Arbuscular mycorrhizal fungi in El Palmar National Park (Entre Rios Province, Argentina) – a protected reserve

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Velázquez M. S., Biganzoli F. & Cabello M. N. (200x) Arbuscular mycorrhizal fungi in El Palmar National Park (Entre Rios Province, Argentina) – a protected reserve – Sydowia XX (X): pp–pp.

There is considerable interest in documenting the biodiversity of plants together with the associated microorganisms. We investigated the species composition and colonisation patterns of arbuscular mycorrhizal fungi (AMF) in five vegetation types in El Palmar National Park, Entre Rios, Argentina. The plant communities consisted of: gallery forest, grassland, marsh, palm forest and scrubland. The roots of 103 plant species of 42 families were examined for AMF colonisation. Ninety-three plant species were found to be colonized. *Arum*-type mycorrhizal colonisation occurred in 74 species, *Paris*-type in three species, three species had a mix of both *Paris*- and *Arum*-type. It was not possible to determine the colonisation type for 13 plant species. A total of 46 taxa of AMF, including the genera *Glomus* (15 taxa), *Acaulospora* (14 taxa), *Scutellospora* (8 taxa), *Gigaspora* (5 taxa), *Archaeospora* (1 taxon), *Entrophospora* (1 taxon), *Pacispora* (1 taxon) and *Paraglomus* (1 taxon), were identified. A higher diversity was observed in the grassland and palm forest, mainly due to a high proportion of Acaulosporaceae and Gigasporaceae. El Palmar National Park contains a well-established, highly diverse, native community of AMF. Consequently, the park may act as a place for *in situ* conservation of Glomeromycota.

Keywords: arbuscular mycorrhizal fungi, *Arum*-type, *Paris*-type, protected areas.

Mycorrhizas constitute the absorbing root systems of approximately 82% of land plants (Wang & Qiu 2006) and are a key component in the maintenance of plant diversity in natural ecosystems (Read 1991). Their ubiquity alone makes them an important component of soil microbial biomass, and they are directly involved in crucial processes at the plant-soil interface as well as in plant community structure and productivity (Grime *et al.* 1987, Klironomos *et al.* 2000).

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Studies on the mycorrhizal status of plants growing in undisturbed areas are fundamental for improving the knowledge of mycorrhizal biology and diversity (Allen 1991) as well as for establishing revegetation programs (Johnston & Ryan 2000). Consequently, the undisturbed area within El Palmar National Park represents an ideal sampling area for this type of study because, with over 700 vascular plant species it is one of the floristically most diverse national parks in Argentina (Biganzoli *et al*. 2001). The park includes one of the remaining populations of the palm *Butia yatay* (Mart.) Becc., in the region. According to the article N° 8 from the Convention on Biological Diversity, concluded at Río de Janeiro on 5 de June 1992 (www.cbd.int 2009), El Palmar National Park can serve, as an important place to conserve plant biodiversity together with the associated microorganisms.

Within the 10 families and 14 genera of the phylum Glomeromycota (Schüssler 2009), two AM types, *Arum* and *Paris*, have been identified based on morphological characteristics (Smith & Smith 1996, 1997). The *Arum*-type is defined on the basis of an extensive intercellular phase of hyphal growth in the root cortex and development of terminal arbuscules on intracellular hyphal branches; the *Paris*-type is defined by the absence of the intercellular phase, the presence of extensive intracellular hyphal coils, and arbuscules as intercalary structures on the coils.

The mycorrhizal status of plants in El Palmar National Park has been unknown up to present. The objectives of the present study were: i) to survey the distribution of *Arum*- and *Paris*-type AMs in this protected area; and ii) to identify the species of AMF from spores within soil samples.

Materials and Methods

Study area

The study area is located at El Palmar National Park, Entre Ríos province, Argentina (31º 50’ S, 58º 17’ W). The climate is temperate with a mean annual temperature of 18.9 °C. Mean annual rainfall is about 1300 mm and water deficit occurs frequently in the summer time (Goveto 2005).

The Entisoles and Inceptisoles soils, which possess a very limited sequence of horizons and a scarce manifestation of pedogenetic processes, are the predominant ones in the park. The materials that constitute the base upon which these different soils have been generated are from fluvial origin (van der Sluijs 1971, Bertolini 1995).

