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Original article

Penicillium gercinae and *Penicillium stangiae* (Eurotiomycetes, Ascomycota), two new species from soil in Brazil

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ABSTRACT

Two new *Penicillium* species are reported from soil of an agroforestry system and an Atlantic Rainforest fragment in Pernambuco, Northeast Brazil. Using a polyphasic approach, combining partial β-tubulin (*BenA*), calmodulin (*CaM*), internal transcribed spacer (ITS), and RNA polymerase II second largest subunit (*RPB2*) gene sequences and morphological characters, *Penicillium gercinae* sp. nov. (section *Ramigena*, series *Georgiensia*), and *Penicillium stangiae* sp. nov. (section *Lanata-Divaricata*, series *Dalearum*) are described. Descriptions based on morphological features are provided. *Penicillium gercinae* is phylogenetically closely related to *P. georgiense*; however, *P. gercinae* does not grow on Czapek yeast extract (CYA) and has monoverticillate and biverticillate conidiophores. *Penicillium stangiae* is phylogenetically closely related to *P. penarojense* and *P. vanderhammenii*. As diagnostic features, *P. stangiae* had slower growth on CYA (28-30 mm in 7 days) and on DG18 (6-7 mm in 7 days) and lacked the production of acid on creatine agar. This research contributes to the expansion of knowledge on the taxonomy and diversity of *Penicillium* species in Brazil.

Keywords: Aspergilloides, *Lanata-Divaricata*, phylogeny, *Ramigena*, soil mycobiota, taxonomy

Introduction

Penicillium species can occur in a wide range of habitats and it is considered one of the most common genus of fungi able to grow on culture media (Visagie *et al.* 2016). In the last decade, species of this genus have been described from specific habitats or substrates (Houbraken *et al.* 2016; Barbosa *et al.* 2018; 2020; Heo *et al.* 2019; Park *et al.* 2019; Ramos *et al.* 2021). In studies on filamentous fungi in soil, *Penicillium* is among the genera with largest number of species and highest densities (Costa *et al.* 2017; Arias Mota & Abarca 2020; Alves *et al.* 2021), contributing to this substrate with the solubilizing of nutrients for plants (Visagie *et al.* 2014).

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Penicillium has been extensively revised owing to new taxonomic insights (mainly based on molecular data) and the introduction of the single-name nomenclature system in fungi (Houbraken & Samson 2011; Visagie *et al*. 2014; Houbraken *et al*. 2020). In the most recent overview of Eurotiales, *Penicillium* species were allocated on 2 subgenera, 32 sections, and 89 series (Houbraken *et al*. 2020).

Penicillium subgenus *Aspergilloides* comprises species with conidiophores in which at least a portion of the stipes terminate in a vesicular swelling (Houbraken *et al*. 2011), with 19 sections, including *Ramigena* and *Lanata-Divaricata*. According to Houbraken *et al*. (2020), section *Ramigena* comprises two series, *Georgiensia* and *Ramigena,* and includes species in which monoverticillate conidiophores are evident; however, divaricate branching at various levels without an arrangement pattern is consistently observed. Section *Lanata-Divaricata* comprises five series, *Dalearum*, *Janthinella*, *Oxalica*, *Rolfsiorum,* and *Simplicissima,* and includes fast-growing species with large, spreading colonies (Houbraken *et al*. 2020). The species on this section form mostly strongly divaricate conidiophores, with terminal, subterminal and intercalate metulae. In the latter, there are intercalary monoverticillate conidiophores. In addition, the terminal cluster often consists of prolongation of the main axis (Houbraken *et al*. 2020).

The present describes two new *Penicillium* species from soils with different land use management systems in Brazil.

Materials and methods

Study area

Soil samples were obtained from an Atlantic Rainforest fragment in Paudalho city, Pernambuco, Brazil (7°5712″S, 35°0626″W) (Fig. 1A). The vegetation is classified as dense ombrophilous forest, characterized by evergreen forest with a canopy of up to 15 m with emergent trees reaching 40 m in height, and dense shrub vegetation comprising arborescent ferns, bromeliads, and palms. Climbing plants, epiphytes (bromeliad and orchids), and ferns are also abundant (Alves *et al*. 2021). Soil samples were also collected from an agroforestry system (AFS) in the same city (7°5727″S, 35°0624″W) (Fig. 1B). This system is going through an agroecological transition process since 2013,

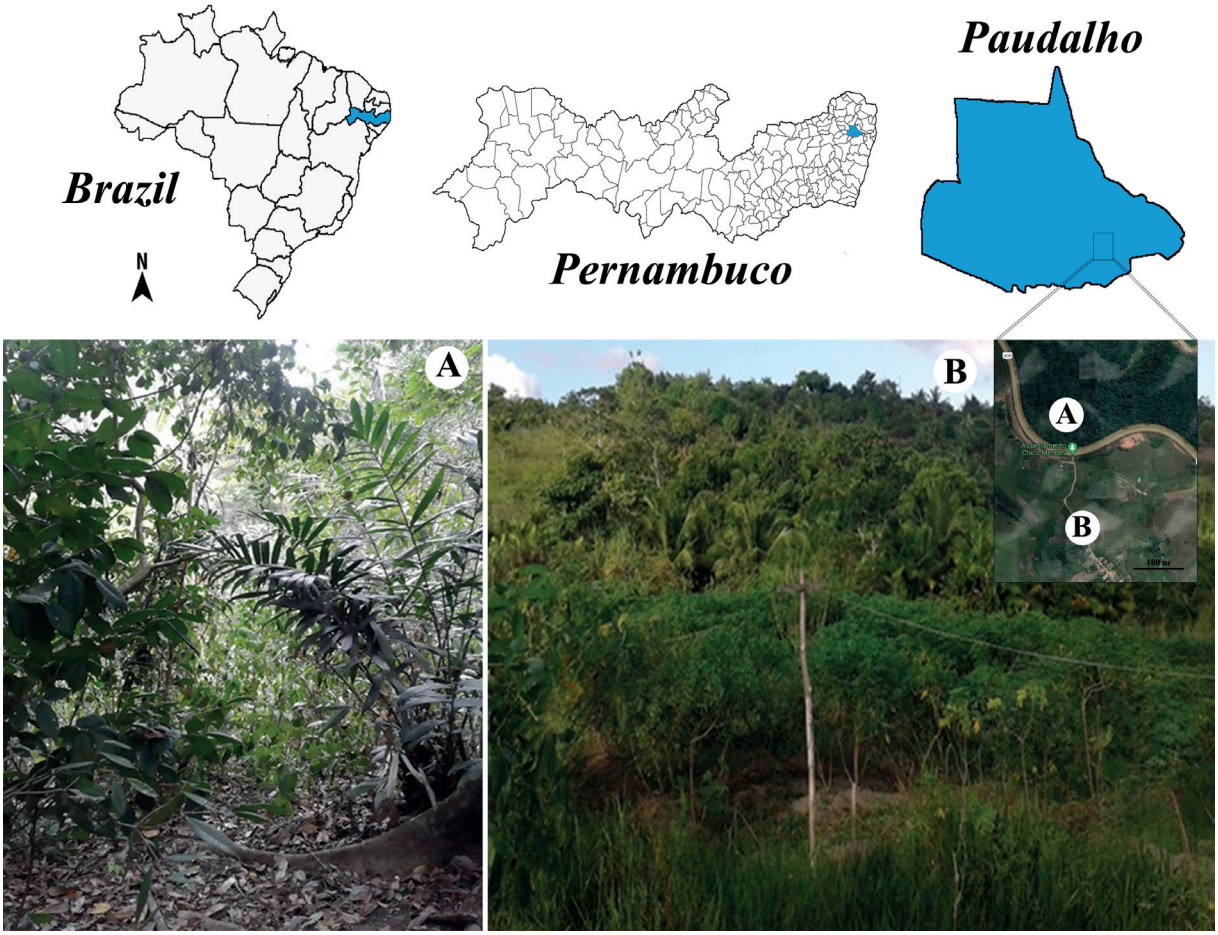

Figure 1. Locations of the soil samples collected in the Atlantic Rainforest (**A**) and in the agroforestry system (**B**) for isolation of *Penicillium* specimens.

with an unproductive sugarcane monoculture being replaced with fruit trees and crops (Alves *et al.* 2021).

