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Amazonian diversity of *Penicillium* Link (Eurotiomycetes, Ascomycota) in the culture collection of the National Institute of Amazonian Research, with description of a new species

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ABSTRACT

Penicillium Link is a widely known genus of ascomycetes, with a worldwide distribution, mainly known for its use in the food industry and production of mycotoxins. Considering the importance of culture collections as repositories of microbial biodiversity, we carried out a polyphasic taxonomic review of fungi of the genus *Penicillium* preserved in the Collection of Microorganisms of Agrosilvicultural Interest at the National Institute for Amazonian Research (CMAI-INPA) in Brazil, which hosts several deposits of *Penicillium* without specific identification. A total of 150 strains were reactivated for macro and micromorphological analyses and DNA extraction, amplification and sequencing. The strains were represented by nine species distributed among the sessions *Citrina* (*P. citrinum*), *Charlesia* (*P. chermesinum*), *Chrysogena* (*P. chrysogenum*, *P. rubens*), *Aspergilloides* (*P. glabrum*), *Sclerotiorum* (*P. meliponae*), *Fasciculata* (*P. palitans*, *P. polonicum*) and *Roquefortorum* (*P. paneum*). One strain is herein described as *Penicillium carneiroi* sp. nov., a new species in the section *Exilicaulis*. We present images of the new species and compare it with other morphologically close species. The polyphasic taxonomic review carried out in this study showed a previously unknown diversity for the Amazon region, and that microorganism repositories may contain new species or records for the region.

KEYWORDS: Amazon, Aspergillaceae, phylogeny, polyphasic taxonomic review

Diversidade amazônica de *Penicillium* Link (Eurotiomycetes, Ascomycota) na coleção de culturas do Instituto Nacional de Pesquisas da Amazônia, com descrição de uma nova espécie

RESUMO

Penicillium Link é um gênero de ascomicetos amplamente conhecido, com distribuição mundial, conhecido principalmente por seu uso na indústria alimentícia e produção de micotoxinas. Considerando a importância das coleções de culturas como repositórios da biodiversidade microbiana, realizamos uma revisão taxonômica polifásica de fungos do gênero *Penicillium* preservados na Coleção de Microrganismos de Interesse Agrossilvicultural do Instituto Nacional de Pesquisas da Amazônia (CMAI-INPA) no Brasil, que abriga vários depósitos de *Penicillium* sem identificação específica. Um total de 150 linhagens foi reativado para análises macro e micromorfológicas e extração, amplificação e sequenciamento de DNA. As linhagens foram representadas por nove espécies distribuídas entre as sessões *Citrina* (*P. citrinum*), *Charlesia* (*P. chermesinum*), *Chrysogena* (*P. chrysogenum*, *P. rubens*), *Aspergilloides* (*P. glabrum*), *Sclerotiorum* (*P. meliponae*), *Fasciculata* (*P. palitans*, *P. polonicum*) e *Roquefortorum* (*P. paneum*). Uma linhagem é aqui descrita como *Penicillium carneiroi* sp. nov., uma nova espécie na seção *Exilicaulis*. Apresentamos imagens da nova espécie e a comparamos com outras espécies morfologicamente próximas. A revisão taxonômica polifásica realizada neste estudo mostrou uma diversidade previamente desconhecida para a região amazônica, e que repositórios de microrganismos podem conter novas espécies ou registros para a região.

PALAVRAS-CHAVE: Amazônia, Aspergillaceae, filogenia, revisão taxonômica polifásica

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INTRODUCTION

Biological collections serve as systematic repositories for various types of biological materials, including type specimens (Oliveira et al. 2019). These collections house organisms, or parts thereof, in an *ex-situ* environment, outside their natural habitats, offering strains for research, educational or biotechnological purposes, as well as acting as database for biogeography and biological conservation (Kamenski et al. 2016). Collections are organized based on the origin and taxonomic identification of each organism, and must prioritize accessibility, quality, longevity, integrity and interoperability of the collection data (Oliveira et al. 2019). They provide essential information on biodiversity, which serves as a basis for scientific and technological development (DiEuliis et al. 2016).

The preservation and availability of biological collections are of paramount importance, particularly given Brazil's immense biodiversity and alarming rates of species extinction (Santos et al. 2018). While many biological collections consist of deceased organisms or parts thereof, such as herbaria and zoological collections, microorganism collections house and preserve living organisms (Oliveira et al. 2019). This unique characteristic demands significant financial resources and specialized professionals, as these collections are responsible for maintaining the biological raw material in a viable state (Akaniro et al. 2023). This, in turn, enables future studies exploring both genetic and metabolic diversity.

The National Institute for Amazonian Research (Instituto Nacional de Pesquisas da Amazônia - INPA) stands as a key institution for Amazonian biodiversity studies, epitomized by its scientific collections that trace back to 1954 with the establishment of the INPA Herbarium (Fernandes 2020). Among these, the Collection of Microorganisms of Agrosilvicultural Interest (CMAI-INPA) harbors more than 1830 cultures of xylophagous and phytopathogenic fungi with notable economic and biotechnological potential.

Penicillium Link. is a widely known genus of fungi, primarily known for its applications in the food and pharmaceutical industries (Visagie et al. 2014). This genus encompasses cosmopolitan species, playing pivotal roles in ecosystems and biotechnological processes. Most species act as decomposers on soil and can produce a variety of mycotoxins (Kemboi et al. 2020). Few organisms are competent in degrading wood due to the lack of an enzymatic apparatus to access its most refractive components, such as lignin, hemicellulose and cellulose (Riley et al. 2014). *Penicillium* species, despite having restricted enzymatic capacity in wood degradation, exhibit high ecological plasticity, employing generalist strategies characterized by fast to moderate growth and colonization (Akaniro et al. 2023). They dominate in conditions of high stress and/or disturbance, opportunistically utilizing resources available from wood-decaying environments (Baltierra-Trejo et al. 2016; Janusz et al. 2017).

Several strains from CMAI-INPA are partially identified, and only through morphological characteristics. While morphological identification is necessary, it has limitations, particularly for *Penicillium*, as many of its species exhibit cryptic diagnostic characters, leading to inconsistent identification (Visagie et al. 2014). This inconsistency, in turn, results in an underestimation of the Amazonian diversity and ecosystem services provided by these organisms. Therefore, it is crucial to conduct a taxonomic revision of the material at CMAI-INPA using a polyphasic approach to establish and maintain a comprehensive database containing accurate information for research purposes, benefiting both national and international researchers. In this context, a subset of *Penicillium* strains stored in the CMAI-INPA collection from 1983 to 2022 was evaluated using polyphasic taxonomy, which combines the use of genetic data, and macro and microscopic characteristics in order to update the diversity of this genus present in the collection and contribute to the microbial diversity of the Amazonian region of Brazil.

MATERIAL AND METHODS

Strain selection and reactivation

For this study, 150 strains deposited as *Penicillium* in the CMAI-INPA collection were selected, after examining their morphological characteristics. The strains were maintained using the continuous subculture preservation method (Guimarães et al. 2014). The taxonomic review was conducted at the Mycology Department at Universidade Federal de Pernambuco - UFPE (Recife, Brazil).

