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OCORRÊNCIA, DISTRIBUIÇÃO E ECOLOGIA DE MACROFUNGOS E MIXOMICETOS EM UM CARSTE NEOTROPICAL.

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Orientador

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MARIANA ARAÚJO MOREIRA

OCORRÊNCIA, DISTRIBUIÇÃO E ECOLOGIA DE MACROFUNGOS E MIXOMICETOS EM UM CARSTE NEOTROPICAL

OCCURRENCE, DISTRIBUTION AND ECOLOGY OF MACROFUNGI AND MYXOMYCETES IN A NEOTROPICAL KARST

Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ecologia Aplicada, área de concentração em Ecologia e Conservação de Recursos Naturais em Paisagens Fragmentadas e Agroecossistemas, para a obtenção do título de Mestre.

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RESUMO GERAL

Os manuscritos apresentados nesta dissertação destacam a importância do estudo da biodiversidade em ambientes cársticos, com ênfase em macrofungos e mixomicetos, organismos ainda não registrados em cavernas no Brasil. As áreas cársticas são caracterizadas por relevo formado pela dissolução de rochas solúveis como calcário e dolomito, apresentam feições com condições ambientais distintas, como tipo de substrato, umidade e luminosidade, que criam micro-habitats específicos. Esses ambientes são ricos e variados, abrigando espécies adaptadas, muitas vezes restritas a esses locais. O objetivo principal da dissertação foi avaliar a ocorrência, distribuição e ecologia de dois grupos de organismos, os macrofungos e os mixomicetos, que, embora compartilhem uma origem comum, são atualmente classificados de forma distinta. O primeiro manuscrito, já publicado, descreve o primeiro registro de mixomicetos em cavernas do Brasil. Foram coletadas três espécies na região cárstica de Pains: *Arcyria denudata*, *Hemitrichia calyculata* e *Metatrichia vesparia*. Esses organismos ameboides, conhecidos por seu ciclo de vida peculiar, habitam ambientes terrestres úmidos e desempenham um papel ecológico significativo na decomposição e no ciclo de nutrientes, sendo fundamentais para a dinâmica dos ecossistemas. O segundo manuscrito relata uma nova ocorrência de *Schizophyllum commune* na Gruta Furnas, Rio de Janeiro, abordando sua morfologia, filogenia e adaptação. Também inclui uma lista de verificação de nomes publicados do gênero e a designação de um lectótipo para *S. commune*. O terceiro manuscrito aborda uma lacuna na pesquisa sobre macrofungos em ambientes subterrâneos, avaliando as variáveis ambientais responsáveis pela presença e ausência desses organismos e sua influência na distribuição no ambiente cárstico. A inclusão desses organismos nas estratégias de conservação é crucial, pois atuam como indicadores do funcionamento dos ecossistemas. Sua diversidade pode ser uma ferramenta valiosa para monitorar a qualidade de habitats, especialmente em áreas com alto valor para conservação.

Palavras- chave: ecologia; macrofungos; mixomicetos; caverna.

ABSTRACT

The manuscripts presented in this dissertation highlight the importance of studying biodiversity in karst environments, with an emphasis on macrofungi and myxomycetes, organisms not yet recorded in caves in Brazil. Karst areas are characterized by relief formed through the dissolution of soluble rocks such as limestone and dolomite, presenting features with distinct environmental conditions, such as substrate type, humidity, and light, which create specific microhabitats. These environments are rich and varied, harboring species that are often adapted and restricted to these locations. The main objective of the dissertation was to evaluate the occurrence, distribution, and ecology of two groups of organisms, macrofungi and myxomycetes, which, although sharing a common origin, are currently classified separately. The first manuscript, already published, describes the first record of myxomycetes in caves in Brazil. Three species were collected in the karst region of Pains: *Arcyria denudata*, *Hemitrichia calyculata*, and *Metatrichia vesparia*. These amoeboid organisms, known for their peculiar life cycle, inhabit humid terrestrial environments and play a significant ecological role in decomposition and nutrient cycling, being fundamental to ecosystem dynamics. The second manuscript reports a new occurrence of *Schizophyllum commune* in Gruta Furnas, Rio de Janeiro, addressing its morphology, phylogeny, and adaptation. It also includes a checklist of published names in the genus and the designation of a lectotype *for S. commune.* The third manuscript addresses a gap in research on macrofungi in subterranean environments, evaluating the environmental variables responsible for the presence and absence of these organisms and their influence on distribution in karst environments. The inclusion of these organisms in conservation strategies is crucial, as they act as indicators of ecosystem functioning. Their diversity can be a valuable tool for monitoring habitat quality, especially in areas with high conservation value.

Keywords: ecology; macrofungi; myxomycetes; cave.

INDICADORES DE IMPACTO

A dissertação, inserida no contexto ambiental, evidencia o registro da diversidade de macrofungos e mixomicetos em regiões cársticas do Brasil, destacando a importância das cavernas como ambientes com características ambientais únicas, que favorecem a adaptação de espécies a essas condições específicas. O estudo resultou no primeiro registro de mixomicetos (organismos ameboides) em cavernas brasileiras, além do primeiro registro da espécie *Schizophyllum commune*, que apresentou características morfológicas distintas em relação às espécies epígeas, sugerindo uma possível adaptação ao ambiente subterrâneo. Também foram registradas 111 espécies de macrofungos em áreas cársticas, incluindo matas e cavernas na região de Pains, uma área extremamente ameaçada pela exploração mineral e agricultura. Trata-se de pesquisas pioneiras e de grande relevância global sobre esses organismos em ambientes cársticos. A pesquisa contou com a colaboração de importantes pesquisadores, como o Professor Doutor Felipe Wartchow e a Professora Doutora Laise de Holanda Cavancanti, cujas contribuições foram essenciais para a identificação, descrição e aprofundamento do entendimento sobre os organismos estudados, além de facilitarem a identificação de lacunas no conhecimento. O estudo ressaltou a importância de investigar esses organismos para preencher as lacunas no conhecimento sobre a biodiversidade e ecologia dos ambientes subterrâneos. Além de ampliar a compreensão das interações ecológicas e da biodiversidade subterrânea, essa pesquisa fornece ferramentas valiosas para a conservação e o monitoramento de impactos ambientais, contribuindo de forma significativa para a preservação desses habitats frágeis e ricos em diversidade biológica.

IMPACT INDICATORS

The dissertation, set within an environmental context, highlights the record of macrofungi and myxomycetes diversity in karst regions of Brazil, emphasizing the importance of caves as environments with unique environmental characteristics that favor the adaptation of species to these specific conditions. The study resulted in the first record of myxomycetes (amoeboid organisms) in Brazilian caves, as well as the first record of the species *Schizophyllum commune*, which showed distinct morphological characteristics compared to epigean species, suggesting a possible adaptation to the subterranean environment. Additionally, 111 species of macrofungi were recorded in karst areas, including forests and caves in the Pains region, an area severely threatened by mineral exploitation and agriculture. These are pioneering studies of global relevance regarding these organisms in karst environments. The research benefited from the collaboration of important researchers, such as Professor Felipe Wartchow and Professor Laise de Holanda Cavancanti, whose contributions were essential for the identification, description, and deepening of the understanding of the studied organisms, as well as facilitating the identification of knowledge gaps. The study emphasized the importance of investigating these organisms to fill the gaps in knowledge about the biodiversity and ecology of subterranean environments. In addition to enhancing the understanding of ecological interactions and subterranean biodiversity, this research provides valuable tools for conservation and monitoring environmental impacts, significantly contributing to the preservation of these fragile habitats rich in biological diversity.

SUMÁRIO

PRIMEIRA PARTE

INTRODUÇÃO GERAL

INTRODUÇÃO GERAL

Esta dissertação investiga a diversidade de dois grupos de organismo, macrofungos e mixomicetos em áreas cársticas no Brasil. Isso inclui se esses organismos ocorrem no ambiente subterrâneo, quais suas características e quais condições podem influenciar em suas ocorrências nesses ecossistemas. Embora os organismos estudados possam se adaptar a viverem no ambiente subterrâneo, não podem ser consideradas espécies troglóbias, ou seja, aquelas espécies que se adaptam ao ambiente cavernícola e vivem estritamente nesse ambiente.

Para contextualizar o estado atual da pesquisa sobre o tema citado acima, foram elaborados três manuscritos. O primeiro manuscrito avaliou a primeira ocorrência de organismos ameboides em duas cavernas na região cárstica de Pains, município de Minas Gerais, Brasil. O segundo descreveu a primeira ocorrência de uma espécie de *S. commune* em ambientes subterrâneos a nível mundial. O terceiro manuscrito investigou se os fatores ambientais influenciam a presença de macrofungos em áreas cársticas e se as cavernas podem atuar como filtros ambientais

O primeiro manuscrito, ou Artigo I, escrito e publicado conforme as normas da “Revista Brasileira de Espeleologia”, apresentou o primeiro registro de três espécies de mixomicetos em cavernas brasileiras. As espécies *Arcyria denudata* (registrada na caverna Angá dos Negros II), *Hemitrichia calyculata* e *Metatrichia vesparia* (ambas na caverna Zé Brega) foram encontradas em madeira em decomposição. Essas cavernas estão localizadas na região cárstica de Pains, no centro-oeste de Minas Gerais. As amostras coletadas foram fixadas e identificadas por meio de análises morfológicas e microscopia. Embora essas espécies sejam comumente encontradas em áreas de mata e florestas, este é o primeiro registro delas em cavernas situadas em zonas afóticas. Mixomicetos são considerados organismos curiosos e complexos, com um ciclo de vida com duas fases bem distintas do ponto de vista morfológico e fisiológico, além de desempenharem um papel importante como bioindicadores em áreas preservadas. O estudo também incluiu uma revisão bibliográfica sobre o registro de mixomicetos em cavernas de outras localidades, destacando registros anteriores em cavernas da Espanha, como relatado por Galán e colaboradores (Galán & Nieto, 2010, 2022; Galán *et al*., 2011, 2018, 2021a, 2021b, 2022; Galán & Rivas, 2019; Galán, 2015).

O segundo manuscrito, ou Artigo II, elaborado conforme as normas do periódico ‘Criptogamie, Mycologie’, apresenta a descrição de uma nova coleção de basidiomas da espécie *Schizophyllum commune*, encontrada na Gruta Furnas, no estado do Rio de Janeiro. O estudo detalha a morfologia diferenciada do espécime, comparando-a com outras 20 espécies do gênero provenientes de diversas localidades, com base em artigos científicos e registros de herbários. Além disso, foram realizadas análises filogenéticas envolvendo 36 espécies do gênero *Schizophyllum*, permitindo uma compreensão mais aprofundada da posição taxonômica de *S. commune* dentro do grupo. Esse trabalho contribui significativamente para o entendimento da diversidade morfológica e filogenética do gênero em ambientes subterrâneos.

O terceiro manuscrito, ou Artigo III, elaborado conforme as normas do periódico "Fungal Ecology", apresenta uma pesquisa sobre a ecologia de macrofungos na região cárstica de Pains, Minas Gerais. O estudo comparou a riqueza e a composição de macrofungos em quatro cavernas e suas respectivas áreas de mata, utilizando transectos e 240 quadrantes como unidades amostrais. Foram coletados macrofungos pertencentes aos filos Basidiomycota e Ascomycota. Além disso, foram analisadas variáveis ambientais, como temperatura, umidade, luminosidade e características dos substratos, com o objetivo de avaliar a influência dessas variáveis na riqueza e composição dos macrofungos dentro e fora das cavernas. Este é o primeiro estudo no Brasil a documentar a ocorrência de macrofungos em um ambiente cárstico, contribuindo significativamente para o entendimento da ecologia desses organismos em habitats subterrâneos.

Os macrofungos são fungos que formam esporocarpos visíveis a olho nu, com formas complexas e variadas (Pradhan *et al.*, 2013). Eles atuam principalmente como decompositores, degradando matéria orgânica, como folhas e madeira morta, e reciclando nutrientes essenciais para o solo. Com ampla distribuição geográfica e alta diversidade, especialmente em florestas tropicais, esses fungos se adaptaram à produção de esporos, o que facilita sua propagação e garante seu sucesso em diversos ecossistemas (Richards *et al.*, 2017).

Nos ambientes cavernícolas, os fungos têm grande importância devido ao seu potencial para bioexploração e aplicações biotecnológicas, além da possibilidade de descoberta de novas espécies. Eles desempenham um papel crucial na cadeia trófica, contribuindo para o equilíbrio ecológico desses ambientes e sendo úteis no monitoramento da qualidade ecológica, especialmente em habitats importantes para a preservação da biodiversidade (Nieves-Rivera *et al.*, 2009).

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SEGUNDA PARTE

ARTIGOS

ARTIGO I

FIRST RECORDS OF MYXOMYCETES IN BRAZILIAN CAVES

ARTIGO I

Este manuscrito foi elaborado, seguindo as diretrizes estabelecidas pela *Revista Brasileira de Espeleologia* - RBEsp. Aceito em 09 de dezembro de 2024 e publicado em 20 de dezembro de 2024.

FIRST RECORDS OF MYXOMYCETES IN BRAZILIAN CAVES

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RESUMO: O objetivo deste artigo é relatar os primeiros registros de mixomicetos em cavernas brasileiras, com base em três espécimes coletados na área cárstica do município de Pains, localizado no estado de Minas Gerais, sudeste do Brasil. Os espécimes, todos pertencentes à família Trichiaceae (Trichiales, Myxogastromycetidae), foram identificados como *Arcyria denudata* (L.) Wettst., *Hemitrichia calyculata* (Speg.) M.L. Farr e *Metatrichia vesparia* (Batsch) Nann.-Bremek. ex G.W. Martin & Alexop Notavelmente, *Hemitrichia* Rostaf. e *Metatrichia* Ing não haviam sido anteriormente documentados em ambientes de cavernas ao redor do mundo. Embora todas as três espécies sejam cosmopolitas, não haviam sido registradas na região centro-oeste de Minas Gerais ou em ambientes de cavernas globalmente.

Palavras-chave: Amoebozoa, Mixomicetos, Cavernas, Carste, Trichiaceae.

ABSTRACT: The aim of this article is to report the first records of myxomycetes in Brazilian caves, based on three specimens collected in the karst area of the municipality of Pains, located in the state of Minas Gerais, southeastern Brazil. The specimens, all belonging to the Trichiaceae family (Trichiales, Myxogastromycetidae), were identified as *Arcyria denudata* (L.) Wettst., *Hemitrichia calyculata* (Speg.) M.L. Farr and *Metatrichia vesparia (*Batsch) Nann.-Bremek. ex G.W. Martin & Alexop. Notably, *Hemitrichia* Rostaf. and *Metatrichia* Ing have not previously been documented in cave environments around the world. While all three species are cosmopolitan, they had not been recorded in the central-west region of Minas Gerais or in cave environments globally.

Key-words: Amoebozoa, Myxomycetes, Caves, Karst, Trichiaceae.

INTRODUCTION

For many years, myxomycetes were classified into different groups based on newly discovered characteristics, being included in the Animal Kingdom, the Plant Kingdom, and even the Fungi Kingdom (Lado & Eliasson, 2022). Molecular evidence indicates that myxomycetes are an evolutionarily ancient group, belonging to the "crown" clade of eukaryotes, and that the group Mycetozoa, to which they belong, is monophyletic (Rollins & Stephenson, 2011). Currently, myxomycetes are classified in the Kingdom Protist (Amoebozoa) and are considered amoeboid, unicellular, and heterotrophic organisms. They have a complex life cycle, which includes an amoeboflagellate, uninucleate, and haploid phase that functions as isogametes, giving rise to the zygote and, subsequently, the multinucleate plasmodial mass. This plasmodium exhibits motility and actively seeks out food sources such as bacteria, fungi, and other organic matter (Novozhilov et al., 2022; Schnittler, 2001).

Depending on environmental conditions and the stage of maturation, the plasmodium, initially negative phototropic, moves to brighter locations and undergoes intense physiological and morphological changes. During this process, all its biomass is directed towards the formation of one or more sporocarps, where the only living part is the spores. In the fruiting body stage, myxomycetes become fixed to the substrate and acquire an appearance quite similar to some ascomycetes and basidiomycetes (Keller et al., 2022). These sporocarps produce resilient spores that, upon release and dispersion, typically by wind or animal vectors (Schnittler, 2001), encounter favorable conditions for germination. This germination process leads to the formation of new amoeboflagellates and subsequently, the zygote and plasmodium thereby initiating a new life cycle.

The class Myxomycetes is widely distributed, with 1100 species (Lado, 2005-2024) often exhibiting preferences for specific environmental factors such as temperature, humidity, and nutrient availability. These organisms have been found in all terrestrial ecosystems, across different climates and vegetation zones, including dryland or restrictive ecosystems, as the Brazilian Caatinga and Mangroves, being particularly abundant in warm-temperate and tropical forests (Vaz et al., 2017; Cavalcanti & Agra, 2019; Hosokawa et al., 2019).

Myxomycetes occupy a variety of microhabitats (Keller et al., 2022), and can be found on aerial or ground litter, leaves (foliicolous), decaying wood (lignicolous), the bark of living trees (corticicolous), succulent plants (succulenticolous), inflorescences (floricolous), mosses (muscicolous), basidiomes (myceticolous), lichens (lichenicolous) snow banks (nivicolous), and herbivore dung (fimicolous). In addition to performing a variety of important ecological functions, such as nutrient mineralization, myxomycetes play a crucial role as regulators of bacterial populations (Coûteaux & Darbyshire, 1998).

The unique microclimatic conditions and types of substrates found in subterranean environments, which usually differ from those in surrounding external environments, are likely the primary factors driving the formation of exclusively cave-dwelling communities (Mammola, 2019). These communities are primarily composed of detritivorous species, with the organic resources available in these environments being predominantly allochthonous, originating from the external environment. These resources play a crucial role in determining the diversity within the ecosystem (Ferreira et al., 2000).

Both bacteria and protozoa, including myxomycetes, play fundamental roles in the dynamics of cave ecosystems. They actively contribute to the production and decomposition of organic matter, making these resources available to other species within the cave invertebrate communities (Galán, 2015). Furthermore, air currents in subterranean environments can transport spores from the external environment, serving as a crucial mechanism for the dispersal and establishment of microorganisms such as fungi and slime molds (Vanderwolf et al., 2013).

Few studies highlight the presence of myxomycetes in subterranean environments. The documentation of Mycetozoa species in caves of the Iberian Peninsula was first reported through research conducted in northern Spain (Galán & Nieto, 2010, 2022; Galán et al., 2010, 2011, 2018, 2021a, 2021b, 2022; Galán & Rivas, 2019). Apart from the material found in the Urdallue cave in Artikutza, where plasmodia and aethalia of *Fuligo septica* (L.) F.H. Wigg. were recorded (Galán, 2015), other specimens were not identified and are considered potential new cave-dwelling species, possibly belonging to the orders Trichiales and Physarales. These bright yellow microorganisms were found on rocks and speleothems. They are macroscopic and cosmopolitan protozoa commonly found on rocks, soils, and decaying wood in temperate and humid forests. They were also discovered in leaf litter near cave entrances, on speleothems, and on walls in aphotic zones.

