

## Research Paper

## Occurrence of arbuscular mycorrhizas and dark septate endophytes in pteridophytes from a patagonian rainforest, Argentina

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Arbuscular mycorrhizas (AM) are one of the most widespread types of symbiotic associations. Pteridophytes occupy an important position in the evolution of vascular plants. However, their mycorrhizal state remains poorly understood. The aim of this work was to describe the general mycorrhizal status and the occurrence of dark septate endophytes (DSE) in the pteridophytic flora of a Valdivian temperate forest in Patagonia, Argentina. First, the roots of nine terrestrial species representing six families were examined, and this information was then compared with other surveys concerning the occurrence of AM in other pteridophytic species within the same Valdivian temperate forest. Arbuscular mycorrhizas were recorded in 98.6% of the samples analyzed in this work and all of them corresponded to the *Paris*-type morphology. Dark septate endophytes were also present within the roots of all terrestrial species. A comparison to published results in other ferns and lycophytes that have been studied in this Valdivian temperate forest (161 sporophytes, 21 species and 10 families) was made. Clear differences in colonization patterns between eusporangiate/leptosporangiate and epiphytic/terrestrial species became evident and are discussed.

**Keywords:** Mycorrhizas / *Paris*-type / Dark septate fungi / Valdivian temperate rainforest / Ferns and lycophytes / Leptosporangiate and eusporangiated ferns

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### Introduction

Plant roots are colonized by several kinds of fungi, some of them being beneficial for plant growth. According to different authors [1, 2], land colonization by plants may not have been possible without fungal symbionts. Among these symbiotic fungi are those that form arbuscular mycorrhizas (AM), the most widespread type of mycorrhizas. Arbuscular mycorrhizal fungi form interactions with the roots of most land plants, in which the host gains essential nutrients from

the fungus in exchange for fixed carbon [3, 4]. Due to the important contribution of AM to soil structure, plant nutrition and productivity, diversity and structure of plant communities are strongly influenced by these organisms [5].

Arbuscular mycorrhiza morphology can be divided into two principal types, *Arum* and *Paris*. The *Arum*-type association is characterized by extensive intercellular hyphae between cortical cells and short lateral branches that form arbuscules within the cells. In contrast, in *Paris*-type colonization the fungi spread directly from cell to cell forming intracellular hyphal coils that frequently have intercalary arbuscules [6, 7]. In addition to these two main morphological types, an *Intermediate*-type colonization has also been described [7, 8].

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Despite it is still not clear which factors determine *Arum* and/or *Paris*-type development, some suggestions have been made. According to some authors, anatomical characters of the host roots [9, 10] and plant genotype [11, 12] considerably influence the development of AM morphologies. Dickson [8] and Kubato *et al.* [13] indicated that AM colonization type is a consequence of the interaction between both, plant and fungus. It has also been suggested that *Paris*-type associations might be more advantageous for plants growing slowly and/or in low-nutrient and high-stress environmental conditions because their rate of spread within roots is slower than that of the *Arum*-type, keeping the energy supply to the fungi at a manageable level [9, 10, 14, 15]. This idea is supported by *Arum*-type mycorrhizas being most abundant in agricultural crops (which are fast growing plants), whereas the *Paris*-type has been found to be most frequent in plants growing in natural ecosystems [7, 12, 15, 16]. However, Allen *et al.* [17] found abundant *Arum*-type colonization in addition to *Paris*-type in different perennial plants from the arctic tundra, meaning that both types of morphologies may be present in plants growing in the same extreme environment. Even more, these authors observed both types of morphologies within the same plant but depending on their position within the root. Therefore, it becomes evident that examination of a wider range of plants from different habitats is needed to completely understand the occurrence and functioning of *Arum*- and *Paris*-type AM [16].

Endophytic fungi are ubiquitous in plants and are the most likely source of new plant–fungus associations [18]. That is why increased attention has recently been given to a group of miscellaneous fungi designated as dark septate endophytes (DSE), which are frequent root colonizers of several plant species in different environments. These conidial fungi are characterized by melanized, regularly-septate hyphae and microsclerotia. They penetrate living plant roots without causing any apparent negative effect. The ecological role of DSE is currently unresolved, but their widespread occurrence in different ecosystems, their potential to function as plant growth promoters and to provide defenses against herbivores and pathogens suggest that these endophytes are a significant component of plant communities, as it has been described by different authors [17, 19–21]. Considering DSE when studying mycorrhizas might yield valuable information about these fungi and their interaction with other root colonizers.

Some phylogenetic studies have revealed a basal dichotomy within vascular plants, separating the lycop-

phytes from the euphyllophytes. The last comprise two major clades: the spermatophytes (seed plants) and the monilophytes (horsetails and ferns). Plants included in the lycophyte and monilophyte clades are spore-bearing or “seed-free”, and because of this common feature their members have been lumped together in a paraphyletic group named “seedless vascular plants”, “seed-free plants” [32] or Pteridophytes. These plants occupy an important position in the origin and evolution of vascular plants [22, 23]. They dominated land flora since the Silurian, throughout the Devonian and Carboniferous, and remain as a major component of many ecosystems to this day [24]. They have evolved remarkable adaptations to extreme environments, from tropical to cold temperate regions, lowland to alpine zones and xeric to aquatic conditions [25]. Nevertheless, mainly because of their low commercial value [26], these plants have largely been ignored and their AM status remains poorly understood [22, 23, 27], together with the role that AM fungi may play in their biogeographical and ecological distribution [28]. Some authors have registered the occurrence of AM interactions in ferns, but most of them did not describe the morphological type present in the studied species [20, 23, 26–30]. According to Zhang *et al.* [22], ferns included in the order Filicales (monilophytes) usually have *Paris*-type associations, while Kessler *et al.* [31] and Smith and Smith [7] stated that both *Arum* and *Paris*-type are present in this group of pteridophytes.

