UNIVERSIDADE FEDERAL DO PARANÁ

BÁRBARA FANAYA MAYRHOFER

BIOPROSPECÇÃO DE FUNGOS ENDOFÍTICOS DA PLANTA MEDICINAL *Vochysia divergens* DA SERRA DO AMOLAR PARA A PRODUÇÃO DE METABÓLITOS SECUNDÁRIOS COM ATIVIDADE ANTIMICROBIANA

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Venho informar a mudança de título da dissertação de mestrado da aluna Bárbara Fanaya Mayrhofer sob minha orientação, a pedido da banca examinadora:

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Título novo: BIOPROSPECÇÃO DE FUNGOS ENDOFÍTICOS DA PLANTA MEDICINAL *Vochysia divergens* DA SERRA DO AMOLAR PARA A PRODUÇÃO DE METABÓLITOS SECUNDÁRIOS COM ATIVIDADE ANTIMICROBIANA

Atenciosamente,

Profa. Dra. Chirlei Glienke

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RESUMO

O Pantanal é um bioma brasileiro rico em biodiversidade, a qual está diretamente relacionada com sua característica sazonalidade. Uma região do Pantanal ainda muito pouco explorada e bioprospectada é a Serra do Amolar. Entre os maiores desafios da atualidade está o surgimento de microrganismos com resistência antimicrobiana, assim, novos compostos para tratar esses microrganismos tornam-se necessários. Uma possível solução para este problema é a exploração de metabólitos secundários produzidos por endófitos associados às plantas medicinais, os quais produzem uma gama diversificada de compostos bioativos. O objetivo deste trabalho foi bioprospectar fungos endofíticos da planta medicinal *Vochysia divergens* localizada na Serra do Amolar para a produção de metabólitos com atividade antimicrobiana e atuar na conservação da comunidade endofítica *ex-situ*. Foram coletadas folhas e pecíolos de 18 plantas da espécie *V. divergens* da Serra do Amolar para o isolamento de endófitos. Os fungos isolados foram classificados pelas características morfológicas e identificados em nível de gênero ou espécie por identificação molecular. Um representante de cada morfogrupo foi utilizado para produção de extratos e avaliada a atividade antimicrobiana contra fungos fitopatógenos e bactérias clínicas resistentes. Foram isolados 293 endófitos, formando 91 morfogrupos. *Diaporthe* e *Colletotrichum* foram os gêneros mais frequentes dentre os morfogrupos. Na avaliação contra os fitopatógenos contra *Colletotrichum abscissum*, quatro extratos dos endófitos *Diaporthe amolarii* sp. nov., *Nemania primolutea, Xylaria arbuscula* e *Anthostomelloides forlicesenica* apresentaram inibição do crescimento micelial (ICM) superior ($p<0,001$) ao fungicida controle positivo. O extrato produzido por *X. arbuscula* também apresentou atividade contra *Phyllosticta citricarpa* e o extrato de *A. forlicesenica* mostrou-se ativo contra *Fusarium graminearum.* Na avaliação contra bactérias clínicas 19 extratos apresentaram concentração inibitória mínima (MIC) inferior a 100 µg/ml contra *Staphylococcus aureus* resistente à meticilina e um deles também contra *Acinetobacter baumanii*. Estes foram selecionados para análise frente às bactérias clínicas *Klebsiella pneumoniae* carbapenemase (KPC), *Pseudomonas aeruginosa* e *Enterococcus* sp. resistente à vancomicina (VRE), destacando-se os extratos produzidos pelos isolados *Neopestalotiopsis egyptiaca Diaporthe vochysiae*, *Neopestalotiopsis* sp., *Cladosporium* sp. complexo *cladosporioides* e *Paecilomyces* sp. com os mais baixos valores de concentração inibitória e bactericida mínima. Neste estudo demonstramos a importante fonte de metabólitos secundários bioativos com potencial biotecnológico que os microrganismos endofíticos representam, também apresentamos a grande diversidade de endófitos da região da Serra do Amolar-Pantanal, incluindo novas espécies nunca isoladas. Além disso contribuímos para a conservação *ex-situ* dessa biodiversidade, permitindo estudos futuros de aplicação biotecnológica com estes isolados.

Palavras-chave: Fungos endofíticos, bioprospecção, fitopatógenos, bactérias clínicas.

ABSTRACT

The Pantanal is a Brazilian biome rich in biodiversity, which is directly attributed to its typical seasonality. A Pantanal region still little explored and bioprospected is the Serra do Amolar. Among the biggest challenges faced today is the emergence of microorganisms with antimicrobial resistance, thus, new compounds to treat these microorganisms become necessary. A possible solution to this problem is the exploration of secondary metabolites produced by endophytes associated with medicinal plants, which produce a diverse range of bioactive compounds. The objective of this work was to bioprospect endophytic fungi of the medicinal plant *Vochysia divergens* located in Serra do Amolar for the production of metabolites with antimicrobial activity and to act in the *ex-situ* conservation of endophytic community. Leaves and petioles were collected from 18 plants of the species *V. divergens* from Serra do Amolar for the isolation of endophytes. The isolated fungi were classified by morphological characteristics and identified at the level of genus or species by molecular identification. One representative of each phenotype was used to produce extracts and the antimicrobial activity against phytopathogenic fungi and resistant clinical bacteria was evaluated. Were isolated 293 endophytes, forming 91 phenotypes. *Diaporthe* and *Colletotrichum* were the most frequent genera among the phenotypes. In the evaluation against phytopathogens against *Colletotrichum abscissum*, four extracts of the endophytes *Diaporthe amolarii* sp. nov., *Nemania primolutea*, *Xylaria arbuscula* and *Anthostomelloides forlicesenica* presented mycelial growth inhibition (ICM) superior ($p < 0.001$) to the positive control fungicide. The extract produced by *X. arbuscula* also showed activity against *Phyllosticta citricarpa* and the extract of *A. forlicesenica* was active against *Fusarium graminearum*. In the evaluation against clinical bacteria, 19 extracts showed a minimum inhibitory concentration (MIC) of less than 100 µg/ml against methicillin-resistant *Staphylococcus aureus* and one of them also against *Acinetobacter baumanii*. These were selected for analysis against the clinical bacteria *Klebsiella pneumoniae* producing carbapenemase (KPC), *Pseudomonas aeruginosa* and *Enterococcus* sp. vancomycin resistant (VRE), with emphasis on extracts produced by *Neopestalotiopsis egyptiaca*, *Diaporthe vochysiae*, *Neopestalotiopsis* sp., *Cladosporium* sp. *cladosporioides* and *Paecilomyces* sp. with the lowest values of minimum inhibitory and bactericidal concentration. In this study we demonstrate the important source of bioactive secondary metabolites with biotechnological potential that endophytic microorganisms represent, we also present the great diversity of endophytes from the Serra do Amolar-Pantanal region, including new species never before isolated. In addition, we contribute to the *ex-situ* conservation of this biodiversity, allowing future studies of biotechnological application with these isolates.

Keywords: Endophytic fungi, bioprospecting, phytopathogens, clinical bacteria.

SUMÁRIO

1 INTRODUÇÃO

O Brasil é conhecido por abrigar uma enorme biodiversidade, a qual está distribuída em seis biomas: Amazônia, Caatinga, Cerrado, Mata Atlântica, Pampa e Pantanal. O Pantanal é localizado no centro da América do Sul, compreendendo no Brasil os estados do Matogrosso e Matogrosso do Sul, além da Bolívia e Paraguai. A região encontra-se na Bacia Hidrográfica do rio Alto Paraguai e tem como principal característica a variação sazonal com períodos de cheia e seca (JUNK et al., 1989). Segundo o Ministério do Meio Ambiente (BRASIL, 2017), o Pantanal é uma das maiores extensões úmidas contínuas do planeta e possui uma enorme riqueza de espécies, cuja diversidade está fortemente relacionada à sazonalidade, o que representa uma importante fonte de recursos naturais com potencial para exploração. No Pantanal, é estimado que apenas 5% das plantas possuem características fisiológicas que as tornam capazes de sobreviver por longos períodos em regiões alagadas (ARIEIRA; DA CUNHA, 2006). A planta *Vochysia divergens*, popularmente conhecida como Cambará, está entre essas espécies, tornando-se, portanto, dominante na região. O cambará é uma planta que há muitos anos vem sendo utilizada pela medicina popular na produção de chás e xaropes para tratar resfriados, tosse, pneumonia e doenças gastrointestinais (ARIEIRA; DA CUNHA, 2006; GOS et al., 2017; HOKAMA, 2017). Tradicionalmente, plantas com propriedades medicinais servem de fonte de novos compostos bioativos para o tratamento de diversas doenças, porém o isolamento e purificação destes compostos pode ser dificultoso e gerar impactos sobre estratégias que visam a conservação do meio ambiente (CHAPLA et al., 2014; SAVI et al., 2019b). Dessa forma, a busca por outras fontes naturais, como os microrganismos endofíticos em associação a plantas medicinais, vem ganhando importância e consequentemente sendo mais explorada.

Endófitos são definidos como microrganismos capazes de colonizar tecidos internos de plantas hospedeiras sem causar danos, podendo ser isolados a partir de diversos tipos de tecidos vegetais desinfectados superficialmente (PETRINI et al., 1993; ARAÚJO et al., 2014). Esses microrganismos apresentam um grande potencial biotecnológico a ser estudado,

pois representam fontes de metabólitos secundários bioativos que podem ser isolados e utilizados para a produção de novas drogas antimicrobianas (GLIENKE et al., 2012; STROBEL; DAISY, 2003). De forma curiosa, embora os microrganismos endofíticos apresentem interação simbiótica com seus hospedeiros, por estarem relacionados com a colonização de hospedeiros específicos podem vir a produzir metabólitos secundários derivados desses hospedeiros. Um bom exemplo já descrito dessa produção mútua de compostos entre endófito e planta hospedeira é o composto paclitaxel, atualmente produzido pelo fungo endofítico *Taxomyces adreanae,* mas tendo sido inicialmente isolado da planta do gênero *Taxus* sp. (STIERLE; STIERLE, 2015).

Estudos recentes mostraram resultados promissores quanto à diversidade de microrganismos endofíticos isolados da planta *V. divergens,* assim como achados significativos do potencial antimicrobiano de extratos produzidos por esses isolados (GOS et al., 2017; HOKAMA, 2017; NORILER, et al., 2018, 2019; SAVI et al., 2018, 2019a). Muitos desses estudos foram realizados na região do Pantanal próxima ao rio Miranda, área de destino para prática de ecoturismo em decorrência das condições hidrogeológicas da região (PEREIRA, 2004). Porém, sabe-se que o aumento do número de visitantes em parques, reservas biológicas e estações ecológicas pode dificultar a conservação desses locais (TOCANTINS, 2006). A região da Serra do Amolar, foco deste estudo, é uma área de preservação ambiental gerida pela Rede de Proteção e Conservação da Serra do Amolar (RPCSA), onde se encontra um dos maiores patrimônios biológicos do Brasil. A diversidade biológica é importante para o equilíbrio do ecossistema, e sua conservação depende da troca genética e da movimentação da biota, sendo tais fatores mantidos justamente por meio da conservação do ambiente (MOREIRA, 2011). Assim, em localidades onde se observa uma ação antropológica significativamente baixa, espera-se uma biodiversidade mais conservada e com potencial importante para trabalhos de bioprospecção de microrganismos com atividade biológica (MOREIRA, 2011).

Dentre os problemas atuais envolvendo a saúde pública e agricultura está o desenvolvimento de resistência de microrganismos patogênicos. (DOS SANTOS et al., 2015; MORANDI et al., 2009). A seleção de espécies resistentes está associada ao uso excessivo e muitas vezes em doses subletais de

fungicidas, e ao uso exagerado e inapropriado de antibióticos, muitas vezes atrelado à imprecisão no diagnóstico de doenças infecciosas (DOS SANTOS et al., 2015; MORANDI et al., 2009). No ano de 2017, a OMS (Organização Mundial da Saúde) (WHO, 2017) publicou uma lista das bactérias que necessitam de novos antibióticos em caráter de urgência, e entre elas encontram-se em prioridade alta *Staphylococcus aureus* resistente à Meticilina e *Enterococcus faecium* resistente à Vancomicina, e em prioridade crítica *Acinetobacter baumanii, Pseudomonas aeruginosa* e espécies da família *Enterobacteriaceae* resistentes à carbapenêmicos. Dentre os microrganismos fitopatogênicos de grande importância para a agricultura estão os fungos das espécies *Colletotrichum abscissum* e *Phyllosticta citricarpa,* responsáveis por provocar doenças em citros, e *Fusarium graminearum*, agente patogênico de milho e cereais (BALDASSARI et al., 2006; BROWN et al., 2012; LIMA et al., 2011). Tais microrganismos também vêm apresentando resistência aos fungicidas usados no cultivo agrícola, gerando uma importante perda na produção e um impacto econômico para os produtores e para o país, quando se trata de exportação do produto (PACCANARO et al., 2017; SILVA et al., 2017; TONIAL et al., 2017). Dessa forma, a bioprospecção de endófitos oferece a possibilidade de descoberta de novos compostos com atividade inibitória contra tais microrganismos patogênicos e que possam ser utilizados na terapia de doenças e controle de pragas (DEEPIKA et al., 2016).

A Serra do Amolar está localizada ao norte do estado do Mato Grosso do Sul, na divisa com o estado do Mato Grosso e com a Bolívia, e sua rica biodiversidade atrai o turismo ecológico e pesqueiro que coloca em risco a conservação dessa biodiversidade. Além de conhecer e bioprospectar essa diversidade endofítica, é urgente o desenvolvimento de ações visando a sua conservação *ex-situ*, antes que a exploração turística, agropecuária e acidentes ambientais, como as recentes queimadas, tragam um dano irreparável de redução da biodiversidade local. Portanto, este trabalho tem como objetivos bioprospectar microrganismos endofíticos isolados da planta medicinal *Vochysia divergens* da região da Serra do Amolar no Pantanal sul-matogrossense para a busca de metabólitos secundários que possuam ação antimicrobiana. Essa bioprospecção também visa atuar na conservação da diversidade microbiota por meio do depósito dos

isolados na coleção de culturas da Rede Paranaense de Coleções Biológicas Taxonline na UFPR (cmrp-taxonline.com).

2 OBJETIVOS

2.1 OBJETIVO GERAL

Este projeto tem como objetivo geral:

- Bioprospectar fungos endofíticos da planta medicinal *Vochysia divergens* da Serra do Amolar para a produção de metabólitos secundários com atividade antimicrobiana e atuar na conservação *ex-situ* da comunidade endofítica.

2.2 OBJETIVOS ESPECÍFICOS

- Isolar fungos endofíticos de folhas e pecíolos da planta medicinal *Vochysia divergens* da Serra do Amolar – Pantanal no Brasil.
- Identificar os endófitos isolados por análise morfológica e filogenética.
- Avaliar o potencial dos endófitos e dos metabólitos secundários produzidos por eles contra fungos fitopatógenos e bactérias patogênicas humanas.
- Atuar na conservação dessa diversidade realizando o depósito dos isolados na coleção de culturas da Rede Paranaense de Coleções Biológicas Taxonline na UFPR.

Na sequência, os dados desta dissertação serão apresentados no formato de artigo, sendo dividido em dois capítulos, os quais serão submetidos em revista de fator de impacto A4 ou superior.

CAPÍTULO 1

Diversity of endophytic fungi of Serra do Amolar-Pantanal in Brazil and their potential to produce secondary metabolites with antifungal activity against phytopathogens

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ABSTRACT

The Pantanal is a Brazilian biome rich in biodiversity and the Serra do Amolar region is still poorly studied in terms of its endophytic community, as well as the potential source of bioactive compounds. In addition, this biome, and especially the Serra do Amolar, recently suffered from forest fires putting this biodiversity at risk. Therefore, in this study, endophytic fungi of the medicinal plant *Vochysia divergens* from Serra do Amolar, an endemic species from the Pantanal, were isolated and bioprospected. We have analyzed the secondary metabolites produced by endophytes and act in *ex-situ* conservation of this endophytic community. Leaves and petioles from 18 specimens of the medicinal plant *V. divergens* were collected and 293 endophytes were isolated and grouped by morphological characteristics in 91 phenotypes. One representative of each

phenotype was used for the production of extracts and for taxonomic identification, and belong to 26 families and 32 different genera, with *Diaporthe* and *Colletotrichum* being the most commonly identified genera. In this study, we isolated two new species and described the new species *Diaporthe amolarii*. Four extracts showed inhibition of mycelial growth of the phytopathogenic fungus *Colletotrichum abscissum* superior ($p < 0.001$) to the fungicide control. The isolates that produced such extracts were identified as belonging to the species *Diaporthe amolarii* (new species described in this study), *Xylaria arbuscula, Nemania primolutea* and *Anthostomelloides forlicesenica*. The extract produced from *X. arbuscula* also presented moderate activity against the phytopathogen *Phyllosticta citricarpa,* and the extract from *Anthostomelloides forlicesenica* showed activity against the phytopathogen *Fusarium graminearum*. Therefore, in our study we report a great diversity of endophytes from Serra do Amolar in Brazil, including new species never isolated, producing promising secondary metabolites with biological activity against phytopathogens. Such extracts should be further explored to describe the active compounds and their mode of action. Finally, a very important contribution of this study was to act in the *ex-situ* conservation of the biodiversity of Serra do Amolar, allowing future studies and biotechnological applications of these endophytes, adding value to Brazilian biodiversity, and demonstrating the importance in the conservation of such biomes.

Keywords: Endophytes, secondary metabolites, *Vochysia divergens*, Pantanal, phytopathogens

INTRODUCTION

One of the biggest challenges facing world agriculture is the control of diseases caused by pathogenic microorganisms (FONTES; VALADARES-INGLIS, 2020). Their rapid dissemination capacity and ability to affect crop productions have a negative impact on the country's agriculture, bringing expressive economic losses (FONTES; VALADARES-INGLIS, 2020). Crop protection management includes, among many practices, the application of chemical pesticides, which has increased during the past decades without bringing a significant and proportional reduction in crop losses (OERKE, 2006). In addition, the large-scale use of pesticides and fungicides to contain phytopathogens is also responsible for several problems, including the resistance of some pathogenic microorganisms (MORANDI, 2009). Among these harmful organisms, fungi of the genera *Colletotrichum*, *Phyllosticta* and *Fusarium* are of great importance. *Colletotrichum abscissum* is known to be the causative agent

of post-bloom fruit drop (PFD) disease in citrus, often observed when the rainy season coincides with the citrus bloom (LIMA et al., 2011; SILVA et al., 2017). Another important citrus pathogen is the species *Phyllosticta citricarpa*, the epidemiological agent of citrus black spot (CBS) disease, which occurs more frequently in subtropical areas such as Brazil, being considered in some regions of the country the greatest threat to citrus production (BALDASSARI et al., 2006; TONIAL et al., 2017). *Fusarium graminearum* is the main epidemiological agent of *Fusarium* head blight (FHB), a disease that affects the production and quality of cereals such as barley, wheat and maize, also being harmful to humans and animals when the crop is contaminated with mycotoxins (BROWN et al., 2012; PACCANARO et al., 2017).

Considering the development of resistance to fungicides in common use, the consequent difficulty in controlling these pathogens and the impacts caused by them, the search for new compounds with biological activity becomes increasingly necessary. A possible solution is to search for natural compounds from medicinal plants or associated endophytic microorganisms. Traditionally, plants with medicinal properties serve as a source of new bioactive compounds for use in various purposes, but the isolation and purification of these compounds can be difficult and cause impact on environmental conservation strategies. In this scenario, other sources of natural compounds, such as endophytic microorganisms, may be an alternative. Endophytes are known to be able to colonize the internal tissues of plants without causing damage to their host (PETRINI et al., 1993; ARAÚJO et al., 2014). In this symbiosis relationship, endophytes receive protection and nutrients and in return produce secondary metabolites that serve as protection and resistance to pathogens for the host (SAVI et al., 2019b).

Brazil is a country with enormous biodiversity, divided into six biomes. One of them is the Pantanal, which is located in the states of Mato Grosso and Mato Grosso do Sul (in addition to Bolivia and Paraguay) and has a remarkable characteristic related to its seasonal variability of periods of floods and droughts. According to the Brazilian Ministry of Environment, the Pantanal is one of the largest continuous wetlands in the world. This biome has an enormous richness of species and its diversity is strongly related to the seasonality of the region, representing an important source of natural resources with potential for exploration. The plant *Vochysia divergens* (Cambará) belongs to the group of about 5% of the tree species of the Pantanal capable of surviving in regions long flooded due to their physiological characteristics, becoming dominant in that area. In addition, it has medicinal properties that have been used in popular medicine to produce teas and syrups for the treatment of colds, coughs, pneumonia and gastrointestinal diseases (ARIEIRA; DA CUNHA, 2006).

Serra do Amolar is an area of environmental preservation in the Pantanal biome, located to the north of the state of Mato Grosso do Sul, on the border with the Mato Grosso and Bolivia, where one of the largest biological heritage sites in Brazil is found. Unlike other regions of the Pantanal where some bioprospecting studies of endophytes from the *Vochysia divergens* plant have already been

carried out by our research group (GOS et al., 2017; NORILER et al., 2018; SAVI et al., 2015, 2018), the Serra do Amolar region is still poorly studied in relation to its endophytic community and its potential source of natural bioactive compounds. In addition, this region has been increasingly sought after as a destination for ecological tourism, consequently increasing anthropological activity, and recently suffering from fires, which places the conservation of its biodiversity at risk. Thus, the aims of this study are bioprospecting of endophytic fungi of the medicinal plant *Vochysia divergens* from Serra do Amolar to produce secondary metabolites with biological activity against the phytopathogens *Colletotrichum abscissum*, *Phyllosticta citricarpa* and *Fusarium graminearum* and act in the *ex-situ* conservation of the endophytes, making such isolates available for future studies and biotechnological applications.

MATERIAL AND METHODS

Plant material

Leaves and petioles from 18 specimens of the medicinal plant *Vochysia divergens* were collected in February 2019 in the Serra do Amolar, a region belonging to the Brazilian biome Pantanal, located in the state of Mato Grosso do Sul – Brazil, close to the border with the state of Mato Grosso – Brazil and Bolivia. The collection was carried out with the assistance of the Serra do Amolar Institute (https://institutoserradoamolar.org.br) along the Paraguay River (18º29’54.7”S 57º27’16.5”W, other collection points presented in supplementary material table S1) (figure 1), site that is approximately 260 km from the collection site identified in the study by Noriler et al. (2018). The collected samples were stored in plastic bags and kept refrigerated until processing.

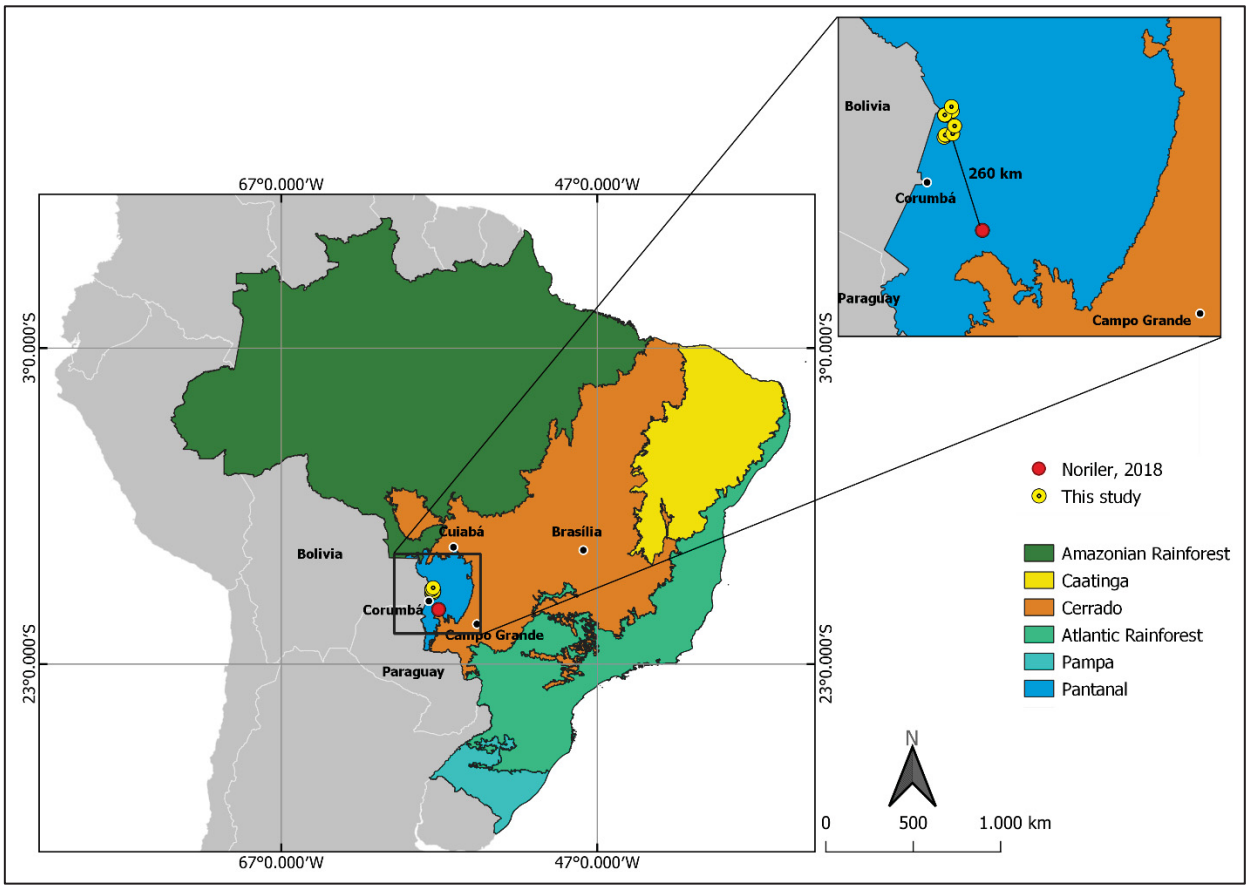


Figure 1: MAP OF BRAZIL SHOWING THE DIVISION BY BIOMES AND THE SAMPLE COLLECTION PLACE. The magnified box shows the points of collection of leaves and petioles of *Vochysia divergens* used in this study (yellow dots) in contrast to the point of collection of leaves and petioles of the same plant in the previous study carried out by Noriler et al. (2018) (red dot) with approximately 260 km of distance between them.
Source: The authors.

Fungal isolation

After collection, 5 leaves and 5 petioles from each plant without marks or injuries were used to isolate endophytic fungi. In order to eliminate the epiphytic microorganisms, a surface disinfection protocol was used, as described by Petrini (1986) and adapted by Noriler et al. (2018). Then, the leaves and petioles were fragmented into 5 pieces of 8x8 mm and placed on petri dishes containing potato dextrose agar (PDA) pH 5.8 with the addition of Tetracycline (50 µg/ml). The plates were incubated at 28ºC for up to 30 days and the fungal growth was checked daily, and the emerging mycelia were transferred to a new PDA plate pH 7.8 and stored for later use.

The isolates were grouped according to their macromorphological characteristics, such as colony color, aspect, and growth rate in PDA culture medium. One representative of each phenotype was randomly selected for molecular identification by DNA sequencing and bioprospecting assays. A pure

culture from each representative was obtained using the single spore culture method according to Gilchrist Saavedra et al. (2006). The isolates were deposited in the Paraná Microbiological Collections Network (CMRP) (https://www.cmrp-taxonline.com), at the Federal University of Paraná, Brazil.

Molecular identification

Genomic DNA was extracted from mycelia grown for 2-3 days over PDA medium at 28 ºC, according to Raeder and Broda (1985) and Glienke (1999). After the extraction, DNA concentration and quality were assessed using electrophoresis on 1% agarose gel. Subsequently, partial regions of five loci were amplified according to the needs of each genus and species to be identified. The internal transcribed spacer region (ITS) of the rDNA was amplified using the primers V9G (DE HOOG; GERRITS VAN DEN ENDE, 1998) and ITS4 (WHITE et al., 1990), a part of beta-tubulin gene (*tub2*) was amplified with primers T1 and T22 (O'DONNELL; CIGELNIK, 1997) or T1 (O'DONNELL; CIGELNIK, 1997) and Bt2b (GLASS; DONALDSON, 1995). For the portion of translation elongation factor 1-alpha (*tef1*) gene the primers EF1-728F and EF1-986R (CARBONE; KOHN, 1999) were used, the partial histone H3 (*his3*) gene was amplified with the primers CYLH3F (CROUS et al., 2004b) and H3-1b (GLASS; DONALDSON, 1995) and for the portion of the actin (*act*) gene primers ACT-512F e ACT-786R (CARBONE; KOHN, 1999) were used.

The PCR reactions were performed for a final volume of 12.5 µL (1X reaction buffer, 0,2 µM of forward primer, 0,2 µM of reverse primer, 1,5 mM of M_gCl_2, 0,2 mM of dNTPs, 0,05 U/Ul of Taq Polymerase). For all isolates, the PCR conditions for ITS were the same: an initial step of 5 min at 94ºC, followed by 35 cycles of 30 sec at 94ºC, 30 sec at 48ºC and 1 min at 72ºC, followed by a final step of 7 min at 72ºC. For the isolates that belong to the Xylariaceae family, the PCR conditions of *act* and *tub2* genes were performed according to Hsieh et al. (2005). For the *Diaporthe* genus, the PCR conditions varied according to the amplified gene, for the *tef1* the conditions used were the same as described by Gomes et al. (2013). For the *tub2* gene, the conditions were: initial step of 5 min at 94ºC, followed by 40 cycles of 30 sec at 95ºC, 50 sec at 58ºC and 1 min at 72ºC, with a final extension of 5 min at 72ºC, and for the *his3* gene the conditions were 5 min at 95ºC, 35 cycles of 30 sec at 95ºC, 30 sec at 55ºC and 2 min at 72ºC, with a final extension of 5 min at 72ºC. The PCR products were purified using the enzymes *Exo1* and *FastAP* (GE Healthcare, USA) and the BigDye® Terminator Kit v3.1 was used for the sequencing reaction. The product of this reaction was purified by the Sephadex G50 polymer, and the sequencing was read in an automatic sequencer ABI3500® (Applied Biosystems, Foster City, CA, USA).

The chromatograms obtained were inspected using MEGA 6.06 (TAMURA et al., 2011) and BioEdit (HALL, 1999). The sequences were compared with those

available in the NCBI/GenBank database (National Center for Biotechnology Information - http://www.ncbi.nlm.nih.gov/BLAST/) using the BLAST Tool and compared with type strains obtained from the MycoBank (http://www.mycobank.org/) and Westerdijk Fungal Biodiversity Institute (http://www.westerdijkinstitute.nl/) databases (tables 1 and 2). Phylogenetic analyses were performed with the sequences that correspond to the type or authentic strains and those generated by this study. The alignments of the DNA sequences were made using the Maftt software (KATOH; TOH, 2008; https://mafft.cbrc.jp/alignment/server/) and verified manually in the MEGA 6.06 software. Phylogeny was performed by Bayesian Inference analysis using MrBayes v3.2.6 x86 (RONQUIST et al., 2012) via CIPRES Science Gateway (MILLER et al., 2011). This analysis was performed using two parallel runs with one cold and three heated chains each, using the number of generations needed to reach split frequencies of ≤0.01 and a sampling frequency set to every 100 generations. The posterior probability values were calculated after discarding the first 25% of the generated trees as burn-in. Resulting trees were plotted in FigTree v.1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/). The substitution modes were selected for each gene using JModelTest (DARRIBA *et al.*, 2012). All sequences obtained were deposited at GenBank, and the access codes are listed in Tables 1 and 2.

Table 1: List of taxa, references and GenBank accession numbers of strains of *Diaporthe* included in the study

		GenBank accession no.[2]				
Species	**Collection number[1]**	***ITS***	***tub2***	***tef1***	***his3***	**Reference**
Diaporthe amolarii	**CMRP4997**					**This study**
Diaporthe sp.	**CMRP5029**					**This study**
D. anacardii	CBS 720.97*	KC343024	KC343992	KC343750	KC343508	Gomes et al. (2013)
D. anacardii	CBS 144610	MK442578	-	MK442692	-	Crous et al. (2019)
D. canthii	CBS 132533*	JX069864	KC843230	KC843120	-	Du et al. (2016)
D. cinerascens	CBS 719.96	KC343050	KC344018	KC343776	KC343534	Gomes et al. (2013)
D. cissampeli	CBS 141331/ CPC 27302*	KX228273	KX228384	-	KX228366	Crous et al. (2013)
D. cytosporella	CBS 137020*	KC843307	KC843221	KC843116	MF418283	Udayanga et al. (2014)
D. dorycnii	MFLUCC 17-1015*	KY964215	KY964099	KY964171	-	Dissanayake et al. (2017)
D. elaeagni	CBS 504.72	KC343064	KC344032	KC343790	KC343548	Gomes et al. (2013)
D. elaeagni-glabrae	LC4806	KX986780	KX999213	KX999172	KX999252	Gao et al. (2017)
D. elaeagni-glabrae	CGMCC 3.18287/ LC4802*	KX986779	KX999212	KX999171	KX999251	Gao et al. (2017)
D. hickoriae	CBS 145.26*	KC343118	KC344086	KC343844	KC343602	Gomes et al. (2013)
D. inconspicua	LGMF922	KC343124	KC344092	KC343850	KC343608	Gomes et al. (2013)
D. inconspicua	CBS 133813*	KC343123	KC344091	KC343849	KC343607	Gomes et al. (2013)
D. macintoshii	BRIP 55064a*	KJ197289	KJ197269	KJ197251	-	Thompson et al. (2015)
D. maytenicola	CPC 21896/ CBS 136441*	KF777157	KF777250	-	-	Crous et al. (2013)
D. oncostoma	CBS 100454	KC343160	KC344128	KC343886	KC343644	Gomes et al. (2013)
D. oncostoma	CBS 589.78	KC343162	KC344130	KC343888	KC343646	Gomes et al. (2013)
D. phillipsi	CAA 817	MK792305	MN000351	MK828076	MK871445	Hilário et al. (2020)

D. phillipsi	CAA 818	MK792307	MN000352	MK828078	MK871447	Hilário et al. (2020)
D. psoraleae	CPC 21634/ CBS 136412*	KF777158	KF777251	KF777245	-	Crous et al. (2013)
D. pterocarpi	MFLUCC 10-0571	JQ619899	JX275460	JX275416	-	Udayanga et al. (2012)
D. saccarata	CBS 116311*	KC343190	KC344158	KC343916	KC343674	Gomes et al. (2013)
D. stictica	CBS 370.54	KC343212	KC344180	KC343938	KC343696	Gomes et al. (2013)
D. velutina	LC4421/ CGMCC 3.18286*	KX986790	KX999223	KX999182	KX999261	Gao et al. (2017)
D. velutina	PSCG 134	MK626918	MK691243	MK654853	MK726205	Guo et al. (2020)

[1]Collection – Type strains included in analysis are indicated with *. Strains marked in bold were those generated by this study.
Culture collections abbreviations: BRIP = Australian plant pathogen culture collection, Queensland, Australia; CAA = Personal Culture Collection of Artur Alves, Universidade de Aveiro, Portugal; CBS = Culture Collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CGMCC = China General Microbiological Culture Collection; CMRP = Microbiological Collections of Paraná Network, Federal University of Paraná, Curitiba, Brazil; CPC = Culture Collection of Pedro Crous, housed at CBS; LC = Working collection of Lei Cai, housed at Institute of Microbiology, CAS, China; LGMF = Culture Collection of Laboratory of Genetics of Microorganisms, Federal University of Paraná, Curitiba, Brazil; MFLUCC = Mae Fah Luang University Culture Collection;
[2]GenBank - *ITS:* internal transcribed spacers and intervening 5.8S nrDNA; *tef1*: translation elongation factor 1-α; *tub2:* partial beta-tubulin gene; *his3:* partial histone H3 gene.
- : Sequence not available

Table 2: List of taxa, reference and GenBank accession numbers of strains of the family Xylariaceae included in the study.

		GenBank accession no.[2]			
Species	**Collection number[1]**	***ITS***	***tub2***	***act***	**Reference**
Anthostomelloides brabeji	CBS 110128*	EU552098	-	-	Jaklitsch and Voglmayr (2012)
A. forlicesenica	**CMRP5045**				**This study**
	CMRP4993				**This study**
	CMRP4983				**This study**
A. krabiensis	MFLUCC 15-0678a	KX305927	-	-	Tibpromma et al. (2017)
A. leucospermi	CBS 110126*	EU552100	-	-	Marincowitz et al. (2008)
A. proteae	CBS 110127*	EU552101	-	-	Marincowitz et al. (2008)
Anthostomelloides sp.	**CMRP4992**				**This study**
	CMRP4988				**This study**
	CMRP5050				**This study**
Biscogniauxia arima	YMJ 122	EF026150	AY951672	AY951784	Hsieh et al. (2005)
Nemania abortiva	BISH 467	GU292816	GQ470219	GQ374123	Hsieh et al. (2010)
N. abortiva	ATCC MYA-4108	FJ172270	-	-	Houseknecht et al, 2016
N. aenea var. aureolatum	ATCC 60819	AF201704	-	-	Pinto-Sherer and Chapela et al, 2000
N. beaumontii	HAST 405	GU292819	GQ470222	GQ389694	Hsieh et al. (2010)
N. bipapillata	HAST 90080610	GU292818	GQ470221	GQ389693	Hsieh et al. (2010)
N. chestersii	JF04024	-	DQ840089	-	Tang et al. (2009)
N. diffusa	HAST 91020401	GU292817	GQ470220	GQ389692	Hsieh et al. (2010)
N. illita	YMJ 236	EF026122	EF025608	EF025593	Ju and Hsieh (2007)
N. macrocarpa	WSP 265	GU292823	GQ470226	GQ389698	Hsieh et al. (2010)
N. macrocarpa	CBS 109567	MH862830	-	-	Vu et al. (2019)
N. maritima	HAST 89120401	GU292822	GQ470225	GQ389697	Hsieh et al. (2010)
N. phetchaburiensis	MFLU 16-1185	MN047124	-	-	Dayarathne et al. (2020)

N. plumbea	6540	JQ846087	-	-	Tan and Guo, 2012
N. plumbea	JF-TH-04-01	DQ641634	-	-	Tang et al. (2007)
N. pouzarii	ATCC 2612	KC477228	-	-	Stadler et al. (2013)
N. primolutea	**CMRP4987**				**This study**
N. primolutea	YMJ 91102001	EF026121	EF025607	EF025592	Ju and Hsieh (2007)
N. serpens	HAST 235	GU292820	GQ470223	GQ389695	Hsieh et al. (2010)
N. serpens	N20A	AJ390431	-	-	Sánchez-Ballesteros et al. (2000)
N. serpens var. macrospora	N21A	AJ390433	-	-	Sánchez-Ballesteros et al. (2000)
N. serpens var. serpens	CBS 659.70	MH859890	-	-	Vu et al. (2019)
N. viridis	MFLU 17-2600*	MN047123	-	-	Dayarathne et al. (2020)
Xylaria acuminatilongissima	HAST 95060506/ HAST 623*	EU178738	GQ502711	GQ853046	Ju and Hsieh (2007)
X. adscendens	J.D.R. 865	GU322432	GQ487709	GQ438746	Hsieh et al. (2010)
X. adscendens	HAST 570	-	GQ487708	GQ438745	Hsieh et al. (2010)
X. acuta	5220	JQ862676	JX868537	-	Chen et al. (2013)
X. aethiopica	YJM 1136	MH790445	MH785221	MH785223	Fournier et al. (2018)
X. allantoidea	HAST 94042903	GU324743	GQ502692	GQ452377	Hsieh et al. (2010)
X. amphithele	HAST 529	GU300083	GQ478218	GQ408905	Hsieh et al. (2010)
X. apoda	HAST 90080804	GU322437	GQ495930	GQ438751	Hsieh et al. (2010)
X. arbuscula	**CMRP5054**				**This study**
X. arbuscula	CBS 126415	KY610394	KX271257	-	Wendt et al. (2018)
X. arbuscula	HAST 89041211	GU300090	GQ478226	GQ421286	Hsieh et al. (2010)
X. areolata	HAST 543	GU300080	GQ478215	GQ408902	Hsieh et al. (2010)
X. atrodivaricata	HAST 95052001	EU178739	GQ502713	GQ853048	Ju and Hsieh (2007)
X. atrosphaerica	HAST 91111214	GU322459	GQ495953	GQ452363	Hsieh et al. (2010)
X. badia	HAST 95070101	GU322446	GQ495939	GQ449235	Hsieh et al. (2010)
X. bambusicola	WSP 205*	EF026123	AY951762	AY951873	Hsieh et al. (2010)
X. bambusicola	JDR 162	GU300088	GQ478223	GQ408910	Hsieh et al. (2005)
X, berteri	JDR 256	GU324750	GQ502698	GQ455442	Hsieh et al. (2010)
X, berteri	YMJ 90112623	GU324749	AY951763	AY951874	Hsieh et al. (2005)
X. botuliformis	YMJ 89091627	MN089652	MN095400	MN095398	Ju and Hsieh (2020)
X. brunneovinosa	HAST 720/ 95060505*	EU179862	GQ502706	GQ853041	Ju and Hsieh (2007)
X. carpophila	CBS 453.72	MH860527	-	-	Vu et al. (2019)
X. castorea	PDD 600	GU324751	GQ502703	GQ455447	Hsieh et al. (2005)
X. cf. castorea	HAST 91092303	GU324752	GQ502704	GQ455448	Hsieh et al. (2010)
X. cirrata	HAST 664	EU179863	GQ502707	GQ853042	Hsieh et al. (2010)
X. coccophora	HAST 786	GU300093	GQ487701	GQ421289	Hsieh et al. (2010)
X. coprinicola	1145	HM585020	HM585018	HM585017	Ju et al. (2011)
X. cornu-damae	CBS 724.69	MH859400	-	-	Vu et al. (2019)
X. cranioides	HAST 226	GU300075	GQ478210	GQ398233	Hsieh et al. (2010)
X. crozonensis	HAST 398	GU324748	GQ502697	GQ455441	Hsieh et al. (2010)
X. cubensis	JDR 860	GU324748	GQ502700	GQ455444	Hsieh et al. (2010)
X. cubensis	GENT 159	-	GQ502702	GQ455446	Hsieh et al. (2010)
X. culleniae	JDR 189	GU322442	GQ495935	GQ455443	Hsieh et al. (2010)
X. curta	HAST 494	GU322444	GQ495937	GQ449233	Hsieh et al. (2010)
X. curta	HAST 92092022	GU322443	GQ495936	GQ438757	Hsieh et al. (2010)
X. digitata	HAST 919	GU322456	GQ495949	GQ449245	Hsieh et al. (2010)