These studies emphasize the presence of these "giant amoebae" in subterranean environments, particularly due to the type of substrate they inhabit and their ecological role. Most were found on speleothems rich in chemoautotrophic bacteria, making this habitat conducive to completing their subterranean life cycle without relying on surface nutrients. As Mycetozoa are known bacterivores, Galán & Nieto (2022) suggested that they can be considered bioindicators of bacteria involved in rock corrosion and the creation or destruction of secondary mineral deposits in caves.

In a study conducted by Nieves-Rivera (2003) in the Rio Camuy cave system in Puerto Rico, the presence of protozoa from the phylum Myxomycota was documented. Sporangia of *Arcyria* sp., *Comatricha* sp., and *Stemonitis* cf. *herbatica* Peck. were found on various substrates, including bat bones, decomposing wood, and leaf litter.

Another group of amoebae closely related to myxomycetes, the dictyostelids, have been discovered in caves throughout North America, as well as in Puerto Rico and Bahamas. Researchers have identified a total of 14 species of dictyostelids and 4 species of *Protostelium* of thriving on various substrates within these cave systems (Waddell 1982; Reeves et al., 2000; Landolt et al., 2006). Similar to myxomycetes, these organisms form fruiting bodies, but their spores are enclosed in a mucilaginous mass, which restricts their dispersal; bats, amphibians, and some invertebrates facilitate the dispersal of dictyostelid spores through ingestion and defecation (Landolt et al., 2006). These findings underscore caves as environments that provide conducive conditions for these protozoa to thrive and complete their life cycles.

Protozoa in general, and myxomycetes in particular, have been underrepresented in studies of cave environments; nevertheless, they may play an important role as intermediaries in the food chain of subterranean ecosystems. By consuming bacteria, these organisms provide a vital food source for invertebrates inhabiting caves, particularly troglobitic species that depend on diets rich in bacteria and fungi (Galán et al., 2010; Galán & Nieto, 2010; Galán, 2011).

The aim of this article is to document the first observations of myxomycetes in caves in Brazil, using specimens collected from the karst region of Pains municipality. This study forms part of a larger project investigating the ecology of macrofungi in the karst areas of Pains, Minas Gerais. The primary objective of this project is to explore how environmental filters influence species distribution in subterranean habitats, emphasizing the crucial role of these organisms in ecosystem dynamics and their importance for the conservation of karst areas.

METHODOLOGY

Study area

Specimens were documented on pieces of wood and tree trunks within two caves in the karst region of Pains, situated in central-western Minas Gerais (Meyer et al., 2022). The climate in this area is humid subtropical, characterized by a dry season from May to August and a rainy season from September to March. The region lies within the São Miguel River Basin and the Ribeirão dos Patos Basin, both significant tributaries of the São Francisco River (Figure 1).

The regional landscape showcases diverse karst formations, featuring carbonate rock outcrops that create distinct features such as sinkholes, caves, residual massifs, lapiaz, cliffs, crevices, and joints (Lucon et al., 2020). Vegetation cover forms a mosaic of phytophysiognomies, encompassing forested areas and open spaces, with Seasonal Semideciduous Forest prevalent at lower elevations and among the outcrops (Melo et al., 2013). In more eroded zones, an additional open phytophysiognomy known as open karst formation is evident. Presently, the region boasts 3,000 registered caves, representing the highest concentration of caves in Latin America (CANIE, 2024).

Figure 1- Map of the karst area of Pains, including the location of the Brega and Angá dos Negros II caves.

The two caves where myxomycetes were recorded are Brega and Angá dos Negros II (Figure 2). Brega cave stretches linearly for approximately 1,208 meters with entrances located at opposite ends of the main cavity passage. While most of this cave is dry, there is a lower level where the water table is accessible, maintaining water year-round with fluctuations during the rainy season. This section also accumulates significant organic matter of plant origin, transported by runoff and deposited in the cave's lower regions.

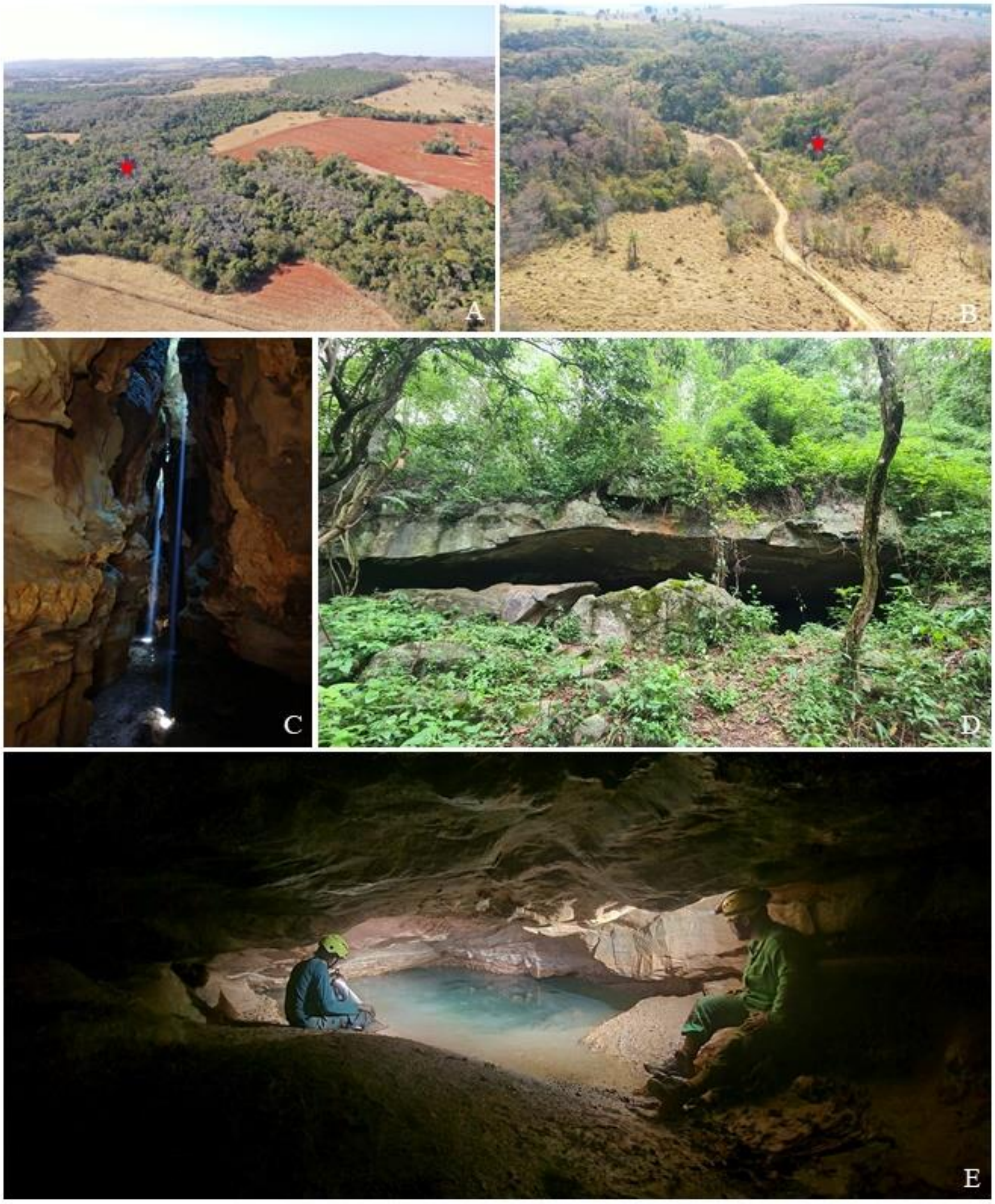

Figure 2- A. Location of the Angá dos Negros II Cave area, B. Location of the Brega Cave area, C. Entrance of the Angá dos Negros II Cave, D-E. Entrance and conduit of the Brega Cave. Photos by Rodrigo L. Ferreira and Marconi Souza Silva.

In contrast, Angá dos Negros II cave is much smaller, measuring about 100 meters in length. It features three entrances: two horizontal and one vertical (skylight), facilitating the entry of plant materials into the cave through gravitational forces.

Sampling, analysis, identification and herborization

The collected specimens were placed in cardboard boxes along with data sheets specifying substrate type, coloration, collection date, temperature, light exposure, and humidity at the collection site. Subsequently, they were transported to the laboratory where they underwent dehydration in an oven at temperatures ranging from 40 to 50°C. This process prepared them for macroscopic and microscopic analysis of the sporocarps to facilitate their identification.

The macroscopic and microscopic characteristics of the sporangia were analised following the methodology described by Parentes & Cavalcanti (2023). The specimens were identified using keys, illustrations, and descriptions provided by Farr (1976) and Poulain et al. (2011). Exsiccates were deposited in the ESAL Herbarium at the Federal University of Lavras under the numbers: ESAL33567, ESAL33568 and ESAL33569.

Taxonomic nomenclature adheres to Lado (2005-2024), and distribution within Brazil is referenced from BFG (2022) and Cavalcanti & Agra (2024). State acronyms follow the standards established by IBGE (2021). Color codes for sporocarps are based on the Kornerup & Wanscher color chart (1978).

RESULTS AND DISCUSSION

The specimens of myxomycetes discovered in the Brega and Angá dos Negros II caves in Pains belong to the genera *Arcyria* F.H. Wigg., *Hemitrichia* Rostaf., and *Metatrichia* Ing, all classified under the family Trichiaceae, order Trichiales (Figures 3-5). These genera are known to occur in diverse Brazilian biomes.

Arcyria denudata (L.) Wettst.

Wood fragments were found near a skylight in Angá dos Negros II Cave, suggesting they originated from the upper part of the limestone outcrop and were likely introduced into the cave by gravitational forces. However, the site was shielded from direct light and rainfall and numerous recently sporulated sporocarps of *Arcyria denudata* were found on the decomposing wood.

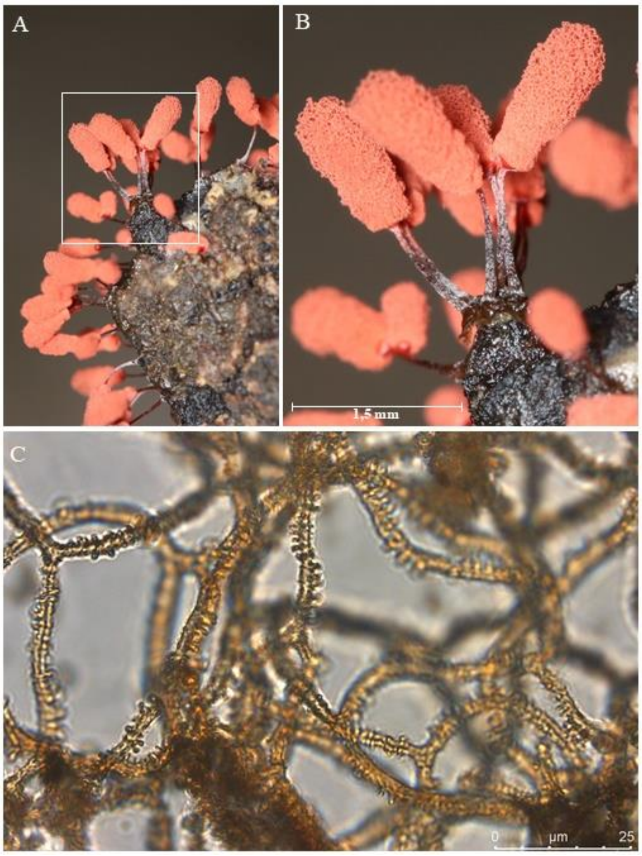

Figure 3- A-B. *Red sporangia of Arcyria denudata.* C. *Microscopy showing capillitium and spores. Photos by Rodrigo L. Ferreira and Laise de Holanda Cavalcanti.*

The sporangia displayed typical characteristics of the species (Farr, 1976): red sporotheca (B8), cylindrical stalks pedicels that were dark and about half the total height of the sporocarp, containing subglobose cysts measuring 10-17 µm in diameter; the capillitium was abundant, elastic, and firmly attached to the calyculus (Figure 3). The distinguishing feature was a shallow calyculus instead of the funnel-shaped one more commonly observed in the species, which still fits within the description by Martin & Alexopoulos (1969).

This species has a cosmopolitan distribution (Martin & Alexopoulos, 1969), documented across Brazil's North (AM, AP, PA, RO, RR), Northeast (AL, BA, CE, MA, PB, PE, PI, RN, SE), Midwest (DF, GO, MS, MT), Southeast (MG, RJ, SP), and South (PR, RS, SC) regions, encompassing all of the country's phytogeographical domains (Agra et al., 2024a). It is commonly found on decomposing logs and branches in humid forests, as well as on live tree trunks, aerial litter, and basidiomes (Costa et al., 2014). There are no documented occurrences of *A. denudata* in caves in the global literature, and only Nieves-Rivera (2003) reports the presence of an unidentified species of the genus in Puerto Rico.

Hemitrichia calyculata **(Speg.) M.L. Farr.**

Few sporocarps, indicating relatively older sporulation, were found on decomposing wood in Brega Cave. These fragments were located near the water table observed in the lower passage of the cave, where a significant accumulation of decomposing plant material occurs during rainy periods. Various filamentous fungi were also observed, some growing diffusely on the plant fragments.

The sporangia, yellow in color (A8), exhibited typical morphological characteristics of the species, including a deep calyculus, a stalk about half the total height, elastic capillitium completely detached from the edges of the calyculus, and filaments adorned with spirals (Figure 4).

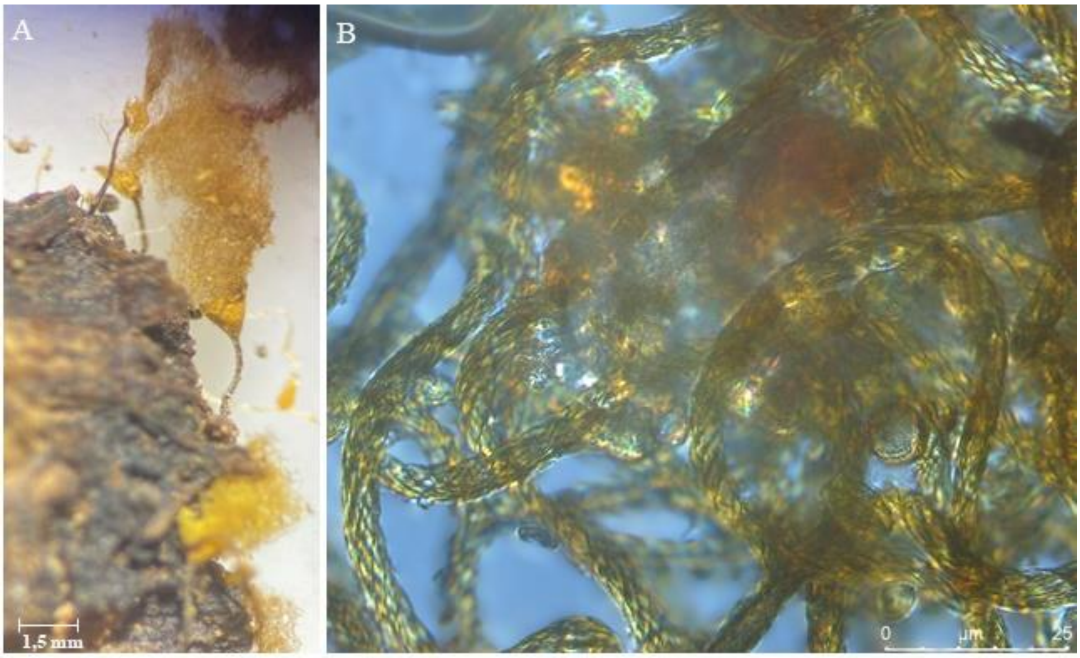

Figure 4- A. Yellow sporangia of Hemitrichia calyculata, showing the membranous calyculus and elastic capillitium, B. Microscopy of capillitium and spores. Photos by Mariana A. Moreira and Laise de Holanda Cavalcanti.

This species has a cosmopolitan distribution, with a high frequency in tropical regions (Farr, 1976; Poulain et al., 2011). Agra et al. (2024b) documented its occurrence across the North (AM, RR), Northeast (AL, BA, CE, MA, PB, PE, PI, RN, SE), Midwest (DF, GO, MS, MT), Southeast (RJ, SP), and South (RS, SC) regions of Brazil, spanning all biomes. Cavalcanti & Agra (2024) listed *H. calyculata* among the species found in Minas Gerais without specifying exact locations. It inhabits various microhabitats within different Brazilian phytophysiognomies, sporulating on forest floor litter, aerial litter, dead logs and branches, live tree bark, and basidiomes (Costa et al., 2014). No records were found in the global literature of *Hemitrichia* species occurring in caves.

***Metatrichia vesparia* (Batsch) Nann.-Bremek. ex G.W. Martin & Alexop.**

Small clusters of sporangia were collected on decomposing wood within Brega Cave, where *H. calyculata* also sporulated, in similar habitat conditions: located near the observed water table in the cave's lower passage, with a notable accumulation of decomposing plant material. The sporangia observed were aged, short- stalked, obovate, grouped closely together, and dark reddish-brown (E8), with a ruptured cartilaginous peridium facilitating expansion of the elastic capillitium (Figure 5). Filaments were adorned with spirals and characteristic spines of the species (Farr, 1976; Poulain et al., 2011).

Figure 5- A. Gregarious clusters of sporangia of Metatrichia vesparia, some of them showing the strongly elastic capillitium. B. Microscopy of capillitium and spores. Photos by Mariana A. Moreira and Laise de Holanda Cavalcanti.

This species exhibits a broad distribution in temperate regions, appearing less common in tropical and Southern Hemisphere climates, likely cosmopolitan (Martin & Alexopoulos, 1969; Farr, 1976). In Brazil, it has been recorded in the North (AM, RO, RR), Northeast (AL, BA, PB, PE, PI, RN, SE), Midwest (DF, MS), Southeast (RJ, SP), and South (PR, RS, SC) regions, across all biomes (Lima & Cavalcanti, 2017; Agra et al., 2024c). Agra's unpublished thesis (2017) includes occurrences in Cerrado areas of Mato Grosso, Barra do Garças, and Santo Antônio do Monte, Minas Gerais.

The genus *Metatrichia* encompasses six species, three of which are found in Brazil (Lado, 2005-2024; Agra et al., 2024c). Despite its extensive distribution within Brazilian territory, *M. vesparia* is not typically among the most common species, sporulating on dead logs, branches, forest litter, and dung of the wild rabbit *Sylvilagus brasiliensis* L. (Bezerra et al., 2008; Costa et al., 2014). No publications were found in the global literature regarding the presence of *Metatrichia* species in cave environments.

Additionally, sporangia of *Arcyria cinerea* (Bull.) Pers., and an unidentified species from the order Physarales were documented on tree trunks in the forested area near Angá dos Negros II cave through photographs (Figure 6). Species of both orders are frequently found in the various ecosystems of Brazil and in other countries of the Neotropics (Lado & Basanta, 2008; BFG, 2022).

