



Two new *Penicillium* section *Sclerotiorum* species from sugarcane soil in Brazil

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Abstract

Two new species were isolated during a survey on the mycobiota of soil of sugarcane fields in a deforested area of Atlantic Forest in Pernambuco, northeastern Brazil. Using a polyphasic approach, combining ITS, partial β -tubulin, calmodulin, and RPB2 gene sequences and morphological features, we described the new species *Penicillium barbosae* sp. nov. (URM 7705^T) and *Penicillium limae* sp. nov. (URM 7706^T), both belonging to section *Sclerotiorum*, series *Adametziarum*. Descriptions based on morphological features are provided and these data show that the species differ from their phylogenetically closely related relatives. Both new species produce monoverticillate conidiophores and globose to subglobose shaped conidia. *Penicillium barbosae* is phylogenetically related to *P. bilaiae*; however, *P. barbosae* attains a colony diameter of 37–38 mm at 25 °C on Czapek yeast extract agar (CYA), is unable to grow at 30 and 37 °C, and produces sclerotia on oatmeal agar. In contrast, the colony diameter of *P. bilaiae* on CYA at 25 °C is 25–33 mm and is able to grow at 30 °C and 37 °C, and sclerotia production is not reported. *Penicillium limae* is related to *P. restingae*. The former species does not grow at 37 °C, in contrast to the latter. Furthermore, *P. limae* grows faster on CYA (22–32 mm vs 18–27 mm), malt extract agar (23–32 vs 16–23 mm), and dichloran 18% glycerol agar (35–40 vs 9–16 mm). The description of these new species increases our knowledge on *Penicillium* biodiversity in tropical agricultural soils.

Keywords *Aspergillaceae* · Biodiversity · Soil fungi · Tropical ecosystem

Introduction

Soil is one of nature's most complex ecosystems and one of the most diverse substrates on earth, resulting from the interactions of climate, relief, organisms, and organic material,

being an excellent microbial habitat, housing several microorganisms, including fungi (Prade et al. 2007; Abreu and Pfenning 2008; Ponge 2015). Fungi are found in communities usually ranging from 10⁴ to 10⁶ colony forming units per gram of soil (Blackwell 2011) and experience less competition in acidic environments (Brandão 1992).

Brazil is the largest producer of sugarcane (*Saccharum officinarum* L.) in the world and this crop is mainly used for sugar and ethanol production (Cheavegatti-Gianotto et al. 2011; Bordonal et al. 2018). In the northeastern region of Brazil, areas of the Atlantic Forest biome were deforested to give rise to the cultivation of sugarcane (Moraes et al. 2016) and the coastal zone of the Pernambuco state is an ideal region for this monoculture due to its favorable climatic and soil condition (Pereira and Alves 2007).

A limited number of studies have analyzed the fungi present in soil of sugarcane plantations in Brazil. Barros (2012) evaluated the fungal community present in soils of sugarcane plantations in the Northeast region and reported the presence of the genera *Aspergillus*, *Fusarium*,

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Penicillium, *Phytophthora*, and *Trichoderma*. Romão-Dumaresq et al. (2016) studied fungi in roots and the rhizosphere of two varieties of sugarcane in the state of São Paulo, and observed that the genus *Penicillium* was one of the most prevalent genera in the soil analyzed. Ramos et al. (2018) evaluated the species diversity of *Penicillium* and *Talaromyces* in sugarcane soils in Pernambuco state, in the Northeast region of Brazil. On a total of 1344 isolates, 1108 belonged to *Penicillium* and 236 to *Talaromyces*.

Penicillium has an important role in various natural processes and its ubiquitous presence has been reported in several studies. *Penicillium* species occupy a wide range of habitats, including soil environments (such as forest soil, beach soil, cultivated soil, desert soil), and species are also associated with plants and food. Several new species were discovered during ecological and biodiversity studies of specific substrates around the world (e.g., Houbraken et al. 2016; Barbosa et al. 2018, 2020). Fungal spores, like those of *Penicillium*, are air dispersed and can be deposited in the soil. However, due to their nutritional requirements and large enzymatic apparatus, *Penicillium* species have been frequently isolated from various soils, including many diverse tropical ecosystems (e.g., Cruz et al. 2013; Barbosa et al. 2016), where they actively participate in biogeochemical cycles (Visagie et al. 2014).

The genus *Penicillium* has been extensively revised in the last decade, due to new taxonomic insights and the introduction of a single name nomenclature system in fungi (Houbraken and Samson 2011; Visagie et al. 2014; Houbraken et al. 2020). In the most recent overview of the order *Eurotiales*, the genus *Penicillium* was subdivided into two subgenera, 32 sections and 89 series, among them section *Sclerotiorum* (Houbraken et al. 2020). This section was introduced by Houbraken and Samson as section *Sclerotiora* (2011) and typified with *P. sclerotiorum*. Most species in this section share the production of yellow and/or orange mycelium and have an orange or reddish colony reverse and bright-colored sclerotia (Visagie et al. 2013). The number of species described in this section has increased in the last decade and currently 36 species are accepted (Houbraken et al. 2020; Choi et al. 2021). Accurate identification of *Penicillium* species nowadays relies on partial β -tubulin (*BenA*) sequencing and these data are ideally supplemented with phenotypic and extrolite data (Visagie et al. 2014).

The aim of the present study was to describe two new *Penicillium* species belonging to section *Sclerotiorum*. The strains were isolated during a study of the mycobiota from sugarcane soil in the Northeast region of Brazil (Ramos et al. 2018). The phylogenetic position of the species in section *Sclerotiorum* is determined using a multigene phylogeny and species descriptions are provided.

Materials and methods

Strains

Soil samples were collected in 2014 and 2015 in an agricultural area of the Engenho Trapiche (8° 35' 21" S, 35° 6' 55" W), located in the municipality of Sirinhaém, along the southern coast of Pernambuco (Fig. 1). The soil was classified as dystrophic yellow latosol/very clayey (Saldanha et al. 2007) and analysis of the samples was performed as described in Ramos et al. (2018). The strains (Table 1) that represent the new species described in the present study were reported previously by Ramos et al. (2018). Following the recommendation of Barbosa et al. (2020), the strains were deposited in the Micoteca URM culture collection and the holotypes (slide preparation) in the URM Mycology Herbarium, both housed at the Federal University of Pernambuco, Recife, Brazil.

