Aspergillus, *Penicillium*, and *Talaromyces* (*Eurotiales*) in Brazilian caves, with the description of four new species

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Abstract: The study of the Brazilian cave mycobiota has revealed a rich but highly diverse assemblage of fungi, with *Aspergillus*, *Penicillium*, and *Talaromyces* being the most frequently reported genera. The present study investigated the airborne fungi and fungi obtained from the bodies of bats, guano, and the soil/sediment from the caves Urubu (in the Atlantic Forest) and Furna Feia (in the Caatinga dryland forest) in the Northeast region of Brazil. Fungal strains were identified based on morphological features and multilocus phylogenetic analyses of ITS, beta-tubulin (*BenA*), calmodulin (*CaM*), and RNA polymerase II second largest subunit (*RPB2*) sequences. A total of 86 isolates were obtained, representing *Aspergillus* (34), *Penicillium* (20), *Talaromyces* (2), and 30 isolates belonging to other genera that will be reported on elsewhere. These isolates were identified as 18 *Aspergillus*, nine *Penicillium*, and one *Talaromyces* species. Eight of the species identified are reported for the first time from a cave environment. Four species showed unique morphological features and phylogenetic relationships, and are newly described. These include two new species of *Aspergillus* (*A. alvaroi sp. nov.* and *A. guanovespertilionum sp. nov.*), one of *Penicillium* (*P. cecavii sp. nov.*), and one of *Talaromyces* (*T. potiguarorum sp. nov.*). Our study increases the awareness and known richness of the Brazilian and global fungal diversity found in caves.

INTRODUCTION

The order *Eurotiales* encompasses important and globally well-known genera such as *Aspergillus*, *Penicillium*, and *Talaromyces*, with species that can be isolated from a wide range of substrates and hosts (Houbraken & Samson 2011, Tsang *et al*. 2018, Houbraken *et al*. 2020, Visagie *et al*. 2024). The taxonomy of these genera has been extensively studied, and the number of described species has increased each year. Visagie *et al*. (2024) provided an updated accepted species list for these genera, accepting 453 *Aspergillus*, 553 *Penicillium*, and 203 *Talaromyces* species. They also provided a new subgeneric classification at subgenus level, with the 453 *Aspergillus* species classified into six subgenera, 28 sections, 88 series, and the 553 *Penicillium* species classified into two subgenera, 32 sections, and 101 series. The number has increased significantly over the last years, as these groups have an established taxonomy, biotechnological

potential, the potential to be pathogens of animals and to cause agricultural losses (Sun *et al*. 2020, Tan *et al*. 2022, Cañete-Gibas *et al*. 2023, Sobol *et al*. 2023).

Aspergillus, *Penicillium*, and *Talaromyces* species produce many conidia that facilitate dispersion (Visagie *et al*. 2014) and are found in diverse substrates (Yilmaz *et al*. 2016, Barbosa *et al*. 2020, Houbraken *et al*. 2020, Visagie *et al*. 2024) including in caves worldwide (Vanderwolf *et al*. 2013, Dominguez-Moñino *et al*. 2021, Wasti *et al*. 2021, Nuankaew *et al*. 2022), Ogórek *et al*. 2022. However, there have been few taxonomic diversity studies in Brazilian caves (Cunha *et al*. 2020, Alves *et al*. 2022), highlighting the importance of mycological studies in these environments. In recent years, studies have documented the presence of *Eurotiales* species in caves worldwide, *e.g.* USA (Raudabaugh *et al*. 2021), Malaysia (Wasti *et al*. 2021), Spain (Jurado *et al*. 2021, Martin-Pozas *et al*. 2022), and Azerbaijan (Mazina *et al*. 2023). Some studies introduced new species,

e.g. one *Aspergillus* sp. in Botswana (Visagie *et al.* 2021), three *Aspergillus* spp. in China (Zhang *et al.* 2020), one *Penicillium* sp. in Canada (Visagie *et al.* 2020), two *Talaromyces* spp. in Thailand (Nuankaew *et al.* 2022), and one *Aspergillus* sp. and one *Talaromyces* sp. in Brazil (Alves *et al.* 2022).

Despite the presence of many caves in Brazil (*ca.* 23 378, with 4 390 in the Caatinga dry forest and 4 711 in the Atlantic Forest - ICMBio-CECAV, 2022), fungal surveys from these environments are scarce. However, existing records suggest that Brazilian caves harbour a diversity of fungi still largely unknown to science, with records of rare and new species. This was observed in a study by Cunha *et al.* (2020), who evaluated the mycobiota of a bat cave in the Caatinga and found that *Aspergillus* accounted for the greatest number of species (12), followed by *Penicillium* (five), *Cladosporium* (three), and *Talaromyces* (three). Similarly, in another bat cave in the Northeast region of Brazil, Pereira *et al.* (2022) reported the richness of eight *Cladosporium* species from the air and described *C. cavernicola* and *C. pernambucoense* as new. Carvalho *et al.* (2022) also analysed the association of fungi with ectoparasitic flies of bats and reported the predominance of *Aspergillus* (three species). Recently, Alves *et al.* (2022) studied the air and soil/sediment from a cave in the Caatinga and identified 17 genera of *Ascomycota*, with the largest number of taxa included in *Aspergillus* (13 species); the authors also described the new species *A. lebretii* and *T. cavernicola* found in this cave. These examples demonstrate the importance of caves for fungal diversity discovery, including for new species.

The presence of such large numbers of bats and the large amount of guano they produce influences the many organisms living in bat caves. Bat guano is an excellent source of nutrients for the fungal community (Poulson 1972, Dimkić *et al.* 2021, Reis *et al.* 2023). In fact, previous work on the bat-guano-fungus interaction in Brazilian bat caves has shown they are hotspots for fungal richness and diversity, remaining largely unstudied (Cunha *et al.* 2020, Pereira *et al.* 2022). Therefore, the aim of this study was to advance our knowledge of the richness of *Aspergillus*, *Penicillium*, and *Talaromyces* species isolated from different substrates (*e.g.* air, sediment, bodies of bats, and bat guano) in two bat caves in the Northeast region of Brazil, including the description of four new species from these caves.

MATERIALS AND METHODS

Sampling

The cave Urubu (10°43'58.1"S, 37°09'56.0"W) is located in the municipality of Divina Pastora, state of Sergipe, in the Atlantic Forest, in a region with a humid tropical climate. The cave Furna Feia (05°02'12''S, 37°33'37''W), is located in the Furna Feia National Park, state of Rio Grande do Norte, in the Caatinga dry forest, with the climate being that of the semi-arid region of the Brazilian Northeast (ICMBio 2020) (Fig. 1).
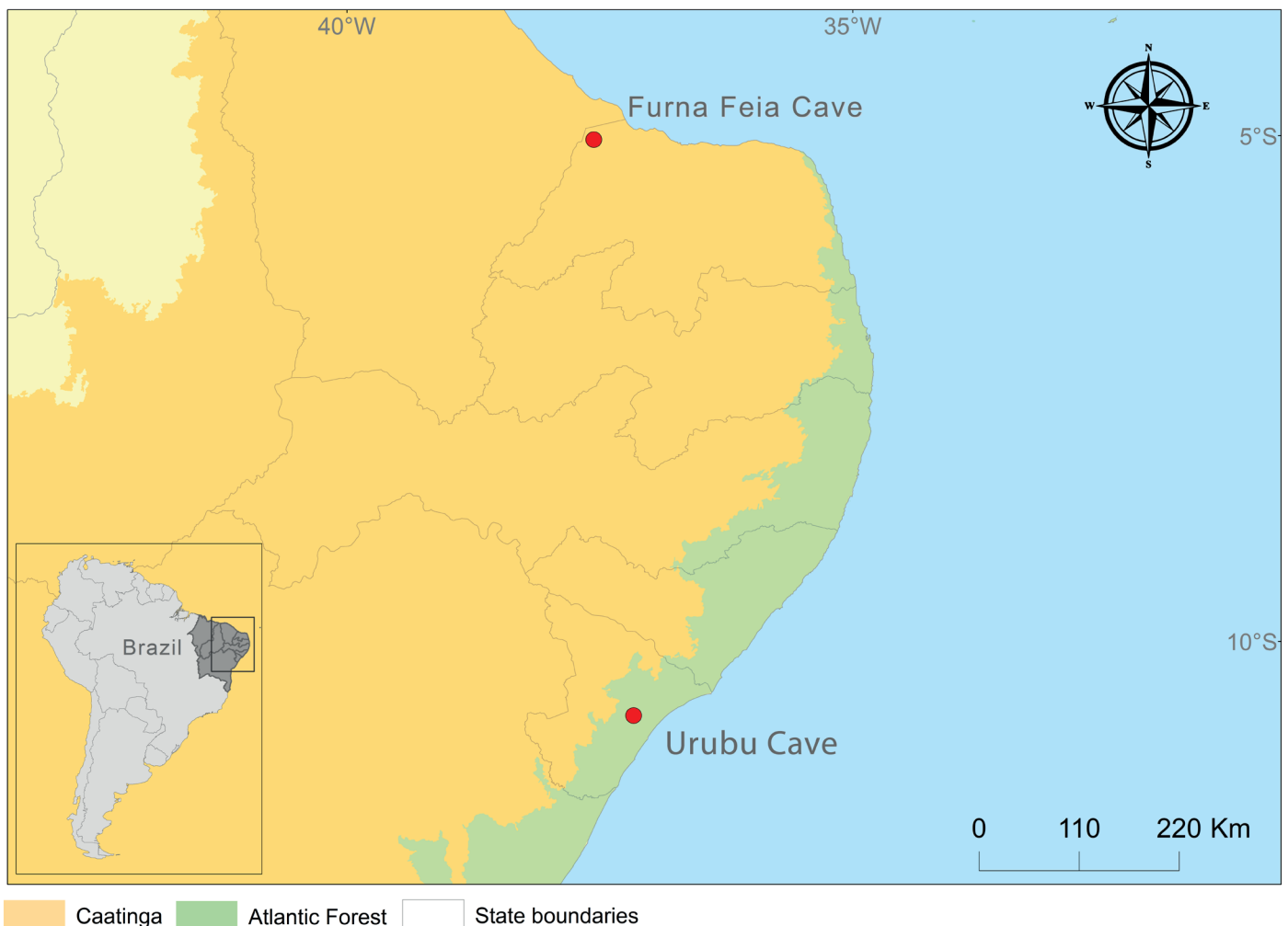

Fig. 1. Geographic location of the Urubu cave (state of Sergipe) and the Furna Feia cave (state of Rio Grande do Norte) in the Northeast region of Brazil.