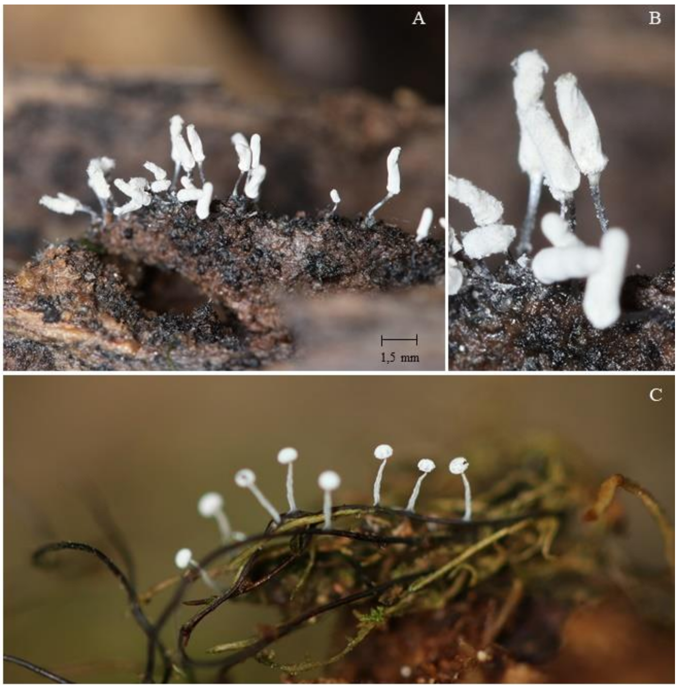

Figure 6- Photographic record of two species of Myxomycetes, sporulated on tree trunks in the forested area near Angá dos Negros II Cave A-B. Arcyria cinerea. C. Sporangia of unidentified specie of Physarales. Photos by Rodrigo Lopes Ferreira.

Myxomycetes in caves

Minas Gerais stands out as one of Brazil's most taxonomically diverse states, boasting a rich variety of taxa ranging from seed plants (11,432 species) to lycophytes, ferns (728 species), hornworts, mosses, and liverworts (785 species), as reported by BFG (2022). However, the Flora and Funga of Brazil (2024) lists only seven families, 12 genera, and 15 species of myxomycetes recorded in the state, notably excluding genera like *Hemitrichia* and *Metatrichia*. A thorough review by Cavalcanti & Agra (2024) expanded this count to 48 species, encompassing 15 genera and three families. This included records from Agra's thesis (2017), which documented *A. denudata*, *H. calyculata*, and *M. vesparia* in Cerrado areas near Santo Antônio do Monte, approximately 200 km from Pains.

The presence of Trichiaceae representatives in caves was first documented by Nieves-Rivera (2003), who found sporangia of an unidentified *Arcyria* species on bat bones and decomposing wood in Puerto Rico's Rio Camuy Cave Park (Table 1). More recently, Galan & Nieto (2022) observed unidentified specimens, probably belonging to the order Trichiales, in Gipuzkoa caves.

The specimens discovered in the Brega and Angá dos Negros II caves mark the first global published records of *A. denudata*, *H. calyculata*, and *M. vesparia* in cave environments, providing initial insights into the presence of myxomycetes in Brazilian cave ecosystems.

Reports of Mycetozoa taxa in caves are notably rare, likely due to the significant scarcity or complete absence of organic matter in the deeper parts of these subterranean environments. These organisms are found more frequently and abundantly in temperate forests, but they can also inhabit arid environments, such as cold or hot deserts like the Atacama, semi-arid regions like the Caatinga, and seasonally dry areas like the Cerrado. It is crucial to recognize that the references cited in Table 1 likely do not encompass all publications mentioning myxomycetes in caves, given the diverse array of specialists and the various journals where their findings may appear. Moreover, due to their lesser-known status, these organisms are sometimes only mentioned at higher taxonomic levels in publications. In some instances, such as Landolt et al. (1992), even the title, keywords, or abstract may not explicitly indicate a new record of myxomycetes occurring in cave environments.

TAXON	SUBSTRATE*	COUNTRY	SOURCE**
Trichiales Trichiaceae *Arcyria* cf	B; W	Puerto Rico: Camuy, Camuy River Caves National Park. La Catedral, Clara de Empalma and Sumidouro Caves.	3

TAXON	SUBSTRATE*	COUNTRY	SOURCE**
Trichiales Trichiaceae *Arcyria denudata*	W	Brazil: Minas Gerais, Pains. Angá dos Negros Cave.	15
Trichiales Trichiaceae *Hemitrichia calyculata*	W	Brazil: Minas Gerais, Pains. Brega Cave.	15
Trichiales Trichiaceae *Metatrichia vesparia*	W	Brazil: Minas Gerais, Pains. Brega Cave.	15
Physarales Physaraceae *Fuligo séptica*	R, L	Spain: Navarra, Goizueta, Artikutza, Urdallue Caves.	7
Physarales Didymiaceae *Didymium trachysporum*	SMC	USA: Maryland, Belstsville. Whitings Neck Cave.	2
Stemonitales Stemonitaceae *Comatricha* cf	L	Puerto Rico: Camuy, Camuy River Caves National Park. La Catedral, Clara de Empalma and Sumidouro Caves.	3
Stemonitales Stemonitaceae *Stemonitis* cf *herbatica*	W	Puerto Rico: Camuy, Camuy River Caves National Park. La Catedral, Clara de Empalma and Sumidouro Caves.	3
Myxomycetes	S	Mexico: Morelos, Emiliano Zapata. Salitre Cave.	1
Mycetozoa Myxomycetes ? Trichiales	R	Spain: Basque Country, Gipuzkoa. Caves:.Leizarãn; Aixa, Igitegi, Montxon, Tortuga; Urkita 2, Meru	4,5,6,8

TAXON	SUBSTRATE*	COUNTRY	SOURCE**
		4., Akaitz txiki 1 and 2; Txispiri and Ibarrondo.	
Mycetozoa	R	Spain: San Sebastian, Errera. Txoritokieta 1 and 2 Caves.	10
Mycetozoa Trichiales and Physarales	R	Spain: Navarra, Serra de Urbasa. Lezeaundi Cave.	9
Mycetozoa Trichiales	R	Spain: Navarra, Aralar. Kolosobarne Cave.	12
Mycetozoa	R	Spain: Basque Country, Gipuzkoa. Aitzelarko; Burnigurutze 1 and 2 Caves.	11,13
?Mycetozoa	R	Spain: Andaluzia, Malaga, Hoyo Conique de Archidona system caves.	14
Unidentified plasmodium.	SG	Puerto Rico: Mayagüez, Mona Island, beach of Pájaros. El Cabalo/Pájaros and Uvero Caves.	3

Table 1- Records of Mycetozoa in underground environments around the world.

*Substrate: W – Decomposing wood. L – Leaf litter. B – Bat bones. S – Soil. SG – Soil enriched with bat guano. SMC – Soil in a moist chamber. R – Rock and speleothems.

**Source: 1 – Hoffman et al. 1986. 2 – Landolt et al. 1992. 3 – Nieves-Rivera 2003. 4– Galán et al. 2010. 5 – Galán & Nieto 2010. 6 – Galán 2011. 7– Galán 2015. 8 – Galán et al .2018. 9 – Galán & Rivas 2019. 10 – Galán et al. 2021a. 11 – Galán et al. 2021b. 12 – Galán & Nieto 2022. 13 – Galán et al. 2022.14 – Martinez Rodríguez et al. 2023. 15 – This work.

It is important to emphasize that the karst region of Pains represents one of Brazil's most significant conflicts between the preservation of speleological heritage and the environmental impacts of mining. The caves in this region are under considerable threat from limestone extraction and improper land use, resulting in irreversible environmental damage and endangering the high diversity of cave-restricted species, many of which are endemic to single caves (Ferreira et al., 2022; Galvão & Costa, 2022).

This study is the first to explore the occurrence of myxomycetes in karst areas in Brazil. These pioneering efforts provide a foundation for future research in this area, contributing to the monitoring of these organisms in specific environments such as caves.

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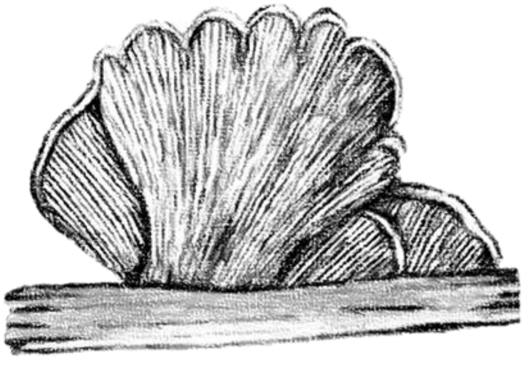
ARTIGO II

BRINGING LIGHT INTO THE DARKNESS: A NEW RECORD OF *SCHIZOPHYLLUM COMMUNE* (AGARICALES: BASIDIOMYCOTA) FROM A BRAZILIAN CAVE AND CLARIFYING THE TYPE SPECIES OF THE GENUS *SCHIZOPHYLLUM*



ARTIGO II

Este manuscrito foi elaborado em sua versão preliminar, seguindo as diretrizes estabelecidas pela revista *Cryptogamie,Mycologie.*

BRINGING LIGHT INTO THE DARKNESS: A NEW RECORD OF *SCHIZOPHYLLUM COMMUNE* (AGARICALES: BASIDIOMYCOTA) FROM A BRAZILIAN CAVE AND CLARIFYING THE TYPE SPECIES OF THE GENUS *SCHIZOPHYLLUM*

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ABSTRACT

Schizophyllum commune is the type species of *Schizophyllum*. The species has a wide distribution over the world growing in diverse substrate including humans. Now, we report interesting specimens of *S. commune* from aphotic zone of a Brazilian cave that include pure white basidiomata sometimes presenting a lateral stipe. We also provide a checklist of *Schizophyllum* names published with a tentative investigation on their type specimens, including *S. commune*, that has its lectotype designed here.

KEY WORDS: *Schizophyllum*, Cave, Neotropics, type.

RÉSUMÉ

Schizophyllum commune est l'espèce type du genre *Schizophyllum*. Cette espèce est largement distribuée dans le monde et se développe sur divers substrats, y compris chez les humains. Nous rapportons ici des spécimens intéressants de *S. commune* provenant de la zone aphotique d'une grotte brésilienne, caractérisés par des basidiomates d'un blanc pur, parfois dotés d'un stipe latéral. Nous fournissons également une liste des noms publiés pour le genre *Schizophyllum*, accompagnée d'une enquête préliminaire sur leurs spécimens types, y compris *S. commune*, dont le lectotype est désigné ici.

MOTS CLÉS: *Schizophyllum*, Grotte, Néotropiques, Types.

INTRODUCTION

Schizophyllum Fr. is a very old fungus name, being one of the most cited genera (Bhunjun *et al.* 2024). It was originally described as having radiating lamellae that are longitudinally bi-splitted with involute margin, and coriaceous subsessile to didimiate pileus with involute margin (Fries 1815), with *S. comune* Fr. as type species (Fries 1821; He *et al*. 2019). Dillenius (1719) considered *Agaricus* Dill. mostly for lignicolous species with pleurotoid habit and reduced stipe, and mentioned in the page 192 'Agaricus villosus, lamellis sinuosis & invicem implexis, Ad Fagos' (Agaricus villose, lamellae sinous and in turning twisted, on oak). It might by the oldest mention of a putative fungus that now belongs to *Schizophyllum*, as already mentioned by Essig (1922) and Watling & Sweeney (1971).

Species of *Schizophyllum*, as observed in many macrofungi, have significant ecological importance for maintaining the life cycle in various ecosystems by acting as decomposers of organic matter (Essig 1922). They also have notable biotechnological applications, as the use in ethanol production and some of their metabolites possess anticancer properties (Cooke 1961; Schmidt 1980; Ghosh *et al.* 2005; Horisawa *et al.* 2015; Liu *et al.* 2015). The genus also has medical relevance, occasionally infecting humans (Batista *et al.* 1955, Oliveira *et al*. 2017, Cavanna *et al.* 2019), as well as veterinary interest (Kano *et al*. 2002),, being a source of schizophyllin, a neutral extracellular polysaccharide with significant antitumor and immunobiological activities (Zhang *et al.* 2013).

Linder (1933) was the first monograph on the genus, reporting six species: *S. comune*, *S. brevilamellatum* Linder, *S. fasciatum* Pat., *S. leprieurii* Linder, *S. radiatum* Fr., and *S. umbrinum* Berk. Cooke (1961) considered the same names, but with the epiteth '*leprieurii*' in the genus *Phaeoschizophyllum* W.B. Cooke and description of *S. brasiliense* W.B. Cooke and *S. palmatum* Jungh. ex W.B. Cooke. However, *S. album* Rick and *S. variabile* Sorokīn were not studied by them. Additional species was included by Nakasone (1996), who found *S. amplum* (Lév.) Nakasone, a fungus with almost smooth hymenium, as belonging to the same clade together sequences of *S. commune* and *S. fasciatum*.

In Brazil, four species are known: *S. umbrinun* from Pará (Berkeley 1851); the enigmatic *S. album*, with an uncertain location, likely from Rio Grande do Sul (Rick 1919); and *S. brasiliensis*, which is a rare species from the state of Goiás (Cooke 1961); and the widely distributed *S. commune* (Assunção *et al.* 2010). All these species have been found in superficial environments. However, subterranean environments also may eventually harbor macrofungi species (Nováková 2009; Vanderwolf *et al.* 2013), although these environments are still extremely neglected in terms of studies on these organisms. Cave environments (hypogean) have some striking differences compared to surface environments (epigean), including the permanent absence of light, greater temperature stability, and high humidity (Culver & Pipan 2019; Mammola 2019). The absence of light, in turn, prevents the development of photoautotrophic organisms, which drastically reduces productivity in these environments (rarely accomplished by chemoautotrophic organisms) (Barton 2006). Thus, cave environments also tend to be quite limiting in terms of trophic resource availability.

A recent expedition in caves in the state of Rio de Janeiro (southeast Brazil) revealed the presence of *Schizophyllum* specimens in the aphotic zone of a carbonate cave. These specimens exhibited some quite distinctive characteristics compared to other species in the genus, such as the complete absence of pigments and the presence of a stipe in older basidiomata, different from *S. commune*. Therefore, in this study, we describe this new collection of *S. commune*, discussing its morphology, phylogenetic relationships with some other species in the genus, and providing a brief discussion on its occurrence in subterranean habitat and potential threats to this species.

MATERIALS AND METHODS

Typification

For checking the status of typification, depository fungal names were first consulted. Then, looking for the original publication, herbaria database and finally consulting the curators were done to obtain accurate information on the respective type specimen or similar original material. The typification status of each validly published name (when possible) was based on the Shenzhen's Code (Turland *et al*. 2018).

Study area

The basidiomata of *S. commune* were discovered in Furnas Cave (also known as Perazzo Cave – 21°33'16.22''S, 41°58'6.92''W), located in Cambuci municipality, Rio de Janeiro State, southeastern Brazil (Fig. 1). This cave lies within the Mantiqueira Province (Almeida *et al.* 1981), a geological formation that runs parallel to the Atlantic coast of southeastern and southern Brazil. The province extends in a NE-SW direction for over 3,000 km, from southern Bahia to Uruguay. Within this province, the Cambuci Domain (Heilbron 2008), where the cave is located, is characterized by garnet-biotite gneisses interspersed with lenses of dolomitic and calc-silicate marbles.

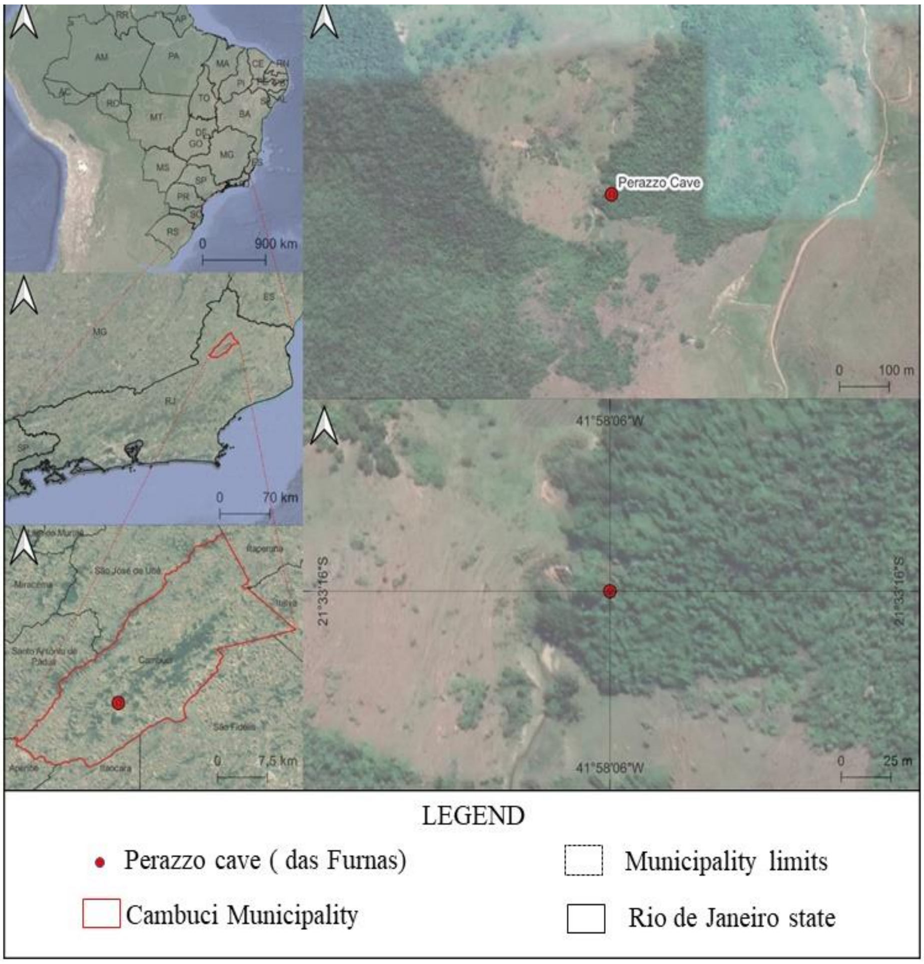

Fig. 1. Map of the Perazzo Cave (das Furnas) locality in the municipality of Cambuci, Rio de Janeiro.

Furnas Cave is one of the largest caves in Rio de Janeiro State, with a linear development of 192 m and a vertical relief of nearly 12 m. A perennial watercourse flows through the cave, shaping sedimentary deposits and creating abrasive features in the host rock (Fig. 2). The cave’s main entrance is located at an elevation of 145 m. The cave features four entrances, three of which are relatively small. Two of these are positioned upstream near the sinkhole where the watercourse enters the cave. The third entrance is located at the end of a dry, secondary conduit close to the main entrance. The largest and primary entrance marks the resurgence point of the watercourse. The cave contains various substrates, ranging from rounded clasts on the floor (Fig. 2B) to exposed bedrock carved by the watercourse (Fig. 2C). As the watercourse flows through a vegetated area (Fig. 2A), plant-derived organic material is transported into the cave and deposited at different locations.