Isolation

Soil samples were stored in plastic bags and processed as described by Barbosa *et al*. (2016). At the laboratory, 25 g of each soil sample was suspended in 225 mL of sterile distilled water and 1 mL of this suspension was transferred to a test tube containing 9 mL of sterile distilled water. This procedure was repeated until a 10^{-3} dilution was achieved. An 1 mL aliquot of this final dilution was poured on Petri dishes with Sabouraud Agar (AS) (40 g L^{-1} dextrose, 10 g L^{-1} peptone, and 15 g L^{-1} agar, pH 5.5) with chloramphenicol (0.017 g mL^{-1}) and Bengal rose (0.05 g L^{-1}). Each dilution was conducted in triplicate. Petri dishes were incubated at 27 ± 2 °C for 10 days. After incubation, the colonies were purified until single colonies were obtained (Alves *et al*. 2021). Among the 100 fungal strains obtained from the soil, 25 belonged to *Penicillium* (Alves *et al*. 2021), and two of those were new species. The strains of the new species were deposited at the Micoteca URM Profa. Maria Auxiliadora Cavalcanti culture collection and the holotypes (slide preparation) at the Herbário Pe. Camille Torrend, both at the Federal University of Pernambuco, Recife, Brazil (Barbosa *et al*. 2020).

Morphologic analysis

The strains were inoculated at three points on culture plates containing Czapek yeast extract agar (CYA), CYA supplemented with 5 % NaCl (CYAS), yeast extract sucrose agar (YES), creatine agar (CREA), Czapek agar (CZ), dichloran 18 % glycerol agar (DG18), malt extract agar (MEA, Oxoid), and oatmeal agar (OA) (Samson *et al*. 2010). All strains were incubated at 25 °C for 7 days. Additional CYA and MEA plates were incubated at 15 °C, 30 °C, and 37 °C. Colony diameters were measured after 7 days of incubation, and colony characteristics (presence of soluble pigments, exudates, obverse and reverse colors, and color of mycelium) were recorded, Rayner (1970). Microscopic observations were made using a microscope *Nikon Eclipse Ni* equipped with a photo camera *Nikon Digital Sight DSFi2*. Images were captured using *software NIS-elements software* (Nikon) and converted to format *Tagged Image File Format* (tiff) to preserve high definition graphics for color raster images. These observations were performed for colonies grown on MEA, and the presence of a sexual stage was investigated in CYA, MEA and OA cultures maintained at 25 °C for at least 40 days. Lactic acid (80 %) was used for mounting, and 96 % ethanol was used to remove excess conidia (Barbosa *et al*. 2018).

Phylogenetic analyses

DNA extraction was performed using the DNA Wizard Genomic DNA Purification Kit, following the manufacturer's recommendations. The β-tubulin (*BenA*), calmodulin (*CaM*), internal transcribed spacer (ITS), and RNA polymerase II second largest subunit (*RPB2*) gene regions were amplified using the primers Bt2a and Bt2b, CMD5 and CMD6, ITS1 and ITS4, and RPB2-5F and RPB2-7CR, respectively (Houbraken *et al*. 2019), with PCR conditions as described by Visagie *et al*. (2014). The PCR products were purified using the Exosap illustrative enzyme ExoProStar™ 1-Step (GE Healthcare Life Sciences, Little Chalfont, UK) and sequenced at the LABCEN/CCB sequencing platform at the UFPE (Recife, Brazil) using the same primers. The electropherograms were analyzed using Sequencher 4.7 (Gene Codes, Ann Arbor, MI, USA), from which the consensus nucleotide sequences were obtained and exported as FASTA files.

DNA sequence alignment and phylogenetic analyses

The sequences obtained were aligned with sequences of *Penicillium* section *Ramigena* and section *Lanata-Divaricata* available in the NCBI database, through alignment in the MAFFT v.7 online interface (Katoh & Standley 2013) (http://mafft.cbrc.jp/alignment/server/) and manual adjustment. For section *Lanata-Divaricata*, *P. glabrum* and *P. oxalicum* were used as outgroups; for section *Ramigena*, *Penicillium* sections *Charlesia* and *Eremophila* were used as outgroups (Visagie *et al.* 2016). The generated DNA sequences were deposited in GenBank under the accession numbers listed in Table 1. The phylogenies from the *BenA*, *CaM*, ITS, and *RPB2* sequences were analyzed both separately and combined. MrBayes 3.2.6 (Ronquist *et al*. 2012) was used to generate phylogenies based on Bayesian inference with 5000000 generations, a sampling frequency of 1000 generations, and a burn-in fraction of 25 %. The best-fit models of nucleotide substitution, implementing the Bayesian information criterion, were estimated separately for each gene region using MrModeltest 2.3 (Nylander 2004). Phylogenetic reconstructions using maximum likelihood analysis were performed on the CIPRES Science Gateway portal (Miller *et al*. 2010) using RAxML-HPC2 on XSEDE (8.2.10) with default parameters (Stamatakis 2014). Phylogenetic trees were plotted using FigTree 1.4.3 (Rambaut 2009). The phylogenetic relationship between the strains and the accepted species of sections *Ramigena* and *Lanata-Divaricata* were determined by analysis of single and concatenated sequence datasets of four loci (*BenA*, *CaM*, ITS, and *RPB2*).

Results

Phylogeny

Penicillium gercinae sp. nov. belongs to the series *Georgiensia* (Fig. 2). Strains URM 8348 and URM 8383, both belonging to this species, formed a sister clade related to *P. georgiense* with high statistical support in the single gene phylogenies (ITS 1 pp, 100 % bs; *BenA* 1 pp, 100 % bs;

Table 1. Sequences of strains obtained by NCBI (National Center for Biotechnology Information) used for phylogenetic analyses of *Penicillium* section *Lanata-Divaricata* with emphasis on the series *Dalearum* and section *Ramigena*.