DNA extraction, amplification and sequencing

Fungal biomass was obtained from the cultures grown on Petri dishes with SDA, kept at 25 °C for up to seven days in the dark. For genomic DNA extraction, Promega's Wizard Genomic DNA Purification Kit was used, following the manufacturer's recommendations. After extraction, polymerase chain reactions (PCR) were performed. Initially, parts of the *BenA* (primers Bt2a and Bt2b) and *CaM* (primers CMD5 and CMD6) genes were amplified as described by Visagie et al. (2014). For the putative new species, additional ITS barcodes (ITS1, 5.8S rDNA, and ITS2; primers V9G and LS266) were generated (Table 1) using the methods described by Samson et al. (2014). The PCR reactions were performed with a total volume of 12.5 µl containing 4.25 µl ddH_2O, 6.25 µl Taq PCR Super Mix (Cellco Biotec), 1 µl DNA template, and 0.5 µl of each forward and reverse primer (10 pM) with the primers thermal cycling programs used for amplification were those recommended by Visagie et al. (2014). The amplicons were evaluated on a 1% agarose gel with SYBR Safe DNA Gel Stain (Invitrogen), and purified using the Exonuclease I and Alkaline Phosphatase

enzymes contained in the EXO+SAP kit (Cellco Biotec), following the manufacturer's recommendations. The purified products were sent to sequencing, using the same primers, on the Multiuser Platform for Sequencing and Gene Expression at the Center for Biological Sciences at UFPE. Newly generated sequences were submitted to the National Center for Biotechnology Information (NCBI) nucleotide database (https://www.ncbi.nlm.nih.gov/).

Table 1. *Penicillium* strains from the Collection of Microorganisms of Agroforestry Interest of the National Institute for Amazonian Research (CMAI-INPA) (collection identification code CMINPA) identified in the present study and their original information. Locality: AM = Amazonas state (Brazil); PA = Pará state (Brazil); Mato Grosso = Mato Grosso state (Brazil). The new species described in here is marked in bold.

CMINPA number	Deposit identification	Current identification	Deposit year	Substrate	Locality
18	*Penicillium* sp.	*P. rubens*	2003	Wood	Balbina-AM
21	*Penicillium* sp.	*P. rubens*	1983	Wood	Balbina-AM
34	*Penicillium* sp.	*P. citrinum*	1985	Wood	Balbina-AM
38	*Penicillium* sp.	*P. rubens*	1985	Termite nest	Balbina-AM
56	*Penicillium* sp.	*P. rubens*	1985	Wood	Belém-PA
71	*Penicillium* sp.	*P. rubens*	1987	Wood	INPA-AM
100	*Penicillium* sp.	*P. rubens*	1987	Wood	INPA-AM
107	*P. verruculosum*	*P. paneum*	1987	Wood	INPA-AM
111	*P. implicatum*	*P. citrinum*	1987	Wood	INPA-AM
117	*P. lividum*	*P. rubens*	1987	Wood	INPA-AM
122	*P. minioluteum*	*P. rubens*	1987	Wood	INPA-AM
124	*P. lividum*	*P. rubens*	1987	Wood	INPA-AM
127	*Penicillium* sp.	*P. citrinum*	1987	Wood	INPA-AM
145	*P. glabrum*	*P. glabrum*	1988	Wood	INPA-AM
156	*P. lividum*	*P. rubens*	1988	Wood	Manaus-AM
161	*P. citrinum*	*P. rubens*	1988	Wood	Manaus-AM
162	*P. citrinum*	*P. rubens*	1988	Wood	Manaus-AM
190	*Trichoderma*	*P. rubens*	1988	Wood	Manaus-AM
191	*Penicillium* sp.	*P. rubens*	1988	Wood	Manaus-AM
260	*P. verruculosum*	*P. rubens*	1993	Wood	Manaus-AM
280	*P. lividum*	*P. rubens*	1993	Wood	Manaus-AM
282	*P. citrinum*	*P. rubens*	1993	Wood	Manaus-AM
286	*Penicillium* sp.	*P. rubens*	1993	Wood	Manaus-AM
292	*Trichoderma*	*P. rubens*	2011	Wood	Manaus-AM
307	*Penicillium* sp.	*P. rubens*	1993	Wood	Manaus-AM
313	*Aspergillus*	*P. rubens*	2003	Wood	Manaus-AM
317	*P. lividum*	*P. rubens*	1993	Wood	Manaus-AM
334	*P. citrinum*	*P. rubens*	1994	Leaf litter	Embrapa-AM
344	*P. citrinum*	*P. citrinum*	1995	Wood	Manaus-AM
465	*P. citrinum*	*P. rubens*	1995	Unknown	Embrapa-AM
483	*P. lividum*	*P. rubens*	2000	Unknown	Manaus-AM
489	*P. commune*	*P. rubens*	2000	Wood	Manaus-AM
494	*P. verruculosum*	*P. rubens*	2000	Leaf litter	Manaus-AM
599	*Trichoderma*	*P. rubens*	2002	Wood	Manaus-AM
607	*P. citrinum*	*P. rubens*	2002	Wood	Manaus-AM
609	*Trichoderma*	*P. rubens*	2002	Wood	Manaus-AM
624	*Penicillium* sp.	*P. citrinum*	2002	Wood	Manaus-AM
625	*Penicillium* sp.	*P. rubens*	2002	Wood	Manaus-AM
665	*Penicillium* sp.	*P. rubens*	2003	Wood	Manaus-AM
673	*P. citrinum*	*P. rubens*	2003	Wood	Manaus-AM
675	*Penicillium* sp.	*P. rubens*	2003	Wood	Manaus-AM
679	*Penicillium* sp.	*P. rubens*	2003	Wood	Manaus-AM
712	*P. griseafulvum*	*P. rubens*	2003	Wood	Manaus-AM
718	*Penicillium* sp.	*P. rubens*	2003	Wood	Manaus-AM

