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MDD

Mycological Diversity Description II

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ABSTRACT

Here, *Diaporthe myracrodruonis* is introduced as new species from Brazil, isolated as endophyte from *Myracrodruon urundeuva*. *Asterina mandaquiensis* is epitypified and ilustrated for the first time. *Serpula similis* is reported as new to the Neotropics, while *Perenniporia centrali-africana* is reported for the first time as endophyte and *Preussia africana* as endophyte from *Spondias tuberosa* in Caatinga in Brazil.

Keywords: Asterinales, Boletales, *CaM*, Diaporthales, ITS rDNA, LSU rDNA, Pleosporales, Polyporales, *tef1-α*, TUB2

Perenniporia centrali-africana *Decock & Mossebo, Sytematics and Geography of Plants 71 (2): 608 (2001)*

(Fig. 1)

Culture characteristics: Colonies on PDA growing up to 50 × 55 mm diam. after 10 days at 25 °C, cottony, without pigment, surface white, reverse plate white. Colonies on MEA 45 × 50 mm diam., cottony, surface white, reverse plate white. *Hyphal system* dimitic to trimitic, generative hyphae with clamp-connection, thin-walled, hyaline, branched, 2–3 µm wide; skeletal hyphae thick-walled, unbranched, hyaline, slightly dextrinoid in Melzer's reagent, 2–4 µm wide. Connective hyphae branched and sinuous, thin, without clamp-connection, 2 µm wide. Chlamydospore-like structures terminal or intercalary, smooth, globose to sub-globose, 10 × 5 µm. Reproductive structures basidia and basidiospores absent.

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Material examined: **BRAZIL, Pernambuco:** Petrolândia, isolated as endophyte in leaves of *Citrullus lanatus* crops (Cucurbitaceae), 25 July 2016, *R.M.F. Silva* (strain: URM 7859; GenBank MH330690).

Notes — *Perenniporia* Murrill is a cosmopolitan genus with the ability to colonize various habitats and substrates and has been reported as endophytes in several plants, such as stems of *Theobroma gileri* (Evans *et al*. 2003), leaves of palm (Pinruan *et al*. 2010) and leaves of bamboo (Zhou *et al*. 2017). The type material of *P. centrali-africana* was first reported on dead wood of an angiosperm in Cameroon (Decock & Mossebo, 2001). Since the original description, there has been only one other report of this species in the world, which was found on decaying wood in Brazil (Crous *et al*. 2017). *Perenniporia centrali-africana* was identified based on phylogenetic analysis using ITS rDNA sequences, and is reported here for the first time as endophyte. In addition, chlamydospore-like structures are also reported for the first time for the species. The sequence obtained showed 99 % identity with other sequences deposited as *P. centrali-africana* (GenBank: KX584433). The phylogenetic analysis resolved our sequence as closely related to other sequences of *P. centrali-africana* (Fig. 2).

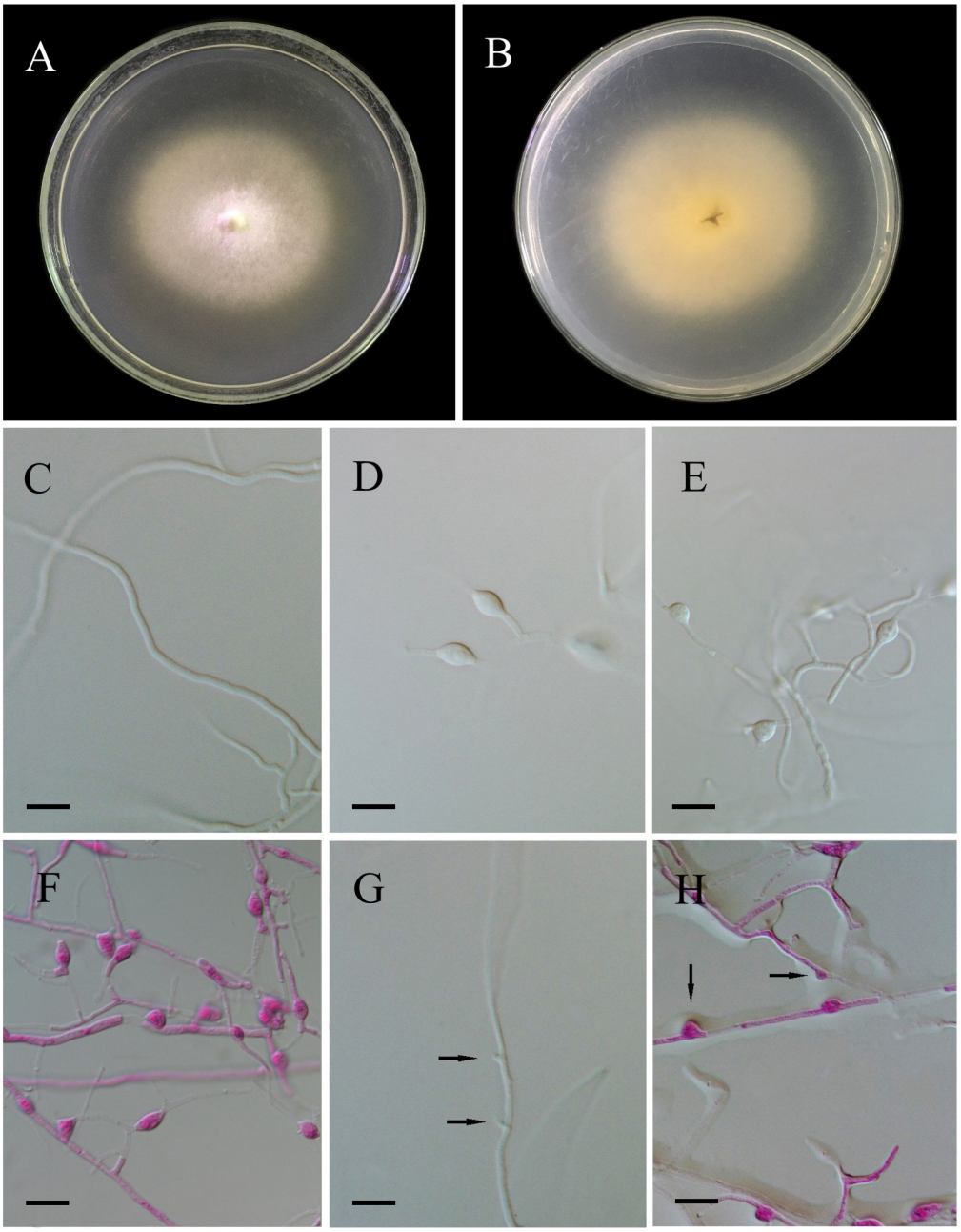

Figure 1. *Perenniporia centrali-africana*. **A-B.** Culture on PDA after 10 days (B. reverse); **C.** Skeletal hyphae. **D-F.** Chlamydospores-like structures. **G-H.** Generative hyphae with clamp connection (arrowed). Scale bars: 10 μm.

