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Original Article

Molecular characterization of fungal species in bats from Brazil's Atlantic Forest

Caracterização molecular de espécies fúngicas em morcegos da Mata Atlântica brasileira

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Abstract

Molecular identification of fungal species associated with insectivorous bats provides critical insights into their role as reservoirs of fungi, including potential pathogens. This is the first study to performed molecular identification of fungal species in insectivorous bats captured from the Atlantic Forest of southern Brazil. *Aspergillus sydowii* and *Penicillium crustosum* were frequently isolated from *Myotis* sp., with *P. crustosum* also dominating samples from *Molossus molossus*, alongside *Aspergillus subalbidus*. *Cladosporium* spp. were prevalent in *Eptesicus diminutus*, while *Sarocladium* was uniquely associated with *Tryroptera tricolor*. Notably, several identified species are known pathogens of humans, animals, and plants. Fungal species in bats remain poorly understood, representing a significant gap in the field of fungal ecology. This gap is particularly notable in Brazilian biomes, where research on this topic is still scarce. Efforts should focus on minimizing habitat loss for bats and mitigating forest disturbances to reduce their interactions with humans, crops, and other animals. Such measures are essential to prevent the dispersal of fungal pathogen spores and safeguard ecological and public health.

Keywords: insectivorous bats, Santa Catarina, mammals Chiroptera, fungi.

Resumo

A identificação molecular de espécies de fungos associadas a morcegos insetívoros fornece informações críticas sobre o seu papel como reservatórios de fungos, incluindo potenciais patógenos. Este é o primeiro estudo a realizar a identificação molecular de espécies fúngicas em morcegos insetívoros capturados na Mata Atlântica do sul do Brasil. *Aspergillus sydowii* e *Penicillium crustosum* foram frequentemente isolados de *Myotis* sp., com *P. crustosum* também presente em amostras obtidas de *Molossus molossus*, juntamente com *Aspergillus subalbidus*. *Cladosporium* spp. foram predominantes em *Eptesicus diminutus*, enquanto *Sarocladium* foi associado exclusivamente com *Tryroptera tricolor*. Notavelmente, várias espécies identificadas são conhecidos patógenos de humanos, animais e plantas. As espécies de fungos em morcegos permanecem pouco compreendidas, representando uma lacuna significativa no campo da ecologia fúngica. Essa lacuna é particularmente notável nos biomas brasileiros, onde as pesquisas sobre o tema ainda são escassas. Os esforços devem centrar-se na minimização da perda de habitat dos morcegos e na mitigação das perturbações florestais para reduzir as suas interações com seres humanos, culturas e outros animais. Tais medidas são essenciais para prevenir a dispersão de esporos de fungos patógenos e salvaguardar a saúde pública e ecológica.

Palavras-chave: morcegos insetívoros, Santa Catarina, mamíferos Chiroptera, fungos.

1. Introduction

Brazil hosts approximately 15% of the world's bat diversity, with 186 species, 68 genera, and nine families (Garbino et al., 2024; Upham, 2025). Bats (Chiroptera) exhibit remarkable ecological and behavioral diversity, characteristics that make them important hosts and reservoirs in the transmission cycles of various potentially

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zoonotic pathogens. In Brazil, zoonotic agents such as rabies virus, *Bartonella* spp., *Histoplasma capsulatum*, and *Leishmania* spp. have already been detected in bat populations (Castelo-Branco et al., 2023). Although various microorganisms have been isolated from bats, studies specifically investigating their association with fungal species remain relatively scarce. Some research has reported the presence of fungal genera such as *Aspergillus*, *Penicillium*, and *Cladosporium* in bats (Shapiro et al., 2015; Furtado et al., 2021, 2024; Ludwig et al., 2021; Pereira et al., 2022), but information on fungal species with opportunistic pathogenic potential remains limited, particularly in Brazilian biomes.

