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Investigando comunidades de eucariotos associados a plantas de ilhas oceânicas brasileiras utilizando DNA *metabarcoding*

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Defesa de dissertação apresentada ao Programa de Pós-graduação em Botânica da Universidade de Brasília como requisito necessário para obtenção do título de Mestre em Botânica.

Orientadora: Prof. Dra. Micheline Carvalho Silva

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“In nature nothing exists alone”.

Rachel Carson

RESUMO

Ilhas oceânicas constituem laboratórios naturais para o estudo da colonização, da biogeografia e das interações entre plantas e microrganismos. Porém, a diversidade de comunidades de eucariotos associadas às plantas nesses ambientes permanece pouco conhecida. No Oceano Atlântico, o Arquipélago de São Pedro e São Paulo (ASPSP) e o complexo insular Trindade–Martin Vaz representam os sistemas insulares mais isolados da costa brasileira. O presente estudo teve como objetivo caracterizar e comparar as comunidades eucarióticas associadas a duas espécies de Cyperaceae desses conjuntos insulares, utilizando DNA *metabarcoding* e sequenciamento de alto rendimento (HTS). No ASPSP, o *Fimbristylis cymosa*, uma espécie de Cyperaceae recentemente registrada se proliferando, teve sua identidade confirmada por reconstrução filogenética. Com base no marcador ITS2, foram obtidas 89 variantes de sequência *amplicon* (ASVs), *Ascomycota* foi o filo dominante, seguido por *Basidiomycota*, sendo *Hortaea werneckii*, *Dimorphiseta obtusa* e *Aspergillus terreus* os táxons atribuídos mais abundantes. Destaca-se a detecção de potenciais patógenos de plantas e animais, bem como de táxons com distribuições biogeográficas restritas ou previamente não documentadas. No complexo Trindade-Martin Vaz, o *metabarcoding* de *Cyperus appendiculatus* var. *atlanticus* resultou na identificação de 411 ASVs, distribuídas em quatro reinos e nove filos, com predomínio de fungos em riqueza e abundância, incluindo linhagens não-Dikarya e outros grupos crípticos. Os resultados evidenciam que ilhas oceânicas do Atlântico atuam como reservatórios de uma diversidade eucariótica associada às plantas inesperadamente elevada, ressaltando a importância de integrar perspectivas microbianas aos estudos de biogeografia insular e conservação.

Palavras-chave: Cyperaceae; DNA ambiental; Fungi; Ilha Trindade; Arquipélago São Pedro e São Paulo.

ABSTRACT

Oceanic islands provide exceptional natural laboratories for studying colonization, biogeography, and plant–microbe interactions, yet the diversity of plant-associated eukaryotic communities in such systems largely unexplored. In the Atlantic Ocean, the equatorial São Pedro and São Paulo Archipelago (SPSPA) and the subtropical Trindade-Martin Vaz insular complex represent the most isolated insular systems off the Brazilian coast. Using a DNA metabarcoding approach and high-throughput sequencing (HTS), we characterize the eukaryotic communities associated with two sedge species. On SPSPA, *Fimbristylis* sp. has been found recently proliferating. Phylogenetic analyses confirmed the species as *Fimbristylis cymosa*. Based on marker ITS2, 89 ASVs (amplicon sequence variants) were detected. *Ascomycota* was the dominant phylum, followed by *Basidiomycota*. *Hortaea werneckii, Dimorphiseta obtusa* and *Aspergillus terreus* were the were the dominant assigned taxa. Notably, we detected potential plant and animal pathogens and taxa with restricted or previously undocumented biogeographical distributions. In the Trindade–Martin Vaz complex, metabarcoding of *Cyperus appendiculatus* var. *atlanticus* yielded 411 ASVs, with taxa spanning four eukaryotic kingdoms and nine phyla, with fungi dominating in richness and abundance. Several taxa linked to potential plant and animal pathogens, as well as non-Dikarya and other cryptic fungal lineages, were detected, many of which have restricted or poorly documented distributions. Together, these findings demonstrate that Atlantic islands act as reservoirs of unexpected plant-associated eukaryotic diversity and emphasize the importance of integrating microbial perspectives into island biogeography and conservation research.

Keywords: Cyperaceae; environmental DNA; Fungi; São Pedro e São Paulo Archipelago; Trindade Island.

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LISTA DE ABREVIAÇÕES E SIGLAS

ASPSP	Arquipélago São Pedro e São Paulo
ASVs	Amplicon sequence variants / variantes de sequência de amplicon
eDNA	Environmental DNA / DNA ambiental
TMV	Complexo insular Trindade-Martin Vaz
POIT	Posto Oceanográfico da Ilha da Trindade
SPSPA	São Pedro São Paulo Archipelago

SUMÁRIO

INTRODUÇÃO GERAL

Ilhas oceânicas abrigam uma biodiversidade singular, em grande parte resultante da dispersão oceânica das espécies, já que muitas se formaram *in situ* e nunca estiveram conectadas a massas continentais (Cowie & Holland, 2006). O isolamento geográfico das ilhas favorece o processo de endemismo, como também torna as espécies endêmicas mais vulneráveis em relação às espécies continentais, devido à sua distribuição geográfica restrita e única, à especificidade de suas interações com o ambiente (Walter, 2004), e, ainda, por apresentarem menor variabilidade genética (Frankham, 1997). Dessa forma, a biodiversidade das ilhas oceânicas é muito sensível a perturbações ambientais, tornando esses ambientes prioritários para ações de conservação e estudo científico.

As ilhas oceânicas possuem importante valor científico, socioeconômico, ambiental e estratégico, visto a singularidade de seus ecossistemas, biodiversidade e geologia, e do potencial para estudos climáticos e oceanográficos, entre outros (Marinha do Brasil, 2026). A partir da Convenção das Nações Unidades sobre os Direitos do Mar, a Ilha da Trindade e o Arquipélago São Pedro e São Paulo (ASPSP) representam relevantes extensões da Zona Econômica Exclusiva Brasileira e todo conhecimento científico gerado nesses ambientes serve de base para a manutenção da soberania nacional (CIRM, 2026; Rezende *et al.*, 2023).

As ilhas oceânicas brasileiras abrangem cinco conjuntos insulares – os arquipélagos de Fernando de Noronha, São Pedro e São Paulo, Martin Vaz, a Ilha da Trindade e o Atol das Rocas – todos isolados do continente e de origem vulcânica, com exceção de São Pedro e São Paulo (Serafini *et al.*, 2010). O grupo insular formado pela Ilha da Trindade e o Arquipélago Martin Vaz é o mais distante da costa brasileira. Sua origem ocorreu há cerca de três milhões de anos, quando uma fratura no leito oceânico da placa sul-americana provocou intensa atividade vulcânica, formando uma grande cadeia de montanhas e vulcões submarinos, denominada Cadeia Vitória-Trindade (Serafini *et al.*, 2010).

A Ilha da Trindade está permanentemente ocupada pela Marinha do Brasil, que criou o Posto Oceanográfico da Ilha da Trindade (POIT) em 1957. No entanto, a presença humana na ilha data da época das Grandes Navegações, tendo se intensificado a partir de 1700 (Secretaria da Comissão Interministerial para os Recursos do Mar, 2017). A expedição de Edmund Halley introduziu cabras e outros animais domésticos, ato que levou à drástica descaracterização da vegetação da ilha. A única espécie arbórea nativa (*Colubrina glandulosa* Perkins) ocupava cerca de 85% da área da ilha e quase foi extinta (Serafini *et al.*, 2010). Relatos históricos sugerem que a cobertura florestal da ilha tenha sido reduzida a 10% da original (Gasparini, 2004). Atualmente, a samambaia-gigante (*Cyathea delgadii* Sternb) é a espécie dominante do estrato arbóreo da ilha, ocorrendo nas encostas acima de 400 m no lado sul da ilha, criando a chamada "floresta nebular de samambaias gigantes" (Faria *et al*. 2021). A maior parte da ilha até os 400 m de altitude é caracterizada por uma vegetação herbácea, com 60% da área coberta por Poaceae e Cyperaceae. (Faria *et al*. 2021). A espécie *Cyperus appendiculatus* var. *atlanticus* domina as comunidades dos locais mais baixos da ilha (Alves, 1998). A espécie se enquadra na categoria "Vulnerável" (VU) na Lista Vermelha da flora brasileira (CNCFlora, 2025).

Trindade abriga quase 200 espécies de plantas terrestres, sendo 11 delas endêmicas (Rezende *et al*., 2023), dentre elas *Cyperus appendiculatus* var. *atlanticus* (Hemsl.) Kük será foco deste estudo. A espécie *Cyperus atlanticus* foi descrita por Hemsley em 1884 como endêmica da Ilha da Trindade e Martin Vaz. Kükenthal (1936) trata *C. appendiculatus* como única espécie incluindo as variedades: *C. appendiculatus* var. *appendiculatus*, *C. appendiculatus* var. *atlanticus*, *C. appendiculatus* var. *noronhae*, *C. appendiculatus* var. *gordonii*. A circunscrição indefinida da espécie revela que estudos das populações das ilhas oceânicas são relevantes.

O Arquipélago Martin Vaz possui três ilhas principais situadas a 48 km de Trindade e distribuídas ao longo de 2,7 km (CIRM, 2017). A maior delas, ilha Martin Vaz, possui cerca de 600 m de comprimento, 175 m de altura e menos de 0,75 km², enquanto as ilhas do Norte e do Sul são muito menores e ficam a cerca de 200 m e 1.200 m de distância da ilha Martin Vaz, respectivamente (Alves *et al*. 2009). Em Martin Vaz ocorrem apenas duas espécies de plantas vasculares, *C. appendiculatus* var. *atlanticus* e *Bulbostylis nesiotis*, que são endêmicas do complexo insular Trindade-Martin Vaz (TMV) (Alves *et al*. 2009).

O Arquipélago de São Pedro e São Paulo (ASPSP) é formado por 15 rochedos distante 945 km da costa brasileira (Serafini *et al*., 2010). Localiza-se na região equatorial do Atlântico, possui área emersa de aproximadamente 13 mil m², de topografia irregular e altitude máxima de 18 m (Motoki *et al*., 2009). Charles Darwin, durante sua viagem a bordo do H.M.S. Beagle, foi o primeiro a perceber a singular geologia do arquipélago quando constatou que sua origem não era vulcânica (Darwin, 1891). De fato, o ASPSP é o único afloramento de rochas onde ocorre a exposição *in situ* do manto abissal acima do nível do mar. Além disso, o arquipélago é resultado de um rápido soerguimento tectônico e o ponto culminante chegou ao nível do mar há cerca de 50 a 70 mil anos (Motoki *et al*., 2009). Devido às suas condições inóspitas, o arquipélago permaneceu isolado até 1982. A partir da Terceira Convenção das Nações Unidas sobre o Direito do Mar, foi iniciado um programa de ocupação e pesquisa do arquipélago, que passou a ser permanentemente ocupado com a construção da primeira Estação Científica construída em 1998 pela Marinha do Brasil (CIRM, 2025; Serafini *et al*. 2010). No ano de 2018, foram criadas unidades de conservação de proteção integral para esses conjuntos insulares: o Monumento Natural do Arquipélago de São Pedro e São Paulo e o Monumento Natural das Ilhas de Trindade, Martin Vaz e do Monte Columbia (Brasil, 2018).

O isolamento geográfico e a origem relativamente recente de Trindade e, especialmente do ASPSP, é fonte de interesse para estudos científicos sobre colonização de longa distância, migração e processos biogeográficos insulares (Rezende *et al*., 2023). No que se refere ao Arquipélago São Pedro e São Paulo, a flora terrestre é naturalmente escassa devido a sua pequena extensão territorial. No entanto, há uma lacuna de conhecimento acerca de quais espécies ali existem e em que abundância. Quando visitado por Darwin, em 1832, o arquipélago não abrigava nenhuma planta. Ele sugere que os primeiros habitantes dessas terras recém-formadas nos oceanos devem ter sido invertebrados associados a penas de aves que ali pousaram (Darwin, 1860). Mais recentemente, foram observadas plantas vasculares a partir de fotografias aéreas (Alves & Castro, 2006). Posteriormente, foram reportadas duas fanerógamas: *Cyperus* sp. (Cyperaceae) e *Boerhavia* sp. (Nyctaginaceae). Segundo relatos de pescadores, essas plantas foram introduzidas por eles e foram as únicas que sobreviveram após algumas tentativas de implantar outras espécies, como coqueiro e milho (Pereira *et al*., 2010). Em 2022, novas expedições ao arquipélago resultaram na coleta de amostras de uma Cyperaceae.

Na natureza, as plantas fornecem nichos para o crescimento e a proliferação de diversos e abundantes microrganismos, como algas, bactérias, fungos, protistas, nematoides e vírus (Trivedi *et al*., 2020), além de outras plantas. Essa comunidade microbiana, conhecida como microbiota da planta, compreende microrganismos benéficos, neutros e patogênicos. A microbiota desempenha importantes funções em relação à saúde e produtividade da planta (Deiner *et al*., 2017) e em ciclos biogeoquímicos globais (Philippot *et al*., 2009). As micorrizas evidenciam a importância das interações planta-microbiota, uma vez que as associações de fungos micorrízicos com algas verdes foram essenciais na evolução das plantas terrestres há cerca de 700 milhões de anos (Heckman *et al*., 2001). Importante destacar que as comunidades microbianas associadas a plantas não são randômicas, mas são estruturadas mostrando uma organização filogenética (Carlström *et al*., 2019). Portanto, ao ampliar a gama funcional e a capacidade metabólica da planta hospedeira, essa complexa comunidade microbiana costuma ser referida como o segundo genoma da planta (Deiner *et al*., 2017).