X. discolour	YMJ 1280/HAST 131023*	JQ087405	JQ087414	JQ087408	Ju et al. (2012)
X. ellisii	NB-623	MN218820	-	-	Ibrahim et al. (2020)
X. enterogena	HAST 785	GU324736	GQ502685	GQ452370	Hsieh et al. (2010)
X. enteroleuca	CBS 128357	MH864898	-	-	Vu et al. (2019)
X. escharoidea	HAST 658	EU179864	GQ502709	GQ853044	Hsieh et al. (2010)
X. eucalypti	CPC 36723*	MN562127	MN556841	-	Crous et al. (2019)
X. feejeensis	HAST_565	GU322452	GQ495945	GQ449241	Hsieh et al. (2010)
X. feejeensis	HAST 92092013	GU322454	GQ495947	GQ449243	Hsieh et al. (2010)
X. fimbriata	HAST 491	GU324753	GQ502705	GQ853040	Hsieh et al. (2010)
X. fissilis	HAST 367	GU300073	GQ470231	GQ398231	Hsieh et al. (2010)
X. frustulosa	HAST 771	GU322450	GQ495943	GQ449237	Hsieh et al. (2010)
X. frustulosa	HAST 92092010	GU322451	GQ495944	GQ449240	Hsieh et al. (2010)
X. cf. glebulosa	HAST 431	GU322462	GQ495956	GQ452366	Hsieh et al. (2010)
X. globosa	HAST 775	GU324735	GQ502684	GQ452369	Hsieh et al. (2010)
X. grammica	HAST 479	GU300097	GQ487704	GQ427197	Hsieh et al. (2010)
X. griseosepiacea	HAST 641	EU179865	GQ502714	GQ853049	Hsieh et al. (2010)
X. haemorrhoidalis	HAST 89041207	GU322464	GQ502683	GQ452368	Hsieh et al. (2010)
X. cf. heliscus	HAST 88113010	GU324742	GQ502691	GQ452376	Hsieh et al. (2010)
X. hongkongensis	GDGM40058*	KF926669	-	-	Tang et al. (2014)
X. hypoxylon	CBS 122620	KY610407	KX271279	-	Wendt et al. (2018)
X. hypoxylon	HAST 152	GU300096	GQ260187	GQ427196	Hsieh et al. (2010)
X. ianthinovelutina	HAST 553	GU322441	GQ495934	GQ438755	Hsieh et al. (2010)
X. intracolorata	HAST 90080402	GU324741	GQ502690	GQ452375	Hsieh et al. (2010)
X. intraflava	HAST 725*	EU179866	GQ502718	GQ853053	Hsieh et al. (2010)
X. juruensis	HAST 92042501	GU322439	GQ495932	GQ438753	Hsieh et al. (2010)
X. laevis	HAST 419	GU324746	GQ502695	GQ455439	Hsieh et al. (2010)
X. laevis	HAST 95072910	GU324747	GQ502696	GQ455440	Hsieh et al. (2010)
X. lechatii	YMJ 780	JQ087406	JQ087415	JQ087409	Ju et al. (2012)
X. liquidambaris	HAST 93090701	GU300094	GQ487702	GQ421290	Hsieh et al. (2010)
X. longissima	IRAN 2268C	KP218906	-	-	Hashemi et al. (2015)
X. luteostromata var. macrospora	HAST 508	GU324739	GQ502688	GQ452373	Hsieh et al. (2010)
X. mali	CBS 385.35	KU683769	KU684205	KU684107	U'Ren et al. (2016)
X. meliacearum	JDR 148	GU300084	GQ478219	GQ408906	Hsieh et al. (2010)
X. microceras	HAST 414	GU300086	GQ478221	GQ408908	Hsieh et al. (2010)
X. montagnei	HAST 495	GU322455	GQ495948	GQ449244	Hsieh et al. (2010)
X. multiplex	HAST 580	GU300098	GQ487705	GQ427198	Hsieh et al. (2010)
X. multiplex	JDR 259	GU300099	GQ487706	GQ438743	Hsieh et al. (2010)
X. muscula	HAST 520	GU300087	GQ478222	GQ408909	Hsieh et al. (2010)
X. nigripes	HAST 653	GU324755	GQ502710	GQ853045	Hsieh et al. (2010)
X. ochraceostroma	HAST 401	EU179869	GQ502717	GQ853052	Hsieh et al. (2010)
X. oligotoma	HAST 784	GU300092	GQ487700	GQ421288	Hsieh et al. (2010)
X. ophiopoda	HAST 93082805	GU322461	GQ495955	GQ452365	Hsieh et al. (2010)
X. oxyacanthae	JDR 859	GU322434	GQ495927	GQ438748	Hsieh et al. (2010)
X. palmicola	PDD 604	GU322436	GQ495929	GQ438750	Hsieh et al. (2010)
X. papulis	HAST 89021903	GU300100	GQ487707	GQ438744	Hsieh et al. (2010)
X. phyllocharis	HAST 528	GU322445	GQ495938	GQ449234	Hsieh et al. (2010)
X. plebeja	HAST 91122401	GU324740	GQ502689	GQ452374	Hsieh et al. (2010)

X. polymorpha	MUCL 49884	KY610408	KX271280	-	Wendt et al. (2018)
X. polymorpha	JDR 1012	GU322460	GQ495954	GQ452364	Hsieh et al. (2010)
X. regalis	HAST 290	GU324745	GQ502694	GQ452379	Hsieh et al. (2010)
X. regalis	HAST 92072001	GU324744	GQ502693	GQ452378	Hsieh et al. (2010)
X. ripicola	KA11-0060-1	KM817199	-	-	Kim et al. (2016)
X. schweinitzi	HAST 92092023	GU322463	GQ495957	GQ452367	Hsieh et al. (2010)
X. scruposa	HAST 497	GU322458	GQ495952	GQ452362	Hsieh et al. (2010)
X. sicula f. major	HAST 90071613	GU300081	GQ478216	GQ408903	Hsieh et al. (2010)
X. striata	HAST 304	GU300089	GQ478224	GQ421284	Hsieh et al. (2010)
X. teffairii	HAST 421	GU324737	GQ502686	GQ452371	Hsieh et al. (2010)
X. teffairii	HAST 90081901	GU324738	GQ502687	GQ452372	Hsieh et al. (2010)
X. tentaculata	KA13-1325	KM077164	-	-	Kim et al. (2016)
X. tuberoides	HAST 475	GU300074	GQ478209	GQ398232	Hsieh et al. (2010)
X. vaporaria	CBS 386.35	MH855714	-	-	Vu et al. (2019)
X. venosula	YMJ 94080508	EF026149	EF025617	EF025602	Hsieh et al. (2010)
X. venustula	HAST 88113002	GU300091	GQ487699	GQ421287	Hsieh et al. (2010)
X. xylarioides	CBS 127883	MH864741	-	-	Vu et al. (2019)

[1]Collection – Type strains included in analysis are indicated with *. Strains marked in bold were those generated by this study.
Culture collections abbreviations: BRIP = Australian plant pathogen culture collection, Queensland, Australia; CAA = Personal Culture Collection of Artur Alves, Universidade de Aveiro, Portugal; CBS = Culture Collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CGMCC = China General Microbiological Culture Collection; CMRP = Microbiological Collections of Paraná Network, Federal University of Paraná, Curitiba, Brazil; CPC = Culture Collection of Pedro Crous, housed at CBS; LC = Working collection of Lei Cai, housed at Institute of Microbiology, CAS, China; LGMF = Culture Collection of Laboratory of Genetics of Microorganisms, Federal University of Paraná, Curitiba, Brazil; MFLUCC = Mae Fah Luang University Culture Collection;
[2]GenBank - *ITS:* internal transcribed spacers and intervening 5.8S nrDNA; *tub2:* partial beta-tubulin gene; *act*: partial actin gene.
- : Sequence not available

Morphological characterization/taxonomy

The descriptions presented here are based on the macromorphological characteristics in different culture media and micromorphological characteristics based on sporulated colonies in cultures, which formed asexual structures. For the isolate of the genus *Diaporthe*, the following culture conditions were used: PDA pH 5.5, oatmeal agar (OA) and 2% malt extract (MEA) with and without the addition of sterile pine needles and autoclaved leaves of *Schinus terebinthifolius* and incubated at 25ºC in a period of 12h of light and 12h in the dark as described by Gomes et al. (2013). Colony diameter measurements were determined at 25ºC in the dark in PDA pH 5.5, OA and MEA media, with measurements taken 3, 4, 5 and 7 days after inoculation. After 15 days the colors of the colonies were described (verse and reverse) using the color charts of Rayner (1970).

For the isolates of the Xylariaceae family, the culture conditions were SME medium (KENERLEY; ROGERS, 1976) and OA medium with and without sterilized pine needles and autoclaved leaves of *Schinus terebinthifolius*, incubated at 28ºC in a period of 12h light and 12h dark.

Evaluation of the biological activity of secondary metabolites

Small scale extracts production from endophytic fungi

The 91 selected endophytic fungi were used in the production of extracts to evaluate the biological activity by fermentation in liquid medium. Initially, the strains were grown in PDA pH 5.8 for 7 days at 28ºC. Then, three mycelial discs (6 mm) were added in Erlenmeyer flasks (250 ml) containing 100 ml of liquid Malt Extract medium (SCHULZ et al., 2002) and incubated under constant agitation (180 rpm) at 28ºC for 10 days. After this period, the cultures were filtered-off using Whatmann filter paper to remove the mycelium, the fermented liquid was subjected to extraction with 1% of the Amberlite® XAD-16N polymer and kept for another day under constant agitation (180 rpm). Then, the resin was separated from the filtrate, washed with distilled water, and eluted in methyl alcohol (V x V) so that the secondary metabolites adsorbed by the resin were extracted by the solvent. Finally, the solvent was evaporated *in vacuo* at 45ºC and the dry crude extract was obtained.

Biological activity of extracts against phytopathogenic fungi

The antifungal activity of the extracts from the endophytes was evaluated in a screening test against the phytopathogenic fungus *Colletotrichum abscissum* (CMRP704). The experiment was carried out with 100 µL of each extract diluted in methanol (10 mg/ml) and spread on a Petri dish containing PDA pH 5.8 using a Drigalski spatula, followed by a mycelial disc (6 mm) of the phytopathogen inoculated in the center of the plate. The commercial fungicide Carbendazim (Derosal®) was used as a positive control, and pure methanol as a negative control. The plates were incubated at 24ºC for 7 days, at which time the diameter of the colonies was measured and compared with that of both control plates (SAVI et al., 2011). This experiment was carried out in triplicates and the results were analyzed using the ANOVA statistical test in the GraphPrism v. 6.01. The treatments presenting mycelial growth inhibition higher than the positive control (fungicide Carbendazim) were also evaluated against the phytopathogens *Fusarium graminearum* (CT STALK21) and *Phyllosticta citricarpa* (CMRP06), using the same methodology described above, with the following modifications: for *F. graminearum*, the temperature and incubation period used were 28ºC and 4 days, for *P. citricarpa* the incubation period used was 21 days.

In addition, crude extracts showing promising activity against one or more pathogens were selected to be produced on a large scale. Once the fractions were obtained, each of them was evaluated following the same protocol described above.

Large scale production and fractionation of extracts

The fungal extract from small scale production that showed promising results in the biological activity tests was produced on a large scale (5 L to 10 L) under the same conditions applied before. This large-scale production was carried out for the chemical fractionation of crude extracts. The crude extract (1.38 g) was fractionated in a 25 x 750 mm C18 silica gel column and eluted in a 0-100% acetonitrile (ACN) gradient in water. The solvents of the obtained fractions were removed by sample concentrators, lyophilization or rotary evaporator.

RESULTS

Serra do Amolar is a rich biome and source of several new endophytic species of fungi isolated from *Vochysia divergens*

A total of 293 cultivable endophytic fungi were isolated from leaves and petioles fragments collected from 18 plants of *Vochysia divergens*. The isolates were grouped into 91 phenotypes according to their morphological characteristics. The 91 isolates representing each phenotype were identified at the genus and/or species level using the NCBI BLAST Tool and phylogenetic analysis by Bayesian Inference using the ITS sequence and additional genes (*act*, *tef1*, *tub2* and *his3*) when necessary to complete the identification. All identified phenotypes belong to the Phylum Ascomycota within three classes: Eurotiomycetes, Dothideomycetes and Sordariomycetes. The dominant class was Sordariomycetes corresponding to 71% of the isolates, and the dominant orders in the Sordariomycetes class were Xylariales (32%), Diaporthales (25%) and Glomerellales (20%). The isolates belong to 26 families and 32 different genera, with *Diaporthe* and *Colletotrichum* being the most frequent (figure 2). Six isolates were identified at the family level because they were not clustered with any type species of the Chaetomiaceae family, and there is still some inaccuracy as to the position of the genera in the phylogeny in this family (table 3).

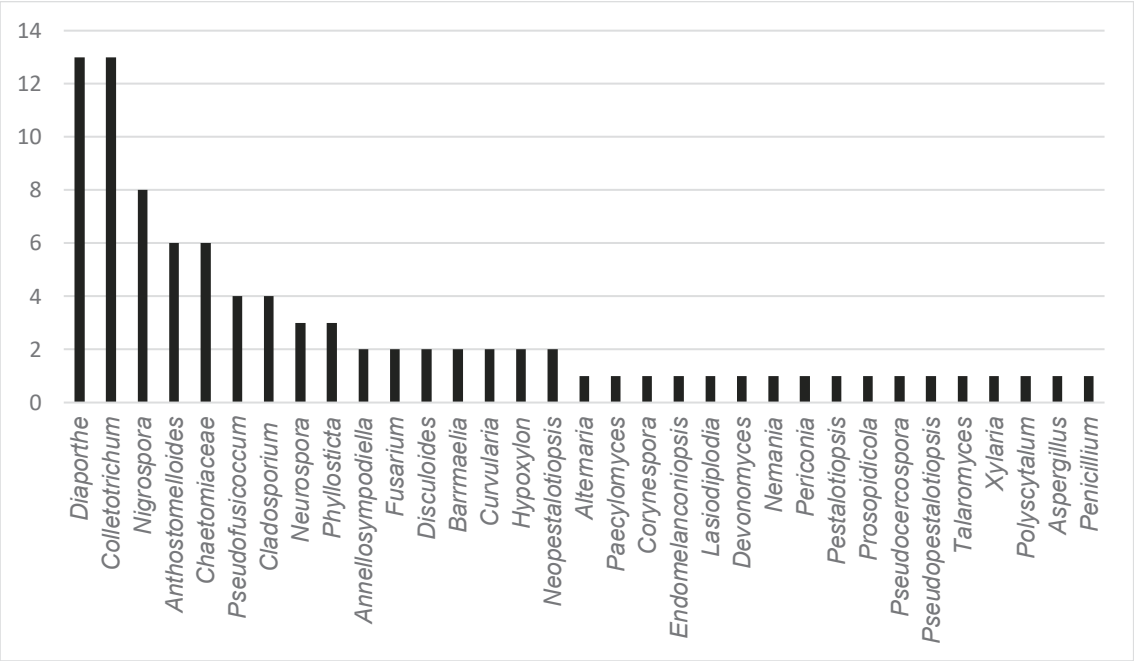


Figure 2: Number of endophytes of each genus and family isolated from leaves and petioles of plant *Vochysia divergens* in the present study.

Table 3- Taxonomic classification of each phenotype of endophytic fungi isolated in this study from *Vochysia divergens*

Taxa					
Class	**Order**	**Family**	**Genus**	**Species**	**Phenotype**
Dothideomycetes (24%)	Botryosphaeriales	Botryosphaeriaceae	*Lasiodiplodia*	*Lasiodiplodia pontae*	65
		Phyllostictaceae	*Phyllosticta*	*Phyllosticta capitalensis*	1,2,70
		Pseudofusicoccaceae	*Pseudofusicoccum*	*Pseudofusicoccum stromaticum*	8,9,16,68
		Endomelanconiopsidaceae	*Endomelanconiopsis*	*Endomelanconiopsis* sp.	62
	Cladosporiales	Cladosporiaceae	*Cladosporium*	*Cladosporium* sp. *cladosporioides* complex	25,46,71,72
	Mycosphaerellales	Mycosphaerellaceae	*Annellosympodiella*	*Annellosympodiella* sp.	28,29
			Mycosphaerella	*Devonomyces* sp.	30
			Pseudocercospora	*Pseudocercospora* sp.	31
	Pleosporales	Corynesporacaceae	*Corynespora*	*Corynespora* sp.	79
		Periconiaceae	*Periconia*	*Periconia* sp.	49
		Pleosporaceae	*Alternaria*	*Alternaria* sp.	75
			Curvularia	*Curvularia* sp.	41,60
Sordariomycetes (71%)	Amphisphaeriales	Pestalotiopsidaceae	*Neopestalotiopsis*	*Neopestalotiopsis* sp.	76
				Neopestalotiopsis egyptiaca	15
			Pestalotiopsis	*Pestalotiopsis* sp.	56
		Sporocadaceae	*Pseudopestalotiopsis*	*Pseudopestlotiopsis* sp.	86
	Diaporthales	Cryphonectriaceae	*Aurantiosacculus*	*Aurantiosacculus* sp.	26,82
		Diaporthaceae	*Diaporthe*	*Diaporthe* sp.	18,23,36,37,52,53
				Diaporthe amolarii	59
				Diaporthe cf heveae 1	57
				Diaporthe infertilis	87
				Diaporthe vochysiae	11,12,50,55
		Prosopidicolaceae	*Prosopidicola*	*Prosopidicola* sp.	45
		Erythrogloeaceae	*Disculoides*	*Disculoides* sp.	26,82
	Glomerellales	Glomerellaceae	*Colletotrichum*	*Colletotrichum gigasporum*	63
				Colletotrichum sp. *boninense* complex	54,58,81

				Colletotrichum sp. *gloeosporioides* complex	4,19,51,69,83,85
				Colletotrichum sp. *acutatum* complex	22,9
	Hypocreales	Nectriaceae	*Fusarium*	*Fusarium* sp.	10,39
	Sordariales	Sordariaceae	*Neurospora*	*Neurospora* sp.	20,21,40
		Chaetomiaceae	*Chaetomiaceae* sp.		32,43,44,88,89,91
	Xylariales	Apiosporaceae	*Nigrospora*	*Nigrospora* sp.	3,13,14,61,64,66,67
				Nigrospora brasiliensis	5
		Barrmaeliaceae	*Barrmaelia*	*Barrmaelia* sp.	27,38
		Hypoxylaceae	*Hypoxylon*	*Hypoxylon* sp.	7,78
		Xylariaceae	*Anthostomelloides*	*Anthostomelloides forlicesenica*	17,47,74
				Anthostomelloides sp.	34,42,80
			Nemania	*Nemania primolutea*	33
			Xylaria	*Xylaria arbuscula*	84
Eurotiomycetes (4%)	Eurotiales	Aspergillaceae	*Aspergillus*	*Aspergilus* sp. sect. *Nigri*, ser. *Japonici*	6
			Penicillium	*Penicillium* sp. sect. *Citrina*	73
		Trichocomaceae	*Talaromyces*	*Talaromyces* sp. sect. *Talaromyces*	24
		Thermoascaceae	*Paecilomyces*	*Paecilomyces* sp.	77

To identify the isolate of the genus *Diaporthe* at the species level, an analysis of the partial sequence of *tef1* with 1033 pb and 314 taxa corresponding to the type and representative strains was performed, being more informative for better resolution of the genus. The Bayesian Inference analysis showed the CMRP4997 and CMRP5029 strains in clade 1 (figure S1) composed of 16 species of *Diaporthe*. From the data obtained in the previous analysis with all species of the genus, a multilocus analysis was carried out, comprising 1678 pb of *tef1*, *tub2* and *his3* partial sequences with the 16 species contained in clade 1 (figure S1). Bayesian Inference analysis showed the CMRP4997 and CMRP5029 strains present in a single branch (supported by 0.999 probability) different from the other species present in the clade. However, due to the long length of the branch, probably these strains do not belong to the same species. Therefore, we assign only the CMRP4997 strain to the new species (*Diaporthe amolarii* sp. nov) and the classification of the CMRP5029 strain remains to be resolved, being here named *Diaporthe* sp. (figure 3).

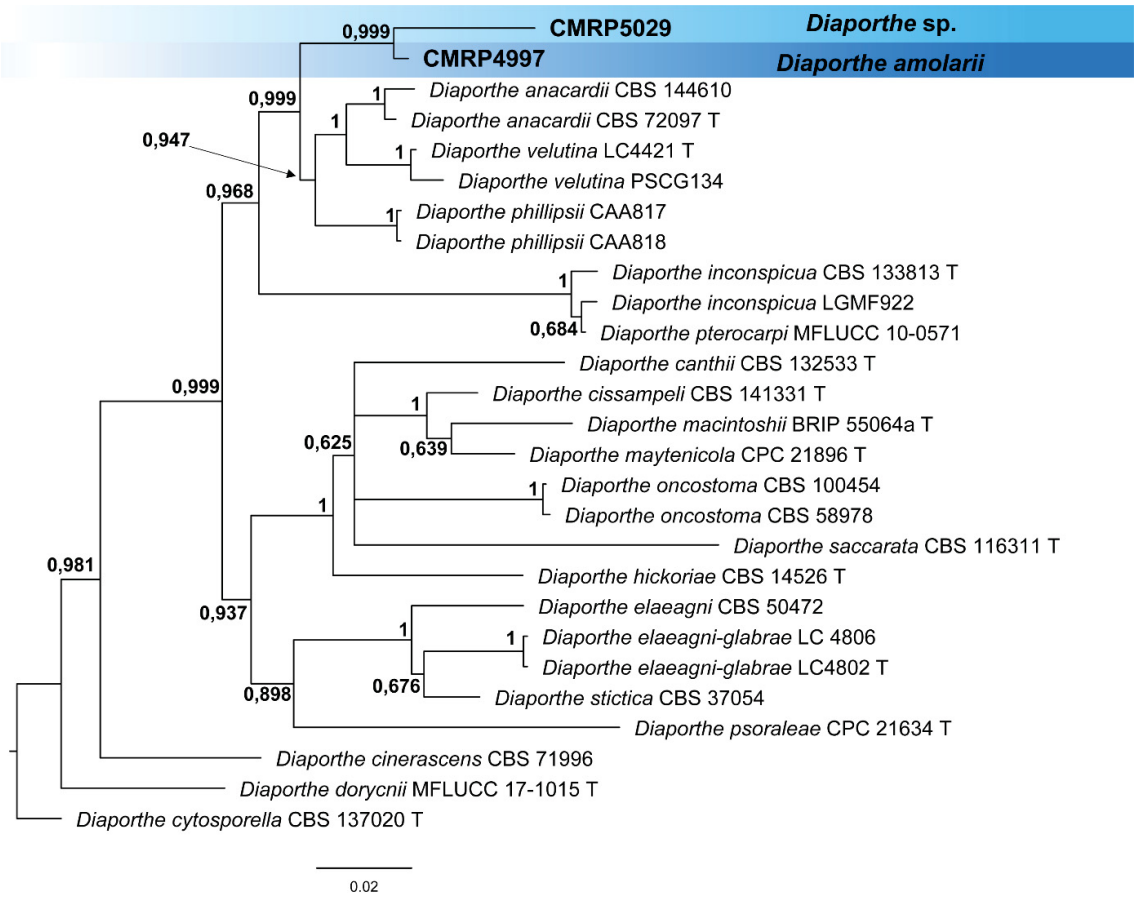


Figure 3: Bayesian Inference tree of *Diaporthe* species from clade 1 (supplementary material) based on multiple alignment of *tef1*, *tub2* and *his3* partial sequences. The data matrix had 25 taxa and 1678 characters. The species *Diaporthe cytosporella* (CBS 137020) was used as outgroup. Strains marked with a “T” correspond to type sequences. Bayesian posterior probabilities equal or greater than 0.50 are presented next to each node. The scale bar of 0.01 represents the number of changes. The sequence of the isolates here studied are presented with its isolation code (CMRP4997 and CMRP5029) highlighted in bold.

In addition, images of the colony macromorphology were obtained in different media, as well as the micromorphological characteristics obtained from sporulating cultures that formed asexual structures are showed in figure 4.

Taxonomy

Diaporthe amolarii Mayrhofer & Glienke, sp. nov Mycobank XX. Fig. 4

Etymology: Named after the Pantanal region where it was collected, Serra do Amolar.

Sporulation on PDA and OA. *Conidiomata* pycnidial globose, conical or irregular, solitary or aggregated, exposed on PDA medium surface, dark brown to black, cream translucent conidial drops exuded from the ostioles, 170–350 µm diameter. *Conidiogenous cells* hyaline and subcylindrical, tapering towards the apex 12.2–15.1 x 2–3.5 µm. *Alpha* conidia common, hyaline, fusiform, biguttulate, 6.7-12.2 x 2-4 µm, mean ± SD = 9.74 ± 1.44 x 3.10 ± 0.46 (n = 50). *Beta* conidia spindle-shaped, aseptate, smooth, hyaline, mostly curved towards one end 26.4-35.1 x 0.3-1.5 µm, mean ± SD = 30.26 ± 2.50 x 0.89 ± 0.29 (n = 50) and *gamma* conidia absent (figure 4).

Culture characteristics: Colonies covering dish after 15 days in the dark at 25ºC. Colonies on flat PDA, aerial mycelium with cotton texture, white to pale yellow on the surface, colonies reaching 79 mm in diameter after 7 days at 25 °C; reverse brown. In flat OA, aerial mycelium with fluffy texture in the center, white on the surface, colonies reaching 79 mm in diameter; reverse light yellow. In the flat MEA, aerial mycelium with cotton texture, white to pale yellow on the surface, colonies reaching 73 mm diameter; reverse yellow to brown forming concentric rings (figure 4).

Specimen examined: Brazil, Serra do Amolar, Pantanal, Mato Grosso do Sul (18°15'37.8"S 57°27'37.4"W), endophytic species isolated from petiole of *Vochysia divergens* (popular name Cambará), February 2019, C. Glienke. Holotype: (CÓDIGO HERBARIUM UFPR) Herbarium of the Department of Botany code, Federal University of Paraná, ex-type culture CMRP4997 (Microbiological Collections of Paraná Network at Federal University of Paraná).

Notes — Endophytic isolate from a medicinal plant in Brazil.

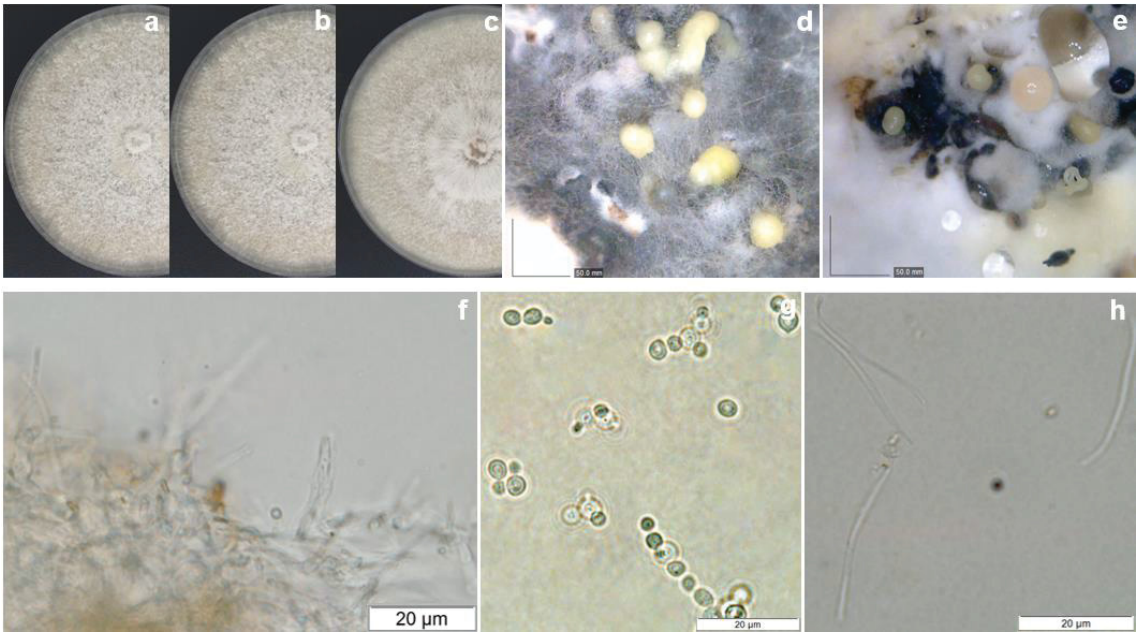


Figure 4: *Diaporthe amolarii* (CMRP4991). a-c: Colonies at 15 days on PDA, MEA and OA, respectively. d-e: Conidiomata sporulating on OA and MEA. f: Conidiogenous cells. g: Alpha conidia. h: Beta conidia. Bars: f-h 20 µm.

The isolate of the genus *Nemania* was identified at the species level by multilocus analysis that comprised 2868 pb of the partial sequences of ITS, *act* and *tub2* of all type and representative strains with GenBank available sequences. The Bayesian Inference analysis (figure 5) showed the strain CMRP4987 in the same branch (0.999 posterior probability) as the species *N. primolutea* (YMJ 91102001), sharing 96% similarity (Identities = 540/565). Thus, the CMRP4987 isolate is confirmed belonging to the species *N. primolutea*. In addition, images of the colony macromorphology in different media and the micromorphological characteristics of sporulating cultures that formed asexual structures were obtained (figure 6).

In order to identify the isolate of the genus *Xylaria* at the species level, a multilocus analysis was also carried out, comprising 3724 pb of the partial sequences of ITS, *act* and *tub2* of all type and representative strains with GenBank available sequences. The Bayesian Inference analysis (figure 7) showed the CMRP5054 strain in the same branch (0.999 posterior probability) as the species *X. arbuscula* (CBS 126415), with which it shares 99% similarity. Therefore, the CMRP5054 strain can be confirmed as belonging to the species *X. arbuscula*. For the identification of the isolates of the genus *Anthostomelloides* at the species level, an analysis of the ITS region was performed, comprising 750 pb of all type and representative sequences available. The Bayesian Inference analysis (figure 8) showed the CMRP4993, CMRP4983 and CMRP5045 in the same branch (0.956 posterior probability) as the species *A. forlicesenica* (MFLUCC 14-0558), which shares 87% similarity (Identities = 249/284), 88% (Identities = 504/570) and 92% (Identities = 523/571) respectively. And the

CMRP4992, CMRP4988 and CMRP5050 strains are present in a single branch different from the other species, being thus characterized as a new species. However, as these strains did not produce reproduction structures, we called them as *Anthostomelloides* sp. nov.

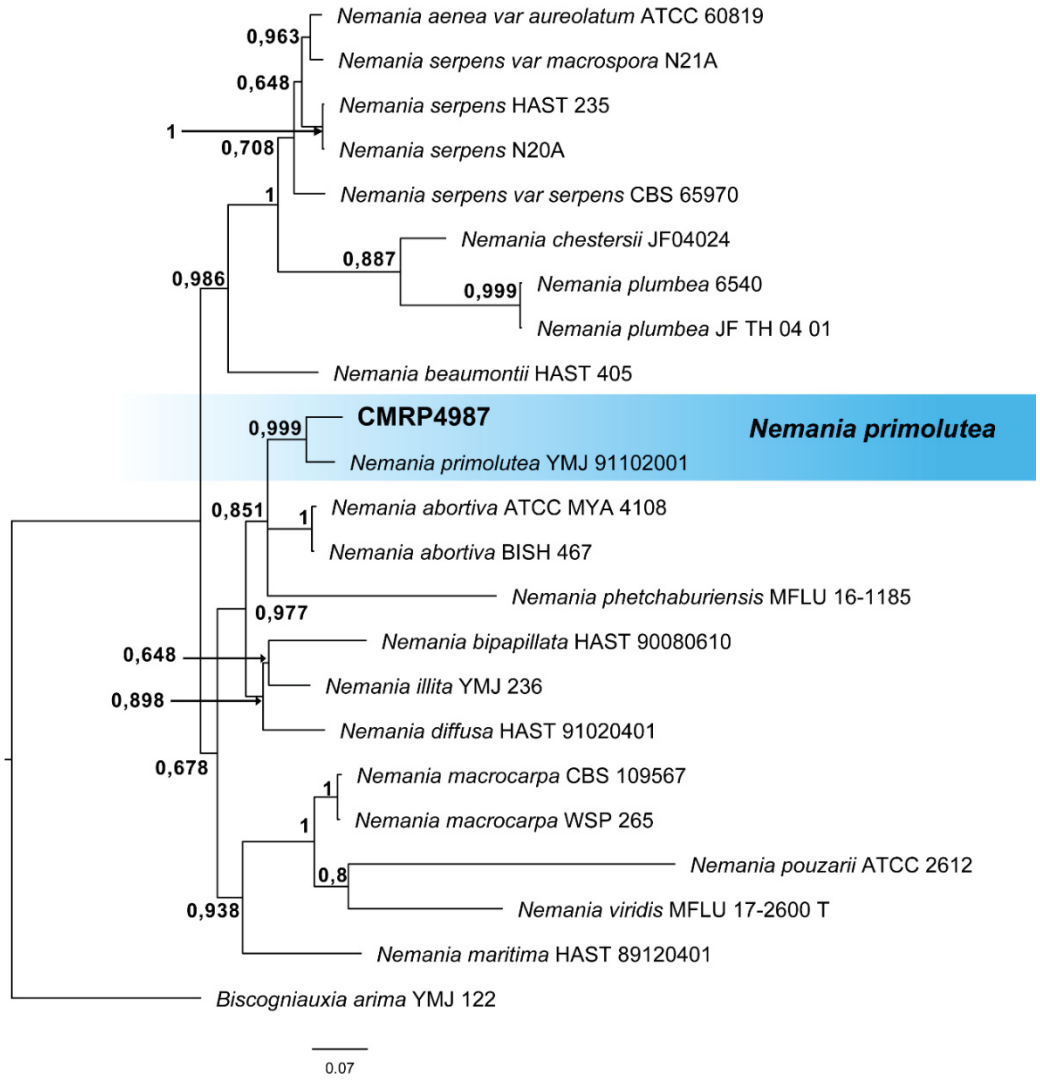


Figure 5: Bayesian Inference phylogenetic tree of *Nemania* species based on multiple alignment of ITS, *act* and *tub2* partial sequences. The data matrix had 23 taxa and 2868 characters. The species *Biscogniauxia arima* (YMJ 122) was used as outgroup. Strains marked with a "T" correspond to type sequences. Bayesian posterior probabilities equal or greater than 0.50 are presented next to each node. The scale bar of 0.07 represents the number of changes. The sequence of the isolate here studied is presented with its isolation code (CMRP4987) highlighted in bold.

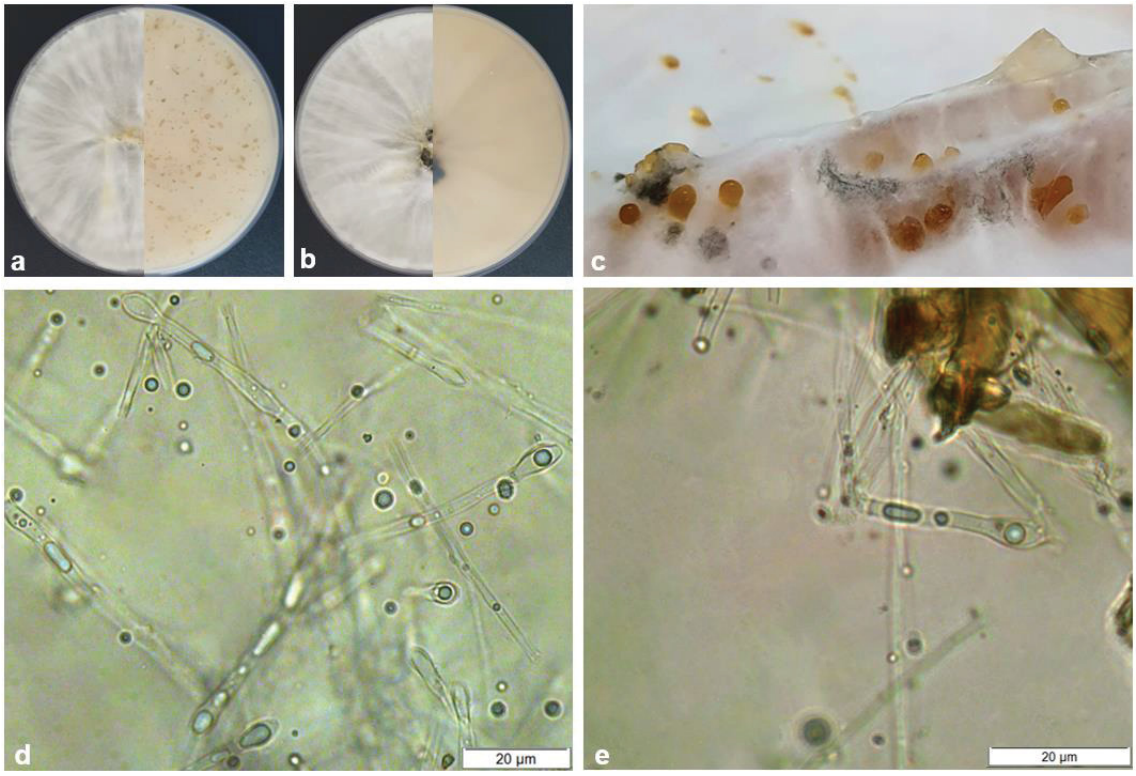


Figure 6: *Nemania primolutea* (CMRP4987). a-b: Colonies (surface and reverse) at 21 days on OA and SME respectively. c: Conidiomata sporulating on PDA with pinne needles. d-e: Ascospores.

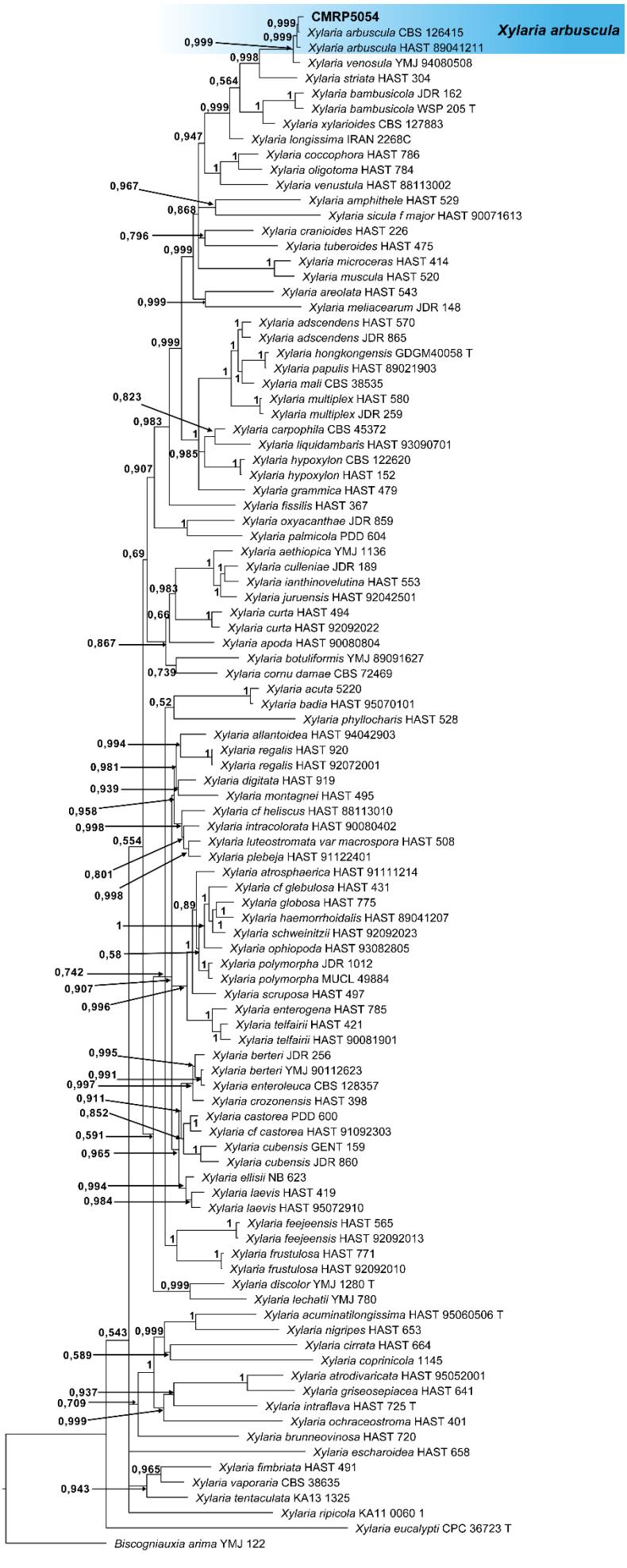

CMRP5054
Xylaria arbuscula
Xylaria arbuscula CBS 126415
Xylaria arbuscula HAST 89041211
Xylaria venosula YMJ 94080508
Xylaria striata HAST 304
Xylaria bambusicola JDR 162
Xylaria bambusicola WSP 205 T
Xylaria xylarioides CBS 127883
Xylaria longissima IRAN 2268C
Xylaria coccophora HAST 786
Xylaria oligotoma HAST 784
Xylaria venustula HAST 88113002
Xylaria amphithele HAST 529
Xylaria sicula f major HAST 90071613
Xylaria cranioides HAST 226
Xylaria tuberoides HAST 475
Xylaria microceras HAST 414
Xylaria muscula HAST 520
Xylaria areolata HAST 543
Xylaria meliacearum JDR 148
Xylaria adscendens HAST 570
Xylaria adscendens JDR 865
Xylaria hongkongensis GDGM40058 T
Xylaria papulis HAST 89021903
Xylaria mali CBS 38535
Xylaria multiplex HAST 580
Xylaria multiplex JDR 259
Xylaria carpophila CBS 45372
Xylaria liquidambaris HAST 93090701
Xylaria hypoxylon CBS 122620
Xylaria hypoxylon HAST 152
Xylaria grammica HAST 479
Xylaria fissilis HAST 367
Xylaria oxyacanthae JDR 859
Xylaria palmicola PDD 604
Xylaria aethiopica YMJ 1136
Xylaria culleniae JDR 189
Xylaria ianthinovelutina HAST 553
Xylaria juruensis HAST 92042501
Xylaria curta HAST 494
Xylaria curta HAST 92092022
Xylaria apoda HAST 90080804
Xylaria botuliformis YMJ 89091627
Xylaria cornu damae CBS 72469
Xylaria acuta 5220
Xylaria badia HAST 95070101
Xylaria phyllocharis HAST 528
Xylaria allantoidea HAST 94042903
Xylaria regalis HAST 920
Xylaria regalis HAST 92072001
Xylaria digitata HAST 919
Xylaria montagnei HAST 495
Xylaria cf heliscus HAST 88113010
Xylaria intracolorata HAST 90080402
Xylaria luteostromata var macrospora HAST 508
Xylaria plebeja HAST 91122401
Xylaria atrosphaerica HAST 91111214
Xylaria cf glebulosa HAST 431
Xylaria globosa HAST 775
Xylaria haemorrhoidalis HAST 89041207
Xylaria schweinitzii HAST 92092023
Xylaria ophiopoda HAST 93082805
Xylaria polymorpha JDR 1012
Xylaria polymorpha MUCL 49884
Xylaria scruposa HAST 497
Xylaria enterogena HAST 785
Xylaria telfairii HAST 421
Xylaria telfairii HAST 90081901
Xylaria berteri JDR 256
Xylaria berteri YMJ 90112623
Xylaria enteroleuca CBS 128357
Xylaria crozonensis HAST 398
Xylaria castorea PDD 600
Xylaria cf castorea HAST 91092303
Xylaria cubensis GENT 159
Xylaria cubensis JDR 860
Xylaria ellisii NB 623
Xylaria laevis HAST 419
Xylaria laevis HAST 95072910
Xylaria feejeensis HAST 565
Xylaria feejeensis HAST 92092013
Xylaria frustulosa HAST 771
Xylaria frustulosa HAST 92092010
Xylaria discolor YMJ 1280 T
Xylaria lechatii YMJ 780
Xylaria acuminatilongissima HAST 95060506 T
Xylaria nigripes HAST 653
Xylaria cirrata HAST 664
Xylaria coprinicola 1145
Xylaria atrodivaricata HAST 95052001
Xylaria griseosepiacea HAST 641
Xylaria intraflava HAST 725 T
Xylaria ochraceostroma HAST 401
Xylaria brunneovinosa HAST 720
Xylaria escharoidea HAST 658
Xylaria fimbriata HAST 491
Xylaria vaporaria CBS 38635
Xylaria tentaculata KA13 1325
Xylaria ripicola KA11 0060 1
Xylaria eucalypti CPC 36723 T
Biscogniauxia arima YMJ 122
0.06

Figure 7: Bayesian Inference phylogenetic tree of *Xylaria* species based on multiple alignment of ITS, *act* and *tub2* partial sequences. The data matrix had 101 taxa and 3724 characters. The species *Biscogniauxia arima* (YMJ 122) was used as outgroup. Strains marked with a "T" correspond to type sequences. Bayesian posterior probabilities equal or greater than 0.50 are presented next to each node. The scale bar of 0.06 represents the number of changes. The sequence of the isolate here studied is presented with its isolation code (CMRP5054) highlighted in bold.