Bats have limited exposure to sunlight and typically inhabit thermally stable, moist environments, which provide an ideal habitat for fungal growth, thereby enhancing their capacity to harbor and spread fungal pathogens to humans, animals and plants (Liu et al., 2024). This may occur because bats constantly transition between caves, forests, croplands, and human settlements, and diverse fungal pathogens can be transferred between these sites (Karunarathna et al., 2023). Emerging fungal pathogens are rapidly becoming a major threat to wildlife (Cheng et al., 2021). Moreover, some fungal species can act as human pathogens that cause infections in immunocompromised hosts (Chiu et al., 2005; Shabankarehfard et al., 2017) or as plant pathogens, leading to crop loss (Vico et al., 2014; Duduk et al., 2020). Bats can act as vectors for pathogenic fungi, a role that should not be underestimated. Understanding how they function as potential vectors is crucial for mitigating the associated risks. However, gaining this understanding will require further research into the influence of species-specific traits, demographic factors, seasonal variations, and landscape structure (Liu et al., 2024).

Understanding the fungal species present in bats is crucial for several reasons: a) to support the conservation of bat populations, as some fungi may have pathogenic potential for these animals and others; b) to assess the health risks posed to humans, given that many bats roost in human structures; and c) to evaluate the possible exposure of bats to plant pathogens they may inadvertently transport, particularly in fragmented forest habitats and expanding agricultural areas.

Despite their ecological importance, the presence of fungal species, including pathogenic fungi, in bats remain poorly understood, particularly in Brazilian biomes, where research is limited. Recent studies suggest that the bat skin mycobiome is highly dynamic, influenced by geography and bat species (Kearns et al., 2023). However, little is known about the potential for bats to harbor pathogenic fungi that could pose risks to animals, plants, and human health. Thus, the present study aims to conduct the first molecular analysis of fungal species isolated from the rostral region of insectivorous bats captured in the tropical Atlantic Forest of southern Brazil, identifying potential fungal pathogens and exploring their ecological and health-related implications.

2. Materials and Methods

2.1. Bats capture and ethics

This study was carried out in the São Francisco Reserve (28°38'51" S, 49°36'32" W), situated in the municipality of Nova Veneza, a subtropical Atlantic Forest area in southern Santa Catarina. The São Francisco Reserve is a mountainous massif with vegetation of the Atlantic Forest and is dominated by Dense Ombrophilous Forests. More than 1,500 ha belongs to the Reserve area, and it is connected to the Aguaí State Biological Reserve (Reserva São Francisco, 2024).

Sampling was conducted between April and September 2022, with two to three consecutive nights of sampling each month. Bats were captured using 10 mist nets (two 12 × 3 m; four 9 × 3 m; and four 6 × 3 m), which were installed in animal transit areas such as vegetation, trails, abandoned roads, forest edges, near water bodies, and within banana plantations. The nets were opened at dusk and remained active for six hours. Nets were checked every 20 minutes to minimize stress on captured individuals. After capture, bats were placed in cloth bags and transported to the field station, where taxonomic identification, biometric measurements, and fungal sample collection were performed. Field sampling was carried out under a license from SISBIO (53718-1) and was approved by the Committee of Ethics for Use of Animals in Experiments from Universidade do Extremo Sul Catarinense (CEUA - 26/2022). Biosafety protocols followed those recommended by the Brazilian Society for the Study of Chiropterans (Moratelli et al., 2020).

2.2. Fungi isolation

A sample of the rostral region of each bat was obtained using a sterile swab moistened with 0.9% sterile saline solution. The swab was lightly spread on the surface of the bat rostral region and added to a tube containing sterile saline solution (Shapiro et al., 2015; Furtado et al., 2021). One tube was used per bat, ensuring that samples were processed individually. The test tubes were placed in a thermal box with ice and refrigerated until they were transported to the laboratory.

Each tube containing the swab was vigorously agitated and an aliquot of each sample was spread on the surface of PDA containing chloramphenicol with Drigalski spreader using the spread-plate method. The PDA medium was incubated at 27 °C ± 2 °C in the dark for seven days. Filamentous fungal colonies were counted (Furtado et al., 2021).

Fungal colonies have been previously identified based on their macromorphological characteristics. The morphologically distinct colonies were subcultured on PDA culture medium at the same time and temperature mentioned above for genera identification, totaling 16 isolates strains. These were sub-cultured on malt extract agar (MEA), 25% glycerol nitrate agar (GN25), and Czapek yeast extract agar (CYA) media and incubated at 25 °C for seven days. Microculture techniques were used for fungal identification. The fungi were grown between three to five days at a temperature of 25 °C and then examined

under a light microscope (100× and 400× magnifications). Morphological identification of the fungi was performed according to the available taxonomic keys and guides (Raper and Fennell, 1965; Pitt, 1981; Varga et al., 2007; Pitt and Hocking, 2009).

