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Arbuscular mycorrhizal and dark septate fungi are not common in roots of epiphytic pteridophytes of a transitional forest area in South Brazil

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) and dark septate fungi (DSF) are symbionts that are associated with the roots of plants, including epiphytic lycophytes and ferns. Paris-type mycorrhiza and glomoid structures are the most common forms of colonization in these plants. This work aimed to evaluate the occurrence of these symbionts in the roots of epiphytic lycophytes and ferns as well as the diversity of AMF spores recovered from substrate associated with the roots of eleven species. Roots of *Asplenium gastonis, Campyloneurum aglaolepis, C. nitidum, Niphidium crassifolium, Pecluma pectinatiformis, Phlegmariurus mandiocanus, Pleopeltis hirsutissima, P. pleopeltifolia* and Selaginella *microphylla* had hyphae and vesicles typical of AMF colonization, but not arbuscules. *Campyloneurum nitidum, Pecluma pectinatiformis, Phlegmariurus mandiocanus, Pleopeltis pleopeltifolia* and *Selaginella microphylla* had melanized hyphae and microsclerotia typical of DSF. All species colonized by DSF were also colonized by AMF. Seventeen spore morphotypes of AMF were identified, of which six were acaulosporoid and eleven glomoid. *Glomus* aff. *formosanum* and Acaulospora aff. *lacunosa* were the most abundant and frequent species. Epiphytic lycophytes and ferns host concurrently AMF and DSF but colonization is scanty in their roots. For the first time, acaulosporoid spores and intraradical vesicles are reported for this group of plants.

Keywords: ferns, Glomeromycotina, lycophytes, mixed rain forest, native species, semideciduous forest

Introduction

In the forest, the light gradient is vertically stratified and some epiphytic species are exposed to high radiation while others grow in almost complete shade (Graham & Andrade 2004). Epiphytes have several adaptations for storing water and nutrients that enable them to occupy the forest canopy, including pseudobulbs (Orchidaceae), succulent roots, stems and leaves (Gesneriaceae, Piperaceae, Cactaceae, Orchidaceae), velamen (Orchidaceae, Araceae), and water-storing leaf rosettes (Bromeliaceae). Some ferns are poikilohydric (Hymenophyllaceae; *Pleopeltis*), others are covered with scales to reduce evapotranspiration (Fraga *et al.* 2008).

Arbuscular mycorrhizae occur in roots of angiosperms and gymnosperms, as well as some bryophytes, lycophytes and ferns (Souza *et al.* 2010), and are recognized as being responsible for the conquest of land (Remy *et al.* 1994; Taylor *et al.* 1995). In this type of association, more than one species of fungus can colonize the roots, and a single species of mycorrhizal fungi can colonize more than one plant species, separately or simultaneously (Chilvers *et al.* 1987; Wagg *et al.* 2008).

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Unlike what has been observed in terrestrial plants, where arbuscular mycorrhizal symbiosis is extremely common, the biological importance of this in plants occupying other niches is less clear. The epiphytic habitat poses different demands to plants compared to the terrestrial habitat and the slow growth rate in lycophytes and ferns (Nervo *et al.* 2019) should affect the relationship with the fungi. For forest epiphytes that naturally grow below the canopy, where there is less light, access to water depends on rainfall and relative humidity, and the availability of inorganic nutrients is influenced by morphological and chemical properties of the bark of the host tree (Reinert 1998).

Arbuscular mycorrhizal structures (Paris and intermediate morphotypes) have been observed in both the roots and gametophytes of lycophytes and ferns, which help them obtain water and mineral nutrients from environment (Muthukumar & Prabha 2013; Muthukumar *et al.* 2014). The leptosporangiate ferns are less dependent on symbiosis than those of early-diverging lineages are, and epiphytic species are unlikely hosts for mycorrhizal fungi because of the environment where they grow (Gemma *et al.* 1992; Lehnert *et al.* 2009). Despite that, colonization may reach percentages close to 60 % and 80 % in ferns and lycophytes, respectively (Muthukumar & Prabha 2013; Muthukumar *et al.* 2014; Muthuraja *et al.* 2014).

