THIAGO OLIVEIRA CONDÉ

SHEDDING LIGHT ON THE FUNGAL DARKNESS: CAVE FUNGI FROM THE SOUTHERN ESPINHAÇO MOUNTAIN RANGE OF MINAS GERAIS

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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Nothing in biology makes sense except in the light of evolution
(Theodosius Dobzhansky)

ABSTRACT

CONDÉ, Thiago Oliveira, D.Sc., Universidade Federal de Viçosa, March, 2023. **Shedding light on the fungal darkness: cave fungi from the Southern Espinhaço Mountain Range of Minas Gerais**. Adviser: Olinto Liparini Pereira.

Interest in cave fungal diversity is flourishing because it may represent a reservoir of new species and metabolites. However, the mycobiota remains poorly studied in the underground environment, especially in Neotropical regions. During surveys that aimed to investigate the fungal diversity in quartzite and karst caves in the Southern Espinhaço Mountain in Brazil, six *Chaetomiaceae* isolates were obtained from different cave substrates. Five taxonomical novelties of *Chaetomiaceae* in Brazilian caves were discovered based on phylogenetic analyses using DNA sequences from ITS, LSU, *TUB*, and *RPB2* genes. *Chaetomium meridionalense*, *Pseudohumicola alba* and *Pseudohumicola lutea* are new species found in Gruta da Extração and Gruta Velha Nova caves. *Parahumicola* is introduced as a new genus representing a novel phylogenetic lineage with unique morphological characteristics in the family *Chaetomiaceae*. This new monotypic genus is typified by *P. guana,* which was found in a bat guano sample in the Gruta Monte Cristo cave. Furthermore, this is the first report of *Collariella bostrychodes* in a neotropical cave. Overall, these findings emphasize that Brazilian caves constitute an untapped source of fungal resources.

Keywords Five new taxa. Biospeleology. *Chaetomium*. *Parahumicola*. *Pseudohumicola*. *Sordariales*.

RESUMO

CONDÉ, Thiago Oliveira, D.Sc., Universidade Federal de Viçosa, março de 2023. **Shedding light on the fungal darkness: cave fungi from the Southern Espinhaço Mountain Range of Minas Gerais**. Orientador: Olinto Liparini Pereira.

O interesse na diversidade fúngica em cavernas está crescendo porque pode representar um reservatório de novas espécies e metabólitos. No entanto, a micobiota permanece pouco estudada no ambiente subterrâneo, especialmente em regiões neotropicais. Durante pesquisas que visavam investigar a diversidade fúngica em cavernas de quartzito e calcário no sul da Serra do Espinhaço, no Brasil, foram obtidos seis isolados de *Chaetomiaceae* de diferentes substratos de caverna. Cinco novidades taxonômicas de *Chaetomiaceae* em cavernas brasileiras foram descobertas com base em análises filogenéticas usando sequências de DNA dos genes ITS, LSU, TUB e RPB2. *Chaetomium meridionalense*, *Pseudohumicola alba* e *Pseudohumicola lutea* são novas espécies encontradas nas cavernas Gruta da Extração e Gruta Velha Nova. *Parahumicola* é introduzido como um novo gênero representando uma nova linhagem filogenética com características morfológicas únicas na família *Chaetomiaceae*. Este novo gênero monotípico é tipificado por *P. guana*, que foi encontrado em uma amostra de guano de morcego na caverna Gruta Monte Cristo. Além disso, este é o primeiro relato de *Collariella bostrychodes* em uma caverna neotropical. Em geral, essas descobertas enfatizam que as cavernas brasileiras constituem uma fonte inexplorada de recursos fúngicos.

Palavras-chave: Cinco novos táxons. Bioespeleologia. *Chaetomium*. *Parahumicola*. *Pseudohumicola*. *Sordariales*.

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SUMMARY

1 GENERAL INTRODUCTION

1.1 The Southern Espinhaço Mountain Range

The Espinhaço Mountain Range is a mountain system that spans 1200 km of Brazilian territory and is located between the states of Bahia and Minas Gerais. It is mostly composed of Proterozoic quartzite rocks (2.5 billion to 541 million years ago) (Silveira et al., 2016; Hasui et al., 2012). The southern portion of the Espinhaço Mountain Range is located in the state of Minas Gerais, in the ecotone between two biomes considered global hotspots for biodiversity conservation, the Atlantic Forest and the Cerrado, and is also classified as a Biosphere Reserve by UNESCO in 2005 (Silveira et al., 2016; Myers et al., 2000). The predominant vegetation in this region is rocky field characterized by herbaceous-shrub formations associated with litholic soils and is considered a center of plant diversity and endemism (Cardoso et al., 2021; Rapini et al., 2008). A more detailed investigation of the taxonomic diversity will provide valuable information about the mycobiota present in the cave environment in the Southern Espinhaço Mountain Range, given the taxonomic potential for the discovery of new species and unexplored biotechnological resources.

1.2 The cave environment

Caves are natural underground cavities that are characterized by their stability, including constant temperature, high humidity, low availability of nutrients, absence of light in deeper parts and little photosynthetic activity (Joshi and Chettri, 2019; Poulson and White, 1969). Because organic carbon is scarce in these environments, the organic matter that enters the system is typically of allochthonous origin, meaning it originates from outside the cave. Carbon can enter cave environments through watercourses that flow into the cave, water percolating through fissures and fractures in the rock, air currents that transport particulate matter, as well as the activity of cave-dwelling animals such as bats, invertebrates, and humans (Gabriel and Northup, 2013; Taylor et al., 2013; Barton and Jurado, 2007). Chemoautotrophy also plays a critical role in maintaining these ecosystems by fixing carbon and releasing energy, with bacteria being the primary actors in this process (Wu et al., 2015; Gabriel and Northup, 2013). Additionally, fungi are also members of the cave microbiota.

Fungi are one of the most diverse groups of organisms within the Eukarya domain. These organisms play a crucial role in the underground ecosystem by contributing to rock and

mineral bioweathering, decomposing organic matter, transforming nitrogen and phosphorus, degrading recalcitrant compounds, and serving as a food source for cave fauna (Wiseschart and Pootanakit, 2020; Zhang et al., 2021; Joshi and Chettri, 2019; Nováková, 2009).

1.3 Fungal diversity in cave environments

It is estimated that there are approximately 2.2 to 3.8 million species of fungi on the planet, and only about 120,000 species, or approximately 8%, are formally described (Hawksworth and Lücking, 2017). One reason for the low knowledge of fungal diversity is that habitats located in tropical regions are still poorly studied (Hawksworth and Lücking, 2017). Another important factor is that the diversity of these organisms is higher in the tropics compared to temperate regions (TEDERSOO et al., 2014). Therefore, studies that include little-explored habitats in tropical regions, such as caves, are extremely important to fill existing gaps in the global inventory of fungal species.

Ascomycota is the most representative phylum among fungi described in caves, with *Aspergillus* and *Penicillium* being the most frequently found genera (Zhang et al., 2021; Zhang et al., 2017; Vanderwolf et al., 2013). Recently, 13 species of the *Penicillium* genus were reported in caves in Canada, including the description of a new species, *P. speluncae* (Visagie et al., 2020). Zhang et al. (2021) identified 73 species of *Penicillium* and 35 species of *Aspergillus*, as well as describing three new species, *A. limoniformis*, *A. phialiformis* and *A. phialosimplex*. *Aspergillus* and *Penicillium* were also abundant in air samples in caves in Brazil (Cunha et al., 2020; Taylor et al., 2013), Spain (Docampo et al., 2011; Docampo et al., 2010), and Slovakia (Nováková, 2009). The presence of *Aspergillus* spores can be an indicator of air quality within a cave, as some species are considered pathogenic or opportunistic, such as *A. fumigatus*, the most common etiological agent of pulmonary aspergillosis (Kousha et al., 2011).

Research on fungi in caves has been focused on *Histoplasma capsulatum* the causative agent of histoplasmosis in humans (Rocha-SILVA et al., 2014), and *Pseudogymnoascus destructans* the causative agent of white-nose syndrome in bats (Sharma et al., 2019). However, other studies have been carried out with the intention of exploring the taxonomic diversity of fungi in subterranean environments and have contributed to a better understanding of the real fungal biodiversity in these places. Vanderwolf et al. (2013) provided a list of 1029 species of fungi divided into 518 genera that have been documented in subterranean environments worldwide.

Since then, new studies have been conducted to provide more information about fungal diversity in the cave ecosystem. Jiang et al. (2017) described three new species of fungi from the genus *Cephalotrichum* among 510 isolates obtained from a limestone cave in China. Zhang et al. (2020) and Zhang et al. (2017) identified 856 species and described 53 new fungal species isolated from thirteen limestone caves in southwest China. These results demonstrate the diversity of fungi present in caves and the enormous potential for identifying new species previously unknown.

1.4 Speleomycology in Brazil

The study of fungi in caves (speleomycology) is still in its early stages in Brazil. Castrillón et al. (1976) reported on fungi in cave environments in the country for the first time, isolating fungi from the genera *Aspergillus*, *Penicillium*, *Verticillium*, *Cephalosporium*, *Fusarium*, *Geotrichum*, and the dermatophyte fungus *Arthroderma amazonicum* in caves in the Amazon region. Due to the use of caves for human activities, such as ecotourism and religious practices, some studies have focused on evaluating air quality inside caves, with a particular interest in identifying pathogenic fungal species. Taylor et al. (2013) found a large number of fungal species, including several species of the genus *Aspergillus*, after the most intense period of visitation to the Gruta Lapa Nova in Minas Gerais. In addition, various studies have reported the presence of *H. capsulatum* in Brazilian caves (Almeida et al., 2019; Rocha-Silva et al., 2014; Dias et al., 2011; Zancopé-Oliveira et al, 2005). During an expedition to the Gruta do Tamboril in Minas Gerais, eight researchers were infected after inhaling *H. capsulatum* spores from bat guano present in the cave (Rocha-Silva et al., 2014).

On the other hand, other studies have sought to explore the biotechnological potential of cave mycobiota. Souza et al. (2016) isolated cave fungi capable of producing pigments such as oosporin, dihydrotrichodimerol, and orvaectano, which have several properties with potential applications in the industry. In addition, fungi isolated from Brazilian caves have demonstrated the ability to produce antioxidant substances (Tavares et al., 2018); compounds that inhibit the protease, phospholipase, and haemolytic and thrombolytic activities of snake venom (Cardoso et al., 2019); cellulose degradation enzymes (De Paula et al., 2019; De Paula et al., 2016); and the enzyme tannase (Melo et al., 2013). Additionally, an isolate of *A. flavus* exhibited resistance to the antifungal drug amphotericin B, possibly related to competition for resources with other organisms present in the cave (Taylor et al., 2017).

1.5 Taxonomical studies of the cave fungal biota in Brazil

Cunha et al. (2020) found a great fungal diversity associated with air, guano, and bats samples in Meu Rei Cave in the Caatinga biome in Pernambuco. In total, 59 taxa belonging to the phyla Ascomycota (50), Basiodiomycota (8), and Mucoromycota (1) were identified through sequencing the ITS (internal transcribed spacer) region of rDNA (Cunha et al., 2020). The genus *Aspergillus* presented the highest number of taxa (12), followed by *Penicillium* (5), *Cladosporium* (3), and *Talaromyces* (3) (Cunha et al., 2020). A high diversity of fungi was isolated from bats' oral cavity, fur, and wing membrane, showing the importance of these organisms as agents of dispersal of fungi from the epigean environment to the caves (Cunha et al., 2020; Ogórek et al., 2020; Vanderwolf et al., 2013). Other studies have explored the mycobiota associated with caves in the Caatinga biome. In the Furna do Morcego bat cave, two new species and six reports of known species belonging to the genus *Cladosporium* were found (Pereira et al., 2022). Carvalho et al. (2022) found remarkable fungal diversity associated with obligate ectoparasitic bat flies in the same cave. They described two new species belonging to the order Pleosporales, and the genus *Aspergillus* was found to be the most abundant in that cave. Alves et al. (2022) investigated the fungal richness present in the Abrigo do Letreiro cave, also located in the Caatinga biome. They found 41 species belonging to 17 genera of Ascomycota and two of Basidiomycota in samples of air and sediment. Additionally, they described one new genus and six new species of ascomycetous fungi inhabiting that cave.

Taxonomical studies of cave fungi in Minas Gerais are scarce and none have focused on the study of fungi belonging to the family Chaetomiaceae. In this study, we aim at describing new fungal taxa belonging to the family Chaetomiaceae found in three caves in the Southern Espinhaço Mountain Range in Minas Gerais, Brazil.

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2 SINGLE CHAPTER

Shedding light on the fungal darkness: a new genus and four new species in the family *Chaetomiaceae* from Brazilian neotropical caves revealed by multi-gene phylogenetic analyses

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Shedding light on the fungal darkness: a new genus and four new species in the family *Chaetomiaceae* from Brazilian neotropical caves revealed by multi-gene phylogenetic analyses

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ABSTRACT

Interest in cave fungal diversity is flourishing because it may represent a reservoir of new species and metabolites. However, the mycobiota remains poorly studied in the underground environment, especially in Neotropical regions. During surveys that aimed to investigate the fungal diversity in quartzite and karst caves in the Southern Espinhaço Mountain in Brazil, six *Chaetomiaceae* isolates were obtained from different cave substrates. Five taxonomical novelties of *Chaetomiaceae* in Brazilian caves were discovered based on phylogenetic analyses using DNA sequences from ITS, LSU, *TUB*, and *RPB2* genes. *Chaetomium meridionalense*, *Pseudohumicola alba* and *Pseudohumicola lutea* are new species found in Gruta da Extração and Gruta Velha Nova caves. *Parahumicola* is introduced as a new genus representing a novel phylogenetic lineage with unique morphological characteristics in the family *Chaetomiaceae*. This new monotypic genus is typified by *P. guana,* which was found in a bat guano sample in the Gruta Monte Cristo cave. Furthermore, this is the first report of *Collariella bostrychodes* in a neotropical cave. Overall, these findings emphasize that Brazilian caves constitute an untapped source of fungal resources.

Keywords 5 new taxa · Biospeleology · *Chaetomium* · *Parahumicola* · *Pseudohumicola* · *Sordariales*

INTRODUCTION

Caves are subsurface natural cavities with unique characteristics, such as constant temperature, high humidity, low nutrient availability, lack of light in deeper parts, and, subsequently, little photosynthetic activity (Poulson and White 1969; Joshi and Chettri 2019). The underground environment is highly influenced by the epigean environment. Water streams, water percolating cracks and fractures of rocks, air currents, plants, and activities of humans and other animals, including bats, are external vectors that play an important role in the cave ecosystem (Barton and Jurado 2007; Gabriel and Northup 2013; Taylor et al. 2013; Wu et al. 2015).

Fungi are abundant in the cave environment (Cunha et al. 2020; Zhang et al. 2021). They can play important roles in the subsurface ecosystem, contributing to the weathering of rocks and minerals, organic matter decaying, nitrogenous and phosphorus cycling and degradation of recalcitrant compounds, besides serving as food to the cave fauna (Nováková et al. 2018; Joshi and Chettri 2019; Wiseschart and Pootanakit 2020; Zhang et al. 2021). Approximately 2,000 fungal species have been reported in the underground environment worldwide, mainly from genera *Aspergillus* and *Penicillium*, which were the most abundant (Vanderwolf et al. 2013; Zhang et al. 2021). Other fungi, including *Chaetomiaceae* (e.g., *Arcopilus*, *Botryotrichum*, *Chaetomium,* *Collariella*, *Dichotomopilus*, *Humicola,* *Staphylotrichum*, *Trichocladium* and others) were isolated from cave environments (Vanderwolf et al. 2013; Zhang et al. 2021). Two species in this family, *Collariella quadrum* and *Staphylotrichum limonisporum* were discovered in karst caves in China (Zhang et al. 2017b).

The taxonomy of *Chaetomiaceae* has been disputed for a long time. Species delimitation has usually focused on the observation of sexual structures, such as asci, ascospores, the presence of germ pores on ascospores, type of ascomatal hairs and ascomatal wall (von Arx et al. 1984, 1986). Nowadays, *Chaetomiaceae* taxonomic studies are based on a polyphasic approach, using morphological characteristics and multi-gene phylogenetic analyses of DNA sequences of the rDNA internal transcribed spacer (ITS), 28S large subunit (LSU), partial β-tubulin (*TUB*) and second largest subunit of RNA polymerase II (*RPB2*). This trend resulted in various perithecia-producing species, originally placed in *Chaetomium*, are now classified in different genera, such as *Amesia*, *Arcopilus*, *Botryotrichum*, *Collariella*, *Dichotomopilus*, *Humicola*, *Ovatospora*, *Thermochaetoides*, *Trichocladium*, *Xanthiomyces* and others (Wang et

al. 2016a, 2019b, a, 2022). Currently, the family *Chaetomiaceae* includes 50 genera and 275 species (Wang et al. 2022).

Members of the *Chaetomiaceae* are ubiquitous. Most species are saprobes and can occur in a broad range of habitats, such as soil, air, compost, animal dung, plants tissues, seeds and indoor environments (Rodríguez et al. 2002; Wang et al. 2016a, b; Zhang et al. 2017a, b, 2021; Sousa et al. 2020). They can also be found as endophytes (Noumeur et al. 2020; Mehrabi et al. 2020) or as opportunistic pathogens of humans (Ryan et al. 2021). Some members of the *Chaetomiaceae* are also known as remarkable secondary metabolite producers with antimicrobial, antioxidant, anticancer or cytotoxic properties (Wang et al. 2017; Jiang et al. 2021; Promgool et al. 2021; Tavares et al. 2022). They have the potential to be used in several applications, such as biomass degradation for biofuel production (Wanmolee et al. 2016), biological control of diseases (Pothiraj et al. 2021), or as biofertilizers (Lang et al. 2012).