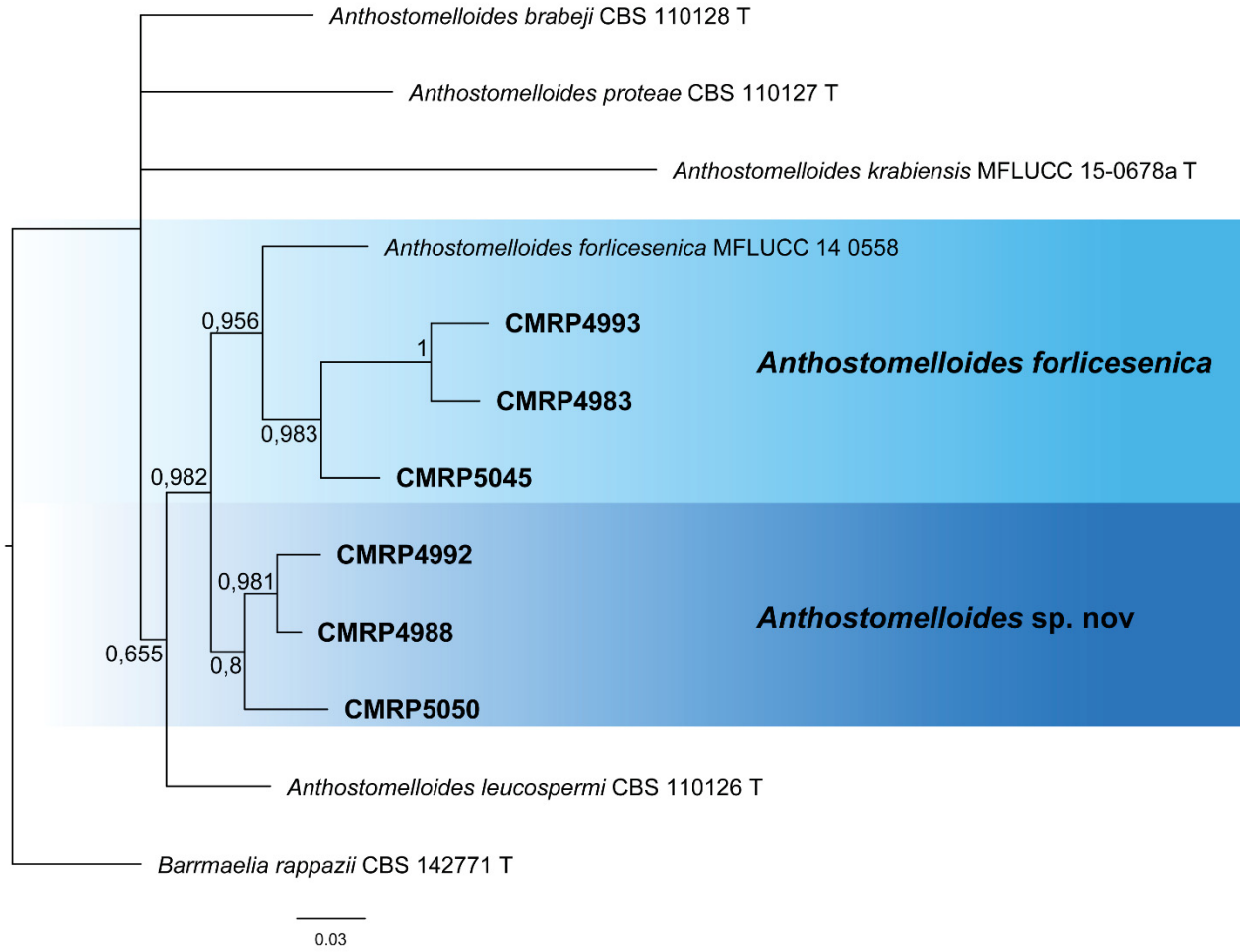
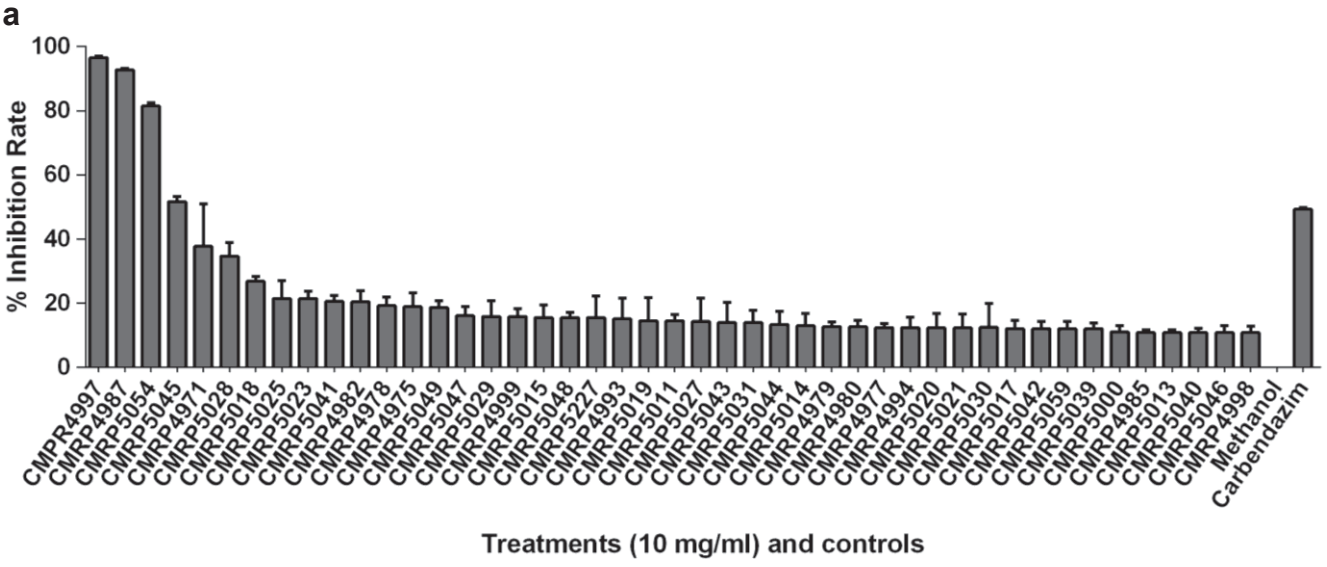


Figure 8: Bayesian Inference phylogenetic tree of *Anthostomelloides* species based on alignment of ITS partial sequences. The data matrix had 12 taxa and 750 characters. The tree was rooted to a sequence of *Barrmaelia rappazii* (CBS 142771). Names marked with a "T" correspond to the type strains. Bayesian posterior probabilities equal or higher than 0.50 are presented next to each node. The scale bar of 0.03 represents the number of changes. The sequences of the isolates studied here are presented with their isolation code (CMRP4992, CMRP4988, CMRP5050, CMRP4993, CMRP4983 and CMRP5045) highlighted in bold.

Endophytic fungi from Serra do Amolar produce secondary metabolites with biological activity against phytopathogenic fungi

Extracts produced by 91 endophytes were evaluated for antifungal activity via the percentage of mycelial growth inhibition of the citrus phytopathogen *C. abscissum* (figure 9). Among the tested extracts, three of them showed greater growth inhibition than the commercial fungicide Carbendazim (mycelial growth inhibition – MGI: 49.3%) (figure 9a and 9b). These extracts were produced by the strains *Diaporthe amolarii* sp. nov. CMRP4997 (MGI: 96.5%), *Nemania primolutea* CMRP4987 (MGI: 92.7%) and *Xylaria arbuscula* CMRP5054 (MGI: 81.4%). In addition to these 3 extracts, the extract produced by *Anthostomelloides forlicesenica* CMRP5045 also showed an excellent mycelial growth inhibition (MGI: 51.6%), despite not being statistically different from the fungicide control.

Based on that, these four extracts with highest growth inhibition in the screening phase were again evaluated for their antifungal activity against the phytopathogenic fungi *Fusarium graminearum* and *Phyllosticta citricarpa* (figure 10). Here the extracts showed lower growth inhibition against *F. graminearum*. However, the extract of the CMRP5054 strain *Xylaria arbuscula* is highlighted for presenting a moderate inhibition over the mycelial growth of *P. citricarpa* (MGI: 62.8%).



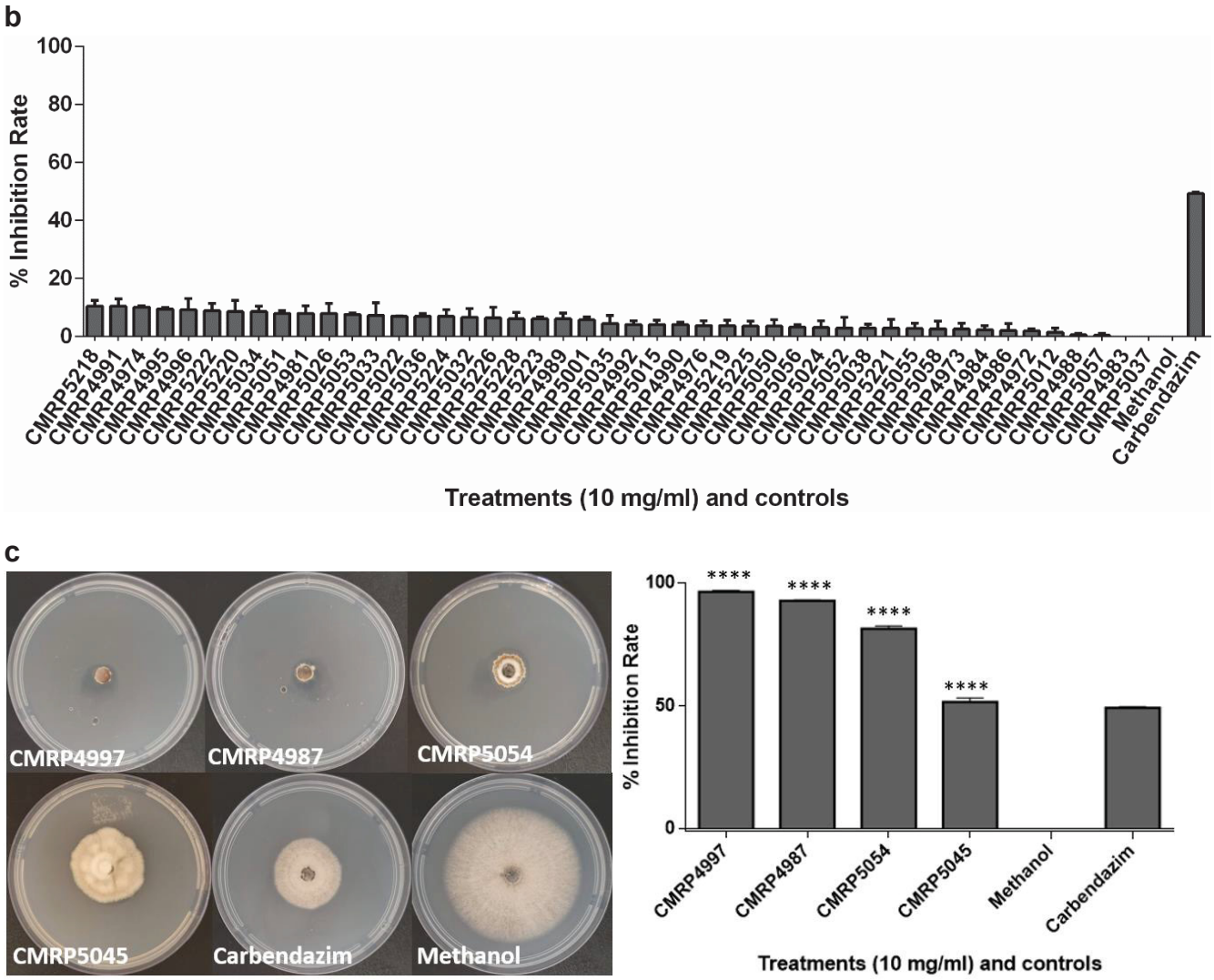


FIGURE 9: MEAN OF THE MYCELIAL GROWTH INHIBITION OF PHYTOPATHOGEN *Colletotrichum abscissum* (CMRP704) AFTER EXPOSURE TO EXTRACTS PRODUCED BY ENDOPHYTES. **a** and **b**: Mean of mycelial growth inhibition (in %) of the phytopathogen *Colletotrichum abscissum* in the presence of 100 µl of the extracts obtained by the cultivation endophytic fungi. **c**: Mycelial growth inhibition test plates and mean of mycelial growth inhibition (in %) of phytopathogen *C. abscissum* from extracts with the highest growth inhibition strain *Diaporthe amolarii* sp. nov. CMRP4997 (MGI: 96.5%), *Nemania primolutea* CMRP4987 (MGI: 92.7%), *Xylaria arbuscula* CMRP5054 (MGI: 81.4%) and *Anthostomelloides forlicesenica* CMRP5045 (MGI: 51.6%) compared to the negative and positive controls (Methanol MGI: 0.00% and Carbendazim MGI: 49.32%).

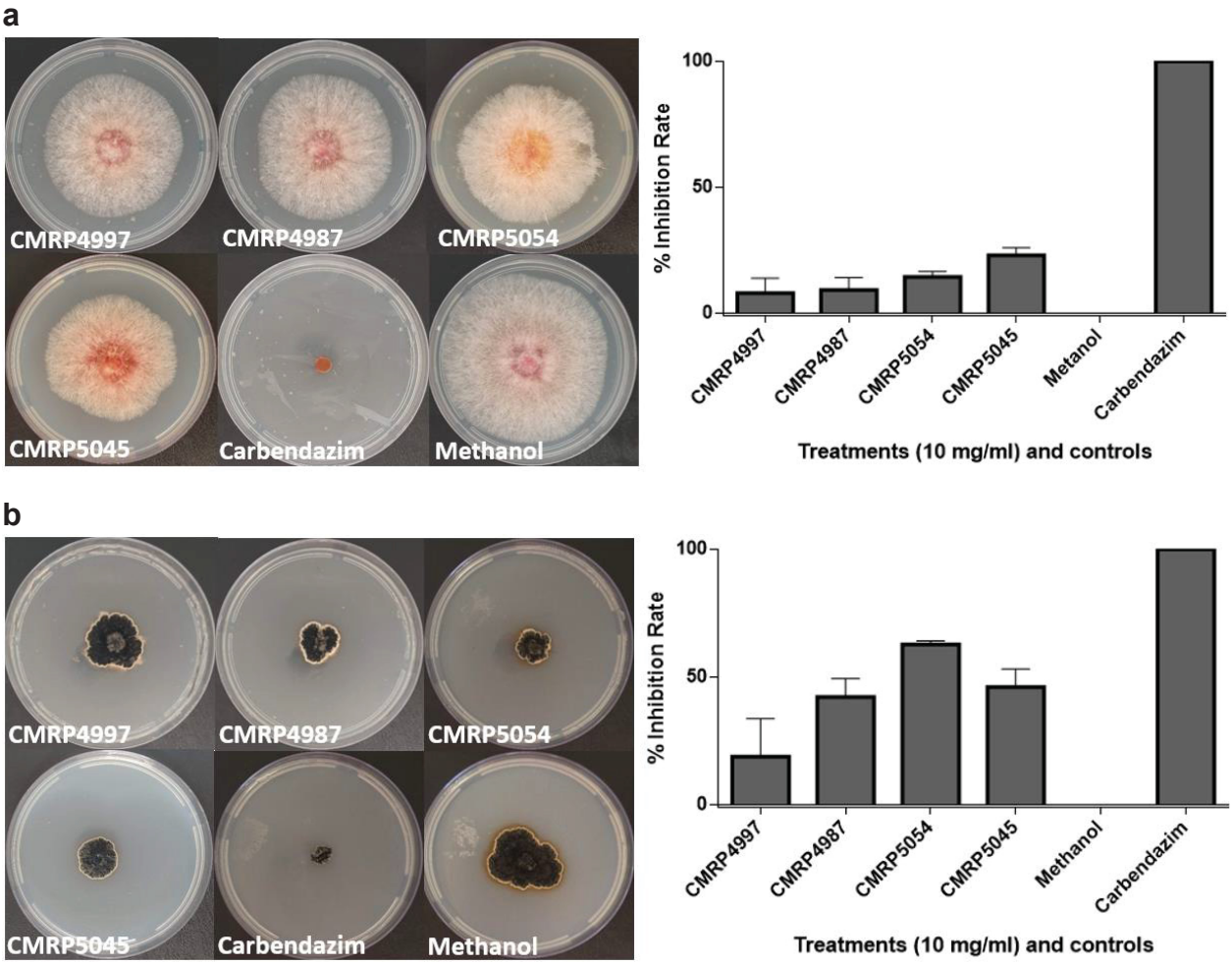


FIGURE 10: MEAN OF THE MYCELIAL GROWTH INHIBITION OF PHYTOPATHOGEN *Fusarium graminearum* (CT STALK21) AND *Phyllosticta citricarpa* (CMRP06) AFTER EXPOSURE TO EXTRACTS SELECTED IN SCREENING TEST. **a**: Mycelial growth inhibition test plates and mean of mycelial growth inhibition (in %) of the phytopathogen *Fusarium graminearum* in presence of 100 µl of the extracts from strains *Diaporthe* sp. nov. CMRP4997 (MGI: 8.0%), *Nemania primolutea* CMRP4987 (MGI: 9.2%), *Xylaria arbuscula* CMRP5054 (MGI: 14.4%) and *Anthostomelloides forlicesenica* CMRP5045 (MGI: 23.0%), compared to the controls (Methanol MGI: 0.0% and Carbendazim MGI: 100.0%). **b**: Mycelial growth inihibition test plates and mean of mycelial growth inhibition (in %) of phytopathogen *Phyllosticta citricarpa* in presence of 100 µl of the extracts from strains *Diaporthe amolarii* sp. nov. CMRP4997 (MGI: 18.9%), *Nemania primolutea* CMRP4987 (MGI: 42.1%), *Xylaria arbuscula* CMRP5054 (MGI: 62.8%) and *Anthostomelloides forlicesenica* CMRP5045 (MGI: 46.2%), compared to the controls (Methanol MGI: 0.0% and Carbendazim MGI: 100.0%).

Diaporthe amolarii sp. nov. CMRP4997 is a new species producing compounds with biological activity

Based on the antifungal activity assay against the phytopathogens, the extract produced by the *Diaporthe amolarii* sp. nov. CMRP4997 strain showed the highest inhibition over of the citrus pathogen *C. abscissum* and was selected for a large-scale production of extracts and subsequently fractionation.

Approximately 1.38 g of crude extract was obtained from a 5 L fermentation culture. In fractionation, 25 fractions were obtained by column chromatography, of which the fractions F18 (7.3 mg) and F19 (6.3 mg) showed the highest mycelial growth inhibition of the citrus pathogen *C. abscissum*, 24.1% and 26.2% respectively (figure 11 and figure S2).

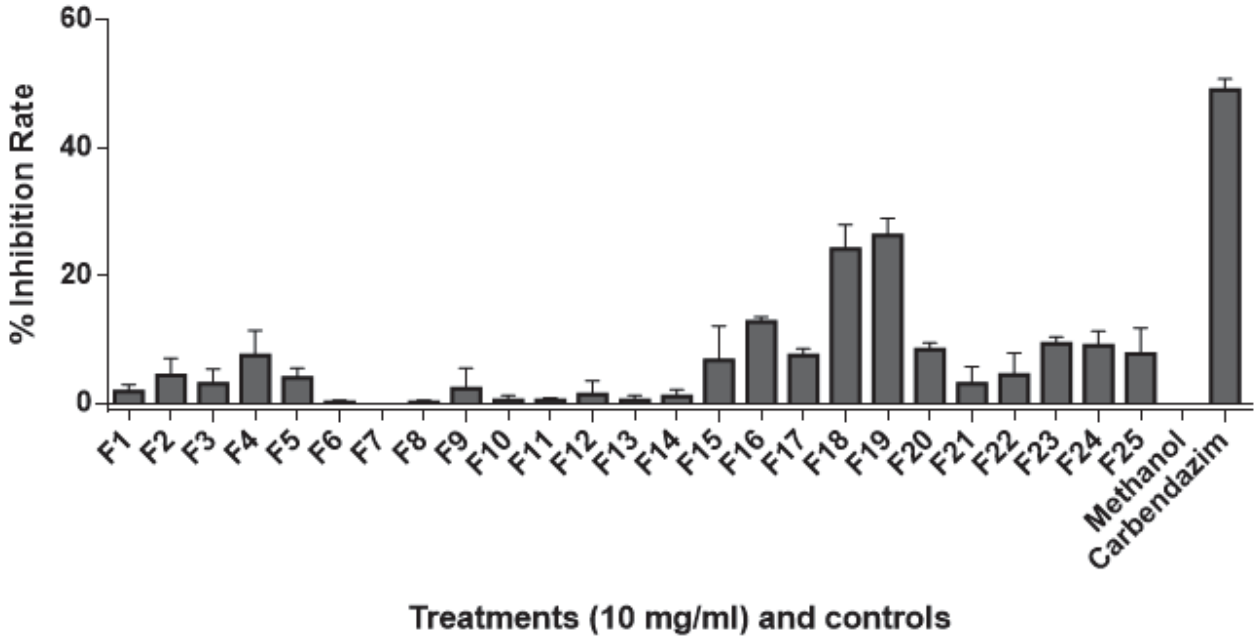


FIGURE 11: MEAN MYCELIAL GROWTH INHIBITION (IN %) OF PHYTOPATHOGEN *Colletotrichum abscissum* AFTER EXPOSURE TO THE 25 EXTRACT FRACTIONS PRODUCED BY CMRP4997 STRAIN *Diaporthe amolarii* sp. nov.

DISCUSSION

Serra do Amolar, a region of environmental conservation managed by the Serra do Amolar Protection and Conservation Network, has an enormous wealth of biodiversity still little explored, largely due to the isolation and the consequent difficulty in accessing the area (ECOTRÓPICA, 2003; MOREIRA, 2011). In addition to the need for knowledge and conscious exploration of the natural resources of this region, actions aiming at the *ex-situ* conservation of biodiversity are increasingly necessary, since the anthropological action and the recent fires that have occurred can have an irreparable effect, reducing biodiversity in the Serra do Amolar region. The *ex-situ* conservation of microorganisms, carried out with the isolates obtained in this study, is done by isolating such organisms and stored them in culture collections, where they will preserved and available for future studies (SMITH et al., 2008).

The isolation data presented in this study show a great diversity of endophytic fungi associated with the medicinal plant *V. divergens*, obtaining 293 isolates and these grouped into 91 phenotypes. Among the representatives of the phenotypes, all isolates belong to the Phylum Ascomycota, being separated into the classes Eurotiomycetes, Dothideomycetes and Sordariomycetes. Sordariomycetes was the dominant class and the dominant orders were Xylariales, Diaporthales and Glomerellales. Furthermore, the 91 isolates studied belong to 26 families and 32 different genera, with *Diaporthe* and *Colletotrichum* being the most commonly identified genera. These results corroborate the findings of previous studies that also performed the isolation of endophytes from the plant *V. divergens*.

In a study by Noriler et al. (2018) 777 endophytic fungi were isolated from leaves and petioles of the plant *V. divergens* also collected in the Pantanal, but in another region: close to the Miranda river. The isolates belonged mainly to the Phylum Ascomycota, but also to Basidiomycota. Sordariomycetes was the isolated dominant class, similar to that observed in the present study, and the dominant orders, despite being the same ones obtained in the present study, Diaporthales was the most dominant among the three. Also, as in the present study, *Diaporthe* was the most dominant genus. The genus *Diaporthe* belongs to the family Diaporthaceae and is composed of hundreds of species (CHEPKIRUI and STADLER, 2017) and has recently been considered a paraphyletic genus (GAO et al., 2017). This genus is distributed worldwide and its species are able to colonize the most varied hosts in different associations, occurring as endophytes, plant pathogens and saprobes (GOMES et al., 2013; UDAYANGA et al., 2011). Among the endophytic fungi, the genus *Diaporthe* is one of the most commonly isolated in different host plants, and also frequently related to the production of secondary metabolites that present the most varied biological activities (CHEPKIRUI and STADLER, 2017; GOMES et al., 2013). Most of the bioactive metabolites recently described from isolates of the genus *Diaporthe* were obtained from endophytes associated with host plants with medicinal properties (CHEPKIRUI and STADLER, 2017). Recent studies have demonstrated antifungal activity of *Diaporthe* endophytes isolated from medicinal plants against citrus phytopathogens such as *C. abscissum* and *P. citricarpa* (NORILER et al., 2018; SAVI et al., 2020; TONIAL et al., 2017), which corroborates the high antifungal activity results obtained by the new species *Diaporthe amolarii* sp. nov. described by this study.

Over the years, due to the need to explore compounds from natural sources, many bioprospecting studies have shown the potential for producing secondary metabolites of endophytic microorganisms (GOS et al., 2017; NORILER et al., 2018; SAVI et al., 2015; SAVI et al., 2019). Likewise, in the present work we report four extracts produced by endophytic fungi that inhibited the mycelial growth of the phytopathogen *C. abscissum*: *Diaporthe amolarii* sp. nov. CMRP4997 (MGI: 96.5%), *Nemania primolutea* CMRP4987 (MGI: 92.7%), *Xylaria arbuscula* CMRP5054 (MGI: 81.4%) and *Anthostomelloides forlicesenica* CMRP5045 (MGI: 51.6%). The first three, in fact, inhibited the mycelial growth of the phytopathogen in a percentage higher than that observed by the positive

control (fungicide Carbendazim). The postbloom fruit drop (PFD) is a disease that affects citrus production caused predominantly by the fungus *Colletotrichum abscissum* (SILVA et al., 2017). Citrus crops are mainly affected during the bloom season, where necrotic lesions are observed on the petals, premature fruit drop and calyx retention are the main symptoms (PINHO; LOPES; PEREIRA, 2015). In Brazil, this disease is responsible for large losses in production, especially in the state of São Paulo, where a large part of citrus culture is concentrated (PINHO; LOPES; PEREIRA, 2015; SILVA *et al.*, 2017). Therefore, finding extracts that have activity against this pathogen is of great importance. Due to the results obtained, the crude extract of the CMRP4997 strain of the new species *Diaporthe amolarii* was selected for large-scale production and fractionation. In this process, 25 fractions were obtained, which were evaluated against the phytopathogen *C. abscissum*, and among them fractions F18 and F19 showed the highest mycelial growth inhibition rates (24.1% and 26.2%). Only the analysis performed after the fractionation of the extract does not allow to infer about the characteristics of the compounds present in the fractions that showed promising results. Therefore, further studies should be carried out with such fractions, in order to determine their active compounds and their mode of action.

In addition to the excellent results observed against *C. abscissum* with this new species of *Diaporthe*, the results obtained with the extracts produced from the fermentation with the species *Nemania primolutea* CMRP4987 (MGI: 92.7%), *Xylaria arbuscula* CMRP5054 (MGI: 81.4%) and *Anthostomelloides forlicesenica* CMRP5045 (MGI: 51.6%) also are important. These 3 genera belong to the family Xylariaceae. Fungi of the Xylariaceae family are normally of cosmopolitan distribution and mainly endophytes, although they are also found as phytopathogens. They are known to be important sources of secondary metabolites with biological activity (EDWARDS et al., 2003; KUHNERT et al., 2014; PETRINI, 1986; STADLER, 2011). However, there are few reports on the production of bioactive metabolites specifically from the species *Nemania primolutea, Xylaria arbuscula* and the genus *Anthostomelloides*.

The species *N. primolutea*, described by Ju et al. (2005), was first isolated from dead trunk of *Artocarpus communis* in the province of Tai-tung County, Lan-yu, Taiwan. So far, few studies have demonstrated biological activity of isolates of the species *N. primolutea*. In study carried out by Tan et al. (2020) extracts produced by 9 endolichenic fungi isolated from *Parmotrema rampoddense* were evaluated against clinical bacteria. One of the 9 isolates was identified as *N. primolutea* and was partially active against *Enterococcus faecalis* and active against *Staphylococcus aureus*. In addition to this, in a study performed by Idris et al. (2019) the lignolytic activity of previously isolated fungi was evaluated, one of which was identified as *N. primolutea*. Despite the isolate of *N. primolutea* having a low lignolytic activity, that study was the first to demonstrate this type of activity for the species.

The species *X. arbuscula* described by Saccardo, 1878 and has recently been studied by a Brazilian research group (AMARAL et al., 2017; AMARAL et al., 2014; AMARAL, 2009). In these studies, the biosynthesis of the compound

Cytochalasin B was observed from the endophyte *X. arbuscula* isolated from healthy tissues of the plant *Cupressus lusitanica*. Such compound is a mycotoxin that has biological activity causing nuclear cell extrusion, inhibition of HIV-1 protease and antibiotic and cytotoxic activity, in addition to also being related to the symbiosis relationship between endophyte and its host (AMARAL et al., 2017; AMARAL et al., 2014; AMARAL, 2009). As the data of antifungal activity presented by the isolate of the species *X. arbuscula* in the present study were obtained by the evaluation of the crude extract, it cannot be said about its composition nor if there is the presence of the compound Cytochalasin B.

The genus *Anthostomelloides* was introduced to the Xylariaceae family by Tibpromma et al. (2017) from the isolation of the species *A. krabiensis*, which was also described in the same study, from dead leaves of *Pandanus odorifer* (Forssk.) collected in Thailand. After the description of the genus some species that had been described belonging to the genus *Anthostomella*, recently considered as polyphyletic, were reclassified, and now belong to the genus *Anthostomelloides*. Until now, the species *A. brabeji*, *A. forlicesenica*, *A. krabiensis*, *A. leucospermi* and *A. proteae* are accepted in the Mycobank database (accessed in February 2021). In a study realized by Amorim et al. (2016) the endophyte of *Paepalanthus planifolius* identified as *Anthostomella brabeji* was used to produce the extract and consequent purification of its compounds. The identified compounds were evaluated for their antibacterial and antifungal activity. In the test, known compounds named siccayne and eutypinol had greater action against *Staphylococcus aureus*, compound 6-hydroxy-2,2-dimethyl-5,6,7,8-tetrahydro-7,8-epoxycroman-4-one and eutypinol showed the best results against *Salmonella setubal*. In addition, siccayne present the best activity against the yeast *Candida albicans*.

Another very promising result obtained in the present work was the extract produced by *Xylaria arbuscula* CMRP5054 strain reducing the mycelial growth of the phytopathogen *Phyllosticta citricarpa* by more than 60%. This pathogen is the epidemiological agent of the Citrus black spot (CBS) disease that affects citrus cultivation, occurring mainly in subtropical regions (GLIENKE et al., 2011; WULANDARI et al., 2009). The disease is characterized by lesions on the skin of the fruit, which can lead to premature fall and generate depreciation of the fruits value and reduce crop productivity (SANTOS et al., 2016; WULANDARI et al., 2009). CBS is a quarantine disease, which means that fruits from contaminated regions cannot be exported to areas without reports of the disease, such as Europe (GLIENKE et al., 2011; TONIAL et al., 2017). In addition, the presence of fungicide residues in fruits is another factor that prevents exports, causing even more economic impacts for producers. Therefore, this extract will be used in future studies in order to assess whether and which active compounds could control the growth of the fungus and the consequent production of ascospores on leaves fallen in the field. Such sexual spores are the main source of spread of the disease in the field, since they are carried by the wind and when adhered to the fruit surfaces, they germinate and develop the lesions.

Although no extract has shown excellent results against the phytopathogen *Fusarium graminearum*, the rates of mycelial growth inhibition observed are not negligible, mainly with the application of the extract produced from the fermentation of the *Anthostomelloides forlicesenica* CMRP5045 (MGI: 23.0%), since this is a pathogen difficult to control. In addition, often, the most important in controlling this pathogen is the search for compounds that reduce the production of mycotoxins, called as anti-mycotoxigenic (PAGNUSSATT et al., 2014). *F. graminearum* is the main cause of Fusarium head blight (FHB) disease in wheat and barley and know to produce toxins, such as fumonisins, zearalenone and trichothecenes, and the availability of efficient control measures is urgent. Therefore, in future studies, we should explore such extracts in the search for compounds that reduce the production of such mycotoxins, in addition to inhibiting the mycelial growth of the pathogen. In addition, it is important to note that the present study is the first to demonstrate an antifungal activity of extracts produced by one endophyte of the genus *Anthostomelloides* against the phytopathogens *C. abscissum*, *P. citricarpa* and *Fusarium graminearum*.

As a conclusion, in our study we report this important region of the Pantanal in Brazil, called Serra do Amolar, as a place with a great diversity of endophytes, including new species never described. In addition, extracts from four endophytic fungi showed promising secondary metabolites with biological activity against phytopathogens, such as *Colletotrichum abscissum*, *Phyllosticta citricarpa* and *Fusarium graminearum*, and should be further explored to describe the active compounds and their mode of action. Finally, a very important contribution of this study was to act in the *ex-situ* conservation of the biodiversity of Serra do Amolar, allowing future studies and biotechnological applications of these endophytes, adding value to Brazilian biodiversity, and demonstrating the importance in the conservation of such biomes.

BIBLIOGRAPHY

AMARAL, L. S. *et al.* An HPLC evaluation of cytochalasin D biosynthesis by Xylaria arbuscula cultivated in different media. **Natural Product Communications**, v. 9, n. 9, p. 1279–1282, 2014.

AMARAL, L. S. *et al.* Biosynthesis and mass spectral fragmentation pathways of 13C and 15N labeled cytochalasin D produced by Xylaria arbuscula. **Journal of Mass Spectrometry**, , v. 52, n. 4, p. 239–247, 2017.

AMARAL, Luciana Silva. Análise de metabólitos secundários produzidos por fungos endofíticos associados à Cupressus lusitanica. p. 164, 2009.

AMORIM, M. R. *et al.* Compounds of Anthostomella brabeji, an Endophytic Fungus Isolated from Paepalanthus planifolius (Eriocaulaceae). v. 27, n. 6, p. 1048–1054, 2016.

ARAÚJO, W. L. et al. **Microrganismos endofíticos: Aspectos teóricos e práticos de isolamento e caracterização.** 2a ed- Santarém: UFOPA, 2014.

ARIEIRA, J.; DA CUNHA, C. N. Fitossociologia de uma floresta inundável monodominante de Vochysia divergens Pohl (Vochysiaceae), no Pantanal Norte, MT, Brasil. **Acta Botanica Brasilica**, v. 20, n. 3, p. 569–580, 2006.

BALDASSARI, R. B.; REIS, R. F.; DE GOES, A.. Susceptibility of fruits of the “Valência” and “Natal” sweet orange varieties to Guignardia citricarpa and the influence of the coexistence of healthy and symptomatic fruits. **Fitopatologia Brasileira**, v. 31, n. 4, p. 337–341, 2006.

BROWN, N. A.; ANTONIW, J.; HAMMOND-KOSACK, K. E. The predicted secretome of the plant pathogenic fungus Fusarium graminearum: A refined comparative analysis. **PLoS ONE**, v. 7, n. 4, 2012.

CARBONE, I.; KOHN, L. M. A method for designing primer sets for speciation studies in filamentous ascomycetes. **Mycologia**, v. 91, n. 3, p. 553–556, 1999.

CHEN, J. *et al.* Diversity and Taxonomy of Endophytic Xylariaceous Fungi from Medicinal Plants of Dendrobium (Orchidaceae). **PLoS ONE**, v. 8, n. 3, 2013.

CHEPKIRUI, C.; STADLER, M. The genus Diaporthe: a rich source of diverse and bioactive metabolites. **Mycological Progress**, v. 16, n. 5, p. 477–494, 2017.

CROUS, P. W. *et al.* Fungal planet description sheets: 154-213. **Persoonia: Molecular Phylogeny and Evolution of Fungi**, v. 31, n. January 2014, p. 188–296, 2013.

CROUS, P.W. *et al.* New and Interesting Fungi. 2. **Fungal Systematics and Evolution**, v. 3, n. June, p. 171–184, 2019.

CROUS, P. W. *et al.* Fungal Planet description sheets : 951 – 1041. p. 223–425, 2019.

CROUS, P. W. *et al.* Calonectria species and their Cylindrocladium anamorphs: Species with sphaeropedunculate vesicles. **Studies in Mycology**, v. 50, n. 2, p. 415–430, 2004.

DARRIBA, D. *et al.* JModelTest 2: More models, new heuristics and parallel computing. **Nature Methods** , v. 9, n. 8, p. 772, 2012.

DAYARATHNE, M.C. *et al.* Morpho-molecular characterization of microfungi associated with marine based habitats. **Mycosphere**, v. 11, n. 1, p. 1–188, 2020.

DE HOOG, G. S.; GERRITS VAN DEN ENDE, A. H. G. Molecular diagnostics of clinical strains of filamentous Basidiomycetes. **Mycoses**, v. 41, n. 5–6, p. 183–189, 1998.

DISSANAYAKE, A. J. *et al.* Molecular phylogenetic analysis reveals seven new Diaporthe species from Italy. **Mycosphere**, v. 8, n. 5, p. 853–877, 2017.

DU, Z. *et al.* Phylogeny and morphology reveal two new species of Diaporthe from Traditional Chinese Medicine in Northeast China. **Phytotaxa**, v. 269, n. 2, p. 090–102, 2016.

EDWARDS, R. L. *et al.* The Xylariaceae as phytopathogens. **Recent Research Development Plant Science**, v. 1, n. January, p. 1–19, 2003.

FONTES, E. M. G.; VALADARES-INGLIS, M. C. **Controle biológico de pragas da agricultura**. 2020.

FOURNIER, J. *et al. Xylaria aethiopica* sp. nov. – a new pod-inhabiting species of *Xylaria* (Xylariaceae) from Ethiopia. **Ascomycete.org**, v. 10, n. 5, p. 209–215, 2018.

FUNDAÇÃO ECOTRÓPICA, Plano de Manejo das RPPN'S Acurizal, Penha e Dorochê, Fundação de Apoio à Vida nos Trópicos, Cuiabá-MT, 2003.

GAO, Y. *et al. Diaporthe* is paraphyletic. **IMA Fungus**, v. 8, n. 1, p. 153–187, 2017.

GILCHRIST-SAAVEDRA, L.; FUENTES-DÁVILA, G.; MARTÍNEZ-CANO, C.; LÓPEZ-ATILANO, R.M.; DUVEILLER, E.; SINGH, R.P. **Practical guide to the identification of selected diseases of wheat and barley**. 2nd ed. CIMMYT, Mexico, D.F, 2006.

GLASS, N. L.; DONALDSON, G. C. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. **Applied and Environmental Microbiology**, v. 61, n. 4, p. 1323–1330, 1995.

GLIENKE, C. ***Guignardia citricarpa* Kiely**: Análise Genética, Cariotípica e Interação com o Hospedeiro, Escola Superior de Agricultura Luiz de Queiroz. 1999.

GLIENKE, C. *et al.* Endophytic and pathogenic *Phyllosticta* species, with reference to those associated with Citrus Black spot. **Persoonia: Molecular Phylogeny and Evolution of Fungi**, v. 26, p. 47–56, 2011.

GOMES, R. R. *et al. Diaporthe*: A genus of endophytic, saprobic and plant pathogenic fungi. **Persoonia: Molecular Phylogeny and Evolution of Fungi**, v. 31, p. 1–41, 2013.

GOS, F. M. W. R. *et al.* Antibacterial Activity of Endophytic Actinomycetes Isolated from the Medicinal Plant *Vochysia divergens* (Pantanal, Brazil). **Frontiers in Microbiology**, 2017.

GUO, Y.S. *et al.* High diversity of *Diaporthe* species associated with pear shoot

canker in China. **Persoonia - Molecular Phylogeny and Evolution of Fungi**, p. 132–162, 2020.

