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Occurrence of arbuscular mycorrhizal fungi on King George Island, South Shetland Islands, Antarctica

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ABSTRACT

Arbuscular mycorrhizal fungi make up an important ecological niche in ecosystems, and knowledge of their diversity in extreme environments is still incipient. The objective of this work was to evaluate the density and diversity of arbuscular mycorrhizal fungi in the soil of King George Island in the South Shetland Islands archipelago, Antarctica. For that, soil and roots of *Deschampsia antarctica* were collected at the brazilian research station in Antarctica. The spore density, species diversity and mycorrhizal colonization in the roots were evaluated. There was a low density of spores (27.4 ± 17.7) and root mycorrhizal colonization (6 ± 5.1%), which did not present statistical difference. Four species of arbuscular mycorrhizal fungi were identified, distributed in two genera: three species of the genus Glomus (*Glomus* sp1, *Glomus* sp2 and *Glomus* sp3) and one of the genus *Acaulospora*, which was identified at species level (*Acaulospora mellea*). Greater soil diversity was verified with pH 5.9 and phosphorus concentration of 111 mg dm^{-3}, occurring two species of genus *Glomus* and *A. mellea*. Based on literature data, this may be the first record of this species of *Acaulospora mellea* in Antarctic soils, colonizing *D. antarctica* plants.

Key words: Antarctica, *Deschampsia antarctica*, low temperatures, mycorrhizal fungi.

INTRODUCTION

Antarctica is the largest ice reserve in the world, with an area of 14 million km^2 and about 95% of the continent covered by ice that concentrates approximately 70% of the fresh water of the planet. The region is considered the global thermal regulator, due to its direct influence on the climate that controls the atmospheric and oceanic circulation, being one of the few terrestrial habitats without anthropic interferences (Delille et al. 2004).

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The Antarctic continent presents extreme edaphoclimatic limitations, which regulate the biodiversity of plants and organisms in the soil. The limiting conditions in this region condition a limited flora, composed almost exclusively of lichens, bryophytes and two species of vascular plants of angiosperms, one Caryophyllaceae *Colobanthus quitensis* (Kunth) Bartl. and one Poaceae *Deschampsia antarctica* Desv. which predominates on the surfaces of the large rocky blocks (Lewis-Smith and Poncet 1985, Gonçalves et al. 2008). The low number of plant species limits the diversity of organisms and microorganisms (still little known), which regulate the flow of energy, maintaining the balance in this ecosystem (Parniske 2008).

Microorganisms are widely distributed in all terrestrial ecosystems, and are studied according to their importance and accessibility, being grouped according to their characteristics, functions and genetic similarities. In the evolutionary sequence of eukaryotes, the group of microorganisms that form the most intimate and durable symbiosis in nature are arbuscular mycorrhizal fungi (AMFs) (Allen 1996, Smith and Read 2008). This symbiosis is reported in fossil evidence, which shows the presence of AMF structures associated with *Notophytum krauselii* in Antarctic soil as early as the Triassic period of approximately 400 million years (Harper et al. 2015).

The AMF establish a mutualistic symbiotic association with the roots of most species of plants, consisting in symbiosis more widespread in nature, it is found in nearly all ecosystems (Siqueira et al. 2007, Sousa et al. 2010). Despite being an important and common symbiosis, the study of diversity, biology and ecology of AMFs in inhospitable conditions of pressure and temperature are still incipient. The greatest abundance of biodiversity information of microorganisms in extreme conditions is attributed to culturable fungi, as in a study of the soil of the Antarctic region (Arenz and Blanchette 2011).

The composition and dynamics of AMF communities have a strong impact on the structure and diversity of plant communities, both in natural ecosystems and managed (Jeffries et al. 2003). The greatest diversity of species of AMFs, can assist in the survival of plant species under biotic stress conditions and abiotic (Smith and Read 2008, Hiiesalu et al. 2014). In extreme regions with arid environments, cold and windy with terrestrial habitats covered by ice and snow for several months of the year are usually found in poor soil nutrients, which limits the development of vegetation (Duc et al. 2009). In environments of high latitudes, which condition selective pressure on plants and, consequently, reduces the formation of mycorrhizal symbiosis (Newsham et al. 2009).