The number of studies describing the fungal diversity in Brazilian caves is growing. Cunha et al. (2020) found high fungal diversity in air, guano and bat samples in the Meu Rei bat cave. Pereira et al. (2022) proposed two new *Cladosporium* species and Carvalho et al. (2022) encountered two new fungal species in ectoparasitic bat flies in the Furna do Morcego bat cave, located at the Caatinga dry forest. In addition, one genus and six new species of fungi were found in the Abrigo do Letreiro cave (Alves et al. 2022). However, the fungal diversity in neotropical caves from the Southern Espinhaço Mountain Range, Brazil, is still unknown.

Several studies carried out in Brazilian caves only used morphological characteristics or low-resolution genes, such as ITS, for fungal identification. This hampers a more accurate species identification of the cave mycobiota, especially in the *Chaetomiaceae*, which includes several species complex accommodating cryptic species. Thus, phylogenetic studies using DNA sequences from different loci can help to solve this problem. This work is part of several surveys that aim at describing the fungal diversity in caves of the Southern Espinhaço Mountain Range, Brazil. Here, we focused on *Chaetomiaceae* isolates found in three caves. Five taxonomical novelties and a new report in the neotropics in the *Chaetomiaceae* are described based on multi-gene molecular phylogeny and morphological analyses.

MATERIAL AND METHODS

Sampling

The Southern Espinhaço Mountain Range is located in the central region of Minas Gerais state, in Brazil. The annual mean temperature ranges from 18 to 19 °C and the annual rainfall is 1250–1550 mm (Verdi et al. 2015). Three caves were included in this study: Gruta da Extração (18°16'54.4"S 43°30'56.7"W), Gruta Monte Cristo (18°17'48.3"S 43°33'39.4"W) and Gruta Velha Nova (18°16'27"S 44°6'18"W) (Fig. 1). Gruta da Extração and Gruta Monte Cristo are quartzite caves located in the Diamantina municipality, while Gruta Velha Nova is a limestone cave in the Monjolos municipality.

Airborne fungi, sediment, rocks and leaf litter were collected in the three caves, in September 2019. Airborne fungi were sampled by using the Koch sedimentation method (Borda et al. 2004; Kuzmina et al. 2012). Three sediment samples were collected at a shallow depth of 1–5 cm at each sampling site, after surface layer removal. Fragments of rocks were collected at different sites if obviously showing fungal colonisation. Leaf litter was collected, when available, at the cave entrances. Additionally, one sample of bat guano was collected on the floor of the Gruta Monte Cristo. The samples were stored at room temperature, before being taken to the Laboratório de Micologia e Etiologia de Doenças Fúngicas de Plantas, Universidade Federal de Viçosa, Minas Gerais, Brazil. The sampling was authorized by the Ministério do Meio Ambiente (MMA)/Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) (SISBIO number 70978-1).

Fungal isolation

The serial dilution method was used for the fungal isolation of rocks and sediment samples (Zhang et al. 2015, 2017b). Briefly, 1 g of each sample was suspended in 9 mL of sterile water. The suspensions were vigorously homogenised using a vortex, and serial dilutions of different concentrations were obtained. Two hundred microliters of each dilution were spread onto plates of Dichloran rose Bengal agar (DRBC) and potato dextrose agar (PDA) containing chloramphenicol with three replicates. Rock samples were processed as described by Ruibal et al. (2005), with some modifications. Leaf litter samples were washed in tap water, according to Ruiz et al. (2005). Thereafter, the samples were incubated in a

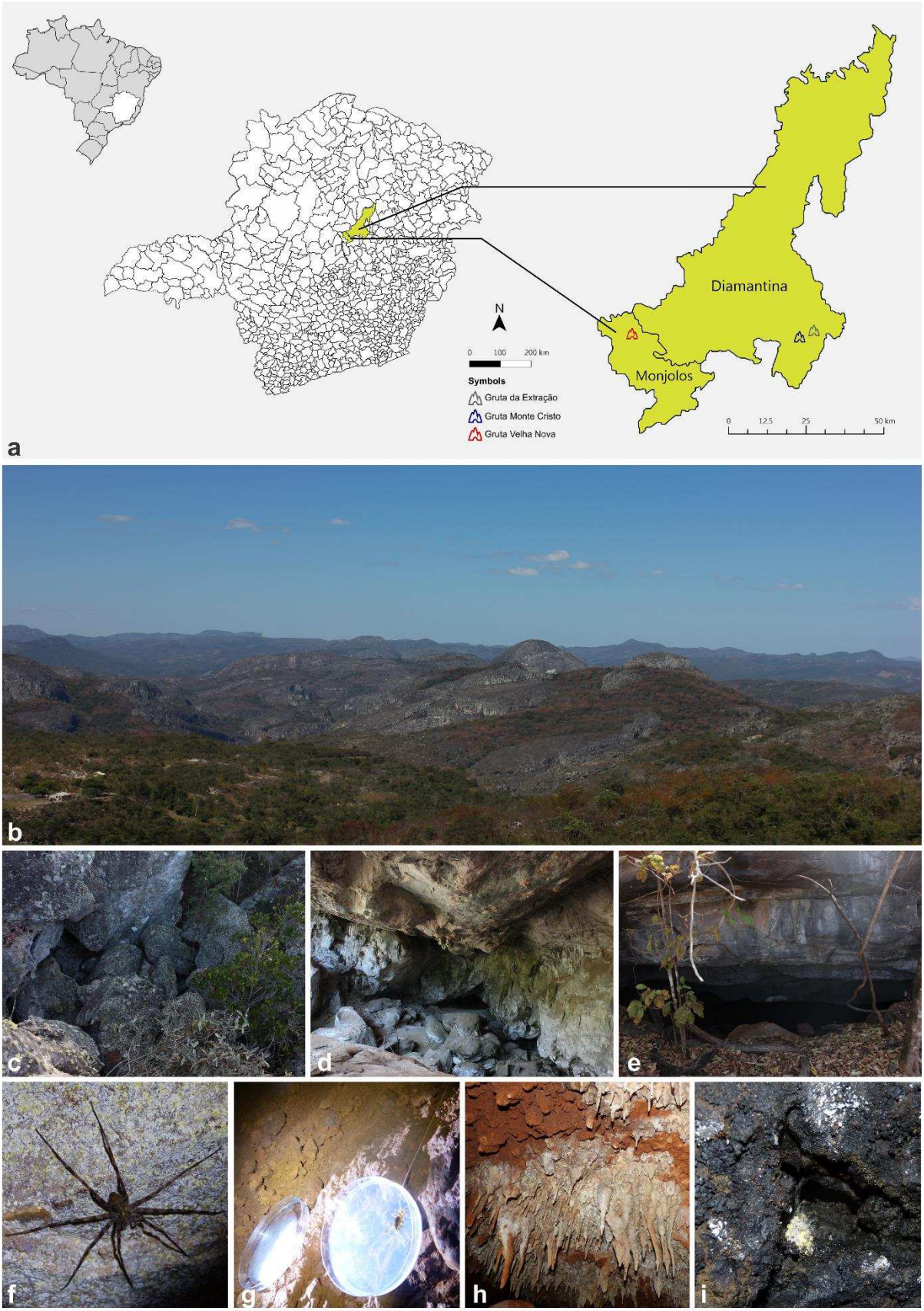

Fig. 1 Caves sampled in the Southern Espinhaço Mountain Range, Minas Gerais state, Brazil. **a** Geographical location of the caves sampled. **b** View of landscape from one of the sampled caves. **c, d, e** Entrances to the Gruta da Extração, Gruta Monte Cristo and Gruta Velha Nova caves, respectively. **f** A **s**pider in the Gruta da Extração cave. **g** A troglobiont insect feeding from a potato-dextrose-agar plate in the Gruta Velha Nova cave. **h** Stalactites. **i** Bat guano

moist chamber, and fungal reproductive structures were transferred to new PDA dishes daily, for 30 days. Sporulating fungi in the bat guano sample were directly transferred to fresh PDA plates under a stereoscope microscope. All plates (airborne fungi, bat guano, leaf litter, rock, and soil), were incubated at 25 °C, in the dark, for approximately one month. Periodically, single colonies were transferred onto new PDA plates. Pure cultures were obtained using the hyphal tip method (Tuite 1969). All fungal isolates were stored in 10 % glycerol at -80 °C. Living cultures representing ex-type strains or representative isolates were deposited in the "Coleção Octávio Almeida Drummond" (COAD) culture collection. Metabolically inactive cultures representing the holotype of new species were deposited in the Herbarium VIC. Both collections are located at the Universidade Federal de Viçosa.

DNA isolation, PCR and phylogenetic analyses

Total genomic DNA extractions were performed by using biomass harvested from PDA colonies incubated from 7–10 days at 25 °C, in the dark, using the Wizard® Genomic Purification Kit (Promega) protocol, with some modifications described by Pinho et al. (2013). PCR amplifications were carried out using the following primers: ITS5 (White et al. 1990) and LR6 (Vilgalys and Hester 1990) for the internal transcribed spacer 1 and 2 regions and intervening 5.8S rDNA region (ITS) and nuclear 28S rDNA region (LSU); fRPB2-5F2 (Sung et al. 2007) and fRPB2-7cR (Liu et al. 1999) or RPB2AM-1bf and RPB2AM-7R (Miller and Huhndorf 2005) for the RNA polymerase second largest subunit (*RPB2*); T1 (O'Donnell and Cigelnik 1997) and Bt2b (Glass and Donaldson 1995) or Tub4RD (Groenewald et al. 2013) for the partial beta-tubulin gene region (*TUB*). PCR conditions were set as follows: initial denaturation step at 94 °C for 2 min; followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 30 s, elongation at 72 °C for 30 s, and a final elongation step at 72 °C for 2 min. Sequencing of amplicons was performed at Macrogen Inc. (South Korea). Consensus sequences for each set of primers were assembled using DNA Baser Sequence Assembly software (Heracle BioSoft).

Sequence datasets were constructed using newly generated sequences in this work and representative sequences of species within *Chaetomiaceae* generated in previous studies (Online Resource 1). Sequence alignments were performed using MAFFT v.7 (Katoh and Standley 2013) and manually optimised with MEGA v7 (Kumar et al. 2016). Individual alignments for each locus were constructed. Also, combined concatenated alignments using ITS, LSU, *RPB2* and *TUB* were constructed using Sequence Matrix 1.8 (Vaidya et al. 2011).

Phylogenetic analyses were performed using Bayesian inference (BI) and maximum likelihood (ML). BI analyses were performed using MrBayes 3.2.7 (Ronquist et al. 2012) within the CIPRES Science Gateway Portal (Miller et al. 2010). The best nucleotide substitution model was calculated using MrModelTest 2.3 (Nylander 2004), according to Akaike Information Criterion (AIC). Two Markov Chain Monte Carlo (MCMC) samplings were run simultaneously for 10×10^6 generations, sampled every 1,000 generations, resulting in 20,000 trees. The first 4 000 trees (25 %) were discarded in the burn-in phase, and the remaining 16 000 trees were utilised for calculating posterior probabilities (pp). ML analyses were run using IQTREE v. 2.2.0 (Minh et al. 2020) with 1,000 bootstrap (bs) replicates performed using ultrafast bootstrapping (Hoang et al. 2018), and the best nucleotide substitution model for each region was calculated using ModelFinder (Kalyaanamoorthy et al. 2017) according to the corrected Akaike Information Criterion (AICc). The resulting trees were visualized in FigTree v. 1.4.3 (Rambaut 2018) and exported to graphics programs. The ML trees were used with bs and pp shown for branches to present phylogenetic results.

Morphological studies

Macroscopic and microscopic characteristics of fungal isolates identified as *Chaetomiaceae* were recorded. Colony morphology characteristics, *i.e.*, colony diameter, colour, texture and soluble pigment production, were recorded using four different media: cornmeal agar (CMA), malt extract agar (MEA), oatmeal agar (OA) and potato carrot agar (PCA) (Crous et al. 2009; Samson et al. 2010). Petri dishes of 90 mm were inoculated in three equidistant points and incubated for 7 days at 25 °C in the dark. Colour names used in the descriptions follow Rayner (1970). Plates were placed in a lightbox and photographs were taken using a Nikon D3400 camera. Asexual spores were described from slide cultures as described by Riddell (1950). In addition, slides were prepared using the inclined coverslip method (Kawato and Shinobu 1959; Nugent et al. 2006). Microscopic slides of sexual structures were mounted under a dissecting microscope using a fine needle to transfer ascomata into the mounting medium. The ascomata were gently squashed to crack and release asci and ascospores. All structures were mounted in lactoglycerol or Shear's solution. Microphotographs were taken 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. Microscopic descriptions were recorded using OA and sometimes CMA or PCA.

RESULTS

Isolates

Six strains belonging to the *Chaetomiaceae* were isolated from the three caves studied in the Southern Espinhaço Mountain Range, Brazil. After morphological and multi-gene phylogenetic analyses, one isolate (COAD 3129) was identified as *Collariella bostrychodes.* The other five strains were found to represent taxonomical novelties in the *Chaetomiaceae*. Two isolates (COAD 3451 and COAD 3122) belonged to *Chaetomium*, two others (COAD 3126 and COAD 3127) to *Pseudohumicola*, and one isolate (COAD 3110) could not be assigned to any known genus in the family.

Phylogenetic analyses

Table 1 presents the alignment length of individual and concatenated analyses and the best nucleotide substitution model for BI and ML analyses used for each DNA region.

Table 1. Number of alignment positions and best nucleotide substitution models selected for Bayesian inference (BI) and maximum-likelihood (ML) analyses.

	***Chaetomiaceae* phylogeny**			***Chaetomium* phylogeny**			***Pseudohumicola* phylogeny**		
		Best model			**Best model**			**Best model**	
Partition	**Length**	**BI**	**ML**	**Length**	**BI**	**ML**	**Length**	**BI**	**ML**
ITS	708	GTR+I+G	GTR+F+I+G4	534	GTR+I+G	TNe+R2	543	GTR+I+G	TIM2e+R2
LSU	570	GTR+I+G	TIM3e+R3	565	GTR+I+G	TNe+R2	532	GTR+I	TIM+F+R2
TUB	1081	GTR+I+G	TPM2+F+I+G4	726	HKY+I+G	TN+F+I+G4	852	GTR+I+G	GTR+F+I+G4
RPB2	531	GTR+I+G	TIM+F+I+G4	598	GTR+I+G	TN+F+I+G4	798	GTR+I+G	TPM2+F+I+G4
concatenated	2890			2423			2725		

The five strains in the *Chaetomiaceae* were identified using phylogenetic analyses of the individual *RPB2* and *TUB2* genes (Online resource 2, 3, 4 and 5) and the concatenated dataset (Figs. 1, 2 and 3). Isolate COAD 3129 was resolved in the *Collariella bostrychodes* clade (Fig. 2). This isolate was found in a leaf litter sample in the Gruta Velha Nova cave. Isolates COAD 3451 and COAD 3122 clustered next to *Chaetomium globosum* and *C. microthecia* and monophyletic relationship of the three species was strongly supported (bs = 100 and pp = 1) (Figs. 2,3). Both were isolated from leaf litter samples, the former was found in the Gruta da Extração cave, the latter in the Gruta Velha Nova cave. Isolates COAD 3126