Like mycorrhizal fungi, another group of common root symbiotic fungi, the dark septate fungi (DSF), presumably affects their hosts in various ways (Jumpponen 2001). These fungi have intense dark pigmentation and form septate hyphae and microesclerotia that grow inter- and intracellularly in the plant cortex (Barrow & Aaltonen 2001). These fungi are widely distributed, are common in harsh environments, and may occur as saprophytic, mutualistic symbiotic or pathogenic species (Barrow & Aaltonen 2001; Mandyam & Jumpponen 2005).

Mixed colonization of DSF and AMF are common in land vascular plants (Fuchs & Haselwandter 2004; Cázares *et al.* 2005; Muthukumar *et al.* 2006; Postma *et al.* 2007; Muthukumar *et al.* 2014). Some authors suggest that DSF can establish associations similar to mycorrhizal fungi, acting as promoters of plant growth, especially by facilitating the absorption of nutrients like phosphorus and nitrogen (Scervino *et al.* 2009; Chen *et al.* 2010), including the production of extracellular enzymes capable of hydrolyzing complex compounds of carbon, nitrogen and phosphorus (Mandyam & Jumpponen 2005). Additionally, it has been suggested that the melanization of fungal cell walls and consequent production of dark hyphae, could be a factor that contributes to the adaptation and survival of the host plant under adverse or stressful conditions, since the melanin would play an important role in elimination of oxygen radicals generated during abiotic stress (Redman *et al.* 2002).

Knowledge about interactions between roots of epiphytic lycophytes and ferns and fungi is yet fragmentary because most of the studies was made in a few localities around the world and analysed a small number of species. Therefore, this study aims to evaluate the occurrence of AMF and DSF in roots of epiphytic lycophytes and ferns from a forest fragment of an ecotonal region (mixed rain forest and semideciduous forest). In addition, the diversity of glomerospores found around roots was also assessed.

Materials and methods

Study area

At the boundary between the second and third Paranaense plateaus is a transitional vegetation between mixed rain forest and semideciduous forest. The vegetation comprises, simultaneously *Araucaria angustifolia* (Bertol.) Kuntze (Araucariaceae), *Dicksonia sellowiana* Hook. (Dicksoniaceae) and large volume of epiphytes, characteristics of mixed rain forest in addition to *Aspidosperma polyneuron* Müll. Arg. (Apocynaceae), *Bougainvillea glabra* Choisy (Nyctaginaceae) and *Cabralea canjerana* (Vell.) Mart. (Meliaceae), typical species of the semideciduous forest (Medri *et al.* 2002; Dettke *et al.* 2008). This vegetation formation has an irregular canopy between 15 and 20 m and emergent trees up to 30 m, whose trunks have thick bark and sustain robust branches and a large, sparsely branched canopy (Veloso 1992). In the tree-shrub understory, there is an herbaceous layer where herbs, ferns, palms and epiphytes are scarce (Torezan 2002).

Located on the banks of the Tibagi River, the study area covers the municipalities of Telêmaco Borba (24°19'28" S - 50°36'59" W) and Ortigueira (24°13'30" S - 50°55'42" W), in the central-eastern region of the state of Paraná, at 1,150 m elevation, inside a transition zone between the subtropical and tropical climate (Cfa/Cfb), with averages annual temperatures and rainfall of 19.5 °C and 1700 mm, respectively (Mendonça & Danni-Oliveira 2002). The forest responds to the seasonal rhythm and in the unfavorable period of the year, the canopy trees shed their leaves, resulting in greater variation and light availability for the understory species, which affects the forest dynamics (Gandolfi 2003).

Plant collection and field data recovery

Plants were collected randomly within three years (2011-2013), mainly during spring and summer (September to March). Pteridophytes were carefully removed from the phorophyte using a knife. Information regarding to locality and environmental features where epiphytes were found, was noted. Four specimens of each species were collected in different sites/phorophytes along the area. These were placed in individual plastic bags and taken to laboratory, where the roots were separated from the rhizomes with the aid of a razor blade and preserved in 70 % alcohol for nearly one month. Rhizomes and leaves were herborized and two species of lycophytes and nine of ferns were identified by

Paulo Henrique Labiak Evangelista (Universidade Federal do Paraná). After that, the plants were submitted at the Herbarium of the Universidade Estadual de Maringá (HUEM). Table 1 summarizes information on the plant species investigated.

