UNIVERSIDADE FEDERAL DO PAMPA

ALICE LEMOS COSTA

ASSOCIAÇÕES ECOLÓGICAS DE FUNGOS COM A FLORA E A FAUNA EM ZONA RIPÁRIA NO ESTADO DO RIO GRANDE DO SUL

São Gabriel - RS
2024

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Tese apresentada ao Programa de Pós-Graduação em Ciências Biológicas *stricto sensu* da Universidade Federal do Pampa, como requisito parcial para a obtenção do Título de Doutora em Ciências Biológicas.

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“Toda a forma de vida é uma manifestação de Deus e está sob os nossos cuidados. Proteja o que é seu - sua fauna sua flora. As plantas e os animais embelezam a terra. São úteis ao homem e representam a riqueza da Pátria. Nunca se deve mutilar, destruir ou deixar que destruam estes bens. Vamos amar nossos animais domésticos. Vamos dar aos selvagens a paz que eles têm direito. Permitamos que enfeitem nossas florestas. Vamos amar os pássaros puros e belos, cantando nas ramagens, voando alegres no espaço ilimitado, como verdadeiros símbolos de liberdade!”

São Francisco de Assis

RESUMO

Os fungos estão presentes em praticamente todos os ambientes e desempenham papéis fundamentais na ciclagem nutricional. Muitas das associações dos fungos ocorrem de maneira harmônica. Em zonas ripárias os fungos interagem com o solo, com a flora e com a fauna, principalmente devido a abundância do recurso hídrico. Compreender os tipos de associações e a bionomia dessas interações são de suma importância. Assim, este estudo objetivou determinar através de dados provindos de coletas e análises realizadas nas zonas ripárias do Rio Vacacaí - São Gabriel/RS, Vale do Rio Pardo - Vera Cruz/RS e Rio dos Sinos - São Francisco de Paula/RS, os tipos de associações desempenhadas pelos fungos e suas relações com a flora e fauna. Para tanto, no Capítulo I é apresentado um levantamento da família Pluteaceae nos biomas brasileiros com ênfase nas ocorrências em zonas ripárias lóticas e lênticas, suas relações e homologias em relação aos tipos de substratos nativos e exóticos utilizados. No Capítulo II foram catalogadas 25 espécies de Agaricales com ocorrência em zona ripária lêntica, onde foram mensurados os teores dos macronutrientes e micronutrientes dos fungos tendo como substrato *Eucalyptus grandis*. No Capítulo III é apresentada uma revisão bibliográfica global sobre a utilização dos fungos como recurso nutricional pelas Aves silvestres, assim como os benefícios já estudados da suplementação fúngica junto a alimentação das Aves de corte. No Capítulo IV se reporta o primeiro registro de micofagia do pássaro *Molothrus bonariensis* com o cogumelo *Macrolepiota banariensis* em uma área ripária dulcícula. No Capítulo V apresenta-se o primeiro registro do ciclo de vida e micofagia de *Zygothrica candens* com o cogumelo *Oudemansiella cubens* em uma zona ripária lótica. No Capítulo VI é relatada a associação de seis espécies de formigas da casta operária de Attini utilizando como recurso alimentar, e sua atuação como agentes dispersores de esporos de fungos Agaricales em zona ripária dulcícula. Os dados dispostos neste estudo auxiliam em uma melhor compreensão dos tipos de associações dos fungos com a flora e a fauna em zona ripária no Rio Grande do Sul, Brasil.

Palavras-Chave: Micofagia; Ciclagem nutricional; Interações ecológicas.

ABSTRACT

Fungi are present in practically all environments and play fundamental roles in nutritional cycling. Many of the fungal associations occur harmoniously. In riparian zones, fungi interact with the soil, flora, and fauna, mainly due to the abundance of water resources. Understanding the types of associations and the bionomics of these interactions is very much importance. Thus, this study aimed to determine, through data from collections and analyses carried out in the riparian zones of the Rio Vacacaí - São Gabriel/RS, Vale do Rio Pardo - Vera Cruz/RS, and Rio dos Sinos - São Francisco de Paula/RS, the types of associations carried out by fungi and their relationships with flora and fauna. To this purpose, Chapter I presents a survey of the Pluteaceae family in Brazilian biomes with an emphasis on occurrences in lotic and lentic riparian zones, their relationships, and homologies concerning the types of native and exotic substrates used. In Chapter II, 25 species of Agaricales occurring in the lentic riparian zone were cataloged, where the levels of macronutrients and micronutrients of the fungi were measured using *Eucalyptus grandis* as a substrate. Chapter III presents a global literature review on the use of fungi as a nutritional resource by wild birds, as well as the already studied benefits of fungal supplementation in the diet of poultry. Chapter IV reports the first record of mycophagy of the bird *Molothrus bonariensis* with the mushroom *Macrolepiota bonariensis* in a freshwater riparian zone. Chapter V presents the first record of the life cycle and mycophagy of *Zygothrica candens* with the mushroom *Oudemansiella cubensis* in a lotic riparian zone. In Chapter VI, the association of six species of ants from the Attini worker caste is reported, used as a food resource, and their action as dispersing agents of Agaricales fungal spores in the lentic riparian zone. The data provided in this study help to better understand the types of associations among fungi, flora, and fauna in the riparian zone at Rio Grande do Sul, Brazil.

Keywords: Mycophagy; Nutritional cycling; Ecological interactions.

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LISTA DE ABREVIATURAS E SIGLAS

$\mu g\ g^{-1}$ – Microgramas grama.

μm – Micrometro.

cm – Centímetro.

$Cmol_c\ L^{-1}$ – Centimol.

FLONA – Floresta Nacional de São Francisco de Paula.

GPS - Sistema de Posicionamento Global.

IB – Inferência Bayesiana.

IP – Inferência de Parcimônia.

KOH – Hidróxido de Potássio.

m^2 – Metro quadrado.

$mg\ L^{-1}$ – Miligramas.

ML – Máxima Verossimilhança.

mm – Milímetros.

MP – Máxima Parcimônia.

NCBI – National Center for Biotechnology of Information

SUMÁRIO

1 INTRODUÇÃO E REVISÃO DE LITERATURA

1.1 Características gerais do Reino Fungi

Presentes nos ambientes terrestres, marinhos, dulçaquícolas e aéreos, os fungos são organismos unicelulares e multicelulares, com distribuição global, desde as regiões polares às temperadas e tropicais (AZEVEDO; BARATA, 2018). Popularmente conhecidos como leveduras, bolores, cogumelos, dentre outros, são abundantes e formam um grupo distinto. A maioria dos fungos são encontrados de forma inconspícua devido a sua pequena morfologia e forma de vida junto ao solo. Porém, alguns subgrupos formam basidiomas, corpos que emergem da matéria habitada durante sua reprodução, tonando-se visíveis (SINGER, 1986).

A biodiversidade fúngica é subestimada, sendo referida na literatura como algo entorno de 2,2 a 3,8 milhões de espécies, das quais 120 mil foram completamente identificadas, o que representaria em inferências cerca de 3% a 8% das espécies realmente existentes no planeta. Sua divisão em grupos ocorre de acordo com suas características moleculares, morfológicas e fisiológicas (HAWKSWORTH; LÜCKING, 2017). Para a classificação taxonômica dos fungos *sensu stricto*, também são considerados os organismos sésseis, eucariotos, majoritariamente aeróbios, de produção e excreção enzimática, parede celular constituída de polissacarídeos, tendo como principais o esterol o e ergosterol. Também, as estruturas vegetativas e reprodutivas dos fungos são amplamente utilizadas para sua classificação (WANG *et al.*, 2020). Essas características auxiliam para a principal divisão nos filos Crytridiomycota, Neocallimastigomycota, Blastocladiomycota, Zoopagomycota, Glomeromycota, Mucoromycota, Ascomycota e Basidiomycota (HAWKSWORTH; LUCKING, 2017).

A variação de formas, tamanhos, cores, compostos químicos, aromas, habitats, hábitos, dentre outros, é um indicativo da grande variedade e alta adaptabilidade dos fungos ao ambiente aos quais estão inseridos (AZEVEDO; BARATA, 2018). Com reprodução complexa, a grande maioria dos fungos realiza propagação por meiósporos, ocorrendo também casos de propagação por mitósporos de forma vegetativa. Já o crescimento fúngico é influenciado por fatores abióticos, tais como temperatura, umidade, concentração nutricional e pH (HAWKSWORTH; LÜCKING, 2017). Nesses casos, as condições ambientais desempenham um papel chave para o desenvolvimento e crescimento fúngico.

1.2 Associações harmônicas envolvendo fungos

As associações são formas de relacionamentos entre os seres vivos que compartilham o mesmo ambiente. No caso dos fungos, ocorre o compartilhamento de praticamente todos os ecossistemas. A dinâmica como essas relações se organizam irá determinar o tipo de interação. Por exemplo, nas relações harmônicas, benefícios mútuos ocorrem sem que necessariamente as espécies estejam conectadas fisicamente, e nestes casos nenhum dos organismos envolvidos na interação é prejudicado (CERQUEIRA; FERREIRA, 2021). Nas relações de mutualismo, vantagens benéficas também estão presentes de forma interespecífica. Ainda, a protocooperação, o comensalismo e o inquilinismo completam os tipos de associações harmônicas dos fungos com os demais seres vivos (DOUGLAS, 2021).

Nos mais diversificados ecossistemas os fungos integram essas relações, devido ao seu grande potencial como cicladores da matéria orgânica. Os fungos podem realocar novamente macronutrientes e micronutrientes aos ciclos bioquímicos. Esses tipos de interações, quando harmônicas, trazem benefícios para os organismos envolvidos (CERQUEIRA; FERREIRA, 2021). No geral, a interação cicladora dos fungos é uma das principais bases subsidiárias para os ciclos ecológicos com a flora e consecutivamente da fauna (CHEN *et al*., 2018).

Dentre as relações dos fungos com o meio ambiente, a degradação dos compostos provindos da matéria orgânica é desempenhada pela maioria dos fungos saprófitos. O champignon, *Agaricus bisporus* (Lange) Imbach é um exemplo, onde além de degradar compostos orgânicos, também é considerado uma das espécies comestíveis mais exploradas na culinária (WALKER; WHITE, 2017).

Os líquens desempenham papel simbiótico, e nesses casos, a associação é benéfica para ambos os parceiros. Tanto o micobionte quanto o ficobionte só conseguem sobreviver devido a sua interação, como no caso de *Chrysothrix chlorina* (Ach.) JRLaundon (1981), um líquen encontrado principalmente em ambientes úmidos cuja associação envolve um ascomiceto e uma alga verde (RAJAPRABU; PONMURUGAN, 2022).

Para as relações de comensalismo dos fungos, outros seres vivos são utilizados sem que ocorra prejuízo. Um exemplo deste tipo é a relação de *Laboulbenyomycetes* com artrópodes em geral, o fungo habita antenas, peças bucais e outras estruturas externas do corpo dos artrópodes, sendo eliminados junto ao processo de ecdise (HAELEWATERS *et al*., 2021).

1.3 Interações dos fungos com a flora

No ecossistema terrestre, os fungos foram por muito tempo integrados ao Reino Plantae, principalmente devido ao seu modo de vida séssil, estruturas similares ao das plantas, e forma de obtenção nutricional por absorção (WANG *et al*., 2020). No entanto, através de registros fósseis, os fungos são datados durante o período câmbrico (542-488 milhões de anos), ou seja, antes da datação das plantas terrestres (BRUNDRETT, 2002). Muitos dos Filos e Classes de fungos da modernidade são datados desde o período carbonífero (318-299 milhões de anos), com registros variando desde líquens a hifas fossilizadas (LEUNG, 2017).

Uma das principais formas de interação dos fungos com as plantas é por simbiose, com importância significativa para o crescimento e desenvolvimento vegetal. É citado que cerca de 90% das plantas necessitam deste tipo de associação fúngica para sua sobrevivência (DOUGLAS, 2021). Nesses casos os fungos atuam como subsidiários secundários, tendo como exemplo a ação micorrízica de *Laccaria fraterna* (Sacc.) Pegler, 1965, com espécies arbóreas de *Eucalyptus*, onde o fungo se beneficia ao utilizar os fotossintatos da planta, enquanto a planta amplia a sua área de captação nutricional via micorriza do fungo (CAMPI *et al*., 2017). Nessa premissa, o emaranhado filamentoso micelial é uma rede conectiva, que interliga diversas espécies na rizosfera (FORTUNA, 2020). Por exemplo, *Armillaria ostoyae* (Peck, 1900) se expande por uma área de 890 hectares nas Montanhas Blue, região leste de Oregon - EUA, considerado um dos maiores organismos fúngicos vivos já descobertos no mundo (BURDSALL; VOLK, 2008).

No entanto, não são apenas as micorrizas que têm importância substancial na associação fungo-planta, os fungos lignícolas auxiliam na realocação dos nutrientes provindos da madeira novamente para o solo (SILVA *et al*., 2016). Um exemplo desta é *Phanerochaete chrysosporium* (Karst, 1889), um potente decompositor de madeira que é amplamente utilizado pela indústria, devido ao seu aparato enzimático que degrada moléculas complexas como a lignina e a celulose (YANG *et al*., 2019). Neste contexto, os fungos estão envolvidos nos principais ciclos dos nutrientes, realocando no solo através da biodegradação componentes nutricionais importantes, tais como o carbono, cálcio, magnésio, enxofre, fósforo, potássio, dentre outros (WALKER; WHITE, 2017).

1.4 Interações dos fungos com a fauna

Os fungos atuam constantemente em diversas interações com a fauna (KUHAR *et al*., 2018). Além disso, muitos fungos possuem grande importância nutricional, servindo como fonte de alimento para insetos, répteis, aves, mamíferos e humanos (AZEVEDO; BARATA, 2018; ATILA *et al*., 2021). Muitas espécies fúngicas nativas possuem associações desconhecidas ou pouco analisadas com a fauna. No estudo de Costa *et al*. (2022a) foi reportado o primeiro registro de micofagia envolvendo o pássaro chupim (*Molothrus bonariensis* Gmelin, 1788) com o cogumelo *Macrolepiota bonariensis* (Singer, 1949), ambas nativas do bioma Pampa.

Glaucomys sabrinus (Shaw, 1801), o único esquilo voador nativo do continente americano foi registrado coletando, secando e armazenando cerca de 722 táxons de Ascomycota, 429 Basidiomycota, 81 Zygomycota, 4 Chytridiomycota, 1 Glomeromycota e 44 táxons fúngicos não identificados (WHEATLEY, 2007; CLOUTIER *et al*., 2019). Um estudo realizado em zona ripária no estado do Rio Grande do Sul, localidades do Rio Vacacaí, Rio do Sinos e Vale do Rio Pardo apresentou de forma inédita a utilização de Agaricales por 6 espécies de formigas da classe operária que utilizaram os cogumelos como alimento, assim como estocaram os mesmos em seus formigueiros (COSTA *et al*., 2022b). Sendo importante ressaltar que para a fauna, a micofagia é muito pouco estudada, sendo um novo campo para futuras análises.

Outras interações são desempenhadas pelos fungos de forma indireta com a fauna, tais como os rizomorfos ou cordões miceliais de *Brunneocorticium* sp., *Crinipellis* sp., *Marasmius* sp. e *Micromphale* sp. que foram identificados como matéria prima dos ninhos de mais de 37 famílias de pássaros, todos nativos da região tropical (SINGER, 1986; KEREKES *et al*., 2009; PETERSEN; HUGHES, 2016; KOCH *et al*., 2018). Essas interações reforçam que a associação fungo-fauna pode ser mais constante e longínqua do que se pensava. No estudo de Costa *et al*.

(2023a) foi catalogado além do uso como recurso alimentar, o ciclo de vida de *Zygothrica candens* (Burla, 1956) utilizando o cogumelo *Oudemansiella cubensis* (Berk. and M.A. Curtis) R.H. Petersen, 2010 como local de ovoposição, eclosão, crescimento e alimentação de todas as fases larvais. Essas e outras formas de associações da fauna junto aos fungos pode auxiliar na compreensão de como essas relações ocorrem, quais são os tipos de interações, assim como quais as vantagens e desvantagens dessas relações.

1.5 Os fungos e as zonas riparias rio-grandenses

Para as zonas ripárias, as comunidades de fungos desempenham como principal característica a interação entre o solo (rizosfera), a flora (vegetação), a fauna (animais) com a água (recurso mineral) (LIND *et al*., 2019). Essas zonas incorporam várias formas, desde pastagens, campos abertos, florestas, zonas húmidas ou até mesmo não vegetativas, situadas próximas a uma acumulação significativa de água lótica ou lêntica (MARTINI, 2020). Devido à disponibilidade contínua de umidade nessas zonas, os fungos estão constantemente presentes, e atuando principalmente junto a matéria orgânica (MORRIS *et al*., 2021).

Nesses ambientes, a razão carbono/nitrogênio desempenhada pelos fungos é um dos ciclos mais importantes para a vegetação local (HUNGRIA; NOGUEIRA, 2022). No estudo de Costa *et al.* (2023b) ao analisar 25 espécies de fungos Agaricales em uma zona ripária lêntica do Rio Vacacaí, município de São Gabriel, a média da proporção carbono/nitrogênio encontrada foi de 13:1. Esses valores representam o potencial de degradação dos fungos ao substrato ao qual estão associados. Para as zonas ripárias, a ciclagem da matéria orgânica é a principal fonte nutricional, onde a realocação dos carbonatos e nitrogenados são a base para o crescimento, manutenção e regeneração dessas áreas de preservação ambiental permanente (HUNGRIA; NOGUEIRA, 2022). Além disso, os fungos comestíveis são um recuso nutricional para a fauna local (COSTA *et al.*, 2023b)

As relações entre espécies nativas e exóticas nas zonas ripárias também interferem na dinâmica (HUNGRIA; NOGUEIRA, 2022). Muitas espécies de fungos nativos mostram adaptações ao substrato arbóreo exótico e vice e versa. Em uma análise envolvendo espécies de Pluteaceae, foram catalogados indivíduos junto às bacias hidrográficas do Rio do Sinos, Rio Guaíba e Rio Santa Maria. Neste estudo, *Pluteus elvaniae* (Wartchow, 2018) e *Pluteus subfibrillosus* (Singer, 1956), endêmicos do Brasil, foram registrados utilizando como substrato espécies arbóreas nativas e exóticas, enquanto outros pluteoides estavam restritos aos substratos nativos e endêmicos, tais como *Astrocaryum aculeatissimum* (Schott) Burret (COSTA *et al.* 2022c). No entanto, as comunidades fúngicas possuem muitas interações não esclarecidas ou até mesmo desconhecidas nessas zonas, demonstrando a necessidade de estudos que busquem compreender os tipos de interações que os fungos desempenham com a flora e a fauna, visando principalmente a preservação dessas áreas.

2 OBJETIVO

2.1 Objetivo Geral

Determinar a diversidade e os tipos de associações dos fungos em zonas ripárias no estado do Rio Grande do Sul, com ênfase nas relações harmônicas, buscando contribuir com a caracterização de seus grupos em aspectos de distribuição, taxonomia, interações e formas de relações com a flora e a fauna.

2.2 Objetivos Específicos

- Analisar a diversidade de fungos em zona ripária, caracterizando os grupos de acordo com sua distribuição e levantamento taxonômico georreferenciado.
- Descrever as associações fúngicas com ocorrência em zona ripária da flora e fauna para compreender as suas interações.
- Reconstruir através de dados morfológicos e moleculares os relacionamentos que expressem a história evolutiva do grupo, com ênfase em suas homologias derivadas.
- Avaliar como fungos se associam a flora de zona ripária, buscando compreender quais são as suas formas de interações em relação a ciclagem nutricional.
- Compreender os tipos e finalidades das associações dos fungos com a fauna, visando a descrição dessas associações com ênfase nas relações harmônicas.

3 METODOLOGIA

3.1 Local da amostragem e coleta

Os exemplares foram coletados em ambiente natural de zona ripária no estado do Rio Grande do Sul, Brasil (Figura 1). Delimitados próximos a corpos d'água lótico e lêntico em São Gabriel, localidade do Rio Vacacaí (-30°21’56’’S e -54°18’48’’W); São Francisco de Paula, localidade do Rio dos Sinos (-29°25’22’’S e -50°23’1’’W); e Vera Cruz, localidade do Vale do Rio Pardo (-29º42'53”S e -52º30'20”W).

Para a amostragem o método de Levantamento Rápido (LR), utilizado para vegetação arbórea (WALTER et al., 2006), foi adaptado para a coleta dos fungos. Para a localização das coordenadas georreferenciadas o aplicativo MAPS.ME (https://maps.me/) foi utilizado. O material fúngico, a amostragem do substrato provindo da flora, assim como indivíduos da fauna e/ou excrementos e fluidos foram coletados sob licenças do SISBIO n° 78538-1, n° 78538-2 e n° 79049-1.

3.2 Preservação e identificação dos exemplares

Para os fungos, as coletas foram realizadas de acordo com Putzke & Putzke (2017), onde cada espécime foi coletado junto a uma camada do substrato. Posteriormente, foram armazenados individualmente em recipientes plásticos e desidratados em estufa a 40°C. A identificação procedeu através da chave de identificação para Fungos Agaricales do Brasil disposta em Putzke & Putzke (2017) e dados disponíveis na plataforma Index Fungorum (http://www.indexfungorum.org/).

Para as espécies da flora associadas ou com interações indiretas junto aos fungos, o Guia de Identificação da Flora do Rio Grande do Sul (https://floradigital.ufsc.br/) foi utilizado. O material biológico provindo da fauna, tais como dejetos fecais foram armazenados sob refrigeração - 4°C em recipientes plásticos. Para os artrópodes, os mesmos foram armazenados em álcool 70% sob refrigeração - 4°C em tubos Falcon 5 ml ou Eppendorf 1 ml, visando a preservação das partes moles dos fungos acopladas externamente, ou dispostas internamente dentro dos indivíduos. Para a identificação dos artrópodes as chaves de Fisher & Cover (2007), Baccaro *et al*. (2015) e Mendes *et* al. (2021) foram utilizadas. Para a identificação dos demais indivíduos da fauna, o Guia de Identificação da Fauna do

Rio Grande do Sul (https://www.ufrgs.br/faunadigitalrs/) foi utilizado como base, e para as Aves, a identificação procedeu de acordo com Borrelli *et al.* (2020).

Para ambas os grupos da flora e fauna, registros em vídeos e fotografias foram realizados para auxiliar na posterior identificação, assim como a consulta técnica com especialistas de cada grupo e/ou área. Foram analisadas características macroscópicas e para as estruturas microscópicas, foram utilizados os microscópios ópticos Olympus CH-2 e Zeiss Discovery V20. Todos os espécimes coletados foram armazenados no Laboratório de Taxonomia de Fungos da Universidade Federal do Pampa, campus São Gabriel, Rio Grande do Sul, Brasil.

3.3 Reconstruções filogenéticas

Matrizes com marcadores morfológicos e moleculares foram geradas com porções selecionadas considerando a sua disponibilidade durante a busca blast na plataforma GenBank (SAYERS, 2020). A heterogeneidade nas taxas de evolução desses genes foi avaliada, assim como o sinal filogenético dos mesmos. Apenas os resultados com valor E menor que e^{-10} foram considerados relevantes e incorporados às análises. Para as espécies que possuíam descrições de estruturas morfológicas macro e microscópicas, foram elaboradas matrizes binárias com o auxílio do programa Mesquite v.2.0 (MADDISON; MADDISON, 2007).

As sequências tratadas foram posteriormente analisadas por Inferência de Parcimônia (IP) no TNT v.1.5 (GOLOBOFF; CATALANO, 2016); Máxima Verossimilhança (ML) no RAxML-GUI v.1.5 (SILVESTRO; MICHALAK, 2012), e Inferência Bayesiana (IB) no MrBayes v. 3.2 (RONQUIST et al., 2012).

3.4 Análises do conteúdo nutricional dos fungos

Os fungos coletados em associação com a flora foram analisados em relação ao seu conteúdo nutricional. Para as coletas envolvendo os fungos com até 3 cm de diâmetro duplicatas foram coletadas, enquanto para os demais espécimes de diâmetro maior, apenas uma unidade foi coletada. A análise dos macronutrientes incluiu carbono (C), nitrogênio (N), cálcio (Ca), magnésio (Mg), fósforo (P) e potássio (K). Os micronutrientes analisados foram zinco (Zn), cobre (Cu), enxofre (S), boro (B), ferro (Fe) e manganês (Mn). Os teores de nutrientes foram analisados seguindo as determinações de Lutz (1985), e a relação C:N foi calculada a partir dos resultados da análise química, segundo Mantovani *et al*. (2007).

3.5 Estatísticas dos dados

Os dados relacionados as interações dos fungos com a flora e fauna foram computados e submetidos a análise variância ANOVA, quando os valores de F se mostraram significativos, foi aplicado o Teste Tukey a 5% de significância (ESTAT, 1994). Para os dados multivariados o programa BioEstat v.5.3 (SANTOS, 2007) foi utilizado.

4 APRESENTAÇÃO DA PESQUISA E ANÁLISE DOS RESULTADOS

Os resultados deste trabalho são apresentados na forma de capítulos. Capítulo I: "Diversity and Ecological Associations of Pluteaceae (Fungi, Agaricomycetes, Agaricales) in Brazilian Biomes", publicado em 28 de agosto de 2022, Brazilian Journal of Environment, ISSN: 2595-4431, DOI: https://doi.org/10.5281/zenodo.7321817, carta de consentimento no Anexo I.

Capítulo II: "A relationship between fungi (Basidiomycota, Agaricomycetes, Agaricales) and nutrient content in riparian area of reforestation with *Eucalyptus grandis* W. Hill ex Maiden (Myrtaceae) in southern Brazil", publicado em 30 de novembro de 2023, Hoehnea, ISSN: 2236-8906, DOI: https://doi.org/10.1590/2236-8906e262023, carta de consentimento no Anexo II.

Capítulo III: "Aves and Fungi interactions in a review global of mycophagy and its associations in wildlife and industry", submetido em 26 de junho de 2023, aceito para publicação em 14 de dezembro de 2023, Rodriguésia, ISSN: 2175-7860, comprovação do aceite no Anexo III.

Capítulo IV: "The bird shiny cowbirds (*Molothrus bonariensis*) in a relationship interesting of mycophagy with the mushroom *Macrolepiota bonariensis* in the brazilian Pampa biome", publicado em 24 de maio de 2022, Studies in Multidisciplinary Review, ISSN 2764-4782, DOI https://doi.org/10.55034/smrv3n2-011, com carta de consentimento no Anexo IV.

Capítulo V: "First report of *Zygothrica candens* Burla, 1956 (Diptera, Drosophilidae) in mycophagic association with the mushroom *Oudemansiella cubensis* (Berk. and M.A. Curtis) R.H. Petersen, 2010 (Agaricales, Physalacriaceae) in southern Brazil", publicado em 30 de dezembro de 2022, Brazilian Journal of Biology, ISSN: 1678-4375, DOI: https://doi.org/10.1590/1519-6984.267871, carta de consentimento no Anexo V.

Capitulo VI: "Mycophagy of Attini Ants (Hymenoptera, Formicidae, Myrmicinae) with Agaricales Mushrooms (Basidiomycota, Agaricomycetes) at Riparian Zone in Southern Brazil", publicado em 30 de novembro de 2022, Brazilian Journal of Animal and Environmental Research, ISSN: 2595-573X, DOI: https://doi.org/10.34188/bjaerv5n4-039, carta de consentimento no Anexo VI.

5 CAPÍTULOS

5.1 Capítulo I

Título: Diversity and Ecological Associations of Pluteaceae (Fungi, Agaricomycetes, Agaricales) in Brazilian Biomes

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Diversity and Ecological Associations of Pluteaceae (Fungi, Agaricomycetes, Agaricales) in Brazilian Biomes

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ABSTRACT

The Pluteaceae family is recorded in 10 Brazilian states, containing lignicolous, terricolous and humicolous species that perform fundamental functions as saprobes. This study aimed to contribute to a better understanding of the interactions and ecological importance of the family. For this, through a synthesis of taxonomic, morphological, and molecular data, the phylogenetic relationships of the group were reconstructed by Maximum Likelihood and Maximum Parsimony. Georeferenced data were used to elucidate the distribution of the species in Brazilian biomes. Phylogenetically, the family integrated a monophyletic clade, where the genera *Pluteus* and *Volvariella* were internally related as sister clades. In relation to the ecology of Pluteaceae, the Southeastern Region was very important, containing about 32% of the family species occurring near watersheds. 62% of the species of the group have occurrence restricted to the Atlantic Forest biome, and many of them are associated with native arboreal substrates, such as *Roupala* sp., *Dicksonia* sp., *Clitoria* sp., and *Astrocaryum aculeatissimum*. In general, the largely lignicolous family acts in the ecosystems as nutrient cycles, mainly related to wood. Also, species of the family with colonization in litterfall and terricolous represented unique interactions involving native arboreal groups and occurrences in areas of intense anthropic action, such as agriculture. Thus, with the compiled data in this study, it was possible to infer that Pluteaceae is directly associated with the regeneration of Brazilian biomes, actively helping in their maintenance.

Keywords: Evolutionary relationships, Ecology of Agaricales, Ecosystems.

Diversidade e Associações Ecológicas de Pluteaceae (Fungi, Agaricomycetes, Agaricales) nos Biomas Brasileiros

RESUMO

A família Pluteaceae é registrada em 10 estados brasileiros, contendo espécies lignícolas, terrícolas e húmicas que desempenham papéis fundamentais como sapróbios. Este estudo teve como objetivo contribuir para uma melhor compreensão das interações e da importância ecológica da família. Para tanto, através de uma síntese de dados taxonômicos, morfológicos e moleculares, as relações filogenéticas do grupo foram reconstruídas por Máxima Verossimilhança e Máxima Parcimônia. Os dados georreferenciados foram utilizados para elucidar a distribuição das espécies nos biomas brasileiros. Filogeneticamente, a família integrou um clado monofilético, onde os gêneros *Pluteus* e *Volvariella* se relacionaram internamente como clados irmãos. Em relação à ecologia de Pluteaceae, a Região Sudeste foi muito importante, contendo cerca de 32% das espécies da família ocorrendo próximas a bacias hidrográficas. 62% das espécies do grupo possuem ocorrência restrita ao bioma Mata Atlântica, e muitas delas estão associadas a substratos arbóreos nativos, tais como *Roupala* sp., *Dicksonia* sp., *Clitoria* sp. e *Astrocaryum aculeatissimum*. Em geral, a família é em grande parte lignícola e atua nos ecossistemas como cicladores de nutrientes, principalmente os relacionados à madeira. Além disso, espécies da família de colonização em serapilheira e terrícolas apresentaram interações únicas envolvendo grupos arbóreos nativos, e ocorrências em áreas de intensa ação antrópica como a da agricultura. Assim, com os dados compilados neste estudo, foi

Costa, A.L., Lopes, C.F., Ferraz, K.R., Heberle, M.A., Putzke, J. (2022). Diversity and Ecological Associations of Pluteaceae (Fungi, Agaricomycetes, Agaricales) in Brazilian Biomes. **Brazilian Journal of Environment**, v.10, n.2, p.219-239.

possível inferir que Pluteaceae está diretamente associada à regeneração dos biomas brasileiros, auxiliando ativamente na sua manutenção.

Palavras-Chaves: Relações evolutivas, Ecologia de Agaricales, Ecossistemas.

1. Introduction

Agaricales include the fungi popularly known as mushrooms, being a temporary phase called basidiome that emerges on the soil surface during the reproductive cycle (Azevedo & Barata, 2018). This important structure also underlies most of the taxonomic classification of its group, through its morphological, anatomical, and microchemical characters (Putzke & Putzke, 2017). However, phylogeny is increasingly contributing to a better understanding of the evolutionary relationships of these individuals (James et al., 2006). With approximately 364 species (Ainsworth, 2008), Pluteaceae (Agaricales, Basidiomycota) contains distinctive macromorphological features for the agaricoids fungi, such as the presence of a well-developed volva in some species of *Volvariella*, annulus absent as in *Pluteus* or rarely present as in *Chamaeota*, also have a pink to pinkish-brown spore print (Putzke & Wartchow, 2008).

In the Brazil, about 104 species (including cf. and var.) of Pluteaceae have already been listed (Putzke & Wartchow, 2008; Maia et al., 2015; Putzke & Putzke, 2018). Many of them after revisions were reported as similar or not belonging to their taxonomic designation (Menolli, 2013; Menolli et al., 2015; Menolli & Capelari, 2016). The family is composed of four distinct genera: *Pluteus*, *Volvariella*, *Chamaeota*, and recently by *Volvopluteus* segregated from *Volvariella* (Singer, 1986; Ainsworth, 2008; Justo et al., 2011). Among the main microscopic features, hymenial cystidia are usually present, metuloid or not metuloid with thick-walled, inverse hymenophore trama, basidiospores globose to ellipsoid, thick-walled, smooth, inamyloid, and cynaphilic (Singer, 1986).

In relation to the ecology of the family, in Brazil many are associated with native vegetation, composed of forests and woodlands, also in riparian zones (Menolli et al., 2015). The species are saprobic with lignicolous, terricolous, and humicolous habits, occurring in 10 Brazilian states: Amazonas - AM, Bahia - BA, Mato Grosso - MT, Minas Gerais - MG, Pará - PA, Paraná - PR, Rio de Janeiro - RJ, Rio Grande do Sul - RS, Rondônia - RO, and São Paulo - SP (Putzke & Wartchow, 2008; Maia et al., 2015; Putzke & Putzke, 2018). For the members of this group, the genera *Pluteus* and *Volvariella* have been distributed in three of the six Brazilian biomes (Amazon, Atlantic Forest, and Pampa). Most *Pluteus* species grow on wood (trunks and branches) or litterfall, while *Volvariella* on the soil (Menolli & Capelari, 2016).

The designation lignicolous for the family marks an important ecological interaction, even though there are exceptions, most species of Pluteaceae grow on wood causing its decomposition (de Mattos, 2020). Thus, they act in the cycling go organic matter through its reintegration into the ecosystem (Lonsdale et al., 2008; Carvalho et al., 2022). Carbon is the main source of the relationship between fungi and vegetation (Baldrian et al., 2016). However, this interaction depends of vectors, such as the type of vegetation, soil conditions, and species diversity (Abrego & Salcedo, 2014; Walthert & Meier, 2017; Copot & Tănase, 2019). Also, geographic, climatic, precipitation, and temperature conditions directly influence the communities of saprobe fungi (Tedersoo et al., 2014; Borgmann-Winter et al., 2022).

