UNIVERSIDADE FEDERAL DE ALAGOAS
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CIRO RAMON FÉLIX DOS SANTOS SILVA

Microbiologia da filosfera: Explorando a biodiversidade, ecologia e taxonomia no sistema leveduras-bromélias em escala global e no semiárido brasileiro

MACEIÓ - ALAGOAS
Março/2023

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Tese apresentada ao Programa de Pós-Graduação em Diversidade Biológica e Conservação nos Trópicos, Instituto de Ciências Biológicas e da Saúde, Universidade Federal de Alagoas, como requisito para obtenção do título de Doutor em CIÊNCIAS BIOLÓGICAS, área de concentração em Conservação da Biodiversidade Tropical.

Orientadora: Profª. Drª. Melissa Fontes Landell

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Tese aprovada em 16 de março de 2023.

Dr.(ª) Presidente – Melissa Fontes Landell/UFAL
(orientadora)

Dr. (ª) Paula Benevides de Morais

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Dr. (ª) André Rodrigues

Dr. (ª) Carlos Augusto Rosa

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Dedico esse trabalho a vida e memória do meu amado pai, Cícero José da Silva.

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O cientista planta tâmaras, a ciência as colhe.

(Reinterpretação autoral do ditado árabe)

RESUMO

Regiões secas (*Drylands*) são áreas com índices de aridez ≤0,65. Nesse tipo de ambiente, as chuvas são escassas e ocorrem em pulsos (eventos raros de super-disponibilidade de recursos). Durante a seca, a demanda nutricional e metabólica é diminuída. Desta maneira, quanto maior o tempo de seca, maior o reservatório nutricional acumulado no ambiente. O tamanho do reservatório influencia a intensidade da resposta ao pulso de água, ou seja, existe uma 'memória' ambiental do evento de chuva anterior. Outro exemplo de ecossistema com frequente déficit de água é a filosfera (parte aérea das plantas). Esse ambiente intermedia a relação da planta com ambiente e é uma fonte megadiversa de microrganismos. A filosfera de bromélias, plantas tipicamente neotropicais, abrigam diversos grupos microbianos, incluindo leveduras. Entretanto, o conhecimento sobre leveduras em bromélias é disperso e ainda incipiente. Os objetivos do presente estudo foram: 1) Compilar, sintetizar e compreender a extensão do conhecimento sobre leveduras da filosfera de bromélias em escala global. 2) Verificar o efeito da chuva e da memória ambiental sobre a diversidade e estrutura taxonômica, filogenética e funcional das leveduras da filosfera de bromélias na Caatinga. 3) Caracterizar a possível nova espécie *Carlosrosaea xxxxxxx*. Para tanto, a literatura sobre leveduras em bromélias foi sistematicamente revisada. Ademais, leveduras foram isoladas regularmente durante dois anos a partir de bromélias na Reserva Particular do Patrimônio Natural (RPPN) Tocaia, no município de Santana do Ipanema, Sertão alagoano. Na produção acadêmica das últimas três décadas foi encontrado registro de mais de 180 espécies de leveduras na filosfera de bromélias, distribuídas em quatro compartimentos: flores, frutos, folhas e tanques. Cerca de 70% das espécies ocorreram exclusivamente em um único compartimento e apenas 2% foram comuns a todos. Mais de 20 novas espécies de leveduras foram descritas em bromélias nesse período e ao menos 50 espécies mostraram algum potencial biotecnológico. Quanto ao efeito das chuvas, a diversidade-alfa não diferiu significativamente entre períodos sazonais. Entretanto, a composição taxonômica divergiu em mais de 70%, embora tenha se mantido funcionalmente estável. A chuva, seca e a memória ambiental tiveram pouca influência nas métricas de diversidade. No entanto, tiveram relação com a estrutura da comunidade. A análise das regiões ITS e D1/D2 do gene 26S rRNA de oito isolados da Caatinga indicou uma possível nova espécie de levedura com afinidade ao gênero *Carlosrosaea*. Diante disso, propõe-se uma nova espécie de *Carlosrosaea* para agrupar os isolados citados. Os resultados trazem informações sobre a diversidade e dinâmica das leveduras da filosfera de bromélias e como ela se relaciona com os pulsos de chuva, seca, sazonalidade e efeito de memória. Compreendendo o efeito da chuva e da seca na microbiota da filosfera, principalmente em regiões secas, pode estimar como a comunidade microbiana das folhas será afetada pelas mudanças climáticas que alteram os ciclos seco-úmido e, consequentemente, como isso se relaciona com o hospedeiro.

Palavras-chave: Caatinga, Regiões Secas, pulsos, filoplano, aridez.

ABSTRACT

Drylands are areas characterized by aridity indexes ≤0.65. In this type of environment, rainfall is decisive for biological dynamics and occurs in pulses (rare events of overavailability of resources). During drought, nutritional and metabolic demand is decreased. Thus, the longer the dry period, the greater the nutritional reservoir accumulated in the environment. Reservoir size influences the strength of the response to the water pulse. That is, there is an environmental 'memory' of the previous rainfall event. Another example of an ecosystem with a frequent water deficit is the phyllosphere (the aerial part of plants). This environment is one of the largest sources of microorganisms on the planet. The phyllosphere of bromeliads, typically neotropical plants, harbor several microbial groups, including yeasts. However, knowledge about yeasts in bromeliads is scattered and still incipient. In this study our objectives were: 1) Compile, synthesize and understand the extent of knowledge about yeasts from the bromeliad phyllosphere. 2) To verify the effect of rain and environmental memory on the diversity and taxonomic, phylogenetic and functional structure of yeasts from the phyllosphere of bromeliads in the Caatinga. 3) Propose the description of the new species *Carlosrosaea xxxxxxx* sp. nov. To this end, the literature on yeasts in bromeliads was systematically reviewed. Furthermore, yeasts were regularly isolated for two years from bromeliads in the Private Natural Heritage Reserve (RPPN) Tocaia, in the municipality of Santana do Ipanema, Alagoas. In the academic production of the last three decades, records of more than 180 species of yeasts were found in the phyllosphere of bromeliads, distributed in four compartments: flowers, fruits, leaves and tanks. About 70% of the species occurred exclusively in a single compartment and only 2% were common to all. More than 20 new yeast species were described in bromeliads during this period and at least 50 species showed some biotechnological potential. As for the effect of rainfall, alpha-diversity did not differ significantly between seasonal periods. However, the taxonomic composition differed by more than 70%, although it was functionally stable. Rain, drought and environmental memory had little influence on diversity metrics. However, they showed a relationship with the structure of the community. The analysis of the ITS and D1/D2 regions of the 26S rRNA gene of eight Caatinga isolates indicated a possible new yeast species with affinity to the genus *Carlosrosaea*. Therefore, a new species of *Carlosrosaea* is proposed to group the cited isolates. Our results bring information about the diversity and dynamics of yeasts in the bromeliad phyllosphere and how it relates to rainfall pulses, drought, seasonality and memory effect. Furthermore, we aggregate information about the tolerance of the phyllosphere yeast community to seasonal changes, from a taxonomic, phylogenetic and functional point of view. By understanding the effect of rain and drought on the phyllosphere microbiota, especially in dry regions, we can think about how the microbial community of the leaves will be affected by climate changes that alter the dry-wet cycles and, consequently, how this relates to the host.

Keywords: Caatinga, Drylands, pulses, phyllosphere, aridity.

SUMÁRIO

1. APRESENTAÇÃO

Regiões secas como a Caatinga cobrem mais de 40% da superfície continental no planeta. Essas regiões são frequentemente secundarizadas pelo Estado e pela ciência. Não é incomum que muitos representantes dessas regiões estejam subamostrados, sobretudo quando comparados aos seus equivalentes úmidos. A Caatinga não foge à regra e frequentemente recebe menos atenção comparada a seus pares úmidos como a Mata Atlântica e a Amazônia. Esse descaso tem componentes sociais e culturais, seja pelo ecossistema ocorrer no Nordeste do país, uma região historicamente descriminada e negligenciada, ou pelo mito que foi construído no senso comum que a Caatinga é um ambiente com pouca biodiversidade.

Na maior parte do ano este ecossistema tem um aspecto seco, com troncos retorcidos e plantas sem folhas. Esse cenário que, durante a maior parte do tempo, diverge das paisagens verdes e exuberantes que habitam o imaginário popular quando se pensa em biodiversidade foi um lastro importante para a construção do mito entorno da Caatinga. Por outro lado, nas últimas décadas, um grande esforço tem sido empenhado em compreender a Caatinga e sua biodiversidade. Regiões secas proveem diversos serviços ecossistêmicos fundamentais como a manutenção dos níveis globais de carbono atmosférico e do clima mundial. Em um cenário de emergência climática como o atual, esses serviços ecossistêmicos se tornam centrais no debate científico e no interesse público.

A disponibilidade de água, sobretudo em regiões secas, afeta todos os tipos de organismos, em todos os níveis de organização ecológica. Tanto macro quanto micro-organismos são afetados, porém, geralmente os organismos microscópicos em ambiente natural (não-clínico) são menos estudados que os macroscópicos. Micro-organismos prestam várias funções relevantes como fixação de carbono e nitrogênio, ciclagem de nutrientes e diversas outras funções que se dá no âmbito das associações mutualísticas com outros grupos, como a manutenção da saúde do hospedeiro e o sucesso reprodutivo. A insipiência dos estudos sobre micro-organismos gera uma

grande lacuna de conhecimento que diminui a compreensão do funcionamento do ecossistema de processos e funções importantes desempenhadas pela microbiota.

Diversos grupos microbianos têm sido encontrados em ambientes áridos, muitos deles com adaptações para tolerar forte radiação solar, estresse oxidativo e déficit hídrico. Entre os grupos estão bactérias, arqueas, microalgas, fungos filamentosos e leveduras. Além do solo, as plantas são substratos importantes para os micro-organismos, vários grupos são capazes de interagir positivamente com plantas produzindo hormônios vegetais, além de ter atividade antagônica a patógenos. A grande ocorrência de micro-organismos em plantas pode ser exemplificada com os fungos. Esse grupo é um dos mais diversos do mundo. Estima-se que existam entre 2,2 e 3,8 milhões de espécies, porém, conhece-se apenas cerca de 5% das espécies. Nos grandes bancos de dados micológicos como o *Mycobank*, a maioria dos registros de fungos concentra-se em substratos vegetais, sobretudo em folhas.

Fungos podem ser subdivididos segundo diversos critérios, um deles separa fungos em filamentosos e leveduras. Leveduras consistem em um grupo formado por várias linhagens de fungos que convergiram para um estado majoritariamente leveduriforme, ou seja, unicelular. Esse grupo de fungos unicelulares está distribuído em diversos ambientes e substratos como animais, plantas, solo e água. Possuem importância biotecnológica (ex. na produção de alimentos e bebidas fermentadas), clínica (causando fungemias como as espécies *Candida albicans* e *Cryptococcus neoformans*) e ambiental (sendo responsáveis por funções ecossistêmicas fundamentais para a manutenção da vida no planeta como a ciclagem de nutrientes).

Plantas tropicais são uma das maiores fonte de micro-organismos. Além disso, são um dos mais importantes, talvez o maior, substrato para descoberta de novas espécies de fungos. Entre os grupos de plantas tropicais mais diversos e endêmicos do Brasil está a família Bromeliaceae. Essa família se distribui entre ambientes úmidos como a Mata Atlântica até regiões semiáridas como a Caatinga. Bromélias são consideradas mosaicos complexos e diversos para leveduras, além de um modelo ecológico importante para regiões tropicais.

Diante da insipiência que ainda persiste nos estudos sobre microbiota ambiental, principalmente de leveduras, em regiões secas, este estudo objetivou: 1) Sintetizar o conhecimento sobre leveduras em bromélias, elucidando padrões e processos ecológicos, lacunas do conhecimento e potencial biotecnológico; 2) Avaliar a relação dos pulsos de chuvas e da sazonalidade nos padrões de diversidade funcional, taxonômica e filogenética de leveduras associadas à bromélias de uma região de Caatinga, no estado de Alagoas; 3) Caracterizar molecular e fisiologicamente uma possível espécie nova de leveduras associadas à bromélias, contribuindo para o conhecimento sobre a biodiversidade do bioma Caatinga.

2. REVISÃO DA LITERATURA

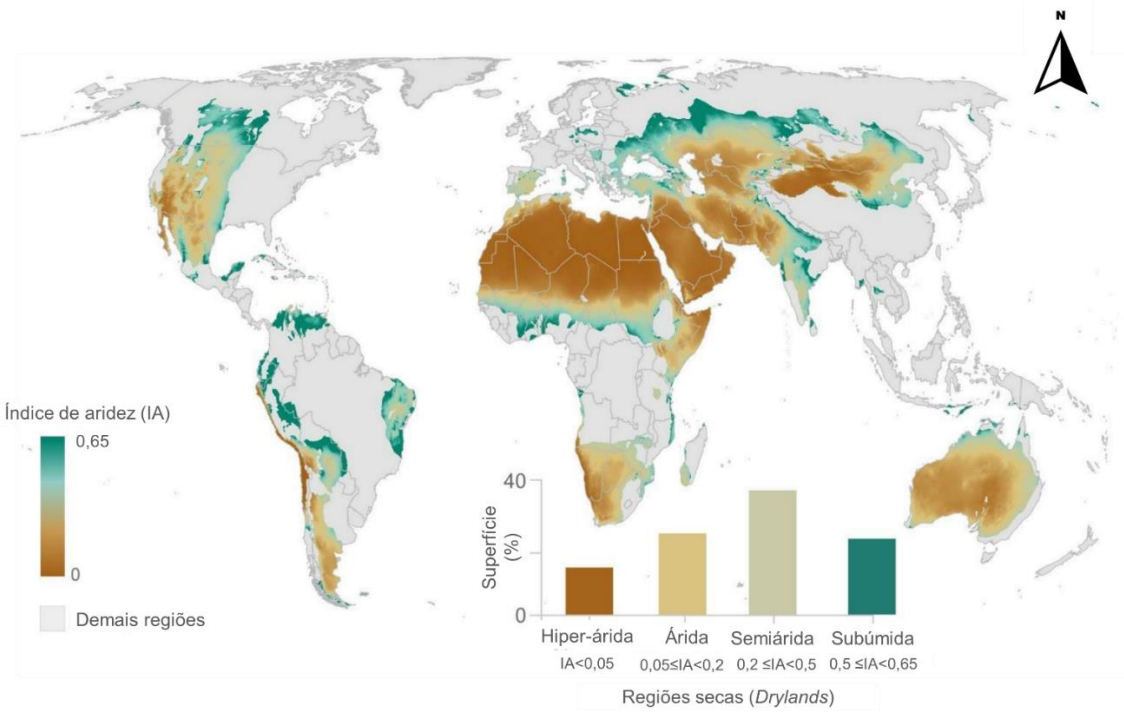
2.1. Regiões Secas, Florestas Sazonalmente Secas e Caatinga

Regiões secas não-polares (*drylands*) cobrem 41% da superfície continental do planeta, o equivalente a aproximadamente 6 bilhões de hectares, e são consideradas ecossistemas frágeis, com biodiversidade única. Esses ambientes fornecem importantes serviços ecossistêmicos no ciclo global do carbono, do nitrogênio, da água e na regulação do clima (HUANG et al., 2016; MAESTRE et al., 2016, 2021). Além disso, essas regiões abrigam 38% da população humana, 35% da biodiversidade global e 20% da diversidade de plantas (MAESTRE et al., 2021). De forma simples (e incompleta), pode-se dizer que regiões secas são áreas com baixa precipitação anual.

Existem vários critérios que podem ser empregados na classificação das regiões secas e seus subtipos. Classicamente, são divididas em hiperáridas, áridas e semiáridas, conforme o volume anual de chuvas (VAC), que não ultrapassa os 500 mm (NOY-MEIR, 1973). Nas regiões hiperáridas o VAC < 25 mm, nas áridas é 25 mm ≤ VAC < 250 mm, e nas semiáridas 250 mm ≤ VAC < 500 mm (HUANG et al., 2016). Essa definição é considerada incompleta por ter como base apenas o regime de chuva como critério delimitatório. Outra maneira que pode ser usada para identificar subtipos de regiões secas é a classificação climática de Köppen–Geiger que atualmente emprega características climáticas, chuva e temperatura para subdividir o clima do mundo em 31 categorias (KOTTEK et al., 2006). O clima árido (tipo B), segundo a classificação de Köppen–Geiger, pode ser subdividido em quatro: clima desértico quente (BWh), clima desértico frio (BWk), clima de estepe quente (BSh) e clima de estepe frio (BSk) (KOTTEK et al., 2006). Além disso, mesmo que os biomas não tenham limites tão fixos e precisos, sabe-se que os principais biomas que constituem as regiões secas são as Savanas, Pradarias e Desertos (HUANG et al., 2016; SAFRIEL et al., 2005). As diversas maneiras que podem ser usadas para classificar e definir as regiões secas contribuem para a compreensão desses ecossistemas por pontos de vista distintos. Sobrepondo essas visões pode-se construir uma compreensão mais completa desse tipo de ambiente.

Nas duas últimas décadas, o índice de aridez (IA) tem sido usado como uma boa ferramenta para classificação das regiões secas (HUANG et al., 2016). O IA é gerado pela razão entre a VAC média e demanda evaporativa anual média (expressa pela evapotranspiração potencial (PET) média anual), um valor de IA < 1 indica déficit de umidade. Segundo esse critério, regiões secas são áreas com IA < 0,65. É algo lógico imaginar que as regiões secas não são homogêneas e o IA permite classificá-las em quatro subtipos que diferem quanto a limitação de água, são eles: hiperáridas (IA < 0,05), áridas (0,05 ≤ IA < 0,2), semiárida (0,2 ≤ IA < 0,5), e subúmidas secas (0,5 ≤ IA < 0,65), ocupando respectivamente, 6,6%, 10,6%, 15,2% e 8,7% da área do planeta (Figura 1) (HUANG et al., 2016; SAFRIEL et al., 2005)**.**

Figura 1- Regiões secas em todo o mundo de acordo com índice de aridez (IA=VAC média/PET anual média).



Fonte: Modificado a partir de (MAESTRE et al., 2021).

Um fator que adiciona camadas de complexidade sobre ‘o que é’ uma região seca é que algumas dessas regiões podem receber grandes montantes de chuva durante parte do ano, um fato contraintuitivo quando pensa-se em regiões secas. Nesse contexto pode-se inserir as Florestas Tropicais Sazonalmente Secas (FTSS), esse tipo de bioma é classicamente reconhecido como uma floresta tropical com um peculiar regime de estações chuvosas e secas. Nas FTSS, a VAC varia entre 250-2000 mm e uma forte estação seca (meses com ≤100 mm de chuva) que pode durar seis meses ou mais. As FTSS podem representar mais de 40% de todas as florestas tropicais e subtropicais (ALLEN et al., 2017; BECKNELL; KISSING KUCEK; POWERS, 2012; PULLA et al., 2015). Quanto a esse bioma, a ciência mal arranhou a superfície do conhecimento, num período de 60 anos (entre 1945 e 2004) apenas 14% da produção científica confiável sobre Florestas Neotropicais tratavam de FTSS (PULLA et al., 2015).

A Caatinga constitui uma região semiárida e a maior FTSS da América do Sul, com área de ~1 milhão de km² cobrindo aproximadamente 10% do território brasileiro e 50% da região Nordeste (DA SILVA; LEAL; TABARELLI, 2017; MACHADO; LOPES, 2004; MORO et al., 2016; SANTOS et al., 2011). É formada por um mosaico de vegetação arbustivo-espinhosa decídua e manchas de floresta seca. A caatinga possui 135 unidades geoambientais e nove ecorregiões, além de ao menos 13 fitofisionomias (DA SILVA et al., 2017). Nesse ambiente, a pluviosidade é baixa (entre 240 e 1500mm por ano), e cerca de metade da área da Caatinga possui uma VAC média <750 mm e, em algumas áreas, <500 mm (LEAL et al., 2005; MORO et al., 2016; SANTOS et al., 2011). A estação chuvosa concentra-se em três meses consecutivos durante o ano e em alguns pontos 60% da VAC pode ocorrer em um único mês, o que gera um grande contraste na paisagem, como pode ser exemplificado na Figura 2 (MENEZES et al., 2012). Por outro lado, os períodos anuais de seca podem variar entre 7 e 11 meses. Além disso, os períodos de chuva podem variar entre os anos, gerando extensos períodos de seca contínua (LEAL et al., 2005; MACHADO; LOPES, 2004). Cerca de 28 milhões de pessoas vivem na Caatinga, sendo esta uma das regiões semiáridas mais populosas e biodiversas do mundo (DA SILVA; LEAL; TABARELLI, 2017).

Figura 2- Contraste paisagístico entre o período seco e chuvoso na Caatinga. Registro realizado na RPPN Tocaia, Sertão alagoano, município de Santana do Ipanema.

Fonte: Elaborado pelo autor (2022).

Por sua paisagem composta por plantas caducas durante grande parte do ano, longos períodos de seca, troncos secos e retorcidos e uma flora com muitos espinhos, construiu-se um mito no imaginário popular onde a Caatinga era um ambiente pobre em diversidade biológica. Desta maneira, esse domínio foi durante muito tempo negligenciado pelo Estado e pela ciência (LEAL et al., 2005). Nas últimas décadas

houve um esforço para desconstruir a imagem da Caatinga como um ambiente pobre, e o avanço dos estudos já contabilizam 3347 espécies de plantas, 548 de aves, 386 de peixes, 276 de formigas, 183 de mamíferos, 164 de fungos micorrízicos, 98 de anfíbios e 79 de répteis (DA SILVA et al., 2017; FERNANDES; CARDOSO; DE QUEIROZ, 2020; WINAGRASKI et al., 2019). Além disso, a taxa de endemismo é variável a depender do grupo em questão, sendo, por exemplo de 6% em mamíferos, 15,7% em plantas e 52,9% em peixes (DA SILVA et al., 2017; FERNANDES; CARDOSO; DE QUEIROZ, 2020).

Dentre os 'Biomas' brasileiros, a Caatinga é o menos estudado e com um número insuficiente de área protegidas, com apenas cerca de 7,4% da região com algum nível de proteção, entretanto boa parte não possui financiamento adequado (DA SILVA et al., 2017; LEAL et al., 2005; SANTOS et al., 2011). No ano de 2009, a Caatinga tinha uma perda de área superior a 45% e evidências que indicavam um aumento na velocidade do desmatamento, com a agropecuária se apresentando como uma das principais responsáveis (MMA, 2011). Menos de uma década depois a área da Caatinga modificada por ação humana é de cerca de 63,6% (DA SILVA et al., 2017) confirmando a tendência de 2009.

Na Caatinga, os períodos de seca são eventos normais que acontecem periodicamente, entretanto, secas longas historicamente causam prejuízos para as pessoas da região, gerando, por exemplo insegurança alimentar grave (MARENGO et al., 2011, 2018). Um dos fatores que contribuem para esses eventos de seca é o *El Niño*, um fenômeno climático-oceânico global caracterizado pelo aumento da temperatura da superfície do mar e alteração dos ventos alísios na região do Pacífico Equatorial. Esse processo afeta os regimes de chuva em regiões tropicais e de latitudes médias, porém não são capazes de explicar sozinhos os eventos de seca da Caatinga (KANE, 1989; MARENGO et al., 2011, 2018; MARENGO; TORRES; ALVES, 2016). Por outro lado, períodos chuvosos podem ser regulados entre outros fatores pelo fenômeno 'contrário', o *La ninã*. Porém, os eventos de *La ninã* estão ficando mais escassos e espaçados, enquanto as secas estão se tornando mais longas e severas (MARENGO et al., 2011; MARENGO; TORRES; ALVES, 2016).

As mudanças climáticas e o aquecimento global estão afetando todo o planeta, e na Caatinga a predição é que as mudanças aumentem o déficit de chuvas e consequentemente aumente a aridez, podendo levar a processos de desertificação e expansão de regiões áridas e semiáridas (HUANG et al., 2016; SAFRIEL et al., 2005). O aquecimento global que tem sido intensificado pela ação humana potencialmente pode gerar uma série de alterações no clima, e consequentemente, na biodiversidade, funções e serviços ecossistêmicos. Uma preocupante projeção indicava um aumento médio de 2 a 4 °C na temperatura e uma diminuição de 20% nas chuvas até 2100 (MARENGO et al., 2011; MARENGO; TORRES; ALVES, 2016). Entretanto, as atividades humanas têm acelerado esse processo e é possível que a marca de 4 °C de incremento na temperatura seja alcançada ainda na década de 2060 (MARENGO et al., 2018). O nordeste brasileiro é uma das regiões mais vulneráveis a mudanças climáticas, colocando em risco sua segurança hídrica, energética e alimentar (MARENGO et al., 2018). O bioma Caatinga é considerado um ambiente susceptível à aridificação e desertificação (cerca de 94% do semiárido nordestino é susceptível a desertificação). Ademais, já se observa em nível global uma tendência de expansão de áreas semiáridas (HUANG et al., 2016).

2.2. Ecologia de regiões secas

Diversos fatores bióticos e abióticos governam o funcionamento dos ecossistemas secos. Entre os abióticos, a quantidade de chuvas e a temperatura são centrais e alteram cadeias tróficas, ciclagem de nutrientes e padrões de coexistência de espécies (MAESTRE et al., 2016; NOY-MEIR, 1973; SCHWINNING et al., 2004). Nesses ecossistemas, a água pode estar direta ou indiretamente relacionada ao fluxo de energia, alterando o controle estomático, taxa de fotossíntese e respiração. Esses processos são intimamente relacionados à disponibilidade de água, sobretudo com insuficiência hídrica, condição que é dominante em regiões secas (COLLINS et al., 2014; NOY-MEIR, 1973). Além disso, a água também tem influência indireta em outras formas de transferência de matéria e energia como a herbivoria e carnivoria. Isso porque em ecossistemas áridos, a taxa de consumo de alimentos está muitas vezes

acoplada a disponibilidade de água no próprio alimento ou na água disponível para consumo (NOY-MEIR, 1973).

A disponibilidade de água é um fator limitante à vida, essa essencialidade é real e intuitivamente mais pronunciada em ambientes secos. Segundo Noy-meir (1973), os três atributos mais importantes para a compreensão do funcionamento dos ecossistemas secos são: i) a precipitação é escassa e, portanto, um fator dominante no controle dos processos biológicos. ii) Nesses ambientes a precipitação ocorre de forma ocasional e modesta. iii) A chuva nesse tipo de ambiente é variável e imprevisível. Ao indicar esses atributos centrais, Noy-Meir define desertos (e regiões secas) como ecossistemas controlados pela água e com chuvas escassas, reduzidas e imprevisíveis. Nesses ambientes, as chuvas geralmente não ultrapassam 2 mm, além disso, é estimado entre 10-50 dias chuvosos por ano, distribuídos entre 3-15 eventos de chuva, dos quais 5 ou 6 possuem magnitude para gerar efeitos biologicamente significativos (COLLINS et al., 2014; NOY-MEIR, 1973).

Ainda que a água não explique necessariamente todo o espectro de relações ecológicas em ambientes secos, inegavelmente ela afeta todos, seja de forma direta ou não (COLLINS et al., 2014). A relação entre funcionamento ecossistêmico em terras secas e a água vem sendo debatida há décadas, o prisma de conhecimento sobre esse tema indica que fatores diversos podem moldar a resposta do ambiente aos influxos de água (MAESTRE et al., 2016; SCHWINNING et al., 2004). Alguns destes fatores são: tipos funcionais de plantas, escala, tamanho e frequência das chuvas, limiares de resposta, textura e umidade prévia do solo (AUSTIN et al., 2004; CHESSON et al., 2004; MAESTRE et al., 2016; NOY-MEIR, 1973; OGLE; REYNOLDS, 2004; REYNOLDS et al., 2004). Por exemplo, solos com maior granulometria e/ou maior quantidade de matéria orgânica tendem a reter mais e por mais tempo a água, o que aumenta a chance dessa água ser usada por micro-organismos e plantas (MAESTRE et al., 2016; REYNOLDS et al., 2004; SCHWINNING et al., 2004). Por outro lado, solos mais arenosos permitem uma absorção mais rápida, desta forma, mesmo um grande volume de chuvas pode não ter efeito biológico efetivo (REYNOLDS et al., 2004).

Pode-se considerar que tudo que é consumido ou utilizado por um organismo, e durante esse processo deixa de estar disponível no meio, pode ser considerado um recurso (BEGON; TOWNSEND, 2020). Mudanças na disponibilidade dos recursos podem alterar o funcionamento dos ecossistemas em vários níveis de organização ecológica (SCHWINNING et al., 2004). No contexto da dinâmica de pulsos, pode-se entender um pulso como um evento efêmero e raro de superabundância de recursos (HOLT, 2008; YANG et al., 2008). Um pulso de recurso é um evento historicamente singular, ou seja, um evento de disponibilidade intensamente maior ao esperado para um determinado ambiente, não necessariamente promove relações adaptativas, mas sim respostas comportamentais e fisiológicas incomuns. Em suma, pulsos são fundamentalmente uma perturbação que gera um estado alternativo transiente (HOLT, 2008). Em resposta a essa repentina mudança da disponibilidade de recursos (pulso), as espécies podem alterar de forma intensa suas dinâmicas populacionais (ex. um pico de recrutamento de sementes ou de taxa metabólica microbiana). Esse uso do pulso de recurso pode ser direto ou indireto e tem potencial para afetar os processos em toda a comunidade e ecossistema em um efeito *bottom-up*, começando com a atividade microbiana e produção primária e, secundariamente, chegando aos níveis mais altos das cadeias. Além disso, o efeito *bottom-up* é seguido por um abrupto decaimento *top-down* (HOLT, 2008; YANG et al., 2008). Embora os pulsos de recursos sejam eventos de curta duração por definição, podem ser gatilhos importantes que iniciam processos poderosos e com efeitos ecológicos persistentes mesmo muito tempo depois do fim do pulso (AUSTIN et al., 2004; YANG et al., 2008).

Diversos ecossistemas respondem ao componente de pulsos, porém a disponibilidade de recurso acima do comum é o que caracteriza os pulsos dependendo da variação natural do ambiente em discussão (YANG et al., 2008). Em regiões secas, na maior parte do ano a situação é de déficit hídrico e, nesses ambientes, as chuvas ocorrem de forma escassa, reduzida e imprevisível. Portanto, pode-se considerar que as entradas de chuvas nesse sistema acontecem em ‘pulsos’ de curta duração (COLLINS et al., 2014; NOY-MEIR, 1973). Além dos efeitos diretos pelo acesso a água, os pulsos de chuva em ambientes secos podem gerar outros efeitos como à

translocação de nutrientes por percolação e escoamento, a disrupção da superfície do solo, afetando a disponibilidade de nutrientes, além da produção, deposição e decomposição de matéria orgânica (AUSTIN et al., 2004; COLLINS et al., 2014).

Pela natureza imprevisível das chuvas em regiões secas, esses ambientes apresentam flutuações abruptas entre seco-úmido em escala de dias ou até horas. Esse tipo de ciclo é comum em diversos ambientes e pode ocorrer em diversas escalas temporais, porém, tendem a ser mais abruptos em regiões secas altamente sazonais, como florestas tropicais decíduas. O ciclo seco-úmido afeta fortemente os processos microbianos e nutricionais do solo, incluindo mineralização de C e N, desnitrificação, volatilização de amônia e biomassa microbiana. Além disso, a maior umidade contribui em última instância para diminuição dos reservatórios de C e N (AUSTIN et al., 2004).

Os longos períodos de seca em *drylands* gera acúmulo de substratos minerais e orgânicos, pois o crescimento de plantas e micro-organismos é restrito durante esse período e há pouca demanda de nutrientes e menor taxa de decomposição e ciclagem (COLLINS et al., 2014). Por exemplo, o acúmulo de N ocorre majoritariamente no período seco, pois a difusão dos íons tende a ser restrita a meios aquosos e os ‘sumidouros’ de nitrogênio estão comprometidos nesse período (AUSTIN et al., 2004). Esse acúmulo leva a uma rápida e intensa taxa de mineralização e desnitrificação durante as fases iniciais do ciclo úmido. Desta forma, ciclos seco-úmido mais frequentes podem acentuar a perda de carbono e nitrogênio dos reservatórios do solo (AUSTIN et al., 2004; SCHWINNING et al., 2004).

Esse sistema de acúmulo e reservatórios podem ser diversos (ex. água, carbono e nitrogênio) e influencia as respostas ecossistêmica às novas precipitação, isso porque dependendo do tamanho do reservatório acumulado a resposta ambiental será diferente. Em certo nível, há uma “memória” ambiental dos eventos de precipitação anteriores. Essa memória, portanto, é uma das chaves para a compreensão da sensibilidade ambiental, sobretudo nos padrões intrasazonais (SCHWINNING et al., 2004). Por exemplo, a duração de um período seco antes de um evento de chuva determina o tamanho do reservatório acumulado de matéria orgânica e N,

consequentemente, o tamanho da atividade de desnitrificação gerada pelo pulso de chuva (AUSTIN et al., 2004). Além disso, a duração da seca afeta também a prontidão fisiológica das plantas para o uso da água (OGLE; REYNOLDS, 2004; REYNOLDS et al., 2004; SCHWINNING et al., 2004). O efeito de memória torna difícil compreender a dinâmica ambiental considerando um único evento de chuva, ou numa escala de precipitação sazonal ou anual total (SCHWINNING et al., 2004). Isso porque a reposta a um pulso de chuva está relacionada não apenas a intensidade do pulso, mas também a distância temporal do último pulso ou a frequência dos ciclos seco-úmido.

Em ecossistemas que não experimentam um forte déficit hídrico, os processos de decomposição são predominantemente biológicos. Entretanto, em ecossistemas secos, principalmente nos períodos de baixa atividade microbiana, a decomposição da matéria orgânica pode ser mediada por fatores físico-químicos. Na superfície, a radiação solar e os ventos degradam, fragmentam e redistribuem nutrientes (MAESTRE et al., 2016). Essa maior contribuição de fatores físico-químicos na decomposição em regiões secas faz com que haja um potencial maior de decomposição do que de produção primária. Isso porque nesse contexto de limitação de água a produção primária é mais afetada que a decomposição (COLLINS et al., 2014). Ainda assim, os micro-organismos são agentes essenciais na dinâmica de ambientes secos. Micro-organismos do solo em latência (criptobióticos) podem responder a eventos de chuva pequenos e frequentes, que são insuficientes para gerar um efeito significativo em plantas, e assim podem dar início em processos de ciclagem e decomposição que aumentam a disponibilidade nutricional no solo (AUSTIN et al., 2004).

Em ecossistemas com limitação de água, a mineralização e imobilização de N por micro-organismos do solo tendem a ser regulados por três mecanismos: i) a relação C:N, ii) eficiência do uso de N pela microbiota (quantidade de N necessária para produzir uma unidade de biomassa microbiana C) e iii) a eficiência do crescimento microbiano (fração do C orgânico usado pelo micro-organismo para produzir biomassa) (AUSTIN et al., 2004). Dentre esses fatores, o que provavelmente mais impacta o balanço entre mineralização e imobilização de N é a relação C:N. Dependendo do

substrato a relação C:N varia substancialmente. Para a biomassa microbiana a relação é cerca de 4:1, em raízes fica entre 30:1 até 70:1 e em material lenhoso chega a 100:1 (AUSTIN et al., 2004). Desta maneira, espera-se que valores baixos de C:N elevem as taxas metabólicas microbianas e favoreçam a mineralização de N, enquanto valores elevados da relação desviem as rotas para a imobilização (AUSTIN et al., 2004).

Por sua vez, a eficiência do uso de N é essencialmente o inverso da relação C:N da biomassa microbiana e é determinada em grande parte pela estrutura da comunidade microbiana do solo (AUSTIN et al., 2004). As bactérias produzem biomassa com uma relação C:N mais baixa do que os fungos e assim imobilizam mais N por unidade de C assimilado, do que comunidades dominadas por fungos. Por outro lado, fungos são mais tolerantes a dessecação que bactérias, pois geralmente toleram menor atividade de água. Por isso, a abundância relativa de fungos tende a aumentar em comunidades de ambientes áridos e semiáridos durante o período seco. A resposta de mineralização ou imobilização durante os ciclos de umidade tendem a ser determinada pela taxa de crescimento relativo entre bactérias e fungos (AUSTIN et al., 2004). Como os solos das terras áridas são cronicamente secos, os fungos, em vez das bactérias, podem dominar os processos de decomposição e transformação de N, além de gerar associações simbióticas com produtores primários, por exemplo, micorrizas (COLLINS et al., 2014).

2.3. Micro-organismos associados às plantas

Plantas possuem associação com micro-organismos tanto em suas estruturas subterrâneas quanto nas aéreas (KOSKELLA, 2020; VORHOLT, 2012). A parte subterrânea, que compreende a interface solo-planta, é denominada rizosfera (BRINGEL; COUÉE, 2015; KOSKELLA, 2020). Por sua vez, ainda que exista certo grau de confusão na definição, a parte aérea das plantas, que compreende todas as estruturas da interface atmosfera-planta, é denominada de filosfera (KOSKELLA, 2020; LINDOW; BRANDL, 2003; VORHOLT, 2012). A filosfera inclui folhas (filoplano), frutos (carposfera), flores (antosfera, incluindo pólen e néctar), caule (caulosfera), e reservatórios de água (fitotelma) (BRINGEL; COUÉE, 2015; KOSKELLA, 2020;

LEVEAU, 2019; LIU; BRETTELL; SINGH, 2020; VACHER et al., 2016; VORHOLT, 2012). Nos diferentes tecidos das plantas, os micro-organismos podem colonizar o interior (endofíticos) ou na superfície (epifíticos). Também é comum que a mesma espécie de micro-organismo possa ser encontrada em ambos os habitats (FONSECA; INÁCIO, 2006; VORHOLT, 2012). Além disso, pode-se agrupar os componentes da microbiota vegetal em transientes (micro-organismos efêmeros, que estão temporariamente associados) e residentes (micro-organismos muito frequentes, fortemente associados) (FONSECA; INÁCIO, 2006). Esses ambientes sustentam a maior diversidade de micro-organismos do planeta, com representantes de diversos grupos como bactérias, arqueas, vírus, fungos filamentosos, leveduras, microalgas, protozoários e nematoides (GOFFREDI; JANG; HAROON, 2015; KOSKELLA, 2020; LINDOW; BRANDL, 2003; THAPA; PRASANNA, 2018; VACHER et al., 2016; VORHOLT, 2012).

A maioria dos estudos sobre a microbiota das plantas se concentra na região da rizosfera (BRINGEL; COUÉE, 2015; KOSKELLA, 2020). Nessa região, a abundância microbiana é maior que na parte aérea das plantas (ANDREOTE; GUMIERE; DURRER, 2014; KOSKELLA, 2020; VORHOLT, 2012). Essa constatação sugere de forma errada que a microbiota da filosfera é de alguma maneira menos importante. Entretanto, a filosfera é a interface ecológica que permeia a relação entre o ambiente e a planta (KOSKELLA, 2020; VACHER et al., 2016). Diversos estudos têm enumerado contribuições essenciais da microbiota da filosfera em processos de defesa, patogenicidade, aquisição de nutrientes, crescimento, sucesso reprodutivo e evolução das plantas (KOSKELLA, 2020; LEVEAU, 2019; RODRIGUEZ et al., 2009; THAPA; PRASANNA, 2018). Por exemplo, a microbiota do néctar é capaz de alterar padrões de preferência de polinizadores, elevando ou diminuindo a frequência de visitação floral, e até mesmo alterando a preferência de diferentes guildas, o que tem impacto direto no sucesso reprodutivo e evolução vegetal (CANTO; HERRERA; RODRIGUEZ, 2017; SCHAEFFER et al., 2017). Além disso, a microbiota pode agir como antagonista a fitopatógenos, mediando a saúde da planta, ou facilitando a infecção por meio da produção de enzimas que degradam a cutícula da planta (BUCK, 2002; LEVEAU,

2019). Por exemplo, em experimento onde uma esterase produzida pela levedura *Pseudozyma antarctica* foi aspergida sobre plantas de tomate e infectada com o fitopatógeno *Botrytis cinerea*, a infecção foi mais grave (UEDA et al., 2018). Isso não indica que a levedura seja patogênica, mas que ela pode ser um potencial facilitador da infecção.

Estima-se que a filosfera represente 60% da biomassa de todos os organismos do planeta (KOSKELLA, 2020). Esse ambiente é extremo, dinâmico, heterogêneo e frequentemente oligotrófico (KOSKELLA, 2020; LEVEAU, 2019; VORHOLT, 2012). Além da elevada exposição à radiação solar e das oscilações bruscas de fatores de estresse como temperatura, oxidação, disponibilidade de nutrientes e água, outro ponto crítico é a camada de cutina, que forma a cutícula da planta (KOSKELLA, 2020; LEVEAU, 2019; MEYER; LEVEAU, 2012; VORHOLT, 2012). A cutícula da planta é uma camada lipídica formada por ácidos graxos de cadeia longa e chega a representar 15% do peso seco das folhas (BRINGEL; COUÉE, 2015). Essa camada protege as folhas e auxilia no controle hídrico, é também a primeira barreira para o estabelecimento dos micro-organismos da filosfera (LINDOW; BRANDL, 2003). A cutícula tem a capacidade de modelar a microbiota através de variações em sua composição química e arquitetura (e.g. rugosidade e tricomas), alterando padrões microbianos de colonização, fixação, composição e abundância (LEVEAU, 2019; LINDOW; BRANDL, 2003; REISBERG et al., 2013; VACHER et al., 2016; VORHOLT, 2012).

A cutícula da planta diminui a evaporação, retenção de água na superfície, lixiviação de metabólitos e difusão de nutrientes, gerando um ambiente adverso e com elevado estresse osmótico (BEATTIE; LINDOW, 1995a; BRINGEL; COUÉE, 2015; LINDOW; BRANDL, 2003; VACHER et al., 2016; VORHOLT, 2012). Por outro lado, a limitação da lixiviação também evita a perda precoce de nutrientes e colabora para manutenção destes nas regiões epifíticas (WHIPPS et al., 2008). Nesse sentido, para a microbiota filosférica, a cutícula pode ser interpretada ao mesmo tempo como uma vantagem e uma desvantagem. Pois, se por um lado gera estresse osmótico, por outro, preserva reservas nutricionais importantes.

Cada compartimento da filosfera (e.g. filoplano, antosfera, carposfera) possui uma microbiota distinta e particular (ABDELFATTAH et al., 2019; COLEMAN-DERR et al., 2016; FÉLIX et al., 2022; LEVEAU, 2019; LIU; HOWELL, 2021). O genótipo é um importante estruturador de comunidades microbianas associados a plantas (LEVEAU, 2019; SAPKOTA et al., 2015). Um mesmo indivíduo possui, por óbvio, um mesmo genótipo. Entretanto, esse genótipo é expresso de forma diferente em cada um dos compartimentos vegetais, o que pode explicar parte das diferenças entre as comunidades (LEVEAU, 2019). Entre os compartimentos da filosfera, o filoplano é o maior e um dos mais estudados (FONSECA; INÁCIO, 2006; KOSKELLA, 2020). A superfície das folhas do mundo soma uma área de aproximadamente 1 milhão de km², o equivalente ao dobro da superfície do planeta Terra (BRINGEL; COUÉE, 2015). É estimado que as folhas abriguem em média 10^6-10^7 células de bactérias por cm² e em valores totais cerca de 10^{26} células (LINDOW; BRANDL, 2003; VORHOLT, 2012). Considerando que essa estimativa não considera células de fungos e demais micro-organismos, o filoplano é de fato um dos maiores habitats microbianos do mundo. Além disso, estima-se que 96% das bactérias da filosfera sejam comensais, portanto não possuem efeito sobre a saúde do hospedeiro, enquanto 2% são patogênicas e 2% promovem crescimento e possuem funções benéficas ao hospedeiro (LINDOW; LEVEAU, 2002).

A configuração microclimática adversa e com constantes flutuações da filosfera demanda aos micro-organismos transpor uma série de barreiras e gargalos ambientais e ecológicos para colonizar esse ambiente (BEATTIE; LINDOW, 1995a; LINDOW; BRANDL, 2003; THAPA; PRASANNA, 2018; VORHOLT, 2012). Micro-organismos da filosfera geralmente interagem com esse ambiente por meio de duas estratégias básica. A primeira é a tolerância, que está relacionada a capacidade de suportar as condições ambientais extremas da filosfera, por exemplo, produzindo pigmentos fotoprotetivos e exopolissacarídeos (EPS) que auxiliam na tolerância à radiação UV e dessecação (BEATTIE; LINDOW, 1995a; THAPA; PRASANNA, 2018). A segunda é a estratégia de prevenção ou resguardo, que tem como cerne empregar mecanismos que evitem a direta exposição dos micro-organismos aos estressores

ambientais da filosfera. E essa pode ser, por exemplo, uma estratégia empregada por micro-organismos que crescem de forma endofítica (BEATTIE; LINDOW, 1995a; THAPA; PRASANNA, 2018). De certo, essas estratégias não são mutuamente excludentes, ou seja, os micro-organismos podem possuir as duas simultaneamente (BEATTIE; LINDOW, 1995a).

Segundo Kinkel (1997), a comunidade microbiana da filosfera é regulada por quatro processos populacionais; i) imigração, ii) emigração, iii) crescimento (geração) e iv) morte. Para Vellend (2010), os mecanismos que regulam as comunidades ecológicas são diversos, mas todos podem ser sintetizados em apenas quatro tipos de processos: seleção, deriva, especiação e dispersão. Nesse contexto, a seleção reflete diferenças na capacidade adaptativa (fitness); a deriva se refere as mudanças estocásticas nas abundâncias relativas das espécies em uma comunidade, a especiação se refere ao surgimento de novas espécies e dispersão trata do movimento dos organismos no ambiente (VACHER et al., 2016; VELLEND, 2010).

Um vasto número de fatores ambientais, ecológicos e evolutivos pode atuar sobre os processos que estruturam as comunidades da filosfera. Alguns dos fatores que alteram dispersão e colonização dos micro-organismos da filosfera envolvem as chuvas que promovem a remoção daqueles já presentes e trazem outros da atmosfera e do dossel adjacente; os ventos que podem transportar esporos e/ou micro-organismos na forma de bioaerosol e depositá-los sobre vegetais; animais (e.g. insetos e aves) que funcionam como vetores e transportam micro-organismos entre diferentes plantas e compartimentos. A microbiota pode ser passada por transferência vertical, onde a parental transmite a microbiota para as sementes (KOSKELLA, 2020; LEVEAU, 2019; VACHER et al., 2016; VORHOLT, 2012). A comunidade epifítica é afetada pela sazonalidade, fatores climáticos, genótipo e ontogênese da planta (BRINGEL; COUÉE, 2015; FONSECA; INÁCIO, 2006; VACHER et al., 2016; VORHOLT, 2012). Além dos fatores externos, as interações bióticas entre os micro-organismos que regulam os padrões de coexistência também estruturam a microbiota (VACHER et al., 2016).

A principal fonte de nutrientes orgânicos para os micro-organismos da filosfera (sobretudo das folhas) são os exsudados disponibilizados pelas plantas, enquanto os nutrientes inorgânicos são geralmente obtidos do meio externo, por exemplo, pela deposição dos ventos (FONSECA; INÁCIO, 2006; KEMLER et al., 2017). Desta forma, a fisiologia da planta possui um papel vital na disponibilidade nutricional e, consequentemente, na estrutura da comunidade microbiana. Duas vias estão relacionadas ao transporte de carboidratos nas plantas, a via simplástica e a apoplástica. Na via simplástica, o movimento de carboidratos se dá entre os citoplasmas célula-a-célula. Por outro lado, a via apoplástica, depende do movimento da sacarose nos espaços extracelulares, o apoplasto. Nesse tipo de transporte, alguns açúcares presentes no apoplasto não são carregados para o floema e difundem-se através da cutícula, um mecanismo essencial para a disponibilização de nutrientes para o filoplano. Esse mecanismo não se restringe a disponibilização de açúcares e pode transportar outros nutrientes orgânicos e inorgânicos (VACHER et al., 2016). Além disso, enquanto a composição de carboidratos no interior das folhas é dominada por sacarose, na superfície os açucares menores são mais frequentes como a glicose e frutose (VACHER et al., 2016).

2.4. A relação da microbiota da filosfera com a água

A filosfera é a porta da frente na interação planta-ambiente, é ela que recebe em primeira mão os efeitos do ambiente externo. A microbiota da filosfera, portanto, recebe esses estressores juntamente com o hospedeiro. Uma das condições mais desafiadoras e mais comuns que os micro-organismos enfrentam na filosfera é o estresse por déficit hídrico. Mesmo em ambientes mais úmidos, a cutícula é uma superfície hidrofóbica e com baixa molhabilidade (capacidade de um líquido em manter contato com uma superfície sólida), e quanto maior a cerosidade, maior é a dificuldade para a permanência da água nas regiões epifíticas como as folhas, gerando um ambiente com pouca água disponível e com grande pressão osmótica pelo acúmulo de metabólitos. Portanto, mecanismos de osmoproteção são vitais para a sobrevivência na filosfera, podem incluir acúmulo e/ou síntese de solutos que invertem a direção da pressão osmótica para dentro das células (BEATTIE; LINDOW, 1995b; THAPA;

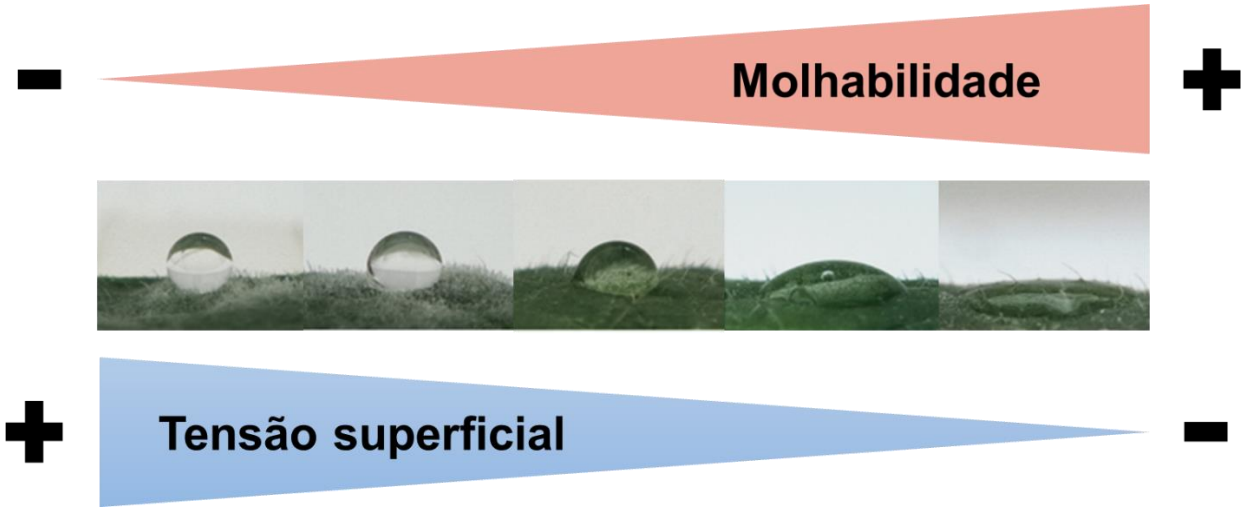
PRASANNA, 2018). A trealose, por exemplo, é um osmorregulador importante e comumente usado como estratégia de sobrevivência pela bactéria *Pseudomonas syringae* (THAPA; PRASANNA, 2018).