Evaluation of root colonization by AMF and DSF

The roots were washed in water, and then cleared in 10 % KOH, acidified with 5 % HCl and stained with trypan blue (Phillips & Hayman 1970). Initially, an assessment was made of the roots under a stereoscopic microscope, and the segments where there were points of fungal colonization were separated and observed under light microscopy. The number of points was recorded and the characteristic AMF (intra-radical hyphae, extra-radical hyphae, vesicles, and arbuscules) and DSF (dark hyphae, hyaline hyphae, microesclerotia) structures were identified. Colonization frequency in the root system (F %) was estimated according to Trouvelot *et al.* (1986).

AMF spore isolation and taxonomic identification

Glomerospores were extracted from 50 g of substrate near the roots, after natural drying, using the wet sieving technique (Gerdemann & Nicolson 1963) followed by centrifugation in 50 % sucrose solution (Jenkins 1964). Under a stereomicroscope, only intact and non-parasitized spores were separated and mounted on semi-permanent slides with resin with polyvinyl alcohol-lactic acid-glycerol (PVLG) or PVLG plus Melzer's solution (Morton *et al.* 1993). After mounting, the slides were kept in a drying oven (± 50 °C) for thirty hours.

Taxonomical identification was based on morphological characteristics of the spores and their wall layers composition, both observed under light microscopy. Original descriptions available on the Arthur Schüßler's website (http://www.amf-phylogeny.com/) and descriptions contained on the International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (https://invam.wvu.edu/) and Department of Plant Pathology, University of Agriculture Szczezin (http://www.zor.zut.edu.pl/Glomeromycota_2/Home.html) were used as support to identify the species.

Statistical analysis

Data were initially evaluated for linearity (visual inspection of residues), normality (Shapiro-Wilk) and homoscedasticity (Levene). As the data did not met the assumptions for parametric statistical analysis, they were subjected to a spearman correlation coefficient analysis. The adopted degree for significant differences was 5 %.

Table 1. Epiphytic lycophytes and ferns collected from a transitional area of Mixed Forest and Semidecidual Forest in South of Brazil and some morphological and ecological data. Plants are deposited at the herbarium of the Universidade Estadual de Maringá (HUEM)*.

Pteridophytes	Phorophyte				Environment
Family/Species	Voucher*	Stem/Root	Group[1]	Position	
Aspleniaceae					
Asplenium gastonis Fée	20367	erect/wiry	holoepiphyte	median trunk, inner crown	shade, partial light
Lycopodiaceae					
Phlegmariurus mandiocanus (Raddi) B. Øllg.	20388	erect/wiry	C-holoepiphyte	lower trunk, median trunk	shade, humid
Polypodiaceae					
Campyloneurum aglaolepis (Alston) de la Sota	24266	short-creeping/wiry	holoepiphyte	median trunk, inner crown	shade, humid
Campyloneurum nitidum (Kaulf.) C. Presl	24249	short-creeping/wiry	F-holoepiphyte	median trunk, inner crown	shade, humid
Microgramma squamulosa (Kaulf.) de la Sota	20467	long-creeping/wiry	C-holoepiphyte	inner crown, outer crown	light, shade
Microgramma vacciniifolia (Langsd. &Fisch.) Copel.	24248	long-creeping/wiry	C-holoepiphyte	inner crown, crown base	light
Niphidium crassifolium (L.) Lellinger	28134	long-creeping/wiry	C-holoepiphyte	lower trunk, median trunk	shade, humid
Pecluma pectinatiformis (Lindm.) M. G. Price	24287	short/long-creeping/wiry	C-holoepiphyte	median trunk	light
Pleopeltis hirsutissima (Raddi) de la Sota	24289	short-creeping/wiry	C-holoepiphyte	inner crown	light, humid
Pleopeltis pleopeltifolia (Raddi) Alston	24243	short-creeping/wiry	C-holoepiphyte	inner crown, median trunk	light
Selaginellaceae					
Selaginella microphylla (Kunth.) Spring	22237	prostrate/wiry	A-holoepiphyte	lower trunk	shade, humid

[1]C: characteristic; F: facultative; A: accidental; according to Bonnet *et al.* (2011)

Results and discussion

Almost all epiphytic species found in this study (except *Microgramma vacciniifolia* and *Niphidium crassifolium*) has distribution limited to South America (Michelon & Labiak 2013). Previous studies had been made in other continents where vegetation and climatic zones are too different. Studies evaluating the presence of AMF and DSF in epiphytic pteridophytes in South America were already made in Argentina (Fernández *et al.* 2010; Lugo *et al.* 2018), but in Brazil, despite the high biodiversity and cases of endemism in the most biomes, including Atlantic Forest (Freitas *et al.* 2016), it is a not reality.