The landscape of the park is conformed by a mosaic of vegetation types, including forests along rivers and streams, tall grassland on humid alluvial plains, xeric steppes on sandy outcrops, and scrublands and palm savannas dominated by *B. yatay* on the uplands (Movia & Menvielle 1994). Based on physiographic and floristic characters, we identified five distinctive vegetation types at El Palmar National Park: gallery forest (GF), grassland (GRA), marsh (MAR), palm forest (PF), and scrubland (SCR). The sampling design consisted of three replicate of each vegetation type. Within each site (5 vegetation types x 3 replicates), three representative soil and root samples were collected (45 samples) and stored in zip-lock bags for transport to the laboratory, where they were stored in a refrigerator at 4 C until processed. Specimens of at least three plant species per site were collected for analysis.

Root samples

One hundred and three plant species were selected and their abundance at each vegetation type was estimated. Plants were collected during flowering and taxonomically identified (www.darwin.edu.ar). Roots were cleared and stained according to Phillips & Hayman (1970). Ten root segments taken from composite root samples were examined for each plant species from a vegetation type. Designation of *Arum*-type or *Paris*-type was made according to the description given by Smith & Smith (1997).

AMF spore diversity

AMF were studied by spore extraction from soil samples. 100 g soil (dry weight) of each sample was wet-sieved and decanted according to Gerdemann & Nicolson (1963) and the modified sucrose density gradient centrifugation method of Walker *et al.* (1982).

For taxonomic identification, fungal spores were mounted in either polyvinyl-lactic acid-glycerine (PVLG) (Koske & Tessier 1983) or PVLG mixed 1:1 (vol/vol) using Melzer's reagent (Brundrett *et al.* 1994) and examined using Leitz Dialux 20EB light microscope. Specimens were compared with voucher material (Germplasm Bank of the Institute Spegazzini, La Plata, Argentina, International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (INVAM, USA, http://invam.cafwvu.edu), Blaszkowski (http://agro.ar.szczecin.pl/~jbłaszkowski/)). Vouchers were deposited in the Herbarium at the Spegazzini Institute (LPS), La Plata, Argentina.

Results

AM colonisation

A total of 103 plant species (42 families and 69 genera) were assessed for AM colonization. Eighty-eight of the 103 species were native to the Park and 15 were exotic (Fig. 1). Plant species examined are given in Figure 1 together with their taxonomic position according to the Angiosperm Phylogeny Group Classification (APG II 2003), their habitats, and the associated AM types. Species with AM accounted for

88,5% of the total number of species sampled. The percentage of plants colonised by AMF was high in the palm forest (96%), grassland (94%), scrubland (91%) and marsh (90%) habitats, with a lower level (70%) in the gallery forest.

Characteristic morphological structures observed in colonised plants were appressoria, arbuscules, coils, auxiliary cells, vesicles and external and internal hyphae depending on the plant species (Figs. 2 A-H). Seventy-four plant species belonging to different families (Fig. 1) were colonized by *Arum*-type AMF (Fig. 2 B). This colonisation type was dominant in the vegetation from the park and was associated with both terrestrial plant species and marsh plants that grow in soils subject to flooding. The mycorrhizal colonisation of *Mutisia coccinea*, *Prunus persica* and *Daphnopsis racemosa* (Fig. 2 C) exhibited the typical *Paris*-type coils, with arbuscules that were always simple and terminal. The *Paris*-type was recorded in terrestrial plants only. In *Commelina diffusa, C. erecta* and *Heimia salicifolia*, three terrestrial species, an intermediate *Arum-Paris*-type colonisation was found. It was not possible to differentiate between the *Paris*-type or the *Arum*-type for thirteen species of terrestrial and marshy habits. Ten species without AMF colonisation were recorded from terrestrial, marshy, aquatic, and epiphytic habits (see Fig. 1).

Fungal communities

Considering the five vegetation types studied at El Palmar National Park, 46 AM fungal taxa belonging to the genera *Acaulospora* (14 species), *Archaeospora* (1 species), *Entrophospora* (1 species), *Gigaspora* (5 species), *Glomus* (15 species), *Scutellospora* (8 species), *Pacispora* (1 species) and *Paraglomus* (1 species) were identified (Tab. 1). The highest diversity was observed in the families Glomeraceae and Acaulosporaceae (33% and 30% respectively) followed by Gigasporaceae (28%).