Species/series	Strain	Substrate and locality	ITS	BenA	CaM	RPB2
Series *Dalearum*						
P. abidjanum	CBS 51374	Soil, Japan	GU981618	GU981666	n.a	n.a
	CBS 246.67	Holotype; Savannah soil, Ivory Coast	GU981582	GU981650	MN969234	JN121469
P. amphipolaria	CBS 140997	Type; underside of leaves of a variety of understory plants, Panama	KT887872	KT887833	KT887794	MN969177
	CBS 140966	peat moss factory, Québec, Canadá	KT887839	KT887800	KT887761	n.a
	CBS 140997	Holotype; underside of leaves of a variety of understory plants, Panama	KT887872	KT887833	KT887794	n.a
	CBS 140965	peat moss factory, Québec, Canadá	KT887838	n.a	KT887760	n.a
P. austrosinense	NN072022	Acidic soil, Hainan, China	KY495003	KY495112	KY494943	n.a
	NN072318	Holotype; Acidic soil, Hainan, China	KY495007	KY495116	MN969328	KY495061
	NN072385	Acidic soil, Hainan, China	KY495017	KY495126	KY494957	KY495070
P. arvense	CBS 513.74	Soil, Japan	n.a	n.a	n.a	KF296415
P. daleae	CBS 211.28	Type; Unknow, Poland	GU981583	GU981649	MN969251	KF296427
	NN072144	Acidic soil, Hainan, China	KY495005	KY495114	KY494945	n.a
	NN072175	Acidic soil, Hainan, China	KY495006	KY495115	KY494946	KY495060
P. griseopurpureum	CBS 406.65	Neotype; Soil under Pinus, Lancashire, UK	KF296408	KF296467	MN969261	KF296431
P. guaibinense	23EM8	Ex-type; soil from the Guaibim sandbank, Brazil	MH674389	MH674391	MH674393	n.a
	KUMCC18-0199	Soil, China	MT152336	MT161680	MT178248	MT384371
P. jianfenglingense	NN044148	Soil, Guangxi, China	KY494984	KY495093	KY494924	KY495044
	NN072384	Holotype; Soil, China	KY495016	KY495125	MN969334	KY495069
	NN072479	Acidic soil, Hainan, China	KY495031	KY495140	KY494971	KY495082
P. penarojense	CBS 113132	Leaf litter exposed 6 months, mature forest, Peña Roja, Colombia	GU981572	GU981644	n.a	n.a
	CBS 113133	Leaf litter exposed 6 months, mature forest, Peña Roja, Colombia	GU981569	GU981645	n.a	n.a
	CBS 113178	Type; Leaf litter exposed 6 months, mature forest, Peña Roja, Colombia	GU981570	GU981646	n.a	n.a
P. rubriannulatum	NN044145	Soil, China	KY494983	KY495092	KY494923	KY495043
	NN072456	Holotype; Soil, China	KY495029	KY495138	MN969336	KY495080
P. stangiae	URM 8347	Type; Soil of Atlantic Forest, Brazil	MW648590	MW646388	MW646390	MW646392
	URM 6020	Soil of Atlantic Forest, Brazil	OK148738	OK148701	OK148703	n.a

Table 1. Cont.

Species/series	Strain	Substrate and locality	ITS	BenA	CaM	RPB2
P. singorense	CBS 138214	Ex-type; indoor house dust, Thailand	n.a	n.a	n.a	MN969138
	DTO 131I8	indoor house dust, Thailand	KJ775673	KJ775166	KJ775402	n.a
	CBS 138214	Ex-type; indoor house dust, Thailand	KJ775674	KJ775167	KJ775403	n.a
P. vanderhammenii	CBS 116296	leaf litter exposed in a litter bag, Colombia	GU981573	GU981648	n.a	n.a
	CBS 126216	Holotype; leaf litter exposed in a litter bag, Colombia	GU981574	GU981647	MN969308	KF296458
P. vasconiae	CBS 339.79	Type; Soil, Spain	GU981599	GU981653	MN969309	MN969144
P. viridissimum	NN053344	Soil, China	KY494991	KY495100	KY494931	KY495049
	NN072081	Holotype; Soil, China	KY495004	KY495113	MN969339	KY495059
P. zonatum	CBS 992.72	Holotype; Soil, Beaufort, North Carolina	GU981581	GU981651	MN969315	KF296461
Section *Ramigena*						
P. capsulatum	CBS 301.48	Type; Optical instrument, Panama	JX841240	MN969375	MK451594	JN406582
	MRC1625	Salami, South Africa	n.a	MK951852	MN031423	n.a
P. cyaneum	CBS 315.48	Type; oil-field, France	AF033427	JX091552	KP735540	JN406575
	CBS 267.29	Ex type; unknown source, Belgium	NR121341	JX091557	n.a	EF634445
	PPRI 25893	wood in mine, South Africa	MK951935	MK451274	MK951884	n.a
P. eremophilum	CBS 123361	Type; moldy prunes, Australia	GU733341	KY709170	KY611931	KY611970
P. georgiense	GR 68	*Allium cepa* L., South Korea	KJ614385	KJ614384	n.a	n.a
	NRRL 35507	Soil dilution plate; sporulating on heads of *A. niger*, Georgia, USA	EF422850	EF506222	EF506238	n.a
	NRRL 35508	Soil dilution plate; sporulating on heads of section *Nigri* species, Texas, USA	EF422851	EF506224	EF506240	n.a
	NRRL 35509	Type; Soil dilution plate; sporulating on heads of *A. niger*, Georgia, USA	EF422852	EF506223	EF506239	KM089734
P. gercinae	**URM 8348**	**Type; soil of Atlantic Forest, Brazil**	**MW648591**	**MW646389**	**MW646391**	**MW646393**
	URM 8383	**soil of Atlantic Forest, Brazil**	**OK148739**	**OK148702**	**OK148704**	**OK148705**
P. hispanicum	CBS 691.77	Ex-type; Citrus lemon fruit, Spain.	JX841247	KJ834456	MN969264	JN406539
	DTO 268D8	indoor house dust, Micronesia	KJ775639	KJ775132	n.a	n.a
P. ornatum	CBS 190.68	Soil, Japan	KC411687	KJ834479	MN969282	JN121451
P. ramusculum	DTO 040-G4	Unknow	n.a	KM088715	KM089100	KM089487
	CBS 251.56	Type; Unknow	NR121326	EU427269	EU427278	EU427260

n.a, not available

CaM 1 pp, 100 % bs; *RPB2* 1 pp, 100 % bs) (Fig. 2) and the combined phylogram (1 pp, 100 % bs) (Fig. 3). *Penicillium stangiae* sp. nov. belongs to the series *Dalearum* (Fig. 4). Strains URM 6020 and URM 8347 of this species formed a well-supported clade with *P. vanderhammenii* and *P. penarojense* in the *BenA* (1 pp, 100 % bs), ITS (0.99 pp, 89 % bs) (Fig. 4) and the combined phylogeny (1 pp, 98 % bs) (Fig. 5). Analysis of *CaM* and *RPB2* sequences could not resolve the phylogenetic position of *Penicillium stangiae* sp. nov. in the same clade with *P. vanderhammenii* and *P. penarojense*. In the *CaM* phylogeny (1 pp, 100 % bs), URM 6020 and URM 8347 took a basal position to a large clade that also included *P. vanderhammenii* and *P. penarojense* (Fig. 4) and, in the *RPB2* phylogeny (1 pp, 99 %), URM 8347 took a basal position to a large clade that included *P. vanderhammenii* and *P. penarojense* (Fig. 4). An overview of the length of each dataset and the most optimal substitution model for each dataset is presented in Table 2.

After the introduction of the two new species, the number of accepted species in section *Ramigena has increased* to seven and that in *Lanata-Divaricata* to 77. The morphology of the new species was compared with that of phylogenetically closely related species, and the details in this regard are presented in the "Notes" and "Discussion" section. Descriptions containing details of the distinguishing characteristics are provided in the "Taxonomy" section below.