Abundant ferns and lycophytes are present among the exuberant vegetation of the Valdivian temperate rainforests of South America. The distribution of these forests in Argentina is reduced and restricted to some few places in Patagonia. Previous studies carried out within this Valdivian region documented that *Equisetum bogotense* HBK and *Lycopodium paniculatum* Desv. ex Poir. (a horsetail and a lycophyte that were previously lumped together in a group named “fern allies”) were facultatively mycorrhizal with an *Intermediate*-type association [33], while epiphytic ferns were non-mycorrhizal [34]. Terrestrial pteridophytes are abundant in the understory of Valdivian temperate forests, but their mycorrhizal behaviour is poorly known, since only some *E. bogotense* and *L. paniculatum* specimens growing in the forest soil have been analysed.

This work aimed to analyze the general mycorrhizal status and the occurrence of DSE in the pteridophytic flora of one of the Valdivian temperate forests of Argentina. For this purpose, it was necessary to describe the AM and DSE colonization in sporophytes of terrestrial fern species. A final comparison between this information and that included in the previously men-

tioned surveys also conducted in this forest [33, 34] is made and discussed.

## Materials and methods

The Subantarctic Province (Subantarctic Domain, Antarctic Region) is situated in the southern part of Argentina and Chile. The Valdivian district corresponds to the most humid district of this province. It occupies a narrow strip of ~75000 km<sup>2</sup> which runs from 37° 20' to 47°00' S latitude between the Eastern slope of the Andes and the Pacific Ocean, lying mostly in Chile and extending into a small part of Argentina [35].

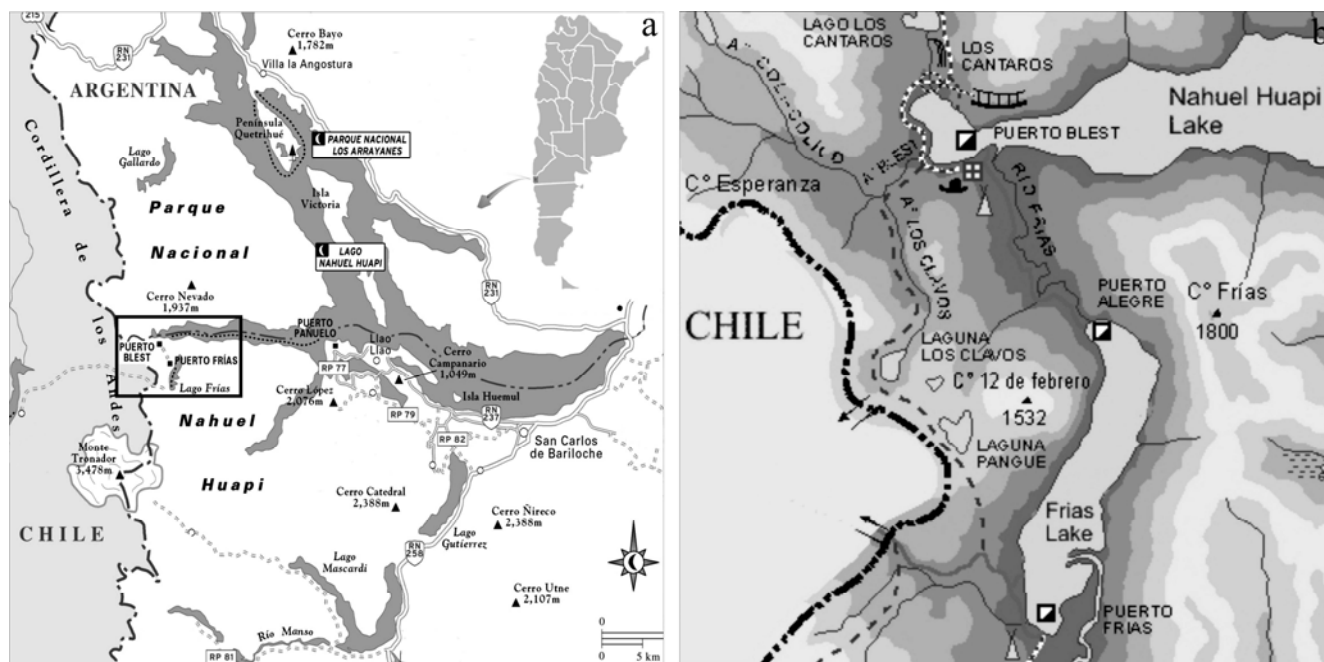
Puerto Blest (41°02' S, 71°49' W; 750 m elevation) is part of the reduced and fragmented Valdivian region of Argentina. It comprises an area of 3.6 km<sup>2</sup> and it is located within the Nahuel Huapi National Park, Río Negro Province, Patagonia (Fig. 1). The annual average temperature is 9 °C and the average annual rainfall is 3000 mm, which makes it one of the rainiest places in the country. In this hydrophilic forest, evergreen species are the most abundant and the dominant tree species is the southern beech *Nothofagus dombeyi* (Mirb.) Oerst. (Fagaceae). The understory is very shaded because of the persistent and dense forest canopy, and it comprises a great diversity of plants, including several fern species [35, 36].