Thirty two species of Glomeromycota were counted in GRA, 30 species in PF, 26 species in SCR, 25 species in MAR, and 23 species in GF.

Discussion

One hundred and three plant species belonging to 42 families were analyzed for AM colonisation. About eighty-eight per cent were colonized by AMF which is in agreement with the findings of Wang & Qiu (2006).

The *Arum*-type was dominant in all plant species analyzed from the El Palmar National Park. This type has generally been regarded as the most common AMF morphology in natural communities (Brundrett & Kendrick 1990*a*, 1990*b*). A relationship between AMF morphology and the ecology of the plant has been previously suggested. O'Connor

Order	Family	Species	Habits	AM type
Polypodiales	Polypodiaceae	*Microgramma vacinifolia*	E	-
	Pteridaceae	*Adiantopsis chlorophylla*	M	*Arum*
		Adiantum lorentzii	T	n.i.
		A. raddianum	T	n.i.
Equisetales	Equisetaceae	*Equisetum giganteum*	M	*Arum*
Gnetales	Ephedraceae	*Ephedra tweediana*	T	n.i.
Magnoliales	Magnoliaceae	*Magnolia* sp*	T	n.i.
Alismatales	Lemnaceae	*Lemna minor*	A	-
Liliales	Liliaceae	*Nothoscordum gracile*	T	*Arum*
		N. montevidensis	T	*Arum*
	Ponteridaceae	*Eichornia crassipes*	A	-
	Smilacaceae	*Smilax campestris*	T	*Arum*
Arecales	Arecaceae	*Butia yatay*	T	*Arum*
Commelinales	Commelinaceae	*Commelina diffusa*	T	*Arum*/ *Paris*
		C. erecta	T	*Arum*/ *Paris*
Poales	Cyperaceae	*Cyperus eragrostis*	M	*Arum*
		C. esculentus	T	n.i.
		C. reflexus	M	n.i.
		C. virens	M	*Arum*
		Eleocharis bonariensis	M	*Arum*
		E. contracta	M	*Arum*
		E. filiculmis	M	*Arum*
	Juncaceae	*Juncus bufonius*	M	*Arum*
		J. capillaceus	M	n.i.
		J. densiflorus	M	*Arum*
	Poaceae	*Agrostis alba**	T	*Arum*
		Aristida circinalis	T	-
		A. jubata	T	n.i.
		Briza calotheca	T	*Arum*
		B. lindmanii	T	*Arum*
		B. macrostachya	T	*Arum*
		*B. minor**	T	*Arum*
		Bromus auleticus	T	*Arum*
		*Lolium multiflorum**	T	*Arum*
		Melica rigida	T	*Arum*
		M. sarmentosa	T	*Arum*
		Rottboellia selloana	T	n.i.
		Paspalum exaltatum	T	*Arum*
		Spartina alterniflora	M	-

Order	Family	Species	Habits	AM type
Caryophylalles	Amaranthaceae	*Alternanthera kurtzii*	M	n.i.
Celastrales	Celastraceae	*Maytenus ilicifolia*	T	*Arum*
Fabales	Fabaceae	*Adesmia bicolor*	T	*Arum*
		A. incana	T	*Arum*
	Polygalaceae	*Monnina resedoides*	T	*Arum*
Fagales	Thymelaeaceae	*Daphnopsis racemosa*	T	*Paris*
Malpighiales	Euphorbiaceae	*Croton gnaphalii*	T	*Arum*
		Phyllantus niruri	T	*Arum*
		P. stipulatus	T	*Arum*
Rosales	Rosaceae	*Prunus persica**	T	*Paris*
Oxalidales	Oxalidaceae	*Oxalis conorrhiza*	T	*Arum*
Brassicales	Brassicaceae	*Capsella bursa pastoris**	T	*Arum*
Malvales	Malvaceae	*Pavonia hastata*	T	-
		P. sepium	T	-
		Wissadula glechomaefolia	T	*Arum*
Sapindales	Sapindaceae	*Allophylus edulis*	T	n.i.
	Meliaceae	*Melia azederach**	T	*Arum*
Violales	Passifloraceae	*Passiflora chrysophylla*	T	*Arum*
		P. caerulea	T	*Arum*
	Violaceae	*Hybanthus parviflorus*	T	*Arum*
Geraniales	Geraniaceae	*Geranium dissectum**	T	-
Myrtales	Lythraceae	*Heimia salicifolia*	T	*Arum/Paris*
	Myrthaceae	*Psidium incanum*	T	*Arum*
		P. luridum	T	*Arum*
Ericales	Sapotaceae	*Pouteria salicifolia*	T	-
Lamiales	Lamiaceae	*Hyptis floribunda*	T	*Arum*
		H. lappacea	T	*Arum*
	Oleaceae	*Fraxinus**	T	*Arum*
		*Ligustrum lucidum**	T	n.i.
		*L. sinense**	T	*Arum*
	Plantaginaceae	*Plantago brasiliensis*	T	*Arum*
	Verbenaceae	*Lippia alba*	T	*Arum*
		L. arechavaletae	T	*Arum*
Solanales	Solanaceae	*Petunia integrifolia*	T	*Arum*
Gentianiales	Asclepiadaceae	*Asclepias curassavica**	T	*Arum*
		A. mellodora	T	*Arum*

