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Original Article

Hidden biodiversity associated with *Pyrrhobryum spiniforme* (Hedw.) Mitt (Bryophyta, Rhizogoniaceae) from an Atlantic Rain Forest fragment in Brazil, a molecular approach

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ABSTRACT

The Brazilian Atlantic Rainforest is amongst the most threatened ecosystems on the planet, with only about 7.5 % of its original vegetation left. It is highly fragmented and with less than 50 % of its remnants protected in Protected Areas. This highly threatened biome houses a species diversity higher than the Amazon, and its high level of diversity and endemism place this forest among the top biodiversity hotspots. Mosses are known to house many other species that seek shelter in their carpets and tuffs. This diverse community associated with mosses is sometimes referred to as the Bryosphere, and studies focusing on this cryptic community are still rare. A tool that allows a fast diversity survey, especially for neglected groups, is paramount in times when the deforestation rates are ever-increasing. In this study, we applied DNA metabarcoding for the first time to investigate cryptic diversity in assemblages present in moss carpets of *Pyrrhobryum spiniforme* (Hedw.) Mitt in one Atlantic Rainforest fragment in Santa Catarina state, Brazil. With the use of two markers (16S and ITS2), we found DNA from 606 taxa from four kingdoms and nine phyla.

KEYWORDS: environmental DNA, eukaryote, metabarcoding, prokaryote, subtropical, Santa Catarina

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Introduction

Originally with about 1.3 million km², the Brazilian Atlantic Rainforest is amongst the most threatened ecosystems on the planet (Colombo and Joly 2010). Nowadays, the estimate is that only about 7.5 % of the original Atlantic Forest is left, highly fragmented, and less than 50 % of those fragments' remnants are protected in Conservation Units (Ribeiro *et al.*, 2009; Roder *et al.*, 2023). The Brazilian Coast is a heavily populated area, and for this reason, its rainforests are under severe pressure (Costa *et al.*, 2011). This still poorly known and highly threatened biome houses a species diversity higher than the Amazon, especially for bryophytes (Costa *et al.*, 2011; Flora e Funga do Brasil, 2024). Its high level of diversity and endemism make this forest among the top biodiversity hotspots (Myers *et al.*, 2000). This highly threatened biome is not only menaced by its drastic area reduction but also by climatic changes (Colombo & Joly, 2010); last but not least, the existing laws are not so effective in protecting the ecosystem (Varjabedian, 2010; Meira *et al.*, 2016).

Costa *et al.* (2011) listed 892 Bryophyta (moss) species in Brazil, from which about 700 are from the Atlantic Rainforest, the richest bryophyte domain of Brazil, housing about 80 % of Brazilian moss species with levels of endemism as high as 20 % (Costa *et al.*, 2011; Flora e Funga do Brasil, 2024). This biome is also highly relevant in the Neotropics, being, after the Andes and Central America, the third in species richness of bryophytes (Gradstein *et al.*, 2001). Santa Catarina is a state in southern Brazil inserted in the Atlantic Rainforest biome, housing 340 moss species, about 48 % of the biome and 38 % of the country moss species (Costa *et al.*, 2011; Flora e Funga do Brasil, 2024), however, the state remains poorly collected, and bryophyte studies are still scarce (Costa, 2009; Remor *et al.*, 2021).

Mosses are known to house many other species that seek shelter in carpets and tuffs formed by many species (Glime, 2017). Organisms usually hard to see and understudied, such as micro-invertebrates, micro-arthropods, algae, fungi, bacteria, and protozoans, are known to inhabit moss carpets (Glime, 2017; Câmara *et al.*, 2021a). This diverse community associated with mosses is sometimes referred to as the Bryosphere (Lindo & Gonzalez, 2010). However, there are very few studies focusing on understanding this cryptic community associated and community characterizations are still rare (Richard *et al.*, 1994). Câmara *et al.* (2021a) used for the first time molecular tools to survey for cryptic diversity associated with moss carpets in Antarctica and found 263 taxa in five kingdoms and 33 phyla living on a small moss carpet fragment in one of the most extreme environments on Earth. For the highly diverse Brazilian Atlantic Rainforest, there is no data on such communities, and virtually nothing is known about cryptic diversity associated with moss carpets.