HALL, T. A. **BioEdit4.8**. Raileigh, 1997-2001. 1 arquivo (11,5M); Disponível em: http://www.mbio.ncsu.edu/bioedit.html BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windons 95/98/NT.

HASHEMI, S.A. *et al.* A new *Xylaria* species from Iran. **Mycologia Iranica**, v. 2, n. 1, p. 1–10, 2015.

HILÁRIO, S. *et al. Diaporthe* species associated with twig blight and dieback of Vaccinium corymbosum in Portugal, with description of four new species. **Mycologia**, v. 112, n. 2, p. 293–308, 2020.

HSIEH, H. M. *et al.* Phylogenetic status of *Xylaria* subgenus *Pseudoxylaria* among taxa of the subfamily Xylarioideae (Xylariaceae) and phylogeny of the taxa involved in the subfamily. **Molecular Phylogenetics and Evolution**, v. 54, n. 3, p. 957–969, 2010.

HSIEH, H. M.; JU, Y. M.; ROGERS, J. D. Molecular phylogeny of *Hypoxylon* and closely related genera. **Mycologia**, v. 97, n. 4, p. 844–865, 2005.

IBRAHIM, A. *et al.* Metabolomic-guided discovery of cyclic nonribosomal peptides from *Xylaria ellisii* sp. nov., a leaf and stem endophyte of *Vaccinium angustifolium*. **Scientific Reports**, v. 10, n. 1, p. 1–17, 2020.

IDRIS, I. R. *et al.* Screening of potential lignin-degrading fungi from the tropical forest for lignocellulose biotreatment. **IOP Conference Series: Earth and Environmental Science**, v. 308, n. 1, p. 0–17, 2019.

JU, Y. M. *et al.* New and interesting penzigioid *Xylaria* species with small, soft stromata. **Mycologia**, v. 104, n. 3, p. 766–776, 2012.

JU, Y. M.; HSIEH, H. M. *Xylaria* species associated with nests of *Odontotermes formosanus* in Taiwan. **Mycologia**, v. 99, n. 6, p. 936–957, 2007.

JU, Y. M.; HSIEH, H. M.; HE, X. S. *Xylaria coprinicola*, a new species that antagonizes cultivation of coprinus comatus in china. **Mycologia**, v. 103, n. 2, p. 424–430, 2011.

JU, Y. M.; ROGERS, J. D.; HSIEH, H. M. New *Hypoxylon* and *Nemania* species from Costa Rica and Taiwan. **Mycologia**, v. 97, n. 2, p. 562–567, 2005.

KATOH, K.; TOH, H. Recent developments in the MAFFT multiple sequence alignment program. **Briefings in Bioinformatics**, v. 9, n. 4, p. 286–298, 2008.

KENERLEY, C. M.; ROGERS, J. D. On *Hypoxylon* serpens in Culture. **Mycologia**, v. 68, n. 3, p. 688, 1976.

KIM, C. S. *et al.* New records of *Xylaria* species in Korea: *X. ripicola* sp. nov. and *X. tentaculata*. **Mycobiology**, v. 44, n. 1, p. 21–28, 2016.

KUHNERT, E. *et al.* New *Hypoxylon* species from Martinique and new evidence on the molecular phylogeny of *Hypoxylon* based on ITS rDNA and β-tubulin data. **Fungal Diversity**, v. 64, n. 1, p. 181–203, 2014.

LIMA, W. G. *et al. Colletotrichum gloeosporioides*, a new causal agent of citrus post-bloom fruit drop. **European Journal of Plant Pathology**, v. 131, n. 1, p. 157–165, 2011.

MARINCOWITZ S., CROUS P. W., GROENEWALD J. Z., WINGFIELD M.J. Microfungi occurring on Proteaceae in the fynbos. **CBS biodiversity series; 7**. Utrecht, the Netherlands: CBS Fungal Biodiversity Centre, 2008.

MILLER, M. A.; PFEIFFER, W.; SCHWARTZ, T. The CIPRES science gateway: A community resource for phylogenetic analyses. **Proceedings of the TeraGrid 2011 Conference: Extreme Digital Discovery, TG'11**, 2011.

MORANDI, M. A. B. *et al.* Controle biológico de fungos fitopatogênicos. **Informe Agropecuário**, v. 30, p. 73-82, 2009.

MOREIRA, V. F. **Rede de Proteção e Conservação da Serra do Amolar: Rompendo fronteiras para a conservação do Pantanal.** Dissertação, Universidade Federal do Mato Grosso do Sul, Corumbá, 2011.

NORILER, S. A. *et al.* Bioprospecting and structure of fungal endophyte communities found in the Brazilian biomes, pantanal, and Cerrado. **Frontiers in Microbiology**, 2018.

O'DONNELL, K.; CIGELNIK, E.. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. **Molecular Phylogenetics and Evolution**, v. 7, n. 1, p. 103–116, 1997.

OERKE, E. C. Centenary Review: Crop losses to pests. **Journal of Agricultural Science**, n. 144, p. 31–43, 2006.

PACCANARO, M. C. *et al.* Synergistic Effect of Different Plant Cell Wall–Degrading Enzymes Is Important for Virulence of *Fusarium graminearum*. **Molecular Plant-Microbe Interactions**, v. 30, n. 11, p. 886–895, 2017.

PAGNUSSATT, F. A. *et al.* Technological and nutritional assessment of dry pasta with oatmeal and the microalga *Spirulina platensis*. **Brazilian Journal of Food Technology**, v. 17, n. 4, p. 296–304, 2014.

PETRINI, O. Taxonomy of Endophytic Fungi of Aerial Plant Tissues. **Microbiology of the Phyllosphere**. Cambridge: Cambridge University Press, 175–187. 1986.

PETRINI, O. et al. Ecology, metabolite production, and substrate utilization in endophytic fungi. **Natural Toxins**, v. 1, n. 3, p. 185–196, 1993.

PINHO, D. B.; LOPES, U. P.; PEREIRA, O. L. *Colletotrichum abscissum*. **Fungal Planet**, v. 34, n. 357, p. 236–237, 2015.

RAEDER, U.; BRODA, P. Rapid preparation of DNA from filamentous fungi. **Letters in Applied Microbiology**, v. 1, n. 1, p. 17–20, 1985.

RANYER, R. W. **A mycological colour chart**. Commonwealth Mycological Institute, Kew, UK. 1970.

RONQUIST, F. *et al.* Mrbayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. **Systematic Biology**, v. 61, n. 3, p. 539–542, 2012.

SÁNCHEZ-BALLESTEROS, J. *et al.* Phylogenetic Study of *Hypoxylon* and Related Genera Based on Ribosomal ITS Sequences. **Mycologia.** v. 92, n. 5, p.

964–977, 2000.

SANTOS, P. J. C. D. *et al. Diaporthe endophytica* and *D. terebinthifolii* from medicinal plants for biological control of *Phyllosticta citricarpa*. **Microbiological Research**, v. 186–187, p. 153–160, 2016.

SAVI, D.; ALUIZIO, R.; GLIENKE, C. Brazilian Plants: An Unexplored Source of Endophytes as Producers of Active Metabolites. **Planta Medica**, 2019.

SAVI, D. C. *et al.* Antitumor, Antioxidant and Antibacterial Activities of Secondary Metabolites Extracted By Endophytic Actinomycetes Isolated From *Vochysia Divergens*. **International Journal of Pharmaceutical, Chemical & Biological Sciences**, v. 5, n. 1, p. 347–356, 2015.

SAVI, D. C. *et al. Phaeophleospora vochysiae* Savi & Glienke sp. nov. Isolated from *Vochysia divergens* Found in the Pantanal, Brazil, Produces Bioactive Secondary Metabolites. **Scientific Reports**, 2018.

SAVI, D. C. *et al.* Dihydroisocoumarins produced by *Diaporthe cf. heveae* LGMF1631 inhibiting citrus pathogens. **Folia Microbiologica**, v. 65, n. 2, p. 381–392, 2020.

SCHULZ, B. *et al.* Endophytic fungi: A source of novel biologically active secondary metabolites. **Mycological Research**, v. 106, n. 9, p. 996–1004, 2002.

SILVA, A. O. *et al.* Identification of *Colletotrichum* species associated with postbloom fruit drop in Brazil through GAPDH sequencing analysis and multiplex PCR. **European Journal of Plant Pathology**, v. 147, n. 4, p. 731–748, 2017.

SMITH, D. *et al.* The Ex Situ Conservation of Microorganisms : Aiming At a Certified Quality Management. **Encyclopedia of Life Support Systems**, n. January, 2008.

STADLER, M. Importance of secondary metabolites in the Xylariaceae as parameters for. **Current Research in Environmental & Applied Mycology**, v. 1, n. 2, p. 75–133, 2011.

STADLER, M. *et al.* The Xylariaceae as model example for a unified nomenclature following the “One Fungus-One Name” (1F1N) concept.

Mycology, v. 4, n. 1, p. 5–21, 2013.

TAMURA, K. *et al.* MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. **Molecular Biology and Evolution**, v. 28, n. 10, p. 2731–2739, 2011.

TAN, M. A. *et al.* Biodiscovery of antibacterial constituents from the endolichenic fungi isolated from *Parmotrema rampoddense*. **3 Biotech**, v. 10, n. 5, p. 1–7, 2020.

TANG, A. M. C.; JEEWON, R.; HYDE, K. D. Phylogenetic relationships of *Nemania plumbea* sp. nov. and related taxa based on ribosomal ITS and RPB2 sequences. **Mycological Research**, v. 111, n. 4, p. 392–402, 2007.

TANG, A. M. C.; LAM, R. Y. C.; LEUNG, M. W. K. *Xylaria hongkongensis* sp. nov. from an urban tree. **Mycotaxon**, v. 128, n. August, p. 37–40, 2014.

THOMPSON, S. M. *et al.* Green and brown bridges between weeds and crops reveal novel *Diaporthe* species in Australia. **Persoonia: Molecular Phylogeny and Evolution of Fungi**, v. 35, n. 1, p. 39–49, 2015.

TIBPROMMA, S. *et al. Anthostomelloides krabiensis* gen. Et sp. Nov. (xylariaceae) from *Pandanus odorifer* (pandanaceae). **Turkish Journal of Botany**, v. 41, n. 1, p. 107–116, 2017.

TONIAL, F. *et al.* Biological activity of *Diaporthe terebinthifolii* extracts against *Phyllosticta citricarpa*. **FEMS Microbiology Letters**, v. 364, n. 5, p. 1–7, 2017.

U'REN, J. M. *et al.* Contributions of North American endophytes to the phylogeny, ecology, and taxonomy of Xylariaceae (Sordariomycetes, Ascomycota). **Molecular Phylogenetics and Evolution**, v. 98, p. 210–232, 2016.

UDAYANGA, D. *et al.* Species limits in *Diaporthe*: molecular re-assessment of *D. citri, D. cytosporella, D. foeniculina and D. rudis*. **Persoonia**, v. 32, p. 83–101, 2014.

UDAYANGA, D. *et al.* Multi-locus phylogeny reveals three new species of Diaporthe from Thailand. **Cryptogamie, Mycologie**, v. 33, n. 3, p. 295–309, 2012.

UDAYANGA, D. *et al.* The genus *Phomopsis*: Biology, applications, species concepts and names of common phytopathogens. **Fungal Diversity**, v. 50, n. September, p. 189–225, 2011.

VU, D. *et al.* Large-scale generation and analysis of filamentous fungal DNA barcodes boosts coverage for kingdom fungi and reveals thresholds for fungal species and higher taxon delimitation. **Studies in Mycology**, v. 92, n. October, p. 135–154, 2019.

WENDT, L. *et al.* **Resurrection and emendation of the Hypoxylaceae, recognised from a multigene phylogeny of the Xylariales**. 2018.

WHITE, T.J. *et al.* **Amplification and Direct Sequencing of Fungal Risosomal RNA Genes for Phylogenetics**: Academic Press, Inc., 1990. ISSN 08953988.v. 2

WULANDARI, N. F. *et al. Phyllosticta citriasiana* sp. nov., the cause of Citrus tan spot of *Citrus maxima* in Asia. **Fungal Diversity**, v. 34, n. January, p. 23–39, 2009.

APÊNDICE 1
Supplementary Material

Diversity of endophytic fungi of Serra do Amolar-Pantanal in Brazil and their potential to produce secondary metabolites with antifungal activity against phytopathogens

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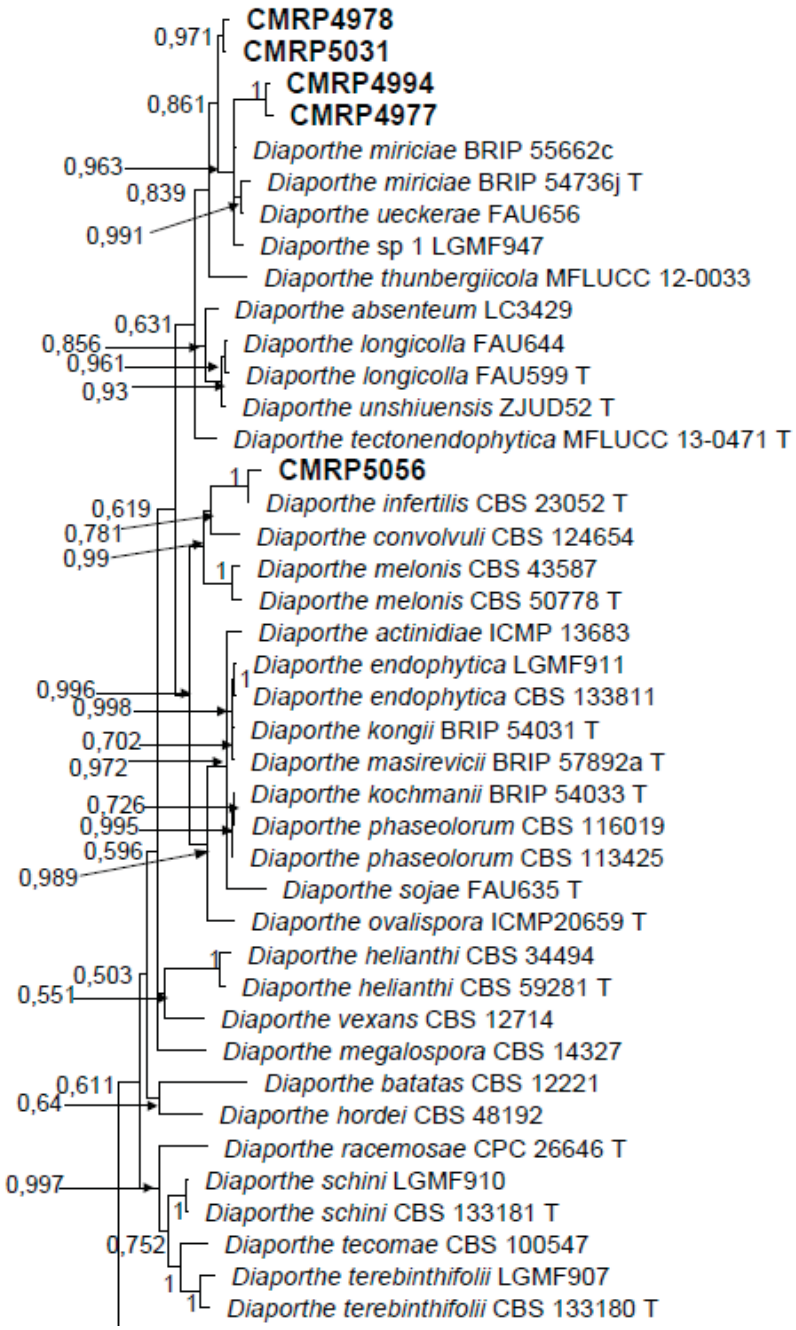
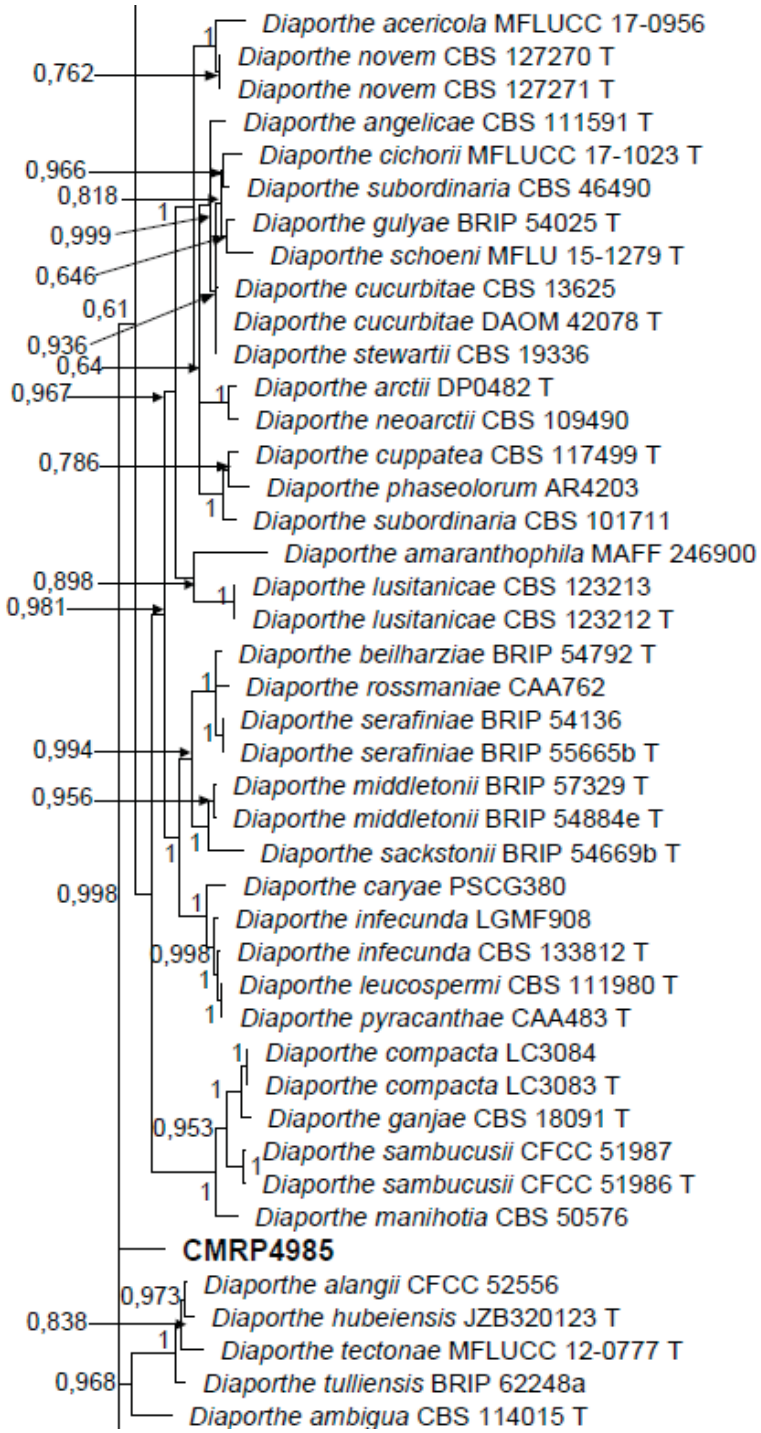


Figure S1: Bayesian Inference phylogenetic tree of *Diaporthe* species based on alignment of *tef1* partial sequence. The data matrix had 314 taxa and 1033 characters. The species *Diaporthella corylina* (CBS 121124) was used as outgroup. Strains marked with a “T” correspond to type sequences. Bayesian posterior probabilities equal or greater than 0.50 are presented next to each node. The scale bar of 0.2 represents the number of changes. The sequence of the isolate here studied are presented with its isolation code (CMRP4997; CMRP5029; CMRP5033; CMRP4985; CMRP5056; CMRP4977; CMRP4994; CMRP5031; CMRP4978) highlighted in bold.



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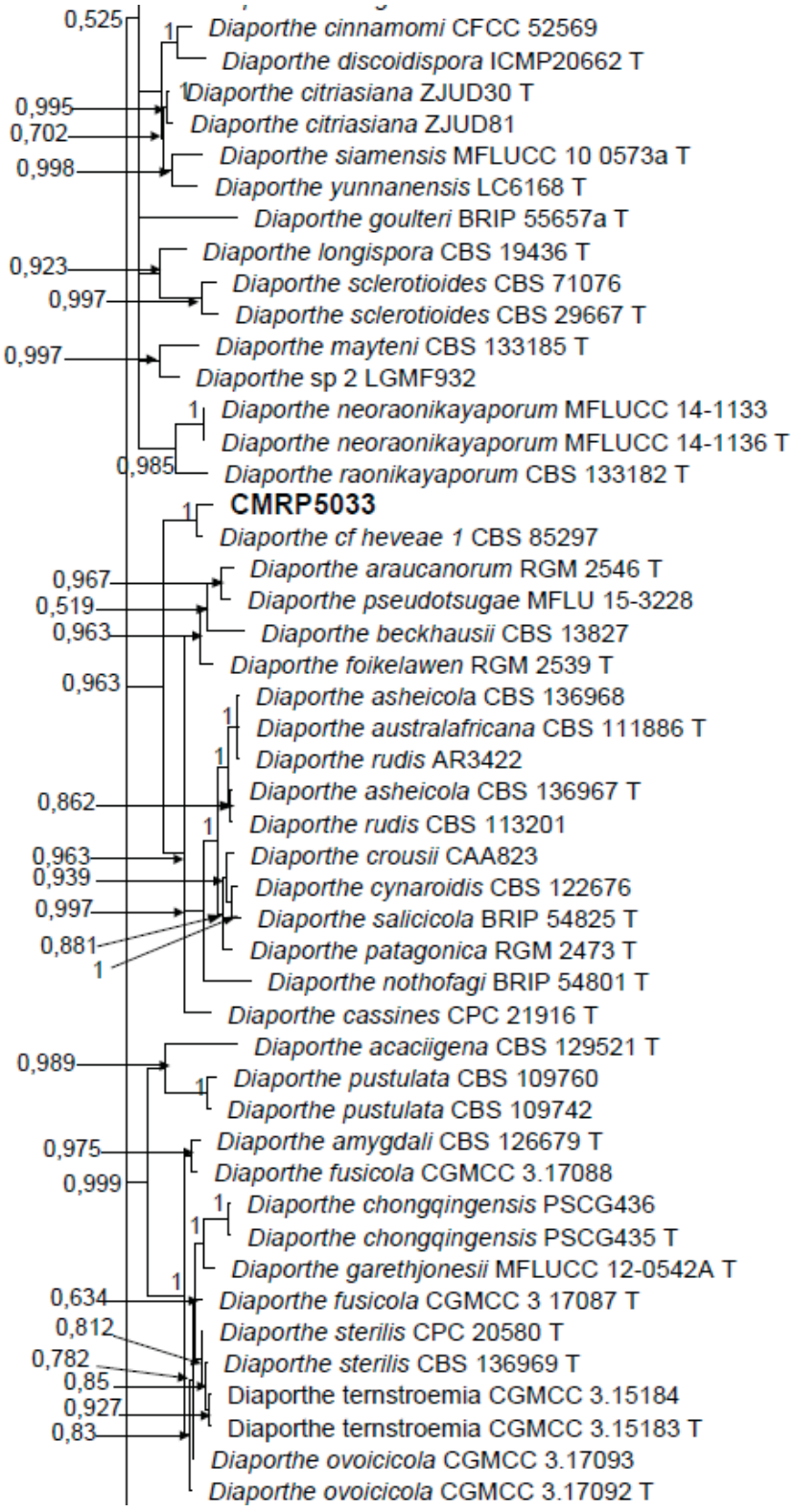

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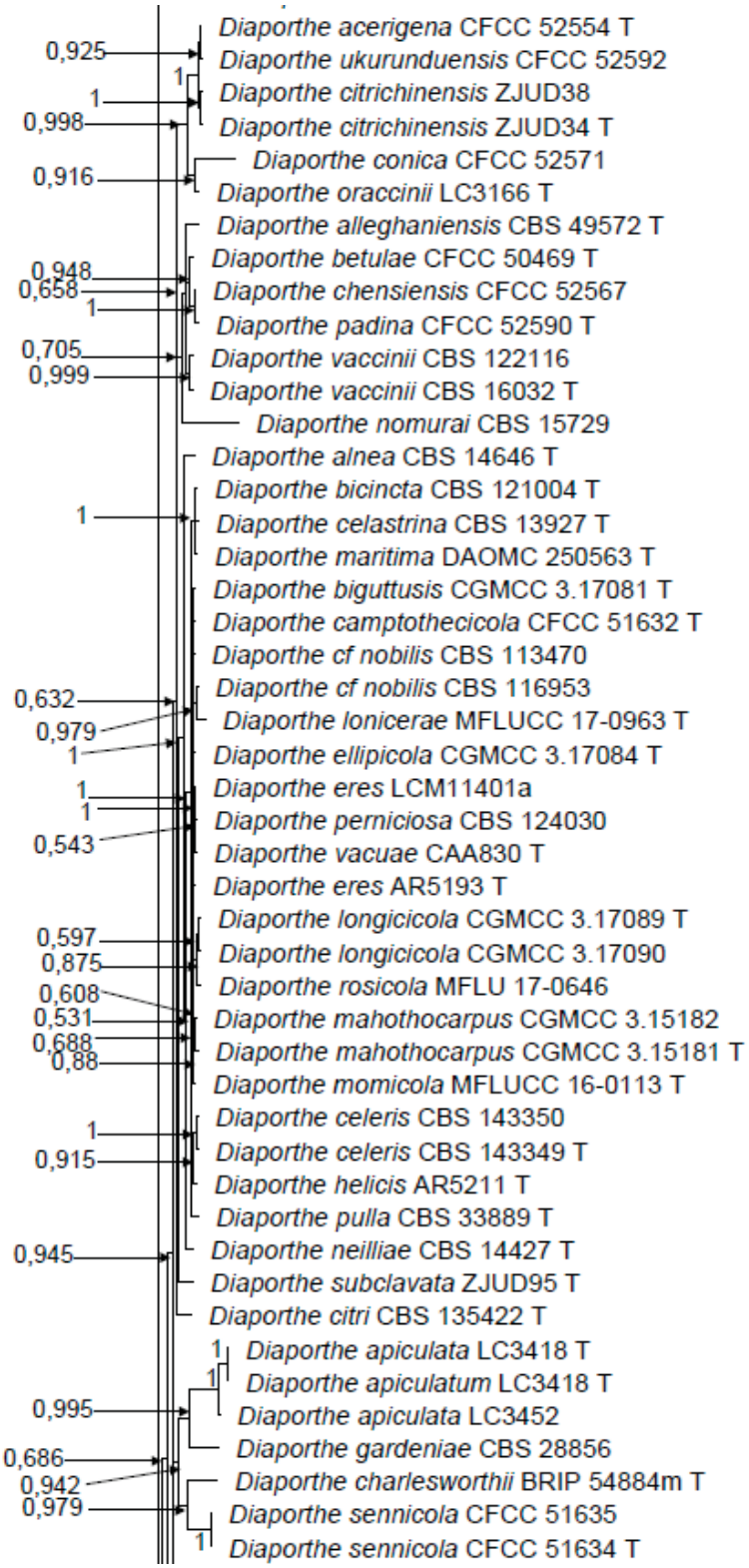

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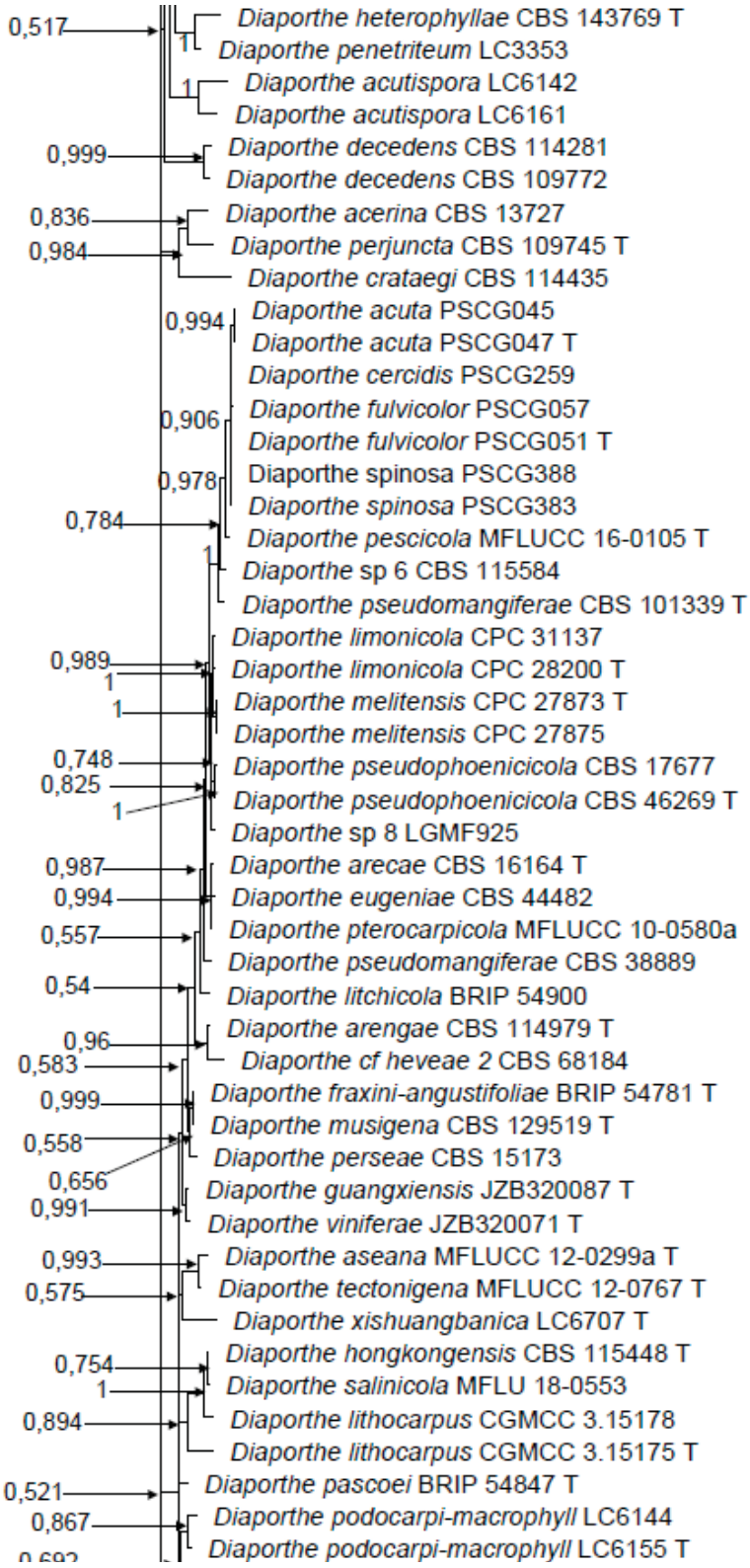

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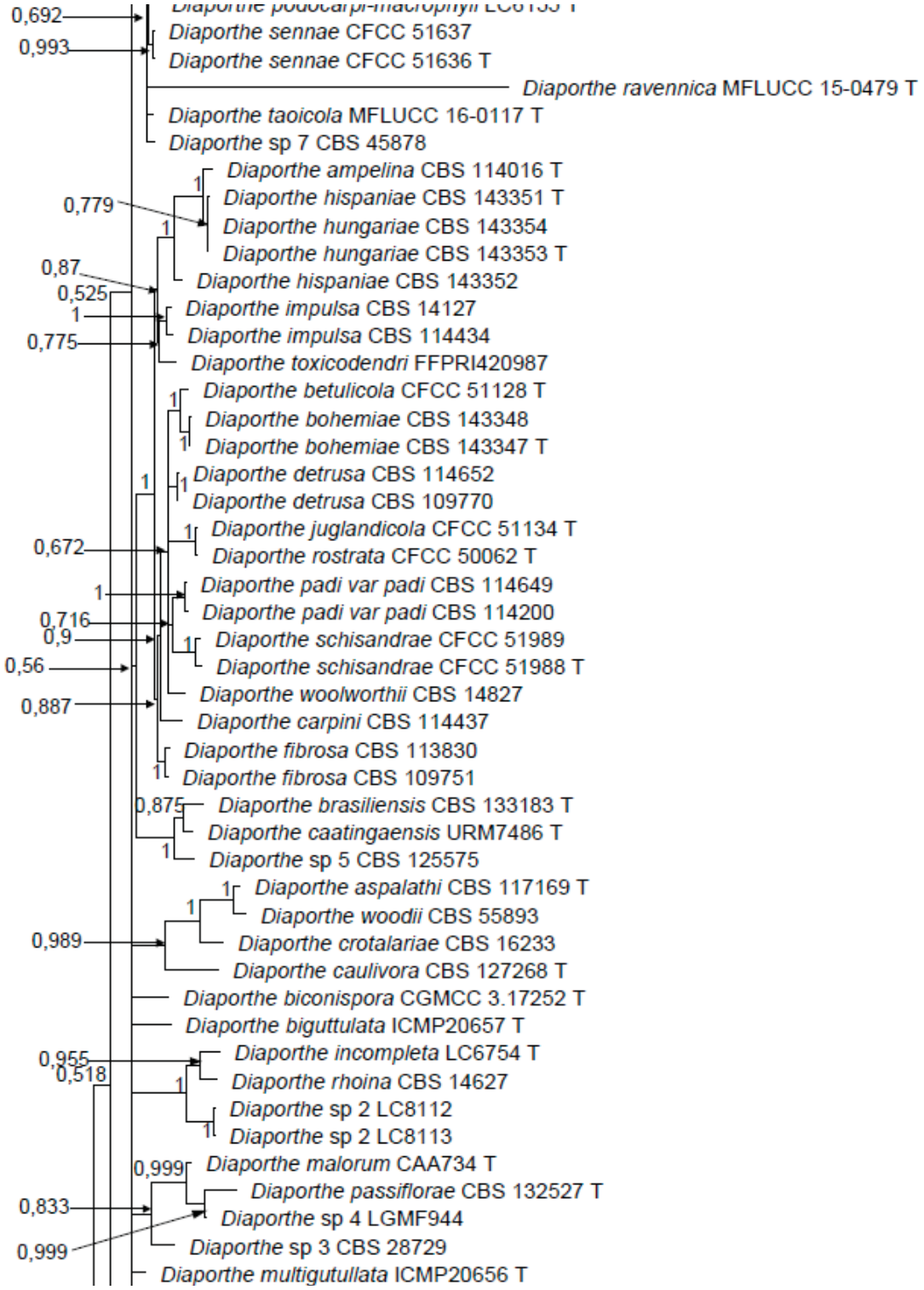

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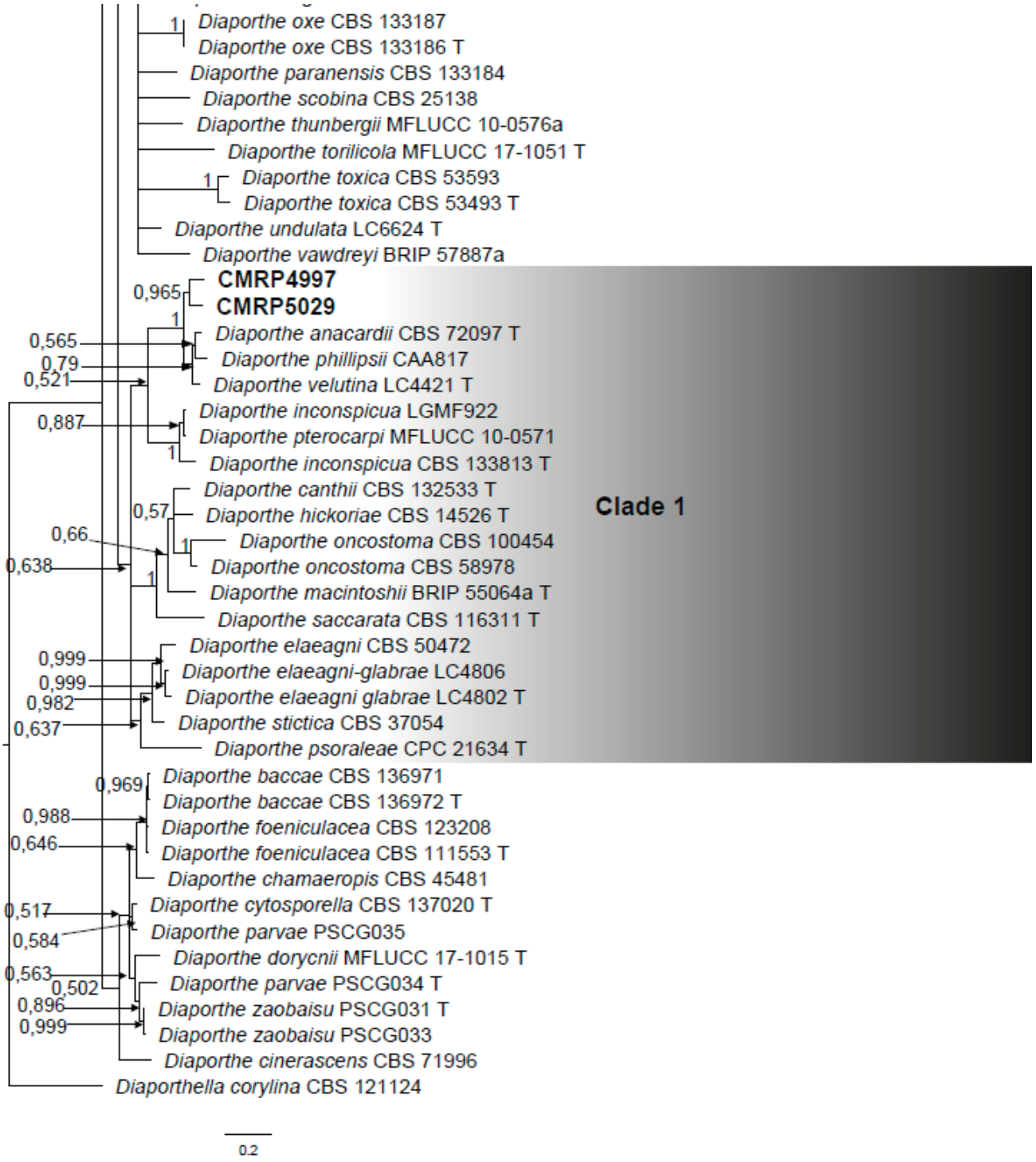

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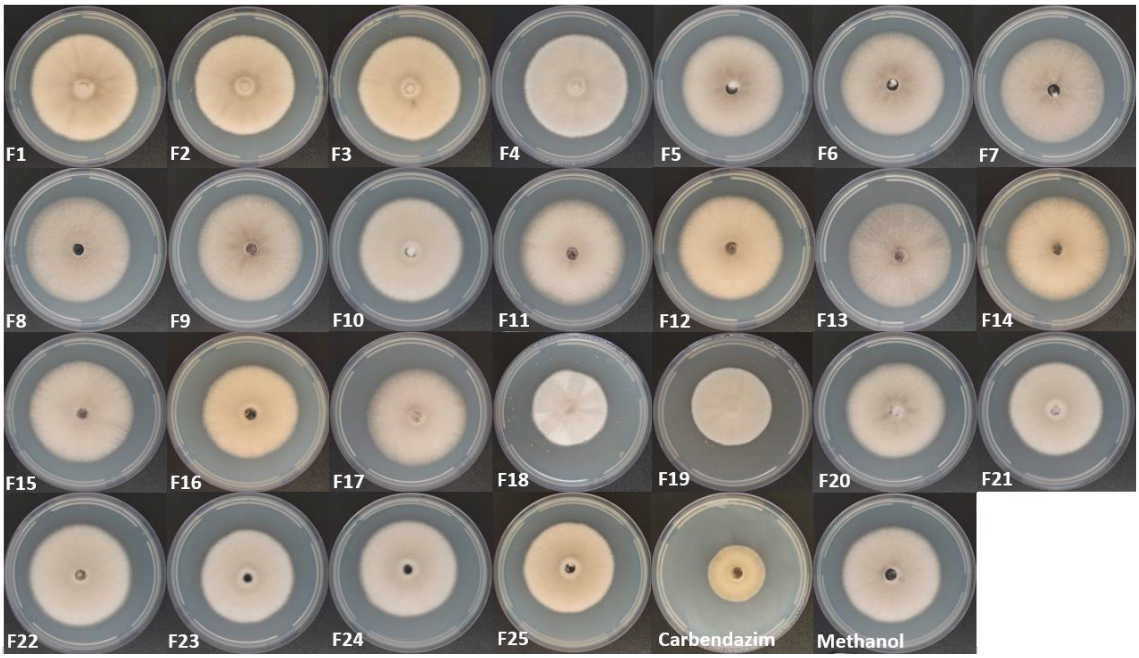

FIGURE S2: MEAN OF THE MYCELIAL GROWTH INHIBITION RATE OF PHYTOPATHOGEN *Colletotrichum abscissum* AFTER EXPOSURE TO EXTRACT FRACTIONS PRODUCED BY THE CMRP4997 STRAIN. Mycelial growth inhibition test plates of each tested fractions and both controls (Carbendazim and Methanol).

Table S1- Sample collection sites

Plant	**Coordinates**
Plant 1	18º29'54.7"S 57º27'16.5"W
Plant 2	18°29'54.5"S 57°27'20.0"W
Plant 3	18°30'22.2"S 57°27'56.1"W
Plant 4	18°29'06.6"S 57°27'05.6"W
Plant 5	18°28'59.3"S 57°27'13.5"W
Plant 6	18º28'10.5"S 57º22'10.2"W
Plant 7	18°15'37.8"S 57°27'37.4"W
Plant 8	18°15'39.4"S 57°27'35.6"W
Plant 9	18°15'45.1"S 57°26'11.3"W
Plant 10	18°15'42.3"S 57°27'29.9"W
Plant 11	18°15'25.6"S 57°27'42.0"W
Plant 11.2	18°15'29.7"S 57°27'33.6"W
Plant 12	18°13'00.0"S 57°22'32.0"W
Plant 13	18°22'55.8"S 57°20'55.8"W
Plant 14	18°22'55.8"S 57°20'55.8"W
Plant 15	18°15'35.5"S 57°27'28.7"W
Plant 16	18°15'36.3"S 57°27'28.3"W
Plant 17	18°10'04.5"S 57°23'01.0"W

CAPÍTULO 2

Endophytic fungi from Serra do Amolar-Pantanal produce secondary bioactive metabolites against multidrug-resistant bacteria

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ABSTRACT

The search for new compounds to control multidrug-resistant bacteria is increasingly necessary due to the constant appearance of resistance. In view of the ability that endophytic microorganisms have to produce active metabolites, they represent potential sources of new compounds for pharmaceutical use. In 2019, our research group isolated 293 endophytic fungi from the medicinal plant *Vochysia divergens* from Serra do Amolar-Pantanal in Brazil, and found great diversity of endophytes, some of which produced extracts with activity against phytopathogenic fungi. Considering these results, the present study aimed to bioprospect this collection of endophytic fungi in the search for secondary metabolites with antimicrobial activity against multidrug-resistant clinical pathogenic bacteria. The isolates that showed promising results were identified by DNA sequencing analysis. From these, a screening test resulted in 19 extracts showing a minimum inhibitory concentration (MIC) below 100 µg/ml against Methicillin-resistant *Staphylococcus aureus* (MRSA) and one against *Acinetobacter baumanii*. These extracts were selected and evaluated against the clinical pathogens *Klebsiella pneumoniae* producing Carbapenemase (KPC), *Pseudomonas aeruginosa* and Vancomycin-resistant *Enterococcus* sp. (VRE). Of these, 3 extracts showed MIC less than 100 µg/ml against VRE. Among the extracts evaluated against all bacteria, the five that presented the lowest MIC and MBC were produced from isolates identified as *Neopestalotiopsis egyptiaca*, *Diaporthe vochysiae*, *Neopestalotiopsis* sp., *Cladosporium* sp. *cladosporioides* complex and *Paecilomyces* sp. In conclusion, this study demonstrated that

endophytic microorganisms represent an important source of secondary metabolites with biotechnological potential for the development of new antimicrobials to control multi-resistant bacteria. In addition, we isolated many endophytic species from the medicinal plant *V. divergens* from Serra do Amolar. These strains are preserved in the CMRP Cultures collection of the Federal University of Paraná and can be used in future studies.

