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**DIVERSIDADE MORFOLÓGICA E GENÉTICA EM DIFERENTES
ESPÉCIES DE MUSGOS DA ILHA NELSON (ANTÁRTICA)**

DISSERTAÇÃO DE MESTRADO

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**DIVERSIDADE MORFOLÓGICA E GENÉTICA EM DIFERENTES
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Orientador: Prof. Dr. Valdir Marcos Stefenon

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RESUMO

A Antártica é o continente mais inóspito do planeta, sua flora consiste basicamente em líquens, algas, fungos e briófitas, o continente tem apenas 14 milhões de km² e a maior parte recoberta por gelo. O verão é curto e frio, com chuvas e precipitação acentuada de neve. Estas condições, em conjunto com um inverno escuro e prolongado, limitam a ocorrência de espécies vegetais na região, uma vez que estas condições dificultam o ciclo reprodutivo. A Ilha Nelson é uma ilha de 19 quilômetros de extensão e 11 quilômetros de largura, situada a sudoeste da Ilha do Rei George nas Ilhas Shetland do Sul, Antártica. As diferenças fenotípicas e genéticas entre as populações de briófitas podem fornecer informações sobre o processo adaptativo de cada população particular, relacionada com a pressão ambiental a que estão expostos. Para determinar a importância da dispersão de propágulos reprodutivos neste padrão de diferenciação, estudos genéticos baseados em marcadores microssatélites foram testados em nosso laboratório. Esta dissertação apresenta dados referentes à diversidade morfológica populacional de três musgos da Ilha Nelson: *Andreae gainii* Cardot. (Andreaeaceae), *Ptychostomum pseudotriquetrum* (Hedw.) J. R. Spence & H.P. Ramsay ex Holyoak & N. Pedersen (Bryaceae) e *Polytrichum juniperinum* Hedw. (Polytrichaceae). Aspectos ecológicos e ambientais relacionados à variação morfológica foram discutidos. E apresenta também os resultados de uma análise molecular baseada em marcadores microssatélites, de sete populações de *P. juniperinum* coletadas na Ilha Nelson. Foram discutidas as barreiras para fluxo gênico e a diversidade intrapopulacional da espécie.

Palavras-chave: musgos, microssatélites, variação morfológica, variação genética

ABSTRACT

Antarctica is the most hostile continent on the planet. Its flora consists primarily of lichens, algae, fungi and bryophytes. The continent has only 14 million km², mostly covered by ice. The summer is short and cold, with rains and severe snowfall. These conditions, together with a long dark winter limit the occurrence of plant species in the region, since these conditions inhibit the reproductive cycle. The Nelson Island is a 19 miles long and 11 miles wide island, located southwest of King George Island in the South Shetland Islands, Antarctica. Phenotypic and genetic differences among populations of bryophytes can provide information about the adaptive process of each particular population in relation to environmental stress to which they are exposed. To determine the importance of the dispersion of reproductive propagules this pattern of differentiation, genetic studies based on microsatellite markers were tested in our laboratory. This dissertation presents data concerning the population morphological diversity of three mosses Nelson Island: *Andreae gainii* Cardot. (Andreaeaceae), *Ptychostomum pseudotriquetrum* (Hedw.) JR Spence & Ramsay HP ex Holyoak & N. Pedersen (Bryaceae) and *Polytrichum juniperinum* Hedw. (Polytrichaceae). Ecological and environmental aspects related to morphological variation were discussed. It also presents the results of molecular analysis based on microsatellite markers of seven populations of *P. juniperinum* collected on Nelson Island. Barriers to gene flow and intrapopulational diversity of the species were discussed.

Key-words: moss, microsatellites, morphological variation, genetic variation

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APRESENTAÇÃO

Na seção **INTRODUÇÃO GERAL**, consta uma revisão da literatura que apresenta o estado da arte dos temas abordados nesta dissertação.

A metodologia utilizada e os resultados obtidos neste trabalho estão apresentados sob a forma de artigos científicos, os quais se encontram na seção **MANUSCRITOS**. Nessa seção constam as seções: Material e Métodos, Resultados, Discussão e Referências Bibliográficas.

A seção **CONCLUSÕES GERAIS** encontra-se no final desta dissertação, e apresenta interpretações e comentários gerais sobre os resultados dos manuscritos presentes neste trabalho.

As **REFERÊNCIAS** referem-se somente às citações que aparecem nos itens **INTRODUÇÃO GERAL** e **CONCLUSÕES GERAIS** desta dissertação.

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1. INTRODUÇÃO GERAL

1.1. Antártica e Estudos Antárticos

A Antártica é o mais meridional dos continentes e um dos menores, com uma superfície de catorze milhões de quilômetros quadrados (FIGURA 1a). Sua formação se deu pela separação do antigo supercontinente Gondwana há aproximadamente 100 milhões de anos e seu resfriamento aconteceu nos últimos 35 milhões de anos (CYGAN, 1981). A Antártica compreende todas as terras localizadas abaixo do paralelo 60°. Foi o último continente a ser descoberto pelo homem e, por isso, o conhecimento dos recursos naturais e biológicos está ainda em fase inicial de exploração. É o continente mais frio, mais seco, com a maior média de altitude e maior índice de ventos fortes do planeta. A temperatura mais baixa da Terra (-89,2 °C) foi registrada na Antártica (RAKUSA-SUSZCZEWSKI, 2002; CYGAN, 1981; KOVALSKI, 1985). Para Pereira & Putzke (1994), estas condições, em conjunto com as impostas pelo inverno escuro e prolongado limitam a ocorrência de espécies vegetais na região, especialmente plantas com flor, uma vez que estas condições impedem o ciclo reprodutivo.

O continente Antártico apresenta uma das condições climáticas mais extremas entre os ambientes do mundo a respeito da temperatura (média anual de 0°C a 2°C na Antártica marítima e de -30°C a -65°C na região central; BEDNAREK OCHYRA et al., 2000, ROZEMA et al., 2005), a incidência de luz (apenas 15-20 semanas de incidência de luz por ano; OCHYRA et al., 1998), a disponibilidade de água (precipitação anual de 300-500mm, principalmente como a neve; ROZEMA et al., 2005) e incidência de raios UV (ROZEMA et al., 2005).

Este continente rodeia o Pólo Sul e é cercado pelo Oceano Antártico, que fica entre o Oceano Pacífico e o Atlântico. Devido ao frio intenso com ventos violentos, esta região, permanentemente coberta pelo gelo, possui condições desfavoráveis para quase todo meio de vida. Atualmente, são 29 países que possuem bases científicas instaladas na Antártica. Aproximadamente mil cientistas fazem diversos experimentos na região e o Brasil é um destes países. As principais dificuldades para o crescimento dos vegetais na Antártica são os fortes ventos, a curta espessura do solo e a limitada quantidade de luz solar, durante o inverno (AVÉROUS, 1993; CAPOZOLI, 1991). Fisicamente, ela é dividida em duas partes pelos

montes Transantárticos perto do estreitamento entre o mar de Ross e o mar de Weddell: a Antártica Oriental, ou Maior, e a Antártica Ocidental, ou Menor, porque correspondem aproximadamente aos hemisférios ocidental e oriental em relação ao meridiano de Greenwich. Ela divide o continente em Antártica Oriental, com planícies, colinas baixas e a geleira Lambert (a maior do mundo) e Antártica Ocidental, com arquipélagos ligados pela cobertura de gelo permanente. As banquisas formadas por água do mar congelado se confundem com o contorno do continente (RIFFENBURGH, 2007).

Os estudos geológicos da Antártica foram dificultados pelo fato de quase todo o continente ser coberto permanentemente por uma grossa camada de gelo. Entretanto, novas técnicas como o sensoriamento remoto começaram a revelar as estruturas por debaixo do gelo. Porém, a maior riqueza mineral da Antártica é a sua água. Cerca de 90% da reserva de água doce potável está congelada no continente (AVÉROUS, 1993; CAPOZOLI, 1991).

A Ilha Nelson é uma ilha de 19 quilômetros de extensão e 11 quilômetros de largura, situada a sudoeste da Ilha do Rei George nas Ilhas Shetland do Sul, Antártica (Figura 1b). A Ilha Nelson está localizada em $62^{\circ} 18' S$ $59^{\circ} 03' W$. O nome Ilha Nelson remonta a pelo menos 1821 e está agora estabelecido por uso internacional. A estação polar de Eco-Nelson (tcheca) está localizada na Ilha Nelson (SCAR, 2014).

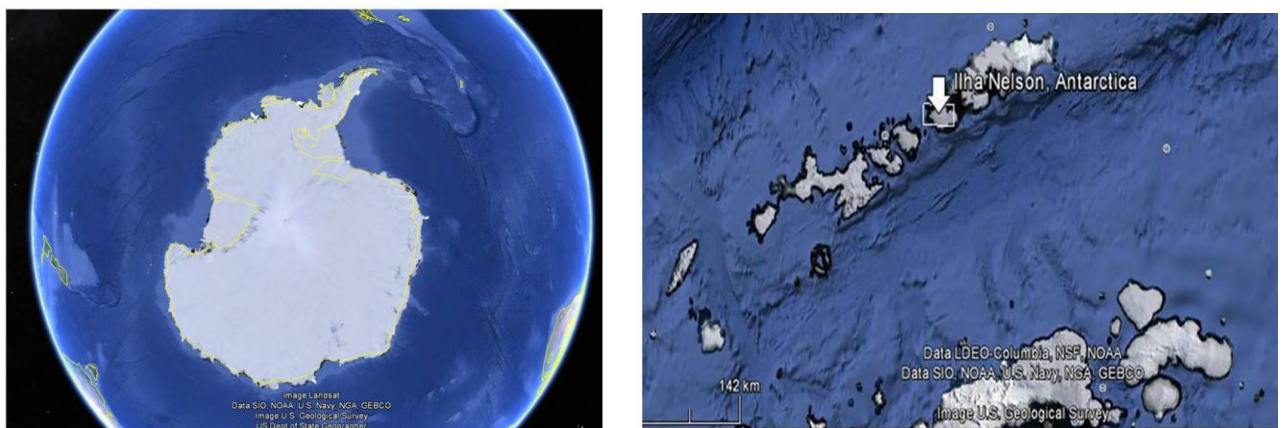


FIGURA 1 - a) Continente Antártico; b) no detalhe a Ilha Nelson, que está situada nas Ilhas Shetland do Sul.

