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Unravelling the fungal darkness in a tropical cave: richness and the description of one new genus and six new species

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Abstract: Caves are special environments that harbour an incredible diversity of life, including fungal species. Brazilian caves have been demonstrated to be biodiversity hotspots for known and unknown fungal species. We investigated the richness of culturable fungi in a tropical cave in Brazil by isolating these microorganisms from the sediment and air. The fungal abundance of colony-forming units (CFUs) was 3 178 in sediment and 526 in air. We used morphological features and phylogenetic analyses of actin (*actA*), calmodulin (*cmdA*), internal transcribed spacer regions and intervening 5.8S rRNA (ITS), large subunit (LSU) rDNA, RNA polymerase II second largest subunit (*rpb2*), translation elongation factor 1-alpha (*tef1*), and β-tubulin (*tub2*) genes to identify these isolates. Forty-one species belonging to 17 genera of *Ascomycota* and two of *Basidiomycota* were identified, and the genus *Aspergillus* was most commonly observed in the cave (13 taxa). Twenty-four species were found in sediment (16 exclusives) and 25 species were found in air (17 exclusives). In this study, we introduced a new genus (*Pseudolecanicillium gen. nov.*) in the family *Cordycipitaceae* and six new species (14 % of the total taxa identified) of fungal isolates obtained from sediment and air: *Aspergillus lebretii sp. nov.*, *Malbranchea cavernosa sp. nov.*, *Pseudohumicola cecavii sp. nov.*, *Pseudolecanicillium caatingaense sp. nov.*, *Talaromyces cavernicola sp. nov.*, and *Tritirachium brasiliense sp. nov*. In addition, we built a checklist of the fungal taxa reported from Brazilian caves. Our results highlight the contribution of Brazilian caves to the estimation of national and global fungal diversity.

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INTRODUCTION

Fungi are cosmopolitan organisms that inhabit aquatic and terrestrial environments (Peay *et al*. 2016). Fungal species also inhabit extreme environments (Tiquia-Arashiro & Grube 2019, Coleine *et al*. 2022), and caves could be one of these extreme environments in which fungal species play key ecological roles (Ferreira *et al*. 2000, Nieves-Rivera 2003, Nováková 2009). Caves are natural subterranean cavities that may have a different lithological origin (*e.g.* carbonate and ferruginous caves, Travassos 2019) and harbour a special subterranean biota, with several species of fauna and flora in the speleological environment (Ogórek *et al*. 2013, Piló & Auler 2013). In this subterranean environment, various species have been reported and described as troglobites, troglophiles, and trogloxenes (Zhang *et al*. 2017, 2021). Troglobites are those living only in the subterranean environments (called hypogeal), the troglophiles live in the epigeal (external) and hypogeal environment, completing their life cycle inside or outside the caves, and trogloxenes depend on the epigean as part of their life cycle (Holsinger & Culver 1988, Pinto-da-Rocha 1993, Bento *et al*. 2021). Caves have a typical environment with low variations in abiotic conditions such as light, temperature, air current, and relative humidity (Lobo & Boggiani 2013).

Cave fungal diversity has been reported over the years (Vanderwolf *et al*. 2013) with an emphasis on airborne fungi, a group whose spores are disseminated by the air (Flores & Onofre 2010). Cave environments have higher chances to harbour

special fauna, flora, and microorganisms (including fungi) that have a high capacity to tolerate abiotic and biotic factors (Nováková 2009, Bastian *et al*. 2010, Ogórek *et al*. 2013). Several studies have been conducted in caves to determine their fungal diversity (Vanderwolf *et al*. 2013, Cunha *et al*. 2020, Zhang *et al*. 2021), and recently, this hypogeal diversity was reported to have originated in the surface environment (Zhang *et al*. 2018).

The Brazilian territory has approximately 22 809 known caves, but this is estimated to be 10 % of the total number of caves in Brazil, emphasising the importance of speleological heritage in the country (Jansen *et al*. 2012, ICMBio/CECAV 2022). The North-eastern region of Brazil currently has approximately 4 303 known caves distributed in nine states, most of which are in Bahia (1 694) and Rio Grande do Norte (1 365) (ICMBio/CECAV 2022). According to Auler & Zogbi (2005), the North-eastern region of Brazil has high potential for new cave discoveries. This region is mainly characterised by the Caatinga domain, a part of the dry diagonal of South America, and is recognised as one of the most diverse dry forests in the world (Silva *et al*. 2017). Bat caves, that is, caves harbouring exceptionally high bat populations (some with more than 100 000 bats), are known in the North-eastern region of Brazil (Otálora-Ardila *et al*. 2019), and their mycobiome has recently been unravelled (Cunha *et al*. 2020, Pereira *et al*. 2022). Due to the presence of thousands of bats in these caves, there is a large deposition of guano, which is a special substrate for the growth of fungal species (Ogórek 2016).

The cave environment frequently harbours rare species of fungi, or even species not yet described, mainly in association with minimally explored substrates, which have great potential for the description of new species (Taylor *et al*. 2013, Cunha *et al*. 2020). Nearly 2 000 species of fungi are currently known from caves (see Zhang *et al*. 2021), but only 180 fungal taxa have been reported from Brazilian caves (Ferreira *et al*. 2000, Pedro & Bononi 2007, Taylor *et al*. 2013; Taylor *et al*. 2014, Paula *et al*. 2016, Hornick 2017, Crous *et al*. 2018, Fonseca *et al*. 2019, Cunha *et al*. 2020, Carvalho *et al.* 2022, Pereira *et al*. 2022). Less than 12 caves in Brazil have had their mycobiome studied, indicating a large knowledge gap on cave fungal diversity in such a megadiverse country. The most recent study on fungi from a Brazilian cave (Cunha *et al*. 2020) showed 59 taxa belonging to 39 genera obtained from the air, guano, and body of bats from a bat cave in the Caatinga. Pereira *et al*. (2022) surveyed another bat cave in the same region and reported eight *Cladosporium* spp., including the description of two new species. Carvalho *et al.* (2022) studied culturable fungi from bat ectoparasites of a cave in the Caatinga and described new species in *Allophoma* and *Pyrenochaetopsis*. Such studies highlight the great potential for new fungal discoveries in the country, and a potentially higher contribution to global fungal diversity. Moreover, these studies emphasized the importance of the mycobiome for cave environments and the conservation of subterranean environments (Cunha *et al*. 2020).

In this study, we investigated the cultivable fungal richness in sediment and air in a tropical cave in the Caatinga dry forest in Brazil. Based on our data and a literature review, we built a checklist of fungal taxa reported from Brazilian caves since the first known study in 1963. Our data may also help Brazilian environmental authorities plan conservation policies for the tourist trade.

MATERIALS AND METHODS

Study area

The Abrigo do Letreiro cave is located in Furna Feia National Park (5°4'14.88''S and 37°32'1.51''W), between the municipalities of Baraúna and Mossoró in the state of Rio Grande do Norte, North-eastern Brazil (Fig. 1). The national park was created in 2012 to protect the conservation area of a speleological complex and the Caatinga dry forest. The park has an area of approximately 8 494 ha and 25 322 ha of a buffer zone, totalling 33 816 hectares (Brasil 2012). The region's climate is BWh' (arid and hot), according to the Köppen's classification (Dubreuil *et al*. 2018), and is characterised by strong insolation with high temperatures throughout the year (average annual temperature: 27.5 °C), relative humidity of approximately 70 %, and annual precipitation around 673.9 mm (ICMBio 2020).

The cave has a limestone lithology and is formed by a single chamber of approximately 64 m of horizontal projection and an altitude of approximately 154 m, with two entrances at its ends and a skylight near the centre (Sobrinho *et al*. 2016). The morphology of the Abrigo do Letreiro cave facilitates the flow of air from the external environment, which indicates that it is a cavity with a high level of energy (Heaton 1986). The Abrigo do Letreiro cave has a very common tree in areas of the Caatinga dry forest, *Erythrina velutina* (*Fabaceae*), at the centre of the cave in a portion where there is a skylight. In addition to its ecological and speleological importance, it has anthropological significance because several cave paintings with geometric traditions and symbolist styles are distributed along the walls and ceiling of the cave (Bento 2013) (Fig. 2).

The collection was authorised by the Ministério do Meio Ambiente (MMA)/Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) (SISBIO number 54274).

Sampling points

The cave was treated as a single chamber and three collection points were chosen from the cave's main entrance: the first was 5.7 m (point 1) from the entrance, followed by the second 5.2 m (point 2), and the third 14 m (point 3). The points were chosen based on easy access to the cave environment and the abiotic conditions observed during the collection. Point 1 had a small colony of bats (*Peropteryx macrotis*), point 2 was located near the skylight, and point 3 was located deep within the cave (Fig. 1).

Isolation of airborne fungi

Airborne fungi were collected, using the sedimentation methodology, on a culture medium contained in Petri dishes, as described by Cunha *et al*. (2020). At each sampling point, three 90 mm Petri dishes containing Sabouraud dextrose (SAB) agar supplemented with chloramphenicol (100 mg/L) were used for fungal isolation. The plates were placed equidistant from each other, 1 m from the cave floor, and opened for 20 min. After exposure, the plates were closed, identified, packaged, and transported to the laboratory. Plates were incubated at 28 °C for up to 14 d in the dark. Fungal colonies were observed daily and counted. Selected colonies were isolated, purified, and preserved for further identification.

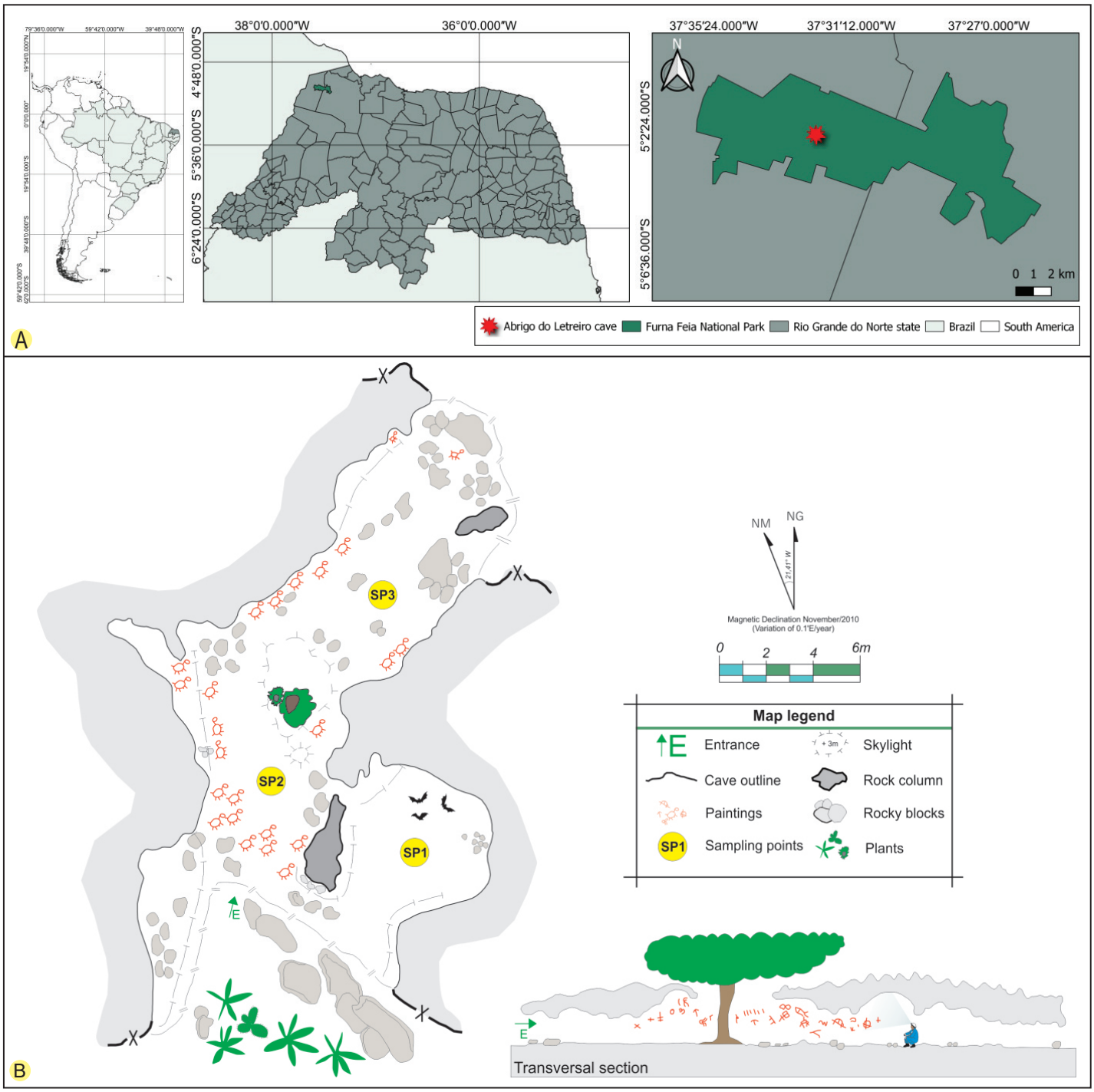

Fig. 1. A. The geographical location of the Abrigo do Letreiro cave in the Furna Feia National Park, Brazil. **B.** Cave sketch showing the sampling points (SP1, SP2 and SP3). The cave sketch was adapted from one drawn by the CECAV/ICMBio-MMA, Brazil.

Isolation of fungi from the sediment of the cave

Approximately 10 g of cave superficial sediment was collected at each sampling point using sterilised 50 mL centrifuge tubes. The sediment at Point 1 was composed of soil, bat guano, and rodent faeces (*Kerodon rupestris*), Point 2 contained soil and rodent faeces (*K. rupestris*), and Point 3 was composed of soil and bat guano. In the laboratory 1 g of each sampled sediment was placed in a 250 mL Erlenmeyer flask containing 9 mL of distilled and sterilised water plus chloramphenicol (0.1 mg/L). The flasks were manually shaken and used to make dilutions of 10^{-2}, 10^{-3}, and 10^{-4}, from which 1 mL was transferred to Petri dishes with Brain and Heart Infusion (BHI) agar and SAB media plus chloramphenicol (100 mg/L). Petri dishes were incubated at 28 °C for at least 7 d in the dark, and representative isolates of the total fungal colonies grown were chosen and purified using SAB plus chloramphenicol (100 mg/L) (Cunha *et al*. 2020).

Fungal isolates

Ex-type cultures are deposited in the *University Recife Mycology* (URM) culture collection (*Micoteca URM Profa. Maria Auxiliadora Cavalcanti*, WDCM 604), and holotypes (as permanent slide preparations) in the URM fungarium (*Herbário URM Pe. Camille Torrend*) at the Universidade Federal de Pernambuco (UFPE), Recife, Brazil (Barbosa *et al*. 2020). In

Fig. 2. Abrigo do Letreiro cave in the Furna Feia National Park, Brazil. **A.** Outside skylight view and the tree *Erythrina velutina* (*Fabaceae*). **B.** Inside skylight view and the tree *E. velutina*. **C.** Sampling point 2 and cave paintings with geometric tradition and symbolist style distributed along the walls and ceiling of the cave. **D.** A small colony of bats (*Peropteryx macrotis*) at the sampling point 1. **E.** Petri dishes used to sample airborne fungi at the sampling point 2. Photos were taken by D.M. Bento.

addition, all isolates are deposited in the working collection of the Laboratório de Taxonomia e Biotecnologia Utilizando Fungos housed at the Micoteca URM/UFPE and ex-type strains and representative isolates in the working collection FCCUFG of Jadson D.P. Bezerra housed at the Laboratório de Micologia of the Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, Brazil.

