* where coils were very abundant. Entry points were found in all 22 species (Fig. 1D). Only 6 species had arbuscules (Table 2). Typical arbuscules of *Arum* type were observed in *Polylepis australis* (Fig. 1E) and *Huperzia saururus*; whereas arbusculate coils typical of the *Paris* type were observed in *Gentianella achalensis* (Fig. 1F), *Blechnum penna-marina*, and *Eryngium agavifolium*.

DSE were observed in most root samples colonized by AM fungi. Melanized loose hyphae grew along the root surface of all species where DSE colonization was observed (Fig. 1G). The thickened, melanized hyphae formed a mass

of densely packed, dark hyphae filling epidermal and outer cortical cells. These structures were typical of what has been described as microsclerotia (Fig. 1H).

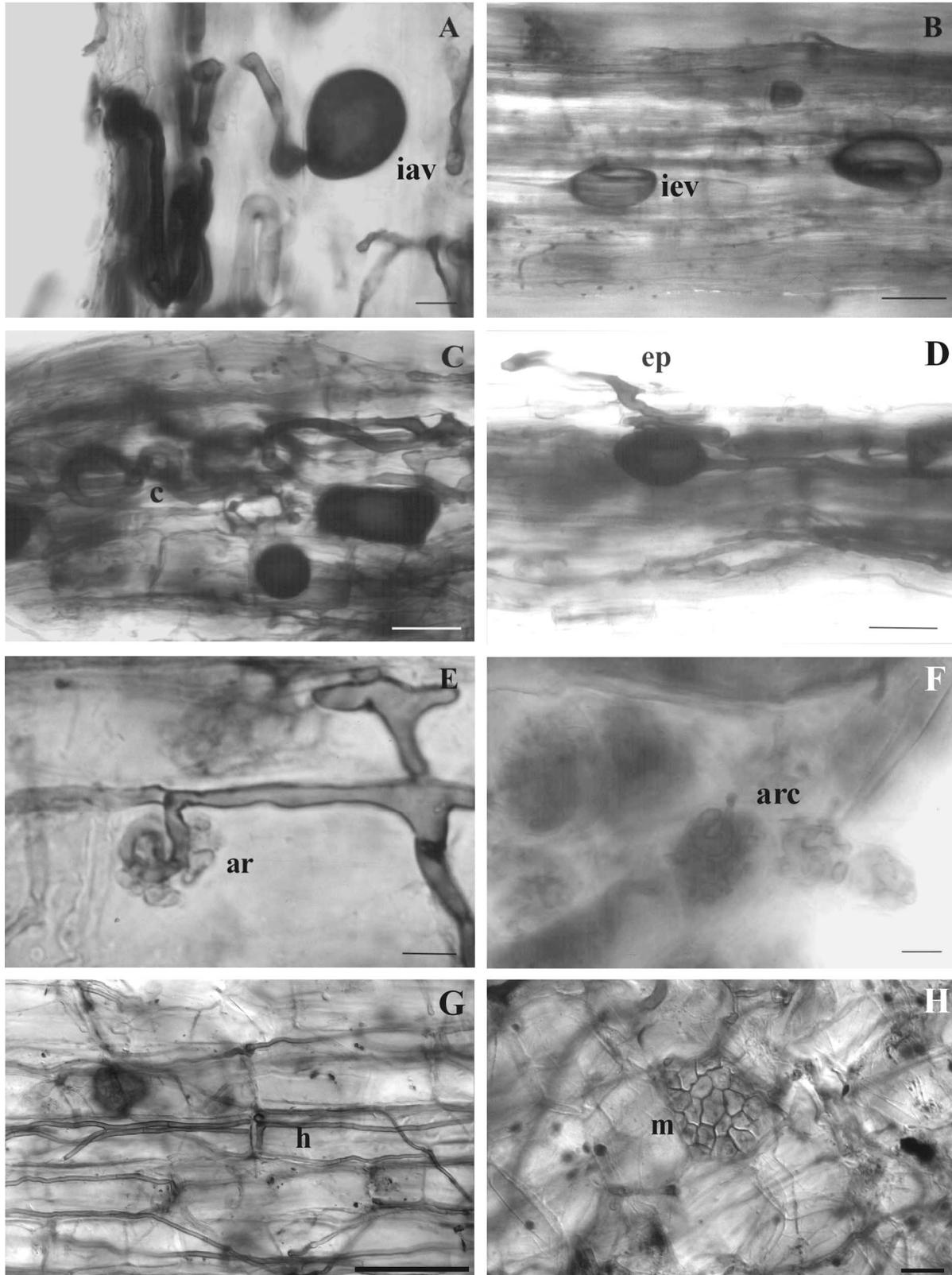
The morphology of AM was found to be *Arum* and *Paris* type. The *Arum* type was found in 5 species belonging to 3 families, in contrast, the *Paris* type was found in 12 species belonging to 8 families. The intermediate type defined as *Arum–Paris* or *Paris–Arum* according to predominant structures was found in 4 species belonging to 3 families. Only in *Carex fuscua* could the pattern not be defined, because we only observed inter and intracellular hyphal colonization (Table 2).