Alguns fatores podem alterar a disponibilidade de água na filosfera, tais como o envelhecimento do hospedeiro e o regime de chuvas (VACHER et al., 2016). O envelhecimento vegetal tende a alterar características físico-químicas na cutícula, que passa a ter maior molhabilidade e permeabilidade, aumentando a adesão da água e a disponibilidade de nutrientes (OSO et al., 2021; VACHER et al., 2016). Por outro lado, o efeito da chuva nas comunidades da filosfera ainda não está elucidado, enquanto alguns estudos sugerem um incremento na abundância (ALLARD et al., 2020), outros, sugerem um decréscimo (ALLARD et al., 2020; KINKEL, 1997). Há ainda estudos que indicam a ausência de efeito da chuva na comunidade da filosfera (STONE; JACKSON, 2021). É possível que a frequência e intensidade das chuvas também tenham relação com a resposta microbiana (KINKEL, 1997). Sabe-se que as chuvas alteram as taxas de colonização e dispersão, promovendo lavagem das folhas e retirada de micro-organismos, ao mesmo tempo que transferem para a planta outros micro-organismos da atmosfera (LEVEAU, 2019; MORRIS, 2001; VACHER et al., 2016).

Pela natureza oligotrófica e adversa da filosfera, esse ambiente tende a selecionar micro-organismos capazes de ‘remodelá-lo’ para acessar os recursos. Por exemplo, estreitando a cutícula, produzindo hormônios, enzimas, exopolissacarídeos (EPS) e biossurfactantes (BEATTIE; LINDOW, 1995b; LEVEAU, 2019). Algumas dessas estratégias são capazes de aumentar e/ou facilitar o acesso à água e a tolerância a seca do micro-organismo. Por exemplo, o fitopatógeno *P. syringae* pode aumentar a molhabilidade e a disponibilidade local de água na folha liberando biossurfactantes (KOSKELLA, 2020). Devido à natureza hidrofóbica da cutícula, o aumento da molhabilidade desse ambiente pode permitir a solubilização e difusão de nutrientes à medida que permite uma maior adesão da água (Figura 3) (LINDOW; BRANDL, 2003). Folhas muito cerosas possuem pouca molhabilidade e mesmo se houver água disponível através da umidade ou da chuva, dificilmente se formará um filme de água. A formação desse filme de água, além de promover disponibilidade

hídrica para a comunidade microbiana na folha, altera rapidamente o pH e redistribui nutrientes na região epifítica (MORRIS, 2001).

Figura 3 - Esquema indicando como a diminuição da tensão superficial e aumento da molhabilidade, que pode ser mediado por biossurfactantes, permite um maior contato da água com superfícies hidrofóbicas como as folhas.



Fonte: Elaborado pelo autor com imagens de He et al.(2021).

Biossurfactantes são moléculas quimicamente diversas produzidas por vários grupos microbianos que tem como principal característica ser anfipáticas, ou seja, possuem uma região polar e uma apolar. Essas moléculas possuem numerosas atividades como: diminuição de tensão superficial, aumento da molhabilidade em superfícies hidrofóbicas, emulsificação entre líquidos imiscíveis e solubilização de hidrocarbonetos (BEATTIE; LINDOW, 1995b; OSO et al., 2021; THAPA; PRASANNA, 2018; ZEISLER-DIEHL; BARTHLOTT; SCHREIBER, 2020). A produção de enzimas que degradam a cutícula e de biossurfactantes tem sido proposta como um mecanismo que pode aumentar a permeabilidade nas folhas e facilitar a movimentação de bactérias no filoplano (DOAN; LEVEAU, 2015; LEVEAU, 2019; LINDOW; BRANDL, 2003; OSO et al., 2021). Assim, a produção de biossurfactantes pode ser uma ferramenta importante

para que micro-organismos epifíticos alterem seu habitat para explorá-lo com maior eficiência (LEVEAU, 2019; LINDOW; BRANDL, 2003).

Por sua vez, a produção de EPS, formação de uma matriz extracelular e biofilme podem auxiliar a comunidade microbiana da filosfera, conferindo, entre outros atributos, tolerância a dessecação. Muitas vezes os EPSs que formam a matriz são higroscópicos (capazes de reter água), conferindo vantagem num ecossistema com déficit hídrico. Além disso, a matriz gerada pode dar gênese a um biofilme microbiano, que é uma estratégia onde populações ou comunidades microbianas formam um complexo e estruturado agregado celular, capaz de responder de forma coordenada a pressões ambientais (OSO et al., 2021; THAPA; PRASANNA, 2018; ZEISLER-DIEHL; BARTHLOTT; SCHREIBER, 2020).

2.5. Leveduras

Leveduras constituem um grupo artificial formado por várias linhagens de fungos com convergência na regulação de mecanismos moleculares que permitem a manutenção de um estado unicelular (NAGY et al., 2014). Apesar de serem predominantemente unicelulares, algumas espécies alternam entre fase unicelular e filamentosa, podendo transitar entre fases intermediárias através da formação de pseudohifas e aglomerados celulares, sendo conhecidas como *yeast-like,* fungos dimórficos ou fungos semelhantes a leveduras (BASTIDAS; HEITMAN, 2009; BOEKHOUT et al., 2011). Assim como nos demais fungos, a parede celular das leveduras também é formada por polissacarídeos estruturais microfibrilares, representados principalmente por quitinas e β-glicanas, responsáveis por conferir rigidez e resistência à parede. Outros compostos como β homo- e hétero-polissacarídeos, proteínas, pequenos lipídeos e sais inorgânicos também são encontrados na parede e atuam como elementos de sedimentação (ORTIZ-CASTELLANOS; RUIZ-HERRERA, 2015).

Fungos podem ser monomórficos ou dimórficos, apresentado transições morfológicas reguladas por fatores moleculares e diferentes gatilhos ambientais (BASTIDAS; HEITMAN, 2009; NAGY et al., 2014). Os mecanismos internos

e externos que desencadeiam essas mudanças morfológicas são diversos e podem apresentar ligação com a fase do ciclo de vida do organismo, fatores nutricionais, evolutivos e com estresses ambientais (ex. temperatura) (BASTIDAS; HEITMAN, 2009; NAGY et al., 2014). A transição entres estes estágios morfológicos pode estar associada a processos patogênicos em algumas espécies, como em *Candida albicans*, que é capaz de mudar (*shift*) para um estado filamentoso que beneficia a adesão às mucosas do hospedeiro (BASTIDAS; HEITMAN, 2009; COOPER, 2011). A linha que separa um estado morfológico de outro (unicelular, dimórfico ou filamentoso) é um terreno cinzento e ainda sem consenso. Provavelmente a morfologia é um continuum, desta forma a nomenclatura estática e categórica utilizada para denominá-los possui caráter utilitário e pedagógico. Além disso, considerando que todos os fungos (ou ao menos a maioria das linhagens) possuem as ferramentas moleculares necessários para a gênese e manutenção do estado unicelular (NAGY et al., 2014), é provável que espécies que hoje são consideradas estritamente filamentosas possam ser encontradas no futuro em um estado leveduriforme e vice-versa.

A principal forma de reprodução das leveduras é assexuada, via brotamentos, fissão ou produção de esporos ejetáveis (balistosporos). Porém, podem apresentar estágios de reprodução sexual sem a produção de corpos de frutificação (KURTZMAN, 2011a, 2011b). Em leveduras a poliploidia é um fenômeno comum (BOEKHOUT et al., 2011; ROBERTS; OLIVER, 2011), o aumento no número da ploidia pode estar relacionado a uma resposta a pressões ambientais. A poliploidia permite que o organismo tenha um maior número de genes disponíveis em seu “arsenal”, que por sua vez, pode influenciar seu *fitness* (VOORDECKERS et al., 2015). Outro ponto é que a poliploidia tem nuances biotecnológicas, por exemplo, muitas linhagens poliploides têm sido aplicadas em processos fermentativos por serem superiores em desempenho e mais tolerantes a estresses do processo industrial como valores extremos de temperatura e pH (SELMECKI et al., 2015).

Filogeneticamente, leveduras são agrupadas em dois filos do reino Fungi: Ascomycota e Basidiomycota (sub-reino Dikarya) (KURTZMAN, 2011a, 2011b). O filo Ascomycota possui três subfilos: Pezizomycotina, Taphrinomycotina e

Saccharomycotina (KURTZMAN, 2011a, 2011b). A sinapomorfia que une o filo dos ascomicetos é a formação de esporos meióticos em ascos, conhecidos como ascósporos (SCHOCH et al., 2009). O filo Basidiomycota também possui três subfilos, são eles: Pucciniomycotina, Agaricomycotina e Ustilaginomycotina (KURTZMAN, 2011a, 2011b). A sinapomorfia que une o grupo são esporos meióticos 'nus' (basidiósporos), sem asco, que se originam de uma estrutura em forma de haste (basídio) (BOEKHOUT et al., 2011).

As leveduras são organismos heterotróficos e as enzimas extracelulares possuem papel fundamental em sua nutrição. São elas que hidrolisam macromoléculas e deixam os nutrientes disponíveis para que a célula possa absorvê-los, e desta forma, direta ou indiretamente mediam os processos de decomposição (ORTIZ-CASTELLANOS; RUIZ-HERRERA, 2015). Leveduras são importantes decompositores nos ecossistemas e um dos primeiros grupos a colonizar substratos ricos em nutrientes (FONSECA; INÁCIO, 2006; GANTER; MORAIS; ROSA, 2017; STARMER; LACHANCE, 2011). Esses organismos estão distribuídos ao redor do planeta, presentes em múltiplos habitats e nichos, associados a plantas, animais e até mesmo a outros fungos. Estão presente em substratos como água doce e salgada, solo e atmosfera, em ambientes naturais e urbanos, úmidos e desérticos (BOEKHOUT et al., 2022; ROSA; PÉTER, 2006; STARMER; LACHANCE, 2011).

Leveduras são capazes de formar biofilme e, por exemplo, alterar a textura do solo, contribuindo consideravelmente para a biomassa microbiana destes. Muitas leveduras são capazes de produzir fatores que estimulam o crescimento das plantas e antagonizar com fitopatógenos (BOTHA, 2011; BRINGEL; COUÉE, 2015; MOLLER; LERM; BOTHA, 2016). As várias interações ecológicas que leveduras podem participar incluem amensalismo, competição, predação, mutualismo e até mesmo como parasitas de outros fungos (BOTHA, 2011). Um bom exemplo é que alguns líquens podem ser constituídos de associação mutualísticas entre macrofungos, leveduras e algas (SPRIBILLE et al., 2016). Em relação ao padrão biogeográfico de leveduras, Tedersoo et al. (2014) demonstraram que em nível global, leveduras de solo diminuem sua

diversidade à medida que se afastam dos trópicos, seguindo o padrão geral de distribuição de espécies, que se aplica também para macro-organismos.

Várias espécies de *Papiliotrema* (anteriormente classificadas como *Cryptococcus*) podem produzir cápsulas polissacarídicas que conferem resistência à dessecação (FONSECA; INÁCIO, 2006; KEMLER et al., 2017). Além disso, características como a produção de pigmentos podem gerar uma vantagem competitiva em um ambiente exposto a diversos estresses oxidativos, como a superfície das folhas. Estes pigmentos são diversos, mas são muitas vezes carotenóides que são amplamente encontrados em membros de Sporidiobolales (por exemplo, *Rhodotorula* e *Sporobolomyces*), ou melanina, produzida por leveduras negras como *Aureobasidium*, *Hortaea* e *Valentiella* (BEZERRA et al., 2022; FONSECA; INÁCIO, 2006; KEMLER et al., 2017). Algumas espécies de *Sporobolomyces* e *Bullera* produzem balistosporos, esporos ejetáveis com função de dispersão (FONSECA; INÁCIO, 2006; KEMLER et al., 2017). Leveduras basidiomicetos são geralmente mais generalistas e capazes de assimilar diversas fontes de carbono (politróficas), tendo vantagem em ambientes oligotróficos. Portanto, são frequentes e dominantes na filosfera, principalmente associados diretamente à cutícula (FÉLIX et al., 2022; FONSECA; INÁCIO, 2006; HAGLER et al., 1993; KEMLER et al., 2017).

Leveduras da filosfera tendem a ocorrer em folhas em uma abundância média de 10^3 a 10^5 CFU/g, relativamente baixo comparado a bactéria (GOUKA; RAAIJMAKERS; CORDOVEZ, 2022). Fatores com a disponibilidade de nutrientes, e genótipo do hospedeiro podem afetar a abundância de leveduras da filosfera (GOUKA; RAAIJMAKERS; CORDOVEZ, 2022). A montagem da comunidade de leveduras da filosfera envolve uma gama de fatores estocásticos e determinísticos. Por exemplo, a colonização possui facetas estocásticas e ocorre principalmente através de vetores como a chuva, os ventos e animais (majoritariamente insetos e aves). Por outro lado, a seleção envolve processos determinísticos relacionados a espécies do hospedeiro e ao *fitness* das espécies ou linhagens de levedura colonizadora (ANDREWS et al., 1987; BLACKWELL, 2017; KINKEL, 1997; MITTELBACH et al., 2015; VACHER et al., 2016; VELLEND, 2010). A abundância de leveduras da filosfera pode ser afetada por diversos

fatores. Alguns estudos sugerem que a sazonalidade e a senescência do hospedeiro incrementam essa abundância (FONSECA; INÁCIO, 2006; GLUSHAKOVA; CHERNOV, 2004, 2007; GOUKA; RAAIJMAKERS; CORDOVEZ, 2022).

Leveduras da filosfera apresentam uma ampla diversidade de interações com o hospedeiro e com outros micro-organismos. Na interação com o hospedeiro, as leveduras podem estimular o crescimento vegetal produzindo hormônios como o ácido indolacético e elicitando as defesas da planta através das vias do ácido jasmônico, ácido salicílico e etileno (GOUKA; RAAIJMAKERS; CORDOVEZ, 2022) . Em interações com micro-organismos a produção de toxinas-*killer* pode oferecer vantagem na competição sobre outras leveduras (GOUKA; RAAIJMAKERS; CORDOVEZ, 2022). Leveduras possuem a capacidade se acessar e assimilar uma gama de compostos orgânicos, bem como, de tolerar e degradar compostos tóxicos (micotoxinas e antifúngicos), essas habilidades conferem vantagens competitivas por espaço e nutriente sobre outros grupos de micro-organismos como bactérias e fungos filamentosos (GOUKA; RAAIJMAKERS; CORDOVEZ, 2022).

Há uma grande lacuna em relação a riqueza de fungos no mundo. Uma estimativa recente é de que existam entre 2,2 e 3,8 milhões de espécies de fungos, porém o número de espécies corretamente descritas é de aproximadamente 120.000 (de 3,15 a 5,45% do total estimado) (HAWKSWORTH; LÜCKING, 2017). Em relação a leveduras, a estimativa é de cerca de 200.000 espécies (BOEKHOUT et al., 2022) e a diversidade de leveduras atualmente descrita está entre 2.200 e 2.300 espécies (BOEKHOUT et al., 2022; YURKOV et al., 2021), pouco mais de 1% da riqueza total estimada.

Alguns dos maiores reservatórios de novidades taxonômicas de fungos filamentosos e leveduras são plantas vasculares (sobretudo na América do Sul), insetos e solo (BOEKHOUT et al., 2022; HAWKSWORTH; LÜCKING, 2017). Nas últimas décadas houve aumento no número de espécies de leveduras descritas. Entre os anos de 1952-1984 a taxa de descrição era de 10 espécies por ano, entretanto, nos últimos anos esse número tem estado acima de 60 espécies (BOEKHOUT et al., 2022). Além

disso, Li et al. (2020), descreveram mais de 100 espécies em um único estudo, o que indica o avanço dessa área. Alguns dos ecossistemas que continuam subamostrados são os ambientes áridos e gélidos, porém, esses parecem promissores para a descoberta de novas espécies (BOEKHOUT et al., 2022).

2.6. Bromélias

Bromeliaceae é uma família de plantas quase exclusivamente neotropical reconhecida, entre outros fatores, por sua grande diversidade, alto endemismo e por fornecer diversos serviços ecossistêmicos de apoio à biodiversidade e regulação hídrica (BENZING, 2000; LADINO et al., 2019). A família é composta por 3500 espécies, 50 gêneros e 8 subfamílias. Bromélias possuem distribuição por diversos países Sul e Centro-americanos, chegando a ocorrer até o Sudeste da América do Norte. Apenas uma espécie é encontrada fora das américas, a espécie *Pitcarnia feliciana*, que ocorre na Costa Oeste da África (BENZING, 2000; MANETTI; DEIAPORTE; LAVERDE, 2009). A espécie de bromélia mais conhecida e com maior importância econômica é *Ananas comosus* (abacaxi) que é a única espécie da família cultivada extensivamente para alimentação (MANETTI; DEIAPORTE; LAVERDE, 2009). Bromélias atraem o interesse científico devido às suas associações com vários grupos de macro e micro-organismos, que variam de invertebrados e anfíbios a vírus, bactérias e leveduras como ilustrado na Figura 4 (GOFFREDI; JANG; HAROON, 2015; LANDELL et al., 2015; LEROY et al., 2016, 2017).

Cerca de metade dos gêneros de bromélias e mais da metade das subfamílias contém espécies capazes de formar tanques de água no centro da roseta, uma estrutura chamada fitotelma (LADINO et al., 2019; MALES; GRIFFITHS, 2017). Esse tanque de água contribui, por exemplo, para a reprodução de diversas espécies animais, tanto vertebrados como anfíbios, quanto invertebrados como artrópodes (BENZING, 2000; LADINO et al., 2019). Dependendo da espécie e das condições ambientais, o fitotelma pode acumular de 0,015 a 45 L de água em um único indivíduo (ZOTZ et al., 2020), e cerca de 50.000 L em um hectare (LADINO et al., 2019). A capacidade de formar o fitotelma faz com que bromélias, sob determinadas condições,

consigam alterar a dinâmica hídrica local (LADINO et al., 2019). Bromélias podem funcionar como um ecossistema insular em microescala (FRANK; LOUNIBOS, 1987), e talvez também como refúgio em épocas de seca, principalmente para organismos aquáticos ou fortemente dependentes da água.

Figura 4- Ilustração de um corte longitudinal em uma bromélia capaz de formar tanque (fitotelma).

Fonte: (BOND, 1975).

Inovações evolutivas, estratégias ecofisiológicas, além de diversos mecanismos de captação de água, explicam o amplo espectro de ocorrência das bromélias. O grande espectro ambiental de bromélias vai desde o seu hábito que pode ser epífito, terrestre ou saxícola (rupícolas) e sua distribuição, que ocorre de 0 a 4000 metros de altitude, de florestas seca a úmidas e de ambientes tropicais a temperados (BENZING, 2000; LADINO et al., 2019; MALES; GRIFFITHS, 2017). As espécies exclusivamente epífitas possuem adaptações fisiológicas e morfológicas que permitem sobreviver sem contato com o solo, absorvendo água e nutrientes apenas através de tricomas nas folhas (BASÍLIO et al., 2015; BENZING, 2000). Nestas espécies, com destaque para as do gênero *Tillandsia*, as raízes perdem total ou parcialmente a função de absorção e passam a ter função de fixação ao substrato.

Traços importantes que contribuíram para o sucesso evolutivo das bromélias são: os tricomas absorventes das folhas, o surgimento do metabolismo fotossintético tipo CAM (Metabolismo ácido das crassuláceas), a capacidade de retenção de água no tanque e a suculência (BENZING, 2000; MALES; GRIFFITHS, 2017). Essas características são importantes para a tolerância à seca e, possivelmente, para a colonização de ambientes xerófilos e de altitude. Males e Griffiths (2017) combinaram a filogenia das bromélias com cinco tipos funcionais, são eles: C_3 terrestres, C_3 tanque-epífitas, CAM atmosférica-epífitas, CAM terrestres e CAM tanque-epífitas. Entre esses tipos funcionais, foram observadas variações morfológicas, ecológicas e fisiológicas. Por exemplo, existem diferenças em características relacionadas à tolerância à seca, como massa de água por unidade de área e potencial osmótico em pleno turgor (MALES; GRIFFITHS, 2017). Além disso, esses grupos funcionais são indicadores indiretos de maior ou menor dependência da raiz para absorção de água. Em termos simples, as epífitas atmosféricas CAM são as mais adaptadas para absorção através de tricomas foliares, enquanto plantas C_3 terrestre são as mais dependentes da absorção das raízes (BENZING, 2000; MALES; GRIFFITHS, 2017).

As inflorescências de bromélias constituem um habitat complexo constituído por vários compartimentos como sépalas, pétalas e brácteas florais. Essas estruturas variam muito em toda a família em formas e cores, além do néctar que varia em

composição e concentração de açúcar (BENZING, 2000; KRÖMER et al., 2008). A família Bromeliaceae possui um amplo espectro de polinizadores, desde insetos, aves, morcegos e até roedores, mas é em grande parte polinizada por beija-flores (SIQUEIRA-FILHO; LEME, 2006). Algumas bromélias têm inflorescências discretas, mas a grande maioria tem inflorescências coloridas, grandes e vistosas, além de produzir grandes quantidades de néctar, uma característica incomum em angiospermas e que explica sua associação predominante com polinizadores vertebrados (BENZING, 2000; BERNARDELLO, 1992; WOLOWSKI; FREITAS, 2015).

3. MÉTODOS

3.1. Caracterização da área de estudo

As amostras de folhas foram coletadas de bromélias da espécie *Bromelia laciniosa* Mart. Ex Schult. & Schult.f (Bromeliaceae, Bromelioideae), uma espécie endêmica da Caatinga (FERREIRA; FABRICANTE; SIQUEIRA-FILHO, 2015). É usada na medicina popular e suas folhas, flores e frutos utilizados no tratamento de cólicas infantis, diarreia, febre, icterícia, caspa e hepatite (DE ALBUQUERQUE et al., 2007). As coletas foram realizadas ao longo de um transecto de 5 × 200 metros na Reserva Particular do Patrimônio Natural Tocaia (RPPN Tocaia), no município de Santana do Ipanema, Alagoas-Brasil, numa área de Caatinga (9°23'08.9"S e 37 °15'22.8"W). Nessa região, as chuvas ocorrem de forma irregular, com volume anual de 500 a 700 mm. A RPPN Tocaia possui uma área de aproximadamente 20 hectares e altitude máxima de cerca de 400 metros, é composta principalmente por uma fisionomia arbórea. O clima predominante é do tipo semiárido Bsh (Köppen).

3.2. Coleta do material

Foram realizadas 12 coletas. Destas, seis no período seco (outubro, novembro e dezembro de 2020 e de 2021) e seis no período chuvoso (maio, junho e julho de 2021 e de 2022). Em cada coleta, cinco indivíduos de bromélias adultas e aparentemente saudáveis foram amostrados aleatoriamente ao longo do transecto

previamente estabelecido. De cada indivíduo de bromélia coletado foram retiradas 3 folhas escolhidas ao acaso, totalizando 60 indivíduos e 180 folhas coletadas durante as 12 coletas. As folhas foram armazenadas em sacos plásticos estéreis e transportadas em temperatura ambiente (27±2 °C) por aproximadamente 4 h até o Laboratório de Diversidade Molecular da UFAL. No laboratório, as folhas foram armazenadas a 10-15 °C e o processamento foi realizado em no máximo 24 h após a coleta do material em campo.

3.3. Processamento das amostras

O processamento das amostras seguiu metodologia modificada a partir de Landell, Mautone e Valente (2006). As folhas passaram por uma lavagem inicial com água destilada estéril para remoção de possíveis contaminantes como poeira e outros materiais exógenos. Em seguida, contemplando as 3 folhas coletadas de cada planta, fragmentos foliares entre 5 e 20 cm² foram cortados aleatoriamente até se obter uma área total de 120 cm². Sequencialmente a esse procedimento, a massa dos 120 cm² de folha foi aferida para cada amostra. Os fragmentos das folhas foram alocados em um frasco Erlenmeyer de volume igual a 250 mL contendo 75 mL de água destilada estéril. Em seguida esse frasco foi agitado por 30 min a 180 RPM e temperatura ambiente. Após a agitação, o produto de lavagem foi recolhido em um frasco estéril e os fragmentos de folhas preservados no frasco Erlenmeyer. Após retirar a água da primeira lavagem, adicionou-se 75 mL de solução de Tween 80 0,5% e o material foi novamente agitado sob o mesmo tempo, rotação e temperatura. O produto da segunda lavagem foi então agregado ao da primeira, formando assim um *pool*. Uma fração de 100 µL deste *pool* foi semeada em duplicata em placas de Petri contendo meio Ágar YM modificado (0,3% extrato de levedura, 0,3% extrato de malte, 0,5% peptona bacteriológica, 1% glicose, 2% ágar, 0,04% cloranfenicol, pH 4,0) nas concentrações de 10^0 e 10^{-1}. As placas foram incubadas a 25-28 °C e observadas diariamente durante 10 dias para o isolamento das leveduras.

3.4. Obtenção e armazenamento dos isolados

Colônias representando morfotipos distintos de leveduras foram delimitadas considerando características como cor, margem, textura, forma e elevação da colônia. Representantes desses morfotipos foram isolados em meio Ágar YEPD (2% glicose, 1% peptona bacteriológica, 0,5% extrato de levedura e 2% ágar). Para verificar se as colônias isoladas eram realmente de leveduras, utilizou-se microscopia óptica de luz nos aumentos de 40 e 100x. Todas os isolados de levedura encontrados durante os 10 dias de observação foram preservadas em 1) meio Ágar GYMP (2% de glicose, 2% de extrato de malte, 0,5% de extrato de levedura, 0,2% de fosfato de sódio monobásico e 2% de ágar), 2) caldo GYMP modificado (1% de glicose, 1% de extrato de malte, 0,25% de extrato de levedura e 0,1% de fosfato de sódio monobásico) e 3) caldo GYMP contendo volume final de 30% de glicerol. Os meios 1 e 2 foram armazenados entre 5 e 15 °C e o meio 3 entre -10 e -20 °C.

3.5. Análise de traços funcionais

Traços metabólicos como a capacidade de fermentar glicose, assimilar diferentes fontes de carbono, produzir enzimas extracelulares e surfactantes/emulsificantes foram avaliadas para se criar um perfil funcional para cada isolado.

3.5.1. Fermentação de glicose

A capacidade dos isolados de leveduras fermentar glicose foi avaliada utilizando tubos de ensaio com meio básico para fermentação de glicose (0,75% de peptona bacteriológica, 0,45% de extrato de levedura e 2% glicose) (KURTZMAN et al., 2011). Tubos de *Durham* invertidos foram posicionados dentro dos tubos de ensaio, os isolados que fermentaram glicose produziram gás carbônico que se acumulou no interior dos tubos de *Durham*, indicando resultado positivo para o teste. Esse teste foi graduado em grau 1, 2 ou 3 dependendo de quanto gás se armazenou no tubo, sendo que no grau 3 o tubo estava totalmente preenchido por gás. O teste durou 21 dias, com leituras diárias nos primeiros cinco dias, e semanais a partir do sétimo dia.

3.5.2. Assimilação de fontes de carbono

O teste de assimilação de fontes de carbono foi aplicado para determinar a capacidade das leveduras utilizarem diferentes fontes de carbono no crescimento. Para o inóculo, células de leveduras foram diluídas em 2 mL de água destilada estéril até atingir uma concentração aproximada de 10^5 células/mL, o que equivale ao grau 1 do cartão de Wickerham. Após isso, foram incubadas em temperatura ambiente (27±2 °C) entre 24-48 h para que suas reservas fossem exauridas, diminuindo a chance de um falso positivo. O perfil de assimilação foi avaliado semanalmente, durante 21 dias incubados a 22-25 °C seguindo Kurtzman et al. (2011). As fontes de carbono utilizadas foram celobiose, D-arabinose, galactose, glicose, glicerol, inulina, rafinose, ramnose e xilose. Os meios de cultura para os testes de assimilação das fontes de carbono consistiam em 0,67% de *Yeast Nitrogen Base* (YNB), 2% de ágar ultrapuro Difco® e 0,5% da fonte de carbono especificada (exceto, Rafinose 1%).

3.5.3. Produção de hidrolases extracelulares

A capacidade dos isolados produzirem seis enzimas hidrolíticas extracelulares foi avaliada: amilase, caseinase, celulase, esterase, lipase e pectinase. O diâmetro dos halos de hidrólise e das colônias de cada isolado foram medidos para avaliar a produção de enzimas extracelulares através da atividade enzimática (pz), aplicada a equação modificada do $\boldsymbol{pz} = \left|\left(\frac{\boldsymbol{Dc}}{\boldsymbol{Dc+Dh}}\right) - \mathbf{1}\right|$**.** Onde pz representa a atividade enzimática, Dh e Dc são, respectivamente, o diâmetro do halo de hidrólise e o diâmetro da colônia. A padronização do inóculo para avaliação da produção enzimática foi realizada utilizando a turbidez. As células dos isolados foram diluídas em 2 mL de água destilada estéril até atingir uma concentração aproximada de 10^5 células/mL (i.e., grau 1 do cartão de Wickerham). Em seguida, o inóculo foi aplicado em forma de ponto no meio de cultura utilizando o carimbo replicador (método de *replica plating*). As placas de petri contendo os meios para cada enzima foram analisadas após um período de 7-10 dias de incubação a 25-28 °C.

A atividade amilolítica foi avaliada usando meio de cultura Ágar amido modificado (0,5% amido solúvel, 0,5% peptona bacteriológica, 0,5% extrato de

levedura, 0,05% sulfato de magnésio, 0,001% sulfato de ferro, 0,001% cloreto de sódio, 1,5% ágar). Após o período de incubação, uma solução de lugol a 1% foi adicionada à placa para visualização do halo de hidrólise. O isolado com atividade amilolítica apresentou um halo que não é corado por lugol e permanece claro (BUZZINI; MARTINI, 2002; CARRASCO et al., 2012; MAUTONE et al., 2010).

A atividade celulolítica foi avaliada usando o meio Ágar CMC modificado (0,5% carboximetilcelulose (CMC), 0,1% nitrato de sódio, 0,1% fosfato monobásico de potássio, 0,1% cloreto de potássio, 0,05% sulfato de magnésio, 0,05% extrato de levedura, 0,1% glicose e 1,7% ágar). Para visualização do resultado, adicionou-se solução de vermelho Congo 0,1% às placas, que foram deixadas em repouso com a solução por 40 min. Em seguida, as placas foram lavadas com solução de NaCl 1M. Em caso de resultado positivo para hidrólise de CMC, a formação de um halo alaranjado foi observada (BUZZINI; MARTINI, 2002; CARRASCO et al., 2012).

A atividade de esterase foi avaliada em meio Ágar Tween 80 modificado (2,5% tween 80, 0,1% glicose, 1% peptona bacteriológica, 0,5% cloreto de sódio, 0,1% cloreto de cálcio e 2% ágar). Após o período de incubação, os isolados que hidrolisaram o substrato apresentaram um halo formado por um precipitado esbranquiçado (BUZZINI; MARTINI, 2002; CARRASCO et al., 2012).

A atividade da protease foi avaliada em meio Ágar Caseína modificado (1% caseína, 0,5% glicose e 2% ágar pH 7,0). Após o período de incubação, os isolados produtores de caseinase apresentaram um halo esbranquiçado (BUZZINI; MARTINI, 2002; CARRASCO et al., 2012).

A atividade da pectinase foi avaliada usando o meio Ágar Pectina modificado (0,67% de base de nitrogênio de levedura (YNB), 1% de pectina cítrica, 1% de glicose e 1,8% de ágar, pH 7,0). Após o período de incubação, solução de brometo de hexadeciltrimetilamônio (CTAB) a 1% foi adicionada à placa. Os isolados produtores apresentaram um halo mais translúcido ao redor da colônia (BUZZINI; MARTINI, 2002; CARRASCO et al., 2012).

3.5.4. Produção de surfactantes/emulsificantes

A atividade tensoativa foi avaliada por meio do teste de Parafilm® M. Os isolados foram cultivados durante 48 h em caldo YEPD em temperatura ambiente (27±2 °C), em seguida 20 µL do cultivo foi adicionado em triplicata sobre fragmentos de parafilme M e mantidos em repouso por 1 min. Após esse tempo, as gotas formadas pela aplicação do cultivo na superfície hidrofóbica do parafilme tiveram seus diâmetros aferidos. Além disso, o formato da gota foi categorizado conforme seu espalhamento. Foram utilizados três controles: caldo YEPD estéril, água destilada estéril (ambos controles negativos) e solução de SDS 1% (controle positivo). Apresentar um diâmetro maior e uma gota mais plana são indícios que a tensão superficial da gota foi diminuída, indicando atividade tensoativa.

Para verificar a atividade bioemulsificante, foi usado o índice de emulsão com estabilidade após 24 h (IE_{24}). Os isolados foram cultivados em 2 mL de caldo YEPD por 48 h, à temperatura ambiente (27±2 °C). Após o crescimento, 2 mL de querosene foi adicionada ao cultivo e agitada com auxílio de um vórtex por 2 min e mantida em repouso por 24 h. Em seguida foram aferidos a altura total do cultivo e a altura da emulsão em centímetros. O IE_{24} foi obtido a partir da equação $\boldsymbol{IE_{24}} = \left(\frac{\boldsymbol{ALT_{emulsão}}}{\boldsymbol{ALT_{total}}}\right) \times \mathbf{100}$, ou seja, a proporção da altura da emulsão em relação à altura total do cultivo, em porcentagem.

3.6. Identificação molecular dos isolados

3.6.1. Extração de DNA genômico

Para a identificação molecular dos isolados, o DNA genômico foi extraído utilizando o protocolo para preparação em pequena escala de DNA de leveduras modificado, originalmente proposto por Sambrook e Russel (2001). Para tal, inicialmente as culturas puras dos isolados foram inoculadas em caldo YEPD durante 2-3 dias a 22-25°C. Após, as células foram precipitadas em tubos de microcentrífuga de 1,5 mL a 10000 x g durante 5 min. Após a centrifugação, o sobrenadante foi descartado e adicionou-se 1 mL de água destilada estéril e homogeneizado. Em seguida, as células

foram novamente centrifugadas sob mesma rotação e tempo. Ao final, o sobrenadante foi novamente descartado.

Foi adicionado ao precipitado de células obtidos no passo anterior 500 µL de solução de lise (0,15 M de NaCl, 50 mM de Tris-HCl pH 8, 10 mM de ácido etilenodiamino tetra-acético (EDTA) pH 8 e 2% de dodecil sulfato de sódio-SDS) e homogeneizado por agitação em *vortex.* Em seguida, os tubos contendo o homogeneizado foram incubados em banho-maria a 65°C por 1 h. Logo após retirar os tubos, adicionou-se imediatamente 200 µL de acetato de potássio 5M pH 4.8, a reação foi homogeneizada por 30 s e incubada em banho de gelo por 30 min. Em seguida, os tubos foram centrifugados a 14000 x g por 5 min e o sobrenadante foi transferido para outro tubo de microcentrífuga. O tubo foi novamente centrifugado a 14000 x g por 5 min e o sobrenadante foi transferido para outro tubo sem utilizar micropipeta. Seguindo, adicionou-se 600µl de isopropanol absoluto conservado a -20 °C. Os tubos foram gentilmente agitados por inversão durante 5 min e mantidos a -20 ºC de um dia para outro. Os tubos foram então centrifugados a 14000 x g durante 20 min e o sobrenadante foi descartado. Um volume de 500 µl de etanol 70% conservado a -20 °C foi adicionado e os tubos foram novamente centrifugados a 14000 x g por 5 min. Ao término o sobrenadante foi descartado e o precipitado passou por secagem a temperatura ambiente (27±2 °C) e foi posteriormente solubilizado em 50 µL de tampão Tris-EDTA (TE) numa proporção de 1 para 1, pH 7,4. A qualidade da extração de DNA foi aferida posteriormente em gel de agarose (0,8%) diluída em tampão TAE 1X (Solução mãe 50X = 242 g TRIS base, 57,1 mL ácido acético glacial e 100 mL de solução de EDTA 0,5 M pH 8,0, em 1 L de água destilada) (COBBAN et al., 2016).

3.6.2. PCR, sequenciamento e análise das sequências

As regiões D1/D2 do gene 26S LSU do rRNA (~600pb) foram amplificadas pela técnica de reação em cadeia da polimerase (PCR) utilizandos os oligonucleotídeos iniciadores NL-1 (5’- GCA TATC AAT AAG CGG AGG AAA AG -3’) e NL-4 (5’- GG TCC GTG TTT CAA GAC GG -3’) (FELL et al., 2000; KURTZMAN; ROBNETT, 1998). Para amplificar a região do espaçador interno transcrito (*Internal Transcrit Spacer* - ITS) do rRNA foram utilizados os oligonucleotídeos iniciadores ITS-1 (5’-TCC GTA GGT GAA

CCT GCG G-3') e ITS-4 (5'-TCC TCC GCT TAT TGA TAT GC-3') (FELL et al., 2000; SCHOCH et al., 2012). Os parâmetros da reação foram estabelecidos seguindo Landell et al. (2010) e o produto da reação foi observado em uma eletroforese usando gel de agarose (1%) diluído em tampão TAE 1X. Os produtos (25 µL) foram purificados através de uma nova precipitação utilizando isopropanol absoluto (20 µL) e acetato de sódio 3M (5 µL). O material foi centrifugado durante 20 min a 13.000 G, o sobrenadante foi descartado e substituído por etanol 70% (50 µL), centrifugado novamente e mais uma vez o sobrenadante foi descartado. Após a secagem do precipitado, o material foi suspenso em 15 µL de água ultrapura. Para o sequenciamento foi utilizado o método de Sanger através do protocolo do Laboratório de Genética Molecular Humana da Universidade Federal de Pernambuco em um sistema de sequenciamento automatizado ABI 3130 *Genetic Analyzer* utilizando o polímero *BigDye* v3.1 e POP7 (*Life Technologies*). Os consensos das sequências foram gerados no software *Staden Package* (STADEN et al., 2000) e MEGA X. Em seguida, as sequências obtidas foram comparadas com outras depositadas no banco de dados do *GenBank* no site do *National Center for Biotechnology Information* (NCBI) utilizando a ferramenta *Basic Local Alignment Search Tool* (BLAST) e o algoritmo BLASTn (ALTSCHUL et al., 1997).

3.7. Caracterização das possíveis espécies novas

Para a caracterização das possíveis espécies novas de leveduras foi empregado o modelo de taxonomia polifásica, incluindo caracterização e análises moleculares e fenotípicas. A informação molecular foi obtida através do sequenciamento das regiões D1/D2 e ITS do rRNA (*vide* secção anterior). A caracterização fenotípica seguiu os protocolos descritos por Kurtzman et al. (2011).

3.7.1. Assimilação de fontes de carbono e nitrogênio

O processo para obtenção do padrão de assimilação de fontes de C e N dos isolados de espécies não-descritas encontradas seguiu protocolo descrito na seção *3.5.2.* O meio base para as fontes de nitrogênio consistia em 1,17% de *Yeast Carbon Base* (YCB) e 2% de ágar ultrapuro Difco®, a concentração de cada fonte de nitrogênio é indicada no Quadro 1, bem como a lista de todas as fontes utilizadas.

Quadro 1- Lista de Fontes de carbono e de nitrogênio

Fontes de carbono		Fontes de nitrogênio
Glicose*	L-arabinitol	Peptona*,[d]
Ácido galacturônico[a]	N-acetilglicosamina (NAG)	
Ácido succínico [a]	Maltose	Cadaverina[c]
Amido	Manitol	
Celobiose	Melezitose	Creatina[d]
Citrato	Melibiose	
D-arabinose	Rafinose	Creatinina
L-arabinose	Ramnose	
Dulcitol	Ribitol	Etilamina[c]
Eritritol	Ribose	
Galactose	Sacarose[b]	Lisina[e]
Glicerol	Salicina	
Glucanato	Sorbitol	Nitrato[d]
Inositol	Trealose	
Inulina	Tween 20	Nitrito[c]
Lactato	Tween 80	
Lactose	Xilose	

*** Controle positivo**
[a] O pH foi ajustado para 7,0 antes da esterilização.
[b] A fonte de carbono foi adicionada após a esterilização para evitar degradação.
[c] Avaliada através do método de *pour plate* (concentração final da fonte 0,064%).
[d] Concentração final da fonte 0,078%.
[e] Concentração final da fonte 0,056%.

3.7.2. Formação de compostos amiloides extracelulares

A capacidade dos isolados formarem compostos amiloides extracelulares foi verificada após 21 dias de crescimento em 22-25 °C em meio básico para glicose (0,67% de YNB, 2% de ágar e 0,5% glicose). Após esse período, uma solução de lugol 1% foi adicionada sobre as colônias. Os isolados que produziram compostos amiloides formaram colônias com coloração azul e verde escuros (KURTZMAN et al., 2011).

3.7.4. Testes de produção de urease e reação ao Diazônio Azul B (DBB)

A avaliação da produção de urease e da capacidade do isolado de reagir ao corante DBB foram utilizados como indicadores presuntivos de afinidade aos filos Ascomycota ou Basidiomycota (KURTZMAN et al., 2011). Para o teste foi usado ágar ureia inclinado em tubos de ensaio (1,17% YCB, 1% de ureia, 2% de ágar e fucsina ácida), a ureia previamente esterilizada foi adicionada apenas após a esterilização pois é termossensível. Colônias puras foram semeadas no meio e observadas diariamente ao longo de três dias. Os isolados que foram capazes de produzir urease, hidrolisam a ureia produzindo amônia que, por sua vez, basifica o meio de cultura e neutralizaram a fucsina ácida, fazendo o meio perder a coloração rosa-arroxeada e ficar esbranquiçado. Após os três dias de leitura do teste de urease, os tubos foram incubados a 60 ºC durante 16 h. Após esse período, 1 mL de solução de DBB diluído em Tris-HCl 1 M pH 7,0 foi adicionada aos tubos. Os isolados foram considerados positivos para a reação quando a colônia apresentou uma coloração avermelhada em contato com a solução e, portanto, foram considerados com afinidade basidiomicética.

3.7.5. Testes de tolerância

3.7.5.1. Tolerância osmótica

Para verificar a tolerância osmótica, os isolados foram semeados e Ágar YEPD modificado contendo 50% de glicose (2% de ágar, 50% de glicose, 1% de peptona, 0,5% de extrato de levedura). O meio foi autoclavado durante 10 min a 110 °C. Além deste, YEPD modificado contendo 5 e 10% de NaCl também foi usado para verificar a tolerância osmótica dos isolados (KURTZMAN et al., 2011).

3.7.5.2. Ácido acético

Foi verificada a tolerância dos isolados de crescer em meio contendo 1% de ácido acético. Para tal, foi usado meio YEPD modificado contendo 1% de ácido acético glacial (KURTZMAN et al., 2011). O ácido só foi adicionado após esterilização do meio de cultura base.

3.7.5.3. Resistência a cicloheximida

A capacidade dos isolados em tolerar crescimento em meio contendo ciclohexamida foi verificada em meio básico para glicose (0,67% de YNB e 0,5% de glicose) com concentração final de 0,1 e 0,01% de cicloheximida (KURTZMAN et al., 2011). A cicloheximida foi acrescida no meio após a esterilização pois é termossensível. A leitura foi realizada após 7 dias de incubação a 22-25 °C.

3.8. Caracterização micromorfológica

Os isolados foram caracterizados morfologicamente seguindo Kurtzman et al. (2011). Para verificar a possível produção de pseudohifas, hifas verdadeiras e/ou estruturas sexuais, os isolados foram semeados em Ágar Batata Dextrose (BDA), Ágar Fubá, Ágar Extrato de Malte e Ágar YEPD. Os isolados foram incubados a 22-25 °C e observados semanalmente por 21 dias.

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4. CAPÍTULO 1 - Different plant compartments, different yeasts: the example of the bromeliad phyllosphere

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Take Away:

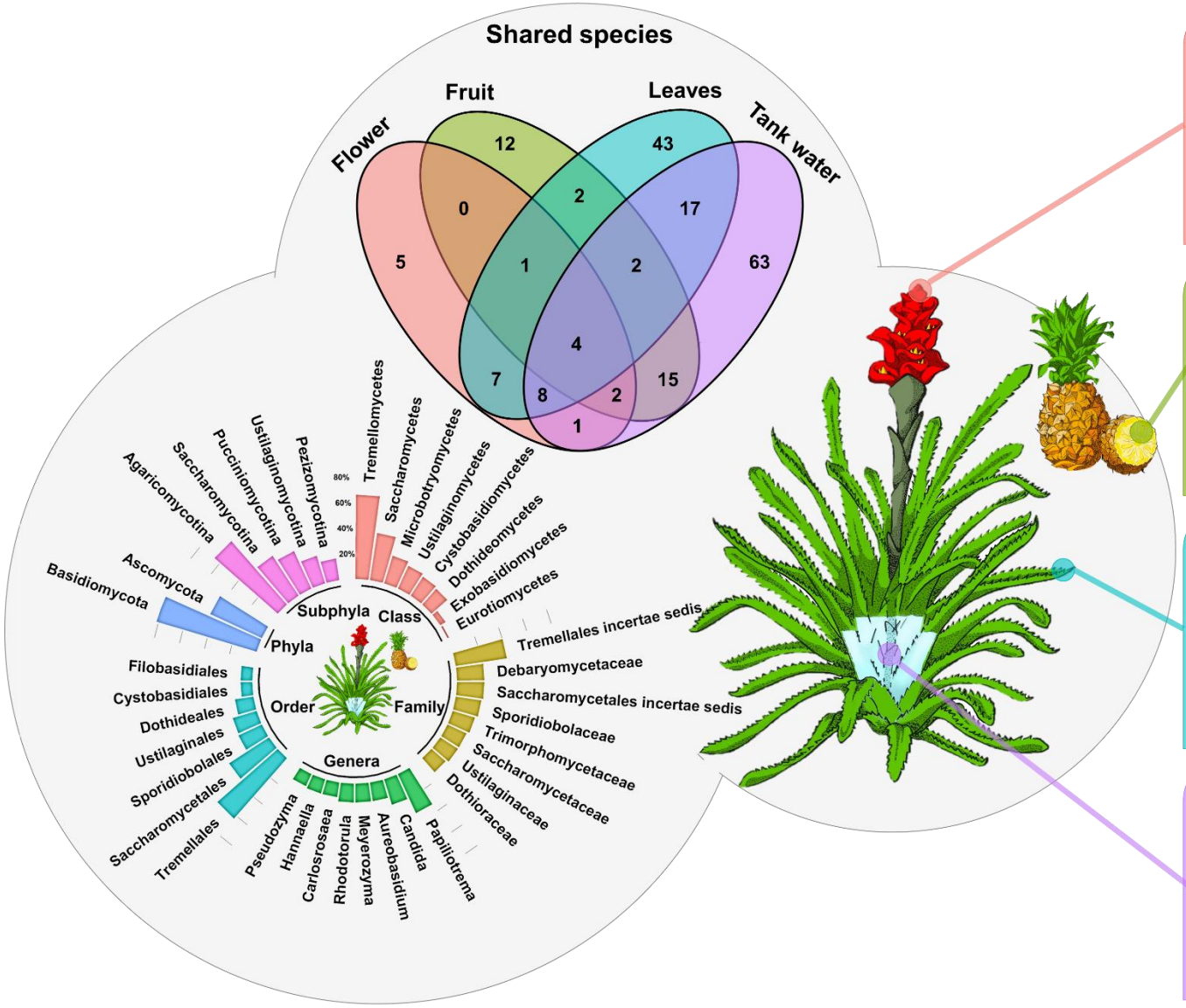
- Bromeliads are a great model for studies of yeast-plant interactions.
- More than 180 yeast species have already been recorded in bromeliads.
- Bromeliads compartments have distinct yeast communities.
- Bromeliad subfamilies and functional types were inconclusive in yeast structuring.
- Yeasts from bromeliads can find industrial application in biotechnology.

Abstract

The plant phyllosphere is one of the largest sources of microorganisms, including yeasts. In bromeliads, the knowledge of yeasts is dispersed and still incipient. To understand the extent of our knowledge on the subject, this review proposes to compile and synthesize existing knowledge, elucidating possible patterns, biotechnological and taxonomic potentials, bringing to light new knowledge, and identifying information gaps. For such, we systematically review scientific production on yeasts in bromeliads using various databases. The results indicated that the plant compartments flowers, fruits, leaves, and water tank (phytotelma) have been studied when focusing on the yeast community in the bromeliad phyllosphere. More than 180 species of yeasts and yeast-like fungi were recorded from the phyllosphere, 70% were exclusively found in one of these four compartments and only 2% were shared among all. In addition, most of the community had a low frequency of occurrence, and approximately half of the species had a single record. Variables such as bromeliad subfamilies and functional types, as well as plant compartments, were statistically significant, though inconclusive and with low explanatory power. At least 50 yeast species with some biotechnological potentials have been isolated from bromeliads. More than 90% of these species were able to produce extracellular enzymes. In addition, other biotechnological applications have also been recorded. Moreover, new species have been described, though yeasts were only exploited in approximately 1% of the existing bromeliads species, which highlights that there is still much to be explored. Nevertheless, it appears that we are still far from recovering the completeness of the diversity of yeasts in this host. Furthermore, bromeliads proved to be a good ecological model for prospecting new yeasts and for studies on the interaction between plants and yeasts. In addition, the yeast community diverged among plant compartments, establishing bromeliads as a microbiologically complex and heterogeneous mosaic.

Keywords: leaf, flower, fruit, phytotelma, systematic review and Bromeliaceae.

Graphical abstract



❖ Flowers (Anthoplane)

The majority reported genera from flowers were unispecific, only three grouped more than one yeast species. The only species exclusive and non-sigleton was *Pseudohyphozyma bogoriensis*.

❖ Fruits

All bromeliad fruit yeasts were isolated from pineapple (*Ananas comosos*). Most were from fermenting ascomycetes. Fruits were the only ones without record the Ustilaginomycotina subphylum.

❖ Leaves

The three richest classes account for 70% of yeast species from leaves. The leaves were where there was the highest prevalence of the phylum Basidiomycota.

❖ Tank water (Phytotelma)

Bromeliad tank water was the compartment with the greatest richness. Almost two thirds of the species are of the orders Saccharomycetales or Tremellales. Basidiomycota was the richest phylum.