In this form, due to the significance of Pluteaceae for Brazilian biomes, this study aimed to contribute to a better understanding of the interactions and ecological importance of the family. For this, a review was performed in the form of a synthesis involving taxonomic, morphological, molecular, and biogeographic data, where distinct characters of the group bionomy were elucidated.

2. Material and Methods

2.1 Bibliographical search and database

For the species of Pluteaceae with occurrence in Brazil (Table 1), a search bibliographic was made containing data of public digital platforms and printed works. The following keywords were used: Pluteaceae, taxonomic description, and new species. The data available in Putzke & Putzke (2018) for the family were used as the guide for research.

Table 1 - Pluteaceae species with occurrence in Brazil with molecular and geographical data.

Species	Access code	BRA states	References
Pluteus albostipitatus	HM562130.1	PR, RS, AM, SP	Menolli et al. (2015)
**Pluteus amazonicus*	KF937355.1	AM	Singer (1961)
**Pluteus anomocystidiatus*	-	PR	Menolli (2013); Menolli et al. (2015)
Pluteus angustisporus	-	PR	Menolli (2013)
Pluteus aquosus	-	RS	Wartchow et al. (2006)
Pluteus argentinensis	-	MG, SP	Rosa & Capelari (2009); Menolli (2013); Dias & Cortez (2013)
**Pluteus aureolatus*	KM983680.1	AM	Menolli (2013)
**Pluteus aureovenatus*	HM562160.1	SP	Menolli et al. (2010)
Pluteus beniensis	JQ065029.1	PR, RS	Wartchow et al. (2006); de Meijer (2006); Menolli & Capelari (2016)
**Pluteus brunneocrinitus*	KM983692.1	SP	Menolli (2013); Menolli et al. (2015)
Pluteus brunneopictus	JF908623.1	RS	Menolli & Capelari (2016)
**Pluteus cebolinhae*	KM983713.1	SP	Menolli (2013); Menolli et al. (2015)
Pluteus cervinus	-	RS, PR	Menolli (2013); Dias & Cortez (2013)
Pluteus chrysophlebius	HM562088.1	PR	Menolli et al. (2010); Menolli (2013); Menolli et al. (2015)
Pluteus chusqueae	-	PR	Menolli (2013)
**Pluteus crassocystidiatus*	-	PR	Menolli et al. (2015)
**Pluteus crassus*	MW050978.1	PR	de Meijer (2006); Menolli (2013)
Pluteus crinitus	KM983691.1	AM	Menolli (2013); Menolli et al. (2015)
Pluteus cubensis	-	PR	Rosa et al. (2003); Xavier-Santos et al. (2004); de Meijer (2006); Rosa & Capelari (2009); Menolli et al. (2015)
**Pluteus densifibrillosus*	HM562159.1	SP	Menolli et al. (2010)
Pluteus diptychocystis	KM983674.1	SP, RN	Menolli (2013); Menolli & Capelari (2016)
Pluteus dominicanus	FJ816665.1	SP	Menolli et al. (2010)
Pluteus eludens	HM562185.1	PR	Menolli (2013)
**Pluteus elvaniae*	-	RS	Wartchow (2018)
Pluteus cf. *fastigiatus*	-	AM	Menolli & Capelari (2016)
Pluteus cf. *fernandezianus*	JQ065028.1	RS	Menolli (2013)
Pluteus fibulatus	-	PR, RS	Menolli (2013)
**Pluteus fluminensis*	FJ816664.1	RJ, PR	Menolli et al. (2010); Menolli & Capelari (2016)
Pluteus fuligineovenosus	FJ816662.1	SP	Menolli & Capelari (2016)
Pluteus cf. *fuliginosus*	-	SP	Menolli & Capelari (2016)
Pluteus fusconigricans	-	PR	Menolli (2013)

Pluteus glaucotinctus	HM562157.1	PR, SP	Wartchow et al. (2006) Menolli & Capelari (2008); Menolli (2013)
Pluteus glaucus	-	SP	Stijve & de Meijer (1993); Guzmán & Guevera (1998)
Pluteus globiger	JQ065030.1	RS, PR	Wartchow et al. (2006); Dias & Cortez (2013)
**Pluteus halonatus*	-	AM	Menolli et al. (2015)
Pluteus harrissi	-	SP, MG, PR	de Meijer (2006); Rosa & Capelari (2009); Menolli (2013)
Pluteus haywardii	-	MG	Rosa & Capelari (2009);
**Pluteus hispidulopsis*	-	RN	Menolli et al. (2015)
Pluteus homolae	-	SP	Menolli (2013)
**Pluteus hylaeicola*	-	PA, PR	Menolli (2013); Menolli et al. (2015)
Pluteus iguazuensis	-	RS, PR, AM	de Meijer (2006); Drehmel et al. (2008); Menolli (2013); Menolli & Capelari (2016)
Pluteus jamaicensis	FJ816657.1	SP	Menolli et al. (2010)
**Pluteus karstedtiae*	KM983682.1	SP, RJ	Menolli (2013)
Pluteus longistriatus	HM562172.1	SP, RJ, PR	Menolli et al. (2010); Menolli (2013)
Pluteus maculosipes	-	PR	Menolli (2013)
Pluteus meridionalis	KJ009767.1	SP	Menolli et al. (2014)
Pluteus nanus	KF306030.1	RS	Menolli & Capelari (2016)
**Pluteus necopinatus*	KM983693.1	RJ	Menolli (2013)
**Pluteus neochrysaegis*	-	PR	Menolli (2013)
Pluteus nigrolineatus	FJ375245.1	RS	Wartchow et al. (2006)
**Pluteus paucicystidiatus*	-	SP	Menolli (2013)
Pluteus phlebophorus	HM562039.1	RS	Singer (1961); Menolli & Capelari (2016)
Pluteus pluvialis	-	RS	Menolli & Capelari (2016)
Pluteus pulverulentus	GU551943.1	PR	de Meijer (2006); Menolli & Capelari (2016)
**Pluteus puttemansii*	HM562164.1	SP	Menolli et al. (2010)
Pluteus riberaltensis	HM562162.1	SP	de Meijer (2006); Menolli et al. (2010);
Pluteus rimosellus	-	PR	Menolli (2013)
Pluteus rimosoaffinis	KM983706.1	BA, SP, PR, RS	de Meijer (2006); Menolli (2013)
Pluteus riograndensis	-	PR, RS	Raithelhuber (1991); Menolli & Capelari (2016)
Pluteus salicinus	JF908625.1	PR	Stijve & de Meijer (1993); de Meijer (2006)
Pluteus sapiicola	KM983707.1	PR	Menolli (2013)
Pluteus striatocystis	-	PR	Menolli (2013)
**Pluteus subfibrillosus*	-	RJ, PR, RS	Menolli et al. (2014)
**Pluteus sublaevigatus*	-	SP, PR	Menolli et al. (2010); Menolli (2013)
Pluteus umbrionalbidus	-	RS, PR, MG, SP	Singer (1958); Rosa & Capelari (2009); Menolli et al. (2010)
Pluteus variipes	-	SP	Menolli & Capelari (2016)
**Pluteus varzeicola*	-	AM	Singer (1961)
Pluteus velutinus	KR022027.1	PA	Menolli (2013)
Pluteus viscidulus	HM562110.1	RS	Singer (1961)

Pluteus xylophilus	HM562163.1	RS, SP, PR	de Meijer (2006); Menolli et al. (2010); Menolli (2013)
Volvariella bombycina	HM562212.1	RS, SP, PR	Sobestiansky (2005); de Meijer (2006); Menolli & Capelari (2008)
Volvariella cubensis	-	PE	Wartchow (2009)
Volvariella earlei	HM246497.1	PR	de Meijer (2006)
Volvariella gloiocephala	JN182873.1	RS, PR	de Meijer (2006)
**Volvariella heterospora*	-	SP	Menolli & Capelari (2008)
Volvariella macrospora	-	AM	Singer (1961)
**Volvariella nullicystidiata*	-	SP	Menolli & Capelari (2008)
**Volvariella oswaldoi*	-	PE	Batista (1957); Putzke (1994)
Volvariella perciliata	EU920672.1	SP, RS	de Meijer (2006); Menolli & Capelari (2008)
Volvariella pusilla	HM246494.1	RS	Singer (1961)
**Volvariella rondoniensis*	-	RO, PR	de Meijer (2006)
Volvariella speciosa	-	PE	Batista (1957)
Volvariella cf. *striata*	-	PE	de Meijer (2006)
Volvariella taylori	-	PR	de Meijer (2006)
Volvariella volvacea	HM367073.1	PR	de Meijer (2006)

Note: Accession numbers with prefixes are available in National Center for Biotechnology Information (NCBI) GenBank (www.ncbi.nlm.nih.gov). Occurrence/sampling location according to references. (-) Indicates species without molecular data but with computed morphological data (see methodology in 2.2). (*) Indicates species with occurrence only in Brazil. Source: Authors (2021).

2.2 Molecular and taxonomic data

For the species of Pluteaceae, a search of molecular data was performed in the National Center for Biotechnology Information (NCBI) GenBank (https://www.ncbi.nlm.nih.gov/). The markers were selected according to their availability for species during the *blast* search. After a preliminary evaluation, parts of the 5.8S, 18S and ITS1-2 genes were tested considering their mutations rates. In total, 44 species of Pluteaceae in a matrix with 529 characters were selected (Table 1). As outgroups, *Amanita campinaranae*, *Amanita craseoderma*, and *Amanita lippiae* were used due to their phylogenetic proximity with the family (Matheny et al., 2006). All sequences were aligned in CodonCode Aligner v.3.7 (Richterich, 2004) with the option *muscle* and *gap* activated.

A matrix with morphological characters according to the bibliography was elaborated. The data available in Putzke & Putzke (2018) was used for the build of the matrix. The following morphological characters were used: presence or absence of volvulus; with or without annulus; diameter and form of the pileus; lamellae form and insertion; size and form of the stipe; morphology of spores and basidia; the arrangement of the lamella trama; cortical layer type; fibulae; habit; habitat, and substrate. The matrix integrated 88 species (three outgroups) and 73 binary characters. In the program Mesquite v.2.7 (Maddison & Maddison, 2007) the data were compilated.

The molecular and morphological data were concatenated in SequenceMatrix v.1.8 (Vaidya et al., 2011). Constructed matrix was used for Maximum Parsimony (MP) *bootstrap* in TNT v.1.5 (Goloboff, et al., 2008) with “Traditional” searches for more parsimonious trees starting at 1.000 replications with the TBR algorithm, keeping 100 cladograms at each step of the heuristic search (*hold* = 100). In TNT, the nodes containing the synapomorphies were analyzed using the “*Collapse*” function. For Maximum Likelihood (ML), conducted in RAxML-GUI v.1.5 (Silvestro & Michalak, 2012) with GTR model, keep 200 rounds calculated with 1.000 repetitions for the fast *bootstrap* reconstruction of the more likelihood, using the “*New Rapid Hill-Climbing”* algorithm (Stamatakis et al., 2008), and ML search activated. ML tree was used as the master tree, and therefore its topology and branch lengths were used for the presentation of the relationships built in this study.

2.3 Georeferencing of specimens

A file in the Excel program (Walkenbach, 2010) containing the geographical origin of the specimens described to Brazil and their geographical coordinates in decimal (latitude and longitude) was prepared, according to the data available in the literature (Table 1). The proportions of species distributions in biomes were computed, and the percentages were calculated in Excel by Fávero & Belfiore (2017) model. The projection from the place of occurrence of specimens in Brazilian biomes was performed in the program R v.3.6 (Ihaka & Gentleman, 1996). For the plotting of data, the packages "*phytools*" and "*mapdata*" (Revell, 2012; Becker & Wilks, 2016) were used.

3. Results

3.1 Phylogenetic analysis of the dataset

The survey conducted to Pluteaceae occurring in Brazil compiled 85 species from family and three outgroups in a matrix with 602 characters (molecular and morphological data). *Pluteus* and *Volvariella* genera with occurrence in Brazil had moderate to high MP and ML *bootstrap* support in phylogeny, according to the relationships (Figure 1).

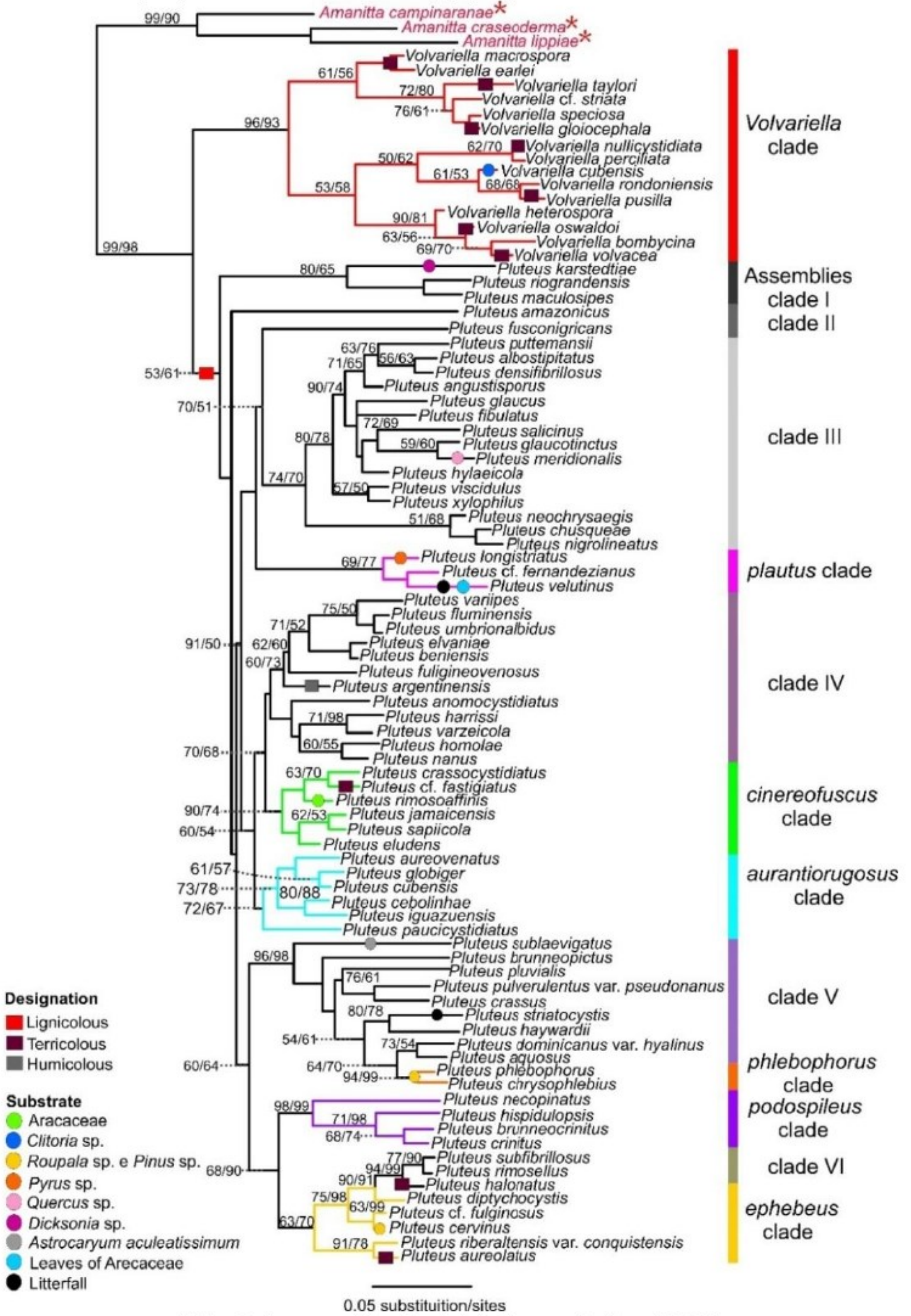
The clade *Volvariella* was listed as internally monophyletic, maintaining high support (ML = 96% and BP = 93%), and externally as clade sister of *Pluteus* (Figure 1). Due to the internal topology of the group not being well resolved in the literature, the precepts by Justo et al. (2011) were maintained according to the identification key for the Brazilian species of *Volvariella* (Putzke & Putzke, 2018).

Pluteus clade was related as monophyletic with moderate support (ML = 53% and MP = 61%). However, many of the relationships among closely related species have been recovered internally. Clades already described in the literature maintained high *bootstrap* support for the species belonging to the sections: *Volvariella* (ML = 96% and MP = 93%), *plautus* (ML = 69% and MP = 77%), *cinerofuscus* (ML = 90% and MP = 74%), *aurantiorugosus* (ML = 72% and MP = 76%), *phlebophorus* (ML = 94% and MP = 99%), *podospileus* (ML = 98% and MP = 99%), and *ephebeus* (ML = 63% and MP = 70%). *Pluteus* assemblages containing the remaining species reconstructed clades with moderate to high *bootstrap* support (Figure 1).

Synapomorphies related to the habit, habitat, and substrate were recovered according to the inferred description for each species, and many of them integrated the same section and share some designation. The clades of assemblies III, IV, V, and VI, *cinerofuscus*, *aurantiorugosus*, *phlebophorus*, *podospileus*, and *ephebeus* were part of the lignicolous. The presence of terricolous species among the lignicolous clades demonstrates the emergence of this behavior more than once during the evolutionary history of the group, according to breach length in the IV and VI assemblages, in *Volvariella*, *cinerofuscus*, and *ephebeus* clades (Figure 1).

Overall, speciation processes in relation to the arboreal association character were dispersed in the phylogeny, where most of the designated species formed paraphyletic and polyphyletic internal subclades, such as *P. karstedtiae* associated with *Dicksonia* sp. (Dicksoniaceae); *P. meridionalis* to *Quercus* sp. (Fagaceae); *P. longistriatus* to *Pyrus* sp. (Rosaceae); *P. cervinus*, *P. chrysophlebius* and *P. phlebophorus* with *Roupala* sp. (Proteaceae) and *Pinus* sp. (Pinaceae); *V. cubensis* to *Clitoria* sp. (Fagaceae); *P.rimosoaffinis* to Aracaceae; and *P. sublaevigatus* to *Astrocaryum aculeatissimum* (Arecaceae). Among the other associations, the arboreal species were not identified in the literature, being described only as branches and trunks of Gymnosperms or Angiosperms (Figure 1).

Figure 1 - ML master tree with reconstructed phylogenetic relationships. Supports obtained in *bootstrap* Maximum Likelihood (ML) and Maximum Parsimony (MP) with values next to the branches.



Note: Outgroups with red asterisk. Source: Authors (2021).

Some clades showed speciation in relation to substrate type and maintained close relationships, such as *phlebophorus* (ML = 94% and MP = 99%), which grows on branches and trunks of *Roupala* sp. and *Pinus* sp. However, some species such as *P. cervinus* maintained distant relationships even though they shared the same substrate type. Other similarities involving distant species of litterfall such as *P. striatocystis* and *P. velutinus* related within the pluteoid clade, but with distinct recent ancestry.

3.2 Distribution and characteristics of the species in the biomes

Among the Pluteaceae species analyzed, about 81% occur only in a single biome, 14% occur in two distinct biomes, and only 5% occur in three of the six Brazilian biomes. 62% of species occur only in the Atlantic Forest, 8% only in the Pampa, and 9% only in the Amazon. 17% occur in both Atlantic Forest and Pampa, 2% in the Amazon and Atlantic Forest, 2% occur in all three biomes (Atlantic Forest, Amazon, and Pampa), and there are not species that share occurrence in the Pampa and Amazon. It is important to emphasize the lack of occurrence records of Pluteaceae in the Cerrado, Pantanal, and Caatinga (Figure 2).

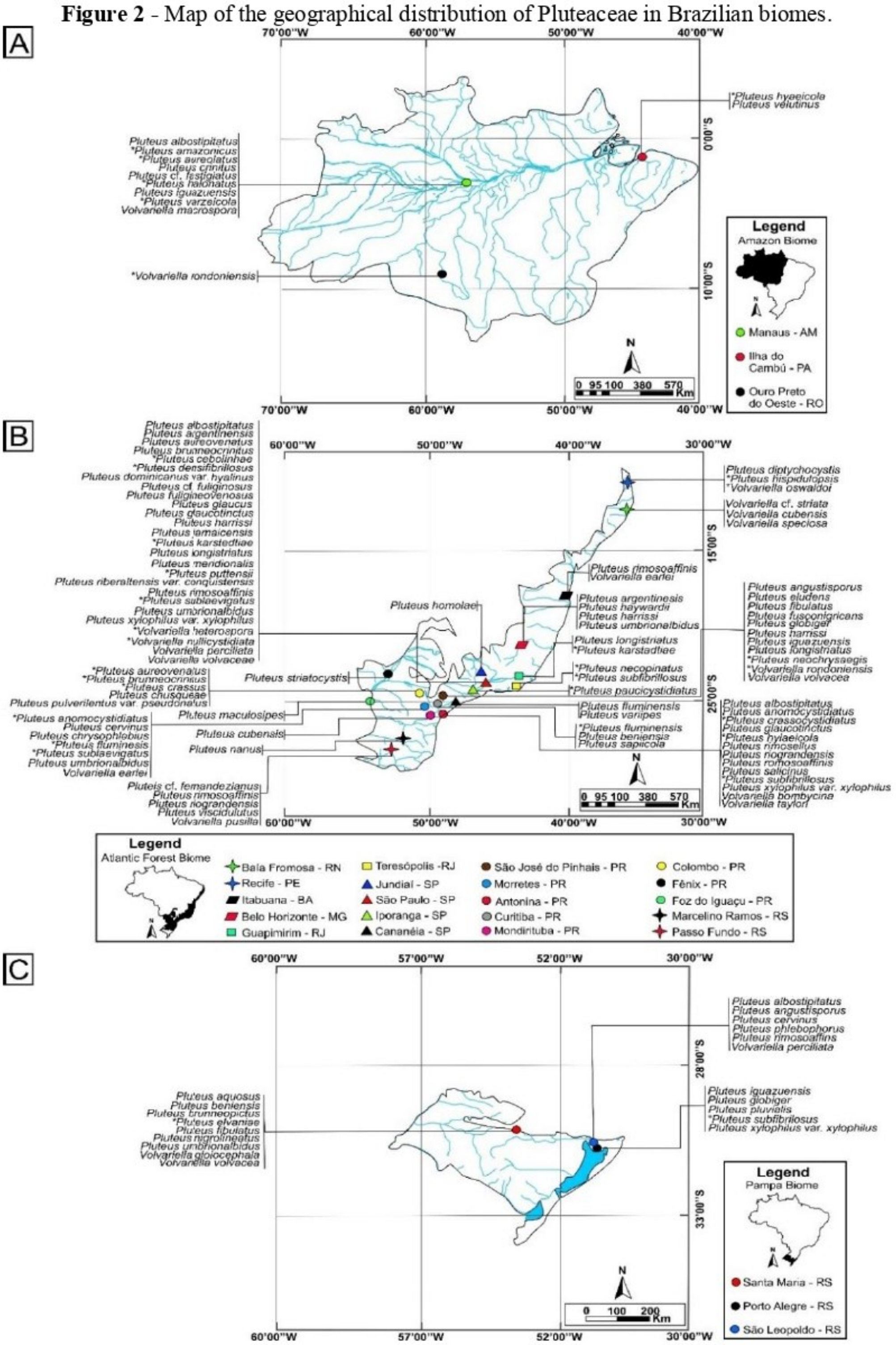
The Southeastern and Southern Regions of Brazil, composed of Atlantic Forest and a portion of the Pampa biome, were the Brazilian regions with the highest occurrence and diversity of species of Pluteaceae. In these regions, 32% of the species of the family occurred near waterways and hydrographic basins that are important to the region, such as in Parque Estadual das Fontes do Ipiranga - SP, Parque Estadual da Cantareira - SP, and Reserva Natural Rio Cachoeira in Antonina - PR. For these species, 50% were restricted to these regions (Figure 2).

In the Northeastern Region, the Atlantic Forest domain was found a higher occurrence of restricted and phylogenetically close species of Pluteaceae, such as *V. oswaldoi*, *V. cubensis*, *V. speciosa*, and *V.* cf. *striata*. While the Amazon biome showed a higher occurrence of restricted species, but distantly related as *V. macrospora*, *P. amazonicus*, *P. velutinus*, *P. varzeicula*, *P.* cf. *fastigiatus*, *P. crinitus*, *P. holonatus*, and *P. aureolatus* (Figure 2).

The Pampa biome and Atlantic Forest showed the occurrence of most phylogenetically close species with the concomitant distribution. Among them *P. cervinus*, *P. subfibrilosus*, *P. beniensis*, *P. umbrionalbidus*, *P. fibulatus*, *P. angustisporus*, *P. albostipiatus*, *P. iguazuensis*, and *P. globiger* that had occurred in both the biomes, are closely related and have a lignicolous habit. As well as *V. nullicystidiata*, *V. perciliata*, *V. speciosa*, and *V.* cf. *striata*, which are terricolous species (Figures 1 and 2).

Generalist species of occurrence in all three biomes and different localities maintained distant relationships but internally integrated into the pluteoid clade. Being them *P. iguazuensis*, *P. rimozoafins*, *P. umbrionalbidus*, *P. longistriatus*, *P. harrissi*, *P. xylophilus*, and *P. albostipiatus*, with exception of *V. volvacea*, which is part of the sister clade *Volvariella* (Figures 1 and 2).

Figure 2 - Map of the geographical distribution of Pluteaceae in Brazilian biomes.



Note: * Indicates endemic species from Brazil. Amazon Biome (A). Atlantic Forest Biome (B). Pampa Biome (C). Map follows with the main water resources. Source: Authors (2021).

4. Discussion

4.1 Diversity of Pluteaceae near watercourses

The Parque Estadual das Fontes do Ipiranga - SP is an Atlantic Forest fragment is integrated by riparian zone that protects the water resources of Riacho do Ipiranga (Tanus et al., 2012). In this location the species *P. meridionalis*, *P. riberaltensis*, *P.* cf. *fuliginosus*, *P. argentinensis*, and *P. aureovenatus* endemic from Brazil had an occurrence record (Rosa & Capelari, 2009; Menolli et al., 2010; Dias & Cortez, 2013; Menolli, 2013; Menolli & Capelari, 2016). In a study conducted by Leal & Gugliotta (2008) in the region, 108 species of Basidiomycetes were registered, but none of them belonged to Pluteaceae. However, the major ecological importance listed for the region was at the fungal succession involved in the action that transforming source of organic matter, dynamizing the trophic chain at the area. Thus, it is possible to infer that the pluteoid group plays this same ecological role since they are designated also saprobic.

Integrating part of Serra da Cantareira, the Parque Estadual da Cantareira - SP Atlantic Forest domain, were registered endemic species, such as *P. brunneocrinitus*, *P. dominicanus* var. *hyalinus*, *P. jamaicensise*, and *P. fuligineovenosus* that occur only in this biome. Some the species endemic from Brazil also were found in area as *P. densifibrillosus*, *V. heterospora*, and *V. nullicystidiata* (de Meijer, 2006; Menolli & Capelari, 2008; Menolli et al., 2010; Menolli, 2013; Menolli et al., 2015; Menolli & Capelari, 2016). Containing micro-watersheds of Ribeirão Santa Inês, Ribeirão das Águas Claras, Ribeirão São Pedro, Rio Baquirivu, Rio Cabuçu, and Córrego Cabuçu (Brasil, 2006), riparian zones along waterways of region play roles in water drainage, nutrient absorption and retention, and also serve as a filter for metropolitan air pollution (Feitosa & Ribeiro, 2005). For the fungal community of this area, in a study involving 23 species of wood-degrading Basidiomycota pointed that the macronutrients: nitrogen, potassium, calcium, magnesium, sulfur, and phosphorus; and micronutrients: boron, chlorine, molybdenum, copper, iron, zinc, and manganese; were more abundant in this soil due to fungal presence (Hamaguchi & Vitali, 2014). The Pluteaceae species that occur in this region are also lignicolous. Therefore, the group also acts in this area as nutrient cycling, mainly connected to the wood.

The Reserva Natural Rio Cachoeira in Antonina - PR, Atlantic Forest domain, has recorded most of the restricted pluteoid species in biome single. *P. rimosoaffinis*, *P. crassocystidiatus*, *P. anomocystidiatus*, *P. salicinus*, *V. bombycine*, and *V. taylori* were recorded in this area. Also, the local shown greater pluteoid diversyty containing species generalist as *P. albostipitatus*, *P. glaucotinctus*, *P. rimosellus*, *P. subfibrillosus*, *P. xylophilus*, *P. riograndensis*, and *P. hylaeicola* that is endemic from Brazil (Raithelhuber, 1991; Sobestiansky, 2005; de Meijer, 2006; Menolli & Capelari, 2008; Menolli, 2013; Menolli et al., 2014; Menolli et al., 2015; Menolli & Capelari, 2016). The 8.600 hectare reserve consists of Dense Alluvial Ombrophylous Forest in Rio das Cachoeiras and its effluents, which is going through a regeneration period due to the high anthropic action of the past (IBGE, 2020). In this aspect, the saprobe fungi assist at various levels during the forest regeneration process (Xavier & de Holanda, 2015). Abrahão et al. (2019) when analyzing the abundance of macrofungi in this region, inferred that 30% of the 796 species collected were associated with the riparian zone, listed as having the highest diversity when compared to the Semideciduous Forest. Borba et al. (2015) showed that the diversity of Basidiomycetes, including species of Pluteaceae in Atlantic Forest in Parque Natural Municipal Nascentes do Garcia, Blumenau - SC, was related to microclimate and vegetation type, both important factors for the diversity of this group in the riparian zone at local.

In general, the Atlantic Forest biome exhibited a diverse group of Pluteaceae species occurring near important watersheds and water resources. The biome has high rates of endemism, not only for Pluteaceae, but also for many other fungal species (Putzke & Putzke, 2017). The regions mentioned above come from anthropic action and significant habitat loss (IBGE, 2020). Even with public policies that seek its preservation, there is a need for further studies aimed at the importance of their bionomy. In this premise, fungi act by providing nutritional raw material mainly those related to cycling processes, acting directly on the regeneration of the environment (Leal & Gugliotta, 2008).

4.2 Pluteoid interactions with arboreal species

Pluteaceae is a family lignicolous and little proportion terricolous, where the *Volvariella* clade contained most of the species with occurrence on the soil (Singer, 1986; Putzke & Wartchow, 2008; Maia et al., 2015; Putzke & Putzke, 2018). For the Brazilian species, distinct specifications occur because some besides growing on wood (Gymnosperms and Angiosperms), also cause its decomposition, such as the clade *phlebophorus*, *podospileus*, *ephebeus*, *aurantiorugosus*, and *cinerofuscus* (de Meijer, 2006; Menolli & Capelari, 2008; Menolli, 2013; Menolli et al., 2014; Menolli et al., 2015; Menolli & Capelari, 2016). Others have relationships specific with substrates, such as *P. meridionalis* which grows on branches of *Quercus* sp. (Menolli et al., 2014). The pluteoid species have an important function in the cycling of matter from the native arboreal (Kew, 2018). At the Atlantic Forest fragment site, it contributes to carbon cycling (Baldrian et al., 2016), assisting in the homeostasis of the ecosystem.

P. cervinus grows on branches and trunks of *Roupala* sp. (native) and *Pinus* sp. (exotic) (Menolli, 2013), occurring in two distinct biomes and sharing between them the same substrate type. In Pampa and in Atlantic Forest, both the local contained artificial forests of *Pinus* sp. that were introduced for reforestation (Vasquez et al., 2007) due to its rapid growth and adaptation to acidic soils (da Silva et al., 2018). Also, *P. phlebophorus* and *P. chrysophlebius* share these same substrates in these biomes (Miranda-Melo et al., 2007; Menolli et al., 2010; Menolli, 2013). In this premise, Xavier & Holanda (2015) reports that for these regions, lignicolous fungi act at various stages of decomposition and have a positive correlation in the regeneration of native vegetation.

Containing a distribution along the Brazilian coast in the Atlantic Forest domain, *Astrocaryum aculeatissimum* is the substrate associated with endemic species from Brazil *P. sublaevigatus* (Menolli et al., 2010; Menolli, 2013). At southeast coast, the native Brazilian palm acts as an ecological filter (Brancalion et al., 2010). Its fruits are consumed for small rodents, such as *Dasyprocta leporina* (agouti), *Trinomys iheringi* (rodents), and *Sciurus aestuans* (squirrel) (Oliveira et al., 2018). These consumers of the fruit are also largely its dispersers, aiding in the wide distribution of the species throughout the coast. Being that for *P. sublaevigatus*, the wide distribution of the host species may have aided in its occurrence in long of the Brazilian coast. Tedersoo et al. (2014) report that the distribution of the host arboreal species is directly related to its fungus-associated distribution.

P. longistriatus have as substrate *Pyrus* sp., popularly known in Brazil as pera or common pear (Menolli et al., 2010; Menolli, 2013). The fruit tree is exotic of nutritional and commercial value, introduced in Brazil of Asian origin. The pera acclimates well to the forests of the southeast, and its abundance is associated with its cultivation and dispersal of the seeds through the massive consumption of the fruit by the fauna (Veasey et al., 2011). Edman & Jonsson (2001) infer that the saprobic fungi are capable of numerous adaptations, as spores can travel up to 500 km and develop in a completely new location if conditions are suitable, especially when the substrate is also abundant.

P. karstedtiae had occurrences recorded in the Atlantic Forest domain. The pluteoid is endemic from Brazil and has as substrate *Dicksonia* sp. (Menolli, 2013). This genus contains about 20 cosmopolitan species, but some are native to Central and South America, and are endangered, such as *Dicksonia sellowiana* (Large & Braggins, 2004). In this form, fungi as *P. karstedtiae* that develop on branches and fallen leaves on the ground can help control soil quality, such as humidity, nitrogen, phosphorus, and carbon concentration, which are essential for the beneficial development of these plants (Sariyildiz, 2015).