2.3. Molecular identification

Aspergillus, *Penicillium*, and *Mucor* isolates were selected for identification at the species level based on previous macro-and micromorphological identifications. Eighteen isolates were grown in Malt Extract Agar (MEA) medium at 25 °C for 5 days, and genomic DNA was extracted using the DN*easy* Plant Mini Kit (QIAGEN, Hilden, Germany), according to the manufacturer's protocol. The quality and quantity of the materials were evaluated using a BioDrop UV-Vis spectrophotometer (BioDrop Ltd., Cambridge, UK). The primer sets used are described in Table 1, PCR conditions for amplification of the loci internal transcribed spacer (ITS), beta-tubulin (BenA), and calmodulin (CAM) were followed according to their respective protocols (White et al., 1990, Glass and Donaldson, 1995; Hong et al., 2006).

The amplicons were purified using the ExoSap-IT enzyme (USB Corporation, Cleveland, Ohio, USA) and sent to the Central Laboratory of High-Performance Technologies in Life Sciences (LaCTAD – UNICAMP) for sequencing. The sequences were assembled and edited using *Geneious*®6.0.6 software (Biomatters, Auckland, New Zealand), and polymorphisms and mutations were confirmed by chromatogram analysis. For *Cladosporium* and *Sarocladium* isolates, adequate sequences for identification were not obtained.

2.4. Phylogenetic analysis

Phylogenetic analysis was conducted using the PAUP 4.0b10 program, based on *maximum likelihood* analysis. For the identification of *Aspergillus* species, the combined ITS + BenA datasets were used for the identification of *Penicillium* species, the combined ITA + CAM was used for the identification of *Mucor* species, the ITS locus was used. Reference sequences were obtained from the National Center for Biotechnology Information (NCBI) database. Multiple sequence alignments were performed using ClustalW (Geneious Plugin). All of the sequences generated in this study were deposited in GenBank (NCBI), and the accession numbers are shown in Tables S1 to S7.

Clade stability was verified using *bootstrap* of 1000 replicates (Swofford, 2002). Appropriate out-groups were selected for each phylogenetic analysis. The trees were visualized using the FigTree v. 1.4 program (Rambaut, 2013).

2.5. Data analysis

For the relative frequency, the following formula was used: sample number, reported as the fungal type, divided by the total number of samples (bat), and multiplied by 100 to obtain a percentage. The relative abundance of each fungus in the sample was calculated using the colony number as an abundance metric and multiplied by 100 to obtain the percentage.

3. Results

A total of 21 bats belonging to four insectivorous *taxa* were captured from an Atlantic Forest remnant in the southern region of Santa Catarina (Brazil): *Myotis* sp. (Kaup, 1829) (N=7, 1 male and 6 females), *Molossus molossus* (Pallas, 1766) (N=7, 2 males and 5 females), *Eptesicus diminutus* (Osgood, 1915) (N=6, 2 males and 4 females), and *Tryroptera* tricolor (Spix, 1823) (N=1, male) (Table 2). Notably, all bats in the study hosted fungi, resulting in a prevalence of 100%. The fungi with the largest number of colonies in each taxon were selected for molecular identification.

Of the 16 fungal isolates cultured, five genera were identified in the rostral region of the bat species: *Aspergillus* (N=7), *Penicillium* (N=5), *Cladosporium* (n=3), *Mucor* (N=2), and *Sarocladium* (N=1). Adequate sequences for identification were obtained from 14 of them and were identified to the species level. To confirm the morphological identification, sequencing was performed on the *Aspergillus*, *Penicillium* and *Mucor* isolates. For the *Cladosporium* and *Sarocladium* isolates, adequate sequences for identification were not possible.

The most frequent and abundant fungal species isolates were *Aspergillus sydowii* (section *Versicolores*) and *Penicillium crustosum* (section *Fasciculata*), shown in Figures 1 and 2, respectively. Strains FML 241 and FML 244, both obtained from *Myotis sp*., were identified as *A. sydowii* based on phylogenetic analysis using combined ITS and BenA loci, with bootstrap support values greater than 70% (Figure 1). *Aspergillus amoenus* (FML 242) and *A. austroafricanus* (FML 243), also from *Myotis* sp. (section

Table 1. Primers used for amplification and sequencing.

