An Acad Bras Cienc (2024) 96(Suppl. 1): e20230251 DOI 10.1590/0001-3765202420230251
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

MICROBIOLOGY

Endophytic fungi associated with *Araucaria angustifolia* (Bertol.) Kuntze

DANIELI B. GROFF, JÉSSICA MARMENTINI, ANDRÉ LUIZ GAGLIOTI, PAULO ROBERTO DA SILVA & ADRIANA KNOB

Abstract: The diversity of endophytes and their ecological relationships with the endangered conifer *Araucaria angustifolia* (a critically endangered species) are unrevealed. This study aimed to characterize the diversity of endophytic fungi associated with *A. angustifolia*. To this end, we analyzed 90 fragments from five individuals collected from a mixed localized fragment in Guarapuava-PR, Brazil. The total DNA of 61 morphotypes was extracted and the Internal Transcribed Spacer (ITS) region was amplified and sequenced. The sequence analysis allowed the identification of 37 genera belonging to the phylum Ascomycota and the classes Eurotiomycetes, Dothideomycetes, and Sordariomycetes, divided into 11 orders and 13 families. Most of the isolated fungi belonged to the Sordariomycetes class (40%) and to the *Xylaria* genus (14%), while Eurotiomycetes was the minority class within the community. Our results reveal the high endophytic richness supporting the life cycle of *A. angustifolia* and reinforce the necessity for the conservation of this conifer, as many genetic resources can be lost owing to its irrational exploration.

Key words: Brazilian biome, diversity, endophytic, fungi.

INTRODUCTION

The *Araucaria* forest is the main original forest type in southern Brazil, while it also occurs in some states in the southeastern region and northeastern Argentina (Carlucci et al. 2021). It is characterized by the presence of the conifer *Araucaria angustifolia*, commonly known as Paraná pine or Brazilian pine (Campanili & Schaffer 2010). *Araucaria angustifolia* is a gymnosperm that belongs to the order Pinales, class Pinopsida, and family Araucariaceae. Despite its apparent structural simplicity, the *Araucaria* forest has an enormous richness of species of the most varied taxa (Ribeiro et al. 2009).

Like many other Brazilian environments, the *Araucaria* forest suffers a sharp and continuous process of fragmentation. Originally, the *Araucaria* forest covered 20 million hectares in southern and southeastern Brazil (Campanili & Schaffer 2010). The intense anthropogenic pressure on the *Araucaria* forest reduced it to 12.6% of its original area by 2005 (Ribeiro et al. 2009), which applies to this day (Rezende et al. 2018, Carlucci et al. 2021). For this reason, *A. angustifolia* is considered a critically endangered species according to the International Union for Conservation of Nature (Thomas 2013).

It is important to study the *Araucaria* forest not only because this ecosystem has been reduced to small fragments, but also because it contains significant genetic resources that are still unknown to man (Solórzano-Filho & Kraus 1999). The extinction of *A. angustifolia* has resulted in serious consequences for the ecosystem, involving the loss of animal and plant diversity

as well as microorganisms, many of which are still undescribed and possibly associated with this valuable plant species (Ribeiro & Cardoso 2012). For this reason, knowledge on the diversity of organisms associated with the *Araucaria* forest is necessary to stimulate the generation and dissemination of scientific and technological knowledge, in order to promote the conservation of its remnants and stimulate sustainable management.

Endophytic fungi are microorganisms that inhabit plant organs and tissues. Such fungi are distributed by colonizing intercellular spaces at some stage or throughout their life cycle, without causing apparent symptoms of disease or damage related to their presence in the host (Packiam & Dhakshinamoorthy 2021).

Endophytic species are of great interest to researchers because they can increase the adaptive value of the host species, help in their development, support them during adverse conditions, promote their stress tolerance, decrease the development of pathogens, and/or inhibit herbivory by producing metabolites (Jia et al. 2016, Alam et al. 2021, Packiam & Dhakshinamoorthy 2021, Jha et al. 2023).

The diversity of fungal endophytes is high, with more than 22 genera found among plant species (Hamzah et al. 2018, Segaran & Sathiavelu 2019). Knowledge on this group of organisms can elucidate the evolutionary history of plants and fungi (Kusari et al. 2014). Furthermore, as the diversity of endophytic fungi is influenced by plant species and environmental factors, the isolation of these fungi from previously unexplored plant communities may present great potential for the discovery of unique and undescribed secondary metabolites.

To the best of our knowledge, the fungal studies in *A. angustifolia* are focused on phytopathogenic fungi (Hodges & May 1972, Butin & Peredo 1986, Mendes et al. 2010), mycorrhizal fungi (Moreira et al. 2003, 2007, 2012, Silva et al. 2009, Zandavalli et al. 2008, Vilcatoma-Medina et al. 2018), and saprotrophic fungi (da Silva et al. 2021). However, to date, the diversity of endophytic fungi associated with *A. angustifolia* has not yet been assessed. The drastic reduction in the vegetation cover of the *Araucaria* forest indicates that it is an area of extreme biological importance, highlighting its priority in research involving inventorying, conservation, and the rational exploitation of the remaining species. Therefore, we aimed to describe endophytic fungi associated with *A. angustifolia*.

