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Discovering the cave mycobiota of the Brazilian Cerrado

Ana Flávia Leão
Doctor Scientiae

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Thesis submitted to the Agricultural Microbiology Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

Adviser: Olinto Liparini Pereira

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“Porque sou eu que conheço os planos que tenho para vocês, diz o Senhor, planos de fazê-los prosperar e não de causar dano, planos de dar a vocês esperança e um futuro”. Jeremias 29,11

Hoje encerro uma longa caminhada em busca do tão sonhado título de Doutora. Uma caminhada com vários tropeços, lutas e lágrimas, mas que hoje colho as alegrias da conquista. Primeiramente agradeço a Deus pelo dom da vida e Virgem Maria por toda intercessão nessa jornada. Agradeço aos meus pais Dalva e Geraldo, exemplos de determinação para que seus filhos sempre alcançassem seus sonhos. Esta conquista de hoje é fruto do trabalho deles em sempre ensinar e incentivar a estudar e a partir dos estudos construir um futuro melhor. E um agradecimento especial aos meus irmãos Fabiana e Geraldo Junior, minhas certezas de que mesmo com as diferenças estaremos sempre juntos compartilhando as conquistas da vida.

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ABSTRACT

LEÃO, Ana Flávia, D.Sc., Universidade Federal de Viçosa, July, 2025. **Discovering the cave mycobiota of the Brazilian Cerrado**. Adviser: Olinto Liparini Pereira.

Caves are unique environments and have become hotspots for mycology. In recent years, studies on cave fungi have highlighted these environments as a new frontier for expanding knowledge of the mycological world. In this sense, the objective of this work was to study cultivable filamentous fungi present in different caves and cavities of the Serra do Espinhaço Meridional (SEM) of Minas Gerais. In Chapter 1, two new species of the genus Amphichorda are described. Amphichorda sp. nov. 1 was isolated from samples of animal dung, sediments and samples of particles suspended in the air in the marble caves Gruta da Viola and Gruta Teto de Seixos, located in Serra do Cipó – part of SEM. Amphichorda sp. nov. 2 was isolated from samples of animal dung collected in two caves on the outskirts of the municipality of Cuiabá – Mato Grosso. These species are the first coprophilous species of the genus Amphichorda described in caves in Brazil. Chapter 2 presents the diversity of filamentous fungi found in the Curral de Pedras cave – Conceição do Mato Dentro, MG. From samples of soil, leaf litter, rock, animal dung and airborne particles, 421 filamentous fungi were isolated, identified by sequencing the ITS region in 78 genera, within 59 families, 31 orders, 12 classes, 7 subphyla and 4 phyla. Chapter 3 discusses the diversity of filamentous fungi found in ferruginous cavities located in Serra da Ferrugem – Conceição do Mato Dentro, MG. A total of 361 filamentous fungi were isolated from samples of airborne particles, sediments, leaf litter, roots and rocks. The fungi were identified by sequencing the ITS region in 70 genera, 46 families, 26 orders, 10 classes, 6 subphyla and 4 phyla. Chapter 4 describes the taxonomic novelties, including a new genus Speluncomyces, and seven new species: Speluncomyces lunatus, Cylindromonium brasiliense, Sesquicillium flavum, Paraneoaraneomyces sp. nov, Tolypocladium sp. nov., Sarocladium sp. nov. and Rachicladosporium sp. nov. These discoveries demonstrate the mycological richness of Brazilian caves that has yet to be explored and reinforce the need for further studies.

Keywords: Fungi; Serra do Espinhaço; Biospeleology

RESUMO

LEÃO, Ana Flávia, D.Sc., Universidade Federal de Viçosa, julho de 2025. **Descobrindo a micobiota de cavernas do Cerrado Brasileiro**. Orientador: Olinto Liparini Pereira.

As cavernas são ambientes únicos e vem se tornado hotspots para micologia. Nos últimos anos, estudos sobre fungos cavernícolas têm destacado esses ambientes como uma nova fronteira para a expansão do conhecimento do mundo micológico. O objetivo deste trabalho foi estudar fungos filamentosos cultiváveis presentes em diferentes cavernas e cavidades da Serra do Espinhaço Meridional (SEM) de Minas Gerais. No Capítulo 1 são descritas duas novas espécies do gênero Amphichorda. Amphichorda sp. nov. 1 foi isolada a partir de amostras de fezes de animais, sedimentos e amostras de partículas suspensas no ar nas cavernas de mármore Gruta da Viola e Gruta Teto de Seixos, situadas na Serra do Cipó – parte da SEM. Amphichorda sp. nov. 2 foi isolada a partir de amostras de fezes de animais coletadas em duas cavernas nos arredores do município de Cuiabá – Mato Grosso. Essas espécies são as primeiras espécies coprofilias pertencentes ao gênero Amphichorda descritas em cavernas no Brasil. No Capítulo 2 é apresentada a riqueza fungos filamentosos encontrada na caverna Curral de Pedras – Conceição do Mato Dentro, MG. A partir de amostras de solo, serapilheira, rocha, fezes de animais e partículas suspensas no ar, foram isolados 421 fungos filamentos, identificados a partir sequenciamento da região ITS em 78 gêneros, dentro de 59 famílias, 31 ordens, 12 classes, 7 subfilos e 4 filos. No Capítulo 3 se discute sobre a riqueza de fungos filamentosos encontrados em cavidades ferruginosas situadas na Serra da Ferrugem – Conceição do Mato Dentro, MG. Foram isolados 361 fungos filamentosos a partir de amostras de partículas suspensas no ar, sedimentos, serapilheira, raízes e rochas. Os fungos foram identificados por meio do sequenciamento da região ITS em 70 gêneros, 46 famílias, 26 ordens, 10 classes, 6 subfilos e 4 filos. No Capítulo 4 são descritas as novidades taxonômicas sendo um novo gênero na família Bionectriaceae (Speluncomyces) e a nova espécie tipo Speluncomyces lunatus, e seis novas espécies: Cylindromonium brasiliense, Sesquicillium flavum, Paraneoaraneomyces sp. nov, Tolypocladium sp. nov., Sarocladium sp. nov. e Rachicladosporium sp. nov. Essas descobertas mostram a riqueza micológica ainda inexplorada das cavernas brasileiras e reforçam a necessidade de mais estudos.

Palavras-chave: Fungos; Serra do Espinhaço; Bioespeleologia

SUMMARY

GENERAL INTRODUCTION

Caves can be classified on natural openings resulting from physical actions and chemical reactions on rocks. Their size is related to their origin and the constituent rock (formed together with the rock or after the formation of the rock by dissolution of the same) (Popović et al. 2015; Park et al. 2020). Caves with larger dimensions and speleological ornamentation are generally formed by limestone rocks, which have greater porosity and consequently greater dissolution of the rock by water, when compared to caves of iron, quartzite and siliciclastic formations (Popović et al. 2015; Park et al. 2020). Caves are underground ecosystems with invaluable values both for their unique speleology and for being shelters for various animals, and for holding an important part of history, telling through cave paintings how ancient civilizations lived (Bastian et al. 2010; Martin-Sanchez et al. 2014; Pereira et al. 2022).

Caves have unique characteristics such as spatial delimitation, absence of direct sunlight, temperature and humidity with little or no variation, scarcity of nutrients and a high concentration of minerals (Zhang et al. 2021; Biagioli et al. 2023; Poli et al. 2024). These characteristics classify the caves as an extreme environment for the development of life, the microbiota found in these environments is unique and has great potential for discovering new fungal species and species, including taxa with potential biotechnological use, such as the production of compounds with antifungal and antibiotic activities (Zhang et al. 2021; Jiang et al. 2023; Poli et al. 2024).

Fungi are one of the main groups found in the cave microbiota. Initially, their study was mainly linked to the occurrence of some diseases, such as white-nose syndrome and histoplasmosis. White-nose syndrome is caused by the fungus *Pseudogymnoascus destructans*, which grows in the eyes and nose of hibernating bats, leading to their death. It is estimated that this disease has decimated thousands of bats and caused billions in losses to agriculture (Jurado et al. 2010; Vanderwolf et al. 2013; Zhang et al. 2021). *Histoplasma capsulatum* is another fungus with great relevance in caves; it is the causal agent of histoplasmosis, a pulmonary infection with lethal potential in humans (Jurado et al. 2010; Silva et al. 2013; Vanderwolf et al. 2013). In a mycological study conducted in Tamboril Cave, Brazil, aimed at assessing the safety of opening the site for speleotourism, four researchers contracted histoplasmosis after collecting samples (Silva et al. 2013).

There are more than 26 000 known caves in Brazil, but knowledge about cave mycology is still incipient (Ministério do Meio Ambiente 2025; Prazeres et al. 2025). The first report of a cave fungus in Brazil was made in 1976 who isolated several fungi from soil samples from a cave in the Amazon rainforest, in the state of Amazonas (Castrillón et al. 1976). Since then, several studies have reported the diversity of fungi in Brazilian caves. In the RM3 cave located in the Iron Quadrangle (Minas Gerais), 32 fungal species were identified in sediment samples (Taylor et al. 2014). In the Brazilian Caatinga (Pernambuco), 37 fungal genera — distributed among the phyla Ascomycota, Basidiomycota, and Mucoromycota — were identified from air, guano, and bat mycobiota samples (Cunha et al. 2020). *Aspergillus guanovespertilionum* and *Penicillium cecavii* were described from samples of guano and spores present in the air of the Urubu Cave (Sergipe), in the Atlantic Forest (Lima et al. 2024).

Minas Gerais is the Brazilian state with the largest number of caves registered at the National Center for Cave Research and Conservation - ICMBIO/CECAV, with 12,911 caves corresponding to almost 50% of all known caves in the country (Ministério do Meio Ambiente, 2025). Minas Gerais is also home to the Southern Espinhaço Mountain Range, one of the main Brazilian geological formations, marked by several rocky outcrops, enormous diversity and high species endemism (Alves et al. 2022; Brina et al. 2025). Recently, studies developed in Serra do Espinhaço demonstrated the potential for discoveries of new fungal species, with a new genus (*Parahumicola*) and seven new species being reported in three different caves (Condé et al. 2023; Dutra et al. 2023; Leão et al. 2024). Given these findings, the objective of this work was: to describe two new species of the genus *Amphichorda* (Chapter 1); to know the mycological diversity present in the quartzite cave Curral de Pedras, Conceição do Mato Dentro - MG (Chapter 2); to know the mycological diversity of caves and ferruginous cavities of Serra da Ferrugem, Conceição do Mato Dentro - MG (Chapter 3); and to describe a new genus and new fungal species isolated from samples collected in caves (Chapter 4).

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CHAPTER 1

Two new species of coprophilous *Amphichorda* (*Bionectriaceae*, *Hypocreales*) in caves from the Cerrado, Brazil

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Two new species of coprophilous *Amphichorda* (*Bionectriaceae*, *Hypocreales*) in caves from the Cerrado, Brazil

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Abstract

Amphichorda (*Bionectriaceae*) is a diverse fungal genus that harbors coprophilic and entomopathogenic species from marine sediments and cave environments. Caves are mycological hotspots and a new frontier for the discovery of new fungal species. The objective of this study was to describe two new species of the genus *Amphichorda* isolated from animal dung samples found in caves. The samples were collected from marbles caves in Serra do Cipó, Minas Gerais, and sandstone caves in Chapada dos Guimarães municipality, Mato Grosso, Brazil. After fungal isolation, genomic DNA was extracted, and the ITS, LSU, *TEF* and *TUB* genes were sequenced. Phylogenetic analyses indicated that the isolates did not belong to any species previously described in the literature this for genius. Thus, we describe *Amphichorda* sp. nov. 1 and *Amphichorda* sp. nov. 2, two new coprophilic species of the genus *Amphichorda*.

Keywords: Ascomycota, biospeleology, molecular phylogeny, Serra do Espinhaço, taxonomy

Introduction

The genus *Amphichorda* is typified by *A. felina*, which was isolated from cat dung in France and was originally classified in the genus *Clavaria* (Fries 1825). Subsequently, based on incorrect lectotypification, it was transferred to the genus *Isaria* because of the production of synnemata (De Hoog 1972), and later moved to *Beauveria* (*Cordycipitaceae*) because of its morphological resemblance to the holoblastic conidiogenous cells (Carmichael et al. 1981; Hodge et al. 2005). However, conidiogenous cells with an apical denticulate rachis, which are common in *Beauveria* species, are absent in *A. felina* (Carmichael et al. 1981). Moreover, the phylogenetic trees of the introduced species *A. cavernicola* and *A. guana* showed clear divergence from other *Beauveria* species in *Cordycipitaceae* (Zhang et al. 2017, 2021). Based on phylogenetic analyses using rDNA regions, Guerra-Mateo et al. (2023) proposed the inclusion of *Amphichorda* in the family *Bionectriaceae*, which was later confirmed in the

studies by Leão et al. (2024) and Wang et al. (2024) that demonstrated its close relationship with the genus *Hapsidospora*. This reclassification was further supported by Wang et al. (2025), who demonstrated a close phylogenetic relationship between *Amphichorda* and *Ovicillium*, reinforcing its placement within *Bionectriaceae*.

Amphichorda comprises ten species, most of which exhibit a coprophilous lifestyle, including *A. cavernicola* (from bird dung), *A. coprophila* (chipmunk, porcupine and rabbit dung), *A. excrementa* (animal dung), *A. felina* (cat, porcupine, and rabbit dung), *A. guana* (bat guano and rabbit dung), *A. kunmingensis* (animal dung), and *A. guizhouensis* (bird feces) (Zhang et al. 2017, 2021; Guerra-Mateo et al. 2023; Wang et al. 2024; Wang et al. 2025). Notably, some *Amphichorda* species are reported exclusively in cave environments. For instance, *A. cavernicola* (found in bird feces) (Zhang et al. 2017) *A. guana* (found in bat guano) (Zhang et al. 2021), *A. yunnanensis* (from the wing surfaces of the bat *Rhinolophus affinis*) (Liu et al. 2023) in caves from China and *A. monjolensis* (from a potato-dextrose-agar plate exposed to a cave atmosphere, which was consumed by an insect) in a cave in Brazil (Leão et al. 2024).

Caves are unique ecosystems with spatial delimitation, partial or total absence of direct sunlight, little variation in temperature, and a limited amount of available nutrients (Poulson and White 1969; Zhang et al. 2017). These characteristics classify these environments as challenging for life; however, studies have demonstrated that caves can be hotspots for fungal discoveries and species with biotechnological potential (Zhang et al. 2021; Leão et al. 2024; Barbosa et al. 2025). For example, the cave-dwelling species *A. guana* produces the bioactive compounds with antifungal and anti-inflammatory activities (Liang et al. 2021; Jiang et al. 2023).

Brazil has a noteworthy number of caves in its territory, with 26,046 caves registered, of which 12,911 are located in the state of Minas Gerais and 585 in Mato Grosso (CECAV 2025). The Cerrado (Brazilian savanna) is the second largest biome in Brazil, spanning 25% (approximately 2 million km^2) of its territory (IBGE 2019). Owing to the increasing human impact on this diverse and pristine ecosystem, the Cerrado is considered a global hotspot for biodiversity conservation (Myers et al. 2000). Many Brazilian caves are in this biome which accounts for 12 008 of the registered caves (CECAV 2025), and only 18 caves have been studied for their micobiota (Prazeres et al 2025). In three caves located in the Serra do Espinhaço Meridional of Minas Gerais, a new fungal genus (*Parahumicola*) and seven new species were

described (*Parahumicola guana, Pseudohumicola alba, Pseudohumicola lutea, Chaetomium meridionalense, Cladosporium diamantinense, Cladosporium speluncae* and *Amphichorda monjolensis*) (Condé et al. 2023; Dutra et al. 2023; Leão et al. 2024).

In the context of increasing interest in cave-dwelling fungi and the underexplored fungal diversity of Brazilian caves, surveys were conducted in marble caves in Minas Gerais and quartzite caves in Mato Grosso. Samples of animal dung and other substrates showing apparent *Amphichorda* synnemata were collected. Based on four-locus phylogenetic analyses, two new species of *Amphichorda* were identified. Detailed morphological descriptions and comparative analyses with other known species of the genus were also performed.

Material and methods

Caves

Sampling was performed in caves from the Brazilian states of Mato Grosso and Minas Gerais. In Mato Grosso, sampling was performed in the Aroe Jari and Pobe Jari sandstone caves, both located in the municipality of Chapada dos Guimarães. Aroe Jari cave with 1 550m of extension, while Pobe Jari is approximately 500 m. Both caves are formed by sandstone rocks and are situated at an altitude of approximately 700 m. In the state of Minas Gerais, sampling was conducted in Gruta da Viola and Gruta Teto de Seixos caves, both located in the municipality of Santana Riacho. These caves are formed in marble rocks. Gruta da Viola has a horizontal length of 615 m, and is situated at an altitude of 867 m, while Gruta Teto de Seixos has a horizontal length of 500 m, and is situated at an altitude of 874 m. Both caves are located within the Morro da Pedreira protected area, which serves as a buffer zone surrounding Serra do Cipó National Park. Sampling was authorized by the Ministério do Meio Ambiente /Instituto Chico Mendes de Conservação da Biodiversidade (MMA/ICMBio) (SISBIO 90329-2).

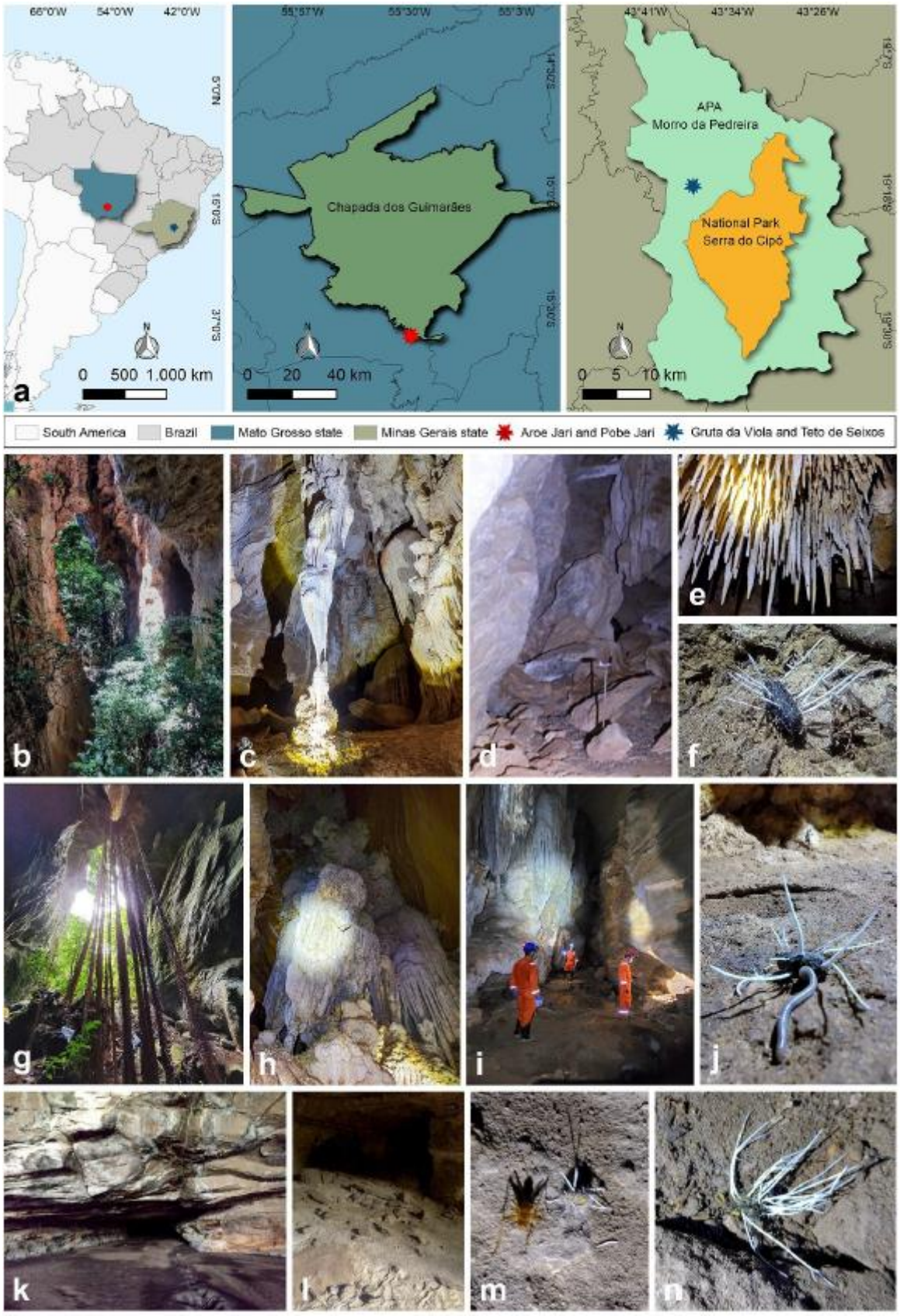

Figure 1 – Caves sampled in the Cerrado biome, Brazil. **a**. Geographical location of the sampled caves. **b.** Entrance of Gruta Teto de Seixos. **c–d.** Interior of the Gruta Teto de Seixos cave. **e.** Stalactites. **f.** Synnemata of *Amphichorda* on animal dung in Gruta Teto de Seixos cave. **g.** Entrance of Gruta da Viola cave. **h–i.** Interior of Gruta da Viola

cave. **j.** Myriapod feeding on animal dung colonized by *Amphichorda* in Gruta da Viola cave. **k–l.** Interior of Aroe Jari and Pobi Jari caves, respectively. **m.** An insect near an animal dung colonized by *Amphichorda* in Aroe Jari cave. **n.** Animal dung with *Amphichorda* synnemata in Pobe Jari cave.

Fungal collection and isolation

Airborne fungi were collected using the Koch sedimentation method (Kuzmina et al. 2012), in which 90 mm Petri dishes containing Dicloran Rose Bengal Chloramphenicol Agar (DRBC) were exposed at 1.5 m from the cave floor for 15 min in the cave environment. The plates were then closed, sealed, and stored in thermal boxes until transportation. Animal dung showing apparent *Amphichorda* colonization were collected at different points and stored in 50 mL Falcon tubes. Approximately 50 g of sediment samples were collected after removal of a 1 cm superficial layer and stored in sterilized zip-lock bags. Leaf litter was manually collected from inside the cave and stored in a paper bag. Samples were stored under refrigeration in thermal boxes, transported to the Laboratório de Micologia e Etiologia de Doenças Fúngicas de Plantas (MICOLAB) in the Universidade Federal de Viçosa (UFV), Minas Gerais, and refrigerated until processing.

The serial dilution method was used for fungal isolation from animal dung and sediment samples, according to Condé et al. (2023). Additionally, small fragments of *Amphichorda* synnemata were directly isolated from animal dung on Potato Dextrose Agar (PDA) amended with chloramphenicol (2 µg/mL). Leaf litter samples were processed as described by Condé et al. (2023). Pure cultures were obtained using the hyphal tip methodology (Tuite, 1969), which were stored in 10% glycerol at –20 °C, in sterilized distilled water at room temperature (Castellani, 1939), and in silica gel (Dhingra and Sinclair, 1995) and deposited in the working collection of the and deposited in the collection of the MICOLAB/UFV. Ex-types cultures were deposited in the Coleção Octávio Almeida Drummond (COAD) culture collection, while dried cultures representing the holotype of new species were deposited in the Herbarium VIC, both collections hosted at UFV.

DNA extraction, PCR and sequencing

Total genomic DNA was extracted from isolates grown in PDA at 25 °C in the dark for 7 d, using the Wizard® Genomic Purification Kit (Promega) following the protocol described by Custódio & Pereira (2025) with some modifications. Briefly, the samples were incubated at 65 °C for 15 min in a dry bath, and then 3 µL of RNA Solution (RNAase) was added and incubated in a dry bath at 37 °C for 15 min. After this period, the solution was cooled to –20 °C for 5 min, 300 µL of Protein Precipitation Solution was added, mixed by vortexing at high speed

for 10 s, and centrifuged for 5 min at 14,000 rpm. Subsequently, 600 µL of the supernatant was transferred to a 1.5 mL microtube containing 600 µL of chilled isopropanol. The solution was manually shaken for 1 min and stored at −20 °C for 40 min. The solution was centrifuged at 14,000 rpm for 5 min, the supernatant was discarded, followed by two washes with 600 µL of chilled 70% ethanol, manual shaking for 1 min, and centrifugation at 14,000 rpm for 5 min. After discarding the ethanol, the microtube containing the pellet was placed to drain on paper towels for 30 s and the ethanol was completely evaporated in a dry bath at 45 °C for a period of 5 to 10 min (depending on the amount of ethanol in the microtube). After drying the pellet, 100 µL of DNA Rehydration Solution was added, shaken manually for 1 min, and incubated in a dry bath at 65 °C for 60 min.

The genomic regions targeted for PCR amplification were the internal transcribed spacers 1 and 2 and intervening 5.8S rDNA region (ITS), nuclear 28S rDNA region (LSU), RNA polymerase second largest subunit (*RPB2*), translation elongation factor 1-alpha gene (*TEF*) and partial beta-tubulin gene region (*TUB*), using the primers and cycling conditions described by Leão et al. (2024). DNA amplicons were purified and sequenced by Macrogen Inc., South Korea (http://www.macrogen.com).

Phylogenetic analyses

The sequences were edited and assembled, and contigs were constructed using FinchTV v.1.4.0 software. The BLASTn® algorithm (blast.ncbi.nlm.nih.gov/Blast.cgi) was used to confirm the taxonomic identity of the isolates at the genus level, and a database of published sequences for the genus *Amphichorda* was constructed (Table 1). The sequences from each DNA region were aligned using MAFFT v.7 (https://mafft.cbrc.jp/alignment/server/index.html) and manually optimized using MEGA v.10 (Kumar et al. 2018). Individual alignments were concatenated using Sequence Matrix 1.8 (Vaidya et al. 2011). Phylogenetic trees of individual and concatenated datasets (ITS, LSU, *TEF* and *TUB*) were constructed using Bayesian Inference (BI) and maximum-likelihood (ML) analyses.

For BI analysis, the best nucleotide substitution model was calculated using MrModelTest 2.3 (Nylander, 2004), according to the Akaike Information Criterion. MrBayes v.3.2.7 (Ronquist et al. 2012) software was used for tree construction. Two runs were conducted simultaneously. Four Markov Chain Monte Carlo (MCMC) chains were conducted simultaneously in each run, and trees were randomly initialized for up to 10,000,000 generations. Trees were sampled every 1,000 generations, resulting in 10,001 trees per run. The

first 2,500 trees were discarded from the analysis. Subsequently, posterior probability values (Rannala & Yang, 1996) were determined from the consensus tree using the remaining trees. For ML analysis, the IQTREE v. 2.1.3 (Nguyen et al. 2015) was used. Ultrafast bootstrapping (Hoang et al. 2018) with 10,000 replicates was used, and the best nucleotide substitution model for each region was estimated using ModelFinder (Kalyaanamoorthy et al. 2017). Phylogenetic trees were visualized using FigTree v. 1.4.3 (Rambaut 2018) and exported to editing software. All sequences obtained in this work were deposited in the GenBank database (http://www.ncbi.nlm.nih.gov) and the accession numbers are listed in Table 1.

Table 1 - List of species and sequences used in phylogenetic analyses. Species and sequences obtained in this study are presented in bold.

Species	Strain	Genbank accession number				Reference
		ITS	LSU	*TEF*	*TUB*	
***Amphichorda* sp. nov. 1**	**COAD 3997^{T}**	**PV635513**	**PV635496**	**PV645727**	**PV645733**	**This study**
***Amphichorda* sp. nov. 1**	**COAD 3998**	**PV635512**	**PV635495**	**PV645728**	**n.d**	**This study**
***Amphichorda* sp. nov. 1**	**CDA4617**	**PV635501**	**n.d**	**n.d**	**n.d**	**This study**
***Amphichorda* sp. nov. 1**	**CDA4618**	**PV635502**	**n.d**	**n.d**	**n.d**	**This study**
***Amphichorda* sp. nov. 1**	**CDA4619**	**PV635503**	**n.d**	**n.d**	**n.d**	**This study**
***Amphichorda* sp. nov. 1**	**CDA4621**	**PV635504**	**n.d**	**n.d**	**n.d**	**This study**
***Amphichorda* sp. nov. 1**	**CDA4622**	**PV635505**	**n.d**	**n.d**	**n.d**	**This study**
***Amphichorda* sp. nov. 1**	**CDA4623**	**PV635506**	**n.d**	**n.d**	**n.d**	**This study**
***Amphichorda* sp. nov. 1**	**CDA4624**	**PV635507**	**n.d**	**n.d**	**n.d**	**This study**
***Amphichorda* sp. nov. 1**	**CDA4625**	**PV635508**	**n.d**	**n.d**	**n.d**	**This study**
***Amphichorda* sp. nov. 1**	**CDA4626**	**PV635509**	**n.d**	**n.d**	**n.d**	**This study**
***Amphichorda* sp. nov. 1**	**CDA4628**	**PV635510**	**n.d**	**n.d**	**n.d**	**This study**
***Amphichorda* sp. nov. 1**	**CDA4629**	**PV635511**	**n.d**	**n.d**	**n.d**	**This study**
***Amphichorda* sp. nov. 2**	**COAD 3999^{T}**	**PV635499**	**PV635494**	**PV645729**	**PV645732**	**This study**
***Amphichorda* sp. nov. 2**	**COAD 4000**	**PV635500**	**PV635493**	**PV645730**	**PV645731**	**This study**
Amphichorda cavernicola	CGMCC3.19571^{T}	MK329056	MK328961	MK335997	MK336083	Zhang et al. (2021)
Amphichorda cavernicola	LC12481	MK329057	MK328962	MK335998	MK336084	Zhang et al. (2021)
Amphichorda cavernicola	LC12485	MK329058	MK328963	MK335999	MK336085	Zhang et al. (2021)
Amphichorda coprophila	CBS 247.82	MH861494	MH873238	OQ954487	OQ981138	Guerra-Mateo et al. (2023)
Amphichorda coprophila	CBS 424.88	OQ942929	OQ943166	OQ954488	OQ981139	Guerra-Mateo et al. (2023)
Amphichorda coprophila	CBS 173.71	AY261368	MH871833	OQ954489	OQ981140	Guerra-Mateo et al. (2023) & Wang et al. (2025)
Amphichorda excrementa	YFCC AECCS848 ^{T}	n.d	OR913439	OR917446	n.d	Wang et al. (2024)
Amphichorda excrementa	CBS 110.08	MH854578	OQ943168	OQ954492	OQ981143	Guerra-Mateo et al. (2023) & Wang et al. (2025)

Amphichorda felina	CBS 250.34	MH855498	OQ943167	OQ954490	OQ981141	Guerra-Mateo et al. (2023) & Wang et al. (2025)
Amphichorda felina	CBS 648.66	OQ942930	MH870575	OQ954491	OQ981142	Guerra-Mateo et al. (2023)
Amphichorda guana	CGMCC3.17908 T	KU746665	KU746711	KX855211	KU746757	Zhang et al. 2017
Amphichorda guana	CGMCC3.17909	KU746666	KU746712	KX855212	KU746758	Zhang et al. 2017
Amphichorda guizhouensis	GMBC 3005 T	PQ726815	PQ726840	PQ758605	n.d	Wang et al. (2025)
Amphichorda guizhouensis	GMBC 3006	PQ726816	PQ726841	PQ758606	n.d	Wang et al. (2025)
Amphichorda kunmingensis	YFCC AKYYH8414 T	n.d	OR913438	OR917448	n.d	Wang et al. (2024)
Amphichorda kunmingensis	YFCC AKYYH8487	n.d	OR913437	OR917449	n.d	Wang et al. (2024)
Amphichorda kunmingensis	CBS 312.50	MH856641	MH868150	OQ954493	OQ981144	Guerra-Mateo et al. (2023) & Wang et al. (2025)
Amphichorda littoralis	FMR 19404 T	OQ942924	OQ943161	OQ954482	OQ981133	Guerra-Mateo et al. (2023)
Amphichorda littoralis	FMR 19611	OQ942926	OQ943163	OQ954484	OQ981135	Guerra-Mateo et al. (2023)
Amphichorda littoralis	FMR 20067	OQ942927	OQ943164	OQ954485	OQ981136	Guerra-Mateo et al. (2023)
Amphichorda monjolensis	COAD 3124 T	OQ288256	OQ288260	OR454090	OQ405043	Leão et al. (2024)
Amphichorda monjolensis	COAD 3125	OQ288257	n.d	n.d	n.d	Leão et al. (2024)
Amphichorda monjolensis	COAD 3120	OQ288258	n.d	n.d	n.d	Leão et al. (2024)
Amphichorda yunnanensis	KUMCC 21-0416 T	n.d	n.d	OR025975	n.d	Liu et al. (2023)
Amphichorda yunnanensis	KUMCC 21-0415	ON426824	n.d	OR025976	n.d	Liu et al. (2023)
Amphichorda yunnanensis	KUMCC 21-0414	ON426823	n.d	OR025977	n.d	Liu et al. (2023)
Hapsidospora chrysogena	CBS 144.62^{T}	OQ429645	OQ055551	OQ470953	n.d	Hou et al. (2023)
Hapsidospora flava	CBS 596.70^{T}	OQ429649	OQ055555	OQ470957	n.d	Hou et al. (2023)
Hapsidospora stercoraria	CBS 516.70^{T}	OQ429662	OQ055568	OQ470970	n.d	Hou et al. (2023)
Ovicillium asperulatum	CBS 130362^{T}	OQ429756	OQ055655	OQ471082	n.d	Hou et al. (2023)
Ovicillium attenuatum	CBS 399.86^{T}	OQ429757	OQ055656	OQ471083	n.d	Hou et al. (2023)
Ovicillium pseudoattenuatum	GMBC 3007^{T}	PQ726817	PQ726842	PQ758607	n.d	Hou et al. (2023)
Bulbithecium ammophilae	CBS 178.78^{T}	OQ429504	OQ055415	OQ470793	n.d	Hou et al. (2023)
Bulbithecium arxii	CBS 737.84^{T}	OQ429505	OQ055416	OQ470794	n.d	Hou et al. (2023)
Bulbithecium ellipsoideum	CBS 993.69^{T}	OQ429507	OQ055418	OQ470796	n.d	Hou et al. (2023)
Alloacremonium ferrugineum	CBS 102877^{T}	OQ429495	OQ055406	OQ470785	n.d	Hou et al. (2023)
Alloacremonium humicola	CBS 613.82^{T}	OQ429496	OQ055407	OQ470786	n.d	Hou et al. (2023)
Acremonium alternatum	CBS 407.66^{T}	OQ429442	OQ055353	OQ470739	n.d	Hou et al. (2023)

Acremonium chlamydosporium	CBS 414.76^{T}	OQ429450	OQ055361	OQ470748	n.d	Hou et al. (2023)
Acremonium egyptiacum	CBS 114785^{T}	OQ429456	OQ055362	OQ470749	n.d	Hou et al. (2023)
Clonostachys rosea	CBS 710.86^{T}	OQ910774	OQ911133	OQ944786	OQ982801	Zhao et al. (2023)
Clonostachys solani	CBS 697.88^{T}	OQ910828	OQ911187	OQ944838	OQ982847	Zhao et al. (2023)
Clonostachys farinosa	CBS 364.78^{T}	OQ910614	OQ910973	OQ944626	OQ982649	Zhao et al. (2023)

Morphological studies

Macroscopic characteristics of the colonies were observed using PDA, synthetic nutrient-poor agar (SNA), and oatmeal agar (OA) for 14 days at 25 ºC in the dark. The size, length, color, and appearance of the colonies were carefully observed and recorded. Color names used in the descriptions follow Rayner (1970). Microscopic slides were prepared using the microculture technique (Riddell, 1950). Small fragments of *Amphichorda* synemmata were grown in small blocks of PDA, SNA, and OA culture medium, covered with sterilized coverslips, and incubated for 7 days in the dark. Subsequently, the coverslips were transferred to a slide and mounted with lactoglycerol. Microphotographs of the reproductive structures were captured using an Olympus BX53 compound microscope equipped with an Olympus Q-Color5 digital camera. At least 30 measurements of relevant morphological features were obtained using the cellSens Dimension 1.9 software.