Tradicionalmente, a identificação taxonômica de microrganismos envolve seu isolamento e cultivo usando diferentes meios de cultura, juntamente à caracterização morfológica e requisitos fisiológicos e nutricionais da espécie. No entanto, técnicas cultura-dependentes conseguem capturar somente uma porção ínfima da diversidade de microrganismos naturalmente presentes em uma amostra (Abdelfattah *et al*., 2018). Assim, várias técnicas moleculares independentes de cultura têm sido empregadas na ecologia microbiana. O uso de tecnologias como o sequenciamento de alto rendimento (HTS - *High Throughput Sequencing*) permite a identificação de milhões de sequências em uma amostra, revelando até mesmo espécies crípticas (Turner *et al*., 2013).

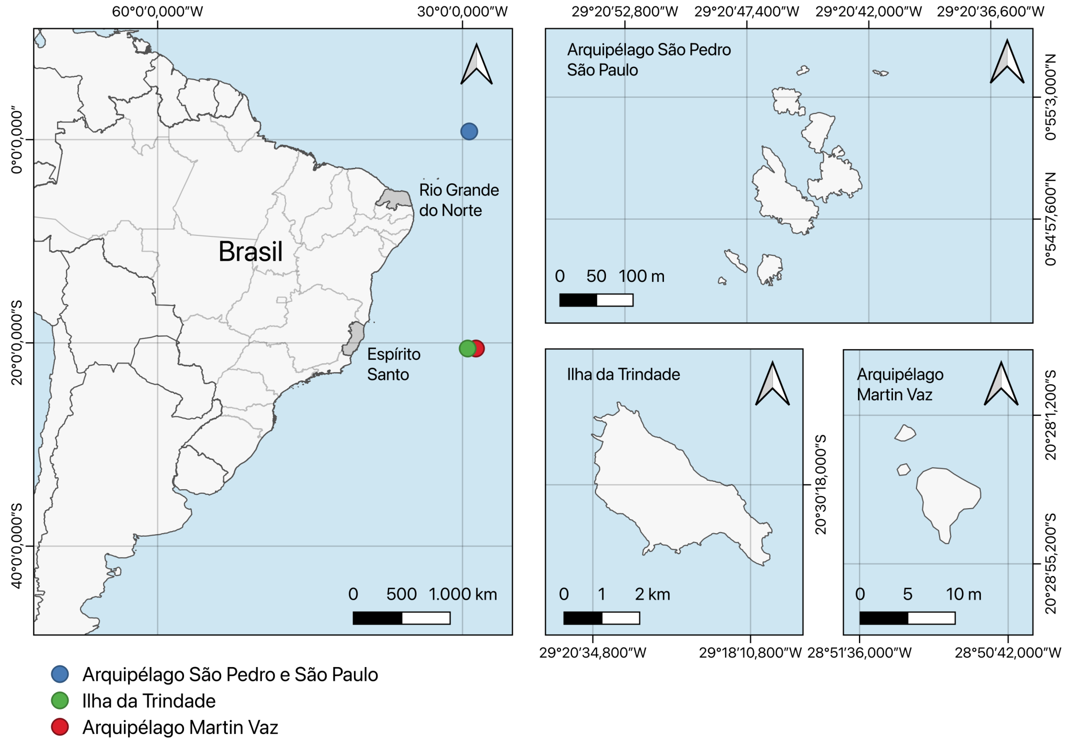

Figura 1. Localização da Ilha da Trindade, Arquipélago Martin Vaz e do Arquipélago São Pedro e São Paulo.

A técnica de DNA *metabarcoding* viabiliza a realização de levantamentos de biodiversidades com o uso de uma amostra ambiental para identificar um grande número de potenciais espécies (Taberlet *et al*., 2012). Dessa forma, tem sido possível caracterizar comunidades microbianas a partir de amostras ambientais complexas e fornecer novos detalhes sobre como elas respondem a fatores ambientais, o que tem tido grande impacto no estudo e uso de microrganismos associados a plantas (Abdelfattah *et al.*, 2018). As análises por *metabarcoding* permitem o estudo de praticamente todos os táxons microbianos em um determinado ambiente, o que é definido como microbiota.

Embora o termo microbiota seja usado indistintamente com microbioma, existem distinções no uso desse termo. O microbioma compreende o estudo de todo material genético microbiano recuperado do ambiente, conhecido por *shotgun metagenomics,* fornecendo informações sobre a composição e função da comunidade. A microbiota refere-se à composição taxonômica da comunidade microbiana determinada por *metabarcoding*, simplesmente revelando quais organismos estão ali (Ursell *et al.*, 2012). O uso de *metabarcoding* para caracterizar uma comunidade microbiana engloba cinco passos principais: amostragem; extração do DNA; amplificação; sequenciamento; e análise dos dados (Abdelfattah *et al.*, 2018).

A diversidade de organismos associados a plantas endêmicas em ilhas oceânicas é desconhecida. Esses organismos podem ser únicos e apresentarem vias metabólicas distintas, podendo propiciar a produção de diferentes substâncias úteis em processos biotecnológicos. Há potencial para aumentar a qualidade e produtividade de culturas agrícolas a partir do estudo dos mecanismos por meio dos quais as plantas controlam a seu microbiota, e os mecanismos que a microbiota usa para controlar a saúde das plantas (Deiner *et al.*, 2017).

Caracterizar a microbiota de plantas insulares pode fornecer detalhes de como ocorre a seletividade dos microrganismos e em que grau processos biogeográficos, estocásticos e determinísticos influenciam a formação da estrutura, comunidade e função das comunidades da microbiota. Sabe-se que a planta hospedeira exerce maior influência seletiva sobre os microrganismos associados do que as características ambientais (Wei *et al.*, 2024). Também é possível identificar similaridades na composição microbiana de plantas cogenéricas, ainda que muito distantes geograficamente, como observado por Le Roux *et al.* (2021).

Esta pesquisa faz parte do projeto "Microbiologia Marinha: utilização biotecnológica de microrganismos do Oceano Atlântico Sul e Ilhas Oceânicas do Brasil", com apoio dos Programa de Pesquisas Científicas na Ilha da Trindade (PROTRINDADE) e do Programa Arquipélago de São Pedro e São Paulo (PROARQUIPELAGO).

Diante do exposto, este estudo objetiva inventariar as comunidades de microrganismos associadas a plantas de ilhas oceânicas brasileiras utilizando a técnica de DNA *metabarcoding*.

Assim, será possível conhecer a comunidade de organismos eucarióticos que estão associados às plantas e explorar similaridades e diferenças na composição dessas comunidades em relação a qual sua planta hospedeira e onde ocorrem.

OBJETIVOS GERAIS

Verificar a diversidade de organismos associados a *C. appendiculatus* var. *atlanticus*, planta endêmica da Ilha Martin Vaz e da Ilha da Trindade, e ao *F. cymosa* do Arquipélago São Pedro e São Paulo.

OBJETIVOS ESPECÍFICOS

Inventariar a diversidade de organismos potencialmente associados a *Cyperus appendiculatus* var. *atlanticus* da Ilha Martin Vaz e da Ilha da Trindade, por meio da técnica de DNA *metabarcoding*;

Inventariar a biodiversidade de organismos potencialmente associados a *Fimbristylis cymosa* do Arquipélago de São Pedro e São Paulo, por meio da técnica de DNA *metabarcoding*;

Caracterizar e comparar as comunidades presentes nas folhas, raízes e inflorescências das plantas de interesse desse trabalho;

Comparar a diversidade de organismos associados a *Cyperus appendiculatus* var. *atlanticus* em relação aos locais amostrados - Ilha Martin Vaz e Pico do Desejado e POIT na Ilha da Trindade.

RESULTADOS

Capítulo 1: Eukaryotic communities associated with plant species *Fimbristylis* sp. on the extreme Equatorial São Pedro and São Paulo Archipelago assessed using DNA metabarcoding

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Abstract

The São Pedro and São Paulo archipelago (SPSPA) is a unique equatorial oceanic outcrop in the Atlantic Ocean, offering a natural laboratory for studying island colonization under geographic isolation. Among the few plants living in SPSPA, there is the sedge *Fimbristylis* sp., which, like other plants, can shelter diverse associated organisms on and in its tissues and potentially enhancing and regulating beneficial processes for them. Using high-throughput DNA metabarcoding, we carried out a pilot investigation of eukaryotic communities associated with a sedge species, recently proliferating on the archipelago. Phylogenetic analyses confirmed the sedge species as *Fimbristylis cymosa*. Additionally, based on marker ITS2, 89 distinct eukaryotic ASVs were assigned. Notably, our study detected potential plant and animal pathogens and taxa with restricted or previously undocumented biogeographical distributions. Our results highlight the archipelago's role as a reservoir of unexpected eukaryotic diversity and emphasize the need for continued exploration of oceanic island ecosystems.

Key words: Atlantic Ocean, environmental DNA, extremophiles, taxonomy.

Introduction

The São Pedro and São Paulo Archipelago (SPSPA), a group of 15 rocky islets located 945 km off the Brazilian coast, in the Equatorial Atlantic Ocean. It has emerged area of about 13,000 m^2 with irregular topography and maximum altitude of 18 meters (Motoki *et al.*, 2009). The climate of the equatorial Atlantic Ocean is influenced by the Intertropical Convergence Zone (ITCZ), which directly affects rainfall patterns in northeastern Brazil. At SPSPA, rainfall is concentrated between January and May, while the rest of the year is comparatively dry (Viana *et al.*, 2009)

During his voyage aboard the H.M.S. Beagle, Charles Darwin first recognized the unique geology of the archipelago noting its non-volcanic origin. (Darwin, 1891). The SPSPA is the only outcrop where the abyssal mantle is exposed above sea level. It formed through rapid tectonic uplift, with the highest point reaching sea level around 50,000 to 70,000 years ago (Motoki *et al.*, 2009). Due to its inhospitable conditions, the archipelago remained isolated until 1982, when, following the Third United Nations Convention on the Law of the Sea, a program of occupation and scientific research in the archipelago was established. In 1998, the Brazilian Navy built the first Scientific Station, and since then, the archipelago has been continuously occupied by scientists and military personnel. (Serafini *et al.* 2010; CIRM, 2026).

Due to its small size, the archipelago's terrestrial biodiversity is extremely restricted. When Darwin visited the islets in 1832, he reported the absence of terrestrial plants growing in the islet (Darwin, 1860). Records of terrestrial vegetation are relatively recent. Aerial photographs suggest the presence of *Canavalia obtusifolia* DC. occurring at the top of lighthouse in the main islet (Alves, R. J. V. & Castro, J. W. de A., 2006). In August 2007, two phanerogams were collected and identified as *Fimbristylis cymosa* R. Br. (UFP043516) and *Boerhavia coccinea* Mill. (UFP047517). According to fishermen, these plants introduced by them and were the only ones that survived after attempts to plant other species, such as coconut trees and corn (Pereira *et al.*, 2010). However, there is still a knowledge gap between the species that currently inhabit the area and their respective abundances. Additionally, the geographic isolation and relatively recent formation of the SPSPA make it an ideal place to study long-distance colonization, migration, and island biogeography (Rezende *et al.*, 2023). The archipelago acts as a true laboratory of natural selection, and has been classified as a priority area for the conservation of biological diversity (MMA/SBF, 2002).

In nature, plants host a diverse and abundant community of microorganisms, including algae, bacteria, fungi, protists, nematodes, and viruses, collectively known as the plant microbiota (Trivedi *et al.*, 2020). This community comprises beneficial, neutral, and pathogenic organisms and plays a crucial role in plant health, productivity (Berendsen *et al.*, 2012), and global biogeochemical cycles (Philippot *et al.*, 2009). The microbiota is often referred to as the plant's "second genome" due to its ability to expand the host plant's metabolic capabilities (Berendsen *et al.*, 2012).

The extinction of any plant species can trigger extinction cascades of its associated organisms and species that depend on them (Brodie *et al.*, 2014; Koh *et al.*, 2004). Documenting the diversity of plant-associated organisms is highly practical for conservation, particularly given that most of plant-associated arthropods and microfungi remain undescribed. (Hawksworth & Rossman, 1997; Larsen *et al.*, 2017). Traditionally, microbial identification relies on culturing and morphological or physiological characterization. However, culture-based methods recover only a small fraction of the natural microbial diversity (Abdelfattah *et al.*, 2018). To overcome this, culture-independent molecular techniques, such as high-throughput sequencing (HTS), are used in microbial ecology and enable the detection of millions of sequences per sample, including cryptic species (Turner *et al.*, 2013). The diversity of organisms associated with plants on oceanic islands remains largely unknown.

Previous reports about the plants inhabiting the archipelago are scarce and lacking species level information. To confirm the identity of the Cyperaceae found there, we perform a phylogenetic reconstruction using the ITS marker, which has proven efficient in exploring relationships within Cyperaceae (Ghamkhar *et al.*, 2003). Moreover, we investigate the diversity of eukaryotic organisms associated with this community of plants in the São Pedro and São Paulo archipelago employing the ITS2 molecular marker for DNA metabarcoding.

Methods

Study site and sampling

The São Pedro and São Paulo archipelago is located at 00º56'N - 29º22'W (Fig. 1). It is considered one of the smallest sets of oceanic islands in the world. Of the 15 islets that make up the archipelago, Belmonte islet is the largest and harbors a lighthouse and a scientific station. The archipelago is in the Intertropical Convergence Zone, characterized by light winds, intense cloud cover, high precipitation and low evaporation rate (Serafini *et al.*, 2010). The archipelago's sedges grow in ornithogenic soil pockets, under extreme conditions, such as high salinity, absence of fresh water, high temperatures and intense solar radiation (Gonçalves *et al.*, 2025).