Collections in cave Urubu were made during the dry season. For cave Furna Feia, collections were made during the dry and rainy seasons. Isolates of *Aspergillus*, *Penicillium* and *Talaromyces* were obtained from the air, bodies of bats, sediment, and guano. Collection was authorised by the Ministry of the Environment (MMA)/Chico Mendes Institute for Biodiversity Conservation (ICMBio) (SISBIO number 68992–3) and the UFPE's Ethics Committee on Animal Care (permit number 114/2019).

Fungal isolations

Isolations of airborne fungi in caves were made using a passive sedimentation methodology as reported in Cunha *et al*. (2020). Briefly, at each sampling point, three 90-mm-diam Petri dishes containing Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol (0.1 mg/L) were placed equidistant from each other, one metre from the cave floor, and were left open for 20 min. After which the plates were closed, taken to the laboratory and were incubated at 28 °C until 7 d in the dark. Colony-forming units (CFU) were counted and representative isolates of the total fungal colonies grown were taken and purified using SDA.

Approximately 10 g of cave sediment or bat guano (insectivorous, omnivorous, and hematophagous) were collected at each sampling point. Isolations were made using a dilution series. One gram of each sediment or bat guano was added to a 250 mL Erlenmeyer flask containing 9 mL distilled water sterilised with chloramphenicol (0.1 mg/L). The flasks were shaken manually, and dilutions of 10^{-2}, 10^{-3}, and 10^{-4} were prepared, from which 1 mL was transferred to Petri dishes containing Brain Heart Infusion agar (BHI) and SDA with chloramphenicol (0.1 mg/L). Petri dishes were incubated at 28 °C for at least 7 d in the dark. Colony-forming units (CFU) were counted, and isolates were selected, purified, and preserved for later identification (Cunha *et al*. 2020).

Fungal isolations were also made from three bat species, *Furipterus horrens* (*Chiroptera*: *Furipteridae*) (eight individuals), *Pteronotus gymnonotus* (seven individuals), and *Pteronotus personatus* (six individuals) (*Chiroptera*: *Mormoopidae*) captured in the same caves. Bats were captured inside the caves between 4:00 pm and 4:30 pm, using a hand net. Samples were collected from three microhabitats on the bat body: oral cavity, fur (belly and back), and wing membrane (ventral and dorsal surfaces). Samples were collected using sterile swabs pre-moistened with sterilised water plus chloramphenicol (0.1 mg/L). Swabs were then individually placed in sterilised 15 mL conical centrifuge tubes containing water plus chloramphenicol (0.1 mg/L), labelled, stored chilled, and shipped for processing. Conical centrifuge tubes were shaken, and 2 mL of the solution were used to inoculate Petri dishes containing BHI and SDA. Petri dishes containing BHI were incubated at 28 °C and plates containing Sabouraud dextrose agar at 37 °C, both for at least 7 d in the dark. Inspection of fungal growth was observed daily, and all the colonies were isolated and purified using SDA.

Morphology

Morphological analyses of our isolates were performed according to Samson *et al*. (2010). Briefly, strains were inoculated at three points on culture media contained in 90-mm-diam Petri dishes using a suspension of conidia: Czapek yeast extract agar (CYA), malt extract agar (MEA), oat agar (OA), Czapek agar (CZ), CYA supplemented with 5 % NaCl (CYAS), creatine agar (CREA), sucrose agar with yeast extract (YES), dichloran 18 % glycerol agar (DG18), and malt extract yeast extract 10 % glucose 12 % NaCl agar (MY10-12). Media with NaCl and glucose (G) gradients (CYA 15 % NaCl, MEA NaCl 5 %, 10 %, 15 %, 20 %, and MEA G 25 %, 30 %, 35 % and 40 %) were prepared as described by Tanney *et al*. (2017). To determine culture characteristics (growth rate, colony texture, pigmentation, and exudates), Petri dishes were incubated at 25 °C for 7 d in the dark. Colours and alphanumeric codes were described using the Rayner colour chart (Rayner 1970). Microscopic observations were performed on cultures grown on MEA. A Nikon Eclipse Ni microscope, equipped with a Nikon DS-Fi2 camera using NISElements AR v. 4.20 software and a Leica DM2500 optical microscope were used to capture the photos, which were later edited in Adobe Illustrator v. 5.1.

The representative and ex-type cultures are deposited in the URM culture collection (Micoteca URM Profa. Maria Auxiliadora Cavalcanti, WDCM 604) and permanent slides (holotypes) in the URM fungarium (Herbário Pe. Camille Torrend), both at the Federal University of Pernambuco (UFPE), Recife, Brazil (see Barbosa *et al*. 2020).

DNA extraction, PCR, sequencing, and phylogenetic analyses

Genomic DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega), according to the manufacturer's recommendations. The internal transcribed spacer including the 5.8S rDNA regions (ITS), β-tubulin (*BenA*), calmodulin (*CaM*), and RNA polymerase II second-largest subunit (*RPB2*) genes were amplified using the primer pairs ITS1/ITS4 (White *et al*. 1990), Bt2a/Bt2b (Glass & Donaldson 1995), CMD5/CMD6 (Hong *et al*. 2006), and rpb2-5F2/frpb2-7cR (Liu *et al*. 1999, Sung *et al*. 2007). Amplification conditions followed those described by Barbosa *et al*. (2020). The PCR products obtained were purified using the Exonuclease/Alkaline Phosphatase mix (Cellco Biotec, Brazil), according to the manufacturer's instructions, and subsequently sent for bidirectional sequencing with the same primers using the BigDye® Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems Life Technologies, Carlsbad, CA, USA) at the Plataforma Multiusuários de Sequenciamento of the UFPE, Recife.

Sequences were edited and assembled using the SeqMan v. 10.0.1 and initial identifications were made using BLASTn comparing against NCBI GenBank database. Newly generated sequences were deposited in GenBank (Table 1). A reference sequence dataset for each genus was compiled from our newly generated sequences and type or reference materials found in previously published papers (*e.g.* Houbraken *et al*. 2011, 2020, Samson *et al*. 2014, Visagie *et al*. 2014, Yilmaz *et al*. 2014, Anelli *et al*. 2018, Sun *et al*. 2020, Zhang *et al*. 2020, Alves *et al*. 2022, Tan *et al*. 2022, Wang & Zhuang 2022a, b, Zhang *et al*. 2023). The datasets were aligned using the default settings of the MAFFT v. 7 online software (https://mafft.cbrc.jp/alignment/software/), and alignments were manually edited using MEGA v. 7 (Tamura *et al*. 2013). Individual alignments, along with the combined dataset, were analysed based on maximum likelihood analysis (ML) using RAxML-HPC BlackBox v. 8.2.12 (Stamatakis & Rougemont 2008) in the CIPRES Science Gateway online platform (https://www.phylo.org/index.php/) (Miller *et al*. 2010). In addition, the combined datasets were analysed using Bayesian inference (BI) analysis performed using MrBayes v. 3.2.7a (Ronquist *et al*. 2012) in the CIPRES Science Gateway. The

Table 1. Species isolated from air, bodies of bats, sediment, and guano from Urubu cave (state of Sergipe, Atlantic Forest) and Furna Feia cave (state of Rio Grande do Norte, Caatinga) in the Northeast region of Brazil and GenBank accession numbers for isolates obtained in our study. S = species first record in a Brazilian cave. B = species first record in a cave environment.

Species	Record	Strains/isolates	Section	Substrates/hosts	GenBank accession numbers			
					ITS	BenA	CaM	RPB2
Aspergillus alvaroi sp. nov.	S/B	URM 8660	*Terrei*	Sediment	PP034171	PP150741	PP150749	PP187788
		URM 8661	*Terrei*	Sediment	PP034172	PP150742	PP150750	PP187789
Aspergillus aureolatus	B	B18	*Nidulantes*	Bat body	–	PP067965	–	–
Aspergillus bertholletiae		URM 8658	*Flavi*	Omnivorous bat guano	PP033023	PP067970	PP150731	–
		URM 8659	*Flavi*	Omnivorous bat guano	PP033024	PP067971	PP150732	–
Aspergillus calidoustus	B	B13	*Usti*	Bat body	–	PP067978	–	–
Aspergillus flavus		FF39	*Flavi*	Sediment	–	PP067972	–	–
Aspergillus guanovespertilionum sp. nov.	S/B	URM 8662	*Polypaecilum*	Haematophagous bat guano	PP034169	PP150739	PP150747	PP187792
		URM 8663	*Polypaecilum*	Haematophagous bat guano	PP034170	PP150740	PP150748	PP187793
Aspergillus montevidensis	S/B	FF34	*Aspergillus*	Sediment	–	PP067975	–	–
		FF38	*Aspergillus*	Sediment	–	PP067976	–	–
		FF310	*Aspergillus*	Sediment	–	PP067977	–	–
Aspergillus pseudonomiae	B	A35	*Flavi*	Air	–	PP067973	–	–
Aspergillus subalbidus	B	B15	*Candidi*	Bat body	–	PP098272	–	–
		B17	*Candidi*	Bat body	–	PP098273	–	–
		CO10	*Candidi*	Omnivorous bat guano	–	PP098274	–	–
		CO11	*Candidi*	Omnivorous bat guano	–	PP098275	–	–
		FF41	*Candidi*	Sediment	–	–	PP150733	–
		FF52	*Candidi*	Sediment	–	–	PP150734	–
Aspergillus sydowii		B3	*Nidulantes*	Bat body	–	PP067966	–	–
		B4	*Nidulantes*	Bat body	–	PP067967	–	–
		CO1	*Nidulantes*	Omnivorous bat guano	–	PP067968	–	–
		CO13	*Nidulantes*	Omnivorous bat guano	–	PP067969	–	–
Aspergillus tamarii		Oi3	*Flavi*	Insectivore bat guano	–	PP067974	–	–
Aspergillus tritici	S/B	FF22	*Candidi*	Sediment	–	PP098270	–	–
		FF26	*Candidi*	Sediment	–	PP098271	–	–
Aspergillus ustus		Oi6	*Usti*	Insectivore bat guano	–	–	PP150735	–
Aspergillus wentii		A14	*Cremei*	Air	–	PP098276	–	–
		A28	*Cremei*	Air	–	PP098277	–	–
		B11	*Cremei*	Bat body	–	PP098278	–	–
Aspergillus sp. 1		A22	*Circumdati*	Air	–	PP158212	–	–
Aspergillus sp. 2		Ci2B	*Candidi*	Isectivorous bat guano	–	PP158210	–	–

Table 1. (Continued).