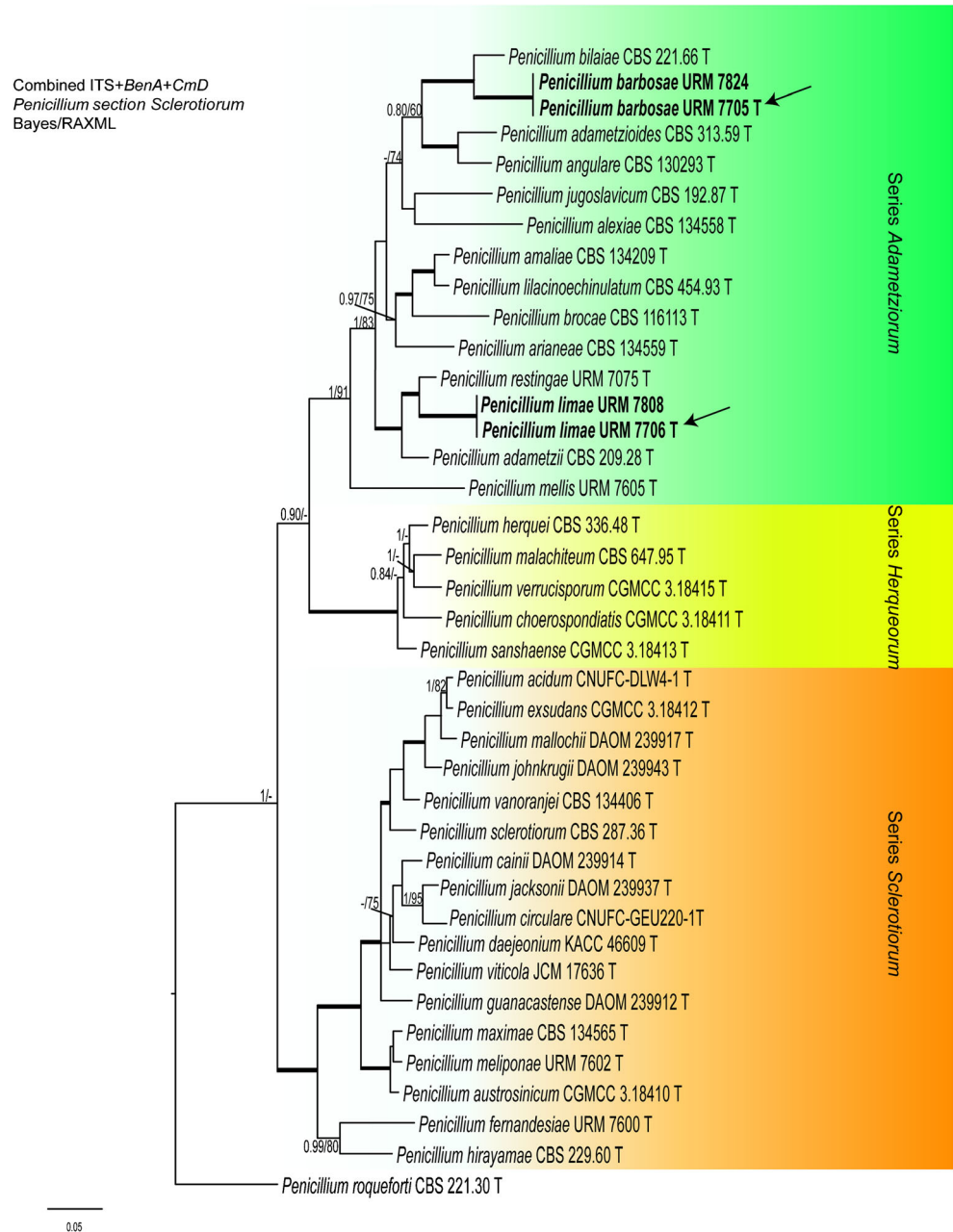
Morphological analysis

For morphological analysis, the strains were inoculated in three equidistant points on creatine sucrose agar (CREA) medium, Czapek yeast extract agar (CYA), CYA supplemented with 5% NaCl (CYAS), dichloran 18% glycerol agar (DG18), malt extract agar (MEA), oatmeal agar (OA), and yeast extract sucrose agar (YES). All agar media were incubated at 25 °C for 7 days. The composition of media followed Samson et al. (2010). Additional CYA and MEA plates were incubated at 5, 30, and 37 °C. Colony diameters were measured after 7 days of incubation and colony characteristics were recorded. Microscopic observations were made from colonies grown on MEA. The presence of a sexual stage was investigated in cultures incubated on CYA, MEA, and OA and incubated for at least 40 days at 25 °C. Lactic acid (60%) was used as a mounting fluid and 96% ethanol was used to remove excess conidia. A Nikon SMZ25 dissecting microscope and a Zeiss AxioImager.A2 with differential interference contrast (DIC) microscope, both equipped with Nikon DS-Ri2 cameras, were used to capture digital images using the software NIS-Elements D v4.50. The size, shape, and pigmentation of microscopic features were recorded. The mycological color chart by Rayner (1970) was used.

DNA isolation, PCR, and sequencing

Genomic DNA extractions were made from 7-day-old colonies grown on MEA using the Promega DNA isolation kit (Wizard Genomic DNA Purification Kit). Polymerase chain reaction (PCR) amplification of the ITS barcode (ITS1, 5.8S rDNA, and ITS2), *BenA*, calmodulin (*CaM*), and *RPB2* gene regions was performed using the methods described by Visagie et al. (2014). PCR products were purified using the Exosap illustrative enzyme ExoProStar™ 1-Step (GE

Fig. 1 Phylogenetic position of new the *Penicillium* species in the section *Sclerotiorum* based on a combined dataset containing ITS, and partial *BenA* and *CaM* sequences. The new species *P. barbosae* sp. nov. (URM 7705^T) and *P. limae* sp. nov. (URM 7706^T) are shown in bold and indicated with arrow in the green clade of series *Adametziorum*



Healthcare Life Sciences) and sequenced on a LABCEN/CB sequencing platform at UFPE (Recife, Brazil) using the same primers. The electropherograms were analyzed using the BioEdit software version 7.2.5 (<https://bioedit.software.informer.com/7.2/>) from which the consensus nucleotide sequences were obtained.

Phylogenetic analysis

Sequence datasets were generated by combining the newly generated sequences with reference (preferably ex-type) sequences from previous studies deposited and stored the nucleotide database at NCBI (GenBank) (Table 1). The sequences

were aligned using MAFFT v.7 (Kato and Standley 2013) and manually optimized using MEGA v. 6.06 (Tamura et al. 2013). Initially, the positioning of the new species in section *Sclerotiorum* was analyzed with a concatenate dataset with ITS, *BenA*, and *CaM* sequences (TreeBase 28142). After this initial analysis, more comprehensive ITS, *BenA*, *CaM*, and *RPB2* sequence datasets for series *Adametziorum* were generated and analyzed (TreeBase 28143). The combined datasets for section *Sclerotiorum* and series *Adametziorum* were made by concatenating the individual alignments using Mesquite v. 3.04 (Maddison and Maddison 2016). Phylogenetic trees were constructed using maximum likelihood analyses (ML) using RAXML-HP v. 8.2.8 (Stamatakis 2014) BlackBox with

Table 1 Strains used for phylogenetic analyses of *Penicillium* section *Sclerotiorum* with emphasis in the series *Adametziorium*