MATERIALS AND METHODS

Samples and isolation of endophytic fungi

In order to isolate endophytic fungi, samples were collected from five *A. angustifolia* trees in Guará district, Guarapuava-PR (25°22'2.917"S 51°17'25.138"W). This area is a fragment of a mixed ombrophilous forest that covers approximately 300 ha. According to the Köppen-Geiger classification, the region is in the Cfb climate zone (mesothermal, humid throughout the year, and mild summer) (Maack 1981). The identification of *A. angustifolia* in site was possible because of the crown characteristics of the genus and this species.

In total, five *A. angustifolia* trees, approximately 10 m high and with no symptoms of disease, were chosen. From each individual, six branches (younger terminal part) were harvested by pruning shears and stored separately (for each tree) in plastic bags. Immediately after collection, the samples were taken to the laboratory for the isolation of endophytic fungi. Surface disinfection was performed for each sample. Initially, each of the six branches of each tree was cut into 10 cm pieces and the needles were removed. Then, the stems were washed in water and neutral detergent with a soft brush

and rinsed in running water and distilled water. The branches were then placed in a laminar flow chamber and subjected to the methodology described by Sun et al. (2011).

After superficial disinfection, the branches were rinsed in sterile distilled water three times and placed on a sterile paper towel to dry. We distributed 100 µL of the water used for the last rinse in a Petri dish containing Sabouraud agar medium supplemented with chloramphenicol (0.05 g L^{-1}) and used it as the negative control. Three 0.5 cm fragments were obtained from each of the branches using tweezers and a sterile scalpel, totaling 90 fragments. These were later arranged equidistantly in Petri dishes with Sabouraud agar medium supplemented with chloramphenicol. The plates were incubated in an oven at 28 °C (±2) in the dark for up to 25 days. The fungi that developed from the plant tissue fragments were collected and placed on plates containing Sabouraud medium until pure colonies were obtained. The strains obtained were grouped into different morphotypes and stored at 4 °C, according to Castellani (1939). All fungi were registered in the Brazilian National System of Genetic Heritage Management and Associated Traditional Knowledge (SisGen) under the number AF5F498.

Molecular identification: DNA extraction, PCR amplification, and sequencing

Distinct fungal morphotypes were molecularly identified. Each strain was cultured in Czapeck medium supplemented with 1% dextrose with a pH of 5.6 and cultivated for 5 days at 28 °C (± 2). After cultivation, the fungal mycelia were filtered through a vacuum pump using sterile filter paper and washed with sterile ultrapure water. Immediately after filtration, the mycelia were macerated using a mortar and pestle with liquid nitrogen to obtain a very fine powder. The cetyltrimethylammonium bromide (CTAB) method proposed by Doyle & Doyle (1987) was used for DNA extraction.

After DNA extraction, amplification was performed using a thermocycler (Mastercycler®, Eppendorf, USA). The PCR amplification of the ITS1–5.8S–ITS2 DNA region was achieved in one fragment using ITS5 forward (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS4 reverse (5'-TCCTCCGCTTATTGATATGC-3') primers, as described by White et al. (1990). The PCR amplification mix of the ITS5-ITS4 comprised approximately 20 ng genomic DNA, 1× BiolaseTM buffer with 1.0 mM $MgCl_2$ (Bioline, London, UK), 10 µM of each primer (forward and reverse), 0.25 mM of each dNTP, and 1 unit of BiolaseTM DNA polymerase (Bioline). The reaction volume was adjusted with ddH_2O to a final volume of 20 µL. The amplification conditions were as follows: initial denaturation at 94 °C for 2 min; 30 cycles of 30 s at 95 °C (denaturation), 60 s at 50 °C (annealing), 60 s at 72 °C, and a final extension at 72 °C for 7 min.

The PCR products were purified using QIAquick PCR purification spin columns (Qiagen) and quantified using a NanoDrop 2000 spectrophotometer with a NanoDrop 2000/2000c (Thermo Fisher Scientific, Inc.).

The sequencing reactions were performed in 10 µL reactions using BigDye Terminator sequencing reagents and protocols (Applied Biosystems, Foster City, California, USA). The data were collected on an ABI-Prism 3500 automated sequencer (Applied Biosystems) by ACTGene Molecular Analyses at the Universidade Federal do Rio Grande do Sul. All sequences were deposited in GenBank.

BLAST (www.blast.ddbj.nig.ac.jp/) was used to verify the homology of the sequences obtained with those deposited in the GenBank NCBI database. Identification at the species level was performed when the sequences obtained presented, in relation to the homologous

reference sequence, coverage greater than 80% and similarity greater than 97%. In addition, when the sequences obtained had homologies with more than 10 different species, the classification was performed only at the genus level (Raja et al. 2017).

Phylogenetic analysis

The phylogenetic tree was constructed using Bayesian analysis (MrBayes v.3.1.2). We used 37 sequences obtained from this study and included more than 25 sequences from the GenBank database. Outgroup taxa belonging to the species *Neurospora crassa*_AY681193 were used.

RESULTS

We obtained 102 filamentous fungi isolates from 90 fragments of *A. angustifolia* sampled. No growth of yeast colonies was observed. At the end of the morphological analysis, 61 morphologically distinct lineages were identified. Of these, one morphotype was selected for the sequencing of the ITS region of the rDNA gene.