Results

Fungal isolates

In this study we focused only on the identification of *Amphichorda* isolates. Seventeen isolates belonging to *Amphichorda* were obtained from samples collected in the caves studied (Table 2). One isolate was obtained from Aroe Jari cave and one from Pobe Jari cave from the animal dung samples. In Gruta da Viola, seven isolates were obtained from animal dung, and one isolate from plates exposed to collect airborne particles. In Gruta Teto de Seixos cave, five isolates were obtained from animal dung, one isolated from the sediment, and one isolated from leaf litter.

Table 2 – Description of the isolates found in relation to the sample of origin, place where the sample was collected and cave.

Cave	Sample type	Collection site	Isolate code

Gruta da Viola	Airborne particles collected by Koch sedimentation	Hall located to the left approximately 42 m from the entrance	CDA4619
	Animal dung and sediment	Room located on the right side approximately 15 m from the entrance	CDA4617, CDA4620
	Animal dung	The last room of the cave, approximately 60 m from the entrance	CDA4618
	Animal dung with *Amphichorda* synemmata	Room located on the left side of the cave approximately 12 m from the main entrance.	COAD 3998 (direct isolation), CDA4621, CDA4622 and CDA4628
Teto dos Seixos	Animal dung with *Amphichorda* synemmata	Main hall of the cave approximately 9 m from the entrance.	COAD 3997 (direct isolation), CDA4623, CDA4624, CDA4625 and CDA4629
	Mycelial growth in leaf litter	Main hall approximately 10 m from the entrance, close to the left wall of the cave	CDA4626
	Sediment	Upper hall on the right side approximately 60 m from the entrance	CDA4627
Aroe Jari	Sediment sample	Main hall	COAD 3999
Pobe Jari	Animal dung with *Amphichorda* synemmata	Main hall	COAD 4000

Phylogenetic analyses

The concatenated alignment of the genus *Amphichorda* had a size of 2,689 characters, including gaps (ITS = 603, LSU = 866, *TEF* = 917 and *TUB* = 301). For BI analysis, the best nucleotide substitution models were GTR+G for ITS and *TUB*, GTR+I+G for LSU and *TEF*. For ML, they were GTR+F+G4 for ITS, TIM3+F+I+R3 for LSU and *TEF* and TIM3+F+G4 for *TUB*.

Based on phylogenetic analyses, the isolates found in this study did not group with any known species and clustered into two novel clades. The isolates COAD 3997 and COAD 3998 formed a monophyletic clade with good phylogenetic support and were sister to *A. monjolensis* (0.97 bs and 94 pp) (Figure 2). Isolates COAD 3999 and COAD 4000 clustered in a unique monophyletic clade from other described species, with high phylogenetic support (0.97 bs and 90 pp), forming a basal branch of the clade formed by the isolates COAD 3997, COAD 3998

and *A. monjolensis* (Figure 2). The other *Amphichorda* sp. nov. 1 isolates were identified only by the phylogeny of the ITS region.

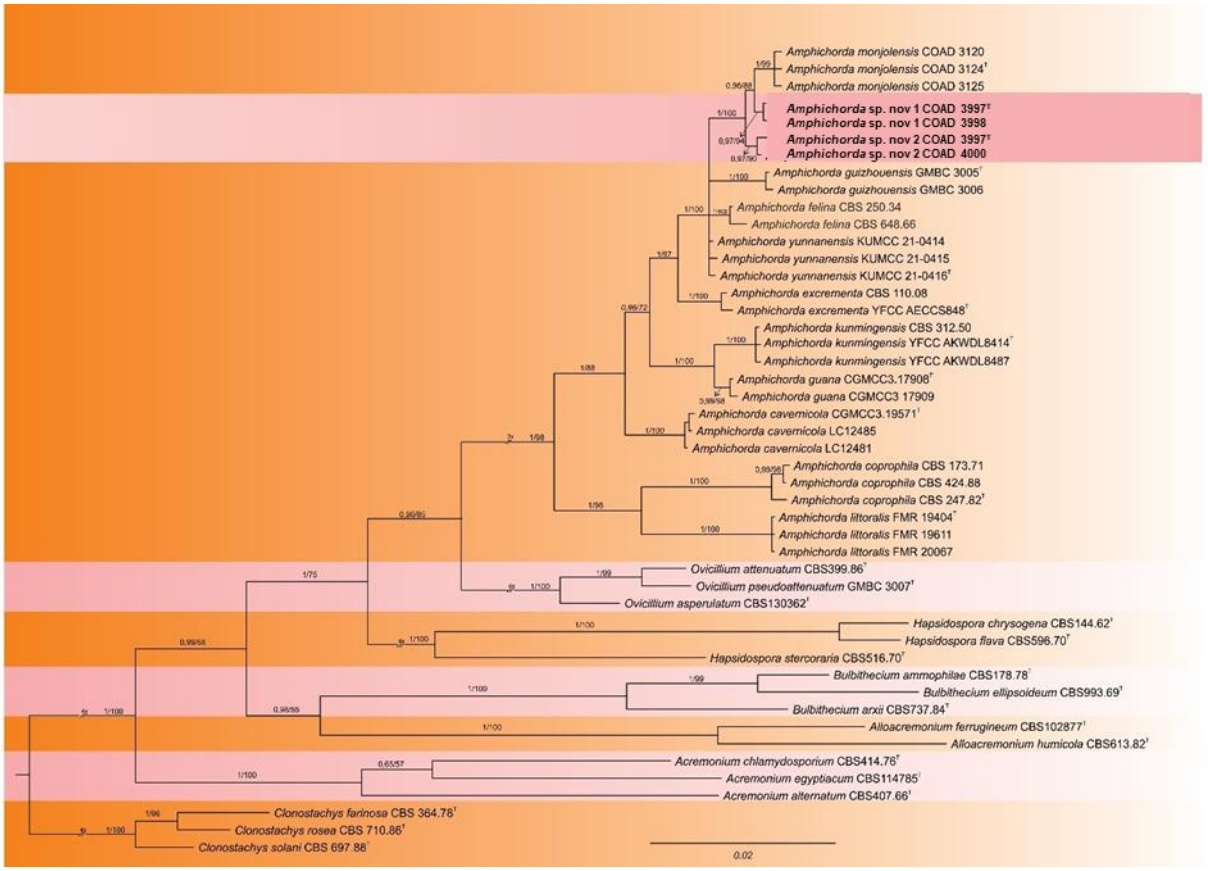

Figure 2 – Bayesian inference and maximum likelihood consensus tree inferred from combined multiple sequence alignments of ITS, LSU, *TEF* and *TUB*. Bayesian posterior probability support values from MCMC (BYPP, first value) and Bootstrap maximum likelihood (ML, second value) analyses are presented above the nodes. The tree is rooted by *Clonostachys rosea* CBS 710.86^{T}, *Clonostachys solani* CBS 697.88^{T} and *Clonostachys farinosa* CBS 364.78^{T}. Ex-type strains are indicated with "T". Newly generated sequences are indicated in bold.

Therefore, we introduce *Amphichorda* sp. nov. 1 sp. nov.for isolates COAD 3997 and COAD 3998, and *Amphichorda* sp. nov. 2 sp. nov. for isolates COAD 3999 and COAD 4000 (Figure 2). When comparing the nucleotide composition (Table 3) and morphology (Table 4) of our species with other species described in the genus, we found significant differences that support our proposal. These new reports increase the number of species described within the genus to 12.

Table 3 – Comparison table of nucleotide composition between proposed species and described species in the genus.

Species	ITS			LSU			*TEF*			*TUB*		
	per-site mutations	gaps	identity	per-site mutations	gaps	identity	per-site mutations	gaps	identity	per-site mutations	gaps	identity
						***Amphichorda* sp. nov. 1 COAD 3997^{T}**						
***Amphichorda* sp. nov. 2 COAD 3999^{T}**	**538/539**	**0**	**99%**	**857/861**	**0**	**99%**	**927/927**	**0**	**100%**	**285/289**	**2**	**99%**
Amphichorda monjolensis COAD 3124^{T}	533/535	0	99%	1068/1071	1	99%	822/826	0	99%	296/305	8	97%
Amphichorda cavernicola CGMCC3.19571^{T}	496/506	1	98%	855/862	1	99%	892/910	0	98%	281/296	7	95%
Amphichorda coprophila CBS 247.82^{T}	475/500	5	95%	877/891	0	98%	753/782	0	96%	277/301	9	92%
Amphichorda excrementa YFCC AECCS848^{T}	-	-	-	814/819	1	99%	856/871	0	98%	-	-	-
Amphichorda felina CBS 250.34	535/539	0	99%	792/796	0	99%	951/960	0	99%	290/300	7	97%
Amphichorda guana CGMCC3.17908^{T}	512/532	2	96%	845/848	0	99%	872/884	0	99%	290/305	9	95%
Amphichorda guizhouensis GMBC 3005^{T}	529/539	0	98%	885/891	8	99%	944/953	0	99%	-	-	-
Amphichorda kunmingensis YFCC AKYYH8414^{T}	-	-	-	817/822	1	99%	845/858	0	98%	-	-	-
Amphichorda littoralis FMR 19404^{T}	534/545	4	98%	839/853	0	98%	891/930	1	96%	276/303	9	91%
Amphichorda yunnanensis KUMCC 21-0416^{T}	544/552	2	99%	-	-	-	927/937	3	99%	-	-	-
						***Amphichorda* sp. nov. 2 COAD 3999^{T}**						
***Amphichorda* sp. nov. 1 COAD 3997^{T}**	**538/539**	**0**	**99%**	**857/861**	**0**	**99%**	**927/927**	**0**	**100%**	**285/289**	**2**	**99%**
Amphichorda monjolensis COAD 3124^{T}	532/535	0	99%	1065/1071	1	99%	822/826	0	99%	284/289	2	98%
Amphichorda cavernicola CGMCC3.19571^{T}	495/506	1	98%	853/862	1	99%	887/905	0	98%	275/289	3	95%
Amphichorda coprophila CBS 247.82^{T}	474/500	5	95%	877/891	0	98%	747/776	0	96%	270/291	4	93%
Amphichorda excrementa YFCC AECCS848^{T}	-	-	-	814/818	1	99%	852/867	0	98%	-	-	-
Amphichorda felina CBS 250.34	534/539	0	99%	792/796	0	99%	945/954	0	99%	283/290	2	98%
Amphichorda guana CGMCC3.17908^{T}	514/534	6	96%	843/848	0	99%	872/884	0	99%	278/290	3	96%
Amphichorda guizhouensis GMBC 3005^{T}	528/539	0	98%	885/891	0	99%	938/947	0	99%	-	-	-
Amphichorda kunmingensis YFCC AKYYH8414^{T}	-	-	-	817/822	1	99%	841/854	0	98%	-	-	-
Amphichorda littoralis FMR 19404^{T}	533/545	4	98%	839/853	0	98%	885/924	1	96%	266/289	3	92%
Amphichorda yunnanensis KUMCC 21-0416^{T}	543/552	2	98%	-	-	-	921/931	3	99%	-	-	-

Taxonomy

Amphichorda **sp. nov. 1** A.F. Leão, D.O. Ramos, T.O. Condé, F.A Custódio & O.L. Pereira, **sp. nov.** Figure 3. MycoBank no.: MB 860071.

Etymology: The epithet refers to the name of the state, Minas Gerais, where the cave is located.

Habitat and distribution: saprophytically on airborne particles, animal dung, leaf litter and sediments in the caves Gruta Teto de Seixos and Gruta da Viola, only known from the type locality in Santana do Riacho, Minas Gerais state, Brazil.

Holotypus: BRAZIL, Santana do Riacho municipality, Minas Gerais state, Gruta Teto de Seixos cave, isolated from animal dung, March 2024, collected by D.R. Oliveira, T.O. Condé, F.A Custódio, J.A. Oliveira, O.L. Pereira and isolated by A.F. Leão (Holotype VIC 49620, preserved as dried culture; culture ex-type: COAD 3997).

ITS barcode: PV635513. Secondary markers: LSU: PV635496, *TEF*: PV645727, *TUB* PV645733 and *RPB2* PV645734.

Additional specimens examined: Brazil, Minas Gerais state, Gruta da Viola and Teto dos Seixos, collected in animal dung sample, airborne particles and sediment, March 2024, collected by D.R. Oliveira, T.O. Condé, F.A Custódio, J.A. Oliveira, O.L. Pereira and isolated by A.F. Leão. Living culture COAD 3998, CDA4617, CDA4618, CDA4619, CDA4620, CDA4621, CDA4622, CDA4623, CDA4624, CDA4625, CDA4626, CDA4627, CDA4628, CDA4629.

Description: *Hyphae* hyaline, smooth-walled, branched. Asexual morph: *Synnemata* white color, emerging from the central part of the colonies on PDA and OA, cylindrical, with branched apex. (Description made in the middle OA) *Conidiophores* hyaline, arising laterally from hyphae, cylindrical, straight or slightly curved, containing one or more conidiogenic cells. *Conidiogenous cells* arising laterally from hyphae or from conidiophores, cylindrical, straight or curved, flask-shaped, 4.1–7.6 × 1.3–2.9 ($\overline{x}$ = 5.7 × 2.0 µm, n = 30). *Conidia* holoblastic, ellipsoidal, hyaline, smooth, often attached of conidiogenous cells 3.0–4.1 × 2.3–2.9 ($\overline{x}$ = 3.5 × 2.5 µm, n = 30). Sexual morph: Undetermined.

Culture characteristics: (after 14 days at 25ºC in the dark) Colonies on PDA reaching 22–29 mm diam., raised with a concave and undulate edge, aerial mycelia moderately produced at the

center, color rosy buff (61); reverse saffron (10). Colonies on SNA reaching 9–12 mm diam., umbonate, undulated edge, aerial mycelia sparse to absent, without synnemata production, color white; reverse salmon (41). Colonies on OA reaching 24–33 mm diam., flat or effuse, lobate edge, aerial mycelia dense, radially arranged synnemata and profusely produced, translucid; reverse rosy buff (61).

Notes: Based on multilocus phylogenetic analyses (ITS, LSU, *TEF* and *TUB*) *Amphichorda* sp. nov. 1 formed a distinct clade with statistical support (bs = 0.97 and pp = 94) closely related with *A. monjolensis* and *Amphichorda* sp. nov. 2 (Figure 2). Pairwise sequence comparisons showed differences between *Amphichorda* sp. nov. 1 and *A. monjolensis* and *Amphichorda* sp. nov. 2 (Table 3). The morphological characteristics also differed between *Amphichorda* sp. nov. 1 from the other species in this clade. Compared to *A. monjolensis*, colonies of *Amphichorda* sp. nov. 1 were slightly smaller on PDA (22–29 mm *vs*. 24–31 mm) and SNA (9–12 mm *vs*.13–17 mm), and larger on OA (24–33 mm *vs*. 17–21 mm and differed in coloration on SNA (white *vs*. translucid) and OA (translucid *vs*. white). The conidiogenous cells of *Amphichorda* sp. nov. 1 were longer but slightly shorter in width (4.1–7.6 × 1.3–2.9 µm *vs*. 3.1–6.1 × 2.7–5.1 µm) and conidia were slightly bigger (3.0–4.1 × 2.3–2.9 µm *vs*. 2.8–3.7×1.8–2.9 µm) than those of *A. monjolensis*. Compared to *Amphichorda* sp. nov. 2, colonies of *Amphichorda* sp. nov. 1 were smaller on SNA (9–12 mm *vs*. 11.5–20 mm) and OA (24–33 mm *vs*. 29–39 mm) and differed in coloration on OA (colorless *vs*. white). The conidiogenous cells of *Amphichorda* sp. nov. 1 were larger (4.1–7.6 × 1.3–2.9 µm *vs*. 3.6–6.5 × 1.8–2.8 µm) than those of *Amphichorda* sp. nov. 2 (Table 4).

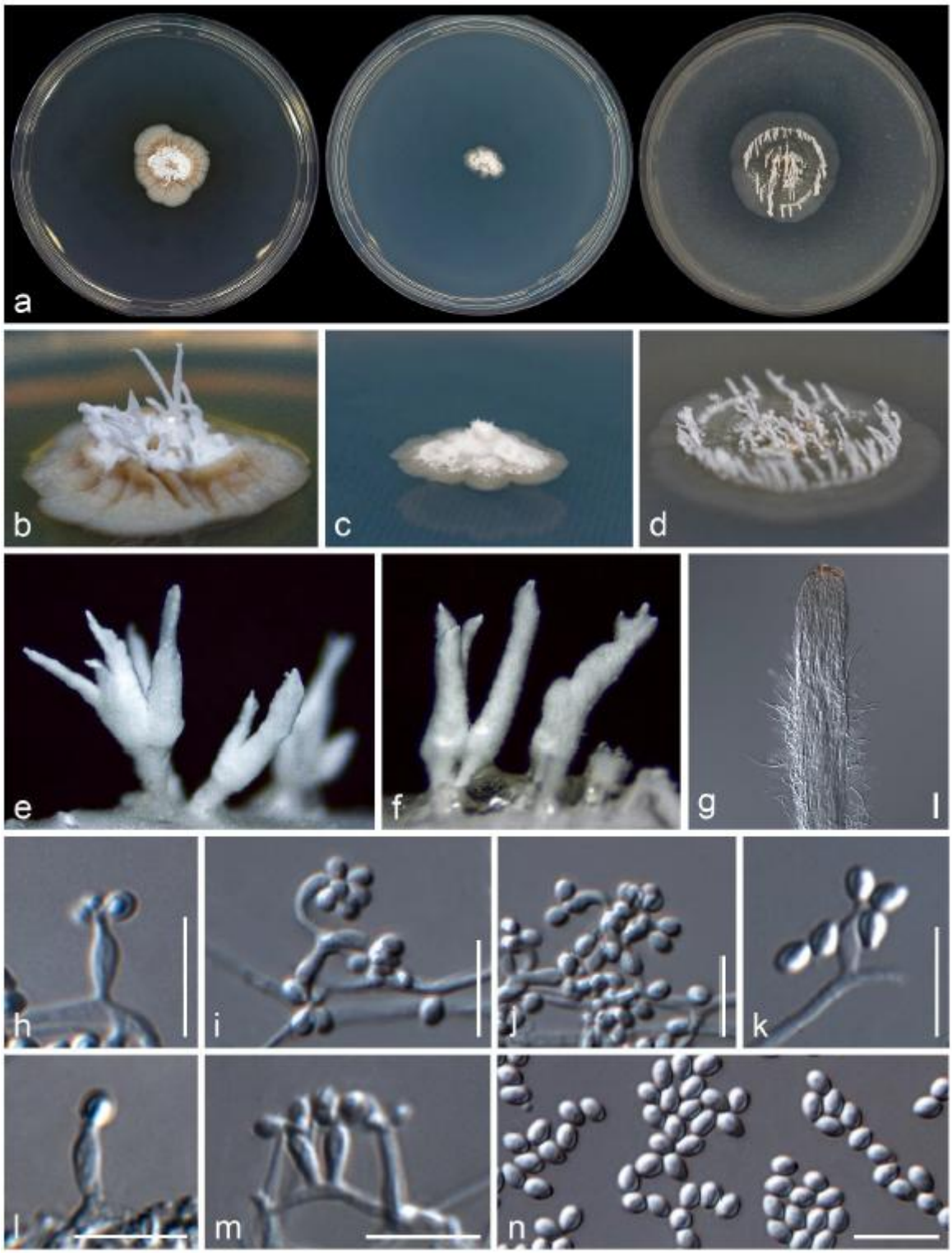

Figure 3 – *Amphichorda* sp. nov. 1 COAD 3997. **a.** Colonies from left to right on PDA, SNA and OA after 14 days at 25 °C in the dark. **b–d.** Texture of colonies on PDA, SNA, and OA, respectively. **e–f.** Synnemata growing on OA. **g.** Synnemata on microscopic view. **h–m**. Conidiophores and conidiogenous cells. **n.** Conidia. Scale bars: g = 20 µm; f–i = 10 µm

Amphichorda **sp. nov. 2** F.A. Custodio, A.F. Leão, T.O. Condé & O.L. Pereira, **sp. nov.** Figure 4. MycoBank no.: MB 860078.

Etymology: The epithet refers to the name of the state, Mato Grosso, where the cave is located.

Habitat and distribution: saprophytically on animal dung in the caves Aroe Jari and Pobe Jari, only known from the type locality in Chapada dos Guimarães, Mato Grosso state, Brazil.

Type: BRAZIL, Chapada dos Guimarães municipality, Mato Grosso state, Pobe Jari cave, isolated from animal dung, October 2023, collected by F.A. Custódio, and isolated by A.F. Leão & F.A. Custódio (Holotype: VIC 49621, preserved as dried culture; culture ex-type: COAD 3999).

ITS barcode: PV635499. Secondary markers: LSU: PV635494, *TEF*: PV645729, *TUB*: PV645732 and *RPB2*: PV645736.

Additional specimens examined: BRAZIL, Mato Grosso, Aroe Jari cave, collected from animal dung sample, October 2023, collected by F.A. Custódio, and isolated by A.F. Leão, F.A. Custódio (Culture ex-type: COAD 4000).

Description: *Hyphae* hyaline, smooth-walled, branched. Asexual morph**:** *Synnemata* emerging from the central part of the colonies, cylindrical and presenting branched apex in on PDA, OA and SNA. (Description made in the middle OA) *Conidiophores* hyaline, cylindrical, straight or slightly curved. *Conidiogenous cells* cylindrical straight or curved, flask-shaped, arising laterally from hyphae or conidiophores, 3.6–6.5 × 1.8–2.8 ($\overline{x}$ = 5.1 × 2.2 µm, n = 30). *Conidia* holoblastic, obovoid, occasionally ellipsoidal, hyaline, smooth, 3.1–4 × 1.9–3.6 µm ($\overline{x}$ = 3.5 × 2.4 µm, n = 30). Sexual morph: Undetermined.

Culture characteristics: (after 14 days at 25ºC in the dark) Colonies on PDA reaching 20–27 mm diam., convex or dome-shaped, undulate edge, aerial mycelia dense in the center with emerging synnemata, color buff (45); reverse buff (45). Colonies on SNA, reaching 11.5–20 mm diam., flat or effuse, undulate edge, aerial mycelia sparse to absent with a single synnema, colour white; reverse rosy buff (61). Colonies on OA, reaching 29–39 mm diam., flat or effuse, entire edge, aerial mycelia moderate, radial growth of the colonies with branched tipped synnemata emerging from the median rings of the colony, color white; reverse rosy buff (61), with growth halos.

Notes: Four-loci phylogenetic analyses (ITS, LSU, *TEF* and *TUB*) showed that *Amphichorda* sp. nov. 2 formed a distinct clade with statistical support (bs = 0.97 and pp = 90) closely related to *Amphichorda* sp. nov. 1 and *A*. *monjolensis* (Figure 2). Pairwise sequence comparisons showed differences between *Amphichorda* sp. nov. 2 and *A*. *monjolensis* and *Amphichorda* sp. nov. 1 (Table 3). Morphological differences between *Amphichorda* sp. nov. 2 and *Amphichorda* sp. nov. 1 are listed in the latter notes. Compared to *A*. *monjolensis*, colonies of *Amphichorda* sp. nov. 2 were smaller on PDA (20–27 mm *vs*. 22–29 mm), larger on OA (29–39 mm *vs*. 17–21 mm), and differed in coloration on SNA (white *vs*. translucid). The conidiogenous cells of *Amphichorda* sp. nov. 2 were slightly shorter in width (3.6–6.5 × 1.8–2.8 µm *vs*. 3.1–6.1 × 2.7–5.1 µm) and conidia were slightly bigger (3.1–4 × 1.9–3.6 µm *vs*. 2.8–3.7×1.8–2.9 µm) (Table 4).

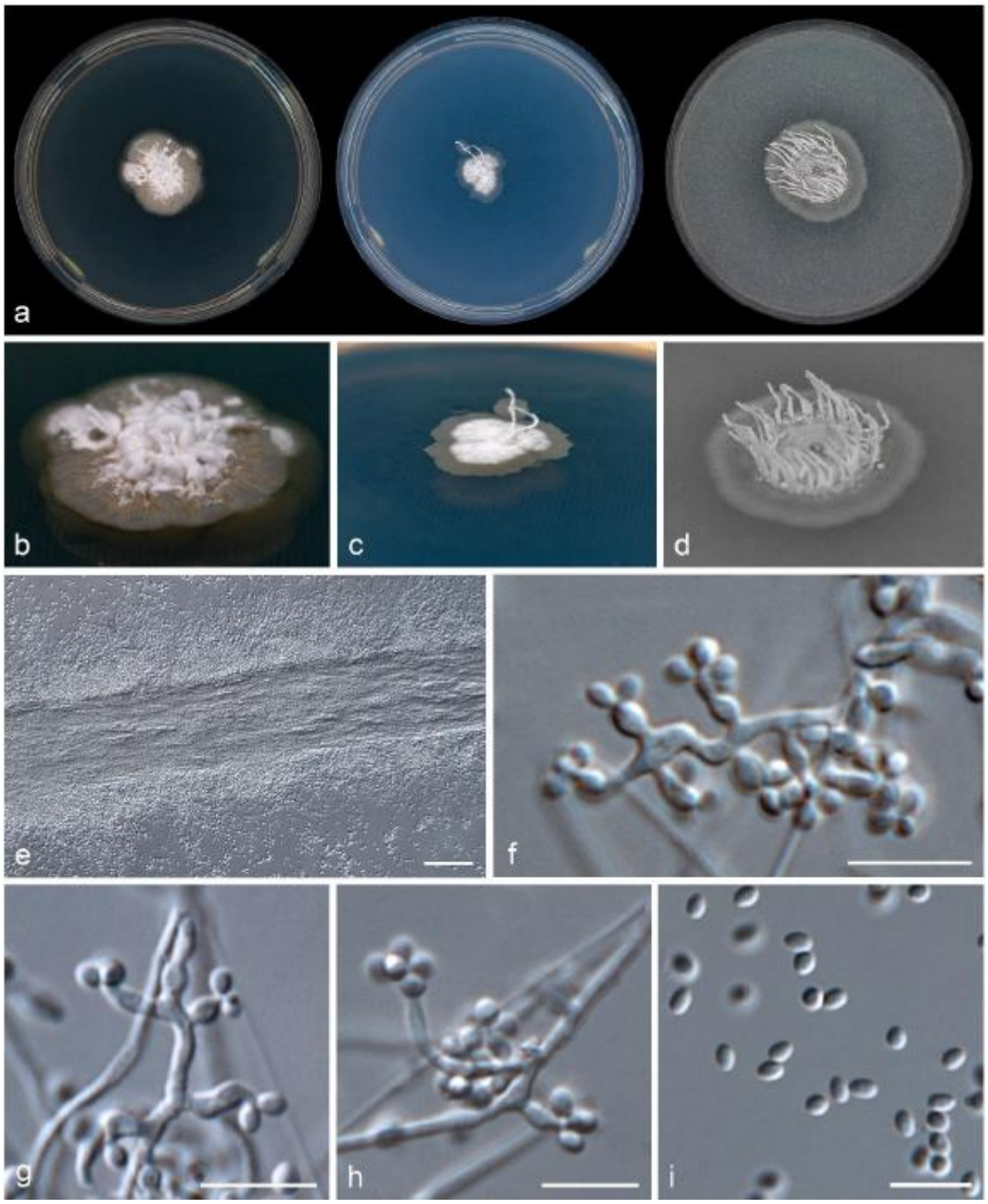

Figure 4 – *Amphichorda* sp. nov. 2 COAD 3999. **a.** Colonies from left to right on PDA, SNA and OA after 14 days at 25 °C in the dark. **b**–**d.** Texture of colonies on PDA, SNA, and OA, respectively **e.** Synnemata on microscopic view. **f–h**. Conidiophores and conidiogenous cells. **i.** Conidia. Scale bars: e = 50 µm; f–i = 10 µm

Table 4 – Comparative table of morphology between proposed species and species described in the genus.

