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Endophytic *Diaporthe* species from Brazil

L.O. Ferro^{1,2#}, J.D.P. Bezerra^{2,3,4#*}, T.M. da Silva⁴, C.S. de Oliveira³, S. dos S. Nascimento^{1,2}, L.M. Paiva¹, X. Fan⁵, P.W. Crous^{6,7,8}, C.M. Souza-Motta^{1,2}

¹Departamento de Micologia Prof. Chaves Batista, Universidade Federal de Pernambuco, Av. Prof. Moraes Rego, s/n, Centro de Biociências, Cidade Universitária, CEP: 50670-901, Recife, PE, Brazil

²Programa de Pós-Graduação em Biologia de Fungos, Universidade Federal de Pernambuco, Av. Prof. Moraes Rego, s/n, Centro de Biociências, Cidade Universitária, CEP: 50670-901, Recife, PE, Brazil

³Laboratório de Micologia, Departamento de Biociências e Tecnologia, Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Rua 235, s/n, Setor Universitário, CEP: 74605-050, Goiânia, GO, Brazil

⁴Programa de Pós-Graduação em Biologia da Relação Parasito-Hospedeiro, Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Rua 235, s/n, Setor Universitário, CEP: 74605-050, Goiânia, GO, Brazil

⁵The Key Laboratory for Silviculture and Conservation of Ministry of Education, Beijing Forestry University, 7 Beijing 100083, China

⁶Westerdijk Fungal Biodiversity Institute, Uppsalalaan 8, Utrecht, 3584 CT, The Netherlands

⁷Department of Biochemistry, Genetics & Microbiology, Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa

⁸Microbiology, Department of Biology, Utrecht University, Padualaan 8, Utrecht, 3584 CH, The Netherlands

#These authors contributed equally

*Corresponding author: jadsonbezerra@ufg.br

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Abstract: *Diaporthe* species can inhabit various hosts with different lifestyles and live as endophytes, pathogens, and saprobes. Our study analysed 180 endophytic *Diaporthe* isolates from *Miconia* sp. in the Atlantic Forest, *Brosimum gaudichaudii* in the Brazilian savanna (Cerrado), and *Anacardium occidentale* in the Caatinga forest and Cerrado in Brazil. Based on multi-locus phylogenetic analyses [β -tubulin (*tub2*), internal transcribed spacer regions and intervening 5.8S rRNA (ITS), translation elongation factor 1-alpha (*tef1*), calmodulin (*cmdA*), and histone (*his3*)] and morphological features, we are introducing seven new species (*D. azevedoi*, *D. catimbauensis*, *D. coracoralinae*, *D. luizorum*, *D. pedratalhadensis*, *D. samambaiaensis*, and *D. vargemgrandensis*) and reporting seven known species (*D. fructicola*, *D. inconspicua*, *D. infertilis*, *D. paranensis*, *D. raonikayaporum*, *D. schini*, and *D. ueckeri*). We also included a morphological description of *D. infertilis* and synonymised *D. lutescens*, *D. pseudoinconspicua*, and *D. samaneae* under *D. inconspicua*; *D. neoraonikayaporum* under *D. raonikayaporum*; and *D. passifloricola*, *D. rosae*, and *D. vochysiae* under *D. ueckeri*, based on limited nucleotide differences among DNA sequence data and overlapping morphological features. Our results highlight the importance of including endophytic isolates in the phylogeny of *Diaporthe*, and show how these data expand the geographic distribution and host relationships of known species.

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INTRODUCTION

Endophytic fungi belong to a highly diverse, polyphyletic group, defined by their colonisation niche, and are microorganisms that inhabit plant tissues in part or throughout their life cycle without causing damage to their hosts (Hardoim *et al.* 2015, Dastogeer *et al.* 2017). Their biotechnological potential makes endophytes a promising group in the search for new molecules, but also highlights the lack of studies that reveal the full complexity of the interactions and understanding of the diversity of these organisms (Zheng *et al.* 2016, Rana *et al.* 2019). Interactions in the plant microbiome highlight the ecological importance of endophytes and make it difficult to distinguish them from other

groups, such as phytopathogenic fungi, which are didactic terms and dependent on a set of biotic and abiotic environmental factors (Azevedo 2000, Brader *et al.* 2017). Endophytic lifestyles can vary from mutualistic, saprophytic, to plant pathogenic. It also depends on the stage at which the interaction occurs, as an endophytic species can be considered a latent pathogen or harmless endophyte (Sheibani-Tezerji *et al.* 2015). Several fungal genera are commonly encountered in studies of endophytic communities, including *Colletotrichum*, *Diaporthe*, and *Fusarium*, some of which can also cause serious plant diseases (Hardoim *et al.* 2015, Suryanarayanan *et al.* 2018).

The genus *Diaporthe* (*Diaporthaceae*, *Diaporthales*, *Sordariomycetes*) (syn. *Phomopsis*) was established by Fuckel

(1867), with *D. alnea* as the type species. *Diaporthe* species are cosmopolitan and frequently found in leaves, branches, fruits, and seeds of diverse plants, living as pathogens, saprobes, and endophytes (Udayanga et al. 2014, Fan et al. 2015, Marin-Felix et al. 2019a, Hilário et al. 2020). Previously, *Diaporthe* species were believed to be host-specific, and their descriptions were mainly based on host plant and morphological features, resulting in an inflation of species names (Rehner & Uecker 1994, Santos et al. 2010, Udayanga et al. 2011). Many *Diaporthe* species lack host selectivity and can act as plant pathogens, causing root and fruit rot, dieback, cankers, leaf spots, and blights (Santos et al. 2011, Gramaje et al. 2012, Fan et al. 2018, Bai et al. 2023); however, they can also be found as endophytes (Hardoim et al. 2015, Pádua et al. 2018, Ferro et al. 2023).

The identification of *Diaporthe* species has changed over the years from being based on unique host species and morphology to a polyphasic taxonomy. Currently, the identity of *Diaporthe* species is mainly based on combined phylogenetic inferences using the internal transcribed spacer regions and intervening 5.8S rRNA (ITS), calmodulin (*cmdA*), histone (*his3*), translation elongation factor 1- α (*tef1*), and β -tubulin (*tub2*), that can provide good resolution in related *Diaporthe* species (Gomes et al. 2013, Huang et al. 2013, Dissanayake et al. 2020, Gao et al. 2021). The paraphyletic origin of *Diaporthe* has been reported based on single or combined genes (Gao et al. 2017, Huang et al. 2021). Consequently, Norphanphoun et al. (2022) proposed the use of species complexes to facilitate the delimitation of *Diaporthe* species, proposing 13 species complexes that can be used as broader descriptors, and thus help in the difficult taxon resolution that still exists within the genus.

Diaporthe species are among the most frequently encountered endophytic fungi (Garcia et al. 2012, Gomes et al. 2013, Vaz et al. 2014, Oliveira et al. 2014, Orlandi et al. 2017, Pádua et al. 2018). In Brazil, studies on important biodiversity conservation units have contributed to an increase in reported fungal endophyte diversity (Savi et al. 2019, Rosa 2021). Among the Brazilian biomes, the Atlantic Forest is considered a global biodiversity hotspot (Leitman et al. 2015) with high plant endemism (Nery et al. 2023). Vaz et al. (2014) studied plants of the family *Myrtaceae* and found a broad distribution of *Sordariomycetes* fungi, including *Diaporthe*. Similar results were observed in *Begonia* spp., with *Diaporthe* being the second most frequent genus (Correia et al. 2018). Recently, Ferro et al. (2023) reported *Diaporthe* to be one of the most abundant fungal genera among endophytes isolated from leaves of *Miconia* sp. (*Melastomataceae*) in a conservation unit in the Atlantic Forest. Caatinga and Cerrado are two other Brazilian biomes that share similar climatic conditions and are seasonally affected by dry conditions (Pennington et al. 2000, Mayle 2004). Both biomes constitute a large proportion of the biodiversity reservoirs in Brazil and have received considerable attention in studies investigating their fungal endophyte diversity (Bezerra et al. 2013, 2019, Noriler et al. 2018). For example, in the Caatinga forest, a new *Diaporthe* species (*D. caatingaensis*) was described as a cactus endophyte (Crous et al. 2016a). Later, Pádua et al. (2018) assessed the endophytic fungal community of *Myracrodruon urundeuva* and Oliveira et al. (2020) studied endophytes from *Poincianella pyramidalis* and identified *Diaporthe* as the most common genus. Other *Diaporthe* species (*D. myracrodruonis*, *D. pseudoinonspicua*, and *D. poincianellae*) were introduced as endophytic fungi in the Caatinga forest (Crous et al. 2018a, b, Silva et al. 2019b). In the Cerrado biome,

studies have demonstrated that *Diaporthe* is the most frequent genus among endophytes in endemic and medicinal plants (Ferreira et al. 2017, Noriler et al. 2018), and new species (e.g. *D. cerradensis*) have also been introduced in the genus based on endophytic isolates (Iantas et al. 2021). However, robust analyses of endophytic *Diaporthe* in Brazilian biomes are still lacking, and our study provides important information on the diversity and host relationships of these fungi in Brazil.

Diaporthe species are used in accessing endophytic fungal communities associated with several plants, and the importance of the debate on fungus-plant associations and measures of possible impacts of endophytes and latent pathogens in forestry and crop species should be investigated (Crous & Groenewald 2005), mainly when they are widely regarded as harmless endophytes. From 2018 to 2022, we collected endophytic *Diaporthe* isolates from plant species of the families *Anacardiaceae*, *Melastomataceae*, and *Moraceae* from three biomes in Brazil. The aim of this study was to identify endophytic *Diaporthe* species based on multi-locus phylogenetic analyses (*cmdA*, *his3*, ITS, *tef1*, and *tub2*) and morphology.

MATERIALS AND METHODS

Endophytic isolates

We analysed 180 endophytic *Diaporthe* isolates obtained between 2018 and 2022 from the leaves and branches of *Miconia* sp. (*Melastomataceae*), *Anacardium occidentale* (*Anacardiaceae*), and *Brosimum gaudichaudii* (*Moraceae*) growing in three Brazilian biomes (Atlantic Forest, Caatinga, and/or Cerrado). For *Miconia* sp., leaves were collected from a conservation unit of the Atlantic Forest (09°15'26.8"S, 36°25'53.7"W), as described by Ferro et al. (2023). Leaves and branches of *A. occidentale* were collected from a conservation unit of the Brazilian tropical dry forest (Caatinga), Parque Nacional do Catimbau, Buíque city, Pernambuco state (8°36'35"S, 37°14'40"W) and in an area of the Brazilian savannah (Cerrado), Reserva Ecológica Vargem Grande, Pirenópolis city, Goiás state (15°48'14.256"S, 48°55'6.204"W). For the medicinal plant *B. gaudichaudii*, leaves and branches were collected from the arboretum (an area of Cerrado) of the Escola de Agronomia of the Universidade Federal de Goiás (UFG), Campus Samambaia, Goiânia city, Goiás state (16°35'58.5"S, 49°16'45.8"W) (Fig. 1). Plant collection and isolation of endophytic fungi were performed as described by Bezerra et al. (2015). The collections were authorised by the Ministério do Meio Ambiente (MMA)/Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) (permission numbers: 63010, 76547, and 76547).

Representative endophytic isolates and ex-type cultures are deposited in the University Recife Mycology (URM) culture collection (Micoteca URM Profa. Maria Auxiliadora Cavalcanti) at the Universidade Federal de Pernambuco (UFPE) (Recife, Brazil) and holotypes in the UFG herbarium (Herbário UFG) at the UFG (Goiânia, Brazil) as permanent slide preparations. The holotype of *D. samambaiaensis* (URM 8913, metabolically inactive culture) is deposited in the URM culture collection. In addition, all endophytic isolates are deposited in the working collection of the Laboratório de Micologia Ambiental at the Centro de Biociências of the UFPE and in the working collection FCCUG of Jadson D.P. Bezerra housed at the Laboratório de Micologia (LabMicol) of the Instituto de Patologia Tropical e Saúde Pública (IPTSP) of the UFG.

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from 7-d-old cultures of endophytic fungi grown on potato dextrose agar (PDA) using the Wizard® Genomic DNA Purification Kit (Promega Corporation), following the manufacturer's instructions. PCR amplifications for part of β -tubulin (*tub2*), internal transcribed spacer region (ITS) of the nrDNA operon and intervening 5.8S rRNA gene, translation elongation factor 1-alpha (*tef1*), calmodulin (*cmdA*), and histone (*his3*) were performed using primers and PCR conditions as listed in Table S1. Sequencing and sequence editing were performed as described by Bezerra *et al.* (2017) and Silva *et al.* (2019a). The sequences obtained in this study were deposited in the GenBank database of the NCBI (Table S2).