| Species	Record	Strains/isolates	Section	Substrates/hosts	GenBank accession numbers			
					ITS	BenA	CaM	RPB2
Aspergillus sp. 3		FF24	*Candidi*	Sediment	–	PP158211	–	–
Aspergillus sp. 4	S/B	Oi4	*Restricti*	Insectivorous bat guano	PP033025	–	–	–
Penicillium cecavii sp. nov.	S/B	URM 8656	*Cinnamopurpurea*	Air	PP034173	PP150743	PP150751	PP187791
		URM 8657	*Cinnamopurpurea*	Isectivorous bat guano	PP034174	PP150744	PP150752	PP187790
Penicillium chermesinum	B	M33	*Charlesia*	Air	–	PP098279	–	–
Penicillium cinnamopurpureum	S/B	MP35	*Cinnamopurpurea*	Air	–	PP098280	–	–
Penicillium citrinum		A11	*Citrina*	Air	–	PP098281	–	–
		A12	*Citrina*	Air	–	PP098282	–	–
		A33	*Citrina*	Air	–	PP098283	–	–
		A34	*Citrina*	Air	–	PP098284	–	–
		N14	*Citrina*	Bat body	–	PP098285	–	–
		FF23	*Citrina*	Sediment	–	PP098286	–	–
		FF312	*Citrina*	Sediment	–	PP098287	–	–
		B7	*Citrina*	Bat body	–	PP098288	–	–
		Oi6B	*Citrina*	Isectivorous bat guano	–	–	PP150736	–
		A16	*Citrina*	Air	–	PP098289	PP150737	–
		B21	*Citrina*	Bat body	–	PP098290	PP150738	–
Penicillium copticola	S/B	M18	*Lanata-Divaricata*	Air	–	PP098291	–	–
Penicillium echinulonalgiovense		MP59	*Lanata-Divaricata*	Air	–	PP098292	–	–
Penicillium sp. 1		WO1	*Lanata-Divaricata*	Omnivorous bat guano	–	PP158213	–	–
Penicillium sp. 2		FF42	*Lanata-Divaricata*	Sediment	–	PP158214	–	–
Penicillium sp. 3		OO2	*Sclerotiorum*	Omnivorous bat guano	–	PP191131	–	–
Talaromyces potiguarorum sp. nov.	S/B	URM 8664	*Talaromyces*	Insectivorous bat guano	PP034175	PP150745	PP150753	PP187794
		URM 8665	*Talaromyces*	Insectivorous bat guano	PP034176	PP150746	PP150754	PP187795
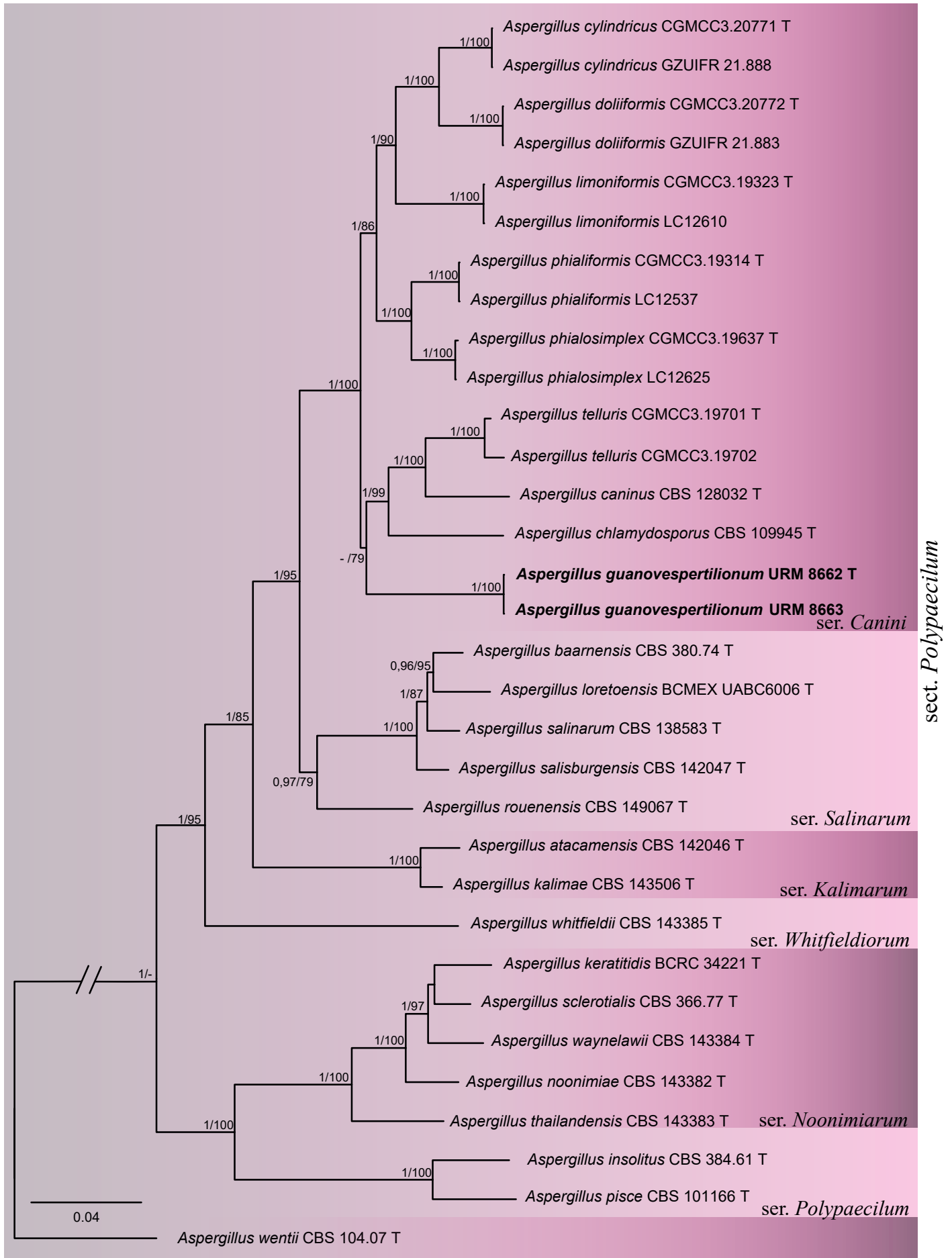
Fig. 2. Bayesian phylogenetic tree using ITS-*BenA-CaM-RPB2* sequences from species included in *Aspergillus* section *Polypaecilum*. The new species described in this study (*Aspergillus guanovespertilionum* URM 8862) is highlighted in **bold.** Ex-type strains = T. Values for ML-BS ≥ 70 % and BPP ≥ 0.95 are included next to nodes. The tree was rooted to *Aspergillus wentii* CBS 104.07.
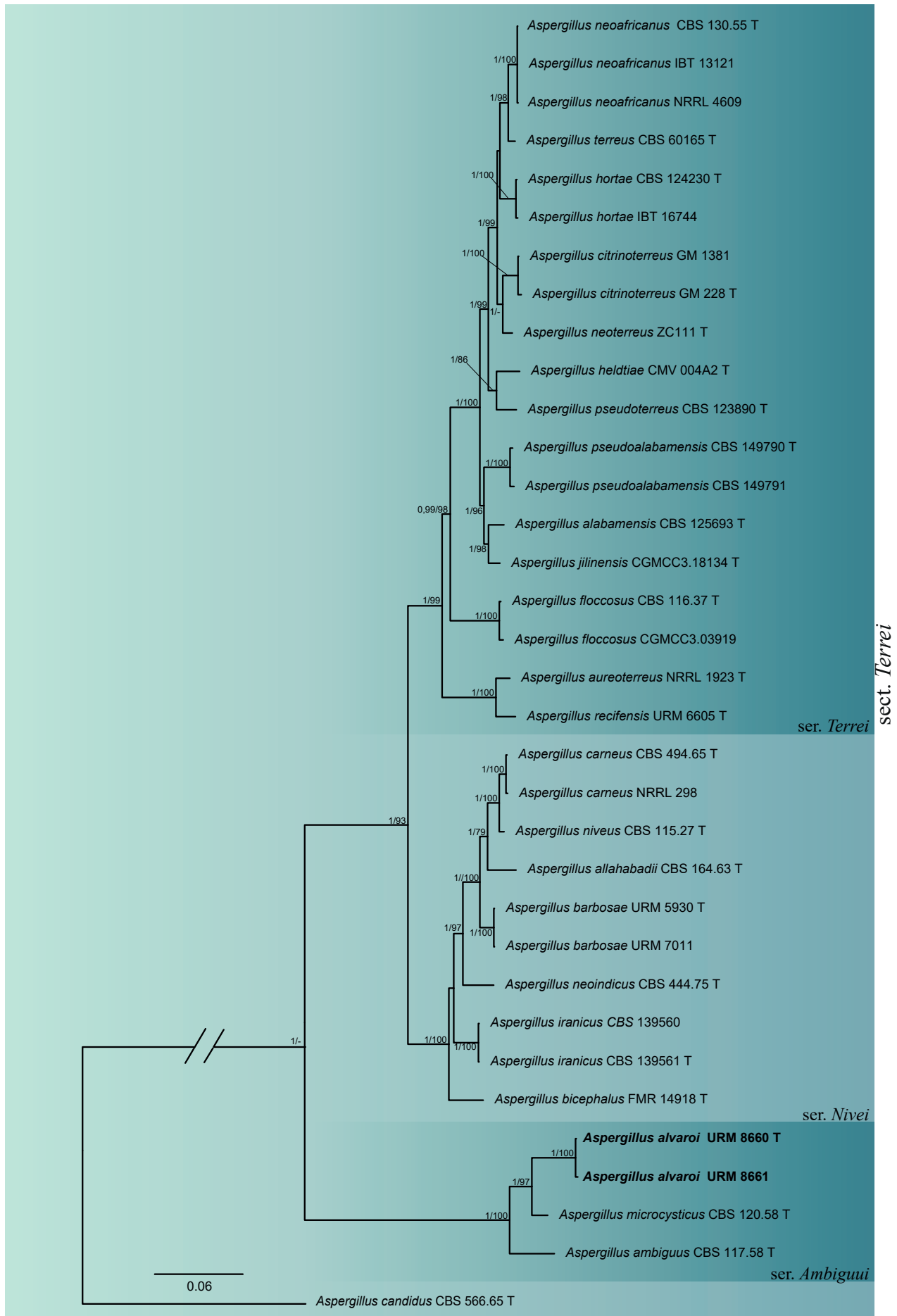
FUSE