Recent developments in molecular biology have allowed considerable advances in the assessment of molecular diversity in environmental samples. DNA metabarcoding by high-throughput sequencing (HTS) represents a new and efficient method for the detection of DNA from rare species (Rippin *et al.*, 2018; Ruppert *et al.*,2019; Câmara *et al.*, 2021a;b), including spores and resting stages, which are typically not detected in morphological surveys. Rippin *et al.* (2018) estimated its efficiency to be about 11 times higher than a traditional morphological approach. The use of such a tool has been very successful as a survey tool and has been used by our research group investigating Antarctic diversity (Câmara *et al.*, 2021a;b; 2022a; 2023; 2024; Carvalho-Silva *et al.*, 2021) and also in the South Atlantic Trindade Island (Câmara *et al.*, 2022b). In the current study, we applied HTS for the first time to investigate cryptic diversity in assemblages present in moss carpets of *Pyrrhobryum spiniforme* (Hedw.) Mitt., a widely distributed moss species in the Atlantic Rainforest fragment in Santa Catarina state, Brazil.

Materials and Methods

Sampling and species identification

Pyrrhobryum spiniforme was chosen for being easy to find and identify, and because it forms big tuffs and consequently likely holds moisture and provides good shelter for other organisms. Identification was made using slides and a compound microscope. Collections were made in a protected area known as Refugio de Vida Silvestre Municipal Meiembipe (Wildlife Refuge of Meiembipe County) in Florianópolis, Santa Catarina state, Brazil (Fig. 1). The region has 5,972 ha, being the largest county protected area in the state, and is covered by Atlantic Rainforest (Brasil *et al.*, 2024). Three samples (tuffs) of about 3cm³, apart about 10 cm from each other, were collected under sterile conditions using sterilized gloves and sealed in sterile plastic bags (Whirl Pack®/US) and kept frozen (-20 °C) until DNA was extracted under sterile conditions at the Microbiology Biology laboratory at the University Federal de Minas Gerais. Voucher specimens of the collected moss species were deposited at the University of Brasília Herbarium (UB) under the collection number Câmara, PEAS 5055b.

DNA extraction and sequencing

Total DNA was extracted using the FastDNA Spin Kit for Soil (MPBIO, Ohio, USA), following the manufacturer's instructions. DNA quality was analyzed by agarose gel electrophoresis (1 % agarose in 1 Tris Borate-EDTA) and then quantified using the Quant-iT ™ PicoGreen dsDNA Assay gen). We selected the internal transcribed spacer 2 (ITS2) of the nuclear ribosomal DNA (Chen *et al.*, 2010; Richardson *et al.*, 2015; Câmara *et al.*, 2022a;b) as barcode, as it has been widely used to identify a diverse range of eukaryotic organisms including fungi, animals, protozoans, chromists, and plants (Ruppert *et al.*, 2019), and has proved effective in recent studies of bryosphere diversity by our group (Câmara *et al.*, 2021a; Rosa *et al.*, 2020; Ogaki *et al.*, 2021; Carvalho-Silva *et al.*, 2021; Câmara *et al.*, 2022a,2022b; 2023; 2024). For Bacteria and Archaea, we used the 16S rRNA gene V3-V4 region (Herlemann *et al.*, 2011), also used with success by our group (Câmara *et al.*, 2021a; b; Câmara *et al.*, 2022b). PCR-amplicons were generated using the primers by White *et al.* (1990) and Klindworth *et al.* (2013), and were sequenced commercially using high-throughput sequencing by Macrogen Inc. (South Korea) on an Illumina MiSeq sequencer (3×300 bp).

All raw sequence data generated in this study have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject PRJNA1312491, titled 'Hidden biodiversity associated with Pyrrhobryum spiniforme from an Atlantic Rain Forest, Brazil'. The data, corresponding to three biological replicates, are accessible under the following individual sample accession numbers: SAMN50872868, SAMN50872869, and SAMN50872870 for the ITS amplicon sequences, and SAMN50872936, SAMN50872937, and SAMN50872938 for the 16S amplicon sequences. This deposition ensures data availability and facilitates the reproducibility and verifiability of the study's findings.