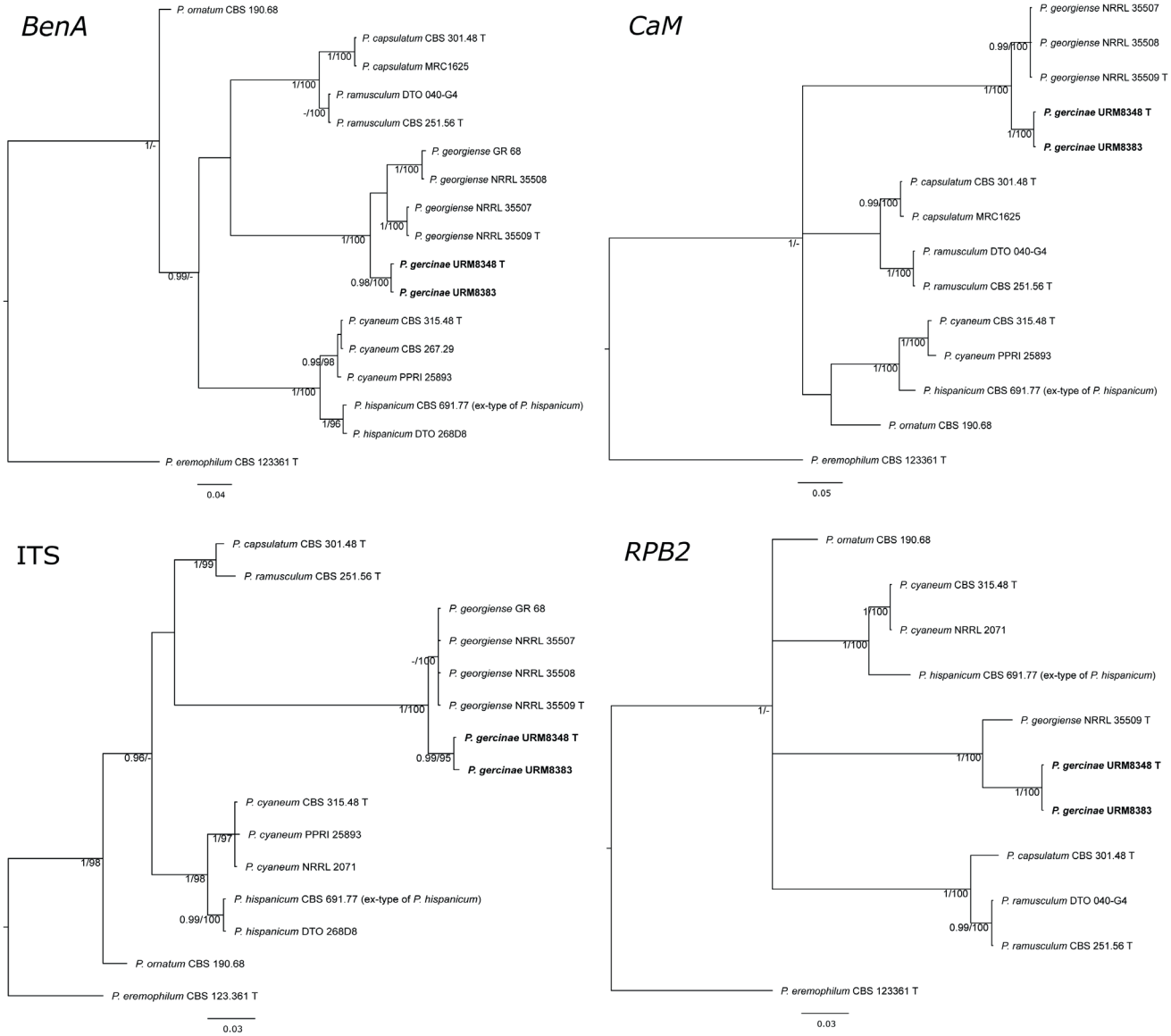

Figure 2. Single-gene phylogenies based on partial β-tubulin (BenA), calmodulin (CaM), internal transcribed spacer (ITS), and RNA polymerase II second largest subunit (RPB2) sequences. Sequences including several strains in the series *Georgiensia*. The new species *P. gercinae* sp. nov. (URM 8383 and URM 8348 T) are highlighted in bold. T = Type.

Table 2. Length (bp), variable positions (pb and %) in the alignments, and nucleotide substitution models used in phylogenetic analyses.

Information about alignment dataset		Section *Ramigena* (Series Georgiensia)	Section *Lanata- Divaricata* (Series *Dalearum*)
BenA	Length (bp)	425	397
dataset	Variable positions (pb and %)	175 (41.17 %)	123 (30.98 %)
	Nucleotide substitution model (BI)	HKY+G	GTR+I+G
CaM	Length (bp)	502	427
dataset	Variable positions (pb and %)	210 (41.83 %)	165 (38.64 %)
	Nucleotide substitution model (BI)	GTR+G	GTR+I+G
ITS dataset	Length (bp)	584	484
	Variable positions (pb and %)	127 (21.74 %)	41 (8.47 %)
	Nucleotide substitution model (BI)	GTR+G	GTR+I+G
RPB2	Length (bp)	914	756
dataset	Variable positions (pb and r%)	253 (27.68 %)	170 (22.48 %)
	Nucleotide substitution model (BI)	SYM+I+G	GTR+I+G
Combined	Length (bp)	2349	2064
dataset	Variable positions (pb and %)	765 (32.57 %)	499 (24.18 %)