Species/series	Strain	Substrate and locality	ITS	<i>BenA</i>	<i>CaM</i>	<i>RPB2</i>
Series <i>Adametziorium</i>						
<i>P. adametzii</i>	CBS 209.28 T = ATCC 10407 = IMI 039751 = NRRL 737 = DTO 190-A8	Soil under conifers, Poznan, Poland	JN714929	JN625957	KC773796	JN121455
<i>P. adametzioides</i>	CBS 313.59 T = ATCC 18306 = IMI 068227 = NRRL 3405	Soil, Japan	JN686433	JN799642	JN686387	JN406578
	DTO 78-A7 = IBT 23667	Unknown	KC773825	KC773775	KC773800	n.a.
	DTO 78-A9 = IBT 27906	Unknown	KC773826	KC773776	KC773801	n.a.
	DTO 78-F2 = IBT 10870	Unknown	KC773827	KC773777	KC773802	n.a.
<i>P. alexiae</i>	CBS 134558 T = DTO 118H8	Quercus suber forest soil, Tunisia	KC790400	KC773778	KC773803	KX961291
<i>P. amaliae</i>	CBS 134209 T = DTO 183F3 = DAOM 241034 = CV1875	<i>Protea repens</i> infructescence, Struisbaai, South Africa	JX091443	JX091563	JX141557	KX961292
	CBS 134211 = DTO 181C6 = CV 204	<i>Protea repens</i> infructescence, Stellenbosch, South Africa	JX091444	JX091560	JX141554	KC470027
	CBS 134555 = DTO 181A5 = DAOM 241032 = CV 112	<i>Protea repens</i> infructescence, Stellenbosch, South Africa	JX091441	JX091559	JX141553	n.a.
	CBS 134212 = DTO 181F7 = DAOM 241031 = CV 401	<i>Protea repens</i> infructescence, Stellenbosch, South Africa	JX091440	JX091558	JX141556	KC470032
<i>P. angulare</i>	CBS 130293 T = IBT 27051 = NRRL 28157	Polypore on dead conifer stump, New Mexico, USA	AF125937	KC773779	KC773804	JN406554
	DTO 190-B8	Soil, Spanderswoud, Netherlands	KC773829	KC773780	KC773805	n.a.
	DTO 41-A2	Soil, Poland	KC773830	KC773781	KC773806	n.a.
	DTO 41-E6	Soil, Poland	KC773831	KC773782	KC773807	n.a.
<i>P. arianae</i>	CBS 134559 T = DTO 20B8	Soil, Spanderswoud, Netherlands	KC773833	KC773784	KC773811	KX961294
<i>P. barbosa</i>	URM 7705 T	Sugarcane soil, Pernambuco, Brazil	MW191494	MG452818	MW183245	LR898886
	URM 7824	Sugarcane soil, Pernambuco, Brazil	MW191495	MG452819	MW183246	LR898887
<i>P. bilaiae</i>	CBS 221.66 T = ATCC 22348 = IMI 113677 = NRRL 3391	Soil, Kiev, Ukraine	JN714937	JN625966	JN626009	JN406610
	CBS 330.90	Unknown	KC773834	KC773785	KC773812	n.a.
	DTO 181D8 = CV255	Mite inside <i>Protea repens</i> infructescence, Stellenbosch, South Africa	JX091437	JX091565	JX141560	n.a.
<i>P. brocae</i>	CBS 116113 T = IBT 26293 = NRRL 31472	Coffee berry borer feces, Tapachula, Chiapas, Mexico	AF484398	KC773787	KC773814	JN406639
<i>P. jugoslavicum</i>	CBS 192.87 T = IMI 314508	Seed of <i>Helianthus annuus</i> , former Yugoslavia	KC773836	KC773789	KC773815	JN406618
<i>P. lilacinoechinulatum</i>	CBS 454.93 T = ATCC 18309 = IMI 068211	Soil, Japan	AY157489	KC773790	KC773816	KX961293
	CBS 134563 = DTO 17E2	Soil, Spanderswoud, Netherlands	n.a.	KC773791	KC773817	n.a.
	CBS 134560 = DTO 42A2	Soil, Poland	n.a.	KC773793	KC773819	n.a.
<i>P. limae</i>	URM 7706 T	Sugarcane soil, Pernambuco, Brazil	MW191493	MG452820	MW183244	LR898888
	URM 7808	Sugarcane soil, Pernambuco, Brazil	FR997883	FR997876	FR997877	FR997878

Table 1 (continued)

Species/series	Strain	Substrate and locality	ITS	<i>BenA</i>	<i>CaM</i>	<i>RPB2</i>
<i>P. mellis</i>	URM 7605 T = CBS 142499	Honey of <i>Melipona scutellaris</i> , Recife, Pernambuco, Brazil	MN431398	MN969417	MN969327	LT854652
	URM 7611	Inside nest of <i>Melipona scutellaris</i> , Recife, Pernambuco, Brazil	MF278317	LT882629	LT882634	LT854652
	RB 9	Inside nest of <i>Melipona scutellaris</i> , Recife, Pernambuco, Brazil	MF278318	LT882625	LT882630	LT882635
<i>P. reconvexovulosi</i>	CCDCA 11500	Leaf litter — Brazil	n.a.	MN497417	MN497418	n.a.
<i>P. restingae</i>	URM 7075 T = CBS 140379	Soil from the Guaibim sandbank, Bahia, Brazil	KF803355	KF803349	KF803352	MN969134
	URM 7070 = 7EM2	Soil from the Guaibim sandbank, Bahia, Brazil	KF803354	KF803348	KF803351	n.a.
	URM 7072 = 44EM8	Soil from the Guaibim sandbank, Bahia, Brazil	KF803353	KF803347	KF803350	n.a.
Series <i>Herqueorum</i>						
<i>P. choerospondiatis</i>	CGMCC 3.18411 T	On fruits of <i>Choerospondias axillaris</i> , Hunan Province, Hengyang City, Hengyang County, Goulou town, Goulou Mountain National Forest Park, China	KX885063	KX885043	KX885053	KX885034
<i>P. herquei</i>	CBS 336.48 T = ATCC 10118 = IMI 28809 = NRRL 1040	Leaf of <i>Agauria pirifolia</i> , France	JN626101	JN625970	JN626013	JN121494
<i>P. malachiteum</i>	CBS 647.95 T = IBT 17515	Soil, Japan	KC773838	KC773794	KC773820	MN969125
<i>P. sanshaense</i>	CGMCC 3.18413 T	Hainan Province, Sansha City, Xisha Islands, Yongxing Island, China	KX885070	KX885050	KX885060	n.a.
<i>P. verrucisporum</i>	CGMCC 3.18415 T	Soil, Hunan Province, Chenzhou City, Yizhang county, Mangshan National Nature Reserve, China	KX885069	KX885049	KX885059	KX885040
Series <i>Sclerotiorum</i>						
<i>P. acidum</i>	JMRC SF:013659 = CNUFC-DLW4-1	Plant debris in water sample, Republic of Korea, Jeonnam Province, Gwangju	KY587441	KY587439	KY587442	KY587446
<i>P. austrosinicum</i>	CGMCC 3.18410 T	Rotten fruit, Guangdong Province, Shaoguan City, Shixing County, Chebaling National Nature Reserve, Xianrendong Village, China	KX885061	KX885041	KX885051	KX885032
<i>P. cainii</i>	DAOM 239914 T	Nuts of <i>Juglans nigra</i> , Niagara, Canada	JN686435	JN686366	JN686389	MT156346
<i>P. circularae</i>	CNUFC-GEU220-1T	Forest soil, Geumsan Park, Jeju Island, Republic of Korea	n.a.	MK481057	MK481061	MK481053
<i>P. daejeonium</i>	KACC 46609	<i>Penicillium</i> rot from grape fruits, Daejeon, Yuseong-gu, Republic of Korea	JX436489	JX436493	JX436491	n.a.
<i>P. exsudans</i>	CGMCC 3.18412 T	Rotten fruit, Guangdong Province, Shaoguan City, Shixing County, China	KX885062	KX885042	KX885052	KX885033
<i>P. fernandesiae</i>	URM 7600 T = CBS 142500	Inside nest of <i>Melipona scutellaris</i> , Recife, Pernambuco, Brazil	MF278314	MN969416	LT854649	LT854654
<i>P. guanacastense</i>	DAOM 239912 T	Gut of the caterpillar <i>Eutelia</i> sp. reared on leaves of <i>Spondias mombin</i> , Santa Rosa, Costa Rica	JN626098	JN625967	JN626010	KX961295
<i>P. hirayamae</i>	CBS 229.60 T = ATCC 18312 = IMI 078255 = NRRL 143	Milled rice, Thailand	JN626095	JN625955	JN626003	JN121459
<i>P. jacksonii</i>	DAOM 239937 T	Forest soil, Queensland, Australia	JN686437	JN686368	JN686391	n.a.
<i>P. johnkrugii</i>	DAOM 239943 T	Forest soil, Langkawi, Kedah, Malaysia	JN686447	JN686378	JN686401	n.a.
<i>P. mallochii</i>	DAOM 239917 T	Caterpillar on <i>Spondias mombin</i> , Santa Rosa, Costa Rica	JN626104	JN625973	JN626016	KX961296
<i>P. maximae</i>	CBS 134565 T = NRRL 2060	Weathering treated cellophane, Florida, USA	EU427298	KC773795	KC773821	MN969126
<i>P. meliponae</i>	URM 7602 T = CBS 142495	Honey of <i>Melipona scutellaris</i> , Recife, Pernambuco, Brazil	MF278315	MN969418	LT854648	LT854653