Fungal identification

Morphology

Strains of the new genus and species were grown on various culture media. For *Aspergillus* and *Talaromyces* species, the isolates were cultivated on malt extract agar (MEA), Czapek agar (CZ), Czapek yeast extract agar (CYA), CYA supplemented with 5 % NaCl (CYAS), dichloran 18 % glycerol agar (DG18), oatmeal agar (OA), yeast extract sucrose agar (YES) and creatine sucrose agar (CREA) (Samson *et al*. 2010). All agar media were incubated at 25 °C for 7 d in the dark. Additional CYA and MEA plates were incubated at 10, 30, and 37 °C and the colony diams were measured after 7 d of incubation in the dark. Microscopic observations were made of colonies grown on MEA (Samson *et al*. 2010). For *Malbranchea* species and the new genus, isolates were subcultured on potato dextrose agar (PDA), OA, and synthetic nutrient-deficient agar (SNA) and incubated in the dark at 25 °C for 14 or 28 d (Zhang *et al*. 2021). The isolates of *Pseudohumicola* were cultivated on PDA, OA, SNA, and MEA and incubated at 25 °C for 14 d in the dark (Zhang *et al*. 2017, Wang *et al*. 2019). For *Tritirachium* species, the isolate was cultivated on CYA, MEA, OA, PDA, SNA, and YES and incubated at 25 °C for 7 d in the dark (Bezerra *et al*. 2020). Colours of colonies were evaluated using the colour charts of Rayner (1970). Lactic acid (60-80 %) was used as the mounting fluid, and 96 % ethanol was used to remove excess conidia. For microscopic analysis, at least 15–30 reproductive structures were analysed to describe each new species. The Nikon Eclipse Ni microscope, equipped with Nikon DS-Fi2 camera, was used to capture images using NIS-Elements AR v. 4.20 software and photos were later edited.

DNA extraction, PCR amplification, and sequencing

Total genomic DNA was extracted using the Wizard® Genomic DNA Purification Kit (Promega Corporation, Madison, WI, USA), according to the manufacturer's protocol. Actin (*actA*), calmodulin (*cmdA*), internal transcribed spacer regions and intervening 5.8S rRNA (ITS), large subunit (LSU) rDNA, RNA polymerase II second largest subunit (*rpb2*), translation elongation factor 1-alpha (*tef1*), and β-tubulin (*tub2*) genes were amplified using the primer pairs ACT-512F and ACT-783R (Carbone & Kohn 1999), CMD5 and CMD6 (Hong *et al*. 2006), ITS1 and ITS4 (White *et al*. 1990), LR0R and LR5 (Vilgalys & Hester 1990), rpb2-5F2 and frpb2-7cR (Liu *et al*. 1999, Sung *et al*. 2007), 983F and 2218R (Rehner & Buckley 2005), EF-728F and EF-986R (Carbone & Kohn 1999), and Bt2a and Bt2b (Glass & Donaldson 1995), respectively. PCR amplification, sequencing, and sequence analysis were performed as described by Bezerra *et al*. (2017a, b) and Barbosa *et al*. (2020). The DNA sequences generated in this study have been deposited in GenBank (Supplementary Table S1).

Phylogenetic analyses

Phylogenetic analyses were performed using our sequences with reference sequences retrieved from the GenBank database, following recently published papers (*e.g. Aspergillus* – Houbraken *et al*. 2020, *Cordycipitaceae* – Mongkolsamrit *et al*. 2020 and Wang *et al*. 2020, *Malbranchea* – Rodríguez-Andrade *et al*. 2021, *Pseudohumicola* – Wang *et al*. 2022, *Talaromyces* – Zhang *et al*. 2021 and Wang & Zhuang 2022, *Tritirachium* – Bezerra *et al*. 2020). Sequences were aligned using the online tool MAFFT v. 7 (Katoh *et al*. 2013) and manually edited using MEGA v.7 (Kumar *et al*. 2016). Initially, each alignment, along with the combined dataset, was analysed based on maximum likelihood (ML) analysis using RAxML-HPC BlackBox v. 8.2.12 (Stamatakis *et al*. 2008) at the CIPRES Science Gateway (Miller *et al*. 2010). Later, the combined datasets were analysed based on Bayesian inference (BI) analysis conducted using MrBayes v. 3.2.7a (Ronquist *et al*. 2012) with XSEDE at the CIPRES Science Gateway. BI analysis was conducted with 1×10^6 generations and a burning value of 25 %, with chains sampled every 1 000 generations, and ML analysis with 1 000 bootstrap replicates. The best nucleotide model for BI analysis was estimated using MrModelTest v. 2.3 software (Nylander 2004), and the GTR + I + G model was used for all ML analyses. Phylogenetic trees were visualised using FigTree software (Rambaut 2010). Values ≥ 0.95 BI posterior probability (BPP) and 70 % ML bootstrap support (ML-BS) are shown near the nodes. The alignments were deposited in TreeBASE (study ID 29511).

Data analyses

The number of colonies (colony-forming units, CFU) was considered to be the fungal abundance, and the values were analysed based on ANOVA using the *F* test with a probability level of 5 %, and Tukey's test was applied for significant results (Cunha *et al*. 2020). Airborne fungi and those obtained from the sediment were evaluated as the total number of CFUs and richness of fungal species (Cunha *et al*. 2020).

Checklist of fungal species in Brazilian caves

Based on the study by Vanderwolf *et al*. (2013), we conducted an active search for papers reporting mycological studies in Brazilian caves using the keywords "Brazilian cave" and "cave fungi in Brazil" in scientific and popular platforms (*e.g.* Web of Science, Scopus, SciELO, and Portal de Periódicos da CAPES). The obtained results were treated, and fungal taxa names were extracted from these papers to build a checklist of mycospeleological studies in Brazil. In this checklist, we observed the Brazilian state/region where the study was conducted, years of publication, and fungal names that were taxonomically checked using the Index Fungorum and MycoBank databases.

RESULTS

General fungal abundance and richness

The abundance of cultivable fungi in Abrigo do Letreiro cave was determined based on the number of colony forming-units (CFUs) at each sampling point (Supplementary Table S2). The fungal abundance was 526 CFU in the air, with point 3 having the highest number of CFU (200); however, no significant difference was observed among the numbers of CFU at each point (F = 0.6145). The number of 3 178 CFU were found in the sediment, with point 1 having the highest number (1 254 CFU); however, no significant difference was observed among the numbers of CFU at each point (F = 2.0075). Seventy-seven isolates (40 from air and 37 from sediment) were selected among UFC observed

and identified based on morphology (*e.g*., genus level) and DNA sequence analyses. These isolates were identified as belonging to 19 genera of *Ascomycota* and *Basidiomycota*, with the phylum *Ascomycota* having the greatest number of taxa (39); only two taxa were *Basidiomycota*. In *Ascomycota* (17 genera), *Aspergillus* had the largest number of taxa (13), followed by *Cladosporium* (4) and *Penicillium* (5). Fourteen other ascomycetous genera were represented by at least one or two taxa (*e.g. Edenia* and *Humicola*). In *Basidiomycota*, the identified isolates were *Sympodiomycopsis* (from the air) and *Tritirachium* (from the sediment) (Table 1).

Airborne fungi

Morphological and phylogenetic analyses of the DNA sequences identified 40 isolates of airborne fungi belonging to 25 species (Table 1). Considering the sampling points defined from the main entrance of the cave, point 1 had the highest richness (12 taxa), followed by points 2 (eight) and 3 (six). *Aspergillus* was the most representative genus in *Ascomycota* with nine taxa, being present at all sampling points. Other genera commonly reported as airborne in several environments (including caves), such as *Cladosporium*, *Penicillium*, and *Talaromyces*, were also identified in our study. Interestingly, at point 1, we identified isolates of the new genus described below in the family *Cordycipitaceae* (a family with entomopathogenic species) and a keratinophilic fungus (*Malbranchea*). At points 2 and 3, we found isolates reported as phytopathogenic fungi (*Cercospora* and *Lasidiodiplodia*), and at point 2, a representative of *Candida*. At point 3, we found a representative of the genus *Edenia* which was first isolated from plants. Nine airborne fungal isolates are described below as belonging to one new genus and five species, representing 20 % of the identified taxa.

Fungi from sediment

Thirty-seven fungal isolates obtained from cave sediment were identified as twenty-four species and taxa belonging to 10 genera (Table 1). Nine of the genera were *Ascomycota*, with *Aspergillus* being the most commonly reported (eight taxa), and *Tritirachium* was the only basidiomycete genus found in the sediment. The sediment from point 1 had the greatest number of species (12), followed by points 2 (11) and 3 (three). Only *Aspergillus sydowii* (points 1 and 3) and *Cladosporium oxysporum* (points 2 and 3) were found at more than one collection point. Nine isolates from the sediments were identified as eight putative new species (33 % of the identified taxa).

Brazilian checklist of mycospeleological studies

We identified 13 mycospeleological studies in Brazil published between 1963 and 2022 (Supplementary Table S3). Among the five macro regions of Brazil, one study was conducted in the Amazon (North), seven in the South-east region, five in the North-east region, and no studies were found in the South and Central West regions. Among the fungal taxa, the largest number were *Ascomycota*, with the genera *Aspergillus*, *Candida*, *Cladosporium*, and *Penicillium* the most commonly reported. A few taxa were included in the *Basidiomycota* and *Mucoromycota* phyla. In these studies, only three new species were introduced based on fungal isolates obtained from the cave environment. Our study enriches this list with a new genus and six new fungal species found in Brazilian caves.

Phylogeny of new species

BLASTn searches using sequences of the 12 isolates, which are introduced below as new species, showed the relationship among our DNA sequences and others deposited in GenBank, which helped us to build specific alignments for each genus having a new species and based on previous studies. Details of the combined datasets (number of species/sequences and length of the datasets (bp)) and the best nucleotide models for BI analysis are shown in Supplementary Table S4. In the phylogenetic analysis, using a combined matrix of *cmdA-tub2-rpb2* sequences, *Aspergillus* isolates (URM 8450 and URM 8451 from the air) were grouped as an independent lineage (BPP = 1 and BS-ML = 100) with *A. europaeus* as the closest species in the series *Wentiorum* of *Aspergillus* section *Cremei* (Fig. 3). Three *Malbranchea* isolates (URM 8445 and URM 8443 from the air; isolate R23 from the sediment) are introduced here as new species based on ITS-LSU phylogenetic inference that placed them as a well-supported clade (BPP = 1 and BS-ML = 100) and related to *M. chinense* (Fig. 4). Two *Pseudohumicola* isolates (URM 8444 from the air and isolate R35 from the sediment) were placed in the ITS-*tub2-rpb2* tree as a unique lineage (BPP = 1 and BS-ML = 100), with *P. pulvericola* and *P. semispiralis* as related species (Fig. 5). Two other isolates (URM 8446 and URM 8447) were obtained from the air and placed in the ITS-LSU-*rpb2-tef1* tree as an unknown and well-defined lineage (BPP = 1 and BS-ML = 100) in the family *Cordycipitaceae*, and are introduced below as a new genus and species (Fig. 6). The fifth new species was introduced based on two *Talaromyces* isolates (URM 8448 and URM 8449) found in the air, and phylogenetically clustered as an independent lineage (ITS-*cmdA-tub2*: BPP = 1 and BS-ML = 100) in the *T. pinophilus* species complex of *Talaromyces* section *Talaromyces* (Fig. 7 and Supplementary Figs S1 and S2). Sequences from one isolate of *Tritirachium* URM 8535, obtained from the sediment, grouped as an independent lineage in the ITS alignment (BPP = 1 and BS-ML = 100) having *Tritirachium batistae* as related species (Fig. 8).

TAXONOMY

Aspergillus lebretii V.C.S. Alves, J.D.P. Bezerra & R.N. Barbosa, ***sp. nov*.** MycoBank MB 846119. Fig. 9.

Etymology: *lebretii* reflects the name of Michel Le Bret, founder of the Brazilian Society of Speleology.

Suborder classification: *Eurotiales*, *Aspergillaceae*.

Infrageneric classification: subgenus *Cremei*, section *Cremei*, series *Wentiorum.*

Typus: **Brazil**, Rio Grande do Norte state, Furna Feia National Park, Abrigo do Letreiro cave, 5°4'14.88''S, 37°32'1.51''W, as an airborne fungus, 5 Mar. 2020, *V.C.S. Alves & D.M. Bento* (**holotype** URM 95150, culture ex-type URM 8451 = FCCUFG 09 = isolate F32).

Culture characteristics (25 °C, 7 d, in the dark): MEA: 26–27 mm diam; mycelium white to buff (45); margins entire, centrally raised, non-sulcate, colony texture cottony, sclerotia absent, poor sporulation, conidial colour *en masse* indeterminate, exudate and pigment soluble absent; reverse saffron (10). CYA: 27–28 mm diam; mycelium white to buff (45), centre slightly elevated, radially sulcate, colony texture cottony, entire to regular margins, sclerotia

absent, poor sporulation, conidial colour *en masse* indeterminate, exudate and pigment soluble absent; reverse primrose (66). CZ: 21–23 mm diam; mycelium white to buff (45); margins entire, centrally raised, non-sulcate, colony texture cottony, entire to

Table 1. Fungal taxa richness. List of fungal taxa isolated from air and sediment in the Abrigo do Letreiro cave, Furna Feia National Park, Rio Grande do Norte, North-eastern region, Brazil. P = fungal taxon present (observed) and – = fungal taxon absent (not observed).

Taxon	Record[a]	Air			Sediment		
Collection points		**Point 1**	**Point 2**	**Point 3**	**Point 1**	**Point 2**	**Point 3**
Ascomycota							
Alternaria jacinthicola	S	–	–	P	–	–	–
Amesia sp.		–	–	–	–	P	–
Aspergillus alboluteus		–	–	–	P	–	–
Aspergillus brunneoviolaceus		–	–	P	–	–	–
Aspergillus cf. niger		–	P	–	–	–	–
Aspergillus dimorphicus	S	–	P	–	–	P	–
Aspergillus eburneocremeus	S	–	–	–	–	P	–
Aspergillus germanicus		–	P	P	–	–	–
Aspergillus lebretii sp. nov.	S	–	–	–	–	–	–
Aspergillus neoniger		P	–	–	–	–	–
Aspergillus sp.		–	–	–	–	P	–
Aspergillus sp. section *Aspergillus*		–	–	–	P	–	–
Aspergillus stellatus		P	–	–	–	P	–
Aspergillus sydowii		P	–	–	P	–	P
Aspergillus tubingensis		–	P	–	P	–	–
Candida sp.		–	P	–	–	–	–
Cercospora vignigena	S	P	–	–	–	–	–
Cercospora cf. canescens	S	P	–	–	–	–	–
Cladosporium oxysporum		P	–	–	–	P	P
Cladosporium subuliforme		–	–	–	–	–	P
Cladosporium tenuissimum		–	–	P	–	P	–
Cladosporium xanthocromaticum	S	P	–	–	–	–	–
Edenia gomezpompae	G, S	–	–	P	–	–	–
Gymnascella hyalinospora		–	–	–	P	–	–
Humicola lutea	S	–	–	–	P	–	–
Humicola sp.		–	–	–	–	P	–
Lasiodiplodia iranensis	G, S	–	P	P	–	–	–
Leptosphaerulina sp.		–	P	–	–	–	–
Malbranchea cavernosa sp. nov.	S	P	–	–	P	–	–
Neocosmospora sp.		–	–	–	P	–	–
Ovatospora sp.	G	–	–	–	P	–	–
Pseudohumicola cecavii sp. nov.	G, S	P	–	–	P	–	–
Pseudolecanicillium caatingaense gen. et sp. nov.	G, S	P	–	–	–	–	–
Penicillium citrinum		–	–	–	–	P	–
Penicillium copticola		–	–	–	P	–	–
Penicillium sp. section *Fasciculata*		–	–	–	–	P	–
Penicillium sp. section *Paradoxa*		–	–	–	–	P	–
Penicillium guanacastense		–	P	–	–	–	–
Talaromyces cavernicola sp. nov.	S	P	–	–	–	–	–
Basidiomycota							
Sympodiomycopsis paphiopedili	G, S	–	P	–	–	–	–
Tritirachium brasiliense sp. nov.	S	–	–	–	P	–	–

[a] G = genus and S = species first record in a cave environment.

regular margins, sclerotia absent, poor sporulation, conidial colour *en masse* indeterminate, exudate and pigment soluble absent; reverse primrose (66). YES: 39–41 mm diam; mycelium white, margins entire, centrally crateriform, radially sulcate, colony texture cottony, entire to regular margins, sclerotia absent, sparse sporulation, conidial colour *en masse* cinnamon (62), exudate and soluble pigments absent, reverse ochreous (44). OA: 14–15 mm diam; centrally raised (convex), non-sulcate, colony texture cottony, entire to regular margins, white to buff (45) coloured mycelium, sclerotia absent, poor sporulation, conidia in undetermined mass, no exudate and soluble pigments; reverse primrose (66). CYAS: 39–40 mm diam; mycelium white, elevated colonies (convex), non-sulcate, colony texture cottony, entire to regular margins, sclerotia absent, sparse sporulation, conidial colour *en masse* buff (45), exudate and soluble pigments absent; reverse saffron (10). CREA: slightly positive for acid production. *Colonies at 30 °C, 7 d in the dark:* MEA: 20–21 mm diam; CYA: 22–23 mm diam; no growth was observed at 37 °C and 10 °C.