Phylogenetic analyses

Phylogenetic analyses were performed using our sequences and reference sequences from ex-type strains retrieved from GenBank. Sequences were aligned using the MAFFT v. 7 online interface (Kato & Standley 2013) and manually edited using MEGA v. 7 software (Kumar *et al.* 2016). For a preliminary analysis, we constructed a large alignment using all *tub2* sequences from our isolates and available ex-type or reference sequences following recent publications on *Diaporthe* (e.g., Gomes *et al.* 2013, Luo *et al.* 2022, Norphanphoun *et al.* 2022, Lambert *et al.* 2023) and additional sequences according to the results of the BLASTn search (Table S2). After this first analysis, we selected representative isolates from the clades

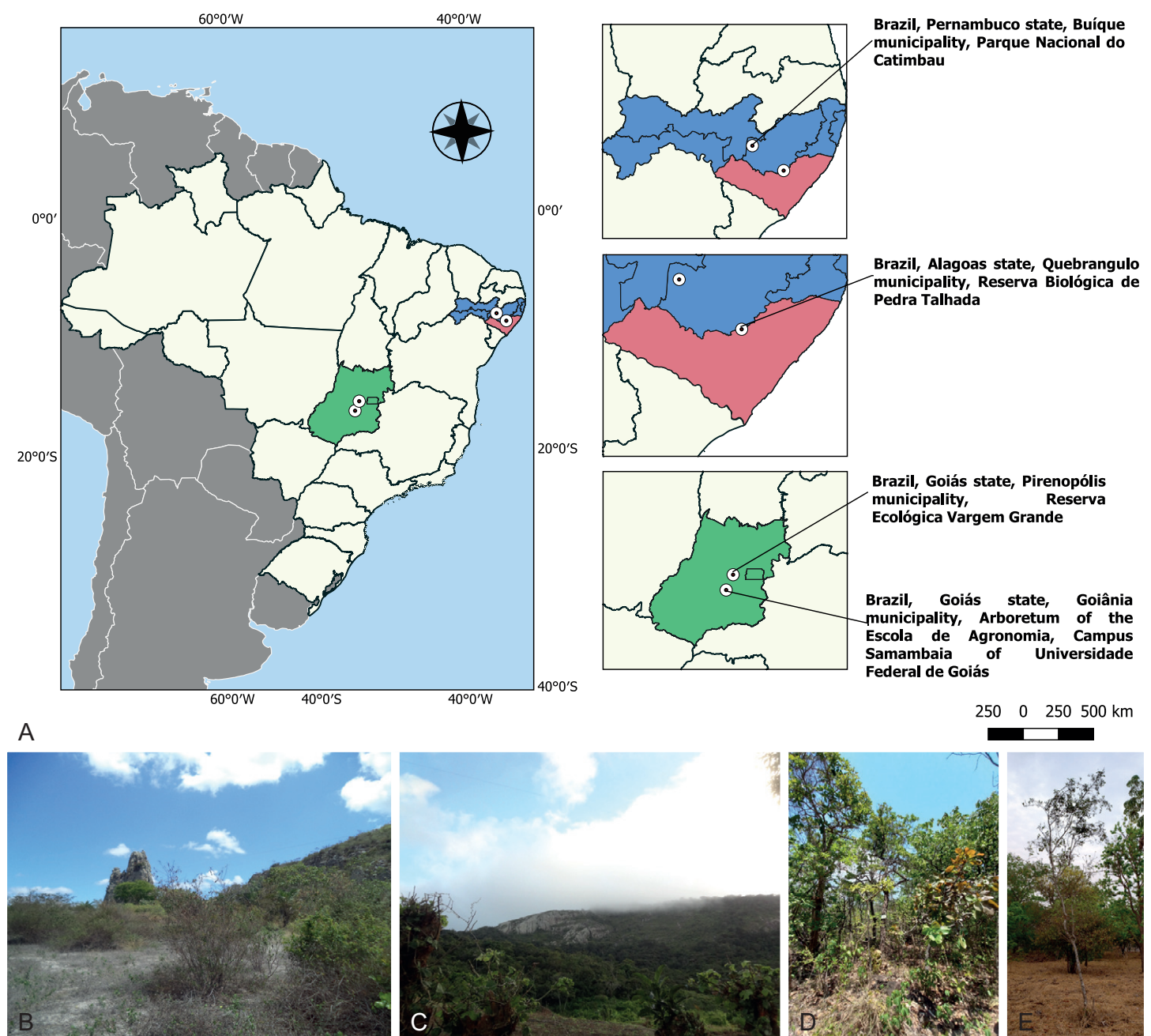


Fig. 1. A. The geographic location of the plant collection sites in Brazilian biomes for the isolation of endophytic *Diaporthe* species. **B.** Caatinga. **C.** Atlantic Forest. **D, E.** Cerrado.

in which our endophytes were placed to amplify *his3*, ITS, *cmdA*, and *tef1* and constructed single alignments for each gene. Highly supported clades of single-locus trees (including only the previously selected phylogenetically related species) were compared to verify phylogenetic agreement among each individual tree. Subsequently, the alignment of each gene was combined into a matrix of five markers. The combined dataset was first analysed based on maximum likelihood (ML) analysis using IQ-TREE v. 1.6.12 software (Nguyen *et al.* 2015) and RAxML HPC BlackBox v. 8.2.12 (Stamatakis *et al.* 2014). The IQ-TREE ML analysis involved 5 000 replications and the ultrafast bootstrap (UFboot2) method was used to calculate branch support (Hoang *et al.* 2018). The ModelFinder software, included in IQ-TREE v. 1.6.12 (Kalyaanamoorthy *et al.* 2017), was used to determine the partitioning strategy and models based on the Akaike information criterion for the dataset using five predefined data blocks (*tub2*, ITS, *tef1*, *cmdA*, and *his3*). The RAxML-HPC BlackBox ML analysis was performed using the default system options in the CIPRES Science Gateway (Miller *et al.* 2010; <http://www.phylo.org/>) with 1 000 bootstrap replicates. The combined dataset was also analysed based on Bayesian inference (BI) conducted using MrBayes v. 3.2.7a (Ronquist *et al.* 2012) in the CIPRES Science Gateway, using the same models and partitions as in the ML analysis (IQ-TREE). The BI analysis was conducted with 1×10^7 generations and a burning value of 25 %, with chains sampled every ten million generations. Individual alignments were analysed based on ML analysis using RAxML-HPC BlackBox v. 8.2.12 and BI analysis.

The resulting phylogenetic trees were visualised using FigTree v. 1.4.4 (Rambaut 2012). Values ≥ 0.95 BI posterior probability (BPP) and bootstrap support (BS) from IQ-TREE UFboot2-BS and RAxML-BS analyses (both BS ≥ 70 %) were plotted near the nodes. The final combined alignment was deposited in TreeBASE (Study ID S31148) and Figshare (doi: 10.6084/m9.figshare.25594161).

Morphology of new species

Endophytic isolates of the new *Diaporthe* species were subcultured on PDA and on water agar (WA) with the inclusion of sterilised host plant tissue on the culture medium surface and incubated at 28 °C in a 12-h light/dark cycle to stimulate sporulation. Cultures that did not sporulate after 1 mo were incubated at 5 °C for further sporulation attempts. The reproductive structures were mounted in lactic acid (85 %), and 30 measurements were taken for conidia and conidiogenous cells and at least 10 for conidiomata. Macromorphological characteristics and colony growth were observed and described on PDA (90-mm-diam Petri dish) after 2 wk of growth at 25 °C in the dark. Colony colour (surface and reverse) was described using the colour chart of Rayner (1970).

RESULTS

Phylogenetic analyses

Our first analysis of *tub2* sequences included 180 sequences from our isolates and 337 sequences from ex-type strains or reference materials retrieved from GenBank. Based on this analysis (Fig. S1), the endophytic *Diaporthe* isolates were positioned in 14 clades, comprising seven clades of known species and seven clades of unknown species, which are proposed as new here.

The endophytic species were positioned in 14 clades belonging to two species complexes (*D. sojae* species complex and *D. oncostoma* species complex), as proposed by Norphanphoun *et al.* (2022). Based on our analyses, we are newly introducing the *D. ocotae* species complex to include two new species and two known species that were not placed in any of the previously known species complexes (Norphanphoun *et al.* 2022).

After building the *tub2* matrix with all sequences of our endophytic *Diaporthe* isolates, along with reference sequences, we selected representatives (54 isolates) of the clades/species with the greatest success in the amplification of other genes to build the combined alignment. In the *tub2* matrix, one of the clades/species was represented by many isolates (95) from the studied collections of endophytic *Diaporthe* isolates. The combined matrix of five genes (*tub2*, ITS, *tef1*, *cmdA*, and *his3*) consisted of 232 sequences, including the outgroup *Diaporthella corylina* (CBS 121124), with 3 379 characters, including gaps (*tub2*: 1–1 008, ITS: 1 009–1 571, *tef1*: 1 572–2 146, *cmdA*: 2 147–2 761, and *his3*: 2 762–3 379), and our isolates clustered into 14 species/clades analysed in two known species complexes, along with a new species complex proposed here. For the ML analysis, the best tree score and final optimisation likelihood value were $-\ln = -68673.723061$ and the estimated base frequencies were: A = 0.207289, C = 0.331503, G = 0.237515, and T = 0.223693, with substitution rates of AC = 1.133800, AG = 3.575569, AT = 1.291865, CG = 0.893045, CT = 4.714306, and GT = 1.000000 and the distribution shape parameter gamma: $\alpha = 0.912841$. The models used for IQ-TREE ML and BI were GTR+I+G (*tub2*, ITS, *tef*, and *his3*) and HKY+I+G for *cmdA*. GTR+I+G was used for RAxML-HPC BlackBox ML analysis. In this matrix, we included representative species to show the phylogenetic relationships of the other *Diaporthe* species complexes to which we did not have related isolates/species (Fig. 2). For these phylogenetic inferences, we constructed individual trees for each of the studied genes (Figs S2–S6).

The five loci analysis has been shown to be the best for resolving our species (Fig. 2). Some of the phylogenetic lineages varied in position among the genes analysed, with some genes being more informative than others when analysed individually. The *tef1*, *cmdA*, and *his3* genes provided a topology with higher similarity when compared to the combined matrix (Fig. 2, Figs S4–S6). The *tub2* and *his3* delivered more information and were the most powerful markers for resolving all (100 %) of the 14 species analysed (Figs S1, S2, S6). The *tef1* resolved 13 (93 %) of the 14 species analysed, only having difficulty in separating *D. fructicola* from the related species (Fig. S4). The *cmdA* resolved 12 (86 %) of the 14 species analysed, having difficulty in separating *D. vargemgrandensis* sp. nov. and *D. schini* from the related species (Fig. S5). The ITS was the least informative gene, separating only eight (57.15 %) of the 14 species analysed, having difficulty in separating *D. catimbauensis* sp. nov., *D. fructicola*, *D. inconspicua*, *D. luizorum* sp. nov., *D. schini*, and *D. vargemgrandensis* sp. nov. from the related species (Fig. S3).

Some of our isolates were grouped into three clades with known species, forming unique and well-supported clades that could not be distinguished among species (Figs S2–S6). Thus, after the inclusion of our endophytic isolates and through analyses of individual genes and a combined matrix, in addition to small differences in DNA sequences and overlapping morphological features, we are synonymising *D. lutescens*, *D. pseudoinconspicua*, and *D. samaneae* under *D. inconspicua*; *D. neoraonikayaporum* under *D. raonikayaporum*; and *D.*