Gene	Primer name	Primer sequence (5'3')	References
β-tubulin *(BenA)*	*BT2A*	GGTAACCAAATCGGTGCTGCTTTC	Glass and Donaldson (1995)
	BT2B	ACCCTCAGTGTAGTGACCCTTGGC	
Internal Transcribed Spacer *(ITS)*	*ITS 1*	TCCGTAGGTGAACCTGCGG	White et al. (1990)
	ITS 4	TCCTCCGCTTATTGATATGC	
Calmodulin *(CaM)*	*CMD5*	CCGAGTACAAGGAGGCCTTC	Hong et al. (2006)
	CMD6	CCGATAGAGGTCATAACGTGG	

Table 2. Fungi isolate identification obtained of the bats species captured from an Atlantic Forest remnant, Southern region of Santa Catarina, Brazil.

Bats	Fungi					
Taxa*	**Isolates****	**Species identification**		**Nº. colonies*****	**RA(%)[a]**	**RF(%)[b]**
Myotis sp. (N=7)	*Aspergillus* sp. FML 241	*Aspergillus* section Versicolores	*A. sydowii*	80	43	29
				120		
	Aspergillus sp. FML 242		*A. amoenus*	20	4	14
	Aspergillus sp. FML 243		*A. austroafricanus*	30	6	14
	Aspergillus sp. FML 244		*A. sydowii*	10	2	14
	Penicillium sp. FML 256	*Penicillium* section Fasciculata	*P. crustosum*	140	45	29
				70		
Molossus molossus Pallas, 1766 (N=7)	*Mucor* sp. FML 252	*Mucor* group *racemosus*	*M lusitanicus*	30	14	4
	Mucor sp. FML 253	*Mucor* group *hiemalis*	*M. merdicola*	30	14	4
	Aspergillus sp. FML 245	*Aspergillus* section *Candidi*	*A. candidus*	30	14	4
	Aspergillus sp. FML 246		*A. subalbidus*	220	14	27
	Penicillium sp. FML 248	*Penicillium* section Fasciculata	*P. crustosum*	450	29	56
	Penicillium sp. FML 250		*P. crustosum*	10		
	Penicillium sp. FML 249	*Penicillium* section *Cinnamopurpureum*	*P. incoloratum*	40	14	5
Eptesicus diminutus Osgood, 1915 (N=6)	*Cladosporium* isolates	*Cladosporium* isolates[c]	*Cladosporium* isolates	20	67	71
				10		
				10		
				10		
	Penicillium sp. FML 251	*Penicillium* section *Citrina*	*P. cairnsense*	10	17	14
	Aspergillus sp. FML 247	*Aspergillus* section Candidi	*A. subalbidus*	10	17	14
Thyroptera tricolor Spix, 1823 (N=1)	*Sarocladium* isolate	*Sarocladium* isolate[c]	*Sarocladium* isolate	30	100	100

Note: [a]relative abundance; [b]relative frequency; [c]Adequate sequences for identification were not obtained. *Taxa: Prevalence of fungal isolates was 100% across all bat species. **Isolates: Sixteen fungal genera were identified. ***Nº colonies: Fungi obtained (nasal region) with the largest number of colonies in each taxon was taken for molecular identification.

Versicolores), were grouped with FML 241 and FML 244 in the same phylogenetic tree and therefore were included in the same figure.

Figure 2 shows that isolates FML 256, FML 248, and FML 250 belong to *Penicillium* section *Fasciculata*. The phylogeny based on ITS and CAM loci combined, showed that isolate FML 256, obtained from Myotis sp. was *Penicillium crustosum*. Isolates FML 248 and FML 250, obtained from *M. molossus*, grouped together, but with no bootstrap support (< 70%); nevertheless, these two isolates were clustered within the *P. crustosum* lineage, and with high bootstrap support.

Additional isolates were identified through phylogenetic analyses presented in the Supplementary Material (Figures S1-S5). These include *Aspergillus candidus* (FML 245) and A. subalbidus (FML 246, 247) from *M. molossus* and *Eptesicus diminutus*, respectively (section Candidi); as well as *Penicillium incoloratum* (FML 249, section Cinnamopurpureum) and *P. cairnsense* (FML 251, section Citrina). Additionally, two isolates obtained from M. molossus (FML 252 and FML 253) were identified as *Mucor lusitanicus* and *Mucor merdicola*, respectively, based on phylogenetic inference using the ITS locus.