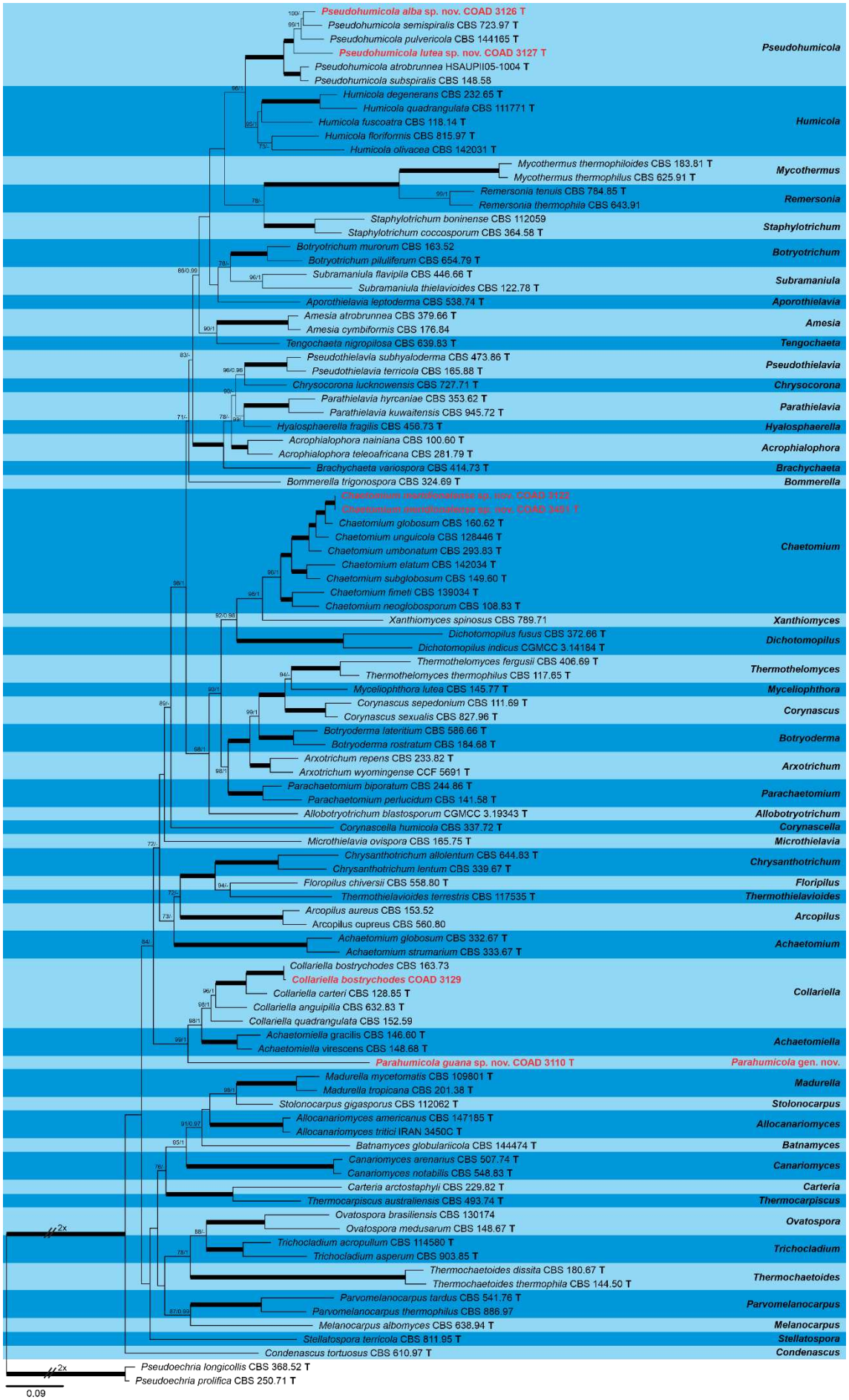
Pseudohumicola alba sp. nov. COAD 3126 T
Pseudohumicola semispiralis CBS 723.97 T
Pseudohumicola pulvericola CBS 144165 T
Pseudohumicola lutea sp. nov. COAD 3127 T
Pseudohumicola atrobrunnea HSAUPII05-1004 T
Pseudohumicola subspiralis CBS 148.58
Pseudohumicola
Humicola degenerans CBS 232.65 T
Humicola quadrangulata CBS 111771 T
Humicola fuscoatra CBS 118.14 T
Humicola floriformis CBS 815.97 T
Humicola olivacea CBS 142031 T
Humicola
Mycothermus thermophiloides CBS 183.81 T
Mycothermus thermophilus CBS 625.91 T
Mycothermus
Remersonia tenuis CBS 784.85 T
Remersonia thermophila CBS 643.91
Remersonia
Staphylotrichum boninense CBS 112059
Staphylotrichum coccosporum CBS 364.58 T
Staphylotrichum
Botryotrichum murorum CBS 163.52
Botryotrichum piluliferum CBS 654.79 T
Botryotrichum
Subramaniula flavipila CBS 446.66 T
Subramaniula thielavioides CBS 122.78 T
Subramaniula
Aporothielavia leptoderma CBS 538.74 T
Aporothielavia
Amesia atrobrunnea CBS 379.66 T
Amesia cymbiformis CBS 176.84
Amesia
Tengochaeta nigropilosa CBS 639.83 T
Tengochaeta
Pseudothielavia subhyaloderma CBS 473.86 T
Pseudothielavia terricola CBS 165.88 T
Pseudothielavia
Chrysocorona lucknowensis CBS 727.71 T
Chrysocorona
Parathielavia hyrcaniae CBS 353.62 T
Parathielavia kuwaitensis CBS 945.72 T
Parathielavia
Hyalosphaerella fragilis CBS 456.73 T
Hyalosphaerella
Acrophialophora nainiana CBS 100.60 T
Acrophialophora teleoafricana CBS 281.79 T
Acrophialophora
Brachychaeta variospora CBS 414.73 T
Brachychaeta
Bommerella trigonospora CBS 324.69 T
Bommerella
Chaetomium mengfeiense sp. nov. COAD 3122
Chaetomium mengfeiense sp. nov. COAD 3451 T
Chaetomium globosum CBS 160.62 T
Chaetomium unguicola CBS 128446 T
Chaetomium umbonatum CBS 293.83 T
Chaetomium elatum CBS 142034 T
Chaetomium subglobosum CBS 149.60 T
Chaetomium fimeti CBS 139034 T
Chaetomium neoglobosporum CBS 108.83 T
Chaetomium
Xanthiomyces spinosus CBS 789.71
Xanthiomyces
Dichotomopilus fusus CBS 372.66 T
Dichotomopilus indicus CGMCC 3.14184 T
Dichotomopilus
Thermothelomyces fergusii CBS 406.69 T
Thermothelomyces thermophilus CBS 117.65 T
Thermothelomyces
Myceliophthora lutea CBS 145.77 T
Myceliophthora
Corynascus sepedonium CBS 111.69 T
Corynascus sexualis CBS 827.96 T
Corynascus
Botryoderma lateritium CBS 586.66 T
Botryoderma rostratum CBS 184.68 T
Botryoderma
Arxotrichum repens CBS 233.82 T
Arxotrichum wyomingense CCF 5691 T
Arxotrichum
Parachaetomium biporatum CBS 244.86 T
Parachaetomium perlucidum CBS 141.58 T
Parachaetomium
Allobotryotrichum blastosporum CGMCC 3.19343 T
Allobotryotrichum
Corynascella humicola CBS 337.72 T
Corynascella
Microthielavia ovispora CBS 165.75 T
Microthielavia
Chrysanthotrichum allolentum CBS 644.83 T
Chrysanthotrichum lentum CBS 339.67 T
Chrysanthotrichum
Floropilus chiversii CBS 558.80 T
Floropilus
Thermothielavioides terrestris CBS 117535 T
Thermothielavioides
Arcopilus aureus CBS 153.52
Arcopilus cupreus CBS 560.80
Arcopilus
Achaetomium globosum CBS 332.67 T
Achaetomium strumarium CBS 333.67 T
Achaetomium
Collariella bostrychodes CBS 163.73
Collariella bostrychodes COAD 3129
Collariella carteri CBS 128.85 T
Collariella anguipilia CBS 632.83 T
Collariella quadrangulata CBS 152.59
Collariella
Achaetomiella gracilis CBS 146.60 T
Achaetomiella virescens CBS 148.68 T
Achaetomiella
Parahumicola guana sp. nov. COAD 3110 T
Parahumicola gen. nov.
Madurella mycetomatis CBS 109801 T
Madurella tropicana CBS 201.38 T
Madurella
Stolonocarpus gigasporus CBS 112062 T
Stolonocarpus
Allocanariomyces americanus CBS 147185 T
Allocanariomyces tritici IRAN 3450C T
Allocanariomyces
Batnamyces globulariicola CBS 144474 T
Batnamyces
Canariomyces arenarius CBS 507.74 T
Canariomyces notabilis CBS 548.83 T
Canariomyces
Carteria arctostaphyli CBS 229.82 T
Carteria
Thermocarpiscus australiensis CBS 493.74 T
Thermocarpiscus
Ovatospora brasiliensis CBS 130174
Ovatospora medusarum CBS 148.67 T
Ovatospora
Trichocladium acropullum CBS 114580 T
Trichocladium asperum CBS 903.85 T
Trichocladium
Thermochaetoides dissita CBS 180.67 T
Thermochaetoides thermophila CBS 144.50 T
Thermochaetoides
Parvomelanocarpus tardus CBS 541.76 T
Parvomelanocarpus thermophilus CBS 886.97
Parvomelanocarpus
Melanocarpus albomyces CBS 638.94 T
Melanocarpus
Stellatospora terricola CBS 811.95 T
Stellatospora
Condenascus tortuosus CBS 610.97 T
Condenascus
Pseudoechria longicollis CBS 368.52 T
Pseudoechria prolifica CBS 250.71 T
2x
2x
0.09

Fig. 2 Maximum-likelihood tree of *Chaetomiaceae* based on a concatenated dataset of ITS, LSU, *RPB2* and *TUB* gene regions. Isolates found in this study are shown in bold red. Ex-type isolates are marked with ''**T**''. Only bootstrap (bs) values ≥ 70% and posterior probabilities (pp) ≥ 0.95 are shown at branches (''-'' means no statistical support). The branches that presented full statistical support (bs = 100% and pp = 1) are thickened. The tree is rooted with *Pseudoechria longicollis* CBS 368.52 and *Pseudoechria prolifica* CBS 250.71 (*Schizotheciaceae*)

and 3127 clustered into the *Pseudohumicola* clade (Figs. 2,4). Both isolates clustered separately and represent two new species in this genus. Isolate COAD 3126 is sister to *P. semispiralis* with high statistical support (bs = 99 and pp = 0.95). In addition, when comparing DNA sequences of *RPB2* and *TUB* gene regions, our putative new species and *P. semispiralis* exhibited per site mutations of 796/830, no gaps (identity = 96 %) for *RPB2*, and 626/669, with 10 gaps (identity = 93.6 %) for *TUB*. They can be morphologically differentiated, as described in the taxonomy section. The isolate COAD 3127 forms a basal clade with isolate COAD 3126, *P. pulvericola*, and *P. semispiralis* (bs = 94 and pp = 1). Both *Pseudohumicola* isolates were encountered in the Gruta Velha Nova cave and isolated from PDA plates exposed to the air of the cave. Isolate COAD 3110 could not be assigned to any recognized genus in the *Chaetomiaceae* when the *RPB2* and *TUB* genes were individually analysed (data not shown), besides using the concatenated dataset (Fig. 2). Thus, we propose the inclusion of a new genus to accommodate this isolate. The isolate COAD 3110 was found in a bat guano sample collected in the Gruta Monte Cristo cave.

TAXONOMY

Chaetomium Kunze, Mykol. Hefte 1: 16. 1817.

Notes: *Chaetomium sensu stricto* is the largest genus in the *Chaetomiaceae* and species are characterized by its globose, ellipsoidal to ovate or obovate and ostiolate ascomata. The ascomatal wall is composed of *textura intricata* or *epidermoidea*, with few species of *textura angularis*. Ascomatal hairs are diverse, but most species have verrucose surfaces. Asci are clavate or fusiform, bearing 8 ascospores, biseriate or irregularly arranged. Ascospores are limoniform to globose and bilaterally flattened. Acremonium-like conidiophores can be found only in a few species (Wang et al. 2016b).

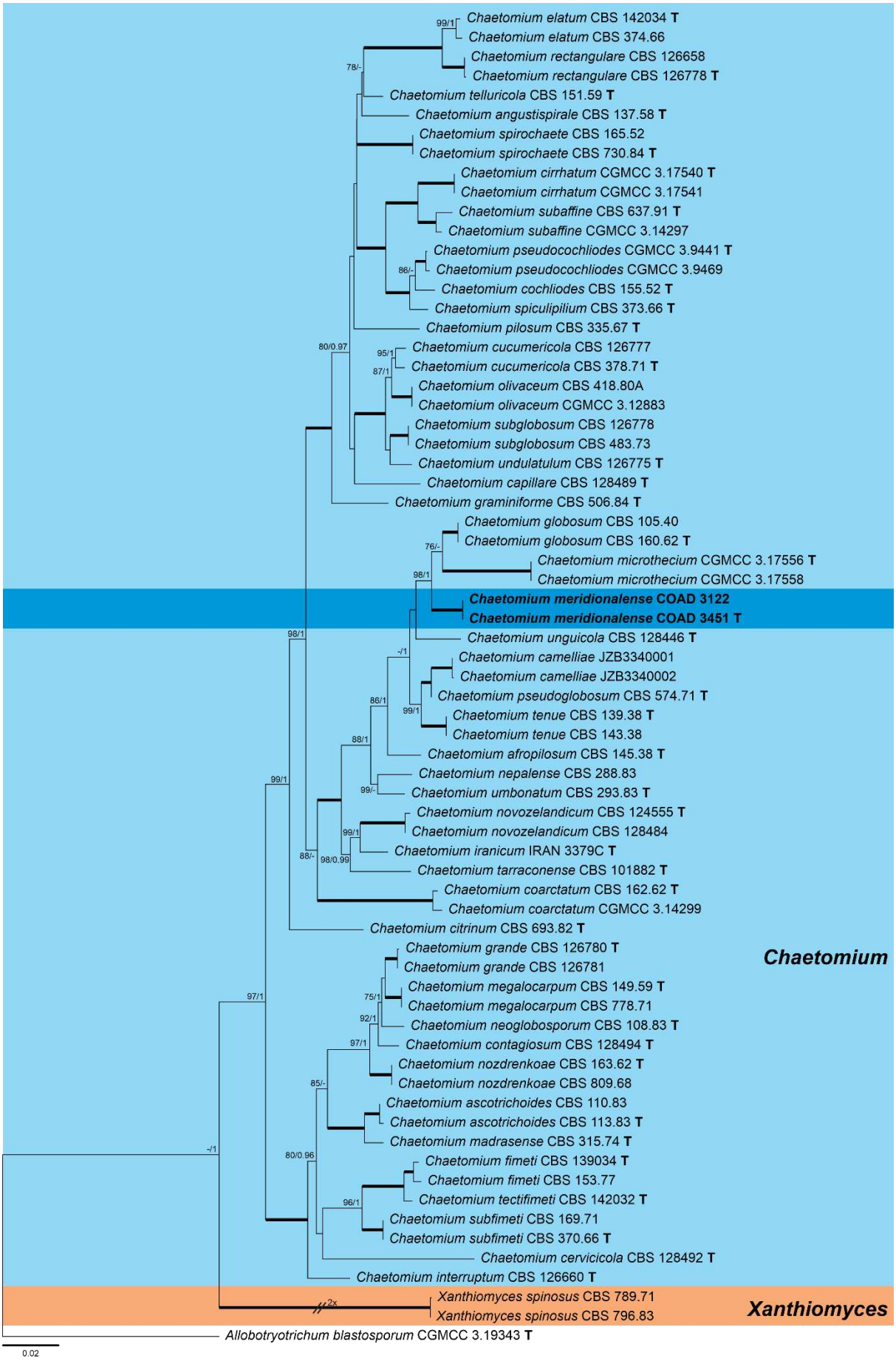
Chaetomium elatum CBS 142034 T
Chaetomium elatum CBS 374.66
Chaetomium rectangulare CBS 126658
Chaetomium rectangulare CBS 126778 T
Chaetomium telluricola CBS 151.59 T
Chaetomium angustispirale CBS 137.58 T
Chaetomium spirochaete CBS 165.52
Chaetomium spirochaete CBS 730.84 T
Chaetomium cirrhatum CGMCC 3.17540 T
Chaetomium cirrhatum CGMCC 3.17541
Chaetomium subaffine CBS 637.91 T
Chaetomium subaffine CGMCC 3.14297
Chaetomium pseudocochliodes CGMCC 3.9441 T
Chaetomium pseudocochliodes CGMCC 3.9469
Chaetomium cochliodes CBS 155.52 T
Chaetomium spiculipilium CBS 373.66 T
Chaetomium pilosum CBS 335.67 T
Chaetomium cucumericola CBS 126777
Chaetomium cucumericola CBS 378.71 T
Chaetomium olivaceum CBS 418.80A
Chaetomium olivaceum CGMCC 3.12883
Chaetomium subglobosum CBS 126778
Chaetomium subglobosum CBS 483.73
Chaetomium undulatulum CBS 126775 T
Chaetomium capillare CBS 128489 T
Chaetomium graminiforme CBS 506.84 T
Chaetomium globosum CBS 105.40
Chaetomium globosum CBS 160.62 T
Chaetomium microthecium CGMCC 3.17556 T
Chaetomium microthecium CGMCC 3.17558
Chaetomium meridionalense COAD 3122
Chaetomium meridionalense COAD 3451 T
Chaetomium unguicola CBS 128446 T
Chaetomium camelliae JZB3340001
Chaetomium camelliae JZB3340002
Chaetomium pseudoglobosum CBS 574.71 T
Chaetomium tenue CBS 139.38 T
Chaetomium tenue CBS 143.38
Chaetomium afropilosum CBS 145.38 T
Chaetomium nepalense CBS 288.83
Chaetomium umbonatum CBS 293.83 T
Chaetomium novozelandicum CBS 124555 T
Chaetomium novozelandicum CBS 128484
Chaetomium iranicum IRAN 3379C T
Chaetomium tarraconense CBS 101882 T
Chaetomium coarctatum CBS 162.62 T
Chaetomium coarctatum CGMCC 3.14299
Chaetomium citrinum CBS 693.82 T
Chaetomium grande CBS 126780 T
Chaetomium grande CBS 126781
Chaetomium megalocarpum CBS 149.59 T
Chaetomium megalocarpum CBS 778.71
Chaetomium neoglobosporum CBS 108.83 T
Chaetomium contagiosum CBS 128494 T
Chaetomium nozdrenkoae CBS 163.62 T
Chaetomium nozdrenkoae CBS 809.68
Chaetomium ascotrichoides CBS 110.83
Chaetomium ascotrichoides CBS 113.83 T
Chaetomium madrasense CBS 315.74 T
Chaetomium fimeti CBS 139034 T
Chaetomium fimeti CBS 153.77
Chaetomium tectifimeti CBS 142032 T
Chaetomium subfimeti CBS 169.71
Chaetomium subfimeti CBS 370.66 T
Chaetomium cervicicola CBS 128492 T
Chaetomium interruptum CBS 126660 T
Xanthiomyces spinosus CBS 789.71
Xanthiomyces spinosus CBS 796.83
Allobotryotrichum blastosporum CGMCC 3.19343 T
Chaetomium
Xanthiomyces
0.02

Fig. 3 Maximum-likelihood tree of *Chaetomium* and *Xanthiomyces* based on a concatenated dataset of ITS, LSU, *RPB2* and *TUB* gene regions. Isolates of the new species found in this study are shown in bold. Ex-type isolates are marked with ‘‘**T**’’. Only bootstrap (bs) values ≥ 70% and posterior probabilities (pp) ≥ 0.95 are shown at branches (‘‘-’’ means no statistical support). The branches that presented full statistical support (bs = 100% and pp = 1) are thickened. The tree is rooted with *Allobotryotrichum blastosporum* CGMCC 3.1943