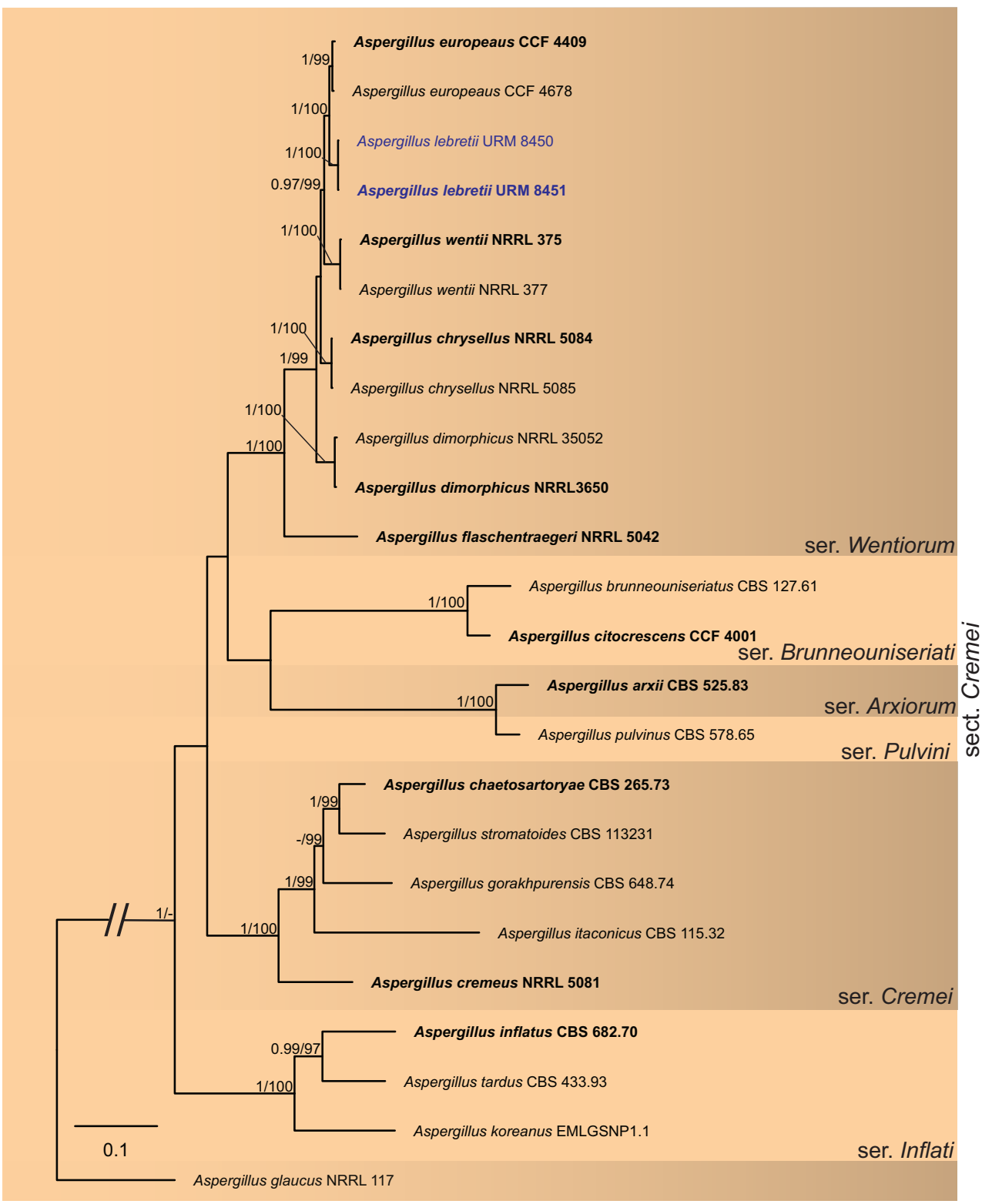

Fig. 3. Bayesian phylogenetic tree using sequences of *cmdA-tub2-rpb2* of species included in *Aspergillus* section *Cremei*. The new species described in this study (*Aspergiilus lebretii*) is highlighted in blue. Ex-type strains are in bold. Values for ML-BS ≥70 % and BPP ≥0.95 are included near nodes. The tree was rooted to *Aspergillus glaucus* NRRL 117.

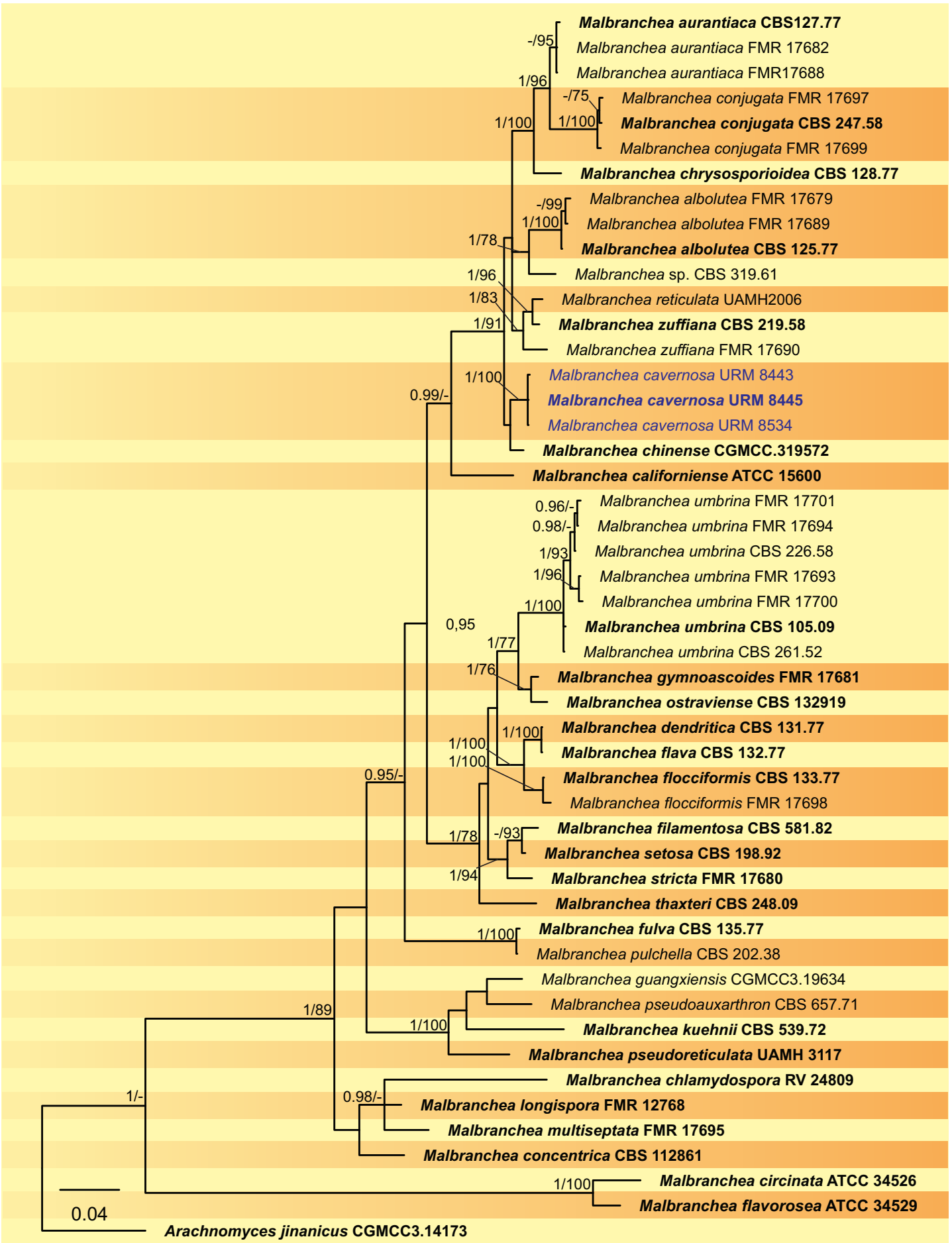

Fig. 4. Bayesian phylogenetic tree using sequences of ITS-LSU of species included in *Malbranchea*. The new species described in this study (*Malbranchea cavernosa*) is highlighted in blue. Ex-type strains are in bold. Values for ML-BS ≥70 % and BPP ≥0.95 are included near nodes. The tree was rooted to *Arachnomyces jinanicus* CGMCC3.14173.

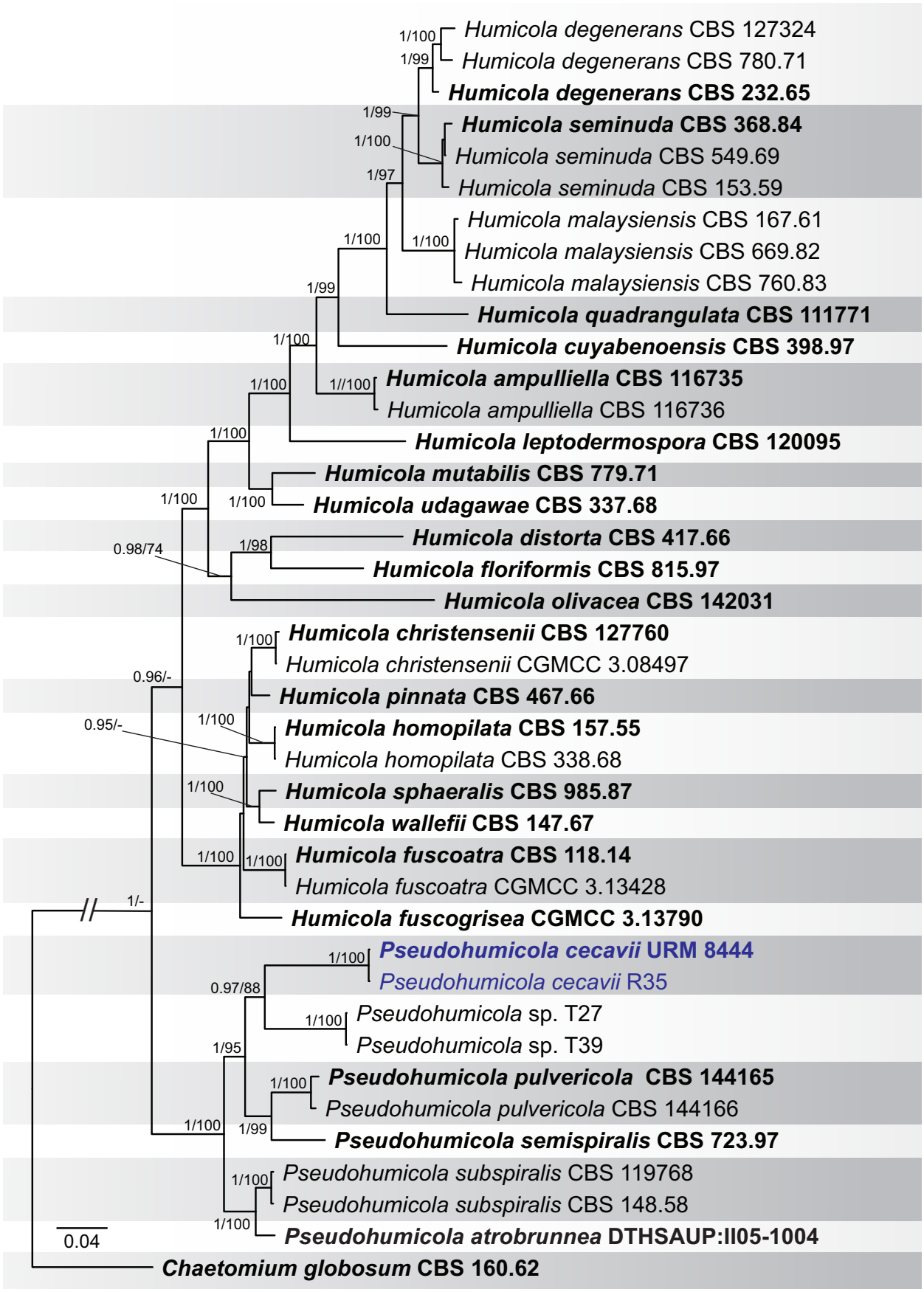

Fig. 5. Bayesian phylogenetic tree using sequences of ITS-*rpb2*-*tub2* of species included in *Humicola* and *Pseudohumicola*. The new species described in this study (*Pseudohumicola cecavii*) is highlighted in blue. Ex-type strains are in bold. Values for ML-BS ≥70 % and BPP ≥0.95 are included near nodes. The tree was rooted to *Chaetomium globosum* CBS 160.62.

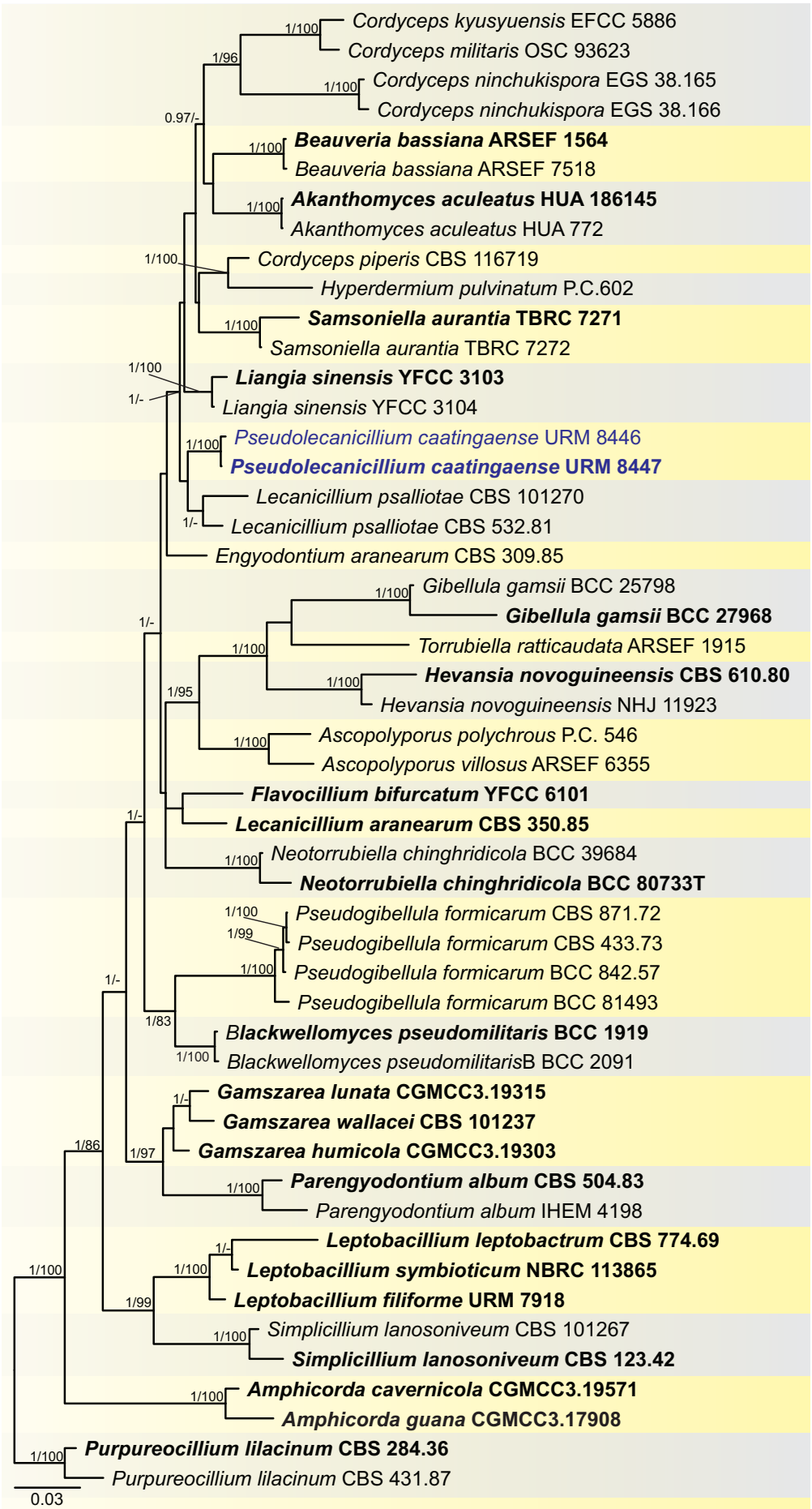

Fig. 6. Bayesian phylogenetic tree using sequences of ITS-LSU-*rpb2-tef1* of species included in the family *Cordycipitaceae*. The new genus and species described in this study (*Pseudolecanicillium caatingaense*) are highlighted in blue. Ex-type strains are in bold. Values for ML-BS ≥70 % and BPP ≥0.95 are included near nodes. The tree was rooted to *Purpureocillium lilacinus* CBS 284.36 and CBS 431.87.