Species	Host/Substrate	Color and diameter (mm) of colonies			Conidiophores	Conidiogenous cells (µm)	Conidia shape and size (µm)	Reference
		PDA	SNA	OA				
***Amphichorda* sp. nov. 2**	Animal faeces collected in sandstone cave	White and cream, 20–27	White and cream, 11.5–20	White, 29–39	Hyaline, cylindrical, straight or slightly curved	Cylindrical straight or curved, flask-shaped, arising laterally from hyphae or conidiophores, 3.6–6.5 × 1.8–2.8 ($\bar{x}$ = 5.1 × 2.2 µm, n = 30).	Holoblastic, obovoid, occasionally ellipsoidal, smooth, 3.1–4 × 1.9–3.6 ($\bar{x}$ = 3.5 × 2.4 µm, n = 30).	**This study**
***Amphichorda* sp. nov. 1**	Animal faeces collected marble in cave	White and cream, 22–29	White, 9–12	Colorless, 24–33	Hyaline, arising laterally in the hyphae, cylindrical, straight or slightly curved, containing one or more conidiogenic cells	Arising laterally from hyphae or conidiophores, cylindrical straight or curved, flask-shaped, 4.1–7.6 × 1.3–2.9 ($\bar{x}$ = 5.7 × 2.0 µm, n = 30).	Holoblastic, ellipsoidal, smooth, often attached of conidiogenous cells 3.0–4.1 × 2.3–2.9 ($\bar{x}$ = 3.5 × 2.5 µm, n = 30).	**This study**
A. monjolensis	PDA plate consumed by an insect in a cave	Whites to cream, 24–31	Colorless, 13–17	White, 17–21	Cylindrical, bearing one or more conidiogenous cells, straight or slightly bent, solitary or synnematous, sometimes branched	Flask-shaped, straight or irregularly bent, 3.1–6.1 × 2.7–5.1	Holoblastic, 2.8–3.7×1.8–2.9	Leão et al. (2024)
A. cavernícola	Bird faeces; soil; plant debris; animal faeces; bat guano collected in Karst cave	Cream yellow, 9–15	White, 9–13	White, 18–22	Cylindrical, straight or slightly curved, occasionally branched	Fusiform or ellipsoidal, straight or irregularly bent, 4.5–8.0 × 2.0–3.0	Broadly elipsoidal to subglobose, 2.5–4.0 × 2.0–3.5	Zhang et al. (2021)
A. coprophila	Chipmunk, rabbit and porcupine dung	Orange to brownish orange, 22–24	Light yellow, 5–10	Light yellow, 34–40	Straight or flexuous, unbranched, bearing lateral or terminal conidiogenous cells, arranged singly or in whorls	Flask-shaped, usually with a strongly bent neck, 6–10 × 2–2.5	Subglobose to somewhat ellipsoidal, 3.5–5.5 × 2–3	Guerra-Mateo et al. (2023)
A. excrementa	Animal faeces	White to cream, 42–44	n.d	n.d	Cylindrical, straight or slightly curved, occasionally branched	Occasionally solitary, mostly in whorls of 2–3, basal portion cylindrical or flask-shaped, usually curved, 4.1–13.9 × 1.3–2.1	Globose to elliptical 1.7–3.0 × 1.2–2.5	Wang et al. (2024)

A. felina	Pupa of *Anaitis efformata*; rabbit dung; mouldy leaves; porcupine dung; cat dung	white to creamywhite, 36–38			Straight	Solitarily or in small groups, consisting of a swollen, flask-shaped or curved, occasionally elongate basal part, 1.5–8.5 × 1.8–2.9	Subglobose, ellipsoidal or ovoidal, sometimes with a pointed base, 2.5–4.7 × 2–3.5	Wang et al. (2024)
A. guana	Bat guano collected in a Karst cave	Yellowish white, 14–18	White to yellow, 13–21	n.d	Straight or slightly curved	Fusiform or ellipsoidal, straight or irregularly bent, 7–10 × 2–3	Broadly ellipsoid to subglobose, 4.5–5.5 × 3.5–5	Zhang et al. (2017)
A. guizhouensis	Animal faeces	White to pinkish, 40–42	n.d	n.d	Cylindrical, straight or slightly curved, occasionally branched	Basal portion cylindrical or flask-shaped, erect or irregularly curved, tapering abruptly towards the apex, 6.0–20.8 × 1.8–3.7	Subglobose to ellipsoidal, 2.6–4.0 × 1.8–2.6	Wang et al. (2025)
A. kunmingensis	Animal faeces	White to pale grey, 52–54	n.d	n.d	n.d	Solitary, occasionally in simple whorls, basal portion cylindrical or fusiform, straight or irregularly bent, 6.1–17.5 × 1.4–2.9	Globose to elliptical 2.3–4.2 × 1.6–3.0	Wang et al. (2024)
A. littoralis	Sediments; fragment of floating rubber tire	Greenish yellow, 20	Greenish yellow, 9–14	Greenish yellow, 30–32	Straight or flexuous, commonly unbranched, bearing lateral or terminal conidiogenous cells, arranged singly or in whorls of 2–4	Flask-shaped, usually with a strongly bent neck, 6–10 (–11.5) × 1.5–2	Subglobose, 3–4 × 2.5–3	Guerra-Mateo et al. (2023)
A. yunnanensis	Wing surfaces of *Rhinolophus*	White, 15–38	-	-	Cylindrical, straight or slightly curved, branched	Monoblastic to polyblastic, ampulliform to flaskshaped, 4–12 × 1–4	Globose to oval, slightly ellipsoid, 2–5 × 2–4	Liu et al. (2023)

Discussion

Based on morphological and phylogenetic analyses, we described two new coprophilous species namely *Amphichorda* sp. nov. 1 and *Amphichorda* sp. nov. 2. *Amphichorda* sp. nov. 1 is the first fungal species ever described from a marble cave worldwide and the second Amphichorda species reported from caves in Minas Gerais. *Amphichorda* sp. nov. 2, on the other hand, represents the first fungal species described from a cave in the state of Mato Grosso.

The *Amphichorda* sp. nov. 1 and *Amphichorda* sp. nov. 2 species described here are closely related and, together with A. monjolensis, formed a unique and well-supported clade of Brazilian species within the genus, as revealed by phylogenetic analyses of the four loci (ITS, LSU, TEF, and TUB) (Figure 2). *Amphichorda monjolensis* was the first species of the genus described in Brazil, in Gruta Velha Nova cave, (Leão et al. 2024) which is located around 200 Km from Gruta Teto de Seixos and Gruta da Viola caves where *Amphichorda* sp. nov. 1 was isolated, these sister species were described from caves located in the same mountain range in the Serra do Espinhaço Meridional in the state of Minas Gerais (Leão et al. 2024). In contrast, *Amphichorda* sp. nov. 2, which was clustered externally to the species mentioned above, was obtained from sandstone caves located around to 1500 Km in the Mato Grosso state, indicating possible geographical isolation between these species from the Brazilian clade.

The type isolate of *Amphichorda* sp. nov. 1 was obtained through direct isolation from an animal dung sample collected in the Gruta Teto de Seixos cave, but other isolates were found in leaf litter and sediment from the same cave and in airborne particles, animal dung and sediment from Gruta da Viola cave (Table 2). The diversity of substrates colonized by *A. mireirensis* his substrate diversity reflects the ecological plasticity already observed in other Amphichorda species, which have been recovered from various substrates including bat wings, bird feces, marine sediment, and airborne particle samples (Zhang et al. 2017; 2021; Guerra-Mateo et al. 2023; Liu et al. 2023; Leão et al. 2024; Wang et al. 2024; Wang et al. 2025). Gruta Teto de Seixos and Gruta da Viola caves are part of a complex of marble caves located in Morro da Pedreira - Serra do Espinhaço Meridional. These caves were reported by Souza et al. 2011 and 2019, and this is the first mycological study conducted in these caves and the first Brazilian fungal species described from a marble lithology cave.

The type isolates of *Amphichorda* sp. nov. 1 and *Amphichorda* sp. nov. 2 were isolated from animal dung collected from the sampled caves. Many species of this genus exhibit a

coprophilous lifestyle, including *A. cavernicola*, *A. coprophila, A. excrementa*, *A. felina, A. guana*, *A. guizhouensis* and *A. kunmingensis* (Zhang et al. 2017; 2021; Guerra-Mateo et al. 2023; Wang et al. 2024; Wang et al. 2025). Animals can use caves during part of their life cycle (trogloxenes), such as bats that use caves for breeding, roosting or hibernating (McClure et al. 2020; Barros and Bernard 2023; Vargas-Mena et al. 2025), or during their entire life cycle, as in many specialized troglobitic species (Trajano 2000). Therefore, animal dung can accumulate in caves, constituting a good source of organic matter in oligotrophic environments. The species *A. cavernicola* and *A. guana*, were isolated from bird feces and bat guano, respectively, in karst caves in China (Zhang et al. 2017, 2021). In this scenario, these *Amphichorda* species appear to have developed the capability to colonize and utilize this substrate to thrive in harsh environments such as caves.

Amphichorda sp. nov. 1 isolates were also obtained from cave air, leaf litter, and soil samples in the Gruta da Viola and Gruta Teto de Seixos caves, which are approximately 50 m apart. We hypothesized that fungal conidia dispersion may be promoted by airflow or animal movement from one cave to another. Mycological studies have demonstrated that fungi are largely found in airborne particles, bat guano, and soil in caves (Taylor et al. 2013; Ogórek et al. 2016; Cunha et al. 2020; Dominguez-Moñino et al. 2021; Borzęcka et al. 2021). A high abundance of fungi was found in airborne and bat guano samples from the touristic cave Lapa Nova in Brazil (Taylor et al. 2013). Found a high abundance of airborne fungi and in soil was found in the Meu Rei bat cave in Pernambuco, Brazil (Cunha et al. 2020). Fungi have also been isolated from the body surfaces of bats in Brazilian caves, demonstrating the importance of these animals in fungal dispersion in underground environments (Cunha et al. 2020; Carvalho et al. 2022; Lima et al. 2024).

In addition to the taxonomic novelties described in this study, the newly described Amphichorda species may also have biotechnological potential. Although the genus has a relatively recent and dynamic taxonomic history, some species have already demonstrated the ability to produce bioactive compounds. For instance, A. felina (NRRL 66746), isolated from animal dung in the United States, was shown to produce cyclosporin C, a compound with antifungal activity (Xu et al. 2018). Moreover, another strain of A. felina (SYSU-MS7908), obtained from the marine ascidian Styela plicata, has been reported to synthesize metabolites with anti-inflammatory effects and moderate inhibitory activity against the enzyme acetylcholinesterase (Jiang et al. 2021, 2023; Yuan et al. 2022). These findings highlight the

genus Amphichorda as a promising source of bioactive metabolites, particularly those isolated from unique and extreme environments such as caves and marine habitats.

In Brazil, more than 26,000 caves are known (CECAV 2025), but only 30 studies on Brazilian mycological diversity are available (Prazeres et al. 2025). This gap between the number of known caves and the studies carried out shows that Brazilian caves are mycological hotspots and are potential sources of new taxa. Until 2023, only a few studies have reported mycological studies in caves in Minas Gerais (Taylor et al. 2013, 2014; Souza et al. 2016). Subsequently, taxonomic studies conducted in three caves described one new genus and seven new species (Condé et al. 2023; Dutra et al. 2023; Leão et al. 2024). This study emphasizes the importance of describing *Amphichorda* sp. nov. 1 and *Amphichorda* sp. nov. 2 to expand our knowledge of mycobiota in Brazilian caves.

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CHAPTER 2

Mycobiota of the quartzite cave in the Southern Espinhaço Mountains – Minas Gerais.

Article for publication in accordance with the standards of International Journal of Speleology

Mycobiota of the quartzite cave in the Southern Espinhaço Mountains – Minas Gerais.

Abstract

Caves are unique environments with singular characteristics and are home to an inconceivable diversity of fungi, with completely unknown species and species with great biotechnological potential. In recent years, studies on cave fungi have highlighted these environments as a new frontier for expanding knowledge of the mycological world. In this sense, the objective of this study was to investigate the presence of cultivable filamentous fungi present in the ferruginous quartzite cave Curral de Pedras - Conceição do Mato Dentro, located in the Serra do Espinhaço Meridional region of Minas Gerais. Samples of soil, litter, rock, animal dung and particles suspended in the air of the cave were collected to isolate the fungi. A total of 421 isolates were obtained through sequencing of the ITS region and were identified at the genus level by comparison in the NCBI database. The isolates were grouped into 78 genera, within 59 families, 31 orders, 12 classes, 7 subphyla and 4 phyla. More than 50% of the isolates were identified as belonging to the genera *Penicillium* and *Cladosporium.* The results obtained reveal a great diversity of filamentous fungi present in the Curral de Pedras cave, highlighting the potential for discovering new species and contributing to the mycological knowledge of Brazilian caves.

Key words: Fungi, diversity, ITS, *Penicillium* and *Cladosporium*.

Introduction

Caves are environments between rocks, with a limited space, absence of direct sunlight, little availability of organic matter and a unique microclimate (Docampo et al., 2011; Auler, 2015; Zhang et al., 2021). Caves have been present in history since the dawn of the human species, serving as shelters and accompanying evolution, today they are known for speleotourism, both for their speleological formations and for housing records of human history (Martin-Sanchez et al., 2014; Salazar-Hamm et al., 2025).

The unique characteristics of caves have always generated an interest in knowing the cave fauna and better understanding the ecology of these environments (Cunha et al., 2020; Prazeres et al., 2025). In recent decades, research has focused on cave mycology and its great potential for discovering new species and the biotechnological potential of these species (Docampo et al., 2011; Paula et al., 2019; Kozlova and Mazina 2020; Poli et al., 2024).

Currently, more than 1626 species of fungi and 644 genera (Zhang et al., 2021) have been reported in cave environments around the world, and it is estimated that this number is still very small, mainly due to the lack of studies in tropical environments (Vanderwolf et al., 2013; Zhang et al., 2021). In Brazil, the first report of cave fungi was carried out by Castrillón et al. (1976) with isolates from caves in the Amazon region, and after almost half a century, only thirty studies were carried out in Brazilian caves, recording the occurrence of 292 fungal genera (Prazeres et al., 2025).Considering the significant number of more than 26 thousand caves registered in Brazil (Ministério do Meio Ambiente, 2025), Brazilian cave mycological knowledge is still very low (Prazeres et al., 2025).

In the state of Minas Gerais, which is home to almost 50% of Brazilian caves, only nine studies have been carried out inventorying the mycology (Condé et al., 2023; Prazeres et al., 2025). Some of these studies were carried out in the Serra do Espinhaço Meridional region, where a new genus and seven new fungal species have already been described (Condé et al., 2023; Dutra et al., 2023; Leão et al., 2024). Given the recent mycological discoveries and the incipience of studies on mycobiota in many caves in this region, the present study aimed to carry out a survey of the cultivable mycobiota present in the Curral de Pedras Cave – Conceição do Mato Dentro, located in the Serra do Espinhaço Meridional in Minas Gerais – Brazil.

Material and methods

Study site

The Curral de Pedras Cave is a cave formed by quartzite rocks (Auler, 2015), located in the district of Santo Antônio do Norte, municipality of Conceição do Mato Dentro – Minas Gerais (18°46'47.1"S 43°31'12.4"W) (Figure 1a). The cave is formed by two conduits: the main conduit with approximately 70 meters in length and a secondary conduit lateral with less vertical development with approximately 60 meters in length (Figure 1b-f). The main conduit has a continuous flow of water throughout its length, leaving the cave environment very humid (Figure 1e).

Collection

In the Curral de Pedras Cave, samples of animal dung (AD), soil (SO), leaf litter (LL), airborne particles (AP) and fungal mycelia growing on the rock (MR) (Figure 2) were collected at six points in the cave described in Table 1. The samples were collected on September 27, 2022. The research was authorized by license number: 82187-1 ICMBio/CECAV.

Table 1. Description of the collection points with details of the number of samples collected.

Collection point description	**Sample type**	**Sample number**
P1 – Main conduit – from the entrance to 10 meters after the entrance. Photic zone.	Airborne particles	6
	Soil	1
	Leaf litter	1
P2 – Main conduit – from 10 meters from the entrance to approximately 40 meters from the entrance. A transition area that receives only indirect light.	Airborne particles	6
	Soil	2
	Leaf litter	2
	Fungal mycelia on the rock	4
	animal dung	1
P3 – Main conduit – from 40 meters from the entrance to the end of the cave. Photic zone.	Airborne particles	6
	Leaf litter	1
P4 – Secondary conduit – from the entrance to 3 meters from the entrance, photic zone.	Airborne particles	3
	Soil	1
P5 – Secondary conduit – approximately 25 meters from the entrance, aphotic zone.	Airborne particles	3
	Soil	1
	animal dung	1
P6 – Secondary conduit – approximately 50 meters from the entrance, aphotic zone.	Airborne particles	3
	Soil	2
	animal dung	1

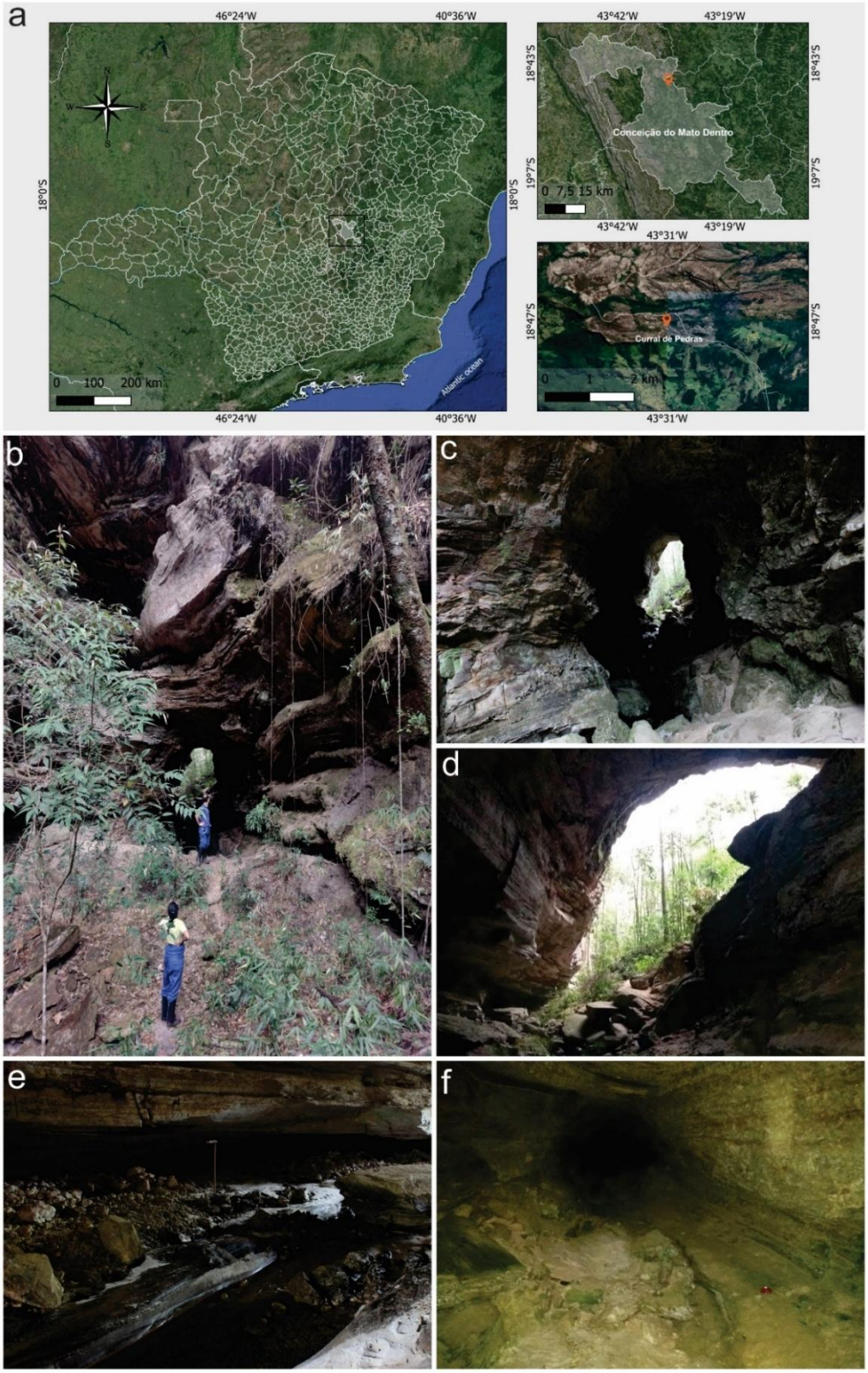

Figure 1. Curral de Pedras Cave – Conceição do Mato Dentro, Minas Gerais – Brazil. a) Map of the cave location. b) Cave entrance. c) Main conduit showing its two openings. d) View of the cave exit. e) Highlight of the semi-aphotic zone of the main cave (P2). f) Entrance of the secondary conduit, highlighting the airborne spore collection plates exposed on the cave floor.

The airborne particles collection was performed using the Koch sedimentation methodology (Kuzmina et al., 2012). Plates with the culture media Potato Dextrose Agar (PDA) added with chloramphenicol (50 μg/mL $^{-1}$), Dicloran Glycerol Agar (DG-18) and Dicloran Rose Bengal Chloramphenicol (DRBC) were exposed to the environment for 15 min. In the main conduit, a 1-meter-high support was used to standardize the collection (Figure 2g-h), while in the secondary conduit the plates were placed directly on the cave floor; the lower vertical development (approximately 1 m) did not allow the use of the support (Figure 1f).

To collect the soil samples, a superficial cleaning was performed, aiming to eliminate bulky substrates; approximately 15 grams of soil was collected in the layer of 1 to 5 cm in depth and stored in Falcon tubes with a capacity of 50 mL (Figure 2b) (Condé et al., 2023). The SE samples were stored in paper bags (Leão-Ferreira et al., 2013). Approximately 10 grams of animal dung was collected and stored in 50 ml Falcon tubes (Figure 2f). For the RO samples, a sterile swab was used to sample the fungal mycelium present on the rock and store it in test tubes containing 0.9% saline solution (Figure 2c-e).

All samples were stored in thermal boxes and later transported to the Laboratório de Micologia e Etiologia de Doenças Fúngicas de Plantas da Universidade Federal de Viçosa, Viçosa – MG, for isolation of cave fungi.

Isolation

The isolation of fungi present in the soil and animal dung samples was performed following the methodology described by Condé et al., (2023). One hundred microliters of each dilution of the soil and animal dung samples, and two hundred microliters of the collection solution of the fungal mycelia growing on the rock samples were spread on plates containing the PDA, Dicloran Glycerol Agar and Dicloran Rose Bengal Chloramphenicol media, with duplicates for each medium. For colony growth, the AR samples, and the dilutions of soil, fungal mycelia growing on the rock and animal dung were incubated at 25ºC, in the dark, and every two days the isolation of the growing colonies was performed on a new PDA plate.

For the isolation of fungi present in the leaf litter samples, the washing technique in running water was used (Castañeda-Ruiz 2005). The samples were washed in running water for one hour, dried on autoclaved paper towels and stored in humid chambers (polystyrene boxes containing filter paper moistened with sterilized water). Over the course of 30 days, the growth of fungal reproductive structures was observed under a stereoscopic microscope and, through the direct isolation method, these structures were transferred to a plate containing PDA medium.

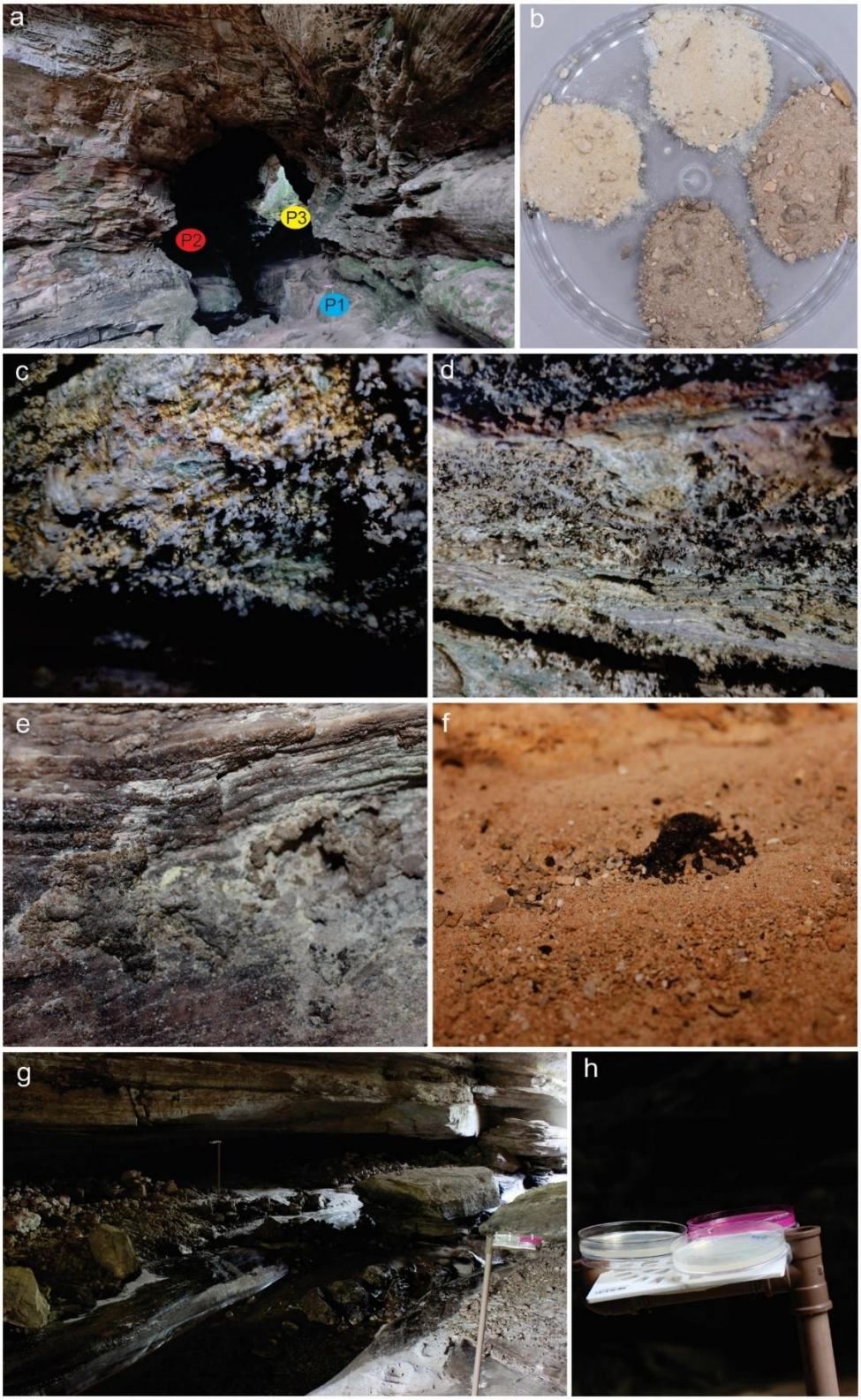

Figure 2. Samples from the Curral de Pedras Cave – Conceição do Mato Dentro, Minas Gerais – Brazil. a: Main conduit of the cave, showing where the collection points were (description of the points in Table 1), b: Soil samples collected, showing the difference in tones between the collection points, c – e: Fungal colonization apparent on the cave wall, (MR sample), f: animal dung sample, g: Photograph of the collection of airborne spores with the aid of a support, h: Detail of the culture media during the exposure for collection of airborne spores in the cave.

Fungi preservation

Using the hyphal tip methodology (Tuite 1969), pure cultures of all isolates were obtained and preserved in 10% glycerol at -20 °C, in sterilized distilled water at room temperature (Castellani 1939). All isolates were stored in the cave fungi collection of the Laboratory of Mycology and Etiology of Fungal Diseases of Plants of the Federal University of Viçosa.

DNA extraction, amplification and sequencing

To collect the fungal mycelium and extract the total genomic DNA, the isolates were grown in PDA medium at 25 °C for 7 days, in the dark. The extraction was performed using the commercial Wizard Genomic DNA Purification kit (Promega®) and following the protocol described by Chapter 1, with the following changes for when the DNA presented high viscosity at the end of the extraction: 300 µL of 5% Chelex solution was added and vortexed for 30 seconds, after centrifugation at 14000 rpm for 5 minutes. 200 µL of the supernatant was transferred to a new 1.5 mL tube containing 500 µL of cold isopropanol and 200 µL of 7.5% ammonium acetate, the mixture was homogenized by vertexing for 30 seconds and kept at -20º for 60 minutes. After the samples were centrifuged at 14000 rpm for 10 minutes, the supernatant was carefully discarded to keep the DNA pellet, which was washed with 600 µL of 70% ethanol, by manually inverting the tube for 2 min. To discard the ethanol, the samples were centrifuged at 14,000 rpm for 5 minutes and then dried in a dry bath at 45 °C for 10 min. The pellet was resuspended in 50 µL of DNA Rehydration solution and kept in a dry bath at 65 °C for 1 hour.

To identify the isolates, amplification of the internal transcribed spacer regions 1 and 2, together with the 5.8S subunit (ITS), was performed using the primers ITS5 (White et al., 1990) and LR6 (Vilgalys and Hester 1990). Polymerase Chain Reactions (PCR) were performed using Taq DNA polymerase Platinum PCR SuperMix (Life Technologies®) or Taq PhireTM Hot Start II (ThermoScientific®). PCR reactions were prepared containing 18 µL of Platinum PCR SuperMix, 0.4 µL of each primer (10 mM), or 0.4 µL of Taq PhireTM Hot Start II, 10 µL of PhireTM Hot Start II buffer solution, 6.4 µL of nuclease-free water and 1 µL of each primer (10 mM). For both reactions, 1.2 µL of DNA (25 ng µL-1) was used. The PCR conditions for the Platinum PCR SuperMix were: initial DNA denaturation at 94°C for 120 seconds, followed by 35 cycles of denaturation at 94°C for 30 seconds, primer annealing at 50°C for 30 seconds followed by DNA extension at 72°C for 120 seconds and a final extension at 72°C for 120 seconds. The PCR conditions for the PhireTM Hot Start II began with initial DNA denaturation

at 98°C for 120 seconds, followed by 35 cycles of denaturation at 98°C for 5 seconds, primer annealing at 55°C for 5 seconds followed by DNA extension at 72°C for 40 seconds and a final extension at 72°C for 60 seconds.

The PCR products were stained with GelRed (Biotium Inc.®) and analyzed by 1% agarose gel electrophoresis at 80 Volts for 40 min in 1g mL^{-1} Tris-acetate-EDTA buffer. The amplified fragment size and purity were verified under ultraviolet light. The PCR products were sent for purification and sequencing to Macrogen Inc., South Korea (http://www.macrogen.com). And the ITS region sequence editing was performed using FinchTV v.1.4.0 software.

Identification

The BLAST algorithm available on the National Center for Biotechnology Information (NCBI) platform (https://www.ncbi.nlm.nih.gov/) was used to identify isolates at the genus level. The sequences obtained in this study were compared with sequences deposited in the database, using the Sequences from type material filter, to compare only with sequences considered to be the type of each species, that is, sequences that passed an accuracy test and have a very low level of error. Isolates with a similarity index greater than 95% with sequences of the same genus available in the database were considered as a species related to the genus. On the other hand, isolates that presented a similarity index lower than 95% were not grouped with any genus and were grouped only within the family that presented similarity. For the final count of genera in this work, isolates that were grouped only at the family level and in this family no isolate was grouped to a genus, for this family one genus was counted, considering the principle that future works will identify these isolates within a genus in this family.

Results

We obtained 421 fungal isolates, where 230 were from samples collected at the main conduit (collection points P1, P2 and P3) and 191 from the secondary conduit samples (collection points P4, P5 and P6) (Figure 3A). There were 147 isolates from the airborne particles samples (100 from the main conduit and 47 from the secondary conduit), 122 isolates from the soil samples (47 from the main conduit and 75 from the secondary conduit), 106 isolates from the animal dung samples (33 from the main conduit and 73 from the secondary

conduit), 36 isolates from the leaf litter samples and 10 isolates from the fungal mycelia growing on the rock samples (Figure 3A).

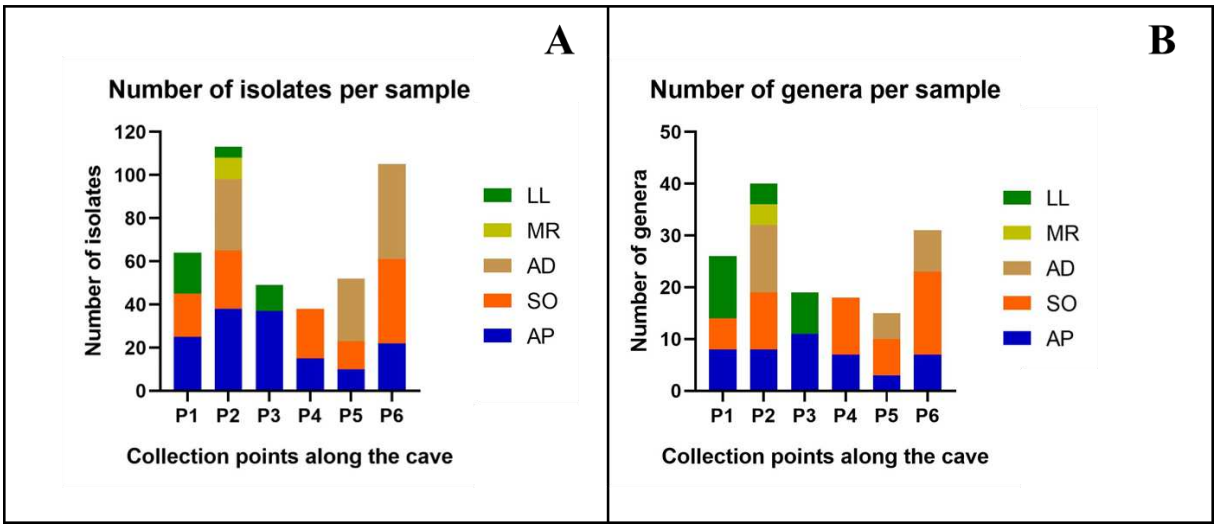

Figure 3. Graphs showing the number of isolates and genera from Curral de Pedras Cave. A: Number of isolates within each sample divided by collection points throughout the cave. B: Number of genera found within each sample divided by collection points throughout the cave. Animal dung (AD), soil (SO), leaf litter (LL), airborne particles (AP) and fungal mycelia growing on the rock (MR).

The isolates from the airborne particles samples were grouped into 25 genera within the phyla *Ascomycota*, *Mucoromycota* and *Basidiomycota* (Table 2, Figure 3B, Figure 4). The genera *Cladosporium* and *Penicillium* presented the largest number of isolates in the AR samples, with 47 and 42 isolates, respectively (Table 2, Figure 4). In the soil samples, the isolates were distributed in 33 genera, notably *Penicillium* (48 isolates) and *Cladosporium* (11 isolates) (Table 2, Figure 4) having the largest number of isolates in the phylum *Ascomycota*, *Umbelopsis* (5 isolates) and *Absidia* (2 isolates) in the phylum *Mucoromycota*, and the genera *Peniophora* and *Apiotrichum* (with 2 isolates each) in the phylum Basidiomycota (Table 2, Figure 4).