When considering the higher relative frequency (RF) and abundance (RA) of the fungal species, *A. sydowii* (section Versicolores) had RF and RA values of 29% and 43%, and *P. crustosum* (section Fasciculata) had RF and RA values of 29% and 45%. These were both obtained from the Myotis sp. (Table 2). *P. crustosum* was the species with higher RF (56%) and RA (29%) values obtained from

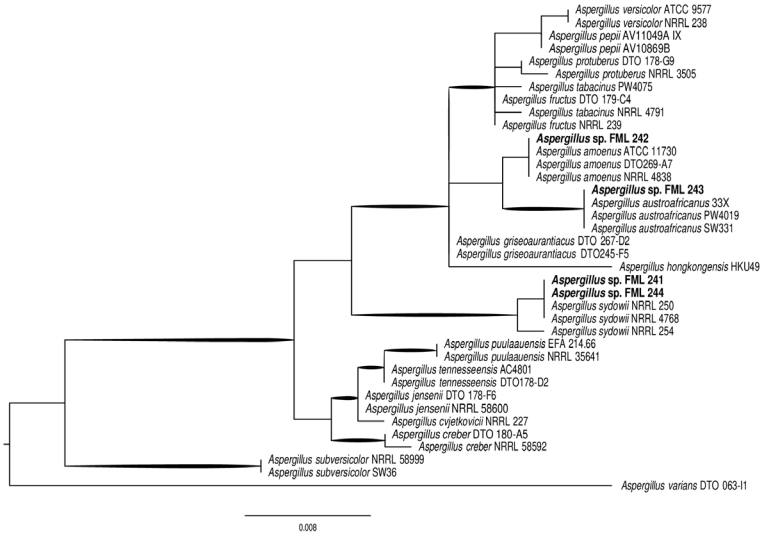

Figure 1. Phylogeny inferred by the ITS and BenA loci combined, data set referring to *Aspergillus* section *Versicolores*. Bootstrap values > 70% are designated as branches in bold in the phylogenetic trees, in which the species *Aspergillus varians* was used as an outgroup.