Data analyses and taxa identification

Quality analysis was carried out using BBDuk v. 38.87 in BBmap software (Bushnell, 2014) with the following parameters: Illumina adapters removed (Illumina artifacts and the PhiX Control v3 Library); ktrim ¼ l; k ¼ 23; mink ¼ 11; hdist ¼ 1; minlen ¼ 50; tpe; tbo; qtrim ¼ rl; trimq ¼ 20; ftm ¼ 5; maq ¼ 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, turning paired-end fastq files into merged and removing 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 databases (SILVA, PLANiTS2, UNITE); the sequence similarity threshold was 97 %. 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 8.3 (Abarenkov *et al.*, 2020). Finally, the remaining unclassified ASVs were filtered and aligned against the filtered NCBI non-redundant nucleotide sequences (nt) database (October 2021) 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". Taxonomic assignments were performed using MEGAN6 (Huson *et al.*, 2016). For simplicity, we henceforth refer to the assigned ASVs as "taxa". For comparative purposes, we consider reads as a proxy for abundance (Deiner *et al.*, 2017; Hering *et al.*, 2018; Câmara *et al.*, 2021a; b; 2022a; b; Rosa *et al.*, 2020; Carvalho-Silva *et al.*, 2021). Rarefaction curves were generated using the software PAST 3.26 (Hammer *et al.*, 2001).

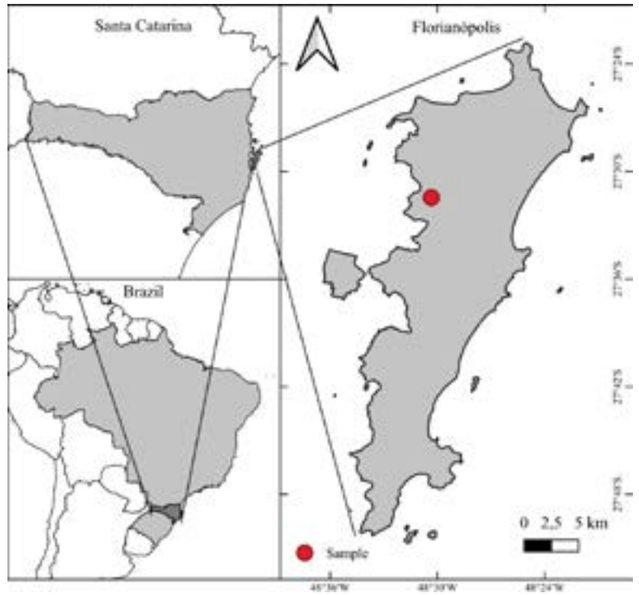

Figure 1. Map showing the sampling site for collecting moss species *Pyrrhobryum spiniforme* (Hedw.) Mitt (Bryophyta, Rhizogoniaceae), from an Atlantic Rain Forest fragment in Florianópolis, Santa Catarina, Brazil.

Results

For ITS2, 286,416 sequences were generated, with 245,709 sequences remaining after filtering. Among the filtered sequences, 110,953 were classified as non-fungal eukaryotes, corresponding to 48 taxa from four kingdoms, 31 Viridiplantae (Fig. 2), four Metazoa, 14 Chromista and Protozoa (Fig. 3), and nine phyla: Ciliophora, Cercozoa, Chlorophyta, Marchantiophyta, Bryophyta, Magnoliophyta, Arthropoda, Nematoda, and Rotifera (Suppl. Table 1). In contrast, 135,141 sequences were identified as fungi, representing 240 taxa across multiple phyla, including Ascomycota, Basidiomycota, Chytridiomycota, Mortierellomycota, Mucoromycota, Olpidiomycota, Rozellomycota, Blastocladiomycota, and Zoopagomycota (Fig. 4) (Suppl. Table 2).

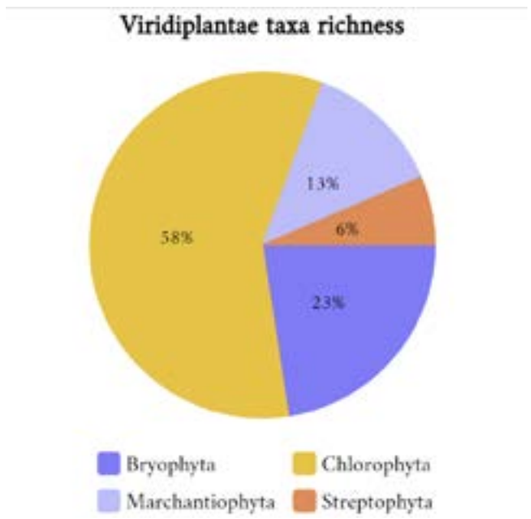

Figure 2. Taxa richness of Viridiplantae (ITS2) sequences from samples of *Pyrrhobryum spiniforme* (Hedw.) Mitt (Bryophyta, Rhizogoniaceae) mats, collected in an Atlantic Rainforest fragment, Florianópolis, Santa Catarina, Brazil.