The isolates obtained from the animal dung samples were identified in 21 genera (Table 2, Figure 3B), with representatives in the phyla *Ascomycota*, *Mucoromycota*, *Basidiomycota*, and *Zoopagomycota*. Within the *Ascomycota* phylum, there was a predominance of isolates in the *Penicillium* genus (with 51 isolates), while in the *Mucoromycota* phylum, the genera *Mucor*, *Isomucor* and *Absidia* were found (Table 2, Figure 4). *Apiotrichum* was the only genus identified in the *Basidiomycota* phylum, and in the *Zoopagomycota* isolates could not be resolved at the genus level and were grouped under *Basidiobolaceae.* (Table 2).

From the fungal mycelia growing on the rock samples, the isolates were identified in the genera *Penicillium*, *Cladosporium*, *Apiospora* and *Pestalotiopsis*, all belonging to the *Ascomycota* phylum (Table 2, Figure 4). The leaf litter samples presented a richness of 22 genera (Table 2, Figure 3B), but only the genera *Clonostachys* (4), *Penicillium* (3), and *Thyronectria* (2) had more than one isolate found (Table 2, Figure 4). Penicillium and Cladosporium were the most frequently isolated genera, with 149 and 69 isolates, respectively. (Table 2, Figure 4).

Through the comparison of the sequences in the NCBI database, the 421 isolates were identified in 78 genera, grouped within 59 families, 31 orders, 12 classes, 7 subphyla and 4 phyla. The phylum *Ascomycota* grouped 91.2% (384 isolates) of the total isolates, followed by *Mucoromycota* 5.7% (24 isolates), *Basidiomycota* 2.4% (10 isolates) and *Zoopagomycota* 0.7% (3 isolates) (Table 2, Figure 5). Regarding the number of genera, the result was similar to the number of isolates with the phylum *Ascomycota*, grouping 85.7% of the genera (68 genera of the 78), followed by the phylum *Mucoromycota* with 7.8% (5 genera), *Basidiomycota* with 5.2% (4 genera) and *Zoopagomycota* with 1.3% (1 genus) (Table 2).

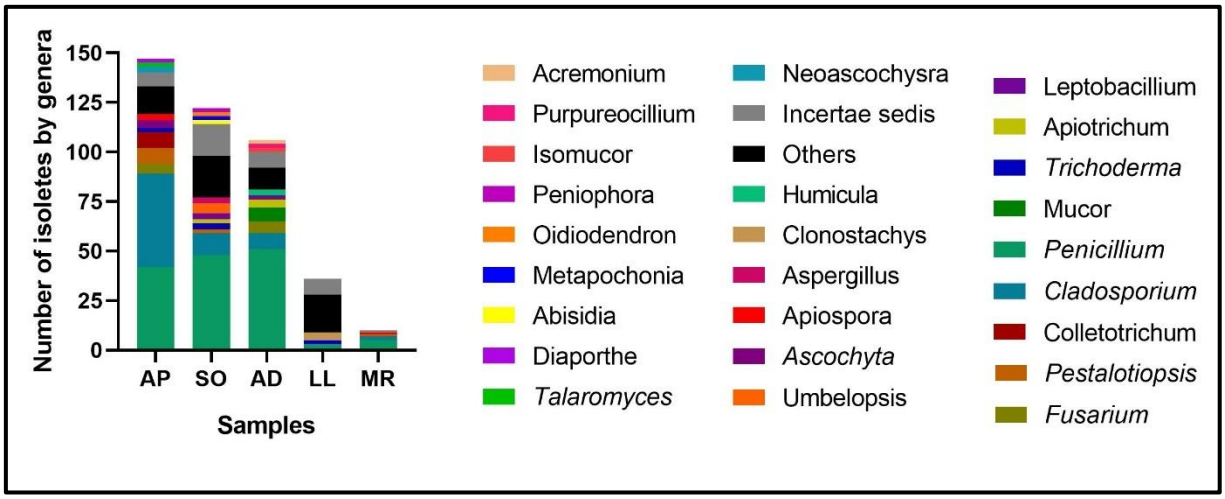

Figure 4. Graph of the quantity of isolate identified by genus, within each sample. The "Others" column contains genera that had only one isolate. The "Incertae sedis" are isolates that were not possible to identify at the genus level. Animal dung (AD), soil (SO), leaf litter (LL), airborne particles (AP) and fungal mycelia growing on the rock (MR).

Table 2. Quantity of isolates found per sample, collection points within the cave, and classification according to genus, family, order, class, subphylum and phylum.

F[1]	S[2]	Class	Order	Family	Genus	Isolates[3]
A	1	*Dothideomycetes*	*Botryosphaeriales*	*Botryosphaeriaceae*	*Neofusicoccum*	AP – P2(1)
			Capnodiales	*Capnodiaceae*	*Chaetocapnodium*	SO – P6(1)
			Cladosporiales	*Cladosporiaceae*	*Cladosporium*	AP – P1(9); P2(13); P3(9); P4(4); P5(2); P6(10)
						SO – P2(3); P4(1); P5(1); P6(3)
						LL – P1(1)
						MR – P2(2)
						AD – P2(4); P6(3)
					Rachicladosporium	AP – P3(1)
			Dothideales	*Saccotheciaceae*	*Aureobasidium*	SO – P4(1)
			Massarineae	*Didymosphaeriaceae*	*Montagnula*	SO – P6(1)
			Mycosphaerellales	*Mycosphaerellaceae*	*Cercospora*	AP – P3(1)
					Mycosphaerella	AP – P3(1)
				Teratosphaeriaceae	*	AP – P3(1); P6(1)
						SO – P4(1)
			Pleosporales	*	*	AP – P3(1)
				Didymellaceae	*	AP – P1(1)
					Ascochyta	AP – P1(1); P3(1); P4(2)
						AD – P2(1)
					Heterophoma	SO – P4(1)
					Neoascochyta	AP – P1(3)
					Paraboeremia	AP – P5(1)
				Paraboeremia	*Paracamarosporium*	SO – P1(1)
					Paraphaeosphaeria	AP – P4(1)
					Pseudopithomyces	SO – P5(1)

		Nigrogranaceae	*	AP – P3(1)
		Periconiaceae	*Periconia*	SO – P1(1)
				LL – P1(1)
		Phaeosphaeriaceae	*Phaeosphaeria*	AP – P1(1)
			Setophoma	AP – P6(1)
		Pleosporaceae	*Bipolaris*	AP – P4(1)
	Venturiales	*Sympoventuriaceae*	*	LL – P3(2)
Eurotiomycetes	*Chaetothyriales*	*Herpotrichiellaceae*	*	SO – P4(1)
	Eurotiales	*Aspergillaceae*	*Aspergillus*	SO – P2(1); P4(1); P6(1)
				AD – P2(1)
			Penicillium	AP – P1(2); P2(13); P3(10); P4(5); P5(7); P6(5)
				SO – P1(10); P2(10); P4(11); P5(4); P6(13);
				LL – P1(3)
				MR – P2(5)
				AD – P2(12); P5(20); P6(19)
		Trichocomaceae	*Talaromyces*	AP – P2(1); P6(1)
	Onygenales	*Onygenaceae*	*Chrysosporium*	SO – P2(1)
Leotiomycetes		*Myxotrichaceae*	*Oidiodendron*	LL – P3(1)
				SO – P6(2)
	Helotiales	*Dermateaceae*	*	LL – P3(1)
		Hamatocanthoscyphaceae	*	LL – P2(1)
		Neolauriomycetaceae	*Lareunionomyces*	LL – P2(1)
		Pezizellaceae	*	LL – P3(1)
	Thelebolales	*Thelebolaceae*	*	LL – P2(1)
Sordariomycetes	*Chaetosphaeriales*	*Chaetosphaeriaceae*	*	LL – P1(1)
	Diaporthales	*Diaporthaceae*	*	AP – P1(1)
			Diaporthe	AP – P3(1); P4(1)

		Valsaceae	*Cytospora*	AP – P2(1)
	Glomerellales	*Glomerellaceae*	*Colletotrichum*	AP – P3(7); P6(1)
	Hypocreales	*	*	SO – P6(1)
				AD – P6(1)
			Acremonium	AD – P2(2)
				SO – P6(1)
		Bionectriaceae	*Clonostachys*	LL – P1(3); P3(1)
			Chlamydocillium	AD – P2(1)
			Ovicillium	AD – P2(1)
		Clavicipitaceae	*	AP – P6(1)
				SO – P1(1); P2(1); P5(1)
				LL – P3(1)
			Metapochonia	AD – P2(1)
				SO – P5(1); P6(1)
		Cordycipitaceae	*Lecanicillium*	AD – P2(1)
			Leptobacillium	LL – P1(1)
				SO – P2(3)
				AD – P6(2)
		Hypocreaceae	*Trichoderma*	AP – P2(1); P3(1)
				SO – P1(1); P4(1); P5(1)
				LL – P3(1)
		Nectriaceae	*	LL – P1(2); P3(2)
				SO – P6(1)
			Calonectria	LL – P1(1)
			Fusarium	AP – P1(5)
				AD – P6(6)
			Xenoacremonium	SO – P6(1)
		Ophiocordycipitaceae	*	SO – P2(1); P6(1)

		Purpureocillium	AD – P2(1); P6(1)
	Sarocladiaceae	*Sarocladium*	LL – P1(1)
			AD – P2(1)
	Stachybotryaceae	*Sirastachys*	LL – P1(1)
Microascales	*Microascaceae*	*Microascus*	SO – P6(1)
		Pseudallescheria	SO – P2(1)
Sordariales	*Chaetomiaceae*	*Arcopilus*	SO – P6(1)
		Humicola	SO – P1(1)
			AD – P2(1)
Togniniales	*Togniniaceae*	*Phaeoacremonium*	AP – P1(1)
Vermiculariopsiellales	*Vermiculariopsiellaceae*	*Vermiculariopsiella*	LL – P3(1)
Xylariales	*	*	LL – P1(1)
	Apiosporaceae	*Apiospora*	AP – P1(1); P2(2)
			MR – P2(1)
	Beltraniaceae	*Beltrania*	LL – P3(1)
	Diatrypaceae	*Diatrypella*	SO – P4(1)
	Hypoxylaceae	*	SO – P6(1)
	Microdochiaceae	*	LL – P2(1)
	Sporocadaceae	*	LL – P2(1)
		Bartalinia	LL – P1(1)
		Pestalotiopsis	AP – P2(5); P3(2); P6(1)
			SO – P6(2)
			MR – P2(1)
	Xylariaceae	*	AP – P2(1)
			MR – P2(1)
			SO – P1(2); P2(1); P4(5)
		Xylaria	SO – P6(1)
	Zygosporiaceae	*	LL – P1(1)

	2	*Saccharomycetes*	*Saccharomycetales*	*Debaryomycetaceae*	*	AD – P2(1)
					Meyerozyma	SO – P2(1)
						AD – P2(1)
B	3	*Agaricomycetes*	*Polyporales*	*Irpicaceae*	*	AP – P6(1)
			Russulales	*Peniophoraceae*	*Peniophora*	SO – P2(1); P6(1)
		Tremellomycetes	*Trichosporonales*	*Trichosporonaceae*	*Apiotrichum*	SO – P2(1); P6(1)
						AD – P6(4)
	4	*Exobasidiomycetes*	*Exobasidiales*	*Brachybasidiaceae*	*	SO – P2(1)
	5	*Mortierellomycetes*	*Mortierellales*	*Mortierellaceae*	*Mortierella*	SO – P4(1)
M	6	*Mucoromycetes*	*Mucorales*	*Backusellaceae*	*	AD – P5(1); P6(1)
				Cunninghamellaceae	*	AD – P5(1)
					Absidia	SO – P4(1); P5(1)
						AD – P5(1)
				Mucoraceae	*	AD – P2(1); P5(1)
					Isomucor	AD – P6(2)
					Mucor	AD – P5(3); P6(4)
		Umbelopsidomycetes	*Umbelopsidales*	*Umbelopsidaceae*	*Umbelopsis*	AP – P4(1)
						SO – P2(1); P4(1); P5(3)
Z	7	*Basidiobolomycetes*	*Basidiobolales*	*Basidiobolaceae*	*	AD – P2(1); P5(2)

F1 = Phyla: A – *Ascomycota*; B – *Basidiomycota*; M – *Mucoromycota*; Z – *Zoopagomycota*.

S2 = Subphyla: 1 – *Pezizomycotina*; 2 – *Saccharomycotina*; 3 – *Agaricomycotina*; 4 – *Ustilaginomycotina*; 5 – *Mortierellomycotina*; 6 – *Mucoromycotina*; 7 – *Entomophthoromycotina*.

AD – samples of animal dung, SO – soil, LL – leaf litter, AP – airborne particles, MR – fungal mycelia growing on the rock

[3] - P1, P2, P3, P4, P5 and P6 – collection points, description in Table 1. ( ) – number of isolates *Incertae sedis

Discussion

Studies demonstrate the influence of the external environment on the dispersion of fungal spores at cave entrances (Shapiro and Pringle 2010; Taylor et al., 2013; Poli et al., 2024;) and the same can be reported when comparing the number of isolates obtained from the AR samples collected in the main conduit and secondary conduit. The main conduit of the Curral de Pedras Cave has a greater vertical development, allowing human visitation, two considerable openings that allow a large air flow and the presence of a continuous flow of water. These factors are reported as influencing the dispersion of spores in caves (Cunha et al., 2020; Biagioli et al., 2024; Poli et al., 2024), which resulted in the greater number of isolates obtained in the main conduit compared to the secondary conduit. In secondary conduct, collection point P6, the most internal part of the conduct, was the one that presented the largest number of secondary conduit isolates. A similar case was reported by Cunha et al., (2020), who, when researching spores present in the air of the Meu Rei cave (Pernambuco, Brazil), also reported a greater number of fungal isolates in the rooms furthest from the entrance. The innermost points of the caves are not as influenced by the external environment, but they are environments generally chosen by animals, especially bats that act as dispersers of fungal spores (Cunha et al., 2020). Comparing the 25 genera found in the airborne particles samples, only the genera *Cladosporium*, *Penicillium*, *Talaromyces*, *Diaporthe*, *Pestalotiopsis* and *Ascochyta* were found in the two conduits (Table 2), showing that there is a difference in fungal genera between the cave conduits (Table 2). Approximately 60% of the isolates from the airborne particles samples were grouped into the *Cladosporium* and *Penicillium* these genera are the most reported in studies of cave air spores (Vanderwolf et al., 2013; Cunha et al., 2020; Zhang et al., 2021; Pereira et al., 2022).

In this study, soil samples, with 228 isolates, and animal dung samples, with 421 isolates, accounted for more than 50% of the isolates obtained (Table 2, Figure 3). These samples have higher organic matter contents when compared to other samples found in caves, and the decomposition of available organic carbon in these types of samples is essential for fungal growth (Adetutu et al., 2011; Biagioli et al., 2024; Salazar-Hamm et al., 2025). Almost all isolates from the phyla *Basidiomycota*, *Mucoromycota*, and *Zoopagomycota* came from these samples (two isolates came from air samples), demonstrating the preference of species belonging to these phyla for substrates richer in organic material (Vanderwolf et al., 2013; Zhang et al., 2021). Cave soil samples and animal dung samples, when found in caves, are the

types of samples that result in the highest amounts of fungal isolates (Taylor et al., 2013; Kozlova and Mazina 2020; Zhang et al., 2021; Alves et al., 2022).

Analyzing the soil samples collected at the main conduit, collection point P2 was the one that presented the largest number of isolates and the greatest richness of genera (Figure 3B). Coincidentally, it was at this point that the greatest evidence of the presence of animals that may contribute to the increase in the content of available organic matter and influence the cave mycobiota was observed (Docampo et al., 2011). P6 at the secondary conduit, the innermost point of the cave, presented the largest number of isolates and the greatest richness of genera within the soil samples. The increase in fungi in sediments collected at deeper points in the caves was also reported by Paula et al., (2016) in Gruta do Catão (Bahia, Brazil), while Taylor et al., (2014) in the RM3 iron cave (Minas Gerais, Brazil) identified a decrease in the number of fungi present in soil samples as they moved away from the cave entrance.

The animal dung samples were the only ones that presented isolates distributed in the four phyla found in this study (*Ascomycota, Basidiomycota, Mucoromycota* and *Zoopagomycota*) (Table 2, Figure 3). Among the 21 genera found, only the genera *Penicillium, Mucor, Fusarium* and *Apiotrichum* were found in the two cave conduits (main conduit and secondary conduit); this diversity may be due to compositional differences between the samples (Cunha et al., 2020). The animal dung samples also presented isolates belonging to the genera *Isomucor, Lecanicillium, Ovicillium* and *Purpureocillium* that were not found in other samples from the cave (Table 2, Figure 4), this fact is due to the higher nutrient content found in these types of samples that favors fungal growth (Kozlova and Mazina 2020). In our research the genus *Penicillium* was representing almost 50% of all isolates found in this guano sample and, only one isolate was found in the genus *Aspergillus* (Table 2, Figure 3 and 4). This result for Penicillium corroborates the observation in the guano from Meu Rei cave (Pernambuco, Brazil) by Cunha et al., (2020), however these authors also observed the genus *Aspergillus* as very common in the samples. Generally, guano and animal dung are cave samples related to pathogenic fungi, such as *Histoplasma capsulatum* (Silva et al., 2013), but these samples have great potential for discovering new fungal species and genera. One the new genus *Parahumicola* and the new species *P. guana* from a guano sample collected in the Gruta Monte Cristo cave – Diamantina (Condé et al., 2023).

Of the 10 isolates obtained from fungal mycelia growing on the rock samples, 70% belonged to the genera *Penicillium* and *Cladosporium* (Table 2), genera already reported in cave rock samples (Vanderwolf et al., 2013). The *Penicillium* genus has been reported to produce

bioactive compounds that contribute to rock biomineralization (Man et al., 2015; Tavares et al., 2018; Agrawal et al., 2024).

The isolates from the leaf litter samples were distributed into 22 genera belonging to the phylum *Ascomycota* (Table 2, Figure 3). Unlike the other samples, the SE samples did not show a predominance of the *Penicillium* and *Cladosporium* genera (Table 2, Figure 3 and 4). The isolation methodology used, where the leaf litter is placed in a humid chamber and the growing fungal growth structures are selected, may have contributed to this fact. Isolates of the genera *Beltrania, Bartalinia, Calonectria, Clonostachys, Lareunionomyces, Sirastachys*, and *Vermiculariopsiella* were only found in the litter samples (Table 2, Figure 3), which may be one of the routes for fungi to enter the cave environment (Vanderwolf et al., 2013; Zhang et al., 2021). *Chaetomium meridionalense* (Condé et al., 2023) was the first species described from a sample of cave litter from a cave located in the Serra do Espinhaço Meridional of Minas Gerais, showing its still unknown mycological potential.

Isolates belonging to the phylum *Ascomycota* were dominant in this study, grouping more than 90% of the isolates and 80% of the genera found (Table 2, Figure 3 and 4). This phylum is the most representative in reports on fungal diversity in caves (Vanderwolf et al., 2013; Zhang et al., 2021; Biagioli et al., 2023; Biagioli et al., 2024;). In thirteen caves in China, more than 80% of isolates were also found within the phylum Ascomycota (Zhang et al., 2021).

In the Curral de Pedras cave, the isolates belonging to the Basidiomycota phylum came from the soil and animal dung samples and represented less than 3% of the total isolates found. The low number of fungi belonging to the phylum Basidiomycota in caves is observed in this study (Table 2), which may be due to the fact that it is a phylum with species that present greater difficulties in laboratory cultivation and are related to substrates with greater nutrient availability (Vanderwolf et al., 2013; Kozlova and Mazina 2020; Zhang et al., 2021).

In the Curral de Pedras Cave, the genera *Penicillium* and *Cladosporium* grouped more than 50% (218) of the isolates found, with isolates coming from all types of samples and from all collection points (Table 2, Figure 3). These genera are widely distributed throughout the cave, largely due to the fact that they are cosmopolitan genera, with characteristics that allow them to adapt widely to different substrates such as soil, insects, and plant material (Adetutu et al., 2011; Vanderwolf et al., 2013; Zhang et al., 2021; Poli et al., 2024). Interestingly, only four isolates belonging to the genus *Aspergillus* were found in the soil and animal dung samples collected at P1 (Table 2, Figure 3). A continuous flow of water that provides high humidity to

the environment was possibly the reason for the low number of isolates in this genus, since the *Aspergillus* is a genus associated with dry environments, and excess humidity has a negative effect on its growth (Kozlova and Mazina 2020; Poli et al., 2024).

The observation that fungal genera are heterogeneously distributed throughout caves is a fact observed in Curral de Pedras Cave: only 25 of the 78 genera (Table 2, Figure 4) found in the study were isolated in more than one sample type or at more than one collection point. This fact can be explained by several factors, such as the chemical composition of the rock, the availability of organic matter, the presence or absence of light, and the flow of air currents, which can influence the distribution of fungi throughout a cave (Shapiro and Pringle 2010; Docampo et al., 2011; Kozlova and Mazina 2020; Alves et al., 2022).

The genera *Apiospora, Ascochyta, Calonectria, Cytospora, Heterophoma, Isomucor, Lareunionomyces, Ovicillium, Rachicladosporium, Vermiculariopsiella* and *Xenoacremonium* found in this study are being reported for the first time in the cave environment (Vanderwolf et al., 2013; Zhang et al., 2021). These reports of genera not yet reported in the literature and of the new species identified in the Curral de Pedras cave highlight the potential for discovering new species within the isolates from the cave and the still unknown potential of Brazilian caves.

Conclusion

A total of 421 fungal isolates were found in the Curral de Pedras Cave and wealth of 78 genera was found in the Curral de Pedras cave, and twelve genera *Apiospora, Ascochyta, Calonectria, Cytospora, Heterophoma, Isomucor, Lareunionomyces, Speluncomyces, Ovicillium, Rachicladosporium, Vermiculariopsiella* and *Xenoacremonium* are being reported for the first time in the cave environment in Brazil and worldwide. These discoveries underscore the ecological and taxonomic importance of Brazilian caves and emphasize the urgent need for further mycological research in these still poorly explored ecosystems.

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CHAPTER 3

Mycobiota of ferruginous cavities in Serra da Ferrugem.

Article for publication in accordance with the standards of International Journal of Speleology

Mycobiota of ferruginous cavities in Serra da Ferrugem

Abstract

The knowledge of cave mycology has aroused interest both for the discovery of new fungal taxa and for its biotechnological potential. The objective of this study was to identify cultivable filamentous fungi present in ferruginous cavities of Serra da Ferrugem - Conceição do Mato Dentro, MG, one of the protected areas of ferruginous rocky fields of Serra do Espinhaço Meridional. To isolate the fungi, samples of airborne particles, sediments, leaf litter, roots and rocks were collected in a cave and four cavities along Serra da Ferrugem. From the samples, 361 filamentous fungi were isolated, from which total genomic DNA was extracted, amplified and the ITS region sequenced and were identified at the genus level by comparison in the NCBI database. Fungi were identified in 70 genera, 46 families, 26 orders, 10 classes, 6 subphyla and 4 phyla. The genera *Devonomyces*, *Mycosphaerella*, *Acrodontium*, *Scolecobasidium*, *Verruconis*, *Nagrajchalara*, *Arcuatospora*, *Chloridium*, *Thozetella*, *Colletotrichum*, *Nectriopsis*, *Leptobacillium*, *Calonectria*, *Cylindromonium*, *Volutella*, *Vermiculariopsiella*, *Castanediella*, *Hansfordia*, *Subramaniomyces*, and *Beltraniella* found in this study are being reported in cave literature for the first time. Knowledge of cave mycology in Brazil is still incipient, especially when it comes to ferruginous areas threatened by the great interest of mining. The wealth of genres found in this study shows the importance of knowing and studying cave environments further.

Keywords: Caves, fungi, Serra do Espinhaço, Biospeleology.

Introduction

The cave environment is a unique ecosystem, with limited space between rocks that provides no direct sunlight, little variation in temperature and humidity, low availability of organic matter, and a high concentration of minerals (Popović et al., 2015; Zhang et al., 2021; Salazar-Hamm et al 2025). The concentration of available minerals is influenced by the rock that forms the caves, and can vary among carbonate, siliciclastic and ferruginous rocks, or a combination of these lithologies (Nola & Bacellar 2021; Lima et al 2023; Salazar-Hamm et al 2025).

Currently, more than 26 thousand caves are registered at the Centro Nacional de Pesquisa e Conservação de Cavernas (ICMBIO/CECAV) in Brazil (Ministério do Meio Ambiente 2025), and of this total, approximately 14% are caves formed by ferruginous rocks. Iron caves are generally composed of intact banded iron formations and ferruginous cangas, which makes these caves more resistant to chemical and mechanical weathering, leading to many of them being of small sizes when compared to limestone caves (Hornick et al., 2017; Calapa et al., 2021; Parker et al., 2022).

Mining is one of the main threats to the cave environment. Currently, 11,397 caves are registered in areas with mining applications or concessions in Brazil (Ministério do Meio Ambiente2025; Salazar-Hamm et al 2025), putting at risk an ecosystem that is still little known, both in terms of the flora above this system and the troglobitic fauna, and especially the micobiota (Nola & Bacellar 2021; Lima et al., 2023; Salazar-Hamm et al 2025). In Brazil, only 30 caves have been studied for their mycobiota, highlighting the gap between the number of known caves and the mycological knowledge about them (Prazeres et al., 2025).

The Serra da Ferrugem is an area of ferruginous rocky field, located within the Serra do Espinhaço Meridional of Minas Gerais, protected by municipal legislation, where there are several caves and cavities and close to an area with potential iron ore mining (Conceição do Mato Dentro (MG) 2007; Dias & Madeira Filho 2020; Alves et al., 2022(a); Brina et al., 2025;). Recently, a new tree species,*Mezilaurus conceicionensis (*Lauraceae), endemic to the area and not yet listed threatened, was described from Serra da Ferrugem (Alves et al., 2022; Brina et al., 2025). The objective of this study was to understand the cultivable mycology in ferruginous caves and cavities of Serra da Ferrugem, municipality of Conceição do Mato Dentro - Minas Gerais and to contribute to expanding knowledge about Brazilian cave mycology, especially in ferruginous areas.

Material and methods

Study location

The Serra do Espinhaço is one of the main Brazilian rock formations, marked by several rocky outcrops, enormous diversity and high species endemism (Alves et al., 2022(a); Brina et al., 2025). It is composed of rocks of different lithologies that form a landscape of quartzite and ferruginous rocky fields. Serra da Ferrugem is one of the few areas of protection of ferruginous rocky fields in Serra do Espinhaço (Brina et al., 2025).

Serra da Ferrugem is an area of over 860 hectares formed by iron rocks and iron cangas, and was protected by municipal law Nº. 1916, of August 21, 2007, of the municipality of Conceição do Mato Dentro - MG (Conceição do Mato Dentro (MG) 2007; Dias & Madeira Filho 2020). The area is now called the Unidade de Conservação Municipal Monumento Natural Serra da Ferrugem and is fully protected to minimize the impacts caused by mining in the municipality of Conceição do Mato Dentro, Minas Gerais (Dias & Madeira Filho 2020).

Collection

To isolate the fungi, samples of airborne particles (AP) were collected from inside the ferruginous cavities, soil samples (SO), leaf litter samples (LL), roots that were present inside the cave (RO), and samples of fungal mycelia that were growing on the rocks (MR). The collections were carried out on September 29, 2022, in five different ferruginous cavities spread along the Serra da Ferrugem (Figure 1). The description of the cavities and samples collected in each is presented in Table 1. The research was authorized by license number: 82187-1 ICMBio/CECAV.

Table 1. Description of the cavities where the collections took place and the samples collected in each cavity.

Cave identification	Cave description	Quantity of samples
CSF 0804	Small cavity measuring 1.80 meters in diameter, located on the slope of Serra da Ferrugem, is difficult to access. It is surrounded by high-altitude rocky field vegetation, providing a large amount of leaf litter to the cavity. The presence of many insects (bees, wasps and hornets) was also observed inside the cavity.	3 airborne particles 1 leaf litter 2 fungal mycelia growing on the rock 1 soil
CMN 16	Cave with 11 meters of development, but access was only possible up to 5 meters, due to the reduction in the width of the conduit and a large number of blocks knocked down on the cave floor. Presence of bats and other animals inside the cave.	12 airborne particles 1 leaf litter 2 fungal mycelia growing on the rock 1 soil 2 roots
CFS 0600	Cavity with maximum development of 2 meters, little vegetation around and presence of many spiders.	3 airborne particles 1 leaf litter 2 fungal mycelia growing on the rock 1 roots
CFS 0386	Cavity with little development, measuring a maximum of 2.5 meters in depth and with a lot of vegetation around it.	1 leaf litter
CFS 0388	Cavity with a maximum development of 2.5 meters in ferruginous laterite, with the presence of collapsed blocks.	1 leaf litter 1 soil

The collections were performed in each cavity according to the availability of samples found. To collect the airborne particles samples, the Koch sedimentation methodology (Kuzmina et al., 2012) was used, which consists of exposing plates with culture media for 15 min. Three types of culture media were used: Potato Dextrose Agar (PDA) added with chloramphenicol (50 µg/mL), Dicloran Glycerol Agar and Dicloran Rose Bengal Chloramphenicol. The collection of soil samples was performed after a superficial cleaning, aiming to eliminate bulky substrates, collecting approximately 15 grams of soil in the layer of 1 to 5 cm in depth and conditioned in Falcon tubes with a capacity of 50 mL. For the leaf litter samples, the plant material found inside the cavities was collected and placed in paper bags (Leão-Ferreira et al., 2013). For the fungal mycelia growing on the rock samples, a sterile swab was used to smear the fungus present on the rock and then the swab was placed in test tubes containing 0.9% saline solution. The roots samples were collected by cutting thin roots of trees growing on the rock inside the cavity with scissors and placed in paper bags. All samples were placed in thermal boxes and later transported to the Laboratório de Micologia e Etiologia de Doenças Fúngicas de Plantas ate the Universidade Federal de Viçosa, Viçosa – MG, for isolation of the cave fungi.

Isolation

To isolate the fungi present in the soil samples, the serial dilution methodology described by Condé et al., (2023) was performed. One hundred microliters of dilution of each concentration samples soil and two hundred microliters sample collection solution for fungal mycelia growing on the rock, was spread over plates containing PDA, Dicloran Glycerol Agar and Dicloran Rose Bengal Chloramphenicolmedia, with duplicates for each medium. For colony growth, the airborne particles samples and the dilutions of the soil and fungal mycelia growing on the rock samples were incubated at 25ºC, in the dark for 30 days, and every two days the growing colonies were transferred to a new PDA plate.

The leaf litter samples were washed in running water for one hour, dried on autoclaved paper towels, and stored in humid chambers (polystyrene boxes containing filter paper moistened with sterilized water) (Castañeda-Ruiz 2005). Over the course of 30 days, the growth of fungal reproductive structures was observed under a stereoscopic microscope, and these structures were transferred to a plate containing PDA medium.

The isolation of fungi endophytically present in the roots samples was performed using the extinction dilution method (Collado et al., 2007; Leite et al., 2013; Oliveira et al., 2024). The roots were washed in running water, superficially disinfected in a 70% alcohol and Tween

80 solution for 1 min, followed by a 2.5% sodium hypochlorite solution for 5 min and finally in a 0.85% NaCl solution for 2 min. After that, the roots were crushed in a blender with a 0.85% NaCl solution and filtered through sieves with mesh sizes of 500 and 212 µm. The particles retained on the 212 µm sieve were resuspended with 0.85% NaCl in a falcon tube and centrifuged at 7000×g for 5 min. The supernatant was discarded, 20 mL of 0.1% carboxymethyl cellulose (CMC) solution was added and centrifuged at 7000×g for 15 min. The supernatant was discarded again, 20 mL of the 0.1% carboxymethyl cellulose was added and the dilutions 1:2, 1:4, 1:8, 1:16 and 1:32 were prepared, 150 µl of the dilutions were plated in Yeast extract-Malt extrac culture medium, with duplicates for each dilution. The plates were incubated at 25 ºC, in the dark, and for 30 days the growing colonies were transferred daily to a PDA plate.