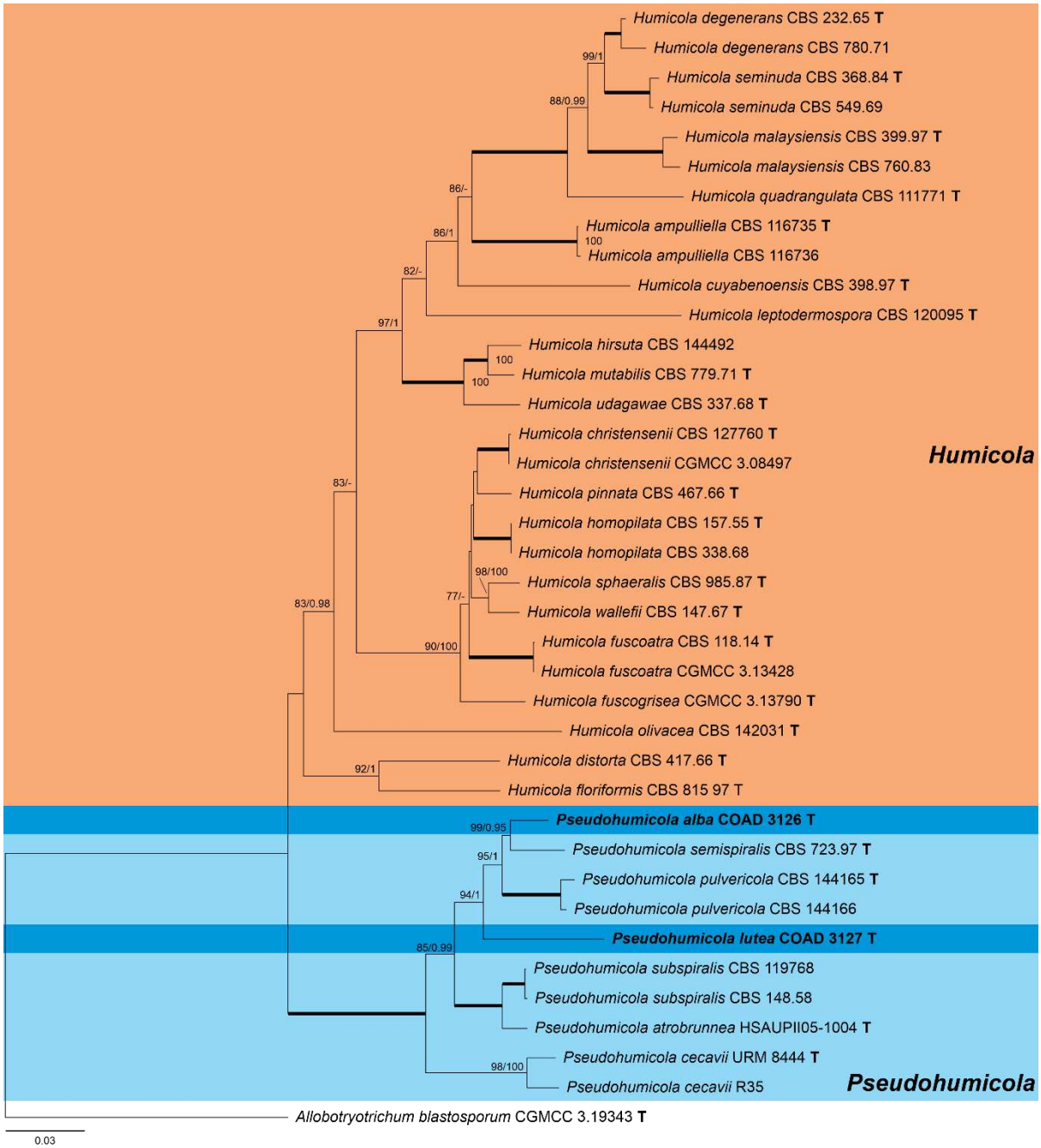

Fig. 4 Maximum-likelihood tree of *Pseudohumicola* and *Humicola* based on a concatenated dataset of ITS, LSU, *RPB2* and *TUB* gene regions. Isolates of the new species found in this study are shown in bold. Ex-type isolates are marked with ‘‘**T**’’. Only bootstrap (bs) values ≥ 70% and posterior probabilities (pp) ≥ 0.95 are shown at branches (‘‘-’’ means no statistical support). The branches that presented full statistical support (bs = 100% and pp = 1) are thickened. The tree is rooted with *Allobotryotrichum blastosporum* CGMCC 3.1943

Chaetomium meridionalense T.O. Condé, A.F. Leão & O.L. Pereira, **sp. nov.** Figure 5. Mycobank –.

Etymology: The epithet refers to the Portuguese name of the Mountain Range, which is "Serra do Espinhaço Meridional".

Type: **Brazil**, Minas Gerais state, Diamantina municipality, Gruta da Extração cave, isolated from leaf litter, Jul. 2019, collected by O.L. Pereira, isolated by T.O. Condé (holotype VIC 47581, ex-type culture COAD 3451).

Description: *Ascomata* superficial, ostiolate, olivaceous buff to greenish olivaceous in reflected light, subglobose or ellipsoidal, 259–477 µm high × 190–344 µm diam, homothallic. *Ascomatal wall* surface brown with *textura intricata* and *epidermoidea*. *Terminal hairs* dark brown, verrucose, undulate, becoming thinner at the tips, erect or flexuous near the base, 2.6–4.4 µm wide. *Lateral hairs* erect or slightly flexuous, 2.5–4.4 µm wide. *Asci* fasciculate and clavate, with eight biseriate ascospores, spore-bearing part 24–35 × 10–16 µm, stalks 14–43 µm, evanescent. *Ascospores* fawn to brown when mature, smooth, limoniform, bilaterally flattened, one apical germ pore, (8.8–)10–10.6(–11.3) × (6.5–)8–8.6(–9.3) × (4.5–)5.7–6.3(–6.8) µm. *Asexual morph* unknown.

Culture characteristics (7 d at 25 °C in the dark): Colonies on OA over 70 mm diam, spreading rapidly; edge entire; aerial hyphae white and sparse when young, then olivaceous grey, greenish olivaceous and vinaceous grey owing to the aggregation of ascomata and exudates; reverse iron grey. Colonies on CMA attaining 55–61 mm diam; edge irregular, fimbriate; aerial hyphae white to buff, sparse, olivaceous grey owing to ascomata formation at the centre of the colony; reverse buff to honey; soluble pigment absent. Colonies on MEA attaining 51–58 mm diam; edge entire or slightly crenate; aerial hyphae white and sparse when young, becoming more or less thick at the centre, little ascomata production; reverse ochreous to fulvous; soluble pigment absent. Colonies on PCA attaining 50–60 mm diam; edge lobate; translucent and sparse aerial hyphae, becoming grey olivaceous due to intense ascomata aggregation; reverse buff to grey olivaceous; soluble pigment absent.

Other materials examined: **Brazil**, Minas Gerais state, Monjolos municipality, Gruta Velha Nova cave, isolated from leaf litter, Sep. 2019, collected by O.L. Pereira and isolated by A.F. Leão, T.O. Condé, and Y.L.G. Dutra (living culture COAD 3122).

Notes: *Chaetomium meridionalense* is phylogenetically related to *C. globosum* and *C. microthecia.* The ascomata of *C. meridionalense* (259–477 × 190–344 µm) are bigger than that of *C. globosum* CBS 160.62 (160–300 × 135–250 µm) and *C. microthecia* CGMCC 3.17556

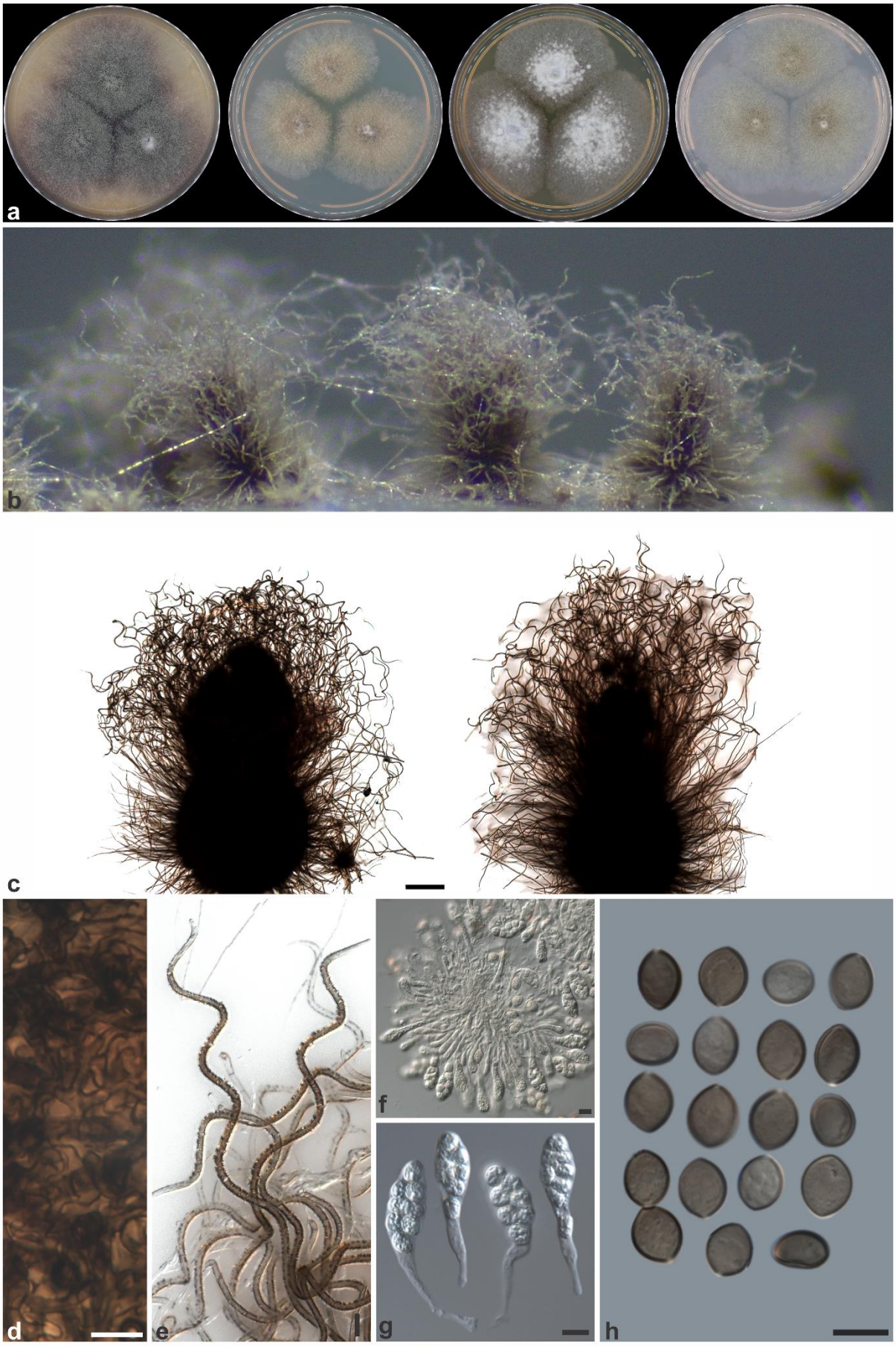
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Fig. 5 *Chaetomium meridionalense* COAD 3451 **a** Colonies on OA, CMA, MEA and PCA from left to right after 7 days at 25 °C in the dark. **b** Side view of ascomata on CMA. **c** Ascomata under a light microscope. **d** Surface of ascoma wall. **e** Upper part of ascomatal hairs. **f, g** Asci with ascospores. **h** Ascospores. Scale bars: c = 100 μm; d–h = 10 μm

(120–200 × 100–160 μm). *Chaetomium meridionalense* has an ascomatal wall consisting of *textura intricata* and *epidermoidea* and paler and slightly larger ascospores. *Chaetomium meridionalense* produces vinaceous grey exudates on OA, while *C. globosum* CBS 160.62 produces luteous to orange exudates on the same medium. Ascospores of *C. meridionalense* resemble pale and larger ascospores of *C. globosum* var. *flavoviride* MUCL 39526 and CBS 145.61. Based on multi-gene phylogenetic analyses, *C. meridionalense* represents a new and well-supported species in the genus *Chaetomium*.

Collariella bostrychodes (Zopf) X. Wei Wang & Samson, Stud. Mycol. 84:179. 2016.
Basionym: *Chaetomium bostrychodes* Zopf, Abh. Bot. Ver. Prov. Brandenburg 19:173. 1877.
Description and illustration: Wang et al. (2016a).
Material examined: **Brazil**, Minas Gerais state, Monjolos municipality, Velha Nova cave, isolated from leaf litter collected near the cave entrance, Sep. 2019, collected by O.L. Pereira and isolated by A.F. Leão, T.O. Condé and Y. L. G. Dutra (living culture COAD 3129).
Notes: *Collariella bostrychodes* was originally named *Chaetomium bostrychodes* until Wang et al. (2016a) established *Collariella*. *Collariella* is monophyletic and characterized by ascomata, with a darkened collar around the ostiolar pore, and limoniform to quadrangular ascospores, bilaterally flattened, with an apical germ pore. *Collariella bostrychodes* isolates have been reported in caves in Israel (Vanderwolf et al. 2013) and China (Zhang et al. 2017b, 2021). To the best of our knowledge, this is the first report of this species in a neotropical cave.

Pseudohumicola X.Wei Wang, P.J. Han, F.Y. Bai & Houbraken, Stud. Mycol. 101: 96. 2022.
Notes: Based on molecular dating evidence, Wang et al. (2022) segregated the genus *Humicola* into two clades in which *Pseudohumicola* has emerged. *Pseudohumicola* is characterized by the presence of aleurioconidia-like conidia which are pigmented, thick-walled, arising laterally, intercalary or terminally on conidiogenous cells without differentiation from hyphal cell (Wang et al. 2022). Acremonium-like phialides and hyaline conidia and ascomata with coiled hairs are present in some species.

Pseudohumicola alba T.O. Condé, A.F. Leão & O.L. Pereira, **sp. nov.** Figure 6.

Mycobank –.

Etymology: The name refers to the white colonies produced by this species.

Type: **Brazil**, Minas Gerais state, Monjolos municipality, Velha Nova cave, isolated from PDA plate exposed to the air of the cave, Sep. 2019, collected by A.F. Leão & T.O. Condé and isolated by A.F. Leão, T.O. Condé & Y.L.G. Dutra (holotype VIC 47582, ex-type culture COAD 3126).

Description: *Somatic hyphae* hyaline. *Aleurioconidia-like* conidia produced laterally or intercalary on hyphae, sometimes on short branches of terminal hyphae, globose to subglobose and obovoid, with a truncated base, single-celled, thick-walled, olivaceous to brown, (8.3–)9.6–10.4(–12.6) × (7–)8.8–9.6(–10.4) µm. *Acremonium-like conidiophores* arising laterally from hyphae, unbranched, aseptate or sometimes septate, conidiogenous cells phialidic, (10.6–)12.5–15.5(–21.3) µm long, (1.7–)2.2–2.6(–3.2) µm wide near the base of phialides. *Conidia* phialidic, hyaline, born in basipetal chains or forming false heads, aseptate, obovoid to truncate, sometimes cylindrical, (2.7–)3.1–3.3(–4) × (1.2–)1.5–1.7(–2) µm. *Sexual morph* absent.

Culture characteristics (7 d at 25 °C in the dark): Colonies on OA reaching 31–37 mm diam; edge entire, translucent; aerial mycelium floccose, thick, white; reverse vinaceous buff; soluble pigment absent. Colonies on CMA reaching 34–37 mm diam; reverse entire, translucent; aerial mycelium floccose, thick, white; reverse buff, grey olivaceous to olivaceous; soluble pigment absent. Colonies on MEA reaching 27–33 mm diam; edge entire, translucent; aerial mycelium floccose, thick, white; reverse pale luteous and cinnamon; soluble pigment absent. Colonies on reaching PCA 35–40 mm diam; edge entire, translucent; aerial mycelium floccose, thick, buff to white; reverse rosy buff and grey olivaceous; soluble pigment absent.

Notes: *Pseudohumicola alba* produced both aleurioconidia-like conidia and acremonium-like synanamorph in slide cultures. This species is phylogenetically closer to *P. semispiralis*. Conidia of both species differ in shape (globose to subglobose and sometimes obovoid, with a truncated base *vs.* globose to oblate and sometimes ovoid) and colour (olivaceous to brown *vs.* hyaline, subhyaline to olivaceous). In addition, *P. alba* COAD 3126 produced an acremonium-like synanamorph, which is absent in *P. semispiralis*. The absence of ascomata in *P. alba* is also a characteristic that differs from *P. semispiralis*. *Pseudohumicola alba* COAD 3126 produced a thick aerial mycelium on all culture media tested, without producing soluble pigments, while *P. semispiralis* produces a thick aerial mycelium only on MEA with apricot to sienna exudates and ochreous to umber pigments on OA and CMA (Wang et al. 2019b). Colonies of *P. alba* also exhibited slower growth in OA, CMA and MEA, compared to *P.*

semispiralis. Colonies of *P. alba* resemble *P. pulvericola* by producing thick aerial mycelium on all media tested. Both species produce aleurioconidia-like conidia and acremonium-like morphs. However, aleurioconidia-like conidia of *P. alba* are olivaceous to brown, while *P. pulvericola* produces olivaceous to dark brown conidia. *Pseudohumicola pulvericola* forms a basal clade with *P. alba* in the multi-gene phylogeny.