P. rimosoaffinis grow on branches of Arecaceae (Menolli & Capelari, 2016). In general, these palms are distributed all over the country, are fruitful, and they also have economic importance in the manufacture of fibers (Baker & Dransfield, 2016). These species realized important interactions with saprophytic fungi for example, of the 14 genera described for Diatrypaceae (Ascomycetes, Xylariales), 9 were reported in associated with species of Arecaceae in South America (Konta, 2020). The occurrence of *P. rimosoaffinis* in Pampa and Atlantic Forest biomes, at diversified points corroborates with the theory of Vanderwal (2017), that infer the influence of fungal composition linked to the abundance of host arboreal species. In their study, the author links the abundance of saprobes *Bulgaria inquinans* (Phacidiaceae) and *Daedalea quercina* (Fomitopsidaceae) to the distribution of *Quercus* sp. at a forest in Romênia (Vanderwal, 2017).

V. cubensis shown occurrence only in the Atlantic Forest domain and has as substrate *Clitoria* sp. (Wartchow, 2009), native from tropical and subtropical forests (Gondim et al., 2005). For this arboreal species, the conservation status in Brazil has been aggravating in recent years, where some are threatened with extinction, such as *Clitoria froes* (IUCN, 2021). This factor may be related to the occurrence of *V. cubensis*, mainly to its endemism in the Northeast Region of the country. According to Hottola & Siitonen (2008), the density index of endemic trees and the associated saprobic leads to the conclusion that the greater the endemism of the plant, the lower the fungal occurrence.

In the Atlantic Forest and Pampa biomes, pluteoid species have mainly native Brazilian tree species as substrate. For the two biomes the fragmentation and habitat loss is constant, which is an aggravating factor for their conservation status (Vasquez et al., 2007). In the Atlantic Forest the urbanization, and in Pampa the agriculture and livestock are examples of anthropic actions in these ecosystems (IBGE, 2020). Thus, the species of Pluteaceae with specific substrate, such as those mentioned above, act together with the host tree species, mainly linked to nutrients from the same. In this form, they guarantee cycling and the return of these nutrients to the soil, which can later be used by new seedlings.

4.3 Pluteoid associations with litterfall

The litterfall substrate is unique and contains a large amount of macro and micronutrients compared to branches or trunks (Costa et al., 2015). Only two pluteoid species were reported associated with this substrate in Brazil. However, according to dos Santos et al. (2015), the macrofungi that are capable of colonizing litterfall are highly adapted to chemically varying conditions, which underscores their importance in these ecosystems.

P. velutinus is endemic from the Amazon domain grows on leaf sheaths of Arecaceae (Menolli, 2013). These palms have many associations with Basidiomycetes fungi. In the study carried in the Amazon biome by Mendoza et al. (2018) involving 12 fungal families, some species were associated with diverse portions of this plant, such as *Auricularia auricula-judae* (Auriculariaceae) to the trunk, *Hexagonia hydnoides* (Polyporaceae) to the branches, and *Mycena zephirus* (Mycenaceae) on leaves. In this context, the interaction of fungal species in relation to the diversity of the arboreal substrate can be associated with the trophic speciation of the group and its adaptation to the habitat (Albuquerque et al., 2010). For other samples described of *P. velutinus*, as in Italia, the species has been reported colonizing branches of *Quercus cerris* (Ferisin & Dovana, 2016), and in Russia on branches of *Castanea sativa* (Kalinina et al., 2020). In general, *P. velutinus* grow on wood (Pradeep et al., 2012), and in Brazil to Amazon biome the report on litterfall of palm trees is unique (Menolli, 2013).

P. striatocystis was the only specimen pluteoid in the Central-Western Region recorded in litterfall at the Atlantic Forest (Menolli, 2013). Inserted in an area that preserves one of the last remnants of the tropical forest due to high anthropic action (Scherer & Bispo, 2011). For the world, this is the second occurrence, the first registration was effectuated in Africa (Pegler, 1977), where *P. striatocystis* also was reported growing in litterfall. Santana et al. (2017) infer that the tropical forest ecosystems form a very varied substrate due to the climatic conditions of temperature and humidity, and this factor influences the broad fungal colonization. In the study of dos Santos et al. (2015) involving a checklist of fungi with occurred in tropical forests, the authors inferred that the litterfall fungi are the vastest group in distribution at intercontinental tropical regions.

However, the two species of Pluteaceae recorded in association with litter play an essential role as saprobes. Only one had a record in the Amazon domain, which holds the largest equatorial forest in the world (IBGE, 2020). In terms of conservation, the Amazon biome suffers large-scale habitat losses, in the face of the advance of deforestation linked to public development policies (Mendoza et al., 2018). The occurrence of pluteoid species in this region, as well as in the Atlantic Forest domain can assist in policies aimed at the conservation of both biomes since they are unprecedented records for Brazil, as well as for the world.

4.4 Soil nutrient cyclers

P. argentinensis grows in the soil and on dicotyledons humus (Rosa & Capelari, 2009; Menolli, 2013; Dias & Cortez, 2013). This important characteristic of the species reinforces an ecological association already reported by Nobre et al. (2010) when analyzing macrofungal species associated with wood humus, where the substrate was rich in modified lignin. The lignicolous fungi contain enzymes capable of degrading complex compounds, such as lignin and sometimes also hemicellulose and cellulose (Junior et al., 2021). The humus resulting from this important process can remain in the soil for hundreds of years and is essential for the continued renewal of the flora since this substrate increases the aeration, nutrient, and water absorption capacity of the soil (Abreu et al., 2022).

P. cf. *fastigiatus*, *P. aureolatus*, and *P. halonatus* with occurrence in Amazon biome were recorded growing solitary on soil (Menolli, 2013; Menolli et al., 2015; Menolli & Capelari, 2016). Medrado et al. (2019) infer that soil colonization is influenced by several biotic and abiotic factors, such as climate, soil chemical characteristics, and plant species growing in the area. In our reconstructions, the phylogenetic relationships among these species showed close. According to Mummey et al. (2010), the habitat of fungi may be a primary factor determinant for the structuration from your community. Corroborated with this theory, *P. aureolatus*, *P. halonatus*, and *P.*cf. *fastigiatus* were the only of the genus *Pluteus* with terricolous habitat. However, these soil saprobes are part of a large group of systematic fungi, with succession layers in diversified periods that change according to the seasons of the year. This characteristic helps in their constant performance in the nutritional maintenance of the soil (Oliveira et al., 2019).

Volvariella clade is predominantly terricolous with a large distribution in Atlantic Forest and Pampa biomes. *V. nullicystidiata, V. earlei, V. taylori, V. gloiocephala*, and *V. volvacea* occurs in both the biomes at more than one locality (de Meijer, 2006; Menolli & Capelari, 2008). Figueiredo et al. (2020) detailed the importance of terricolous macrofungi in the Atlantic Forest in the extreme south of Bahia. In the study, 77 of the sampled species (Basidiomycota and Ascomycota), 51% were associated with secondary vegetation in the middle stage of regeneration. According to the Secretaria do Meio Ambiente (Brasil, 2017), the fungi in the region perform an ecological action associated with the regeneration of the flora.

The presence of fungi in the soil brings numerous benefits, mainly related to carbon cycling (Baldrian et al., 2016). In the Brazilian biomes that suffer from anthropic action, the deconstruction of plant organic biomass by the enzymatic action of fungi is one of the most active interactions in these ecosystems (Floudas et al., 2012). *V. macrospora* was found in clay soil (Singer, 1961) and was reported in actuation as a nutrient drain and in aerating of the soil (Bononi et al. 2008).

In Atlantic Forest, the species endemic from Brazil *V. oswaldoi* had an occurrence registered on substrates of cotton crop culture of genera *Gossypium* sp. (Malvaceae) (Batista, 1957; Putzke, 1994). *V. pusilla* in the same biome also was found in agricultural soil (Singer, 1961). The saprobic fungi interacted in agricultural soil is inferred to Carrenho et al. (2007) as an aid in the degradation of organic matter and nutrient transport, also in energy flow and pathogen control. In a study conducted with the fungi *Hysterangium incarceratum* (Hysterangiaceae), *Laccaria fraterna* (Hydnangiaceae), and *Pisolithus tinctorius* (Sclerodermataceae), that have mycorrhizal associations in agricultural areas, a simulation of the levels of heavy metals (Al, As, Cd, Cr, Ni, and Pb) was performed by Ray et al. (2005), inferring to these fungi the accumulation of these metals benefiting the soil. *Thelephora terrestris* (Thelephoraceae) and *Suillus bovinus* (Suillaceae) associated with the culture of *Pinus sylvestris* also have been evaluated, and all species shown actuation as protectors on copper levels (Van-Tichelen et al., 2001; Ray et al., 2005).

For pluteoid species that have the soil as substrate, there is an important interaction related to the cycling of organic matter from agriculture. In Brazil, mainly in the Amazon, Atlantic Forest and Pampa biomes, areas of intercropped cultivation occur (IBGE, 2020). Thus, the fungal presence is a natural aid since the fungus rapidly degrades the rest of the organic matter, returning nutrients to the soil that will serve as a nutritional basis for the new crop.

5. Conclusion

Pluteaceae is a group lignicolous fungi, mainly the members of the genus *Pluteus*. The pluteoid act helping in the cycling of wood compounds that are difficult to degrade. Also, the terricolous *Volvariella* are saprobes that play key ecological roles on the soil. The data compiled in the literature and the relationships built in this study showed that the species of the family with occurrence in Brazil performed ecological importance unique for the biomes.

The Atlantic Forest biome showed a great diversity of species described, containing interactions ecological important to the regions near watercourses. Association pluteoid specifics with the native arboreal Brazilian and distribution of group showed how much the fungal-arboreal interaction is important for these regions. Terricolous fungal of the family present in areas of intense anthropic action play important actions that should be better analyzed in a future study. Thus, it is possible to infer that the family is actively associated with the cycling of nutrients and the regeneration of the flora in Brazilian biomes.

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5.2 Capítulo II

Título: A relationship between fungi (Basidiomycota, Agaricomycetes, Agaricales) and nutrient content in riparian area of reforestation with *Eucalyptus grandis* W. Hill ex Maiden (Myrtaceae) in southern Brazil

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Article

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A relationship between fungi (Basidiomycota, Agaricomycetes, Agaricales) and nutrient content in riparian area of reforestation with *Eucalyptus grandis* W. Hill ex Maiden (Myrtaceae) in southern Brazil

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ABSTRACT – (A relationship between fungi (Basidiomycota, Agaricomycetes, Agaricales) and nutrient content in riparian area of reforestation with *Eucalyptus grandis* W. Hill ex Maiden (Myrtaceae) in southern Brazil). Due the tolerance in soil degraded, *Eucalyptus* is widely used in reforestation area. This study aims to evaluate the fungi that use *Eucalyptus grandis* W. Hill ex Maiden as substrate in reforestation area in southern Brazil. Fungi were identified and macronutrient and micronutrient contents were evaluated in order to understand the relationship between the fungi and the substrate. There were 200 specimens found, categorized into 25 species belonging to 10 families of Agaricales (Basidiomycota, Fungi). Substrates used by fungi were branches, roots, stems, humus, and soil. Macronutrients mean level found in fungi followed the order Ca>K>P>Mg, and micronutrients S>Fe>Mn>Cu/B>Zn. C:N ratio mean was 13:1, associated with substrate degradation potential, since the enzymatic production of fungi is affected by disposition of these nutrients. The data obtained in this study allowed a better understanding of fungi associated with the exotic arboreal substrate, and their nutritional significance in reforestation area.
Keywords: Agaricales, associations, Myrtaceae, nutrient, reforestation

RESUMO – (Uma relação entre fungos (Basidiomycota, Agaricomycetes, Agaricales) e teor de nutrientes em área ripária de reflorestamento com *Eucalyptus grandis* W. Hill ex Maiden (Myrtaceae) no Sul do Brasil). Devido à tolerância em solos degradados, *Eucalyptus* é amplamente utilizado em áreas de reflorestamento. Este estudo teve como objetivo avaliar os fungos que utilizam *Eucalyptus grandis* W. Hill ex Maiden como substrato em uma área de reflorestamento no sul do Brasil. Para compreender a relação dos fungos com o substrato, os mesmos foram identificados e os teores de macronutrientes e micronutrientes foram avaliados. Foram encontrados 200 espécimes divididos entre 25 espécies pertencentes a 10 famílias de Agaricales (Basidiomycota, Fungi). Os substratos utilizados pelos fungos foram galhos, raízes, caules, húmus e solo. Os teores médios de macronutrientes encontrados nos fungos seguiram a ordem Ca>K>P>Mg, e os micronutrientes S>Fe>Mn>Cu/B>Zn. A relação C:N foi de 13:1, associada ao potencial de degradação do substrato, uma vez que a produção enzimática dos fungos é afetada pela disposição desses nutrientes. Os dados obtidos neste estudo auxiliam em uma melhor compreensão dos fungos associados ao substrato arbóreo exótico e sua importância nutricional na área de reflorestamento.
Palavras-chaves: Agaricales, associações, Myrtaceae, nutriente, reflorestamento

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Introduction

The *Eucalyptus* (Myrtaceae) is an arboreal genus that is frequently used in afforestation and reforestation operations because of its high tolerance in degraded soils (Njouonkou *et al*. 2021). In Rio Grande do Sul State this genus plays a significant role to economy, primarily in silviculture area (Echer *et al*. 2015). Soils that have been damaged by anthropic action, *Eucalyptus* is often used as a pioneer species for planting due their tolerance the acid pH and salinity (Taiz *et al*. 2018). However, it is an exotic species in Brazil, introduced in Rio Grande do Sul State in middle of XIX century (Trentim *et al*. 2014).

A limited number of studies reported ecological role of fungi in relation to substrates of exotic tree species, as well as their role in nutrients recirculation. According to Manzato *et al*. (2020), capable fungi to use exotic trees substrate are very important to biodegradation of this organic matter type. Fungi associated with these trees can break down and extract carbon compounds, including lignin, cellulose, and hemicellulose (de Araujo *et al*. 2018, Martins *et al*. 2018). The carbon/nitrogen ratio can provide insight into how native fungi diversity is associated with exotic tree species (Bononi *et al*. 2017, Njouonkou *et al*. 2021). Agaricales fungi have carbon-to-nitrogen ratio mean between 10:1 and 15:1 (Stoffella & Kahn 2001). A study conducted by D'Agostini *et al*. (2011) with fungi that decompose wood, the C:N ratio was noted as a factor in the time needed to breakdown organic matter in part due to the time required to produce decomposing enzymes, such as cellulase. The priming effect is attributed to the ability of fungi to decompose organic matter through the availability of fresh carbon (Fontaine *et al*. 2011). Nitrogen also interacts with decomposition system once the availability interferes with the diversity of fungi (Liao *et al*. 2020). Understanding the carbon-to-nitrogen ratio is essential since it is linked with type of organic matter and the decomposition process by fungi. In this context, size population, as well as the capacity of enzyme production and mineralization is related the fungi action in nutrient cycle, once the lignicolous species possess enzymatic apparatus which is designed to extract these compounds from wood, and terrestrial are involved in decomposition organic matter deposited in the soil (Bahram & Netherway 2022).

Macronutrients and micronutrients are acquired by fungi from substrate to which they grow or are attached in combination with the capacity to extract these nutrients (Silva-Neto *et al*. 2022). Therefore, the ability of fungi to absorb and retain certain nutrients is essential to comprehend how to occur nutritional cycle. In study with edible fungi, Malinowski *et al*. (2021) report that some species contained essential nutritional content. Edible fungi contain macronutrients and micronutrients suitable for human and animal consumption. They are functional foods because containing around of 20-35% proteins, 5-10% essential amino acids, vitamins and minerals, as well as low lipid levels (Altaf *et al*. 2020).

This study investigated fungi-plant-substrate association in a riparian reforestation area in southern Brazil with *Eucalyptus grandis* W. Hill ex Maiden monoculture. To understand this association, the study also analyzed the nutritional potential and chemical composition of Agaricales associated with this type of exotic substrate.

Material and methods

Study area – During the period 2021-2022 the collection was conducted at Fundação Estadual de Pesquisa Agropecuária (FEPAGRO), São Gabriel, Rio Grande do Sul State, Brazil (-30°20'13"S and -54°15'49"W). In the region, there is a weir of 37 m^2 protected by a reforestation area of 336 m^2 composed of *Eucalyptus grandis* W. Hill ex Maiden trees implemented in the year 2000. Previously, the area was planted with soy, and the same is still occurring in the farms in its surroundings. Temperatures oscillated from 3 to 35°C, with a mean of 10°C in winter, 18°C in autumn, 23°C in spring, and 30°C in summer. The average monthly precipitation ranges from 4 to 152 mm, with relative humidity from 19% to 70% (Embrapa 2022).

Sample – Fungi were collected under license SISBIO 79049-1 using the Rapid Survey method (Walter & Guarino 2006) with the modifications: perimeters of lines L1, L2, and L3 were subdivided according with limits of Brazilian riparian zones. Line 1 (active water channel) in the first 30 m from the water's edge. Line 2 (flood plain) for another 30 m from the end of the perimeter of L1. Line 3 (filter area) for another 30 m beyond the end of L2 (Brasil 1976). During the collection, a 5-minute interval was maintained, all specimens found were gathered up, and each line (L1, L2, and L3) there was one collector. The expeditions were seasonal with a total collection time of approximately 24 hours. To collect fungi from branches and stems of trees 1 cm diameter and 1 mm thick woody portion was removed near the basidiome. In the case of fungi close to the roots exposed on the soil surface, 1 cm in diameter and 1 mm thick were removed, and for this collection, the structure was followed up to the plant (de Araujo *et al*. 2018). For fungi found on soil, about 10 g were collected together with the basidiome. Samples were placed in paper bags and then dehydrated at 40°C for 48 hours.

Taxonomic identification – Fungal species were identified using the taxonomic key for Agaricales from Brazil (Putzke & Putzke 2017, 2019). Microscopy analyzes were conducted through cuts on the basidiomata lamellae and placing them on slides, which were then rehydrated with 3% KOH solution. The Olympus DP53 optical microscope was used to observe microstructures, such as spores, basidia, and hyphae.

Nutritional content – The dry mass of fungi collected was analyzed in relation to the nutritional levels. For species with pileus diameters smaller than 5 cm, duplicates were separated, while for species with larger diameters, only one unit was reserved. Analysis of macronutrients included carbon (C), nitrogen (N), calcium (Ca), magnesium (Mg), phosphorus (P), and potassium (K). The micronutrients analyzed were zinc (Zn), copper (Cu), sulfur (S), boron (B), iron (Fe), and manganese (Mn). Nutrient contents were analyzed following the determinations by Lutz (1985), and the C:N ratio was calculated using the results from chemical analysis, according to Mantovani *et al*. (2007). The analyzes were performed at Laboratório de Solos da Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Rio Grande do Sul State, Brazil.

Statistics – Nutritional contents of fungi were computed and quantified using the BioEstat v.2.0 program in percentage terms (Ayres & Junior 2000). The means of nutritional content obtained with each species were submitted to Analysis of Variance (ANOVA) and compared by Tukey test at 5% level of error probability with ESTAT version 2 software (Estat 1994).

Results

The diversity of fungi found in the study area comprised 25 species associated with *Eucalyptus grandis* W. Hill ex Maiden, developing on branches (1), roots (1), stems (3), humus (7), and on the soil (13), totaling 200 specimens. In total, 10 families of Agaricales were found. Records of fungi included the occurrence of six species new to Rio Grande do Sul State, as well as 21 species associated with *E. grandis* for the first time (table 1 and figure 1).

Nutritional chemical analyzes of all mushrooms showed macronutrient content in following order Ca>K>P>Mg, and for micronutrients S>Fe>Mn >Cu/B/Zn. It was not possible to detect any differences in the concentration of Cu, B, or Zn. Terricolous fungi (soil and humus), when compared to lignicolous fungi (stem and branches), exhibited higher levels of macronutrients and micronutrients (table 2).

The carbon-to-nitrogen ratio (C: N) is summarized in Table 3. As result of this study, the C:N ratio of the species varied from 16.2 to 11.00 in C content, and from 1.58 to 0.66 in N content, with 13:1 mean. Considering total value of dry mass, C represented 93% and N 7%. Different terricolous fungi showed oscillations from 14.2 to 11.08 in C, and 1.43 to 0.81 in N. Oscillations between 14.70 and 11.08 in C, and 1.06 to 0.75 in N was found in humicolous fungi. In the case of lignicolous fungi, values on stems ranged from 12.90 to 10.90 in C, and 1.02 to 0.78 in N, while values on branches ranged from 12.98 to 9.79 in C, and 1.08 to 0.78 in N. Values associated with roots ranged between 15.1 and 12.20 in C, and 0.98 to 0.78 in N.

Table 1. Agaricales fungi found in the riparian area of reforestation with *Eucalyptus grandis* W. Hill ex Maiden, São Gabriel, Rio Grande do Sul State, Brazil.

Family	Species	Substrate	N°	FR	Occurrence	Reference
Agaricaceae	*Agaricus rufoaurantiacus* Heinem, 1961	Soil	8	*	PE, RS, and SP	Pegler 1997, Costa *et al.* 2022
Entolomataceae	*Clitopilus scyphoides* Singer, 1946	Soil	8	*	PR and RS	Singer 1961, de Meijer 2006
Entolomataceae	*Entoloma* sp. (Fr.) P. Kumm,1871	Soil	2	-	WD	Putzke & Putzke 2017
Hydnangiaceae	*Laccaria fraterna* (Sacc.) Pegler, 1965	Soil	48	*	PR and RS	Putzke 1999, Meijer 2008
Hymenogastraceae	*Galerina* sp. Earle, 1909	Humus	2	-	WD	Putzke & Putzke 2019
Hymenogastraceae	*Gymnopilus earlei* Murrill, 1913	Humus	2	*	MT, PR, and RS	Araujo 1984, Sobestiansky 2005, Bononi *et al*. 2017
Hymenogastraceae	**Gymnopilus junonius* (Fr.) PD Orton, 1960	Stem	2	*	AM	Silva 2015
Hymenogastraceae	*Gymnopilus pampeanus* (Speg.) Singer, 1951	Stem	13	*	RS and SP	Singer 1953, Pleigler 1997, Sobestiansky 2005
Hymenogastraceae	**Gymnopilus paraenses* (Berk.) Pegler, 1988	Humus	2	*	PA	Araujo 1984

Table 1. Cont.

Family	Species	Substrate	N°	FR	Occurrence	Reference
Hymenogastraceae	*Gymnopilus subtropicus* Hesler, 1969	Root	5	-	PR, PB, and RS	Sobestiansky 2005, Meijer 2008, Magnago *et al.* 2015
Hymenogastraceae	**Gymnopilus zenkeri* (Henn.) Singer, 1951	Humus	6	*	RJ	Albuquerque 2006
Lycoperdaceae	**Apioperdon pyriforme* (Schaeff.) Vizzini, 2017	Soil	11	*	SP	Baseia 2005
Lycoperdaceae	*Lycoperdon perlatum* Pers, 1796	Soil	6	*	PE, RS, and SP	Baseia 2005, Cortez *et al.* 2013
Mycenaceae	*Mycena galericulata* (Scop.) Gray, 1821	Soil	12	*	RS	Rick 1961
Pluteaceae	*Pluteus cervinus* (Schaeff.) P. Kumm, 1871	Humus	16	*	PR and RS	Singer 1956, Cortez & Coelho 2005
Physalacriaceae	*Oudemansiella canarii* (Jungh.) Höhn, 1909	Branches	4	*	MG, RS, and SC	Putzke & Pereira 1988, Rosa & Capelari 2009
Psathyrellaceae	*Coprinellus domesticus* (Bolton) Vilgalys, Hopple & Jacq. Johnson, 2001	Humus	2	*	RS	Cortez & Coelho 2005, Meijer 2006
Psathyrellaceae	*Coprinus lagopus* (Fr.) Fr. 1838	Soil	8	*	PR and RS	Pleigler 1997, Meijer 2008
Psathyrellaceae	**Parasola lactea* (AH Sm.) Redhead, Vilgalys & Hopple, 2001	Soil	3	*	PR	Meijer 2006
Psathyrellaceae	*Psathyrella argillospora* Singer, 1973	Soil	1	*	RS	Singer 1953, Cortez & Coelho 2005, Meijer 2006
Psathyrellaceae	*Psathyrella hypertropicalis* Guzmán, Bandala & Montoya, 1988	Humus	1	*	RS	Singer 1953, Cortez & Coelho 2005
Psathyrellaceae	**Psathyrella murrillii* AH Sm. 1972	Soil	4	*	MT and SP	Pleigler 1997, Bononi *et al.* 2008
Strophariaceae	*Pholiota* sp. (Fr.) P.Kumm, 1871	Stem	18	-	WD	Putzke & Putzke 2019
Strophariaceae	*Stropharia rugosoannulata* Farl. ex Murrill, 1922	Soil	1	*	RS	Pleigler 1997
Tricholomataceae	*Lepista nuda* (Bull.) Cooke, 1871	Soil	15	*	RS	Guerrero & Homrich 1999

Note: Abundance of individuals (N°). First record in *Eucalyptus grandis* W. Hill ex Maiden (FR = *), and data shortage (-). Observation of first occurrence in Rio Grande do Sul State (*) next to the species name. States of occurrence in Brazil: Amazonas (AM), Minas Gerais (MG), Mato Grasso (MT), Pará (PA), Pernambuco (PE), Paraná (PR), Rio Grande do Sul (RS), Santa Catarina (SC), São Paulo (SP). Genus widely distributed in Brazil (WD). Source: Authors (2022).

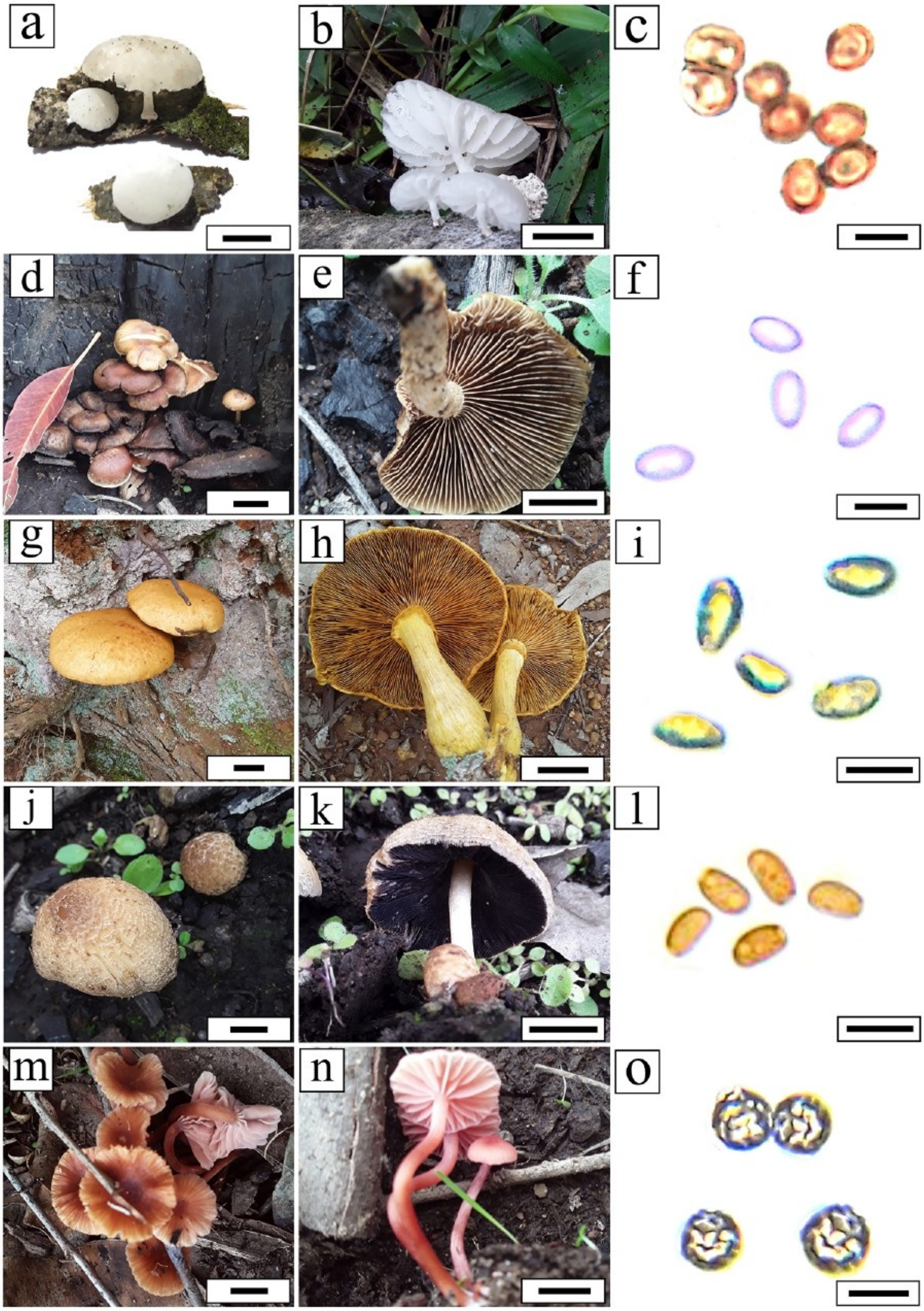

Figure 1. Macrostructures and microstructures of some fungi found in association with *Eucalyptus grandis* W. Hill ex Maiden, São Gabriel, Rio Grande do Sul State, Brazil. *Oudemansiella canarii* (Jungh.) Höhn on branches. a-c. *Pholiota* sp. (Fr.) P.Kumm on stem. d-f. *Gymnopilus junonius* (Fr.) PD Orton on roots. g-i. *Coprinellus domesticus* (Bolton) Vilgalys, Hopple & Jacq. Johnson on humus. j-l. *Laccaria fratera* (Sacc.) Pegler on soil. m-o. Pileus top view (a, b, c, g, and m), bottom view (b, e, h, k, and n), and spores (c, f, i, l, and o). Note: Scales (a, b, d, e, g, h, j, k, m, and n) 25 mm. Scales (c, f, i, l, and o) 10 µm. Source: Authors (2022).

Table 2. Macronutrient and micronutrient contents of fungi with occurrence in riparian area of reforestation with *Eucalyptus grandis* W. Hill ex Maiden, São Gabriel, Rio Grande do Sul State, Brazil.

Species	Substrate	Macronutrients cmol$_c$ L^{-1}					Micronutrientes mg L^{-1}				
		P	K	Ca	Mg	S	Cu	Zn	Fe	Mn	B
Agaricus rufoaurantiacus	Soil	0.48	2.41	5.76	0.04	2.02	0.02	0.01	0.33	0.04	0.03
Clitopilus scyphoides	Soil	0.47	1.98	4.98	0.05	1.99	0.01	0.01	0.47	0.02	0.01
Entoloma sp.	Soil	0.41	2.02	5.44	0.04	2.03	0.01	0.01	0.35	0.04	0.02
Laccaria fraterna	Soil	0.43	2.23	4.68	0.05	2.02	0.02	0.02	0.43	0.05	0.01
Galerina sp.	Humus	0.31	2.07	3.43	0.03	2.01	0.02	0.01	0.37	0.05	0.01
Gymnopilus earlei	Humus	0.35	2.00	3.37	0.03	2.00	0.02	0.01	0.35	0.05	0.01
Gymnopilus junonius	Stem	0.40	1.87	3.86	0.02	1.99	0.02	0.02	0.32	0.03	0.03
Gymnopilus pampeanus	Stem	0.41	1.79	2.98	0.02	1.98	0.02	0.02	0.33	0.03	0.03
Gymnopilus paraenses	Humus	0.32	2.05	3.40	0.03	2.02	0.02	0.01	0.34	0.05	0.01
Gymnopilus subtropicus	Root	0.45	2.04	3.88	0.02	2.03	0.02	0.02	0.32	0.03	0.03
Gymnopilus zenkeri	Humus	0.31	2.00	3.38	0.03	2.01	0.02	0.01	0.36	0.05	0.01
Apioperdon pyriforme	Soil	0.44	2.01	5.02	0.05	2.10	0.01	0.02	0.35	0.06	0.02
Lycoperdon perlatum	Soil	0.48	1.99	4.34	0.05	2.32	0.01	0.01	0.41	0.06	0.01
Mycena galericulata	Soil	0.45	2.03	5.03	0.04	1.98	0.02	0.02	0.44	0.05	0.03
Pluteus cervinus	Humus	0.30	1.97	3.33	0.03	2.09	0.02	0.01	0.38	0.05	0.01
Oudemansiella canarii	Branches	0.41	1.86	3.45	0.02	2.01	0.02	0.02	0.36	0.03	0.03
Coprinellus domesticus	Humus	0.31	1.99	3.34	0.03	2.00	0.02	0.01	0.37	0.05	0.01
Coprinus lagopus	Soil	0.44	2.05	5.00	0.05	2.12	0.02	0.02	0.33	0.05	0.03
Parasola lactea	Soil	0.45	2.09	4.33	0.04	2.00	0.02	0.01	0.43	0.04	0.01
Psathyrella argillospora	Soil	0.42	1.90	4.99	0.05	2.01	0.01	0.02	0.40	0.05	0.03
Psathyrella hypertropicalis	Humus	0.33	2.00	3.35	0.03	2.02	0.02	0.01	0.34	0.05	0.01
Psathyrella murrillii	Soil	0.46	1.99	5.03	0.04	1.98	0.01	0.02	0.34	0.05	0.02
Pholiota sp.	Stem	0.42	1.78	2.95	0.02	2.00	0.01	0.01	0.30	0.03	0.03
Stropharia rugosoannulata	Soil	0.43	2.00	4.90	0.06	1.98	0.01	0.02	0.39	0.04	0.02
Lepista nuda	Soil	0.44	2.07	4.98	0.04	2.02	0.02	0.02	0.40	0.03	0.02

Macronutrients cmol$_c$ L^{-1}

Fungi/Substrate	P	K	Ca	Mg
Soil	0.44 ± 0.02 cA	2.02 ± 0.13 bA	4.99 ± 0.38 aA	0.05 ± 0.00 dA
Humus	0.31 ± 0.01 cB	2.00 ± 0.03 bA	3.36 ± 0.03 aB	0.03 ± 0.00 dB
Stem	0.41 ± 0.01 cA	1.79 ± 0.04 bB	2.98 ± 0.51 aC	0.02 ± 0.00 dB
Branches	0.41 ± 0.00 cA	1.86 ± 0.00 bB	3.45 ± 0.00 aB	0.02 ± 0.00 dB

Micronutrientes mg L^{-1}

	S	Cu	Zn	Fe	Mn	B
Soil	2.02±0.09aA	0.01±0.00dB	0.02±0.00dA	0.40±0.04bA	0.05±0.01cA	0.02±0.00dB
Humus	2.01±0.03aB	0.02±0.00dA	0.01±0.00dB	0.36±0.01bB	0.05±0.00cA	0.01±0.00dB
Stem	1.99±0.01aC	0.02±0.00dA	0.02±0.00dA	0.32±0.01bC	0.03±0.00cB	0.03±0.00dA
Branches	2.01±0.00aB	0.02±0.00dA	0.02±0.00dA	0.36±0.00bB	0.03±0.00cB	0.03±0.00dA

Note: Means ± standard deviation followed in the same lowercase letters in columns and uppercase letters between substrates do not differ significantly by Tukey test at 5% level of error probability. Source: Authors (2022).