1.2. Flora Antártica

As briófitas são plantas criptogâmicas avasculares e relativamente simples, com ampla distribuição geográfica e especialmente predominantes nas regiões tropicais e subtropicais

(LEMOS-MICHEL, 2001). De maneira geral, as briófitas caracterizam-se por seu tamanho pequeno, podendo variar de poucos milímetros até um metro, como ocorre com alguns musgos de hábito pendente e presença de gametófitos talosos ou folhosos, clorofilados e fotossintetizantes (LEMOS-MICHEL, 2001). Apresentam parede celular celulósica e, algumas vezes, cutícula e alternância de gerações bem definida. A geração assexuada e diplóide é representada por um esporófito de vida efêmera, não ramificado, dependente e aderido ao gametófito (geração sexuada), que é haplóide, fotossintetizante, perene e livre, produzindo os órgãos sexuais: anterídios e arquegônios (SCHOFIELD, 1985). São abundantes em ambientes úmidos e sombrios, no interior de matas ou são típicas de áreas urbanas (LEMOS-MICHEL, 2001). Também ocorrem em habitats aquáticos (DELGADILLO & CÁRDENAS, 1990) e em lugares secos e desérticos, já que são adaptadas à dessecação e sobrevivem como esporos dormentes ou gametófitos secos (MARGULIS & SCHWARTZ, 2001). Por tolerarem condições ambientais extremas estão amplamente distribuídas pelo mundo, desde ambientes árticos, tundra e florestas boreais até florestas tropicais, ambientes submersos, cavernas escuras e rochas expostas, tolerando também aspensão de água salgada, mas nunca sendo encontradas em ambiente marinho (DELGADILLO & CÁRDENAS, 1990; FRAHM, 2003).

As briófitas desenvolvem-se nos mais diversos substratos: troncos vivos ou em decomposição, ramos, húmus, superfície de rocha, solos calcáreos, argilosos ou arenosos, folhas vivas e outros materiais orgânicos. Também podem ocorrer sobre telhados, muros e calçadas, além de substratos não usuais, como cascos de tartaruga e calçados velhos (FRAHM, 2003).

As principais formações vegetais encontrados na Antártica são compostas por briófitas, líquens, algas e duas espécies de plantas vasculares (*Deschampsia antarctica* Desv.(Poaceae) e *Colobanthus quitensis* (Caryophyllaceae) Bartl (Kunth)), sendo os musgos um grupo muito relevante para este continente (OCHYRA et al., 2008; PUTZKE e PEREIRA, 2001). Na década de 1980, muitos trabalhos foram elaborados com os musgos antárticos, destacando-se: Kanda (1986), Ochyra (1985), Ochyra & Ochi (1986), Ochi (1982), que descrevem as espécies de musgos para pequenas áreas da Antártica Marítima. Apenas para a Ilha Rei George, Ochyra et al. (1998) registrou 58 espécies de musgos, incluindo espécies do gênero *Andreaea* Hedw., *Bryum* Hedw., *Polytrichum* Hedw., entre outros.

Os musgos são mais numerosos que as hepáticas, possuem maior biomassa no continente, participando de extensas formações e associações, e, portanto, são mais bem estudados. A primeira tentativa de reunir o que havia sido publicado sobre a brioflora foi feita

por Steere (1961), que elaborou uma revisão preliminar das briófitas da Antártica. Greene (1968) apresenta uma lista dos musgos até então conhecidos, porém Robinson (1972) foi quem realizou um dos primeiros trabalhos sobre os musgos da Antártica com chaves para identificação.

Musgos são espécies cosmopolitas encontradas como as principais espécies de plantas na Antártica, devido à sua capacidade de sobreviver em condições ambientais extremas. Assim, estudar a diversidade e a dinâmica populacional de espécies antárticas de musgos pode nos ajudar a prever a dinâmica de populações de espécies pioneiras, sob o efeito das mudanças climáticas e isolamento da população.

As condições extremas tornam o continente Antártico um ambiente propício para se estudar efeitos da radiação ultravioleta sobre diferentes organismos. Sabendo que tais condições são favoráveis ao desenvolvimento de mecanismo de sobrevivência, espécies de plantas da região são em potencial produtoras de agentes fotoprotetores (SMITH *et al.*, 2002; PEREIRA, 2009). A Antártica é o ponto do planeta que mais recebe radiações solares do tipo UV-B, consequência de problemas com a camada de ozônio. Radiações deste tipo são potencialmente nocivas a saúde, danos causado por alta intensidade excedem os níveis de defesa e reparo de qualquer organismo (SCHMITZ-HOERNER & WEISSENBOCK, 2003).

Nesta dissertação, diferentes espécies de musgos antárticos, por serem abundantes no continente, foram estudados: *Polytrichum juniperinum* Hedw., *Ptychostomum pseudotriquetrum* (Hedw.) J.R. Spence & H.P. Ramsay ex Holyoak & N. Pedersen (Bryaceae) e *Andreae gainii*.

O gênero *Polytrichum* é um táxon com distribuição mundial, ocorrendo, além das zonas polares (Ártico e da Antártica), em regiões de clima temperado, nas áreas de terras altas e elevadas dos trópicos e subtropicais. *Polytrichum juniperinum* Hedw. é uma espécie de musgo pioneira com distribuição cosmopolita. No continente Antártico, *P. juniperinum* se limita à Antártica marítima, onde é uma espécie difundida de musgo (OCHYRA, 1998), se desenvolve em áreas livres de gelo, exceto em locais com excesso de umidade (VICTORIA *et al.*, 2009). Esta espécie cresce sobre solo, barranco, rocha ou húmus em locais diretamente expostos ou parcialmente sombreados. Em campo cobre grandes áreas sobre solo e rochas sendo comumente encontrada ao lado de trilhas e estradas. Foi encontrada na maioria das vezes exposta diretamente ao sol. *P. juniperinum* permanece sob gelo durante o inverno e quando ocorre o degelo reinicia seu crescimento durante o verão, raramente forma esporófito. Esta espécie é caracterizada pela margem da lâmina do filídio involuta que recobre a lamela, que é facilmente visualizada sob estereomicroscópio (PERALTA, 2009).

Ptychostomum pseudotriquetrum (Hedw.) J.R. Spence & H.P. Ramsay ex Holyoak & N. Pedersen (Bryaceae) é uma musgo cosmopolita de coloração verde, filídios amontoados e lanceolados e com margem plana levemente serrilhada na ponta. Visto em uma variedade de habitats, tanto em solo como pastagens, em remendos rochosos, casca de árvores, etc. De acordo com (KANDA & IWATSUKI, 1989) quase totalidade dos musgos desta espécie coletados até o momento em camadas profundas de lagos na Antártica continental eram novos e endêmicos à Antártica.

Imura et al. (1994) investigaram a estrutura morfológica de colônias destes musgos na Antártica e discutiram o processo de desenvolvimento das colônias. Mesmo assim, estudos sobre os aspectos dinâmicos das comunidades de musgos ainda permanecem obscuros e nenhum estudo dinâmico das comunidades de musgos no vale apareceu ainda. Este musgo mantém crescimento vigoroso sob a condição úmida (SMITH, 1999).

O sistema de rizóides de *P. pseudotriquetrum* é extenso, com rizóides individuais (SMITH, 1999). Estes desempenham um papel importante na estabilização do solo em que a espécie está estabelecida e também na retenção de umidade. As características morfológicas desta espécie ajudam se estabelecerem um local coberto de areia.

A família Andreaeaceae é monotípica e compreende 50-75 espécies que são comuns em regiões mais frias, na Austrália é representada por 15 espécies (MURRAY, 2012). *Andreaea gainii* é um musgo perene, acrocarpico e é muitas vezes encontrada formando almofadas de coloração preta ou misturas de preto e outros tons escuros (roxo, marrom, bronze, verde ou laranja); cresce em rochas ácidas e em altas altitudes. O gênero é mais diversificado na Tasmânia e *A. gainii* foi relatado pela primeira vez da Austrália.

Eles são pequenos e delicados musgos e as cápsulas são formadas nas pontas dos ramos verticais. Os esporos são liberados da cápsula através de deiscência por fendas longitudinais, em vez de através de um opérculo como em outros musgos (SMITH & DAVISON, 1993). Espécies de Andreaeaceae são epilíticas: *Andreaea* normalmente cresce em granito ou outras rochas ácidas enquanto *Andreaeobryum macrosporum* Steere & B. M. Murray cresce em calcário.

Neste trabalho, foram testadas as seguintes hipóteses:

- I. As populações estudadas são morfológicamente mais diferenciadas, quanto mais distantes da costa elas se encontram.
- II. A diversidade morfológica das populações de musgos analisadas está ligada a adaptação ao microambiente.

- III. A diferenciação morfológica entre populações de *P. juniperinum* é reflexo de diferenciação genética, portanto, relacionado à distância das populações até a costa.

2. OBJETIVOS

2.1. Objetivo Geral

Este trabalho tem por objetivo avaliar a variabilidade fenotípica de populações naturais de *Polytrichum juniperinum*, *Andreae gainii* e *Ptychostommum pseudotriquetrum* da Ilha Nelson, Antártica, visando identificar padrões relacionados com a distribuição geográfica e também avaliar a diversidade genotípica de *P. juniperinum*.

2.2. Objetivos específicos

Estudos baseados somente em caracteres morfológicos a partir de populações crescentes pode refletir plasticidade fenotípica, em vez de padrões adaptativos. Portanto, as comparações morfológicas e genéticas são necessários a fim de evitar conclusões baseadas em plasticidade fenotípica das populações.