Table 1. Continued

CMINPA number	Deposit identification	Current identification	Deposit year	Substrate	Locality
767	*Penicillium* sp.	*P. rubens*	2003	Wood	Manaus-AM
770	*P. fellutanum*	*P. palitans*	2003	Wood	Manaus-AM
778	*Penicillium* sp.	*P. rubens*	2003	Wood	Manaus-AM
783	*P. citrinum*	*P. rubens*	2003	Wood	Manaus-AM
785	*P. citrinum*	*P. rubens*	2003	Wood	Manaus-AM
795	*Penicillium* sp.	*P. rubens*	2003	Wood	Manaus-AM
835	*Penicillium* sp.	*P. rubens*	2003	Wood	Manaus-AM
840	*Penicillium* sp.	*P. rubens*	2003	Wood	Manaus-AM
896	*Penicillium* sp.	*P. rubens*	2003	Wood	Manaus-AM
913	*Penicillium* sp.	*P. rubens*	2003	Wood	Manaus-AM
924	*P. lividum*	*P. rubens*	2003	Wood	Manaus-AM
936	*P. lividum*	*P. rubens*	2004	Wood	Manaus-AM
938	*Penicillium* sp.	*P. chrysogenum*	2004	Wood	Manaus-AM
940	*Penicillium* sp.	*P. rubens*	2004	Wood	Manaus-AM
945	*Penicillium* sp.	*P. rubens*	2004	Wood	Manaus-AM
955	*P. raistrickii*	*P. rubens*	2004	Wood	Manaus-AM
966	*P. implicatum*	*P. citrinum*	2004	Wood	Manaus-AM
969	*Penicillium* sp.	*P. citrinum*	2004	Wood	Manaus-AM
971	*P. implicatum*	*P. rubens*	2004	Wood	Manaus-AM
980	*Penicillium* sp.	*P. rubens*	2004	Wood	Manaus-AM
998	*Penicillium* sp.	*P. rubens*	2004	Wood	Manaus-AM
1001	*P. lividum*	*P. rubens*	2004	Wood	Manaus-AM
1023	*P. lividum*	*P. rubens*	2004	Wood	Manaus-AM
1027	*Penicillium* sp.	*P. rubens*	2004	Wood	Manaus-AM
1061	*Penicillium* sp.	*P. rubens*	2004	Wood	Manaus-AM
1066	*Trichoderma*	*P. rubens*	2004	Wood	Manaus-AM
1073	*Trichoderma*	*P. rubens*	2004	Wood	Manaus-AM
1087	*Penicillium* sp.	*P. rubens*	2004	Wood	Manaus-AM
1088	*P. lividum*	*P. rubens*	2004	Wood	Manaus-AM
1090	*P. citrinum*	*P. rubens*	2004	Wood	Manaus-AM
1096	*P. lividum*	*P. rubens*	2004	Wood	Manaus-AM
1107	*Penicillium* sp.	*P. citrinum*	2004	Wood	Manaus-AM
1116	*Penicillium* sp.	*P. rubens*	2004	Wood	Manaus-AM
1117	*Aspergillus*	*P. rubens*	2004	Wood	Manaus-AM
1122	*P. citrinum*	*P. rubens*	2004	Wood	Manaus-AM
1124	*Penicillium* sp.	*P. rubens*	2004	Wood	Manaus-AM
1142	*Penicillium* sp.	*P. citrinum*	2004	Wood	Manaus-AM
1188	*Penicillium* sp.	*P. rubens*	2006	Ventilation duct	INPA-AM
1193	*Paecilomyces*	*P. rubens*	2006	Ventilation duct	INPA-AM
1196	*Penicillium* sp.	*P. rubens*	2006	Ventilation duct	INPA-AM
1206	*P. lividum*	*P. rubens*	2006	Ventilation duct	INPA-AM
1207	*Penicillium* sp.	*P. rubens*	2006	Ventilation duct	INPA-AM
1208	*Penicillium* sp.	*P. rubens*	2006	Ventilation duct	INPA-AM
1211	*Penicillium* sp.	*P. rubens*	2006	Ventilation duct	INPA-AM
1216	*Paecilomyces*	*P. rubens*	2006	Ventilation duct	INPA-AM
1219	*Talaromyces*	*P. rubens*	2006	Wood	Manaus-AM
1249	*Paecilomyces*	*P. rubens*	2008	Unknown	Unknown
1250	*Trichoderma*	*P. citrinum*	2010	Unknown	Unknown
1274	*Trichoderma*	*P. rubens*	2008	Unknown	Unknown
1309	***Penicillium* sp.**	***P. carneiroi* sp. *nov.***	**2008**	**Wood**	**Paragominas-PA**
1311	*Penicillium* sp.	*P. rubens*	2008	Unknown	Unknown

Table 1. Continued

CMINPA number	Deposit identification	Current identification	Deposit year	Substrate	Locality
1316	*Penicillium* sp.	*P. citrinum*	2008	Unknown	Unknown
1320	*Paecilomyces*	*P. citrinum*	2008	Unknown	Unknown
1323	*P. lividum*	*P. rubens*	2008	Unknown	Unknown
1329	*Penicillium* sp.	*P. rubens*	2008	Unknown	Unknown
1333	*Penicillium* sp.	*P. rubens*	2008	Unknown	Unknown
1338	*Penicillium* sp.	*P. rubens*	2008	Unknown	Unknown
1342	*Penicillium* sp.	*P. rubens*	2008	Unknown	Unknown
1363	*Penicillium* sp.	*P. rubens*	2008	Unknown	Unknown
1372	*Penicillium* sp.	*P. rubens*	2008	Unknown	Unknown
1381	*Penicillium* sp.	*P. rubens*	2008	Unknown	Unknown
1382	*Paecilomyces*	*P. rubens*	2008	Unknown	Unknown
1383	*Paecilomyces*	*P. citrinum*	2008	Unknown	Unknown
1384	*Trichoderma*	*P. chrysogenum*	2008	Unknown	Unknown
1393	*P. citrinum*	*P. rubens*	2008	Unknown	Unknown
1399	*Penicillium* sp.	*P. rubens*	2008	Unknown	Unknown
1416	*Paecilomyces*	*P. rubens*	2008	Unknown	Unknown
1424	*Paecilomyces*	*P. rubens*	2008	Unknown	Unknown
1432	*P. chrysogenum*	*P. rubens*	2008	Unknown	Unknown
1437	*Penicillium* sp.	*P. rubens*	2008	Unknown	Unknown
1454	*Penicillium* sp.	*P. rubens*	2008	Wood	Roraima
1519	*P. citrinum*	*P. rubens*	2011	Basidiomycetes	Manaus-AM
1544	*Paecilomyces*	*P. chermesinum*	2012	Acrylic	INPA-AM
1749	*Penicillium* sp.	*P. citrinum*	2011	Soil	Mato Grosso
1751	*Penicillium* sp.	*P. rubens*	2011	Soil	Mato Grosso
1753	*Penicillium* sp.	*P. rubens*	2011	Soil	Mato Grosso
1754	*Penicillium* sp.	*P. rubens*	2011	Soil	Mato Grosso
1755	*Penicillium* sp.	*P. citrinum*	2010	Soil	Mato Grosso
1759	*Penicillium* sp.	*P. citrinum*	2012	Soil	Mato Grosso
1761	*Penicillium* sp.	*P. rubens*	2011	Soil	Mato Grosso
1807	*Trichoderma*	*P. rubens*	Unknown	Basidiomycetes	Manaus-AM
1898	*Penicillium* sp.	*P. meliponae*	Unknown	Guarana extract	Uruará-PA
1961	*Penicillium* sp.	*P. polonicum*	2022	Moldy bread	Manaus-AM

Phylogenetic analysis and strain deposit

The electropherograms obtained through sequencing, were inspected using the BioEdit software (Hall 2014). After editing, a search was performed for similar sequences deposited in GenBank available at the NCBI, using the BLASTn tool. The sequences were then aligned together with those retrieved (including sequences from ex-type cultures) from the database using the MAFFT v.7 (Yamada et al. 2016) and manually corrected using the MEGA v.7 (Kumar et al. 2016). Then, maximum likelihood analyses were performed with 1000 bootstrap resampling using RAxML-HPC version 8.2.12 9 (Stamatakis 2014) in BlackBox with GTRGAMMA model, adopting default parameters, and Bayesian Inference analysis (1×106 generations and the first 25% of the samples were discarded) in MrBayes 3.2.7a on XSEDE, both implemented in the CIPRES Science Gateway online platform. Nucleotide substitution models were searched through Mr. ModelTest2 2.1.6 (Flouri et al. 2015) on XSEDE via the CIPRES Science Gateway (www.phylo.org), being estimated separately for each alignment and selected according to Akaike Information Criterion (AIC). The combined datasets for section Exilicaulis were made by concatenating the individual alignments using Mesquite v. 3.04 (Maddison and Maddison 2023). The phylogenetic trees obtained were visualized in FigTree v.1.4.0 and edited in CorelDRAW 2017. Bayesian inference (BI) posterior probabilities (pp) and bootstrap (bs) values are included 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.

A representative of each species was deposited in the URM Culture Collection following the protocols established by the collection and holotype (as permanent slide preparations) in the HURM fungarium (Herbário URM Pe. Camille Torrend), both at the Universidade Federal de Pernambuco (UFPE),

Recife, Brazil (Barbosa et al. 2020). Sequences and related annotations used for fungal identification were deposited in the NCBI. The voucher received for each sequence deposited will be associated with the deposit information of the CMAI-INPA and URM collections.