Table 1 (continued)

Species/series	Strain	Substrate and locality	ITS	<i>BenA</i>	<i>CaM</i>	<i>RPB2</i>
<i>P. sclerotiorum</i>	CBS 287.36 T = ATCC 10494 = IMI 040569 = NRRL 2074	Air, Java, Indonesia	JN626132	JN626001	JN626044	JN406585
<i>P. vanoranjei</i>	CBS 134406 T = DIO99H6	<i>Quercus suber</i> forest soil, Tunisia	KC695696	KC695686	KC695691	n.a.
<i>P. viticola</i>	JCM 17636 T	Grape, Yamanashi, Japan	AB606414	AB540174	n.a.	n.a.

n.a., not available

1000 rapid bootstrap inferences via the CIPRES science gateway (<http://www.phylo.org/>) (Miller et al. 2010) adopting default parameters. Bayesian inference (BI) analysis was performed in MrBayes 3.2.2 (Ronquist et al. 2012). In the Bayesian analyses, every 1000 generations were sampled and the first 25% of the samples were discarded. The most suitable substitution model was determined separately for each gene region using jModelTest v. 2.1.7 (Posada 2008). Trees were visualized in FigTree v. 1.1.2 (Rambaut 2009) and edited in Adobe Illustrator v. 5.1. Bayesian inference (BI) posterior probabilities (pp) and bootstrap (bs) values are labelled at the nodes. Branches with full support in Bayesian and ML analyses are thickened. Values below 0.95 pp and 70% bootstrap support are not shown or indicated with a hyphen.

Results

The phylogenetic relationship between the strains and the accepted species of section *Sclerotiorum* was determined by analysis of a concatenated sequence dataset of three loci (ITS, *BenA*, and *CaM*). According to this analysis, both new species belong to series *Adametziorium* (Fig. 1). The phylogenetic relationship within series *Adametziorium* and the concordance between the generated single-gene phylograms was studied using a more comprehensive set of strains (Fig. 2). An overview of each dataset and the most optimal substitution model is given in Table 2.

Strains URM 7705 and URM 7824, both belonging to the new species described here as *P. barbosa*, form a sister clade related to *P. bilaiae* with high statistical support in the single-gene phylogenies (ITS 1.00 pp, 99% bs; *BenA* 1.00 pp, 100% bs; *CaM* 1.00 pp, 99% bs; *RPB2* 1.00 pp, 100% bs) (Fig. 2) and the combined phylogram (1.00 pp, 99% bs) (Fig. 3). URM 7706 and URM 7808 (*Penicillium limae* sp. nov.) are in all phylogenies positioned as a sister to *P. restingae* with high statistical support (ITS 0.99 pp, 75% bs; *BenA* 1.00 pp, 98% bs; *CaM* 1.00 pp, 97% bs; *RPB2* 1.00 pp, 100% bs) (Figs. 2 and 3).

After the introduction of the two new species in this study, the number of accepted species in the section rises to 38. The morphology of the new species was compared with the phylogenetically closely related species, and details are given in Table 3 and in the “Discussion” section. Descriptions containing details of the distinguishing characteristics are provided in the “Taxonomy” section below.

Taxonomy

Penicillium barbosa S. Ramos, R. Cruz, R.N. Barbosa, Houbraken, sp. nov. Fig. 4.

Mycobank MB 837908

Etymology: In honor of Eliane Barbosa, a mycologist from the Micoteca URM, for her contributions to fungal identification.

Type: Brazil: Pernambuco: Sirinhaém, ex sugarcane soil at Trapiche - sugarcane mill factory, December 2014, collected and isolated by S. Ramos. Holotype URM 94474 (slide preparation), deposited in the URM fungarium (Recife, Brazil); ex-type strain URM 7705.

ITS barcode: MW191494. Alternative markers: *BenA* = MG452818; *CaM* = MW183245; *RPB2* = LR898886.

Colony diam, 7 days (mm): CYA 37–38; MEA 39–40; YES 36–37; DG18 35–37; OA 28–30; CYAS 34–35; CREA 17–19; CYA 5 °C no growth; CYA 30 °C no growth; 37 °C no growth.

Colony characters: CYA, 25 °C, 7 days: colonies radially sulcate; margins entire, low, narrow; mycelium white; colony texture slightly floccose; sporulation weak to moderate; conidial color *en masse* pale greenish gray (110); exudate and soluble pigment absent; reverse buff (45) to umber (9). MEA, 25 °C, 7 days: colonies radially sulcate; margins entire, low, narrow; mycelium white; colony texture floccose; sporulation weak to moderate; conidial color *en masse* pale grayish green

Fig. 2 Single-gene phylogenies based on ITS, *BenA*, *CaM*, and *RPB2* sequences including several strains in the series *Adametziorium*. The new species *P. barbosa* sp. nov. (URM 7705^T) and *P. limae* sp. nov. (URM 7706^T) are highlighted in green