Table 3. Carbon-to-nitrogen ratio (C: N) of fungi with occurrence in riparian area of reforestation with *Eucalyptus grandis* W. Hill ex Maiden, São Gabriel, Rio Grande do Sul State, Brazil.

Species	Substrate	Mean	Minimum	Maximum	Mean	Minimum	Maximum
			$\mu g\ C\ g^{-1}$			$\mu g\ N\ g^{-1}$	
Agaricus rufoaurantiacus	Soil	14.35	13.6	15.1	1.08	0.73	1.43
Clitopilus scyphoides	Soil	14.20	12.2	16.2	1.15	0.80	1.50
Entoloma sp.	Soil	12.15	10.1	14.2	1.08	0.73	1.43
Laccaria fraterna	Soil	11.95	10.9	13.0	1.11	0.81	1.41
Galerina sp.	Humus	12.90	14.1	11.7	0.83	0.68	0.98
Gymnopilus earlei	Humus	12.5	14.0	11.0	0.81	0.66	0.96
Gymnopilus junonius	Stem	11.90	10.9	12.9	0.92	0.82	1.02
Gymnopilus pampeanus	Stem	11.25	10.4	12.1	0.83	0.73	0.93
Gymnopilus paraenses	Humus	12.40	10.1	14.7	0.86	0.66	1.06
Gymnopilus subtropicus	Root	13.65	12.2	15.1	0.88	0.78	0.98
Gymnopilus zenkeri	Humus	13.05	11.9	14.2	0.92	0.77	1.07
Apioperdon pyriforme	Soil	13.30	12.5	14.1	1.23	0.88	1.58
Lycoperdon perlatum	Soil	14.65	13.5	15.8	1.13	0.93	1.33
Mycena galericulata	Soil	12.95	11.9	14.0	1.05	0.80	1.30
Pluteus cervinus	Humus	12.90	10.9	14.9	0.90	0.75	1.05
Oudemansiella canarii	Branches	11.38	9.78	12.98	0.93	0.78	1.08
Coprinellus domesticus	Humus	12.90	10.9	14.9	1.12	0.77	1.47
Coprinus lagopus	Soil	12.50	11.0	14.0	1.12	0.87	1.37
Parasola lactea	Soil	11.75	10.4	13.1	1.23	0.98	1.48
Psathyrella argillospora	Soil	13.75	12.5	15.0	1.19	0.84	1.54
Psathyrella hypertropicalis	Humus	13.50	11.8	15.2	0.91	0.76	1.06
Psathyrella murrillii	Soil	12.65	10.9	14.4	0.91	0.76	1.06
Pholiota sp.	Stem	12.70	11.2	14.2	0.93	0.78	1.08
Stropharia rugosoannulata	Soil	13.30	10.9	15.7	1.15	0.80	1.50
Lepista nuda	Soil	13.00	11.8	14.2	1.09	0.89	1.29

Source: Authors (2022).

Discussion

A large number of species were found on soil, compared to other types of substrates. According to Putzke & Putzke (2017) *Agaricus rufoaurantiacus* occupies the interior of forests as its natural habitat. This is the first report of association with *E. grandis*. *Apioperdon pyriforme* and *Lycoperdon perlatum* also grow on soil (Alves & Cortez 2014, Xu *et al.* 2019), and *L. perlatum* has already been associated with *Eucalyptus* spp. in India (Natarajan & Purushothama 1987). This is the first occurrence of *A. pyriforme* in Rio Grande do Sul, as well as association with *E. grandis*.

Clitopilus scyphoides has been observed growing alongside *Eucalyptus globulus* Labill. and *Eucalyptus macarthur* H. Deane & Maiden in Spain (Aragón 2002). Due to the fact that *Clitopilus austroprunulus* Morgado, GM Gates & Noordel, 2012 was registered under *Eucalyptus regnans* F. Muell, *Clitopilus* sp. with *Eucalyptus cladocalyx* F. Muell and *Eucalyptus baxteri* (Benth.) Maiden & Blakely ex J.M.Black; it appears that the genus has an affinity for exotic tree substrates, all records occurred in Australia (Crous *et al.* 2012). Also, *C. austroprunulus* has been associated with *E. regnans* in Africa (Decock 2012).

In Russia, *Entoloma* sp. has been recorded along with *Eucalyptus brassiana* S.T. Blake (Crous *et al.* 2018), and in Australia, the genus has already been identified with *E. baxteri* and *Eucalyptus dunnii* Maiden. Also, *Entolama nipponicum* Kasuya, Nabe, Noordel & Dima, 2019 was cataloged with *E. grandis* in same region (Catcheside 2006, Bahram & Netherway 2022). There is a wide distribution of occurrence of genus in Brazilian biomes (Putzke & Putzke 2017).

Laccaria fraterna is an ectomycorrhiza with record of association involving *Eucalyptus diversicolor* F. Muell., *Eucalyptus globulus* (Labill.), and *Eucalyptus tereticornis* Sm., respectively in India, Scotland, and Australia (Reddy & Natarajan 1995, Dunstan *et al*. 1998, Mason *et al*. 2000). This is the first report of *E. grandis* associated with this fungi.

In this study, *Mycena galericulata* was found on soil next to *E. grandis* and this is report first of association. Species has already an association record in Spain with *E. globulus* (Lorenzo *et al*. 2009). Associated with *Eucalyptus* spp. other species of the same genus, such as *Mycena pseudoinclinata* AH Sm. 1947 in Italy (La Rosa *et al*. 2009), *Mycena neerimensis* Grgur, 1998 in Australia (Grgurinovic 1998), and *Mycena filopes* (Bull.) P. Kumm, 1871 in Brazil (Sobestiansky 2005) have already been found.

This is record first of *Coprinus lagopus* associated with *E. grandis*. However, the fungi already have cataloged with *E. globulus* in Peru (Ordóñez & Rabanal 2019). Furthermore, *Coprinus* sp. has an occurrence record with *Eucalyptus* sp. in Brazil (Manzato *et al*. 2020).

In Paraná a record of *Parasola lactea* was described (Meijer 2006), for Rio Grande do Sul and the association with *E. grandis* this is report first. However, only *Parasola conopilea* (Fr.) Örstadius & Larss, 2008 is cited in association with *Eucalyptus gomphocephala* DC. in Australia (Bougher & Cook 1983).

Psathyrella argillospora, *Psathyrella hypertropicalis,* and *Psathyrella murrillii* do not have known associations with *Eucalyptus*. Additionally, this is the first report of *P. murrillii* occurrence in Rio Grande do Sul State. Only in Costa Rica *Psathyrella ovispora* Deschuyteneer, Heykoop & Moreno, 2019 was associated with *Eucalyptus* spp. (Crous *et al*. 2019).

Lepista nuda was associated to *Eucalyptus fasciculosa* F. Muell in Australia (Catcheside 2006), and *Eucalyptus camaldulensis* Dehnh in Italy (Venturella & La Rocca 2001). Equal to *Stropharia rugosoannulata* is known to occur in agricultural soils (Stamets 2005), but this is record first of both in association with *E. grandis*.

Galerina includes humicolous, terrestrial and lignicolous species (Spahr 2018). In Brazil, the genus is widely distributed and recorded occurrence in Rio Grande do Sul State (Singer 1953). Putzke & Putzke (2019) mention 17 species in Brazil and only *Galerina montivaga* Singer, 1969 it is humicolous. The genus also occurs on branches and litter of *Eucalyptus* spp. (Manzato *et al.* 2020).

Only *Pholiota conissans* (Fr.) MM Moser, 1953; *Pholiota highlandensis* (Peck) Singer, 1952; *Pholiota limonella* (Peck) Sacc. 1887; *Pholiota nameko* (T. Itô) S. Ito & S. Imai 1933; *Pholiota spumosa* (Fr.) Singer, 1948; and *Pholiota squarrosoides* (Peck) Sacc. 1887 has occur register in Brazil. In terms of associations with tree species, *P. nameko* has a record with *Eucalyptus saligna* Sm. in Paraná (Paccola *et al*. 2001), as well as *P. highlandensis* and *Pholiota communus* (Cleland & Cheel) Grgur, 1997 with *Eucalyptus marginata* Donn ex Sm. in Australia (Robinson & Williams 2011).

This study found *Gymnopilus* species directly associated with the stem and root. In roots *Gymnopilus subtropicus* was found. *Gymnopilus earlei* and *Gymnopilus zenkeri* were found in humus mainly close to the roots. *G. zenkeri* has already been associated with *Eucalyptus* sp. in France (Njouonkou *et al*. 2021), *Gymnopilus junonius* with *Eucalyptus* spp. in Uruguay (Barneche *et al*. 2017), *Gymnopilus spectabilis* (Weinm.) AH Sm. 1949 and *G. pampeanus* in India (Kaur & Rather 2015), *G. pampeanus* in Argentina (Colavolpe & Albertó 2014), as well as *Gymnopilus corticophilus* Rees, 1999; *Gymnopilus tomentulosus* Rees, 1999; *Gymnopilus* tasmanicus Rees, 1999; *Gymnopilus eucalyptorum* (Cleland) Singer, 1947; *Gymnopilus tyallus* Grgur, 1997; and *Gymnopilus moabus* Grgur, 1997 in Australia (Rees *et al.* 1999). It is the first occurrence record associated with *E. grandis* and in Rio Grande do Sul State for *G. junonius*, *G. paraensis,* and *G. zenkeri*.

Pluteus cervinus grows on wood humus (Putzke & Putzke 2019). However, this is the first record of association with *E. grandis*. In relation the others species, *Pluteus ludwigii* Ferisin, Justo & Dovana, 2019 has been recorded growing on *E. grandis* humus in Russia (Ordóñez & Rabanal 2019), *Pluteus albotomentosus* Malysheva & Malysheva, 2014 and *Pluteus extremiorientalis* Malysheva & Malysheva, 2014 with *Eucalyptus urophylla* S.T.Blake in Indonesia (Crous *et al*. 2014).

Coprinellus domesticus integrates another new association record with the tree species of this study. Using *Eucalyptus* spp. humus as substrate, *Coprinellus* sp. was cataloged in Australia (Tyagi *et al*. 2019).

Lignicolous *Oudemansiella canarii* grows in stem and branches. It is described growing in the stem of *Eucalyptus* sp. in Argentina (Alberti *et al*. 2021), this is the first record of association with *E. grandis*.

Macronutrient and micronutrient contents analyzed in this study can provide a basis for further analyzes regarding application nutritional of these fungi. Although the fungi studied exhibit associations with *E. grandis*, there are not techniques or standards for cultivating them in exotic tree. *A. rufoaurantiacus, L. fraterna, G. pampeanus, G. paraensis, G. zenkeri, A. pyriforme, L. perlatum, O. canarii, C. lagopus,* and *L. nuda* are classified as non-toxic and potentially edible (Crosier *et al*. 1949, Ruegger *et al*. 2001, Ndong *et al*. 2011, Akatin 2013, Putzke & Putzke 2017, 2018, Sridhar & Karun 2019, Altaf *et al*. 2020). This group not contemplate the fungi cultivated in Brazil for edibility purposes, so a more robust analysis of their potential for consumption is required. Moreover, *G. earlei, G. subtropicus, M. galericulata, P. lactea, P. argillospora, P. hypertropicalis,* and *P. murrillii* do not present sufficient data regarding their edibility or toxicity. There are not data on the edibility of *C. scyphoides, G. junonius, P. cervinus, C. domesticus,* and *S. rugosoannulata*, but levels of toxicity have already reported in studies involving these species (Gabriel *et al.* 1997, Novikova 2001, Hartley *et al.* 2009, Wu *et al.* 2011, Lee *et al.* 2020), and therefore their consumption should not be considered.

In terms of macronutrient, P, K, Ca, and Mg remained within the range already described for edible Agaricales species, such as *Agaricus bisporus* (JE Lange) Imbach, 1946; *Lentinula edodes* (Berk.) Pegler, 1976; and *Pleurotus* spp. (Silva-Neto *et al.* 2022). The contents of these macronutrients were higher in terricolous fungi than in other fungi. Nutritional retention of fungi is related to their availability in the substrate. Neina (2019) demonstrates that Ca is widely distributed in soil, predominantly in Ca^{2+} form, but specific mechanisms in each plant permit Ca to enter in permeable channels. In all species analyzed, Ca was the most abundant macronutrient. This result supports Agrahar-Murugkar & Subbulakshmi (2005) findings in *Calvatia gigantean* (Batsch ex Pers.) Lloyd, *Cantharellus cibarius* Pe. 1821, *Russula integra* (L.) Fr. 1838, *Gomphus floccosus* (Schwein.) Singer, 1945, and *Lactarius quieticolor* Romagn, 1958, which oscillated between 5 and 9 $cmol_c\ L^{-1}$.

The values in K content were consistent with described in literature. Different species of mushrooms evaluated in Poland had similar K contents with this study (Rudawska & Leski 2005). However, Malinowski *et al.* (2021) infer higher level with ranging between 5-20 $cmol_c\ L^{-1}$in *Boletus edulis* Bull. 1782, *Imleria badia* (Fr.) Vizzini, 2014, and *Leccinum scabrum* (Bull.) Gray, 1821.

The P content was similar to described for *A. bisporus* (Beyer 2003). Rudawska & Leski (2005) report Mg content in different mushroom species analyzed did not differ, as well as in the species of fungi analyzed in this study.

Micronutrients S, Fe, Mn, Cu, B, and Zn showed values consistent with previously described in literature (Beyer 2003, Agrahar-Murugkar & Subbulakshmi 2005, Rudawska & Leski 2005, Fidanza *et al.* 2010, Malinowski *et al.* 2021). Rudawska & Leski (2005) in analysis of S content with mushrooms found higher level, as in our results. Nevertheless, high concentrations of S can be toxic to plants (Taiz *et al.* 2018). Minor fluctuation of values of S among the species this study occurred, it is possible to infer that fungi are capable of accumulating this micronutrient.

Mn, Cu, and Zn contents did not show expressive oscillations, with exception of Fe, which obtained the highest value among metals. Fidanza *et al.* (2010) report that metallic micronutrient levels generally remain stable in edible Agaricales. Similarly, the Cu and Zn contents were also less expressive and very small in comparison to other micronutrients analyzed (Fidanza *et al.* 2010). Rudawska & Leski (2005) who analyzed the micronutrient content of mushrooms, presented a similar order of contents found in our data. According to Rudawska & Leski (2005), metallic micronutrients in mushrooms are primarily related to acidic pH of soil. It is important to emphasize that *Eucalyptus* are tolerant to acidic soils (Pinto & de Negreiros 2021). In this study there was not conducted analysis soil, but due to the presence of metals with higher levels in terrestrial fungi, we can infer that the fungi presented these values influenced by the availability of these micronutrients in substrate. Additionally, B content which is considered esterified was found in small concentrations, similar the date by Rudawska & Leski (2005).

Carbon-to-nitrogen ratio (C: N) of analyzed fungi oscillated of 10:1 to 15:1, compatible with values in literature for edible Agaricales species (Stoffella & Kahn 2001). However, none of species found contained C:N ratio values described, thus the values presented are unpublished. Carbon and nitrogen levels in fungi biomass are directly related to the rate of decomposition and mineralization. In study by D'Agostini *et al.* (2011) with *L. edodes*, *Pleurotus ostreatus* (Jacq.) P. Kumm, 1871, and *Agaricus blazei* Murrill, 1945, when analyzing the C:N ratio, discovered that mycelial growth was proportional to this ratio. In this premise, mycelial growth was reduced in proportions greater than 15:1 or less than 10:1 (D'Agostini *et al.* 2011).

Organic matter is main source of C for fungi, but the assimilation of N depends not only of substrate also of fixation by microorganisms (Liao *et al.* 2021). According to Martins *et al.* (2018) *P. ostreatus* obtained rapid degradation of basidiomata when the C:N ratio was lower than 10:1, and generated basidiomata of higher fresh biomass value when the C:N ratio was in the expected range (15:1 to 10:1). In contrast, increasing these proportions for *A. blezei* cultivation was beneficial. Kopytowski (2002) found that the C:N ratio yielded higher quality and productivity in the 20:1 ratio, higher than those found in this study. However, in edible species the C:N ratio does not take into account substrate type, but rather biomass profitability.

Conclusions

Association of fungi with *Eucalyptus grandis* W. Hill ex Maiden substrate it is paramount importance in nutritional cycling at reforestation area. Moreover, our analyzes showed levels of primary and secondary essential nutrients in species found, and these values can be used as a basis for new studies of their nutritional potential. The C:N ratio help in understanding ability of fungi to absorb and recycle nutrients from organic matter, since the enzymatic production of fungi is affected by its disposition. Fungi in this study showed adapted to the exotic tree substrate, their ecological interaction may have played a significant role in restructuring the reforestation area studied.

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Conflicts of interest

There is no conflict of interest.

Author contributions

Alice Lemos Costa: Contribution to sampling; data analysis and interpretation; manuscript preparation; critical revision and adding intellectual content.

Cassiane Furlan-Lopes: Contribution to sampling and manuscript preparation.

Fernando Augusto Bertazzo-Silva: Contribution to sampling and manuscript preparation.

Ana Luiza Klotz-Neves: Contribution to sampling and manuscript preparation.

Kamille Rodrigues Ferraz: Contribution to sampling and manuscript preparation.

Ana Flavia Zorzi: Contribution to sampling and manuscript preparation.

Silvane Vestena: Contribution to sampling; data analysis and interpretation; manuscript preparation; critical revision and adding intellectual content.

Jair Putzke: Contribution to sampling; data analysis and interpretation; manuscript preparation; critical revision and adding intellectual content.

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Aves and Fungi interactions in a review global of mycophagy and its associations in wildlife and industry

Associations of mycophagy Aves-Fungi

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Abstract

Fungi and Aves are present in all ecosystems and interact with a variety of organisms. The purpose of this study was to compile and analyze in the literature the mycophagy and association of birds with fungi to evaluate the aspects of interaction habits and habitat in natural and industrial environments. In this study, 64 species of wild birds were found with documented interactions involving fungi. However, only 32 had the consumed or used-for-nesting fungi species fully identified. In these cases, there is a correlation between the birds' foraging habits and the habitats of fungi. According to the findings of this review study, birds' foraging habits are closely linked to fungi habitats in relation the interactions between the groups. Also, the poultry industry is increasingly using mushrooms as a nutritional supplement due to their benefits. Despite the limited knowledge about the nutritional benefits of these associations in the wild, results from the industry indicate that the benefits would be similar.

Keywords: Avian mycophagy, foraging habits, natural and artificial food, beneficial associations, poultry farming.

Introduction

There are approximately 3.8 million species of fungi that have been cataloged (Hyde *et al*. 2020), with the first appearing 1.2 billion years ago (Berbee & Taylor 2010). Fungi can be found in various ecosystems, including aquatic, terrestrial, and arboreal environments, and they interact with a wide range of organisms, including mammals, arthropods, birds, and plants (Tiquia-Arashiro *et al*. 2019). It is estimated that birds originated approximately 95 million years ago (Lee *et al*. 2014) and currently encompass a total of 10.426 recorded species (Gill *et al*. 2022). Similar to fungi, they have been found in all ecosystems, exhibiting a range of feeding habits from specialists to generalists (Ericson *et al*. 2006). Birds employ various foraging tactics, including aerial, terrestrial, and aquatic environments (Robinson & Holmes 1982). This diversity is further categorized into three main groups: carnivores, herbivores, and omnivores (Beddall 1957).

Several characteristics of fungi, such as shape, size, color, aroma, and texture, contribute to the interaction between prey and predator in mycophagy (Beever & Lebel 2014). Fungal spores can be dispersed through interactions with fauna, consumption, internal or external travel with consumers or travelers, and defecation (O'Malley *et al*. 2013; Horton 2017; Jusino *et al*. 2022). According to Costa *et al*. (2022), *Molothrus bonariensis* (Gmelin, 1788) acts as a dispersing agent of *Macrolepiota bonariensis* in the studied area through mycophagy activities and subsequent defecation.

Many fungi disperse their spores by wind (Golan & Pringle 2017), but most distances considered within reach are minimal, with up to 95% of the spores remaining within one meter of the fungus (Galante *et al*. 2011; Horton *et al*. 2013). Elliott & Vernes (2019) reported a relationship between fungal habitat and the foraging habits of birds. This relationship was observed in the case of *Menura novaehollandiae* (Latham, 1801), which feeds on *Rossbeevera vittatispora*, a soil fungi. Other forms of association between birds and fungi exist in relation to habitat and foraging behavior. For instance, *Colaptes auratus* (Linnaeus, 1758) has been documented interacting with *Fomes fomentarius*, where did the bird show a preference for excavating nests in trees with the presence of the fungi (Martin *et al*. 2004; Edworthy *et al*. 2012; Lorenz *et al*. 2015).

According to Elliott *et al*. (2019), avian mycophagy is more widespread than previously believed. Caiafa *et al*. (2021) demonstrated that several truffle species in Patagonia rely partially on birds for their dispersal, including *Scelorchilus rubecula* (Kittlitz, 1830) and *Pteroptochos tarnii* (King, 1831). However, numerous unanswered questions persist regarding the evolution of mycophagy in birds, the significance of feeding habits in their natural environment, and the correlation between avian feeding and foraging behaviors in relation to fungal habitats. Furthermore, limited knowledge exists regarding the nutritional value of fungi in the diets of birds.

Nutritional supplementation with fungi in poultry farming has been found to be beneficial (Azevedo & Barata 2018). Studies involving broiler chickens (*Gallus gallus* - Linnaeus, 1758) have shown that the inclusion of fungi in feed improves the nutritional quality of meat, intestinal microbiota, immune responses, and antioxidant activity (Bidarnamani *et al*. 2015; Bederska-Lojewska *et al*. 2017; Mahfuz *et al*. 2020; Lima *et al*. 2021). Similar results have also been observed in other types of meat industries, such as duck, goose, quail, and turkey (Bederska-Lojewska *et al*. 2017).

Regardless of whether birds ingest fungi naturally or artificially in the industry, mycophagy occurs. There are few studies that demonstrate the interactions, nutritional benefits, behavior, and foraging of birds that include fungi in their diet. The study aims to compile and analyze literature data on the mycophagy of birds to investigate the habit and habitat aspects of this interaction. Furthermore, it aims to compile the benefits associated with including fungi in poultry industry diets.

Material & Methods

Bibliographic Survey

A bibliographic review was conducted on bird and fungi species involved in mycophagy, which have been published in scientific articles, reports, book citations, and digital public platforms such as Web of Science (https://www.webofscience.com) and Google Scholar (https://scholar.google.com). The keywords "aves," "birds," "fungi," "mushroom," "mycophagy," "fungivory," and "poultry farming" were utilized. Data pertaining to interactions and food in natural or industrial environments were collected. This study cites information from the literature that involves identified or unidentified species, as well as fungi taxonomic groups.

Statistical analysis of the foraging habits and fungi habitat

A file in Excel format (Walkenbach 2010) containing binary data was compiled to analyze the foraging habits of birds and fungi habitats. The graphs were elaborated using the R v.3.6 program (Ihaka & Gentleman 1996) and presented in percentage values (habitat/habit). Birds were classified as terrestrial or arboreal foragers (Gill *et al*. 2022), and the fungi were designated as terrestrial for those growing on soil, and arboreal for those growing on wood (Putzke & Putzke 2017, 2019).

Foraging habit and habitat characteristics were categorized into four categories: Partial Foraging on Ground (PFG); Major Ground Foraging (MGF); Partial Arboreal Foraging (PAF); and Major Arboreal Foraging (MAF). In addition, two positions were tested: Predominant Vertical Position (PVP) and Predominant Horizontal Position (PHP). Two categories of fungi were tested according to their habitat: Terrestrial Habitat (TH) and Arboreal Habitat (AH). The characteristics of species and the percentages were calculated in the model by Fávero & Belfiore (2017).

Results & Discussion

Records of the interaction between birds and fungi in the literature

This bibliographic review study found reports of 64 bird species interacting with fungi in the wild. However, only 32 birds had the consumed fungi species fully identified. A total of 29 families belonging to Bucerotiformes, Casuariformes, Charadriiformes, Dinornithiformes, Galliformes, Passeriformes, Piciformes, Psittaciformes and Trogoniformes were cataloged (Tab. 1). In the literature, the consumption of fungi by birds is commonly described in imprecise ways, not identifying the fungi species involved. Regarding the fungi, only 32 species belonging to 17 families of Cyttariales, Pezizales, Polyporales, Hymenochaetales, Russulales, Boletales, and Agaricales were identified (Tab. 1).

Many incomplete instances of associations between birds and fungi have been documented in the literature. In Table 1, fungi that were not properly identified are classified according to class, order, family, and genus. Despite the need for further evidence to refute or support these claims, it is important to cite them as they provide a foundation for future studies. Additionally, taxonomic identification of fungi can be challenging due to their complexity. Moreover, many cited reports refer to observations made by ornithologists or naturalists regarding feeding behavior or the utilization of fungi as a resource, particularly in relation to nest excavation.

Observational reports comprise the majority of studies on the interaction between birds and fungi. In relation the mycophagy, many reports in the literature describe birds foraging near the presence of fungi. As for nest excavation, the birds are reported using trees with fungi present. The studies that involved DNA sampling and gastrointestinal analysis were not specifically conducted to investigate mycophagy, although they were mentioned due to the significance of the results. The study of this interaction generally requires collaboration because many reports involving birds do not correctly identify the fungi involved. It is difficult to understand how the Aves-Fungi interaction occurs when the fungi are not identified. Which fungi species are consumed by birds, or which fungi are involved in the nest excavation process, for example, remain unclear.

Birds foraging behavior and its fungal associations

A summary of the main relationships between birds' foraging habits and fungi habitats for the identified species is shown in Figure 1. The foraging and mycophagy habits of birds indicate that species of the orders Galliformes and Bucerotiformes consume terrestrial fungi during foraging. The horizontal position on the ground during foraging is predominant in Galliformes, as reported by Schutz *et al*. (2001) and Trupkiewicz *et al*. (2018). In this group, *Gallus gallus* has reportes in mycophagy with *Armillaria gallica*, *Entoloma abortivum*, and *Hypholoma lateritium* (Elliott & Vernes 2019). *Laccocephalum mylittae* has also been associated with Malleefowl (*Leipoa ocellata*), and *Lentinula lateritia* has been found in the diet of Australian brushturkey (*Alectura lathami*) (Benshemesh 1992; Reichelt & May 1997; Simpson 1998, 2000). It is important to emphasize that all the mushrooms mentioned above are edible and grow in soil (Miller & Miller 2006), and that the birds are ground foragers.

Most studied Passeriformes consume terrestrial fungi as part of their diet, with a small proportion consuming arboreal fungi. According to Martin *et al*. (2017), Passeriformes adopt both horizontal and vertical foraging positions, and their habits are highly diverse. *Agaricus campestris* is an edible species that grows in the ground (Putzke & Putzke 2017) and has been associated with the mycophagy behavior of *Corvus brachyrhynchos*, a bird that forages in the soil (Webster 1902; Kilpatrick 2003). Additionally, *Cormobates leucophaea* is an arboreal forager that primarily forages vertically (Christidis *et al*. 2008). The species was reported in mycophagy with *Laetiporus portentosus* (Maurer *et al*. 2017), an edible medicinal mushroom that grows on wood (Fuller *et al*. 2005).

The Psittaciformes are primarily insectivorous and frugivorous, adopting a wide variety of foraging positions. *Alisterus scapularis*, for example, has been observed in a horizontal position during foraging (Plant *et al*. 2020). There have been records of this bird feeding on *Cyttaria gunnii* (Elliott & Elliot 2019), which forms globose structures on the branches of the host tree resembling fruit silhouettes. These structures are also edible and orange in color (Leonard 2017). *Trogon surrucura* (Trogoniforme) is both a frugivore and an insectivore with extensive foraging abilities, both terrestrially and arboreally, in a variety of vertical and horizontal positions (Sarquis *et al*. 2017). Birds of this species has been reported feeding on *Fomes fasciatus*, a fungus that grows on tree trunks (Cockle *et al*. 2012).

To Jusino *et al*. (2015), Piciformes, especially woodpeckers, prefer to excavate trees infected with lignicolous fungi because the excavated material is easily accessible. The compounds lignin and cellulose confer greater resistance to the cell wall of plants, but they are degraded by fungi. The "Tree Selection Theory" proposes that woodpeckers prefer these trees due to the ease of excavation (Jusino *et al*. 2015). During the study conducted with *Picoides borealis*, over 50 species of fungi were associated with the bird's preference for choosing trees to excavate (Jusino *et al*. 2016). However, there is a gap to be filled in relation to mycophagy involving woody fungi in particular within this bird's group. Despite this, it is important to mention a correlation to a preference for excavating nests in places where wood fungus occurs. In the future, this group could be further analyzed regarding the presence or absence of mycophagy activities. However, it is reasonable to infer that they act as dispersal agents since they coexist with the sites where wood fungi are present.

There is a wide variety of behaviors that birds may display during their foraging activities, which can be characterized as opportunistic, incidental, or even compulsory interactions with food sources. Various strategies can be employed, including considering the characteristics of prey biomass (LeBrasseur 1969), the ease of capturing or manipulating prey, the duration of foraging, as well as the presence of predators or competitors during the activity (Brink & Dean 1966). In this regard, one of the advantages of including fungi in birds' diets is the lack of an escape response by the prey (Samson *et al*. 2019). The literature does not describe the specific strategies used for mycophagy, which poses an important question for future research. However, our data collection provides evidence that arboreal foragers consume fungi from the same habitat/substrate, or have some form of association with them, such as when they excavate nests. Conversely, terrestrial foragers tend to include or interact with soil fungi.

Optimal Foraging Theory explains that diet follows certain concepts: I) The occurrence of predators is independent of the abundance of prey; II) The predator may not specialize in unprofitable prey; III) There is not take of food by partial preference; IV) Encounter and capture rates are sequential, and not simultaneous (Helfman 1990). Homogeneous environments in food distribution are rare while heterogeneous environments generally predominate. In order to be effective foragers, species must establish a favorable cost-benefit relationship (Adamík *et al.* 2003). Based on these results, further exploration of bird mycophagy should be undertaken in the future. It is worth noting that Galliformes do not forage in trees but may roost in them. Passeriformes adopt an intermediate position during foraging, often perching pointed downward constantly. Piciformes, Psittaciformes, Bucerotiformes, and Trogoniformes spend most of their time perched but also feed on the ground.

The use of mushrooms in poultry farming

The results of studies on the feeding efficiency of mushrooms in broiler chickens are presented in Table 2. There are some species of mushrooms that are consumed more frequently, such as *Agaricus bisporus*, also known as champignons. Due to its nutritional value, insects, reptiles, birds, mammals and humans consume this species (Azevedo & Barata 2018). Increasingly, mushrooms are becoming popular not only for their nutritional value but also as nutraceuticals used in the meat industry, particularly in poultry (Bederska-Lojewska *et al.* 2017). Studies have shown that mushrooms have a positive effect on feeding, resulting in increased body mass and improved meat quality (Camay 2016; Yan *et al.* 2018; Ilyina *et al.* 2020).

Studies in aviculture indicate promising results when fungi are added to diets. In natural environments, species of fungi that have been reported in mycophagy with birds, as well as with other taxonomic groups, are included in the poultry industry's diet (Azevedo & Barata 2018). For example, *Agaricus* has been reported as food in mycophagy by birds of the Corvidae and Muscicapidae families (Tab. 1). In the industry, the same genus is used to feed Phasianidae and Anatidae (Table 2). Clearly, these avian groups have a significant commercial interest, but *Agaricus* also has associations of mycophagy with other birds in the wild. Unpublished studies have demonstrated the nutritional and immunological benefits of consuming fungal species that are already classified as edible in the diets of avian species (Guo *et al*. 2004; Camay 2016; Mahfuz *et al*. 2020). These results can naturally occur in mycophagy associations of wild birds.

Poultry farming is constantly confronted with the use of antibiotics for bacteria control (Camay 2016). Recent studies have shown an increased use of fungi in pathogen control in recent years, as avian species develop resistance to drugs (Mahfuz *et al*. 2020). An example of this argument is the study by Guo *et al*. (2004), which used extracts of *Lentinula edodes* and *Tremella fuciformis* to control bacterial infection caused by the pathogen *Mycoplasma gallisepticum* in broilers. An effect similar to the antibiotic Apramycin was observed, along with a benefit to intestinal flora when the extract of these fungi was used (Guo *et al*. 2004) (Tab. 2). There has been a description of *Alectura lathami* engaging in mycophagy with *Lentinula lateritia* (Simpson 1998, 2000), a fungus of the same genus, but in the wild (Tab. 1). Although *Tremella fuciformis* is not associated with mycophagy in wild birds, it is an edible species (Stamets 2000).

In studies with broilers, diets supplemented with *Pleurotus ostreatus*, *Agaricus bisporus, Flammulina velutipes*, and *Lentinula edodes* showed health benefits (Tab. 2). Supplementation resulted in improvements in growth, better meat quality, and a reduction in bacterial infections (Camay 2016; Mahfuz *et al*. 2020). Additionally, the inclusion of fungal supplements in broiler feed led to a significant decline in *Escherichia coli* infections in the group with the supplemented diet (Shang *et al*. 2014). In the study by Hines *et al*. (2013), supplementation with mushrooms also led to an increase in the populations of *Lactobacillus* spp. and *Bifidobacterium* spp. which are beneficial to the intestinal tract.

The inclusion of *Agaricus blazei* as a powdered extract in broiler feed resulted in significant reductions in serum cholesterol levels (Fanhani *et al*. 2016). Additionally, the addition of *Pleurotus ostreatus* to broiler diets significantly decreased serum triglyceride concentrations (Toghyani *et al*. 2012). In this premise, Kavyani *et al.* (2012) reported an increase in antibody production in the blood cells of chickens fed these mushrooms compared to the control group. Birds naturally consume mushrooms of this genus during foraging activities (Tab. 1). However, the benefits associated with mycophagy have been studied primarily in the avian industry, but they can also occur in nature.