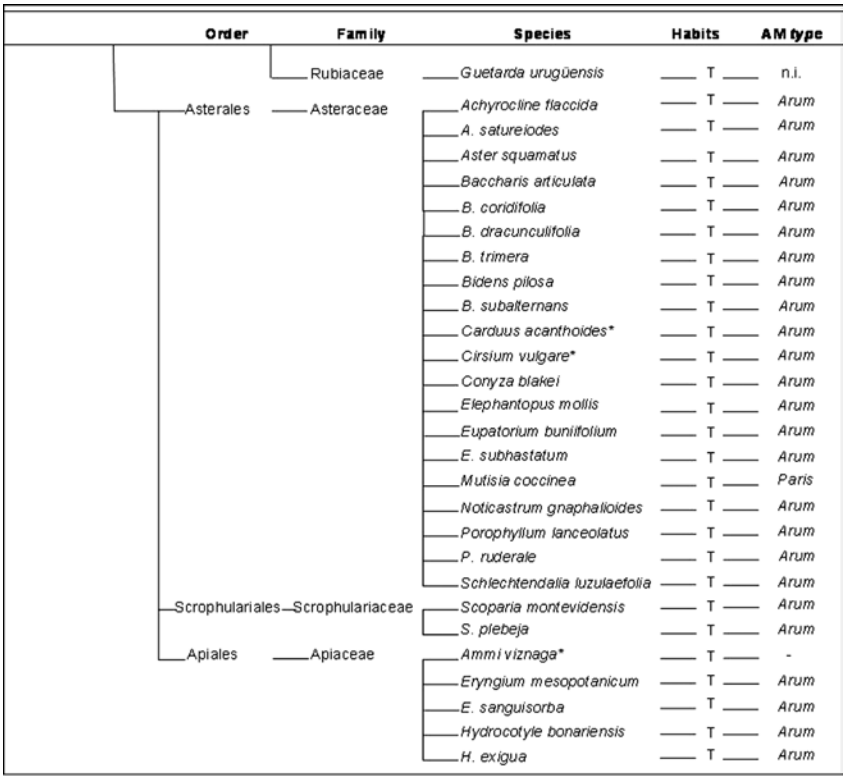

Figure 1. Angiosperm Phylogeny Group Classification (APG II 2003) shown life habits associated with AM types. Asterisks denote exotic plant species. A (aquatic), E (epiphytic), M (marshy), T (terrestrial), – (absent colonisation) and, n.i. (not identified).

et al. (2001) found that the *Arum*-type is dominant in plants that grow under a high solar exposure in a desert area from Australia. Yamato (2004) reported a predominance of the *Arum*-type in the weedy herbs and vines growing in an old abandoned field in Kansai region of Japan. We found *Arum*-type at all five vegetation types, including plant species from the relatively shadey gallery forest.

In this study the *Paris*-type colonisation was found in three species only: *Daphnopsis racemosa*, *M. coccinea,* and *P. Persica*.

Although the formation of *Paris*- or *Arum*-type arbuscular mycorrhizas is primarily under the genetic control of the host plant (Gerdemann 1965, Jacquelinet-Jeanmougin & Gianinazzi-Pearson 1983), and there is a strong relationship between the colonisation type and the identity of plant families (Harley & Smith 1983, Carrillo *et al.* 1992, Fontenla *et al.* 1998, Yamato 2004), there is some evidence that the fungal species may also have some effect (Cavagnaro *et al.* 2001). In our study, both AM colonisation types - *Arum* and *Paris*- were found

Table 1. Species recorded at El Palmar National Park following the AMF classification by Schüssler (2009). X indicates the presence of the species in different vegetation types: GF (gallery forest), GRA (grassland), MAR (marsh), PF (palm forest) and SCR (scrubland).

