

Aspergillus and *Penicillium* from quartzite and ferruginous caves in Minas Gerais

Thiago Oliveira Condé (1), Ana Flávia Leão (1), Fábio Alex Custódio (2) & Olinto Liparini Pereira (2)

(1) Laboratório de Micologia e Etiologia de Doenças Fúngicas, Departamento de Microbiologia, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brasil.

(2) Laboratório de Micologia e Etiologia de Doenças Fúngicas, Departamento de Fitopatologia, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brasil.

Contact: olliveira.thiago@gmail.com and oliparini@ufv.br

Resumo

A ordem Eurotiales compreende gêneros economicamente importantes, como *Aspergillus* e *Penicillium*, amplamente distribuídos em habitats terrestres, incluindo cavernas. O estado de Minas Gerais abriga a maioria das cavernas conhecidas no Brasil; no entanto, sua micobiota cavernícola é pouco estudada. Este estudo investiga a presença de espécies de *Aspergillus* e *Penicillium* em cavernas ferruginosas e quartzíticas da porção sul da Serra do Espinhaço, em Minas Gerais, Brasil. Amostras foram coletadas de vários substratos cavernícolas e analisadas por métodos de filogenia molecular. Foram obtidos 330 isolados, com *Penicillium* sendo o gênero dominante. As análises filogenéticas das regiões gênicas da beta-tubulina e calmodulina identificaram 4 espécies de *Aspergillus* e 14 de *Penicillium*, com 16 espécies potencialmente novas detectadas. Oito espécies foram relatadas em cavernas no mundo pela primeira vez, enquanto três constituem novos registros para cavernas brasileiras. O estudo destaca a importância do estudo da micobiota de cavernas na elucidação da diversidade fúngica e no aprimoramento das estimativas globais de fungos. Além disso, enfatizamos a necessidade de esforços de conservação, já que esses ecossistemas cavernícolas estão ameaçados pela expansão das atividades de mineração na região.

Abstract

The Eurotiales order comprises economically important genera, such as *Aspergillus* and *Penicillium*, that are ubiquitously distributed in terrestrial habitats, including caves. Minas Gerais state harbours most of the caves known in Brazil; however, its cave mycobiota is poorly studied. This study investigates the presence of *Aspergillus* and *Penicillium* species in ferruginous and quartzite caves of the Southern Espinhaço Mountain Range in Minas Gerais, Brazil. Samples were collected from various cave substrates and analysed using molecular phylogenetic methods. A total of 330 isolates were obtained, with *Penicillium* being the dominant genus. Phylogenetic analyses of beta-tubulin and calmodulin gene regions identified 4 *Aspergillus* and 14 *Penicillium* species, with 16 potentially new species detected. Eight species were reported in caves worldwide for the first time, while three were new records for Brazilian caves. The study highlights the importance of cave mycobiota research in uncovering fungal diversity and improving global fungal estimates. It also emphasizes the need for conservation efforts, as these cave ecosystems are threatened by expanding mining activities in the region.

1. Introduction

The order *Eurotiales* comprises diverse and economically important fungal genera such as *Aspergillus* and *Penicillium* (*Aspergillaceae*). *Penicillium* is known for producing penicillin, revolutionising bacterial infection treatment (HOUBRAKEN et al., 2011). *Aspergillus oryzae* is used in food fermentation, while *P. camemberti* and *P. roqueforti* are used in cheese production (COTON et al., 2020; LESSARD et al., 2014; ABE & GOMI, 2007). However, both genera can cause food spoilage, produce harmful mycotoxins, and include pathogenic species (YU et al., 2020; FRISVAD et al., 2019).

Members of the *Eurotiales* order are widely distributed in terrestrial habitats. They are found in indoor environments (VISAGIE et al., 2014b), natural ecosystems (BARBOSA et al., 2020, 2022; VISAGIE et al., 2014a), and caves (VANDERWOLF et al., 2013). *Aspergillus* and *Penicillium* are among the most abundant fungi in caves worldwide (ZHANG et al., 2021). Members of *Eurotiales* in Brazilian caves have been reported in different states, including Goiás (OLIVEIRA et al., 2024a), Minas Gerais (CONDÉ et al., 2022), Pará (NÓBREGA et al., 2024), Pernambuco (CUNHA et al., 2020),

Rio Grande do Norte (ALVES et al., 2022b), and Sergipe (LIMA et al., 2024).