- Determinar se a distância das populações até a costa influencia no tamanho dos gametófitos e filídios das espécies analisadas;
- Caracterizar a diferenciação genética populacional da espécie *P. juniperinum*, em sete pontos de coleta da Ilha Nelson, Antártica, baseada em marcadores microsatélites.
- Determinar se a distância das populações de *P. juniperinum* até a costa influencia nas características genéticas;

3. MANUSCRITOS

O primeiro manuscrito, intitulado “Is the morphometric differentiation among Antarctic mosses populations a response to local microenvironment?”, foi submetido para publicação na revista *Bioscience Journal* (ISSN 1981-3163). O trabalho apresenta dados referentes à diversidade morfológica populacional de três musgos da Ilha Nelson: *Andreae gainii* Cardot. (Andreaeaceae), *Ptychostomum pseudotriquetrum* (Hedw.) J.R. Spence & H.P. Ramsay ex Holyoak & N. Pedersen (Bryaceae) and *Polytrichum juniperinum* Hedw. (Polytrichaceae). Aspectos ecológicos e ambientais relacionados à variação morfológica observada são discutidos.

O segundo artigo, intitulado “Genetic diversity and structure in populations of *Polytrichum juniperinum* from Nelson Island, Antarctica” será submetido para a revista *Polar Biology* (ISSN 0722-4060). Este trabalho apresenta os resultados de uma análise molecular baseada em marcadores microssatélites, de sete populações de *P. juniperinum* coletadas na Ilha Nelson. São discutidas as barreiras para fluxo gênico e a diversidade intrapopulacional da espécie.

3.1. Is the morphometric differentiation among Antarctic mosses populations a response to local microenvironment?

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Abstract: Microclimate, including moisture availability, low temperature and ground-level wind speed, has a major influence on plant development. Regarding wind effects, direct influence in plant growth and increase in cytosolic calcium concentration was already reported. In addition, Antarctic plants may suffer the abrasive effects of wind-blown ice crystals or sand. Expecting that mosses growing at different distances from the coast might display significant phenotypic plasticity as response to the Antarctic maritime winds, the correlation between distance from the coast and morphometric traits of three moss species growing in the Nelson Island, Antarctic Peninsula was evaluated. Significant differentiation concerning gametophyte length and leaf width and length was observed for all species. However, just populations of *A. gainii* revealed a significant correlation of leaf measures and distance to the coast, suggesting phenotypic plasticity of this species to microenvironmental conditions. We suggested that while *A. gainii* presents high plasticity capacity for the microenvironmental conditions of Nelson Island, *P. pseudotriquetrum* and *P. juniperinum* are less plastic species. Further studies may add important information about the effect of other edaphic-climatic events related to this plasticity.

Key words: *Ptychostommum pseudotriquetrum*, *Polytrichum juniperinum*, *Andreae gainii*, Nelson Island, phenotypic plasticity.

Introduction

Over the last 30 years, a substantial increase in the number of scientific publications about the Antarctic continent has been observed with important emphasis in the Biological Sciences (Stefenon *et al.*, 2013). Antarctica is the most untouched region of the world and also one of the most vulnerable to environmental changes, enabling the understanding of the environmental dynamics through systematic studies. In addition, thanks to the minor interference of human actions, Antarctica provides the opportunity to access diversity trends and patterns over a large area where species dynamics are primarily determined by natural processes (Cannone *et al.*, 2013).

The Antarctic continent presents extreme conditions concerning temperature (annual mean from 0°C to 2°C in the maritime Antarctica and from -30°C to -65°C in the central region; Bednarek-Ochyra *et al.* 2000, Rozema *et al.* 2005), light incidence (15 to 20 weeks of light incidence per year in the Northern Maritime Antarctic; Bednarek-Ochyra *et al.* 2000), water availability (annual precipitation of 300-500 mm; Rozema *et al.* 2005) and UV incidence (the Antarctic polar vortex hinders the outer supply of ozone, leading to severe ozone breakdown at the surface of cold polar stratospheric clouds; Rozema *et al.* 2005). In addition to the climatic uniqueness, the Antarctic region is geographically isolated from South America since about 200 million years ago, as consequence of the continental drift in the Eocene (Fogg 1992).

Mosses cover large areas of Antarctic islands and phenotypic variances among populations growing in different microenvironments may provide information about the plasticity related to the environmental pressure to which they are exposed. Microclimate,

including factors such as moisture availability, low temperature and ground-level wind speed, has a major influence on plant growth (Alberdi *et al.* 2002). Concerning ground-level wind, it has been shown that the wind speed has direct influence in the size of the leaves in vascular plants (Whitehead 1962, Whitehead & Luti 1962) and induces the increase in cytosolic calcium concentration (Knight *et al.* 1992). Moreover, the abrasive effects of wind-blown ice crystals or sand may directly affect plants in Antarctica (Alberdi *et al.* 2002). Regarding temperature, protection from wind may play a more important role than temperature *per se* in providing a favorable environment for plant growth in high-latitude ecosystems. Wind speed had a generally negative impact on mean daily temperature (Marion *et al.* 1997).

The wind causes significant effects on photosynthesis. When the air flow passes through the tree and moves the sheets, air is pumped in and out of the stomata, which accelerates the exchange of O₂ and CO₂ (Zhu *et al.*, 2004). It is important in determining phenotypic trees as it affects the morphology of roots, stem, crown and also the distribution of growth (Zhu *et al.*, 2004). Peltola (2006) found that under a regime of wind conditions at constant speed and direction, the mechanical stability of vascular plants is affected by the horizontal force carried by wind and vertical gravitational force, causing an inclination to adapt to the environment, and better positioning for its metabolic activities (light harvesting, water control, etc.). The quantitative analysis of growth is the first item in the assessment of crop production. Therefore, the amount of material contained in the plant, such as stems, roots and fruits, and photosynthetic surface (leaf area), should be measured (Monteiro *et al.*, 2005). According to Goyal *et al.* (2013), leaf area represents the apparatus of light interception for photosynthesis and from that estimate is possible to find some ecophysiological variables such as leaf area ratio, net assimilation rate, rate of growth and development and quantification of variations in growth plants due to genetic or environmental differences.

Therefore, it is expected that as response to the Antarctic maritime winds, mosses growing at different distances from the coast might display significant phenotypic plasticity.

Aiming to test this hypothesis, we evaluated the correlation between distance from the coast and morphometric traits of three moss species growing in the Nelson Island, Antarctica: *Ptychostomum pseudotriquetrum* (Hedw.) J.R. Spence & H.P. Ramsay ex Holyoak & N. Pedersen (*Bryaceae*), *Polytrichum juniperinum* Hedw. (*Polytrichaceae*) and *Andreae gainii* Cardot. (*Andreaeaceae*).

Material and Methods

Samples were collected in the Austral summer 2011 and summer 2012, in ice-free areas of the Nelson Island (Fig. 3.1.1), during the Brazilian Antarctic Mission. This island has an area of about 164.8 km², with a permanent ice cap covering 95% of the island and reaching the ocean along a large part of the island margin (Jiawen *et al.* 1995). Four tufts of *Ptychostomum pseudotriquetrum*, seven tufts of *Polytrichum juniperinum* and four cushions of *Andreae gainii*, were found in the study area and sampled. Each cushion or tuft sampled was considered a single population. *P. juniperinum* was collected in greater numbers because it is more abundant than the other species and easier visualization. The distance from the coast was taken as the shortest linear distance between the population and the margin of the island. Complete gametophytes were collected with substrate, conditioned in plastic bags containing silica gel and maintained in coolers similar to the ambient temperature of Brazil. The specimens were identified using the identification keys provided in Ochyra *et al.* (1998; 2008) and Putzke and Pereira (2001). Vouchers were deposited in the Herbarium of the Universidade Federal do Pampa (HBI), São Gabriel, RS, Brazil. Samples were rehydrated in the laboratory and thirty gametophytes from each population were randomly selected from the

middle of the cushion or tuft for morphometric analyses. The length of each gametophyte was measured using a digital calliper rule (Mitutoyo[®]) and the length and width of twenty leaves from each gametophyte were measured using a stereomicroscope and the software Motic Image Plus[®]. Pair-wise population means were compared using a two-tailed *t*-test and the correlation between the morphometric traits and the distance to the coast were determined using the Pearson correlation index. The significance was determined through a one-tailed *t*-test.

Results

For all species studied, the morphometric measures revealed significant difference among populations ($p < 0.05$; Table 3.1.1). In *P. pseudotriquetrum*, population P43 had the longest gametophytes with 2.8 cm, while population Q72 revealed the smallest gametophytes (1.13 cm). The leaf length ranged from 0.164 cm (population P43) to 0.201 cm (population Q55) and the leaf width ranged from 0.049 (population Q22) to 0.075 cm (population Q72). For *P. juniperinum* the population P96 showed the longest gametophytes with 6.99 cm, while population P34 had shortest gametophytes (2.28 cm). Leaf length ranged from 0.448 cm (population P27) to 0.670 cm (population P96). Leaf width ranged from 0.429 cm (population P63) to 0.595 cm (population P27). For the populations of *A. gainii*, the gametophyte length ranged from 1.38 cm (population Q298) to 2.29 cm (population P198), leaf length ranged from 0.062 cm (population P39) to 0.162 cm (population Q294) and leaf width ranged from 0.055 cm (population Q294) to 0.374 cm (population P194).

High negative significant correlation between morphometric traits and distance to the coast was observed for *A. gainii*, for the width and length of the leaves ($r = -0.92$ and $r = -0.95$, respectively). All other correlations were not significant ($p > 0.05$; Table 3.1.1.).

Discussion

Structured morphological variation among bryophyte populations as observed in this study is infrequent. This significant variability may be effect of the distinct differentiation in niche structure in the Nelson Island, as suggested for *Syrrhopodon leprieurii* Mont. (Calymperaceae) from montane and spatially isolated regions (Pereira *et al.* 2013).

Phenotypic plasticity exhibited by bryophytes may represent an alternative strategy over genetic differentiation to enable growth in a range of environments (Skotnicki *et al.* 2000). Plasticity is usually thought to be an evolutionary adaptation to environmental variation that occurs within the lifespan of an individual organism. If the optimal phenotype in a given environment changes with environmental conditions, then the ability of individuals to express different traits should be advantageous and thus selected for.

