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**Investigando a diversidade biológica de quatro lagos na Ilha James Ross (Antártica)
com uso de DNA *metabarcoding***

Florianópolis

2024

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com uso de DNA *metabarcoding***

Dissertação submetida ao Programa de Pós-Graduação em Fungos, Algas e Plantas da Universidade Federal de Santa Catarina como requisito parcial para a obtenção do título de Mestre em Biologia de Fungos, Algas e Plantas.

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Kauana Beppler de Souza

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DNA *metabarcoding***

O presente trabalho em nível de Mestrado foi avaliado e aprovado, em 08 de março de 2024, pela banca examinadora composta pelos seguintes membros:

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Dedico este trabalho a todos os pesquisadores polares.

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Life finds a way.
(Ian Malcolm, 1993)

RESUMO

A Antártica é o continente mais austral da Terra, ocupando uma área de cerca de 14 milhões de km². Corresponde a cerca de 10% da superfície do planeta, é a principal reserva de gelo (90%) e água doce (70%) do mundo. É uma das regiões mais inexploradas do planeta, com um conjunto de condições extremas como frio intenso, seca e condições muito oligotróficas. Com o aumento da temperatura global e o derretimento das geleiras, ocorre a liberação de água líquida que pode conter material biológico e propágulos viáveis e criar ambientes propícios para que novos organismos que chegam na Antártica se estabeleçam. A Península Antártica (PA) está localizada ao leste do continente antártico e representa a única área do continente (1%) onde se observam áreas com derretimento completo do gelo durante o verão. A Ilha James Ross está localizada no Mar de Weddell, a nordeste da PA, e nesta ilha está localizada a região de Clearwater Mesa, que possui mais de 60 lagos rasos. Os lagos são ambientes extremófilos e intocados, raros, com águas de temperaturas extremas, muito transparentes, recebendo alta incidência de luz solar e raios UV e com baixa disponibilidade de nutrientes, além de serem importantes reservas biológicas para diversos organismos, com a cobertura de gelo derretendo durante o verão e permitindo a entrada de nutrientes e outros diásporos. O DNA *metabarcoding* permite a identificação simultânea do DNA de espécies presentes em amostras ambientais (eDNA), como amostras de solo, água ou outras amostras ambientais. O uso dessa ferramenta facilita a logística do estudo da biodiversidade, com menos idas a campo e menor demanda de pesquisadores e taxonomistas que tenham conhecimento de taxonomia específica, visto que a ferramenta se baseia na comparação das sequências obtidas nas amostras com as sequências disponibilizadas nos bancos de dados. O presente trabalho tem como objetivo investigar e caracterizar as comunidades biológicas de quatro lagos da ilha James Ross utilizando a ferramenta de DNA *metabarcoding*. Para tal, amostras de sedimento dos lagos foram coletadas durante o verão austral 2019/2020 e posteriormente o DNA das amostras foi extraído e sequenciado. Como resultados, obtivemos um total de 393.010 DNA *reads*, representando 107 táxons diferentes, com distribuição em quatro reinos. O reino Plantae foi o mais representativo, com 78 táxons amostrados, seguido por Chromista, com 19 táxons, Protozoa com oito e Animalia com apenas um. Dos táxons amostrados, 20 são novos registros de ocorrência para o continente Antártico e outros cinco possuíam registros apenas para o gênero. Dentre as novas ocorrências estão seis taxa de plantas com flores, o que pode refletir a ação antrópica no continente Antártico. Análises estatísticas com curva de rarefação, Análise de Agrupamento (Cluster), Análise de Correspondência Destendenciada (DCA) e Escala Multidimensional Não-Métrica (NMDS) foram realizadas a fim de relacionar as características físico-químicas e geológicas dos lagos com a sua composição taxonômica, que se mostrou ser heterogênea.

Palavras-chave: Biodiversidade lântica; colonização antártica; diversidade críptica; metagenômica.

ABSTRACT

Antarctica is the southernmost continent on Earth, occupying an area of around 14 million km². It corresponds to around 10% of the planet's surface and is the main reserve of ice (90%) and fresh water (70%) in the world. It is one of the most unexplored regions on the planet, with a set of extreme conditions such as intense cold, drought and very oligotrophic conditions. With the increase in global temperature and the melting of glaciers, liquid water is released that can contain biological material and viable propagules and create favorable environments for new organisms that arrive in Antarctica to establish themselves. The Antarctic Peninsula (AP) is located to the east of the Antarctic continent and represents the only area of the continent (1%) where areas with complete ice melt are observed during the summer. James Ross Island is located in the Weddell Sea northeast of PA, and this island is home to the Clearwater Mesa region, which has more than 60 shallow lakes. Lakes are rare, extremophilic and untouched environments, with waters of extreme temperatures, very transparent, receiving a high incidence of sunlight and UV rays and with low availability of nutrients, in addition to being important biological reserves for various organisms, with the ice cover melting during the summer and allowing nutrients and other diaspores to enter. DNA metabarcoding allows for the simultaneous identification of the DNA of species present in environmental samples (eDNA), such as soil, water or other environmental samples. The use of this tool facilitates the logistics of studying biodiversity, with fewer trips to the field and less demand from researchers and taxonomists who have knowledge of specific taxonomy, as the tool is based on comparing sequences obtained from samples with sequences available in databases. The present work aims to investigate and characterize the biological communities of four lakes on James Ross Island using the DNA metabarcoding tool. To this end, sediment samples from the lakes were collected during the austral summer of 2019/2020 and the DNA from the samples was subsequently extracted and sequenced. As a result, we obtained a total of 393,010 DNA reads, representing 107 different taxa, distributed across four kingdoms. The kingdom Plantae was the most representative, with 78 taxa sampled, followed by Chromista, with 19 taxa, Protozoa with eight and Animalia with just one. Of the taxa sampled, 20 are new records of occurrence for the Antarctic continent and another five had records only for the genus. Among the new occurrences are six taxa of flowering plants, which may reflect human action on the Antarctic continent. Statistical analyzes with rarefaction curve, Cluster Analysis (Cluster), Detrended Correspondence Analysis (DCA) and Non-Metric Multidimensional Scaling (NMDS) were carried out in order to relate the physicochemical and geological characteristics of the lakes with their taxonomic composition, which proved to be heterogeneous.

Keywords: Lentic biodiversity; Antarctic colonization; cryptic diversity; metagenomics.

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LISTA DE ABREVIATURAS E SIGLA

AP	Antarctic Peninsula
DCA	Análise de Correspondência Destendenciada
eDNA	Environmental DNA / DNA ambiental
NMDS	Escala Multidimensional Não-Métrica
OPERANTAR	Operação Antártica
PA	Península Antártica
PROANTAR	Programa Antártico Brasileiro
UFSC	Universidade Federal de Santa Catarina
UnB	Universidade de Brasília

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INTRODUÇÃO GERAL

A Antártica é o continente mais austral da Terra, compreende todas as terras e mares ao sul do paralelo 60°S, ocupando uma área de cerca de 14 milhões km², correspondendo a cerca de 8% da superfície do planeta, e é a principal reserva de gelo (90%) e água doce (70%) do planeta (Convey 2009, Brasil 2014). O continente é ainda o mais isolado (Convey 2001), devido aos processos que começaram durante a separação da Gondwana, que se mantêm através da corrente atmosférica, do Vórtex Polar e da corrente Circumpolar Antártica, responsáveis pelo isolamento térmico e biogeográfico do continente e também pela glaciação da Antártica (Clarke *et al.* 2005, Barnes 2006).

Com menos de 1% do seu território livre de gelo (Convey 2001, Convey *et al.* 2014), foi o último continente a ser descoberto pelo homem (na década de 1820) e nunca teve população permanente. Nos últimos anos o aumento no número de população transitória vêm aumentando devido à instalação das bases científicas dos mais de 58 países signatários do Tratado da Antártica, sendo o continente que menos sofreu com ações antrópicas, o que permite estudar a sua biologia por apresentar um gradiente de condições naturais (Convey 2001). Adicionalmente é também uma das regiões mais inexploradas do planeta, com um conjunto de condições extremas como frio intenso, seca e condições muito oligotróficas (Gonçalves *et al.* 2012; Convey *et al.* 2014). É um continente desolado e isolado, sem suporte para a vida humana, porém é dominado por microorganismos, plantas (Bryophyta) e alguns invertebrados, além de focas, pinguins e outras aves, entre outros animais, que fazem parte da cadeia alimentar marinha (Laybourn-Parry & Wadham 2014). A precipitação média anual do continente varia de menos de 10 mm no Vale Seco de McMurdo a 600 mm ao longo da Península Antártica (Bockheim & Schaefer 2015), enquanto a temperatura varia de -98°C (a menor já observada, mensurada através de satélites de mapeamento infravermelho no leste da Antártica) e 19,8°C (a maior temperatura já registrada, na estação Britânica Signy) (Scambos *et al.* 2018, Turner 2021).

O continente antártico é regido pelo Tratado Antártico, que foi criado em 1959 e entrou em vigor em 1961, o qual regula a atuação dos países na Antártica (Brasil 2014). Na sua criação, 12 países originalmente assinaram o tratado, se tornando membros signatários originais do tratado, ou seja, opinam e decidem sobre o destino do continente (Marinha do Brasil 2016). Percebendo a importância de fazer parte dessa tomada de decisão, o Brasil adere ao tratado em 1975, e implementa o Programa Antártico Brasileiro

(PROANTAR), iniciando suas atividades em 1982, e se tornando em 1983, através da primeira Operação Antártica (OPERANTAR I), membro consultivo do Tratado da Antártica (Marinha do Brasil 2016), ou seja, com direito a voz e voto em todas as decisões sobre a região, privilégio de apenas 29 países. Atualmente a Antártica faz parte do entorno estratégico brasileiro, com motivações geopolíticas e abrindo espaço para que a comunidade científica nacional pudesse realizar atividades no continente (Marinha do Brasil 2016, De Mattos & Câmara 2020).