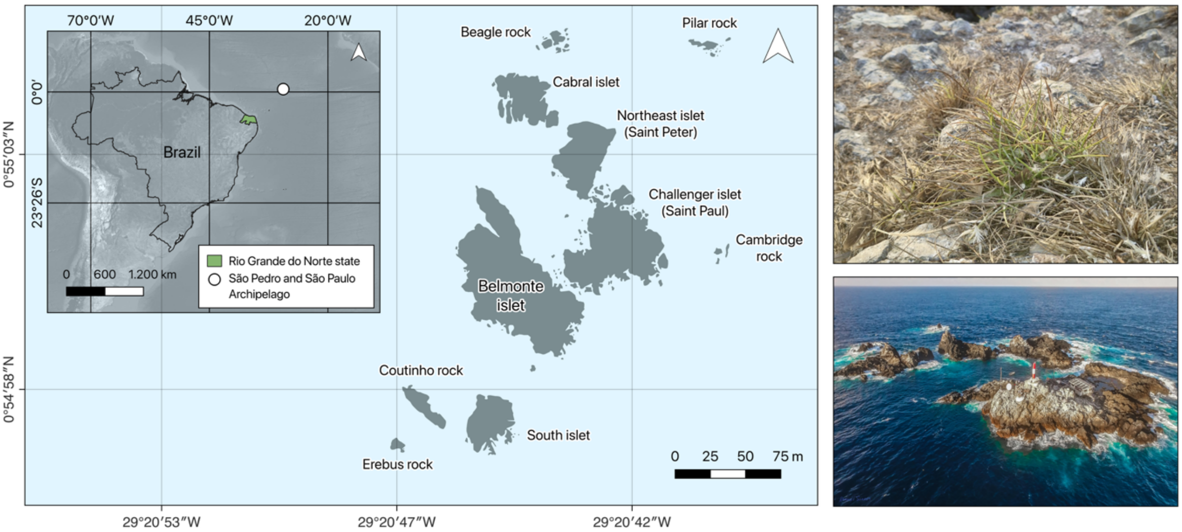

Figure 1. Map of São Pedro and São Paulo Archipelago (SPSPA). Tufts of *Fimbristylis* sp. growing in Belmont islet, on ornithogenic soil (photo by Mariana Reis). Aerial photo showing Belmont islet with lighthouse in front (photo by Marinha do Brasil).

The first records of *Fimbristylis* sp. in SPSPA are herbarium samples collected in 2007 (UFP43516) and 2017 (UB1037075, UB1037079, UB1037080, UB1029112, UB1171762, UB1194432). Leaves from samples UB1029112 and UB1171762 were stored in silica for posterior DNA extraction to perform phylogenic reconstruction. The specimens used for metabarcoding analysis were collected during an expedition in August and September 2022, when three tufts, with leaves and roots, were collected (Table 1). Plant samples were obtained with sterile gloves and tools, kept in sealed sterile plastic bags (Whirl Pack®/ US) and frozen (− 20°C) until DNA extraction. These samples were processed in a flow hood, where roots, leaves and inflorescences were separated and trimmed using sterilized petri dishes, scissors, and tweezers. Whenever possible, the greenest leaves and largest roots were chosen. Voucher specimens (UB0354418) were deposited in the UB herbarium, University of Brasilia, where there are further vouchers to validate taxonomy.

Table 1. Identification, plant individual and tissue type of the six *Fimbristylis cymosa* samples obtained from Belmonte Islet, São Pedro and São Paulo Archipelago.

Sample ID	Plant individual	Tissue type
F1	B	Leaf
F2	C	Leaf
F3	A	Root
F4	B	Root
F5	C	Root
F6	C	Inflorescence

Plant DNA Extraction, Amplification, Sequencing

Total genomic DNA was extracted from silica gel-dried leaves, following Doyle and Doyle (1987) with modifications (Borsch *et al*., 2003). The Internal Transcribed Spacer (ITS) region was amplified with the primers ITS5 and ITS4 (Baldwin *et al*., 1995). The reaction mix for polymerase chain reaction (PCR) with a total volume of 50 µl contained 26.5 ml Milli-Q water, 5 µl of 5X Mg free buffer, 5 µl of 50 mM of MgCl2, 2 µl at 10 mg/ml of BSA, 4 µl of 1 mM dNTP, 2.5 µl of 10 µM of each primer, 2 µl DNA and 0.5 µl Taq polymerase. The extractions and amplifications were carried out in the Plant Molecular Biology Laboratory at the University of Brasília. The PCR amplifications were performed in a thermocycler using 35 cycles at 95°C for 30 sec, and 54°C for the ITS region for 45 sec, and 72°C for 1 min, an initial cycle at 95°C for 1 min, and a final extension at 72°C for 5 min. The purification and sequencing were carried out by Macrogen Inc. (Seoul, Korea). Sequences were deposited in GenBank under the accession numbers listed in Appendix 1.

Phylogenetic reconstruction

We generated two sequences obtained from DNA extraction of samples from SPSPA and added sequences from GenBank obtained with their BLAST search. From these, sequences with uncertain species identification (such as cf.) and duplicates were removed. We retained up to two sequences per species, resulting in 48 sequences. (Appendix 2). Sequences were aligned with MUSCLE 5.1 (Edgar, 2004) on MEGA 12 version 12.0.11 (Kumar, 2024). The trees were rooted using *Abildgaardia* sp. as an outgroup according to Larridon *et al*., (2021).

Maximum Parsimony (MP) analysis was carried out using MEGA 12 version 12.0.11 (Kumar, 2024) with default settings and clade support was evaluated using the non-parametric bootstrap (Felsenstein 1985), with 1,000 replicates. Maximum likelihood (ML) and Bayesian analyses (BI) were performed using PhyML 3.3.20180214 (Guindon *et al*., 2010) and Mr. Bayes v. 3.2.6 (Ronquist *et al*., 2012) plug-ins on Geneious Prime 2025.1.2 (https://www.geneious.com). Clade support was evaluated using the non-parametric bootstrap (Felsenstein 1985), with 1,000 replicates for MP for ML. For Bayesian Inference two parallel Markov Chain Monte Carlo (MCMC) simulations were run for 5 million generations, sampling one tree every 1000 generations. The first 25% of the trees were discarded as burn-in. The BI support was evaluated using posterior probabilities.

Environmental DNA extraction and ITS amplification

Environmental DNA from the plant sample was extracted using the FastDNA Spin Kit for Soil (MPBIO, Ohio, USA). Extractions were carried out under strict sterile conditions to avoid contamination. The sealed bags were only opened inside a previously sterilized flow hood. DNA quality was analyzed using agarose gel electrophoresis (1% agarose in 1 × Trisborate EDTA) and then quantified using the Quanti- iT# Pico Green dsDNA Assay (Invitrogen). Extracted DNA was used as a template for generating PCR amplicons. The internal transcribed spacer 2 (ITS2) of the nuclear ribosomal DNA was used as a DNA barcode for molecular species identification (Chen *et al*., 2010). The PCR-amplicons were generated using the universal primers ITS3 and ITS4 (White *et al*., 1990). The construction of the libraries and amplification of DNA and high-performance sequencing was performed in Illumina Miseq V3 (Illumina, Inc.) at BPI (Botucatu, Brasil), where fragments of approx. 600 bp (2x300) with high quality were obtained.

Metabarcoding data analysis

Quality analysis was carried out using BBDuk v. 38.87 in BBmap software (Bushnell, 2014) with the following parameters: Illumina adapters removing (Illumina artifacts and the PhiX Control v3 Library); ktrim = l; 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 2024.5 (https:// qiime2.org/) for bioinformatics analyses (Bolyen *et al.*, 2019). The qiime2-dada2 plugin was used for filtering, dereplication, turning paired-end fastq files into merged and remove chimeras, using default parameters (Callahan *et al.*, 2016). Taxonomic assignments of ASVs were determined using the qiime2-feature-classifier (Bokulich *et al.*, 2018) classify-sklearn against different curated databases (PLANiTS2, UNITE); the sequence similarity threshold was 99%. For ITS2, firstly, ASVs were classified against the PLANiTS2 database (Banchi *et al.*, 2020). After this step, ASVs that remained unclassified were filtered and classify-sklearn classified against the UNITE Eukaryotes ITS database version 10.0 (Abarenkov *et al.*, 2025). Finally, the remaining unclassified ASVs were filtered and aligned against the filtered NCBI non-redundant nucleotide sequences (nt) database (February 2025) using BLASTn (Camacho *et al.*, 2009) with default parameters; the nt database was filtered with the following keywords: “ITS1”, “ITS2”, “Internal transcribed spacer”, and “internal transcribed spacer”. The abundance table of taxonomic assignments were generated using MEGAN6 (Huson *et al.*, 2016). Sequences will be submitted to GenBank.

For simplicity, we henceforth refer to the assigned ASVs as “taxa”. For comparative purposes, we used the number of reads as a proxy for relative abundance (Deiner *et al.*, 2017; Hering *et al.*, 2018). ASVs with relative abundance > 1% were considered dominant and those < 1% as minor (rare) components of the fungal community.

Species accumulation curves were obtained using the Mao Tao index. All results were obtained with 95% confidence, and bootstrap values were calculated from 1,000 replicates using the sofware PAST 5.2 (Hammer *et al.*, 2001b). Venn diagrams followed Heberle *et al.*, (2015). Information about distribution and habitat was obtained from GBIF (https://www.gbif.org/), GlobalFungi (Vetrovsky *et al.*, 2020), AlgalBase (Guiry, M.G., 2025) and relevant studies in the literature. For Fungi, databases FungalTraits and FUNGuild were also consulted (Põlme *et al.*, 2020; Nguyen *et al.*, 2016).

Results

The sedge *F. cymosa* R. Br. was first observed in the archipelago occurring sparsely around Belmonte islet, and it was first collected and identified in 2007 (UFP043516). During a more recent expedition in August 2022, the species was observed more abundantly and a few individuals were sampled. Now, the community is much less abundant and tufts are smaller, as it was observed in an expedition in December 2025 (Fig. 2).

The species *F. cymosa* is knwon to tolerate drought and salt stress in saltmarshes environments (Peters *et al*., 2025). Although it is a perennial species, the survival and abundance of plant community seem to be continuously affected with sea spray, strong winds, intense solar radiation and other environmental challenges. However, these variations of the archipelago's terrestrial vegetation have never been formally recorded and studied.

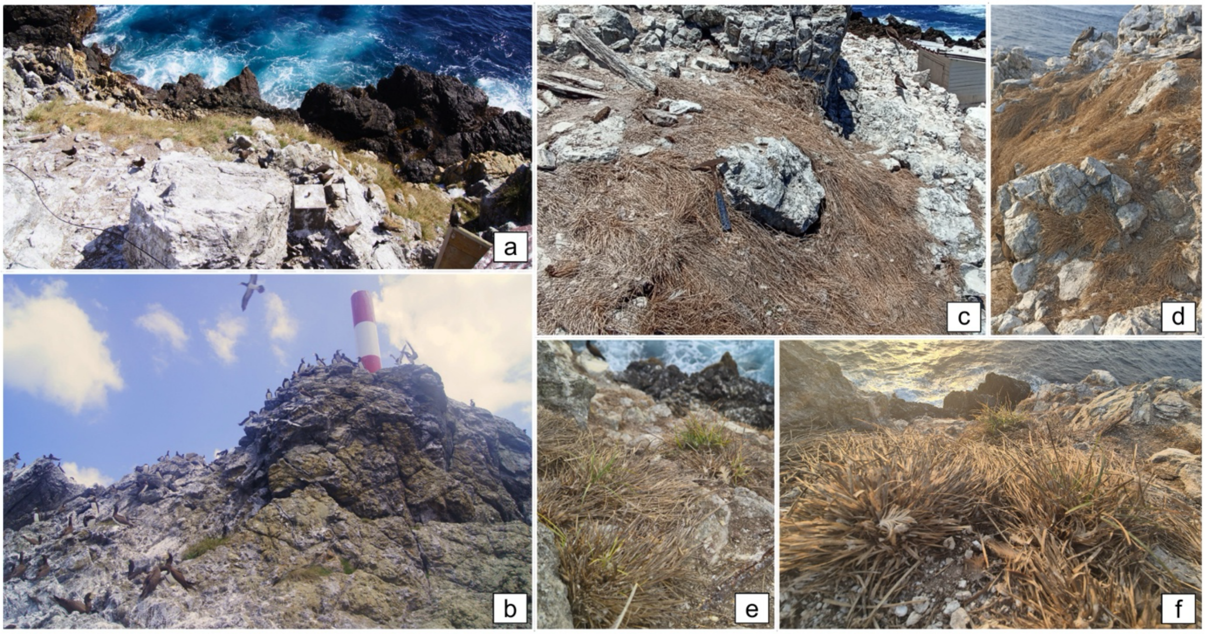

Figure 2. Photos showing the changes of the scarce vegetation present in SPSPA. Photos (a) and (b) from June 2017 by Tamara Dantas; (c) and (d) from 2022 by Eduardo Senra; (e) and (f) from November 2025 by Mariana Reis.

Phylogenetic analysis

This study generated two new sequences, *F. cymosa* (ASPSP) 1 and *F. cymosa* (ASPSP) 2, as seen in Figure 3. These samples formed a supported clade with the GenBank samples of *F. cymosa* in all analysis (MP 100, ML 100, BI 1) (Suppl. Figure 1, 2). Therefore, we can confirm that the sedge found in SPSPA is the species *F. cymosa*. This finding corroborates the identification of herbarium specimens collected in 2007 and 2017 and demonstrates that the plant community of the SPSPA is monospecific, having consisted of a single species for nearly two decades.