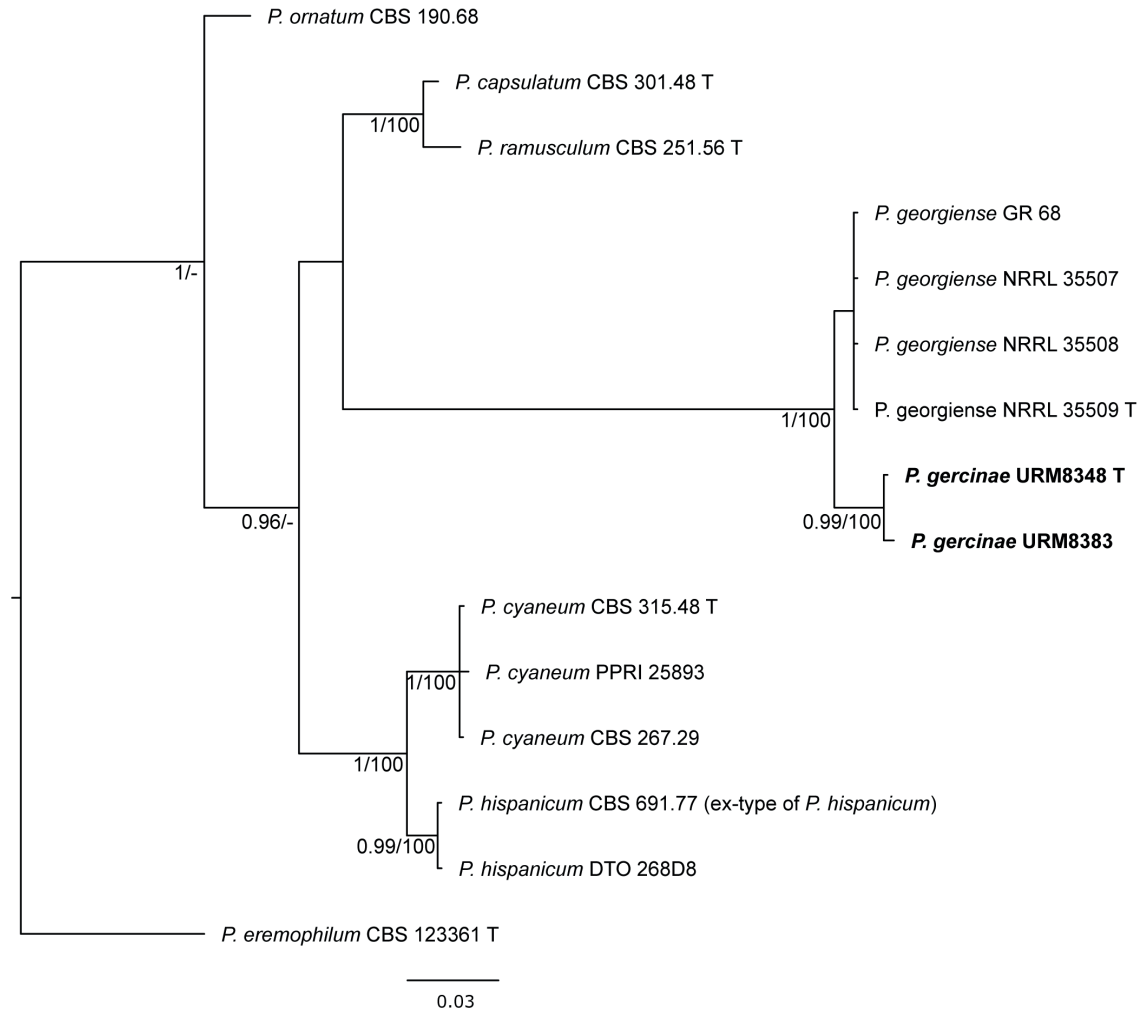

Figure 3. Phylogenetic position of the new *Penicillium* species in the series *Georgiensia* (section *Ramigena*) based on a combined dataset containing β-tubulin (*BenA*), calmodulin (*CaM*), internal transcribed spacer (ITS), and RNA polymerase II second largest subunit (*RPB2*) sequences. The new species *P. gercinae* sp. nov. (URM 8383 and URM 8348 T) are highlighted in bold. T = Type.

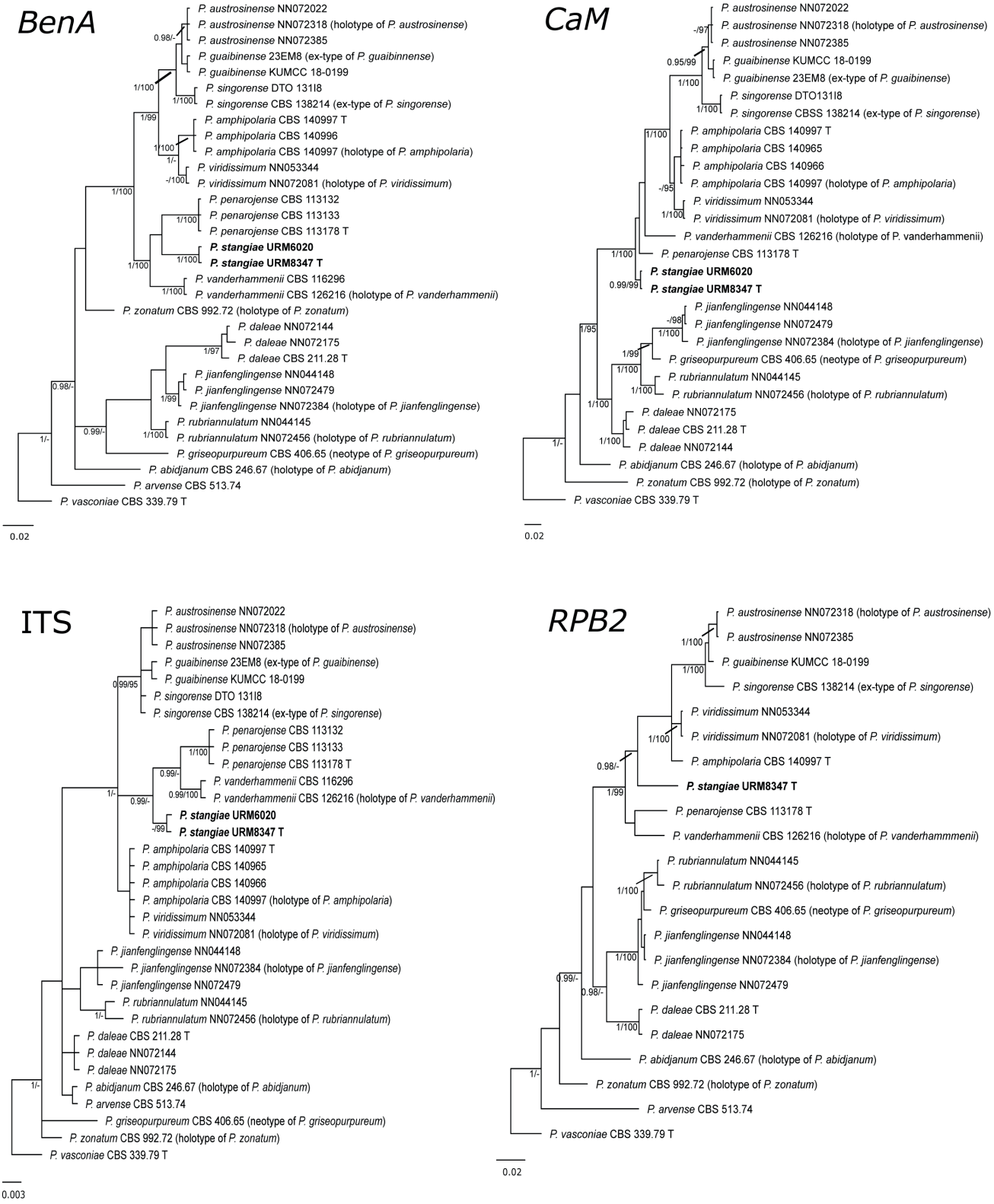

Figure 4. Single-gene phylogenies based on partial β-tubulin (BenA), calmodulin (CaM), internal transcribed spacer (ITS), and RNA polymerase II second largest subunit (RPB2) sequences. Sequences including several strains in the series *Dalearum*. The new species *P. stangiae* sp. nov. (URM 6020 and URM 8347) are highlighted in bold. T = Type.