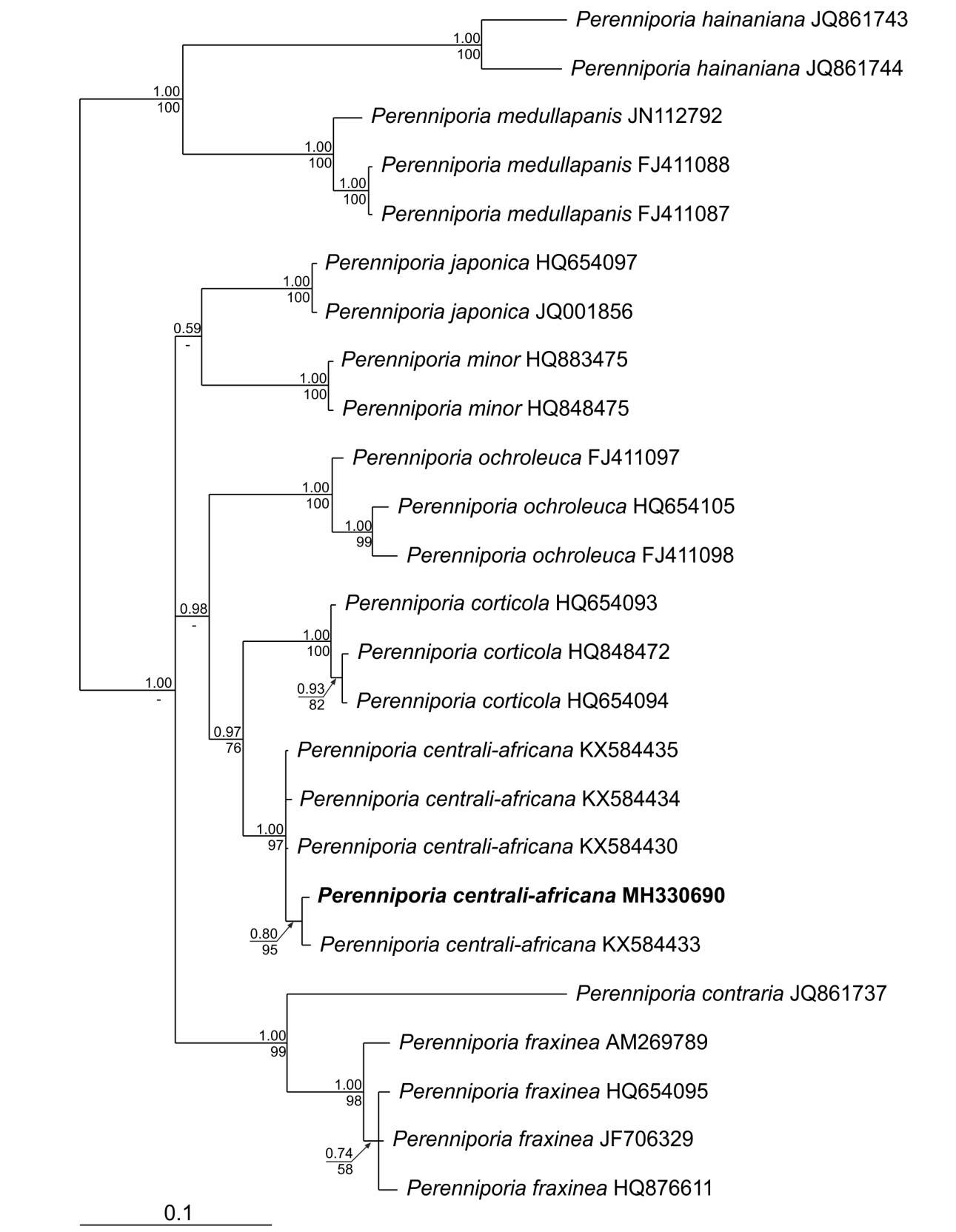

Figure 2. Phylogenetic tree of the *Perenniporia* constructed using ITS rDNA sequences, showing the position of *P. centrali-africana* isolated from watermelon leaves. The sequence obtained in this study is in boldface. Support values are from Bayesian inference and maximum likelihood analyses, respectively.

Serpula similis *(Berk. & Broome) Ginns, Mycologia 63(2): 231 (1971)*

= *Merulius similis* Berk. & Broome, J. Linn. Soc., Bot. 14(no. 73): 58 (1873) [1875]

Description (updated from Ginns 1971): basidiomata effuse, effuse-reflexed to pileate, separable from the substrate, semicircular, up to 4 cm in radius, approximately 2 mm thick at the base, soft. Upper surface pale brown with small darker patches where touched; glabrous, smooth when fresh; slightly irregularly folded or wrinkled due to shrinking when dry. Hymenial surface yellow-brown, brittle, somewhat shiny, with a reticulate to merulioid pattern of shallow, reticulate to irregular, shallow pores, 2–3 pores per mm, becoming more shallow towards margin, tubes brown, dense, fragile (01.–0.3 mm deep). Margin buff to pallid or tan, thick, pruinose or matted tomentose, up to 1 mm wide; rhizomorphs lacking; context pallid, spongy, approximately 1 mm thick, homogeneous. Hyphal system mono- to dimitic, context hyphae interwoven, generative hyphae hyaline, thin-walled, with clamp connections (1.5–7.5 µm wide), skeletal hyphae refractive, thick-walled, often with no lumen, very occasionally branched, nonseptate, often abruptly constricted to smaller diameter (0.4–4 µm wide); crystals numerous in context, scattered but not incrusting hyphae. Basidiospores bright yellow, thick-walled, smooth, ellipsoid to broadly-ovate, adaxially flattened [4–5 (–7) × 3– 3.5 (–4.5) µm], IKI–.

Material examined: **BRAZIL. Amapá**: Porto Grande, Floresta Nacional do Amapá, Sept. 2013, Feb. 2014, Oct. 2014, Feb. 2015, A. Soares (URM 89847, URM 89848, URM 89849, URM 89851, URM 89852). **CAMEROON**. **Campo**: Akok Lowland Rain Forest. Dec 1991, M. Nuñez & L. Ryvarden (O F909008). **GHANA**. **Prov**. **Ashanti Region**: Bibiri Forest Reserve. Oct, 2010. M. M. Apetorgbor (O F505801). **THAILAND**. **Cangwat Chiang Mai**: Amphoe Muang. Aug 1978. T. Schumacher (O F909006). **ZAIRE**. **Irangi (Kivu)**: Primary Rain Forest. Apr 1972. J. Rammeloo (O F909005).