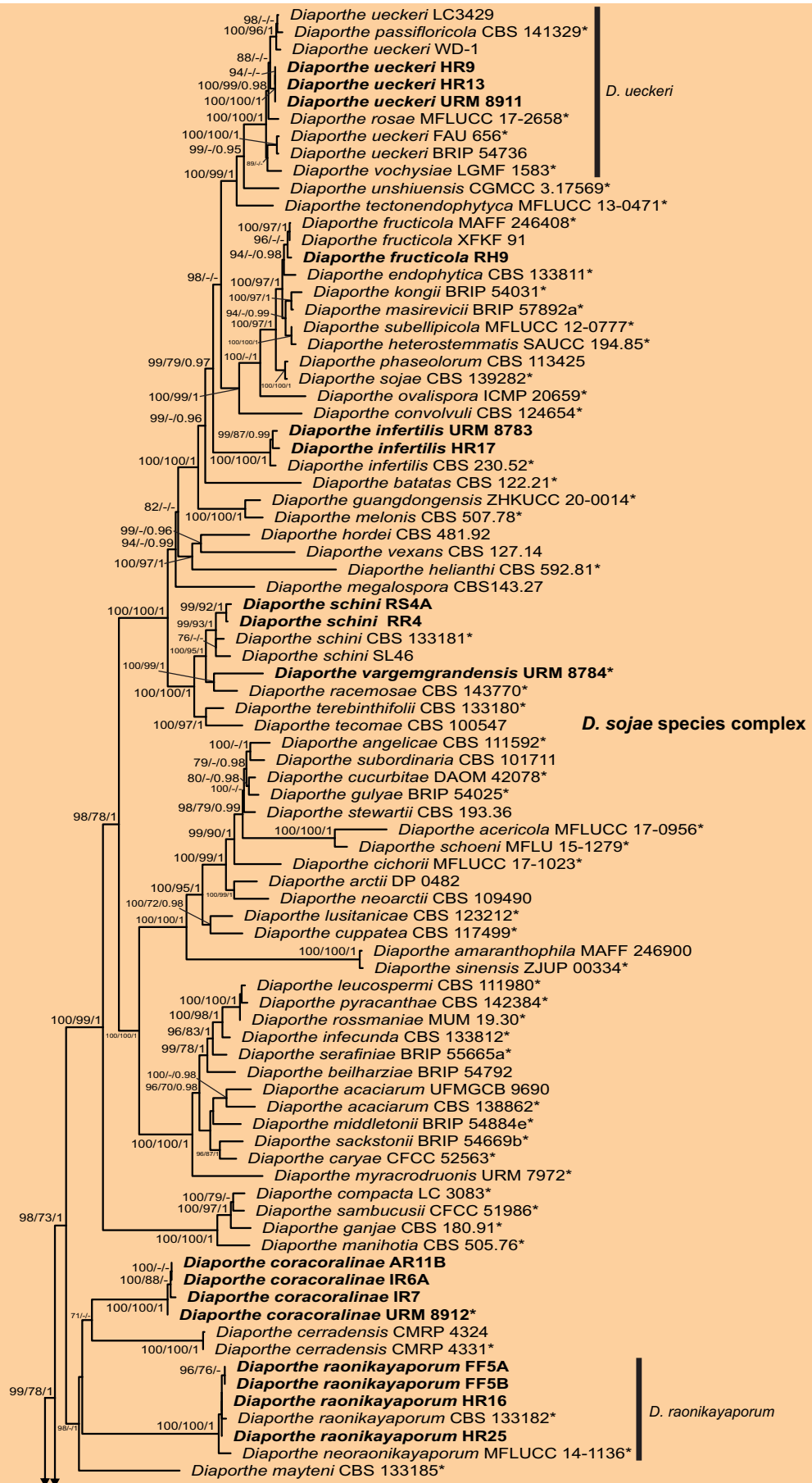


Fig. 2. Maximum Likelihood (ML) phylogenetic tree of the combined *tub2*, ITS, *tef1*, *cmdA* and *his3* sequences of *Diaporthe* species. The species/isolates obtained in this study are in **bold**. Ex-type strains are marked with an asterisk (*). Confidence values for ML-BS $\geq 70\%$ (UFboot2/RAXML) and BPP ≥ 0.95 are included near the nodes and the "-" indicates statistical support below the threshold values. The scale bar represents the expected number of changes per site. The tree was rooted to *Diaporthella corylina* (CBS 121124).

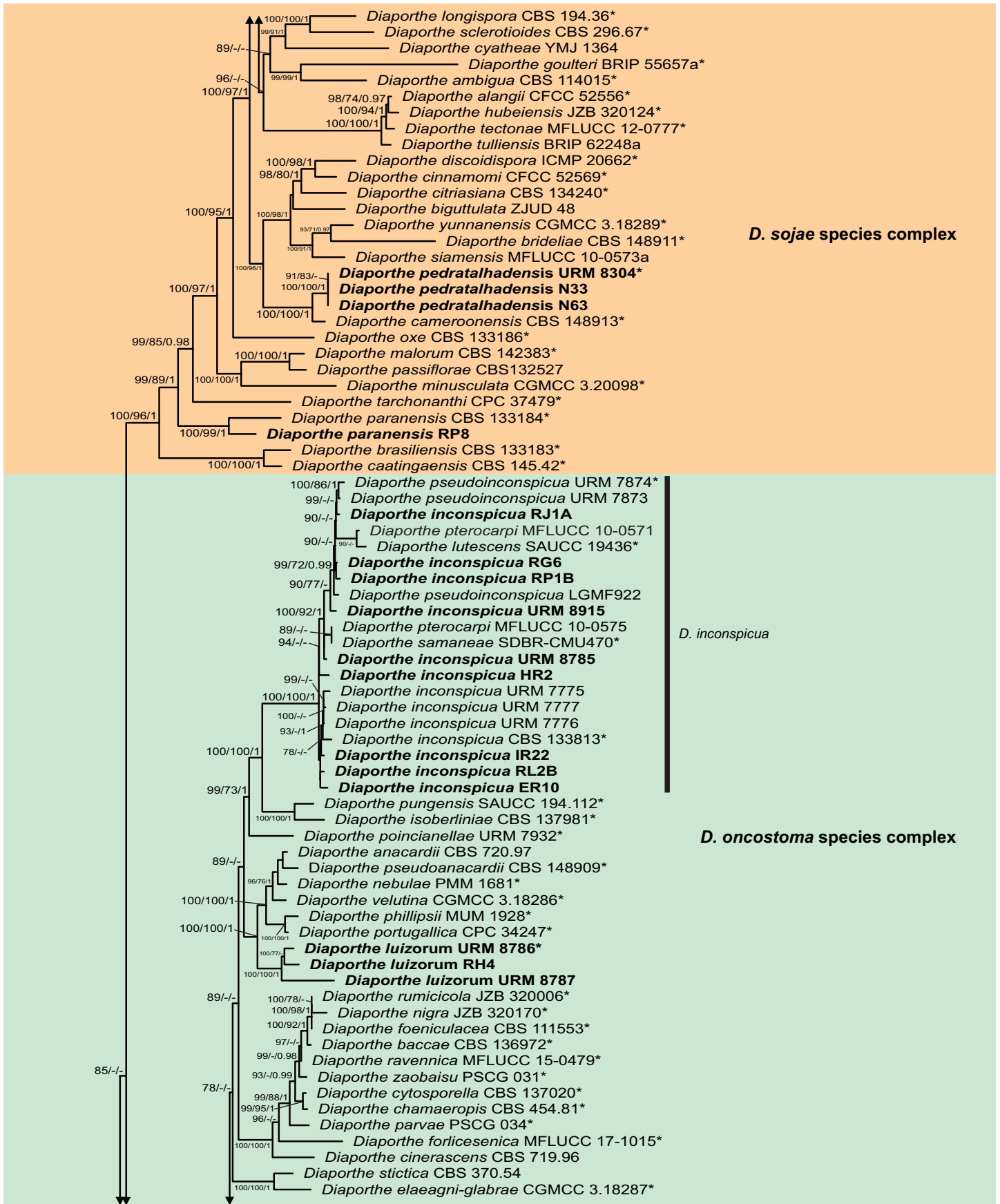


Fig. 2. (Continued).

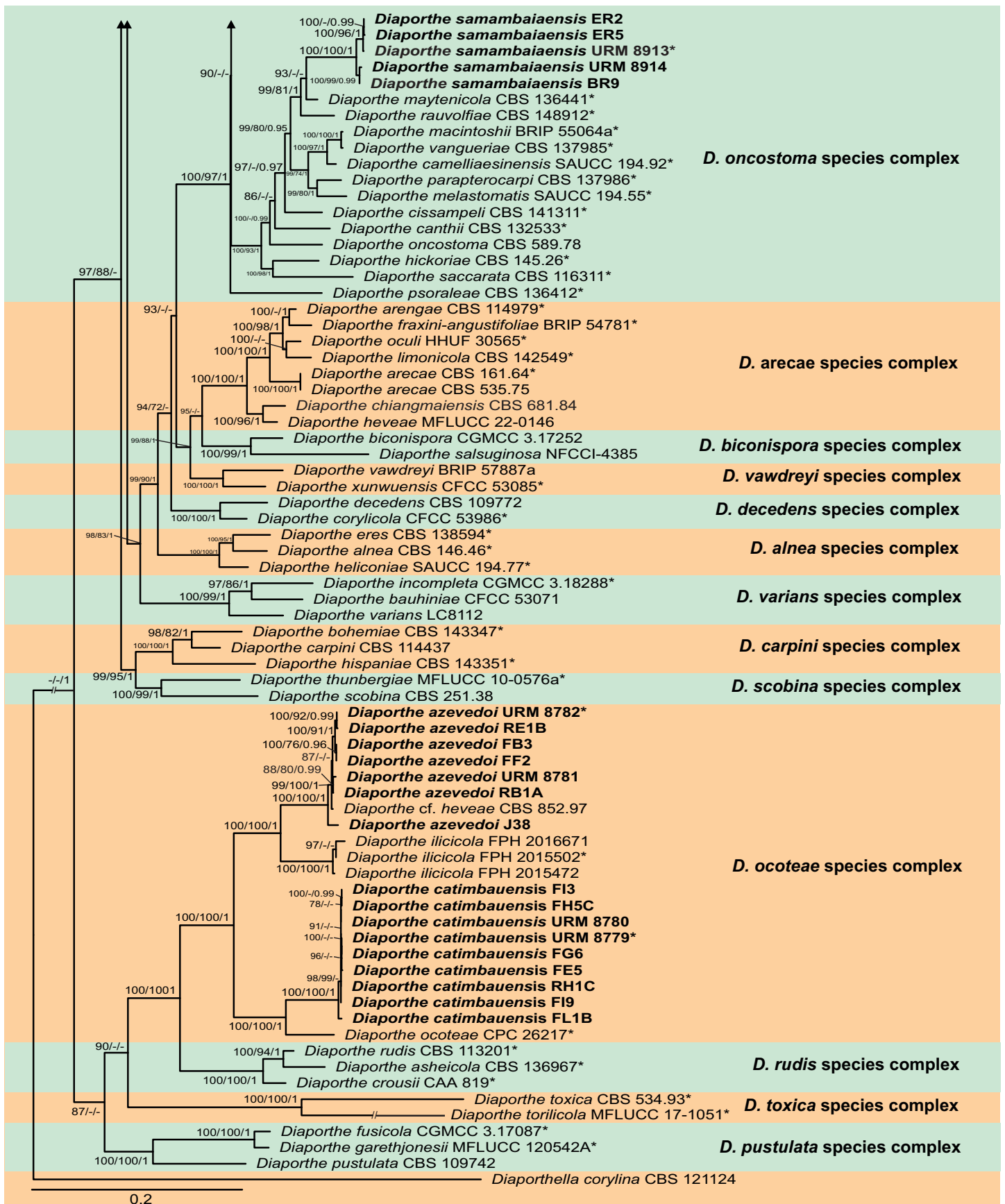


Fig. 2. (Continued).

passifloricola, *D. rosae*, and *D. vochysiae* under *D. ueckeri*.

Furthermore, based on phylogenetic analyses (Fig. 2, Figs S2–S6), we are proposing seven new species (*Diaporthe azevedoi*, *D. catimbauensis*, *D. coracoralinae*, *D. luizorum*, *D. pedratalhadensis*, *D. samambaiaensis*, and *D. vargemgrandensis*) and report the occurrence of seven known species (*D. fruticola*, *D. inconspicua*, *D. infertilis*, *D. paranensis*, *D. raonikayaporum*, *D. schini*, and *D. ueckeri*) among our endophytic isolates, expanding their host relationships and geographic distribution.

Taxonomy

Diaporthe azevedoi L.O. Ferro, Souza-Motta & J.D.P. Bezerra, **sp. nov.** MycoBank MB 852375. Figs 3A, 4A.

Etymology: Named in honour of Prof. dr João Lúcio de Azevedo, a prominent Brazilian scientist who published several studies of endophytic fungi from Brazil.

Conidiomata pycnidial, solitary, agglomerate, superficial and immersed, globose to subglobose, with ostioles exuding light yellow conidial mass, pycnidial wall of *textura angularis*, 251–511.5(–883.5) × 235.5–465(–651) µm. **Conidiophores** reduced to conidiogenous cells. **Conidiogenous cells** enteroblastic, phialidic, hyaline, filiform, cylindrical, 10.5–23(–28) × 1–2(–2.5) µm. **Alpha conidia** hyaline, aseptate, fusiform, occasionally guttulate, acute at both ends, 6.5–7.5 × 2–2.5 µm. **Beta** and **gamma conidia** not observed. **Sexual morph** not observed.

Culture characteristics: Colonies flat, mycelium with irregular margin, centrally sulcate, velvety appearance, centrally smoke grey to olivaceous with fuscous black edges and brown reverse with olivaceous edges; measuring 75 mm diam.

Typus: Brazil, Pernambuco state, Buíque municipality, Parque Nacional do Catimbau (8°36'35"S, 37°14'40"W), isolated as an endophyte from leaves of *Anacardium occidentale* (Anacardiaceae), 11 Nov. 2020, L.O. Ferro & S.S. Nascimento (**holotype** UFG 37189; culture ex-type URM 8782 = FCCUGF 34 = Isolate FA1A).

Additional specimens examined: Brazil, Pernambuco state, Buíque municipality, Parque Nacional do Catimbau (8°36'35"S, 37°14'40"W), isolated occurring endophytically in *A. occidentale*, 11 Nov. 2020, L.O. Ferro & S.S. Nascimento, isolates from leaves: FB3, FD6, FE4A, FE4B, FF2, FG2B, and branches: RB1A, RE1B; Goiás state, Pirenópolis municipality, Reserva Ecológica Vargem Grande (15°48'14.256"S, 48°55'6.204"W), isolated occurring endophytically in *A. occidentale*, 12 Oct. 2022, L.O. Ferro & J.D.P. Bezerra, isolates from leaves: FR3, FR4, FS3, and branches: RL5, URM 8781 = FCCUGF 35, RQ1A, RR1B, RR1C, RO14, RL6; Alagoas state, Quebrangulo municipality, Reserva Biológica de Pedra Talhada (09°15'26.8"S, 36°25'53.7"W), isolated occurring endophytically in leaves of *Miconia* sp. (Melastomataceae), 20 Jul. 2018, L.O. Ferro, isolate J38.