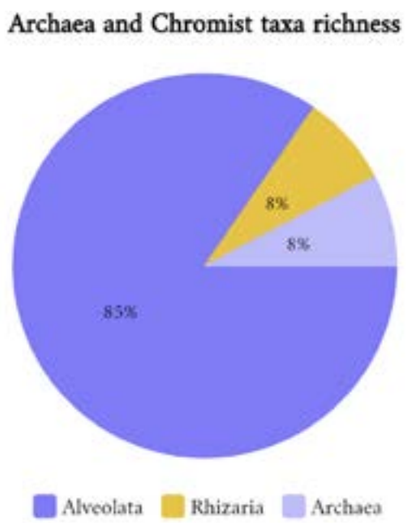

Figure 3. Taxa richness of Archaea and Chromists sequences (ITS2) from samples of *Pyrrhobryum spiniforme* (Hedw.) Mitt (Bryophyta, Rhizogoniaceae) mats, collected in an Atlantic Rainforest fragment, Florianópolis, Santa Catarina, Brazil.

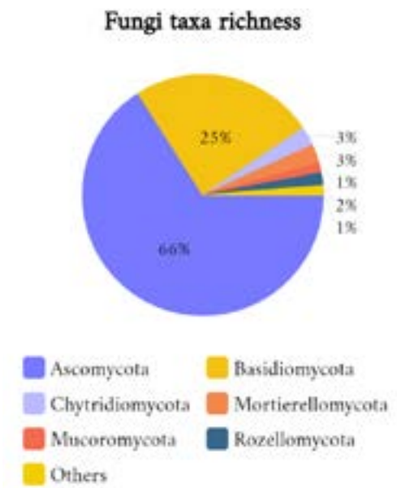

Figure 4. Taxa richness of Fungi sequences (ITS2) from samples of *Pyrrhobryum spiniforme* (Hedw.) Mitt (Bryophyta, Rhizogoniaceae) mats, collected in an Atlantic Rainforest fragment, Florianópolis, Santa Catarina, Brazil.

Concerning the 16S, a total of 342,750 sequences were generated, and 249,869 remained after filtering, corresponding to 558 taxa in 31 phyla, 80 classes, 169 orders, 244 families, and 263 genera (Suppl. Table 3). Sequences belonging to chloroplasts were removed and analyzed separately. Among the 32 observed phyla, four contained more than 70 % of all reads. The phylum Proteobacteria was the most abundant, followed by Actinobacteria, Bacteroidota, and Acidobacteriota (Fig. 5). One of the replicas exhibited 12 reads (less than 0.01 % of all ASVs) belonging to the same ASV assigned to the family Methanomassiliicoccaceae, a methanogenic Archaea belonging to the phylum Thermoplasmatota. The chloroplast sequences belonged to algae and could be assigned to either Viridiplantae (phylum Chlorophyta, genera *Jenufa*, *Watanabea*, and uncultured chlorophyta) or to Chromista (phylum Ochrophyta, genus *Vischeria*).

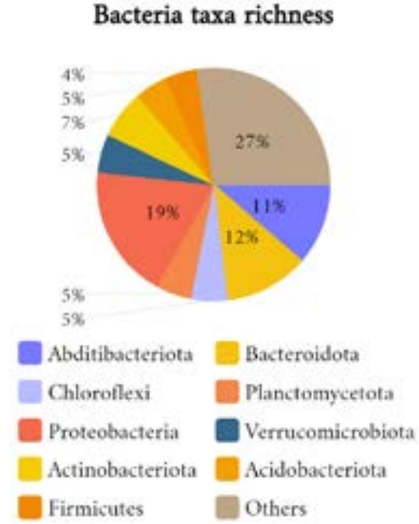

Figure 5. Taxa richness of Bacteria sequences (16S) from samples of *Pyrrhobryum spiniforme* (Hedw.) Mitt (Bryophyta, Rhizogoniaceae) mats, collected in an Atlantic Rainforest fragment, Florianópolis, Santa Catarina, Brazil.