The state of Minas Gerais harbours 11,029 caves, representing almost half of the caves registered in Brazil (CRUZ & COSTA NETO, 2023). Many of these caves are located in the Southern Espinhaço Mountain Range (SEMR) (AULER et al., 2015), including the quartzite and ferruginous caves found in the municipality of Conceição do Mato Dentro and its vicinities. The Monumento Natural da Serra da Ferrugem (MNSF) is an integral conservation unit which preserves an area of approximately 867 ha. However, iron mining activities that surround the MNSF threaten the caves and biodiversity of this pristine environment (DIAS & MADEIRA FILHO, 2020), including its cave mycobiota. This study aimed to identify fungal isolates belonging to the order *Eurotiales* from ferruginous and quartzite caves in the SEMR. Phylogenetic analyses using the DNA sequences of the beta-tubulin and calmodulin gene regions were performed to identify fungal isolates.

2. Methodology

2.1. Study area and sample collection

Samples were collected at three different locations. In Alvorada de Minas (Itapanhoacanga), sampling was performed in one ferruginous cavity. In Conceição do Mato Dentro, sampling was performed in the Gruta Curral de Pedras (quartzite/ferruginous), which is approximately 400m long; and in the MNSF, where sampling was performed in five ferruginous cavities.

Samples of airborne fungal particles, rocks, sediment, leaf litter, roots, and animal dung were collected from the caves. Airborne fungal particles were collected using the Koch sedimentation method (KUZMINA et al., 2012). Petri plates (90 mm) containing Potato Dextrose Agar (PDA) amended with chloramphenicol (2 mg/L), Dichloran-Rose Bengal-Chloramphenicol Agar (DRBC), and Dichloran Glycerol Agar (DG18) culture media were exposed to the cave environment for 15 minutes. Rock samples were collected with the aid of a sterile swab. Sediment samples were collected at a depth of 1–5 cm after removing the surface layer. Leaf litter and root samples were collected and stored in paper bags, while animal dung samples were collected and stored in 50 mL Falcon tubes.

2.2. Fungal isolation

The serial dilution method was used to isolate fungi from rocks, sediments, and animal dung (ZHANG et al., 2017). The root samples were processed as described by OLIVEIRA et al. (2024b). PDA, DRBC, and DG18 culture media were used for fungal isolation. All plates were incubated at 25 °C for 30 days, during which fungal growth was observed daily, and new fungal colonies were periodically transferred to fresh PDA plates. Litter samples were washed in running tap water and then incubated in a humid chamber (CASTAÑEDA-RUIZ et al., 2005), where they were periodically observed under a stereoscopic microscope and reproductive fungal structures were transferred to fresh PDA plates.

Pure cultures were obtained using the hyphal tip method (TUIITE, 1969). Fungal cultures were stored in 2 mL microtubes containing 10% glycerol solution at –20 °C. All isolates were stored in the cave fungal collection of Micolab/UFV.

2.3. DNA isolation, amplification and sequencing

Total genomic DNA was isolated from fungal mycelia grown in PDA for seven days using a commercial Wizard Genomic DNA Purification kit (Promega®). DNA amplification of the internal transcribed spacers 1 and 2 and the intervening 5.8S subunit (ITS) using primer pairs ITS5/LR6 (VILGALYS & HESTER, 1990; WHITE et al., 1990) was performed for all isolates. Subsequently, a smaller set of representative isolates of *Eurotiales* was chosen, and the partial beta-tubulin (*BenA*) and calmodulin (*CaM*) gene regions were amplified using the primers Bt2a/Bt2b (GLASS & DONALDSON, 1995) and CMD5 and CMD6 (HONG et al., 2006), respectively. DNA amplicon sequencing was performed by Macrogen Inc. (South Korea). The sequencing results were visualised and trimmed using FinchTV software (Geospiza Inc.).

2.4. Phylogenetic analyses

A DNA sequence dataset was constructed using *BenA* and *CaM* sequences generated in this study and those stored

in the National Center for Biotechnology Information (NCBI). DNA alignments were performed using MAFFT v.7 (KATO & STANDLEY, 2013). Phylogenetic analyses were performed using maximum likelihood (ML) in IQ-TREE software (MINH et al., 2020), with 10,000 ultrafast bootstrap (BS) replicates. Only BS values of ≥ 90 were plotted at the nodes. Phylogenetic trees were visualized using FigTree v. 1.4.3 and exported to graphics programs for editing.