AMF Taxa	GF	GRA	MAR	PF	SCR
Phylum Glomeromycota Walker & Schussler					
Class Glomeromycetes Cavalier-Smith					
Orders Archaeosporales Walker & Schussler					
Fam. Archaeosporaceae Morton & Redecker					
Archaeospora trappei (Am.&Lind.)Morton & Redecker emend Spain					X
Orders Diversisporales Walker & Schussler					
Fam. Acaulosporaceae Morton & Benny					
Acaulospora bireticulata Rothwell & Trappe		X	X	X	x
A. delicata Walker, Pfeiff. & Bloss	X	X	X	X	x
A. denticulata Sieverd. & Toro	X		X	X	x
A. dilatata Morton	X	X		x	
A. excavata Ingleby & Walker			X	X	X
A. lacunosa		x			
A. laevis Gerd. & Trappe	X	X		x	
A. mellea Spain & Schenck	X	X	X	X	x
A. nicolsonii Walker, Reed & Sanders	X		X	X	x
A. scrobiculata Trappe	X	X		x	
A. spinosa Walker & Trappe	X	X	X	X	x
A. tuberculata Janos & Trappe				x	
Acaulospora sp. 1	X	X		x	
Acaulospora sp. 2	X	X		x	
Fam. Entrophosporaceae Oehl & Sieverd.					
Entrophospora infrequens (Hall) Ames & Scneid.	X	X	X	X	x
Fam. Gigasporaceae Morton & Benny					
Gigaspora candida Bhattacharjee, Mukerji, Tewari & Skoropad		X	X	X	X
G. gigantea (Nicolson & Gerd.) Gerd. & Trappe				X	
G. margarita Becker & Hall				X	
Gigaspora sp 1		X			
Gigaspora sp 2		X			
Scutellospora biornata Spain, Sieverd. & Toro		X	X	X	X
S. calospora (Nicolson & Gerd.) Walker & Sanders		X		X	X
S. coralloidea (Trappe, Gerd. & Ho) Walker & Sanders		X			
S. dipapillosa (Walker & Koske) Walker & Sanders		X	X	X	x

AMF Taxa	GF	GRA	MAR	PF	SCR
S. fulgida Koske & Walker				x	
S. gilmorei (Trappe & Gerd.) Walker & Sanders		X	X	X	x
S. heterogama (Nicolson & Gerd.) Walker & Sanders		X			x
Scutellospora sp. 1		x			
Fam. Pacisporaceae Walker, Blaszk., Schussler & Schwarzott					
Pacispora sp. 1	X	X	X		x
Orders Glomerales Morton & Benny					
Fam. Glomeraceae Pirozynski & Dalpe					
Glomus aggregatum Schenck & Sm.	X		X		x
G. ambisporum Sm. & Schenck	x				
G. claroideum Schenck & Sm. emend Walker & Vestberg	X	X	X	X	X
G. clarum Nicolson & Schenck	X	X	X	X	X
G. constrictum Trappe		X	x		
G. coronatum Giovann.	X	X		x	
G. diaphanum Morton & Walker	X		X		x
G. dimorphicum Boyetchko & tewari	X		X		x
G. etunicatum Becker & Gerd.	X	X	X	X	x
G. fasciculatum (Thaxt) Gerd. & Trappe emend Walker & Koske				x	
G. glomerulatum Sieverd.		X	X	x	
G. intraradices Schenck & Sm.	X		X		
G. microaggregatum Koske, Gemma & Olexia	X	X	X	X	x
G. mosseae (Nicolson & Gerd.) Gerd. & Trappe	X	X	X	X	x
Glomus sp. 1		X	X		x
Orders Paraglomerales Walker & Scussler					
Fam. Paraglomeraceae Morton & Redecker					
Paraglomus laccatum Renker, Blask. & Buscot		X			x

in members of three plant families. Species of the Asteraceae presented *Arum*-type colonisation in all species with the exception of *Mutisia coccinea*, were *Paris*-type colonisation type could be observed. Smith & Smith (1997) reported the co-existence of the two types of colonisation in some plant families. In the case of *Commelina difusa* and *C. erecta* (Commelinaceae) and *Heimia salicifolia* (Lythraceae), we observed the simultaneous development of *Paris*-*Arum* morphological types located in different sites of the same root system. The co-occurrence of these two colonisation types has been reported previously by Bonfante-Fasolo & Fontana (1985) and Kubota *et al*. (2005).