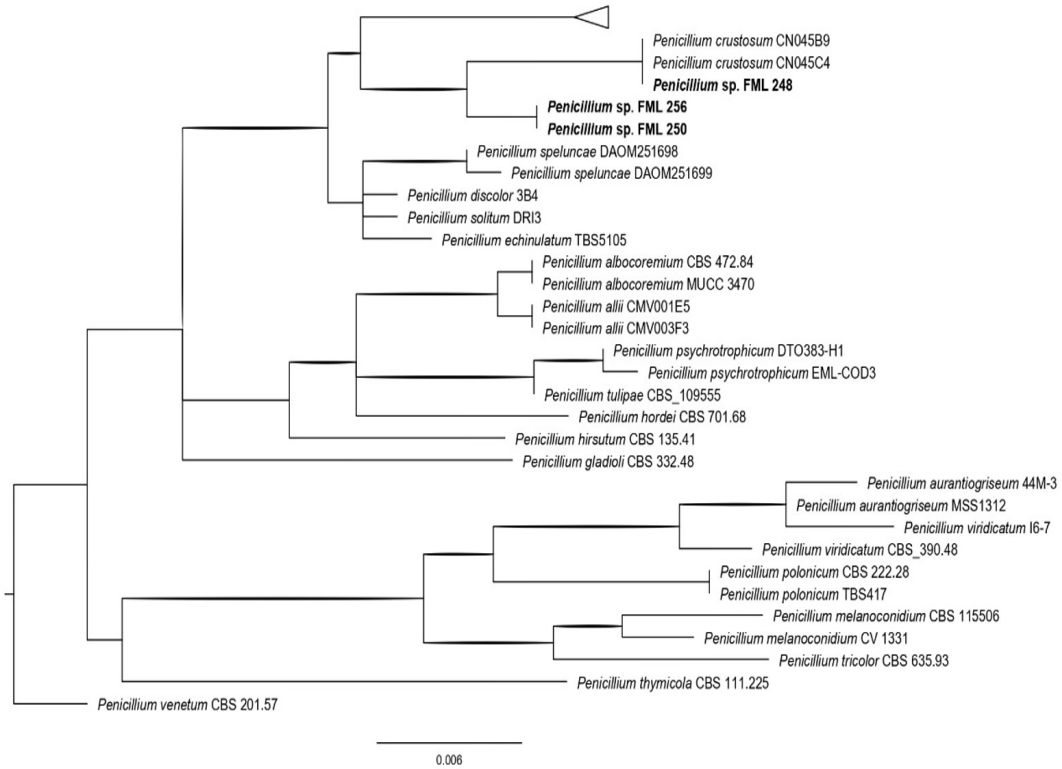

Figure 2. Phylogeny inferred by the ITS and CAM loci combined, data set referring to *Penicillium* section *Fasciculata*. Bootstrap values > 70% are designated as branches in bold in the phylogenetic trees, in which the species *Penicillium venetum* was used as an outgroup.

M. molossus, while *A. subalbidus* (section *Candidi*) had a RF of 27% in *M. molossus*.

Cladosporium isolates had a higher frequency and abundance in *E. diminutus* (RF of 71% and RA of 67%), whereas the *Sarocladium* isolate was the only fungi obtained from *T. tricolor* (RF and RA of 100%).

4. Discussion

The bat *taxa* studied harbored a diverse range of fungal species in their rostral regions, encompassing both resident and transient mycobiota. Bats play a significant role as carriers and dispersers of fungal spores, with variations

influenced by species-specific behaviors and habitats. In our study, the genera *Aspergillus* and *Penicillium* exhibited the greatest diversity among isolated species.

4.1. Aspergillus species and their pathogenic potential

The section *Versicolores* were frequent and abundant in *Myotis* sp., with *A. sydowii* the fungus species most isolated, followed by *A. austroafricanus* and *A. amoenus*. Species from this section are ubiquitous, often isolated from a wide range of substrates, including soil, indoor environments, food, feed, plants, caves, and clinical materials (Pitt and Hocking, 2009; Jurjevic et al., 2012; Zahradnik et al., 2013; Siqueira et al., 2016). Notably, *A. sydowii* has been linked to human onychomycosis and peritonitis (Chiu et al., 2005; Takahata et al., 2008) as well as fungal dermatitis in horses (Lee et al., 2012). Its abundance in *Myotis* sp. highlights its ecological and potentially zoonotic significance, marking the first documentation of this species in the rostral region of insectivorous bats.

In *Molossus molossus*, we isolated species from the section *Candidi*, including *A. subalbidus* and *A. candidus*, which are commonly found in indoor and cave environments, food, and soil (Glässnerová et al., 2022). *Aspergillus candidus* is notable for its involvement in human infections such as invasive aspergillosis (Ribeiro et al., 2005), brain granuloma (Linares, McGarry and Baker, 1971), and onychomycosis (Piraccini et al., 2002; Ahmadi et al., 2012). *Aspergillus candidus* may induce both cellular and humoral responses in animals (Krysinska-Traczyk and Dutkiewicz, 2000). They have also been isolated from birds (Sharma et al., 1971). Recently, *A. candidus* was isolated from the wing surfaces of the bat species *Hipposideros armiger* in the Yunnan province of Kunming, Xishan District, China (Liu et al., 2023). Conversely, *A. subalbidus* has been frequently recovered from bat guano-contaminated soils in Gcwihaba Cave, situated in the Okavango basin, Botswana (Visagie et al., 2021), further suggesting a transient association with bats.

4.2. Penicillium species and mycotoxin production

The genus *Penicillium*, also prominent in our study, has been previously reported in bats (Shapiro et al., 2015; Furtado et al., 2021; Furtado et al., 2024). *P. crustos*um, one of the most frequently isolated species from *Myotis* sp. and *M. molossus*, is a known mycotoxin producer with significant toxic effects on the nervous system (Evans and Gupta, 2007). This species has been isolated from house dust samples collected around the world (Visagie et al., 2014) and recorded in the biomes of the Brazilian Atlantic Forest and the Brazilian Pampa, found mainly in soil samples (Barbosa et al., 2022). It was isolated from the body surface of *Myotis daubentonii* in Flanders, Belgium (Becker et al., 2023) and is also known as a postharvest pathogen of fruit (Vico et al., 2014; Duduk et al., 2020). *Penicillium* genera including sections *Fasciculata* and section *Citrina* were commonly isolated from Canadian bat caves (Visagie et al., 2020).