Among the 61 morphotypes that were subjected to DNA extraction and amplification of the ITS sequence, 24 did not have their DNA amplified or did not have quality sequences. Therefore, further analyses were carried out with the 37 strains for which molecular identification was possible.

Some endophytes were identified at the genus level, whereas others were identified at the species level. Seventeen genera were observed: *Annulohypoxylon*, *Aspergillus*, *Colletotrichum*, *Coniochaeta*, *Diaporthe*, *Fimetariella*, *Fusarium*, *Hypoxylon*, *Mycoleptodiscus*, *Muyocopron*, *Neofusicoccum*, *Neopestalotiopsis*, *Pestalotiopsis*, *Phyllosticta*, *Preussia*, *Trichoderma*, and *Xylaria*. All genera belong to the phylum Ascomycota and to the classes Eurotiomycetes, Dothideomycetes, and Sordariomycetes, and are divided into 11 orders and 13 families (Table I). Figure 1 shows the diversity of the colony morphology (obverse and reverse) of the endophytic fungi that were identified. The results of the sequences demonstrate that the ITS region, considered a DNA barcode for fungi, was not effective in discriminating many of the strains under investigation at the species level.

The phylogenetic tree analysis (Figure 2) revealed the presence of large clades, which correspond to the three fungal classes found (Sordariomycetes, Eurotiomycetes, and Dothideomycetes). Most of the isolated fungi belonged to the Sordariomycetes class (40%) and the *Xylaria* genus (14%). In contrast, Eurotiomycetes was recognized as a minority class within the community, with strains belonging to a single genus, including the *Aspergillus flavus* and *Aspergillus fumigatus* species. The class Dothideomycetes included representatives of two orders, three families, and three genera.

DISCUSSION

This study presents, for the first time, the characterization of a community of cultivable endophytic fungi associated with individuals of *A. angustifolia* in southern Brazil. Compared to studies carried out with other gymnosperms and angiosperms, the number of species described in the present work is significant, considering the sampling effort of 90 fragments, from five individuals (Hormazabal & Piontelli 2009, Correia et al. 2018, Wang et al. 2019, Ferreira et al. 2020).

The endophytic species isolated were placed in 11 different orders, seven of which belonged to the Sordariomycetes class, with emphasis on the order Xylariales and genus *Xylaria*, which comprised the largest number of

Table I. Classification of endophytic fungi associated with *A. angustifolia*.

Endophytic fungi classification					
Code	**Class**	**Order**	**Family**	**Proposed species**	**GeneBank Acession**
W12	Eurotiomycetes	Eurotiales	Aspergillaceae	*Aspergillus fumigatus*	MY996900
W23				*Aspergillus fumigatus*	MY996901
W44				*Aspergillus flavus*	MY996902
W16	Dothideomycetes	Pleosporales	Sporormiaceae	*Preussia mínima*	MY996903
W37		Botryosphaeriales	Botryosphaeriaceae	*Neofusiococcum* sp.	MY996904
W56				*Phyllosticta capitalensis*	MY996905
W411				*Phyllosticta capitalensis*	MY996906
W43				*Phyllosticta capitalensis*	MY996907
W19	Sordariomycetes	Coniochaetales	Coniochaetaceae	*Coniochaeta velutina*	MY996908
W26		Hypocreales	Hypocreaceae	*Trichoderma koningiopsis*	MY996909
W42			Nectriaceae	*Fusarium* sp.	MY996910
W48		Glomerellales	Glomerellaceae	*Colletotrichum* sp.	MY996911
W57		Muyocopronales	Muyocopronaceae	*Mycoleptodiscus* sp.	MY996912
W18				*Muyocopron* sp.	MY996913
W32		Diaporthales	Diaporthaceae	*Diaporthe* sp.	MY996914
W312				*Diaporthe* sp.	MY996915
W45				*Diaporthe serafinie*	MY996916
W41				*Diaporthe* sp.	MY996917
W28		Amphisphaeriales	Pestalotiopsidaceae	*Neopestalotiopsis* sp.	MY996918
W13				*Pestalotiopsis* sp.	MY996919
W24				*Pestalotiopsis* sp.	MY996920
W211				*Neopestalotiopsis* sp.	MY996921
W27		Xylariales	Hypoxylaceae	*Annulohypoxylon stygium*	MY996922
W11				*Hypoxylon* sp.	MY996923
W311				*Hypoxylon* sp.	MY996924
W315				*Hypoxylon* sp.	MY996925
W213			Xylariaceae	*Xylaria* sp.	MY996926
W114				*Xylaria* sp.	MY996927
W218				*Xylaria* sp.	MY996928
W313				*Xylaria berteri*	MY996929
W214				*Xylaria* sp.	MY996930
W310				*Xylaria* sp.	MY996931
W47				*Xylaria* sp.	MY996932
W15			Hypoxylaceae	*Hypoxylon* sp.	MY996933
W39			Xylariaceae	*Xylaria berteri*	MY996934
W111				*Xylaria* sp.	MY996935
W410		Sordariales	Lasiosphaeriaceae	*Fimetariella rabenhorstii*	MY996936