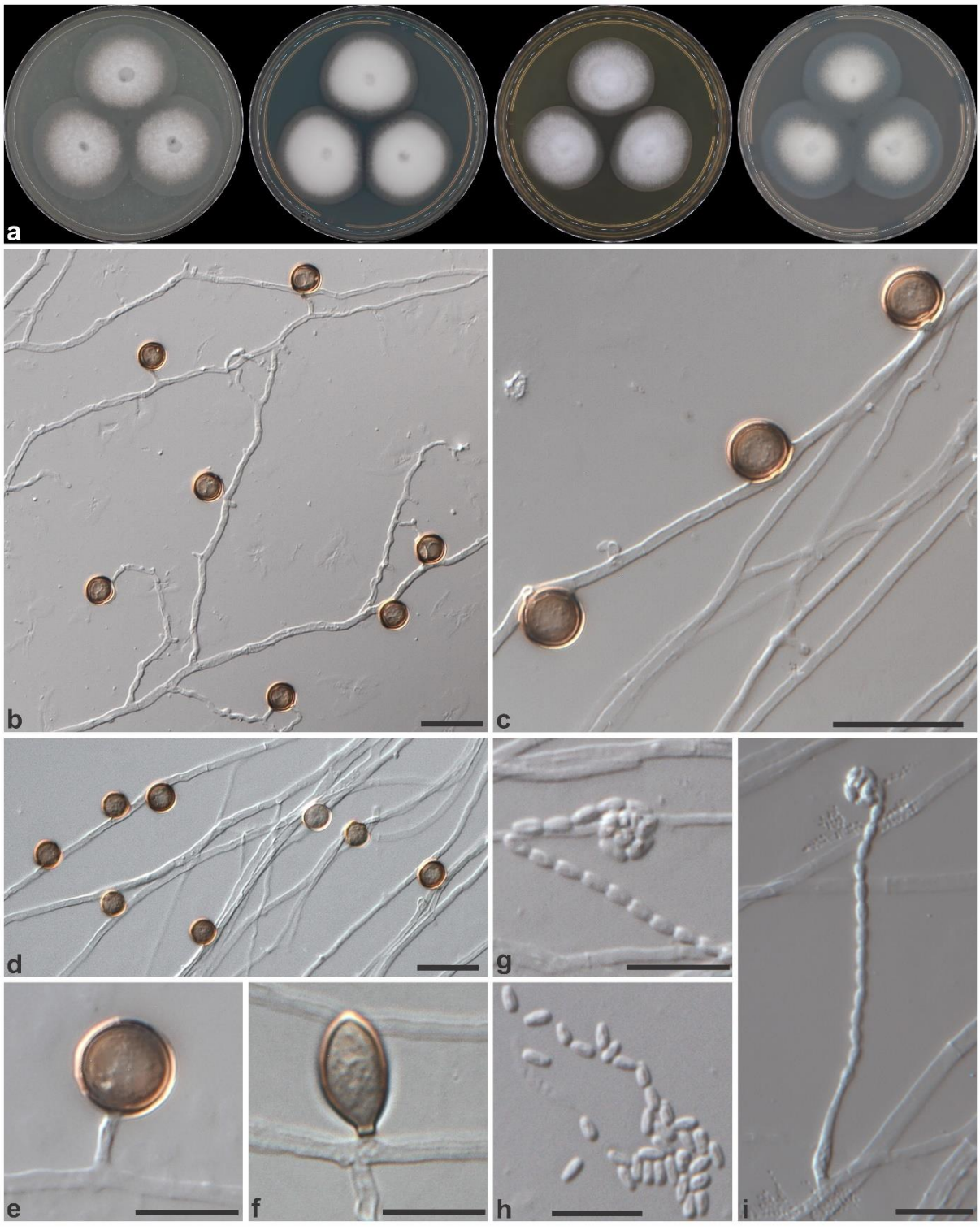

Fig. 6 *Pseudohumicola alba* COAD 3126. **a** Colonies on OA, CMA, MEA and PCA from left to right after 7 days at 25 °C in the dark. **b, c, d** Aleurioconidia-like conidia and hyphae. **e, f** Aleurioconidia-like conidia. **g, h** Hyaline conidia. **i** Acremonium-like conidiophore and hyaline conidia. Scale bars: b–d = 20 µm; e–i = 10 µm

Pseudohumicola lutea T.O. Condé, A.F. Leão & O.L. Pereira, **sp. nov.** Figure 7.

Mycobank –.

Etymology: The epithet refers to the luteous exudate produced by this species.

Type: **Brazil**, Minas Gerais state, Monjolos municipality, Velha Nova cave, isolated from PDA plate exposed to the air of the cave, Sep. 2019, collected by A.F. Leão & T.O. Condé and isolated by A.F. Leão, T.O. Condé & Y.L.G. Dutra (holotype VIC 47583, ex-type culture COAD 3127).

Description: *Somatic hyphae* hyaline. *Aleurioconidia-like conidia* produced laterally or intercalary on hyphae, sometimes on short branches of terminal hyphae, solitary or in clusters of two or more, globose to subglobose and occasionally cylindrical or pyriform, single-celled, vinaceous buff to olivaceous, with a brown thick wall when mature, (7.5–)10.8–11.8(–14.5) × (7.2–)10.4–11.2(–12) µm. *Acremonium*-like conidiophores or phialidic conidia not found. *Asexual morph* absent.

Culture characteristics (7 d at 25 °C in the dark): Colonies on OA attaining 38–42 mm diam; edge entire; aerial hyphae sparse, white, becoming amber to luteous due to exudate production, aleurioconidia-like conidia formed sparsely; reverse luteous to pale luteous. Colonies on CMA attaining 32–35 mm diam; edge entire; texture floccose, aerial mycelium white at the edge, becoming olivaceous to pale olivaceous grey due to intense aleurioconidia-like conidia production; reverse greenish olivaceous to olivaceous black and buff edge; soluble pigment absent. Colonies on MEA attaining 20–24 mm diam; edge crenate, translucent; texture membranous, olivaceous to greenish olivaceous with cottony white or buff mycelium at the centre; reverse olivaceous to grey olivaceous; soluble pigment absent. Colonies on PCA attaining 38–42 mm diam; edge entire, translucent; filamentous mycelium abundant, white, reaching the top of the cover plate, citrine soluble pigment around the edge of the colony; reverse citrine to pale luteous.

Notes: *Pseudohumicola lutea* COAD 3127 formed a basal clade to *P. alba*, *P. pulvericola* and *P. semispiralis*. Isolate COAD 3127 only produced aleurioconidia-like conidia. *Pseudohumicola lutea* is morphologically similar to *P. atrobrunnea* by its olivaceous conidia, with a thick and brown wall occasionally born in chain or clusters of 2 or 3. Based on multi-gene phylogenetic analyses, it was found that *P. lutea* and *P. atrobrunnea* grouped into distinct lineages. Moreover, *P. lutea* is the only species in the genus that produces amber to luteous

exudates on OA, slowly growing and membranous olivaceous to greenish olivaceous colonies on MEA and filamentous aerial mycelium with citrine exudates on PCA.

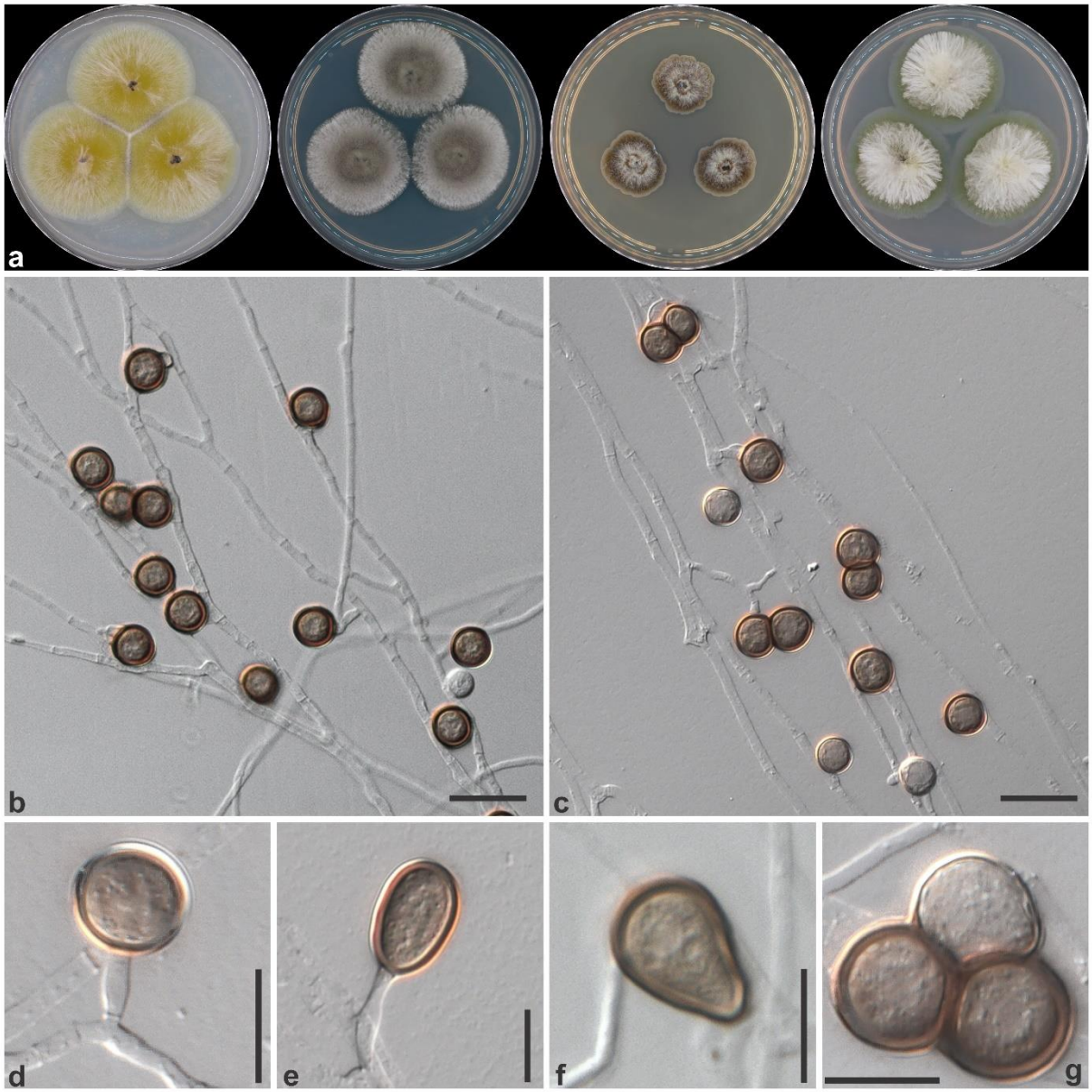

Fig. 7 *Pseudohumicola lutea* COAD 3127. **a** Colonies on OA, CMA, MEA and PCA from left to right after 7 days at 25 °C in the dark. **b, c** Aleurioconidia-like conidia and hyphae. **d, e, f, g** Aleurioconidia-like conidia. Scale bars: b–c = 20 µm; d–g = 10 µm

Parahumicola T.O. Condé, Y.L.G. Dutra & O.L. Pereira, **gen. nov.**

Mycobank –.

Etymology: The name refers to the morphological similarity to humicola-like conidia produced by the type species of this genus.

Type species: *Parahumicola guana* T.O. Condé, Y. L. G. Dutra & O.L. Pereira.

Notes: The novel genus *Parahumicola* produces aleurioconidia, as defined by Hambleton et al. (2005), that resemble humicola-like conidia but are considerably bigger. Aleurioconidia of *Parahumicola* are born in somewhat differentiated conidiophores, have a truncated base, prominent apex and a conspicuous germ pore. Ascomata are unknown. The colonies of *Parahumicola* are olivaceous grey, as recorded in all the tested culture media. According to the phylogenetic analyses using four loci, *Parahumicola* is related to the genera *Achaetomiella* and *Collariella*.

Parahumicola guana T.O. Condé, Y.L.G. Dutra & O.L. Pereira **sp. nov.** Figure 8.

Mycobank –.

Etymology: The epithet name refers to the substrate from which this fungus was isolated.

Type: **Brazil**, Minas Gerais state, Diamantina municipality, Gruta Monte Cristo cave, isolated from bat guano found on the floor of the cave, Sep. 2019, collected by T.O. Condé and isolated by Y. L. G. Dutra (holotype VIC 47486, ex-type culture COAD 3110).

Description: *Somatic hyphae* hyaline. *Conidiophores* hyaline, born laterally from hyphae, unbranched or occasionally branched, cylindrical, tapering toward the base or clavate, aseptate or septate, 6.9–24.8 µm long, 3.8–5.7 µm wide near top. *Aleurioconidia* single celled, solitary or in clusters of two or three, globose, subglobose or ellipsoidal, with a truncated base and sometimes with a protruding apex, single-celled, thick-walled, with a conspicuous germ pore, olivaceous to brown or dark brown when mature, rhexolytic when seceding, (12.2–)16–19.4(–26.8) × (10.3–)12.3–14.3(–17.8) µm. *Sexual morph* not observed.

Culture characteristics (7 d at 25 °C in the dark): Colonies on OA reaching 37–42 mm diam; edge entire, translucent; without aerial mycelia, colonies translucent near the edge, fuscous or olivaceous grey at the centre owing to intense sporulation; reverse olivaceous grey; soluble pigment absent. Colonies on CMA reaching 50–52 mm diam; entire, translucent; without aerial mycelia, colonies translucent near the edge, olivaceous buff at the outer region, olivaceous grey or iron grey at the centre due to intense aleurioconidia-like conidia production; reverse olivaceous grey; soluble pigment absent. Colonies on MEA reaching 51–52 mm diam; edge entire, translucent; sparse white aerial mycelia at the centre of the colony, translucent near the edge, olivaceous buff at the outer region, olivaceous grey or iron grey at the centre due to intense sporulation; reverse iron grey; soluble pigment absent. Colonies on PCA reaching 41–43 diam mm; edge entire, translucent; without aerial mycelia, colony translucent near the edge, olivaceous or olivaceous grey at the centre of the colony due to aleurioconidia production; reverse olivaceous grey; soluble pigment absent.

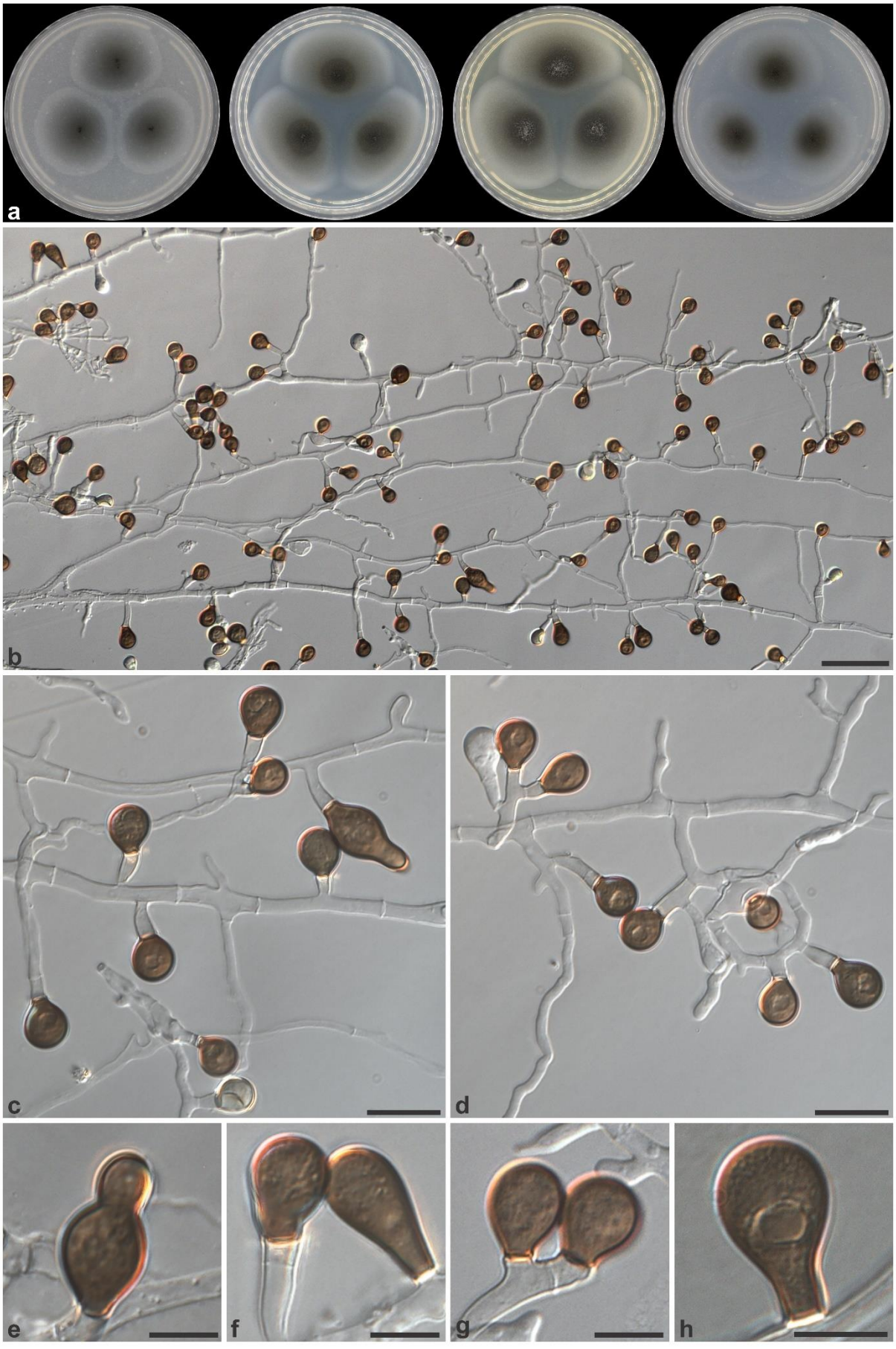
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Fig. 8 *Parahumicola guana* COAD 3110. **a** Colonies on OA, CMA, MEA and PCA from left to right after 7 days at 25 °C in the dark. **b, c, d** Aleurioconidia and hyphae. **e, f, g, h** Aleurioconidia. Scale bars: b = 50 µm; c–d = 20 µm; e–h = 10 µm

Notes: *Parahumicola guana* COAD 3110 produces aleurioconidia resembling those formed by *Humicola* in respect to pigmentation and shape. However, the conidia of *P. guana* are considerably larger (12.2–)16–19.4(–26.8) × (10.3–)12.3–14.3(–17.8) µm compared to those measured by Wang et al. (2019b), and have a truncated base, protruding apex and a conspicuous germ pore. Moreover, aleurioconidia-like conidia of *Parahumicola* are born from conidiophores, unlike *Humicola*. These characteristics represent a unique type of conidia in the *Chaetomiaceae*.