1. INTRODUTION

There are variations on the phyllosphere concept, nonetheless, in general, it is the entire interface between plants and the atmosphere, including leaves (phylloplane), fruits (carposphere), flowers (anthosphere), stems (caulosphere), and waterscape (phytotelma) (Bringel and Couée, 2015; Vacher et al., 2016). The phyllosphere is an extreme, dynamic, and heterogeneous environment, often oligotrophic. Besides the traditional exposure and sudden fluctuations of stress factors, such as temperature, solar radiation, oxidation, the availability of nutrients and water, another critical point is the cutin layer, which forms the plant's cuticle. The plant's cuticle protects the leaves and assists in water control. It also decreases the evaporation and leaching of metabolites, limits the availability of nutrients, and creates an extreme and adverse environment (Bringel and Couée, 2015; Vorholt, 2012). Nowadays, it is well established that plants are microbiologically structured environments, rich in diversity, and ecologically complex (Redford et al., 2010; Vorholt, 2012). Each plant compartment (e.g. leaves, flowers, fruits, etc.) can and often do have distinct microbial communities that can vary in space, time, and ontogenetically (Abdelfattah et al., 2019; Fonseca and Inácio, 2006; Liu and Howell, 2021).

Bromeliaceae is an almost exclusively neotropical plant family recognized, among other factors, for its great diversity (approximately 3,500 species, 50 genera and 8 subfamilies), high endemism and for providing several ecosystem services in support of biodiversity and water regulation (Benzing, 2000; Ladino et al., 2019). Different than other members of angiosperms, bromeliads are mostly pollinated by vertebrates, mainly hummingbirds, but there is a long list of possible pollinators which involve groups such as bats, rodents and bees (Benzing, 2000; Siqueira-Filho and Leme, 2006; Tschapka and Von Helversen, 2007). Bromeliad inflorescences are mostly large and colorful, produce large volumes of nectar, with these traits reinforcing their relationship with vertebrate pollinators (Benzing, 2000; Siqueira-Filho and Leme, 2006; Wolowski and Freitas, 2015). Furthermore, they present a wide spectrum of colonized environments, lifestyles and ecophysiological types, occupying environments ranging from tropical to temperate climate regions, from 0 to 4,000 m of altitude and from rainy to dry forests

(Benzing, 2000; Crayn et al., 2015; Ladino et al., 2019; Males and Griffiths, 2017). Another important element about Bromeliaceae is that several species can accumulate water in a structure called phytotelma, a region in the center of the rosette. This water tank contributes, for example, to the reproduction of several mosquito species, including some vectors of human diseases such as *Aedes aegypti*, *A. albopictus*, *Culex* sp. and *Haemagogus* sp. (Benzing, 2000; Ladino et al., 2019). Among the species of mosquitoes associated with bromeliads, a minority is a vector of certain human diseases (less than 6%) (Ladino et al., 2019). Bromeliads function as a microscale island ecosystem (Frank and Lounibos, 1987), and perhaps also as a refuge in dry times, mainly, but not exclusively, for aquatic or heavily water-dependent organisms.

The microbiota associated with bromeliads has been studied for over a century (Frank and Lounibos, 1987), and the associated microbial groups include bacteria, archaea, viruses, filamentous fungi, yeasts, basal fungi (Chytridiomycota) and several other microeukaryotes (Goffredi et al., 2015; Leroy et al., 2016; Louca et al., 2017; Morais et al., 2020). In addition, vascular plants are one of the greatest sources of fungal diversity (Hawksworth and Lücking, 2017). For example, in the MycoBank database, the substrate with the highest number of records are leaves. These associated communities are modulated and/or regulated by a complex network of physicochemical, climatic, ecological, geographic factors and host-related factors such as senescence and genotype (Fonseca and Inácio, 2006; Vacher et al., 2016; Vorholt, 2012; Whipps et al., 2008).

Yeasts are a group formed by several fungal isolates with a convergent regulatory evolution for the maintenance of the unicellular phenotype (Nagy et al., 2014). These organisms are known to be among the first to establish themselves in nutrient-rich environments (Fonseca and Inácio, 2006; Ganter et al., 2017; Starmer and Lachance, 2011) and to occupy a wide spectrum of habitats distributed across the planet, whether associated with plants and animals, water, soil or the atmosphere (Rosa and Péter, 2006; Starmer and Lachance, 2011). Yeasts have been used for millennia in beverage and food fermentation processes, with the most well-known species being Saccharomyces cerevisiae - the “baker's yeast” (Rosa and Péter, 2006). Although some

yeast species are human pathogens, such as *Candida albicans* and *Cryptococcus neoformans* (Rosa and Péter, 2006), they represent a small portion of this group. For instance, several yeast types associated with plants present antagonistic activity to pathogens and are capable of producing plant hormones that stimulate plant growth (Buck, 2002; Limtong and Koowadjanakul, 2012; Marques et al., 2021). It has recently been estimated that there are currently approximately 1,600 described yeast species (Vu et al., 2016). However, this number may probably exceed 2300 species (Yurkov et al., 2021).

For about 30 years several generations of scientist have been studying yeasts associated with bromeliads. During this period, most studies have focused on yeast diversity and on the description of new species. Thus, through a systematic review we collected almost three decades of data on yeasts in bromeliads, we analyzed the diversity, frequency and structure of yeasts in bromeliads in different plant compartments (leaves, flowers, fruits and tank water) and considered other variables such as the bromeliad subfamily and functional type. Moreover, to understand the extent of our knowledge on the subject, we compile and synthesize existing knowledge, besides bringing to light new knowledge. In addition, we synthesized knowledge on new species and the biotechnological potential of bromeliad yeasts.

2. METHODS

2.1. Search in databases and data collection

We explored four databases to compile the bibliographic material used in this study: PubMed (https://pubmed.ncbi.nlm.nih.gov/), Scielo (https://www.scielo.org/), Scopus (https://www.scopus.com/) and Web of Science (https://www.webofscience.com/). The search terms (yeast* OR micro-fung* OR microfung* OR "unicellular fung*") AND (bromelia* OR pineapple OR ananas) were applied in a standardized way, searching for titles, abstracts and keywords on papers throughout the months of September and October 2021. The results were downloaded into bibtex files and concatenated into a single file. The online tool texmed

(https://www.bioinformatics.org/texmed/) was used to access the bibtex file from PubMed. In addition, manual searches were carried out on Google Scholar (https://scholar.google.com/).

2.2. Bibliography screening: inclusion and exclusion criteria

The papers files were imported into the R statistical software (R Team, 2016) using the revtools package (Westgate, 2019), duplicate references were removed. Following the reading of abstracts or complete material, the next step involved verifying whether the references met the following criteria: (1) Only studies focused on yeasts associated with bromeliads, whether in the fields of biodiversity, ecology, taxonomy, or application in biotechnological processes. However, articles that only used yeast counts as an indicator of food (pineapple fruit) contamination were excluded. (2) Non-English language papers were disregarded. (3) Only papers published in scientific journals were considered. Sources such as publications in congresses, symposia, or books were not incorporated. (4) Papers focused only on yeasts from industrialized juices and wines were also excluded. (5) All studies should be available online.

2.3. Research questions

- What is the community of yeasts associated with bromeliads, how is it structured among the plant compartments and what are the potential causal actors that shape yeast communities?
- What new yeast species associated with bromeliads have been described?
- What are the main biotechnological properties found in yeast from bromeliads?

2.4. Data extraction, tabulation, and synthesis

The units used were defined considering the scope of the study on a sample of bromeliad species, the compartments and sampling sites, as outlined in Figure 1. Thus, our work unit consisted of each Delimited Unit of Analysis (DUA). The DUAs were used to incorporate part of the variation in various information layers of the analyzed papers (e.g. hosts, substrates and sampling sites). The studies available in the literature have often different methodologies and vary in numbers of samples, hosts, compartments and sampling sites, DUAs are a way to standardize the analysis, although

there are biases involved. The papers were grouped into four categories according to their work focus (each study could be placed in more than one category):

1. Diversity and ecology - for articles with a focus on describing the local diversity of yeasts, which may or may not relate to ecological issues.

2. Taxonomy - papers focusing on the description of new species or taxonomic groups.

3. Biotechnology - studies that sought to apply yeasts in some biotechnological process and/or prospect some product or service of these isolates that could have some biotechnological application.

4. Others- when the paper did not fit into any of the previously mentioned categories.

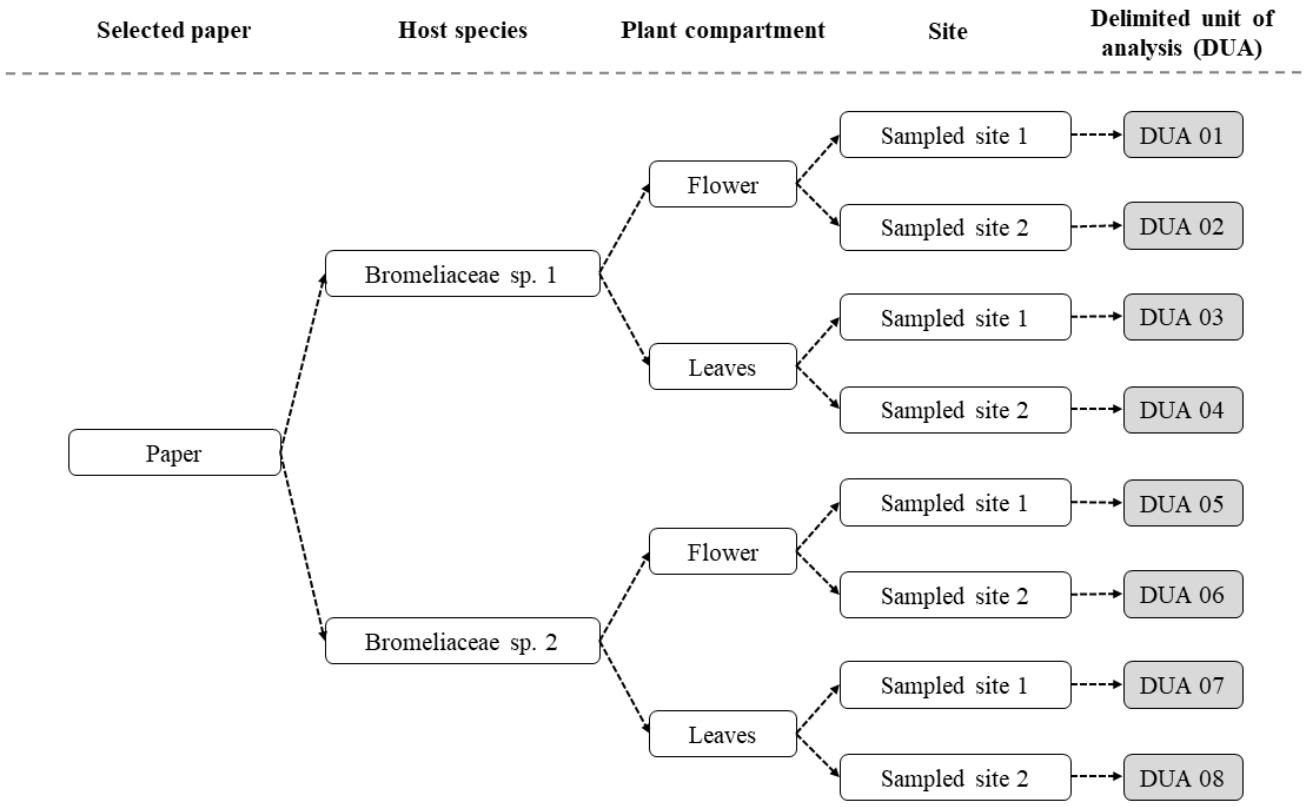


Fig. 1- Schematic drawing exemplifying how the units used were delimited. The delimited units of analysis (DUAs) would be related to the number of bromeliad species, compartments, and sampling sites from each paper.

To analyze the community of yeasts in bromeliads, the following information was collected from the papers: the lists of species, host name, compartment type (flowers, fruits, leaves or water tank), yeast identification method (molecular or phenotypic), culture medium isolation, collection site and biotechnological products or services produced/provided by yeasts. Yeast species with dubious, inconclusive, or incomplete identification were disregarded (except for biotechnology studies, where the identification at the genus level was also computed). The names of yeast species were revised using the MycoBank database (https://www.mycobank.org/) and specialist bibliography, e.g. (Kurtzman, 2011; Liu et al., 2015; Wang et al., 2016, 2015). From the correct names of yeast species, other taxonomic levels were assigned (Phylum, Subphylum, Class, Order, Family and Genus). The taxonomically correct names of bromeliads were verified on the Global Biodiversity Information Facility-GBIF (https://www.gbif.org/) and the subfamilies assigned through consultation on the Bromeliad Society International- BSI (https://www.bsi.org/new/taxonomy/). Furthermore, functional types were assigned to bromeliads in accordance with (Males and Griffiths, 2017).

2.5. Statistical analysis

Alpha-diversity indices and estimators (Simpson's index, Dominance index and Chao1) and Whittaker beta-diversity were calculated in the PAST software (Hammer et al., 2001), using the frequency of occurrence of species in DUAs (see the previous topic) in bromeliad compartments. The frequency of occurrence was calculated using the expression F_{oc}=(f×100)/D, where 'F_{oc}' is the frequency of occurrence of the group in question (e.g. species, genus, etc), 'f' is the count of the occurrence of the group in the DUAs (in total or in a specific compartment), and 'D' is the total number of the DUAs considered (for the entire phylosphere D=164, for flowers D=21, fruits D=18, leaves D=100 and water tanks D=25). An analysis of variance (ANOVA) was performed to verify whether there was a significant difference among the alpha-diversity indicators cited among the plant compartments. In addition, the normality of the data was verified

by applying the Shapiro-Wilk normality test, while Tukey's multiple comparisons test was applied afterward to verify the pair-by-pair difference between the compartments, with all these analyses being performed in the R software (R Team, 2016). The collector curve was also plotted using the frequency of species in the DUAs in the different compartments. The rarefaction and extrapolation (by Chao1) methods were used with 100 bootstrap pseudo-replications. This analysis and the design of the figure were carried out in R software using the packages iNEXT (Hsieh et al., 2016) and ggplot2 (Wilke, 2016).

The BETADISPER analysis, 'Multivariate homogeneity of groups dispersions (variances)', in R software was carried out to verify the similarity of the sample units dispersion between functional types (CAM tank-epiphytes, CAM atmospheric epiphyte, CAM terrestrial and C_3 tank-epiphytes), subfamilies (Bromelioideae, Tillandsioideae, and Pitcairnioidaeae), compartment types (flowers, fruits, leaves, and water tank) and identification methods (phenotypic or molecular). When a variable with more than two levels was tested, we used the permutation test of the homogeneity of multivariate dispersions (PERMDISP) to verify the paired significance between the groups. In this regard, the Vegan package (Dixon, 2003) was used to generate a Principal Coordinate Analysis (PCoA) applying Jaccard distance and a Multivariate Permutation Analysis (PERMANOVA), employing with 999 permutations to verify the probability of the evaluated groups (e.g. functional types) to be found at random. These data were plotted using the ggplot2 package and the difference between the centroid distances was verified with an ANOVA and Tukey's test. For the BETADISPER, PERMANOVA and PERMDISP, only the DUAs from the 20 papers in the category Diversity and ecology were used. This specific data was used as adding studies from the other categories could bias the heterogeneity of the scope of the papers.

3. RESULTS

A total of 665 papers were identified from all databases. Following the removal of duplicate papers, 383 unique references remained. After applying all filters

and criteria, 43 studies published between 1993 and 2021 were selected to be reviewed (Table 1 and Supplementary Figure 1). Twenty papers were grouped under the category Diversity and ecology, nineteen in the category Taxonomy, fourteen in the category Biotechnology, and four in the category Other. From all studies, 164 Delimited Units of Analysis (DUAs) were established (see method).

3.1. Bromeliad species and yeasts

Forty-four bromeliad species identified were recorded, most within the subfamily Bromelioideae (70%), followed by Tillandsioideae (30%). Representatives of Pitcairnioidaeae were identified, but none with a conclusive identification, being named only as Dyckia sp. and Encholirium spp. Furthermore, four functional types were recorded in these bromeliads: C_3 tank-epiphytes (15%), CAM atmospheric epiphyte (15%), CAM tank-epiphytes (46%) and CAM terrestrial (24%). The bromeliads with the highest yeast richness recorded were *Vriesea minarum* L.B. Sm. (n = 46 species), *Ananas comosus* (L.) Merr. (n = 44) and *Bromelia karatas* L. (n = 41).

3.2. Yeasts from bromeliad phyllosphere

From all studies in the sample, 181 yeasts and yeast-like species from four bromeliad compartments were mentioned: flowers, fruits, leaves, and water tank (Table 2). These species were grouped into 88 genera, 36 families, 20 orders, 8 classes and 5 subphyla of Basidiomycota and Ascomycota (Tables 2 and 3). Approximately 13% (n = 24) of the species found belong to the genus Candida, followed by Papiliotrema (n = 9; 5%), Rhodotorula (n = 9; 5%), and Hannaella (n = 8; 4%). Other prominent groups are the families Saccharomycetaceae (n = 27; 15%), Sporidiobolaceae (n = 16; 9%) and Ustilaginaceae (n = 12; 7%), which together group 30% of the species. Similarly, about 80% of all yeast species recorded are inserted in four orders, as follows: Saccharomycetales (n = 69; 38%), Tremellales (n = 46; 25%), Sporidiobolales (n = 16; 9%) and Ustilaginales (n = 14; 8%). Two-thirds of the species belong to Saccharomycotina and Agaricomycotina subphyla. Overall, the Basidiomycota phylum had more species than the Ascomycota (58 and 42%, respectively).

Most of the yeast species showed a low occurrence, as 46% were singletons (species occurring in only one DUA) and 65% were singletons or doubletons. Furthermore, about 97% of yeast species were present in less than 10% of the DUAs. The only species with a frequency ≥10% were *Papiliotrema laurentii* (13%), *Papiliotrema flavescens* (12%), *Meyerozyma guilliermondii* (12%), *Candida intermedia* (11%), *Pseudozyma hubeiensis* (11%) and *Aureobasidium pullulans* (10%) (Figure 2A). Combined, at least one of these five species occurred in more than a third of all units analyzed. Regarding the most frequent genera, only eight of the 88 registered had a frequency of occurrence greater than 10%: *Papiliotrema* (34%), *Candida* (21%), *Aureobasidium* (15%), *Meyerozyma* (15%), *Rhodotorula* (15%), *Carlosrosaea* (12%), *Hannaella* (12%) and *Pseudozyma* (10%). At least one of these genera occurred in 73% of all sampled units (Figure 2B).

In addition, 52% of the species occurred in only one bromeliad species, while only ten yeast species had a frequency of occurrence higher or equal than 20% on 44 bromeliad species: *P. laurentii* (39% occurrence), *C. intermedia* (36%), *P. flavescens* (31%), *Ps. hubeiensis* (30%), *Naganishia albida* (27%), *Papiliotrema leoncinii* (25%), *A. pullulans* (23%), *Hannaella pagnoccae* (23%), *M. guilliermondii* (25%) and *Carlosrosaea hohenbergiae* (20%). Combined, at least one of these species occurred in more than 90% of the bromeliad species recorded.

Table 1- List of articles inserted and analyzed in the review.

Paper title	Defined category	Reference
Yeasts and coliform bacteria of water accumulated in bromeliads of mangrove and sand dune ecosystems of southeast Brazil	Diversity	Hagler et al., 1993
A preliminary note on yeast communities of bromeliad-tank waters of Rio de Janeiro, Brazil	Diversity	Araujo et al., 1998
The influence of air pollution on the phyllosphere microflora composition of *Tillandsia leaves* (Bromeliaceae)	Biotechnology and Diversity	Brighigna et al., 2000
Microbial antagonists control postharvest black rot of pineapple fruit	Biotechnology and Diversity	Reyes et al., 2004
Candida bromeliacearum sp. nov. and *Candida ubatubensis* sp. nov., two yeast species isolated from the water tanks of *Canistropsis seidelii* (Bromeliaceae)	Taxonomy	Ruivo et al., 2005
Biodiversity of yeasts associated to bromeliads in Itapuã Park, Viamão/RS	Diversity	Landell et al., 2006
Farysizyma gen. nov., an anamorphic genus in the Ustilaginales to accommodate three novel epiphytic basidiomycetous yeast species from America, Europe and Asia	Taxonomy	Inácio et al., 2008
Cryptococcus bromeliarum sp. nov., an orange-coloured basidiomycetous yeast isolated from bromeliads in Brazil	Taxonomy	Landell et al., 2009
Autochthonous yeasts associated with mature pineapple fruits, freshly crushed juice and their ferments; and the chemical changes during natural fermentation	Biotechnology and Diversity	Chanprasartsuk et al., 2010
Comparative analysis of fungal growth in commercially and laboratory prepared fruit juices – Using orange and pineapple as a case study	Diversity	Udota and Urua, 2010
First report of bacterium and yeasts associated with pineapple fruit collapse in Espirito Santo State, Brazil	Diversity	Korres et al., 2010
Taxonomic structure of the yeasts and lactic acid bacteria microbiota of pineapple (*Ananas comosus* L. Merr.) and use of autochthonous starters for minimally processing	Diversity and Other	Di Cagno et al., 2010
Candida aechmeae sp. nov. and *Candida vrieseae* sp. nov., novel yeast species isolated from the phylloplane of bromeliads in Southern Brazil	Taxonomy	Landell et al., 2010
Candida krusei and *Kloeckera apis* inhibit the causal agent of pineapple fusariosis, *Fusarium guttiforme*	Biotechnology and Diversity	Korres et al., 2011
Pineapple wine fermentation with yeasts isolated from fruit as single and mixed starter cultures	Biotechnology	Chanprasartsuk et al., 2012
Bandoniozyma gen. nov., a genus of fermentative and non-fermentative Tremellaceous yeast species	Taxonomy	Valente et al., 2012
Kazachstania bromeliacearum sp. nov., a yeast species from water tanks of bromeliads	Taxonomy	Araujo et al., 2012
Comparison of methods for identification of yeasts isolated during spontaneous fermentation of freshly crushed pineapple juices	Diversity and Other	Chanprasartsuk et al., 2013

Kazachstania rupicola sp. nov., a yeast species isolated from water tanks of a bromeliad in Brazil	Taxonomy	Safar et al., 2013
Hagleromyces gen. nov., a yeast genus in the Saccharomycetaceae, and description of *Hagleromyces aurorensis* sp. nov., isolated from water tanks of bromeliads	Taxonomy	Sousa et al., 2014
Hannaella pagnoccae sp. nov., a tremellaceous yeast species isolated from plants and soil	Taxonomy	Landell et al., 2014
The diversity and extracellular enzymatic activities of yeasts isolated from water tanks of *Vriesea minarum*, an endangered bromeliad species in Brazil, and the description of *Occultifur brasiliensi*s f.a., sp. nov	Biotechnology, Diversity and Taxonomy	Gomes et al., 2015
Enzymatic activity and susceptibility to antifungal agents of brazilian environmental isolates of *Hortaea werneckii*	Diversity and Other	Formoso et al., 2015
Phenotypic and molecular diversity of *Meyerozyma guilliermondii* isolates isolated from food and other environmental niches, hints for an incipient speciation	Other	Corte et al., 2015
Bullera vrieseae sp. nov., a tremellaceous yeast species isolated from bromeliads	Taxonomy	Landell et al., 2015
Kockovaella libkindii sp. nov., a yeast species isolated from water tanks of bromeliad	Taxonomy	Gomes et al., 2016
Papiliotrema leoncinii sp. nov. and *Papiliotrema miconiae* sp. nov., two tremellaceous yeast species from Brazil	Taxonomy	Pagani et al., 2016
Isolation of *Saccharomyces cerevisiae* from pineapple and orange and study of metal's effectiveness on ethanol production	Biotechnology	Nasir et al., 2017
Pineapple (*Ananas comosus* L. Merr.) wine production in Angola: Characterisation of volatile aroma compounds and yeast native flora	Biotechnology and Diversity	Dellacassa et al., 2017
Carlosrosaea hohenbergiae sp. nov. and *Carlosrosaea aechmeae* sp. nov., two tremellaceous yeasts isolated from bromeliads in north-eastern Brazil	Taxonomy	Félix et al., 2017
Occultifur plantarum f.a., sp. nov., a novel cystobasidiomycetous yeast species	Taxonomy	Khunnamwong et al., 2017
Pattersoniomyces tillandsiae gen. et comb. nov.: linking sexual and asexual morphs of the only known smut fungus associated with Bromeliaceae	Taxonomy	Piątek et al., 2017
Probiotic potential of yeasts isolated from pineapple and their use in the elaboration of potentially functional fermented beverages	Biotechnology and Diversity	Amorim et al., 2018
Fungal Planet description sheets: 716–784	Taxonomy	Crous et al., 2018
Fungal Planet description sheets: 868–950	Taxonomy	Crous et al., 2019
The natural lipolytic yeast *Candida* sp. Rmutsb-27 isolated from pineapple for treatment of cooking oil contaminated wastewater	Biotechnology	Tangsombatvichit et al., 2020
Richness and biotechnological potential of the yeast community associated with the bromeliad phylloplane in the Brazilian Neotropical Forest	Biotechnology and Diversity	Navarro et al., 2020

Yeast in plant phytotelmata: Is there a core community in different localities of rupestrian savannas of Brazil?	Biotechnology and Diversity	Morais et al., 2020
Changes of quality of minimally-processed pineapple (*Ananas comosus*, var. queen victoria) during cold storage: Fungi in the leading role	Diversity	Leneveu-jenvrin et al., 2020
Vishniacozyma alagoana sp. nov. a tremellomycetes yeast associated with plants from dry and rainfall tropical forests	Taxonomy	Félix et al., 2020
Plant growth promoting traits of yeasts isolated from the tank bromeliad *Vriesea minarum* L.B. Smith and the effectiveness of *Carlosrosaea vrieseae* for promoting bromeliad growth	Biotechnology	Marques et al., 2021
Plant endophytic yeasts *Pichia fermentans* and *Meyerozyma caribbica* improve growth, biochemical composition, haematological parameters and morphology of internal organs of premature *Barbonymus gonionotus*	Biotechnology and Diversity	Islam et al., 2021
Behind the nectar: the yeast community in bromeliads inflorescences after the exudate removal	Diversity	Félix et al., 2021

Table 2- Number of yeast taxa found at taxonomic levels in each compartment of bromeliads.

	Species	**Genera**	**Family**	**Order**	**Class**	**Subphyla**	**Phyla**
Total bromeliads	181	88	36	20	8	5	2
Flowers	28	22	13	7	6	5	2
Fruits	38	23	12	7	6	4	2
Leaves	84	44	23	13	7	5	2
Tank water	111	61	27	16	8	5	2

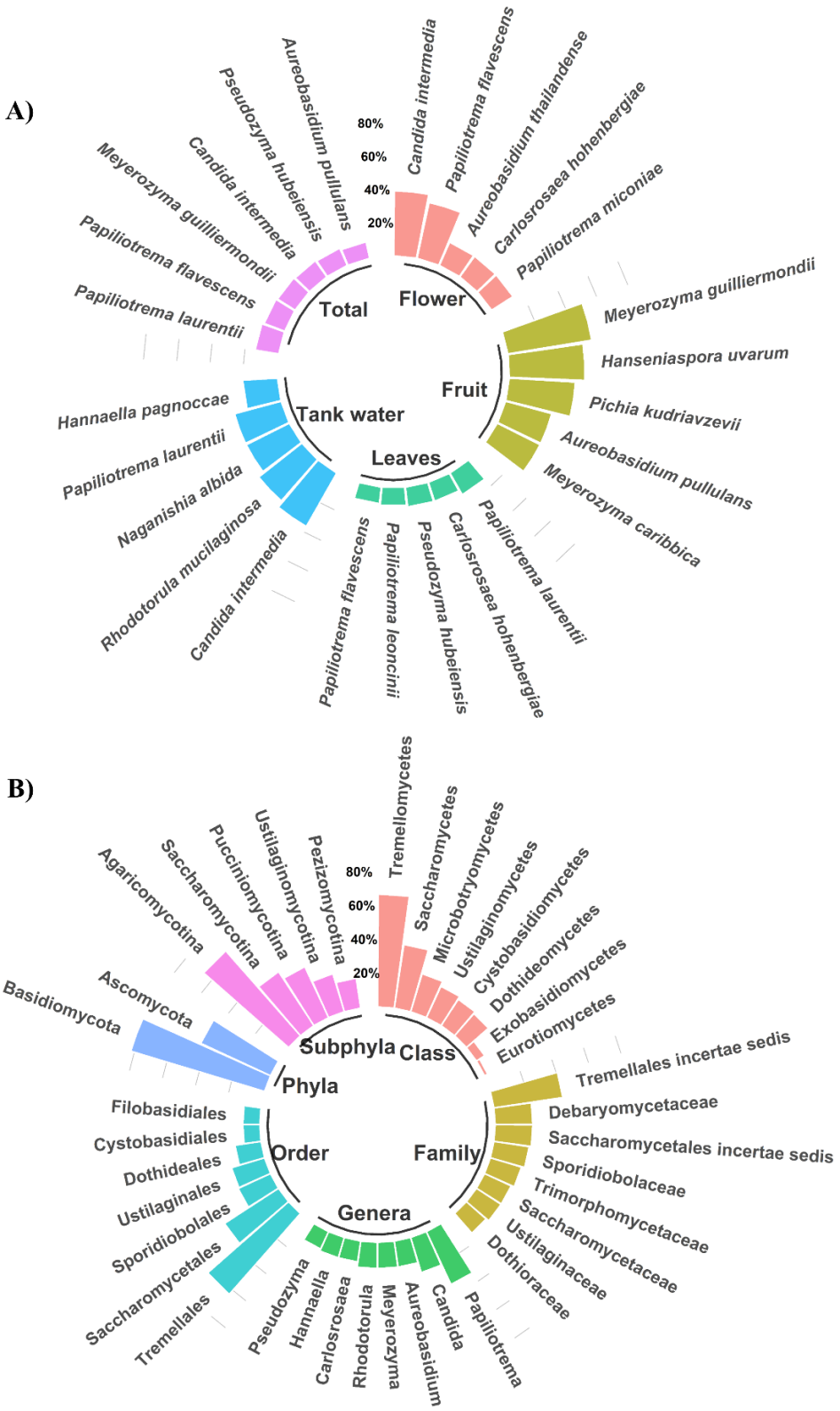


Fig. 2- Barplot showing the frequency of occurrence of the groups. A, frequency of taxonomic groups in the bromeliad phyllosphere. B, the most frequent species in the compartments and in all phyllosphere.

3.3. Yeasts from bromeliad compartments

The diversity and composition of yeast species varied among bromeliad compartments. For example, regarding diversity (estimated with Simpson's index), total richness (estimated with Chao 1) and dominance, leaves and water tanks were significantly more diverse, richer and with communities less dominant than flowers and fruits ($p<0.05$). On the other hand, these same variables did not vary significantly between flowers and fruits and between leaves and water tanks ($p>0.05$), as shown in Figure 3A-C. However, the number of singletons (species occurring in only one unity) found in each compartment was proportionally similar to water tanks (57%), leaves (52%), fruits and flowers (both 50%).

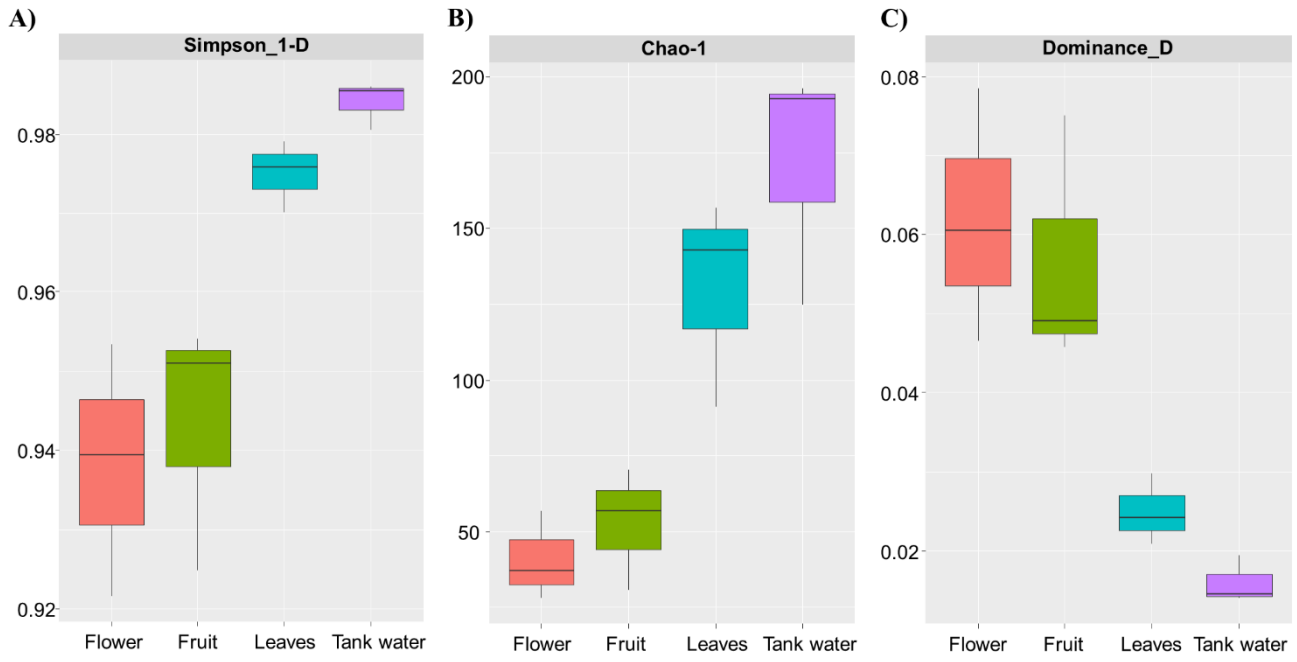


Fig. 3- Boxplots showing Simpson's alpha diversity index (A), total richness estimated by Chao-1 (B) and the dominance of yeast communities (C) among the bromeliad phyllosphere compartments.

Furthermore, more than 70% (n = 129) of the species were recorded exclusively in a single compartment (Figure 4B). Only four species were shared among all compartments: *A. pullulans*, *Debaryomyces hansenii*, *M. guilliermondii* and *Rhodotorula glutinis*. In terms of the community composition, the most distinct

compartments were fruits and leaves, which shared only eight species and had a Whittaker beta-diversity of 0.85 (Figure 4A). Observing the compartments separately, it is possible to observe that the most exclusive species, in terms of the richness of each compartment, was water tank (62%), followed by leaves (51%), fruit (32%) and flower (18%). The collector curve corroborates the estimates of Chao 1 and indicates that the diversity is even greater than that found, mainly in water tanks and leaves (Figure 4C and Supplementary Figure 2).

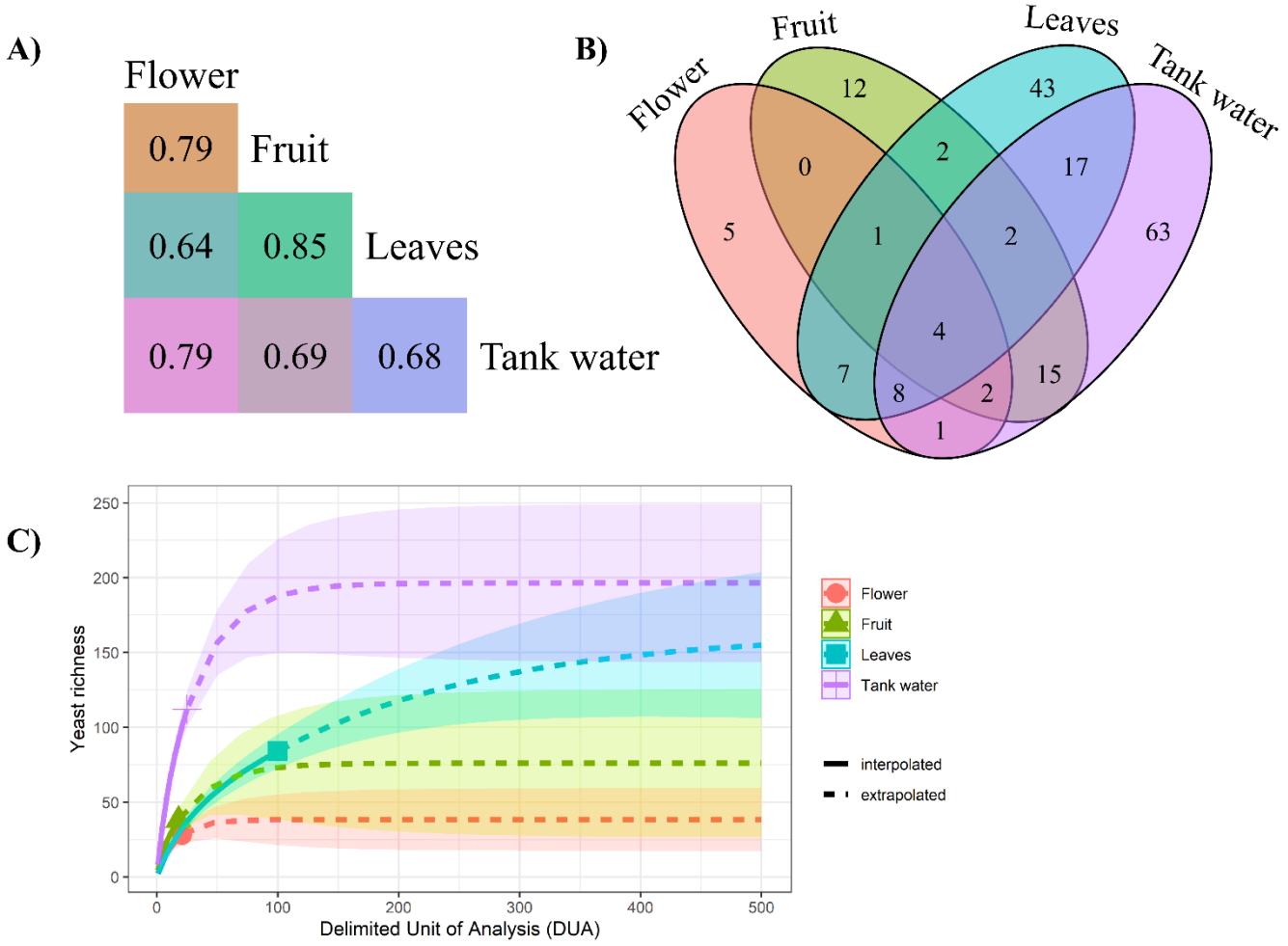


Fig. 4- (A), Whittaker's beta-diversity between the different compartments. (B), the Venn diagram illustrates the sharing of species among the different compartments of the bromeliad phyllosphere. (C), Extrapolation rarefaction curve. The curve was elaborated with 1000 pseudoreplica bootstrap and the extrapolation was performed with the Chao1 estimator.

Moreover, depending on the plant compartment analyzed, the species most frequently found may vary. For example, the most frequent yeasts on flowers were *C. intermedia* (38%) and *P. flavescens* (33%), on fruits were *M. guilliermondii* (56%), *Hanseniaspora uvarum* (50%) and *Pichia kudriavzevii* (44%), while on leaves they were *P. laurentii* (14%), *Ps. hubeiensis* (11%), *Ca. hohenbergiae* (11%) and *P. leoncinii* (10%) and on water tanks they were *C. intermedia* (36%), *Rhodotorula mucilaginosa* (32%), *P. laurentii* (28%) and *N. albida* (28%).

3.3.1. Yeasts in bromeliad flowers

The only exclusively non-singleton species found in flowers was *Pseudohyphozyma bogoriensis*. Moreover, only three genera have more than one yeast species: *Papiliotrema* (n = 4 species; 14%), *Sporobolomyces* (n = 3; 11%) and *Aureobasidium* (n = 2; 7%), and approximately 86% of the reported genera were unispecific. The Sporidiobolaceae family was the most represented (n = 5 species, 18%). As for orders, classes and other hierarchical groups, no major differences were found in the entire phyllosphere. However, in flowers, the subphylum Pucciniomycotina stands out (n = 8; 29%), mainly when compared with the class of Microbotryomycetes (n = 7; 25%) containing the genus *Sporobolomyces, Colacogloea, Pseudohyphozyma, Rhodosporidiobolus,* and *Rhodotorula*. The phylum Basidiomycota recorded most of the species, accounting for 19 species (69%).

3.3.2. Yeasts in bromeliad fruits

Candida sorboxylosa, *Pi. kudriavzevii*, *Pichia fermentans* and *Saccharomyces cerevisiae* were exclusively isolated from fruits and presented a relatively high frequency in this compartment. Furthermore, *Candida* (n = 4 species; 11%), *Rhodotorula* (n = 4; 11%), Pichia (n = 4; 11%), *Hanseniaspora* (n = 3; 8%), *Starmerella* (n = 2; 6%) and *Meyerozyma* (n = 2; 6%) were the genera with more than one reported species. Different than the other compartments, many species of the Saccharomycetaceae family (n = 14; almost 40% of all species in this compartment), as well as the order Saccharomycetales (n = 26 species; 72%) were present in fruits. This is consequently reflected in the prevalence of species in the subphylum Saccharomycotina and the

Ascomycota phylum, respectively 72 and 78%. Furthermore, among all compartments analyzed in this study, fruits were the only ones without any representative of the Ustilaginomycotina subphylum.

3.3.3. Yeasts in bromeliad leaves

Several species were frequently and exclusively found in leaves: *Farysia itapuensis, Genolevuria bromeliarum, Papiliotrema siamense, Queiroziella brasiliensis, Sporobolomyces salmonicolor* and *Symmetrospora suhii*. Moreover, the five most representative genera were *Papiliotrema* (n = 7; 9%), *Hannaella* (n = 6; 7.5%), Candida (n = 5; 6%), *Rhodotorula* (n = 4; 5%) and *Aureobasidium* (n = 4; 5%). Together, these five genera represented a third of the total species in leaves. The families with highest number of species were Sporidiobolaceae (n = 8; 10%) and Trimorphomycetaceae (n = 5; 6%). However, in terms of order, Tremellales had 31 species (almost 40% of the total), followed by Saccharomycetales (n = 12; 15%) and Sporidiobolales (n = 8; 10%). The three richest classes account for 70% of yeasts in leaves: Tremellomycetes (n = 34; 43%), Saccharomycetes (n = 12; 15%) and Microbotryomycetes (n = 10; 12%). The subphyla Agaricomycotina and Pucciniomycotina grouped two thirds of the yeast species. Among all compartments, leaves had the highest prevalence of the phylum Basidiomycota, representing 79% of the species.

3.3.4. Yeasts in bromeliad water tank

Bromeliad water tank was the compartment with the greatest richness. At least five species that were not singletons or doubletons were found exclusively in water tanks: *Kazachstania bromeliacearum, Kwoniella heveanensis, Occultifur brasiliensis, Papiliotrema rajasthanensis,* and *Starmerella magnoliae*. The genus *Kwoniella* was found exclusively in water tanks. In addition, *Candida* and *Rhodotorula* were the richest genera (n = 21 species; 12% and n = 8 species; 7%). Twenty-seven families are registered in this compartment, being Saccharomycetaceae (n = 18; 16%), Ustilaginaceae (n = 11; 10%) and Sporidiobolaceae (n = 10; 9%). Combined, these families compose more than a third of the total yeast species. Almost two-thirds of the species belong to the orders Saccharomycetales (n = 49; 43%) and Tremellales (n = 24;

21%). This is also true for the classes Saccharomycetes (n = 49; 43%) and Tremellomycetes (n = 29; 25%) and for the subphyla Saccharomycotina (n = 49; 43%) and Agaricomycotina (n = 29; 25%). Basidiomycota was the richest phylum, comprising 54% of the species.

3.4. Yeast community structure in bromeliads

3.4.1. Definition of units

From the 20 articles in the category Diversity and ecology, 103 DUAs were defined considering bromeliad species/compartment/paper/sampled site (Figure 1). In this data group, 171 yeast species were recorded. Among the DUAs, 20 corresponded to flowers (19%), 16 to fruits (16%), 54 to leaves (52%), and 13 to water tanks (13%). In 28 units, classical methods (phenotypic) for the identification of yeasts and other molecular methods were used. Our analyses (PERMANOVA) indicated that the methods (phenotypic or molecular) are data classes that in fact exert some explanatory power on the data set, and that the groups formed have little chance of being found by chance ($p=0.001$). However, the low explanatory power shows a low correlation between these classes and the community structure ($R^2=0.06$). The PERMDISP result was also significant ($p=0.004$), indicating a heterogeneous dispersion between groups formed by samples with different identification methods. The group formed by samples identified with molecular methods had higher variance, probably an effect of cryptic species that are a methodological artifact of phenotypic methods. Therefore, the analyses were prepared with all units, without discriminating the types of identification methods, but also with the data of each identification method separately (Table 4).

3.4.2. Community structure

The results found herein were not conclusive, as the statistical significance of the classes varied depending on the data set evaluated (Table 4). Furthermore, even when the result showed statistical significance, the low explanatory values of R^2 indicate a low correlation between the variables and community structure. Bromeliad compartments and functional types were the categories with the highest average explanatory power (although the values were low). One of the reasons for the low

explanatory power can be the large variance in some classes, for example in leaves and in CAM tank-epiphytes. Comparing the classes of each category pair-by-pair (without discriminating the types of identification method), the three subfamilies registered diverged significantly from each other (p-value was always ≤ 0.003). However, regarding the functional types, only CAM tank-epiphyte bromeliads diverged significantly from C_3 tank-epiphytes (p=0.023) and from CAM atmospheric epiphyte (p=0.002). In addition, CAM atmospheric epiphyte bromeliads diverged marginally from CAM terrestrial plants (p=0.056). Nonetheless, in compartments, the leaves were the only class that diverged significantly from flowers (p=0.01) and fruits (p=0.003). When comparing the distance of the centroids of each type of compartment (flowers, fruits, leaves and water tanks) to evaluate internal heterogeneity, leaves were the compartment with the highest variance (Figure 5). The difference in the variance of the groups was significant (p<0.0001) and when compared pair-by-pair, leaves diverged significantly from fruits (p=0.0001), water tanks (p=0.0009) and showed a marginally significant difference in relation to flowers (p=0.064).

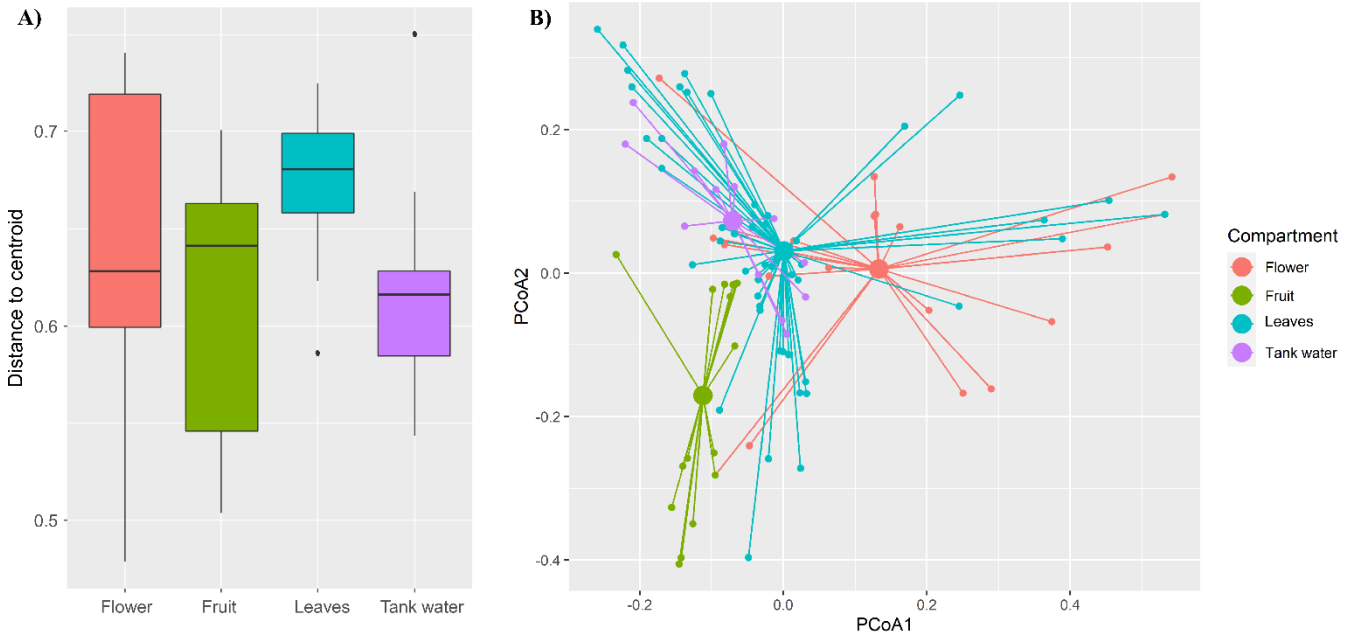


Fig. 5- (A) Boxplot of the distances of the points on the PCoA in relation to the centroid (variance) of the compartments and bromeliads. (B) Principal Coordinate Analysis (PCoA) applying Jaccard distance from the yeast community on bromeliads, the points refer to the Delimited Units of Analysis (DUAs) used in the analysis, and the central point of each class is the centroid.

3.5. New yeast species in bromeliads

A total of 22 new yeast species described were recorded (7 ascomycetes and 15 basidiomycetes) associated with 30 species of bromeliads (Supplementary Table 1). The genera with the highest number of new species described are *Candida* (n = 4) and *Carlosrosaea* (n = 3). In addition, Tremellales and Saccharomycetales are the most recorded orders, presenting 10 and 7 new species, respectively. The new species described are grouped into 4 subphyla, Agaricomycotina (n = 10 species), Saccharomycotina (n = 7), Pucciniomycotina (n = 3) and Ustilaginomycotina (n = 2).

The new species were described associated with leaves (n = 10), water tanks (n = 8), with some new species found in both substrates (n = 3). Only the species *Ca. hohenbergiae* was described as associated with leaves and flowers. The bromeliad species with the highest number of new yeast species described were *V. minarum* L.B.Sm. (n = 6), *Vriesea friburgensis* Mez (n = 4) and *Werauhia gigantea* (Mart. ex Schult. & Schult.f.) J.R.Grant (n = 4); both belong to the subfamily Tillandsioideae. *P. leoncinii* was the species associated with the highest number of bromeliad species (10 species).