Keywords: Endophytes, secondary metabolites, *Vochysia divergens*, Pantanal, antibacterial activity

INTRODUCTION

The increasing development of antimicrobial resistance in pathogenic bacteria is one of the most urgent problems faced by health care services worldwide. Factors such as indiscriminate prescription and use of antibiotics, as well as failures and delay in diagnostic of infections contribute to the current cases of antimicrobial resistance (COSTELLOE et al., 2010; DOS SANTOS et al., 2015). In 2017, the World Health Organization (WHO, 2017) published a list showing some of the pathogenic bacteria resistant to antibiotics, which represent a major threat to public health worldwide and should be considered a priority for the search and development of new drugs. The list presents 12 bacteria or families of bacteria, divided into groups according to the urgency of developing new antibiotics. Among the bacteria listed as high priority are *Enterococcocus faecium* Vancomycin-resistant and *Staphylococcus aureus* Methicillin and Vancomycin-resistant, and in critical priority the bacteria *Acinetobacter baumanii* carbapenem-resistant, *Pseudomonas aeruginosa* Carbapenem-resistant and *Enterobacteriaceae* Carbapenem-resistant and ESBL-producing.

Nature represents a great source of compounds still little explored. Plants, especially those known for their medicinal properties, play an important role in the search for bioactive compounds, as they are not only sources of natural compounds with biological activity, but also are repositories of endophytic microorganisms. The isolation and purification of plant compounds in adequate yield for use become problematic considering the environmental conservation strategies (DOS SANTOS et al., 2015; SAVI et al., 2019; YU et al., 2010). Therefore, the exploitation of microorganisms capable of producing bioactive compounds for pharmaceutical use becomes more promising.

Endophytic microorganisms are known for having the ability to colonize during all or part of their life cycle internal plant tissues without causing symptoms

to the host plants, this association being classified as symbiotic (PETRINI et al., 1993; ARAÚJO et al., 2014). Inside the host, the endophyte is able to provide nutrients, assist in obtaining essential molecules from the environment, and also promote the defense of the host plant against phytopathogenic organisms by producing bioactive secondary metabolites (VENIERAKI et al., 2017). Due to the ability to produce metabolites with biological activity and considering the enormous diversity of species estimated to exist and yet to be explored, endophytes can be great sources of study in the discovery of new compounds for pharmaceutical use (GLIENKE et al., 2012; STROBEL; DAISY, 2003; YU et al., 2010).

In 2019, our research group isolated 293 endophytic fungi of the medicinal plant *Vochysia divergens* from Serra do Amolar-Pantanal in Brazil, and found great diversity of species, some of which produced extracts with activity against phytopathogenic fungi (MAYRHOFER et al, 2021). In the present study we aim at bioprospecting this collection of endophytic fungi to search for secondary metabolites with antimicrobial activity against clinical multidrug-resistant pathogenic bacteria.

MATERIAL AND METHODS

Organisms

The 91 endophytic fungi used in this study were previously obtained from leaves and petioles of 18 specimens of the medicinal plant *Vochysia divergens* located in Serra do Amolar, Pantanal region, in Brazil (MAYRHOFER et al, 2021). These isolates were identified at the genus level by sequencing the ITS region of rDNA (Table S1) and are preserved in the Microbiological Collections of Paraná Network (CMRP) (https://www.cmrp-taxonline.com), at the Federal University of Paraná, Brazil.

Evaluation of the biological activity of secondary metabolites

Extracts production from endophytic fungi

Crude extracts were obtained from the 91 fungal strains in this study through fermentation in liquid medium, following the conditions proposed by MAYRHOFER et al. (2021).

Biological activity of extracts against clinical bacteria

Initially, a screening test was performed to evaluate the antibacterial activity of the extracts against two clinical resistant bacteria, a Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Acinetobacter baumanii*. The activity was measured using the Minimal Inhibitory Concentration (MIC) assay in 96-well micro dilution plates, each well containing 100 µL of Muller-Hinton broth. A 10 µL of bacterial suspension (previously activated) was prepared at a concentration of 0.5 on the McFarland scale and 50 µL of each crude extract diluted in methanol (10 mg/ml) in serial dilutions were both also added. The controls consisted in wells inoculated with only the culture medium, with the culture medium plus the bacteria suspension and with only the solvent used in the extracts dilution (methanol), serving as sterility control, positive control and negative control, respectively. The antibiotics Oxacilin and Vancomycin for gram positive bacteria and Gentamicin and Streptomycin for gram negative bacteria were also used as controls. The plates were incubated at 37ºC for 24h and after this period bacterial growth was observed. MIC is characterized by the lowest concentration of the extract capable of inhibiting bacterial growth, which is represented by the first well where no turbidity of the medium is observed (DOS SANTOS *et al.*, 2015).

From the screening test with all 91 extracts, those with MIC values below 100 µg/ml for one or both tested bacteria were selected. The 19 selected extracts were tested again using the MIC methodology against *Klebsiella pneumoniae* producing Carbapenemase (KPC), *Pseudomonas aeruginosa* and Vancomycin-resistant *Enterococcus* sp. (VRE). In addition to the MIC, the selected extracts were also evaluated for their Minimal Bactericidal Concentration (MBC). The assay was carried out after obtaining the minimal inhibitory concentration, 50 µL of the content of the wells where growth has not been observed were inoculated in a Mueller-Hinton plate. The plates were incubated at 37ºC for 24h and after this period, bacterial growth was observed. The plate corresponding to the lowest concentration of the extract that does not show bacterial growth indicates MBC, that is, such concentration of the extract was able to inhibit not only bacterial growth, but also eliminate it completely (DOS SANTOS *et al.*, 2015).

Molecular identification

The 19 strains providing the extracts with better results in the screening test against the pathogenic bacteria were used for identification at the species level, when possible. The amplification and sequencing of partial regions of four loci were carried out according to the needs for each genus and species to be identified by phylogenetic analysis. Partial sequences of beta-tubulin gene (*tub2*) were amplified with primers Bt2a and Bt2b (GLASS; DONALDSON, 1995), the portion of translation elongation factor 1-alpha (*tef1*) gene was amplified with the primers EF1-728F and EF1-986R (CARBONE; KOHN, 1999), the partial histone H3 (*his3*) gene was amplified with the primers CYLH3F (CROUS et al., 2004) and H3-1b (GLASS; DONALDSON, 1995) and for the portion of the actin (*act*) gene the primers ACT-512F e ACT-786R (CARBONE; KOHN, 1999) were used.

The PCR reactions were performed for a final volume of 12.5 µL (1X reaction buffer, 0,2 µM of forward primer, 0,2 µM of reverse primer, 1,5 mM of M_gCl_2, 0,2 mM of dNTPs, 0,05 U/Ul of Taq Polymerase). For the *Lasiodiplodia* genus, the PCR conditions for *tef1* were the same as described as Ismail et al. (2012) and for *tub2* were as in Slippers et al. (2013). For *Colletotrichum* the PCR conditions for *act* gene were carried out according to Weir et al. (2012). The PCR conditions varied for the *Diaporthe* genus, according to the amplified gene, for *tef1* the conditions used were the same as described by Gomes et al. (2013). For the *tub2* gene, the conditions were: initial step of 5 min at 94ºC, followed by 40 cycles of 30 sec at 95ºC, 50 sec at 58ºC and 1 min at 72ºC, with a final extension of 5 min at 72ºC, and for the *his3* gene the conditions were 5 min at 95ºC, 35 cycles of 30 sec at 95ºC, 30 sec at 55ºC and 2 min at 72ºC, with a final extension of 5 min at 72ºC. The PCR products were purified using *Exo1* and *FastAP* enzymes (GE Healthcare, USA) and for the sequencing reaction the BigDye® Terminator Kit v3.1 was used. The products of the reactions were purified by the Sephadex G50 polymer, and the sequencing was read in an automatic sequencer ABI3500® (Applied Biosystems, Foster City, CA, USA).

The chromatograms obtained were inspected in MEGA 6.06 (TAMURA et al., 2011) and BioEdit (HALL, 1999). The sequences were compared with those available in the NCBI/GenBank database (National Center for Biotechnology Information - http://www.ncbi.nlm.nih.gov/BLAST/) using the BLAST Tool and compared with type strains obtained in MycoBank (http://www.mycobank.org/) and Westerdijk Fungal Biodiversity Institute (http://www.westerdijkinstitute.nl/) databases (list of taxa used in the phylogenetic analysis is in the supplementary material). Phylogenetic analyses were performed with type or authentic strains and those sequences generated by this study. The alignments of the DNA sequences were made using the Maftt software (KATOH; TOH, 2008; https://mafft.cbrc.jp/alignment/server/) and verified manually in MEGA 6.06 software. Phylogeny was performed by Bayesian Inference analysis using MrBayes v3.2.6 x86 (RONQUIST et al., 2012) via CIPRES Science Gateway (MILLER et al., 2011). This analysis was performed using two parallel runs with one cold and three heated chains each, using the number of generations needed to reach split frequencies of ≤0.01 and a sampling frequency set to every 100 generations. The posterior probability values were calculated after discarding the first 25% of the generated trees as burn-in. Resulting trees were plotted in FigTree v.1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/). The substitution modes were selected for each gene using JModelTest (DARRIBA et al., 2012).

RESULTS

Endophytic fungi produce secondary metabolites with activity against resistant gram-positive and gram-negative clinical bacteria

Among 91 extracts evaluated in the screening test, 19 presented MIC values below 100 µg/ml against methicillin-resistant *S. aureus* (MRSA) and one of them also against *A. baumanii* (table 1, highlighted in black). These extracts were also evaluated against the clinical pathogens *Klebsiella pneumoniae* producing Carbapenemase (KPC), *Pseudomonas aeruginosa* and vancomycin-resistant *Enterococcus* sp. (VRE) and used in assays to determine MBC against the 5 pathogens (MRSA, VRE, *A. baumanii*, *P. aeruginosa* and KPC) (table 2). All of these 19 endophytes were then identified by multilocus phylogenetic analysis (Table 2; figures 1 – 5, S2-S11).

Among the 19 extracts selected in the screening test, 5 extracts produced by endophytes (Table 2, highlighted in black) stood out for presenting high activity against at least, one pathogen: *Neopestalotiopsis egyptiaca* CMRP4981 against MRSA (MIC: 13 µg/ml; MBC: 40 µg/ml), VRE (MIC: 4.3 µg/ml; MBC: 13 µg/ml) and KPC (MIC and MBC: 1,100 µg/ml), *Diaporthe vochysiae* CMRP4978 against MRSA (MIC: 13 µg/ml), VRE (MIC: 13 µg/ml) and *A. baumanii* (MIC: 40 µg/ml), *Neopestalotiopsis* sp. CMRP5000 against MRSA (MIC: 13 µg/ml) and VRE (MIC: 13 µg/ml), *Cladosporium* sp. *cladosporioides* complex CMRP5016 against MRSA (MIC: 13 µg/ml) and *Paecilomyces* sp. CMRP5047 against MRSA (MIC: 13 µg/ml). These results are better explained below.

Diaporthe vochysiae (CMRP4978)

The extract produced by this endophyte showed a low minimum inhibitory concentration (MIC: 13 µg/ml) against gram-positive bacteria methicillin-resistant *S. aureus* and *Enterococcus* sp. (MIC: 13 µg/ml) and presented the lowest MIC among all extracts evaluated against the important gram-negative bacteria *A. baumanii* (MIC: 40 µg/ml). Given the importance of these results, this strain was identified at the species level, based on the partial sequence of *tef1* with 1033 pb and using 314 taxa corresponding to all the type or representative strains available. The Bayesian Inference analysis showed the CMRP4978 strain in clade 1 (figure S1) composed of 11 species of *Diaporthe*. Then, a multilocus analysis was carried out, comprising 1707 pb of *tef1*, *tub2* and *his3* partial sequences with the 11 species contained in clade 1. The Bayesian Inference analysis showed CMRP4987 strain clustered (supported by 0.999 probability) with the type strain of the species *D. vochysiae* (LGMF1583) and other three isolates obtained in the present study (CMRP4977, CMRP5031 and CMRP4994) that showed no activity against the pathogens tested. Then, these strains were identified as being the species *D. vochysiae* (figure 1).

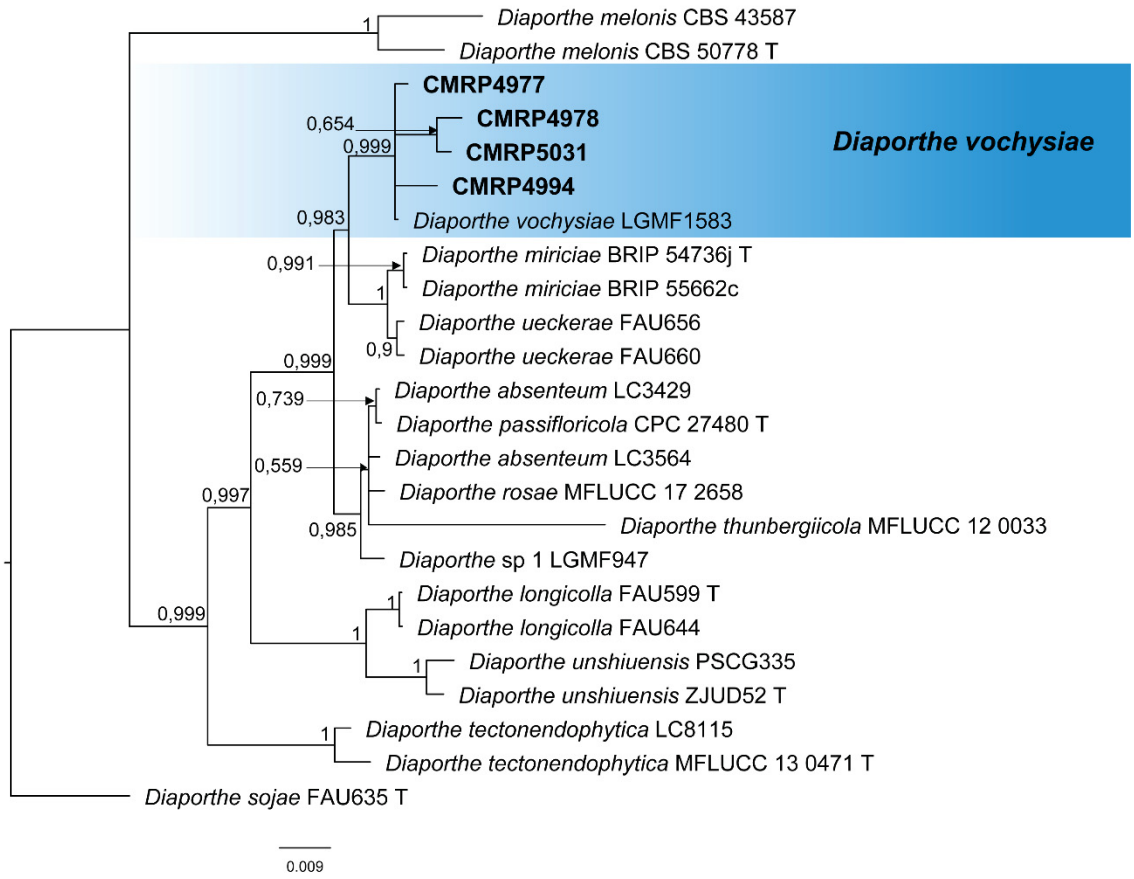

Figure 1: Bayesian Inference phylogenetic tree of *Diaporthe* species of the clade 1 (supplementary material) based on multiple alignment of *tef1*, *tub2* and *his3* partial sequences. The data matrix had 24 taxa and 1708 characters. The species *Diaporthe sojae* (FAU 635) was used as outgroup. Strains marked with a "T" correspond to type strains. Bayesian posterior probabilities equal or greater than 0.50 are presented next to each node. The scale bar of 0.009 represents the number of changes. The sequence of the isolates here studied are presented with its culture collection code (CMRP4977; CMRP4978; CMRP5031; CMRP4994) highlighted in bold.

Neopestalotiopsis egyptiaca (CMRP4981)

The extract produced by this strain showed the lowest values of inhibitory and bactericidal concentration against the gram-positive bacteria MRSA (MIC: 13 µg/ml; MBC: 40 µg/ml) and VRE (MIC: 4.3 µg/ml; MBC: 13 µg/ml). Although this extract shows only moderate activity against the producing carbapenemase gram-negative bacteria *Klebsiella pneumoniae* (MIC: 1,100 µg/ml; MBC: 1,100 µg/ml), it was the best found in the present study against this important pathogen. This extract also presented moderate activity against another important gram-negative bacteria *A. baumanii* (MIC: 122 µg/ml; MBC: 1100 µg/ml).

In view of the importance of these results, CMRP4981 strain was identified at the species level by multilocus analysis that comprised 2150bp of the partial sequences of the *tef1* and *tub2* genes of all type or representative strains with available sequences. The Bayesian Inference analysis (figure 2) showed the CMRP4981 strain in the same branch (0.883 posterior probability) where the type

strain of the species *N. egyptiaca* (COAD2167) is found. Thus, the CMRP4981 was identified as *N. egyptiaca*.

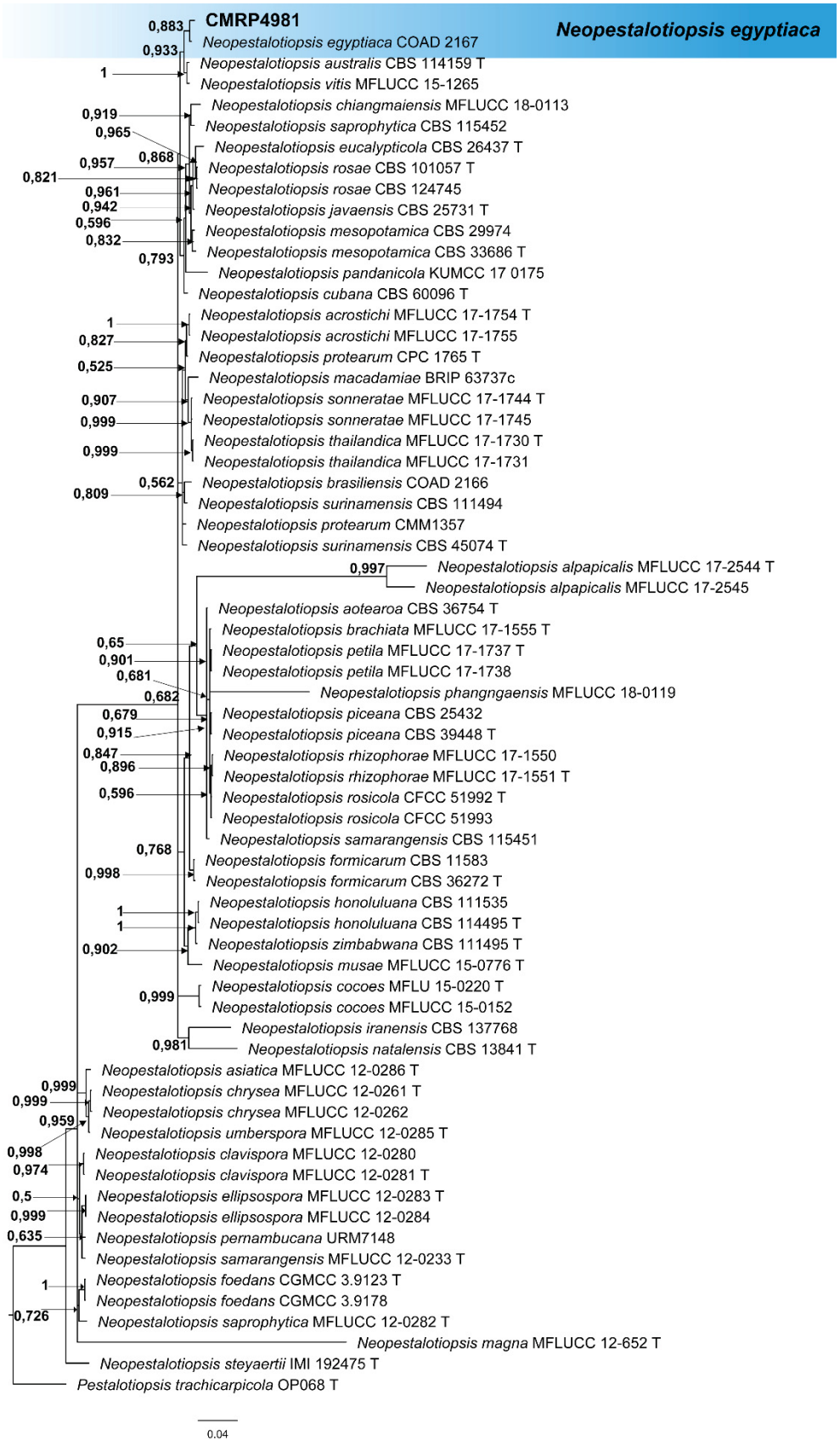

Figure 2: Bayesian Inference phylogenetic tree of *Neopestalotiopsis* species based on multiple alignment of *tef1* and *tub2* partial sequences. The data matrix had 66 taxa and 2150 characters. The species *Pestalotiopsis trachicarpicola* (OP068) was used as outgroup. Strains marked with a "T" correspond to type sequences. Bayesian posterior probabilities equal or greater than 0.50 are presented next to each node. The scale bar of 0.04 represents the number of changes. The sequence of the isolate here studied is presented with its culture collection code (CMRP4981) highlighted in bold.

***Neopestalotiopsis* sp (CMRP5000)**

Another species of the genus *Neopestalotiopsis* produced an extract showing low MIC (13 µg/ml) against the gram-positive bacteria MRSA and VRE. However, as *tef1* sequencing has so far failed, this strain has been identified as *Neopestalotiopsis* sp. (figure 3). Therefore, species level identification is still in progress.

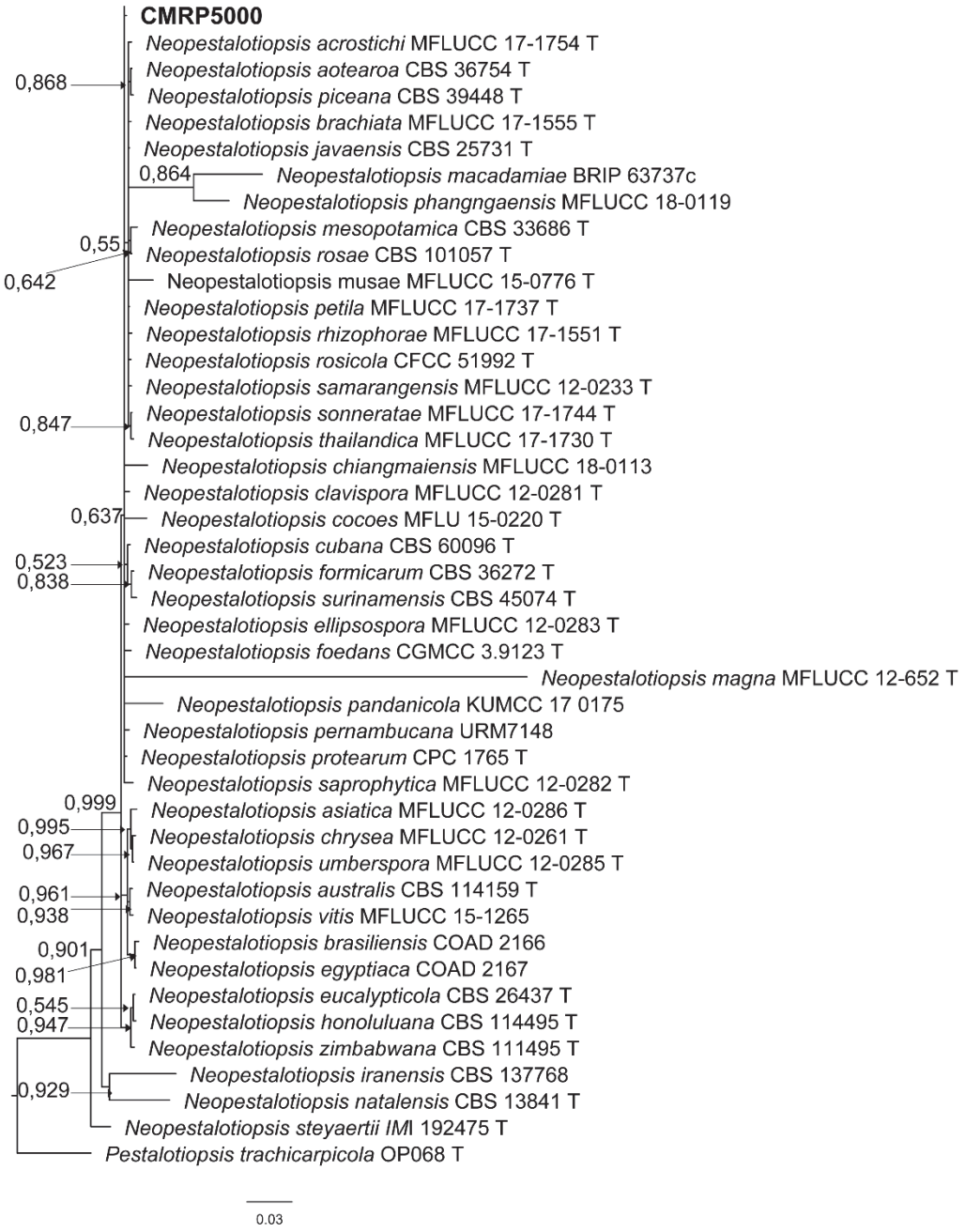

Figure 3: Bayesian Inference phylogenetic tree of *Neopestalotiopsis* species based on multiple alignment of ITS and *tef1* partial sequences. The data matrix had 45 taxa and 1800 characters. The species *Pestalotiopsis trachicarpicola* (OP068) was used as outgroup. Strains marked with a "T" correspond to type sequences. Bayesian posterior probabilities equal or greater than 0.50 are presented next to each node. The scale bar of 0.03 represents the number of changes. The sequence of the isolate here studied is presented with its culture collection code (CMRP5000) highlighted in bold.

***Cladosporium* sp. nov. (*cladosporioides* complex) (CMRP5016)**

The extract produced by this strain presented MIC of 13 µg/ml and MBC of 122 µg/ml against the gram-positive bacteria MRSA.

To try to identify this isolate at the species level, a multilocus analysis was carried out, comprising 2019 pb of the partial sequences of ITS, *tef1* and *act* of

all type or representative strains available. The Bayesian Inference analysis (figure 4) showed that the CMRP5016 strain belongs to the *Cladosporium cladosporioides* species complex, in a single branch (0.961 posterior probability) with another strain (CMRP5226) of this present study (figure 4). As these two strains were not clustered with other species of the *Cladosporium cladosporioides* complex, we suggest them as a new species. However, as these strains did not produce reproductive structures, they were called *Cladosporium* sp. nov. *cladosporioides* complex.

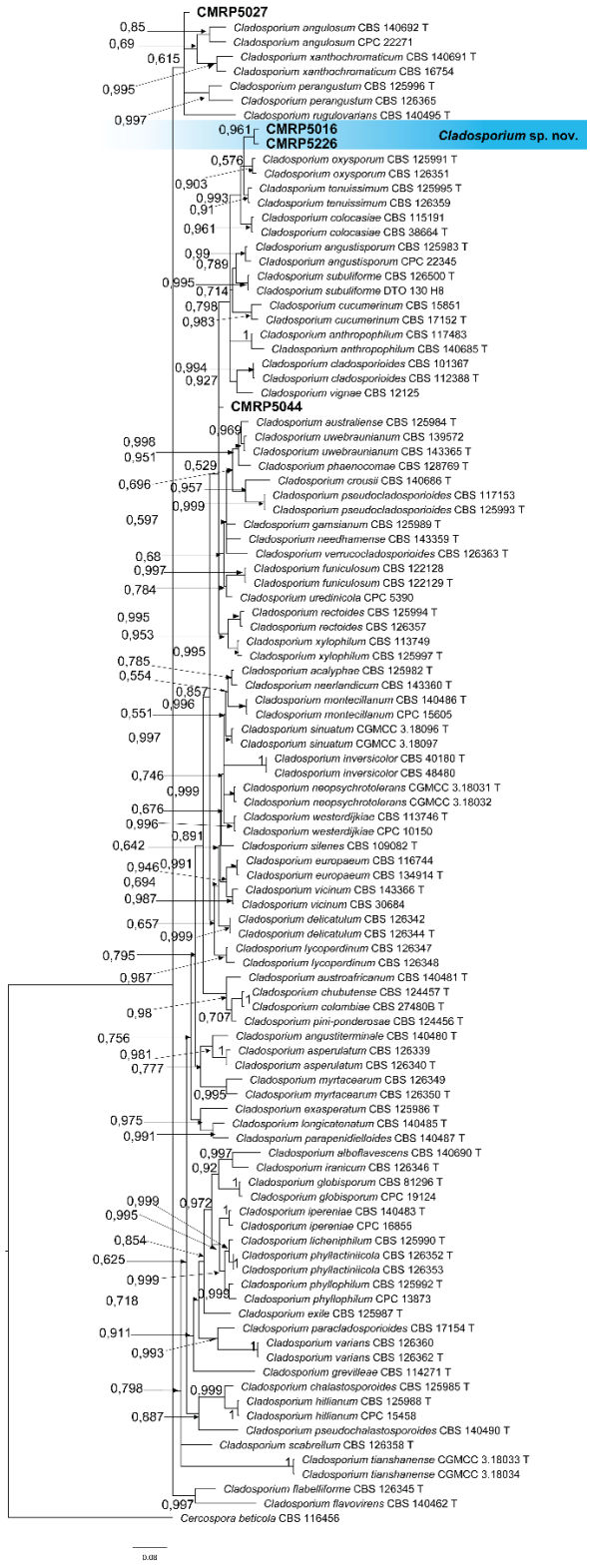

Figure 4: Bayesian Inference phylogenetic tree of *Cladosporium cladosporioides* species complex based on multiple alignment of ITS, *tef1*, and *act* partial sequences. The data matrix had 104 taxa and 2019 characters. The species *Cercospora beticola* (CBS 116456) was used as outgroup. Strains marked with a "T" correspond to type sequences. Bayesian posterior probabilities equal or greater than 0.50 are presented next to each node. The scale bar of 0.08 represents the number of changes. The sequence of the isolates here studied are presented with its culture collection code (CMRP5027; CMRP5016; CMRP5226; CMRP5044) highlighted in bold.

***Paecilomyces* sp. (CMRP5047)**

The extract produced from this strain also presented activity against the gram-positive bacteria MRSA (MIC of 13 µg/ml; MBC of 122 µg/ml).

The isolate of the genus *Paecilomyces* was identified at the genus level by a multilocus analysis that comprised 1604 pb of the partial sequences of ITS and *tub2* genes of all type or representative strains with available sequences. The Bayesian Inference analysis (figure 5) showed that the CMRP5047 strain was grouped with the species *P. subglobosus* and *P. variotii*, and therefore, a single species has not yet been assigned, and we called it *Paecilomyces* sp.

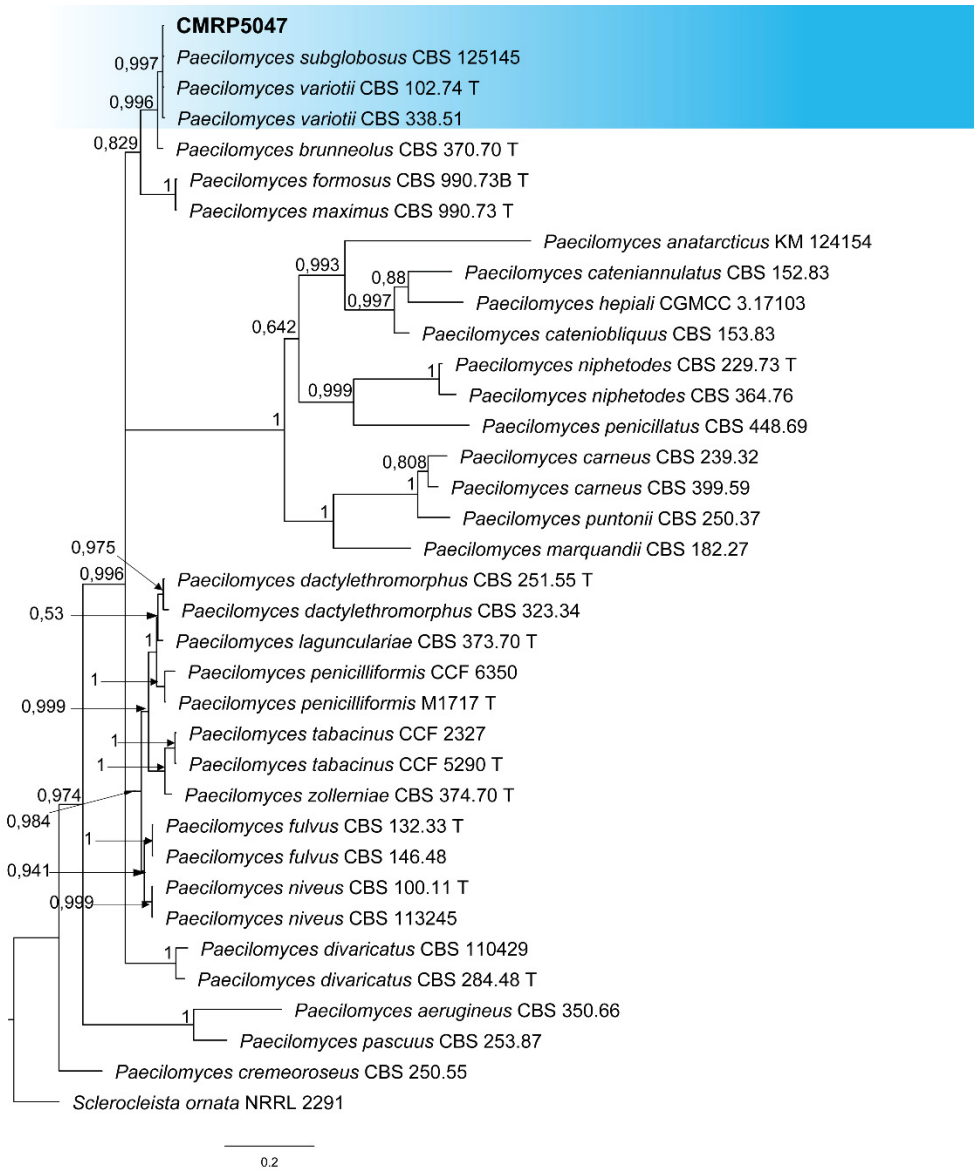

Figure 5: Bayesian Inference phylogenetic tree of *Paecilomyces* species based on multiple alignment of ITS, and *tub2* partial sequences. The data matrix had 33 taxa and 1604 characters. The species *Sclerocleista ornata* (NRRL 2291) was used as outgroup. Strains marked with a "T" correspond to type sequences. Bayesian posterior probabilities equal or greater than 0.50 are presented next to each node. The scale bar of 0.2 represents the number of changes. The sequence of the isolate here studied is presented with its culture collection code (CMRP5047) highlighted in bold.

In addition, the other 14 extracts also showed activity against MRSA with MIC of 40 µg/ml (table 2) and were identified by multilocus phylogenetic analysis as: *Aspergillus* sp. (Subgen. *Circundati*, sect. *Nigri*, ser. *Japonici*) (CMRP5218), *Neurospora sublineolata* (CMRP5024) and *Neurospora* sp. (CMRP5014), *Periconia ignaria* (CMRP5028), *Colletotrichum* sp. *boninense* complex (CMRP5034), *Curvularia* sp. (CMRP5035), *Lasiodiplodia pontae* (CMRP4998 and CMRP5051), *Colletotrichum* sp. *gloeosporioides* complex (CMRP4999 and CMRP5001), *Penicillium* sp. sect. *Citrina* ser. *Citrina* (CMRP5227), *Pestalotiopsis*

sp. (CMRP5055), Chaetomiaceae (CMRP5059) and *Annellosympodiella* sp. (CMRP5020). The phylogenetic trees that show the identification of these isolates are presented in the supplementary material (figures S2-S11).

Table 1 - Minimum Inhibitory Concentrations of the extracts produced by the endophytes in screening test

Identification	Collection nº	MRSA[1] MIC[3] µg/ml	*A. baumanii*[2] MIC µg/ml	Identification	Collection nº	MRSA MIC µg/ml	*A. baumanii* MIC µg/ml
Diaporthe vochysiae	**CMRP4978**	13 µg/ml	40 µg/ml	*Diaporthe infertilis*	CMRP5056	122 µg/ml	1,100 µg/ml
Neopestalotiopsis egyptiaca	**CMRP4981**	13 µg/ml	122 µg/ml	*Mycosphaerella* sp.	CMRP5021	122 µg/ml	3,300 µg/ml
Cladosporium sp.	**CMRP5016**	13 µg/ml	1,100 µg/ml	*Anthostomelloides* sp.	CMRP5045	122 µg/ml	3,300 µg/ml
Neopestalotiopsis sp.	**CMRP5000**	13 µg/ml	1,100 µg/ml	*Barrmaelia* sp.	CMRP5023	122 µg/ml	>3,300 µg/ml
Paecilomyces sp.	**CMRP5047**	13 µg/ml	1,100 µg/ml	*Diaporthe vochysiae*	CMRP4994	122 µg/ml	>3,300 µg/ml
Neurospora sp.	**CMRP5014**	40 µg/ml	122 µg/ml	*Curvularia* sp.	CMRP4991	366 µg/ml	366 µg/ml
Colletotrichum sp. *gloeosporioides* complex	**CMRP4999**	40 µg/ml	366 µg/ml	*Anthostomelloides* sp.	CMRP4993	366 µg/ml	366 µg/ml
Aspergillus sp. subgen. *Circundati*, sect. *Nigri*, ser. *Japonici*	**CMRP5218**	40 µg/ml	1,100 µg/ml	*Colletotrichum* sp. *gloeosporioides* complex	CMRP4974	366 µg/ml	1,100 µg/ml
Annellosympodiella sp.	**CMRP5020**	40 µg/ml	1,100 µg/ml	*Nigrospora* sp.	CMRP4979	366 µg/ml	1,100 µg/ml
Neurospora sublineolata	**CMRP5024**	40 µg/ml	1,100 µg/ml	*Nigrospora* sp.	CMRP4980	366 µg/ml	1,100 µg/ml
Periconia ignaria	**CMRP5028**	40 µg/ml	1,100 µg/ml	*Colletotrichum* sp. *gloeosporioides* complex	CMRP4984	366 µg/ml	1,100 µg/ml
Colletotrichum sp. *boninense* complex	**CMRP5034**	40 µg/ml	1,100 µg/ml	*Diaporthe* sp.	CMRP4985	366 µg/ml	1,100 µg/ml
Penicillium sp. subgen. *Aspergilloides*, sect. *Citrina*, ser. *Citrina*	**CMRP5227**	40 µg/ml	1,100 µg/ml	*Barrmaelia* sp.	CMRP5018	366 µg/ml	1,100 µg/ml
Curvularia sp.	**CMRP5035**	40 µg/ml	1,100 µg/ml	*Nemania primolutea*	CMRP4987	366 µg/ml	1,100 µg/ml
Lasiodiplodia pontae	**CMRP4998**	40 µg/ml	1,100 µg/ml	*Diaporthe* sp.	CMRP4989	366 µg/ml	1,100 µg/ml
Pseudopestalotiopsis sp.	**CMRP5055**	40 µg/ml	1,100 µg/ml	Fusarium sp.	39-P4P5P2	366 µg/ml	1,100 µg/ml
Chaetomiaceae sp.	**CMRP5059**	40 µg/ml	1,100 µg/ml	*Polyscytalum* sp.	48-P13F2F2	366 µg/ml	1,100 µg/ml
Colletotrichum sp. *boninense* complex	**CMRP5051**	40 µg/ml	3,300 µg/ml	*Diaporthe vochysiae*	CMRP5031	366 µg/ml	1,100 µg/ml
Colletotrichum sp. *gloeosporioides* complex	**CMRP5001**	40 µg/ml	3,300 µg/ml	*Nigrospora* sp.	CMRP5039	366 µg/ml	1,100 µg/ml
Prosopidicola sp.	CMRP5026	122 µg/ml	366 µg/ml	*Phyllosticta capitalensis*	CMRP5043	366 µg/ml	1,100 µg/ml
Nigrospora sp.	CMRP5041	122 µg/ml	366 µg/ml	*Colletotrichum* sp.	CMRP5053	366 µg/ml	1,100 µg/ml
Xylaria arbuscula	CMRP5054	122 µg/ml	366 µg/ml	Chaetomiaceae sp.	CMRP5057	366 µg/ml	1,100 µg/ml
Fusarium sp.	CMRP5012	122 µg/ml	1,100 µg/ml	Chaetomiaceae sp.	CMRP5058	366 µg/ml	1,100 µg/ml
Diaporthe vochysiae	CMRP4977	122 µg/ml	1,100 µg/ml	*Colletotrichum* sp. *acutatum* complex	CMRP5228	366 µg/ml	1,100 µg/ml
Neurospora sp.	CMRP5013	122 µg/ml	1,100 µg/ml	*Colletotrichum* sp. *gloeosporioides* complex	CMRP4995	366 µg/ml	3,300 µg/ml

Talaromyces sp. sec. *Talaromyces*	CMRP5015	122 µg/ml	1,100 µg/ml
Disculoides sp.	CMRP5017	122 µg/ml	1,100 µg/ml
Annellosympodiella sp.	CMRP5019	122 µg/ml	1,100 µg/ml
Pseudocercospora sp.	CMRP5022	122 µg/ml	1,100 µg/ml
Chaetomiaceae sp.	CMRP4986	122 µg/ml	1,100 µg/ml
Anthostomelloides sp.	CMRP4988	122 µg/ml	1,100 µg/ml
Colletotrichum sp. *acutatum* complex	CMRP5222	122 µg/ml	1,100 µg/ml
Anthostomelloides sp.	CMRP4992	122 µg/ml	1,100 µg/ml
Cladosporium sp.	CMRP5027	122 µg/ml	1,100 µg/ml
Colletotrichum sp. *boninense* complex	CMRP5030	122 µg/ml	1,100 µg/ml
Pestalotiopsis sp.	CMRP5032	122 µg/ml	1,100 µg/ml
Diaporthe amolarii	CMRP4997	122 µg/ml	1,100 µg/ml
Nigrospora sp.	CMRP5036	122 µg/ml	1,100 µg/ml
Nigrospora sp.	CMRP5040	122 µg/ml	1,100 µg/ml
Pseudofusicoccum stromaticum	CMRP5042	122 µg/ml	1,100 µg/ml
Cladosporium sp.	CMRP5226	122 µg/ml	1100 µg/ml
Cladosporium sp.	CMRP5044	122 µg/ml	1,100 µg/ml
Alternaria sp.	CMRP5046	122 µg/ml	1,100 µg/ml
Hypoxylon sp.	CMRP5048	122 µg/ml	1,100 µg/ml
Corynespora sp.	CMRP5049	122 µg/ml	1,100 µg/ml
Anthostomelloides sp.	CMRP5050	122 µg/ml	1,100 µg/ml
Diaporthe sp.	CMRP5029	1,100 µg/ml	366 µg/ml
Phyllosticta capitalensis	CMRP4971	1,100 µg/ml	1,100 µg/ml
Phyllosticta capitalensis	CMRP4972	1,100 µg/ml	1,100 µg/ml
Nigrospora sp.	CMRP4973	1,100 µg/ml	1,100 µg/ml
Nigrospora brasiliensis	CMRP4975	1,100 µg/ml	1,100 µg/ml
Hypoxylon sp.	CMRP5011	1,100 µg/ml	1,100 µg/ml
Pseudofusicoccum stromaticum	CMRP4976	1,100 µg/ml	1,100 µg/ml
Pseudofusicoccum stromaticum	CMRP5219	1,100 µg/ml	1,100 µg/ml
Pseudofusicoccum stromaticum	CMRP4982	1,100 µg/ml	1,100 µg/ml
Anthostomelloides sp.	CMRP4983	1,100 µg/ml	1,100 µg/ml
Diaporthe sp.	CMRP5220	1,100 µg/ml	1,100 µg/ml
Colletotrichum sp. *acutatum* complex	CMRP5221	1,100 µg/ml	1,100 µg/ml
Diaporthe sp.	CMRP4990	1,100 µg/ml	1,100 µg/ml
Chaetomiaceae sp.	CMRP5025	1,100 µg/ml	1,100 µg/ml
Chaetomiaceae sp.	CMRP5224	1,100 µg/ml	1,100 µg/ml
Diaporthe sp.	CMRP4996	1,100 µg/ml	1,100 µg/ml
Colletotrichum sp. *boninense* complex	CMRP5033	1,100 µg/ml	1,100 µg/ml
Endomelanconiopsis sp.	CMRP5037	1,100 µg/ml	1,100 µg/ml
Colletotrichum gigasporum	CMRP5038	1,100 µg/ml	1,100 µg/ml
Disculoides sp.	CMRP5052	1,100 µg/ml	1,100 µg/ml

[1]Methicilin resistant *Staphylococcus aureus*, [2]*Acinetobacter baumanii*, [3] Minimal Inhibitory Concentration.
Strains marked in bold had their extracts selected to be evaluated against *Klebsiella pneumoniae* producing Carbapenemase (KPC), *Pseudomonas aeruginosa* and Vancomycin resistant *Enterococcus* sp. (VRE).