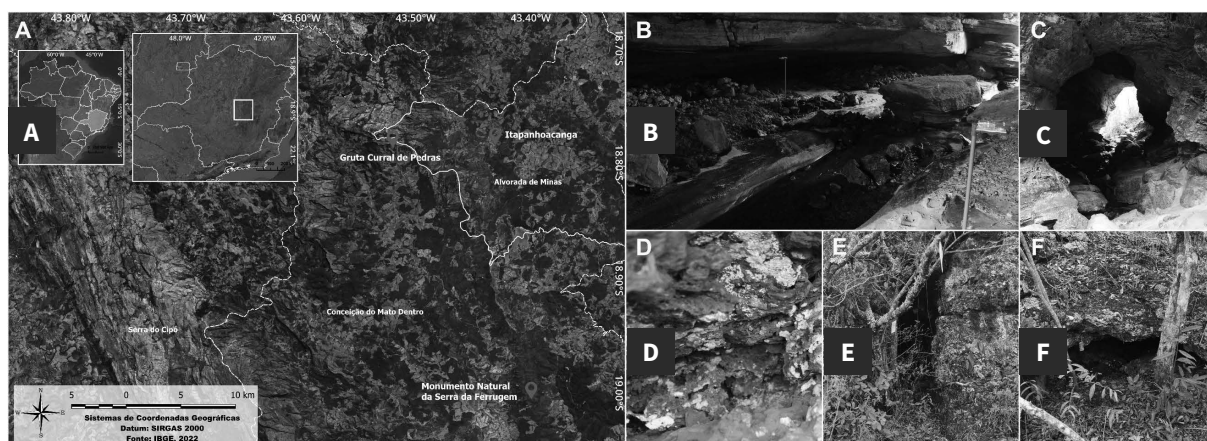


Figure 1: A: Location of the caves analysed in this study. B–C: Gruta Curral de Pedras airborne fungi sampling and cave entrance. D: Fungal colonisation of cave walls in Itapanhoacanga. E–F: Ferruginous cavities in Monumento Natural da Serra da Ferrugem.

3. Results

A total of 330 isolates belonging to *Aspergillus* and *Penicillium* were found in the caves sampled, of which 15 were identified as *Aspergillus* and 315 as *Penicillium*, according to ITS sequencing. From the samples

analysed, 143 isolates were recovered from sediments, 80 from airborne particles, 52 from animal dung, 39 from rocks, 11 from leaf litter, and five from plant roots (Fig. 2A)

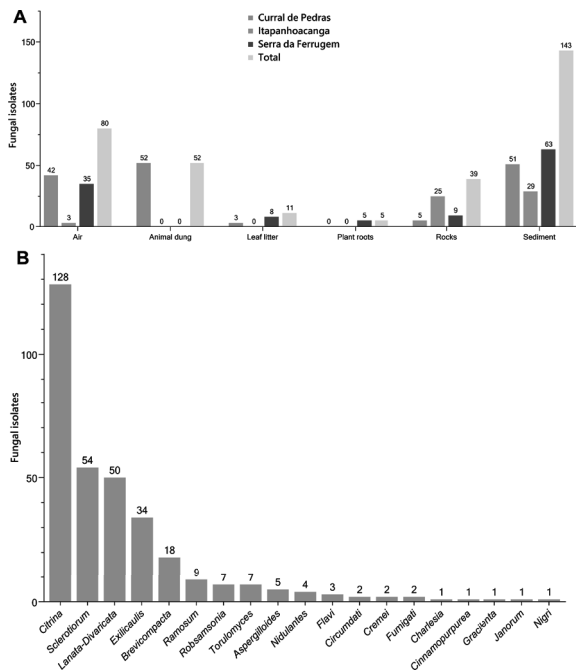


Figure 2: Statistics of *Aspergillus* and *Penicillium* isolates obtained in this study. **A:** Number of isolates obtained from different substrates in the three localities. **B:** Abundance of fungal isolates obtained by section in *Aspergillus* and *Penicillium*.

Of these, 153 isolates were recovered from the Gruta Curral de Pedras, 120 from the Serra da Ferrugem caves, and 57 from Itapanhoacanga cave. In the caves analysed, section *Citrina* was the most abundant with 128 isolates, followed by *Sclerotium*, *Lanata-Divariata*, *Exilicaulis*, and *Ramosum* with 54, 50, 34, and 18 isolates, respectively (Fig. 2B). The other sections are depicted in Figure 2B.

Phylogenetic trees were constructed using a small subset of isolates which were subjected to DNA sequencing of more phylogenetically informative regions, *i.e.* *BenA* and *CaM* (Figs 2 and 3). Four species of *Aspergillus* were identified, namely *A. bertholletiae* (sect. *Flavi*), *A. stramenius* (sect. *Fumigati*), *A. tubingensis* (sect. *Nigri*), and *A. versicolor* (sect. *Nidulantes*), whereas strains CF 3851 and CF 4138, belonging to sections *Circumdati* and *Flavi*, respectively, could not be identified to the species level (Fig. 3).