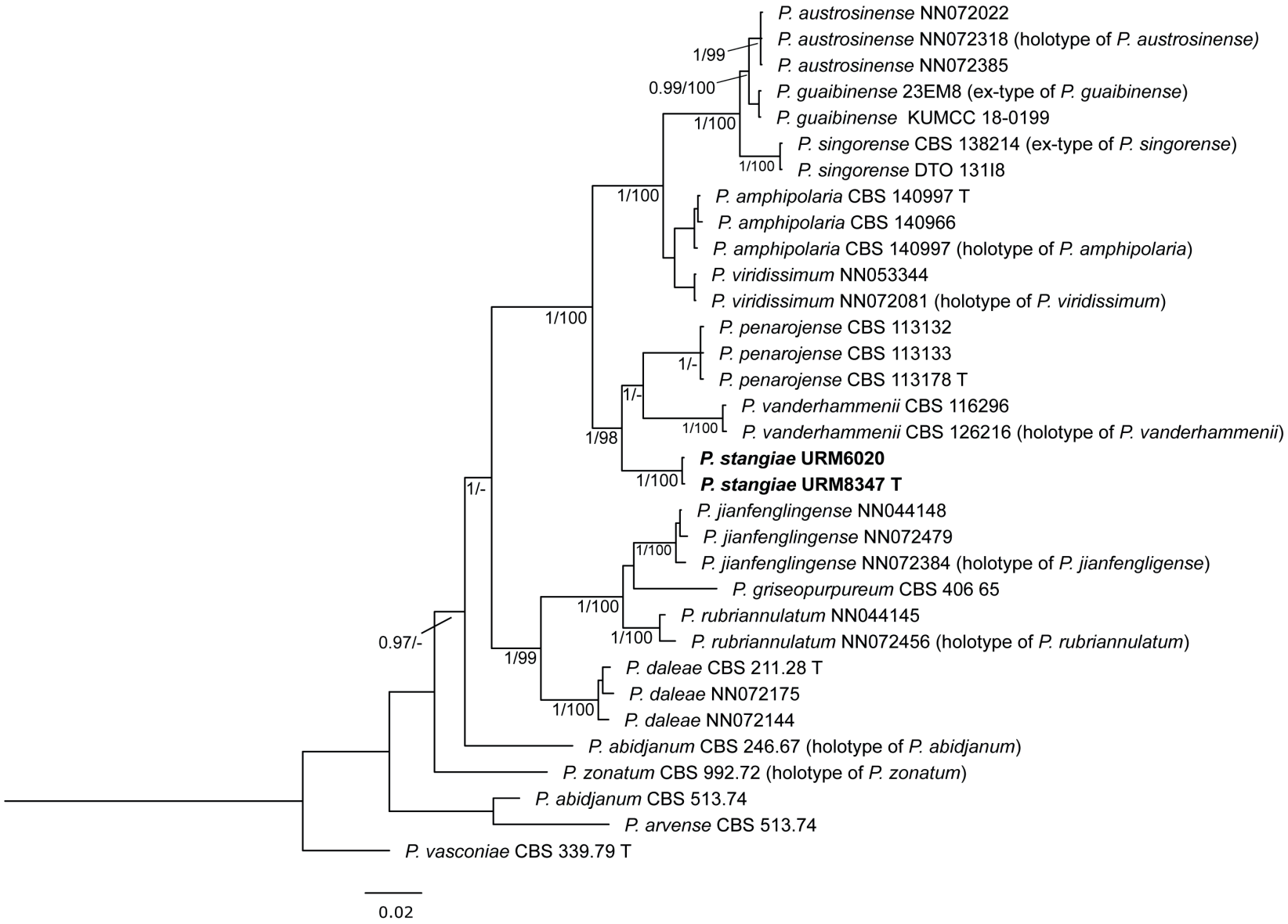

Figure 5. Phylogenetic position of the new *Penicillium* species in the series *Dalearum* (section *Lanata-Divaricata*) based on a combined dataset containing β-tubulin (*BenA*), calmodulin (*CaM*), internal transcribed spacer (ITS), and RNA polymerase II second largest subunit (*RPB2*) sequences. The new species *P. stangiae* sp. nov. (URM 6020 and URM 8347) are highlighted in bold. T = Type.

Taxonomy

Penicillium gercinae A.L. Alves & P.V. Tiago, **sp. nov.** (Fig. 6)

MycoBank MB 841260;

In: subgenus *Aspergilloides*, section *Ramigena*, series *Georgiensia*.

Type specimen. BRAZIL, Paudalho, Pernambuco state, isolated from soil, March 02, 2018, A.L. Alves, URM 94476, Holotype (slide preparation); ex-type living culture, URM 8348.

Etymology. *gercinae*. In honor of Gercina, the farmer who owns the AFS where the soil sample from which the isolated fungus was collected.

Diagnosis: No growth on CYA, CYAS and CZ. Conidiophores monoverticillate or biverticillate.

ITS barcode: MW648591. Alternative identification markers: *BenA* = MW646389, *CaM* = MW646391, *RPB2* = MW646393.

Colony diameter (7 d, in mm): CYA 25 °C no growth; MEA 25 °C 35-38; YES 25 °C 15-18; DG18 25 °C 6-8; OA 25 °C 23-25; CYAS 25 °C no growth; CZ 25 °C no growth; CREA 25 °C 5-8; CYA 15 °C no growth; CYA 30 °C no growth; CYA 37 °C no growth; MEA 15 °C no growth; MEA 30 °C 14-16; MEA 37 °C no growth.

Colony characters: CYA 25 °C: no growth MEA, 25 °C: margin plane, with center raised; mycelium white; texture velvety; sporulation weak to moderate; conidial color *en masse* pale greenish gray (110); exudate absent; soluble pigment absent; reverse ochreous (44). YES, 25 °C: margins low, radially sulcate, raised at the center; mycelium white; texture velutine; conidial color *en masse* olivaceous gray (121); exudate absent; soluble pigment absent, reverse dull green (70). DG18, 25 °C: colonies radially sulcate, slightly raised at center; margins low, entire; mycelium white; texture velvety; sporulation weak; conidial color *en masse* olivaceous black (108); exudate absent; soluble pigment absent; reverse olivaceous (48). OA, 25 °C: plane; margins fasciculate; mycelium dull green (70); texture floccose; sporulation sparse; conidial color *en masse* dull green (70); exudate absent; soluble pigment absent; reverse colorless. CYAS, 25 °C: no growth CZ, 25 °C: no growth. CREA, 25 °C: good growth, acid production absent.

Micromorphology: *conidiophores* monoverticillate or biverticillate, *stipe* smooth, light brown (15-) 30-100 (-190) × 2-3 µm; *vesicle* 2-3 µm; *metulae* 10-12× 2-3 µm; *phialides* ampulliform, 6 per stipe, 5-10 × 2-3 µm; *conidia* smooth walled, subellipsoid, 1-3 µm, the young conidia are less pigmented and smaller in average width/length.

Additional material examined: BRAZIL, Paudalho, Pernambuco, from soil of agroforestry system, March 01, 2018, A.L. Alves (URM 8383).

Notes: Phylogenetic analyses placed *Penicillium gercinae* in the same clade as *P. georgiense*, which belongs to the series *Georgiensia*, a basal branch distinct from the main clade of section *Ramigena*. However, unlike *P. gercinae*, *P. georgiense* can grow on CYA. *P. gercinae* differs from *P. georgiense* by its rapid growth on MEA (35-40 *vs*. 21-33 mm); *P. gercinae* have monoverticillate and biverticillate conidiophores, whereas *P. georgiense* have only biverticillate conidiophores. Conidiophores of *P. gercinae* are longer than that of *P. georgiense* (30-100 µm *vs*. 15-30 µm). The vesicle diameter of *P. gercinae* is smaller than that of *P. georgiense* (2-3 µm *vs*. 6 µm). The metulae length of *P. gercinae* is shorter than that commonly observed in *P. georgiense* (10-12 µm *vs*. 15 µm). *P. georgiense* have less phialides per stipe than *P. gercinae* (3-12 per stipe *vs*. 3 or 6 per stipe). The spore shape and size of *P. gercinae* is different from those of *P. georgiense* (subellipsoid and 1-3 µm *vs*. ellipsoidal to subspherical and 3-4 µm). These comparisons were made according to the description of *P. georgiense* by Peterson & Horn (2009) in the same cultivation conditions.

Penicillium stangiae A.L. Alves & P.V. Tiago, **sp. nov.** (Fig. 7)

MycoBank MB 841261;

In: subgenus *Aspergilloides*, section *Lanata-Divaricata*, series *Dalearum*.

Type specimen. BRAZIL, Paudalho, Pernambuco state, on soil of Atlantic Rainforest, March 02, 2018, A.L. Alves, URM 94477, Holotype (slide preparation); ex-type living culture, URM 8347.

Etymology. *stangiae*. In honor of Dorothy Mae Stang, an American-Brazilian nun who supported social movements for agrarian reform and sustainable development.

Diagnosis: Slow growing colonies on CYA and DG18 at 25 °C. This species produces monoverticillate and divaricate conidiophores and can not grow on CYA and MEA, on both 15 °C and 30 °C.

ITS barcode: MW648590. Alternative identification markers: *BenA* = MW646388, *CaM* = MW646390, *RPB2* = MW646392.