Although all populations evaluated in this study grow in the same sub-Antarctic island, such morphometric differences may have an adaptive significance. For *A. gainii*, the population with the smallest gametophyte length revealed the largest leaves for both traits measured. This suggests compensation towards extending exposure to light. Higher exposure to light radiation maximizes photosynthesis. Therefore, an increase in leaf surface supporting more exposure to light is linked to competitive strategies. When the plants produce more photosynthates they can invest in processes such as growth and maintenance, enhancing survival rates (Andrade *et al.* 2013). Moreover, snow cover in the Antarctic is an important microclimatic factor in protecting plants from windblown ice and sand particles (Alberdi *et al.* 2002). During the growing season, the moss populations are suitable to these elements in the ice-free areas. Thus, this phenotypic plasticity may be related to the response of *A. gainii* against the sand particles and salt-spray deposition from the ocean, carried by winds.

Populations growing near the coast may have their leaves damaged more frequently as effect of these events. Considering that these leaf length and width revealed significant negative correlation with distance to the coast, i.e., as near from the coast, as larger the leaves, such bigger leaves guarantee higher photosynthetic area, even after injury.

In addition to photosynthesis compensation, such correlation may be a direct response to wind speed. Plantlets of *Helianthus annuus* L. and *Zea mays* L. growing under controlled conditions at lower wind speed develop greater leaves than plantlets growing at higher wind speed (Whitehead 1962, Whitehead & Luti 1962). In East Antarctica, Ma *et al.* (2010) demonstrated that the wind speed near the surface increases from 6.4 m/s in the coast up to 9.0 m/s about 420 m far from the coast. Also the intensification of the surface slope increases the wind speed, reaching 10 m/s in a slope of 4.5 m/km. Although we do not have such measures in our study area, if we consider the same pattern for wind speed in Nelson Island, the populations more distant from the coast should experience winds with higher speed and, corroborating our morphometric data, present smaller leaves as response to this microenvironment.

Another evidence of the plasticity potential of *A. gainii* as response to microenvironments is related to the life form of populations. Victoria *et al.* (2009) have shown that *A. gainii* populations from King George Island grow as tufts (straight main axis with similar branches, parallel to the main axis) or as cushions (axis coming from a central point, growing radially), depending on the colonized substrata. Over soil or fine rock particles, *A. gainii* populations grow as tufts, while over emergent rocks or their particles, this species grow as cushions.

For *P. pseudotriquetrum* and *P. juniperinum*, no correlation was observed between gametophyte and leaf size or between morphometric measures and distance to island margin. Therefore, the morphometric differences observed among populations of these species may

also be plasticity reaction to microenvironments, but not as response to the maritime winds. These species also present in Nelson Island the same life form (tufts) observed in King Georg Island by Victoria *et al.* (2009), suggesting that they lack the same plasticity capacity that *A. gainii* revealed in this survey.

Concluding, the results of our study suggest the existence of significant plasticity in *A. gainii* populations as response to local microenvironments in Nelson Island. Additional studies can add important information about the effect of other edaphic-climatic events related to this plasticity, as well as the existence of such plasticity at a more broad level, sampling populations in adjacent islands of the Antarctic Peninsula and in the Continental Antarctica. Further studies in Antarctica can also highlight the plasticity capacity of *P. pseudotriquetrum* and *P. juniperinum* in response to microenvironments.

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References

- ALBERDIA, M., BRAVOB, L.A., GUTIE´RREZC, A., GIDEKELC, A. & CORCUERA, L.J. 2002. Ecophysiology of Antarctic vascular plants. *Physiologia Plantarum*, 115, 479–486.
- ANDRADE, E.A., BARBOSA, M.E.A. & DEMETRIO, G.R. 2013. Density-dependent

- morphological plasticity and trade-offs among vegetative traits in *Eichhornia crassipes* (Pontederiaceae). *Acta Amazonica*, 43, 455-460.
- BEDNAREK-OCHYRA, H., VÁNA, J., OCHYRA, R. & LEWIS-SMITH, R.I. 2000. *The liverwort flora of Antarctica*. Cracow: Polish Academy of Sciences, Institute of Botany, 236pp.
- CANNONE N., CONEVEY P. & GUGLIELMIN M. 2013. Diversity trends of bryophytes in continental Antarctica. *Polar Biology*, 36, 259-271.
- FOGG, G.E. 1992. *A History of Antarctic Science*. Cambridge: Cambridge University Press, 483pp.
- JIAWEN, R., DAHE, Q., PETIT, J.R., JOUZEL, J., WENTI, W., CHEN L., XIAOJUN, W., SONGLIN, Q. & XIAOXIANG, W. 1995. Glaciological studies on Nelson Island, South Shetland Islands, Antarctica. *Journal of Glaciology*, 41, 408-412.
- KNIGHT, M.R., SMITH, S.M. & TREWAVAS, A.J. 1992. Wind-induced plant motion immediately increases cytosolic calcium. *Proceedings of the National Academy of Sciences of the USA*, 89, 4967-4971.
- MA, Y., BIAN, L., XIAO, C., ALLISON, I. & ZHOU, X. 2010. Near surface climate of the traverse route from Zhongshan Station to Dome A, East Antarctica. *Antarctic Science*, 22, 443-459.
- MARION, G.M., HENRY, G.H.R., FRECKMAN, D.W., JOHNSTONE, J., JONES, G., JONES, M.H., LÉVESQUE, E., MOLAU, U., MØLGAARD, P., PARSONS, A.N., SVOBODA, J. & VIRGINIA R.A. 1997. Open-top designs for manipulating field temperature in high-latitude ecosystems. *Global Change Biology*, 3, 20-32.
- MONTEIRO, J. E. B. A. 2005. Estimação da área foliar do algodoeiro por meio de dimensões e massa das folhas. *Bragantina*, v.64, n.1, p.15-24.
- OCHYRA, R. 1998. *The moss flora of King George Island, Antarctica*. Cracow: W. Szafer Institute of Botany, Polish Academy of Sciences, 279pp.
- OCHYRA, R., LEWIS-SMITH, R.I. & BEDNAREK-OCHYRA, H. 2008. *The illustrated moss flora of Antarctica*. Cambridge: Cambridge University Press, 685pp.
- PELTOLA, H. M. 2006. Mechanical Stability of Trees Under Static Loads. *American Journal of Botany*, V. 90, N. 10, P. 1501-1511.
- PEREIRA, M.R., DAMBROS, C.S. & ZARTMAN, C.E. 2013. Will the real *Syrrhopodon leprieurii* please stand up? The influence of topography and distance on phenotypic variation in a widespread Neotropical moss. *The Bryologist*, 116, 58-64.

- PUTZKE, J. & PEREIRA, A.B. 2001. *The Antarctic Mosses, with special references to the Shetland Islands*. Canoas: Ed. ULBRA, 196pp.
- ROZEMA, J., BOELEN, P. & BLOKKER, P. 2005. Depletion of stratospheric ozone over the Antarctic and Arctic: Responses of plants of polar terrestrial ecosystems to enhanced UV-B, an overview. *Environmental Pollution*, 137, 428-442.
- SKOTNICKI, M.L., NINHAMI, J.A. & SELKIRK, P.M. 2000. Genetic diversity, mutagenesis and dispersal of Antarctic mosses - a review of progress with molecular studies. *Antarctic Science*, 12, 363-373.
- STEFENON, V.M., ROESCH, L.F.W. & PREREIRA, A.B. 2013. Thirty years of Brazilian research in Antarctica: ups, downs and perspectives. *Scientometrics*, 95, 325-331.
- VICTORIA, F.C., PEREIRA, A.B. & COSTA, D.P. 2009. Life-forms of moss species in defrosting areas of King George Island, South Shetland Islands, Antarctica. *Bioscience Journal*, 25, 151-160.
- WHITEHEAD, F.H. 1962. Experimental studies of the effect of wind on plant growth and anatomy. II. *Helianthus annuus*. *New Phytologist*, 61, 59-62.
- WHITEHEAD, E.H. & LUTI, R. 1962. Experimental studies of the effect of wind on plant Growth. I, *Zea Mays*. *New Phytologist*, 61, 56-58.
- ZHU, J.J.; LIU, Z.; LI, X.; MATSUZAKI, T.; GONDA, Y. 2004. Review: effects of wind on trees. *Journal of Forestry Research*, v. 15, n.2, p. 153-160.

Figure 3.1.1. Geographic location of sampled populations of *P. pseudotriquetrum*, *P. juniperinum* and *A. gainii* in ice-free areas of the Nelson Island, Antarctic Peninsula, Antarctica. Images obtained from Google Earth®.