Table 2 – Minimum Inhibitory (MIC) and Bactericidal Concentrations of 19 extracts selected in screening test

Isolates identification	Collection nº	Gram-positive				Gram-negative					
		MRSA[1]		VRE[2]		*A. baumanii*[3]		*P. aeruginosa*[4]		KPC[5]	
		MIC[6] µg/ml	MBC[7] µg/ml	MIC µg/ml	MBC µg/ml	MIC µg/ml	MBC µg/ml	MIC µg/ml	MBC µg/ml	MIC µg/ml	MBC µg/ml
Neopestalotiopsis egyptiaca	CMRP4981	**13**	**40**	**4,3**	**13**	122	1,100	3,300	>3,300	**1,100**	**1,100**
Diaporthe vochysiae	CMRP4978	**13**	1,100	**13**	3,300	**40**	>3,300	1,100	>3,300	3,300	>3,300
Neopestalotiopsis sp.	CMRP5000	**13**	122	**13**	122	1,100	3,300	1,100	>3,300	3,300	>3,300
Cladosporium sp. *cladosporioides* complex	CMRP5016	**13**	122	366	3,300	1,100	3,300	1,100	3,300	3,300	>3,300
Paecilomyces sp.	CMRP5047	**13**	122	366	1,100	1,100	3,300	1,100	3,300	3,300	>3,300
Aspergillus sp. Subgen. *Circundati*, sect. *Nigri*, ser. *Japonici*	CMRP5218	40	40	40	40	1,100	3,300	1,100	>3,300	3,300	>3,300
Neurospora sp.	CMRP5014	40	366	40	122	122	3,300	1,100	>3,300	3,300	>3,300
Annellosympodiella sp.	CMRP5020	40	122	366	1,100	1,100	3,300	1,100	3,300	3,300	>3,300
Neurospora sublineolata	CMRP5024	40	122	40	122	1,100	3,300	1,100	>3,300	3,300	>3,300
Periconia ignaria	CMRP5028	40	122	40	122	1,100	3,300	1,100	3,300	1,100	3,300
Colletotrichum sp. *boninense* complex	CMRP5034	40	122	122	122	1,100	3,300	1,100	3,300	3,300	>,3300
Curvularia sp.	CMRP5035	40	1,100	40	3,300	1,100	1,100	1,100	3,300	3,300	3,300
Lasiodiplodia pontae	CMRP4998	40	122	122	3,300	1,100	3,300	1,100	>3,300	3,300	>3,300
Colletotrichum sp. *gloeosporioides* complex	CMRP4999	40	122	40	1,100	366	3,300	3,300	>3,300	3,300	>3,300
Penicillium sp. Subgen. *Aspergilloides*, sect. *Citrina*, ser. *Citrina*	CMRP5227	40	366	366	1,100	1,100	3,300	1,100	3,300	3,300	>3,300
Colletotrichum sp. *boninense* complex	CMRP5051	40	122	40	366	3,300	3,300	1,100	>3,300	3,300	>3,300
Colletotrichum sp. *gloeosporioides* complex	CMRP5001	40	122	40	122	3,300	3,300	3,300	>3,300	3,300	>3,300
Pseudopestalotiopsis sp.	CMRP5055	40	366	40	122	1,100	1,100	1,100	3,300	3,300	3,300
Chaetomiaceae sp.	CMRP5059	40	366	366	3,300	1,100	>3,300	1,100	>3,300	3,300	>3,300
Methanol		>3,300	>3,300	>3,300	>3,300	>3,300	>3,300	>3,300	>3,300	>3,300	>3,300
Gentamicin		-	-	-	-	36	36	4	4	328	986

Streptomycin		-	-	-	-	1,850	1,850	200	200	200	1,850
Oxacillin		11,100	33,300	3,700	33,300	-	-	-	-	-	-
Vancomycin		1,6	1,6	5	136	-	-	-	-	-	-

[1]Methicilin resistant *Staphylococcus aureus*, [2]Vancomycin resistant *Enterococcus*, [3]*Acinetobacter baumanii*, [4]*Pseudomonas aeruginosa* and [5]*Klebsiella pneumoniae* producing carbapenemase
[6]Minimal Inhibitory Concentration, [7]Minimal Bactericidal Concentration
The most promising MIC and MBC results are marked in bold and underlined

DISCUSSION

Traditionally, plants with medicinal properties serve as a source of bioactive compounds for the treatment of various diseases, however, obtaining these compounds can impact the environment conservation (SAVI et al., 2019b). Thus, new strategies, such as the search for natural compounds from the secondary metabolism of endophytic microorganisms, are a solution. Endophytic microorganisms have the characteristic of being able to colonize internal tissues of plants without causing apparent damage to the host (PETRINI et al., 1993; ARAÚJO et al., 2014) and have been reported as great potential producers of compounds with antibiotic, antifungal, antioxidant, antiparasitic and cytotoxic activity (GOS et al., 2017; NORILER, et al., 2018; SANTOS, et al., 2016; SAVI, et al., 2015; SAVI et al., 2019a). It is estimated that approximately 2.8 million infections caused by resistant bacteria occur in the United States alone each year, these infections are related to high rates of morbidity and mortality and high costs in healthcare, leading to about 35,000 deaths per year (MORRIS; CERCEO, 2020). Therefore, the need to search for new compounds for the development of new antimicrobial drugs, especially for those resistant microorganisms recently listed by WHO as priorities (WHO, 2017; WILLYARD, 2017), becomes increasingly important and necessary. These data reinforce the importance of the results presented here, since 19 extracts produced from the fermentation of endophytic fungi were able to inhibit the growth with low MIC values of one or more resistant bacteria for which new antimicrobial drugs are urgently needed.

The genus *Neopestalotiopsis* has been described by Maharachchikumbura et al. (2014) in a study that, based on morphological and phylogenetic characteristics, divided the genus *Pestalotiopsis* into three genera: *Pestalotiopsis*, *Neopestalotiopsis* and *Pseudopestalotiopsis*. *Neopestalotiopsis* is frequently reported as a phytopathogen and few studies to date report endophytes of the genus related to biological activity. In a study by Tanapichatsakul et al. (2019), 11 extracts produced by endophytes were evaluated for antimicrobial activity. The extract produced by the isolate *Neopestalotiopsis* sp. MFLUCC 15-1130 stood out for presenting broad spectrum antimicrobial activity against six bacterial and two fungal species in relation to other extracts and controls, in addition to having high antioxidant activity. Such activity was attributed by the authors to the detection of a high concentration of eugenol in the extract. The present study demonstrated two isolates of the genus *Neopestalotiopsis* with inhibitory activity against MRSA, VRE and KPC. The isolate *Neopestalotiopsis* sp. (CMRP5000) showed activity against MRSA and VRE with considerably low minimum inhibitory concentration (MIC: 13 µg/ml for both bacteria). Furthermore, this is the first study that demonstrates an extract produced by an endophytic isolate of the species *N. egyptiaca* (CMRP4981) with high antibacterial activity against MRSA (MIC: 13 µg/ml; MBC: 40 µg/ml) and VRE (MIC: 4.3 µg/ml; MBC: 13 µg/ml) and a moderate activity against KPC (MIC: 1,100 µg/ml; MBC: 1,100 µg/ml).

The *Diaporthe* genus has worldwide distribution and its species are able to colonize the most varied hosts in different associations, occurring as endophytes, phytopathogens and saprobes (GOMES et al., 2013; UDAYANGA et al., 2011). Among endophytic fungi, the genus *Diaporthe* presents a high frequency of isolation among varied host plants, in addition to being frequently related to the production of secondary bioactive metabolites (CHEPKIRUI; STADLER, 2017; GOMES et al., 2013). Often, bioactive metabolites are obtained from isolates of the genus in relation to plants with medicinal properties (CHEPKIRUI; STADLER, 2017). Recent studies have shown antibacterial activity of *Diaporthe* endophytes isolated from medicinal plants against human pathogenic bacteria. A study by Medeiros et al. (2018) evaluated the antimicrobial activity of the crude extract and two compounds (diaporthin and orthosporin) produced by the *Diaporthe terebinthifoli* isolate against *Escherichia coli*, *Micrococcus luteus*, *Saccharomyces cerevisiae*, methicillin-sensitive *Staphylococcus aureus*, and MRSA. As a result, the diaporthin compound obtained higher activity compared to orthosporin. In addition, the crude extract showed activity against MRSA, which according to the authors suggests the production of other compounds with activity. In a study by Noriler et al. (2019) a new species of *Diaporthe* was described and identified as *D. vochysiae* (LGMF1583), which was isolated from the plant *Vochysia divergens* from the Brazilian Pantanal biome. Secondary metabolites produced by this strain were characterized and evaluated for their biological activity against resistant bacteria. As a result, one of the new compounds produced by the strain, identified as vochysiamide b, showed considerable antibacterial activity against KPC with a minimum inhibitory concentration of 80 µg/ml. The present study is the second to isolate *D. vochysiae* strain from the medicinal plant *Vochysia divergens* from the Brazilian Pantanal biome, however it is the first time that this species has been isolated in the Serra do Amolar region in the Pantanal. The search for similarity of sequences in the genbank revealed that, so far, this species has not been isolated from any other host or place, suggesting that this is an endemic species in the Pantanal. In addition, the considerable antibacterial activity against MRSA (MIC: 13 µg/ml; MBC: 40 µg/ml), VRE (MIC: 13 µg/ml) and *A. baumanii* (MIC: 40 µg/ml) of the crude extract produced by the CMRP4978 strain *D. vochysiae* in this study, reinforces the biotechnological potential of this species against multi-drug resistant human pathogenic bacteria. Since the strain evaluated in this study showed antibacterial activity against pathogens different from those observed in the LGMF1583 strain *D. vochysiae* by Noriler et al. (2019), we suggest that the CMRP4978 strain produces compounds different from those produced by LGMF1583. Thus, a future study with the objective of characterizing and evaluating the compounds produced by CMRP4978 is necessary to elucidate the issue related to the antibacterial activity observed by the species *D. vochysiae*.

The genus *Cladosporium* was identified by Link (1815), has a cosmopolitan distribution and is found in association with various types of plants, both as endophyte or phytopathogen (BENSCH et al., 2012). To date, few studies have demonstrated the biological activity of isolates of the genus *Cladosporium* against human pathogenic bacteria. In a study by Khan et al. (2016) the crude

extract, the fractions and the purified compounds produced by an isolate identified as *Cladosporium* sp. were evaluated against gram-positive and gram-negative bacteria and the authors reported moderate and promising activities. In this study, an isolate identified as *Cladosporium* sp. *cladosporioides* complex (CMRP5016) showed antibacterial activity with low minimum inhibitory concentration against MRSA (MIC: 13 µg/ml).

The genus *Paecilomyces* was described by Bainier (1907) and is characterized by being close to *Penicillium* species, but with morphological differences. This genus has a global distribution and can be isolated from several sources, such as marine environments, soil, insects, and plants (Li et al., 2020). Some studies have shown biological activity of secondary metabolites produced by species of the genus *Paecilomyces*, which suggests a great biotechnological potential (Li et al., 2020). In a study conducted by Liu et al. (2011) compounds produced by isolates of the species *P. variotii* against resistant bacteria were evaluated and, moderate activity was observed against *S. aureus* (MRSA). Another study by Mosadeghzad et al. (2013) also evaluated the compounds of the crude extract produced by an isolate identified as *Paecilomyces* sp. against MRSA, *Escherichia coli*, *Aeromonas hydrophila* and *Candida albicans*. The crude extract showed activity against all pathogens, while the purified compounds showed activity only against MRSA. These results corroborate the findings obtained in this present study, with inhibitory activity of the isolate identified as *Paecilomyces* sp. (CMRP5047) against MRSA (MIC: 13 µg/ml).

In conclusion, in our study, we demonstrated that endophytic microorganisms represent an important source of secondary metabolites with biotechnological potential for the development of new antimicrobials for the control of multidrug resistant bacteria. Future studies with the objective of identifying and purifying the active compounds of the extracts presented here, are necessary. In addition, we isolated a large number of species as endophytes of the medicinal plant *V. divergens* from Serra do Amolar and preserved them in the CMRP Culture collection of the Federal University of Paraná that will be available for future studies.

BIBLIOGRAPHY

ARAÚJO, W. L. et al. **Microrganismos endofíticos: Aspectos teóricos e práticos de isolamento e caracterização.** 2a ed- Santarém: UFOPA, 2014.

BAIRIER, G. Mycothèque de l'École de Pharmacie. XI. Paecilomyces, genre nouveau de Mecédinées. **Bulletin de la Société Mycologique de France**. 23:26-27. 1907.

BENSCH, K. *et al.* The genus cladosporium. **Studies in Mycology**. v. 72, p. 1–401, 2012.

CARBONE, I.; KOHN, L. M. A method for designing primer sets for speciation studies in filamentous ascomycetes. **Mycologia**, v. 91, n. 3, p. 553–556, 1999.

CHEPKIRUI, C.; STADLER, M. The genus *Diaporthe*: a rich source of diverse and bioactive metabolites. **Mycological Progress**, v. 16, n. 5, p. 477–494, 2017.

COSTELLOE, C. *et al.* Effect of antibiotic prescribing in primary care on antimicrobial resistance in individual patients: Systematic review and meta-analysis. **BMJ (Online)**, v. 340, n. 7756, p. 1120, 2010.

CROUS, P. W. *et al.* Calonectria species and their Cylindrocladium anamorphs: Species with sphaeropedunculate vesicles. **Studies in Mycology**, v. 50, n. 2, p. 415–430, 2004.

DARRIBA, D. *et al.* JModelTest 2: More models, new heuristics and parallel computing. **Nature Methods**, v. 9, n. 8, p. 772, 2012.

DOS SANTOS, I. P. *et al.* Antibacterial activity of endophytic fungi from leaves of *Indigofera suffruticosa* Miller (Fabaceae). **Frontiers in Microbiology**, v. 6, n. MAY, p. 1–7, 2015.

GLASS, N. L.; DONALDSON, G. C. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. **Applied and Environmental Microbiology**, v. 61, n. 4, p. 1323–1330, 1995.

GLIENKE, C *et al.* Antimicrobial Activity of Endophytes from Brazilian Medicinal Plants. **Antimicrobial Agents**, 2012.

GOMES, R. R. *et al. Diaporthe*: A genus of endophytic, saprobic and plant pathogenic fungi. **Persoonia: Molecular Phylogeny and Evolution of Fungi**, v. 31, p. 1–41, 2013.

GOS, F. M. W. R. *et al.* Antibacterial Activity of Endophytic Actinomycetes Isolated from the Medicinal Plant *Vochysia divergens* (Pantanal, Brazil). **Frontiers in Microbiology**, 2017.

HALL, T. A. **BioEdit4.8**. Raileigh, 1997-2001. 1 arquivo (11,5M); Disponível em: http://www.mbio.ncsu.edu/bioedit.html BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windons 95/98/NT.

ISMAIL, A. M. *et al. Lasiodiplodia* species associated with dieback disease of mango (*Mangifera indica*) in Egypt. **Australasian Plant Pathology**, v. 41, n. 6, p. 649–660, 2012.

KATOH, K.; TOH, H. Recent developments in the MAFFT multiple sequence alignment program. **Briefings in Bioinformatics**, v. 9, n. 4, p. 286–298, 2008.

KHAN, Md Imdadul Huque *et al.* Cytotoxic and antibacterial naphthoquinones from an endophytic fungus, *Cladosporium* sp. **Toxicology Reports**, v. 3, p. 861–865, 2016.

LI, X. Q. *et al.* A Systematic Review on Secondary Metabolites of *Paecilomyces* Species: Chemical Diversity and Biological Activity. **Planta Medica**, v. 86, n. 12, p. 805–821, 2020.

LINK, H. F. Observationes in ordines plantarum naturales 2. **Magazin der Gesellschaft Naturforschenden Freunde Berlin**. 7: 25-45. 1816.

LIU, J. *et al.* Antibacterial polyketides from the jellyfish-derived fungus *Paecilomyces variotii*. **Journal of Natural Products**, v. 74, n. 8, p. 1826–1829, 2011.

MAHARACHCHIKUMBURA, S. S. N. *et al.* Pestalotiopsis revisited. **Studies in Mycology**, v. 79, n. 1, p. 121–186, 2014.

MEDEIROS, A. G. *et al.* Bioprospecting of *Diaporthe terebinthifolii* LGMF907 for antimicrobial compounds. **Folia Microbiologica**, v. 63, n. 4, p. 499–505, 2018.

MILLER, M. A.; PFEIFFER, W.; SCHWARTZ, T. The CIPRES science gateway: A community resource for phylogenetic analyses. **Proceedings of the TeraGrid 2011 Conference: Extreme Digital Discovery, TG'11**, 2011.

MORRIS, S.; CERCEO, E.. Trends, epidemiology, and management of multi-drug resistant gram-negative bacterial infections in the hospitalized setting. **Antibiotics**, v. 9, n. 4, p. 1–20, 2020.

MOSADEGHZAD, Z. *et al.* Chemical components and bioactivity of the marine-derived fungus *Paecilomyces* sp. Collected from Tinggi Island, Malaysia. **Chemistry of Natural Compounds**, v. 49, n. 4, p. 621–625, 2013.

NORILER, S. A. *et al.* Bioprospecting and structure of fungal endophyte communities found in the Brazilian biomes, pantanal, and Cerrado. **Frontiers in Microbiology**, 2018.

NORILER, S. A. *et al.* Vochysiamides A and B: Two new bioactive carboxamides produced by the new species *Diaporthe vochysiae*. **Fitoterapia**, v. 138, n. May, p. 104273, 2019.

PETRINI, O. et al. Ecology, metabolite production, and substrate utilization in endophytic fungi. **Natural Toxins**, v. 1, n. 3, p. 185–196, 1993.

RONQUIST, F. *et al.* Mrbayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. **Systematic Biology**, v. 61, n. 3, p. 539–542, 2012.

SANTOS, P. J. C. D. *et al. Diaporthe endophytica* and *D. terebinthifolii* from medicinal plants for biological control of *Phyllosticta citricarpa*. **Microbiological Research**, v. 186–187, p. 153–160, 2016.

SAVI, D. C. *et al.* Antitumor, Antioxidant and Antibacterial Activities of Secondary Metabolites Extracted By Endophytic Actinomycetes Isolated From *Vochysia divergens*. **International Journal of Pharmaceutical, Chemical & Biological Sciences**, v. 5, n. 1, p. 347–356, 2015.

SAVI, D. C. *et al.* Secondary metabolites produced by the citrus phytopathogen *Phyllosticta citricarpa*. **Journal of Antibiotics**, v. 72, n. 5, p. 306–310, 2019a.

SAVI, D. C.; ALUIZIO, R.; GLIENKE, Chirlei. Brazilian Plants: An Unexplored Source of Endophytes as Producers of Active Metabolites. **Planta Medica**, v. 85, n. 8, p. 619–636, 2019b.

SLIPPERS, B. *et al.* Phylogenetic lineages in the botryosphaeriales: A systematic and evolutionary framework. **Studies in Mycology**, v. 76, p. 31–49, 2013.

STROBEL, G.; DAISY, B. Bioprospecting for microbial endophytes and their natural products. **Microbiology and molecular biology reviews : MMBR**, v. 67, n. 4, p. 491–502, 2003.

TAMURA, K. *et al.* MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. **Molecular Biology and Evolution**, v. 28, n. 10, p. 2731–2739, 2011.

TANAPICHATSAKUL, C. *et al.* Production of eugenol from fungal endophytes Neopestalotiopsis sp. and *Diaporthe* sp. isolated from *Cinnamomum loureiroi* leaves . **PeerJ**, v. 7, p. e6427, 2019.

UDAYANGA, D. *et al.* The genus *Phomopsis*: Biology, applications, species concepts and names of common phytopathogens. **Fungal Diversity**, v. 50, n. September, p. 189–225, 2011.

VENIERAKI, A.; DIMOU, M.; KATINAKIS, P. Endophytic fungi residing in medicinal plants have the ability to produce the same or similar pharmacologically active secondary metabolites as their hosts. **Hellenic Plant Protection Journal**, v. 10, n. 2, p. 51–66, 2017.

WEIR, B. S.; JOHNSTON, P. R.; DAMM, U. The *Colletotrichum gloeosporioides* species complex. **Studies in Mycology**, v. 73, p. 115–180, 2012.

WILLYARD, C. The drug-resistant bacteria that pose the greatest health threats. **Nature**, v. 543, n. 7643, p. 15, 2017.

WHO. **WHO publishes listo of bacteria for which new antibiotics are urgentely needed.** World Health Organization. 2017. Disponível em: <https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed> Acesso em: 10 jan. 2021.

YU, H. *et al.* Recent developments and future prospects of antimicrobial metabolites produced by endophytes. **Microbiological Research**, v. 165, n. 6, p. 437–449, 2010.

APÊNDICE 2

Supplementary Material

Endophytic fungi from Serra do Amolar-Pantanal produce secondary bioactive metabolites against multidrug-resistant bacteria

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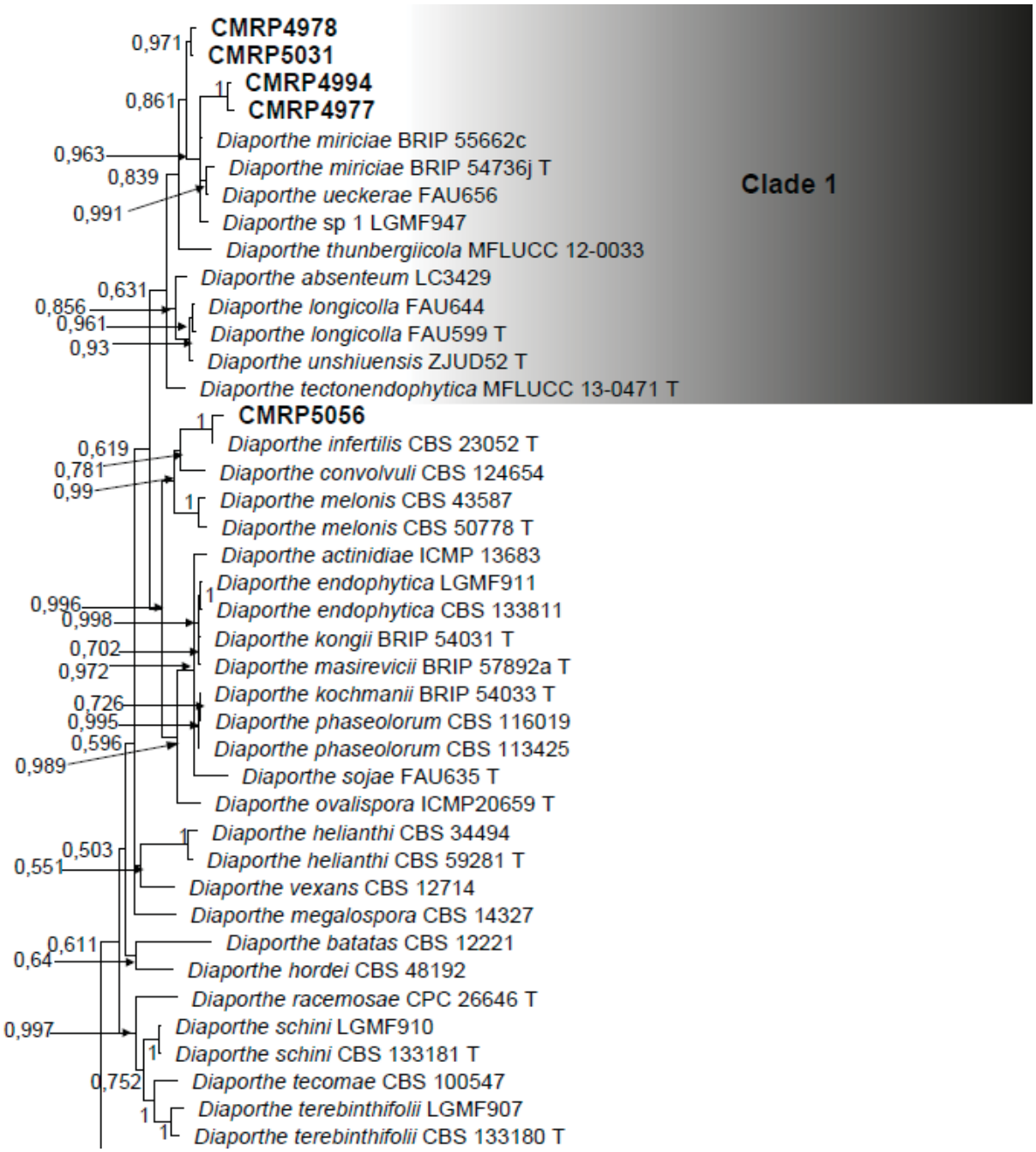
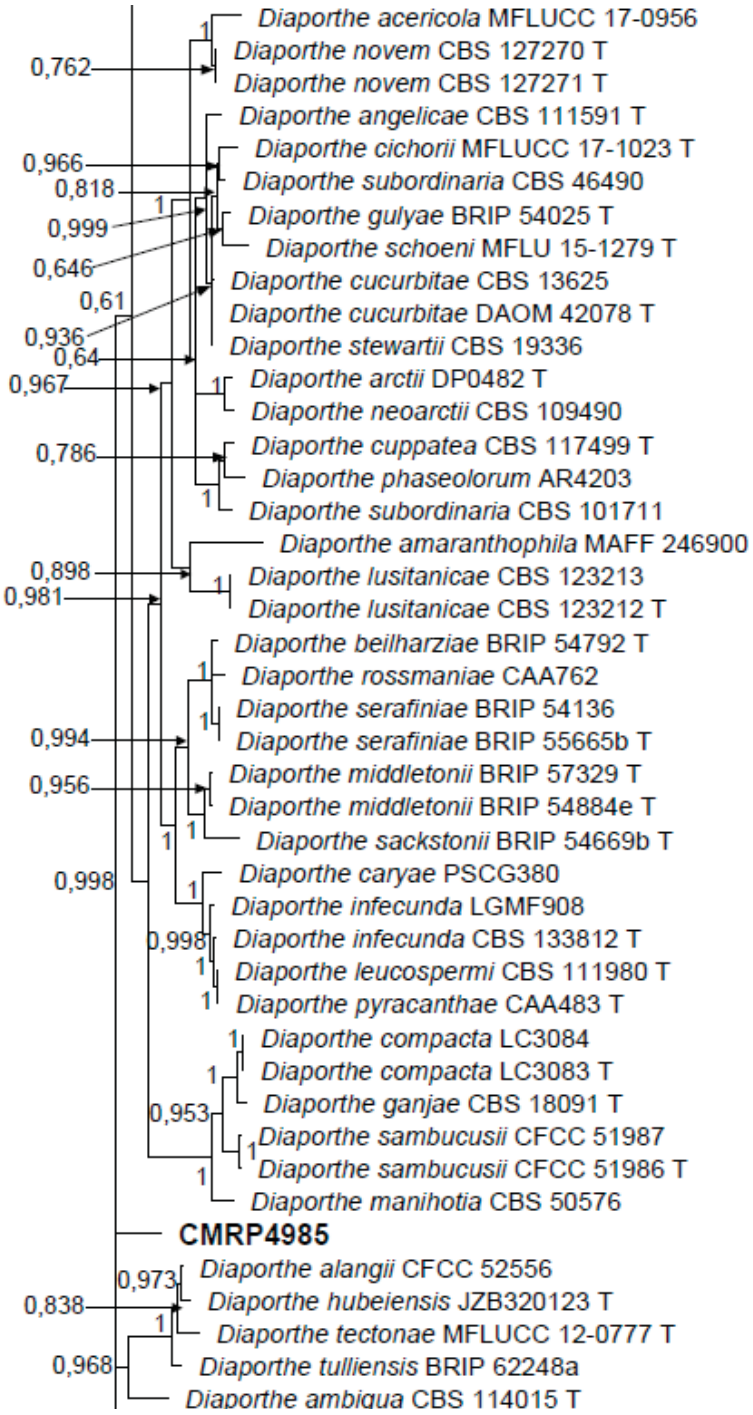


Figure S1: Bayesian Inference phylogenetic tree of *Diaporthe* species based on alignment of *tef1* partial sequence. The data matrix had 314 taxa and 1033 characters. The species *Diaporthella corylina* (CBS 121124) was used as outgroup. Strains marked with a “T” correspond to type sequences. Bayesian posterior probabilities equal or greater than 0.50 are presented next to each node. The scale bar of 0.2 represents the number of changes. The sequence of the isolates here studied are presented with its isolation code (CMRP4997; CMRP5029; CMRP5033; CMRP4985; CMRP5056; CMRP4977; CMRP4994; CMRP5031; CMRP4978) highlighted in bold.



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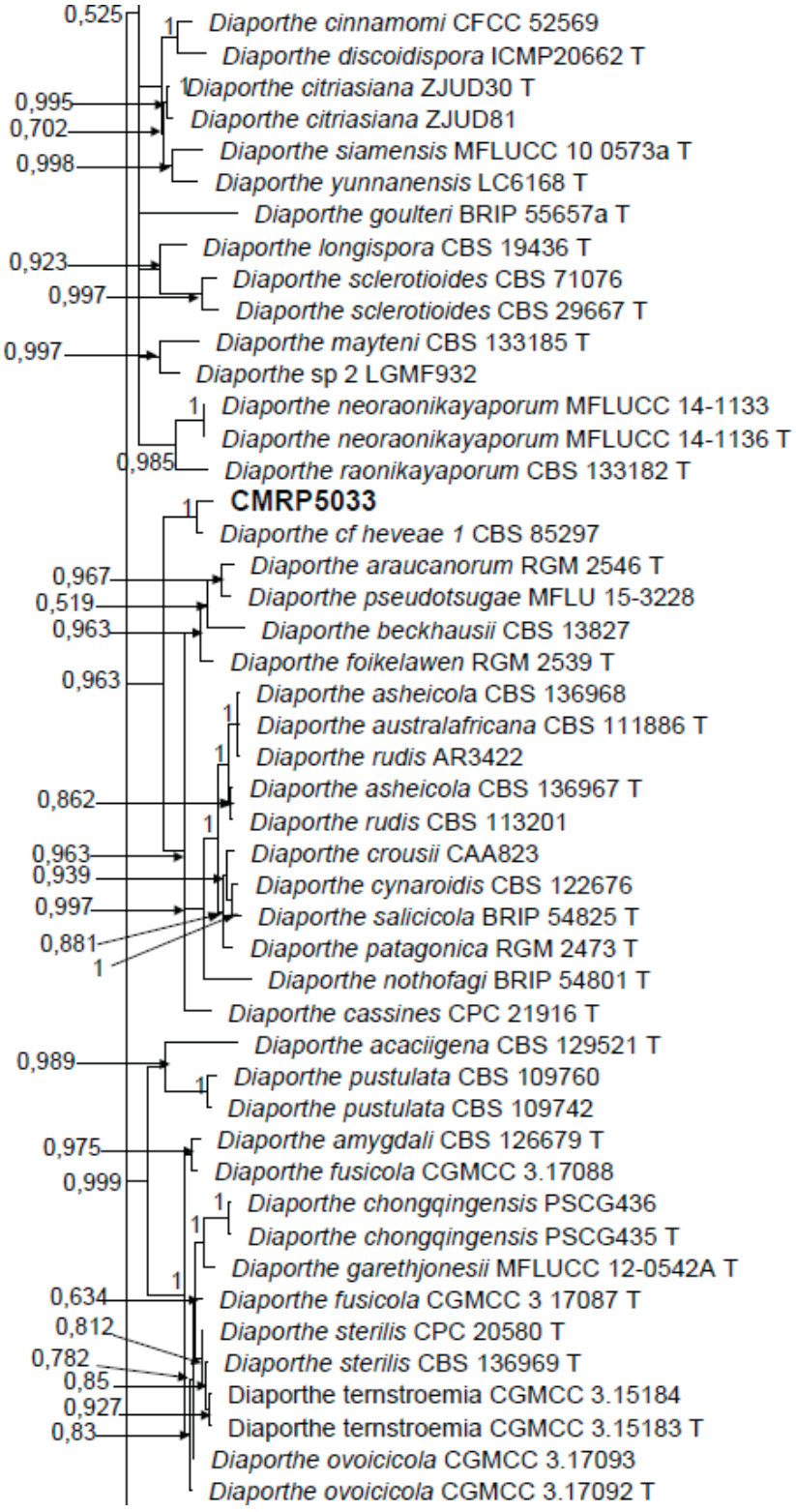

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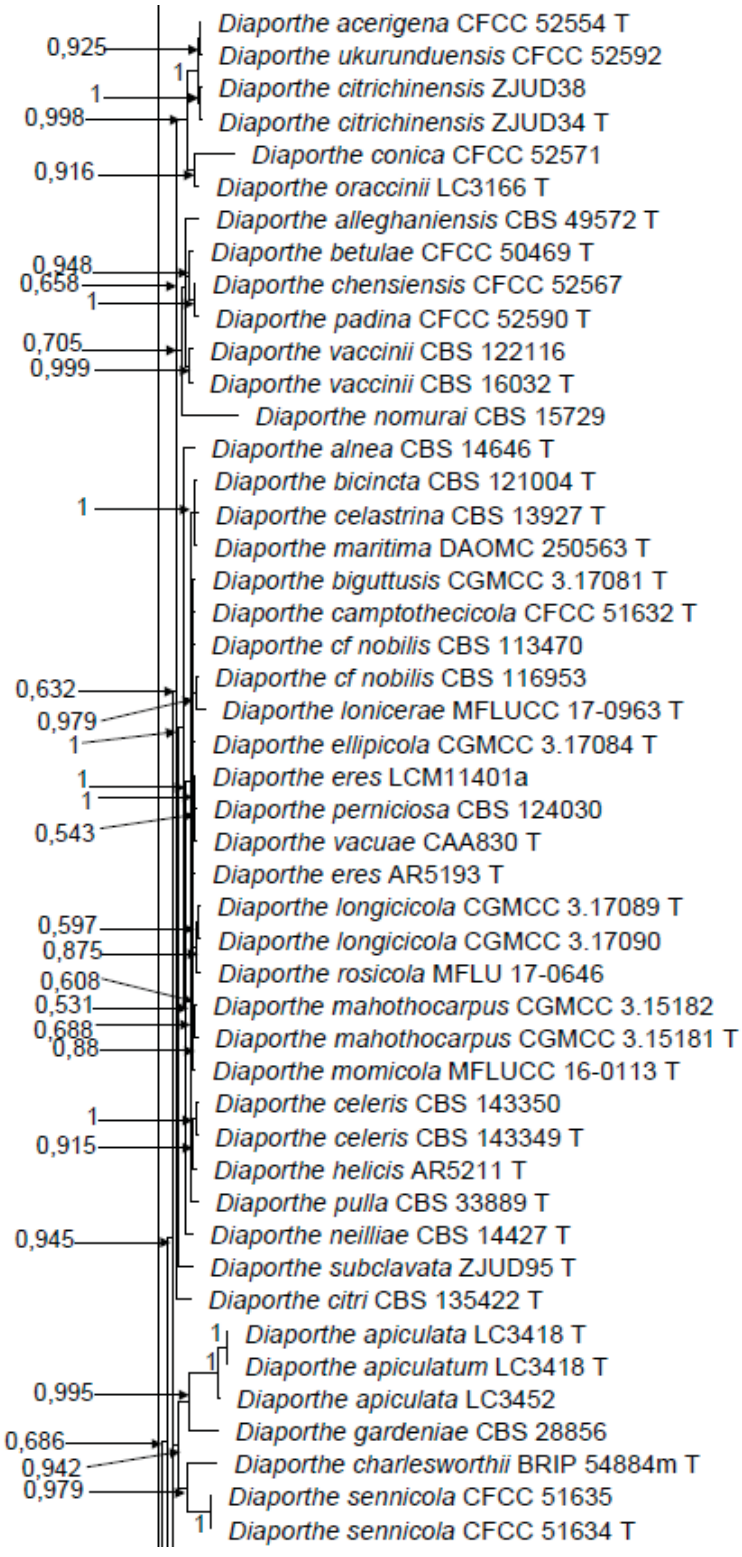

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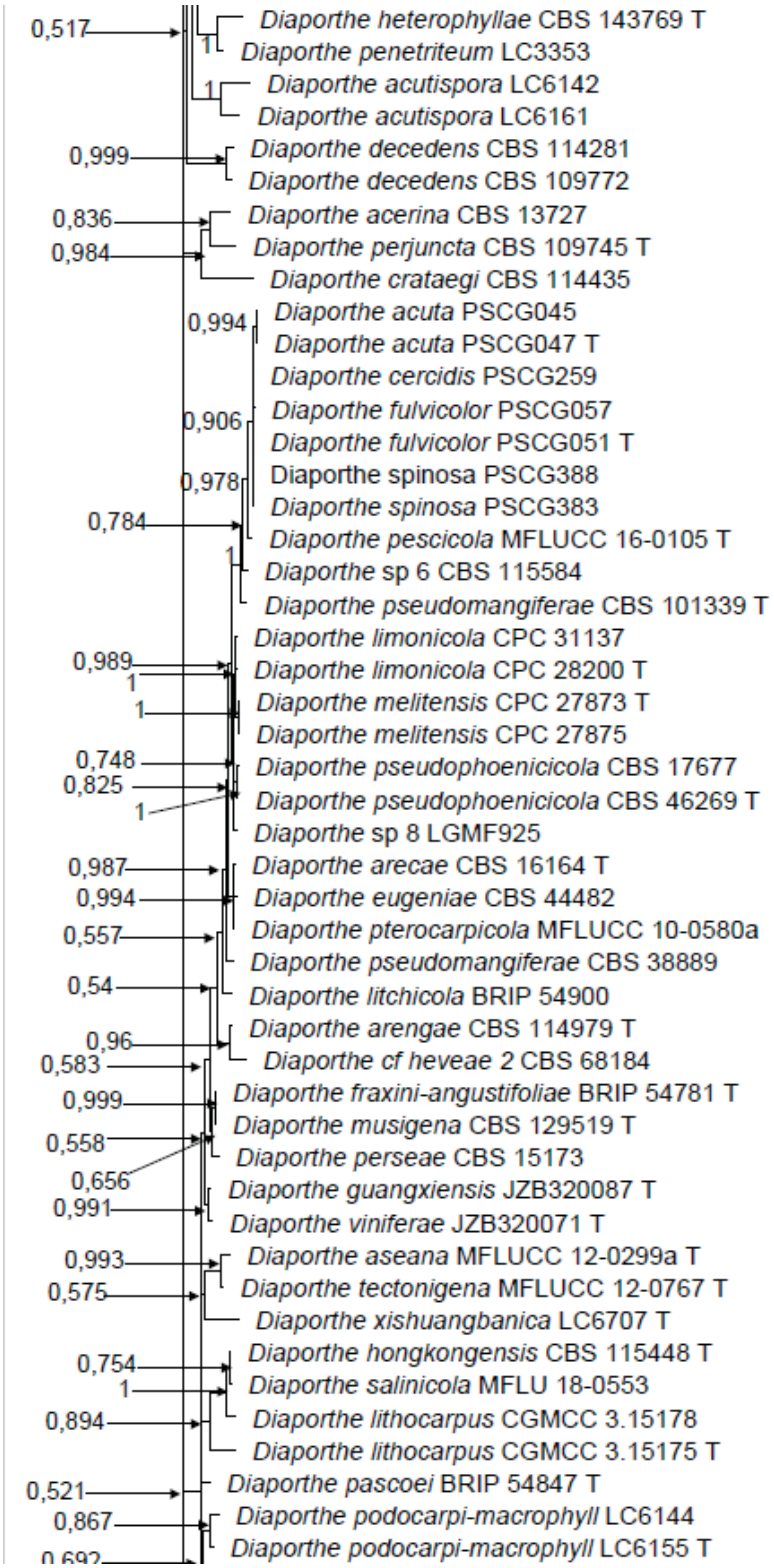