Culture and micromorphological analysis

After reactivation, fungi were checked through the analysis of macroscopic characters (colony color, texture, shape, margin and presence of soluble pigments and exsudates) and microscopic characters (somatic and reproductive microstructures), based on the specialized literature (e.g., Pitt 1979; Visagie et al. 2014; Barbosa et al. 2018). To confirm the already known species, the characteristics were verified on malt extract agar (MEA) and czapek yeast autolyzed agar (CYA), and compared with the description in the literature. For the analyses of the new species, the protocol recommended by Visagie et al. (2014) and Houbraken et al. (2020) was used. The isolates were inoculated at three equidistant points on 90 mm Petri dishes containing the following culture media: Czapek yeast agar (CYA), CYA supplemented with 5% NaCl (CYAS), malt extract agar (MEA), czapek agar (Cz), dichloran 18% glycerol agar (DG18), yeast extract sucrose agar (YES), oatmeal agar (OA) and creatine sucrose agar (CREA), and incubated in the dark for 7 days at 25 ºC, with additional CYA and MEA plates incubated at 15 and 37 ºC. The color names and codes used in descriptions followed Rayner (1970) and are indicated in parentheses in the description.

Microstructure analyses were carried out from cultures grown in MEA in the dark for 7 days at 25 ºC. The slides were mounted with 60% lactic acid + 70% ethanol and observed under a Leica DM2500 microscope. Images were captured using flexacam C3 and processed using Leica Application Suite X 3.8.1.2 software. For scanning electron microscopy (SEM), a fragment of the fungal culture was removed and fixed in a 2 mL Eppendorf tube in a glutaraldehyde solution for a period of 24 hours. Then, the samples were dehydrated in an increasing series of ethanol and subjected to drying using the critical point equipment Bal-Tec/CPD-030 (Critical Point Dryer), and were subsequently covered by gold in high vacuum conditions for metallization. The images were viewed using a Tescan Vega 3 scanning electron microscope. Each sample was examined at different magnification levels in order to capture the best images of the microstructures.

RESULTS

Phylogeny of the CMAI-INPA *Penicillium* strains

To provide an overview of the results, the phylogenetic relationships between species and strains stored in the CMAI-INPA collection and belonging to various sections of the genus *Penicillium* were investigated using *BenA* sequences (Figure 1). In total, the *BenA* alignments included 187 sequences derived from CMAI-INPA strains, as well as sequences from publicly available ex-type cultures. We identified 20 strains that belonged to genera other than *Penicillium* and, therefore, were excluded from further analysis in this study.

The 127 *Penicillium* strains of CMAI-INPA were identified as belonging to the following sections: *Citrina* (*P. citrinum* Thorn, 1919; 16 strains), *Charlesia* (*P. chermesinum* Biourge, 1923; 1 strain), *Chrysogena* [*P. chrysogenum* Thorn (1910); 2 strains, and *P. rubens* Biourge (1910); 102 strains], *Aspergilloides* [*P. glabrum* (Wehmer) Westling (1911); one strain], *Sclerotiorum* (*P. meliponae* Barbosa, Souza-Motta, Oliveira & Houbraken, 2018; 1 strain), *Fasciculata* (*P. palitans* Westling, 1911; 1 strain, and *P. polonicum* Zalessky, 1927; 1 strain), and *Roquefortorum* (*P. paneum* Frisvad 1996; 1 strain). Additionally, we generated and analyzed calmodulin sequences for representative isolates of each species previously identified based on beta tubulin sequence and morphology (Figure 2). One lineage (CMINPA1309) did not cluster with any known species and is thus here described as a new species in the section *Exilicaulis*, series *Citreonigra*.

The phylogenetic relationships of this strain were studied using single-gene phylogenies (Figure 3) and a concatenated three-gene sequence dataset (Figure 4). Unfortunately, we were unable to generate RPB2 sequences despite numerous attempts and the use of different protocols. Phylogenetic reconstruction of the section *Exilicaulis* was performed using *BenA* (407 bp), *CaM* (473 bp), and *ITS* (547 bp) sequences. The most optimal substitution models used for Bayesian Inference (BI) analysis were TrNef+I for *BenA*, TrNef+G for *CaM*, and TrN for ITS, while GTRGAMMA+I was used for all Maximum Likelihood (ML) analyses. The results from the concatenated analysis show that the investigated strains form distinct lineages within the section *Exilicaulis*, which is consistent with the results from the single-gene analysis.

Morphology of the *Pencillium* strains

In comparison with literature data, the main macromorphological divergence observed in this study concerned the *P. meliponae* lineage in the collection, which differed from the original description, mainly in relation to colors (Barbosa et al. 2018). This difference in color may be due to the different culture medium used, as well as to an infraspecific natural color variation (Visagie et al. 2014). *Penicillium paneum* differed from the literature mainly in the growth rate in both CYA and MEA, which was much lower in the strain studied by Frisvad and Samson (2004).

Our strains of *P. chermesinum* on CYA at 25 ºC showed a slower growth rate and the absence of exudate, which can be explained by the difference in observation time of 7 days in our study. All variations observed in our strains of *P. citrinum*, *P. chrysogenum*, *P. glabrum* and *P. polonicum* had already been described in the literature (Visagie et al. 2014; de Camargo et al. 2022) (Figures 5 and 6, Table 2).