In poultry farming, the study is also exploring the use of fungi diets in other species, such as ducks (*Anser anser*), geese (*Cairina moschata*), quail (*Coturnix coturnix*), and turkeys (*Meleagris gallopavo*). The results of fungi supplementation in these groups are presented in Table 2. In general, both the fungal and avian industries are developing products which can provide low-cost feed and better nutritional value (Bederska-Lojewska *et al*. 2017).

The main benefits associated with the inclusion of fungi in poultry farming are improved nutritional retention, enhanced performance of the intestinal tract, and increased production of antibodies. There are variations in physiological responses among poultry species due to the diversity and composition of mushrooms in their diets. Further research is crucial to investigate dosage, preparation methods, and the inclusion of new fungal species in avian diets. Currently, there are only study results demonstrating how avians respond nutritionally to the inclusion of fungi in their diet. Considering that mycophagy occurs naturally, these studies could serve as a foundation for future research.

Conclusion

The association of birds with fungi in their diet or interactions, such as nest building, is important for natural ecosystems and should be further explored in the future. Numerous benefits have been mentioned regarding the inclusion of mushrooms in poultry farming, which can also be applicable for studying wild species based on their potential. There are several unidentified species of fungi reported in these associations, and inaccurate reports should be reviewed in the future. Conversely, research in the poultry industry has demonstrated the advantages of supplementing avian diets with fungi.

Considering the presence of fungi in all ecosystems, especially before the emergence of birds, suggests that mycophagy may have been very ancient. Additionally, the foraging behavior of birds should be analyzed in relation to the occurrence of fungi to gain a deeper understanding of the correlation. Several intrinsic and extrinsic factors may contribute to a better understanding of the interaction between birds and fungi in feeding. Avian associations with mycophagy represent an emerging field, and this review can serve as a foundation for further studies in this area.

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Tables and Figures legends

Table 1 – Records in the literature involving bird species interacting or consuming fungi.

Note: Name of avian species according to Gill *et al*. (2022), and fungal species according to IndexFungorum (http://www.indexfungorum.org/names/names.asp) and MycoBank (https://www.mycobank.org/). *Fungal associations detected in the nests of birds. Source: Authors (2023).

Table 2 – Efficacy of fungi in the diet of poultry farming species (Broiler, Ducks, Goose, Quail, and Turkey). The fungal supplementary was included in powdered extracts in the ration.

Note: Name of avian species according to Gill *et al*. (2022), and fungal species according to IndexFungorum (http://www.indexfungorum.org/names/names.asp) and MycoBank (https://www.mycobank.org/). Source: Authors (2023).

Figure 1 – Interactions of birds with fungi. The pizza graphs show the percentages for each listed category and the silhouettes of the birds are represented according to the species in this study. The arrows indicate the trend of horizontal or vertical position during foraging. Predominant Vertical Position - PVP; Predominant Horizontal Position - PHP. Legends Aves: Partial Foraging in Ground - PFG; Major Ground Foraging - MGF; Partial Arboreal Foraging - PAF; Major Arboreal Foraging - MAF. Legends Fungi: Terrestrial Habitat - TH; Arboreal Habitat - AH. *Picidae (Piciformes) with fungal associations detected in the nests of birds. Source: Authors (2023).

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Table 1 – Records in the literature involving bird species interacting or consuming fungi.

Families and species of birds	Families, species, or taxonomic groups of fungi	Method	References
Acanthizidae	**Unidentified species**		
Pycnoptilus floccosus	Small mushrooms	Observation	Zwart 1973
Alaudidae	**Pyronemataceae**		
Alaemon alaudipes	*Phaeangium lefebvrei*	Observation	Alsheikh & Trappe 1983
Ammomanes cinctura			
Ammomanes deserti			
Eremophila bilopha			
Eremophila alpestris			
Galerida cristata			
Galerida theklae			
Climacteridae	**Fomitopsidaceae**		
Cormobates leucophaea	*Laetiporus portentosus*	Observation	Maurer *et al*. 2017
Cacatuidae	**Unidentified species**		
Calyptorhynchus funereus	Possibly mold	Observation	Taylor & Mooney 1990
Casuariidae	**Unidentified species**		
Dromaius novaehollandiae	Possibly *Lycoperdon* sp. and *Bovista* sp.	Gastrointestinal analysis	Simpson 1998
Casuarius casuarius	Possibly Polypores	Gastrointestinal analysis	Crome 1976, Simpson 1998
Climacteridae	**Unidentified species**		
Climacteris picumnus	Possibly filamentous fungi and Agaricaceae	Observation	Doerr & Doerr 2002
Columbidae	**Unidentified species**		
Alopecoenas beccarii	Unidentified fungi	Observation	Weeks *et al*. 2017
Hemiphaga novaeseelandiae	Possibly *Cyttaria* spp.	Observation	Rawlings 1956, Clout *et al*. 1986, O'Donnell &

			Dilks 1994
Corvidae	**Agaricaceae**		
Corvus brachyrhynchos	*Agaricus campestris*	Observation	Webster 1902
Cracidae	**Unidentified species**		
Crax daubentoni	Unidentified fungus	Gastrointestinal analysis	Bertsch & Barreto 2008
	Marasmiaceae		
Perisoreus canadenses	*Pleurocybella porrigens*	Observation	Bailey 1904, Alsheikh & Trappe 1983
	Unidentified species		
Perisoreus infaustus	Unidentified fungus	Gastrointestinal analysis	Andreev 1978
Dinornithidae	**Unidentified species**		
Dinornis robustus	Possibly *Armillaria* sp., *Cortinarius* sp. and *Inocybe* sp.	DNA analysis	Boast *et al*. 2018
Furnariidae	**Unidentified species**		
Coryphistera alaudina	Mushrooms	Observation	Olrog 1955
Glareolidae	**Unidentified species**		
Cursorius cursor	Mushrooms	Observation	Alsheikh & Trappe 1983
Icteridae	**Agaricaceae**		
Molothrus bonariensis	*Macrolepiota bonariensis*	Observation and scat microanalysis	Costa *et al*. 2022a
Megalapterygidae	**Unidentified species**		
Megalapteryx didiformis	Possibly *Armillaria* sp., *Cortinarius* sp., and *Inocybe* sp.	DNA analysis	Boast *et al*. 2018
Megapodiidae	**Omphalotaceae**		
Alectura lathami	*Lentinula lateritia*	Observation	Simpson 1998, 2000
	Polyporaceae		
Leipoa ocellata	*Laccocephalum mylittae*	Observation	Benshemesh 1992, Reichelt & May 1997
	Unidentified species		

Alectura lathami	Unidentified fungus	Observation	Elliott & Vernes 2021
Leipoa ocellata	Possibly *Mycena* spp., *Laccocephalum* sp., and *Paxillus* sp.	Observation	Benshemesh 1992, Reichelt & May 1997
Meliphagidae	**Unidentified species**		
Ptilotula penicillata	Unidentified fungus	Gastrointestinal analysis	Mckeown 1934
Menuridae	**Boletaceae**		
Menura novaehollandiae	*Rossbeevera vittatispora*	Scat microanalysis	Elliott & Vernes 2019
	Unidentified species		
Menura novaehollandiae	Possibly *Agaricus* sp., *Descolea* spp., *Hypholoma* sp., *Hysterangium* spp., *Scleroderma* sp., *Russulaceae,* and unidentified fungus	Scats microanalysis	Elliott & Vernes 2019
Muscicapidae	**Agaricaceae**		
Erithacus rubecula	*Agaricus campestris*	Observation	Hastings & Mottram 1914
Odontophoridae	**Unidentified species**		
Callipepla californica	Possibly *Suillus* spp.	Observation	Alsheikh & Trappe 1983
Colinus virginianus	Possibly *Rhizopogon* sp. and mushrooms	Gastrointestinal analysis	Laessle & Frye 1956, Miller 1969
Petroicidae	**Physalacriaceae**		
Petroica longipes	*Armillaria limonea*	Observation	Medway 2000
	Unidentified species		
Eopsaltria australis	Possibly *Gymnomyces* sp.	Observation	Simpson 2000
Phasianidae	**Physalacriaceae**		
Gallus gallus	*Armillaria gallica*	Observation	Elliott & Vernes 2019
	Entolomataceae		
	Entoloma abortivum		
	Strophariaceae		
	Hypholoma lateritium		

	Unidentified species		
Bonasa umbellus	Fungus; mushrooms, Basidiomycetes, Agaricaceae, possibly *Lactarius* spp., *Melanoleuca* spp., and *Russula* spp.	Observation and gastrointestinal analysis	Kittams 1943, Brown 1946, Stewart 1956, Miller 1969, Mcgowan 1973, Rose & Parker 1983, Tanney & Hutchison 2011
Meleagris gallopavo	Mushrooms and *Tuber* sp.	Gastrointestinal analysis	Miller 1969, Alsheikh & Trappe 1983
Lagopus lagopus	Unidentified fungus	Gastrointestinal analysis	Williams *et al*. 1980
Canachites canadensis	Basidiomycetes	Observation	Ellison 1966, Pendergast & Boag 1970, Franceschi & Boag 1991
Phasianus colchicus	Mushrooms	Gastrointestinal analysis	Dutton & Bolen 2000
***Picidae**	**Cerrenaceae**		
Colaptes auratus	*Cerrena unicolor*	Observation	Dennis 1969, Skutch 1969, McClelland *et al*. 1979, Miller *et al*. 1979, Raphael & White 1984, Keisker 1986, Harestad & Keisker 1989, Dobkin *et al*. 1995, Saab & Dudley 1998, Steeger & Dulisse 2002, Aitken & Martin 2004, Martin *et al*. 2004, Blanc & Martin 2012, Edworthy *et al*. 2012, Lorenz *et al*. 2015
	Fomitopsidaceae		
	Fomes fomentarius	Observation	
	Polyporaceae		
	Phellinus rimosus	Observation	
	Hymenochaetaceae		
	Phellinus pini	Observation	
Dryocopus lineatus	**Fomitopsidaceae**		
	Fomes fasciatus		
	Polyporaceae	Observation	Kilham & O'Brien 1979, Cockle *et al*. 2012
	Perenniporia martia		
Dryocopus pileatus	**Fomitopsidaceae**		
	Fomes fomentarius	Observation	Conner *et al*. 1976, McClelland *et al*. 1979, Miller *et al*. 1979, Keisker 1986, Harestad & Keisker
	Postia sericeomollis	Observation	
	Hymenochaetaceae		

	Phellinus igniarius	Observation	1989, Parks *et al*. 1997, McClelland & McClelland 1999, Steeger & Dulisse 2002, Hartwig *et al*. 2004, Martin *et al*. 2004, Blanc & Martin 2012, Edworthy *et al*. 2012
Dryobates pubescens	**Fomitopsidaceae**		Conner *et al*. 1976, McClelland *et al*. 1979, Keisker 1986, Runde & Capen 1987, Harestad & Keisker 1989, Steeger & Dulisse 2002, Aitken & Martin 2004, Martin *et al*. 2004, Blanc & Martin 2012
	Fomes fomentarius	Observation	
	Hymenochaetaceae		
	Phellinus igniarius	Observation	
	Polyporaceae		
	Trametes versicolor	Observation	
Leuconotopicus borealis	**Polyporaceae**	Observation	Steirly 1957, Lay & Russell 1970, Conner *et al*. 1976, Jackson 1977, Conner & Locke 1982, Conner & O'Halloran 1987, Hooper *et al*. 1991, Rudolph & Conner 1991, Conner *et al*. 1994, Jusino *et al*. 2014, 2015, 2016
	Daedaleopsis confragosa		
	Peniophoraceae		
	Peniophora cinerea		
	Peniophora incarnata		
	Hymenochaetaceae		
	Phellinus pini		
	Fomitopsidaceae		
	Postia sericeomollis (Romell) Jülich, 1982		
	Stereaceae		
	Stereum complicatum		
	Polyporaceae		
	Trametes versicolor		
Leuconotopicus villosus	**Fomitopsidaceae**		Kilham 1968, 1971, 1979, Conner *et al*. 1976, McClelland *et al*. 1979, Miller *et al*. 1979,
	Fomes fomentarius	Observation	
	Hymenochaetaceae		
	Phellinus igniariu	Observation	

			Raphael & White 1984, Keisker 1986, Harestad & Keisker 1989, Saab & Dudley 1998, Steeger & Dulisse 2002, Aitken & Martin 2004, Martin *et al*. 2004, Blanc & Martin 2012, Edworthy *et al*. 2012, Lorenz *et al*. 2015
	Hymenochaetaceae		
Sphyrapicus nuchalis	*Phellinus igniarius* *Phellinus tremulae*	Observation	Crockett & Hadow 1975, Daily 1993, Dobkin *et al*. 1995, Hart & Hart 2001, Steeger & Dulisse 2002, Aitken & Martin 2004, Martin *et al*. 2004, Losin *et al*. 2006, Blanc & Martin 2012, Edworthy *et al*. 2012
Sphyrapicus thyroideus	*Phellinus tremulae*	Observation	Crockett & Hadow 1975, McClelland *et al*. 1979, Miller *et al*. 1979, Raphael & White 1984
	Fomitopsidaceae		
Sphyrapicus varius	*Fomes fomentarius* **Hymenochaetaceae** *Phellinus igniarius* *Phellinus tremulae* **Polyporaceae** *Trametes versicolor* *Trichaptum biforme*	Observation	Kilham 1968, 1971, 1979, McClelland *et al*. 1979, Miller *et al*. 1979, Keisker 1986, Runde & Capen 1987, Harestad & Keisker 1989, Savignac & Machtans 2006
	Unidentified species		
Picoides borealis	Possibly *Coniophora* sp., *Daedaleopsis* sp., *Exidia* sp., *Peniophora* sp., *Phlebia* sp., *Porodaedalea* sp., *Postia* sp., *Stereum* sp., *Trametes* sp., and unidentified fungus	Observation	Steirly 1957, Lay & Russell 1970, Ligon 1971, Conner *et al*. 1976, Jackson 1977, Conner & Locke 1982, Conner & O'Halloran 1987, Hooper 1988, Hooper *et al*. 1991, Rudolph & Conner 1991,

			Conner *et al*. 1994, Rudolph *et al*. 1995, Harding 1997, Jusino *et al*. 2014, 2015, 2016
Prunellidae	**Pyronemataceae**		
Prunella atrogularis	*Phaeangium lefebvrei*	Observation	Alsheikh & Trappe 1983
Psittacidae			
Enicognathus ferrugineus	Possibly *Cyttaria* sp.	Observation	Díaz & Kitzberger 2006, Díaz *et al*. 2012
Psittaculidae	**Cyttariaceae**		
Alisterus scapularis	*Cyttaria gunnii*	Observation	Elliott & Vernes 2019
Micropsitta bruijnii	Fungi and gelatinous fungus	Gastrointestinal analysis	Rand 1942, Greensmith 1975, Schodde 1977, Ivereigh 2001, Sam *et al*. 2017, Weeks *et al*. 2017
Ptilonorhynchidae	**Tricholomataceae**		
Ptilonorhynchus violaceus	*Lepista nuda*	Observation	Elliott & Marshall 2016
Turdidae	**Strophariaceae**		
Turdus merula	*Hypholoma lateritium*	Observation	Hastings & Mottram 1914
Trogonidae	**Ganodermataceae**		
	Ganoderma tornatum		
	Fomitopsidaceae		
Trogon surrucura	*Fomes fasciatus*	Observation	Cockle *et al*. 2012
	Meripilaceae		
	Rigidoporus ulmarius		
Upupidae	**Pyronemataceae**		
Upupa epops	*Phaeangium lefebvrei*	Observation	Alsheikh & Trappe 1983
	Polyporaceae		
Leipoa ocellata	*Laccocephalum mylittae*	Observation	Benshemesh 1992, Reichelt & May 1997
Rhinocryptidae	**Unidentified species**		
Scelorchilus rubecula	Possibly *Amylascus* sp.,	Fecal and DNA	Caiafa *et al*. 2021

	Austropaxilus sp., *Cortinarius* sp., *Cystangium* sp., *Descolea* sp., *Elaphomyces* sp., *Gautieria* sp., *Hysterangium* sp., *Inocybe* sp., *Lactarius* sp., and *Ruhlandiella* sp.	analyses	
Pteroptochos tarnii	Possibly *Amylascus* sp., *Cortinarius* sp., *Cystangium* sp., *Descolea* sp., *Elaphomyces* sp., *Gautieria* sp., *Hysterangium* sp., *Inocybe* sp., *Melanogaster* sp., *Ruhlandiella* sp., and *Russula* sp.	Fecal and DNA analyses	Caiafa *et al*. 2021

Note: Name of avian species according to Gill *et al*. (2022), and fungal species according to IndexFungorum (http://www.indexfungorum.org/names/names.asp) and MycoBank (https://www.mycobank.org/). *Fungal associations detected in the nests of birds. Source: Authors (2023).

Table 2 – Efficacy of fungi in the diet of poultry farming species (Broiler, Ducks, Goose, Quail, and Turkey). The fungal supplementary was included in powdered extracts in the ration.

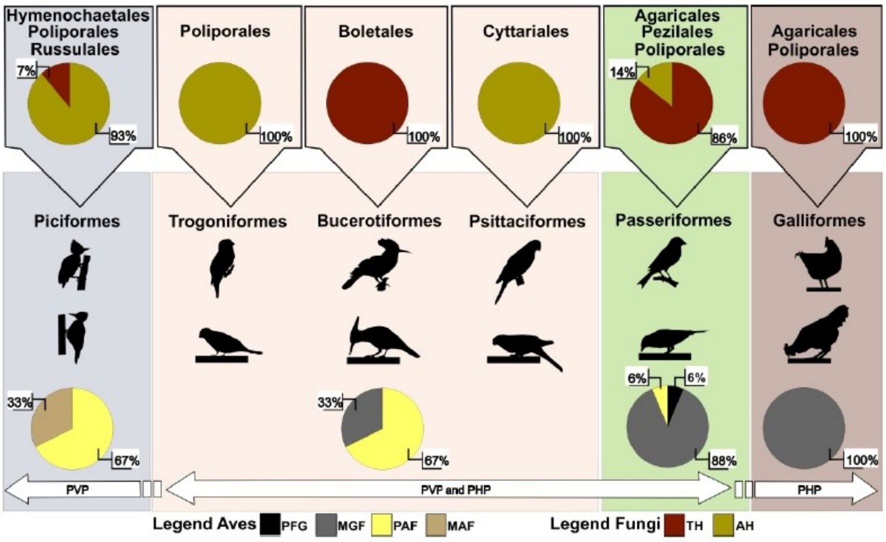
Avian families and species	Fungi families and species	Main results analyzed with inclusion in the diet	References
Phasianidae	**Agaricaceae**		
Gallus gallus	*Agaricus bisporus*	Tendency for increased body, better meat quality, better beneficial intestinal flora (*Lactobacilli* spp. and *Bifidobacteria* spp.), protective and antioxidant activity.	Giannenas *et al*. 2010a, Giannenas *et al*. 2010b, 2011, Kavyani *et al*. 2012, Mršić *et al*. 2013, Bidarnamani *et al*. 2015, Nirmala *et al*. 2017
	Agaricus blazei	Lower total serum of cholesterol concentration and increase in the production of antibodies against Newcastle disease virus.	Fanhani *et al*. 2016
	Agaricus brasiliensis	Tendency for increased body and better meat quality.	Guimarães *et al*. 2014
	Agaricus subrufescens	Action to combat the protozoan *Eimeria* spp.	Lima *et al*. 2021
	Cordycipitaceae		
	Cordyceps militaris	Lower blood lipid levels and higher levels of *Bifidobacteria* spp.	Willis *et al*. 2013, Chanjula & Cherdthong *et al*. 2018
	Physalacriaceae		
	Flammulina velutipes	Antioxidant action, nutritional improvements in the quality of meat, eggs, and stability against yolk lipid oxidation.	Chumkam & Jintasataporn 2011, Sohn *et al*. 2015, Yan *et al*. 2018, Mahfuz *et al*. 2020

Polyporaceae		
Fomitella fraxinea	Protection against lost weight during viral infections and reduced oocyst shedding.	Dalloul *et al*. 2006
Ganoderma lucidum	Tendency for increased body, better meat quality and higher levels of *Bifidobacteria* spp.	Ogbe *et al*. 2013, Willis *et al*. 2007, Willis *et al*. 2013, Ilyina *et al*. 2020
Hericiaceae		
Hericium caput-medusae	Antioxidant action, reduced population of *Salmonella* spp. and *Escherichia coli*, higher levels of *Lactobacilli* spp. and *Bifidobacteria* spp.	Shang *et al*. 2014, Mahfuz *et al*. 2020
Laetiporaceae		
Laetiporus sulphureus	Tendency for increased body, better meat quality and increase of level antibody production.	Ilyina *et al*. 2020
Omphalotaceae		
Lentinus edodes	Tendency for increased body, bactericidal action against *Mycoplasma gallisepticum*, better beneficial intestinal flora and higher levels of *Bifidobacteria* spp.	Guo *et al*. 2004, Willis *et al*. 2013
Tremellaceae		
Tremella fuciformis	Tendency for increased body, bactericidal action against *Mycoplasma gallisepticum* and better beneficial intestinal flora.	Guo *et al*. 2004
Pleurotaceae		
Pleurotus ostreatus	Tendency for increased body, better meat quality, antibacterial	Demeterová *et al*. 2012, Kavyani *et al*. 2012, Toghyani *et al*. 2012,

		action, triglyceride lower, increased antibody production, anti-protozoan action against *Eimeria* spp. and increase of level antibody production against the influenza virus.	Fard *et al*. 2014, Abro *et al*. 2016, Camay 2016
	Pleurotus sajor caju	Hepatoprotective, tendency for increased body and better meat quality.	Yogeswari *et al*. 2012
Anatidae	**Pleurotaceae**		
Anser anser	*Pleurotus ostreatus*	Increased body weight and better meat flavor and acceptability	Chang *et al*. 2016
	Pleurotus eryngii	Tendency for increased body and better carcass nutritional quality characteristics	Shuliang *et al*. 2016
Cairina moschata	**Agaricaceae**		
	Agaricus bisporus	Better carcass nutritional quality characteristics, higher levels of growth performance and carcass yields	Asadi-Dizaji *et al*. 2014, Vargas-Sánchez *et al*. 2019
	Ganodermataceae		
	Ganoderma lucidum	Reduced *Eimeria* spp. oocyst, increased antibody titers and better nutritional quality of eggs	Liu *et al*. 2016, Al-Zuhariy & Hassan 2017, Al-Tikriti & Al-Douri 2019
Phasianidae	**Omphalotaceae**		
	Lentinus edodes	Better nutritional quality of eggs	Nuraini *et al*. 2019
Cortunix cortunix	**Pleurotaceae**		
	Pleurotus ostreatus	Better carcass nutritional quality characteristics, antioxidant activity, decreased cholesterol	Asadi-Dizaji *et al*. 2017, Vargas-Sánchez *et al*. 2018, Vargas-Sánchez *et al*. 2019

		and triglyceride in blood, increased antibody titers against influenza disease virus and reduced *Eimeria* spp. oocyst	
Phasianidae	**Agaricaceae**		
Meleagris gallopavo	*Agaricus bisporus*	Antioxidant protective activity, higher levels of *Lactobacilli* spp. and *Bifidobacteria* spp., reduced population of *Escherichia coli*.	Giannenas *et al*. 2011

Note: Name of avian species according to Gill *et al*. (2022), and fungal species according to IndexFungorum (http://www.indexfungorum.org/names/names.asp) and MycoBank (https://www.mycobank.org/). Source: Authors (2023).



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5.4 Capítulo IV

Título: The bird shiny cowbirds (*Molothrus bonariensis*) in a relationship interesting of mycophagy with the mushroom *Macrolepiota bonariensis* in the brazilian Pampa biome

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The bird shiny cowbirds *(Molothrus bonariensis)* in a relationship interesting of mycophagy with the mushroom *Macrolepiota bonariensis* in the brazilian Pampa biome

0 pássaro chupim-gaudério *(Molothrus bonariensis)* em uma interessante relação de micofagia com o cogumelo *Macrolepiota bonariensis* no bioma Pampa brasileiro

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ABSTRACT

Bird-fungi associations are ecologically important, and the species of these groups that occur in the Pampa biome region have different characteristics. The Shiny Cowbirds *(Molothrus bonariensis* — Icteridae) is a ground foraging bird that cohabits vegetation of grasslands. Some mushrooms of this biome have considerably large and edible basidiomes, such as species of genus *Macrolepiota* (Agaricaceae). Therefore, the objective of this study was to contribute to the first description of mycophagy for the Pampa biome, including the species *Molothrus bonariensis* and *Macrolepiota bonariensis*. Data were submitted to a test of variance ANOVA and Tukey test at a 5% significance level. For the quantitative multivariate data, the test of Kiviat was elaborated. The test of Schumacher and Eschmeyer was used to determine the degree of interaction of the birds with the mushrooms. 60 hours of observations were realized with a flock of bird containing approximately 76 individuals in the study area. 30 fresh scat samples and 10 mushrooms that showed pecks signals were collected from the area georeferenced. Microscopic analysis of scat of birds revealed basidiospores of mushroom. For the record of foraging events, the mycophagy of birds was higher in zones with more occurrence of mushrooms. The results assist in better understand this important mycophagy interaction between the Shiny Cowbird and the mushroom *M. bonaerensis*. The study confirms that bird-fungal mycophagy associations are more widespread than previously thought.

Keywords: ecological interactions, avian natural diet, ornithology, mycology.

RESUMO

As associações pássaro-fungo são ecologicamente importantes, e as espécies desses grupos que ocorrem na região do bioma Pampa possuem características diferentes. O Chupim-gaudério (*Molothrus bonariensis* – Icteridae) é um pássaro forrageiro terrestre que coabita a vegetação dos campos. Alguns cogumelos deste bioma possuem basidiomas consideravelmente grandes e comestíveis, como os das espécies do gênero *Macrolepiota* (Agaricaceae). Assim, o objetivo deste estudo foi contribuir para a primeira descrição de micofagia para o bioma Pampa, incluindo as espécies *Molothrus bonariensis* e *Macrolepiota bonariensis*. Os dados foram submetidos ao teste de variância ANOVA e teste Tukey ao nível de 5% significância. Para os dados quantitativos multivariados, foi elaborado o teste Kiviat. O teste de Schumacher e Eschmeyer foi utilizado para determinar o grau de interação dos pássaros com os cogumelos. Foram realizadas 60 horas de observações com um bando de pássaros contendo aproximadamente 76 indivíduos na área de estudo. 30 amostras de fezes frescas e 10 cogumelos que apresentaram sinais de bicadas foram coletados da área georreferenciada. A análise microscópica das fezes dos pássaros revelou basidiósporos do cogumelo. Para o registro dos eventos de forrageamento, a micofagia dos pássaros foi maior nas zonas com a maior ocorrência dos cogumelos. Os resultados auxiliaram em uma melhor compreensão dessa importante interação de micofagia entre o Chupim-gaudério e o cogumelo *M. bonaerensis*. O estudo confirma que as associações de micofagia entre pássaros e fungos são mais difundidas do que se pensava anteriormente.

Palavras-chave: interações ecológicas, dieta natural de aves, ornitologia, micologia.

1 INTRODUCTION

Interactions between birds and fungi have an important ecological function in diverse ecosystems (ELLIOTT *et al*., 2019). The predominant presence of fungi in virtually all existing habitats provides directly or indirectly their interaction with a lot of organisms (PEAY *et al*., 2008). Containing an enormous morphological diversity of basidiomes and robust mechanisms for the dispersal of their spores, fungal strategies range from long distance dispersal by wind to the active and passive dispersal by animal (MAIA *et al*., 2015; HORTON, 2017). These advantages of dispersal by mycophagy may have evolutionarily led this group to develop distinct morphological and physiological processes mainly related to their dispersers (HORTON, 2017).

Studies have shown that with birds, selection pressures is directly related to environmental factors. For example, birds that forage on the ground are more likely to eat mushrooms that grow in the soil (KOENIG; DICKINSON, 2004). This fact was inferred by Webster (1902), who observed foraging *Corvus brachyrhynchos* eating *Agaricus campestris*. In addition, the forager *Menura novaehollandiae* was observed feeding with *Rossbeevera vittatispora* (ELLIOTT; VERNES, 2019), supporting this theory.

These interactions are not unique to ground, the observation reported by Maurer *et al*. (2017) with the arboreal forager *Cormobates leucophaea* eating the ligninolytic species *Laetiporus portentosus*, may be an example of feeding habit linked to the type foraging. In general, these facts help to understand Optimal Foraging Theory (OFT), in which the generalist predator seeks to minimize energy expenditure by expanding its food sources rather than maximizing energy expenditure by seeking specific food sources (KAMIL; KREBS, 2012). Thus, coevolution between predator and prey becomes profitable once a new adaptation against the predator requires a lot of energy (YAHNKE, 2006).

In Brazil, the Pampa biome occupies an area of 63% of the state of Rio Grande do Sul (IBGE, 2019). Species found in this region are adapted to a temperate subtropical and temperate climate with four well-characterized seasons, predominant vegetation of grasslands, sparse shrub, and tree formation (BERRETTA, 2021). Natural grasslands are typical and the main economic activity is livestock. Also, it is a source of staple food for more than 20 million cattle and sheep (LUCCA; AREND, 2020). The main characteristics of soil include a sandy and clayey texture, due to the origin of the sedimentary rock (SEVERO, 2020).

In the Pampa biome, the mushroom *Macrolepiota bonaerensis* (Agaricaceae) and the bird popularly known as Shiny Cowbird (*Molothrus* *bonariensis* – Icteridae) were recorded (ALVES *et al*., 2016; PUTZKE; PUTZKE, 2017). Both are associated with the vegetation of the grasslands in Pampa. The fungal species has main characteristics the edibility, grows gregarious or solitary in the soil, has to 20 cm in diameter, and nutmeg or floury smell (PUTZKE; PUTZKE, 2017). The bird species is resident, with approximately 17 to 21 cm long, omnivorous, gregarious, and ground forager (DE PIACENTINI *et al*., 2015).

To contribute unpublished data on the interaction between birds and fungi involving the Shiny Cowbirds and mushroom *M. bonariensis*, this study aimed to describe the foraging behavior of this bird and the inclusion of mushroom in its natural diet.

2 METHODS

2.1 GEOREFERENCING OF THE STUDY AREA

The study area included 2 ha, located in São Gabriel, Rio Grande do Sul state, Brazil, in Casa Branca farm (latitude -30°19'53''S and longitude -54°17'18''W). The Pampa biome predominant in the region was composed of grasslands and pasture, with border riparian vegetation. Two artificial lentic water pond forms were delimited as the center of the study area. A 90 m perimeter was established around the water bodies. The application MAPS.ME GPS (https://maps.me) was used to divisions of the three zones studied (A, B, and C). The zones were divided according to the legislation for riparian zones in Brazil (BRASIL, 1976). Zone A (active water channel) extends in the first 30 m from the edge of the water. Zone B (flood plain) extended for another 30 m from the end of the perimeter of zone A. Zone C (filter area) extending for another 30 m beyond the end of zone B (Figure 1).

Figure 1. Artificial water pond with location and georeferencing of the study area. Replica of the actual GPS image available in Google Earth (https://earth.google.com). Division of zones A, B and C according to the legislation applicable to riparian zones from Brazil (BRASIL 1976).

Source: Author (2021).

2.2 RAPID SURVEY OF MUSHROOMS AND OBSERVATION OF BIRDS FORAGING BEHAVIOR

The Rapid Survey methodology used for tree vegetation (RATTER *et al.*, 2001) was adapted for quantitative sampling and basidiome collection. Due to the short period of exposure of basidiomes of *Macrolepiota* indicated in the literature among April and May only (SOBESTIANSKY, 2005; ROTHER; SILVEIRA, 2008; ALVES *et al.*, 2016), the survey occurred among March and May 2021. The number of mature basidiomes was cataloged and georeferenced in each zone (A, B, and C). According to the identification key for *Macrolepiota* with occurrences in Rio Grande do Sul state (PUTZKE; PUTZKE, 2017), based on the findings of more species of the genus in the study area, photographic images and notes were made on morphological characters such as color, diameter and texture of the pileus, the base of the stipe, and type of ring.

Shiny Cowbirds species were identified using Guia de Aves do Rio Grande do Sul (JACOBS; FENALTI, 2020). Bird foraging behavior was observed in a georeferenced area located 50 m from the endpoint of zone C. This space was stipulated aiming at non-human interference (ROMANOWSKI; ZMIHORSKI, 2008). Bushnell 10x42 binocular with long-range lenses was used for observations. Results related to foraging behavior were based on observations, and was assumed that all foraging observations should be treated as independent observations (WEIMERSKIRCH *et al.*, 2009).

Succeeding the birds returned to the place of the overnight stay, the basidiomes with signs of pecking were collected under license SISBIO nº 79049-1, dried at 40°C, and stored in paper bags for taxonomic identification (PUTZKE; PUTZKE, 2017). Subsequently, they were deposited at the Laboratório de Taxonomia de Fungos LATAF in Universidade Federal do Pampa campus São Gabriel, Rio Grande do Sul state, Brazil. In the collection zones, the fresh scats on soil, leaves, and fence were collected and stored under refrigeration (BORRELLI *et al*. 2020).

2.3 MUSHROOM AND SCATS MICROSCOPY

For the mushrooms showing signs of pecking, analyzes macroscopic and microscopic characters were performed using the key for Agaricales from Brazil (PUTZKE; PUTZKE, 2017). Histological cuts of lamellae and the cortical layer of the pileus were performed. The cuts were rehydrated in 5% KOH and arranged on the slide, covered with a coverslip. The method for microbial analysis of the scats was adapted as follows: placed in a Falcon 50 ml tube a portion of 0.5 g of scats for 1 hour in a solution containing ethanol (70% concentrated) diluted with water and two drops of liquid detergent (BORRELLI *et al*., 2020). The liquid was removed to the 5 ml mark, and the rest was macerated with the help of a stick. Two drops of the solution were deposited in the slide and covered with a coverslip. The Olympus DP53 optical microscopic was used for both procedures.