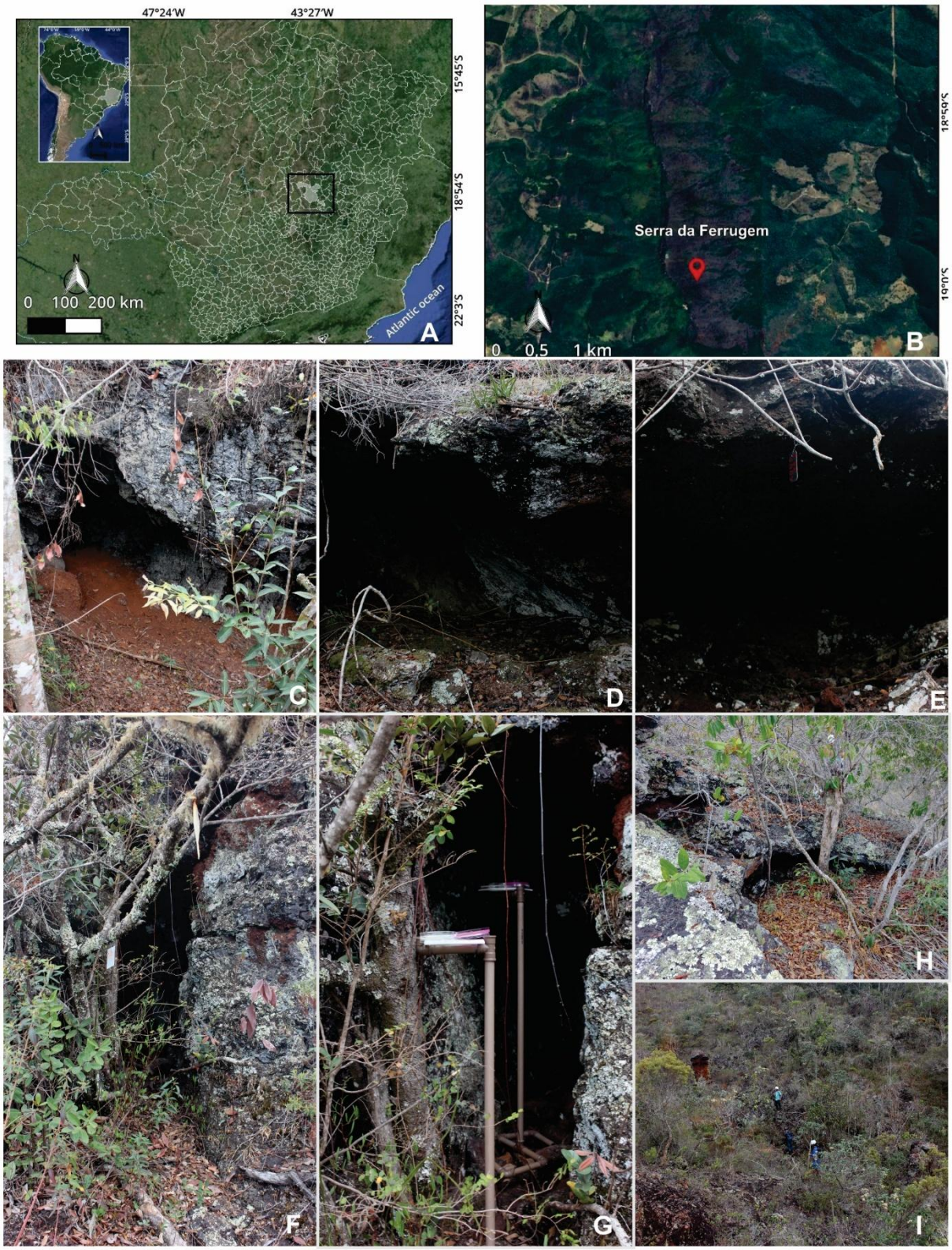

Figura 1 – A-B: Location of Serra da Ferrugem; C: Cavity CFS 0388; D: Cavity CFS 0386; E: Cavity CSF 0804; F: Cave CMN 16; G: Cave CMN highlighting structures for collecting airborne particles samples; H: Cavity CFS 0600; I: Serra da Ferrugem.

Fungi preservation

Through the hyphal tip methodology (Tuite 1969) pure cultures of all isolates were obtained and these were preserved in 10% glycerol at -20 °C, in sterilized distilled water at room temperature (Castellani 1939). All isolates were stored in the cave fungi collection of the Laboratory of Mycology and Etiology of Fungal Diseases of Plants of the Federal University of Viçosa.

DNA extraction, amplification and sequencing

The isolates were grown in PDA medium over cellophane at 25°C for 7 days, in the dark, for collection of fungal mycelium and extraction of total genomic DNA. The extraction was performed using the commercial Wizard Genomic DNA Purification kit (Promega®) and following the protocol described in chapter 1, with the changes described in chapter 2.

The identification of the isolates was performed by amplifying the internal transcribed spacer regions 1 and 2, together with the 5.8S subunit (ITS), using the methodology described in chapter 2. The PCR products were stained with GelRed (Biotium Inc.®) and analyzed by electrophoresis in a 1% agarose gel at 80 Volts for 40 min in 1 g mL^{-1} Tris-acetate-EDTA buffer. The size of the amplified fragment and purity were verified under ultraviolet light. The PCR products were sent for purification and sequencing to Macrogen Inc., South Korea (http://www.macrogen.com). The sequence editing of the ITS region was performed using FinchTV v.1.4.0 software.

Identification

The BLAST algorithm available on the National Center for Biotechnology Information (NCBI) platform (https://www.ncbi.nlm.nih.gov/) was used to identify isolates at the genus level. The sequences obtained in this study were compared with sequences deposited in the database, using the Sequences from type material filter, to compare only with sequences that have undergone an accuracy process and have a very low error level. Isolates with a similarity index greater than 95% with sequences of the same genus available in the database were considered as a species related to the genus. On the other hand, isolates that presented a similarity index lower than 95% were grouped only within the family that presented similarity. In the final count of genera in this study, isolates that were grouped at the family level and in this family no isolate was grouped with a genus, the family was counted as a genus, assuming that this isolate belongs to a genus (not identified) within the family.

Results

From the samples collected in the Serra da Ferrugem (SF) cavities, a total of 361 cultivable filamentous fungi were isolated, 101 from the airborne particles samples, 51 from the roots samples, 18 from the fungal mycelia growing on the rock samples, 85 from the leaf litter samples and 106 from the soil samples (Figure 2A, Table 2). The isolates were identified and distributed in 70 genera, 46 families, 26 orders, 10 classes, 6 subphyla and 4 phyla (Figure 2A, Table 2). Within the *Ascomycota* phylum, the genera *Penicillium* with 119 isolates, *Trichoderma* with 43, *Cladosporium* with 36 and *Talaromyces* with 13 presented the largest numbers of isolates (Figure 2A, Table 2). The *Zoopagomycota* phylum had only two isolates that could not be identified at the genus level, being grouped in the *Kickxellaceae* family (Table 2).

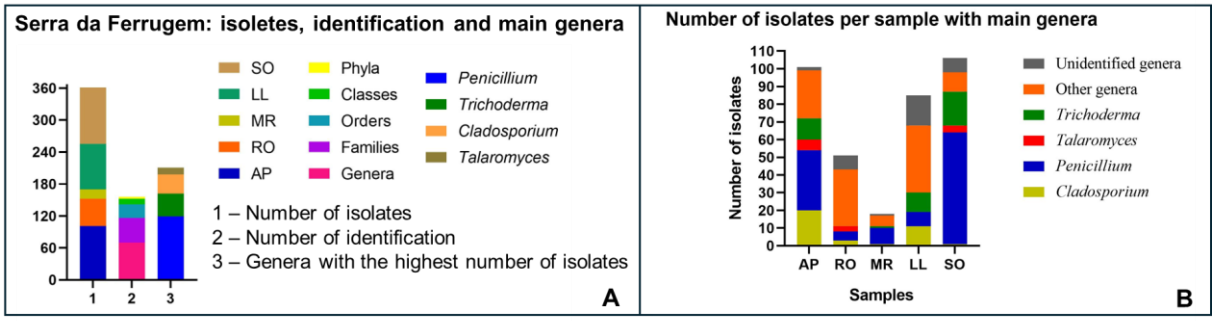

Figure 2 – A Number of isolates per sample collected at Serra da Ferrugem cave, overall identification number and main genera found; B Number of isolates within each genus divided by sample. AP - samples of airborne particles, SO - soil samples, LL - leaf litter, RO - roots that were present inside the cave, MR - fungal mycelia that were growing on the rocks.

In the airborne particles samples, approximately 65% of isolates belonged to the genera *Penicillium* (34 isolates), *Cladosporium* (20 isolates) and *Trichoderma* (12 isolates), (Figure 2B, Table 1). Only two isolates from the airborne particles samples did not belong to the phylum *Ascomycota*, one in the genus *Entomortierella* of the phylum *Mucoromycota* and another with an unidentified genus in the family *Polyporaceae*, of the phylum *Basidiomycota* (Table 2).

Endophytic isolates from the roots samples were grouped into 8 identified genera and another 6 unidentified genera within families that did not present any isolates in another genus (Figure 2B, Table 2). Almost 22% of the isolates from the roots samples were identified as belonging to the same family *Thyridariaceae*, and it was not possible to identify the genus. Only *Penicillium*, *Purpureocillium*, *Cladosporium*, *Talaromyces* and *Diaporthe* had more than one isolate (Figure 2B, Table 2).

In the fungal mycelia growing on the rock samples, all isolates were identified in the phylum *Ascomycota*, with *Penicillium* being the genus that grouped 50% of the isolates, and only *Fusarium* had more than one isolate (Figure 2B, Table 2). Two isolates from the fungal mycelia growing on the rock were not identified at the genus level; one isolate was grouped only in the order *Capnodiales*, being the only isolate of the order, and another in the family *Mycosphaerellaceae* (Table 2).

The leaf litter samples presented a greater diversity of genera among the samples, with 24 genera identified and 5 families that presented isolates without identified genera, totaling 29 different genera (Figure 2B, Table 2). The only isolates of the *Zoopagomycota* phylum in this study came from the SE samples. Approximately 50% of the genera identified from the leaf litter samples had only one isolate. *Trichoderma* and *Cladosporium* were the genera with the most isolates, 11 each, followed by *Penicillium* with 8 (Figure 2B, Table 2).

In the soil samples, of the 14 genera identified, only 4 had more than one isolate, with the *Penicillium* genus grouping almost 60% of the isolates, with 63 individuals. Followed by the genera *Trichoderma*, *Talaromyces* and *Sesquicillium* with 19, 4 and 2 isolates respectively (Figure 2B, Table 2).

From the CSF 0804 cavity, 62 fungal isolates were obtained, 10 from airborne particles and fungal mycelia growing on the rock samples each, 6 from the leaf litter and 36 from the soil samples (Figure 3A, Table 3). The isolates were grouped into 17 genera, 15 families, 14 orders, 8 classes, 4 subphyla and 3 phyla. Approximately 95% of isolates belonged to the *Ascomycota* phylum, with the *Penicillium* (30), *Trichoderma* (10) and *Cladosporium* (6) as the most common genera (Figure 3B, Table 3). The *Basidiomycota* phylum had only one isolate in this cavity, which was grouped within the *Polyporaceae* family, and the *Mucoromycota* phylum grouped one isolate in the *Absidia* genus and another in the *Umbelopsidaceae* family.

In the CMN16 cave, 175 isolates were obtained 71 from the airborne particles samples, 31 from the roots, 3 from the fungal mycelia growing on the rock, 17 from the leaf litter and 53 isolates from the soil samples (Figure 3A, Table 3). These isolates were identified in 39 genera, 29 families, 15 orders, 7 classes, 4 subphyla and 3 phyla. Only one isolate was identified in the *Basidiomycota* phylum, in the *Irpex* genus. In the *Mucoromycota* phylum, 9 isolates and three genera were identified, *Entomortierella*, *Mucor* and *Umbelopsis* with one isolate each, and another six isolates were identified as belonging to the *Cunninghamellaceae* family. In the *Ascomycota* phylum, the genera *Penicillium*, *Cladosporium*, *Trichoderma* and *Talaromyces* grouped more than 60% of the isolates belonging to the phylum (Figure 3B, Table 3).

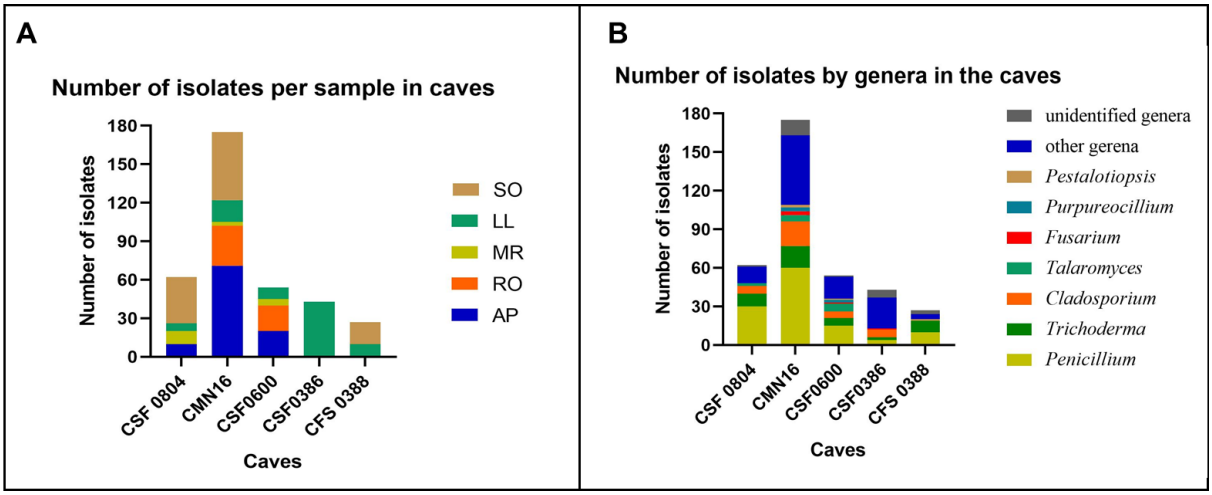

Figure 3 – A Number of isolates per sample in caves; B Number of isolates by main genera in the caves AP - samples of airborne particles, SO - soil samples, LL - leaf litter, RO - roots that were present inside the cave, MR - fungal mycelia that were growing on the rocks.

From the samples collected in cavity CSF0600, 54 filamentous fungi were isolated, with the airborne particles and roots samples having 20 isolates each, 9 isolates from the leaf litter samples and 5 from the fungal mycelia growing on the rock samples (Figure 3A, Table 3). The isolates were identified in 20 genera, of which 7 were unidentified, 19 families, 11 orders, 5 classes, 2 subphyla and 2 phyla. In the *Basidiomycota* phylum, only one isolate was identified at the genus level (*Fomitopsis*), while the other three isolates of the phylum were each grouped into a different family (*Meruliaceae*, *Phaneroetaceae* and *Peniophoraceae*). Within the phylum Ascomycota, the genera with more than one isolate were: *Penicillium* (15), *Talaromyces* (6), *Trichoderma* (6), *Cladosporium* (5), and *Purpureocillium* (2) (Figure 3B, Table 3).

In cavity SF0386, only one leaf litter sample was collected from which it was possible to isolate 43 filamentous fungi (Figure 3A, Table 3). The isolates were identified in 20 genera, with 4 unidentified and only 9 with more than one isolate. The isolates were distributed in 14 families, 11 orders, 6 classes, 3 subphyla and 3 phyla. In the phyla *Mucoromycota* and *Zoopagomycota* it was not possible to identify the genera of the isolates, being grouped in the families *Umbelopsidaceae*, with one isolate, and *Kickxellaceae*, with two isolates, respectively. In the phylum *Ascomycota*, which grouped 93% of the isolates, the genera *Cladosporium* (6 isolates) and *Penicillium* (4 isolates) were the ones that presented the highest numbers of isolates. The genera *Acrodontium*, *Thozetella*, *Trichoderma*, *Calonectria*, *Volutella*, *Hansfordia* and *Subramaniomyces* had two isolates each (Figure 3B, Table 3).

In cavity CFS 0388, 27 filamentous fungi were isolated, 10 from the leaf litter sample and 17 from the soil sample (Figure 3A, Table 3). All isolates were grouped into the phylum

Ascomycota and subphylum *Pezizomycotina*, within 3 classes, 6 orders, 6 families and 6 genera. *Penicillium* (10 isolates) and *Trichoderma* (9 isolates) were the genera with the highest numbers of isolates, followed by *Vermiculariopsiella* (2 isolates). The other genera identified (*Verruconis*, *Chloridium* and *Pestalotiopsis*) had only one isolate (Figure 3B, Table 3).

Table 2 - General classification of all isolates found in Serra da Ferrugem – Conceição do Mato Dentro, Brazil, by genus, family, order, class, subphylum and phylum, and identification of the source samples. Airborne particles (AP), soil (SO), leaf litter (LL), roots (RO), samples of fungal mycelia that were growing on the rocks (MR).

Phylum	Subphylum	Class	Order	Family	Genus	Samples type	Isolates
Ascomycota	*Pezizomycotina*	*Dothideomycetes*				RO	1
						LL	1
			Capnodiales			MR	1
			Cladosporiales	*Cladosporiaceae*	*Cladosporium*	AP	20
						RO	3
						MR	1
						LL	11
						SO	1
			Dothideales	*Saccotheciaceae*		AP	1
			Mycosphaerellales	*Mycosphaerellaceae*		MR	1
					Devonomyces	LL	1
					Mycosphaerella	LL	1
					Pseudocercospora	AP	1
				Teratosphaeriaceae		AP	1
						LL	1
					Acrodontium	LL	2
					Euteratosphaeria	AP	1
					Neocatenulostroma	AP	2
			Myriangiales	*Elsinoaceae*		AP	1
			Pleosporales	*Didymellaceae*	*Ascochyta*	MR	1
						AP	2
				Didymosphaeriaceae	*Chromolaenicola*	AP	1
					Paracamarosporium	LL	1
						RO	1

			Paraphaeosphaeria	MR	1
		Pleosporaceae	*Alternaria*	AP	2
			Bipolaris	AP	2
			Curvularia	AP	1
		Thyridariaceae		RO	11
	Venturiales	*Sympoventuriaceae*		LL	2
			Scolecobasidium	LL	1
			Verruconis	LL	1
Eurotiomycetes	*Eurotiales*	*Aspergillaceae*	*Aspergillus*	SO	1
				AP	1
			Penicillium	AP	34
				RO	5
				MR	9
				LL	8
				SO	63
		Trichocomaceae		RO	1
			Talaromyces	AP	6
				RO	3
				SO	4
	Phaeomoniellales	*Phaeomoniellaceae*		LL	1
Leotiomycetes				LL	1
		Pseudeurotiaceae		LL	1
	Erysiphales	*Erysiphaceae*		LL	1
	Helotiales			LL	1
			Nagrajchalara	LL	1
		Dermateaceae		RO	3
		Hyphodiscaceae		RO	5

Sordariomycetes	*Calosphaeriales*	*Pleurostomataceae*	*Pleurostoma*	SO	1
	Chaetosphaeriales	*Chaetosphaeriaceae*		LL	7
				RO	1
			Arcuatospora	LL	1
			Chloridium	LL	1
			Thozetella	LL	2
	Diaporthales	*Diaporthaceae*		RO	5
				LL	1
			Diaporthe	RO	2
		Schizoparmaceae	*Coniella*	AP	1
		Valsaceae	*Cytospora*	SO	1
	Glomerellales	*Glomerellaceae*	*Colletotrichum*	LL	2
	Hypocreales			LL	1
		Bionectriaceae	*Clonostachys*	SO	1
			Nectriopsis	LL	1
			Sesquicillium	SO	2
		Cordycipitaceae	*Leptobacillium*	LL	2
		Hypocreaceae	*Trichoderma*	AP	12
				MR	1
				LL	11
				SO	19
		Nectriaceae	*Calonectria*	LL	2
			Cylindromonium	LL	2
			Fusarium	AP	2
				MR	2
				LL	1
			Volutella	LL	2

				Ophiocordycipitaceae		SO	1
					Purpureocillium	AP	1
						RO	4
			Sordariales	*Chaetomiaceae*	*Arcopilus*	SO	1
			Vermiculariopsiellales	*Vermiculariopsiellaceae*	*Vermiculariopsiella*	LL	2
			Xylariales		*Castanediella*	LL	1
					Hansfordia	LL	2
					Subramaniomyces	LL	2
				Apiosporaceae	*Nigrospora*	AP	1
				Beltraniaceae	*Beltraniella*	LL	1
				Microdochiaceae	*Selenodriella*	RO	1
				Sporocadaceae		LL	1
						AP	1
					Bartalinia	AP	1
					Neopestalotiopsis	AP	1
						MR	1
					Pestalotiopsis	AP	3
						SO	1
	Saccharomycotina	*Saccharomycetes*	*Saccharomycetales*	*Debaryomycetaceae*		LL	1
Basidiomycota	*Agaricomycotina*	*Agaricomycetes*	*Polyporales*	*Fomitopsidaceae*	*Fomitopsis*	RO	1
				Irpicaceae	*Irpex*	RO	1
				Meruliaceae		RO	1
				Phanerochaetaceae		RO	1
				Polyporaceae		AP	1
			Russulales	*Peniophoraceae*		RO	1
Mucoromycota	*Mortierellomycotina*	*Mortierellomycetes*	*Mortierellales*	*Mortierellaceae*	*Entomortierella*	AP	1
	Mucoromycotina	*Mucoromycetes*	*Mucorales*	*Cunninghamellaceae*		SO	6

					Absidia	SO	1
				Mucoraceae	*Mucor*	SO	1
						SO	1
		Umbelopsidomycetes	*Umbelopsidales*	*Umbelopsidaceae*		LL	1
					Umbelopsis	SO	1
Zoopagomycota	*Kickxellomycotina*	*Kickxellomycetes*	*Kickxellales*	*Kickxellaceae*		LL	2

Table 3 – Classification of isolates into genus, family, order, class, subphylum and phylum, and identification of source samples. Table divided by cavities collated in Serra da Ferrugem – Conceição do Mato Dentro, Brazil. Airborne particles (AP), soil (SO), leaf litter (LL), roots (RO), samples of fungal mycelia that were growing on the rocks (MR)

Phylum	**Subphylum**	**Class**	**Order**	**Family**	**Genus**	**Samples type**	**Number of isolates**
Cavidade CSF 0804							
Ascomycota	*Pezizomycotina*	*Dothideomycetes*	*Capnodiales*			MR	1
			Cladosporiales	*Cladosporiaceae*	*Cladosporium*	AP	4
						MR	1
						SO	1
			Dothideales	*Saccotheciaceae*		AP	1
			Mycosphaerellales	*Mycosphaerellaceae*		MR	1
					Devonomyces	LL	1
			Pleosporales	*Didymellaceae*	*Ascochyta*	MR	1
		Eurotiomycetes	*Eurotiales*	*Aspergillaceae*	*Aspergillus*	SO	1
					Penicillium	AP	3
						MR	6
						LL	1
						SO	20
				Trichocomaceae	*Talaromyces*	SO	2

			Phaeomoniellales	*Phaeomoniellaceae*		LL	1
		Leotiomycetes	*Erysiphales*	*Erysiphaceae*		LL	1
		Sordariomycetes	*Glomerellales*	*Glomerellaceae*	*Colletotrichum*	LL	1
			Hypocreales	*Bionectriaceae*	*Sesquicillium*	SO	1
				Hypocreaceae	*Trichoderma*	AP	1
						SO	9
	Saccharomycotina	*Saccharomycetes*	*Saccharomycetales*	*Debaryomycetaceae*		LL	1
Basidiomycota	*Agaricomycotina*	*Agaricomycetes*	*Polyporales*	*Polyporaceae*		AP	1
Mucoromycota	*Mucoromycotina*	*Mucoromycetes*	*Mucorales*	*Cunninghamellaceae*	*Absidia*	SO	1
		Umbelopsidomycetes	*Umbelopsidales*	*Umbelopsidaceae*		SO	1
Caverna CMN16							
Ascomycota	*Pezizomycotina*	*Dothideomycetes*				RO	1
			Cladosporiales	*Cladosporiaceae*	*Cladosporium*	AP	12
						RO	3
						LL	4
			Mycosphaerellales	*Mycosphaerellaceae*	*Mycosphaerella*	LL	1
					Pseudocercospora	AP	1
				Teratosphaeriaceae		AP	1
						LL	1
					Euteratosphaeria	AP	1
					Neocatenulostroma	AP	2
			Myriangiales	*Elsinoaceae*		AP	1
			Pleosporales	*Didymellaceae*	*Ascochyta*	AP	1
				Didymosphaeriaceae	*Chromolaenicola*	AP	1
					Paracamarosporium	LL	1

				RO	1
			Alternaria	AP	2
		Pleosporaceae	*Bipolaris*	AP	2
			Curvularia	AP	1
		Thyridariaceae		RO	10
			Aspergillus	AP	1
				AP	23
				RO	1
		Aspergillaceae	*Penicillium*	MR	1
				LL	1
				SO	34
				AP	2
		Trichocomaceae	*Talaromyces*	RO	1
				SO	2
				LL	1
Leotiomycetes		*Pseudeurotiaceae*		LL	1
	Helotiales	*Dermateaceae*		RO	3
Sordariomycetes	*Calosphaeriales*	*Pleurostomataceae*	*Pleurostoma*	SO	1
	Chaetosphaeriales	*Chaetosphaeriaceae*		LL	2
		Diaporthaceae		RO	5
	Diaporthales		*Diaporthe*	RO	2
		Schizoparmaceae	*Coniella*	AP	1
		Valsaceae	*Cytospora*	SO	1
	Hypocreales	*Bionectriaceae*	*Clonostachys*	SO	1
			Sesquicillium	SO	1

				Hypocreaceae	*Trichoderma*	AP	9
						MR	1
						LL	3
						SO	4
				Nectriaceae		LL	1
					Fusarium	AP	2
						MR	1
				Ophiocordycipitaceae		SO	1
					Purpureocillium	AP	1
						RO	2
			Xylariales		*Castanediella*	LL	1
				Apiosporaceae	*Nigrospora*	AP	1
				Microdochiaceae	*Selenodriella*	RO	1
				Sporocadaceae		AP	1
					Bartalinia	AP	1
					Neopestalotiopsis	AP	1
					Pestalotiopsis	AP	2
Basidiomycota	*Agaricomycotina*	*Agaricomycetes*	*Polyporales*	*Irpicaceae*	*Irpex*	RO	1
Mucoromycota	*Mortierellomycotina*	*Mortierellomycetes*	*Mortierellales*	*Mortierellaceae*	*Entomortierella*	AP	1
	Mucoromycotina	*Mucoromycetes*	*Mucorales*	*Cunninghamellaceae*		SO	6
				Mucoraceae	*Mucor*	SO	1
		Umbelopsidomycetes	*Umbelopsidales*	*Umbelopsidaceae*	*Umbelopsis*	SO	1
Cavidade CSF0600							
Ascomycota	*Pezizomycotina*	*Dothideomycetes*	*Cladosporiales*	*Cladosporiaceae*	*Cladosporium*	AP	4
						LL	1

			Pleosporales	*Didymellaceae*	*Ascochyta*	AP	1
				Didymosphaeriaceae	*Paraphaeosphaeria*	MR	1
				Thyridariaceae		RO	1
			Venturiales	*Sympoventuriaceae*	*Scolecobasidium*	LL	1
		Eurotiomycetes	*Eurotiales*	*Aspergillaceae*	*Penicillium*	AP	8
						RO	4
						MR	2
						LL	1
				Trichocomaceae		RO	1
					Talaromyces	AP	4
						RO	2
		Leotiomycetes	*Helotiales*	*Hyphodiscaceae*		RO	5
		Sordariomycetes	*Chaetosphaeriales*	*Chaetosphaeriaceae*		RO	1
			Diaporthales	*Diaporthaceae*		LL	1
			Hypocreales	*Cordycipitaceae*	*Leptobacillium*	LL	1
				Hypocreaceae	*Trichoderma*	AP	2
						LL	4
				Nectriaceae	*Fusarium*	MR	1
				Ophiocordycipitaceae	*Purpureocillium*	RO	2
			Xylariales	*Sporocadaceae*	*Neopestalotiopsis*	MR	1
					Pestalotiopsis	AP	1
Basidiomycota	*Agaricomycotina*	*Agaricomycetes*	*Polyporales*	*Fomitopsidaceae*	*Fomitopsis*	RO	1
				Meruliaceae		RO	1
				Phaneroetaceae		RO	1
			Russulales	*Peniophoraceae*		RO	1

Cavidade CSF0386							
Ascomycota	*Pezizomycotina*	*Dothideomycetes*	*Cladosporiales*	*Cladosporiaceae*	*Cladosporium*	LL	6
			Mycosphaerellales	*Teratosphaeriaceae*	*Acrodontium*	LL	2
			Venturiales	*Sympoventuriaceae*		LL	2
		Eurotiomycetes	*Eurotiales*	*Aspergillaceae*	*Penicillium*	LL	4
		Leotiomycetes	*Helotiales*			LL	1
					Nagrajchalara	LL	1
		Sordariomycetes	*Chaetosphaeriales*	*Chaetosphaeriaceae*		LL	3
					Arcuatospora	LL	1
					Thozetella	LL	2
			Glomerellales	*Glomerellaceae*	*Colletotrichum*	LL	1
			Hypocreales			LL	1
				Bionectriaceae	*Nectriopsis*	LL	1
				Cordycipitaceae	*Leptobacillium*	LL	1
				Hypocreaceae	*Trichoderma*	LL	2
				Nectriaceae		LL	1
					Calonectria	LL	2
					Fusarium	LL	1
					Volutella	LL	2
			Xylariales		*Hansfordia*	LL	2
					Subramaniomyces	LL	2
				Beltraniaceae	*Beltraniella*	LL	1
				Sporocadaceae		LL	1
Mucoromycota	*Mucoromycotina*	*Umbelopsidomycetes*	*Umbelopsidales*	*Umbelopsidaceae*		LL	1
Zoopagomycota	*Kickxellomycotina*	*Kickxellomycetes*	*Kickxellales*	*Kickxellaceae*		LL	2

Cavidade CFS 0388							
Ascomycota	*Pezizomycotina*	*Dothideomycetes*				LL	1
			Venturiales	*Sympoventuriaceae*	*Verruconis*	LL	1
		Eurotiomycetes	*Eurotiales*	*Aspergillaceae*	*Penicillium*	LL	1
						SO	9
		Sordariomycetes	*Chaetosphaeriales*	*Chaetosphaeriaceae*		LL	2
					Chloridium	LL	1
			Hypocreales	*Hypocreaceae*	*Trichoderma*	LL	2
						SO	7
			Vermiculariopsiellales	*Vermiculariopsiellaceae*	*Vermiculariopsiella*	LL	2
			Xylariales	*Sporocadaceae*	*Pestalotiopsis*	SO	1

Discussions

In the airborne particles samples collected from the Serra da Ferrugem (SF) cavities, there was a predominance of the genera *Penicillium*, *Cladosporium* and *Trichoderma* (Figure 2, Table 2). These genera are frequently reported in cave environments worldwide, a pattern likely related to their high adaptability and large spore production , which facilitates dispersion (Zhang et al., 2021; Poli et al., 2024).

The soil samples yielded the highest number of isolates in this study, followed by the airborne particles samples (Figure 2, Table 2) generally this substrate is the one that presents the most isolates in caves (Vanderwolf et al., 2013; Zhang et al., 2021). Soil is among the most favorable substrates for fungal colonization in caves due to its higher organic matter content. The decomposition of this available organic carbon is essential for fungal growth and maintenance of the cave ecosystem (Adetutu et al., 2011; Biagioli et al., 2024).

The genus *Pencillium* was the most frequently isolated taxon (Figure 2, Table 2), consistent with its widespread occurrence in caves globally (Vanderwolf et al., 2013; Zhang et al., 2021). Analyzing soil and sediments from the ferruginous cave RM3, also found the genus *Pencillium* to be the most diverse in the cave Taylor et al., (2014). In addition to *Pencillium*, the genera *Talaromyces* and Trichoderma were dominant in soil samples from the Gruta do Catão cave in Bahia state, Brazil (Paula et al., 2016). Although *Aspergillus* is also widely reported in cave sediments, only one isolate of this genus was recovered in our study.

The genera *Devonomyces*, *Mycosphaerella*, *Acrodontium*, *Scolecobasidium*, *Verruconis*, *Nagrajchalara*, *Arcuatospora*, *Chloridium*, *Thozetella*, *Colletotrichum*, *Nectriopsis*, *Leptobacillium*, *Calonectria*, *Cylindromonium*, *Volutella*, *Vermiculariopsiella*, *Castanediella*, *Hansfordia*, *Subramaniomyces*, and *Beltraniella,* were found only in leaf litter samples, and some of these genera are being reported for the first time in the cave literature (Vanderwolf et al., 2013; Zhang et al., 2021). Leaf litter can be a major source of fungal entry into the cave environment (Vanderwolf et al., 2013; Zhang et al., 2021) and is also a sample with great potential for the discovery of new fungal species *Chaetomium meridionalense* (Condé et al., 2023) was previously reported from litter samples collected in caves of the Southern Espinhaço Mountainsof Minas Gerais.

Although roots are not widely studied in cave environments, mycorrhizal fungi have been reported in shallow caves, but their identification has not been successful (Lamont &

Lange 1976; Jasinska et al., 1996; Vanderwolf et al., 2013; Poelman et al., 2021). Poelman et al., (2021) conducted the first study of fungi in aeroponic roots in caves. They analyzed the mycobiota present in the samples using cultivation-independent methods and found a diversity of genera, ranging from endophytes to mycorrhizal fungi to plant pathogens. Of the diversity of genera found by Poelman et al., (2021), only the genus *Cladosporium* was also found in our study. The genus *Selenodriella,* exclusively in airborne particles samples in this study, is also reported here for the first time in the cave environment (Vanderwolf et al., 2013; Zhang et al., 2021).

Of the 361 isolates identified, approximately 95% belongedhe phylum *Ascomycota*, which is consistent with previous studies that report this phylum as the most dominant in cave ecosystems due to its cosmopolitan nature and broad substrate adaptability (Figure 2, Table 2) (Vanderwolf et al., 2013; Zhang et al., 2021; Biagioli et al., 2024). In this study, *Penicillium* was the most abundant genus, although only two *Aspergillus* isolates were obtained. In the ferruginous cave RM3 in Nova Lima, Minas Gerais, both genera were more abundant (Taylor et al., 2014). The genera *Aspergillus* and *Penicillium* are the genera with the most reports in caves both worldwide and in Brazil (Vanderwolf et al., 2013; Zhang et al., 2021; Alves et al., 2022).