3.6. Bromeliad yeasts in biotechnological processes

In the analyses, at least 50 species of yeasts were found isolated from bromeliads capable of being used in biotechnological processes in the industrial, agricultural, and/or environmental sectors (Supplementary Table 2). The industrial potential of yeasts isolated from bromeliads is mainly the production of extracellular enzymes such as amylase, cellulase, esterase, pectinase, protease, and xylanase. More than 90% of the listed species with some biotechnological potentials were able to produce at least one of these enzymes. The enzymes produced by the largest number of yeast species were respectively protease, pectinase and xylanase. However, other biotechnological applications were also registered, such as the probiotic activity of *Meyerozyma caribbica*, the potential in the production of biodiesel from Candida sp., and the production of fermented beverages by *Hanseniaspora guilliermondii, Hanseniaspora*

opuntiae, H. uvarum, M. caribbica, M. guilliermondii, Saccharomyces cerevisiae-like and *Saccharomycodes ludwigii*. Among agricultural and related applications are the promotion of plant growth by *Carlosrosaea vrieseae* and the production of traits related to plant growth, such as the production of indoleacetic acid (IAA), siderophores and the ability to solubilize phosphate. In addition, yeasts from bromeliads have also shown their value as a potential source of animal nutrition, such as *M. caribbica* and *S. ludwigii*, which were used as feed in fish farming. In the environmental sector, *A. pullulans, Candida* spp., *Cryptococcus* spp. and *Sporobolomyces* spp. were used as potential air quality indicators. In addition, *Candida* sp. showed potential in bioremediation, specifically related to the treatment of oil-contaminated environments.

Table 3- Yeasts taxa, hosts and compartments recorded in the review.

Yeast species	Host species	Flower	Fruit	Leaves	Water tank	Reference
Ascomycota (Phyla)						
Pezizomycotina (Subphyla)						
Aureobasidium leucospermi	*Ananas comosus* (L.) Merr.		×			Chanprasartsuk et al., 2012
	Aechmea leptantha (Harms) Leme & J.A.Siqueira			×		Navarro et al., 2020
Aureobasidium melanogenum	*Aechmea leptantha* (Harms) Leme & J.A.Siqueira			×		Navarro et al., 2020
Aureobasidium pullulans	*Tillandsia caput-medusae* É.Morren			×		Brighigna et al 2000
	Tillandsia schiedeana Steud.			×		Brighigna et al 2000
	Ananas comosus (L.) Merr.		×			Chanprasartsuk et al., 2010; 2013
	Aechmea froesii (L.B.Sm.) Leme & J.A.Siqueira	×				Félix et al., 2021
	Aechmea tomentosa Mez	×				Félix et al., 2021
	Vriesea minarum L.B.Sm.				×	Gomes et al., 2015
	Neoregelia cruenta (Graham) L.B.Sm.				×	Hagler et al., 1993
	Quesnelia quesneliana (Brongn.) L.B.Sm.				×	Hagler et al., 1993
	Bromelia karatas L.				×	Morais et al., 2020
	Billbergia sp.			×		Navarro et al., 2020
	Canistrum improcerum Leme & J.A.Siqueira			×		Navarro et al., 2020
Aureobasidium thailandense	*Aechmea froesii* (L.B.Sm.) Leme & J.A.Siqueira	×				Félix et al., 2021
	Aechmea tomentosa Mez	×				Félix et al., 2021
	Billbergia sp.	×				Félix et al., 2021
	Aechmea froesii (L.B.Sm.) Leme & J.A.Siqueira			×		Navarro et al., 2020
	Aechmea leptantha (Harms) Leme & J.A.Siqueira			×		Navarro et al., 2020

	Aechmea tomentosa Mez			×		Navarro et al., 2020
	Aechmea werdermannii Harms			×		Navarro et al., 2020
	Ananas comosus (L.) Merr.			×		Navarro et al., 2020
	Billbergia sp.			×		Navarro et al., 2020
	Hohenbergia stellata Schult. & Schult.f.			×		Navarro et al., 2020
Endosporium aviarium	*Vriesea minarum* L.B.Sm.				×	Morais et al., 2020
Exophiala placitae	*Vriesea minarum* L.B.Sm.				×	Morais et al., 2020
Hortaea werneckii	*Aechmea leptantha* (Harms) Leme & J.A.Siqueira			×		Formoso et al., 2015; Navarro et al., 2020
Talaromyces amestolkiae	*Ananas comosus* (L.) Merr.		×			Leneveu-jenvrin et al., 2020
Saccharomycotina (Subphyla)						
Candida aechmeae	*Aechmea recurvata* (Klotzsch) L.B.Sm.			×		Landell et al., 2010
	Billbergia nutans H.Wendl.			×		Landell et al., 2010
Candida albicans	*Bromelia karatas* L.				×	Morais et al., 2020
	Ananas comosus (L.) Merr.		×			Udota and Urua, 2010
Candida bromeliacearum	*Canistropsis seidelii* (L.B.Sm. & Reitz) Leme			×		Ruivo et al., 2005
Candida buenavistaensis	*Encholirium* sp.				×	Morais et al., 2020
Candida diddensiae	*Werauhia gigantea* (Mart. ex Schult. & Schult.f.) J.R.Grant				×	Landell et al., 2006
Candida duobushaemulonis	*Bromelia karatas* L.				×	Morais et al., 2020
Candida glabrata	*Bromelia karatas* L.				×	Morais et al., 2020
	Encholirium sp.				×	Morais et al., 2020
Candida glucosophila	*Quesnelia arvensis* (Vell.) Mez				×	Araujo et al., 1998
Candida heveicola	*Vriesea minarum* L.B.Sm.				×	Morais et al., 2020
Candida intermedia	*Aechmea nudicaulis* (L.) Griseb.				×	Araujo et al., 1998
	Nidularium procerum Lindm.				×	Araujo et al., 1998
	Quesnelia arvensis (Vell.) Mez				×	Araujo et al., 1998
	Vriesea procera (Mart. ex Schult. & Schult.f.) Wittm.				×	Araujo et al., 1998

	Aechmea costantinii (Mez) L.B.Sm.	×				Félix et al., 2021
	Aechmea froesii (L.B.Sm.) Leme & J.A.Siqueira	×				Félix et al., 2021
	Aechmea fulgens Brongn.	×				Félix et al., 2021
	Aechmea leptantha (Harms) Leme & J.A.Siqueira	×				Félix et al., 2021
	Billbergia sp.	×				Félix et al., 2021
	Canistrum alagoanum Leme & J.A.Siqueira	×				Félix et al., 2021
	Canistrum aurantiacum É.Morren	×				Félix et al., 2021
	Vriesea minarum L.B.Sm.				×	Gomes et al., 2015
	Quesnelia quesneliana (Brongn.) L.B.Sm.				×	Hagler et al., 1993
	Bromelia karatas L.				×	Morais et al., 2020
	Encholirium sp.				×	Morais et al., 2020
	Araeococcus chlorocarpus (Wawra) Leme & J.A.Siqueira			×		Navarro et al., 2020
Candida jaroonii	*Canistrum aurantiacum* É.Morren			×		Navarro et al., 2020
Candida leandrae	*Bromelia karatas* L.				×	Morais et al., 2020
Candida melibiosica	*Vriesea minarum* L.B.Sm.				×	Gomes et al., 2015
Candida membranifaciens	*Vriesea minarum* L.B.Sm.				×	Gomes et al., 2015
	Ananas comosus (L.) Merr.		×			Nasir et al., 2017
Candida nivariensis	*Ananas comosus* (L.) Merr.		×			Chanprasartsuk etal., 2013
	Bromelia karatas L.				×	Morais et al., 2020
	Encholirium sp.				×	Morais et al., 2020
Candida orthopsilosis	*Bromelia karatas* L.				×	Morais et al., 2020
	Encholirium sp.				×	Morais et al., 2020
Candida parapsilosis	*Bromelia karatas* L.				×	Morais et al., 2020
	Encholirium sp.				×	Morais et al., 2020
	Araeococcus chlorocarpus (Wawra) Leme & J.A.Siqueira			×		Navarro et al., 2020
	Canistrum alagoanum Leme & J.A.Siqueira			×		Navarro et al., 2020

	Hohenbergia ridleyi (Baker) Mez			×		Navarro et al., 2020
Candida pseudointermedia	*Bromelia karatas* L.				×	Morais et al., 2020
	Encholirium sp.				×	Morais et al., 2020
Candida sinolaborantium	*Aechmea fulgens* Brongn.			×		Navarro et al., 2020
Candida soli	*Quesnelia quesneliana* (Brongn.) L.B.Sm.				×	Hagler et al., 1993
Candida sorboxylosa	*Ananas comosus* (L.) Merr.		×			Chanprasartsuk et al., 2010
Candida tropicalis	*Aechmea nudicaulis* (L.) Griseb.				×	Araujo et al., 1998
	Nidularium procerum Lindm.				×	Araujo et al., 1998
	Quesnelia arvensis (Vell.) Mez				×	Araujo et al., 1998
	Ananas comosus (L.) Merr.		×			Chanprasartsuk et al., 2010; 2013; Udota and Urua, 2010
Candida ubatubensis	*Vriesea minarum* L.B.Sm.				×	Gomes et al., 2015; Morais et al., 2020
	Canistropsis seidelii (L.B.Sm. & Reitz) Leme			×		Ruivo et al., 2005
Candida vrieseae	*Werauhia gigantea* (Mart. ex Schult. & Schult.f.) J.R.Grant			×		Landell et al., 2010
Clavispora lusitaniae	*Ananas comosus* (L.) Merr.		×			Amorim et al 2018
Cyberlindnera saturnus	*Quesnelia arvensis* (Vell.) Mez				×	Araujo et al., 1998
Debaryomyces hansenii	*Neoregelia cruenta* (Graham) L.B.Sm.				×	Araujo et al., 1998
	Quesnelia arvensis (Vell.) Mez				×	Araujo et al., 1998
	Aechmea recurvata (Klotzsch) L.B.Sm.	×				Landell et al., 2006
	Billbergia nutans H.Wendl.			×		Landell et al., 2006
	Tillandsia gardneri Lindl.	×				Landell et al., 2006
	Vriesea procera (Mart. ex Schult. & Schult.f.) Wittm.			×		Landell et al., 2006
	Ananas comosus (L.) Merr.		×			Udota and Urua, 2010
Dipodascus albidus	*Aechmea recurvata* (Klotzsch) L.B.Sm.	×				Landell et al., 2006
Diutina rugosa	*Neoregelia cruenta* (Graham) L.B.Sm.				×	Araujo et al., 1998
	Quesnelia arvensis (Vell.) Mez				×	Araujo et al., 1998

Galactomyces candidus	*Ananas comosus* (L.) Merr.		×			Udota and Urua, 2010
Hagleromyces aurorensis	*Bromelia karatas* L.				×	Morais et al., 2020
	Bromelia karatas L.			×		Sousa et al., 2014
Hanseniaspora guilliermondii	*Ananas comosus* (L.) Merr.		×			Dellacassa et al., 2017
	Bromelia karatas L.				×	Morais et al., 2020
Hanseniaspora opuntiae	*Ananas comosus* (L.) Merr.		×			Chanprasartsuk et al., 2010; 2013; Dellacassa et al., 2017
	Bromelia karatas L.				×	Morais et al., 2020
Hanseniaspora uvarum	*Ananas comosus* (L.) Merr.		×			Chanprasartsuk et al., 2010; 2013; Dellacassa et al., 2017
	Aechmea fulgens Brongn.	×				Félix et al., 2021
	Cryptanthus dianae Leme	×				Félix et al., 2021
	Vriesea minarum L.B.Sm.				×	Gomes et al., 2015
	Ananas comosus (L.) Merr.		×			Udota and Urua, 2010
Kazachstania africana	*Aechmea nudicaulis* (L.) Griseb.				×	Araujo et al., 1998
Kazachstania bromeliacearum	*Aechmea nudicaulis* (L.) Griseb.				×	Araujo et al., 2012
	Neoregelia cruenta (Graham) L.B.Sm.				×	Araujo et al., 2012
	Nidularium procerum Lindm.				×	Araujo et al., 2012
	Quesnelia quesneliana (Brongn.) L.B.Sm.				×	Araujo et al., 2012
	Vriesea procera (Mart. ex Schult. & Schult.f.) Wittm.				×	Araujo et al., 2012
Kazachstania rupicola	*Vriesea minarum* L.B.Sm.				×	Safar et al., 2013; Gomes et al., 2015
Kloeckera apiculata var. apis	*Ananas comosus* (L.) Merr.		×			Korres et al., 2011
Kluyveromyces aestuarii	*Aechmea nudicaulis* (L.) Griseb.				×	Araujo et al., 1998
Kluyveromyces marxianus	*Quesnelia quesneliana* (Brongn.) L.B.Sm.				×	Hagler et al., 1993
	Ananas comosus (L.) Merr.		×			Udota and Urua, 2010
Kodamaea ohmeri	*Vriesea minarum* L.B.Sm.				×	Gomes et al., 2015
Lachancea thermotolerans	*Vriesea procera* (Mart. ex Schult. & Schult.f.) Wittm.				×	Araujo et al., 1998

Metschnikowia fructicola	*Tillandsia geminiflora* Brongn.			×		Landell et al., 2006
Metschnikowia hawaiiensis	*Tillandsia gardneri* Lindl.			×		Landell et al., 2006
Metschnikowia koreensis	*Vriesea minarum* L.B.Sm.				×	Gomes et al., 2015
Meyerozyma caribbica	*Ananas comosus* (L.) Merr.		×			Amorim et al 2018; Leneveu-jenvrin et al., 2020; Islam et al., 2021
	Vriesea minarum L.B.Sm.				×	Gomes et al., 2015
	Bromelia karatas L.				×	Morais et al., 2020
	Encholirium sp.				×	Morais et al., 2020
Meyerozyma guilliermondii	*Ananas comosus* (L.) Merr.		×			Reyes et al., 2004; Chanprasartsuk et al., 2010; 2013; Dellacassa et al., 2017; Di Cagno et al., 2010
	Aechmea muricata (Arruda) L.B.Sm.	×				Félix et al., 2021
	Aechmea werdermannii Harms	×				Félix et al., 2021
	Vriesea minarum L.B.Sm.				×	Gomes et al., 2015
	Quesnelia quesneliana (Brongn.) L.B.Sm.				×	Hagler et al., 1993
	Bromelia karatas L.				×	Morais et al., 2020
	Encholirium sp.				×	Morais et al., 2020
	Aechmea froesii (L.B.Sm.) Leme & J.A.Siqueira			×		Navarro et al., 2020
	Aechmea fulgens Brongn.			×		Navarro et al., 2020
	Billbergia sp.			×		Navarro et al., 2020
	Canistrum alagoanum Leme & J.A.Siqueira			×		Navarro et al., 2020
Pichia fermentans	*Ananas comosus* (L.) Merr.		×			Udota and Urua, 2010; Chanprasartsuk et al. 2013; Islam et al., 2021
Pichia kudriavzevii	*Ananas comosus* (L.) Merr.		×			Chanprasartsuk et al., 2010; 2013; Korres et al., 2011
Pichia membranifaciens	*Aechmea nudicaulis* (L.) Griseb.				×	Araujo et al., 1998
	Nidularium procerum Lindm.				×	Araujo et al., 1998
	Vriesea procera (Mart. ex Schult. & Schult.f.) Wittm.				×	Araujo et al., 1998

	Ananas comosus (L.) Merr.		×			Chanprasartsuk et al., 2013
	Quesnelia quesneliana (Brongn.) L.B.Sm.				×	Hagler et al., 1993
Pichia occidentalis	*Ananas comosus* (L.) Merr.		×			Chanprasartsuk et al., 2010
Priceomyces melissophilus	*Tillandsia gardneri* Lindl.			×		Landell et al., 2006
Saccharomyces cerevisiae	*Ananas comosus* (L.) Merr.		×			Udota and Urua, 2010; Leneveu-jenvrin et al., 2020
Saccharomycodes ludwigii	*Ananas comosus* (L.) Merr.		×			Chanprasartsuk et al., 2010; 2013
Saturnispora silvae	*Vriesea minarum* L.B.Sm.				×	Gomes et al., 2015
Schwanniomyces etchellsii	*Quesnelia arvensis* (Vell.) Mez				×	Araujo et al., 1998
	Vriesea procera (Mart. ex Schult. & Schult.f.) Wittm.				×	Araujo et al., 1998
Schwanniomyces occidentalis	*Neoregelia cruenta* (Graham) L.B.Sm.				×	Araujo et al., 1998
Schwanniomyces vanrijiae	*Nidularium procerum* Lindm.				×	Araujo et al., 1998
	Tillandsia gardneri Lindl.			×		Landell et al., 2006
Starmerella apicola	*Ananas comosus* (L.) Merr.		×			Chanprasartsuk et al., 2010
Starmerella magnoliae	*Quesnelia arvensis* (Vell.) Mez				×	Araujo et al., 1998
	Vriesea procera (Mart. ex Schult. & Schult.f.) Wittm.				×	Araujo et al., 1998
Starmerella stellata	*Ananas comosus* (L.) Merr.		×			Chanprasartsuk et al., 2013
Torulaspora delbrueckii	*Aechmea froesii* (L.B.Sm.) Leme & J.A.Siqueira	×				Félix et al., 2021
	Neoregelia cruenta (Graham) L.B.Sm.				×	Hagler et al., 1993
	Quesnelia quesneliana (Brongn.) L.B.Sm.				×	Hagler et al., 1993
	Bromelia karatas L.				×	Morais et al., 2020
	Ananas comosus (L.) Merr.		×			Udota and Urua, 2010
Wickerhamiella jalapaonensis	*Aechmea fulgens* Brongn.	×				Félix et al., 2021
Wickerhamiella sorbophila	*Quesnelia quesneliana* (Brongn.) L.B.Sm.				×	Hagler et al., 1993
Wickerhamomyces anomalus	*Quesnelia arvensis* (Vell.) Mez				×	Araujo et al., 1998
	Ananas comosus (L.) Merr.		×			Dellacassa et al., 2017
Yamadazyma mexicana	*Quesnelia arvensis* (Vell.) Mez				×	Araujo et al., 1998

Yarrowia lipolytica	*Quesnelia arvensis* (Vell.) Mez				×	Araujo et al., 1998
	Ananas comosus (L.) Merr.		×			Chanprasartsuk et al., 2010; 2013
Zygossacharomyces bailii	*Ananas comosus* (L.) Merr.		×			Chanprasartsuk et al., 2010; 2013
	Tillandsia gardneri Lindl.			×		Landell et al., 2006
Basidiomycota (Phyla)						
Agaricomycotina (Subphyla)						
Atelosaccharomyces hudeloi	*Nidularium procerum* Lindm.				×	Araujo et al., 1998
	Quesnelia quesneliana (Brongn.) L.B.Sm.				×	Hagler et al., 1993
Bulleribasidium variabile	*Quesnelia arvensis* (Vell.) Mez				×	Araujo et al., 1998
Bulleromyces albus	*Bromelia antiacantha* Bertol.			×		Landell et al., 2006
	Tillandsia geminiflora Brongn.			×		Landell et al., 2006
Carcinomyces nordestinensis	*Bromelia antiacantha* Bertol.			×		Crous et al., 2019
Carlosrosaea aechmeae	*Aechmea costantinii* (Mez) L.B.Sm.			×		Félix et al., 2017; Navarro et al., 2020
Carlosrosaea hohenbergiae	*Aechmea fulgens* Brongn.	×		×		Félix et al., 2017, 2021; Navarro et al., 2020
	Canistrum alagoanum Leme & J.A.Siqueira	×				Félix et al., 2017, 2021
	Aechmea leptantha (Harms) Leme & J.A.Siqueira			×		Félix et al., 2017; Navarro et al., 2020
	Hohenbergia ramageana Mez			×		Félix et al., 2017
	Tillandsia sp.			×		Félix et al., 2017; Navarro et al., 2020
	Canistrum aurantiacum É.Morren			×		Navarro et al., 2020
	Hohenbergia ridleyi (Baker) Mez			×		Navarro et al., 2020
	Hohenbergia stellata Schult. & Schult.f.			×		Navarro et al., 2020
	Tillandsia chapeuensis Rauh			×		Navarro et al., 2020
Carlosrosaea vrieseae	*Tillandsia gardneri* Lindl.			×		Landell et al., 2015
	Vriesea friburgensis Mez				×	Landell et al., 2015
	Vriesea minarum L.B.Sm.				×	Landell et al., 2015

Cystofilobasidium macerans	*Neoregelia cruenta* (Graham) L.B.Sm.				×	Hagler et al., 1993
	Quesnelia quesneliana (Brongn.) L.B.Sm.				×	Hagler et al., 1993
Dimennazyma cisti-albidi	*Aechmea froesii* (L.B.Sm.) Leme & J.A.Siqueira	×				Félix et al., 2021
Dioszegia hungarica	*Neoregelia cruenta* (Graham) L.B.Sm.				×	Hagler et al., 1993
	Quesnelia quesneliana (Brongn.) L.B.Sm.				×	Hagler et al., 1993
	Vriesea friburgensis Mez			×		Landell et al., 2006
	Vriesea procera (Mart. ex Schult. & Schult.f.) Wittm.			×		Landell et al., 2006
	Werauhia gigantea (Mart. ex Schult. & Schult.f.) J.R.Grant			×		Landell et al., 2006
Fellomyces borneensis	*Aechmea werdermannii* Harms			×		Navarro et al., 2020
	Neoregelia gigas Leme & L.Kollmann			×		Navarro et al., 2020
Fellomyces fuzhouensis	*Vriesea procera* (Mart. ex Schult. & Schult.f.) Wittm.			×		Landell et al., 2006
Fellomyces penicillatus	*Vriesea minarum* L.B.Sm.				×	Gomes et al., 2015
Fellomyces polyborus	*Dyckia* sp.			×		Landell et al., 2006
Genolevuria bromeliarum	*Tillandsia gardneri* Lindl.			×		Landell et al., 2009
	Vriesea friburgensis Mez			×		Landell et al., 2009
	Vriesea procera (Mart. ex Schult. & Schult.f.) Wittm.			×		Landell et al., 2009
Goffeauzyma gastrica	*Quesnelia quesneliana* (Brongn.) L.B.Sm.				×	Hagler et al., 1993
Hannaella kunmingensis	*Hohenbergia stellata* Schult. & Schult.f.			×		Navarro et al., 2020
Hannaella luteola	*Vriesea minarum* L.B.Sm.				×	Gomes et al., 2015
	Tillandsia gardneri Lindl.	×				Landell et al., 2006
	Tillandsia geminiflora Brongn.			×		Landell et al., 2006
	Bromelia karatas L.				×	Morais et al., 2020
	Encholirium sp.				×	Morais et al., 2020
	Aechmea fulgens Brongn.			×		Navarro et al., 2020
	Neoregelia gigas Leme & L.Kollmann			×		Navarro et al., 2020
Hannaella oryzae	*Araeococcus chlorocarpus* (Wawra) Leme & J.A.Siqueira			×		Navarro et al., 2020

Hannaella pagnoccae	*Vriesea minarum* L.B.Sm.			×	×	Landell et al., 2014; Gomes et al., 2015
	Bromelia karatas L.				×	Landell et al., 2014; Morais et al., 2020
	Encholirium sp.				×	Landell et al., 2014; Morais et al., 2020
	Tillandsia geminiflora Brongn.			×		Landell et al., 2014
	Werauhia gigantea (Mart. ex Schult. & Schult.f.) J.R.Grant			×		Landell et al., 2014
	Aechmea leptantha (Harms) Leme & J.A.Siqueira			×		Navarro et al., 2020
	Aechmea muricata (Arruda) L.B.Sm.			×		Navarro et al., 2020
	Araeococcus chlorocarpus (Wawra) Leme & J.A.Siqueira			×		Navarro et al., 2020
	Hohenbergia stellata Schult. & Schult.f.			×		Navarro et al., 2020
Hannaella phetchabunensis	*Tillandsia* sp.			×		Navarro et al., 2020
Hannaella siamensis	*Aechmea leptantha* (Harms) Leme & J.A.Siqueira			×		Navarro et al., 2020
Hannaella sinensis	*Vriesea minarum* L.B.Sm.				×	Gomes et al., 2015
Hannaella zeae	*Bromelia karatas* L.				×	Morais et al., 2020
	Encholirium sp.				×	Morais et al., 2020
Kockovaella libkindii	*Vriesea minarum* L.B.Sm.				×	Gomes et al., 2016
Kockovaella sacchari	*Neoregelia gigas* Leme & L.Kollmann			×		Navarro et al., 2020
Kwoniella dendrophila	*Bromelia karatas* L.				×	Morais et al., 2020
Kwoniella heveanensis	*Bromelia karatas* L.				×	Morais et al., 2020
	Encholirium sp.				×	Morais et al., 2020
	Vriesea minarum L.B.Sm.				×	Morais et al., 2020
Kwoniella mangroviensis	*Bromelia karatas* L.				×	Morais et al., 2020
	Encholirium sp.				×	Morais et al., 2020
Naganishia albida	*Neoregelia cruenta* (Graham) L.B.Sm.				×	Araujo et al., 1998
	Nidularium procerum Lindm.				×	Araujo et al., 1998
	Quesnelia arvensis (Vell.) Mez				×	Araujo et al., 1998

	Vriesea procera (Mart. ex Schult. & Schult.f.) Wittm.				×	Araujo et al., 1998
	Neoregelia cruenta (Graham) L.B.Sm.				×	Hagler et al., 1993
	Quesnelia quesneliana (Brongn.) L.B.Sm.				×	Hagler et al., 1993
	Aechmea recurvata (Klotzsch) L.B.Sm.			×		Landell et al., 2006
	Bromelia antiacantha Bertol.			×		Landell et al., 2006
	Tillandsia gardneri Lindl.			×		Landell et al., 2006
	Tillandsia geminiflora Brongn.			×		Landell et al., 2006
	Vriesea friburgensis Mez			×		Landell et al., 2006
	Werauhia gigantea (Mart. ex Schult. & Schult.f.) J.R.Grant			×		Landell et al., 2006
	Ananas comosus (L.) Merr.		×			Reyes et al., 2004
Papiliotrema flavescens	*Aechmea froesii* (L.B.Sm.) Leme & J.A.Siqueira	×		×		Navarro et al., 2020; Félix et al., 2021
	Aechmea muricata (Arruda) L.B.Sm.	×				Félix et al., 2021
	Araeococcus chlorocarpus (Wawra) Leme & J.A.Siqueira	×		×		Navarro et al., 2020; Félix et al., 2021
	Billbergia sp.	×				Félix et al., 2021
	Canistrum aurantiacum É.Morren	×				Félix et al., 2021
	Cryptanthus dianae Leme	×				Félix et al., 2021
	Vriesea minarum L.B.Sm.				×	Gomes et al., 2015; Morais et al., 2020
	Bromelia karatas L.				×	Morais et al., 2020
	Encholirium sp.				×	Morais et al., 2020
	Aechmea costantinii (Mez) L.B.Sm.			×		Navarro et al., 2020
	Aechmea leptantha (Harms) Leme & J.A.Siqueira			×		Navarro et al., 2020
	Aechmea werdermannii Harms			×		Navarro et al., 2020
	Canistrum improcerum Leme & J.A.Siqueira			×		Navarro et al., 2020
	Hohenbergia stellata Schult. & Schult.f.			×		Navarro et al., 2020
Papiliotrema laurentii	*Neoregelia cruenta* (Graham) L.B.Sm.				×	Hagler et al., 1993; Araujo et al., 1998

	Vriesea minarum L.B.Sm.				×	Gomes et al., 2015; Morais et al., 2020
	Quesnelia quesneliana (Brongn.) L.B.Sm.				×	Gomes et al., 2015
	Bromelia antiacantha Bertol.			×		Landell et al., 2006
	Tillandsia crocata (É.Morren) N.E.Br.	×		×		Landell et al., 2006
	Tillandsia gardneri Lindl.			×		Landell et al., 2006
	Tillandsia geminiflora Brongn.			×		Landell et al., 2006
	Werauhia gigantea (Mart. ex Schult. & Schult.f.) J.R.Grant			×		Landell et al., 2006
	Bromelia karatas L.				×	Morais et al., 2020
	Encholirium sp.				×	Morais et al., 2020
	Aechmea froesii (L.B.Sm.) Leme & J.A.Siqueira			×		Navarro et al., 2020
	Aechmea fulgens Brongn.			×		Navarro et al., 2020
	Aechmea leptantha (Harms) Leme & J.A.Siqueira			×		Navarro et al., 2020
	Ananas comosus (L.) Merr.			×		Navarro et al., 2020
	Canistrum alagoanum Leme & J.A.Siqueira			×		Navarro et al., 2020
	Canistrum aurantiacum É.Morren			×		Navarro et al., 2020
	Tillandsia chapeuensis Rauh			×		Navarro et al., 2020
Papiliotrema leoncinii	*Aechmea fulgens* Brongn.			×		Pagani et al., 2016
	Aechmea leptantha (Harms) Leme & J.A.Siqueira			×		Pagani et al., 2016
	Aechmea recurvata (Klotzsch) L.B.Sm.			×		Pagani et al., 2016
	Bromelia antiacantha Bertol.			×		Pagani et al., 2016
	Bromelia sp.			×		Pagani et al., 2016
	Canistrum alagoanum Leme & J.A.Siqueira			×		Pagani et al., 2016
	Canistrum aurantiacum É.Morren			×		Pagani et al., 2016
	Tillandsia crocata (É.Morren) N.E.Br.				×	Pagani et al., 2016
	Tillandsia gardneri Lindl.			×		Pagani et al., 2016
	Vriesea friburgensis Mez			×		Pagani et al., 2016

	Werauhia gigantea (Mart. ex Schult. & Schult.f.) J.R.Grant			×		Pagani et al., 2016
Papiliotrema mangalensis	*Aechmea fulgens* Brongn.			×		Navarro et al., 2020
	Cryptanthus dianae Leme			×		Navarro et al., 2020
Papiliotrema miconiae	*Aechmea leptantha* (Harms) Leme & J.A.Siqueira	×				Félix et al., 2021
	Canistrum alagoanum Leme & J.A.Siqueira	×				Félix et al., 2021
	Canistrum aurantiacum É.Morren	×				Félix et al., 2021
	Aechmea werdermannii Harms			×		Navarro et al., 2020
Papiliotrema nemorosa	*Aechmea costantinii* (Mez) L.B.Sm.	×				Félix et al., 2021
	Aechmea froesii (L.B.Sm.) Leme & J.A.Siqueira	×				Félix et al., 2021
	Vriesea minarum L.B.Sm.				×	Gomes et al., 2015; Morais et al., 2020
	Bromelia karatas L.				×	Morais et al., 2020
	Encholirium sp.				×	Morais et al., 2020
	Cryptanthus zonatus (Vis.) Beer			×		Navarro et al., 2020
	Tillandsia chapeuensis Rauh			×		Navarro et al., 2020
Papiliotrema rajasthanensis	*Vriesea minarum* L.B.Sm.				×	Gomes et al., 2015
	Bromelia karatas L.				×	Morais et al., 2020
	Encholirium sp.				×	Morais et al., 2020
Papiliotrema siamense	*Ananas comosus* (L.) Merr.			×		Navarro et al., 2020
	Cryptanthus dianae Leme			×		Navarro et al., 2020
	Tillandsia kegeliana Mez			×		Navarro et al., 2020
Papiliotrema terrestris	*Bromelia karatas* L.				×	Morais et al., 2020
	Encholirium sp.				×	Morais et al., 2020
Phaeotremella foliacea	*Quesnelia arvensis* (Vell.) Mez				×	Araujo et al., 1998
Piskurozyma taiwanensis	*Aechmea froesii* (L.B.Sm.) Leme & J.A.Siqueira			×		Navarro et al., 2020
Rhynchogastrema complexa	*Bromelia karatas* L.				×	Morais et al., 2020
	Ananas comosus (L.) Merr.			×		Valente et al., 2012

	Neoregelia cruenta (Graham) L.B.Sm.			×		Valente et al., 2012
Saitozyma flava	*Quesnelia quesneliana* (Brongn.) L.B.Sm.				×	Hagler et al., 1993
	Vriesea minarum L.B.Sm.				×	Morais et al., 2020
	Bromelia karatas L.			×		Navarro et al., 2020
Saitozyma podzolica	*Cryptanthus dianae* Leme	×				Félix et al., 2021
	Vriesea minarum L.B.Sm.				×	Gomes et al., 2015
	Bromelia karatas L.				×	Morais et al., 2020
	Aechmea froesii (L.B.Sm.) Leme & J.A.Siqueira			×		Navarro et al., 2020
	Aechmea muricata (Arruda) L.B.Sm.			×		Navarro et al., 2020
	Aechmea werdermannii Harms			×		Navarro et al., 2020
	Ananas comosus (L.) Merr.			×		Navarro et al., 2020
	Araeococcus chlorocarpus (Wawra) Leme & J.A.Siqueira			×		Navarro et al., 2020
Solicoccozyma aeria	*Quesnelia quesneliana* (Brongn.) L.B.Sm.				×	Hagler et al., 1993
Tremella fuciformis	*Cryptanthus dianae* Leme			×		Navarro et al., 2020
Tremella globispora	*Ananas comosus* (L.) Merr.		×			Chanprasartsuk et al., 2010; 2013
	Bromelia karatas L.				×	Morais et al., 2020
	Vriesea minarum L.B.Sm.				×	Morais et al., 2020
Trichosporon bergelli	*Aechmea nudicaulis* (L.) Griseb.				×	Araujo et al., 1998
Vanrija humicola	*Vriesea friburgensis* Mez			×		Landell et al., 2006
Vishniacozyma alagoana	*Aechmea leptantha* (Harms) Leme & J.A.Siqueira	×				Félix et al., 2021
	Aechmea froesii (L.B.Sm.) Leme & J.A.Siqueira			×		Félix et al., 2020; Navarro et al., 2020
	Bromelia antiacantha Bertol.			×		Félix et al., 2020
	Hohenbergia stellata Schult. & Schult.f.			×		Félix et al., 2020; Navarro et al., 2020
	Aechmea fulgens Brongn.			×		Navarro et al., 2020
Vishniacozyma taibaiensis	*Billbergia* sp.			×		Navarro et al., 2020

	Cryptanthus dianae Leme			×		Navarro et al., 2020
Vonarxula javanica	*Billbergia* sp.			×		Navarro et al., 2020
Pucciniomycotina (Subphyla)						
Colacogloea diffluens	*Aechmea leptantha* (Harms) Leme & J.A.Siqueira	×				Félix et al., 2021
	Tillandsia chapeuensis Rauh			×		Navarro et al., 2020
Curvibasidium nothofagi	*Encholirium* sp.				×	Morais et al., 2020
Cyrenella elegans	*Aechmea froesii* (L.B.Sm.) Leme & J.A.Siqueira			×		Navarro et al., 2020
	Aechmea tomentosa Mez			×		Navarro et al., 2020
Cystobasidium calyptogenae	*Encholirium* sp.				×	Morais et al., 2020
Cystobasidium laryngis	*Vriesea minarum* L.B.Sm.				×	Gomes et al., 2015
Cystobasidium minutum	*Quesnelia arvensis* (Vell.) Mez				×	Araujo et al., 1998
	Quesnelia quesneliana (Brongn.) L.B.Sm.				×	Hagler et al., 1993
	Tillandsia gardneri Lindl.			×		Landell et al., 2006
	Vriesea friburgensis Mez			×		Landell et al., 2006
	Werauhia gigantea (Mart. ex Schult. & Schult.f.) J.R.Grant			×		Landell et al., 2006
Erythrobasidium hasegawianum	*Ananas comosus* (L.) Merr.		×			Chanprasartsuk et al., 2010; 2013
	Vriesea minarum L.B.Sm.				×	Gomes et al., 2015
	Encholirium sp.				×	Morais et al., 2020
Microbotryozyma collariae	*Bromelia karatas* L.				×	Morais et al., 2020
Occultifur brasiliensis	*Vriesea minarum* L.B.Sm.				×	Gomes et al., 2015
	Bromelia karatas L.				×	Morais et al., 2020
Occultifur externus	*Bromelia karatas* L.				×	Morais et al., 2020
	Encholirium sp.				×	Morais et al., 2020
	Vriesea minarum L.B.Sm.				×	Morais et al., 2020
	Tillandsia chapeuensis Rauh			×		Navarro et al., 2020
Occultifur plantarum	*Bromelia karatas* L.	×				Félix et al., 2021

	Canistrum aurantiacum É.Morren	×				Félix et al., 2021
	Neoregelia cruenta (Graham) L.B.Sm.				×	Khunnamwong et al., 2017
	Aechmea werdermannii Harms			×		Navarro et al., 2020
Pseudohyphozyma bogoriensis	*Aechmea fulgens* Brongn.	×				Félix et al., 2021
	Araeococcus chlorocarpus (Wawra) Leme & J.A.Siqueira	×				Félix et al., 2021
Queiroziella brasiliensis	*Aechmea leptantha* (Harms) Leme & J.A.Siqueira			×		Crous et al., 2018
	Tillandsia geminiflora Brongn.			×		Crous et al., 2018
	Werauhia gigantea (Mart. ex Schult. & Schult.f.) J.R.Grant			×		Crous et al., 2018
	Aechmea leptantha (Harms) Leme & J.A.Siqueira			×		Navarro et al., 2020
Rhodosporidiobolus poonsookiae	*Cryptanthus dianae* Leme			×		Navarro et al., 2020
Rhodosporidiobolus ruineniae	*Aechmea muricata* (Arruda) L.B.Sm.	×				Félix et al., 2021
	Cryptanthus dianae Leme	×				Félix et al., 2021
	Encholirium sp.				×	Morais et al., 2020
Rhodosporidium diobovatum	*Vriesea minarum* L.B.Sm.				×	Gomes et al., 2015
Rhodotorula aurantiaca	*Quesnelia arvensis* (Vell.) Mez				×	Araujo et al., 1998
	Vriesea procera (Mart. ex Schult. & Schult.f.) Wittm.				×	Araujo et al., 1998
	Neoregelia cruenta (Graham) L.B.Sm.				×	Hagler et al., 1993
	Aechmea recurvata (Klotzsch) L.B.Sm.			×		Landell et al., 2006
	Tillandsia gardneri Lindl.			×		Landell et al., 2006
	Tillandsia geminiflora Brongn.			×		Landell et al., 2006
	Vriesea procera (Mart. ex Schult. & Schult.f.) Wittm.			×		Landell et al., 2006
	Werauhia gigantea (Mart. ex Schult. & Schult.f.) J.R.Grant			×		Landell et al., 2006
	Ananas comosus (L.) Merr.		×			Reyes et al., 2004
Rhodotorula babjevae	*Neoregelia cruenta* (Graham) L.B.Sm.				×	Hagler et al., 1993
	Quesnelia quesneliana (Brongn.) L.B.Sm.				×	Hagler et al., 1993

Rhodotorula glutinis	*Aechmea nudicaulis* (L.) Griseb.				×	Araujo et al., 1998
	Neoregelia cruenta (Graham) L.B.Sm.				×	Hagler et al., 1993; Araujo et al., 1998
	Quesnelia quesneliana (Brongn.) L.B.Sm.				×	Hagler et al., 1993
	Tillandsia crocata (É.Morren) N.E.Br.	×				Landell et al., 2006
	Vriesea procera (Mart. ex Schult. & Schult.f.) Wittm.			×		Landell et al., 2006
	Ananas comosus (L.) Merr.		×			Reyes et al., 2004
Rhodotorula graminis	*Quesnelia quesneliana* (Brongn.) L.B.Sm.				×	Hagler et al., 1993
Rhodotorula lactosa	*Tillandsia geminiflora* Brongn.			×		Landell et al., 2006
Rhodotorula mucilaginosa	*Neoregelia cruenta* (Graham) L.B.Sm.				×	Araujo et al., 1998
	Nidularium procerum Lindm.				×	Araujo et al., 1998
	Quesnelia arvensis (Vell.) Mez				×	Araujo et al., 1998
	Ananas comosus (L.) Merr.		×			Chanprasartsuk et al., 2010; 2013; Leneveu-jenvrin et al., 2020
	Vriesea minarum L.B.Sm.				×	Gomes et al., 2015; Morais et al., 2020
	Bromelia karatas L.				×	Morais et al., 2020
	Encholirium sp.				×	Morais et al., 2020
Rhodotorula paludigena	*Vriesea minarum* L.B.Sm.				×	Morais et al., 2020
Rhodotorula taiwanensis	*Vriesea minarum* L.B.Sm.				×	Morais et al., 2020
	Aechmea leptantha (Harms) Leme & J.A.Siqueira			×		Navarro et al., 2020
Rhodotorula toruloides	*Ananas comosus* (L.) Merr.		×			Chanprasartsuk et al., 2013
	Encholirium sp.				×	Morais et al., 2020
Sporobolomyces carnicolor	*Aechmea froesii* (L.B.Sm.) Leme & J.A.Siqueira	×				Félix et al., 2021
Sporobolomyces roseus	*Bromelia antiacantha* Bertol.			×		Landell et al., 2006
	Tillandsia gardneri Lindl.			×		Landell et al., 2006
	Tillandsia geminiflora Brongn.	×				Landell et al., 2006
	Tillandsia stricta Sol. ex Ker Gawl.			×		Landell et al., 2006

	Werauhia gigantea (Mart. ex Schult. & Schult.f.) J.R.Grant			×		Landell et al., 2006
	Ananas comosus (L.) Merr.		×			Udota and Urua, 2010
Sporobolomyces salmonicolor	*Bromelia antiacantha* Bertol.			×		Landell et al., 2006
	Tillandsia geminiflora Brongn.			×		Landell et al., 2006
	Vriesea friburgensis Mez			×		Landell et al., 2006
	Werauhia gigantea (Mart. ex Schult. & Schult.f.) J.R.Grant			×		Landell et al., 2006
Sporobolomyces shibatanus	*Bromelia antiacantha* Bertol.			×		Landell et al., 2006
	Tillandsia crocata (É.Morren) N.E.Br.	×				Landell et al., 2006
	Tillandsia geminiflora Brongn.			×		Landell et al., 2006
	Vriesea friburgensis Mez			×		Landell et al., 2006
Symmetrospora marina	*Vriesea minarum* L.B.Sm.				×	Morais et al., 2020
	Aechmea leptantha (Harms) Leme & J.A.Siqueira			×		Navarro et al., 2020
	Aechmea werdermannii Harms			×		Navarro et al., 2020
	Hohenbergia stellata Schult. & Schult.f.			×		Navarro et al., 2020
Symmetrospora suhii	*Aechmea cephaloides* J.A.Siqueira & Leme			×		Navarro et al., 2020
	Aechmea leptantha (Harms) Leme & J.A.Siqueira			×		Navarro et al., 2020
	Canistrum aurantiacum É.Morren			×		Navarro et al., 2020
	Cryptanthus burle-marxii Leme			×		Navarro et al., 2020
Symmetrospora symmetricus	*Hohenbergia ridleyi* (Baker) Mez			×		Navarro et al., 2020
Yunzhangia sonckii	*Vriesea friburgensis* Mez			×		Landell et al., 2006
Ustilaginomycotina (Subphyla)						
Anomalomyces panici	*Vriesea minarum* L.B.Sm.				×	Gomes et al., 2015; Morais et al., 2020
Anomalomyces yakirrae	*Vriesea minarum* L.B.Sm.				×	Morais et al., 2020
Anthracocystis elionuri	*Bromelia karatas* L.				×	Morais et al., 2020
Anthracocystis everhartii	*Bromelia karatas* L.				×	Morais et al., 2020

Cintractia samanensis	*Vriesea minarum* L.B.Sm.				×	Morais et al., 2020
Farysia itapuensis	*Dyckia* sp.			×		Inácio et al., 2008
	Tillandsia gardneri Lindl.			×		Inácio et al., 2008
	Tillandsia geminiflora Brongn.			×		Inácio et al., 2008
	Vriesea friburgensis Mez			×		Inácio et al., 2008
	Vriesea procera (Mart. ex Schult. & Schult.f.) Wittm.			×		Inácio et al., 2008
Gjaerumia minor	*Vriesea minarum* L.B.Sm.				×	Morais et al., 2020
Jaminaea rosea	*Cryptanthus zonatus* (Vis.) Beer			×		Navarro et al., 2020
Kalmanozyma brasiliensis	*Encholirium* sp.				×	Morais et al., 2020
Langdonia jejuensis	*Vriesea minarum* L.B.Sm.				×	Morais et al., 2020
Meira miltonrushii	*Aechmea werdermannii* Harms			×		Navarro et al., 2020
	Hohenbergia ridleyi (Baker) Mez			×		Navarro et al., 2020
Meira nashicola	*Aechmea froesii* (L.B.Sm.) Leme & J.A.Siqueira			×		Navarro et al., 2020
Microstroma bacarum	*Neoregelia cruenta* (Graham) L.B.Sm.				×	Araujo et al., 1998
	Tillandsia crocata (É.Morren) N.E.Br.			×		Landell et al., 2006
Moesziomyces antarcticus	*Neoregelia cruenta* (Graham) L.B.Sm.				×	Hagler et al., 1993
	Quesnelia quesneliana (Brongn.) L.B.Sm.				×	Hagler et al., 1993
	Hohenbergia stellata Schult. & Schult.f.			×		Navarro et al., 2020
Moesziomyces aphidis	*Aechmea leptantha* (Harms) Leme & J.A.Siqueira	×		×		Félix et al., 2021; Navarro et al., 2020
	Vriesea minarum L.B.Sm.				×	Gomes et al., 2015
	Araeococcus chlorocarpus (Wawra) Leme & J.A.Siqueira			×		Navarro et al., 2020
	Bromelia karatas L.			×		Navarro et al., 2020
	Canistrum alagoanum Leme & J.A.Siqueira			×		Navarro et al., 2020
	Hohenbergia stellata Schult. & Schult.f.			×		Navarro et al., 2020
Pattersoniomyces tillandsiae	*Canistrum improcerum* Leme & J.A.Siqueira			×		Piątek et al., 2017; Navarro et al., 2020

	Tillandsia flabellata Baker			×		Piątek et al., 2017
	Tillandsia leiboldiana Schltdl.			×		Piątek et al., 2017
	Vriesea minarum L.B.Sm.				×	Piątek et al., 2017
Pseudozyma hubeiensis	*Araeococcus chlorocarpus* (Wawra) Leme & J.A.Siqueira	×		×		Navarro et al., 2020; Félix et al., 2021
	Cryptanthus dianae Leme	×		×		Navarro et al., 2020; Félix et al., 2021
	Vriesea minarum L.B.Sm.				×	Gomes et al., 2015; Morais et al., 2020
	Bromelia karatas L.				×	Morais et al., 2020
	Encholirium sp.				×	Morais et al., 2020
	Aechmea froesii (L.B.Sm.) Leme & J.A.Siqueira			×		Navarro et al., 2020
	Aechmea werdermannii Harms			×		Navarro et al., 2020
	Ananas comosus (L.) Merr.			×		Navarro et al., 2020
	Billbergia sp.			×		Navarro et al., 2020
	Canistrum alagoanum Leme & J.A.Siqueira			×		Navarro et al., 2020
	Canistrum improcerum Leme & J.A.Siqueira			×		Navarro et al., 2020
	Hohenbergia stellata Schult. & Schult.f.			×		Navarro et al., 2020
	Tillandsia chapeuensis Rauh			×		Navarro et al., 2020
	Tillandsia kegeliana Mez			×		Navarro et al., 2020
Sympodiomycopsis paphiopedili	*Aechmea leptantha* (Harms) Leme & J.A.Siqueira			×		Navarro et al., 2020
Sympodiomycopsis yantaiensis	*Aechmea froesii* (L.B.Sm.) Leme & J.A.Siqueira			×		Navarro et al., 2020
Ustilago sparsa	*Bromelia karatas* L.				×	Morais et al., 2020

4. DISCUSSION

4.1. Diversity

It is not the first time that bromeliads are mentioned as an environment rich in yeast diversity, e.g. (Morais et al., 2020; Navarro et al., 2020). However, these results present a more comprehensive and precise view of the size of this richness, as well as how it is distributed among the different compartments of the phyllosphere. The richest taxonomic groups (species number) in the bromeliad phyllosphere are groups commonly found in other plants, especially in leaves (Fonseca and Inácio, 2006), such as genera *Candida, Papiliotrema, Rhodotorula, Hannaella*, and the orders Saccharomycetales, Tremellales, Sporidiobolales and Ustilaginales. Regarding the frequency of occurrence of the genera, *Papiliotrema, Candida, Aureobasidium, Meyerozyma, Rhodotorula, Carlosrosaea, Hannaella* and *Pseudozyma* were the most frequent in bromeliads. At least one of these genera was found in about 70% of the analyzed units. The classically dominant genera in the phyllosphere are *Aureobasidium, Cryptococcus, Rhodotorula* and *Sporobolomyces* (Fonseca and Inácio, 2006; Vacher et al., 2016). Even today, it is not uncommon to mention *Cryptococcus* as one of the most frequent genera in plants (Vacher et al., 2016). Nevertheless, most species previously grouped in this genus have been reclassified and are distributed in other genera, such as *Kwoniella, Naganishia, Papiliotrema, Saitozyma* and *Vishniacozyma* (Liu et al., 2015). At least 17 species previously grouped in the genus *Cryptococcus* were transferred to *Papiliotrema* (Liu et al., 2015). Including species known to be frequent in plant substrates such as *P. flavescens, P. laurentii* and *P. nemorosus* (Fonseca and Inácio, 2006). We reported genera that are conventionally found in the phyllosphere, though often not very expressive. For instance, ascomycete yeasts tend to be less frequent in the phyllosphere than basidiomycetes (Fonseca and Inácio, 2006). Nonetheless, in bromeliad flowers (Félix et al., 2021), fruits (Chanprasartsuk et al., 2010), leaves (Landell et al., 2006) and water tanks (Araujo et al., 1998; Morais et al., 2020), the most frequent groups found include the genus *Candida*, but also *Meyerozyma* and *Hanseniaspora*. Still, the genus *Pseudozyma* has already been frequently recorded in bromeliad water tanks in the Brazilian savanna (Morais et al., 2020). It stood out also as one of the most common genera in bromeliads in Northeastern Brazil, both in leaves and in flowers (Félix et al., 2021; Navarro et al., 2020). Nevertheless,

Pseudozyma was the most frequent in other Poales plants (e.g. rice and maize) (Nasanit et al., 2016, 2015). Another recurrent genus in bromeliads is *Carlosrosaea*, which currently has five described species, all associated with plant substrates. To date, three of them are exclusively associated with bromeliads: *Ca. vrieseae, Ca. aechmeae* and *Ca. hohenbergiae* (Félix et al., 2021).