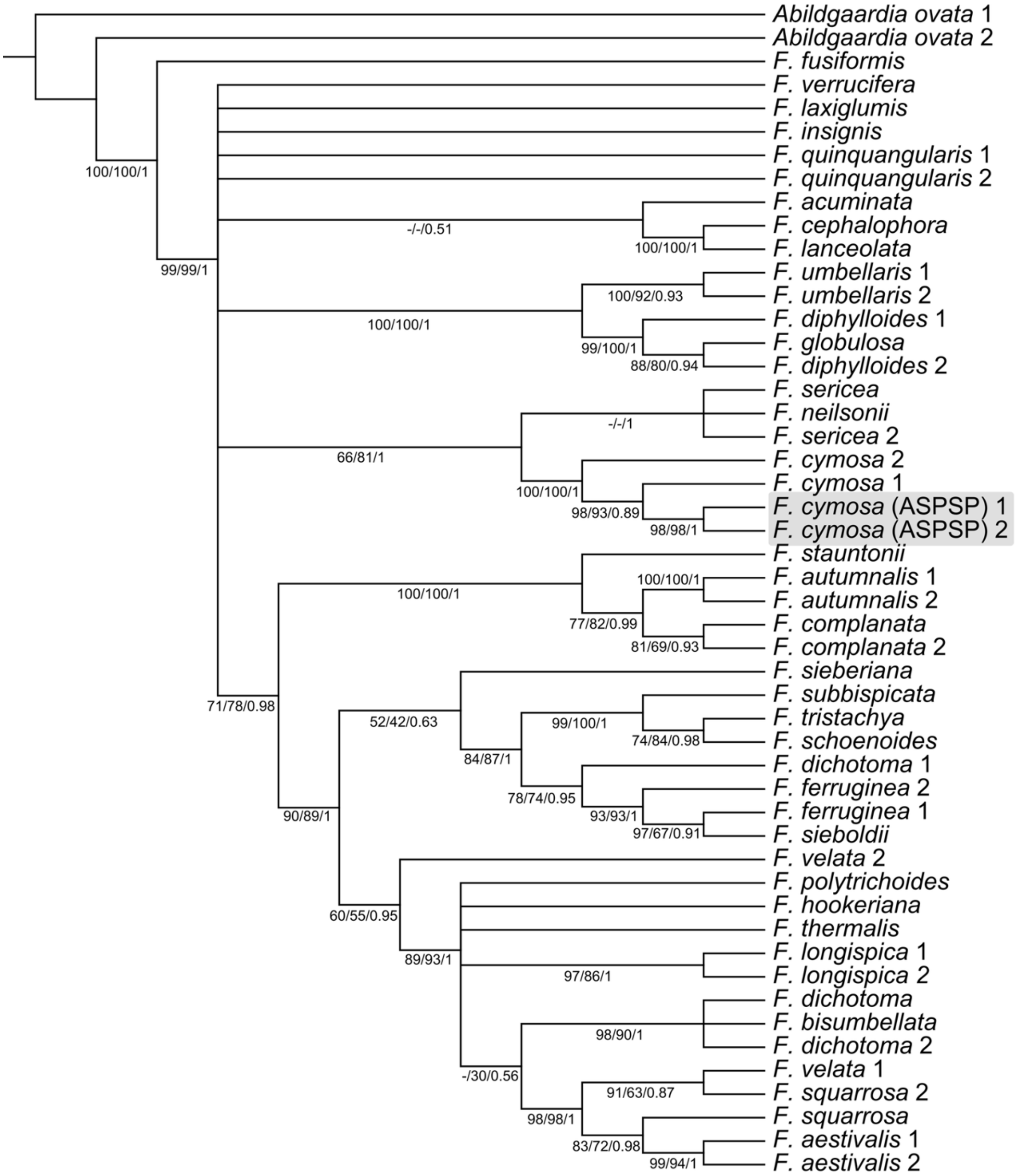

Figure 3. Cladogram obtained from Bayesian Inference based on ITS region data. Values below branches are bootstrap support for Maximum Parsimony Maximum Likelihood and Bayesian Posterior Probabilities, respectively. Sequences obtained from samples of São Pedro e São Paulo Archipelago indicated in grey box.

Associated organisms

A total of 366,457 ITS2 paired-end DNA reads were generated in the sequencing run and 305,024 reads remained for taxonomic classification after cleaning and denoising. These remaining sequences represented 89 ASVs (amplicon sequence variants) (Suppl. Table 1). A total of 311 reads could not be assigned to any taxonomic rank (Table 2).

Table 2. Number of sequences present in samples of *Fimbristylis cymosa* from São Pedro e São Paulo Archipelago (SPSPA), after quality filtering and classified by each database.

Samples	Total
Raw sequences	366,457
Cleaned and filtered	305,024
UNITE	259,081
PLANiTS2	678
GenBank	45,265
Unassigned	311

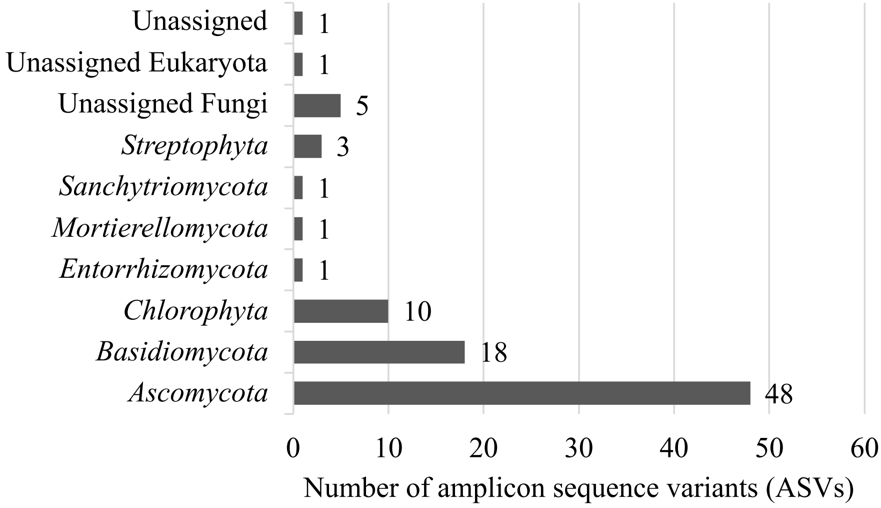

Figure 4. Number of amplicon sequence variants (ASVs) at phylum level identified associated with *Fimbristylis cymosa* from São Pedro e São Paulo Archipelago (SPSPA). Taxonomy classification follows (Tedersoo *et al.*, 2018).

From all samples, the classified ASVs included two kingdoms (Fungi and Viridiplantae) and seven phyla (*Ascomycota, Basidiomycota, Chlorophyta, Entorrhizomycota, Mortierellomycota, Sanchytriomycota* and *Streptophyta*) (Fig. 4). Fungi was the most abundant kingdom with 74 ASVs, followed by Viridiplantae with 13 ASVs. Out of the 89 classified ASVs, 36 (40%) could be identified at species level and 30 (34%) at genus level. Twenty-two (25%) ASVs could only be assigned to higher taxonomic levels (phylum, class and family) and may therefore represent taxa not found in the consulted databases or currently undescribed species.

The rarefaction curves calculated for all samples, except for F6, reached a plateau indicating sufficient sampling effort (Fig. 5). This suggests that the analyzed sequences accurately represent the sequence diversity present in the study environments, except for sample 85-ITS. This sample's rarefaction curve indicates that obtained reads can potentially classify more taxa if sequencing is executed again.

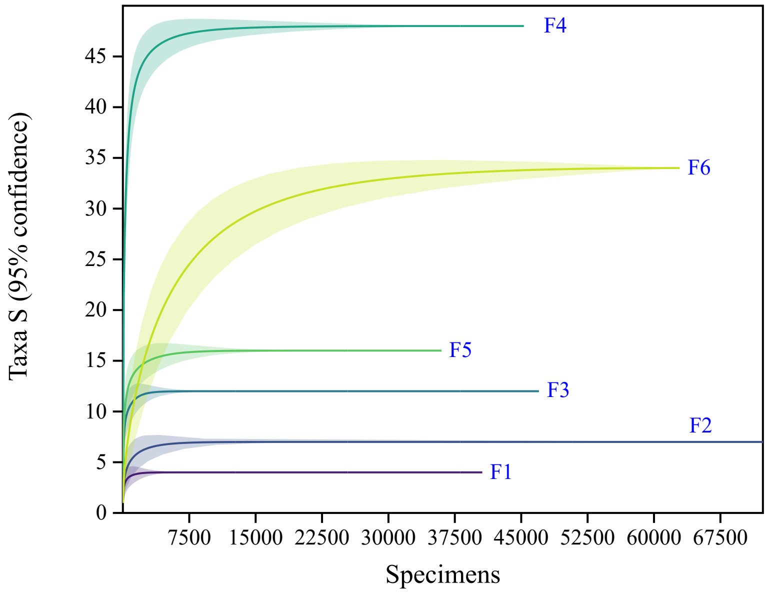

Figure 5. Rarefaction curves (Mao Tao index) for the eukaryotic communities detected from each of the six *Frimbristylis cymosa* samples obtained from the São Pedro and São Paulo Archipelago.

Fungi was the dominant kingdom detected in these eukaryotic communities, with *Ascomycota* as the dominant phylum, followed by *Basidiomycota*, *Entorrhizomycota*, *Mortierellomycota* and *Sanchytriomycota*, in rank order. Thirteen ASVs were classified as dominant (relative abundance ≥ 1%), which included the taxa *Hortaea werneckii*, *Dimorphiseta obtusa*, *Aspergillus terreus*, *Poaceascoma* sp., uncultured fungus, *Phaeosphaeria* sp., *Eonema pyriforme, Natipusillaceae*, *Ypsilina graminea, Alternaria* sp., *Ramalinaceae* sp., *Marasmius* sp. and *Chaetomiaceae* sp., in rank of abundance. Together these fungi represent 94% of all DNA reads. The most abundant species were *H. werneckii* with 142,634 reads (47%), *D. obtusa* with 44,096 (14%) and *A. terreus* with 41,493 (14%).

The kingdom Viridiplantae compromised 899 reads (0,3% of total reads) classified in 13 ASVs. Sequences of 2 phyla were detected, *Chlorophyta* and *Streptophyta*. Among *Chlorophyta*, 9 taxa were found: *Prasiola* sp., *Ignatius tetrasporus*, *Stichococcus bacillaris*, *Desmococcus olivaceus*, *Trebouxia potteri*, *Chlorellaceae* sp., *Trebouxia jamesii*, *Tetracystis tetraspora* and *Scenedesmus vacuolatus*, in rank of abundance.

Some classified taxa have not been previously recorded in South America or the Atlantic Ocean, such as *Clorophyta* species: *Scenedesmus vacuolatus*, *T. tetraspora, T. jamesii, T. potteri*, *I. tetrasporus*; and Fungi: *Leptogium antarcticum*, *Tetramelas chloroleucus*, *Eonema pyriforme*, *Kondoa yuccicola*, *Mycena abramsii*, *Lecania glauca*, *Tricladium curvisporum* and *Ypsilina graminea*.

Roots, leaves and inflorescence

The roots yielded the most taxa followed by the inflorescence and leaves (Fig. 6). Only *H. werneckii*, *D. obtusa*, *Curvularia tuberculata* and *Wallemia* sp. were found in all environments. Additionally, *I. tetrasporus*, *Penicillium* sp., *A. terreus*, *Natipusillaceae* sp. and *Xenomyrothecium tongaense* were present in both roots and inflorescences. No taxa were exclusive to the leaves.

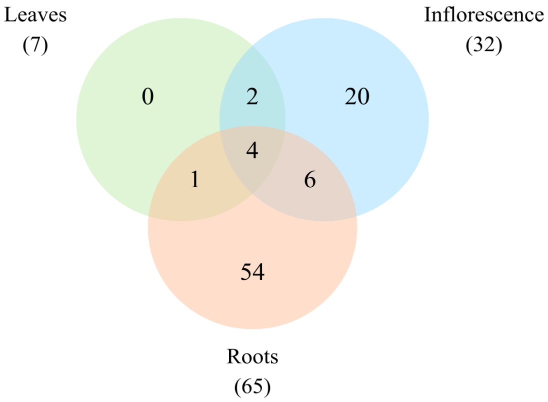

Figure 6. Venn diagram showing number of assigned taxa associated with roots, leaves and inflorescence of *Frimbristylis cymosa* from São Pedro e São Paulo Archipelago.

Discussion

Our phylogenetic results indicate that the sedge occurring is SPSPA is the species *F. cymosa.* This perennial sedge is a halophytic and pantropical species and it occurs in coastal areas or areas with maritime influence of the tropical regions of the world (WFO, 2026), including all coastal states of Brazil (Peters *et al.* 2025, POWO, 2025) and others islands as Fernando de Noronha (RB1398740, RB01400195, CEN00069755, UFRN00017492). However, it remains unclear how and when these plants arrived in the archipelago. Local fishermen believe that the species may have been introduced unintentionally through construction sand transported during the building of the scientific station. Since the inauguration of the first station in 1998, recurrent wave impact caused structural damage, leading to its replacement by a new station constructed in 2007. (CIRM, 2026). Comparative population genomic analysis between continental specimens and those currently found in SPSPA may help clarify their origins.

Previous studies revealed a diverse and complex fungal community in soil samples from the SPSPA (Gonçalves *et al.*, 2025). The root associated community has a higher diversity of taxa, with representants from seven phyla. Fewer taxa were found in association with leaves and flowers, with 4 phyla assigned (Fig. 6). This can be explained as the aerial surface of plants is relatively nutrient poor and experiences much more dynamic conditions. Temperature, moisture, and radiation fluctuate throughout the day and night (Turner *et al.*, 2013). Interestingly, the inflorescence yielded significantly more taxa (32) than the leaves (7), although it is also an aerial organ.

The communities associated with *F. cymosa* in SPSPA, as identified through DNA metabarcoding, encompass 7 phyla based on data generated using a single marker (ITS2). These findings highlight the high diversity associated with the only plant found in this isolated archipelago. Intriguingly, one of the most abundant classified ASVs corresponds to an unknown fungus (2,9% of total reads). This finding may indicate the presence of a previously undescribed taxon, or it may simply reflect the current incompleteness of reference databases.