In the genus *Penicillium*, 14 strains and 14 species were identified, whereas the other 17 strains, representing 14 species, could not be assigned to any known species (Fig. 4). Isolates identified were *P. fusisporum*, *P. brevicompactum*, *P. nothofagi*, *P. paxilli*, *P. steckii*, *P. shearii*, *P. melinii*, *P. cf. sichuanense*, *P. cf. carajasense*, *P. cf. stangiae*, *P. viridissimum*, *P. virgatum*, *P. cf. xyleborini*, and *P. cf. austriaca* (Fig. 4). *Penicillium* isolates were distributed in sections *Aspergilloides*, *Brevicompacta*, *Charlesia*, *Citrina*, *Exilicaulis*, *Gracilenta*, *Lanata-Divariata*, *Ramosum*, *Robsamsonia*, *Sclerotium*, and *Torulomyces* (Fig. 4).

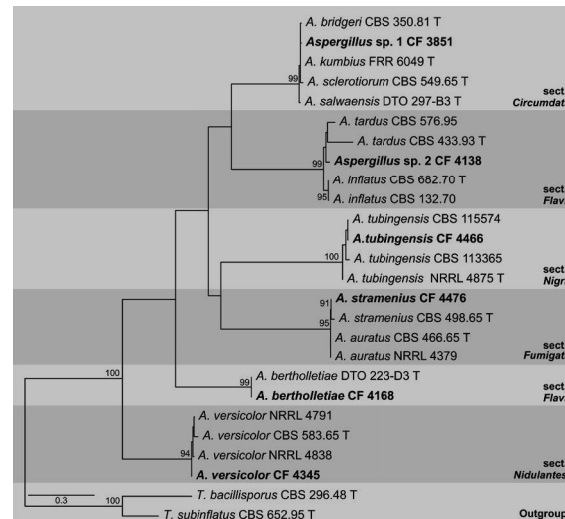


Figure 3: Maximum-likelihood phylogenetic tree of *Aspergillus* based on *CaM* sequences. Isolates found in this study are shown in bold. Ex-type isolates are marked with “T”. Only bootstrap (bs) values $\geq 90\%$ are shown at branches. The tree is rooted with *T. bacillisporus* CBS 296.48 and *T. subinflatus* CBS 652.95.

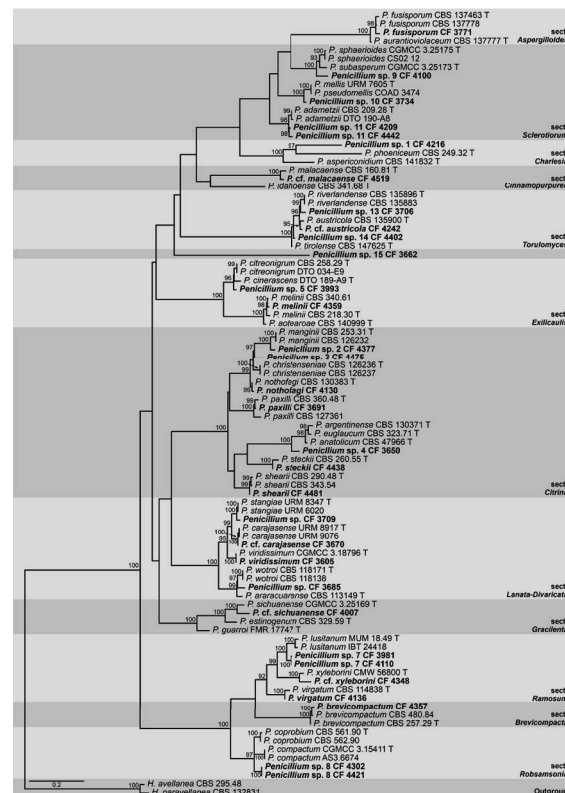


Figure 4: Maximum-likelihood phylogenetic tree of *Penicillium* based on *BenA* sequences. Isolates found in this study are shown in bold. Ex-type isolates are marked with “T”. Only bootstrap (bs) values $\geq 90\%$ are shown at branches. The tree is rooted with *H. avellanea* CBS 295.48 and *H. paravellanea* CBS 132831.

The isolates that could not be identified may represent new species of *Aspergillus* and *Penicillium*. They will be further analysed using a polyphasic approach, combining multigene molecular phylogeny and morphological analyses of vegetative and reproductive structures, to determine whether these isolates represent new species. Table 1 depicts the species found in *Aspergillus* and *Penicillium* according to phylogenetic analyses of the *CaM* and *BenA* sequences.