DISCUSSION

In this study, a new genus and four new species isolated from Brazilian caves are proposed in the *Chaetomiaceae*. Based on morphology and multi-gene phylogenetic analyses using ITS, LSU, *RPB2* and *TUB* markers, we introduce *Chaetomium meridionalense*, *Pseudohumicola alba*, *Pseudohumicola lutea*, and *Parahumicola guana*. Moreover, *Collariella bostrychodes* is first reported from neotropical caves. The best results for delimiting species were obtained using *TUB* and *RPB2* gene regions. The rDNA regions ITS and LSU were not suitable for the identification of most species. This is in agreement with Wang et al. (2016b, 2022), who suggested *TUB* and *RPB2* as secondary DNA barcodes for the identification of species in the *Chaetomiaceae*.

According to Wang et al. (2016b), the genus *Chaetomium sensu stricto* can be subdivided into three phylogenetic distinct clades, which reflect the morphological differences between them. *Chaetomium meridionalense* is placed within group II corresponding to the *C. globosum* clade, which exhibits relatively small and limoniform ascospores and terminal ascomatal hairs with flexuous to undulate or slightly coiled shapes. *Chaetomium meridionalense* isolates COAD 3154 and COAD 3122 have shown distinct colony morphology on all growth media examined. They were found in two different caves with distinct lithologies. Isolate COAD 3154 was found in a quartzite cave and exhibited a larger production of exudates and ascomata than COAD 3122, which was isolated in a limestone cave.

A recent study introduced *Pseudohumicola* to accommodate species phylogenetically distinct from *Humicola* (Wang et al. 2022). Based on DNA sequences and morphology, isolates COAD 3126 and COAD 3127 are proposed as two new species within this genus, *P. alba* and

P. lutea. Both species were phylogenetically distinct from other species in the genus. Also, morphological characteristics such as the colour and shape of conidia and colony characteristics on different culture media were informative to separate them from other species in the genus. Recent work from Alves et al. (2022) found *P. cecavii,* a new species of *Pseudohumicola*, isolated from air and sediment samples in the Abrigo do Letreiro cave, located in the Caatinga dry forest in Brazil. Thus, our study expands the number of extant *Pseudohumicola* species to seven, of which three were isolated from Brazilian caves (Alves et al. 2022; Wang et al. 2022).

The genus *Parahumicola* clustered in a phylogenetic lineage sharing a common ancestor with *Achaetomiella* and *Collariella*. *Achaetomiella* was introduced by von Arx (1970) and was later transferred to *Collariella.* Recently, Wang et al. (2022) based on molecular dating analyses, resurrected *Achaetomiella* as a sister genus to *Collariella*. *Collariella* is characterised by a darkened collar around the ostiolar pore of ascomata, while *Achaetomiella* species lack this feature. Interestingly, isolate COAD 3110 only produced conidia and failed to produce ascomata on all culture media examined. This is intriguing since the production of conidia by *Achaetomiella* and *Collariella* species is unknown. We speculate that isolate COAD 3110 may be heterothallic or exclusively able to reproduce sexually in nature. However, asexual sporulation only was also described for *Acrophialophora* (except a few species), *Botryoderma*, *Remersonia*, *Myceliophthora*, some *Humicola* and *Pseudohumicola* species (Wang et al. 2019b, a, 2022).

Several species of *Chaetomiaceae* have been isolated from cavernicolous substrates, such as cave air, walls, guano, sediment and wood, among others (Vanderwolf et al. 2013; Cunha et al. 2020; Zhang et al. 2021). The isolates found in this study were collected from different substrates in the three caves sampled. *Chaetomium meridionalense* and *Collariella bostrychodes* were isolated from leaf litter collected in the Gruta da Extração and Gruta Velha Nova caves. *Pseudohumicola alba* and *P. lutea* were isolated from PDA plates exposed to the Gruta Velha Nova cave air. *Parahumicola guana* was found in a guano sample collected on the floor of the Gruta Monte Cristo cave. *Chaetomiaceae* isolates were not detected in any of the rock and soil samples. These results corroborate that fungal community composition in caves can be affected by external vectors. Vectors can be plant debris transported by water or wind into caves, airflow transporting particulate material from the epigean environment and the activity of animals, such as bats, using caves as a shelter and depositing faeces on the cave floor (Ogorek et al. 2016; Zhang et al. 2017b; Cunha et al. 2020). According to Zhang et al. (2018), new species of fungi found in karst caves in China are unlikely troglobitic organisms, but rather travellers from the epigean environment. They have used molecular dating evidence and stated

that the geological age of caves is too short for fungal speciation. Therefore, the fungal species found in our study may have originated from the epigean environment.

We are aware that monotypic species might be a problem since the infraspecific variation is not adequately represented. However, the monotypic species proposed in this work are distinct from other known species by both morphology and multi-gene phylogenetic analyses. The description of these new taxa helps to increase knowledge about the recently proposed and still poorly known genus *Pseudohumicola* (Wang et al. 2022). Moreover, the description of the novel genus *Parahumicola* increases the diversity of conidia morphology known in *Chaetomiaceae*. New surveys in the caves where these species were found are needed to retrieve more isolates.

Caves are complex and fragile ecosystems possessing highly specialized fauna (Trajano 2000) and microbiota (Zhang et al. 2017b, 2021). Human activities inside caves, such as religious demonstrations and non-regulated ecotourism, may negatively impact their biodiversity (Taylor et al. 2013). Also, these pristine environments are threatened by the expansion of economic activities, such as mining, in areas where protected caves are already established (Abessa et al. 2019; Piló et al. 2022). The present study expands the record of fungi in the global cave ecosystem. Further surveys will allow us to identify the diversity linked to other fungal taxa in the Southern Espinhaço Range, Brazil.

Declarations

Ethics approval The sampling was authorized by the Ministério do Meio Ambiente (MMA)/Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) (SISBIO number 70978-1).

Consent to participate Not applicable.

Consent for publication Not applicable.

Availability of data and materials DNA sequences generated in this work are available at GenBank (https://www.ncbi.nlm.nih.gov/genbank/). DNA alignments are provided in the Electronic Supplementary Material.

Competing interests The authors declare no competing interests.

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Author's contribution Thiago Oliveira Condé performed sampling, laboratory work, data analyses and writing. Ana Flávia Leão, André Wilson Campos Rosado and Yan Lucas Gomes Dutra performed sampling, laboratory work and data analyses. Olinto Liparini Pereira, Soraya de Carvalho Neves and Lucio Mauro Soares Fraga performed sampling, data analyses and facilitated funding acquisition. Maria Catarina Megumi Kasuya and Olinto Liparini Pereira supervised and designed the research project. All authors reviewed the manuscript.

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APPENDIX

Online resource 1 List of species and sequences used in the phylogenetic analyses. Species and sequences obtained in this study are shown in bold.

Species	**Culture accession**	**Substrate/Host**	**Genbank accession number**			
			ITS	**LSU**	***TUB***	***RPB2***
Achaetomiella gracilis	CBS 146.60^{T}	Soil	KX976648	KX976743	KX976990	KX976842
Achaetomiella virescens	CBS 148.68^{T}	Agricultural soil	KX976654	KX976749	KX976996	KX976848
Achaetomium globosum	CBS 332.67^{T}	Rhizosphere	KX976570	KX976695	KX976911	KX976793
Achaetomium strumarium	CBS 333.67^{T}	Soil	AY681204	AY681170	AY681238	KC503254
Acrophialophora nainiana	CBS 100.60^{T}	Farm soil	MK926793	MK926793	MK926893	MK876755
Acrophialophora teleoafricana	CBS 281.79^{T}	Soil	MK926795	MK926795	MK926895	MK876757
Allobotryotrichum blastosporum	CGMCC 3.19343^{T}	Roots of *Saccharum officinarum*	MN215716	MN215554	MN329887	MN255397
Allocanariomyces americanus	CBS 147185^{T}	Human right hip subcutaneous tissue	MT902181	MT902391	MT904876	MT904877
Allocanariomyces tritici	IRAN 3450C^{T}	Seed endophyte of *Triticum boeoticum*	MT568839	MT568842	MT568850	MT568845
Amesia atrobrunnea	CBS 379.66^{T}	Mouldy mattress	JX280771	JX280666	KX976916	KX976798
Amesia cymbiformis	CBS 176.84	Tent rope	KX976577	KX976702	KX976919	KX976801
Aporothielavia. leptoderma	CBS 538.74^{T}	Soil	NR_16421	NG_067253	MZ343025	MZ342986
Arcopilus aureus	CBS 153.52	–	KX976582	KX976707	KX976924	KX976806
Arcopilus cupreus	CBS 560.80	Dung of moose	KX976584	KX976709	KX976926	KX976808
Arxotrichum repens	CBS 233.82^{T}	Soil	MK919282	MK919282	MK919396	MK919338
Arxotrichum wyomingense	CCF 5691^{T}	Soil	LT968153	LT968143	LT971393	–
Batnamyces globulariicola	CBS 144474^{T}	Roots of *Globularia alypum*	MT075917	MT075917	MT075919	MT075918
Bommerella trigonospora	CBS 324.69^{T}	Soil	–	MZ351419	MZ343022	MZ342984
Botryoderma lateritium	CBS 586.66^{T}	Soil mixed with leaf litter	MK919287	MK919287	MK919401	MK919343
Botryoderma rostratum	CBS 184.68^{T}	Sandy soil	MK919288	MK919288	MK919402	MK919344
Botryotrichum murorum	CBS 163.52	–	KX976591	KX976716	KX976933	KX976815
Botryotrichum piluliferum	CBS 654.79^{T}	Pastry	KX976597	KX976722	KX976939	KX976821
Brachychaeta variospora	CBS 414.73^{T}	Soil	MK926797	MK926797	MK926897	MK876759
Canariomyces arenarius	CBS 507.74^{T}	Desert soil	MK926798	MK926798	MK926898	KM655438
Canariomyces notabilis	CBS 548.83^{T}	Litter of *Phoenix canariensis*	MK926802	MK926802	MK926902	MK876763
Carteria arctostaphyli	CBS 229.82^{T}	*Arctostaphylos uva-ursi*	MK926807	MK926807	MK926907	MK876767
Chaetomium afropilosum	CBS 145.38^{T}	–	KT214574	KT214605	KT214751	KT214675

Species	Culture accession	Substrate/Host	Genbank accession number			
			ITS	LSU	*TUB*	*RPB2*
Chaetomium angustispirale	CBS 137.58^{T}	*Fraxinus* sp.	JN209862	JN209862	JN256141	KF001824
Chaetomium ascotrichoides	CBS 113.83^{T}	*Gossypium humitectum*	KC109752	KC109752	KC109770	KF001832
Chaetomium ascotrichoides	CBS 110.83	Soil	KC109753	KC109753	KC109771	KF001833
Chaetomium camelliae	JZB3340001^{T}	Leaf of *Camellia sinensis*	MT535751	MT535749	MT535533	MT535537
Chaetomium camelliae	JZB3340002	Leaf of *Camellia sinensis*	MT535752	MT535750	MT535534	MT535538
Chaetomium capillare	CBS 128489^{T}	Animal hair	KT214583	KT214614	KT214760	KT214686
Chaetomium cervicicola	CBS 128492^{T}	Neck of *Homo sapiens*	KT214558	KT214592	KT214735	KT214662
Chaetomium chirrhatum	CGMCC 3.17540^{T}	Decaying paper	KP336792	KP336841	KP336890	KT149508
Chaetomium chirrhatum	CGMCC 3.17541	Decaying paper	KP336793	KP336842	KP336891	KT149509
Chaetomium citrinum	CBS 693.82^{T}	Rice field soil	KT214587	KT214617	KT214764	KT214691
Chaetomium coarctatum	CBS 162.62^{T}	Seed of *Cappanula medium*	JN209863	JN209863	JN256142	KF001802
Chaetomium coarctatum	CGMCC 3.14299	Dead stem of unknown plant	JN209924	JN209924	JN256194	KF001804
Chaetomium cochliodes	CBS 155.52^{T}	Animal dung	KC109754	KC109754	KC109772	KF001811
Chaetomium cochliodes	CGMCC 3.9440	Tuber of *Panax notoginseng*	JN209866	JN209866	JN256145	KF001814
Chaetomium contagiosum	CBS 128494^{T}	Cornea of *Homo sapiens*	KT214555	KT214589	KT214732	KT214659
Chaetomium cucumericola	CBS 378.71^{T}	–	KT214579	KT214610	KT214756	KT214680
Chaetomium cucumericola	CBS 126777	Petiole of *Cucumis sativus*	HM365247	HM365247	KT214757	KT214681
Chaetomium elatum	CBS 142034^{T}	Cardboard	KX976612	KX976733	KX976954	KX976832
Chaetomium elatum	CBS 374.66	Decomposing leaf	KC109758	KC109758	KC109776	KF001820
Chaetomium fimeti	CBS 139034^{T}	Soil	KT214559	KT214593	KT214736	KT214663
Chaetomium fimeti	CBS 153.77	–	KT214561	KT214594	KT214738	KT214664
Chaetomium globosum	CBS 160.62^{T}	Compost	KT214565	KT214596	KT214742	KT214666
Chaetomium globosum	CBS 105.40	Mouldy book	KT214566	KT214597	KT214743	KT214667
Chaetomium globosum	CBS 147.60	Raincoat	JN209909	JN209909	JN256179	KF001793
Chaetomium graminiforme	CBS 506.84^{T}	*Acer* sp.	KT214584	KT214615	KT214761	KT214687
Chaetomium grande	CBS 126780^{T}	Leaf of *Triticum aestivum*	HM365253	HM365253	HM365273	KT214657
Chaetomium grande	CBS 126781	Straw of *Triticum aestivum*	KT214554	KT214588	KT214731	KT214658
Chaetomium interruptum	CBS 126660^{T}	Seed of *Triticum aestivum*	HM365246	HM365246	KT214741	KT214665

Species	Culture accession	Substrate/Host	Genbank accession number			
			ITS	LSU	*TUB*	*RPB2*
Chaetomium iranicum	IRAN 3379C^{T}	Insect	–	–	MN520421	MT273944
Chaetomium madrasense	CBS 315.74^{T}	Rhizosphere of *Pennisetum typhoides*	KC109751	KC109751	KC109769	KF001831
Chaetomium megalocarpum	CBS 149.59^{T}	Leaf of *Ficus carica*	KC109744	KC109744	KC109762	KF001828
Chaetomium megalocarpum	CBS 778.71	Humus-rich soil	KC109747	KC109747	KC109765	KF001827
Chaetomium meridionalense	**COAD 3451^{T}**	**Leaf litter from a cave**	**ON989660**	**ON979679**	**ON988187**	**OP131292**
Chaetomium meridionalense	**COAD 3122**	**Leaf litter from a cave**	**ON989661**	**ON979680**	**ON988188**	**OP131291**
Chaetomium microthecium	CGMCC 3.17556^{T}	Plant	KP336785	KP336834	KP336883	KT149505
Chaetomium microthecium	CGMCC 3.17558	Plant	KP336787	KP336836	KP336885	KT149507
Chaetomium neoglobosporum	CBS 108.83^{T}	Green leaf of *Triticum aestivum*	KC109750	KC109750	KC109768	KF001825
Chaetomium nepalense	CBS 288.83	Soil	MH861591	MH873316	–	MZ342983
Chaetomium novozelandicum	CBS 124555^{T}	Dead decaying twig	KT214576	KT214607	KT214753	KT214677
Chaetomium novozelandicum	CBS 128484	Scalp of *Homo sapiens*	KT214578	KT214609	KT214755	KT214679
Chaetomium nozdrenkoae	CBS 163.62^{T}	Soil	KT214556	KT214590	KT214733	KT214660
Chaetomium nozdrenkoae	CBS 809.68	Greenhouse soil	KT214557	KT214591	KT214734	KT214661
Chaetomium olivaceum	CBS 418.80A	Nilgai dung	JN209914	JN209914	JN256184	KF001806
Chaetomium olivaceum	CGMCC 3.12883	Camel dung	JN209911	JN209911	JN256181	KF001807
Chaetomium pilosum	CBS 335.67^{T}	Grain of *Triticum aestivum*	KT214586	FJ666356	KT214763	FJ666387
Chaetomium pseudocochliodes	CGMCC 3.9441^{T}	Roots of *Panax notoginseng*	JN209925	JN209925	JN256195	KF001816
Chaetomium pseudocochliodes	CGMCC 3.9469	Rhizosphere of *Panax notoginseng*	JN209926	JN209926	JN256196	KF001815
Chaetomium pseudoglobosum	CBS 574.71^{T}	–	KT214573	KT214604	KT214750	KT214674
Chaetomium rectangulare	CBS 126778^{T}	Leaf of *Hordeum vulgare*	HM365239	HM365239	HM365285	KT214688
Chaetomium rectangulare	CBS 126658	Stem of *Hordeum vulgare*	HM365240	HM365240	HM365286	KT214689
Chaetomium spiculipilium	CBS 373.66^{T}	Decaying vegetable debris	KC109756	KC109756	KC109774	KF001809
Chaetomium spirochaete	CBS 730.84^{T}	Animal dung	JN209921	JN209921	JN256191	KF001819
Chaetomium spirochaete	CBS 165.52	Animal dung	KT214585	KT214616	KT214762	KT214690
Chaetomium subaffine	CBS 637.91^{T}	Cereal	JN209929	JN209929	JN256199	KF001817
Chaetomium subaffine	CGMCC 3.14297	Unknown plant stem	JN209928	JN209928	JN256198	KF001818
Chaetomium subfimeti	CBS 370.66^{T}	Paper and vegetable material	KT214562	FJ666354	KT214739	FJ666385