2.4 STATISTICAL ANALYSIS OF DATA

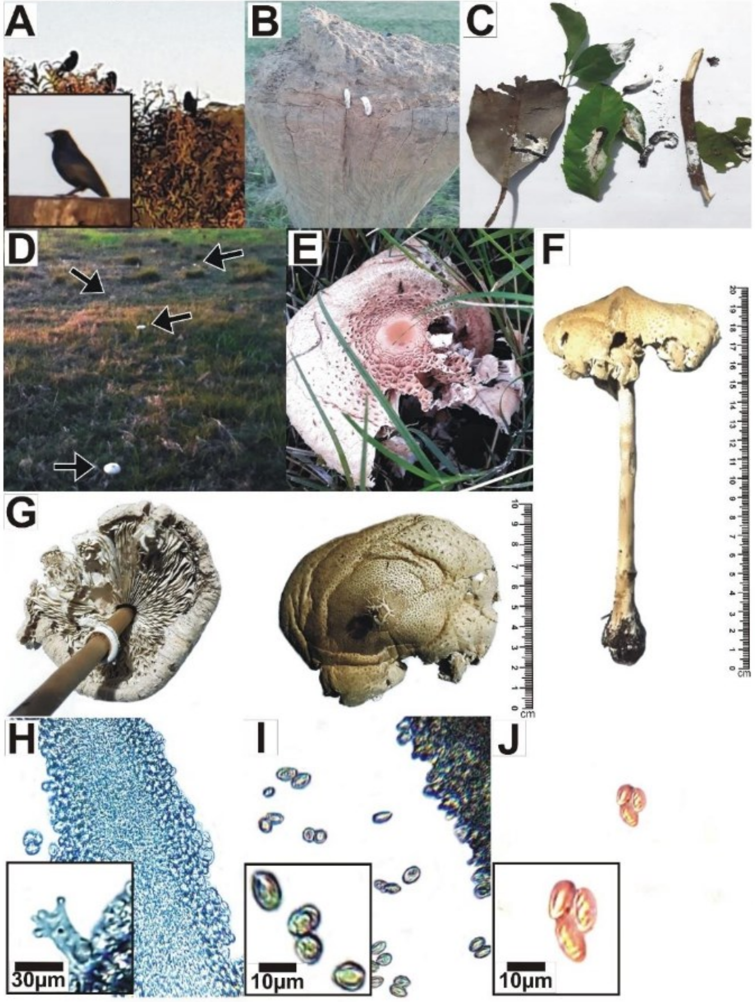
The data of foraging activity was measured in moments of occurrence of Shiny Cowbirds in Zones A, B, and C. Bird number that had been occurred in each zone, the birds that had eaten mushrooms, and the interactions of individual foraging were cataloged. The number of mushrooms available per zone and the number of units with pecking signs were submitted to analysis of variance ANOVA and Tukey test at the 5% significance level (ESTAT, 1994). For quantitative multivariate data, the test by Kiviat diagram was elaborated (WANG *et al*., 2015). The method of Schumacher and Eschmeyer was used for population estimation (EDWARDS; EBERHARDT, 1967), and determine interactions between bird foraging and mushroom abundance. The analyzes were elaborated in the BioEstat v.5.3 program (SANTOS, 2007).

3 RESULTS

In the study area, 10 visits were realized between March and April 2021. Observations were conducted in alternated days, according to rain precipitation, and totaled about of 60 observation hours. Low grasslands due to intense cattle activity during the hot season contributed to record of the foraging birds. A flock of Shiny Cowbirds containing approximately 76 individuals was observed in the delimited zones (Figure 2-A). A sample of 30 fresh scats was collected during the observations, and the scats were collected after the visitation of the birds (Figure 2 B-C).

Two species of *Macrolepiota* were found at the georeferenced site, but only *Macrolepiota bonaerensis* showed peck signs and was collected for analysis (Figure 2 D-G). The key of Putzke and Putzke (2017) was used to view the morphological macroscopic and microscopic characteristics, also to identify the mushroom species (Figure 2 H-I). Microscopic analysis of scats sampled birds contained basidiospores of the mushroom (Figure 2-J).

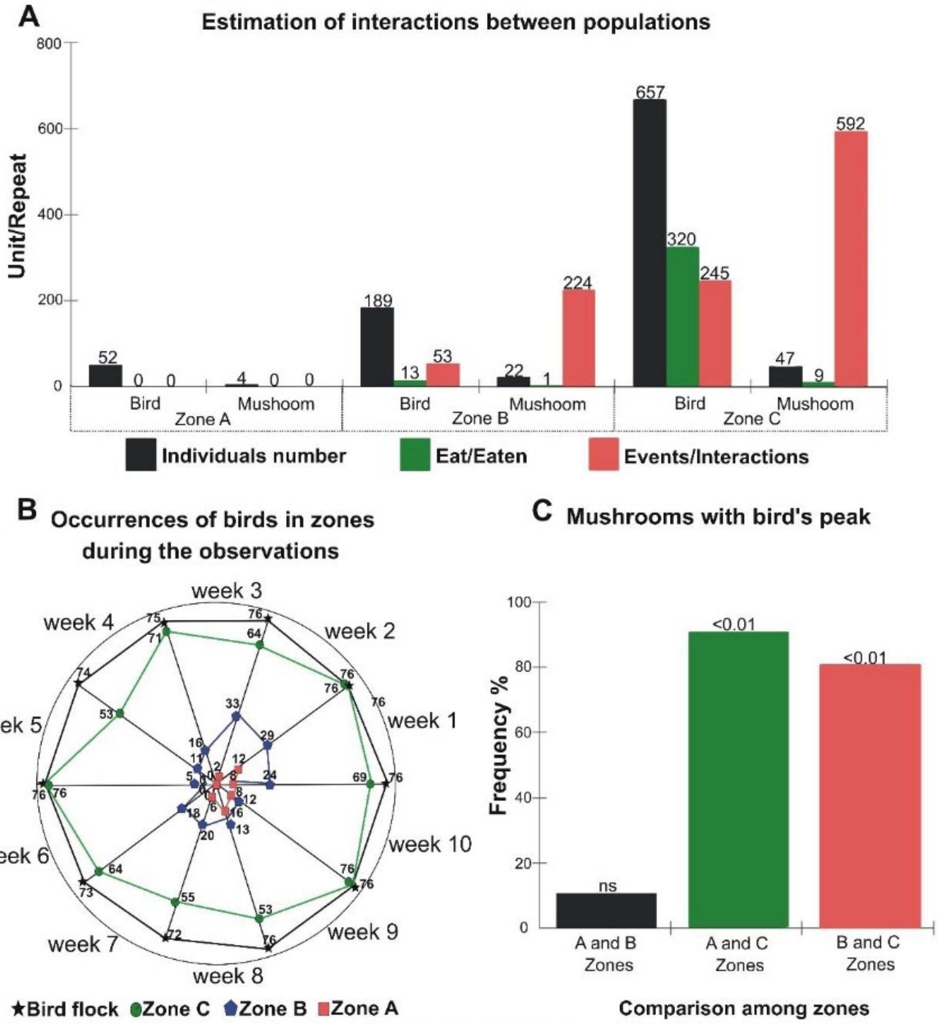
Figure 2. Analyzed material of species of this study: *Molothrus bonariensis* and *Macrolepiota bonaerensis*. Shiny Cowbirds species (A). Scats material collected (B-C). Zone C with arrows indicating the place occurrence of mushrooms (D). Pileus at the collection place with signs of bird's beak (E). Basidiome collected (F). Superficial and lamellar views of the mushroom pileus with pecking signs (G). Lamellar trama with 4-spored basidia and basidiospores (H-I). Basidiospores founding in the bird's scats (J).



Source: Author (2021).

Four mushrooms were georeferencing in zone A during the observations. Even occurring the foraging behavior near this place, the birds did not include those mushrooms in their diet. In zone B, 22 mushrooms were found and only one mushroom was collected containing signs of pecks. Zone C was the region with the more distance of artificial water ponds and near the riparian vegetation. In this zone, 47 mushrooms were registered and nine showed signs of pecks (Figure 3-A).

Figure 3. Statistical analysis of species of this study: *Molothrus bonariensis* and *Macrolepiota bonaerensis*. Schumacher and Echmeyer test (A). Diagram of Kiviat (B). Analysis of variance ANOVA and Tukey-test with 5% probability (C). Inferences were made during the 60 hours of observation. Ns – no significant values.



Source: Author (2021).

The parameters related to the mushrooms and birds by Schumacher and Echmeyer test indicated significant differences in the interaction of birds in each zone (A, B, and C), and the number of mushrooms that had pecks. Results showed that the greater the availability of mushrooms in each zone, more units of mushroom served as food for the birds. Thus, higher were the interaction indices of birds with the mushrooms during the foraging. There was no record of bird foraging events with mushrooms in zone A, where 5% of the mushrooms in this study were georeferenced. However, 17% of foraging events occurred in zone B, which accounted for 27% of interactions between birds together at 28%

of georeferenced mushrooms. Zone C accounted for 83% of the foraging events, maintaining 73% of all bird-mushroom interactions, totaling 67% of georeferenced mushrooms (Figure 3 A).

The spatial distribution of species per zone (A, B, and C) and the temporal series of cyclic characters showed that the birds had a higher occurrence in zone C. In the third and fifth, and after in the third and seventh weeks respectively, zones B and A had a decline in the presence of birds. Nevertheless, the first and third, and later along the seventh and tenth weeks, were the periods with the highest occurrence of Shiny Cowbird flock in zones B and A (Figure 3-B).

The comportment of parameter alpha chosen a *priori* revealed that among the zones A and C, B and C differences occurred, principally influenced by the behavior of birds during foraging activity (Figure 3-C). Inside each zone, such as in C, can be seen the birds move quickly (10 times per minute) among the mushrooms. Also, subgroups with few individuals performed this behavior repetitively.

4 DISCUSSION

The results shown the first description of the inclusion of mushroom *Macrolepiota bonaerensis* in the natural diet of the Shiny Cowbird. In general, flocks of these birds feed on insects, seeds, and fruits. The bird also has a habit of digging into cattle scats and following them in search of insects and ticks (DE PIACENTINI *et al*., 2015). This is the first description of the inclusion of mushrooms as a food source for this bird and the pioneering description of this interaction bird-fungi. The interactions is the first from South America in the Pampa biome, and the second in the world to use the method of scats microscopy analysis in addition to the foraging observations, the first was performed by Elliott *et al*. (2019).

In general, mycophagy bird-fungi is little studied, although there is a lot of interest of mycologists and ornithologists in clarifying these relationships. The foraging behavior of Shiny Cowbirds shown in observations was standard. The use of space and the association of small subgroups in independent food search were already cited in the literature (KATTAN *et al*., 2016). It is possible to infer that these birds develop those strategies with the inclusion of mushrooms in the diet and did not change their foraging behavior, keeping flocking consistent.

To Maia *et al*. (2015), the mycophagy for birds occurs naturally, because some individuals may tend to stay in the intensive feeding areas, as was the case in zone C. Also, some occasionally went for these zones in alternated moments. This behavior was seen during the observations. The interaction of birds with the mushrooms showed that as greater the abundance of food, the greater the permanence of birds in the zone. This factor corroborates with the theory by Kamil and Krebs (2012), where the optimal foraging is the one that has low energetic value and more food available.

The abundance of mushrooms directly influenced their inclusion in the birds diet. Differences of mushrooms in each zone occurred but were higher from zone A to zone C in ascending order. Zone A formed an active water channel, and these conditions were not ideal for *M. bonaerensis* (ROTHER; SILVEIRA, 2008). Zones B and C showed better conditions, being that zone B is a flood plain, and zone C is a filter area, both ideal for the occurrence of *M. bonaerensis* (SOBESTIANSKY, 2005; ALVES et al., 2016). To Berretta (2021) the conditions in these regions, principally the riparian zone, as in zone C, generally have more species diversity. Consequently, the findings help to highlight the importance of preserving riparian zones, whether of arboreal vegetation or vegetation of grasslands.

After birds visit, the georeferencing corroborated in the identification of mushrooms that had pecking. In this perspective, Koenig and Dickinson (2004) infer that the bird-fungi interaction is more favorable when both have similar habits/habitats. The soil mushrooms are more likely to integrate the diet of ground forager birds (HORTON, 2017). Webster (1902) related in your study the inclusion of *Agaricus campestris* in the diet of *Corvus brachyrhynchos* in North America, where both the mushroom and the bird have habitats/habits respectively of soil and ground forager. The study of Elliott and Vernes (2019) with *Rossbeevera vittatispora* (grown on soil) and *Menura novaehollandiae* (ground forager) in Australia also reinforces this theory.

In Australia, another example was announced by Maurer *et al*. (2017), however with different habits/habitats when observing the *Cormobates leucophaea*, a treecreeper forager that included in its diet *Laetiporus portentosus*, which is ligninolytic fungal that grow in substrate of trunks. These factors probably are relational to the predator-prey coevolution (YAHNKE, 2006), and weighing the implications of Optimal Foraging Theory (KAMIL; KREBS, 2012), that in future can be better studied.

M. bonaerensis grow directly on soil and open areas (PUTZKE; PUTZKE, 2017). The checking of mushrooms during the observations and scats analysis enabled to prove that Shiny Cowbirds eat *M. bonaerensis*, once that bird has not ingested the mushroom spores otherwise. In this premise, 30 fresh scats samples from various individuals were used in our analysis, and in all of them spores of mushrooms were found. However, studies further should be conducted to reinforce this result, once the mycophagy was related to the specific flock with occurrence in São Gabriel city. Despite both species being distributed in the same region, they are replicated for all Pampa biome extensions (ALVES *et al*., 2016, PUTZKE; PUTZKE, 2017).

Bird behavior to include in their diet this mushroom is not restricted only to geographical localization. This can be linked to many factors, for example, mammals that frequently eat fungi detect them by smell and visual cues (VERNES *et al*., 2015). In this point, *M. bonaerensis* has a large pileus (8 - 10 cm diameter) bell-shaped to flattened, of whitish to hazelnut color with nutmeg or floury smell (PUTZKE; PUTZKE, 2017). Therefore, these other factors need to be explored, involving also other species.

5 CONCLUSION

The results in relation to the food association of Shiny Cowbird (*Molothrus bonariensis*) with the mushroom *Macrolepiota bonaerensis* assist to understand the mycophagy of these birds. The distribution, foraging behavior, and availability of the mushroom as a food resource, demonstrate the importance of both species to the Pampa biome. More research needs to be done to analyze the nutritional value and dispersion potential, among other questions of bird-fungi associations. In this form, the results shown that the mycophagy association bird-fungi is more present than previously thought.

ACKNOWLEDGEMENTS

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5.5 Capítulo V

Título: First report of *Zygothrica candens* Burla, 1956 (Diptera, Drosophilidae) in mycophagic association with the mushroom *Oudemansiella cubensis* (Berk. and M.A. Curtis) R.H. Petersen, 2010 (Agaricales, Physalacriaceae) in southern Brazil

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Notes and Comments

First report of *Zygothrica candens* Burla, 1956 (Diptera, Drosophilidae) in mycophagic association with the mushroom *Oudemansiella cubensis* (Berk. and M.A. Curtis) R.H. Petersen, 2010 (Agaricales, Physalacriaceae) in southern Brazil

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Mycophagy associations in Diptera can be divided into four distinct groups based on the lifestyle of each species. Primary mycophagists feed exclusively on fungi (basidiome or mycelium). Secondary mycophagists are called saprobes and feed on decaying hosts. Detritivores may feed on much decaying biological material, in this case they may oviposit on basidiome. Predators occur in association with all fungal structures, and their larvae are mainly predatory in the last stage (Santa-Brígida et al. 2012). For a better understanding of mycophagous flies, the selection of habit depends of fungi occurrence (Bunyard, 2018). In addition, basidiomata only form with humidity and temperature specific to each species (Putzke and Putzke, 2017). An important point is that the larval phase of Diptera is relatively short (Yamashita and Hijii, 2007), which may well coincide with the period of basidiome occurrence.

Gottschalk et al. (2009) and Bunyard (2018) infer in their studies that mycophagous flies of Drosophilidae are associated with more than 31 species of mushrooms, some toxic such as *Amanita muscaria* (L.) Lam (Agaricaceae), including *Drosophila fallen* (Fallen, 1823), *Leucophenga varia* (Walker, 1849), *Mycodrosophila dimidiate* (Loew, 1862), and *Zygothrica hypandriata* (Burla, 1956). In addition, Bunyard and Foote (1990) reported that the life cycle of *Drosophila putrida* (Sturtevant, 1916) was associated with the mushroom *Oudemansiella radicata* (Relhan) Singer (Physalacriaceae).

Oudemansiella cubensis (Berk. & MA Curtis) RH Petersen (2010) has a fleshy pileus and is described in Brazil, Costa Rica, Cuba, Dominican Republic, Ecuador, Colombia, and Argentina (Petersen et al., 2008; Wartchow et al., 2010). The species is mentioned as native in South America (Putzke and Putzke, 2017). There are no known cases of mycophagous flies associated with this mushroom in the literature. There are no reports of mycophagous of feeding habits to *Zygothrica candens* (Burla, 1956), and this is the first report involving *O. cubensis*. Also, the fly is distributed only in Brazil and Ecuador, considered native to South America (Tidon et al., 2022). Thus, the present study aimed to describe the first record of mycophagy and the association of *Z. candens* with the mushroom *O. cubensis* in southern Brazil.

The study was conducted in September 2021 in the National Forest (FLONA) in São Francisco de Paula, Rio Grande do Sul, Brazil (-29°25’22”S and -50°23’11”W), a conservation unit of approximately 1,606.00 hectares of native Atlantic Forest, with fragments of dense ombrophilous forest and mixed ombrophilous forest. Samples were collected in the Centenarian Araucaria Trail. Four basidiomata of mushroom with signs of mycophagy growing on the substrate of *Araucaria angustifolia* Bertol. (Kuntze), and five adult flies in the basidiomata were collected to identification. The mushrooms and flies were collected under license SISBIO n° 78538-1.

Basidiomata were dried at 40°C and stored in paper bags for taxonomic identification using the key of Putzke and Putzke (2017). Drosophilids were preserved in tubes containing 70% ethyl alcohol and identified based on external morphology and genitalia (male and female) according to Burla (1956) and Grimaldi (1987). Both samples were examined with optical microscope Olympus DP53 and Zeiss Discovery V20. Subsequently, the samples were deposited at the Laboratório de Taxonomia de Fungos in Universidade Federal do Pampa, Campus São Gabriel, Rio Grande do Sul, Brazil.

As taxonomic characters, *O. cubensis* has a pileus 30-120 mm in diameter, grayish white. Surface of the pileus with scales and glutinous. Fleshy context with grayish-white lamellae of 1.5 mm, adnate. Stipe white, fibrillose-squamous with a base of 30-140 × 2-20 mm (Figure 1A). White or cream spores with 10-16 µm, globose to subglobose, smooth and thick-walled, inamyloid, hyaline. Basidia with 50-120 × 18-30 µm, clavate and tetrasporic.

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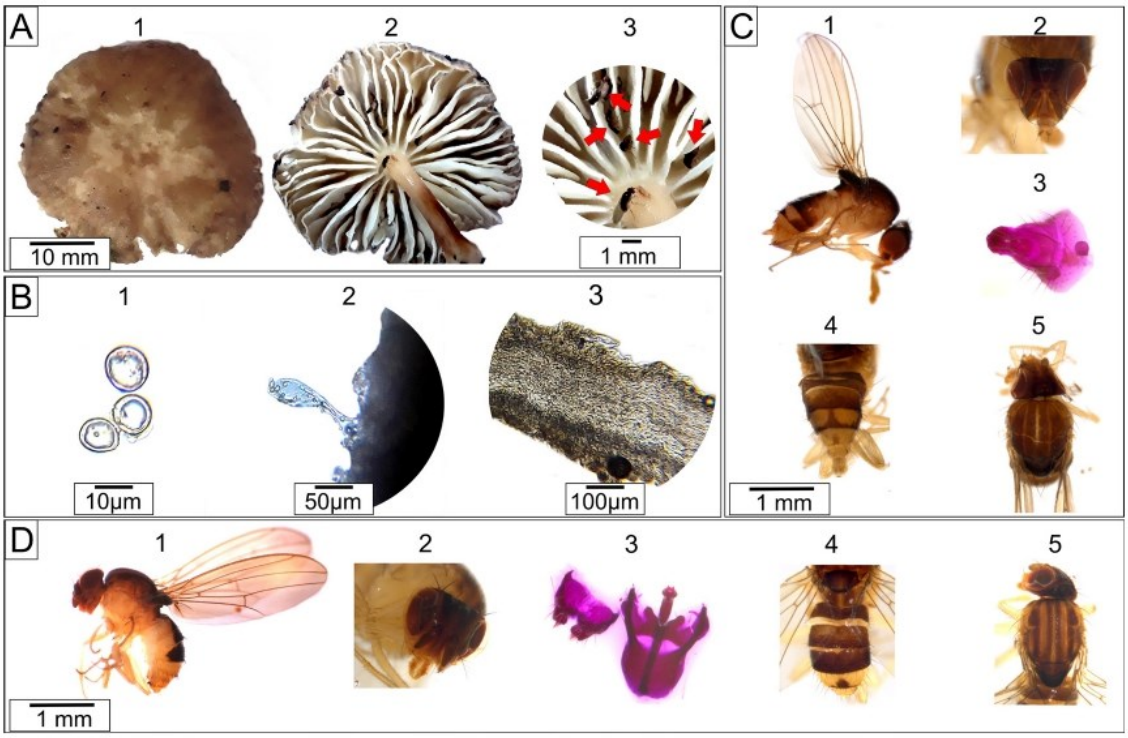

Figure 1. Macro and microstructures of the species analyzed in this study. *Oudemansiella cubensis*: pileus top view 1-A, bottom view 2-A, arrows indicating adult flies found in the basidiome 3-A, spores 1-B, basidium 2-B, lamellar trama 3-B. *Zygothrica candens* female: lateral view 1-C, frontal view head 2-C, genitalia (oviscapt valve + spermathecal capsule) 3-C, dorsal view abdomen 4-C, dorsal view thorax 5-C. *Zygothrica candens* male: lateral view 1-D, frontal view head 2-D, genitalia (hypandrium in V-shaped and pregonites + phallus and phallapodeme) 3-D, dorsal view abdomen 4-D, dorsal view thorax 5-D.

Pleurocystidia and cheilocystidia numerous with 70-200 × 15-50 µm, versiform (Figure 1B). Pileipellis formed by piriform or subglobose elements. Context formed by broad filamentous hyphae. Cortical layer of the stipe formed by thin filamentous hyphae. Grows on wood. Distribution in Brazil in Porto Alegre city - Rio Grande do Sul state (Putzke and Pereira, 1988), and São Paulo city - São Paulo state (Singer, 1989). In São Francisco de Paula city - Rio Grande do Sul state this is the first record of the species.

During the collection of material, individuals in holometabolous development stages (embryos, larvae, pupae and adults) of *Z. candens* were found in the lamellar region of *O. cubensis*. According to Burla (1956) as taxonomic characters, *Z. candens* has head brown, eyes red, pedicel and scape brown, total length 18-25 mm. Orbital and bristles plates are brown. Front and face ochre with ocellar tringle occupying approximately 1/2 of the frontal length. Thorax with dark and light bands brownish-brown, scutellum brown and slighter in the margins, legs yellow. Wings hyaline, without distinct spots with veins straight. Abdomen yellow with brown bands on tergites. Terminalia in U-shaped form with frontal lobes small. Hypandrium in V-shaped form containing many growth lines. Phallapodeme short in lateral view (Figure 1C and D). Distribution in Brazil in Boracéia city - São Paulo state (Val and Kaneshiro, 1988) and Itatiaia city - Rio de Janeiro state (Burla 1956; Wheeler, 1970). There is already a case for the São Francisco de Paula city - Rio Grande do Sul state (Gauterio et al., 2020).

In relation to the characteristics of the interaction of mycophagy between fly and mushroom, the mushroom has shown signs of predation in the lamellar portions of the basidiome. In the analyzes of internal structures of digestive system of the fly, spores mushroom were found in the hindgut. External structures, such as wings, legs, thorax and abdomen also showed attached spores.

The study reports the first observation of mycophagy association of *Z. candens* with *O. cubensis* in southern Brazil. The natural population of *Zygothrica* has already been described in Rio Grande do Sul state (Valer et al., 2016; Gauterio et al., 2020). However, the occurrence of *Z. candens* on *O. cubensis* is new. This fact can be associated with fungi species whose known distribution from southern region of the country (Petersen et al., 2008; Wartchow et al., 2010).

Neotropical mycophagous flies are also represented in the Drosophilidae. Bunyard (2018) studied three mycophagous flies feeding on *Amanita* sp., which is considered a toxic species. Nevertheless, drosophilids are not only associated with toxic mushrooms. Other Agaricomycetes, belonging to the families Agaricaceae and Boletaceae (Bunyard, 2007), as well as *Oudemansiella* sp. (Physalacriaceae) have already been described in the literature in mycophagy associations with drosophilids (Bunyard and Foote, 1990). The latter are classified as non-toxic mushrooms (Putzke and Putzke, 2017). In the study by Gauterio et al. (2020) with analyzes of the phylogenetic relationships of *Zygothrica* and other genera of drosophilids associated with fungi, it was inferred that the neotropical group has a lineage close with neotropical fungi of Auriculariales and Agaricales. In these two cited orders, the use of the fly as a breeding site and the presence of its larvae are reported in the fungi, but the taxonomic information on the fungi was not mentioned. However, this factor could be an of the most important indicators of this association.

In studies conducted in the Brazilian Amazon involving arthropods associated with edible mushrooms, Amaringo-Cortegano et al. (2013) found a relationship among *O. cubensis* and individuals of Poduromorpha and Siphonophorida. The latter order was identified in 87% of the collected samples containing mycophagous individuals. However, the authors did not report individuals of drosophilids. Doge et al. (2015) reported that resource variability is the main factor for the Drosophilidae population size. In addition, fleshy basidiomata such as *O. cubensis* are reported to be preferred by the mycophagous flies (Santa-Brígida et al. 2012). According to Gauterio et al. (2020), mycophagous flies and fungi coevolved. Moreover, most flies already analyzed are polyphagous and complete their life cycle in basidiomata (Bunyard, 2018). Another important factor is the presence of soft tissues in Agaricales, macro and micronutrients, and the tolerance of mycophagous flies to fungal toxins, as concluded by Yamashita and Hijii (2007). Thus, even if *Z. candens* have occurrence in this region, it is more feasible to infer that the fly uses the mushroom as a temporal resource. In this sense, the mushroom is not the main responsible for the maintenance of its guild richness. As a benefit, the fly disperses the spores of this mushroom to distances reachable by its flight.

The adaptation of the fly in using a spatially unpredictable temporal resource such as the basidiome may be the key that determines the success of species presence in the region. Also, the attractiveness of the mushroom resulting both from the quality of the substrate, as in the case of *A. angustifolia* trees, and the natural influence of the FLONA are intrinsically involved in this association. However, *O. cubensis* is considered an arboreal host, with wood as its main substrate (Putzke and Putzke, 2017), so the occurrence of the fungi is not restricted to the distribution of *A. angustifolia*. According to Bunyard (2007, 2018), it is unpredictability that drives the mechanisms of speciation in drosophilids. The occurrence of the fly and the mycophagy associated with the entire life cycle of the studied individuals, which includes oviposition, growth phases and return of the adult individual, are possibly interconnected. Therefore, further analysis should be conducted, considering that the description of this mycophagy association is unprecedented for both species and has only been described in southern Brazil.

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5.6 Capítulo VI

Título: Mycophagy of Attini Ants (Hymenoptera, Formicidae, Myrmicinae) with Agaricales Mushrooms (Basidiomycota, Agaricomycetes) at Riparian Zone in Southern Brazil

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Mycophagy of Attini Ants (Hymenoptera, Formicidae, Myrmicinae) with Agaricales Mushrooms (Basidiomycota, Agaricomycetes) at Riparian Zone in Southern Brazil

Micofagia de Formigas Attini (Hymenoptera, Formicidae, Myrmicinae) com Cogumelos Agaricales (Basidiomycota, Agaricomycetes) em Zonas Ripárias no Sul do Brasil

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ABSTRACT

Attini ants (Hymenoptera, Formicidae, Myrmicinae) have great diversity in exploiting food resources. However, little is known about the mycophagy involving Agaricales fungi (Basidiomycota, Agaricomycetes). Moreover, these associations are of paramount importance in riparian zones, as the interaction among soil, fauna and flora is fundamental to the maintenance of these environments. Thus, the aim of this study was to describe cases of mycophagy between ants and fungi in order to understand how these associations occur in riparian zones. To this, collections were made between 2021-2022 in the valleys of the Rio Vacacaí, Rio dos Sinos and Rio Pardo in the state of Rio Grande do Sul, Brazil. The samples were analyzed for characters macro and microscopic and identified taxonomically. Mycophagy activities of six species of ants worker were cataloged, among them *Acromyrmex niger*, *Acromyrmex versicolor*, *Tranopelta gilva*, *Tranopelta subterranea*, *Pheidole flavens* and *Mycetosoritis hartmanni*, which included in their diet the mushrooms *Agaricus rufoaurantiacus*, *Macrocybe titans*, *Agrocybe underwoodii*, *Dactylosporina steffenii*, *Lepiota micropholis* and *Neopaxillus echinospermus*, respectively. The observed interactions demonstrate the dispersal of fungal spores through ant body structures, such as legs, antennae, thorax and abdomen; basidiomata as aliment source, such as pileus, lamellae and stipe; and generalized and specialized levels of predation on various fungi structures. Our results demonstrate unprecedented mycophagous relationships and show that Agaricales fungi can be considered a food source for Attini in riparian zones in Brazil south.

Keywords: Food resource, spore dispersing agents, ecological interactions.

RESUMO

As formigas Attini (Hymenoptera, Formicidae, Myrmicinae) apresentam grande diversidade na exploração de recursos alimentares. No entanto, pouco se sabe sobre a micofagia envolvendo fungos Agaricales (Basidiomycota, Agaricomycetes). Além disso, essas associações são de suma importância em zonas ripárias, pois a interação entre solo, fauna e flora é fundamental para a manutenção desses ambientes. Assim, o objetivo deste estudo foi descrever casos de micofagia entre formigas e fungos a fim de entender como essas associações ocorrem em zonas ripárias. Para isso, foram feitas coletas entre 2021-2022 nos vales do Rio Vacacaí, Rio dos Sinos e Rio Pardo no estado do Rio Grande do Sul, Brasil. As amostras foram analisadas quanto aos caracteres macro e microscópicos, e identificadas taxonomicamente. Foram catalogadas as atividades de micofagia de seis espécies de formigas operárias, dentre elas *Acromyrmex niger*, *Acromyrmex versicolor*, *Tranopelta gilva*, *Tranopelta subterranea*, *Pheidole flavens* e *Mycetosoritis hartmanni*, que incluíam em sua dieta os cogumelos *Agaricus rufoaurantiacus*, *Macrocybe titans*, *Agrocybe underwoodii*, *Dactylosporina steffenii*, *Lepiota micropholis* e *Neopaxillus echinospermus*, respectivamente. As interações observadas demonstram a dispersão de esporos dos fungos através das estruturas do corpo das formigas, tais como pernas, antenas, tórax e abdômen; estruturas dos basidiomas como fonte alimentar, tais como pileo, lamelas e estipe; e níveis generalizados e especializados de predação em várias estruturas dos cogumelos. Nossos resultados demonstram relações micofágicas inéditas, e mostram que os fungos Agaricales podem ser considerados como fonte de alimento para Attini em zonas ripárias no sul do Brasil.

Palavras-chave: Recurso alimentar, agentes de dispersão de esporos, interações ecológicas.

1 INTRODUCTION

The ant species Attini (Hymenoptera, Formicidae, Myrmicinae) is one of the most successful groups of Arthropods, both in terms of diversity and abundance. The group has a high social complexity and a great exploration ability in a variety of food niches (Houadria and Menzel, 2020). Food from various parts of plants, such as leaves, seeds, fruits, and roots; also multiple kinds, such as carrion, manure, and others, are part of a wide variety of dietary habits of this group (Lanan, 2014). Many nutritional forms are used to some extent by ants, and consequently, some become specialists and others generalists in terms of their diet (Csata and Dussutour, 2019).

Little is known about the inclusion of fungi in ant diet, nor about the types of mycophagy habit involving the ants with Agaricales Fungi (Basidiomycota, Agaricomycetes) (Epps and Penick, 2018). Except for *Euprenolepis* ants, with reports of mycophagy in the Old World (Claridge and Trappe, 2005; Witte and Maschwitz, 2008; Von-Beeren *et al*. 2014), in addition to a select group of Attini endemic to the New World and known to cultivate fungi (de Fine *et al*. 2014). For these fungiculture ants, five types of agricultural systems are cataloged: lower agriculture, coral-fungus agriculture, yeast agriculture, generalized higher agriculture, and leafcutter agriculture (Branstetter *et al.* 2017).

In terms of fungal structures associated with ant feeding, gongylidia (swollen hyphae), hyphae tissues in general (pileus and stipe), spores, and mycelium are the most commonly reported (Epps and Penick, 2018). However, even with few studies on mycophagy in Hymenoptera, other Arthropod orders such as Acari, Coleoptera, Collembola, Diptera, Lepidoptera, and Orthoptera have already had important inferences regarding the inclusion of fungi in their diet (Schigel, 2012; Dash *et al*., 2018; Innocenti and Sabatini, 2018; Pollierer *et al*., 2020; Tozlu *et al*., 2022; Wakil *et al*., 2022). As nutritional importance of fungi, regarding the benefits of their consumption by arthropods, mainly minerals, vitamins and fats are listed (Biedermann and Vega, 2020).

In contrast, there are several reports of ants visiting the basidiomata of various fungi, mainly in relation to foraging and predation of larvae attached to the fungal tissues (Branstetter *et al.* 2017; Epps and Penick, 2018; Pereira *et al.* 2020). However, it is unclear whether the visit is limited to hunting larvae, or whether mycophagy also occurs. It is believed that many ants spread fungal spores even when there is no mycophagy because the spores can attach to the ants' body structures and travel with them short and long distances (Witte and Maschwitz, 2008; Von-Beeren *et al*. 2014; Epps and Penick, 2018).