Figure 2: Locality of Perazzo cave (A), Mains conduit (B), Watercourse secundary conduit (C).

The basidiomata of *S. commune* were found on a log along the lateral portion of the cave’s main conduit. This log was situated in an elevated section, wedged between fallen blocks and the cave wall, approximately 25 meters from the main entrance. The log was located within the aphotic zone. Several basidiomata were observed in various stages of fruiting.

While the cave shows signs of occasional human visitation, its interior remains relatively well-preserved. However, the surrounding environment has experienced significant alteration, with large areas of Atlantic Forest replaced by pastures.

MORPHOLOGICAL STUDY

Macroscopic descriptions are based on the analysis of the photos and dried specimens, with measurements using an It-Blue LE-1998 digital caliper. Color codes follows Kramer (2004). Microscopic observations were made from free hand sectioned dried specimens and mounted in 3% KOH and floxin solutions, as well Melzer' reagent and plain lactic acid. Presentation of basidiospores data follows the slightly modified methodology by Tulloss *et al.* (1992). Measurements and statistics are based on 30 spores. Abbreviations include **L**(**W**) = average basidiospores length (width), Q = the length: width ratio range as determined from all measured basidiospores, and **Qm** = the Q value averaged from all basidiospores measured. Herbarium codes follow Thiers (continuously updated).

MOLECULAR STUDY

DNA extraction, PCR, and sequencing

For the DNA extraction process, 1g of fungal tissue was macerated using liquid nitrogen and distributed into microtubes. Then, a lysis buffer (Tris pH 8.0 200 mM, EDTA pH 8.0 50 mM, SDS 2%, NaCl 250 mM, proteinase K 100 µg/ml) was added. The mixture was manually agitated for 15 minutes (keeping the mixture cold) and then incubated at 65ºC for another 15 minutes. One volume of phenol-chloroform (1/1) was added for deproteinization, and the mixture was gently agitated manually while keeping it cold. The tubes were centrifuged at 10,000 rpm for 10 minutes at 4°C, and the upper phase was collected. The procedure was repeated once with phenol-chloroform (1/1) and once with chloroform-isoamyl alcohol (24/1). After the final deproteinization step with chloroform-isoamyl alcohol, the supernatant was distributed into microtubes, 1 volume of cold isopropanol was added, and the tubes were kept in a freezer for half an hour. Subsequently, the tubes were centrifuged at 12,000 rpm at 4ºC for 30 minutes. The supernatant was discarded, and the pellet was washed with 70% ethanol. After removing the excess ethanol, the tubes were incubated in a dry bath at 55ºC until the pellet was dry. The DNA was resuspended in 50 µl of TE (Abreu *et al.* 2023).

The DNA purity was analyzed by spectrophotometry using the Nanodrop One (Nanodrop Technologies, Wilmington, DE, USA) equipment (260/280 nm). The DNA was purified using

MAGTIVIO® metal beads. DNA integrity was confirmed by electrophoresis on a 0.8% agarose gel with 1X TAE buffer (Pylro *et al.* 2014).

The samples were subjected to PCR using the ITS1/ITS4 primer pairs (White *et al.* 1990), conserved for the rDNA region. The amplification was performed in a thermal cycler with the following cycling conditions: i) initial denaturation at 94°C for five minutes; ii) 36 cycles of 94°C for 30 seconds, annealing at 57°C for 30 seconds, and extension at 72°C for 1 minute and 30 seconds; and iii) final extension at 72°C for 5 minutes. At the end of the reaction, the PCR product was stored at -4°C. A negative control without DNA was also used in the PCR amplifications. After the amplifications, electrophoresis was performed on a 0.8% agarose gel under UV light following staining with Red Gel on the iBright transilluminator (Invitrogen®). The amplified products were purified using MAGTIVIO® metal beads.

The sequence quality and chromatography analysis was performed with Sequencher (Nishimura 2000).

Phylogenetic Analysis

The dataset includes a sequence from the specimen collected in this study, as well as thirty-six sequences obtained from GenBank and the following studies: Robledo et al. (2014), Siqueira et al. (2016), Oliveira et al. (2017), Pimenta et al. (2017), Carreño-Ruiz et al. (2019), and Sammut et al. (2019) listed in table 1.

Table 1. Species names, references, and GenBank accession numbers. The GenBank accession number of the sequence generated in this study is in bold.

Specie	Voucher	Locality	ITS1/ ITS2/ 5.8S	References
Schizophyllum commune	**ESAL33570**	**Brazil**	**PQ587110**	**This Study**
Schizophyllum commune	CCG010	México	MF554592.1	Carreno et al. (2019)
Schizophyllum commune	ITA286	Peru	ON426450.1	GenBank
Schizophyllum commune	CCG011	México	MF554593.1	Carreno et al. (2019)
Schizophyllum commune	CCG012	México	MF554594.1	Carreno et al. (2019)
Schizophyllum commune	CBS476.64	Espanha	LT217531.1	Siqueira et al. (2016)
Schizophyllum commune	IPEC1083H	Brazil	KU255858.1	Oliveira et al. (2015)
Schizophyllum commune	PD21	Brazil	KX668572.1	Pimenta et al. (2017)
Schizophyllum commune	UFMGCB 9619	Brazil	KX788176.1	Furbino et al. (2018)

Specie	Voucher	Locality	ITS1/ ITS2/ 5.8S	References
Schizophyllum commune	URM8772	Brazil	PP873655.1	GenBank
Schizophyllum commune	BCC22128	Thailand	FJ372688.1	Robledo et al. (2014)
Schizophyllum commune	BCC26414	Thailand	FJ372690.1	Robledo et al. (2014)
Schizophyllum commune	M34	China	HM595559.1	Robledo et al. (2014)
Schizophyllum commune	2IV_2_2	Thailand	FJ372688.1	Robledo et al. (2014)
Schizophyllum commune	BCC26414	Thailand	FJ372690.1	Robledo et al. (2014)
Schizophyllum commune	JBRI-M22-010	South Korea	OP345206.1	GenBank
Schizophyllum commune	MA56	Thailand	GQ254677.1	Robledo et al. (2014)
Schizophyllum sp.	PDD-103380	China	KF727408.1	Robledo et al. (2014)
Schizophyllum sp.	HFJAU0085	China	MN622803.1	Robledo et al. (2014)
Schizophyllum sp.	S39	Pakistan	KY636376	Robledo et al. (2014)
Schizophyllum radiatum	UTHSCDI14-3	Espanha	LT217541.1	Siqueira et al. (2016)
Schizophyllum radiatum	CBS_301.32	Panamá	AY571060.1	Robledo et al. (2014)
Schizophyllum radiatum	DO90	China	KP050645	Robledo et al. (2014)
Schizophyllum radiatum	CCG016	México	MK585511.1	Carreno-Ruiz et al. (2019)
Schizophyllum umbrinum	A-FL02.1	USA	AF249391.1	Robledo et al. (2014)
Schizophyllum umbrinum	MUCL43017	Espanha	LT217560.1	Siqueira et al. (2016)
Schizophyllum umbrinum	BDNA2316	Brazil	KR261612	Sammut et al. (2019)
Schizophyllum cf. umbrinum	ROBLEDO 2478	Brazil	KM098067	Robledo et al. (2014)
Schizophyllum amplum	NH1803	Spain	DQ097353	Sammut et al. (2019)
Schizophyllum amplum	ALV14009	Spain	MH013190	Sammut et al. (2019)
Schizophyllum amplum	CBS_182.83	Netherlands	L43381.1	Sammut et al. (2019)
Schizophyllum amplum	CBS_285.88	Netherlands	L43382.1	Carreno-Ruiz et al. (2019)
Schizophyllum leprieurii	ROBLEDO1313	Brasil	KM098065.1	Robledo et al. (2014)
Schizophyllum fasciatum	CBS_267.60	Mexico	L43385.1	Robledo et al. (2014)
Schizophyllum fasciatum	CBS_267.60	USA	LT217559.1	Siqueira et al. (2016)
Porodisculus pendulus	-	USA	AY572009.1	Robledo et al. (2014)
Fistulina hepática	REG593	USA	AY571038.	Robledo et al. (2014)

The sequences were aligned and adjusted with MAFFT and using E-INS-I algorithm (Katoh & Standley 2013), and checked with MEGA 11.0 software (Tamura *et al*. 2021). Genetic distances were estimated, and a substitution model was chosen for the dataset to perform the phylogenetic analyses. Maximum likelihood was inferred by best model using JModelTest2 (Darriba *et al*. 2012) and RAxML v.8 (Stamatakis 2014) with GTRGAMMA as model for bootstrapping phase (rapid bootstrap analysis with 1000 replicates), and implemented in CIPRES (Miller *et al.* 2010). Bayesian inference analysis was conducted using MrBayes software (Ronquist & Huelsenbeck 2003) implemented in TOPALi (Milne *et al.* 2004) with model HKY-G substitution model (Hasegawa *et al.* 2003). The graphical editing of the generated phylogenetic trees was done using FigTree (http://tree.bio.ed.ac.uk/). The sequence is deposited in GenBank under accession PQ587110.

RESULTS

Typification

Schizophyllum commune Fr. [as '*Schizophyllus communis*'], Observ. mycol. 1: 103. 1815; sactioned by Fries (1821: 330) and conserved against *Agaricus alneus* (Linnaeus 1753: 1176).
Syntypes (see Art. 9.6) listed in the sanctioned work by Fries (1821: 330): Vaillant (1727: Pl. 10 Fig. 7), Schäffer (1763: Pl. 246), Bulliard (1788: Pl 346), Sowerby (1797: Pl. 183).
Lectotypus hic designatus: Bulliard (1788, Pl. 346).
MycoBank typification number: MBT208403

Notes: The genus and species was only erected by Fries (1815: 103) and later sanctioned by Fries (1821: 330-331, Art. F.3.2). Although he cited many illustrations, it is not certain that Fries used these plates for it. Anyway, our case is similar to the one seen in the example 2 of the Art. 9.1 of the code. However, we can consider all of the plates mentioned by him as syntypes, and the one published by Bulliard the lectotype of *S. commune*.

In his sanctioning work, he also mentioned the protologue of *Agaricus alneus* L. in Linnaeus (1753: 1176), and description in Persoon (1801: 485). Battarra (1759: Pl. 38 Fig. D) also might corresponds to another syntype, but Fries indicated the Fig 'D' (capital d). Actually, the better figure that matches with *S. commune* in a strict sense is the 'Figure d' (note that is a lowercase d) in this Battarra's Plate 38.

Schizophyllum album Rick, Brotéria, sér. bot. 17: 111 (1919)
Typification: unknown.

Notes: The species was described as having white lanose pileus and stipe, on which is yellowish downward; centrally inserted and radicant stipe; decurrent lamellae with united (= junctus; joined/junction/juncture, connected/united together, Cash 1965, Stern 1983) margin, and varioulsy anastomosed, ‘with internal cavity’ (Rick 1919); and growing on soil. It is very uncertain that this fungus represents a member of *Schizophyllum*, but a very probably a lentinoid fungus.

Schizophyllum alneum (L.) J. Schöt. in Cohn, Krypt. -Fl. Schlesien (Breslau) 3.1(33–40): 553. 1889.
Basionym: *Agaricus alneus* L., Sp. Pl.: 1176. 1753.
Typification: unknown; Linnaeus (1753) indicated ‘Fl. Svec. no. 1083’ as type, but the specimen is unknown to us at moment.

Schizophyllum amplum (Lév.) Nakasone, Mycologia 88(5): 771. 1996.
Basionym: *Cyphella ampla* Lév., Annls Sci. Nat., Bot., sér. 3 9: 126. 1848.
Typification: unknown; Léveillé (1848) nor Nakasone (1996) indicated any type, original specimens or illustration for this name. Probably a neotype will be required.

Schizophyllum brevilamellatum Linder, Am. J. Bot. 20: 559. 1933.
Holotype: Venezuela, 1900, leg. Lieut. W Robinson (FH 781502).
Notes: Although Linder (1933) did not explicitly designate it as type, the image of exsiccate FH 781502 provided by the herbarium manager, Genevieuve E. Tocci, align clearly with his Plate 33 Fig. 1. Therefore, this exsiccate is the holotype of *S. brevilamellatum* (Art. 9.1b).

Schizophyllum commune f. ***stipitatum*** L. Krieg., Mycologia 14: 47. 1922.
Typification: The holotype of this form was not preserved (Krieger 1922).

Schizophyllum egelingianum Ellis & Everh., Bull. Torrey Bot. Club 22: 439. 1895.
Typification: Holotype (NY 776278), according to the herbarium’s virtual collection (https://sweetgum.nybg.org/science/vh/specimen-details/?irn=875217); known from Mexico (Ellis & Everhart 1895).

Schizophyllum exiguum Miq., Bull. Sci. Phys. Nat. Néerl.: 455. 1839.
Typification: unknown; the specimen was collected on palm from Surinam (Miquel 1839).

Schizophyllum fasciatum Pat., J. Bot., Paris 1: 170. 1887.

Lectotype designed by Cooke (1961: 590): Mexico, Vera Cruz, VIII.1864, leg Sallé (FH 781583).

Typification details: the type, discovered from an unknown locality in Mexico, was putatively deposited in PC (Patouillard 1887), with apparent isotype in the herbarium S (https://herbarium.nrm.se/specimens/F345455). Unfortunately, we did not receive any information from herbarium PC regarding this species and *S. mexicanum* (see below).

Also consulting the webpage of the Herbarium PC database (https://science.mnhn.fr/institution/mnhn/collection/pc/item/search) we don't find any specimen with this information. Linder (1933: 552) already had mentioned that the majority of the analysed exsiccates by him were housed in FH. The herbarium manager G.E. Tocci kindly sent image of the suppose type specimen of *S. fasciatum*. Since the Patouillard (1887) informed PC as the institution where the type is supposedly deposited, it is clear that Cooke (1961) did not find this specimen, but only the ones deposited in FH.

Schizophyllum flabellare Fr., Adami Afzelii fungi Guineenses ...: fig. 11. 1837.

Notes: reference not seen by the authors, but mentioned in Fries (1936-1838: 403) in the Plate XI, Fig. 25.

Schizophyllum leprieurii Linder, Am. J. Bot. 20: 561. 1933.

Holotype: French Guiana, Leprieur 1003, ex. herb. Richard, in Patouillard Herbarium (FH 781501).

Notes: As observed with *S. brevilamellatum*, the image sent by the FH herbarium manager align clearly with (Linder 1933, Pl. 33 Fig. 7). Therefore, this exsiccate is considered the holotype of *S. leprieurii* (Art. 9.1b).

Schizophyllum lobatum Went, Ber. Dt. Bot. Ges. 14: 158. 1896.

Lectotypus hic designatus: Went (1896, Pl. 12 Figs. 1-7).

MycoBank typification number: MBT215677

Schizophyllum mexicanum Pat., J. Bot., Paris 1: 171. 1887.

Isotype: México, Cuxtla, 1856, leg. Sallé (FH 781504 as '*S. fasciatum* var. *mexicanum* Pat.').

Notes: Patouillard (1887) reported in his protologue that it was deposited in the Herbarium PC. In addition to FH specimen, Cooke (1961) also examined material from PC, although we cannot find any information. In this case, Cooke's tentativeness of typification failed because he cited the "type" from both PC and FH. Unfortunately, prior to the conclusion of this manuscript, we didn't receive any information from the curator of the herbarium PC to check this information.

Schizophyllum mexicanum var. ***incisum*** Pat. in Patouillard & Gaillard, Bull. Soc. Mycol. Fr. 4: 24. 1888.
Isotype: Venezuela, San Fernando du Atabapo, IX.1887, A. Gaillard 1969 (FH 00000)
Typification: not informed (Patouillard & Gaillard 1888), with an apparent isotype in the herbarium S (https://herbarium.nrm.se/specimens/F345602).

Schizophyllum miia (Scop.) Fr. [as '*mya*'], Epicr. Syst. Mycol. (Upsaliae): 403. 1836-1838.
Basionym: *Agaricus miia* Scop., Annus hist.-nat. 4: 147 (1770)
Lectotype hic designatus: Scopoli (1770, Pl. 1 Fig. 4).
MycoBank typification number: MBT560477
Notes: Fries (1836-1838) recombined the species without seeing the specimen.
Schizophyllum multifidum (Batsch) Fr., in Berkeley & Broome, J. Linn. Soc., Bot. 14(73): 46. 1873 (1875).
Basionym: *Agaricus multifidus* Batsch, Elench. fung. (Halle): 173. 1783.
Lectotypus hic designatus: Batsch (1783, Fig. 126a-f).
MycoBank typification number: MBT215416

Schizophyllum murrayi Massee, J. Bot., Lond. 30: 164. 1892.
Typification: The holotype is deposited in the Herbarium K (Elliot s.n.) (https://www.indexfungorum.org/Names/NamesRecord.asp?RecordID=205532). Massee (1895) described a *Schizophyllum*, but according to his Plate 322, Figures 13-14 and discussion suggest a pleurotoid fungus with brownish lamellae and equinulated subglobose "vinose" basidiopspores, suggesting a member of *Crepidotus* (Fr.) Staude (Singer 1986).

Schizophyllum palmatum Jungh. ex W.B. Cooke, Mycologia 53: 592. 1961.
Holotype: Japan, no locality, no date, P.F. von Siebold s.n. (L 0059218). Details in https://data.biodiversitydata.nl/naturalis/specimen/L%20%200059218

Schizophyllum ratiatum Fr., Nova Acta R. Soc. Scient. Upsal., Ser. 3 1(1): 41. 1851 (1855).
Replaced synonym: ***Agaricus radiatus*** Sw., Prodr.: 148. 1788. (Nom. illegit., Turland et al. 2018 Art. 53.1, Art. F.3.4) against ***Agaricus radiatus*** Bolton, Hist. Fung. Halifax (Huddersfield) 1: 39. 1788. (Sanctioned by Fries 1821: 313-314).
Typification: Fries (1855) indicated Swartz (1788), who described *A. radiatus* from Jamaica, but it cannot be used due corresponds an illegitimate not sanctioned name by Fries (1821). However, Fries (1855: 41) based the description in a material collected 'Ad truncos ad Mirador in regno Mexicano copiose. Liebman'. The holotype was noted by Linder (1933: 558) as 'Mexico: Mirador, Liebmann, authentic material' within which the "microscopic characters of the specimens from the two localities agree in all details". Unfortunately, we haven't additional details on this specimen.