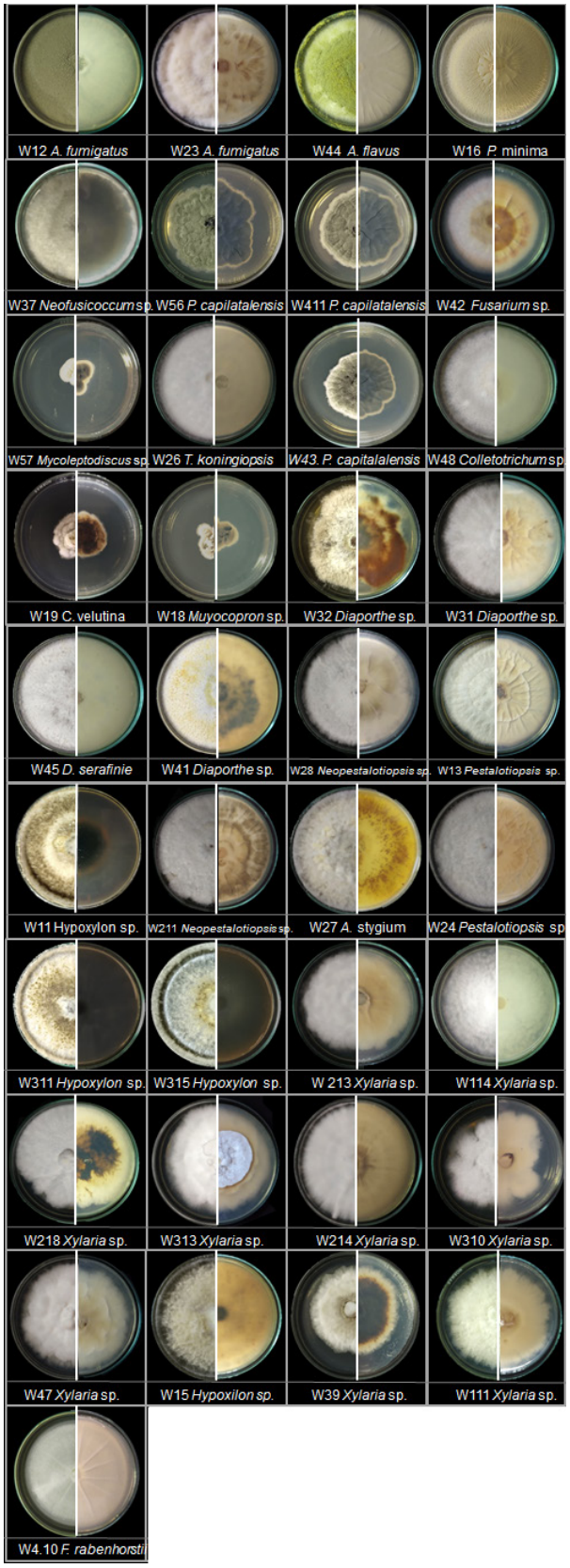

Figure 1. Diversity of the colony morphology (obverse and reverse) of the endophytic fungi associated with *A. angustifolia* cultivated in Sabouraud agar at 25 °C, for 14 days.

species. The *Xylaria* genus is one of the most diverse within the family Xylariaceae, with approximately 600 described species (Helaly et al. 2018). *Xylaria* species are typical examples of endophytes isolated from fragments of apparently healthy plants, and are predominant in these diversity studies (Vaz et al. 2014). In Brazil, these endophytes have also been reported to be abundant in other plants, such as *Eucalyptus microcorys*, *Vochysia divergens*, and *Stryphnodendron adstringens* (Lacerda et al. 2018, Noriler et al. 2018). According to Okane et al. (2012), fungi of the genus *Xylaria* are generalists and can colonize a wide variety of plants.

Fungi of the genera *Diaporthe* and *Pestalotiopsis* were also abundant, and both have already been reported as endophytic fungi in other studies (Banhos et al. 2014, Noriler et al. 2018); however, these fungi, along with others belonging to genera *Neofusicoccum*, *Fusarium*, *Phyllosticta*, and *Colletotrichum*, are considered pathogens of plants such as soybean, sunflower, pepper, bean, and coffee (Nguyen et al. 2009, Shetty et al. 2011, Lazarotto et al. 2014, Udayanga et al. 2015, Batista et al. 2019). Of these, *Fusarium* and *Neofusicoccum* have already been reported as pathogens of *A. angustifolia* (Auer & Grigoletti Junior 1997, Dalmas et al. 2013).

Endophytic relationships depend on the balance of antagonism between the fungus and its host. Throughout infection, mutualism, commensalism, and parasitism interactions can be established, and owing to this, fungi considered phytopathogenic, such as *Fusarium* and *Colletotrichum*, have already been isolated as endophytes, conferring benefits to host plants (Nandhini et al. 2018, Huang et al. 2020). In

addition to the habit changes that occur because of balanced antagonism, genetic alterations that result in the conversion of parasites to non-parasites have already been observed (Freeman & Rodriguez 1993, Hacquard et al. 2016).

Many of the genera described herein have previously been reported as endophytes of other plants, including native Brazilian plants. Similarly, 10 genera described in the present study, including *Xylaria*, *Diaporthe*, and *Pestalotiopsis* were also associated with angiosperm species *Vochysia divergens* Pohl. and *Stryphnodendron adstringens* (Mart.) Coville belonging to the Brazilian Pantanal and Cerrado biomes, respectively (Noriler et al. 2018). Six genera associated with *A. angustifolia* were associated with *Myrcia guianensis* (Aubl.) DC. found in the Amazon (Banhos et al. 2014).