Notes: *Diaporthe azevedoi* (URM 8782) is phylogenetically placed in a clade with *D. cf. heveae* (CBS 852.97) and other endophytic isolates from our study. *Diaporthe heveae* was isolated from the branches of *Hevea brasiliensis* in Sri Lanka (Petch 1906) and the isolate *D. cf. heveae* (CBS 852.97) was deposited together with another isolate (CBS 681.84), both obtained from *Hevea brasiliensis* (Brazil and India, respectively) (Gomes et al. 2013). However, similarly as shown by Gomes et al. (2013), in our study, these isolates clustered in different clades, not being conspecific. Gomes et al. (2013) pointed out that the isolates deposited in the CBS collection were sterile and they did not solve the taxonomy of these isolates, naming both *Diaporthe cf. heveae*. In another study, the isolate *D. cf. heveae*

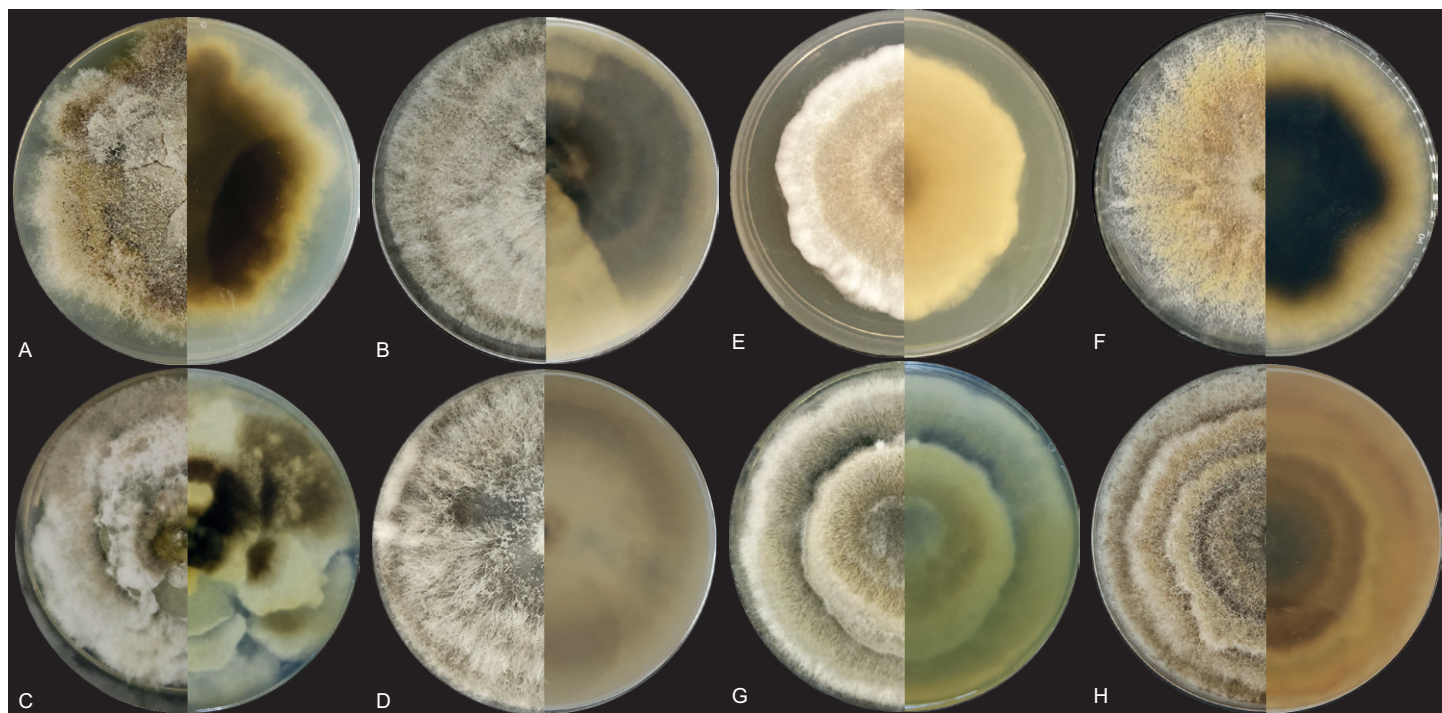


Fig. 3. Colonies of *Diaporthe* species on PDA after 7 d at 25 °C in the dark (surface and reverse). **A.** *Diaporthe azevedoi* URM 8782. **B.** *Diaporthe catimbauensis* URM 8779. **C.** *Diaporthe coracoralinae* URM 8912. **D.** *Diaporthe infertilis* URM 8783. **E.** *Diaporthe luizorum* URM 8786. **F.** *Diaporthe pedratalhadensis* URM 8304. **G.** *Diaporthe samambaiaensis* URM 8913. **H.** *Diaporthe vargemgrandensis* URM 8784.

(CBS 681.84) clustered with *D. heveae* (MFLUCC 22-0146), isolated from the leaf spots of *Nephrolepis cordifolia* in Thailand (Seifollahi *et al.* 2023), and was treated as *D. heveae*. However, Pereira *et al.* (2023) studied the *D. arecae* species complex and identified the isolate CBS 681.84 as *D. chiangmaiensis*. In our study, we obtained sporulating isolates that appeared to be conspecific with CBS 852.97, and we introduced the new species, *D. azevedoi*. This new species is phylogenetically related to *D. ilicicola* (FPH2015502 = CBS 144318) isolated from the fruit of *Ilex verticillata* × *Ilex serrata* obtained in the USA (Lin *et al.* 2018), and their nucleotide pairwise sequences differ by 19 bp for *tub2*, 20 bp for ITS, and 33 bp for *his3*; no sequences of *D. ilicicola* are available for *cmdA* and *tef1*. Morphologically, *D. azevedoi* has smaller fusoid conidia (6.5–7.5 × 2–2.5 µm) and *D. ilicicola* has larger (7.13–9.43 × 2.91–4.16 µm) ovoid to ellipsoid conidia (Lin *et al.* 2018). In addition, *D. ilicicola* (FPH2015502 = CBS 144318) was not included in the analysis performed by Norphanphoun *et al.* (2022), and as this clade is related to *D. catimbauensis* sp. nov. (URM 8779), *D. ocoteae* (CPC 26217), and *D. ilicicola* (FPH 2015502), we included all of them in the *Diaporthe ocoteae* species complex introduced here.

Diaporthe catimbauensis L.O. Ferro, Souza-Motta & J.D.P. Bezerra, *sp. nov.* MycoBank MB 852376. Figs 3B, 4B.

Etymology: The name refers to the Parque Nacional do Catimbau, a conservation unit of the Caatinga forest where the endophyte was first isolated.

Conidiomata pycnidial, solitary, agglomerate, superficial on plant tissues placed on culture medium surface, globose to subglobose, with ostioles exuding light cream conidial mass, short neck occasionally observed, pycnidial wall of *textura angularis*, (90–)279–446.5(–465) × 185–409(–465) µm. **Conidiophores** reduced to conidiogenous cells. **Conidiogenous cells** enteroblastic, phialidic, hyaline, subcylindrical to cylindrical, attenuate towards the apex, occasionally a septum may be observed, 9.5–14 × 1–2 µm. **Alpha conidia** hyaline, aseptate, fusiform, occasionally guttulate, a hilum-like protuberance at base occasionally observed, 2.5–4.5(–6.5) × 2 µm. **Beta** and **gamma conidia** not observed. **Sexual morph** not observed.

Culture characteristics: Colonies covering Petri dish with sparse aerial mycelium, flat, velvety appearance, surface smoke grey and reverse dark grey at centre and grey edges.

Typus: **Brazil**, Pernambuco state, Buíque municipality, Parque Nacional do Catimbau (8°36'35"S, 37°14'40"W), isolated as an endophyte from branches of *Anacardium occidentale* (*Anacardiaceae*), 11 Nov. 2020, L.O. Ferro & S.S. Nascimento (**holotype** UFG 37190; ex-type culture URM 8779 = FCCUFG 36 = Isolate RG2).

Additional specimens examined: **Brazil**, Pernambuco state, Buíque municipality, Parque Nacional do Catimbau (8°36'35"S, 37°14'40"W), isolated occurring endophytically in *A. occidentale*, 11 Nov. 2020, L.O. Ferro & S.S. Nascimento, isolates from leaves: FA1B–FA1E, FA2A, FA2B, FA3A, FA3B, FA5–FA8, FA10, FA12, FB1A–FB1D, FB2A, FB2B, FB4, FC1A, FC1B, FC2, FC4, FD1A–FD1C, FD2A, FD3–FD5, FE1A–FE1C, FE5, FG1A–FG1D, FG4A, FG4B, FG6, FH1A–FH1D, FH3, FH5C, FH6, FI1B, FI1D, FI2A, FI2B, FI3, FI4A–FI4C, FI6, FI8, FI9, and branches: RA1A, RA2A, RA2B, RA4A, RA4B, RB1B, URM 8780 = FCCUFG 37, RB2B, RB5, RC1A, RC1B, RC2A, RC2B, RC3A, RC3B, RD1A, RD1C–RD1E, RD2A–RD2D, RE1C,

RH1C, RH1E, RH1F; Goiás state, Pirenópolis municipality, Reserva Ecológica Vargem Grande (15°48'14.256"S, 48°55'6.204"W), occurring endophytically in *A. occidentale*, 12 Oct. 2022, L.O. Ferro & J.D.P. Bezerra, isolates from leaves: FL1A–FL1C, FR2, FS5 and branches: RN4.

Notes: Phylogenetically, *Diaporthe catimbauensis* (URM 8779) is positioned as a single lineage related to *D. ocoteae* (CPC 26217), which was isolated from the leaves of *Ocotea obtusata* (*Lauraceae*) in France (Crous *et al.* 2016b), and their nucleotide pairwise sequences differ by 29 bp for *tub2* and 11 bp for ITS; no sequences of *D. ocoteae* are available for *cmdA*, *his3*, and *tef1*. Morphologically, *D. catimbauensis* has larger pycnidia (279–446.5 × 185–409 µm) than *D. ocoteae* (300 µm diam) and the conidiophores of *D. catimbauensis* are not branched and produce smaller conidia (2.5–4.5 × 2 µm) than those of *D. ocoteae* (9–10 × 2.5–3 µm) (Crous *et al.* 2016b). In a study of *Diaporthe* species complexes, Norphanphoun *et al.* (2022) placed *D. ocoteae* as a singleton; however, because of its relationship with our new species, we are proposing the *Diaporthe ocoteae* species complex, including *D. catimbauensis* (URM 8779), *D. ocoteae* (CPC 26217), *D. azevedoi* (URM 8782), and *D. ilicicola* (FPH2015502 = CBS 144318). *Diaporthe catimbauensis* was the most common species in the study, representing more than 50 % of our isolates, and was found to be associated with *A. occidentale* growing in two Brazilian biomes (Caatinga and Cerrado).

Diaporthe coracoralinae L.O. Ferro, Souza-Motta & J.D.P. Bezerra, *sp. nov.* MycoBank MB 852377. Figs 3C, 4C.

Etymology: Named in honour of Cora Coralina (Ana Lins dos Guimarães Peixoto, 1889–1985), a poet from Goiás state, considered one of the most important writers of Brazilian literature.

Conidiomata pycnidial, solitary, agglomerate, superficial on culture medium surface, globose, subglobose, ostiolate, pycnidial wall of *textura angularis*, 105–135 × 70.5–88 µm. **Conidiophores** reduced to conidiogenous cells. **Conidiogenous cells** enteroblastic, phialidic, hyaline, filiform, cylindrical, (5.5–)8–9 × 1.5–2(–2.5) µm. **Alpha conidia** hyaline, aseptate, fusiform, guttulate, a hilum-like protuberance at base occasionally observed, 5–6(–7) × 2–2.5(–3) µm. **Beta** and **gamma conidia** not observed. **Sexual morph** not observed.

Culture characteristics: Colonies covering Petri dish, concentric with velvety appearance, centre isabelline and white, reverse honey.

Typus: **Brazil**, Goiás state, Goiânia municipality, arboretum (didactic area of the Cerrado) of the Escola de Agronomia, Campus Samambaia of Universidade Federal de Goiás (16°35'58.5"S, 49°16'45.8"W), isolated as an endophyte from branches of *Brosimum gaudichaudii* (*Moraceae*), 10 Oct. 2020, T.M. da Silva & J.D.P. Bezerra (**holotype** UFG 37191; ex-type culture URM 8912 = FCCUFG 38 = Isolate IR4).