Table 1: List of *Aspergillus* and *Penicillium* species found in the caves studied using *BenA* and *CaM* gene regions

Species	Section	Isolate	Cave substrate	Locality	Species	Section	Isolate	Cave substrate	Locality
<i>Aspergillus</i> sp. 1	<i>Circumdati</i>	CF 3851	Air	Serra da Ferrugem	<i>P. cf. carajasense</i>	<i>Lanata-Divaricata</i>	CF 3670	Sediment	Serra da Ferrugem
<i>Aspergillus</i> sp. 2	<i>Flavi</i>	CF 4138	Sediment	Curral de Pedras	<i>P. cf. stangiae</i>	■ <i>Lanata-Divaricata</i>	CF 3709	Sediment	Serra da Ferrugem
<i>A. bertholletiae</i>	<i>Flavi</i>	CF 4168	Sediment	Curral de Pedras	<i>P. viridissimum</i>	■ <i>Lanata-Divaricata</i>	CF 3605	Air	Serra da Ferrugem
<i>A. stramenius</i>	■ <i>Fumigati</i>	CF 4476	Sediment	Itapanhoacanga	<i>Penicillium</i> sp. 6	<i>Lanata-Divaricata</i>	CF 3685	Cave wall	Serra da Ferrugem
<i>A. tubingensis</i>	<i>Nigri</i>	CF 4466	Cave wall	Itapanhoacanga	<i>P. virgatum</i>	<i>Ramosum</i>	CF 4136	Sediment	Curral de Pedras
<i>A. versicolor</i>	<i>Nidulantes</i>	CF 4345	Animal dung	Curral de Pedras	<i>P. cf. xyleborini</i>	■ <i>Ramosum</i>	CF 4348	Animal dung	Curral de Pedras
<i>P. fusisporum</i>	■ <i>Aspergilloides</i>	CF 3771	Plant roots	Serra da Ferrugem	<i>Penicillium</i> sp. 7	<i>Ramosum</i>	CF 3981	Air	Curral de Pedras
<i>P. brevicompactum</i>	<i>Brevicompacta</i>	CF 4357	Air	Curral de Pedras	<i>Penicillium</i> sp. 7	<i>Ramosum</i>	CF 4110	Air	Curral de Pedras
<i>Penicillium</i> sp. 1	<i>Charlesia</i>	CF 4216	Leaf litter	Curral de Pedras	<i>Penicillium</i> sp. 8	<i>Robsamsonia</i>	CF 4302	Animal dung	Curral de Pedras
<i>P. nothofagi</i>	■ <i>Citrina</i>	CF 4130	Sediment	Curral de Pedras	<i>Penicillium</i> sp. 8	<i>Robsamsonia</i>	CF 4421	Animal dung	Curral de Pedras
<i>P. paxilli</i>	● <i>Citrina</i>	CF 3691	Cave wall	Serra da Ferrugem	<i>Penicillium</i> sp. 9	<i>Sclerotiorum</i>	CF 4100	Sediment	Curral de Pedras
<i>P. steckii</i>	● <i>Citrina</i>	CF 4438	Sediment	Itapanhoacanga	<i>Penicillium</i> sp. 10	<i>Sclerotiorum</i>	CF 3734	Sediment	Serra da Ferrugem
<i>P. shearii</i>	<i>Citrina</i>	CF 4481	Sediment	Itapanhoacanga	<i>Penicillium</i> sp. 11	<i>Sclerotiorum</i>	CF 4209	Cave wall	Curral de Pedras
<i>Penicillium</i> sp. 2	<i>Citrina</i>	CF 4377	Animal dung	Curral de Pedras	<i>Penicillium</i> sp. 11	<i>Sclerotiorum</i>	CF 4442	Sediment	Itapanhoacanga
<i>Penicillium</i> sp. 3	<i>Citrina</i>	CF 4475	Sediment	Itapanhoacanga	<i>P. cf. austriaca</i>	■ <i>Torulomyces</i>	CF 4242	Animal dung	Curral de Pedras
<i>Penicillium</i> sp. 4	<i>Citrina</i>	CF 3650	Sediment	Serra da Ferrugem	<i>Penicillium</i> sp. 12	<i>Torulomyces</i>	CF 3706	Sediment	Serra da Ferrugem
<i>Penicillium</i> sp. 5	<i>Exilicaulis</i>	CF 3993	Air	Curral de Pedras	<i>Penicillium</i> sp. 13	<i>Torulomyces</i>	CF 4402	Sediment	Curral de Pedras
<i>P. melinii</i>	● <i>Exilicaulis</i>	CF 4359	Sediment	Curral de Pedras	<i>Penicillium</i> sp. 14	undefined	CF 3662	Sediment	Serra da Ferrugem
<i>P. cf. sichuanense</i>	■ <i>Gracilenta</i>	CF 4007	Air	Curral de Pedras					

■ = first report in caves worldwide; ● = first report in Brazilian caves.