Riparian zones are permanent protected areas where the presence of vegetation, mycota, and fauna play unique ecological interactions related to their conservation (Brasil, 2012). In this respect, little is known about the activity of ants in these areas, as well as the fungal community. Nevertheless, nutrient cycling for fungi is an important process that takes place in all environments (White *et al.* 2018). The benefits cited for ants include supporting plant reproduction, improving the nutrient quality of the soil, and aerating the soil, among others (Tuma *et al.* 2020).

In this form, to better understand the mycophagy associations that occur in different riparian zones in southern Brazil, this study aimed to describe unique cases of mycophagy involving *Acromyrmex niger* (Smith, 1858), *Acromyrmex versicolor* (Pergande, 1893), *Tranopelta gilva* (Mayr, 1866), *Tranopelta subterranea* (Mann, 1916), *Pheidole flavens* (Roger, 1863) and *Mycetosoritis hartmanni* (Wheeler, 1907), who have included in their diet *Agaricus rufoaurantiacus*, *Macrocybe titans*, *Agrocybe underwoodii*, *Dactylosporina steffenii*, *Lepiota micropholis* and *Neopaxillus echinospermus*, respectively. Also, to analyze how these interactions occur and the levels of mycophagy performed by the group, in order to aid in their understanding.

2 MATERIAL AND METHODS

Sample location

In the period 2021-2022, visits were realized to riparian zones in Rio Vacacaí, locality of São Gabriel (Point A, latitude -30°21'56"S and longitude -54°18'48"W); Rio dos Sinos, locality of São Francisco de Paula (Point B, latitude -29°25'22"S and longitude -50°23'1"W); and in the Vale do Rio Pardo, locality of Vera Cruz (Point C, latitude -29º42'53 "S and longitude -52º30'20 "W). All points located in Rio Grande do Sul state, southern Brazil.

Collection and taxonomic identification of specimens

The Rapid Survey method used for tree vegetation (Walter and Guarino, 2006) was adapted to collect basidiomata with signs of mycophagy. The fungal material with the ants was collected under license from SISBIO n°78538-1. For the mushrooms, collections were performed according to Putzke and Putzke (2017), where each specimen was collected with the substrate. The basidiomata were stored individually in plastic containers and dehydrated at 40°C. Identification of Agaricales mushrooms was performed according to the key for Agaricales Fungi of Brazil available in Putzke and Putzke (2017) and data available on the Index Fungorum platform (http://www.indexfungorum.org/).

The ants found in basidiomata were stored in tubes with 70% alcohol. The keys of Fisher and Cover (2007) and Baccaro *et al*. (2015) were used for identification, as well as the data available on the AntWiki platform (http://www.antwiki.org/). Specimens were analyzed according to their macromorphological and micromorphological characteristics using the Olympus CH-2 optical microscope. All collected specimens (mushrooms and ants) were stored in the Laboratório de Taxonomia de Fungos of Universidade Federal do Pampa, São Gabriel campus, Rio Grande do Sul state, Brazil.

Statistical analyzes of the data

ANOVA test was conducted under the model of variables with the "predation location" factor and predation levels for the "amount" factor, conducted in the form of data percentage (Sabin and Stafford, 1990). Comparisons among the means of spore quantity, as well as of each individual were conducted under the LSMeans model (Lenth and Lenth, 2018), applying the Test-Tukey at 5% significance using the Estat v.2.0 program (Estat, 1994).

3 RESULTS AND DISCUSSION

For the Attini six species of workers ants were cataloged involved in mycophagy activities: *Acromyrmex niger*, *Acromyrmex versicolor*, *Tranopelta gilva*, *Tranopelta subterranea*, *Pheidole flavens* and *Mycetosoritis hartmanni*. The ants were associated with six species of Agaricales: *Agaricus rufoaurantiacus*, *Macrocybe titans, Agrocybe underwoodii*, *Dactylosporina steffenii, Lepiota micropholis* and *Neopaxillus echinospermus* respectively. After the identification of the specimens, two taxonomic keys were prepared, one for the identification of the mushrooms and the other for identifying the ants, both involved in mycophagy.

Key of identification of Agaricales Fungi involved in mycophagy

1a. Hymenophore with free or adnate lamellae, context not gelatinized. Spores amyloid or inamyloid, smooth or angular, without hilar appendage, globose to ellipsoid, nodose-subangular or subangular. Annulus often present. Cortical layer of pileus formed by a palisadoderm or hymenioderm, sometimes epithelial with prostrate hyphae…………...…………………………………………………………………...…**2**

1b. Hymenophore with adnate or decurrent lamellae, gelatinized context. Spores inamyloid, globose equinate with hilar appendage, smooth thin-walled. Annulus absent. Cortical layer himeniform or sub-hymeniform, with smooth elements (Physalacriaceae)……………………………………………***Dactylosporina steffenii***

2a. Spores varying from yellow to fuscous-brown, tobacco-brown or clay-brown. Germinative pore present or absent …………………...………………..……..……..…**3**

2b. Spores white to cream colored, inamyloid subglobose to ovoid, thin-walled. Germinative pore absent (Callistosporiaceae)……………..……..…***Macrocybe titans***

3a. Cortical layer of the pileus formed by a palisadoderm or hymenioderm, which may also be epithelial, or formed by spherocysts with prostrate hyphae. Hymenophore trama regular to irregular, never bilateral or inverse (Agaricaceae)…………………..…..**4**

3b. Cortical layer of pileus formed by a cellular epithelium or by subisodiametric spherocysts, hymenophore trama regular (Strophariaceae) or inverse (Paxillaceae)…..**5**

4a. Basidiome does not change color in KOH. Spores inamyloid dark-toned, brown, thick-walled ovoid, germinative pore absent. Absent clamp connections. Pileus 2 - 5 cm in diameter, with recurved orange-brown scales………..***Agaricus rufoaurantiacus***

4b. Basidiome does not change color when bruised. Spores pseudoamyloid light-toned, white, ovoid not truncate at base, thin-walled, germinative pore absent. Absent clamp connections. White pileus up to 1.5 cm in diameter containing white scales……………………………………………………….....***Lepiota micropholis***

5a. Pileus up to 20 mm in diameter, ochraceous brown. Cystidia usually more than 55 µm long. Spores ellipsoid thick-walled and smooth, containing a truncated germinative pore…………………………...…………………...………..……***Agrocybe underwoodii***

5b. Pileus up to 40 mm in diameter, yellowish brown or orange-gold. Annulus imperceptible to absent. Spores subglobose to globose, thick-walled inamyloid, germinative pore absent……………………...……......…..***Neopaxillus echinospermus***

Key of identification of Attini worker ants involved in mycophagy

1a. Mesosoma covered with non-flexible, mostly homogeneous spines, hairs or bristles………………………………………………………………………...………...**2**

1b. Mesosoma covered with short, widely spaced hairs; long hairs may occur, but then flexible and largely heterogeneous…………………………………….………………**4**

2a. Body and head with rigid, incised, erect spines. Head usually containing a pair of dorsal tubercles. Frontal lobes well developed, partially or completely covering the insertion of the antennae, clearly separated by the posterior median portion of the clypeus (*Acromyrmex*)………………………………...………………………………..**3**

2b. Body and head with conspicuous erect bristles, often stiff and curled. Head without the presence of dorsal tubercles. Frontal lobes poorly developed and not expanded laterally to cover sides of head, but then enlarged and reaching anterior margin of clypeus in full-face view (*Mycetosoritis*)……………...……...***Mycetosoritis hartmanni***

3a. Eyes and mandibles covered by a uniform opaque integument. Mandible subtly prolonged. Tubercles on back of gaster grouped in four longitudinal and sometimes imperceptible series, containing varying thicknesses that are shorter or of some size like that of the spines……………………………………………….***Acromyrmex niger***

3b. Eyes and apical edge of mandibles more blackish than integument. Mandible prolonged and robust. Tubercles on the back of the gaster randomly distributed, median and short, of varying thickness, but shortened on each side of the anterior margin………………………………………………………..***Acromyrmex versicolor***

4a. Mandibles with 4 or more teeth that gradually increase in size toward the apex. Propodeum with projections and spines (*Pheidole*)………………...…***Pheidole flavens***

4b. Mandibles with 4 to 5 teeth, not increasing toward the apex, but may contain differentiated preapical tooth. Propodeum without projections or spines (*Tranopelta*)………………………………………………………………………....**5**

5a. Promesonotum and propodeum slightly convex, continuous in lateral view. Scap of antennae not extending beyond vertexal border. Eyes equidistant between ocelli and clypeal margin, small and faceted…………………………….…....***Tranopelta gilva***

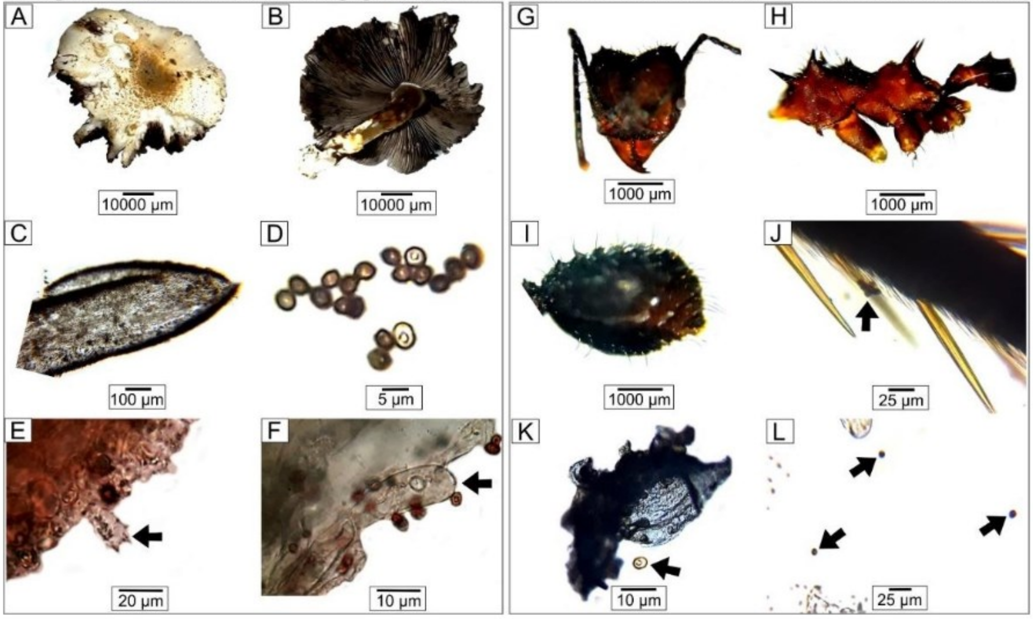
5b. Promesonotum and propodeum strongly convex in lateral view. Scap of antennae surpassing vertexal edge. Eyes closer to posterior margin of clypeus than to ocellus, amphagi and prominent……………………………………...***Tranopelta subterranea***

Taxonomic description of species involved in mycophagy

Agaricus rufoaurantiacus Heinem and *Acromyrmex niger* (Smith, 1858)

The fungus has a pileus 2 - 3 cm in diameter, convex and umbonate. Pileus pale cream with orange-brown scales (Figure 1 A). Lamellae free and membranous, proximal and brown (Figure 1 B). Stipe 2.5 - 4 cm in diameter, central, cylindrical and inflated at base with recurved squamules (Figure 1 B). Hymenophoreal trama regular (Figure 1 C). Annulus present, membranous, superior and near the pileus. Spores 4 - 5 x 2.5 - 3.7 µm, ellipsoid to ovoid, brown, smooth, thick-walled (Figure 1 D). Basidia 12.5 - 18 x 5 - 6.5 µm, clavate to sub-fusoid with 2 to 4 sterigmata (Figure 1 E). Pleurocistidia absent. Cheilocystidia 13. 5 - 20 x 5 - 7.5 µm, inflated-clavate, with thin wall (Figure 1 F). Cortical layer trichodermal, with narrow and parallel terminal hyphae. Absent clamp connections. Grows on soil. Distribution in Brazil: the occurrence in Rio Grande do Sul in the Pampa biome is unprecedented, being mentioned for Brazil by Pegler (1997) only for the states of São Paulo and Pernambuco in the Atlantic Forest biome.

Figure 1 - *Agaricus rufoaurantiacus* (A-F) and *Acromyrmex niger* (G-L). Pileus top view (A). Basidiome with lamellae (B). Lamellar trama (C). Spores (D). Basidium (E). Cheilocystidium (F). Frontal view of head (G). Lateral view of thorax (H). Side view of gaster (I). Spores attached to leg (J). Posterior region of intestine with spore (K). Cut of the posterior region of the intestine containing spores (L). Arrows indicate the structures described. Source: Authors (2022).



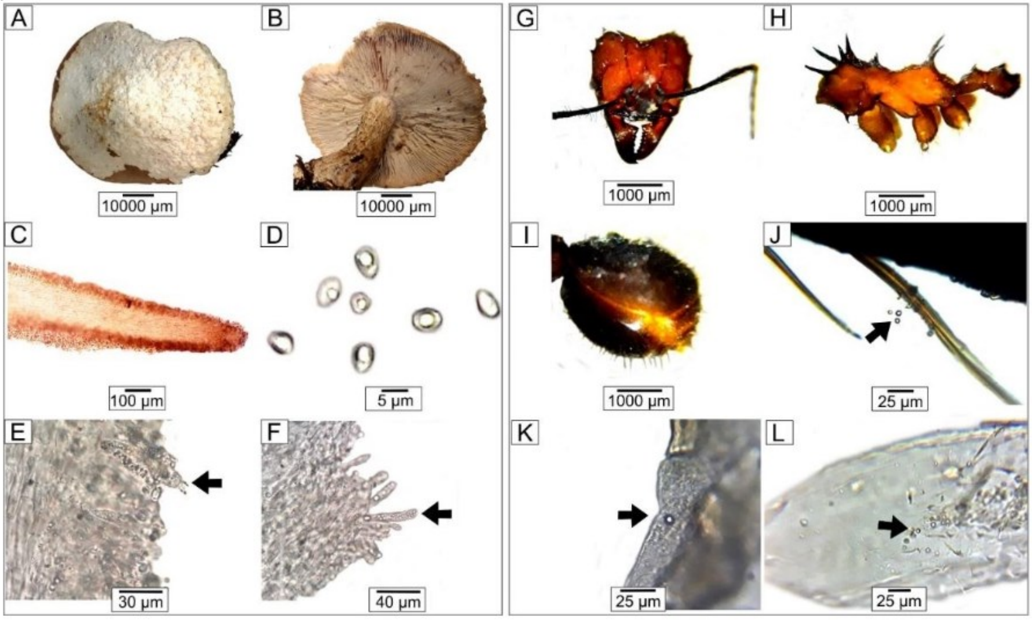
The ant has a supraocular spine present, a pair of defined dorsal spines, small eyes, and a short extension on the mandible (Figure 1 G). Tegument opaque with uniform coloration. Postpetiole articulated, first segment of gaster rigid, does not flex over mesosoma. Thorax with 3 or 4 pairs of smooth spines (Figure 1 H). Tubercles on dorsum of gaster grouped in four longitudinal and sometimes imperceptible series (Figure 1 I). Spores of the fungus were found in external body structures of the ants, arranged in the head, thorax and abdomen region, also among spines, antennae and legs (Figure 1 J). Internally, spores were found in the gut (Figure 1 K and L). Distribution in Brazil: in the Rio Grande do Sul, in the Pampa biome, *A. ninger* has already been described (Loeck *et al*. 2003). Also, in the Atlantic Forest (Garcia *et al*. 2011), Cerrado (Vaz-de-Mello *et al*. 1998), Pantanal and Amazon (Gonçalves, 1961), being considered a native species of the Neotropical region.

***Macrocybe titans* (H.E. Bigelow & Kimbr.) Pegler, Lodge & Nakasone and *Acromyrmex versicolor* (Pergande, 1893)**

The fungi present pileus 8 - 50 cm in diameter, convex to flat. Pileus surface ochraceous, with grayish margin (Figure 2 A). Lamellae strongly sinuate, grayish white and proximal (Figure 2 B). Stipe 6 - 15 cm of diameter, cylindrical to subclavate, containing small squamules (Figure 2 B). Hymenophoreal trama regular (Figure 2 C). Annulus absent. Spores 5.5 - 7.0 × 4.0 - 5.0 µm, subglobose to ovoid with thin walls (Figure 2 D). Basidia 25 - 38 × 6.5 - 10 µm, narrowly clavate containing 4 sterigmas (Figure 2 E). Pseudocystidia scattered 35 - 50 × 7 - 10 µm, fusoid with narrow, filiform apex, with thin walls (Figure 2 F). Cortical layer of the pileus subhymenial, not gelatinized, narrow, interwoven thin-walled hyphae with conspicuous clamp connections. Grows directly on soil. Distribution in Brazil: in Rio Grande do Sul the species already has a record of occurrence in the Atlantic Forest biome (Singer, 1990). Occur in the states of Paraná, Atlantic Forest biome (Battistin and Picciola, 2015).

The ant has an absent supraocular spine, a wide and shallow frontal canal, eyes and apical border of the mandible black, large and triangular mandible (Figure 4 G). Tegument opaque, postpetiole articulated, first segment of gaster rigid, does not flex over mesosoma, thorax with 3 or 4 pairs of smooth long spines (Figure 2 H). Tubercles on the dorsum of the gaster grouped in lateral series, arranged in irregular rows (Figure 2 I). Spores of fungi were found in the region of the head, thorax and legs (Figure 2 J). In the gut spores were found (Figure 2 K and L). Distribution in Brazil: in the Atlantic Forest biome, the species has no cataloged occurrence, as well as for Brazil. There are only records of occurrence for North and Central America (Fowler, 1988; Julian and Fewell, 2004), and a single occurrence in South America for Guyana (Fernández and Sendoya, 2004), being considered a species native to the Neo-Arctic region.

Figure 2 - *Macrocybe titans* (A-F) and *Acromyrmex versicolor* (G-L). Pileus top view (A). Basidiome with lamellae (B). Hymenophoral trama (C). Spores (D). Basidium (E). Pseudocystidia (F). Frontal front view of head (G). Lateral view of thorax (H). Side view of gaster (I). Spores attached to leg (J). Posterior region of intestine with spore (K). Cut of the posterior region of the intestine containing spores (L). Arrows indicate the structures described. Source: Authors (2022).

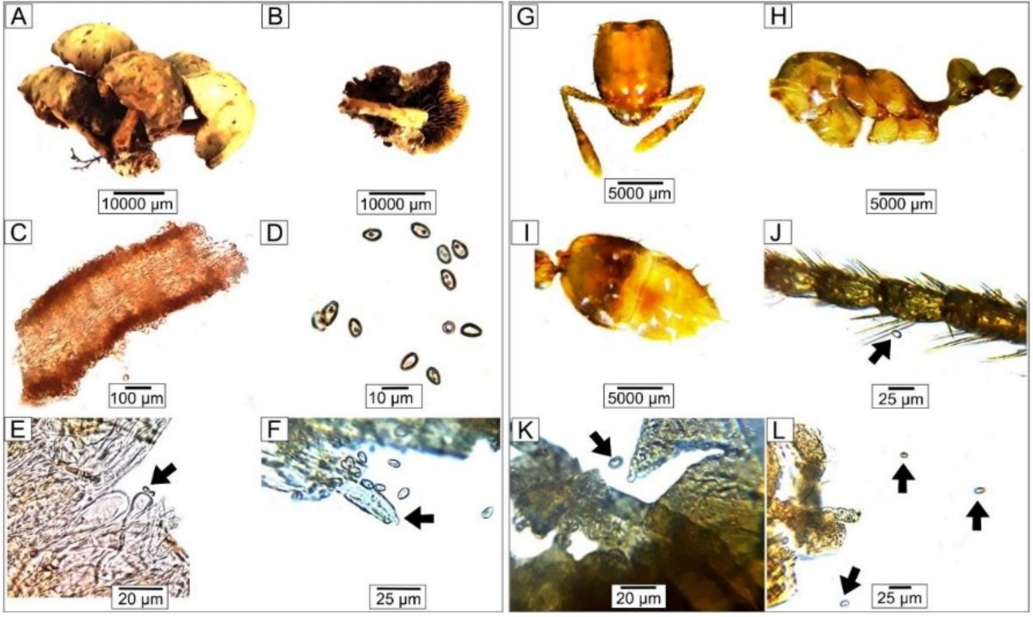


Agrocybe underwoodii (Murrill) Singer and *Tranopelta gilva* (Mayr, 1866)

The pileus of fungi with up to 20 mm in diameter, convex to flattened shape with an ochraceous brown hue (Figure 3 A). Lamellae adnate, close, broad with yellowish tones. Stipe 25 - 30 x 2 - 3 mm, central with slightly enlarged base. Annulus absent or hardly noticeable (Figure 3 B). Hymenophoreal trama regular (Figure 3 C). Spores 8 - 11.5 × 6 - 7 µm, ellipsoid thick-walled and smooth, with truncated germinal pore (Figure 3 D). Basidia 18 - 20 × 3 - 5 µm, smooth, thick-walled with 2 - 4 sterigmate. Pleurocystidia 55 - 70 × 15 - 23 µm, broad, thin-walled, hyaline, clavate (Figure 3 E). Cheilocystidia 25 - 55 x 9 - 18 µm of diameter, ventricose at base and narrowed apex up to 8 µm in diameter (Figure 3 F). Cortical layer of the pileus formed by a cellular epithelium containing subglobose to pyriform elements with 22 - 30 x 12 - 18 µm. Clamp connections present. Specimens were found growing on substrate of *Araucaria angustifolia* (Bertol.) Kuntze, 1898. Distribution in Brazil: in Rio Grande do Sul the description is unprecedented. However, in Atlantic Forest biome the species already has occurrence for the state of Paraná (Watling, 1992; Meijer, 2008; Coimbra, 2015).

The ant contains a rectangular head, diminutive faceted, equidistant eyes. Antennal scrobes absent. Mandibles small, smooth with sheen and longitudinal striations (Figure 3 G). Propodeum and promesonotum continuous in lateral view, weakly convex and interrupted by a metanotal groove (Figure 3 H). Gaster ranging from translucent to white, containing fine, short, shiny, whitish and flexible pilosity (Figure 3 I). Mushroom spores were found in the region of the antennae and legs (Figure 3 J). Internally, spores were also found in the gut (Figure 3 K and L). Distribution in Brazil: in the Atlantic Forest biome the species already has a cataloged occurrence (Fernandez, 2003), as well as for the Pantanal and Cerrado (Demétrio *et al*. 2017), Amazonia (Ryder *et al*. 2007) and Caatinga (Prado *et al*. 2019), being considered native to the Neotropical region.

Figure 3 - *Agrocybe underwoodii* (A-F) and *Tranopelta gilva* (G-L). Pileus top view (A). Basidiome with lamellae (B). Hymenophoral trama (C). Spores (D). Basidium and pseudocystidia (E). Cheilocystidia (F). Front view of head (G). Lateral view of thorax (H). Lateral view of gaster (I). Spore attached to the leg (J). Section of the gut with spore (K-L). Arrows indicate the structures described. Source: Authors (2022).

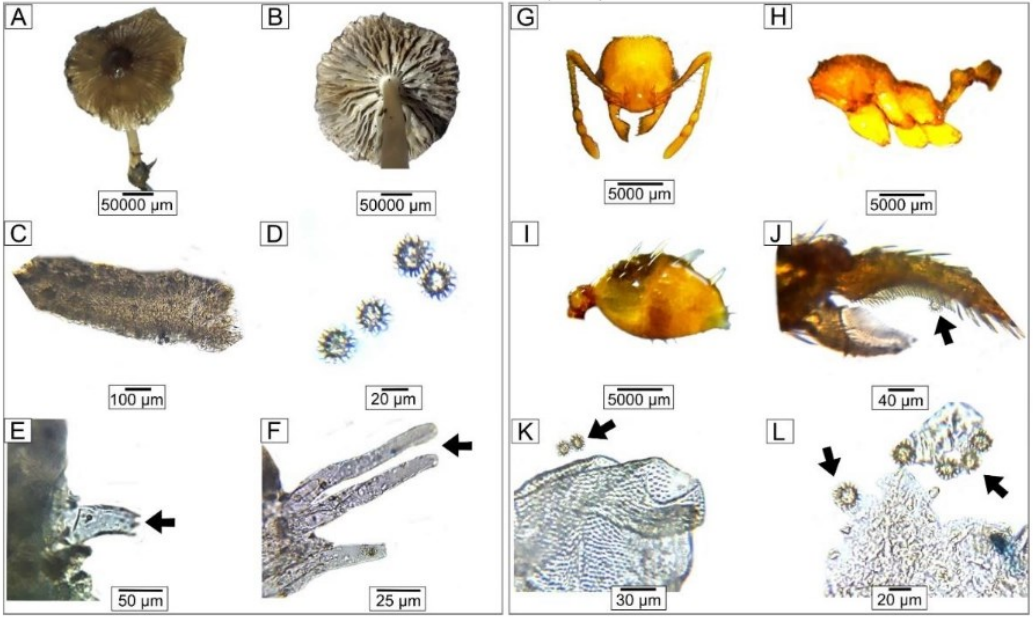


Dactylosporina steffenii **(Rick) Dörfelt and** ***Tranopelta subterranea*** **(Mann, 1916)**

The fungi exhibit pileus 3.5 - 10 cm in diameter, umbrinous, dark gray or grayish in color, convex to flattened in shape (Figure 4 A). Lamellae adnate, white, sub-close to distant. Stipe clear, broad at base showing pseudorriza (Figure 4 B). Hymenophoreal trama regular with filamentous and septate hyphae (Figure 4 C). Spores 15 - 23 x 14 - 21 µm (including spines), globose, equinate with large hilar appendage. Spines 1.5 - 3.5 µm long, inamyloid (Figure 4 D). Basidia 40 - 60 x 10 - 20 µm, hyaline, usually containing four sterigma (Figure 4 E). Thin-walled hyaline cheilocystidia 30 - 40 x 15 - 22 µm, thin-walled, hyaline, vesicular to filamentous or nearly clavate. Pleurocystidia 80 - 160 x 15 - 50 µm, ampulaceous, mucronate to subcapitate, thin-walled, sometimes with resinous incrustations at the apex (Figure 4 F). Cortical layer of the pileus formed by globose cells 15 - 30 x 8 -10 µm, with palisade hymeniodermal terminal cells. Present clamp connections. Grow in soil and its pseudorriza extends more than 10 cm deep into the soil. Distribution in Brazil: the species has been described for all state of Rio Grande do Sul (Atlantic Forest and Pampa biome) (Putzke and

Pereira, 1988), Pantanal, Cerrado and Caatinga (Valões-Araújo and Wartchow, 2021), and for the Amazon (Wartchow *et al*. 2010).

Figure 4 - *Dactylosporina steffenii* (A-F) and *Tranopelta subterranea* (G-L). Pileus top view (A). Basidiome with lamellae (B). Lamellar trama (C). Spores (D). Basidium (E). Pleurocystidia (F). Front view of head (G). Lateral view of thorax (H). Lateral view of gaster (I). Spore attached to the leg (J). Posterior region of intestine with spores (K and L). Arrows indicate the structures described. Source: Authors (2022).



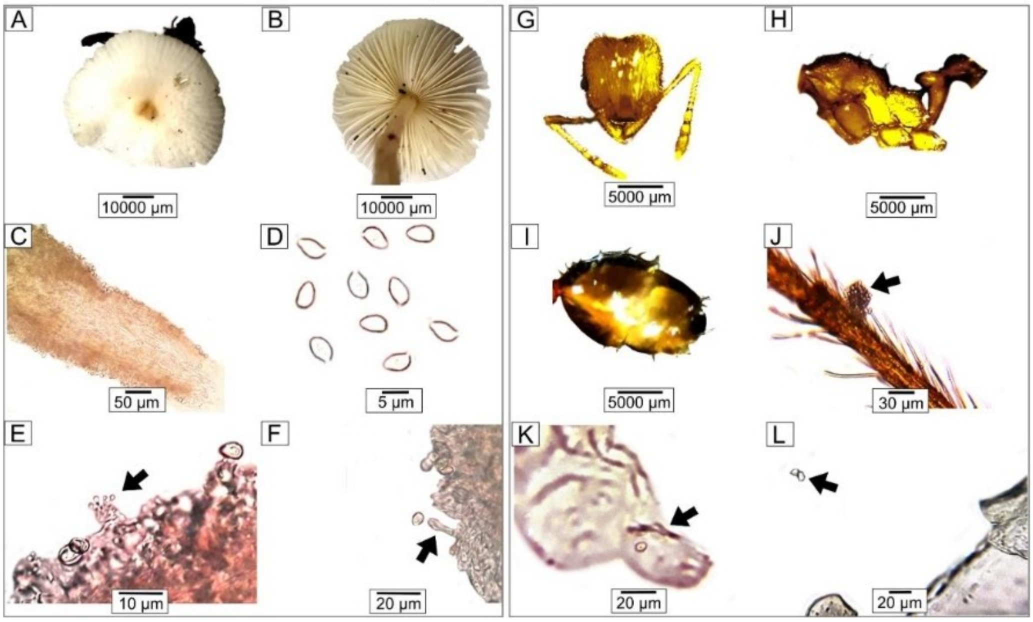
The ant has wider than long head, moderate and faceted eyes. Antennal scrobes absent. Mandibles smooth, shiny and longitudinally carinae. Five robust teeth with apical and subapical ones larger than the rest (Figure 4 G). Propodeum and promesonotum strongly convex in lateral view, containing deep metanotal sulcus (Figure 4 H). Gaster translucent, containing almost imperceptible striations in the posterior region, long and flexible erect hairs of whitish color (Figure 4 I). Spores were found in the region of the antennae and legs (Figure 4 J). In the gut, mushroom spores were also found (Figure 4 K and L). Distribution in Brazil: in the Pampa biome the occurrence record is unpublished, containing records only for the Pantanal (Demétrio *et al*. 2017) and Amazonia (Mertl *et al*. 2012), where it is considered native.

Lepiota micropholis (Berk. & Broome) Sacc. and *Pheidole flavens* (Roger, 1863)

The fungi has a pileus up to 1.5 cm in diameter, convex and slightly umbonate, white in color, slightly squamous near the striated margin (Figure 5 A). Lamellae close free, white in color. Stipe 3.8 - 4 x 0.2 - 0.5 µm, white and thin near apex. Annulus present and evanescent (Figure 9 B). Hymenophoreal trama regular (Figure 5 C). Spores 4.9 - 6.5 x 4 - 5 µm, pseudoamyloid, ovoid, smooth, thin-walled and hyaline, without germinal pore (Figure 5 D). Basidia 20 - 30 x 8 - 12 µm, clavate and tetrasporate (Figure 5 E). Cheilocystidia 30 - 40 x 10 - 15 µm, clavate, thin-walled hyaline (Figure 5 F). Pileus cortical layer tricodermal. Grows on soil. Distribution in Brazil: in the state of Rio Grande do Sul, in Pampa biome the description is unprecedented. It has been described for Brazil only for the Atlantic Forest biome in São Paulo by Pegler (1997).

The ant has a rectangular head longer than wide and a rugoreticulum between the eyes and the antennal fossa, eyes moderate and faceted. Antennal scrobes absent. Mandibles with four teeth or more that gradually increase in size toward apex (Figure 5 G). Propodeum and promesonotum with projections and short spines, propodeal spine well developed (Figure 5 H). Gaster shiny, smooth, containing flexible whitish hairs (Figure 5 I). Spores were found in the region of the antennae, legs and abdomen (Figure 5 J). Internally, in the gut mushroom spores were found (Figure 5 K and L). Distribution in Brazil: it has distribution in American continent, considered native to the Neotropical region. Its distribution is cataloged for the Pampa biome by Casadei-Ferreira *et al*. (2020), Atlantic Forest (Sarnat *et al*. 2014), Pantanal (Silva, 2018), Cerrado and Caatinga (Moreira *et al*. 2020).

Figure 5 - *Lepiota micropholis* (A-F) and *Pheidole flavens* (G-L). Pileus top view (A). Basidiome with lamellae (B). Trama lamellar (C). Spores (D). Basidium (E). Cheilocystidia (F). Front view of head (G). Lateral view of thorax (H). Lateral view of gaster (I). Spores attached to the leg (J). Posterior region of intestine with spore (K and L). Arrows indicate the structures described. Source: Authors (2022).

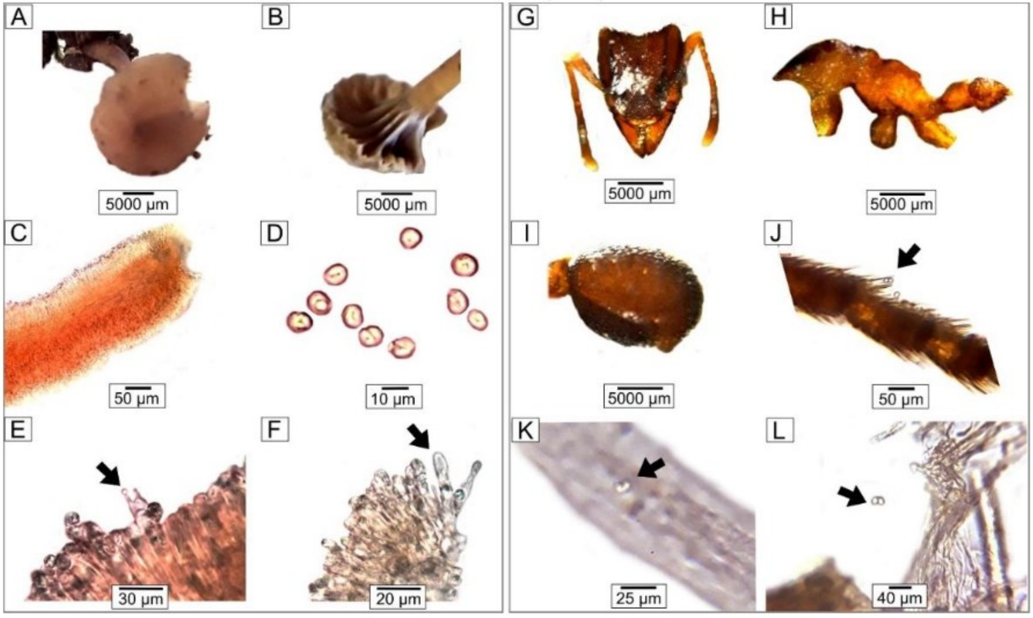


***Neopaxillus echinospermus* (Speg.) Singer and *Mycetosoritis hartmanni* (Wheeler, W.M., 1907)**

The fungi has pileus up to 40 mm in diameter, flattened or flattened to infundibuliform, dark yellow brown or reddish orange in color, with lobed to wavy margins (Figure 5 A). Lamellae decurrent, distant to subdistant with brown coloration and concolorous border. Stipe 20 - 40 x 2 - 3.5 µm, cylindrical, keeled at apex region, bulbous base pale yellowish-brown and smooth. Ring absent (Figure 5 B). Hymenophoreal trama subregular (Figure 5 C). Spores 6.5 - 9 x 6.5 - 8.5 µm, subglobose to globose, thick walled. pale brown, inamyloid in coloration (Figure 5 D). Basidia tetrasporate and rarely bisporate of 28 - 40 x 8 - 15 µm (Figure 5 E). Cheilocystidia 18.5 - 32 x 6.5 - 16 µm, vesiform thin-walled hyaline and pale (Figure 5 F). Pleurocystidia absent. Cortical layer of the hymenophoreal pileus subregular. Present clamp connections. Grows on soil. Distribution in Brazil: in the state of Rio Grande do Sul Pampa biome this description is unpublished. However, the species has records for the Atlantic Forest biome in the states of Rio Grande do Sul, Paraná and São Paulo (Singer and Digilio, 1951; Singer, 1964; Watling and Meijer, 1997; Silva-Filho *et al*. 2018).