Other sections, such as *Cinnamopurpureum* and *Citrina*, were exclusively isolated from *M. molossus* and *E. diminutus*, specifically *P. incoloratum* and *P. cairnsense*. These fungi are commonly found in soil and food, raising concerns about their potential mycotoxin production in agricultural areas frequented by bats (Houbraken et al., 2011; Barbosa et al., 2020).

4.3. Transient and opportunistic fungal species in bats

Mucor species, specifically *M. merdicola* and *M. lusitanicus*, have also been found on *M. molossus*. Members of the *Mucor circinelloides* complex, including *M. lusitanicus* are among the *Mucor* species most frequently isolated from clinical sources and are involved in infections (Alvarez et al., 2009; Wagner et al., 2020). Some species have been isolated from bat carcasses in caves (Karunarathna et al., 2020). *Mucor hiemalis* and *M. racemosus*, for example, were the most abundant species obtained from bat carcasses (mainly *Myotis* sp.) found in a cave in southern Italy (Voyron et al., 2011). This species has also been reported in immunocompromised patients with subcutaneous zygomycosis (Desai et al., 2013). It is an opportunistic fungal infection with a high mortality rate that infects a wide range of substrates from bread to human skin tissue (Ribes et al., 2000). Mucorales enter the human host through inhalation, percutaneous inoculation, or ingestion (Ribes et al., 2000) and typically affect immunocompromised patients (Parfrey, 1986; Lee et al., 1999; Prabhu and Patel, 2004). These fungi may be associated with soil and are found transiently in bats. Bats can carry these fungi into urbanized zones, especially *M. lusitanicus*, which can cause diseases in immunocompromised individuals.

The genus *Cladosporium* was most abundant in *E. diminutus*, a species adapted to both natural and urban habitats. *Cladosporium* species are common in soil and air and have been linked to cutaneous phaeohyphomycosis in bats (Souto et al., 2023). Additionally, their association with bat ectoparasites underscores the need for further investigation into the role of these vectors in fungal transmission (Haelewaters et al., 2018). *Cladosporium sphaerospermum* and *Cladosporium cladosporioides* are the most frequently isolated fungi from bat caves in Brazil (Cunha et al., 2020). In Brazil, the *Cladosporium* genera have already been isolated from nasal hair and the rostral region of the molossid bat (Shapiro et al., 2015; Furtado et al., 2021, 2024).

In the present study, *Sarocladium* was the only found in *T. tricolor*. *Sarocladium strictum* is a widespread soil saprotroph that can cause diseases in various plant and fungal hosts (Kuris et al., 2014). This fungus has already been detected in White Stork *Ciconia ciconia* nests and displays strong affinities to the soil environment owing to favorable conditions, such as pH, organic matter content, and air-water conditions (Błońska et al., 2021). *Sarocladium strictum* was isolated from the airborne "Nietoperek" Bat Reserve in Western Poland (Kokurewicz et al., 2016). When considering that *T. tricolor* is usually found inside curled leaves of plants such as *Heliconia*, *Musa*, *Calathea*, and *Phenakospermum*, *Sarocladium* could be brought by insects or dust from the ground that are present in them. However, to the best of our knowledge, no study has demonstrated this species as a possible pathogen of bats or other animals when associated with their mycobiota.

4.4. Future directions and conservation efforts

The diversity of fungal species found in bats from an Atlantic Forest remnant in southern Brazil, as evidenced by this study and our previous work (Furtado et al., 2021, 2024), underscores the complex interactions between bats, their habitats, and their associated mycobiota. In this study, we identified some fungi, such as *A. sydowii* and *P. crustosum*, which are known to have pathogenic potential. These fungi may pose a risk to public health, particularly to immunocompromised individuals, highlighting the importance of monitoring fungal communities associated with wildlife.

Environmental disturbances, such as deforestation, habitat fragmentation, and urban expansion, can alter the dynamics of fungal transmission between bats, their environments, and humans. Bats serve as carriers of both resident and transient fungal species, potentially dispersing these pathogens across natural and anthropized landscapes, which could lead to the introduction of pathogenic fungi into human-occupied areas (Karunarathna et al., 2023).