GenBank accession numbers: URM 89847: ITS MH458401, LSU MH404846; URM 89851: ITS MH453543, LSU MH453544.

Notes: This is the first record of *S. similis* from the Neotropics. It was originally described from Sri Lanka and it was previously reported from tropical regions in Africa, Southeast Asia and Australia (Skrede *et al*. 2011). In the phylogenetic tree, the specimens of *S. similis* from Brazil clustered with specimens from Australia and Thailand with strong support (100 %/1.00). The specimens from Brazil (Fig. 3) are characterized by distinctly pileate basidiomata, white to pale cream (when fresh) and yellow to dark orange (when dried). The hyphal system is monomitic, with generative hyphae with larges clamps (3–5 mm). These morphological features are slightly different from the original description, which reports an effused basidioma and a dimitic hyphal system. Although many studies describe *S. similis* as having an effused basidioma (Ginns 1971; Carlier *et al*. 2004), analysis from the material deposited in the Herbarium of the University of Oslo (O) revealed that some specimens from Africa are also pileate (O F505801; O F909008). Initially, the Brazilian specimens were thought to be a new species due to these morphological differences, but the molecular data showed that they represent only a variation of the species.

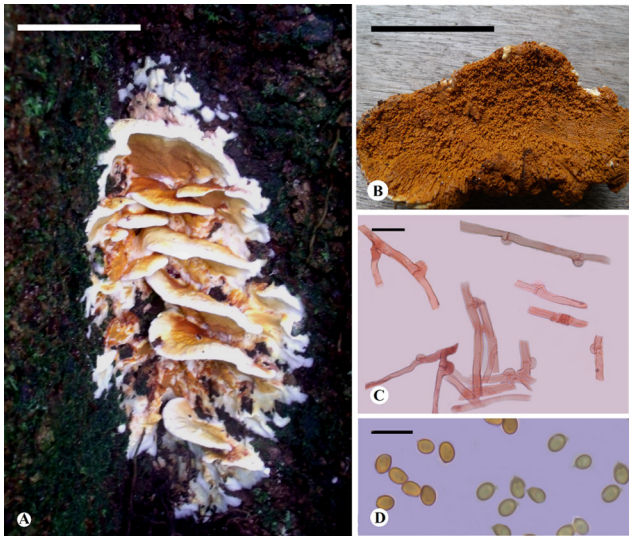

Figure 3. *Serpula similis* **(**URM 89847): **A.** Fresh basidiomata. **B.** Hymenial Surface. **C.** Generative hyphae with clamp connections. **D.** Yellow basidisopores. Scale bars: a, b=10 cm. c, d= 5 µm. Figure: A.M.S. Soares and R. Alvarenga. The macro- (morphology, color, consistency, size) analyses were performed on both fresh and dehydrated basidiomata. Free-hand sections from hymenium and context were visualized under optical microscopy using 3 % KOH + 1 % phloxine, and Melzer's reagent (Ryvarden 1991) Color designation followed Watling (1969).

Serpula costaricensis M. Mata & Ryvarden is another similar species. As in *S. similis*, the basidioma is distinctly pileate; however, it is larger and more robust and has ellipsoid, bigger basidiospores [6–7 (8) × 4–5 µm] (Mata & Ryvarden 2007). *Serpula fuscescens*, the only species reported to Brazil so far, has encrusted cystidia and subglobose to ovoid basidiospores [(4.5–) 5–6 × 4–5 µm] (Nakasone 2008).

As showed in the phylogenetic tree, *S. similis* is closely related to the *S. tignicola* (Harmsen) M.P. Christ. with high statistical support (89/1.00) (Fig. 4). *Serpula tignicola* is a European species and differs from *S. similis* by the resupinate basidioma with folded to raduloid hymenium and rhizomorphs (Harmsen 1952; Christiansen 1960). Besides, these two species show considerable genetic difference, as can be seen from the branches in the phylogram (Fig. 4). This particular attribute was also noticed by Carlier *et al*. (2004) in their analyses using only nLSU and by Skrede *et*