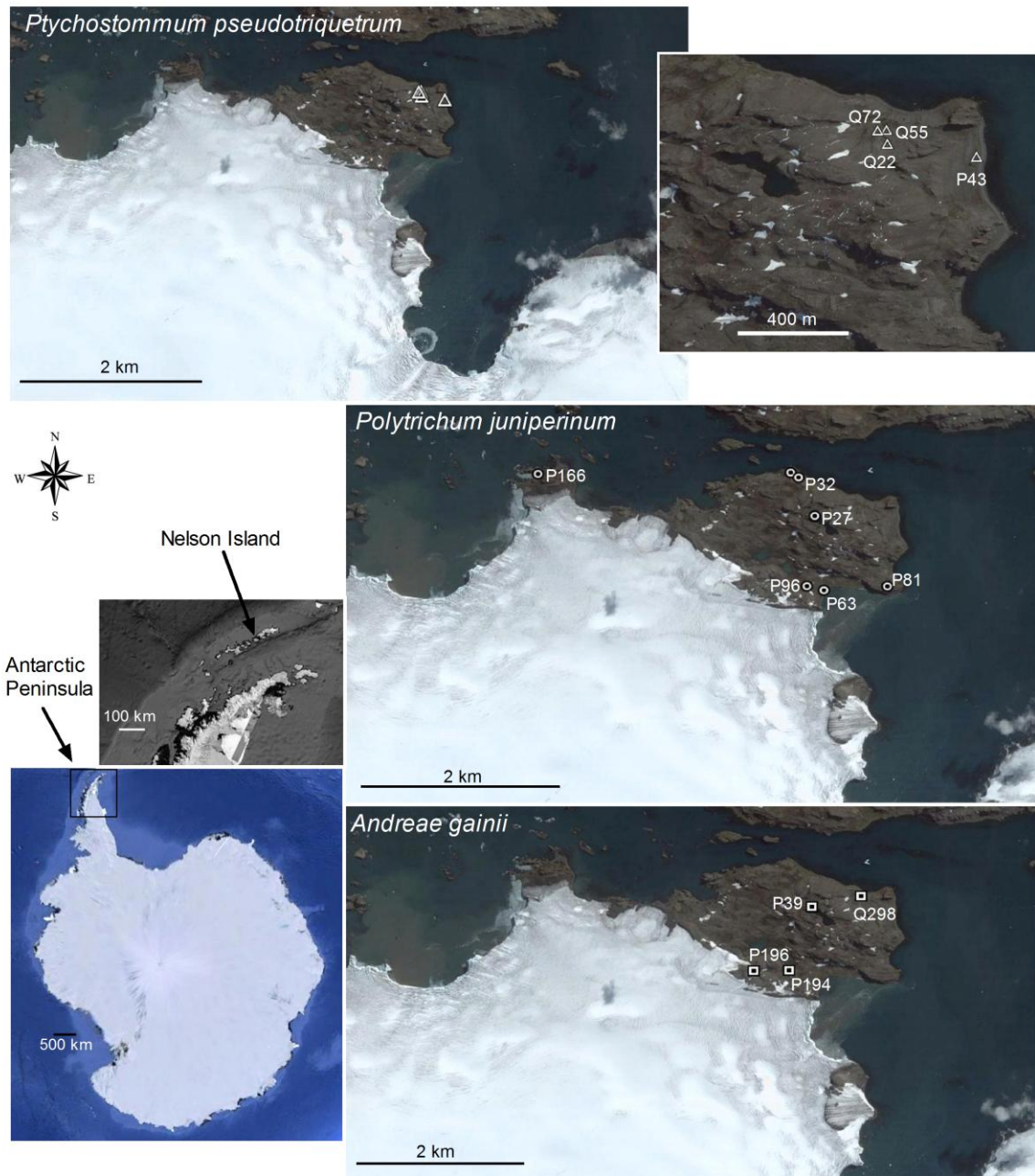


Table 3.1.1. Mean measurements and standard deviation of the gametophyte length, leaf length and leaf width for each population and each species. The Pearson correlation index (r) between each morphometric traits and the distance of populations to the island margin is also presented.

<i>Ptychostomum pseudotriquetrum</i>			
	Gametophyte Length (cm)	Leaf Length (mm)	Leaf Width (mm)
Q22	2.22(± 0.230) ^A	0.187(± 0.042) ^B	0.049(± 0.016) ^C
P43	2.45(± 0.237) ^A	0.164(± 0.032) ^B	0.062(± 0.018) ^B
Q55	1.44(± 0.363) ^B	0.201(± 0.037) ^A	0.063(± 0.013) ^B
Q72	1.13(± 0.152) ^B	0.182(± 0.028) ^B	0.075(± 0.015) ^A
r	0.02 ($p = 0.48$)	0.65 ($p = 0.17$)	-0.68 ($p = 0.15$)
<i>Polytrichum juniperinum</i>			
	Gametophyte Length (cm)	Leaf Length (mm)	Leaf Width (mm)
P27	3.043(± 0.580) ^D	0.448(± 0.098) ^C	0.595(± 0.136) ^A
P32	5.816(± 1.290) ^B	0.615(± 0.090) ^A	0.594(± 0.134) ^A
P34	2.283(± 0.522) ^E	0.506(± 0.087) ^B	0.506(± 0.103) ^A
P63	3.350(± 0.665) ^D	0.459(± 0.065) ^C	0.429(± 0.115) ^B
P81	2.850(± 0.592) ^E	0.494(± 0.093) ^C	0.469(± 0.138) ^B
P96	6.990(± 1.328) ^A	0.670(± 0.141) ^A	0.499(± 0.113) ^B
P166	4.836(± 2.089) ^C	0.485(± 0.096) ^C	0.574(± 0.162) ^A
r	-0.01 ($p = 0.49$)	-0.08 ($p = 0.43$)	0.40 ($p = 0.18$)
<i>Andreae gainii</i>			
	Gametophyte Length (cm)	Leaf Length (mm)	Leaf Width (mm)
P39	1.52(± 0.179) ^B	0.062(± 0.011) ^C	0.024(± 0.005) ^B
P194	1.57(± 0.219) ^B	0.097(± 0.049) ^B	0.037(± 0.006) ^A
P196	2.29(± 0.473) ^A	0.092(± 0.017) ^B	0.035(± 0.007) ^A
Q294	1.38(± 0.169) ^B	0.162(± 0.019) ^A	0.055(± 0.032) ^C
r	0.41 ($p = 0.29$)	-0.95 ($p = 0.02$)	-0.92 ($p = 0.04$)

Values followed by the same letter in the column are not statistically different at $\alpha = 5\%$

Genetic diversity and structure in populations of *Polytrichum juniperinum* from Nelson Island, Antarctica

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Abstract: The continent Antarctic is the coldest, driest, with the highest average altitude and higher index of strong winds on the planet. Antarctica is divided into East Antarctica and West Antarctica. About 90% of the continent is situated in East Antarctica. The main vegetation types in Antarctica are composed of bryophytes, lichens, algae and two species of vascular plants. In this study, we discuss the analysis of the genetic characteristics of natural populations of *Polytrichum juniperinum* collected in plant communities of melting areas of Nelson Island. Samples of *P. juniperinum* were collected at seven different points of Nelson Island, Antarctic. Whereas gametophytes are haploid, the set of alleles amplified in each of the microsatellite loci for each individual was identified as a haplotype and the data from all subjects were evaluated by a molecular variance analysis, Principal Coordinates Analysis and Shannon Diversity Index, using GenAlEx 6.5 software. From three microsatellite loci, five haplotypes were identified and variation is occurring within only one population. In genetic levels, the present study revealed a large intra-population differentiation. There is great morphological and genetic differentiation in *P. juniperinum*, but there is no relationship between the two analyzes, this suggests that gene flow between neighboring populations is small. The possibility exists that mutations have occurred to explain the diversity among populations or mating populations, although the literature does not show the existence of fertile individuals on the Antarctic continent.

Key words: moss, haploid, genetic diversity

Introduction

The Antarctic continent presents one of the most extreme climatic conditions among the world environments, concerning temperature (annual mean from 0°C to 2°C in the maritime Antarctica and from -30°C to -65°C in the central region; BEDNAREK-OCHYRA et al. 2000, ROZEMA et al. 2005), light incidence (e.g. only 15 - 20 weeks of light incidence per year in the Northern Maritime Antarctic; BEDNAREK-OCHYRA et al., 2000), water availability (annual precipitation of 300-500 mm, mainly as snow; ROZEMA et al. 2005) and UV incidence (the Antarctic polar vortex hinders the outer supply of ozone, leading to severe ozone breakdown at the surface of cold polar stratospheric clouds; ROZEMA et al., 2005). In order to survive in such environment, Antarctic fauna and flora have to be adapted to such conditions. This continent is composed by two main regions: the continental Antarctica and the Antarctic Peninsula. In comparison to the continental Antarctica, the Antarctic Peninsula presents a somewhat more suitable environment, with higher level of biodiversity. While the continental Antarctica has only 15 species of mosses recorded (SMITH, 1984), just for the King Georg Island, Ochyra et al. (1998) recorded 58 species of mosses, including widespread species of genus *Andreaea* Hedw., *Bryum* Hedw. and *Polytrichum* Hedw.

The geological study of Antarctica have been hampered by the fact that almost the whole continent is permanently covered with a thick layer of ice. However, new techniques such as remote sensing begun to reveal the structures beneath the ice. However, the greatest mineral wealth of Antarctica is its water. About 90% of the amount of fresh drinking water is frozen on the continent (AVEROUS, 1993; CAPOZOLI, 1991). The Nelson Island is an island 19 miles long and 11 miles wide, located southwest of King George Island in the South Shetland Islands, Antarctica. The Nelson Island is located at 62° 18'S 59° 03'W. The name

Nelson Island dates back to at least 1821 and is now established for international use. A polar station Eco-Nelson (Czech Republic) is located on Nelson Island (SCAR, 2014).

Bryophytes are developed in various substrates: living or decaying trunks, branches, humus, surface rock, limestone, clay or sandy soils, fresh leaves and other organic materials. Can also occur on roofs, walls and pavements, and unusual substrates such as turtle shells and old shoes (FRAHM 2003). The main vegetation types found in Antarctica are composed of bryophytes, lichens, algae and two species of vascular plants (*Deschampsia antarctica* Std. (Poaceae) and *Colobanthus quitensis* (Caryophyllaceae) Bartl (Kunth)), and mosses a very important group for this continent (OCHYRA et al., 2008; PUTZKE and PEREIRA, 2001).

The genus *Polytrichum* is a taxon with worldwide distribution, occurring, in the both polar zones (Arctic and Antarctic), in regions of temperate climate, and in the highlands and some elevated hills of the tropics and subtropics. Studies about morphological and genetic variation in species of the genus *Polytrichum* have been performed in the northern Hemisphere (van der VELDE and BIJLSMA, 2000; van der VELDE et al., 2001; van der VELDE and BIJLSMA, 2003; WILSON and PROVAN, 2003), revealing the patterns of genetic structure, morphological adaptation and phylogeographical patterns of this taxon. However, it has been proposed that because of the extreme plasticity of moss gametophytes in Antarctica, morphological markers are likely unreliable indicators of genetic variability in this continent and the use of genetic markers could avoid the problem of environmental influence on morphological traits (SELKIRK et al., 1997).