Discussion

Data generated by metabarcoding studies are highly dependent on the quality of the consulted databases. It is known that such databases increase on an everyday basis, but still, the new records should be taken carefully, as many Brazilian taxa may be absent from such databases, which may cause a hit on a closer relative present. The same applies to the unknown taxa reported here, as they could either represent taxa not present in databases or undescribed taxa new to science. On the other hand, organisms may have much wider distributions than previously considered, especially poorly known ones like some protozoans and microalgae. Data obtained by Câmara *et al.* (2023), when sampling across a 40º latitudinal gradient, from Rio de Janeiro (Brazil) all the way to King George Island in Antarctica, suggests that diaspores do travel long distances and may be responsible for some odd records found here (see also Gama *et al.*, 2016; Mota de Oliveira *et al.*, 2022; Câmara *et al.*, 2024). It is also important to notice that we are reporting the presence of DNA and not necessarily the presence of a viable organism; dead cells and pollen do contain DNA, but are not going to become a new viable organism.

Concerning Prokaryotes is of notice that the bacterial taxa assemblage found in *P. spiniforme* shows high taxonomic richness and metabolic potential, indicating the presence of bacteria involved in carbon, nitrogen, and sulfur cycles. Cyanobacteria present in the assemblage can perform oxygenic photosynthesis, and some of the identified genera belong to nitrogen fixers, such as *Nostoc* Vaucher ex Bornet & Flahault, 1886, *Leptolyngbya* Anagnostidis & Komárek, 1988, and *Cyanothece* Komárek, 1976. Among the genera with higher relative abundance, there are the heterotrophic acidophiles, such as *Acidiphilium* Harrison 1981(Proteobacteria), an "uncultured Acidobacteriaceae" (Acidobacteria), and the genus *Bryobacter* Kulichevskaya et al. 2010, which was isolated from *Sphagnum* L. peat bog (Kulichevskaya *et al.*, 2010). The assemblage also contains the methanotrophic genus *Roseiarcus* Kulichevskaya et al. 2014 (Proteobacteria) and methanogenic archaea belonging to the family Methanomassiliicoccaceae, suggesting the occurrence of methane cycling in this environment. Methanomassiliicoccaceae belongs to the phylum Thermoplasmatales and can be found in peat bogs and sewage sludge (Ino *et al.*, 2013; Weil *et al.*, 2021). Sulfur oxidizers can also be identified, and sequences belonging to the bacteria *Rhodoferax* Hiraishi et al. 1992 were detected. This genus is commonly found in stagnant water, and some of the species are also photoheterotrophic bacteria (Jin *et al.*, 2020; Latysheva *et al.*, 2012).

Concerning Fungi, the most dominant fungal sequences assigned in association with *P. spiniforme* clumps were taxa identified at high taxonomic levels and with worldwide distribution. However, *Pseudogymnyoascus pannorum* (Link) Minnis & D.L.Lidner, *Mortierella* sp., *Massaria* sp., *Lachnellula* sp., *Ceratobasidium* sp., and *Mycena* sp. were identified at least at the genus level. The genus *Pseudogymnoascus* Raillo, Zentralbl (1929) (anamorphic form of *Geomyces*) is frequently found in soils from Arctic, alpine, temperate, and Antarctic regions (Mercantini *et al.*, 1989; Lorch *et al.*, 2013; Minnis & Lindner, 2013; Rosa *et al.*, 2020). *Pseudogymnoascus* species colonize habitats with different carbon sources and can be particularly abundant in habitats characterized by lower temperatures (Arenz & Blanchette, 2011). *Pseudogymnoascus* detection calls attention to the pathogenic species for bats, *P. destructans* (Blehert & Gargas) Minnis & D.L. Lindner, that kill bats, causing the white-nose syndrome (WNS) in bats in temperate regions (Lorch *et al.*, 2011). In tropical environments, Camara *et al.* (2022b) detected the DNA of *Pseudogymnoascus* in forest soils on the isolated Brazilian Trindade Island, South Atlantic. *Mortierella* Coem., Bull (1863) includes about 100 species (Wagner *et al.*, 2013), which often occur in different soil types (Kirk *et al.*, 2008). Despite its occurrence in cold environments, Camara *et al.* (2022a) detected the DNA of the *Mortierella* genus in tropical soil on Trindade Island, Brazil.