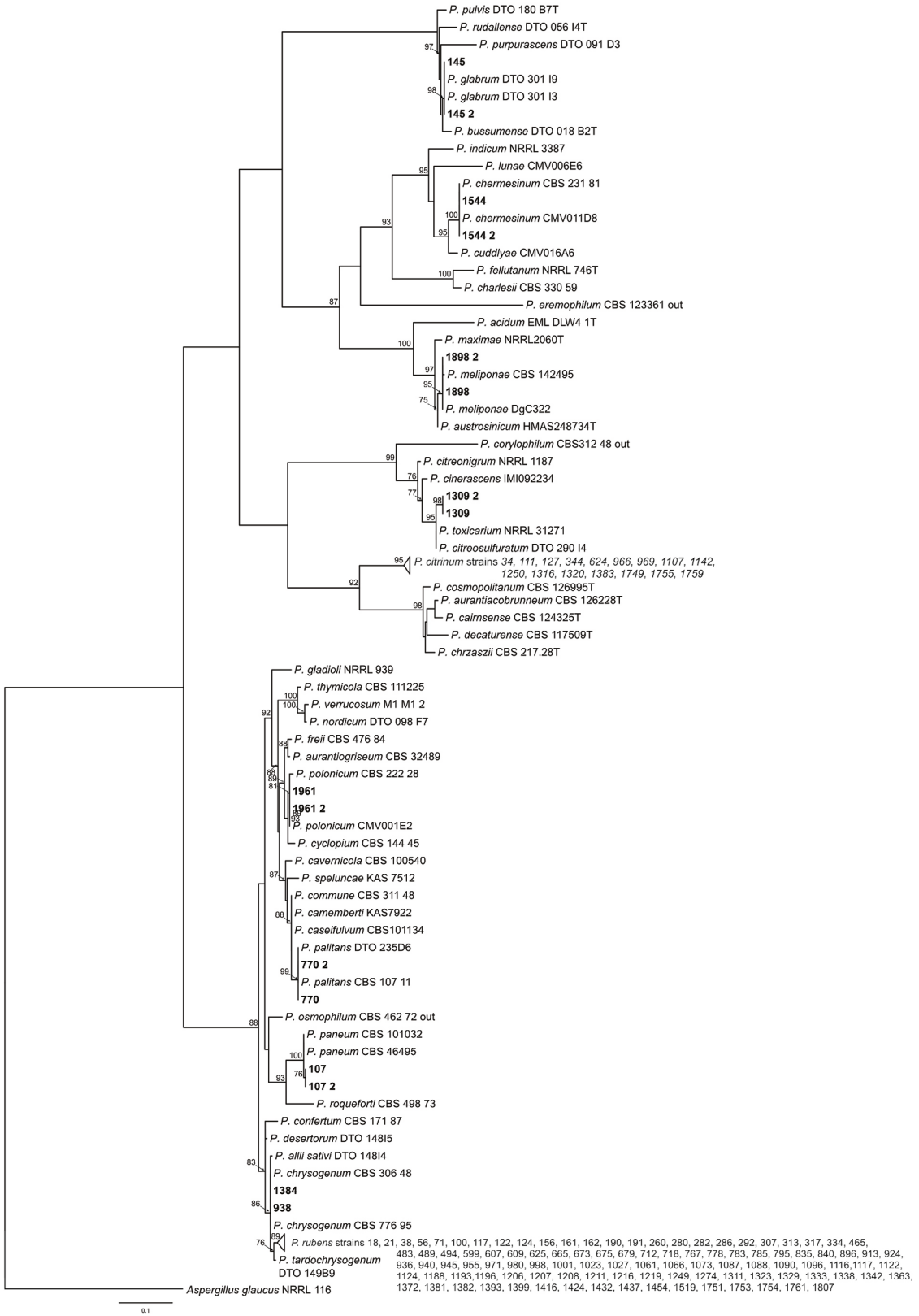

Figure 1. Phylogenetic tree with all *Penicillium* lineages deposited in the Collection of Microorganisms of Agrosilvicultural Interest at INPA (CMAI-INPA) based on maximum likelihood analysis obtained by the *BenA* loci. Bootstrap support score values above 70% are indicated at the nodes. Branches in bold indicate new sequences of the studied material. The tree is rooted to *Aspergillus glaucus* NRRL 116.

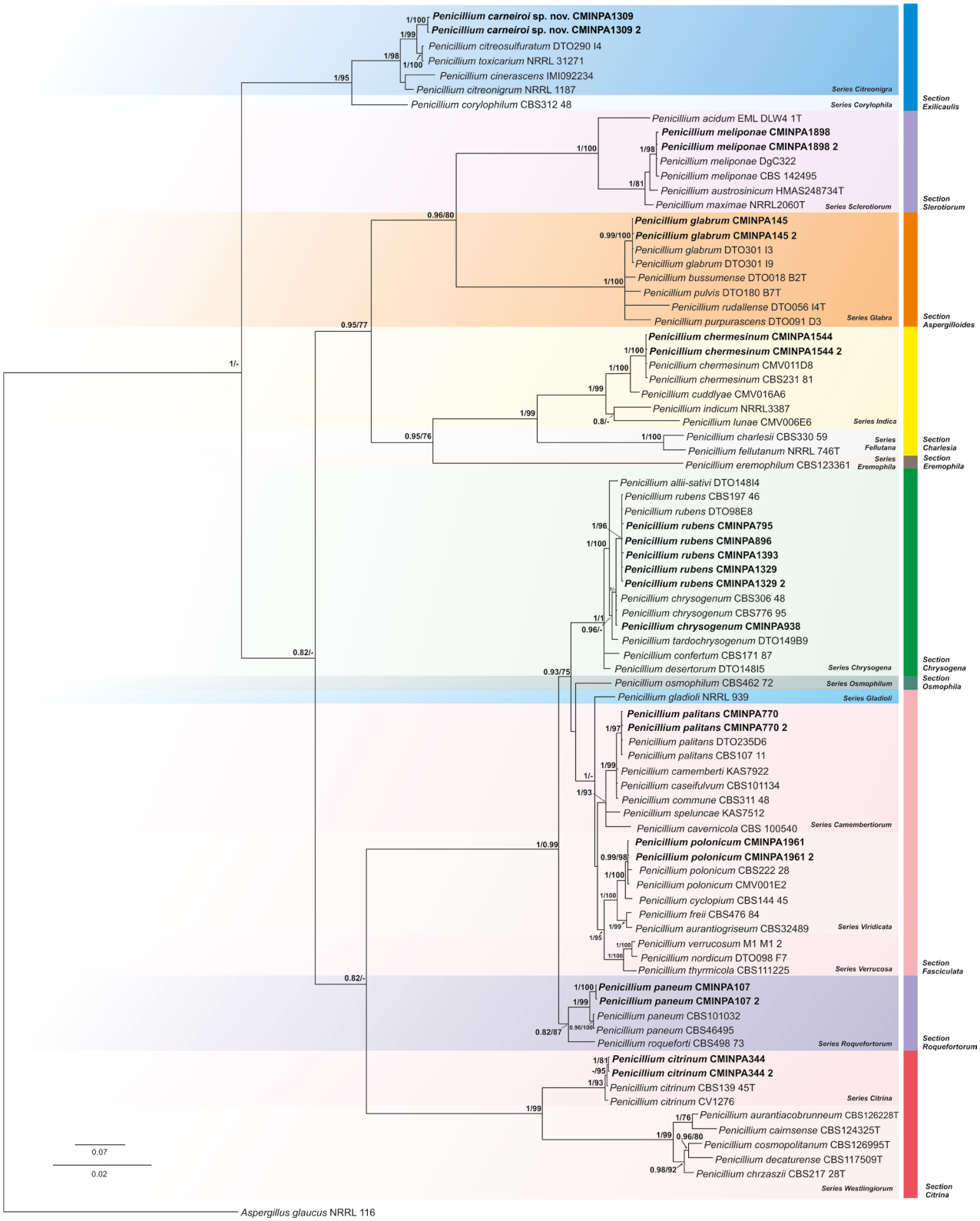

Figure 2. Phylogenetic tree of *Penicillium* species of the Collection of Microorganisms of Agrosilvicultural Interest at INPA (CMAI-INPA) based on Bayesian inference and maximum likelihood analysis obtained by the combined *BenA* and *CaM* loci. Branch lengths are proportional to phylogenetic distance. Bayesian posterior probability/bootstrap support score values above 70% are indicated on the nodes. Branches in bold indicate new sequences from the studied material. The tree is rooted to *Aspergillus glaucus* NRRL 116.