Of the total, six potential animal pathogens were identified: *Alternaria* sp., *A. terreus*, *Fusarium* sp., *H. werneckii*, *Madurella tropicana* and *Penicillium* sp. and two potential animal parasites - *Rhinocladiella* sp. and *Penicillium* sp. Fungal infections in birds are common and serious systemic diseases, and members of the genera *Aspergillus* sp. are frequently isolated pathogens (Garcia *et al.*, 2007; Hubálek, 2004). *Aspergillus* sp. and *Penicillium* sp. have been reported as pathogens of *Sula leucogaster*, one of the three seabirds breeding in the archipelago (Zampieri *et al*. 2015)

Fourteen potential plant pathogens were also identified: *Alternaria* sp., *C. tuberculata*, *Fusarium* sp., *Microdochium phragmitis*, *Neodevriesia strelitziicola*, *Penicillium* sp., *Phaeosphaeria* sp., *Zasmidium* sp., *Zymoseptoria* sp., *M. abramsii*, *Entorrhiza* sp., *Cadophora* sp., *Herpotrichia* sp. and *Marasmius* sp. *Curvularia tuberculata* (*Ascomycota*, *Pleosporales*) is a plant pathogen, reported for some economically important plants (de Luna *et al*., 2002). It is also known as an opportunistic human pathogen (Vasikasin *et al*., 2019). *H. werneckii* (*Ascomycota*, *Capnodiales*) is a black yeast known to be extremely halotolerant and occur in some of the most extreme conditions on the planet, from the deep sea to the Atacama desert (Vaupotič & Plemenitaš, 2007; Gostinčar *et al*., 2023). Recently, this species was also found in abyssal core sediments collected at a 4444 m depht in the equatorial Atlantic, about 439 km south of SPSPA (Gontijo *et al*., 2025). Moreover, this species is the etiological agent of tinea nigra, a superficial cutaneous fungal infection (Sutton *et al*., 2009).

Dimorphiseta obtusa (*Ascomycota*, *Hypocreales*) is a soil saprotroph. *Dimorphiseta* species have been found associated with the rhizosphere soil and plant leaves (Liang *et al*., 2019; Lombard *et al*., 2016). *Aspergillus* (*Ascomycota*, *Eurotiales*) species are ubiquitous, saprophytic fungi that play crucial roles in global carbon and nitrogen cycles. Several *Aspergillus* species have a notable impact on public health both beneficially being used for industrial applications and negatively as pathogens and source of mycotoxins, such as aflatoxin that contaminate common crops. *A. terreus* is pathogenic to plants, animals and humans, especially in immunocompromised individuals. *Aspergillus* representatives produce tiny hydrophobic conidia that easily enter and disperse in the air, and survive for long periods in various substrates and environmental conditions (Abdel-Azeem *et al*., 2016; Dagenais & Keller, 2009; Davis & Porter, 2006).

The presence of *Wallemia* sp. and *Wallemia hederae* indicates that the archipelago represents a potential source of extremophilic organisms. The genus *Wallemia* (*Basidiomycota*, *Wallemiales*) compromises the most xerotolerant, xerophilic and halophilic species worldwide. They are filamentous food-borne pathogenic fungi, found in osmotically challenging environments (Zajc & Gunde-Cimerman, 2018). Curiously, to date, *W. hederae* has only been isolated from non-saline substrates (Jančič *et al*. 2016). As demonstrated by (Gonçalves *et al*., 2025), the SPSPA environment has exceptionally elevated sodium saturation levels even for marine and coastal environments. The fungus *Antarctomyces psychrotrophicus* is one of the only two species described to this genus, which were originally described from Antarctica (de Menezes *et al*., 2017; Stchigel *et al*., 2001). Recent studies of soil samples from the oceanic Trindade Island, using a metabarcoding approach, also identified this species, marking the first record of this species outside Antarctica (Camara *et al*., 2022). The assignment of this species in SPSPA may indicate that the real distribution is larger than previously thought and its dispersion may be facilitated by migratory birds.

Interestingly, a few species with restricted distribution were assigned. Two lichenized fungi - *Lecania glauca* and *Leptogium antarcticum* – are only known in Antarctica (Vetrovsky *et al*., 2020). *Eonema pyriforme* (Agaricales, Basidiomycota) is a wood saprotroph only reported for Europe, Russia and Mexico. Two other fungi phyla were found associated with the roots: *Mortierellomycota* (*Linnemannia sclerotiella*) and *Entorrhizomycota* (unknown *Entorrhizaceae*). *Mortierellaceae* species are saprobe soil fungi able to grow under nutrient-poor (oligotrophic) conditions (Bergero *et al*., 1999). They are also known to be plant-endophytes with a dual lifestyle and often are found associated to bacteria (Zhang *et al*., 2020; Telagathoti *et al*., 2021). *Entorrhizomycetes* are plant parasites that form galls on the roots of plants in the Juncaceae and Cyperaceae families (Bauer *et al*., 2015).

The fungal community associated with *F. cymosa* was found to be very distinct from the soil fungal community revealed by Gonçalves *et al*. (2025) in the archipelago, with only 10 taxa in common: *H. werneckii*, *A. terreus*, *N. strelitziicola*, *Spiromastigoides asexualis*, *X. tongaense*, *M. abramsii*, *Penicillium* sp., *Fusarium* sp., *Neodevriesia* sp. and *Wallemia* sp. This might indicate that some organisms are being selected by the plant host. It has been shown that fungal endophytes can symbiotically confer stress tolerance to plants, an essential strategy that could explain how *F. cymosa* has been able to withstand the polyextreme environment of SPSPA (Rodriguez *et al*., 2008).

Though not many taxa were found, the assigned Chlorophyta represent five families (Chlorellaceae, Ignatiaceae, Prasiolaceae, Scenedesmaceae and Trebouxiaceae) and tree orders (Chlorophyceae, Trebouxiophyceae and Ulvophyceae). The most abundant taxon is an unknown *Prasiola*, though it was only found in a single root sample. *Prasiola* species are cosmopolitan and are found in terrestrial, freshwater and marine conditions (Guiry, M.G., 2025). Following, *Ignatius tetrasporus* is the only species of its genus, found in terrestrial environments and freshwater and it has only been recorded for the United States and Russia

(Guiry, M.G., 2025). The Trebouxiophyceae *Stichococcus bacillaris* is a cosmopolitan terrestrial alga, but with no records for Brazil or any Atlantic oceanic islands (Guiry, M.G., 2025). *Desmococcus olivaceus* is a Chlorophyceae with aeroterrestrial and chasmoendolithic habit and widespread distribution (Guiry, M.G., 2025; Gustavs *et al*., 2011). Two *Trebouxia* species were assigned but neither had previously been recorded in South America or the Atlantic Ocean. Interestingly, *Trebouxia* is a common lichen photobiont genus, but it has also been detected in air and rain samples (Cáliz *et al*., 2018; Dillon *et al*., 2020; Dong *et al*., 2019). The leaves samples didn't have any Viridiplantae, indicating that the shade and humidity provided by the soil might be essential for the presence of plant associated algae.

In conclusion, our study revealed a diverse eukaryotic community, especially in association with the roots of *F. cymosa.* The presence of taxa such as *H. weneckii* and *Wallemia* sp. indicate that the SPSPA may be a potential source of extremophilic fungi. However, potential animal pathogens were also assigned, indicating the need of caution and monitoring with visitors in the archipelago. It is important to note that the assignment of taxa based on the sampling of environmental DNA sequencing does not confirm the presence of viable propagules or active organisms, and the accuracy of the identifications relies on the quality and completeness of databases. Sampling during the rainy season, between March and April, would greatly contribute to further investigate the core associated organisms to *Fimbristylis cymosa*. Additional studies using metatranscriptomic analysis or culturing approaches, such as Geremia *et al*. (2025), are fundamental to better understand the complex community associated with plants in such an isolate and hostile environment, such as São Pedro and São Paulo Archipelago.

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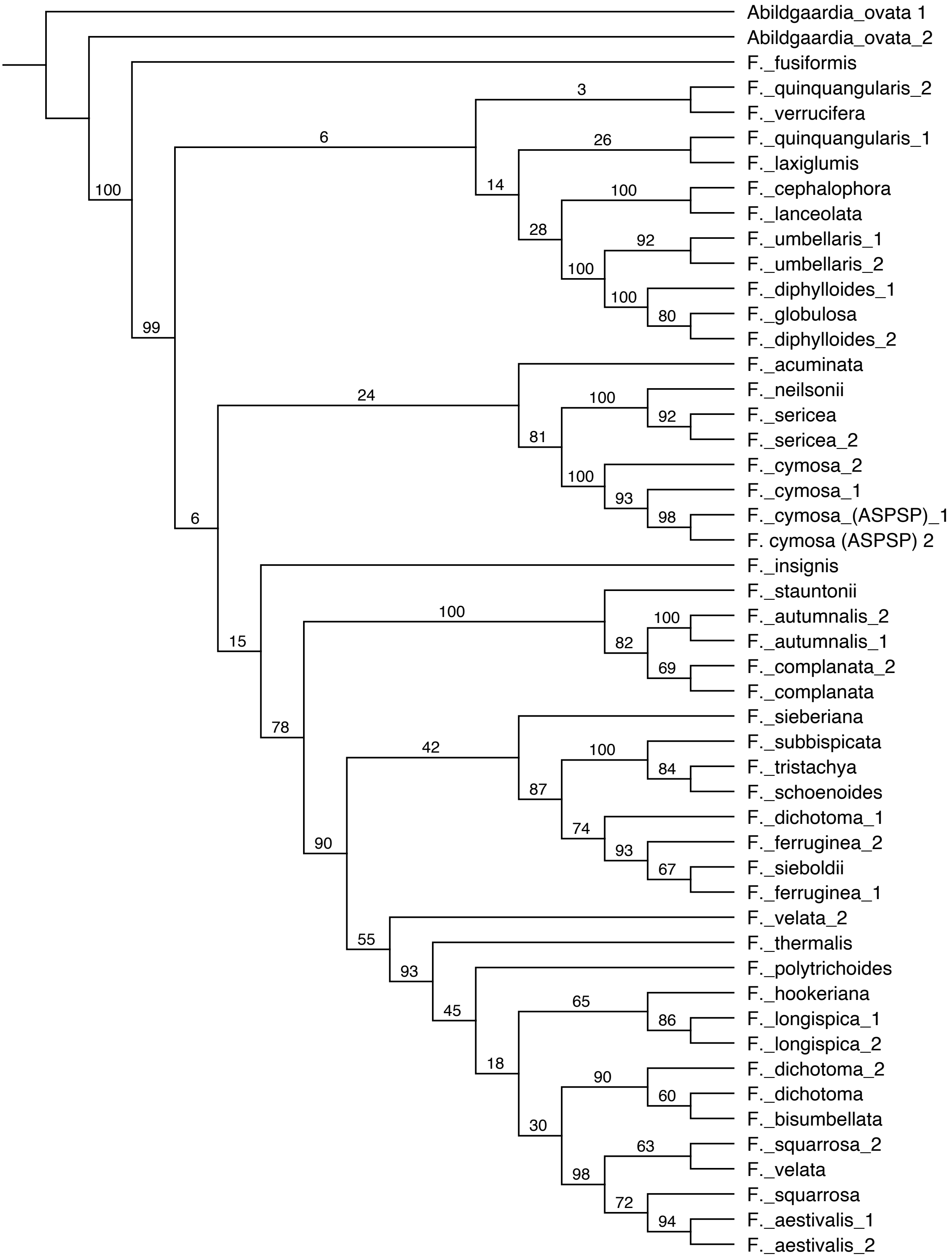
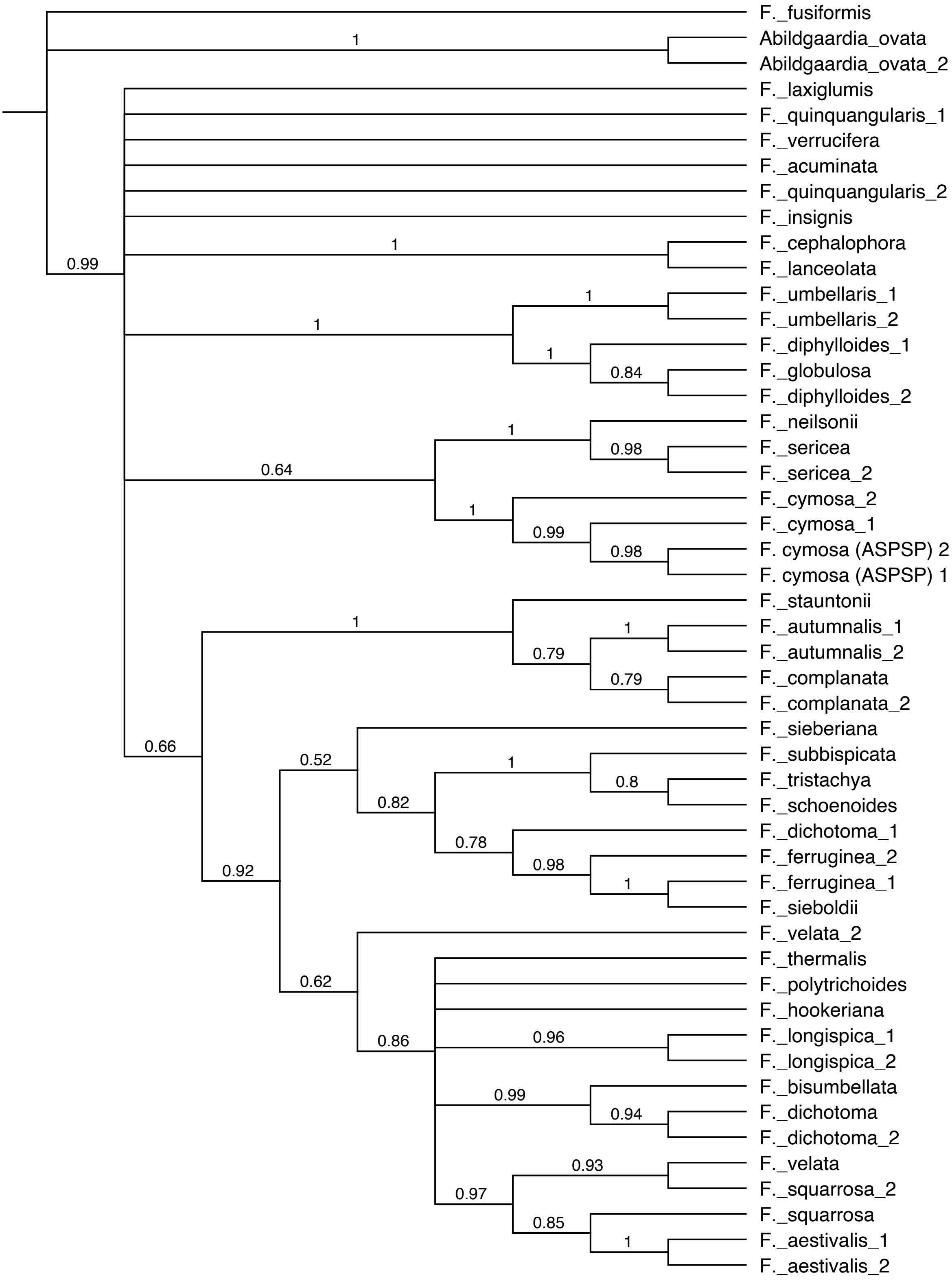


Supplementary Figure 1. Maximum Likelihood.