Despite the importance of mycorrhizal symbiosis in environments under limiting conditions, the number of information regarding the evolution of this association, its ecological dynamics and the biological structure of AMFs in the Antarctic Continent are scarce. Considering the great relevance of this ice reserve to global balance, knowledge of the diversity and biological structure in this habitat is fundamental to a better understanding of the dynamics within this ecosystem, making possible the elaboration of a preservation strategy for this natural resource.

The presence of *D. antarctica* is frequent in this region of Antarctica, including in places where the megafauna of mammals and birds is concentrated, and certainly this species of plant assists in the presence of this biodiversity above and below the ground (Gonçalves et al. 2008). Therefore, the present study objective to evaluate the occurrence of arbuscular mycorrhizal fungi (AMFs), from soil from King George Island, South Shetland Islands archipelago, Antarctica.

MATERIALS AND METHODS

This study was conducted in six geo-referenced areas (latitude of 62°4'58.8''S and longitude of 58°23'24.4''O and altitude of 2-19 m) on King George Island, South Shetland Islands archipelago, Antarctica. The sampling area is located on the Keller Peninsula, Admiralty Bay, whose region temperatures are extremely low, with an annual average varying around -10°C. The areas of collection of samples are located near the Antarctic Station Commander Ferraz, of the Brazilian Antarctic Program (PROANTAR). In the collection areas there is no structured soil system, as normally we know in tropical and temperate regions, but rather a cluster of particulate rock/sandy material in different sizes under larger rocks, where the aerial part of the plants is allocated. For all collection areas, it was collected the soil (fine-particulate rocks) adhered to the rhizospheric systems of the plants, which is about 10 cm deep.

The soil and root samples, with ten replicates in each area, were collected from the rhizospheric region of *Deschampsia antarctica* Desv., an endemic species in this region, that occur from the coast of the island to the highest areas near the bare rock or in ice formations (Gielwanowska et al. 2005, Gonçalves et al. 2008).

The samples were collected, identified, conditioned and sent to the laboratory for analysis where they were stored in a cold camera at 4ºC. Then the analysis and characterization of the soil chemical attributes were carried out.

The evaluation of the density of mycorrhizal fungi spores (AMFs) was performed by the wet sieving technique as Gerdemann and Nicolson (1963). The diversity of species was defined based on morphological characteristics of the spores (color, size and number of walls), according to the description of the INVAM (2016, http://www.invam.wvu.edu/2016).

The mycorrhizal colonization rates were obtained using 1g of fine roots per replicate were washed clarified KOH (10%, v/v) and stained with trypan blue in lactoglycerol (0.05%, v/v), according to Koske and Gemma (1989), determining the colonization by the technique of intersection in reticulated plates (Phillips and Hayman 1970, Giovannetti and Mosse 1980).

Data were submitted to analysis of variance, using the significance level of 5%. The values for the percentage of mycorrhizal colonization and number of spores were transformed into Log (X). Afterwards, the means were compared by the test (Tukey 5%), using the statistical program SISVAR (Ferreira 2011).

RESULTS AND DISCUSSION

Biodiversity studies have shown the cosmopolitan character of the AMFs in the most diverse habitats (Smith and Read 2008). The present study reinforces this assertion by showing the existence of this fungus group in soil samples and roots of the *D. antarctica* species on King George Island (Table I and Figure 1). These data show the occurrence of AMF species colonizing *D. antarctica* plants in this region of Antarctica.

Mycorrhizal colonization and spore density of AMF did not show a significant difference ($p \leq 0.05$) between the studied areas, whose mean density ranged from 10 to 33 AMF spores in 50g of soil and from 3.5 to 8.8% for mycorrhizal colonization (Table I). In spite of having a widespread occurrence in most ecosystems, arbuscular mycorrhizal fungi are influenced by biotic factors (metabolism and physiology of AMF species and host species) and abiotic factors (pH, phosphorus content and temperature), which interfere with the multiplication of spores and in the process of root colonization.

In the present study the extreme conditions of the area were possibly the factors that conditioned

TABLE I
Evaluation of the study areas, determining the number of spores, mycorrhizal colonization, AMFs species diversity, and chemical characterization of the studied soil.