## Sample collection and analysis

Sporophytes of terrestrial ferns were sampled by random walk between late spring 2005 and early autumn 2006. In order to complete the general analyses of the mycorrhizal status of these plants, conspecific specimens that were exceptionally growing on other substrates, such as trunks and rocks, were also collected.

Sporophytes were carefully removed, stored in individual plastic bags and kept at 4 °C. Roots were separated from the rest of the plant and carefully cleaned under a stereoscopic microscope (Olympus SZ30) to remove all root pieces belonging to other plants. Samples were stained using a modified Phillips and Hayman [37] method [see 33, 34]. Once stained, ten root pieces of approximately 1 cm length were randomly selected from each sample and mounted in glycerin on a microscope slide. For each specimen, three slides (30 root pieces = ~30 cm) were made and examined with a light microscope (Olympus BX40). Typical AM structures (intra or intercellular hyphae, vesicles, coils and arbuscules) were used for their classification into *Arum* or *Paris*-type [7, 8] and for quantifying mycorrhizal colonization [17, 20, 33, 34].

For quantification, slides were observed with a Olympus BX40 microscope using a Plan 20× N.A. 0.40 objective. Root length colonized by AM fungi was estimated according to the intersect method described by McGonigle *et al.* [38]. In each slide no less than 100 fields were



**Figure 1.** Geographical location of the study area. a) Nahuel Huapi National Park and Puerto Blest area indicated with a rectangle; b) Detailed Puerto Blest region.

quantified (being 320 the average number of fields quantified per sample). Percentages of root length colonized by AM were analyzed using a One-way-ANOVA and Duncan Post-hoc Test. Data was first normalized using square transformation. If DSE were detected, their presence was recorded.

Typical structures corresponding to AM and DSE were documented as brightfield images which were captured with a digital camera (Sony Exwave HAD) and Image-Pro Plus 4.1.0.0. analysis software for Windows.

### General mycorrhizal status analyzes

A Multiple Correspondence Analysis (MCA) was conducted to analyze the relationships of the following active variables among all the ferns and lycophytes studied up to the moment in Puerto Blest: “family” (10 categories), “AM colonization percentage” (6 categories: 1 = 0%, 2 = 1–20%, 3 = 21–40%, 4 = 41–60%, 5 = 61–80%, 6 = 81–100%) and “habitat” (3 categories: terrestrial, epiphytic, saxicolous). The variable “species” (21 categories) was considered as supplementary. This comparison let us describe the general mycorrhizal status of the entire pteridophytic flora present in this Valdivian temperate forest.

## Results

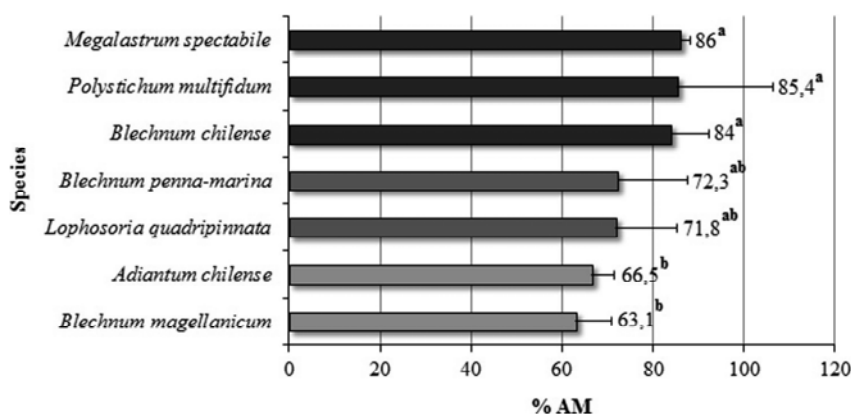
### Occurrence of arbuscular mycorrhizas

A total of 58 terrestrial sporophytes corresponding to nine species that usually grow in the soil were collected. In addition, one epiphytic and ten saxicolous specimens belonging to six of these species were found. The collected sporophytes were included in the Blechnaceae (66%), Dicksoniaceae (11%), Dryopteridaceae (10%), Pteridaceae (9%), Gleicheniaceae (2%) and Hymenophyllaceae (2%) families (Table 1), which correspond to leptosporangiated ferns in the order Filicales. All the species have been previously reported for Puerto Blest [36, 39].

Presence of AM was recorded in most of the specimens (98.6%), with the exception of one *Hymenophyllum dentatum* sporophyte (Table 1). Arbuscules were found in all the colonized samples. Due to the absence of intercellular hyphae and the presence of extensive coils of intracellular hyphae within cortical cells, from which arbuscules were formed as intercalary structures, the AM observed in these plants were classified as Paris-type. Arbuscular mycorrhizal fungi were observed entering into the cortical cells directly through

**Table 1.** Percentage of AM colonization in ferns of Puerto Blest. N°: number of sporophytes collected; AM%: mean and standard deviation corresponding to the colonization values recorded per species and habitat; (range): the lowest and the highest colonization values recorded per species and habitat if more than one sporophyte were sampled; \*: one of the samples was positive but non-quantified because material was insufficient; \*\*: samples not statistically analyzed because only one specimen was found.