Schizophyllum umbrinum Berk., Hooker's J. Bot. Kew Gard. Misc. 3: 15. 1851.
Lectotypus hic designatus: Brazil, Pará, Caripi, no date, Spruce s.n. [K(M) 231987].
MycoBank typification number: MBT205848
Notes: Cooke (1961: 592) performed a putative lectotypification when referred to 'Wright 64, Cuba (K)' as type with the mention "A collection by Wright from Filantropia, Cuba, at FH is labeled "authentic."". Consulting the virtual specimens database of the herbarium FH (https://herbtrack.science.kew.org/accession/232001) the only mention of this collection number is *Agaricus aureobrunneus* Berkeley & M. A. Curtis (FH 601849), published by Berkeley & Curtis (1869). An additional specimen housed in the herbarium NY (NY 776279) is also marked as 'type' but the exact the information on the location is confused because sheet presents many labels informed Cuba, Surinam, Peru, Nicaragua, Brazil, 'Spruce', 'Wright' (https://sweetgum.nybg.org/science/vh/specimen-details/?irn=875222).
Actually, no Cuban specimen is treated in the protologue. Berkeley (1851) mentioned the following regions in the protologue: Caripi (Spruce), Vera Cruz (Galeotti) and Xalapa (Mr. Harries). The specimens of these places clearly correspond to syntypes (Art. 9.6). During our consultation to Kew Herbarium webpage, we found in the virtual collection (https://herbtrack.science.kew.org/search) the registration of the following exsiccates marked as isotypes: K(M) 231986, K(M) 231987, K(M) 231988, K(M) 231989, K(M) 231990, and K(M) 231991. Indeed, the exsiccate K(M) 231988 is the most complet, on which also includes 1849 as the year of the collection. Lee Davies (pers. comm.) informed that, among the specimens mentioned above, K(M) 231986 and K(M) 231989 are marked as belonging to 'Herb. Berk.' and one of them might be the holotype. Asking for images, the K fungarium

curator Ms. Issy Miles-Bunch (pers. corresp.) sent a image of the sheet containing all required exsicates. After careful examination we found K(M) 231990 as the most similar specimen to the one depicted in Berkeley (1851: Plate 1, Figure 1a-right). The Plate 1, Figure 1a-left is also somewhat very similar to exsicatum K(M) 231987, mostly in the format of the broken tree bark, that is chosen here as the lectotype of *S. umbrinum*.

Schizophyllum variabile Sorokīn, Revue Mycol., 12 (no. 45): 10. 1890.
Typification: Turkmenistan, Ak-Rabat, Boukharie, 15.viii.1879, anon.
Lectotypus hic designatus: Sorokīn (1890, Pl. XXXVII, fig. 404 and Pl. XXXVIII, figs. 405-407).
MycoBank typification number: MBT205601
Cooke (1961: 596) mentioned that only analysed illustration on herbarium sheet deposited in NY. We request information about this illustration and L. Briscoe (pers. corresp.) sent the image. Observing it is different?equal? to the published plate. Thus the illustration deposited in the herbarium NY is to choose as the lectotype of *S. variabile*.

Phylogenetic Analysis

The phylogenetic tree with Bayesian inference (BI) revealed significant genetic diversity within the genus *Schizophyllum*, with well-supported clades grouping isolates based on genetic similarities (Fig.3). Additionally, it showed a clear separation between different species of the genus *Schizophyllum* and the outgroup *Porodisculus pendulus* (Fr.) Murrill and *Fistulina hepatica* (Schaeff.) With.

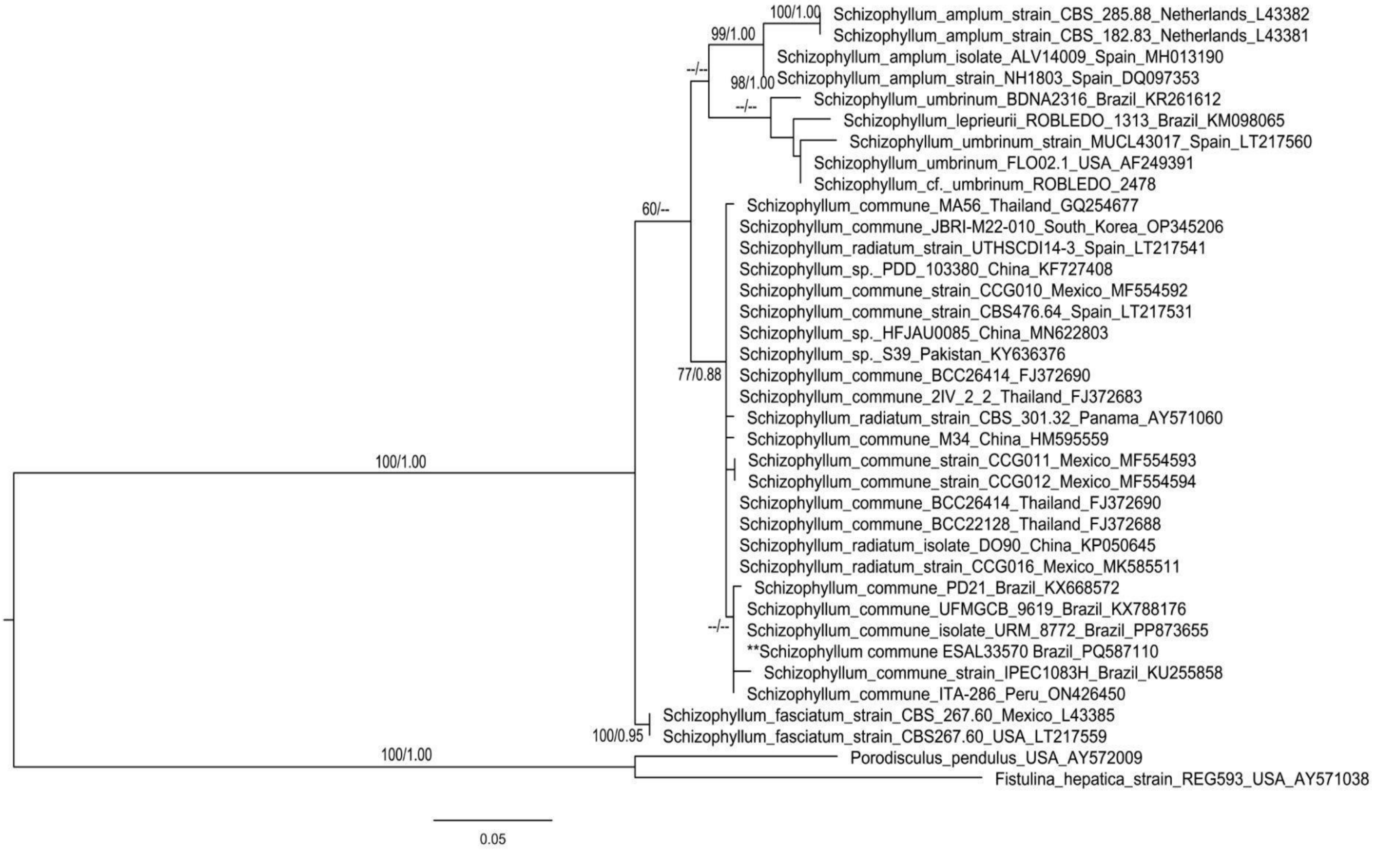

Fig.3. Phylogenetic tree of *Schizophyllum* based on ML and BI analyses for the nrDNA-ITS region. Sequences generated in this study are in bold. Bootstrap support values (MLB≥0.60 - right) and Posterior Probabilities values (BPP≥0.80 - left) are shown above the supported branches. Sequences *Fistulina hepatica* and *Porodisculus pendulus* (DQ867114; DQ867114) were used as outgroup taxa.

The diversity within *S. commune* reflects geographic and possibly ecological variations, suggesting that the species is genetically diverse and widely distributed.

The phylogeny suggests that the *Schizophyllum commune* ESAL isolate shares a close relationship with isolates from tropical and subtropical regions.

The clade containing *S. commune* ESAL has moderate support (77%), suggesting that the internal relationships within this group may not be fully resolved.

Close species, such as *S. fasciatum* and *S. amplum*, are separated from *S. commune*, indicating clear phylogenetic distinction.

The study of morphology and habitat of these isolates, combined with phylogenetic information, can provide insights into local adaptations and the intraspecific diversity of *S. commune*.

Family Schizophylaceae Quél. (1888)

Genus *Schizophyllum* Fr. (1815)

Schizophyllum commune Fr. [as ‘*Schizophyllus communis*’], Observ. Mycol. 1: 103. 1815; per Fr., Systema Mycologicum 1: 330. 1821.

Material examined: Brazil, Rio de Janeiro, Cambuci, Perazzo Cave (das Furnas), marble lithology, conduit with subterranean stream and decomposing organic matter, 21°33'16"S and 41°58'6"W, 145 m a.s.l., remnant Atlantic Forest with pasture areas, 17 February 2024, M.A. Moreira & R.L. Ferreira s/n (ESAL 33570**,** JPB 67195). GenBank: PQ587110.

Basidiomata small in size, mostly pleurotoid. **Pileus** up to 7–11 mm wide and 6–11.5 mm in diam., subsessile to shallowly cupulate then convex to spatulate in pleurotoid specimens; surface lanose to sometimes glabrescent, dry opaque, white (oac909), unchanging; margin entire, inrolled in young basidiomes then incurved to slightly downturned in older specimen, sometimes very narrowly lobed; context coriaceous, very thin, white, unchanging. **Hymenophore** formed by close to subclose splitted-lamellae, narrowly separated along the edge, sometime forked, radiating fom the same point in the laterally inserted stipe or in rays from the pileus center in the cupulate specimens, pale cream (oac794, 795); very narrow; edge smooth, involuted and obtuse, even in splitted parts. **Stipe** 4.5–7.5 × 1.2–2.5 mm, even or tapering downward; surface lanose to sometimes slightly glabrescent near base, white (oac909), unchanging; context solid, 0.5 mm thick, unchanging (Fig. 4) **Odor** woody in dried specimens; taste not performed. **Spore print** not obtained.

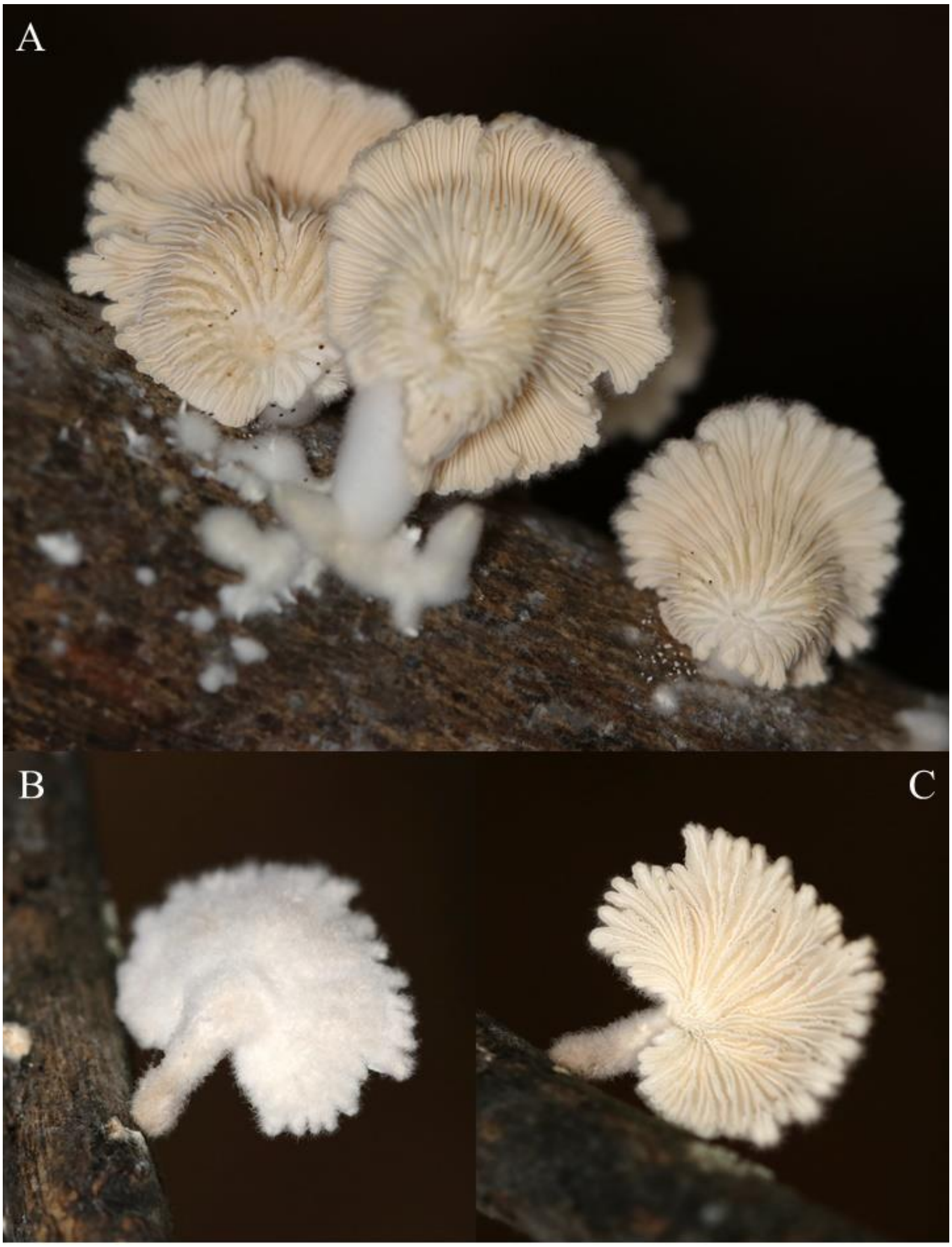

Fig. 4. A-C. Photos of fresh basidiomata of *Schizophyllum commune* on decomposing wood in the Perazzo Cave (das Furnas). Photo by Rodrigo Lopes Ferreira.

Basidiospores (4.8–)5.5–7.1(–7.3) × (2.3–)2.5–3 µm (L = 6.3 µm; W = 2.7 µm; Q = (1.83–)1.92–2.80 (–2.83); Qm = 2.35), inamyloid, hyaline in 3% KOH, cylindrical to sometimes elongate, sometimes adaxially concave in side view, smooth, thin-walled, filled with small gutules; hilar appendix small, sublateral. Basidia 20.5–25 × 3.5–4.5 µm, slender-clavate, colorless, 4-spored, clamped. Basidioles abundant, subcylindric to narrowly clavate. Hymenial cystidia absent. Subhymenium with hyphae 2–3.5 µm wide, colorless, mostly thin-walled, to tickening to 0.5 µm thick. Hymenophoral trama with descendent construction, filamentous hyphae upto 3–7 µm wide, colorless, walls thickening to 0.5–1 µm; skeletal hyphae to 4 µm wide, thick-walled with wall 0.8–2 µm thick; lamellae edge with abundant projecting filamentous hyphae. Pileus context consisting densely packed with many parallel to sub-radial hyphae 4.5–6.5 µm wide, thick walled 1.5–1.8 µm thick, mostly as skeletal hyphae. Pileus surface made o loosely arranged exclusive skeletal hyphae 2–4.5 µm wide, thick-walled (0.5–)0.8–1.5 µm thick, with anticlinal/interwoven direction, hyaline. Clamp connections abundant and conspicuous in all examined tissues (Fig. 5).

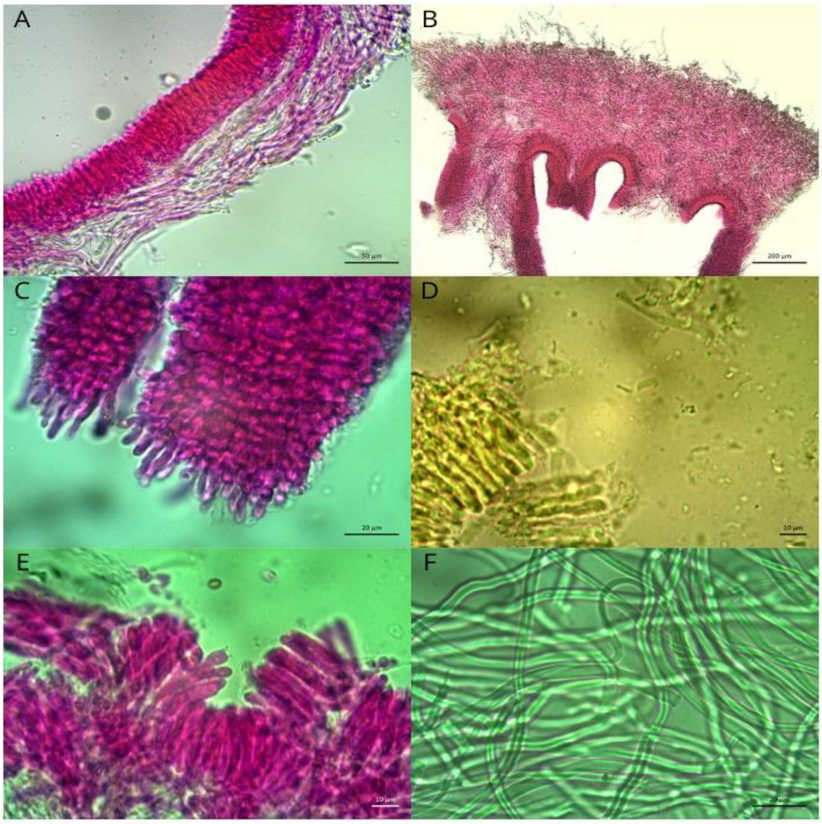

Fig.5. A-F Microscopic characteristics of Brazilian specimens of *Schizophyllum commune* in the cave. A. Hymenium and lamella trama. B. Transversal section. C. Terminal hyphoid elements of the lamella edge. D. Basidiospore under Melzer's reagent. E. Basidium e Basidioles. F. Elements of the lanose pileus surface.

Habitat: gregarious on branch of indeterminate tree in an lighless cave zone.

Known distribution: widespread in all continents (Bhunjun et al. 2024).

Remarks: new collection of *S. comune* is recognized in the field by its small, pure white basidiomes, sometimes stipitate and radiating from the same punctual lamellae. Microscopically, the narrow basidiospores measuring 5.5–7.1 × 2.5–3 µm, with Qm = 2.35, and the presence of skeletal hyphae are consistent with the species concept. However, *S. comune* exhibits high phenotypic plasticity, displaying a high morphology distinction into this species

(Marian *et al.* 2024). Other enigmatic species also share somewhat similar morphology, but the lack of preserved basidiomata impedes a better comparison.

Schizophyllum radiatum Fr. was described as having a thin, coriaceous, suborbicular pileus, sessile or with a short stipe, and flabelliform, widely lobed or digitated, sessile, substipitate, or stipitate by the elongation of the pileus margin, with colors ranging from white, white-gray, or brownish (Fries 1855). Differently from Carreño-Ruiz et al. (2019), our phylogeny shows that they are not different species based on our ITS phylogenetic analysis.

Schizophyllum variabile, a rare taxon from Ak-Rabat (Turkmenistan) and growing on branches of *Juniperus* '*kokanica*', also present a well developed eccentric stipe on which the lamellae radiata and pilose pileus surface, that might represent a phenetically close species. This entity has some features that can be useful to segregate the species from *S. comune*, as follow: (1) the brown hymenophore; (2) the frequent young basidiomata growing from the pileal margin of older ones; and (3) although the size of the basidiospores is not mentioned, we can infer from the drawings that they are distinctly wider, with est. Q = 1.58–1.75 (–2.00), and est. Qm = 1.73, n = 5 (Sorokīn 1890).