Areas*	pH	P	K	Ca	Mg	OMS	no. of spores	colonization	AMFs Species
	H2O	--mg dm^{-3}--		--cmolc dm^{-3}--		g kg^{-1}	50 mL dm^{-3}	%	
1	5.5	223	216	2.60	4.60	21,1	32 a	7.1 a	*Acaulospora mellea* *Glomus* sp1
2	5.6	144	168	3.60	4.60	20,0	10 a	8.8 a	*Glomus* sp3
3	5.8	93	124	2.00	3.60	18,2	31 a	4.1 a	*Glomus sp3*
4	5.4	129	144	2.00	4.30	21,0	30 a	5.4 a	*Acaulospora mellea*
5	5.5	96	108	7.70	6.10	18,9	33 a	4.8 a	*Glomus* sp2
6	5.9	111	128	13.70	9.70	20,7	13 a	3.5 a	*Acaulospora mellea* *Glomus* sp1 *Glomus* sp2

*Mean followed by the same letter in the column, do not differ between them by the Tukey test at 5% of significance. OMS: Organic matter soil.

the low spore density and the establishment of symbiosis (mycorrhizal colonization), which may be reduced or even not occurring (DeMars and Boerner 1995). The high levels of phosphorus observed in the areas (93.05 to 222.73 mg dm^{-3}) are consistent with study by Cury et al. (2015) and low temperature and plant species diversity may have affected mycorrhizal symbiosis (Table I).

The very low temperature inhibits the enzyme activity, twinning of spores and mycelium growth of AMF in the soil, limiting the mycorrhizal colonization. This has been verified in a study by Upson et al. (2008), where the low temperature conditions had a low 10% mycorrhizal colonization in roots of *D. antarctica* plants in Southern Georgia.

The reduction of the activity of the AMF under conditions of thermal stress has been evidenced in other studies, as observed in the region of the Shetland Islands in the parallel 63S, with the presence of AMFs (arbuscules) in roots of *D. antarctica* (Upson et al. 2008). This result corroborates this study, which were also observed AMF structures (vesicles and hyphae) colonizing plants of *D. antarctica* in parallel 62S (Figure 1).

The functional compatibility between the mycorrhizal symbiosis and the phosphorus level in the soil solution controls the kinetic parameters of the absorption and influx of P to the plants, and may favor or not the establishment of symbiosis (Silveira 1990, Silveira and Cardoso 2004). The phosphorus can inhibit or completely limit the formation of symbiosis, causing different responses of AMF in the growth of host plants (Parniske 2008, Smith and Read 2008). This is well documented in tropical and subtropical soils, however, to the soil in the study area there is no information relating to these interactions.

The high P content observed in this study may have influenced spore production and extraradicular mycelial growth of AMF in the soil (Siqueira et al. 2007). However, the magnitude of these effects is influenced by the host species and environmental factors, in particular the irradiation (Smith and Read 1997), which is an important factor and

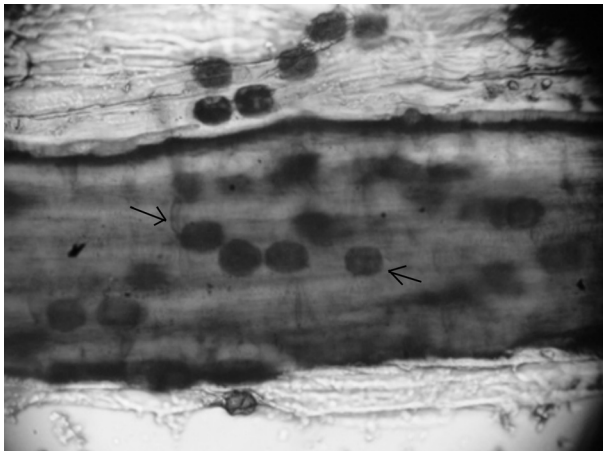

Figure 1 - Root colonized by arbuscular mycorrhizal fungi (AMFs), observed during the evaluation of the roots of *D. antarctica*. The arrows indicate the presence of vesicles (storage structures) inside the roots.