Fig. 3. Bayesian phylogenetic tree using ITS-*BenA-CaM-RPB2* sequences from species included in *Aspergillus* section *Terrei*. The new species described in this study (*Aspergillus alvaroi* URM 8660) is highlighted in **bold.** Ex-type strains = T. Values for ML-BS ≥ 70 % and BPP ≥ 0.95 are included next to nodes. The tree was rooted to *Aspergillus candidus* CBS 566.65.
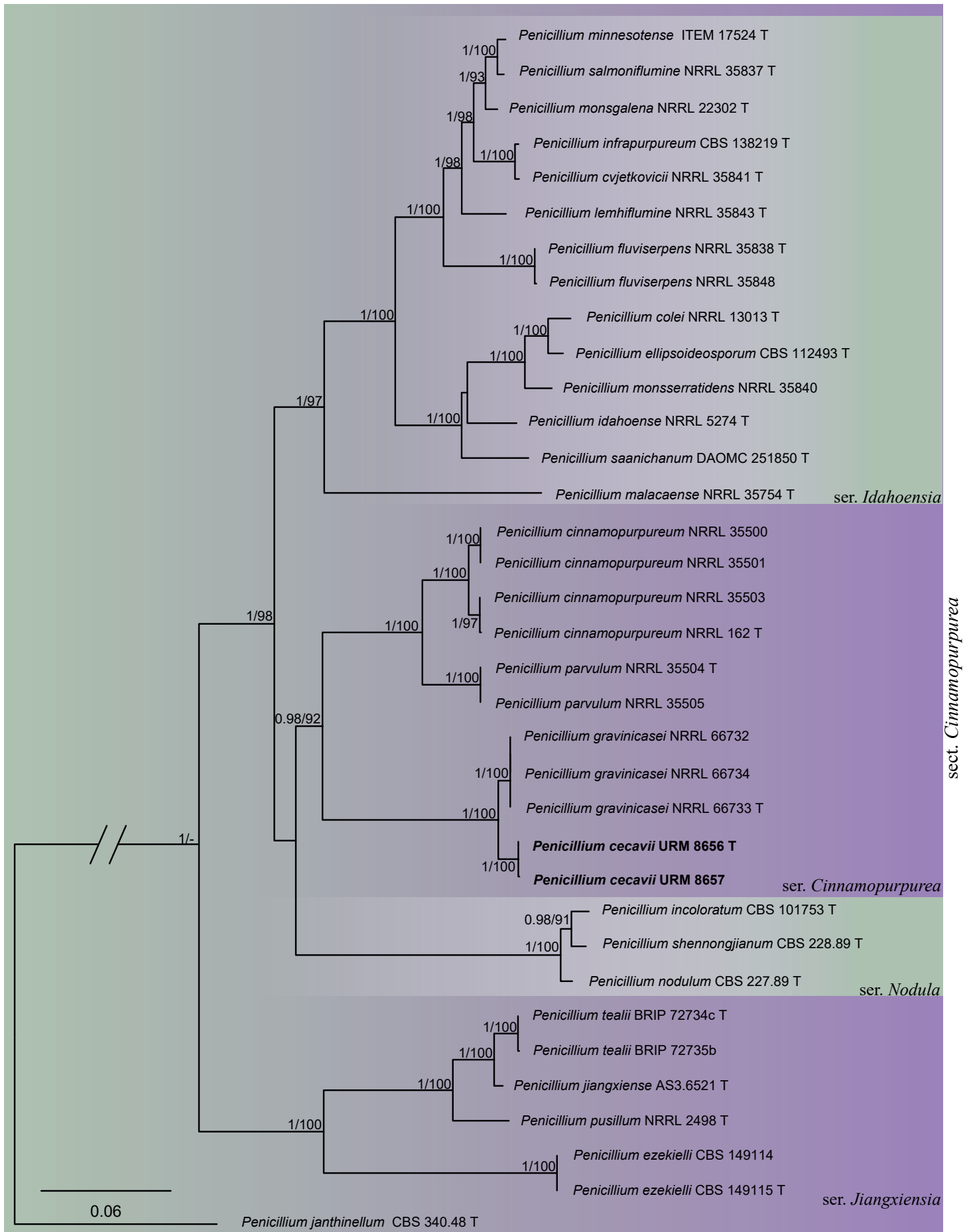
Fig. 4. Maximum likelihood tree using ITS-*BenA-CaM-RPB2* sequences from species included in *Penicillium* section *Cinnamopurpurea*. The new species described in this study (*Penicillium cecavii* URM 8656) is highlighted in **bold**. Ex-type strains = T. Values for ML-BS ≥ 70 % and BPP ≥ 0.95 are included next to nodes. The tree was rooted to *Penicillium janthinellum* CBS 340.48.

BI analysis was performed with 1×10^6 generations and a burn-in of 25 %, with chains sampled every 1 000 generations, and ML analysis with 1 000 bootstrap replicates. The best nucleotide model for BI analysis was estimated using MrModelTest v. 2.3 (Nylander 2004), and the GTR + I + G model was used for all ML analyses. The phylogenetic trees were visualised in FigTree v. 1.1.2 (Rambaut 2010), and edited in Adobe Illustrator v. 5.1. Bootstrap support values (BS-ML) greater than or equal to 70 % and Bayesian posterior probabilities (BPP) equal to or greater than 0.95 are shown above/below-supported nodes. Final alignments were deposited in TreeBASE (study S30837).

RESULTS

In total we obtained 86 isolates, of which 56 were identified as *Aspergillus*, *Penicillium*, and *Talaromyces*, and 30 belonging to other genera (*e.g. Blastobotrys, Cladosporium, Circinella, Fusarium, Hyphopichia, Humicola, Lecanicillium, Malbranchea, Metarhizium, Ovatospora, Pestalotiopsis,* and *Rhizomucor*) that will be reported on elsewhere. Among the 56 isolates, 18 were from guano, 14 from air, 10 from bats, and 14 from cave soil/sediment. At generic level, 34 isolates belong to *Aspergillus*, 20 to *Penicillium*, and two to *Talaromyces*. *Aspergillus* was the most diverse genus, with 18 species identified that belong to 10 sections (Table 1). Nine *Penicillium* species that belong to five sections were identified, and *Talaromyces* was represented by one species. Based on our analyses, four new species were discovered and are described below in the Taxonomy section, namely, two *Aspergillus* (*A. alvaroi sp. nov.* and *A. guanovespertilionum sp. nov.*), one *Penicillium* (*P. cecavii sp. nov.*), and one *Talaromyces* (*T. potiguarorum sp. nov.*) species. Of the 26 species identified, *A. montevidensis, A. tritici, P. cinnamopurpureum,* and *P. echinulonalgiovense*, along with the four new species, are newly reported from cave environments.

Phylogenetic analyses

Details of the combined datasets [number of species/sequences and length of datasets (bp)] and the best nucleotide models for ML and BI analyses are shown in Supplementary Table S1.

Section *Polypaecilum* — Using a combined matrix of ITS, *BenA*, *CaM*, and *RPB2*, isolates URM 8662 and URM 8663 grouped as an independent lineage (BPP = 1 and BS-ML = 100 %) in section *Polypaecilum* series *Canini*, as the closest relative of *A. chlamydosporus, A. caninus* and *A. telluris* (Fig. 2; Supplementary Fig. S1).
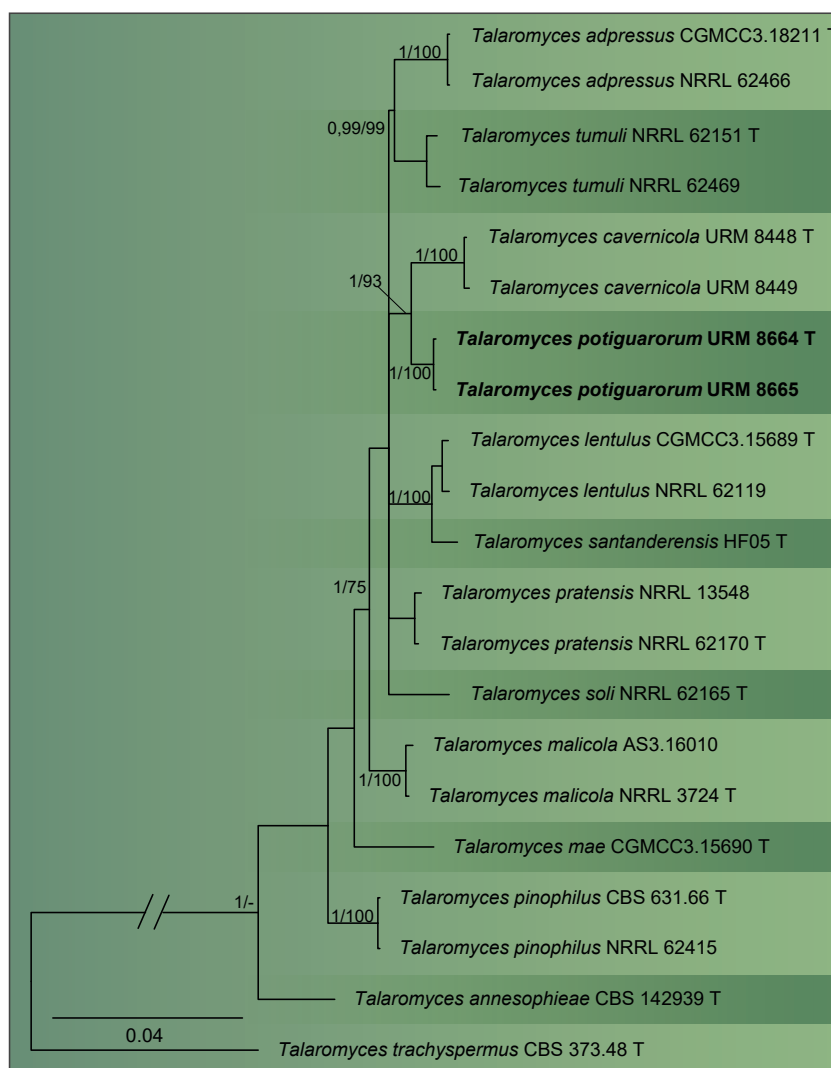