Em 1998 é assinado o Protocolo ao Tratado da Antártica sobre proteção ao meio ambiente, também conhecido como Protocolo de Madri, que foi criado em 1991 e visa a proteção integral do continente, durante 50 anos. Através desse tratado, a Antártica recebeu o status de “Reserva Natural Internacional dedicada à Ciência e à Paz” (Marinha do Brasil 2016). Através deste protocolo e do Tratado da Antártica em si, os países que desejam ser membros signatários do Tratado e possuir o poder de decidir sobre o futuro da maior reserva de água doce do mundo, grandes reservas de petróleo, gás natural, minério e recursos pesqueiros que constituem o continente antártico devem realizar ali pesquisa científica de qualidade (Marinha do Brasil 2016). Além dos recursos naturais, a Antártica é também de grande importância para a manutenção do clima global (Farmer & Cook 2013), que, se alterado, poderá gerar impactos diretos para a sociedade brasileira (De Mattos & Câmara 2020).

O aumento de temperatura vem sendo observado por todo o globo, mas poucas regiões do planeta apresentam um aquecimento tão rápido quando a Península Antártica (PA) (Convey 2001, Turner *et al.* 2005, Glasser *et al.* 2014), e apesar de algumas regiões da porção continental da Antártica registrarem uma diminuição da temperatura, a PA vem apresentando um aumento geral da temperatura nos últimos 50 anos (Farmer & Cook 2013, Turner *et al.* 2016) o que pode ocasionar grandes efeitos na vegetação, fauna e funga associada (Câmara *et al.* 2022). Há alguns anos, o continente Antártico ainda conseguia resistir à colonização de novas espécies, por haver pouquíssima atividade antrópica no continente e também pela presença da Corrente Circumpolar Antártica, que de certa forma, mantém o continente isolado do resto do mundo (Clarke *et al.* 2006, Galera *et al.* 2018). Além disso, os diásporos que conseguiam chegar ao continente encontravam condições muito extremas, o que dificultava a colonização (Pearce *et al.* 2016). Porém, o constante aumento da temperatura no continente e no mundo causa um aumento na disponibilidade de água líquida e aumento na frequência pluviométrica, que somado ao aumento no número de pessoas que visitam o continente, através do turismo

e através das bases de pesquisa instaladas na Antártica, a região se torna cada vez mais favorável para a chegada de novos microrganismos e propágulos e à colonização de novas espécies (Convey 2001, Pearce *et al.* 2009, Galera *et al.* 2018, Rückamp *et al.* 2011). Esse aumento no número de diásporos que chega ao continente e seu sucesso na colonização, poderá aumentar o número de invasões biológicas e coloca em risco a flora, fauna e funga local (Galera *et al.* 2018; Pearce *et al.* 2009). O turismo antártico iniciou por volta de mais de um século atrás (Headland 1994) e vem se intensificando a partir do ano de 1990 (Vereda & Jensen 2020) também pode ser responsável pela chegada de novos propágulos no continente, que podem acabar se estabelecendo e alterando o ecossistema local e outras consequências ambientais que podem ter impacto global (Kariminia *et al.* 2013).

Como já citado, a Antártica é a maior reserva de gelo do mundo, reserva esta que foi sendo formada a partir do acúmulo de neve que não derreteu ao longo dos anos e foram comprimidas por uma nova camada de neve, o que vem ocorrendo ao longo de milhares de anos, podendo ser o habitat de muitos organismos e servir como um registro químico de climas e ambientes passados (Abyzov 1993, Gunde-Cimerman *et al.* 2003, Convey *et al.* 2009). Com as mudanças climáticas, o aumento da temperatura global e o derretimento das geleiras, ocorre a liberação de água líquida que pode conter material biológico e propágulos viáveis (Câmara *et al.* 2022) e a expansão das áreas livres de gelo também dará origem a muitos habitats potenciais novos e também aumentará a conectividade entre as áreas livre de gelo, o que permitirá que as espécies nativas ocupem novos espaços, mas também que propágulos que estão chegando na Antártica se estabeleçam (Chown & Convey 2007, Chown *et al.* 2012, Lee *et al.* 2017).

A Península Antártica (PA) está localizada ao leste do continente Antártico e representa a única área do continente (1%) onde se observa o derretimento total do gelo durante o verão (Union 2010). A história da PA conta com diversos eventos de glaciação e deglaciação, e essa deglaciação, juntamente com as mudanças climáticas, tiveram por consequência a criação de áreas sem gelo, formações de solo, lagos e ilhas (Braun & Gossmann 2002, Cook *et al.* 2005, Ochyra *et al.* 2008). A PA e os agrupamentos de ilhas próximas são chamados de Antártica Marítima, onde as áreas livres de gelo abrigam cerca de 380 lagos subglaciais (Laybourn-Parry & Wadham 2014).

Entre os ambientes extremófilos e intocados que existem na Antártica, os lagos são bastante peculiares. São, na sua maioria, rasos, com águas de temperaturas extremas, muito transparentes, o que permite uma alta incidência de luz e raios UV, além da baixa disponibilidade de nutrientes, e por apresentarem uma geologia bem distinta entre si,

acabam tendo propriedades químicas bastante variáveis (Gonçalves 2011, Gonçalves 2012). São também importantes reservas biológicas para diversos organismos (Gonçalves 2022). Diferentemente dos lagos de latitudes mais baixas, os lagos da Antártica são prístinos e ainda não sofreram com ações humanas diretas e são importantes centros de biomassa, biodiversidade e de ciclagem biogeoquímica (Laybourn-Parry & Wadham 2014). Há diferença nas dinâmicas dos lagos da Antártica Continental e Marítima. Nos lagos da Antártica Continental, que são lagos cobertos por gelo, não existe uma variação muito grande nos gradientes físicos e químicos, enquanto os lagos da região marítima, onde a temperatura é um pouco mais elevada, a cobertura de gelo desses lagos derrete durante o verão, permitindo a entrada de nutrientes e outros diásporos, mostrando também importantes alterações na composição das espécies desses lagos (Villaescusa *et al.*, 2010).

A Ilha James Ross possui cerca de 2500 km², e esta localizada no Mar de Weddell, a nordeste da Península Antártica entre as coordenadas 63°47'S-64°27'1S e 57°05'W-58°24'W (Björck 1996, Láska 2011). Abrigada pela PA dos ventos oestes, responsáveis por trazerem as massas de ar úmido, James Ross possui cerca de 80% do seu território coberto por gelo, com o clima severo, com a média de temperatura no nível do mar se mantendo entre -7 a -10°C ao longo do ano, e no verão elas se mantêm um pouco abaixo de 0°C, permitindo que James Ross seja uma área de permafrost contínuo (Björck 1996, Láska 2011). A precipitação da parte norte da Ilha é de menos de 150 mm por ano, mas é um local onde comumente neva (Björck 1996). A região de Clearwater Mesa, localizada na Ilha James Ross, é uma região que possui mais de 60 lagos rasos e lagoas, como pode ser observado na Figura 1, que permitem o estudo de regiões que ainda não sofreram ações antrópicas (Lecomte *et al.* 2020). Os lagos da região de Clearwater Mesa são lagos que estão sobre rochas do Grupo Vulcânico de James Ross, datados do Plioceno, e são compostos principalmente por basaltos alcalinos e brechas de haloclastia palagonitizada, cobertas com depósitos glaciais com clastos basálticos (Smellie *et al.* 2013). Apesar do hiato existente no aquecimento, observado no início do século XXI, as previsões da Rota de Concentração Representativa (do inglês, *Representative Concentration Pathway* - RCP) para o ano de 2100 indicam um maior aquecimento e secagem pronunciada nas áreas livres de gelo da Ilha James Ross, devido ao descongelamento e retração das geleiras, afetando ativamente os riachos, lagos e as comunidades existentes nesses ecossistemas (Roman *et al.* 2019).



Fig. 1. Localização da região de Clearwater Mesa em: **a)** Arquipélago James Ross; **b)** porção centro-norte da Ilha James Ross. **c)** Fotografia aérea da região, que foi utilizada para gerar o mapa. **d)** Principais características morfogeológicas e hidrográficas da região. Fonte: Imagem retirada de Roman et al., 2019.

A fim de compreender como os organismos são dispersados e colonizam as poucas regiões da Antártica na qual eles conseguem chegar e se estabelecer, é importante ter o conhecimento prévio da composição atual do local. Alguns trabalhos tradicionais de avaliação da diversidade da vegetação Antártica já foram feitos (Ochyra *et al.* 2008) e são considerados grandes contribuições para o conhecimento que temos atualmente sobre a biodiversidade Antártica, porém para esse tipo de trabalho é necessário que o pesquisador tenha um conhecimento prévio da taxonomia específica sobre diversos grupos de organismos, além de demandar uma logística muito complexa para coletar o material. O trabalho científico na Antártica depende da colaboração entre as nações e o pesquisador precisa fazer parte de algum grupo de pesquisa Antártica para conseguir o suporte

logístico e ter sua pesquisa aprovada pelos protocolos e convenções propostos no Tratado da Antártica (Laybourn-Parry & Wadham 2014). Desta forma, com o avanço da tecnologia, novas ferramentas de identificação biológica vêm sendo empregadas, como por exemplo a ferramenta ferramenta de DNA barcoding (Hebert *et al.* 2003, Hollingsworth *et al.* 2009) e DNA *metabarcoding* (Taberlet *et al.* 2012). Essas técnicas fornecem muitos dados, que quando utilizados de forma adequada podem proporcionar informações a respeito da distribuição de espécies e da composição de organismos nas comunidades, de forma padronizada (Taberlet *et al.* 2012).

O DNA barcoding realiza a identificação molecular através do uso de marcadores padronizados e específicos para os diferentes táxons, sendo uma técnica que permite identificar organismos em qualquer estágio de vida e até mesmo de amostras degradadas (Savolainen *et al.* 2005). Porém, uma limitação desta técnica é que ela permite a análise de apenas uma amostra por vez, que deve ser o mais pura possível para obter a interpretação mais fiel possível dos dados (Hebert *et al.* 2003), não sendo a mais adequada para o estudo de identificação de uma maior gama de organismos (Ratnasingham *et al.* 2007).