Schizophyllum album from South Brazil is similar in the lanose pileus and stipe, but very different in many aspects: (1) the basidiomata grow on soil, (2) the stipe is centrally attached and is radicant and yellowish downward; and (3) the lamellae are decurrent with united (= junctus; joined/junction/juncture, connected/united together, Cash 1965, Stern 1983) margin, varioulsy anastomosed, 'with internal cavity' (Rick 1919). It is uncertain that this fungus represents a member of *Schizophyllum*, but very probably a lentinoid fungus.

Schizophyllum palmatum from Japan was also described as withe (but also ashen) dry basidiomata with 'suborbicular to flabelliform with a lobate to deeply palmately cleft margin' pileus. However, primarily differs in the sessile or subsessile to laterally substipitate, and much smaller basidiospores 3.2-4.0 × 1.5-2.0 µm (Cooke 1961: 592-593).

Schizophyllum umbrinum Berk (1851), material from Cuba analyzed by Linder (1933), has small, thick, and deeply lobed basidiomes, either subsessile or laterally stipitate, with a rough stipe, if present, bearing white hairs, and found solitary or gregarious on dead wood. It is easily distinguished from other species by its brown color. *Schizophyllum leprieurii*, described by Linder (1933) from French Guiana, might corresponds to a synonym of *S. umbrinun* in terms of color, basidiome size, context structure, and phylogeny.

Schizophyllum fasciatum Pat. and *S. mexicanum* are synonyms and were described as having a thin pileus, sessile to short stipitate, densely hairy, with alternating light neutral gray and dark light or dark brown zones (Patouillard 1887). It is found on dead wood and/or parasitizing sugarcane.

NOTES

This new collection is unique within *S. commune* due to the whitish coloration of its basidiomes. In general, white to hyaline organisms, especially arthropods, are often considered adaptations for living in subterranean environments (Sket 2008). However, regarding the observed fungus, there is limited information to conclude whether the white basidiomes are related to a troglomorphic adaptation of these specimens. Further molecular studies of the collected specimens are needed to define their phylogenetic position within the genus.

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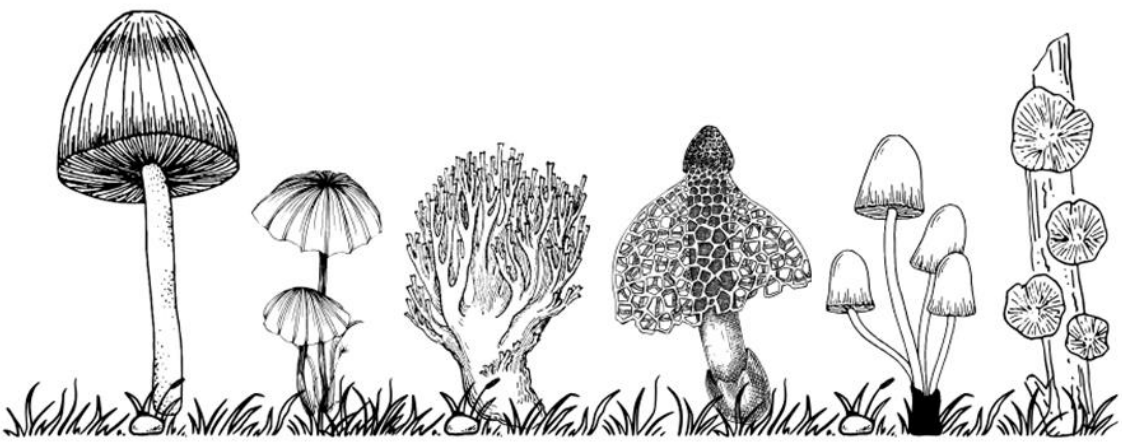
ARTIGO III

FACTORS DETERMINING THE RICHNESS AND COMPOSITION OF MACROFUNGI IN A NEOTROPICAL KARST IN SOUTH AMERICA: ENVIRONMENTAL FILTERS INFLUENCING THE SELECTION OF MACROFUNGI IN CAVES AND SURROUDINGS



ARTIGO III

Este manuscrito foi elaborado em sua versão preliminar, seguindo as diretrizes estabelecidas pela revista *Fungal Ecology*.

Factors determining the richness and composition of macrofungi in a neotropical karst in South America: environmental filters influencing the selection of macrofungi in caves and surroudings?

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Abstract

Macrofungi are fundamental components of tropical ecosystems, playing a crucial role in nutrient cycling. However, there is limited information regarding their presence and diversity in karst areas and caves in Brazil, and studies addressing these organisms on a global scale are scarce. This study assessed the richness and composition of macrofungi from the Basidiomycota and Ascomycota phyla, as well as the environmental factors influencing their presence in a significant karst region in Minas Gerais, Brazil. The results indicated that the richness and composition of macrofungi are not directly related to the two environments (caves and their respective forests) but are primarily influenced by the type of substrate in which the fungi develop. Despite environmental differences, karst systems provide favorable conditions for macrofungal growth. Additionally, caves, despite being oligotrophic environments, act as ecological filters, selecting specific macrofungal species capable of establishing in these habitats.

Keywords: Macrofungal community, caves, Karst. Basidiomycota, Ascomycota.

INTRODUCTION

Karst areas are exceptional environments, distinguished by geological landscapes shaped through the dissolution of rocks such as limestone and dolomite. This process gives rise to unique features, including cave systems, sinkholes, and subterranean rivers (Melo et al., 2013). Each karst feature is characterized by distinct environmental attributes, such as variations in substrate availability and type, as well as climatic conditions. In regions like southeastern Brazil, vegetation cover forms a mosaic of landscapes, comprising both forested and open areas. Dense forests typically dominate the base of the rocky formations, while a drier, more open secondary vegetation thrives on the rocky surfaces (Melo et al., 2013).

Cave environments (hypogean) exhibit distinct characteristics when compared to surface environments (epigean). Key differences include the permanent absence of light in areas far from cave entrances, greater climatic stability marked by constant temperatures, and consistently high humidity levels. The lack of sunlight prevents the development of photoautotrophic organisms, resulting in significantly reduced primary productivity within these ecosystems. In some rare instances, this limited productivity is supported by chemoautotrophic organisms (Barton, 2006; Culver & Pipan, 2019; Mammola, 2019). Therefore, these systems face severe constraints on the availability of organic resources (Mammola, 2019). Cave communities predominantly consist of generalist detritivorous species, relying heavily on organic matter of allochthonous origin. These external resources are crucial for sustaining the biodiversity integral to subterranean ecosystems (Ferreira et al., 2000).

These unique characteristics create microhabitats that sustain a restricted and highly specialized biodiversity, specifically adapted to extreme conditions. Such environments facilitate the formation of distinct ecological niches and act as environmental filters, favoring species with specialized traits that allow them to colonize and thrive in these settings (Howarth, 1983; Poulson & White, 1969). Although much of the research on subterranean biodiversity has centered on animals, other groups also inhabit these ecosystems and face similar environmental challenges and constraints. For example, Bebber & Chaloner (2022) demonstrated that fungi can independently adapt to climatic, edaphic, and biotic conditions and resources. However, evidence from saprotrophic fungi suggests a potential correlation between specialization and climatic variations.

Macrofungi, distinguished by their production of visible sporocarps, play pivotal roles in forest ecosystems. As decomposers, they break down organic matter and recycle essential nutrients back into the soil, maintaining its fertility (Pradhan et al., 2013). Additionally, macrofungi significantly influence forest dynamics by forming symbiotic associations with the roots of 80% to 90% of trees in tropical, temperate, and boreal forests, thereby fostering ecological balance (Arnolds, 1992; Niego et al., 2023). Their contribution to nutrient cycling is vital for ensuring the availability of resources necessary for plant growth. This process enhances forest health and productivity, which, in turn, supports higher species diversity (Zotti et al., 2013).

Macrofungi are widely distributed geographically, demonstrating remarkable tolerance to diverse environmental conditions and thriving across a range of ecosystems. Their exceptional diversity, particularly in tropical forests, can be attributed to the evolution of adaptive strategies such as spore production, which enhances their dispersal and ability to colonize new habitats, ensuring their long-term evolutionary success (Richards et al., 2017).

In South America, biomes like the Amazon and Atlantic Forest stand out as hotspots for soil fungal diversity. In contrast, regions with extreme conditions, such as deserts and polar areas, exhibit significantly lower fungal diversity due to the stringent limitations imposed by their arid or cold environments (Niskanen et al., 2023).

According to Nováková (2009), fungi are commonly found in subterranean environments, associated with a variety of substrates such as sediments, bat guano, decomposing organic matter, speleothems, and even as spores present in air and water. A comprehensive review on the global distribution of major fungal species in caves identified a total of 1,029 species, with the phyla Ascomycota and Basidiomycota (macrofungi) being the most dominant (Vanderwolf et al., 2013). In these ecosystems, fungi play critical roles as decomposers or parasites, releasing nutrients that can be utilized by other organisms. Together with bacteria, fungi form a primary food source for protozoa and invertebrates in cave ecosystems (Cubbon, 1976).

Despite their ecological importance and sensitivity to environmental changes, the diversity and ecological roles of fungi in karst areas remain poorly understood. This study seeks to address this gap by analyzing the richness and composition of macrofungi in two distinct environments, caves and their adjacent forested areas. Additionally, it investigates how environmental factors influence their occurrence, aiming to establish connections between these interconnected habitats.

MATERIALS AND METHODS

Study area

The study was carried out in the karst region of Pains, situated in the central-western part of Minas Gerais state, Brazil. This area is characterized by Neoproterozoic limestones and dolomites from the Bambuí Group (Piló & Cruz, 2022). The region features predominantly subterranean drainage systems, including sinkholes, resurgences, canyons, dolines, and other residual formations typical of karst landscapes (Timo & Travassos, 2023). With more than 2,600 caves, it currently holds the distinction of having the highest concentration of caves in South America (CECAV, 2021).

The region lies within the Cerrado biome, extending into the innermost boundaries of the Atlantic Forest (IBGE, 2004). The local climate is classified as "Cwa" under the Köppen system, denoting a subtropical climate with dry, mild winters and hot, humid summers (Alvares et al., 2013).

The research was conducted in a series of carbonate outcrops within an area known as Angá dos Negros. Four caves were sampled, chosen for their entrances, which facilitate the deposition of organic matter via gravity and surface runoff. These caves are dry and exhibit varying degrees of light exposure across different zones (Figure 1).

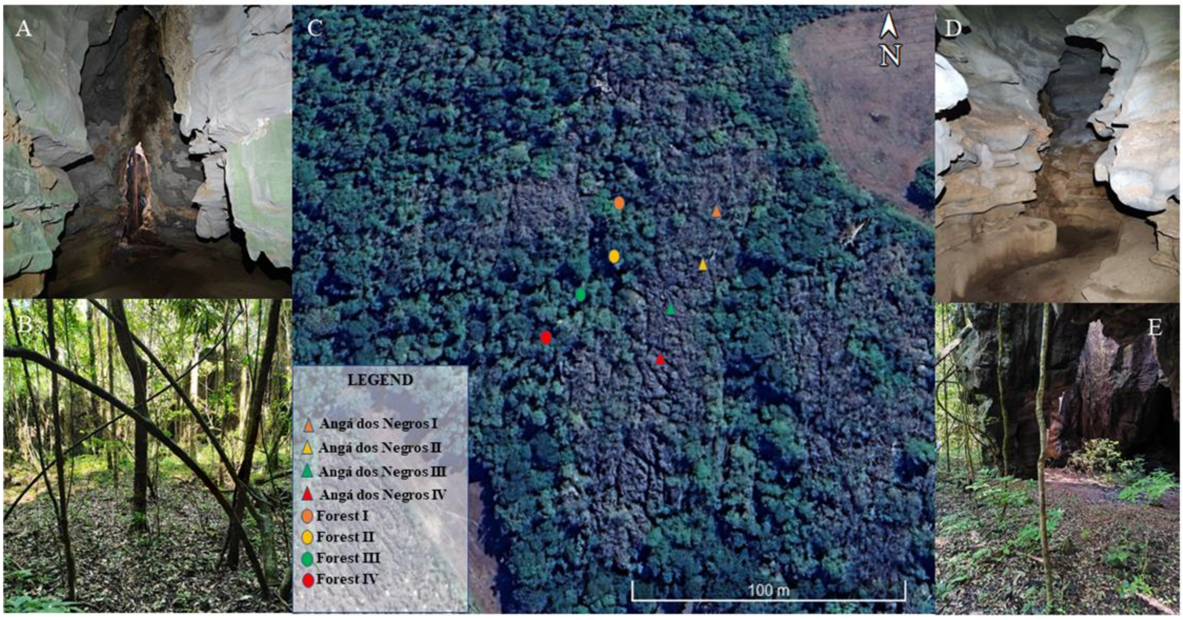

Figure 1: Pains karst area: forest area I (A), Angá dos Negros II cave (B), entrance to Angá dos Negros I cave (C), Angá dos Negros III cave (D), location of collection points (E).

Sampling design

Collections were carried out during two field campaigns in February and April 2024. Sampling was conducted in four caves (Angá dos Negros I, II, III, and IV) and their respective forested areas adjacent to the cave entrances (Table 1).

Table 1: Geographical coordinates and vegetation of the investigation sites.

COLLECTION LOCATIONS	LONGITUDE	LATITUDE	LITHOLOGY/VEGETATION
Angá dos negros cave I	45°39'38.04"W	20°26'9.40"S	Limestone
Angá dos negros cave II	45°39'37.63"W	20°26'10.57"S	Limestone
Angá dos negros cave III	45°39'37.65"W	20°26'12.56"S	Limestone
Angá dos negros cave IV	45°39'38.03"W	20°26'12.92"S	Limestone
Forest area I	45°39'39.71"W	20°26'9.26"S	Semideciduous seasonal forest
Forest area II	45°39'38.40"W	20°26'10.69"S	Semideciduous seasonal forest
Forest area III	45°39'38.84"W	20°26'12.53"S	Semideciduous seasonal forest
Forest area IV	45°39'38.71"W	20°26'13.19"S	Semideciduous seasonal forest

For this study, both basidiocarps and ascocarps were collected. Transects of 100 meters were established within each cave and their corresponding forested areas. Sampling was performed using 1 m² quadrants, which served as sampling units. These quadrants were arranged in triads, with a minimum spacing of 5 meters between them, spanning from the deepest part of the cave to the more distant epigean regions beyond the entrance (Figure 2).

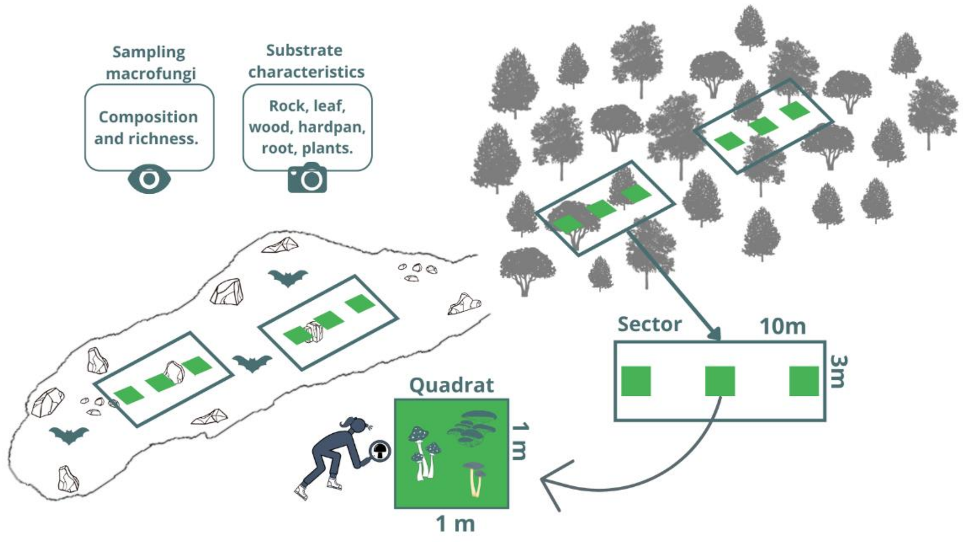

Figure 2: Infographic showing sampling methodology for biotic and abiotic data in the Pains karst area, using quadrants and transects as standardized sampling areas. (Adapted from Souza-Silva et al., 2021)

Data collection and identification

Each quadrant was thoroughly inspected, and basidiocarps and ascocarps larger than 0.1 cm in diameter were carefully collected using a cutting tool. Samples were taken from various substrates, including soil, leaves, branches, roots, and wood. The collected specimens were photographed in situ using a digital camera and a metric scale to document features such as coloration and size, which may change after collection. Specimens were then placed in paper bags, transported for dehydration, and subsequently deposited in the ESAL Herbarium at the Federal University of Lavras.

Macrofungi were identified to the lowest possible taxonomic level and categorized into morphotypes. Field observations focused on macroscopic characteristics, including the color, shape, surface, and margin of the pileus; the color, density, and features of the lamellae; and the size, surface, and color of the stipe. In the laboratory, microscopic traits were analyzed using a ZEISS stereomicroscope.

Environmental variables

The 240 sampling units were georeferenced using GPS, with measurements of relative humidity and temperature recorded. These data were collected using a digital thermo-hygrometer positioned near the ground and left in place for at least five minutes to ensure accurate readings. Rainfall data for the municipality of Pains were obtained from the INPE website.

In the forested areas, light entry was estimated by assessing canopy openness. Photographs were taken approximately 1.30 meters above the ground, always in the early morning to avoid direct sunlight, which could interfere with the computational analysis. The images used to determine canopy openness were processed using the GLAMA software (Gap Light Analysis Mobile Application, Tichý, 2014). In the caves, three distinct light zones were identified: photic, characterized by direct sunlight; disphotic, characterized by indirect sunlight; and aphotic, with a total absence of sunlight.

The organic and inorganic substrates within each quadrant were characterized using photographs taken at chest height, with the camera positioned parallel to the ground. In the laboratory, the percentage composition of each substrate type was analyzed using Image J software.

Data analysis

To evaluate species richness across different zones (epigean, photic, disphotic, and aphotic), we conducted an ANOVA followed by pairwise Tukey tests. To explore the similarities and differences in macrofungi composition between cave environments and adjacent epigean areas, we used Non-Metric Multidimensional Scaling (nMDS) plots and performed an Analysis of Similarity (ANOSIM) to assess the significance of groupings formed by caves and forests. The nMDS plots visually represented group differences, while ANOSIM statistically validated these differences. All analyses were performed using the vegdist function from the "vegan" package in R, with the plots generated using the "ggplot2" package.

To investigate the effects of environmental factors (substrate composition) on species richness in caves and their surrounding epigean environments, we employed Generalized Linear Mixed Models (GLMMs) based on variables quantified within each sampling quadrant. Poisson distributions were used as the response variable in the GLMMs, with the sampling environment included as a random effect (Zuur et al., 2009). Variable selection was carried out using the dredge function from the MUMIN package, testing all possible combinations of variables in the full model, including the null model. The final model was constructed by averaging all models with a delta < 2.