There is evidence to suggest that the phyllosphere community is more resilient and tolerates disturbances generated by rough stresses, such as rainfall. In fact, it is more influenced by long-term events, for example, seasonality (Stone and Jackson, 2021). On the other hand, there are also works that point to more immediate changes in the phyllosphere community, mainly on fruit surfaces, in response to disruptive events (Allard et al., 2020). However, this pattern is not absolute and may vary depending on the plant species and compartment studied (Allard et al., 2020). The aerial part of plants is a dynamic environment mainly regulated by four population processes: i) immigration, ii) emigration, iii) growth (generation), and iv) death (Fonseca and Inácio, 2006; Kinkel, 1997). Specifically in bromeliads, the microbial community can vary taxonomically and be functionally stable, even at small spatial scales (Louca et al., 2017). Most species recorded in the bromeliad phyllosphere had a low frequency of occurrence: 65% had at most two occurrences in the analyzed units, 52% occurred in only one host species, while 70% occurred in a single compartment of the phyllosphere (flowers, fruits, leaves or water tank). Only five yeast species (3% of the total) had a frequency of occurrence greater than 10%: *P. laurentii, P. flavescens, M. guilliermondii, C. intermedia, Ps. hubeiensis* and *A. pullulans*. The literature corroborates the low frequency of occurrence of yeasts in the phyllosphere, with most species found presenting a frequency of occurrence lower than 10%. Depending on the study, most may have a frequency lower than 1% (Glushakova et al., 2014; Glushakova and Chernov, 2010, 2007; Limtong and Kaewwichian, 2015; Nasanit et al., 2016). In bromeliads, the low frequency of occurrence and the large number of singletons have already been recorded in flowers, leaves and water tanks (Félix et al., 2021; Gomes et al., 2015; Morais et al., 2020; Navarro et al., 2020). This highlights that the richness of the phyllosphere tends to be mostly originated from rare groups. For example, the contribution of rare bacteria to the alpha and beta-diversity of the Phragmites australis phyllosphere is significantly greater than the contribution of abundant ones

(Zhou et al., 2019). If the intense dynamic of the phyllosphere accelerates the population processes that regulate it, the taxonomic shift of the phyllosphere can explain the low frequency of occurrence of the species, as a large part of the community members would be frequently changing. Nonetheless, this does mean that yeasts from the bromeliad phyllosphere are mostly transient. On the contrary, it shows that the community is dynamic and in a frequent turnover. Recent results point to a microbiota-sharing relationship among phyllosphere compartments and air, with leaves being the largest source of microorganisms. The air in this relationship could be compared to a microorganism 'stock' fed mainly by the plant compartments. Thus, the microbiota originating from the plant compartments is available and can, in a second moment, recolonize these compartments (Abdelfattah et al., 2019).

4.2. Yeasts in the different bromeliad compartments

Fonseca and Inácio (2006) mention that the phyllosphere yeast community tends to be more similar among leaves, intact fruits and flowers (when nectar is disregarded), especially concerning species composition and the prevalence of basidiomycetes yeasts. This prediction arises from the idea that these habitats are analogous as they share a similar configuration, since the interaction between the epiphytic microbiota and the plant cuticle is necessary in all these compartments. Our result partly confirms this statement, as among the compartments analyzed, the most similar communities were flowers and leaves; the only ones in which it is mandatory that yeasts interact directly with the cuticle. For instance, in fruits and water tanks, the microbial communities can interact with the plant without necessarily having any direct contact with the cuticle, as the tanks create an aquatic environment and used fruit samples are mainly dismantled, as discussed ahead. Regarding the diversity, leaves and water tanks were significantly more diverse than fruits and flowers. Furthermore, the composition was very distinct: 70% of all yeast species were exclusive to one of the compartments and only 2% were shared among all. The community structure followed the pattern and diverged between compartments, but inconclusively. However, it could be safely observed that leaves were the most heterogeneous compartment compared to the others. Using independent methods of cultivation, similar standards have been recently recorded, with few fungal OTUs shared among grape vineyards, fruit and flowers. In some

studies, the proportion of shared OTUs is as low as 0.3%, reinforcing how these compartments can be distinct (Abdelfattah et al., 2019; Liu and Howell, 2021).

4.3. Flowers

Flowers had the lowest richness of yeast species among the compartments, as well as the lowest proportion of exclusive species (18%). Moreover, they were the compartment with a community similarily related to leaves. It is important to emphasize that this compartment is, to date, the least studied within the bromeliad phyllosphere. Only one study focused on bromeliad flowers, and another sporadically collected a sample thereof. The most frequent species in this compartment were also common in leaves and tanks: *P. flavescens* and *C. intermedia*. Recent studies highlight that the most frequent species in flowers can vary greatly depending on the host and even between studies (Félix et al., 2021). Furthermore, only *Ph. bogoriensis* was a non-singleton exclusive to the flowers compartment. It was possible to observe that species richness in groups such as the genus *Sporobolomyces*, the class Microbotryomycetes and the subphylum Pucciniomycotina increased in flowers in relation to the other compartments. Abdelfattah et al. (2019) found a significantly greater abundance of *Sporobolomyces* in flowers than in other vine compartments. It is evident that to tolerate the extreme conditions of the phyllosphere, microorganisms must have efficient and versatile strategies that enable the colonization of this habitat. One of the explanations for the prevalence of some of the groups found relies on certain traits that make them more tolerant to phyllosphere stress. For example, when regarding the ability to produce pigments that are related to the tolerance to UV radiation, these pigments can be diverse, but they are often carotenoids that are widely found in members of Sporidiobolales (e.g. *Rhodotorula* and *Sporobolomyces*), or melanin, produced by black yeasts such as *Aureobasidium* and *Hortaea* (Fonseca and Inácio, 2006; Kemler et al., 2017). Furthermore, some species of *Sporobolomyces* and *Bullera* produce ballistospores, which are ejectable spores that have a dispersal function (Fonseca and Inácio, 2006; Kemler et al., 2017). Much of the literature usually mentions flowers as an environment dominated by ascomycetes yeasts and that the basidiomycetes yeasts found in this compartment are usually contaminating groups (Canto et al., 2017; Pozo et al., 2011). However, our results corroborate the

statement that when disregarding exudates such as nectar, at least in bromeliad flowers, most of the community consists of basidiomycetes. Nevertheless, the prevalence of basidiomycetes has been previously reported in hexose-dominant nectar flowers and flowers pollinated by birds (Mittelbach et al., 2015).

The anthoplane is a term that we will use to refer to the plant compartment that was analyzed in the study carried out by Félix et al. (2021), which is defined as the surface of the floral tissue after the removal (at least in a large part) of the floral exudates and consequently of the microbial community that accompanies them. So far, only Félix et al. (2021) has discussed this compartment in bromeliads as a unit (or subunit). Although Landell et al. (2006) have briefly isolated anthoplane yeasts, there was no mention of a distinction between the anthoplane and the anthosphere. Studies of yeast in flowers are commonly performed in anthosphere communities without distinguishing between the tissue surface and exudates, e.g. (Canto et al., 2017; Mittelbach et al., 2015; Pozo et al., 2011). There is not enough information in the literature to prove this, though the general idea is that the anthoplane is a subcompartment of the anthosphere. Thus, all microorganisms in the anthoplane are part of the anthosphere, but the opposite is not true. According to this logic, the prevalence of basidiomycetous yeasts would be characteristic of the anthoplane, as it has a similar configuration to the phylloplane (leaf), and of ascomycetes in the anthosphere, mainly due to the presence of nectar and other exudates that benefit specialists such as members of Metschnikowiaceae (Morris et al., 2020). This highlights that flowers are a complex substrate, with greater resemblance to a mosaic with several compartments instead of a single homogeneous compartment.

4.4. Fruits

Fruits are a complex and heterogeneous environment from a microbiological point of view (Ganter et al., 2017). For example, in pineapple fruits yeast density varies from 5.01 ± 0.05 to 4.0 ± 0.6 log CFU/g^{-1}. Density is higher in the fruit's outer ring and it decreases as the innermost regions are measured (Di Cagno et al., 2010). Similar to leaves, fruits are often dominated by oligotrophic and non-fermentative species of basidiomycete yeasts, mainly in immature and/or intact fruits (Fonseca and Inácio, 2006; Maksimova et al., 2009; Péter et al., 2017). However, among the bromeliad compartments, fruits were the most dissimilar to the leaves and

the only that showed the dominance of ascomycetes (78% of the species in this compartment), mainly from the Saccharomycetaceae family. A plausible explanation is that yeasts from bromeliad fruits were always perforated from ripe pineapple fruits (*Ananas comosus* (L.) Merr.), on various substrate types (fermented fruit, pulp, or peels). In addition, enrichment methods were used, which usually overrepresent ascomycetes yeasts. Basidiomycetes yeasts are usually more generalist and capable of assimilating several carbon sources (polytrophic), having an advantage in oligotrophic environments. Therefore, they are frequent and dominant in the phyllosphere, mainly directly associated with the cuticle (Félix et al., 2021; Fonseca and Inácio, 2006; Hagler et al., 1993; Yurkov, 2017).

Furthermore, in fruits, the natural process of senescence that leads to disruption, and later decay, mostly promotes changes in basidiomycetes, oligotrophic and non-fermentative communities, as well as in mostly ascomycetes, copiotrophic predominant communities and with fermenting yeasts (Ganter et al., 2017; Péter et al., 2017). This issue is strengthened as we observe that the most frequent yeasts in bromeliad fruits were ascomycetes fermenters, being frequently found in fruits (Ganter et al., 2017) such as *M. guilliermondii, H. uvarum* and *Pi. kudriavzevii*. Species related and exclusive to this bromeliad compartment, such as *C. sorboxylosa, Pi. kudriavzevii, Pi. fermentans* and *Saccharomyces cerevisiae* are also all fermenters. In turn, plant ascomycetes yeasts, mainly members of Saccharomycetaceae, tend to be specialists (assimilate a more restricted number of carbon sources), fast-growing and dominate copiotrophic environments (rich in simple sugars) such as nectar and ripe or decaying fruits (Félix et al., 2021; Fonseca and Inácio, 2006; Ganter et al., 2017; Péter et al., 2017). For example, approximately 85% of yeasts considered to be nectivores are fast-growing specialist ascomycetes (Pozo et al., 2011). While the surface-associated community of immature and/or intact fruit tends to undergo community displacements, the damaged or fermented fruit community tends to be more recurrent and stable (Fonseca and Inácio, 2006; Péter et al., 2017). For instance, in pineapple fruits, *M. guilliermondii* was the species that dominated the first days of fermentation, while towards the end of the process, the species *H. uvarum* became dominant. This pattern remained in independent collections in two continents (Chanprasartsuk et al., 2010). Moreover, a large number of yeasts capable of producing killer toxins have been found in fruits (mainly

decayed) (Ganter et al., 2017; Starmer and Lachance, 2011), which highlights the competitive character of the habitat, typical of copiotrophic environments (Félix et al., 2021).

4.5. Leaves

The surface of the leaves (phylloplane) is one of the largest habitats in the world and it has an estimated total surface area of 1 billion km²; equivalent to twice the surface area of the Earth (Bringel and Couée, 2015). Bromeliad leaves can vary in several aspects, from length, shape, color, as well as in terms of the serration of the edge. For example, while members of the genus Bromelia have a densely serrated edge, members of the subfamily Tillandsioideae have smooth edges (Benzing, 2000). Some species of bromeliads depend on the absorption of leaves to access water and nutrients, which reinforces the importance of the associated microbiota for the processes of decomposition and nutrient cycling (Leroy et al., 2016). In addition to the most common environmental disturbers that can affect the leaf yeast community such as rain, wind, and solar radiation (Fonseca and Inácio, 2006), other external factors can impact the community, e.g. epiphytic yeasts can be completely inhibited by air pollution on bromeliad leaves epiphytes of the genus Tillandsia (Brighigna et al., 2000). Most of the organic compounds made available to the epiphytic microbiota are made available spontaneously or non-spontaneously by the plant itself, while inorganic compounds are usually from an external source (Fonseca and Inácio, 2006; Kemler et al., 2017). As already mentioned, the phyllosphere (especially the phylloplane) is an extreme and oligotrophic environment, and this may be one of the factors that generate its great diversity. Copiotrophic environments (rich in simple sugars) are usually marked by a rapidly growing specialist community that, under competitive pressure, can diminish local diversity, and oligotrophic environments are marked by populations with lower abundances and higher diversity (Cray et al., 2013; Lachance, 2013; Félix et al. 2021). Furthermore, it is known that the yeast species shift is larger in these compartments in relation to the others (Fonseca and Inácio, 2006).

Compared to the other compartments analyzed (flowers, fruits, and water tank), leaves were the most heterogeneous (variance) and this compartment is the most perennial structures among the others, which can corroborate the heterogeneity

recorded. It is possible that the turnover of species, which can occur for seasonal reasons, host senescence, deterministic or stochastic factors, cause the community to vary over time more intensely than in structures that are restricted to a seasonal or reproductive period. Bromeliad leaves were one of the most diverse plant compartments and, as expected, the basidiomycetes were dominant. The genera *Papiliotrema* and *Carlosrosaea* stood out in this compartment in terms of species richness and the frequency of occurrence. The most frequent species in bromeliad leaves were all basidiomycetes: *P. laurentii, Ps. hubeiensis, Ca. hohenbergiae* and *P. leoncinii*. Finally, one of the patterns described regarding the richness of yeasts in bromeliad leaves indicates the existence of a significant and positive correlation between the diversity of bromeliads and associated yeasts. This pattern is probably a result of the greater diversity of hosts, which generates a more heterogeneous environment with a greater diversity of available niches (Navarro et al., 2020). However, no significant relationship was found between the beta-diversity of bromeliads and yeasts, which may indicate that there is no species-specific relationship between hosts and yeasts (Navarro et al., 2020).

4.6. Water tank

About half of the bromeliad genera and more than half of the subfamilies contain species capable of forming water tanks (phytotelma) (Ladino et al., 2019; Males and Griffiths, 2017). Depending on the species and environmental conditions, the phytotelm can accumulate from 0.015 to 45 L of water in a single individual (Zotz et al., 2020), and about 50,000 L in a hectare (Ladino et al., 2019). However, this compartment is an adverse environment mainly because it is ephemeral and dependent on external conditions, such as rain. Furthermore, evidence suggests less fluctuation in water conditions in plants with larger tanks and that accumulate more water (Zotz et al., 2020), although it is not known how this affects the phytotelm yeasts. The yeast richness in tanks was the highest among the compartments, closely followed by leaves. Moreover, more than half of the yeast species in bromeliad water tanks were basidiomycetes. The prevalence of basidiomycetes is probably related to the ability of this group to use different sources of nutrition (polytrophy) and to colonize oligotrophic environments, such as the case of water tanks. The polytrophic capacity of bromeliad phytotelma yeasts has already been

verified. According to Hagler et al. (1993), more than half of the yeasts found assimilated more than 20 different carbon sources. It is already known that the microbial community in the tank can vary geographically, interspecifically, and even intraspecifically (Hagler et al., 1993; Louca et al., 2017; Morais et al., 2020). There is evidence to indicate that this environment is extremely variable taxonomically, but at least in bacteria, it is functionally stable (Louca et al., 2017). The most frequent species in this compartment were *C. intermedia, R. mucilaginosa, P. laurentii* and *N. albida*. All these species are cosmopolitan, but often associated with plant substrates (Rosa and Péter, 2006).

4.7. Community structure

Evolutionary innovations, ecophysiological strategies, besides several water harvesting mechanisms, explain the wide spectrum of occurrence of bromeliads (Benzing, 2000; Crayn et al., 2015; Males and Griffiths, 2017). We can mention the absorbent trichomes of the leaves, the emergence of photosynthetic metabolism type CAM (Crassulacean Acid Metabolism), the capacity to retain water in the tank, and succulence as factors that contributed to the evolutionary success of bromeliads (Benzing, 2000; Crayn et al., 2015; Males and Griffiths, 2017). These traits are important for drought tolerance and, possibly, for the colonization of xerophic environments and high altitudes. Males and Griffts (2017) combined the phylogeny of bromeliads with five functional types previously described, based on the photosynthetic pattern and the water absorption mechanism associated with the bromeliad habit, namely: C_3 terrestrials, C_3 tank epiphytes, CAM atmospheric epiphytes, CAM terrestrials, and CAM tank epiphytes. Among these functional types, morphological, ecological and physiological variations were observed. In addition, there are differences in traits related to drought tolerance, such as water mass per unit area and osmotic potential at full turgor, for example (Males and Griffiths, 2017). Furthermore, these functional groups are indirect indicators of greater or lesser dependence on the root for water absorption. Simply speaking, CAM atmospheric epiphytes are the most adapted for absorption through leaf trichomes, while C_3 terrestrial plants are the most dependent on root absorption (Benzing, 2000; Leroy et al., 2016; Males and Griffiths, 2017). We observed that bromeliad subfamilies, functional types and compartments were shown to have some effect on the structure

of the associated yeast community. Nonetheless, the result was inconclusive since there was a variation in the results depending on the data analyzed. However, even considering the possibility of the significant results being maintained, the explanatory power of the variables was low. The R^2 of the bromeliad subfamily ranged from 3 to 11%, from 7 to 15.6% for the compartments, and from 6.8 to 15.3% for the functional types. The low values of R^2 in these categories may be associated with some classes that had high variance in the compartments, leaves were significantly more heterogeneous (greater variance) than flowers, fruits and water tanks. As previously mentioned, this high heterogeneity in leaves may be related to the shift of species that tends to be large in the phylloplane (Fonseca and Inácio, 2006).

4.8. New Yeast Species

More than twenty new yeast species were described from bromeliads between 2005 and 2020. Several studies on bromeliads have recorded many undescribed species associated with these plants. In bromeliad tanks, Gomes et al. (2015) found 10 new yeast species (28% of the total recorded by the authors); from leaves, Navarro et al. (2020) recorded 32 new species (38% of the total registered); and from flowers, Félix et al. (2021) registered six possible undescribed species (22% of the total registered). Considering all species recorded in bromeliads (n = 181), more than a tenth of these species had as initial isolation substrate bromeliads. Little is known about the diversity of existing fungi, only around 5%. Especially in South America, vascular plants are the largest reservoir on the planet for the discovery of new fungi, (Hawksworth and Lücking, 2017). A positive correlation was found between the number of bromeliad hosts and yeast species (Navarro et al., 2020), and about 44 species of bromeliads have been studied so far regarding the yeast community, which is equivalent to only 1% of the richness of bromeliads described. It is evident that we have barely scratched the surface in the knowledge of bromeliad-associated yeasts and that the number of described and undescribed species is even greater than we realize.

4.9. Biases

Some biases can influence this study, mainly because here we have gathered an array of information generated by methodologically heterogeneous

studies. Some possible sources of bias include the criteria used to obtain and select the articles, as well as the choice of databases, besides the different culture media and methodologies used in isolation. Furthermore, different studies showed different sample sizes and the number of hosts analyzed. Another source of bias is the yeast identification method. Older studies tend to use phenotypic identification methods that are known to underestimate the richness, ignoring cryptic species. In addition, some studies, in this case always focusing on fruits (all about pineapples), collected samples available in artificial environments such as markets or plantations. While this methodological difference is a disadvantage because it reduces the explanatory power of the analyses carried out, it is also an opportunity to discuss how these problems can be solved in future studies. For instance, classical (phenotypic) identification may underestimate richness and generate artificial results, but with increasingly more accessible molecular methods and next-generation sequencing (NGS) this type of problem tends to become less frequent.

The culture medium used to seed the samples can be chosen according to a variety of goals and rationales. Depending on the research, it may be a result of legacy issues. For example, the medium that has historically been used in a laboratory, or it may be just because it is the medium that is currently available. Whatever the reason, it is well known that choosing a methodology is, to some degree, choosing tolerable biases, as every methodology has its limitations and problems. For example, in the isolation of yeasts from bromeliads, several culture media have already been used: Fungal Plate Count Agar (PCA), Malt Extract Agar (MEA), Potato Dextrose Agar (PDA), Sabouraud Dextrose Agar (SDA), Wort Agar, Yeast Malt Agar (YM Agar) and Yeast Extract Peptone Dextrose Agar (YEPD Agar). In addition to this variety of media used, there may also be modifications such as acidification and the addition of antibiotics such as chloramphenicol, streptomycin and oxytetracycline. Most studies on bromeliads use YM Agar medium, often with the addition of some antibiotics. The other means mentioned are less often used. In addition to these points, the present study did not consider geoclimatic variables, which can be important predictors of the microbial community, as the special distribution of the studies evaluated was strongly asymmetric, with several gaps and most studies carried out in Brazil (about 65%), mostly in Atlantic Forest areas.

Another point to consider is that the data used in this study were collected until October 2021 and since then other studies have been published expanding the diversity of known yeasts in bromeliads. For example, Nutaratat et al. (2022) described the new genus *Savitreella* and the species *Savitreella phatthalungensis* and *Goffeauzyma siamensis* associated with pineapple leaves. In addition, the species *Sa. phatthalungensis* is the first taphrinomycete yeast recorded in bromeliads. Similarly, Bezerra et al. (2022) described *Valentiella maceioensis*, the new genus and species of black yeast-like associated with bromeliad leaves. Another work that recently described yeasts associated with bromeliad water tank and leaves was Navarro et al. (2022) who described *Tremella ananatis* and *Tremella lamprococc*i.

5. CONCLUSIONS

Overall, it was possible to verify the great richness of yeasts associated with bromeliads. Nevertheless, it seems that we are still far from recovering the fullness of the diversity of yeasts in this substrate. However, with the frequent modernization of independent cultivation techniques, this goal may not be so far reaching. Moreover, the predominant yeast community from the bromeliad phyllosphere was similar to what is expected from the phyllosphere in general, though several expected patterns such as the prevalence of basidiomycetes in fruits were not confirmed. However, this is likely associated with the type of material and method applied in isolation. The yeast community diverged among compartments, thus establishing bromeliads as a complex and heterogeneous environment. Leaves and tanks were the most diverse compartments, which may be associated with the fact that leaves are heterogeneous environments (a point that was reaffirmed in this analysis) and tanks are ephemeral environments subject to large fluctuations. Furthermore, both environments are oligotrophic, which can be a factor that increases the richness of associated species.

The structure of the yeast community was related to the variables analyzed (functional bromeliad types, bromeliad subfamilies and compartment types), but all were low explanatory power. As already known, the dynamic of the

phyllosphere microbiota is modulated by several factors, both deterministic and stochastic. This complex network of factors must be evaluated with more precise experimental designs so that the patterns can be deeply elucidated. The functional types of bromeliads and compartments seem to be useful and promising variables, which can be used as explanatory units in future studies. Nevertheless, the data used herein had several biases such as the variation in sample size, host and method of yeasts identification. Thus, in future studies, these variables should be controlled in an experimental design that allows verifying the real influence of the variables verified herein. Furthermore, further studies are needed to confirm the influence of scale on the processes and patterns of yeasts in bromeliads.

Bromeliads proved to be a potential substrate for prospecting new yeast species and a precious though still little explored source of biotechnological raw material. From the data collected, it was evident that there is still much to be explored in bromeliads. For instance, no studies were found on nectar. Among the data already available, those on flowers are still very incipient. In these last three decades (since the 90s) of studies on yeasts in bromeliads, most of the works focused on the descriptive survey of communities. The importance of this knowledge framework is undeniable, as these works provided the scientific community with all the knowledge obtained to date. It is necessary to take the next steps and increase the number of studies that seek to understand the processes that structure the epiphytic community and not just verify the patterns.

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5. CAPÍTULO 2 - Diversity, rainfall pulses and memory effect: A look at yeast-plant system from Brazilian Tropical Semiarid Dryland

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Abstract

In drylands, rainfall occurs in pulses (rare events of resource over-availability) and the drought that precedes it affects the intensity of the biological response during the pulse (memory effect). The phyllosphere is the plant aboveground region, it's the gateway to plant-environment interaction and a microbial megadiverse environment. In both ecosystems, water deficit is recurrent and has wide implications for biological communities. Here, we aimed to verify the rainfall, seasonality and environmental memory effect on the diversity and community structure of phyllosphere yeasts in a Brazilian dryland. While taxonomic, phylogenetic and functional alpha-diversity did not differ significantly between seasonal periods, rain, drought and memory had little influence on alpha-diversity but were significantly related to community structure. The taxonomic composition, although functionally stable, diverged significantly over seasons and presented high turnover values. Most of the functional traits did not differ between periods, however there was a tendency towards greater frequency during the rainy season. Our results bring insights into phyllosphere yeasts dynamics in a tropical dryland and how it relates to rainfall pulses, seasonality, and memory effect. In addition, by understanding the effect of dry-wet cycles on the microbiota of the semiarid phyllosphere, we can predict effects on host health due to microbial dysbiosis.

Keywords: Caatinga, seasonality, bromeliad, leaf, phyllosphere and turnover.

Introduction

Drylands are fundamentally regions with an aridity index (AI) < 0.65, that is, with a high water-deficit (Noy-Meir 1973; Huang *et al.* 2016). These areas, which cover more than 41% of the planet and are considered fragile ecosystems, generally have unique biodiversity and provide important ecosystem services in the global carbon, water and nitrogen cycles, and climate regulation (Huang *et al.* 2016; Maestre *et al.* 2016, 2021). According to Noy-Meir (1973), the three most important attributes for understanding the drylands ecosystem functioning are: i) The precipitation is scarce and, therefore, a dominant factor in the control of biological processes, ii) occurs occasionally and modestly, iii) and is variable and unpredictable.

In the context of pulse dynamics, a pulse is defined as an ephemeral event of resource overabundance (Holt 2008; Yang *et al.* 2008; Collins *et al.* 2014). The pulses are a perturbation that generates a transient alternate state (Holt 2008). In response to resource pulses, species can alter their dynamics (e.g. a spike in seed recruitment or microbial metabolic rate) (Holt 2008; Yang *et al.* 2008; Collins *et al.* 2014). Although, resource pulses are short-lived events, they can be important triggers that initiate powerful processes with persistent ecological effects long after the pulse ends (Austin *et al.* 2004; Yang *et al.* 2008). In drylands, it can be considered that rainfall inflows occur in 'pulses' (Noy-Meir 1973; Collins *et al.* 2014). The rainfall pulse can influence other processes such as the translocation of nutrients, disruption of the soil surface, redox patterns and the organic matter production, deposition and decomposition (Austin *et al.* 2004; Collins *et al.* 2014).

The long periods of drought in drylands create reservoirs of mineral and organic substrates because growth of plants and microorganisms is limited during this time, generating little demand for nutrients, and lower rates of decomposition and cycling (Austin *et al.* 2004; Holt 2008). This reservoir system can be diverse (e.g. water, carbon and nitrogen) and influence ecosystem responses to future precipitation, since depending on the size of the accumulated reservoir, environmental response will be different (Austin *et al.* 2004; Collins *et al.* 2014). For example, the duration of a dry period before a rain event determines the size of the accumulated reservoir of organic matter and N inorganic. Consequently, this can

shape the size of denitrification activity generated by rainfall (Austin *et al.* 2004; Reynolds *et al.* 2004). At some level, there is an environmental 'memory' of previous precipitation events, which is one of the keys to understanding the dryland environmental sensitivity, especially in intra-seasonal patterns (Reynolds *et al.* 2004; Schwinning *et al.* 2004).

The phyllosphere (aerial part of plants) receives the effects of the external environment and mediates its relationship with the plant (Vacher *et al.* 2016; Koskella 2020). Furthermore, this habitat is home to one of the most diverse microbiotas on the planet, which includes groups such as bacteria, archaea, viruses, filamentous fungi, yeasts, microalgae, protozoa and nematodes (Vorholt 2012; Goffredi, Jang and Haroon 2015; Vacher *et al.* 2016; Thapa and Prasanna 2018; Koskella 2020). One of the most challenging and common conditions that phyllosphere microorganisms face is water-deficit stress. Even in more humid environments, the cuticle (the layer that covers the plant) is a hydrophobic surface with low wettability. It is difficult for water to remain in the epiphytic regions (especially in the leaves), generating an environment with little water available and high osmotic pressure due to accumulation of metabolites (Beattie and Lindow 1995; Lindow and Brandl 2003; Vorholt 2012; Bringel and Couée 2015; Vacher *et al.* 2016).

Microorganisms of the phyllosphere have essential functions for plants, acting in processes of defense, pathogenicity, nutrient acquisition, growth, reproductive success and evolution (Rodriguez *et al.* 2009; Thapa and Prasanna 2018; Leveau 2019; Koskella 2020). For example, phyllosphere yeasts can act in nutrient-cycling processes, antagonize phytopathogens and produce plant hormones that stimulate growth (Buck 2002; Limtong and Koowadjanakul 2012). Because of the oligotrophic nature of the phyllosphere, this environment tends to select microorganisms capable of 'remodeling' it to access resources. For example, narrowing the cuticle, producing hormones, enzymes, exopolysaccharides (EPS) and biosurfactants (Beattie and Lindow 1995; Leveau 2019). Some of these strategies can increase and/or facilitate access to water and drought tolerance, such as the production of biosurfactants that increase leaf wettability (Beattie and Lindow 1995; Leveau 2019; Koskella 2020). Events of intense taxonomic variation are recorded in the phyllosphere microbiome, but recent evidence indicates the existence of a functional core (Louca *et al.* 2017;

Lajoie, Maglione and Kembel 2020). Therefore, plants may have active mechanisms to select essential functional groups, often related to the metabolism of amino acids, nucleotides, carbohydrates, and energy (Lajoie, Maglione and Kembel 2020). Morevoer, as the prevailing condition in drylands is water deficit, fungi, rather than bacteria, can dominate the processes of N decomposition and transformation, in addition to generating symbiotic associations with primary producers (Austin *et al.* 2004; Collins *et al.* 2014).

This study makes relevant contributions to the knowledge of microbial diversity in drylands, as well as some functional traits that may represent ecological and biotechnological insights, such as the production of enzymes and biosurfactants. Additionally, we address the following question: How do the dynamics of rainfall pulses and drought (rainfall volume, storm or single-rain events, seasonality and memory) affect the yeast community structure and diversity of the bromeliad phyllosphere from Brazilian semiarid dryland? Based on this question, the following hypotheses were tested: H^{i}- Heavier and more continuous rainfall, and longer periods of drought preceding the rain event (memory effect) increase yeast diversity (taxonomic, phylogenetic and functional) and abundance. In view of this, the diversity and abundance of yeasts were greater during the rainy season. H^{ii}- The rainfall, drought duration, memory effect and seasonality modulate the structure of taxonomic, phylogenetic and functional of the phyllosphere yeast community. H^{iii}- Furthermore, the seasonal periods present a distinct taxonomic and phylogenetic structure but are functionally similar.

Methods

Model plant

The plant model species was *Bromelia laciniosa* Mart. ex Schult. & Schult.f (Bromeliaceae, Bromelioideae), an endemic species of the semiarid dryland located in Northeast Brazil (Caatinga) (Ferreira, Fabricante and Siqueira-Filho 2015). Its leaves, flowers and fruits are popularly used in the treating child colic, diarrhea, fever, jaundice, dandruff and hepatitis (de Albuquerque *et al.* 2007). It is a typically terrestrial plant insert in the terrestrial CAM (crassulacean acid metabolism) functional group (Males and Griffiths 2017).

Study area description

The collections were carried out in the Tocaia Reserve (RPPN Tocaia), municipality of Santana do Ipanema, Alagoas-Brazil, located in the semi-arid region of northeastern Brazil (Caatinga) (9°23'08.9"S and 37°15'22.8"W) along a transect of 5 × 200 meters. The rainfall occurs irregularly, with an annual volume of 500 - 700 mm. The RPPN Tocaia has an area of approximately 20 hectares and a maximum altitude of about 400 meters, it is mainly composed of an arboreal physiognomy. The predominant climate is semi-arid type Bsh (Köppen).

Collections of plant material

Twelve collections were carried out, six in the dry season (October, November and December 2020 and 2021) and six in the rainy season (May, June and July 2021 and 2022). In each collection, five individuals of adult and apparently healthy bromeliads were randomly sampled along the previously established transect. Three leaves were randomly selected from each plant individual collected, making a total of 60 individuals, and 180 leaves collected during the collections. The leaves were sealed in sterile plastic bags, then transported at room temperature (27±2 °C) for approximately 4 h to the laboratory. In the laboratory, the leaves were stored at 10-15 °C and processing was carried out within a maximum of 24 h after the material was collected in the field.

Samples processing

The leaves underwent an initial wash with sterile distilled water to remove contaminants such as dust and other exogenous materials. Then, leaf fragments between 5 and 20 cm^2 were randomly cut contemplating the 3 leaves collected, until a total area of 120 cm^2 was obtained, and the mass was measured, which was allocated in a 250 mL Erlenmeyer flask containing 75 mL of sterile distilled water. The water containing the leaf fragments was stirred for 30 min at 180 RPM. After the shaking, the washing product was collected in a sterile flask and the leaf fragments were preserved in an Erlenmeyer flask. After removing the water from the first wash, 75 mL of a 0.5% solution of Tween 80 was added and stirred again under the same time and rotation. The product of the second wash was then added to that of the first,

forming a pool. A 100 µL fraction of this product was seeded in petri dishes containing modified YM Agar medium (0.3% yeast extract, 0.3% malt extract, 0.5% bacteriological peptone, 1% glucose, 2% agar, 0.04% chloramphenicol, pH 4.0) in the dilution of 10^0 and 10^{-1}, in duplicate. The plates were incubated at 25-28 °C and observed daily during 10 days for yeast isolation.

Isolation and storage of yeasts

The different yeast morphotypes, delimited considering colonial characteristics such as color, margin, texture, shape, and elevation, were isolated on YEPD agar medium (2% glucose, 1% bacteriological peptone, 0.5% yeast extract, and 2% agar). All yeast isolates obtained during 10 days of isolation were preserved in GYMP medium (2% glucose, 2% malt extract, 0.5% yeast extract, 0.2% monobasic sodium phosphate, and 2% agar) for further analysis.

Functional traits

The assimilation profile was estimated during 21 days of growth based on the ability of the isolates to grow using a single carbon source following Kurtzman *et al.* (2011). The carbon sources used were cellobiose, D-arabinose, galactose, glucose, glycerol, inulin, raffinose, rhamnose, and xylose. In addition, the ability of the isolates to ferment glucose was verified to produce extracellular hydrolytic enzymes such as amylase, caseinase, cellulase, esterase, lipase, and pectinase, and to produce biosurfactants.

The diameter of the halos and colonies of each isolate were measured to evaluate the production of extracellular enzymes, and the modified pz equation was used: $\mathbf{pz} = \left|\left(\frac{\mathbf{Dc}}{\mathbf{(Dc+Dh)}}\right) - \mathbf{1}\right|$. Where pz represents the enzymatic activity, Dh and Dc are, respectively, the diameter of the hydrolysis halo and the diameter of the colony.

The inoculum standardization for the evaluation of enzyme production was performed using turbidity as a reference to quantify the number of cells. Cells of the isolates were diluted in 2 mL of sterile distilled water until reaching an approximate concentration of 10^5 cells/mL, equivalent to degree 1 of the Wickerham card. Then

the inoculum was applied as dot on the culture medium using the replicator stamp. The Petri dishes were analyzed after 7-10 days of incubation at 25-28 °C.

The amylolytic activity was evaluated using a modified starch agar culture medium (0.5% soluble starch, 0.5% bacteriological peptone, 0.5% yeast extract, 0.05% magnesium sulfate, 0.001% iron sulfate, 0.001% sodium chloride, 1.5% agar). After the incubation period, a 1% Lugol solution was applied to the plate to visualize the hydrolysis halo. The isolate with amylolytic activity shows a halo not stained by Lugol and remains clear (Buzzini and Martini 2002; Mautone *et al.* 2010; Carrasco *et al.* 2012).

The cellulolytic activity was evaluated by modified CMC agar (0.5% carboxymethylcellulose (CMC), 0.1% sodium nitrate, 0.1% monobasic potassium phosphate, 0.1% potassium chloride, 0.05% magnesium sulfate, 0.05% yeast extract, 0.1% glucose and 1.7% agar). The result was visualized with a 0.1% solution of Congo red added to the plates, and after 40 min the plates were washed with 1M NaCl solution. Isolates capable of hydrolyzing CMC showed an orange halo (Buzzini and Martini 2002; Carrasco *et al.* 2012).

The esterase activity was evaluated using modified a Tween 80 agar medium (2.5% tween 80, 0.1% glucose, 1% bacteriological peptone, 0.5% sodium chloride, 0.1% calcium chloride, and 2% agar). After the incubation period, the isolates that hydrolyzed the substrate showed a halo formed by a whitish precipitate halo (Buzzini and Martini 2002; Carrasco *et al.* 2012).

The protease activity was evaluated using a modified casein agar medium (1% casein, 0.5% glucose, and 2% agar pH 7.0). After the incubation period, the caseinase-producing isolates showed a whitish halo (Buzzini and Martini 2002; Carrasco *et al.* 2012).

The pectinase activity was evaluated using a modified pectin agar medium (0.67% Yeast Nitrogen Base (YNB), 1% citrus pectin, 1% glucose, and 1.8% agar, pH 7.0). After the incubation period, 1% hexadecyltrimethylammonium bromide (CTAB) was added to the plate. The producer isolates showed a clear halo around the colony (Buzzini and Martini 2002; Carrasco *et al.* 2012).

The surfactant/emulsifying activity was evaluated using the emulsion index with stability after 24 h (IE_{24}). For testing, isolates were cultured for 48 hours at 22-25 °C in YEPD broth. To verify the activity, after growth in 2 mL of YEPD broth, 2 mL of kerosene was added to the culture, stirred for 2 minutes in the tubes and then left to rest for 24 h. The total height of the crop and the height of the emulsion in centimeters were then measured. The IE_{24} was obtained from the equation $\mathbf{IE_{24}} = \left(\frac{\mathbf{Hight_{emulsion}}}{\mathbf{Hight_{total}}}\right) \times \mathbf{100}$, that is, the proportion of the emulsion height in relation to the total height of the crop, in percentage.

Molecular identification of yeasts

The total genomic DNA was extracted following the protocol for the small-scale preparation of modified yeast DNA, originally proposed by (Sambrook and Russel 2001). Then, the D1/D2 region of the LSU gene rRNA was amplified by the Polymerase Chain Reaction (PCR) technique using the primers NL-1 and NL-4 (Kurtzman and Robnett 1998; Fell *et al.* 2000). The PCR parameters followed (Landell *et al.* 2010). The amplicons were sequenced using the Sanger method and identified by comparing them to the GenBank database using the Basic Local Alignment Search Tool (BLAST) on the International Nucleotide Sequence Collaboration Databases website using the BLASTn algorithm (Altschul *et al.* 1997).

Statistical analysis

The colonies were counted to estimate the yeast abundance considering each yeast morphotype isolated from the samples, expressed in colony-forming units per square centimeter (CFU/cm²). Taxonomic diversity was estimated using Hill's numbers, which allow assigning weights to abundances based on the q factor: q0 (richness/greater weight for rare species), q1 (typical values) and q2 (which assigns greater weight to more abundant species). The Average Taxonomic Distinctness (AvTD) index (Clarke and Warwick 1998) was estimated using the Vegan package (Dixon 2003). The AvTD was calculated using phylogenetic nodes of the genera, family, order, class, subphylum, and phylum, undefined groups were considered polytomies. The complete classification of each species was obtained from the Mycobank website (https://www.mycobank.org) and, when necessary, from

specialized literature. The functional richness (FRic), functional dispersion (FDis), and functional evenness (FEve) indices were estimated using the yeast abundances in the samples and the proportional frequency of analyzed functional traits (trait frequency/species richness) by the FD package (Laliberté *et al.* 2014). Whittaker's beta-diversity was estimated in the Vegan package (Dixon 2003) using the mean abundances of the species in the seasonal periods. Beta-diversity fractionation to identify the contribution of nestedness and turnover to beta-diversity was implemented using Jaccard index and binary data (presence and absence) for taxonomic, phylogenetic, and functional composition in the betapart package (Baselga and Orme 2012).

The explanatory environmental variables used to assess the effect of the rainfall and drought regime in the community were: i) Storm volume in millimeters, this variable refers to the most recent rainfall event in consecutive days before each sampling. According to Reynolds et al. (2004), in certain cases greater and/or longer rainfall is necessary for any biological effect to be noticeable. ii) Volume of the most recent rainy day in millimeters, this variable refers to the most recent rain event before each collection, and these are mostly single-day events. iii) The memory effect accounted for from the most recent rain event. This variable considers the number of days of drought that preceded the most recent rainfall event. iv) Rainy days in the month, this variable considers the number of rainy days in the thirty days preceding each sampling. v) Volume of accumulated rainfall in the month (mm). This variable indicates the accumulated rainfall in the thirty days preceding each sampling. The rainfall values were collected by the HidroWeb tool, which integrates the National Water Resources Information System (SNIRH) from the Brazilian national water agency (ANA) database. The code of the consulted hydrological station is 00937032.

To check the possible relationship among the explanatory environmental variables on the abundance and alpha-diversity indices (taxonomic, phylogenetic, and functional), we implemented a generalized linear model (GLM) using negative binomial distribution in the MASS package (Ripley *et al.* 2013). For these analyses, the plant individuals were used as an analytical unit. Bromeliad AT33 was excluded from analysis as no yeast isolate was obtained from this individual. To verify the

multivariate relationship of explanatory variables with the structure taxonomic and phylogenetic (by composition and abundance data), and functional (by composition and proportional frequency data) of yeast communities, a series of Canonical Analysis of Principal coordinates (CAP) was implemented using the Bray-Curtis distance in the Vegan package (Dixon 2003). To avoid errors and bias, all models were built avoiding inserting autocollinear variables in the same model. The possible autocollinearity of the explanatory variables was verified by the variance inflation factors (VIF).

The effect of seasonality on community diversity and structure was tested considering each of the twelve collections as analytical units over time. To verify the ability of the seasonal periods to group and differentiate samples using the taxonomic, phylogenetic or functional traits of the community was employed a Multivariate Permutation Analysis (PERMANOVA). The residuals method was employed under the full model with 999 permutations by a Principal Coordinate Analysis (PCoA) applying Bray-Curtis distance. Additionally, a similarity percentage (SIMPER) analysis using Bray-Curtis distance and 999 permutations was implemented with species abundance data in seasonal periods. The SIMPER analysis indicated that the elements (variables) that most contributed to the dissimilarity between seasonal periods using taxonomic, phylogenetic or functional information. In addition, the analysis also indicated which of these groups showed significantly different abundance (in the case of taxonomic and phylogenetic data) and frequency (in the case of functional traits) between periods. All analyzes were performed in the R software (R Team 2021).

Results

Diversity from plant-yeast system in Brazilian dryland

From bromeliad leaves were recorded a richness of 79 species, 43 genera, 27 families and 20 orders of yeasts and yeast-like fungi. Species from phylum Basidiomycota totaled 70% (n=56) (50% Agaricomycotina, 32% Pucciniomycotina and 18% Ustilaginomycotina) and from phylum Ascomycota 30% (n=24) (50% Saccharomycotina, 46% Pezizomycotina and 4% Taphrinomycotina) (Supplementary Table 1). The richness in periods (dry and rainy) was identical: 50 species. The

proportion between Basidiomycota and Ascomycota species remained stable regardless of the period.

The average yeast richness and standard deviation was 3.9±2.1 species per plant, ranging from 0 to 9 yeast species in a single individual. Only one of the sixty plants sampled did not register any yeast. More than half of the species (55%) were singletons (species with a single record) and 75% were singletons or doubletons. The most frequently recorded species in samples were *Aureobasidium thailandense* (36%) and *Occultifur brasiliensis* (25%). The yeast abundance average per plant individual was 9.53×10² CFU/cm² (i.e. 4.62×10³ CFU/g) and the mean abundance per yeast isolate was 1.88×10² CFU/cm² (i.e. 8.99×10² CFU/g). The non-singleton or non-doubleton species with the highest mean abundance were *Candida blankii* (4.48×10² CFU/cm²) and *Saitozyma ninhbinhensis* (4.12×10² CFU/cm²). Additionally, 37% of the isolates found had abundance values lower than 1.0×10² CFU/cm² and 85% less than 3.0×10² CFU/cm². Less than 2% of the isolates showed an abundance greater than 1.0×10³ CFU/cm². Among the functional traits evaluated, the most frequent was the assimilation raffinose, inulin and glucose, followed by the production of the extracellular hydrolases (esterase, lipase and cellulase) (Figure 1, Supplementary Figure 1). On the other hand, as less frequent, the emulsification and fermentations abilities were observed. The least frequent traits were the production of the extracellular hydrolase pectinase and amylase (Figure 1, Supplementary Figure 1).

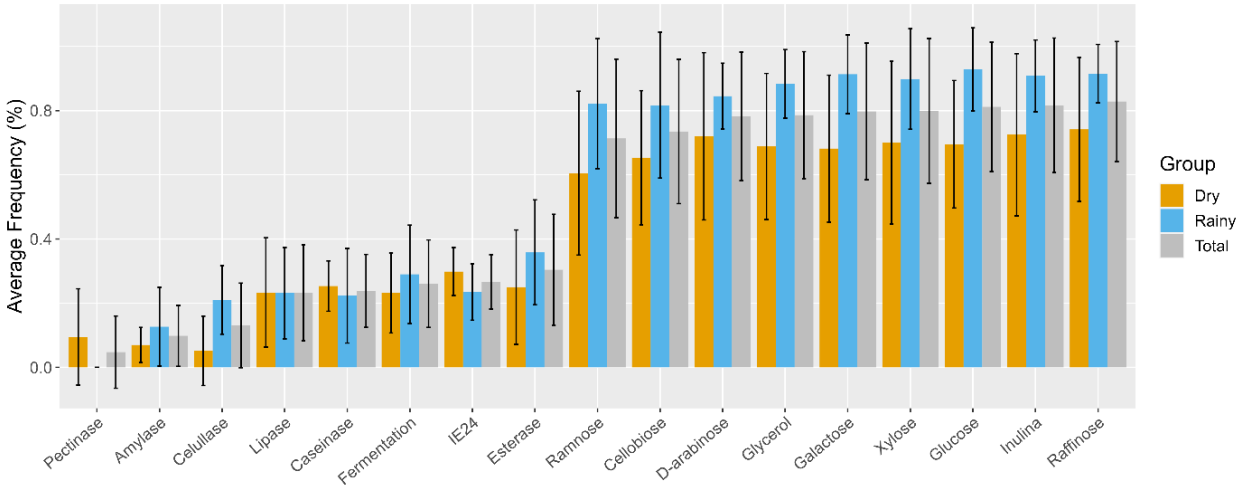


Figure 1- Frequency proportional to species richness of functional traits in each bromeliad sample. Values are shown for each seasonal period and combined data (Total). The black bar indicates the standard deviation.

How do drought, rainfall and memory effect yeast diversity and abundance?

Contrary to the initial hypothesis, the most recent rainfall volume, the number of dry days the community was exposed to, the number of rainy days in a month and the monthly rainfall volume were not significant predictors for any of the analyzed response variables. The memory effect (dry time that preceded the last rain) did not show a significant relationship with most diversity or abundance metrics, except for FRic (Functional richness). However, the relationship between memory and FRic was negative, that is, the longer the period of drought that precedes the rain, the lower the functional richness found in the yeast community (α=-0.03, R^2= 0.08, p-value=0.011). The volume of the most recent consecutive rain (storm) was positively and significantly related to the q0 component (species richness) (α=0.008, R^2= 0.14, p-value=0.002) and with FRic (α=0.03, R^2= 0.26, p-value<0.0001). But it showed no relationship with the other diversity metrics or with the abundance of yeasts.

Taxonomic, phylogenetic and functional yeast community structure

Among the explanatory variables, the storm volume showed a significant relationship with the taxonomic (p-value=0.001, R^2=0.05) and phylogenetic (p-value=0.002, R^2=0.08), but not with functional community structure (Figure 2). The memory effect was relevant only in the taxonomic structure (p-value=0.03, R^2=0.04). Also, the most recent rainfall volume was not related to the structure of the yeast community in any of its facets. Only the rainy days in a month (p-value=0.007, R^2= 0.37), monthly rainfall volume (p-value=0.005, R^2= 0.41) and drought days duration (p-value=0.001, R^2= 0.53) showed a significative relationship only with the functional structure of the community (Figure 2 and Supplementary Figure 1).

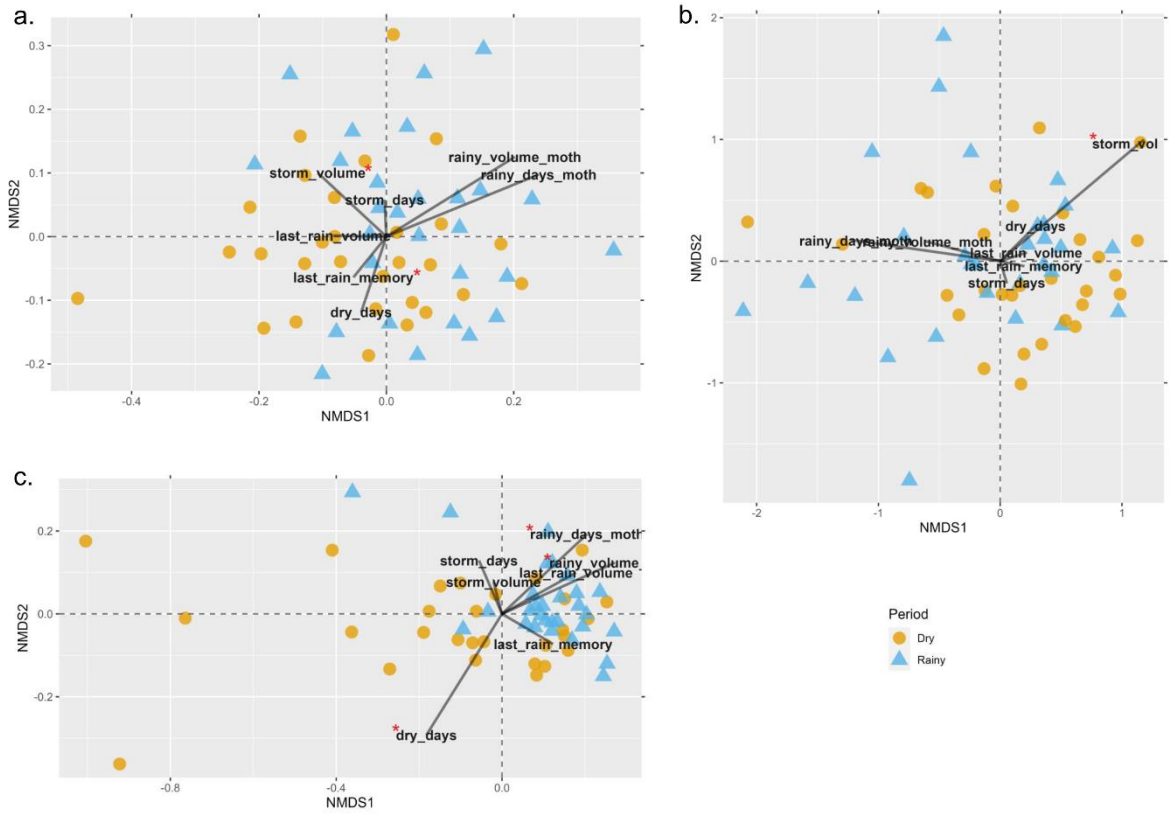


Figure 2- Non-metric multidimensional scaling (NMDS) using data on species abundance (a). The abundance of phylogenetic groups (genera, family, order, class, subphylum, and phylum) (b). Values of frequency of the functional traits (c). All ordination analysis were constructed by Bray-Curtis distance, and the vectors marked with a red asterisk indicate represent the variables that showed a significant relationship with the structure of the community by Canonical Analysis of Principal coordinates (CAP) result.