Massaria De Not. 1844 (*Massariaceae*, *Ascomycota*) includes 20 known species and displays worldwide distribution (Kirk *et al.*, 2008), with occurrences confined to the northern temperate climate zone, but also detected in Asia (Voglmayr & Jaklitsch, 2011). According to Voglmayr and Jaklitsch (2011), *Massaria* species are majoritarian decomposers and display weak pathogenicity or opportunistic growth. *Lachnellula* (Lachnaceae, Ascomycota) includes 40 known species with worldwide occurrence, mainly in temperate environments (Kirk *et al.*, 2008). According to Oguchi (1981), *Lachnellula* P. Karst. 1884 can shelter species that cause cancer disease on various conifers. *Ceratobasidium* D.P. Rogers 1935 (Cantharellales, Basidomycota) comprises 18 species reported as saprotrophic and endophyte-orchids ecological role, but includes also facultative plant pathogens (anamorphic form *Rhizoctonia* DC.1815) able to attack commercially important crops (Kirk *et al.*, 2008). *Mycena* (Pers.) Roussel (1806) (Agaricales, Basidiomycota) represents a large fungal genus with about 500 species known and worldwide distribution (Kirk *et al.*, 2008). *Mycena* includes species with a strong saprotrophic ecological role in the environment, but also representatives reported as plant pathogens have been discovered (Desjardin et al., 2008). Furthermore, few taxa are able to form ectomycorrhizal relationships with a host (Thoen *et al.*, 2020).

Concerning the Viridiplantae, all green algae listed here are widespread in freshwater, but none have been cited for Santa Catarina yet. *Chlamydomonas* Ehrenberg, 1833 is a huge genus with more than 500 species worldwide (Bicudo & Menezes, 2017), with 14 species listed in Brazil, but *C. leiostraca* (Strehlow) H.Ettl is not one of them. *Chloromonas* Gobi 1899-1900 is a widespread genus with about 150 species, including two in Brazil (*C. frigida* (Skuja) Gerloff & Ettl and *C. pumilo* Ettl). *Chloromonas fonticola* (R.Brabez) Gerloff & Ettl has not been previously cited for Brazil. *Apatococcus* F.Brand, 1925 is a genus of seven widespread species not previously recorded for Brazil. *Elliptochloris perforata* Hoffmann & Kostikov is known only from Belgium and Chile (Guiry & Guiry, 2023). The genus has eight widely distributed species, but none has been cited in Brazil. *Prasiola* Meneghini, 1838, is a worldwide widespread genus with 49 species, being two from Brazil: a marine and endemic *P. minuta* Dickie (Taylor, 1931) and *P. velutina* (Lyngbye) Trevisan (Freitas & Loverde-Oliveira, 2013), none from Santa Catarina. *Trentepohlia* includes 54 species worldwide and 14 species in Brazil, but none of them are cited in SC. *Coccomyxa* Schmidle, 1901 is a genus with 34 species, none reported from Brazil. With two species, the genus *Dictyochloropsis* Geitler, 1966 occurs in arctic North America, Europe, Australia, New Zealand, and Asia, but has not previously been cited in Brazil. *Symbiochloris* Skaloud, Friedl, A.Beck & Dal Grande, 2016 is a genus with 10 species, all known only from Europe, the Arctic, Asia, and the Canary Islands; the species cited here for the first time in Brazil. *Coelastrella* Chodat, 1922 is a genus with 16 species, and *Coelastrella terrestris* (=*Scotiellopsis terrestris* (Reisigl) Puncochárová & Kalina) has been cited for Rio de Janeiro, Brazil (Menezes, 2010). *Chloroidium* Nadson, 1906 has 11 widespread species, including nearby Uruguay and Argentina (Guiry & Guiry, 2023), but is here cited for the first time in Brazil.

Amongst the Marchantiophyta (Liverworts), all reported taxa are pantropical and widely distributed in Brazil, with three highly speciose genera (*Cololejeunea* (Spruce) Steph., *Drepanolejeunea* (Spruce) Steph., and *Riccardia* Gray) and *Cryptolophocolea* L.Söderstr., Crand.-Stotl., Stotler & Váňa (*Chiloscyphus* Corda) extremely abundant in the Atlantic Rainforest. Two of them (*Cololejeunea* and *Drepanolejeunea*) are abundant on leaves and rarely occur on other bryophytes like *Pyrrhobryum*, and the other two are frequent and abundant on soil (*Riccardia* and *Cryptolophocolea*). *Cololejeunea* is a genus with 22 species in Brazil, of which eight have been cited for the Santa Catarina Rainforest: *C. antillana* Pócs, *C. cardiocarpa* (Mont.) A. Evans, *C. diaphana* A. Evans, *C. obliqua* (Nees & Mont.) Schiffn., *C. subcardiocarpa* Tixier, *C. subscariosa* (Spruce) R.M. Schuster, *C. surinamensis* Tixier, and *C. verwimpii* Tixier. *Drepanolejeunea* is a genus with 18 species in Brazil, of which four have been cited for Santa Catarina: *D. araucariae* Steph., *D. campanulata* (Spruce) Steph., *D. mosenii* (Steph.) Bischl. and *D. orthophylla* (Nees & Mont.) Bischl. *Riccardia* is a genus with 12 species in Brazil, of which four are reported for Santa Catarina: *R. cataractum* (Spruce) Schiffn., *R. glaziouvii* (Spruce) Meenks, *R. hymenophytioides* (Spruce) Meenks, *R. multifida* (L.) S.F. Gray, *R. schwaneckei* (Steph.) Pagán. *Chiloscyphus perissodontus* (Spruce) J.J. Engel & R.M. Schust. (= *Lophocolea perissodonta* (Spruce) Steph.) is also reported for Santa Catarina, it is a synonym of *Cryptolophocolea martiana* (Nees) L. Söderstr., Crand.-Stotl. & Stotler. The presence of liverworts associated with moss clumps could refer to spores, propagules (maybe taken by the wind), or even motile gametes moving on water films.