The conservation of native habitats is, therefore, essential not only for preserving bat biodiversity but also for maintaining ecological balance and minimizing the emergence of fungal-related diseases. It is important to ensure that sufficient intact habitat is available to meet species needs, including foraging and roosting sites. Additionally, effective mitigation strategies should be employed during the development of buildings and bridges to prevent the formation of novel community aggregations (Sutherland et al., 2020), which could facilitate pathogen transmission.

To minimize the risk of pathogen spillover, it is crucial that bats have access to natural habitats that reduce interactions between agricultural and natural areas. Managing bat populations in agricultural landscapes using bat houses could help reduce the need for pesticides, thereby decreasing their environmental impact. These bat houses could be designed to suit the scale of agricultural areas, and incorporating buffer zones with shorter vegetation in forest ecosystems may discourage species that host diverse fungi (Liu et al., 2024). This strategy could also attract bat species that forage in open areas, reducing direct contact with crops.

Our findings emphasize the critical importance of habitat conservation and the need for continued research into the ecological and epidemiological roles of bats in fungal dispersion.

5. Conclusion

Different fungi species were identified depending on the bat species captured. Notably, some species have been recognized as pathogens in humans (*A. sydowii*, *A. candidus*, and *M. lusitanicus*), animals (*A sydowii*), and plants (*P. crustosum*). *A. sydowii* and *P. crustosum* were frequently and abundantly isolated from *Myotis* sp., with *P. crustosum* also prevalent in *M. molossus*. Although these species have been reported for the first time in the rostral region of insectivorous bats, further monitoring of these microorganisms is necessary to better understand the risk of pathogen transmission between bats and other fauna, as well as the potential impact on human health.

It is crucial to implement conservation strategies aimed at protecting the natural habitats of bats, particularly in Brazilian biomes where fungal biodiversity in bats remains underexplored. Habitat loss and forest fragmentation not only threaten biodiversity but also increase the risk of contact between bats, humans, and agricultural crops, facilitating the spread of pathogenic fungal spores.

Therefore, preserving natural habitats and reducing interactions between agricultural zones and surrounding ecosystems is essential. Continued research into the ecological and epidemiological interactions between bats and fungi is important for developing effective public health and biodiversity protection measures.

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Data Availability Statement

The data is available upon request to the corresponding author.

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Supplementary Material

Supplementary material accompanies this paper.

Figure S1. Phylogeny inferred by the ITS and BenA loci combined, data set referring to *Aspergillus* section *Candidi*. Bootstrap values > 70% are designated as branches in bold in the phylogenetic trees, in which the species *Aspergillus yunnanensis* was used as an outgroup.

Figure S2. Phylogeny inferred by the ITS and CAM loci combined, data set referring to *Penicillium* section *Cinnamopurpureum*. Bootstrap values > 70% are designated as branches in bold in the phylogenetic trees, in which the species *Penicillium gravinicasei* was used as an outgroup.

Figure S3. Phylogeny inferred by the ITS and CAM loci combined, data set referring to *Penicillium* section *Citrina*. Bootstrap values > 70% are designated as branches in bold in the phylogenetic trees, in which the species *Penicillium euglacum* was used as an outgroup.

Figure S4. Phylogeny inferred by the ITS locus, data set referring to *Mucor* group *racemosus*. Bootstrap values > 70% are designated as branches in bold in the phylogenetic trees, in which the species *Mucor mousanensis* was used as an outgroup.

Figure S5. Phylogeny inferred by the ITS locus, data set referring to *Mucor* group *hiemalis*. Bootstrap values > 70% are designated as branches in bold in the phylogenetic trees, in which the species *Mucor piriformis* was used as an outgroup.

Table S1. Isolates included in the study and their origins - genus *Aspergillus* section *Versicolores*

Table S2. Isolates included in the study and their accession numbers - genus *Aspergillus* section *Candidi*

Table S3. Isolates included in the study and their accession numbers - genus *Penicillium* section *Fasciculata*

Table S4. Isolates included in the study and their accession numbers - genus *Penicillium* section *Cinnamopurpureum*

Table S5. Isolates included in the study and their accession numbers - genus *Penicillium* section *Citrina*

Table S6. Isolates included in the study and their accession numbers - genus *Mucor* section *Racemosus*

Table S7. Isolates included in the study and their accession numbers - genus *Mucor* section *Hiemalis*

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