Taxon	Habitat	N°	AM% (range)
<b>Blechnaceae</b>			
<i>Blechnum chilense</i>	Saxicolous	2	91.6 ± 4.9 (88.1–95.1)
	Terrestrial	16	80.6 ± 8.1 (67.7–97.3)
<i>Blechnum magellanicum</i>	Epiphytic	1	54.2 ± 7.1
	Saxicolous	2*	68.3 ± 4.1
<i>Blechnum penna-marina</i>	Terrestrial	1	66.9 ± 9.0
	Saxicolous	1	86.6 ± 6.2
	Terrestrial	23	71.6 ± 15.2 (24.4–91.3)
<b>Dicksoniaceae</b>			
<i>Lophosoria quadripinnata</i>	Terrestrial	8	71.8 ± 13.6 (49.6–89.0)
<b>Dryopteridaceae</b>			
<i>Megalastrum spectabile</i>	Saxicolous	2	85.5 ± 3.0 (83.4–87.6)
	Terrestrial	1	87.1 ± 7.1
<i>Polystichum multifidum</i>	Saxicolous	1	54.5 ± 12.0
	Terrestrial	3	95.7 ± 5.1 (89.9–99.6)
<b>Gleicheniaceae</b>			
<i>Gleichenia quadripartita</i> **	Terrestrial	1	66.3 ± 7.9
<b>Hymenophyllaceae</b>			
<i>Hymenophyllum dentatum</i> **	Terrestrial	1	0
<b>Pteridaceae</b>			
<i>Adiantum chilense</i>	Saxicolous	2	61.3 ± 6.2 (56.9–65.7)
	Terrestrial	4	69.0 ± 1.4 (67.5–70.9)



**Figure 2.** Mean and standard deviation of the percentages of mycorrhizal colonization in ferns of Puerto blest. a, b: colonization values followed by different superscript letter are significantly different.

the root epidermis (Fig. 2). Hypha corresponding to these fungi and associated with typical AM colonization were also detected within root hairs, meaning that it would be possible that they first penetrate into them and then spread into the root cortex (Fig. 2).

The percentage of root length colonized differed among species and families (Table 1, Fig. 3), being higher than 50% in almost all the samples (except for one *Blechnum penna-marina* specimen) (Table 1). When seven out of the nine species were considered for the ANOVA test (excluding *Gleichenia quadripartita* and the non-mycorrhizal *H. dentatum* for being represented by only one specimen), it was observed that the mean colonization values corresponding to *B. chilense*, *M. spectabile* and *P. multifidum* were significantly greater than those of *A. chilense* ( $p = 0.021$ ;  $p = 0.013$ ;  $p = 0.008$  respectively) and *B. magellanicum* ( $p = 0.009$ ;  $p = 0.005$ ;  $p = 0.003$ ). *Lophosoria quadripinnata* and *B. penna-marina* had intermediate colonization percentages so they did not show significant differences with any of the species in the other two groups (Fig. 3). The colonization level of the only collected *G. quadripartita* sporophyte was similar to the value obtained for the species in the group with the low percentages. At family level, the highest mean colonization value corresponded to the Dryopteridaceae (Table 2).

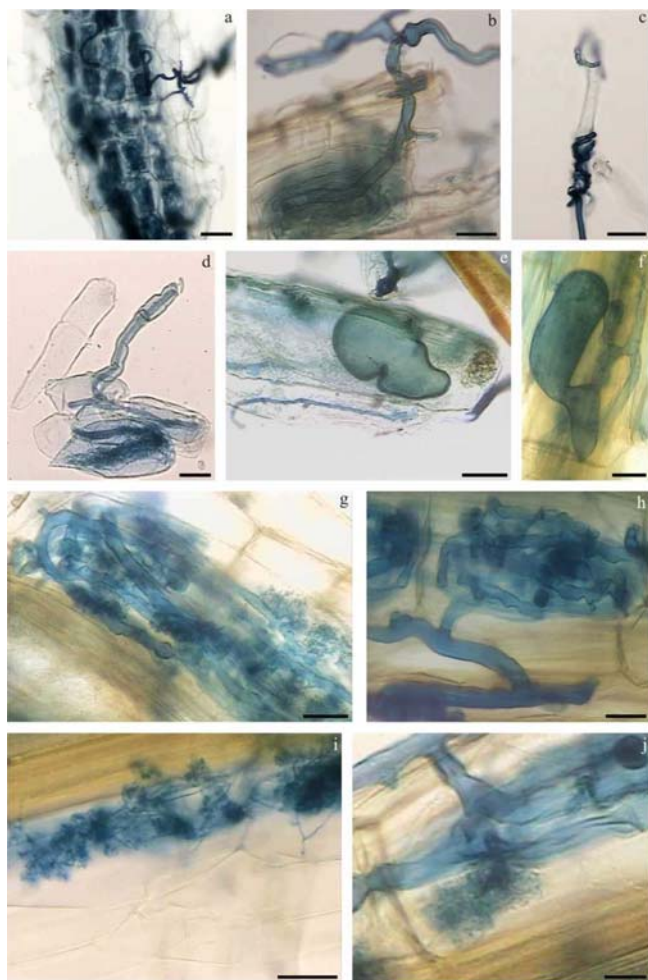
### Dark Septate Endophytes (DSE)

Root systems of all the sporophytes analyzed in this study were also colonized by DSE. These fungi were present even in *H. dentatum*, the only non-mycorrhizal specimen collected. The pattern of DSE colonization was similar among the samples, and the standardized vocabulary proposed by Jumpponen and Trappe [19] will be used to describe it.