The Antarctic continent is the most untouched region of the world but is also among the most vulnerable to global environmental changes. Thanks to these conditions, this continent enables the understanding of the environmental dynamics through systematic studies and provides the opportunity to access diversity trends and patterns over a large area where species dynamics are primarily determined by natural processes (CANNONE et al.,

2013). As consequence, a substantial increase in the number of scientific publications in the Antarctic continent has been observed over the last 30 years (STEFENON et al., 2013) with important emphasis observed in the Biological Sciences.

This study relied on morphometric and genetic records from populations of *Polytrichum juniperinum* from Nelson Island, Antarctica, aiming to answer three main questions: (1) are populations of *P. juniperinum* from Nelson Island originated from a single introgression, presenting, therefore, very high similarity? (2) Are individual gametophytes from each population clones from asexual reproductive events occurred in each tuft? (3) Does morphometric differentiation among Antarctic populations of *P. juniperinum* reflect genetic differentiation?

Material and Methods

Samples were collected in the Austral summer 2011-2012, in ice-free areas of the Nelson Island (FIGURE 1), during the Brazilian Antarctic Mission. This island has an area of about 164.8 km², with a permanent ice cap covering 95% of the island and reaching the ocean along a large part of the island margin (JIAWEN et al., 1995). Seven populations of *Polytrichum juniperinum*, were found in the study area and sampled. Complete gametophytes were collected with substrate, conditioned in plastic bags containing silica gel and maintained at room temperature. Vouchers were deposited in the Herbarium of the Universidade Federal do Pampa (HBI), São Gabriel, RS, Brazil.

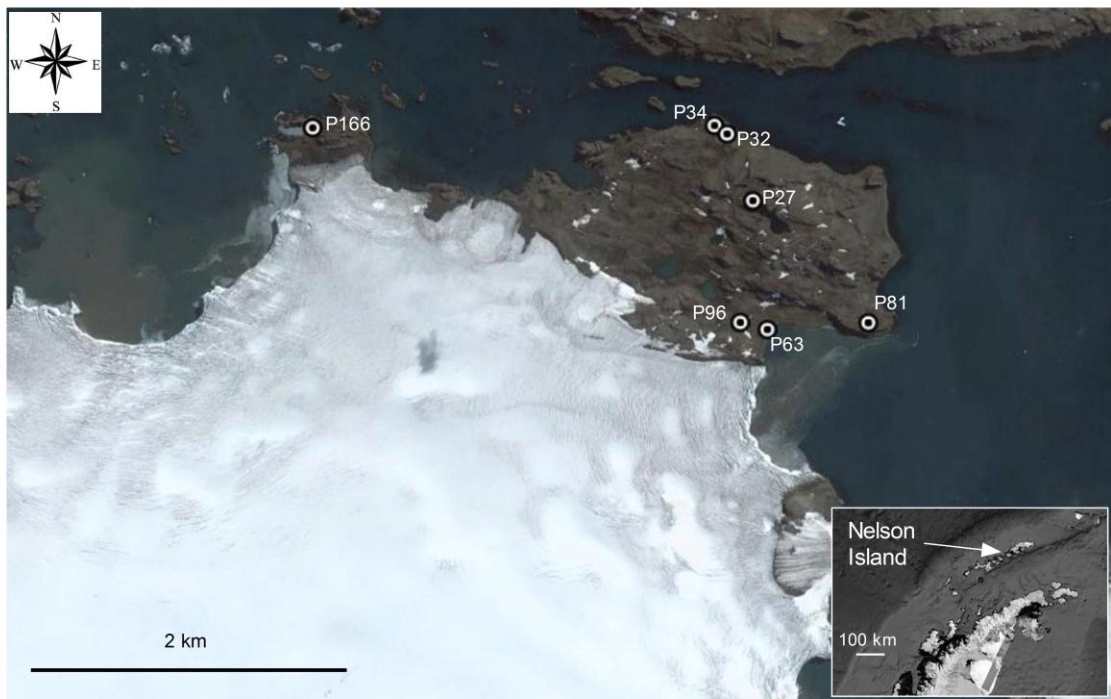


FIGURE 3.2.1. - The sampling points of *Polytrichum juniperinum* on Nelson Island, Antarctica. Font Google Earth™

The samples were analyzed at the Center for Interdisciplinary Research in Biotechnology - CIPBiotec, Campus São Gabriel da UNIPAMPA and For the morphometric analysis, samples were rehydrated in the laboratory and 30 gametophytes from each population were randomly selected from the middle of the tufts for morphometric analyses. The length of each gametophyte was measured using a digital calliper rule (Mitutoyo®) and the length and width of twenty leaves from each gametophyte were measured using a stereomicroscope and the software Motic Image Plus®. Pair-wise population means were compared using a two-tailed *t*-test.

For the genetic analysis, 24 gametophytes were randomly selected within each population, washed with distilled water and disrupted individually into collection microtubes using the Tissue Lyser (Qiagen®) grinder with 3 mm tungsten beads. Total DNA was extracted using the DNeasy 96 Plant Kit (Qiagen®), following the instructions of the

manufacturer. Genomic DNA was stained with GelRed[®] (Biotium), checked under UV light and diluted to a concentration of 10 ng/ μ L and deposited at -20°C until use.

Seven microsatellite loci developed based on expressed sequence tag regions of the moss *Physcomitrella patens* (VICTORIA et. al. 2011) were employed in this study. The PCR amplification of all loci was performed in aT1000 Thermal Cycler (Bio-Rad[™]) in 13.5 μ L reactions, comprising 2.0 μ L template DNA, 1.25 μ M of each primer and 6.25 μ L of GoTaq[®] Green Master Mix (Promega). All fragments were separated on 3% agarose gel, stained with GelRed[®] (Biotium), checked under UV light and photographed using a digital camera. The PCR protocol consisted of an initial denaturing step of 5 min at 95°C, followed by a touchdown procedure with 11 cycles of 30 sec at 94°C, 1 min at 62°C (-1°C per cycle) and 30 sec at 72°C, followed by 35 cycles in using the annealing temperature of 52°C and a final extension step of 10 min at 72°C.

Since gametophytes are the haploid phase of the mosses, just one allele is amplified in each locus. Therefore, the genotype of each gametophyte was determined as a haplotype and each single combination of alleles amplified was considered an individual haplotype. The haplotype frequency ($pHap_n$) and the Shannon index of diversity ($I = -\sum p_i \times \log_2 p_i$) were computed for each population. The inter-population genetic structure was determined through an analysis of molecular variance (AMOVA, EXCOFFIER et al. 1992) and a multivariate analysis (PCoA) based on the pairwise Shannon index of diversity (I). All analyses were performed using the software GenAlEx 6.5 (PEAKALL and SMOUSE, 2006).

Results

For the morphometric analysis, population P96 showed the longest gametophytes with 6.99 cm, while population P34 had shortest gametophytes (2.28 cm). Leaf length ranged from

0.448 cm (population P27) to 0.670 cm (population P96). Leaf width ranged from 0.429 cm (population P63) to 0.595 cm (population P27). All measures are summarized in Table 3.2.1.

TABLE 3.2.1.: Mean measurements and standard deviation of the gametophyte length, leaf length and leaf width for each population of *P. Juniperinum* from Nelson Island, Antarctica.

	Gametophyte Length (cm)	Leaf Length (mm)	Leaf Width (mm)
P27	3.043(±0.580) ^D	0.448(±0.098) ^C	0.595(±0.136) ^A
P32	5.816(±1.290) ^B	0.615(±0.090) ^A	0.594(±0.134) ^A
P34	2.283(±0.522) ^E	0.506(±0.087) ^B	0.506(±0.103) ^A
P63	3.350(±0.665) ^D	0.459(±0.065) ^C	0.429(±0.115) ^B
P81	2.850(±0.592) ^E	0.494(±0.093) ^C	0.469(±0.138) ^B
P96	6.990(±1.328) ^A	0.670(±0.141) ^A	0.499(±0.113) ^B
P166	4.836(±2.089) ^C	0.485(±0.096) ^C	0.574(±0.162) ^A

Values followed by the same letter in the column are not statistically different at $\alpha = 5\%$

From the seven tested microsatellite loci, just three revealed reliable amplifications in at least nine gametophytes within each population. Therefore, the genetic data was evaluated for three loci and from nine to 23 gametophytes from each population (TABLE 3.2.2). The genetic analysis revealed a total of four different haplotypes among the 103 genotyped gametophytes. Haplotype 1 was found in populations P27, P32, P34 and P81, haplotype 2 in populations P27, P34 and P81, haplotype 3 was observed in populations P63 and P96, and the haplotype 4 was found in populations P63, P96 and P166 (TABLE 3.2.1). Two populations (P32 and P166) presented only one haplotype (H₁ and H₄), respectively (TABLE 3.2.2). Because single haplotypes were observed in populations P32, P34, P63, P96 and P166, the Shannon diversity index was $I = 0.0$. For population P27, which presented three haplotypes, the computed Shannon diversity index was $I = 0.614$.

According to the AMOVA, the variation among populations is $\Phi_{ST} = 0.69$ ($p = 0.001$). The PCoA analysis based on pair-wise Φ_{ST} (FIGURE 2) reflected the total identity of

populations P63 and P96 and the high differentiation of populations P166 and P32 in relation to the others.

TABLE 3.2.2.: Genetic diversity estimations and haplotype frequencies for seven populations of *P. juniperinum*.

	P27	P32	P34	P63	P81	P96	P166
N	16	14	18	14	9	14	7
N_{hap}	2	1	2	2	2	2	1
uh	0.125	0.000	0.366	0.264	0.222	0.264	0.000
$pHap_1$	0.938	1.000	0.778	0.000	0.889	0.000	0.000
$pHap_2$	0.063	0.000	0.222	0.000	0.111	0.000	0.000
$pHap_3$	0.000	0.000	0.000	0.143	0.000	0.143	0.000
$pHap_4$	0.000	0.000	0.000	0.857	0.000	0.857	1.000

N = number of haplotypes; N_{hap} = effective number of haplotypes; uh = unbiased diversity; $pHap$ = haplotype frequency.