Among the mosses (Bryophyta), taxa found are associated with terrestrial habitats and belong to taxonomically rich groups. The Order Hypnales is the biggest order of pleurocarpous mosses with more than 5,000 species widespread all over the globe, including the tropics and remote regions like Antarctica. *Callicostella* (Müll.Hal.) Mitt. is a pantropical genus with seven species reported from Brazil, all previously reported from Santa Catarina. *Syrrhopodon* Schwägr. contains 25 widespread species in Brazil, being six found in Santa Catarina: *S. elongatus* Sull. *S. gaudichaudii* Mont., *S. incompletus* Schwägr., *S. parasiticus* (Brid.) Besch., *S. prolifer* Schwägr., and *S. tortilis* Hampe. According to Pursell (2007), *Fissidens exilis* Hedw. is a species from Europe, introduced in North America (http://www.efloras.org/florataxon.aspx?flora_id=1&taxon_id=10342); the name was excluded from Neotropics and is morphologically similar to *Fissidens pellucidus* Hornsch. a common species in Brazil. *Fissidens* Hedw. is a large genus with 65 species in Brazil and 24 in Santa Catarina. *Mnium hornum* Hedw. is a doubtful name that is associated with the genus *Pelargonium* L'Hér., with a very common American species, *P. rhynchophorum* (Harv.) T.J.Kop. *Seligeria* Bruch & Schimp. is a genus with about 67 species from temperate North America and Europe, not being cited to Brazil; however, the family Seligeriaceae has two species endemics from the Rio de Janeiro Atlantic Rainforest (*Blindia magellanica* Schimp. ex Müll. Hal. and *Brachydontium notorogenes* W.R Buck & Schaf.-Verw.). The occurrence of *Seligeria* is probably associated with *Dicranella* Ulrich, 1894 subgenus *Seligeria*, since *Dicranella* is a very undersampled genus in databases.

Amongst the flowering plants (Magnoliophyta), there were very few records. *Brassica* L. is a genus of edible plants (e.g, cabbage, cauliflower, radish) commonly found worldwide, including Brazil and Santa Catarina, and could indicate anthropogenic impact. *Deschampsia* P.Beauv. is a grass genus with more than 100 species from Africa, North America, Central America, South America, and Antarctica (tropicos.org). *Deschampsia caespitosa* (L.) Beauv. is common in South Brazil, including Santa Catarina (Longhi-Wagner, 2015), *D. parvula* (Hook. f.) Desv. is a common species in Chile and Argentina.

Concerning the Metazoa

The few taxa found are due to the use of ITS2 and 16S, markers usually not well-suited for this kind of investigation. *Cletocamptus* Schmankevitsch, 1875 is a widespread genus of Copepoda with 29 species (Gómez *et al.*, 2017). According to Gómez *et al.* (2004), they can be found in estuaries and coastal lagoons, as well as in freshwater habitats. There are at least three species reported in Brazil (*C. levis* Gomez, 2005; *C. nudus* Gomez, 2005, and *C. sinaloensis* Gómez, Fleeger, Rocha-Olivares & Foltz, 2004), but none from Santa Catarina. *Ptenothrix* C.Börner, 1906 is a widespread genus of springtail already reported in Brazil but not from Santa

Catarina (Bellinger *et al.*, 2023). *Lecane bulla* (Goose, 1851) is a widespread rotifer species in Brazil (Castanha *et al.*, 2015).