A partir do surgimento do DNA *metabarcoding*, as análises dos organismos de uma comunidade passa a ter resultados mais robusto, complementando a técnica de DNA *barcoding*. As duas se diferem em relação a tecnologia de sequenciamento que utilizam e nos objetivos específicos que elas possuem (Comtet *et al.* 2015). Enquanto o DNA barcoding utiliza uma amostra pura para identificar uma única espécie, o DNA metabarcoding utiliza amostra de uma comunidade ambiental para identificar um grande número de espécies (Taberlet *et al.* 2012). O DNA *metabarcoding* utiliza amostra de uma comunidade ambiental para identificar um grande número de espécies (Taberlet *et al.* 2012). Permite a identificação simultânea do DNA de espécies presentes em amostras ambientais (eDNA), como amostras de sedimentos de solo, água ou outras (Fahner *et al.* 2016), e através de dados moleculares de uma célula ou de indivíduos inteiros, identificar os organismos que viveram ou ainda vivem naquele local (Ficetola *et al.* 2008). Porém, o sucesso de ambas as técnicas, *barcoding* e *metabarcoding*, depende da qualidade dos bancos de dados que serão consultados, onde, por vezes, constarão os vouchers de espécies que foram previamente identificadas de forma tradicional, tiveram seu DNA sequenciado e foram associadas aos seus *barcodings* (Ratnasingham & Herber 2007), o que torna o trabalho de identificação tradicional essencial.

Diante do exposto, este estudo visa diminuir a lacuna do conhecimento acerca da biodiversidade encontrada na Ilha James Ross, através das amostras de DNA ambiental, utilizando a técnica de DNA *metabarcoding*, visto que tais comunidades permanecem pouco estudadas, em especial com o uso de ferramentas moleculares. Não conhecemos bem a diversidade e os fatores que influenciam a estrutura das comunidades de lagos Antárticos.

OBJETIVOS

Objetivo geral

O objetivo geral deste trabalho é investigar e caracterizar as comunidades biológicas lênticas em quatro diferentes lagos na ilha James Ross (Mar de Weddell, Antártica), que são os lagos Adriana, Lília, Cecília e Soledad, utilizando a ferramenta DNA *metabarcoding*.

Objetivos específicos

- Identificar e comparar a diversidade biológica do sedimento de quatro lagos da ilha James Ross;
- Relacionar os organismos encontrados com a presença humana na Antártica;
- Investigar a relação dessa biodiversidade com as características físico-químicas e diferenças de localização dos lagos;
- Inventariar e averiguar se há espécies exóticas ou que tenham algum potencial patogênico;
- Averiguar se existem espécies que possam ter aplicações biotecnológicas.

DESENVOLVIMENTO

CAPITULO 1: INVESTIGATING THE BIOLOGICAL DIVERSITY OF FOUR LAKES ON JAMES ROSS ISLAND (ANTARCTICA) USING DNA METABARCODING

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Introduction

Antarctica is the southernmost continent on Earth, comprising all land and seas south of the parallel 60°S, occupying an area of around 14 million km², corresponding to around 8% of the planet's surface, and is the main reserve of ice (90%) and 70% of the planet's freshwater (Convey 2009, Brasil 2014). With less than 1% of its territory being ice-free (Convey 2001, Convey *et al.* 2014), it's also one of the most unexplored regions on the planet, with a set of extreme conditions such as intense cold, drought and conditions that are very oligotrophic (Gonçalves *et al.* 2012, Convey *et al.* 2014).

The increase in temperature has been observed across the globe, but few regions of the planet experience warming as quickly as the Antarctic Peninsula (AP) (Convey 2001, Turner *et al.* 2005, Glasser *et al.* 2014), increase that has been observed in the last 50 years (Farmer & Cook 2013, Turner *et al.* 2016), which can cause effects on vegetation, fauna and fungi associated at the site (Câmara *et al.* 2022). With the climate change, the increase in global temperature and the melting of glaciers, liquid water is released and that may contain biological material and viable propagules (Câmara *et al.* 2022). Furthermore, the melting of glaciers also ends up creating new ice-free areas, which are possible new habitats for native species and also for new propagules that manage to reach Antarctica, in addition to creating connectivity between existing ice-free

areas, allowing the exchange of species between them (Chown & Convey 2007, Chown *et al.* 2012; Lee *et al.* 2017).

The Antarctic Peninsula is located in the east of the Antarctic continent and represents the only area of the continent (1%) with where total ice melt occurs during the summer (Union, 2010). The AP and nearby island clusters are called Maritime Antarctica, where ice-free areas are home to about 380 subglacial lakes (Laybourn-Parry & Wadham 2014). Antarctic lakes are extremophile and almost untouched environments. They are, mostly, shallow, with very cold waters, very transparents, which means that the incidence of light and UV rays is quite high, they have low availability of nutrients, and because they have a different geological origin, the chemical properties of the lakes are quite variable (Gonçalves 2011, Gonçalves 2012). Antarctic lakes are pristine and have suffered little from direct human actions and are important centers of biomass, biodiversity and biogeochemical cycling (Laybourn-Parry & Wadham 2014), which allows studying the biodiversity of practically untouched places.

Some traditional work to learn about Antarctica's moss diversity has already been done (Ochyra *et al.* 2008) and are considered great contributions to what we know about the diversity of Antarctica, but for this type of work to be carried out is necessary for the researcher to have a great deal of prior knowledge about many groups of organisms, in addition to the logistics for collecting material for this type of study is much more complex. Thus, with the advancement of technology, new biological identification tools have been used, such as DNA *metabarcoding* (Taberlet *et al.* 2012), which, if used appropriately, can provide valuable information on the distribution of species and the composition of organisms in communities in a standardized way (Taberlet *et al.* 2012). DNA *metabarcoding* uses a sample from an environmental community to identify a large number of taxons (Taberlet *et al.* 2012), allowing the simultaneous identification of the DNA present in environmental samples (eDNA), such as soil, water or air samples (Fahner *et al.* 2016), and through data from cells or whole organisms, identify the organisms that lived or still live in that location (Ficetola *et al.* 2008), it also allows the identification of species that are difficult to identify traditionally, through spores, cysts, pollen or seed, in addition to reducing the number of specialized taxonomists and trips to the field (Deiner *et al.* 2017).

That said, the present work, aimed the investigation of James Ross Island lakes using the DNA *metabarcoding* techniques, aiming to answer questions such as which organisms are present in these lakes, if these organisms can be related with the human

presence in Antarctica, inventory and check whether there are exotic species or species with pathogenic potential or that may have biotechnological applications, and also correlate the taxonomic composition of the lakes with their physicochemical and geological characteristics

Methods

Study site

The James Ross Island is approximately 2500 km², located at the Weddell Sea, northeast of the AP between coordinates 63°47'S-64°27'S e 57°05'W-58°24'W (Björck 1996, Láska 2011). It has around 80% of its territory covered by ice, with a harsh climate and temperatures that remain between -7 and -10°C, staying slightly below 0°C during the summer, which allows it to be an area with continuous permafrost (Björck 1996, Láska 2011). The precipitation in the northern part of the Island is less than 150 mm per year, but drifting snow is a common phenomenon (Björck 1996). The Clearwater Mesa region, located on James Ross Island, is a region that has more than 60 shallow lakes (Lecomte *et al.* 2020). The subject of this work is four lakes (Lília, Cecília, Soledad and Adriana) located in the Clearwater Mesa region, at James Ross Island, northeast of the AP. The lakes are shown in figure 2.

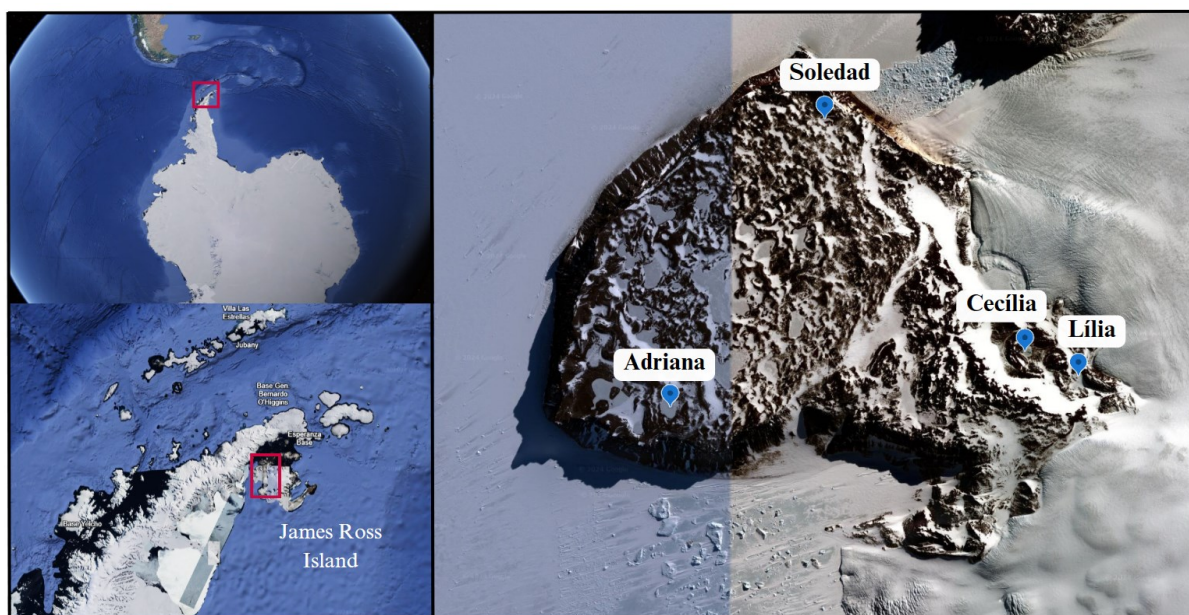


Fig. 2. Location of the lakes in James Ross Island. **a.** and **b.** Satellite images obtained from Google Earth Pro, **a)** indicating the location of Antarctica and the Antarctic Peninsula (inside the red square); **b)** James Ross Island (indicated within the red square); **c)** Lakes Lília, Soledad, Cecília and Adriana, indicated by

the blue points in the image, located on James Ross Island. Source: Images prepared by the author using the Google Earth tool and edited for better viewing

Lakes Soledad, Adriana and Cecilia have a similar distance from each other, with Lake Lília being closer to Lake Cecilia, as seen in Figure 3. Despite having similar distances, the lakes were chosen because they are positioned at very different points from each other, more or less close to glaciers, with greater or lesser influence from the sea, sheltered from winds and depth. Furthermore, there is the logistical issue of ensuring that the samples are carried out properly.