Colony diameter (7 d, in mm): CYA 25 °C 28-30; MEA 25 °C 28-29; YES 25 °C 36-37; DG18 25 °C 6-7; OA 25 °C 35-36; CYAS 25 °C 8-10; CZ 25 °C 34-35; CREA 25 °C 13-14; CYA 15 °C no growth; CYA 30 °C no growth; CYA 37 °C no growth; MEA 15 °C no growth; MEA 30 °C no growth; MEA 37 °C no growth.

Colony characters: CYA 25 °C radially plane; margins entire, low, narrow; mycelium white; texture cottony; conidial color *en masse* pale grayish green (68); exudate and soluble pigment absent; reverse primrose (64); MEA, 25 °C: plane; margins entire, low, narrow; mycelium white; texture floccose; sporulation weak to moderate; conidial color *en masse* pale greenish gray (110); exudate absent; soluble pigment absent, reverse sepia (63) to honey (64). YES 25 °C radially sulcate, margins low, entire; mycelium white; texture velvety; sporulation weak; conidial color *en masse* white in bord and hazel (88) at center; exudate absent;

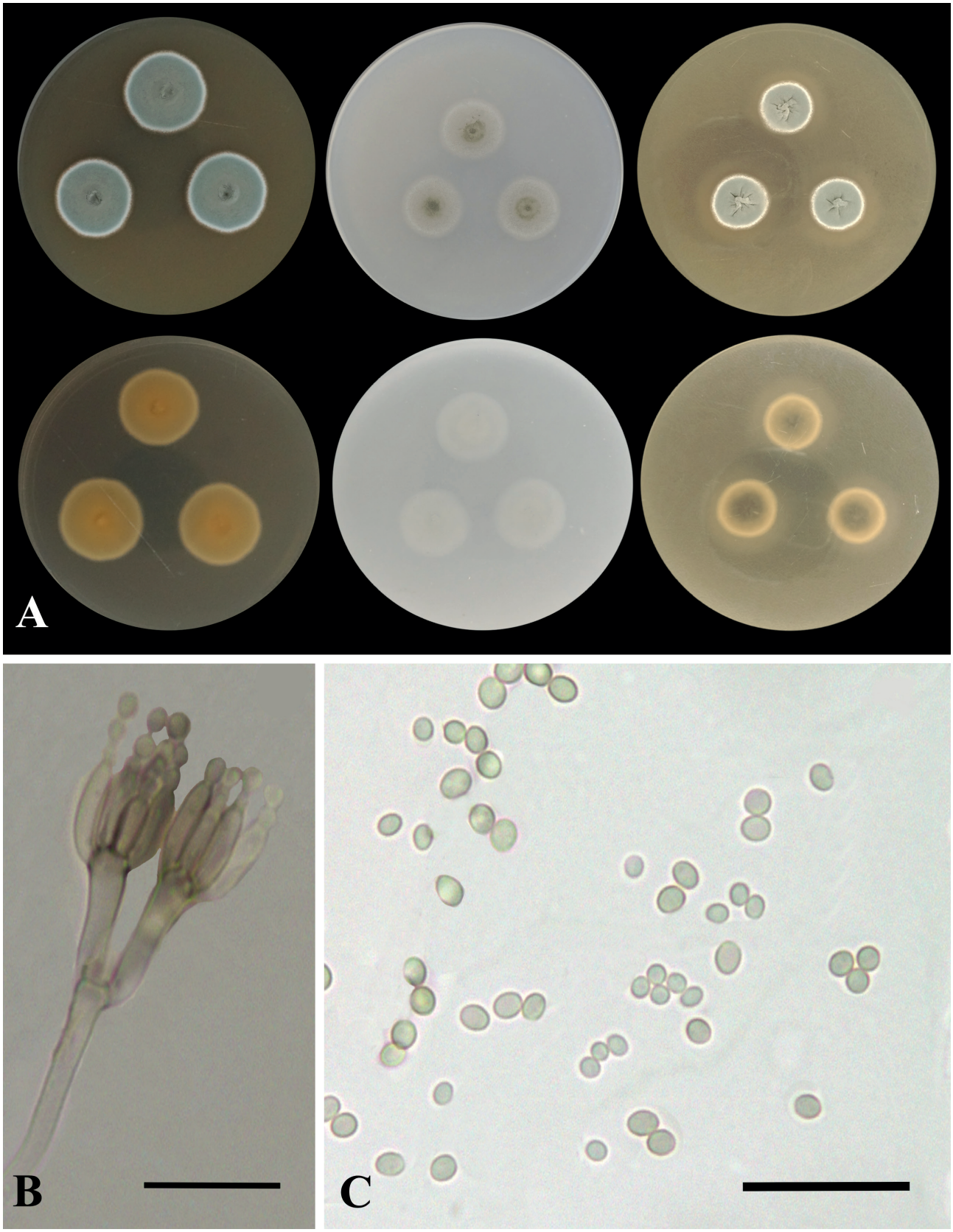

Figure 6. *Penicillium gercinae* sp. nov. **A** Verse (Up) and reverse (Down) of colonies on MEA, on OA and on YES. **B** Conidiophore. **C** Conidia. Scale bars: 12 µm **(B)**; 13 µm **(C)**.