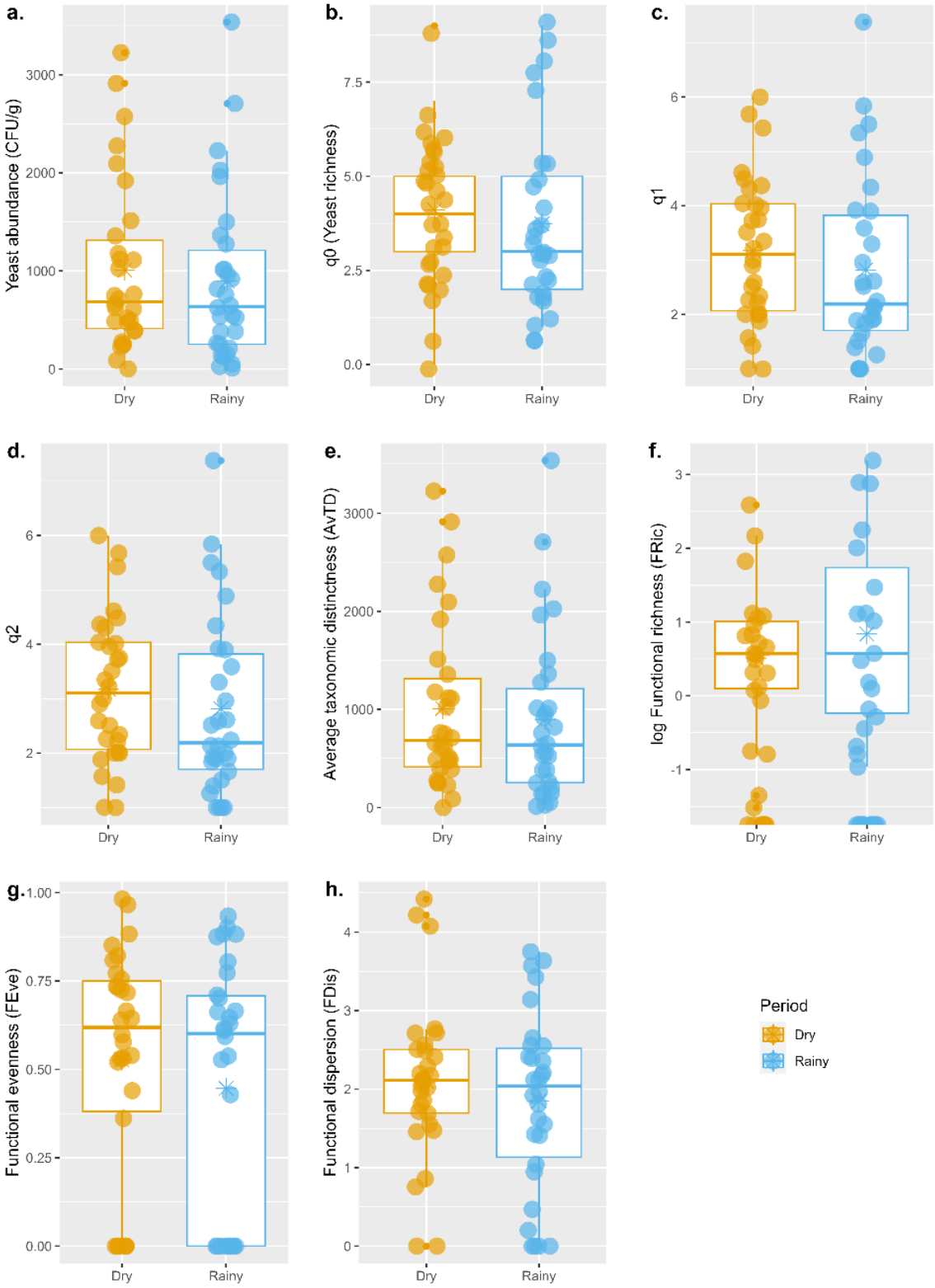
The seasonal periods and yeasts

The Hill's components (q0, q1 and q2) used to estimate taxonomic diversity did not differ significantly between season periods (p-value>0.5). The same happened in the estimators of phylogenetic diversity (Average taxonomic distinctness – AvTD, p-value =0.1) and functional diversity (FRic, FDis, and FEve, p-value>0.51) (Figure 3). The yeast abundance per plant was 9% lower in the rainy season (9.29×10^2 CFU/cm²) when compared to the dry season (1.0×10^3 CFU/cm²) and this difference was not significant (p=0.23). No species occurred in all collections carried out in both seasons. The species *A. thailandense* and *C. blankii* were the only ones present in all the collections of the rainy period and *Papiliotrema laurentii* and *Tremella ananatis* were the only ones to be present in all the collections of the dry period. Additionally, only 25% (n=20) of the species were shared between seasons, and 37.5% (n=30) were exclusively recorded in each.

Considering species composition and abundance data, the yeast community structure varied between seasonal periods (p-value=0.002, R^2=0.15), but not when considering phylogenetic (p-value=0.09) or functional (p-value=0.57) data. This taxonomic difference between periods was also observed in the Whittaker beta-diversity values: taxonomic (w=0.58), phylogenetic (w=0.35) and functional (w=0.03). Considering occurrence (binary) data, 99.5% of the beta-diversity observed in yeast species and phylogenetic groups between seasonal periods was generated by the species replacement component (turnover). However, the functional beta-diversity was negligible, represented fully by the nestedness component.

The abundance of no species differed significantly between seasonal periods, however the species that most contributed to the dissimilarity between periods were *O. brasiliensis* (8% contribution, more abundant in the dry period) and *C. blankii* (8% contribution, more abundant in the rainy season) (Supplementary Table 2). Among the phylogenetic groups, the group that diverged significantly was the genus *Candida* and related groups such as: the order Saccharomycetales, the class Saccharomycetes, and the subphylum Saccharomycotina (p-value≤0.013) (Supplementary Table 3). The genus *Candida* was the most abundant in the rainy season, which led to an increase in the other related phylogenetic groups.

The assimilation of rhamnose was more frequent in the rainy season and was the trait that most contributed to the functional dissimilarity between periods, however it did not differ significantly between dry and rainy seasons. The traits that varied significantly between seasonal periods were galactose assimilation (p-value=0.038) and cellulase production (p-value=0.024), both more frequent in the rainy season (Figure 1, Supplementary Table 4). Even though the majority did not demonstrate significant differences, 76% of the evaluated functional traits were more frequent in the rainy season. Only the production of lipase, pectinase and caseinase enzymes and the ability to emulsify (IE_{24}) were most frequent in the dry period. There was no difference in the number of traits expressed per species between seasonal periods, neither when comparing core species (present in both periods), and when comparing exclusive species (p-value≥0.5). Most of the functional traits evaluated were trophic, related to the acquisition and use of nutrients. The results indicate that there is no difference in the polytrophy of the species between the seasonal periods.

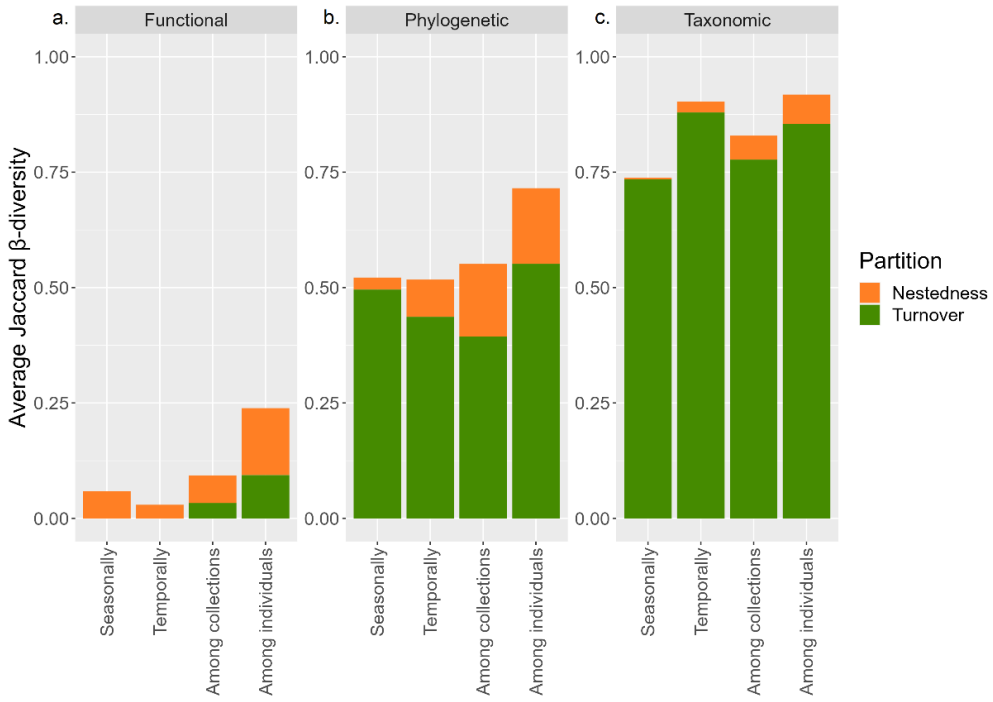


421

422 **Figure 3**- Boxplots with the variances of abundance (a) and alpha-diversity metrics of taxonomic (b, c
423 and d), phylogenetic (e), and functional (f, g and h) between seasonal periods. The asterisk indicates
424 the average.

425 ***Beta-diversity partitions***

426 Taxonomic beta-diversity was higher compared to phylogenetic and functional
427 in all scales analyzed: seasonal, temporal, among collections and plant individuals
428 (Figure 4). Except for the functional facet, turnover accounted for most of the beta-
429 diversity observed across all scales. In all different facets, beta-diversity was higher
430 among bromeliad individuals and the most significant contribution of nestedness to
431 beta-diversity was observed; however, this participation was always minor compared
432 to turnover in general. In all observed scales, only functional beta-diversity showed a
433 more significant contribution from nestedness than turnover. Furthermore, the
434 contribution of nestedness to beta-diversity was lower on the seasonal scale,
435 gradually increased on the temporal scale, among collections, and reached the
436 highest contribution among individuals (Figure 4). This trend was the opposite in
437 functional beta-diversity and seasonally and temporally the contribution of
438 nestedness is absolute.



439

440 **Figure 4-** Jaccard beta-diversity values (binary data), at different scales: Seasonal, Temporal
441 (between the four quarterly collection blocks), Among collections and Among plant individuals. In
442 addition, we also indicate the beta diversity partitions, mostly turnover is the most representative
443 partition. However, functional beta-diversity is dominated by nestedness.

Discussion

Yeasts from Caatinga dryland

Certain groups of microorganisms are more associated with one substrate than with others. In the case of yeasts, the phylum Basidiomycota is prevalent in leaves, intact fruits and flowers surfaces, when nectar is disregarded (Fonseca and Inácio 2006). In addition, it is expected that the community will change over the seasonal and ontogenetic periods. In summer (mainly in temperate environments), the genera *Papiliotrem*a, *Rhodotorula*, and *Sporobolomyces* are overrepresented, mainly due to factors such as temperature, sunlight, and day length (Glushakova and Chernov 2004, 2010; Fonseca and Inácio 2006; Vacher *et al.* 2016; Kemler *et al.* 2017). On the other hand, Fonseca and Inácio (2006) cite evidence that in the wettest and coldest months, *Cryptococcus* spp. (currently, *Papiliotrema* and others), mainly *Papiliotrema laurentii*, is prevalent. Several species from *Cryptococcus/Papiliotrema*, can produce polysaccharide capsules that confer resistance to desiccation (Fonseca and Inácio 2006; Kemler *et al.* 2017). In addition, traits such as the production of pigments can generate a competitive advantage in an environment exposed to various oxidative stresses, such as the surface of leaves. Pigment producers such as *Rhodotorula* and *Sporobolomyces* (carotenoids), and *Aureobasidium* (melanin) are persistent in the phylloplane (Fonseca and Inácio 2006; Kemler *et al.* 2017).

In most studies, the seasonal dynamics of phyllosphere microbiota (including yeasts) are developed in temperate environments, where sudden changes in climatic and physiological conditions mark the seasons. In the tropical environment, the maximum productivity is marked by the rainy season and the minimum by the dry season. In addition, temperatures do not vary as much between seasons. In temperate environments, fungal diversity metrics and abundance tend to vary over seasonal periods due to a dependence on the hydrothermal regime and the ontogenetic period of the host (Glushakova and Chernov 2004, 2010; Fonseca and Inácio 2006). The senescence process favors the appearance of damage to the cutin, making the nutritive components of the leaves more available, decreasing the hydrophobicity of the leaf surface, and potentially decreasing the concentration of

antimicrobial substances. This explains the peak of diversity and abundance of the epiphytic microbiota during autumn (Inácio *et al.* 2002; Glushakova and Chernov 2004, 2010; Fonseca and Inácio 2006). Some hypotheses claim, that one of the reasons for the increase in the leaf community in autumn may be higher humidity and milder temperatures (Inácio *et al.* 2002; Fonseca and Inácio 2006). In tropical environments, community drivers can vary. For bacteria, the leaf content of the water and phosphorus influences the community's abundance and structure, while for fungi, the leaf aluminum content is significant (Vacher *et al.* 2016). However, it is not easy to extrapolate these associations to all communities in the phyllosphere.

Our data indicate an increase in the frequency and abundance of the groups such as Basidiomycota in the dry period, especially the species *O. brasiliensis*, *P. laurentii* and *T. ananatis*. On the other hand, the groups that stand out in the rainy season are the phylum Ascomycota, the genus *Candida* and species such as *A. thailandense* and *C. blankii*. Other studies have observed that in plants, mainly in leaves: the proportion of Ascomycota reaches its maximum in autumn and its minimum between late winter and early spring (Glushakova and Chernov 2010; Abu-Ghosh, Droby and Korine 2014). In the phyllosphere of mosses of the genus *Sphagnum* in swamp (wetter) ascomycetes yeasts were more abundant than in forest (lower humidity), 18.4 and 10.95% of the abundance, respectively (Kachalkin and Yurkov 2012).

In a dryland system, an increase in the proportion of basidiomycetes yeasts in relation to ascomycetes was observed in drier periods (Abu-Ghosh, Droby and Korine 2014). In summer, basidiomycetes reached 86% of the species of the phyllosphere. As the ascomycete species increased in wetter periods, the frequency of phyla was the same during this period (Abu-Ghosh, Droby and Korine 2014). However, considering the whole mycobiome of the soil, greater aridity (greater water deficit) promotes dominance of fungi of the phylum Ascomycota (Tedersoo *et al.* 2014; Maestre *et al.* 2016). Our data did not show differences in the proportion of ascomycetes in the rainy or dry season, but that some species and groups of ascomycetes are more frequent and/or abundant in the rainy period. It is possible that the proportion increase between phyllosphere ascomycetes and basidiomycetes in more humid seasonal periods observed in several works is characteristic of

temperate environments. In the tropical environment, the yeast phylum proportion tends to be similar between seasonal periods, as well as taxonomic diversity. This statement is supported by the study by Gomes *et al*. (2015), who found similar diversity of yeasts in bromeliad tanks. However, the composition of the community was quite different between periods.

Does rain and drought affect yeasts on leaves?

In drylands, water availability is considered the central factor in controlling biological activity. Furthermore, the leaf surface is a hydrophobic region subject to intense water limitation. Some factors, such as host age and rainfall regime, can change water availability in the phyllosphere (Vacher *et al.* 2016). Plant aging tends to change physicochemical characteristics in the cuticle, which becomes more wettable and permeable, increasing water adhesion and nutrient availability (Vacher *et al.* 2016; Oso *et al.* 2021). Very waxy leaves have poor wettability; even if water is available through humidity or rain, a film of water will hardly form. In addition to promoting water availability for the microbial community on the leaf, the formation of this water film quickly changes the pH and redistributes nutrients in the epiphytic region (Morris 2001). It is possible that the frequency and intensity of rainfall are also related to the microbial response (Kinkel 1997). Rain fall is known to alter colonization and dispersion rates, washing the leaves and removing microorganisms, while transferring other microorganisms from the atmosphere to the plant (Morris 2001; Vacher *et al.* 2016; Leveau 2019).

In this way, we expected that the rains would contribute strongly to the phyllosphere yeast community diversity. However, differing from the initial hypothesis, rain did not present significant influence. Only storm volume and memory effect had any significant relationship with certain diversity metrics. Storm volume positively influenced functional and species richness, and memory effect was related only to functional richness. Although, in the case of memory effect, the relationship was negative, diverging from our hypothesis.

Studies on epiphytic yeasts in the desert suggest that these microorganisms are resistant to various abiotic stresses, mainly of oxidative nature (Abu-Ghosh, Droby and Korine 2014). However, in experimental studies with grasses,

intermediate drought conditions did not significantly affect the phyllosphere bacterial community, but when drought was intensified, diversity tended to decrease (Bechtold *et al.* 2021). It is plausible that longer droughts than the one we have recorded (a maximum of 58 days without any rain) could affect the epiphytic microbiota. In the phyllosphere of many plants, short-term rainfall events tend not to result in changes in microbiota diversity and abundance. For example, in tomato leaves (Allard, Ottesen and Micallef 2020) and macrophyte leaves (Stone and Jackson 2021) rainfall did not influence the richness and abundance of the bacteria communities in the short term. In contrast, in tomato fruits rainfall increased the richness few days later until reaching close to the richness recorded before the rain (Allard, Ottesen and Micallef 2020). There is evidence that rainfall and other long-term factors, such as seasonality, influence the microbial community of the phyllosphere more than short-term factors (Chen *et al.* 2021; Stone and Jackson 2021; Yan *et al.* 2022).

Taxonomic, phylogenetic and functional structures of the community

Rainfall metrics did not influence, or not as expected, facets of yeast diversity while environmental variables proved to be relevant for the organization of communities. Interestingly, the volume of the most recent storm was significantly related to the taxonomic and phylogenetic structure of the community. While the most recent rainfall, mostly a single-rain event, showed no significant relationship with the data. This may be an indication that in this environment, a semiarid dryland, not only the pulse dynamics, but also the constancy of the rain is relevant to the relationships and structures of the community. Reynolds et al. (2004) argues about minimum thresholds necessary for rain events to be reflected in biological effects. In drylands, rainfall events generally do not exceed 2 mm, in more arid drylands it is estimated between 10-50 rainy days per year, distributed between 3-15 rain events, of which 5 or 6 have the magnitude to generate effects biologically significant (Noy-Meir 1973; Collins *et al.* 2014).

According to Kinkel (1997), the microbial community of phyllosphere is regulated by four population processes: i) immigration, ii) emigration, iii) growth (generation) and iv) death. For Vellend (2010), the mechanisms that regulate ecological communities are diverse, but all of them can be synthesized in just four

types of processes: selection, drift, speciation and dispersion. In this context, selection reflects differences in fitness, drift refers to stochastic changes in the relative abundances of species in a community, speciation refers to the emergence of new species, and dispersion deals with the movement of organisms in space (Vellend 2010; Vacher *et al.* 2016).The memory effect was significant for the taxonomic structure, reinforcing that these variables are more relevant for the structure of the community than for diversity. The functional structure was unrelated to variables such as most recent rainfall, memory, or storm volume. On the other hand, it was strongly related to drought days, monthly volume of rainfall and monthly rainy days.

Other studies, mostly in temperate environments, indicate a gradual increase in yeast diversity in leaves from seasonal periods with a peak of diversity in autumn (Glushakova and Chernov 2004, 2007, 2010). One mechanism that justifies this is the milder temperatures, lower humidity, and more nutrients made available by plants (Fonseca and Inácio 2006; Gouka, Raaijmakers and Cordovez 2022). However, this seasonal cycle tends to occur mainly in short-lived plants and in temperate environments. In our study, the model plants were bromeliads, a tropical group of slow-growing and long-lived plants that do not lose leaves seasonally (Benzing 2000; Ladino *et al.* 2019). We expected that water availability would be translated into greater nutrient availability and diversity during the rainy season, therefore increasing alpha-diversity in all its facets: taxonomic, phylogenetic and functional. However, our hypothesis was falsified, the diversity and abundance of yeasts did not vary significantly between dry and rainy seasons. Gomes et al. (2015), found the same pattern when analyzing yeast diversity in bromeliad tanks in Brazil, the yeast community showed stable diversity values and varied compositionally.

Stability in taxonomic alpha-diversity may explain the stability in other diversity facets like functional. The relationship between biodiversity and ecosystem functioning is robustly supported by evidence from observational and experimental studies, therefore, more diverse biological communities are expected to present greater diversity of ecosystem functions(Cardinale *et al.* 2012). The links that mediate this relationship are diverse and not fully understood. Some of these mechanisms are the complementary niche partitioning (when several species

complement each other spatio-temporally in resource use), the effect of species identity (when specific species have a disproportionate functional role being crucial to ecosystem functioning) and facilitation (when species facilitate the activities of others by improving ecosystem functioning) (Daam *et al.* 2019). The functional diversity of yeasts was similar between seasonal periods, and this may be a causal relationship with the also “stable” taxonomic diversity. However, we cannot determine whether these factors are just correlated.

Beta-diversity

Between seasonal periods, our data point to a large and significant taxonomic beta-diversity of yeasts, a modest and non-significant phylogenetic variation and a strong functional stability. In *Typha latifolia* macrophyte leaves, a high seasonally bacteria beta-diversity was found, but related to nestedness and not turnover (Stone and Jackson 2021). Host species can influence microbial composition and structure in the phyllosphere (Bechtold *et al.* 2021). In another example, the organization of endophytic and epiphytic bacterial communities differs. The epiphytic community appears to be more widely distributed among distinct species, and the endophytic community tends to be particular to each host species (Yao *et al.* 2020).

Similar to our results, endophytic fungi from the phyllosphere and rhizosphere of cacti from Caatinga dryland showed a trend towards stability in species richness, but with intense turnover (Ferreira-Silva *et al.* 2021). Even after being subjected to rain events epiphytic bacteria in tomato leaves showed stability in OTUs richness and temporal variation in community composition (Allard, Ottesen and Micallef 2020). The associated bacterial community can exhibit significant taxonomic variation in bromeliads, even at a few meters of spatial scales (Louca *et al.* 2017). This pattern was also observed in yeasts in bromeliad phytotelma between seasonal periods, with similar diversity values (Gomes *et al.* 2015). Recent studies suggest that, even using culture-independent approaches, communities can vary substantially by sharing few OTUs between different plants (Yan *et al.* 2022). Multiple variables may influence the discrepancies among studies, both the type of approach (culture- dependent or independent), and factors such as the microbial group studied and the scale of the study.

Several studies point to the phyllosphere as a dynamic environment with an equally dynamic and diverse community with significant taxonomic variation in both bacteria and fungi (Andrews *et al.* 1987; Kinkel 1997; Vacher *et al.* 2016; Louca *et al.* 2017; Stone and Jackson 2021). We observed a high taxonomic and phylogenetic beta-diversity (almost entirely generated by turnover) in all observed scales: seasonal, temporal, among collections and among plant individuals. However, the functional beta-diversity was low and represented by nestedness. The turnover of epiphytic species is well documented and may have several causes: seasonality, senescence, host type, and temporal and spatial variation (Inácio *et al.* 2002; Glushakova and Chernov 2004, 2010; Fonseca and Inácio 2006; Peñuelas *et al.* 2012). This is not the first study to record a large taxonomic variation associated with the stability of functional groups (or traits). Louca et al. (2017) verified a large spatial turnover of bacterial species in bromeliad tanks while maintaining functional groups. Lajoie et al. (2020) reported that the most abundant (and most consistent) functional groups in the phyllosphere microbiota of several tree species are related to metabolism (about 45%), the main ones being the metabolism of amino acids, nucleotides, carbohydrates, and energy. This functional stability may indicate the existence of a phyllosphere functional core (Lajoie, Maglione and Kembel 2020). There is a discussion about taxonomic and functional decoupling, where the taxonomic structure can vary significantly while keeping its functional configuration stable. Nevertheless, the discussion revolves mainly around prokaryotes, with few mentions of eukaryotic organisms (Louca *et al.* 2018).

Stochastic and deterministic mechanisms are important for assembling biological communities, including the phyllosphere microbiota (Vellend 2010; Vacher *et al.* 2016; Gouka, Raaijmakers and Cordovez 2022). The high turnover of species and phylogenetic groups indicates high community dynamics at different scales and a high rate of extinction and colonization. Colonization has stochastic facets and occurs mainly through vectors such as rain, wind and animals (mostly insects and birds. On the other hand, selection involves deterministic processes related to hosting species and the fitness of colonizing species or isolates (Andrews *et al.* 1987; Kinkel 1997; Vellend 2010; Mittelbach *et al.* 2015; Vacher *et al.* 2016; Blackwell 2017). The results indicate a stochastic and intense colonization dynamic; however,

non-random selection processes are based on functional traits. It is reflected in the high taxonomic turnover and high functional stability observed.

Functional traits from phyllosphere yeasts

As mentioned, there were no significant differences in relation to the components of functional diversity between periods. The more expressed traits in the phyllosphere were the carbon source assimilation, principally plant-derived oligo and monosaccharides like raffinose, glucose and xylose, but also polysaccharides like inulin. In yeasts isolated from flag leaves of wheat carbohydrates (xylose, D-mannitol e N-acetyl-D-glucosamine) were the most frequent nutrients assimilated, followed by polymers and organic acids (Gouka *et al.* 2022). One of the characteristics that allows yeasts to conquer different habitats, including the phyllosphere, is their ability to use a wide spectrum of carbon and nitrogen sources (polytrophy). In the study by Hagler et al. (1993), more than half of the yeast community recovered from the phytotelma of bromeliads were able to assimilate more than 20 different carbon sources. The main source of organic nutrients for microorganisms in the phyllosphere (especially leaves) are exudates provided by plants, while inorganic nutrients are generally obtained from the external environment, for example, by wind deposition (Fonseca and Inácio 2006; Kemler *et al.* 2017). In this way, plant physiology plays a vital role in nutritional availability and, consequently, in the structure of the microbial community (Vacher *et al.* 2016). In dry environments, the dynamics of rain pulses generate biological responses in plants. Thus, it is expected that in rainy seasons the availability and diversity of organic compounds for microbiota becomes greater.

Yeasts are heterotrophic organisms and extracellular enzymes play a fundamental role in their nutrition. They hydrolyze macromolecules and make nutrients available for the cell to absorb, thus directly or indirectly mediating decomposition (Fonseca and Inácio 2006). We observed that esterase was the most frequently produced among the evaluated hydrolases. Esterases and other hydrolases such as cellulase produced by fungi, are important in the flow of carbon in environments (Treseder *et al.* 2018). On the other hand, the production of cuticle-degrading enzymes and biosurfactants has been proposed as a mechanism that can increase permeability in leaves and facilitate the movement of bacteria in the

phylloplane (Lindow and Brandl 2003; Doan and Leveau 2015; Leveau 2019; Oso *et al.* 2021). Furthermore, *in vitro*, esterases produced by the epiphytic yeast species *Pseudozyma antarctica* was able to affect the plant's cutin and influence its water dynamics (Ueda *et al.* 2015). Furthermore, esterase produced by *P. antarctica* aggravated the infection caused by *Botrytis cinerea* in tomato plants (Ueda *et al*. 2018). It does not indicate that the yeast is pathogenic but may be a facilitator of the infection.

In our research, few traits were individually expressed with significative difference when compared dry and rainy seasons. However, a clear pattern is that most of the traits analyzed were more frequently expressed during the rainy season. That corroborates with the idea that rains generate greater nutritional diversity for microorganisms in the phyllosphere. Interestingly, among the few traits that were most frequently expressed in the dry period is the emulsion index (IE_{24}). This index is indicative of one of the activities of a surfactant. Biosurfactants are chemically diverse molecules produced by various microbial groups whose main characteristic is amphipathic; they have a polar and a non-polar region. These molecules have numerous activities such as: decreasing surface tension, increasing wettability on hydrophobic surfaces, emulsifying between immiscible liquids, and solubilizing hydrocarbons (Beattie and Lindow 1995; Thapa and Prasanna 2018; Zeisler-Diehl, Barthlott and Schreiber 2020; Oso *et al.* 2021). Thus, the production of biosurfactants can be an important tool for epiphytic microorganisms to change their habitat and increase the availability of water and nutrients in the phyllosphere (Lindow and Brandl 2003; Leveau 2019). For example, the phytopathogen *Pseudomonas syringae* can increase the leaf's wettability and local water availability by releasing biosurfactants (Koskella 2020). The higher frequency of emulsion is evidence that in the studied dryland (Caatinga), during the drier period, phyllosphere yeasts increase the production of biosurfactants to improve surface wettability and increase access to water. However, more data and studies are needed to evaluate this hypothesis.

Conclusions

Given this, our results bring unprecedented insights into the dynamics of phyllosphere yeast microbiota from tropical dryland and how it relates to rainfall

pulses, drought, seasonality and memory effect. Furthermore, our study added information about the persistent alpha-diversity of the phyllosphere yeast community to seasonal changes, from a taxonomic, phylogenetic and functional perspective. Contrary to what is usually observed in seasonal studies in temperate regions, the yeast community showed stability in diversity and seasonal variation in terms of composition. Indicating a possible tropical seasonal pattern that may differ from the temperate pattern, where there is usually a change in diversity depending on the season. By understanding the effect of rain and drought on the phyllosphere microbiota, especially in drylands, we can think about how the microbial community of the leaves will be affected by the changes in the dry-wet cycles that can be caused by climate change. The phyllosphere microbiota is an important factor for the resilience and maintenance of plant health. Consequently, this community's dysbiosis can influence the host's health. In addition, our results indicate trends in the production of products of biotechnological interest such as enzymes and emulsifiers in different seasons. This information can serve as a guide for bioprospecting in future studies.

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Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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6. CAPÍTULO 3 - *Carlosrosaea xxxxxxx* sp. nov., a new tremellomycetes yeast from Brazilian Seasonally Dry Tropical Forest (Caatinga)

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Abstract

Bromeliads are tropical plants that are a substrate rich in yeast taxonomic novelties. In a study carried out in a semi-arid region in Northeastern Brazil, yeast isolates were obtained from leaves of the *Bromelia laciniosa*. By analyzing the regions of the D1/D2 domain of LSU rRNA and the Internal Transcribed Spacer (ITS) region of rRNA, six isolates showed an affinity with the genus *Carlosrosaea*. The dissimilarity with the already described species and the phylogenetic reconstruction indicates that these isolates constitute a new *Carlosrosaea* species. Therefore, we propose the new tremellomycetes yeast *Carlosrosaea xxxxxxx* sp. nov. (Trimorphomycetaceae, Basidiomycota). According to genetic analyses, *C. xxxxxxx* sp. nov. differs from the *C. vrieseae* by 18-19 bp substitutions in D1/D2 and 50 bp substitutions in ITS and from the *C. foliicola* by 22-23 bp substitutions on D1/D2 and 43 bp substitutions on ITS. Furthermore, *C. xxxxxxx* sp. nov. differs from *C. vrieseae* and *C. foliicola* by the ability to produce extracellular starch-like compounds. The holotype of *C. xxxxxxx* sp. nov. is CBS XXXX, deposited at Mycobank under code MBXXXX.

Keywords: Caatinga, bromeliad, semiarid, Tremellales, Basidiomycota.

Introduction

Drylands cover 41% of the Earth's continental surface. They are characterized by a water deficit that can vary from severe to moderate, with aridity indexes ≤0.65. These ecosystems are threatened by climate change and human occupation. In addition, they are microbiologically undersampled, with unique biodiversity and provide important ecosystem services in the global cycle of carbon, nitrogen, and water [1–3]. Depending on the intensity of aridity, Drylands can be divided into hyper-arid, arid, semi-arid, and sub-humid [1]. The Caatinga is a semi-arid dryland that occupies 1/10 of the Brazilian territory (~1 million km²) and forms the largest Seasonally Dry Forest in South America [4–6]. In this environment, rainfall is low (between 240 and 1500 mm per year) and is concentrated in three consecutive months [7, 8]. In some points in the Caatinga about 60% of the annual volume of rainfall can occur in a single month [9].

The Caatinga is home to 3,347 plant species, about 16% of which are endemic [10]. One of the groups of plants that stands out in this environment is the Bromeliaceae family. This group of plants is almost exclusively neotropical, only one species is found outside the Americas, the species *Pitcarnia feliciana*, which occurs on the West Coast of Africa [11], a biological vestige of when these continents were connected. The family comprises 3,500 species, 50 genera and 8 subfamilies [11, 12]. About half of the bromeliad genera and more than half of the subfamilies contain species capable of forming water tanks (phytotelm) [12, 13]. Bromeliads can function as a microscale island ecosystem and perhaps also as a refuge, particularly in times of drought [14].

The phyllosphere (aerial part of plants) includes leaves (phyloplane), fruits (carposphere), flowers (anthosphere, including pollen and nectar), stems (caulosphere), and water reservoirs (phytotelm). These environments support one of the greatest diversity of microorganisms on the planet, with representatives of diverse groups such as bacteria, archaea, viruses, filamentous fungi, yeasts, microalgae, protozoa and nematodes [15–19]. Some of the largest reservoirs of taxonomic novelties of filamentous fungi and yeasts are vascular plants (especially in South America), insects and soil [3, 20–22]. There is a large gap in relation to the richness of fungi in the world, a recent estimate is that there are between 2.2 and 3.8

million species of fungi, but the number of species correctly named and described is around 120,000 (from 3.15 to 5.45% of the estimated total) [20]. Regarding yeasts, the estimate is around 200,000 species [3] and the diversity of yeasts currently described is between 2,200 and 2,300 species, just over 1% of total wealth [3, 23].

The genus *Carlosrosaea* (Trimorphomycetaceae, Tremellales, Basidiomycota) is among the groups of yeasts frequently associated with plants. This genus was proposed by Liu et al. [24] to receive the species *Carlosrosaea vriesea*, isolated from bromeliads in Brazil and previously allocated in the genus *Bullera* [25]. Currently, there are five species described in *Carlosrosaea*. The species *Carlosrosaea hohenbergiae* and *Carlosrosaea aechmeae* were described associated with bromeliads from Brazil [26]; and *Carlosrosaea foliicola* and *Carlosrosaea simaoensis* associated with leaves from China [27].

During studies carried out in bromeliads from Northeast Brazil, eight yeast isolates, through molecular information of the D1/D2 domain of LSU rRNA and the Internal Transcribed Spacer (ITS) region of rRNA, indicated affinity with the genus *Carlosrosaea*. The analyzes indicated that these isolates differ from the five species already described for the group. Therefore, we propose the description of the new tremellomycetes yeasts *Carlosrosaea xxxxxxx* sp. nov., the fourth species of the group originating from bromeliads from Brazil.

Material and methods

Sampling area

Between 2017 and 2022, leaves of the species *Bromelia laciniosa* Mart. ex Schult. & Schult.f (Bromeliaceae, Bromelioideae) were collected at Tocaia Private Heritage Reserve (RPPN Tocaia), in the municipality of Santana do Ipanema, Alagoas, Northeast Brazil (9°23'08.9"S and 37°15 '22.8"W). The RPPN Tocaia has an area of approximately 20 hectares, a maximum altitude of about 400 meters, and is mainly composed of trees.

Plant collection, yeast isolation and maintenance

Leaves of apparently healthy adult individuals were collected, stored in sterile plastic bags, and transported to the laboratory at room temperature for

approximately 4 h. In the laboratory, the leaves were stored at 10-15 °C, and processing was carried out within 24 h after collecting the material in the field. For the processing of the samples, the leaves underwent an initial wash with sterile distilled water to remove possible contaminants such as dust and other exogenous materials. Then, leaf fragments between 5 and 20 cm^2 were randomly cut until a total area of 120 cm^2 was obtained. Sequentially, the leaf fragments were placed in a 250 mL Erlenmeyer flask containing 75 mL of sterile distilled water. Then this flask was shaken for 30 min at 180 RPM and at room temperature (27±2 °C). After stirring, the wash product was collected in a sterile flask and the leaf fragments were kept in the Erlenmeyer flask. After removing and storing the product from the first wash, 75 mL of sterile Tween 80 solution (0.5% concentration) was added to the flask and the material was stirred again at the same time, rotation and temperature. In the end, the products of the two washes were aggregated, thus forming a pool. A 100 µL fraction of this pool was seeded in duplicate on Petri dishes containing modified YM agar medium (0.3% yeast extract, 0.3% malt extract, 0.5% bacteriological peptone, 1% glucose, 2% agar, 0.04% chloramphenicol, pH 4.0) at concentrations of 10^0 and 10^{-1}.

The plates were incubated at 25-28 °C and observed daily for 10 days for yeast isolation on YEPD agar medium (2% glucose, 1% bacteriological peptone, 0.5% yeast extract, and 2% agar). Yeast isolates were preserved in tubes containing GYMP agar medium (2% glucose, 2% malt extract, 0.5% yeast extract, 0.2% monobasic sodium phosphate, and 2% agar) and GYMP broth containing final volume of 30% glycerol.

Morphological and physiological characterization

All isolates were morphologically and physiologically characterized by Kurtzman et al. [28]. The carbon and nitrogen assimilation pattern were measured in solid medium using the replica plating technique. To verify the possible production of pseudo-hyphae, true hyphae and/or sexual structures, the isolates were sown on potato dextrose agar (PDA), corn meal agar (CMA) and malt extract agar (MEA) at 22-25 °C and observed for 21 days.

Molecular analysis

For molecular identification of isolates, genomic DNA was extracted using the protocol for small-scale preparation of yeast DNA modified, originally proposed by Sambrook and Russel [29]. From this, the D1/D2 regions of the 26S LSU and the Internal Trancrit Spacer (ITS) of the rRNA were amplified via PCR. Using primers NL-1 (5'- GCA TATC AAT AAG CGG AGG AAA AG -3') and NL-4 (5'- GG TCC GTG TTT CAA GAC GG -3'); and ITS-1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS-4 (5'-TCC TCC GCT TAT TGA TAT GC-3') [30–32]. The reaction parameters were established following Landell et al. [33]. For sequencing, the Sanger method was used through the protocol facilities from the *Plataforma Multiusuária de Sequenciamento de* DNA at the *Laboratório de Bioinformática e Biologia Evolutiva of the Universidade Federal de Pernambuco* in an automated sequencing system ABI 3130 Genetic Analyzer using the polymer BigDye v3.1 and POP7 (Life Technologies). Sequence consensus was generated using the Staden Package software [34] and MEGA X. Then, the sequences obtained were compared with others deposited in the GenBank database on the National Center for Biotechnology Information (NCBI) website using the Basic Local Alignment Search Tool (BLAST) and the BLASTn algorithm [35]. To estimate phylogenetic relationships, trees were constructed using D1/D2 regions and ITS applying neighbor-joining method. To reconstruct the phylogenetic relationships, gaps were excluded, and the Kimura two-parameter model was applied to correct the genetic distances [36]. Branch robustness was estimated with the bootstrap resampling method using 1000 pseudoreplicates [37].

Results and Discussion

Novel species delineation and identification

Eight yeast isolates were isolated from bromeliad leaves of *Bromelia laciniosa* Mart. ex Schult. & Schult.f (Bromeliaceae, Bromelioideae) from the Caatinga biome, a semi-arid Dryland inserted in Northeast Brazil (Table 1). Analysis of the D1/D2 regions of the 26S LSU and the Internal transcribed Spacer (ITS) of the rRNA indicated that these isolates have affinity with the genus *Carlosrosaea*, and diverge from type material of all currently described species: *C. vrieseae* (18-19 bp substitutions in D1/D2 and 50 in ITS), *C. hohenbergiae* (20-21 bp substitutions in

D1/D2 and 54 in ITS), *C. aechmeae* (22-23 bp substitutions in D1/D2 and 44 in ITS), *C. foliicola* (22-23 bp substitutions on D1/D2 and 43 on ITS), and *C. simaoensis* (21-23 bp substitutions on D1/D2 and 46 on ITS) (Fig. 1). Therefore, we propose the species *C. xxxxxxx* sp. nov. to accommodate these isolates. The isolates of *C. xxxxxxx* sp. nov. differ from each other by 0-1 bp in the D1/D2 domain and have identical ITS sequences. Furthermore, *C. xxxxxxx* sp. nov. differs from *C. foliicola*, *C. simaoensis* and *C. vrieseae* by the ability to produce extracellular starch-like compounds and from *C. aechmeae* by the ability to assimilate sucrose (Table 2).

Table 1- Information on *Carlosrosaea xxxxxxx* sp. nov. isolates: Culture collection code, GenBank sequence accession number and date of collection.

Culture collection deposit code			**Genbank access code**		**Collection date**
CBS	**UFMG**	**UFAL**	**ITS**	**D1D2**	
		BRT303		MH909017	September 21, 2017
		BRT537			October 19, 2021
		BRT586			November 23, 2021
	UFMG-CM-	BRT651			May 24, 2022
CBS XX^T	UFMG-CM^T	BRT659^T			May 24, 2022
		BRT663			May 24, 2022
		BRT696			June 23, 2022
		BRT714			July 19, 2022

Table 2- Selection of physiological/biochemical characteristics that differentiate *Carlosrosaea xxxxxxx* sp. nov. from other species of the genus.

Characteristic	***C. aechmeae*** [a]	***C. foliicola*** [b]	***C. hohenbergiae*** [a]	***C. simaoensis*** [b]	***C. vrieseae*** [c]	***C. xxxxxxx***
Carbon source						
Inulin	+	-	+	-	V	D,w
Sucrose	-	+	V	+	V	+
Glycerol	-	+	-	+	V	-
Meso-erythritol	-	+	V	-	-	-
Succinic acid	-	D,w	V	w	V	-
Citrate	-	D,w	-	+	V	-
D-gluconate	-		+		+	w
L-arabinitol	+		+			w
DL-lactate	-	+	-		V	w
Tween 20	-		V		+	w
Nitrogen source						
Cadaverine	+	-	-	-	+	w
Lysine	+	-	+	-	+	w
Starch-like compounds	+	-	+	-	-	+

[a] Data obtained from Félix et al. [26].
[b] Data obtained from Li et al. [27].
[c] Data obtained from Landell et al. [25].

Carlosrosaeae is a monophyletic genus in the Trimorphomycetaceae family. The genus is well supported by analysis of the D1/D2 and ITS rRNA regions, and it has affinity with the genus *Vishniacozyma* and the clade *Tremella sensu stricto* (Fig. 1). The Trimorphomycetaceae family appears monophyletic from the analysis of the LSU rRNA region, but its support via bootstrap is not robust [24]. The genus *Vishniacozyma* and *Tremella* occur in diverse substrates such as soil, plants and marine environments. In addition, these genera can be found in arctic climate, humid and semi-arid tropical climates [38–41].

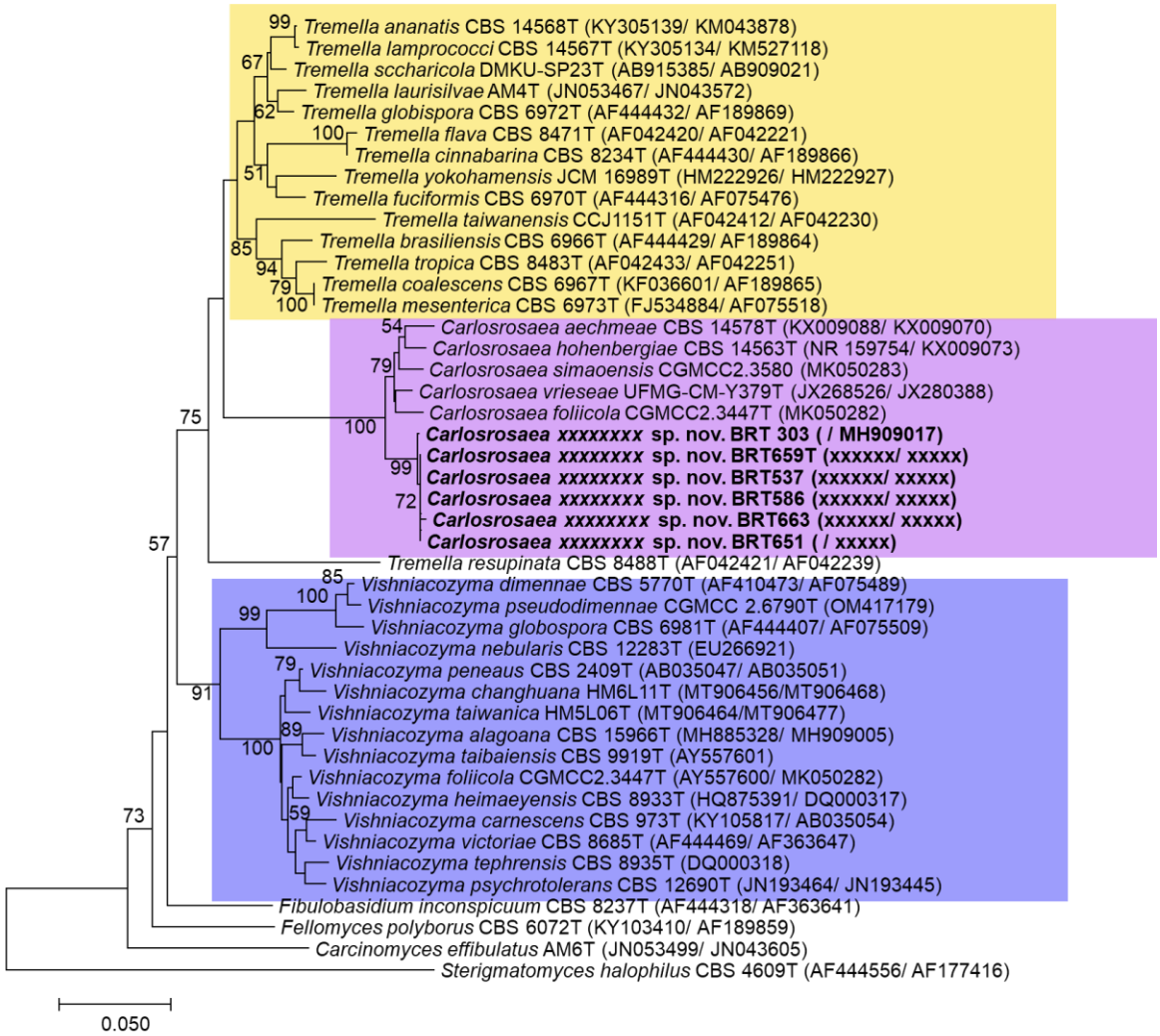

Fig. 1- Phylogenetic tree of *Carlosrosaea xxxxxxx* sp. nov obtained by neighbour-joining (Kimura two-parameter distance method) analysis of the concatenated D1/D2 and ITS regions. Bootstrap values ≥50% are show. Bar, 0.05 substitutions per nucleotide position.

Bromeliads are an important substrate for prospecting new yeast isolates and species [42]. More than 180 species of yeasts have already been registered in bromeliads and, together with the phytotelmata, the leaves are one of the most diverse substrates of these plants [42]. All described species of the genus *Carlosrosaea* (Tremellales, Basidiomycota) has association with plant substrates, mainly to bromeliads, with records of at least 13 species of these plants, in flowers, phytotelma and leaves [25–27, 42–44]. The species *C. vrieseae* can stimulate plant growth (evaluated in bromeliads) producing indoleacetic acid (IAA), phosphate solubilization, siderophore production and antagonism to phytopathogens [45]. Furthermore, *Carlosrosaea* spp. isolates produced extracellular enzymes such as amylase, cellulase, pectinase, and protease [44]. It indicates, in addition to an important biotechnological potential, a possible ecological role of the genus in stimulating plant growth.

Description of *Carlosrosaea xxxxxxx* sp. nov.

Carlosrosaea xxxxxxx

After 3 days on YEPD broth at 22–25 ºC, yeast cells are globose, subglobose with unipolar or bipolar buds (2.4–3.9 × 3.0–5.1 µm) (Fig. 2a). The colonies are cream to yellowish-white, smooth, and creamy (Fig. 2b). In Dalmau plates after 4 weeks on cornmeal agar, were not observed pseudohypha or hypha formation. Glucose fermentation ability is negative and ballistoconidia production is absent. The species can grow in the presence of 0.01% cycloheximide, but not at 0.1%. The ability to grow in medium containing 10 and 16% NaCl was observed, but it was not able to grow in the presence of 50% glucose. There was no growth in the presence of 1% acetic acid. The urease activity, the reaction of diazonium blue B and the starch-like compounds production are positive. The following carbon compounds are assimilated: D-glucose, inulin (slow), sucrose, raffinose, melibiose, D-galactose, lactose (weak), trehalose, maltose (slow), melezitose, soluble starch (variable), cellobiose (weak), L-rhamnose (weak), D-xylose, L-arabinose, D-arabinose (slow), D-ribose, D-ribitol (slow), D-mannitol (weak), D-glucitol (weak), *myo*-inositol (weak), D-gluconate (weak), D-glucosamine (weak), N-acetylglucosamine (weak), D-galacturonic acid (weak), xylitol (variable), L-arabinitol (weak), DL-lactate (weak), Tween 20 (weak) and Tween 80 (variable). The following carbon compounds are not

assimilated: salicin, glycerol, meso-erythritol, succinic acid and citrate. The assimilated nitrogen compounds were nitrite (weak), cadaverine (weak) and lysine (weak). The following nitrogen compounds are not assimilated: nitrate, creatinine, creatine, ethylamine.

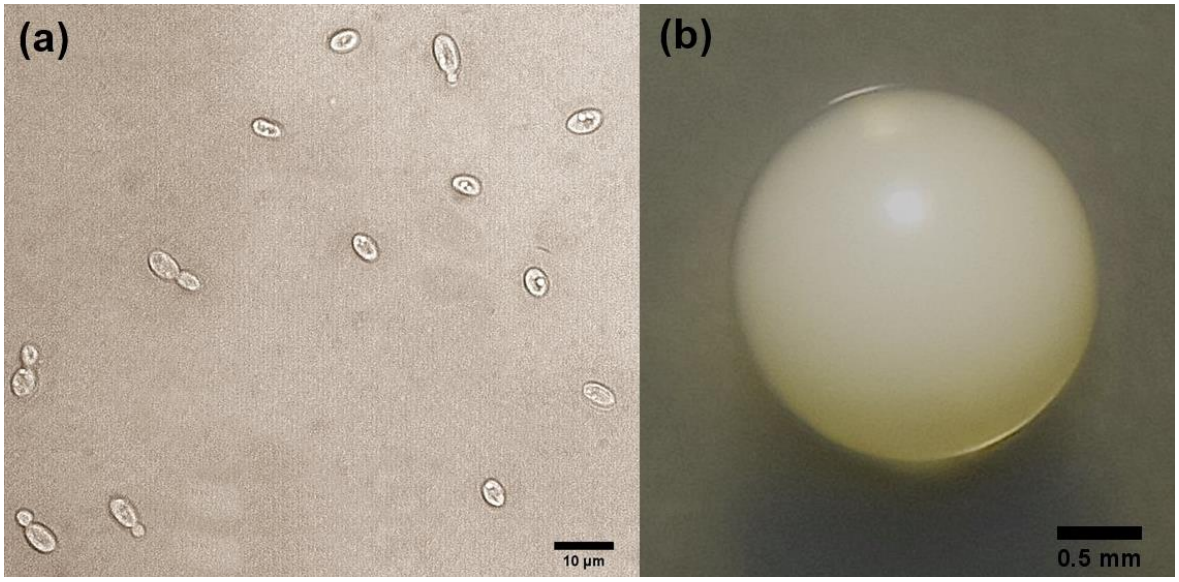

Fig. 2- Cellular (a) and colonial (b) morphology of *C. xxxxxxx* sp. nov. grown in YEPD for 48 h at 22-25 °C.