Additional specimens examined: **Brazil**, Goiás state, Goiânia municipality, arboretum (didactic area of the Cerrado) of the Escola de Agronomia, Campus Samambaia of the Universidade Federal de Goiás (16°35'58.5"S, 49°16'45.8"W), isolated as an endophyte from branches of *B. gaudichaudii* (*Moraceae*), 10 Oct. 2020, T.M. da Silva & J.D.P. Bezerra, isolates FCCUFG 39 = AR11B, AR16, IR3B, IR6A, IR7, IR17, IR19,

IR20; Goiás state, Pirenópolis municipality, Reserva Ecológica Vargem Grande (15°48'14.256"S, 48°55'6.204"W), isolated as an endophyte from branches of *A. occidentale* (Anacardiaceae), 12 Oct. 2022, L.O. Ferro & J.D.P. Bezerra, isolate RN6.

Notes: *Diaporthe coracoralinae* (URM 8912) is phylogenetically related to *D. cerradensis* (CMRP4331), which was also isolated as an endophyte from the leaves of *Stryphnodendron adstringens* of the Cerrado in Brazil (Iantas et al. 2021), and their nucleotide pairwise sequences differed by 25, 16, 53, 40, and 37 bp for *tub2*, ITS, *tef1*, *cmdA*, and *his3*, respectively. Morphologically, no beta conidia were observed for *D. coracoralinae* and both species also differ in the size of conidiogenous cells, which are smaller in *D. coracoralinae* (8–9 × 1.5–2 µm) than in *D. cerradensis* (12.2–15.1 × 2–3.5 µm) (Iantas et al. 2021). We placed our new species within the *Diaporthe sojae* species complex.

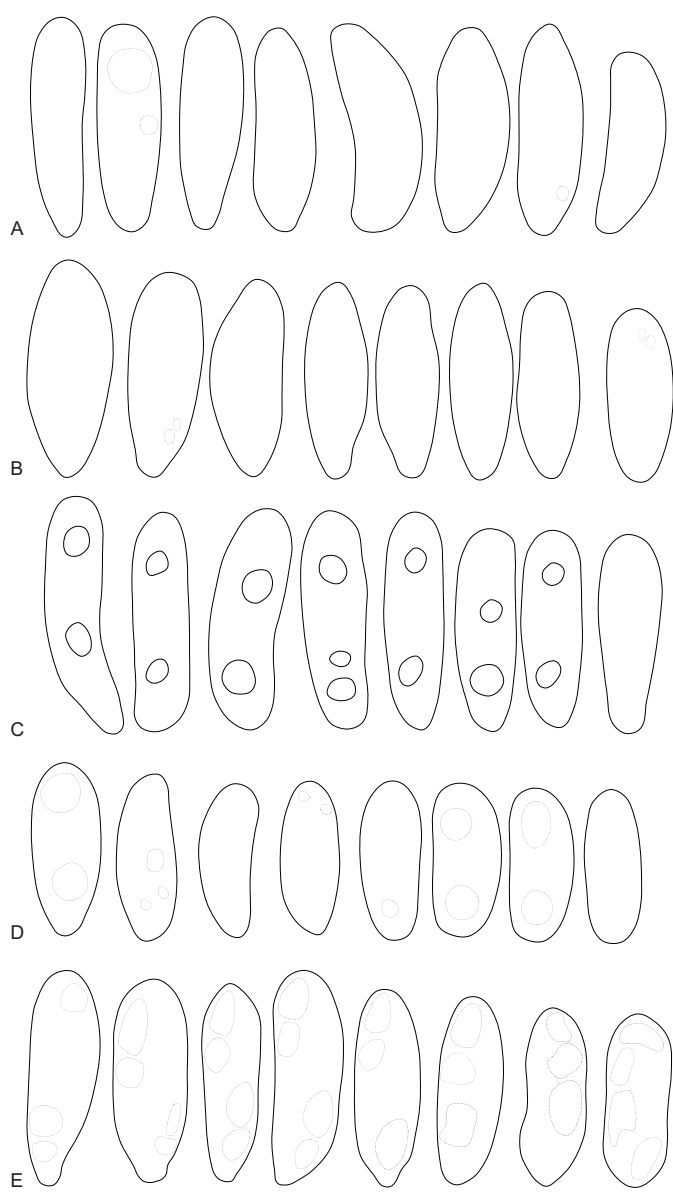


Fig. 4. Alpha conidia of *Diaporthe* species. **A.** *Diaporthe azevedoi* URM 8782. **B.** *Diaporthe catimbauensis* URM 8779. **C.** *Diaporthe coracoralinae* URM 8912. **D.** *Diaporthe infertilis* URM 8783. **E.** *Diaporthe luizorum* URM 8786. Scale bar = 10 µm.

Diaporthe fructicola Minosh. et al., *Persoonia* **42**: 409. 2019.

See Crous et al. (2019) for illustrations and descriptions.

Typus: Japan, Tokyo, Hahajima, on the fruit of *Passiflora edulis* × *P. edulis* f. *flavicarpa* (Passifloraceae), Jun. 2015, T. Ono HM15-390 (holotype TNSF-54762; ex-type culture MAFF 246408).

Distribution and hosts: Brazil: *Anacardium occidentale* (this study); Japan: *Passiflora edulis* × *P. edulis* f. *flavicarpa* (Crous et al. 2019, Minoshima et al. 2020).

Specimen examined: Brazil, Pernambuco state, Buíque municipality, Parque Nacional do Catimbau (8°36'35"S, 37°14'40"W), isolated as an endophyte from branches of *A. occidentale* (Anacardiaceae), 11 Nov. 2020, L.O. Ferro & S.S. Nascimento, isolate FCCUG 40 = RH9.

Notes: *Diaporthe fructicola* was proposed by Crous et al. (2019) and was isolated from *Passiflora edulis* × *P. edulis* f. *flavicarpa* in Japan. Minoshima et al. (2020) also reported that this species causes fruit rot in passion fruit. This is the first report of *D. fructicola* as an endophyte.

Diaporthe inconspicua R.R. Gomes et al, *Persoonia* **31**: 23. 2013. Synonyms: *Diaporthe pseudoinconspicua* T.G.L. Oliveira et al., *Persoonia* **40**: 275. 2018.

Diaporthe lutescens S.T. Huang et al., *MycKeys* **77**: 81. 2021.

Diaporthe samaneae Monkai & S. Lumyong, *J. Fungi* **9**: 14. 2023.

Conidiomata pycnidial, solitary or aggregated, superficial to embedded on PDA or associated with infected leaf tissue, erumpent, dark brown to black, globose, 65–954 × 100–742 µm with ostioles exuding yellowish conidial mass. **Conidiophores** hyaline, smooth, branched or not, ampulliform, straight to sinuous, 7.5–31.5 × 1–3 µm. **Conidiogenous cells** enteroblastic, phialidic, cylindrical to subcylindrical or ampulliform, tapering towards the apex, bifurcated, prominent collar, (2–)5–14.5 × 1.5–2.5(–3) µm. **Paraphyses** absent. **Alpha conidia** hyaline, smooth, fusiform, biguttulate, subtruncated at the base, 5–11 × 1.5–3(–3.5) µm. **Beta conidia** hyaline, aseptate, filiform, straight to curved, base subtruncate, widened towards the apex, (17.5–)18–29 × 1–2 µm. **Gamma conidia** and **sexual morph** not observed.

Typus: Brazil, on petiole of *Maytenus ilicifolia*, Jul. 2007, R.R. Gomes (holotype CBS H-21102; ex-type culture LGMF 930 = CPC 20306 = CBS 133813).

Distribution and hosts: Brazil: *Maytenus ilicifolia* (Gomes et al. 2013), *Atriplex nummularia* (Queiroz et al. 2017), *Vellozia gigantea* (Ferreira et al. 2017), *Poincianella pyramidalis* (Crous et al. 2018a, Bezerra et al. 2018), *Copaifera langsdorffii* (Carvalho et al. 2021), *Anacardium occidentale* (this study), *Brosimum gaudichaudii* (this study); South Africa: *Cyclopia* sp. (Smit et al. 2021); Uruguay: *Pyrus communis* (Sessa et al. 2018).

Specimens examined: Brazil, Pernambuco state, Buíque municipality, Parque Nacional do Catimbau (8°36'35"S, 37°14'40"W), isolated as an endophyte from branches of *A. occidentale*, 11 Nov. 2020, L.O. Ferro & S.S. Nascimento, isolate RG6; Goiás state, Pirenópolis municipality, Reserva Ecológica Vargem Grande (15°48'14.256"S, 48°55'6.204"W), isolated occurring endophytically in *A. occidentale*, 12

Oct. 2022, L.O. Ferro & J.D.P. Bezerra, isolates from branches: RJ1A, RL2B, RP1B and leaves: URM 8785 = FCCUG 41; Goiás state, Goiânia municipality, arboretum (didactic area of the Cerrado) of the Escola de Agronomia, Campus Samambaia of the Universidade Federal de Goiás (16°35'58.5"S, 49°16'45.8"W), isolated as an endophyte from branches of *B. gaudichaudii* (Moraceae), 10 Oct. 2020, T.M. da Silva & J.D.P. Bezerra, isolates URM 8915 = FCCUG 42, ER10, HR2, IR22.

Notes: *Diaporthe inconspicua* was introduced by Gomes *et al.* (2013) based solely on phylogenetic analyses, and Bezerra *et al.* (2018) provided a morphological description for this species. Previously, *Diaporthe pterocarpi* was introduced by Udayanga *et al.* (2012), with *Phomopsis pterocarpi* (Hughes 1953) as basionym. However, Udayanga *et al.* (2012) chose an isolate from Thailand to serve as epitype for the name, which is morphologically distinct from the holotype from Togo. Hughes (1953) described *P. pterocarpi* as having conidiomata (100 × 200 µm), conidiophores (10 × 2–3 µm), and conidia (6–9 × 2.5–3 µm), differing from the material proposed by Udayanga *et al.* (2012), with conidiomata (65–100 × 100 µm), conidiophores (10–15 × 1–2 µm), and conidia (5–)6–7(–9) × (2–)2.5(–3) µm. In addition, the typification designated by Udayanga *et al.* (2012) was not Code compliant [Art. 9.9 (Shenzhen)]. The oldest valid name for this clade, therefore, is *D. inconspicua*. Later, three species (*D. lutescens*, *D. pseudoinconspicua*, and *D. samaneae*) were introduced and placed as phylogenetic neighbours to the clade containing *D. inconspicua* and the isolates named as *D. pterocarpi*. *Diaporthe pseudoinconspicua* was proposed for endophytes from Brazil (Crous *et al.* 2018b) and *D. lutescens* was isolated from leaves of *Chrysalidocarpus lutescens* (Sun *et al.* 2021). Recently, Monkai *et al.* (2023) introduced *D. samaneae* for a saprobic fungus on dead wood of *Samanea saman* in Thailand. Interestingly, we included our isolates (nine), compared them with other endophytic isolates from Brazil (Bezerra *et al.* 2018, Crous *et al.* 2018b), and demonstrated that they were conspecific with sequences of the ex-type strains of the species synonymised here. Based on the limited nucleotide differences among sequences of different genes and in our analyses of individual and combined genes (Fig. 2, Figs S1–S6), this clade remained as a unique lineage. In addition, morphological descriptions were not useful in separating the treated species, confirming our decision to synonymise *D. lutescens*, *D. pseudoinconspicua*, *D. samaneae*, and the isolates named as “*D. pterocarpi*” under *D. inconspicua*, the first valid species name in this clade. A revised description is included to expand the circumscription of *D. inconspicua* and allow an easy morphological and phylogenetic distinction from related species.

Diaporthe infertilis Guarn. & Crous, *IMA Fungus* 8: 327. 2017. Figs 3D, 4D.

Conidiomata pycnidial, solitary, agglomerate, superficial on plant tissues placed on culture medium surface, globose, ostiolate, short neck occasionally observed, pycnidial wall of *textura angularis*, 186–279 µm wide. **Conidiophores** reduced to conidiogenous cells. **Conidiogenous cells** enteroblastic, phialidic, hyaline, filiform, cylindrical, 8.5–9.5(–14) × 1–1.5(–2) µm. **Alpha conidia** hyaline, aseptate, fusiform, guttulate, a hilum-like protuberance at base occasionally observed, 4–5.5 × 2–2.5 µm. **Beta** and **gamma conidia** not observed. **Sexual morph** not observed.

Culture characteristics: Colonies flat, covering Petri dish after 2 wk, non-sulcate, cottony appearance, aerial mycelium smoke grey and reverse hazel.

Typus: Suriname, Paramaribo, decaying fruit of *Citrus sinensis*, Apr., 1932, N.J. van Sochtelen (**holotype** CBS H-23179; ex-type culture CBS 230.52).

Distribution and hosts: Brazil: *Glycine max* (Gomes *et al.* 2013), *Mikania glomerata* (Polonio *et al.* 2015), *Anacardium occidentale* (this study), *Brosimum gaudichaudii* (this study); Italy: unknown host (Gomes *et al.* 2013); Suriname: *Citrus sinensis* (Guarnaccia & Crous 2017).