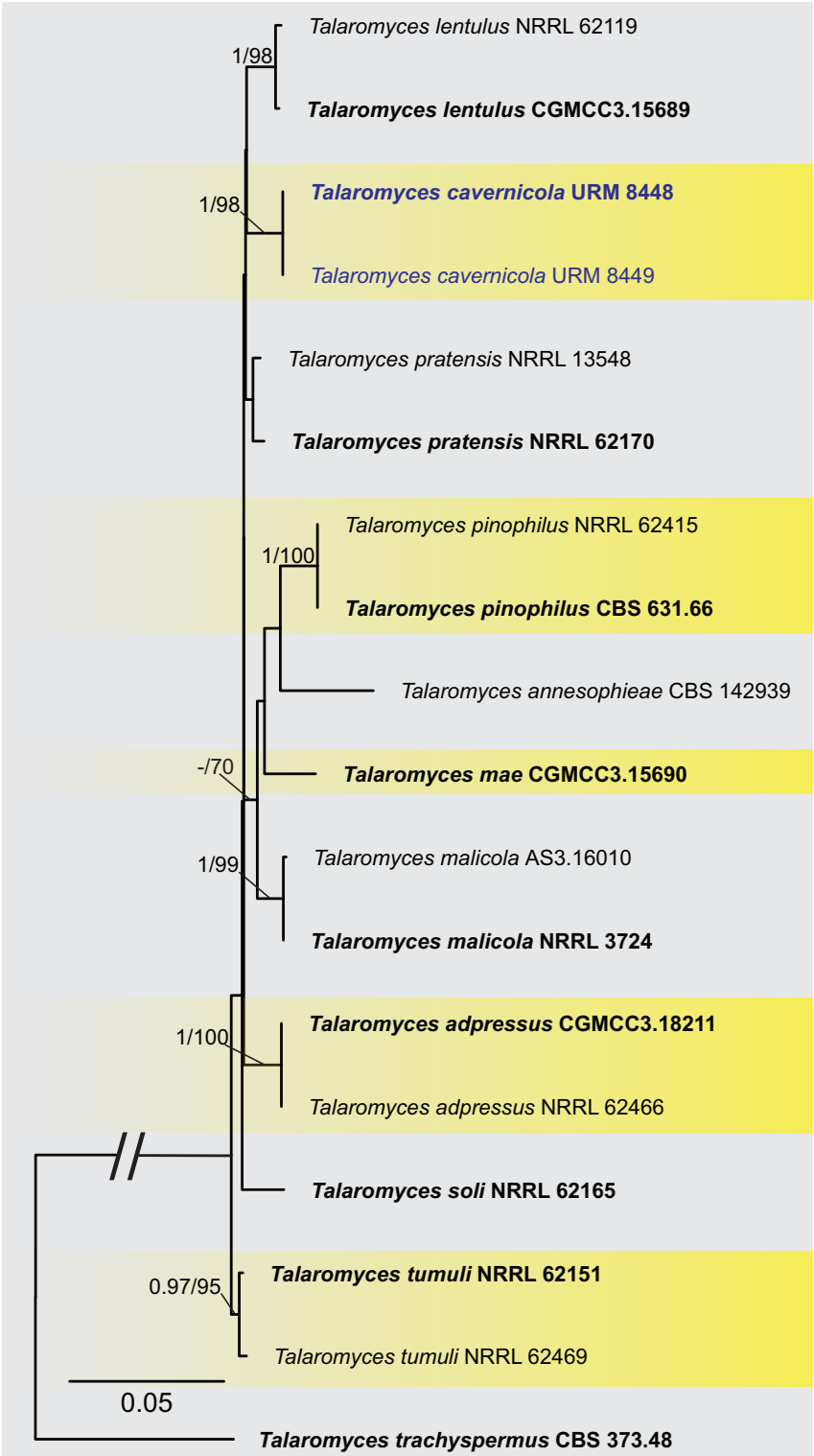

Fig. 7. Maximum likelihood tree using sequences of *cmdA-tub2-rpb2* of species included in *Talaromyces* section *Talaromyces*. The new species described in this study (*Talaromyces cavernicola*) is highlighted in blue. Ex-type strains are in bold. Values for ML-BS ≥70 % and BPP ≥0.95 are included near nodes. The tree was rooted to *Talaromyces trachyspermus* CBS 373.48.

Conidiophores biseriate, stipes, hyaline, smooth-walled, aseptate and occasionally septate, 558–604.5(–930) × 8–13.5 µm, occasionally longer. *Vesicle* globose and occasionally subglobose, 16–40 µm. *Metulae* wedge-shaped, occasionally septate, 13.5–16 × 5–5.5 µm. *Phialides* ampulliform, (7.5–)8–8.5 × (4.5–)5–5.5 µm. *Conidia* globose, yellow to yellowish-brown, smooth to slightly roughened, 2.5–4.5 µm. *Hülle cells* and *sclerotia* were absent. *Sexual morph* not observed.

Additional material examined: **Brazil**, Rio Grande do Norte state, Furna Feia National Park, Abrigo do Letreiro cave, 5°4'14.88''S, 37°32'1.51''W, as an airborne fungus, 5 Mar. 2020, *V.C.S. Alves & D.M. Bento*, URM 8450 = FCCUFG 10 = isolate F23.

Notes: BLASTn searches using ITS sequences showed that *Aspergillus lebretii* has a high identity (99–100 %) to species in the series *Wentiorum* of *Aspergillus* section *Cremei*. The *tub2*

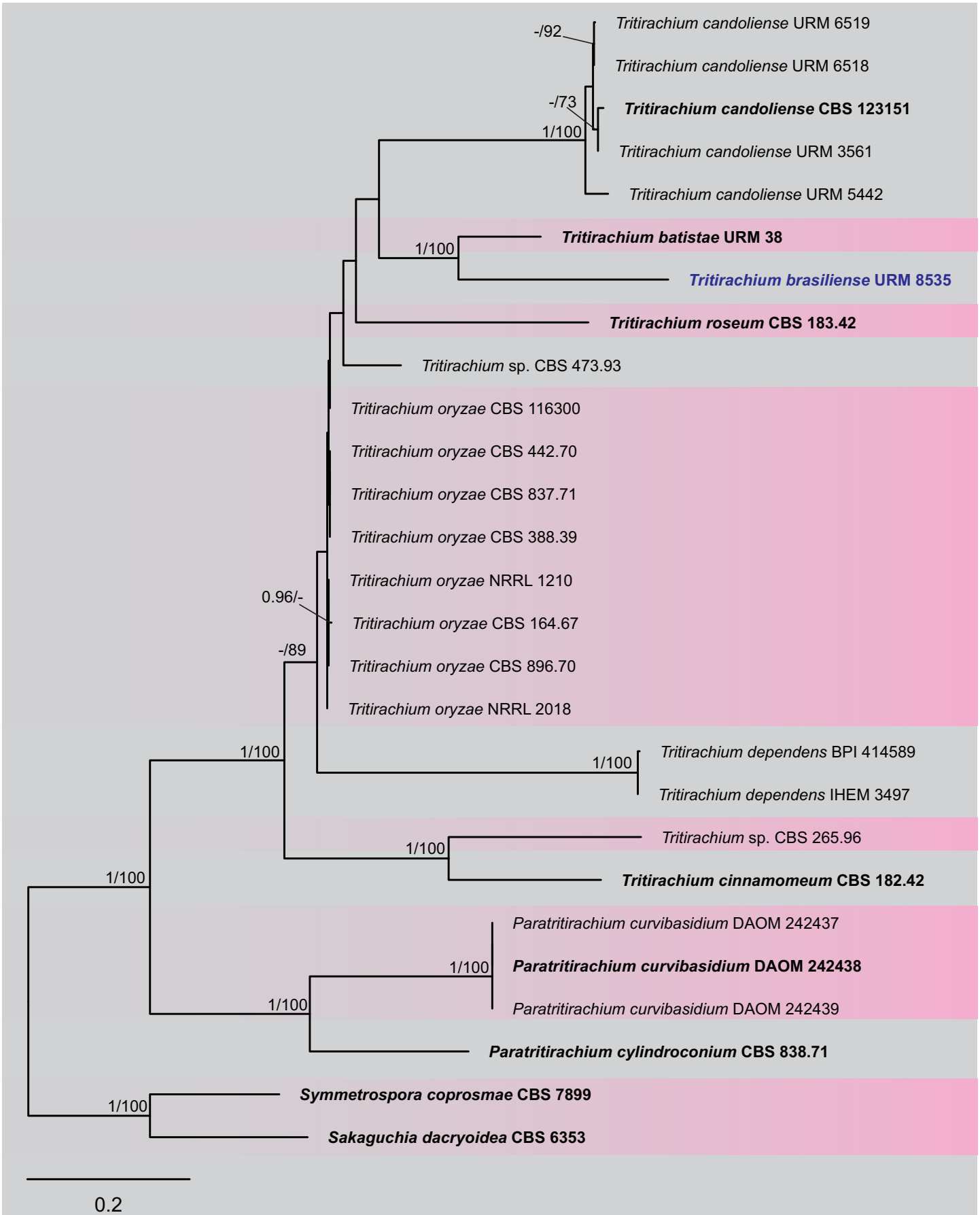

Fig. 8. Maximum likelihood tree using sequences of ITS of species included in *Tritirachium* and related genera. The new species described in this study (*Tritirachium brasiliense*) is highlighted in blue. Ex-type strains are in bold. Values for ML-BS ≥70 % and BPP ≥0.95 are included near nodes. The tree was rooted to *Sakaguchia dacryoidea* CBS 6353 and *Symmetrospora coprosmae* CBS 7899.

Fig. 9. *Aspergillus lebretii* URM 8451. **A.** Colonies from left to right (top row) MEA, CYA, CZ, YES, CYAS and OA; (bottom row) MEA, CYA, CZ, YES, CYAS reverse and CREA, after 7 d at 25 °C in the dark. **B–G.** Conidiophore and conidia. **H.** Conidia. **I.** Schematic line drawing of *Aspergillus lebretii*. Scale bars: B = 100 µm; C–E = 50 µm; F, G = 25 µm; H = 10 µm.

sequences of *A*. *lebretii* showed an identity of 98.08 % to 98.17 % with the ex-type strain of *A*. *europaeus* CCF 4409 and 95.91 % to 96.15 % with the ex-type strains of *A*. *chrysellus* NRRL5084 and *A*. *dimorphicus* NRRL 3650. The *cmdA* sequences of *A*. *lebretii* showed an identity of 96.55 %–97.5 % with the ex-type strain of *A. europaeus* CCF 4409, and 91.54 % to 96 % identity with the ex-type strains of *A*. *chrysellus* NRRL5084 and *A*. *dimorphicus* NRRL 3650. The *rpb2* sequences of *A*. *lebretii* showed an identity of 98.31 % with the ex-type strain of *A. europaeus* CCF 4409 and 97.40 % with *A*. *dimorphicus* NRRL 3650. Phylogenetic analyses based on a combined dataset *tub2-cmdA-rpb2* indicated that *A*. *lebretii* forms a unique, well supported lineage related to *A. europaeus* CCF 4409 (Fig. 3). Based on a nucleotide pairwise comparison of *tub2-cmdA-rpb2*, *A*. *lebretii* differed from the ex-type culture of *A*. *europaeus* CCF 4409 by 1/535 bp of ITS, 6/421 of *tub2,* 10/618 bp of *cmdA* and 19/884 bp of *rpb2*. Macromorphologically, *A*. *lebretii* differs from *A*. *europaeus* by having colonies of cottony texture, poor sporulation, reverse of colonies saffron colour, and absence of soluble pigments in MEA, whereas in *A*. *europaeus*, the colonies have a granular texture, sporulation occurs on almost the entire colony surface, and the reverse of colonies is yellow to bright yellow and there is the production of yellow soluble pigments. On CYA, the colonies of *A*. *lebretii* showed low growth (27–28 mm diam) and cottony texture, whereas *A*. *europaeus* colonies were larger (32–45 mm diam) and velvety to floccose textures. In addition, *A*. *lebretii* shows weak production of acidic compounds in CREA, whereas *A*. *europeaus* shows intensive production (Hubka *et al.* 2016). Micromorphologically, *A*. *lebretii* commonly has long stipes [558–604.5(–930) × 8–13.5 µm, occasionally longer] than *A*. *europeaus* (300–750 × 7–15 µm); vesicles in *A*. *lebretii* are globose and occasionally subglobose (16–40 µm) and *A*. *europeaus* has vesicles piriform or globose (11–44 µm); conidia in *A*. *lebretii* are globose, pigmented and slightly smaller (2.5–4.5 µm) and *A*. *europaeus* has conidia variable in shape, colourless when young and pigmented with age, and slightly larger (3.5–5 × 3–4.5 µm) (Hubka *et al*. 2016).

Malbranchea cavernosa V.C.S. Alves, R.A. Lira, Souza-Motta & J.D.P. Bezerra, ***sp. nov*.** MycoBank MB 846121. Fig. 10.

Etymology: *cavernosa* reflects the location, a cave, where the species was first isolated.

Suborder classification: *Onygenales*, *Malbrancheaceae*.

Typus: **Brazil**, Rio Grande do Norte state, Furna Feia National Park, Abrigo do Letreiro cave, 5°4'14.88''S, 37°32'1.51''W, as an airborne fungus, 5 Mar. 2020, *V.C.S. Alves & D.M. Bento* (**holotype** URM 95151, culture ex-type URM 8445 = FCCUFG 13 = isolate E27).

Culture characteristics (25 °C, 4 wk, in the dark): PDA: 65–67 mm diam, centrally raised, lobed and irregular margins, velutinous, non-sulcate, ochreous (44); exudates or soluble pigments were not observed; reverse scarlet (5) to orange (7). OA: 63–65 mm diam, flat, lobed, and irregular margins, velutinous, non-sulcate, ochreous (44) to apricot (42), centre fawn (87); exudate hyaline and abundant and soluble pigments are absent; reverse sienna (8) to orange (7). SNA: 29–30 mm diam, flat, lobed margins, cottony, non-sulcate, ochreous (44) to saffron (10); exudates or soluble pigments were not observed; reverse saffron (10) to ochreous (44). Sporulation at 10–14 d.

Hyphae hyaline, septate, 1–1.5(–2) µm wide. *Sexual morph*: *Ascomata* abundant, solitary or in clusters, globose, white at first and becoming orange to brown when mature, (232–) 251–325(–335) µm diam. *Peridial hyphae* rough, thick walled, septate, pale brown, branched forming a reticuloperidium, terminated by spine-like projection, 2–6.5 µm wide. *Appendages* with a curved tip, smooth, aseptate, 456–576(–688) × 3–4.5 µm. *Asci* 8-spored, globose, hyaline, 5.5–6.5 µm. *Ascospores* were globose to subglobose, smooth-walled to roughened, 2.5–3.5 × 2–2.5 µm. *Asexual morph* not observed.

Additional material examined: **Brazil**, Rio Grande do Norte state, Furna Feia National Park, Abrigo do Letreiro cave, 5°4'14.88''S, 37°32'1.51''W, as an airborne fungus, 5 Mar. 2020, *V.C.S. Alves & D.M. Bento*, URM 8443 = FCCUFG 14 = isolate E13; Rio Grande do Norte state, Furna Feia National Park, Abrigo do Letreiro cave, 5°4'14.88''S, 37°32'1.51''W, isolated from sediment, 5 Mar. 2020; *R.A. Lira & D.M. Bento*, URM 8534 = isolate R23.