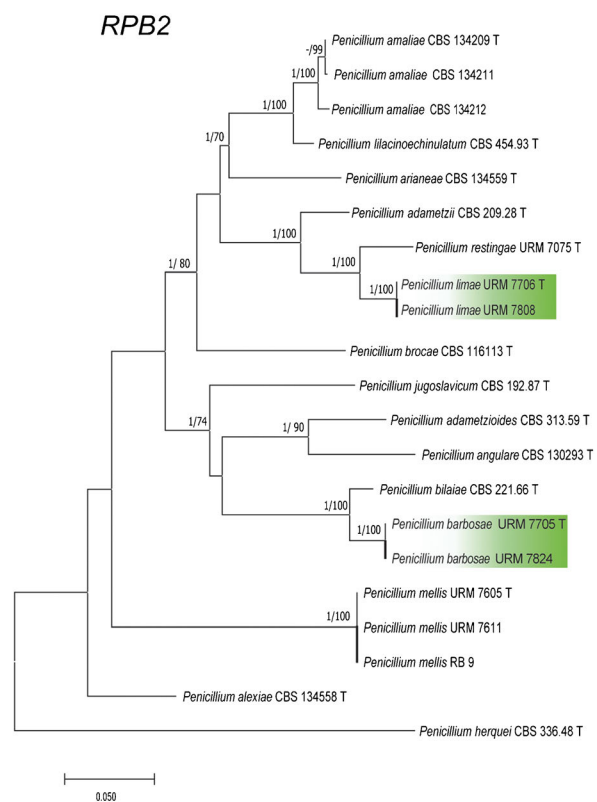
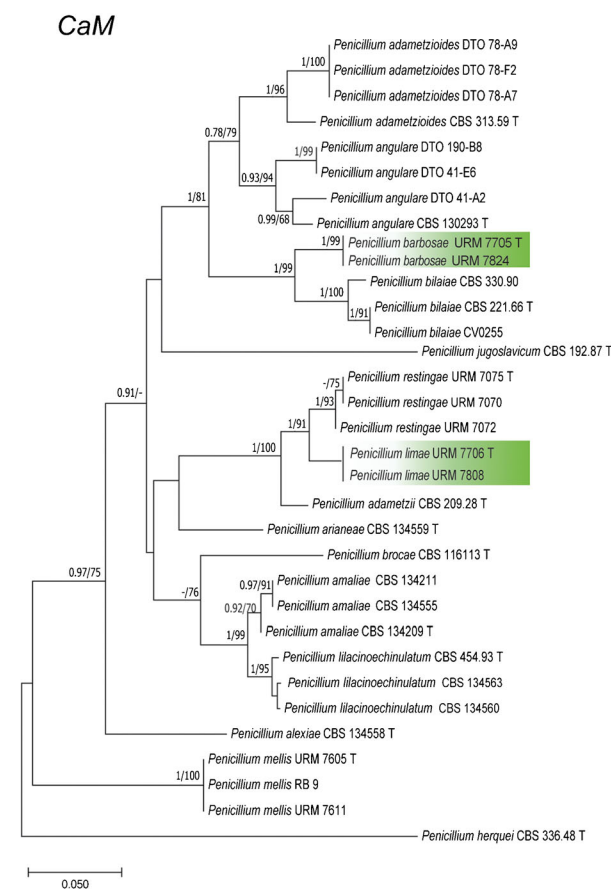
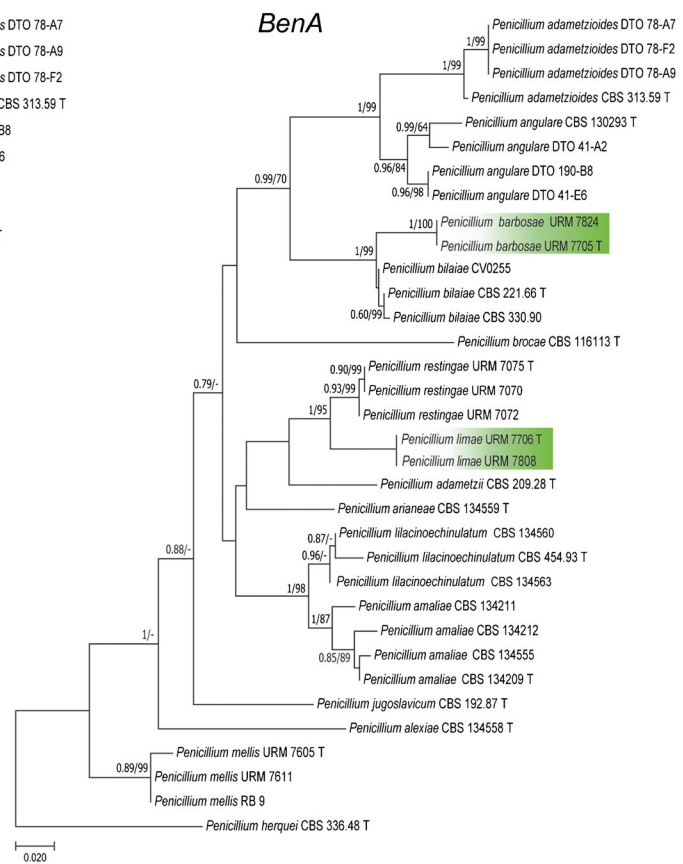
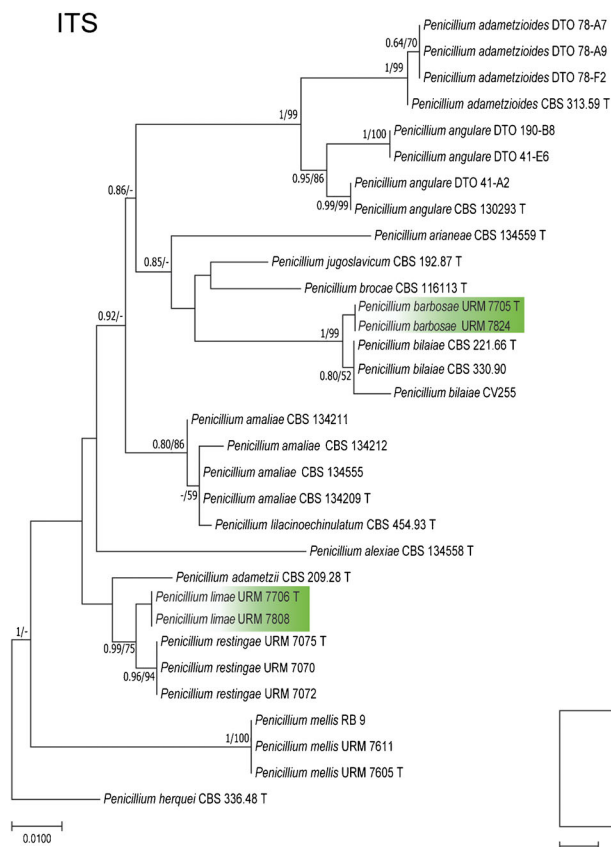


Table 2 Sequence datasets and models used in the phylogenetic analyses

	ITS (bp)	Model	<i>BenA</i> (bp)	Model	<i>CaM</i> (bp)	Model	<i>RPB2</i> (bp)	Model	Total length (bp)
Section <i>Sclerotiorum</i>	536	GTR+G	376	GTR+G	456	TrN+G	–	–	1368
Series <i>Adametziorium</i>	516	GTR+G	357	GTR+G	370	TrN+G	818	TIMeF+I+G	2061

(110); exudate absent or present as clear droplets; soluble pigment absent, reverse pale luteous (11) to orange (7). YES, 25 °C, 7 days: colonies radially sulcate slightly, raised at center; margins entire, low, narrow; mycelium white; colony texture floccose; sporulation moderate; conidial color *en masse* grayish green (110); exudate absent; soluble pigment absent, reverse pale luteous (11) to orange (7). DG18, 25 °C, 7 days: colonies plane, slightly raised at center; margins low, entire; mycelium white; colony texture slightly floccose; sporulation weak; conidial color *en masse* grayish green (110); exudate absent; soluble pigment absent; reverse straw (46). OA, 25 °C, 7 days: Colonies radially sulcate, entire; margins

regular; mycelium smoke gray (105); colony texture velvety; sporulation sparse; conidial color *en masse* mouse gray (118); exudate absent; soluble pigment absent; reverse straw (46) to umber (9). CYAS, 25 °C, 7 days: colonies radially sulcate slightly raised at center; margins entire, low, narrow; mycelium white; colony texture floccose; sporulation moderate; conidial color *en masse* grayish green (110); exudate absent; soluble pigment absent, reverse straw (46) to umber (9). CREA, 25 °C, 7 days: good growth, acid production absent.