Fig. 5. Bayesian phylogenetic tree using *BenA-CaM-RPB2* sequences from species included in *Talaromyces* section *Talaromyces*. The new species described in this study (*Talaromyces potiguarorum* URM 8664) is highlighted in **bold**. Ex-type strains = T. Values for ML-BS ≥ 70 % and BPP ≥ 0.95 are included next to nodes. The tree was rooted to *Talaromyces trachyspermus* CBS 373.48.
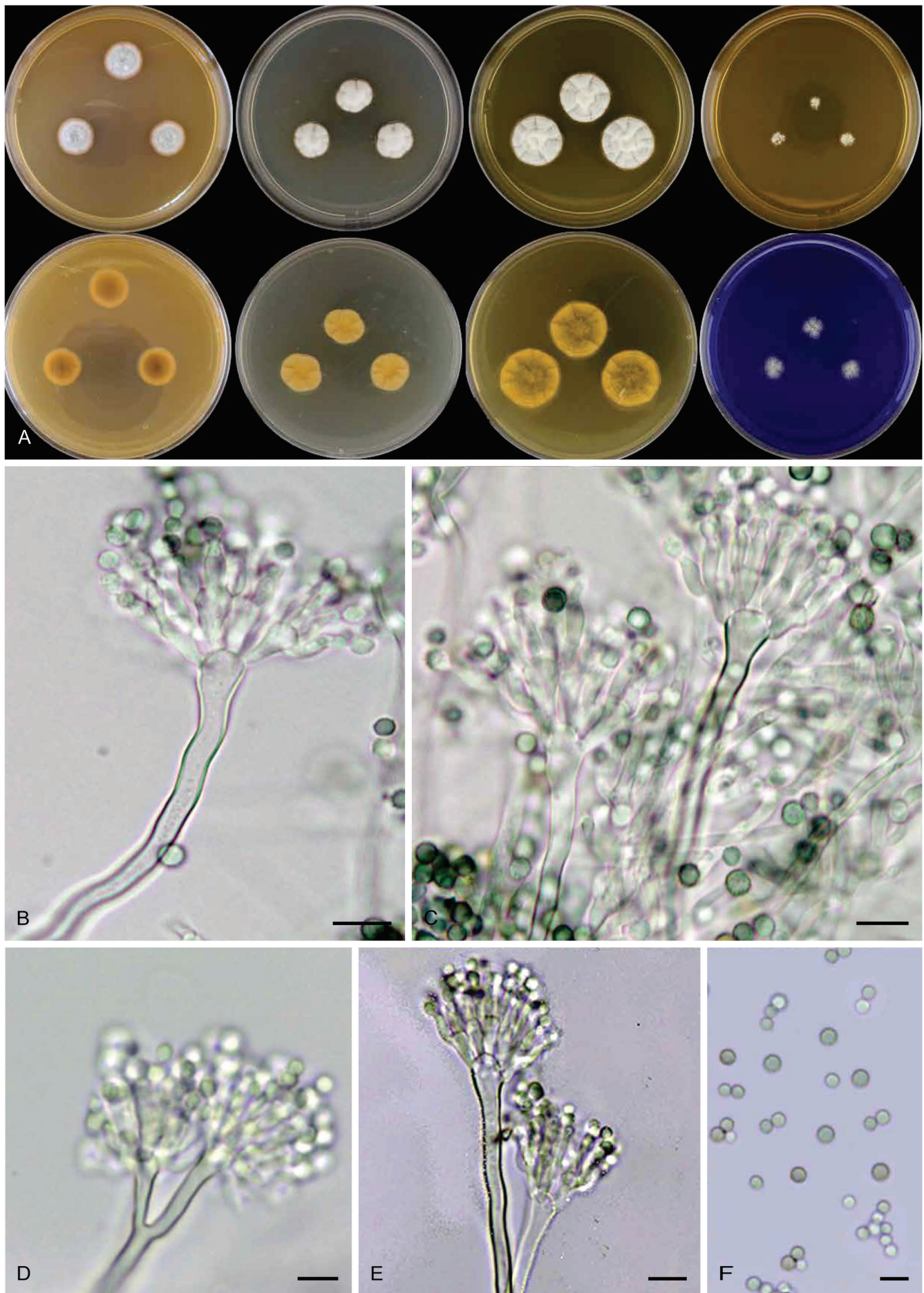
Fig. 6. *Aspergillus alvaroi* URM 8660, ex-type. **A.** Colonies from left to right (top row) MEA, CYA, YES, and DG18; (bottom row) reverse MEA, reverse CYA and reverse YES and CREA. **B–E.** Conidiophores and conidia. **F.** Conidia. Scale bars = 10 μm.

Section *Terrei* — Our phylogenetic analysis using a combined matrix of ITS, *BenA*, *CaM*, and *RPB2* resolved isolates URM 8660 and URM 8661 as a fully supported independent lineage (BPP = 1 and BS-ML = 100 %) in the section *Terrei* series *Ambigui*, with *A. ambiguus* and *A. microcysticus* its closest relatives (Fig. 3; Supplementary Fig. S2).

Section *Cinnamopurpurea* — In our ITS, *BenA*, *CaM*, and *RPB2* matrix, URM 8656 and URM 8657 were resolved as an independent lineage (BPP = 1 and BS-ML = 100 %) in section *Cinnamopurpurea* series *Cinnamopurpurea*, with *P. gravinicasei* its closest relative (Fig. 4; Supplementary Fig. S3).

Section *Talaromyces* — Based on our phylogenetic analysis using *BenA*, *CaM*, and *RPB2*, URM 8664 and URM 8665 belong to section *Talaromyces* as a unique species, closely related to *T. cavernicola* in the *T. pinophilus* species complex (Fig. 5; Supplementary Figs S4, S5).

Taxonomy

Aspergillus alvaroi J.M.S. Lima, R.N. Barbosa, J.D.P. Bezerra & Souza-Motta, ***sp. nov.*** MycoBank MB 851901. Fig. 6.

Etymology: In honour of the first author's grandfather, Álvaro Francisco da Silveira (in memoriam), who was a science teacher for 30 years in primary schools of the state of Pernambuco, Brazil.

Infrageneric classification: subgenus *Circumdati*, section *Terrei*, series *Ambigui*.

Typus: **Brazil**, Rio Grande do Norte state, Furna Feia cave (05°02′12′′S, 37°33′37′′W), isolated from sediment collected in a cave, Apr. 2020, *J.M.S. Lima* & *D. Bento* [**holotype** URM 95547 (slide preparation) is deposited in the URM fungarium (Recife, Brazil), culture ex-type URM 8660].

Conidiophores biseriate, stipes smooth, slight green pigmentation, aseptate, 31–390 × 2–4 μm. *Vesicle* piriform, 5–7.5 μm. *Metulae* 4–8 × 2.5–4 μm. *Phialides* 5–8.5 × 2–4 μm. *Conidia* globose to subglobose, smooth, greenish, 2.5–5 × 2.5–5 μm. Accessory conidia were not observed.

Colony diameter (7 d, in mm, in the dark): CYA 13–16; CYA 15 °C no growth; CYA 30 °C 24–25; CYA 37 °C 22–24; CYAS 15–16; CZ 9–10; CREA 10–11; DG18 11–13; MEA 15–16; MEA 15 °C no growth; MEA 30 °C 18–20; MEA 37 °C 20–22; OA 13–14; YES 20–21.

Culture characteristics: CYA 25 °C, 7 d: Colonies convex with ridges on margins; margins wavy; mycelium white, glaucous (73) along the margins; texture velutinous; sporulation poor; exudates absent, soluble pigments absent; reverse buff (45) to hazel (88). CYAS 25 °C, in 7 d: Colonies convex with furrows; margins entire; mycelium white; texture velutinous; sporulation poor; exudates absent; soluble pigments absent; reverse buff (45) to greyish sepia (106). CZ 25 °C, in 7 d: Colonies flat; margins wavy; mycelium inconspicuous; sporulation absent; exudates absent; soluble pigments absent; reverse slightly buff (45). CREA 25 °C, in 7 d: Poor growth, without acid production. DG18 25 °C, 7 d: Colonies crateriform; margins entire; mycelium white; texture velutinous to lightly floccose; sporulation strong; conidia colour *en masse* glaucous, grey (19); exudate absent; soluble pigments absent; reverse buff (45). MEA, 25 °C, in 7 d: Colonies slightly umbonate; margins entire; mycelium white; texture velutinous; sporulation along the margins; conidia colour *en masse* glaucous, sky blue (93); exudates absent; soluble pigments absent; reverse buff (45) to olivaceous (48) to umber (9). OA 25 °C, in 7 d: Colonies flat; margins entire; mycelium white; texture floccose; sporulation strong; conidia colour *en masse* dark bluish green (24); exudates absent; soluble pigments absent; reverse pistachio green (92). YES 25 °C, in 7 d: Colonies crateriform with grooves along the margins; margins entire; mycelium white, glaucous (73) along margins; texture velutinous; sporulation poor; exudates absent; soluble pigments absent; reverse fawn (87) to hazel (88).

Additional material examined: **Brazil**, Rio Grande do Norte state, Furna Feia cave (05°02′12′′S, 37°33′37′′W), isolated from sediment collected in a cave. Apr. 2020, *J.M.S. Lima* & *D. Bento* (culture URM 8661).

Notes: The phylogenetic analysis based on a combined ITS rDNA, *BenA*, *CaM*, and *RPB2* sequence dataset resolved *A. alvaroi sp. nov.* as an independent lineage related to *A. microcysticus* and *A. ambiguus* in section *Terrei* and series *Ambigui* (Fig. 3). Morphologically, conidia of *A. alvaroi* are greenish and globose like those of *A. microcysticus* (Sappa 1955). The conidiophores of *A. alvaroi* measure up to 390 μm in length, while those of *A. microcysticus* are 200 μm in length (Sappa 1955), and those of *A. ambiguus* are up to 240 μm in length (Klich 1993). *Aspergillus ambiguus* produces rough conidia on MEA (Klich 1993), while *A. alvaroi* produces smooth conidia. *Aspergillus microcysticus* produces amber exudates on CZ at 25 °C (Sappa 1955), while this characteristic was not observed in any culture of *A. alvaroi*.

Aspergillus guanovespertilionum J.M.S. Lima, R.N. Barbosa, J.D.P. Bezerra & Souza-Motta, ***sp. nov.*** MycoBank MB 851902. Fig. 7.

Etymology: Refers to the substrate — bat guano — from where this species was isolated.

Infrageneric classification: subgenus *Polypaecilum*, section *Polypaecilum*, series *Canini*.

Typus: **Brazil**, Sergipe state, Urubu cave (10°43′58.1″S, 37°09′56.0″W), isolated from hematophagous bat guano collected in a cave, Aug. 2019, *J.M.S. Lima* & *E. Barbier* [**holotype** URM 95545 (slide preparation) is deposited in the URM fungarium (Recife, Brazil), culture ex-type URM 8662].

Conidiophores solitary phialides borne laterally or terminally on vegetative hyphae. *Phialides* monophialidic, hyaline, cylindrical, 1.5–13 × 0.5–2.5 μm. *Conidia* solitary or in small heads, and occasionally in short chains, hyaline, smooth, subglobose, ellipsoidal or pear-shaped, 3.5–5 × 2.5–5.5 μm diam. *Chlamydospores* on long, branched stalks, hyaline, smooth and thick-walled, globose, subglobose and ellipsoidal, 10.5–21 μm diam.