Initial colonization consisted in superficial narrow, septate and more frequently melanized runner hyphae or loose hyphal networks. Occasionally, runner hyphae were branched at a 90° angle to the main hypha (Fig. 4a). Superficial hyphae were sometimes extending from the root surface and associated with asexual fungal structures (Fig. 4b). At the point where DSE penetrated into the cortical cells, they formed different types of appressoria (swollen structures preceding penetration) (Fig. 4c, d). As it was observed for AM, these fungi were also capable of passing through root hairs (Fig. 4e, f). Once into the epidermal layer the fungi tended to coil (Fig. 4g, h). Then, the fungus passed through adjoining epidermal cell walls by usually forming narrow penetration tubes which arise from appressorium-like structures (Fig. 4i). While the fungi were growing intercellularly, they developed microsclerotia (intracellular groups of rounded, closely packed, thick-walled and usually darkly pigmented fungal cells), which sometimes occupied the entire volume of the cell (Fig. 4i, j). A distinguishing feature of the DSE present in these plants was the occurrence of “cerebriform structures” (Fig. 4k, l). Dark septate fungi forming all these structures had straight (Fig. 4a, e), moniliform (Fig. 4f) or irregularly lobed hyphae (Fig. 4h) which occasionally got stained with Trypan blue. The root stele was not colonized.

### Review of the pteridophyte mycorrhization in the studied area

Table 2 shows a summary of all the ferns and lycophytes studied in this Valdivian temperate forest up to the moment. A total of ten families, 21 species and 161 specimens were considered for this analysis, using published data from Fernández *et al.* [38, 39] and the present study.



Arbuscular mycorrhizas were found in ten out of the 21 species (47.6%). The two eusporangiate species (9.5% out of the total number of species analyzed) were found to be facultatively mycorrhizal with an *Intermediate*-type AM morphology. Among the leptosporangiate species, eight were consistently colonized by *Paris*-type AM while 11 had a non-mycorrhizal behavior.

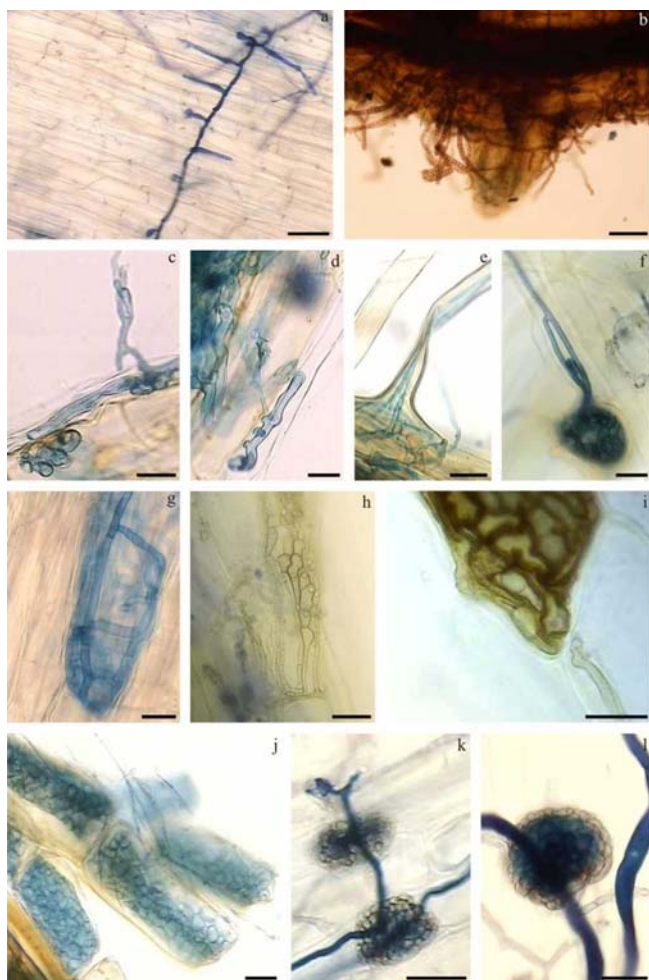
When all the information was analyzed using a Multiple Correspondence Analysis (MCA), three groups are clearly defined (Fig. 5). Group 1 includes the eusporangiate families, Lycopodiaceae and Equisetaceae, which are associated with the lowest mean colonization values. Group 2 comprises all the species corresponding to

**Figure 3.** Arbuscular mycorrhizas structures corresponding to *Paris*-type morphology observed in ferns of Puerto Blest. a) Intensive AM colonization and entry point through the root cortex in *Polystichum multifidum*; b) Hypha entering the root of *Adiantum chilense* and coiling within the cortex; c) Hypha growing around a root hair of *P. multifidum*; d) AM fungus passing through a root hair of *Megalastrum spectabile*; e, f) Irregular vesicles observed in *Lophosoria quadripinnata* and *Blechnum magellanicum*, respectively; g) Coil that occupies the major part of the cell volume in *B. pennamariana* and intercalary arbuscules; h) Coils and arbuscules in *B. chilense* with hyphae passing from one cell to the other; i, j) Arbuscules present in *Gleichenia quadripartita* and *B. chilense* respectively. Scale bars: a: 200  $\mu$ m, b: 20  $\mu$ m, c: 10  $\mu$ m, d–i: 20  $\mu$ m, j: 10  $\mu$ m.