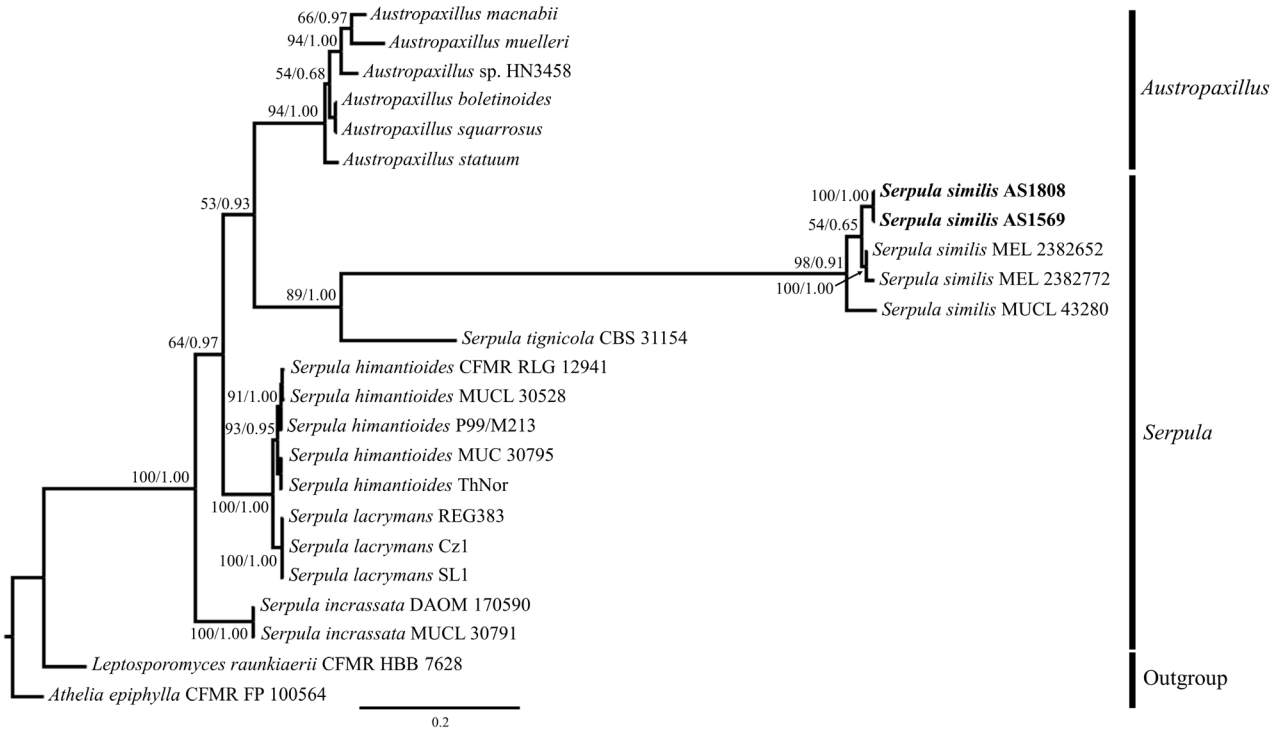

Figure 4. Phylogenetic tree inferred from ITS and LSU rDNA gene sequences using Bayesian and maximum likelihood analyses with the GTR + G evolution model retrieved by PartitionFinder v1.1.0 (Lanfear *et al*. 2012). Bayesian and likelihood trees showed the same topology displayed here. Branch lengths reflect estimated number of 0.2 changes per site. Bayesian analysis was performed with MrBayes 3.2.1 software (Ronquist *et al*. 2012) for 10 million generations with four Markov chains. Likelihood analysis was performed with the same evolutionary model under 1000 rearrangements using RAxML (Stamatakis 2014). Bootstrap support (BS) values after 1000 rearrangements and posterior probabilities are indicated on tree nodes. Branches with PP > 0.95 and BS from 90 % are considered as strongly supported.

al. (2011), using five molecular markers. The latter study suggested that the long branch indicates an increased rate of molecular evolution in this lineage.

Phylogenetically, *S. similis* and *S. tignicola* are distantly related to the clade where *S. lacrymans*, the type species of *Serpula*, is placed, indicating that they belong to a different genus. However, additional morphological and molecular analyses of *Serpula s.l.* through the inclusion of additional specimens and markers, are necessary for a better circumscription of the genus.

Asterina mandaquiensis *Henn., Hedwigia 48: 12. 1908.*

MycoBank MB 223742, Figs. 5–6

Description: Colonies epiphyllous, circular, single to confluent, black, 0.5–4 mm diam. Hyphae straight to slightly flexuous, branching unilaterally or alternately, ferruginous to brown, septate, hyphal cells cylindrical, 4.5–5 µm diam. (2–3.5 µm), smooth. Appressoria numerous, entire, sessile, lateral, alternate to unilateral, never opposed, ovate, unicellular, facing forward, 6–10 × 5–7 µm, brown, penetration peg central on the appressorial cell. Ascomata superficial, thyriothecia, scutiform, on top of mycelial mat, circular, single to confluent, fringed at margins, randomly distributed in the colony, 142–218 µm diam. (200–250 µm diam.), opening by a central star-shaped fissure, dark brown to blackish; wall of textura radiate, cells isodiametric to cylindrical. Pseudoparaphyses cylindrical, filiform, septate, unbranched, hyaline to yellowish, up to 1.5 µm wide. Asci bitunicate in structure, fissitunicate, disposed as an upright palisade layer, ovoid, 8-spored, hyaline, 50–65 × 25–35 µm (35–50 × 20–30 µm). Ascospores cylindrical to ellipsoid, ends rounded, straight to arched, 1-septate, constricted at the median septum, hyaline, becoming brown at maturity, smooth, 22.5–27.5 × 7.5–10.5 µm (20–25 × 8–10 µm). Asexual morph not observed. Measurements of the structures in the original description are in parentheses.

Material examined: **BRAZIL. Minas Gerais**: Viçosa, Mata da Biologia (20°45'32.375"S 42°51'44.154"W), on living leaves of *Eugenia uniflora* L. (Myrtaceae), May 2012, *A.L. Firmino* (VIC42824, epitype designated here).

GenBank accession number: LSU MH780924; ITS MH780973

Notes — The specimen described above was collected in the state of Minas Gerais on living leaves of *Eugenia uniflora*.

The original material was described by Hennings (1908) based on material from the Mandaqui, a neighbourhood in São Paulo city, Brazil, on leaves of the same host. Mandaqui is part of Serra da Cantareira, and the type material was collected by Martio in 1903, however, with the extensive expansion of the city of São Paulo, the native vegetation was totally destroyed, making it impossible to recollect this material at the same place. This species is illustrated here for the first time.

Phylogenetic analyses reveal that the *A. mandaquiensis* is well segregated from the other members of the family Asterinaceae (Fig. 6).

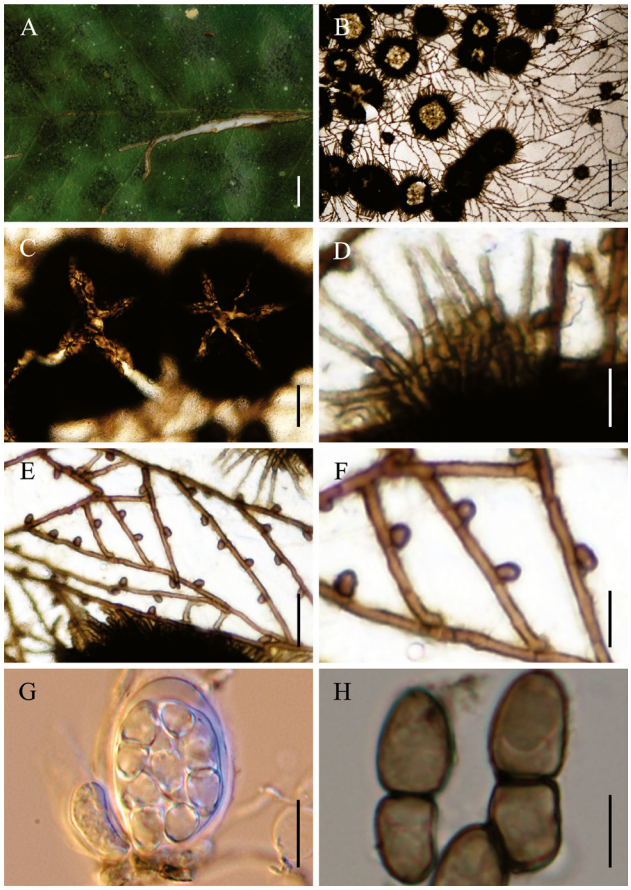

Figure 5. *Asterina mandaquiensis* (VIC42824, epitype). **A.** Colonies on leaf surface. **B.** Colony with open thyriothecia and surface mycelium. **C.** Thyriothecia opened by a central star-shaped fissure. **D.** Thyriothecium fringed at margins. **E.** Branched mycelium with appressoria. **F.** Alternate to unilateral appressoria. **G.** Ovoid ascus with immature hyaline ascospores. **H.** Brown and smooth mature bicelular ascospores. **Scale bars:** A= 4 mm; B= 200 µm; C-E= 50 µm; D-F–G= 20 µm; H= 10 µm.