Amaranthaceae, Brassicaceae, Commelinaceae, Cyperaceae, Juncaceae and Scrophulariaceae families have generally been reported as non-mycotrophic (Harley & Harley 1987, Brundett 1991, Brundrett *et al.* 1995, Smith & Read 1997, Fontenla *et al.* 2001). We have found a colonisation with arbuscular fungi in some species. However, as known from literature, non-mycotrophic plants can become "facultatively mycotrophic" due to stress caused by unfavourable ecological conditions (Tao *et al.* 2004, Lovera & Cuenca 2006, Becerra *et al.* 2007). In fact, though El Palmar National Park water shortage constitutes an aggravating factor (Goveto 2005), many of these families are frequent in this type of vegetation where water is not a limiting factor (e.g. marsh). Therefore, the non-mycotrophic conditions of these families should be revised.

Ferns from Pteridaceae presented AMF colonisation. The presence of arbuscules was confirmed in *Adiantopsis chlorophylla*. AMF fern colonisation has been recorded previously (Harley & Harley 1987, Newman & Reddell 1987, Godoy *et al.* 1994, Zhao 2000). In the presented study colonisation was not observed in *Microgamma vaciinifolia*, an epiphytic fern that habitually grows on *B. yatay*. These results agree with observations made by Bermudes & Benzina (1989) and Lesica & Antibus (1990), who did not find AM colonisation in epiphytic ferns grown on Arecaceae. Nadarajah & Nawawi (1993) did not find AM colonisation on epiphytic species grown on palms in Malaysia. In lower pteridophytes, Zhang *et al.* (2004) reported *Paris*-type colonisation in *Equisetum hiemale* and *E. ramosissimum* (Equisetaceae). In our research, *Arum*-type colonisation was observed in *E. giganteum*.

Only 10 of the plant species analyzed did not have AM colonisation. The absence of AMs was not related to environmental patterns or to the taxonomic family they belonged to.

Up to now, in Argentina, 46 taxa of Glomeromycota were found (Cabello & Irrazabal 2004). In this work we have found 11 arbuscular fungal species for the first time in Argentina. The El Palmar National Park has a high diversity of AMF in comparison with other areas in Argentina. The richness of the species found in El Palmar National Park is not surprising due to it is one of the most floristically diverse national parks with more than 700 species of vascular plants (Biganzoli et al. 2001).

Glomus and *Acaulospora* were the dominant AMF genera in the studied area. The genus *Acaulospora* has been recorded associated with plants in natural ecosystems (Li *et al.* 2003, Zhao *et al.* 2003, Tao *et al.* 2004), whereas *Glomus* species have been reported as dominant in agrosystems (Zhang *et al.* 1994, Vestberg 1995, Oehl *et al.* 2003, Gai *et al.* 2004, Schalamuk *et al.* 2006). A large number of species belonging to Gigasporaceae were also recorded. In general, members from this family prefer soils with high sand content (Schenck *et al.* 1975, Koske 1981), like those of the El Palmar National Park. Notwithstand-