**Table 2.** Comparison of the mycorrhizal status of all the ferns and lycophytes present in Puerto Blest according to their taxonomy, evolutionary stage and habit. **F:** Facultatively mycorrhizal (% of sporophytes colonized by AM); **NM:** Non-mycorrhizal; **C:** constantly mycorrhizal, meaning that AM were observed in all the specimens. For more details see \* Fernández *et al.* [33] and \*\* Fernández *et al.* [34].

Clade	Evolutionary stage	Family (N° species)	Examined samples	N° Sporophytes per habitat			Arbuscular mycorrhizas		
				Epiphyte	Terrestrial	Saxicolous	Status	Morphological type	Mean colonization value (%)
Lycophytes	Eusporangiate	Lycopodiaceae (1)*	14	2	12	–	F (43)	<i>Intermediate</i>	9.5 $\pm$ 15.3
Monylophytes	Eusporangiate	Equisetaceae (1)*	12	–	12	–	F (67)	<i>Intermediate</i>	8.6 $\pm$ 7.8
	Leptosporangiate	Aspleniaceae (1)**	10	8	2	–	NM	–	0
		Blechnaceae (3)	46	1	40	5	C	<i>Paris</i>	72 $\pm$ 10.2
		Dryopteridaceae (2)	7	–	4	3	C	<i>Paris</i>	85.7 $\pm$ 0.43
		Gleicheniaceae (1)	1	–	1	–	C	<i>Paris</i>	66.3 $\pm$ 7.9
		Hymenophyllaceae (9)**	49	32	17	–	NM	–	0
		Dicksoniaceae (1)	8	–	8	–	C	<i>Paris</i>	71.8 $\pm$ 13.6
		Polypodiaceae (1)**	8	8	–	–	NM	–	0
Pteridaceae (1)	6	–	4	2	C	<i>Paris</i>	66.4 $\pm$ 5		
<b>Total</b>		<b>10 families (21 species)</b>	<b>161</b>	<b>51</b>	<b>100</b>	<b>10</b>			





**Figure 4.** Colonization pattern by dark septate endophytes. a) Runner hyphae branched at a 90° angle to the main hypha in *Adiantum chilense*; b) Net of superficial hyphae on roots of *Hymenophyllum dentatum*; c, d) Appressoria-like and penetrating hyphae in roots of *Megalastrum spectabile* and *Blechnum magellanicum* respectively; e, f) Hyphae of DSE colonizing the root hairs of *A. chilense* and *Gleichenia quadripartita*; g) DSE coiling within the cortical cells of *Lophosoria quadripinnata*; h) Microsclerotia in roots of *B. pennamariana*; i) Hyphae passing through adjoining epidermal cell walls by forming narrow penetration tubes which arise from appressorium-like structures and associated microsclerotium in *B. magellanicum*; j) Groups of rounded, closely packed, fungal cells that occupies the entire volume of the root cells of *Polystichum multifidum*; k, l) Cerebriform structures corresponding to DSE in *B. chilense*. Scale bars: a–b: 200 µm, c–k: 20 µm, l: 10 µm.

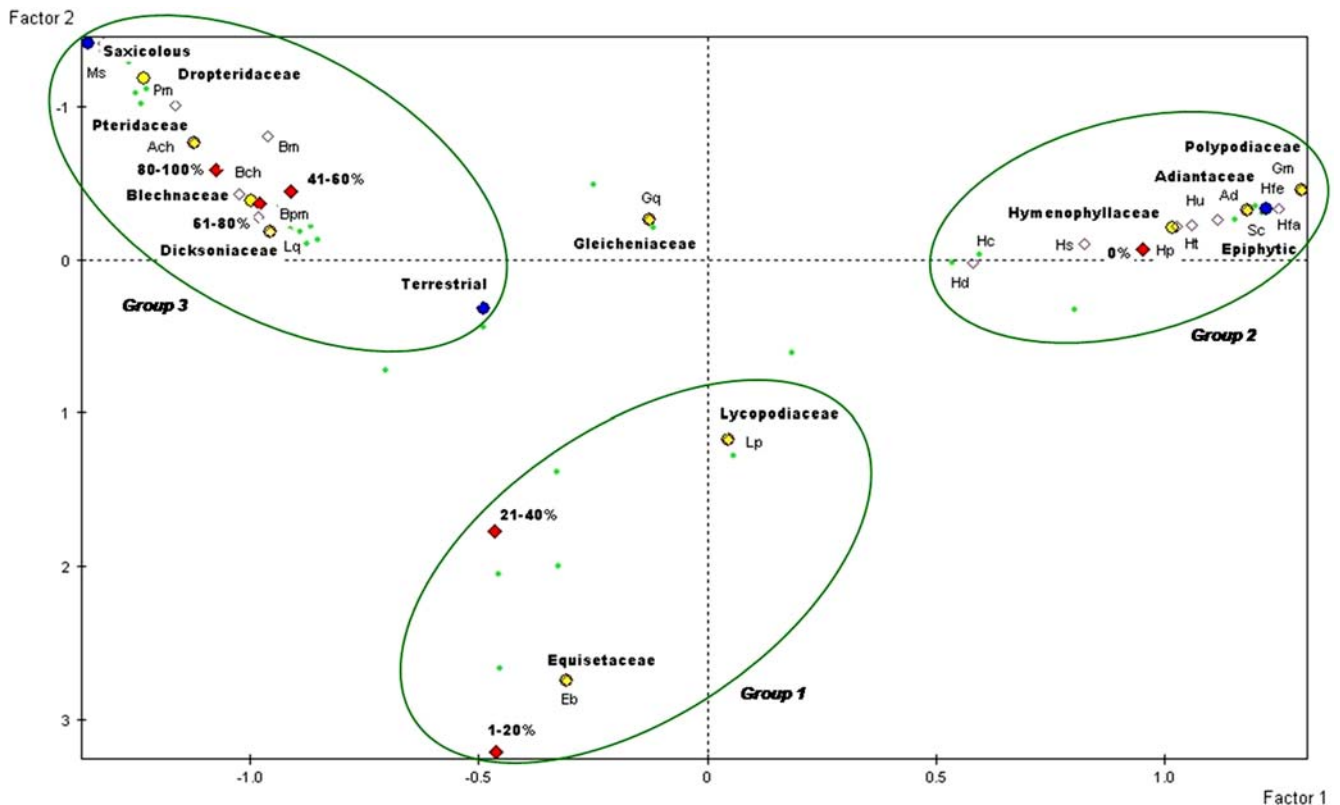
the families Polypodiaceae, Adiantaceae and Hymenophyllaceae, which are characterized for being epiphytic and non-mycorrhizal. Within Group 3 are all the families which are typically terrestrial (and their corresponding species). This last group is also associated with the highest colonization values.