4. Discussion

Caves are environments characterised by partial or total absence of light, high humidity, stable temperature, and limited amount of organic matter (except for bat caves) (BARTON; JURADO, 2007; POULSON; WHITE, 1969). Despite these challenging conditions, fungi can thrive in these ecosystems, and many studies have reported the presence of these organisms, including the discovery of new species and genera (ALVES et al., 2022b; CONDÉ et al., 2023; ZHANG et al., 2017). According to estimates of fungal diversity in caves worldwide, nearly 2,000 species of fungi have been reported in these environments (VANDERWOLF et al., 2013; ZHANG et al., 2021), of which *Aspergillus* and *Penicillium* are often the most abundant genera.

Based on phylogenetic analyses, we identified four species in the genus *Aspergillus* and 14 species in *Penicillium* from ferruginous and quartzite caves in Minas Gerais. Additionally, we identified 16 fungal lineages that may represent new species in these genera. Cunha et al., (2020) found that *Aspergillus* and *Penicillium* were predominately isolated from different substrates in the Meu Rei bat cave in Pernambuco. Caves in Minas Gerais also harbour *Aspergillus* and *Penicillium* (CONDÉ et al., 2022; TAYLOR et al., 2014; TAYLOR et al., 2013). In the Lapa Nova cave, these genera were the most abundant in air and bat guano samples (TAYLOR et al., 2013), including species found in our study, such as *A. versicolor* and *P. brevicompactum*. In the RM3 ferruginous cave located in the Iron Quadrangle, these genera were also abundant, and *A. fumigatus*, the most common causal agent of pulmonary aspergillosis, was isolated (TAYLOR et al., 2014). Although we did not find *A. fumigatus* in our survey, we identified one strain, *A. stramenius* (CF 4476), which belonged to the

same section as *A. fumigatus* (section *Fumigati*).

In this study, eight species are reported in caves worldwide for the first time, namely *A. stramenius*, *P. fusisporum*, *P. nothofagi*, *P. cf. sichuanense*, *P. cf. stangiae*, *P. viridissimum*, *P. cf. xyleborini*, and *P. cf. austriaca*. Additionally, three species are reported in Brazilian for the first time, namely *P. paxilli*, *P. steckii*, and *P. melinii*. Interestingly, *P. stangiae* is a species discovered in a soil sample from the Atlantic Forest in Pernambuco (ALVES et al., 2022a), whereas our strain CF 3709 was also found in the soil of Serra da Ferrugem, which is located in the Cerrado biome, thus expanding the known distribution of this fungus in Brazil.

We identified phylogenetic lineages of *Aspergillus* and *Penicillium* that may represent new species. For example, isolate CF 3662 could not be identified in any section of *Penicillium*, and further analyses will determine whether this isolate represents a new section in the genus. Studies have revealed new taxa of fungi in caves from Minas Gerais, including the discovery of a new fungal genus and seven new species (CONDÉ et al., 2023; DUTRA et al., 2023; LEÃO et al., 2024), which emphasise the potential of caves to harbour undiscovered fungi.

Ferruginous caves in the MNSR are threatened because of the expansion of mining activities in the region (DIAS; MADEIRA FILHO, 2020). Therefore, studies on cave mycobiota are important to uncover the fungi that inhabit these pristine and threatened environments, which helps to improve global fungal estimates. This finding reinforces the need to include fungi in conservation efforts, which can lead to the protection of fungal species and subterranean environments.

5. Conclusion

In this study, we provide information on the distribution of fungi belonging to *Aspergillus* and *Penicillium* in the caves of Minas Gerais. Additionally, we provide the perspective for finding new species of *Eurotiales* fungi in Brazilian ferruginous and quartzite caves. These reports on *Aspergillus* and *Penicillium* in Brazilian caves are important to improve our knowledge of cave biodiversity, as fungi have historically

been neglected in this field.

These discoveries are of utmost importance for filling the knowledge gap in current estimates of fungal species on Earth. Currently, these estimates indicate that less than 10% of the 2–3 million fungal species inhabit our planet. Therefore, studies on cave mycobiota can help to enhance these numbers.

Acknowledgements

This work was supported by the TCCE ICMBio/Vale: Speleological Compensation, in the Term of Commitment between Vale S.A and the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), with operational management carried out by the Instituto Brasileiro de Desenvolvimento e Sustentabilidade (IABS), Coordenação de Aper-

feiçoamento de Pessoal de Nível Superior (CAPES, Financial Code 001), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) and the Fundação Arthur Bernardes (FUNARBE).