Preussia africana *Arenal, Plantas & Peláez, Fungal Diversity 20: 6. 2005.*

Fig. 7

Description: *Ascomata* scattered, partially immersed in culture media. *Pseudothecia* scattered and isolated, mostly below the mycelium and partially immersed in culture, globose to pyriform, ostiolate, brown to dark brown, (52–) 91–99(–143) × 52–78(–104) µm in diameter. *Peridium* dark brown, glabrous, pseudoparenchymatous in surface view, membranaceous, coriaceous. *Pseudoparaphyses* filiform, septate, interspersed with asci. *Asci* bitunicate, without opening, apex rounded, 39–47(–65) × (13–)15.5–18 µm, short and robust stipe up to 9 × 5 µm, cylindrical-clavate, eight-spored. *Ascospores* uniseriate to biseriate, cylindrical, dark brown; four-celled, cells separable at the central septa with shallow constrictions, rounded apex, 26–28.5 × 5–5.5 µm; gelatinous sheath hyaline and narrow.

Cultures (in the dark, 25 °C, 2 wk): Colonies on PDA attaining 13 mm diam. and on MEA 29 mm diam. Texture floccose, initially white to light cream in color, occasionally submerged, reverse from dark brown to black (according to the color charts of Rayner 1970).

Material examined: *Preussia africana*. **BRAZIL. Pernambuco:** Petrolina, isolated as an endophyte from leaves of *Spondias tuberosa* Arruda (Anacardiaceae), May 2017, *V.M. Svedese* and *L.C. Oliani* (strain: URM 7936 = isolate 63V).

GenBank accession number: ITS MH220329.1.

Notes — The genus *Preussia* was proposed by Fuckel (1867) to accommodate species with cleistothecioid ascomata and associated with plant debris, wood or soil (Kruys & Wedin 2009). The relationship between *Preussia* and *Sporormiella* has been questioned by several authors. They are considered synonyms due to their similar morphological features and results of phylogenetic analyses (Kruys & Wedin 2009; Mapperson *et al*. 2014; Kruys 2015; Crous *et al*. 2018). Only *Preussia*, along with five other accepted genera, is included in the family Sporormiaceae in the Outline of Ascomycota: 2017 by Wijayawardene *et al*. (2018). The species treated herein, *P. africana*, was proposed by Arenal *et al*. (2005) based on morphological and molecular analyses of a series of isolates of the genus *Preussia* from different substrates, such as goat dung, zebra dung and *Viburnum tinus* L. leaves. Furthermore, *P. africana* has been isolated as an endophytic fungus from different plant species (Brum *et al*. 2012; Gonzalez-Menendez *et al*. 2017). Phylogenetic analysis (Fig. 8) using the ITS rDNA sequence of our isolate placed it in the same clade with sequences from the type material and other sequences deposited as *P. africana*. The morphological analysis of our isolate, obtained as endophyte, differs in some regards with the description of the type material, mainly concerning pseudothecia diameter (180–290 µm), and size of asci (94–110 × 15–17 µm) and ascospores (32.5–44 × 4–7 µm) (Arenal *et al*. 2005). This is the first report of *P. africana* isolated as endophytic fungus from leaves of *S. tuberosa* (Anacardiaceae) in Caatinga forest in Brazil.