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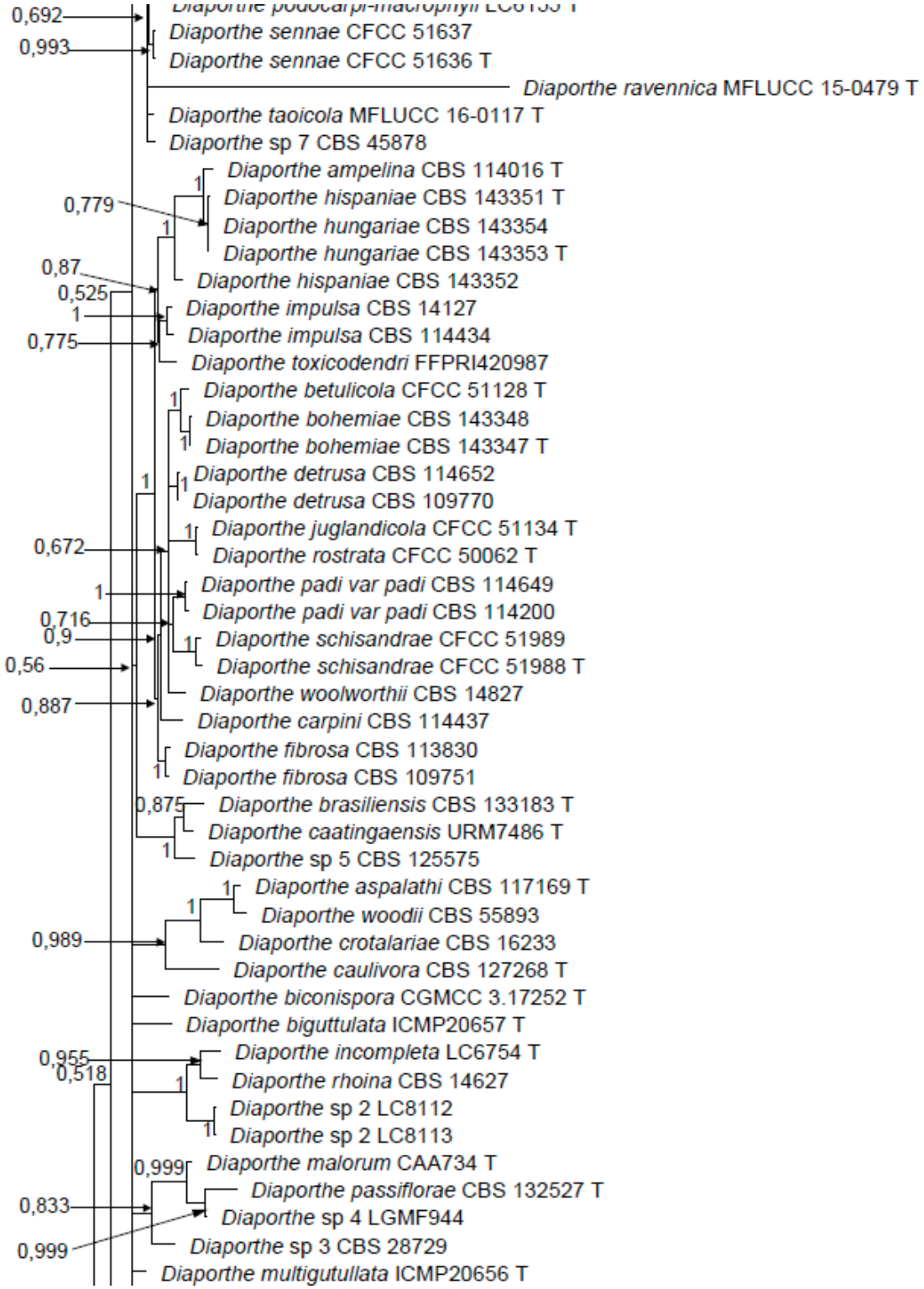

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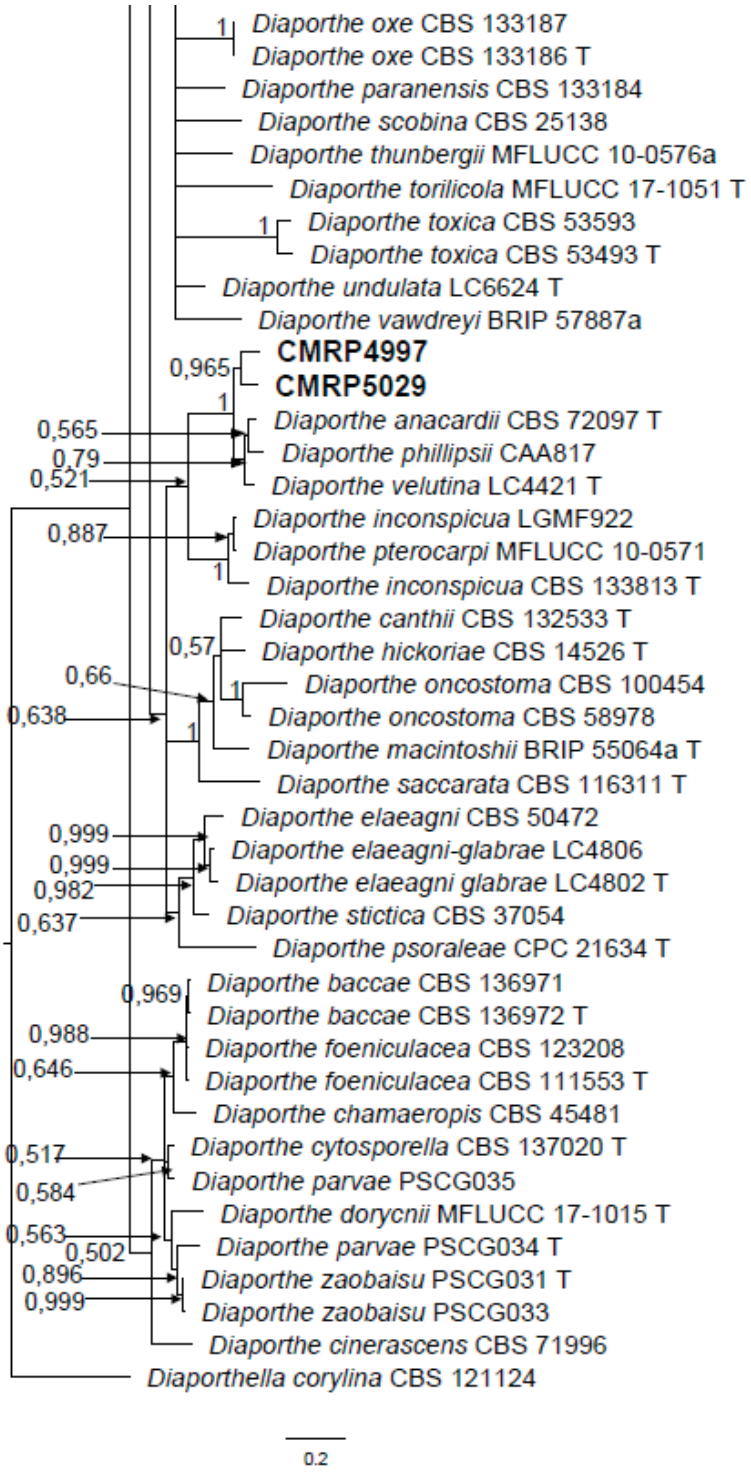

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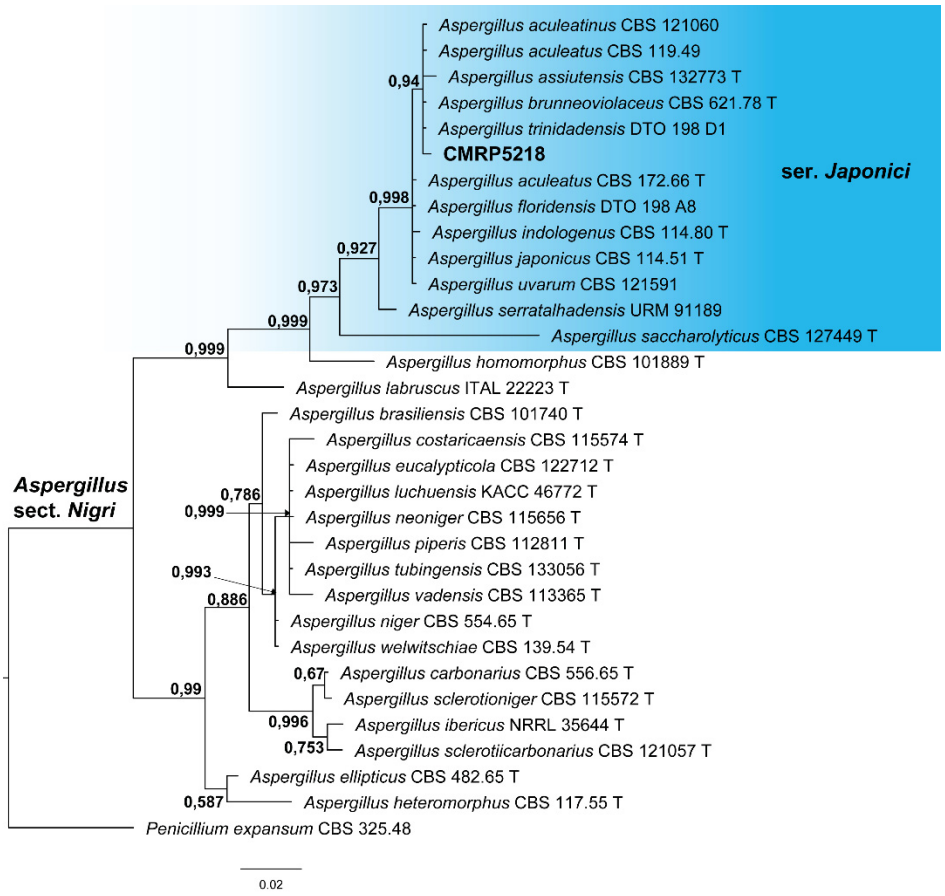


Figure S2: Bayesian Inference phylogenetic tree of *Aspergillus* sect. *Nigri* species based on alignment of ITS, partial sequence. The data matrix had 32 taxa and 641 characters. The species *Penicillium expansum* (CBS 32.548) was used as outgroup. Strains marked with a “T” correspond to type sequences. Bayesian posterior probabilities equal or greater than 0.50 are presented next to each node. The scale bar of 0.02 represents the number of changes. The sequence of the isolate here studied is presented with its isolation code (CMRP5218) highlighted in bold.

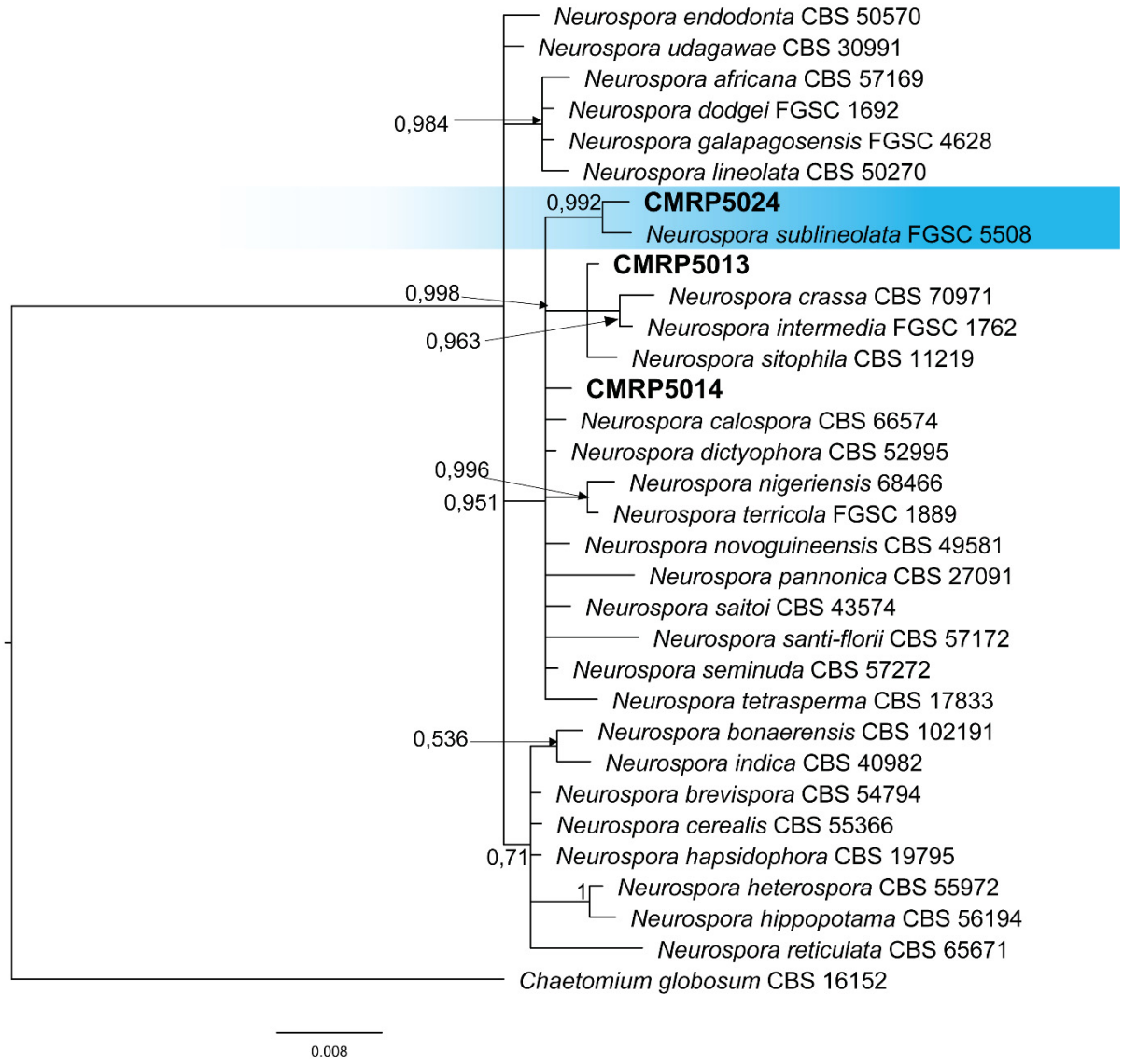


Figure S3: Bayesian Inference phylogenetic tree of *Neurospora* species based on the alignment of ITS partial sequence. The data matrix had 32 taxa and 633 characters. The species *Chaetomium globosum* (CBS 16.152) was used as outgroup. Strains marked with a “T” correspond to type sequences. Bayesian posterior probabilities equal or greater than 0.50 are presented next to each node. The scale bar of 0.008 represents the number of changes. The sequence of the isolates here studied are presented with its culture collection code (CMRP5024; CMRP5013; CMRP5014) highlighted in bold.

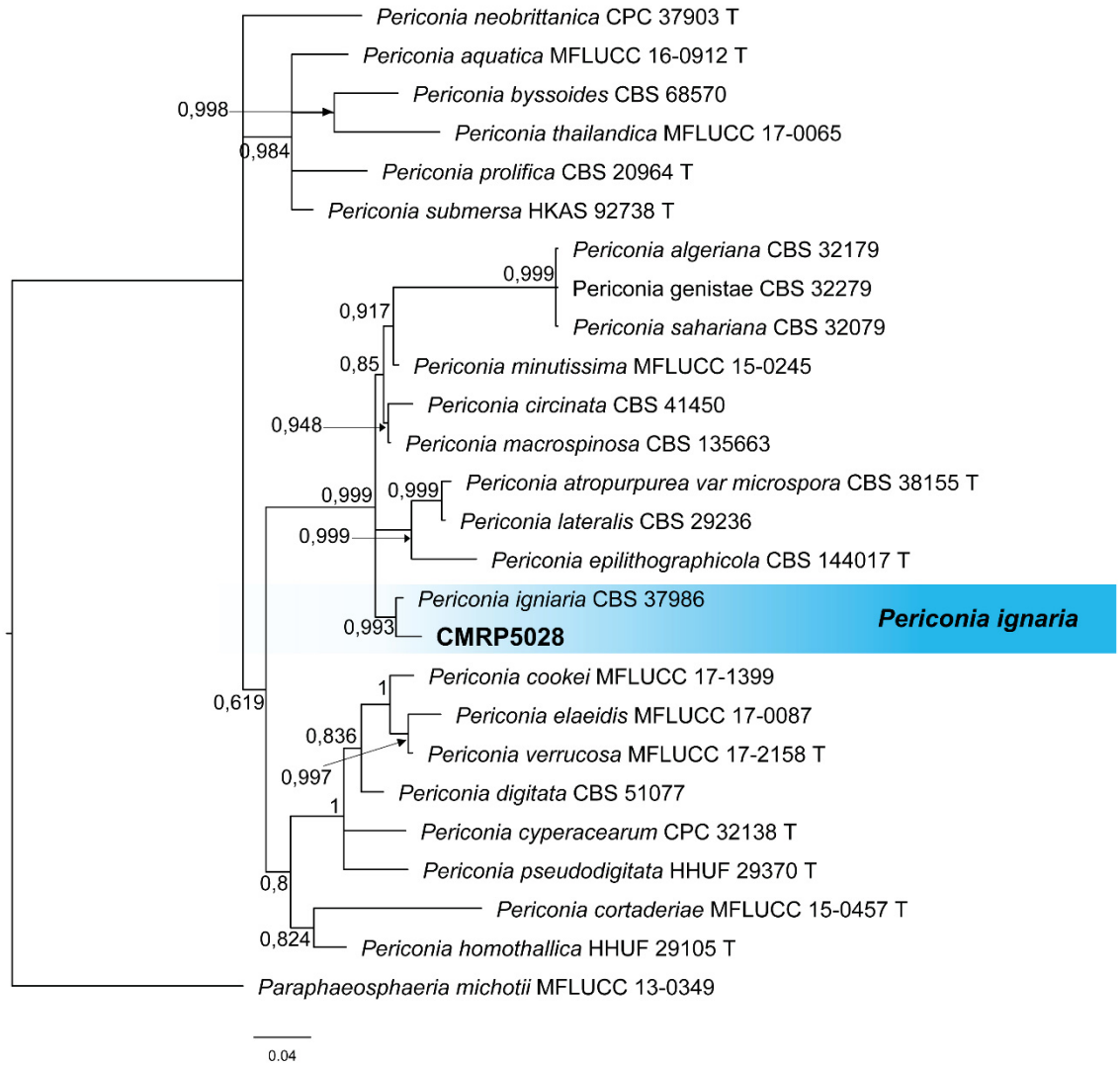


Figure S4: Bayesian Inference phylogenetic tree of *Periconia* species based on the alignment of ITS partial sequence. The data matrix had 26 taxa and 690 characters. The species *Paraphaeosphaeria michotii* (MFLUCC 13-0349) was used as outgroup. Strains marked with a "T" correspond to type sequences. Bayesian posterior probabilities equal or greater than 0.50 are presented next to each node. The scale bar of 0.04 represents the number of changes. The sequence of the isolate here studied is presented with its culture collection code (CMRP5028) highlighted in bold.

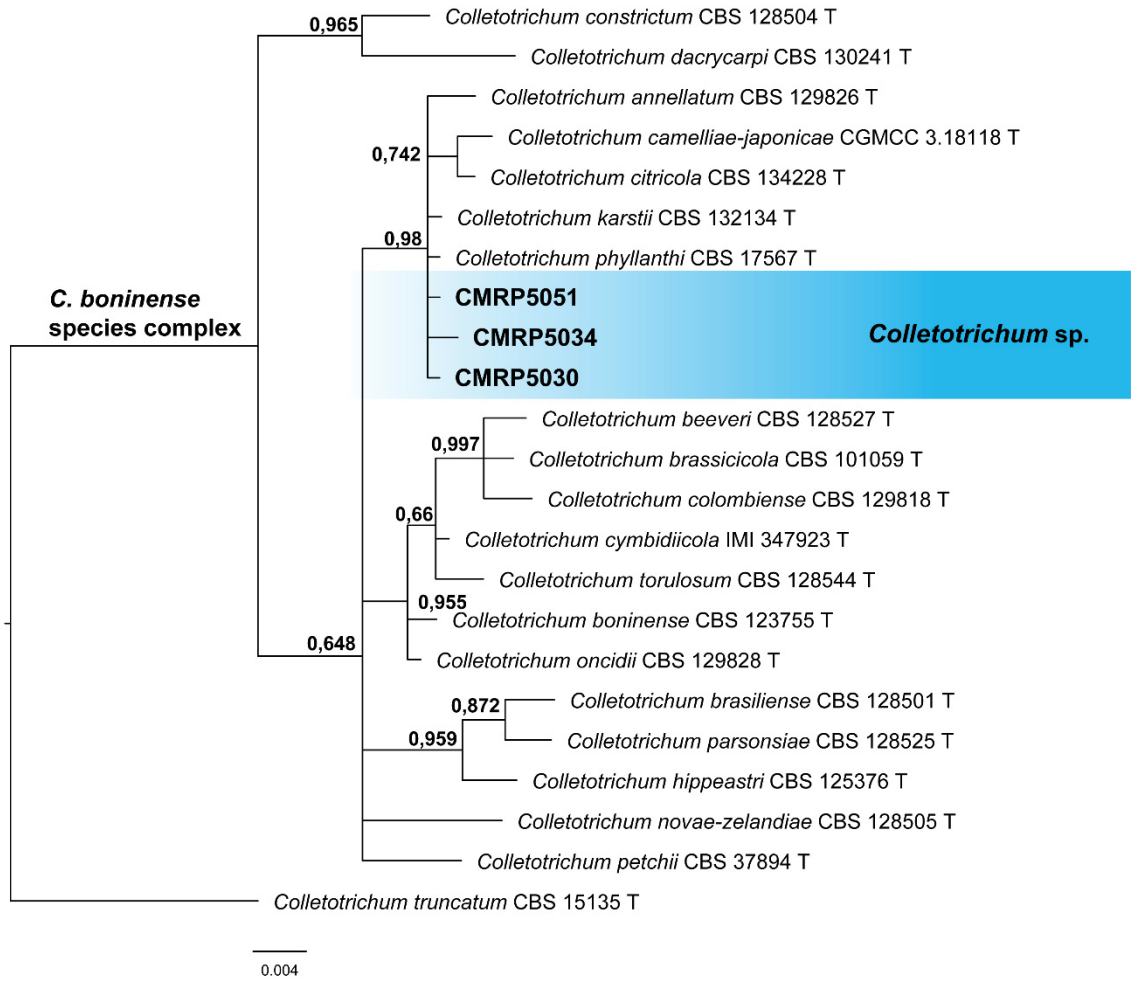
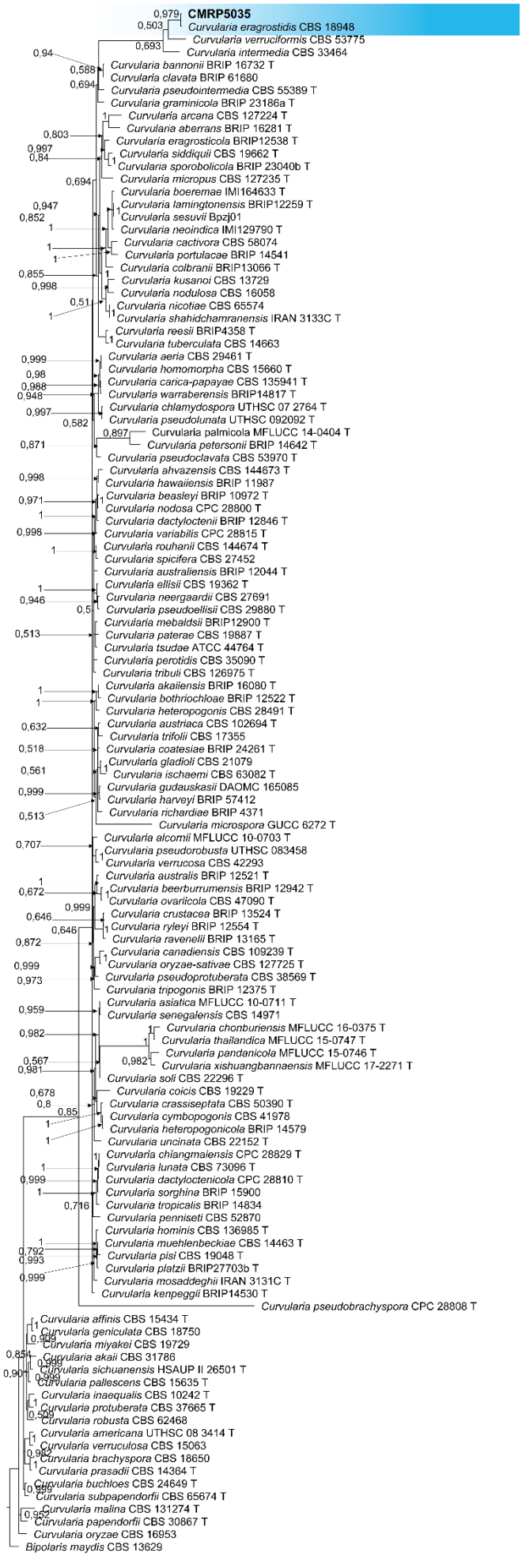


Figure S5: Bayesian Inference phylogenetic tree of *Colletotrichum boninense* complex species based on alignment of ITS partial sequence. The data matrix had 23 taxa and 560 characters. The species *Colletotrichum truncatum* (CBS 15135) was used as outgroup. Strains marked with a "T" correspond to type sequences. Bayesian posterior probabilities equal or greater than 0.50 are presented next to each node. The scale bar of 0.004 represents the number of changes. The sequence of the isolates here studied are presented with its culture collection code (CMRP5030; CMRP5034; CMRP5051) highlighted in bold.


CMRP5035
Curvularia eragrostidis CBS 18948
Curvularia verruciformis CBS 53775
Curvularia intermedia CBS 33464
Curvularia bannonii BRIP 16732 T
Curvularia clavata BRIP 61680
Curvularia pseudointermedia CBS 55389 T
Curvularia graminicola BRIP 23186a T
Curvularia arcana CBS 127224 T
Curvularia aberrans BRIP 16281 T
Curvularia eragrosticola BRIP12538 T
Curvularia siddiquii CBS 19662 T
Curvularia sporobolicola BRIP 23040b T
Curvularia micropus CBS 127235 T
Curvularia boeremae IMI164633 T
Curvularia lamingtonensis BRIP12259 T
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Curvularia kusanoi CBS 13729
Curvularia nodulosa CBS 16058
Curvularia nicotiae CBS 65574
Curvularia shahidchamranensis IRAN 3133C T
Curvularia reesii BRIP4358 T
Curvularia tuberculata CBS 14663
Curvularia aeria CBS 29461 T
Curvularia homomorpha CBS 15660 T
Curvularia carica-papayae CBS 135941 T
Curvularia warraberensis BRIP14817 T
Curvularia chlamydospora UTHSC 07 2764 T
Curvularia pseudolunata UTHSC 092092 T
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Curvularia nodosa CPC 28800 T
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Curvularia coatesiae BRIP 24261 T
Curvularia gladioli CBS 21079
Curvularia ischaemi CBS 63082 T
Curvularia gudauskasii DAOMC 165085
Curvularia harveyi BRIP 57412
Curvularia richardiae BRIP 4371
Curvularia microspora GUCC 6272 T
Curvularia alcornii MFLUCC 10-0703 T
Curvularia pseudorobusta UTHSC 083458
Curvularia verrucosa CBS 42293
Curvularia australis BRIP 12521 T
Curvularia beerburrumensis BRIP 12942 T
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Curvularia crustacea BRIP 13524 T
Curvularia ryleyi BRIP 12554 T
Curvularia ravenelii BRIP 13165 T
Curvularia canadiensis CBS 109239 T
Curvularia oryzae-sativae CBS 127725 T
Curvularia pseudoprotuberata CBS 38569 T
Curvularia tripogonis BRIP 12375 T
Curvularia asiatica MFLUCC 10-0711 T
Curvularia senegalensis CBS 14971
Curvularia chonburiensis MFLUCC 16-0375 T
Curvularia thailandica MFLUCC 15-0747 T
Curvularia pandanicola MFLUCC 15-0746 T
Curvularia xishuangbannaensis MFLUCC 17-2271 T
Curvularia soli CBS 22296 T
Curvularia coicis CBS 19229 T
Curvularia crassiseptata CBS 50390 T
Curvularia cymbopogonis CBS 41978
Curvularia heteropogonicola BRIP 14579
Curvularia uncinata CBS 22152 T
Curvularia chiangmaiensis CPC 28829 T
Curvularia lunata CBS 73096 T
Curvularia dactyloctenicola CPC 28810 T
Curvularia sorghina BRIP 15900
Curvularia tropicalis BRIP 14834
Curvularia penniseti CBS 52870
Curvularia hominis CBS 136985 T
Curvularia muehlenbeckiae CBS 14463 T
Curvularia pisi CBS 19048 T
Curvularia platzii BRIP27703b T
Curvularia mosaddeghii IRAN 3131C T
Curvularia kenpeggii BRIP14530 T
Curvularia pseudobrachyspora CPC 28808 T
Curvularia affinis CBS 15434 T
Curvularia geniculata CBS 18750
Curvularia miyakei CBS 19729
Curvularia akaii CBS 31786
Curvularia sichuanensis HSAUP II 26501 T
Curvularia pallescens CBS 15635 T
Curvularia inaequalis CBS 10242 T
Curvularia protuberata CBS 37665 T
Curvularia robusta CBS 62468
Curvularia americana UTHSC 08 3414 T
Curvularia verruculosa CBS 15063
Curvularia brachyspora CBS 18650
Curvularia prasadii CBS 14364 T
Curvularia buchloes CBS 24649 T
Curvularia subpapendorfii CBS 65674 T
Curvularia malina CBS 131274 T
Curvularia papendorfii CBS 30867 T
Curvularia oryzae CBS 16953
Bipolaris maydis CBS 13629
0.2

Figure S6: Bayesian Inference phylogenetic tree of *Curvularia* species based on multiple alignment of ITS, *tef1* and *gapdh* partial sequences. The data matrix had 122 taxa and 3442 characters. The species *Bipolaris maydis* (CBS 13.629) was used as outgroup. Strains marked with a "T" correspond to type sequences. Bayesian posterior probabilities equal or greater than 0.50 are presented next to each node. The scale bar of 0.2 represents the number of changes. The sequence of the isolate here studied is presented with its culture collection code (CMRP5035) highlighted in bold.

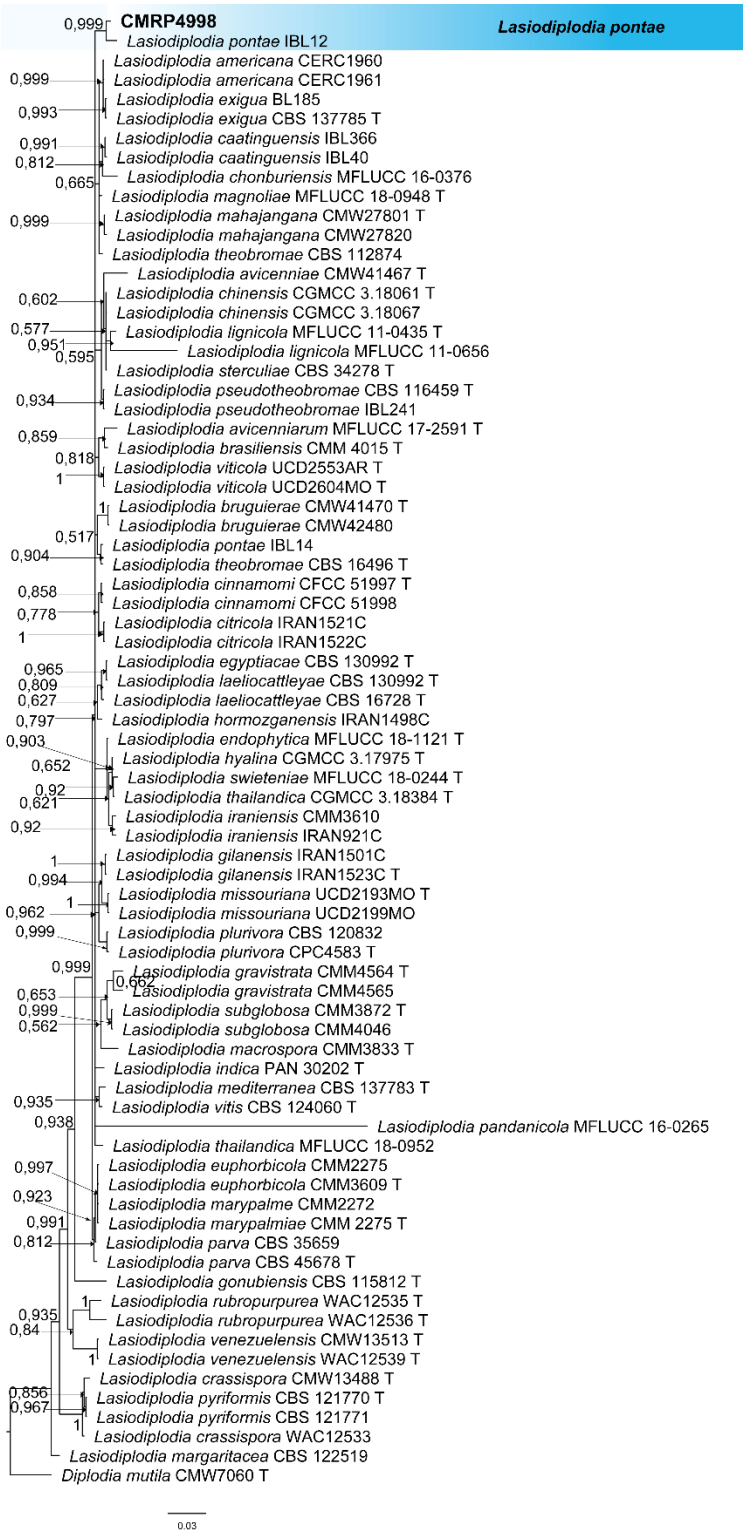

Figure S7: Bayesian Inference phylogenetic tree of *Lasiodiplodia* species based on multiple alignment of ITS, *tef1* and *tub2* partial sequences. The data matrix had 76 taxa and 2126 characters. The species *Diplodia mutila* (CMW 7060) was used as outgroup. Strains marked with a “T” correspond to type sequences. Bayesian posterior probabilities equal or greater than 0.50 are presented next to each node. The scale bar of 0.03 represents the number of changes. The sequence of the isolate here studied is presented with its culture collection code (CMRP4998) highlighted in bold.

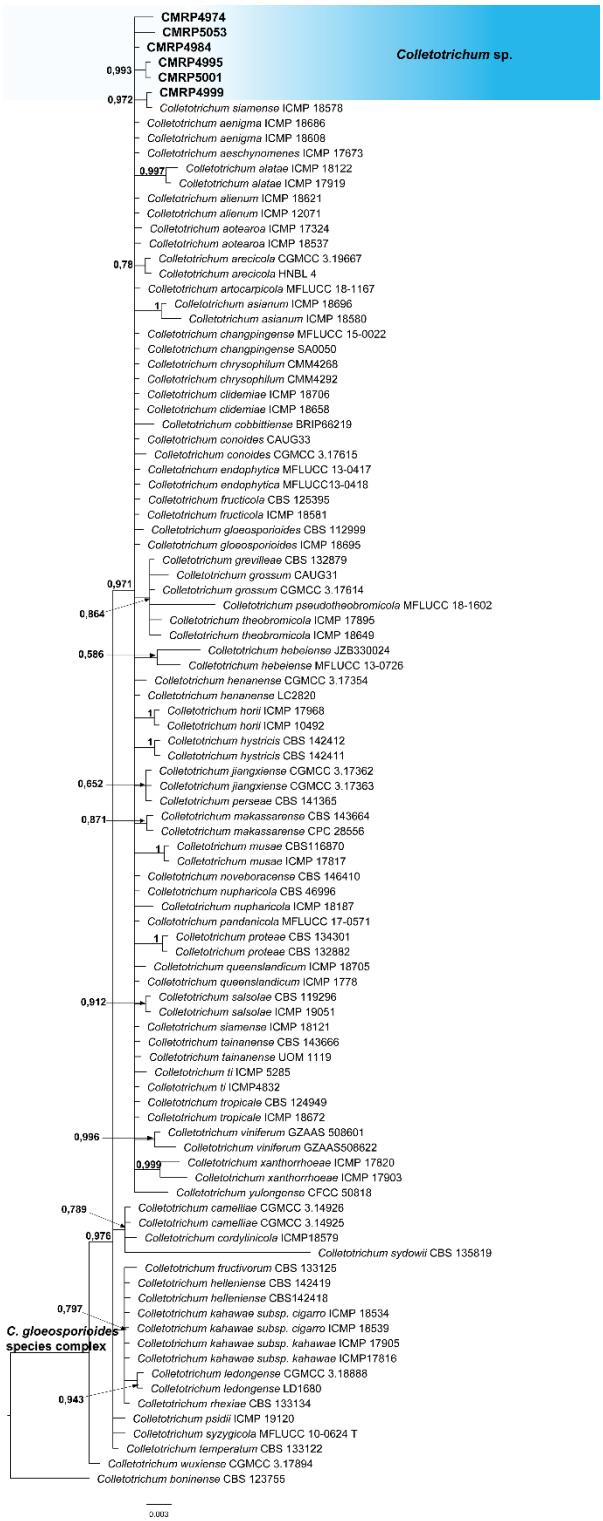

Figure S8: Bayesian Inference phylogenetic tree of *Colletotrichum gloeosporioides* complex species based on multiple alignment of ITS, *tub2*, *act*, *gapdh* partial sequences. The data matrix had 98 taxa and 2162 characters. The species *Colletotrichum boninense* (CBS 123755) was used as outgroup. Strains marked with a "T" correspond to type sequences. Bayesian posterior probabilities equal or greater than 0.50 are presented next to each node. The scale bar of 0.03 represents the number of changes. The sequence of the isolates here studied are presented with its culture collection code (CMRP4984; CMRP495; CMRP5001; CMRP4999) highlighted in bold.

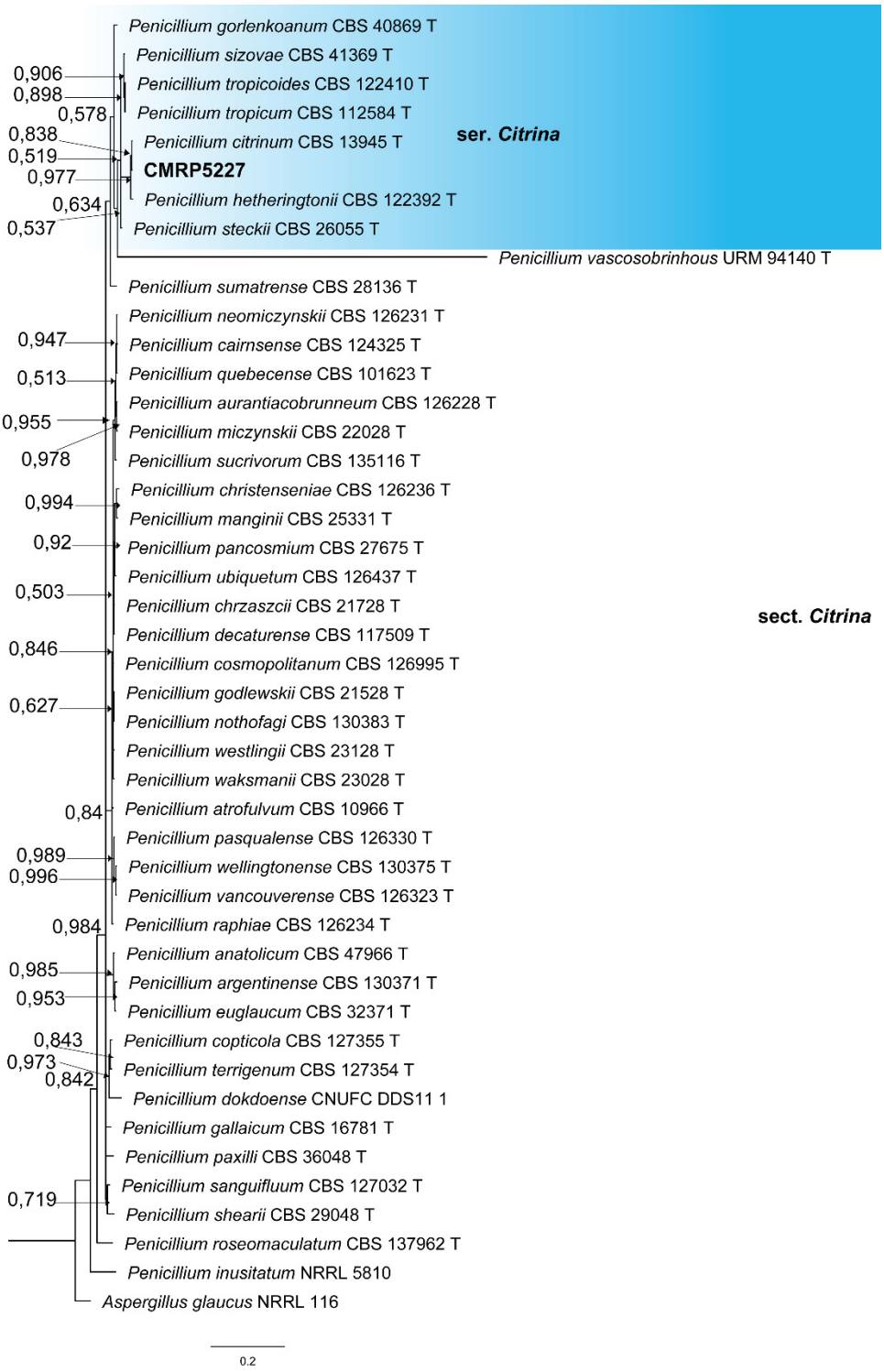

Figure S9: Bayesian Inference phylogenetic tree of *Penicillium* sect. *Citrina* species based on the alignment of ITS partial sequence. The data matrix had 45 taxa and 899 characters. The species *Aspergillus glaucus* (NRRL 166) was used as outgroup. Strains marked with a "T" correspond to type sequences. Bayesian posterior probabilities equal or greater than 0.50 are presented next to each node. The scale bar of 0.2 represents the number of changes. The sequence of the isolate here studied is presented with its isolation code (73-P11.2F1F1) highlighted in bold.