Supplementary Figure 2. Maximum Parsimony.

Appendix 1. Voucher information (voucher number, herbarium, locality) and GenBank accession numbers of the *Fimbristylis* specimens newly sequenced for the present study.

Fimbristylis cymosa R.Br., Dantas 966, UB, Arquipélago de São Pedro e São Paulo, Pernambuco, Brazil; *Fimbristylis cymosa* R.Br., Dantas 967, UB, Arquipélago de São Pedro e São Paulo, Pernambuco, Brazil.

Appendix 2. GenBank accession numbers of sequences used in phylogenetic reconstruction.

Abildgaardia ovata 1 (AB180719); *Abildgaardia ovata* 2 (LC576032); *F. acuminata* (LS999542); *F. aestivalis* 1 (AB250626); *F. aestivalis* 2 (JX644879); *F. autumnalis* 1 (AB250627); *F. autumnalis* 2 (JX644880); *F. bisumbellata* (MH808753); *F. cephalophora* (AY506777); *F. complanata* 1 (AB250628); *F. complanata* 2 (JX644881); *F. cymosa* 1 (AB250629); *F. cymosa* 2 (AY506798); *F. dichotoma* (LS999545); *F. dichotoma* 1 (AB250630); *F. dichotoma* 2 (MH808754); *F. diphylloides* 1 (AB250632); *F. diphylloides* 2 (LC655279); *F. ferruginea* 1 (AB250633); *F. ferruginea* 2 (AB250634); *F. fusiformis* (KY652919); *F. globulosa* (JX644885); *F. hookeriana* (LS999549); *F. insignis* (LS999550); *F. lanceolata* (AY506786); *F. laxiglumis* (AY506785); *F. longispica* 1 (AB250635); *F. longispica* 2 (AB250636); *F. neilsonii* (AY506784); *F. polytrichoides* (AY506796); *F. quinquangularis* 1 (LS999552); *F. quinquangularis* 2 (MH808756); *F. schoenoides* (LS999554); *F. sericea* 1 (ON908442); *F. sericea* 2 (AB250640); *F. sieberiana* (AY506801); *F. sieboldii* (JX644884); *F. squarrosa* 1 (AB250641); *F. squarrosa* 2 (JX644887); F. stauntonii (JX644888); *F. subbispicata* (AB250642); *F. thermalis* (MF964172); *F. tristachya* (AY506802); *F. umbellaris* 1 (LS999555); *F. umbellaris* 2 (LS999556); *F. velata* 1 (AB250643); *F. velata* 2 (MK352460); *F. verrucifera* (AB250644).

Capítulo 2: Uncovering the hidden eukaryotic community of *Cyperus appendiculatus* var. *atlanticus* (Hemsl.) Kük from Trindade Island and Martin Vaz Archipelago with DNA metabarcoding

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Abstract

The Trindade–Martin Vaz insular complex in the South Atlantic Ocean represents Brazil's most remote oceanic island system and a natural laboratory for studying plant-associated microbial diversity. Oceanic islands represent natural laboratories for studying plant-associated microbial diversity, yet such communities remain poorly explored. Using a DNA metabarcoding approach and high-throughput sequencing (HTS), we characterize eukaryotic assemblages associated with *Cyperus appendiculatus* var. *atlanticus*, an endemic sedge dominating herbaceous vegetation on both Trindade and Martin Vaz islands. We detect 665,052 DNA reads assigned to 411 amplicon sequence variants (ASVs). These taxa spanned four eukaryotic kingdoms and nine phyla, with Fungi dominating the assemblages in terms of richness and abundance. Several taxa associated with potential plant and animal pathogens were detected, alongside non-Dikarya fungal lineages and other cryptic taxa. Overall, our results reveal a highly diverse eukaryotic community associated with *C. appendiculatus* var. *atlanticus* and highlight the Trindade and Martin Vaz islands as reservoirs of previously undocumented microbial diversity.

Key words: environmental DNA, taxonomy, South Atlantic islands

Introduction

Trindade Island is situated in the South Atlantic, about 1,140 km from the Brazilian coast, and along with the Martin Vaz Archipelago, forms Brazil's most remote insular group. These islands originated around three million years ago due to volcanic activity along the Vitória-Trindade Ridge. Trindade covers 9.28 km², rises 620 m above sea level, and is surrounded by ocean depths reaching 5,800 m (Alves, 1998; Serafini *et al.*, 2010). Since 1957, when the Brazilian Navy created the Oceanographic Post of Trindade Island (POIT), Trindade Island has been permanently occupied (Gasparini, 2004).

The native vegetation of Trindade was largely destroyed by introduced animals, especially goats that were brought to the island in the 18th century. Today, Trindade hosts nearly 200 terrestrial plant species, 11 of which are endemic (Rezende *et al.*, 2023). The island's only native tree (*Colubrina glandulosa* Perkins) once covered 85% of the island but was nearly eradicated (Gasparini, 2004; Serafini *et al.*, 2010). Today, the most conspicuous vegetation type is the "giant fern forest", formed by the scaly tree fern *Cyathea delgadii* Pohl. ex Sternb. and located above 400 m on the island's southern slopes (Faria *et al.*, 2021). Below 400 m, the island is dominated by herbaceous vegetation, with 60% covered by Poaceae and Cyperaceae (Faria *et al.*, 2021). *Bulbostylis nesiotis* (Hemsl.) C.B.Clarke dominates higher elevations, while *Cyperus appendiculatus* var. *atlanticus* (Hemsl.) Kük. is found in lower areas (Alves, 1998). Both are listed as Vulnerable (VU) in Brazil's Red List (Prieto *et al.* 2012; CNCFlora, 2025.).

The Martin Vaz Archipelago (20º28'S, 28º50'W), located 48 km from Trindade, consists of three small islands spread across 2.7 km. The largest, Martin Vaz Island, is 600 m long, 175 m high, and under 0.75 km² in area. Only two vascular plants are known there - *C. appendiculatus* var. *atlanticus* and *B. nesiotis* - both endemic to the Trindade-Martin Vaz complex (TMV) (Alves *et al.*, 2009).

In nature, plants host a diverse and abundant community of microorganisms, including algae, bacteria, fungi, protists, nematodes, and viruses, collectively known as the plant microbiota (Trivedi *et al.*, 2020). This community comprises beneficial, neutral, and pathogenic organisms and plays a crucial role in plant health, productivity, and global biogeochemical cycles (Philippot *et al.*, 2009). The microbiota is often referred to as the plant's "second genome" due to its ability to expand the host plant's metabolic capabilities (Deiner *et al.*, 2017).

Traditionally, microbial identification relies on culturing and morphological or physiological characterization. However, culture-based methods recover only a small fraction of the natural microbial diversity (Abdelfattah *et al.*, 2018). To overcome this, culture-independent molecular techniques, such as high-throughput sequencing (HTS), are used in microbial ecology and enable the detection of millions of sequences per sample, including cryptic species (Turner *et al.*, 2013).

Microorganisms, like plants and animals, also show biogeographic patterns. However, unlike the extensive knowledge on island biogeography of higher organisms, we have limited understanding of the distribution of microorganisms within and among islands (Li *et al.*, 2020). It is also known that plants recruit their associated organisms. Here, we aim to describe and compare the community of microorganisms associated with the leaves, inflorescences and roots of *Cyperus appendiculatus* var. *atlanticus*, endemic plant species from the insular complex Trindade-Martin Vaz.

Methods

Study site and sampling

Trindade Island (20º30'S, 29º20'W) is situated in the South Atlantic, about 1,140 km from the city Vitória, Brazil, and 2,400 km from the west coast of Africa (Fig. 1). Along with the Martin Vaz Archipelago, it forms Brazil's most remote insular group. These islands originated around three million years ago due to volcanic activity along the Vitória-Trindade Ridge. Trindade covers 9.28 km^2, rises 620 m above sea level, and is surrounded by ocean depths reaching 5,800 m (Alves, 1998; Serafini *et al.*, 2010). It is predominantly rugged and is considered the most heterogeneous and topographically varied Brazilian volcanic island. (Almeida, Fernando F.M. de, 2002; Schaefer CEGR & Oliveira FS, 2015). Martin Vaz is the largest island of a small archipelago situated 48 km east of Trindade Island at 20°28′S and 28°50′W. Its vegetation is scarce, but it is an important nidification site for several marine birds (Alves, 1998).

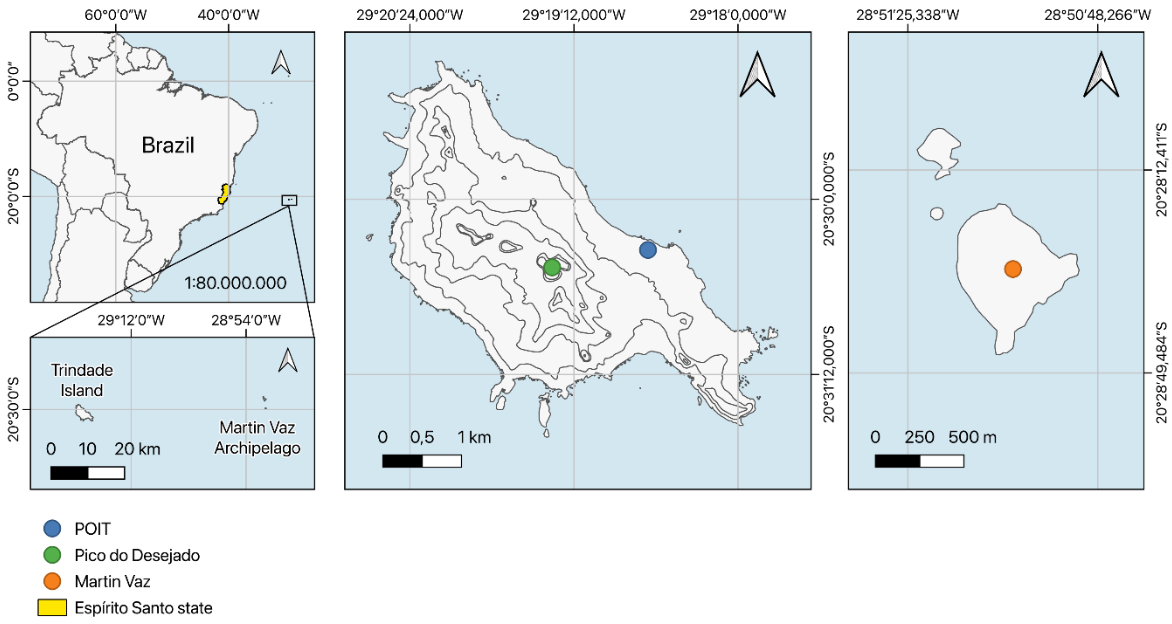

Figure 1. Location of the Trindade-Martin Vaz oceanic insular complex and detailed view of Trindade Island and Martin Vaz Archipelago.

Fifteen individuals of *Cyperus appendiculatus* var. *atlanticus*, with leaves, inflorescences, and roots, were collected in an expedition in May 2024 in Trindade and Martin Vaz islands. For Trindade Island, five samples were obtained at Desejado Hill and six at POIT (Posto Oceanográfico da Ilha da Trindade), and four samples were obtained in Martin Vaz Island (Fig. 1, Table 1). Plant samples were collected with sterile gloves and tools and kept in sealed sterile plastic bags (Whirl Pack®/ US) and frozen (− 20 °C) until DNA extraction. The samples were processed in a flow hood, where pieces of roots, inflorescences and leaves were trimmed using sterilized scissors and tweezers. Whenever possible, the greenest leaves and largest roots were chosen.

Table 1. Identification, plant individual and tissue type of the six *Fimbristylis cymosa* samples obtained from Trindade and Martin Vaz islands.