Specimens examined: Brazil, Goiás state, Pirenópolis municipality, Reserva Ecológica Vargem Grande (15°48'14.256"S, 48°55'6.204"W), isolated as endophyte from branches of *A. occidentale* (Anacardiaceae), 12 Oct. 2022, L.O. Ferro & J.D.P. Bezerra, isolate URM 8783 = FCCUG 43; Goiás state, Goiânia municipality, arboretum (didactic area of the Cerrado) of the Escola de Agronomia, Campus Samambaia of the Universidade Federal de Goiás (16°35'58.5"S, 49°16'45.8"W), isolated as endophyte from branches of *B. gaudichaudii* (Moraceae), 10 Oct. 2020, T.M. da Silva & J.D.P. Bezerra, isolate FCCUG 44 = HR17.

Notes: *Diaporthe infertilis* (CBS 230.52) was proposed by Guarnaccia & Crous (2017) isolated from decomposing *Citrus sinensis* in Suriname. Previously, Gomes *et al.* (2013) named this isolate (CBS 230.52) as *D. citri*, but with new molecular analyses, *D. citri* clustered in a different clade (Udayanga *et al.* 2014) and the isolate CBS 230.52 was later named *D. infertilis* (Guarnaccia & Crous 2017). Guarnaccia & Crous (2017) did not have success in inducing sporulation in this isolate, and thus *D. infertilis* (CBS 230.52) was identified based only on multi-locus phylogenetic analyses. Some endophytes isolated from *A. occidentale* and *B. gaudichaudii* (Cerrado) showed high identity and phylogenetic relationship with the ex-type strain of *D. infertilis* (CBS 230.52), for which we are providing a morphological description.

Diaporthe luizorum L.O. Ferro, Souza-Motta & J.D.P. Bezerra, *sp. nov.* MycoBank MB 852378. Figs 3E, 4E.

Etymology: Named in honour of the parents (Luiz de Gonzaga and Luiza) of the first author of this paper.

Conidiomata pycnidial, solitary, agglomerate, superficial on plant tissues placed on culture medium surface, globose to subglobose, with ostioles exuding light cream conidial mass, pycnidial wall of *textura angularis*, 279–418.5(–465) × (186–)279–418(–465) µm. **Conidiophores** reduced to conidiogenous cells. **Conidiogenous cells** enteroblastic, phialidic, hyaline, filiform, slightly ampulliform, subcylindrical to cylindrical, attenuate towards the apex, (5–)6–12(–14.5) × 1–2.5 µm. **Alpha conidia** hyaline, aseptate, fusiform, guttulate, acute at apex, a hilum-like protuberance at base occasionally observed, (4.5–)5.5–6.5(–7.5) × 2–2.5(–3) µm. **Beta** and **gamma conidia** not observed. **Sexual morph** not observed.

Culture characteristics: Colony measuring 68 mm, slightly irregular margin, cottony with buff colour at the centre, white edges, reverse umber to buff.

Typus: Brazil, Pernambuco state, Buíque municipality, Parque Nacional do Catimbau (8°36'35"S, 37°14'40"W), isolated as an endophyte from leaves of *Anacardium occidentale* (Anacardiaceae), 11 Nov. 2020, L.O. Ferro & S.S. Nascimento (**holotype** UFG 37192; ex-type culture URM 8786 = FCCUFG 45 = Isolate FG2A).

Additional specimens examined: Brazil, Pernambuco state, Buíque municipality, Parque Nacional do Catimbau (8°36'35"S, 37°14'40" W), isolated as an endophyte from branches of *A. occidentale*, 11 Nov. 2020, L.O. Ferro & S.S. Nascimento, isolate RH4; Goiás state, Pirenópolis municipality, Reserva Ecológica Vargem Grande (15°48'14.256"S, 48°55'6.204"W), isolated occurring endophytically in *A. occidentale* (Anacardiaceae), 12 Oct. 2022, L.O. Ferro & J.D.P. Bezerra, isolates from branches: URM 8787 = FCCUFG 46 and leaves: FJ4C, FP2, FS2; Goiás state, Goiânia municipality, arboretum (didactic area of the Cerrado) of the Escola de Agronomia, Campus Samambaia of the Universidade Federal de Goiás (16°35'58.5"S, 49°16'45.8"W), isolated as an endophyte from branches of *B. gaudichaudii* (Moraceae), 10 Oct. 2020, T.M. da Silva & J.D.P. Bezerra, isolates IR6B, IR29, and IR2.

Notes: *Diaporthe luizorum* (URM 8786) is phylogenetically related to a clade with *D. anacardii* (CBS 720.97), *D. phillipsii* (MUM 1928), *D. portugallica* (CPC 34247), *D. pseudoanacardii*

(CBS 148909), *D. nebulae* (PMM 1681), and *D. velutina* (LC4421). In this clade, *D. luizorum* and *D. pseudoanacardii* (Lambert et al. 2023) are the only endophytic species. Morphologically, *D. luizorum* has smaller conidiogenous cells [(5–)6–12(–14.5) × 1–2.5 µm] than *D. phillipsii* (19.5 × 1.4 µm) and *D. pseudoanacardii* [7–28 × 1–3.5(–4) µm], and they also differ by the absence of beta conidia that are readily observed in other related species. The closest phylogenetic species, *D. phillipsii* (MUM 1928) and *D. portugallica* (CPC 34247), differ from *D. luizorum* (URM 8786) by 16 and 15 bp for *tub2*, 12 and 20 bp for ITS, 18 and 17 bp for *tef1*, 30 and 32 bp for *cmdA*, and 17 and 18 bp for *his3*, respectively. *Diaporthe luizorum* belongs to the *Diaporthe oncostoma* species complex.

Diaporthe paranensis R.R. Gomes et al., *Persoonia* **31**: 28. 2013.

See Gomes et al. (2013) for illustrations and descriptions.

Typus: Brazil, Paraná, Colombo, endophytic species isolated from petiole of *Maytenus ilicifolia* (popular name Espinheira Santa, Celastraceae), Jul. 2007, R.R. Gomes (**holotype** CBS H-21099; ex-type culture CBS 133184).

Distribution and hosts: Brazil: *Maytenus ilicifolia* (Gomes et al. 2013), *Prunus persica* (de Souza et al. 2021), *Anacardium occidentale* (this study).

Specimen examined: Brazil, Goiás state, Pirenópolis municipality, Reserva Ecológica Vargem Grande (15°48'14.256"S, 48°55'6.204"W), isolated as an endophyte from branches of *A. occidentale*, 12 Oct. 2022, L.O. Ferro & J.D.P. Bezerra, isolate FCCUFG 47 = RP8.

Notes: *Diaporthe paranensis* was proposed by Gomes et al. (2013) as an endophytic fungus isolated from *Maytenus ilicifolia* in Brazil. Later, de Souza et al. (2021) reported this species causing peach rot in Brazil. Here we expand the known host range, lifestyle, and geographical distribution of this species.

Diaporthe pedratalhadensis L.O. Ferro, Souza-Motta & J.D.P. Bezerra, *sp. nov.* MycoBank MB 852379. Figs 3F, 5A, B.

Etymology: The name refers to the Reserva Biológica de Pedra Talhada (REBIO de Pedra Talhada), a conservation unit of the Atlantic Forest where the endophyte was first isolated.

Conidiomata pycnidial, solitary, agglomerate, superficial on culture medium and on plant tissues placed on culture medium surface, globose to subglobose, ostiolate, conidial mass light cream, evanescent, pycnidial wall of *textura angularis*, 214–279(–325.5) × 186–232.5 µm. **Conidiophores** reduced to conidiogenous cells. **Conidiogenous cells** enteroblastic, phialidic, hyaline with a base slightly pigmented, filiform, slightly ampulliform, subcylindrical to cylindrical, attenuate towards the apex, (8–)13.5 × 3 µm. **Alpha conidia** hyaline, aseptate, fusoid, guttulate, acute at apex, a hilum-like protuberance at base occasionally observed, (5.5–)8(–9.5) × 3(–4) µm. **Beta conidia** hyaline, aseptate, filiform, straight to curved, curved at apex, truncate base, (22–)32.5 × 1.5(–3). **Gamma conidia** and **sexual morph** not observed.

Culture characteristics: Colonies measuring 60–65 mm, cottony, presence of exudates, colour varying from olivaceous, primrose and white, reverse dark brick to straw.

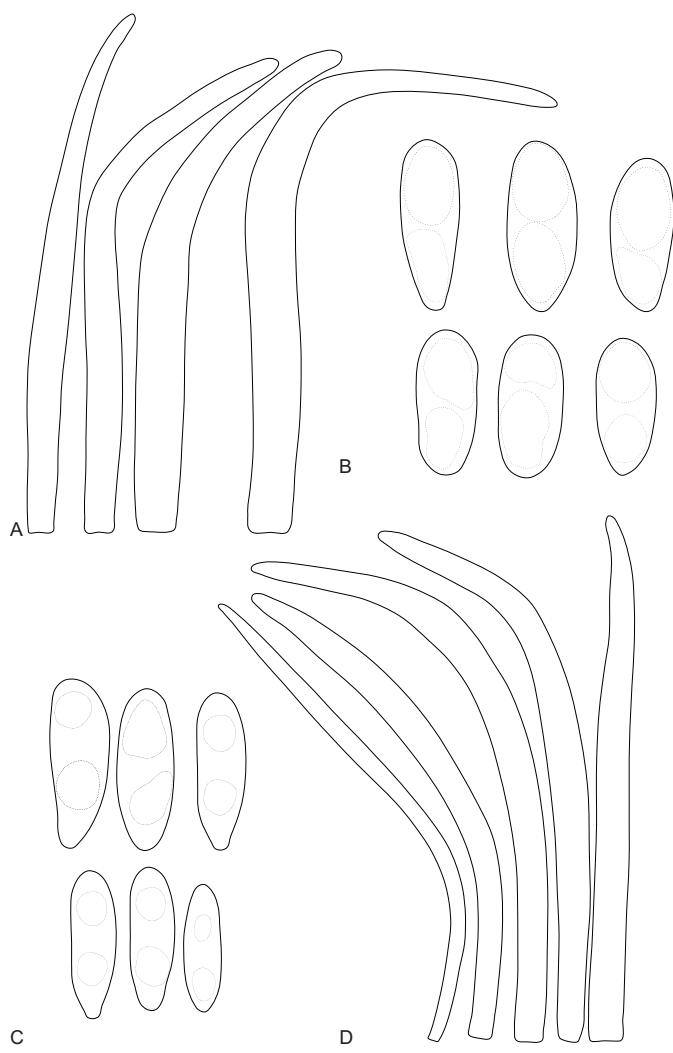


Fig. 5. Alpha and beta conidia of *Diaporthe* species. **A, B.** *Diaporthe pedratalhadensis* URM 8304. **C, D.** *Diaporthe vargemgrandensis* URM 8784. Scale bar = 10 µm.

Typus: Brazil, Alagoas state, Quebrangulo municipality, Reserva Biológica de Pedra Talhada (09°15'26.8"S, 36°25'53.7"W), as an endophyte from leaves of *Miconia* sp. (*Melastomataceae*), 20 Jul. 2018, L.O. Ferro (**holotype** UFG 37193; ex-type culture URM 8304 = FCCUFG 48 = Isolate N23).

Additional specimens examined: Brazil, Alagoas state, Quebrangulo municipality, Reserva Biológica de Pedra Talhada (09°15'26.8"S, 36°25'53.7"W), as an endophyte from the leaves of *Miconia* sp. (*Melastomataceae*), 20 Jul. 2018, L.O. Ferro, isolates FCCUFG 49 = N63 and N33.

Notes: *Diaporthe pedratalhadensis* (URM 8304) has *D. cameroonensis* (CBS 148913) as a related species which was isolated as an endophyte from *Atractogynne gabonii* in Cameroon (Lambert *et al.* 2023), and their sequences differ in 13 bp for *tub2*, 11 bp for ITS, 5 bp for *tef1*, 4 bp for *cmdA* and 10 bp for *his3*. Morphologically, *D. pedratalhadensis* has larger alpha conidia (8 × 3 µm) than *D. cameroonensis* (4.5–6 × 1.5–2.5 µm), and has beta conidia that were not observed in *D. cameroonensis* (Lambert *et al.* 2023). We include *D. pedratalhadensis* in the *Diaporthe sojae* species complex.

Diaporthe raonikayaporum R.R. Gomes *et al.*, *Persoonia* **31**: 31. 2013.

Synonym: *Diaporthe neoraonikayaporum* Doilom *et al.*, *Fungal Diversity* **82**: 16. 2017.

Conidiomata pycnidial, scattered or aggregated, globose or subglobose to conical or ampulliform, dark brown to black, immersed, 110–200 × 50–130 µm [also observed (690–)950–1 045(–1 190) × (805–)995–1 110(–1 285) µm], elongated black neck of 40–140 µm long, ostiolate, with conidial mass, initially hyaline to yellowish, becoming white to cream or pink conidial droplets exuding from central ostioles, thick-walled cells of *textura angularis* comprising of two cell layers, black to brown. *Conidiophores* hyaline, branched above the septa, tapering towards the apex to unbranched, ampulliform to subcylindrical, filiform, 1–3-septate, smooth, dense, straight or slightly curved, 15–23 × 1.3–3 µm. *Conidiogenous cells* enteroblastic, phialidic, hyaline, cylindrical to subcylindrical, filiform, straight to curved, tapering towards the apex, collarette not flared, periclinal thickening prominent, 2.5–9 × 1.5–3 µm. *Alpha conidia* hyaline, oblong to ellipsoid, apex bluntly rounded, base obtuse to subtruncate, bi- to multiguttulate, 5–7 × 2–3 µm. *Beta conidia* filiform, hyaline, flexible to slightly curved, aseptate, base subtruncate, 16–18 × (1–)1.5(–2) µm. *Gamma conidia* aseptate, hyaline, smooth, fusoid, with, bi-guttulate to multi-guttulate, apex acutely rounded, base subtruncate, (7–)8.5–11(–13) × (1–)2–2.5 µm. *Sexual morph* not observed.