## Discussion

In Argentina there are three hotspots of pteridophytic diversity: two subtropical humid centers in the northwest and northeast of the country, and a temperate humid center in southern part of Argentina, which overlaps with the Valdivian temperate rainforest region. The southern temperate center, where 79 species corresponding to 33 genera have been described [39], has the lowest pteridophytic diversity, but the highest number of endemisms [40]. The three surveys considered in the present work include a total of 21 species and 12 genera, which correspond to 27% of the species and 36% of the genera cited for the southern temperate center. The highest percentage of sporophytes corresponded to the Hymenophyllaceae and Blechnaceae (Table 2). These findings are in agreement with Ponce *et al.* [40], who established that they are the most common families in the southern temperate center, and that *Blechnum* and *Hymenophyllum* are among the richest genera of Argentina.

Arbuscular mycorrhizas were found in ~48% of the species studied in Puerto Blest. It is interesting to notice that the members of the family Hymenophyllaceae lack AM despite the presence of inocula in the soil, which has been indirectly demonstrated by the occurrence of AM in adjacent plants and by Chaia *et al.* [43], who reported that in Puerto Blest the AM soil infective capacity ranges from 0.6 to 1.7 IU/g soil. Two of the species colonized by AM are eusporangiate, facultative mycorrhizal, with an *Intermediate*-type AM, while eight are leptosporangiate (Filicales), mostly terrestrial and consistently colonized by *Paris*-type AM. These findings are in agreement with Smith and Smith [7], who established that representatives of the Filicales mostly have *Paris*-type colonization pattern.

It has been proposed that “primitive” fleshy and relatively thick roots of eusporangiate pteridophytes are more highly colonized by AM than the “advanced” fine roots of the leptosporangiate pteridophytes (Boullard’s hypothesis [44]), so that facultative mycorrhizal associations are considered to be a feature of relatively “advanced” ferns (Filicales). This fact would imply that evolution in pteridophytes is accompanied by a decrease in mycotrophy [18, 23, 29, 41, 44]. However, the results obtained in this study shows that leptosporangiate ferns can be extensively colonized by AM. Furthermore, the existence of these fungi in all the sporophytes included in typically terrestrial families suggests that in Puerto Blest terrestrial leptosporangiate ferns tend to be consistently mycorrhizal. These results accord with Lee *et al.* [26] and do not support Boullard’s



**Figure 5.** Results of the Multiple Correspondence Analyses. The categorical variables and their corresponding categories were a) Species (Lp: *Lycopodium paniculatum*, Eb: *Equisetum bogotense*, Ad: *Asplenium dareoides*, Gm: *Grammitis magellanica*, Hc: *Hymenophyllum cuneatum*, Hd: *Hymenophyllum dentatum*, Hfa: *Hymenophyllum falklandicum*, Hfe: *Hymenophyllum ferrugineum*, Hp: *Hymenophyllum pectinatum*, Hs: *Hymenophyllum seselifolium*, Ht: *Hymenophyllum tortuosum*, Hu: *Hymenophyllum umbratile*, Sc: *Serpillopsis caespitosa*, Bch: *Blechnum chilense*, Bm: *Blechnum magellanicum*, Bpm: *Blechnum penna-marina*, Ms: *Megalastrum spectabile*, Pm: *Polystichum multifidum*, Gq: *Gleichenia quadripartita*, Lq: *Lophosoria quadripinnata* and Ach: *Adiantum chilense*); b) Family; c) AM colonization percentages and d) Habit.

hypothesis. West *et al.* [28] also found differences between evolutionary stages. They compared different pteridophyte species in terms of *Glomus* richness and found fewer species within the roots of eusporangiate than of leptosporangiate species. Altogether, this information suggests that mycorrhizal leptosporangiate species have higher colonization levels and harbor more diverse mycorrhizal communities than “ancient” eusporangiate species.