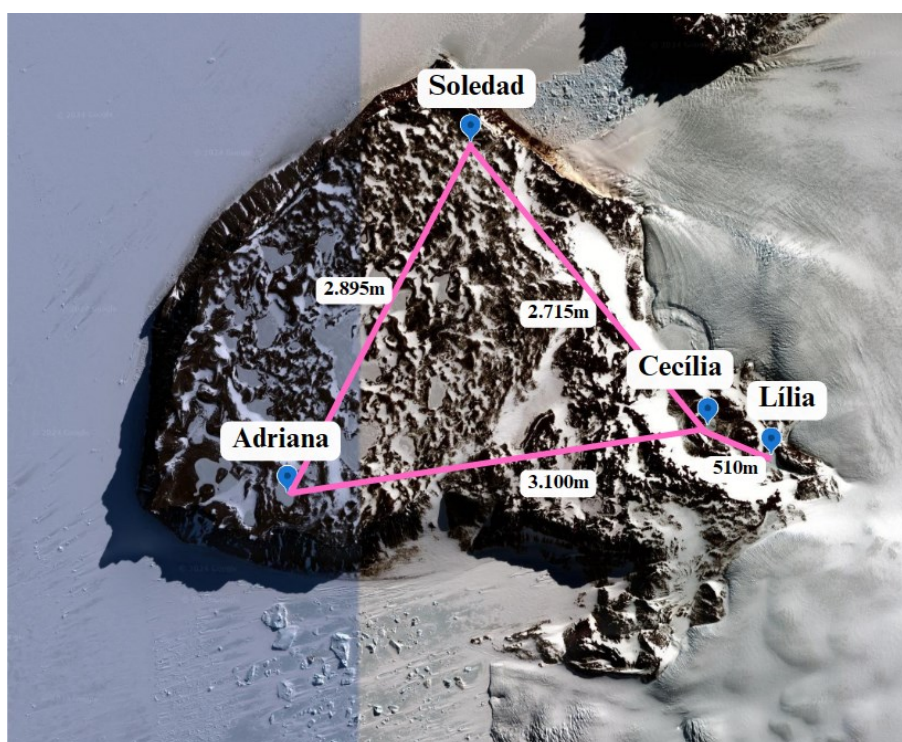


Fig. 3. Location of the lakes in James Ross Island and the distance between each lake. Source: Image prepared by the author using the Google Earth tool and edited for better viewing.

Sediment sampling

The collection and processing techniques, in addition to the physicochemical analysis of the sediments were carried out as mentioned in Gonçalves *et al.* (2022), as they are the same samples. The sediments were collected during the austral summer of 2019/2020, and were collected and stored immediately in Whirl-pak bags (Nasco, Ft. Atkinson, WI) in triplicate. Approximately 500 g of sediments were sampled from three different points on the littoral region of each lake, separated by approximately 50 m from each other and

at a depth of 20-50 cm. Each sediment sample was immediately sealed into the bags and frozen at -20 °C, kept like this until the moment of DNA extraction, which was carried out at the University of Brasília (UnB) plant molecular lab.

Metabarcoding

Subsamples were taken from the core in triplicates under strict contamination control conditions. Total DNA was extracted from the samples using the FastDNA Spin KIT for Soil Kit (MPBIO, Ohio, USA), following the manufacturer's instructions. DNA quality was analyzed by agarose gel electrophoresis and then quantified using the Quanti-iT™ PicoGreen dsDNA Assay (Invitrogen). The DNA chosen molecular marker used to identify the species was the nuclear ribosomal DNA internal transcribed spacer 2 (ITS2), using the universal primers ITS3 and ITS4 (White *et al.* 1990). Library construction and DNA amplification were performed using the Herculase II Fusion DNA Polymerase Nextera XT Index Kit V2, following the Illumina 16S metagenome library preparation and sequencing protocol (Part #15044223, Rev. B). The sequence pairing (2 x 300 pb) was performed on the MiSeq platform (Illumina) by Macrogen Inc. (South Korea).

Metagenomic analyzes

The data analysis was also carried out in accordance with what was mentioned in the article by Gonçalves (2022). Quality analysis was carried out using BBDuk v. 38.87 in BBmap software (Bushnell 2014), with the following parameters: Illumina adapters removing (Illumina artefacts and the Phix Control v3 Library); ktrim = 1; k = 23; mink = 11; hdist = 1; minlen = 50; tpe; tbo; qtrim = rl; trimq = 20; ftm = 5; maq = 20. The remaining sequences were imported to QIIME2 version 2021.4 (<https://qiime2.org/>) for bioinformatics analyses (Bolyen *et al.* 2019). The qiime2-dada2 plugin was used for filtering, dereplication, turn paired-end fastq files into merged, and remove chimeras, using default parameters (Callahan *et al.* 2016). Taxonomic assignments were determined for amplicon sequence variants (ASVs) in four steps. First, ASVs were classified using the qiime2-feature-classifier (Bokulich *et al.* 2018) classify-sklearn against the database PLANiTS2 (Banchi *et al.* 2020). Second, the unclassified ASVs are contrasted with the UNITE Eukaryotes ITS database version 8.3 (Abarenkov *et al.* 2020). The remaining

unclassified ASVs were filtered and aligned against the filtered NCBI (NCBI, 1988) non-redundant nucleotide sequences (nt) database (October, 2021) using BLASTn (Camacho *et al.* 2009) with default parameters; the nt database was filtered using the following keywords: “ITS1”, “ITS2”, “Internal transcribed spacer”, and “internal transcribed spacer”. Finally, output files from BLASTn (Camacho *et al.* 2009) were imported to MEGAN6 (Huson *et al.* 2016) and taxonomic assignments were performed using the “megan-nucl-Jan2021.db” mapping file with default parameters and trained with Naive Bayes classifier and a confidence threshold of 98.5%. From these database comparisons, a taxa abundance table was generated.

Ecological analyzes

To better understand this distribution of species and make a comparison with the characteristics of the lakes, statistical analyzes were carried out using the Past4Project program (Hammer & Harper 2023). To observe whether the sediment collections that were carried out for the lakes reflect the biodiversity of taxa in these lakes, rarefaction curves were made. The Past4Project software, version 4.16 (Hammer & Harper, 2024) also was used to generate Simpson and Shannon diversity indices. To analyze the similarity in the taxonomic composition of the lakes, Cluster Analysis was used, using unweighted paired group method using arithmetic averages (UPGMA) as an algorithm and dice (Sorensen Index) as a similarity index and Detrended Correspondence Analysis (DCA) to corroborate with the Cluster Analysis, and to correlate the physicochemical characteristics of the lakes with the biodiversity found in them, Non-metric Multidimensional Scaling (NMDS) was used. To compare the taxa composition of the different lakes, a Venn diagram was generated using the VENNY 2.1.0 website.

Results

A total of 1.188.187 raw sequences were obtained, 397.756 for lake Adriana, 387.523 for lake Cecília, 233.749 for lake Lília and 169.159 for lake Soledad. After the BBDuk cleanup sequences and QIIME2, the number of sequences for lake Adriana was 333.409, for lake Cecília it was 331.036, for Lake Lília it was 177.083 and for Lake Soledad it was 136.946, totalizing 978.474 DNA reads. The results obtained and cited here are illustrated in Table I. The DNA sequences found for the Fungi kingdom totaled

110,741, and have already been analyzed and published by Gonçalves *et al.* (2022), and are therefore not included in this work. As Lake Soledad only had two sediment samples, we chose to standardize and randomly exclude samples from the other lakes so that everyone would have two samples each.

Table I. Total DNA reads after each “cleaning” step of the obtained sequences. After QIIME2, these are the total sequences used in the analyzes of this work. Samples represented with * are those that were excluded to standardize the analyses.

Samples	Total reads obtained	After BBDuk	After QIIME2 – TOTAL READS USED
Lago Adriana MP129*	82979	79644	70135
Lago Adriana MP186	100185	94410	85070
Lago Adriana MP187*	111715	102713	93344
Lago Adriana MP188	102877	95105	86680
Lago Cecilia MP189*	80680	75356	70247
Lago Cecilia MP190	116038	107392	101193
Lago Cecilia MP191*	92823	85553	79673
Lago Cecilia MP192	97982	86760	81186
Lago Lilia MP133*	80299	73931	58950
Lago Lilia MP134	78892	72962	59532
Lago Lilia MP135	74558	71063	59493
Lago Soledad MP136	88121	79401	69482
Lago Soledad MP138	81038	74923	67703

After refining the data obtained and standardizing the samples studied, a total of 393,010 DNA reads were found in sediment samples from the four lakes, representing 107 taxa, distributed across four kingdoms. The rarefaction curves indicates that the sediment samples from the four lakes have reached the asymptote, indicating that the samples have been reached and that the samples well represent the biodiversity of the four lakes (Figure 4).