Typus: Brazil, Pará, Redenção, endophytic species isolated from leaf of *Spondias mombin* (*Anacardiaceae*), Jul. 2007, K. Rodriguez (**holotype** CBS H-21097; ex-type culture CBS 133182).

Distribution and hosts: Brazil: *Spondias mombin* (Gomes *et al.* 2013), *Anacardium occidentale* (this study), *Brosimum gaudichaudii* (this study); Thailand: *Tectona grandis* (Doilom *et al.* 2017).

Specimens examined: Brazil, Pernambuco state, Buíque municipality, Parque Nacional do Catimbau (8°36'35"S, 37°14'40"W), isolated as an endophyte from leaves of *A. occidentale*, 11 Nov. 2020, L.O. Ferro &

S.S. Nascimento, isolates FF5A, FF5B; Goiás state, Goiânia municipality, arboretum (didactic area of the Cerrado) of the Escola de Agronomia, Campus Samambaia of the Universidade Federal de Goiás (16°35'58.5" S, 49°16'45.8"W), isolated as an endophyte from branches of *B. gaudichaudii* (*Moraceae*), 10 Oct. 2020, T.M. da Silva & J.D.P. Bezerra, isolates FCCUFG 50 = HR16 and FCCUFG 51 = HR25.

Notes: *Diaporthe raonikayaporum* was proposed by Gomes *et al.* (2013) for endophytes obtained from the leaves of *Spondias mombin* in the Amazon Forest (Brazil). In our phylogenetic inferences (Fig. 2, Figs S1–S6), the clade of *D. raonikayaporum* contained six isolates, including four isolates from our study and the ex-type of *D. neoraonikayaporum* found in *Tectona grandis* in Thailand (Doilom *et al.* 2017). These species appeared phylogenetically and morphologically indistinguishable, and therefore *D. neoraonikayaporum* is synonymised under *D. raonikayaporum*. In the present study, *D. raonikayaporum* was also reported to be an endophyte in the Caatinga and Cerrado biomes, thereby expanding its geographical distribution and host range. A revised description is included to expand the circumscription of *D. raonikayaporum* and allow for morphological and phylogenetic distinctions from related species.

Diaporthe samambaiaensis L.O. Ferro, Souza-Motta & J.D.P. Bezerra, **sp. nov.** MycoBank MB 852380. Fig. 3G.

Etymology: The name refers to the Campus Samambaia of the Universidade Federal de Goiás, where the fungus was first collected.

Cultures non-sporulating on PDA and WA. *Diaporthe samambaiaensis* (URM 8913) differs from its closest phylogenetic neighbour, *D. maytenicola* (CBS 136441), by unique fixed alleles in two loci based on alignments of the independent loci: ITS positions 104 (C), 110 (–), 111 (C), 116 (A), 182 (C), 404 (C), 415 (T), 433 (T), 436 (T), 438 (C), 439 (G), 469 (G), and 514 (C); *tub2* positions 332 (A), 349 (G), 425 (–), 432 (A), 437 (G), 521 (C), 527 (T), 533 (T), 545 (G), 550 (A), 553 (T), 568 (T), 576 (G), 578 (C), 581 (T), and 614 (C); and from *D. rauvolfiae* (CBS 148912) by unique fixed alleles in five loci based on alignments of the independent loci: ITS positions 90 (T), 104 (C), 110 (–), 111 (C), 116 (A), 12 (T), 134 (A), 135 (A), 153 (C), 182 (C), 206 (C), 404 (C), 415 (T), 428 (T), 434 (T), 435 (C), 436 (T), 437 (C), 438 (–), 439 (G), 442 (G), 445 (A), 461 (C), 469 (G), 491 (C), and 514 (C); *tub2* positions 332 (A), 336 (A), 349 (G), 366 (A), 369 (C), 370 (C), 400 (C), 425 (–), 432 (A), 437 (G), 448 (G), 449 (T), 521 (C), 527 (T), 533 (T), 545 (G), 550 (A), 553 (T), 563 (A), 568 (T), 576 (G), 581 (T), and 613 (G); *cmdA* positions 46 (T), 52 (C), 80 (G), 86 (C), 123 (A), 134 (C), 135 (C), 136 (T), 137 (C), 148 (T), 157 (A), 168 (G), 169 (A), 184 (A), 206 (A), 208 (A), 217 (A), 227 (C), 388 (C); *his3* positions 156 (G), 162 (C), 165 (C), 174 (C), 175 (A), 179 (G), 197 (C), 202 (C), 203 (T), 206 (C), 207 (9T), 208 (A), 209 (C), 291 (T), and 373 (T); *tef1* positions 81 (–), 82 (–), 143 (C), 163 (T), 164 (A), 167 (T), 170 (C), 177 (C), 180 (T), 195 (C), 205 (T), 213 (C), 222 (C), 234 (C), 238 (G), 244 (G), 252 (T), 258 (C), 259 (A), 266 (T), 277 (T), 280 (T), 281 (G), 288 (T), and 303 (C).

Culture characteristics: Colonies covering Petri dish, aerial mycelium, presence of exudates, sandy appearance, hyaline to straw, reverse iron grey at the centre and edges olivaceous buff. Cultures non-sporulating on PDA and WA.

Typus: Brazil, Goiás state, Goiânia municipality, arboretum (didactic area of the Cerrado) of the Escola de Agronomia, Campus Samambaia of the Universidade Federal de Goiás (16°35'58.5"S, 49°16'45.8"W), isolated as endophyte from branches of *Brosimum gaudichaudii* (Moraceae), 10 Oct. 2020, T.M. da Silva & J.D.P. Bezerra (**holotype** URM 8913, preserved as metabolically inactive culture; ex-type culture URM 8913 = FCCUG 52 = Isolate AR9).

Additional specimens examined: Brazil, Goiás state, Goiânia municipality, arboretum (didactic area of the Cerrado) of the Escola de Agronomia, Campus Samambaia of the Universidade Federal de Goiás (16°35'58.5"S, 49°16'45.8"W), isolated as an endophyte from branches of *B. gaudichaudii* (Moraceae), 10 Oct. 2020, T.M. da Silva & J.D.P. Bezerra, isolates URM 8914 = FCCUG 53, AR1, AR7, AR15, BR9, ER2–ER5, IR15.

Notes: *Diaporthe samambaiaensis* (URM 8913) clusters in a strongly supported monophyletic clade containing 11 non-sporulating isolates from the Brazilian savannah (Cerrado), placed in the *D. oncostoma* species complex (Norphaphoun et al. 2022). Our species is phylogenetically related to *D. maytenicola*, which was isolated from the leaves of *Maytenus acuminata* var. *acuminata* in South Africa (Crous et al. 2013). *Diaporthe samambaiaensis* differs from its closest phylogenetic neighbour, *D. maytenicola*, by unique fixed alleles based on alignments of each locus: 13 bp for ITS and 16 bp for *tub2*; no sequences of *D. maytenicola* are available for *cmdA*, *his3*, and *tef1* and from *D. rauvolfiae* by unique fixed alleles based on alignments of each locus: 26 bp for ITS, 23 bp for *tub2*, 19 bp for *cmdA*, 15 bp for *his3*, and 25 bp for *tef1*.

Diaporthe schini R.R. Gomes et al., *Persoonia* **31**: 32. 2013.

See Gomes et al. (2013) for illustrations and descriptions.

Typus: Brazil, Paraná, Curitiba, endophytic species isolated from the leaf of *Schinus terebinthifolius* (Anacardiaceae), Jul. 2007, J. Lima (**holotype** CBS H-21093; culture ex type CBS 133181).

Distribution and hosts: Brazil: *Schinus terebinthifolius* (Gomes et al. 2013), *Anacardium occidentale* (this study); Ecuador: *Solanum quitoense* (Dávila et al. 2018).

Specimens examined: Brazil, Goiás state, Pirenópolis municipality, Reserva Ecológica Vargem Grande (15°48'14.256"S, 48°55'6.204"W), isolated as an endophyte from branches of *A. occidentale* (Anacardiaceae), 12 Oct. 2022, L.O. Ferro & J.D.P. Bezerra, isolates FCCUG 54 = RS4A and FCCUG 55 = RR4.

Notes: *Diaporthe schini* was proposed by Gomes et al. (2013) for endophytes from *Schinus terebinthifolia* in Brazil. In our study, we found that this species lives endophytically in the same family (Anacardiaceae), expanding its geographic distribution and host relationships in Brazilian biomes. *Diaporthe schini* has also been reported in the diseased leaves of *Solanum quitoense* in the Amazon region of Ecuador (Dávila et al. 2018).

Diaporthe ueckeri Udayanga & Castl. [as 'ueckerae'], *Fungal Diversity* **119**: 401. 2014.

Synonyms: *Diaporthe passifloricola* Crous & M.J. Wingf, *Persoonia* **36**: 395. 2016.

Diaporthe rosae Samarak & K.D. Hyde, *Fungal Diversity* **89**: 185. 2018.

Diaporthe vochysiae Noriler et al., *Fitoterapia* **138**: 104273. 2019.

Conidiomata pycnidial, semi-immersed, scattered or aggregated in host tissue or culture medium, erumpent at maturity, globose to pyriform, 35–367 × 80–328 µm, with elongate or short neck, 90–300 µm long, ostiolate with conidial mass predominantly yellow, and yellow to reddish brown, walls parenchymatous or thick of *textura angularis*, consisting of 3–6 layers of medium brown of *textura angularis*. *Conidiophores* hyaline, smooth, branched or unbranched, ampulliform, long, slender, subcylindrical to cylindrical, (9–)10–50 × 1.5–4 µm. *Conidiogenous cells* enteroblastic, phialidic, cylindrical, terminal, slightly tapering towards apex, 7–20 × 0.5–3(–5) µm, collarette occasionally present and flared, with slightly periclinal thickening. *Paraphyses* absent. *Alpha conidia* aseptate, hyaline, smooth, fusoid-ellipsoid or oval, often biguttulate, base subtruncate, (2.5–)3–8(–8.5) × (1.5–)2–4 µm. *Beta conidia* occasionally found, spindle-shaped, aseptate, smooth, hyaline, base truncate, tapering from the lower third towards the apex, curved, (20–)22–25(–27) × 0.5–× 1.5(–2) µm. *Gamma conidia* and *sexual morph* not observed.

Typus: USA, Oklahoma, on crown of *Cucumis melo*, F.A. Uecker (**holotype** BPI 748011; ex-type culture FAU 656 = CBS 139283).

Distribution and hosts: Brazil: *Costus spiralis* (Soares et al. 2018), *Vochysia divergens* (Noriler et al. 2019, lantás et al. 2021), *Stryphnodendron adstringens* (lantás et al. 2021), *Copaifera pubiflora* (Carvalho et al. 2021), *Miconia* sp. (Ferro et al. 2023), *Anacardium occidentale* (this study), *Brosimum gaudichaudii* (this study); China: *Camellia* sp. (Gao et al. 2016), *Michelia shiluensis* (Yi et al. 2018), *Citrus reticulata* (Chaisiri et al. 2021), *Citrus grandis* (Dong et al. 2021), *Eucalyptus citriodora* (Liao et al. 2023); Colombia: *Glycine max* (López-Cardona et al. 2021); Malaysia: *Passiflora foetida* (Crous et al. 2016b), *Hyophorbe lagenicaulis* (Guo et al. 2023), *Nepenthes ampullaria* (Wong et al. 2018), *Mangifera indica* (Lim et al. 2019); Taiwan: *Hylocereus undatus* (Wang et al. 2022); Thailand: *Magnolia champaca* and *Senna siamea* (Perera et al. 2018), *Rosa* sp. (Wanasinghe et al. 2018), *Nephelium lappaceum* (Huanraluek et al. 2022); USA: *Cucumis melo* (Udayanga et al. 2015);

Specimens examined: Brazil, Goiás state, Goiânia municipality, arboretum (didactic area of the Cerrado) of the Escola de Agronomia, Campus Samambaia of the Universidade Federal de Goiás (16°35'58.5"S, 49°16'45.8"W), isolated as an endophyte from branches of *B. gaudichaudii* (Moraceae), 10 Oct. 2020, T.M. da Silva & J.D.P. Bezerra, isolates URM 8911 = FCCUG 56, CF5, ER8, FR2, GR3, GR4, HR9, HR1B, HR13, HR23; Goiás state, Pirenópolis municipality, Reserva Ecológica Vargem Grande (15°48'14.256"S, 48°55'6.204"W), isolated as an endophyte from leaves of *A. occidentale*, 12 Oct. 2022, L.O. Ferro & J.D.P. Bezerra, isolate FCCUG 57 = FR6.