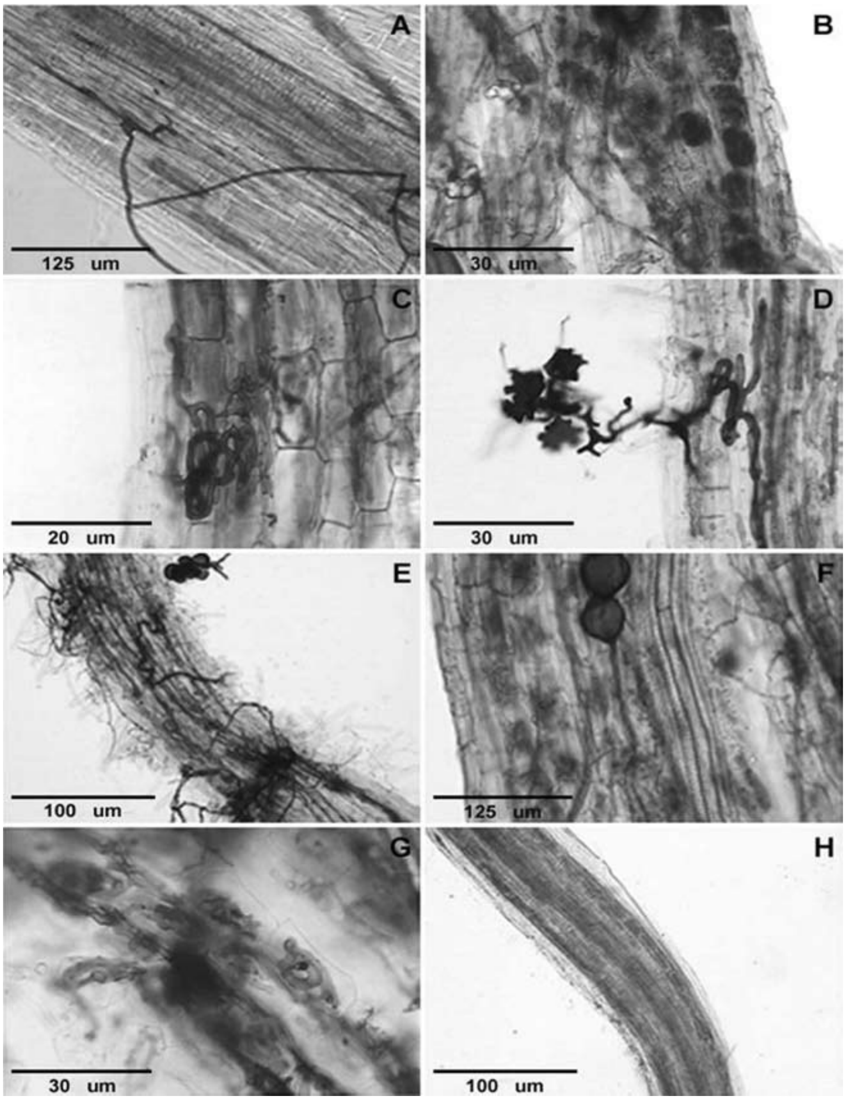

Figure 2. Arbuscular mycorrhizal fungi in cleared and stained roots. (A) Apresorium in *Eryngium megapotanicum*; (B) Arbuscules in *Butia yatay*; (C) Coils in *Daphnopsis racemosa*; (D) Auxiliary cells in *Bidens pilosa*; (E) External mycelium in *Monnina resedoides*; (G) *Paris*-type colonisation in *Daphnopsis racemosa*; (H) Non-colonised root in *Geranium dissectum*.

ing, members of Gigasporaceae were escarsed in marsh and absent from the gallery forest. Although we did not specifically quantify ligh interception by tree in the gallery forest, this vegetation type has one of the shaded under-canopies. This, coupled the grain of vascular plant diversity (high diversity for the vegetation type but low diverse above a point in the ground) and frequent flooding events probably influence Gigasporaceae development.

The highest percentages of plant species colonised were correlated with the vegetation types where we found the highest fungal richness. The AMF fungal diversity (46 taxa) in El Palmar National Park, was associated with 42 plant families. The *Arum*-type colonisation pattern was present in 90% of the species examined. This agrees with results of Fracchia *et al.* (2009) who found that the *Arum*–type was most frequent in species examined in the Chaco Serrano woodlands of Argentina However, these results contrast with other reports for Argentina. The *Paris*–type AM colonisation was reported as being dominant in species in the Yungas forests (Becerra *et al.* 2007) and in *Polylepis* woodlands (Menoyo *et al.* 2007).

Further work is reequired in other regions of Argentina to determine the overall frequency of these AM types.

Acknowledgments

We thank Dr. Larry Peterson for critical reading of the manuscript. The National Parks authorities provided permits to do this study in El Palmar National Parks. This research is part of the 11/N527 UNLP and PICT 2007 01233 projects. A grant from Comisión de Investigaciones Científicas de la provincia de Buenos Aires (CIC) supported this research. Velázquez S. is recipient of a fellowship from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and Cabello M. from is a researcher of (CIC).

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(Manuscript accepted 7 May 2010; Corresponding Editor: M. Kirchmair)