Notes: Based on BLASTn searches, the ITS sequences of *Malbranchea cavernosa* have a low identity of 96.10 % with the ex-type strain of *M. chinense* CGMCC 3.19572 and with *Auxarthron thaxteri* CBS 297.63 (a species synonymized with *Malbranchea umbrina*, Rodríguez-Andrade *et al*. 2021). The LSU sequences of *M. cavernosa* showed high identity (99.23 %) with the ex-type strain of *M. chinense* CGMCC 3.19572. The *tub2* sequences of *M. cavernosa* showed low identity (92.94 %) with the sequences of *M*. *chinense* CGMCC 3.19572. The *tef1* sequences of *M*. *cavernosa* showed low identity (95.94 %) with the sequences of *M*. *chinense* CGMCC 3.19572 and *M*. *guangxiensis* LC12464 (94.64 %.) Phylogenetic analyses based on a combined dataset of ITS-LSU rDNA sequences, showed that *M. cavernosa*, is a unique and well supported lineage related to *M. chinense* CGMCC 3.19572 (Fig. 4). Based on a pairwise nucleotide comparison of ITS-LSU, *M. cavernosa* differs from that of *M*. *chinense* with 19/712 bp of ITS and 4/833 bp of LSU. Macromorphologically, colonies of *M. cavernosa* differ from that of *M*. *chinense* by fast growth on all culture media (PDA: 18–23 mm diam; OA: 18–23 mm diam; SNA: 21–25 mm diam in *M*. *chinense*) (Zhang *et al*. 2021). Micromorphologically, *M. cavernosa* differs from *M*. *chinense*, which only has an asexual morph, by having only a sexual morph. *Malbranchea cavernosa* morphologically resembles *Malbranchea ostraviensis*, which differs from the size of the ascoma (300–400 µm in *M*. *ostraviensis*) and by peridial hyphae that are sometimes dichotomously branched in *M*. *ostraviensis*. The appendages of *M. cavernosa* are slightly larger and wider than those of *M*. *ostraviensis* (350–600 × 2.8–3.2 µm); asci in *M. cavernosa* are globose (5.5–6.5 µm) and smaller than those of *M*. *ostraviensis*, which are globose, subglobose or pyriform (7.4–8.7 × 6.6–7.7 µm); ascospores of *M. cavernosa* are globose to subglobose and in *M*. *ostraviensis* are oblate (2.5–3 µm) (Hubka *et al*. 2013) and they are not phylogenetically related.

Pseudohumicola cecavii V.C.S. Alves, R.A. Lira, Souza-Motta & J.D.P. Bezerra, ***sp. nov*.** MycoBank MB 846122. Figs 11, 12.

Etymology: *cecavii* reflects the name of the Brazilian federal institution devoted to the study and conservation of caves, the Centro Nacional de Pesquisa e Conservação de Cavernas, whose acronym is CECAV.

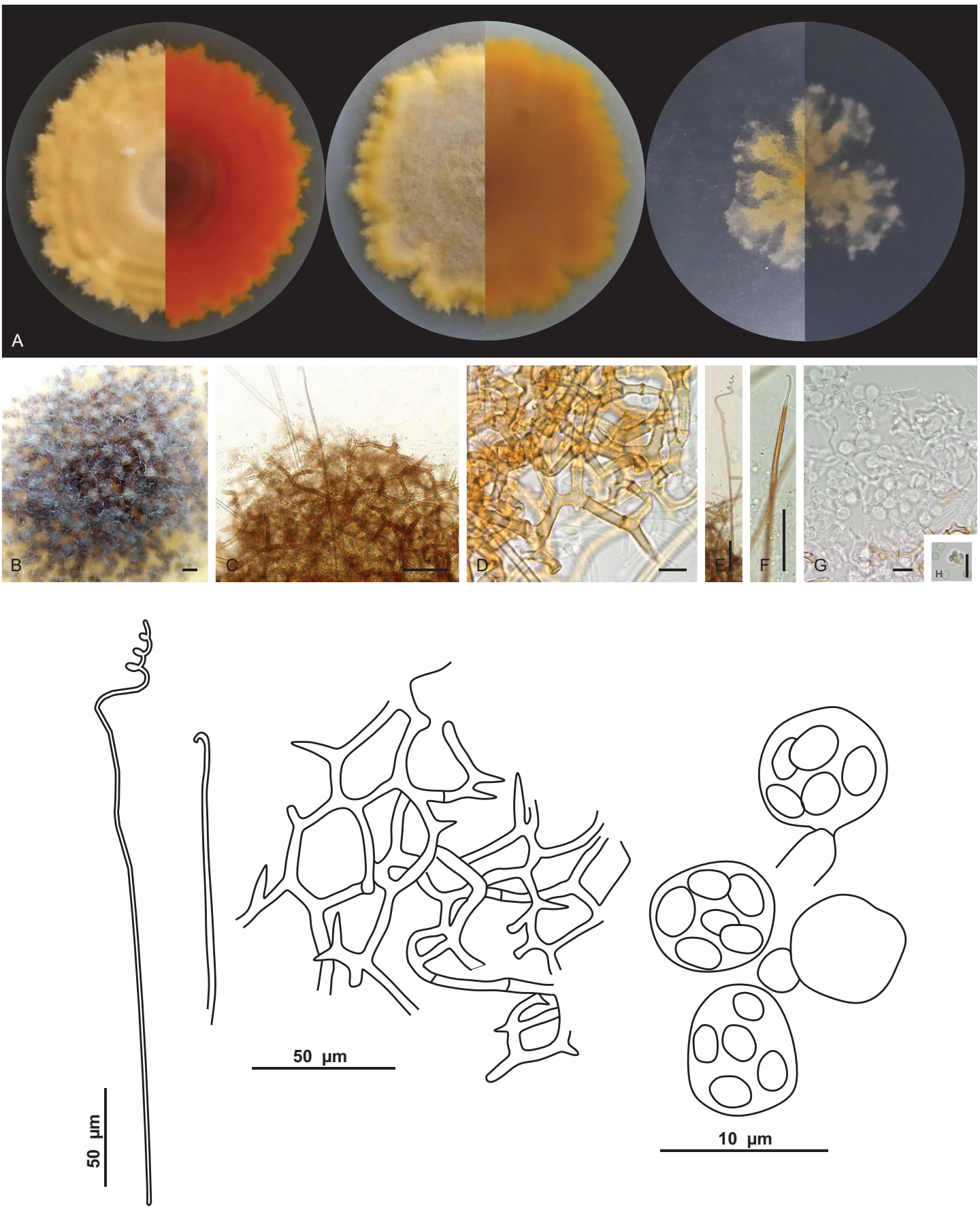

Fig. 10. *Malbranchea cavernosa* URM 8445. **A.** Colonies on PDA, OA and SNA after 4 wk at 25 °C in the dark. **B.** Ascoma. **C.** Ascoma gymnothecium. **D.** Reticuloperidium terminated by spine-like projection. **E, F.** Appendages. **G.** Asci. **H.** Ascospores. **I.** Schematic line drawing of *Malbranchea cavernosa*. Scale bars: B = 300 µm; C, E, F = 50 µm; D, G, H = 10 µm.

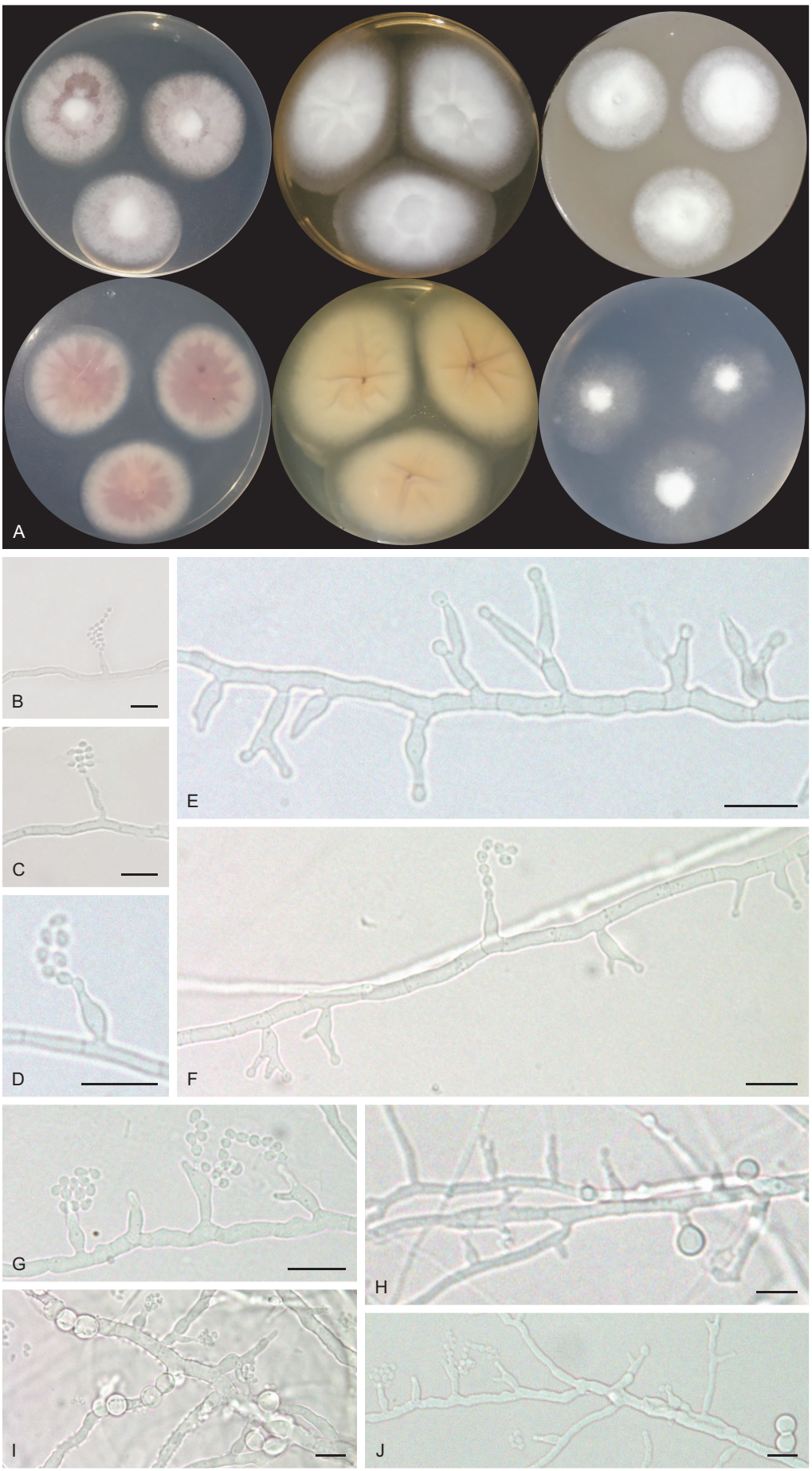

Fig. 11. *Pseudohumicola cecavii* URM 8444. **A.** Colonies from left to right (top row) PDA, MEA and OA; (bottom row) PDA reverse, MEA reverse and SNA after 7 d at 25 °C in the dark. **B–G.** Conidiophore, conidiogenous cells and conidia. **H–J.** Conidiophore, conidiogenous cells, conidia and chlamydospores. Scale bars = 10 µm.

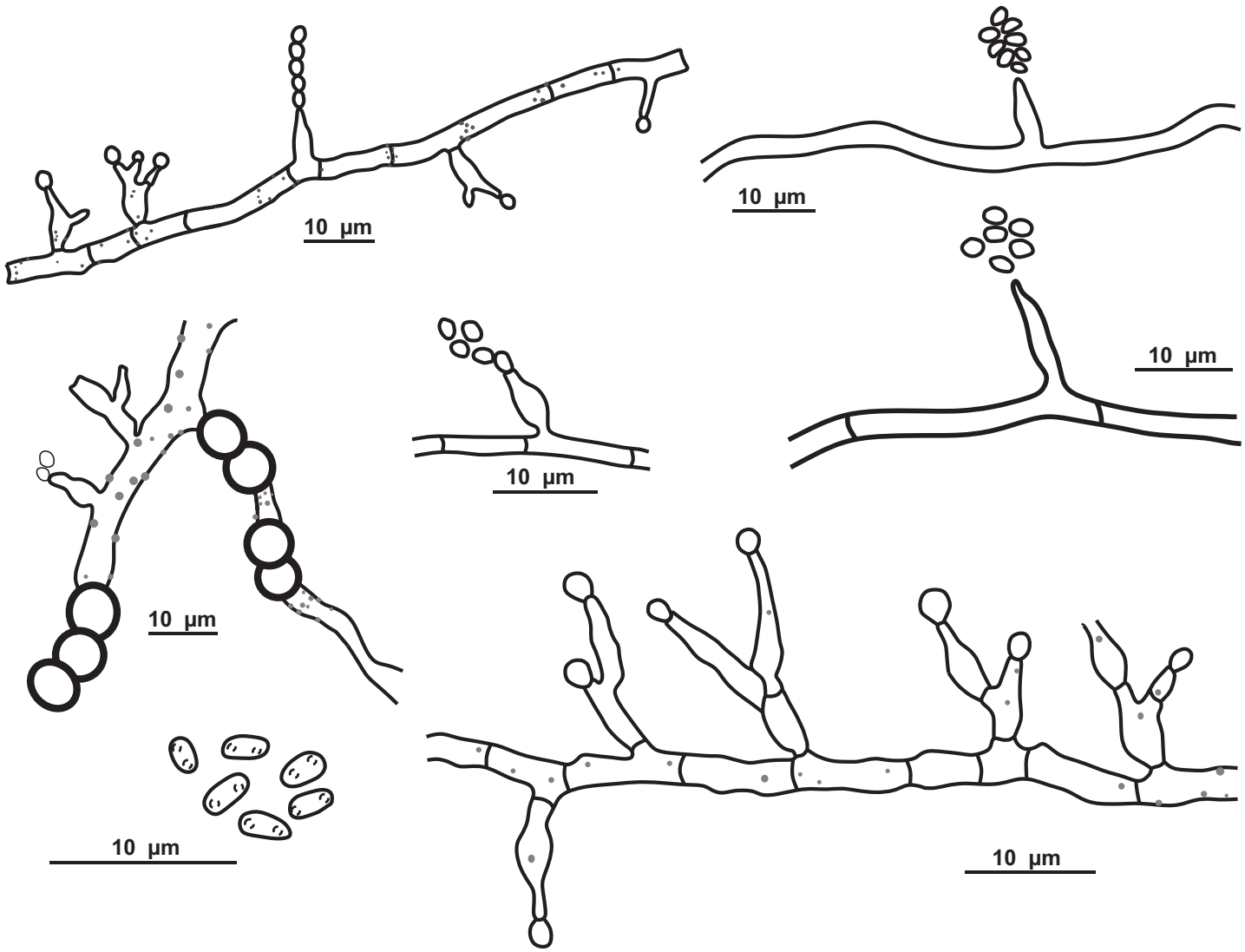

Fig. 12. Schematic line drawing of *Pseudohumicola cecavii* URM 8444.

Suborder classification: *Sordariales*, *Chaetomiaceae*.

Typus: **Brazil**, Rio Grande do Norte state, Furna Feia National Park, Abrigo do Letreiro cave, 5°4'14.88''S, 37°32'1.51''W, as an airborne fungus, 5 Mar. 2020, *V.C.S. Alves & D.M. Bento* (**holotype** URM 95154, culture ex-type URM 8444 = FCCUFG 17 = isolate E21).

Culture characteristics (25 °C, 2 wk, in the dark): PDA: 34–35 mm diam, white to rosy vinaceous (58), cottony, centrally raised, entire to regular margin, radially sulcate, exudate, and soluble pigments absent; reverse rosy vinaceous (58) to buff (45). MEA: 30–35 mm diam, white, cottony, centrally raised, entire to regular margin, radially sulcate, exudate and soluble pigments absent; reverse ochreous (44). OA: 29–30 mm diam, white mycelium, cottony, centrally crateriform, entire to regular margin, non-sulcate, exudate and soluble pigments absent; reverse saffron (10). SNA: (25–)30–32 mm diam, white mycelium, cottony, centrally raised, filiform margin, non-sulcate, hyaline exudate, and soluble pigments absent; reverse buff (45).

Hyphae hyaline, septate, branched and tortuous, 1–3 µm wide. *Conidiogenous cells* phialidic, cylindrical to ampuliform, 4.5–7.5 × 1.5–3 µm. *Conidia* globose to subglobose, chains or slime heads, guttulate, 2–3 × 1–1.5(–2) µm. *Chlamydospores* globose, occasionally subglobose, intercalary and terminal, occasionally in chains, 5–7 µm. *Sexual morph* not observed.