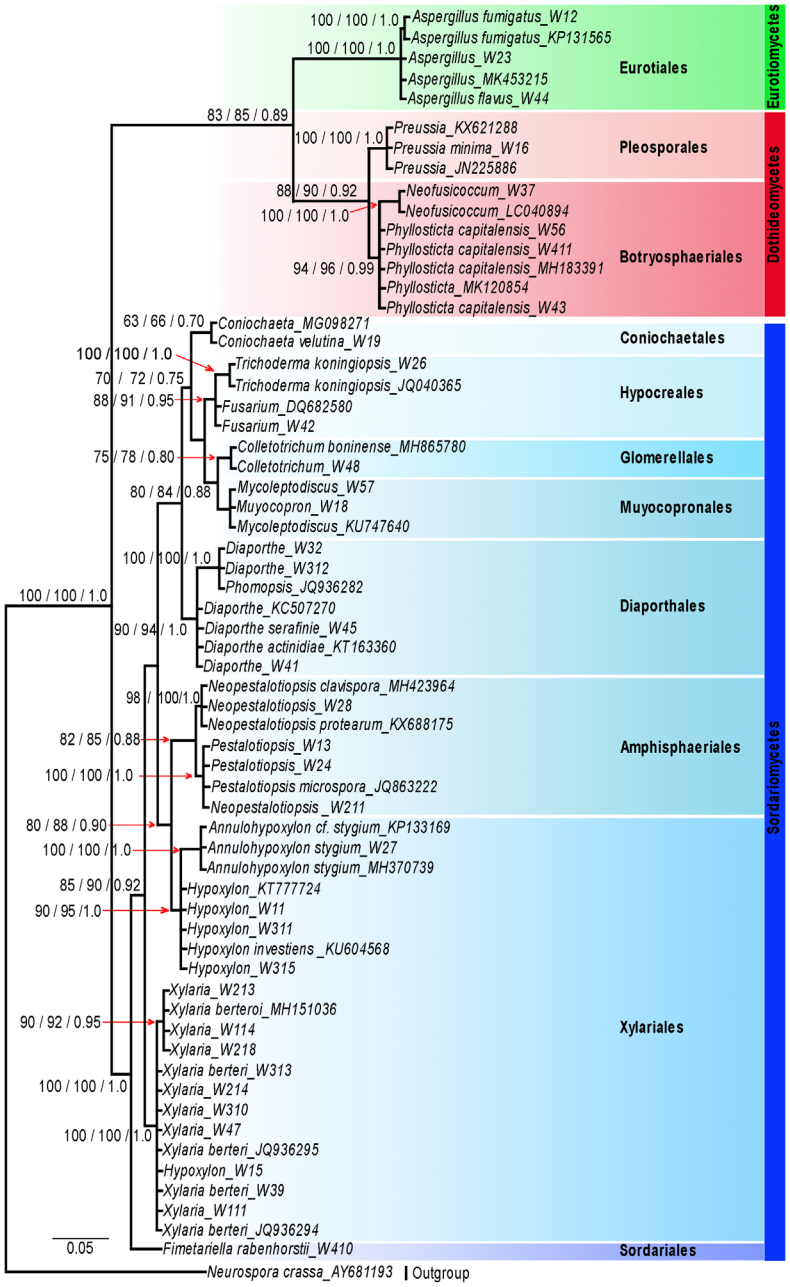

Figure 2. Phylogenetic tree of the ITS region nucleotide sequences of endophytic fungi associated with *A. angustifolia*. The tree was built with Bayesian analysis. The Bootstrap values are based on 1000 replicate runs, shown as percent. *Neurospora crassa*_AY681193 was used as the outgroup. The strains codes follows the name of fungal species.

A. fumigatus strains were previously isolated from the leaves of *Erythrophleum fordii* Oliver, a legume species of the Fabaceae family (Shi et al. 2015). *Colletotrichum* sp. is well recognized as an endophyte of a wide variety of plants distributed worldwide, including members of the Amaryllidaceae, Orchidaceae, Proteaceae, and Solanaceae families (Weir et al. 2012). Until now, there has been only one record of *Fimetariella rabenhorstii* in Brazil and is described as an endophytic of *S. adstringens* (Carvalho et al. 2012). These findings highlight that the genera of endophytic fungi found in the present study have the capacity to develop in a wide diversity of hosts.

Researchers have found it difficult to understand the evolutionary process of these microorganisms and their adaptation to the hosts (Gladieux 2018), as the diversity of endophytic fungi is influenced by the physiology of the host, environmental factors, and interactions with other organisms. Research shows that the community can vary in hosts from different places, is distinguished in different parts of the plant, and can be seasonally modified (Shi et al. 2016, Bowman & Arnold 2018). It is believed that endophytic fungi have host specificity, as observed in phytopathogenic fungal strains. However, it is difficult to understand this characteristic, which requires a larger number of plants to be sampled, in addition to the fact that specificity also occurs below the species level (Arnold 2007).