The holotype of *C. xxxxxxx* sp. nov. CBS XXXX, is preserved in a metabolically inactive state in the CBS Yeast Collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands and in the Collection of Microorganisms; in the Cells of Federal University of Minas Gerais (*Coleção de Microrganismos e Células da Universidade Federal de Minas Gerais*- UFMG), Belo Horizonte, Minas Gerais, Brazil as the strain UFMG-CM-XXX; and in the Mycotheque of the Molecular Diversity Laboratory of the Federal University of Alagoas (*Micoteca do Laboratório de Diversidade Molecular da Universidade Federal de Alagoas* - UFAL), as the strain BRT659. Mycobank number is XXXXXX. Additional isolates are indicated in Table 1.

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7. DISCUSSÃO GERAL

Plantas são fontes importantes de micro-organismos e, ao mesmo tempo, os micro-organismos que formam a microbiota das plantas são importantes nos processos ecológicos e evolutivos dos vegetais (KOSKELLA, 2020; MEYER; LEVEAU, 2012; MORRIS, 2001; VACHER et al., 2016; VORHOLT, 2012). Uma fração ínfima da microbiota do planeta é conhecida, dependendo do grupo, apenas 1% do total (BOEKHOUT et al., 2022; HAWKSWORTH; LÜCKING, 2017). Conhecer a biodiversidade se faz cada vez mais necessário, e em um cenário de mudanças climáticas, a interação planta-microbiota pode permitir que os hospedeiros estendam seus limites de tolerância, entretanto, para compreender as minúcias e o real potencial desse e de outros mecanismos é necessário conhecer os grupos microbianos e suas funções.

Bromélias são plantas tropicais ricas em diversidade de leveduras (MORAIS; DE SOUSA; ROSA, 2020; NAVARRO et al., 2020). Mais de 180 espécies de leveduras já foram registradas em bromélias. Grupos de leveduras frequentemente encontrados na filosfera de bromélias envolvem gêneros como *Candida, Rhodotorula, Hannaella, Aureobasidium, Meyerozyma, Carlosrosaea* e *Pseudozyma*. Grupos anteriormente inserido no gênero *Cryptococcus* e que atualmente estão distribuídos em outros gêneros estão entre os mais frequentes da filosfera, são eles: *Kwoniella, Naganishia, Papiliotrema, Saitozyma* e *Vishniacozyma* (LIU et al., 2015). As espécies que mais se destacaram na filosfera de bromélias foram *Papiliotrema laurentii* e *Papiliotrema flavescens*. Muitos desses grupos já possuem registros como membros dominantes da filosfera, principalmente em folhas (FONSECA; INÁCIO, 2006).

Além disso, essa diversidade de leveduras se distribui por vários compartimentos da filosfera como folhas, flores, frutos e tanques. Cada um desses compartimentos possui uma comunidade de leveduras distinta, que responde a estrutura única de cada compartimento. A interação com a cuticula pode ser um modulador importante da estrutura da comunidade e isso pode explicar por que leveduras em folhas, frutos intactos e flores (quando o néctar é desconsiderado) tende a ser estruturalmente semelhantes (FONSECA; INÁCIO, 2006). Plantas frequentemente apresentam comunidades microbianas distintas a depender do

compartimento estudado (ABDELFATTAH et al., 2019; LIU; HOWELL, 2021). Leveduras em bromélias parecem seguir o mesmo padrão e divergir em configuração dependendo do compartimento.

Não há consenso sobre o efeito das chuvas nas comunidades microbianas da filosfera. A microbiota pode se apresentar mais resilientes a eventos de chuva de curto prazo e não manifestar alterações significativas (e.g., STONE; JACKSON, 2021), ou responder de maneira mais imediata a eventos disruptivos de chuva (e.g., ALLARD; OTTESEN; MICALLEF, 2020). A filosfera é um ambiente dinâmico e sujeito a flutuações em muitos fatores ambientais. Micro-organismos da filosfera em ambientes áridos possuem camadas de sobreposição de estresse hídrico, uma gerada pela cutícula da planta que dificulta a lixiviação de metabólitos e limita a molhabilidade, e outra pelo próprio ambiente árido que na maior parte do tempo se encontra em déficit hídrico (KOSKELLA, 2020; MAESTRE et al., 2016; NOY-MEIR, 1973; OSO et al., 2021; VACHER et al., 2016).

Em ambientes árido, as chuvas não essenciais e controlam os processos biológicos (MAESTRE et al., 2016; NOY-MEIR, 1973). É de se esperar que nesses ambientes a microbiota da filosfera responda aos pulsos de chuva, principalmente porque quantidades pequenas de chuva geralmente são suficientes para elicitar a comunidade microbiana em regiões secas (REYNOLDS et al., 2004). Entretanto, a comunidade de leveduras em folhas de bromélias na Caatinga apresentou pouca influência da chuva na diversidade, ao mesmo tempo que a sazonalidade mostrou um efeito importante na estrutura da comunidade.

As leveduras são organismos heterotróficos e as enzimas extracelulares desempenham um papel fundamental na sua nutrição, elas hidrolisam macromoléculas e disponibilizam nutrientes para que a célula utilize. Dentre as enzimas avaliadas, a esterase foi a mais produzida por leveduras da filosfera de bromélias. As esterases e outras hidrolases, como a celulase produzida por fungos, são importantes no fluxo de carbono nos ambientes (TRESEDER et al. 2018). *In vitro*, as esterases produzidas pela espécie de levedura epífita *Pseudozyma antarctica* foram capazes de afetar a cutícula da planta e influenciar sua dinâmica hídrica (UEDA et al. 2015). Além disso, essa mesma esterase foi capaz de agravar a infecção causada por *Botrytis cinerea* em tomateiros (Ueda et al. 2018).

Em bromélias, não se observou diferenças significativas em relação aos componentes da diversidade funcional entre os períodos. Os traços mais expressos na filosfera foram relacionados a assimilação de fontes de carbono, principalmente oligo e monossacarídeos derivados de plantas como rafinose, glicose e xilose, mas também polissacarídeos como inulina. A capacidade de assimilar uma grande gama de compostos é uma das características que permite às leveduras conquistar diferentes habitats. Hagler et al. (1993), indicaram que leveduras de tanque de bromélias possuem uma natureza politrófica, mais da metade da comunidade de leveduras recuperada assimilou mais de 20 diferentes fontes de carbono.

O índice de emulsão (IE_{24}) foi um dos poucos traços testados que foi mais frequente durante o período seco em leveduras na Caatinga. Este índice é indicativo de uma das atividades de um surfactante. Biossurfactantes são moléculas quimicamente diversas produzidas por vários grupos microbianos cuja principal característica é uma estrutura anfipática. Essas moléculas têm inúmeras atividades, como: diminuir a tensão superficial, aumentar a molhabilidade em superfícies hidrofóbicas, emulsificar líquidos imiscíveis e solubilizar hidrocarbonetos (BEATTIE; LINDOW, 1995; THAPA; PRASANNA, 2018; ZEISLER-DIEHL; BARTHLOTT; SCHREIBER, 2020; OSO et al., 2021). A produção de biossurfactantes pode ser uma ferramenta importante para microrganismos epífitos aumentarem o acesso a água e nutrientes (LINDOW; BRANDL, 2003; LEVEAU, 2019). A maior frequência de emulsão durante a seca pode indicar que na Caatinga esse traço é favorecido durante períodos secos e promove um aumento do acesso à água para as leveduras epifíticas. No entanto, mais dados e estudos são necessários para avaliar a validade dessa hipótese.

8. CONSIDERAÇÕES FINAIS

Este estudo contribuiu com o conhecimento em biodiversidade e ecologia de leveduras da filosfera de bromélias. Corroboramos que plantas são substratos ricos em micro-organismos e que bromélias possuem potencial para prospecção de leveduras nos campos de: novidades taxonômicas, matéria-prima biotecnológica e funcionamento ecossistêmico. Além disso, constata-se que ainda há uma grande

lacuna sobre biodiversidade de leveduras em bromélias, e principalmente sobre os processos que esses micro-organismos desempenham.

É evidente que ainda há muito a ser explorado nas bromélias. Atualmente, nenhum estudo sobre leveduras de néctar de bromélias foi desenvolvido, mesmo com a estrutura singular da família Bromeliaceae possui, como o grande espectro de polinizadores e de composição química do néctar. Entre os anos 1993-2021, a maioria dos estudos sobre leveduras em bromélias se concentrou no levantamento descritivo de comunidades. A importância desse referencial de conhecimento é inegável. Entretanto, é necessário dar os próximos passos e aumentar o número de estudos que buscam entender os processos que estruturam a comunidade de leveduras e os mecanismos funcionais providos por essa comunidade e não apenas verificar os padrões.

Os resultados trazem informações sem precedentes sobre a dinâmica da microbiota de levedura em uma região semiárida importante como a Caatinga. O presente estudo acrescenta informações sobre a tolerância da comunidade de leveduras da filosfera a mudanças sazonais, de uma perspectiva taxonômica, filogenética e funcional. Além disso, ao entender o efeito da chuva e da seca na microbiota da filosfera, podemos pensar em como a comunidade microbiana das folhas será afetada pelas mudanças nos ciclos seco-úmido que podem ser causadas pelas mudanças climáticas. A microbiota da filosfera é um fator importante para a resiliência e manutenção da fitossanidade, consequentemente, a disbiose dessa comunidade pode influenciar na saúde do hospedeiro.

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ANEXOS

Material suplementar (Capítulo 1, secção 4)

Supplementary Table 1- New yeast species that have been described associated with bromeliads.

Phyla Subphyla	Yeast species	Host	Site	Substrate	Ref.
Ascomycota					
Saccharomycotina					
	Candida aechmeae	***Aechmea recurvata and Billbergia nutans***	**Itapuã State Park, Viamão- Brazil**	**Phylloplane**	**(Landell et al., 2010)**
	Candida bromeliacearum	***Canistropis seidelii***	**Picinguaba, Serra do Mar State Park- Brazil**	**Phytotelma**	**(Ruivo et al., 2005)**
	Candida ubatubensis	***C. seidelii***	**Picinguaba, Serra do Mar State Park- Brazil**	**Phytotelma**	**(Ruivo et al., 2005)**
	Candida vrieseae	***Vriesea gigantea***	**Itapuã State Park, Viamão- Brazil**	**Phytotelma**	**(Landell et al., 2010)**
	Hagleromyces aurorensis	***Bromelia karatas***	**Aurora do Tocantins, Tocantins State- Brazil**	**Phytotelma**	**(Sousa et al., 2014)**
	Kazachstania bromeliacearum	***Quesnelia quesneliana, Nidularium procerum, Neoregelia cruenta, Aechmea nudicaulis and Vriesia procera***	**Mangrove of Coroa Grande (Poço das Antas) and Sand dune area of Maricá- Brazil**	**Phytotelma**	**(Araujo et al., 2012)**
	Kazachstania rupicola	***Vriesea minarum***	**Serra da Piedade region, Caeté city- Brazil**	**Phytotelma**	**(Safar et al., 2013)**
Basidiomycota					
Agaricomycotina					
	Carcinomyces nordestinensis	***Bromelia antiacantha***	**Tocaia reserve, Santana do Ipanema- Brazil**	**Phylloplane**	**(Crous et al., 2019)**
	Carlosrosaea aechmeae	***Aechmea constantinii***	**Murici ecological reserve, Murici- Brazil**	**Phylloplane**	**(Felix et al., 2017)**
	Carlosrosaea hohenbergiae	***Hohenbergia ramageana, Tillandsia sp. Portea leptantha, Canistrum alagoanum and Aechmea fulgens***	**Serra da Barriga, União dos Palmares- Brazil; Serra da Saudinha, Maceió- Brazil and Murici ecological reserve, Murici- Brazil**	**Phylloplane and Anthosphera**	**(Felix et al., 2017)**
	Carlosrosaea vrieseae	***V. minarum, Vriesea friburgensis and Tillandsia gardneri***	**Serra da Piedade region, Caeté city- Brazil and Itapuã State Park, Viamão- Brazil**	**Phylloplane and Phytotelma**	**(Landell et al., 2015)**
	Genolevuria bromeliarum	***V. procera, V. friburgensis and T. gardneri***	**Itapuã State Park, Viamão- Brazil**	**Phylloplane**	**(Crestani et al., 2009)**
	Hannaella pagnoccae	***V. gigantea, Tillandsia geminiflora, V. minarum, Encholirium sp. and B. karatas***	**Itapuã State Park, Viamão- Brazil; South of Brazil, Aurora do Tocantins, Tocantins State- Brazil and Serra da Piedade region, Caeté city- Brazil**	**Phylloplane and Phytotelma**	**(Landell et al., 2014)**
	Kockovaella libkindii	***V. minarum***	**Serra da Piedade region, Caeté city- Brazil**	**Phytotelma**	**(Gomes et al., 2016)**
	Papiliotrema leoncinii	***Tillandsia crocata, B. antiacantha, T. gardneri, V. gigantea, A. recurvata, V. friburgensis, C. alagoanum, A. fulgens, Canistrum aurantiacum, P. leptantha and Bromelia sp.***	**Itapuã State Park, Viamão- Brazil; Murici ecological reserve, Murici- Brazil; Tocaia reserve, Santana do Ipanema- Brazil and Pedra Talhada Biological Reserve, Quebrangulo- Brazil**	**Phylloplane**	**(Pagani et al., 2016)**
	Rhynchogastrema complexa	***N. cruenta* and *Ananas comosus***	**Hsinchu, Taiwan and Marica Resting, Rio de Janeiro-Brazil**	**Phylloplane**	**(Valente et al., 2012)**
	Vishniacozyma alagoana	***Aechmea froesii, B. antiacantha, Hohenbergia stellate and unidentified Bromeliaceae***	**Serra da Barriga, União dos Palmares- Brazil; Serra da Caiçara, Maravilha- Brazil; Tocaia Reserve, Santana do Ipanema- Brazil**	**Phylloplane**	**(Félix et al., 2020)**
Pucciniomycotina					
	Occultifur brasiliensis	***Vriesea minarum***	**Serra da Piedade region, Caeté city- Brazil**	**Phytotelma**	**(Gomes et al., 2015)**
	Occultifur plantarum	***N. cruenta***	**Coastal dune habitat, Maricá- Brazil**	**Phylloplane**	**(Khunnamwong et al., 2017)**
	Queiroziella brasiliensis	***P. leptantha, T. geminiflora and V. gigantea***	**Serra da Barriga, União dos Palmares- Brazil and Itapuã State Park, Viamão- Brazil**	**Phylloplane**	**(Crous et al., 2018)**
Ustilaginomycotina					
	Farysia itapuensis	***V. friburgensis, V. procera, T. gardneri, T. geminiflor and Dyckia sp.***	**Itapuã State Park, Viamão- Brazil**	**Phylloplane**	**(Inácio et al., 2008)**
	Pattersoniomyces tillandsiae	***Canistrum improcerum, V. minarum, Tillandsia leiboldiana, Tillandsia flabellata***	**Vera Cruz- Mexico; Valleé de Cordova- Mexico; Honduras; Guatemala; Serra da Saudinha, Maceió- Brazil and Serra do Cipó, Santana do Riacho- Brazil**	**Phylloplane and Phytotelma**	**(Piątek et al., 2017)**

Supplementary Table 2- Yeast species isolated from bromeliads with some biotechnological potential registered and the paper reference.

Yeast taxon	Production of indoleacetic acid (IAA)[a]	Lipase activity[b]	Production of fermented beverages	Phosphate solubilization	Siderophore production	Antagonism to phytopathogens	Promotion of plant growth[c]	Use as animal feed[d]	Air quality bioindicator	Probiotic potential	Production of extracellular enzymes					
											Amylase	**Cellulase**	**Esterase**	**Pectinase**	**Protease**	**Xylanase**
Anomalomyces panici	(Morais et al., 2020)			(Morais et al., 2020)	(Morais et al., 2020)						(Gomes et al., 2015)	(Gomes et al., 2015)			(Gomes et al., 2015)	(Gomes et al., 2015)
Aureobasidium leucospermi											(Navarro et al., 2020)	(Navarro et al., 2020)	(Navarro et al., 2020)	(Navarro et al., 2020)		
Aureobasidium pullulans	(Morais et al., 2020)								(Brighigna et al., 2000)		(Gomes et al., 2015)	(Gomes et al., 2015)		(Gomes et al., 2015)	(Gomes et al., 2015)	(Gomes et al., 2015)
Aureobasidium thailandense												(Navarro et al., 2020)	(Navarro et al., 2020)	(Navarro et al., 2020)		
Candida intermedia	(Morais et al., 2020)			(Morais et al., 2020)	(Morais et al., 2020)										(Gomes et al., 2015)	
Candida melibiosica	(Morais et al., 2020)			(Morais et al., 2020)	(Morais et al., 2020)						(Gomes et al., 2015)	(Gomes et al., 2015)			(Gomes et al., 2015)	(Gomes et al., 2015)
Candida membranifaciens	(Morais et al., 2020)			(Morais et al., 2020)	(Morais et al., 2020)										(Gomes et al., 2015)	
Candida spp.		(Tangsombatvichit et al., 2020)							(Brighigna et al., 2000)							(Gomes et al., 2015)
Candida ubatubensis	(Morais et al., 2020)			(Morais et al., 2020)	(Morais et al., 2020)											
Carlosrosaea sp.											(Navarro et al., 2020)	(Navarro et al., 2020)		(Navarro et al., 2020)	(Navarro et al., 2020)	
Carlosrosaea vrieseae	(Morais et al., 2020)			(Morais et al., 2020)	(Morais et al., 2020)		(Marques et al., 2021)									
Colacogloea sp.	(Morais et al., 2020)			(Morais et al., 2020)	(Morais et al., 2020)											
Cystobasidium laryngis											(Gomes et al., 2015)			(Gomes et al., 2015)	(Gomes et al., 2015)	
Cryptococcus spp.						(Reyes et al., 2004)			(Brighigna et al., 2000)		(Gomes et al., 2015)			(Gomes et al., 2015)	(Gomes et al., 2015)	
Dioszegia sp.	(Morais et al., 2020)			(Morais et al., 2020)							(Gomes et al., 2015)					(Gomes et al., 2015)
Fellomyces penicillatus	(Morais et al., 2020)			(Morais et al., 2020)	(Morais et al., 2020)						(Gomes et al., 2015)			(Gomes et al., 2015)	(Gomes et al., 2015)	
Fellomyces sp.											(Gomes et al., 2015)	(Gomes et al., 2015)				
Hannaella pagnoccae	(Morais et al., 2020)			(Morais et al., 2020)								(Gomes et al., 2015)				(Gomes et al., 2015)
Hannaella sinensis																(Gomes et al.,

																	2015)
Hanseniaspora guilliermondii			(Dellacassa et al., 2017)														
Hanseniaspora opuntiae			(Dellacassa et al., 2017)														
Hanseniaspora uvarum			(Chanprasartsuk et al., 2012, 2010; Dellacassa et al., 2017)												(Gomes et al., 2015)		
Kazachstania rupicola	(Morais et al., 2020)				(Morais et al., 2020)											(Gomes et al., 2015)	
Kloeckera apiculata var. apis						(Korres et al., 2011)											
Kockovaella libkindii	(Morais et al., 2020)																
Kodamaea ohmeri	(Morais et al., 2020)			(Morais et al., 2020)	(Morais et al., 2020)										(Gomes et al., 2015)	(Gomes et al., 2015)	(Gomes et al., 2015)
Meira sp.	(Morais et al., 2020)			(Morais et al., 2020)	(Morais et al., 2020)												(Gomes et al., 2015)
Metschnikowia koreensis																(Gomes et al., 2015)	
Metschnikowia koreensis	(Morais et al., 2020)			(Morais et al., 2020)	(Morais et al., 2020)												
Meyerozyma caribbica			(Amorim et al., 2018)				(Islam et al., 2021)	(Amorim et al., 2018)							(Gomes et al., 2015)		
Meyerozyma guilliermondii	(Morais et al., 2020)		(Chanprasartsuk et al., 2010; Dellacassa et al., 2017)		(Morais et al., 2020)	(Reyes et al., 2004)				(Gomes et al., 2015; Navarro et al., 2020)	(Navarro et al., 2020)	(Navarro et al., 2020)	(Gomes et al., 2015)	(Gomes et al., 2015)			
Meyerozyma sp.	(Morais et al., 2020)			(Morais et al., 2020)	(Morais et al., 2020)												
Moesziomyces aphidis										(Gomes et al., 2015)				(Gomes et al., 2015)	(Gomes et al., 2015)	
Myriangium sp.	(Morais et al., 2020)			(Morais et al., 2020)					(Gomes et al., 2015)	(Gomes et al., 2015)			(Gomes et al., 2015)	(Gomes et al., 2015)	(Gomes et al., 2015)		
Occultifur brasiliensis	(Morais et al., 2020)			(Morais et al., 2020)	(Morais et al., 2020)				(Gomes et al., 2015)	(Gomes et al., 2015)			(Gomes et al., 2015)	(Gomes et al., 2015)	(Gomes et al., 2015)		
Papiliotrema flavescens	(Morais et al., 2020)				(Morais et al., 2020)				(Gomes et al., 2015)				(Gomes et al., 2015)	(Gomes et al., 2015)	(Gomes et al., 2015)		
Papiliotrema laurentii	(Morais et al., 2020)			(Morais et al., 2020)	(Morais et al., 2020)				(Gomes et al., 2015)	(Gomes et al., 2015)				(Gomes et al., 2015)	(Gomes et al., 2015)		
Papiliotrema nemorosa	(Morais et al., 2020)			(Morais et al., 2020)	(Morais et al., 2020)				(Gomes et al., 2015)	(Gomes et al., 2015)			(Gomes et al., 2015)	(Gomes et al., 2015)	(Gomes et al., 2015)		
Papiliotrema rajasthanensis	(Morais et al., 2020)			(Morais et al., 2020)	(Morais et al., 2020)								(Gomes et al., 2015)	(Gomes et al., 2015)	(Gomes et al., 2015)		
Papiliotrema sp.	(Morais et al., 2020)			(Morais et al., 2020)													

Pattersoniomyces tillandsiae	(Morais et al., 2020)		(Morais et al., 2020)										
Pichia fermentans						(Islam et al., 2021)							
Pichia kudriavzevii					(Korres et al., 2011)								
Pseudozyma hubeiensis													(Gomes et al., 2015)
Pseudozyma sp.												(Gomes et al., 2015)	
Rhodosporidium diobovatum	(Morais et al., 2020)		(Morais et al., 2020)	(Morais et al., 2020)							(Gomes et al., 2015)	(Gomes et al., 2015)	(Gomes et al., 2015)
Rhodotorula mucilaginosa												(Gomes et al., 2015)	
Rhodotorula spp.	(Morais et al., 2020)		(Morais et al., 2020)		(Reyes et al., 2004)								
Saccharomyces cerevisiae		(Nasir et al., 2017)											
Saccharomycodes ludwigii		(Chanprasartsuk et al., 2012)											
Saitozyma flava								(Navarro et al., 2020)	(Navarro et al., 2020)		(Navarro et al., 2020)	(Navarro et al., 2020)	
Saitozyma podzolica	(Morais et al., 2020)		(Morais et al., 2020)					(Gomes et al., 2015)			(Gomes et al., 2015)	(Gomes et al., 2015)	(Gomes et al., 2015)
Saturnispora silvae	(Morais et al., 2020)		(Morais et al., 2020)	(Morais et al., 2020)								(Gomes et al., 2015)	
Sporobolomyces spp.							(Brighigna et al., 2000)						
Symmetrospora marina								(Navarro et al., 2020)	(Navarro et al., 2020)		(Navarro et al., 2020)	(Navarro et al., 2020)	
Tremella fuciformis										(Navarro et al., 2020)	(Navarro et al., 2020)		
Wickerhamomyces anomalus		(Dellacassa et al., 2017)											

a- From the absence or presence of L-Tryptophan.
b- Potential treatment of oil contamination and biodiesel production.
c- *In vitro*.
d- Used in fish farming.

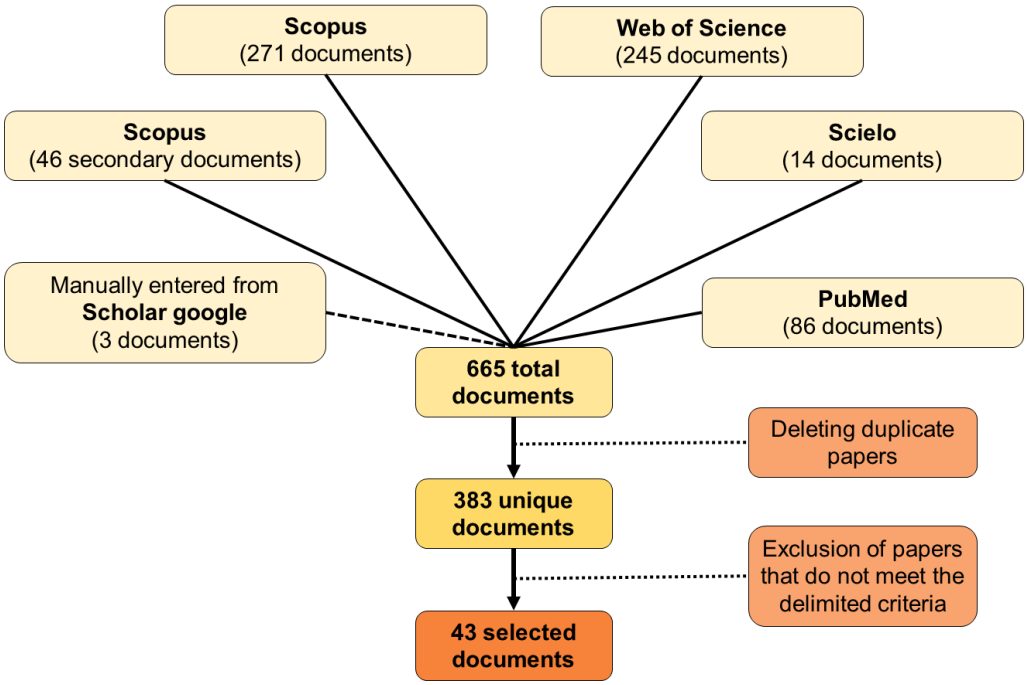


Fig. Supple. 1- Scheme of the search, sorting and selection of papers used in the analyses.

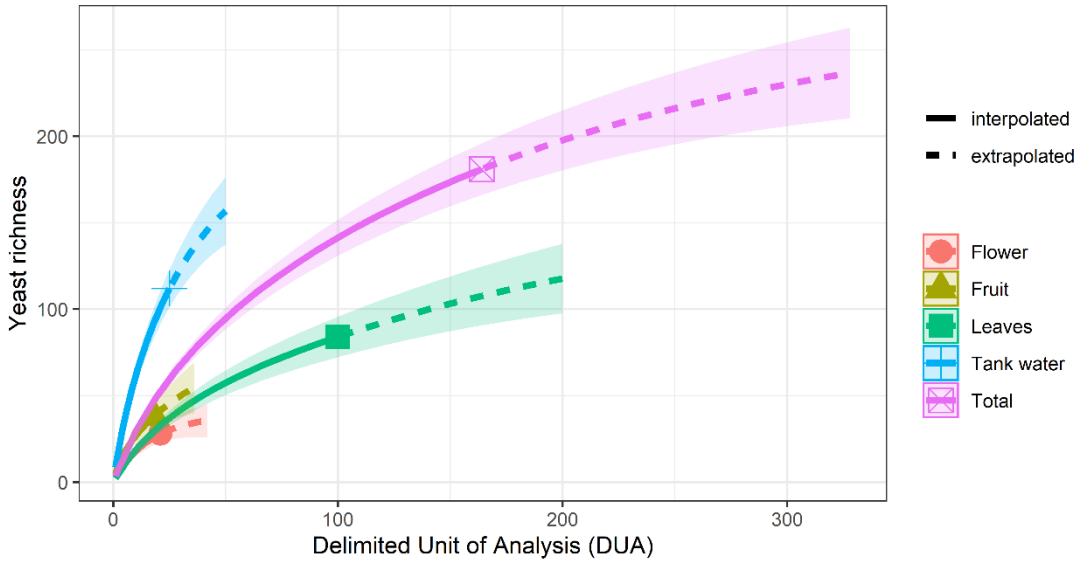


Fig. Supple. 2- Extrapolation-rarefaction curve considering data from all bromeliad phylosphere compartments. The curve was elaborated with 1000 pseudoreplica bootstrap and the extrapolation was performed with the Chao1 estimator.

Material suplementar (Capítulo 2, secção 5)

Supplementary Table 1- Species recorded in the study, as well as their frequency of occurrence and average abundance considering the samples (individuals of bromeliads) as the analytical unit.

Phyla	Subphyla	Species	Dry		Rainy	
			Frequency (%)	Average abundance (CFU/g)	Frequency (%)	Average abundance (CFU/g)
Ascomycota						
	Pezizomycotina					
		Aureobasidium melanogenum	3.3	125.0	3.4	12.5
		Aureobasidium thailandense	4.0	251.6	55.2	87.1
		Aureobasidium tremulum	3.3	12.5	3.4	125.0
		Chaetosphaeria sp.			3.4	250.0
		Exophiala bergeri	3.3	125.0		
		Exophiala spinifera	1.0	191.7	13.8	106.3
		Hortaea werneckii	3.3	2625.0		
		Parapyrenis conica			3.4	125.0
		Pseudosydowia sp.			3.4	75.0
		Selenophoma sp.	6.7	125.0		
		Tricellula aurantiaca			3.4	125.0
	Saccharomycotina					
		Candida blankii	6.7	100.0	24.1	547.3
		Candida diddensiae			3.4	250.0
		Candida orthopsilosis	3.3	125.0		
		Candida parapsilosis			6.9	125.0
		Candida sp. 1	3.3	125.0		
		Candida sp. 2	3.3	25.0	6.9	43.8
		Candida sp. 3			6.9	971.9
		Candida sp. 4			3.4	140.6
		Hanseniaspora opuntiae			3.4	125.0
		Meyerozyma guilliermondii	16.7	96.3	2.7	233.3

		Starmerella ilheusensis			3.4	68.8
		Yueomyces sp.	3.3	12.5		
	Taphrinomycotina					
		Taphrina sp.	3.3	125.0		
Basidiomycota						
	Agaricomycotina					
		Carcinomyces nordestinensis	3.3	125.0	1.3	375.0
		Carlosrosaea sp. 1	3.3	125.0	3.4	125.0
		Carlosrosaea sp. 2	6.7	187.5	17.2	425.0
		Carlosrosaea sp. 3			3.4	125.0
		Fellomyces penicillatus			3.4	125.0
		Hannaella phetchabunensis			6.9	356.3
		Hannaella siamensis	3.3	125.0	6.9	65.6
		Hannaella sinensis	3.3	62.5		
		Hannaella taiwanensis			13.8	225.0
		Hannaella zeae	3.3	375.0		
		Kwoniella dejecticola	3.3	87.5		
		Kwoniella dendrophila			3.4	12.5
		Kwoniella heveanensis	16.7	145.0		
		Kwoniella mangrovensis	3.3	125.0		
		Papiliotrema flavescens	6.7	21.9	6.9	75.0
		Papiliotrema laurentii	36.7	152.8	13.8	182.8
		Papiliotrema miconiae			6.9	81.3
		Papiliotrema rajasthanensis	3.3	125.0	3.4	25.0
		Papiliotrema sp. 1	3.3	250.0		
		Papiliotrema sp. 2			3.4	87.5
		Rhynchogastrema noutii	3.3	125.0		
		Saitozyma flava			6.9	43.8
		Saitozyma ninhbinhensis	1.0	412.5		
		Saitozyma podzolica	1.0	115.6	3.4	406.3
		Tremella ananatis	33.3	360.0	17.2	203.8
		Tremella sp.			3.4	50.0
		Vishniacozyma alagoana	16.7	197.5	6.9	125.0

Pucciniomycotina					
	Boekhoutia sp.			3.4	125.0
	Colacogloea sp.	3.3	125.0		
	Cyrenella elegans			3.4	125.0
	Cystobasidium keelungensis	3.3	125.0		
	Cystobasidium sp.	3.3	125.0		
	Erythrobasidium sp.	3.3	125.0		
	Halobasidium xiangyangense			3.4	375.0
	Hasegawazyma sp.	6.7	125.0	3.4	37.5
	Occultifur brasiliensis	36.7	434.1	31.3	229.2
	Occultifur externus	3.3	125.0		
	Occultifur plantarum	3.3	12.5	3.4	12.5
	Rhodosporidiobolus ruineniae	1.0	87.5		
	Rhodotorula mucilaginosa	6.7	131.3		
	Rhodotorula paludigena	3.3	62.5		
	Rosettozyma sp.	6.7	125.0		
	Sakaguchia oryzae			3.4	131.3
	Symmetrospora marina			6.9	56.3
	Symmetrospora suhii	3.3	125.0	13.8	209.4
Ustilaginomycotina					
	Anthracocystis anthracoideispora			3.4	125.0
	Kordyana sp.			3.4	125.0
	Microstroma sp. 1			6.9	125.0
	Microstroma sp. 2	6.7	68.8		
	Moesziomyces antarcticus	3.3	12.5		
	Moesziomyces aphidis	3.3	12.5		
	Pseudozyma hubeiensis			3.4	375.0
	Pseudozyma pruni	3.3	125.0		
	Pseudozyma tsukubaensis	3.3	125.0		
	Ustilago maydis			3.4	12.5

Supplementary Table 2- Results of the similarity percentage analysis (SIMPER) between seasonal periods, the analysis was performed with 999 permutations and considering the taxonomic data (species abundance) and the twelve collections as sample units.

Species	Contribution to average dissimilarity	Standard deviation (sd)	Average to sd ratio	Average abundance Dry	Average abundance Rainy	Cumulative contribution	p-value
Occultifur externus	0.08443	0.08171	1.0333	159.17	68.75	0.099	0.795
Candida blankii	0.0831	0.148	0.5614	6.67	127.71	0.196	0.128
Aureobasidium thailandense	0.06777	0.08241	0.8224	100.62	46.46	0.276	0.238
Tremella sp.	0.05485	0.04805	1.1417	120	33.96	0.34	0.416
Candida sp. 3	0.05319	0.12471	0.4265	0	64.79	0.402	0.288
Carlosrosaea sp. 3	0.04791	0.05608	0.8544	12.5	70.83	0.458	0.334
Saitozyma podzolica	0.03096	0.05525	0.5603	41.25	0	0.495	0.334
Kordyana sp.	0.02969	0.06765	0.4389	87.5	0	0.53	0.892
Microstroma sp. 1	0.02893	0.04056	0.7133	16.04	46.67	0.564	0.791
Papiliotrema miconiae	0.02528	0.0184	1.3737	56.04	24.38	0.593	0.499
Yueomyces sp.	0.02312	0.0235	0.9839	32.92	8.33	0.62	0.202
Carlosrosaea sp. 1	0.02127	0.03606	0.5899	4.17	37.5	0.645	0.782
Hannaella zeae	0.021	0.02641	0.7952	0	30	0.67	0.068
Kwoniella mangrovensis	0.0193	0.03404	0.5671	24.17	0	0.692	0.209
Fellomyces penicillatus	0.01751	0.0223	0.7854	19.17	14.17	0.713	0.63
Hannaella siamensis	0.01724	0.04104	0.4202	0	23.75	0.733	0.396
Taphrina sp.	0.01634	0.02165	0.7549	4.17	27.92	0.752	0.338
Hannaella phetchabunensis	0.01126	0.02725	0.4132	0	12.5	0.765	0.262
Sakaguchia oryzae	0.0111	0.01851	0.5995	7.4	13.54	0.778	0.6
Hanseniaspora opuntiae	0.00921	0.0213	0.4324	12.5	0	0.789	0.577
Candida diddensiae	0.00751	0.01816	0.4132	0	8.33	0.798	0.262
Colacogloea sp.	0.00751	0.01816	0.4132	0	8.33	0.807	0.262
Rhodotorula mucilaginosa	0.0066	0.01461	0.4518	8.75	0	0.815	0.527
Saitozyma flava	0.00653	0.00963	0.6781	8.33	0	0.822	0.235

Papiliotrema sp. 2	0.00635	0.01471	0.4319	8.33	0	0.83	0.561
Pseudozyma pruni	0.00616	0.01436	0.4288	0	12.5	0.837	0.56
Carlosrosaea sp. 2	0.00582	0.01026	0.5673	4.17	4.17	0.844	0.699
Microstroma sp. 2	0.00563	0.00856	0.6573	0	8.33	0.85	0.217
Hannaella sinensis	0.00535	0.00851	0.6292	4.17	4.38	0.857	0.77
Candida parapsilosis	0.0052	0.01227	0.424	0	8.33	0.863	0.466
Hortaea werneckii	0.00503	0.00754	0.668	8.33	1.25	0.869	0.423
Papiliotrema laurentii	0.00398	0.00518	0.7695	1.46	5	0.878	0.273
Moesziomyces antarcticus	0.00369	0.00856	0.431	4.58	0	0.883	0.586
Papiliotrema rajasthanensis	0.00366	0.00868	0.4221	0	5.42	0.887	0.402
Rhodotorula paludigena	0.00347	0.00577	0.6002	8.75	0	0.891	0.375
Candida sp. 4	0.0034	0.0081	0.4202	0	4.69	0.895	0.396
Candida sp. 1	0.00335	0.00778	0.431	4.17	0	0.899	0.586
Cystobasidium sp.	0.00335	0.00778	0.431	4.17	0	0.903	0.586
Occultifur plantarum	0.00335	0.00778	0.431	4.17	0	0.907	0.586
Rhodosporidiobolus ruineniae	0.00335	0.00778	0.431	4.17	0	0.911	0.586
Tremella ananatis	0.00335	0.00778	0.431	4.17	0	0.915	0.586
Rosettozyma sp.	0.00318	0.00736	0.4319	4.17	0	0.918	0.561
Candida orthopsilosis	0.00307	0.0071	0.4324	4.17	0	0.922	0.577
Boekhoutia sp.	0.00303	0.0072	0.4202	0	4.17	0.925	0.396
Chaetosphaeria sp.	0.00303	0.0072	0.4202	0	4.17	0.929	0.396
Kwoniella dejecticola	0.00303	0.0072	0.4202	0	4.17	0.932	0.396
Ustilago maydis	0.00303	0.0072	0.4202	0	4.17	0.936	0.396
Candida sp. 2	0.00299	0.00602	0.4976	0.83	2.92	0.94	0.451
Papiliotrema sp. 1	0.00298	0.00544	0.5485	4.17	0.83	0.943	0.764
Starmerella ilheusensis	0.00283	0.00644	0.4389	8.33	0	0.946	0.887
Selenophoma sp.	0.00273	0.00644	0.424	0	4.38	0.95	0.466
Pseudosydowia sp.	0.0026	0.00614	0.424	0	4.17	0.953	0.466
Cyrenella elegans	0.00248	0.00569	0.4351	4.17	0	0.956	0.652
Erythrobasidium sp.	0.00248	0.00569	0.4351	4.17	0	0.959	0.652
Exophiala bergeri	0.00248	0.00569	0.4351	4.17	0	0.961	0.652
Parapyrenis conica	0.00243	0.00584	0.416	0	2.92	0.964	0.306
Symmetrospora suhii	0.00243	0.00483	0.503	0	3.75	0.967	0.268

Aureobasidium tremulum	0.00239	0.00459	0.5196	0.42	4.17	0.97	0.65
Anthracocystis anthracoideispora	0.00205	0.00479	0.4288	0	4.17	0.972	0.56
Cystobasidium keelungensis	0.00205	0.00479	0.4288	0	4.17	0.975	0.56
Halobasidium xiangyangense	0.00205	0.00479	0.4288	0	4.17	0.977	0.56
Hasegawazyma sp.	0.00205	0.00479	0.4288	0	4.17	0.98	0.56
Saitozyma ninhbinhensis	0.00182	0.0043	0.424	0	2.92	0.982	0.466
Kwoniella dendrophila	0.00173	0.00399	0.4351	2.92	0	0.984	0.652
Aureobasidium melanogenum	0.00163	0.00313	0.521	4.17	0.42	0.986	0.903
Pseudozyma hubeiensis	0.00156	0.00368	0.424	0	2.5	0.987	0.466
Symmetrospora marina	0.00143	0.00338	0.424	0	2.29	0.989	0.466
Exophiala spinifera	0.00141	0.00322	0.4389	4.17	0	0.991	0.887
Meyerozyma guilliermondii	0.00141	0.00322	0.4389	4.17	0	0.992	0.887
Pseudozyma tsukubaensis	0.00141	0.00322	0.4389	4.17	0	0.994	0.887
Tricellula aurantiaca	0.00139	0.00334	0.416	0	1.67	0.996	0.306
Hannaella taiwanensis	0.00124	0.00285	0.4351	2.08	0	0.997	0.652
Rhynchogastrema noutii	0.00071	0.00161	0.4389	2.08	0	0.998	0.887
Papiliotrema flavescens	0.00054	0.00094	0.5766	0.42	0.42	0.999	0.584
Kwoniella heveanensis	0.00035	0.00083	0.416	0	0.42	0.999	0.306
Occultifur brasiliensis	0.00034	0.00078	0.431	0.42	0	0.999	0.586
Vishniacozyma alagoana	0.00026	0.00061	0.424	0	0.42	1	0.466
Moesziomyces aphidis	0.00014	0.00032	0.4389	0.42	0	1	0.887
Yueomyces sp.	0.00014	0.00032	0.4389	0.42	0	1	0.887

Supplementary Table 3- Results of the similarity percentage analysis (SIMPER) between seasonal periods, the analysis was performed with 999 permutations and considering the phylogenetic data (taxa abundance) and the twelve collections as sample units.

Taxon	Contribution to average dissimilarity	Standard deviation (sd)	Average to sd ratio	Average abundance Dry	Average abundance Rainy	Cumulative contribution	p-value
Basidiomycota	3.85E-02	2.29E-02	1.68E+00	5.85E+02	4.35E+02	0.064	0.673
Ascomycota	3.27E-02	2.39E-02	1.37E+00	2.61E+02	3.54E+02	0.118	0.191
Saccharomycetales	2.72E-02	2.49E-02	1.09E+00	3.23E+01	2.70E+02	0.163	0.013
Saccharomycetes	2.72E-02	2.49E-02	1.09E+00	3.23E+01	2.70E+02	0.208	0.013
Saccharomycotina	2.72E-02	2.49E-02	1.09E+00	3.23E+01	2.70E+02	0.254	0.013
Candida	2.46E-02	2.67E-02	9.20E-01	1.58E+01	2.17E+02	0.294	0.017
Tremellales	1.99E-02	1.45E-02	1.37E+00	3.51E+02	2.78E+02	0.368	0.758
Tremellomycetes	1.99E-02	1.45E-02	1.37E+00	3.51E+02	2.78E+02	0.401	0.758
Agaricomycotina	1.99E-02	1.45E-02	1.37E+00	3.51E+02	2.78E+02	0.434	0.758
Pucciniomycotina	1.94E-02	1.46E-02	1.33E+00	2.21E+02	1.27E+02	0.466	0.866
Pezizomycotina	1.90E-02	1.63E-02	1.17E+00	2.24E+02	8.44E+01	0.498	0.356
Cystobasidiomycetes	1.77E-02	1.42E-02	1.25E+00	1.89E+02	1.23E+02	0.527	0.947
Dothideomycetes	1.61E-02	1.46E-02	1.10E+00	2.01E+02	5.35E+01	0.554	0.299
Cystobasidiaceae	1.50E-02	1.29E-02	1.17E+00	1.72E+02	8.17E+01	0.579	0.904
Cystobasidiales	1.50E-02	1.29E-02	1.17E+00	1.72E+02	8.17E+01	0.603	0.904
Occultifur	1.46E-02	1.37E-02	1.07E+00	1.64E+02	6.92E+01	0.628	0.813
Trimorphomycetaceae	1.26E-02	1.16E-02	1.09E+00	6.95E+01	9.56E+01	0.648	0.802
Dothideales	1.12E-02	1.36E-02	8.18E-01	1.14E+02	5.35E+01	0.667	0.356
Aureobasidium	1.11E-02	1.37E-02	8.10E-01	1.05E+02	5.10E+01	0.685	0.325
Dothioraceae	1.11E-02	1.37E-02	8.09E-01	1.14E+02	5.10E+01	0.704	0.343
Carlosrosaea	9.26E-03	9.92E-03	9.34E-01	1.67E+01	7.92E+01	0.719	0.381
Tremella	8.91E-03	8.02E-03	1.11E+00	1.20E+02	3.56E+01	0.734	0.468
Tremellaceae	8.91E-03	8.02E-03	1.11E+00	1.20E+02	3.56E+01	0.749	0.468

Hannaella	7.28E-03	1.08E-02	6.76E-01	1.88E+01	5.81E+01	0.776	0.58
Saitozyma	7.01E-03	9.18E-03	7.64E-01	5.28E+01	1.65E+01	0.787	0.396
Papiliotrema	6.13E-03	4.30E-03	1.43E+00	7.00E+01	3.85E+01	0.797	0.647
Hortaea	4.95E-03	1.13E-02	4.39E-01	8.75E+01	0.00E+00	0.806	0.88
Teratosphaeriaceae	4.95E-03	1.13E-02	4.39E-01	8.75E+01	0.00E+00	0.814	0.88
Capnodiales	4.95E-03	1.13E-02	4.39E-01	8.75E+01	0.00E+00	0.822	0.88
Meyerozyma	4.82E-03	6.76E-03	7.13E-01	1.60E+01	4.67E+01	0.83	0.775
Debaryomycetaceae	4.82E-03	6.76E-03	7.13E-01	1.60E+01	4.67E+01	0.838	0.775
Vishniacozyma	3.85E-03	3.92E-03	9.84E-01	3.29E+01	8.30E+00	0.845	0.22
Bulleribasidiaceae	3.85E-03	3.92E-03	9.84E-01	3.29E+01	8.30E+00	0.851	0.22
Kwoniella	3.73E-03	5.38E-03	6.93E-01	3.12E+01	4.00E-01	0.857	0.054
Carcinomyces	3.55E-03	6.01E-03	5.90E-01	4.20E+00	3.75E+01	0.863	0.77
Carcinomycetaceae	3.55E-03	6.01E-03	5.90E-01	4.20E+00	3.75E+01	0.869	0.77
Microbotryomycetes	3.30E-03	3.20E-03	1.03E+00	3.21E+01	0.00E+00	0.874	0.056
Ustilaginomycotina	3.26E-03	3.47E-03	9.38E-01	1.38E+01	2.96E+01	0.88	0.671
Symmetrospora	3.11E-03	4.07E-03	7.65E-01	4.20E+00	3.17E+01	0.885	0.289
Symmetrosporaceae	3.11E-03	4.07E-03	7.65E-01	4.20E+00	3.17E+01	0.89	0.289
Exophiala	2.96E-03	3.68E-03	8.03E-01	2.33E+01	1.42E+01	0.9	0.768
Herpotrichiellaceae	2.96E-03	3.68E-03	8.03E-01	2.33E+01	1.42E+01	0.905	0.768
Chaetothyriales	2.96E-03	3.68E-03	8.03E-01	2.33E+01	1.42E+01	0.91	0.768
Eurotiomycetes	2.96E-03	3.68E-03	8.03E-01	2.33E+01	1.42E+01	0.915	0.768
Ustilaginaceae	2.05E-03	2.98E-03	6.87E-01	9.20E+00	1.71E+01	0.918	0.848
Ustilaginales	2.05E-03	2.98E-03	6.87E-01	9.20E+00	1.71E+01	0.922	0.848
Ustilaginomycetes	2.05E-03	2.98E-03	6.87E-01	9.20E+00	1.71E+01	0.925	0.848
Halobasidium	1.88E-03	4.54E-03	4.13E-01	0.00E+00	1.25E+01	0.928	0.258
Sporidiobolaceae	1.80E-03	2.42E-03	7.43E-01	1.96E+01	0.00E+00	0.931	0.272
Sporidiobolales	1.80E-03	2.42E-03	7.43E-01	1.96E+01	0.00E+00	0.934	0.272
Sordariomycetes	1.68E-03	3.02E-03	5.59E-01	0.00E+00	1.25E+01	0.937	0.126
Exobasidiomycetes	1.68E-03	2.35E-03	7.16E-01	4.60E+00	1.25E+01	0.94	0.697
Pseudozyma	1.63E-03	2.30E-03	7.07E-01	8.30E+00	1.25E+01	0.942	0.824

Chaetosphaeria	1.25E-03	3.03E-03	4.13E-01	0.00E+00	8.30E+00	0.944	0.258
Chaetosphaeriaceae	1.25E-03	3.03E-03	4.13E-01	0.00E+00	8.30E+00	0.947	0.258
Chaetosphaeriales	1.25E-03	3.03E-03	4.13E-01	0.00E+00	8.30E+00	0.949	0.258
Erythrobasidiales	1.22E-03	1.34E-03	9.14E-01	1.25E+01	1.20E+00	0.951	0.215
Microstroma	1.20E-03	1.61E-03	7.43E-01	4.60E+00	8.30E+00	0.953	0.696
Microstromataceae	1.20E-03	1.61E-03	7.43E-01	4.60E+00	8.30E+00	0.955	0.696
Microstromatales	1.20E-03	1.61E-03	7.43E-01	4.60E+00	8.30E+00	0.957	0.696
Rhodosporidiobolus	1.10E-03	2.44E-03	4.52E-01	8.80E+00	0.00E+00	0.958	0.561
Rosettozyma	1.09E-03	1.61E-03	6.78E-01	8.30E+00	0.00E+00	0.96	0.249
Rosettozymaceae	1.09E-03	1.61E-03	6.78E-01	8.30E+00	0.00E+00	0.962	0.249
Rosettozymales	1.09E-03	1.61E-03	6.78E-01	8.30E+00	0.00E+00	0.964	0.249
Cystobasidium	9.70E-04	1.45E-03	6.69E-01	8.30E+00	0.00E+00	0.965	0.322
Hasegawazyma	8.40E-04	1.26E-03	6.68E-01	8.30E+00	1.20E+00	0.967	0.427
Rhodotorula	7.00E-04	1.22E-03	5.72E-01	1.08E+01	0.00E+00	0.969	0.41
Saccharomycetaceae	5.90E-04	8.73E-04	6.75E-01	4.00E-01	6.50E+00	0.97	0.249
Taphrina	5.60E-04	1.30E-03	4.31E-01	4.20E+00	0.00E+00	0.971	0.6
Taphrinaceae	5.60E-04	1.30E-03	4.31E-01	4.20E+00	0.00E+00	0.972	0.6
Taphrinales	5.60E-04	1.30E-03	4.31E-01	4.20E+00	0.00E+00	0.973	0.6
Taphrinomycetes	5.60E-04	1.30E-03	4.31E-01	4.20E+00	0.00E+00	0.974	0.6
Taphrinomycotina	5.60E-04	1.30E-03	4.31E-01	4.20E+00	0.00E+00	0.975	0.6
Rhynchogastrema	5.30E-04	1.23E-03	4.32E-01	4.20E+00	0.00E+00	0.976	0.593
Rhynchogastremaceae	5.30E-04	1.23E-03	4.32E-01	4.20E+00	0.00E+00	0.977	0.593
Boekhoutia	5.00E-04	1.20E-03	4.20E-01	0.00E+00	4.20E+00	0.978	0.382
Kordyana	5.00E-04	1.20E-03	4.20E-01	0.00E+00	4.20E+00	0.978	0.382
Tricellula	5.00E-04	1.20E-03	4.20E-01	0.00E+00	4.20E+00	0.979	0.382
Brachybasidiaceae	5.00E-04	1.20E-03	4.20E-01	0.00E+00	4.20E+00	0.98	0.382
Calloriaceae	5.00E-04	1.20E-03	4.20E-01	0.00E+00	4.20E+00	0.981	0.382
Chionosphaeraceae	5.00E-04	1.20E-03	4.20E-01	0.00E+00	4.20E+00	0.982	0.382
Agaricostilbales	5.00E-04	1.20E-03	4.20E-01	0.00E+00	4.20E+00	0.983	0.382
Exobasidiales	5.00E-04	1.20E-03	4.20E-01	0.00E+00	4.20E+00	0.983	0.382

Helotiales	5.00E-04	1.20E-03	4.20E-01	0.00E+00	4.20E+00	0.984	0.382
Agaricostilbomycetes	5.00E-04	1.20E-03	4.20E-01	0.00E+00	4.20E+00	0.985	0.382
Leotiomycetes	5.00E-04	1.20E-03	4.20E-01	0.00E+00	4.20E+00	0.986	0.382
Selenophoma	4.70E-04	1.07E-03	4.39E-01	8.30E+00	0.00E+00	0.987	0.88
Sakaguchia	4.60E-04	1.07E-03	4.24E-01	0.00E+00	4.40E+00	0.987	0.437
Sakaguchiaceae	4.60E-04	1.07E-03	4.24E-01	0.00E+00	4.40E+00	0.988	0.437
Sakaguchiales	4.60E-04	1.07E-03	4.24E-01	0.00E+00	4.40E+00	0.989	0.437
Parapyrenis	4.30E-04	1.02E-03	4.24E-01	0.00E+00	4.20E+00	0.99	0.437
Requienellaceae	4.30E-04	1.02E-03	4.24E-01	0.00E+00	4.20E+00	0.99	0.437
Xylariales	4.30E-04	1.02E-03	4.24E-01	0.00E+00	4.20E+00	0.991	0.437
Colacogloea	4.10E-04	9.49E-04	4.35E-01	4.20E+00	0.00E+00	0.992	0.672
Erythrobasidium	4.10E-04	9.49E-04	4.35E-01	4.20E+00	0.00E+00	0.993	0.672
Erythrobasidiaceae	4.10E-04	9.49E-04	4.35E-01	4.20E+00	0.00E+00	0.993	0.672
Heterogastridiaceae	4.10E-04	9.49E-04	4.35E-01	4.20E+00	0.00E+00	0.994	0.672
Heterogastridiales	4.10E-04	9.49E-04	4.35E-01	4.20E+00	0.00E+00	0.995	0.672
Anthracocystis	3.40E-04	7.98E-04	4.29E-01	0.00E+00	4.20E+00	0.995	0.538
Cyrenella	3.40E-04	7.98E-04	4.29E-01	0.00E+00	4.20E+00	0.996	0.538
Fellomyces	3.40E-04	7.98E-04	4.29E-01	0.00E+00	4.20E+00	0.996	0.538
Hanseniaspora	3.40E-04	7.98E-04	4.29E-01	0.00E+00	4.20E+00	0.997	0.538
Cuniculitremaceae	3.40E-04	7.98E-04	4.29E-01	0.00E+00	4.20E+00	0.997	0.538
Pseudosydowia	2.60E-04	6.14E-04	4.24E-01	0.00E+00	2.50E+00	0.999	0.437
Saccotheciaceae	2.60E-04	6.14E-04	4.24E-01	0.00E+00	2.50E+00	0.999	0.437
Starmerella	2.40E-04	5.63E-04	4.24E-01	0.00E+00	2.30E+00	1	0.437
Moesziomyces	8.00E-05	1.30E-04	6.10E-01	8.00E-01	0.00E+00	1	0.403
Ustilago	4.00E-05	1.02E-04	4.24E-01	0.00E+00	4.00E-01	1	0.436
Yueomyces	2.00E-05	5.40E-05	4.39E-01	4.00E-01	0.00E+00	1	0.877

Supplementary Table 4- Results of the similarity percentage analysis (SIMPER) between seasonal periods, the analysis was performed with 999 permutations and considering the functional data (traits frequency) and the twelve collections as sample units.