Micromorphology: Conidiophores monovercillate. Stipes smooth-walled, (20–)80–130(–170) × 2–3 μm, vesiculate, up to 7 μm, sometimes non-vesiculate. Phialides

Fig. 3 Combined phylogeny based on ITS, *BenA*, *CaM*, and *RPB2* sequences, including several strains in the series *Adametziorium*. The new species *P. barbosa* sp. nov. (URM 7705^T) and *P. limae* sp. nov. (URM 7706^T) are highlighted in green

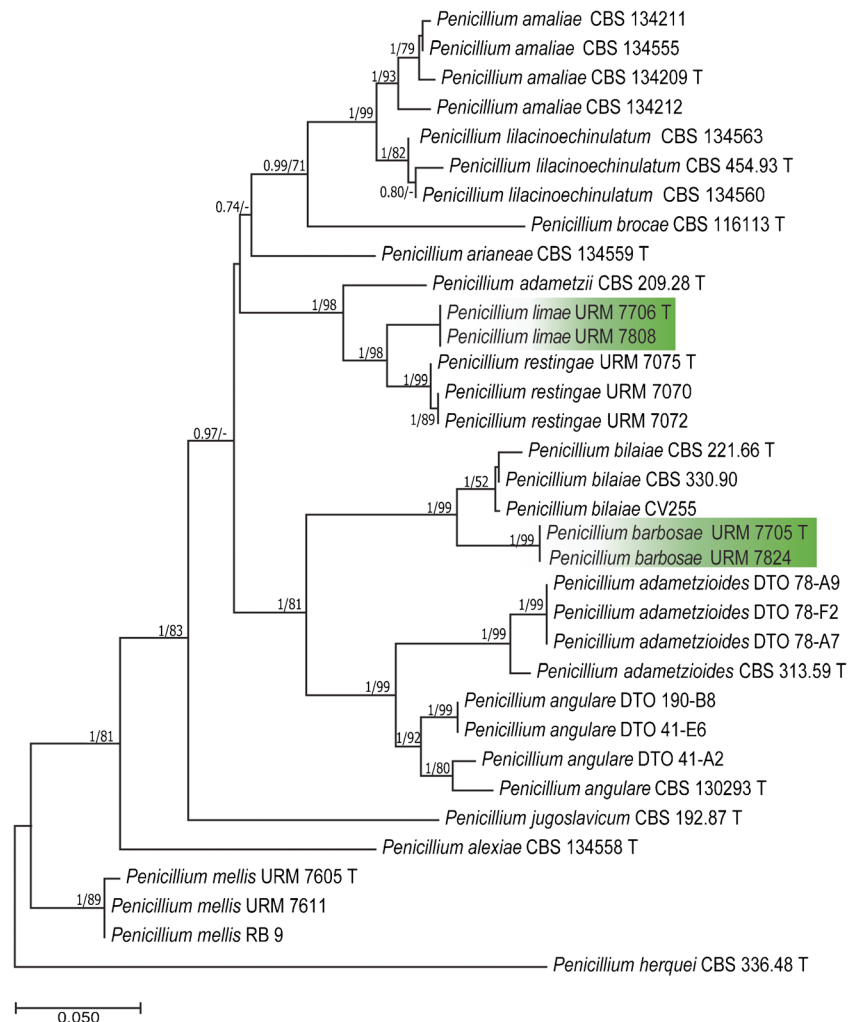


Table 3 Comparison of most important colony and microscopic characters of the newly described species and their phylogenetically closest relatives

Species name/ strain number	Colony diameter after 7 days (in mm)							Colony characters		Conidia	
	CYA 25 °C	CYA 30 °C	CYA 37 °C	MEA 25 °C	YES 25 °C	DG18 25 °C	CYAS 25 °C	Acid on CREA	Sclerotia	Ornamentation	Shape
<i>P. barbosa</i> URM 7705	37–38	NG	NG	39–40	36–37	35–37	34–35	Absent	Abundantly (on OA)	Echinulate	Globose to subglobose
<i>P. bilaiae</i> ¹ CBS 221.66	25–33	25–30	13–15	25–28	29–31	20–22	25–29	Strong	Absent	Rough	Globose to subspheroid
<i>P. limae</i> URM 7706	22–32	31–33	NG	23–32	36–37	35–40	29–30	Absent	Absent	Finely roughened	Globose to subglobose
<i>P. restingae</i> ² URM 7075	18–27	n/a	23–33	16–23	35–42	30–35	33–35	n/a	Absent	Finely roughened	Globose
<i>P. adamezi</i> ¹ CBS 209.28	27–35	27–37	10–16	28–35	27–36	9–16	18–21	Absent	Absent	Smooth to finely rough	Globose to subglobose

NG, no growth; *n/a*, not available in original description. ¹ Data from Visagie et al. (2014). ² Data from Crous et al. (2014). Growth rate data for *P. restingae* on YES, DG18, and CYAS was generated in this study

ampulliform, (8–10–11) × 2–3 μm. Conidia globose to subglobose, echinulate, 2.5–3 μm. Sclerotia abundantly produced on OA, near substrate and covered by conidiophores, hard, pale brown, globose to subglobose, (100–)200–350 μm.

Additional material examined: URM 7824 (ITS: MW191495, *BenA*: MG452819, *CaM*: MW183246, *RPB2*: LR898887), from sugarcane soil at Trapiche - sugar cane mill factory, December 2014, S. Ramos.

Notes: *Penicillium barbosa* is phylogenetically most closely related to *P. bilaiae*. Both species predominantly produce monoverticillate conidiophores and roughened, globose to subglobose conidia. However, these species can be distinguished by their growth rates on CYA incubated at 30 and 37 °C. The former species is unable to grow at these temperatures, while the latter can (Visagie et al. 2013). Furthermore, sclerotia are produced by *P. barbosa* on OA, and these structures are not reported in *P. bilaiae*.

Penicillium limae S. Ramos, R. Cruz, C. Souza-Motta, N. Tinti, **sp. nov.** Fig. 5.

Mycobank MB 837909

Etymology: In honor of Prof. Dr. Nelson Lima, head of the fungal culture collection, Micoteca da Universidade do Minho (MUM), Braga — Portugal, for his contribution to mycology in Brazil, and especially to the Mycology Department of UFPE.

Type: Brazil: Pernambuco: Sirinhaém, ex sugarcane soil at Trapiche - sugarcane mill factory, November 2014, collected and isolated by S. Ramos. Holotype 94475 (slide preparation), deposited in the URM fungarium (Recife, Brazil); ex-type strain URM 7706.