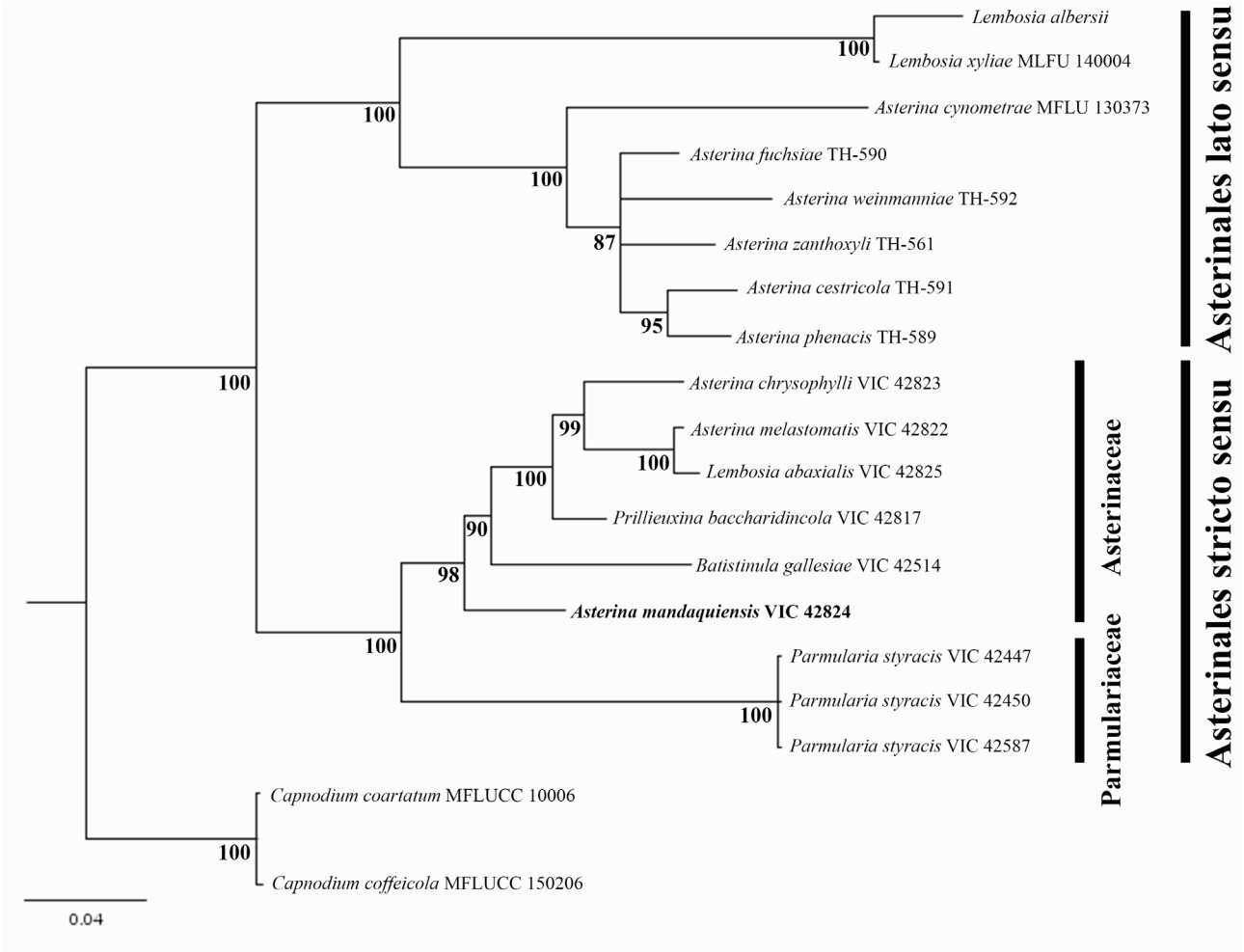

Figure 6. Phylogenetic tree obtained from Bayesian Inference analysis using the sequences of the LSU rDNA obtained from data set of 17 taxa including representatives of Asterinales *stricto sensu*, *lato sensu* and the epitype of *Asterina mandaquiensis* (highlighted in bold). *Capnodium coaratum* MFLUCC 10006 and *Capnodium coffeicola* MFLUCC 150206 were used as outgroup.

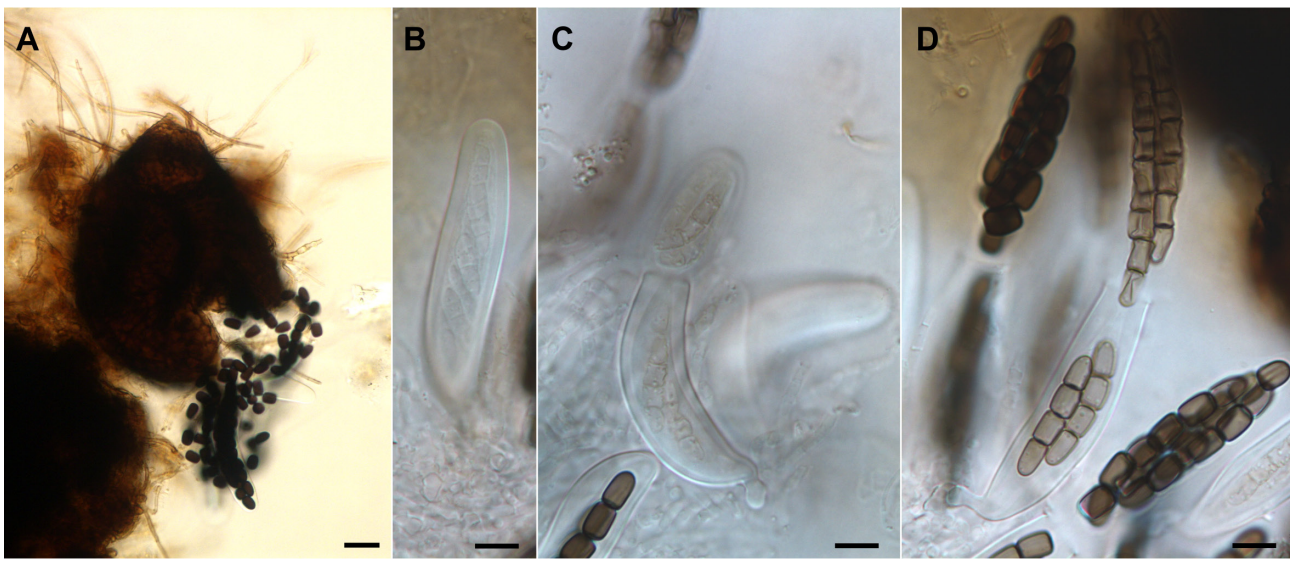

Figure 7. *Preussia africana* (URM 7936). **A.** Ascoma, asci and ascospores. **B-C.** Developing asci with young ascospores. **D.** Asci and ascospores. Scale bars: A: 20 µm; B-D: 10 µm.