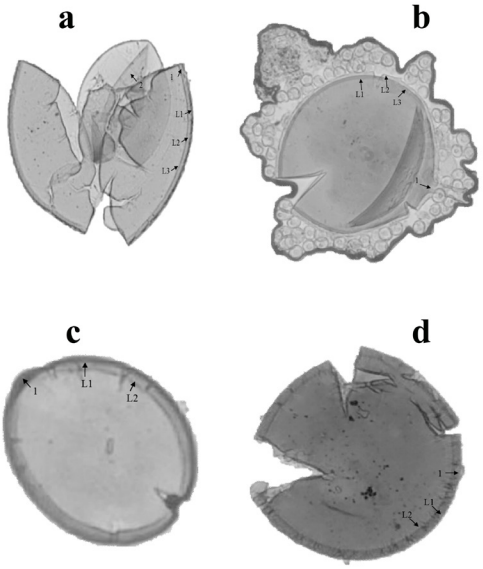

Figure 2 - Species of arbuscular mycorrhizal fungi (AMFs) identified in the study areas, **(a)** *Acaulospora mellea* – spore without ornamentation, presence of scar (1a), reaction with melzer (2a) and wall composition with three layers (L1, L2 and L3); **(b)** *Glomus sp1* – glomus type spore (1b), without ornamentation, hyaline and with three layers on the wall (L1, L2 and L3); **(c)** *Glomus sp*2 – small scale spore of 100µm, glomoide type (1c), without ornamentation, with thick layer of two layers (L1 and L2); **(d)** *Glomus sp3* – large spore in 100µm scale, glomid type (1d), without ornamentation, with thick wall and two layers (L1 and L2).

can influence even more than the light intensity, being able to promote high levels of mycorrhizal colonization of the roots due to the photosynthetic activity of the plant and the availability of photosynthates to the AMFs. However, luminosity directly interferes with the rate of colonization and sporulation of fungi (Parniske 2008). In addition, the AMF species depend on the photosensitivity of the plants, which regulates the photosynthetic activity, being able to determine the diversity of plant species in low light intensity environments.

In the studied areas four AMF species were recovered, whose identification is based on morphological characteristics according to the International Collection of Arbuscular Mycorrhizal Fungi Culture (INVAM) (Table I and Figure 2). These results show that species of these two genera present greater tolerance to the adverse conditions of soil and climate, especially *Acaulospora mellea* present in 3 of the 6 studied areas. Other previous studies, Cabello et al. (1994) has also demonstrated the presence of *Glomus antarcticum*, associated with *D. antarctica* plants in the Antarctic Western Penisula.

The low plant diversity of the studied region is possibly associated with temperature conditions, which influenced the frequency and occurrence of AMFs in the studied region (Newsham et al. 2009, Silveira and Cardoso 2004). Another important factor verified in the areas of this study was the presence of soil mesofauna species, especially mites and nematodes. These beings are permanent heterotrophic in these areas, which can be found throughout the year in regions of low temperatures (Arenz and Blanchette 2011). The presence of nematode species in Antarctic soil has been reported in a study since 1984 by Gray and Lewis Smith (1984) and their functions need to be better understood.

In total, four AMFs species were identified, distributed in two genera: three species belonging to the genus *Glomus* (*Glomus* sp1, *Glomus* sp2

and *Glomus* sp3) and one species of the genus *Acaulospora*, which was identified at the species level (*Acaulospora mellea*), based on morphological patterns of the spore wall (Figure 2). This result reinforces the potential of these genera to present a wide geographic distribution, even in adverse conditions as presented in this study.

Even though forming one of the most important symbioses in nature, the AMFs are still poorly exploited in adverse temperature and pressure environments, and there is a greater range of information regarding the distribution and abundance of other groups of microorganisms, as observed for fungus cultivars in soil in the Antarctic region of Antarctic Peninsula (Arenz and Blanchette 2011). Based on data from the literature, this study is certainly one of the first studies to report the presence of the species *Acaulospora mellea*, colonizing *D. antarctica* in this geographical location of the 62S parallel of King George Island.

The results found in the present study reinforce that the AMFs are present in different terrestrial environments. And certainly this group of fungi plays important ecological roles in the regions of Antarctica (Arenz and Blanchette 2011). However, there is a need for research to determine what the diversity of AMFs in these regions and their ecological function, their contribution to the development of *D. antarctica* plants, and how climate change is affecting the distribution of this important symbiosis in the region of Antarctica. This demonstrates the importance of deepening the research, within the scope of PROANTAR, on the occurrence of mycorrhiza associated with vascular plant species in the region of the so-called Maritime Antarctica, where the South Shetland Islands archipelago is located.

CONCLUSIONS

The studied areas showed low spore density and mycorrhizal colonization of the roots of *D. antarctica* plants.

Three species of *Glomus* and one *Acaulospora mellea* were recovered in the soil.

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