Additional material examined: **Brazil**, Rio Grande do Norte state, Furna Feia National Park, Abrigo do Letreiro cave, 5°4'14.88" S, 37°32'1.51" W, isolated from sediment, 5 Mar. 2020, *R.A. Lira & D.M. Bento*, isolate R35.

Notes: Based on BLASTn searches of ITS sequences, *Pseudohumicola cecavii* has a high identity (99.79 %) to a sequence deposited as "*Humicola* sp. 1002-FVD3 1" which was obtained from the frugivorous guano of a Brazilian bat (Cunha *et al*. 2020), amongst other sequences of the recently introduced genus *Pseudohumicola* (Wang *et al*. 2022). The *rpb2* sequence had low identity (93.72 %) with *P. atrobrunnea* HSAUP II05-1004 isolated from soil, and the *tub2* sequence also had low identity (89.24 %, *P. subspiralis* CBS 148.58 isolated from leaf fragments in soil) (Wang *et al*. 2019). Phylogenetic analyses of a combined ITS-*rpb2*-*tub2* alignment placed our sequences as a unique lineage in a clade encompassing *P. pulvericola* and *P. semispiralis* and related to other *Pseudohumicola* isolates (T27 and T39) obtained during this study (Fig. 5). However, we were not able to obtain new cultures during the subculturing process

of these two isolates which also appeared as a putative new phylogenetic species. Macromorphologically, *P. cecavii* differs from *P. pulvericola* which has faster colony growth on OA (51–57 mm diam) and MEA (46–52 mm diam), and from *P. semispiralis* (OA: 36–42 mm diam, producing a soluble pigment ochreous to umber; MEA: 39–45 mm diam, producing a soluble pigment) (Wang *et al*. 2019). Micromorphologically, *P. cecavii* differs from *P. pulvericola* which has thick-walled and larger conidia (2.5–4 × 1–2(–2.5) µm) and larger conidiophores/conidiogenous cells (13–30 × 1–3 µm). *Pseudohumicola cecavii* mainly differs from *P. semispiralis* by its only known sexual morph and production of conidia usually lateral on the hyphae (Wang *et al*. 2019).

Pseudolecanicillium V.C.S. Alves, Souza-Motta & J.D.P. Bezerra, ***gen. nov.*** MycoBank MB 846123.

Etymology: Reflects the phylogenetic relationship with species previously described in *Lecanicillium* and related genera.

Suborder classification: Hypocreales, *Cordycipitaceae*.

Hyphae septate, hyaline, smooth. *Conidiophores* long, lecanicillium-like, wider at the base, tapering towards the tip, reduced to conidiogenous cells, and semimacronematous or somewhat differentiated with conidiogenous cells arranged solitary or in 2–4 verticills. *Conidiogenous cells* phialidic, discrete, wider at the base and tapering gradually towards the tip, straight to slightly flexuous, terminally and laterally in the conidiophores, mono or polyphialidic, proliferating sympodially, and forming a rachis-like structure in the upper region, with multiple denticules, producing solitary or short chains of conidia. *Conidia* dry, solitary or in short chains, ellipsoid to slightly fusiform, apices acute, smooth, aseptate, hyaline. *Chlamydospores* were not observed. *Sexual morph* not observed.

Type species: Pseudolecanicillium caatingaense V.C.S. Alves, Souza-Motta & J.D.P. Bezerra

Pseudolecanicillium caatingaense V.C.S. Alves, Souza-Motta & J.D.P. Bezerra, ***sp. nov.*** MycoBank MB 846124. Figs 13, 14.

Etymology: *caatingaense* reflects the location, the Caatinga tropical dry forest, where the species was first isolated in Brazil.

Typus: **Brazil**, Rio Grande do Norte state, Furna Feia National Park, Abrigo do Letreiro cave, 5°4'14.88''S, 37°32'1.51''W, as an airborne fungus, 5 Mar. 2020, *V.C.S. Alves & D.M. Bento* (**holotype** URM 95152, culture ex-type URM 8447 = FCCUFG 15 = isolate E34).

Culture characteristics (25 °C, 2 wk, in the dark): PDA: 74–76 mm diam, white, cottony, centrally raised, entire to regular margin, non-sulcate, exudate, and soluble pigments absent; reverse white to creamish. OA: 70–72 mm diam, white, cottony, centrally raised, entire to regular margin, non-sulcate, exudate, and soluble pigments absent; reverse creamish to straw (46). SNA: 74–76 mm diam, white, cottony, centrally raised, entire to regular margin, non-sulcate, exudate, and soluble pigments absent; reverse white to creamish. Sporulation within 7–10 d.

Hyphae 1–2 µm wide, septate, hyaline, and smooth. *Conidiophores* long, straight to flexuous, lecanicillium-like, wider at the base, tapering towards the tip, reduced to conidiogenous cells, and semimacronematous or somewhat differentiated with conidiogenous cells arranged solitary or in 2–4 verticills. *Conidiogenous cells* phialidic, discrete, wider at the base and tapering gradually towards the tip, straight to slightly flexuous, terminally and laterally in the conidiophores, mono or polyphialidic, proliferating sympodially, and forming a rachis-like structure in the upper region, with multiple denticules, producing solitary or short chains of conidia, 10–18.5 × 1–1.5 µm. *Conidia* dry, solitary or in short chains, ellipsoid to slightly fusiform, apices acute, smooth, aseptate, hyaline, 2–4.5 × 1.5–2 µm. *Chlamydospores* were not observed. *Sexual morph* not observed.

Additional material examined: **Brazil**, Rio Grande do Norte state, Furna Feia National Park, Abrigo do Letreiro cave, 5°4'14.88''S, 37°32'1.51''W, as an airborne fungus, 5 Mar. 2020, *V.C.S. Alves & D.M. Bento*, URM 8446 = FCCUFG 16 = isolate E31.

Notes: BLASTn searches using ITS sequences of *Pseudolecanicillium caatingaense* demonstrated low identity (95.03 %) to a sequence deposited as *Blackwellomyces pseudomilitaris* TBRC 3662 isolated from *Lepidoptera* (larvae) (Mongkolsamrit *et al*. 2020), amongst other sequences of *Cordyceps* and *Lecanicillium*. The *tef1* sequence had low identity (95.36 %) to a sequence deposited as *L. psalliotae* CBS 532.81, isolated from soil (Sung *et al*. 2007). The LSU sequences showed high identity (99 %) with sequences deposited as *Lecanicillium* species (*e.g. Lecanicillium fusisporum* CBS 164.70), *tub2* had low identity (89.67 %) with *L. tenuipes* CBS 658.80, and the *rpb2* sequences also had low identity (83.95 %) to the ex-type strain of *L. fungicola* var. *aleophilum* CBS 992.69. Phylogenetic analysis of the ITS-LSU-*rpb2*-*tef1* matrix identified sequences of the new genus and species as a unique and well supported lineage in the family *Cordycipitaceae* (Fig. 6) and related to sequences deposited as *L. psalliotae* (CBS 101270 and CBS 532.81). Cordycipitoid fungi have been widely studied over the years, and the systematic position of some taxa in the family *Cordycipitaceae* have also been resolved based on multigene phylogenetic analyses (Wang *et al*. 2020). Recently, new genera were introduced in this family (*e.g. Flavocillium*, *Gamszarea*, *Jenniferia*, *Liangia*, and *Polystromomyces*) (Wang *et al*. 2020; Zhang *et al*. 2021; Mongkolsamrit *et al*. 2022). *Pseudolecanicillium caatingaense* mainly differs from the related genera by having phialides proliferating sympodially and forming a rachis-like structure in the upper region and with multiple denticules; and by having conidia ellipsoid to slightly fusoid and apices acute.

Talaromyces cavernicola V.C.S. Alves, J.D.P. Bezerra & R.N. Barbosa, ***sp. nov.*** MycoBank MB 846125. Fig. 15.

Etymology: *cavernicola* reflects the location, a cave, where the species was first isolated.

Suborder classification: *Eurotiales*, *Trichocomaceae*.

Infrageneric classification: section *Talaromyces*.

Typus: **Brazil**, Rio Grande do Norte state, Furna Feia National Park, Abrigo do Letreiro cave, 5°4'14.88''S, 37°32'1.51''W, as an airborne fungus, 5 Mar. 2020, *V.C.S. Alves & D.M. Bento* (**holotype** URM 95155, culture ex-type URM 8448 = FCCUFG 11 = isolate E35A).

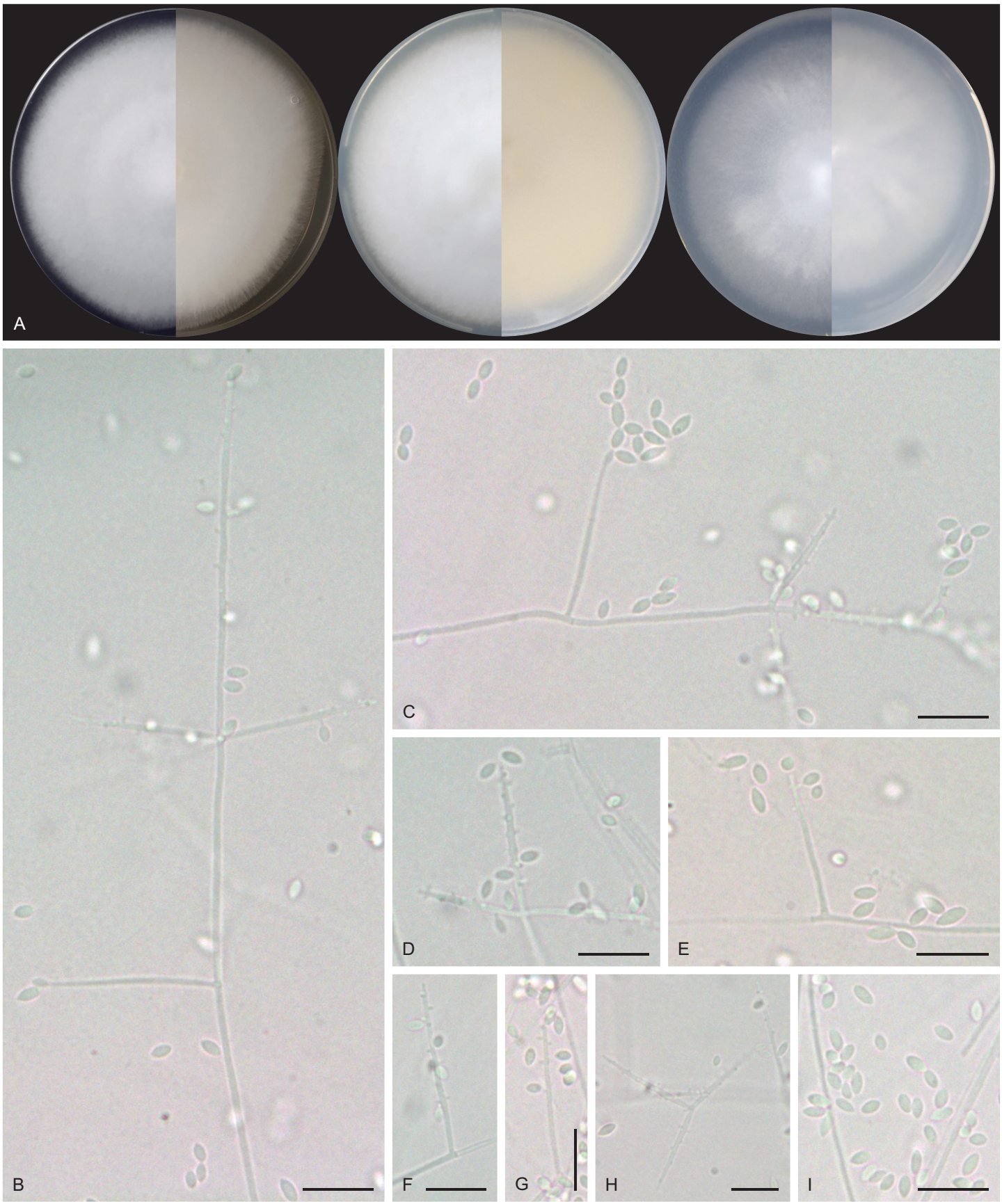

Fig. 13. *Pseudolecanicillium caatingaense* URM 8447. **A.** Colonies on PDA, OA and SNA after 2 wk at 25 °C in the dark. **B, C.** Conidiophores, conidiogenous cells and conidia. **D–H.** Details of conidiogenous cells and conidia. **I.** Conidia. Scale bars = 10 µm.

Culture characteristics (25 °C, 7 d, in the dark): MEA: 35–41 mm diam, mycelium pure yellow (14) to greenish yellow (16), entire to regular margins colonies slightly elevated at the centre, non-sulcate, colony texture velutinous, sclerotia absent, sparse sporulation, conidial colour *en masse* greyish green (50), colourless exudates in small droplets, soluble pigments absent; reverse umber (9) to ochreous (44). CYA: 31–35 mm diam, mycelium pure yellow (14) to yellow green (71), colony plane,

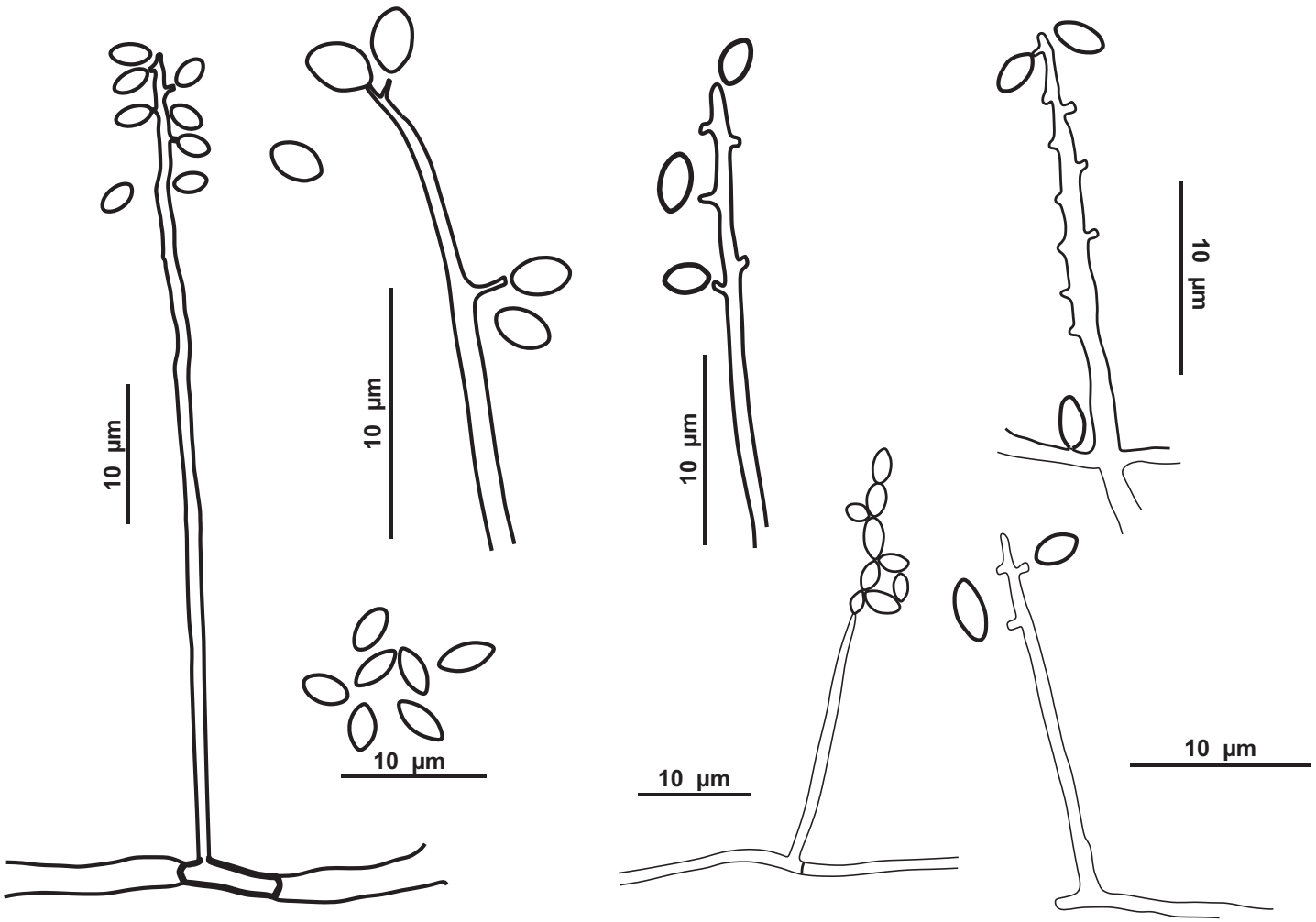

Fig. 14. Schematic line drawing of *Pseudolecanicillium caatingaense* URM 8447.

radially sulcate, regular to entire margins, sclerotia absent, sparse sporulation, conidial colour *en masse* yellow green (71), exudates in small to large droplets of hyaline to cinnamon (62), soluble pigments absent; reverse sienna (8) to ochreous (44). CZ: 18–19 mm diam, mycelium pure yellow (14), margins entire to regular umbonate, radially sulcate, colony texture velutinous, sclerotia absent, poor sporulation, conidial colour *en masse* indeterminate, colourless exudates in small droplets, soluble pigments absent; reverse saffron (10) to salmon (41). YES: (40–) 45–47 mm diam, mycelium pure yellow (14) to yellow-green (71), colonies slightly elevated at the centre, non-sulcate, colony texture cottony, entire to regular margins, coloured mycelium, sclerotia absent, sparse sporulation, conidial colour *en masse* yellow-green (71), soluble pigments and exudate absent; reverse sienna (8) to ochreous (44). OA: 24–28 mm diam, flat, non-sulcate, colony texture cottony, entire to regular margins, citrine green (67) to yellow green (71) mycelium, sclerotia absent, poor sporulation, conidia in undetermined mass, exudates in small droplets of yellowish colour, soluble pigments absent; reverse primrose (66). DG18: 4–7 mm diam, mycelium white, colonies umbonate, non-sulcate, colony texture cottony, entire to regular margins, sclerotia absent, poor sporulation, conidial colour *en masse* indeterminate, soluble pigments and exudate absent; reverse saffron (10) to umber (9). CREA: low acid production. *Colonies at 30 °C, 7 days, in the dark:* MEA: 49–50 mm diam; CYA: 34–35 mm diam; *colonies at 37 °C:* MEA: 27–28 mm diam; CYA: 34–38 mm diam; no growth was observed at 10 °C.