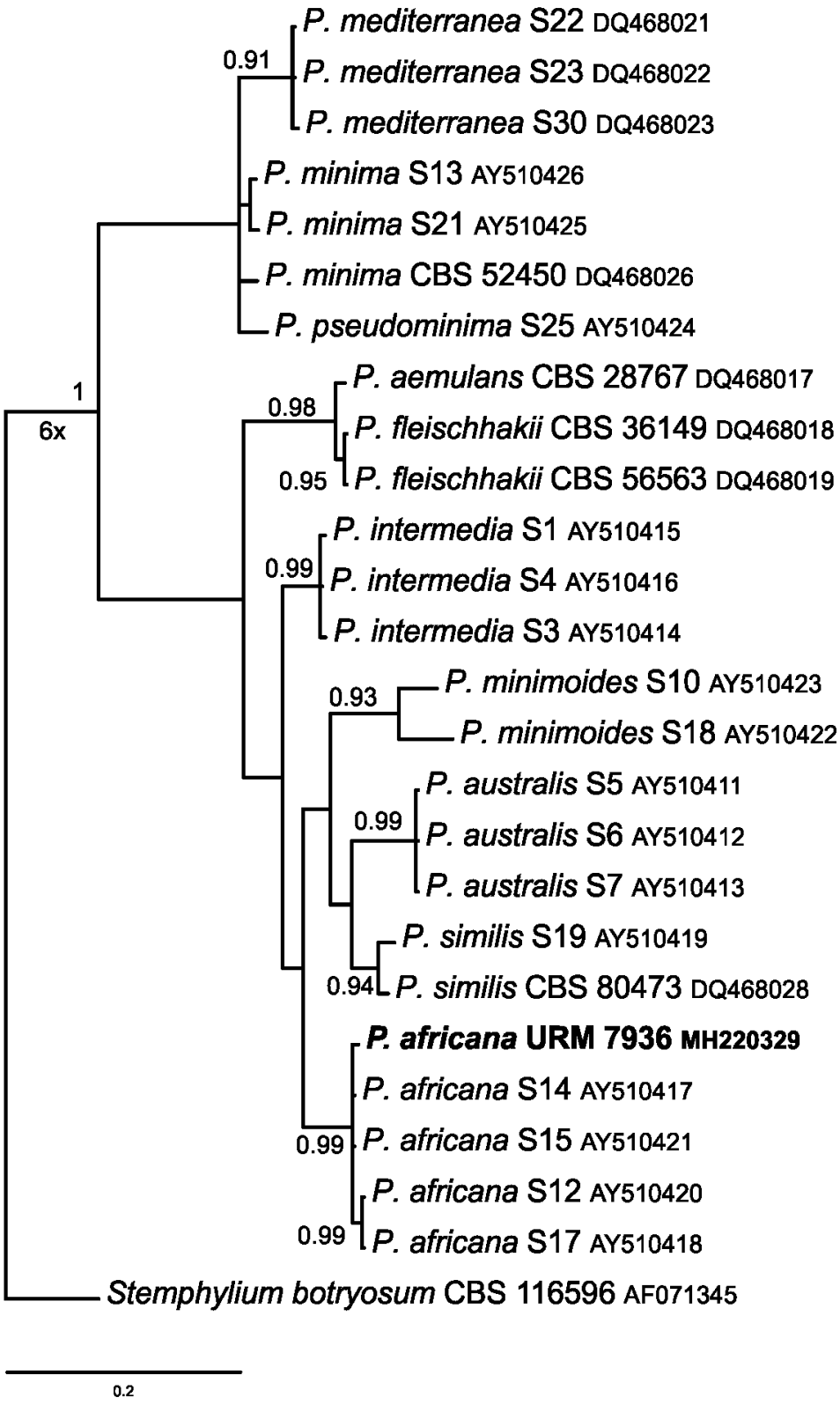

Figure 8. Bayesian inference tree obtained using ITS rDNA sequences of 10 representative species of *Preussia* performed in MrBayes on XSEDE in the CIPRES science gateway (Miller *et al*. 2010) using the model SYM+G as estimated by the MrModelTest v. 2.3 (Nylander 2004). The new sequence obtained in this study is in bold. *Stemphylium botryosum* (CBS 116596) was used as an outgroup.

Diaporthe myracrodruonis *A.P.S.L. Pádua, T.G.L. Oliveira, Souza-Motta, Fan & J.D.P. Bezerra, sp. nov.*

MycoBank MB 828865, Fig. 9

Etymology: The name refers to the host genus from which it was isolated, *Myracrodruon*.

Description: *Conidiomata* pycnidial in culture, globose to subglobose with thin wall, solitary or aggregated, dark brown to black, (424–)636–954(–975) × (286–)318–795(–901) µm, with whitish to light cream conidial drops exuding from the ostioles. *Conidiogenous cells* phialidic, hyaline, smooth wall, occasionally branched, straight to sinuous, thick at base tapering towards the apex, moderately curved apex, (5–)6–9(–11) × 1.5–2(–2.5) µm. *Alpha conidia* aseptate, hyaline, smooth, fusoid or elongated ellipsoid attenuating toward both ends, 2.5–3.5(–4) × 1–1.5 µm. *Beta conidia* sickle-shaped, aseptate, hyaline, smooth, truncated base, curved apex slightly tapered, 18–20(–24) × 0.7–1 µm. *Sexual morph* not observed.

Cultures (in the darkness, 25 °C): Colonies on PDA attaining 9 cm diam., with formation of pycnidia after 15 days. Aerial mycelium of floccose texture, initially white becoming yellowish to brown, with reverse from yellowish to light brown.

Type: **BRAZIL. Pernambuco:** Serra Talhada (7°57'21.38"S 38°17'43.92"W), isolated as endophyte from leaves of *Myracrodruon urundeuva* Allemão (Anacardiaceae), July 2016, A.P.S.L. Pádua (Holotype URM 92587; Culture ex-type URM 7972).

GenBank accession number: URM 7972 (ex-type): ITS MK205289, CaM MK205290, *tef1-α* MK213408, TUB2 MK205291.

Notes — The genus *Diaporthe* has been widely revised, with new species frequently being described (Gomes *et al*. 2013; Gao *et al*. 2015; Yang *et al*. 2018). The strain URM 7972 was isolated from leaves of *Myracrodruon urundeuva* in Brazil (Pádua *et al*. 2018) and identified as *Diaporthe myracrodruonis* sp. nov. The ex-type strain URM 7972 clustered as an independent lineage in the current phylogenetic tree (Fig. 10). BLASTn searches using ITS sequence of *D. myracrodruonis* demonstrated 96 % similarity to *D. infecunda* (LGMF940, GenBank KC343133.1). Based on CaM sequence, *D. myracrodruonis* is 98 % identical to *D. infecunda* (CBS 133812, GenBank KC343368.1). For *tef1-α*, the new species has low identity (95–96 %) to sequences deposited as *D. beilharziae*, *D. middletonii* and *D. infecunda*, and 99 % similarity to sequences deposited as *D. infecunda* (GenBank MG265941 and MG265940). The TUB2 sequence of *D. myracrodruonis*