Arbuscular mycorrhizal colonization varied among the species, although most species had colonization levels from 26% to 50%. None of the species had low colonization values (1%–5%), and only 3 species always had more than 75% of the roots colonized by AM (Table 2). We were unable to find a pattern in the percentage of colonization per functional type. Colonization in herbaceous plants varied from 6% to 75% and in shrubs from 6% to 100%. Ferns and grasses had high and less variable colonization rates, between 26%–50% and 51%–75%, respectively (Table 2).

Discussion

Our results showed the predominance of plants colonized by AM fungi in sparse *Polylepis* woodlands of Central Argentina. These results are in agreement with observations in other forest ecosystems (i.e., Godoy et al. 1994; Andrade et al. 2000; Onguene and Kuyper 2001; Wubet et al. 2003) and are consistent with the assumption that AM associations are

Fig. 1. Patterns of arbuscular mycorrhizal (AM) fungi and dark septate endophyte (DSE) root colonization of native plants in *Polylepis* woodlands, Central Argentina: (A) intracellular vesicle (iav) in *Oreomyrrhis andicola*; (B) intercellular vesicles (iev) in *Polylepis australis*; (C) Coils (c) in *Polypodium bryopodum*; (D) entry points (ep) in *Gamochaeta americana*; (E) arbuscules (ar) in *Polylepis australis*; (F) arbusculate coils (arc) in *Gentianella achalensis*; (G) melanized hyphae (h) of DSE fungi present within epidermal cells in *Berberis hieronymi*; (H) microsclerotium (m) in an outer cortical cell in *Eryngium agavifolium*. Scale bars in A, F and H = 10 μ m; in B and G = 50 μ m; in C and D = 15.6 μ m; and in E = 5 μ m.



the most widespread mycorrhizae in the plant kingdom (Smith and Read 1997; Cairney 2000).

The mycorrhizal status of *Eryngium agavifolium*, *Oreomyrrhis andicola*, *Achyrocline satureioides*, *Gamochaeta americana*, *Berberis hieronymi*, *Polystichum montevidense*, *Gentianella achalensis*, *Lepechinia meyenii*, *Satureja odora*, *Huperzia saururus*, *Festuca tucumanica*, *Polypodium bryopodum*, *Polylepis australis*, and *Bartsia crenoloba* are reported for the first time for this forest ecosystem. The remaining plant species had been previously reported as associated with AM (Rothwell and Vogel 1982; Godoy et al. 1994; Fontenla et al. 2001; Lugo et al. 2003).

The role of DSE in the ecosystem is not clearly understood. These fungi are abundant in soils and colonize a broad range of families, but the relationship between host plant and DSE range from mutualistic to parasitic depending on the species and growing conditions of the host plants (Haselwandter and Read 1982; Jumpponen and Trappe 1998; Jumpponen 2001). In our study, DSE were present in most root systems, along with AM fungi (Table 2). This co-occurrence has also been observed in a diversity of plant species (Jumpponen and Trappe 1998; Cazares et al. 2005).

Ericoid (ER) mycorrhizae were observed in *Gaultheria poeppigii*, which belongs to the family Ericaceae. This species, also reported by Urcelay (2002), showed a tripartite association (AM–DSE–ER). No other mycorrhizal types were observed in any of the plants, which is consistent with what has been found in similar communities (Allen 1991; Fontenla et al. 2001; Hartnett and Wilson 2002).

In agreement with other studies, species belonging to the same family tended to have the same mycorrhizal behaviour and colonization type (Read and Haselwandter 1981; Harley and Harley 1987; Newman and Reddell 1987; Gemma et al. 1992; Hildebrand-Vogel et al. 1992; Koske et al. 1992; Smith and Smith 1997; Zhao 2000). Several exceptions to this general behaviour were observed. For instance, the *Paris*-type colonization in Asteraceae and the presence of *Arum*- and *Paris*-type colonization in Apiaceae (Table 2) are not in agreement with the reports of Smith and Smith (1997). These authors did not find intermediate types among both colonization types, as we observed in *Lepechinia meyenii*, *Satureja odora*, *Bartsia crenoloba*, and *Deyeuxia hieronymi*. In this study, we observed a dominance of *Paris*-type AM colonization among herbs, shrubs, grasses, and ferns. Similar results were found in other natural ecosystems (Smith and Smith 1997; Yamato and Iwasaki 2002). However, it is important to emphasize that AM morphology is dependent upon individual plant species, the fungi colonizing it, and environmental factors such as soil and nutrient conditions. As Dickson (2004) stated, the *Arum* and *Paris* type are the morphological extremes of a continuum. Thus, exceptions to the general rules are expected.

Regarding the extent of AM colonization, the reported variations may be due to the different plant species that exhibit diverse abilities to establish mycorrhizal associations (Smith and Read 1997). Nevertheless, the colonization values were considerably higher than most reported (Read and Haselwandter 1981; Dhillon et al. 1995; Lugo et al. 2003).

These results must be considered preliminary, since samples were obtained only in sparse woodlands, and they cover only a small proportion of the plant diversity of these for-

ests. Nevertheless, our study woodlands should be good reference sites, as these trees presented relatively good seed viability (Renison et al. 2004) and in situ regeneration (personal observations). Furthermore, this is the first report published on the mycorrhizal status of some of the species examined. Considering the predominance of AM associations in *P. australis* woodlands and the potential benefits that these associations may bring to plant establishment, AM should receive special attention in management, conservation, and reforestation of these woodlands.

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