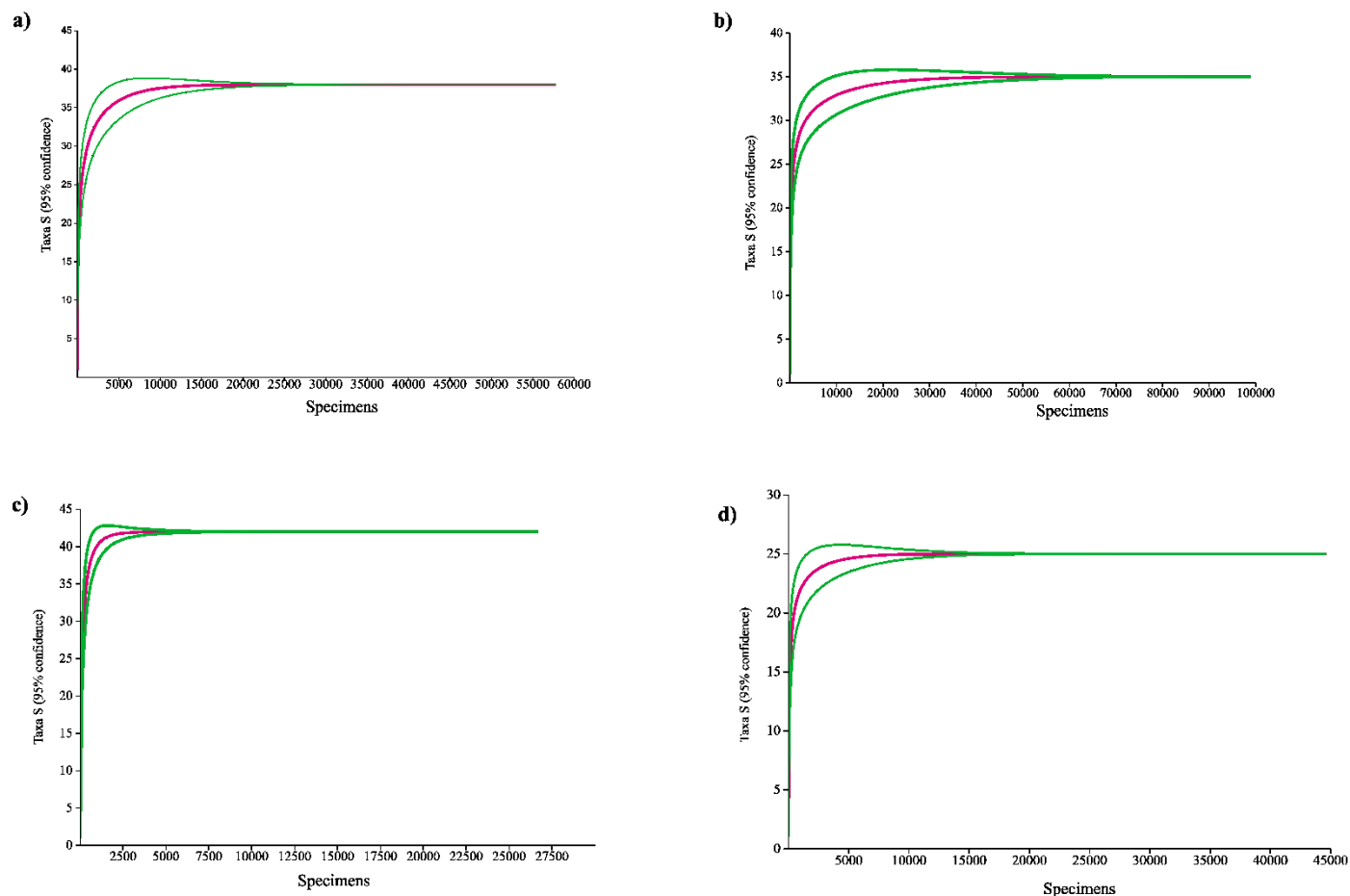


Fig. 4. Rarefaction curves of the lakes: **a)** Adriana; **b)** Cecilia; **c)** Lilia and **d)** Soledad. Green lines represent 95% of confidence limits. Source: Graphics created by the author using the Past4Project tool.

Our results indicated that the kingdom with the greatest representation was Plantae, with 78 taxa, comprised of two phyla followed by Chromista with 19, comprised of four phyla, Protozoa with 8 and Animalia with one. A total of 20 taxa are new records for Antarctica. A list of sampled taxa and their distribution was prepared and can be seen in Table II. The classification follows Leliaert *et al.* (2012) for Viridiplantae and Cavalier-Smith (1998) for Chromista, Protista and Metazoa, and the plant classification system follows APG IV (2016).

Table II. Abundance of the number of reads of the taxa sampled in the four lakes of James Ross Island, Weddel Sea, Antarctica. Habitat: Ep = elliptical; F = fresh water; M = marine; P = parasite; S = soil; Se = sediment; T = terrestrial; W = cosmopolitan. Distribution: A = Antarctica; Ae = endemic to Antarctica; Af = Africa; Ar = Arctic; As = Asia; Au = Australia; B = Bipolar; CA = Central America; E = Europe; NA = North America; NZ = New Zealand; SA = South America; U = Ubiquitous. Species that had no previous record for Antarctica were marked with *, and species that only had a genus record are marked with **.

TAXA	Habitat	Distribution	Number of Reads						
			Adriana	Cecilia	Lilia	Soledad			
KINGDOM ANIMALIA									
Order Diptera			0	8	0	0			
KINGDOM CHROMISTA									
Phylum Ochrophyta (Bacillariophyta)			0	7	125	0			
Order Chromulinales									
<i>Monochrysis</i> sp. *	M	E, NA	1217	0	0	0			
Order Haptorida			704	5	0	0			
<i>Fuscheria</i> sp.	M	A	0	476	0	0			
Order Nassulida									
<i>Parafurgasonia</i> sp.	Se	Af	0	4145	0	0			
Order Hymenostomatida			0	0	85	0			
<i>Tetrahymena</i> sp.	F	W	0	0	201	0			
Order Philasterida									
<i>Homalogastra</i> sp.	M	A	572	688	215	0			
Class Oligohymenophorea incertae sedis			8487	211	1117	147			
Order Cyrtophorida			0	1970	295	394			
Class Spirotrichea			2538	0	0	0			
Order Sporadotrichida			3226	2318	2205	2357			
<i>Halteria</i> sp.	T, F	A, E, NA	0	408	0	0			
Family Oxytrichidae			8866	1978	50	458			
<i>Cyrtohymena</i> sp.	A	M	0	0	590	0			
<i>Gonostomum</i> sp.	T	A, As, Au, E, NA, SA	1361	0	0	0			

Order Pavloales						
<i>Diacronema lutheri</i> (Droop) Bendif & Véron	M	E, NA	155	0	0	0
Class Oomycetes			0	0	536	0
KINGDOM PROTOZOA						
Order Vannellidae			0	0	0	109
Order Cercomonadida			0	367	0	0
<i>Eocercomonas uvella</i> D.Bass & Cavalier-Smith *	S	A	1259	0	0	0
Class Aphelidea			0	34	13	0
Class Ichthyosporea			24	644	377	161
<i>Neovahlkampfia damariscottae</i> (F.C.Page) Sus.Brown & Jonckheere	F, P, T	W	1099	0	0	0
<i>Allovahlkampfia</i> sp.	T, F	W	382	39	99	74
<i>Apusomonas proboscidea</i> A.G.Alexeieff *	T, F	Au, E	0	0	46	15
KINGDOM PLANTAE			0	222	59	0
Divisão Bryophyta						
Family Bryaceae						
<i>Bryum pseudotriquetrum</i> (Hedw.) G.Gaertn., B.Mey. & Scherb.	T	B	0	9722	0	0
Family Pottiaceae						
<i>Hennediella heimii</i> (Hedw.) R.H. Zander	T	B	0	124	4527	0
Divisão Chlorophyta			7742	28	533	10
Class Chlorophyceae			1579	34	251	525
Family Aphanochaetaceae						
<i>Aphanochaete</i> sp. *	F	W	0	20594	58	1117

Family Chaetophoraceae						
<i>Diplosphaera</i> sp.	F	W	14	0	530	0
<i>Stigeoclonium variabile</i> Nägeli **	F	Af, As, NZ, E, NA	0	0	0	104
Order Chlamydomonadales			9	10020	374	5464
Family Characiochloridaceae						
<i>Chlamydomodium starrii</i> (Fott) Ettl & Gärtner	T	A, Ar, E, Af	272	443	425	8832
Family Chlamydomonadaceae			400	0	0	0
<i>Chlamydomonas bacca</i> Ettl *	F	E	0	0	186	0
<i>Chlamydomonas cf. latifrons</i> Nygaard	F	E	144	0	0	0
<i>Chlamydomonas noctigama</i> Korschikov	F	E, NA	0	0	140	0
<i>Chlamydomonas raudensis</i> Ettl	F	A, E	87	21	0	0
<i>Chlamydomonas splendida</i> L.Péterfi	F	E	2639	0	0	0
<i>Chlamydomonas</i> sp.	F, M	A, E, NA	0	0	151	0
<i>Chloromonas subdivisa</i> (Pascher & Jahoda) Gerloff & Ettl	F	E	0	0	124	0
<i>Chloromonas</i> sp.	T, F	Af, Ar, As, Au, E, NA, NZ, SA	12	0	0	0
Family Chlorococcaceae			0	116	0	9297
<i>Chlorococcum macrostigmatum</i> R.C.Starr	T	NA	0	14303	0	0
<i>Chlorococcum microstigmatum</i> P.A.Archibald & Bold	T	E, NA	5080	7442	761	6158
<i>Chlorococcum</i> sp.	T	E, NA	757	0	422	1457
<i>Tetracystis</i> sp.	T, F	E, NA	55	0	0	21

Family Dunaliellaceae						
<i>Dunaliella acidophila</i> (Kalina) Masjuk*	F	E	56	0	0	0
Family Volvocaceae						
<i>Gonium</i> sp.	F	W	28	0	0	0
Family Chlorosarcinaceae						
<i>Neochlorosarcina negevensis</i> (Friedmann & Ocampo-Paus) Shin Watanabe	T	As	155	0	22	0
Family Bracteacoccaceae						
<i>Bracteacoccus bullatus</i> Fuciková, Flechtner & L.A.Lewis	T	A, E	0	28	1609	0
Family Dictyococcaceae						
<i>Dictyococcus</i> sp. **	F, S	Ar, As, Au, E, SA	0	0	98	0
Family Mychonastaceae						
<i>Mychonastes ovahimbae</i> Krienitz, C.Bock, Dadheech & Proschold *	F	Af	0	1510	0	0
<i>Mychonastes</i> sp.	F, M	Af, E, NA, SA	0	4852	0	0
<i>Mychonastes</i> sp.2	F, M	Af, E, NA, SA	473	0	0	263
Family Scenedesmaceae						
<i>Ankyra judayi</i> (G.M.Smith) Fott *	F	As, E, NA, SA	0	87	0	0
<i>Chodatodesmus australis</i> Sciuto, Verleyen, Moro & La Rocca	T, F	Ae	586	268	159	4584
<i>Desmodesmus costatogranulatus</i> (Skuja) E.Hegewald	F	E, NA, NZ, SA	0	1769	0	401
Family Tetrasporaceae						