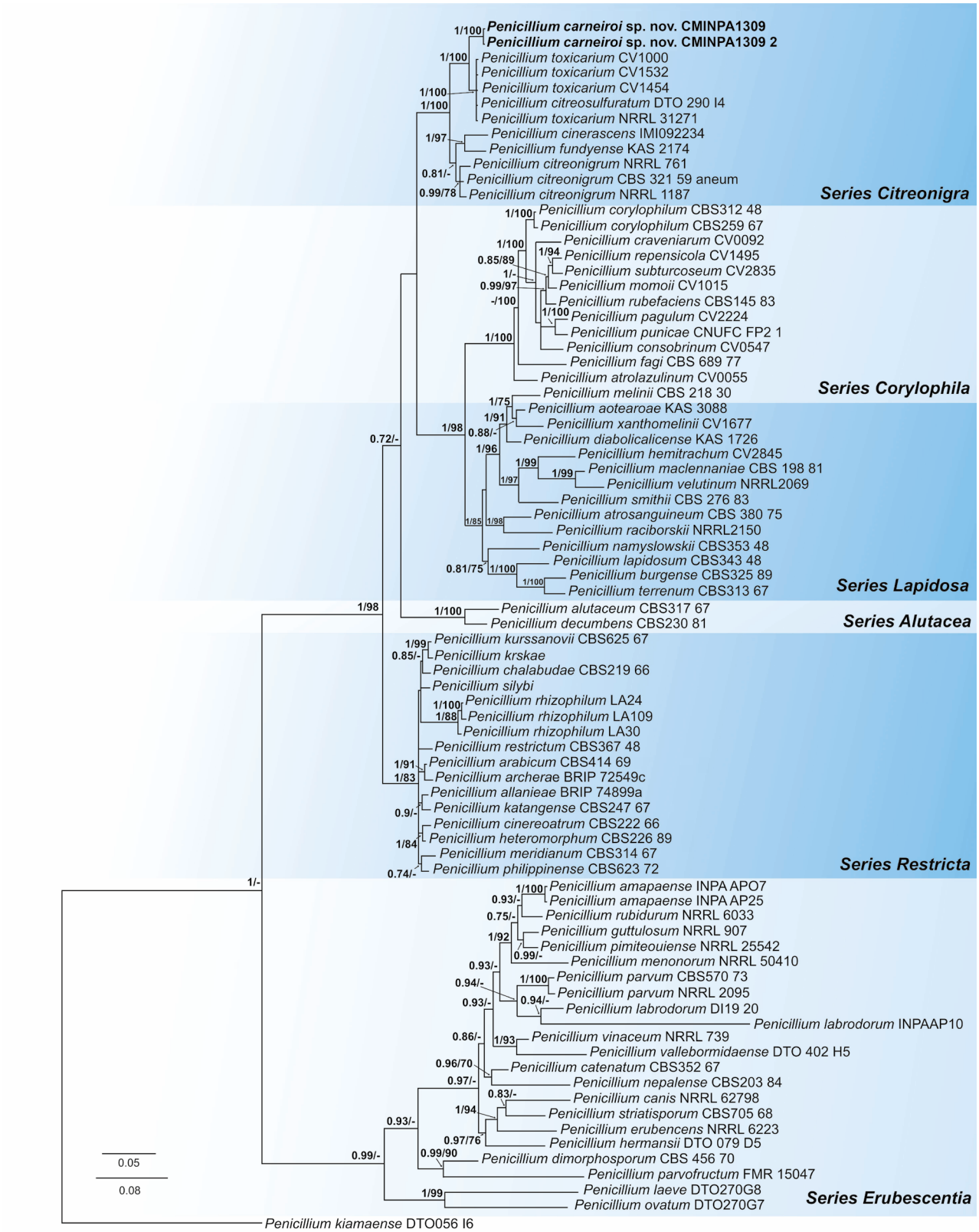

Figure 3. Phylogenetic tree of *Penicillium* section *Exilicaulis* based on Bayesian inference and maximum likelihood analyses of the combined *BenA* and *CaM* loci. Branch lengths are proportional to phylogenetic distance. Bayesian posterior probability/bootstrap support score values above 70% are indicated on the nodes. Branches in bold indicate new sequences from the studied material. The tree is rooted to *Penicillium kiamaense* DTO056 16.

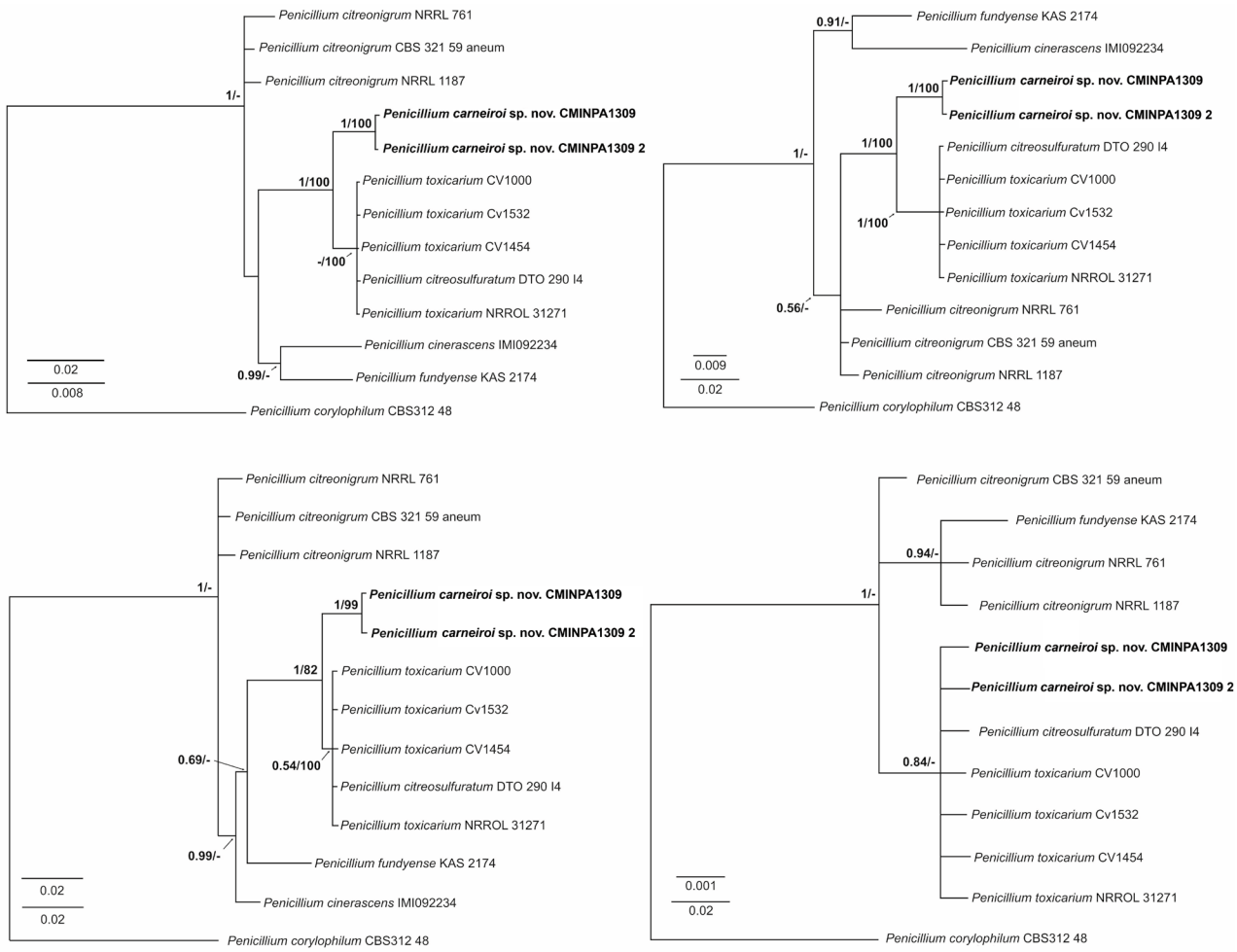

Figure 4. Phylogenetic tree of *Penicillium* series *Citreonigra* based on Bayesian inference analysis (above) and maximum likelihood analysis (below), obtained by the combined *BenA* and *CaM* loci (above, left), *CaM* loci (above, right), *BenA* (below, left) and ITS (below, right). Bayesian posterior probability/bootstrap support score values above 70% are indicated on the nodes. Branches in bold indicate new sequences from the studied material. The trees are rooted to *Penicillium corylophilum* CBS31248.

Table 2. Main morphological characteristics of *Penicillium* strains from the Collection of Microorganisms of Agroforestry Interest at INPA (CMAI-INPA) identified in the present study, on CYA at 25 °C.