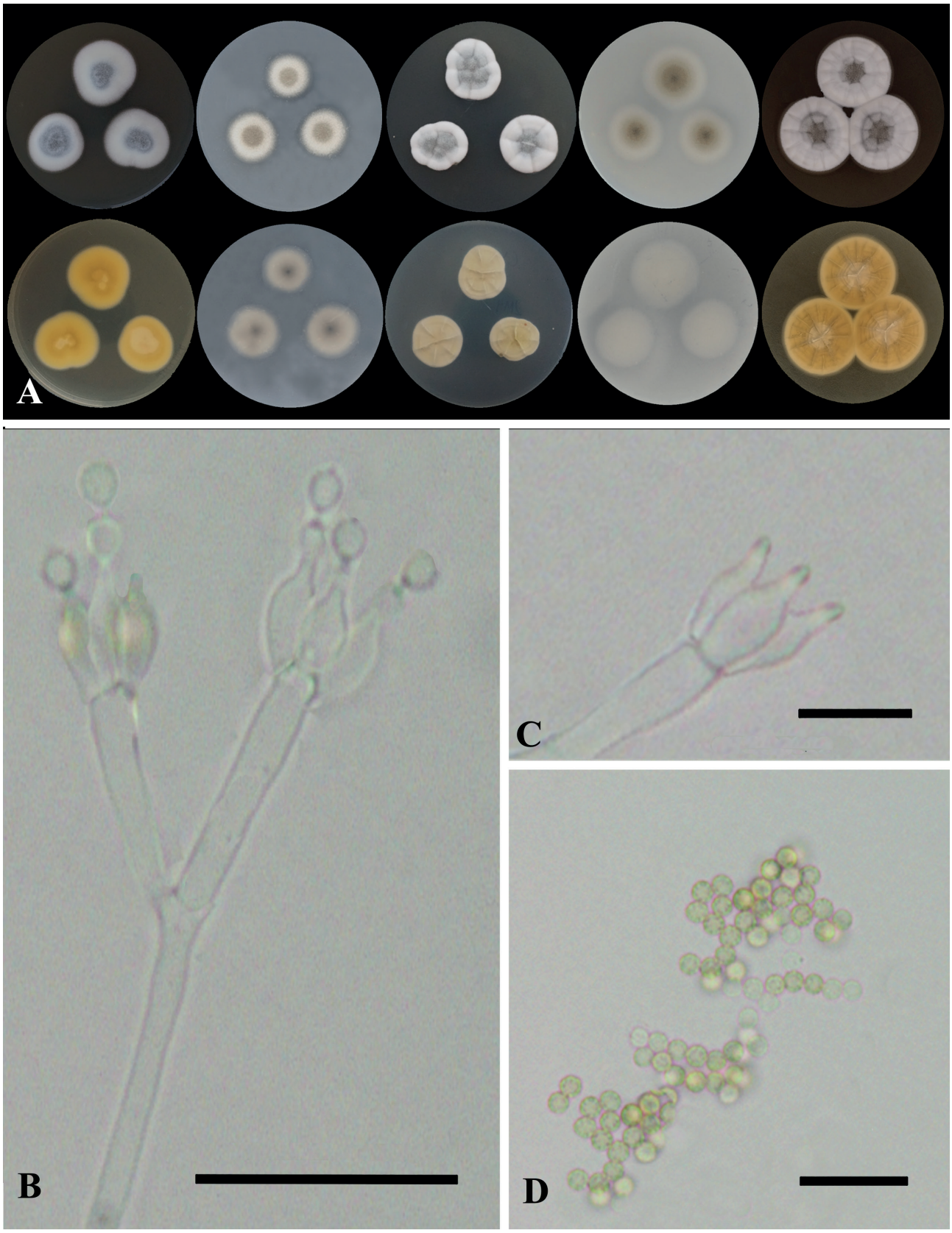

Figure 7. *Penicillium stangiae* sp. nov. **A** Verse (Up) and reverse (Down) of colonies on MEA, on CZ, on CYA, on OA and on YES, respectively. **B-C** Conidiophore. **D** Conidia. Scale bars: 30 µm **(B)**; 15 µm **(C)**; 10 µm **(D)**.

soluble pigment absent, reverse cinnamon (62). DG18, 25 °C: radially sulcate, slightly raised at the center; margins low, entire; mycelium white; texture velvety; sporulation weak; conidial color *en masse* greenish gray (110); exudate absent; soluble pigment absent; reverse olivaceous (48). OA, 25 °C: plane; margins regular; mycelium white; texture floccose; sporulation sparse; conidial color *en masse* hazel (88); exudate absent; soluble pigment absent; reverse white. CYAS, 25 °C: radially sulcate, raised at center; margins entire, low; mycelium white; texture velvety; sporulation moderate; conidial color *en masse* white; exudate absent; soluble pigment absent, reverse buff (45). CZ, 25 °C: radially sulcate, slightly raised at center; margins entire, low, narrow; mycelium white; texture velvety; sporulation moderate; conidial color *en masse* hazel (88); exudate absent; soluble pigment present, reverse olivaceous (48). CREA, 25 °C: good growth, acid production absent.

Micromorphology: *Conidiophores* monoverticillate and divaricate, *stipes* smooth, hyaline, (17.5-) 20-40 (-100) × 1.5-2.5 µm; *vesicle* 2-2.7 µm; *metulae* divergent, 2 per stipe, 12.5-15 × 2-3 µm; *phialides* ampulliform, 3 or 6 per metula or stipe, 5-7.5 × 2 µm; *conidia* echinulate, globose, 1-2 µm diam.

Additional material examined: BRAZIL, Recife, Pernambuco state, isolated from soil of Dois Irmãos Park, March 2010, R.C. Ramos (URM 6020).

Notes: Phylogenetic analyses resolved *Penicillium stangiae*, classified in section *Lanata-Divaricata* series *Dalearum*, within the same clade as *Penicillium penarojense* and *Penicillium vanderhammenii*; however, *P. penarojense* and *P. vanderhammenii* exhibited faster growth on CYA at 25 °C (40-45 mm and 42-49 mm, respectively) than *P. stangiae* (28-30 mm). Some strains of *P. penarojense* and strains of *P. vanderhammenii* produce acid compounds on CREA, whereas strains of *P. stangiae* do not. Colony diameter of *P. penarojense* and *P. vanderhammenii* on DG18 is larger than that of *P. stangiae* (20-25 mm and 15-20 mm, respectively, *vs*. 6-7 mm). *P. penarojense* and *P. vanderhammenii* possess monoverticillate and biverticillate conidiophores, whereas *P. stangiae* have monoverticillate, divaricate conidiophores: those of *P. penarojense* and *P. vanderhammenii* conidiophores are longer than those of *P. stangiae* (200-600 µm and 200-800 µm, respectively, *vs*. 20-40 µm). Metulae of *P. penarojense* are longer than that of *P. stangiae* (15-25(-30) µm *vs*. 12-15 µm), and the former have more phialides per metulae than the latter (4-8 per metulae *vs*. 3-4 per metulae); phialide of *P. penarojense* and *P. vanderhammenii* are longer than that of *P. stangiae* (6.0-9.0 µm, 7.0-9.0 µm, and 5-7 µm, respectively). Both *P. penarojense* and *P. stangiae* have globose conidia. These comparisons were made according to the description of *P. georgiense* by Houbraken *et al*. (2011) in the same cultivation conditions.

Discussion

Several new species of *Penicillium* were described in Brazil recently, some of them isolated from soil (Crous *et al*. 2014; Taniwaki *et al*. 2015; Crous *et al*. 2019; Ramos *et al*. 2021). We introduce *P. gercinae* in the section *Ramigena* and *P. stangiae* in the section *Lanata-Divaricata,* both species from soil in the Atlantic Forest environment.

The new species *P. gercinae* belongs to the series *Georgiensia* and produces monoverticillate and biverticillate conidiophores, indicating a relationship with other taxa of the small section *Ramigena*. A sexual state was not observed in the new species described in the present study. *P. gercinae* is phylogenetically close to *P. georgiense,* but it does not grow on CYA nor produce acid on CREA, and has globose conidia. *P. georgiense* is no longer a sole, basal species in section *Charlesia* clade (Sun *et al*. 2021), being actually supported in clade of section *Ramigena* (Houbraken *et al*. 2020) and, until now, form the series *Georgiensia* with *P. gercinae*.

Penicillium stangiae belongs to this series *Dalearum* in the section *Lanata-Divaricata,* and produces monoverticillate, biverticillate, and divaricate conidiophores, colonies in shades of green, smooth walled, globose to subglobose conidia. Sexual morphs were not observed in cultures. Several features observed in new species are in compliance with the characteristics features for this series. As in Houbraken *et al*. (2020), the series *Dalearum* has species with moderately fast growing colonies, conidial color *en masse* dull green, bluish green to gray-green; conidiophores monoverticillate, biverticillate, and divaricate, occasionally terverticillate, conidia variable shaped, and variable growth at 37 °C.

The present study is the first report of a new species in section *Ramigena*, found in the soil of an agroforestry system in Northeast Brazil. Most species in section *Ramigena* have been isolated from organic substrates (soil, leaf litter, and wood) (Tab. 1). It is noteworthy that agroecological transition areas contain microorganisms that favor the use of carbon sources in the soil, thus making this nutrient available to plants during the agroecological transitional process, promoting soil fertility (Hecht 2018). In the present study, we report for the first time, in the soil of the Atlantic Rainforest fragment in Northeast Brazil, a new species in section *Lanata-Divaricata* series *Dalearum*. Species in this section have an affinity for acidic soils and are acid-tolerant (Diao *et al*. 2018; Alves *et al*. 2021). In response to phosphorus deficiency in soil and recalcitrant lignin-rich organic matter, fungi can release organic acids and enzymes for nutrient acquisition, developing the rhizosphere and enzymatic processes that promote tolerance to low pH in plants (Deepa *et al*. 2010; Fujii 2014). The results of the current study contribute to the taxonomic knowledge of *Penicillium* at soil of different land use.

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