Colonization by AMF and DSF was verified in the selected plants (Fig. 1), with the first group of fungi occurring in a greater number of species than the second. The majority of epiphyte species colonized by the AMF occur associated with the trunk and the internal part of the phorophyte crown (Tab. 1), places used by terrestrial animals that transit between the floor and the canopy, a condition that favors the introduction of mycorrhizal propagules (Lehnert & Kessler 2016). Five species of epiphytes showed dual colonization and a moderate correlation (Spearman Rank $R = 0.51$; $p \leq 0.05$) was observed between the frequencies of AMF and DSF in roots. Coexistence of these symbionts are known for all groups of plants, including pteridophytes; nevertheless,

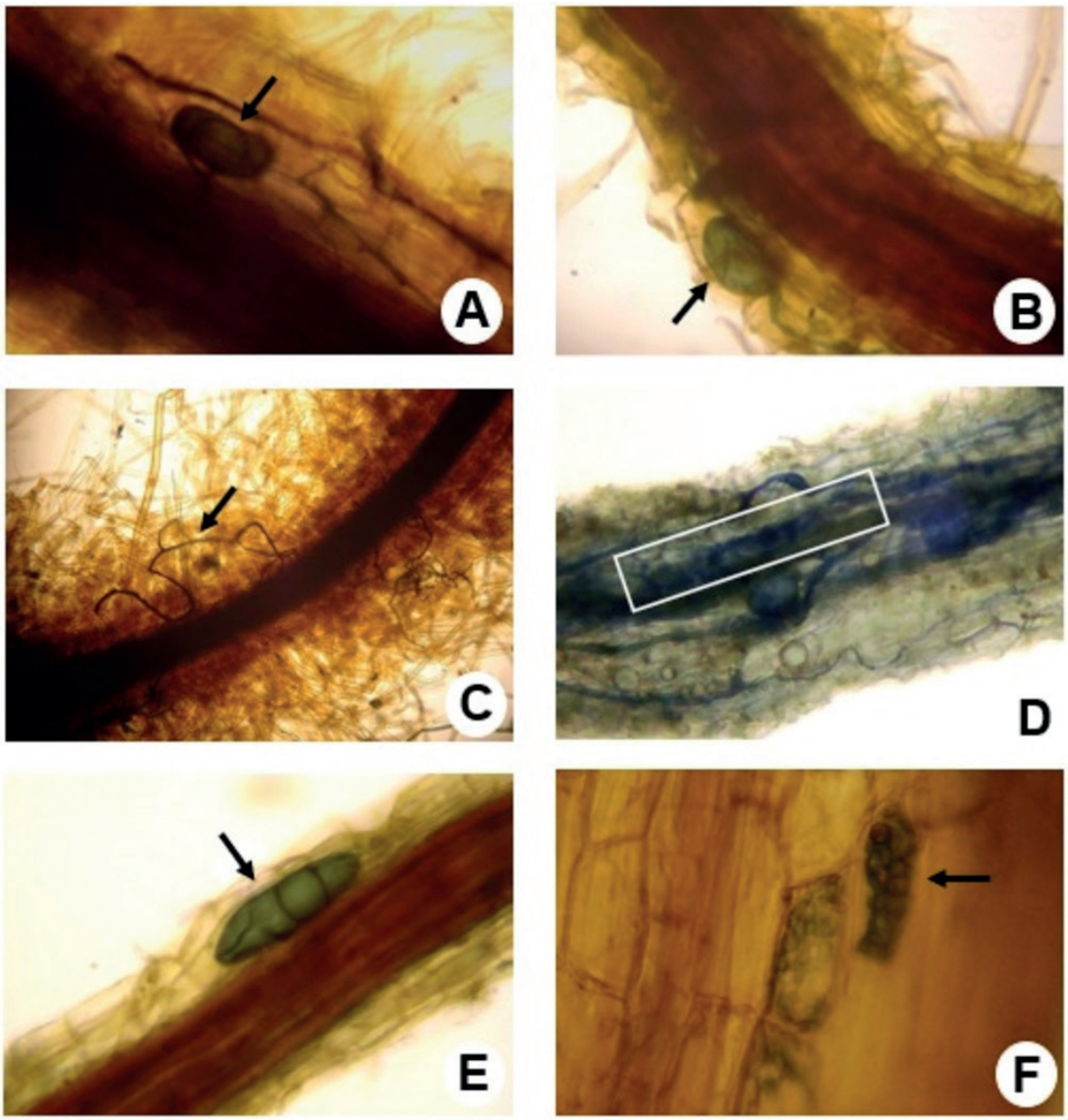

Figure 1. Details (arrows) of root colonization of epiphytic lycophytes and ferns by AMF and DSF. **A**) acaulosporoid vesicle in *Niphidium crassifolium*; **B**) glomoid vesicle in *Asplenium gastonis*; c) extra-radical hyphae of AMF in *Pleopeltis hirsutissima*; **D**) degenerating AMF arbuscules in *Niphidium crassifolium*; **E**) and **F**) microsclerotium of DSF in *Pecluma pectinatiformis* and *Phlegmariurus mandiocanus* respectively.

it is an unusual event in epiphytic lycophytes and ferns (Muthukumar & Prabha 2013; Muthukumar *et al.* 2014; Lara-Pérez *et al.* 2015).

Typical structures of arbuscular mycorrhizal colonization were observed in nine species but not all the examined individuals of a species were colonized (Tab. 2). Considering the frequency of occurrence (F %), four groups of species were recognized: 1) not colonized by AMF (0 %): *Microgramma squamulosa*, *M. vacciniifolia*); 2) marginally colonized (25 %): *Campyloneurum aglaolepis*, *Pleopeltis pleopeltifolia*, *P. hirsutissima*, *Selaginella microphylla*); 3) occasionally colonized (50 %): *Asplenium gastonis*, *Pecluma pectinatiformis*, *Phlegmariurus mandiocanus*); widely colonized (≥75 %): *Niphidium crassifolium*, *Campyloneurum nitidum*).

Extra-radical hyphae linked to the epidermis were observed in five of the nine colonized species; intra-radical hyphae and vesicles were observed in all species colonized. Two vesicle morphologies were detected, acaulosporoid and glomoid (Fig. 1A, B) which matches the groups of AMF species found near the roots (Tab. 2). Arbuscules were not found, although they have been observed in other species of pteridophytes, including epiphytes (Dickson *et al.* 2007; Muthuraja *et al.* 2014). In the absence of arbuscules, hyphal coils and arbusculate coils usually transfer nutrients to ferns (Dickson *et al.* 2007), but these were also not observed in this study. The absence of such structures may indicate that the mycorrhizal symbiosis is or, in that circumstance, was functionally neutral (Brundrett 2009).