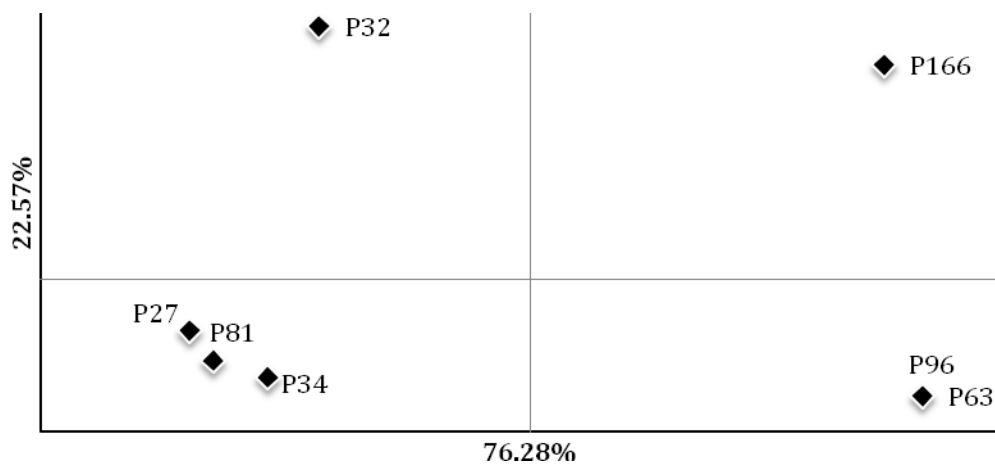


FIGURE 3.2.2. - Principal coordinates analysis demonstrating the Euclidean distance between populations of *P. juniperinum* analyzed in this study.

Discussion

Structured morphological variation among bryophyte populations as observed in this study is infrequent. This significant variability may be effect of the distinct differentiation in

niche structure in the Nelson Island, as suggested for *Syrrhopodon leprieurii* Mont. (Calymperaceae) from montane and spatially isolated regions (PEREIRA *et al.* 2013).

Although all populations evaluated in this study grow in the same sub-Antarctic island, such morphometric differences may have an adaptive significance. Phenotypic plasticity exhibited by bryophytes may represent an alternative strategy over genetic differentiation to enable growth in a range of environments (SKOTNICKI *et al.*, 2000). Plasticity is usually thought to be an evolutionary adaptation to environmental variation that occurs within the lifespan of an individual organism. If the optimal phenotype in a given environment changes with environmental conditions, then the ability of individuals to express different traits should be advantageous and thus selected for.

Accordingly to the significant morphometric differentiation observed, high among population genetic differentiation was recorded in this study, representing 69% of the total genetic variation. Gametophytes are the dominant haploid vegetative stage in mosses and sexual recombination only occurs by means of the sporophytes. In Antarctica, sporophyte production is rare and vegetative dispersal seems the most likely means of local spread and establishment of new populations (SKOTNICKI *et al.*, 2000). Therefore, such high among populations differentiation may be effect of mutational events. Since the sexual reproduction seems a rare event in Antarctic mosses, Skotnicki *et al.* (2000) suggested that such within-population may be caused by immigration, establishment of propagules from different origins and by mutagenesis.

Because Antarctic vegetation has historically experienced some of the lowest levels of UV radiation on Earth, it has been hypothesized that it might show particular susceptibility to the elevated UV radiation resulting from recent ozone depletion (CLARKE and ROBINSON 2008). As consequence of this exposure, high levels of mutation may be occurring in Antarctic mosses. Since growth occurs from a single apical cell in mosses, somatic mutations

in a distinct cell are passed on to all subsequent cells of that stem (SKOTNICKI et al., 2000). The occurrence of haplotypes shared for different populations may be effect of dispersal of asexual propagules or gametophyte fragments and establishment of new colonies.

Similar results concerning among populations genetic differentiation in Antarctic moss populations were recorded through genetic markers. In *Sarconeurum glaciale* (C.Bryhn.) Card. et Bryhn, isozyme and RAPD markers revealed high genetic differentiation among populations sampled in different sites of the Continental Antarctica (SELKIRKI et al., 1997; SKOTNICKI et al., 1999). Populations of *Ptychostommum pseudotriquetrum* (ex *Bryum pseudotriquetrum*) revealed moderate to high genetic differentiation among sampled populations, including some populations completely identical for RAPD markers (Skotnicki et al. 1998). In *Pohlia nutans* (Hedw.) Lindb., high genetic differentiation among populations from Antarctic Peninsula and populations from the Continental Antarctica was found, while very high similarity was observed among the continental populations (SKOTNICKI et al. 2002). The genetically divergent populations of *P. juniperinum* from Nelson Island likely are originated from different introgressions or resulting from somatic mutations.

Sporophytes of *P. juniperinum* have not been observed in Nelson Island and, like other Antarctic mosses, this species appears to reproduce only asexually, by dispersal of vegetative propagules. Results obtained from Clarke et al. (2009) demonstrate that mutations occur in a detectable level in Antarctic buds of *Ceratodon purpureus* (Hedw.) Brid., suggesting that mutations are the most probable source of genetic alterations in the Antarctic environmental conditions. Within population genetic diversity was observed in five out the seven populations, suggesting the occurrence of mutational events. Mutation has also been proposed as the origin of within population genetic diversity in other Antarctic moss species (SELKIRKI et al. 1997, SKOTNICKI et al. 1998, 1999) studied with RAPD markers.

European populations of *P. juniperinum* studied by Van der Velde & Bijlsma (2000)

revealed high genetic diversity for isozyme loci (mean $H = 0.127$) and moderate differentiation among populations ($F_{ST} = 0.241$). Comparing to results obtained for other species of *Polytrichum* studied in the same survey, the authors justified the genetic pattern observed in *P. juniperinum* as result of predominance of sexual reproduction occurring in the studied populations. The absence of diversity observed in populations P32 and P166 and the lack of sporophytes records in populations of *P. juniperinum* from Nelson Island suggest the predominance of asexual reproduction in this species in Antarctica.

Regarding the relationship between morphometric and genetic differentiation of populations, the low correlation between genetic and morphometric pair-wise differentiation suggests that the morphological differentiation observed among these seven populations is not correlated with the genetic differentiation observed. Pairs of populations that are genetically identical revealed significantly different morphometric traits. It likely results from the fact that morphological differentiation may be related with adaptation to microenvironment while the genetic markers evaluated in this study, although originated from EST regions, may be related to other metabolic functions in *P. juniperinum*, not to gametophyte length and leaves size.

Referencias Bibliográficas

Avérous, P., 1993. Au Bout Du Monde, L'Antarctique. Editora Augustus, pp.120, 1993.

Bednarek-Ochyra, H.; Vána, J.; Ochyra, R.; Lewis-Smith, R. I., 2000. The liverwort flora of Antarctica. Polish Academy of Sciences, Institute of Botany, Cracow.

Capozoli, U., 1991. Antártida – A Última Terra. Editora USP, pp.384.

- Cannone N., Conevey P. & Guglielmin M. 2013. Diversity trends of bryophytes in continental Antarctica. *Polar Biology*, 36, 259-271.
- Clarke, L. J., Ayre, D. J. & Robinson, S. A., 2009. Genetic structure of East Antarctic populations of the moss *Ceratodon purpureus*. *Antarctic Science* 21, 51-58.
- Delgadillo M., C. & Cárdenas S., A., 1990. Manual de Briofitas. 2 ed. Cuadernos del Instituto de Biología 8. Universidad Nacional Autonoma de Mexico, México, D.F.
- Excoffier L, Smouse PE, Quattro J. M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, 131, 479–491.
- Frahm, J-P., 2003. Manual of Tropical Bryology. *Tropical Bryology* 23: 1-196.
- Jiawen, R., Dahe, Q., Petit, J. R., Jouzel, J. Wenti, W. Chen, L., Xiaojun., Songlin, Q. and W. Xiaoxiang. 1995. Glaciological studies on Nelson Island, South Shetland Islands, Antarctica. *J. Glaciol.* 41 (138), 408-412.
- Lemos-Michel, E., 2001. Hepáticas Epífitas sobre o pinheiro-brasileiro no Rio Grande do Sul. Editora da Universidade, Porto Alegre, 191 p.
- Margulis, L. & Schwartz, K., 2001. Cinco Reinos - um guia ilustrado dos filós da vida na Terra. 3 ed. Guanabara Koogan, Rio de Janeiro.
- Ochyra. R.; Bednarek-Ochyra, H.; Lewis-Smith, R. I., 1998. 170 years of research of the Antarctic moss flora. In: Głowacki P, Bednarek J. (eds), *Polish Polar Studies*. Institute of Geophysics of the Polish Academy of Sciences, Warszawa, 159–177.
- Ochyra R, Lewis-Smith RI, Bednarek-Ochyra H., 2008. The illustrated moss flora of Antarctica. Cambridge, Cambridge University Press, 685.
- Peakall, R.; Smouse, P. E., 2006. GenAEx 6: genetic analysis in Excel. Population genetic software for teaching and research *Molecular Ecology Notes* 6: 288–295.