There are relatively few early land plant fossils that show AM colonization, but fossil records apparently indicated that mycorrhizas occurring in earliest land plants were more similar to the *Arum*-type [2, 45], suggesting that this type of AM could be the more ancient type of mycorrhizas [46]. Among the species analyzed in Puerto Blest, only *E. bogotense* and *L. paniculatum* have an *Intermediate*-type AM, with arbuscules corresponding to the *Arum*-type morphology [see 33, 34]. Intra and intercellular hyphae, vesicles and arbuscules such as those observed in *E. bogotense* were also recorded in other

*Equisetum* species from Western and Arctic Canada [20]. Thus, regarding evolutionary stages, it could be said that the occurrence of an *Intermediate*-type AM in the more ancient eusporangiate pteridophytes and the presence of *Paris*-type AM in the advanced leptosporangiate Filicales only (Table 2), supports this hypothesis and the fact that evolutionary stages play an important role in determining the mycorrhizal colonization in pteridophytes.

As it was mentioned before, there is currently no consistent experimental evidence to address the question of whether AM morphology is determined by the fungus, the plant, the interaction, and/or the environment [8–13]. One possibility that has been suggested by different authors [9, 10, 14–16] is that the *Paris*-type morphology keeps the energy supply to the fungi at a suitable level, minimizing ‘energy costs’, so that it would be more beneficial for plants growing slowly and/or under high-stress conditions. The fact that ferns are mostly perennial plants, with long life cycles and



slow growth rates [47], that usually grow in stressful habitats (mainly because of lack of light), would be consistent with this idea. Nevertheless, *Arum*-type AM were also found in different slow-growing perennial plants from the arctic tundra, which spend several months in darkness [17]. This controversial information indicates that further studies are needed in order to better understand how biotic and abiotic factors influence colonization pattern of AM fungi in this Valdivian temperate forest.

In Table 2 and in the performed MCA (Fig. 5), it can be observed that species comprised in typically epiphytic families are non-mycorrhizal, while those included in terrestrial families have the highest colonization percentages. A similar tendency has been documented by other authors who established that epiphytic species are non-mycorrhizal or have significantly less colonization than terrestrial and saxicolous species [18, 27, 29, 31, 48]. It is important to mention that mycorrhizae were not completely absent in epiphytic ferns, terrestrial species occasionally growing in epiphytic habitats had AM. These findings support the idea that the distinction between non-mycorrhizal and mycorrhizal states is often species specific, with some phylogenetic groups being more likely to be colonized [31, 49]. Thus, there are different explanations for the phenomenon observed in this study: it is possible that the substrate in addition to environmental conditions influence the mycorrhizal status of the examined species, but it is also plausible that genetic factors play a considerable role in shaping fern–fungal associations in these plants, as was described by West *et al.* [28]. Moreover, it is probable that all these factors are interacting at the same time in a complex system that we still have to elucidate, so further studies are needed in order to determine which of them are more likely to be affecting endorhizal fungi (AM and DSF).

Dark septate endophytes have been reported in several plant species from different habitats, including some ferns [19, 20, 31, 41, 42]. The presence of these fungi in all the species considered in this work accords with such studies and demonstrates that DSE are ubiquitous in pteridophytes of Puerto Blest. Some basic structures seem to be constant for DSE colonization regardless of the host species, such as superficial networks and inter- and intracellular hyphae. Notwithstanding DSE microsclerotia have been described in other ferns and lycophytes [20, 41], as far as we are aware cerebriform microsclerotia have been observed only in pteridophytes growing in this Valdivian temperate rainforest [33, 34]. In most of the species analyzed in these three surveys DSE and/or AM hypha were

seen in root hairs. This phenomenon has been documented in a few other pteridophytes [41, 42], but it has not been previously reported for other vascular plant species in the Patagonian region.

These results in addition to other studies which documented different kinds of fungi within the root system of the same plant species [16, 17, 21, 30], indicate that ferns and lycophytes can be colonized by a variety of fungi, as was suggested by Winther *et al.* [50]. The fact that Hodson *et al.* [20] found that roots of different *Equisetum* species were commonly colonized by different endorhizal fungi, such as AM, DSE, hyaline septate endophytes and some other fungi which form structures similar to those of ectomycorrhizas (Hartig net and mantle), also sustains this idea.

### Concluding remarks

According to our findings, ferns and lycophytes of the Valdivian temperate region or Argentina can be colonized by DSE and AM. It seems that the mycorrhizal status (facultative, consistently mycorrhizal or non-mycorrhizal) and the morphological type (*Intermediate* or *Paris*) are related to evolutionary stages (eusporangiate or leptosporangiate) (Table 2, Fig. 5), as discussed by Zhao [23]. Eusporangiate species are facultatively mycorrhizal, present the lowest colonization percentages (Fig. 5, Group 1) and have an *Intermediate*-type AM [33]. Among leptosporangiate ferns, epiphytic species are non-mycorrhizal (Fig. 5, Group 2), whereas terrestrial species tend to be consistently mycorrhizal (Fig. 5, Group 3) with a *Paris*-type colonization pattern [34].

This review of the pteridophytic flora present in a Valdivian temperate forest of Patagonia pointed out that there are diverse and complex factors contributing to the mycorrhizal status of ferns and lycophytes (habit, environmental conditions, root morphology). Since the mycorrhizal status of these plants also seems to be related to their systematic position, it would be possible to use this feature as a phylogenetic marker [23, 26, 29]. It remains the aim of further studies to identify the fungal species forming these plant–fungal interactions and to analyze how they interact among themselves and with the host plant.

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