Colony diameter (7 d, in mm, in the dark): CYA 12–14; CYA 15 % NaCl no growth; DG18 8–9; MEA 10–12; MEA 37 °C no growth; MEA NaCl 5 % 13–14; MEA NaCl 10 % 2–5; MEA NaCl 15 % 2–7;
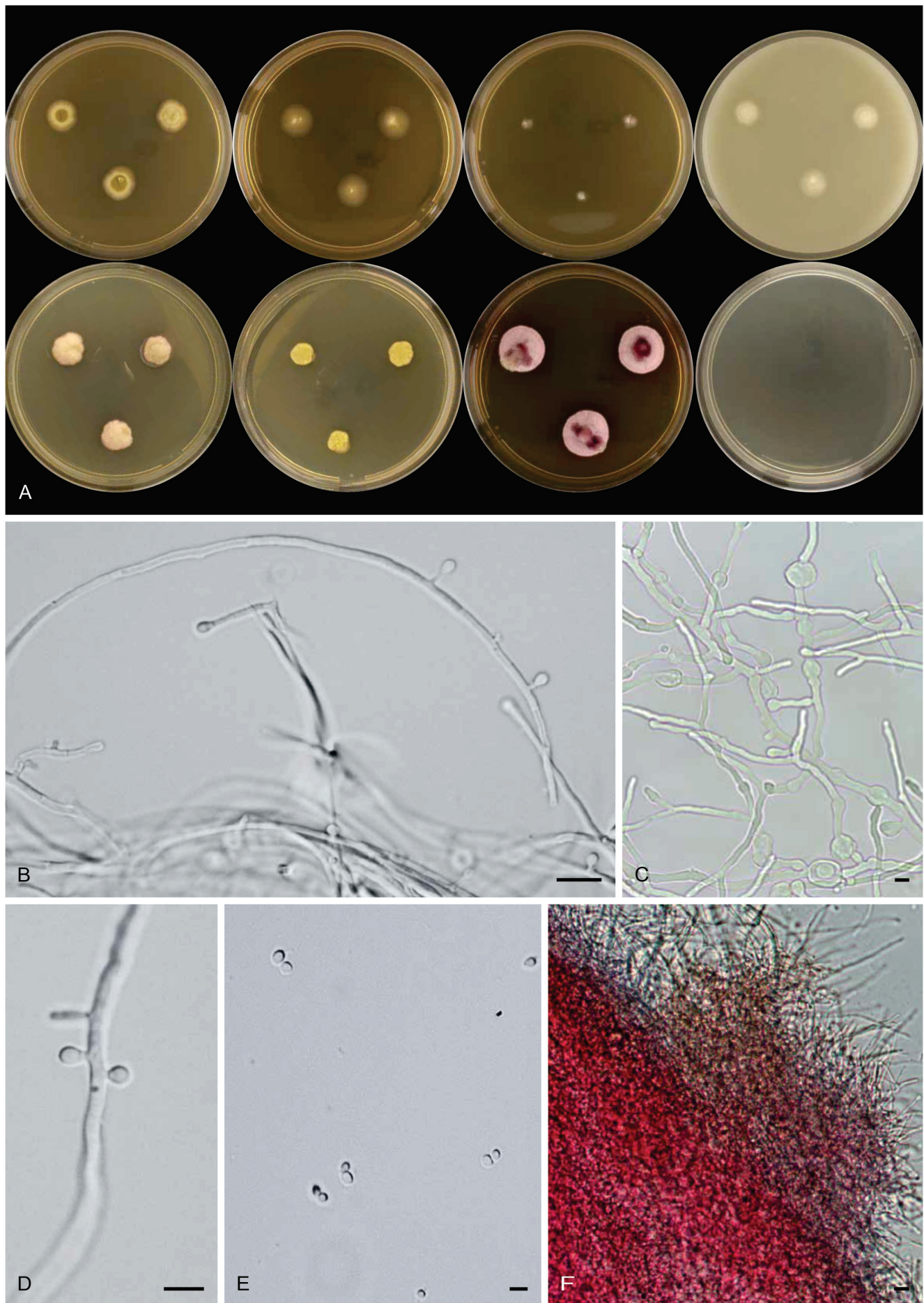
Fig. 7. *Aspergillus guanovespertilionum* URM 8662, ex-type. **A.** Colonies from left to right (top row) MEA, MEA 40 % G, MEA 20 % NaCl, and OA; (bottom row) CYA, DG18, YES and CYA 5 % NaCl. **B, D, E.** Phialides and conidia. **C.** Chlamydospores. **F.** Mycelium (hyphae). Scale bars = 10 μm.

MEA NaCl 20 % 3–5; MEA G 25 % 15–16; MEA G 30 % 12–13; MEA G 35 % 12–14; MEA G 40 % 11–12; MY10-12 10–11; OA 8–9; YES 17–18.

Culture characteristics (25 °C, 7 d): CYA: Colonies convex; margins wavy; mycelium white to citrine green (67); texture floccose; sporulation absent; exudates absent; soluble pigments absent; reverse rosy vinaceous (58). DG18: Colonies flat; margins wavy; mycelium pale luteous (11); texture velutinous; sporulation absent; exudates absent; soluble pigments absent; reverse ochreous (44). MEA: Colonies flat; margins entire; mycelium inconspicuous; texture velutinous; sporulation poor in the centre, pale luteous (11); exudates absent; soluble pigments absent; reverse saffron (10) to umber (9). MEA 25 % G: Colonies flat; margins entire; mycelium inconspicuous; texture velutinous; sporulation poor in the centre; conidia colour *en masse* pale luteous (11); exudates absent; soluble pigments absent; reverse ochreous (44). MEA 30 % G: Colonies flat; margins entire; mycelium inconspicuous; texture velutinous; sporulation poor in the centre; conidia colour *en masse* pale luteous (11); exudates absent; soluble pigments absent; reverse ochreous (44). MEA 35 %: Colonies flat; margins entire; mycelium inconspicuous; texture velutinous; sporulation poor in the centre; conidia colour *en masse* pale luteous (11); exudates absent; soluble pigments absent; reverse ochreous (44). MEA 40 % G: Colonies flat; margins entire; mycelium inconspicuous; texture velutinous; sporulation poor in the centre; conidia colour *en masse* pale luteous (11); exudates absent; soluble pigments absent; reverse ochreous (44). MY10-12: Colonies slightly raised; margins entire; mycelium white; texture floccose; sporulation absent; exudates absent; soluble pigment vinaceous (57); reverse salmon (41) to vinaceous (57). OA: Colonies flat; margins entire; mycelium white; texture floccose; sporulation absent; exudates absent; soluble pigments absent; reverse white. YES: Colonies convex; margins entire; mycelium greyish-rose (55) to dark vinaceous (82) in the centre; texture floccose; sporulation poor; exudates absent; soluble pigments blood coloured (3); reverse red (2) to blood coloured (3).

Additional material examined: **Brazil**, Sergipe state, Urubu cave (10°43′58.1″S, 37°09′56.0″W), isolated from hematophagous bat guano collected in a cave, Aug. 2019, *J.M.S. Lima* & *E. Barbier* (culture URM 8663).

Notes: Phylogenetic analyses based on ITS rDNA, *BenA*, *CaM*, and *RPB2* sequences indicated that *A. guanovespertilionum sp. nov.* is a unique lineage in section *Polypaecilum* series *Canini*, and resolves closest to *A. chlamydosporus*, *A. caninus*, and *A. telluris* (Fig. 2). Morphologically, *A. guanovespertilionum* and *A. chlamydosporus* have low sporulation capacity and produce chlamydospores. The chlamydospores of *A. guanovespertilionum* are larger than those of *A. chlamydosporus* (10.5–21 *vs* 5–8 × 4–7 μm), and *A. guanovespertilionum* has smooth conidia, while *A. chlamydosporus* has smooth to rough conidia (Gené *et al.* 2003). *Aspergillus guanovespertilionum* differs in slower growth rates on OA at 25 °C (8–9 *vs* 11–13 mm) compared to *A. chlamydosporus*. Differently from *A. chlamydosporus*, *A. caninus*, and *A. telluris*, *A. guanovespertilionum* does not have the ability to grow at 37 °C. In addition, *A. guanovespertilionum* produced a red soluble pigment in YES and MY10-12, while *A. chlamydosporus*, *A. caninus*, and *A. telluris* do not produce

soluble pigments of this colour (Tanney *et al.* 2017, Sun *et al.* 2020).

Penicillium cecavii J.M.S. Lima, R.N. Barbosa, J.D.P. Bezerra & Souza-Motta, ***sp. nov.*** MycoBank MB 851903. Fig. 8.

Etymology: Reflects the name of the Brazilian federal institution dedicated to the conservation and study of caves, the Centro Nacional de Pesquisa e Conservação de Cavernas (National Centre for Research and Conservation of Caves), whose acronym is CECAV.

Infrageneric classification: subgenus *Aspergilloides*, section *Cinnamopurpurea*, series *Cinnamopurpurea*.

Typus: **Brazil**, Sergipe state, Urubu cave (10°43′58.1″S, 37°09′56.0″W), isolated from the air in a cave, Aug. 2019, *J.M.S. Lima* & *E. Barbier* [**holotype** URM 95543 (slide preparation) is deposited in the URM fungarium (Recife, Brazil), culture ex-type URM 8656].

Conidiophores monoverticillate, occasionally biverticillate, stipes smooth, 20.5–101.5 × 2–2.5 μm. *Vesicle* 4.5–5 μm. *Phialides* 2–5, ampulliform, 5.5–8.5 × 1.5–3 μm. *Conidia* smooth-walled, greenish, globose, 1.5–2.5 μm diam.

Colony diameter (7 d, in mm, in the dark): CYA 20–21; CYA 15 °C 5–8; CYA 30 °C 18–22; CYA 37 °C no growth; CYAS 20–21; CZ 10–12; CREA 5–10; DG18 17–18; MEA 16–18; MEA 15 °C 4–5; MEA 30 °C 15–18; MEA 37 °C no growth; OA 12–13; YES 20–22.