Conidiophores biverticillate, stipes septate, hyaline to pigmented, smooth to roughened, commonly long, 21–420 × 3–4 µm. *Metulae* 3–5, 8–10 × 2.5–3.5 µm. *Phialides* 2–4, acerose, 8–9 × 2–4 µm. *Conidia* globose to subglobose, greenish, ornamented and occasionally smooth, 2 × 1.5–2 µm. *Sclerotia* absent. *Sexual morph* not observed.

Additional material examined: **Brazil**, Rio Grande do Norte state, Furna Feia National Park, Abrigo do Letreiro cave, 5°4'14.88''S and 37°32'1.51''W, as an airborne fungus, 5 Mar. 2020, *V.C.S. Alves & D.M. Bento*, URM 8449 = FCCUFG 12 = isolate E35B.

Notes: Based on BLASTn searches, the ITS sequences of *Talaromyces cavernicola* have a high identity (99.62–99.81 %) with species of *Talaromyces* section *Talaromyces*. The *tub2* of *T. cavernicola* showed 98.26 % identity with the ex-type strain of *T. pratensis* and 98 % to 98.5 % identity with the sequences of *T. stollii* NRRL62122, *T. lentulus* NRRL62143, *T. tumuli* NRRL62151, and *T. sayulitensis* NRRL62203. The *cmdA* sequence of *T. cavernicola* showed low identity (96.39%) with the ex-type strain of *T. pinophilus* CBS 631.66. The *rpb2* sequences of *T. cavernicola* showed an identity of 98.75% with the ex-type strain of *T. pinophilus* CBS 631.66. Phylogenetic analyses based on a combined dataset *tub2-cmdA-rpb2* showed that *T. cavernicola* is a unique, well supported lineage related to *T. soli* NRRL62165 (Fig. 7 and Supplementary Figs S1 and S2). Based on a nucleotide pairwise comparison of *tub2-cmdA-*

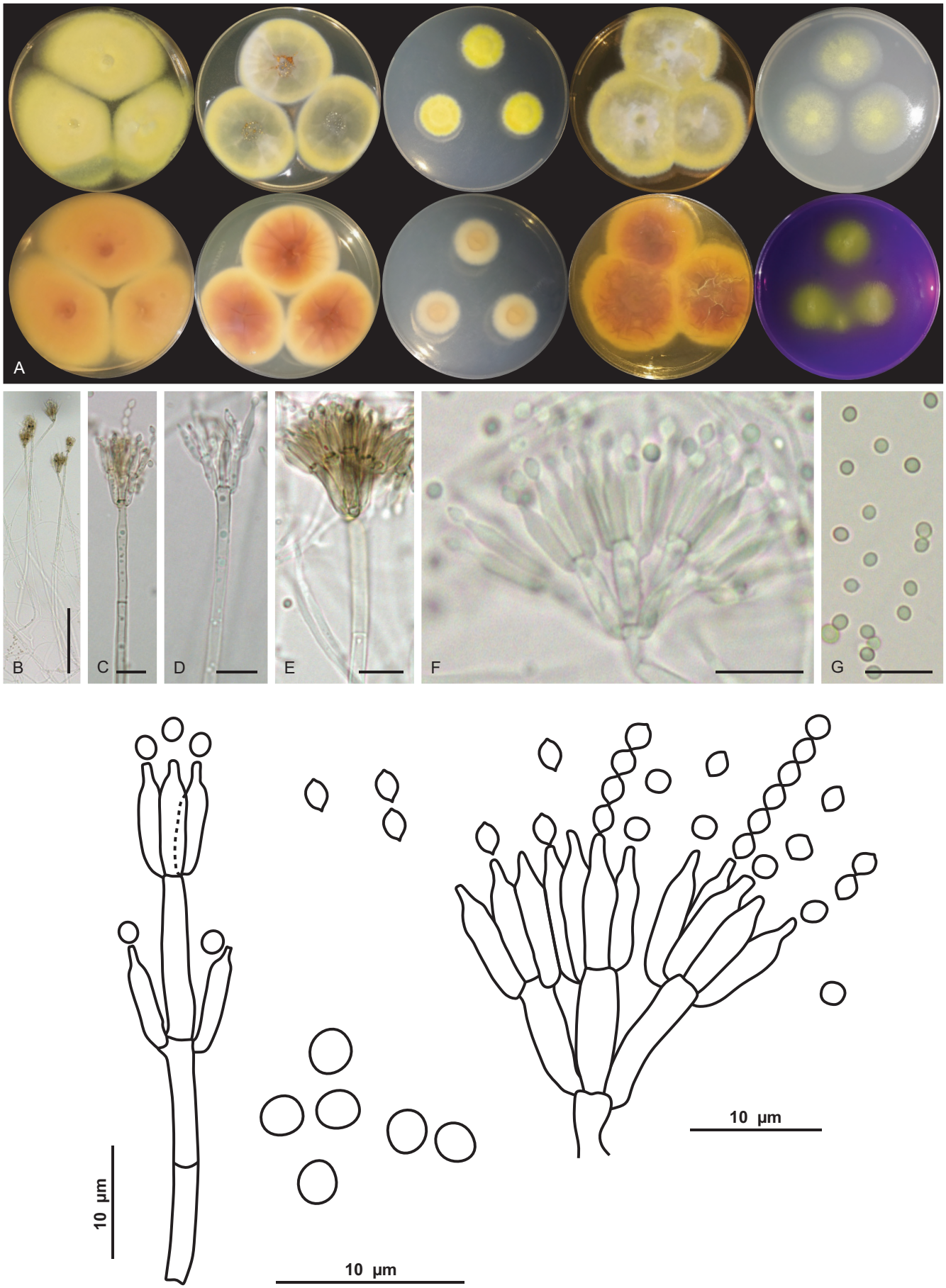

Fig. 15. *Talaromyces cavernicola* URM 8448. **A.** Colonies from left to right (top row) MEA, CYA, CZ, YES and OA; (bottom row) MEA, CYA, CZ, YES reverse and CREA after 7 d at 25 °C in the dark. **B–F.** Conidiophore and conidia. **G.** Conidia. **H.** Schematic line drawing of *Talaromyces cavernicola*. Scale bars: B = 100 µm; C–G = 10 µm.

rpb2, *T. cavernicola* differs from *T. soli* by 6/512 bp for *tub2*, 16/533 bp for *cmdA* and 19/753 bp for *rpb2*; no differences were observed in ITS. Macro morphologically, colonies of *T. cavernicola* differ from those of *T. soli* by having small growth on DG18 (4–7 mm diam in *T. cavernicola* and 7–14 days in *T. soli*). On OA, colonies of *T. cavernicola* were smaller (24–28 mm diam) than that of *T. soli* (23–43 mm diam). On CREA, *T. cavernicola* showed low production of acidic compounds, whereas *T. soli* showed moderate production of acidic compounds (Peterson & Jurjević 2019). Micromorphologically, *T. cavernicola* has longer conidiophores (21–420 × 3–4 µm) than *T. soli* [(5–)30–200(–320, rare) × (2.5–)3–4(–4.5) µm]; metulae in *T. cavernicola* are slightly smaller [(8–10 × 2.5–3.5 µm) and in verticils of 3–5], while in *T. soli* metulae are 8–12(–14) × 2.5–4 µm and in verticils of (3–)5–9(–11); phialides in *T. cavernicola* are smaller (8–9 × 2–4 µm and in verticils of 2–4) than in *T. soli* (9–11(–20) × 2.5–3(–3.5) µm and in verticils of (3–)5–9(–11)); and conidia smaller (2 × 1.5–2 µm) in *T. cavernicola* than *T. soli* [2.5–3.5(–5.5) × 2.5–3.5(–4.5) µm] (Peterson & Jurjević 2019).

Tritirachium brasiliense R.A. Lira, V.C.S. Alves, Souza-Motta & J.D.P. Bezerra, ***sp. nov.*** MycoBank MB 846126. Figs 16, 17.

Etymology: *brasiliense* reflects the name of the country, Brazil, where the fungus was firstly isolated from the sediment of a cave.

Suborder classification: *Tritirachiales, Tritirachiaceae*.

Typus: **Brazil**, Rio Grande do Norte state, Furna Feia National Park, Abrigo do Letreiro cave, 5°4'14.88''S, 37°32'1.51''W, isolated from sediment, 5 Mar. 2020, *R.A. Lira & D.M. Bento* (**holotype** URM 95153, culture ex-type URM 8535 = FCCUFG 18 = isolate R11).

Culture characteristics (25 °C, 7 d, in the dark): PDA: 5–7 mm diam, mycelium rosy buff (61), wavy margin, slightly raised, without furrows, colony texture velvety, exudate and soluble pigments absent; reverse hazel (88). MEA: 4–6 mm diam, mycelium pale vinaceous (85), wavy margin, slightly raised colony, without furrows, colony texture velvety, exudate and soluble pigments absent; reverse dark brick (60) to fawn (87). OA: 5–9 mm diam, mycelium pale vinaceous (85), irregular margins, flat, not furrowed, colony texture velvety, exudate and soluble pigments absent; reverse buff (61). SNA: 6–9 mm diam, mycelium rosy vinaceous (58), irregular margins, flat, not furrowed, colony texture velvety, exudate and soluble pigments absent; reverse cinnamon (62) to park brick (60). YES: 7–11 mm diam, white, entire margins slightly wavy, convex, without furrows, cottony texture, exudate and soluble pigments absent; reverse cinnamon (62) and the centre isabelline (65). CYA: 6–8 mm de diam, mycelium pale purplish grey (127), irregular margins, raised, without furrows, colony texture velvety, exudate and soluble pigments absent; reverse hazel (88) and the centre isabelline (65).

Hyphae pale brownish, smooth walled, 1–2 µm wide. *Conidiophores* reddish brown, lighter towards the base, smooth and somewhat thick-walled, 100–140 × 1.5–2 µm, slightly tapering towards the tip, bearing mainly on the upper part 3–8 whorls conidiogenous cells and occasionally a side branch which bears (1–)3–4 conidiogenous cells. *Conidiogenous cells* consisting of an elongate basal part, slightly swollen below the middle, tapering towards the tip, 9–20 × 1–1.5, and a well-developed geniculate part. *Conidia* reddish brown, smooth, thin-walled, globose to subglobose, rarely with a slightly apiculate base, 2–3 × 1–3 µm. *Chlamydospores* not observed. *Sexual morph* not observed.

Notes: Based on BLASTn searches of ITS sequence, *Tritirachium brasiliense* had a low identity (90.70 %) with *Tritirachium oryzae* TO-BRK2. The LSU sequence showed low identity with the sequences of *Tritirachium batistae* URM 38 (96.56 %) and *T. oryzae* TO-BRK2 (95.62 %). The *rpb2* sequences also showed low identity (85.12 %) with sequences of *T. batistae* URM 38. Phylogenetic analyses of ITS sequences placed the new species as a unique lineage related to *T. batistae* URM 38 (Fig. 8). *Tritirachium brasiliense* differs from *T. batistae* by differences in colony colour on the PDA, MEA, CYA, YES, and OA culture media; in *T. brasiliense* the predominant colour is pale vinaceous to rosy buff and in *T. batistae* the predominant colour is white to greyish. The size of colonies also differs between *T. brasiliense* and *T. batistae* which has larger colonies sizes. The texture of colonies in *T. brasilense* is predominantly velvety, except on YES, while in *T. batistae* the cottony texture was predominantly observed. Conidiophores of *T. brasiliense* are slightly reddish and larger (100–140 µm) than those of *T. batistae* that are brownish with maturity and smaller (9–23.5 µm). The conidia of *T. brasiliense* are reddish brown and in *T. batistae* they are hyaline (Bezerra *et al*. 2020).

DISCUSSION

Studies on fungi in caves have been conducted to investigate different substrates and hosts (Zhang *et al*. 2021). Our study observed high fungal abundance in the sediment and air of the Abrigo do Letreiro cave in the Caatinga drylands of Northeast Brazil at all sampling points. Morphological features and phylogenetic analyses indicated 41 species belonging to 17 genera of *Ascomycota* and two genera of *Basidiomycota*, with *Aspergillus* being the most commonly observed genus in the cave (13 taxa). One new genus and six species were described in our study. Despite the growing quest to unravel the mycobiota of the cave environment, there is still a knowledge gap regarding the fungi living in these environments, especially when associated with special caves, such as the caves of the Caatinga dry forest in Brazil (Cunha *et al*. 2020, Pereira *et al*. 2022).