<i>Paulschulzia pseudovolvox</i> (P.Schulz) Skuja	F	AS, As, Au, E	0	0	152	0
Family Radiococcaceae						
<i>Neocystis mucosa</i> Krienitz, C.Bock, Nozaki & M.Wolf	F	A, E	0	0	782	0
Class Trebouxiophyceae						
Order Chlorellales						
			11	307	2943	0
Family Chlorellaceae						
			8790	310	2353	15
Family Chlorellaceae						
			0	0	157	0
<i>Catena viridis</i> Chodat *	F	Af, E	17	0	0	0
<i>Chlorella pituita</i> C.Bock, Krienitz & Pröschold			0	180	315	0
<i>Chlorella</i> sp.			0	0	1422	0
<i>Xerochlorella minuta</i> (J.B.Petersen) Mikhailyuk & P.M.Tsarenko **	T,F	E	0	0	46	0
<i>Didymogenes palatina</i> Schmidle	F	As, E, NZ, SA	10129	0	0	0
<i>Micractinium</i> sp.	F	W	0	81	0	429
<i>Mucidosphaerium</i> sp. *	F	W	0	0	169	0
Family Oocystaceae						
<i>Oocystella oogama</i> Hindák*	F	E	89	0	0	46
Order Microthamniales						
			0	0	475	0
Family Prasiolaceae						
<i>Stichococcus bacillaris</i> Nägeli	W	Ar, E, SA	0	7	284	0
<i>Stichococcus</i> sp.	W	Ar, E, SA	15	15	814	0
Family Koliellaceae						
<i>Koliella sempervirens</i> (Chodat) Hindák	F	E	0	123	561	0
<i>Raphidonema nivale</i> Lagerheim	F	A, As, Au, E, NZ, SA	0	0	6809	0

Family Prasiolaceae						
<i>Desmococcus spinocystis</i> Gärtner & Ingolic	T	E	12	0	2306	50
Family Coccomyxaceae						
<i>Coccomyxa</i> sp.	F	A, As, Au, E, NA, NZ, SA	0	0	253	0
Family Trebouxiaceae						
<i>Trebouxia arboricola</i> Puymaly **	Ep, F, S, Se	Ar, As, E, SA	18	6	0	0
<i>Trebouxia asymmetrica</i> Friedl & Gärtner	T	E	29	65	42	17
Family Ulvales incertae sedis						
<i>Paulbroadya prostrata</i> (Broady & Ingerfeld) Darienko & Pröschold *	Ep	A	0	10	0	0
Family Kornmanniaceae						
<i>Pseudendoclonium</i> <i>submarinum</i> Wille	M	W	84	0	0	0
<i>Pseudendoclonium</i> sp.	F, M, T	Ar, As, Au, E, NA	5232	10683	186	265
Family Chlorocystidaceae						
<i>Desmochloris mollenhaueri</i> Darienko, Friedl & Pröschold **	Se	A	1674	122	208	3229
Family Hazeniaceae						
<i>Hazenia</i> sp.1	F	A, As, Au, CA, E, NA, SA, NZ	0	0	41	271
<i>Hazenia</i> sp.	F	A, As, Au, CA, E, NA, SA, NZ	80	384	0	4595
Family Monostromataceae						
<i>Protomonostroma</i> sp.	M	A, E, NA	1985	809	867	1217

Family Planophilaceae						
<i>Planophila bipyrenoidosa</i> Reisigl	T	E	207	3260	622	7853
<i>Planophila</i> sp.	F, M	Ar, As, E, NA	5957	6296	653	2761
Family Sarcinofilaceae						
<i>Sarcinofilum mucosum</i> (Broady) Darienko & Pröschold *	F	E	46	0	0	139
Family Tupiellaceae						
<i>Tupiella speciosa</i> Darienko & Pröschold *	F	E	0	392	0	507
Family Ulotrichaceae						
			4266	8060	2372	10000
<i>Chlorothrix</i> sp.	T	E	29584	17089	160	373
Class Magnoliopsida						
			4	0	0	0
Family Apiaceae						
<i>Petroselinum crispum</i> (Mill.) Fuss *	T	SA, CA, NA	0	0	66	0
Family Plumbaginaceae						
<i>Limonium</i> sp.*	T	As, Af, Au, E, NA	0	0	63	0
Family Fabaceae						
<i>Glycine soja</i> Siebold & Zucc.*	T	W	0	0	45	0
Family Rubiaceae						
<i>Diodella teres</i> (Walter) Small*	T	As, CA, NA, SA	0	9	0	0
Family Myrtaceae						
<i>Eucalyptus fulgens</i> Rule*	T	W	0	0	40	0
Family Cannabaceae						
<i>Cannabis sativa</i> L. *	T	W	22	0	0	0

UNKNOWN	24380	11002	6429	6189
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The taxon with the highest number of reads in the kingdom Plantae was *Chlorothrix* sp. (47.206 reads), and it was also one that presented the highest number of reads among all the kingdoms. For Chromista, the taxon with the highest number of reads was the family Oxytrichidae (11.352), which is the highest rank achieved; for Protozoa, the taxon with the highest number of reads was *Eocercomonas uvella* (1.259 reads); for Animalia, only one taxon was sampled, which was identified as Order Diptera. From the reads obtained, 48,000 DNA reads, however, could not be correlated with any sequence, remaining as “unknown”, corresponding to around 12.21% of the total reads.

Diversity indices vary between the four lakes, as can be seen in Table III.

Table III: Diversity indices for the four lakes sampled in this work.

Diversity indices	Lakes			
	Adriana	Cecilia	Lilia	Soledad
Number of DNA reads	128989	136564	47553	79904
Number of taxa	38	35	42	25
Simpson's (dominance)	0,8578	0,8894	0,9959	0,8785
Shannon	2,385	2,47	2,767	2,364
Evenness	0,2857	0,3379	0,379	0,4251

Through the Venn Diagram, seen in Figure 5, we observed that 21 of the taxa were found in all four lakes, but the distribution of species varies, with pairs of lakes sharing at least one specie between them, and some presenting species that were sampled only there, being Lilia the lake with the largest number of exclusive taxa.

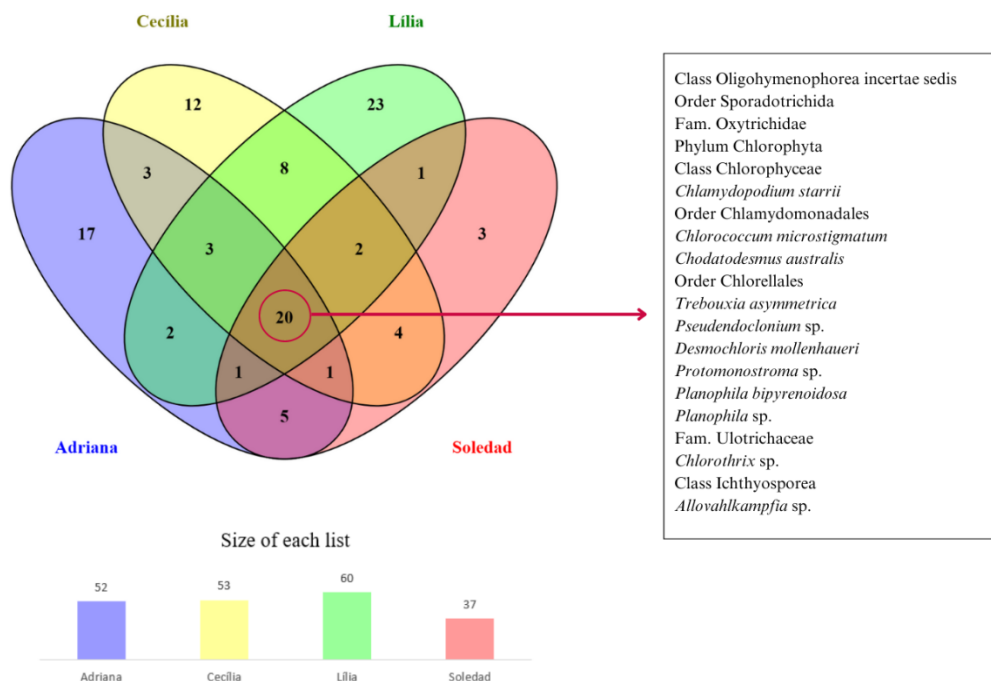


Fig. 5. Venn diagram showing the distribution of taxa obtained in sediment samples from lakes Adriana, Cecilia, Lilia and Soledad. In the box positioned on the right, the taxa that were sampled in all four lakes are indicated. Source: prepared by the author.

Physicochemical and geographical characteristics of the lakes

As can be seen in Table IV, lakes Adriana, Cecilia, Lilia and Soledad have slightly different characteristics, mainly those related to its geological features, such as altitude and depth. The chemical composition also varies a little, with the sandy loam textural class dominating in 3 of lakes, with the exception of Lakes Cecilia which has the Loam textural class, which also has the highest percentage of silt among the lakes. The pH of the four lakes remains above 8, indicating that lakes are alkalines, and the acidity potential remains very close to 0, or even reaching this value.

Table IV: Physicochemical parameters and geomorphological characters of lakes Adriana, Cecilia, Lilia and Soledad.