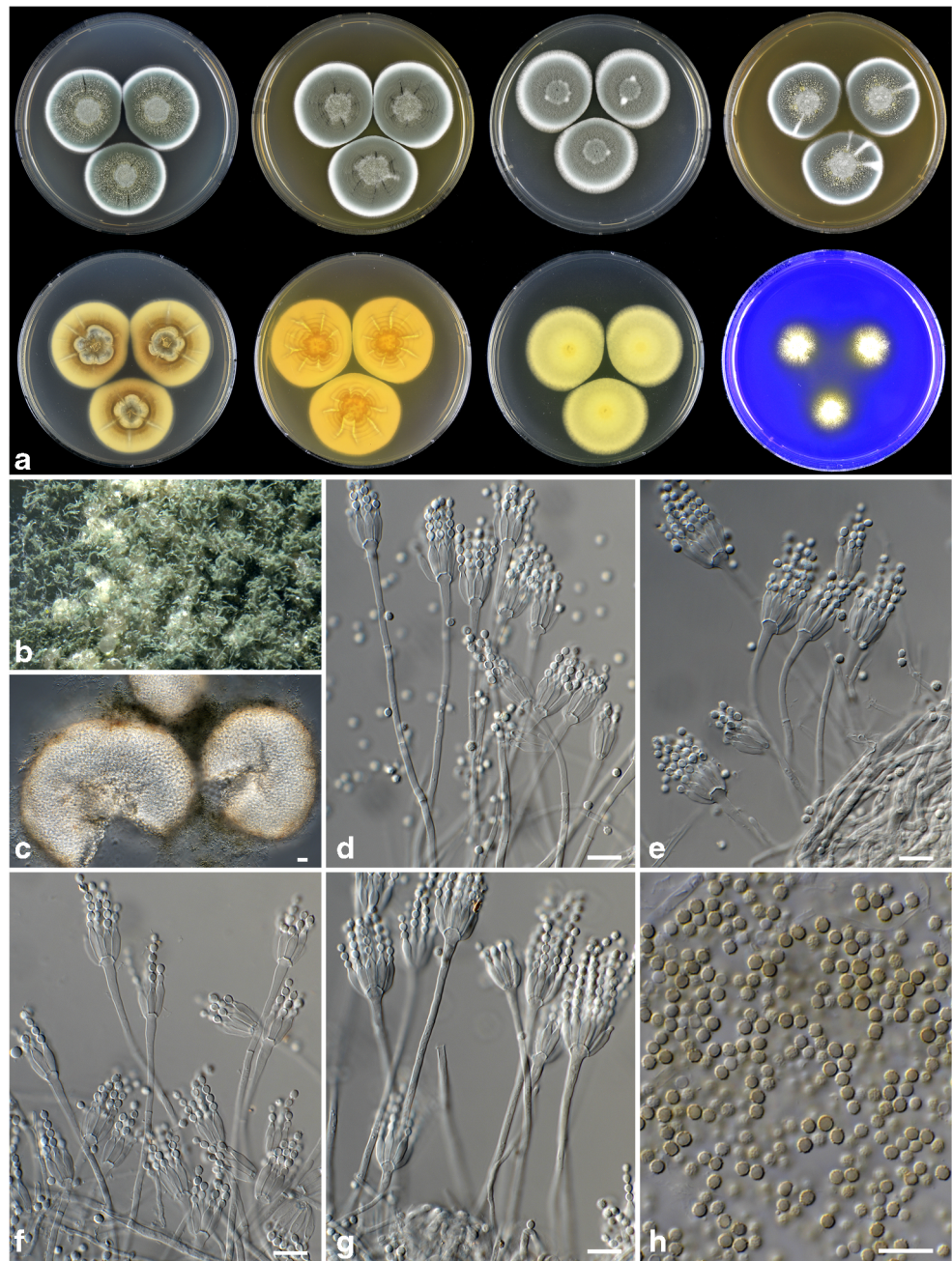
ITS barcode: MW191493. Alternative markers: *BenA* = MG452820; *CaM* = MW183244; *RPB2* = LR898888.

Colony diam, 7 days (mm): CYA 22–32; MEA 23–32; YES 36–37; DG18 35–40; OA 30–48; CYAS 29–30; CREA 12–20; CYA 5 °C no growth; CYA 30 °C 31–33; CYA 37 °C no growth.

Colony characters: CYA, 25 °C, 7 days: colonies radially sulcate; margins entire, low, narrow; mycelium white; colony texture floccose; sporulation sparse to moderate; conidial color *en masse* pale greenish gray (110); exudate and soluble pigment absent; reverse straw (46). MEA, 25 °C, 7 days: colonies plane, radially sulcate; margins entire, low, narrow; mycelium white; colony texture floccose; sporulation sparse to moderate; conidial color *en masse* greenish gray (110); exudate present as clear droplets; soluble pigment absent, reverse straw (46) to umber (9). YES, 25 °C, 7 days: colonies radially sulcate; margins entire, low, narrow; mycelium white; colony texture floccose; sporulation sparse; conidial color *en masse* indeterminate; exudate absent; soluble pigment absent, reverse straw (46). DG18, 25 °C, 7 days: colonies plane, radially sulcate raised at center; margins low, entire; mycelium white; colony texture floccose; sporulation sparse to moderate; conidial color *en masse* pale greenish gray (110); exudate absent; soluble pigment absent; reverse buff (45). OA, 25 °C, 7 days: colonies radially sulcate, entire; margins regular; mycelium white; colony texture floccose; sporulation sparse; conidial color *en masse* pale greenish gray (110); exudate absent; soluble pigment absent; reverse buff (45). CYAS, 25 °C, 7 days: colonies radially sulcate slightly raised at center; margins entire, low, narrow; mycelium white; colony texture floccose; sporulation sparse; conidial color *en masse* indeterminate; exudate absent; soluble pigment absent, reverse buff (45). CREA, 25 °C, 7 days: good growth, acid production absent. No growth on CYA at 5 °C and 37 °C.

Micromorphology: Conidiophores monoverticillate. Stipes smooth walled, 10–30(–70) × 1.5–2.5 μm, mostly

Fig. 4 Morphological characters of *Penicillium barbosae*. **a** Colonies from left to right, CYA, YES, DG18, and MEA verse (top row) and CYA, YES, DG18 reverse, and CREA (bottom row) at 25 °C. **b** Colony texture. **c** Sclerotia produced on OA. **d–g** Conidiophores. **h** Conidia. Scale bars 10 µm



non-vesiculate, sometimes present, up to 6 µm. Phialides ampulliform, $6.5\text{--}7.5 \times 1.7\text{--}2.5$ µm. Conidia globose to subglobose, finely roughened, $2\text{--}2.5\text{--}(3)$ µm. Sclerotia or ascmata not observed.

Additional material examined: URM 7808 (ITS: FR997883, *BenA*: FR997876, *CaM*: FR997877, *RPB2*: FR997878), from sugarcane soil at Trapiche - sugar cane mill factory, December 2014, S. Ramos.