For the first time, we have described the apparent broad endophytic fungal diversity of *A. angustifolia*. The revealed community included 17 genera in the phylum Ascomycota. Among these, there was a high prevalence of *Xylaria* species. This study opens perspectives to bioprospecting as a strategy for conservation and sustainable use of *A. angustifolia*, a promising source of undiscovered and potentially useful genetic resources.

Acknowledgments

This study was financed by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES) - Finance Code 001.

REFERENCES

ALAM B, LǏ J, GĚ Q, KHAN MA, GŌNG J, MEHMOOD S, YUÁN Y & GǑNG W. 2021. Endophytic fungi: from symbiosis to secondary metabolite communications or vice versa? Front Plant Sci 12: 791033.

ARNOLD AE. 2007. Understanding the diversity of foliar endophytic fungi: progress, challenges, and frontiers. Fungal Biol Rev 21: 51-66.

AUER CG & GRIGOLETTI JUNIOR A. 1997. Doenças registradas em *Araucaria angustifolia* e *Pinus* spp. nos estados do Paraná e de Santa Catarina. Colombo EMBRAPA-CNPF 31: 1-3.

BANHOS EF, SOUZA AQL, ANDRADE, JC, SOUZA ADL, KOOLEN HHF & ALBUQUERQUE PM. 2014. Endophytic fungi from *Myrcia guianensis* at the Brazilian Amazon: Distribution and bioactivity. Braz J Microbiol 45: 153-161.

BATISTA RO, LEITE TS, NICOLI A, CARNEIRO JES, CARNEIRO PCS, PAULA JUNIOR TJ & QUEIROZ MV. 2019. Research article infection and colonization of common bean by EGFP transformants of *Fusarium oxysporum* f. sp. *phaseoli*. Genet Mol Res 18: GMR18370.

BOWMAN EA & ARNOLD AE. 2018. Distributions of ectomycorrhizal and foliar endophytic fungal communities associated with *Pinus ponderosa* along a spatially constrained elevation gradient. Am J Bot 105: 687-699.

BUTIN H & PEREDO HL. 1986. Hongos parasitos em coniferas de America Del Sur, con especial referencia a Chile. Biblioth Mycol 101: 10-100.

CAMPANILI M & SCHAFFER WB. 2010. Mata Atlântica: manual de adequação ambiental. Série Biodiversidade, 35. Brasília: Ministério do Meio Ambiente, 96 p.

CARLUCCI MB, MARCILIO-SILVA V & TOREZAN JM. 2021. The Southern Atlantic Forest: use, degradation, and perspectives. In: MARQUES MCM & GRELLE CEV (Eds), The Atlantic Forest: history, biodiversity, threats and opportunities of the megadiverse forest, Switzerland: Springer Nature, p. 91-111.

CARVALHO CR ET AL. 2012. The diversity, antimicrobial and anticancer activities of endophytic fungi associated with the medicinal plant *Stryphnodendron adstringens* (Mart.) Coville (Fabaceae) from the brazilian savannah. Symbiosis 57: 95-107.

CASTELLANI A. 1939. Viability of some pathogenic fungi in distilled water. J Trop Med Hyg 42: 225-225.

CORREIA AML, LIRA SP, ASSIS MA & RODRIGUES A. 2018. Fungal endophyte communities in *Begonia* species from the Brazilian Atlantic Rainforest. Curr Microbiol 75: 441-449.

DA SILVA SS, COSTA LA & GUSMÃO LFP. 2021. DIVERSITY of saprotrophic filamentous fungi on *Araucaria angustifolia* (Bertol.) Kuntze (Brazilian pine). Braz J Microbiol 52: 1489-1501.

DALMAS FR, ASTARITA L, DEFIPPIS L, MAGEL E, FIEDLER H-P & BAUER R. 2013. Growth inhibition of an *Araucaria angustifolia* (Coniferopsida) fungal seed pathogen, *Neofusicoccum parvum*, by soil streptomycetes. BMC Microbiolol 13: 168.

DOYLE JJ & DOYLE JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bull 19: 11-15.

FERREIRA MC, ASSIS JCS & ROSA LH. 2020. Diversity of endophytic fungi associated with *Carapichea ipecacuanha* from a native fragment of the Atlantic Rain Forest. S Afr J Bot 5: 1-5.

FREEMAN S & RODRIGUEZ RJ. 1993. Genetic conversion of a fungal plant pathogen to a nonpathogenic, endophytic mutualist. Science 260: 75-78.

GLADIEUX P. 2018. What makes a specialized endophyte special? Mol Ecol 27: 3037-3039.

HACQUARD S ET AL. 2016. Survival trade-offs in plant roots during colonization by closely related beneficial and pathogenic fungi. Nat Commun 7: 1-13.

HAMZAH TNT, LEE SY, YIDAHAT A, TERHEM R, FARIDAH-HANUM I & MOHAMED R. 2018. Diversity and characterization of endophytic fungi isolated from the tropical mangrove species, *Rhizophora mucronata*, and identification of potential antagonists against the soil-borne fungus, *Fusarium solani*. Front Microbiol 9: 1707.

HELALY SE, THONGBAI B & STADLER M. 2018. Diversity of biologically active secondary metabolites from endophytic and saprotrophic fungi of the ascomycete order Xylariales. Nat Prod Rep 35: 992-1014.