The vesicles are usually formed after the arbuscules (Montero *et al.* 2019), and as these have a half-life ranging from two to twelve days (Gadkar *et al.* 2001), it is believed that arbuscules were not observed because they had already degenerated. The dark coloration of the roots and the lack of a complete clearing of the cortex (Fig. 1C) may have made it difficult to see the arbuscules, especially if they were in an advanced degree of senescence. In the thinner roots, clearing was more efficient and mycorrhizal colonization could be more easily evidenced. In some regions of these roots, structures resembling degenerating arbuscules were observed (Fig. 1D), but the small incidence of these does not enable us to confirm their identity.

The frequency of the mycorrhizal colonization of the root segments was low, ranging from 3.33 to 16.67 %, similarly to the values found by Lara-Pérez *et al.* (2015), but much lower than those observed in epiphytic lycophytes and ferns of other studies (Fernández *et al.* 2010; Muthukumar & Prabha 2013; Muthukumar *et al.* 2014; Muthuraja *et al.* 2014). Factors such as availability of infective propagules, root morphology, host plant identity, growth rate and degree of mycorrhizal dependence may have contributed to the low colonization percentages found in this study. The use of different methods (grid-line intersect, root slide technique, magnified intersections) in other studies for assessing root colonization by AMF could also contribute to these differences.

Absence or low frequency of mycorrhizal propagules in the atmosphere are factors that can restrict the establishment of the association. The vertical dispersion of AMF depends on animals, moving from the ground to the canopy (Mangan & Adler 2002), or wind (Chaudhary *et al.* 2020), but the second condition is expected to be fairly inefficient inside a forest (Egan *et al.* 2014). In this inventory, the low number of spores of AMF (Tab. 3) could be due to both the limited entry of propagules into the tree crowns, fact recognized as critical by Muthukumar & Prabha (2013), and restriction of colonization and sporulation mediated by plants through shortening transfer of photoassimilates to the fungi.