Culture characteristics: CYA 25 °C, 7 d: Colonies crateriform, slightly furrowed; margins entire, regular; mycelium white; texture velutinous to lightly floccose; sporulation dense; conidia colour *en masse* greenish grey (110); exudate absent; soluble pigment absent; reverse brown vinaceous (84) to sepia (63). CYAS 25 °C, 7 d: Colonies flat; margins entire, regular; mycelium white; texture floccose; sporulation dense; conidia colour *en masse* greenish grey (110); exudate absent; soluble pigment absent; reverse primrose (66) to dark brick (60). CZ 25 °C, 7 d: Colonies flat; margins irregular; mycelium discrete lavender grey (125); texture velutinous; sporulation moderate; conidia colour *en masse* malachite green (72); exudate absent; soluble pigment absent; reverse vinaceous buff (86) to fawn (87). CREA 25 °C, 7 d: moderate growth, acid not produced. DG18 25 °C, 7 d: Colonies convex; margins entire, regular; mycelium white; texture cottony; sporulation indeterminate along margins; conidia colour *en masse* indeterminate to greenish, glaucous (91); exudate absent; soluble pigment absent; reverse salmon (41) to pale olivaceous grey (120), umber (9). MEA 25 °C, 7 d: Colonies flat; margins entire, regular; mycelium white; texture velutinous; sporulation dense; conidia colour *en masse* greenish grey (110); exudate absent; soluble pigment absent; reverse dull green (70) to fawn (87). OA 25 °C, 7 d: Colonies flat; margins entire, regular; mycelium white; texture velutinous to lightly floccose; sporulation dense; conidia colour *en masse* greenish grey (110); exudate absent; soluble pigment absent. YES 25 °C, 7 d: Colonies moderately deep, radially and concentrically sulcate; margins entire, regular; mycelium white; texture velutinous to lightly floccose; sporulation dense; conidia colour *en masse* greenish grey (110); exudate absent; soluble pigment absent; reverse buff (45) to hazel (88).
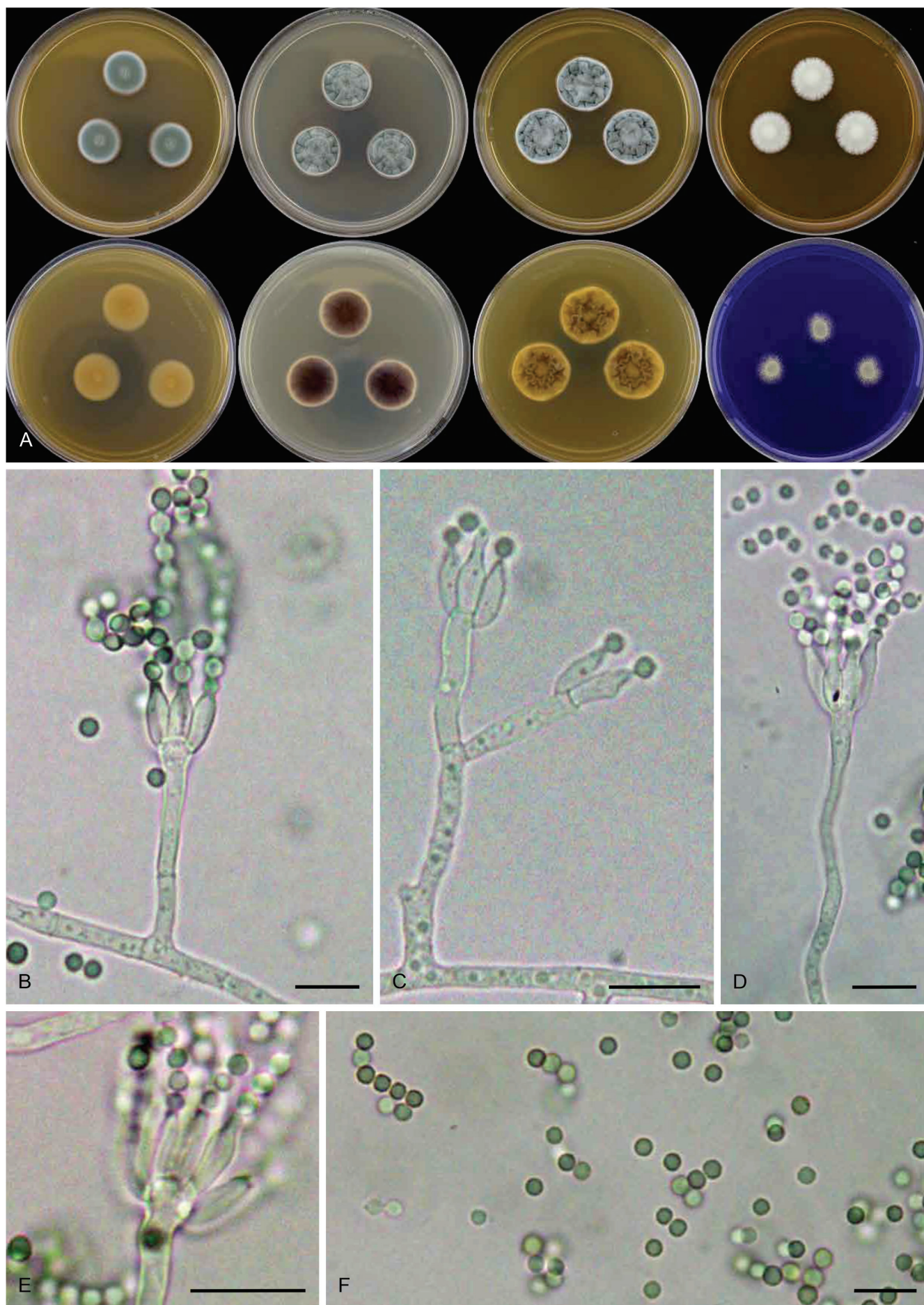
Fig. 8. *Penicillium cecavii* URM 8656, ex-type. **A.** Colonies from left to right (top row) MEA, CYA, YES, and DG18; (bottom row) reverse MEA, reverse CYA and reverse YES and CREA. **B–E.** Conidiophores and conidia. **F.** Conidia. Scale bars = 10 μm.

Additional material examined: **Brazil**, Sergipe state, Urubu cave (10°43′58.1″S, 37°09′56.0″W), isolated from the air of a cave, Aug. 2019, *J.M.S. Lima* & *E. Barbier* (culture URM 8657).

Notes: Multi-locus phylogenetic analyses (Fig. 4) indicate that *P. cecavii sp. nov.* forms a unique and well-supported lineage closely related to *P. gravinicasei* in section *Cinnamopurpurea* and series *Cinnamopurpurea*. According to Houbraken *et al*. (2020), conidiophores of the species in this section are commonly monoverticillate and shorter than 50 μm in length; however, *P. cecavii* also presented conidiophores of 20.5–101.5 μm in length. *Penicillium cecavii* differs from *P. gravinicasei* in a faster growth rate in CYA at 25 °C (20–22 *vs* 17–18 mm) and in the absence of exudates in CYA and OA (Anelli *et al*. 2018).

Talaromyces potiguarorum J.M.S. Lima, R.N. Barbosa, J.D.P. Bezerra & Souza-Motta**, *sp. nov*.** MycoBank MB 851904. Fig. 9.

Etymology: In reference to the Potiguara indigenous people of the state of Rio Grande do Norte in Brazil where the species was first collected.

Infrageneric classification: section *Talaromyces.*

Typus: **Brazil**, Rio Grande do Norte state, Furna Feia cave (05°02′12′′S, 37°33′37′′W), isolated from insectivorous bat guano, in Aug. 2019, *J.M.S. Lima* & *E. Barbier* [**holotype** URM 95549 (slide preparation) is deposited in the URM fungarium (Recife, Brazil), culture ex-type URM 8664].

Conidiophores biverticillate, hyaline to slight green pigmentation; stipes smooth-walled, 27–261 × 2–2.5 μm. *Metulae* 3–5, 7.5–10.5 × 2.5 μm. *Phialides* 3–4, acerose, 10.5–13 × 2.5 μm. *Conidia* smooth-walled, globose to subglobose, greenish, 2–3 × 2–3 μm. *Ascomata* not observed.

Colony diameter (7 d, in mm, in the dark): CYA 35–36; CYA 15 °C no growth; CYA 30 °C 40–44; CYA 37 °C 25–27; CYAS no growth; CZ 31–34; CREA 27–28; DG18 8–9; MEA 37–40; MEA 15 °C no growth; MEA 30 °C 45–50; MEA 37 °C 25–26; OA 28–30; YES 34–38.

Culture characteristics: CYA 25 °C, 7 d: Colonies flat; margins entire; mycelium pure yellow (14) or pale luteous (11); texture velutinous; sporulation sparse; conidia colour *en masse* indeterminate; exudates absent; soluble pigments absent; reverse salmon (41) to umber (9). CZ 25 °C, in 7 d: Colonies flat; margins branched; mycelium inconspicuous lavender grey (125); texture floccose; sporulation absent; exudates absent; soluble pigments absent; reverse pale. CREA 25 °C, in 7 d: Strong growth, strong acid production. DG18 25 °C, 7 d: Colonies flat; margins entire; mycelium white; texture floccose; sporulation moderate; conidia colour *en masse* pistachio green (92) to pure yellow (14); exudates absent; soluble pigments absent; reverse salmon (41) to ochreous (44). MEA, 25 °C, 7 d: Colonies flat; margins entire; mycelium white to sulphur yellow (15) with citrine (13) in the centre; texture floccose; sporulation poor in the centre; conidia colour *en masse* greenish olivaceous (90); exudates absent; soluble pigments absent; reverse ochreous (44). OA 25 °C, 7 d: Colonies flat; margins entire; mycelium citrine green (67) to (15) sulphur yellow; texture floccose to funiculose; sporulation poor in the centre; conidia colour *en masse* greenish olivaceous (90); exudates absent; soluble pigments absent; reverse straw coloured (46). YES, 25 °C 7 d: Colonies flat, sulcate; margins entire; mycelium white to citrine green (67); texture floccose; sporulation absent; exudates absent; soluble pigments absent; reverse salmon (41) to umber (9).

Additional material examined: **Brazil**, Rio Grande do Norte state, Furna Feia cave (05°02′12′′S, 37°33′37′′W), isolated from insectivorous bat guano collected in a cave, Aug. 2019, *J.M.S. Lima* & *E. Barbier* (culture URM 8665).

Notes: Our multi-locus phylogenetic analysis (Fig. 5) indicates that *T. potiguarorum sp. nov.* forms a unique and well-supported lineage with *T. cavernicola* as its closest relative in the *T. pinophilus* species complex. Morphologically, *T. potiguarorum* differs from *T. cavernicola* in the length of its phialides (13 *vs* 9 μm), in the absence of exudates on CYA and OA at 25 °C, and faster growth rate on CZ (31–34 *vs* 18–19 mm diam) (Alves *et al*. 2022). In addition, on OA, *T. potiguarorum* has a colony with a floccose to funiculose texture, while *T. cavernicola* has a cottony texture.

DISCUSSION

This is the first mycological study of the Urubu and Furna Feia bat caves, located in the Atlantic Forest and Caatinga dry forest biomes, respectively, in the Northeast region of Brazil. The data presented here contribute to the inclusion of new information on the richness of *Aspergillus*, *Penicillium*, and *Talaromyces* in Brazilian biomes (Barbosa *et al*. 2020, Barbosa *et al*. 2022). *Aspergillus* was the most frequent (63 %) and had the highest number of taxa on all substrates, followed by *Penicillium* (32 %) and *Talaromyces* (3 %). Our results are similar to those of previous studies that have also reported *Aspergillus*, *Penicillium*, and *Talaromyces* as the most abundant fungi in cave environments worldwide (Nováková 2009, Vanderwolf *et al*. 2013, Man *et al*. 2015, Zhang *et al*. 2017, Chlebicki & Jakus 2019, Cunha *et al*. 2020, Visagie *et al*. 2020, Zhang *et al*. 2020, Jurado *et al*. 2021, Sanchez-Moral *et al*. 2021, Visagie *et al*. 2021, Alves *et al*. 2022).