To assess the influence of environmental variables on the composition of fungal communities in caves, we conducted distance-based redundancy analysis (dbRDA) using the dbrda function from the "vegan" package (Legendre & Anderson, 1999; Oksanen et al., 2013). Bray-Curtis distances were employed for species composition, and Gower distances were used for trait composition (Legendre & Anderson, 1999). dbRDA, as a constrained ordination method, provides ecologically meaningful insights into community structure. Multicollinearity among predictor variables was evaluated using Variance Inflation Factors (VIFs), with VIF > 10 indicating high collinearity (O'Brien, 2007). The overall significance of the final model was assessed using an analysis of variance (ANOVA).

RESULTS

Richness and composition of macrofungi

A total of 1,098 macrofungi were sampled across 240 quadrants, encompassing four caves and their adjacent epigean areas. The specimens belonged to two phyla: Basidiomycota, represented by 967 basidiocarps distributed across 104 morphospecies, 39 genera, and 26 families; and Ascomycota, represented by 131 ascomata distributed across 7 morphospecies, 3 genera, and 3 families (Table 2; Figures 3 , 4).

Table 2: Taxonomic diversity and distribution of macrofungi in the karst area of Pains, located in the central-west region of Minas Gerais, Brazil. The study includes four caves and their respective forest areas: Angá dos Negros I (AN1), Angá dos Negros I (AN2), Angá dos Negros III (AN3), Angá dos Negros IV (AN4), Forest I (FT1), Forest II (FT2), Forest III (FT3), and Forest IV (FT4).

PHYLUM/FAMILY/TÁXONS	AN1	AN2	AN3	AN4	FT1	FT2	FT3	FT4
BASIDIOMYCOTA								
AGARICACEAE								
Agaricus sp.						+		+
Basidio imaturo		+			+		+	
Cystolepiota sp.						+		
Calvatia sp.								+
Clorophylum sp.						+		
Entoloma sp.1					+			
Entoloma sp.2	+				+			
Entoloma luteosplendidum						+		
Lepiota sp.1					+			+
Lepiota sp.2						+		+
Lepiota sp.3					+			
Lepiota cristata	+							
Leucoagaricus sp.1					+		+	
Leucoagaricus sp.2					+		+	+
Leucoagaricus sp.3		+			+	+	+	+
Leucoagaricus sp.4					+			
Leucoagaricus sp.5					+	+		
Leucocoprinus sp.1							+	
Leucocoprinus sp.2						+		
Leucocoprinus sp.3								+
Leucocoprinus cf. *brunneoluteus*					+			
Leucocoprinus cretaceus								+
Macrolepiota sp.					+		+	+
AMANITACEAE								
Zhuliangomyces sp.							+	
CREPIDOTACEAE								
Crepidotus sp.						+		
Simocybe sp.					+			

PHYLUM/FAMILY/TÁXONS	AN1	AN2	AN3	AN4	FT1	FT2	FT3	FT4
GANODERMATACEAE								
Ganoderma sp.								+
GEASTRACEAE								
Geastrum saccatum					+	+		
HYGROPHORACEAE								
Cotylidia sp.					+			
Hygrocybe sp.1								+
Hygrocybe sp.2							+	
HYMENOGASTRACEAE								
Phaecollybia sp.					+			
INOCYBACEAE								
Inocybe sp.						+		
LENTARIACEAE								
Lentaria cf.								
MARASMIACEAE								
Marasmius cf. *capillaris*					+	+		
Marasmius haematocephalus		+			+	+	+	+
Marasmius cf. *siccus*								+
Marasmius sp.1					+	+	+	
Marasmius sp.2						+	+	
Marasmius sp.3							+	
Marasmius sp.4		+				+		
Marasmius sp.5								+
Marasmius sp.6								+
Tetrapyrgos sp.								+
MERULIACEAE								
Cymatoderma sp.						+		
MYCENACEAE								
Hemimycena sp.1		+	+	+		+	+	
Hemimycena sp.2		+					+	
Hemimycena sp.3			+				+	
Hemimycena sp.4			+					
Mycena sp.1				+			+	
Mycena sp.2							+	+
Mycena sp.3								+
OMPHALOTACEAE								
Collybiopsis sp.1					+		+	
Collybiopsis sp.2								+

PHYLUM/FAMILY/TÁXONS	AN1	AN2	AN3	AN4	FT1	FT2	FT3	FT4
Collybiopsis sp.3								+
Collybiopsis sp.4								+
Gymnopus sp.1								+
Gymnopus sp.2							+	
Gymnopus sp.3								+
Gymnopus sp.4								+
POLYPORACEAE								
Coriolopsis cf. *caperata*								+
Daedaleopsis sp.								+
Favolus sp.1					+			
Favolus sp.2		+						
Favolus sp.3							+	
Lentinus sp.1								+
Lentinus sp.2							+	
Lentinus cf. *crinitus*			+				+	
Poroid sp.1						+		
Poroid sp.2							+	
Poroid sp.3					+			
Poroid sp.4							+	
Poroid sp.5								+
Poroid sp.6					+			
Poroid sp.7						+		
Poroid sp.8					+			
Poroid sp.9								+
PHALLACEAE								
Phallus indusiatus					+			
PHYSALACRIACEA								
Oudemansiella steffenii					+			
PLEUROTACEAE								
Pleurotus sp.					+			
PLUTEACEAE								
Pluteus sp.1							+	
Pluteus sp.2							+	
PSATHYRELACEAE								
Coprinellus sp.					+			
Psathyrella sp.1					+			+
Psathyrella sp.2		+		+	+			
Psathyrella sp.3						+		

PHYLUM/FAMILY/TÁXONS	AN1	AN2	AN3	AN4	FT1	FT2	FT3	FT4
Psathyrella sp.4						+		
Psathyrella sp.5		+				+	+	+
Psathyrella sp.6								+
PTERULACEAE								
Pterullicium cf. sp.	+							
Pterulla sp.						+		+
POROTHELEACEAE								
Gerronema cf. *strombodes*							+	
SCHIZOPHYLLACEAE								
Schizophyllum commune					+			
SCLERODERMATACEAE								
Scleroderma sp.1						+		
STROPHORIACEAE								
Deconica sp.					+			
Gymnopilus sp.1						+		
Gymnopilus sp.2						+		
TRICHOLOMATACEAE								
Lepista sp.							+	
ASCOMYCOTA								
SARCOSCYPHACEAE								
Phillipsia crispata	+							
XYLARIACEAE								
Entonaema sp.	+							
Xylaria cf. *ianthinovelutina*			+		+	+	+	
Xylaria cf. *longipes*	+						+	
Xylaria cf. *polimorpha*					+			
Xylaria sp.1				+				
Xylaria sp.2	+	+						

Figure 3: Some species found in forest areas in the karst region of Pains, Brazil: *Cotylidia* sp. (A), *Leucoagaricus* sp.5 (B), *Lepista* sp. (C), *Lentaria* cf. sp. (D) *Gerronema* cf. *strombodes* (E), *Xylaria* cf. *longipes* (F), *Oudemansiella steffenii* (G), *Leucoagagricus* sp.2 (H), *Psathyrella* sp.2 (I), *Leucocoprinus* cf. *brunneoluteus* (J), *Geastrum saccatum* (K), *Higrocybe* sp.2 (L), *Marasmius haematocephalus* (M), *Marasmius siccus* (N), *Lentinus* sp.1 (O), *Calvatia* sp. (P), *Leucoagaricus lilaceus* (Q), *Coriolopsis* cf. *caperata* (R), *Marasmius* sp.5 (S), *Scleroderma* sp.2 (T), *Pterula* sp. (U), *Collybiopsis* sp.3 (V) *Agaricus* sp. (W), *Phallus indusiatus* (X).

Figure 4: Some species found in the Angar dos Negros I, II, III and IV caves in the Pains karst region, Brazil: *Pterulicium* cf. sp. (A), *Entonaema liquescens* (B), *Phillipsia crispata* (C), *Hemimycena* sp.2 (D), *Hemimycena* sp.3 (E), *Xylaria* sp.1 (F), *Entoloma* sp.2 (G), *Psathyrella* sp.5 (H), *Lentinus* cf *crinitus*, (I), *Lepiota* cf. *cristata* (J), *Xylaria* cf. *ianthinovelutina* (K).

Species richness was considerably lower in caves (6.5 ± 2.6) compared to the surrounding epigean environments (31.5 ± 2.6) (Figure 4).

Significant differences in taxonomic diversity were observed between the two habitats. In caves, the most prevalent families were Mycenaceae (Basidiomycota), represented by 6 species, and Xylariaceae (Ascomycota), represented by 5 species (Figure 5). In the forested areas, the most diverse families were Agaricaceae (22 species), Polyporaceae (16 species), Marasmiaceae (10 species), and Omphalotaceae (8 species).

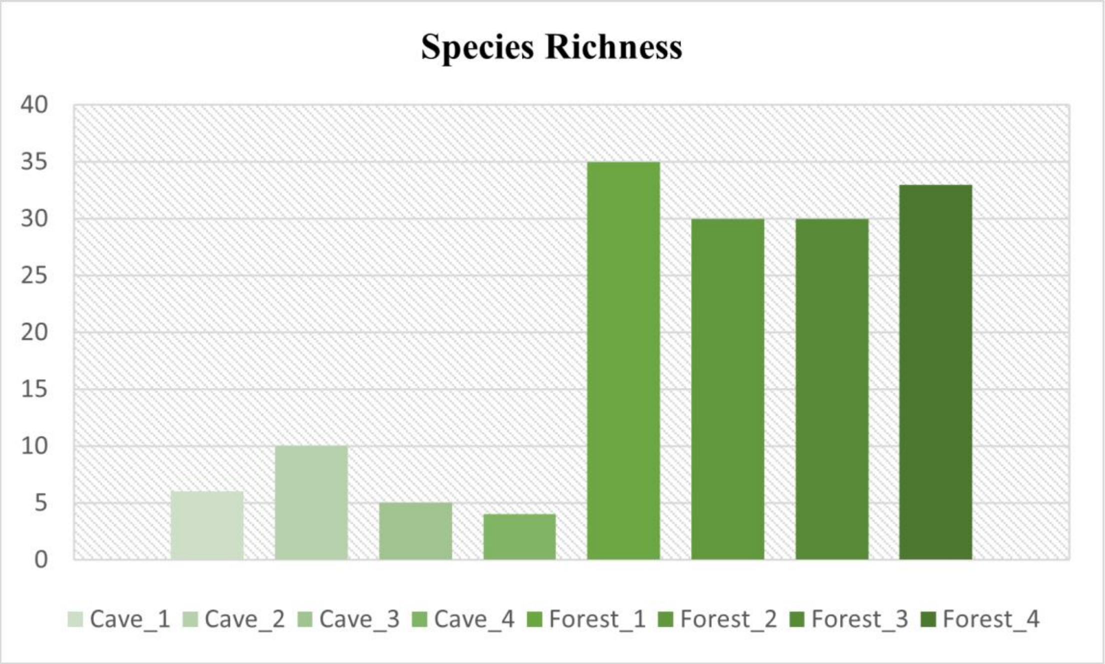

Figure 4: Richness of macrofungi sampled in two distinct areas, caves and forest, in the karst region of Pains, Minas Gerais municipality.

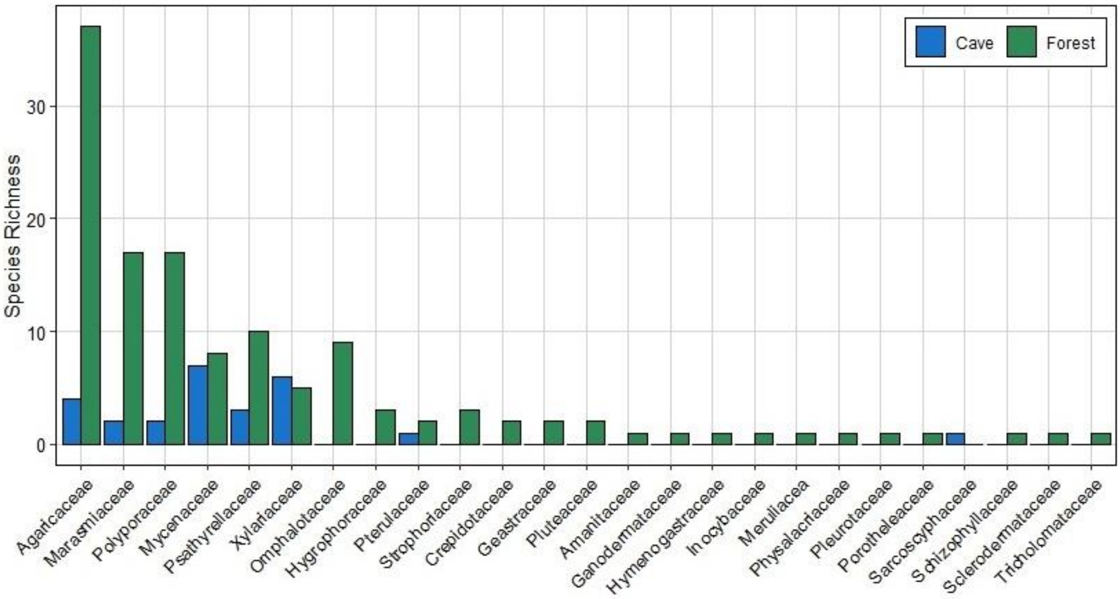

Figure 5: Richness of macrofungal families in caves and forests.

Additionally, seven families were shared between both environments (epigean and hypogean): Agaricaceae, Marasmiaceae, Polyporaceae, Mycenaceae, Psathyrellaceae, Xylariaceae, Pterulaceae and Porotheleaceae. Among these, Agaricaceae emerged as the most species-rich family in both habitats, though it was significantly more abundant in the forested areas. In caves, species richness was more limited, with many families represented by only one or two species.

Epigean zones, characterized by forested areas with direct light exposure, were compared to the different cave illumination zones (photic, disphotic, and aphotic). Notably, canopy openness values in the epigean areas did not significantly influence the analyses (Figure 6).

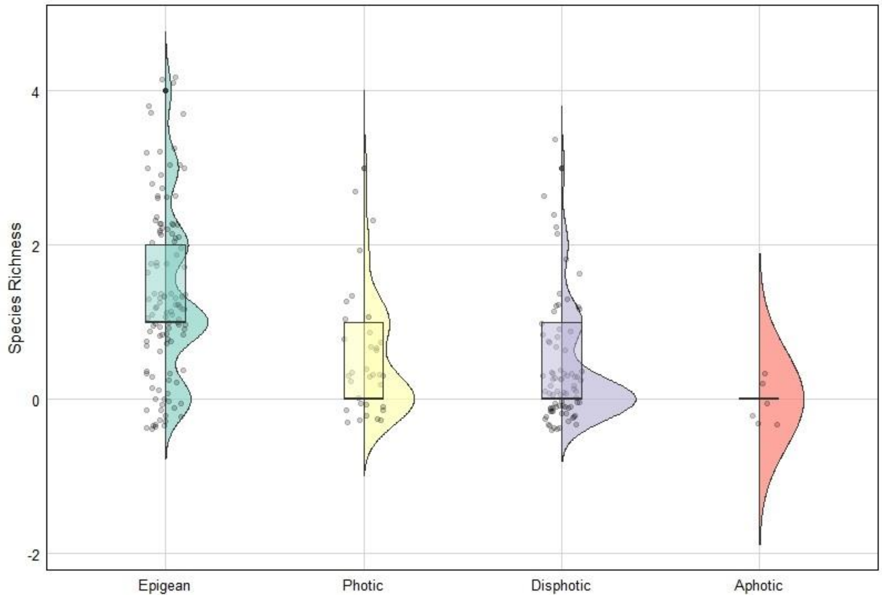

Figure 6: Species richness in different light zones: Forest areas (epigean) with constant light and caves with different levels of light incidence (photic, dysphotic and aphotic).

The mean species richness in quadrants located in epigean zones was 1.36 ± 1.11, compared to the photic zones of the caves, which had a mean richness of 0.51 ± 0.75. In the disphotic zones, the mean richness was 0.39 ± 0.71, while no species were observed in the aphotic zones. Species richness was significantly higher in epigean zones compared to all cave zones ($p < 0.05$). However, no significant differences in richness were detected between the photic and disphotic zones within the caves.

Influence of environmental variables in richness and composition

When analyzing the MDS plots, a distinct differentiation in the composition of macrofungi species was evident between the four sampled caves and the forest environments. ANOSIM analysis confirmed a statistically significant difference in fungal composition between these two habitat types ($R = 0.4115$; $p = 0.022$).

Forested areas (surrounding epigean zones) clustered together, indicating a more similar species composition among these sites (Figure 7).

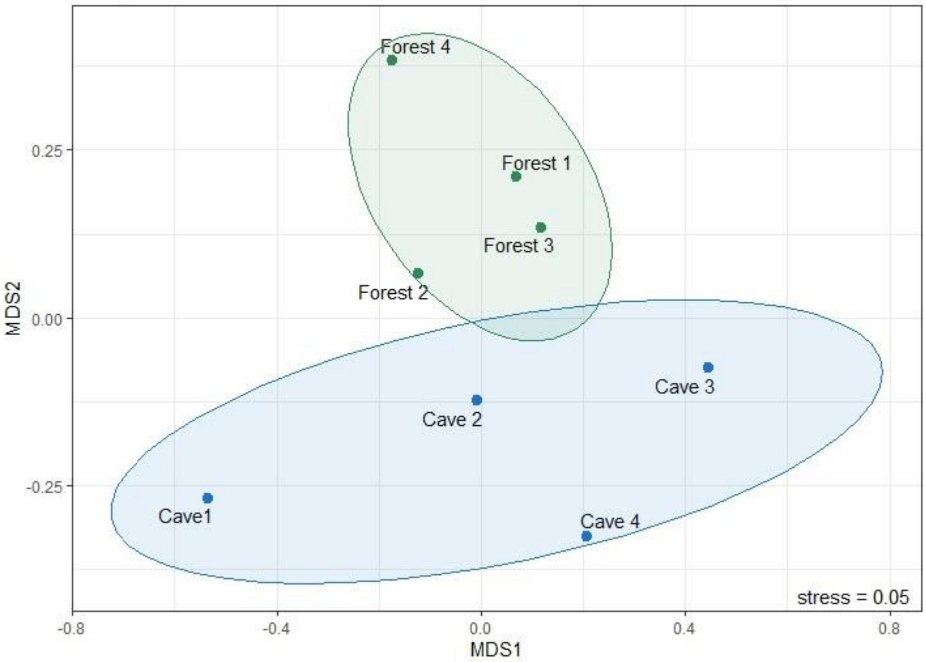

Figure 7: NMDS analysis based on the Jaccard index showing the dissimilarity in macrofungal species composition between four forest areas (green points) and four caves (blue points).

Conversely, the caves formed a separate, distinct cluster, highlighting a lower degree of similarity in species composition within the caves but a clear divergence from the forested areas.

The GLMM, which accounted for the total fungal species richness in the studied environments and the substrates quantified in each sampling unit, explained approximately 37% of the total variance (Figure 8). The model identified significant positive effects of wood and root substrates on species richness, suggesting that their presence enhances macrofungal diversity. In contrast, "hardpan/soil" had a significant negative impact on species richness, indicating that these substrates reduce fungal diversity. The variables rock, MOV (moist organic vegetation), and MOM (moist organic matter) did not significantly influence species richness (GLMER; $z = 7.85$, $p < 0.001$, $R^2 = 0.48$).