Species	Colony Diameter in CYA25 (mm)	Exudate	Soluble pigment	Type of conidiophore	Conidial shape	Size of conidia (µm)
P. citrinum	28 × 31	Greenish	Present	Biverticillate and monoverticillate	Globose	2.5 × 3.0
P. chermesinum	31 × 34	Absent	Absent	Monoverticillate	Ellipsoid	2.0 × 2.5
P. chrysogenum	30 × 38	Yellowish	Absent	Biverticillate and terverticillate	Globose to subglobose	2.5 × 3.0
P. glabrum	38 × 45	Absent	Absent	Monoverticillate	Globose to subglobose	3.0 × 3.5
P. meliponae	30 × 25	Orange	Present	Monoverticillate	Globose	2.0 × 3.0
P. palitans	25 × 30	Hyaline	Absent	Terverticillate	Globose to subglobose	3.5 × 4.0
P. paneum	19 × 22	Absent	Absent	Terverticillate	Globose	3.2 × 5.0
P. polonicum	23 × 29	Hyaline	Present	Biverticillate	Globose	3.5 × 4.0
P. rubens	30 × 36	Yellowish	Absent	Biverticillate and terverticillate	Globose to subglobose	2.5 × 3.5

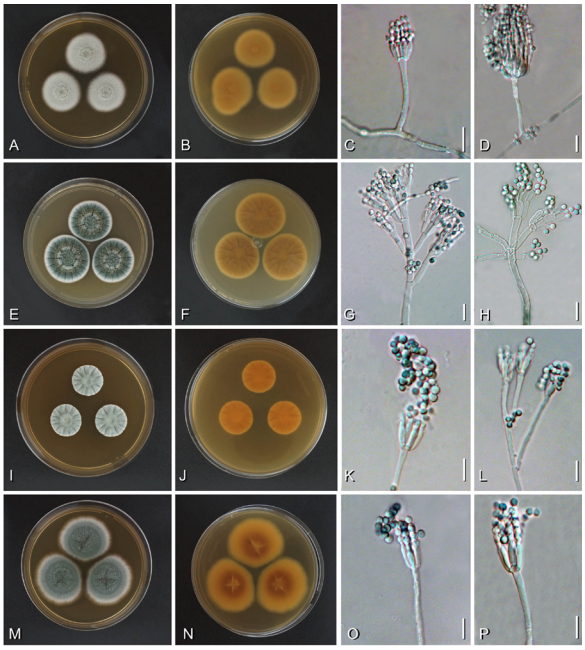

Figure 5. Diversity of *Penicillium* in the Collection of Microorganisms of Agrosilvicultural Interest at INPA (CMAI-INPA). *Penicillium chermesinum* (CMINPA1544): colonies on MEA, verse (**A**) and reverse (**B**), and conidiophores (**C** and **D**); *P. chrysogenum* (CMINPA938): colonies in CYA, verse (**E**) and reverse (**F**), and conidiophores (**G** and **H**); *P. citrinum* (CMINPA344): colonies in MEA, verse (**I**) and reverse (**J**), and conidiophores (**K** and **L**); *P. glabrum* (CMINPA145): colonies in MEA, verse (**M**) and reverse (**N**), and conidiophores (**O** and **P**). Scale bars = 10 µm.

Description of the new species

Penicillium carneiroi S.S.P. Sousa, R.N. Barbosa & R.F.R. Melo sp. nov. (Figure 7)

MycoBank: MB851566

Subgenus *Aspergilloides* section *Exilicaulis* series *Citreonigra* (Houbraken et al. 2020).

Typification: BRAZIL, Paragominas, Pará State, from wood, October 31, 2008, HURM 95730 (holotype - permanent slide), HURM 95731 (Isotype) (Culture ex-type living culture, URM 8348 = CMINPA 1309). GenBank: ITS = PP060440; *BenA* = PP068048; *CaM* = PP068049.

Diagnosis*: Peninicillium carneiroi* sp.nov. can be recognized by the absence of soluble pigment in MEA and CYA, small conidiophores, smooth-walled to finely rough-walled spores and absence of growth in CYA and MEA at 37 ºC. Multigene analysis confirms the species as unique.

Description*:* Colony diam, 7 days, in mm: CYA 25 ºC 19–22; CYA 15 ºC 13–14; CYA 37 °C no growth; MEA 25 ºC 16–18; MEA 15 ºC 13–15; MEA 37 ºC no growth; CYAS 25 ºC 9–13; DG 25 ºC 18 15–16; CZ 25 ºC 15–16; YES 25 ºC 29–30; OA 25 ºC 23–24; CREA 25 ºC 9–11. CYA 25 ºC 7 days: Colonies round, raised, no sulcate, mycelia white, slightly mealy to velvety, margins entire, degree of sporulation moderate, conidia *en masse* greenish yellow (15)

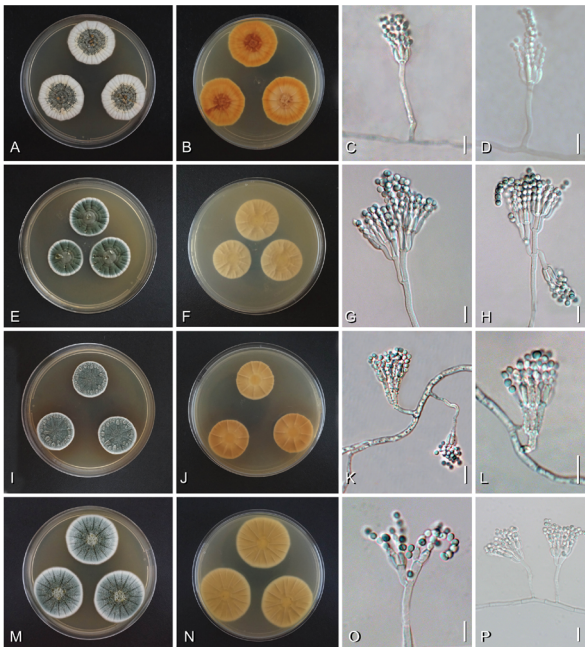

Figure 6. Diversity of *Penicillium* in the Collection of Microorganisms of Agrosilvicultural Interest at INPA (CMAI-INPA). *Penicillium meliponae* (CMINPA1898): colonies in CYA, verse (**A**) and reverse (**B**), and conidiophores (**C** and **D**); *P. palitans* (CMINPA770): colonies in CYA, verse (**E**), reverse (**F**) and conidiophores (**G** and **H**); *P. polonicum* (CMINPA1961): colonies in CYA, verse (**I**), reverse (**J**) and conidiophores (**K** and **L**); *P. rubens* (CMINPA1329): colonies in CYA, verse (**M**), reverse (**N**) and conidiophores (**O** and **P**). Scale bars = 10 µm.