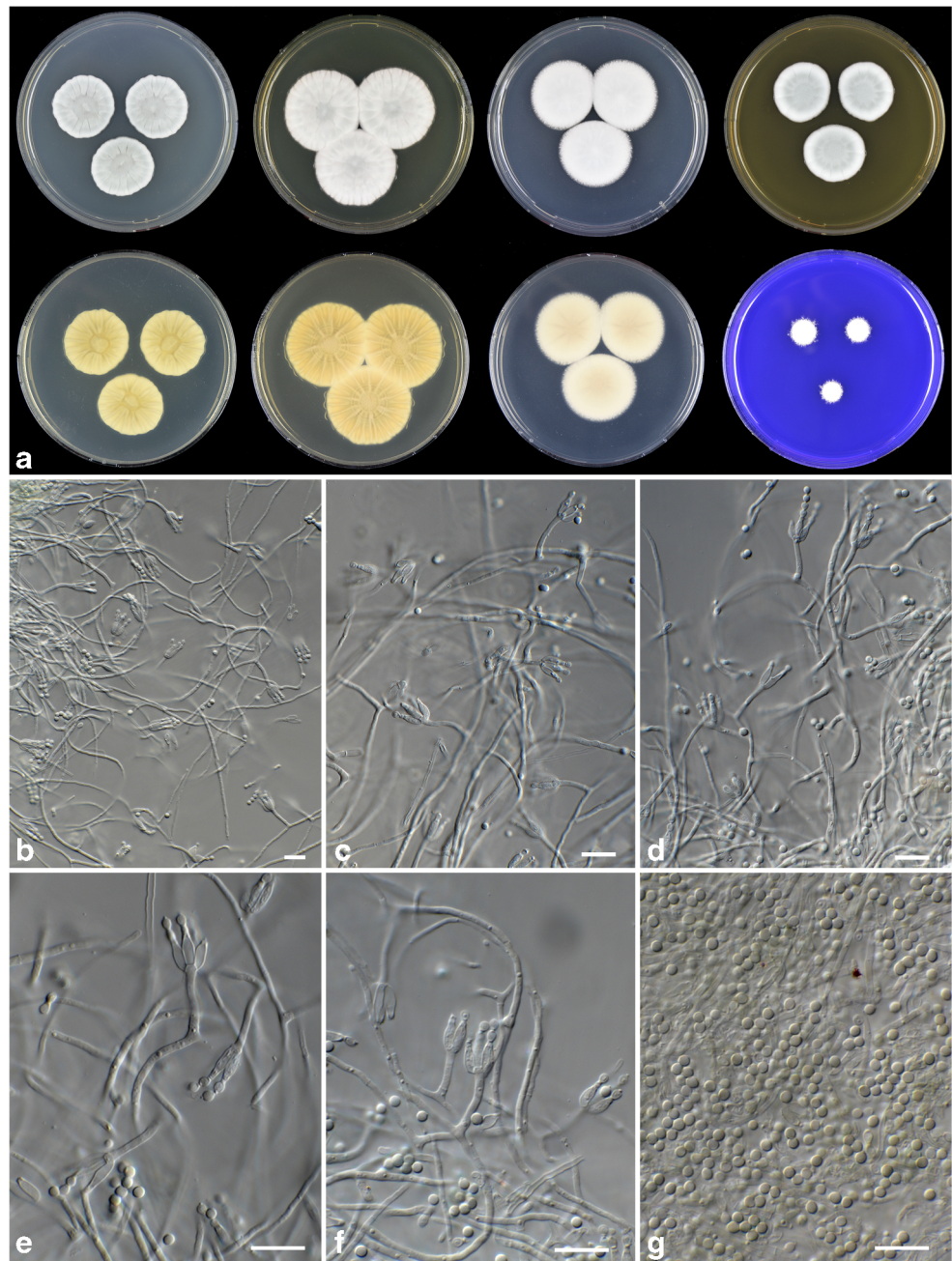
Notes: *Penicillium limae* is phylogenetically most closely related to *P. restingae*. These species (*P. limae* vs *P. restingae*) differ by the fast growth on CYA (22–32 vs 18–27 mm), MEA (23–32 vs 16–23 mm) (Crous et al. 2014), DG18 (35–40 vs 30–35) and CYAS (29–30 vs 33–

35). *Penicillium limae* does not grow on CYA at 37 °C, while *P. restingae* does (23–33 mm).

Discussion

The genus *Penicillium* is of great importance to several scientific fields, is one of the most used fungi in biotechnology, and has great economic impact on human life, e.g., by the production of bioactive compounds such as antibiotics (penicillin, Houbraken et al. 2011) and mycotoxins (Perrone and Susca 2017). Species of this genus occur in a wide variety of habitats including soil, air, indoor environments, and food (Visagie

Fig. 5 Morphological characters of *Penicillium limae*. **a** Colonies from left to right, CYA, YES, DG18, and MEA verse (top row) and CYA, YES, DG18 reverse, and CREA (bottom row) at 25 °C. **b–f** Conidiophores. **g** Conidia. Scale bars 10 μm



et al. 2014). An infrageneric classification system is traditionally used in *Penicillium*, and currently 32 sections and 89 series are accepted (Houbraken et al. 2020). Section *Sclerotiorum* belongs to the subgenus *Aspergilloides* and includes three series (*Adametziorum*, *Herqueorum*, and *Sclerotiorum*). This section is phylogenetically well-defined, with several recent studies introducing new species (e.g., Visagie et al. 2013; Wang et al. 2017; Barbosa et al. 2018; Wanasinghe et al. 2018; Crous et al. 2019; Hyde et al. 2019; Choi et al. 2021). With the exception of the species of series *Herqueorum* (*P. choerospondiatis*, *P. herquei*, *P. malachiteum*, *P. sanshaense*, and *P. verruciosporum*), all

other species belonging to this section produce predominantly monoverticillate conidiophores. Specific phenotypic characters that define the three series of the section could not be identified; however, species classified in series *Sclerotiorum* generally produce orange colonies and lack strongly colored, soluble pigments such as those generally observed in the species of series *Adametziorum*. The new species *P. barbosa* and *P. limae* belong to series *Adametziorum* and produce monoverticillate conidiophores, colored colonies, and sclerotia, indicating a relationship with other taxa of section *Sclerotiorum*. No sexual state was observed in the new species described in our study.

Series *Adametziorum* species are commonly isolated from soil around the world. In Brazil, three taxa of this series have been recorded, *P. mellis* from honey samples collected in Pernambuco state (Barbosa et al. 2018), *P. restingae* from soil (Crous et al. 2014), and *P. reconvexovelosoi* from leaf litter (Crous et al. 2019), the last two were collected from sand dunes in Guaibim, Bahia state. It is interesting to mention that these three reports, and also the two new species described here, were isolated in states located in the Northeast region of Brazil. Cruz et al. (2013) and Barbosa et al. (2016, 2020) reported several species of *Penicillium* section *Sclerotiorum* species in soils in Brazil; thus, our record reinforces the idea that soil is a good substrate for the isolation of *Penicillium* species. Some species can actively participate in biogeochemical cycles (Visagie et al. 2014). On the other hand, others might just be dispersed and latently present in the substrate. It is important to highlight that studies on the diversity and ecology of *Penicillium* and other genera in Brazilian tropical soils are still scarce, despite the country harboring an important component of global biodiversity (Cruz et al. 2013).

The diversity and interactions between fungi and sugarcane, one of the most important crops in Brazil, have rarely been studied. Romão-Dumaresq et al. (2016), based on a culture dependent strategy, determined the structure and diversity of the fungal community (root endophytes and rhizosphere) associated with two varieties of sugarcane in Brazil. They isolated 2236 fungal colonies, which were subsequently identified using ITS barcoding, and these data showed that the phylum Ascomycota was predominated present, and the most frequent genera were *Penicillium* (33.3%), *Fusarium* (16.9%), *Aspergillus* (7.2%), and *Trichoderma* (4.4%). These strains were unfortunately not confidentially identified at the species (or series) level. The interactions among microorganisms and plant roots are essential for the nutrition, growth, and productivity of the plant (Ortiz-Castro et al. 2009). The description of these new species adds to our knowledge of biodiversity and distribution of these organisms in soils of tropical ecosystems.

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Author contribution SMSR, RC, and NTO prepared the project and guided the experiment; SMSR and RC collected the material and executed the specified methodology; SMSR, RC, and RNB wrote the text; SMSR, RC, RNB, and JH identified the species; ARM and RNB made the phylogenetic trees; all authors revised the text.

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Data Availability Strains deposited in URM Collection, sequences in GenBank/ENA/NCBI, alignments of phylogenies in TreeBASE, and names in MycoBank.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare no competing interests.

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