Parameters	Lakes			
	Lilia	Cecilia	Adriana	Soledad
Lake geological characteristics (ROMAN <i>et al.</i>, 2019)				
Location	64°01'44.4" S; 57°39'05.4" W	64°01'43.3 S; 57°39'48.3" W	64°01'58.0" S; 57°43'35.5"W	64°00'37.8" S; 57°41'56.5" W
Altitude (meters above sea level)	101	67	265	215
Total area (m ²)	~16300	6857	18548	5027
Perimeter (m)	749	322	513	380
Distance to coastline (m)	1835	1412	537	419
Depth (m)	21	4,5	0,3	0,5
Lake shape	Elongated N-S	Elongated N-S	Circular	Elongated E-W
Sediment physical parameters				
Clay (%)	10	25	29	32
Silt	17	57	31	41
Coarse sand	54	12	12	9
Fine sand	19	6	28	18
Textural class	Sand-loam	Silt-loam	Clay-loam	Clay-loam
Sediment chemical parameters				
pH in H ₂ O	8,8	8,7	9,1	8,4
Exchangeable P – mg/dm ³	87,3	5,6	56,4	116,9
Sum of exchangeable bases Ca+K+Mg (SB) – cmol _c /dm ³	6,35	7,60	8,40	5,80
Percentage of base saturation (PBS) - %	100,0	95,8	100,0	89,8
H+Al - potential acidity - cmol _c /dm ³	0,00	0,33	0,00	0,66
Cation exchange capacity at pH 7 (CEC) - cmol _c /dm ³	6,35	7,93	8,40	6,46
Total organic carbon (TOC) – dag/kg	0,45	0,7	0,54	0,93
Micronutrient Fe – mg/dm ³	220,2	63,0	100,2	186,4
Micronutrient Mn - mg/dm ³	27,9	78,6	126,6	69,7

Cluster and DCA analyses were carried out in order to test the similarity between lakes, based on the composition of taxa present in them. The result can be seen in figure 6.



Fig. 6. On the left, Cluster Analysis and on the right the Detrended Correspondence Analysis (DCA), showing the similarity in the composition of the lakes. Source: prepared by the author using the PAST4 tool.

The Non-metric Multidimensional Scaling was performed to correlate the physical and chemical parameters sampled from the sediments with the number of reads and taxa found in the four lakes, as can be seen in Figure 7.

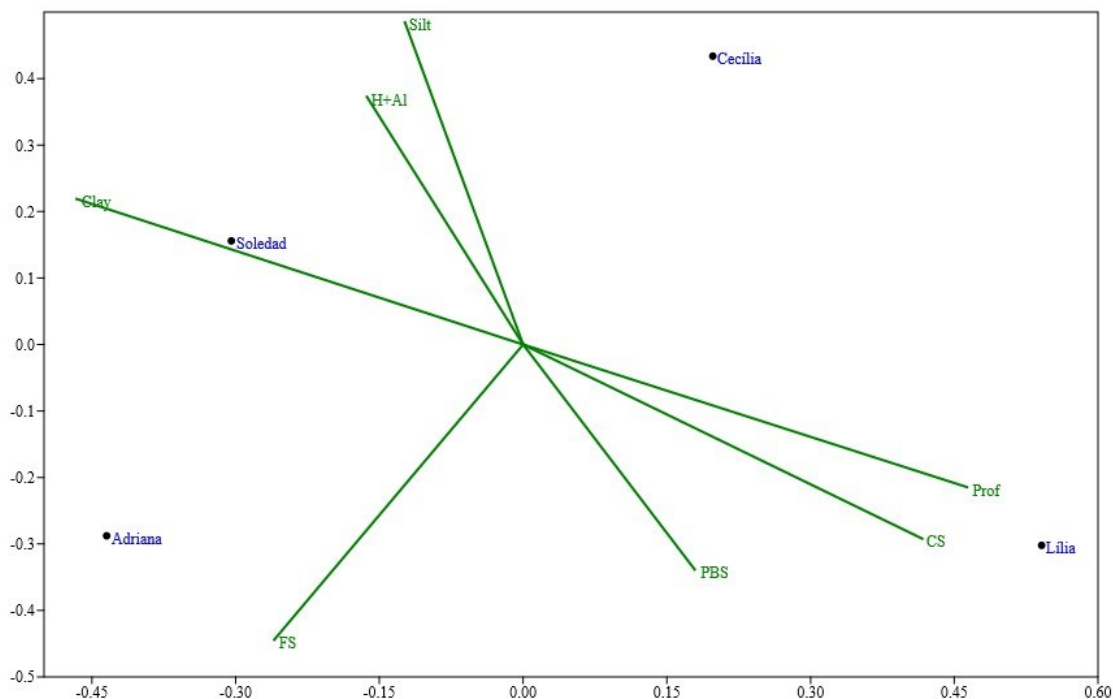


Fig. 7. Non-metric Multidimensional Scaling showing the similarity between the lakes, correlating the taxa composition with the physicochemical characteristics of the lakes. Prof = depth (m); Silt = silt; CS = coarse sand; Clay = percentage of clay (%) FS = fine sand; PBS = percentage of base saturation (%); H+Al = H+Al acidity potential (cmolc/dm³). Source: prepared by the author using the Past4Project tool.

Discussion

The lakes studied are extremophilic and almost untouched environments, mostly shallow, with waters very transparent, receiving a high incidence of sunlight and UV rays and with low availability of nutrients (Gonçalves 2011, Gonçalves 2012). They are also important biological reserves for several organisms (Gonçalves 2022). Due to the logistical difficulties in collecting material and the need for prior knowledge of the taxonomy of different groups to carry out classical identification studies, the DNA metabarcoding tool has recently been used to understand the biodiversity of different locations, in a standardized way (Taberlet *et al.* 2012, Câmara *et al.* 2021, Câmara *et al.* 2022, De Souza *et al.* 2022, Gonçalves 2022).

Our work found taxa distributed across four kingdoms, with the one with the highest number of taxa being Plantae, with 78 taxa found, followed by the Chromista with 19 taxa, Protozoa with eight taxa sampled and Animalia, with only one taxon found. Around 12.21% of the reads obtained in this work could not be correlated with any taxon, remaining as “unknown”, (Table II). These results may be related of database quality and even the lack of knowledge and classical taxonomy studies for these taxa, or they may in fact represent new taxa that have not been described yet. In this way, we highlight the need and importance of classical taxonomy work, which is responsible for feeding the databases, allowing this type of work, using DNA metabarcoding, to be developed with greater precision. It is important to highlight that the presence of the DNA of the taxa reported in this work does not imply that the organisms are living in the study site or that there are viable propagules, it only indicates that their DNA, in some way, managed to reach the lakes sampled, either by wind, by human action or the action of other animals, or in some other way.

Of the 107 taxa found, 20 of them, around 18,7%, are new records to Antarctica, and 5 taxa had only the genus cited in other publications. Taxons from the Kingdom Chromista and Green algae are those with the most previous records for the continent. Of the taxa that already had previous records for the Antarctic continent, 44 of them were mentioned by the Câmara (2017, 2018, 2020, 2021, 2022), which presents the vast majority of their work carried out with samples made around the South Shetland Islands, located on the opposite site of the Antarctic Peninsula, compared to the lakes of James Ross Island, sampled in this work.

Chromista Kingdom

Among the Chromista, new records include *Monochrysis* sp., (Ochrophyta) a genus of ciliates comprising six freshwater species (Guiry & Guiry 2022), reported for the Arctic and Europe, but never before reported for the Antarctic continent.

Tetrahymena (Ciliophora) is a genus with around 41 species and already recorded in the Antarctic (only the species *T. rostrata* (Kahl) Corliss) (Kerpner 1999, Santovito 2017). The genus is a good model for studies of biotechnological applications and for “quick, reliable, sensitive and inexpensive bioassays” (Santovito 2017). The discovery of the telomerase enzyme was based on studies carried out with this genus, and

earned the Nobel Prize in Physiology and Medicine for Elizabeth Blackburn, Carol Greider and Jack Szostak in 2009.

Plantae Kingdom

There were 17 new records for Antarctica, in addition to some species of which only the genus had been recorded for Antarctica. The genus *Aphanochaete*, a green algae, comprises around 10 species not previously recorded for Antarctica (Guiry & Guiry 2022). The species *Chlamydomonas bacca* (green algae), *Dunaliella acidófila* (microalgae), *Tupiella speciosa* (green algae) and *Oocystella oogama* (microalgae) are European species without previous records for Antarctica, however, the genus *Dunaliella* is described in Pick, U. (1999) as very resistant to extreme environmental conditions. *Mychonastes ovahimbae* (green algae) is reported only from Africa, and other species of this genus have been studied as alternative sources for the production of biodiesel (Yuan *et al.* 2011). The species *Ankyra judayi* (green algae) is a widespread freshwater species and its southernmost occurrence record was reported in New Zealand, it appears to be a very resistant species to UVA and UVB and is a good source of food for zooplankton (Cabrera 1997). The species *Catena viridis* (microalgae) is distributed in Europe and Africa, and which, together with the species *C. detoniana*, found in the freshwaters of Spain (Guerrero 1964), are the only two species of this genus. The genus *Mucidosphaerium* (microalgae) comprises only 4 species, with a distribution almost restricted to Europe, with the exception of the species *M. pulchellum*, which has a wider distribution across the globe and despite being a species of Antarctic origin is also distributed in Europe, *Sarcinofilum mucosum* (microalgae) has no previous record for the continent. The species *Stigeoclonium variabile* (green algae) has no previous record for Antarctica, but the genus *Stigeoclonium* (*S. variabile* Nägeli ex Kützing) has already been reported previously for Antarctica (Fonseca *et al.* 2021), the same happens for the species *Trebouxia arboricola* (green algae) (Câmara 2020, Câmara 2021, Câmara 2022) and *Desmochloris mollenhaueri* (green algae) (Câmara 2020). The genus *Trebouxia* has previously been cited as records for Antarctica (Câmara 2020, Câmara 2021, Câmara 2022), but the species *T. arboricola* had not been reported for Antarctica, being a freshwater species from the Arctic. A species of the genus *Dictyococcus* (microalgae), found in this work, *D. pseudovarians*, was previously cited by Andreyeva & Kurbatova (2014), where it was reported as the first record for Antarctica. The same occurs with the species *Xerochlorella minuta* (green algae) (previously *Dictyosphaerium minutum*

J.B.Petersen), which has no previous record for this species, but another species (*D. chorelloides* (Naumann) Komárek et Perman) of the same genus was mentioned previously (Andreyeva & Kurbatova 2014). The species *Desmochloris mollenhaueri* has its first record of occurrence for Antarctica reported here, being a soil species that was thought to be endemic to South Africa, but it is a species relatively close to *D. halophile*, which has already been reported for Antarctica (Câmara 2020, Câmara 2021). The species *Bracteacoccus bullatus* (green algae) is apparently good for producing vegetable oil at low temperatures and has a high level of unsaturated fatty acids (Lukavsky 2023).