HODGES CS & MAY LC. 1972. A root disease of pine, Araucaria and Eucalyptus in Brazil caused by a new species of *Cylindrocladium*. Phytopathology 62: 898-901.

HORMAZABAL E & PIONTELLI E. 2009. Endophytic fungi from Chilean native gymnosperms: antimicrobial activity against human and phytopathogenic fungi. World J Microbiol Biotechnol 25: 813-819.

HUANG L-Q, NIU Y-C, SU L, DENG H & LYU H. 2020. The potential of endophytic fungi isolated fron cucurbit plants for biocontrol of soilborne fungal diseases of cucumber. Microbiol Res 231: 1-11.

JHA P, KAUR T, CHHABRA I, PANJA A, PAUL S, KUMAR V & MALIK T. 2023. Endophytic fungi: hidden treasure chest of antimicrobial metabolites interrelationship of endophytes and metabolites. Frontiers Microbiol 14: 1227830.

JIA M, CHEN L, XIN HL, ZHENG CJ, RAHMAN K, HAN T & QIN LP. 2016. A friendly relationship between endophytic fungi and medicinal plants: a systematic review. Front Microbiol 9: 7-906.

KUSARI S, SINGH S & JAYABASKARAN C. 2014. Biotechnological potential of plant-associated endophytic fungi: hope versus hype. Trends Biotechnol 32: 297-303.

LACERDA LT, GUSMÃO LFP & RODRIGUES A. 2018. Diversity of endophytic fungi in *Eucalyptus microcorys* assessed by complementary isolation methods. Mycol Progr 17: 719-727.

LAZAROTTO M, BOVOLINI MP, MUNIZ MFB, HARAWAKA R, REINIGER LRS & SANTOS AF. 2014. Identification and characterization of pathogenic Pestalotiopsis species to pecan tree in Brazil. Pesqui Agropecu Bras 49: 440-448.

MAACK R. 1981. Geografia Física do Estado do Paraná, 2nd ed., Rio de Janeiro: José Olympio, 450 p.

MENDES MAS, URBEN AF, DIANESE JC, SILVA, VL, FERREIRA MASV, SANCHEZ M & SANTOS LPT. 2010. Fungos relatados em plantas no Brasil. In: MENDES MAS, URBEN AF & DIANESE JC (Eds), Fungos em plantas no Brasil, Brasília: Embrapa, p. 749-830.

MOREIRA M, BARETTA D & CARDOSO EJBN. 2012. Doses de fósforo determinam a prevalência de fungos micorrízicos arbusculares em Araucaria angustifolia. Cienc Florest 22: 813-820.

MOREIRA M, BARETTA D, TSAI SM, GOMES-DA-COSTA SM & CARDOSO EJBN. 2007. Biodiversity and distribution of arbuscular mycorrhizal fungi in *Araucaria angustifolia* forest. Sci Agr 64: 393-399.

MOREIRA M, TRUFEM SFB, GOMES-DA-COSTA SM & CARDOSO EJBN. 2003. Arbuscular mycorrhizal fungi associated with *Araucaria angustifolia* (Bert.) O. Ktze Mycorrhiza 13: 211-215.

NANDHINI M, RAJINI SB, UDAYASHANKAR AC, NIRANJANA SR, LUND OS, SHETTY HS & PRAKASH HS. 2018. Diversity, plant growth promoting and downy mildew disease suppression potential of cultivable endophytic fungal communities associated with pearl millet. Biol Control 127: 127-138.

NGUYEN PTH, PETTERSSON OV, OLSSON P & LIJEROTH E. 2009. Identification of *Colletotrichum* species associated with anthracnose disease of coffee in Vietnam. Eur J Plant Pathol 127: 73-87.

NORILER SA, SAVI DC, ALUIZIO R, PALÁCIO-CORTES AM, POSSIEDE YM & GLIENKE C. 2018. Bioprospecting and Structure of Fungal Endophyte Communities Found in the Brazilian Biomes, Pantanal, and Cerrado. Front Microbiol 9: 1526.

OKANE I, SRIKITIKULCHAI P, TABUCHI Y, SIVICHAI S & NAKAGIRI A. 2012. Recognition and characterization of four Thai xylariaceous fungi inhabiting various tropical foliages as endophytes by DNA sequences and host plant preference. Mycoscience 53: 122-132.

PACKIAM KK & DHAKSHINAMOORTHY M. 2021. Endophytic fungi: A review of survival strategies that influence the biodiversity studies associated with biopotential products. In: SHARMA VK, SHAH MP, PARMAR S & KUMAR A (Eds), Bio-prospects in sustainable agriculture, environment and nano-technology, San Diego: Academic Press, p. 1-15.

RAJA HA, MILLER AN, PEARCE CJ & OBERLIES NH. 2017. Fungal identification using molecular tools: a primer for the natural products research community. J N Prod 80: 756-770.

REZENDE CL, SCARANO FR, ASSAD ED, JOLY CA, METZGER JP, STRASSBURG BBN, TABARELLI M, FONSECA GA & MITTERMEIER RA. 2018. From hotspot to hopespot: An opportunity for the Brazilian Atlantic Forest. Perspect Ecol Conserv 16: 208-214.