Notes: *Diaporthe ueckeri* was introduced by Udayanga et al. (2015) for a fungus on the crowns of *Cucumis melo* in the USA. Gao et al. (2016) synonymised *D. miriciae* under *D. ueckeri*, and three species (*D. passifloricola*, *D. rosae*, and *D. vochysiae*) were later described and clustered as phylogenetic neighbours of *D.*

ueckeri. *Diaporthe passifloricola* was introduced for a fungus collected on leaf spots of *Passiflora foetida* in Malaysia (Crous *et al.* 2016b), *D. rosae* was described for a saprobic fungus on the dead pedicel of *Rosa* sp. in Thailand (Wanasinghe *et al.* 2018), and *D. vochysiae* was proposed for endophytes from *Vochysia divergens* in Brazil (Noriler *et al.* 2019). We added sequences from our endophytic isolates to the phylogenetic analyses, and the clade containing the species mentioned above appeared to be phylogenetically indistinguishable (Fig. 2, Figs S1–S6). Furthermore, morphological features were not useful in separating the species treated in this clade; therefore, we decided to synonymise *D. passifloricola*, *D. rosae*, and *D. vochysiae* under *D. ueckeri*. In our study, we expanded the host and geographic distribution of *D. ueckeri* and provided an expanded circumscription.

Diaporthe vargemgrandensis L.O. Ferro, Souza-Motta & J.D.P. Bezerra, *sp. nov.* MycoBank MB 852381. Figs 3H, 5C, D.

Etymology: The name refers to the Vargem Grande Ecological Reserve, a conservation unit of the Cerrado where the endophyte was first isolated.

Conidiomata pycnidial, solitary, agglomerate, superficial on plant tissues placed on culture medium surface, globose to subglobose, ostiolate, conidial mass hyaline, pycnidial wall of *textura angularis*, 232.5–260.5(–418.5) × 149–232.5(–279) µm. **Conidiophores** reduced to conidiogenous cells. **Conidiogenous cells** enteroblastic, phialidic, hyaline, filiform, subcylindrical to cylindrical, attenuate towards the apex, 7.5–10.5 × 2–2.5 µm. **Alpha conidia** hyaline, aseptate, fusiform, guttulate, acute at apex, a hilum-like protuberance at base occasionally observed, (5–)6–6.5 × (1.5–)2–2.5 µm. **Beta conidia** hyaline, aseptate, filiform, straight to curved, curved at apex, truncate base, (12–)14–18.5(–25) × 1–1.5 µm. **Gamma conidia** and **sexual morph** not observed.

Culture characteristics: Colonies covering Petri dish, flat with concentric zones, velvety appearance, discretely sulcate at the centre, smoke grey and zones honey to ochreous with whitish edges, reverse amber to sienna.

Typus: **Brazil**, Goiás state, Pirenópolis municipality, Reserva Ecológica Vargem Grande (15°48′14.256″S, 48°55′6.204″W), isolated as an endophyte from branches of *Anacardium occidentale* (Anacardiaceae), 12 Oct. 2022, L.O. Ferro & J.D.P. Bezerra (**holotype** UFG 37194; ex-type culture URM 8784 = FCCUGF 58 = Isolate RN3A).

Additional specimen examined: **Brazil**, Goiás state, Pirenópolis municipality, Reserva Ecológica Vargem Grande (15°48′14.256″S, 48°55′6.204″W), isolated as an endophyte from branches of *A. occidentale*, 12 Oct. 2022, L.O. Ferro & J.D.P. Bezerra, isolate FCCUGF 59 = LF135.

Notes: *Diaporthe vargemgrandensis* (URM 8784) is related to a single strain of *D. racemosae* (CPC 26645) isolated from the diseased tissue of *Euclea racemosa* in South Africa (Marin-Felix *et al.* 2019b), and their pairwise nucleotides differed by 2 bp for *tub2*, 8 bp for ITS, 10 bp for *tef1*, 24 bp for *cmdA*, and 42 bp for *his3*. Morphologically, *D. vargemgrandensis* has slightly larger conidiogenous cells (7.5–10.5 × 2–2.5 µm) than *D. racemosae*

(5.5–8 × 1–2 µm) and has beta conidia, which are not observed in *D. racemosae* (Marin-Felix *et al.* 2019b). We placed *D. vargemgrandensis* in the *Diaporthe sojae* species complex.

DISCUSSION

In this study, 180 endophytic *Diaporthe* isolates were collected from *A. occidentale* (Anacardiaceae), *B. gaudichaudii* (Moraceae) and *Miconia* sp. (Melastomataceae) in three Brazilian biomes (Atlantic Forest, Caatinga, and Cerrado). Multigene analyses revealed that the isolates were distributed across 14 species of *Diaporthe*, including seven new species (*D. azevedoi*, *D. catimbauensis*, *D. coracoralinae*, *D. luizorum*, *D. pedratalhadensis*, *D. samambaiaensis*, and *D. vargemgrandensis*) and seven known species (*D. fructicola*, *D. inconspicua*, *D. infertilis*, *D. paranensis*, *D. raonikayaporum*, *D. schini*, and *D. ueckeri*). Three of the known species were positioned in clades with other related species, with few differences in their DNA sequences and overlapping morphological characteristics. Therefore, we treated *D. pseudoinconspicua*, *D. lutescens*, and *D. samanae*, and the isolates named as “*D. pterocarp*” as synonyms under *D. inconspicua* (Gomes *et al.* 2013, Crous *et al.* 2018a, Sun *et al.* 2021, Monkai *et al.* 2023). In addition, *D. neoraonikayaporum* was synonymised under *D. raonikayaporum* (Gomes *et al.* 2013, Doilom *et al.* 2017) and *D. passifloricola*, *D. rosae*, and *D. vochysiae* were synonymised under *D. ueckeri* (Crous *et al.* 2016b, Wanasinghe *et al.* 2018, Noriler *et al.* 2019). Our results highlight the important richness of *Diaporthe* species isolated as endophytes from Brazilian biomes and their ability to live in different habitats and hosts.

Among the *Diaporthe* species identified in this study, *D. catimbauensis* *sp. nov.* was the most common, representing 52.75 % of the isolates. Another species, *D. fructicola*, to the best of our knowledge, is reported for the first time as an endophyte from Brazil. The endophytic species obtained in this study have also been reported as saprophytes and phytopathogens. For example, *D. infertilis* was isolated from decomposing *Citrus sinensis* (Guarnaccia & Crous 2017) and *D. paranensis* causing peach tree rot (de Souza *et al.* 2021), showing that previously reported pathogenic *Diaporthe* species may also be endophytes in Brazilian biomes. This fact reinforces the need to investigate the mycobiome, in which many pathogens may live as endophytes or latent pathogens (Crous & Groenewald 2005). The non-selectivity of hosts for *Diaporthe* species was also demonstrated in our study, where the same species was found in more than one host and in different biomes. For example, *D. azevedoi* *sp. nov.* was isolated from *A. occidentale* in the Caatinga and Cerrado forests, and from *Miconia* sp. in the Atlantic Forest. Similarly, *D. catimbauensis* *sp. nov.* and *D. luizorum* *sp. nov.* were isolated from *A. occidentale* in the Caatinga and Cerrado regions. Further study including tropical/endophytic isolates will help us uncover the host specificity for the newly introduced species.

Diaporthe is known as a paraphyletic group of fungi, and the definition of species is still complex and often difficult to resolve, making this genus a good example for understanding distribution and plasticity in lifestyle and taxonomy (Gao *et al.* 2017). Recent morphological and multi-locus phylogenetic analyses have contributed to delimiting new *Diaporthe* species and reclassifying them (Udayanga *et al.* 2012, Gomes *et al.* 2013, Fan *et al.* 2018). Based on these analyses, species groups have

already been reported to be species complexes, for example, *Diaporthe sojae* and *Diaporthe arecae* species complexes (Huang *et al.* 2015, Udayanga *et al.* 2015). Recently, Norphanphoun *et al.* (2022) analysed sequences from 535 strains using a combined matrix of five genes and proposed the inclusion of 13 species complexes to facilitate the understanding of *Diaporthe* species. Our isolates grouped into two *Diaporthe* species complexes, *Diaporthe sojae* and *Diaporthe oncostoma* species complexes (Norphanphoun *et al.* 2022). Furthermore, two new species (*D. azevedoi* and *D. catimbauensis*) were introduced, along with a new complex, the *Diaporthe ocoteae* species complex to accommodate them.

Similar to other genera (e.g. *Colletotrichum*) (Damm *et al.* 2013, Hilário *et al.* 2021), the difficulty in resolving some well-related *Diaporthe* species may have a distinct understanding with the inclusion of new species, known species with recent molecular data, and the addition of isolates in less representative phylogenetic clades or species, thus minimising taxonomic confusion (Fan *et al.* 2018, Hilário *et al.* 2021). Hongsanan *et al.* (2023) provided a detailed review of accepted or presumed *Diaporthe* species and suggested taxonomic changes for some species, demonstrating the importance of evaluating *Diaporthe* based on highly robust and updated analyses. With the addition of our isolates to the multigene analyses presented here, we were able to resolve species that had a doubtful relationship among the clades, proposing, through robust phylogenetic support, a new treatment for very closely related species, helping to solve some of the complexities that still exist among *Diaporthe* species.

Our study showed a wide richness of endophytic *Diaporthe* isolates/species in plants from the Atlantic Forest, Caatinga, and Cerrado biomes in Brazil, reinforcing the idea that natural areas harbour endophytic mycodiversity that is still underestimated. Similarly, as highlighted by Xiao *et al.* (2023) in a large study of *Diaporthe* species in China, additional novel species are likely to be found in the future among endophytic isolates from Brazilian biomes. Our data also contribute to the knowledge of the geographic distribution, host relationships, and estimation of *Diaporthe* diversity, providing important information about fungal species in the ecoregions of Brazil.

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Fig. S1. Maximum Likelihood (ML) phylogenetic tree of the *tub2* sequences (big tree). The species/isolates obtained in this study are in **bold**. Ex-type strains are marked with an asterisk (*). Confidence values for ML-BS $\geq 70\%$ (RAxML) and BPP ≥ 0.95 are included near the nodes and the “-” indicates statistical support below the threshold values. The scale bar represents the expected number of changes per site. The tree was rooted to *Diaporthella corylina* (CBS 121124).

Fig. S2. Maximum Likelihood (ML) phylogenetic tree of the representative *tub2* sequences (small tree). The species/isolates obtained in this study are in **bold**. Ex-type strains are marked with an asterisk (*). Confidence values for ML-BS $\geq 70\%$ (RAxML) and BPP ≥ 0.95 are included near the nodes and the “-” indicates statistical support below the threshold values. The scale bar represents the expected number of changes per site. The tree was rooted to *Diaporthella corylina* (CBS 121124).

Fig. S3. Maximum Likelihood (ML) phylogenetic tree of the representative ITS sequences. The species/isolates obtained in this study are in **bold**. Ex-type strains are marked with an asterisk (*). Confidence values for ML-BS $\geq 70\%$ (RAxML) and BPP ≥ 0.95 are included near the nodes and the “-” indicates statistical support below the threshold values. The scale bar represents the expected number of changes per site. The tree was rooted to *Diaporthella corylina* (CBS 121124).

Fig. S4. Maximum Likelihood (ML) phylogenetic tree of the representative *tef1* sequences. The species/isolates obtained in this study are in **bold**. Ex-type strains are marked with an asterisk (*). Confidence values for ML-BS $\geq 70\%$ (RAxML) and BPP ≥ 0.95 are included near the nodes and the “-” indicates statistical support below the threshold values. The scale bar represents the expected number of changes per site. The tree was rooted to *Diaporthella corylina* (CBS 121124).

Fig. S5. Maximum Likelihood (ML) phylogenetic tree of the representative *cmdA* sequences. The species/isolates obtained in this study are in **bold**. Ex-type strains are marked with an asterisk (*). Confidence values for ML-BS $\geq 70\%$ (RAxML) and BPP ≥ 0.95 are included near the nodes and the “-” indicates statistical support below the threshold values. The scale bar represents the expected number of changes per site. The tree was rooted to *Diaporthella corylina* (CBS 121124).

Fig. S6. Maximum Likelihood (ML) phylogenetic tree of the representative *his3* sequences. The species/isolates obtained in this study are in **bold**. Ex-type strains are marked with an asterisk (*). Confidence values for ML-BS $\geq 70\%$ (RAxML) and BPP ≥ 0.95 are included near the nodes and the “-” indicates statistical support below the threshold values. The scale bar represents the expected number of changes per site. The tree was rooted to *Diaporthella corylina* (CBS 121124).

Table S1. Gene regions used in this study, respective primer pairs, PCR protocols, and references.

Table S2. GenBank accession numbers of sequences of *Diaporthe* species used in the phylogenetic analyses. Isolates and sequences obtained in this study are given in **bold**.