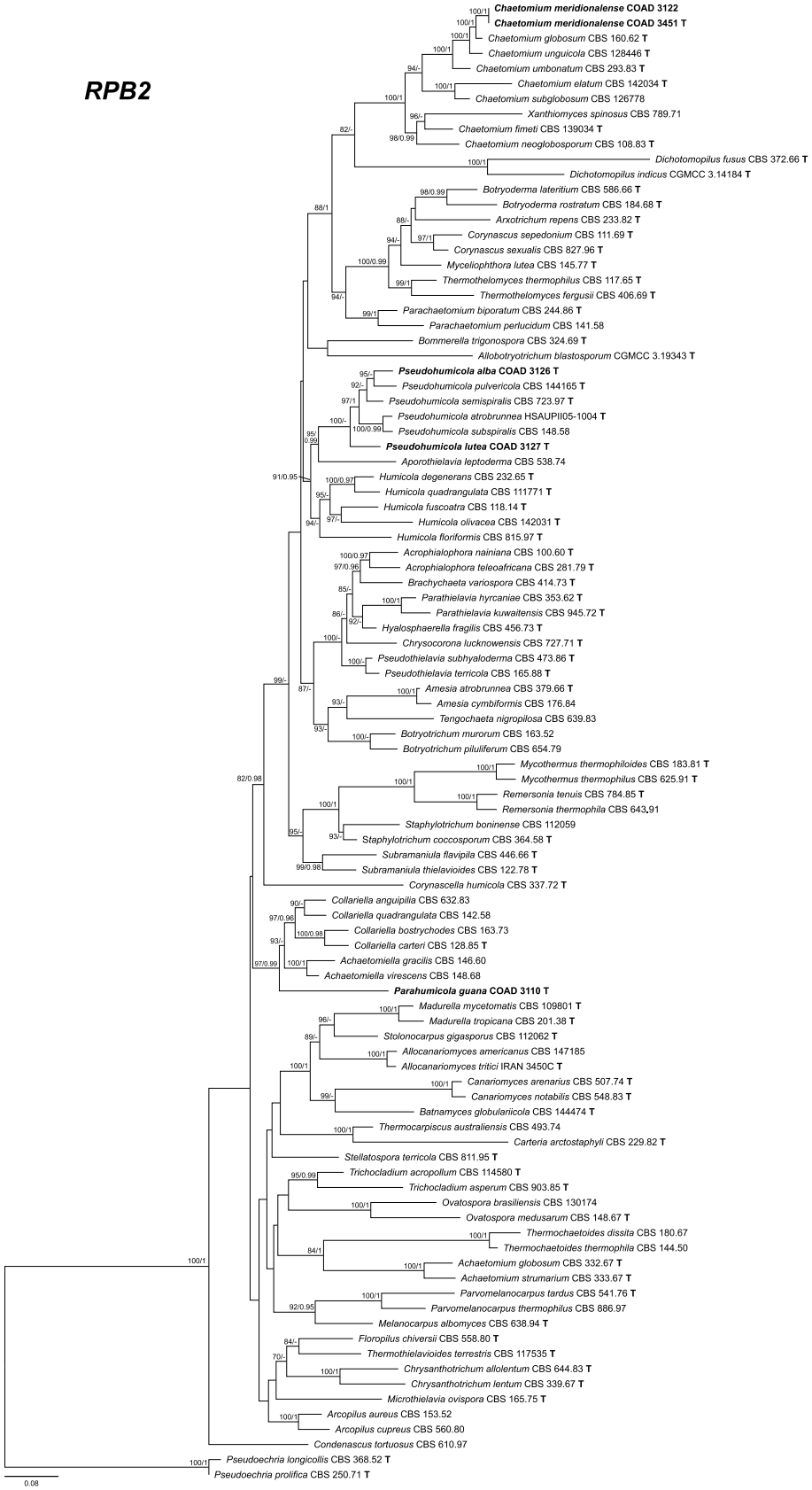
Species	Culture accession	Substrate/Host	Genbank accession number			
			ITS	LSU	*TUB*	*RPB2*
Chaetomium subfimeti	CBS 169.71	Soil	KT214563	FJ666357	KT214740	FJ666388
Chaetomium subglobosum	CBS 149.60^{T}	Dead herbaceous stem	JN209930	JN209930	JN256200	KF001808
Chaetomium subglobosum	CBS 483.73	*Eriobotrya japonica*	KT214581	KT214612	KT214758	KT214684
Chaetomium tarraconense	CBS 101882^{T}	Soil	–	–	MZ343005	MZ342964
Chaetomium tectifimeti	CBS 142032^{T}	Dust	KX976640	KX976737	KX976982	KX976836
Chaetomium telluricola	CBS 151.59^{T}	Soil	KT214582	KT214613	KT214759	KT214685
Chaetomium tenue	CBS 139.38^{T}	–	KT214568	KT214599	KT214745	KT214669
Chaetomium tenue	CBS 143.38	–	KT214572	KT214603	KT214749	KT214673
Chaetomium umbonatum	CBS 293.83^{T}	Soil	KT214575	KT214606	KT214752	KT214676
Chaetomium undulatulum	CBS 126775^{T}	Leaf of *Hordeum vulgare*	HM365251	HM365251	HM365279	KT214682
Chaetomium undulatulum	CBS 126776	Leaf of *Triticum aestivum*	HM365250	HM365250	HM365278	KT214683
Chaetomium unguicola	CBS 128446^{T}	Nail of *Homo sapiens*	KT214567	KT214598	KT214744	KT214668
Chrysanthotrichum allolentum	CBS 644.83^{T}	Soil	MK926808	MK926808	MK926908	MK876768
Chrysanthotrichum lentum	CBS 339.67^{T}	Soil	MK926809	MK926809	MK926909	MK876769
Chrysocorona lucknowensis	CBS 727.71^{T}	Dung of deer	MK926813	MK926813	MK926913	MK876773
Collariella anguipilia	CBS 632.83^{T}	Dung of rabbit	MZ334721	MZ351424	MZ343028	MZ342989
Collariella bostrychodes	CBS 163.73	Dung of antelope	KX976641	KX976738	KX976983	KX976837
Collariella bostrychodes	**COAD 3129**	**Leaf litter from a cave**	**ON989664**	**ON979683**	**ON988191**	–
Collariella carteri	CBS 128.85^{T}	Air	KX976647	KX976742	KX976989	KX976841
Collariella quadrangulata	CBS 142.58	Soil	KX976650	KX976745	KX976992	KX976844
Condenascus tortuosus	CBS 610.97^{T}	Soil	MK926817	MK926817	MK926917	MK876777
Corynascella humicola	CBS 337.72^{T}	Soil	KX976656	KX976751	KX976998	KX976850
Corynascus sepedonium	CBS 111.69^{T}	Soil	HQ871751	KX976777	KX977027	KX976892
Corynascus sexualis	CBS 827.96^{T}	Soil	MK919295	MK919295	MK919409	MK919352
Dichotomopilus fusus	CBS 372.66^{T}	Leaf litter	KX976660	KX976754	KX977002	KX976859
Dichotomopilus indicus	CGMCC 3.14184^{T}	Rhizosphere of *Panax notoginseng*	GU563367	GU563360	JF772453	KX976861
Floropilus chiversii	CBS 558.80^{T}	Dung of moose	MK926818	MK926818	MK926918	MK876778
Humicola ampulliella	CBS 116735^{T}	Discarded sock	LT993568	LT993568	LT993487	LT993649

Species	Culture accession	Substrate/Host	Genbank accession number			
			ITS	LSU	*TUB*	*RPB2*
Humicola ampulliella	CBS 116736	Soil	LT993569	LT993569	LT993488	LT993650
Humicola christensenii	CBS 127760^{T}	Soil	LT993571	LT993571	LT993490	LT993652
Humicola christensenii	CGMCC 3.08497	–	LT993572	LT993572	LT993491	LT993653
Humicola cuyabenoensis	CBS 398.97^{T}	Rain forest	LT993573	LT993573	LT993492	LT993654
Humicola degenerans	CBS 232.65^{T}	Soil under mixed forest	LT993574	LT993574	LT993493	LT993655
Humicola degenerans	CBS 780.71	Termite mound	LT993575	LT993575	LT993656	LT993494
Humicola distorta	CBS 417.66^{T}	*Populus tremuloides* dead leaf	LT993577	LT993577	LT993496	LT993658
Humicola floriformis	CBS 815.97^{T}	Fallen leaves	LT993578	LT993578	LT993497	LT993659
Humicola fuscoatra	CBS 118.14^{T}	Soil	LT993579	LT993579	LT993498	LT993660
Humicola fuscoatra	CGMCC 3.13428	Soil	LT993580	LT993580	LT993499	LT993661
Humicola fuscogrisea	CGMCC 3.13790^{T}	Soil	LT993581	LT993581	LT993500	LT993662
Humicola hirsuta	CBS 144492^{T}	Soil	MZ334726	MZ351425	MZ343013	MZ342974
Humicola homopilata	CBS 157.55^{T}	Filter paper in soil	LT993582	LT993582	LT993501	LT993663
Humicola homopilata	CBS 338.68	–	LT993583	LT993583	LT993502	LT993664
Humicola leptodermospora	CBS 120095^{T}	Forest soil	LT993584	LT993584	LT993503	LT993665
Humicola malaysiensis	CBS 399.97^{T}	*Elaeis guineensi*	LT993586	LT993586	LT993505	LT993667
Humicola malaysiensis	CBS 760.83	Soil	LT993587	LT993587	LT993506	LT993668
Humicola mutabilis	CBS 779.71^{T}	Soil	LT993588	LT993588	LT993507	LT993669
Humicola olivacea	CBS 142031^{T}	Dust	LT993589	LT993589	LT993508	LT993670
Humicola pinnata	CBS 467.66^{T}	Deadwood	LT993590	LT993590	LT993509	LT993671
Humicola quadrangulata	CBS 111771^{T}	Soil	LT993593	LT993593	LT993512	LT993674
Humicola seminuda	CBS 368.84^{T}	Soil	LT993594	LT993594	LT993513	LT993675
Humicola seminuda	CBS 549.69	Soil under *Thuja occidentalis*	LT993596	LT993596	LT993515	LT993677
Humicola sphaeralis	CBS 985.87^{T}	Soil	LT993598	LT993598	LT993517	LT993679
Humicola udagawae	CBS 337.68^{T}	–	LT993601	LT993601	LT993520	LT993682
Humicola wallefii	CBS 147.67^{T}	Soil	LT993602	LT993602	LT993521	LT993683
Hyalosphaerella fragilis	CBS 456.73^{T}	Rhizosphere of *Pennisetum typhoideum*	KX976693	KX976791	KX977042	MK876779
Madurella mycetomatis	CBS 109801^{T}	Foot mycetoma of a woman	MK926820	MK926820	MK926920	MK876781

Species	Culture accession	Substrate/Host	Genbank accession number			
			ITS	LSU	*TUB*	*RPB2*
Madurella pseudomycetomatis	CBS 129177^{T}	Mycetoma of a man's lower jaw	MK926821	MK926821	MK926921	MK876782
Melanocarpus albomyces	CBS 638.94^{T}	Chicken nest straw	KX976679	KX976773	KX977021	KX976886
Microthielavia ovispora	CBS 165.75^{T}	Root of *Avena sativa*	MK926826	MK926826	MK926926	MK876787
Myceliophthora lutea	CBS 145.77^{T}	Hay	HQ871775	KM655351	KX977026	KX976891
Mycothermus thermophiloides	CBS 183.81^{T}	Soil	LT993603	LT993603	LT993684	LT993522
Mycothermus thermophilus	CBS 625.91^{T}	Chicken nest straw	LT993604	LT993604	LT993685	LT993523
Ovatospora brasiliensis	CBS 130174	Soil	KX976682	KX976780	KX977030	KX976895
Ovatospora medusarum	CBS 148.67^{T}	Soil	KX976684	KX976782	KX977032	KX976897
Parachaetomium biporatum	CBS 244.86^{T}	Soil	MK919303	MK919303	MK919417	MK919360
Parachaetomium perlucidum	CBS 141.58^{T}	Dead herbaceous stem	MK919308	MK919308	MK919422	MK919365
Parahumicola guana	**COAD 3110^{T}**	**Bat guano from a cave**	**ON989659**	**OP108407**	**OP131290**	**ON995381**
Parathielavia hyrcaniae	CBS 353.62^{T}	Sand dune soil	KM655329	KM655368	KX977043	KM655401
Parathielavia kuwaitensis	CBS 945.72^{T}	Desert soil	KM655332	KM655371	KX977044	KM655404
Parvomelanocarpus tardus	CBS 541.76^{T}	Cotton jacket	KX976681	KX976775	KX977023	KX976888
Parvomelanocarpus thermophilus	CBS 886.97	Soil	KM655350	MH874288	MZ343037	KM655434
Pseudoechria longicollis	CBS 368.52^{T}	Deteriorating material	MK926847	MK926847	MK926947	MK876809
Pseudoechria prolifica	CBS 250.71^{T}	Dung of *Cobus defassa*	MK926848	MK926848	MK926948	MK876810
Pseudohumicola alba	**COAD 3126^{T}**	**Air from a cave**	**ON989662**	**ON979681**	**ON988189**	**ON995382**
Pseudohumicola atrobrunnea	HSAUPII05-1004^{T}	Soil	LT993570	LT993570	LT993489	LT993651
Pseudohumicola cecavii	URM 8444^{T}	Air from a cave	ON862932	–	OP672391	OP722570
Pseudohumicola cecavii	R35	Sediment from a cave	–	–	OP672392	OP722571
Pseudohumicola lutea	**COAD 3127^{T}**	**Air from a cave**	**ON989663**	**ON979682**	**ON988190**	**ON995383**
Pseudohumicola pulvericola	CBS 144165^{T}	Dust	LT993591	LT993591	LT993510	LT993672
Pseudohumicola pulvericola	CBS 144166	Dust	LT993592	LT993592	LT993511	LT993673
Pseudohumicola semispiralis	CBS 723.97^{T}	Paper	LT993597	LT993597	LT993516	LT993678
Pseudohumicola subspiralis	CBS 148.58	Leaf fragments in soil	LT993599	LT993599	LT993518	LT993680
Pseudohumicola subspiralis	CBS 119768	Soil	LT993600	LT993600	LT993519	LT993681
Pseudothielavia subhyaloderma	CBS 473.86^{T}	Forest soil	MK926833	MK926833	MK926933	MK876794

Species	Culture accession	Substrate/Host	Genbank accession number			
			ITS	**LSU**	***TUB***	***RPB2***
Pseudothielavia terricola	CBS 165.88^{T}	Barren soil	KX976694	KX976792	KX977045	MK876795
Remersonia tenuis	CBS 784.85^{T}	Dung of horse	LT993609	LT993609	LT993690	LT993528
Remersonia thermophila	CBS 643.91	Compost	LT993610	LT993610	LT993691	LT993529
Staphylotrichum boninense	CBS 112059	Twig	LT993616	LT993616	LT993697	LT993535
Staphylotrichum coccosporum	CBS 364.58^{T}	Soil	LT993620	LT993620	LT993701	LT993539
Stellatospora terricola	CBS 811.95^{T}	Paddy soil	MK926835	MK926835	MK926935	MK876797
Stolonocarpus gigasporus	CBS 112062^{T}	Dung of *Camelus dromedarius*	MK926836	MK926836	MK926936	MK876798
Subramaniula flavipila	CBS 446.66^{T}	Dead leaves	KP862600	KP970647	KP900706	KP900669
Subramaniula thielavioides	CBS 122.78^{T}	Dung of nilgai	KP862597	KP970654	KP900708	KP900670
Tengochaeta nigropilosa	CBS 639.83^{T}	Soil from a *Pinus* forest	MZ334730	–	MZ343029	MZ342990
Thermocarpiscus australiensis	CBS 493.74^{T}	Nesting material of incubator bird	KM655339	KM655378	MZ343024	KM655419
Thermochaetoides dissita	CBS 180.67^{T}	Straw of *Typha*	MK919319	MK919319	MK919433	MK919375
Thermochaetoides thermophila	CBS 144.50^{T}	Decaying wheat straw	MK919314	MK919314	MK919428	KM655436
Thermothelomyces fergusii	CBS 406.69^{T}	Mushroom compost	HQ871794	KX976776	KX977024	MK919378
Thermothelomyces termophilus	CBS 117.65^{T}	Dry pasture soil	MK919331	MK919331	MK919445	MK919387
Thermothielavioides terrestris	CBS 117535^{T}	Soil	MK926837	MK926837	MK926937	MK876799
Trichocladium acropullum	CBS 114580^{T}	Soil	LT993626	LT993626	LT993707	LT993545
Trichocladium asperum	CBS 903.85^{T}	Acidic soil	LT993632	LT993632	LT993713	LT993551
Xanthiomyces spinosus	CBS 789.71	Culture of algae	MH860357	MZ351429	MZ343034	MZ342995
Xanthiomyces spinosus	CBS 796.83	Straw	MZ334724	MZ351430	MZ343035	MZ342996