To date, there is no information related to the genera *Arcuatospora*, *Ascochyta*, *Beltraniella*, *Calonectria*, *Castanediella*, *Chromolaenicola*, *Coniella*, *Cylindromonium*, *Cytospora*, *Devonomyces*, *Entomortierella*, *Euteratosphaeria*, *Nagrajchalara*, *Nectriopsis*, *Neocatenulostroma*, *Pseudocercospora*, *Selenodriella*, *Sesquicillium*, *Subramaniomyces*, *Thozetella*, *Vermiculariopsiella* and *Verruconis*, which are present in the cave environment around the world, and this work is the first report. A global compilation of fungal genera reported in caves was published by Vanderwolf et al., (2013) and updated by Zhang et al., (2021) totaling more than 600 genera. Our study contributes new records to this growing listAnother genus found in this work is *Neopestalotiopsis*, to date the only report of this genus in the cave environment was carried out by Liu et al., (2021) who described the species *Neopestalotiopsis cavernicola*, collected in Gem Cave in China, making this the first report of the genus in a cave in Brazil.

Conclusions

The Brazilian cave mycobiota remains poorly understood and underexplored. This study highlights the rich mycological diversity found in the ferruginous caves of Serra da Ferrugem. A total of 361 cultivable filamentous fungi were obtained and identified across 70 different genera, and this work reports, for the first time, the occurrence of 22 genera (*Arcuatospora*, *Ascochyta*, *Beltraniella*, *Calonectria*, *Castanediella*, *Chromolaenicola*, *Coniella*, *Cylindromonium*, *Cytospora*, *Devonomyces*, *Entomortierella*, *Euteratosphaeria*, *Nagrajchalara*, *Nectriopsis*, *Neocatenulostroma*, *Pseudocercospora*, *Selenodriella*, *Sesquicillium*, *Subramaniomyces*, *Thozetella*, *Vermiculariopsiella* and *Verruconis*) of fungi in the cave environment, reinforcing the importance of caves as mycological hotspots. The expansion of mycological research in these environments not only contributes to biodiversity inventories but may also reveal taxa of ecological, biotechnological, or pharmaceutical relevance.

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CHAPTER 4

Description of new fungal species

Article accepted for publication - According to the Fungal Diversity Journal standard

Fungal diversity notes 2020–2125: taxonomic and phylogenetic contributions to freshwater fungi and other fungal taxa – *Speluncomyces lunatus*, *Cylindromonium brasiliense* and *Sesquicillium flavum.*

Fungal diversity notes 2025 extra edition: taxonomic and phylogenetic contributions to freshwater fungi and other fungal taxa – *Paraneoaraneomyces* sp. nov*, Tolypocladium* sp. nov*, Sarocladium* sp. nov.and *Rachicladosporium* sp. nov.

CHAPTER 4.1

Description of new fungal species

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According to the Fungal Diversity Journal standard

Fungal diversity notes 2020–2125: taxonomic and phylogenetic contributions to freshwater fungi and other fungal taxa – *Speluncomyces lunatus*, *Cylindromonium brasiliense* and *Sesquicillium flavum*

Fungal diversity notes 2020–2125: taxonomic and phylogenetic contributions to freshwater fungi and other fungal taxa

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Hypocreales Lindau, Nat. Pflanzenfam., Teil. I (Leipzig) 1(1): 343 (1897)

Bionectriaceae Samuels & Rossman, Stud. Mycol. 42: 15 (1999)

Notes: Rossman et al. (1999) introduced Bionectriaceae, which includes 26 genera with perithecial (21) or cleistothecial (5) ascomata. Later, they confirmed the monophyly of Bionectriaceae using the phylogenetic analysis of LSU sequence. Hyde et al. (2020d) and Wijayawardene et al. (2022b) further accepted 47 genera in Bionectriaceae. However, Hou et al. (2023) reported 39 monophyletic genera based on multi-locus phylogenetic analysis of combined ITS, LSU, *rpb2*, and *tef1-α*. The genus *Sesquicillium* was reinstated as a sister genus to *Clonostachys* (Zhao et al. 2023), and *Amphichorda* was transferred from Cordycipitaceae to Bionectriaceae (Guerra-Mateo et al. 2023). Currently, this family comprises approximately 41 monophyletic genera, including several acremonium-like fungi and other asexual morphs, such as beauveria-like, clonostachys-like, verticillium-like, and sesquicillium-like (Summerbell et al. 2011; Hou et al. 2023; Guerra-Mateo et al. 2023; Zhao et al. 2023).

Sesquicillium W. Gams, Acta Bot. Neerl. 17: 455 (1968)

Notes: Gams (1968) introduced the genus *Sesquicillium* to accommodate fungi having verticillate conidiophores with intercalary phialides and sparsely branched verticils. Schroers (2001) applied a broader concept to clonostachys-like, sesquicillium-like, and verticillium-like anamorphs, and synonymised several genera with *Clonostachys*, including *Sesquicillium*.

However, Zhao et al. (2023) reinstated *Sesquicillium* to accommodate fungi that exhibit penicillate conidiophores, having 1–2 intercalary phialides below a single terminal phialide. Based on a multi-locus phylogenetic analysis, *Sesquicillium* is sister to *Clonostachys* (Zhao et al. 2023). Both genera occupy different ecological niches in nature, and species of *Sesquicillium* produce mononematous conidiophores and reduced perithecial stroma superficially on leaves, whereas *Clonostachys* spp. can form sporodochia and well-defined stroma and occur endophytically in woody plants (Zhao et al. 2023).

Sesquicillium flavum F.A. Custódio, A.F. Leão, T.O. Condé & O.L. Pereira, ***sp. nov.***

Index Fungorum number: IF850535; *Facesoffungi number*: FoF 15036; Fig. 92

Etymology: the epithet refers to the yellowish pigment produced by this fungus on culture media.

Holotype: VIC 49485

On soil from a ferruginous cave. **Sexual morph**: undetermined. **Asexual morph**: on SNA, *mycelia* septate, branched, smooth-walled, hyaline, 2–5 µm wide. *Conidiophores* 42–115 × 2–4 µm ($\overline{x}$ = 77 × 3 µm, n = 10), micronematous, mononematous, monomorphic, subcylindrical, arising from the agar surface or from aerial mycelia, sometimes from coils formed by mycelia, 1–4 verticillate, divergent branches, hyaline, smooth-walled. *Conidiogenous cells* enteroblastic, polyphialidic, adpressed or divergent; *stipes* 30–79 × 2–4 µm; *penicilli* 43–105 × 17–51 µm; *terminal phialides* solitary, cylindrical, clavate or lageniform, adpressed or divergent, tapering towards the apex (4.9–)7.3–8.6(–12.2) µm long, (1.4–)1.9–2.3(–2.9) µm wide at base, (2.2–)2.7–3(–3.3) µm at widest point, (0.8–)1.1–1.2(–1.6) µm wide near aperture; *intercalary phialides* below solitary terminal phialide, cylindrical, with subapical pegs 0.8–2.6 µm long, sometimes arranged in whorls, (5.7–)7.1–8.2(–10.3) × 2.5–3.5 µm. *Conidia* (4–)4.7–5.1(–5.8) × (3–)3.3–3.6(–4.1) µm ($\overline{x}$ = 4.9 × 3.4 µm, n = 20), subglobose to ellipsoidal, aseptate, hyaline, smooth-walled, with rounded ends, with median hilum or sometimes slightly laterally displaced.

Culture characteristics: (incubated for 14 days at 25 ºC in 12 h near UV light) colonies on PDA reaching 31–52 mm diam, fimbriate edge, flat, cottony, with some membranous areas, aerial mycelia dense, without sporulation, white colour on surface, amber colour (47) on reverse, yellowish soluble pigment (Rayner 1970). Colonies on MEA reaching 30–61 mm diam,

fimbriate edge, flat, aerial mycelia cottony, moderate, hyphal tufts growing irregularly, sporulation intense, white colour on surface, straw (46) on reverse. Colonies on OA reaching 32–65 mm diam, fimbriate edge, flat, cottony, moderate aerial mycelia with irregular hyphal tufts, sporulation intense, white colour on obverse, straw (46) on reverse, yellowish soluble pigment. Colonies on SNA reaching 22–25 mm diam, fimbriate edge, flat, translucid, aerial mycelia sparse, sporulation intense, smoke grey colour (105) on surface and reverse.

Material examined: Brazil, Minas Gerais, Conceição do Mato Dentro, Serra da Ferrugem (19º0'16.096"S 43º23'39.034"W) (Cave CMN 16), in soil from a ferruginous cave, 29 September 2022, A.F. Leão, T.O. Condé, F.A. Custódio & O.L. Pereira (VIC 49485, **holotype**); ex-type living culture, COAD 3667. *ibid*., (Cave CSF 0804), in soil from a ferruginous cave, 29 September 2022, A.F. Leão; T.O. Condé, F.A. Custódio & O. L. Pereira, living culture, COAD 3668.

GenBank accession numbers: COAD 3667 = ITS: OR669010, *rpb2*: OR678546, *tef1-α*: OR678545, *tub2*: OR678547; COAD 3668 = ITS: OR669011, LSU: OR669011, *rpb2*: OR678549, *tef1-α*: OR678548, *tub2*: OR678550.

Notes: multi-locus phylogenetic analysis of combined ITS, LSU, *rbp2*, and *tef1-α* reveals that *Sesquicillium flavum* (COAD 3667 and COAD 3668) forms a new and well-supported lineage that is related to *S. lasciadis* and *S. candelabrum* (Fig. 93). Morphologically, *S. flavum* can be distinguished from *S. candelabrum* in producing only solitary terminal phialides (Fig. 92c–e), whereas, *S. candelabrum* has terminal phialides arranged in whorls (Zhao et al. 2023). *Sesquicillium flavum* differs from *S. lasiacidis* in producing subglobose, shorter and wider conidia (4–)4.7–5.1(–5.8) × (3–)3.3–3.6(–4.1) µm (Fig. 92f, g), whereas, *S. lasiacidis* exhibits ellipsoidal, longer and narrower conidia (5.6–)6.4–7.6(–8.2) × (1.8–)2.2–2.8(–3.2) µm (Schroers 2001). *Sesquicillium* spp. are widely reported in soil and decaying organic matter (Schroers 2001), and our new species has also been found in a soil sample collected from a ferruginous cave.

Fig. 92 ***Sesquicillium flavum*** (VIC 49485, **holotype**). **a** Colonies, from left to right, top row = PDA, MEA, OA, and SNA; bottom row = PDA, MEA, and OA. **b** Conidiophores in aerial mycelia on OA. **c**–**e** Penicillate conidiophores bearing intercalary and terminal phialides. **f** Conidiophores arising from coils formed by mycelia. **g** Conidia. Scale bars: **c**–**e** = 20 μm, **f**, **g** = 10 μm.

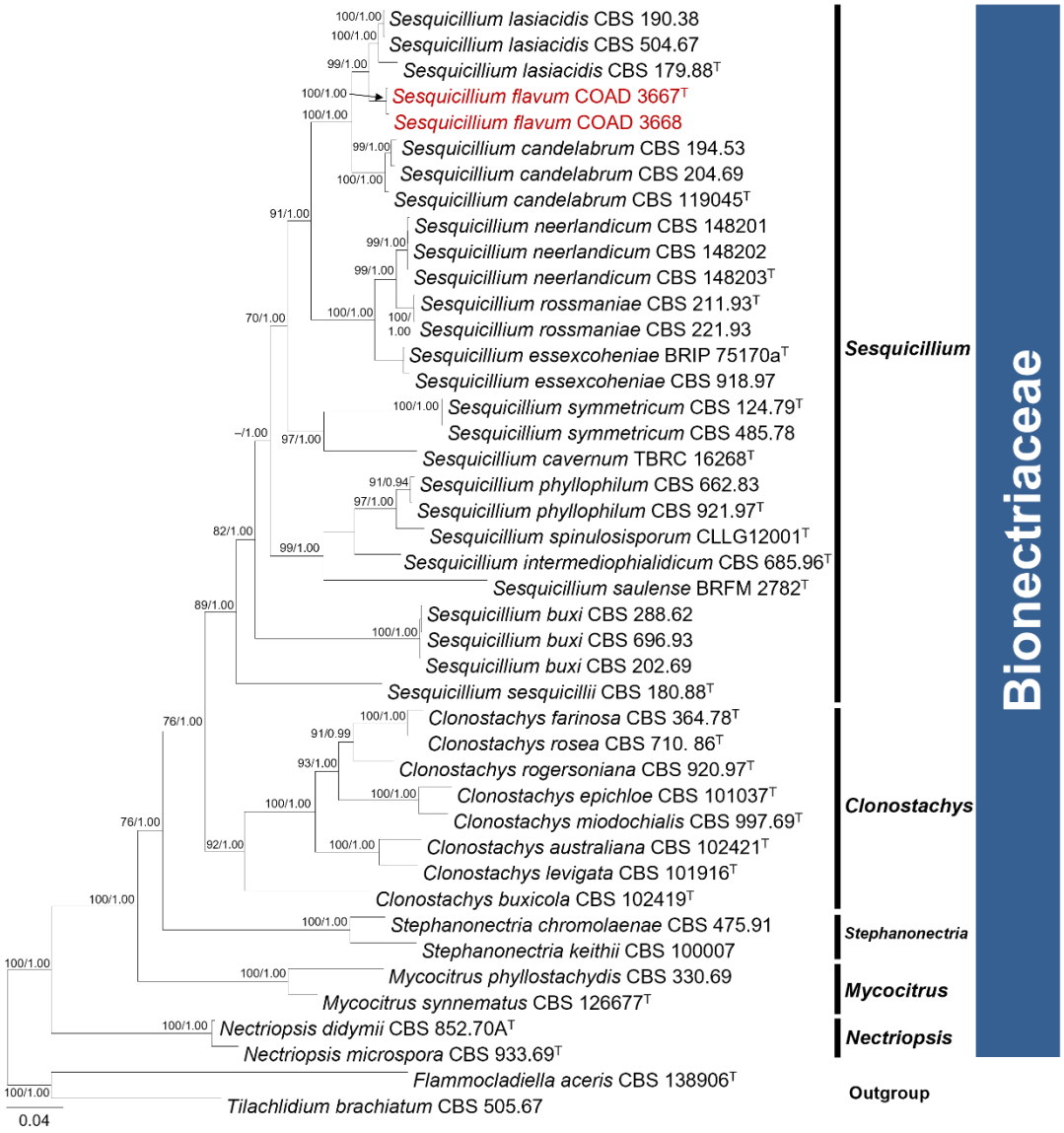

Fig. 93 Maximum likelihood consensus tree inferred from the combined ITS, LSU, *rpb2*, *tef1-α* and *tub2* multiple sequence alignments. Bootstrap support values for maximum likelihood (ML, first value) equal to or greater than 70% and Bayesian posterior probabilities from MCMC analyses (BYPP, second value) equal to or greater than 0.90 are given above the nodes. The scale bar indicates expected changes per site. The tree is rooted to *Flammocladiella aceris* CBS 138906 (Flammocladiellaceae) and *Tilachlidium brachiatum* CBS 505.67 (Tilachlidiaceae). Ex-type strains are indicated with "^{T}". The newly generated sequences are indicated in red.

Clavicipitaceae (Lindau) Earle, Contributions from the United States National Herbarium 6: 170 (1901)

Notes: Clavicipitaceae is a highly diverse family in the Hypocreales, comprising 50 genera and more than 500 species (Robert et al. 2013). Some members within the family are well-known entomopathogens (*Metarhizium* spp. and *Pochonia* spp.), plant pathogens (*Claviceps* spp.), or grass symbionts (*Epichloe* spp.) (Mongkolsamrit et al. 2020; Gao et al. 2021). Based on molecular phylogenetic analyses, Sung et al. (2007a) divided Clavicipitaceae into three clades, two of which are now represented by *Cordycipitaceae* and *Ophiocordycipitaceae*.

Speluncomyces A.F. Leão, T.O. Condé, F.A. Custódio & O.L. Pereira, ***gen. nov.***

Index Fungorum number: IF850531; *Facesoffungi number*: FoF 15037

Etymology: name refers to *spelunca*, meaning cave in Latin, in reference to the collection location, where the new fungus was discovered.

Saprobic on leaf litter found in cave. **Sexual morph**: undetermined. **Asexual morph**: On PDA, *mycelia* septate, branched, smooth-walled, forming bundles, hyaline. *Conidiophores* micronematous, mononematous, erect, cylindrical, straight or flexuous, arising from hyphae or ropes formed by the mycelia, unbranched, septate, sometimes reduced to conidiogenous cells, hyaline. *Conidiogenous cells* enteroblastic, monophialidic, integrated, terminal, smooth-walled, cylindrical to subulate, hyaline with inconspicuous periclinal thickening and collarette at conidiogenous loci. *Conidia* allantoid to lunate, thin- and smooth-walled, aseptate, hyaline, arranged in slimy heads.

Type species: *Speluncomyces lunatus* A.F. Leão, T.O. Condé, F.A. Custódio & O.L. Pereira

Notes: the monotypic genus *Speluncomyces* is introduced herein to accommodate an asexual taxon *S. lunatus* in Clavicipitaceae, which was collected from leaf litter in a ferruginous cave. Phylogenetic analyses of the combined ITS, LSU*, rpb2*, and *tef1-α* sequence dataset show that *Speluncomyces* forms an independent lineage in Clavicipitaceae, closely related to *Pseudometarhizium* (Fig. 97). *Pseudometarhizium* also produces monophialidic conidiogenous cells and hyaline conidia but can be distinguished from *Speluncomyces* by producing conidial

chains and by the shape of conidia (Chen et al. 2022). Clavicipitaceae includes acremonium-like fungi that produce monophialides, and conidia arranged in slimy heads, such as *Paraneoaraneomyces*, *Pochonia*, and *Subuliphorum* (Hou et al. 2023; Zhang et al. 2023b). The novel genus *Speluncomyces* differs from *Paraneoaraneomyces*, *Pochonia*, and *Subuliphorum* in the absence of phialides arising from hyphae regimental, chlamydospores, and verticillate conidiophores, respectively.

Speluncomyces lunatus A.F. Leão, T.O. Condé, F.A. Custódio & O.L. Pereira, ***sp. nov.***

Index Fungorum number: IF850532; *Facesoffungi number*: FoF 15034; Fig. 96

Etymology: in reference to the lunate-shaped conidia produced by this fungus.

Holotype: VIC 49484

Saprobic on leaf litter from a ferruginous cave. **Sexual morph**: undetermined. **Asexual morph**: on PDA, *mycelia* septate, branched, smooth-walled, forming bundles, hyaline, 0.75–1.5 µm diam. *Conidiophores* 10–73.5 × 0.9–1.8 µm ($\overline{x}$ = 27.9 × 1.3 µm, n = 20), solitary or aggregated, erect, flexuous, arising from hyphae, 1–3-septate, sometimes reduced to conidiogenous cells, unbranched, hyaline. *Conidiogenous cells* 7.5–25.5 × 0.8–1.5 µm ($\overline{x}$ = 19.1 × 1.1 µm, n = 20), enteroblastic, monophialidic, integrated, determinate, terminal, cylindrical to subulate, flexuous, with inconspicuous periclinal thickening and collarette at conidiogenous loci, smooth-walled, hyaline. *Conidia* 2.0–7.5 × 1–1.5 µm ($\overline{x}$ = 14.7 × 2.3 µm, n = 20), allantoid to lunate, aseptate, hyaline, thin- and smooth-walled, arranged in slimy heads.

Culture characteristics: (incubated for 14 days at 25 ºC in 12 h near UV light) colonies on PDA reaching 16–18 mm diam, radially striate with entire edge, raised with concave edge, aerial mycelia cottony, dense, profuse, aerial sporulation, white on surface, saffron (10) on reverse (Rayner 1970). Colonies on MEA reaching 14–15 mm diam, flat, entire edge, centre concave with raised margin, aerial mycelia cottony dense, profuse, aerial sporulation, white colour on surface, saffron (10) on reverse. Colonies on OA reaching 23–25 mm diam, flat to slightly umbonate, entire edge, aerial mycelia cottony dense, aerial sporulation, white on surface, salmon (41) on reverse. Colonies on SNA reaching 14–16 mm diam, average size 15 mm diam, entire edge, aerial mycelia cottony, sparse, aerial sporulation, salmon (41) on surface and reverse.

Material examined: Brazil, Minas Gerais state, Conceição do Mato Dentro municipality, Curral de Pedras cave (18º46'48.205"S 43º31'13.627"W), isolated on leaf litter from a ferruginous cave, 27 September 2022, A.F. Leão, T.O. Condé, F.A. Custódio & O.L. Pereira (VIC 49484, **holotype**); ex-type living culture, COAD 3666.

GenBank accession numbers: ITS: OR669009, LSU: OR669009, *rpb2*: OR678544, *tef1-α*: OR678543.

Notes: according to the multi-locus phylogenetic analysis using ITS, LSU, *rpb2*, and *tef1-α*, *Speluncomyces lunatus* COAD 3666 forms a new and well-supported lineage in the Clavicipitaceae closest to *Pseudometarhizium araneogenum* and *P. lepidopterorum* (Fig. 97). The new species produces allantoid to lunate conidia, arranged in slimy heads (Fig. 96f–i), completely differing from *P. araneogenum* and *P. lepidopterorum*, that have shorter and fusiform conidia formed in chains (Chen et al. 2022). Interestingly, *P. araneogenum* and *P. lepidopterorum* are parasites of spiders and pupa of Leptidopera, while *S. lunatus* was found saprophytically on a leaf litter sample from a ferruginous cave.

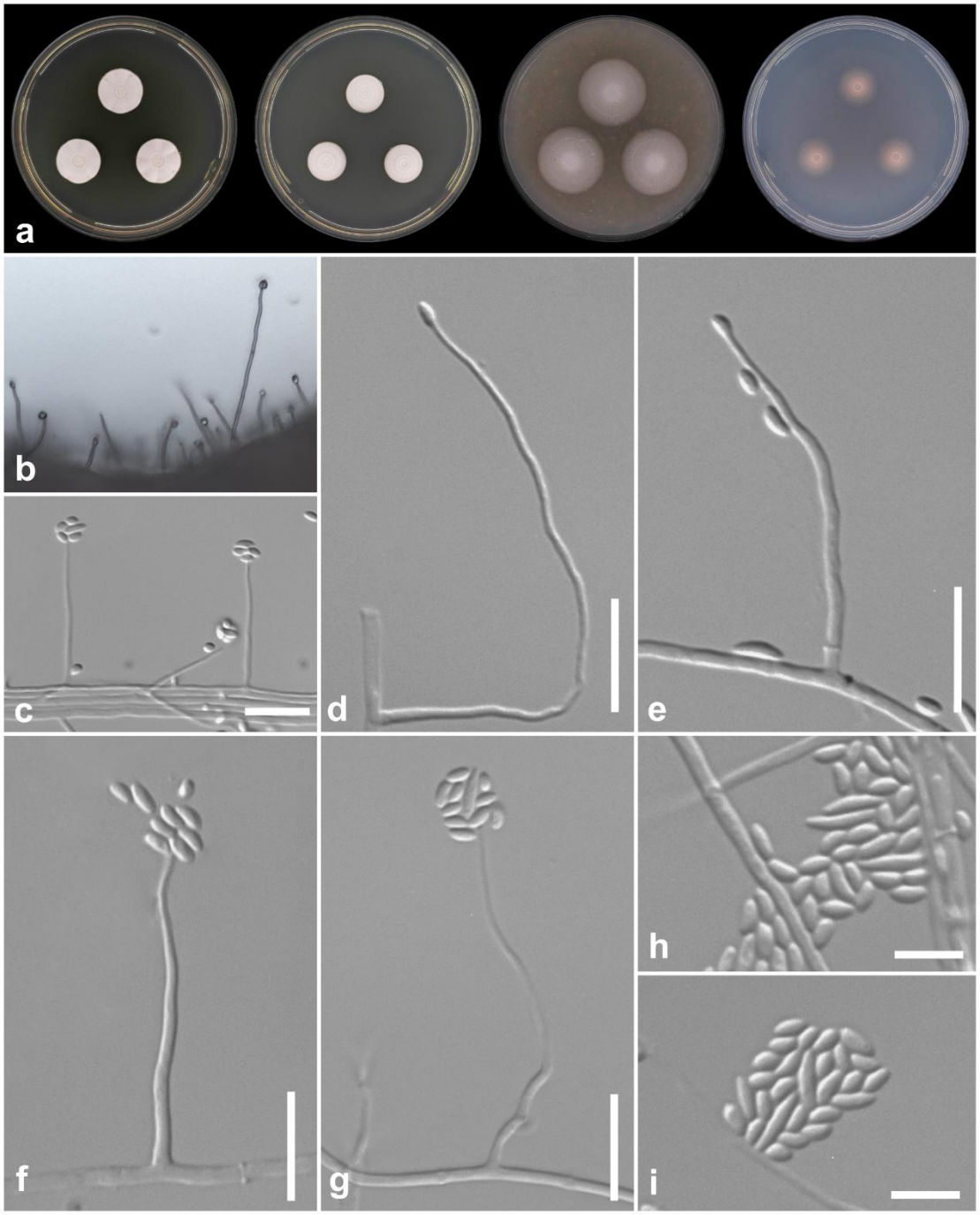

Fig. 96 ***Speluncomyces lunatus*** (VIC 49484, **holotype**). **a** Colonies, from left to right, on PDA, MEA, OA, and SNA. **b**–**g** Conidiophores. **h**, **i** Conidia. Scale bars: **c**–**g** = 10 µm, **h**, **i** = 5 µm.

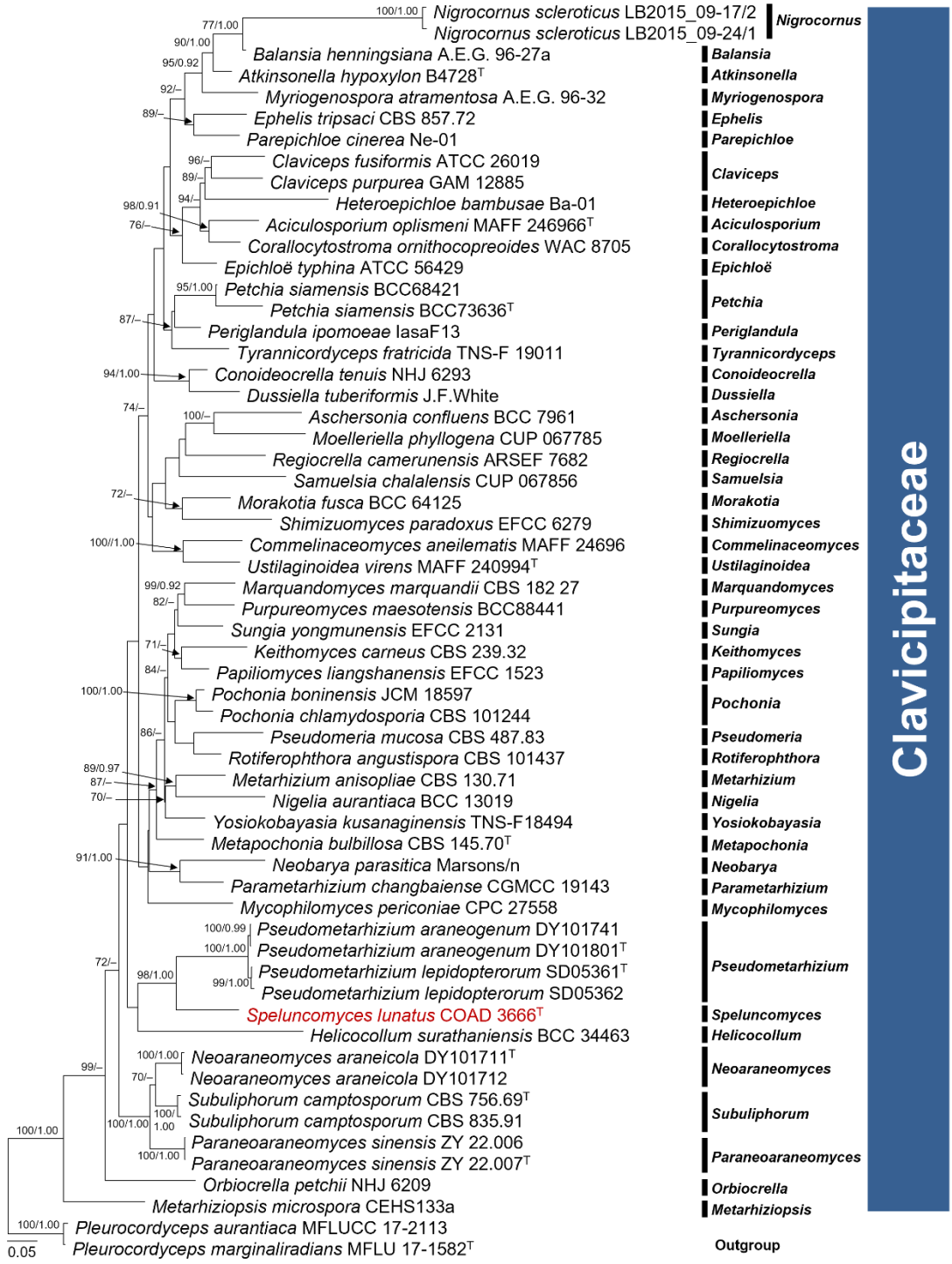

Fig. 97 Maximum likelihood consensus tree inferred from the combined ITS, LSU, *rpb2*, and *tef1-α* multiple sequence alignments. Bootstrap support values for maximum likelihood (ML, first value) equal to or greater than 70% and Bayesian posterior probabilities from MCMC analyses (BYPP, second value) equal to or greater than 0.90 are given above the nodes. The scale bar indicates expected changes per site. The tree is rooted to *Pleurocordyceps aurantiaca* MFLUCC 17-2113 and *P. marginaliradians* MFLU 17-1582. Ex-type strains are indicated with "T". The newly generated sequences are indicated in red.

Niessliaceae Kirschst., Annales Mycologici 37: 89 (1939)

Notes: Niessliaceae was introduced by Kirschstein (1939) and currently comprises 26 genera, including the type genus *Niesslia* (Wijayawardene et al. 2020; Hou et al. 2023). The sexual morphs are characterized by perithecial ascomata, unitunicate asci with an inamyloid apical ring, and hyaline, ellipsoidal to fusiform ascospores (Hyde et al. 2020d). The asexual morphs can be hyphomycetous that are characterized by thin-walled, hyaline, unbranched conidiophores with a short, distinct collarette, straight, smooth phialides, and oval to ellipsoid, 0–1-septate, hyaline conidia. In addition, the asexual morphs can also be coelomycetous showing dark brown, globose or subglobose, submerged conidiomata, hyaline, branched, septate conidiophores, and oval to ellipsoid, hyaline, 0–2-septate conidia (Hyde et al. 2020d).

Cylindromonium Crous, Persoonia 43: 313 (2019)

Notes: *Notes*: *Cylindromonium* was introduced by Crous et al. (2019) with a generic name referring to its cylindrical conidia produced in unbranched conidiophores. Currently, eight species have been described in *Cylindromonium*, *viz*., *C. alloxyli*, *C. dirinariae*, *C. eugeniicola*, *C. everniae*, *C. lichenicola*, *C. lichenophilum*, *C. panamaense* and *C. rhabdosporum* (Crous et al. 2019, 2020, 2021, 2023a, 2024; Ohmaki et al. 2023). Species within the genus are lichenicolous, mycophilic, or saprophytic (Crous et al. 2023a; Hou et al. 2023). *Cylindromonium* was initially placed in Nectriaceae by Crous et al. (2019). However, based on multi-locus phylogenetic analysis of combined ITS, LSU, *rpb2*, and *tef1-α*, this genus has now been circumscribed in Niessliaceae (Hou et al. 2023). *Trichonectria* is considered as the teleomorph of *Cylindromonium*, and this connection has been demonstrated by Ohmaki et al. (2023). *Cylindromonium* is polyphyletic; its taxonomy requires further examination (Hou et al. 2023).

Cylindromonium brasiliense T.O. Condé, F.A. Custódio, A.F. Leão & O.L. Pereira, ***sp. nov.***

Index Fungorum number: IF850533; *Facesoffungi number*: FoF 15035; Fig. 98

Etymology: the epithet refers to Brazil, the country of origin of this fungus.

Holotype: VIC 49487

Saprobic on leaf litter from a ferruginous cave. **Sexual morph**: undetermined. **Asexual morph**: on OA, *mycelia* septate, branched, smooth-walled, hyaline, 1–2 µm wide.

Conidiophores (18.5–)26–35(–54.5) × 1.5–2 µm ($\overline{x}$ = 30.4 × 1.8 µm, n = 10), micronematous, mononematous, erect, flexuous, arising from hyphae, 1–2-septate, sometimes reduced to conidiogenous cells, unbranched, hyaline, thin-walled. *Conidiogenous cells* (18–)26–34(–38) × 1.5–2 µm ($\overline{x}$ = 30.1 × 1.8 µm, n = 10), enteroblastic, monophialidic, integrated, determinate, terminal, subcylindrical, flexuous, tapering towards the apex, sometimes with periclinal thickening and non-flared collarette at the conidiogenous loci, hyaline, smooth-walled. *Conidia* (12–)14–15.5(–17) × 2–3 µm ($\overline{x}$ = 14.7 × 2.3 µm, n = 20), cylindrical, with obtuse ends, 1-septate, hyaline, thin-walled, smooth-walled, solitary or aggregating in mucoid packets of two or more conidia, with a protruding hilum.