The ant has a broad, rectangular head, without the presence of tubercles and with poorly developed frontal lobes. Eyes moderate and faceted. Mandibles sclerotized, robust with attenuated, slightly curved apical portion and slender apical tooth (Figure 5 G). Bristles along dorsum diminutive, conspicuous erect, often stiff and curled, propodeal spine poorly developed (Figure 5 H). Gaster blackened, covered with short, distant, almost imperceptible bristles distributed in transverse rows (Figure 5 I). Mushroom spores were found on the legs (Figure 5 J). Internally, spores were found in the gut (Figure 5 K and L). Distribution in Brazil: this is the first record of occurrence of the species for the Pampa biome. However, for the Cerrado (Sosa-Calvo *et al*. 2009) and Atlantic Forest (Sosa-Calvo *et al*. 2017) biomes the species already has occurrence records.

Figure 6 - *Neopaxillus echinospermus* (A-F) and *Mycetosoritis hartmanni* (G-L). Pileus top view (A). Basidiome with lamellae (B). Trama lamellar (C). Spores (D). Basidium (E). Cheilocystidia (F). Front view of head (G). Lateral view of thorax (H). Lateral view of gaster (I). Spores attached to the leg (J). Posterior region of intestine with spore (K and L). Arrows indicate the structures described. Source: Authors (2022).



Mycophagic associations between ants and mushrooms

About 70 individuals of *Acromyrmex niger* showed associations with *Agaricus rufoaurantiacus* on the soil surface during the daytime at point A. At the site, 7 basidiomata were found in the area and 4 contained signs of mycophagy. The main parts of the basidiomata involved in predation were the pileus surface, lamellae and stipe. Mycophagous associations have already been described for *Acromyrmex niger* with other Agaricales fungi, including *Leucoagaricus gongylophorus*, where besides preying on all parts of the basidiome, it is also associated with its cultivation as a fungiculture (Aylward *et al*. 2013). For *Agaricus rufoaurantiacus*, this is the first record of association with this ant species. However, in the study by Wartchow *et al*. (2008), unidentified nests of leafcutter ants were found near basidiomata of this mushroom in the Atlantic Forest biome in Pernambuco.

About 20 individuals of *Acromyrmex versicolor* showed mycophagy associations with *Macrocybe titans* on the soil surface at point C at night. In the area, two basidiomata of *Macrocybe titans* were found there, and only 1 showed signs of predation. In the mushroom, only the surface of the pileus showed signs of mycophagy, including a small portion of the lamellar margin region. For *Acromyrmex versicolor*, the title of fungiculture is frequent and connected to the survival of their nests (Clark and Fewell, 2014). Also, *Leucocoprinus* sp. has already been reported in fungiculture association with this ant species (Bizarria *et al*. 2022). For the *Macrocybe titans*, there are already records of occurring near nests of unidentified leafcutter ants in Costa Rica (Pegler *et* *al*. 1998; Mueller and Mata, 2001). Nevertheless, this is the first report of mycophagy between the two species of this study.

Approximately 7 individuals of *Tranopelta gilva* were found during the day at point B in mycophagy activity with *Agrocybe underwoodii* on the soil surface. A total of 5 basidiomata of *Agrocybe underwoodii* were found in the region and 3 specimens showed signs of mycophagy, only the bordon of the lamellae showed signs of predation. Although *Tranopelta gilva* is cited as a fungiculture ant (Fernandez, 2003), there are no known cases in the literature with Agaricales fungi. For *Agrocybe underwoodii*, this is the first report of mycophagy associated with ants. However, there are citations for the genus from Russia (Rayner *et al*. 1985) and Malaysia (Fung and Tan, 2022) with unidentified fire ants.

About 16 individuals of *Tranopelta subterranea* showed associations with *Dactylosporina steffenii* at point A on the soil surface during the day. In the area, 3 basidiomata of *Dactylosporina steffenii* were found and 2 showed signs of mycophagy. Of the parts of the basidiomata involved in predation, only the lamellar region was affected. Although there is evidence in the literature of possible interaction with filamentous fungi (Delabie *et al*. 2000), there are no records of mycophagic interactions of *Tranopelta subterranea* with Agaricales fungi, which is a novelty for both the ant and the fungi. However, for *Oudemansiella*, a taxonomic classification that precedes *Dactylosporina*, there are records of unidentified ants collecting their basidiomata in Manaus in Amazon biome (Amaringo-Cortegano *et al*. 2013).

Only 3 individuals of *Pheidole flavens* showed mycophagous activity with *Lepiota micropholis* at point A on the soil surface during the day. In the area, 4 basidiomata of *Lepiota micropholis* were found, but only 1 showed signs of mycophagy. Basidiome structures showing signs of predation included the surface of pileus and lamellae portions. For both species, the association of mycophagy is unprecedented. However, for *Pheidole*, there is evidence in the literature of association with filamentous fungi (Wilson, 2003). For *Lepiota*, associations with fungal gardens of leafcutter ant species of *Acromyrmex*, *Atta*, *Apterostigma*, *Cyphomyrmex*, and *Trachymyrmex* are known (Hervey *et al*. 1977).

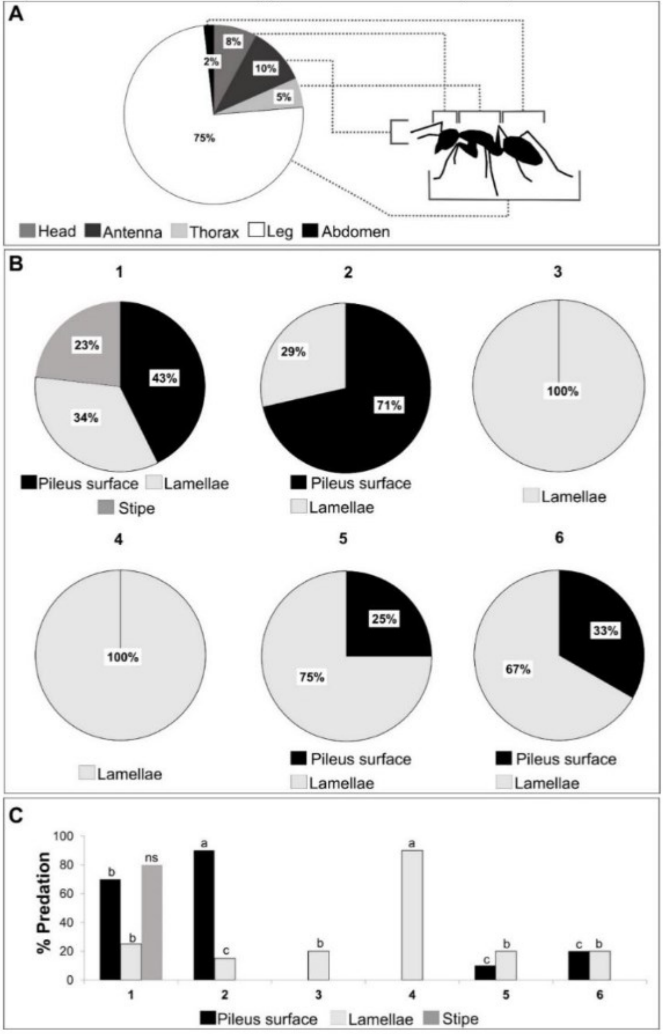
Approximately 2 individuals of *Mycetosoritis hartmanni* showed mycophagous activity with *Neopaxillus echinospermus* on the soil surface at point A during the day. In the area, 3 basidiomata of *Neopaxillus echinospermus* were found and 1 showed signs of mycophagy. The surface part of the pileus and a small part of the lamellar margin showed signs of predation. For *Mycetosoritis hartmanni*, mycophagy associations with fungal gardens are recorded in the literature, including *Escovopsis* and *Trichoderma* (Sanchez-Peña, 2010; Solomon *et al*. 2011), but both belong to the Ascomycota. For Agaricales fungi, the ant has a description of association with *Lepiota* (Batra and

Batra, 1967). For *Neopaxillus echinospermus*, the report of mycophagous association is unpublished.

Levels of interaction and predation of ants with the mushrooms

The present study reports the interaction of six ant species in mycophagous activities with six Agaricales fungi. Our results describe novel associations with ants already cited in literature as fungiculture and not cited. For the ants, the report is similar once that of the activities mycophagy involved fungi with and without a description of the application in Attini fungiculture. The interactions involving these associations are summarized in Figure 7.

Figure 7 - Potential of spore dispersal, mycophagy, and predation activity of ants associated with mushrooms. Percentage of ants body structures that contained the fungi spores (A). Percentage of structures of basidiomata that were eaten by the ants (B). Comparison of the level of predation among the ants in relation to the structures of mushroom. Divergent letters in the graphs indicate significant differences with a *P-value* of 0.05% (C). Note: ns indicates non-significant value. *Acromyrmex niger* (1). *Acromyrmex versicolor* (2). *Tranopelta gilva* (3). *Tranopelta subterranean* (4). *Pheidole flavens* (5). *Mycetosoritis hartmanni* (6). Source: Authors (2022).



In general, it could be seen that the ants not only eat but also carry parts of the fungi to their nests. Several parts of the basidiomata were associated with predatory activities, such as the surface of the pileus, the lamellae, and the stipe. According to Von-Beeren *et al*. (2014), ant harvesting performed directly on basidiomata affects spore dispersal and has a significant positive impact on the local fungal community, as does ant harvesting of seeds, which affects the plant community (Nelsen *et al*. 2018). Under this premise, the ant structures that contained the most fungal spores followed in descending order: legs, antennae, head, thorax, and abdomen (Figure 7 A).

Several ant genera have been described in the literature as fungal spore dispersers, including the members of this study: *Acromyrmex* (Aylward *et al*. 2013; Clark and Fewell, 2014), *Tranopelta* (Delabie *et al*. 2000; Fernandez, 2003), *Pheidole* (Wilson, 2003), and *Mycetosoritis* (Sanchez-Peña, 2010; Solomon *et al*. 2011). However, little is known about the forms of interaction and mycophagy. Thus, the data presented help in questions relevant to the known Attini mycophagy, describing predation standards and reported forms of association.

Studies show that certain ants visit the basidiomata to capture larvae of arthropods in general and that many are predatory in the basidiome itself (Witte and Maschwitz, 2008). Ants are also known to collect fungi to grow them in their nests (Epps and Penick, 2018). However, the main point of contention is whether or not ants benefit nutritionally from fungal structures, such as the surface of the pileus, lamellae and stipe. According to de Moura and Okura (2022), basidiomata are rich in proteins, carbohydrates and dietary fiber, which can vary of 9 - 40%, 23 - 70%, and 12 - 40%, respectively, depending of specimen. Thus, our study demonstrates optimization of the Attini-Mycophagous interaction, because the cataloged ants not only collected parts of the fungi but also disseminated their spores and fed on them as shown in Figures 1-7.

All structures of the basidiomata were eaten by ants in descending order: lamellae, pileus surface, and stipe (Figure 7 B). Each species studied exhibited specific characteristics. *Acromyrmex niger* showed generalist mycophagy preying on all parts of the basidiome, while *Acromyrmex versicolor* showed a preference for the lamellae and also surface pileus part. *Tranopelta gilva* and *Tranopelta subterranea* showed a preference only the lamellar portions of the basidiomata. *Pheidole flavens* and *Mycetosoritis hartmanni* showed more interest in the lamellae and later in the surface pileus portion.

Acromyrmex niger and *Acromyrmex versicolor* had a high percentage of predation compared to the other species of this study (Figure 7 C). According to Clark and Fewell (2014), *Acromyrmex* has a collective foraging pattern that is fast and efficient. This is evidence of optimization of mycophagous strategy, as they were also the species that benefited most from the diverse structures of basidiomata. According to Wartchow *et al*. (2008), the species of this genus tend to be generalists in terms of feeding, which would explain the diversity of structures integrated into mycophagy. Therefore, it can be concluded that mycophagy is generalized in these species.

For *Tranopelta gilva* and *Tranopelta subterranea* there are descriptions of generalist dietary habits (Rayner *et al*. 1985), particularly in relation to the diversity of decomposing organic matter (Fernandez, 2003). For the samples of this study, specifications related to mycophagy were cataloged, the mycophagy occurred preferably in lamellae region of the basidiomata (Figure 7 C). These data are consistent with the reports of Fung and Tan (2022), in which more than 20 species of edible mushroom were implicated in predation by fire ants in lamellae region, including individuals of *Tranopeltra*. Thus, is possible to infer that occur a specialization of mycophagy on the lamellae regions involving these ants.

Pheidole flavens has a diet that is classified as generalist (Wilson, 2003), while *Mycetosoritis hartmanni* is considered a fungiculture (Sanchez-Peña, 2010; Solomon *et al*. 2011). Regarding the mycogaphy of Agaricales fungi, both showed a percentage of predation in the lamellae region and on the pileus surface (Figure 7 C). In this case, it is possible to infer a generalist pattern with respect to the parts of the fungi that were used in their diet. Corroborated with the theory of Branstetter *et al*. (2017), who inferred the generalist feeding success for both genera from the fact that they do not only use photosynthetic food as a food source.

Attini ants are known for their generalist diet (Lanan, 2014). However, few data are available on the diet of fungi, and these data refer only to a selected group of fungiculture ants (Witte and Maschwitz, 2008). In a controlled laboratory study with individuals of *Aphaenogaster*, a generalist ant group known for its carnivorous habits, Epps and Penick (2018) infer that not only was prey (larvae) collected in basidiomata, but that workers also collected pieces of mushroom free of prey, chewed the same, and deposited them in their nests. In the nest, the workers continued the process of macerating the basidiome pieces and distributing them all over the colony. In our study, only worker ants were identified, but our observations and sampling were limited to the soil surface. Further studies could elucidate the mycophagous associations of these species and their internal interactions within the colony, as well as possible cultivator groups that have not yet been described.

The species this study inhabit a very important ecosystem, there are many unknown interactions in riparian zones (Vidon *et al*. 2019). As previously described in the literature, fungi in mycorrhizal associations integrate a nutritional mycelial network, and among the species studied, *Dactylosporina steffenii* forms pseudorriza (Putzke and Putzke, 2017). In this sense, the diversity of ants active in these zones is underestimated, and they could play an important role in the interaction plants-fungi-soil. Another important point to study is the spread and maintenance of the fungal network that plays an active role in the nutrient cycling of these environments. In this case, the dispersal of spores over short or long distances by ants could be an important issue for the local fungal community.

4 CONCLUSION

From the findings related to the ants, it can be deduced that mycophagous Attini is an underestimated group that contradicts the idea that they use basidiomata only as hunting grounds or act only as disseminators of fungal spores. Each ant showed peculiarities in relation to the associated fungi, preferences structures and the levels of predation in basidiomata. In the case of Agaricales fungi, our results revealed new interactions that will serve as a basis for further studies, especially in relation to nest dynamics associated with mycophagy and fungiculture.

However, it is possible to conclude that these interactions in the riparian zones studied may favor either the predator or the fungus grower, the prey or the culture. In addition, the ability of ants to exploit a temporally limited resource such as basidiomata may be related to their success in food diversity. Our study reveals a diverse social potential of mycophagous, as specificities related to food resource exploitation were cataloged. It can be concluded that Attini-Mycophagous integrate an important group in the riparian zones of southern Brazil.

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6 CONSIDERAÇÕES FINAIS

Para as associações dos fungos com a flora, foram reconstruídas neste estudo relações fungo-substrato envolvendo marcadores moleculares, caracteres morfológicos, e tipos de substratos associados com Pluteaceae de distribuição nos biomas brasileiros. Dentre as principais associações descritas, estão o uso de substratos nativos como *Dicksonia* sp. (xaxim) por pluteoides lignícolas, assim como a ocorrência do grupo em zonas riparias importantes do estado do Rio Grande do Sul. Algumas espécies apresentaram preferências generalizadas, utilizando tanto a vegetação nativa quanto exótica.

Em relação as iterações nutricionais, foram dispostos os teores de macro e micro nutrientes de 25 espécies de Agaricales associados a *Eucalyptus grandis* W. Hill. em uma área de reflorestamento ripária dulcícola. A relação carbono/nitrogênio destes macrofungos foi de 13:1, destacando-se o papel importante destes macrofungos à ciclagem nutricional provinda da matéria vegetal exótica.

Para a fauna, associações inéditas foram descritas envolvendo os fungos como um recurso alimentar ou de outras formas, como por exemplo, a construção de ninhos. Foram compiladas na forma de uma revisão dados envolvendo 64 espécies de aves interagindo direta ou indiretamente com 32 espécies fúngicas identificadas, e muitas outras não identificadas, indicando uma área de grande potencial para novas pesquisas. Descreveu-se o hábito micofágico do pássaro *Molothrus bonariensis* (chupim-guadério) com o cogumelo *Macrolepiota bonariensis*. Também, o ciclo de vida e micofagia de *Zygothrica candens* (mosca) com o cogumelo *Oudemansiella cubensis*. O uso dos fungos nativos como recurso de alimento e armazenagem, além dos níveis de predação e potencial de dispersão dos esporos por formigas Attini da casta operária.

Todos estes achados são de suma importância para a compreensão das interações desempenhadas pela flora-fauna-fungo em zonas ripárias no estado do Rio Grande do Sul. Nossos achados auxiliam em uma melhor compreensão dos tipos de associações ecológicas envolvendo esses grupos, com ênfase nas relações harmônicas. Os achados inéditos descritos podem servir como base para novos estudos e aprofundamento dos principais mecanismos fisiológicos que atuam nessas associações, e que auxiliam na manutenção dessas importantes áreas de preservação ambiental.

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ANEXOS

Anexo I

OPEN
JOURNAL
SYSTEMS
ISSN: 2595-4431

Brazilian Journal of Environment

Revista Brasileira de
Meio Ambiente

Declaração

Brazilian Journal of Environment, ISSN 2595-4421, declara que o artigo intitulado "**Diversity and Ecological Associations of Pluteaceae (Fungi, Agaricomycetes, Agaricales) in Brazilian Biomes**" de autoria de *Alice Lemos Costa, Cassiane Furlan Lopes, Kamille Rodriguez Ferraz, Marines de Avila Heberle* & *Jair Putzke* que foi publicado na v.10., n.2, p. 219-239 está autorizado para a sua reprodução na integra junto a Tese de Doutorado da primeira autora.

Link do artigo:
https://revistabrasileirademeioambiente.com/index.php/RVBMA/article/view/1241

Dr. Jadson Freire-Silva
Editor-Chefe

Recife, 28 de setembro de 2022

Anexo II

HOEHNEA
INSTITUTO DE BOTÂNICA

Atestado de Publicação

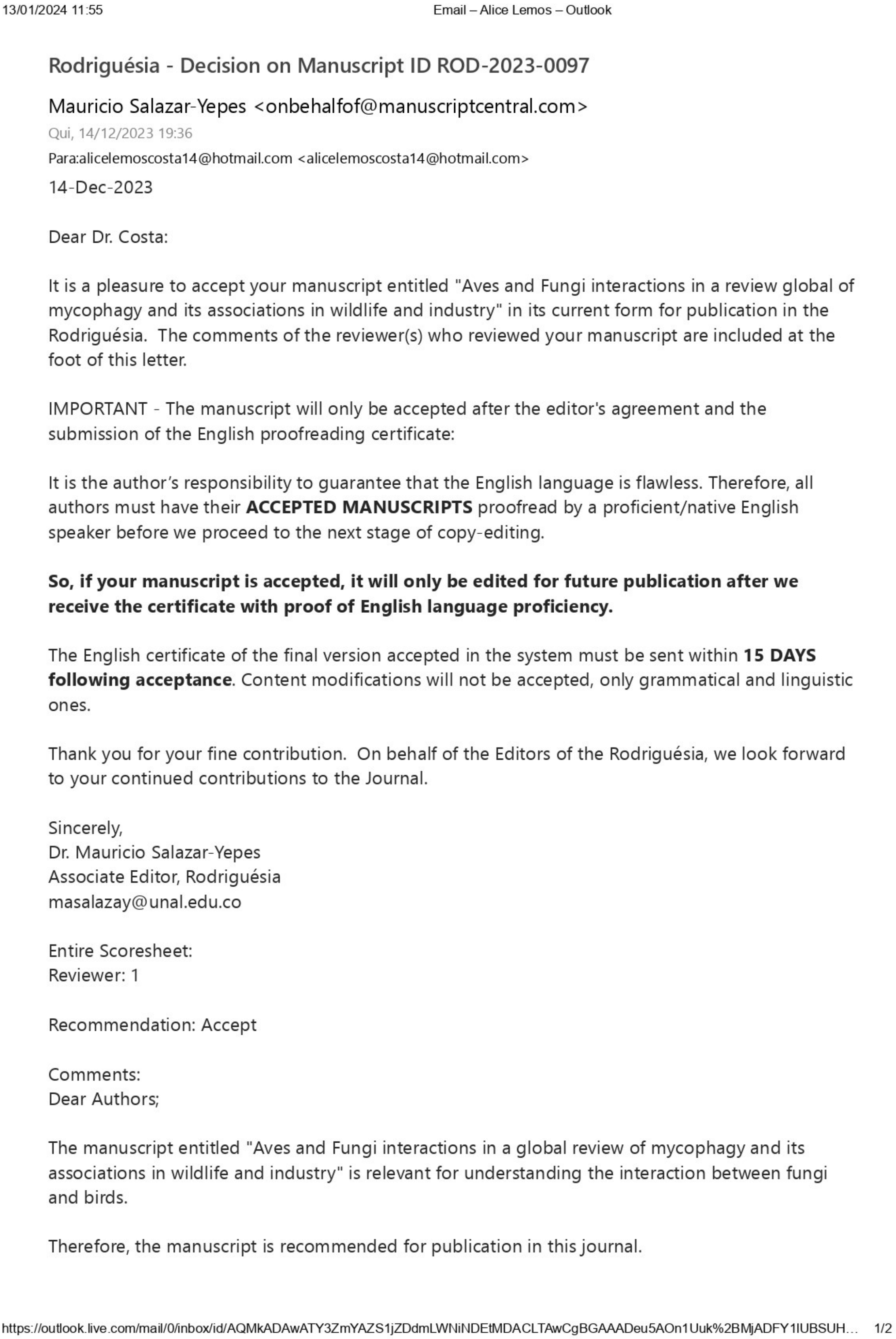
A *Hoehnea*, ISSN 2236-8906, atesta para os devidos fins que o artigo intitulado "A relationship between fungi (Basidiomycota, Agaricomycetes, Agaricales) and nutrient content in riparian area of reforestation with *Eucalyptus grandis* W. Hill ex Maiden (Myrtaceae) in southern Brazil" de autoria de Alice Lemos Costa, Cassiane Furlan-Lopes, Fernando Augusto Bertazzo-Silva, Ana Luiza Klotz-Neves, Kamille Rodrigues Ferraz, Ana Flavia Zorzi, Silvane Vestena & Jair Putzke foi publicado no v. e262023, nº. 50, p. 1-13, DOI: https://doi.org/10.1590/2236-8906e262023 está autorizado para a sua reprodução na integra com as devidas referenciações junto a Tese de Doutorado da primeira autora.

Link do artigo:
https://www.scielo.br/j/hoehnea/a/SHjJFKjPDWKNHvryg9fPSCK/abstract/?lang=en

Dra. Iracema Helena Schoenlein-Crusius
Editor-in-Chief, Hoehnea
editorhoehnea.ipa@sp.gov.br

São Paulo, 11 de dezembro de 2023

Anexo III



13/01/2024 11:55 Email – Alice Lemos – Outlook

Rodriguésia - Decision on Manuscript ID ROD-2023-0097

Mauricio Salazar-Yepes <onbehalfof@manuscriptcentral.com>
Qui, 14/12/2023 19:36
Para:alicelemoscosta14@hotmail.com <alicelemoscosta14@hotmail.com>

14-Dec-2023

Dear Dr. Costa:

It is a pleasure to accept your manuscript entitled "Aves and Fungi interactions in a review global of mycophagy and its associations in wildlife and industry" in its current form for publication in the Rodriguésia. The comments of the reviewer(s) who reviewed your manuscript are included at the foot of this letter.

IMPORTANT - The manuscript will only be accepted after the editor's agreement and the submission of the English proofreading certificate:

It is the author's responsibility to guarantee that the English language is flawless. Therefore, all authors must have their **ACCEPTED MANUSCRIPTS** proofread by a proficient/native English speaker before we proceed to the next stage of copy-editing.

So, if your manuscript is accepted, it will only be edited for future publication after we receive the certificate with proof of English language proficiency.

The English certificate of the final version accepted in the system must be sent within **15 DAYS following acceptance**. Content modifications will not be accepted, only grammatical and linguistic ones.

Thank you for your fine contribution. On behalf of the Editors of the Rodriguésia, we look forward to your continued contributions to the Journal.

Sincerely,
Dr. Mauricio Salazar-Yepes
Associate Editor, Rodriguésia
masalazay@unal.edu.co

Entire Scoresheet:
Reviewer: 1

Recommendation: Accept

Comments:
Dear Authors;

The manuscript entitled "Aves and Fungi interactions in a global review of mycophagy and its associations in wildlife and industry" is relevant for understanding the interaction between fungi and birds.

Therefore, the manuscript is recommended for publication in this journal.

https://outlook.live.com/mail/0/inbox/id/AQMkADAwATY3ZmYAZS1jZDdmLWNiNDEtMDACLTAwCgBGAAADeu5AOn1Uuk%2BMjADFY1IUBSUH… 1/2

Additional Questions:
Does the manuscript contain new and significant information to justify publication?: Not applicable

Does the Abstract (Summary) clearly and accurately describe the content of the article?: Yes

Is the problem significant and concisely stated?: Yes

Are the methods described comprehensively?: Yes

Are the interpretations and conclusions justified by the results?: Yes

Is adequate reference made to other work in the field?: Yes

Please state any conflict(s) of interest that you have in relation to the review of this paper (state "none" if this is not applicable).: None.

Rating:

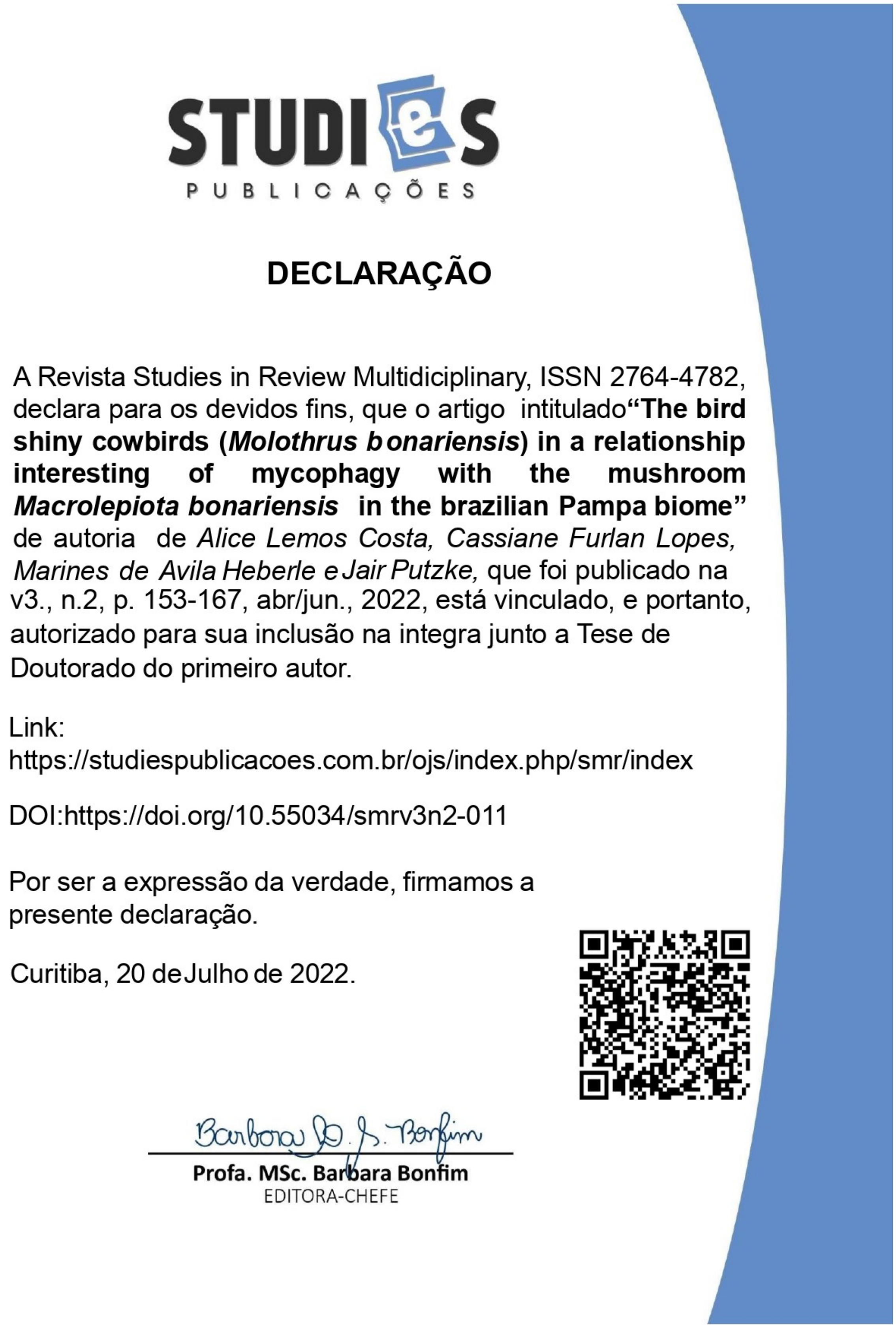
Interest: 2. Good

Quality: 2. Good

Originality: 2. Good

Overall: 2. Good

Anexo IV



STUDIES
PUBLICAÇÕES

DECLARAÇÃO

A Revista Studies in Review Multidiciplinary, ISSN 2764-4782, declara para os devidos fins, que o artigo intitulado **“The bird shiny cowbirds (*Molothrus bonariensis*) in a relationship interesting of mycophagy with the mushroom *Macrolepiota bonariensis* in the brazilian Pampa biome”** de autoria de *Alice Lemos Costa, Cassiane Furlan Lopes, Marines de Avila Heberle e Jair Putzke,* que foi publicado na v3., n.2, p. 153-167, abr/jun., 2022, está vinculado, e portanto, autorizado para sua inclusão na integra junto a Tese de Doutorado do primeiro autor.

Link:
https://studiespublicacoes.com.br/ojs/index.php/smr/index

DOI:https://doi.org/10.55034/smrv3n2-011

Por ser a expressão da verdade, firmamos a presente declaração.

Curitiba, 20 de Julho de 2022.

Barbara D. S. Bonfim

Profa. MSc. Barbara Bonfim
EDITORA-CHEFE

Anexo V

BRAZILIAN
JOURNAL OF
BIOLOGY®
THE INTERNATIONAL JOURNAL ON NEOTROPICAL BIOLOGY

DECLARAÇÃO

Brazilian Journal of Biology, ISSN 1678-4375, declara que o manuscrito intitulado "**First report of *Zygothrica candens* Burla, 1956 (Diptera, Drosophilidae) in mycophagic association with the mushroom *Oudemansiella cubensis* (Berk. and M.A. Curtis) R.H. Petersen, 2010 (Agaricales, Physalacriaceae) in southern Brazil**" de autoria de *A. L. Costa, M. F. Mendes, C. Furlan-Lopes, F. A. Bertazzo-Silva, A. Köhler and J. Putzke* foi publicado v.e267871, n.82, p. 1-4 sob DOI: https://doi.org/10.1590/1519-6984.267871 e está autorizado para a sua reprodução de forma integral junto a Tese de Doutorado da primeira autora.

Link do artigo:
https://www.scielo.br/j/bjb/a/fPTmZjVdDnKH5ghr3PYJfTG/?lang=en

Dra. Takako Matsumura Tundisi
Editora-Chefe

São Carlos, 12 de janeiro de 2023

Anexo VI

Brazilian Journal of Animal and Environmental Research

DECLARAÇÂO

A Brazilian Journal of Animal and Environmental Research, ISSN 2595-573X, editada por Brazilian Journals Publicações de Periódicos e Editora Ltda. (CNPJ 32.432.868 / 0001-57), declara que o artigo **"Mycophagy of Attini Ants (Hymenoptera, Formicidae, Myrmicinae) with Agaricales Mushrooms (Basidiomycota, Agaricomycetes) at Riparian Zone in Southern Brazil"** de Alice Lemos Costa, Cassiane Furlan-Lopes, Fernando Augusto Bertazzo-Silva, Ana Luiza Klotz-Neves, Kamille Rodrigues Ferraz, Andreas Köhler, Jair Putzke, que foi publicado no v.5, n.4, p. 3935-3960, out./dez., 2022, está vinculado, e portanto, autorizado para sua inclusão na integra junto a Tese de Doutorado do primeiro autor.

Link de publicação:
https://ojs.brazilianjournals.com.br/ojs/index.php/BJAER/article/view/54469/40263

DOI:10.34188/bjaerv5n4-039

Como expressão da verdade, assinamos esta declaração.

São José dos Pinhais, 22 de novembro de 2022.

Prof. Dra. Dariane Cristina Catapan
Editora-chefe