Caves with high levels of organic matter, such as guano, are excellent reservoirs of fungi (Wasti *et al*. 2021). In our study, 18 species of *Aspergillus* distributed in 10 sections were identified (*Aspergillus*, *Candidi*, *Circumdati*, *Cremei*, *Flavi*, *Nidulantes*, *Restricti*, *Polypaecilum*, *Terrei*, and *Usti*), having the highest number of fungal isolates in the substrates bat guano and soil/sediment. Bat guano has a unique geochemistry (Wurster *et al*. 2015), as it is initially a moist and alkaline substrate and later becomes more acidic and drier, making it preferable for some microbiological communities (Ferreira & Martins 1999, Moulds 2004). In addition, as highlighted by Karkun *et al*. (2012), the presence of *Aspergillus* and *Penicillium* in guano can also be attributed to a significant amount of partially digested cellulose, lignin, and pectin in the bat's alimentary tract.

In contrast to other studies that showed *Penicillium* as the most common in guano and soil/sediment from caves (Novaková 2009, Man *et al*. 2015, Ogórek *et al*. 2016, Zhang *et al*. 2020, Wasti *et al*. 2021), our results highlighted *Aspergillus* as the most abundant genus. For example, in a cave complex located in an arid region in Azerbaijan, *Aspergillus* was the most common in sediment (Mazina *et al*. 2023). In Brazilian caves, the presence of this genus is noted in different substrates (Taylor *et al*. 2013,
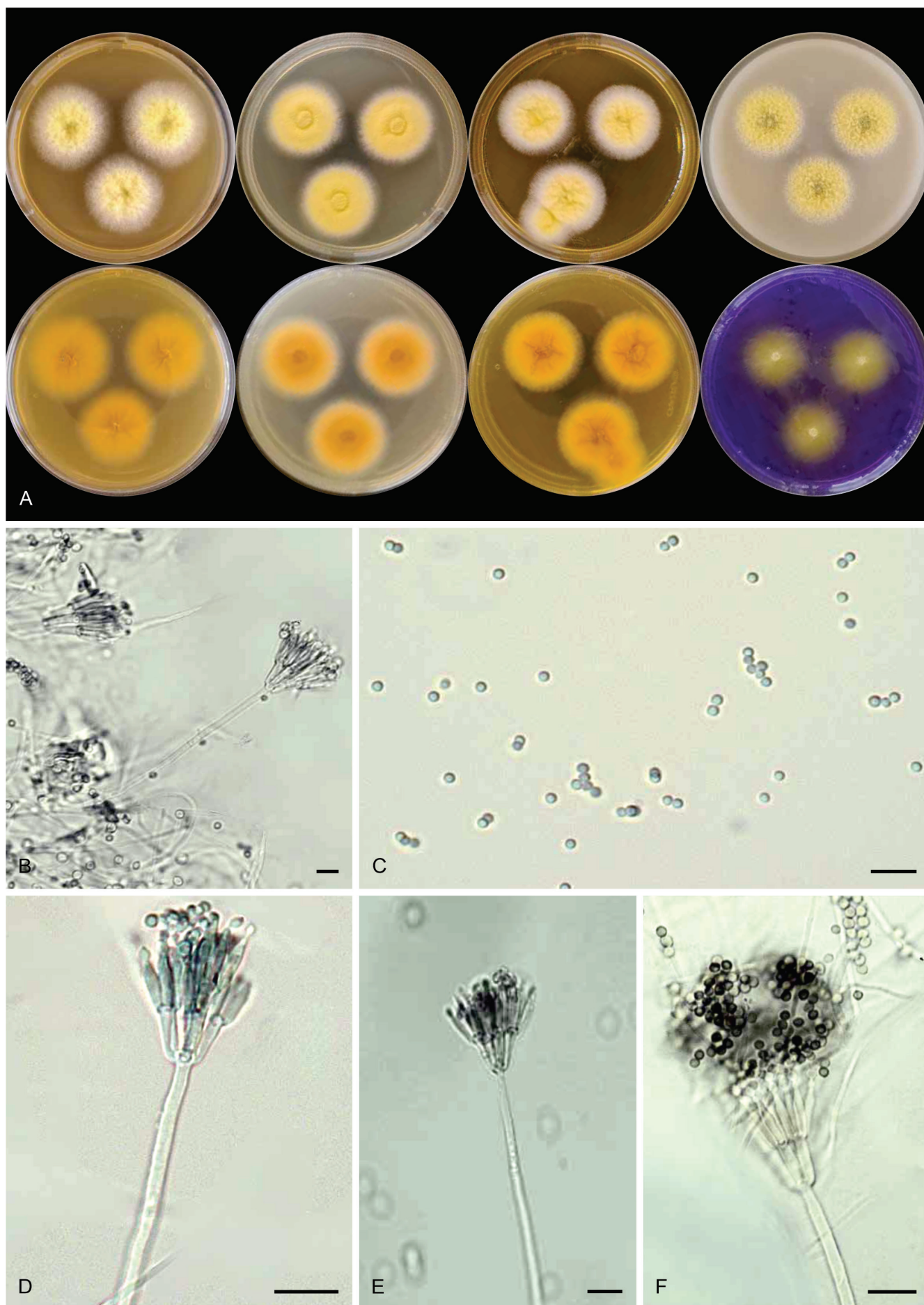
Fig. 9. *Talaromyces potiguarorum* URM 8664, ex-type. **A.** Colonies from left to right (top row) MEA, CYA, YES, and OA; (bottom row) reverse MEA, reverse CYA and reverse YES and CREA. **B, D–F.** Conidiophores. **C.** Conidia. Scale bars = 10 μm.

Paula *et al*. 2016, Alves *et al*. 2022). These results may be due to the fact that species in this genus have the capacity to live in different environmental conditions, including dry environments and substrates (Mishustin & Pushkinskaya 1960, Klich 2002, Barbosa *et al*. 2020). Christensen *et al*. (2000) showed that the occurrence of *Aspergillus* and *Penicillium* in soils varies regionally, with *Aspergillus* being more common in soils of tropical regions.

Penicillium has a wide distribution and is considered to be one of the most common genera in indoor environments (Visagie *et al*. 2014). In caves, this genus is commonly reported in the most varied substrates in countries such as Brazil, Canada, China, and Malaysia (Cunha *et al*. 2020, Visagie *et al*. 2020, Zhang *et al*. 2020, Wasti *et al*. 2021). In our study, 20 isolates of *Penicillium* were identified and grouped into five sections and nine species, the vast majority of which were isolated as airborne fungi from caves. In bat hibernation caves in Poland, Borzecka *et al*. (2021) and Kokurewicz *et al*. (2016) reported that abiotic conditions and bats influenced the concentration of airborne fungi, with the genus *Penicillium* being the most frequent. The production of small conidia that are easily dispersed in the air (Stupar *et al*. 2023), as well as the influence of the external environment (Borzęcka *et al*. 2021), may also be the reason for the predominance of this genus as airborne fungi in some caves. The presence of *P. citrinum* and *P. copticola*, isolated in this study as airborne fungi and from bodies of bats, suggests that bats and air currents in caves may be determining factors in species composition, similar to those observed by Cunha *et al.* (2020) in a Brazilian cave.

Among the species isolated in this study, *A. bertholletiae* was only reported in caves in Brazil and Sri Lanka (Cunha *et al*. 2020, Silva *et al*. 2021), and *P. copticola* in caves in Brazil and China (Zhang *et al*. 2020, Alves *et al*. 2022). The other species of *Aspergillus* and *Penicillium* are commonly reported in association with numerous substrates in caves in different regions, being *A. ustus* considered one of the most common species in the cave environment worldwide (Vanderwolf *et al*. 2013). Another example is *A. flavus*, which was the most abundant species in sediment from a Brazilian cave (Taylor *et al*. 2014) and was also reported as the most dominant species found on bats in Australia (Holz *et al*. 2018). *Aspergillus subalbidus* and *A. sydowii* were the most abundant species in a cave in Botswana (Visagie *et al*. 2021), similar to the results found in our study. In Brazilian caves, there have been few reports of *Talaromyces*, including species obtained from the body of bats (Cunha *et al*. 2020) and from air (Alves *et al*. 2022). In this study, only one species of *Talaromyces* was identified and described as new (*T. potiguarorum*).

Information on the species richness of *Aspergillus*, *Penicillium*, and *Talaromyces* from tropical caves, such as those we have studied in Brazil, has revealed the presence of species mainly restricted to surface environments and with specialised ecological relationships (Cunha *et al*. 2020, Alves *et al*. 2022, Carvalho *et al*. 2022). The data reported here from Brazilian caves may help us to understand the origin of cave fungi (Zhang *et al*. 2018) and also support the argument that caves are a hotspot of mycological diversity, as is observed for obligate stygobitic and troglobitic species (Silva & Ferreira 2016). Our study revealed 26 species of *Aspergillus*, *Penicillium*, and *Talaromyces* in caves, eight of which were reported for the first time in cave environments (*Aspergillus alvaroi sp. nov.*, *Aspergillus guanovespertilionum sp. nov.*, *Aspergillus montevidensis*, *Aspergillus tritici*, *Penicillium cecavii sp. nov.*, *Penicillium cinnamopurpureum*, *Penicillium*

echinulonalgiovense, and *Talaromyces potiguarorum sp. nov.*). The importance of speleomycology lies not only in estimating the national and global diversity of fungi but also in fungal taxonomy and ecology, as well as in the conservation and management of caves with tourist potential in the country.

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

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

Fig. S1. Maximum likelihood trees using an independent dataset of ITS, *BenA*, *CaM*, and *RPB2* of species included in *Aspergillus* section *Polypaecilum*.

Fig. S2. Maximum likelihood trees using an independent dataset of ITS, *BenA*, *CaM*, and *RPB2* of species included in *Aspergillus* section *Terrei*.

Fig. S3. Maximum likelihood trees using an independent dataset of ITS, *BenA*, *CaM*, and *RPB2* of species included in *Penicillium* section *Cinnamopurpurea*.

Fig. S4. Bayesian phylogenetic trees using sequences of *BenA-CaM-RPB2* of species included in *Talaromyces* section *Talaromyces*.

Fig. S5. Maximum likelihood trees using an independent dataset of ITS, *BenA*, *CaM*, and *RPB2* of species included in *Talaromyces* section *Talaromyces*.

Table S1. Details of the combined datasets (number of species/sequences and length of datasets (bp)) and the best nucleotide models for BI analysis.