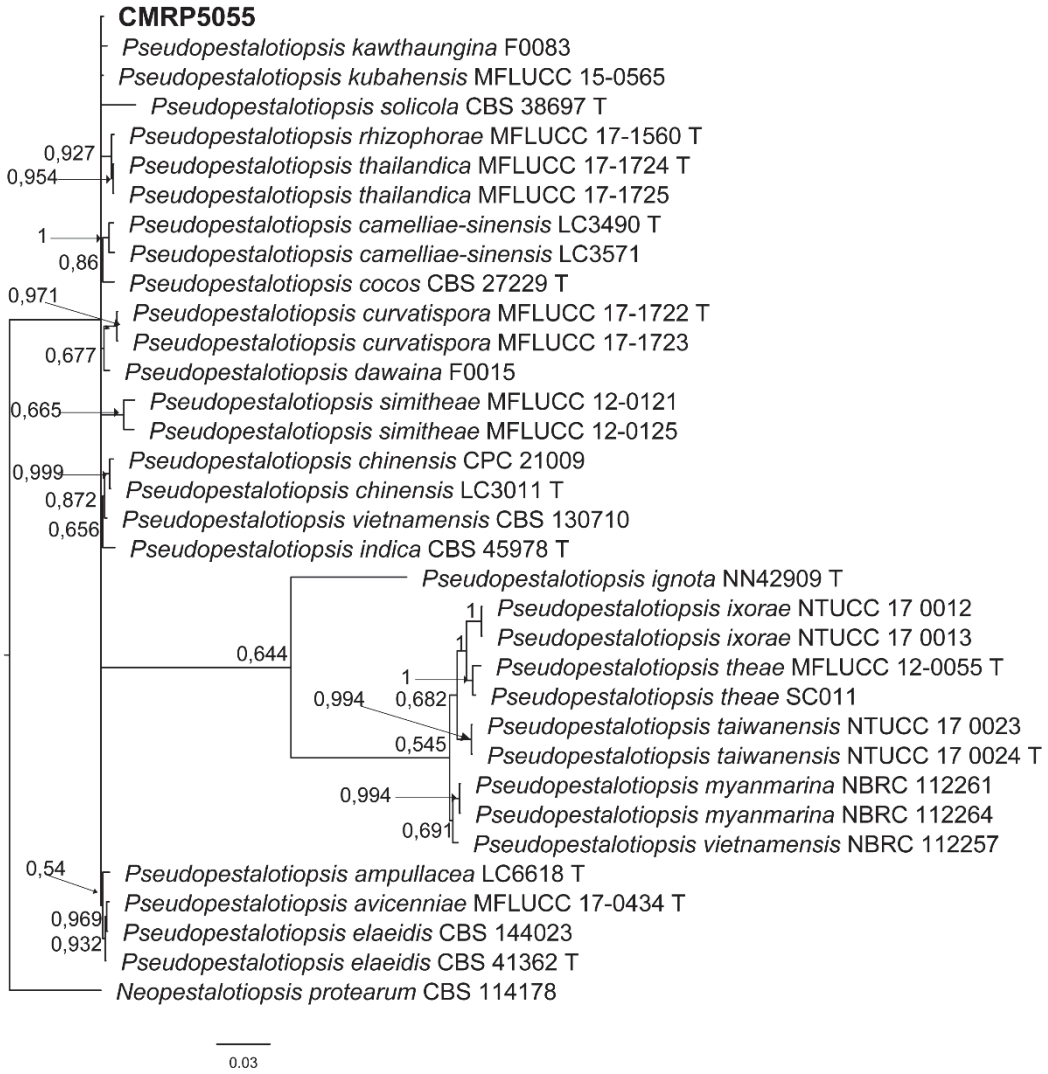

Figure 10: Bayesian Inference phylogenetic tree of *Pseudopestalotiopsis* species based on multiple alignment of ITS, *tef1* and *tub2* partial sequences. The data matrix had 34 taxa and 2447 characters. The species *Neopestalotiopsis protearum* (CBS 114178) was used as outgroup. Strains marked with a “T” correspond to type sequences. Bayesian posterior probabilities equal or greater than 0.50 are presented next to each node. The scale bar of 0.03 represents the number of changes. The sequence of the isolate here studied is presented with its culture collection code (CMRP5055) highlighted in bold.

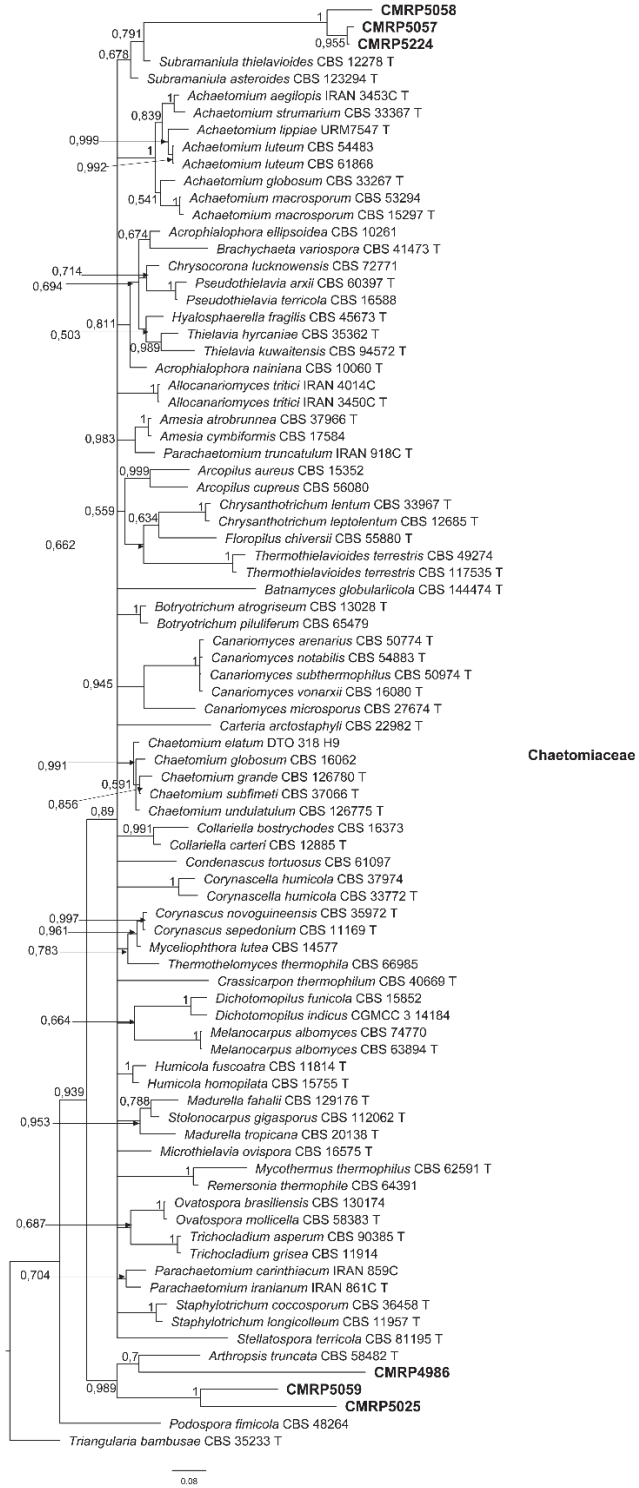

Figure S11: Bayesian Inference phylogenetic tree of menbers of Chaetomiaceae family based on alignment of ITS partial sequence. The data matrix had 34 taxa and 2447 characters. The species *Podospora fimicola* (CBS 48.264) and *Triangularia bambusae* (CBS 35.233) (Podosporaceae) were used as outgroup. Strains marked with a “T” correspond to type sequences. Bayesian posterior probabilities equal or greater than 0.50 are presented next to each node. The scale bar of 0.08 represents the number of changes. The sequence of the isolates here studied are presented with its culture collection code (CMRP5058; CMRP5057; 44-P15F5F1; CMRP4986; CMRP5059; CMRP5025) highlighted in bold.

Table S1- Identification of endophytic fungi isolated from *Vochysia divergens* used for antibacterial activity evaluation

Collection n°	Identification	Collection n°	Identification
CMRP4971	*Phyllosticta capitalensis*	CMRP4993	*Anthostomelloides forlicesenica*
CMRP4972	*Phyllosticta capitalensis*	CMRP5225	*Polyscytalum* sp.
CMRP4973	*Nigrospora* sp.	CMRP5028	*Periconia ignaria*
CMRP4974	*Colletotrichum* sp. *gloeosporioides* complex	CMRP4994	*Diaporthe vochysiae*
CMRP4975	*Nigrospora brasiliensis*	CMRP4995	*Colletotrichum* sp. *gloeosporioides* complex
CMRP5218	*Aspergillus* sp. *Subgen. Circundati, sect.* Nigri*, ser.* Japonici	CMRP4996	*Diaporthe* sp.
CMRP5011	*Hypoxylon* sp.	CMRP5029	*Diaporthe* sp.
CMRP4976	*Pseudofusicoccum stromaticum*	CMRP5030	*Colletotrichum* sp. *boninense* complex
CMRP5219	*Pseudofusicoccum stromaticum*	CMRP5031	*Diaporthe vochysiae*
CMRP5012	*Fusarium* sp.	CMRP5032	*Pestalotiopsis* sp.
CMRP4977	*Diaporthe vochysiae*	CMRP5033	*Diaporthe cf heveae 1*
CMRP4978	*Diaporthe vochysiae*	CMRP5034	*Colletotrichum* sp. *boninense* complex
CMRP4979	*Nigrospora* sp.	CMRP4997	*Diaporthe amolarii*
CMRP4980	*Nigrospora* sp.	CMRP5035	*Curvularia* sp.
CMRP4981	*Neopestalotiopsis egyptiaca*	CMRP5036	*Nigrospora* sp.
CMRP4982	*Pseudofusicoccum stromaticum*	CMRP5037	*Endomelanconiopsis* sp.
CMRP4983	*Anthostomelloides forlicesenica*	CMRP5038	*Colletotrichum gigasporum*
CMRP5220	*Diaporthe* sp.	CMRP5039	*Nigrospora* sp.
CMRP4984	*Colletotrichum* sp. *gloeosporioides* complex	CMRP4998	*Lasiodiplodia pontae*
CMRP5013	*Neurospora sp.*	CMRP5040	*Nigrospora* sp.
CMRP5014	*Neurospora* sp.	CMRP5041	*Nigrospora* sp.
CMRP5221	*Colletotrichum* sp. *acutatum* complex	CMRP5042	*Pseudofusicoccum stromaticum*
CMRP4985	*Diaporthe* sp.	CMRP4999	*Colletotrichum* sp. *gloeosporioides* complex
CMRP5015	*Talaromyces* sp. sec. *Talaromyces*	CMRP5043	*Phyllosticta capitalensis*
CMRP5016	*Cladosporium* sp. *cladosporioides* complex	CMRP5226	*Cladosporium* sp. *cladosporioides* complex
CMRP5017	*Disculoides* sp.	CMRP5044	*Cladosporium* sp. *cladosporioides* complex
CMRP5018	*Barrmaelia* sp.	CMRP5227	*Penicillium sp. (subgen. Aspergilloides, sect. Citrina, ser. Citrina)*

CMRP5019 *Annellosympodiella* sp.
CMRP5020 *Annellosympodiella* sp.
CMRP5021 *Devonomyces* sp.

CMRP5022 *Pseudocercospora* sp.
CMRP4986 Chaetomiaceae sp.
CMRP4987 *Nemania primolutea*
CMRP4988 *Anthostomelloides* sp.

CMRP5222 *Colletotrichum* sp. *acutatum* complex
CMRP4989 *Diaporthe* sp.
CMRP4990 *Diaporthe* sp.
CMRP5023 *Barrmaelia* sp.
CMRP5223 *Fusarium* sp.
CMRP5024 *Neurospora sublineolata*
CMRP4991 *Curvularia* sp.
CMRP4992 *Anthostomeloides* sp.
CMRP5025 Chaetomiaceae sp.
CMRP5224 Chaetomiaceae sp.
CMRP5026 *Prosopidicola* sp.
CMRP5027 *Cladosporium* sp. *cladosporioides* complex

CMRP5045 *Anthostomelloides forlicesenica*
CMRP5046 *Alternaria* sp.
CMRP5000 *Neopestalotiopsis* sp.

CMRP5047 *Paecilomyces* sp.
CMRP5048 *Hypoxylon* sp.
CMRP5049 *Corynespora* sp.
CMRP5050 *Anthostomelloides* sp.

CMRP5051 *Colletotrichum* sp. *boninense* complex
CMRP5052 *Disculoides* sp.
CMRP5053 *Colletotrichum* sp. *gloeosporioides* complex
CMRP5054 *Xylaria arbuscula*
CMRP5001 *Colletotrichum* sp. *gloeosporioides* complex
CMRP5055 *Pseudopestalotiopsis* sp.
CMRP5056 *Diaporthe infertilis*
CMRP5057 Chaetomiaceae sp.
CMRP5058 Chaetomiaceae sp.
CMRP5228 *Colletotrichum* sp. *acutatum* complex
CMRP5059 Chaetomiaceae sp.

3 CONCLUSÃO

Dessa forma, neste estudo demonstramos o potencial biotecnológico de fungos endofíticos isolados da planta medicinal *V. divergens* do bioma brasileiro pantanal para a descoberta de compostos com atividade antifúngica e antibacteriana. Podendo estes compostos, uma vez que purificados e identificados servirem para o controle de fungos fitopatogênicos na agricultura e para o tratamento de doenças causadas por bactérias multirresistentes, os quais carecem de fungicidas e medicamentos atualmente. Além disso, reafirmamos a importância da conservação da comunidade endofítica isolada *ex-situ*, permitindo a preservação e a continuidade dos estudos com os mesmos.

4 REFERÊNCIAS

AMARAL, L. D. S. *et al.* An HPLC evaluation of cytochalasin D biosynthesis by Xylaria arbuscula cultivated in different media. **Natural Product Communications**, v. 9, n. 9, p. 1279–1282, 2014. Disponível em: https://doi.org/10.1177/1934578x1400900914

AMARAL, L. S. *et al.* Biosynthesis and mass spectral fragmentation pathways of 13C and 15N labeled cytochalasin D produced by Xylaria arbuscula. **Journal of Mass Spectrometry**, v. 52, n. 4, p. 239–247, 2017. Disponível em: https://doi.org/10.1002/jms.3922

AMARAL, L. S. Análise de metabólitos secundários produzidos por fungos endofíticos associados à Cupressus lusitanica. p. 164, 2009.

AMORIM, M. R. D. *et al.* Compounds of Anthostomella brabeji, an Endophytic Fungus Isolated from Paepalanthus planifolius (Eriocaulaceae). v. 27, n. 6, p. 1048–1054, 2016.

ARAÚJO, W. L. et al. **Microrganismos endofíticos: Aspectos teóricos e práticos de isolamento e caracterização.** 2a ed- Santarém: UFOPA, 2014.

ARIEIRA, J.; DA CUNHA, C. N. Fitossociologia de uma floresta inundável monodominante de *Vochysia divergens* Pohl (Vochysiaceae), no Pantanal Norte, MT, Brasil. **Acta Botanica Brasilica**, v. 20, n. 3, p. 569–580, 2006.

BAIRIER, G. Mycothèque de l'École de Pharmacie. XI. Paecilomyces, genre nouveau de Mecédinées. **Bulletin de la Société Mycologique de France**. 23:26-27. 1907.

BALDASSARI, R. B.; REIS, R. F.; DE GOES, A. Susceptibility of fruits of the "Valência" and "Natal" sweet orange varieties to *Guignardia citricarpa* and the influence of the coexistence of healthy and symptomatic fruits. **Fitopatologia Brasileira**, v. 31, n. 4, p. 337–341, 2006.

BENSCH, K. *et al.* The genus cladosporium. **Studies in Mycology**. v. 72, p. 1–401, 2012.

BRASIL. Ministério do Meio Ambiente. **Pantanal.** Disponível em: <http://www.mma.gov.br/biomas/pantanal.html> Acesso em: 10 jan. 2021.

BROWN, N. A.; ANTONIW, J.; HAMMOND-KOSACK, K. E. The predicted secretome of the plant pathogenic fungus *Fusarium graminearum*: A refined comparative analysis. **PLoS ONE**, v. 7, n. 4, 2012.

CARBONE, I.; KOHN, L. M. A method for designing primer sets for speciation studies in filamentous ascomycetes. **Mycologia**, v. 91, n. 3, p. 553–556, 1999. Disponível em: https://doi.org/10.1080/00275514.1999.12061051

CHAPLA, V. M. *et al.* Bioactive secondary metabolites from Phomopsis sp., an endophytic fungus from *Senna spectabilis*. **Molecules**, v. 19, n. 5, p. 6597–6608, 2014.

CHEN, J. *et al.* Diversity and Taxonomy of Endophytic Xylariaceous Fungi from Medicinal Plants of Dendrobium (Orchidaceae). **PLoS ONE**, v. 8, n. 3, 2013. Disponível em: https://doi.org/10.1371/journal.pone.0058268

CHEPKIRUI, C.; STADLER, M. The genus Diaporthe: a rich source of diverse and bioactive metabolites. **Mycological Progress**, v. 16, n. 5, p. 477–494, 2017. Disponível em: https://doi.org/10.1007/s11557-017-1288-y

COSTELLOE, C. *et al.* Effect of antibiotic prescribing in primary care on antimicrobial resistance in individual patients: Systematic review and meta-analysis. **BMJ (Online)**, v. 340, n. 7756, p. 1120, 2010.

CROUS, P. W. *et al.* Fungal planet description sheets: 154-213. **Persoonia: Molecular Phylogeny and Evolution of Fungi**, v. 31, n. January 2014, p. 188–296, 2013. Disponível em: https://doi.org/10.3767/003158513X675925

CROUS, P. W. *et al.* New and Interesting Fungi. 2. **Fungal Systematics and Evolution**, v. 3, n. June, p. 171–184, 2019. Disponível em: https://doi.org/doi.org/10.3114/fuse.2020.05.13 Japanese

CROUS, P. W. *et al.* Fungal Planet description sheets : 951 – 1041. p. 223–425, 2019.

CROUS, P. W. *et al.* Calonectria species and their Cylindrocladium anamorphs:

Species with sphaeropedunculate vesicles. **Studies in Mycology**, v. 50, n. 2, p. 415–430, 2004.

DARRIBA, Diego *et al.* JModelTest 2: More models, new heuristics and parallel computing. **Nature Methods**, [*s. l.*], v. 9, n. 8, p. 772, 2012. Disponível em: https://doi.org/10.1038/nmeth.2109

DAYARATHNE, MC *et al.* Morpho-molecular characterization of microfungi associated with marine based habitats. **Mycosphere**, [*s. l.*], v. 11, n. 1, p. 1–188, 2020. Disponível em: https://doi.org/10.5943/mycosphere/11/1/1

DE HOOG, G. S.; GERRITS VAN DEN ENDE, A. H.G. Molecular diagnostics of clinical strains of filamentous Basidiomycetes. **Mycoses**, [*s. l.*], v. 41, n. 5–6, p. 183–189, 1998. Disponível em: https://doi.org/10.1111/j.1439-0507.1998.tb00321.x

DEEPIKA, V.B.; MURALI, T.S.; SATYAMOORTHY, K. Modulation of genetic clusters for synthesis of bioactive molecules in fungal endophytes: A review. **Microbiological Research**, v. 182, p. 125–140, 2016.

DISSANAYAKE, A. J. *et al.* Molecular phylogenetic analysis reveals seven new Diaporthe species from Italy. **Mycosphere**, v. 8, n. 5, p. 853–877, 2017.

DOS SANTOS, I. P. *et al.* Antibacterial activity of endophytic fungi from leaves of *Indigofera suffruticosa* Miller (Fabaceae). **Frontiers in Microbiology**, v. 6, n. MAY, p. 1–7, 2015.

DU, Z. *et al.* Phylogeny and morphology reveal two new species of Diaporthe from Traditional Chinese Medicine in Northeast China. **Phytotaxa**, v. 269, n. 2, p. 090–102, 2016. Disponível em: https://doi.org/10.11646/phytotaxa.336.2.3

EDWARDS, R. L. *et al.* The Xylariaceae as phytopathogens. **Recent Research Development Plant Science**, v. 1, n. January, p. 1–19, 2003.

FONTES, Eliana Maria Gouveia; VALADARES-INGLIS, Maria Cleria. **Controle biológico de pragas da agricultura**. 2020.

FOURNIER, J. *et al. Xylaria aethiopica* sp. nov. – a new pod-inhabiting species of Xylaria (Xylariaceae) from Ethiopia. **Ascomycete.org**, v. 10, n. 5, p. 209–215,

2018. Disponível em: https://doi.org/10.25664/ART-244

GAO, Y. *et al. Diaporthe* is paraphyletic. **IMA Fungus**, v. 8, n. 1, p. 153–187, 2017. Disponível em: https://doi.org/10.5598/imafungus.2017.08.01.11

GLASS, N. L.; DONALDSON, G. C. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. **Applied and Environmental Microbiology**, [*s. l.*], v. 61, n. 4, p. 1323–1330, 1995. Disponível em: https://doi.org/10.1128/aem.61.4.1323-1330.1995

GLIENKE, C. *et al.* Endophytic and pathogenic Phyllosticta species, with reference to those associated with Citrus Black spot. **Persoonia: Molecular Phylogeny and Evolution of Fungi**, v. 26, p. 47–56, 2011. Disponível em: https://doi.org/10.3767/003158511X569169

GLIENKE, C. *et al.* Antimicrobial Activity of Endophytes from Brazilian Medicinal Plants. **Antimicrobial Agents**, 2012.

GOMES, R. R. *et al.* Diaporthe: A genus of endophytic, saprobic and plant pathogenic fungi. **Persoonia: Molecular Phylogeny and Evolution of Fungi**, [*s. l.*], v. 31, p. 1–41, 2013. Disponível em: https://doi.org/10.3767/003158513x666844

GOS, F. M. W. R. *et al.* Antibacterial Activity of Endophytic Actinomycetes Isolated from the Medicinal Plant *Vochysia divergens* (Pantanal, Brazil). **Frontiers in Microbiology**, 2017.

GUO, Y. S. *et al.* High diversity of Diaporthe species associated with pear shoot canker in China. **Persoonia - Molecular Phylogeny and Evolution of Fungi**, p. 132–162, 2020. Disponível em: https://doi.org/10.3767/persoonia.2020.45.05

HALL, T. A. **BioEdit4.8**. Raileigh, 1997-2001. 1 arquivo (11,5M); Disponível em: http://www.mbio.ncsu.edu/bioedit.html BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windons 95/98/NT.

HASHEMI, S. A. *et al.* A new Xylaria species from Iran. **Mycologia Iranica**, v. 2, n. 1, p. 1–10, 2015. Disponível em: http://mi.iranjournals.ir/article_13603_2.html

HILÁRIO, S. *et al.* Diaporthe species associated with twig blight and dieback of

Vaccinium corymbosum in Portugal, with description of four new species. **Mycologia**, [*s. l.*], v. 112, n. 2, p. 293–308, 2020. Disponível em: https://doi.org/10.1080/00275514.2019.1698926

HOKAMA, Y., SAVI, D. C., ASSAD, B., ALUIZIO, R., GOMES-FIGUEIREDO, J., ADAMOSKI, D., et al. (2017). “Endophytic fungi isolated from Vochysia divergens in the pantanal, Mato Grosso do Sul: diversity, phylogeny and biocontrol of Phyllosticta citricarpa” in **Endophytic Fungi: Diversity, Characterization and Biocontrol**, 4th Edn, ed E. Hughes (Hauppauge, NY: Nova), 1–25.

HSIEH, H. M. *et al.* Phylogenetic status of Xylaria subgenus Pseudoxylaria among taxa of the subfamily Xylarioideae (Xylariaceae) and phylogeny of the taxa involved in the subfamily. **Molecular Phylogenetics and Evolution**, v. 54, n. 3, p. 957–969, 2010. Disponível em: https://doi.org/10.1016/j.ympev.2009.12.015

HSIEH, H. M.; JU, Y. M.; ROGERS, Jack D. Molecular phylogeny of Hypoxylon and closely related genera. **Mycologia**, v. 97, n. 4, p. 844–865, 2005. Disponível em: https://doi.org/10.3852/mycologia.97.4.844

IBRAHIM, A. *et al.* Metabolomic-guided discovery of cyclic nonribosomal peptides from Xylaria ellisii sp. nov., a leaf and stem endophyte of Vaccinium angustifolium. **Scientific Reports**, v. 10, n. 1, p. 1–17, 2020. Disponível em: https://doi.org/10.1038/s41598-020-61088-x

IDRIS *et al.* Screening of potential lignin-degrading fungi from the tropical forest for lignocellulose biotreatment. **IOP Conference Series: Earth and Environmental Science**, v. 308, n. 1, p. 0–17, 2019. Disponível em: https://doi.org/10.1088/1755-1315/308/1/012014

ISMAIL, A. M. *et al. Lasiodiplodia* species associated with dieback disease of mango (*Mangifera indica*) in Egypt. **Australasian Plant Pathology**, v. 41, n. 6, p. 649–660, 2012.

JU, Y. M. *et al.* New and interesting penzigioid Xylaria species with small, soft stromata. **Mycologia**, v. 104, n. 3, p. 766–776, 2012. Disponível em: https://doi.org/10.3852/11-313

JU, Y. M.; HSIEH, H. M. Xylaria species associated with nests of Odontotermes formosanus in Taiwan. **Mycologia**, v. 99, n. 6, p. 936–957, 2007. Disponível em: https://doi.org/10.3852/mycologia.99.6.936

JU, Y. M.; HSIEH, H. M.; HE, X. S. Xylaria coprinicola, a new species that antagonizes cultivation of coprinus comatus in china. **Mycologia**, v. 103, n. 2, p. 424–430, 2011. Disponível em: https://doi.org/10.3852/10-215

JU, Y. M.; ROGERS, J. D.; HSIEH, H. M. New Hypoxylon and Nemania species from Costa Rica and Taiwan. **Mycologia**, v. 97, n. 2, p. 562–567, 2005. Disponível em: https://doi.org/10.1080/15572536.2006.11832831

JUNK, W. J.; BAYLEY, P. B.; SPARKS, R. E. The Flood Pulse Concept in River-Floodplain Systems. **Canadian Journal of Fisheries and Aquatic Sciences**, n. September, 1989.

KATOH, K.; TOH, H. Recent developments in the MAFFT multiple sequence alignment program. **Briefings in Bioinformatics**, v. 9, n. 4, p. 286–298, 2008. Disponível em: https://doi.org/10.1093/bib/bbn013

KENERLEY, C. M.; ROGERS, J. D. On Hypoxylon serpens in Culture. **Mycologia**, v. 68, n. 3, p. 688, 1976. Disponível em: https://doi.org/10.2307/3758993

KHAN, M. I. H. *et al.* Cytotoxic and antibacterial naphthoquinones from an endophytic fungus, *Cladosporium* sp. **Toxicology Reports**, v. 3, p. 861–865, 2016.

KIM, C. S. *et al.* New records of Xylaria species in Korea: X. ripicola sp. nov. and X. tentaculata. **Mycobiology**, v. 44, n. 1, p. 21–28, 2016. Disponível em: https://doi.org/10.5941/MYCO.2016.44.1.21

KUHNERT, E. *et al.* New Hypoxylon species from Martinique and new evidence on the molecular phylogeny of Hypoxylon based on ITS rDNA and β-tubulin data. **Fungal Diversity**, v. 64, n. 1, p. 181–203, 2014. Disponível em: https://doi.org/10.1007/s13225-013-0264-3

LI, X. Q. *et al.* A Systematic Review on Secondary Metabolites of *Paecilomyces* Species: Chemical Diversity and Biological Activity. **Planta Medica**, v. 86, n. 12, p. 805–821, 2020.

LIMA, W. G. *et al. Colletotrichum gloeosporioides*, a new causal agent of citrus

post-bloom fruit drop. **European Journal of Plant Pathology**, v. 131, n. 1, p. 157–165, 2011.

LINK, H. F. Observationes in ordines plantarum naturales 2. **Magazin der Gesellschaft Naturforschenden Freunde Berlin**. 7: 25-45. 1816.

LIU, J. *et al.* Antibacterial polyketides from the jellyfish-derived fungus *Paecilomyces variotii*. **Journal of Natural Products**, v. 74, n. 8, p. 1826–1829, 2011.

MAHARACHCHIKUMBURA, S. S. N. *et al.* Pestalotiopsis revisited. **Studies in Mycology**, v. 79, n. 1, p. 121–186, 2014.

MEDEIROS, A. G. *et al.* Bioprospecting of *Diaporthe terebinthifolii* LGMF907 for antimicrobial compounds. **Folia Microbiologica**, v. 63, n. 4, p. 499–505, 2018.

MILLER, Mark A.; PFEIFFER, Wayne; SCHWARTZ, Terri. The CIPRES science gateway: A community resource for phylogenetic analyses. **Proceedings of the TeraGrid 2011 Conference: Extreme Digital Discovery, TG'11**, 2011. Disponível em: https://doi.org/10.1145/2016741.2016785

MORANDI, M. A. B. *et al.* Controle biológico de fungos fitopatogênicos. **Informe Agropecuário**, v. 30, p. 73-82, 2009.

MOREIRA, V. F. **Rede de Proteção e Conservação da Serra do Amolar: Rompendo fronteiras para a conservação do Pantanal.** Dissertação, Universidade Federal do Mato Grosso do Sul, Corumbá, 2011.

MORRIS, S.; CERCEO, E.. Trends, epidemiology, and management of multi-drug resistant gram-negative bacterial infections in the hospitalized setting. **Antibiotics**, v. 9, n. 4, p. 1–20, 2020.

MOSADEGHZAD, Z. *et al.* Chemical components and bioactivity of the marine-derived fungus *Paecilomyces* sp. Collected from Tinggi Island, Malaysia. **Chemistry of Natural Compounds**, v. 49, n. 4, p. 621–625, 2013.

NORILER, S. A. *et al.* Bioprospecting and structure of fungal endophyte communities found in the Brazilian biomes, pantanal, and Cerrado. **Frontiers in**

Microbiology, 2018.

NORILER, S. A. *et al.* Vochysiamides A and B: Two new bioactive carboxamides produced by the new species *Diaporthe vochysiae*. **Fitoterapia**, v. 138, n. May, p. 104273, 2019.

O'DONNELL, K.; CIGELNIK, E. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus Fusarium are nonorthologous. **Molecular Phylogenetics and Evolution**, v. 7, n. 1, p. 103–116, 1997. Disponível em: https://doi.org/10.1006/mpev.1996.0376

OERKE, E. C. Centenary Review: Crop losses to pests. **Journal of Agricultural Science**, n. 144, p. 31–43, 2006. Disponível em: https://doi.org/10.1017/S0021859605005708

PACCANARO, M. C. *et al.* Synergistic Effect of Different Plant Cell Wall–Degrading Enzymes Is Important for Virulence of *Fusarium graminearum*. **Molecular Plant-Microbe Interactions**, v. 30, n. 11, p. 886–895, 2017.

PAGNUSSATT, F. A. *et al.* Technological and nutritional assessment of dry pasta with oatmeal and the microalga Spirulina platensis. **Brazilian Journal of Food Technology**, v. 17, n. 4, p. 296–304, 2014. Disponível em: https://doi.org/10.1590/1981-6723.1414

PEREIRA, M. C. B. **Bacia Hidrográficado rio Miranda: estado da arte.** Campo Grande: UCDB, 2004.

PETRINI, O. et al. Ecology, metabolite production, and substrate utilization in endophytic fungi. **Natural Toxins**, v. 1, n. 3, p. 185–196, 1993.

PINHO, D. B.; LOPES, U. P.; PEREIRA, O. L. Colletotrichum abscissum. **Fungal Planet**, v. 34, n. 357, p. 236–237, 2015.

RAEDER, U.; BRODA, P. Rapid preparation of DNA from filamentous fungi. **Letters in Applied Microbiology**, v. 1, n. 1, p. 17–20, 1985. Disponível em: https://doi.org/10.1111/j.1472-765X.1985.tb01479.x

RONQUIST, F. *et al.* Mrbayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. **Systematic Biology**, v. 61, n. 3, p.

539–542, 2012. Disponível em: https://doi.org/10.1093/sysbio/sys029

SÁNCHEZ-BALLESTEROS, J. *et al.* Phylogenetic Study of Hypoxylon and Related Genera Based on Ribosomal ITS Sequences Maria A . Portal , María Julián , Víctor Rubio , Gerald F . Bills , Jon D . Polishook , Gonzalo Platas , Sagrario Mochales and Fernando Peláez REFERENCES Linked references. **Mycologya**, [*s. l.*], v. 92, n. 5, p. 964–977, 2000.

SANTOS, P. J. C. D. *et al.* Diaporthe endophytica and D. terebinthifolii from medicinal plants for biological control of Phyllosticta citricarpa. **Microbiological Research**, v. 186–187, p. 153–160, 2016. Disponível em: https://doi.org/10.1016/j.micres.2016.04.002

SAVI, D C *et al.* Antitumor, Antioxidant and Antibacterial Activities of Secondary Metabolites Extracted By Endophytic Actinomycetes Isolated From Vochysia Divergens. **International Journal of Pharmaceutical, Chemical & Biological Sciences**, v. 5, n. 1, p. 347–356, 2015. Disponível em: http://search.ebscohost.com/login.aspx?direct=true&db=a9h&AN=115922571&site=ehost-live

SAVI, D. C. *et al. Phaeophleospora vochysiae* Savi & Glienke sp. nov. Isolated from *Vochysia divergens* Found in the Pantanal, Brazil, Produces Bioactive Secondary Metabolites. **Scientific Reports**, 2018.

SAVI, D. C. *et al.* Secondary metabolites produced by the citrus phytopathogen *Phyllosticta citricarpa*. **Journal of Antibiotics**, v. 72, n. 5, p. 306–310, 2019a.

SAVI, D.; ALUIZIO, R.; GLIENKE, C.. Brazilian Plants: An Unexplored Source of Endophytes as Producers of Active Metabolites. **Planta Medica**, 2019b. Disponível em: https://doi.org/10.1055/a-0847-1532

SCHULZ, B. *et al.* Endophytic fungi: A source of novel biologically active secondary metabolites. **Mycological Research**, v. 106, n. 9, p. 996–1004, 2002. Disponível em: https://doi.org/10.1017/S0953756202006342

SILVA, A. O. *et al.* Identification of *Colletotrichum* species associated with postbloom fruit drop in Brazil through GAPDH sequencing analysis and multiplex PCR. **European Journal of Plant Pathology**, v. 147, n. 4, p. 731–748, 2017.

SLIPPERS, B. *et al.* Phylogenetic lineages in the botryosphaeriales: A systematic

and evolutionary framework. **Studies in Mycology**, v. 76, p. 31–49, 2013.

SMITH, D. *et al.* The Ex Situ Conservation of Microorganisms : Aiming At a Certified Quality Management. **Encyclopedia of Life Support Systems**, n. January, 2008.

STADLER, M. Importance of secondary metabolites in the Xylariaceae as parameters for. **Current Research in Environmental & Applied Mycology**, v. 1, n. 2, p. 75–133, 2011. Disponível em: https://doi.org/10.5943/cream/1/2/1

STADLER, M. *et al.* The Xylariaceae as model example for a unified nomenclature following the “One Fungus-One Name” (1F1N) concept. **Mycology**, v. 4, n. 1, p. 5–21, 2013. Disponível em: https://doi.org/10.1080/21501203.2013.782478

STIERLE, A. A.; STIERLE, D. B. Bioactive secondary metabolites produced by the fungal endophytes of conifers. **Natural Product Communications**, v. 10, n. 10, p. 1671–1682, 2015.

STROBEL, G.; DAISY, B.. Bioprospecting for microbial endophytes and their natural products. **Microbiology and molecular biology reviews : MMBR**, v. 67, n. 4, p. 491–502, 2003.

TAMURA, K. *et al.* MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. **Molecular Biology and Evolution**, v. 28, n. 10, p. 2731–2739, 2011. Disponível em: https://doi.org/10.1093/molbev/msr121

TAN, M. A. *et al.* Biodiscovery of antibacterial constituents from the endolichenic fungi isolated from Parmotrema rampoddense. **3 Biotech**, v. 10, n. 5, p. 1–7, 2020. Disponível em: https://doi.org/10.1007/s13205-020-02213-5

TANAPICHATSAKUL, C. *et al.* Production of eugenol from fungal endophytes Neopestalotiopsis sp. and *Diaporthe* sp. isolated from *Cinnamomum loureiroi* leaves . **PeerJ**, v. 7, p. e6427, 2019.

TANG, A. M. C.; JEEWON, R.; HYDE, K. D. Phylogenetic relationships of Nemania plumbea sp. nov. and related taxa based on ribosomal ITS and RPB2

sequences. **Mycological Research**, v. 111, n. 4, p. 392–402, 2007. Disponível em: https://doi.org/10.1016/j.mycres.2007.01.009

TANG, A. M. C.; LAM, R. Y. C.; LEUNG, M. W. K. Xylaria hongkongensis sp. nov. from an urban tree. **Mycotaxon**, v. 128, n. August, p. 37–40, 2014. Disponível em: https://doi.org/10.5248/128.37

THOMPSON, S. M. *et al.* Green and brown bridges between weeds and crops reveal novel Diaporthe species in Australia. **Persoonia: Molecular Phylogeny and Evolution of Fungi**, v. 35, n. 1, p. 39–49, 2015. Disponível em: https://doi.org/10.3767/003158515X687506

TIBPROMMA, S. *et al.* Anthostomelloides krabiensis gen. Et sp. Nov. (xylariaceae) from Pandanus odorifer (pandanaceae). **Turkish Journal of Botany**, v. 41, n. 1, p. 107–116, 2017. Disponível em: https://doi.org/10.3906/bot-1606-45

TONIAL, F. *et al.* Biological activity of *Diaporthe terebinthifolii* extracts against *Phyllosticta citricarpa*. **FEMS Microbiology Letters**, v. 364, n. 5, p. 1–7, 2017.

TOCANTINS, N. **Áreas protegidas e turismo, estudo de caso: Parque Nacional do Pantanal Matogrossense/MT e seu contorno.** Tese, Universidade Federal de São Carlos, 2005.

U'REN, J. M. *et al.* Contributions of North American endophytes to the phylogeny, ecology, and taxonomy of Xylariaceae (Sordariomycetes, Ascomycota). **Molecular Phylogenetics and Evolution**, v. 98, p. 210–232, 2016. Disponível em: https://doi.org/10.1016/j.ympev.2016.02.010

UDAYANGA, D. *et al.* Species limits in Diaporthe: molecular re-assessment of D. citri, D. cytosporella, D. foeniculina and D. rudis. **Persoonia**, v. 32, p. 83–101, 2014.

UDAYANGA, D. *et al.* Multi-locus phylogeny reveals three new species of Diaporthe from Thailand. **Cryptogamie, Mycologie**, v. 33, n. 3, p. 295–309, 2012. Disponível em: https://doi.org/10.7872/crym.v33.iss3.2012.295

UDAYANGA, D. *et al.* The genus Phomopsis: Biology, applications, species concepts and names of common phytopathogens. **Fungal Diversity**, v. 50, n. September, p. 189–225, 2011. Disponível em: https://doi.org/10.1007/s13225-

011-0126-9

VENIERAKI, A.; DIMOU, M.; KATINAKIS, P. Endophytic fungi residing in medicinal plants have the ability to produce the same or similar pharmacologically active secondary metabolites as their hosts. **Hellenic Plant Protection Journal**, v. 10, n. 2, p. 51–66, 2017.

VU, D. *et al.* Large-scale generation and analysis of filamentous fungal DNA barcodes boosts coverage for kingdom fungi and reveals thresholds for fungal species and higher taxon delimitation. **Studies in Mycology**, v. 92, n. October, p. 135–154, 2019. Disponível em: https://doi.org/10.1016/j.simyco.2018.05.001

WEIR, B. S.; JOHNSTON, P. R.; DAMM, U. The *Colletotrichum gloeosporioides* species complex. **Studies in Mycology**, v. 73, p. 115–180, 2012.

WENDT, L. *et al.* **Resurrection and emendation of the Hypoxylaceae, recognised from a multigene phylogeny of the Xylariales**. 2018. ISSN 18618952.v. 17 Disponível em: https://doi.org/10.1007/s11557-017-1311-3

WHITE, T. J. *et al.* **Amplification and Direct Sequencing of Fungal Risosomal RNA Genes for Phylogenetics**. : Academic Press, Inc., 1990. ISSN 08953988.v. 2 Disponível em: https://doi.org/10.1016/B978-0-12-372180-8.50042-1

WHO. **WHO publishes listo of bacteria for which new antibiotics are urgentely needed.** World Health Organization. 2017. Disponível em: <https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed> Acesso em: 10 jan. 2021.

WILLYARD, C. The drug-resistant bacteria that pose the greatest health threats. **Nature**, v. 543, n. 7643, p. 15, 2017.

WULANDARI, N. F. *et al.* Phyllosticta citriasiana sp. nov., the cause of Citrus tan spot of Citrus maxima in Asia. **Fungal Diversity**, v. 34, n. January, p. 23–39, 2009.

YU, H. *et al.* Recent developments and future prospects of antimicrobial metabolites produced by endophytes. **Microbiological Research**, v. 165, n. 6, p. 437–449, 2010.