All the six species of flowering plants found in this work are new occurrences for Antarctica. The species *Petroselinum crispum*, Apiaceae family, commonly known as parsley, originates from the Mediterranean region but is widely cultivated, used in cooking as a seasoning and as a garnish for dishes, in addition to presenting antioxidant properties (Agyare 2017). The genus *Limonium*, Plumbaginaceae family, is made up of almost 400 species that occur naturally in Europe, North and South America, Asia, Africa, Australia and in tropical and temperate zones, frequently inhabits coastal environments and many species are found in salt marshes or on salines, gypsum, or alkaline soil if growing in locations away from the coast, are well known in the international ornament industry, for presenting beautiful flowers that remain colorful for a long time and can also be useful as a dried flower (Morgan & Funnell 2018). *Glycine soja* (soybean), Fabaceae family, is originally from Asia, is an important crop, being used as food and as oil and without prior registration for Antarctica. *Diodella teres* is a species of Rubiaceae originally from North America, it is an invasive plant and its occurrence is frequent in the Center-West and Southeast regions of Brazil (Conceição & Aoyama 2015). The species *Eucalyptus fulgens*, Myrtaceae family, is a tree endemic to Australia, but the genus *Eucalyptus* includes more than 700 species, widely cultivated around the globe for timber, cellulose and oil, and there are records that wood fragments, including *Eucalyptus*, can reach the Antarctic (Lityńska-Zajac 2012), in addition to around 47% of Antarctic garbage being composed of wood (Anfuso *et al.* 2020). Finally, the species *Cannabis sativa*, Cannabaceae family is a species originating from India and is widely cultivated and used for recreational and medicinal uses. The presence of all these plant species in the Antarctic suggests the influence of anthropogenic actions, since close to James Ross Island there are two research stations, Mendel Station (Czech Republic) and Marambio Station (Argentina), which can provide propagules through wind and sea (Catán *et al.* 2020), in addition to the numerous field trips on the island, carried out by many countries

and camps for research on site, which is also carried out by the Brazilian Antarctic Program.

For taxa already recorded on the Antarctic continent, *Bryum pseudotriquetum*, Bryaceae family, is one of the most widely distributed species of Antarctic mosses, being present both in maritime Antarctica and in several locations on continental Antarctica (Câmara 2022).

Protozoa Kingdom

For the Protozoa, the species *Eocercomonas uvella* (unicellular protozoan) appears as a new record for the Antarctic continent, species described in Spain in 2002. The species *Apusomonas proboscidea* (unicellular protozoan) is a European species with no previous record for Antarctica. *Neovahlkampfia damariscottae* (unicellular protozoan) is the only species of this genus never before found in Antarctica, but it may just be a separation and renaming of the genus *Vahlkampfia*, which is previously mentioned by Tysl (2016).

Animalia Kingdom

The only taxon in the kingdom Animalia is the order Diptera, with 8 reads of this order, just for Lake Cecília. There are two native species of diptera in Antarctica, *Belgica antarctica* and *Parochlus steinenii*, in addition to two other invasive species that have already been found on the continent, *Eretmoptera murphyi* and *Trichocera maculupennis* (Kozeretska 2022). Unfortunately we were only able to reach the level of order for this group, since Diptera is a very large group, with more than 159,00 species described. Most “terrestrial” flies actually live close to bodies of water (Courtney 2015), and since with climate change new lakes are appearing in Antarctica, the place ends up becoming a possible habitat where this group could settle. Diptera is a group with great importance for human health and other animals, as it has some disease-transmitting genera, such as *Aedes*, *Anopheles* and *Culex* (Alikhan 2018).

Relationship environmental variables and taxa lakes metagenomic biodiversity

The lake with the highest number of readings sampled was Lake Cecília, followed by Lake Adriana, Soledad and Lília, however, despite presenting a smaller

number of reads, Lake Lília proved to be the most biodiverse, with 42 taxa sampled. Two of the lakes studied in this work, Cecília and Lília, are very close to each other, and also very close to the Blancmange Glacier, which influences their composition by providing organic matter and water for both lakes. Both lakes have a very well-established moss community around them and are fed by melting glaciers, which can serve as a source of organic matter and freshwater, in addition to being located in a region relatively protected from winds, which favor drifting snow accumulation (Gonçalves *et al.* 2022).

Lake Lília is the furthest from the coast among the four lakes studied, and also the one with the largest perimeter and depth, which may explain the greater number of taxa found in this lake compared to the others. Soledad was the lake that presented one of the lowest numbers of reads of taxa found, as seen in Table III, which may be related to its higher potential acidity index (H + Al), higher total organic matter levels (COC), and its location.

Lakes Soledad and Adriana are shallower lakes, both less than 1 m deep, and are maintained by snowmelt. These are also the lakes that are found at higher altitudes (Soledad at 215 m above sea level and Adriana at 265 m), where the vegetation is poorer and are more exposed to winds and evaporation, which constantly changes the conductivity of the water (Gonçalves 2022).

The Cluster Analysis, made with Sorensen-Dice index, is an analysis made based on the similarity of the taxa found in the lakes, ranging from 0 to 1, where 1 would be completely similar data and 0 would be completely different data. For this work, this analysis shows that beta diversity varies in the lakes sampled. Analyzing the dendrogram obtained for this analysis, we see that lakes Soledad and Adriana are placed as more similar to each other (as can be seen in Figure 6) and lakes Lília and Cecília are also more similar to each other, but the nodes do not reach 0.6, which would be an acceptable number to affirm the similarity between the lakes (Gotelli & Ellison 2016), but all lakes had low similarity between them. The fact that this analysis grouped lakes Lília and Cecília together as more similar may be related to the physical proximity of these two lakes. When we analyze the similarity between the lakes, we see that similarity value is below 0.5, showing that, unlike expected, the lakes do not have a similar taxon composition. Due to the environmental barriers faced by organisms to reach Antarctica, we expected that the organisms capable of breaking this barrier and reaching the continent would be the same, or very similar, and in this way the taxonomic composition of sites would also be similar, which has been refuted by Cluster analysis. The Detrended

Correspondence Analysis (DCA) corroborates the results obtained by the Cluster, pointing out the heterogeneity of the composition of the lakes.

The Non-metric Multidimensional Scaling points out that environmental variables, such as percentage of clay (Clay), acidity potential (H + Al), amount of silt (Silt), depth (prof), coarse sand (CS) and fine sand (FS) and percentage of base saturation (PBS), are responsible for the heterogeneity of lake composition. As can be seen in Figure 7, the clay variable is highly correlated with Lake Soledad, which among the lakes studied in this work is the one with the highest percentage of clay among them (32%). Previous studies show that suspended clay alters the composition of phytoplankton communities and grazing and may also be responsible for reducing zooplankton grazing and productivity (Cuker 1987). The amount of coarse sand and depth are environmental variables highly related to Lake Lília. Lília is the deepest among the four, at 21m deep, which ends up influencing the level of solar incidence in the different layers of the water column, also interfering with transparency, oxygen rate, temperature and other parameters, thus reflecting on the taxon composition (Leech 2000, T-Krasznai 2023).

The diversity indices obtained in this work demonstrate a high evenness rate (Simpson's index) ranging from 0,8578 (Lake Adriana) to 0,9959 (Lake Lília), and a high entropy rate within the community (Shannon index), ranging from 2,385 (Lake Adriana) to 2,767 (Lake Lília). The diversity indices in this work are quite similar to those of work carried out in other Antarctic lakes (Câmara *et al.* 2021, De Souza *et al.* 2022) and also in other locations on the continent using other types of eDNA, such soil and rocks samples (Câmara *et al.* 2020, Câmara *et al.* 2022).

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CONCLUSÃO

O uso de DNA *metabarcoding* se mostrou uma ferramenta efetiva para avaliar amostras de sedimentos de lagos. O registro de 20 novos táxons, além das cinco espécies que tiveram apenas o gênero citado anteriormente nos mostra que ainda restam muitos táxons ocorrendo na Antártica, os quais não conhecemos. Cabe aqui ressaltar a importância de trabalhos clássicos para a alimentação e manutenção dos bancos de dados, para fornecer dados sobre as espécies e sequências de alta qualidade que possibilitam que trabalhos como este sejam feitos com maior precisão. Os novos registros para os taxa de plantas com flores também parece refletir o aumento das atividades antrópicas na região, visto que são plantas utilizadas na alimentação, ornamentação e como matéria prima para fabricação de caixas e móveis, e que existem duas estações de pesquisa próximas a James Ross, além das idas à campo e até mesmo acampamentos na Ilha, que apesar de tomarem todos os cuidados necessários com os resíduos gerados, podem acabar deixando propágulos no local.

A presença do DNA dos táxons relatados neste trabalho nas amostras de sedimento dos lagos não implica que estes organismos estejam vivendo no local de estudo ou que existem propágulos viáveis, e sim que o seu DNA, de alguma forma, conseguiu chegar até os lagos amostrados, seja pelo vento, pela ação humana ou de outros animais, ou de alguma outra maneira.

Com relação a composição de espécies de cada lago individualmente e quando comparados entre si, observamos que a composição de espécie entre eles não é homogênea, contrariando o que pensávamos a respeito das espécies conseguirem romper as barreiras físicas para se chegar ao continente Antártico, e também pode ser reflexo da localização e das características, físico-químicas e geomorfológicas, de cada um dos lagos, principalmente profundidade, potencial de acidez, areia fina e grossa, porcentagem de argila e lodo que compõem os diferentes lagos, compo pode ser observado na análise NMDS.

Observamos que algumas espécies originárias de outros países estão chegando na Antártica. São espécies exóticas, que com a maior disponibilidade de áreas livres de gelo podem conseguir se estabelecer na Antártica e com isso trazer problemas para a fauna, flora e funga nativos. Além disso, alguns desses táxons, como do grupo Diptera citado no trabalho, são conhecidos por carrear e difundir doenças. Outras espécies encontradas apresentam aplicações biotecnológicas, como para a produção de

biocombustíveis e de óleos, o que pode ser interessante para a indústria e necessitam de maiores estudos.

Apesar dos resultados obtidos neste trabalho e de como foi dito anteriormente, ainda há muito sobre a composição biológica da Antártica que desconhecemos, o que torna essencial que mais estudos nesta direção sejam feitos.

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