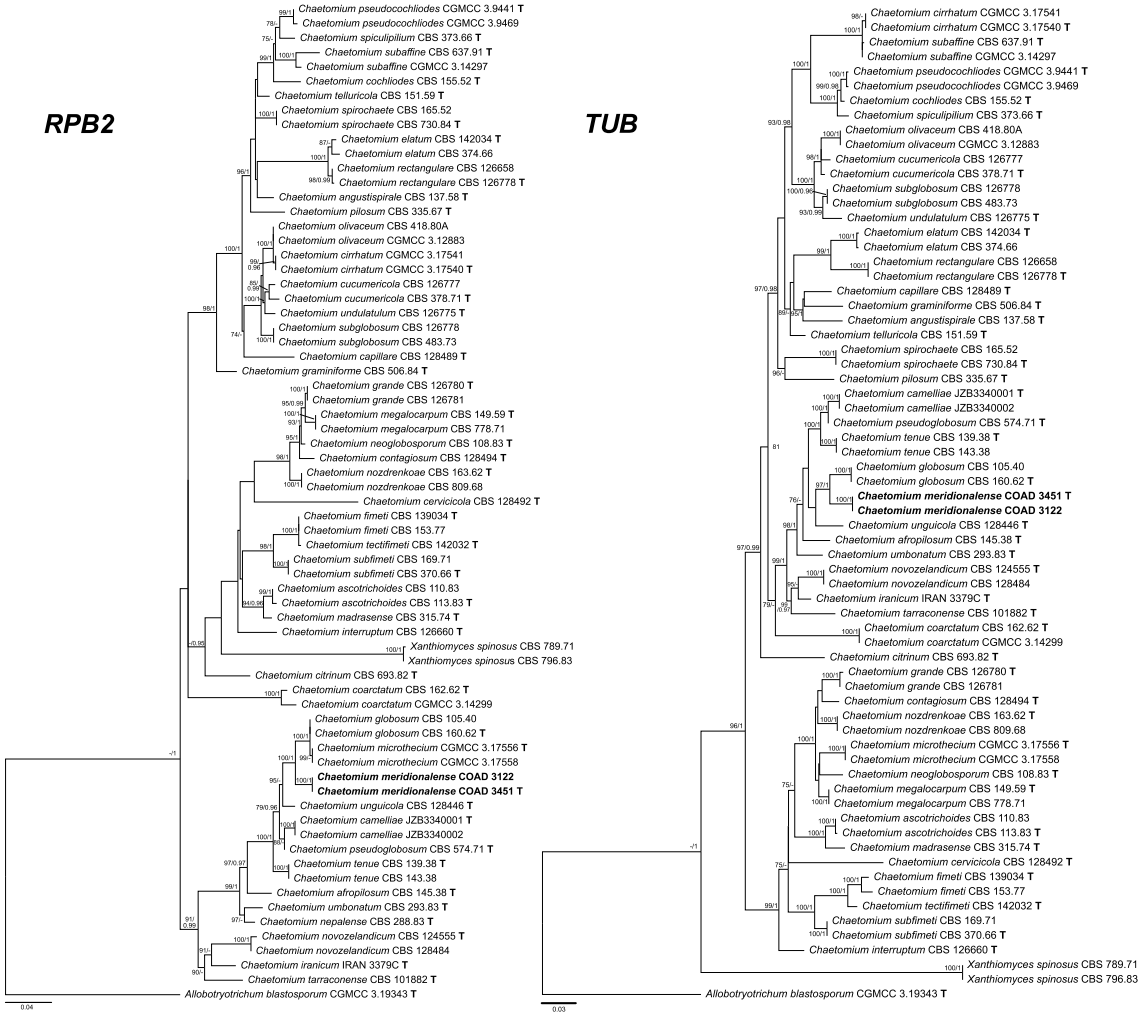
"T" = ex-type strains



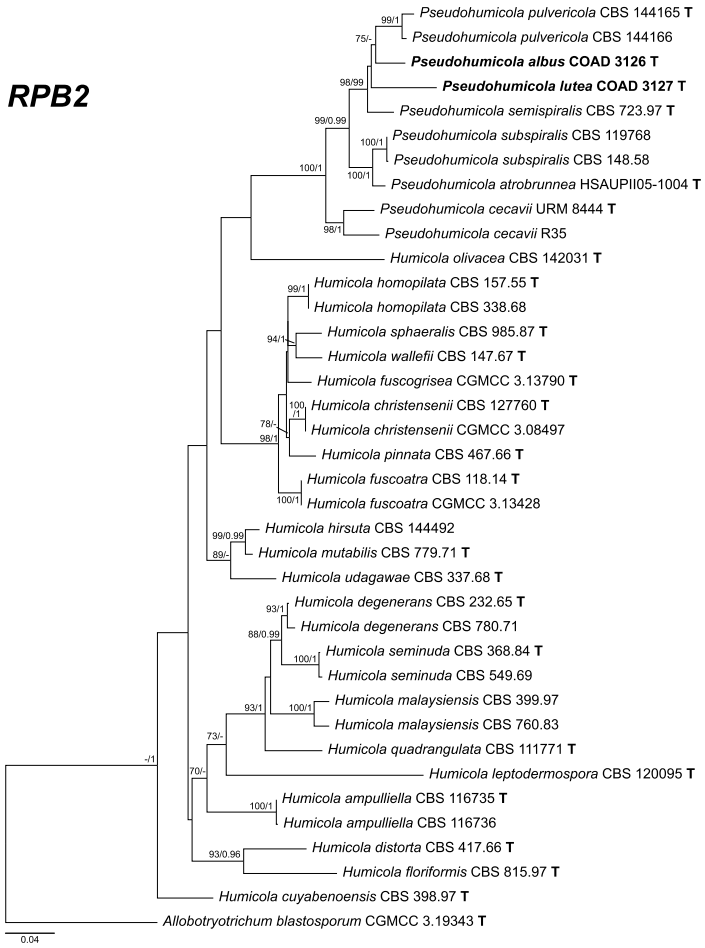
Online resource 2 Maximum-likelihood tree of *Chaetomiaceae* based on the *RPB2* gene region. Isolates found in this study are shown in bold red. Ex-type isolates are marked with ‘‘**T**’’. Only bootstrap (bs) values ≥ 70% and posterior probabilities (pp) ≥ 0.95 are shown at branches (‘‘-’’ means no statistical support). The tree is rooted with *Pseudoechria longicollis* CBS 368.52 and *Pseudoechria prolifica* CBS 250.71 (*Schizotheciaceae*)

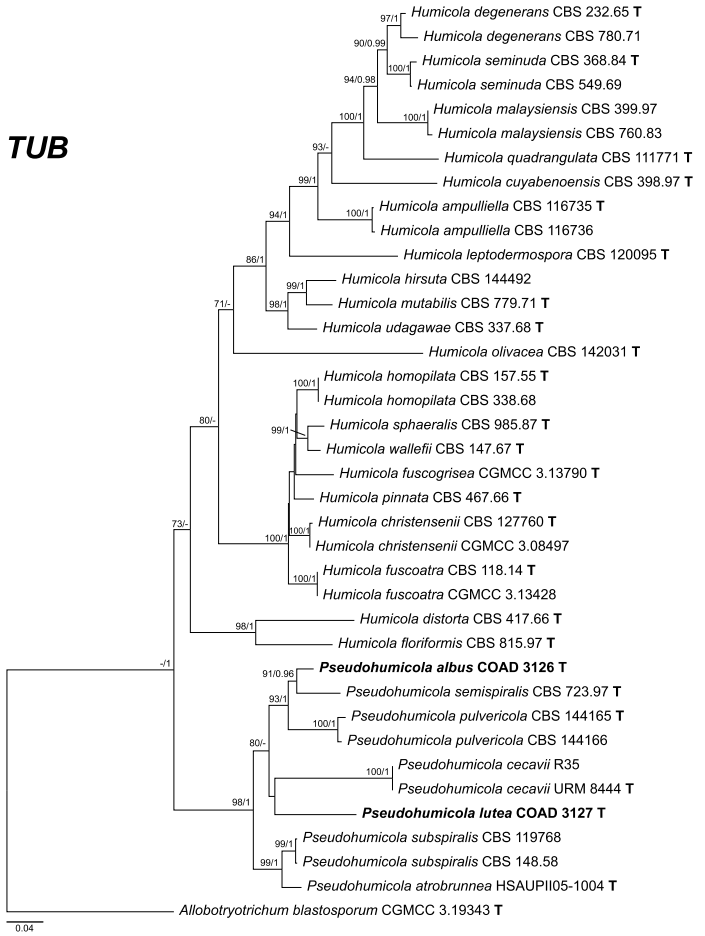

TUB
Pseudohumicola alba COAD 3126 T
Pseudohumicola semispiralis CBS 723.97 T
Pseudohumicola pulvericola CBS 144165 T
Pseudohumicola lutea COAD 3127 T
Pseudohumicola atrobrunnea HSAUPII05-1004 T
Pseudohumicola subspiralis CBS 148.58
Humicola degenerans CBS 232.65 T
Humicola quadrangulata CBS 111771 T
Humicola floriformis CBS 815.97 T
Humicola olivacea CBS 142031 T
Humicola fuscoatra CBS 118.14 T
Staphylotrichum boninense CBS 112059
Staphylotrichum coccosporum CBS 364.58 T
Botryotrichum murorum CBS 163.52
Botryotrichum piluliferum CBS 654.79
Subramaniula flavipila CBS 446.66 T
Subramaniula thielavioides CBS 122.78 T
Tengochaeta nigropilosa CBS 639.83
Amesia atrobrunnea CBS 379.66 T
Amesia cymbiformis CBS 176.84
Mycothermus thermophiloides CBS 183.81 T
Mycothermus thermophilus CBS 625.91 T
Remersonia tenuis CBS 784.85 T
Remersonia thermophila CBS 643.91
Pseudothielavia subhyaloderma CBS 473.86 T
Pseudothielavia terricola CBS 165.88 T
Parathielavia hyrcaniae CBS 353.62 T
Parathielavia kuwaitensis CBS 945.72 T
Hyalosphaerella fragilis CBS 456.73 T
Chrysocorona lucknowensis CBS 727.71 T
Acrophialophora nainiana CBS 100.60 T
Acrophialophora teleoafricana CBS 281.79 T
Brachychaeta variospora CBS 414.73 T
Chaetomium meridionalense COAD 3122
Chaetomium meridionalense COAD 3451 T
Chaetomium globosum CBS 160.62 T
Chaetomium unguicola CBS 128446 T
Chaetomium umbonatum CBS 293.83 T
Chaetomium elatum CBS 142034 T
Chaetomium subglobosum CBS 126778
Chaetomium fimeti CBS 139034 T
Chaetomium neoglobosporum CBS 108.83 T
Dichotomopilus fusus CBS 372.66 T
Dichotomopilus indicus CGMCC 3.14184 T
Xanthiomyces spinosus CBS 789.71
Allobotryotrichum blastosporum CGMCC 3.19343 T
Thermothelomyces fergusii CBS 406.69 T
Thermothelomyces thermophilus CBS 669.85
Myceliophthora lutea CBS 145.77 T
Corynascus sepedonium CBS 111.69 T
Corynascus sexualis CBS 827.96
Botryoderma lateritium CBS 586.66
Botryoderma rostratum CBS 184.68
Arxotrichum repens CBS 233.82
Arxotrichum wyomingense CCF 5691
Parachaetomium biporatum CBS 244.86 T
Parachaetomium perlucidum CBS 141.58
Microthielavia ovispora CBS 165.75 T
Bommerella trigonospora CBS 324.69 T
Collariella bostrychodes CBS 163.73
Collariella bostrychodes COAD 3129
Collariella carteri CBS 128.85 T
Collariella anguipilia CBS 632.83
Collariella quadrangulata CBS 142.58
Achaetomiella gracilis CBS 146.60
Achaetomiella virescens CBS 148.68
Parahumicola guana COAD 3110 T
Floropilus chiversii CBS 558.80 T
Thermothielavioides terrestris CBS 117535 T
Chrysanthotrichum allolentum CBS 644.83 T
Chrysanthotrichum lentum CBS 339.67 T
Corynascella humicola CBS 337.72 T
Achaetomium globosum CBS 332.67 T
Achaetomium strumarium CBS 333.67 T
Arcopilus aureus CBS 153.52
Arcopilus cupreus CBS 560.80
Aporothielavia leptoderma CBS 538.74
Stellatospora terricola CBS 811.95 T
Ovatospora brasiliensis CBS 130174
Ovatospora medusarum CBS 148.67 T
Trichocladium acropollum CBS 114580 T
Trichocladium asperum CBS 903.85 T
Canariomyces arenarius CBS 507.74 T
Canariomyces notabilis CBS 548.83 T
Melanocarpus albomyces CBS 638.94 T
Condenascus tortuosus CBS 610.97
Madurella mycetomatis CBS 109801 T
Madurella tropicana CBS 201.38 T
Stolonocarpus gigasporus CBS 112062 T
Allocanariomyces americanus CBS 147185
Allocanariomyces tritici IRAN 3450C T
Batnamyces globulariicola CBS 144474 T
Parvomelanocarpus tardus CBS 541.76 T
Parvomelanocarpus thermophilus CBS 886.97
Carteria arctostaphyli CBS 229.82 T
Thermocarpiscus australiensis CBS 493.74
Thermochaetoides dissita CBS 180.67
Thermochaetoides thermophila CBS 144.50
Pseudoechria longicollis CBS 368.52 T
Pseudoechria prolifica CBS 250.71 T
0.1

Online resource 3 Maximum-likelihood tree of *Chaetomiaceae* based on the *TUB* gene region. Isolates found in this study are shown in bold red. Ex-type isolates are marked with ‘‘**T**’’. Only bootstrap (bs) values ≥ 70% and posterior probabilities (pp) ≥ 0.95 are shown at branches (‘‘-’’ means no statistical support). The tree is rooted with *Pseudoechria longicollis* CBS 368.52 and *Pseudoechria prolifica* CBS 250.71 (*Schizotheciaceae*)



Online resource 4 Maximum-likelihood tree of *Chaetomium* and *Xanthiomyces* based on an independent dataset of *RPB2* and *TUB* gene regions. Isolates of the new species found in this study are shown in bold. Ex-type isolates are marked with ‘‘**T**’’. Only bootstrap (bs) values ≥ 70% and posterior probabilities (pp) ≥ 0.95 are shown at branches (‘‘-’’ means no statistical support). The tree is rooted with *Allobotryotrichum blastosporum* CGMCC 3.1943


RPB2
Pseudohumicola pulvericola CBS 144165 T
Pseudohumicola pulvericola CBS 144166
Pseudohumicola albus COAD 3126 T
Pseudohumicola lutea COAD 3127 T
Pseudohumicola semispiralis CBS 723.97 T
Pseudohumicola subspiralis CBS 119768
Pseudohumicola subspiralis CBS 148.58
Pseudohumicola atrobrunnea HSAUPII05-1004 T
Pseudohumicola cecavii URM 8444 T
Pseudohumicola cecavii R35
Humicola olivacea CBS 142031 T
Humicola homopilata CBS 157.55 T
Humicola homopilata CBS 338.68
Humicola sphaeralis CBS 985.87 T
Humicola wallefii CBS 147.67 T
Humicola fuscogrisea CGMCC 3.13790 T
Humicola christensenii CBS 127760 T
Humicola christensenii CGMCC 3.08497
Humicola pinnata CBS 467.66 T
Humicola fuscoatra CBS 118.14 T
Humicola fuscoatra CGMCC 3.13428
Humicola hirsuta CBS 144492
Humicola mutabilis CBS 779.71 T
Humicola udagawae CBS 337.68 T
Humicola degenerans CBS 232.65 T
Humicola degenerans CBS 780.71
Humicola seminuda CBS 368.84 T
Humicola seminuda CBS 549.69
Humicola malaysiensis CBS 399.97
Humicola malaysiensis CBS 760.83
Humicola quadrangulata CBS 111771 T
Humicola leptodermospora CBS 120095 T
Humicola ampulliella CBS 116735 T
Humicola ampulliella CBS 116736
Humicola distorta CBS 417.66 T
Humicola floriformis CBS 815.97 T
Humicola cuyabenoensis CBS 398.97 T
Allobotryotrichum blastosporum CGMCC 3.19343 T
0.04


TUB
Humicola degenerans CBS 232.65 T
Humicola degenerans CBS 780.71
Humicola seminuda CBS 368.84 T
Humicola seminuda CBS 549.69
Humicola malaysiensis CBS 399.97
Humicola malaysiensis CBS 760.83
Humicola quadrangulata CBS 111771 T
Humicola cuyabenoensis CBS 398.97 T
Humicola ampulliella CBS 116735 T
Humicola ampulliella CBS 116736
Humicola leptodermospora CBS 120095 T
Humicola hirsuta CBS 144492
Humicola mutabilis CBS 779.71 T
Humicola udagawae CBS 337.68 T
Humicola olivacea CBS 142031 T
Humicola homopilata CBS 157.55 T
Humicola homopilata CBS 338.68
Humicola sphaeralis CBS 985.87 T
Humicola wallefii CBS 147.67 T
Humicola fuscogrisea CGMCC 3.13790 T
Humicola pinnata CBS 467.66 T
Humicola christensenii CBS 127760 T
Humicola christensenii CGMCC 3.08497
Humicola fuscoatra CBS 118.14 T
Humicola fuscoatra CGMCC 3.13428
Humicola distorta CBS 417.66 T
Humicola floriformis CBS 815.97 T
Pseudohumicola albus COAD 3126 T
Pseudohumicola semispiralis CBS 723.97 T
Pseudohumicola pulvericola CBS 144165 T
Pseudohumicola pulvericola CBS 144166
Pseudohumicola cecavii R35
Pseudohumicola cecavii URM 8444 T
Pseudohumicola lutea COAD 3127 T
Pseudohumicola subspiralis CBS 119768
Pseudohumicola subspiralis CBS 148.58
Pseudohumicola atrobrunnea HSAUPII05-1004 T
Allobotryotrichum blastosporum CGMCC 3.19343 T
0.04

Online resource 5 Maximum-likelihood tree of *Pseudohumicola* and *Humicola* based on an independent dataset of *RPB2* and *TUB* gene regions. Isolates of the new species found in this study are shown in bold. Ex-type isolates are marked with ‘‘**T**’’. Only bootstrap (bs) values ≥ 70% and posterior probabilities (pp) ≥ 0.95 are shown at branches (‘‘-’’ means no statistical support). The tree is rooted with *Allobotryotrichum blastosporum* CGMCC 3.1943