References

- ABE, K.; GOMI, K. (2007) Food Products Fermented by *Aspergillus oryzae*. In: GOLDMAN, G. H.; OSMANI, S. A. (Eds.). The Aspergilli: Genomics, Medical Aspects, Biotechnology, and Research Methods. CRC Press, p. 449–460.
- ALVES, A. L. et al. (2022a) *Penicillium gercinae* and *Penicillium stangiae* (*Eurotiomycetes, Ascomycota*), two new species from soil in Brazil. *Acta Botanica Brasilica*, v. 36, p. e2022abb0006.
- ALVES, V. C. S. et al. (2022b) Unravelling the fungal darkness in a tropical cave: richness and the description of one new genus and six new species. *Fungal Systematics and Evolution*.
- AULER, A. et al. (2015) Cavernas da Serra do Espinhaço Meridional.
- BARBOSA, R. D. N. et al. (2020) Brazilian tropical dry forest (Caatinga) in the spotlight: an overview of species of *Aspergillus*, *Penicillium* and *Talaromyces* (*Eurotiales*) and the description of *P. vascosobrinhou* sp. nov. *Acta Botanica Brasilica*, v. 34, n. 2, p. 409–429.
- BARBOSA, R. D. N. et al. (2022) Brazilian Atlantic Forest and Pampa Biomes in the spotlight: an overview of *Aspergillus*, *Penicillium*, and *Talaromyces* (*Eurotiales*) species and the description of *Penicillium nordestinense* sp. nov. *Acta Botanica Brasilica*, v. 36, p. e2021abb0390.
- BARTON, H.; JURADO, V. (2007) What's up down there? Microbial diversity in caves. *Microbe*, v. 2, p. 132–138.
- CASTAÑEDA-RUIZ, R. F. et al. (2005) Microfungi from submerged plant material: *Zelotriadelphia amoena* gen. et sp. nov. and *Vanakripa fasciata* sp. nov. v. 91, p. 339–345.
- CONDÉ, T. O. et al. (2022) What is down there? Diversity of *Penicillium* species isolated from Neotropical caves in Minas Gerais, Brazil. In: II INTERNATIONAL SYMPOSIUM OF AGRICULTURE MICROBIOLOGY. Lavras, Minas Gerais, Brazil: Universidade Federal de Lavras.
- CONDÉ, T. O. et al. (2023) Shedding light on the darkness: a new genus and four new species in the family *Chaetomiaceae* from Brazilian neotropical caves revealed by multi-gene phylogenetic analyses. *Mycological Progress*, v. 22, n. 7, p. 49.
- COTON, E. et al. (2020) *Penicillium roqueforti*: an overview of its genetics, physiology, metabolism and biotechnological applications. *Fungal Biology Reviews*, v. 34, n. 2, p. 59–73.
- CRUZ, J. B.; COSTA NETO, J. F. (2023) Anuário Estatístico do Patrimônio Espeleológico Brasileiro 2022. Ministério do Meio Ambiente e Mudança do Clima, ICMBio/CECAV.
- CUNHA, A. O. B. et al. (2020) Living in the dark: Bat caves as hotspots of fungal diversity. *PLOS ONE*, v. 15, n. 12, p. e0243494.
- DIAS, T. H.; MADEIRA FILHO, W. (2020) PROJETO MINAS-RIO NO ENTORNO DO MONUMENTO NATURAL SERRA DA FERRUGEM. *Cadernos de Estudos Sociais*, v. 35, n. 2.
- DUTRA, Y. L. G. et al. (2023) Two new *Cladosporium* species from a quartzite cave in Brazil. *Brazilian Journal of Microbiology*, v. 54, n. 4, p. 3021–3031.
- FRISVAD, J. C. et al. (2019) Taxonomy of *Aspergillus* section *Flavi* and their production of aflatoxins, ochratoxins and other mycotoxins. *Studies in Mycology*, v. 93, n. 1, p. 1–63.
- GLASS, N. L.; DONALDSON, G. C. (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Applied and Environmental Microbiology*, v. 61, n. 4, p. 1323–1330.
- HONG, S.-B. et al. (2006) Novel *Neosartorya* species isolated from soil in Korea. *International Journal of Systematic and Evolutionary Microbiology*, v. 56, n. 2, p. 477–486.
- HOUBRAKEN, J. et al. (2020) Classification of *Aspergillus*, *Penicillium*, *Talaromyces* and related genera (*Eurotiales*): An overview of families, genera, subgenera, sections, series and species. *Studies in Mycology*, v. 95, p. 5–169.
- HOUBRAKEN, J.; FRISVAD, J. C.; SAMSON, R. A. (2011) Fleming's penicillin producing strain is not *Penicillium chrysogenum* but *P. rubens*. *IMA Fungus*, v. 2, n. 1, p. 87–95.
- KATOH, K.; STANDLEY, D. M. (2013) MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Molecular Biology and Evolution*, v. 30, n. 4, p. 772–780.