Sample ID	Individual	Sample Type	Location	Latitude	Longitude	Altitude (m)	Voucher (UB)
C1	A	Inflorescence	Desejado Hill	20°30'29"S	29°19'43"W	584 m	Faria, 1572A
C2	B	Inflorescence	Desejado Hill	20°30'29"S	29°19'43"W	584 m	Faria, 1572A

C3	C	Leaf	Desejado Hill	20°30'29"S	29°19'43"W	584 m	Faria, 1572A
C4	D	Inflorescence	Desejado Hill	20°30'29"S	29°19'43"W	584 m	Faria, 1572A
C5	D	Leaf	Desejado Hill	20°30'29"S	29°19'43"W	584 m	Faria, 1572A
C6	E	Inflorescence	POIT	20°30'31"S	29°18'46"W	10 m	Faria, 1622A
C7	F	Leaf	POIT	20°30'31"S	29°18'46"W	10 m	Faria, 1578A
C8	F	Root	POIT	20°30'31"S	29°18'46"W	10 m	Faria, 1578A
C9	G	Inflorescence	POIT	20°30'31"S	29°18'46"W	10 m	Faria, 1622A
C10	G	Root	POIT	20°30'31"S	29°18'46"W	10 m	Faria, 1622A
C11	H	Root	POIT	20°30'31"S	29°18'46"W	10 m	Faria, 1578A
C12	I	Leaf	Martin Vaz Island	20°28'26"S	28°51'04"W	160 m	Faria, 1604A
C13	J	Root	Martin Vaz Island	20°28'26"S	28°51'04"W	160 m	Faria, 1604A
C14	K	Root	Martin Vaz Island	20°28'26"S	28°51'04"W	160 m	Faria, 1604A
C15	L	Root	Martin Vaz Island	20°28'26"S	28°51'04"W	160 m	Faria, 1604A

Environmental DNA extraction and ITS amplification

Environmental DNA from the plant sample was extracted using the FastDNA Spin Kit for Soil (MPBIO, Ohio, USA). Extractions were carried out under strict sterile conditions to avoid contamination. The sealed bags were only opened inside a previously sterilized flow hood. DNA quality was analyzed using agarose gel electrophoresis (1% agarose in 1 × TrisborateEDTA) and then quantified using the Quanti- iT# Pico Green dsDNA Assay (Invitrogen). Extracted DNA was used as a template for generating PCR amplicons. The internal transcribed spacer 2 (ITS2) of the nuclear ribosomal DNA was used as a DNA barcode for molecular species identification (Chen *et al*., 2010). The PCR-amplicons were generated using the universal primers ITS3 and ITS4 (White *et al*., 1990). The construction of the libraries and amplification of DNA and high-performance sequencing was performed in Illumina Miseq V3 (Illumina, Inc.) at BPI (Botucatu, Brasil), where fragments of approx. 600 bp (2x300) with high quality were obtained.

Metabarcoding data analysis

Quality analysis was carried out using BBDuk v. 38.87 in BBmap software (Bushnell, 2014) with the following parameters: Illumina adapters removing (Illumina artifacts and the PhiX Control v3 Library); ktrim = l; 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 2024.5 (https:// qiime2.org/) for bioinformatics analyses (Bolyen *et al.*, 2019). The qiime2-dada2 plugin was used for filtering, dereplication, turning paired-end fastq files into merged and remove chimeras, using default parameters (Callahan *et al.*, 2016). Taxonomic assignments of ASVs were determined using the qiime2-feature-classifier (Bokulich *et al.*, 2018) classify-sklearn against different curated databases (PLANiTS2, UNITE); the sequence similarity threshold was 99%. For ITS2, firstly, ASVs were classified against the PLANiTS2 database (Banchi *et al.*, 2020). After this step, ASVs that remained unclassified were filtered and classify-sklearn classified against the UNITE Eukaryotes ITS database version 10.0 (Abarenkov *et al.*, 2025). Finally, the remaining unclassified ASVs were filtered and aligned against the filtered NCBI non-redundant nucleotide sequences (nt) database (February 2025) using Vsearch with default parameters; the nt database was filtered with the following keywords: “ITS1”, “ITS2”, “Internal transcribed spacer”, and “internal transcribed spacer”. Sequences have been submitted to GenBank under the accession numbers XXXXXX.

For simplicity, we henceforth refer to the assigned ASVs as “taxa”. For comparative purposes, we used the number of reads as a proxy for relative abundance (Deiner *et al.*, 2017; Hering *et al.*, 2018). Species accumulation curves were obtained using the Mao Tao index. All results were obtained with 95% confidence, and bootstrap values were calculated from 1,000 replicates using PAST 5.2 (Hammer *et al.*, 2001a) Venn diagrams followed Heberle *et al.*, (2015). Information about distribution and habitat was obtained from GBIF (https://www.gbif.org/), GlobalFungi (Vetrovsky *et al.*, 2020), AlgalBase (Guiry, M.G., 2025) and relevant studies in the literature. For Fungi, databases FungalTraits and FUNGuild were also consulted (Põlme *et al.*, 2020; Nguyen *et al.*, 2016).

To perform diversity analysis, the assigned ASVs were filtered, excluding sequences of host species, known contaminants and sequences that were not assigned above phylum level. The number of DNA reads and relative abundances of the ASVs were used to quantify the taxa present in the samples. ASVs with relative abundance > 1% were considered dominant and those < 1% as minor (rare) components of the community. The relative abundances were used to quantify taxon diversity, richness and dominance, using the following indices: taxon diversity, richness and dominance, using the following indices: (i) Fisher's α, (ii) Margalef's and (iii) Simpson's, respectively. Alpha diversity indices were calculated from ASV abundance tables in R (v. 2026.01.0+392) using the vegan package (Oksanen *et al.*, 2001).

Results

A total of 888,736 reads were generated, of which 665,052 (75%) remained after quality filtering (Table 2). These were assigned to 411 amplicon sequence variants (ASVs) (Suppl. Table 1).

Table 2. Number of sequences present in samples obtained from *Cyperus appendiculatus* var. *atlanticus* from Trindade and Martin Vaz islands, after quality filtering and classified by each database.

Samples	Total
Raw sequences	888,736
Cleaned and filtered	665,052
UNITE	302,137
PLANiTS2	40,241
GenBank	322,674
Unassigned	31,184

After filtering the resulting ASVs by removing known contaminants, sequences of host species and sequences not assigned above phylum level, sequences representative of 382 taxa remained. These represented four Eukaryote kingdoms, of which Fungi included six phyla and 366 taxa, Metazoa one phylum and one taxon, Viridiplantae two phyla and 14 taxa and the clade Stramenopila one taxon (Figure 2). One hundred seventy-five (45,8%) ASVs were identified to species-level and forty-tree (11,2%) ASVs could only be identified to higher taxonomic levels (phylum, class, order and family).

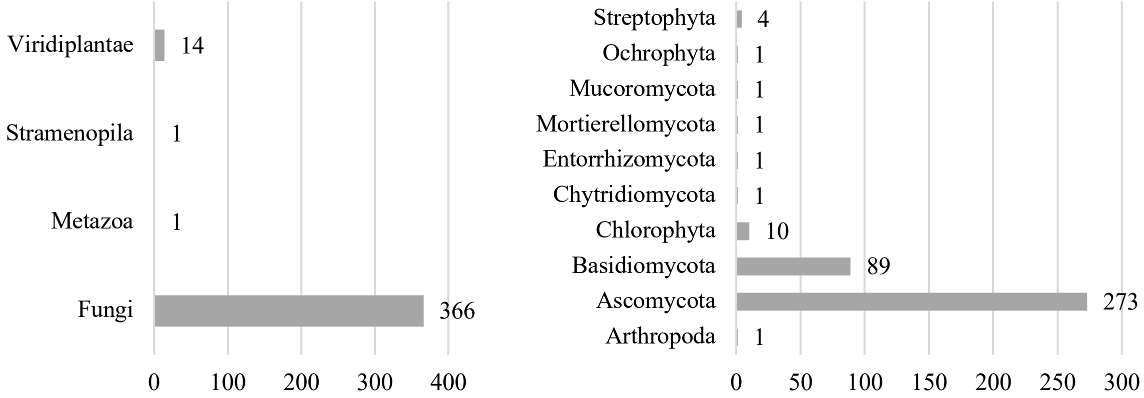

Figure 2. Number of amplicon sequence variants (ASVs) at kingdom (left) and phylum (right) level found associated with *Cyperus appendiculatus* var. *atlanticus* from Trindade and Martin Vaz islands. Taxonomy classification follows (Tedersoo *et al*., 2018).

Twenty-three taxa were classified as dominant (relative abundance ≥ 1%): *Xylodon rhizomorphus*, *Aureobasidium* sp., *Pleosporales* sp., *Aureobasidium tremulum, Neopestalotiopsis* sp., *Papiliotrema terrestres*, *Cladosporium* sp., *Naganishia* sp., *Preussia* sp., *Mycosphaerella* sp., *Talaromyces* sp., *Rhodosporidiobolus lusitaniae, Peniophorella* sp., *Phoma* sp., *Trechispora* sp., *Taphrina deformans*, *Ascomycota*, *Sphaerobolaceae* sp., *Hortaea werneckii*, *Rigidoporus* sp., *Pseudozyma hubeiensis*, *Teratosphaeria syncarpiae* and *Chaetomiaceae* sp. A further 360 ASVs formed rare components of the assigned communities.

Non-fungal diversity compromised sixteen ASVs from three kingdoms (Viridiplantae, Metazoa and Stramenopila) and four phyla (*Chlorophyta*, *Streptophyta*, *Ochrophyta* and *Arthropoda*). Chlorophyta was the most diverse group with 10 ASVs: *Bracteacoccus deserticola*, *Chlamydomonas pygmaea*, *Ignatius tetrasporus*, *Stichococcus* sp., *Tetracystis* sp. present in POIT samples, *Phycopeltis epiphyton*, *Planophila* sp., *Symbiochloris* sp. present in Desejado Hill, and *Myrmecia* sp. present in all three sites. Among Streptophyta, three Lamiales representants were detected - *Eremophila* sp., *Scrophulariaceae* sp. and Lamiales sp. – and one Bryopsida, *Sarmentypnum* sp. Sequences of *Drosophila* sp. (Metazoa, *Arthropoda*) were detected only on one inflorescence sample from Desejado Hill. The diatom *Haslea* sp. (Stramenopila, *Ochrophyta*) was found at all three sites, though with very few reads.

The calculated rarefaction curves reached asymptotes for all assemblages from the three sampling sites, indicating that most of the diversity present was detected (Figure 3). The assemblages displayed high diversity indices, although varying between the samples (Table 3).

Regarding differences across sampling sites, the highest number of ASVs, diversity (Fisher's α), richness (Margalef) and dominance (Simpson's) were detected in the assemblages from Desejado Hill, although it was the site with the least number of DNA reads. POIT generated the highest number of DNA reads but showed the lowest values of diversity (Fisher's α) and richness (Margalef). Among the 382 ASVs assigned, 24 were found in all sampling sites (Fig. 4). Concerning sample types, the number of DNA reads was similar for the three types. However, the leaves generated the highest number of ASVs, Fisher's α, Margalef and Simpson indices, while the inflorescence generated the lowest number of ASVs, Fisher's α and Margalef indices. Among all assigned ASVs, 34 taxa were detected across the three sample types.

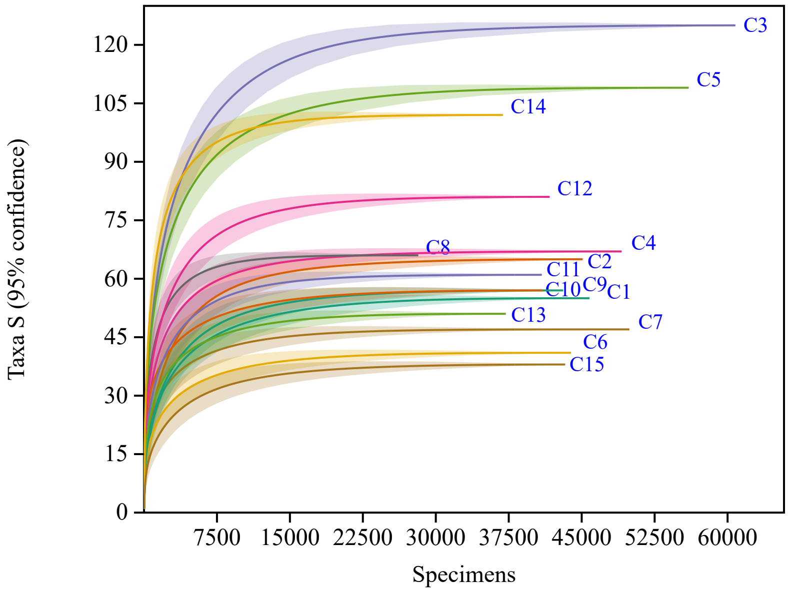

Figure 3. Rarefaction curves obtained from each sampling site**.**

Table 3. Average number of DNA reads, ASVs and diversity indices of assemblages detected in plant samples of *C. appendiculatus* from Trindade and Martin Vaz islands, grouped by sampling location and sample type (n = number of samples).

	Desejado Hill	POIT	Martin Vaz Island	Inflorescence	Leaves	Roots
n	5	6	4	5	4	6
Number of DNA reads	16,709	31,319	30,671	26,907	25,648	26,169
Number of assigned ASVs	72.8	45.8	56	48	77.8	52
Fisher's α	10	5.34	6.97	5.86	10.47	6.46
Margalef	7.43	4.36	5.43	4.7	7.75	5.09
Simpson	0.79	0.74	0.64	0.74	0.78	0.68
Shannon	2.28	1.75	1.77	1.79	2.24	1.85

Location had a stronger effect on assemblage composition, as evidenced by the higher number of site-exclusive taxa compared to those shared across locations. (Fig. 4). Desejado Hill was the location with the most taxa assigned (194), followed by POIT (160) and Martin Vaz Island (136).