Traits	Contribution to average dissimilarity	Standard deviation (sd)	Average to sd ratio	Average bundance Dry	Average bundance Rainy	Ordered cumulative contribution	p-value
Ramnose	0.018078	0.014416	1.254	0.605	0.8217	0.086	0.169
Galactose	0.016903	0.0138	1.2248	0.6817	0.9133	0.167	0.038
Xylose	0.016656	0.015116	1.1019	0.7	0.8983	0.247	0.173
Cellobiose	0.016389	0.011915	1.3755	0.6533	0.8167	0.325	0.272
Glucose	0.016285	0.012515	1.3012	0.695	0.9283	0.403	0.058
Inulina	0.015743	0.013409	1.174	0.725	0.9083	0.478	0.162
Glycerol	0.015555	0.011719	1.3273	0.6883	0.8833	0.553	0.104
D_arabinose	0.014497	0.012264	1.182	0.72	0.845	0.622	0.291
Raffinose	0.014035	0.012752	1.1006	0.7417	0.915	0.689	0.154
Esterase	0.011318	0.007612	1.4868	0.25	0.3583	0.743	0.351
Celullase	0.010855	0.005879	1.8464	0.0517	0.21	0.795	0.024
Lipase	0.009775	0.00649	1.5061	0.2333	0.2317	0.842	0.997
Fermentation	0.008649	0.006469	1.3369	0.2317	0.29	0.883	0.558
Caseinase	0.007192	0.00496	1.4499	0.2533	0.2233	0.918	0.255
IE_{24}	0.006061	0.004289	1.4131	0.2983	0.235	0.947	0.312
Amylase	0.00603	0.004363	1.3821	0.07	0.1267	0.975	0.413
Pectinase	0.005139	0.007482	0.6868	0.095	0	1	0.408

Supplementary Table 5- Synthesis of information on environmental variables and diversity metrics in the twelve collections carried out in the study.

Collection ID	Collection date	Period	Storm volume (mm)	Rain volume (mm)	Rain memory (days)	Drought period (days)	Rainy days in the moth	Rain volume in the moth (mm)	Average yeast abundance (CFU/g)	q0	q1	q2	AvTD	FDis	FRic	FEve
D01	October 7, 2020	Dry	12.2	2.1	1	27	2	5.6	510	3.8	3.21	3.05	76.00	3.18	1.52	0.44
D02	November 10, 2020	Dry	62.7	13	0	4	3	62.7	623.75	4	3.13	2.73	53.95	2.62	4.72	0.60
D03	December 2, 2020	Dry	62.7	6.8	4	21	4	69.5	2412.5	5.4	3.08	2.51	84.89	1.44	9.49	0.55
D04	October 19, 2021	Dry	9.5	13.4	25	23	1	13.4	396.25	3	2.38	2.13	59.57	1.55	1.03	0.48
D05	November 23, 2021	Dry	9.5	13.4	25	58	0	0	1433.75	4.6	3.46	2.98	84.69	2.35	2.16	0.66
D06	December 7, 2021	Dry	9.5	45	63	8	1	45	678.125	2.8	2.02	1.72	76.55	1.45	0.39	0.38
R01	May 18, 2021	Rainy	79.7	0.6	0	1	16	136.2	1318.75	6	4.83	4.21	82.78	2.45	6.01	0.72
R02	June 15, 2021	Rainy	46.2	12.3	1	8	12	72.8	545	3.6	2.47	2.12	66.94	1.46	2.63	0.30
R03	July 20, 2021	Rainy	11	2.3	1	1	15	124.4	800	1.8	1.88	1.62	40.19	1.17	0.46	0.17
R04	May 24, 2022	Rainy	36.2	30.9	9	2	7	99.9	1116.875	5.4	3.95	3.28	87.89	1.88	1.62	0.58
R05	June 23, 2022	Rainy	18.7	7.8	0	1	23	304.6	996.875	4	3.25	2.88	85.61	2.37	1.30	0.65
R06	July 19, 2022	Rainy	18.2	4.3	1	2	22	228.4	611.25	2.6	2.28	2.13	67.36	1.77	0.70	0.32

Supplementary Table 6- List of isolates observed in the study.

ID	Species	Class	Subphyla	Phyla	Collection date	Seasonal period	Abundance (CFU/cm^2)	GenBank number	Collections number
BRT461	*Anthracocystis anthracoideispora*	Ustilaginomycetes	Ustilaginomycotina	Basidiomycota	18/05/2021	Chuvoso	125		
BRT632	*Aureobasidium melanogenum*	Dothideomycetes	Pezizomycotina	Ascomycota	24/05/2022	Chuvoso	12.5		
BRT403	Aureobasidium melanogenum	Dothideomycetes	Pezizomycotina	Ascomycota	02/12/2020	Seco	125		
BRT616	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	07/12/2021	Seco	12.5		
BRT624	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	24/05/2022	Chuvoso	12.5		
BRT700	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	19/07/2022	Chuvoso	12.5		
BRT542	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	23/11/2021	Seco	50		
BRT609	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	07/12/2021	Seco	62.5		
BRT524	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	19/10/2021	Seco	75		
BRT538	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	23/11/2021	Seco	137.5		
BRT633	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	24/05/2022	Chuvoso	212.5		
BRT618	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	07/12/2021	Seco	12.5		
BRT392	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	10/11/2020	Seco	12.5		
BRT440	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	18/05/2021	Chuvoso	12.5		
BRT472	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	15/06/2021	Chuvoso	12.5		
BRT474	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	15/06/2021	Chuvoso	12.5		
BRT482	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	15/06/2021	Chuvoso	12.5		
BRT489	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	15/06/2021	Chuvoso	12.5		
BRT491	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	15/06/2021	Chuvoso	12.5		
BRT494	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	15/06/2021	Chuvoso	12.5		
BRT516	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	19/10/2021	Seco	12.5		
BRT525	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	19/10/2021	Seco	12.5		
BRT594	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	07/12/2021	Seco	12.5		
BRT600	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	07/12/2021	Seco	12.5		
BRT676	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	23/06/2022	Chuvoso	12.5		
BRT623	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	24/05/2022	Chuvoso	18.75		
BRT454	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	18/05/2021	Chuvoso	25		
BRT518	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	19/10/2021	Seco	25		
BRT610	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	07/12/2021	Seco	25		
BRT475	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	15/06/2021	Chuvoso	31.25		
BRT541	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	23/11/2021	Seco	37.5		
BRT557	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	23/11/2021	Seco	37.5		
BRT590	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	07/12/2021	Seco	37.5		
BRT445	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	18/05/2021	Chuvoso	50		

BRT503	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	20/07/2021	Chuvoso	62.5
BRT552	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	23/11/2021	Seco	62.5
BRT602	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	07/12/2021	Seco	68.75
BRT545	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	23/11/2021	Seco	75
BRT480	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	15/06/2021	Chuvoso	87.5
BRT462	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	18/05/2021	Chuvoso	125
BRT510	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	20/07/2021	Chuvoso	125
BRT514	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	20/07/2021	Chuvoso	125
BRT547	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	23/11/2021	Seco	125
BRT548	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	23/11/2021	Seco	125
BRT550	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	23/11/2021	Seco	125
BRT560	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	23/11/2021	Seco	125
BRT568	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	23/11/2021	Seco	125
BRT595	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	07/12/2021	Seco	125
BRT707	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	19/07/2022	Chuvoso	125
BRT705	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	19/07/2022	Chuvoso	131.25
BRT669	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	23/06/2022	Chuvoso	150
BRT562	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	23/11/2021	Seco	250
BRT569	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	23/11/2021	Seco	250
BRT576	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	23/11/2021	Seco	250
BRT597	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	07/12/2021	Seco	250
BRT579	*Aureobasidium thailandense*	Dothideomycetes	Pezizomycotina	Ascomycota	23/11/2021	Seco	500
BRT520	*Aureobasidium tremulum*	Dothideomycetes	Pezizomycotina	Ascomycota	19/10/2021	Seco	12.5
BRT456	*Aureobasidium tremulum*	Dothideomycetes	Pezizomycotina	Ascomycota	18/05/2021	Chuvoso	125
BRT698	*Boekhoutia* sp.	Agaricostilbomycetes	Pucciniomycotina	Basidiomycota	23/06/2022	Chuvoso	125
BRT399	*Candida blankii*	Saccharomycetes	Saccharomycotina	Ascomycota	02/12/2020	Seco	75
BRT505	*Candida blankii*	Saccharomycetes	Saccharomycotina	Ascomycota	20/07/2021	Chuvoso	12.5
BRT506	*Candida blankii*	Saccharomycetes	Saccharomycotina	Ascomycota	20/07/2021	Chuvoso	12.5
BRT507	*Candida blankii*	Saccharomycetes	Saccharomycotina	Ascomycota	20/07/2021	Chuvoso	12.5
BRT493	*Candida blankii*	Saccharomycetes	Saccharomycotina	Ascomycota	15/06/2021	Chuvoso	62.5
BRT673	*Candida blankii*	Saccharomycetes	Saccharomycotina	Ascomycota	23/06/2022	Chuvoso	81.25
BRT467	*Candida blankii*	Saccharomycetes	Saccharomycotina	Ascomycota	18/05/2021	Chuvoso	125
BRT513	*Candida blankii*	Saccharomycetes	Saccharomycotina	Ascomycota	20/07/2021	Chuvoso	125
BRT589	*Candida blankii*	Saccharomycetes	Saccharomycotina	Ascomycota	07/12/2021	Seco	125
BRT701	*Candida blankii*	Saccharomycetes	Saccharomycotina	Ascomycota	19/07/2022	Chuvoso	125
BRT499	*Candida blankii*	Saccharomycetes	Saccharomycotina	Ascomycota	20/07/2021	Chuvoso	2750
BRT706	*Candida diddensiae*	Saccharomycetes	Saccharomycotina	Ascomycota	19/07/2022	Chuvoso	250
BRT596	*Candida orthopsilosis*	Saccharomycetes	Saccharomycotina	Ascomycota	07/12/2021	Seco	125

BRT664	*Candida parapsilosis*	Saccharomycetes	Saccharomycotina	Ascomycota	24/05/2022	Chuvoso	125
BRT662	*Candida parapsilosis*	Saccharomycetes	Saccharomycotina	Ascomycota	24/05/2022	Chuvoso	125
BRT360	*Candida* sp. 1	Saccharomycetes	Saccharomycotina	Ascomycota	07/10/2020	Seco	125
BRT708	*Candida* sp. 2	Saccharomycetes	Saccharomycotina	Ascomycota	19/07/2022	Chuvoso	12.5
BRT374	*Candida* sp. 2	Saccharomycetes	Saccharomycotina	Ascomycota	10/11/2020	Seco	25
BRT702	*Candida* sp. 2	Saccharomycetes	Saccharomycotina	Ascomycota	19/07/2022	Chuvoso	75
BRT435	*Candida* sp. 3	Saccharomycetes	Saccharomycotina	Ascomycota	18/05/2021	Chuvoso	68.75
BRT473	*Candida* sp. 3	Saccharomycetes	Saccharomycotina	Ascomycota	15/06/2021	Chuvoso	1875
BRT674	*Candida* sp. 4	Saccharomycetes	Saccharomycotina	Ascomycota	23/06/2022	Chuvoso	140.625
BRT508	*Carcinomyces nordestinensis*	Tremellomycetes	Agaricomycotina	Basidiomycota	20/07/2021	Chuvoso	125
BRT535	*Carcinomyces nordestinensis*	Tremellomycetes	Agaricomycotina	Basidiomycota	19/10/2021	Seco	125
BRT448	*Carcinomyces nordestinensis*	Tremellomycetes	Agaricomycotina	Basidiomycota	18/05/2021	Chuvoso	500
BRT457	*Carcinomyces nordestinensis*	Tremellomycetes	Agaricomycotina	Basidiomycota	18/05/2021	Chuvoso	500
BRT536	*Carlosrosaea* sp. 1	Tremellomycetes	Agaricomycotina	Basidiomycota	19/10/2021	Seco	125
BRT697	*Carlosrosaea* sp. 1	Tremellomycetes	Agaricomycotina	Basidiomycota	23/06/2022	Chuvoso	125
BRT586	*Carlosrosaea* sp. 2	Tremellomycetes	Agaricomycotina	Basidiomycota	23/11/2021	Seco	125
BRT537	*Carlosrosaea* sp. 2	Tremellomycetes	Agaricomycotina	Basidiomycota	19/10/2021	Seco	250
BRT663	*Carlosrosaea* sp. 2	Tremellomycetes	Agaricomycotina	Basidiomycota	24/05/2022	Chuvoso	250
BRT714	*Carlosrosaea* sp. 2	Tremellomycetes	Agaricomycotina	Basidiomycota	19/07/2022	Chuvoso	250
BRT659	*Carlosrosaea* sp. 2	Tremellomycetes	Agaricomycotina	Basidiomycota	24/05/2022	Chuvoso	375
BRT651	*Carlosrosaea* sp. 2	Tremellomycetes	Agaricomycotina	Basidiomycota	24/05/2022	Chuvoso	625
BRT696	*Carlosrosaea* sp. 2	Tremellomycetes	Agaricomycotina	Basidiomycota	23/06/2022	Chuvoso	625
BRT692	*Carlosrosaea* sp. 3	Tremellomycetes	Agaricomycotina	Basidiomycota	23/06/2022	Chuvoso	125
BRT710	*Chaetosphaeria* sp.	Sordariomycetes	Pezizomycotina	Ascomycota	19/07/2022	Chuvoso	250
BRT587	*Colacogloea* sp.	Microbotryomycetes	Pucciniomycotina	Basidiomycota	23/11/2021	Seco	125
BRT452	*Cyrenella elegans*	Cystobasidiomycetes	Pucciniomycotina	Basidiomycota	18/05/2021	Chuvoso	125
BRT357	*Cystobasidium keelungensis*	Cystobasidiomycetes	Pucciniomycotina	Basidiomycota	07/10/2020	Seco	125
BRT581	*Cystobasidium* sp.	Cystobasidiomycetes	Pucciniomycotina	Basidiomycota	23/11/2021	Seco	125
BRT565	*Erythrobasidium* sp.	Cystobasidiomycetes	Pucciniomycotina	Basidiomycota	23/11/2021	Seco	125
BRT418	*Exophiala bergeri*	Eurotiomycetes	Pezizomycotina	Ascomycota	02/12/2020	Seco	125
BRT646	*Exophiala spinifera*	Eurotiomycetes	Pezizomycotina	Ascomycota	24/05/2022	Chuvoso	93.75
BRT522	*Exophiala spinifera*	Eurotiomycetes	Pezizomycotina	Ascomycota	19/10/2021	Seco	12.5
BRT582	*Exophiala spinifera*	Eurotiomycetes	Pezizomycotina	Ascomycota	23/11/2021	Seco	62.5
BRT496	*Exophiala spinifera*	Eurotiomycetes	Pezizomycotina	Ascomycota	15/06/2021	Chuvoso	81.25
BRT466	*Exophiala spinifera*	Eurotiomycetes	Pezizomycotina	Ascomycota	18/05/2021	Chuvoso	125
BRT654	*Exophiala spinifera*	Eurotiomycetes	Pezizomycotina	Ascomycota	24/05/2022	Chuvoso	125
BRT558	*Exophiala spinifera*	Eurotiomycetes	Pezizomycotina	Ascomycota	23/11/2021	Seco	500
BRT468	*Fellomyces*	Tremellomycetes	Agaricomycotina	Basidiomycota	18/05/2021	Chuvoso	125

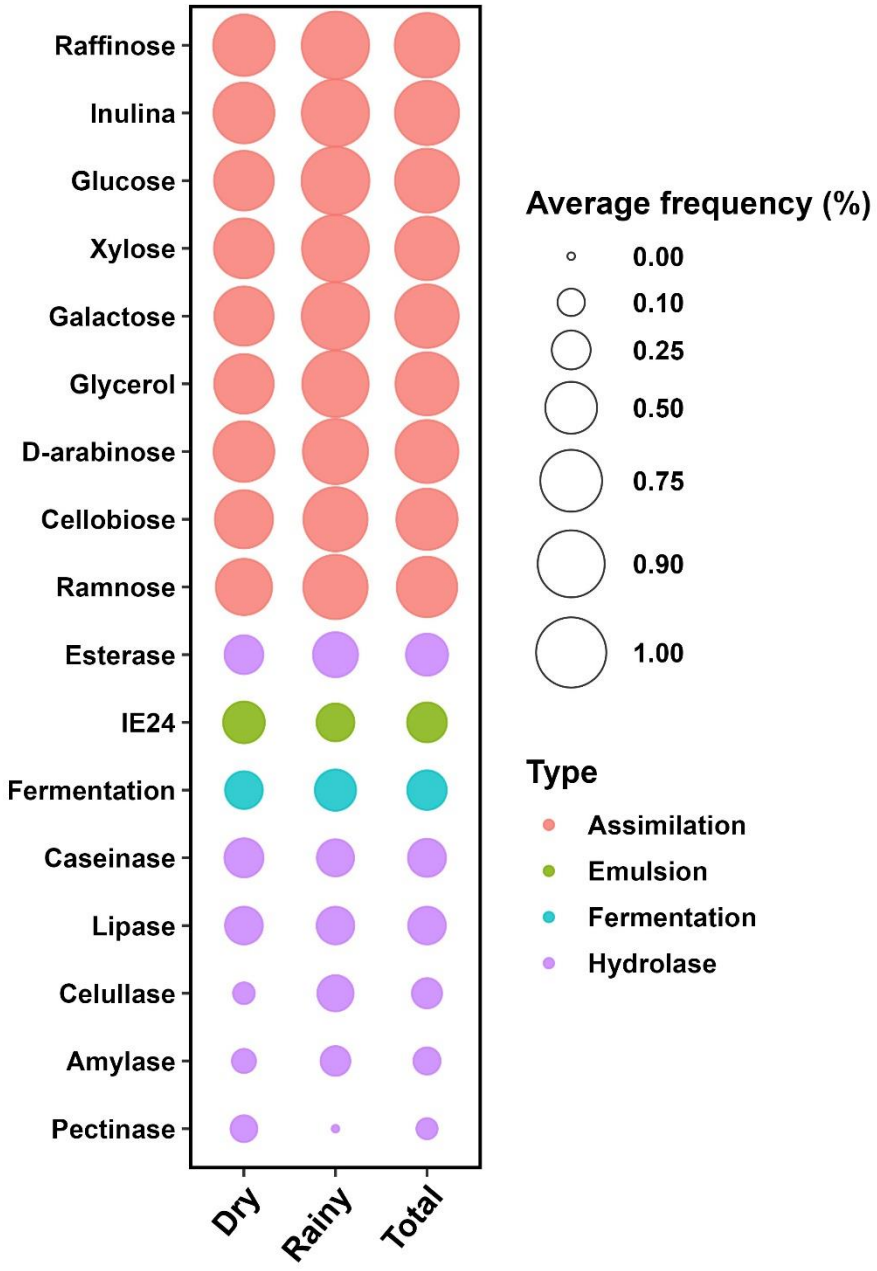
	penicillatus/borneensis/polyborus						
BRT716	*Halobasidium xiangyangense*	Cystobasidiomycetes	Pucciniomycotina	Basidiomycota	19/07/2022	Chuvoso	375
BRT672	*Hannaella phetchabunensis*	Tremellomycetes	Agaricomycotina	Basidiomycota	23/06/2022	Chuvoso	581.25
BRT670	*Hannaella siamensis*	Tremellomycetes	Agaricomycotina	Basidiomycota	23/06/2022	Chuvoso	118.75
BRT483	*Hannaella siamensis*	Tremellomycetes	Agaricomycotina	Basidiomycota	15/06/2021	Chuvoso	12.5
BRT353	*Hannaella siamensis*	Tremellomycetes	Agaricomycotina	Basidiomycota	07/10/2020	Seco	125
BRT553	*Hannaella sinensis*	Tremellomycetes	Agaricomycotina	Basidiomycota	23/11/2021	Seco	62.5
BRT703	*Hannaella taiwanensis*	Tremellomycetes	Agaricomycotina	Basidiomycota	19/07/2022	Chuvoso	75
BRT712	*Hannaella taiwanensis*	Tremellomycetes	Agaricomycotina	Basidiomycota	19/07/2022	Chuvoso	125
BRT458	*Hannaella taiwanensis*	Tremellomycetes	Agaricomycotina	Basidiomycota	18/05/2021	Chuvoso	250
BRT675	*Hannaella taiwanensis*	Tremellomycetes	Agaricomycotina	Basidiomycota	23/06/2022	Chuvoso	450
BRT612	*Hannaella zeae*	Tremellomycetes	Agaricomycotina	Basidiomycota	07/12/2021	Seco	375
BRT438	*Hanseniaspora opuntiae*	Saccharomycetes	Saccharomycotina	Ascomycota	18/05/2021	Chuvoso	125
BRT444	*Hasegawazyma* sp.	Cystobasidiomycetes	Pucciniomycotina	Basidiomycota	18/05/2021	Chuvoso	37.5
BRT368	*Hasegawazyma* sp.	Cystobasidiomycetes	Pucciniomycotina	Basidiomycota	07/10/2020	Seco	125
BRT426	*Hasegawazyma* sp.	Cystobasidiomycetes	Pucciniomycotina	Basidiomycota	02/12/2020	Seco	125
BRT433	*Hortaea werneckii*	Dothideomycetes	Pezizomycotina	Ascomycota	02/12/2020	Seco	2625
BRT694	*Kordyana* sp.	Exobasidiomycetes	Ustilaginomycotina	Basidiomycota	23/06/2022	Chuvoso	125
BRT555	*Kwoniella dejecticola*	Tremellomycetes	Agaricomycotina	Basidiomycota	23/11/2021	Seco	87.5
BRT495	*Kwoniella dendrophila*	Tremellomycetes	Agaricomycotina	Basidiomycota	15/06/2021	Chuvoso	12.5
BRT406	*Kwoniella heveanensis*	Tremellomycetes	Agaricomycotina	Basidiomycota	02/12/2020	Seco	12.5
BRT375	*Kwoniella heveanensis*	Tremellomycetes	Agaricomycotina	Basidiomycota	10/11/2020	Seco	75
BRT515	*Kwoniella heveanensis*	Tremellomycetes	Agaricomycotina	Basidiomycota	19/10/2021	Seco	125
BRT376	*Kwoniella heveanensis*	Tremellomycetes	Agaricomycotina	Basidiomycota	10/11/2020	Seco	137.5
BRT377	*Kwoniella heveanensis*	Tremellomycetes	Agaricomycotina	Basidiomycota	10/11/2020	Seco	375
BRT517	*Meyerozyma caribbica/guilliermondii*	Saccharomycetes	Saccharomycotina	Ascomycota	19/10/2021	Seco	62.5
BRT439	*Meyerozyma caribbica/guilliermondii*	Saccharomycetes	Saccharomycotina	Ascomycota	18/05/2021	Chuvoso	334.375
BRT437	*Meyerozyma caribbica/guilliermondii*	Saccharomycetes	Saccharomycotina	Ascomycota	18/05/2021	Chuvoso	706.25
BRT551	*Meyerozyma caribbica/guilliermondii*	Saccharomycetes	Saccharomycotina	Ascomycota	23/11/2021	Seco	12.5
BRT627	*Meyerozyma caribbica/guilliermondii*	Saccharomycetes	Saccharomycotina	Ascomycota	24/05/2022	Chuvoso	12.5
BRT408	*Meyerozyma caribbica/guilliermondii*	Saccharomycetes	Saccharomycotina	Ascomycota	02/12/2020	Seco	125
BRT509	*Meyerozyma caribbica/guilliermondii*	Saccharomycetes	Saccharomycotina	Ascomycota	20/07/2021	Chuvoso	125

BRT549	*Meyerozyma caribbica/guilliermondii*	Saccharomycetes	Saccharomycotina	Ascomycota	23/11/2021	Seco	125
BRT436	*Meyerozyma caribbica/guilliermondii*	Saccharomycetes	Saccharomycotina	Ascomycota	18/05/2021	Chuvoso	153.125
BRT526	*Meyerozyma caribbica/guilliermondii*	Saccharomycetes	Saccharomycotina	Ascomycota	19/10/2021	Seco	156.25
BRT685	*Microstroma* sp. 1	Exobasidiomycetes	Ustilaginomycotina	Basidiomycota	23/06/2022	Chuvoso	125
BRT354	*Microstroma* sp. 2	Exobasidiomycetes	Ustilaginomycotina	Basidiomycota	07/10/2020	Seco	12.5
BRT350	*Microstroma* sp. 2	Exobasidiomycetes	Ustilaginomycotina	Basidiomycota	07/10/2020	Seco	125
BRT409	*Moesziomyces antarcticus*	Ustilaginomycetes	Ustilaginomycotina	Basidiomycota	02/12/2020	Seco	12.5
BRT355	*Moesziomyces aphidis*	Ustilaginomycetes	Ustilaginomycotina	Basidiomycota	07/10/2020	Seco	12.5
BRT397	*Occultifur brasiliensis*	Cystobasidiomycetes	Pucciniomycotina	Basidiomycota	02/12/2020	Seco	412.5
BRT389	*Occultifur brasiliensis*	Cystobasidiomycetes	Pucciniomycotina	Basidiomycota	10/11/2020	Seco	12.5
BRT534	*Occultifur brasiliensis*	Cystobasidiomycetes	Pucciniomycotina	Basidiomycota	19/10/2021	Seco	12.5
BRT477	*Occultifur brasiliensis*	Cystobasidiomycetes	Pucciniomycotina	Basidiomycota	15/06/2021	Chuvoso	25
BRT415	*Occultifur brasiliensis*	Cystobasidiomycetes	Pucciniomycotina	Basidiomycota	02/12/2020	Seco	75
BRT362	*Occultifur brasiliensis*	Cystobasidiomycetes	Pucciniomycotina	Basidiomycota	07/10/2020	Seco	125
BRT366	*Occultifur brasiliensis*	Cystobasidiomycetes	Pucciniomycotina	Basidiomycota	07/10/2020	Seco	125
BRT419	*Occultifur brasiliensis*	Cystobasidiomycetes	Pucciniomycotina	Basidiomycota	02/12/2020	Seco	125
BRT420	*Occultifur brasiliensis*	Cystobasidiomycetes	Pucciniomycotina	Basidiomycota	02/12/2020	Seco	125
BRT427	*Occultifur brasiliensis*	Cystobasidiomycetes	Pucciniomycotina	Basidiomycota	02/12/2020	Seco	125
BRT447	*Occultifur brasiliensis*	Cystobasidiomycetes	Pucciniomycotina	Basidiomycota	18/05/2021	Chuvoso	125
BRT471	*Occultifur brasiliensis*	Cystobasidiomycetes	Pucciniomycotina	Basidiomycota	18/05/2021	Chuvoso	125
BRT652	*Occultifur brasiliensis*	Cystobasidiomycetes	Pucciniomycotina	Basidiomycota	24/05/2022	Chuvoso	125
BRT683	*Occultifur brasiliensis*	Cystobasidiomycetes	Pucciniomycotina	Basidiomycota	23/06/2022	Chuvoso	125
BRT684	*Occultifur brasiliensis*	Cystobasidiomycetes	Pucciniomycotina	Basidiomycota	23/06/2022	Chuvoso	125
BRT449	*Occultifur brasiliensis*	Cystobasidiomycetes	Pucciniomycotina	Basidiomycota	18/05/2021	Chuvoso	200
BRT645	*Occultifur brasiliensis*	Cystobasidiomycetes	Pucciniomycotina	Basidiomycota	24/05/2022	Chuvoso	212.5
BRT381	*Occultifur brasiliensis*	Cystobasidiomycetes	Pucciniomycotina	Basidiomycota	10/11/2020	Seco	250
BRT384	*Occultifur brasiliensis*	Cystobasidiomycetes	Pucciniomycotina	Basidiomycota	10/11/2020	Seco	250
BRT356	*Occultifur brasiliensis*	Cystobasidiomycetes	Pucciniomycotina	Basidiomycota	07/10/2020	Seco	375
BRT463	*Occultifur brasiliensis*	Cystobasidiomycetes	Pucciniomycotina	Basidiomycota	18/05/2021	Chuvoso	375
BRT446	*Occultifur brasiliensis*	Cystobasidiomycetes	Pucciniomycotina	Basidiomycota	18/05/2021	Chuvoso	625
BRT405	*Occultifur brasiliensis*	Cystobasidiomycetes	Pucciniomycotina	Basidiomycota	02/12/2020	Seco	2762.5
BRT349	*Occultifur externus*	Cystobasidiomycetes	Pucciniomycotina	Basidiomycota	07/10/2020	Seco	125
BRT383	*Occultifur plantarum*	Cystobasidiomycetes	Pucciniomycotina	Basidiomycota	10/11/2020	Seco	12.5
BRT492	*Occultifur plantarum*	Cystobasidiomycetes	Pucciniomycotina	Basidiomycota	15/06/2021	Chuvoso	12.5
BRT411	*Papiliotrema flavescens*	Tremellomycetes	Agaricomycotina	Basidiomycota	02/12/2020	Seco	12.5
BRT486	*Papiliotrema flavescens*	Tremellomycetes	Agaricomycotina	Basidiomycota	15/06/2021	Chuvoso	12.5

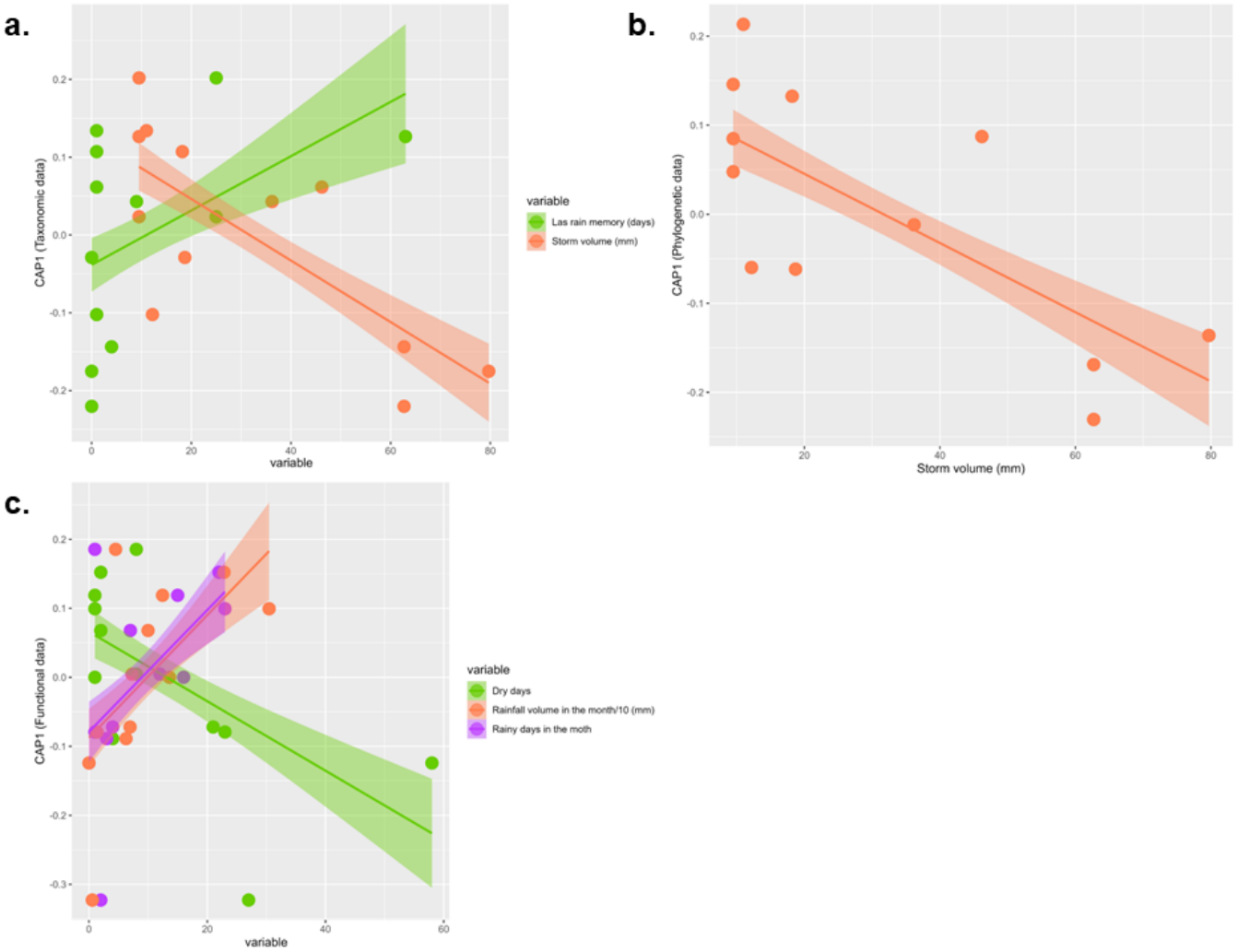
BRT372	*Papiliotrema flavescens*	Tremellomycetes	Agaricomycotina	Basidiomycota	10/11/2020	Seco	31.25
BRT484	*Papiliotrema flavescens*	Tremellomycetes	Agaricomycotina	Basidiomycota	15/06/2021	Chuvoso	50
BRT500	*Papiliotrema flavescens*	Tremellomycetes	Agaricomycotina	Basidiomycota	20/07/2021	Chuvoso	87.5
BRT351	*Papiliotrema laurentii*	Tremellomycetes	Agaricomycotina	Basidiomycota	07/10/2020	Seco	12.5
BRT476	*Papiliotrema laurentii*	Tremellomycetes	Agaricomycotina	Basidiomycota	15/06/2021	Chuvoso	12.5
BRT556	*Papiliotrema laurentii*	Tremellomycetes	Agaricomycotina	Basidiomycota	23/11/2021	Seco	12.5
BRT591	*Papiliotrema laurentii*	Tremellomycetes	Agaricomycotina	Basidiomycota	07/12/2021	Seco	12.5
BRT504	*Papiliotrema laurentii*	Tremellomycetes	Agaricomycotina	Basidiomycota	20/07/2021	Chuvoso	37.5
BRT528	*Papiliotrema laurentii*	Tremellomycetes	Agaricomycotina	Basidiomycota	19/10/2021	Seco	50
BRT599	*Papiliotrema laurentii*	Tremellomycetes	Agaricomycotina	Basidiomycota	07/12/2021	Seco	75
BRT498	*Papiliotrema laurentii*	Tremellomycetes	Agaricomycotina	Basidiomycota	20/07/2021	Chuvoso	100
BRT358	*Papiliotrema laurentii*	Tremellomycetes	Agaricomycotina	Basidiomycota	07/10/2020	Seco	125
BRT359	*Papiliotrema laurentii*	Tremellomycetes	Agaricomycotina	Basidiomycota	07/10/2020	Seco	125
BRT388	*Papiliotrema laurentii*	Tremellomycetes	Agaricomycotina	Basidiomycota	10/11/2020	Seco	125
BRT511	*Papiliotrema laurentii*	Tremellomycetes	Agaricomycotina	Basidiomycota	20/07/2021	Chuvoso	125
BRT543	*Papiliotrema laurentii*	Tremellomycetes	Agaricomycotina	Basidiomycota	23/11/2021	Seco	143.75
BRT481	*Papiliotrema laurentii*	Tremellomycetes	Agaricomycotina	Basidiomycota	15/06/2021	Chuvoso	187.5
BRT410	*Papiliotrema laurentii*	Tremellomycetes	Agaricomycotina	Basidiomycota	02/12/2020	Seco	250
BRT637	*Papiliotrema laurentii*	Tremellomycetes	Agaricomycotina	Basidiomycota	24/05/2022	Chuvoso	268.75
BRT428	*Papiliotrema laurentii*	Tremellomycetes	Agaricomycotina	Basidiomycota	02/12/2020	Seco	625
BRT502	*Papiliotrema miconiae*	Tremellomycetes	Agaricomycotina	Basidiomycota	20/07/2021	Chuvoso	37.5
BRT512	*Papiliotrema miconiae*	Tremellomycetes	Agaricomycotina	Basidiomycota	20/07/2021	Chuvoso	125
BRT488	*Papiliotrema rajasthanensis*	Tremellomycetes	Agaricomycotina	Basidiomycota	15/06/2021	Chuvoso	25
BRT572	*Papiliotrema rajasthanensis*	Tremellomycetes	Agaricomycotina	Basidiomycota	23/11/2021	Seco	125
BRT385	*Papiliotrema* sp. 1	Tremellomycetes	Agaricomycotina	Basidiomycota	10/11/2020	Seco	250
BRT487	*Papiliotrema* sp. 2	Tremellomycetes	Agaricomycotina	Basidiomycota	15/06/2021	Chuvoso	87.5
BRT641	*Parapyrenis conica*	Sordariomycetes	Pezizomycotina	Ascomycota	24/05/2022	Chuvoso	125
BRT621	*Pseudosydowia* sp.	Dothideomycetes	Pezizomycotina	Ascomycota	24/05/2022	Chuvoso	75
BRT460	*Pseudozyma hubeiensis*	Ustilaginomycetes	Ustilaginomycotina	Basidiomycota	18/05/2021	Chuvoso	375
BRT421	*Pseudozyma pruni*	Ustilaginomycetes	Ustilaginomycotina	Basidiomycota	02/12/2020	Seco	125
BRT364	*Pseudozyma tsukubaensis*	Ustilaginomycetes	Ustilaginomycotina	Basidiomycota	07/10/2020	Seco	125
BRT379	*Rhodosporidiobolus ruineniae*	Microbotryomycetes	Pucciniomycotina	Basidiomycota	10/11/2020	Seco	125
BRT554	*Rhodosporidiobolus ruineniae*	Microbotryomycetes	Pucciniomycotina	Basidiomycota	23/11/2021	Seco	12.5
BRT378	*Rhodosporidiobolus ruineniae*	Microbotryomycetes	Pucciniomycotina	Basidiomycota	10/11/2020	Seco	125
BRT402	*Rhodotorula mucilaginosa*	Microbotryomycetes	Pucciniomycotina	Basidiomycota	02/12/2020	Seco	125
BRT592	*Rhodotorula mucilaginosa*	Microbotryomycetes	Pucciniomycotina	Basidiomycota	07/12/2021	Seco	37.5
BRT398	*Rhodotorula mucilaginosa*	Microbotryomycetes	Pucciniomycotina	Basidiomycota	02/12/2020	Seco	100
BRT413	*Rhodotorula paludigena*	Microbotryomycetes	Pucciniomycotina	Basidiomycota	02/12/2020	Seco	62.5

BRT373	*Rhynchogastrema noutii*	Tremellomycetes	Agaricomycotina	Basidiomycota	10/11/2020	Seco	125
BRT367	*Rosettozyma* sp.	Microbotryomycetes	Pucciniomycotina	Basidiomycota	07/10/2020	Seco	125
BRT395	*Rosettozyma* sp.	Microbotryomycetes	Pucciniomycotina	Basidiomycota	10/11/2020	Seco	125
BRT643	*Saitozyma flava*	Tremellomycetes	Agaricomycotina	Basidiomycota	24/05/2022	Chuvoso	12.5
BRT622	*Saitozyma flava*	Tremellomycetes	Agaricomycotina	Basidiomycota	24/05/2022	Chuvoso	75
BRT348	*Saitozyma ninhbinhensis*	Tremellomycetes	Agaricomycotina	Basidiomycota	07/10/2020	Seco	125
BRT363	*Saitozyma ninhbinhensis*	Tremellomycetes	Agaricomycotina	Basidiomycota	07/10/2020	Seco	125
BRT588	*Saitozyma ninhbinhensis*	Tremellomycetes	Agaricomycotina	Basidiomycota	07/12/2021	Seco	987.5
BRT601	*Saitozyma podzolica*	Tremellomycetes	Agaricomycotina	Basidiomycota	07/12/2021	Seco	96.875
BRT431	*Saitozyma podzolica*	Tremellomycetes	Agaricomycotina	Basidiomycota	02/12/2020	Seco	125
BRT640	*Saitozyma podzolica*	Tremellomycetes	Agaricomycotina	Basidiomycota	24/05/2022	Chuvoso	406.25
BRT530	*Saitozyma podzolica*	Tremellomycetes	Agaricomycotina	Basidiomycota	19/10/2021	Seco	125
BRT634	*Sakaguchia oryzae*	Cystobasidiomycetes	Pucciniomycotina	Basidiomycota	24/05/2022	Chuvoso	131.25
BRT404	*Selenophoma* sp.	Dothideomycetes	Pezizomycotina	Ascomycota	02/12/2020	Seco	125
BRT423	*Selenophoma* sp.	Dothideomycetes	Pezizomycotina	Ascomycota	02/12/2020	Seco	125
BRT626	*Starmerella ilheusensis*	Saccharomycetes	Saccharomycotina	Ascomycota	24/05/2022	Chuvoso	68.75
BRT657	*Symmetrospora marina*	Cystobasidiomycetes	Pucciniomycotina	Basidiomycota	24/05/2022	Chuvoso	100
BRT497	*Symmetrospora marina*	Cystobasidiomycetes	Pucciniomycotina	Basidiomycota	15/06/2021	Chuvoso	12.5
BRT451	*Symmetrospora suhii*	Cystobasidiomycetes	Pucciniomycotina	Basidiomycota	18/05/2021	Chuvoso	125
BRT584	*Symmetrospora suhii*	Cystobasidiomycetes	Pucciniomycotina	Basidiomycota	23/11/2021	Seco	125
BRT648	*Symmetrospora suhii*	Cystobasidiomycetes	Pucciniomycotina	Basidiomycota	24/05/2022	Chuvoso	212.5
BRT470	*Symmetrospora suhii*	Cystobasidiomycetes	Pucciniomycotina	Basidiomycota	18/05/2021	Chuvoso	375
BRT369	*Taphrina* sp.	Taphrinomycetes	Taphrinomycotina	Ascomycota	07/10/2020	Seco	125
BRT453	*Tremella ananatis*	Tremellomycetes	Agaricomycotina	Basidiomycota	18/05/2021	Chuvoso	62.5
BRT380	*Tremella ananatis*	Tremellomycetes	Agaricomycotina	Basidiomycota	10/11/2020	Seco	125
BRT566	*Tremella ananatis*	Tremellomycetes	Agaricomycotina	Basidiomycota	23/11/2021	Seco	125
BRT450	*Tremella ananatis*	Tremellomycetes	Agaricomycotina	Basidiomycota	18/05/2021	Chuvoso	68.75
BRT361	*Tremella ananatis*	Tremellomycetes	Agaricomycotina	Basidiomycota	07/10/2020	Seco	125
BRT533	*Tremella ananatis*	Tremellomycetes	Agaricomycotina	Basidiomycota	19/10/2021	Seco	125
BRT583	*Tremella ananatis*	Tremellomycetes	Agaricomycotina	Basidiomycota	23/11/2021	Seco	125
BRT678	*Tremella ananatis*	Tremellomycetes	Agaricomycotina	Basidiomycota	23/06/2022	Chuvoso	137.5
BRT598	*Tremella ananatis*	Tremellomycetes	Agaricomycotina	Basidiomycota	07/12/2021	Seco	225
BRT386	*Tremella ananatis*	Tremellomycetes	Agaricomycotina	Basidiomycota	10/11/2020	Seco	250
BRT387	*Tremella ananatis*	Tremellomycetes	Agaricomycotina	Basidiomycota	10/11/2020	Seco	250
BRT432	*Tremella ananatis*	Tremellomycetes	Agaricomycotina	Basidiomycota	02/12/2020	Seco	250
BRT464	*Tremella ananatis*	Tremellomycetes	Agaricomycotina	Basidiomycota	18/05/2021	Chuvoso	250
BRT713	*Tremella ananatis*	Tremellomycetes	Agaricomycotina	Basidiomycota	19/07/2022	Chuvoso	500
BRT417	*Tremella ananatis*	Tremellomycetes	Agaricomycotina	Basidiomycota	02/12/2020	Seco	875

BRT422	*Tremella ananatis*	Tremellomycetes	Agaricomycotina	Basidiomycota	02/12/2020	Seco	1125
BRT485	*Tremella* sp.	Tremellomycetes	Agaricomycotina	Basidiomycota	15/06/2021	Chuvoso	50
BRT644	*Ustilago maydis*	Ustilaginomycetes	Ustilaginomycotina	Basidiomycota	24/05/2022	Chuvoso	12.5
BRT606	*Vishniacozyma alagoana*	Tremellomycetes	Agaricomycotina	Basidiomycota	07/12/2021	Seco	375
BRT611	*Vishniacozyma alagoana*	Tremellomycetes	Agaricomycotina	Basidiomycota	07/12/2021	Seco	50
BRT455	*Vishniacozyma alagoana*	Tremellomycetes	Agaricomycotina	Basidiomycota	18/05/2021	Chuvoso	125
BRT469	*Vishniacozyma alagoana*	Tremellomycetes	Agaricomycotina	Basidiomycota	18/05/2021	Chuvoso	125
BRT575	*Vishniacozyma alagoana*	Tremellomycetes	Agaricomycotina	Basidiomycota	23/11/2021	Seco	375
BRT407	*Yueomyces* sp.	Saccharomycetes	Saccharomycotina	Ascomycota	02/12/2020	Seco	12.5



Supplementary Figure 1- Frequencies in bromeliad samples in percentage and categories of functional traits expressed by yeasts in different periods, dry and rainy.



Supplementary Figure 2- Relationship of the first component (CAP1) from the Canonical Analysis of Principal coordinates (CAP) and the environmental variables indicating the significant relationships observed.