- KUZMINA, L. YU. et al. (2012) Microbiota of the Kinderlinskaya cave (South Urals, Russia). *Microbiology*, v. 81, n. 2, p. 251–258.
- LEÃO, A. F. et al. (2024) *Amphichorda monjolensis* sp. nov., a new fungal species isolated from a Brazilian limestone cave, with an update on acremonium-like species in *Bionectriaceae*. *Brazilian Journal of Microbiology*.
- LESSARD, M.-H. et al. (2014) Metatranscriptome analysis of fungal strains *Penicillium camemberti* and *Geotrichum candidum* reveal cheese matrix breakdown and potential development of sensory properties of ripened Camembert-type cheese. *BMC Genomics*, v. 15, n. 1, p. 235.
- LIMA, J. M. S. et al. (2024) *Aspergillus*, *Penicillium*, and *Talaromyces* (*Eurotiales*) in Brazilian caves, with the description of four new species. *Fungal Systematics and Evolution*.
- MINH, B. Q. et al. (2020) IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. *Molecular Biology and Evolution*, v. 37, n. 5, p. 1530–1534.
- NÓBREGA, J. P. et al. (2024) Six new *Penicillium* species in the section *Lanata-Divaricata* from a cave in Amazon rainforest, Brazil. *Mycological Progress*, v. 23, n. 1, p. 71.
- OLIVEIRA, J. A.; CUSTÓDIO, F. A.; PEREIRA, O. L. (2024) Cultivable root endophytic fungi associated with *Acrocomia aculeata* and its antagonistic activity against phytopathogenic oomycetes. *Brazilian Journal of Microbiology*.
- OLIVEIRA, P. H. F. et al. (2024) Mapa do tesouro: Riqueza de espécies de *Penicillium* na caverna Lapa do Boqueirão do Cerrado goiano. *Revista Brasileira de Espeleologia-RBEsp*, v. 1, p. 339–369.
- PAULA, C. C. P. D. et al. (2019) High cellulolytic activities in filamentous fungi isolated from an extreme oligotrophic subterranean environment (Catão cave) in Brazil. *Anais da Academia Brasileira de Ciências*, v. 91, n. 3, p. e20180583.
- POULSON, T. L.; WHITE, W. B. (1969) The Cave Environment: Limestone caves provide unique natural laboratories for studying biological and geological processes. *Science*, v. 165, n. 3897, p. 971–981.
- TAYLOR, E. L. S. et al. (2014) Cave Entrance dependent Spore Dispersion of Filamentous Fungi Isolated from Various Sediments of Iron Ore Cave in Brazil: a colloquy on human threats whilecaving. *AMBIENT SCIENCE*, v. 1, n. 1, p. 16–28.
- TAYLOR, E.; RESENDE STOIANOFF, M.; LOPES FERREIRA, R. (2013) Mycological study for a management plan of a neotropical show cave (Brazil). *International Journal of Speleology*, v. 42, n. 3, p. 267–277, 2013.
- TUITE, J. (1969) Plant pathological methods. Fungi and bacteria. Burgess Publishing Company.
- VANDERWOLF, K. et al. (2013) world review of fungi, yeasts, and slime molds in caves. *International Journal of Speleology*, v. 42, n. 1, p. 77–96.
- VILGALYS, R.; HESTER, M. (1990) Rapid genetic identification and mapping

of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology*, v. 172, n. 8, p. 4238–4246.

VISAGIE, C. M. et al. (2014a) Diversity of *Penicillium* section *Citrina* within the fynbos biome of South Africa, including a new species from a *Protea repens* infructescence. *Mycologia*, v. 106, n. 3, p. 537–552.

VISAGIE, C. M. et al. (2014b) *Aspergillus*, *Penicillium* and *Talaromyces* isolated from house dust samples collected around the world. *Studies in Mycology*, v. 78, n. 1, p. 63–139.

WHITE, T. J. et al. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods and applications*, v. 18, n. 1, p. 315–322.

YU, L. et al. (2020) Postharvest control of *Penicillium expansum* in fruits: A review. *Food Bioscience*, v. 36, p. 100633.

ZHANG, Z. F. et al. (2017) Culturable mycobiota from Karst caves in China, with descriptions of 20 new species. *Persoonia - Molecular Phylogeny and Evolution of Fungi*, v. 39, n. 1, p. 1–31.

ZHANG, Z.-F. et al. (2021) Culturable mycobiota from Karst caves in China II, with descriptions of 33 new species. *Fungal Diversity*, v. 106, n. 1, p. 29–136.