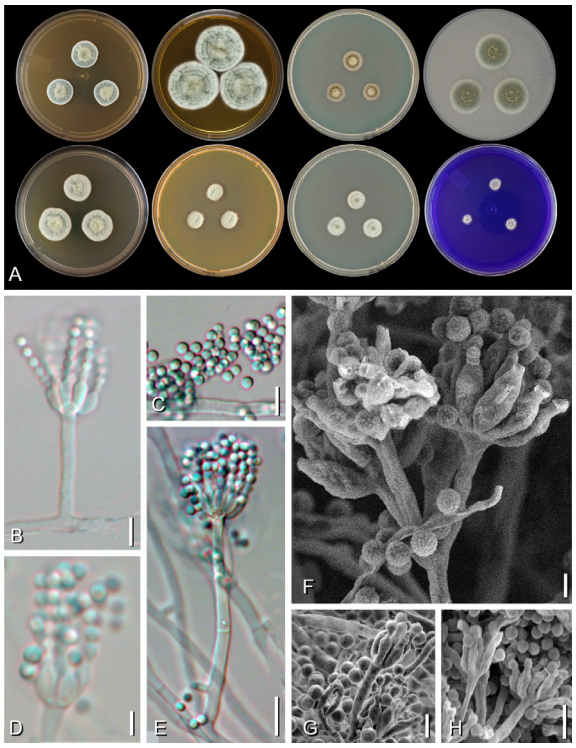

Figure 7. *Penicillium carneiroi* sp. nov.: **A** – Colonies on different culture media after 7 days at 25 °C. Top row (from left to right): MEA, YES, Cz and OA. Bottom row (from left to right): CYA, CYAS, Dg18 and CREA; **B**, **D**-**H** – conidiophores; **C** – conidia. Scale bars = 5 µm.

to dark herbage green (69), exudate composed of several small, yellowish droplets; soluble pigment absent, reverse pure yellow (14) at center becoming paler towards the margin. MEA 25 ºC 7 days: Colonies round, slightly umbonate at the center, no sulcate, mycelia white, velvety, margins entire, sporulation strong, conidia *en masse* greyish yellow green (68) to malachite green (72), exudate composed of several small, hyaline droplets, soluble pigment absent, reverse honey (64) at center, becoming paler towards the margin. CYAS 25 ºC 7 days: Colonies wrinkled, mycelia white, mealy, margins entire, sporulation strong, conidia *en masse* grayish yellow-green (68), exudate absent, soluble pigment absent, reverse honey (64). DG18 25 ºC 7 days: Colonies wrinkled, raised, no sulcate, mycelia white, velvety, margins entire, sporulation moderate, conidia *en masse* white to pale greenish gray (123), exudate absent, soluble pigment absent, reverse amber (47) at center, becoming paler towards the margin. CZ 25 ºC 7 days: Colonies round, slightly umbonate at the center, raised, no sulcate, mycelia white, velvety, margins entire, sporulation moderate, conidia *en masse* greyish yellow green (68), exudate absent, soluble pigment absent, reverse pale luteous (11) to yellow green (71). YES 25 ºC 7 days: Colonies wrinkled, raised, no sulcate, mycelia white, velvety, margins undulate, sporulation moderate, conidia *en masse* greyish yellow green (68), exudate absent, soluble pigment absent, reverse honey (64). OA 25 ºC 7 days: Colonies round, no sulcate, mycelia white, velvety, margins entire, sporulation strong, conidia *en masse* gray olivaceous (107) to greenish gray (110), exudate composed of several small, yellowish droplets, soluble pigment absent, reverse prim rose (66) to grayish yellow green (68). CREA 25 ºC 7 days: acid not produced. **Micromorphology** (7 days at 25 ºC) in MEA, conidiophores monoverticillate, hyaline, stipes smooth-walled, 17.2–48 × 1.2–2.6 µm, usually septate, phialides arising from a swelling at the top of each stipe, in whorls of 3–5(–7) per stipe, ampulliform, 4.7–7.5 × 2.1–2.8 µm; conidia hyaline, smooth-walled to finelly rough-walled, globose, 1.2–2.7 µm in diameter.

Etymology: In homage to Paulo de Berredo Carneiro, one of the creators and founders of INPA.

Notes: *Penicillium carneiroi* sp.nov. is closest to *P. citreosulfuratum* Biourge, 1923 with very similar morphological characteristics which can be differentiated by the absence of both growth in CYA at 37 ºC and soluble pigment in MEA and CYA in *P. carneiroi* sp.nov. Furthermore, *P. carneiroi* sp.nov. produces shorter conidiophores and more rough-walled spores than *P. citreosulfuratum.* Three other species, *P. citreonigrum* Dierckx, 1901, *P. cinerascens* Biourge 1923, and *P. fundyense* Visagie, Clark & Seifert 2016, are also closely related, with similar morphology, being differentiated mainly by phylogeny, a criterion also used in Visagie et al. (2016b). *Penicillium toxicarium* Miyake ex Ramírez became synonymous with *P. citreosulfuratum* in the study carried out by Visagie et al. (2016b).

DISCUSSION

The Amazon region is considered a biodiversity hot spot, with the INPA collections being the largest maintainers of this diversity (Fernandes 2020). At CMAI-INPA, ten species of *Penicillium* were identified that have enormous biotechnological potential, according to literature. Most of the *Penicillium* strains analyzed in this study were isolated from wood, and these species are known to secrete enzymes that are efficient in the degradation process of hemicelluloses and cellulose (Méndez-Líter et al. 2021), therefore, *P. rubens* dominance could be associated with the substrate in which they were found. *Penicillium rubens* is also known worldwide for the production of penicillin (Pathak et al. 2020), which highlights the diversity of secondary metabolites produced by this species, ranging from extremely harmful to health promoters (Abrunhosa et al. 2010), including antifungal, antibacterial, cytotoxic (Zhang et al. 2022), antithrombotic and pro-angiogenic activities (Li et al. 2023). This species has also been isolated from objects and surfaces in spacecrafts, but the effect of microgravity on its growth is still unknown (Hupka et al. 2023; Zea et al. 2018).

The phylogenetic tree obtained in this study resolved the positioning of the lineages in the CMAI-INPA collection into distinct and well-supported clades, both through Bayesian inference and maximum likelihood analyses. The classification obtained among the closest species is in accordance with Houbraken et al. (2020). When analysing each amplified region separately, the ITS region performed poorly as a species-level marker, as was also observed by Visagie and Yilmaz (2023), which recommended using *BenA* and *CaM* instead of ITS species-level classification in *Penicillium*. The separate phylogenies confirmed the individual reliability of *BenA* and *CaM* for the identification of already known species. However, the topology resulting from the concatenated data was the most similar to other published ones on the genus, and is also more indicated when the identification of a new species is involved.

Penicillium carneiroi sp.nov. is phylogenetically distinct in the *Citreonigra* series, but does not present significant morphological differences from the other species in the series, as was observed for *P. fundyense* (Visagie et al. 2016). The latter authors used ITS and *BenA* sequences to identify strains in the *P. fundyense* clade, adopting the phylogenetic species concept, as recommended by Visagie et al. (2016b). This concept was also used to resolve other species in the same section, as in the cases of *P. chalabudae* Visage 2016 (Visagie et al. 2016) and *P. rhizophilum* Ansari, Asgari, Zare & Zamanizadeh 2023 (Ansari et al. 2023).

The scientific collections at INPA are among the largest repositories of *ex-situ* Amazonian biodiversity (Fernandes 2020), but even so their actual potential remains underrepresented due to the large amount of unidentified deposited material, as is the case with the CMAI-INPA. Studies like this are therefore of great importance to further the availability of duly catalogued and reliably identified and authenticated material, to maximize the supports function of collections to scientific research (DiEuliis et al. 2016).

CONCLUSIONS

This study carried out the taxonomic identification at the species level of the *Penicillium* strains in one of the largest fungal culture collections of the Brazilian Amazon region, cooperating significantly with the preparation and maintenance of the collection's database, enabling their use for research purposes. Furthermore, it contributed to the knowledge of fungal biodiversity in the Amazon biome, with the description of a new species for science. Our results allowed us to conclude that *Penicillium rubens* is the dominant species of the genus in wood among the material preserved in the studied collection, and that much of the diversity of fungi is underestimated using only morphological taxonomy tools. We also concluded that microbial diversity repositories lacking a thorough taxonomic review can harbour unknown genetic resources.

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