- Putzke, J. & Pereira, A B., 2001. The Antarctic Mosses, with special references to the Shetland Islands. Canoas. Ed. ULBRA, 196p.
- Rozema, J.; Boelen, P.; Blokker, P., 2005. Depletion of stratospheric ozone over the Antarctic and Arctic: Responses of plants of polar terrestrial ecosystems to enhanced UV-B, an overview 137: 428-442
- Selkirk P.M., Skotnicki M.L., Adam M.B., Connet T. (1997). Genetic variation in Antarctic populations of the moss *Sarconeurum glaciale*. *Polar Biology* 18: 344–350
- Schofield, W.B., 1985. Introduction to Bryology. Macmillan Publishing Company, New York.
- Skotnicki M.L., Ninham J.A., Selkirk P.M. (1998). Genetic diversity in the moss *Bryum argenteum* in Australia, New Zealand and Antarctica. *Bryologist* 101: 412-421
- Skotnicki, M.L., Ninham, J.A. & Selkirk, P.M. (1999). Genetic diversity and dispersal of the moss *Sarconeurum glaciale* on Ross Island, East Antarctica. *Molecular Ecology* 8: 753-762
- Skotnicki, M. L., J. A. Ninham, and P. M. Selkirk. 2000. Genetic diversity, mutagenesis and dispersal of Antarctic mosses—a review of progress with molecular studies. *Antarctic Science*. 12: 363–373.
- Skotnicki M. L., Selkirk P. M., Kitajima E et al. 2002. The first sub-Antarctic plant virus report: stilbocarpa mosaic bacilliform badnavirus (SMBV) from Macquarie Island. *Polar Biol* 26: 1–7.
- Smith R. I. L., 1984. Terrestrial plant biology of the sub-antarctic and Antarctic. In: *Antarctic Ecology* (ed. Laws R. M.), pp. 61-162. Academic Press, London.
- Stefenon, V.M., Roesch, L.F.W. & Pereira, A.B., 2012. Thirty years of Brazilian research in Antarctica: ups, downs and perspectives. *Scientometrics* (on line first).doi: 10.1007/s11192-012-0809-3.
- Van der Velde, M. e Bijlsma, R., 2000. Amount and structure of intra- and interspecific genetic variation in the moss genus *Polytrichum*. *Heredity*85: 328–337

Van Der Velde M. e Bijlsma, R., 2003. Phylogeography of five *Polytrichum* species within Europe. *Biological Journal of the Linnean Society* 78 (2): 203-213

Scar, 2014. Scientific Committee on Antarctic Research. Disponível em <http://www.scar.org/>. Acesso em 12/12/2013.

Van der Velde M. L. van de Zande R. Bijlsma, 2001. Genetic structure of *Polytrichum formosum* in relation to the breeding system as revealed by microsatellites. *Journal of Evolutionary Biology* 14: 288-295.

Victoria F. C.; Maia, L.C.; Oliveira, A. C., 2011. In *silico* comparative analysis of SSR markers in plants. *BMC Plant Biology*, 11:15. doi:10.1186/1471-2229-11-15

Wilson P. J., Provan J, 2003. Effect of habitat fragmentation on levels and patterns of genetic diversity in natural populations of the peat moss *Polytrichum commune*. *Proceedings of the Royal Society of London Series-B* 270: 881–886. doi: 10.1098/rspb.2002.2324

4. CONCLUSÕES GERAIS

Observou-se significativa diferença entre as populações das espécies analisadas com relação ao tamanho do gametófito. A nível morfológico, o presente estudo revelou diferenciação interpopulacional significativa para as características avaliadas: comprimento dos gametófitos, comprimento dos filídios e largura dos filídios. Dados morfológicos e genéticos foram analisados em conjunto, pois mesmo que a espécie ocorra em locais diferentes, poderia haver diferenças devido ao isolamento.

Considerando que a intensidade da luz pode ter efeitos importantes sobre o crescimento do gametófito, este pode ser um fator importante para gerar esta diferenciação nas espécies analisadas e de fato, durante o inverno todas as populações ficam com menor tempo de luz nos dias de inverno. Nas condições climáticas extremas da Antártica, as populações tem baixa disponibilidade de água, fato que pode explicar os tamanhos comparativos menores dos filídios, como já demonstrado em um experimento com *Philonotis fontana* (Hedw.) Brid.

Embora mais estudos experimentais sejam necessários para avaliar o nível de adaptação genética de populações *Polytrichum juniperinum* às condições da Antártica, este estudo revela a existência de características morfológicas relacionadas com a adaptação às condições ambientais e fornece o primeiro passo para destacar esse problema para espécies de plantas do continente antártico. Considerando que a literatura especializada sugere a inexistência de indivíduos férteis de *P. juniperinum* no continente Antártico, foram caracterizados níveis de diferenciação populacional das espécies neste continente, portanto os dados foram correlacionados com a dispersão de propágulos.

Havendo a existência de alguma adaptação morfológica das populações deve ser observado menor crescimento em ambientes menos favoráveis, como é o ambiente Antártico. Como efeito da distância geográfica entre as populações e a dispersão provavelmente limitada de estruturas reprodutivas da espécie, o fluxo gênico deve ser quase ausente entre elas.

5. REFERÊNCIAS BIBLIOGRÁFICAS

- Avérous, P., 1993. Au Bout Du Monde, L'Antarctique. Editora Augustus, pp.120, 1993.
- Bednarek-Ochyra, H.; Vãna, J.; Lewis-Smith, R.I. & Ochyra R., 2000. The liverwort of Antarctica. Polish Academy of Science, Institute of Botany, Cracow, 237p.
- Capozoli, U., 1991. Antártida – A Última Terra. Editora USP, pp.384.
- Cygan B. 1981. Characteristics of meteorological conditions at the Arctowski Station during the summer season of 1979/1980. Pol Polar Res 2:35-46
- Greene, S. W., 1968. Studies in Antarctica bryology: 1. A basic check: list for mosses. Revue Bryologique et Lichénologique, Nouvelle Série 36 (1-2):132-138
- Imura, S., Higuchi, M., Kanda, H. and Iwatsuki, Z, 1994. Structure of moss colonies in the Syowa Station area, Antarctica. Proc. NIPR Symp. Polar Biol., 7, 232-236
- Kanda, H., 1986. Moss communities in some ice-free áreas along the Söya Coast, East Antarctica. Memoirs of Natural Institute of Polar Research, Special Issue., 44: 229-240
- Kanda, H. e Iwatsukzi, 1989. Two aquatic mosses in the lakes near Syowa Station, Continental Antarctica. Hikobia, 10, 293-297.
- Kanda, H. & Ohtani, S., 1991. Morphology of the aquatic mosses collected in lake Yukidori, Langhovde, Antarctica. Proceedings of NIPR Symposium on Polar Biology, 4: 114-122
- Kovalski, D., 1985. Wind structure at the Arctowski Station. Polish Polar Research, v. 20, p. 203-220.
- Murray, B. M., 2012. Australian Mosses Online. 55. *Andreaeaceae*. http://www.anbg.gov.au/abrs/Mosses_online/Andreaeaceae.pdf (2012).
- Ochi, H., 1982. A revision of the Bryoideae, Musci in Southern South America. Journal Faculty Education Tottori University Natural Sciences 31: 11-47.

- Ochyra, R., 1985. On the Antarctic species of the family Orthotrichaceae. *Lindbergia* 11: 141–146.
- Ochyra, R., 1998. The moss flora of King George Island, Antarctica. Polish Academy of Sciences. Cracow, 279p.
- Ochyra, R. & Ochi, H., 1986. New or otherwise interesting species of the genus *Bryum* (Musci, Bryaceae) in the Antarctic. *Acta Botanica Hungarica* 32 (1-4): 209-219.
- Ochyra R, Bednarek-Ochyra H, Lewis-Smith R. I., 1998. 170 years of research of the Antarctic moss flora. In: Głowacki P, Bednarek J. (eds), *Polish Polar Studies*. Institute of Geophysics of the Polish Academy of Sciences, Warszawa, 159–177.
- Ochyra R, Lewis-Smith RI, Bednarek-Ochyra H., 2008. *The illustrated moss flora of Antarctica*. Cambridge, Cambridge University Press, 685.
- Peralta, D.F., 2009. *Polytrichaceae (Polytrichales, Bryophyta) do Brasil*. Tese de Doutorado. Instituto de Botânica, São Paulo. 170p.
- Pereira, A. B. & Putzke J., 1994. Floristic composition of Stinker Point, Elephant Island, Antarctica. *Kor. J. Polar Res.* 5(2): 37-47.
- Pereira, B. K., Rosa, R. M., et al., 2009. Protective effects of three extracts from Antarctic plants against ultraviolet radiation in several biological models. *Journal of Photochemistry and Photobiology B: Biology*, 96, 117–129.
- Putzke, J. & Pereira, A B., 2001. *The Antarctic Mosses, with special references to the Shetland Islands*. Canoas. Ed. ULBRA, 196p.
- Rakusa-Suszczewski, S. 2002. King George Island. South Shetland Islands, Maritime Antarctic. In: Beyer, L.; Bölter, M. (Ed.). *Geocology of Antarctic Ice-Free Coastal Landscapes*. Series: Ecological Studies. Berlin: Springer Verlag. v. 154, p. 23-41.
- Riffenburgh, B., 2007. *Encyclopedia of the Antarctic*. [S.l.]: CRC Press. vol. 2. ISBN 978-0-415-97024-2

- Rozema, J.; Boelen, P. e Blokker, P., 2005. Depletion of stratospheric ozone over the Antarctic and Arctic: Responses of plants of polar terrestrial ecosystems to enhanced UV-B, an overview 137: 428-442.
- Robinson, H. E., 1972. Observations on the origin and taxonomy of the Antarctic moss flora. In: Llano G. A. (ed.) Antarctic terrestrial biology. Antarctic Research Series. Vol. 20. Washington, American Geophysical Union. 163-177.
- Scar, 2014. Scientific Committee on Antarctic Research. Disponível em <http://www.scar.org/>. Acesso em 12/12/2013.
- Schmitz-Hoerner, R. e Weissenböck, G., 2003. Contribution of phenolic compounds to the UV-B screening capacity of developing barley primary leaves in relation to DNA damage and repair under elevated UV-B levels. *Phytochemistry* 64 243–255
- Steere, W. C., 1961. A preliminary review of the bryophytes of Antarctica. In: *Science in Antarctica Part 1. The life science in Antarctica*. Washington, D.C. National Academy of Science – National Research Council, p. 20-33.
- Van Der Velde M e Bijlsma, R., 2003. Phylogeography of five *Polytrichum* species within Europe. *Biological Journal of the Linnean Society* 78 (2): 203-213.
- Victoria F. C., Pereira A. B. e Costa D. P., 2009. Composition and distribution of moss formations in the ice-free areas adjoining the Arctowski region, Admiralty Bay, King George Island, Antarctica. *Iheringia Série Botânica* 64:(1) 81-91.