Concerning the Cromista and Protozoa

Again, the use of different markers could render a more diverse community; most taxa found are also widespread and poorly known. There is a great gap in the knowledge of such organisms in Santa Catarina state, raising the issue of the need to improve the knowledge of poorly known organisms in the Atlantic Rainforest. Also, many taxa were only assigned higher ranks, making it difficult to say much about them.

Comparison with other moss studies

The study of diversity within moss mats using DNA metabarcoding techniques is still a relatively new field, with only a limited number of studies available for comparison. Notably, the research conducted by Câmara *et al.* (2021a), which analyzed a fragment of the transplanted Antarctic moss mat *Sanionia uncinata* (Hedw.) Loeske remains the sole work that provides a basis for comparison. However, some differences hamper a more precise comparison. First, in our current study, we did not use the Cox1 marker used by Câmara *et al.* (2021a) and used an acrocarpous moss, whereas *Sanionia* Loeske is a pleurocarp moss. Other than that, in the moss mat fragment analyzed by Câmara *et al.* (2021a), a total of 263 taxa were identified using the ITS2 marker in conjunction with the 16s and Cox1 markers and our study using only ITS2 and 16S found 846 taxa, what is expected as the rainforest is a much more diverse region than the climatically harsh Antarctica.

From the 263 taxa found in Antarctica by Câmara *et al.* (2021a), 74 taxa were retrieved from ITS2 and Cox1, representing five kingdoms: Chromista, Fungi, Metazoa, Protista, and Viridiplantae. Additionally, 189 taxa were identified through the 16S marker in the transplanted moss mat. In our sample, all kingdoms identified by Câmara (2021a) were present, except Protista. Among the ITS2 sequences analyzed in our study, we identified 14 non-fungal taxa shared with our sample, which included five Chromists, four Metazoans, and five Viridiplantae. The common fungal taxa consisted of only four, which included three Basidiomycetes (specifically *Glaciozyma* sp., *Leucosporidium* sp., and *Mastigobasidium* sp.) and one unknown Chytridiomycetes.

Concerning the bacteria, *P. spiniformes* and *S. uncinata* samples shared a total of 118 taxa, indicating that the Antarctic moss mat shares approximately 55 % of its richness with its subtropical counterpart. Conversely, 38 % of the taxa identified in the subtropical *P. spiniformis* mat were also shared with its Antarctic relative. This suggests that, despite geographical separation, about 15 % of the total bacterial richness found in both moss mats appears to be shared.

Even considering that this is a descriptive and exploratory study, we have recovered a diverse community living in association with the target moss species. Our results suggest that the metabarcoding technique enables a quick and broad taxonomic identification and could be a potential tool for assessing local cryptic diversity. It also provides valuable insights into the connections and unique characteristics of these microenvironments, highlighting ecosystems and taxonomic groups that are often overlooked, especially when taxonomic expertise from so many different groups is missing or hard to find. Future studies could benefit from using different genetic markers and increasing the sampling size.

Supplementary Materials

The following online material is available for this article:

Table S1. Non-fungal taxa found with distribution and DNA reads associated with Pyrrhobryum spiniforme clumps. Eu=Europe, AS=Asia, Au=Australia, NZ=New Zealand, AS= South America, SAfr=South Africa, ME=Middle East, Pa=Pacific, W=Widespread, C=Cosmopolitan.

Table S2. Fungal taxa found and DNA reads associated with Pyrrhobryum spiniforme clumps.

Table S3. 16S, Prokaryote taxa found and DNA reads associated with Pyrrhobryum spiniforme clumps.

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Authors' Contributions
FLVB, MC-S, and PEASC performed the collections, LHR analyzed the fungi data, DFP, FLVB, and FACL performed the bioinformatics, CCB analyzed the bacteria data, and MC-S and PEASC analyzed the non-fungi eukaryotic data.

Conflict of Interest
The authors claim no conflict of interest

Data Availability
All raw sequence data generated in this study have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject PRJNA1312491, titled 'Hidden biodiversity associated with Pyrrhobryum spiniforme from an Atlantic Rain Forest, Brazil'. The data, corresponding to three biological replicates, are accessible under the following individual sample accession numbers: SAMN50872868, SAMN50872869, and SAMN50872870 for the ITS amplicon sequences, and SAMN50872936, SAMN50872937, and SAMN50872938 for the 16S amplicon sequences.

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