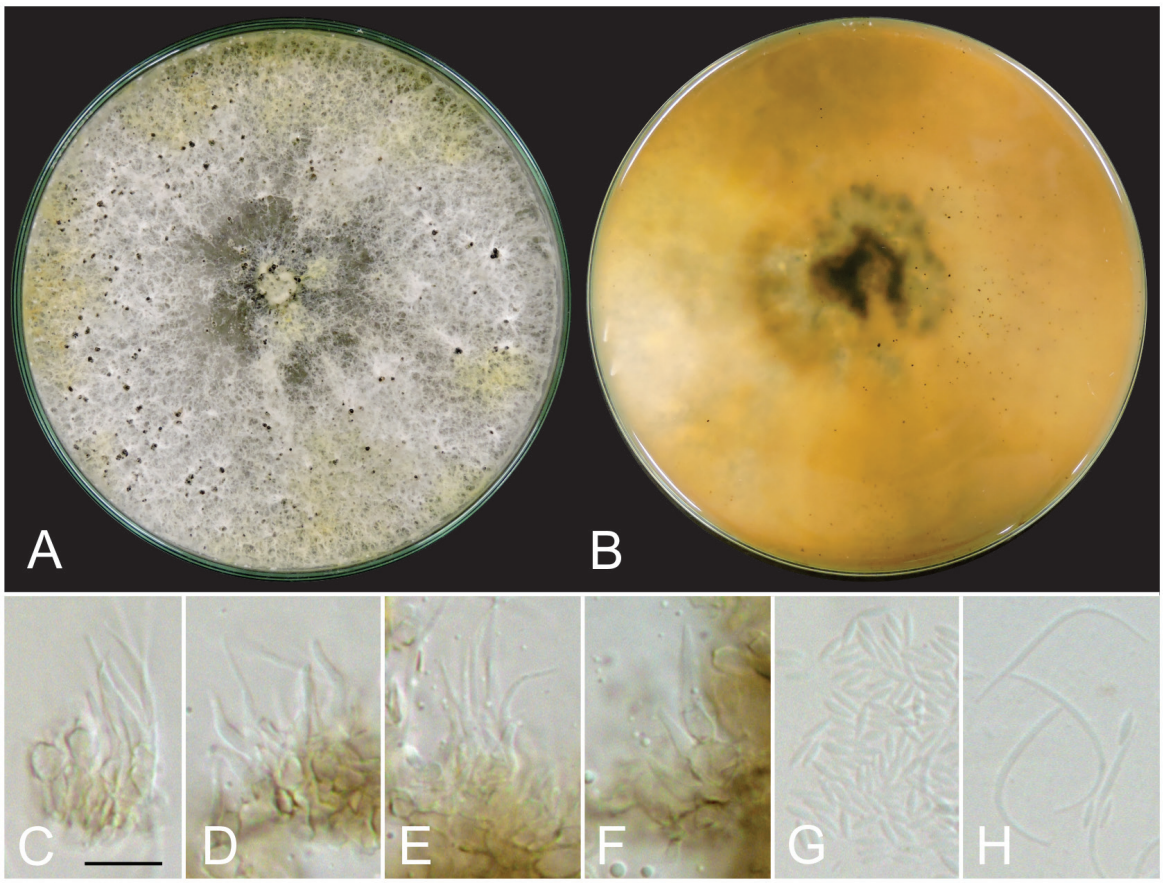

Figure 9. *Diaporthe myracrodruonis* (URM 7972 – ex-type culture). **A-B.** Colony on PDA after 15 days. **C-F.** Conidiogenous cells. **G.** Alpha conidia. **H.** Alpha and beta conidia. Scale bars: 10 µm.

has 96–98 % identity to sequences deposited as *D. acaciarum*, *D. infecunda*, and *D. middletonii*. The most closely related species, *Diaporthe infecunda*, was isolated from medicinal plants in Brazil, and its description was based on phylogenetic analyses using sequences from five genes (Gomes *et al*. 2013). Morphologically, *D. myracrodruonis* differs from *D. beilharzie* in the size of pycnidia (250–300 µm) and alpha (5.5–10 × 2–3 µm) and beta (15–25 × 1.0–1.5 µm) conidia (Tan *et al*. 2013); from *D. acaciarum* in the size of pycnidia (up to 300 µm diam.) and alpha (6–7.5 × 2–3 µm) and beta (20–40 × 1.5–2 µm) conidia (Crous *et al*. 2014); and from *D. middletonii* in the size of pycnidia (up to 300 µm diam.) and alpha (5–8 × 2–3 µm) and beta (20–35 × 1.0–1.5 µm) conidia (Thompson *et al*. 2015). *Diaporthe myracrodruonis* is the first species of *Diaporthe* described as an endophytic fungus from leaves of *Myracrodruon urundeuva* in the Brazilian tropical dry forest (Caatinga).

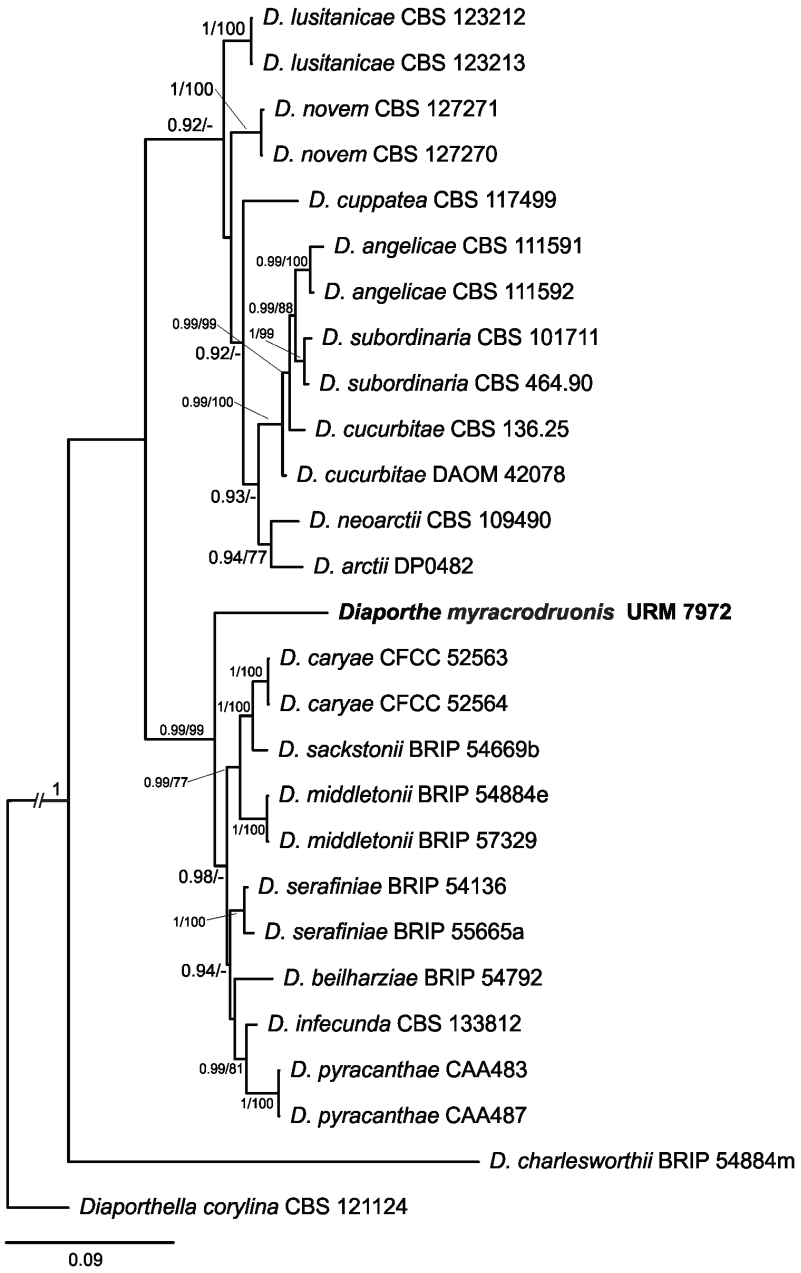

Figure 10. Bayesian inference tree obtained using ITS rDNA, CaM, *tef1-α* and TUB2 sequences of species of *Diaporthe* performed using MrBayes on XSEDE in the CIPRES science gateway with the substitution models GTR+I+G (ITS) and HKY+G (CaM, *tef1-α* and TUB2). The Maximum likelihood analysis using the model GTR+I+G was conducted in RAxML in CIPRES using the combined dataset. BPP and ML-BS above 0.95 and 70 %, respectively, are shown near nodes. The new species is in bold face. *Diaporthella corylina* (CBS 121124) was used as an outgroup.

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