Culture characteristics: (incubated for 14 days at 25 ºC in 12 h near UV light) colonies on PDA reaching 14–19 mm diam, lobate edge, convex with papillate surface, convolute, aerial mycelia sparse to moderate from the edge towards the centre, cottony, dense, odour prominent, luteous colour (12) on surface and on reverse (Rayner 1970). Colonies on MEA reaching 8–11 mm diam, lobate edge, convex with papillate surface, convolute, aerial mycelia sparse to moderate from the edge for colony centre, cottony, dense, odour prominent, pale luteous colour (11) on surface and reverse. Colonies on OA reaching 25–31 mm diam, entire edge to undulate, flat, slightly striate, aerial mycelia sparse to absent, rosy buff colour (61) on surface, buff (45) on reverse. Colonies on SNA reaching 3–4 mm diam, lobate edge, flat, aerial mycelia sparse to absent, vinaceous buff colour (86) on surface and reverse.

Material examined: Brazil, Minas Gerais, Conceição do Mato Dentro, Serra da Ferrugem (Cave CSF 0386) (19º00'07.9"S 43º23'48.4"W), on leaf litter from a ferruginous cave, 29 September 2022, A.F. Leão, T.O. Condé, F.A. Custódio & O.L. Pereira (VIC 49487, **holotype**); ex-type living culture, COAD 3669.

GenBank accession numbers: ITS: OR669012, LSU: OR669012, *rpb2*: OR678552, *tef1-α*: OR678551, *tub2*: OR678553.

Notes: in our multi-locus phylogenetic analysis of ITS, LSU, *rbp2* and *tef1-α*, *Cylindromonium brasiliense* was phylogenetically closest to *C. eugeniicola* (Fig. 99). However, when comparing DNA sequences of ITS, LSU, *rpb2*, and *tef1-α* loci, *C. brasiliense* and *C. eugeniicola* (CBS 146075, type), per-site mutations of 525/540, 2 gaps (identity = 97%) for ITS, 804/818, no gaps (identity = 98%) for LSU, 646/730, no gaps (identity = 88%) for *rpb2*, and 782/808, no gaps (identity = 97%) for *tef1-α* were observed. Morphologically, *C. brasiliense* only exhibits monophialidic conidiophores (Fig. 98e–h), sometimes reduced to

conidiogenous cells, whereas *C. eugeniicola* can produce conidiophores bearing up to four phialides, and slightly larger conidia, (12–)14–15.5(–17) × 2–3 µm (Fig. 98f–i) *vs.* (13–)16–18(–20) × 2(–2.5) µm (Crous et al. 2021). *Cylindromonium brasiliense* produces 1-septate conidia that differs from *C. dirinariae*, *C. everniae*, *C. lichenicola*, *C. lichenophilum*, and *C. rhabdosporum* that produce aseptate conidia (Crous et al. 2019, 2021; Ohmaki et al. 2023). It also differs from *C. panamaense*, which produces multiseptate conidia (Crous et al. 2024). *Cylindromonium brasiliense* most resembles *C. alloxyli* by having similar conidiophores (1–2 septate, unbranched, 30–60 × 2–3 µm) and conidia (1–septate, (14–)15–17(–18) × 2–3 µm), but differs in the presence of collarette in *C. brasiliense* (Crous et al. 2020). In addition, they are found in distant branches in the multi-locus phylogenetic analysis (Fig. 99). Therefore, *C. brasiliense* is established as a novel species within *Cylindromonium*.

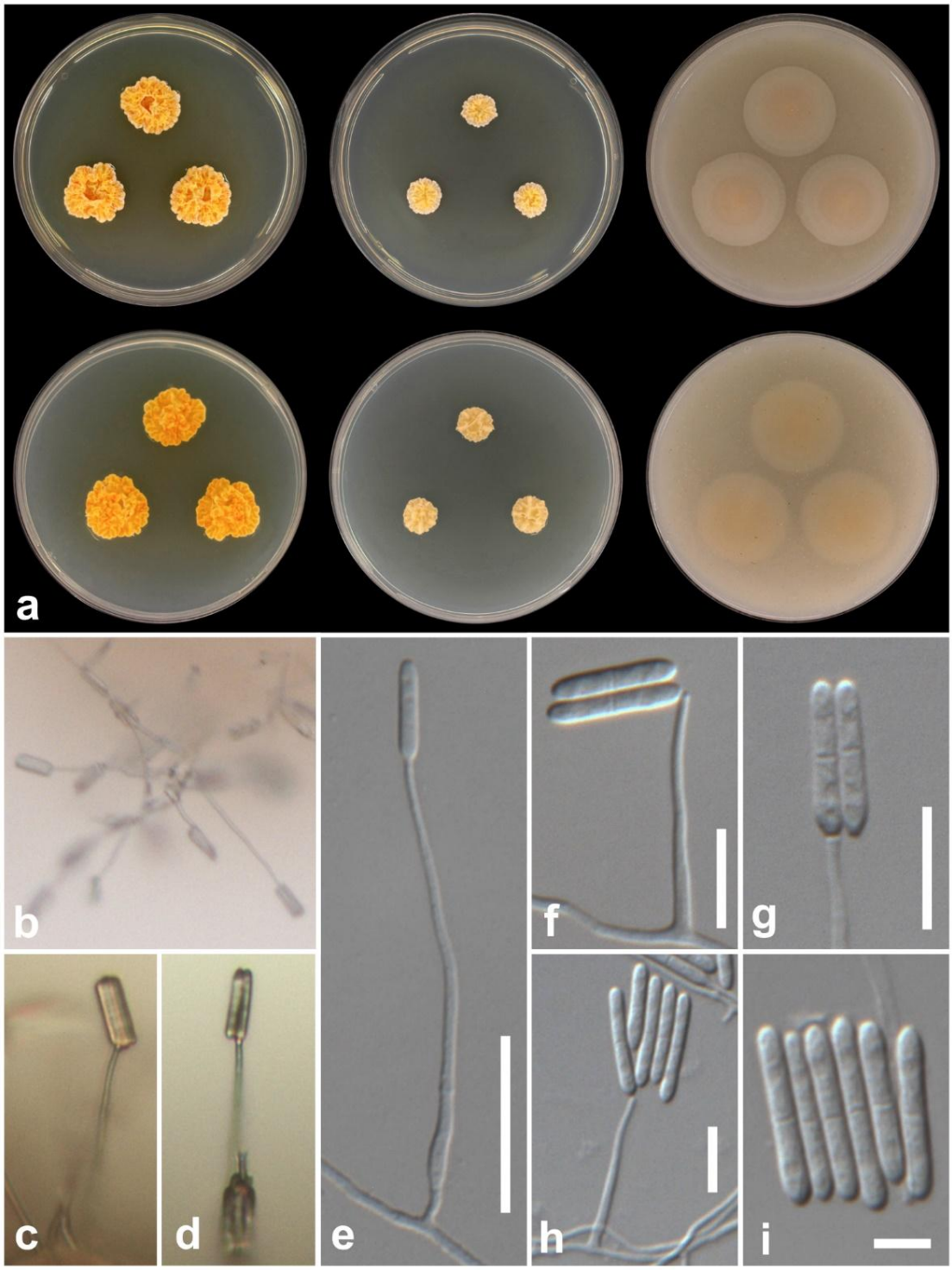

Fig. 98 ***Cylindromonium brasiliense*** (VIC 49487, **holotype**). **a** Colonies, from left to right, top row = PDA, MEA and OA; bottom row = PDA, MEA and OA. **b**–**d** Conidiophores bearing conidia in mucoid packets. **e**–**h** Conidiophores and conidia. **i** Cylindrical conidia. Scale bars: **e** = 20 µm, **f**–**h** = 10 µm, **i** = 5 µm.

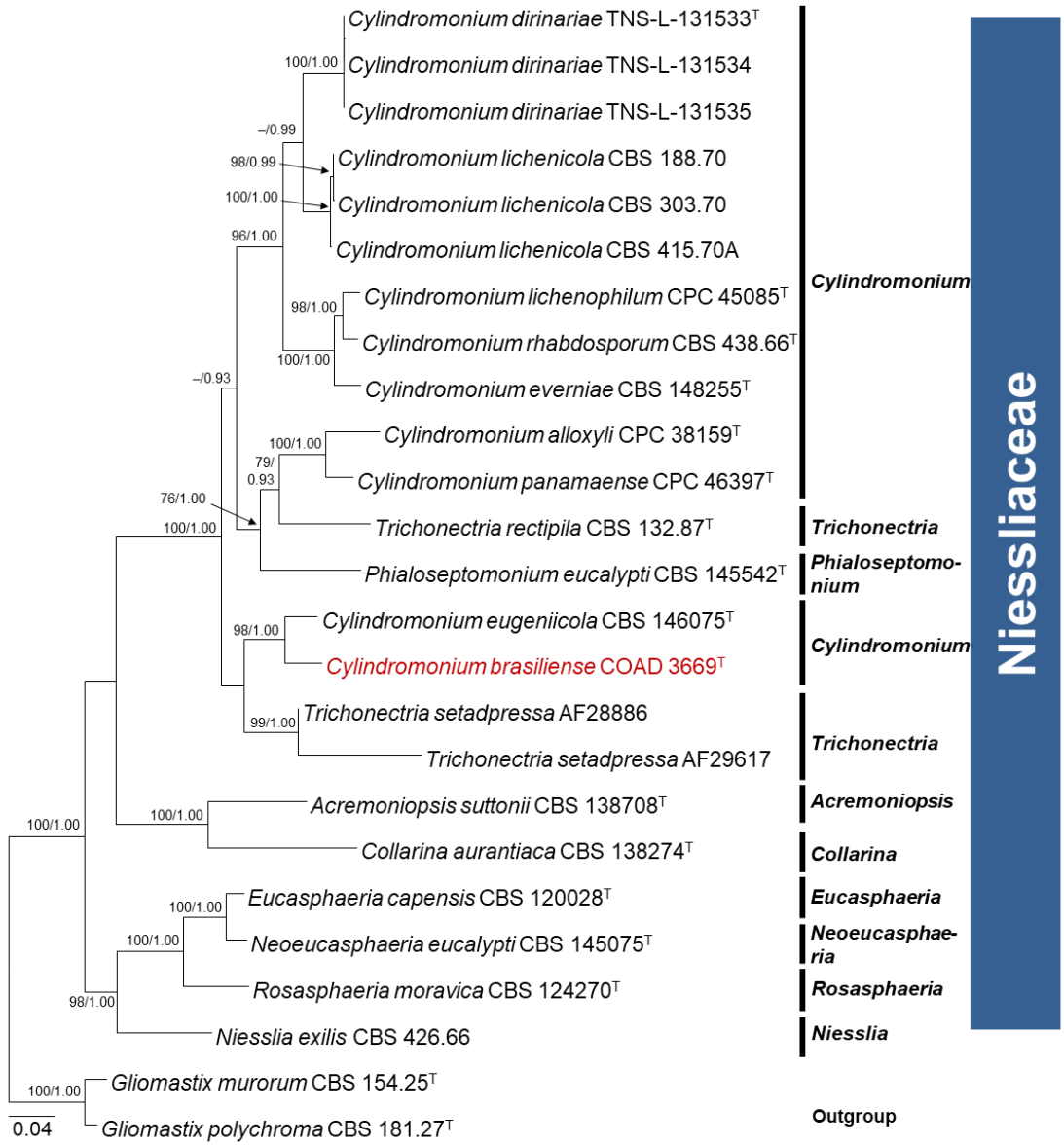

Fig. 99 Maximum likelihood consensus tree inferred from the combined ITS, LSU, *rpb2*, and *tef1-α* multiple sequence alignments. Bootstrap support values for maximum likelihood (ML, first value) equal to or greater than 70% and Bayesian posterior probabilities from MCMC analyses (BYPP, second value) equal to or greater than 0.90 are given above the nodes. The scale bar indicates expected changes per site. The tree is rooted to *Gliomastix murorum* CBS 154.25 and *G. polychroma* CBS 181.27. Ex-type strains are indicated with "T". The newly generated sequences are indicated in red.

Entry by Ana F. Leão[1], Fábio A. Custódio[2], Thiago O. Condé[1], Olinto L. Pereira[2]

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CHAPTER 4.2

Description of new fungal species

Article submitted for publication - According to the Fungal Diversity Journal standard

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Fungal diversity notes 2025 extra edition: taxonomic and phylogenetic contributions to freshwater fungi and other fungal taxa – *Paraneoaraneomyces* sp. nov., *Tolypocladium* sp. nov., *Sarocladium* sp. nov. and *Rachicladosporium* sp. nov.

Fungal diversity notes 2025 extra edition: taxonomic and phylogenetic contributions to freshwater fungi and other fungal taxa

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Hypocreales Lindau, Die Natürlichen Pflanzenfamilien nebst ihren Gattungen und wichtigeren Arten 1 (1): 343 (1897)

Clavicipitaceae (Lindau) Earle, Contributions from the United States National Herbarium 6: 170 (1901)

Notes: The *Clavicipitaceae* is a highly diverse family in the *Hypocreales* comprising 60 genera distributed in more than 500 species (Hyde et al. 2024). Some members of the family are well known as entomopathogens (*Metarhizium* spp., *Pseudometarhizium* spp.), nematophagous (*Pochonia chlamydosporia*), mycoparasites (*Mycophilomyces periconiae*), plant pathogens (*Claviceps* spp.) or grass symbionts (*Epichloë* spp.) (Mongkolsamrit et al. 2020; Gao et al. 2021). Based on molecular phylogenetic analyses, Sung et al. (2007) divided *Clavicipitaceae* into three clades, two of which are now represented by *Cordycipitaceae*, *Ophiocordycipitaceae*. Recently, the family *Polycephalomycetaceae* has been proposed and represents a fourth family of Clavicipitoid fungi (Xiao et al. 2023).

Paraneoaraneomyces Zhi.Y. Zhang & Y.F. Han MycoKeys 98: 113-132 (2023)

Notes: Paraneoaraneomyces was described by Zhang et al. (2023) to accommodate the species *P. sinensis*. This genus is phylogenetically related to *Neoaraneomyces*, which led to its naming as *Paraneoaraneomyces*. Morphologically, this genus can be differentiated from *Neoaraneomyces* and other genera in the family *Clavicipitaceae* by producing cymbiform to reniform conidia attached or forming small globose heads on the phialides and solitary, straight to flexuous phialides arising from aerial or regimental hyphae (Zhang et al. 2023). *Paraneoaraneomyces sinensis* was isolated from green belt soil in China (Zhang et al. 2023).

Paraneoaraneomyces* sp. nov.** F.A. Custódio, A. F. Leão, T.O. Condé & O.L. Pereira, sp. nov.***

Index Fungorum number: IF 859432; Facesoffungi number: FoF xxxx; Fig. xxx

Etymology: Name refers to Brazil, the country of origin of this fungus.

Holotype: VIC 49638

On soil from a ferruginous cave. **Sexual morph:** undetermined. **Asexual morph:** on PDA mycelium septate, branched, smooth-walled, forming bundles, coils, hyaline, 1.0–3.5 µm wide. *Conidiophores* reduced to conidiogenous cells phialidic 16.2–40.9(–137.7) × 0.9–2.2 ($\overline{x}$ = 35.3 × 1.59 µm, n = 21) µm, arinsing laterally or terminally from hyphae, smooth-walled, cylindrical to subulate, solitary, flexuous, and hyaline. *Conidia* 3.9–5.7 × 1–1.6 µm ($\overline{x}$ = 4.7 × 1.3 µm, n = 30) ellipsoidal to cymbiform, smooth-walled, aseptate, sometimes aggregates on slimy heads or the apex of phialides. *Chlamydospores* ellipsoid, subglobose to globose, thick- and smooth-walled, terminal intercalary intercalated, hyaline, 3.5–8.2 × 3.5–8.2 µm.

Culture characteristics: (incubated for 14 days at 25 ºC with photoperiod 12 h light) colonies on PDA reaching 15–20 mm diam, cottony, undulate, convex or dome-shaped, aerial mycelium profuse, white colour on surface and pale luteous (11) on the edges and safrron (10) in the center on reverse (Rayner 1970)**.** Colonies on OA reaching 10–15 mm diam, cottony, entire edge, convex or dome-shaped, aerial mycelium moderate, honey colour (64) with white center on surface and luteous (12) on reverse. Colonies on SNA reaching 13–14 mm diam, cottony, entire edge, low convex, aerial mycelium profuse, white colour on surface and primrose (66) on reverse.

Material examined: **Brazil**, Minas Gerais state, Conceição do Mato Dentro municipality, Curral de Pedras cave (18º46'48.205"S 43º31'13.627"W), isolated from the soil a cave, 27 September 2022, A.F. Leão; T.O. Condé, F.A. Custódio & O. L. Pereira (VIC 49638, **holotype**); ex-type living culture COAD 4024.

GenBank accession numbers: PV727065 (ITS & LSU), PV738216 (*tef1*).

Notes: Paraneoaraneomyces sp. nov. COAD 4024 clustered in a new and well-supported lineage in the genus *Paraneoaraneomyces*, close to *P. sinensis* (Fig. 2). This new species differs morphologically from *P. sinensis* in texture of colonies, slower growth rate and distinct coloration on the reverse side. *Paraneoaraneomyces* sp. nov. produces colonies up to 14 mm on SNA, 20 mm on PDA, and 15 mm on OA, with appearance cottony, convex or dome-shaped, abundant aerial mycelium and distinct pigmentation on the reverse, including saffron and luteous tones. Zhang et al. (2023) observed that *P. sinensis* produces colonies up to 31 mm

on SNA, 37 mm on PDA, 38 mm on OA, and with flat to slightly raised morphology, felty or flocculent texture, regular margins, and predominantly white coloration and with pale yellow or white pigmentation on the reverse. Furthermore, *P. brasiliensis* differs from *P. sinensis* in that it produces chlamydospores and does not produce phialides from regimental hyphae.

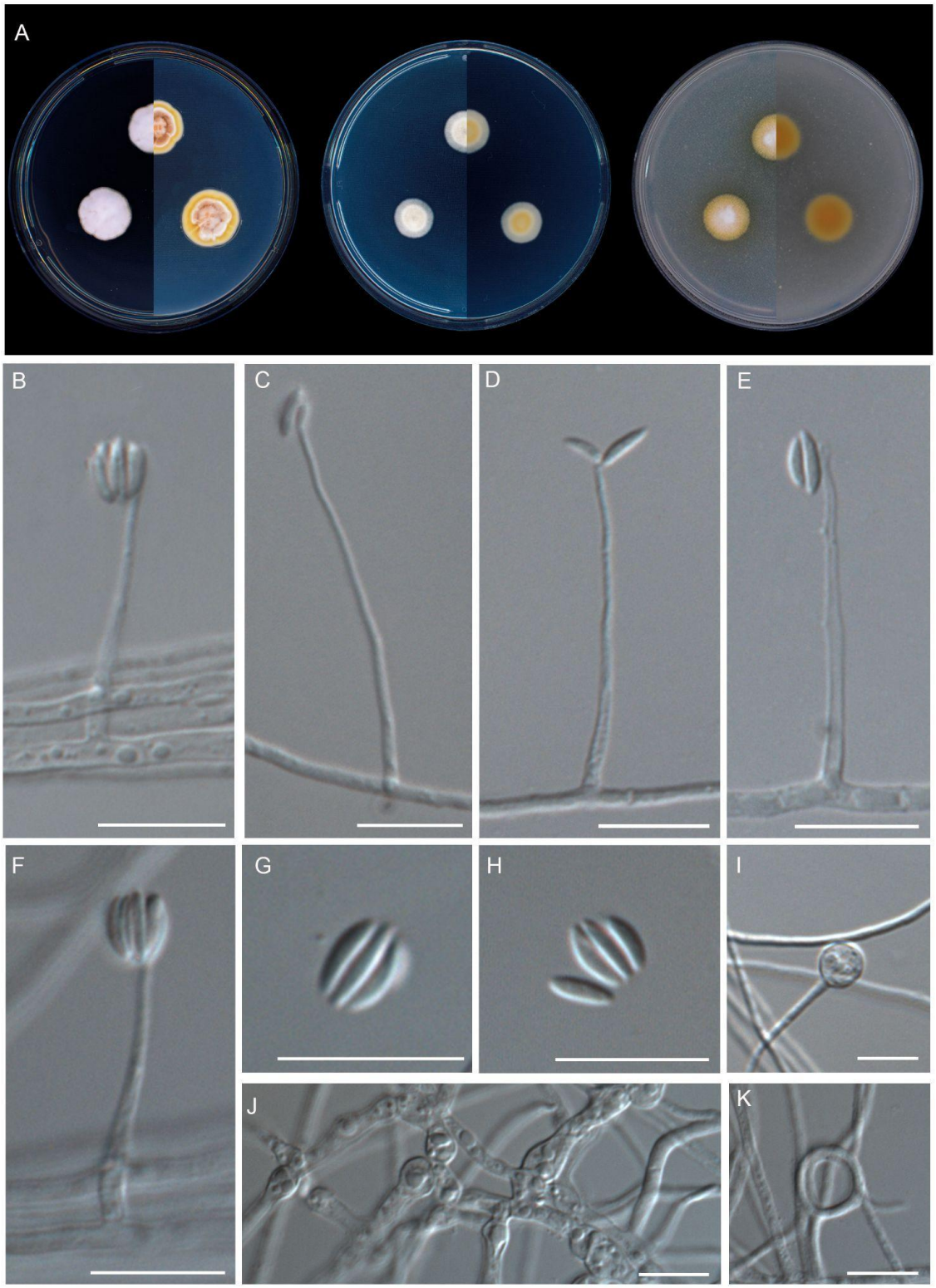

Fig. 1 ***Paraneoaraneomyces*** **sp. nov.** (VIC 49638, **holotype**). **a** Colonies, from left to right, on PDA, SNA and OA after 14 d at 25ºC (upper surface and lower surface). **b**–**g** Conidiophores. **g-h** Conidia. **I-J** Chlamydospores. **K** Coiled hyphae. Scale bars: = 10 µm.

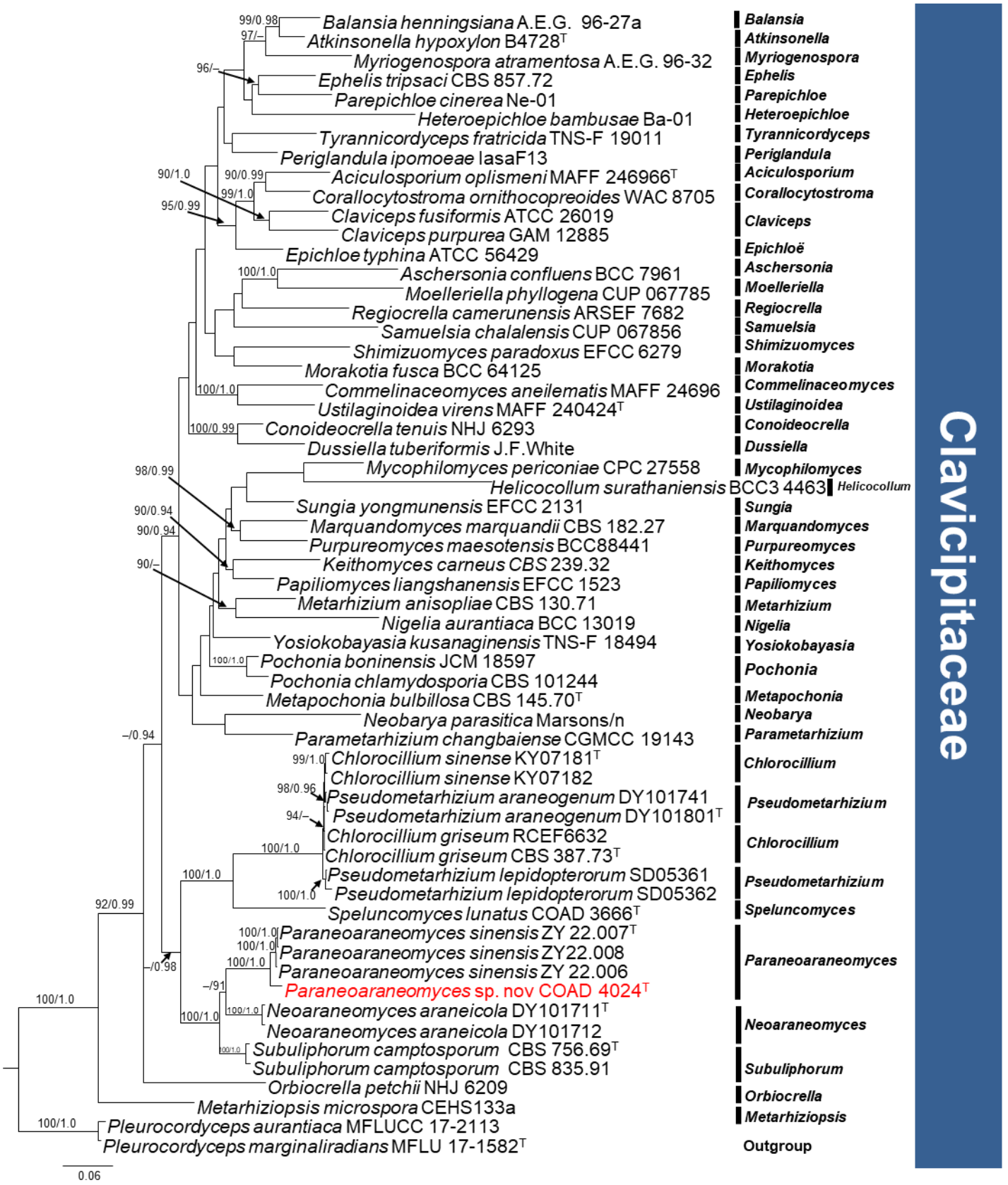

Fig. 2 Maximum likelihood consensus tree inferred from the combined ITS, LSU and *tef1-α* multiple sequence alignments. Bootstrap support values for maximum likelihood (ML, first value) equal to or greater than 70% and Bayesian posterior probabilities from MCMC analyses (BYPP, second value) equal to or greater than 0.90 are

given above the nodes. The scale bar indicates expected changes per site. The tree is rooted to *Pleurocordyceps aurantiaca* MFLUCC 17-2113 and *P. marginaliradians* MFLU 17-1582. Ex-type strains are indicated with "[T]". The newly generated sequences are indicated in red.

Ophiocordycipitaceae G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora Studies in Mycology 57: 5–59 (2007)

Notes: The family *Ophiocordycipitaceae* was proposed by Sung et al. (2007) to accommodate species that were phylogenetically distinct from *Cordycipitaceae* and *Clavicipitaceae*, and most of which presented characteristics of resistant to flexible stromata with dark coloration and production of conidia from branched conidiophores (Sung et al. 2007; Quandt et al. 2014; Yamamoto et al. 2022; Xiao et al. 2023; Song et al. 2024). Based on the type genus *Ophiocordyceps*, the family currently includes the genera *Hirsutella*, *Paraisaria*, *Harposporium*, *Tolypocladium*, *Drechmeria* and *Purpureocillium* (Xiao et al. 2023). It is a diverse family with species of great medical, economic, ecological and cultural importance, generally found in soils and decomposing organic matter in tropical and subtropical regions, and also houses pathogenic species of insects, mycoparasites and endophytes (Quandt et al. 2014; Yamamoto et al. 2022; Song et al. 2024).

Tolypocladium W. Gams, Persoonia 6(2): 185 (1971). emend. C. A. Quandt et al. IMA Fungus 5: 125 (2014).

Notes: *Tolypocladium* was defined by Gams in 1971, to group the anamorphic species *T. cylindrosporum*, *T. geodes* and *T. inflatum* isolated from soil samples, and which presented branched conidiophores, unicellular conidia carried in viscous heads and inflated phialides (Dong et al. 2022; Das et al. 2023; Song et al. 2024). The genus *Tolypocladium* shelters a variety of cosmopolitan species found endophytically in plants, entomopathogenic, mycoparasitic, in soil and in decomposing material (Dong et al. 2022; Das et al. 2023; Soares et al. 2023; Song et al. 2024; Lim et al. 2025). Some species of *Tolypocladium* are also known for their ability to produce bioactive metabolites (Dong et al. 2022; Lim et al. 2025). Currently, there are approximately 60 species described in this genus; however, molecular data are not available for 16 species (Dong et al. 2022; Song et al. 2024).

Tolypocladium **sp. nov.** F.A. Custódio, A.F. Leão, T.O. Condé, & O.L. Pereira, *sp. nov.*

Index Fungorum number: IF 859433; *Facesoffungi number*: FoF XXXX; Fig. xxx

Etymology: Name refers to Serra da Ferrugem, the place of origin of this fungus.

Holotype: VIC 49693.

On soil from a ferruginous cave. **Sexual morph:** undetermined. **Asexual morph:** on PDA mycelium septate, branched, smooth-walled, hyaline, 1.2–3.9 µm wide. *Conidiophores* 7.16–133.2 × 1.3–3.8 µm ($\overline{x}$ = 61.9 × 1.96 µm, n = 30), arinsing laterally or terminally from hyphae, smooth-walled, with 1–8 septa, sometimes reduced to conidiogenous cells, cylindrical to subulate, solitary, flexuous, hyaline, unbrached, sometimes branched. *Conidiogenous cells* phialidic 3.3–26 × 1–2.3 µm ($\overline{x}$ = 13.2 × 1.6 µm, n = 30), hyaline, subulate to langeniform, terminally or laterally on conidiophores, sometimes arinsing laterally from hyphae. *Conidia* 2–4.7 × 1.5–2.6 µm ($\overline{x}$ = 4.7 × 2.6 µm, n = 30), globose to ellipsoidal, smooth-walled, aseptate, hyaline, sometimes aggregates on the apex of phialides.

Culture characteristics: (incubated for 14 days at 25 ºC with photoperiod 12 h light) colonies on PDA reaching 25–46 mm diam, entire edge, convex with papillate surface, aerial mycelium sparse or absent and hyphal tufts in the center of the colony, pale mouse grey colour (117) on surface and olivaceous buff (89) on reverse (Rayner 1970)**.** Colonies on OA reaching 5–48 mm diam, entire edge, flat or effuse, aerial mycelium sparse or absent and hyphal tufts in the center of the colony, colony with a velvety appearance, smoke grey colour (105) on surface and on reverse. Colonies on SNA reaching 26–31 mm diam, undulate, flat or effuse, aerial mycelium profuse; hyphal tufts; dense, aerial sporulation, white colour on surface and on reverse.

Material examined: **Brazil**, Minas Gerais, Conceição do Mato Dentro, Serra da Ferrugem (Cave CMN16) (19º00'07.9"S 43º23'48.4"W), isolated on soil from a ferruginous cave, 29 September 2022, A.F. Leão; T.O. Condé, F.A. Custódio & O. L. Pereira (VIC 49693, **holotype**); ex-type living culture COAD 4025.

GenBank accession numbers: PV727059 (ITS), PV727064 (LSU), PV738214 (*tef1*), PV738217 (*tub2*).

Notes: According to the multilocus phylogenetic analysis of ITS, LSU, and *tef1*, *Tolypocladium* sp. nov. COAD 4025 formed a new phylogenetic lineage close to the clade formed by *T. tropicale* CBS 136897 and *T. sobaeksanense* KNUF 22-014 (bs = 99 and pp = 1) (Fig. 4). Pairwise DNA sequence comparisons between *T. ferruginense* COAD 4025 and *T. tropicale* CBS 136897 exhibited per-site mutations of 457/464, no gaps (identity = 98%) for ITS; 787/788, no gaps (identity = 99%) for LSU; and 853/877, no gaps (identity = 97%) for

tef1; and *T. sobaeksanense* KNUF 22-014 exhibited per-site mutations of 490/498, 1 gap (identity = 98%) for ITS; and 895/917, 1 gap (identity = 98%) for *tef1*. Moreover, *T.* sp. nov. differs morphologically from these species by producing larger conidia and phialides, and by not producing chlamydospores. *Tolypocladium* sp. nov. produces conidia globose to ellipsoidal with 2–4.7 × 1.5–2.6 µm and phialides subulate to langeniform with 3.3–26 × 1–2.3 µm. *Tolypocladium sobaeksanense* produces conidia globose with 2.1 ± 0.1 µm and phialides subcylindrical with 12.2 ± 4.7 × 1.7 ± 0.4 µm (Song et al. 2024). *Tolypocladium tropicale* produces conidia spherical measuring 1.5 ± 0.1 µm and phialides langeniform with 12.2 ± 4.7 × 1.7 ± 0.4 µm (Gazis et al. 2014).