The richness of AMF was modest, and the composition of the communities varied greatly among and within the epiphytic species (Tab. 3). Seventeen species (or morphotypes) were identified, six of acaulosporoid spores and eleven of glomoid spores. *Acaulospora* aff. *lacunosa* and *Glomus* aff. *formosanum* were present in three host

Table 2. Occurrence of fungal structures and mean percentages of colonization in roots of epiphytic lycophytes and ferns collected from a transitional area of Mixed Rain Forest and Semidecidual Forest in the South of Brazil.

Epiphytes	Arbuscular mycorrhizal fungi						Dark septate fungi				
	FO	AP	IH	VE	AR	F%	FO	DH	HH	MC	F%
Asplenium gastonis	2	+	+	+	-	3.33	0	-	-	-	0.00
Campyloneurum aglaolepis	1	-	+	+	-	3.33	0	-	-	-	0.00
Campyloneurum nitidum	3	+	+	+	-	8.89	3	+	-	+	4.44
Microgramma squamulosa	0	-	-	-	-	0.00	0	-	-	-	0.00
Microgramma vacciniifolia	0	-	-	-	-	0.00	0	-	-	-	0.00
Niphidium crassifolium	4	+	+	+	-	8.34	0	-	-	-	0.00
Pecluma pectinatiformis	2	+	+	+	-	16.67	2	+	-	+	10.00
Phlegmariurus mandiocanus	2	-	+	+	-	5.00	1	+	-	+	3.33
Pleopeltis hirsutissima	1	+	+	+	-	6.67	0	-	-	-	0.00
Pleopeltis pleopeltifolia	1	-	+	+	-	3.33	1	+	-	+	6.67
Selaginella microphylla	1	-	+	+	-	3.33	1	+	-	+	6.67

n=4; + presence; - absence; MNS: mean number of spores; FO: frequency of occurrence (when two or more replicates were colonized, means were obtained from these number only); AP: appressorium; IH: intra-radical hyphae; VE: vesicles; AR: arbuscules; DH: dark hyphae; HH: hyaline hyphae; MC: microesclerotia; F % = colonized number of segments/total number of segments x 100.

Table 3. Composition of arbuscular mycorrhizal fungi found in the rhizosphere of epiphytic lycophytes and ferns collected in a transitional area of Mixed Rain Forest and Semidecidual Forest in South of Brazil.

Species of AMF	Epiphytes											TE	ANS
	Ag	Ca	Cn	Ms	Mv	Nc	Pp	Pm	Plp	Plh	Sm		
Acaulospora aff. *cavernata*	-	-	-	-	-	-	8	-	-	-	-	1	1
Acaulospora aff. *colossica*	-	-	-	-	-	-	-	-	1	-	-	1	1
Acaulospora aff. *gedanensis*	-	-	2	-	-	-	-	-	-	-	-	1	2
Acaulospora aff. *lacunosa*	-	-	1	-	-	4	-	-	-	16	-	3	21
Acaulospora morrowiae	-	-	-	-	-	-	3	1	-	-	-	2	4
Acaulospora sp. (*morrowiae* like, M^-)	-	-	-	-	-	-	4	-	-	-	-	1	4
Claroideoglomus aff. *candidum*	-	-	-	-	-	1	-	-	-	-	-	1	1
Claroideoglomus aff. *luteum*	-	-	-	-	-	-	-	-	-	2	-	1	2
Funneliformis aff. *geosporum*	-	-	-	-	-	-	-	-	-	1	-	1	1
Glomus aff. *formosanum*	-	-	9	-	-	6	-	-	-	6	-	3	21
Glomus aff. *rubiformis*	-	-	3	-	-	-	-	-	-	-	-	1	3
Glomus invermaium	-	-	-	-	-	-	-	-	-	1	-	1	1
Glomus macrocarpum	-	-	-	-	1	-	-	-	-	-	-	1	1
Glomus sp. (tubaeforme-like)	-	-	S	-	-	-	-	-	-	-	-	1	+500
Glomus sp. (inner layer M^+)	-	-	1	-	-	1	-	-	-	-	-	2	2
Glomus sp. (spines in inner layer)	-	-	-	-	-	-	-	-	-	4	-	1	4
Glomus sp. (conical pustules)	21	-	-	-	-	-	-	-	-	-	-	1	21
Total number of species	1	-	6	-	1	4	3	1	1	6	-		
Total number of spores	21	-	16	-	1	12	15	1	1	30	-		
Total number of sporocarps	-	-	1	-	-	-	-	-	-	-	-		