RIBEIRO CM & CARDOSO EJBNC. 2012. Isolation, selection and characterization of root-associated growth promoting bacteria in Brazil Pine (*Araucaria angustifolia*). Microbiol Res 167: 69-78.

RIBEIRO CM, METZGER JP, MARTENSEN AC, PONZONI F & HIROTA M. 2009. Brazilian Atlantic Forest: How much is left and how is the remaining forest distributed? Implications for conservation. Biol Conserv 142: 1141-1153.

SEGARAN G & SATHIAVELU M. 2019. Fungal endophytes: A potent biocontrol agent and a bioactive metabolites reservoir. Biocatal Agric Biotechnol 21: 101284.

SHETTY KG, MINNIS AM, ROSSMAN AY & JAYACHANDRAN K. 2011. The Brazilian peppertree seed-borne pathogen, *Neofusicoccum batangarum*, a potential biocontrol agent. Biol Control 56: 91-97.

SHI Y ET AL. 2016. Endophytic fungal diversity and space-time dynamics in sugar beet. Eur J Soil Biol 77: 77-85.

SHI Y-S, ZHANG Y, CHEN X-Z, ZHANG N & LIU Y-B. 2015. Metabolites produced by the endophytic fungus *Aspergillus fumigatus* from the stem of *Erythrophloeum fordii* Oliv. Molecules 20: 10793-10799.

SILVA RF, ANTONIOLLI ZI, LEAL L & SILVA AS. 2009. Ocorrência de fungos micorrízicos em espécies florestais na região central do estado do Rio Grande do Sul. R Bras Agrociência 15: 65-70.

SOLÓRZANO-FILHO JA & KRAUS JE. 1999. Breve história das matas de Araucária. Revista Forest 99: 37-40.

SUN X, GUO L & HYDE KD. 2011. Community composition of endophytic fungi in *Acer truncatum* and their role in decomposition. Fungal Divers 47: 85-95.

THOMAS P. 2013. *Araucaria angustifolia*. The IUCN Red List of Threatened Species Avaliable on: < https://www.iucnredlist.org/species/32975/2829141>. Accessed 06 March 2023.

UDAYANGA D, CASTLEBURY LA, ROSSMAN AY, CHUKEATIROTE E & HYDE KD. 2015. The *Diaporthe sojae* species complex: Phylogenetic re-assessment of pathogens associated with soybean, cucurbits and other field crops. Fungal Biol 119: 383-407

VAZ ABM, DA COSTA AGFC, RAAD LVV & GÓES-NETO A. 2014. Fungal endophytes associated with three South American Myrtae (Myrtaceae) exhibit preferences in the colonization at leaf level. Fungal Biol 118: 277-286.

VILCATOMA-MEDINA C, KASCHUK G & ZANETTE F. 2018. Colonization and spore richness of arbuscular mycorrhizal fungi in Araucaria nursery seedlings in Curitiba, Brazil. Hindawi Int J Agron 2018: 1-6.

WANG L, REN L, LI C, GAO C, LIU X, WANG M & LUO Y. 2019. Effects of endophytic fungi diversity in different coniferous species on the colonization of *Sirex noctilio* (Hymenoptera: Siricidae). Sci Rep 9: 5077.

WEIR BS, JOHNSTON PR & DAMM U. 2012. The *Colletotrichum gloeosporioides* species complex. Stud Mycol 73: 115-180.

WHITE TJ, BRUNS TD, LEE SB & TAYLOR JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: INNIS MA, GELFAND DH, SNINSKY JJ & WHITE TJ (Eds), PCR Protocols, San Diego: Academic Press, p. 315-322.

ZANDAVALLI RB, STÜRMER SL & DILLENBURG RL. 2008. Species richness of arbuscular mycorrhizal fungi in forests with Araucaria in Southern Brazil. Hoehnea 35: 63-68.

How to cite
GROFF DB, MARMENTINI J, GAGLIOTI AL, SILVA PR & KNOB A. 2024. Endophytic fungi associated with *Araucaria angustifolia* (Bertol.) Kuntze. An Acad Bras Cienc 96: e20230251. DOI 10.1590/0001-3765202420230251.

Manuscript received on March 08, 2023;
accepted for publication on May 06, 2024

DANIELI B. GROFF
https://orcid.org/0009-0005-5264-8938

JÉSSICA MARMENTINI
https://orcid.org/0009-0008-2911-7380

ANDRÉ LUIZ GAGLIOTI
https://orcid.org/**0000-0002-2638-4642**

PAULO ROBERTO DA SILVA
https://orcid.org/0000-0003-3190-1461

ADRIANA KNOB
https://orcid.org/**0000-0002-1239-2182**

Universidade Estadual do Centro-Oeste, Departamento de Ciências Biológicas, Alameda Élio Antonio Dalla Vecchia, 838, 85040-167 Guarapuava, PR, Brazil

Correspondence to: **Adriana Knob**
E-mail: knob@unicentro.br

Author contributions

Danieli Ballmann Groff: acquisition of data, analysis and interpretation of data, drafting of manuscript; Jéssica Marmentini: acquisition of data, analysis and interpretation of data, drafting of manuscript; André Luiz Gaglioti: acquisition of data, analysis and interpretation of data; Paulo Roberto da Silva: acquisition of data, critical revision; Adriana Knob: Study conception and design, drafting of manuscript.