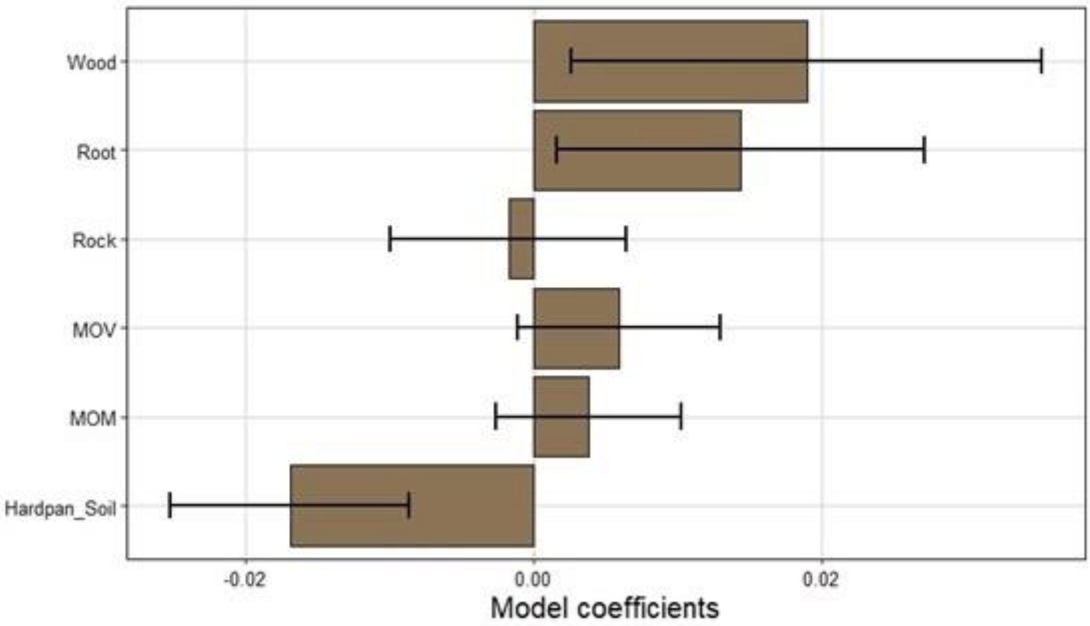

Figure 8: Model coefficients showing the influence of different environmental variables on the species richness of macrofungi in forest and cave environments. The variables tested were: wood, roots, rock, living organic matter (MOV), detritus (MOM), hardpan/soil.

The dbRDA analysis of the influence of substrates on macrofungal communities in the subterranean environment revealed that only the first axis was significant, explaining approximately 19% of the variation. In the final model, the variable MOV was the only factor significantly associated with species composition, showing a stronger link to epigean regions. A distinct separation between hypogean and epigean groups was clearly observed (Figure 9).

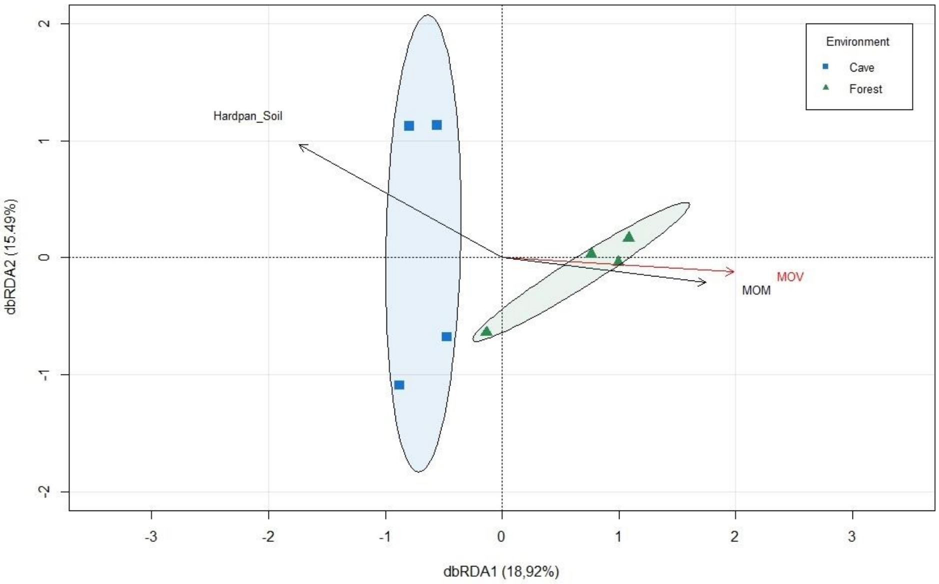

Figure 9: The graph presents a distance-based redundancy analysis (dbRDA) assessing the effects of substrates on macrofungal communities in caves and forest areas.

DISCUSSION

Research on the ecology of macrofungi in forested areas and caves presents significant challenges due to the limited number of available studies and the wide variety of habitats these organisms occupy. The complexity of these environments, coupled with unique conditions such as light availability, humidity, and substrate composition, complicates the establishment of standardized methodologies for data collection and analysis.

Despite these challenges, this study underscores the impressive diversity of macrofungi species in the karst region of Pains. With just two sampling campaigns conducted in a relatively small area, a substantial number of specimens were collected, comparable to results from other studies involving more extensive sampling efforts across larger regions. Notably, species richness was higher in forested areas compared to caves.

This higher richness may be attributed to the edge effect created by the forest's proximity to adjacent agricultural fields, which likely promotes increased spore dispersal. Furthermore, the soil in the region (characterized by elevated moisture levels and nutrient richness resulting from the dissolution of carbonate rocks) combined with the diverse plant life in these fertile soils, likely provides an ideal habitat for macrofungi development.

Variations in sampling methods, including the number of transects, sampling duration, and criteria used for recording macrofungi, can significantly impact the results obtained. Studies with a larger number of transects typically provide a more comprehensive representation of macrofungal communities, while those with limited spatial coverage may underestimate local diversity. Additionally, the duration of sampling plays a critical role: shorter sampling periods may fail to account for seasonal variations or the rapid decomposition of fruiting bodies, especially in dynamic environments like forests and caves.

These methodological inconsistencies hinder comparisons between studies and may result in divergent interpretations of macrofungal richness, composition, and ecological interactions. To overcome these challenges, it is crucial to standardize sampling protocols and thoroughly document collection conditions. This would facilitate a more accurate understanding of macrofungal ecology across diverse environmental contexts.

Research on the inventory and ecology of macrofungi in Minas Gerais remains sparse. Moreover, several studies lack robust and well-defined sampling methodologies. This issue of inconsistent standardization is not confined to Minas Gerais; similar shortcomings are observed nationally. For instance, Pires et al. (2014) conducted monthly collections over one year, whereas Souza & Aguiar (2004) carried out collections over seven months. Nascimento et al.

(2021) did not specify the duration of their sampling, which was limited to the rainy season. While all these studies used trails within forest fragments as sampling areas, they did so without adhering to specific methodological frameworks. Such a lack of standardization may have influenced the recorded species richness, as collections were neither systematic nor guided by targeted criteria.

Conversely, several studies, including those by Braga-Neto et al. (2000), Albuquerque et al. (2004), Silva et al. (2015), Pagin-Claudio et al. (2022), Gibertoni (2008), Komura et al. (2017), Patrício et al. (2021), Antunes (2020), Alves (2020), Lima et al. (2018), Bononi et al. (2008), Cavalcante et al. (2021), and Couceiro & Couceiro (2022), employed standardized sampling methodologies. These approaches involved techniques such as transects, quadrants, plots, specific distances, seasonal analyses, regular sampling intervals, and other well-defined criteria.

Similarly, studies conducted outside Brazil have also adopted standardized methodologies for macrofungi sampling, as seen in the works of Amade (2020), O'Hanlon & Harrington (2012), Andrew et al. (2013), Tuo et al. (2022), Gates et al. (2011), Pradhan et al. (2013), Marzana et al. (2018), Baptista et al. (2010), Jaroszewicz et al. (2021), Angelini et al. (2015), Tibuhwa et al. (2011), and Carvalho (2021).

It has been observed that studies using well-defined methodologies consistently report higher species richness and diversity. Thoughtfully designed techniques, such as employing transects, plots, or quadrants in combination with clear criteria for seasonality, sampling frequency, and spatial coverage, ensure greater representativeness of the data. Conversely, non-standardized methodologies run the risk of underestimating or overestimating species richness, undermining the accuracy of results and complicating cross-study comparisons.

Among studies conducted in Brazil, Rosa & Capelari (2009) identified 109 species of macrofungi in a forest fragment in Minas Gerais without employing a defined sampling methodology. In contrast, Patrício et al. (2020) recorded 24 species in the Palmari Natural Reserve, Amazonas, utilizing unspecified transects in open, high-humidity areas. Compared to these studies, our research, which employed 120 quadrants in a forested area, recorded a higher species richness.

Internationally, Pradhan et al. (2013) documented 120 species in Indian forests using 96 quadrants, with Polyporaceae and Marasmiaceae being the most representative families, also observed in our study area. Similarly, Angelini et al. (2015) in Italy collected 943 individuals and 133 species over a year, prominently featuring the *Mycena* genus, which aligns with findings from other studies. These comparisons highlight that, despite methodological variations, the most representative families and genera consistently appear across different regions, reflecting global patterns in macrofungal diversity.

Richness and composition of Macrofungi in karst areas and caves

This is the first study in Brazil to report the occurrence of macrofungi in a karst environment. However, similar studies have been conducted in other countries, documenting certain genera through both microbial cultures in caves and the observation of fruiting bodies in hypogean environments. Vargas & Restrepo (2020) explored macrofungal biodiversity in a karst system in El Peñon, located in the Colombian Andes. This region features an extensive oak forest, where macrofungi from the phyla Basidiomycota and Ascomycota were recorded.

Several families identified in the forests of the karst region of Pains were also reported in the karst system of El Peñon, Colombia (Vargas & Restrepo, 2020). Notable among these are Agaricaceae, Amanitaceae, Marasmiaceae, and Pleurotaceae, all belonging to the phylum Basidiomycota, as well as Xylariaceae from the phylum Ascomycota. A remarkable example is the species *Xylaria ianthinovelutina*, which was recorded in both the forests and caves of Pains as well as in El Peñon. Furthermore, genera such as *Pleurotus* , *Agaricus* , and *Calvatia* . were common to both studies, highlighting the similarity of macrofungi present in these karst environments despite geographical and ecological differences.

A review study on the global distribution of major fungal species in caves documented 1,029 species, with the phyla Ascomycota and Basidiomycota (macrofungi) being the most representative (Vanderwolf et al., 2013). The study emphasized families such as Polyporaceae, Psathyrellaceae, and Mycenaceae, all within the phylum Basidiomycota and classified as macrofungi. Conducted through cultivation and culture methods, the study revealed a significant occurrence and abundance of these families in some sampled caves. Additionally, these fungi are classified as saprotrophic, particularly associated with the decomposition of wood and leaves.

A study by Nieves & Riviera (2009) documented guanophilic fungal species isolated from samples collected in three caves in Puerto Rico. Among these, several species belonging to the phylum Basidiomycota were identified, including the genera *Lepiota* and *Marasmius*, which were also recorded in the Angá dos Negros I cave in our study. Additionally, species from the genus *Xylaria* (Ascomycota) were observed, with *Xylaria* emerging as the most frequently encountered genus in the caves examined in the karst region of Pains.

Influence of environmental variables on macrofungal richness and composition

Substrates rich in organic matter, such as wood and roots, were the most influential factors driving species richness in both caves and forested areas. In contrast, less favorable or nutrient-poor substrates, such as compacted soil, negatively impacted macrofungal diversity. These findings underscore the pivotal role of substrate quality and availability in shaping macrofungal communities within these environments.

A pronounced dissimilarity in species composition was observed across the different sampled areas. The four forested sites exhibited greater similarity to one another, likely due to the predominance of similar substrate types in these areas. This suggests that variations in substrate types distinctly influence macrofungal communities in caves and forests. In caves, compacted soil (hardpan) had a significant negative effect on species richness, whereas organic substrates, such as moist organic vegetation (MOV) and moist organic matter (MOM), strongly supported macrofungal communities in forested regions.

In forested areas, dead organic matter (MOM), commonly referred to as litter, was identified as the most significant substrate influencing species composition, while "wood" was analyzed separately as an independent substrate.

Previous studies, such as Payton's (1993), have suggested that topography plays a critical role in shaping species diversity within forest ecosystems. In this study, the sampling sites, situated on a karst plateau with elevations ranging from 720 to 723 meters, featured a distinctive landscape of large limestone massifs and specialized vegetation zones (Coelho & Calux, 2022). These geological features likely provide favorable habitats for various macrofungal groups.

Antunes' (2020) study in the Serra da Mantiqueira similarly reported high richness and diversity of mushrooms. While altitude did not directly affect species richness, temporal variation between sampling periods had a significant impact on diversity. Altitude influenced community composition, with species turnover observed along the altitudinal gradient. Furthermore, environmental factors such as soil moisture, relative humidity, and higher temperatures were associated with greater species richness, with higher values of these variables supporting increased diversity.

In the forested areas of our study, we also noted interactions between macrofungi and insects, particularly dipterans and members of the Staphylinidae family (Coleoptera), which directly contribute to ecosystem services (Figure 10). These interactions can have both beneficial and harmful effects. According to Niego et al. (2021) and Biedermann & Vega (2020), some macrofungi and mycophagous insects engage in mutualistic symbioses, wherein insects obtain nutrition in exchange for spore dispersal and protection. Research indicates a strong correlation between fungal community composition and the diversity of mycophagous insects in forest ecosystems.

Additionally, various species of ants, termites, and beetles cultivate macrofungi in fungal gardens, which serve as a critical food source for these insects (Epps & Arnold, 2018; Yamashita & Hijii, 2007).

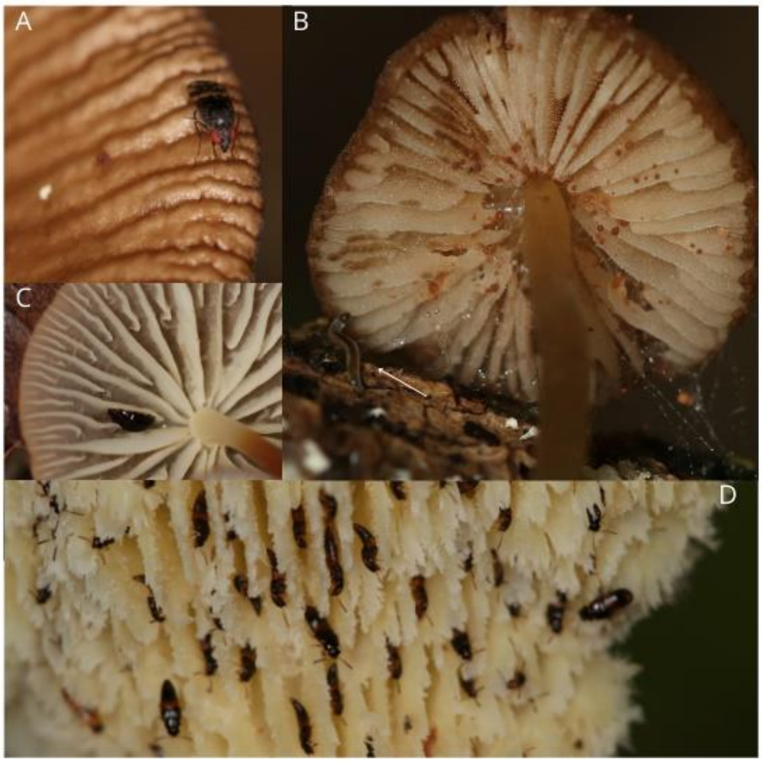

Figure 10: Interactions between macrofungi and insects. Diptera (A), Insect larva (B), Coleoptera (C), Staphylinidae (Coleoptera) (D).

CONCLUSION

This study highlights the importance of karst areas as critical habitats for macrofungi, essential components of forest ecosystems. These organisms play vital roles in ecological processes, and the species richness observed in forested areas reflects the diversity of available substrates. However, macrofungal communities exhibited significant dissimilarity between cave and forest environments, with lower species richness in caves likely due to the predominance of compacted soil (hardpan) and limited organic matter availability.

Most abiotic variables showed no significant influence on species richness in either environment (epigean or hypogean), which may be attributed to the methodology used. Variables such as temperature, humidity, and canopy openness (light availability) were measured during a single instance per quadrant. Considering the relatively rapid fruiting cycles of macrofungi, continuous monitoring over an extended period would likely provide more robust insights into how these factors influence macrofungal presence.

A key issue explored in this study is the role of caves as environmental filters. The findings indicated low species similarity between caves and their surrounding forests, supporting the notion that substrate type is the primary factor determining the presence of macrofungi. Nonetheless, macrofungi can be transported into subterranean environments via allochthonous organic matter and may thrive even with limited resources. According to the Traditional Niche Assembly Theory, species composition is shaped by environmental characteristics that facilitate species establishment in specific locations (Matos et al., 2013). Thus, in addition to the environmental factors analyzed in this study, other unexamined influences may play a role in shaping macrofungal community composition in forests and caves, contributing to the observed low morphospecies similarity between these habitats.

Some of the differences in species composition patterns may be attributed to the dispersal abilities of individuals (Hubbell, 2001; 2006). This underscores the need for future research to incorporate a broader range of environmental variables to identify the niche factors driving the observed low similarity.

Finally, this study emphasizes the importance of investigating biodiversity-rich regions that are increasingly threatened by human activities such as intensive mining, livestock farming, and agriculture. The karst region of Pains, with its remarkable speleological heritage, urgently requires targeted conservation efforts that rely on scientific research to guide preservation initiatives.

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TERCEIRA PARTE

CONSIDERAÇÕES FINAIS

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Esta dissertação destaca a importância dos estudos de biodiversidade em ambientes cársticos, com ênfase em macrofungos e mixomicetos, grupos ainda não explorados nas cavernas brasileiras. Embora muitas cavernas estejam localizadas em Unidades de Conservação, que oferecem proteção, o Brasil possui um vasto patrimônio espeleológico ainda a ser descoberto. A legislação ambiental ainda carece de critérios específicos para classificar a relevância espeleológica, especialmente no que se refere à micobiota, que não é mencionada nos critérios legais.

Os resultados deste trabalho ressaltam o potencial desses ecossistemas para abrigar uma diversidade única, destacando a necessidade de expandir o conhecimento sobre suas dinâmicas ecológicas e funções nos ambientes subterrâneos. As cavernas tropicais analisadas demonstraram uma rica diversidade de macrofungos e mixomicetos, incluindo espécies ainda não registradas em outras cavernas do mundo. No entanto, são necessários estudos mais profundos para compreender o papel desses organismos na dinâmica ecológica desses ecossistemas. Estudos anteriores focaram apenas em grupos taxonômicos específicos, como fungos e leveduras, limitando-se a amostras restritas. Este é o primeiro estudo sobre macrofungos em cavernas tropicais que apresenta dados sobre sua diversidade, incluindo adaptações morfológicas e a influência de variáveis ambientais nos ambientes subterrâneos.