Differences in the spatial distribution of fungi: the role of biotic and abiotic factors

In our study, the abundance of airborne fungi was spatially heterogeneous, being the region furthest from the cave entrance the most representative of the number of CFUs. On the other hand, the highest fungal abundance in sediment was at the point 1 (near the main cave entrance). The point 1 had the highest species richness for both airborne and sediment fungi. Taylor *et al*. (2013) investigated a cave in Minas Gerais state (Brazil) and obtained a different result for the abundance of airborne fungi, which was higher near the entrance; however, the studied cave was highly visited as a tourist attraction. Similar to our results, Cunha *et al*. (2020) demonstrated that the collection points distant from the bat cave entrance had the highest abundance of airborne fungi, which was attributed to the large presence and movement of

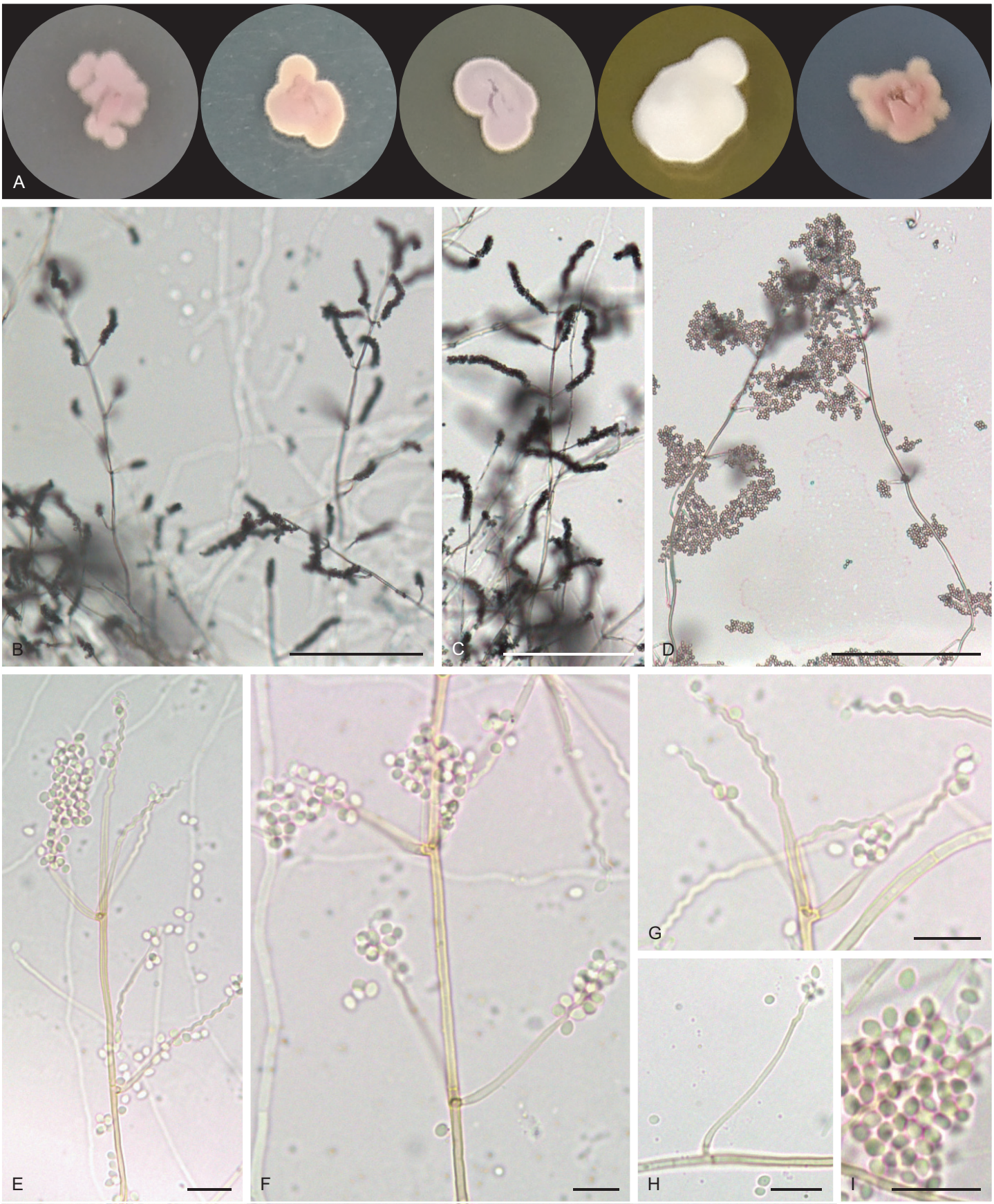

Fig. 16. *Tritirachium brasiliense* URM 8535. **A.** Colonies from left to right OA, PDA, CYA, YES and SNA after 7 d at 25 °C in the dark. **B–F.** Conidiophores, conidiogenous cells and conidia. **G, H.** Details of conidiogenous cells. **I.** Conidia. Scale bars: B–D = 100 µm; E–I = 10 µm.

bats at this sampling point. In contrast, the abundance of fungi in the sediment was higher at a collection point closer to the main entrance (being the closest point of the Abrigo do Letreiro cave), and the deposition of guano at this point of the cave was higher due to a small colony of bats roosting at this point. In a study of fungi from the terrestrial Gruta do Catão (Bahia state,

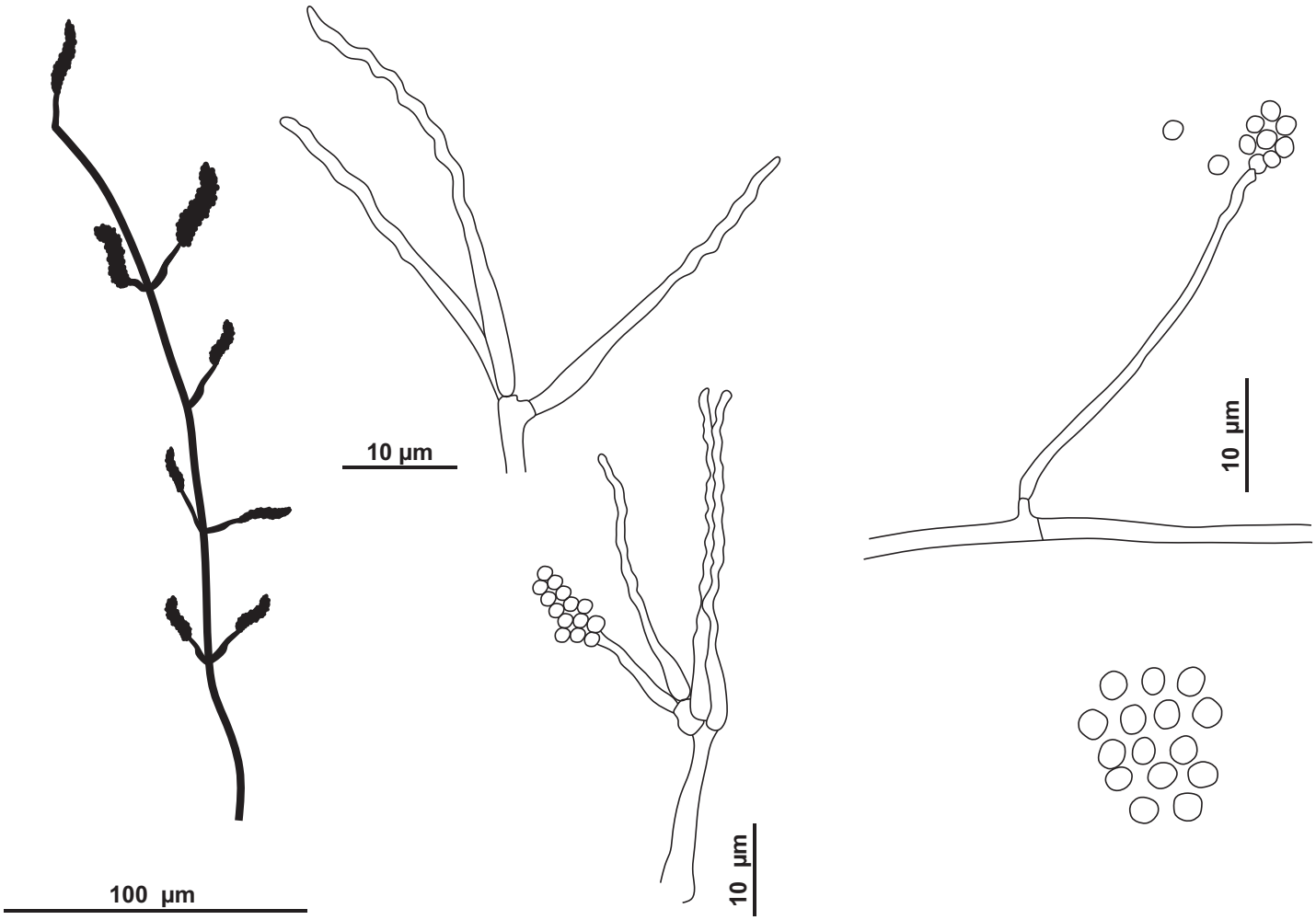

Fig. 17. Schematic line drawing of *Tritirachium brasiliense* URM 8535.

Brazil), similar results were observed regarding the abundance of sediment fungi, which was higher in the closed areas of the cave (Paula *et al*. 2016). In an iron-ore cave in Brazil, Taylor *et al*. (2014) showed that the highest abundance of fungi from sediment was obtained in the twilight zone in the cave, where there was a transition from the entrance to the darkest zone. In addition, Zhang *et al.* (2018) suggested that fungi from caves may have their origin in the surface environment, as the reported fungal species are also known in outside environments, and because the speleogenesis seems to be short for fungal speciation.

Forty-nine fungal taxa were identified in Abrigo do Letreiro cave, with the collection point located near the main entrance and the highest richness of fungi (22 taxa, almost half of the total species identified in our study). The Abrigo do Letreiro cave has a special morphology, that is, a skylight in the middle of the cave where we found a tree species (*Erythrina velutina*, *Fabaceae*) of the Caatinga dry forest (Sobrinho *et al*. 2016); that skylight allows a connection between the hypogeal and the external environments, resembling the same conditions found at the main cave entrance and also has a similar fungal richness shared between these two collection points. The air current, the direct connection with the land surface, and the presence of bats at this sampling point may influence this result because the fungal community can be influenced by factors that are responsible for transporting fungal propagules and consequently causing their dispersion (Vanderwolt *et al*. 2013, Vanderwolf *et al*. 2013b, Johnson *et al*. 2013, Holz *et al*. 2018, Cunha *et al.* 2020).

The mycobiota in caves has a distinct distribution pattern that can be influenced by biotic and abiotic factors, such as the entrances that caves may have, air currents, the presence of bats, and consequently, the deposition of guano (Cunha *et al*. 2020). This fact can be verified with fungal findings that are normally also found in the external environment in association with plants or with insects and other animals, and this was also verified in the cave environment (Zhang *et al*. 2017, Cunha *et al*. 2020). Places with greater deposition of guano and organic material are where greater fungal development can be observed (Taylor *et al*. 2013, Cunha *et al*. 2020). The origin of fungi in caves was demonstrated to be from the external environment (Zhang *et al*. 2018).

Richness and noteworthy records

The fungal isolates were grouped into 19 genera, with *Aspergillus* being the most common, followed by *Penicillium* and *Cladosporium*. These fungi are commonly the most reported taxa in caves around the world, in tropical and subtropical caves, and European countries (Varderwolf *et al*. 2013, Zhang *et al*. 2017, Cunha *et al*. 2020, Zhang *et al*. 2021). Similar to our results, Zhang *et al*. (2017) reported that these genera are most frequently isolated from the air in karst caves in China; similar results were obtained by Kokurewicz *et al*. (2016) in Poland. In Brazil, *Aspergillus* and *Penicillium* species were also more abundant in air samples, bat guano, soil, sediment, and other substrates (see

Supplementary Table S3; Castrillón *et al*. 1976, Taylor *et al*. 2013, Paula *et al*. 2016, Cunha *et al*. 2020). Studying a bat cave in the Caatinga, Cunha *et al*. (2020) obtained the largest number of taxa (12) of *Aspergillus*, five of *Penicillium* and three of *Cladosporium* from different substrates/hosts of the Meu Rei cave (Catimbau National Park, Pernambuco state, Brazil); similar to our results, the genus *Aspergillus* was the most representative in the air of the Meu Rei cave. Recently, Pereira *et al*. (2022) reported the richness of eight *Cladosporium* species, including two new species, from the air in Furna do Morcego cave located in the Caatinga forest in Brazil and Carvalho *et al.* (2022) reported the isolation of 13 fungal species from bat ectoparasites of a cave in the Caatinga with the description of two new species. Taylor *et al*. (2014) and Paula *et al*. (2016) studied the sediments of Brazilian caves and reported *Aspergillus* and *Penicillium* as the most commonly found genera. These genera of fungi can be found widely in environments other than caves, and are very common in the isolation of fungi from various substrates that are considered ubiquitous fungi and adaptable to different environments (Crous *et al*. 2014, Barbosa *et al*. 2016, Diao *et al*. 2018).

We also isolated fungi in the Abrigo do Letreiro cave, which are commonly associated with plants and have been reported as entomopathogens. Cunha *et al*. (2020) also reported isolates of *Diaporthe*, *Aplosporella* (mainly found in plants), and *Beauveria bassiana* (mainly reported as an entomopathogen) in their study of a tropical cave in Brazil. Other fungi are known to have species treated as opportunistic pathogens (*e.g*. *Candida* and *Malbranchea*) were also found in our study. We did not observe the presence of the pathogenic fungus *Histoplasma capsulatum*, probably because of the methods used to isolate this fungal species; however, human infection by this fungus has already been reported in caves of Brazil (Vicentini *et al*. 2012) and it has been reported in cavities in other countries (Varderwolf *et al*. 2013).

New taxa discovered

One new genus and six new species were described for the fungi obtained in our study, accounting for 14 % of the total taxa identified. *Aspergillus lebretii sp. nov.* has been described in the series *Wentiorum* of the section *Cremei* and other species in this section have been related to caves (Vanderwolf *et al*. 2013, Hubka *et al*. 2016) and are commonly found in soil and as food spoilage (Hubka *et al*. 2016); some species are also used in the production of enzymes and organic acids and have been reported as mycotoxin producers and contaminants of cereals (Flannigan 1986, Roehr *et al*. 1992). Interesting, *A. europeaus*, the species mostly related to *A. lebretii*, were collected from soils near European caves, and other isolates were also obtained from the soil in the Czech Republic (Nováková *et al*. 2012, 2014b, Hubka *et al*. 2015) and a new species *A. citoscrescens* was isolated from cave sediment in Spanish Castañar de Ibor cave (Crous *et al*. 2015); *Aspergillus wentii*, the most known species of section *Cremei*, has also been reported in several caves around the world (Vanderwolf *et al*. 2013). Similar to *Aspergillus*, species of *Talaromyces* section *Talaromyces*, where *T. cavernicola sp. nov*. is placed, are commonly found in soil, have biotechnological potential to produce pigments and enzymes, and are reported as opportunistic pathogens in humans (Guevara-Suarez *et al*. 2017, Morales-Oyervides *et al*. 2020).

The new species *Malbranchea cavernosa* was introduced in a genus mainly reported to have keratinophilic potential and has been reported as an opportunistic etiological agent in humans and other animals (Hubka *et al*. 2013). In addition, species of *Malbranchea* are mainly isolated from the soil and excrement of animals and have been previously reported in caves (as *Auxarthron* species, Zhang *et al*. 2021). *Pseudolecanicillium caatingaense gen. et sp. nov*. has been described in the family *Cordycipitaceae*, which includes species mainly found as entomopathogenic fungi of several insects and has great potential for use in biological control (Goettel *et al*. 2008, Mongkolsamrit *et al*. 2020, Wang *et al*. 2020). Some fungi belonging to the family *Cordycipitaceae* have also been reported in caves (Varderwolf *et al*. 2013, Cunha *et al*. 2020, Zhang *et al*. 2021). The fifth new species introduced here, *Pseudohumicola cecavii*, was found in air and sediment with guano from insectivorous bats and excrements of small mammals (*Kerodon rupestris*), and other species in this genus have been reported from different substrates (*e.g.* dung of horse, leaves, paper, and soil) (Wang *et al*. 2019). Species of *Pseudohumicola*, a recently introduced genus (Wang *et al*. 2022), and the related genus *Humicola* have been isolated from caves in several countries (Vanderwolf *et al*. 2013, Zhang *et al*. 2017) and in Brazil, they have also been reported from the guano of frugivore bats (Cunha *et al*. 2020). In addition, some *Humicola* species have the potential as bio-organic fertilisers or biological control organisms for plant diseases (Lang *et al*. 2012). The new species of *Tritirachium*, *T. brasiliense*, was isolated from sediment; species of this genus are reported in different substrates and habitats, such as decaying organic matter and plants (see Bezerra *et al*. 2020) and they have also been reported as human opportunistic pathogens (Martínez-Herrera *et al*. 2015).

As it is an environment little explored in terms of mycobiota studies, the Abrigo do Letreiro cave presented a high richness of airborne and sediment fungi when compared to other caves in Brazil (see Supplementary Table S3), considering that 14 % of the obtained species were described as novel. Caves in the Caatinga dry forest in Brazil have great potential for the discovery of known and unknown fungal species (see Cunha *et al*. 2020, Pereira *et al*. 2022, Carvalho *et al.* 2022 and Supplementary Table S3) which need to be treated as mycodiversity hotspots in the country.

Conflict of interest: The authors declare that there is no conflict of interest.

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Supplementary Material: http://fuse-journal.org/

Fig. S1. Maximum likelihood tree using sequences of *tub2-cmdA-rpb2* of species included in *Talaromyces* section *Talaromyces.*

Fig. S2. Maximum likelihood tree using an independent dataset of ITS, *tub2*, *cmdA*, and *rpb2* of species included in *Talaromyces* section *Talaromyces*.

Table. S1. GenBank accession numbers of sequences obtained in this study (in bold) and sequences from other studies are ordered according to the phylogenetic analyses of the new species described here.

Table. S2. Number of fungal colonies (CFU) from air and sediment.

Table. S3. Checklist of mycoespeleological studies in Brazil.

Table. S4. Details on the combined datasets (number of taxa, sequences, and length of dataset (bp)) and the best–fit models for each partition proposed by MrModelTest.