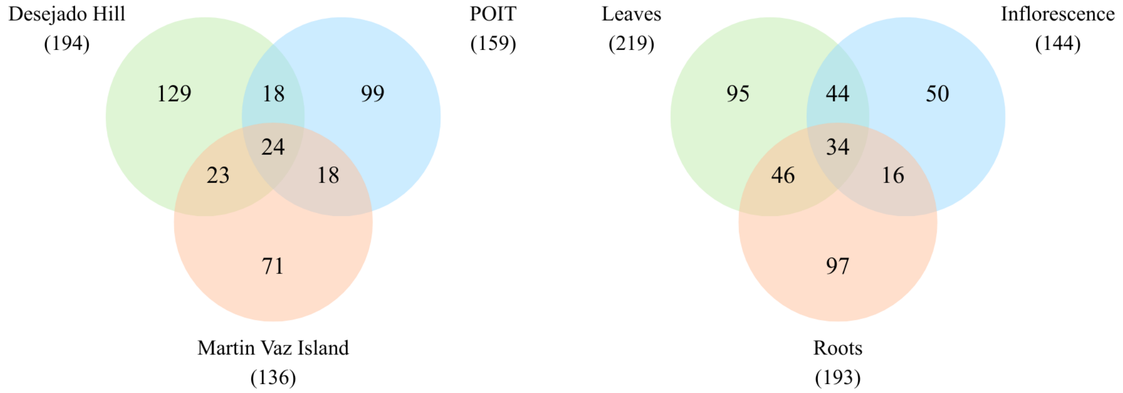

Figure 4. Venn diagram illustrating number of assigned taxa found associated to *C. appendiculatus* across the different sampling sites (left) and number of assigned taxa associated with leaves, inflorescence and roots of *C. appendiculatus* var. *atlanticus* (right).

Discussion

The eukaryotic communities associated with *C. appendiculatus* var. *atlanticus* on the Trindade and Martin Vaz islands revealed unexpectedly high diversity, encompassing nine phyla detected using a single molecular marker (ITS2). These findings emphasize the ecological complexity associated with this common endemic plant species. Despite the high taxonomic richness, it is important to acknowledge that detecting an organism's DNA does not confirm the presence of active organisms or viable propagules. Such findings can be related to encysted forms, spores, pollen or even single cells and dead tissue. Sequence assignment also relies on the quality and completeness of data available in existing reference databases, which contributes to the presence of unassigned or ambiguously classified ASVs.

Fungi dominated the assemblages, represented by six phyla and 366 taxa. *Ascomycota* was the dominant phylum, followed by *Basidiomycota*. This finding is consistent with their central ecological role in plant-associated environments (Bonfante *et al*., 2019).

Representatives from genera known to include potential animal pathogens were detected: *Aspergillus, Cyphellophora, Exophiala, Malassezia, Penicillium, Pseudozyma, Talaromyces, Trichoderma, Aureobasidium, Alternaria* and *Fusarium* (Nguyen *et al*., 2016); as well as potential plant pathogens: *Alternaria, Botryosphaeria, Capnodium, Cladosporium, Colletotrichum, Diaporthe, Exobasidium, Fusarium, Mycosphaerella, Penicillium, Phaeosphaeria, Pyrenochaeta, Stemphylium, Taphrina, Teratosphaeria, Aspergillus, Nectria, Aureobasidium, Cadophora, Trichoderma, Ilyonectria, Neofusicoccum* and *Nigrospora* (Nguyen *et al*., 2016). This suggests that *C. appendiculatus* may act as a reservoir or transient substrate for a wide range of fungal taxa.

Only seven species overlapped with fungal communities previously reported from soil samples in the region (Câmara *et al*. 2022): *Fusarium oxysporum, Malassezia restricta, Mrakia frigida, Papiliotrema flavescens, Rhodotorula mucilaginosa, Saitozyma podzolica* and *Vishniacozyma victoriae*. This limited overlap suggests that plant tissues host specialized communities rather than simply reflecting the surrounding soil microbiota.

Representatives of non-Dikarya fungi were detected: *Entorrhiza* sp. (*Entorrhizomycota*), *Gongronella* sp. (*Mucoromycota*), *Mortierella* sp. (*Mortierellomycota*) and *Rhizophlyctis rosea* (*Chytridiomycota*). These lineages represent cryptic phyla often overlooked by culture-dependent methods. *Entorrhiza* species are associated with host plants in Cyperaceae, and are characterized by obligate biotrophy, a hypogeous lifestyle and spore production within living root cells (Riess *et al*., 2019). *Mortierella* species have been reported as important members of the soil core microbial community (Tedersoo *et al*. 2014, Zhang *et al*. 2019). *Gongronella* fungi are known to produce a variety of bioactive metabolites, while *Rhizophlyctis rosea*, a common soil chytrid, produces a diverse range of enzymes especially effective to degrade plant biomass (Gleason *et al*., 2019).

All ten green algae (Chlorophyta) and the diatom *□asle asp*. (Stramenopila, *Ochrophyta*) identified in this study have not previously been recorded from Trindade Island (GBIF, 2026; Guiry, M.G., 2025). All assigned Chlorophyta are either terrestrial or aeroterrestrial and *Bracteacoccus deserticola*, *Stichococcus* sp., *Symbiochloris* sp. and *Myrmecia* sp. are lichen photobionts (Guiry, M.G., 2025; Veselá *et al*., 2024).

Regarding the Streptophyta detected, sequences assigned to *Scrophulariaceae* sp. and Lamiales sp. were recovered from samples collected at Desejado Hill and POIT, whereas sequences of *Eremophila* sp. were detected at Desejado Hill and Martin Vaz Island. Alves

(1998) reported the occurrence of one *Scrophulariaceae* species (*Verbascum virgatum*) on Trindade Island, but this taxon is no longer recorded in the local flora. The moss *Sarmentypnum* sp. was assigned in samples from Desejado Hill and Martin Vaz Island, but there are no records for this genus or the family *Amblystegiaceae* in the area (Faria *et al.*, 2012).

The only Metazoa assigned in this study was a *Drosophila* sp., detected on one inflorescence sample from Desejado Hill.

Community composition varied across sampling locations, with location exerting a stronger influence than plant tissue type. Unexpectedly, the leaves harbored more taxa than the roots, although the aerial surface of plants is relatively nutrient poor and experiences much more dynamic conditions, with temperature, moisture, and radiation fluctuating throughout the day and night (Turner 2013).

Desejado Hill had the highest richness and diversity indices likely due to more favorable environmental conditions compared to the other two sites. Located in the "giant fern forest", Desejado Hill provides increased shade and moisture, which may promote the establishment and maintenance of plant-associated assemblages. In contrast, assemblages from POIT displayed the lowest diversity indices, probably reflecting the stronger anthropogenic impact in this area. Martin Vaz Island represents the most isolated and pristine sampling site, accessible only by helicopter. Due to its small area of only 0,3 km^2, low richness was expected according to island biogeography theory (Connor & McCoy, 1979). Despite this, Martin Vaz Island exhibited higher diversity indices than POIT, suggesting that reduced human disturbance may play a more important role than island size in structuring these communities.

Most of the identified diversity represented fungal taxa, and the use of additional and more specific molecular markers in future investigations is likely to reveal even greater diversity in other eukaryotic groups. However, it is important to emphasize that the assignment of putative taxonomic identities based on environmental DNA does not confirm the presence of active organisms or viable propagules. Moreover, the accuracy of these identifications is strongly dependent on the quality and completeness of reference databases.

Overall, this study demonstrates that *C. appendiculatus* var. *atlanticus* supports a diverse assemblage of eukaryotes, including cryptic and poorly documented taxa. The associated communities varied among sampling sites and plant tissues, indicating spatial and environmental influences on community composition. These findings reinforce the importance of oceanic islands as reservoirs of hidden biodiversity and highlight the need for further integrative studies combining metabarcoding, functional analyses, and cultivation-based approaches to better elucidate the ecological roles and biogeographical origins of these communities.

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DISCUSSÃO GERAL

Este estudo fornece uma caracterização das comunidades eucarióticas associadas às plantas em dois dos sistemas insulares oceânicos mais remotos do Oceano Atlântico: o Arquipélago de São Pedro e São Paulo (ASPSP) e o complexo insular Trindade-Martin Vaz (TMV). Por meio da aplicação de DNA *metabarcoding*, os resultados revelaram uma ampla diversidade de eucariotos, destacando as ilhas oceânicas como reservatórios de linhagens microbianas crípticas e reforçando a importância de incorporar perspectivas microbianas aos estudos de biogeografia insular e conservação.

As análises filogenéticas e morfológicas confirmaram que a Cyperaceae presente no ASPSP corresponde à espécie *Fimbristylis cymosa*, uma espécie amplamente distribuída nos trópicos, presente em todo o litoral brasileiro e em outras ilhas oceânicas como Fernando de Noronha. No entanto, sua chegada ao arquipélago permanece duvidosa, embora relatos de pescadores da região sugiram uma introdução antrópica acidental por meio da areia utilizada na construção da última estação científica.

Os resultados das análises de DNA ambiental revelam que as comunidades associadas a *F. cymosa* abrangeram sete filos e 89 ASVs, enquanto em TMV, as comunidades associadas a *Cyperus appendiculatus* var. *atlanticus* representam 411 ASVs distribuídos em nove filos e quatro reinos, todos detectados a partir de um único marcador molecular (ITS2). Tanto no ASPSP e TMV, os fungos dominaram em riqueza e abundância, com *Ascomycota* e *Basidiomycota* representando os filos mais diversos.

Até o momento, o reino Fungi na Ilha da Trindade era representado por apenas 29 espécies baseadas em estudos de espécimes (Rezende *et al.*, 2023). Com o uso de DNA *metabarcoding*, cinco filos previamente não reportados e mais 247 táxons (113 identificadas a nível de espécie) foram identificados (Camara *et al.*, 2022).

A estrutura das comunidades variou de acordo com o tecido vegetal e a localização. No ASPSP, as raízes apresentaram maior diversidade, possivelmente refletindo condições micro ambientais mais estáveis e maior disponibilidade de nutrientes em comparação aos tecidos aéreos. Em contraste, no TMV, as folhas abrigaram, de forma inesperada, maior riqueza de táxons do que as raízes, apesar da filosfera ser um ambiente relativamente pobre em nutrientes e sujeito a intensas variações de temperatura, umidade e radiação (Turner, 2013).

Em Trindade, Martin Vaz e ASPSP as comunidades associadas às plantas foram distintas da microbiota do solo analisado em outros estudos (Câmara *et al.*, 2022; Gonçalves *et al.*, 2025), com um número reduzido de táxons compartilhados. Esse padrão sustenta a hipótese de que as plantas exercem um papel ativo na seleção ou filtragem de seus organismos associados. Tal seleção mediada pelo hospedeiro pode ser particularmente relevante em ambientes insulares extremos, nos quais microrganismos simbióticos podem aumentar a tolerância das plantas a estresses abióticos, como salinidade, dessecação, limitação de nutrientes, elevada radiação e condições poliextremas como as do ASPSP.

Um resultado marcante comum a Trindade, Martin Vaz e ASPSP foi a detecção de táxons associados a potenciais patógenos de plantas e animais. Para ASPSP, é importante notar a detecção de *Aspergillus* e *Penicillium*, diante de registros prévios de infecções fúngicas em aves marinhas reprodutoras do arquipélago, ressaltando a necessidade de monitoramento e cautela em um local constantemente ocupado por humanos.

Também foram encontradas linhagens fúngicas extremófilas e crípticas. A detecção de fungos halotolerantes e xerofílicos, como *Hortaea werneckii* e espécies de *Wallemia* no ASPSP, é consistente com os elevados níveis de salinidade e as condições ambientais adversas do arquipélago. Da mesma forma, as plantas hospedeiras *C. appendiculatus* var. *atlanticus* e *F. cymosa* são tolerantes a ambientes halófilos. A presença de fungos não-Dikarya, como *Entorrhizomycota*, *Mortierellomycota*, *Mucoromycota* e *Chytridiomycota*, destaca o potencial do *metabarcoding* para revelar linhagens raramente detectadas por métodos dependentes de cultivo. Alguns táxons apresentaram padrões biogeográficos distintos, incluindo espécies anteriormente consideradas restritas à Antártica ou a outras regiões distantes, sugerindo distribuições mais amplas possivelmente facilitadas por vetores de dispersão de longa distância, como ventos e aves migratórias.

A detecção de diferentes táxons de Chlorophyta associada às raízes de *F. cymosa* amplia ainda mais o espectro de eucariotos associados às plantas nesses sistemas. A ausência de algas nas amostras de folhas sugere que a umidade e o sombreamento proporcionados pelo solo podem ser essenciais para o estabelecimento desses organismos, especialmente sob as condições de intensa radiação e dessecação do ASPSP.

Embora a técnica de DNA *metabarcoding* tenha permitido a detecção de uma ampla diversidade de eucariotos, é importante ressaltar suas limitações. A presença de sequencias de DNA não permite distinguir organismos ativos de propágulos dormentes ou não viáveis, e a resolução taxonômica depende da completude e qualidade das bases de dados. Consequentemente, parte das ASVs detectadas pode representar táxons ainda não descritos ou sem sequências de referência disponíveis. A integração de abordagens de metabarcoding com métodos de cultivo, análises de metatranscriptômica e experimentais podem ajudar a melhor revelar o funcionamento dessas comunidades e como elas interagem com suas plantas hospedeiras.

CONCLUSÃO

Estudos com metabarcoding em ambientes isolados e extremos são importantes meios para investigar a diversidade biológica desses locais. Este trabalho é o primeiro a investigar comunidades de organismos eucariotos encontradas junto a plantas no ASPSP e no TMV. Em síntese, este estudo demonstra que espécies de Cyperaceae presentes em ilhas oceânicas remotas do Atlântico hospedam comunidades eucarióticas diversas e ecologicamente complexas, que não refletem simplesmente a microbiota do solo adjacente. Ao revelar diversidade críptica, organismos extremófilos e táxons potencialmente patogênicos, estes resultados reforçam a importância da microbiota associada às plantas nos ecossistemas insulares e destacam o potencial das ilhas oceânicas para o avanço do conhecimento em biogeografia microbiana, interações hospedeiro–microrganismo e conservação em ambientes extremos.

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