TE: total of epiphytes; ANS: accumulated number of spores (n=4); Ag: *Asplenium gastonis;* Ca: *Campyloneurum aglaolepis;* Cn: *Campyloneurum nitidum;* Ms: *Microgramma squamulosa;* Mv: *Microgramma vacciniifolia;* Nc: *Niphidium crassifolium;* Pp: *Pecluma pectinatiformis;* Pm: *Phlegmariurus mandiocanus;* Plp: *Pleopeltis pleopeltifolia;* Plh: *Pleopeltis hirsutissima;* Sm: *Selaginella microphylla*. $M+$ = positive reaction to Melzer; M^- = negative reaction to Melzer. *S* = sporocarp with more than 500 spores.

species, and *Pleopeltis hirsutissima* and *Campyloneurum nitidum* were the epiphytes that showed the greatest species richness of AMF (six each). In three species, only one spore was found, which might mean accidental presence. Five other species showed 12 to 30 spore communities, with the highest number of spores occurring in the epiphytes with more weakly colonized roots. *Glomus* sp. (tubaeforme-like) and *Glomus* aff. *rubiformis* produce hundreds of spores in sporocarps and, in this inventory, both were found only in one species, *Campyloneurum nitidum*.

In an inventory made by Muthukumar & Prabha (2013), in which they evaluated 54 species of lycophytes and ferns, with different life forms (epiphytes, terrestrial, aquatic and saxicolous) and coming from five sites, a total of nine spores morphotypes (species) of AMF were identified, all belonging to glomoid genera. In our study, spores of *Acaulospora* are for the first time extracted from substrate around the roots of lycophytes and ferns. Although acaulosporoid vesicles were also observed intraradically, it is not possible to affirm that those spores were formed by the root-colonizing fungi.

Root morphology is considered an important factor in establishing the arbuscular mycorrhizal association in terrestrial plants (Baylis 1975; Manjunath & Habte 1991). Lycophytes and ferns of epiphytic habit may present two main morphologies, fat and fleshy roots, usually colonized by fungi (AMF, DSF, Mucoromycotina), or thin and wiry roots, less susceptible to association (Pressel *et al.* 2016). The plants of the present study have roots of the second type, which, in addition to having a smaller diameter and greater mechanical resistance, also have cortical cells impregnated with phenolic compounds and thickened walls (Schneider 2000), which can hinder the entry and formation of intra-radical hyphae.

No relation was found between the occurrence of colonization and the taxonomic groups of epiphytes studied. The small sample size may be a reason for that. Lycopodiaceae, Selaginellaceae, Aspleniaceae and Polypodiaceae had roots colonized by AMF and no relationship trend between percentage of root colonization and growth habit (erect, creeping or climbing stems). However, the higher percentages were observed in plants on low trunk, medium trunk and inner crow, regions most likely used by animals dispersing mycorrhizal propagules.

Roots of five species showed intra-radical structures of dark septate fungi (Fig. 1E, F). The frequencies (F %) of colonization of the root system were low, ranging from 1.67 to 10 % (Tab. 2). *Pecluma pectinatiformis* was the species with the highest frequency of colonization by DSF, followed by *Selaginella microphylla*, and *Pleopeltis pleopeltifolia*. Muthukumar & Prabha (2013) and Lara-Pérez *et al.* (2015) did not find DSF in roots of epiphytic ferns and lycophytes. Conversely, Muthukumar *et al.* (2014) found frequencies that varied from 30 to 80 %.

The data from this study show that colonization of epiphytic lycophytes and ferns roots by AMF and DSF is incipient. In the case of AMF, this can result from a

low inflow of propagules along the phorophyte just as a low mycorrhizal plant dependency, due to the various mechanisms of adaptation to the air environment. Further, it is the first time that the presence of acaulosporid spores and intraradical vesicles was related to this group of plants.

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