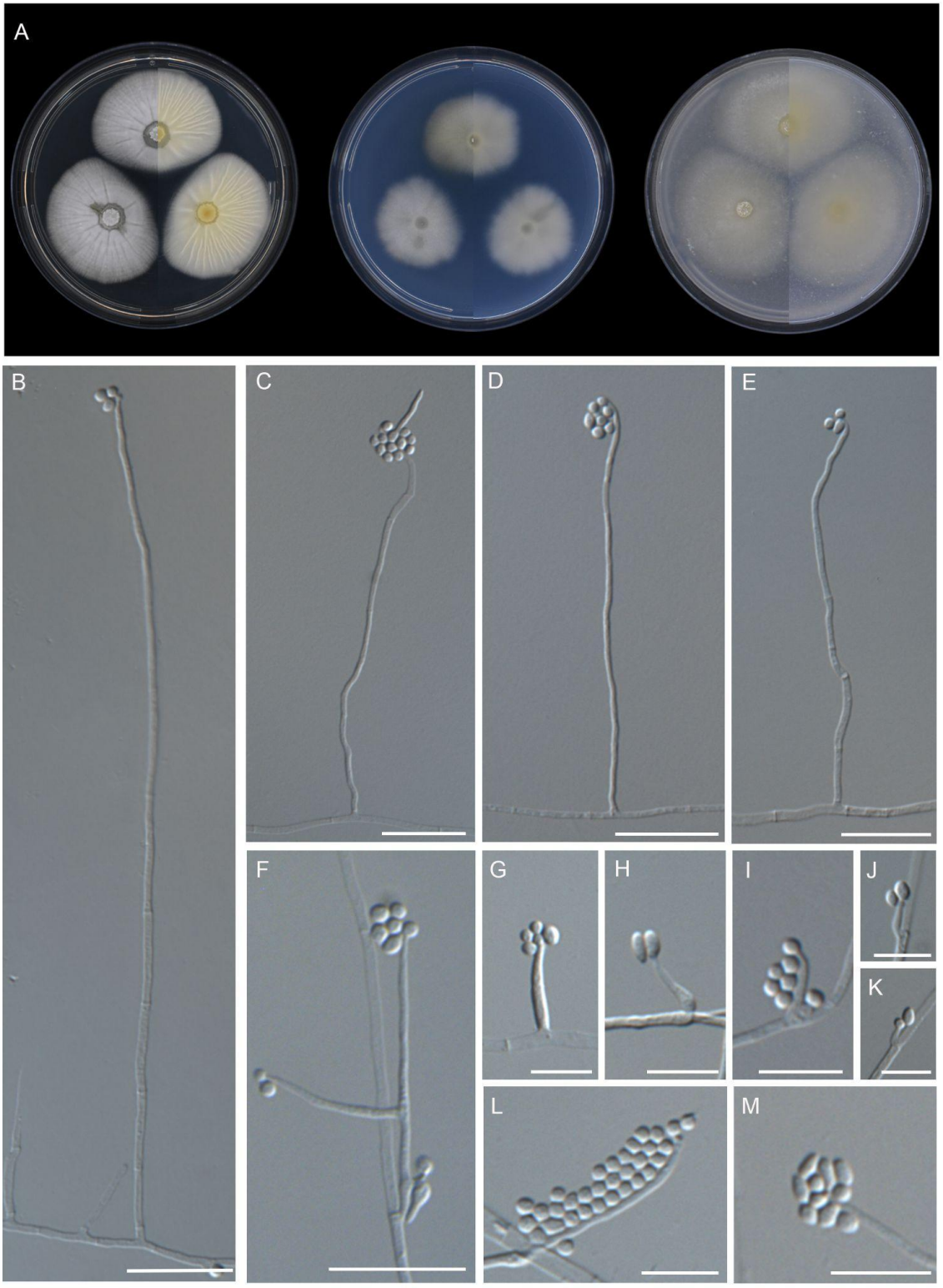

Fig. 3 ***Tolypocladium*** **sp. nov.** (VIC 49693, **holotype**). **a** Colonies, from left to right, on PDA, SNA and OA after 14 d at 25ºC (upper surface and lower surface). **b**–**f** Conidiophores. **g-k** Conidiogenous cells. **l-m** Conidia.Scale bars: **b**–**f** = 20 µm, **g**–**m** = 10 µm.

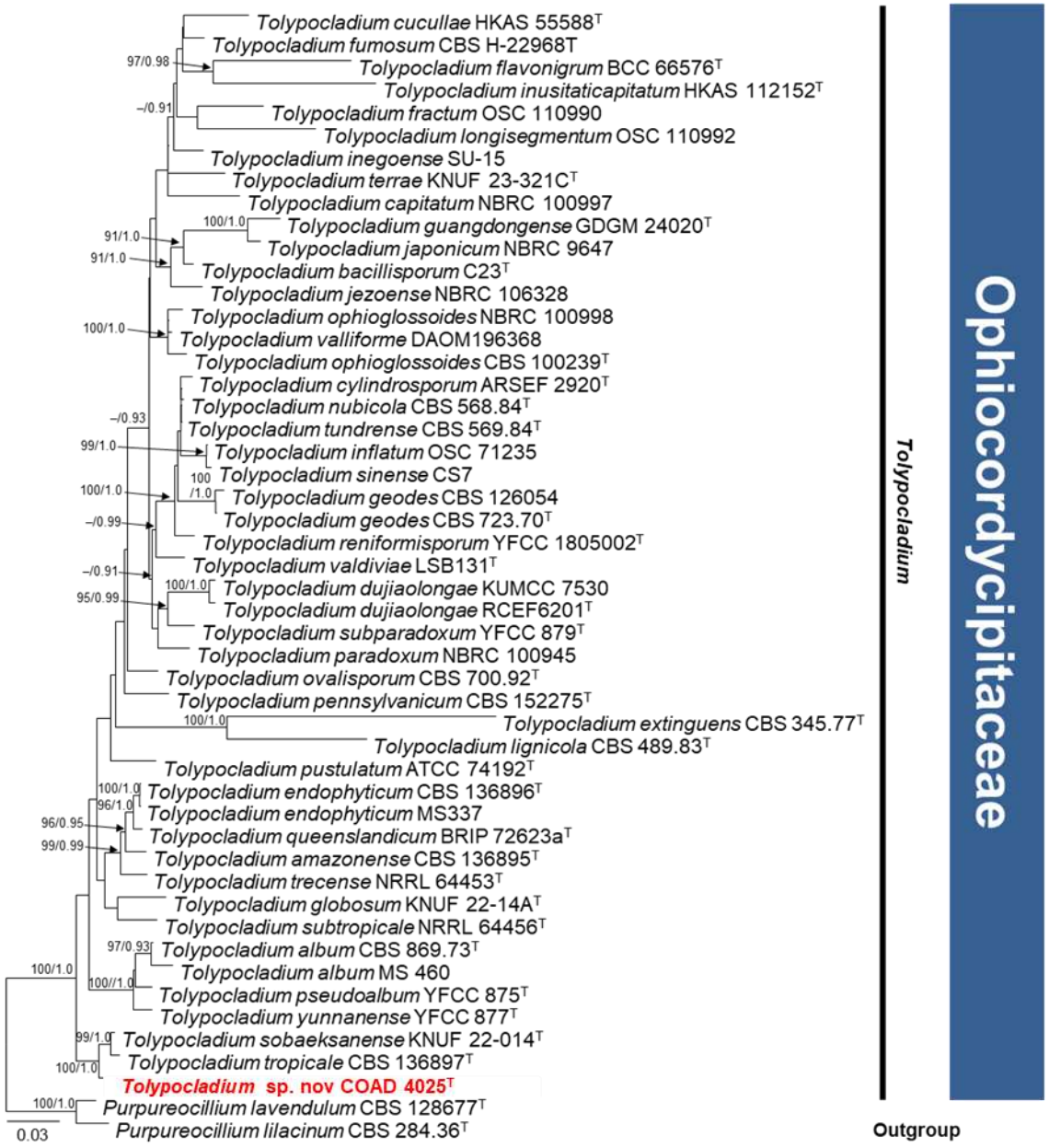

Fig. 4 Maximum likelihood consensus tree inferred from the combined ITS, LSU and *tef1* multiple sequence alignments. Bootstrap support values for maximum likelihood (ML, first value) equal to or greater than 70% and Bayesian posterior probabilities from MCMC analyses (BYPP, second value) equal to or greater than 0.90 are given above the nodes. The scale bar indicates expected changes per site. The tree is rooted to *Purpureocillium lavendulum* CBS 128677 and *Purpureocillium lilacinum* CBS 284.36. Ex-type strains are indicated with "^{T}". The newly generated sequences are indicated in red.

Sarocladiaceae L. Lombard Persoonia 41:343 (2018)

Notes: The family *Sarocladiaceae* is characterized by solitary, branched and elongated phialidic conidiogenous cells, and hyaline conidia arranged in chains or forming false heads (Giraldo et al. 2015; Crous et al. 2018; Phukhamsakda et al. 2020). *Sarocladiaceae* was introduced by Lombard Crous et al. (2018) to accommodate the genera *Sarocladium* and *Parasarocladium* and currently also accommodates the genera *Chlamydocillium* and *Polyphialocladium* (Hou et al. 2023).

Sarocladium W. Gams & D. Hawksw., Kavaka 3: 57. 1976 (1975)

Notes: *Sarocladium* was proposed by Gams and Hawksworth (1975) to accommodate the species *S. oryzae* and *S. attenuatum*, causal agents of rice sheath rot, currently, in addition to phytopathogenic species, the genus contains saprophytic, endophytic, mycoparasitic and human pathogenic species (Anjos et al. 2020; Ou et al. 2020; Phukhamsakda et al. 2020). Thirty-eight species are accepted in the genus, characterized by phialidic conidiogenesis, simple or branched conidiophores, and conidia forming false heads (Anjos et al. 2020; Ou et al. 2020; Phukhamsakda et al. 2020; Hou et al. 2023).

Sarocladium **sp. nov.** T.O. Condé, A.F. Leão, F.A. Custódio & O.L. Pereira, ***sp. nov.***

Index Fungorum number: IF 859434; *Facesoffungi number*: FoF XXXXXX; Fig.xx

Etymology: In honour of the Centro Nacional de Pesquisa e Conservação de Cavernas (CECAV), the Brazilian government institution devoted to the study and conservation of caves.

Holotype: VIC 49665

On animal dung from a ferruginous cave. **Sexual morph**: undeterminated. **Asexual morph:** In OA, hyphae septate, hyaline, branched, and smooth-walled, measuring 0.9–2.19 µm wide. Phialides acircular, hyaline, and smooth-walled, measuring 15.42–29.15 µm long and 1.22–2.78 µm wide at the base ($\overline{x}$ = 20.93 × 1.90 µm, n = 30). Conidia unicellular, smooth-walled, aseptate, ellipsoidal to cylindrical, measuring 2.64–4.66 × 0.88–1.46 µm ($\overline{x}$ = 3.69 × 1.12 µm, n = 30).

Culture characteristics: (incubated for 14 days at 25 ºC in dark) Colonies on PDA reaching 29–32 mm diam, entire edge, raised, aerial mycelium moderate, peach colour (4) on surface and saffron (10) on reverse (Rayner 1970)**.** Colonies on OA reaching 31–38 mm diam, entire edge, flat or effuse, aerial mycelium sparse or absent, rosy buff colour (61) on surface and on reverse. Colonies on SNA reaching 31–36 mm diam, undulate, flat or effuse, aerial mycelium sparse or absent, rosy buff colour (61) on surface and on reverse. Colonies on MEA reaching 16–23 mm diam, undulate, flat or effuse, aerial mycelium sparse or absent, rosy buff colour (61) on surface and on reverse.

Material examined: **Brazil**, Minas Gerais state, Conceição do Mato Dentro municipality, Curral de Pedras cave (18º46'48.205"S 43º31'13.627"W), isolated on animal dung from a ferruginous cave, 27 September 2022, A.F. Leão; T.O. Condé, F.A. Custódio & O. L. Pereira (VIC 49665, **holotype**); ex-type living culture COAD 4055.

GenBank accession numbers: PV727060 (ITS), PV727063 (LSU), PV738215 (*tef1*), PV702313 (*act*).

Notes: According to the multilocus phylogenetic analysis of ITS, LSU, *act*, and *tef1*, *Sarocladium* sp. nov. COAD 4055 is phylogenetically related to *S. strictum* CBS 346.70 (bs = 99 and pp = 1) and *S. mali* ACC 39308 (pp = 0.98) (Fig. 6). Pairwise DNA sequence comparisons between *S.* sp. nov. and *S. strictum* CBS 346.70 exhibited per-site mutations of 509/511, 2 gaps (identity = 99%) for ITS; 799/801, 1 gap (identity = 99%) for LSU; 765/783, no gaps (identity = 98%) for *act* and 800/808, no gaps (identity = 99%) for *tef1*; and *S. mali* ACC 39308 exhibited per-site mutations of 502/506, no gaps (identity = 99%) for ITS; and 700/716, no gaps (identity = 98%) for *act*. *Sarocladium* sp. nov. has smaller phialides (20.93 × 1.90 µm) than those of *S. strictum* (up to 56 × 2.5 µm), and smaller conidial size (2.64–4.66 × 0.88–1.46 µm) than *S. mali* (4.08–4.44 × 1.54–2.04 µm).

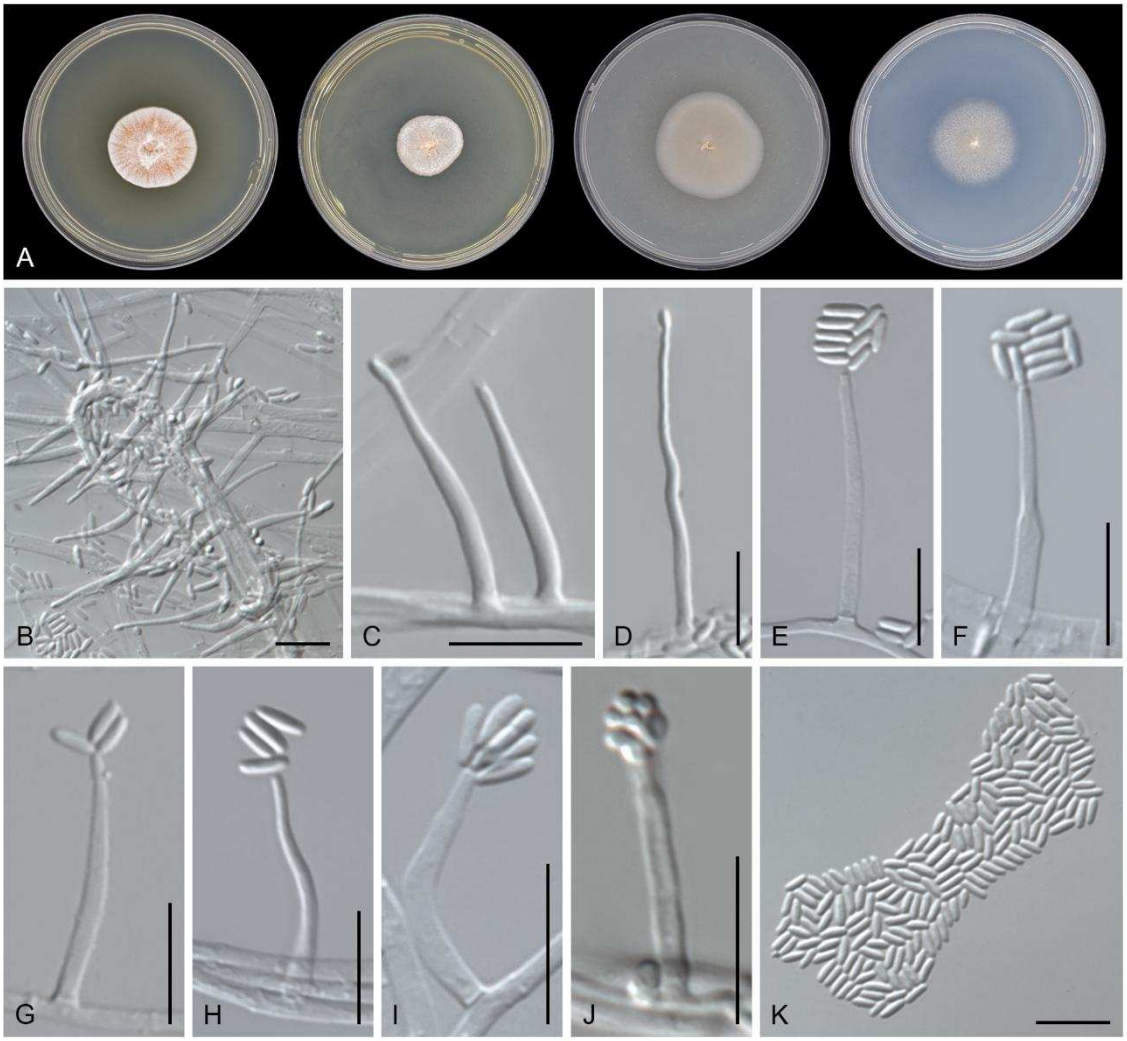

Fig. 5 ***Sarocladium*** **sp. nov.** (VIC 49665, **holotype**). **a** Colonies from left, on MEA, PDA, OA and SNA (upper surface). **b** Conidiophores arising from coiled hyphae. **c**–**j** Conidiophores **k** Conidia. Scale bars: **b**–**k** = 10 µm.

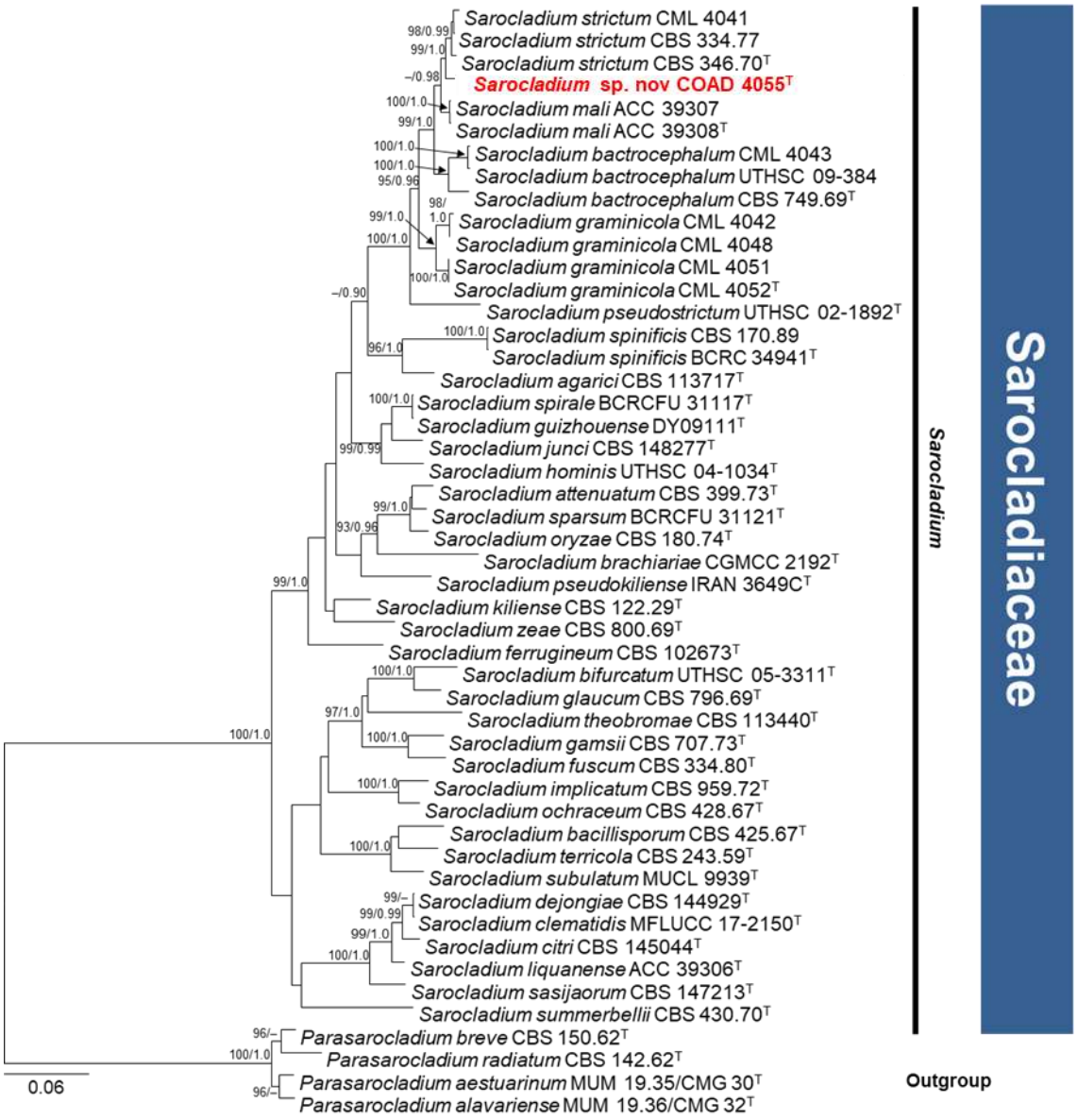

Fig. 6 Maximum likelihood consensus tree inferred from the combined ITS, LSU, *tef1* and *act* multiple sequence alignments. IBootstrap support values for maximum likelihood (ML, first value) equal to or greater than 70% and Bayesian posterior probabilities from MCMC analyses (BYPP, second value) equal to or greater than 0.90 are given above the nodes. The scale bar indicates expected changes per site. The tree is rooted to *Parasarocladium breve* CBS 150.62, *Parasarocladium radiatum* CBS 142.62, *Parasarocladium aestuarinum* MUM 19.35/CMG 30 and *Parasarocladium alavariense* MUM 19.36/CMG 32. Ex-type strains are indicated with "^{T}". The newly generated sequences are indicated in red.

Cladosporiales Abdollahz & Crous, Stud. Mycol. 95: 390 (2020)

Cladosporiaceae Chalm. & R.G. Archibald, The Yearbook of Tropical Medicine and Hygiene 1: 25 (1915)

Notes: *Cladosporiaceae* contains cladosporium-like species characterized by long conidiophores and pigmented conidia in chains, the species are found in a wide variety of environments as saprophytic, endophytic, phytopathogenic and human pathogenic (Abdollahzadeh et al. 2020; Piątek et al. 2023). Based on the type genus *Cladosporium*, the family currently also contains the genera *Cryoendolithus*, *Davidiellomyces*, *Graphiopsis*, *Neocladosporium*, *Rachicladosporium*, *Toxicocladosporium* and *Verrucocladosporium* (Piątek et al 2023).

Rachicladosporium Crous, U. Braun & C.F. Hill, Studies in Mycology 58: 33–56. 2007.

Notes: The genus *Rachicladosporium* was introduced by Crous et al. (2007) to group a species like Cladosporium that produced conidiophores in apical rachis. Described from the species *Rachicladosporium luculiae*, the genus currently houses seventeen morphologically distinct species (*Rachicladosporium africanum, Rachicladosporium alpinum, Rachicladosporium americanum, Rachicladosporium cboliae, Rachicladosporium conostomii, Rachicladosporium corymbiae, Rachicladosporium eucalypti, Rachicladosporium europaeum, Rachicladosporium ignacyi, Rachicladosporium inconspicuum, Rachicladosporium iridis, Rachicladosporium kajetanii, Rachicladosporium luculiae, Rachicladosporium mcclintockiae, Rachicladosporium paucitum, Rachicladosporium pini* and *Rachicladosporium silesianum*) originating from different substrates such as rocks, plants and insects (Piątek et al. 2023).

Rachicladosporium* sp. nov.** A.F. Leão, T.O. Condé, F.A. Custódio, & O.L. Pereira, ***sp. nov.

Index Fungorum number: IF 859435; *Facesoffungi number*: FoF ***; Fig. **

Etymology: Name refers to the place of origin of this fungus, a cave.

Holotype: VIC 49666.

Asexual morph: On MEA, *mycelium* branched, septate hyphae, constricted at septa, pale brown to brown, smooth or sometimes verrucose because of hyphal exudate production,

1.5–3.5 µm wide. *Conidiophores* brown or dark brown, smooth, thick-walled, cylindrical, unbranched, erect or flexuous, up to 200 µm long, 6–7 µm wide, multiseptated. *Conidiogenous cells* terminal, brown or dark brown, smooth, polyblastic, subcylindrical, 9–21 × 3–5 µm; loci terminal, thickened, darkened, refractive, 1.5 µm diam. *Ramoconidia* 0–1 septate, subcylindrical, brown, smooth, 8.5–19.5 × 2–5 µm ($\overline{x}$ = 13.5 × 3,5 µm); hila thickened and refractive, up to 1.5 µm diam. *Conidia* 0–1 septate, ellipsoidal or subcylindrical with truncated base, brown or pale brown, smooth, formed in branched chains of up to 10, (9.5–)11–14(–19) × (4.5–)3(–2) µm; hila thickened and refractive, up to 1.5 µm diam. On PDA, *chlamydospores* formed on intercalary and terminal clusters, brown or dark brown, thick-walled, 0–1 septate or muriformly septate, up to 12 µm diam ($\overline{x}$ = 7.5 µm). **Sexual morph:** undetermined.

Culture characteristics: incubated for 10 days at 25 ºC in dark: colonies on PDA reaching 9–11 mm diam, on OA reaching 9–11mm diam and on MEA reaching 4–11 mm diam. Incubated for 15 days at 25 ºC in dark colonies on PDA reaching 15–17 mm diam, entire edge, convex with papillate surface, aerial mycelium sparse to absent, smoke grey colour (105) on surface and iron grey (122) on reverse (Rayner 1970)**.** Colonies on OA reaching 15–19 mm diam, entire edge, umbonate, aerial mycelium sparse to absent, iron grey colour (122) on surface and on reverse. Colonies on MEA reaching 7–18 mm diam, undulate, umbonate, aerial mycelium moderate, pale olivaceous grey colour on surface (120) and mouse grey (118) on reverse. Incubated for 30 days at 25 ºC in dark colonies on PDA reaching 29–34 mm diam, entire edge, umbonate, aerial mycelium sparse to absent, olivaceous grey colour (121) on surface and iron grey (122) on reverse (Rayner 1970)**.** Colonies on OA reaching 28–33 mm diam, entire edge, umbonate, aerial mycelium moderate, greyish sepia colour (106) and fuscous black (104) on surface and on reverse. Colonies on MEA reaching 16–33 mm diam, undulate, umbonate, aerial mycelium moderate, greyish sepia colour (106) on surface and fuscous black (104) on reverse.

Material examined: **Brazil**, Minas Gerais state, Conceição do Mato Dentro municipality, Curral de Pedras cave (18º46'48.205"S 43º31'13.627"W), isolated from air cave, 27 September 2022, A.F. Leão; T.O. Condé, F.A. Custódio & O. L. Pereira (VIC 49666, **holotype**); ex-type living culture COAD 4056.

GenBank accession numbers: PV727061 (ITS), PV727062 (LSU), PV738218 (*rpb2*).

Notes: According to the multilocus phylogenetic analysis of ITS, LSU, and *rbp2*, *Rachicladosporium* sp. nov. is phylogenetically related to *R. cboliae* (bs=99 and pp=1.0) and

R. silesianum (bs=100 and pp=1.0) (Fig. 8). Pairwise DNA sequence comparisons of *Rachicladosporium* sp. nov. COAD 4056 exhibited per-site mutations of 525/529, 1 gap (identity = 99%) for ITS, 862/863, no gaps (identity = 99%) for LSU, and 692/754, no gaps (identity = 92%) for *rpb2*, with *R. cboliae* CBS 125424; and 534/549, 5 gaps (identity = 97%) for ITS, and 820/824, no gaps (identity = 99%) for LSU, with *R. silesianum* G395. Morphologically, *R.* sp. nov. can be distinguished from *R. cboliae* by producing larger ramoconidia (8.5–19.5 × 2–5 µm *vs*. 7–12 × 3–4 µm), conidia ((9.5–)11–14(–19) × (4.5–)3(–2) µm *vs*. (6–)7– 8(–10) × (2–)2.5(–3) µm)), and chlamydospores (up to 12 µm *vs*. 6 µm) (Crous et al. 2009). Moreover, *Rachicladosporium* sp. nov. produces larger and muriform chlamydospores, differing from those of *R. silesianum*, which are 1-septate and up to 9 µm (Piątek et al. 2023). Additionally, *Rachicladosporium* sp. nov. produces cladosporium-like conidiophores, whereas *R. silesianum* does not. Muriform chlamydospores (Fig. ***h) are also produced by *R. europaeum* (Piątek et al. 2023); however, these species are phylogenetically distant from each other (Fig. ***). This is the first report of this genus in caves worldwide.

Fig. 7 ***Rachicladosporium*** **sp. nov.** (VIC 49666, **holotype**). **a** Colonies from left, on PDA, MEA and OA (upper surface). **b**–**d** Ramoconidia and conidia. **e** Conidia. **f**–**g** Chlamydospores. Scale bars: **b**–**h** = 20 µm.

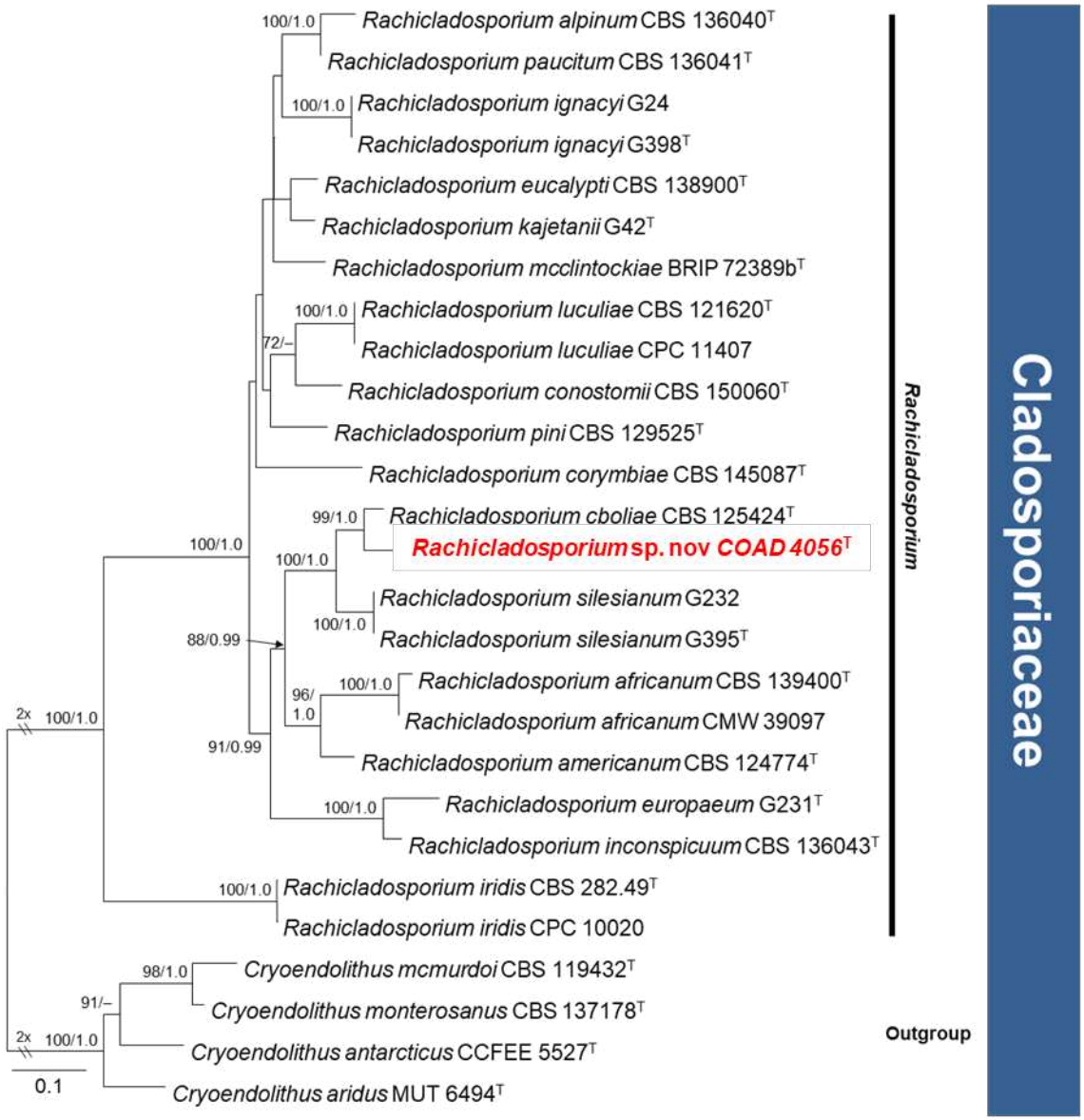

Fig. 8 Maximum likelihood consensus tree inferred from the combined ITS, LSU and *rpb2* multiple sequence alignments. Bootstrap support values for maximum likelihood (ML, first value) equal to or greater than 70% and Bayesian posterior probabilities from MCMC analyses (BYPP, second value) equal to or greater than 0.90 are given above the nodes. The scale bar indicates expected changes per site. The tree is rooted to *Cryoendolithus mcmurdoi* CBS 119432, *Cryoendolithus monterosanus* CBS 137178, *Cryoendolithus antarcticus* CCFEE 5527 and *Cryoendolithus aridus* MUT 6494. Ex-type strains are indicated with "T". The newly generated sequences are indicated in red.

Entry by Ana F. Leão[1], Fábio A. Custódio[2], Thiago O. Condé[1], Olinto L. Pereira[2]

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